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2 **Apoptotic Extracellular Vesicles (ApoEVs) Safeguard Liver Homeostasis and**

3 **Regeneration *via* Assembling an ApoEV-Golgi Organelle**

4 Running title: Liver regulation by apoEVs *via* organelle assembly

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18

1 **Summary**

2 Apoptosis is an integral physiological cell death process that occurs frequently and generates
3 a huge number of apoptotic extracellular vesicles (apoEVs). However, whether apoEVs are
4 necessary for maintaining organ homeostasis remains unclear. Here, we show that circulatory
5 apoEVs engraft in liver and undergo specialized internalization by hepatocytes (HCs) based
6 on surface signature of galactose and *N*-acetylgalactosamine. Furthermore, apoEVs rescue
7 liver injury in apoptotic-deficient *Fas* mutant and *Caspase-3* knockout mice, which is exerted
8 by restoring the featured hepatic ploidy homeostasis. Surprisingly, apoEVs form a chimeric
9 organelle complex with recipient Golgi apparatus *via* SNARE-mediated membrane interaction,
10 which consequently facilitates microtubule organization and HC cytokinesis. Notably, through
11 Golgi recovery and ploidy transition, apoEVs contribute to liver regeneration and protect
12 against acute hepatic failure. Collectively, these results identify a previously unrecognized role
13 for apoEVs and the specific mechanisms by which they safeguard liver homeostasis, and
14 suggest the potential of apoEV-based therapy for liver disorders.

15

16 **Keywords**

17 Apoptosis; extracellular vesicle; liver homeostasis; regenerative therapy; Golgi assembly;
18 ploidy transition

19

1 Introduction

2 Apoptosis is a form of programmed cell death that primarily accounts for routine cell turnover
3 in healthy individuals; it is estimated to occur in the human body at a rate of approximately
4 150 billion cells or roughly 0.4% of total cells per day (Arandjelovic and Ravichandran, 2015;
5 Elliott and Ravichandran, 2016; Morioka et al., 2019). Such physiological cell death is
6 recognized as an integral part of development and homeostatic maintenance, while it also
7 contributes to tissue stress responses and regeneration (Fuchs et al., 2013; Fuchs and Steller,
8 2011). During apoptosis, cells undergo a highly regulated process of disassembly including
9 membrane blebbing and protrusion, which ultimately leads to generation of apoptotic
10 fragments as extracellular vesicles (EVs) (Atkin-Smith et al., 2015; Coleman et al., 2001;
11 Poon et al., 2014). The heterogeneity of these EVs, originally known as apoptotic bodies
12 (apoBDs), has recently begun to be appreciated (Caruso and Poon, 2018). They vary in their
13 mechanisms of production, cargos, and size ranging from 50 nm to several microns; for these
14 reasons, the more general term of apoptotic extracellular vesicles (apoEVs) is now favored
15 (Caruso and Poon, 2018; Dieude et al., 2015; Lynch et al., 2017). Tens of apoEVs can be
16 released per cell, and they have increasingly been noticed as regulators of immune
17 responses, stem cell-mediated tissue turnover and regenerative therapies (Berda-Haddad et
18 al., 2011; Brock et al., 2019; Liu et al., 2018; Liu et al., 2020). However, the essential role of
19 apoEVs in maintaining organ homeostasis remains underdetermined.

20 The liver has long been considered as a major organ for apoptotic cell removal from
21 circulation, a function which is mostly attributed to the resident macrophages, called Kupfer
22 cells (Shi et al., 1996). It is generally believed that efficient phagocytosis is tolerogenic and

1 critically maintains liver homeostasis, whereas defective efferocytosis contributes to a
 2 spectrum of hepatic pathologies due to overwhelming apoptotic material-induced
 3 hyperinflammation (Morioka et al., 2019). Engulfment of apoEVs by liver macrophages can
 4 nevertheless be pro-inflammatory and fibrogenic (Canbay et al., 2003), leaving the detailed
 5 roles played by apoEVs in the liver controversial and elusive. Notably, the liver is also well
 6 known for its extreme and rapid capacity to regenerate after injuries, while loss or impairment
 7 of this regenerative capacity underlies severe or chronic liver damage that may eventually
 8 progress to hepatic failure (Forbes and Newsome, 2016). It has been reported that mice
 9 lacking either caspase-3 or caspase-7, the key apoptotic executors, demonstrate a
 10 substantial defect in liver regeneration after partial hepatectomy (PHx) (Li et al., 2010).
 11 Delayed liver regeneration after PHx has also been documented in Fas-deficient
 12 lymphoproliferative (*Fas^{lpr}*) mice, which show defective Fas-induced apoptosis and resemble
 13 human systemic lupus erythematosus, an autoimmune disease associated with increased
 14 susceptibility to liver disease (Bessone et al., 2014; Desbarats and Newell, 2000;
 15 Watanabe-Fukunaga et al., 1992). In light of these findings, understanding the regulatory
 16 function and mechanisms of apoEVs in the liver will have significant implications for
 17 deciphering principles that underlie hepatic regeneration and establishing novel therapies for
 18 hepatic disease.

19 EVs have emerged as playing pivotal roles in intercellular communication based on their
 20 surface bioactive molecules and various types of cargos including nucleic acids, proteins and
 21 lipids (Pitt et al., 2016; Roy et al., 2018). Given these properties, EVs serve as homeostatic
 22 modulators and can be leveraged in both diagnostics and therapeutics (Pitt et al., 2016; Roy

1 et al., 2018). ApoEVs, however, are a relatively understudied EV population which possess
 2 unique molecular signatures (Dieude et al., 2015; Wickman et al., 2013). Despite the
 3 characterization of EVs and their potent functions, how these vesicles recognize recipient
 4 cells and what processes they undergo in recipient cells are not yet fully understood. The
 5 current model postulates uptake of EVs *via* endocytosis, after which they shuttle within
 6 endosomes, surf on the endoplasmic reticulum and finally fuse with acidified endosomes or
 7 lysosomes for cargo release (Heusermann et al., 2016; Joshi et al., 2020). Nevertheless, we
 8 have limited knowledge of the specific mechanisms that EVs use to target certain recipient
 9 cells and the biological effects that EVs may exert on the recipient endomembrane system.

10 Here, we aimed to investigate the biological properties and related physiological function
 11 of apoEVs in maintenance of organ homeostasis. We discovered that apoEVs are
 12 characterized by specific surface markers which endow them with remarkable liver
 13 engraftment, specialized internalization by hepatocytes (HCs) and functional assembly with
 14 recipient Golgi apparatus. ApoEVs were further revealed to crucially preserve hepatic ploidy
 15 homeostasis, maintain liver integrity, promote liver regeneration after PHx and mediate
 16 therapy against acute liver failure (ALF) in mice. These findings suggest a general
 17 understanding of the characterization, function, mechanisms and application of apoEVs
 18 underlying organ maintenance and regeneration, with potential applications for novel
 19 therapeutics.

20

1 **Results**

2 **Circulatory apoEVs engraft in the liver and safeguard liver homeostasis**

3 ApoEVs used in this study were induced from multiple cell types (stem/stromal cells, immune
4 cells and fibroblasts) and different species (human and mouse) and by various methods
5 (protein kinase inhibition, oxidative stress, mitochondrial permeabilization and Fas
6 crosslinking) targeting both the intrinsic (*i.e.* mitochondrial) and extrinsic (*i.e.* death receptor)
7 pathways of apoptosis (Xu and Shi, 2007). Among these, the apoEVs primarily discussed
8 here are those derived from mesenchymal stem cells (MSCs) after staurosporine (STS)
9 treatment (Liu et al., 2018; Liu et al., 2020). Notably, the protein kinase inhibitor STS has been
10 recognized as an apoptosis inducer *via* both caspase-dependent and caspase-independent
11 pathways (Belmokhtar et al., 2001). Moreover, MSCs represent potent EV donors with
12 immense translational potential, and require the apoptotic process to fulfill their therapeutic
13 effects after transplantation (Galleu et al., 2017; Liu et al., 2020). The protocol was
14 determined according to our previously established methodology for isolating apoBDs (Liu et
15 al., 2018; Liu et al., 2020) with necessary modifications for collecting the general apoEV
16 population more broadly (Figure S1A). STS-induced apoptosis of MSCs was confirmed by
17 morphology (Figure S1B) and terminal deoxynucleotidyl transferase dUTP nick end labeling
18 (TUNEL) analysis (Figure S1C). Notably, the protein yield of collected apoEVs was 10-fold
19 higher than that of exosomes (Exos) released by MSCs in the non-apoptotic culture condition
20 (Figure S1D). ApoEVs demonstrated characteristic morphology (Figure S1E) with a wide size
21 distribution between 100-800 nm in diameter (Figure S1F). Further analyses revealed that the
22 apoEVs expressed apoptotic markers such as cleaved caspase-3 and surface exposures of

1 typical phagocytosing signals (Arandjelovic and Ravichandran, 2015) phosphatidylserine
2 (PtdSer, shown by Annexin V binding), complement component 1q (C1q) and
3 thrombospondin 1 (TSP1) (Figure S1G-J). Nevertheless, apoEVs did not express the nuclear
4 lamina component Lamin B1, a negative marker of EVs (Figure S1J). Therefore, apoEVs
5 were verified with their specialized profiles being discovered.

6 To investigate the potential physiological function of apoEVs, we first examined the
7 biodistribution of circulatory apoEVs by tracing labeled apoEVs after intravenous infusion.
8 Positron emission tomography (PET) scanning of live mice demonstrated that copper
9 radioisotope (^{64}Cu)-labeled apoEVs engraft in liver, among other tissues, as early as 1 h after
10 infusion (Figure 1A). Surprisingly, in a 48 hours period, the ^{64}Cu radioisotope signal indicating
11 apoEVs increasingly aggregated in liver, with approximately 70% of total ^{64}Cu -apoEV signal
12 detected in liver at 48 h; at this point, the liver had 10-fold higher ^{64}Cu -apoEV radiation
13 intensity than any other dissected organ/tissue (Figure 1A). Tracing of apoEVs labeled with
14 the lipophilic fluorescent dye PKH26 confirmed that liver engraftment after infusion proceeded
15 in a manner resembling zero-order elimination kinetics, *i.e.* a constant amount being
16 eliminated per unit time, with a half-time ($t_{1/2}$) of about 8 days (Figure 1B). The linear change
17 indicated that the liver biologically processed or metabolized apoEVs.

18 The above data inspired us to explore whether hepatic apoEVs were indeed associated
19 with any functional implications. To address this issue, we studied two mouse models with
20 deficiency in apoptosis (Liu et al., 2018), specifically *Fas* mutant (*Fas^{mut}*, characterized by
21 functionally deficient *Fas*, previously referred to as the *Fas^{lpr}* mice (Watanabe-Fukunaga et al.,
22 1992)) and *Caspase-3* knockout (*Casp3^{-/-}*) mice. As has been previously established (Xu and

1 Shi, 2007), the Fas ligand (FasL)/Fas pathway is a well-characterized extrinsic apoptosis
 2 pathway, while caspase-3 is critical for the final execution phase of both intrinsic and extrinsic
 3 pathways of apoptosis. We confirmed that *Fas^{mut}* and *Casp3^{-/-}* mice are both defective in
 4 physiological apoptosis, as shown by decreased circulatory and organ-specific cellular
 5 apoptotic rates in freshly isolated peripheral blood mononucleated cells (PBMNCs) (Figure
 6 S1K), bone marrow mononucleated cells (BMMNCs) (Figure S1L) and liver non-parenchymal
 7 cells (NPCs) (Figure S1M). We have also introduced a liver EV isolation protocol (Figure S1N)
 8 (Ishiguro et al., 2019) and applied Annexin V binding to detect a tissue-specific apoEV
 9 population (Liu et al., 2018). The liver EVs were identified based on morphology, particle
 10 distribution, expression of tetraspanins CD9, CD63 and CD81, and negative for Lamin B1
 11 (Figure S1O-Q).

12 A chronic intermittent apoEV infusion protocol at a biweekly interval was designed based
 13 on the hepatic elimination kinetics to replenish hepatic apoEVs (Figure 1C). Notably, systemic
 14 infusion of apoEVs had limited influence on general traits of *Fas^{mut}* mice in terms of body
 15 weight (BW) gain (Figure S2A), food intake (Figure S2B) and daily spontaneous activity
 16 (Figure S2C). Also notable is that despite being recognized as a lymphoproliferative model
 17 (Watanabe-Fukunaga et al., 1992), *Fas^{mut}* mice used in this study (8-16 weeks of age on the
 18 B6 background) did not show inflammatory features and their inflammation level remained
 19 comparable to wild type (WT) upon apoEV infusion (Figure S2D). As expected, we discovered
 20 that *Fas^{mut}* mice possessed less than half amount of liver apoEVs as WT mice, a deficiency
 21 which was recovered by apoEV infusion (Figure 1D). The total liver tissue EV quantity,
 22 however, was unchanged despite *Fas^{mut}* and apoEV infusion (Figure S2E), indicating the

1 ability of the organism to adapt and maintain the tissue EV pool at a constant level.
 2 Importantly, partial loss of apoEVs in the liver of *Fas^{mut}* mice was functionally sufficient to
 3 provoke liver injury, as demonstrated by a reduced fasting liver weight (LW) to BW ratio,
 4 obvious alterations upon gross examination and in histological analysis, and elevated serum
 5 levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Figure
 6 1E-G). ApoEV infusion restored the liver tissue integrity in *Fas^{mut}* mice (Figure 1E-G) without
 7 triggering fibrotic or inflammatory responses (Figure S2F, G). Furthermore, apoEV infusion
 8 also rescued the liver damage developed by *Casp3^{-/-}* mice due to hepatic apoEV deficiency
 9 (Figure S3A-E).

10 In analyzing liver tissue injuries, we noticed with interest that *Fas^{mut}* mice tended to
 11 possess a high percentage of binucleated HCs, and that cytoplasm vacuolization occurred in
 12 a high prevalence in these binucleated HCs (Figure 1F). One cellular characteristics of the
 13 mammalian liver is the polyploidization of HCs, during which individual HCs acquire more than
 14 two sets of chromosomes resulting from the failure of cytokinesis (Wang et al., 2017).
 15 Progressive polyploidization is considered a pathophysiological process in the liver (Gentric et
 16 al., 2015), but the detailed contributions of polyploid HCs to liver function and their regulatory
 17 mechanisms are not fully understood (Wilkinson et al., 2019a; Wilkinson et al., 2019b; Zhang
 18 et al., 2018a). We further evaluated whether apoEVs regulate the HC ploidy status in the liver.
 19 Phalloidin staining revealed HC cell borders and demonstrated that *Fas^{mut}* mice aggregated
 20 more binucleated HCs than mononucleated HCs compared to WT mice, which was rescued
 21 by apoEV infusion (Figure 1H). Moreover, propidium iodide (PI) staining of HC chromatin
 22 showed that *Fas^{mut}* mice possessed reduced diploid (2N) HCs and increased tetraploid (4N)

1 HCs compared to WT mice, which was also rectified by apoEV infusion (Figure 1I). Octoploid
2 (8N) HCs were not significantly influenced in the experimental context. ApoEV infusion also
3 restored the ploidy homeostasis in *Casp3*^{-/-} mouse livers, which developed polyploidization as
4 well (Figure S3F, G). Taken together, these data suggest that apoEVs are indispensably
5 required for safeguarding liver ploidy and functional homeostasis (Figure 1J).

6

7 **Specialized internalization of apoEVs by HCs promotes ploidy transition in the liver**

8 Next, we investigated how apoEVs regulate liver homeostasis. Considering the clues that
9 apoEVs may contribute to the reversal of HC polyploidization, we examined whether HCs,
10 particularly binucleated ones, uptake apoEVs directly for their functional regulation. By using
11 PKH26 to label the membrane of apoEVs before infusion, we confirmed that binucleated HCs
12 in the liver were indeed capable of taking up apoEVs, such that PKH26 fluorescent signals
13 were detected in approximately 40% of HCs at 24 hours after infusion (Figure 2A).
14 PKH26-labeled infused apoEVs were also engulfed by F4/80⁺ liver-resident macrophages
15 (Figure S2H). Given that lipophilic membrane labeling may be xenobiotic and result in loss of
16 membrane integrity, among other drawbacks, we further applied a previously established
17 mitochondria-targeting luminogen, known as DCPy, with aggregation-induced emission (AIE)
18 characteristics (Zheng et al., 2020). This AIE luminogen (AIEgen) is a photosensitizer which
19 generates singlet oxygen (¹O₂) upon light irradiation and induces apoptosis (Zheng et al.,
20 2020; Zheng et al., 2018). We identified that DCPy efficiently labeled apoEVs after apoptotic
21 induction, and that the AIEgen-labeled apoEVs were mainly internalized by HCs after the
22 infusion (Figure 2A, S2H), suggesting that HCs are the primary cellular targets of apoEVs in

1 the liver. To further examine whether the HC uptake of apoEVs occurred as a physiological
 2 event, we applied a parabiosis model in which *Fas^{mut}* apoptosis-deficient mice were
 3 connected with green fluorescent protein (GFP) transgenic mice (*GFP^{+/+}*) to obtain shared
 4 circulatory apoEVs (Liu et al., 2018). Indeed, GFP signals were detected in binucleated HCs
 5 of the *Fas^{mut}* mice (Figure 2B). These data indicated a putative link between internalization of
 6 apoEVs by HCs, particularly binucleated ones, and functional regulation of the liver.

7 We continued to dissect the reasons for and functional results of apoEVs being
 8 selectively taken up by HCs. It has been documented that HCs specifically bind and
 9 internalize galactose (Gal)- or *N*-acetylgalactosamine (GalNAc)-terminating glycoproteins
 10 through the featured asialoglycoprotein receptor (ASGPR) (Ashwell and Harford, 1982), and
 11 that desialylated glycans characterized by Gal and GalNAc residues are exposed on the
 12 surface of apoptotic cells (Dini et al., 1992). Thus, we investigated whether apoEVs use the
 13 sugar recognition system for HC internalization. Using fluorescent lectin conjugates
 14 specifically binding the desialylated residues, we revealed that both Gal and GalNAc were
 15 exposed on the surface of apoEVs; particularly, GalNAc marked over 90% of apoEVs (Figure
 16 2C). Furthermore, GalNAc abundance in apoEVs was higher than in Exos and in source
 17 MSCs when normalized by total membrane labeling fluorescent units, suggesting an
 18 apoEV-specific profile (Figure 2C). To evaluate whether the Gal/GalNAc-ASGPR system is
 19 indeed pivotal to apoEV uptake by HCs, we treated primary cultured HCs with apoEVs under
 20 different conditions with competing exogenous Gal/GalNAc or the ASGPR-specific antibody
 21 (Dini et al., 1992; McVicker et al., 2002) (Figure 2D). Data demonstrated that both the added
 22 monosaccharide mixture and the ASGPR antibody effectively inhibited HC uptake of apoEVs

(Figure 2E). Furthermore, we noticed in this simple short-term culture process that mononucleated diploid HCs became binucleated and polyploidized, and we managed to track the subsequent ploidy transition of tetraploid hepatocytes in particular using a mitogenic stimulation system after sorting (Duncan et al., 2010) (Figure 2F). We discovered that 4N-HCs from *Fas^{mut}* mice rarely underwent complete cytokinesis marked by the microtubule “midbody” structure (Figure 2G) and failed to produce 2N daughter cells (Figure 2H). Intriguingly, apoEV treatment empowered the *Fas^{mut}* 4N-HCs to bypass cytokinesis failure and induced an emergence of 2N populations, indicating ploidy reversal (Figure 2G, H). Collectively, these data suggest that specialized uptake of apoEVs by HCs promotes ploidy transition.

The above results prompted us to investigate whether apoEV-promoted HC ploidy transition is of functional significance to the liver. By sorting and comparing properties of 2N- and 4N-HCs (Figure S4A), we discovered that 4N-HCs possessed lower oxidative metabolic activity but accumulated more reactive oxygen species (ROS) than 2N-HCs, irrespective of the derivation from apoEV deficiency or replenishment conditions (Figure S4B, C). The oxidative stress in 4N-HCs was possibly due to a general decline of expression of antioxidant genes (Figure S4D), potentially underlying the injury-proneness of binucleated HCs in the liver. Notably, apoEV treatment maintained the oxidative phosphorylation (OXPHOS) rate and restrained the oxidative stress of unsorted HCs (Figure S4E, F), which might be attributed to ploidy reversal effects of apoEVs.

To confirm whether apoEVs indeed regulate liver function by targeting HCs to promote ploidy transition, we designed *in vivo* experimental protocols using high-dose intermittent injection of GalNAc at 500 mg/kg to inhibit apoEV uptake by HCs, and low-dose intermittent

1 administration of GalNAc-conjugated small interfering RNAs (siRNAs) for *Anillin* (G-si*Anln*) at
 2 4 mg/kg to specifically inhibit HC cytokinesis (Figure 2I). As previously established, GalNAc
 3 conjugation allows targeted delivery of siRNAs to HCs with limited off-target effects, and
 4 Anillin is an actin-binding protein required for cytokinesis (Zhang et al., 2018a; Zhang et al.,
 5 2018b). We confirmed that only the high-dose GalNAc injection effectively suppressed liver
 6 engraftment of apoEVs (Figure 2J), and that G-si*Anln* administration selectively
 7 down-regulated *Anln* mRNA expression in HCs (Figure 2K). Furthermore, both GalNAc and
 8 G-si*Anln* administration inhibited apoEV restoration of HC ploidy homeostasis in *Fas^{mut}* mice,
 9 leaving lower percentages of mononucleated and diploid HCs in the liver compared to the
 10 control siRNA-injected (G-si*Luc*) *Fas^{mut}* mice with apoEV infusion (Figure 2L, M). Importantly,
 11 hematoxylin and eosin (H&E) staining of liver tissues and serum ALT analysis demonstrated
 12 that either blocking HC uptake of apoEVs or inhibiting HC cytokinesis diminished
 13 apoEV-mediated maintenance of liver integrity under apoptotic deficiency (Figure 2N, O).
 14 These results suggested that internalization of apoEVs by HCs promotes ploidy transition for
 15 liver homeostasis (Figure 2P).

16 We further examined the parabiosis model to investigate whether physiological apoEVs
 17 contribute to liver homeostasis maintenance (Figure S5A). Intermittent injection of GalNAc
 18 was also applied to inhibit HC uptake of apoEVs in *GFP^{+/+}*-paired *Fas^{mut}* mice (Figure S5A, B).
 19 As shown by H&E staining and serum ALT analysis, 2-month parabiosis with healthy *GFP^{+/+}*
 20 mice helped the paired *Fas^{mut}* mice to recover from liver injury, while GalNAc injection
 21 suppressed the effects (Figure S5C, D). Furthermore, parabiosis with *GFP^{+/+}* mice reduced
 22 the percentages of binucleated HCs and promoted emergence of 2N-HCs in the liver of *Fas^{mut}*

1 mice, both of which were also counteracted by GalNAc injection (Figure S5E, F). These data
2 indicate that circulatory endogenous apoEVs maintained liver ploidy and functional
3 homeostasis through HC uptake.

4

5 **ApoEVs interact with recipient Golgi apparatus to form a chimeric organelle complex** 6 **for HC cytokinesis**

7 Next, we aimed to decipher how apoEVs facilitate ploidy transition of HCs. In the previous *in*
8 *vivo* and *in vitro* tracing experiments, we noticed that internalized apoEVs gathered in the
9 perinuclear region of HCs (Figure 2A and 2E). Intriguingly, it has been reported that following
10 endocytosis, the Golgi apparatus receives cargo by a process called backward/retrograde
11 transport (Johannes and Popoff, 2008), while the destination of EVs in recipient cells remains
12 uncertain (Heusermann et al., 2016; Liu et al., 2020). We stained HC Golgi *in vivo* using an
13 antibody against Golgin84 (also known as Golga5), an integral Golgi membrane protein. We
14 discovered that almost all internalized apoEVs in binucleated HCs contact with the Golgi
15 membrane, which occurred rapidly at 24 h post-apoEV infusion or as a physiological event
16 during parabiosis (Figure 3A). To further characterize apoEV interactions with the recipient
17 Golgi, we transfected sorted 4N-HCs in culture with GFP-plasmids labeling
18 *N*-acetylgalactosaminyltransferase 2 (GALNT2), a Golgi-localized enzyme. We found that
19 apoEVs were indeed closely adjacent to and specifically contacted the perinuclear Golgi
20 apparatus in interphase, during which apoEVs and recipient Golgi formed a chimeric
21 three-dimensional structure by apoEVs “inserting” into Golgi “pits”, “bridging” Golgi tubules
22 and circling around Golgi “ends” (Figure 3B). The Golgi apparatus is increasingly

1 acknowledged as a microtubule organizing center (MTOC) which safeguards mitotic spindle
2 formation and cell division (Wei et al., 2015). In addition, microtubules are regulated by
3 post-translational modifications, including acetylation at the lysine 40 (K40) residues of
4 α -tubulin, which indicates stabilization of microtubules for functional execution (Montagnac et
5 al., 2013). We further revealed that perinuclear interactions between apoEVs and Golgi in
6 binucleated HCs were also associated with microtubule organization, particularly formation of
7 acetylated microtubules, which also enriched in the perinuclear region and co-localized with
8 the apoEV-Golgi chimeric structure in the interphase (Figure 3C). Moreover, the intimate
9 spatial and structural correlations among apoEVs, Golgi and acetylated microtubules were
10 also detected in HC cytokinesis with the midbody actually formed by the acetylated
11 microtubules (Figure 3C), suggesting functional contributions to the cytokinesis process.

12 We were particularly interested in the chimeric structure formed by apoEVs and recipient
13 Golgi, and further evaluated whether this interaction had functional implications for Golgi
14 maintenance. By comparing the GALNT2-labeled Golgi morphology in sorted 4N-HCs from
15 WT and *Fas^{mut}* mice, we unexpectedly discovered that whereas the Golgi in WT 4N-HCs
16 exhibited a relatively complete ribbon structure, the Golgi in *Fas^{mut}* 4N-HCs was fragmented
17 and dispersed (Figure 3D). Notably, when tracing labeled apoEVs in *Fas^{mut}* 4N-HCs, the
18 apoEVs enriched in the perinuclear region, where a complete Golgi was ought to be, while
19 maintaining contact with the Golgi fragments or as Golgi “replenishments” (Figure 3D). As the
20 GALNT2 represents a *medial/trans*-Golgi marker, we also performed immunofluorescent (IF)
21 staining of 130 kDa Golgi matrix protein (GM130), a *cis*-Golgi marker. Data demonstrated that
22 the GM130 fluorescence was also dispersed in *Fas^{mut}* 4N-HCs, whereas in *Fas^{mut}* 4N-HCs

1 after apoEV treatment, the GM130-labeled structure was tighter, with internalized apoEVs
2 embedding among the Golgi stacks (Figure 3D). ApoEV contacts with the Golgi and
3 protection of Golgi integrity were also found in *Casp3^{-/-}* 4N-HCs (Figure S3H). These results,
4 especially the apoEV-Golgi structural assimilation, inspired us to hypothesize that integration
5 of apoEVs with the recipient Golgi may form a vesicle-organelle complex.

6 To test this hypothesis, we transfected apoEV source cells (*i.e.* MSCs) and sorted
7 4N-HCs with mCherry- or EGFP-plasmids for enforced expression of the same targets, Golgi
8 reassembly-stacking protein 55 (GRASP55, responsible for stacking of Golgi cisternae
9 located in *medial*-Golgi) and GalT (galactosyltransferase, an enzyme on *trans*-Golgi
10 membranes) (Figure 3E). After collecting apoEVs from the transfected MSCs and treating
11 HCs with the apoEVs, we detected donor GRASP55/GalT integrated with their recipient
12 counterparts (Figure 3E). Indeed, apoEVs carried a spectrum of Golgi proteins with varied
13 abundance, including the Golgi stacking protein GRASP55, the *trans*-Golgi network protein 38
14 (TGN38), Golgin84 for tethering of vesicles, and Syntaxin 5 (Stx5) for docking and fusion of
15 vesicles (Figure 3F). Correspondingly, *Fas^{mut}* HCs derived from apoptosis-deficient conditions
16 showed varying degree of decline in the expression of multiple Golgi proteins, while apoEV
17 treatment in culture replenished the insufficient Golgi proteins (Figure 3F). Rescue of Golgi
18 integrity was further confirmed under electron microscopy, which revealed that the Golgi in
19 *Fas^{mut}* 4N-HCs was mostly dispersed into vesicle forms, whereas distinguishable Golgi stacks
20 were observed after apoEV treatment (Figure 3G). Moreover, when quantified after isolation
21 from cultured HCs, Golgi in the apoEV-deficient *Fas^{mut}* mice lost over 50% of the protein mass,
22 which was recovered by apoEV replenishment (Figure 3H). ApoEV-mediated Golgi recovery

1 was verified in *Casp3*^{-/-} HCs as well (Figure S3I). Also, after using apoEVs derived from
2 *GFP*^{+/+} MSCs, the GFP signal was detected in the isolated HC Golgi (Figure 3I). Taken
3 together, these data suggest that integration of apoEVs with recipient Golgi apparatus
4 safeguarded the structural integrity of the Golgi organelle.

5 We then examined the functional results downstream of apoEV-induced Golgi recovery.
6 First, we confirmed that either α -tubulin or its acetylated form (ac- α -tubulin) was detected in
7 the isolated HC Golgi (Figure 3I), indicating the Golgi was structurally conjugated to
8 microtubules. Notably, *Fas*^{mut} HC Golgi showed a decreased ac- α -tubulin level compared to
9 WT HC Golgi, while apoEV treatment promoted the Golgi-conjugated microtubule acetylation
10 (Figure 3I). These protein expression changes on isolated HC Golgi were consistent with
11 general cellular changes of the 4N-HCs, in which apoEV treatment rescued the diminished
12 perinuclear acetylated microtubules together with the assembled Golgi against *Fas*^{mut}
13 apoptotic deficiency (Figure 3J). To further demonstrate the significance of Golgi recovery for
14 mediating effects of apoEVs, we applied Brefeldin A (BFA), a chemical that is commonly used
15 to disrupt the Golgi organization. We revealed that BFA treatment induced Golgi disassembly
16 despite the perinuclear apoEVs, and it also diminished apoEV-promoted acetylated
17 microtubules and inhibited apoEV-rescued cytokinesis in *Fas*^{mut} 4N-HCs (Figure 3J). Western
18 blot analyses of microtubule acetylation levels in HCs confirmed these results, showing
19 apoEV-upregulated ac- α -tubulin expression in *Fas*^{mut} HCs being suppressed by BFA (Figure
20 3K). Moreover, apoEV application triggered upregulation of a series of downstream cascade
21 proteins for cytokinesis (Barr and Gruneberg, 2007) in *Fas*^{mut} HCs (Figure 3K), such as the
22 inner centromere protein (INCENP, a part of the chromosomal passenger complex regulating

1 cytokinesis), the mitotic kinesin-like protein 1 (MKLP1, also known as KIF23, a motor protein
2 forming the centralspindlin complex with Racgap1), Rac GTPase-activating protein 1
3 (RacGAP1, a microtubule-dependent signal for Rho regulation), and finally Ras homolog
4 family member A (RhoA, the master regulator of contractile ring formation for abscission).
5 Positive effects of apoEVs on the cytokinesis proteins were also diminished by BFA
6 treatments (Figure 3K), resulting in suppressed function of apoEVs to promote 2N-HC
7 emergence during ploidy transition of *Fas^{mut}* 4N-HCs (Figure 3L). Collectively, the above data
8 indicated that apoEVs assembled with recipient Golgi apparatus to form a chimeric organelle
9 structure, which we hereby name as the ApoEV-Golgi Complex (AGC), for driving microtubule
10 acetylation and safeguarding the cytokinesis process of HCs (Figure 3M).

11

12 **ApoEVs use the SNARE mechanism to assemble with Golgi and maintain liver** 13 **homeostasis**

14 Next, we investigated the molecules responsible for AGC assembly. The soluble
15 *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) mechanism is
16 recognized as mediating the final step in vesicle transport, *i.e.* docking and fusion of vesicles
17 with the target membrane compartment, which is dependent on the interactions between
18 specific molecules on the vesicles (the v-SNAREs) and on the target membranes (the
19 t-SNAREs) (Pfeffer, 1996). Currently, there are three known v-SNAREs that mediate
20 endosomal interactions with the Golgi in the retrograde route of transport: vesicle-associated
21 membrane protein 3 (VAMP3, also known as cellubrevin), VAMP4 and Golgi SNARE of 15
22 kDa (GS15) (Johannes and Popoff, 2008). With this knowledge, we examined whether

1 apoEVs, as examples of extracellular vesicles, may adopt the intracellular vesicle/endosomal
2 mechanisms to interact with the Golgi apparatus. IF staining and flow cytometric analyses
3 demonstrated that high percentages of apoEVs expressed VAMP3 and VAMP4 on their
4 surfaces (84.0% positive for VAMP3, 66.7% positive for VAMP4), but virtually none expressed
5 GS15 (less than 10% positive) (Figure 4A). Furthermore, VAMP3 protein expression in
6 apoEVs was even higher than in the source MSCs (Figure 4B). To evaluate whether VAMP3
7 and/or VAMP4 indeed mediate the AGC assembly, we transfected source MSCs with siRNAs
8 for *Vamp3* and/or *Vamp4* and collected the *Vamp*-knockdown apoEVs for functional
9 experiments (Figure 4C). Data demonstrated that in *Fas^{mut}* 4N-HCs, knockdown of *Vamp3*
10 alone was enough to prevent apoEVs from docking with the recipient Golgi without affecting
11 the uptake, and the internalized *Vamp3*-knockdown apoEVs scattered in the cytoplasm away
12 from the Golgi (Figure 4D). Knockdown of *Vamp4* had limited effects on the fate of apoEVs in
13 recipient cells (Figure 4D). We further revealed that *Vamp3*-knockdown apoEVs did not
14 rescue the fragmented Golgi, nor did they restore the diminished acetylated microtubules in
15 *Fas^{mut}* 4N-HCs (Figure 4E). To directly confirm that apoEVs adopted VAMP3 to assemble with
16 the Golgi, we used the isolated Golgi system and detected hVAMP3 when treating HCs with
17 hApoEVs, indicating integration of apoEV-VAMP3 into the recipient Golgi (Figure 4F). These
18 data suggest that VAMP3 was the key factor in apoEV-mediated AGC assembly and
19 functional effects of apoEVs.

20 It has been reported that certain VAMPs, including VAMP3, on synaptic vesicles can be
21 cleaved by Tetanus toxin (TeNT) (McMahon et al., 1993). We verified that VAMP3 on apoEVs
22 was also sensitive to TeNT treatment (Figure 4G), which provided a useful tool for evaluating

1 apoEV-VAMP3 function *in vivo*. Importantly, as shown by Golgin84 IF staining, we discovered
 2 that *in vivo* binucleated HCs in *Fas^{mut}* mice were fragmented, consistent with the dispersed
 3 Golgi of *Fas^{mut}* 4N-HCs in culture (Figure 4H). Furthermore, total Golgi protein mass in *Fas^{mut}*
 4 mouse liver was decreased compared to WT mouse liver, corresponding to the decreased
 5 Golgi protein mass in cultured *Fas^{mut}* HCs (Figure 4I). Notably, chronic intermittent infusion of
 6 apoEVs partially rescued the defective HC Golgi together with the declined liver Golgi in
 7 *Fas^{mut}* mice (Figure 4H, I). These defects were also rectified by long-term parabiosis with
 8 *GFP^{+/+}* mice, based on GalNAC-mediated physiological apoEV internalization (Figure S5G, H).
 9 Expectedly, infusion of *Vamp3*-knockdown apoEVs or TeNT-pretreated VAMP3-cleaved
 10 apoEVs failed to restore Golgi integrity in *Fas^{mut}* mice (Figure 4H, I), confirming VAMP3 as the
 11 critical mediator of apoEV effects. The significance of VAMP3 to apoEV function was further
 12 verified by *Vamp3* knockdown or TeNT pretreatment abolishing the promotion of HC ploidy
 13 reversal and protection against liver injury in *Fas^{mut}* mice by infused apoEVs (Figure 4J-M).
 14 Taken together, these results indicate that apoEVs used the SNARE mechanism to assemble
 15 with Golgi and activate downstream functional cascades to maintain liver homeostasis (Figure
 16 4N).

18 **ApoEV-mediated Golgi recovery and ploidy reversal contribute to liver regeneration**

19 Next, we investigated the implications of apoEV regulation of the liver. The liver is well known
 20 for its remarkable regenerative capabilities, which initiates a coordinated series of
 21 mechanisms to regain its original volume following injury or resections (Forbes and Newsome,
 22 2016). Interestingly, it has been documented that HC proliferation and/or hypertrophy

1 contribute to liver regeneration after PHx, while rapid division of binucleated HCs without
 2 mitosis particularly underlies liver regeneration after 70% PHx, a large-scale surgery after
 3 which the liver can recover (Hori et al., 2011; Mitchell and Willenbring, 2008; Miyaoka et al.,
 4 2012). Moreover, a recent study has demonstrated that the polyploid state restricts HC
 5 proliferation and liver regeneration in mice (Wilkinson et al., 2019b), although controversies
 6 exist (Matsumoto et al., 2020; Wilkinson et al., 2019a; Zhang et al., 2018a). We applied the
 7 commonly used 70% PHx model to examine the participation of apoEVs in the liver
 8 regeneration process (Figure 5A). At 72 h after 70% PHx, when one round of HC cell cycle is
 9 typically finished (Sakamoto et al., 1999), we confirmed regeneration of the remnant liver
 10 tissues to over 60% of the original liver mass, thus guaranteeing the survival of the mice
 11 (Figure 5B). Notably, compared to sham-treated mice that only underwent laparotomy, PHx
 12 mice demonstrated an increase in Annexin V-labeled hepatic apoEVs (Figure 5C), which
 13 might be related to a wave of cellular apoptosis accompanying mitosis of HCs (Sakamoto et
 14 al., 1999), indicating apoEVs being actively involved in the liver regeneration process.
 15 Furthermore, we discovered a slight increase of Golgin84-stained area in the regenerating
 16 liver (Figure 5D), together with a reduced percentage of binucleated HCs (Figure 5E),
 17 suggesting responses of Golgi being associated with apoEV changes and the ploidy transition
 18 of HCs in liver regeneration.

19 To reveal the functional significance of apoEVs to liver regeneration, we established an
 20 80% massive hepatectomy (MHx) mouse model (Figure 5F). Without intervention, mice may
 21 not survive this injury and typically die within 72 h after surgery (Hori et al., 2011). Importantly,
 22 infusion of apoEVs at 24 h prior to surgery substantially improved the survival rate of MHx

1 mice (Figure 5G) and promoted recovery of liver mass at 48 h after surgery (Figure 5H).
 2 Infusion of TeNT-pretreated VAMP3-cleaved apoEVs failed to promote liver regeneration and
 3 improve survival of MHx mice (Figure 5G, H). As expected, infusion of apoEVs significantly
 4 upregulated the Golgi abundance in liver after MHx, and the effects were diminished by TeNT
 5 pretreatments (Figure 5I). Moreover, apoEV infusion accelerated the division of binucleated
 6 HCs for rapid liver regeneration dependent on apoEV-VAMP3 expression (Figure 5J).
 7 Collectively, these data provide proof-of-concept evidence that apoEV-mediated Golgi
 8 recovery and ploidy reversal contribute to liver regeneration (Figure 5K).

9

ApoEVs protect against lethal liver failure through Golgi and ploidy restorations

11 The above findings encouraged us to further unravel the therapeutic potential of apoEVs in
 12 counteracting liver diseases, by applying a clinically relevant model of ALF induced by lethal
 13 acetaminophen (APAP) challenge (Barbier-Torres et al., 2017; Stravitz and Lee, 2019). Due to
 14 overdoses of paracetamol and other medications for pain and fever control, APAP toxicity
 15 represents the principal cause of ALF in developed countries, estimated in the United States
 16 at about 30,000 patients hospitalized per year and accounting for 45.7% of all ALF cases
 17 (Bliesen et al., 2014; Stravitz and Lee, 2019). However, how liver regeneration becomes
 18 overwhelmed or impaired in this circumstance, thus failing to restore the severely damaged
 19 liver, is not fully understood (Forbes and Newsome, 2016). In previous experiments, we
 20 noticed that declined hepatic apoEVs in *Fas^{mut}* mice and *Casp3^{-/-}* mice led to the onset of liver
 21 injury, which did not heal until apoEVs were replenished. We further observed in the lethally
 22 APAP-damaged liver, which again especially demonstrated severely injured binucleated HCs

(Figure 6A-C), that hepatic apoEVs were reduced (Figure 6D). Furthermore, we also found impaired liver Golgi after APAP challenge (Figure 6E), whereas the regenerative division of binucleated cells was not initiated (Figure 6F). These findings indicate that apoEV exhaustion with Golgi and ploidy transition impairments underlie diminished liver regeneration in ALF.

We therefore explored whether apoEV infusion could serve as an effective therapeutic for ALF (Figure 6G). Currently, the antioxidant *N*-acetyl L-cysteine (NAC) is the sole available and standard therapy to treat APAP overdose patients, but it is only effective as an antidote within a short time window of less than 8-12 h after APAP ingestion (Barbier-Torres et al., 2017). In light of this information, we applied apoEV infusion at 8 h post-APAP challenge and compared it to NAC supplied at the same time point (Figure 6G). Data demonstrated that while NAC administration at this time failed to rescue the severe liver damage induced by APAP, apoEVs provoked a surprisingly complete liver protection in surviving mice at 24 h after APAP injection (Figure 6H). Also, the effects of apoEVs were abolished by TeNT pretreatment for VAMP3 cleavage (Figure 6H). Further evaluation of mouse survival revealed that apoEV infusion, but not NAC injection, dramatically lowered the death rates, with effects diminished by TeNT pretreatment (Figure 6I). The ability of apoEVs to ameliorate APAP-induced ALF were attributed to the rescue of liver Golgi and further emergence of mononucleated HCs in liver, together with declined HC apoptosis, which were suppressed after apoEV-VAMP3 being cleaved by TeNT (Figure 6J-L). Collectively, these results suggest that apoEV infusion promotes HC recovery for ALF therapy (Figure 6M).

We took a final step to examine whether the functional molecules we discovered on the surface of STS-induced MSC-derived apoEVs, *i.e.* Gal/GalNAc and VAMP3, were general

1 profiles of apoEVs as functional markers for liver regulation. Based on flow cytometric
2 analyses established above, we tested the exposure of aopEVs to PtdSer (Annexin V-binding),
3 Gal/GalNAc and VAMP3 in the following circumstances: human MSCs (hMSCs) treated with
4 ABT-737, a B-cell lymphoma-2 (Bcl-2) homology domain 3 (BH3)-mimetic that directly triggers
5 permeabilization of the mitochondrial outer membrane to induce the intrinsic pathway of
6 apoptosis (Medina et al., 2020) (Figure S6A); mouse activated T cells with Fas-crosslinking
7 (anti-Fas treatment) to induce the extrinsic pathway of apoptosis, which represents a
8 physiological source of apoEVs (Figure S6B); and STS-treated NIH/3T3 cells, which are a
9 mouse fibroblast cell line representing differentiated cells, another physiological source of
10 apoEVs (Figure S6C). Our data demonstrated that apoEVs collected from the different
11 conditions uniformly exposed PtdSer and were characterized by surface Gal, GalNAc and
12 VAMP3 (Figure S6A-C), suggesting a general apoptotic vesicular signature (AVS) marking a
13 functional “envelope” of apoEVs for liver regulation (Figure S6D).

14

1 Discussion

2 Apoptosis is recognized as an integral physiological cell death process which occurs daily
 3 with a high prevalence and generates a huge number of apoEVs (Arandjelovic and
 4 Ravichandran, 2015; Atkin-Smith et al., 2015; Caruso and Poon, 2018; Fuchs and Steller,
 5 2011). However, whether apoEVs are necessary for maintaining organ homeostasis has
 6 remained unclear. In this study, we aimed to investigate the biological properties and the
 7 related physiological function of apoEVs in organ maintenance. We characterized apoEVs by
 8 their specific AVS and possession of liver engraftment properties. We found that apoEVs were
 9 recognized and internalized by HCs in the liver through the Gal/GalNAc-ASGPR system, in
 10 which they assembled with recipient Golgi apparatus to form a chimeric AGC structure based
 11 on the SNARE mechanism. AGC formation in binucleated HCs then activated downstream
 12 functional cascades including microtubule acetylation and cytokinesis, which were
 13 indispensable for ploidy transition of HCs to safeguard the diploid populations. Ultimately,
 14 apoEVs were of functional significance for maintaining liver homeostasis, promoting liver
 15 regeneration and mediating therapy against ALF. These findings collectively suggest a
 16 previously unrecognized role and mechanism of apoEVs in regulating liver function, shedding
 17 light on the potential of developing apoEV therapy for liver diseases.

18 Apoptosis represents the dominant modality of cell death in the normal homeostasis of
 19 multicellular organisms, and is indispensable not only for necessary cell elimination but also
 20 for regulation of fundamental life processes such as immune reactions, metabolism and
 21 regenerative proliferation (Fuchs et al., 2013; Fuchs and Steller, 2011; Han and Ravichandran,
 22 2011; Morioka et al., 2019). Effects of apoptosis are exerted through either a “passive” mode

1 by efferocytosis of apoptotic “corpses”, during which macrophages exert their own function
2 (Morioka et al., 2018), or a more “active” form that involves controlled release of nucleotides,
3 proteins and metabolites for regulation of phagocytosis and compensatory proliferation
4 (Chekeni et al., 2010; Chera et al., 2009; Medina et al., 2020; Wickman et al., 2013). Among
5 the paracrine signals, apoEVs possess unique properties as subcellular membrane-delimited
6 particles, which encapsulate heterogeneous factors for distant and combinational messaging
7 (Atkin-Smith et al., 2015; Dieude et al., 2015; Liu et al., 2018). We have previously reported
8 that circulating apoEVs maintain stem cell function in bone and ameliorate osteopenia *via*
9 transferring concerted signaling modulators (Liu et al., 2018). Others have documented that
10 apoEVs deliver multiple mitogenic or alarm cues to trigger molecular and functional cascades
11 for epithelial turnover and inflammation (Berda-Haddad et al., 2011; Brock et al., 2019;
12 Wickman et al., 2013). In the present study, we established a regulatory link between
13 hepatic/systemic apoEVs and liver homeostasis, mediated by specific apoptotic vesicular
14 markers for destined engraftment and functional internalization. ApoEV contributions to liver
15 homeostasis further underlie the effects of organismal apoptotic regulation. As far as we know,
16 this is the first evidence for the requirement of apoEVs in organ homeostatic maintenance and
17 reveals the previously unknown, specific mechanisms involved. The heterogeneity of apoEVs
18 introduced by source cells, during the apoptotic process and among the potential subsets of
19 apoEVs will be a critical issue to be addressed in next studies. Future studies will also need to
20 elaborate upon the physiological source and identity of liver-regulating apoEVs, and trace
21 their involvement in liver homeostasis and pathologies.

1 The liver is a vital organ that functions as the primary center for sustaining metabolism
2 and disposing of toxins, and it is also known to clear systemic apoptotic cells through its
3 resident macrophages (Shi et al., 1996). However, as apoptotic materials may overwhelm the
4 phagocytic capacity of liver macrophages (Morioka et al., 2019), it is plausible that other
5 hepatic cell populations contribute to digestion of the apoptotic “meal”. As the major cell type
6 in the liver responsible for its function, HCs account for about 80% of liver weight and about
7 70% of all liver cells; HCs also show the ability to engulf apoptotic cells (Dini et al., 1992;
8 McVicker et al., 2002). In this study, notably, we reveal that HCs actually represent the main
9 target of apoEVs in the liver, particularly for those free of xenobiotic membrane labeling.
10 Furthermore, internalization of apoEVs by HCs induces functional results such as promoting
11 cytokinesis. One possible mechanism enlightened by this study is that apoEVs control Golgi
12 structure formation and function, as intact Golgi promotes microtubule polymerization and
13 organization through the containing of γ -tubulin, the major nucleator of microtubules (Rios et
14 al., 2004), and α -tubulin acetyltransferase 1 (α TAT1), the acetyltransferase specific to the K40
15 residue of α -tubulin (Nakakura et al., 2016), and improved Golgi-derived microtubule
16 organization facilitates cell division and cytokinesis (Wei et al., 2015). These findings provide
17 a new perspective on cell death, indicating that the contents of vesicles produced during
18 apoptosis are naturally required for functional purposes, rather than just being efferocytosed.
19 Intriguingly, other EV populations (e.g. Exos) also engraft in the liver and are involved in liver
20 regulation (Wiklander et al., 2015; Ying et al., 2017), but the underlying reasons why the liver
21 needs EVs are still elusive. Here, we for the first time uncovered that apoEVs indispensably
22 participate in the unique ploidy transition process of HCs, which is characteristically seen in

1 hepatic postnatal development, aging and pathologies (Fortier et al., 2017; Wang et al., 2017).
2 It is notable that non-apoptotic EVs may emerge to maintain a constant liver EV pool upon
3 loss of apoEVs, as indicated in this study, whereas the endogenous source of the
4 non-apoptotic EVs and their functional roles remain unrecognized. However, our findings
5 contribute to explaining the high demand of the liver for apoEVs at the functional level,
6 indicating the apoptotic materials reciprocally potentiate dynamic behaviors of living cells.
7 These data consistent with recent studies by us and others suggesting that diploid HCs play a
8 key role in maintaining liver homeostasis and regeneration (Gentric et al., 2015; Wang et al.,
9 2014; Wilkinson et al., 2019b), although they are relatively low in abundance and their
10 functional roles are still in controversial (Kreutz et al., 2017; Zhang et al., 2018a). Moreover,
11 whether apoEVs exert differential effects on diploid and polyploid HCs, and whether apoEVs
12 realize ploidy reversal of mononucleated polyploid HCs, remain to be investigated. Also to be
13 examined is the responses of aneuploid HCs to apoEVs, which might promote genetic
14 diversity among HCs in the liver and facilitate stress-induced liver adaptation (Duncan et al.,
15 2012; Duncan et al., 2010). Our results pave an avenue for further extensive examinations of
16 apoEV regulation of general HC and liver function, including but not limited to the age-related
17 polyploidization process, the glucose/lipid metabolism and the protein/enzyme synthesis.

18 The eukaryotes possess an ancient and elaborate endomembrane system which is
19 responsible for the biosynthesis and transport of proteins and lipids, as well as for the
20 formation of major intracellular organelles (Field and Dacks, 2009). The endomembrane
21 system is dynamically maintained by endosomal vesicular trafficking in a bi-directional mode:
22 anterograde transport ends with exocytosis and retrograde transport starts with endocytosis,

1 while the Golgi serves as the crucial interface between these two processes (Johannes and
2 Popoff, 2008; Lee et al., 2004). Interestingly, EVs, as products of endosomal or plasma
3 membrane origin, also have close relationships with the endomembrane system for trafficking
4 after internalization (Heusermann et al., 2016; Joshi et al., 2020). In a previous study, we have
5 reported that apoEVs after engulfment regulate recipient lysosomal biogenesis by activating a
6 master transcription factor (Liu et al., 2020). However, it remains unknown whether EVs or
7 apoEVs directly integrate into recipient organelles with functional significance. In this study,
8 we discovered that apoEVs assemble with recipient Golgi and are required for Golgi integrity,
9 which adds new dimensions to the current knowledge in the following respects. First,
10 homeostasis of an organelle or at least a part of the endomembrane system demands
11 extrinsic EV inputs. Second, EVs can be integrally reused as necessary structural units for
12 functional execution. Finally, the Golgi apparatus might actually be a chimeric organelle (or
13 the AGC structure) in certain cells with physiological apoEV internalization. One mechanism
14 revealed in this study is that apoEVs express v-SNAREs and apoEVs use VAMP3 to
15 assemble with Golgi. According to current models, as far as we know, SNARE-mediated
16 membrane contacts may end up with different outcomes, such as a full-collapse fusion or just
17 a kiss-and-run mode of flicker fusion (Smith et al., 2008). Although VAMP3 functionally
18 expresses on apoEVs for finding Golgi, how apoEVs work with Golgi will still be interesting to
19 further examine. Whether other EVs also assemble with recipient organelles and whether
20 apoEVs fuse with endosomes or other membranous organelles remain to be investigated. It is
21 intriguing findings that Golgi integrity is required for liver homeostasis and that Golgi
22 participates in liver regeneration, whereas loss of Golgi contributes to ALF progression.

1 Although the regulatory mechanisms of Golgi assembly and disassembly in organ health and
2 sickness are not fully understood, the involvements of Golgi in stress responses and disease
3 pathogenesis emerge to be noticed (Machamer, 2015; Sbodio et al., 2018). In this regard,
4 apoEV infusion may serve as an effective approach to potentiate healthy Golgi for incoming
5 functional requirements (e.g. in MHx) or rescue defective Golgi (e.g. in ALF). Moreover, as the
6 Golgi apparatus performs multiple important functions such as protein sorting and
7 modification (Lee et al., 2004), it would be significant to decipher the potential role of the Golgi
8 as a cargo- or signaling-processing platform for internalized EVs, which might be linked to the
9 undissected mechanisms apoEVs in promoting microtubule acetylation *via* the Golgi in this
10 study.

11 To cope with a constant influx of noxious stimuli, the liver has developed a remarkable
12 and tightly controlled regenerative capacity in response to parenchymal loss, which
13 nevertheless can be impaired in severe or chronic injury settings (Forbes and Newsome,
14 2016). Thus, liver disease is a primary cause of mortality and morbidity that is also rising
15 globally (Williams et al., 2017). Currently, for end-stage liver disease or failure, the only clinical
16 approach is liver transplantation, but availability is limited by several major challenges
17 including a donor shortage (Stravitz and Lee, 2019). Therefore, there remains an unmet need
18 to rescue patients from lethal liver injuries. In this study, on the basis of previous findings on
19 apoptotic regulation of tissue regeneration (Brock et al., 2019; Li et al., 2010), we unraveled
20 that infusion of allogeneic apoEVs significantly promotes liver regeneration after PHx and can
21 serve as an effective therapeutic for ALF. ApoEV therapy holds great promise for translational
22 applications, especially when the current antidote fails to work in late-stage ALF. Based on the

1 results of this study, we assume that by apoEVs promoting ploidy transition, the ROS
2 toxicity-prone 4N-HCs are rapidly replaced by 2N-HCs for liver protection, while whether this
3 is a form of action of detoxification or regeneration will be tricky to explain. It is notable that
4 with translational advantages, therapeutic apoEVs can be easily obtained at a larger amount
5 than Exos and without ultracentrifuge from cultured MSCs, which possess immense potential
6 for regenerative and modulatory transplantation and have been used extensively in clinical
7 trials for treating a variety of diseases (Galipeau and Sensebe, 2018). Furthermore, given the
8 potential hepatotropic property of apoEVs, engineered chimeric apoEVs can be constructed
9 for targeted delivery of therapeutic molecules or drugs, as we previously established (Dou et
10 al., 2020), which might prove useful for treating complicated chronic diseases of the liver.
11 Nevertheless, the application of apoEVs in treating liver diseases should also be cautious
12 particularly considering that the polyploid state may play a tumor-suppressive role in the liver
13 (Zhang et al., 2018a; Zhang et al., 2018b). Although that apoEVs are kind of safeguarding
14 ploidy homeostasis of the liver without promoting diploid HCs over the physiological status,
15 how apoEVs might balance the effects and risks remain elusive. Taken together, our findings
16 open a window for future translational research on apoEVs to counteract diseases in the liver
17 context.

18

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16

Author contributions

B.S. contributed to the study design and conduct, and the manuscript drafting. R.W. contributed to data acquisition, analysis and interpretation. C.C. and X.K. contributed to data analysis and interpretation. D.W. contributed to hepatectomy experiments. H.C.T. contributed to GalNAc-based hepatocyte ploidy manipulation. Y.W. contributed to the experimental design on Golgi apparatus. Y.L., O.J. and X.C. contributed to labeling of apoEVs by radionuclides.

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8

9 Declaration of Interests

10 The authors have declared that no conflict of interest exists.

11

1 **Figure legends**

2 **Figure 1. Circulatory apoptotic extracellular vesicles (apoEVs) engraft in the liver and**

3 **safeguard liver homeostasis. (A)** Biodistribution of intravenously injected copper isotope

4 (⁶⁴Cu)-labeled apoEVs. Mice underwent whole-body coronal positron emission tomography

5 (PET) at indicated time points. Organs/tissues were dissected and measured for radiation

6 intensity at 48 h. WAT, white adipose tissue; Lv., liver; Sp., spleen; Stm., stomach; Kid., kidney;

7 L., lung; Bw., bowel; H., heart; Fm., femur. Scale bar = 10 mm. *N* = 4 per organ/tissue. **(B)**

8 Tracing of PKH26-labeled apoEVs (red) in the liver, counterstained with DAPI for detecting

9 DNA (blue). ApoEVs were intravenously injected and liver samples were collected at indicated

10 time points. After a single infusion, kinetic changes of apoEVs in the liver were obtained by

11 fluorescent imaging analysis. The half-life of liver-engrafted apoEVs was calculated. Scale

12 bars = 25 μ m. *N* = 6 per time point. **(C)** Schematic diagram showing study design of apoEV

13 infusion in *Fas* mutant (*Fas*^{mut}) mice. ApoEVs were infused intravenously (*i.v.*) at a protein

14 concentration of 5 μ g/g. **(D)** Flow cytometric analysis of Annexin V⁺ liver tissue extracellular

15 vesicles (apoEVs) with corresponding quantification. WT, wild type. *N* = 3 per group. **(E)**

16 Gross view images of liver and quantification of fasting liver weight (LW) to body weight (BW)

17 ratio. Scale bars = 5 mm. *N* = 6 per group. **(F)** Hematoxylin and eosin (H&E) staining of liver

18 tissues in respective periportal vein (PV) and pericentral vein (CV) areas. Hepatic injury

19 scores were examined based on pathological parameters. Scale bars = 50 μ m. *N* = 6 per

20 group. **(G)** Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

21 levels were determined. *N* = 6 per group. **(H)** Representative liver fluorescent images showing

22 hepatocytes (HCs) with different numbers of nuclei (blue, DAPI for DNA) and their cell borders

1 (green, phalloidin for F-actin). # indicates binucleated HCs. Percentages of binucleated and
2 mononucleated HCs were quantified. Scale bars = 25 μ m. *N* = 6 per group. **(I)** After propidium
3 iodide (PI) staining, ploidy distribution of HCs (diploid, 2N; tetraploid, 4N; octoploid, 8N) was
4 analyzed by flow cytometry. *N* = 3 per group. **(J)** Graphical summary demonstrating that
5 apoEVs maintained liver homeostasis in apoptotic deficient conditions. Data represent mean
6 $\pm$ standard deviation. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.0001. Statistical analyses were
7 performed by one-way analysis of variance followed by the Newman-Keuls post hoc tests.

8

9 **Figure 2. Specialized internalization of apoEVs by hepatocytes (HCs) promotes ploidy**

10 **transition. (A)** Tracing of PKH26-labeled (for membrane) or aggregation-induced emission
11 luminogen (AIEgen, for mitochondria)-labeled apoEVs (red) in liver, with phalloidin staining
12 F-actin (green) to show HC cell borders. DAPI was used for counterstaining DNA (blue).
13 ApoEVs were intravenously injected and liver samples were collected 24 h after the infusion.
14 Representative confocal images show binucleated HCs and the neighbored macrophages
15 (M ϕ s). Flow cytometric analyses were performed on freshly isolated primary HCs to detect
16 the fluorescent signals and determine percentages of apoEV-positive HCs. Scale bars = 10
17 μ m. **(B)** Tracing of green fluorescent protein (GFP)-labeled particles (green) in liver of *Fas*
18 mutant (*Fas^{mut}*) mice, with phalloidin staining F-actin (red) to show borders of HC cells. DAPI
19 was used for counterstaining DNA (blue). *Fas^{mut}* mice and *GFP^{+/+}* mice were
20 connected to generate parabiosis model for 2 months. A representative confocal image shows
21 a binucleated HC and a neighboring M ϕ . Scale bar = 10 μ m. **(C)** Representative fluorescent
22 images showed apoEV surface exposure of desialylated residues including galactose (Gal)

1 and *N*-acetylgalactosamine (GalNAc). ApoEVs (labeled by CellMask™ Deep Red, red) were
2 stained with FITC-conjugated lectins (green) specific for Gal or GalNAc binding and
3 aggregation. Flow cytometric analysis was performed to determine percentages of apoEV
4 staining. ApoEV exposure of GalNAc was compared to exosomes (Exos) or to apoEV source
5 mesenchymal stem cells (MSCs) using fluorescent unit (FU, analyzed by a fluorescent reader)
6 over membrane labeling (by CellMask™ Deep Red) FU ratio. Scale bars = 25 μ m. *N* = 3 per
7 group. **(D)** Schematic diagram demonstrates the study design of apoEV uptake by primary
8 cultured HCs. WEM, William's E Medium; BSA, bovine serum albumin; ASGPR,
9 asialoglycoprotein receptor; ab, antibody. ApoEVs were treated at a protein concentration of
10 10 μ g/ml. Gal and GalNAc were added together at 200 mM each, and antibodies were added
11 at 5 μ g/ml. **(E)** Representative fluorescent images show PKH26-labeled apoEVs (red)
12 uptaken by binucleated HCs (DAPI for DNA, blue). White dashed lines depict cell borders of
13 cultured HCs. Flow cytometric analysis was performed to detect the fluorescent signals and
14 determine the percentage of apoEV-positive HCs. Scale bars = 10 μ m. *N* = 5 per group. **(F)**
15 Schematic diagram demonstrates the study design of HC ploidy transition. ApoEV protein
16 concentration was 10 μ g/ml. Representative image shows complete cytokinesis, which is
17 identified by the midbody structure formed by microtubules (MTs, α -tubulin immunostaining,
18 green; counterstained with DAPI, blue). Scale bar = 20 μ m. **(G)** Imaging analysis of complete
19 cytokinesis events. WT, wild type. *N* = 4 per group. **(H)** After propidium iodide (PI) staining,
20 ploidy distribution of HCs (diploid, 2N; tetraploid, 4N; octoploid, 8N) was analyzed by flow
21 cytometry. *N* = 4 per group. **(I)** Schematic diagram demonstrating the study design of *in vivo*
22 HC uptake or HC ploidy intervention upon apoEV infusion. ApoEVs were infused

intravenously (*i.v.*) at a protein concentration of 5 µg/g. GalNAc was injected subcutaneously (s.c.) at 500 mg/kg once per week to inhibit apoEV uptake by HCs. GalNAc-conjugated small interferon RNA (siRNA) against the *Anillin* gene (G-si*Anln*) for inhibiting HC cytokinesis or its luciferase control (G-si*Luc*) was injected at 4 µg/g. **(J)** Tracing of PKH26-labeled apoEVs (red) in the liver. DAPI was used for DNA counterstaining (blue). Scale bars = 25 µm. **(K)** Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of *Anln* mRNA expression in freshly isolated HCs, liver non-parenchymal cells (NPCs) and splenocytes (SPCs) with β -actin as internal control. *N* = 3 per group. **(L)** Representative liver fluorescent images show HCs with different numbers of nuclei (blue, DAPI for DNA) and their cell borders (green, phalloidin for F-actin). # indicates binucleated HCs. Percentages of binucleated and mononucleated HCs were quantified. Scale bars = 25 µm. *N* = 5 per group. **(M)** After PI staining, ploidy distribution of HCs was analyzed by flow cytometry. *N* = 3 per group. **(N)** Hematoxylin and eosin (H&E) staining of liver tissues in periportal vein (PV) areas. Hepatic injury scores were assessed based on pathological parameters. Scale bars = 50 µm. *N* = 5 per group. **(O)** Serum alanine aminotransferase (ALT) levels were determined. *N* = 5 per group. **(P)** Graphical summary demonstrates that apoEV uptake by HCs promotes ploidy transition for liver homeostasis. Data represent mean $\pm$ standard deviation. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.0001; NS, not significant, *P* > 0.05. Statistical analyses were performed using Student's *t* test for two-group analysis or one-way analysis of variance followed by the Newman-Keuls post hoc tests for multiple group comparisons.

21

Figure 3. ApoEVs interact with recipient Golgi apparatus to form a chimeric organelle complex. **(A)** Left: Tracing of aggregation-induced emission luminogen (AIEgen)-labeled apoEVs (red) in liver, with Golgin84 immunostaining for Golgi apparatus (green) and counterstaining with DAPI (blue). ApoEVs were intravenously injected and liver samples were collected at 24 h after the infusion. Right: Tracing of green fluorescent protein (GFP)-labeled particles (green) in the liver of *Fas* mutant (*Fas^{mut}*) mice, with Golgin84 immunostaining for Golgi apparatus (red) and counterstaining with DAPI (blue). *Fas^{mut}* mice and *GFP* transgenic (*GFP^{+/+}*) mice were connected as a parabiosis model for 2 months. Representative confocal images show binucleated HCs. Scale bars = 5 μ m. **(B)** Tracing of PKH26-labeled apoEVs (red) in sorted tetraploid HCs (4N-HCs), with Golgi apparatus being demonstrated by a plasmid of *N*-acetylgalactosaminyltransferase 2 (GALNT2)-GFP (green) and counterstaining with DAPI (blue). Representative 2D confocal images show binucleated HCs and 3D reconstructed images show apoEV contacting Golgi apparatus. Scale bars = 10 μ m (2D) and 500 nm (3D). **(C)** Representative fluorescent images show cultured 4N-HCs in interphase and in cytokinesis with GALNT2-GFP-labeled Golgi (green), PKH26-labeled apoEVs (red), DAPI counterstain (blue), and ac- α -tubulin immunostaining for acetylated microtubules (MTs, white). Scale bars = 10 μ m. **(D)** Representative fluorescent images show cultured 4N-HCs with GALNT2-GFP-labeled Golgi (green, up) or 130 kDa Golgi matrix protein (GM130) immunofluorescent (IF) staining (green, down), PKH26-labeled apoEVs (red), and DAPI counterstain (blue). Imaging analysis was performed to quantify HC percentages with Golgi fragmentation and Golgi with apoEV fluorescence intensity. WT, wild type. Scale bars = 5 μ m. *N* = 4 per group. **(E)** Schematic diagram demonstrates the study design for investigating

1 apoEV-Golgi assimilation. Representative fluorescent images show cultured 4N-HCs with
2 their own Golgi proteins (green) and apoEV-transferred Golgi proteins (red) with DAPI
3 counterstain (blue). GRASP55, Golgi reassembly-stacking protein 55; GalT,
4 galactosyltransferase. Scale bars = 5 μ m. **(F)** Western blot analysis shows multiple Golgi
5 protein expression in apoEVs compared to their source MSCs, and in cultured HCs. TGN38,
6 *trans*-Golgi network protein 38; Stx5, syntaxin 5; GAPDH, glyceraldehyde 3-phosphate
7 dehydrogenase. **(G)** Representative transmission electron microscopy (TEM) images show
8 Golgi ultrastructure in sorted 4N-HCs. Distinguishable Golgi stacks per cell were quantified.
9 Scale bars = 200 nm. *N* = 4 per group. **(H)** Golgi apparatus was isolated from cultured HCs
10 and Golgi protein mass was determined using the BCA method. *N* = 4 per group. **(I)** Western
11 blot analysis showed GFP expression, MT and acetylated MT markers in isolated Golgi.
12 ApoEVs were derived from *GFP*^{+/+} MSCs. GM130 was used as an internal control. **(J)**
13 Representative fluorescent images show cultured 4N-HCs with ac- α -tubulin immunostaining
14 for acetylated MTs (white), GALNT2-GFP-labeled Golgi (green), PKH26-labeled apoEVs (red),
15 and DAPI counterstain (blue). Imaging analysis was performed to quantify acetylated MTs
16 fluorescence intensity and complete cytokinesis events. Ctrl, control; BFA, Brefeldin A, added
17 at 1 nM. Scale bars = 5 μ m. *N* = 4 per group. **(K)** Western blot analysis show MT and
18 expression of multiple mitosis proteins in cultured HCs. INCENP, inner centromere protein;
19 MKLP1, mitotic kinesin-like protein 1; RacGAP1, Rac GTPase-activating protein 1; RhoA, Ras
20 homolog family member A. **(L)** After propidium iodide (PI) staining, ploidy distribution in HCs
21 (diploid, 2N; tetraploid, 4N; octoploid, 8N) was analyzed by flow cytometry. *N* = 4 per group.
22 **(M)** Graphical summary demonstrating apoEV assembly with Golgi to safeguard HC

1 cytokinesis. Data represent mean $\pm$ standard deviation. **, $P < 0.01$; ***, $P < 0.0001$.
 2 Statistical analyses were performed by Student's t test for two-group analysis or one-way
 3 analysis of variance followed by the Newman-Keuls post hoc tests for multiple group
 4 comparisons.

5
 6 **Figure 4. ApoEVs use vesicle-associated membrane protein 3 (VAMP3) to assemble**
 7 **with Golgi. (A)** Representative immunofluorescent images show apoEV (labeled with
 8 CellMask™ Deep Red, red) surface expression of vesicle-localized-soluble
 9 N -ethylmaleimide-sensitive factor attachment protein receptors (v-SNAREs, green) VAMP3, V
 10 VAMP4 and Golgi SNARE of 15 kDa (GS15). Flow cytometric analysis was performed to
 11 determine percentages of positively stained apoEVs. Scale bars = 25 μ m. **(B)** Western blot
 12 analysis show expression of VAMPs in apoEVs compared to their source MSCs. **(C)** Western
 13 blot analysis show expression of VAMPs in apoEVs. Source MSCs were transfected with
 14 small interferon RNAs (siRNAs) as a negative control (siCtrl) for VAMP3 (siVamp3), VAMP4
 15 (siVamp4), and both VAMP3 and VAMP4 (siVamp3/4). Flow cytometric analysis was
 16 performed to determine percentages of positively stained apoEVs after knockdown of VAMPs.
 17 **(D)** Representative images show cultured *Fas* mutant (*Fas^{mut}*) tetraploid hepatocytes
 18 (4N-HCs) with 130 kDa Golgi matrix protein (GM130) immunofluorescent staining (green),
 19 PKH26-labeled apoEVs (red), and DAPI counterstain (blue). White dashed lines depict cell
 20 borders of cultured HCs. Imaging analysis was performed to quantify apoEV uptake and
 21 percentages of Golgi-contacted apoEVs. Scale bars = 10 μ m. $N = 4$ per group. **(E)**
 22 Representative fluorescent images show cultured *Fas^{mut}* 4N-HCs with ac- α -tubulin

1 immunostaining (white), *N*-acetylgalactosaminyltransferase 2 (GALNT2)-GFP-labeled Golgi
2 (green), PKH26-labeled apoEVs (red), and DAPI counterstain (blue). Imaging analysis was
3 performed to quantify HC percentages with Golgi fragmentation and acetylated microtubules
4 (MTs) fluorescence intensity. Scale bars = 5 μ m. *N* = 4 per group. **(F)** Western blot analysis
5 showed human VAMP3 (hVAMP3) expression in isolated HC Golgi. ApoEVs were derived
6 from human MSCs (hApoEVs). GM130 was used as an internal control. **(G)** Western blot
7 analysis showed VAMP3 expression in apoEVs without or with treatment by 250 ng/ml
8 Tetanus toxin (TeNT). **(H)** Representative Golgin84 immunofluorescent staining (green)
9 images of liver Golgi counterstained with DAPI (blue). WT, wild type. Imaging analyses were
10 performed to quantify HC percentages with Golgi fragmentation. Scale bars = 5 μ m. *N* = 4 per
11 group. **(I)** Golgi apparatus was isolated from the liver and Golgi protein mass was determined
12 using the BCA method. *N* = 4 per group. **(J)** Representative liver fluorescent images showed
13 HCs with different nuclei (blue, DAPI for DNA) and their cell borders (green, phalloidin for
14 F-actin). # indicates binucleated HCs. Scale bars = 25 μ m. *N* = 4 per group. **(K)** After PI
15 staining, percentages of binucleated HCs were quantified. Diploid HCs (2N-HCs) were
16 analyzed using flow cytometry. *N* = 4 per group. **(L)** Hematoxylin and eosin (H&E) staining of
17 liver tissues in periportal vein (PV) areas. Hepatic injury scores were examined based on
18 pathological parameters. Scale bars = 50 μ m. *N* = 4 per group. **(M)** Serum alanine
19 aminotransferase (ALT) levels were determined. *N* = 4 per group. **(N)** Graphical summary
20 illustrating that apoEVs use VAMP3 to assemble with Golgi for HC and liver regulation. Data
21 represent mean $\pm$ standard deviation. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.0001; NS, not
22 significant, *P* > 0.05. Statistical analyses were performed by Student's *t* test for two-group

1 analysis or one-way analysis of variance followed by the Newman-Keuls post hoc tests for
2 multiple group comparisons.

3

4 **Figure 5. ApoEV-mediated Golgi recovery and ploidy reversal contribute to liver**

5 **regeneration. (A)** Schematic diagram demonstrates the study design of 70% partial

6 hepatectomy (PHx). **(B)** Gross view images of liver and quantification of liver weight (LW) to

7 body weight (BW) ratio after recovery for 72 h. Scale bars = 5 mm. *N* = 5 per group. **(C)** Flow

8 cytometric analysis showed Annexin V⁺ liver tissue extracellular vesicles (apoEVs) and

9 corresponding quantification. *N* = 3 per group. **(D)** Representative Golgin84

10 immunofluorescent staining (green) images of liver Golgi counterstained with DAPI (blue).

11 Imaging analysis was performed to quantify Golgi area percentages. Scale bars = 5 μ m. *N* = 4

12 per group. **(E)** Representative liver fluorescent images show hepatocytes (HCs) with different

13 numbers of nuclei (blue, DAPI for DNA) and their cell borders (green, phalloidin for F-actin). #

14 indicates binucleated HCs. Percentages of binucleated HCs were accordingly quantified.

15 Scale bars = 25 μ m. *N* = 4 per group. **(F)** Schematic diagram demonstrates the study design

16 of 80% massive hepatectomy (MHx) therapy. ApoEVs at a protein concentration of 5 μ g/g

17 were infused intravenously (*i.v.*) alone or after administration of 250 ng/ml Tetanus toxin

18 (TeNT). **(G)** Kaplan-Meier survival curve of MHx mice. *N* = 10 per group. **(H)** Gross view

19 images of the liver and quantification of LW/BW ratio after recovery for 48 h. Scale bars = 5

20 mm. *N* = 5 per group. **(I)** Representative Golgin84 immunofluorescent staining (green) images

21 of liver Golgi counterstained with DAPI (blue). Imaging analysis was performed to quantify

22 Golgi area percentages. Scale bars = 5 μ m. *N* = 4 per group. **(J)** Representative liver

1 fluorescent images show HCs with different numbers of nuclei (blue, DAPI for DNA) and their
2 cell borders (green, phalloidin for F-actin). # indicates binucleated HCs. Percentages of
3 binucleated HCs were quantified. Scale bars = 25 μ m. $N = 4$ per group. **(K)** Graphical
4 summary demonstrates apoEVs promote Golgi recovery and ploidy reversal for liver
5 regeneration. Data represent mean $\pm$ standard deviation. *, $P < 0.05$; **, $P < 0.01$; ***, $P <$
6 0.0001. Statistical analyses were performed by Student's t test for two-group analysis or
7 one-way analysis of variance followed by the Newman-Keuls post hoc tests for multiple group
8 comparisons.

9

10 **Figure 6. ApoEVs protect against acute acetaminophen (APAP)-induced liver failure**
11 **through Golgi and ploidy restorations. (A)** Schematic diagram demonstrates the study
12 design of APAP-induced acute liver failure (ALF). APAP was injected intraperitoneally (*i.p.*) at
13 1 g/kg. **(B)** Hematoxylin and eosin (H&E) staining of liver tissues. Hepatic injury scores were
14 examined based on pathological parameters. Ctrl, control. Scale bars = 100 μ m. $N = 5$ per
15 group. **(C)** Serum alanine aminotransferase (ALT) levels were determined. $N = 5$ per group.
16 **(D)** Flow cytometric analysis of Annexin V⁺ liver tissue extracellular vesicles (apoEVs) and
17 corresponding quantification. $N = 3$ per group. **(E)** Representative Golgin84
18 immunofluorescent staining (green) images of liver Golgi counterstained with DAPI (blue).
19 Imaging analysis was performed to quantify Golgi area percentages. Scale bars = 5 μ m. $N = 4$
20 per group. **(F)** Representative liver fluorescent images show hepatocytes (HCs) with different
21 numbers of nuclei (blue, DAPI for DNA) and their cell borders (green, phalloidin for F-actin). #
22 indicates binucleated HCs. Percentages of binucleated HCs were quantified. Scale bars = 25

1 μm . $N = 5$ per group. **(G)** Schematic diagram demonstrates the study design of ALF therapy.

2 APAP was injected *i.p.* at 1 g/kg. ApoEVs at protein quantification of 5 $\mu\text{g/g}$ were infused

3 intravenously (*i.v.*) with or without 250 ng/ml Tetanus toxin (TeNT). The antioxidant *N*-acetyl

4 L-cysteine (NAC) was injected *i.p.* at 1 g/kg. **(H)** H&E staining of liver tissues. Hepatic injury

5 scores were examined based on pathological parameters. Scale bars = 100 μm . $N = 5$ per

6 group. **(I)** Kaplan-Meier survival curve of ALF mice. $N = 10$ per group. **(J)** Representative

7 Golgin84 immunofluorescent staining (green) images of liver Golgi counterstained with DAPI

8 (blue). Imaging analysis was performed to quantify Golgi area percentages. Scale bars = 5

9 μm . $N = 4$ per group. **(K)** Representative liver fluorescent images show HCs with different

10 numbers of nuclei (blue, DAPI for DNA) and their cell borders (green, phalloidin for F-actin). #

11 indicates binucleated HCs. Percentages of binucleated HCs were quantified. Scale bars = 25

12 μm . $N = 4$ per group. **(L)** Percentages of apoptotic HCs were quantified by terminal

13 deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. $N = 4$ per

14 group. **(M)** Graphical summary demonstrates apoEV infusion promotes HC recovery for ALF

15 therapy. Data represent mean $\pm$ standard deviation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$;

16 NS, not significant, $P > 0.05$. Statistical analyses were performed by Student's *t* test for

17 two-group analysis, one-way analysis of variance followed by the Newman-Keuls post hoc

18 tests for multiple group comparisons, or the Log-rank test for survival curve comparisons.

19

1 **METHODS**

2 **LEAD CONTACT AND MATERIALS AVAILABILITY**

3 Further information and requests for resources and reagents should be directed to and will be
4 fulfilled by the Lead Contact, Songtao Shi (songtaos@upenn.edu).

6 **METHOD DETAILS**

7 **Animals**

8 The following mouse strains were obtained from the Jackson Laboratory: *Fas^{mut}*
9 (B6.MRL-*Fas^{lpr}*/J, JAX# 000482), *Casp3^{-/-}* (B6N.129S1-*Casp3^{tm1Flv}*/J, JAX# 006233, provided
10 as heterozygotes), *GFP^{+/+}* (C57BL/6-Tg(CAG-EGFP)1Osb/J, JAX# 003291), and C57BL/6J
11 (JAX# 000664) as the WT (Liu et al., 2018). Male mice at ages of 8 or 12 weeks were used.
12 All mice were housed in pathogen-free conditions, maintained on a standard 12-h light-dark
13 cycle, and received food and water *at libitum*. Genotyping was performed by polymerase
14 chain reaction (PCR) using tail samples from mice and primer sequences provided by the
15 Jackson Laboratory. All animal experiments were performed in compliance with the relevant
16 laws, institutional guidelines and ARRIVE guidelines.

18 **Reagents**

19 All antibodies, chemicals, cytokines and plasmids used in this study are listed in the Key
20 Resources Table (Table S1).

22 **Isolation and culture of MSCs**

1 Mouse MSCs were isolated from hind limb bone marrow and cultured, as we previously
2 described (Liu et al., 2018). Briefly, a single suspension of bone marrow cells was seeded at a
3 density of 1.5×10^7 cells per 10 cm culture dish at 37°C in 5% CO₂. Non-adherent cells were
4 removed after 24 h and attached cells were maintained for 16 days in alpha-Minimum
5 Essential Medium (α-MEM) (12571-048, Invitrogen, USA) supplemented with 20% fetal
6 bovine serum (FBS) (17L624, Sigma-Aldrich, USA), 2 mM L-glutamine (35050-061, Invitrogen,
7 USA), 55 μM 2-mercaptoethanol (21985-023, Invitrogen, USA), 100 U/ml penicillin
8 (15140-122, Invitrogen, USA) and 100 μg/ml streptomycin (15140-122, Invitrogen, USA).
9 Colony-forming attached cells were passed twice by trypsin (12605-010, Invitrogen, USA) for
10 further experimental use.

11 Human MSCs were isolated from full-term umbilical cords and cultured, as we previously
12 described (Sun et al., 2010). Briefly, fresh umbilical cords were obtained from informed,
13 healthy mothers in local maternity hospitals of China after normal deliveries and were
14 processed under sterile conditions. The cords were rinsed twice in phosphate buffered saline
15 (PBS) in penicillin and streptomycin, and outer membrane and vessels were removed. The
16 washed cords were then cut into 1 mm² pieces and incubated in low-glucose Dulbecco's
17 modified Eagle's medium (DMEM) (11885-084, Invitrogen, USA) containing 10% FBS
18 (S12450, Atlanta Biologicals, USA), 2 mM L-glutamine (35050-061, Invitrogen, USA), 100
19 U/ml penicillin (15140-122, Invitrogen, USA) and 100 μg/ml streptomycin (15140-122,
20 Invitrogen, USA) at 37°C in a humidified atmosphere of 5% CO₂. Colony-forming attached
21 cells were passed by trypsin (12605-010, Invitrogen, USA) for further experimental use.

22

1 Isolation and culture of T lymphocytes

2 Mouse T cells were derived from spleen and cultured, as we previously described (Sui et al.,
3 2017). Briefly, with ACK Lysing Buffer (10-548E, Lonza Bioscience, Switzerland) was used to
4 remove red blood cells. T cells were isolated and stimulated for 48 h with 3 µg/ml plate-bound
5 anti-mouse CD3 antibody (100340, BioLegend, USA) and 2 µg/ml soluble anti-mouse CD28
6 antibody (102116, BioLegend, USA) in RPMI 1640 medium (21870-076, Invitrogen, USA)
7 supplemented with 10% FBS (17L624, Sigma-Aldrich, USA), 2 mM L-glutamine (35050-061,
8 Invitrogen, USA), 100 U/mL penicillin (15140-122, Invitrogen, USA), and 100 g/ml
9 streptomycin (15140-122, Invitrogen, USA) at 37°C in a humidified atmosphere of 5% CO₂.

10

11 Culture of the cell line

12 NIH/3T3 cell line was purchased from ATCC and was maintained in low-glucose DMEM
13 (11885-084, Invitrogen, USA) supplemented with 10% FBS (17L624, Sigma-Aldrich, USA), 2
14 mM L-glutamine (35050-061, Invitrogen, USA), 100 U/ml penicillin (15140-122, Invitrogen,
15 USA) and 100 µg/ml streptomycin (15140-122, Invitrogen, USA) at 37°C in a humidified
16 atmosphere of 5% CO₂.

17

18 Induction and examination of apoptosis

19 Apoptosis was induced by multiple methods in this study. To induce cell apoptosis *via* both
20 caspase-dependent and caspase-independent pathways, as we previously established (Liu et
21 al., 2018), STS (ALX-380-014, Enzo Life Sciences, USA) was added to serum-free medium at
22 500 nM for 16 h. For oxidative stress-induced apoptosis, as we previously established with

1 minor modifications (Zheng et al., 2018), mitochondrial photodynamic activation was
 2 performed on AIEgen-stained cells in serum-free conditions with ultralow-power light
 3 irradiation at 0.7 mW/cm² for 6 h. To initiate apoptosis *via* the intrinsic pathway, as reported
 4 (Medina et al., 2020), the BH3-mimetic ABT-737 (T2099, TargetMol, USA) which directly
 5 induces permeabilization of the mitochondrial outer membrane was applied at 5 µM for 48 h.
 6 To initiate apoptosis *via* the extrinsic pathway, as previously reported (Medina et al., 2020), T
 7 cells were treated with the CD95/Fas antibody (152803, BioLegend, USA) at 5 µg/ml for
 8 Fas-crosslinking during the 48 h activation. Apoptosis was identified by TUNEL (G3250,
 9 Promega, USA) or Annexin V (BMS500FI-300, Invitrogen, USA) / 7AAD (559925, BD
 10 Pharmingen, USA) staining.

11

12 **Isolation, treatment and infusion of apoEVs**

13 ApoEVs were isolated from medium of apoptotic cells using a sequential centrifugation
 14 method followed by filtering, as we previously established with modifications (Liu et al., 2018).
 15 Briefly, after sequential centrifugation at 800 g for 10 min at 4°C and 2,000 g for 10 min at 4°C
 16 followed by filtering through 5 µm filters, apoptotic cell lysates and debris were removed. Then,
 17 apoEVs were pelleted by centrifugation at 16,000 g for 30 min at 4°C and were diluted in PBS.
 18 Quantification of apoEVs was performed using the nanoparticle tracking analysis (NTA) for
 19 particle numbers and the BCA method (23225, Thermo Scientific, USA) for protein amounts.
 20 For treatment of HCs, apoEVs were added to the culture medium at a protein concentration of
 21 10 µg/ml, with the dosage being determined according to preliminary tests. For intravenous
 22 infusion, apoEVs in PBS were injected *via* the mouse caudal vein at a protein concentration of

1 5 µg/g. For TeNT treatment of apoEVs to cleave VAMP3, TeNT (582243, Merck Millipore,
2 Germany) was added to the apoEV solution at 250 ng/ml and incubated at 37°C for 30 min.
3 ApoEVs were then centrifuged at 16,000 g for 30 min and washed twice with PBS before
4 resuspension.

6 **Labeling of apoEVs**

7 ApoEVs were labeled using multiple methods in this study. ⁶⁴Cu radionucleotide labeling of
8 apoEVs was performed based on modifying our previous method of labeling of liposomes
9 (Sun et al., 2017). Briefly, apoEVs were pelleted and resuspended in metal-free Na₂HPO₄
10 solution (pH = 7.5), into which NOTA-NHS ester (C100, CheMatech, France), a metal chelator,
11 was added at 150 nM and incubated at 4°C overnight. Purification of the conjugated apoEVs
12 was performed by centrifugation and washed with the Na₂HPO₄ solution. ⁶⁴Cu labeling was
13 performed by adding 20-30 mCi ⁶⁴CuCl₂ and the mixture was shaken at 37°C for 30 min
14 followed by purification. The labeling efficiency was confirmed by instant thin-layer
15 chromatography (ITLC) plates with citric acid (0.1 M, pH = 5) as an eluent. For labeling of
16 apoEVs by membrane dyes, either PKH26 (PKH26PCL, Sigma-Aldrich, USA) or CellMask™
17 Deep Red (C10046, Invitrogen, USA) was used according to the manufacturers' instructions.
18 For labeling of apoEVs by the AIEgen, DCPy was synthesized according to our established
19 protocol (Zheng et al., 2020). DCPy was then added to serum-free cell culture medium at a
20 final concentration of 5 µM and incubated for 30 min followed by washes. Cells were then
21 induced apoptosis by light irradiation, as stated above.

22

1 Isolation of Exosomes

2 Exosomes (Exos) were isolated from cultured un-apoptotic cells according to previous
3 protocols with minor modifications (Liu et al., 2015). Briefly, cells were cultured in
4 Exo-depleted medium (complete medium depleted of FBS-derived Exos by overnight
5 centrifugation at 120,000 g) for 48 h. Exos from culture supernatants were isolated by
6 differential centrifugation: 800 g for 10 min, 2,000 g for 10 min, 16,000 g for 30 min and
7 120,000 g for 120 min. Quantification of Exos was performed using the BCA method (23225,
8 Thermo Scientific, USA) for protein amounts.

9

10 Isolation, sorting, culture and ploidy transition of primary HCs

11 Isolation of primary mouse HCs was performed according to previous protocols with minor
12 modifications (Li et al., 2016). Briefly, mouse liver was perfused *via* catheterization of the
13 portal vein using a 24G needle catheter (381212, BD, USA) and a mini-pump machine
14 (13-876-2, Thermo Fisher, USA) under general anesthesia. The liver was perfused firstly with
15 10 ml Hank's balanced salt solution (HBSS) (14175-079, Invitrogen, USA) to remove blood
16 followed by 20 ml HBSS supplemented with 1 mM ethylene
17 glycolbis(aminoethylether)-tetra-acetic acid (EGTA) (E3889, Sigma-Aldrich, USA) to remove
18 the endogenous calcium. Then the liver was perfused with 20 ml HBSS supplemented with 5
19 mM CaCl_2 (223506, Sigma-Aldrich, USA) and 40 $\mu\text{g/ml}$ liberase TM (LIBTM-RO,
20 Sigma-Aldrich, USA) for digestion. All the solutions were kept at 37°C in a water bath. After
21 digestion, the liver was dissected and washed in ice-chilled HBSS, and cells were teased out
22 into high-glucose DMEM (11965-084, Invitrogen, USA) supplemented with 10% FBS (17L624,

1 Sigma-Aldrich, USA), 100 U/ml penicillin (15140-122, Invitrogen, USA) and 100 µg/ml
 2 streptomycin (15140-122, Invitrogen, USA). HCs were then prepared by centrifugation at 50 g
 3 for 5 min at 4°C, filtered through 70 µm nylon strainers, purified by a 49% Percoll solution
 4 (P4937, Sigma-Aldrich, USA), and resuspended in William's E Medium (WEM) with
 5 GlutaMAX™ (32551-087, Invitrogen, USA) containing 10% FBS (17L624, Sigma-Aldrich,
 6 USA), 10 nM dexamethasone (D4902, Invitrogen, USA), 100 U/ml penicillin (15140-122,
 7 Invitrogen, USA) and 100 µg/ml streptomycin (15140-122, Invitrogen, USA). HCs were then
 8 seeded onto collagen-coated dishes (354450, Corning, USA) or coverslips (72295-14,
 9 Electron Microscopy Sciences, USA) and incubated at 37°C in a humidified atmosphere of 5%
 10 CO₂ for 3 h for attachment.

11 For sorting of primary HCs, according to the published protocol (Duncan et al., 2010),
 12 isolated HCs before seeding were resuspended at 2×10⁶/ml for 30 min at 37°C in
 13 high-glucose DMEM (11965-084, Invitrogen, USA) supplemented with 10% FBS (17L624,
 14 Sigma-Aldrich, USA), 10 mM Hydroxyethyl piperazineethanesulfonic acid (HEPES) (H4034,
 15 Sigma-Aldrich, USA), 15 µg/ml Hoechst 33342 (H3570, Invitrogen, USA) and 5 µM reserpine
 16 (R0875, Sigma-Aldrich, USA). The HCs were then sorted for diploid and tetraploid HCs by
 17 flow cytometry and were collected in high-glucose DMEM (11965-084, Invitrogen, USA)
 18 supplemented with 50% FBS (17L624, Sigma-Aldrich, USA) and 10 mM HEPES (H4034,
 19 Sigma-Aldrich, USA). Sorted HCs were pelleted by centrifugation, resuspended and seeded
 20 in WEM with GlutaMAX™ (32551-087, Invitrogen, USA) containing 10% FBS (17L624,
 21 Sigma-Aldrich, USA), 10 nM dexamethasone (D4902, Invitrogen, USA), 100 U/ml penicillin
 22 (15140-122, Invitrogen, USA) and 100 µg/ml streptomycin (15140-122, Invitrogen, USA).

1 For apoEV uptake assays in HC culture, after attachment, HCs were washed twice with
2 HBSS (14175-079, Invitrogen, USA) and medium was changed to WEM with GlutaMAX™
3 (32551-087, Invitrogen, USA) containing 0.2% bovine serum albumin (BSA) (700-100P,
4 Gemini Bio, USA), 100 U/ml penicillin (15140-122, Invitrogen, USA) and 100 µg/ml
5 streptomycin (15140-122, Invitrogen, USA) for following experiments (Li et al., 2016). ApoEVs
6 were added at a protein concentration of 10 µg/ml for 24 h, during which 200 mM Gal (G5388,
7 Sigma-Aldrich, USA) together with 200 mM GalNAc (HY-33212, MedChemExpress, China),
8 or 5 µg/ml antibody for ASGPR (sc-166633, Santa Cruz Biotechnology, USA) or its isotype,
9 were added to inhibit sugar recognition, as stated previously with minor modifications
10 (McVicker et al., 2002).

11 Ploidy transition of cultured HCs was induced in a defined SUM3 medium (Duncan et al.,
12 2010). SUM3 medium was prepared with 75% high-glucose DMEM (11965-084, Invitrogen,
13 USA), 25% Waymouth's medium (11220-035, Invitrogen, USA), 2 mM L-glutamine
14 (35050-061, Invitrogen, USA), 100 U/ml penicillin (15140-122, Invitrogen, USA), 100 µg/ml
15 streptomycin (15140-122, Invitrogen, USA), 10 mM HEPES (H4034, Sigma-Aldrich, USA), 50
16 ng/ml epidermal growth factor (EGF) (E4127, Sigma-Aldrich, USA), 1 µg/ml insulin (I0516,
17 Sigma-Aldrich, USA), 30 nM sodium selenite (S5261, Sigma-Aldrich, USA), 10 µg/ml
18 transferrin (T8158, Sigma-Aldrich, USA), 50 ng/ml somatotropin (869008-M, Sigma-Aldrich,
19 USA) and 1 µM liothyronine (T₃) (16028, Cayman Chemical, USA). After attachment, sorted
20 4N-HCs were washed twice with HBSS and medium was changed to the SUM3 medium,
21 which was refreshed each day for 5 days. According to a protocol established through
22 previous observations (Duncan et al., 2010; Fortier et al., 2017), cytokinesis was analyzed at

72 h by counting the microtubule midbody events, and ploidy status was analyzed at 120 h after collection by trypsin (12605-010, Invitrogen, USA). ApoEVs were added at a protein concentration of 10 µg/ml, and BFA (11861, Cayman Chemical, USA) was applied at 1 nM.

4

5 **Isolation of liver NPCs, PBMNCs and BMMNCs**

6 For isolation of liver NPCs, after liberase perfusion and cell collection, liver cells were
7 centrifuged at 50 g for 5 min at 4°C to remove HCs, and the supernatant was centrifuged at
8 500 g for 5 min at 4°C to pellet NPCs. For PBMNC isolation, whole peripheral blood was
9 extracted from the mouse retro-orbital venous plexus, and cells were isolated by
10 centrifugation at 500 g for 5 min at 4°C followed by being treated with ACK Lysing Buffer
11 (10-548E, Lonza Bioscience, Switzerland) to remove red blood cells. After washing with PBS,
12 PBMNCs were collected by centrifugation at 500 g for 5 min at 4°C. For BMMNC isolation,
13 whole bone marrow cells were treated with ACK Lysing Buffer (10-548E, Lonza Bioscience,
14 Switzerland), washed with PBS, and collected by centrifugation at 500 g for 5 min at 4°C.

15

16 **Isolation of liver tissue EVs and quantification**

17 Liver tissue EV isolation was performed according to a previous protocol with modifications
18 (Ishiguro et al., 2019). Briefly, after liberase perfusion and tissue collection, HCs were
19 removed by centrifugation at 50 g for 5 min at 4°C, and the supernatant was centrifuged at
20 500 g for 5 min at 4°C to pellet NPCs. The supernatant of this step was further centrifuged at
21 2,000 g for 10 min at 4°C to remove tissue debris, filtered through 5 µm filters, and centrifuged
22 at 16,000 g for 30 min at 4°C to pellet EVs. Quantifications of liver tissue EVs were performed

using the NTA for particle numbers and apoEVs were detected by Annexin V-APC labeling (640920, BioLegend, USA).

Transfection of plasmids and siRNAs

Transfection of plasmids and siRNAs was performed according to our previous experience (Liu et al., 2018). A CellLight® Reagent based on a GALNT2 (Golgi-resident enzyme) plasmid for labeling of Golgi apparatus was used according to the manufacturer's instructions (C10592, Invitrogen, USA). The reagent was added to HCs at 5×10^6 particles/ml at the time of medium change after attachment for 24 h incubation. GRASP55-mCherry and GRASP55-EGFP plasmids were used as previously described (Xiang and Wang, 2010; Zhang et al., 2018c). GalT-mCherry (87327, Addgene, USA) and GalT-EGFP (11929, Addgene, USA) plasmids were purchased. HCs were transfected with GRASP and GalT plasmids at 1 µg/ml using the Lipofectamine™ LTX Reagent with PLUS™ Reagent (15338-100, Invitrogen, USA) at the time of medium change for 24 h incubation. siRNAs for mouse VAMP3 (sc-41339, Santa Cruz Biotechnology, USA) and VAMP4 (sc-61767, Santa Cruz Biotechnology, USA) were purchased, with non-targeting siRNA being used as the negative control. MSCs were incubated in Opti-MEM for 6 h (31985-070, Invitrogen, USA) and were transfected with siRNAs at 50 nM for 48 h using the Lipofectamine™ RNAiMAX Reagent (13778-100, Invitrogen, USA).

GalNAc and GalNAc-conjugated siRNA experiments

For *in vivo* experiments, GalNAc (HY-33212, MedChemExpress, China) was injected

1 subcutaneously at 500 mg/kg once per week. GalNAc-conjugated siRNAs were obtained from
2 Alnylam Pharmaceuticals, USA, as 10 mg/ml stocks for G-si*Luc* and G-si*Anln*, as previously
3 reported (Zhang et al., 2018a; Zhang et al., 2018b). The working concentrations of the
4 siRNAs were 4 mg/ml diluted in PBS, and they were subcutaneously injected at 4 mg/kg once
5 per week.

6

7 **PHx surgeries**

8 The PHx procedure was performed under sterile conditions by methods described previously
9 (Hori et al., 2011; Mitchell and Willenbring, 2008). Isoflurane inhalation (R500IP, RWD Life
10 Science, USA) was used to anesthetize the animals. For 70% PHx, the median and left lobes
11 of liver were resected. For 80% MHx, the median, left and right posterior lobes of the liver
12 were resected. Sham operation was performed by laparotomy.

13

14 **ALF modeling and therapy**

15 The mouse ALF model was established by APAP treatment, as stated previously
16 (Barbier-Torres et al., 2017). Mice were fasted overnight, and APAP (A3035, Sigma-Aldrich,
17 USA) was injected intraperitoneally at a lethal dose of 1 g/kg. NAC (A7250, Sigma-Aldrich,
18 USA) was also injected intraperitoneally at 1 g/kg at 8 h post to APAP challenge. Control mice
19 were injected with an equal amount of PBS.

20

21 **Parabiosis**

22 The parabiosis mouse model was established as previously stated (Liu et al., 2018). The mice

1 to be joined in parabiosis were anaesthetized and shaved along the opposite lateral flanks.
 2 Incisions were made on the corresponding lateral aspects from the olecranon to the knee joint
 3 of each mouse. The olecranon and knee joints were each attached by sutures, and the dorsal
 4 and ventral skins were sewed together with continuous suture. Each pair of parabiotic mice
 5 was kept in a single cage after the operation.

6

7 **Gross analyses**

8 Body weights of mice were recorded weekly and food intake was recorded daily. Liver weights
 9 were recorded after sacrifice and gross morphology was characterized. Fasting experiments
 10 were performed after a fasting of 16 h.

11

12 **Cage wheel running**

13 As previously reported (Pistilli et al., 2011), spontaneous activity of mice was monitored using
 14 a cage running wheel system (Columbus Instruments, USA). Computer software (RMCWin,
 15 Delta Computer Systems, USA) specific to the equipment was used to quantify the total
 16 number of wheel revolutions. Data were expressed as distance covered per hour.

17

18 **PET scanning and quantification of radiation intensity**

19 PET scans were performed using an Inveon microPET scanner (Siemens Medical Solutions,
 20 Germany), as we previously documented (Sun et al., 2017). Briefly, mice were anesthetized
 21 using isoflurane at indicated time points after intravenous injection of ⁶⁴Cu-labeled apoEVs
 22 and were scanned under PET. Reconstruction of PET images was done without correction for

1 attenuation or scatter using a 3D ordered subsets expectation maximization algorithm. Image
2 analysis was performed using ASI Pro VMTM software (Siemens Medical Solutions,
3 Germany). At 48 h, mice were sacrificed and multiple organs/tissues were dissected and
4 weighed for biodistribution analysis. The radiation intensity of ^{64}Cu signals was measured in a
5 well gamma-counter (Wallach Wizard, PerkinElmer, USA).

6

7 **Liver histology and IF staining**

8 At sacrifice, liver tissues were rapidly isolated and fixed overnight with 4% paraformaldehyde
9 (PFA) (150146, MP Biomedicals, USA). For histological analyses, samples were dehydrated
10 and embedded in paraffin, and 5 μm serial sections were prepared (RM2125, Leica,
11 Germany). Sections then underwent either H&E staining (3801698, Leica, Germany),
12 Masson's trichrome staining (HT15-1KT, Sigma-Aldrich, USA) or TUNEL (G3250, Promega,
13 USA) staining. For liver damage assessment, H&E staining sections were scored from 0-4 for
14 sinusoidal congestion, vacuolization of the HC cytoplasm and parenchymal necrosis,
15 according to Suzuki's classification (Suzuki et al., 1993). Liver inflammation was scored from
16 0-3 indicating absent, mild, moderate or severe mononuclear and polymorphonuclear
17 infiltrations (Sancho-Bru et al., 2007).

18 For IF staining, liver tissues were rapidly isolated, fixed in 4% PFA, cryoprotected with 30%
19 sucrose, and embedded in optimal cutting temperature (OCT) compound (4583, Sakura
20 Finetek, USA). The specimens were snap-frozen and sectioned into 10 μm sagittal sections
21 (CM1950, Leica, Germany). For F-actin staining of HC borders, as we previously stated
22 (Wang et al., 2014), sections were probed with phalloidin conjugated to either AlexaFluor-488

1 or Rhodamine (R37112/R37110, Invitrogen, USA) according to the manufacturer's
2 instructions, and counterstained with DAPI (ab104139, Abcam, UK). Percentages of
3 binucleated and mononucleated HCs were quantified using ImageJ 1.47 software (National
4 Institute of Health, USA) from at least four microscopic fields. For staining of Golgi apparatus
5 or Kupffer cells, sections were treated with 0.3% Triton X-100 (X100-100ML, Sigma-Aldrich,
6 USA) diluted in PBS for 20 min at room temperature, blocked with 5% BSA (700-100P, Gemini
7 Bio, USA) dissolved in PBS for 1 h at room temperature, and stained with a rabbit anti-mouse
8 Golgin84/Golga5 primary antibody (NBP1-83352, Novus Biologicals, USA) or a rabbit
9 anti-mouse F4/80 primary antibody (ab6640, Abcam, UK) overnight at 4°C at a concentration
10 of 1:100. After washing with PBS, sections were then stained with an Alexa Fluor
11 488-conjugated goat anti-rabbit secondary antibody (A-11008, Invitrogen, USA) for 1 h at
12 room temperature at a concentration of 1:200, and counterstained with DAPI (ab104139,
13 Abcam, UK).

14

15 **Serum aminotransferase analyses and enzyme-linked immunosorbent assay (ELISA)**

16 At sacrifice, whole peripheral blood was extracted from the mouse retro-orbital venous plexus,
17 and serum was isolated by centrifugation at 3,000 g for 15 min at 4°C followed by
18 centrifugation at 12,000 g for 15 min at 4°C. Concentrations of ALT and AST were determined
19 using general chemistry kits (A7526-150 and A7561-150, Pointe Scientific, USA) according to
20 the manufacturer's instructions. Concentrations of tumor necrosis factor-alpha (TNF-α) and
21 interleukin-6 (IL-6) were determined using ELISA kits (430907 and 431307, BioLegend, USA)
22 according to the manufacturer's instructions.

1

2 **Ploidy analyses by PI staining**

3 For detection of ploidy (Zhang et al., 2018b), freshly isolated primary HCs or cultured HCs
4 collected after ploidy transition were fixed at 2×10^6 /ml in 75% ethanol overnight at -20°C. Cells
5 were then incubated with 500 µl of PI/Rnase Staining Buffer (550825, BD Pharmingen, USA)
6 at room temperature for 15 min and analyzed by flow cytometry.

7

8 **IF staining of cells and apoEVs**

9 For IF staining of cytoskeleton and organelles (Joseph et al., 2008; Kolobova et al., 2017),
10 cultured HCs were fixed at 37°C for 15 min with 4% PFA dissolved in the microtubule
11 stabilizing buffer (MTSB), which was composed of 50 mM piperazine-1,4-bisethanesulfonic
12 acid (PIPES) (190257, MP Biomedicals, USA), 5 mM EGTA (E3889, Sigma-Aldrich, USA) and
13 5 mM MgSO_4 (13142-100G, Honeywell, USA) in distilled water (pH = 7.0). After washing with
14 MTSB, cells were permeabilized with ice-cold methanol for 10 min, washed with MTSB, and
15 blocked with 5% BSA (700-100P, Gemini Bio, USA) in MTSB for 1 h at room temperature.
16 Cells were then stained with a mouse anti-mouse GM130 primary antibody (610822, BD
17 Transduction Laboratories, USA) alone or together with a rat anti-mouse α -tubulin primary
18 antibody (MA1-80017, Invitrogen, USA) or a rabbit anti-mouse ac- α -tubulin (acetyl K40)
19 primary antibody (ab179484, Abcam, UK) overnight at 4°C at a concentration of 1:100 in
20 MTSB. After washing with MTSB, cells were then stained with Alexa Fluor 488-conjugated
21 goat anti-mouse secondary antibody (A-11001, Invitrogen, USA) alone or together with Alexa
22 Fluor 647-conjugated goat anti-rat secondary antibody (A-21247, Invitrogen, USA) or Alexa

1 Fluor 647-conjugated goat anti-rabbit secondary antibody (A-21245, Invitrogen, USA) for 1 h
2 at room temperature at a concentration of 1:200 in MTSB, and counterstained by DAPI
3 (ab104139, Abcam, UK).

4 For IF staining of surface markers of apoEVs, after isolation, apoEVs were stained with
5 rabbit anti-human/mouse VAMP3 primary antibody (13640, Cell Signaling Technology, USA),
6 rabbit anti-mouse VAMP4 primary antibody (PA1-768, Invitrogen, USA), mouse anti-mouse
7 GS15 primary antibody (610960, BD Transduction Laboratories, UK), rabbit anti-mouse C1q
8 primary antibody (ab71940, Abcam, UK), or mouse anti-mouse TSP1 primary antibody
9 (sc-59887, Santa Cruz Biotechnology, USA) at 4°C for 1 h at a concentration of 1:100 in PBS.
10 After centrifugation and washing with PBS, apoEVs were then stained with FITC-conjugated
11 goat anti-mouse secondary antibody (405305, BioLegend, USA) or FITC-conjugated donkey
12 anti-rabbit secondary antibody (406403, BioLegend, USA) for 1 h at 4°C at a concentration of
13 1:200 in PBS, and counterstained with CellMask™ Deep Red (C10046, Invitrogen, USA).

14

Annexin V and lectin binding assay

16 Exposure of PtdSer on apoEVs was detected by FITC-conjugated Annexin V (640906,
17 BioLegend, USA) binding at a concentration of 1:100 according to the manufacturer's
18 instructions and counterstained with CellMask™ Deep Red (C10046, Invitrogen, USA).
19 Exposure of Gal or GalNAc on apoEVs, Exos and MSCs was respectively detected by
20 FITC-conjugated peanut lectin (L7381, Sigma-Aldrich, USA) or Alexa Fluor 488-conjugated
21 soybean lectin (L11272, Invitrogen, USA) binding both at a concentration of 1:100 for 1 h at
22 4°C, followed by counterstaining with CellMask™ Deep Red for membrane labeling (C10046,

1 Invitrogen, USA). Fluorescence intensity was determined using a microplate reader (Synergy
2 H1, Bio-Tek, USA) with Gen5 software (Bio-Tek, USA) at 488/517 nm for lectins and 649/666
3 nm for CellMask™ Deep Red.

4

5 **Image acquisition and analyses**

6 Bright-field images of cell morphology, liver H&E, Masson's and TUNEL staining, and
7 fluorescent images of liver engraftment of apoEVs, apoEV uptake *in vitro* and marker
8 expression were obtained using an inverted microscope (Axio Observer 5, Zeiss, Germany).
9 Fluorescent images of liver ploidy, apoEV uptake *in vivo*, Golgi and microtubule analyses
10 were obtained using confocal microscopes (SP5-II, Leica, Germany; or LSM 900, Zeiss,
11 Germany). Z-stacks of images were scanned at high resolution and were processed and
12 reconstructed in 3 dimensions. Volocity software (PerkinElmer, USA) was applied for 3D
13 analysis of apoEV interactions with the Golgi. Quantification of images was carried out with
14 the ImageJ 1.47 software (National Institutes of Health, USA).

15

16 **Flow cytometry**

17 Sorting of HCs was performed using the MoFlo Astrios EQ Cell Sorter (Beckman Coulter,
18 USA) with a 150 µm nozzle. Doublets were eliminated on the basis of pulse width, and ploidy
19 populations were identified by Hoechst 33342-stained DNA contents using an ultraviolet 355
20 nm laser and a 425-440 nm bandpass filter. Ploidy analyses of HCs after PI staining were
21 evaluated using a FACSCalibur Flow Cytometer (BD Biosciences, USA). Analyses of apoEV
22 uptake efficacy, cellular apoptotic rates and apoEV marker expression were performed using

1 a NovoCyte Flow Cytometer (ACEA Biosciences, USA). CellMask™ Deep Red staining was
2 used to eliminate background noise signals when analyzing apoEVs. Flow cytometric data
3 were processed using FlowJo v10 software (FlowJo LLC, USA).

4

5 **Golgi isolation**

6 Golgi apparatus was isolated from mouse liver or cultured HCs by sequential sucrose
7 gradients using a Golgi Isolation Kit (GL0010-1KT, Sigma-Aldrich, USA), based on the
8 protocol we previously established (Tang and Wang, 2015). Isolated Golgi proteins were
9 extracted and the protein mass was determined using the BCA method (23225, Thermo
10 Scientific, USA).

11

12 **Transmission electron microscopy (TEM) analyses**

13 Cultured HCs were collected by trypsin (12605-010, Invitrogen, USA) after 96 h in the ploidy
14 transition protocol. HCs were then fixed with 2.5% glutaraldehyde (G5882, Sigma-Aldrich,
15 USA) and 2.0% PFA (150146, Sigma-Aldrich, USA) solution in 0.1 M sodium cacodylate
16 buffer (pH =7.4) (C0250, Sigma-Aldrich, USA) overnight at 4°C. After subsequent washes, the
17 samples were post-fixed in 2.0% osmium tetroxide (75633-2ML, Sigma-Aldrich, USA) for 1 h
18 at room temperature, and rinsed in distilled water prior to *en bloc* staining with 2% uranyl
19 acetate (22400, Electron Microscopy Sciences, USA). After dehydration through a graded
20 ethanol series, the specimens were infiltrated and embedded in the EMbed-812 resin (14120,
21 Electron Microscopy Sciences, USA). Thin sections were stained with uranyl acetate and lead
22 citrate (22410, Electron Microscopy Sciences, USA), and examined with a JEM-1010 electron

1 microscope (JEOL, Japan) fitted with a digital camera (Hamamatsu Photonics, Japan) and
2 image capture software (AMT Imaging, USA). Interphase 4N-HCs containing a binucleated
3 profile with an intact nuclear envelope were selected to quantify distinguishable Golgi stacks
4 per cell. Golgi stacks were identified using morphological criteria and were quantified using
5 standard stereological techniques, as we previously stated (Souter et al., 1993; Xiang and
6 Wang, 2010).

7 TEM analyses of apoEVs and liver tissue EVs were performed on collected pellets diluted
8 in PBS. Diluted EVs were fixed with 1% glutaraldehyde (G5882, Sigma-Aldrich, USA) for 30
9 min at 4°C, absorbed onto glow-discharged 300-mesh heavy-duty carbon-coated formvar
10 copper grids (ProSciTech, Australia) for 5 min, and the excess liquid was blotted on filter
11 papers. Grids were washed twice with distilled water and negatively stained with 2.5% uranyl
12 acetate (22400, Electron Microscopy Sciences, USA). Wide-field images encompassing
13 multiple vesicles were taken on a JEM-1200EX electron microscope (JEOL, Japan).

14

15 **NTA analyses**

16 The size distribution of apoEVs and liver tissue EVs was measured using NanoSight NS300
17 (Malvern Panalytical, UK) and analyzed with NTA software (Malvern Panalytical, UK).
18 Quantification of liver tissue EVs was also performed using ZetaView instrument S/N 19-447
19 (Particle Metrix, Germany) and analyzed with ZetaView analysis software (Particle Metrix,
20 Germany).

21

22 **RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)**

1 Total RNA was isolated from freshly isolated or cultured cells using the Aurum Total RNA Mini
2 Kit (732-6820, Bio-Rad, USA) according to the manufacturer's instructions. cDNA was
3 synthesized using the Reverse Transcription Kit (4368814, Applied Biosystems, USA).
4 qRT-PCR was performed using SYBR Green Master Mix (B21202, Bimake, USA) on the
5 Real-Time PCR Systems (CFX96, Bio-Rad, USA; or LightCycler 96, Roche Life Science,
6 Swiss). The primers used in this study are listed in Table S2. β -actin was used as the internal
7 control.

8

9 **Western blot assay**

10 Western blot assay was performed on cultured MSCs or HCs, isolated apoEVs or liver tissue
11 EVs, and isolated Golgi after lysis in the RIPA Lysis Buffer System with protease and
12 phosphatase inhibitors (sc-24948, Santa Cruz Biotechnology, USA). Protein levels were
13 quantified using the Pierce™ BCA Protein Assay Kit (23225, Thermo Scientific, USA). A total
14 of 20 μ g protein was separated in the 4%-12% NuPAGE gel (NP0321BOX/NP0322BOX,
15 Invitrogen, USA) and was transferred to 0.2 μ m nitrocellulose membranes (Millipore, USA).
16 The membranes were then blocked with 5% BSA (700-100P, Gemini Bio, USA) at room
17 temperature for 1 h, followed by incubation overnight at 4°C with the following primary
18 antibodies: antibodies to GRASP55 (sc-271840), TGN38 (sc-166594), CD9 (sc-13118), CD63
19 (sc-5275), CD81 (sc-70803), INCENP (sc-376514), RacGAP1 (sc-166477) and RhoA (sc-418)
20 were purchased from Santa Cruz Biotechnology, USA, and were used at concentrations of
21 1:200; antibodies to Stx5 (14151), Caspase 3 (9662), Cleaved caspase-3 (9664), VAMP3
22 (13640) and GAPDH (5174) were obtained from Cell Signaling Technology, USA, and were

1 used at concentrations of 1:1000; the antibody to GM130 (610822) was purchased from BD
 2 Transduction Laboratories, USA, and was used at a concentration of 1:1000; the antibody to
 3 Golgin84 (NBP1-83352) was purchased from Novus Biologicals, USA, and was used at a
 4 concentration of 1:1000; antibodies to α -tubulin (MA1-80017) and VAMP4 (PA1-768) were
 5 purchased from Invitrogen, USA, and were used at concentrations of 1:1000; antibodies to
 6 ac- α -tubulin (ab179484), Lamin B1 (ab133741) and hVAMP3 (ab200657) were purchased
 7 from Abcam, UK, and were used at concentrations of 1:1000; the antibody to MKLP1/KIF23
 8 (DF2573) was purchased from Affinity Biosciences, China, and was used at a concentration of
 9 1:500; antibodies to GFP (SAB2702197) and β -actin (A5441) were purchased from
 10 Sigma-Aldrich, USA, and were used at concentrations of 1:1000. The membranes were then
 11 washed and incubated for 1 h at room temperature with horseradish peroxidase
 12 (HRP)-conjugated secondary antibodies (sc-516102/sc-2357, Santa Cruz Biotechnology,
 13 USA; or 7077, Cell Signaling Technology, USA). Immunoreactive proteins were detected
 14 using the SuperSignal™ West Pico PLUS Chemiluminescent Substrate (34580, Thermo
 15 Scientific, USA), the SuperSignal™ West Femto Maximum Sensitivity Substrate (34095,
 16 Thermo Scientific, USA), and the Autoradiography Film (LabScientific, USA) or the ChemiDoc
 17 MP Imaging System (Bio-Rad, USA).

18

19 **Oxygen consumption assessment**

20 Mitochondrial OXPHOS activity was measured using Mito-ID® O₂ Extracellular Sensor Kit
 21 (ENZ-51044, Enzo Life Sciences, USA) according to the manufacturer's protocol, in which
 22 oxygen consumption was detected based on a fluorescent probe. Briefly, 10 μ L probe was

1 added to cultured HCs, and the fluorescence intensity was read immediately using the
2 microplate reader (Synergy H1, Bio-Tek, USA) with the Gen5 software (Bio-Tek, USA) at
3 380/650 nm kinetically for 90 min, at an interval of 1 min. The mean value of fluorescence
4 intensity of 3 wells per group was determined for the oxygen consumption curves (Lv et al.,
5 2018).

6

7 **ROS determination**

8 Total intracellular ROS contents of cultured HCs were measured using the fluorescent probe
9 2',7'-dichlorofluorescein diacetate (DCFDA) (ab113851, Abcam, UK) (Lv et al., 2018). Briefly,
10 25 mM DCFDA was added to cells and was incubated at 37°C for 30 min. Fluorescence
11 intensity was determined using a microplate reader (Synergy H1, Bio-Tek, USA) with the
12 Gen5 software (Bio-Tek, USA) with an excitation at 488 nm.

13

14 **QUANTIFICATION AND STATISTICAL ANALYSIS**

15 Data are represented as the mean $\pm$ standard deviation (SD) unless otherwise indicated.
16 Statistical significance was evaluated by two-tailed Student's *t* test for two-group comparison,
17 or by one-way analysis of variation (ANOVA) followed by Newman-Keuls post-hoc tests for
18 multiple comparisons using Prism 5.01 software (GraphPad, USA). Log-rank tests were used
19 for Kaplan-Meier survival curve comparisons. Values of *P* < 0.05 were considered statistically
20 significant.

21

22 **MATERIALS AVAILABILITY**

1 This study did not generate new unique reagents.

2

3 **DATA AND CODE AVAILABILITY**

4 This study did not generate/analyze [datasets/code].

5

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1 **Supplementary figure legends**

2 **Figure S1. Identification of extracellular vesicles (EVs) and apoptosis-deficient mouse**

3 **models used in this study. (A)** Schematic diagram demonstrating protocol for isolation of

4 staurosporine (STS)-induced apoptotic extracellular vesicles (apoEVs). **(B)** Representative

5 bright field images showing morphologies of cultured mesenchymal stem cells (MSCs)

6 without or with STS treatment. Ctrl, control. Scale bars = 50 μ m. **(C)** Representative

7 fluorescent images showing terminal deoxynucleotidyl transferase dUTP nick end labeling

8 (TUNEL, green) staining of apoptotic MSCs counterstained with DAPI (blue). Scale bars = 50

9 μ m. **(D)** BCA analysis of the protein yield of apoEVs and exosomes (Exos) from 10^6 MSCs. *N*

10 = 3 per group. **(E)** Representative transmission electron microscopy (TEM) image showing

11 the morphologies of apoEVs. Scale bar = 100 nm. **(F)** Nanosight analysis showing diameters

12 of apoEVs. **(G-I)** Representative fluorescent images show apoEV (labeled by CellMask™

13 Deep Red, red) surface exposure of phosphatidylserine (PtdSer, Annexin V labeling) and

14 expression of complement component 1q (C1q) and thrombospondin 1 (TSP1) (green). Flow

15 cytometric analysis determined percentages of positively stained apoEVs. Scale bars = 25 μ m.

16 **(J)** Western blot showing marker expression in apoEVs and source MSCs. **(K)** Flow

17 cytometric analysis showed apoptosis of peripheral blood mononucleated cells (PBMNCs).

18 PBMNCs from wild-type (WT), *Fas* mutant (*Fas^{mut}*) or *Caspase-3* knockout (*Casp3^{-/-}*) mice,

19 staining with Annexin V and 7AAD. Percentages of Annexin V⁺ cells were quantified as

20 apoptotic rates. ND, not detected. *N* = 3 per group. **(L)** Flow cytometric analysis showed

21 apoptosis of bone marrow mononucleated cells (BMMNCs). BMMNCs from WT, *Fas^{mut}* or

22 *Casp3^{-/-}* mice were stained with Annexin V and 7AAD. Percentages of Annexin V⁺ cells were

1 quantified as apoptotic rates. $N = 3$ per group. **(M)** Flow cytometric analysis showed
 2 apoptosis of liver non-parenchymal cells (NPCs). NPCs from WT, *Fas^{mut}* or *Casp3^{-/-}* mice were
 3 stained with Annexin V and 7AAD. Percentages of Annexin V⁺ cells were quantified as
 4 apoptotic rates. $N = 3$ per group. **(N)** Schematic diagram demonstrating the liver cell and EV
 5 isolation protocol. HCs, hepatocytes. **(O)** Representative TEM image showing the
 6 morphologies of liver EVs. Scale bar = 50 nm. **(P)** Nanosight analysis showing diameters of
 7 liver EVs. **(Q)** Western blot showing marker expression in liver EVs and HCs. Data represent
 8 mean $\pm$ standard deviation. ***, $P < 0.0001$. Statistical analyses were performed by one-way
 9 analysis of variance followed by the Newman-Keuls post hoc tests.

10

11 **Figure S2. Phenotypic profiles of *Fas* mutant (*Fas^{mut}*) mice after apoEV infusion. (A)**
 12 Gross view images of 16-week-old mice and quantification of body weight (BW). WT, wild type.
 13 Scale bars = 1 cm. $N = 6$ per group. ***, $P < 0.0001$, *Fas^{mut}* mice compared to WT mice; NS,
 14 not significant, $P > 0.05$, *Fas^{mut}* mice after apoEV infusion compared to *Fas^{mut}* mice. **(B)**
 15 Quantification of food intake of 16-week-old mice. NS, not significant, $P > 0.05$. **(C)**
 16 Spontaneous activity of 16-week-old mice was assessed by running distance per hour using a
 17 cage running wheel system. Gray area indicates dark phase. $N = 3$ per group. **(D)**
 18 Enzyme-linked immunosorbent assay (ELISA) detected serum levels of tumor necrosis
 19 factor-alpha (TNF- α) and interleukin-6 (IL-6) in 16-week-old mice. NS, not significant, $P > 0.05$.
 20 $N = 6$ per group. **(E)** ZetaView analysis showing liver tissue extracellular vesicles (EVs) and
 21 corresponding quantification. NS, not significant, $P > 0.05$. $N = 3$ per group. **(F)** Masson's
 22 trichrome staining showing liver tissue and corresponding quantification of fibrotic area

1 percentages. Scale bars = 50 μ m. NS, not significant, $P > 0.05$. $N = 6$ per group. **(G)** Hepatic
2 inflammation scores were examined based on pathological parameters. NS, not significant, $P >$
3 0.05. $N = 6$ per group. **(H)** Tracing of PKH26-labeled (for membrane) or aggregation-induced
4 emission luminogen (AIEgen, for mitochondria)-labeled apoEVs (red) in liver, with Kupffer cell
5 membranes stained with F4/80 (green) and counterstaining with DAPI for DNA (blue).
6 ApoEVs were intravenously injected and liver samples were collected at 24 h after the
7 infusion. Representative confocal images showed macrophages. Scale bars = 5 μ m. Data
8 represent mean $\pm$ standard deviation. Statistical analyses were performed by one-way
9 analysis of variance followed by the Newman-Keuls post hoc tests.

10

11 **Figure S3. Caspase-3 knockout (*Casp3*^{-/-}) mice demonstrate hepatic disorders rescued**
12 **by apoEV infusion. (A)** Schematic diagram demonstrates the study design of apoEV infusion
13 in *Casp3*^{-/-} mice. ApoEVs were infused intravenously (*i.v.*) at a protein concentration of 5 μ g/g.
14 **(B)** ZetaView analysis showing liver tissue extracellular vesicles (EVs) and corresponding
15 quantification. WT, wild type. $N = 3$ per group. **(C)** Flow cytometric analysis showing Annexin
16 V⁺ liver tissue EVs (apoEVs) with corresponding quantification. $N = 3$ per group. **(D)**
17 Hematoxylin and eosin (H&E) staining of liver tissues in periportal vein (PV) area. Hepatic
18 injury scores were examined based on pathological parameters. Scale bars = 50 μ m. $N = 4$
19 per group. **(E)** Serum alanine aminotransferase (ALT) levels were determined. $N = 4$ per
20 group. **(F)** Representative liver fluorescent images show hepatocytes (HCs) with different
21 nuclei (blue, DAPI for DNA) and their cell borders (green, phalloidin for F-actin). # indicates
22 binucleated HCs. Percentages of binucleated HCs were accordingly quantified. Scale bars =

1 25 μm . $N = 4$ per group. **(G)** Diploid HCs (2N-HCs) were analyzed by flow cytometry after
 2 propidium iodide (PI) staining. $N = 4$ per group. **(H)** Tracing of PKH26-labeled apoEVs (red) in
 3 sorted tetraploid HCs (4N-HCs), with Golgi apparatus being demonstrated by a plasmid of
 4 *N*-acetylgalactosaminyltransferase 2 (GALNT2)-GFP (green) and counterstaining by DAPI for
 5 DNA (blue). Imaging analysis was performed to quantify HC percentages with Golgi
 6 fragmentation. Scale bars = 5 μm . $N = 4$ per group. **(I)** Golgi apparatus was isolated from
 7 cultured HCs and Golgi protein mass was determined by using the BCA method. $N = 4$ per
 8 group. Data represent mean $\pm$ standard deviation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$;
 9 NS, not significant, $P > 0.05$. Statistical analyses were performed by one-way analysis of
 10 variance followed by the Newman-Keuls post hoc tests.

11

12 **Figure S4. Tetraploid hepatocytes (4N-HCs) are weaker in metabolic activity and more**
 13 **prone to oxidative stress than diploid hepatocytes (2N-HCs).** **(A)** Flow cytometric sorting
 14 of primary mouse HCs based on Hoechst 33342 staining. Representative bright field images
 15 showing morphologies of 2N- and 4N-HCs. Scale bars = 10 μm . **(B)** Oxidative
 16 phosphorylation (OXPHOS) metabolic activities of sorted HCs were analyzed by oxygen
 17 consumption kinetics. WT, wild type; *Fas*^{mut}, *Fas* mutant; apoEVs, apoptotic extracellular
 18 vesicles. $N = 3$ per group. Curves are depicted with mean values. **(C)** Intensity of cellular
 19 reactive oxygen species (ROS) in sorted HCs were analyzed by the 2',7'-dichlorofluorescein
 20 diacetate (DCFDA) probe. $N = 3$ per group. Data represent mean $\pm$ standard deviation. **(D)**
 21 Quantitative real-time polymerase chain reaction (qRT-PCR) showed mRNA expression of
 22 multiple antioxidants in sorted HCs normalized to β -actin. Nfe2l2, nuclear factor erythroid

1 2-related factor 2; Sod2, superoxide dismutase 2; Cat, catalase; Nqo1, NAD(P)H quinone
2 dehydrogenase 1; Gclc, glutamate-cysteine ligase catalytic subunit; Gpx1, glutathione
3 peroxidase 1; Gsr, glutathione reductase. $N = 3$ per group. Data represent mean $\pm$ standard
4 deviation. **(E)** OXPHOS metabolic activities of unsorted primary HCs were analyzed based on
5 oxygen consumption kinetics. $N = 3$ per group. Curves are depicted with mean values. **(F)**
6 Intensity of cellular ROS in unsorted primary HCs was determined by the DCFDA probe. $N = 3$
7 per group. Data represent mean $\pm$ standard deviation. **, $P < 0.01$; ***, $P < 0.0001$. Statistical
8 analyses were performed by Student's t test for two-group analysis or one-way analysis of
9 variance followed by the Newman-Keuls post hoc tests for multiple group comparisons.

10

11 **Figure S5. Circulating extracellular vesicles (EVs) in parabiosis mice contribute to liver**
12 **homeostasis. (A)** Schematic diagram demonstrates the study design of parabiosis using *Fas*
13 mutant (*Fas^{mut}*) mice and green fluorescent protein (GFP) transgenic (*GFP^{+/+}*) mice.
14 *N*-acetylgalactosamine (GalNAc) was injected subcutaneously (s.c.) at 500 mg/kg once per
15 week for inhibiting hepatocyte (HC) uptake of apoEVs. **(B)** Tracing of GFP-labeled particles
16 (green) in liver of *Fas^{mut}* mice, with HC cell borders being stained with phalloidin for F-actin
17 (red) and counterstaining with DAPI for DNA (blue). Representative confocal images show
18 binucleated HCs. Scale bar = 10 μ m. **(C)** Hematoxylin and eosin (H&E) staining of liver
19 tissues in periportal vein (PV) area. Hepatic injury scores were examined based on
20 pathological parameters. Scale bars = 50 μ m. $N = 4$ per group. **(D)** Serum alanine
21 aminotransferase (ALT) levels were determined. $N = 4$ per group. **(E)** Representative liver
22 fluorescent images show HCs with different numbers of nuclei (blue, DAPI for DNA) and their

1 cell borders (green, phalloidin for F-actin). # indicates binucleated HCs. Percentages of
2 binucleated HCs were accordingly quantified. Scale bars = 25 μ m. $N = 4$ per group. **(F)** Diploid
3 HCs (2N-HCs) were analyzed by flow cytometry after propidium iodide (PI) staining. $N = 4$ per
4 group. **(G)** Representative Golgin84 immunofluorescent staining (green) images of liver Golgi
5 counterstained with DAPI (blue). Imaging analysis was performed to quantify Golgi area
6 percentages. Scale bars = 5 μ m. $N = 4$ per group. **(H)** Golgi apparatus was isolated from liver
7 and Golgi protein mass was determined using the BCA method. $N = 4$ per group. Data
8 represent mean $\pm$ standard deviation. **, $P < 0.01$; ***, $P < 0.0001$. Statistical analyses were
9 performed by one-way analysis of variance followed by the Newman-Keuls post hoc tests.

10

11 **Figure S6. ApoEVs are characterized by a general combined surface signature for liver**
12 **regulation. (A-C)** Flow cytometric analyses were performed to determine percentages of
13 positively stained apoEVs by Annexin V for surface exposure of phosphatidylserine (PtdSer),
14 by FITC-conjugated lectins for surface exposure of galactose (Gal) and
15 *N*-acetylgalactosamine (GalNAc), and by a specific antibody for surface expression of
16 vesicle-associated membrane protein 3 (VAMP3). The general apoptotic vesicular signature
17 (AVS) was identified with apoEVs from B-cell lymphoma-2 (Bcl-2) homology domain 3
18 (BH3)-mimetic ABT-737-induced apoptosis (*via* the intrinsic pathway) of human mesenchymal
19 stem cells derived from the umbilical cord (hMSCs, **A**), anti-Fas-induced apoptosis (*via* the
20 extrinsic pathway) of activated mouse T lymphocytes (**B**), and staurosporine (STS)-induced
21 apoptosis of the mouse fibroblast cell line NIH/3T3 (**C**). **(D)** Graphical summary demonstrates
22 the main findings of this study. ApoEVs were characterized by the specific AVS and engrafted

1 in liver. ApoEVs are recognized and uptaken by hepatocytes (HCs) through the
 2 asialoglycoprotein receptor (ASGPR), in which they assemble with the Golgi apparatus to
 3 form a chimeric ApoEV-Golgi Complex (AGC) based on the soluble
 4 *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) mechanism. ApoEV
 5 uptake and AGC assembly activate downstream functional cascades including microtubule
 6 acetylation and cytokinesis of HCs, which are indispensable for ploidy transition of HCs to
 7 safeguard diploid (2N) rather than tetraploid (4N) HC populations. Ultimately, apoEVs are of
 8 functional significance for maintaining liver homeostasis, promoting liver regeneration and
 9 mediating therapy for acute liver failure.

10

1 **Supplementary tables** (For Table S1, see the Key Resources Table)

2 **Table S2. Primers used in this study.**

Mouse genes	Primer sequences
<i>Actb</i> (β -actin)	Forward: 5'-AGCGGTTCCGATGCCCT-3' Reverse: 5'-TTGGCATAGAGGTCTTTACGGATG-3'
<i>Anln</i>	Forward: 5'-CTGAACTCAGGATCCGAAGCAT-3' Reverse: 5'-ACTGGAGTAGCTGAGGCATT-3'
<i>Nfe2l2</i>	Forward: 5'-GGACATGGAGCAAGTTTGGC-3' Reverse: 5'-CCAGCGAGGAGATCGATGAG-3'
<i>Sod2</i>	Forward: 5'-GCCTGCTCTAATCAGGACCC-3' Reverse: 5'-GTAGTAAGCGTGCTCCCACA-3'
<i>Cat</i>	Forward: 5'-CACTGACGAGATGGCACA-3' Reverse: 5'-TGTGGAGAATCGAACGGCAA-3'
<i>Nqo1</i>	Forward: 5'-CGCCTGAGCCCAGATATTGT-3' Reverse: 5'-GCACTCTCTCAAACCAGCCT-3'
<i>Gclc</i>	Forward: 5'-CACATCTACCACGCAGTCAA-3' Reverse: 5'-GGATGGTTGGGGTTTGTCTC-3'
<i>Gpx1</i>	Forward: 5'-CTCGGTTTCCCGTGCAATCA-3' Reverse: 5'-GGTCGGACGTACTTGAGGGA-3'
<i>Gsr</i>	Forward: 5'-CGTCTATGCTGTGGGAGACG-3' Reverse: 5'-GGCTGAAGACCACAGTAGGG-3'