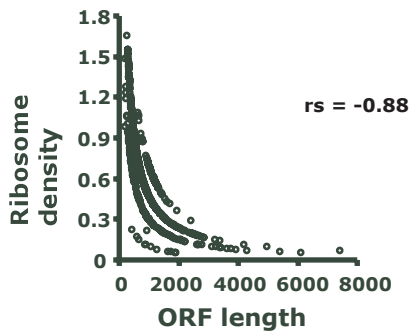


# Supplemental Figure 7

## Examining the possibility of incorrect assignment of number of ribosomes in each fraction.

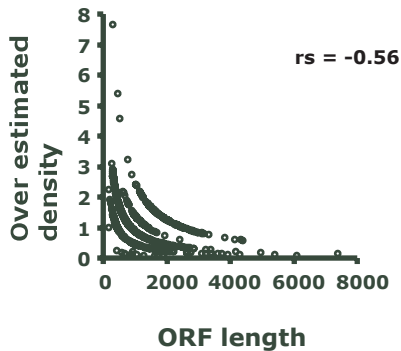
A potential cause for the inverse correlation between ribosome density and ORF length that we investigated was incorrect assignment of number of ribosomes in the fastest sedimenting region of the gradient (fractions 12 to 14), as these regions of the gradient lack single ribosome resolution. To explore this possibility, we analyzed only the well-resolved region of the gradient (A) and applied extreme error margins for the number of ribosomes in each fraction (B). Error may also arise from the use of the peak fraction to calculate the ribosomal density, rather than a weighted average of the signal in each polysomal fraction. We therefore calculated the density values based on a weighted average and analyzed the relation between ORF length and density (c). The Spearman rank correlation ( $r_s$ ) is presented in each case.

### A). Analyzing only the well-resolved region of the gradient (fractions 6-11).



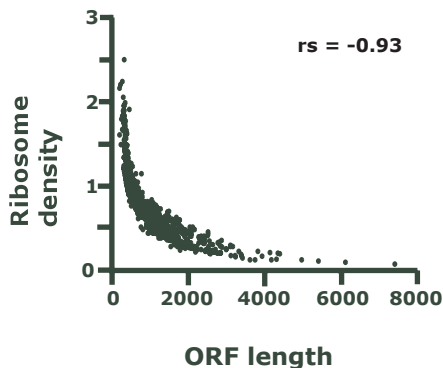
To ensure that the inverse correlation does not arise from low resolution of sedimentation in fractions 12 -14, we excluded the genes that peak in these fractions from the analysis. The inverse correlation is still apparent, with  $r_s = -0.88$ .

### B). Applying extreme error margins for the number of ribosomes in each fraction.



To ensure that the inverse correlation does not arise from wrong assignment of number of ribosomes to each fraction, we applied extremely high error estimates to the number of ribosomes in each fraction: fraction 9 - 4.5 ribosomes instead of 3, fraction 10 - 8.75 (4.7), fraction 11 - 14 (7) and fraction 12 - 25 (10.7). The ribosome numbers for fractions 13 and 14 are irrelevant because no mRNA had peaked in these fractions. If the correlation arose from incorrect assignment of number of ribosomes, this manipulation should have eliminated the correlation. The correlation is reduced ( $r_s = -0.56$ ) as follows from the manipulation applied, yet is not eliminated.

### C). Basing the density calculations on a weighted average across the polysome profile.



To ensure that the inverse correlation does not arise from basing the density calculations on the peak fraction, we calculated the density of each mRNA based on a weighted average of the signal in fractions 6-14. As can be seen, the correlation still holds, and even becomes more significant.

The results from the above analyses strongly suggest that incorrect ribosome number assignment is not the cause of the observed inverse correlation between ribosome density and ORF length.