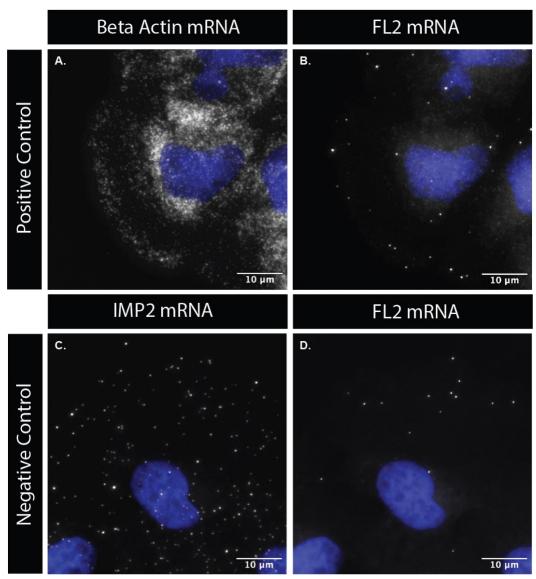
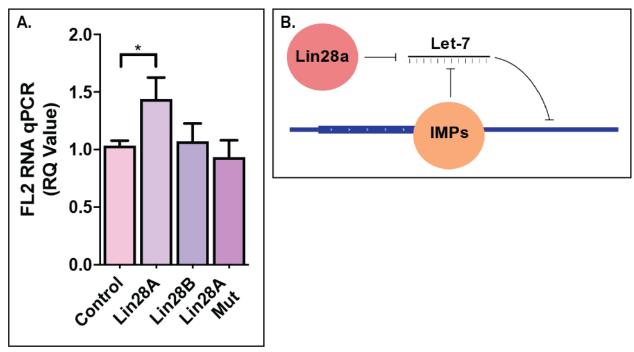


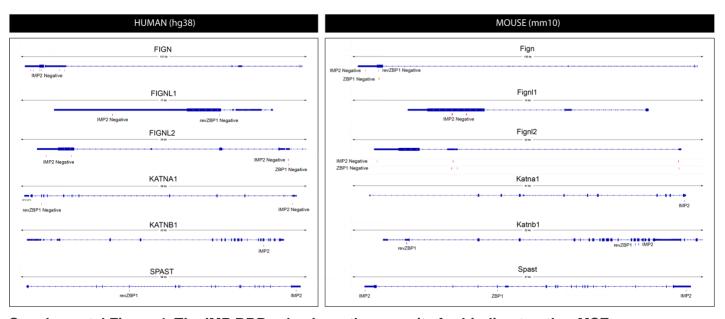
Supplemental Figure 1. Optimization of smFISH for visualization of FL2 mRNA. A) Schematic of the difference between the use of traditional Stellaris smFISH probes and optimized smFISH probes. The Stellaris probes use fluorescently labeled 18-22mer probes that hybridize to the mRNA sequence. The optimized probes use a primary set of 30mer probes that hybridize to the mRNA sequence and a secondary set of fluorescently labeled probes that hybridize to the 20mer overhangs of the primary set. B) FL2 mRNA smFISH in MEFs using Stellaris probes. C) FL2 mRNA smFISH using optimized probes.



Supplemental Figure 2. Comparative smFISH images of control mRNA and FL2 mRNA used for the polarization and dispersion index assay. A) β -actin mRNA used as the positive control. B) FL2 mRNA in the same cell used in panel A. C) IMP2 mRNA used as a negative control. D) FL2 mRNA in the same cell used in panel C.



Supplemental Figure 3. LIN28 indirectly affects FL2 mRNA expression by negatively regulating let-7 miRNA expression. A) FL2 mRNA quantity in N2A cells with 48 hours of LIN28 overexpression. Lin28A Mut is a mutant that cannot negatively regulate let-7 miRNA. B) Schematic of the regulating molecules involved in FL2 mRNA expression.



Supplemental Figure 4. The IMP RBPs also have the capacity for binding to other MSEs . IMP1 (ZBP1) and IMP2 RBP binding motifs within the human (left) and mouse (right) gene sequences of the MSEs, including fidgetin (FIGN, Fign), fidgetin-like 1 (FIGNL1, Fignl1), fidgetin-like 2 (FIGNL2, Fignl2), katanin p60 (KATNA1, Katna1), katanin p80 (KATNB1, Katnb1) and sptastin (SPAST, Spast). IMP1/ZBP1 motifs from (Patel et al, Genes and Development 2013) and IMP2 motifs from (Biswas et al, Nat Com 2019). Positive and negative correspond to DNA strand on which the motif search was performed (all strands correspond to the gene of interest). Motif search limited to exons (thick lines) only, motifs found in introns (thin lines) were excluded as they were unlikely to be present in the mature mRNA.