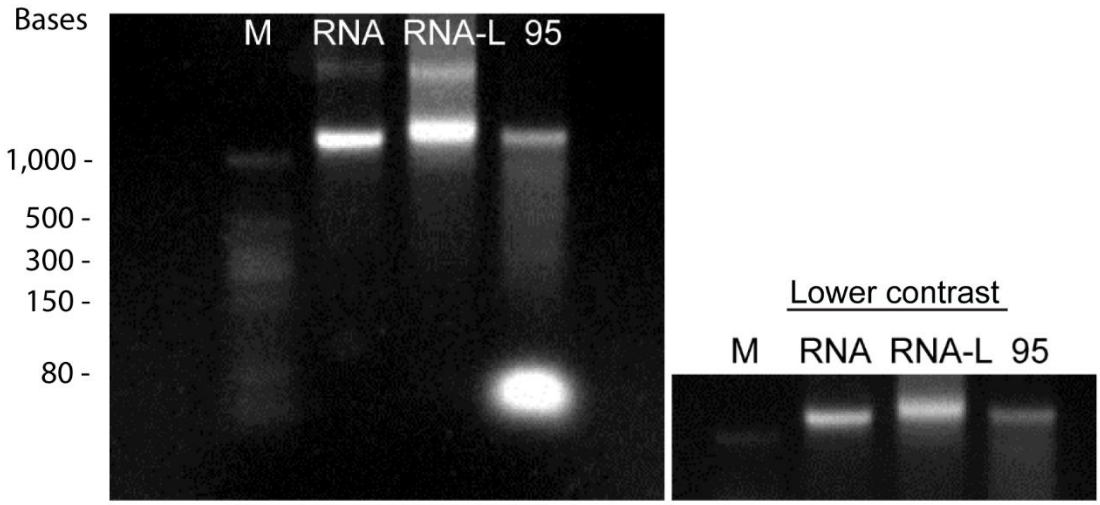
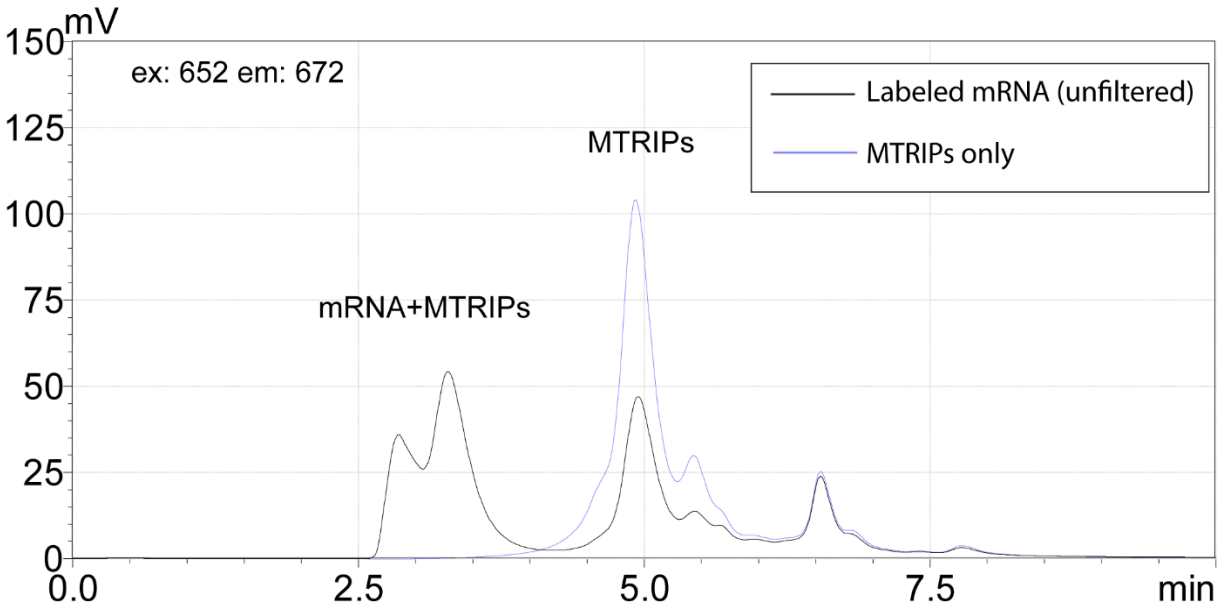
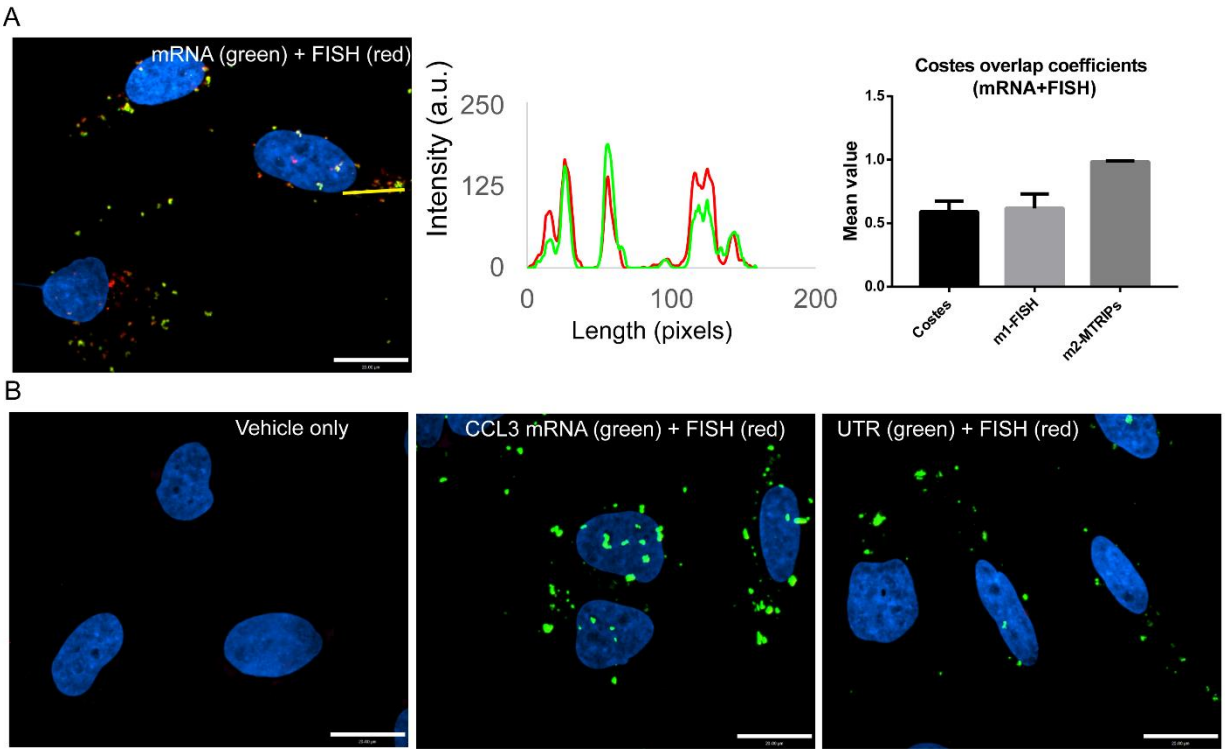
Supplemental Figures



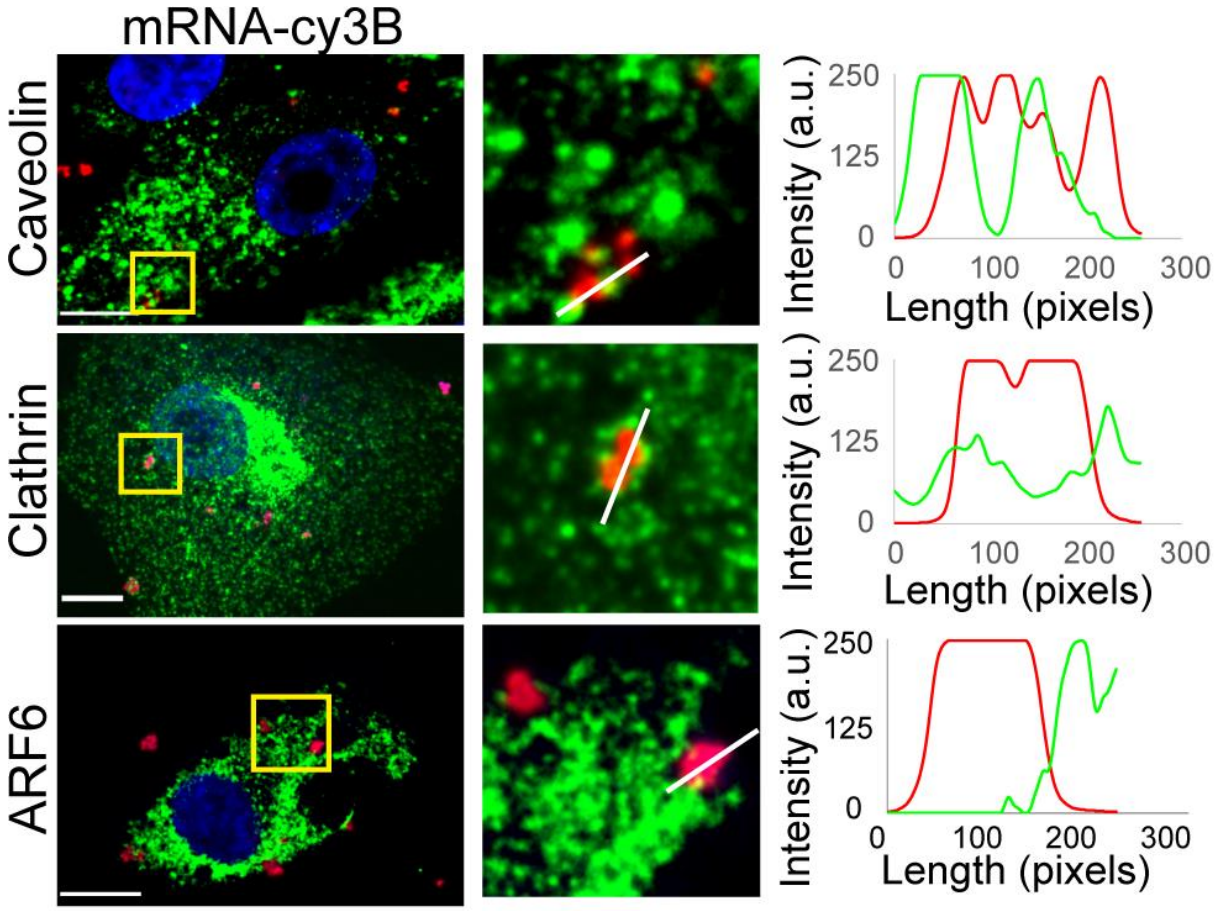
S1: Agarose gel comparing unlabeled mRNA (RNA) and labeled mRNA (RNA-L). Labeled mRNA visibly migrated slower than unlabeled mRNA. Labeled mRNA was heated to 95°C in order to denature MTRIPs resulting in recovery of the band for unlabeled mRNA as well as an additional band at the bottom of the gel for denatured MTRIPs. M indicates Low Range ssRNA Ladder (NEB).



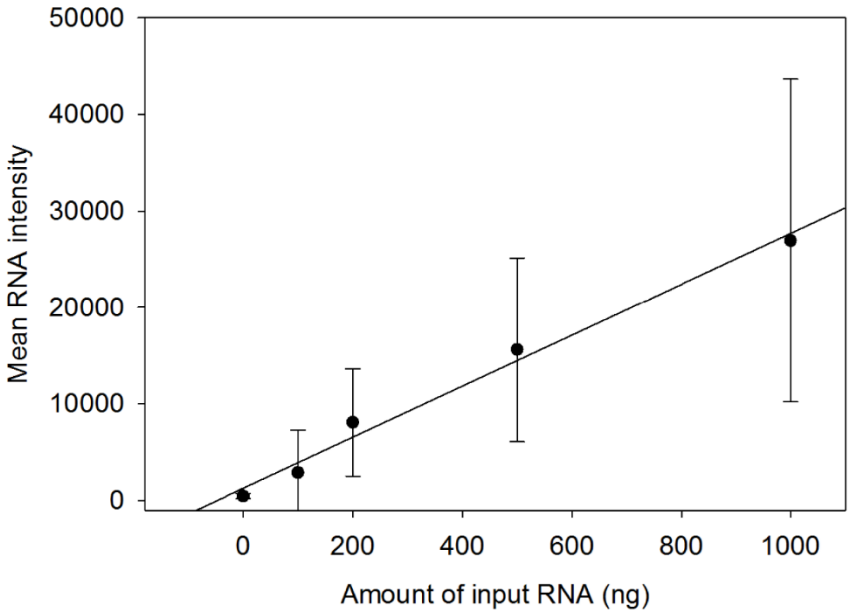
S2: Quantification of degree of labeling of mRNA with MTRIPs by size exclusion chromatography. Dylight-650 labeled mRNA fluorescence was compared to the same molar amount of MTRIPs without mRNA. The degree of labeling is calculated based on the difference in peak fluorescence of the MTRIPs (~5 min) corresponding to the amount of MTRIPs which are bound to the mRNA (~3 min). This corresponds to a degree of labeling of approximately 2 MTRIPs per mRNA.



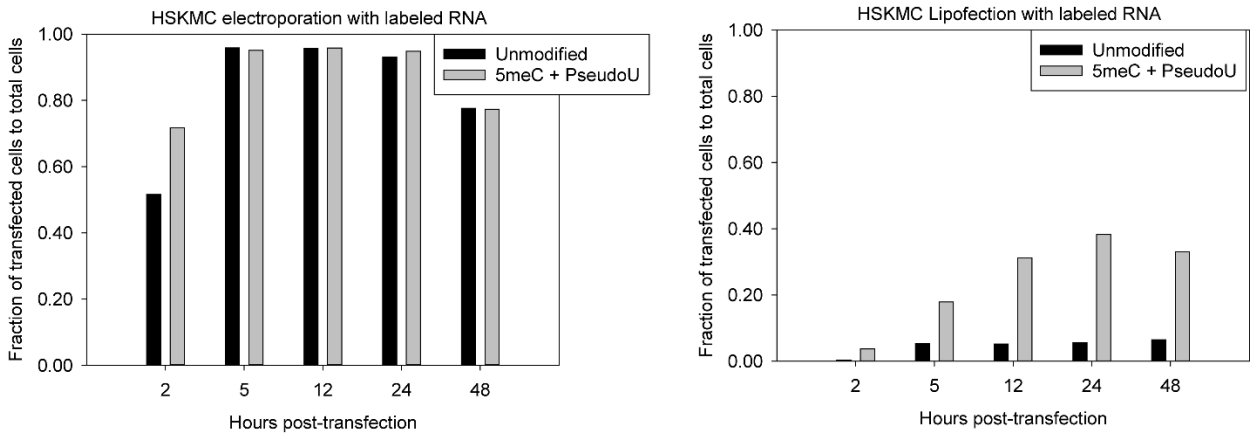
S3: mRNA labeled with Dylight-650 MTRIPs colocalizes with Quasar 570 labeled FISH probes targeted to the coding region of the mRNA in Hela cells. (A) A representative image, line profile (yellow), and Mander's overlap coefficients are plotted (overall Costes, where m1-refers to FISH signal containing MTRIP signal, and m2 refers to MTRIP signal containing FISH signal). (B) There was no detectable FISH signal above background in controls including vehicle only, an mRNA encoding CCL3 containing the same 3'UTR probe binding sites, and a DNA containing the 3' UTR probe binding site only. Images were analyzed in Volocity software with at least 15 cells per condition.



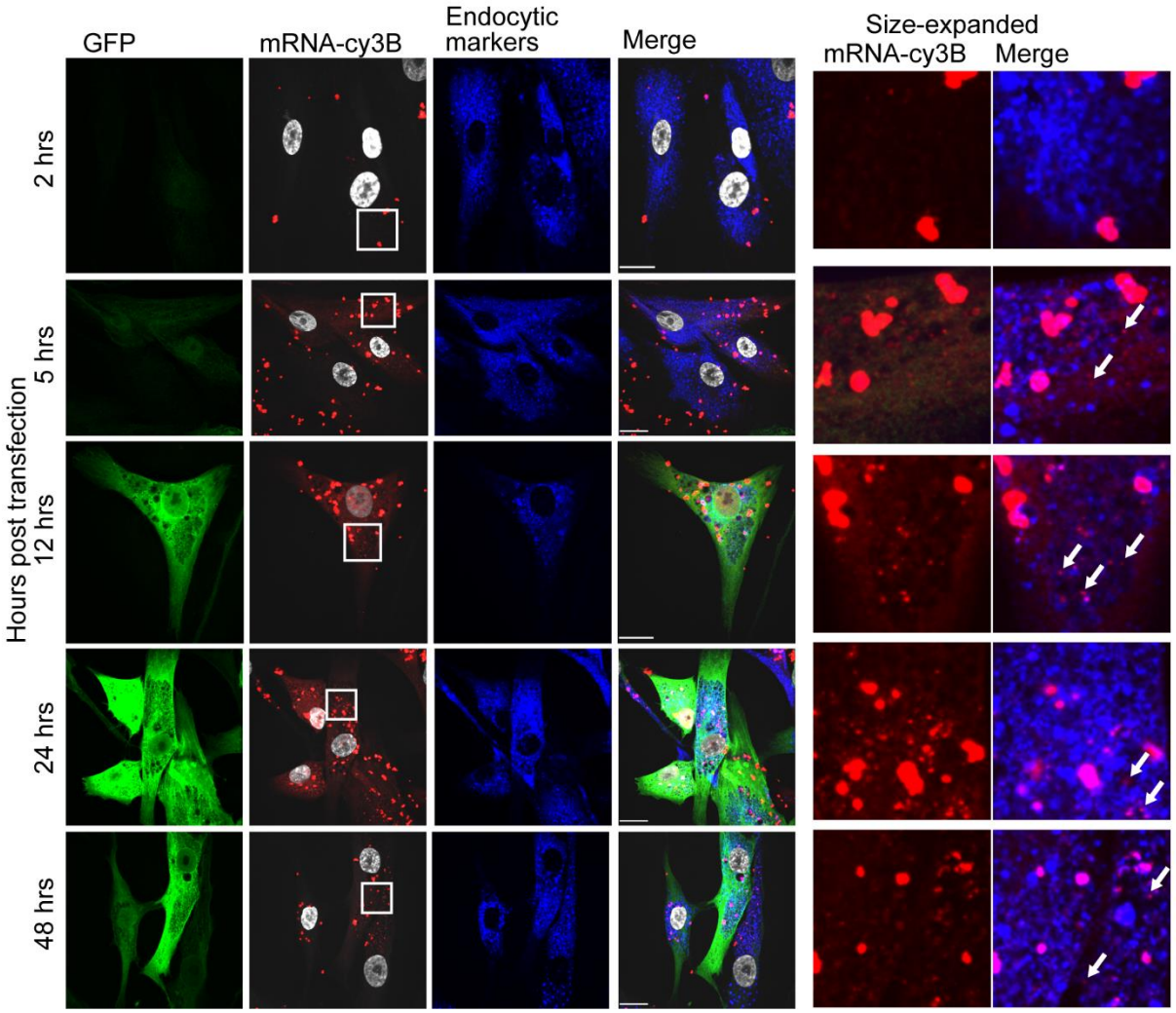
S4: mRNA labeled with MTRIPs and delivered by lipofection allowed characterization of delivery route by costaining with Clathrin light chain, Caveolin, or ARF6. mRNA appeared associated with Clathrin Light Chain and Caveolin containing vesicles, but not with ARF6 (scale bar =11µm). Line profiles as indicated by white lines.



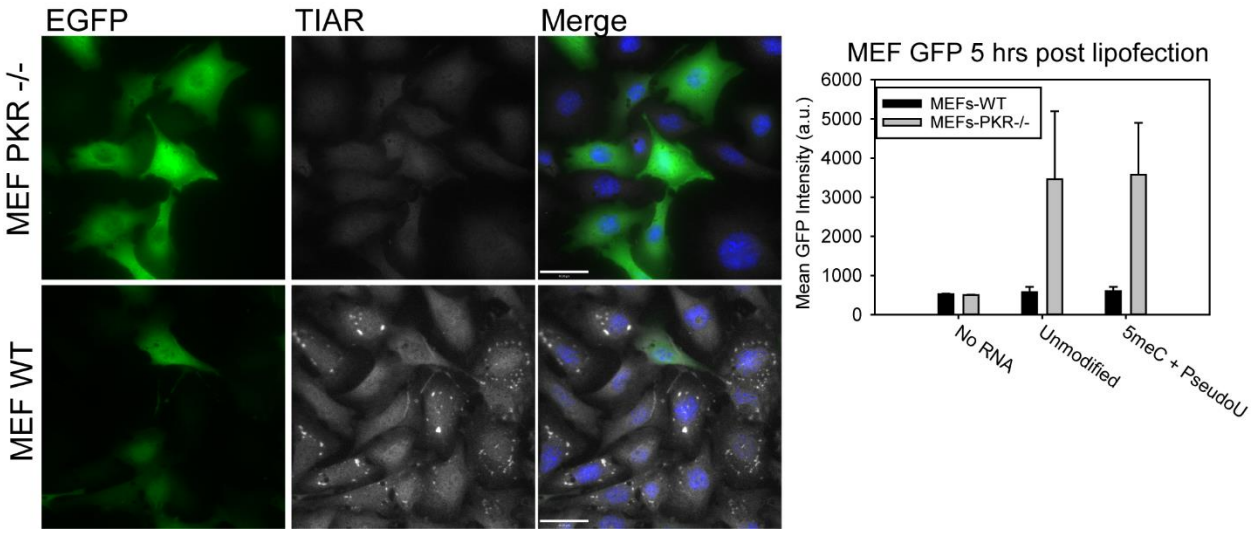
S5: Upon electroporation, mean mRNA intensity scales linearly with increasing amounts of transfected cy3b-labeled mRNA up to 1000ng per 100000 cells. HSkMCs were electroporated with cy3b labeled mRNA, fixed 30 minutes post transfection, and analyzed by flow cytometry.



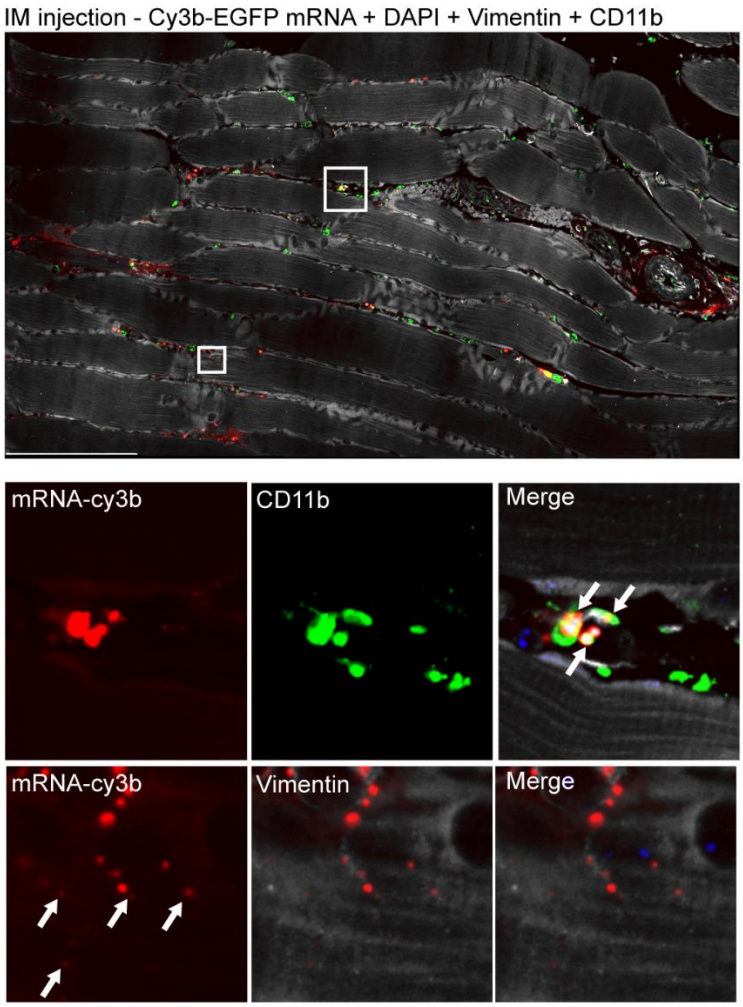
S6: Transfection efficiency of HSkMC cells upon electroporation or lipofection with labeled modified or unmodified EGFP mRNA. “Expressing cells” are characterized by the sum of EGFP intensity greater than 6 times the standard deviation of non-transfected cells. The percent of GFP expressing cells in HeLa and MEF cells upon electroporation or lipofection (data not shown) were typically higher than 90%.



S7: Imaging of lipofected cells fixed and stained with endocytic markers EEA1, CD63, and LAMP1. Representative images of 5meC+PseudoU modified EGFP mRNA lipofected into HSkMCs and fixed at 2, 5, 12, 24 and 48 hours post-transfection. White boxes indicate magnified regions. Due to high dynamic range, contrast enhancement was necessary to visualize small mRNAs and resulted in mRNA granules to appear larger. White arrows indicate single mRNA granules in red which do not overlap with endocytic markers (blue), indicating that the fraction of cytosolic mRNA increases over time. (scale bar = 20µm).



S8: Wild-type MEF cells lipofected with EGFP mRNA produced very little protein 5 hours post transfection due to extensive SG formation with both modified and unmodified mRNA. MEF PKR-/- cells produced similar levels of protein regardless of modification. These cells did not form stress granules. The SG marker TIAR is used for staining of SGs (white) and the mean GFP intensity was quantified for at least 30 cells per sample in 40x widefield images using Volocity software. (bars = S.D of population, scale bar = 20µm)



S9: Visualization of labeled mRNA following IM injection in mouse muscle tissue sections. Cy3b-labeled mRNA (red) was imaged in anterior tibialis muscle sections removed 16 hours post-IM injection. Immunofluorescence staining for anti-cd11b (green) and anti-vimentin (white) is shown. Expanded views of white boxed areas are single-xy planes and have been contrast enhanced differently for visualization. Cells that are CD11b-positive are indicated by arrows, as well as RNA located near the perinuclear region of muscle cells. (scale bar =150µm)

Supplementary Table 1:

Mean, SEM, and P values for treatments in Fig 5B:

RNA values	Lipofectamine (mean+-SEM)	Electroporation (mean+-SEM)	Significantly different? (Mann-Whitney test)
Unmodified mRNA	*346488 ± 43126*	*1073724 ± 96611*	Yes, P<.0001
Modified mRNA	*398804 ± 44168*	*1316282 ± 113595*	Yes, P<.0001
Significantly different? (Mann-Whitney test)	No, P=.4453	No, P=.2346	

EGFP values	Lipofectamine (mean+-SEM) x 10^6	Electroporation (mean+-SEM) x 10^6	Significantly different? (Mann-Whitney test)
Unmodified mRNA	*759 ± 102*	*2826 ± 172*	Yes, P<.0001
Modified mRNA	*2977± 313*	*2402 ± 139*	Yes, P=.0046
Significantly different? (Mann-Whitney test)	Yes, P<0.0001	No, P=0.0699	