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Supplemental information

Identifying multiscale translational safety biomarkers using a network-based systems approach

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Figure S1. TG-based rat liver TXG-MAPr dendrogram, related to Figure 1. A) Module dendrogram as produced by Ward2 clustering algorithm applied to pair-wise Pearson correlations for each module across all treatment conditions. B) Module dendrogram is shown highlighting manual adjustment of branch lengths and the anti-clockwise labelling system of branches.

A

B

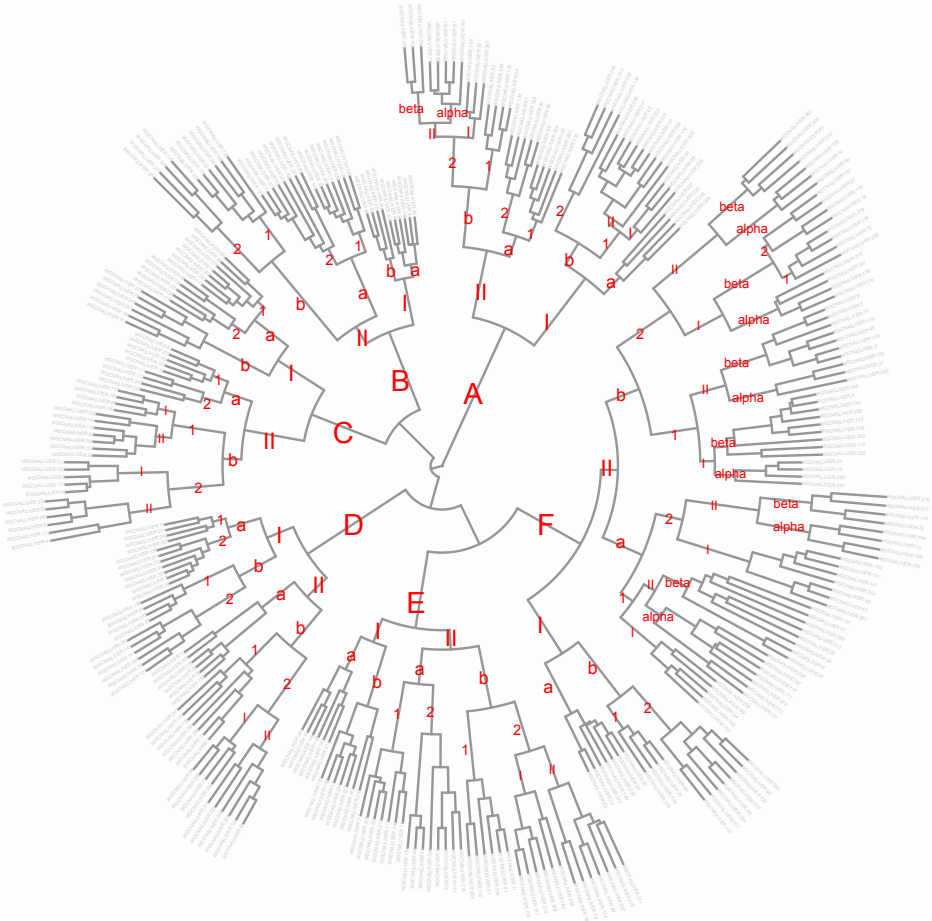
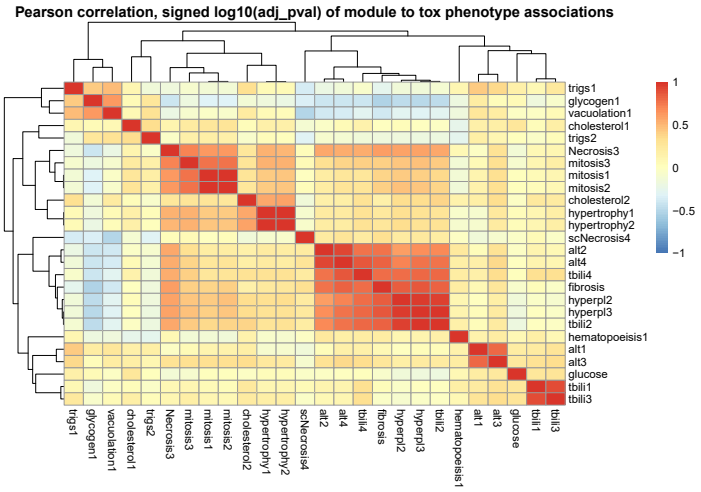
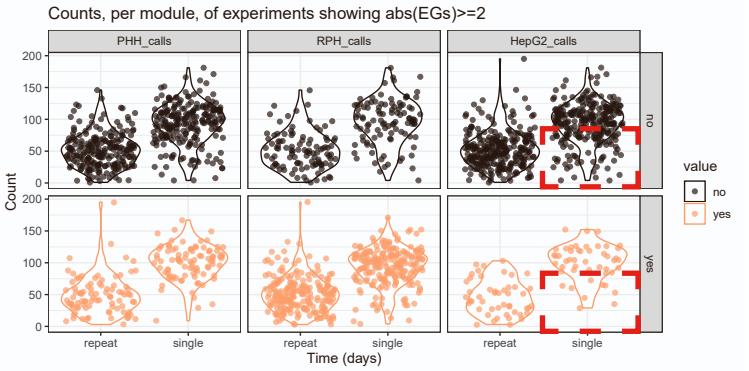


Figure S2. TG-based rat liver TXG-MAPr features, association with toxicity phenotypes and preservation to human in vitro datasets, related to Figure 1. A) Heatmap of Pearson correlation calculated between signed log10(adj_pval) of module to toxicity phenotype associations. B) Scatterplot of the natural logarithm transformed Z-summary of TG-modules towards PHH data (x-axis) and HepG2 data (y-axis). Each dot correspond to a module and the color indicates whether it is associated to a pathology (top 10). The shape indicates Median Rank statistic, the lower the more preserved. C) Violin plots with superimposed scatter plot of, per module, the counts of conditions (compound_dose) showing abs(EGs>=2), distinguishing single exposures (<=1day) or repeat exposure scenarios (> 1day), faceted for the preservation calls in the 3 comparisons (yes: Z_summary >=2, no: Zsummary<2).

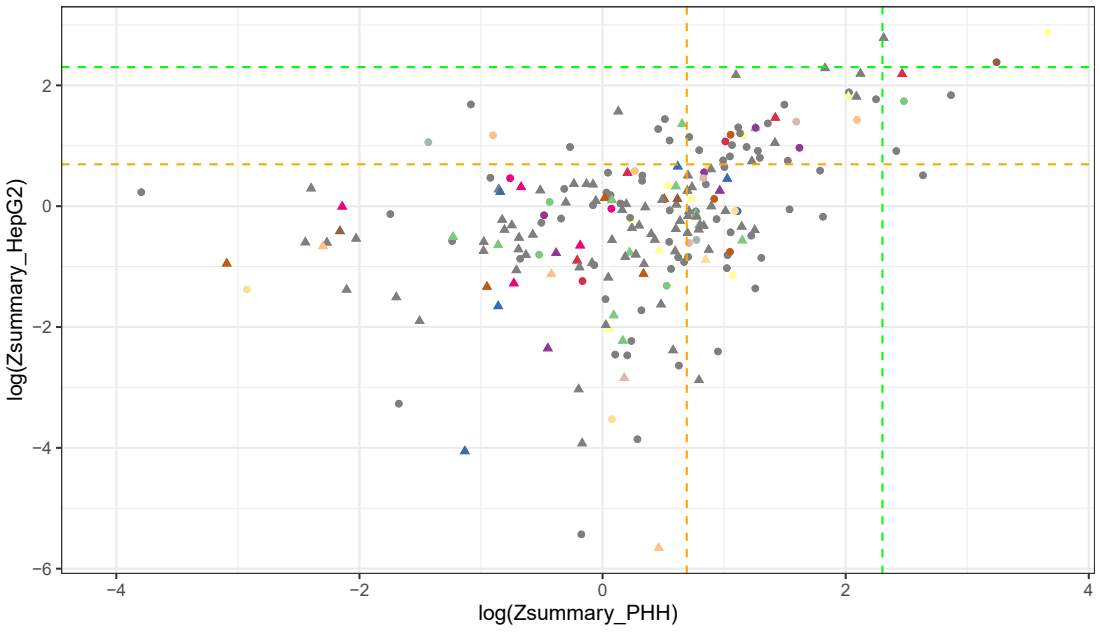
A



C



B

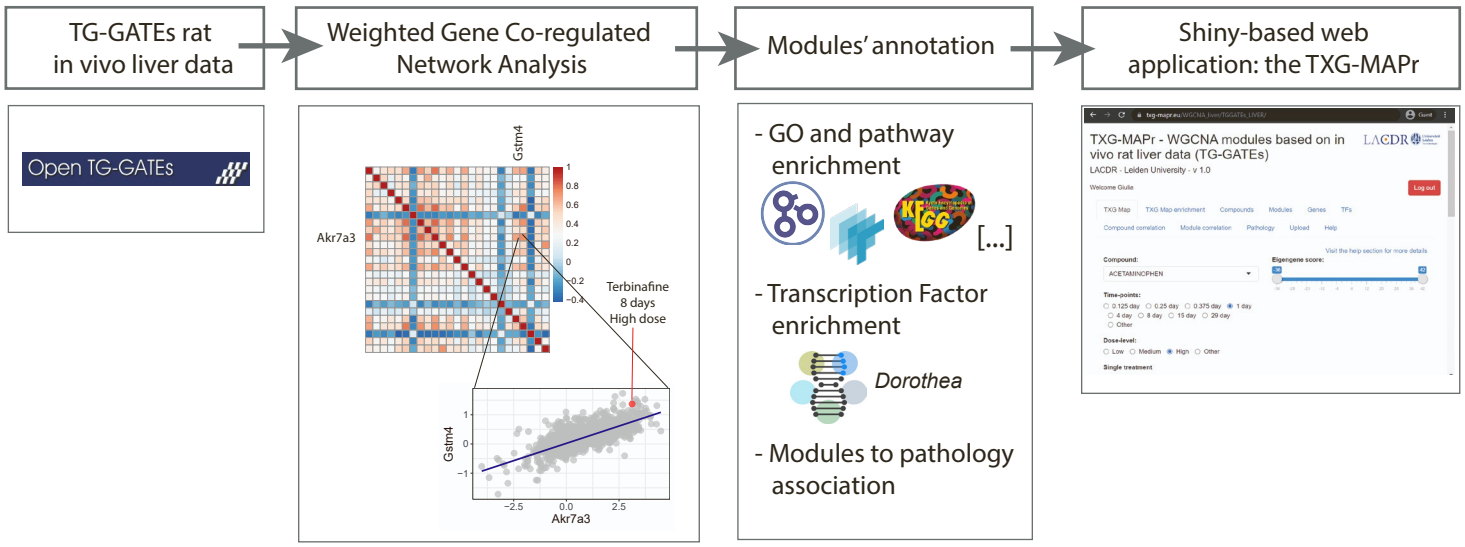


MedianRank1
- MedianRank < 100
- MedianRank >= 100

path
- ALT
- Deposit, glycogen
- Fibrosis
- GLC
- Hematopoiesis, extramedullary
- Hypertrophy
- Increased mitosis
- Necrosis
- Proliferation, bile duct
- Single cell necrosis
- TBIL
- TC
- TG
- Vacuolization, cytoplasmic
- NA

Figure S3. The DILI TXG-MAPr tool, related to Figure 1. A) Conceptual representation on the development of the DILI TXG-MAPr tool, starting from TG-GATEs based WGCNA and Shiny architecture development. B) The DILI TXG-MAPr tools include an environment with multiple tabs, and help page help in tool understanding and data interpretation as well as a dedicated upload function to analyze external datasets. More details about this environment can be found in 18.

A



B

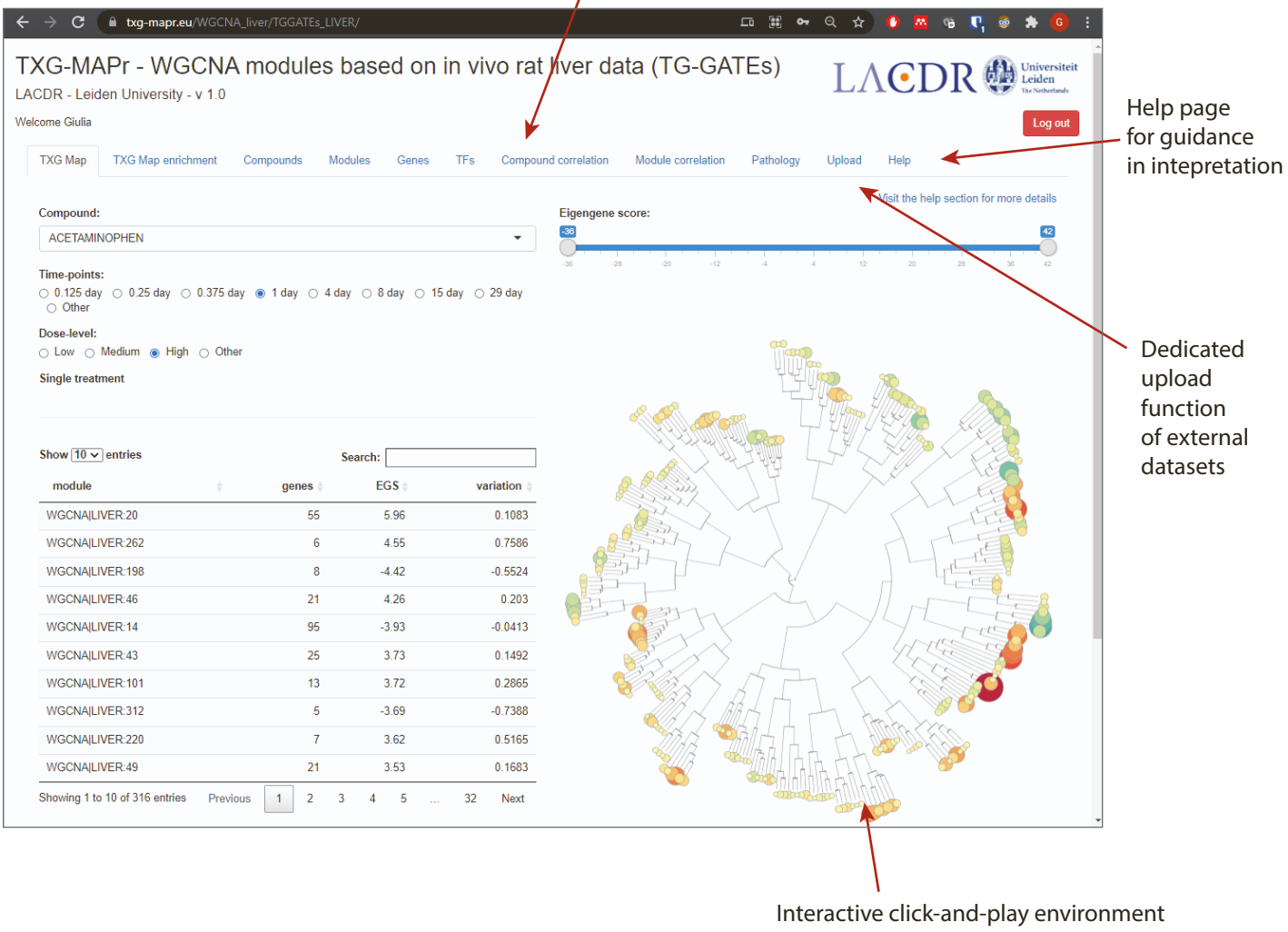
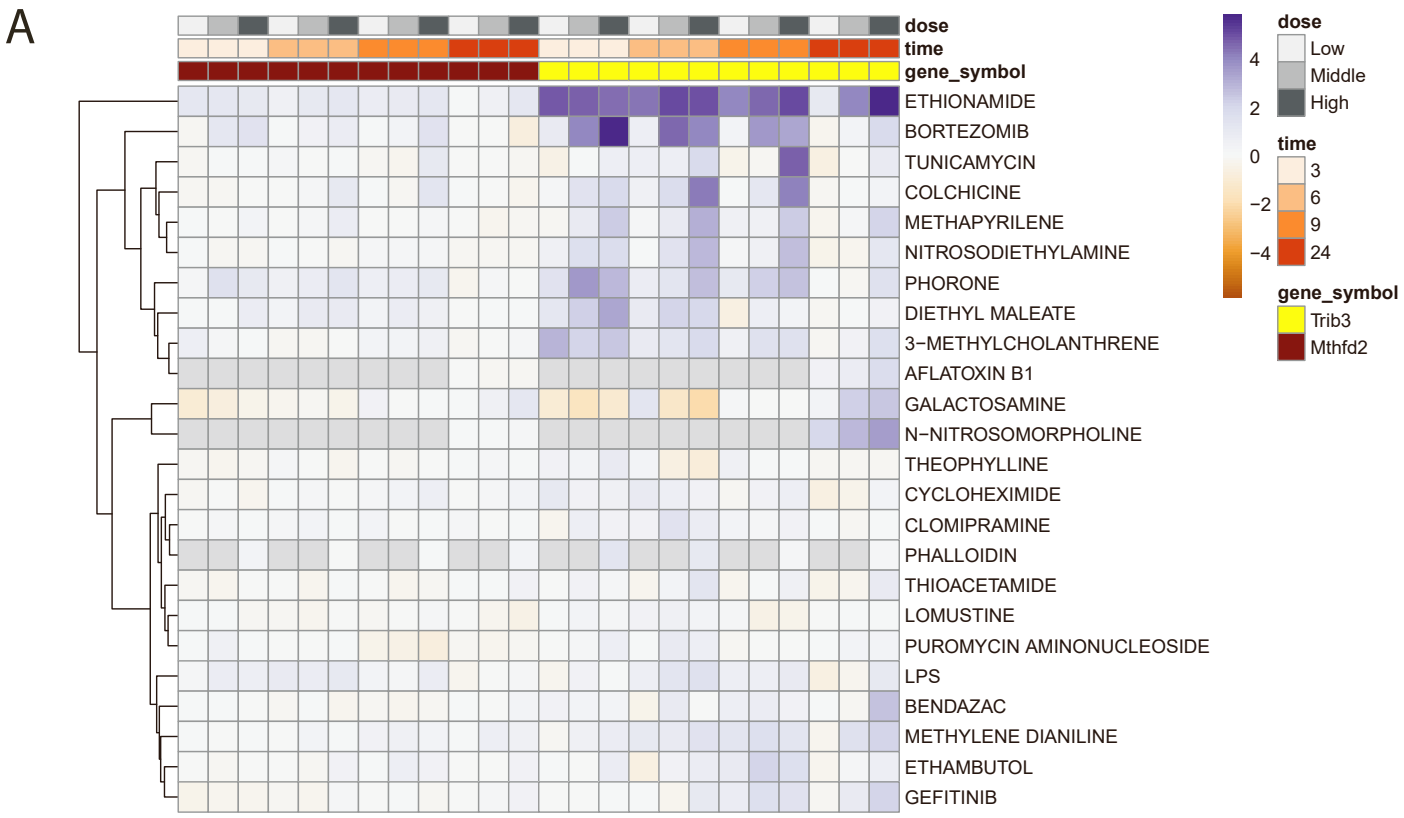


Figure S4. Trib3 and Mthfd2 log2FC across positive single cell necrosis compounds, related to Figure 3. A) Heatmap of Trib3 and Mthfd2 log2FC across all single cell necrosis positive stressors in TG rat in vivo data. B) Scatterplot of rLIV:176 maximum EGs over time and concentration, Mthfd2 (rat) and MTHFD2 (PHH) maximum log2FC over time and concentration, for all TG compounds (x-axis). The size of the circles is proportional to the average score of single cell necrosis in rat, and the color indicated the DILI Rank status, when available.



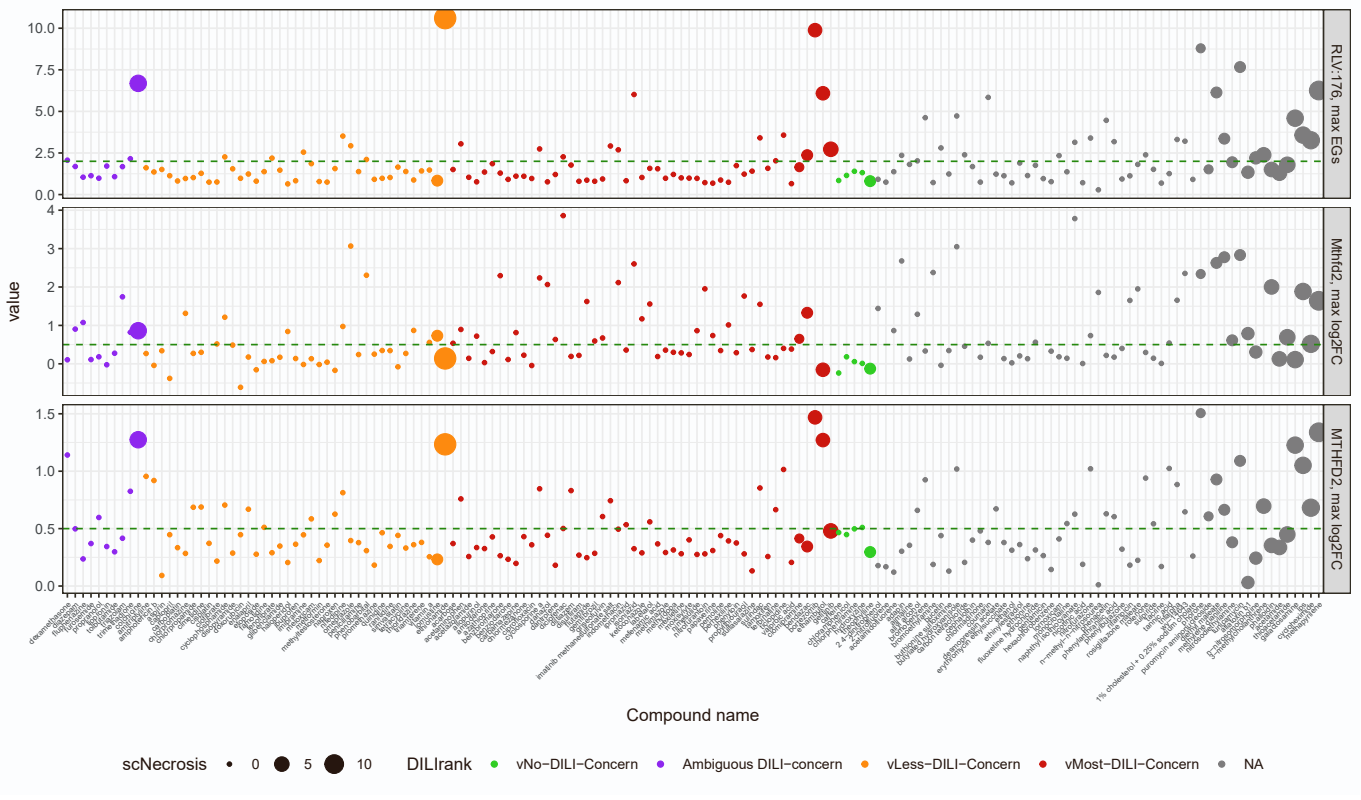


Figure S5. Validation of HepG2 TRIB3-eGFP reporter cell line, related to Figure 4.
Overview of all readouts related to the siRNA knock down experiment of the HepG2 BAC-eGFP TRIB3 cell line exposed to DMSO (0) and different concentrations of tunicamycin (3,6,12,24 µM) at timepoint 0 and 24 hrs, 48 hrs, and 72 hrs after exposure.

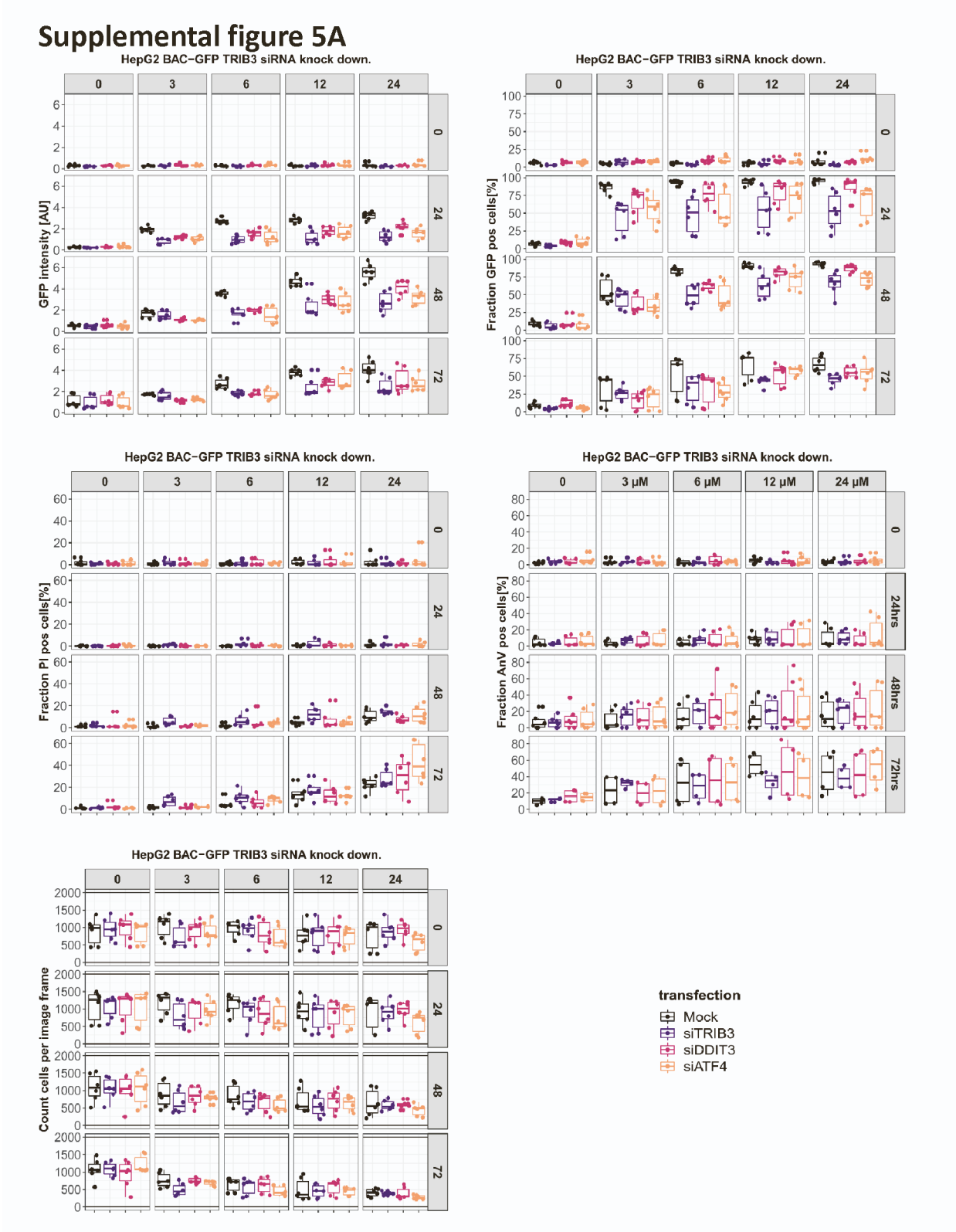
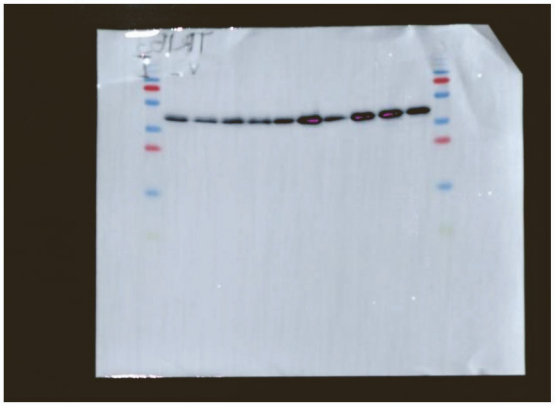


Figure S6. Validation of HepG2 TRIB3-eGFP reporter cell line, related to Figure 4.
HepG2 BAC-eGFP TRIB3 western blots (3 biological replicates) stained with anti-TRIB3 antibody.

HepG2 BAC-GFP TRIB3 Western Blot
Antibody: Anti-TRIB3

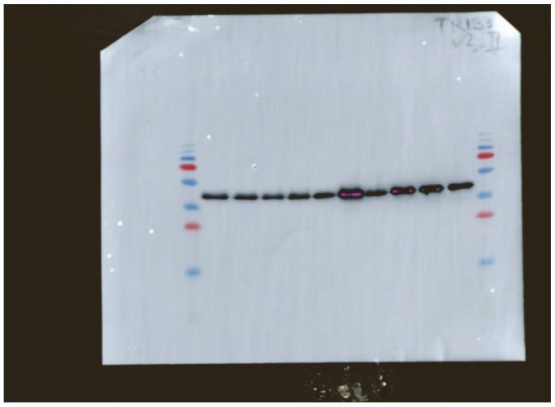
Biological replicate 1



Raw image size marker

Raw image intensity optimized

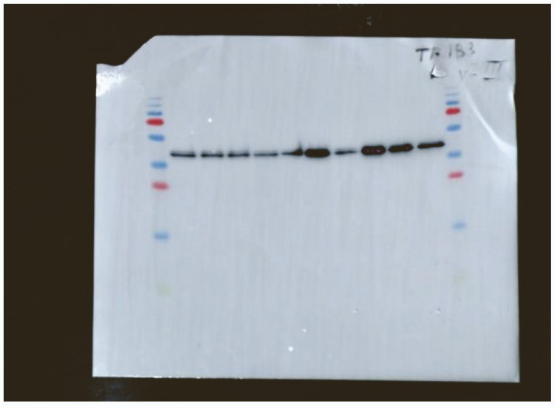
Biological replicate 2



Raw image size marker

Raw image intensity optimized

Biological replicate 3



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Raw image intensity optimized

Slot 1: Medium
Slot 2: DMSO
Slot 3: Tunicamycin 0.024 µM
Slot 4: Tunicamycin 0.24 µM
Slot 5: Tunicamycin 2.4 µM
Slot 6: Tunicamycin 24 µM
Slot 7: Thapsigargin 0.006 µM
Slot 8: Thapsigargin 0.06 µM
Slot 9: Thapsigargin 0.6 µM
Slot 10: Thapsigargin 6 µM

Figure S7. Validation of HepG2 TRIB3-eGFP reporter cell line, related to Figure 4.
HepG2 BAC-eGFP TRIB3 western blots (3 biological replicates) stained with anti-alpha-tubulin antibody.

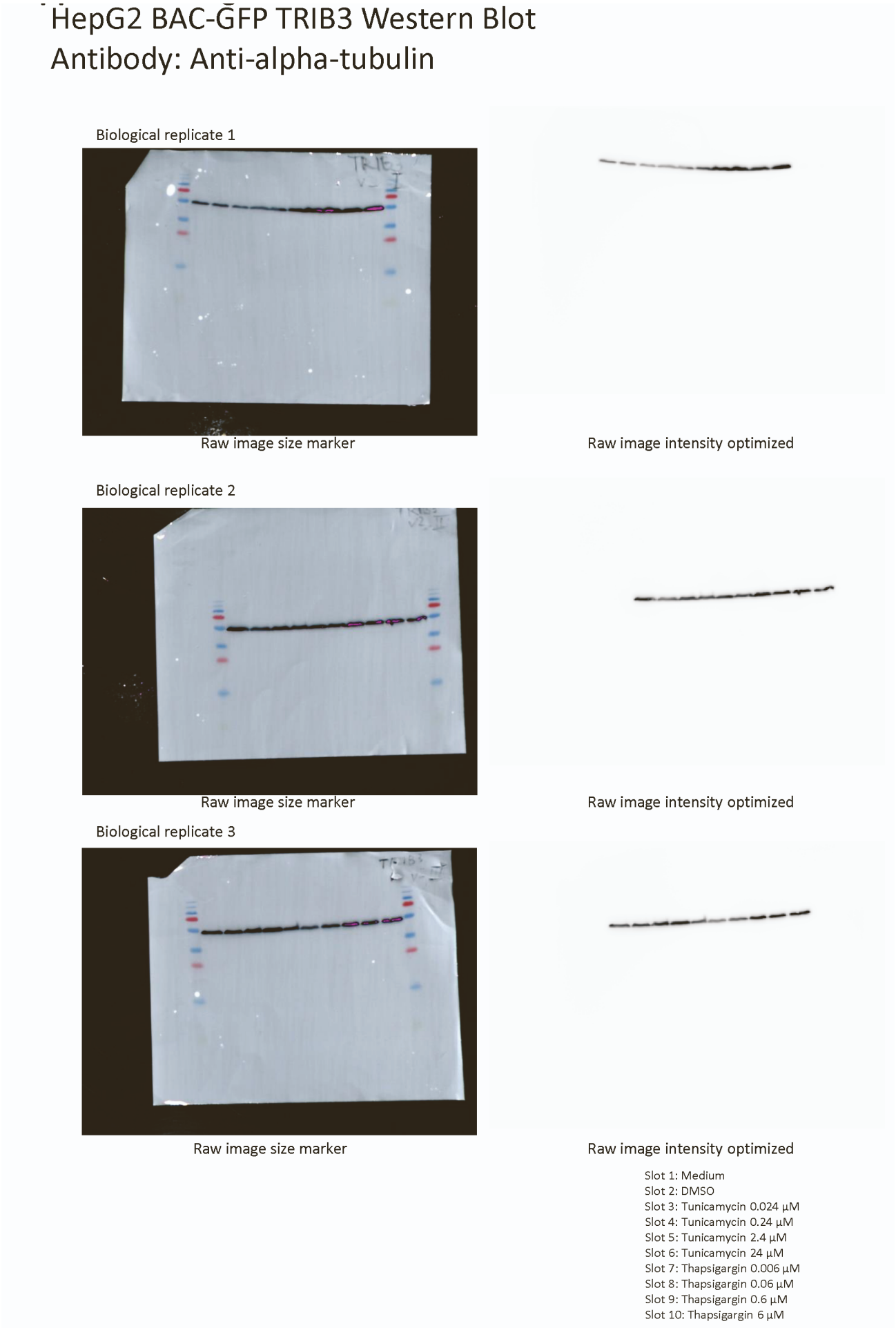
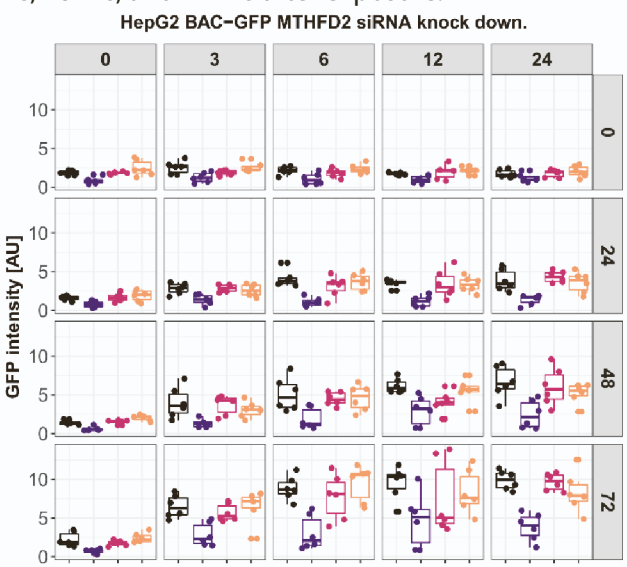
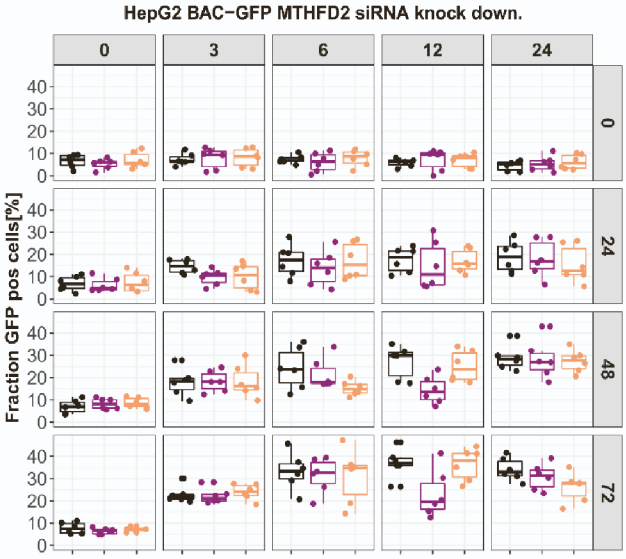
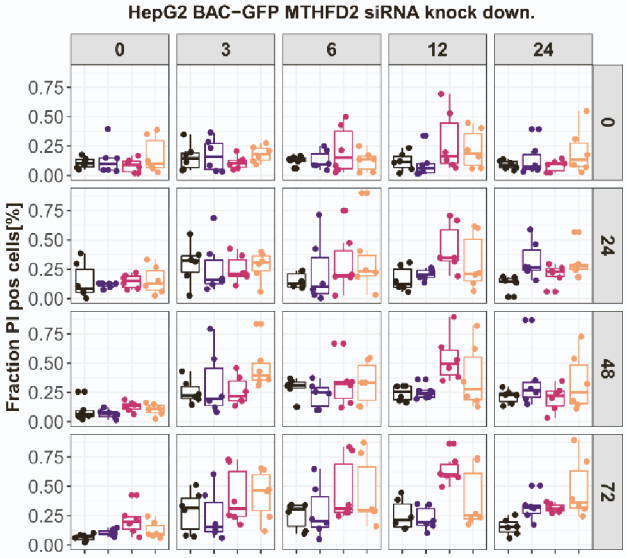
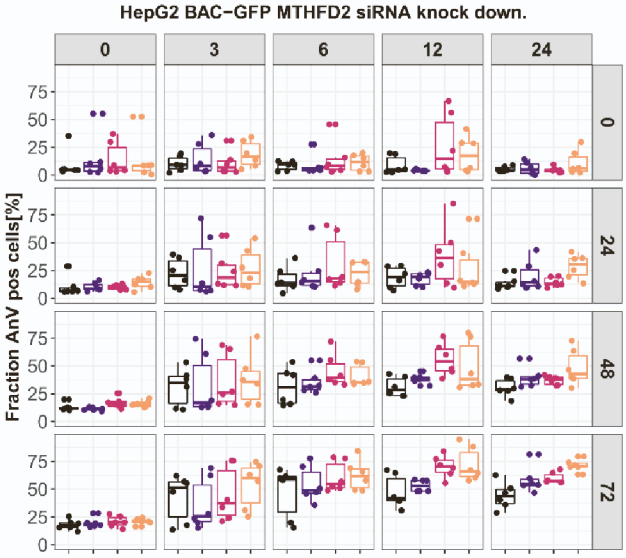


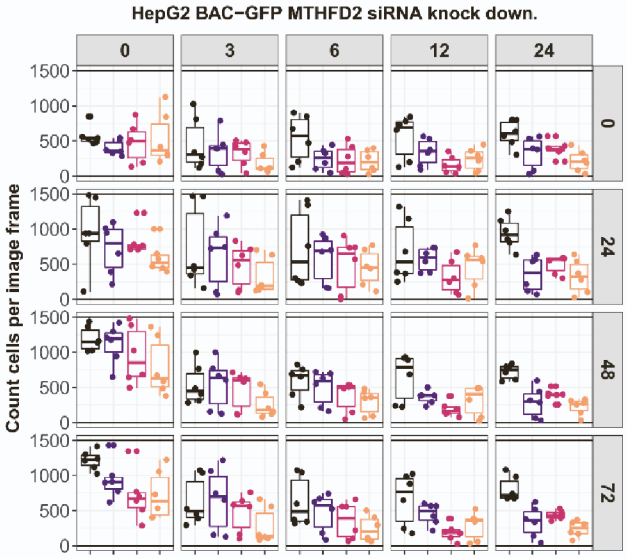
Figure S8. Validation of HepG2 MTHFD2-eGFP reporter cell line, related to Figure 4.
Overview of all readouts related to the siRNA knock down experiment of the HepG2 BAC-eGFP MTHFD2 cell line exposed to DMSO (0) and different concentrations of tunicamycin (3,6,12,24 µM) at timepoint 0 and 24 hrs, 48 hrs, and 72 hrs after exposure.











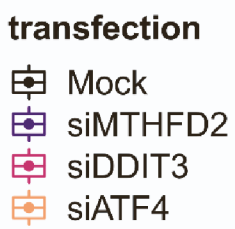
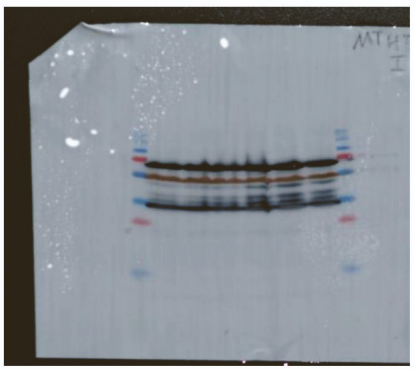


Figure S9. Validation of HepG2 MTHFD2-eGFP reporter cell line, related to Figure 4.
HepG2 BAC-eGFP MTHFD2 western blots (3 biological replicates) stained with anti-MTHFD2 antibody.

HepG2 BAC-GFP MTHFD2 Western Blot
Antibody: Anti-MTHFD2

Biological replicate 1



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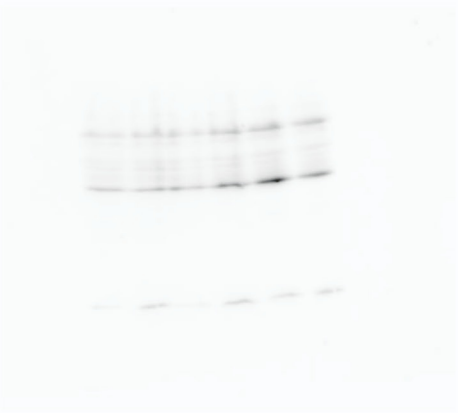
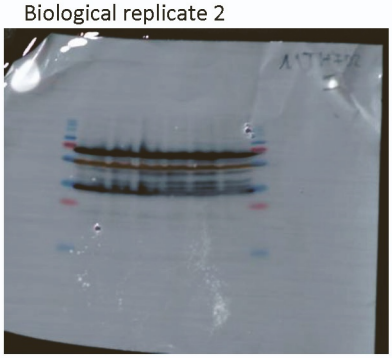


Raw image intensity optimized

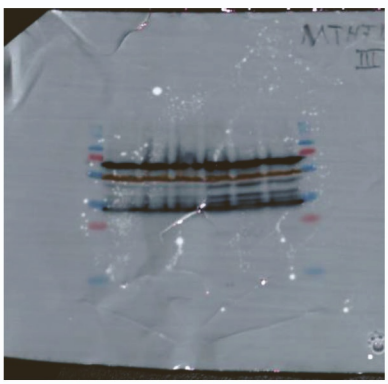
Biological replicate 2



Raw image size marker

Raw image intensity optimized

Biological replicate 3



Raw image size marker

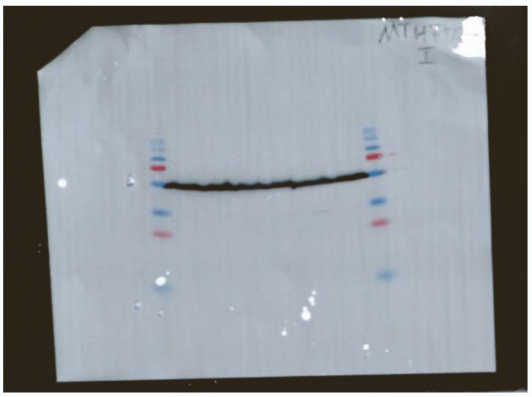
Raw image intensity optimized

Slot 1: Medium
Slot 2: DMSO
Slot 3: Tunicamycin 0.024 µM
Slot 4: Tunicamycin 0.24 µM
Slot 5: Tunicamycin 2.4 µM
Slot 6: Tunicamycin 24 µM

Figure S10. Validation of HepG2 MTHFD2-eGFP reporter cell line, related to Figure 4.
HepG2 BAC-eGFP MTHFD2 western blots (3 biological replicates) stained with anti-alpha-tubulin antibody.

HepG2 BAC-GFP MTHFD2 Western Blot
Antibody: Anti-alpha-tubulin

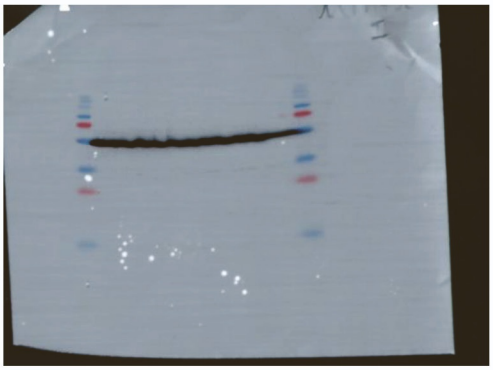
Biological replicate 1



Raw image size marker

Raw image intensity optimized

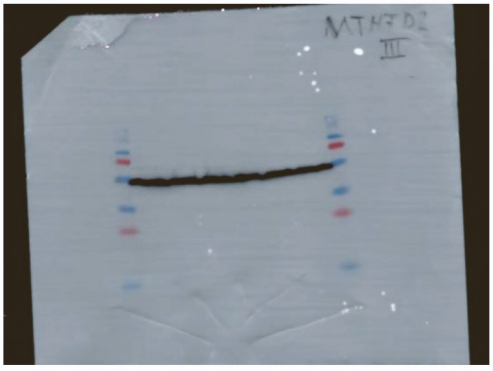
Biological replicate 2



Raw image size marker

Raw image intensity optimized

Biological replicate 3

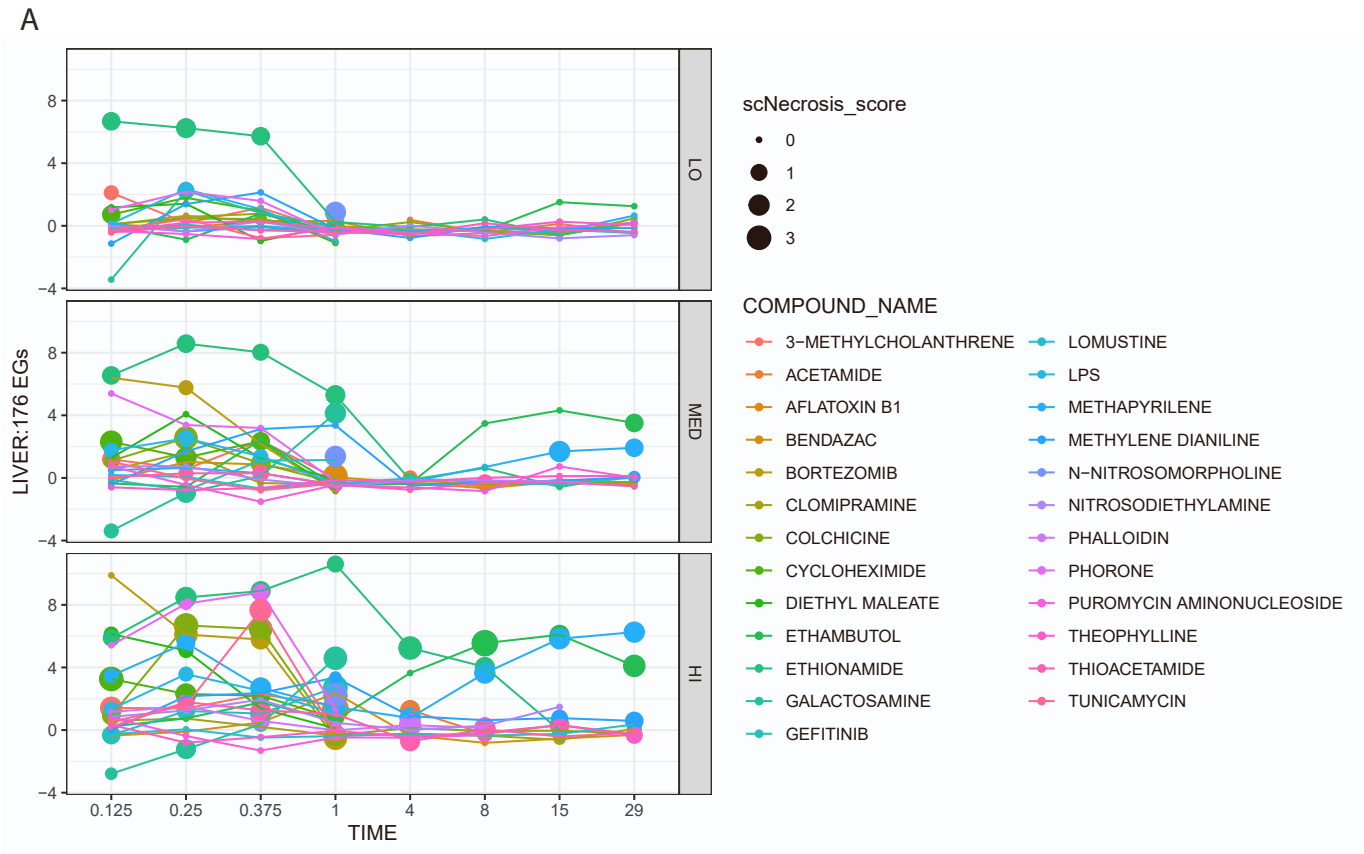


Raw image size marker

Raw image intensity optimized

Slot 1: Medium
Slot 2: DMSO
Slot 3: Tunicamycin 0.024 µM
Slot 4: Tunicamycin 0.24 µM
Slot 5: Tunicamycin 2.4 µM
Slot 6: Tunicamycin 24 µM

Figure S11. Selection of compounds for HepG2 TRIB3-eGFP and HepG2 MTHFD2-eGFP screen, related to Figure 5.
A) Time and dose-response plot of TG:rLIV:176 EGs across compounds inducing single cell necrosis in TG rat in vivo data. The size of each circle indicates the average score of single cell necrosis. B) Scatter plot of Pearson correlations between TG:rLIV:176 EGs and single cell necrosis score (y axis) and between Trib3 rat in vivo log2FC and single cell necrosis score. The color indicate the compounds were included to carry on the screening.



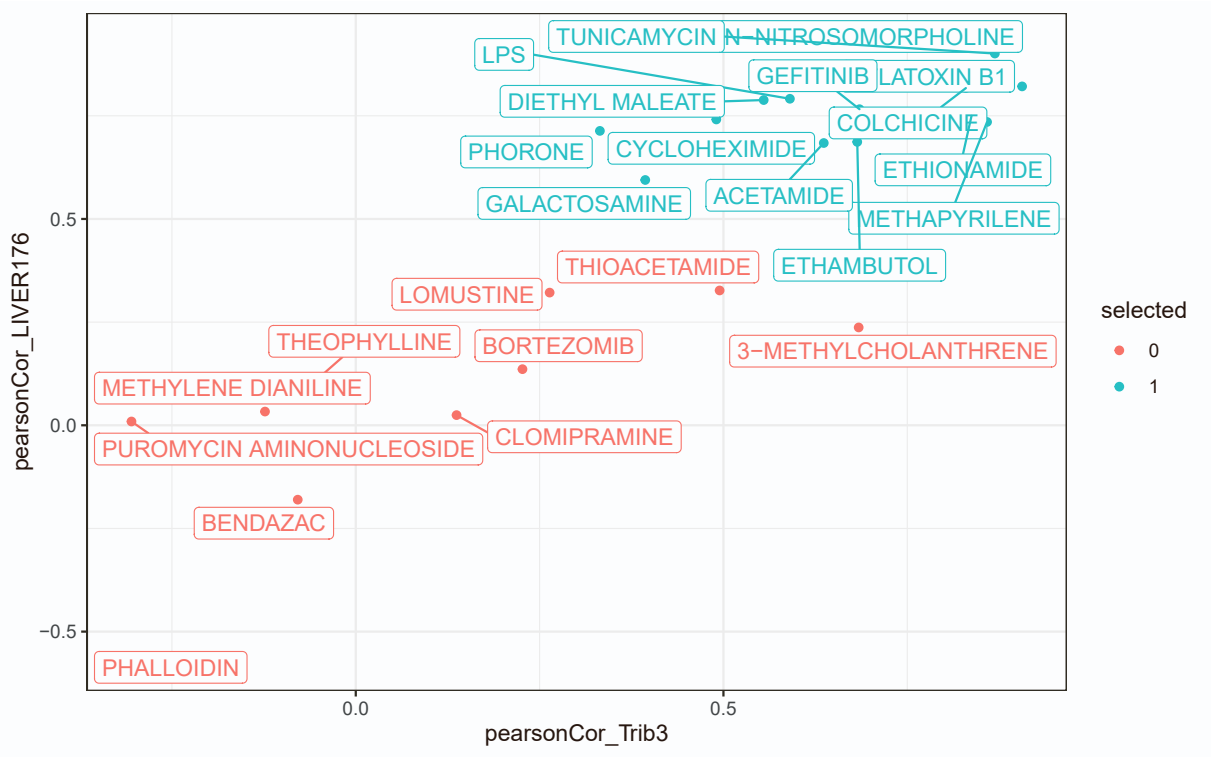


Figure S12. Rat liver and PHH gene expression data for TRIB3, MTHFD2 and DDIT3 for the compounds included in the screening, related to Figure 5. Rat liver and PHH gene expression data for TRIB3, MTHFD2 and DDIT3 for the scNecrosis4 positive compounds included in the screening (A and B), for the scNecrosis4 negative compounds, no other pathologies (C and D), for the scNecrosis4 negative compounds but positive for pathologies, in cluster I (E and F) and cluster II of Figure 5 (G and H). The size of the circles represents the scNecrosis average score.

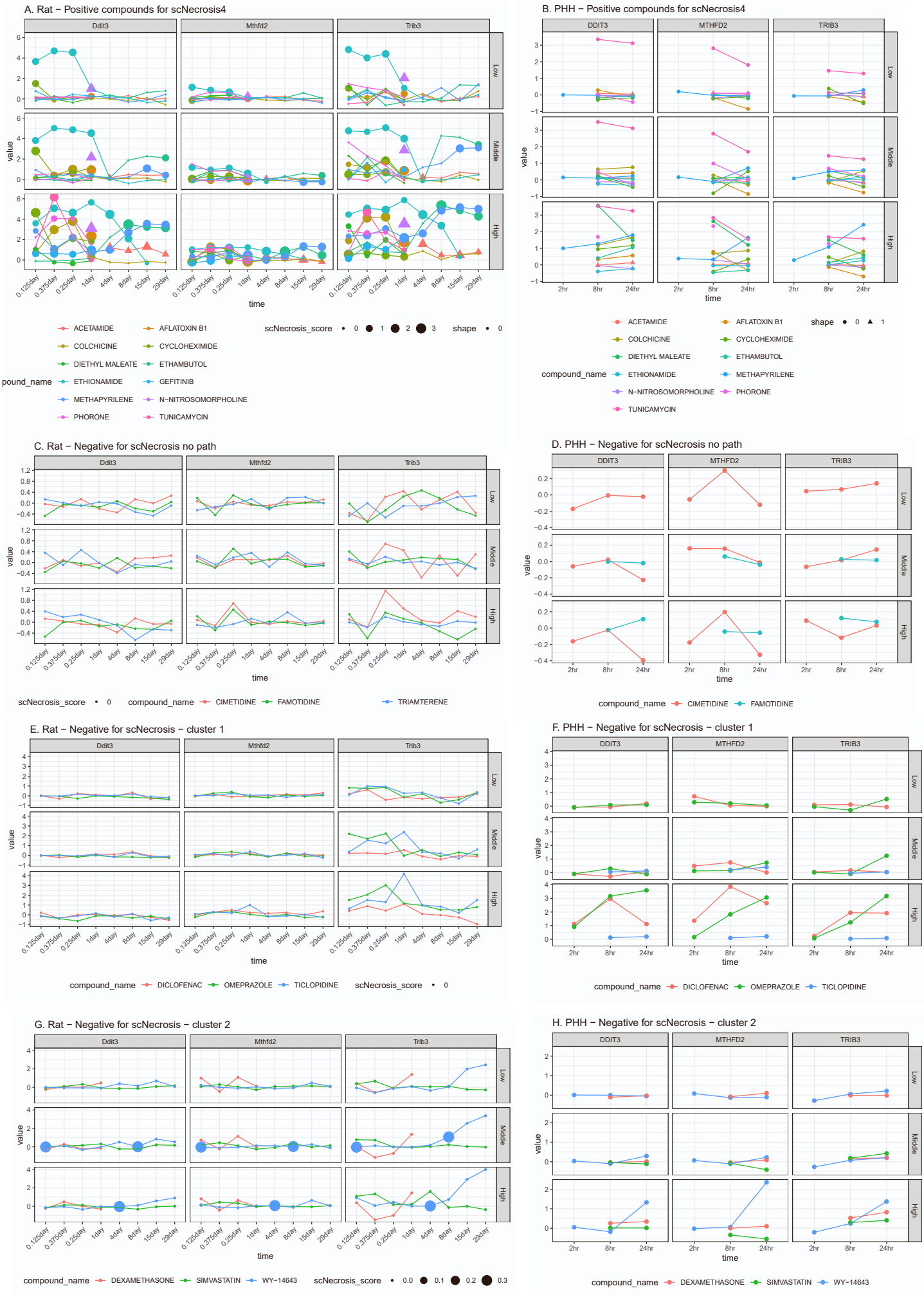


Figure S13. HepG2 TRIB3-eGFP and HepG2 MTHFD2-eGFP compound screening, related to Figure 5. The upper panel shows a direct comparison of the reporter cell lines based on their microscopic images. The lower panel shows an overview of all compounds' activation of the reporter cell lines based on the fraction of eGFP positive cells.

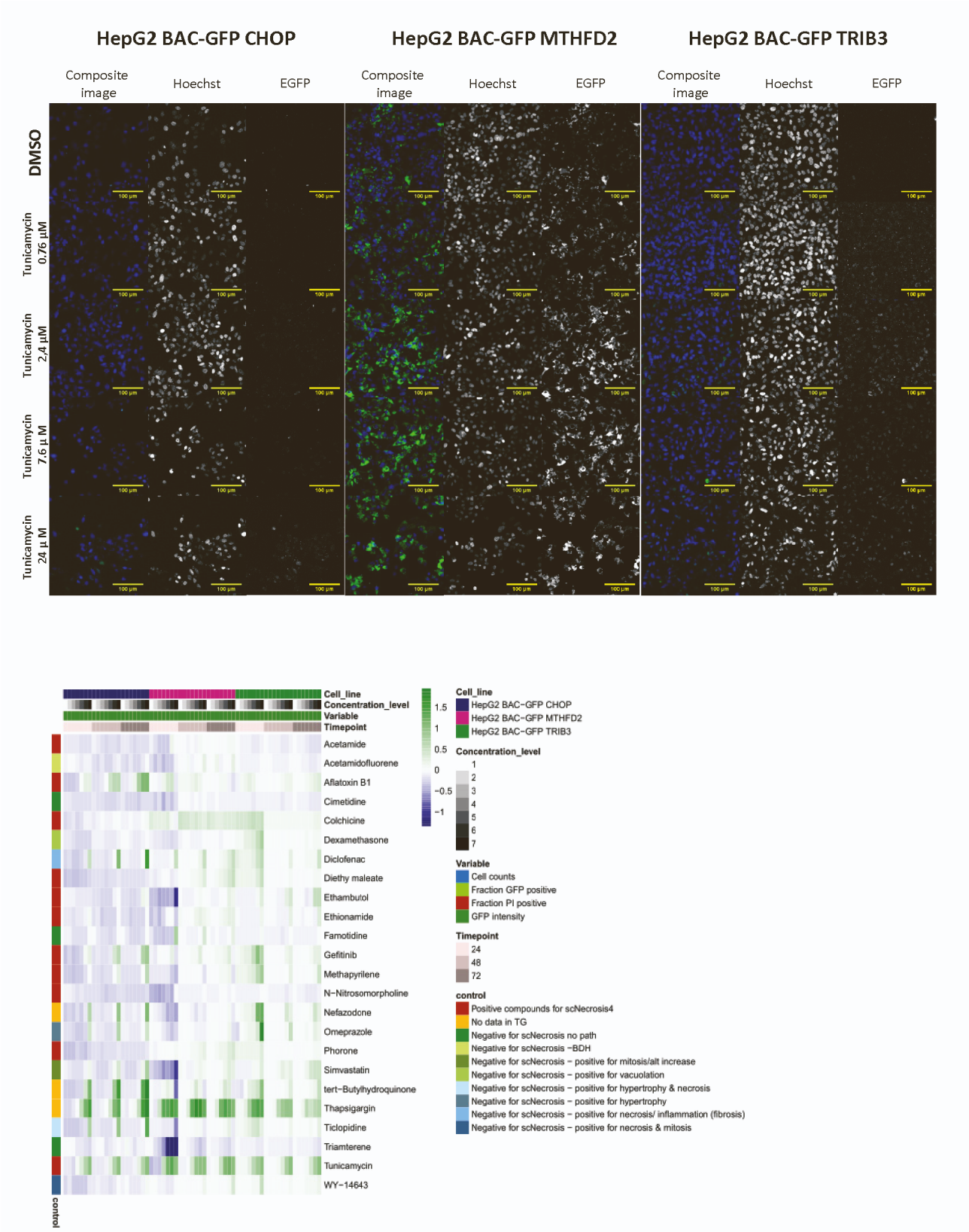


Figure S14. HepG2 TRIB3-eGFP and HepG2 MTHFD2-eGFP compound screening, related to Figure 5. Upper panel contains the normalized eGFP intensity in the nucleus (CHOP, TRIB3) or the cytoplasm (MTHFD2). Middle panel displays the normalized cell count (x1000) compared to the DMSO control of the individual cell line at the individual point. Lower panel show the fraction of PI positive cells compared to the DMSO control.