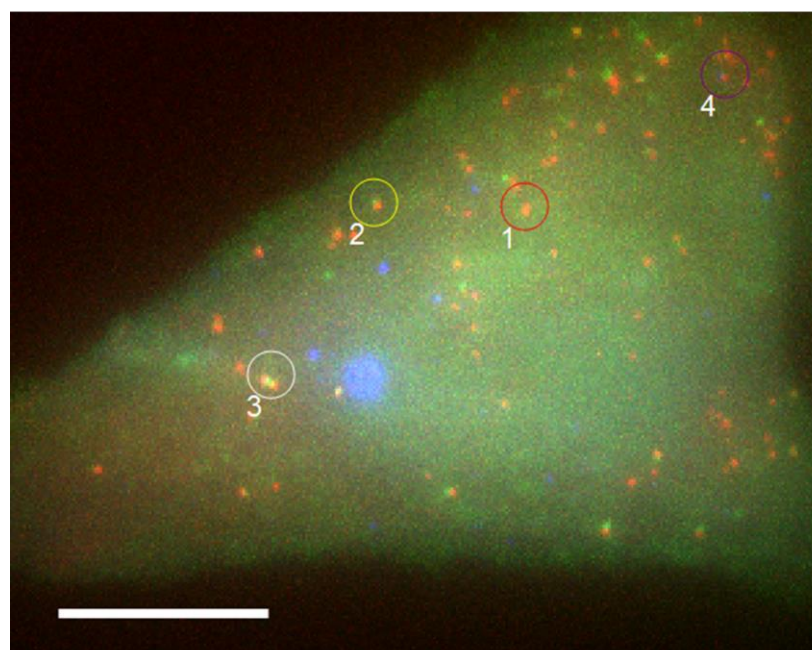
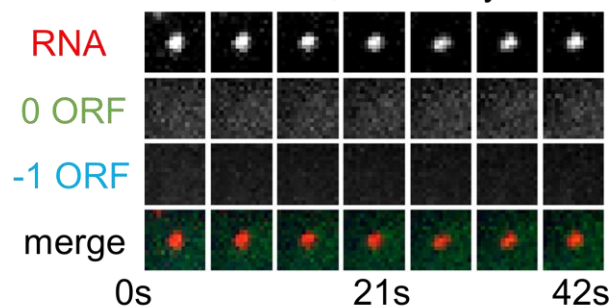


Fig. S1. Accumulation of mature AlexX and XXLb1 proteins from multi-frame tags

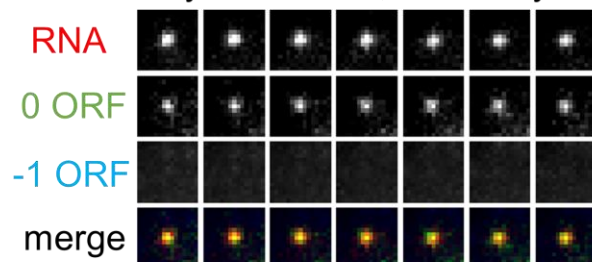
Mature AlexX (upper) and XXLb1 (lower) membrane proteins accumulate in distinct punctae that coalesce to form elongated structures that undulate dynamically. These structures are enriched at the top and bottom of cells and around the edge of the cytoplasm (arrows), consistent with membrane localization. Movie S2 shows the dynamics of these structures. In the +FSS multi-frame reporter, mature AlexX comes from 0 frame canonical translation, while mature XXLb1 comes from -1 frameshifting translation. In the -FSS control reporter, only mature AlexX accumulates, indicating little to no frameshifting. The -FSS (+1 nt) column shows accumulation of XXLb1 when an extra nucleotide is inserted in the -FSS control reporter following the start codon. This extra nucleotide pushes XXLb1 into the 0 frame, demonstrating it can be expressed.



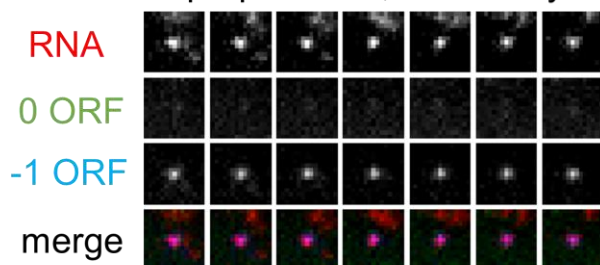
1. red circle, RNA only



2. yellow circle, 0 TS only



4. purple circle, -1 TS only



3. white circle, 0 & -1 TS

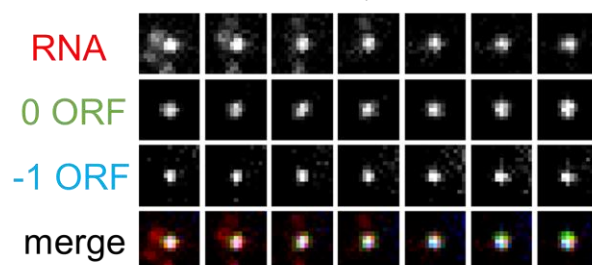


Fig. S2. A cell with all possible types of translation sites

A sample cell exhibiting all four possible translation sites (TS). (1) untranslated TS; (2) 0 frame only TS; (3) 0 and -1 frame translated TS; and (4) -1 frame only TS. Scale bar = 10 μ m. Movie S4 showcases the dynamics of these spots.

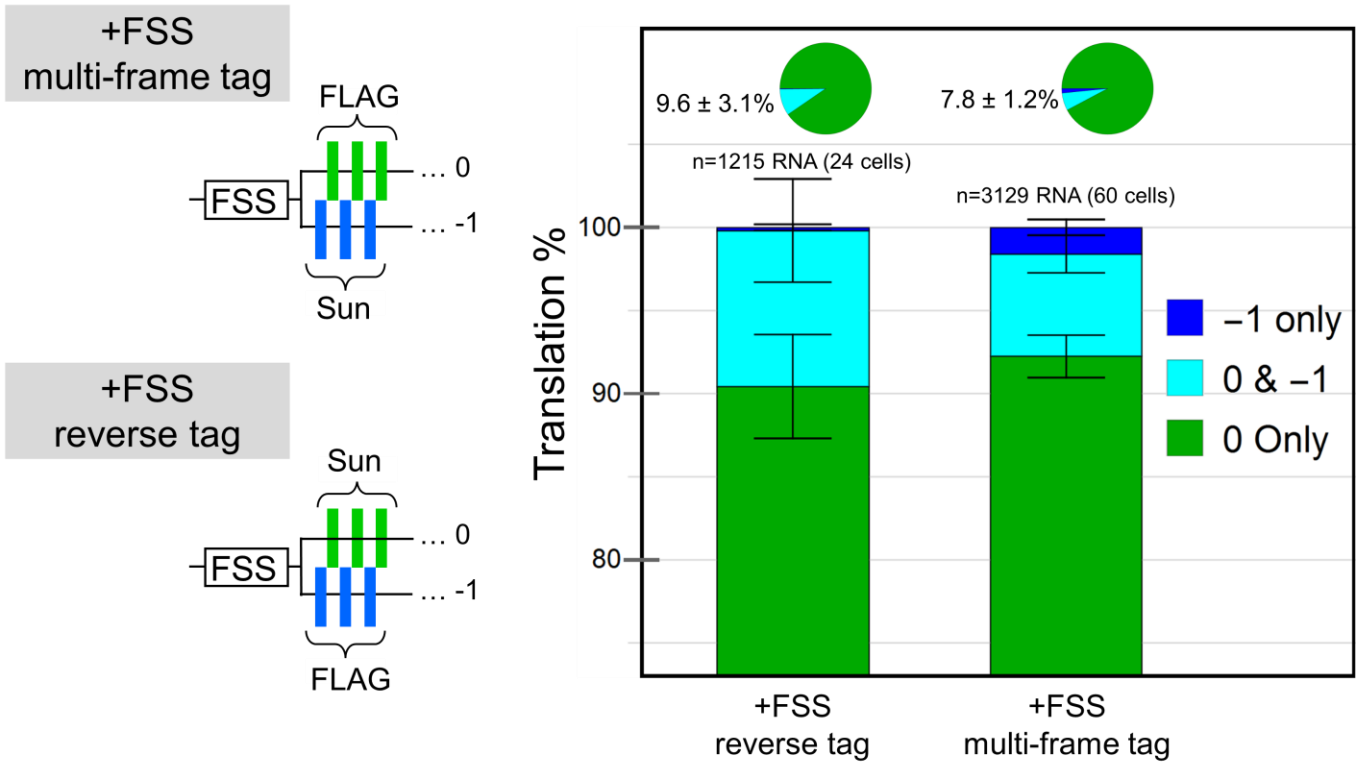


Figure S3. Epitope order in tags has little impact on percentage of frameshifting RNA
 The percentage of RNA translating the -1 frame only (dark blue), the 0 and -1 frames (cyan), and the 0 frame only (green) for the +FSS multi-frame tag and the +FSS reverse tag (in which FLAG and SunTag epitopes are reversed). Pie charts show the percentage of translating species per cell. Error bars represent S.E.M. among cells.

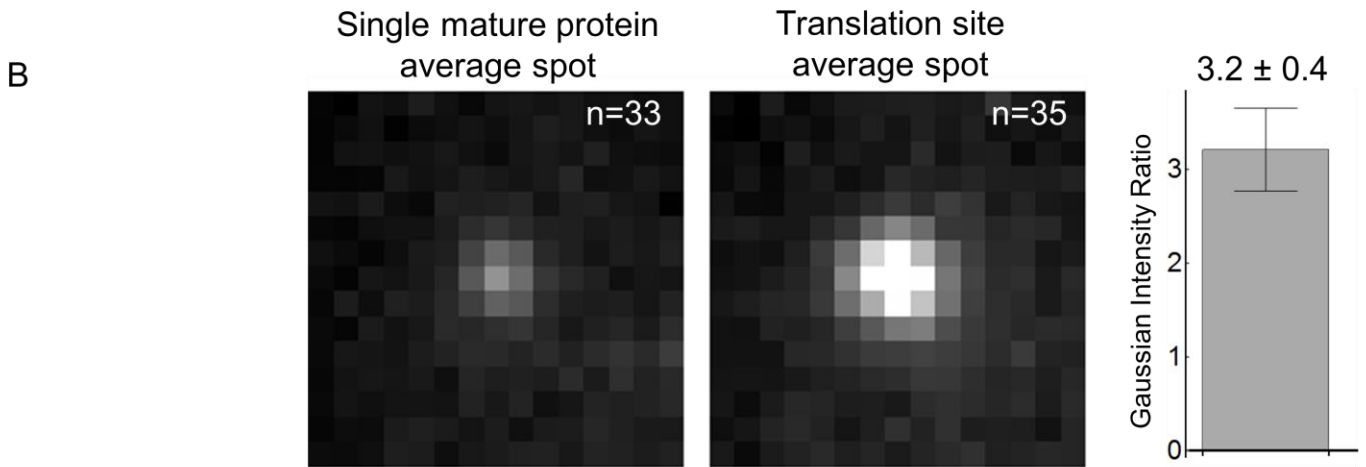
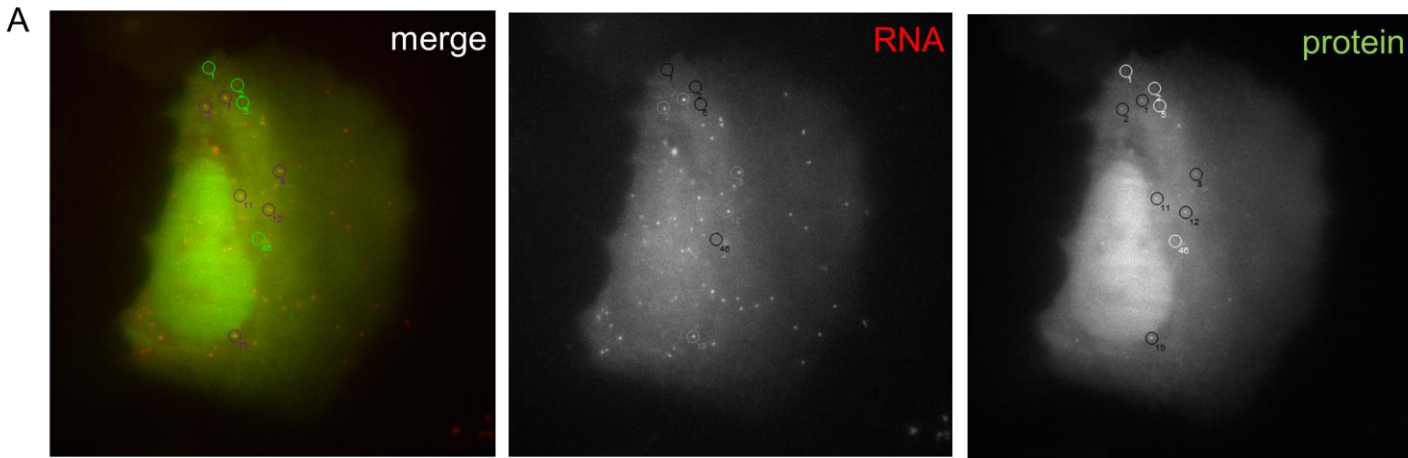


Figure S4. Calibrating translation site intensity to the number of ribosomes

(A) Example cell where single mature membrane proteins were detected (green circles), along with translation sites (co-localized RNA and protein; purple circles). (B) Average images of detected spots (33 mature proteins and 35 translation sites) were fit to a Gaussian to determine their intensity ratio, shown in the bar graph on the right. This ratio provides a calibration factor to convert translation site intensities to the number of ribosomes. The error bar represents the propagated 90% confidence interval from the fit.

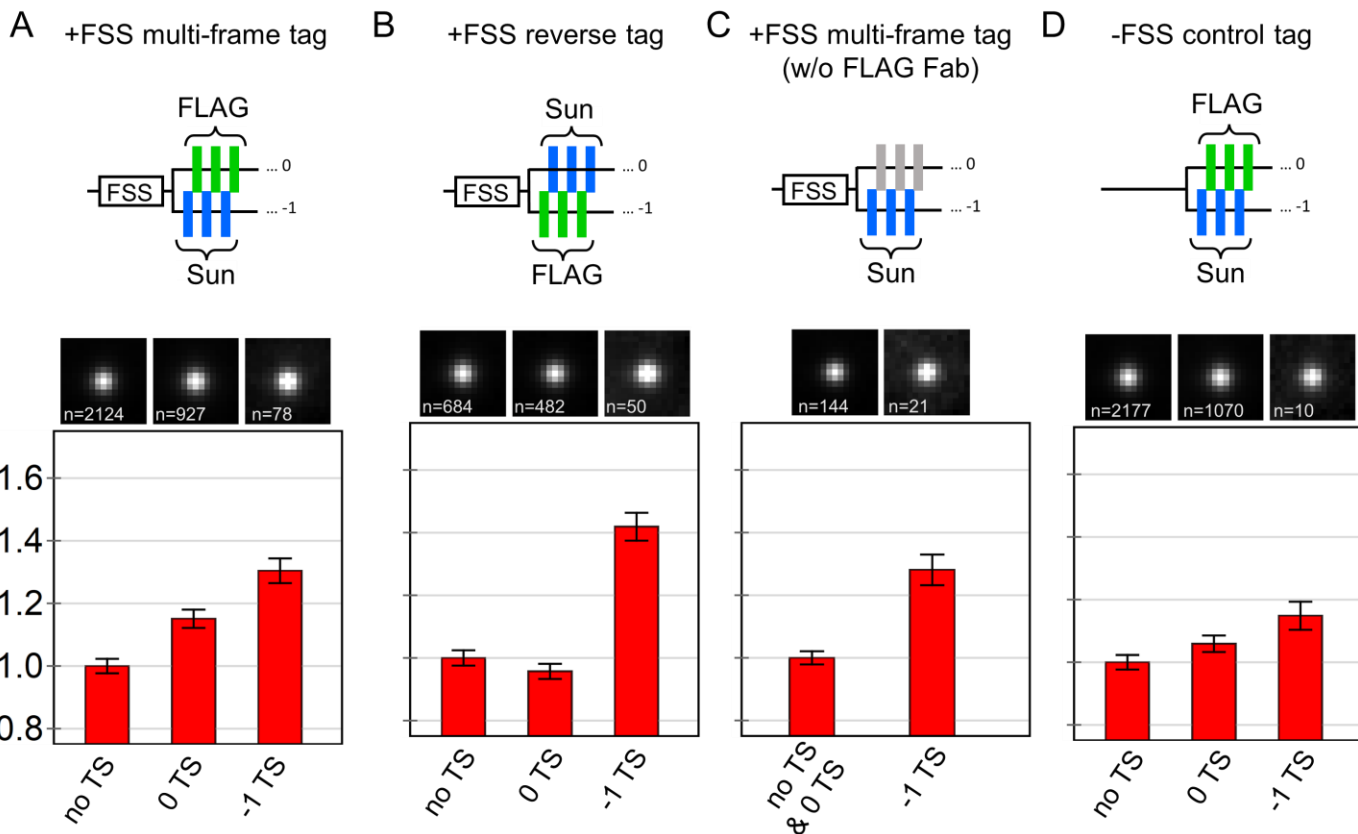


Figure S5. Frameshifting sites have brighter RNA signals

(A-E) Average RNA signals for non-translating RNA (no TS), 0 only translation sites (0 TS), and -1 frameshifting translation sites (-1 TS). The number of RNA used to generate each average image is shown. The bar graphs below show the Gaussian fit intensity (normalized to non-translating RNA, i.e. 0 TS sites). Experiments were done using (A) the +FSS multi-frame tag, (B) the +FSS reverse tag, (C) the +FSS multi-frame tag imaged without anti-FLAG Fab, and (D) the -FSS control tag. Error bars represent the fitted 90% confidence interval. Aside from the -FSS control, frameshifting sites (-1 TS) have significantly brighter RNA signals compared to canonical translation sites (0 TS). Note the frameshifting observed in the -FSS control represents less than 1% of translating RNA and can be considered background frameshifting.

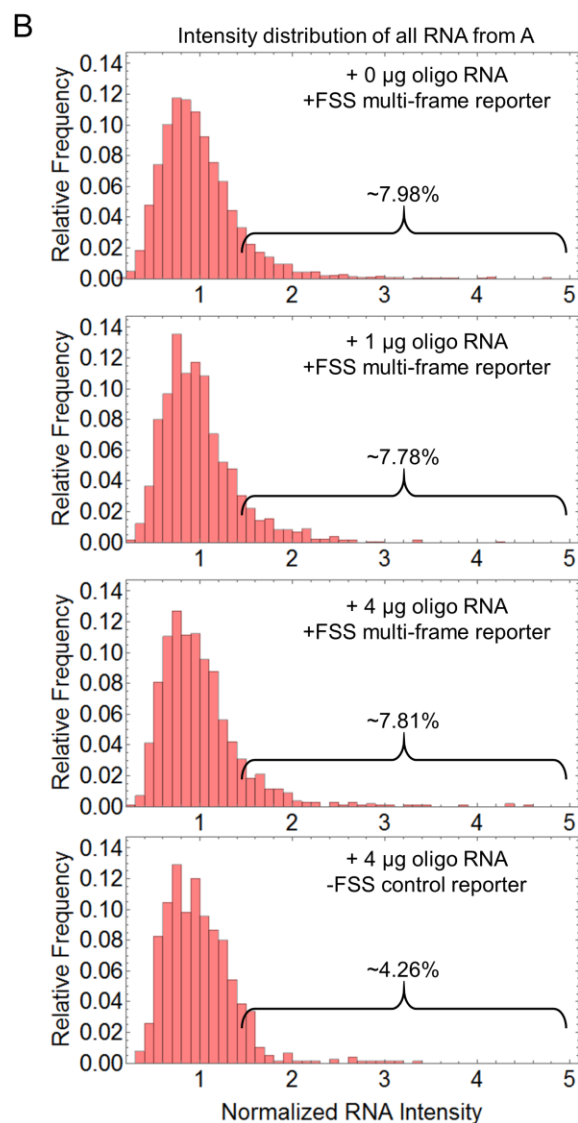
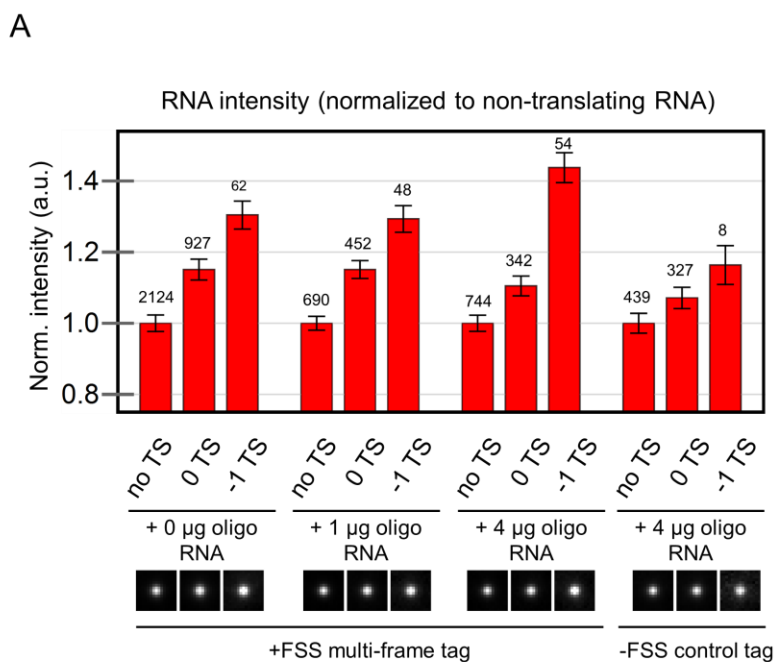
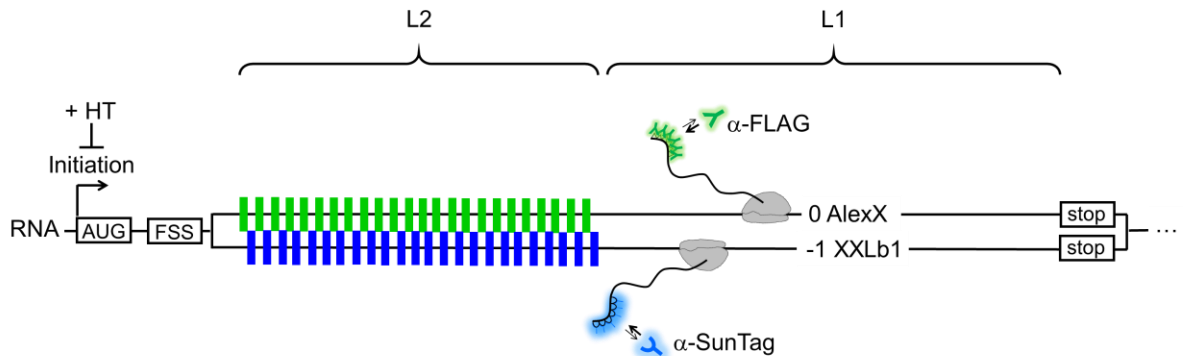


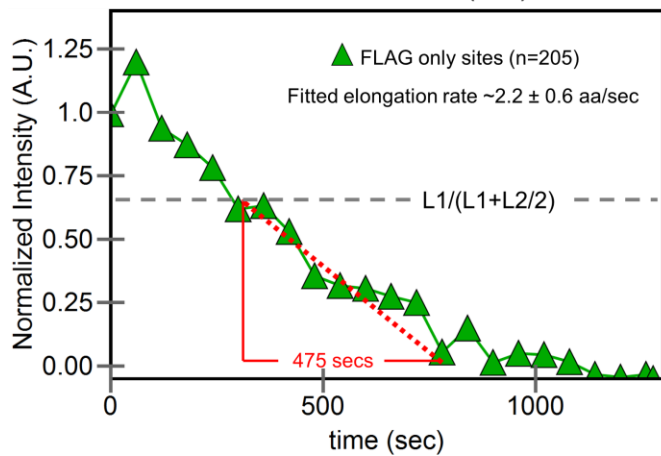
Figure S6. Upon oligo RNA co-transfection, stimulated frameshifting still occurs at bright RNA sites, even though the fraction of bright RNA sites remains unchanged

(A) Average RNA signals from non-translating RNA (no TS), 0-frame only translation sites (0 TS), and frameshifting translation sites (-1 TS) when different concentrations of oligo RNA (0, 1, and 4 μ g) encoding the frameshift sequence were co-transfected into cells with the +FSS multi-frame reporter or the control -FSS reporter. The bar graph above shows the Gaussian fit intensity (normalized to non-translating RNA, i.e. 0 TS sites). (B) The distributions of RNA signals from the four experiments normalized to their mean value. In all +FSS experiments, the fraction of bright RNA (defined here as having an intensity greater than or equal to 1.6 times the mean) remained constant. The -FSS experiment had fewer bright RNA, suggesting the FSS sequence facilitates incorporation into multi-RNA sites.

Ribosomal run-off induced by harringtonine



Ribosome run-off from non-frameshifting translation site (TS)



Ribosome run-off from frameshifting translation site (TS)

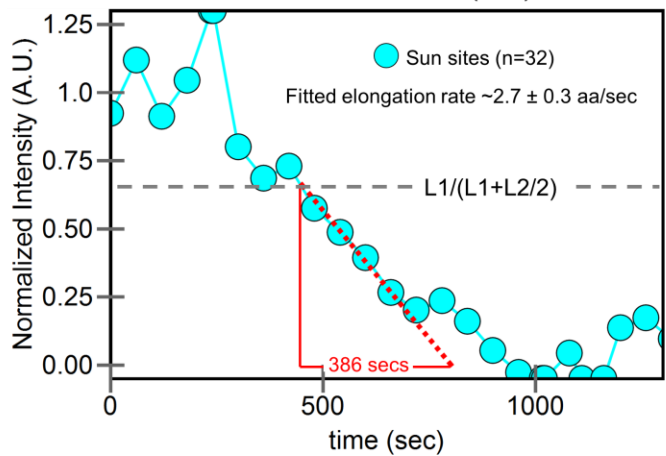
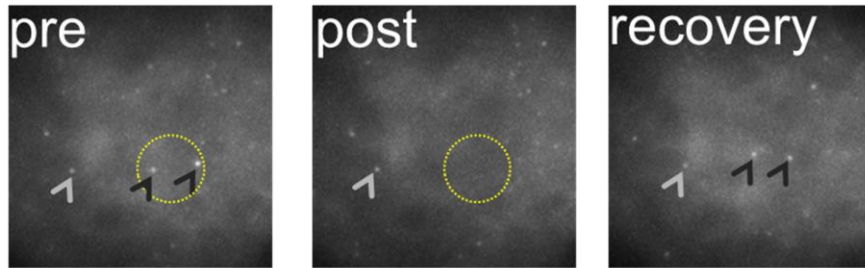
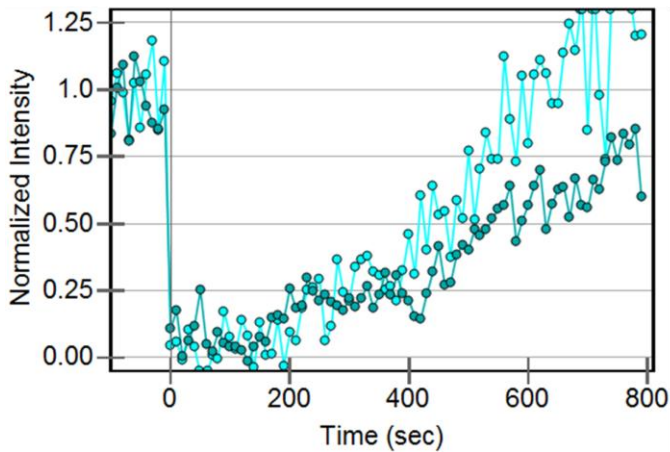


Figure S7. Fits to linear portion of ribosomal run-offs provide elongation rate estimates

The total intensity of detected non-frameshifting and frameshifting translation sites decays with time after addition (at $t = 0$ sec) of the translational initiation inhibitor harringtonine. Data is taken from Fig. 4A. If $L2$ is the length of the tagged portion of the open reading frame and $L1$ is the length of the non-tagged portion, then the linear (post-tag) portion of the decay begins from approximately $L1/(L1+L2/2)$. This portion of the curve provides an estimate of the elongation rate during the part of the run-off where no new epitopes are being translated. Therefore, the run-off fit is independent of the frameshifting kinetics that occur upstream of the epitopes. Curves are normalized to their initial values.



Single RNA FRAP recoveries



Average FRAP recovery

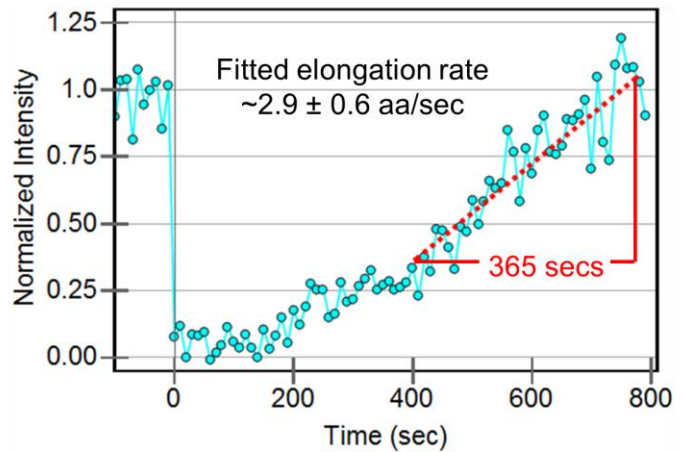


Figure S8. Fluorescence recovery after photobleach of frameshifting sites

(A) Fluorescence recovery after photobleaching (FRAP) experiments were performed at frameshifting translation sites (yellow circle marks the photobleach spot). The fluorescence recovery of the 0-frame signal within these sites was quantified as a function of time, with sample pre, post, and recovery frames shown above. (B) The average FRAP recovery time can be fit to estimate the elongation rate. This rate is similar to what was measured with harringtonine in Fig. S7.

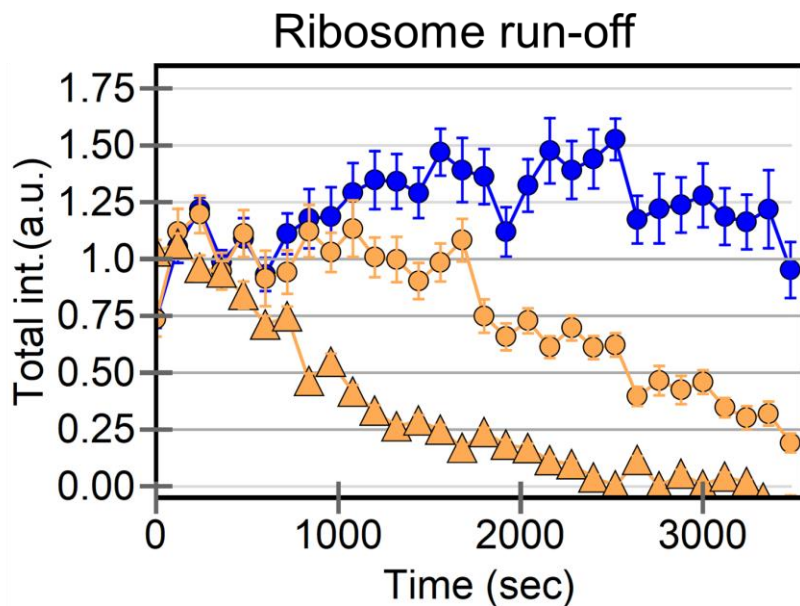
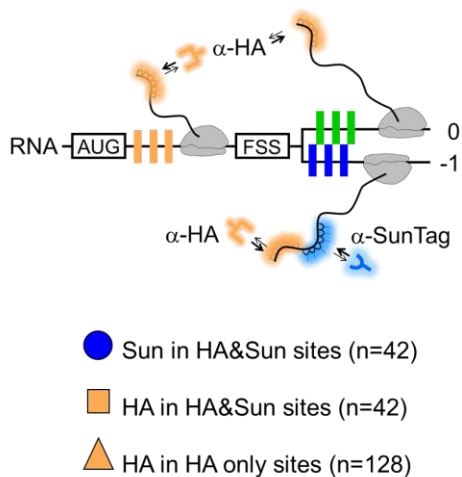
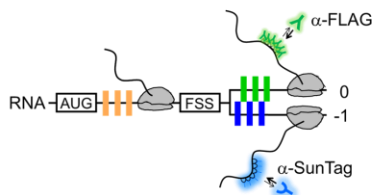


Figure S9. Ribosome run-off from the HA multi-frame tag

Ribosome run-off curve showing Sun signal in HA&Sun sites from the experiment performed in Figure 4B. The Sun signal comes from frameshifted ribosomes that have run past the frameshift sequence (FSS). Error bars represent S.E.M.

▲ FLAG in FLAG only sites (n=158)



■ HA in HA only sites (n=128) from Fig. 4B

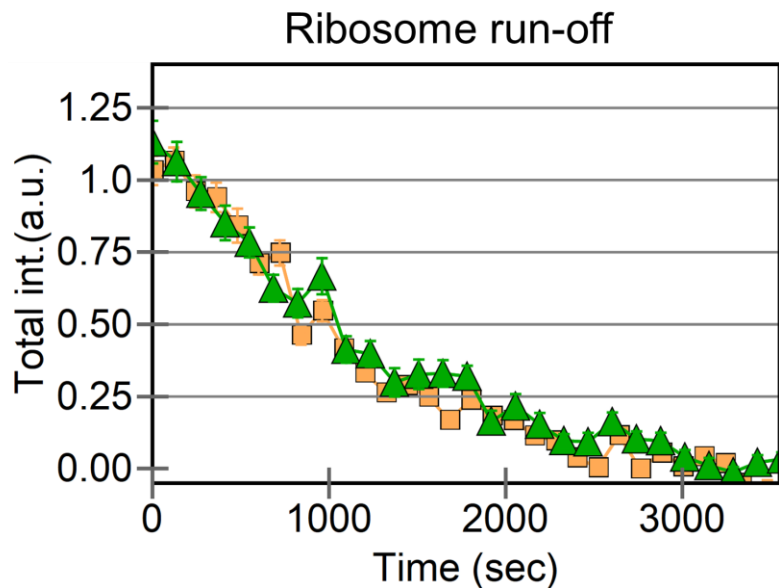
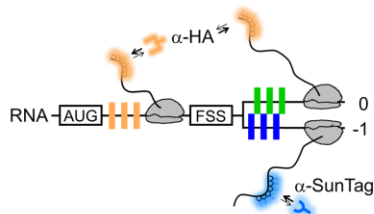


Figure S10. Ribosome run-off from FLAG epitopes in HA multi-frame tag

For completeness, an additional experiment was performed to generate the ribosome run-off curve from FLAG epitopes in the HA multi-frame tag. Because we can only image two epitopes at the same time (since RNA is imaged in a third color), we examined FLAG and Sun epitopes (Sun epitopes were required to distinguish frameshifting and non-frameshifting sites). The FLAG run-off from this experiment is on the right (green triangles). This curve is compared to the run-off curve of HA in HA only sites (orange squares) from Figure 4B. Both experiments represent non-frameshifting sites (no Sun signal detected). Error bars represent S.E.M.

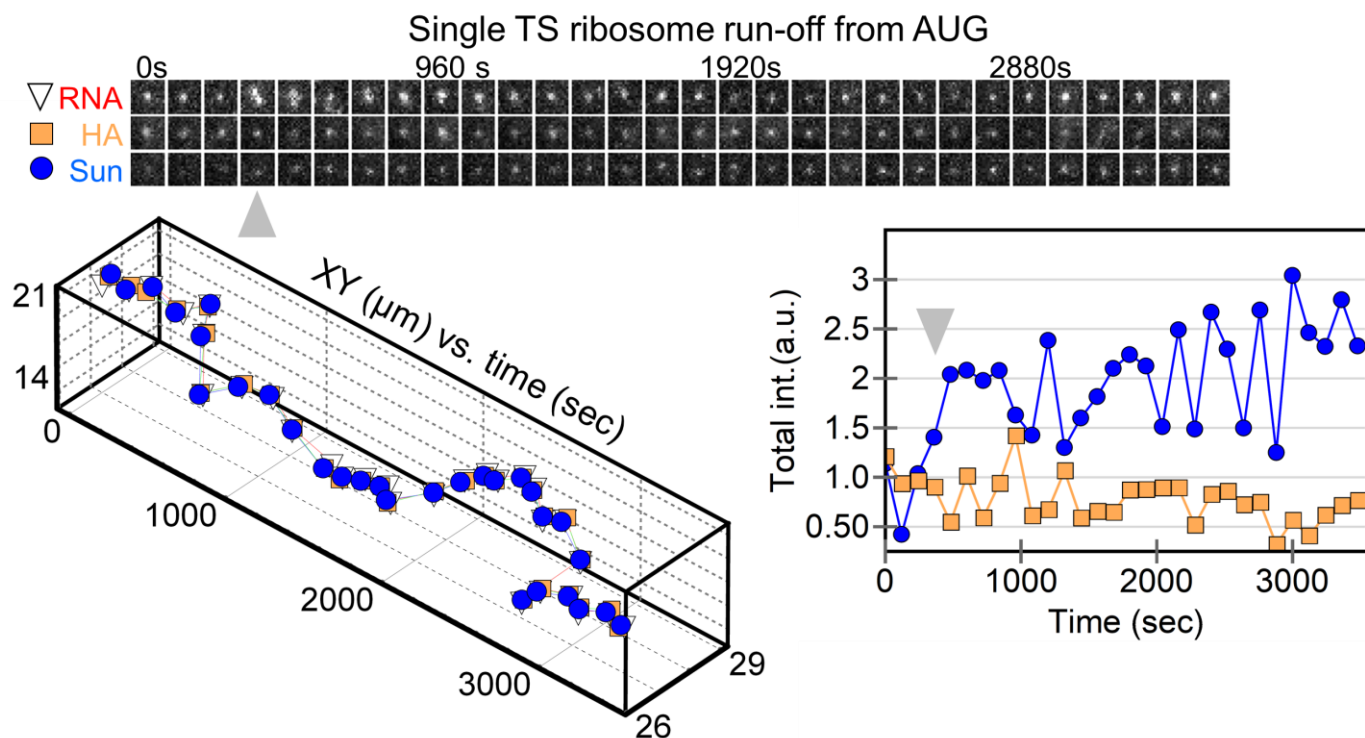


Figure S11. Track of a stimulated frameshifting burst at a single translation site

A single frameshifting translation site encoding the HA multi-frame tag was tracked after harringtonine addition. On the top, a montage of image trims shows the detected RNA-, HA-, and Sun shown signals through time. Below, the positions of the detected signals within the site are plotted through time. On the right, the normalized total intensity of the HA Fab signal (marking all ribosomes) and the SunTag scFv signal (marking frameshifting ribosomes) is plotted through time. At about the fourth timepoint, another non-translating RNA interacts with the frameshifting RNA, leading to a burst of frameshifting signal (Sun). Gray arrows signify a burst of frameshifting, coinciding with multi-RNA interactions.

Bursting model

Constitutive model

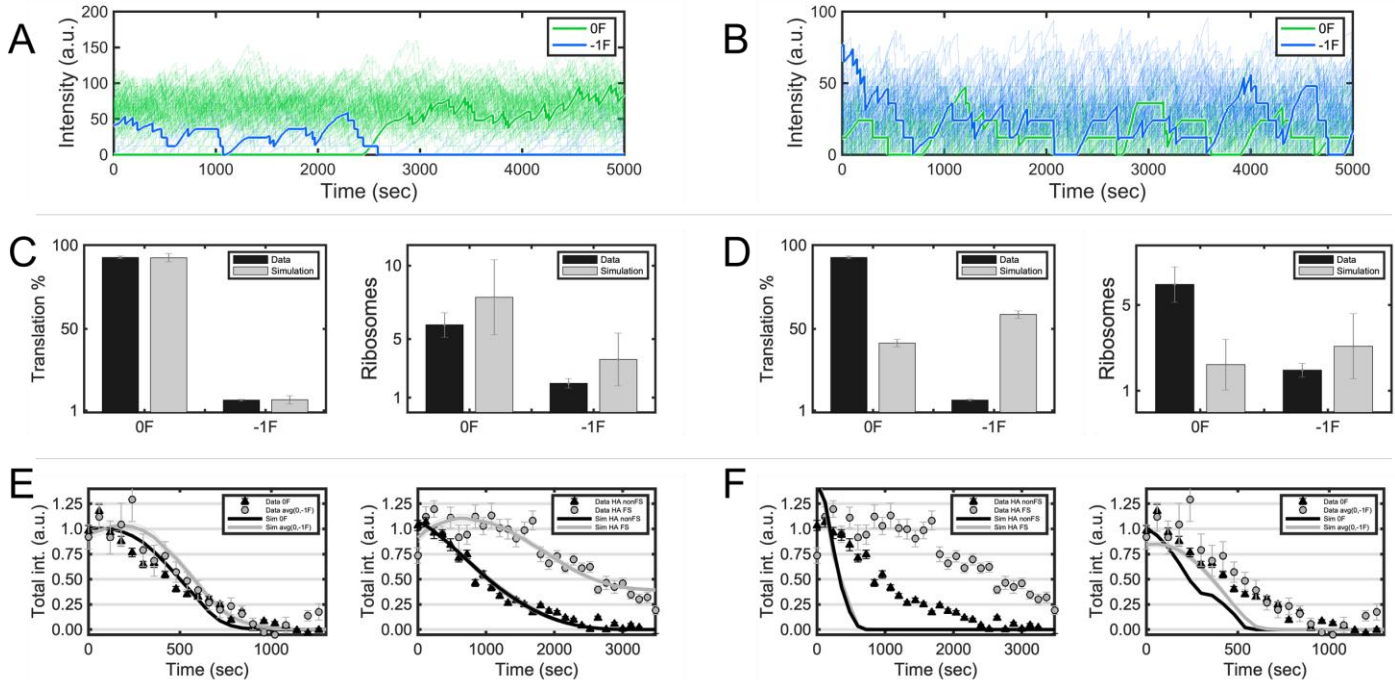


Figure S12. Comparison between the bursting and constitutive models

Model dynamics for the bursting (A,C,E) and constitutive model (B,D,F). Simulations were performed using the best parameter values obtained from the optimization process. (A,B) Simulated time courses representing single molecule fluctuation dynamics from 100 translation sites (a sample trace is shown in bold). Green and blue lines represent the translation of FLAG (0 frame) and Sun (-1 frame) epitopes in the +FSS multi-frame tag, respectively. (C,D) On the left, bar graphs showing the experimental (black) and simulated (gray) percentages of 0 and -1 frame translation. On the right, the number of ribosomes translating either the 0 or -1 frame. (E,F) On the left, simulated run-off (solid lines) from the frameshift sequence (FSS) of all ribosomes in non-frameshifting (black) and frameshifting sites (gray), plotted with data in Fig. 4A (black triangles and gray circles). On the right, simulated run-off (solid lines) from the start site (AUG) of all ribosomes in non-frameshifting (black) and frameshifting sites (gray), plotted with data in Fig. 4B (black triangles and gray circles). Error bars represent the standard error of the mean (S.E.M.). Details can be found in the Supplementary Methods.

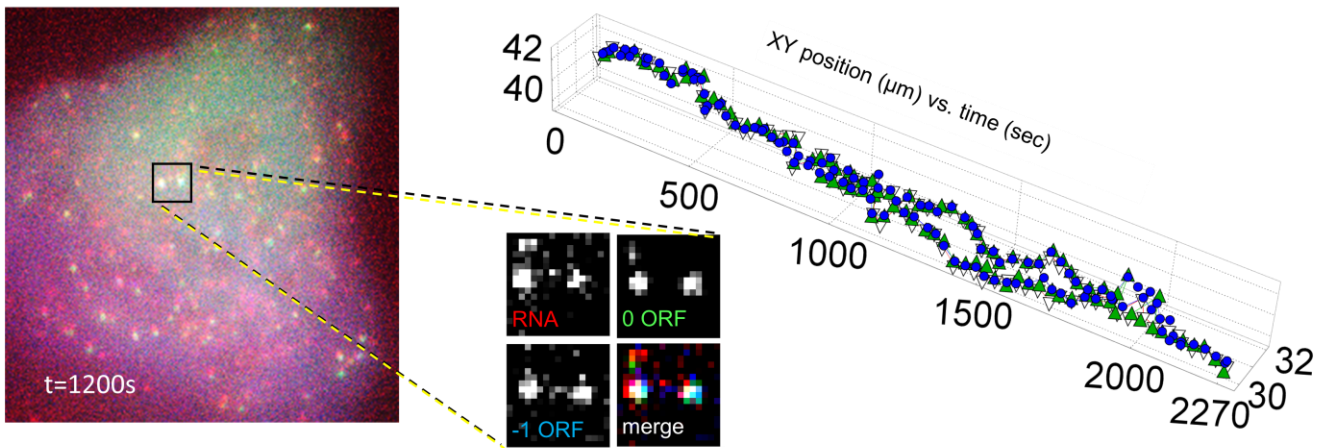


Figure S13. Single frameshifting RNA track persisting for >35 minutes

A sample frameshifting translation site encoding the +FSS 2x multi-frame tag was tracked for >35 minutes. Left shows the cell at the 1200 second time point. The zoom shows the channels separated to highlight two frameshifted RNA that split from a single translation site. On the right, the position of the spot is plotted through time.

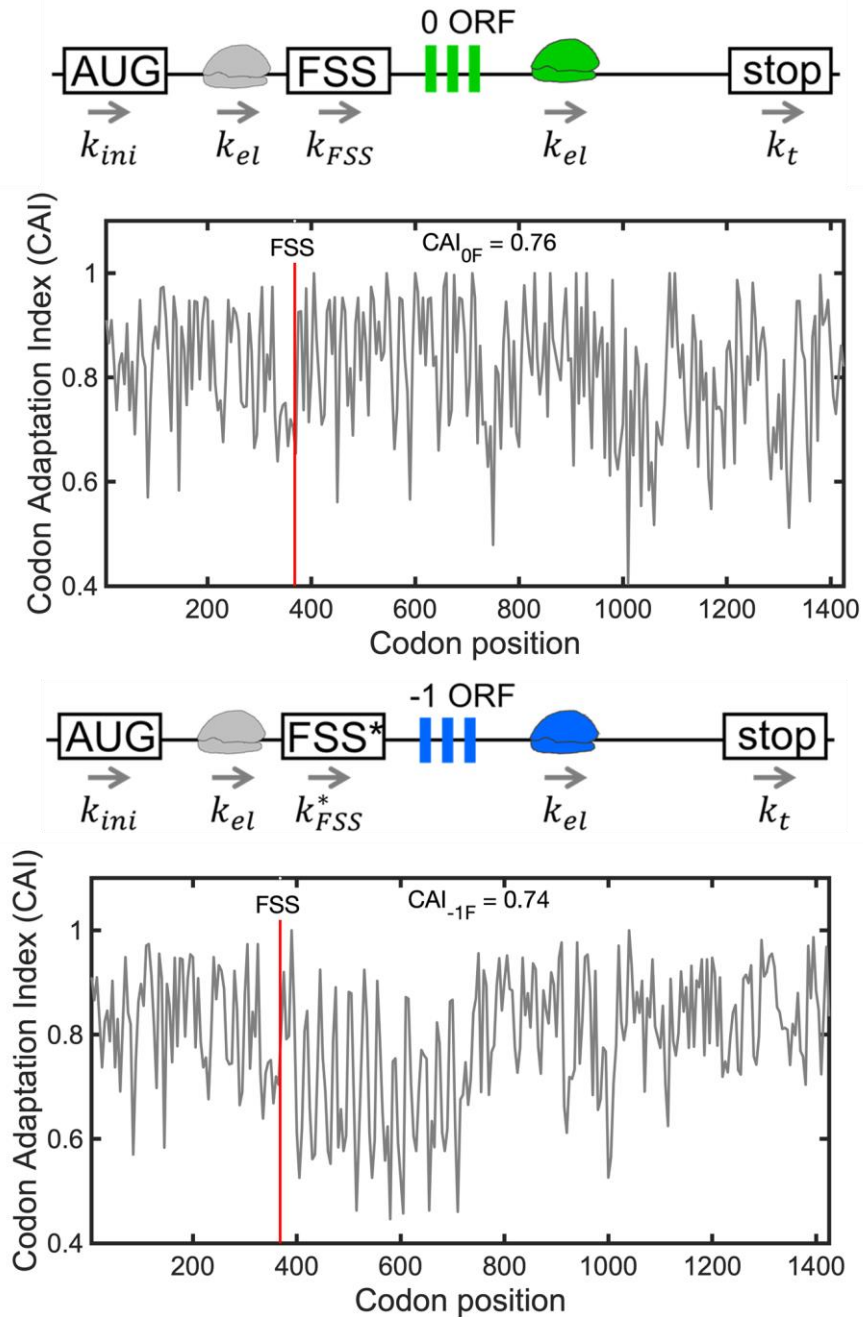


Figure S14. Codon usage plays a minor role in traffic jam formation

The codon adaptation index (CAI) for the 0 frame (top) and -1 frame (bottom) of the +FSS multi-frame tag. The y-axis shows the CAI calculated using the codon frequency in the human genome. The x-axis shows the length of the genes, in codons. In the plots, rare codons have low CAIs and common codons have high CAIs. The vertical red line represents the location of the frameshift sequence (FSS). Similar CAIs are obtained for the sequences in the 0 frame ($CAI_{0F} = 0.76$) and the -1 frame ($CAI_{-1F} = 0.74$).

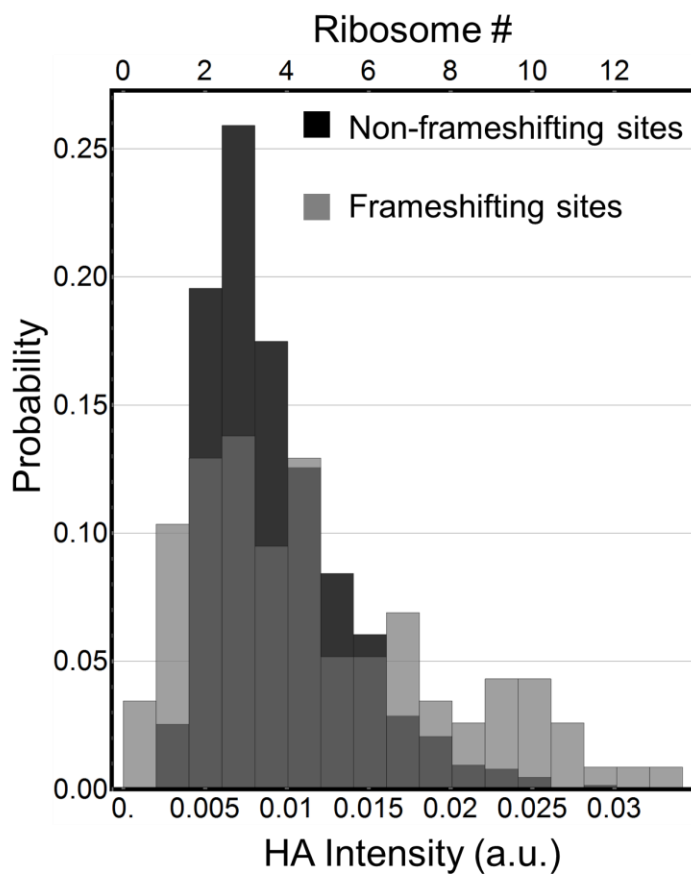


Figure S15. HA signal intensity distributions of non-frameshifting and frameshifting sites
 Intensity distribution of non-frameshifted HA signals (black) versus frameshifted HA signals (gray) produced from the HA multi-frame tag.