Interval-based secretomics unravels acute-phase response in hepatocyte model systems

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Supplemental Figures and Figure Legends S1-S7

Supplemental Files

This article contains supplemental data that are included as separate data files that contain following information:

Supplemental Table 1. Summary of the cumulative secretome data from IL1b and IL6 treated HepG2 and differentiated HepaRG cells.

Supplemental Table 2. Summary of the k-means clustering of the cumulative secretomics data of IL1b and IL6 treated HepaRG cells.

Supplemental Table 3. Summary of the interval-based secretome data from IL1b and IL6 treated differentiated HepaRG cells.

Supplemental Table 4. Summary of the k-means clustering of the interval-based secretomics data of IL1b and IL6 treated HepaRG cells.

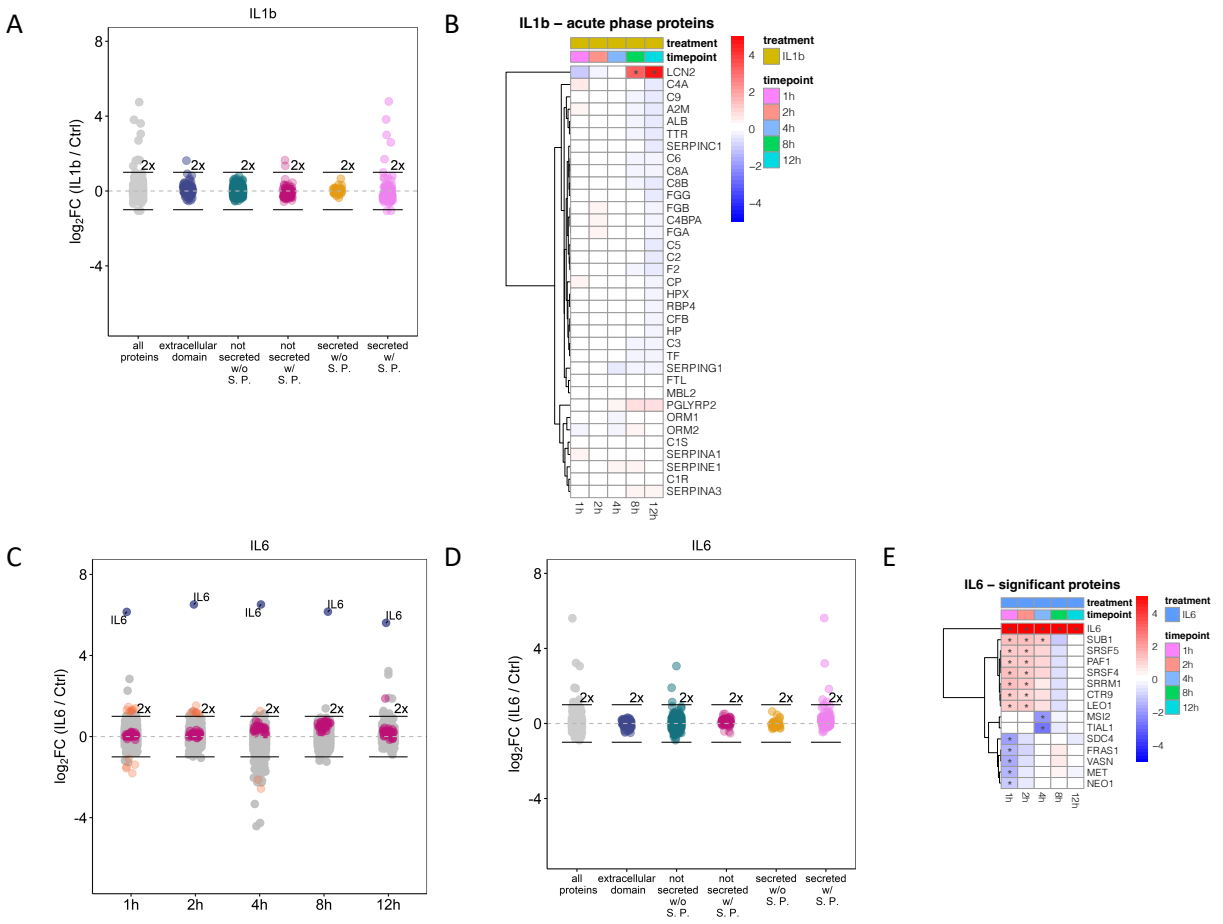
Supplemental Table 5. Summary of IL1b induced cell surface shedding experiment.

Supplemental Table 6. Peptide raw data of the cumulative secretome experiments of IL1b and IL6 treated HepG2 cells

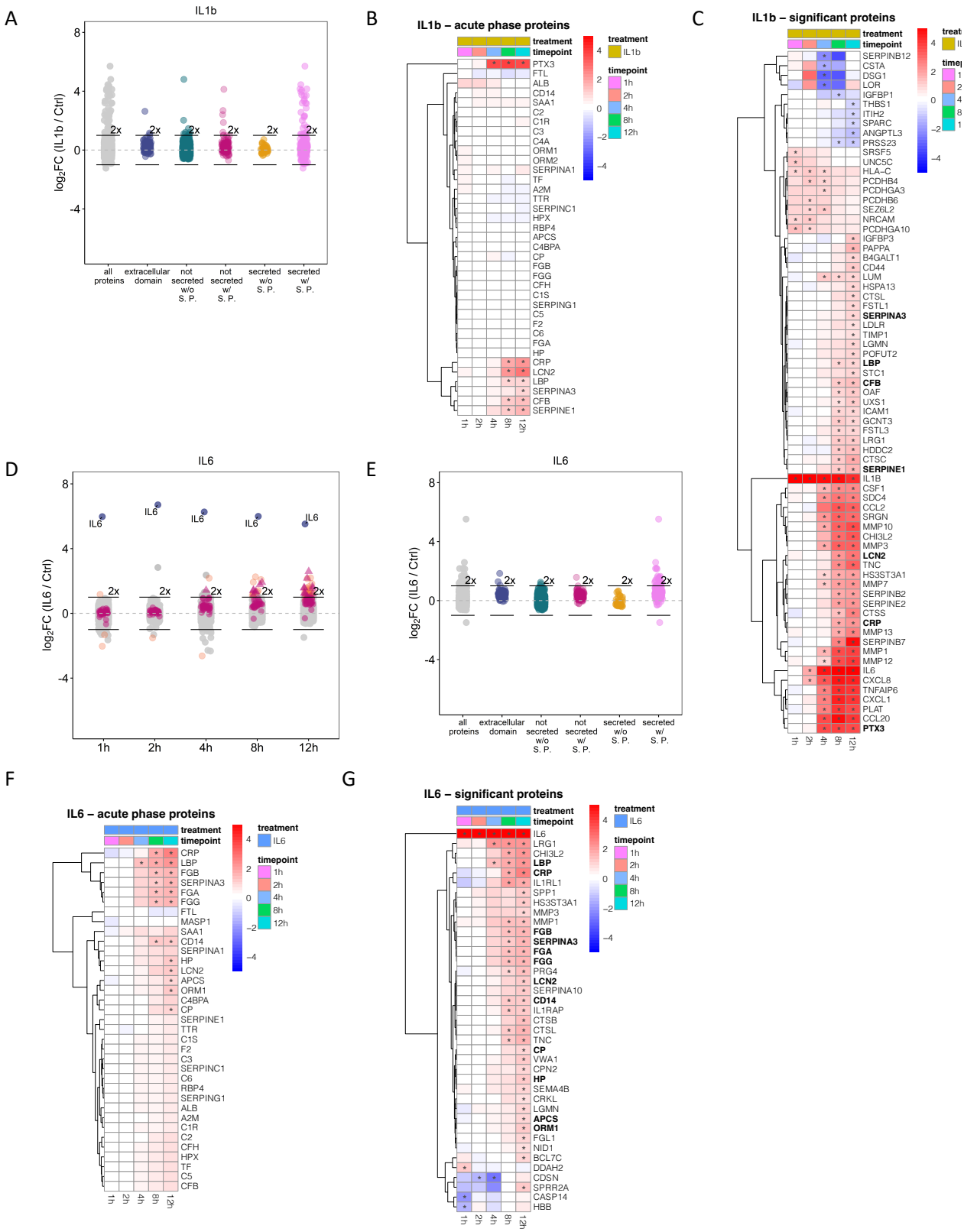
Supplemental Table 7 Peptide raw data of the cumulative secretome experiments of IL1b and IL6 treated differentiated HepaRG cells.

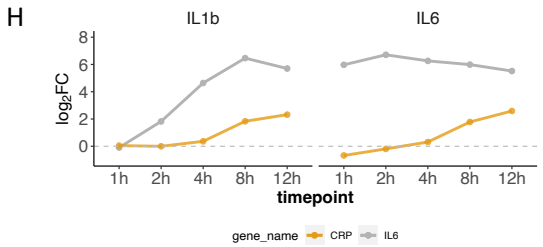
Supplemental Table 8. Peptide raw data of the interval-based secretome experiments of IL1b and IL6 treated differentiated HepaRG cells.

Supplemental Table 9. Peptide raw data of the IL1b induced cell surface shedding experiments.

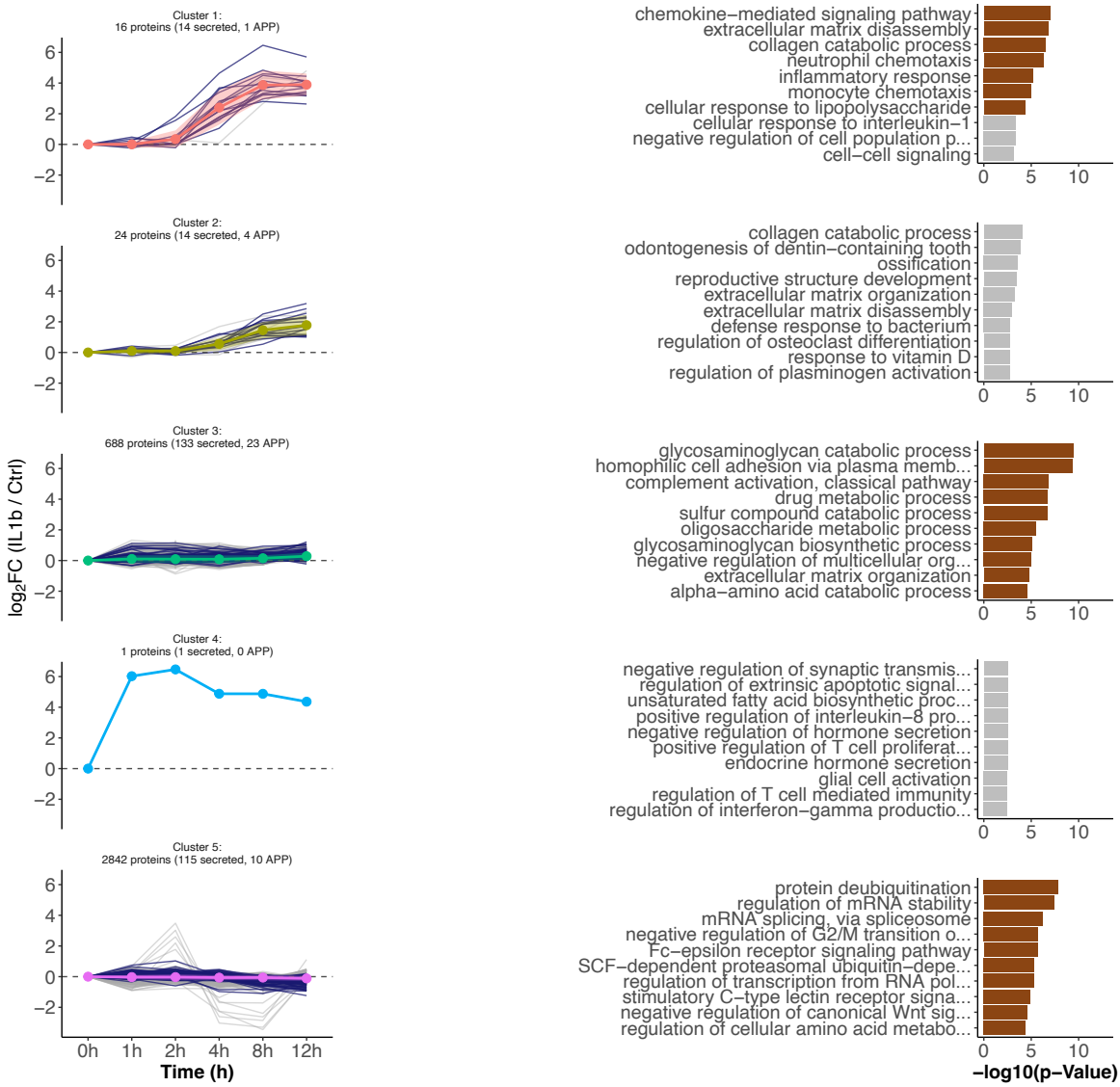


Supplemental Fig. S1 (A) proteins released at 12 h of treatment with IL1b from HepG2 cells grouped by their subcellular location annotation based on UniProt. Grey: all proteins, blue: proteins with an extracellular domain, teal: proteins without a signal peptide (S. P.) and not annotated as secreted, purple: proteins with signal peptide and not annotated as secreted, yellow: proteins without signal peptide and annotated as secreted, pink: proteins with signal peptide and annotated as secreted. 2 fold increase in abundance is indicated by horizontal bars. **(B)** Acute-phase proteins identified in the secretomes of IL1b treated HepG2 cells across different timepoints. Displayed are $\log_2$ fold changes to the respective time matched control. Statistically significant changes are denoted with asterisks (*). **(C)** Secretomics analysis of IL6 treated HepG2 cells. All identified proteins are depicted with their mean $\log_2$ fold change (n=3) against the time matched controls at the indicated time points. Proteins passing the significance thresholds (p(Benjamini Hochberg) < 0.05 and $\log_2$ fold change > 2x standard deviation of the individual treatment) are colored in orange, acute-phase response proteins are colored in purple, IL6 colored in blue and indicated with name. 2 fold increase in abundance is indicated by horizontal bars. **(D)** same as (A) for IL6 treated HepG2 cells. **(E)** significantly released proteins in the secretome of IL6 treated HepG2 cells across different timepoints. Displayed are log2 fold changes to the respective time matched control. Statistically significant changes are denoted with asterisks (*).

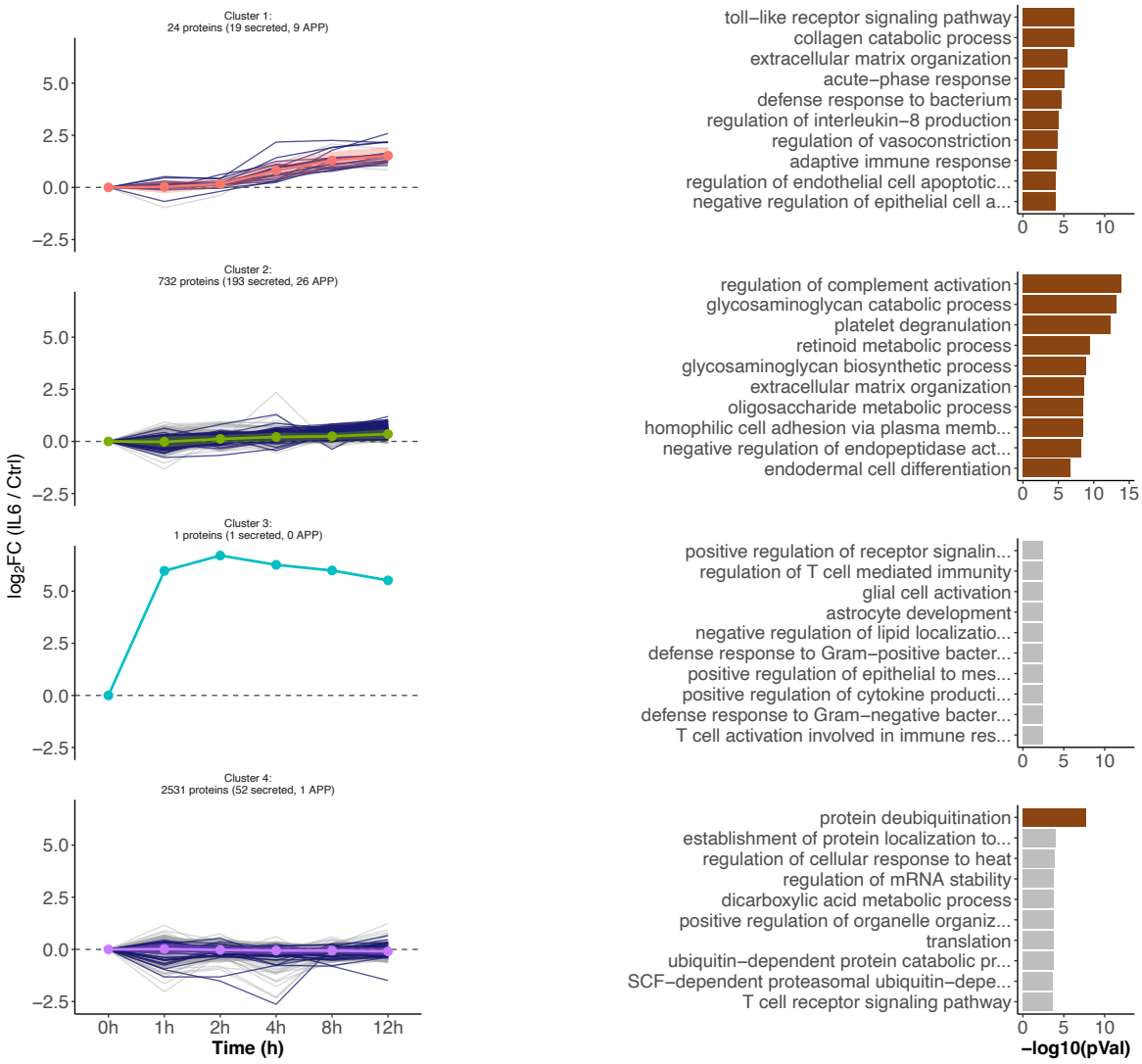

A
IL1b
log2FC (IL1b / Ctrl)
all proteins
extracellular domain
not secreted w/o S. P.
not secreted w/ S. P.
secreted w/o S. P.
secreted w/ S. P.
B
IL1b – acute phase proteins
treatment
timepoint
IL1b
1h
2h
4h
8h
12h
C
IL1b – significant proteins
D
IL6
log2FC (IL6 / Ctrl)
E
IL6
F
IL6 – acute phase proteins
G
IL6 – significant proteins



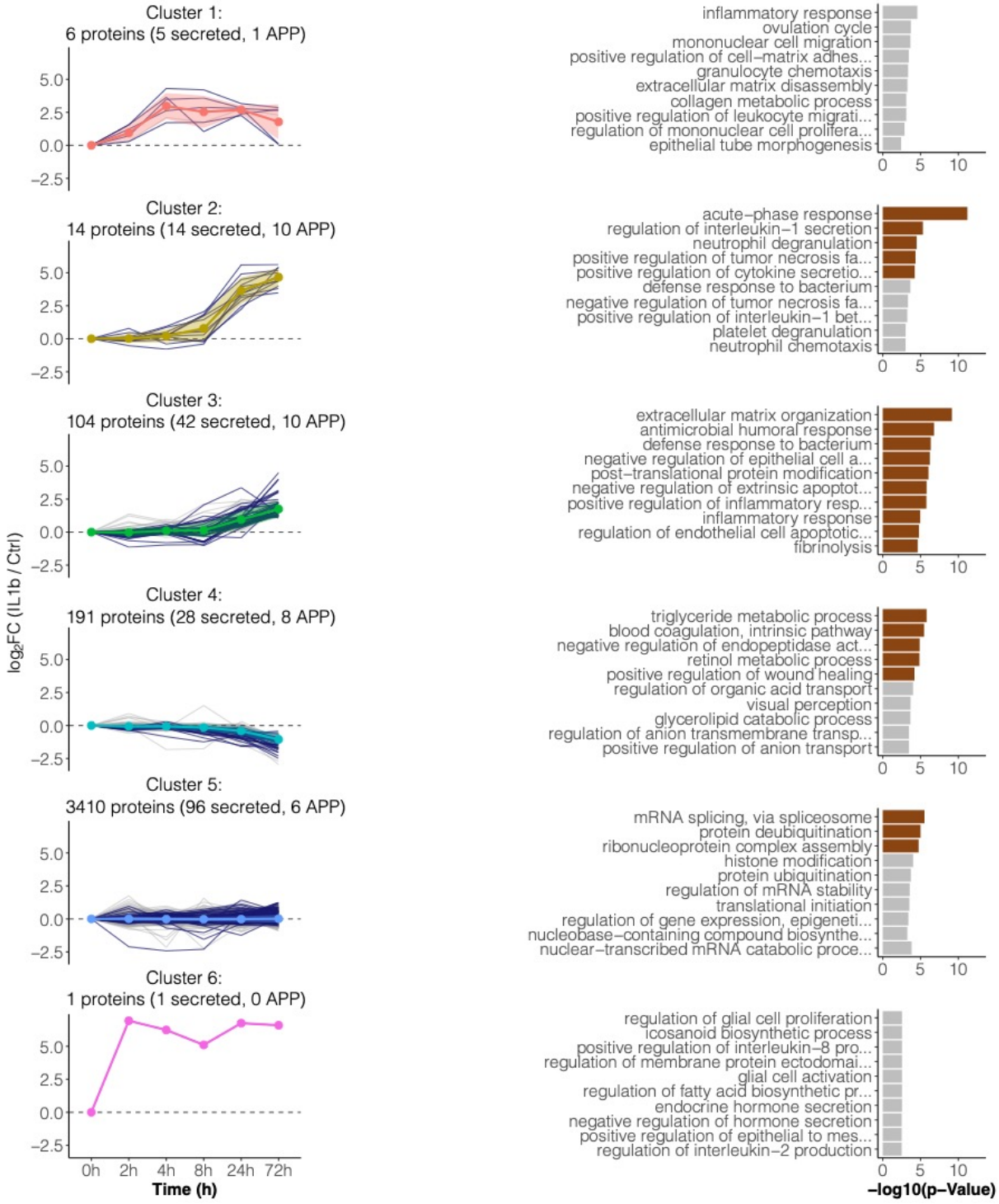
Supplemental Fig. S2 (A) proteins released at 12 h of treatment with IL1b from dHepaRG cells grouped by their subcellular location annotation based on UniProt. Grey: all proteins, blue: proteins with an extracellular domain, teal: proteins without a signal peptide (S. P.) and not annotated as secreted, purple: proteins with signal peptide and not annotated as secreted, yellow: proteins without signal peptide and annotated as secreted, pink: proteins with signal peptide and annotated as secreted. 2 fold increase in abundance is indicated by horizontal bars. **(B)** All acute-phase proteins identified in the secretomes of IL1b treated dHepaRG cells across different timepoints. Displayed are log_2 fold changes to the respective time matched control. Statistically significant changes are denoted with asterisks (*). **(C)** same as (B) showing all significant proteins upon IL1b treatment in dHepaRG cells **(D)** Secretomics analysis of IL6 treated HepaRG cells. All identified proteins are depicted with their mean log_2 fold change (n=3) against the time matched controls at the indicated time points. Proteins passing the significance thresholds (p(Benjamini Hochberg) < 0.05 and log_2 fold change > 2x standard deviation of the individual treatment are colored in orange, acute-phase response proteins are colored in purple, acute-phase proteins passing the significance thresholds are depicted as triangles, IL6 is colored in blue and indicated with name. 2 fold increase in abundance is indicated by horizontal bars. **(E)** same as A for IL6 treated dHepaRG cells. **(F)** and **(G)** same as (B) and (C) for IL6 treated HepaRG cells. **(H)** IL-6 dependent secretion of CRP in dHepaRG cells. CRP and IL6 are depicted with their mean log_2 fold change (n=3) against the time matched controls at the indicated time points for stimulation with IL1b (left panel) and IL6 (right panel)



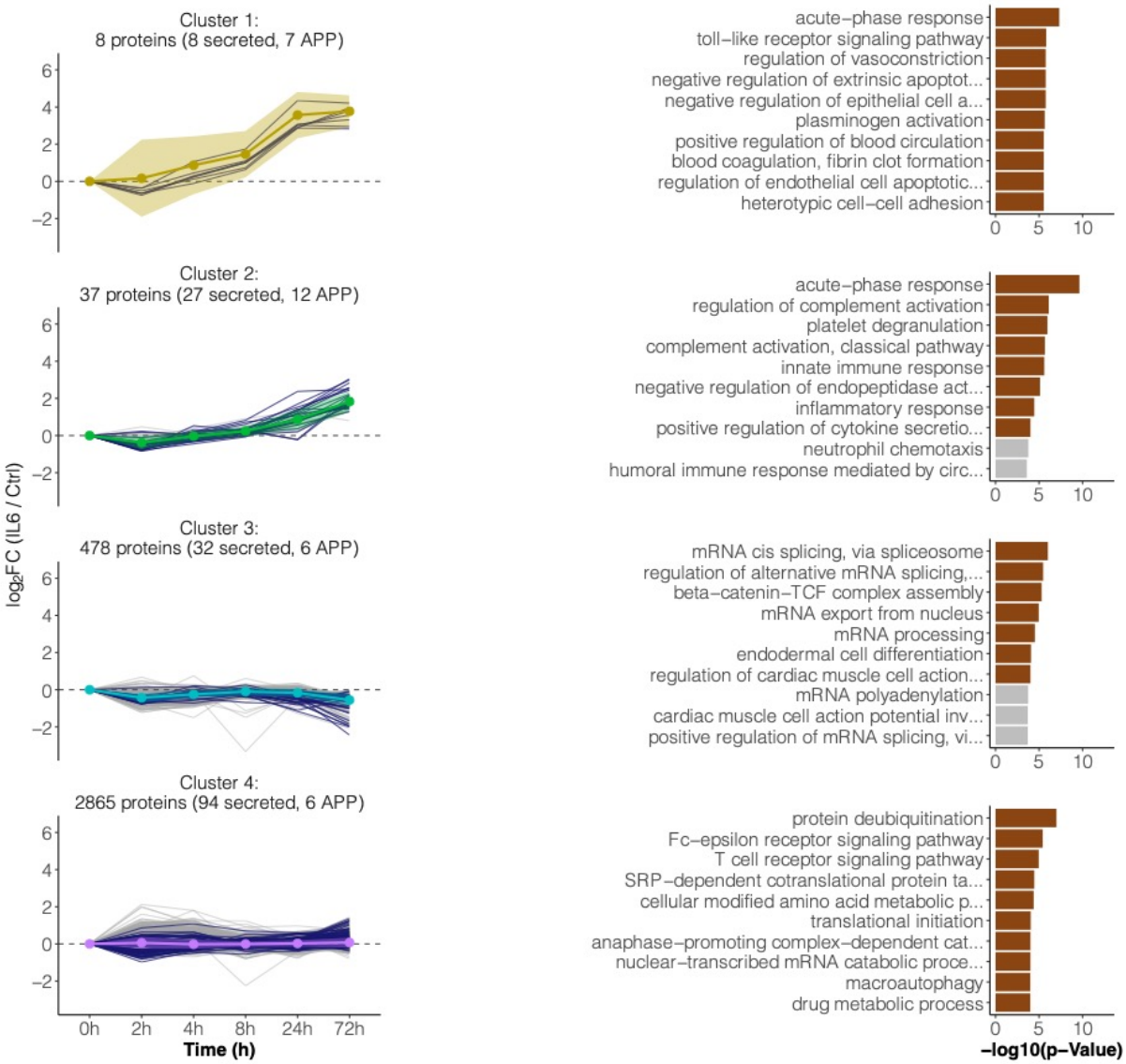
Supplemental Fig. S3 Time resolved k-means cluster analysis of the cumulative secretome of IL1b treated HepaRG cells. Left panel: k-means clustering of all identified. Clustering was performed by using proteins annotated to be secreted as training dataset. Colored ribbons indicate the mean $\log_2$ fold change ± SD in each cluster. Right panel: GO-term enrichment analysis showing the top 10 biological processes for each cluster (brown bars indicate significant GO-terms with p(Benjamini-Hochberg corrected) < 0.05).



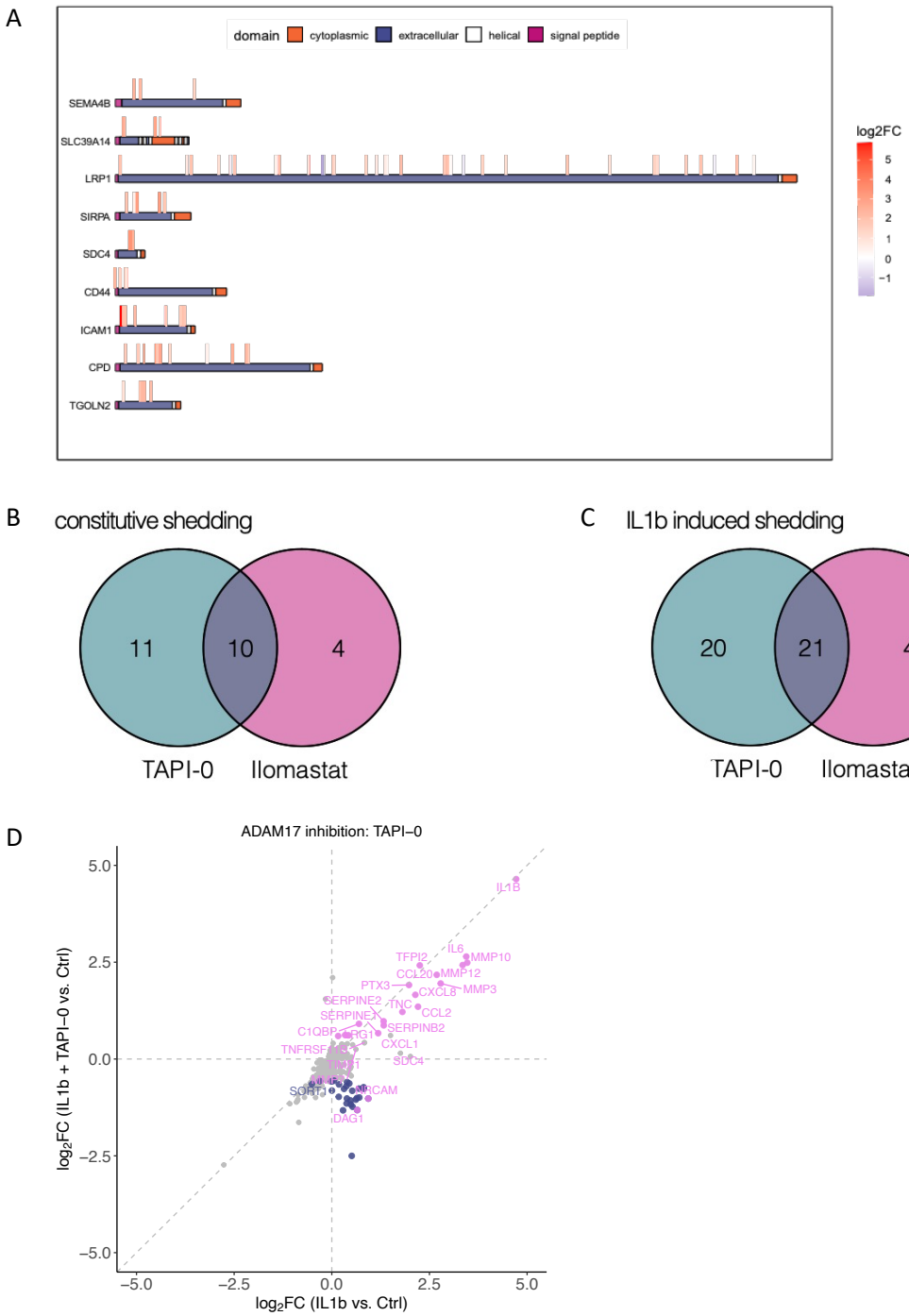
Supplemental Fig. S4 Time resolved k-means cluster analysis of the cumulative secretome of IL6 treated HepaRG cells. Left panel: k-means clustering of all identified. Clustering was performed by using proteins annotated to be secreted as training dataset. Colored ribbons indicate the mean $\log_2$ fold change ± SD in each cluster. Right panel: GO-term enrichment analysis showing the top 10 biological processes for each cluster (brown bars indicate significant GO-terms with p(Benjamini-Hochberg corrected) < 0.05).



Supplemental Fig. S5 Time resolved k-means cluster analysis of the interval-based secretomes of IL1b treated HepaRG cells. Left panel: k-means clustering of all identified. Clustering was performed by using proteins annotated to be secreted as training dataset. Colored ribbons indicate the mean log_2 fold change ± SD in each cluster. Right panel: GO-term enrichment analysis showing the top 10 biological processes for each cluster (brown bars indicate significant GO-terms with p(Benjamini-Hochberg corrected) < 0.05).

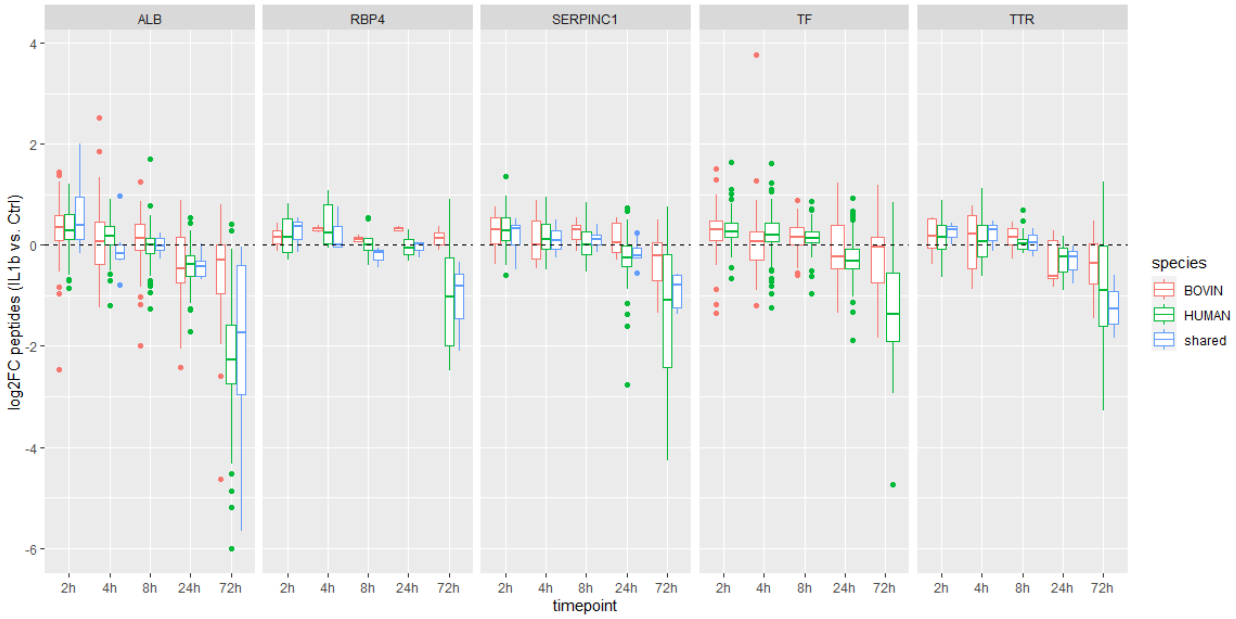


Supplemental Fig. S6 Time resolved k-means cluster analysis of the interval-based secretomes of IL6 treated HepaRG cells. Left panel: k-means clustering of all identified proteins. Clustering was performed by using proteins annotated to be secreted as training dataset. Colored ribbons indicate the mean $\log_2$ fold change ± SD in each cluster. Right panel: GO-term enrichment analysis showing the top 10 biological processes for each cluster (brown bars indicate significant GO-terms with p(Benjamini-Hochberg corrected) < 0.05).



Supplemental Fig. S7 (A) Matching of detected peptides to their corresponding protein sequence of proteins with an extracellular domain that are found in the secretome of IL1b treated HepaRG cells after 72 h. Color of matched peptides denotes the $\log_2$ fold change. **(B)** Venn diagram showing the intersection of transmembrane proteins that are negatively affected upon treatment with Ilomastat or TAPI-0 (constitutive shedding). **(C)** Venn diagram showing the intersection of transmembrane proteins that are negatively affected upon treatment with Ilomastat or TAPI-0 during stimulation of the APR with IL1b (IL1b induced shedding). **(D)** Changes in IL1b dependent protein secretion patterns upon addition of the ADAM17 inhibitor TAPI-0 after 8h treatment. Inhibitors and IL1b were added simultaneously, and supernatants were collected after 8h. The scatter plot visualize the IL1b

dependent and inhibitor affected protein secretion. X-axis displays log_2 protein fold changes of IL1b treated HepaRG cells versus time matched controls to identify IL1b dependent secretion. Y-axis displays log_2 protein fold changes of IL1b and inhibitor treated HepaRG cells versus time matched controls to identify inhibitor-dependent alterations. Transmembrane proteins with an extracellular domain are shown in blue, secreted proteins are shown in pink.



Supplemental Fig. S8. Comparison of log_2 fold changes of peptides from acute-phase proteins. MS raw data of the interval-based IL1b dataset were searched with a combined human-cow database. Distribution of log_2 fold changes comparing the IL1b treated samples with their time matched controls were plotted across all timepoints. Colors denote species membership: red boxplots show unique bovine peptides, green unique human peptides and blue shows shared peptides.