iScience, Volume *24*

Supplemental information

Landscape of functional interactions of human processive ribonucleases revealed by high-throughput siRNA screenings

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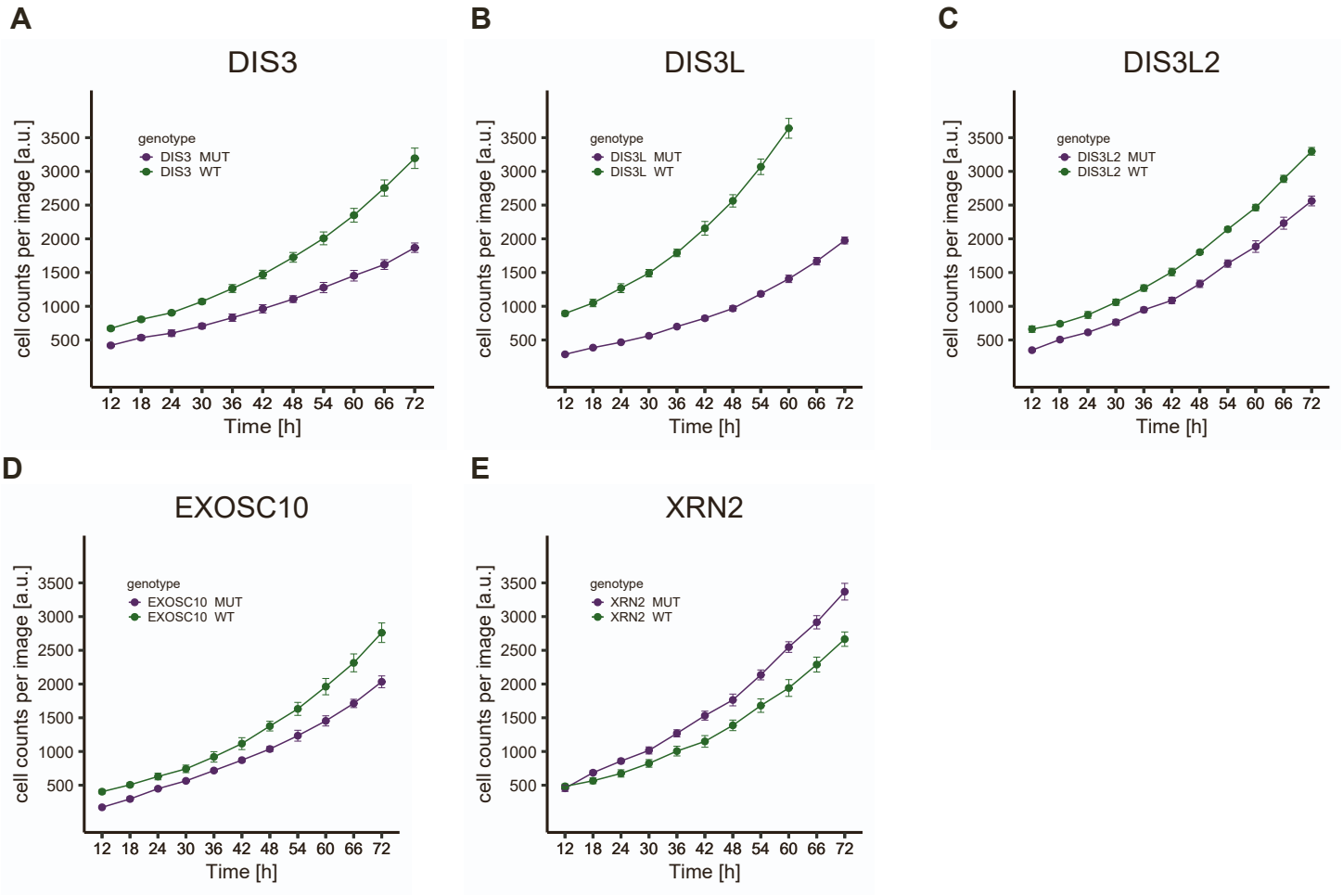


Figure S1

Mutations introduced in processive ribonucleases had different impacts on cell proliferation, related to Figure 1

The graphs represent the cells growth curve plotting cell counts versus time at 6-hour intervals for cells with expression of WT (green) and MUT (violet) version of (A) DIS3, (B) DIS3L, (C) DIS3L2, (D) EXOSC10 and (E) XRN2.
Growth rate was determined using the Incucyte real-time cell imaging system. Results are expressed as mean ± SD of three replicates.

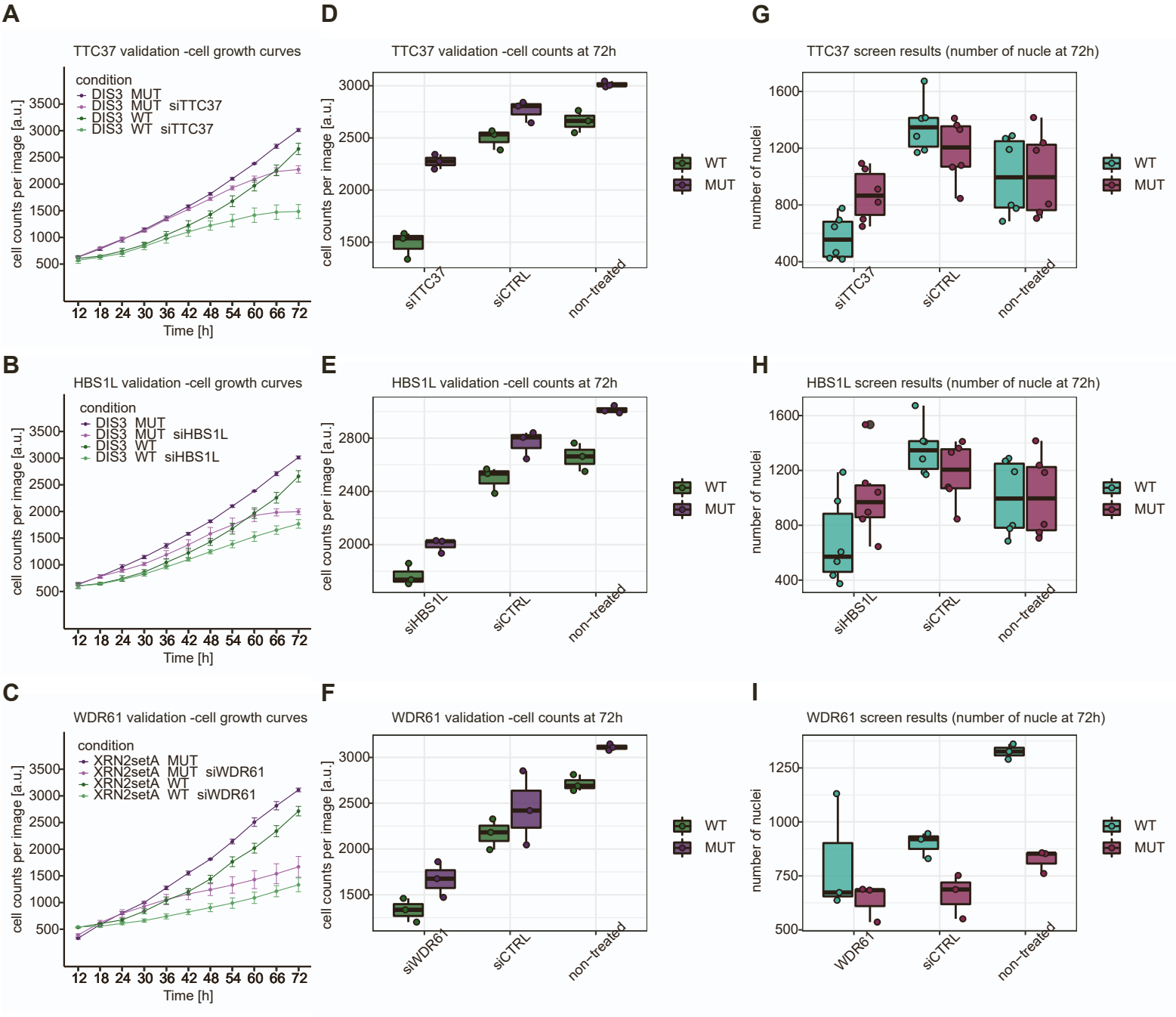


Figure S2

Validation of selected hits of custom core RNA metabolism siRNA screen for human processive ribonucleases, related to Figure 1

A-C. The graphs represent the cells growth curve plotting cell counts versus time at 6-hour intervals for cells with expression of WT (green) and MUT (violet) version of DIS3 (A and B) and XRN2 (C) transfected with siRNA for TTC37, HBS1L or WDR61, respectively. For the control of transfection influence cells were also treated with control siRNA. Growth rate was determined using the Incucyte real-time cell imaging system. Results are expressed as mean ± SD of three replicates.

D-F. The graphs represent results of counting cell number per well at 72 hours post siRNA transfection of cell lines described above. For the statistic data from real-time growth rate measurement at point 72 hours were taken.

G-I. Number of nuclei per cell line expressing wild type or mutated version of query ribonuclease as measured in the primary core metabolism screen. The data represents raw measurements from the screen that were used to calculate relative fitness expressed as a green fraction and further transformed into z-scores.

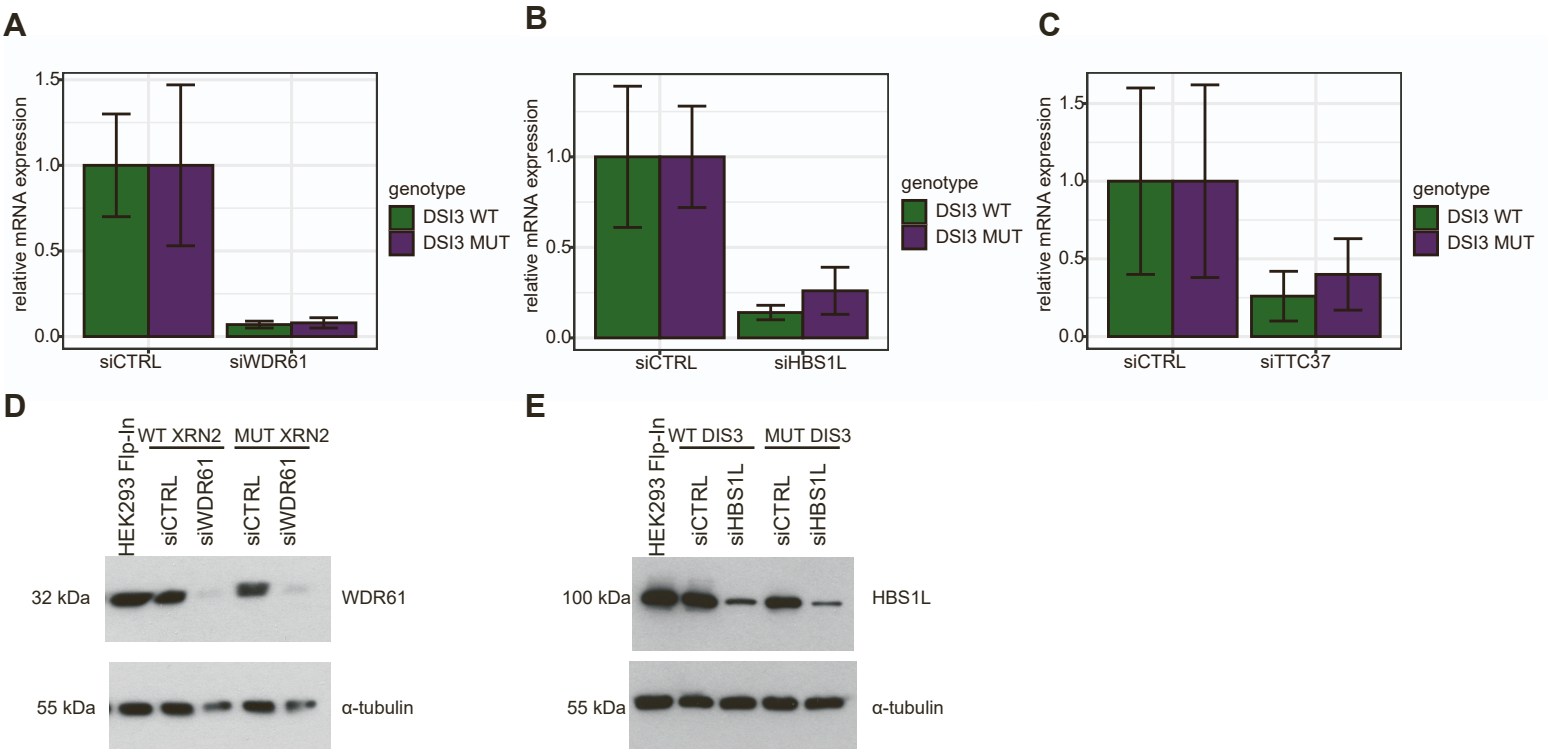


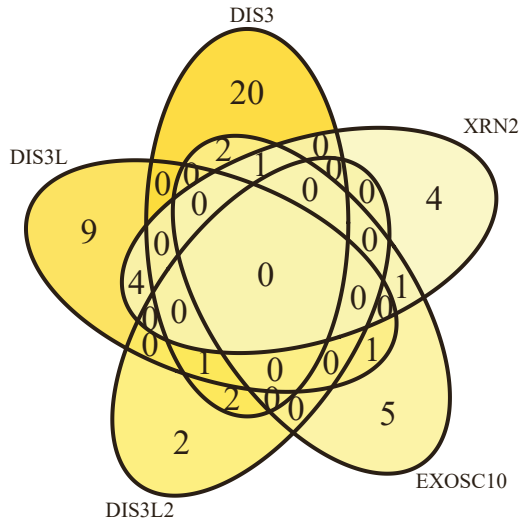
Figure S3

Confirmation of silencing of siRNA used in hit validation of core RNA metabolism results, related to Figures 1 and S2

A-C. Relative expression level of mRNA of WDR61 (A), HBS1L (B), and TTC37 as measured with RT-qPCR. For the normalization, GAPDH was used. The mRNA level was measured in cells with expression of WT (green) and MUT (violet) version of DIS3 (A and B) and XRN2 (C) transfected with siRNA for TTC37, HBS1L or WDR61, respectively.

D,E. Representative images of western blot analysis of WDR61 (D) and HBS1L (E) protein level in cell lines described above. The images are representative of three experiments that were performed in parallel with real-time growth analysis to confirm the transfection efficiency. The level of TTC37 gene protein product was not assayed as we could not find any properly working antibody. As loading control, we were detecting α-tubulin.

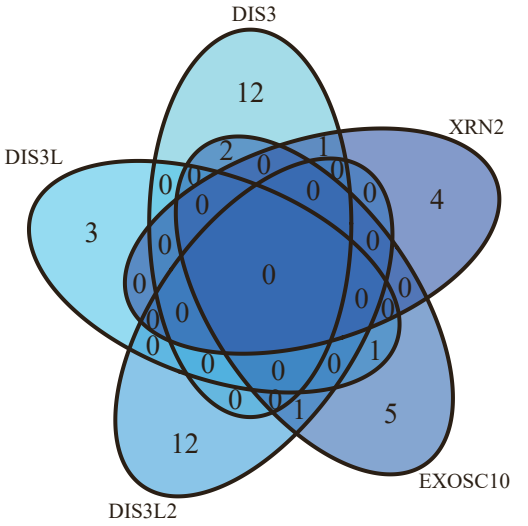
A



B

POS / POS	DIS3	DIS3L	DIS3L2	EXOSC10	XRN2
DIS3		**DDX19B**	THOC2 **DDX19B** NXF1	CPSF1 **POLR2A** THOC1	**POLR2A**
DIS3L			**DDX19B**	DGCR8	DDX39B DHX8 DROSHA SF3B1
DIS3L2					
EXOSC10					**POLR2A** SNRPD3
XRN2					

C



D

NEG / NEG	DIS3	DIS3L	DIS3L2	EXOSC10	XRN2
DIS3					
DIS3L					
DIS3L2					
EXOSC10	EXOSC3 MTREX	SMG8	DDX56		
XRN2	C1D				

E

POS / NEG	DIS3	DIS3L	DIS3L2	EXOSC10	XRN2
DIS3			ZFP36	C1D	
DIS3L					
DIS3L2	DDX56 DDX6 EDC4 NCBP2 TOB1			SNRPD3	SNRPD3 SNRPB PIWI2L
EXOSC10	DDX56 SNRPD1				
XRN2				C1D	

Figure S4

Summary of overlap of negative and positive genetic interactions with main human RNases, identified in the siRNA screen with a custom core RNA metabolism siRNA library, related to Figure 1, Tables S1 and S2

A, C. Venn diagrams representing shared and unique genetic interactions for DIS3, DIS3L, DIS3L2, EXOSC10 and XRN2. The overlap was analyzed separately for negative (A) and positive (C) interactions.

B, D, E. Tables summarizing common interactions for DIS3, DIS3L, DIS3L2, EXOSC10, and XRN2 identified in custom core RNA metabolism siRNA screen. The tables were divided to summarize common negative interactions (B), suppressors (D), and interactions that were opposite for both ribonucleases (E). Genes showing the same genetic interactions with three ribonucleases are in bold, and genes that interact with three nucleases but in a complex way are underlined.

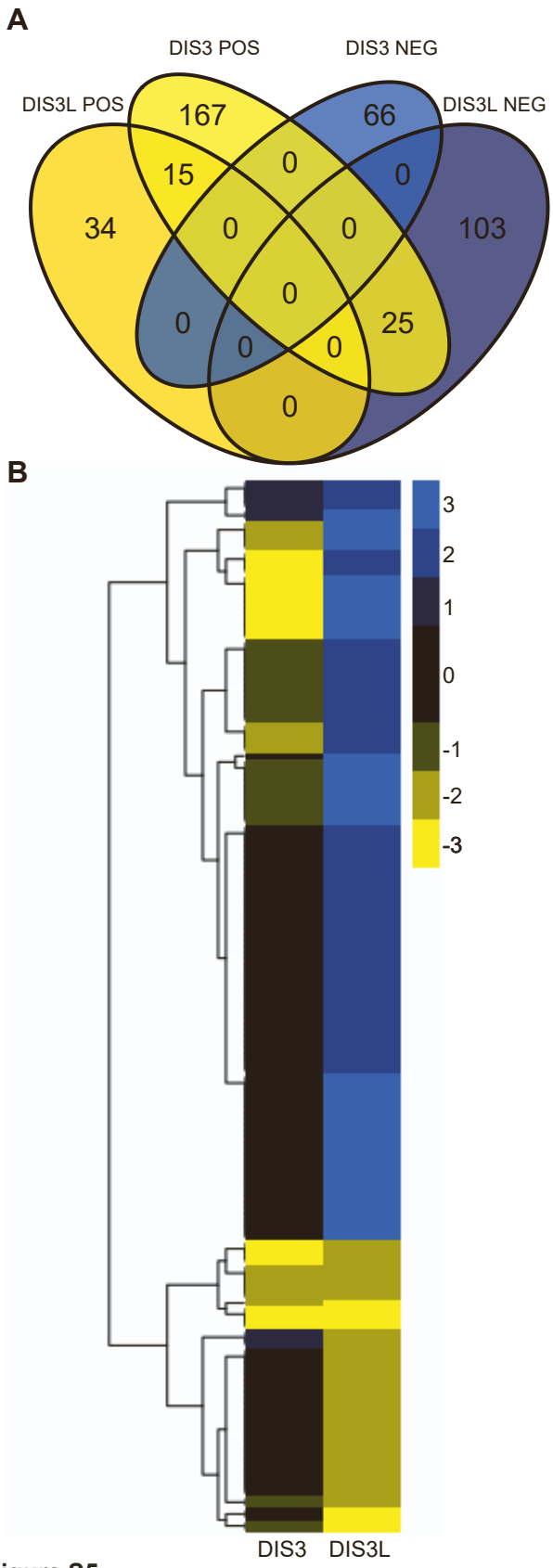


Figure S5

Summary of overlap of negative and positive interactions with DIS3 and DIS3L identified in the siRNA screen with a custom RNA metabolism extended siRNA library, related to Figure 4 and Tables S1 and S5

A. Venn diagrams show shared and unique genetic interactions for DIS3 and DIS3L.

B. Heatmap representation of summarized z* scores of genetic interactions between DIS3 or DIS3L and RNA metabolism genes. The plots represent scores for 410 of 3904 tested genes that were identified as a significant hit for DIS3 or DIS3L. Yellow represents positive hits (suppressors). Blue represents negative hits. Black represents genes that have no interactions.

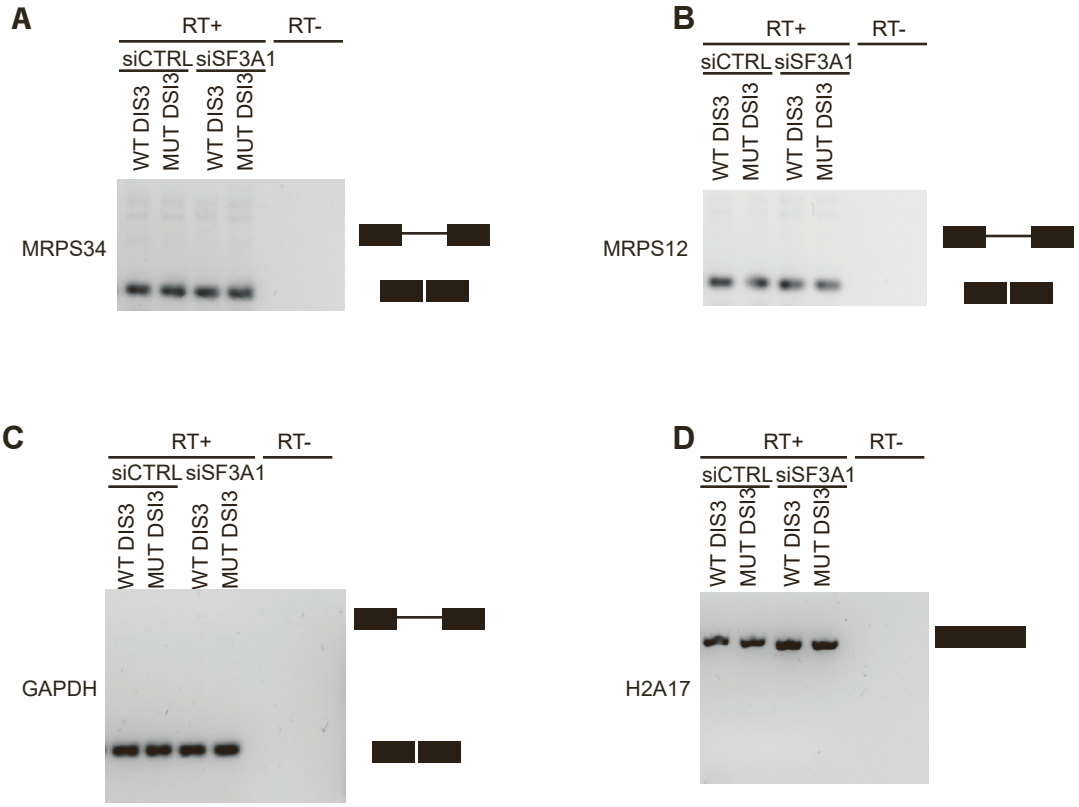


Figure S6

RT-PCR validation of two genes that showed intron retention in cells transfected with SF3A1 siRNA, related to Figure 5

The intron retention level was measured in cells expressing WT or MUT version of DIS3 and transfected with siRNA for SF3A1 or control siRNA for 72hours. The analyzed genes MRPS34 (A) and MRPS12 were showing the IR on the level of PSI -0.2 and could not be confirmed cases. As controls, we performed RT-PCR on a gene that did not show intron retention after SF3A1 depletion GAPDH (C) and a gene that is intronless - H2A17 (D)

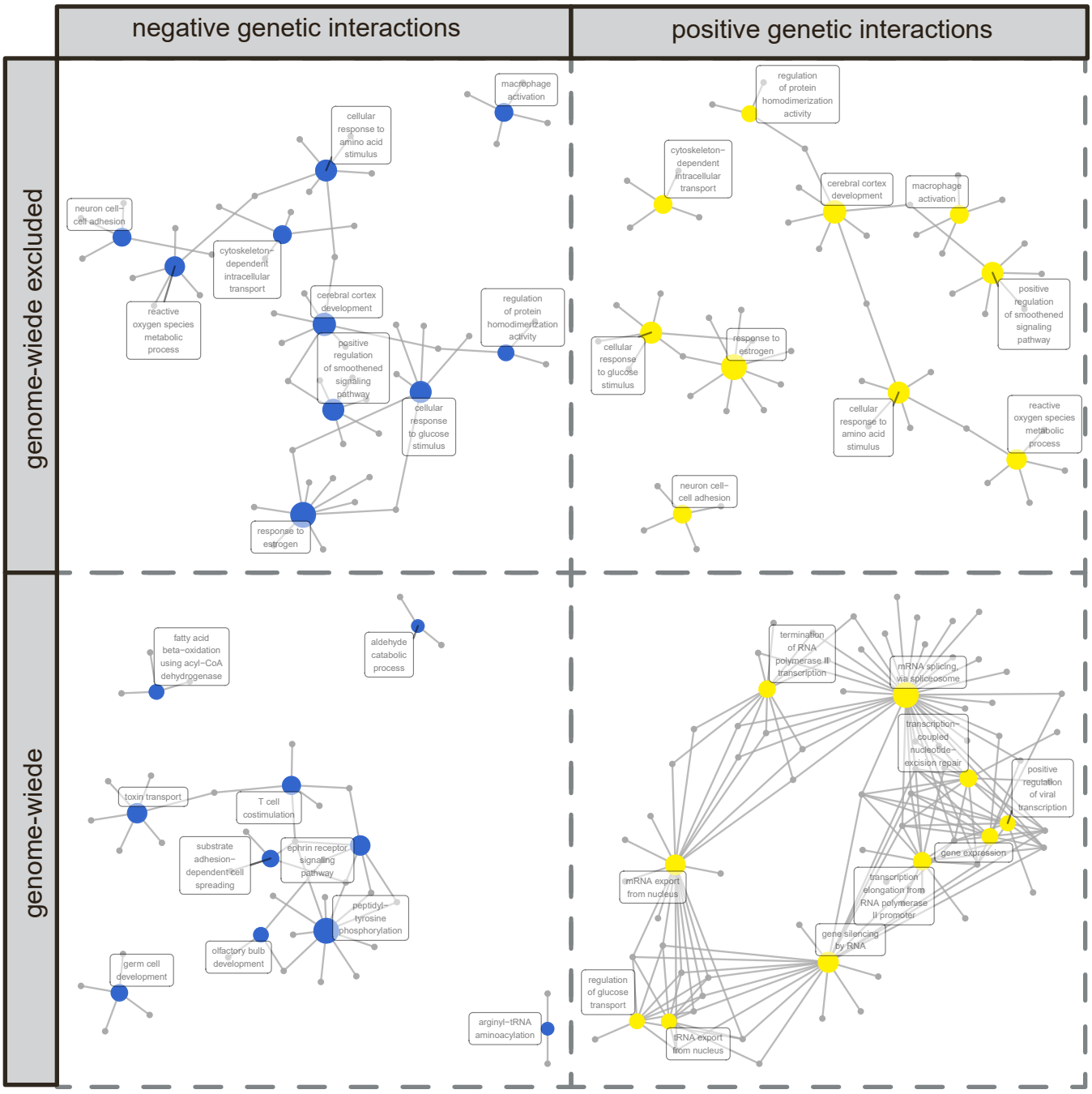


Figure S7

DIS3 genetic interactions are mainly involved in RNA metabolism processes, related to Figure 6 and Table S8

Networks illustrate interactions between biological processes that share genes that were identified in the genome-wide siRNA screen for DIS3 and in the subset of hits from the genome-wide screen that excluded hits identified in the extended RNA metabolism screen. The colored nodes (blue and yellow) represent biological processes that were interconnected and shared the same genes that were identified as synthetic lethal interactions or suppressors, respectively. The networks were constructed in R/Bioconductor based on the associations of hit genes with biological processes according to the DAVID direct GO database.

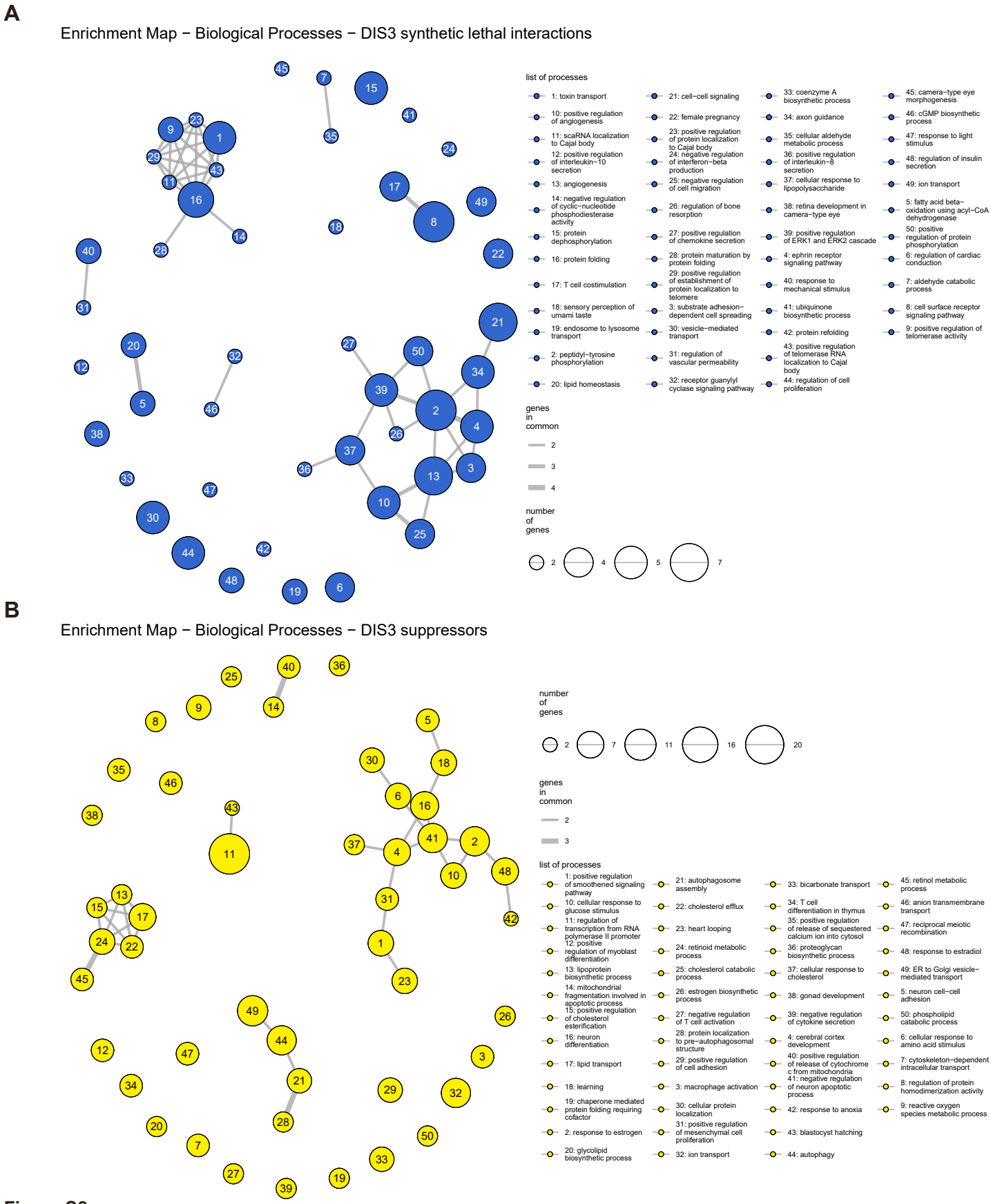


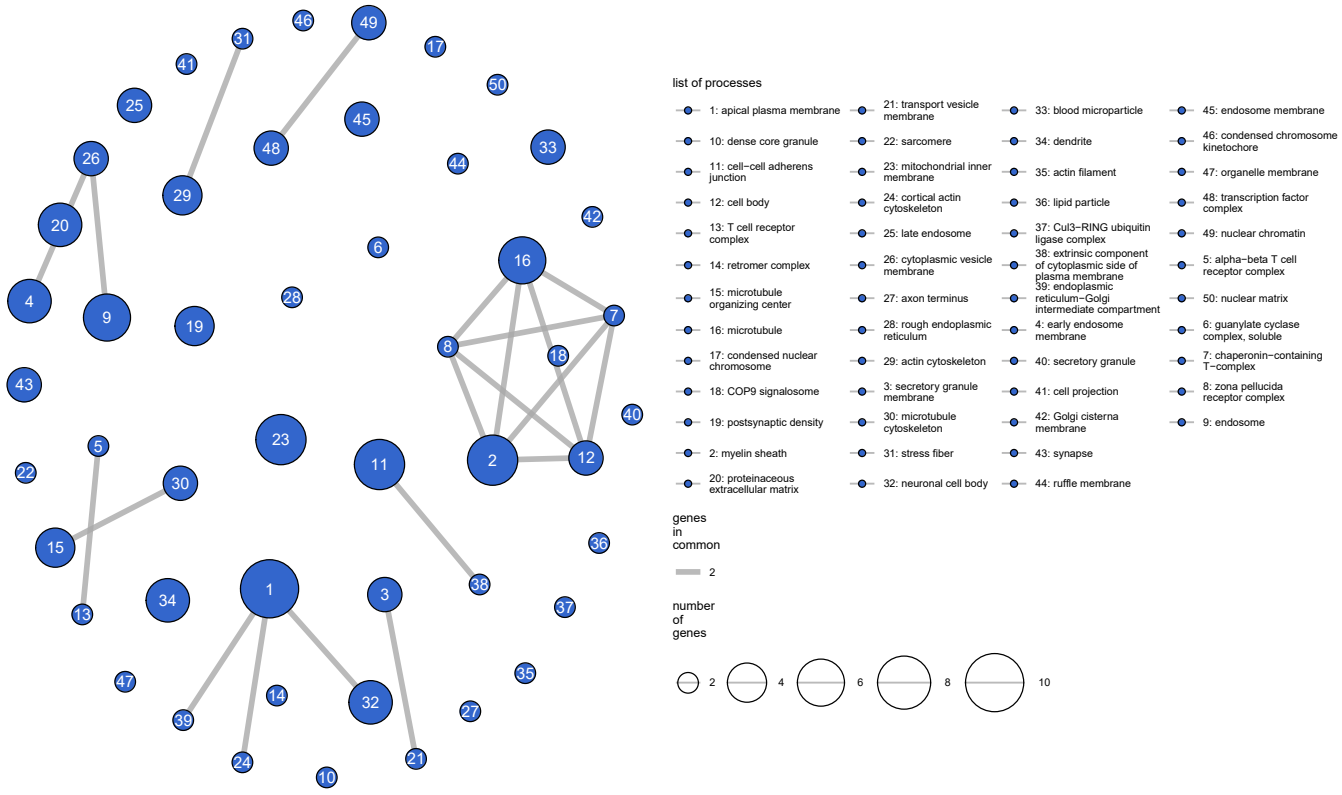
Figure S8

Enrichment map of Biological Processes associated with genes identified as genetic interactors of DIS3, related to Figure 6 and Table S8

A-B. The maps display clusters of biological processes enriched by genes that were identified as (A) negative genetic interactions and (B) suppressors of DIS3. The enrichment was performed on the subset of hits that were identified in genome-wide screen, excluding hits identified in the extended RNA metabolism screen. The networks were constructed in R/Bioconductor based on associations of hit genes with biological processes according to the DAVID direct GO database.

A

Enrichment Map − Cellular Compartment − DIS3 synthetic lethal interactions



B

Enrichment Map − Cellular compartment − DIS3 suppressors

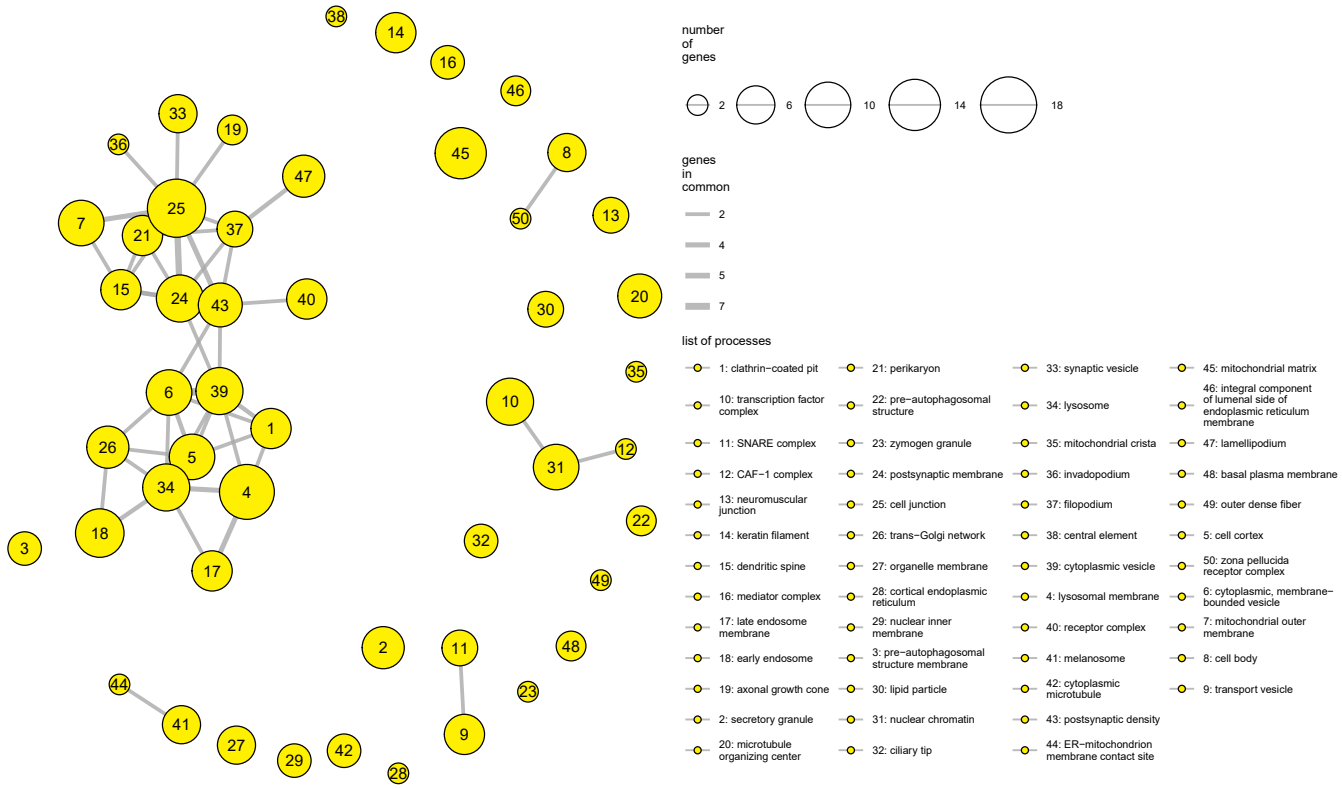


Figure S9

Enrichment map of Cellular Components associated with genes identified as genetic interactions of DIS3, related to Figure 6 and Table S8

A-B. The maps display clusters of cellular components that were enriched by genes identified as (A) negative genetic interactions and (B) suppressors of DIS3. The enrichment was performed on the subset of hits that were identified in the genome-wide screen, excluding hits identified in the extended RNA metabolism screen. The networks were constructed in R/Bioconductor based on associations of hit genes with biological processes according to the DAVID direct GO database.

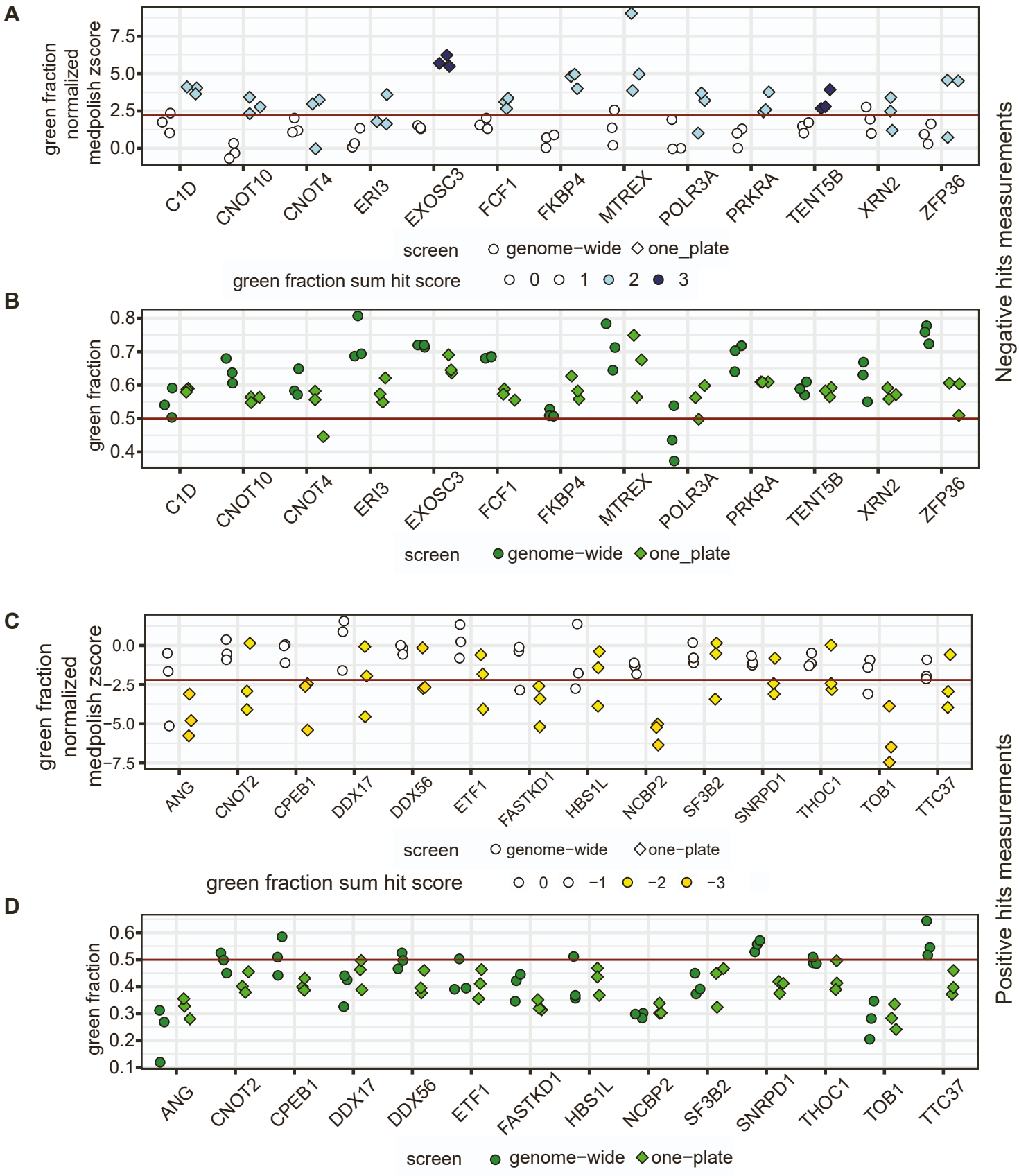


Figure S10

Impact of normalization on hit identification differences between core RNA metabolism siRNA screen and genome-wide siRNA screen for DIS3, related to STAR methods - Quantification and statistics in RNAi screening

The dot plots represent median polish normalized green fractions (quantitative phenotype measure assayed in siRNA screening) (A and C) for genes that were identified as negative (A) and positive (C) hits in core metabolism RNA siRNA screen but not observed as significant hits in genome-wide siRNA screen. For comparison, raw data green fractions (quantitative phenotype measure assayed in siRNA screening) (B and D) for genes that were identified as negative (B) and positive (D).

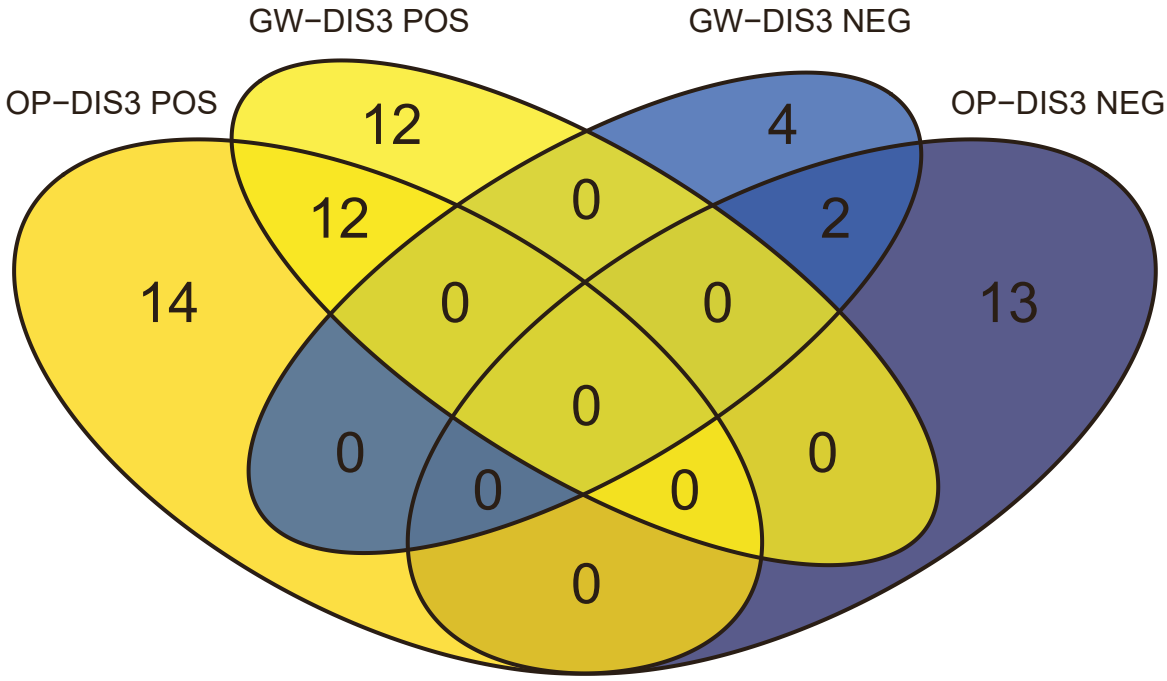


Figure S11

Comparison of core RNA metabolism siRNA screen to genome-wide siRNA screen for DIS3, related to STAR methods - Quantification and statistics in RNAi screening

Venn diagram representing overlap between genes with negative - NEG (blue) and positive - POS (yellow) genetic interaction with DIS3 identified in core RNA metabolism siRNA screen (one-plate; OP) and genome-wide siRNA screen (GW).