



HAL
open science

A digital twin of liver predicts regeneration after drug-induced damage at the level of cell type orchestration

Jieling Zhao, Ahmed Ghallab, Reham Hassan, Steven Dooley, Jan G Hengstler, Dirk Drasdo

► To cite this version:

Jieling Zhao, Ahmed Ghallab, Reham Hassan, Steven Dooley, Jan G Hengstler, et al.. A digital twin of liver predicts regeneration after drug-induced damage at the level of cell type orchestration. 2022. hal-03738207

HAL Id: hal-03738207

<https://inria.hal.science/hal-03738207>

Preprint submitted on 25 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 A digital twin of liver predicts regeneration after drug-induced 2 damage at the level of cell type orchestration

3 Jieling Zhao^{1,2}, Ahmed Ghallab^{1,3}, Reham Hassan^{1,3}, Steven Dooley⁴, Jan G. Hengstler¹, Dirk Drasdo^{1,2,*}

4 ¹Leibniz Research Centre for Working Environment and Human Factors, Technical University of Dortmund (IfADo),
5 Dortmund, Germany; ²Institut National de Recherche en Informatique et en Automatique (INRIA), Saclay, France;
6 ³Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, South Valley University, Qena,
7 Egypt; ⁴Molecular Hepatology Section, Department of Medicine II, Medical Faculty Mannheim, Heidelberg
8 University, Mannheim, Germany

9 *Correspondance: dirk.drasdo@inria.fr (D.D.)

10 ABSTRACT

11 This communication presents a mathematical mechanism-based model of the regenerating liver
12 after drug-induced pericentral lobule damage resolving tissue microarchitecture. The
13 consequence of alternative hypotheses about the interplay of different cell types on
14 regeneration were simulated. Regeneration dynamics has been quantified by the size of the
15 damage-induced dead cell area, the hepatocyte density and the spatial-temporal profile of the
16 different cell types. We use deviations of observed trajectories from simulated system to
17 identify branching points, at which the systems behavior cannot be explained by the underlying
18 set of hypotheses anymore. Our procedure reflects a successful strategy for generating a fully
19 digital liver-twin that, among others, permits to test perturbations from the molecular up to the
20 tissue scale. The model simulations are complementing current knowledge on liver
21 regeneration by identifying gaps in mechanistic relationships and guiding the system towards
22 the most informative (lacking) parameters that can be experimentally addressed.

23 KEYWORDS

24 Digital liver twin; Liver regeneration; Agent-based model; Liver lobule; DILI; Tissue micro-
25 architecture; Cell types; Cell-cell signaling

26 INTRODUCTION

27 The liver is the main detoxifying organ in the human body to remove drugs and toxins from the
28 blood. It is organized in 2-5 lobes (depending on the species), which itself are composed of
29 hexagonal-shaped repetitive histological and functional units, called lobules. Oxygen-rich blood
30 transported via the hepatic artery from the aorta and oxygen-poor blood carried via the portal
31 veins from the intestine mix up in the periphery of the lobule, perfuse it through a network of
32 fenestrated capillaries (called sinusoids) and then drain into the "central vein" (CV).

33 Administration of a hepatotoxic dose of protein alkylating compounds, e.g., acetaminophen
34 (Paracetamol, APAP, 300 mg/kg, i.p. in mouse), or carbon tetrachloride (CCl₄, 1 g/kg, i.p. in
35 mouse), generate a pericentral damage of about 50% of the hepatocytes in the lobule that is
36 subsequently in most cases perfectly regenerated, both histologically and functionally (Zieve et
37 al., 1985, Hoehme et al., 2007, 2010, Ghallab et al., 2016). APAP is the most frequent cause for
38 acute liver failure (Olson et al., 2017) and a "model substance" to study drug-induced acute
39 liver injury and its adverse outcome on cells, organs, and organisms through a series of
40 successive key events, recently discussed in detail in the context of the popular concept of
41 Adverse Outcome Pathways (AOPs) (Leist et al., 2017).

42 CCl₄ is a frequently studied hepatotoxic compound using a different detoxification pathway but
43 generating a very similar spatial-temporal liver tissue damage pattern and downstream
44 regeneration response. Several mechanisms may contribute to the death of hepatocytes.
45 Cytochrome P450-enzymes such as Cyp2e1 and Cyp1a2 (summarized in this work as CYP450)
46 metabolize APAP (or CCl₄) into toxic radicals, which downstream causes oxidative stress,
47 dysfunction of mitochondria, and DNA damage, resulting in mitochondrial membrane
48 permeability transition and hepatocyte death. In addition to these initiation events,
49 inflammatory mediators such as bile acids, cytokines and chemokines, the latter two
50 predominantly secreted by non-parenchymal and immune cells, may critically contribute in the
51 APAP-induced liver injury (Liu et al., 2004, Ghallab et al., 2022).

52 Upon repetitive administration, both compounds generate liver fibrosis, which has led to a wide
53 use of both compounds to study the development of chronic liver disease in rodent models
54 (O'dell et al., 1986, Ghallab et al., 2019, Nevzorova et al., 2020). Chronic liver disease is a
55 consequence of compromised regeneration that is incapable of repairing repetitive tissue
56 injury. Understanding the mechanisms controlling regeneration is a major subject of
57 investigation in order to improve treatment of chronic liver disease and regeneration after
58 acute liver injury, including partial hepatectomy.

59 Liver regeneration is an overly complex process involving many different cell types and factors
60 (Kang et al., 2012, Kisseleva and Brenner, 2021, Michalopoulos and Bhushan, 2021). Upon
61 inflicted liver damage, the injured main hepatic parenchyma cells, namely, hepatocytes start to
62 release factors to trigger an inflammatory response (Calderwood et al., 2016, Li et al., 2020).
63 Liver resident macrophages, namely, Kupffer cells are activated to recruit neutrophils to initiate
64 death of injured hepatocytes (Marra and Tacke, 2014). After liver injury is initiated, hepatic
65 stellate cells (HSCs) migrate into the lesion and become activated through stimulating factors
66 released from damaged hepatocytes, the activated macrophages, the extracellular matrix,
67 neighboring sinusoidal endothelial cells and platelets (Puche et al., 2013, Michalopoulos and
68 Bhushan, 2021). After the liver injury, there is also massive infiltration of bone marrow derived
69 (non-resident) macrophages into the liver to elicit liver impairment and to restore liver integrity
70 at different stages (Tacke and Zimmermann, 2014). Despite extensive studies over decades, the
71 precise orchestration is only partially understood, and the understanding is mainly qualitative.

72 There are alternative sets of hypotheses on how certain factors interplay, and it is not well
73 understood how sensitive the regeneration outcome is regarding a modification of time scales
74 or rate constants. This is difficult to study experimentally for several reasons: (1) the primary
75 motivation for experiments is to understand the regeneration process in human, but
76 experiments in human are per se not permitted and experiments in animal models are subject
77 to close ethical control and do not fully reflect the processes in human; (2) the rate constants
78 and time scales are generally hard to control in animal experiments; and (3) the experiments
79 are resource-intense with regards to personnel, technology, animal numbers and consumables.
80 Hence, a promising strategy may be to formulate alternative sets of mechanisms and
81 implement them on the computer within a virtual liver twin. The consequences of these
82 mechanisms on the regeneration process can be simulated and the most promising
83 perturbations or facilitations proposed for "wet-lab" approval. Such strategy establishes
84 computational model-guided experimentation by identifying the most informative experiment
85 regarding a certain scientific question and can thus accelerate knowledge gain in
86 (patho)physiological processes at all integrated levels (molecular, cellular, tissue, organ,
87 system/body). In the discussed liver twin model, relevance and variability of the interplay of the
88 different factors hypothesized to control liver regeneration after acute damage can be
89 simulated and the outcome can be evaluated. Moreover, the prospective sensitivity of readout
90 parameters of interest can be tested in silico. Theoretical variation of parameters and variables
91 in the model can help to identify those with the largest influence on the observables.

92 In this study, we present a pipeline how multi-level computational modeling may inform
93 experimental strategies to investigate liver regeneration upon toxin-induced acute injury. In the
94 past decades, computational modeling has become more and more important to investigate
95 the possible consequences of complex biological systems interactions from sub-cellular level to
96 tissue level (Knutzdottir et al., 2017, Talman et al., 2019) and integrating molecular signals and
97 mechanical interactions of cells (Thurley et al., 2015, Park et al., 2017, Kim et al., 2018,
98 Stepanova et al., 2021), or directly address the mechanical contributions to biological observed
99 phenomena in multicellular populations (Vishwakarma et al., 2018, Schwarz, 2020). This
100 emerges as the complexity of the possible interactions makes it difficult to infer the
101 consequences of certain mechanisms or perturbations by reasoning alone; in the meantime,
102 even the computational multicellular models call for sophisticated methods of parameter
103 inference (Jagiella et al., 2017). There are many computational studies developed to explore the
104 underlying mechanisms of liver regeneration and disease processes (e.g. Drasdo et al., 2014 and
105 refs therein). Ordinary differential equations were used to describe the dynamics of molecular
106 factors to regulate the distinct functional states of hepatocytes in response to partial
107 hepatectomy (Verma et al., 2019) and to reproduce the patterns of acute drug-induced toxicity
108 (Kuepfer et al., 2018). Models based on set of partial differential equations were developed to
109 mimic the behavior of glucose regulation in liver with diabetes (Hetherington et al., 2012), to
110 investigate the function-perfusion processes of liver cancer (Lambers et al., 2021), and to
111 simulate the drug perfusion in the liver (Schwen et al., 2014, 2016). Friedman and Hao (2017)

112 built a system of partial differential equations representing the network of liver fibrosis to
113 explore the efficacy of potential drugs to block the fibrosis progression. Jerby et al. (2010)
114 constructed a generic model based on network of various molecular data sources for human
115 liver metabolism. Remien et al. (2012) used nonlinear ordinary differential equations to study
116 the liver dysfunction.
117 Wambaugh and Shah (2010) used an agent-based hepatic model integrating a graphical model
118 of the sinusoidal network to study the chemical metabolization in the liver. Dutta-Moscato et
119 al. (2014) used an agent-based model to study the liver inflammation and fibrosis. Ho et al.
120 (2020, 2020a) constructed an *in-silico* pipeline integrating hepatic vessels and blood flow for
121 liver surgical plan. Naik et al. (2014) constructed a multi-level model integrating multiple body
122 compartments to investigate hepatic metabolism and its associated deregulations.

123 We constructed a network describing the intercellular relationship between the major cell
124 types and the biochemical signals in the process of liver regeneration from literature. We
125 embedded this network into our spatial-temporal lobule model and tested the reference case
126 (the normal regeneration process) and different types of perturbations, especially such that are
127 difficult to perform experimentally. Furthermore, we ran perturbation simulations to predict
128 the influence of cell type depletion on liver regeneration. Our model resolves liver
129 microarchitecture, representing each cell individually as a basic modeling element with realistic
130 cell-biomechanics. By comparison with experimental readout parameters, we were able to
131 identify hypotheses that were incompatible with data, and which perturbation experiments
132 critically modify the regeneration process (schematized in Fig. 5).

133 Our model approach presented below hence points towards getting mechanistic insight in liver
134 damage and regeneration with less resources i.e., faster, easier and cheaper, by a simulation
135 step prior to the experiment. The first step could be running simulations with our model that
136 permits identifying whether a certain hypothesized mechanism (that may also be a finding from
137 an *in vitro* experiment) would be expected to explain a certain observation *in vivo*, and/or,
138 whether, dropping (knocking down) this mechanism is expected to change the result. In our
139 work, the “observation” is the successful tissue regeneration compared to its failure. In case of
140 a result change upon knock-out, a corresponding validation experiment *in vivo* may be up-
141 prioritized while in the opposite case (no change expected) it may be dropped. In border-line
142 cases, where either a change or no change is both possible, depending on parameters, the
143 simulations inform about the combinations of parameters at which a change of result is
144 expected. This contributes to turn qualitative descriptions increasingly into quantitative process
145 information including parameterization of each component process, even in such complex
146 situations as tissue regeneration (or degeneration). In order to optimally perform these
147 different functions, guiding experiments, identification of experimental parameters etc., the
148 model needs to be as realistic and quantitative as possible in its key elements, such as
149 representation of tissue architecture, cell biomechanics, and cell-cell interaction, which guided
150 our model choice.

151 We demonstrate this in the discussion section along with hypotheses made in refs. (Krenkel et
152 al., 2014, Marques et al., 2012, Seki et al., 2007, Boulter et al., 2012).

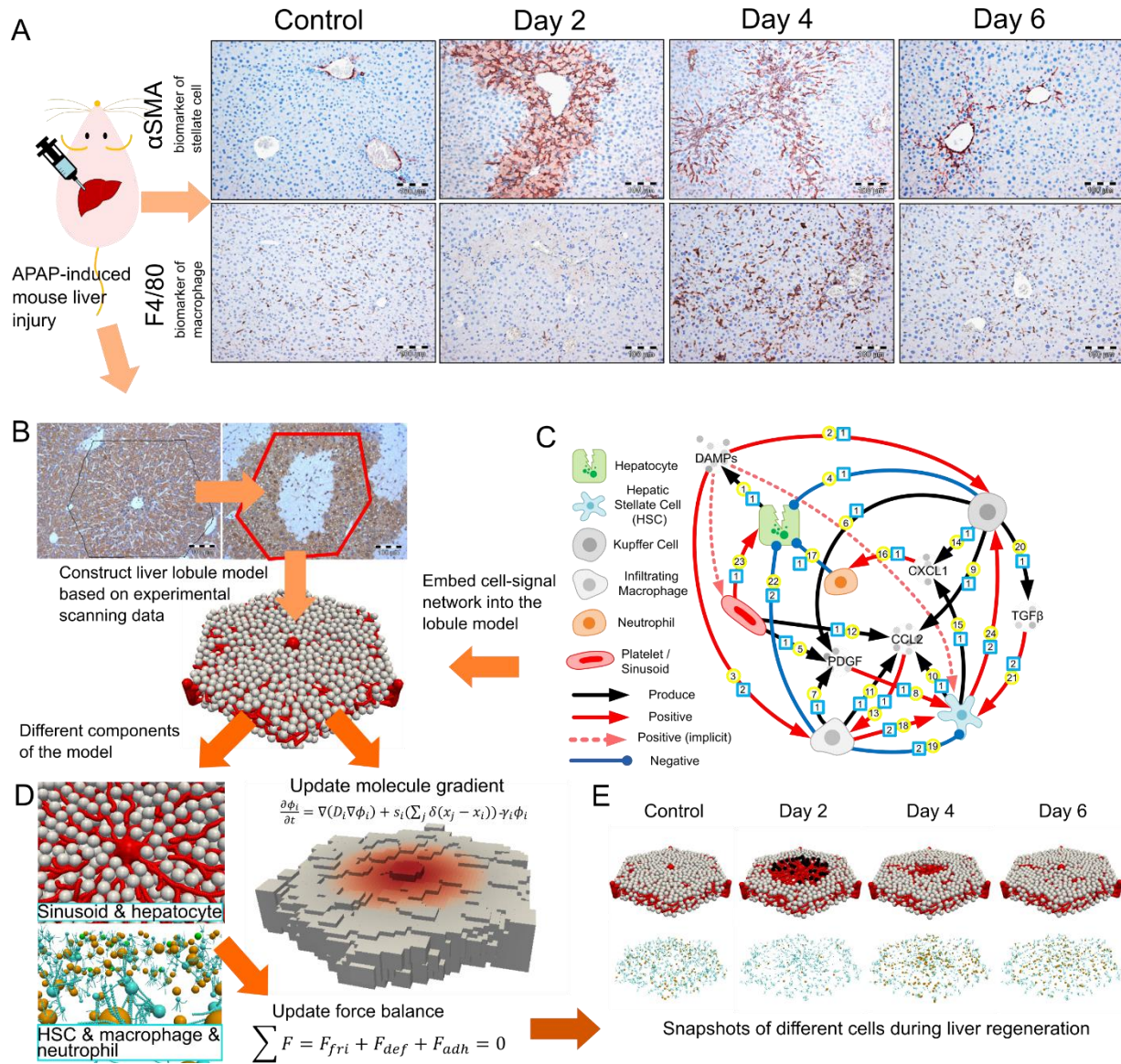
153 Our modeling strategy is also able to guide diagnosis and therapy, if fed with the proper patient
154 data. In so far, our work addresses systems complexity (Steven Hawking: "*I think the next*
155 *century will be the century of complexity*").

156 **RESULTS**

157 **Modeling approach**

158 **Construction of a lobule: Geometry of lobule, choice of cell types, number and distribution.**

159 The chosen tissue unit in this work is the individual hepatic lobule. Following previous work (Fig.
160 1B, Hoehme et al., 2010), modeling was performed in a statistically representative lobule of
161 hexagonal shape, with the central vein in the center of the lobule and portal veins in three of its
162 6 corners. The precise micro-architecture of the lobule was constructed from parameter
163 distributions obtained from 3D liver tissue reconstructions. Upon injection of a hepatotoxin,
164 such as CCl₄ (or APAP), the liver parenchymal cells, namely hepatocytes, in the pericentral
165 region of the lobule die, resulting in a pericentral dead cell area. The starting state of the
166 simulation was defined by labeling pericentral hepatocytes as injured and potential candidates
167 to die. According to the current understanding of hepatotoxicity upon CCl₄ or APAP, the injured
168 hepatocytes are those that are cytochrome P450-enzymes positive and received a critical dose
169 of CCl₄ (or APAP) (Sezgin et al., 2018, Ghallab et al., 2021).



170

171 **Figure 1. Model of multi-cellular lobule system based on experiments.** (A) APAP-
 172 induced liver regeneration on mouse and the spatio-temporal pattern of the different liver
 173 cell types. We have corresponding experimental stainings as reference. (B) A lobule in
 174 hexagonal shape is constructed to study the liver regeneration process upon toxin-induced
 175 acute damage following our previous work (Hoehme et al., 2010). In this study, CCl₄ is used
 176 to induce lobular damage, where the necrotic lesion size reaches the maximum area 2 days
 177 after the injection of CCl₄. (C) The network includes relevant cell types and intercellular
 178 signals contributing to liver regeneration. “Positive” means to activate or to attract a
 179 certain type of cell; “Negative” means to kill, to eliminate, or to deactivate a certain type of
 180 cell. The number in the yellow circle marks the related reference (Table S2). The number in
 181 the cyan square indicates its classification, (cl-)“1” indicates the relationship has literature
 182 support; (cl-)“2” indicates the relationship is discussed as option by literature without
 183 data-based evidence. (D) For the different liver cell types, specific geometric objects are
 184 used to represent their shape (grey: hepatocytes; red: sinusoids; cyan: HSCs; brown:

185 macrophages; green: neutrophils). Cell movement is updated by solving a force-velocity
186 equation according to the respective cell-mechanical properties. The signal gradient is
187 updated by solving a related diffusion equation. (E) Illustration of different cell types at
188 different days after the injury.

189 **Identification of relevant cell types and cell-cell-interaction network**

190 In a next step, relevant cell types were identified and incorporated in the lobule. These
191 comprise hepatocytes, sinusoidal endothelial cells organizing the sinusoidal network, hepatic
192 stellate cells (HSCs), macrophages (both Kupffer cells and infiltrating macrophages), platelets,
193 and neutrophils (Fig. 1C). Two types of interactions were considered: Mechanical interactions
194 by adhesive and repulsive forces as well as mechanical friction forces, and intercellular
195 communication via signaling molecules, including DAMPs (Damage-associated molecular
196 pattern), PDGF (Platelet-derived growth factor), TGF β (Transforming growth factor β), CXCL1 (C-
197 X-C motif chemokine ligand 1), and CCL2 (C-C motif chemokine ligand 2).

198 The interaction network of the different cell types was classified into highly probable “cl-1”
199 (with direct experimental support) or probable “cl-2” (with indirect experimental support) (Fig.
200 1C).

201 There is a huge body of experimental data on specific aspects of the regeneration process that
202 partially looks as alternative or backup mechanisms, which do not significantly modulate the
203 liver regeneration model. We here focus on the plausible selection of those mechanisms
204 emerging from the data that have been implemented in the model. We first detail these
205 mechanism before discussing possible alternatives.

206 In the model, DAMPs are released by the injured hepatocytes (Brenner et al., 2013), e.g. to
207 activate Kupffer cells (cl-1). This is based on the report that injured hepatocytes can release
208 HMGB1 and HSP-79 (both are DAMP signals, Huebener et al., 2015, Calderwood et al., 2016, Li
209 et al., 2020,) to activate Kupffer cells (Martin-Murphy et al., 2010) (cl-1). Platelets are recruited
210 to the site of injury and bind with sinusoidal endothelial cells during the early stage of liver
211 injury (Nowatari et al., 2014, Ramadori et al., 2019) (cl-1) to promote the proliferation of
212 hepatocytes (Meyer et al., 2015) and synthesize PDGF (also synthesized by activated
213 macrophages, Pinzani et al., 1994) (cl-1). PDGF acts on activated HSCs, which display
214 upregulated PDGFR expression to induce proliferation and migration (Yang et al., 2003, Melton
215 and Yee, 2007) (cl-1). In the model, the source of PDGF was simplified as the positions of
216 sinusoids inside the dead region, of activated Kupffer cells and of infiltrating macrophages.
217 TGF β is synthesized by activated Kupffer cells based on the report that TGF β is predominantly
218 expressed in Kupffer cells (De Bleser et al., 1997) (cl-1). TGF β acts on HSCs, which are highly
219 responsive to that cytokine, and in most cases, TGF β is described as prominent driver of
220 activation (e.g., Cai et al., 2018, Fan et al., 2019) (cl-1); there are also cases described, where
221 TGF β is more involved in HSC survival than activation, whereby another factor secreted from
222 infiltrating macrophages is a more prominent driver of activation (Imamura et al., 2005) (cl-2).

223 In the model, two different factors to activate HSCs were assumed: one is TGF β that is provided
224 by activation of ECM deposited Latent TGF β (Fan et al., 2019), subsequently synthesized by the
225 liver non parenchymal cells, that is, activated Kupffer cells, HSCs and LSECs. The other factor is
226 an assumed alternate factor generated by activated infiltrating macrophages. Then, CXCL1 is
227 synthesized by both activated Kupffer cells and HSCs to attract neutrophils (Kisseleva and
228 Brenner, 2007, Marra and Tacke, 2014) (cl-1). CCL2 is synthesized by activated HSCs, Kupffer
229 cells, infiltrating macrophages and sinusoids in the lesion region to attract infiltrating
230 macrophage (cl-1). This is based on the report that activated stellate cells, Kupffer cells,
231 macrophages, endothelial cells secrete CCL2 to control the macrophage infiltration (Baeck et
232 al., 2012, Krenkel et al., 2014).

233 In the model, a subpopulation of Kupffer cells are assumed to be able to eliminate dead
234 hepatocytes based on the observation that dead hepatocytes are engulfed by Kupffer cells
235 (Canbay et al., 2003) (cl-1). Moreover, infiltrating macrophages are assumed to also eliminate
236 dead hepatocytes as previously described (Boulter et al., 2012) (cl-2).

237 We have three additional hypotheses on the function of infiltrating macrophages. The first is to
238 contribute to the activation of HSCs e.g. based on reports that infiltrating macrophages secrete
239 at least one HSC activating factor (Imamura et al., 2005) (cl-2); the second is to induce death of
240 the activated HSCs based on the report that infiltrating macrophages might trigger apoptosis of
241 activated HSCs (Tacke and Zimmermann, 2014) (cl-2); the third is to revert the activated HSCs to
242 the quiescent phenotype (Kisseleva et al., 2012, Troeger et al., 2012, Hassan, 2017) (cl-2). This is
243 probably depending on different macrophage phenotypes/subpopulations, as becomes more
244 and more evident from recent scRNASeq experiments (Willemsen and Winther, 2020).

245 One advantage of in silico modeling is to permit straightforward testing of all different selected
246 regeneration scenarios, which may then serve as a guide to exclude those ones for
247 experimental validation that by the simulations have to be assumed apriori to fail. This is
248 demonstrated below by running simulations switching off or varying interactions labeled as “cl-
249 2”, and therewith test the influence of each of the following interactions (and the combination
250 of some of them) on the process of liver regeneration:

- 251 (1) Infiltrating macrophages eliminate (or do not eliminate) dead hepatocytes (Fig. 1C, no.
252 22, cl-2);
- 253 (2) DAMPs activate (or do not activate) infiltrating macrophages (Fig. 1C, no. 3, cl-2);
- 254 (3) HSCs are activated by TGF β , e.g. from Kupffer cells (no. 21, cl-2), or an alternate factor
255 produced by infiltrating macrophages (no. 18, cl-2);
- 256 (4) HSCs either attract or do not attract Kupffer cells to migrate (no. 24, cl-2);
- 257 (5) Infiltrating macrophages revert activated HSCs to a quiescent phenotype (no. 19, cl-2),
258 or induce cell death of activated HSCs (included in no. 19, cl-2).

259 If interactions (1) – (5) were all excluded, regeneration did not succeed in the model.

260 The model permits to study in how far alternative assumptions on cell-cell interactions,
261 perturbations within each of them, or in selective ones impact on regeneration of the peri-
262 central drug-induced lesion.

263 **State changes of cell types and timings:** The state changes of cell types concern attributes such
264 as activation and deactivation, or initialization for differentiation, as we now know into multiple
265 different functional phenotypes. The cellular consequences can be, among others, migration,
266 reversion from activation, killing, proliferation and death, depending on the specific cell type
267 (specified in table S2), and other parameters.

268 The timing of our simplified scenario was chosen in agreement with our own experiments (as in
269 Fig. 1A) and from published references (Dragomir et al., 2012, Graubardt et al., 2017). For
270 Kupffer cells, indications of a potentially constant cell population size were assumed. To
271 challenge this assumption, we studied both, varying and constant Kupffer cell populations (see
272 details in SI). In our model, the Kupffer cell population is considered as an input parameter and
273 not a readout. Alternately, we could model Kupffer cell number kinetics as appearance and
274 death process (death at days 1-2, appearance and spread from day 2 on), but this was not the
275 purpose of this work.

276 **Model at cell & tissue level; cell geometry:** Hepatocytes, hepatic stellate cells, Kupffer cells and
277 infiltrating macrophages, as well as neutrophils are modelled, each as individual entity. The
278 endothelial cells are not modelled individually, but as part of a network of sinusoids. Each
279 hepatocyte has been approximated by an isotropic, elastic, and adhesive sphere (Fig. 1D, white
280 objects), named "center-based model" (CBM), capable of interacting with other cells or blood
281 vessels by mechanical forces or chemical signals. The sphere can be thought as specifying the
282 region in space where the hepatocyte is localized with overwhelming probability. The CBM has
283 been parameterized by material and cell-kinetic parameters, which permits to readily identify
284 the physiological parameter ranges. Macrophages and neutrophils were equivalently mimicked
285 by a CBM, but with different cell parameters (Fig. 1D, green and brown objects). Different from
286 the former cell types, the HSCs were approximated by an isotropic, elastic, and adhesive sphere
287 with chains of elastic springs emanating from their body to capture their long protrusions (Fig.
288 1D, cyan objects). The sinusoidal network is modeled as semi-flexible chains of spheres that are
289 connected by springs (Fig. 1D, red objects). This design accounts for the fact that vessels resist
290 bending and stretching. The parameters of the sinusoids comprise volume, density and
291 branching orientation, which are sampled from the scanning data of the real liver sinusoidal
292 system (see more details in Hoehme et al., 2010).

293 **Model at cell & tissue level; force balance & cell movement:** Cell and sinusoid movement is
294 computed based on force balance. The model takes into account passive and active forces.
295 Passive forces are friction forces of each cell with its environment (other cells, sinusoids,
296 intercellular medium), deformation and compression forces experienced by a cell, as well as
297 cell-cell and cell-sinusoidal adhesion forces. Cell migration, as it occurs by anchoring of cells in

298 the extracellular matrix, e.g. in the space of Disse, is mimicked as an active force. The precise
299 form of the forces (as detailed in the STAR METHODS) has been chosen to directly represent
300 cell material parameters. For example, the cell-cell interaction force has been approximated by
301 the "Johnson-Kendall-Roberts"(JKR)- force model for homogeneous elastic sticky spheres,
302 which has been shown by micro-pipette experiments (Chu et al., 2005) to quantitatively
303 reproduce the force-distance relation of two cells brought in contact and pulled apart. In
304 conjunction with the friction force, the emerging behavior at the tissue level is viscoelastic.
305 During liver regeneration, cell proliferation causes cell compression, which cannot be properly
306 addressed by standard pair-wise forces (as JKR, Hertz etc.) (Van Liedekerke et al., 2015). To
307 correct for this shortcoming, the JKR-force was modified for small cell-cell distances by a term
308 that accounts for volume compression forces arising from large cell deformation, calibrating
309 with the "Deformable Cell Model (DCM)" (Van Liedekerke et al., 2019).

310 The motion of cells and sinusoids is updated by solving an overdamped stochastic equation of
311 motion, a Langevin equation, which summarizes all forces exerted on them: $\sum F_{fri} + F_{def} +$
312 $F_{adh} = 0$, where F_{fri} , F_{def} , F_{adh} are friction force with the environment, deformation force,
313 and adhesive force with other cells or elements, respectively (Fig. 1D, see more details of each
314 force term in STAR METHODS).

315 **Model at cell & tissue level; molecular interactions:** Cell types may secrete signal molecules
316 that are sensed by other cells. The spread of the molecules is mimicked by a diffusion equation.
317 The cells generating the molecules are represented as source terms in the diffusion equation.
318 Moreover, the equation contains a general first-order kinetics decay term (molecule
319 degradation term in Fig. 1D, see more details of each equation term in STAR METHODS).

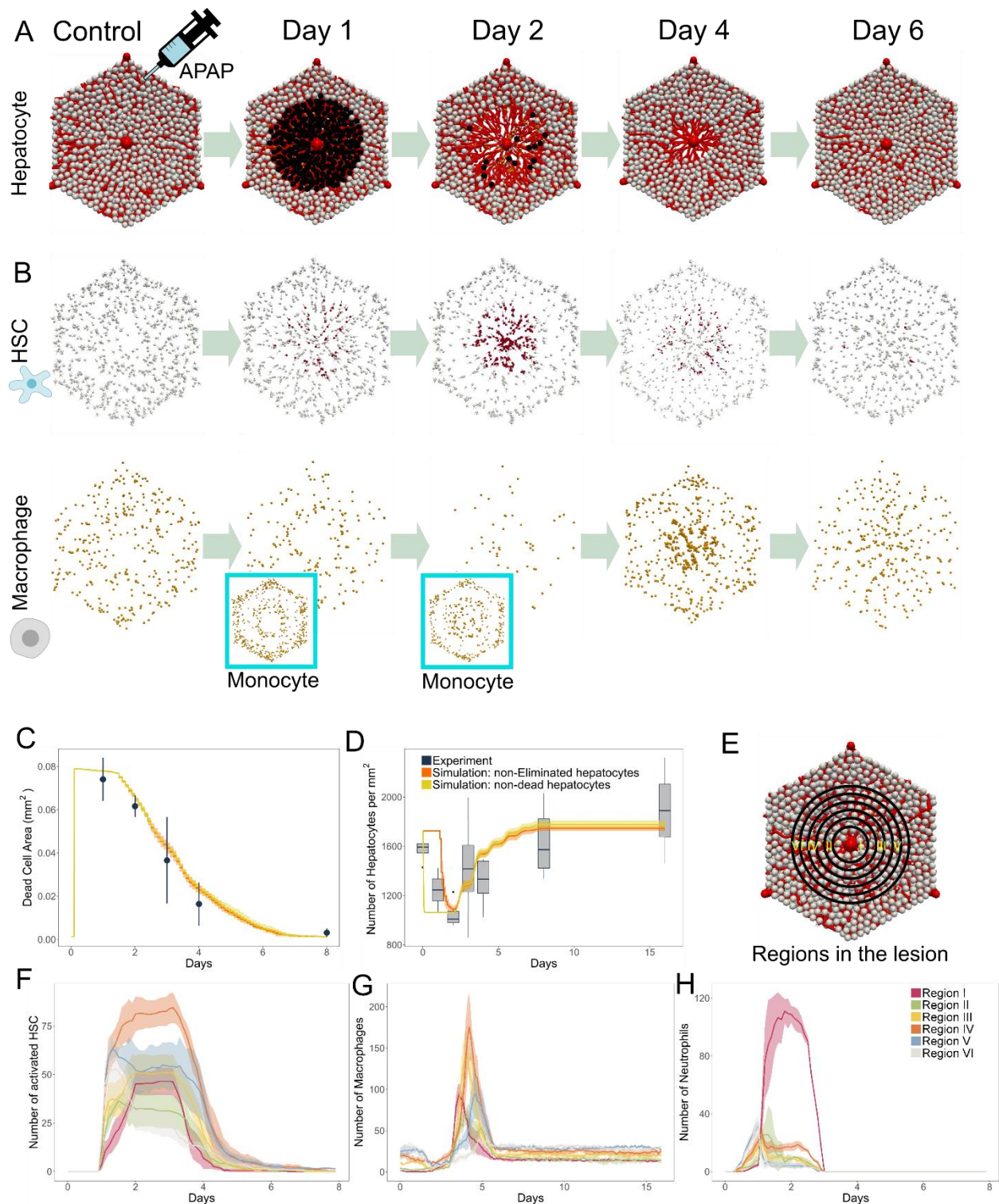
320 **Model parameterization:** Most model parameters and the liver lobule micro-architecture have
321 been chosen as previously developed (Hoehme et al., 2010) (Table S1). The densities of non-
322 parenchymal cells not considered in that reference has been estimated from other published
323 references, as for neutrophils for example, from McDonald et al., 2010, Marques et al., 2014
324 (Table S1).

325 **Definition of a reference model:** The definition of a reference model is to some extent
326 arbitrary. Here, it was guided by choosing all interactions from Fig. 1C that belong to cl-1
327 (consensus), plus all the interactions (1-5) of cl-2, as shown above, and which were verified to
328 result in a functioning regeneration scenario in the simulation (as in Fig. 1E), in agreement with
329 the experimentally observed lesion size and hepatocyte density (the details of interaction label
330 and corresponding reference can be seen in Fig. 1C and Table S1).

331 **Simulated regeneration scenarios with the reference and alternative interaction models**

332 **The reference model:** The simulation starts at day 0, right after injection of the hepatotoxic
333 substance (CCl₄/APAP). Hepatocyte injury is expected to occur in less than an hour, supported
334 by fact that in mice, for example, the highest APAP blood concentration ("Cmax") is reached
335 ~30 minutes after injecting the drug into the peritoneum (Sezgin et al., 2018, Schneider et al.,

336 2021, Schuran et al., 2021). The timing of the processes depicted in Fig. 1C follows the assumed
337 logical order of the subprocesses finally resulting in a functioning regeneration (Fig. S2). In a
338 first step, the direct effect of APAP on Cyp450 positive hepatocytes via NAPQI generation was
339 included. This was realized by constructing a dose-dependent cell death induction in this
340 subpopulation of hepatocytes, and the time (point) at which death occurs. Both parameters are
341 based on experimental data in that the NAPQI-pathway displays a time and dose-dependent (in
342 vitro: concentration dependent).



343

344 **Figure 2. Pattern of liver lobule regeneration (reference model).** (A) Simulated lobule,
 345 consisting of different cell types and the sinusoids over time, taking into account different
 346 simulation scenarios. White spheres are healthy hepatocytes, black spheres are dead
 347 hepatocytes; sinusoids are visualized as red lines. (B) The distribution of HSCs (dark red:
 348 activated; grey: quiescent) and macrophages (brown) over time. The distribution of Ly6C-high

349 monocytes on days 1 and 2 are shown in the blue boxes. Their precise spatial pattern depends
350 on the timepoint of secretion and the range of CCL2. (C and D) Lesion area and number of
351 hepatocytes over time from both, simulations and experiments. (E) Spatial distribution of
352 activated HSCs, macrophages, and neutrophils in the lobule, measured by counting the
353 corresponding cell number over the distance to the central vein (CV). As illustrated, there are
354 six regions considered: Region I ($< 21.4 \mu\text{m}$ to the CV), II (between 21.4 and $42.8 \mu\text{m}$ to the CV),
355 III (between 42.8 and $64.2 \mu\text{m}$ to the CV), IV (between 64.2 and $85.6 \mu\text{m}$ to the CV), V (between
356 85.6 and $107 \mu\text{m}$ to the CV), VI (between 107 and $128.4 \mu\text{m}$ to the CV). (F to H) Number of
357 activated HSCs, macrophages, and neutrophils in all regions over time. Error bars represent the
358 standard deviation of four simulation runs, using different random seeds.

359 In a second step, the timing of the interaction processes not directly depending on NAPQI
360 (these were introduced above, are depicted in Fig. S2 and are based on information found in
361 the references in Table S2 as well as on simulation-calibration) is explained, and related to the
362 spatial-temporal pericentral hepatocyte damage and regeneration process depicted in Fig. 2A.
363 In detail, before drug administration, both HSCs and Kupffer cells are distributed
364 homogeneously in the lobule (Fig. 2B, quiescent/activated HSCs are colored in white/red and
365 Kupffer cells are colored in brown). Up to about 1 hour after drug administration, injured
366 hepatocytes secrete DAMPs that activate Kupffer cells, making them secreting CXCL1, TGF β and
367 CCL2. CXCL1 attracts neutrophils which migrate towards the Kupffer cells. At the same time,
368 sinusoidal endothelial cells/platelets also localized in the region of the prospective lesion are
369 secreting CCL2 and PDGF. PDGF attracts HSCs, which then cause migration of Kupffer cells
370 towards them. Hence, two processes make Kupffer cells preferentially being activated in the
371 prospective dead cell area: firstly, the secretion of DAMPs by hepatocytes in the prospective
372 lesion, which force DAMP concentration to be higher for Kupffer cells in the prospective lesion
373 than outside, and secondly, the fact that the Kupffer cells are attracted by the HSCs, which
374 become activated, stimulated to proliferate and migrate towards the prospective lesion by the
375 gradient of DAMPs, PDGF and chemokines. In-line with Fig. 1A, the population size of Kupffer
376 cells has been modelled to drop after activation until day 2 and recover thereafter. Death has
377 been mimicked by a death rate such that the decay could be qualitatively reproduced, the
378 recovery has been modelled by adding new Kupffer cells with a certain rate. The diffusion
379 length for DAMPs is about 3 cell diameters in 1 hour, so at the short time scale considered, the
380 DAMPs do not spread over the entire lobule. Consequently, neutrophils attracted by CXCL1,
381 migrate almost entirely towards the prospective lesion. Neutrophils in contact with injured
382 hepatocytes are assumed to initiate their death reaching a state in which they become
383 “flagged” for a potential elimination by macrophages after about 1 day. About the same time,
384 TGF β (probably in a first step derived from LTGF β deposits in the ECM adjacent to the damaged
385 hepatocyte area, activated by the dying hepatocytes) activates HSCs making them secrete CCL2
386 and CXCL1 amplifying and/or backing up the effect of Kupffer cells and platelets. Activated HSCs
387 proliferate and secrete extracellular matrix (ECM) (Kisseleva and Brenner, 2021). Infiltrating
388 macrophages are attracted by CCL2 approaching the dead hepatocytes. The infiltrating
389 macrophages are initiated as monocytes with Ly6C-high phenotype, which do not show up in
390 the macrophage population in Fig. 2B. However, those monocytes (inset to Fig. 2B) that are in
391 the range of CCL2 move during the two days towards the hepatocyte damage region. After a

392 period of time between 2 and 3 days, the infiltrated monocytes transform into macrophages
393 with Ly6C-low phenotype (Zigmond et al., 2014, Graubardt et al., 2017). They can phagocytose
394 the dead hepatocytes when they are at Ly6C-high phenotype. Once they adopted the Ly6C-low
395 phenotype, they can phagocytose or revert activated HSCs (Tacke and Zimmermann, 2014). The
396 Kupffer cell population drops in the first two days due to toxic damage (Ritz et al., 2018) (Fig.
397 2B), and recover from day 2 on. The recovered Kupffer cells can, as the Ly6C-low phenotype,
398 phagocytose the dead hepatocytes, as well as the ECM that has been deposited by activated
399 HSCs (after repeated administration of CCl₄ or APAP, ECM deposition becomes significant,
400 Ghallab et al., 2019).

401 This leads to a clearance of the lesion from dead hepatocytes, while it may still be populated
402 with activated HSCs and macrophages (Fig. 2B, day 2). Around day 1.5-2, hepatocytes outside of
403 the dead lesion start to enter S-phase to eventually replace the dead hepatocytes (Hoehme et
404 al., 2010). Sinusoids located in the lesion are among the prime candidates for HGF and EGF
405 secretion, which are both mitogens for hepatocytes (Michalopolous 2010, 2017). After about 3
406 days, macrophages begin to deactivate HSCs. At day 6, the lesion is closed by healthy
407 hepatocytes having replaced the removed (dead) hepatocytes (Fig. 2B). In different simulation
408 runs, we find sporadic dead hepatocytes that are later phagocytosed, while in some simulations
409 the dead hepatocytes have been entirely eliminated by day 6 and the lesion was closed.
410 Infiltrating macrophages have already disappeared. The HSCs become deactivated, and both,
411 HSCs and Kupffer cells redistribute in the lobule (Fig. 2B), the latter accompanied and driven by
412 a change of fate from Ly6C-high phenotype to Ly6C-low phenotype (see details in STAR
413 METHODS).

414 In a next step, the spatial temporal distributions were quantified. For the lesion area and
415 hepatocyte density over time, for which we had quantitative experimental values (Hoehme et
416 al., 2010), the simulation results show a perfect agreement to the experimentally observed
417 values (Figs. 2C, D). In Hoehme et al., 2010, the lesion area was experimentally defined as the
418 area not containing hepatocyte nuclei anymore, while signs of cell death occurred already
419 earlier, but were not considered in the experimental curve (SI to that reference). Referring to
420 the direct effect of NAPQI, the lesion may alternatively be assessed regarding the lobule space
421 not occupied by "healthy" hepatocytes, in which case the dead cell area shows up earlier as in
422 Fig. 2C, different from the previous model that did not take into account the direct effect by
423 NAPQI-detoxification (Hoehme et al., 2010). Following the same line of argument, the
424 hepatocyte density maybe defined as the number of hepatocytes over the area of the lobule (as
425 in Hoehme et al., 2010) or the number of healthy hepatocytes over the area of the lobule. Both
426 measures result in different spatial profiles before 2 days after the injury. In Fig. 2D we depicted
427 the number of healthy hepatocytes, which may be difficult to demarcate from those already
428 damaged and about to die in the experiment.

429 We lacked quantitative values for the other cell types, so estimated their density from
430 published references (Bouwens et al., 1986, Wake, 2006, McDonald et al., 2010, Zigmond et al.,
431 2014) and measured in the simulation the change of their profile, which may be seen as a
432 mainly qualitative model prediction. In order to simulate the dynamic change of the
433 distributions of HSCs, macrophages (both Kupffer cells and infiltrating macrophages), and

434 neutrophils, the lesion region was divided evenly into 6 sub-regions according to their distance
435 to the CV (Fig. 2E), and the number of each type of sinusoidal cell type in each sub-region was
436 counted over time. As shown in Fig. 2F (experimental: Fig. S1D), the number of activated HSCs
437 starts to rise after day 1, probably driven by TGF β , provided by activation of its latent form from
438 the ECM, and subsequently from de novo production by Kupffer cells. This parallels with
439 Kupffer cells beginning to eliminate the bodies of dead hepatocytes (Canbay et al., 2003). The
440 number of activated HSCs is highest in region I, which is located most close to the CV. The
441 number of activated HSCs peaks around day 2, then dropping dramatically due to the
442 interventional effect of Ly6C-low expressing infiltrating macrophages, which induce cell death
443 and/or reversion to quiescence (Ritz et al., 2018, Fischer et al., 2002). NK (natural killer) and
444 NKT (natural killer T) cells also contribute to induce death of activated HSCs (Gao and Radaeva,
445 2013). At around day 6, there are no activated HSCs left in the lobule. As shown in Fig. 2G, the
446 number of macrophages in the pericentral area decreases before day 2, probably from toxic
447 damage upon phagocytosing the dead hepatocytes. After day 3, the Kupffer cell number quickly
448 rises again, due to repopulation of the pericentral area by proliferation and migration of the
449 resident Kupffer cells and/or differentiation from the massively infiltrating macrophages into
450 the lesion. After day 4, the macrophage population in the lesion drops again, this time due to
451 depletion of infiltrating macrophages (Zigmond et al., 2014). After around day 6, the number
452 almost resumes to initial values, e.g. resulting from the Kupffer cells relocating back to their
453 initial distribution. As shown in Fig. 2H, the number of neutrophils rises quickly after day 0,
454 attracted by a CXCL1 gradient and in order to deplete the injured hepatocytes. Recruited
455 neutrophils accumulate in region I and the number rises until day 1. Until day 3, the neutrophil
456 population disappears.

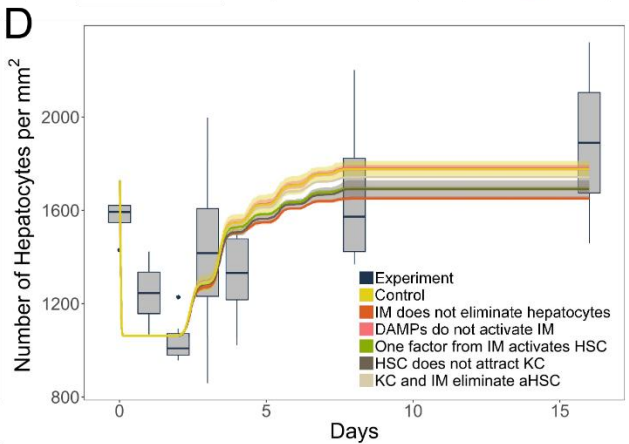
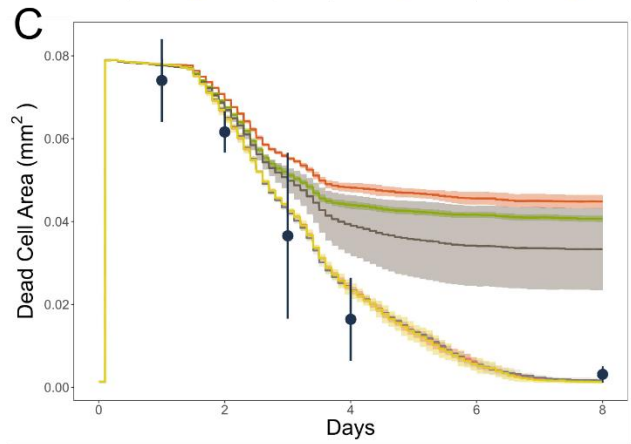
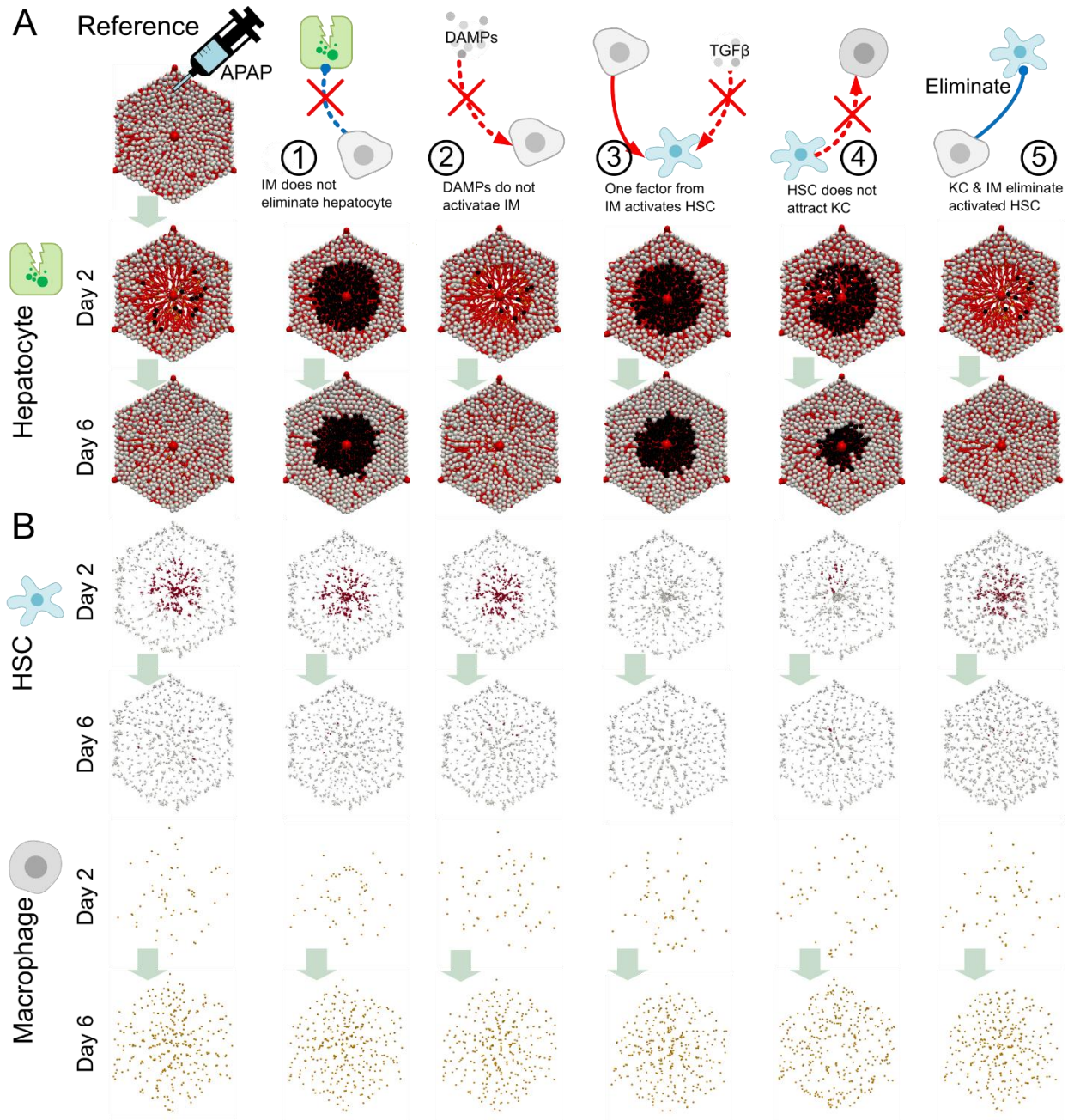
457 Our simulation of the reference case can achieve the expected pattern of normal liver
458 regeneration and agree with the experimentally observed dynamics for the necrotic lesion size
459 and hepatocyte density.

460 Next, we explored the communication between different cell types and factors to achieve liver
461 regeneration after NAPQI induced damage by testing type “2” interactions (probably happening
462 *in vivo*) in the network.

463 **Alternative cell-cell interaction network models:**

464 In a next step variations of the reference model (RM) were considered by individually modifying
465 the following five interactions of the reference network: (1) Infiltrating macrophages cannot
466 (RM: can; no. 22) eliminate the dead hepatocytes; (2) Infiltrating macrophages are not activated
467 by DAMPs (RM: are activated; no. 3); (3) HSCs are activated by another factor, besides TGF β ,
468 which is produced by activated macrophages derived from infiltrating monocytes (RM: TGF β is
469 activating HSC, no. 21; we assumed that this factor has about the same diffusion constant as
470 TGF β); (4) HSCs cannot attract Kupffer cells to migrate (RM: they do, no. 24); (5a) Infiltrating
471 macrophages deplete activated HSCs (Fig. S6A-S6E); (5b) 50% of activated HSCs are
472 depleted/50% are reverted (Fig. S6G) (RM: infiltrating macrophages revert activated HSCs to
473 quiescent phenotype, no. 19).

474 Below, we refer to a model, in which the assumption (k ; $k=1, 2, 3, 4, 5$) has been modified as
475 model. As shown in Fig. 3A, C, D, the alternative models (2) and (5) do not significantly alter the
476 result of the reference model regarding a complete liver regeneration, while the modifications
477 (1), (3), and (4) influence the lesion recovery in that regeneration after 16 days is still
478 incomplete. Fig. 3A displays a single simulation scenario, where dead hepatocytes are colored
479 in black. Fig. 3C shows the average over 4 runs with different random seeds. Among the
480 perturbations (1), (3) and (4), the area of the unhealed lesion is largest for model (1), probably
481 due to the inhibition of phagocytosis of infiltrating macrophages. The Kupffer cells alone are not
482 able to clear dead hepatocytes in time. However, a parameter sensitivity analysis suggests that
483 Kupffer cells equipped with a stronger phagocytosis capacity, by a shorter elimination duration,
484 would alone be able to clear the necrotic area from dead hepatocytes in the experimentally
485 observed time (Fig. S6H; matching with the RM). The importance of infiltrating macrophages in
486 clearing up the lesion is also indicated by the modification implemented as model (3), where
487 HSC activation is depleted (Fig. 3B and Fig. S4D). Due to the lack of activated HSCs in the lesion,
488 the expression level of CCL2 is not sufficient to attract infiltrating macrophages into the lesion
489 to clear up dead hepatocytes (Fig. 3B, Fig. S4C, and Fig. S5B). Similar to model (3), in model (4)
490 the number of Kupffer cells in the lesion is much smaller than that in the reference case, hence
491 less TGF β is produced in the lesion resulting in decreased numbers of activated HSCs (Fig. 3B,
492 Fig. S4D, and Fig. S4C). Again, the expression level of CCL2 is reduced, and as a consequence,
493 fewer infiltrating macrophages are attracted into the lesion, as compared to the reference case
494 (Fig. 3A and Fig. S4C).



496 **Figure 3. Pattern of liver lobule regeneration resulting from alternative interactions.** (A) The
497 regenerating lobule over time under reference and five alternative interactions: (1) Infiltrating
498 macrophages do not eliminate dead hepatocytes; (2) DAMPs do not activate infiltrating
499 macrophages; (3) HSCs are not activated by TGF β but by one factor from infiltrating
500 macrophages; (4) Kupffer cells do not migrate towards HSCs; (5) Kupffer cells and infiltrating
501 macrophages eliminate activated HSCs instead of reverting them to quiescent mode. (B) The
502 distribution of activated HSCs (antibody: α SMA) and macrophages (antibody: F4/80) over time
503 under reference and five perturbed interactions. (C and D) Lesion area and hepatocyte density
504 over time under reference and five alternative interactions.

505 We further tested a model simultaneously implementing assumptions (2) and (5), which did not
506 have significant impact on liver regeneration (Fig. S6F-S6H).

507 Our computational study of these five individually modified interactions suggests two main
508 conclusions: (1) There is a positive feedback loop between Kupffer cells and HSCs, where the
509 presence of HSCs promotes migration of Kupffer cells into the lesion, while increasing numbers
510 of Kupffer cells in the lesion increase the number of activated HSCs, which attract more
511 infiltrating macrophages to help clear up dead hepatocytes; (2) The way to diminish activated
512 HSCs by macrophages, either by engulfment or by phenotype reversion has no significant
513 impact on lesion recovery. Note however, that in case HSCs are depleted, the remaining HSCs
514 would have to proliferate to repopulate the lobule to its original population size. It is now
515 obvious from experimental data that half of the population of activated HSCs is depleted and
516 half of it is reverted, which by itself would more or less lead to repopulation of the liver to the
517 original healthy state (Kisseleva and Brenner, 2021).

518 In a next step, we test how far simulations of perturbation scenarios are suited to guide
519 experiments in a way to permit pinpointing differences between interaction mechanisms at the
520 tissue level, and hence serve to validate the model predictions.

521 **Simulated perturbation experiments: Depletion of non-parenchyma cells**

522 After the simulated testing of the reference model and of variations that differ from the
523 reference model by one interaction, additional perturbation simulations were performed to
524 predict the impact of depletion of cells typically found along the sinusoidal network spaces on
525 liver regeneration. In each of four perturbation simulations (I-IV), one of the four cell types
526 HSCs, Kupffer cells, infiltrating macrophages and neutrophils was depleted individually.

527 A further advantage of the model is that it permits to test hypothetical cases and see how much
528 contribution to an observed effect can be attributed to sub-processes. Both will be discussed
529 below.

530 **Perturbation I: The model predicts that depletion of HSCs results in an unhealed lesion**

531 The depletion of HSCs (Fig. 4A, scenario 1) did not change the size of the lesion induced by the
532 drug and characterized by dead or dying hepatocytes. This turns out to be the same for all cell
533 type depletions, indicating that the lesion size is mainly controlled by the cell death caused

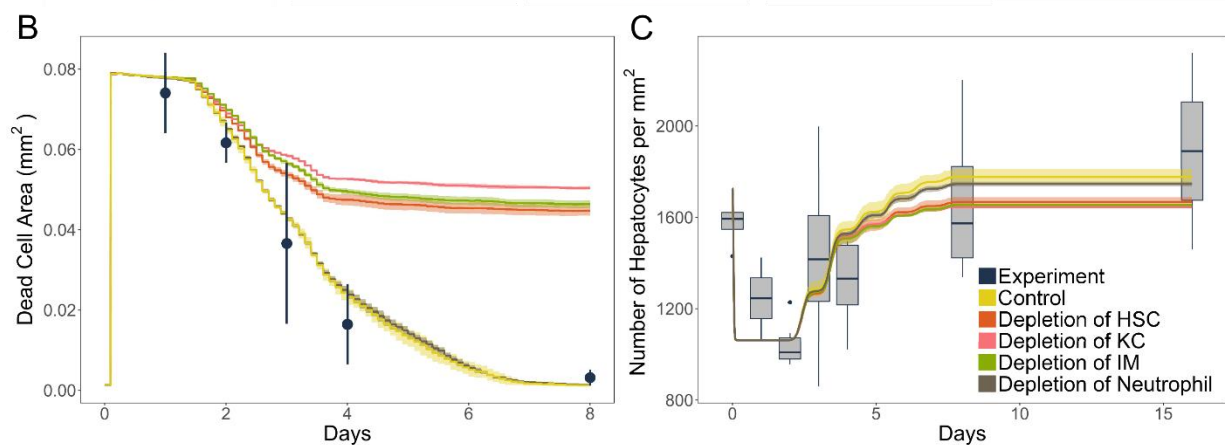
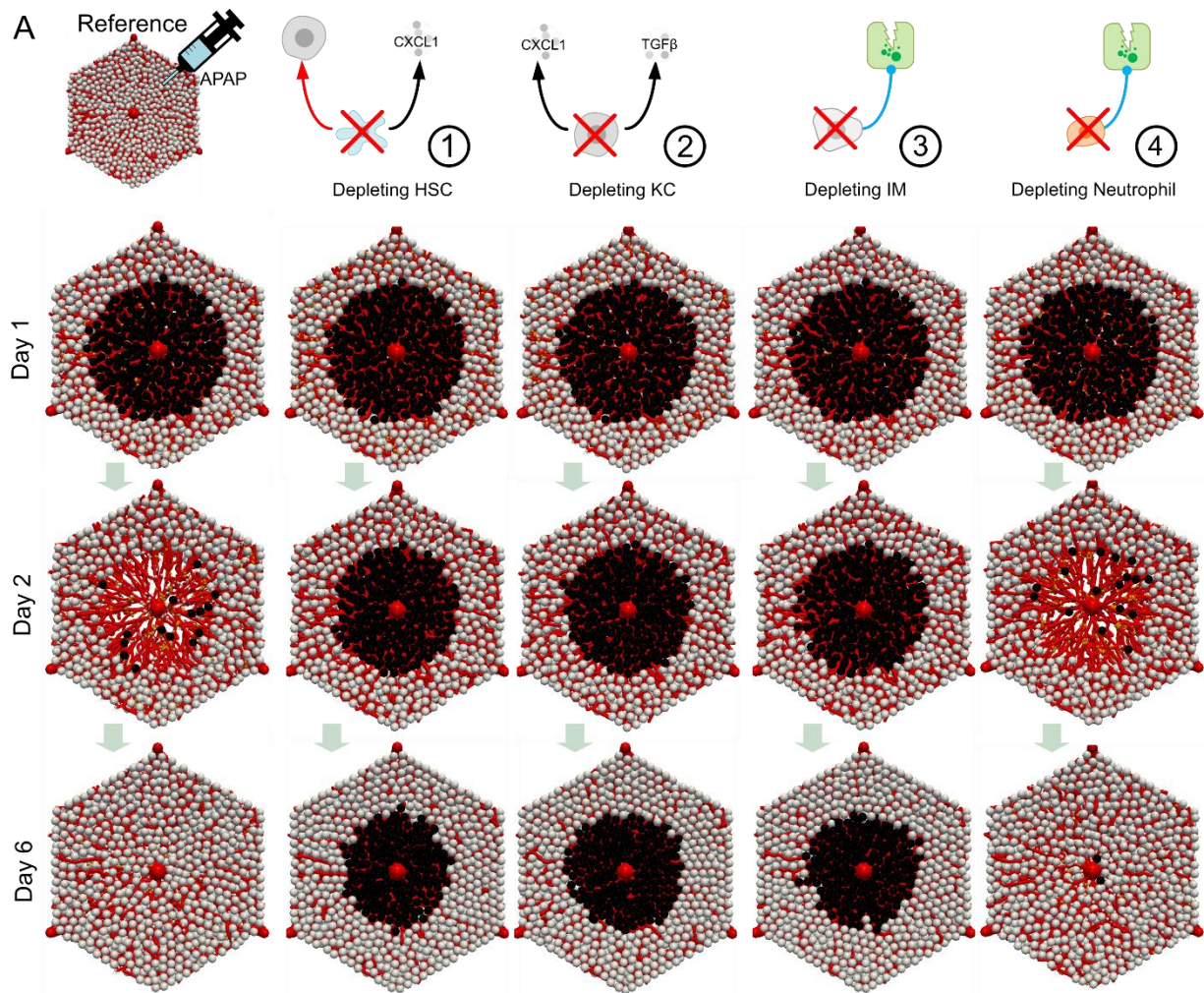
534 downstream of the NAPQI pathway. In the case of HSC depletion, however, the dead
535 hepatocytes are not cleared up. Due to the lack of activated HSCs, CCL2 levels are not sufficient
536 to attract infiltrating macrophages to clean up dead hepatocytes. In addition, due to the
537 absence of HSCs, Kupffer cells do not migrate. Consequently, the number of Kupffer cells in the
538 lesion is smaller than in the reference case. This is similar to the perturbation that disables HSCs
539 to attract Kupffer cells to migrate (scenario 4, Fig. 3A). In general, depletion of HSCs leads to
540 reduced numbers of neutrophils, Kupffer cells, and infiltrating macrophages in the lesion. As a
541 consequence, a large number of dead hepatocytes remains uncleared in the lobule.

542 Our prediction is consistent with a previous study, where the depletion of HSCs resulted in
543 decreased expression levels of CXCL1 and reduced numbers of infiltrating neutrophils (Stewart
544 et al., 2014).

545 **Perturbation II / III: The model predicts that depletion of macrophages abrogates clearance of**
546 **lesion from dead hepatocytes, independent on whether it concerns the Kupffer cells or the**
547 **infiltrating macrophages.**

548 As shown in Fig. 4A (scenario 2), after depletion of Kupffer cells, the necrotic lesion is only
549 marginally cleared up from dead hepatocytes. Due to the lack of Kupffer cells, there is a
550 reduced TGF β signal to fully activate HSCs, leading to qualitatively the same scenario as above
551 after depletion of HSCs. As Kupffer cells are totally missing, phagocytosis by Kupffer cells does
552 not take place, leaving even more dead hepatocytes in the lesion than in the previous case. We
553 further tested if HSCs can be activated even after depletion of Kupffer cells, we found that the
554 lesion can be recovered in time (Fig. S6G). These results indicated the importance of activation
555 of HSCs in regulating lesion recovery.

556 After the depletion of infiltrating macrophages (scenario 3 in Fig. 4A), again a large part of the
557 dead hepatocytes remains in the lobule not being cleared. This is similar to the perturbation of
558 disabling infiltrating macrophages to eliminate dead hepatocytes (Fig. 3A, scenario 1).



559

560 **Figure 4. Pattern of liver lobule regeneration upon depletion of different cell types in**
 561 **the reference model (Fig. 2).** (A) The regenerating lobule over time as reference state and
 562 upon depletion of four sinusoidal cell types: (1) Depletion of HSCs; (2) Depletion of Kupffer
 563 cells; (3) Depletion of infiltrating macrophages; (4) Depletion of neutrophils. (B and C)
 564 Lesion area and hepatocyte density over time in the reference state and upon depletion of
 565 different cell types as indicated.

566 Our prediction is consistent with a previous study of depleting infiltrating macrophages in acute
567 liver injury, which resulted in delayed tissue recovery (You et al., 2013).

568 Because of the (expected) dramatic effect of macrophage depletion on the regeneration result,
569 we refined the assumed perturbation by replacing the dynamic kinetics of Kupffer cell numbers
570 in the course of the regeneration process by assuming a constant population size after the 6-
571 hours time point.

572 For this case (Fig. S8), we found no consequence on the readout parameters at day 6,
573 interestingly indicating that the precise dynamics of the Kupffer cell population may not be a
574 critical determinant of the regeneration process, as long as Kupffer cells are present for being
575 activated by damaged hepatocytes through DAMPs, and to facilitate HSC activation early (about
576 one hour) after drug administration.

577 **Perturbation IV: The model predicts that the depletion of neutrophils has no significant**
578 **influence on liver regeneration**

579 As shown in Fig. 4A, after simulation the depletion of neutrophils, there is neither a significant
580 effect on the generation of damage, nor on the regeneration of the necrotic lesion in
581 comparison to the reference scenario. Therefore, neutrophils might be considered as providing
582 backup mechanisms to induce damage towards epithelial cells, to phagocytose the damaged
583 debris, and to induce cell death in HSC (Kisseleva and Brenner, 2021). This is in-line with ref.
584 (Krenkel and Tacke, 2014), stating that cell death induced by neutrophils in APAP-induced liver
585 injury is not very obvious *in vivo*, even though it can be observed *in vitro* (Marques et al., 2012).

586 This still needs further debate, as an older study of depleting neutrophils during acute liver
587 injury reported protection of the lobule against hepatotoxicity with a significantly reduced
588 centrilobular necrosis area (Liu et al., 2006). As the latter was not the case in our simulations for
589 the hypotheses studied in the main body of this work, it prompted us to investigate a further
590 highly speculative case, that may not be expected in acute liver damage, but perhaps could be
591 obtained by proper experimental manipulations (Fig. S7). Here, we consider regeneration from
592 a necrotic lesion that has not been generated by cell-death due to the NAPQI-pathway. The
593 NAPQI pathway is for example not activated in hepatocytes that lack Cytochrom-P-450
594 enzymes, as occurring during periportalization after repetitive administration of APAP (or CCl₄;
595 Ghallab et al., 2019), and another hepatotoxic insult would be responsible for the multicellular
596 necrotic lesion. In that simulation, for simplicity, we assumed also a circular necrotic lesion that
597 initiates phagocytosis of dead hepatocyte debris by neutrophils and macrophages. Here, we
598 could observe a protective effect against hepatotoxicity upon neutrophil depletion (Fig. S7). The
599 simulations demonstrate that our framework may permit *in silico* tests of hypotheses that may
600 not be present as such in nature, but may be realized by proper engineered manipulations.

601 DISCUSSION

602 In our study, we developed a multi-level computational model of a virtual liver lobule that
603 integrates an intercellular network of major hepatic cells and takes into account cell-cell
604 communication based on selected published knowledge. We applied the model to simulate the
605 liver regeneration process after APAP-induced acute hepatotoxicity and tested documented
606 and hypothesized interactions between cells and signals. Furthermore, we made testable
607 predictions on perturbations that were already performed in previous experimental studies.

608 Our virtual liver twin illustrates that testing or perturbing the intercellular communication
609 between cells and signals allows exploring the mechanism behind liver regeneration and
610 guiding the design of relevant experiments. The *in silico* liver considered here includes reported
611 and hypothesized interactions between cell types, mediated both mechanically and by chemical
612 signals. It permits to study alternative hypotheses and their consequences on the regeneration
613 process. A plausible candidate model implemented the following temporal events: (1) Injured
614 hepatocytes produce DAMPs and are killed via cell stress from APAP detoxification by NAPQI
615 (Fig. S2). Platelets in the lesion produce PDGF; (2) Moreover, Kupffer cells and HSCs are also
616 activated by DAMPs. The so activated HSCs are assumed in agreement with our data to be not
617 detected through α SMA, and migrate towards the gradient of PDGF; (3) activated non-
618 parenchymal cells (and damaged hepatocytes) produce CXCL1, while neutrophils migrate
619 towards the gradient of CXCL1 and induce death in injured hepatocytes; (4) Kupffer cells
620 migrate towards the cluster of HSCs; (5) Activated Kupffer cells produce TGF β to activate
621 another function of HSCs, namely the production of CCL2 to attract infiltrating macrophages
622 into the lesion. We assume that those HSCs are labelled by α SMA; (6) infiltrating macrophages
623 along with Kupffer cells engulf and eliminate the bodies of dead hepatocytes; (7) the healthy
624 hepatocytes surrounding the lesion proliferate and collectively migrate towards the lesion to
625 recover the lost hepatocytes, following a tug-of-war mechanism (Trepap et al., 2009; Hoehme
626 et. al., 2010; 2022); (8) Infiltrating macrophages switch from Ly6C-high phenotype to Ly6C-low
627 phenotype to revert the activated HSCs into quiescent HSCs, whereas activated Kupffer cells
628 switch back to quiescent Kupffer cells; (9) To restore the original cell distribution, we simulated
629 alternative scenarios. Either HSCs previously localized in the lesion may die and be
630 phagocytosed by macrophages, and those outside the lesion could proliferate. Another,
631 perhaps more hypothetical scenario was the migration of quiescent Kupffer cells and HSCs. In a
632 computer simulation comparing both scenarios (not shown) the regeneration of the necrotic
633 hepatocyte lesion turned out to be insensitive to the precise mechanism of restorage of the
634 original cell distribution. During the entire process, different types of hepatic cells interact and
635 collaborate to achieve a perfect liver regeneration in time.

636 An important advantage of this virtual liver is that it permits to investigate the role of individual
637 cell types and different signals by simulations. For example, the presence of one or several
638 factors as well as its interaction with other factors can be depleted completely, weakened or
639 amplified, and the consequence of this modification on the regeneration process and its final
640 outcome can be studied. This may be impossible in an *in vivo* experimental model due to
641 several reasons. Firstly, some modifications are technically not feasible or accompanied with
642 huge effort. Secondly, possible backup mechanisms may hide the effect of a given change such

643 that the direct and indirect consequences are not detangled. Moreover, model simulations may
644 show that certain manipulations are likely not resulting in a significant change of outcome, and
645 therefore are not sufficiently informative to justify the effort of experimental realization,
646 especially in regard to the 3R for animal experimentation. On the other hand, differences in the
647 outcome of experiments and model simulations may indicate so far un-recognized mechanisms
648 that would remain hidden without such simulations of a reference situation.

649 To demonstrate opportunity and power provided by the virtual liver, we simulated a number of
650 perturbation experiments and presented their result as one of three possible basic scenarios, as
651 follows (Fig. 5).

652 **Incomplete regeneration (Fig. 5, blue scenario):** An incomplete regeneration with dead
653 hepatocytes remaining in the drug-induced lesion is predicted if either the infiltrating
654 macrophages are lacking phagocytotic activity (scenario 1 in Fig. 3A), or are completely
655 depleted from the lobule (scenario 3 in Fig. 4A). This suggests that the Kupffer cells, which are
656 the resident macrophages, are insufficient to clear the lesion. This is still the case, even if their
657 dead body elimination time is reduced from 3 hours (Haecker et al., 2002) to 6 minutes (Fig.
658 S6H). The Kupffer cells are observed to engulf the dead hepatocytes (Canbay et al., 2003) while
659 the infiltrating macrophages are hypothesized to remove the dead hepatocytes *in vivo* in ref.
660 (Boulter et al., 2012). Our simulation (scenario 1 in Fig. 3) strongly support this hypothesis as
661 depleting the phagocytosis ability of infiltrating macrophage leads to incomplete regeneration.
662 However, one may argue that if the Kupffer cells have a sufficiently strong ability to phagocytize
663 dead cells, they might in principle clear the lesion alone. Our model permits to specify the
664 parameters under which this would be the case.

665 Seki et. al. (2007) observed *in vitro* that stimulated HSCs attract Kupffer cells to migrate towards
666 them. Within our model we could show the prospective effect of such a mechanism *in vivo*: by
667 depleting Kupffer cells or inhibiting Kupffer cells to migrate towards the concentrated HSCs in
668 the lesion, there are also uncleared dead hepatocytes remaining in the lesion (scenario 4 in Fig.
669 3A and scenario 2 in Fig. 4A), suggesting that the relation observed by Seki et. al. (2007) *in vitro*
670 should indeed be present *in vivo*, not requiring an *in vivo* study in the first place, where the
671 conditions are more complex and difficult to control. I.e., the simulation indicates that in such a
672 case as for the *in vitro*-finding by Seki et. al. (2007) an *in vivo* validation experiment (as it had
673 been done) is promising to perform hence the resources (e.g. time, money, material, personnel)
674 are likely well invested. In that sense, simulations with our model can guide the experimental
675 strategy. When we depleted the HSCs from the lobule or disabled the activation of HSCs, for
676 example through TGF β produced by Kupffer cells, a similar unhealed lobule resulted (scenario 3
677 in Fig. 3A and scenario 1 in Fig. 4A). Together, the simulations suggest that the key to clear up
678 the lesion is to attract a large enough number of macrophages in time to eliminate the dead
679 hepatocytes. This requires the help of HSCs, e.g., to guide the migration of both Kupffer cells
680 and infiltrating macrophages into the lesion.

681 **Reduced or no lesion (Fig. 5, yellow scenario):** The role of neutrophils is complex and
682 controversial, some studies have shown that a lack of neutrophils does not affect the outcome
683 or severity of APAP-induced liver injury (Krenkel and Tacke, 2014), while other studies indicated

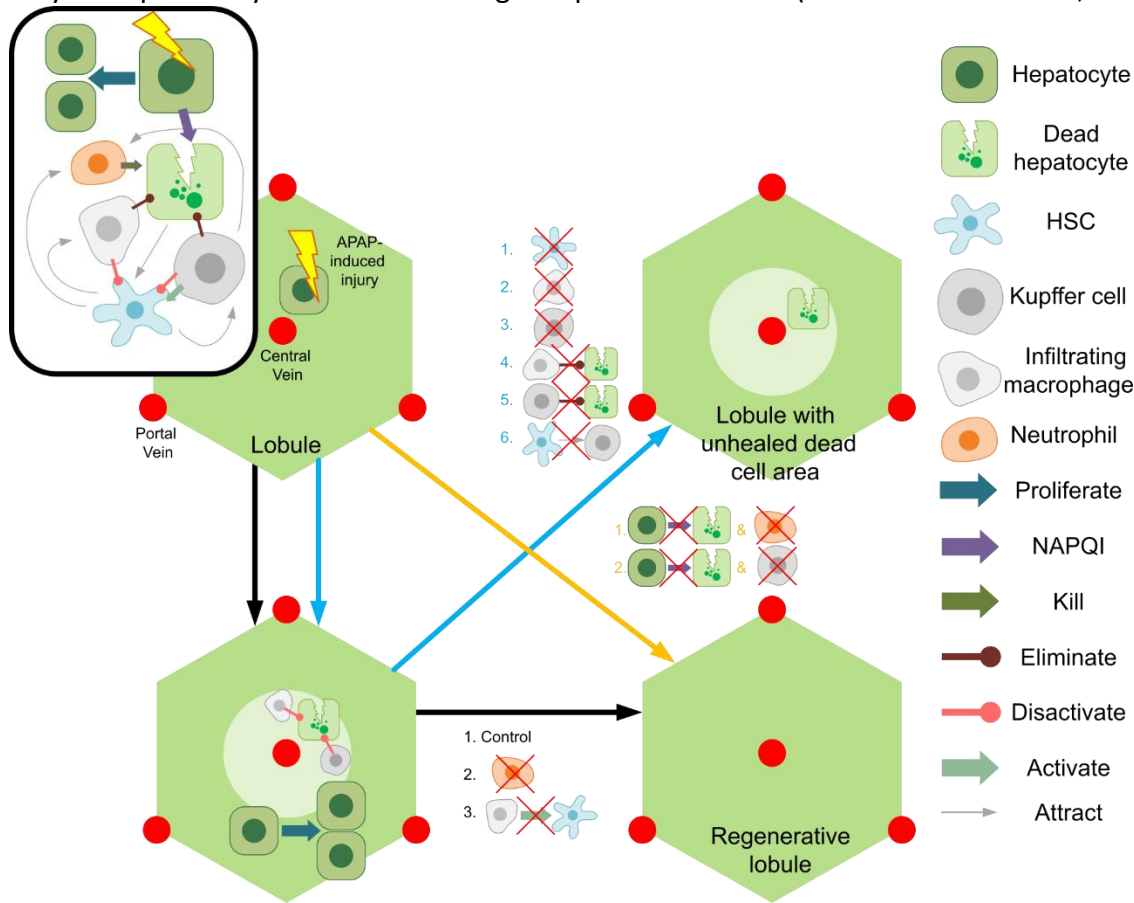
684 that the neutrophils can directly mediate hepatocyte death in APAP-induced liver injury
685 (Marques et al., 2012). We suggested that this might be due to the effect of NAPQI-induced cell
686 death in the recent study (Ghallab et al., 2019). The majority of hepatocytes in the lesion are
687 killed through NAPQI-induced detoxification. Therefore, the cell death induced by neutrophils
688 in APAP-induced liver injury is not very obvious *in vivo* (Krenkel and Tacke, 2014), but can be
689 observed *in vitro* (Marques et al., 2012). To test this assumption, we abrogated NAPQI-induced
690 cell death, in which we found the majority of the hepatocytes in the lesion are killed by
691 neutrophils. This is in agreement with the indication that neutrophils can mediate hepatocyte
692 death. Furthermore, we abrogated NAPQI-induced cell death and depleted neutrophils. The
693 lobule was surprisingly protected with no dead hepatocytes present (scenario 4 in Fig. S7A).
694 This is another example of how our model can be used to test if or/and within which parameter
695 ranges certain hypothesized mechanism have an observed effect.

696 **No effect on lesion generation nor on regeneration (Fig. 5, black scenario):** However, if NAPQI-
697 induced cell death is not affected, the depletion of neutrophils has no significant impact on liver
698 regeneration (scenario 4 in Fig. 4A). This result indicates that the neutrophil-induced cell death
699 is supplementary to the NAPQI-induced cell death. Even if the latter is disabled in the
700 simulation, the neutrophils can still kill the Cyp450 hepatocytes to generate room for the
701 dependent new proliferation generated hepatocytes. In addition, the repopulation of the
702 regenerated liver with quiescent HSCs, subsequent to (1) reversion of activated HSCs back to
703 the quiescent phenotype by macrophages, and (2) inducing cell death of activated HSCs by
704 macrophages or neutrophils, has no significant effect on the lesion regeneration (scenario 5 in
705 Fig. 3A). Furthermore, we also tested the case if HSCs are activated in the early stage (right
706 after the injury). As shown in Fig. S6G, when HSCs are activated first and migrate afterwards or
707 in the case when KCs are depleted, HSCs can still be activated, both have no significant effect
708 on lesion regeneration.

709 In conclusion, we have demonstrated the possible benefit and opportunities of a virtual liver to
710 simulate, in this case, liver regeneration after acute damage in control and perturbation cases,
711 at the level of a lobule and its constituting cell types, in time and space.

712 This may also include situations that can only be attained upon complex engineered
713 manipulations, e.g. to explore potential therapy or protection effects. The model can be further
714 developed and specified to simulate physiological and pathophysiological scenarios of liver and
715 liver diseases. For example, in order to capture complex hepatocyte shapes as it occurs during
716 fatty liver disease, fibrosis, cirrhosis or hepatocellular cancer, the cuboidal hepatocyte model

717 may be replaced by a cell model at higher spatial resolution (Van Liedekerke et al., 2019, 2020).



718

719 **Figure 5. Summary of the construction and application of the virtual liver twin to study liver**
 720 **regeneration after acute damage.**

721 Limitations of the study: A precise determination of the threshold concentrations in the
 722 simulation was not possible as the data were not present, and as the precise orchestration of
 723 molecular factors was and could not be mimicked in great detail. The underlying
 724 parametrisation concept was to choose plausible values out of accessible parameter ranges and
 725 infer parameters by comparison of their effect on the regeneration process. The robustness of
 726 the so determined parameters was studied in simulated sensitivity analyses. Nevertheless, it
 727 cannot be fully excluded that some parameters could in reality be outside of the ranges.
 728 However, this is not critical: the parameters / mechanisms that have been found to critically
 729 modify the regeneration process compared to the experimental observation should be
 730 challenged by additional experiments, whose outcome would then serve to re-calibrate the
 731 model parameter if necessary. Such an iterative procedure – identification of critical
 732 parameters and / or mechanisms by the model simulation – then experimental testing – then
 733 re-calibration of the digital liver twin model, will ultimately lead to a full quantitative digital
 734 twin model of liver generation.

735 In summary, we show the potential of our *in-silico* liver to successfully simulate liver physiology,
 736 and therewith present a promising strategy towards a full digital liver twin, that permits to test

737 perturbations from the molecular up to cell, tissue and body scales. We are not expecting it to
738 replace experiments, but guiding towards the most informative experiments by identifying gaps
739 in mechanistic knowledge. Such a liver twin is a key milestone on the route to guide diagnosis
740 and therapy if fed with patient data. In so far, our work responds on the question of systems
741 complexity.

742 **MATERIALS & METHODS**

743 **EXPERIMENTAL METHOD**

744 **Induction of acute liver injury by acetaminophen**

745 Acetaminophen (APAP)-induced acute liver injury was done in 10-week-old, male C57BL6/n
746 mice (Janvier Labs, France), as previously described (Holland et al., 2022). Briefly, a dose of 300
747 mg/kg APAP was administered intraperitoneally into overnight fasted mice. Liver tissue samples
748 were collected time-dependently, as indicated in the results section, and were processed for
749 immunohistochemistry, as previously described (Campos et al., 2020, Gianmoena et al., 2021).
750 All experiments were approved by the local animal welfare committee (LANUV, North Rhine-
751 Westphalia, Germany, application number: 84-02.04.2016.A279).

752 **Immunohistochemistry**

753 Immunostaining of macrophages and activated hepatic stellate cells was performed in 4 μ m-
754 thick paraffine-embedded liver tissue sections using an autostainer (Discovery Ultra Automated
755 Slide Preparation System, Roche, Germany), as previously described (Schneider et al., 2021).
756 The used antibodies and the staining conditions are summarized in the key resources table.

757 **COMPUTATIONAL MODEL AND METHOD DETAILS**

758 **Agent-based modeling of cells and elements**

759 To capture their approximate shape, hepatocytes, macrophages, and neutrophils are
760 approximated as spheres within a "center-based model"(CBM)-approach, which mimics the
761 forces between cells as forces between their centers (Van Liedekerke et. al. 2015). Within the
762 CBM-concept, the spherical shape does not represent the precise cell shape but region in space
763 where the cell is located at with overwhelming probability. An HSC is modeled as a sphere
764 forming the cell's core body, connected to four semi-flexible chains of spheres; sinusoids are
765 modeled as semi-flexible chains of spheres. Such spheres can for example readily be
766 constructed from inscribing spheres of maximal radius into image volume data sets stained for
767 endothelial cells (e.g., by CD31) (e.g. Rohrschneider et al., 2007). During each time step Δt , the
768 velocity \vec{v}_i of each sphere i is calculated according to one Langevin equation (an overdamped
769 stochastic equation) of motion . The new position of i is then updated to $\vec{p}_i(t) + \vec{v}_i(t)\Delta t$,
770 where $\vec{p}_i(t)$ is the position of i at time t . Since HSCs, macrophages and neutrophils are either
771 all located inside, wrap around, or migrate along sinusoids, we assume that they always remain
772 in contact with a sinusoid edge (for HSC, the core body is sticking on the sinusoid edge).
773 Therefore, the vector $\vec{v}_i\Delta t$ for one HSC, macrophage, or neutrophil i is projected onto the line

774 of sinusoid edge to which i is in contact with. The position of i is then updated as $\vec{p}_i + \vec{e}_i(\vec{v}_i \Delta t \cdot$
 775 $\vec{e}_i)$, where \vec{e}_i is the unit orientation vector of the local tangent to the sinusoid to which i is
 776 connected with. The equations of motion for each type of cell and element are defined below.

777 **Equation of motion for hepatocytes.** Each hepatocyte is represented as a homogeneous
 778 isotropic, elastic, adhesive sphere. It can migrate, grow, divide, and interact with other cells or
 779 sinusoids. The position of hepatocyte i is updated from:

$$780 \quad \Gamma_{ECM,i} \vec{v}_i + \sum_j \Gamma_{i,j} (\vec{v}_i - \vec{v}_j) = \sum_j \vec{F}_{ij} + \vec{F}_{mig,i}, \quad (1)$$

781 where $\Gamma_{ECM,i}$ is the friction coefficient with the extracellular matrix (ECM, which is not explicitly
 782 modeled in this study), $\Gamma_{i,j} = \gamma_{\perp} (\vec{e}_{ij} \otimes \vec{e}_{ij}) + \gamma_{\parallel} (I - \vec{e}_{ij} \otimes \vec{e}_{ij})$ is the friction tensor between cell
 783 i and the sphere j of other cell type (e.g. hepatocyte j) or sinusoid sphere, and \vec{e}_{ij} is the unit
 784 vector from i towards j , \vec{F}_{ij} is the corresponding central repulsion/adhesion interaction force,
 785 $\vec{F}_{mig,i}$ is an (active) migration force of cell i . The central force is computed by (Popov, 2010):

$$786 \quad \vec{F}_{ij} = \left(\frac{4\hat{E}}{3\hat{R}} [a(d_{ij})]^3 - \sqrt{8\pi\sigma\hat{E}[a(d_{ij})]^3} \right) \vec{e}_{ij}, \quad (2)$$

787 where the contact radius $a(d_{ij})$ allows to compute hepatocyte-hepatocyte contact area, and
 788 can be obtained by $d_{ij} = \frac{a^2}{\hat{R}} - \sqrt{\frac{2\pi\sigma}{\hat{E}}}$, \hat{E} and \hat{R} are defined as $\hat{E} = \left(\frac{1-\nu_i^2}{E_i} + \frac{1-\nu_j^2}{E_j} \right)^{-1}$ and
 789 $\hat{R} = \left(\frac{1}{R_i} + \frac{1}{R_j} \right)^{-1}$, with E_i and E_j being the Young's moduli, ν_i and ν_j the Poisson ratios, and R_i

790 and R_j the radii of i and j . The migration force is computed by $\vec{F}_{mig,i} = f_{dir} \vec{e}_i + \sqrt{2\Gamma_{ECM,i}^2 D_i} \cdot$
 791 $\vec{\eta}_i$, where f_{dir} is one constant force magnitude, \vec{e}_i is the unit vector from i towards the central
 792 vein, D_i is the diffusion constant of i , $\vec{\eta}_i$ is an uncorrelated noise term with amplitude
 793 $\langle \eta_{in}(t) \eta_{jm}(t') \rangle = \delta_{ij} \delta_{mn} \delta(t - t')$, t is the time for the current step, t' is the time for the last
 794 step, and $m, n \in (x, y, z)$ denote the coordinates (see more details in Hoehme et. al., 2010).

795 **Equation of motion for macrophages (Kupffer cells and infiltrating macrophages) and**
 796 **neutrophils.** Macrophages and neutrophils i are represented as point objects not interacting
 797 with any other structure but capable of migrating along the sinusoids (if not otherwise stated).
 798 The position of i is updated by solving the following equation:

$$799 \quad \Gamma_{ECM,i} \vec{v}_i + \sum_j \Gamma_{i,j} (\vec{v}_i - \vec{v}_j) = \sum_j \vec{F}_{ij} + \vec{F}_{mig,i}, \quad (3)$$

800 where $\Gamma_{ECM,i}$ is the friction coefficient with the extracellular matrix, $\Gamma_{i,j}$ is the friction tensor
 801 between i and j (same as Eqn. 1), \vec{F}_{ij} is the corresponding central repulsion/adhesion
 802 interaction force, $\vec{F}_{mig,i}$ is the migration force to drive i to migrate. The interaction force in Eqn.
 803 (3) does not play an important role as compared to the densely distributed hepatocytes,
 804 macrophages and neutrophils are distributed much more sparsely in the lobule, and they are
 805 much smaller than hepatocytes.

806 **Equation of motion for HSCs.** The core body of HSCs is modelled as one homogeneous
 807 isotropic, elastic, adhesive sphere (mainly representing the HSC's nucleus) with several semi-
 808 flexible chains of spheres as “arms” (to mimic the long HSC's protruding branches). The position
 809 of one HSC i (Fig. S1C) is updated by solving the following equation of motion:

$$810 \quad \Gamma_{ECM,i} \vec{v}_i + \sum_j \Gamma_{i,j} (\vec{v}_i - \vec{v}_j) = \sum_j \vec{F}_{ij} + \vec{F}_{mig,i} + \sum_k \vec{F}_{ela,ik}, \quad (4)$$

811 where $\Gamma_{ECM,i}$ is the friction coefficient with ECM, $\Gamma_{i,j}$ is the friction tensor between i and
 812 another cell or element j (same as Eqn. 1), \vec{F}_{ij} is the interaction force between i and another
 813 cell or element j (same as Eqn. 2). $\vec{F}_{ela,ik}$ represents elastic force between the head sphere of i
 814 and its connected arm spheres k (see definition in Fig. S1C). $\vec{F}_{mig,i}$ is the migration force to
 815 drive i to migrate. The equation of motion for an arm sphere i' of HSC i (blue sphere in Fig.
 816 S1C) is approximated by:

$$817 \quad \Gamma_{ECM,i'} \vec{v}_{i'} = \sum_{k'} \vec{F}_{ela,i'k'}, \quad (4b)$$

818 where k' denote the connected spheres of arm sphere i' . The arms were represented to permit
 819 representation of the HSC shape as well as of potential direct cell-cell communication through
 820 the arms. However, in reality, these arms are so small that within this work their interaction
 821 with other cells was neglected.

822 **Equation of motion for sinusoids.** Sinusoids are modeled as semi-flexible spheres (Hoehme et
 823 al., 2010). For one sinusoid sphere i , the position of i is updated by solving the following
 824 equation of motion:

$$825 \quad \Gamma_{ECM,i} \vec{v}_i = \sum_j (\Gamma_{\parallel,SE} (\vec{w}_{ij} - \vec{e}_{ij} (\vec{w}_{ij} \cdot \vec{e}_{ij}))) + \vec{F}_{ij} + \sum_k (\Gamma_{\parallel,SS} (\vec{w}_{ik} - \vec{e}_{ik} (\vec{w}_{ik} \cdot \vec{e}_{ik}))) + \vec{F}_{ik} + \vec{F}_{i,ela}, \quad (5)$$

827 where $\Gamma_{ECM,i}$ is the friction coefficient with environment, $\Gamma_{\parallel,SE}$ denotes the friction between
 828 sinusoid sphere i and its interacting sphere, for example hepatocyte j , $\vec{w}_{ij} = \vec{v}_j - \vec{v}_i$ is the
 829 difference of velocity between i and j , \vec{e}_{ij} is the unit direction vector from i towards j , \vec{F}_{ij} is the
 830 interaction force between i and j (same as Eqn. 2), $\Gamma_{\parallel,SS}$ denotes the friction between two
 831 sinusoid spheres i and k , $\vec{w}_{ik} = \vec{v}_k - \vec{v}_i$ is the difference of velocity between i and k , \vec{e}_{ik} is the
 832 unit direction vector from i towards k , \vec{F}_{ik} is the interaction force between i and k , $\vec{F}_{i,ela}$ is the
 833 spring force that arises from the chain connections between spheres belonging to the same
 834 sinusoid.

835 **Gradient of signals to regulate cell behaviors**

836 In our model, each type of molecular signal is produced by one type of cell. The dynamic of
 837 signal i released by cell j is governed by a partial differential equation (PDE):

$$838 \quad \frac{\partial \phi_i}{\partial t} = \nabla(D_i \nabla \phi_i) + s_i (\sum_j \delta(x - x_j)) - \gamma_i \phi_i, \quad (6)$$

839 where ϕ_i is the density of i , D_i is the diffusion coefficient of i , s_i is the production rate of i , x_j
 840 denotes the position of cell j , which produces i , γ_i is the decay rate of i . The center of mass of

841 cell j is set as the origin of the source. The simulation domain Ω is set as a box large enough to
 842 contain the entire lobule. The diffusion process of i inside Ω is assumed to be isotropic and
 843 homogenous. We assume Dirichlet boundary condition $\phi_i(x) = 0$ for $x \in \partial\Omega$.

844 **The cubic system to approximate the signal gradient.** We used a simple cubic system to
 845 approximate the concentration of all signals. The entire lobule is located in a big cube which is
 846 divided into N^3 small element cubes (Fig. S1A, N is number of element cubes on each axis). The
 847 concentration of signal l at cube i, j, k at time t is denoted as $c_{l:i,j,k}^t$. After the time lapse of Δt ,
 848 the concentration is denoted as $c_{l:i,j,k}^{t+\Delta t}$. Then the solution of Eqn. 6 can be explicitly
 849 approximated as:

$$\frac{c_{l:i,j,k}^{t+\Delta t} - c_{l:i,j,k}^t}{\Delta t} =$$

$$850 D_l \left(\frac{c_{l:i-1,j,k}^t - 2c_{l:i,j,k}^t + c_{l:i+1,j,k}^t}{\Delta x^2} + \frac{c_{l:i,j-1,k}^t - 2c_{l:i,j,k}^t + c_{l:i,j+1,k}^t}{\Delta y^2} + \frac{c_{l:i,j,k-1}^t - 2c_{l:i,j,k}^t + c_{l:i,j,k+1}^t}{\Delta z^2} \right) + s_l - \gamma_l c_{l:i,j,k}^t,$$

851 where D_l , s_l , γ_l are the diffusion coefficient, production rate, and decay rate of l . Since we use
 852 the cube as finite element, Δx , Δy , Δz are the same as the width of the element cube. To verify
 853 our cubic system, we tested one simple example of placing one injured hepatocyte which
 854 produces DAMP in the center of the system. Then we solve Eqn. 6 by using our method and the
 855 software deal.II (Arndt et al., 2021) for the numerical solution. As shown in Fig. S1B, our
 856 method can get a relatively good approximation of the solution. (We did not use deal.II directly
 857 as it turned out to be too slow.)

858 **Diffusion rate.** In our model, the diffusion rate of one molecule is scaled approximately as the
 859 inverse of the cube root of the molecular weight following Goodhill, 1997. We take the
 860 diffusion rate of TGF β , $D_{TGF\beta}$ as reference, the diffusion rate D_i molecule i is then
 861 approximated as $D_i = D_{TGF\beta} \left(\frac{w_{TGF\beta}}{w_i} \right)^{1/3}$, where $w_{TGF\beta}$ is the molecular weight of TGF β , w_i is
 862 the molecular weight of i . The diffusion rates of all molecular signals in our model are listed in
 863 Table S1.

864 **Production rate.** The concentrations of above molecules in the in vitro studies in serum or cell
 865 are around 1-20 ng/ml (Al-Alwan et al., 2014, De Donat et al., 2008, Gouwy et al., 2009, Jube et
 866 al., 2012, Sieber et al., 2018). In our model, the concentration unit of molecule is assumed to be
 867 1 which equals to 5 ng/ml. The production rate s_i of molecular i is arbitrarily fixed as 0.1.

868 **Decay rate.** In our model, the decay rate γ_i of molecule i is approximated according to its half-
 869 life time: $\gamma_i = \ln 2 / T_{i,1/2}$, where $T_{i,1/2}$ is the half-life time of molecule i . The decay rate of all
 870 molecular signals in our model is listed in Table S1.

871 **Scenario of lobule regeneration**

872 In our model, the cell behaviors are regulated by certain type of signals. Different types of cells
 873 communicated with each other by responding to specific signals and collaborated to achieve
 874 the clearance of necrotic region and recovery from the toxin-induced injury.

875 **Behaviors of hepatocytes.** We assume that the hepatocytes within $164 \mu\text{m}$ (the lobule lesion
876 size due to CCl_4 -induced injury is taken from Hoehme et al., 2010) from the central vein of the
877 lobule are CCl_4 -induced injured cells. In our model, they are marked as *injured* hepatocytes,
878 which can synthesize DAMPs (Li et al., 2020, Calderwood et al., 2016) to activate, among
879 others, Kupffer cells and infiltrating macrophages (Martin-Murphy et al., 2010, Mihm, 2018).
880 Activated Kupffer cells and activated HSCs can produce CXCL1 to attract neutrophils to migrate
881 into the lesion area (Marra and Tacke, 2014), and CCL2 to attract infiltrating macrophages.

882 We have observed that the majority of the hepatocytes are dead 2 hours after the
883 administration of APAP in vitro (experimental data not shown here). A previous study also
884 showed that neutrophils can induce necrosis of hepatocytes upon administration of APAP
885 (Marques et al., 2012). In our model, we assigned random waiting times for each *injured*
886 hepatocyte i according to one Gamma distribution $\Gamma(\alpha = 5, \beta = 5)$. After the waiting time is
887 up, i is marked as *dead (necrotic)* hepatocyte. The choice of α is because the necrotic pathway
888 triggered for hepatocytes due to AILI (acetaminophen-induced liver injury) involves about 5
889 reactions (Dichamp et al., manuscript in preparation) and the choice of β is to fit the data that
890 almost all hepatocytes are dead 2 hours after the administration of APAP. In addition to APAP,
891 neutrophils can also induce necrosis of hepatocytes. For any *injured* hepatocyte i , if its distance
892 with one neutrophil j satisfies $d_{ij} < r_i + r_j$, it is then marked as *dead (necrotic)* hepatocyte
893 (neutrophil can evoke the necrosis of a hepatocyte, Marra and Tacke, 2014). We assumed that
894 for any dead hepatocyte i enduring for more than 24 hours, it then can be eliminated by
895 macrophages (it takes roughly 24 hours for a dying cell to lose its membrane integrity and
896 collapse into fragments, Maruyama et al., 2001). Since it usually takes 2-4 hours for one
897 macrophage to engulf and degrade foreign objects such as dead cell bodies (Haecker et al.,
898 2002), we simulate the degradation process of one dead body of hepatocyte i if it gets contact
899 with one Kupffer cell or infiltrating macrophage j with Ly6C-high phenotype, such that the
900 distance between them satisfies $d_{ij} < r_i + r_j$, i is removed from the model system, 3 hours
901 after the contact.

902 **Behaviors of Kupffer cells.** We assume that for one Kupffer cell i , if its local concentration of
903 DAMP, $\phi_{DAMP,i}$ is higher than a threshold $\phi_{DAMP,activate}$, i is activated and can synthesize
904 TGF β , CXCL1, CCL2, and PDGF (Pinzani et al., 1994, De Bleser et al., 1997, Marra and Tacke,
905 2014, Krenkel et al., 2014). A previous study has reported that due to its highly stationary
906 behavior, Kupffer cells "are not suited" to migrate to the injury site (Ju and Tacke, 2016), but
907 the presence of HSCs does promote the migration of Kupffer cells towards HSCs (Seki et al.,
908 2007). We assume that one activated Kupffer cell i can migrate towards one activated HSC j if
909 the distance between them satisfies $d_{ij} < r_i + r_j + l_{HSC,branch}$, where $l_{HSC,branch}$ is the length
910 of the HSC branch. A migration force $\vec{F}_{mig,i} = f_{mig,i} \frac{\vec{e}_{ij}}{\|\vec{e}_{ij}\|}$, $f_{mig,i} \sim \mathcal{N}(F_{mig,KC,mean}, F_{mig,KC,sd})$ on
911 i is then added to the equation of motion of cell i , where \vec{e}_{ij} is the orientation unit vector from
912 i to j , $F_{mig,KC,mean}$ and $F_{mig,KC,sd}$ are the mean and standard deviation of migration force
913 magnitude approximated from the mean and standard deviation of migration speed of Kupffer
914 cells (due to the highly stationary behavior of Kupffer cells (Ju and Tacke, 2016), we arbitrarily
915 set a low migration speed for them. We also tested higher and even lower migration speed of

916 Kupffer cells in Fig. S3) as $F_{mig,KC,mean} = \Gamma_{ECM,KC} v_{KC,mean}$, $F_{mig,KC,sd} = \Gamma_{ECM,KC} v_{KC,sd}$ (see
917 value of $v_{KC,mean}$ and $v_{KC,sd}$ in Table S1). Since dead hepatocytes can be engulfed by Kupffer
918 cells (Canbay et al., 2003) and the phagocytosis ability of macrophages is decreased by 50%
919 after it uptakes dead cells (Erwig et al., 1999), we assigned one phagocytosis probability $p_{phag,i}$
920 for each activated Kupffer cell i , where $p_{phag,i}$ is initiated as 1. If the distance between i and
921 one dead hepatocyte j satisfies $d_{ij} < r_i + r_j$, and one random number sampled from uniform
922 distribution $U(0,1)$ is less than $p_{phag,i}$, then i stops moving and remains with hepatocyte j for
923 3 hours to engulf and degrade the dead hepatocyte. $p_{phag,i}$ is then divided by two to mimic the
924 decreased phagocytosis ability of i . If there are more than one dead hepatocyte in contact with
925 i , only one of them is randomly selected as the one to be engulfed by i . After 3 hours, j is
926 removed from the system and i can move again. In our model, we consider two alternative
927 time courses for Kupffer cells. Either their population size drops upon activation, recovering
928 from day 2 on, or the Kupffer cell population remains constant. Since in the latter case the life
929 span of one Kupffer cell is no longer than about 4 days, as reported in Naito et al., 2004, we
930 assume that each Kupffer cell would revert to a quiescent mode after it is activated.

931 **Behaviors of HSCs.** PDGF has been reported to attract HSCs to migrate (Melton and Yee, 2007,
932 Yang et al., 2003). We assume that for one HSC i , if its local concentration of PDGF, $\phi_{PDGF,i}$ is
933 higher than a threshold $\phi_{PDGF,migrate}$, a migration force
934 $\vec{F}_{mig,i} = f_{mig,i} \frac{\nabla \phi_{PDGF,i}}{\|\nabla \phi_{PDGF,i}\|}$, $f_{mig,i} \sim \mathcal{N}(F_{mig,HSC,mean}, F_{mig,HSC,sd})$ is then applied on i , where
935 $F_{mig,HSC,mean}$ and $F_{mig,HSC,sd}$ are the mean and standard deviation of migration force
936 magnitude approximated from the mean and standard deviation of migration speed of HSC
937 (Tangkijvanich et al., 2001) as $F_{mig,HSC,mean} = \Gamma_{ECM,HSC} v_{HSC,mean}$,
938 $F_{mig,HSC,sd} = \Gamma_{ECM,HSC} v_{HSC,sd}$ (see value of $v_{HSC,mean}$, $v_{HSC,sd}$ in table S1). Every HSC is
939 initiated as in *quiescent* mode. TGF β has been reported to activate HSCs, but it was also
940 suggested that it is not TGF β but one factor produced by infiltrating macrophages that activates
941 HSCs (Imamura et al., 2005). Here we assume two alternative mechanisms for HSC activation.
942 One is that HSCs are activated by TGF β produced by Kupffer cells. If the local concentration of
943 TGF β , $\phi_{TGF\beta,i}$ at HSC i is higher than a threshold $\phi_{TGF\beta,activate}$, i is activated into the *activated*
944 mode; the other is that HSCs are activated by one factor produced by infiltrating macrophages
945 (the type of this factor is not known, so we assume it has the same diffusion constant and decay
946 rate of TGF β). If the local concentration of this factor, $\phi_{factor,i}$ at HSC i is higher than the
947 threshold $\phi_{TGF\beta,activate}$, i is activated into *activated* mode. Upon activation, HSCs can
948 synthesize CXCL1 and CCL2 (Kisseleva and Brenner, 2007, Baeck et al., 2012). HSCs remain
949 activated even if TGF β falls below the threshold again. As reported before, activated HSCs are
950 phagocytosed by Ly6C-low phenotype infiltrating macrophages in the later stage of liver
951 regeneration (Tacker and Zimmermann, 2014) (mechanism 1). In addition to phagocytosis, we
952 assumed another mechanism (mechanism 2) for the fate of activated HSCs: Ly6C-low
953 phenotype infiltrating macrophages can revert activated HSCs to the quiescent mode. In our
954 model, these two mechanisms are simulated as following: if hepatocyte $i^{[OB]}$ is in activated $j^{[OB]}$
955 $d_{ij} < r_i + r_j^{[OB]}$ it is eliminated $j^{[OB]}$ under mechanism 1) or reverted to quiescent mode again
956 under mechanism 2). To keep the same density of HSCs after liver regeneration, we assumed
957 that under mechanism 1), once $i^{[OB]}$ is activated, it grows and divides after 24 hours.

958 **Behaviors of infiltrating macrophages.** DAMPs are suggested to activate macrophages (Mihm
959 2018). We assume that for one infiltrating macrophage i , if its local concentration of DAMP,
960 $\phi_{DAMP,i}$ is higher than a threshold $\phi_{DAMP,activate}$, i is activated and can synthesize CCL2, and
961 PDGF (Baeck et al., 2012, Pinzani et al., 1994). During liver regeneration, macrophages infiltrate
962 into the liver following the chemoattract of CCL2 (Baeck et al., 2012). We assume that for one
963 infiltrating macrophage i , if its local concentration of CCL2, $\phi_{CCL2,i}$ is higher than a threshold
964 $\phi_{CCL2,migrate}$, a migration force $\vec{F}_{mig,i} = f_{mig,i} \frac{\nabla \phi_{CCL2,i}}{\|\nabla \phi_{CCL2,i}\|}$, $f_{mig,i} \sim \mathcal{N}(F_{mig,IM,mean}, F_{mig,IM,sd})$ is
965 then applied on i , where $F_{mig,IM,mean}$ and $F_{mig,IM,sd}$ are the mean and standard deviation of
966 the migration force magnitude, approximated from the mean and standard deviation of
967 migration speed of infiltrating macrophages (Grabher et al., 2007) as $F_{mig,IM,mean} =$
968 $\Gamma_{ECM,IM} v_{IM,mean}$, $F_{mig,IM,sd} = \Gamma_{ECM,IM} v_{IM,sd}$ (see value of $v_{IM,mean}$, $v_{IM,sd}$ in Table S1). Each
969 infiltrating macrophage is initiated as Ly6C-high phenotype, which is responsible of degrading
970 necrotic cells (Tacke and Zimmermann, 2014). After a certain duration it transforms into Ly6C-
971 low phenotype, which is responsible of anti-fibrosis (Ramachandran et al., 2012, Tacke and
972 Zimmermann, 2014). If the distance between one infiltrating macrophage i in Ly6C-high
973 phenotype and one dead hepatocyte j satisfies $d_{ij} < r_i + r_j$, j is removed 3 hours later to
974 mimic the process of engulfment and degradation of apoptotic hepatocytes (Boulter et al.,
975 2012). There is also the same phagocytose probability $p_{phag,i}$ assigned to i to mimic the
976 decreased phagocytosis ability of the macrophage (Erwig et al., 1999). If the distance between
977 one infiltrating macrophage i in Ly6C-low phenotype and one activated HSC j satisfies
978 $d_{ij} < r_i + r_j$, and one random number sampled from $U(0,1)$ is less than $p_{phag,i}$, j is removed
979 or reverted to quiescent mode under mechanisms 1) and 2) of the fate of activated HSC,
980 respectively. If mechanism 1) is used, $p_{phag,i}$ is then halved. The infiltrating macrophages are
981 initiated as monocytes with Ly6C-high phenotype, and after a period of time between 2 and 3
982 days they transform into macrophages with Ly6C-low phenotype (Zigmond et al., 2014,
983 Graubardt et al., 2017). The transforming time from Ly6C-high phenotype to Ly6C-low
984 phenotype is chosen as 72 hours according to the observation from Zigmond et al., 2014 (the
985 mass peak of Ly6C-low phenotype macrophages is at 3 days after the injury). The Ly6C-high
986 phenotype may not be stained by F4/80 (as shown in Fig. 2 of Dragomir et al., 2012). Hence, at
987 day 2 after the injury, F4/80 staining shows only the Ly6C-low phenotype, which are the KCs
988 (Fig. 1A). The lifetime of one infiltrating macrophage in the system is set to be 96 hours
989 (estimated from the observation from the APAP-induced liver injury done by Zigmond et al.,
990 2014, where the mass peak of infiltrating macrophages is at 1 day after the injury and they are
991 hardly to be seen 5 days after the injury). Infiltrating macrophages are added into the lobule
992 system evenly during the first 24 hours. The initial position of one infiltrating macrophage is
993 randomly sampled inside the lobule but outside of the lesion (164 μm away from the central
994 vein) reflecting their approximate distribution at the time when they start to migrate on
995 response to CCL2, whose peak is at about day 1.

996 **Behaviors of neutrophils.** Neutrophils are recruited by CXCL1 at the early stage of liver injury
997 (De Filippo et al., 2013). We assume that for one neutrophil i , if its local concentration of
998 CXCL1, $\phi_{CXCL1,i}$ is higher than a threshold $\phi_{CXCL1,migrate}$, a migration force $\vec{F}_{mig,i} =$

999 $f_{mig,i} \frac{\nabla\phi_{CXCL1,i}}{\|\nabla\phi_{CXCL1,i}\|}, f_{mig,i} \sim \mathcal{N}(F_{mig,Neutrophil,mean}, F_{mig,Neutrophil,sd})$ is then applied on i ,
1000 $F_{mig,Neutrophil,mean}$ and $F_{mig,Neutrophil,sd}$ are the mean and standard deviation of the
1001 migration force magnitude, approximated from the mean and standard deviation of migration
1002 speed of neutrophils (Marques et al., 2014) as
1003 $F_{mig,Neutrophil,mean} = \Gamma_{ECM,Neutrophil} v_{Neutrophil,mean}$,
1004 $F_{mig,Neutrophil,sd} = \Gamma_{ECM,Neutrophil} v_{Neutrophil,sd}$ (see value of $v_{Neutrophil,mean}$, $v_{Neutrophil,sd}$ in
1005 Table S1). If its distance with one injured hepatocyte j satisfies $d_{ij} < r_i + r_j$, j is then marked as
1006 a dead (necrotic) hepatocyte (neutrophils induce the necrosis of hepatocytes during acute liver
1007 injury, Ramaiah and Jaeschke, 2007). The lifetime of one neutrophil in the system is set to be 48
1008 hours (Graubardt et al., 2017). Neutrophils are added into the lobule system evenly during the
1009 first 24 hours. They migrate very quick. In the model, the initial position of each neutrophil is
1010 randomly sampled inside the lobule but outside of the lesion (164 μm away from the central
1011 vein).

1012 **Behaviors of platelets/sinusoids.** Following liver injury, platelets are recruited to the liver and
1013 adhere to the endothelium to generate factors such as PDGF and HGF (Meyer et al., 2015,
1014 Nowatari et al., 2014). In our model, platelets are not explicitly modeled. The sinusoid spheres
1015 within the lesion ($< 164 \mu\text{m}$ away from the central vein) are considered as endothelium
1016 adherent with platelets, which are an additional source of PDGF. The presence of platelet-
1017 adherent sinusoid spheres lasts until day 2 (the count of platelets resumes to normal after day
1018 2, as reported in Stravitz et al., 2017). The promotive effect of platelet released HGF on the
1019 proliferation of hepatocytes is not modeled explicitly. The proliferation of hepatocytes is
1020 modeled by applying a spatio-temporal proliferation pattern extracted from experimental data
1021 on all healthy hepatocytes in the liver according to its location and time (Hoehme et al., 2010).

1022 **Dynamic of signals.** In our model, DAMPs are synthesized by injured and dead hepatocytes;
1023 TGF β is synthesized by activated Kupffer cells; CCL2 is synthesized by activated Kupffer cells,
1024 activated HSCs, activated infiltrating macrophages, and platelet-adherent sinusoids; CXCL1 is
1025 synthesized by activated Kupffer cells and activated HSCs; PDGF is synthesized by platelet-
1026 adherent sinusoids and Kupffer cells. There is also one factor synthesized by activated
1027 infiltrating macrophages. We assume that this factor takes the same diffusion coefficient and
1028 decay rate as TGF β . The simulated sensitivity test of the concentration of each signal to
1029 regulate the behavior of a certain cell type is shown in Fig. S3. The production rate coefficient
1030 for TGF β is multiplied by 5 when its source cell, Kupffer cell is phagocytosing dead hepatocytes,
1031 by taking into account the observation that the TGF β expression level in Kupffer cells incubated
1032 with apoptotic cells is 5-fold higher than those incubated without apoptotic cells (Canbay et al.,
1033 2003).

1034 REFERENCES

1035 Al-Alwan, L.A., Chang, Y., Rousseau, S., Martin, J.G., Eidelman, D.H., Hamid, Q. (2014). CXCL1
1036 inhibits airway smooth muscle cell migration through the decoy receptor duffy antigen receptor
1037 for chemokines. The Journal of Immunology 193, 1416–1426.

- 1038 Amiri, K.I., Richmond, A. (2003). Fine tuning the transcriptional regulation of the CXCL1
1039 chemokine. *Progress in Nucleic Acid Research and Molecular Biology* 74, 1—36.
- 1040 Baeck, C., Wehr, A., Karlmark, K.R., Heymann, F., Vucur, M., Gassler, N., Huss, S., Klussmann, S.,
1041 Eulberg, D., Luedde, T., Trautwein, C., Tacke, F. (2012). Pharmacological inhibition of the
1042 chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic
1043 hepatic injury. *Gut* 61(3), 416—426.
- 1044 Berchiche, Y.A., Gravel, S., Pelletier, M., St-Onge, G., Heveker, N. (2011). Different effects of the
1045 different natural CC chemokine receptor 2b ligands on β -arrestin recruitment, G α i signaling,
1046 and receptor internalization. *Molecular Pharmacology* 79(3), 488—498.
- 1047 Boulter, L., Govaere, O., Bird, T.G., Radulescu, S., Ramachandran, P., Pellicoro, A., Ridgway, R.A.,
1048 Seo, S.S., Spee, B., Van Rooijen, N., Sansom, O.J., Iredale, J.P., Lowell, S., Roskams, T., Forbes,
1049 S.J. (2012). Macrophage derived Wnt signaling opposes Notch signalling in a Numb mediated
1050 manner to specify HPC fate in chronic liver disease in human and mouse. *Nature Medicine* 18,
1051 572—579.
- 1052 Bouwens, L., Knook, D.L., Wisse, E. (1986). Local proliferation and extrahepatic recruitment of
1053 liver macrophages (Kupffer cells) in partial-body irradiated rats. *Journal of Leukocyte Biology*
1054 39, 687—697.
- 1055 Brenner, C., Galluzzi, L., Kepp, O., Kroemer, G. (2013). Decoding cell death signals in liver
1056 inflammation. *Journal of Hepatology* 59, 583—594.
- 1057 Bufen, N., Saitakis, M., Dogniaux, S., Buschinger, O., Bohineust, A., Richert, A., Maurin, M., Hivroz,
1058 C., Asnacios, A. (2015). Human primary immune cells exhibit distinct mechanical properties that
1059 are modified by inflammation. *Biophysical Journal* 108(9), 2181—2190.
- 1060 Cai, X., Li, Z., Zhang, Q., Qu, Y., Xu, M., Wan, X., Lu, L. (2018). CXCL-EGFR-induced kupffer cells
1061 secrete TGF- β 1 promoting hepatic stellate cell activation via the SMAD2/BRD4/C-MYC/EZH2
1062 pathway in liver fibrosis. *Journal of Cellular and Molecular Medicine* 22, 5050—5061.
- 1063 Calderwood, S.K., Gong, J., Murshid, A. (2016). Extracellular HSPs: the complicated roles of
1064 extracellular HSPs in immunity. *Frontiers in Immunology* 7, 159.
- 1065 Campos, G., Schmidt-Heck, W., De Smedt, J., Widera, A., Ghallab, A., Putter, L., et al. (2020).
1066 Inflammation-associated suppression of metabolic gene networks in acute and chronic liver
1067 disease. *Arch. Toxicol.* 94, 205—217.
- 1068 Canbay, A., Feldstein, A.E., Higuchi, H., Werneburg, N., Grambihler, A., Bronk, S.F., Gores, G.J.
1069 (2003). Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine
1070 expression. *Hepatology* 38(5), 1188—1198.
- 1071 Chu, Y., Dufour, S., Thiery, J.P., Perez, E., Pincet, F. (2005). Johnson-Kendall-Roberts theory
1072 applied to living cells. *Physical Review Letters* 94(2): 028102.

- 1073 Davies, J.E., Apta, B.H.R., Harper, M.T. (2018). Cross-reactivity of anti-HMGB1 antibodies for
1074 HMGB2. *Journal of Immunological Methods* 456, 72—76.
- 1075 De Bleser, P.J., Niki, T., Rogiers, V., Geerts, A. (1997). Transforming growth factor- β gene
1076 expression in normal and fibrotic rat liver. *Journal of Hepatology* 26(4), 886—893.
- 1077 De Donatis, A., Comito, G., Buricchi, F., Vinci, M.C., Parenti, A., Caselli, A., Camici, G., Manao, G.,
1078 Ramponi, G., Cirri, P. (2008). Proliferation versus migration in platelet-derived growth factor
1079 signaling. *The Journal of Biological Chemistry* 283(29), 19948—19956.
- 1080 De Filippo, K., Dudeck, A., Hasenberg, M., Nye, E., Van Rooijen, N., Hartmann, K., Gunzer, M.,
1081 Roers, A., Hogg, N. (2013). Mast cell and macrophage chemokines CXCL1/CXCL2 control the
1082 early stage of neutrophil recruitment during tissue inflammation. *Blood* 121(24), 4930—4937.
- 1083 Drasdo, D., Hoehme, S., Hengstler, J.G. (2014). How predictive quantitative modelling of tissue
1084 organisation can inform liver disease pathogenesis. *Journal of Hepatology* 61(4), 951—956.
- 1085 Dragomir, A.D., Sun, R., Choi, H., Laskin, J.D., Laskin, D.L. (2012). Role of Galectin-3 in classical
1086 and alternative macrophage activation in the liver following acetaminophen intoxication. *The*
1087 *Journal of Immunology* 189, 5934—5941.
- 1088 Dutta-Moscato, J., Solovyev, A., Mi, Q., Nishikawa, T., Soto-Gutierrez, A., Fox, I.J., Vodovotz, Y.
1089 (2014). A multiscale agent-based in silico model of liver fibrosis progression. *Frontiers in*
1090 *Bioengineering and Biotechnology* 2, 18.
- 1091 Erwig, L., Gordon, S., Walsh, G.M., Rees, A.J. (1999). Previous uptake of apoptotic neutrophils or
1092 ligation of integrin receptors downmodulates the ability of macrophages to ingest apoptotic
1093 neutrophils. *Blood* 93(4), 1406—1412.
- 1094 Fan, W., Liu, T., Chen, W., Hammad, S., Longerich, T., Hausser, I., Fu, Y., Li, N., He, Y., Liu, C.,
1095 Zhang, Y., Lian, Q., Zhao, X., Yan, C., Li, L., Yi, C., Ling, Z., Ma, L., Zhao, X., Xu, H., Wang, P., Cong,
1096 M., You, H., Liu, Z., Wang, Y., Chen, J., Li, D., Hui, L., Dooley, S., Hou, J., Jia, J., Sun, B. (2019).
1097 ECM1 prevents activation of transforming growth factor β , hepatic stellate cells, and
1098 fibrogenesis in mice. *Gastroenterology* 157(5), 1352—1367.
- 1099 Fischer, R., Cariers, A., Reinehr, R., Häussinger, D. (2002). Caspase 9-dependent killing of
1100 Hepatic Stellate Cells by activated Kupffer cells. *Gastroenterology* 123, 845—861.
- 1101 Friedman, A., Hao, W. (2017). Mathematical modeling of liver fibrosis. *Mathematical*
1102 *Biosciences and Engineering* 14(1), 143—164.
- 1103 Gao, B., Radaeva, S. (2013). Natural killer and natural killer T cells in liver fibrosis. *Biochimica et*
1104 *Biophysica Acta – Molecular Basis of Disease* 1832(7), 1061—1069.
- 1105 Gardin, I., Linhart, N.C., Petiet, A., Bok, B. (1992). Dosimetry at the cellular level of Kupffer cells
1106 after technetium-99m-sulphur colloid injection. *The Journal of Nuclear Medicine* 33(2), 380—
1107 384

1108 Ghallab, A., Celliere, G., Henkel, S.G., Driesch, D., Hoehme, S., Hofmann, U., Zellmer, S., Godoy,
1109 P., Sachinidis, A., Blaszkewicz, M., et al. (2016). Model-guided identification of a therapeutic
1110 strategy to reduce hyperammonemia in liver diseases. *Journal of Hepatology* 64(4), 860—871.

1111 Ghallab, A., Myllys, M., Holland, C.H., Zaza, A., Murad, W., Hassan, R., Ahmed, Y.A., Abbas, T.,
1112 Abdelrahim, E.A., Schneider, K.M., Matz-Soja, M., Reinders, J., Gebhardt, R., Berres, M., Hatting,
1113 M., Drasdo, D., Saez-Rodriguez, J., Trautwein, C., Hengstler, J.G. (2019). Influence of liver
1114 fibrosis on lobular zonation. *Cells* 8(12), 1556.

1115 Ghallab, A., Myllys, M., Friebel, A., Duda, J., Edlund, K., Halilbasic, E., Vucur, m., Hobloss, Z.,
1116 Brackhagen, L., Begher-Tibbe, B., et al. (2021). Spatio-Temporal Multiscale Analysis of Western
1117 Diet-Fed Mice Reveals a Translationally Relevant Sequence of Events during NAFLD Progression.
1118 *Cells* 10(10), 2516.

1119 Ghallab, A., Hassan, R., Hofmann, U., Friebel, A., Hobloss, Z., Brackhagen, L., Begher-Tibbe, B.,
1120 Myllys, M., Reinders, J., Overbeck, N., Sezgin, S., Zühlke, S., Seddek, A., Murad, W.,
1121 Brecklinghaus, T., Kappenberg, F., Rahnenführer, J., González, D., Goldring, C., Copple, I.,
1122 Marchan, R., Longerich, T., Vucur, M., Luedde, T., Urban, S., Canbay, A., Schreiter, T., Trauner,
1123 M., Akakpo, J., Olyae, M., Curry, S., Sowa, J., Jaeschke, H., Hoehme, S., Hengstler, J.G. (2022).
1124 Interruption of bile acid uptake by hepatocytes after acetaminophen overdose ameliorates
1125 hepatotoxicity. *Journal of Hepatology*, accepted in press.

1126 Gianmoena, K., Gasparoni, H., Jashari, A., Gabrys, P., Grgas, K., Ghallab, A., et al. (2021).
1127 Epigenomic and transcriptional profiling identifies impaired glyoxylate detoxification in NAFLD
1128 as a risk factor for hyperoxaluria. *Cell Reports* 36, 109526.

1129 Goodhill, G.J. (1997). Diffusion in axon guidance. *European Journal of Neuroscience* 9, 1414—
1130 1421.

1131 Gouwy, M., Struyf, S., Verbeke, H., Put, W., Proost, P., Opdenakker, G., Van Damme, J. (2009).
1132 CC chemokine ligand-2 synergizes with the nonchemokine G protein-coupled receptor ligand
1133 fMLP in monocyte chemotaxis, and it cooperates with the TLR ligand LPS via induction of CXCL8.
1134 *Journal of Leukocyte Biology* 86, 671—680.

1135 Grabher, C., Cliffe, A., Miura, K., Hayflick, J., Pepperkik, R., Rørth, P., Wittbrodt, J. (2007). Birth
1136 and life of tissue macrophages and their migration in embryogenesis and inflammation in
1137 medaka. *Journal of Leukocyte Biology* 81(1), 263—271.

1138 Graubardt, N., Vugman, M., Mouhadeb, O., Caliori, G., Pasmanik-Chor, M., Reuveni, D.,
1139 Zigmond, E., Brazowski, E., David, E., Chappell-Maor, L., Jung, S., Varol, C. (2017). Ly6Chi
1140 monocytes and their macrophage descendants regulate neutrophil function and clearance in
1141 acetaminophen-induced liver injury. *Frontiers in Immunology* 8, 626.

1142 Haecker, H., Fuermann, C., Wagner, H., Haecker, G. (2002). Caspase-9/-3 activation and
1143 apoptosis are induced in mouse macrophages upon ingestion and digestion of *Escherichia coli*
1144 bacteria. *The Journal of Immunology* 169, 3172—3179.

- 1145 Hassan, R. (2017). Mechanisms of activated hepatic stellate cell removal in acute and chronic
1146 liver injury. Thesis, Justus-Liebig-Universität Giessen.
- 1147 Herant, M., Heinrich, V., Dembo, M. (2005). Mechanics of neutrophil phagocytosis: behavior of
1148 the cortical tension. *Journal of Cell Science* 118, 1789—1797.
- 1149 Hetherington, J., Sumner, T., Seymour, R.M., Li, L., Rey, M.V., Yamaji, S., Saffrey, P.,
1150 Margoninski, O., Bogle, I.D.L., Finkelstein, A., Warner, A. (2012). A composite computational
1151 model of liver glucose homeostasis. I. building the composite model. *Journal of the Royal
1152 Society Interface* 9, 689—700.
- 1153 Ho, H., Yu, H.B., Bartlett, A., Hunter, P. (2020). An in silico pipeline for subject-specific
1154 hemodynamics analysis in liver surgery planning. *Computer Methods in Biomechanics and
1155 Biomedical Engineering* 23(4), 138—142.
- 1156 Ho, H., Dahmen, U., Hunter, P. (2020a). An in silico rat liver atlas. *Computer Methods in
1157 Biomechanics and Biomedical Engineering* 23(10), 597—600.
- 1158 Hoehme, S., Hengstler, J.G., Brulport, M., Schaefer, M., Bauer, A., Gebhardt, R., Drasdo, D.
1159 (2007). Mathematical modelling of liver regeneration after intoxication with CCl₄. *Chemico-
1160 Biological Interactions* 168(1): 74—93.
- 1161 Hoehme, S., Drasdo, D. (2010). A cell-based simulation software for multi-cellular systems.
1162 *Bioinformatics* 26(20), 2641—2642.
- 1163 Hoehme, S., Brulport, M., Bauer, A., Bedawy, E., Schormann, W., Hermes, M., Puppe, V.,
1164 Gebhardt, R., Zellmer, S., Schwarz, M., Bockamp, E., Timmel, T., Hengstler, J.G., Drasdo, D.
1165 (2010). Prediction and validation of cell alignment along microvessels as order principle to
1166 restore tissue architecture in liver regeneration. *Proceedings of the National Academy of
1167 Sciences* 107(23), 10371—10376.
- 1168 Hoehme, S., Boettger, J., Hammad, S., Begher-Tibbe, B., Bucur, P., Vibert, E., Gebhardt, R.,
1169 Hengstler, J.G., Drasdo, D. (2022). A predictive computational model shows that biomechanical
1170 cell cycle progression control can explain liver regeneration after partial hepatectomy. Accepted.
- 1171 Holland, C.H., Ramirez, F., Myllys, M., Hassan, R., Edlund, K., Hofman, U., et al. (2022).
1172 Transcriptomic cross-species analysis of chronic liver disease reveals consistent regulation
1173 between humans and mice. *Hepatol. Commun.* 6, 161—177.
- 1174 Huebener, P., Hernandez, C., Schwabe, R.F. (2015). HMGB1 and injury amplification. *Oncotarget*
1175 6(27), 23048—23049.
- 1176 Imamura, M., Ogawa, T., Sasaguri, Y., Chayama, K., Ueno, H. (2005). Suppression of macrophage
1177 infiltration inhibits activation of hepatic stellate cells and liver fibrogenesis in rats.
1178 *Gastroenterology* 128(1), 138—146.

- 1179 Jagiella, N., Rickert, D., Theis, F.J., Hasenauer, J. (2017). Parallelization and high-performance
1180 computing enables automated statistical inference of multi-scale models. *Cell Systems* 4(2),
1181 194—206.
- 1182 Jerby, L., Shlomi, T., Ruppin, E. (2010). Computational reconstruction of tissue-specific
1183 metabolic models: application to human liver metabolism. *Molecular Systems Biology* 6, 401.
- 1184 Jikine, A., Edelstein-Keshet, L. (2011). A comparison of mathematical models for polarization of
1185 single eukaryotic cells in response to guided cues. *PLoS Computational Biology* 7(4), e1001121.
- 1186 Ju, C., Tacke, F. (2016) Hepatic macrophages in homeostasis and liver diseases: from
1187 pathogenesis to novel therapeutic strategies. *Cellular & Molecular Immunology* 13(3), 316—
1188 327.
- 1189 Jube, S., Rivera, Z., Bianchi, M.E., Powers, A., Wang, E., Pagano, I., Pass, H.I., Gaudino, G.,
1190 Carbone, M., Yang, H. (2012). Cancer cell secretion of the DAMP protein HMGB1 supports
1191 progression in malignant mesothelioma. *Cancer Research* 72(13), 3290—3301.
- 1192 Kang, L., Mars, W.M., Michalopoulos, G.K. (2012). Signals and cells involved in regulating liver
1193 regeneration. *Cells* 1(4), 1261—1292.
- 1194 Kim, M., Silberberg, Y., Abeyaratne, R., Kamm, R.D., Asada, H.H. (2018). Computational
1195 modeling of three-dimensional ECM-rigidity sensing to guide directed cell migration.
1196 *Proceedings of the National Academy of Sciences* 115(3), E390—E399.
- 1197 Kisseleva, T., Brenner, D.A. (2007). Role of hepatic stellate cells in fibrogenesis and the reversal
1198 of fibrosis. *Journal of Gastroenterology and Hepatology* 22, 573—578.
- 1199 Kisseleva, T., Cong, M., Paik, Y., Scholten, D., Jiang, C., Benner, C., Iwaisako, K., Moore-Morris,
1200 T., Scott, B., Tsukamoto, H., et al. (2012). Myofibroblasts revert to an inactive phenotype during
1201 regression of liver fibrosis. *Proceedings of the National Academy of Sciences* 109(24), 9448—
1202 9453.
- 1203 Kisseleva, T., Brenner, D. (2021). Molecular and cellular mechanisms of liver fibrosis and its
1204 regression. *Nature Reviews Gastroenterology & Hepatology* 18, 151—166.
- 1205 Knutsdottir, H., Zmurchok, C., Bhaskar, D., Palsson, E., Nogare, D.D., Chitnis, A.B., Edelstein-
1206 Keshet, L. (2017). Polarization and migration in the zebrafish posterior lateral line system. *PLoS*
1207 *Computational Biology* 13(4), e1005451.
- 1208 Krenkel, O., Mossanen, J.C., Tacke, F. (2014). Immune mechanisms in acetaminophen-induced
1209 acute liver failure. *Hepatobiliary Surgery & Nutrition* 3(6), 331—343.
- 1210 Kuepfer, L., Clayton, O., Thiel, C., Cordes, H., Nudischer, R., Blank, L.M., Baier, V., Heymans, S.,
1211 Caiment, F., Roth, A., Fluri, D.A., Kelm, J.M., Castell, J., Selevsek, N., Schlapbach, R., Keun, H.,
1212 Hynes, J., Sarkans, U., Gmuender, H., Herwig, R., Niederer, S., Schuchhardt, J., Segall, M.,

- 1213 Kleinjans, J. (2018). A model-based assay design to reproduce in vivo patterns of acute drug-
1214 induced toxicity. *Archives of Toxicology* 92(1), 553—555.
- 1215 Lambers, L., Suditsch, M., Wagner, A., Ricken, T. (2021). A multiscale and multiphase model of
1216 function-perfusion growth processes in the human liver. *Proceedings in Applied Mathematics*
1217 and *Mechanics* 20(1), e20200290.
- 1218 Lee, Y., Patel, D., Park, S. (2011). Local rheology of human neutrophils investigated using atomic
1219 force microscopy. *International Journal of Biological Sciences* 7(1), 102—111.
- 1220 Leist, M., Ghallab, A., Graepel, R., et al. (2017). Adverse outcome pathways: opportunities,
1221 limitations and open questions. *Archives of Toxicology* 91: 3477—3505.
- 1222 Li, H., Zhou, Y., Wang, H., Zhang, M., Qiu, P., Zhang, M., Zhang, R., Zhao, Q., Liu, J. (2020).
1223 Crosstalk between liver macrophages and surrounding cells in nonalcoholic steatohepatitis.
1224 *Frontiers in Immunology* 11, 1169.
- 1225 Liu, Z., Govindarajan, S., Kaplowitz, N. (2004). Innate immune system plays a critical role in
1226 determining the progression and severity of acetaminophen hepatotoxicity. *Gastroenterology*
1227 127, 1760—1774.
- 1228 Liu, Z., Han, D., Gunawan, B., Kaplowitz, N. (2006). Neutrophil depletion protects against murine
1229 acetaminophen hepatotoxicity. *Hepatology* 43(6), 1220—1230.
- 1230 Marques, P.E., Amaral, S.S., Pires, D.A., Nogueira, L.L., Soriani, F.M., Lima, B.H.F., Lopes, G.A.O.,
1231 Russo, R.C., Avila, T.V., Melgaco, J.G., Oliveira, A.G., Pinto, M.A., Lima, C.X., De Paula, A.M.,
1232 Cara, D.C., Leite, M.F., Teixeira, M.M., Menezes, G.B. (2012). Chemokines and mitochondrial
1233 products activate neutrophils to amplify organ injury during mouse acute liver failure.
1234 *Hepatology* 56(5), 1971—1982.
- 1235 Marques, P.E., Oliveira, A.G., Pereira, R.V., David, B.A., Gomides, L.F., Saraiva, A.M., Pires, D.A.,
1236 Novaes, J.T., Patricio, D.O., Cisalpino, D., et al. (2014). Hepatic DNA deposition drives drug-
1237 induced liver injury and inflammation in mice. *Hepatology* 61(1), 348—360.
- 1238 Marra, F., Tacke, F. (2014). Roles for chemokines in liver disease. *Gastroenterology* 147, 577—
1239 594.
- 1240 Martin-Murphy, B.V., Holt, M.P., Ju, C. (2010). The role of damage associated molecular pattern
1241 molecules in acetaminophen-induced liver injury in mice. *Toxicology Letters* 192, 387—394.
- 1242 Maruyama, R., Takemura, G., Aoyama, T., Hayakawa, K., Koda, M., Kawase, Y., Qiu, X., Ohno, Y.,
1243 Minatoguchi, S., Miyata, K., Fujiwara, T., Fujiwara, H. (2001). Dynamic process of apoptosis in
1244 adult rat cardiomyocytes analyzed using 48-hour videomicroscopy and electron microscopy.
1245 *American Journal of Pathology* 159(2), 683—691.

- 1246 McDonald, B., Pittman, K., Menezes, G.B., Hirota, S.A., Slaba, I., Waterhouse, C.C.M., Beck, P.L.,
1247 Muruve, D.A., Kubes, P. (2010). Intravascular danger signals guide neutrophils to sites of sterile
1248 inflammation. *Science* 330(6002), 362—366.
- 1249 Melton, A., Yee, H.F. (2007). Hepatic stellate cell protrusions couple platelet-derived growth
1250 factor-BB to chemotaxis. *Hepatology* 45(6), 1446—1453.
- 1251 Meyer, J., Lejmi, E., Fontana, P., Morel, P., Gonelle-Gispert, C., Bühler, L. (2015). A focus on the
1252 role of platelets in liver regeneration: Do platelet-endothelial cell interactions initiate the
1253 regenerative process? *Journal of Hepatology* 63(5), 1263—1271.
- 1254 Mihm, S. (2018). Danger-associated molecular patterns (DAMPs): Molecular triggers for sterile
1255 inflammation in the liver. *International Journal of Molecular Sciences* 19, 3104.
- 1256 Michalopoulos, G.K. (2010). Liver regeneration after partial hepatectomy: critical analysis of
1257 mechanistic dilemmas. *The American Journal of Pathology* 176(1), 2—13.
- 1258 Michalopoulos, G.K. (2017). Hepatostat: Liver regeneration and normal liver tissue
1259 maintenance. *Hepatology* 65(4), 1384—1392.
- 1260 Michalopoulos, G.K., Bhushan, B. (2021). Liver regeneration: biological and pathological
1261 mechanisms and implications. *Nature Reviews Gastroenterology & Hepatology* 18, 40—55.
- 1262 Murphy, K.E., Hall, C.L., Maini, P.K., McCue, S.W., McElwain, D.L.S. (2012). A fibrocontractive
1263 mechanochemical model of dermal wound closure incorporating realistic growth factor
1264 kinetics. *Bulletin of Mathematical Biology* 74, 1143—1170.
- 1265 Naik, A., Rozman, D., Belič, A. (2014). SteatoNet: The first integrated human metaolic model
1266 with multi-layered regulation to investigate liver-associated pathologies. *PLoS Computational*
1267 *Biology* 10(12), e1003993.
- 1268 Naito, M., Hasegawa, G., Ebe, Y., Yamamoto, T. (2004). Differentiation and function of Kupffer
1269 cells. *Medical Electron Microscopy* 37, 16—28.
- 1270 Nakamura, K., Nonaka, H., Saito, H., Tanaka, M., Miyajima, A. (2004). Hepatocyte proliferation
1271 and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice.
1272 *Hepatology* 39(3), 635—644.
- 1273 Nevzorova, Y.A., Boyer-Diaz, Z., Cubero, F.J., Gracia-Sancho, J. (2020). Animal models for liver
1274 disease – A practical approach for translational research. *Journal of Hepatology* 73, 423—440.
- 1275 Nowatari, T., Murata, S., Fukunaga, K., Ohkohchi, N. (2014). Role of platelets in chronic liver
1276 disease and acute liver injury. *Hepatology Research* 44, 165—172.
- 1277 O’Dell, J.R., Zetterman, R.K., Burnett, D.A. (1986). Centrilobular hepatic fibrosis following
1278 acetaminophen-induced hepatic necrosis in an alcoholic. *JAMA* 255(19), 2636—2637.
- 1279 Olson, K.R., Davarpanah, A.H., Schaefer, E.A., Elias, N., Misdraji, J. (2017). Case 2-2017 – An 18-
1280 year-old woman with acute liver failure. *The New England Journal of Medicine* 376: 268—278.

- 1281 Park, J., Holmes, W.R., Lee, S.H., Kim, H., Kim, D., Kwak, M.K., Wang, C.J., Edelstein-Keshet, L.,
1282 Levchenko, A. (2017). Mechanochemical feedback underlies coexistence of qualitatively distinct
1283 cell polarity patterns within diverse cell populations. *Proceedings of the National Academy of*
1284 *Sciences* 114(28), E5750—E5759.
- 1285 Pinzani, M., Milani, S., Grappone, C., Weber JR., F.L., Gentilini, P., Abboud, H.E. (1994).
1286 Expression of platelet-derived growth factor in a model of acute liver injury. *Hepatology* 19(3),
1287 701—707.
- 1288 Popov, V.L. (2010). *Contact mechanics and friction*. Springer.
- 1289 Puche, J.E., Saiman, Y., Friedman, S.L. (2013). Hepatic stellate cells and liver fibrosis.
1290 *Comprehensive Physiology* 3(4), 1473—1492.
- 1291 Ramachandran, P., Pellicoro, A., Vernon, M.A., Boulter, L., Aucott, R.L., Ali, A., Hartland, S.N.,
1292 Snowdon, V.K., Cappon, A., Gordon-Walker, T.T., Williams, M.J., Dunbar, D.R., Manning, J.R.,
1293 Van Rooijen, N., Fallowfield, J.A., Forbes, S.J., Iredale, J.P. (2012). Differential Ly-6C expression
1294 identifies the recruited macrophage phenotype, which orchestrates the regression of murine
1295 liver fibrosis. *PNAS* 109(46), E3186—E3195.
- 1296 Ramadori, P., Klag, T., Malek, N.P., Heikenwalder, M. (2019). Platelets in chronic liver disease,
1297 from bench to bedside. *JHEP Reports* 1, 448—459.
- 1298 Ramaiah, S.K., Jaeschke, H. (2007). Role of neutrophils in the pathogenesis of acute
1299 inflammatory liver injury. *Toxicologic Pathology* 35, 757—766.
- 1300 Remien, C.H., Adler, F.R., Waddoups, L., Box, T.D., Sussman, N.L. (2012). Mathematical
1301 modeling of liver injury and dysfunction after acetaminophen overdose: Early discrimination
1302 between survival and death. *Hepatology* 56(2), 727—734.
- 1303 Ritz, T., Krenkel, O., Tacke, F. (2018). Dynamic plasticity of macrophage functions in diseased
1304 liver. *Cellular Immunology* 330, 175—182.
- 1305 Rohrschneider, M., Scheuermann, G., Hoehme, S., Drasdo, D. (2007). Shape characterization of
1306 extracted and simulated tumor samples using topological and geometric measures. 29th Annual
1307 International Conference of the IEEE Engineering in Medicine and Biology Society, 6271—6277.
- 1308 Rotsch, C., Braet, F., Wisse, E., Radmacher, M. (1997). AFM imaging and elasticity
1309 measurements on living rat liver macrophages. *Cell Biology International* 21(11), 685—696.
- 1310 Schneider, K.M., Elfers, C., Ghallab, A., Schneider, C.V., Galvez, E.J.C., Mohs, A., Gui, W.,
1311 Candels, L.S., Wirtz, T.H., Zuehlke, S., et al. (2021). Intestinal dysbiosis amplifies
1312 acetaminophen-induced acute liver injury. *Cellular and Molecular Gastroenterology and*
1313 *Hepatology* 11(4), 909—933.
- 1314 Schneider, K.M., Candels, L.S., Hov, J.R., Myllys, M., Hassan, R., Schneider, C.V., et al. (2021).
1315 Gut microbiota depletion exacerbates cholestatic liver injury via loss of FXR signaling. *Nat.*
1316 *Metab.* 3, 1228—1241.

1317 Schuran, F.A., Lommetz, C., Steudter, A., Ghallab, A., Wieschendorf, B., Schwinge, D., Zuehlke,
1318 S., Reinders, J., Heeren, J., Lohse, A.W., et al. (2021). Aryl hydrocarbon receptor activity in
1319 hepatocytes sensitizes to hyperacute acetaminophen-induced hepatotoxicity in mice. *Cellular
1320 and Molecular Gastroenterology and Hepatology* 11(2), 371–388. Seki, E., De Minicis, S.,
1321 Österreicher, C.H., Kluwe, J., Osawa, Y., Brenner, D.A., Schwabe, R.F. (2007) TLR4 enhances TGF-
1322 β signaling and hepatic fibrosis. *Nature Medicine* 13(11), 1324–1332.

1323 Schwarz, U.S. (2020). To buckle or not to buckle. *Nature Materials* 19, 8–9.

1324 Schwen, L.O., Krauss, M., Niederal, C., Gremse, F., Kiessling, F., Schenk, A., Preusser, T. (2014).
1325 Spatio-temporal simulation of first pass drug perfusion in the liver. *PLoS Computational Biology*
1326 10(3), e1003499.

1327 Schwen, L.O., Kuefer, L., Preusser, T. (2016). Modeling approaches for hepatic spatial
1328 heterogeneity in pharmacokinetic simulations. *Drug Discovery Today Disease Models* 22, 35–
1329 43.

1330 Seki, E., De Minicis, S., Österreicher, C.H., Kluwe, J., Osawa, Y., Brenner, D.A., Schwabe, R.F.
1331 (2007). TLR4 enhances TGF- β signaling and hepatic fibrosis. *Nature Medicine* 13, 1324–1332.

1332 Sezgin, S., Hassan, R., Zuehlke, S., Kuepfer, L., Hengstler, J.G., Spiteller, M., Ghallab, A. (2018).
1333 Spatio-temporal visualization of the distribution of acetaminophen as well as its metabolites
1334 and adducts in mouse livers by MALDI MSI. *Archives of Toxicology* 92(9), 2963–2977.

1335 Sieber, P., Schaefer, A., Lieberherr, R., Goff, F.L., Stritt, M., Welford, R.W.D., Gatfield, J., Peter,
1336 O., Nayler, O., Luethi, U. (2018). Novel high-throughput myofibroblast assays identify agonists
1337 with therapeutic potential in pulmonary fibrosis that act via EP₂ and EP₄ receptors. *PLOS ONE*
1338 13(11), e0207872.

1339 Soyombo, A.A., DiCorleto, P.E. (1994). Stable expression of human platelet-derived growth
1340 factor B chain by bovine aortic endothelial cells. *The Journal of Biological Chemistry* 269(26),
1341 17734–17740.

1342 Stepanova, D., Byrne, H.M., Maini, P.K., Alarcon, T. (2021). A multiscale model of complex
1343 endothelial cell dynamics in early angiogenesis. *PLoS Computational Biology* 17(1), e1008055.

1344 Stewart, R.K., Dangi, A., Huang, C., Murase, N., Kimura, S., Stolz, D.B., Wilson, G.C., Lentsch,
1345 A.B., Gandhi, C.R. (2014). A novel mouse model of depletion of stellate cells clarifies their role
1346 in ischemia/reperfusion- and endotoxin-induced acute liver injury. *Journal of Hepatology* 60,
1347 298–305.

1348 Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T.M., Serbina, N.V., Lipuma, L., Leiner, I., Li, M.O.,
1349 Frenette, P.S., Pamer, E.G. (2011). Bone marrow mesenchymal stem and progenitor cells induce
1350 monocyte emigration in response to circulating Toll-like receptor ligands. *Immunity* 34(4),
1351 590–601.

- 1352 Stravitz, R.T., Ellerbe, C., Durkalski, V., Reuben, A., Lisman, T., Lee, M.W., the Acute Liver Failure
1353 Study Group. (2017). Thrombocytopenia is associated with multi-organ system failure in
1354 patients with acute liver failure. *Clinical Gastroenterology & Hepatology* 14(4), 613—620.
- 1355 Sun, D., Novotny, M., Bulek, K., Liu, C., Li, X., Hamilton, T. (2011). Treatment with IL-17 prolongs
1356 the half-life of chemokine CXCL1 mRNA via the adaptor TRAF5 and the splicing-regulatory factor
1357 SF2 (ASF). *Nature Immunology* 12(9), 853—860.
- 1358 Tacke, F., Zimmermann, H.W. (2014). Macrophage heterogeneity in liver injury fibrosis. *Journal*
1359 *of Hepatology* 60, 1090—1096.
- 1360 Talman, L., Agmon, E., Peirce, S.M., Covert, M.W. (2019). Multiscale models of infection.
1361 *Current Opinion in Biomedical Engineering* 11, 102—108.
- 1362 Tangkijvanich, P., Tam, S.P., Yee, J.F. (2001). Wound-induced migration of rat hepatic stellate
1363 cells is modulated by endothelin-1 through Rho-kinase-mediated alterations in the acto-myosin
1364 cytoskeleton. *Hepatology* 3(1), 74—80.
- 1365 Thurley, K., Gerecht, D., Friedmann, E., Hofer, T. (2015). Three-dimensional gradients of
1366 cytokine signaling between T cells. *PLoS Computational Biology* 11(4), e1004206.
- 1367 Trepap, X., Wasserman, M.R., Angelini, T.E., Millet, E., Weitz, D.A., Butler, J.P., Fredberg, J.J.
1368 (2009). Physical forces during collective cell migration. *Nature Physics* 5(6), 426—430.
- 1369 Troeger, J.S., Mederacke, I., Gwak, G., Dapito, D.H., Mu, X., Hsu, C.C., Pradere, J., Friedman,
1370 R.A., Schwabe, R.F. (2012). Deactivation of hepatic stellate cells during liver fibrosis resolution
1371 in mice. *Gastroenterology* 143(4), 1073—1083.
- 1372 Van Coillie, E., Van Damme, J., Opdenakker, G. (1999). The MCP/eotaxin subfamily of CC
1373 chemokines. *Cytokine & Growth Factor Reviews* 10(1), 61—86.
- 1374 Van Liedekerke, P., Palm, M.M., Jagiella, N., Drasdo, D. (2015). Simulating tissue mechanics with
1375 agent-based models: concepts, perspectives, and some novel results. *Computational Particle*
1376 *Mechanics* 2(4): 401—444.
- 1377 Van Liedekerke, P., Neitsch, J., Johann, T., Alessandri, K., Nassoy, P., Drasdo, D. (2019).
1378 Quantitative agent-based modeling reveals mechanical stress response of growing tumor
1379 spheroids is predictable over various growth conditions and cell lines. *PLoS Computational*
1380 *Biology* 15(3), e1006273.
- 1381 Van Liedekerke, P., Neitsch, J., Johann, T., Warnt, E., Gonzalez-Valverde, I., Hoehme, S.,
1382 Grosser, S., Kaes, J., Drasdo, D. (2020). A quantitative high-resolution computational mechanics
1383 cell model for growing and regenerating tissues. *Biomechanics and Modeling in*
1384 *Mechanobiology* 19(1), 189—220.
- 1385 Verma, B.K., Subramaniam, P., Vadigepalli, R. (2019). Model-based virtual patient analysis of
1386 human liver regeneration predicts critical perioperative factors controlling the dynamic mode of
1387 response to resection. *BMC Systems Biology* 13(1), 1—15.

- 1388 Vishwakarma, M., Di Russo, J., Probst, D., Schwarz, U.S., Das, T., Spatz, J.P. (2018). Mechanical
1389 interactions among followers determine the emergence of leaders in migrating epithelial cell
1390 collectives. *Nature Communications* 9(1), 1–12.
- 1391 Waltenberger, J., Usuki, K., Fellström, B., Funa, K., Heldin, C. (1992). Platelet-derived endothelial
1392 cell growth factor Pharmacokinetics, organ distribution and degradation after intravenous
1393 administration in rats. *FEBS Letters* 313(2), 129–132.
- 1394 Wake, K. (2006). Hepatic stellate cells: Three-dimensional structure, localization, heterogeneity
1395 and development. *Proceedings of the Japan Academy, Series B* 82(4), 155–164.
- 1396 Wakefield, L.M., Winokur, T.S., Hollands, R.S., Christopherson, K., Levinson, A.D., Sporn, M.B.
1397 (1990). Recombinant latent transforming growth factor β 1 has a longer plasma half-life in rats
1398 than active transforming growth factor β 1, and a different tissue distribution. *The Journal of*
1399 *Clinical Investigation* 86(6), 1976–1984.
- 1400 Wambaugh, J., Shah, I. (2010). Simulating microdosimetry in a virtual hepatic lobule. *PLoS*
1401 *Computational Biology* 6(4), e1000756.
- 1402 Willemsen, L., de Winther, M.P.J. (2020). Macrophage subsets in atherosclerosis as defined by
1403 single-cell technologies. *Journal of Pathology* 250, 705–714.
- 1404 Yang, C., Zeisberg, M., Mosterman, B., Sudhakar, A., Yerramalla, U., Holthaus, K., Xu, L., Eng, F.,
1405 Afdhal, N., Kalluri, R. (2003). Liver fibrosis: insights into migration of hepatic stellate cells in
1406 response to extracellular matrix and growth factors. *Gastroenterology* 124(1), 147–159.
- 1407 Yang, Y., Liao, J., Lin, C., Chang, C., Wang, S., Ju, M. (2012). Characterization of cholesterol-
1408 depleted or -restored cell membranes by depth-sensing nano-indentation. *Soft Matter* 8(3),
1409 682–687.
- 1410 You, Q., Holt, M., Yin, H., Li, G., Hu, C., Ju, C. (2013). Role of hepatic resident and infiltrating
1411 macrophages in liver repair after acute injury. *Biochemical Pharmacology* 86(6), 836–843.
- 1412 Zandarashvili, L., Sahu, D., Lee, K., Lee, Y.S., Singh, P., Rajarathnam, K., Iwahara, J. (2013). Real-
1413 time kinetics of high-mobility group box 1 (HMGB1) oxidation in extracellular fluids studied by
1414 in Situ protein NMR spectroscopy. *Journal of Biological Chemistry* 288(17), 11621–11627.
- 1415 Zieve, L., Anderson, W.R., Dozeman, R., Draves, K., Lyftogt, C. (1985). Acetaminophen liver
1416 injury: sequential changes in two biochemical indices of regeneration and their relationship to
1417 histologic alterations. *The Journal of Laboratory and Clinical Medicine* 105(5), 619–624.
- 1418 Zigmond, E., Samia-Grinberg, S., Pasmanik-Chor, M., Brazowski, E., Shibolet, O., Halpern, Z.,
1419 Varol, C. (2014). Infiltrating monocyte-derived macrophages and resident Kupffer cells display
1420 different ontogeny and functions in acute liver injury. *Journal of Immunology* 193, 344–353.

1421 **ACKNOWLEDGEMENTS**

1422 The authors acknowledge financial support from BMBF LiSyM FKZ: 031L0045, BMBF
1423 LiSyM-Krebs: 031L0257D, ANR iLite, ANR-16-RHUS-0005. A.G. was funded by the German-
1424 Research-Foundation (DFG; GH 276). S.D. was funded by LiSyM Grant PTJ-FKZ: 031L0043.

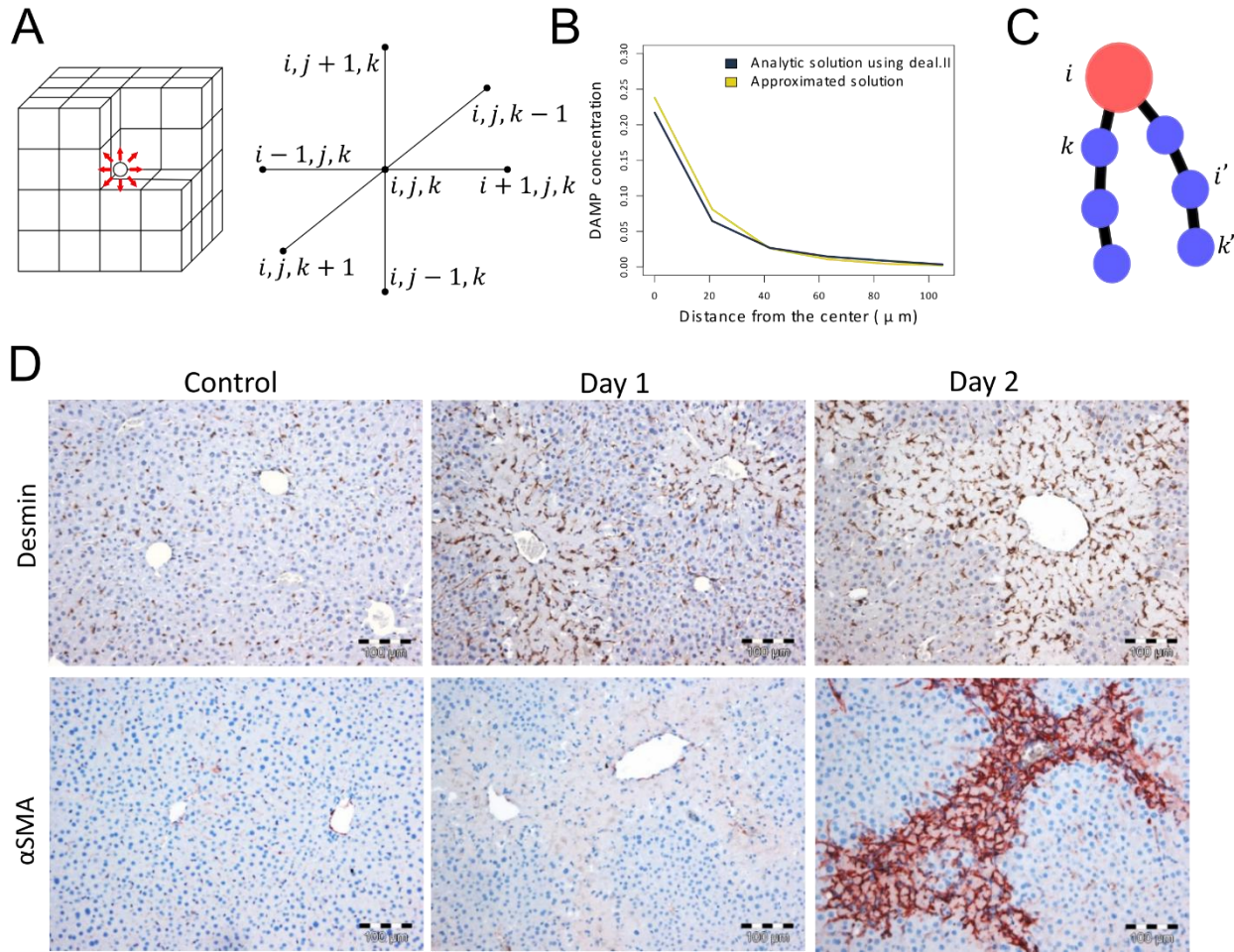
1425 **AUTHOR CONTRIBUTIONS**

1426 The modeling part was performed by D.D. and J.Z., the experimental part by A.G., R.H., J.G.H.

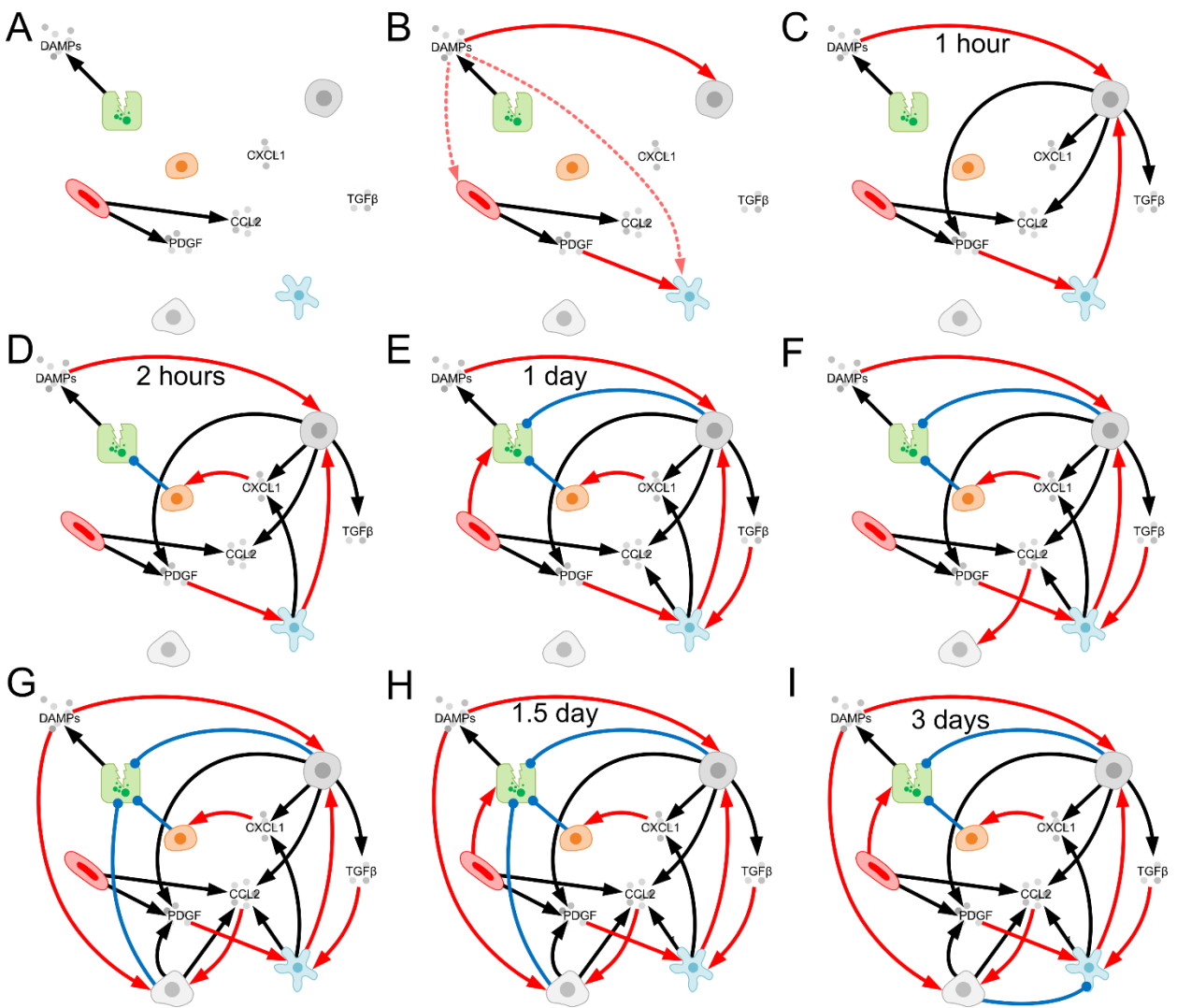
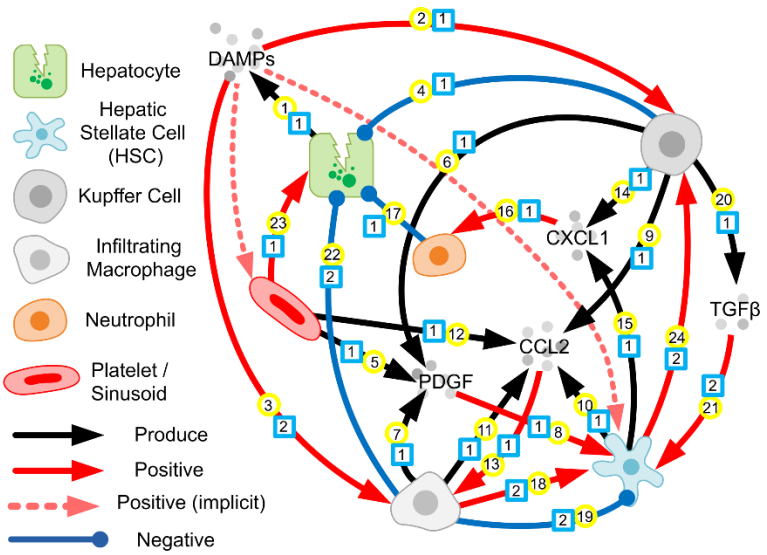
1427 **Conceptualization:** D.D.; **Data curation:** J.Z., A.G., R.H.; **Formal analysis:** J.Z., A.G., D.D., **Funding**
1428 **acquisition:** A.G., S.D., J.G.H., D.D.; **Investigation:** D.D., J.Z.; **Methodology:** D.D., J.Z. (modeling);
1429 A.G., J.G.H. (experimental); **Project administration:** D.D.; **Resources:** D.D., J.G.H.; **Software**
1430 **implementation:** J.Z.; **Supervision:** D.D., A.G.; **Validation:** D.D., J.Z., A.G., S.D., J.G.H.;
1431 **Visualization:** J.Z.; **Writing, Original Draft:** J.Z. and D.D.; **Writing, Review and Editing:** J.Z., A.G.,
1432 S.D., J.G.H., D.D.

1433 **DECLARATION OF INTERESTS**

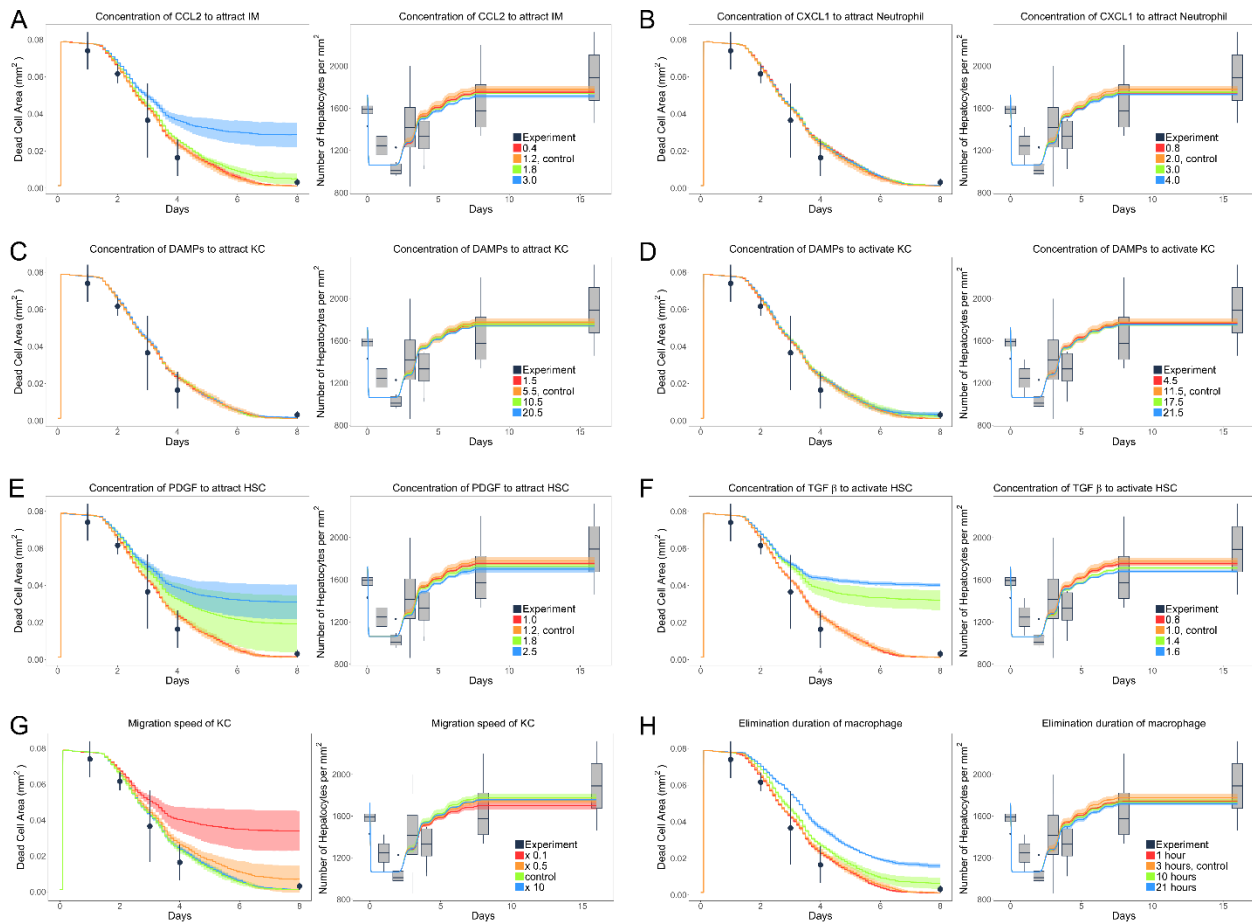
1434 The authors declare no competing interests.



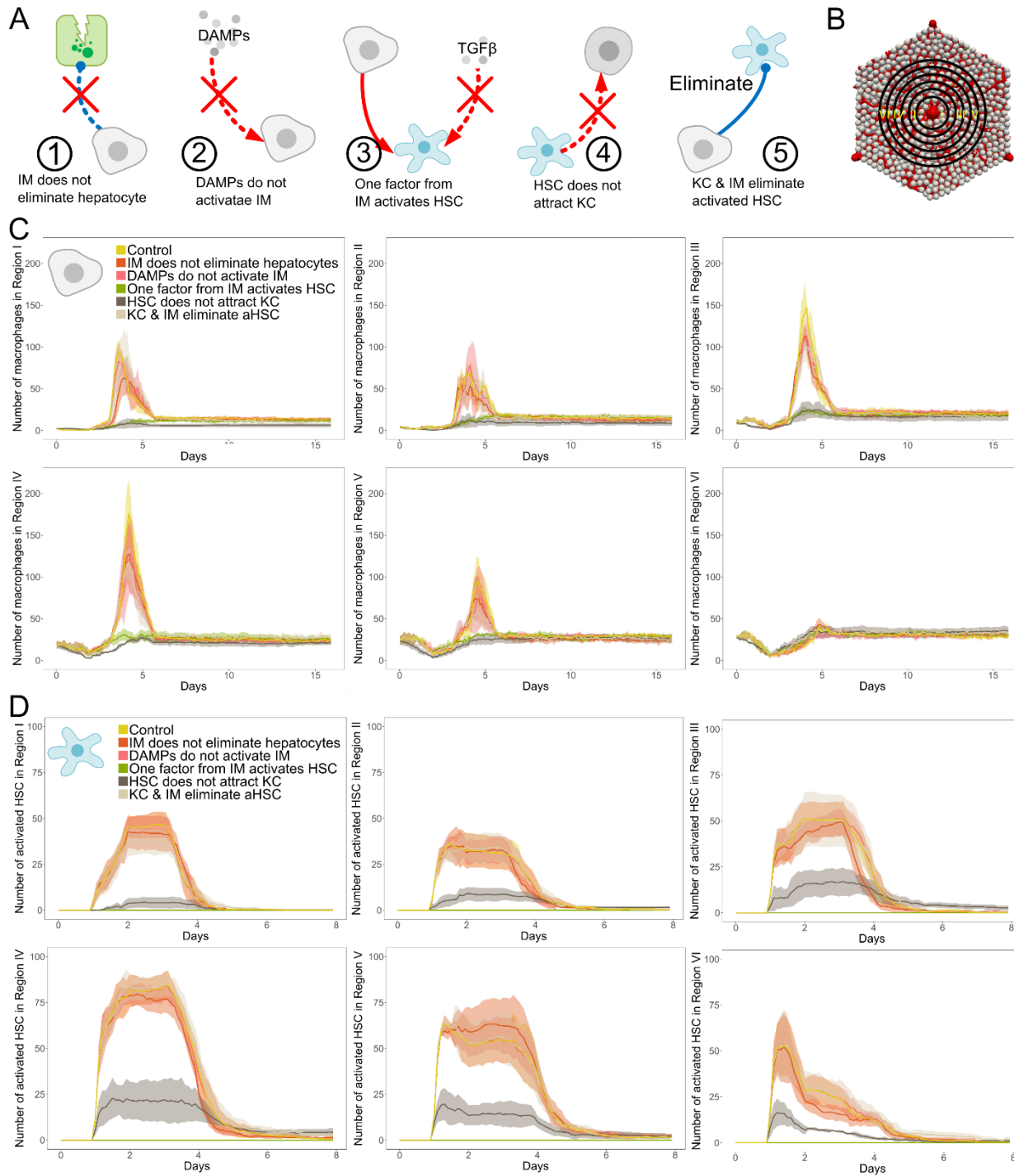
1436
 1437 **Figure S1. The cubic system to simulate the signal gradient and the staining of HSCs before**
 1438 **day 2.** (A) A cubic system is used to approximate the solution of PDE regarding a signal gradient
 1439 in the lobule. (B) Comparison of the approximated solution of the concentration of a DAMP
 1440 released by one hepatocyte with the corresponding analytic solution by using software deal.II
 1441 (Arndt et al., 2021). (C) HSC number i is modeled as one head sphere (red) connected with
 1442 several arms (in the simulation, 5 arms are used while in C only 2 arms are represented for
 1443 simplicity) represented as chains of spheres (blue). There are elastic forces between every
 1444 sphere and its connected neighboring spheres. Then elastic force on i' from k' is defined as
 1445 $\vec{F}_{ela,i'k'} = \vec{e}_{i'k'} \Delta L_{i'k'} E_{HSC} \pi R_{HSC,arm}^2 / L_{i'k'}$, where E_{HSC} is the Young's modulus of HSC,
 1446 $R_{HSC,arm}$ is the radius of the arm spheres (we assume it is 1/3 of the radius of head sphere of
 1447 HSC), $L_{i'k'}$ is the equilibrium length of $i'k'$, $\Delta L_{i'k'}$ is the deviation from $L_{i'k'}$, $\vec{e}_{i'k'}$ is unit vector
 1448 from i' to k' . In the interaction with other cell types, only the head sphere of i (red) is
 1449 considered. (D) The staining of all types of HSCs (Desmin) and activated HSCs (α SMA).



1451 **Figure S2. Time course of the interaction processes in the reference state.** (A-C) About 1 hour
 1452 after drug administration: Injured hepatocytes secrete DAMPs to activate Kupffer cells, which
 1453 secrete CXCL1, CCL2 and TGF β . Platelets secrete PDGF and CCL2. HSCs are attracted by PDGF.
 1454 From activation on, the Kupffer cell population decreases until day 2 due to the cell death,
 1455 before it recovers again. (D) About 2 hours after APAP administration, neutrophils are attracted
 1456 by CXCL1 to induce the death of injured hepatocytes. Kupffer cells migrate towards HSCs. (E-G)
 1457 Around 1 day after APAP administration, HSCs are activated by TGF β and secrete CCL2 and
 1458 CXCL1. Infiltrating macrophages are attracted by CCL2 and activated by DAMPs. Both,
 1459 infiltrating macrophages and Kupffer cells phagocytose dead hepatocytes. (H) Around 1.5 days
 1460 after APAP administration, healthy hepatocytes surrounding the necrotic region proliferate and
 1461 migrate to recover the lesion. (I) Around 3 days after APAP administration, infiltrating
 1462 macrophages switched from Ly6C-high to Ly6C-low phenotype and revert/remove activated
 1463 HSCs.



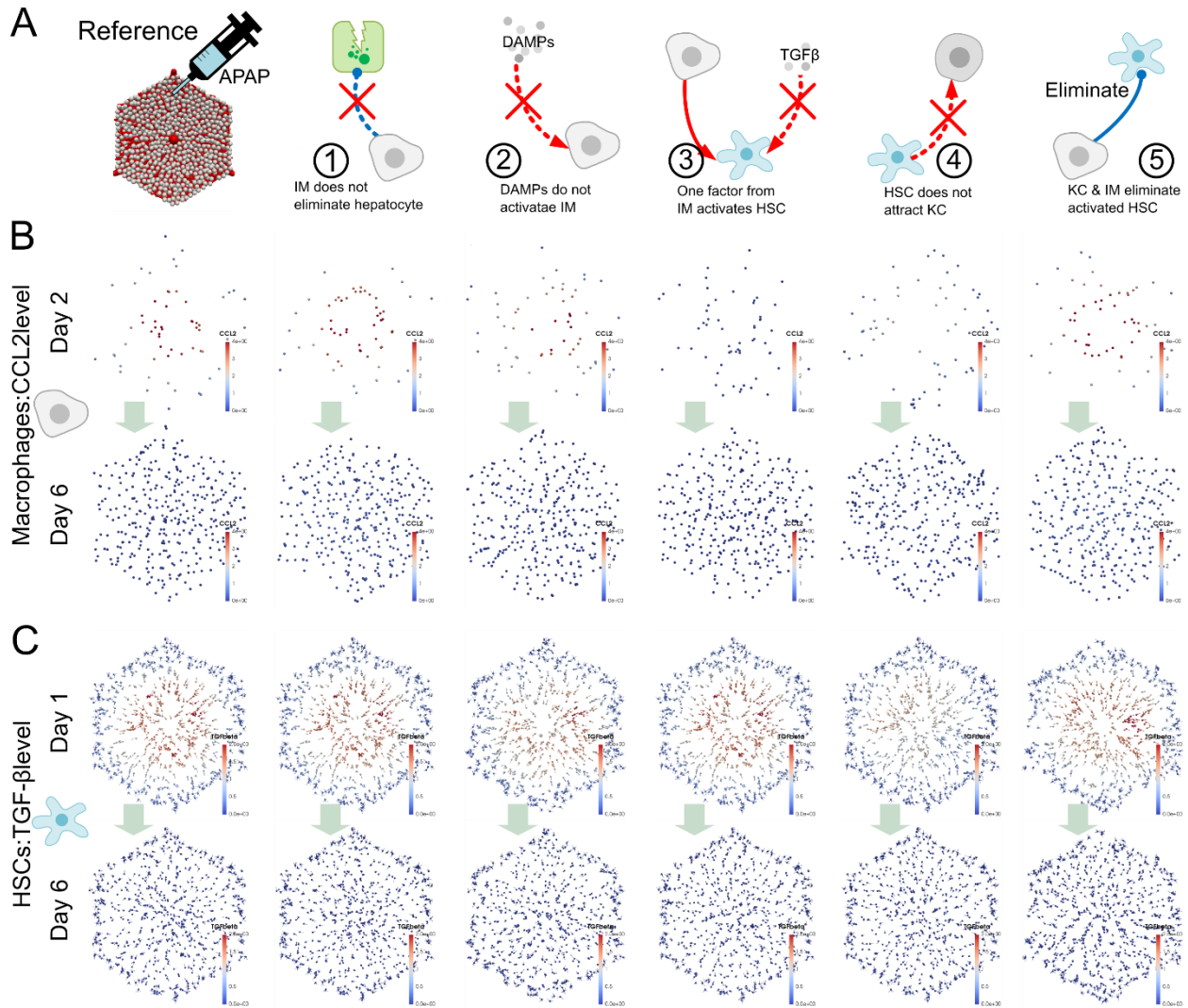
1464 **Figure S3. Sensitivity test of the impact of signal concentrations on liver regeneration.**
 1465 Concentration of different signals: (A) CCL2: attract the infiltration of macrophages. (B) CXCL1:
 1466 attract neutrophils. (C) DAMP: attracts Kupffer cells. (D) DAMP: activates Kupffer cells. (E)
 1467 PDGF: attracts HSCs. (F) TGF β : activates HSCs. (G) Migration speed of Kupffer cells. (H)
 1468 Elimination duration of macrophages.



1470

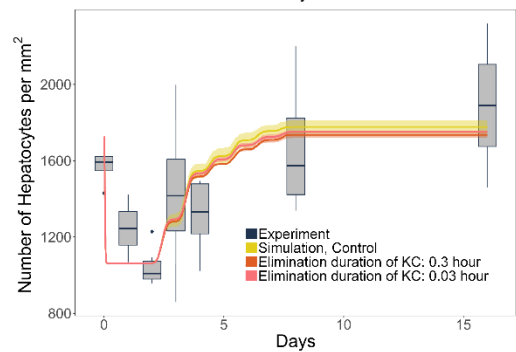
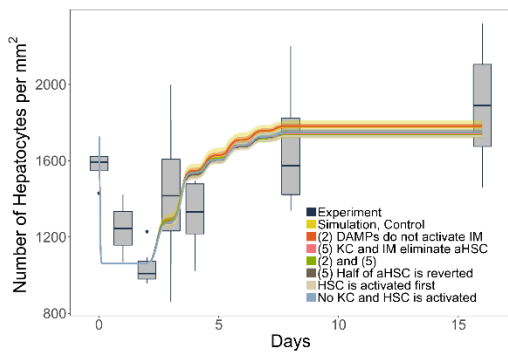
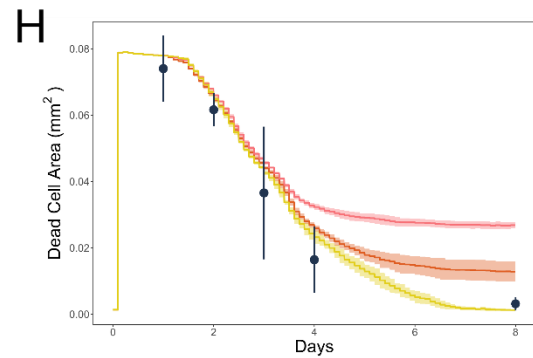
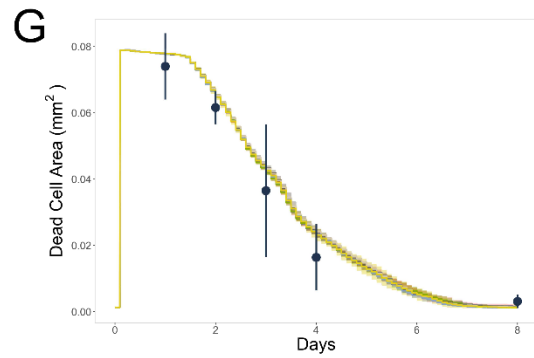
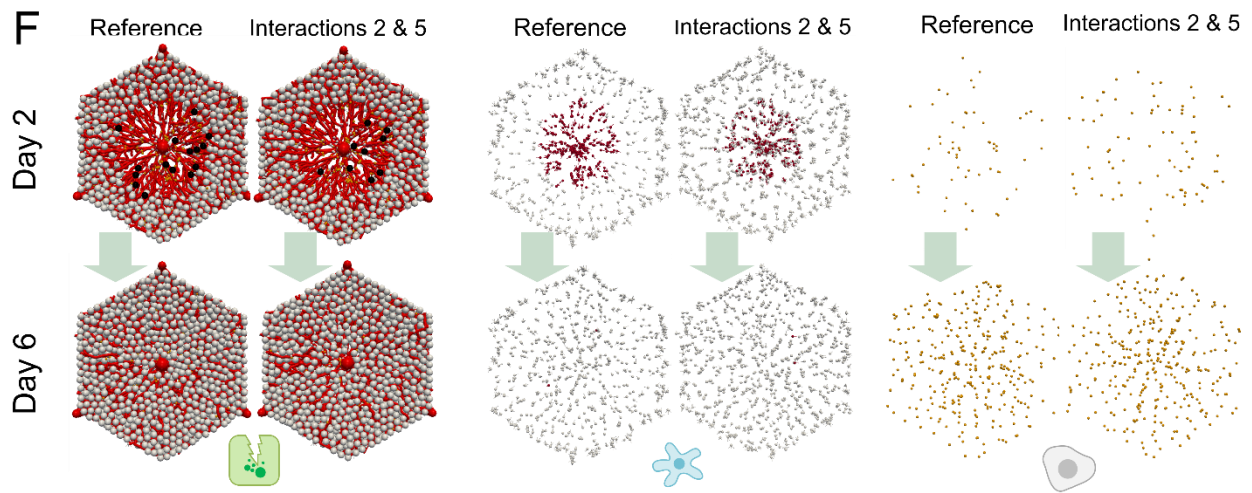
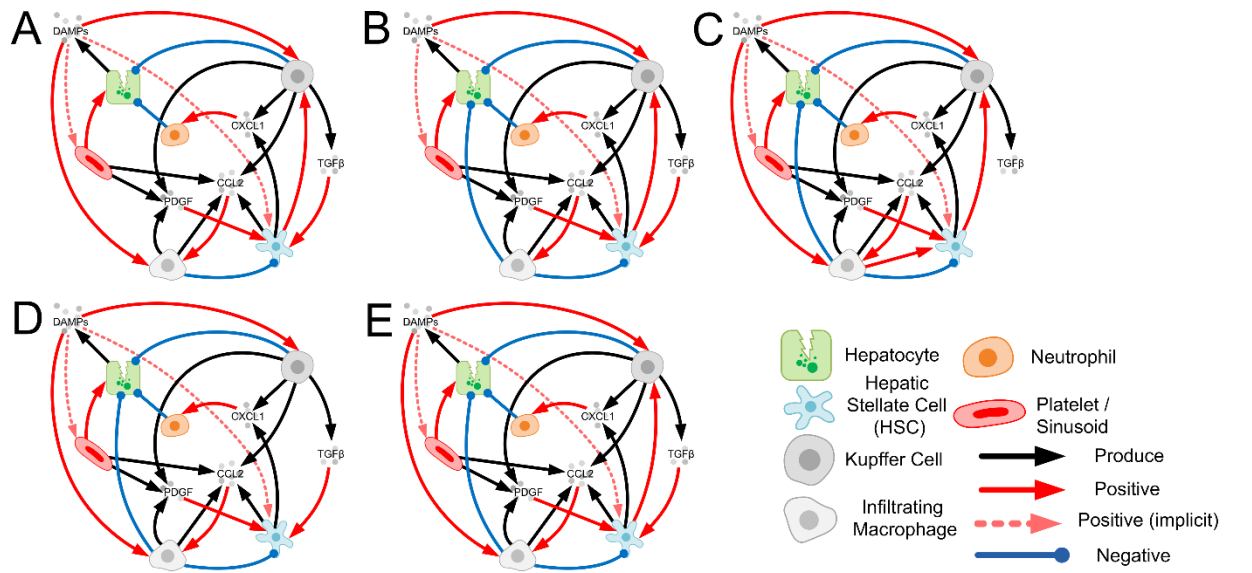
1471 **Figure S4. Number of macrophages and activated HSCs during liver regeneration under**
 1472 **different perturbed interactions.** (A) Illustration of five perturbed interactions. "IMs eliminate
 1473 hepatocytes: no" corresponds to the perturbation that infiltrating macrophages cannot
 1474 eliminate dead hepatocytes; "DAMPs activate IMs: no" corresponds to the perturbation that
 1475 DAMPs cannot activate infiltrating macrophages; "TGF β activates KC HSCs: no" corresponds to the
 1476 perturbation that a factor produced by infiltrating macrophages rather than TGF β activates
 1477 HSCs; "KCs migrate to HSCs: no" corresponds to the perturbation that Kupffer cells cannot

1478 migrate towards HSCs; “IMs eliminate aHSCs” corresponds to the perturbation that infiltrating
 1479 macrophages eliminate the activated HSCs rather than switch them back to the quiescent
 1480 phenotype. (B) Spatial distribution of activated HSCs and macrophages in the lobule, measured
 1481 by counting the corresponding number in six regions according to its distance to the central
 1482 vein. (C and D) Number of macrophages and activated HSCs in each region over time under five
 1483 perturbed interactions. Absolute numbers are estimated from the corresponding cell densities
 1484 taken from literature (Wake, 2006 & Bouwens et al., 1986), which might not match with the
 1485 real cell number in the lobule.

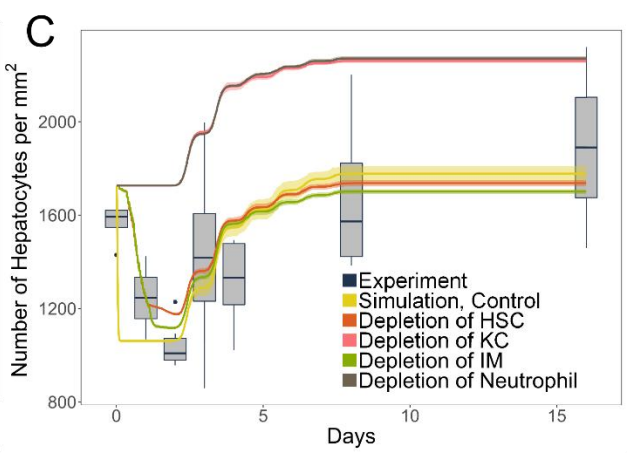
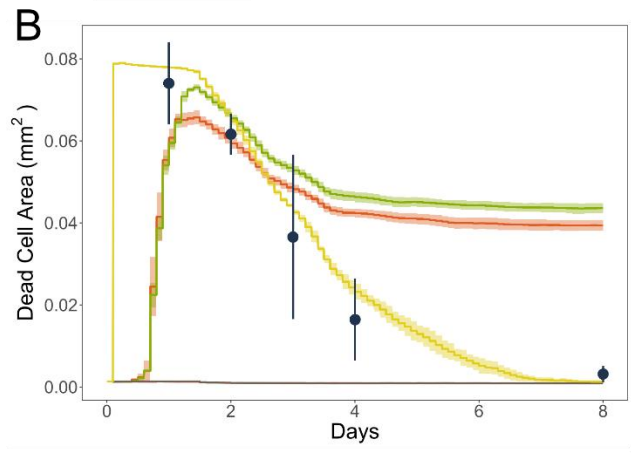
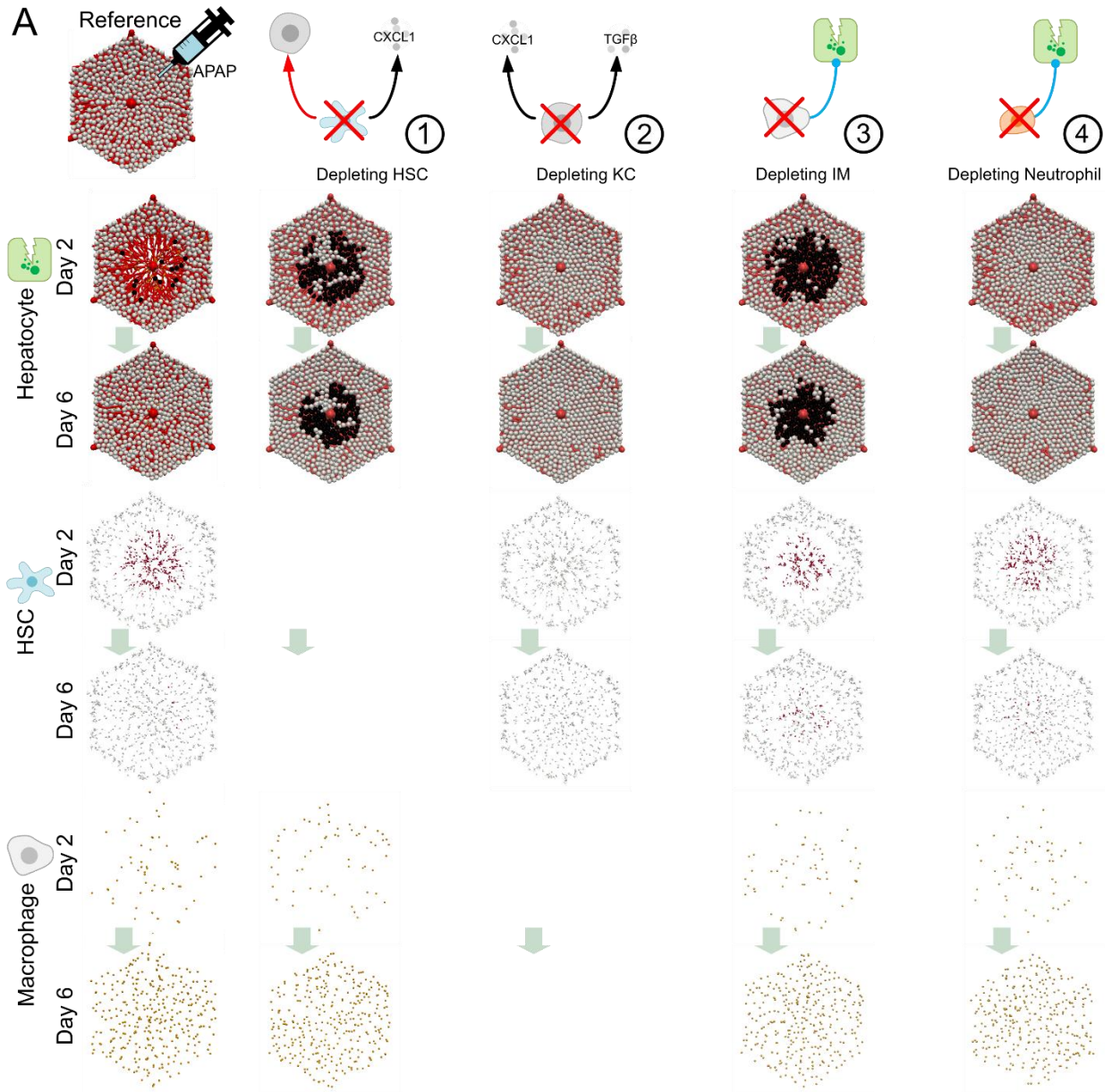


1486

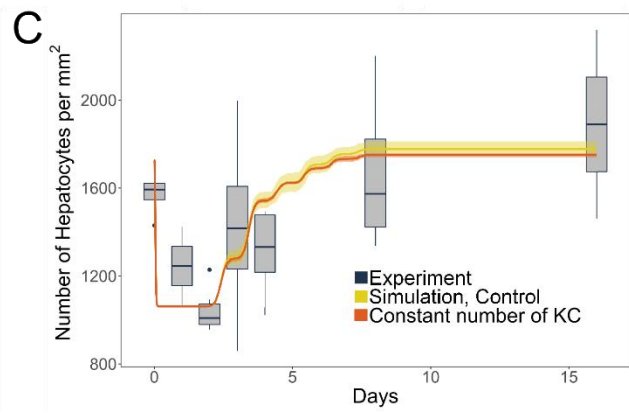
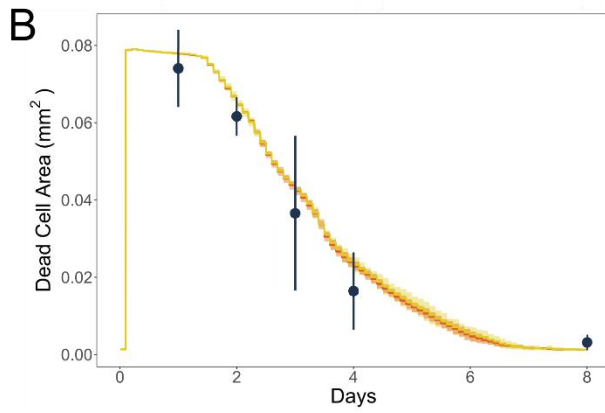
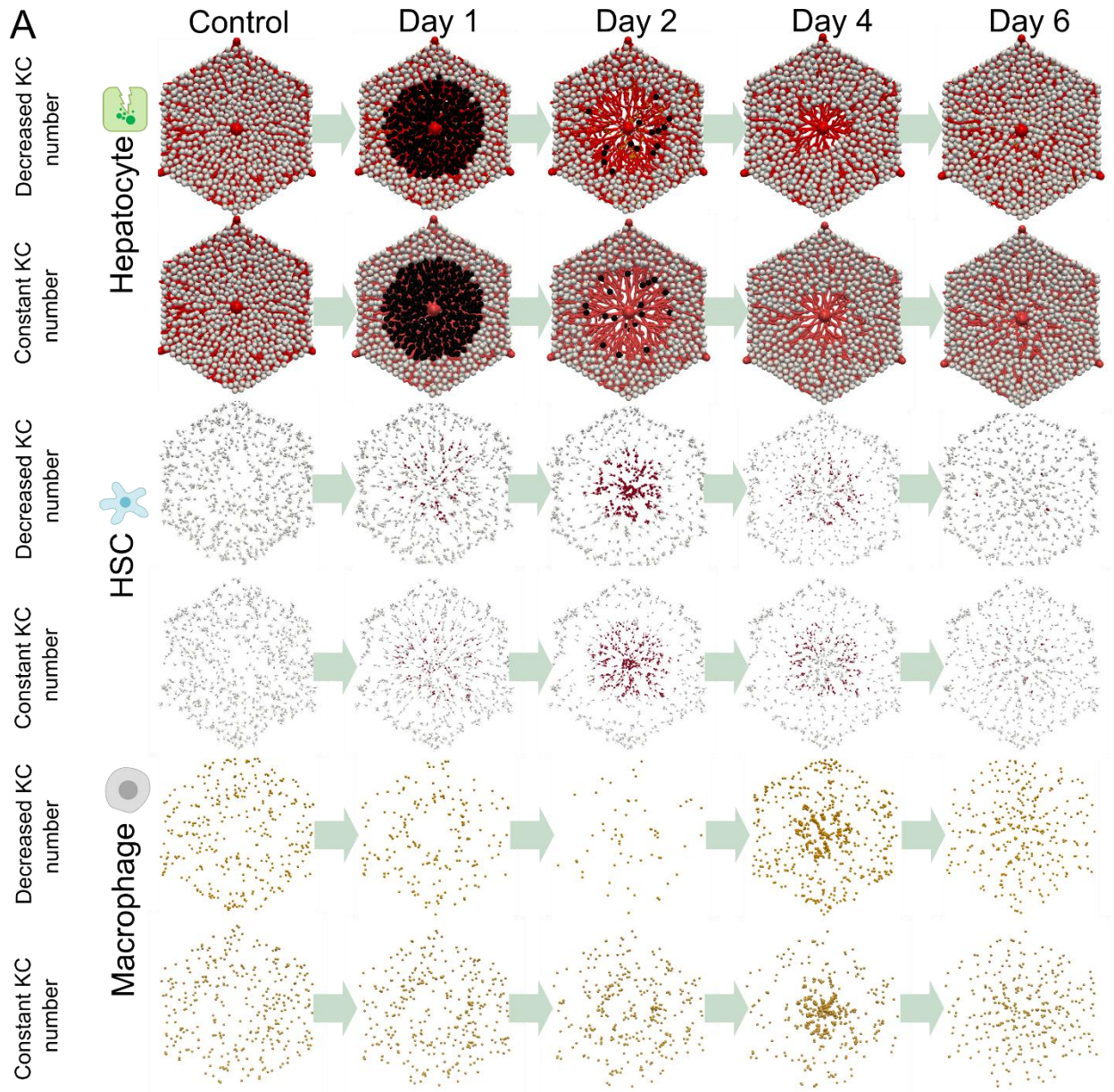
1487 **Figure S5. Molecular expression levels of signaling molecules in the reference state and five**
 1488 **perturbed cases.** (A) Illustration of five perturbed interactions. (B) The local level of CCL2 at
 1489 macrophages. (C) The local level of TGFβ at HSCs.



1491 **Figure S6. Network integrating possible and interpreted interactions.** Network where (A)
1492 infiltrating macrophages do not eliminate the dead hepatocytes; (B) DAMPs do not activate the
1493 infiltrating macrophages; (C) one factor released by infiltrating macrophages (not TGF β)
1494 activates the HSCs; (D) KCs do not migrate towards the HSCs; (E) infiltrating macrophages
1495 eliminate the activated HSCs, are presented. (F) Simulations using the network that integrates
1496 the two interactions that “DAMPs do not activate the infiltrating macrophages” and “infiltrating
1497 macrophages eliminate the activated HSCs”. Snapshots of the different cell types at days 2 & 6
1498 are shown. (G) The corresponding curves of lesion area and hepatocytes density are displayed.
1499 (H) For the interaction “infiltrating macrophages do not eliminate the dead hepatocytes”,
1500 different phagocytosis capacities of the Kupffer cells (elimination duration time) are tested:
1501 lesion area and hepatocytes density are displayed.



1503 **Figure S7. Pattern of regeneration in the liver lobule after abrogating NAPQI and depleting**
1504 **different cell types of.** Here, a hypothetical case of regeneration from a necrotic lesion is
1505 considered that has been alternatively generated by cell-death triggered independent of the
1506 NAPQI-pathway. (A) The regenerating lobule over time of the reference state and upon
1507 individual depletion of four sinusoidal cell types: (1) Depletion of HSCs; (2) depletion of Kupffer
1508 cells; (3) depletion of infiltrating macrophages; (4) depletion of neutrophils. For hepatocytes,
1509 white represents the healthy ones, black represents the dead ones. For HSCs, white represents
1510 the quiescent ones, red represents the activated ones. (B and C) Lesion area and hepatocytes
1511 density over time in the reference state and upon individual depletion of different cell types.
1512 Upon depletion of HSCs, due to the decreased CXCL1 levels, the number of neutrophils
1513 infiltrating into the lesion is reduced, leading to generally reduced numbers of neutrophils,
1514 Kupffer cells, and infiltrating macrophages in the lesion. As consequence, a large number of
1515 dead hepatocytes remains uncleared in the lobule. Depletion of Kupffer cells leads to a non-
1516 necrotic lesion in the lobule. Due to the lack of Kupffer cells, therefore reduced TGF β signal to
1517 trigger the activation of HSCs regarding the secretion of CXCL1, consequently, lower levels of
1518 CXCL1 production, reduced neutrophil attraction and diminished hepatocyte injury. The
1519 depletion of infiltrating macrophages does almost not affect the size of the necrotic lesion, but
1520 only reduces its clearance from dead hepatocytes. After the depletion of neutrophils (Fig. S7A,
1521 scenario 4), since neutrophils are responsible to kill injured hepatocytes, all injured hepatocytes
1522 in the lesion remain non-necrotic if neutrophils are depleted. Therefore, the lesion area
1523 remains to be zero.



1525 **Figure S8.** Pattern of liver regeneration with constant numbers of Kupffer cells. (A) The
 1526 regenerating lobule over time in the reference state and with assuming a constant number of
 1527 Kupffer cells. (B) Dead cells area over time. (C) Hepatocytes density over time.

1528 **Table S1. List of parameters used in the study, related to Figure 1.**

Parameter	Description	Value	Reference
r_{Hep}	Hepatocyte radius	$\sim 10.7 \mu\text{m}$	Estimated from data
r_{sin}	Sinusoid radius	$\sim 2.1 \mu\text{m}$	Estimated from data
r_{HSC}	HSC nucleus radius (head sphere of HSC)	$\sim 2 \mu\text{m}$	Wake, 2006
$l_{HSC,branch}$	HSC branch length	$\sim 12 \mu\text{m}$	Wake, 2006
r_{KC}	Kupffer cell radius	$\sim 4 \mu\text{m}$	Gardin et al., 1992
r_{IM}	Infiltrating macrophage radius	$\sim 6 \mu\text{m}$	Shi et al., 2011
$r_{Neutrophil}$	Neutrophil radius	$\sim 4 \mu\text{m}$	Herant et al., 2005
K_{Hep}	Young's modulus of hepatocyte	$\sim 450 \text{ Pa}$	Hoehme et al., 2010
K_{sin}	Young's modulus of sinusoid	$\sim 600 \text{ Pa}$	Hoehme et al., 2010
K_{HSC}	Young's modulus of HSC	$\sim 700 \text{ Pa}$	Estimated from fibroblast, Yang et al., 2012
K_{KC}	Young's modulus of Kupffer cell	$\sim 3100 \text{ Pa}$	Rotsch et al., 1997
K_{IM}	Young's modulus of infiltrating macrophage	$\sim 1400 \text{ Pa}$	Estimated from immune macrophage, Bafi et al., 2015
$K_{Neutrophil}$	Young's modulus of neutrophil	$\sim 1500 \text{ Pa}$	Lee et al., 2011
ν	Poisson ratio of all cell types	0.4	Hoehme et al., 2010
NA	Cell cycle time of hepatocyte	24 hours	Hoehme et al., 2010
$\Gamma_{ECM,cell}$	Cell-medium friction (all cell types except HSC)	10^8 Ns/m^3	Estimated
$\Gamma_{ECM,HSC}$	HSC-medium friction	10^{10} Ns/m^3	Estimated

$\Gamma_{\parallel,SE}, \Gamma_{\parallel,SS}, \gamma_{\perp}, \gamma_{\parallel}$	Friction between all cell types including sinusoids	10^8 Ns/m^3	Estimated
Den_{HSC}	HSC density in the liver	$\sim 1/70 \mu\text{m}$ of the sinusoid	Wake, 2006
Den_{KC}	Kupffer cell density in the liver	$\sim 2 \times 10^4 / \text{mm}^3$	Bouwens et al., 1986
Den_{IM}	Infiltrating macrophage density in the liver	$\sim 2 \times Den_{KC}$	Estimated from Zigmond et al., 2014
$Den_{Neutrophil}$	Neutrophil density in the liver	$\sim 1.9 \times 10^{-3} / \mu\text{m}^2$ of the lesion	McDonald et al., 2010
$SP_{HSC,mean}$	Mean of migration speed of HSC	$\sim 2.1 \mu\text{m}/\text{hour}$	Tangkijvanich et al., 2001
$SP_{HSC,SD}$	SD of migration speed of HSC	$\sim 0.1 \mu\text{m}/\text{hour}$	Tangkijvanich et al., 2001
$SP_{KC,mean}$	Mean of migration speed of Kupffer cell	$\sim 0.04 \mu\text{m}/\text{min}$	Based on Ju and Tacke, 2016
$SP_{KC,SD}$	SD of migration speed of Kupffer cell	$\sim 0.01 \mu\text{m}/\text{min}$	Based on Ju and Tacke, 2016
$SP_{IM,mean}$	Mean of migration speed of infiltrating macrophage	$\sim 5 \mu\text{m}/\text{min}$	Grabher et al., 2007
$SP_{IM,SD}$	SD of migration speed of infiltrating macrophage	$\sim 1.8 \mu\text{m}/\text{min}$	Grabher et al., 2007
$SP_{Neutrophil,mean}$	Mean of migration speed of neutrophil	$\sim 0.12 \mu\text{m}/\text{second}$	McDonald et al., 2010
$SP_{Neutrophil,SD}$	SD of migration speed of neutrophil	$\sim 0.02 \mu\text{m}/\text{second}$	McDonald et al., 2010
$D_{TGF\beta}$	Diffusion rate of TGF β , estimated from its molecular weight, 25 kDa	$2.6 \times 10^{-11} \text{ m}^2/\text{s}$	Murphy et al., 2012
D_{DAMP}	Diffusion rate of DAMP, estimated from its molecular weight, 28 kDa	$2.5 \times 10^{-11} \text{ m}^2/\text{s}$	Davies et al., 2018
D_{CCL2}	Diffusion rate of CCL2, estimated from its molecular weight, 13 kDa	$3.3 \times 10^{-11} \text{ m}^2/\text{s}$	Van Coillie et al., 1999
D_{PDGF}	Diffusion rate of PDGF, estimated from its molecular weight, 30 kDa	$2.4 \times 10^{-11} \text{ m}^2/\text{s}$	Soyombo and DiCorleto, 1994
D_{CXCL1}	Diffusion rate of CXCL1, estimated from its molecular weight, 21 kDa	$2.8 \times 10^{-11} \text{ m}^2/\text{s}$	Amiri and Richmond, 2003

$\gamma_{TGF\beta}$	Decay rate of TGF β , estimated from its half-life, 120 s	5.8×10^{-3} /s	Wakefield et al., 1990
γ_{DAMP}	Decay rate of DAMP, estimated from its half-life, 1000 s	5.7×10^{-4} /s	Zandarashvili et al., 2013
γ_{CCL2}	Decay rate of CCL2, estimated from its half-life, 1200 s	5.8×10^{-4} /s	Berchiche et al., 2011
γ_{PDGF}	Decay rate of PDGF, estimated from its half-life, 600 s	1.1×10^{-3} /s	Waltenberger et al., 1992
γ_{CXCL1}	Decay rate of CXCL1, estimated from its half-life, 900 s	7.7×10^{-4} /s	Sun et al., 2011
$\phi_{DAMP,migrate}$	Concentration of DAMP to trigger Kupffer cell to migrate	5.5	Estimated
$\phi_{DAMP,activate}$	Concentration of DAMP to activate Kupffer cell	11.5	Estimated
$\phi_{PDGF,migrate}$	Concentration of PDGF to trigger HSC to migrate	1.2	Estimated
$\phi_{TGF\beta,activate}$	Concentration of TGF β to activate quiescent HSC	1.0	Estimated
$\phi_{CXCL1,migrate}$	Concentration of CXCL1 to trigger neutrophil to migrate	2.0	Estimated
$\phi_{CCL2,migrate}$	Concentration of CCL2 to trigger infiltrating macrophage to migrate	1.2	Estimated
NA	Fraction of dying Kupffer cells	$\sim 5/6$	Estimated from Graubardt et al., 2017
$t_{Ly6C^{high} \rightarrow Ly6C^{low}}$	Transforming time from Ly6C-high phenotype to Ly6C-low phenotype for macrophages	~ 72 hours	Estimated from Zigmond et al., 2014
$t_{elimination}$	Engulfment and elimination duration of macrophages	~ 3 hours	Estimated from Haecker et al., 2002
$t_{IM,life}$	Lifetime of infiltrating macrophages	~ 4 days	Estimated from Zigmond et al., 2014
$t_{Neutrophil,life}$	Lifetime of neutrophils	~ 48 hours	Graudardt et al., 2017

1529 The concentration unit of molecules: 1 equals to 5 ng/ml.

1530 **Table S2. Reference of each arrow in Figure 1B, related to Figure 1.**

Label	Reference	Function	Type	Prob.
1	Li et al., 2020, Calderwood et al., 2016	Injured and dead hepatocytes produce DAMPs	p	1
2	Martin-Murphy et al., 2010	DAMPs activate Kupffer cells	+	1
3	Mihm, 2018	DAMPs activate infiltrating macrophages	+	2
4	Canbay et al., 2003	Kupffer cells engulf dead hepatocytes	-	1
5–7	Pinzani et al., 1994	Platelets, active Kupffer cells and infiltrating macrophages produce PDGF	p	1
8	Melton and Yee, 2007, Yang et al., 2003	PDGF attracts HSCs to migrate	+	1
9	Krenkel et al., 2014	Active Kupffer cells produce CCL2	p	1
10–12	Baeck et al., 2012	Platelets, active HSCs and infiltrating macrophages produce CCL2	p	1
13	Baeck et al., 2012	CCL2 attracts infiltrating macrophages to migrate	+	1
14	Marra and Tacke, 2014	Active Kupffer cells produce CXCL1	p	1
15	Kisseleva and Brenner, 2007	Active HSCs produce CXCL1	p	1
16	Marra and Tacke, 2014	CXCL1 attracts neutrophils to migrate	+	1
17	Marra and Tacke, 2014	Neutrophils induce necrosis of hepatocytes	-	1
18	Imamura et al., 2005	Infiltrating macrophages activate HSCs	+	2
19.a	Tacke and Zimmermann, 2014	Infiltrating macrophages eliminate active HSCs	-	1
19.b	Kisseleva et al., 2012 & Troeger et al., 2012	Infiltrating macrophages revert active HSCs to quiescent phenotype	-	2
20	De Bleser et al., 1997	Active Kupffer cells produce TGFβ	p	1
21	Cai et al., 2018, Imamura et al.,	TGFβ activates HSCs	+	2

	2005			
22	Boulter et al., 2012	Infiltrating macrophages eliminate dead hepatocytes	-	2
23	Meyer et al., 2015	Platelets promote proliferation of hepatocytes	+	1
24	Seki et al., 2007	Active HSCs attract Kupffer cells to migrate	+	2

1531 Type: “p” indicates production; “+” indicates positive effect; “-” indicates negative effect.

1532 Prob.: “1” indicates highly probable with direct experimental support; “2” indicates probable

1533 with indirect experimental support.