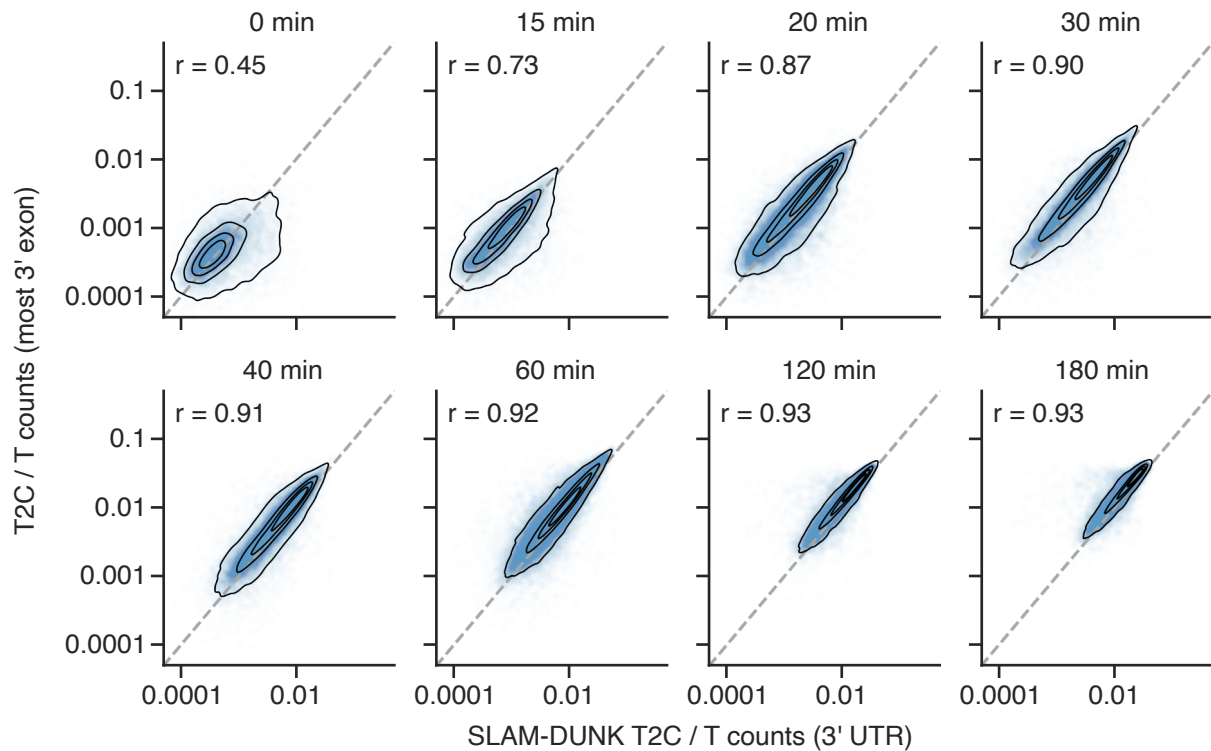


Appendix for  
**Subcellular mRNA kinetic modeling reveals nuclear retention as rate-limiting**

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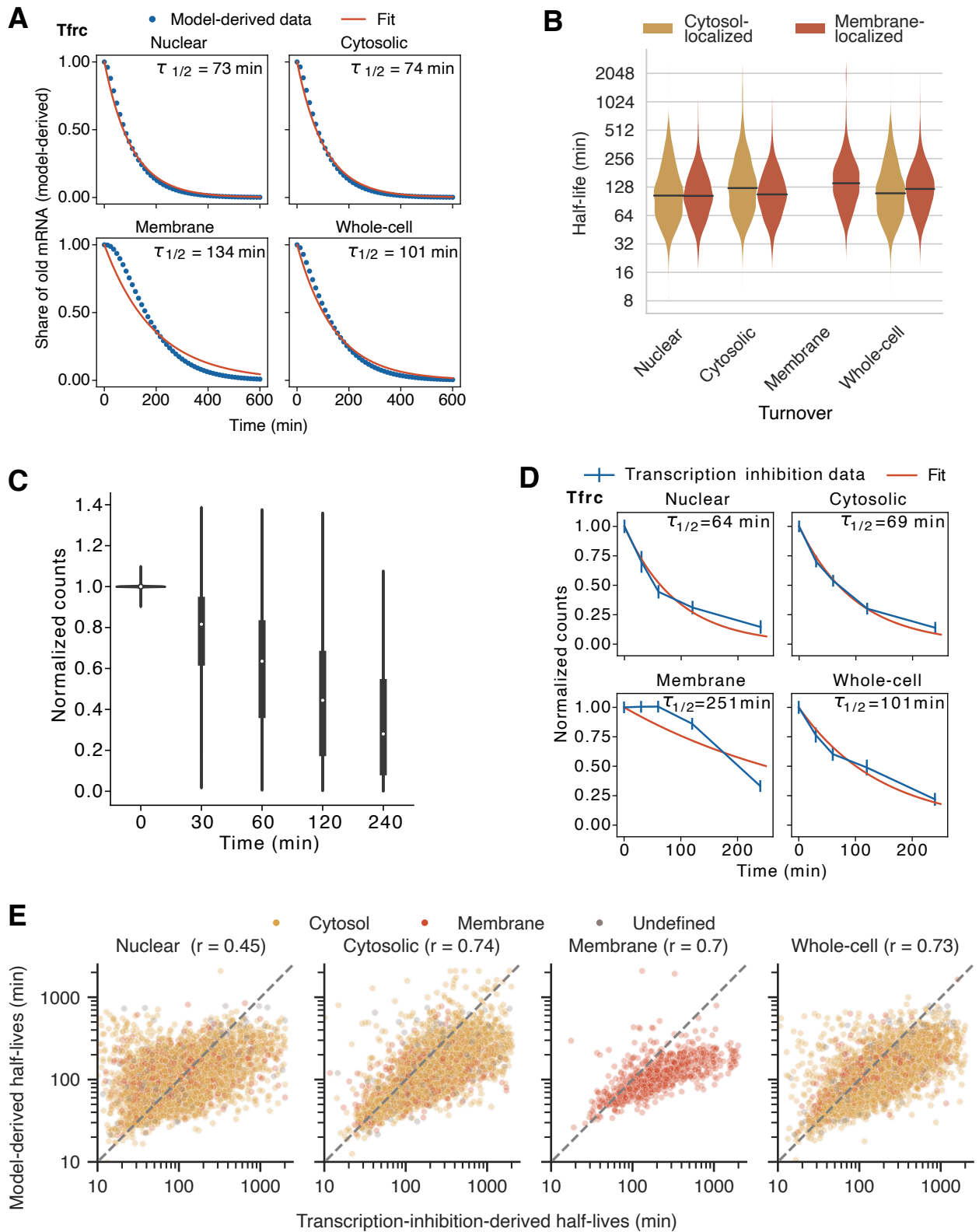
## Appendix Figure S1



## Appendix Figure S1. Mapping and T2C counting comparison to SLAM-DUNK method

Scatterplots of T2C/T count data of all expressed transcripts from nuclear, cytosolic and membrane samples, faceted by timepoints. Labeling data is obtained using our custom analysis pipeline (y-axis, only data from most 3' exon per transcript) and the established SLAM-DUNK method (x-axis), where alignment is done on the 3' UTR only. Spearman rank correlation is shown on top left in each facet. Number of data points from top left ( $t = 0$  min) to bottom right ( $t = 180$  min):  $n = 10897$ ,  $n = 13040$ ,  $n = 42836$ ,  $n = 26550$ ,  $n = 28657$ ,  $n = 69195$ ,  $n = 36652$  and  $n = 36601$ . Most 3' exon T2C and SLAM-DUNK 3' UTR data was filtered to contain at least a T coverage of 1000 and 3000, respectively. Overall, there is very high agreement between the methods and no systematic over- or underestimation visible. For the  $t=0$  min samples the agreement is lower, since the T2C mutations are largely sequencing errors.

## Appendix Figure S2



## Appendix Figure S2. Estimating model-derived pulse-chase-like and transcription inhibition-derived mRNA half-lives

- A. Exemplary fit to determine model-derived, aggregated half-lives, shown for Tfrc. From the subcellular rates, model-derived pulse-chase trajectories are calculated for different compartments (blue dots). To this data, a simple exponential decay model is fit (red line). Best fit values are shown on top right in each parameter facet.
- B. Violin plot showing aggregated half-lives of model-derived parameters for cytosol- (yellow,  $n = 7677$ ) and membrane-localized (red,  $n = 1693$ ) transcripts.
- C. Combined violin and box plots of normalized counts from transcription inhibition experiment using Flavopiridol ( $n = 37297$ , all compartments). x-axis shows time exposed to flavopiridol. White dots depict median values. Lower and upper hinges of box plots correspond to the 25th and 75th percentiles, respectively.
- D. Exemplary fit to determine transcription inhibition-derived half-lives, shown for Tfrc. A simple exponential decay model (red line) was fitted to normalized counts ( $n = 3$  replicates per time point, blue vertical lines indicate standard error). Best fit values are shown on top right in each parameter facet.
- E. Scatterplots of model-derived and transcription inhibition-derived aggregated half-lives of nuclear ( $n = 5979$ ), cytosolic ( $n = 5910$ ), membrane ( $n = 811$ ) and whole-cell ( $n = 6316$ ) compartments. Color indicates localization. Spearman rank correlation is shown in facet title. Dashed, gray line is the identity line.