Appendix Table of Contents
p. 2 - Appendix figure legends
p. 3 - Appendix Figure S1: uORF and oORF presence/number repress translation in all measured mammalian cell types
p. 4 - Appendix Figure S2: Group pictures of reporter-injected embryos
p. 5 - Appendix Figure S3: uORF length distributions in mammals
Appendix Supplemental Figure legends

Appendix Figure S1: uORF and oORF presence/number repress translation in all measured mammalian cell types
Cumulative plots show translation efficiency of CDSs with varying numbers of uORFs (purple) and oORFs (orange) versus transcripts lacking uORFs/oORFs (black) for transcripts expressed at least 0.5RPKM; for all human and mouse samples (See Expanded View Table EV3 for sample key). Statistical comparisons of translation efficiency (1 uORF vs. no uORFs or 1 oORF vs. no oORFs; Two-sided Wilcoxon) for every plot are highly significant (p<0.0005 for all samples shown).

Appendix Figure S2: Group pictures of reporter-injected embryos
(A) Raw group pictures of reporter-injected embryos at 24hpf are shown for each reporter variant with dsRed and GFP fluorescence

Appendix Figure S3: uORF length distributions in mammals
uORFs are shorter than expected by chance - Histograms show the length distribution of all (A) human and (B) mouse uORFs (bin size 10nt). Vertical dotted lines indicate the observed mean length of endogeneous uORFs and the mean length of uORFs obtained by 500x sequence shuffling of human and mouse TLSs (2-sided p=3.04e-164, p=2.09e-302 respectively).
Figure S1
<table>
<thead>
<tr>
<th>No uORF</th>
<th>1uORF</th>
<th>3uORF</th>
<th>1oORF</th>
<th>1uORF (w)</th>
</tr>
</thead>
</table>

Figure S2

(A)
Figure S3

(A) Distribution of human uORF length

(B) Distribution of mouse uORF length