Supplementary Figures

Synchronized necrotic death of attached hepatocytes mediated via gap junctions

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Methods

Chemicals

Allyl alcohol was purchased from Sigma (Tokyo, Japan). A glutathione cell-based detection kit (blue fluorescence) was obtained from Cayman Chemical Company (Ann Arbor, MI USA). Staurosporine was purchased from Wako (Osaka, Japan) and Calcein AM was from Life Technologies (Carlsbad, CA USA). siRNA for Cx26 was obtained from Bonac Corporation (Fukuoka, Japan): Cx26 siRNA1, 5’-CCGGAGACAUGAAAAGAAA-3’

Supplementary Figure 1.

Allyl alcohol, but not staurosporine, caused synchronized death of attached hepatocytes

Primary cultured murine hepatocytes were exposed to (a) 24 μM allyl alcohol or (b) 10 μM staurosporine. TMRM was used as an indicator of cell death. Images were obtained every 10 min until 1,500 min by time-lapse microscopy. For cell couplets, the time of death of one cell in the couplet was plotted on the X axis and that of the other cell on the Y axis.

Supplementary Figure 2.

Knockdown of Cx26 expression by Cx26 siRNA1

(a) Hepatocytes prepared from WT and Cx32 KO mice were incubated with 100 nM control (NT) siRNA or Cx26 siRNA1 (si1) for 13 h. Then Cx26 and GAPDH were detected by western blotting. Full-length blots are presented in Supplementary Figure 6c d.

(b) Hepatocytes were obtained from Cx32 KO mice, and then incubated with either (b) 100 nM control (NT) siRNA or (c) Cx26 siRNA1 for 13 h. After exposure to 10 mM APAP, images were obtained every 10 min until 1,500 min by time-lapse

microscopy. The data were plotted as described for Supplementary Figure 1.

Supplementary Figure 3.

Knockdown of Cx26 expression by Cx26 siRNA in cocultures of female and male hepatocytes

Hepatocytes from male WT and Cx32 KO mice were plated into dishes for 3h, after which female WT and Cx32 KO hepatocytes together with control (NT) siRNA or Cx26 siRNA (Si) were added to the dishes. After 13 h, Cx26 and GAPDH were detected by western blotting. Full-length blots are presented in Supplementary Figure 6e f.

Supplementary Figure 4.

Formation of gap junctions between attached male and female hepatocytes.

Hepatocytes obtained from male WT mice were plated into a dish and incubated for 3h, followed by staining with Calcein AM, a fluorescent dye. After removing free dye, unstained female hepatocytes were added to the same dish and images were obtained every 10 min by time-lapse microscopy. Representative images obtained at

the start of coculture (0 min) and 250 min after addition of unstained hepatocytes are shown.

Supplementary Figure 5.

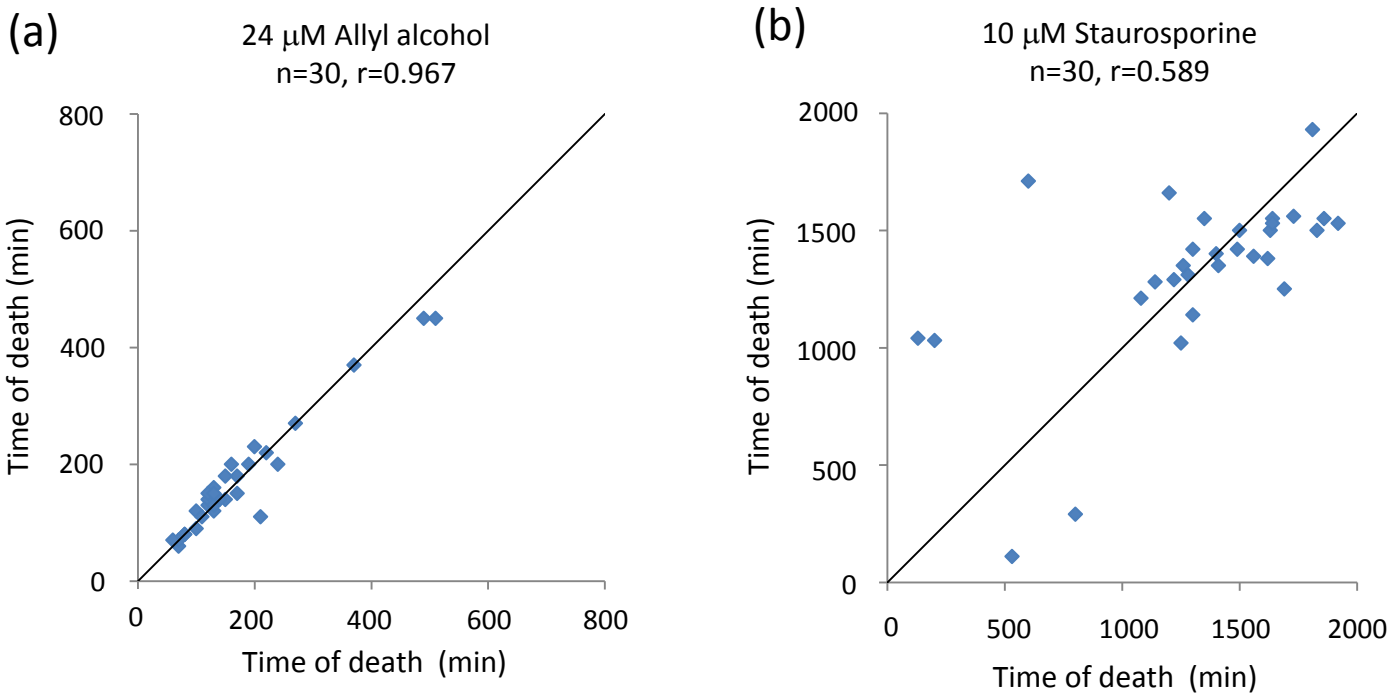
Equal distribution of glutathione (GSH) via gap junctions

Hepatocytes from male and female WT and Cx32 KO mice were plated into dishes and incubated for 3 h. Then WT hepatocytes were incubated with control (NT) siRNA and Cx26 KO hepatocytes were incubated with Cx26 siRNA for 13 h. Glutathione was detected with a glutathione cell-based detection kit (blue fluorescence).

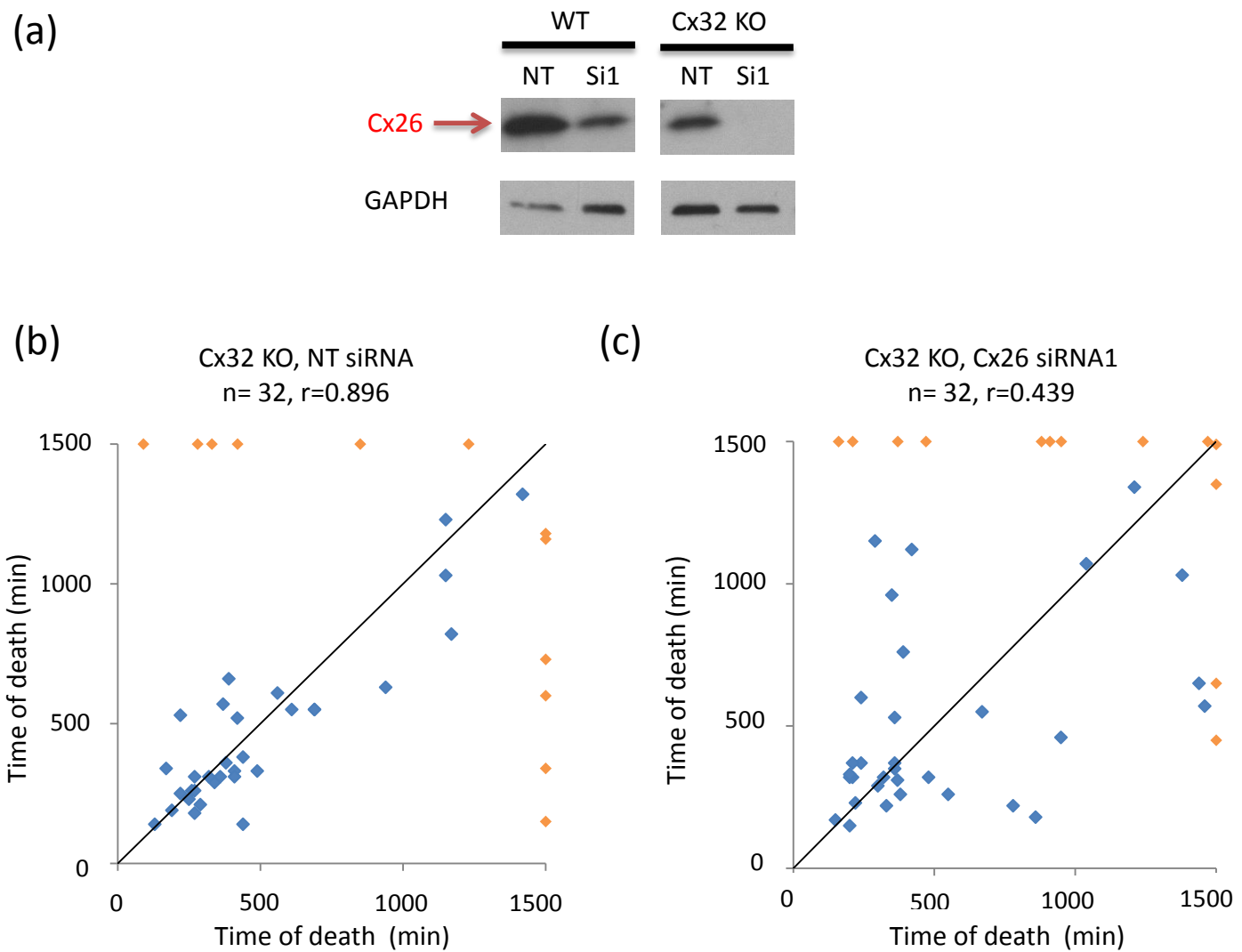
Supplementary Figure 6.

Full length of western blot images of (a, b) Figure 3a, (c, d) Supplementary Figure 2a, (e, f) Supplementary Figure 3.

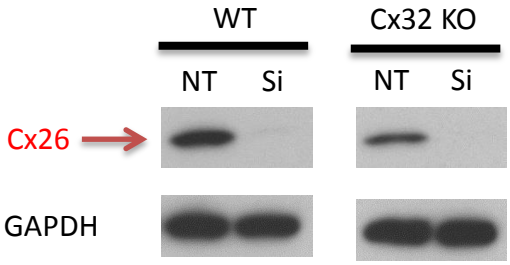
Supplementary Figure 1.



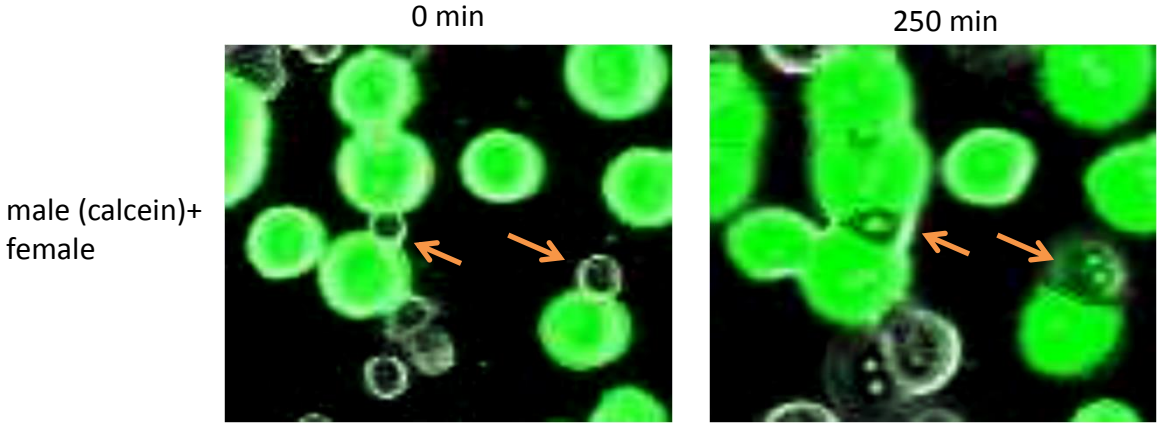
Supplementary Figure 2.



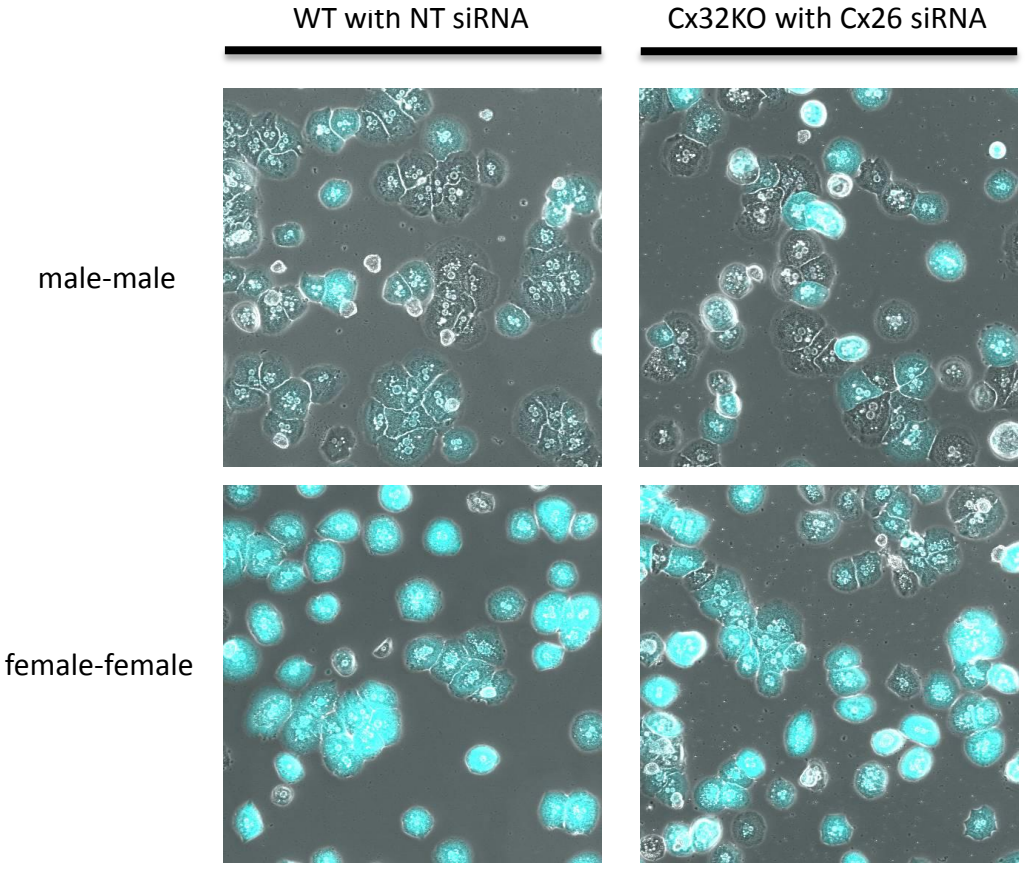
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figure 6.