

Supplemental figure legends

Supplemental figure S1. High efficiency transfection in DRiF-library recipient cell lines 427-TTS and AnTat-TTS. A) Consistency and stability of high transfection efficiencies across clones containing a DRiF-compatible self-cleaving construct inserted at a silent *VSG* locus on a minichromosome. Double-strand break at the target locus is induced by the addition of tetracycline (+Tet). Optimisation of time of double-strand break induction (B) and mass of plasmid DNA (C) in 427-TTS. D) Performance of a similar strategy in EATRO1125 bloodstream-form cells.

Supplemental figure S2. Comparison of measured gene effect sizes and variance in RIT-Seq and DRiF-Seq experiments in Lister 427 and EATRO1125 at different times post-induction of RNAi. RIT-Seq data are from 7435 non-redundant TREU927 genes in (Alsford *et al.* 2011). Genes identified as associated with a significant gain- or loss-of-fitness in previous analysis by DEGSeq ($|Z| > 3.3$) are indicated (red) alongside reanalysis of the data by *a posteriori* noise estimation (blue lines at $\text{fdr} = 0.1$ and 0.01). DRiF-Seq libraries in Lister 427 (16152 genes) or EATRO1125 (14211 genes) show *apNE* fdr only. Gene sets associated with select biological functions are highlighted as in Fig. 4.

Supplemental figure S3. Application of *a posteriori* noise estimation to genome-wide CRISPR data from *Toxoplasma* (Sidik *et al.*, 2016). A) Calculation of Z scores for 8158 targetted genes based on either the sum of counts for all sgRNAs targetting a gene or the median sgRNA effect for each gene. The expected null distribution estimated from the fitted variance of the data is overlaid in red. The number of genes detected by *apNE* as enriched/depleted at $\text{fdr} = 0.1$ or 0.01 are shown in blue, in addition to percentage over-representation of genes in each distribution tail. B) Distribution of 'phenotype' scores (mean $\log_2$ fold change in the normalised counts of the top five scoring sgRNAs for each gene) showing the position of minimum Z-scores (genes inferred by *apNE* to have no effect). C) Volcano plot showing distribution of adjusted p-values for genes calculated by *apNE* against their P6 phenotype score.

Supplemental figure S4. Quantitative enrichment analysis for fitness effect sizes associated with GO Terms in Biological Process, Cellular Component and Molecular Function ontologies. Terms that have fitness effect sizes significantly different (adjusted p-value ≤ 0.05 ; Monte Carlo permutation test) from the average effect size of genes with GO annotation. Circle size indicates number of measured genes associated with each GO term. For clarity, GO descriptors for some terms with lower significance are not shown. Full data are available in Suppl. Data File 8.

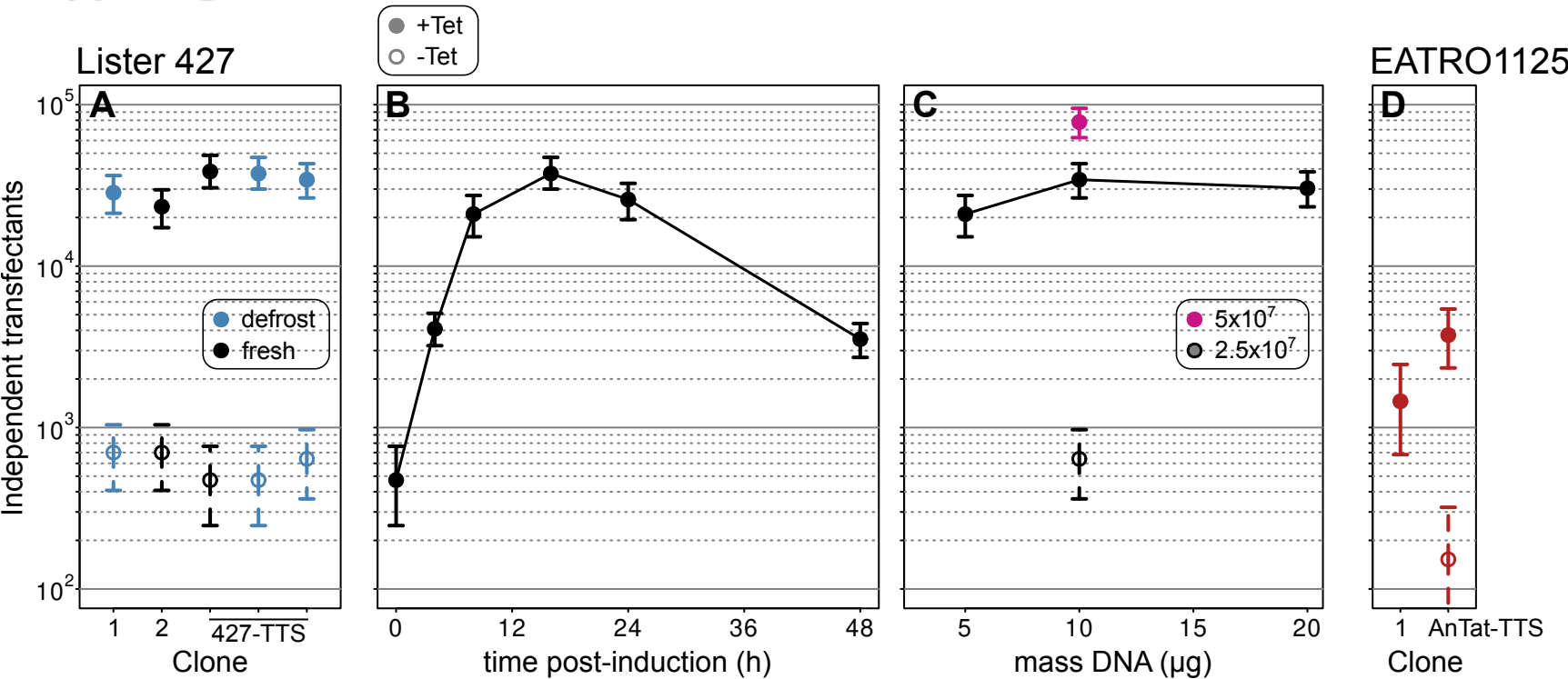
Supplemental figure S5. Distribution of DRiF-Seq fitness effect sizes for gene products localised to different subcellular compartments. Localisation annotation is from the TrypTag genome-wide protein localisation database (Billington *et al.* 2023). Effect sizes are for EATRO1125 at 6 days post-induction. The number of genes associated with each annotation and the proportion with significant fitness cost at $\text{fdr} \leq 0.01$ are indicated above each distribution.

Supplemental figure S6. Comparison of mRNA and protein characteristics with DRiF-Seq fragment fitness cost. All effect sizes are for EATRO1125 at day 6 post-induction. A) Mean effect size distributions for fragments targetting annotated 5'UTR, CDS or 3'UTR of 123 genes with fragments specifically targetting all 3 regions. Data are derived from a remapping of the fragment count data to the TREU927 genome to take advantage of the UTR annotation and only fragments entirely encompassed within each feature are included. B) Relationship between differential fragment effect size (difference between specific fragment effect size and median effect of targetting fragments) and differential GC content of fragment. Fragments targetting 1280 genes associated with large loss-of-fitness (≥ 256 -fold depletion; $\text{fdr} \leq 0.01$) and targetted by ≥ 2 fragments are included. C) Relationship between measured mRNA (*Fadda et al. 2014*) and protein (*Tinti et al. 2019*) turnover in bloodstream-form cells and DRiF-Seq effect size. Correlations for both all genes for which data are available (All) and only those with ≥ 256 -fold depletion at $\text{fdr} \leq 0.01$ (LoF) are shown. Box-whisker plots for 8 quantiles are also shown. D) Proportion of genes with 'hypothetical', 'putative' or full functional annotation in each of 8 quantiles of mRNA half-life. E) Distributions of measured mRNA or protein half-lives for genes with different loss-of-fitness temporal classification (Fig. 8). Circle size above each category shows the number of genes for which data are available.

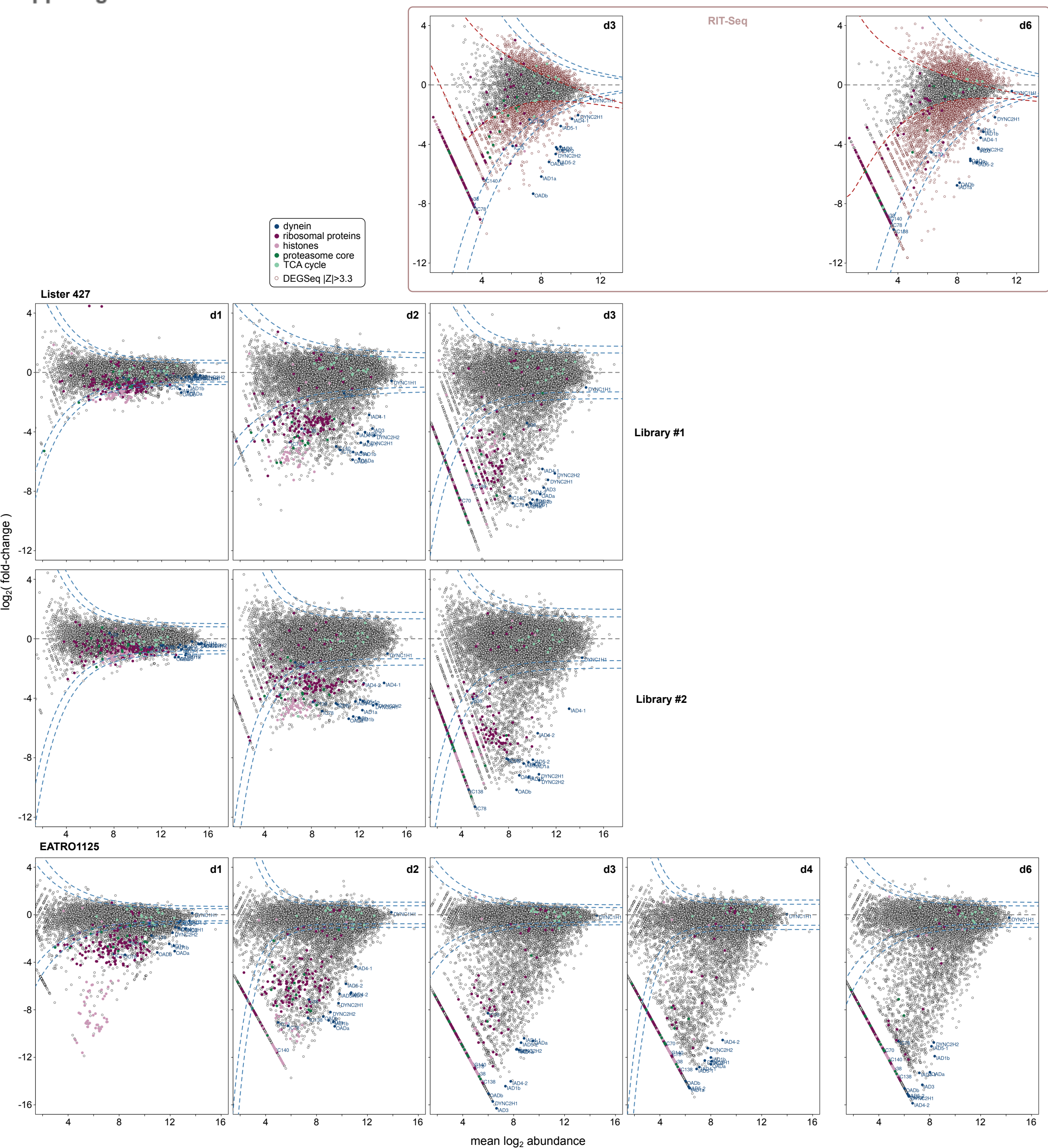
Supplemental figure S7. Validation of differential RNAi effect size inferred from genome-wide DRiF-Seq fitness estimates by individual RNAi. Growth of 2 clonal RNAi lines for RNAi targetting 10 genes in both Lister 427 (L427) and EATRO1125 (AnTat) in the presence and absence of induction of RNAi by tetracycline are shown. For clarity, cumulative cell numbers of Lister 427 cells have been offset by 100-fold. Arrows indicate times during growth where cultures were passaged into fresh medium.

Supplemental figure S8. Quantitative enrichment analysis for timing of loss-of-fitness profiles of GO Terms in Biological Process, Cellular Component and Molecular Function ontologies. GO terms that are significantly enriched (overall p -value ≤ 0.1) are highlighted. Circle size reflects the total number of genes encompassed by each GO term. Overall p -value for terms across timing profiles are the harmonic means of the adjusted p -values for enrichment in each of the 6 timing clusters. Enrichment and timing values are arithmetic means weighted by significance (see Methods). Descriptors are given for terms with significant enrichment. Full data are available in Suppl. Data File 10.

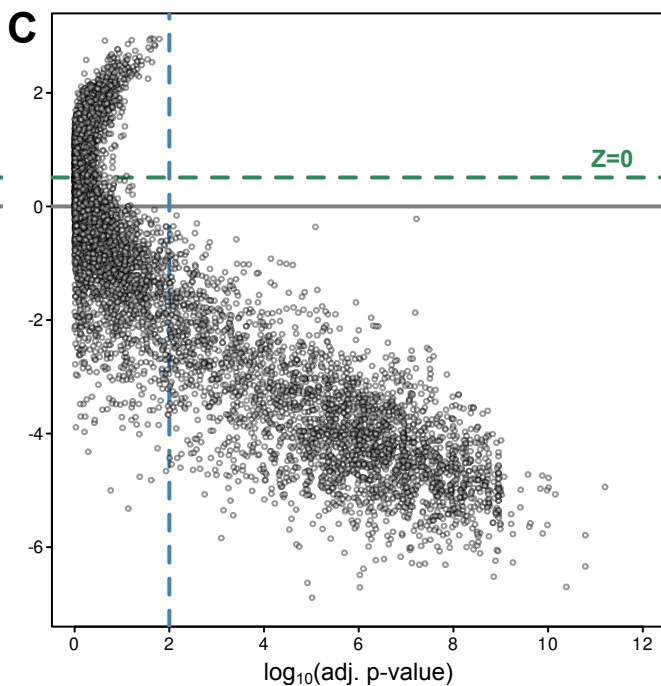
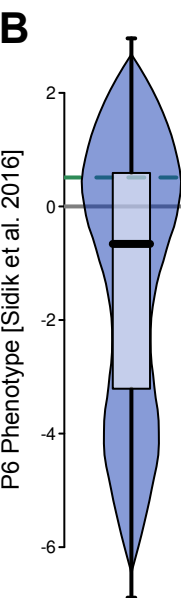
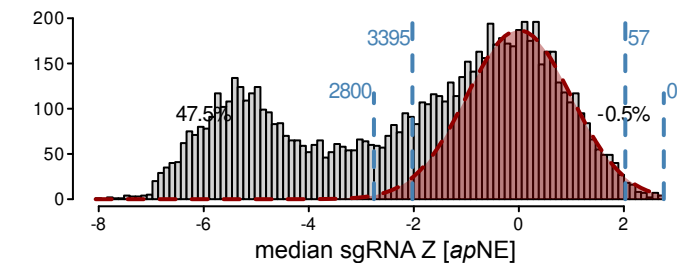
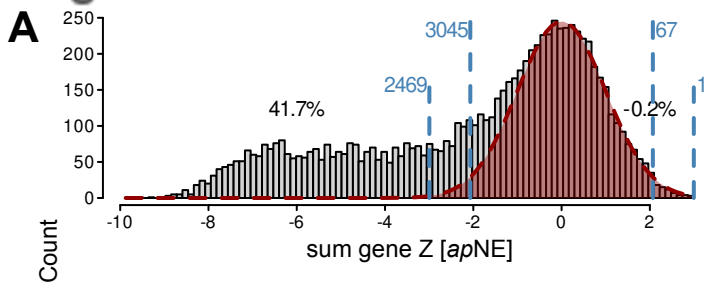
Suppl. Fig. 1



Suppl. Fig. 2



Suppl. Fig. 3



Biological process

Cellular component

Molecular function

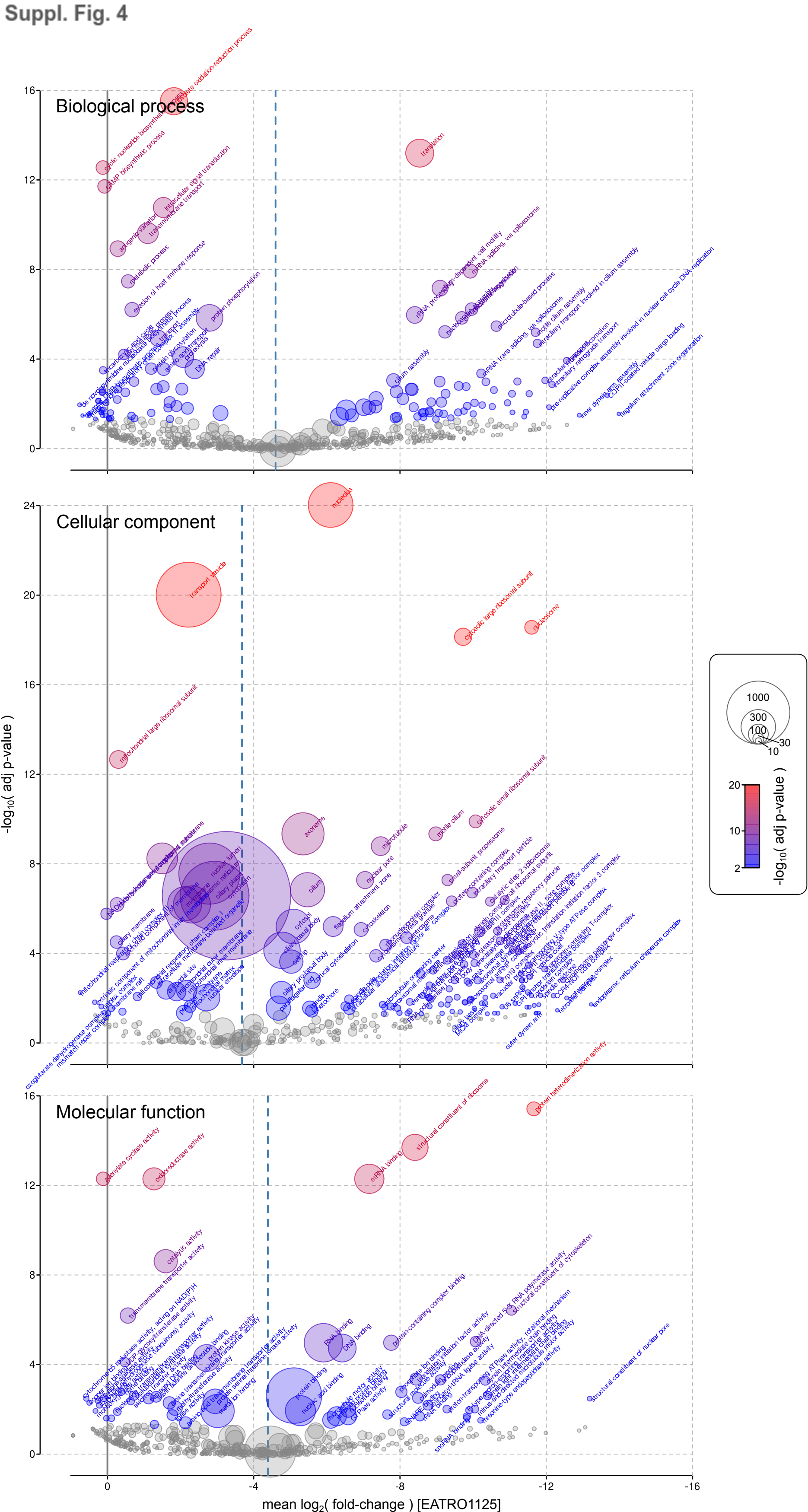
mean $\log_2$ (fold-change) [EATRO1125]

$-\log_{10}(\text{adj p-value})$

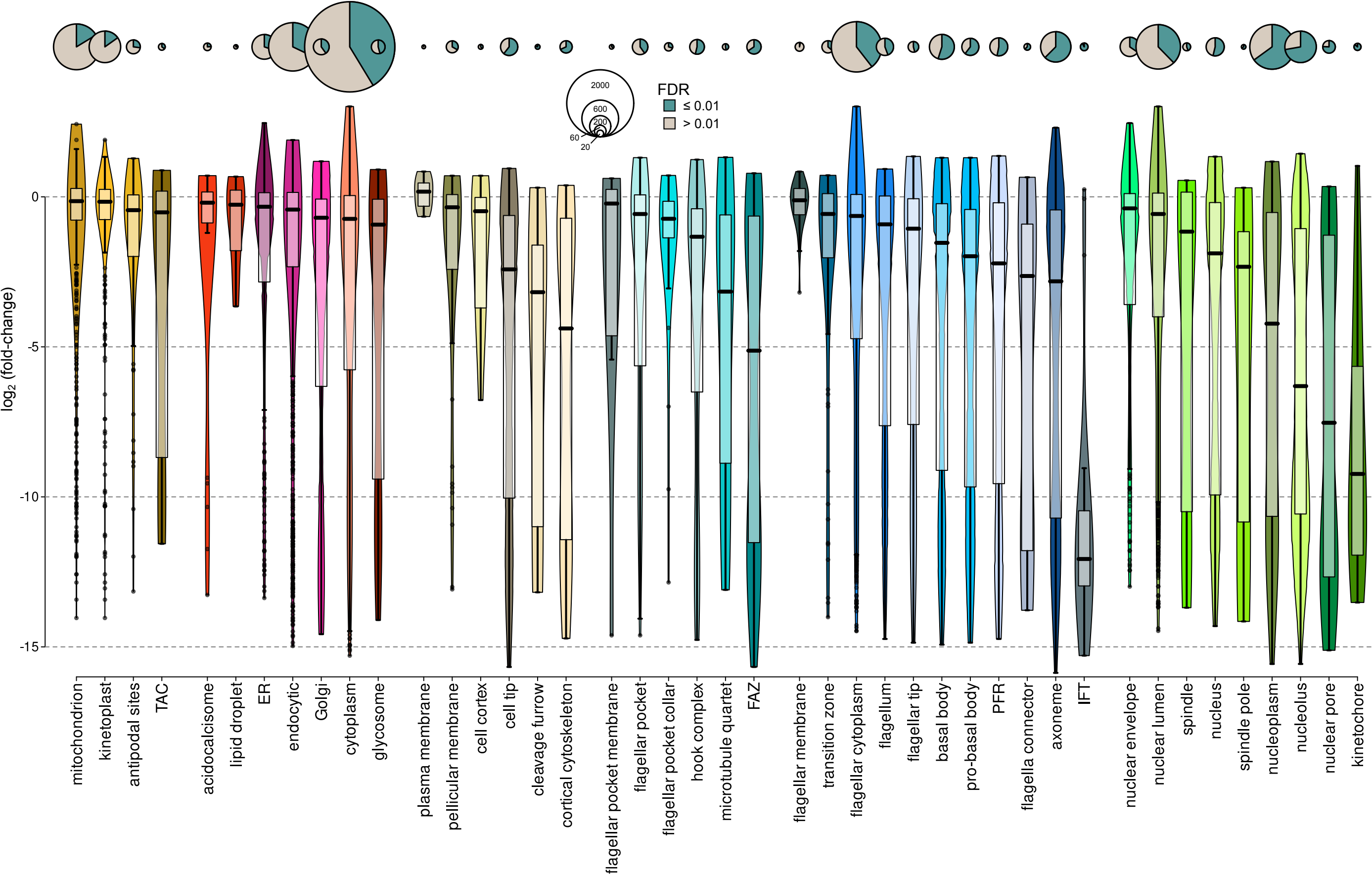
1000
300
100
30
10

20
10
2

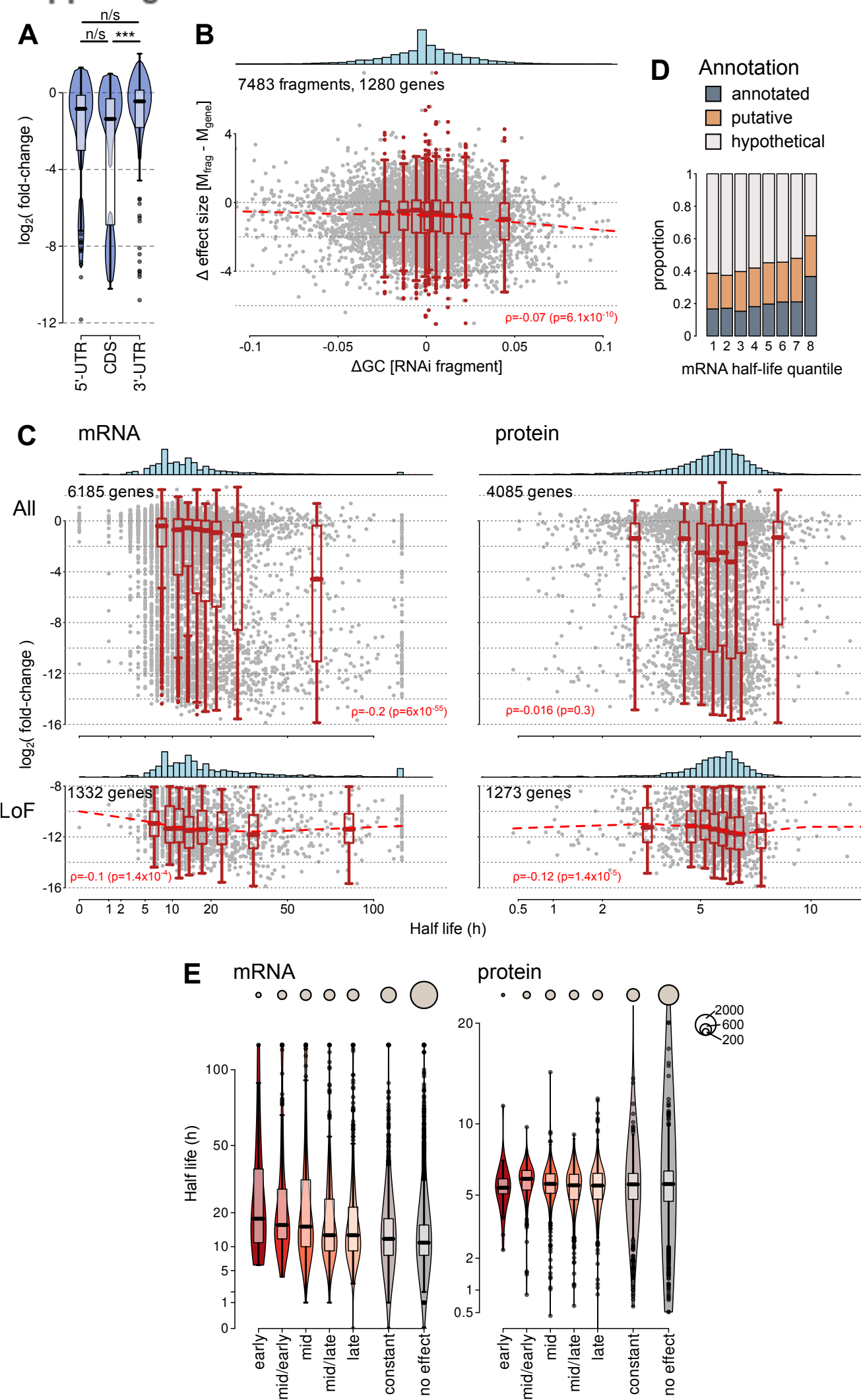
$-\log_{10}(\text{adj p-value})$



Suppl. Fig. 5

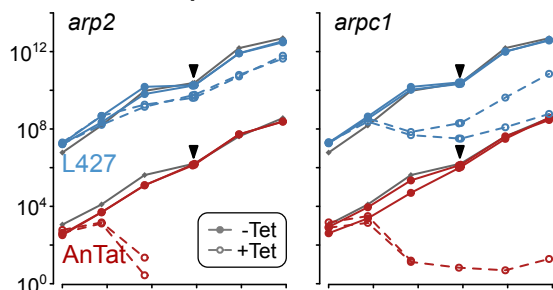


Suppl. Fig. 6

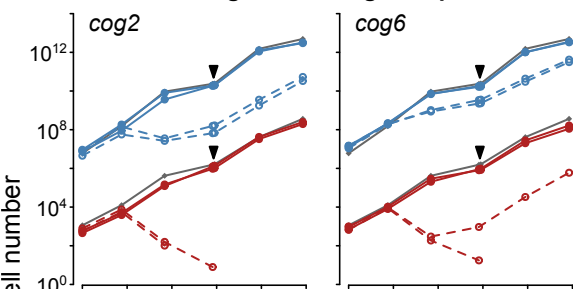


Suppl. Fig. 7

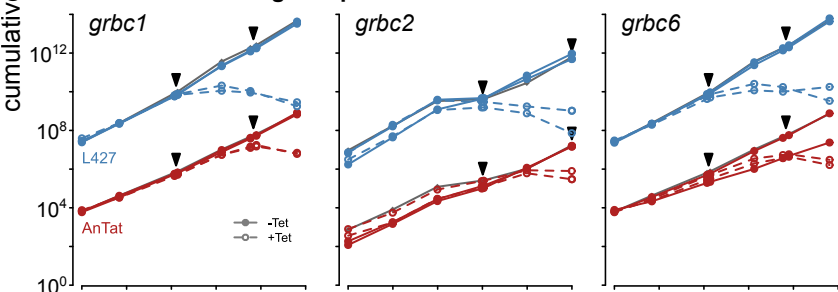
ARP2/3 complex



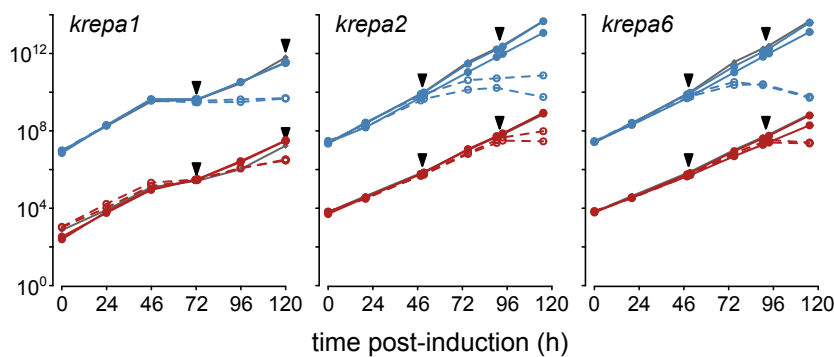
Conserved oligomeric Golgi complex



Guide RNA binding complex



Editosome



- 1 proteasome regulatory particle
- 2 proteasome core complex
- 3 proteasome regulatory particle, lid subcomplex
- 4 proteasome core complex, alpha-subunit complex

