## HMGB1 is a Central Driver of Dynamic Pro-inflammatory Networks in Pediatric Acute Liver Failure induced by Acetaminophen

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#### **Supplementary Figures**

**Fig. S1. Time-dependent release of inflammatory mediators in PALF patients.** Serum samples from PALF spontaneous survivors were assessed for inflammatory mediators and segregated into three sub-groups (APAPo+NAC [n=13 patients], non-APAPo+NAC [n=8 patients], and non-APAPo non-NAC [n=40 patients]) and non-survivors (n=12 patients). Box plots represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles with a line at the median and error bars defining the 10<sup>th</sup> and 90<sup>th</sup> percentiles (significance level set at *P*<0.05, analyzed by Two-Way ANOVA followed by Holm-Sidak *post hoc t*est).

**Fig. S2. Explanation of DyNA output.** A representative DyNA output showing a network of inflammatory mediator connections over a specific time interval. The closed red circles (nodes) represent mediators with at least one connection to another mediator, while open yellow nodes represent mediators with no connections to other mediators. Black lines (edges) represent positive connections linking mediators that move in the same direction, while red edges represent negative connections that link mediators that move in opposite direction.

**Fig. S3. Time-dependent release of inflammatory mediators by HC from C57BL/6 and HC-HMGB1**<sup>-/-</sup> **mice.** Analysis of supernatant concentrations of inflammatory mediators released by freshly isolated primary HC from C57BL/6 and HC-HMGB1<sup>-/-</sup> mice. Cells were treated with 10 mM APAP alone (C57BL/6: n=3, HMGB1<sup>-/-</sup>: n=5 mice) or for 1h followed by 1 mM N-acetylcysteine (NAC) (C57BL/6: n=9, HMGB1<sup>-/-</sup>: n=6 mice) administered at 1-24h following APAP (APAP+NAC) (C57BL/6: n=3, HMGB1<sup>-/-</sup>: n=3 mice). Non-treated HC harvested at the same time points served as controls (C57BL/6: n=12, HMGB1<sup>-/-</sup>: n=11 mice). Results are presented as the mean ± SEM (significance level set at *P*<0.05, analyzed by Two-Way ANOVA followed by Holm-Sidak *post hoc* test).

Suppl. Fig. 1



Suppl. Fig. 1



**GM-CSF** 

Suppl. Fig. 1



IFN- $\alpha$ 2

Suppl. Fig. 1



Suppl. Fig. 1



IL-1RA

Suppl. Fig. 1



**IL-1**β

Suppl. Fig. 1



Suppl. Fig. 1



 $sIL-2R\alpha$ 

Suppl. Fig. 1



Suppl. Fig. 1



Suppl. Fig. 1



Suppl. Fig. 1



Suppl. Fig. 1



Suppl. Fig. 1



Suppl. Fig. 1



IL-12p40

Suppl. Fig. 1



IL-12p70

Suppl. Fig. 1



Suppl. Fig. 1



Suppl. Fig. 1



IL-17A

Suppl. Fig. 1



**IP-10** 

Suppl. Fig. 1



MCP-1

Suppl. Fig. 1



## MIG

Suppl. Fig. 1



MIP-1 $\alpha$ 

Suppl. Fig. 1



**ΜΙΡ-1**β

Suppl. Fig. 1



 $\textbf{TNF-}\alpha$ 

Suppl. Fig. 1



#### HMGB1

Suppl. Fig. 1



NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup>







IFN-γ









**IL-1**β















![](_page_33_Figure_0.jpeg)

24

![](_page_33_Figure_2.jpeg)

Suppl. Fig. 3

![](_page_34_Figure_0.jpeg)

![](_page_34_Figure_1.jpeg)

![](_page_34_Figure_2.jpeg)

24

IL-13

![](_page_34_Figure_4.jpeg)

![](_page_35_Figure_0.jpeg)

![](_page_35_Figure_1.jpeg)

IL-17A

![](_page_36_Figure_1.jpeg)

IP-10

![](_page_36_Figure_3.jpeg)

![](_page_37_Figure_0.jpeg)

MCP-1

![](_page_37_Figure_2.jpeg)

![](_page_38_Figure_0.jpeg)

![](_page_38_Figure_1.jpeg)

**ΜΙΡ-1**α

![](_page_38_Figure_3.jpeg)

#### TNF- $\alpha$

Suppl. Fig. 3

![](_page_39_Figure_1.jpeg)

Time (h) Time (h) Time (h)

1 3 Time (h)

![](_page_40_Figure_0.jpeg)

![](_page_40_Figure_1.jpeg)

NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup>

![](_page_40_Figure_3.jpeg)