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Supplemental Information

The cardiac translational landscape reveals that micropeptides are new players involved in cardiomyocyte hypertrophy

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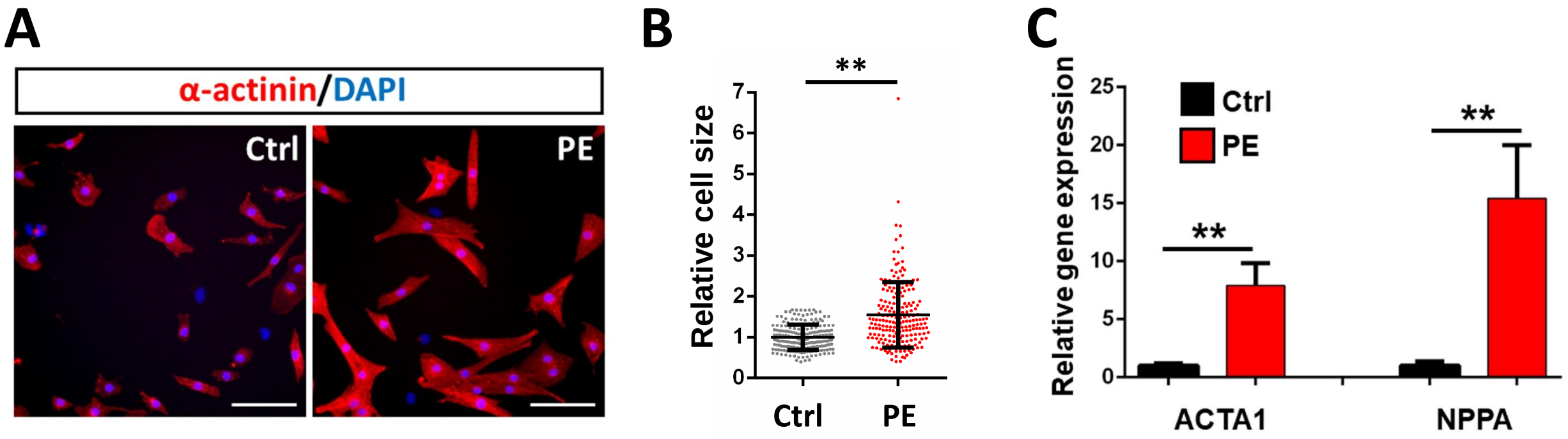
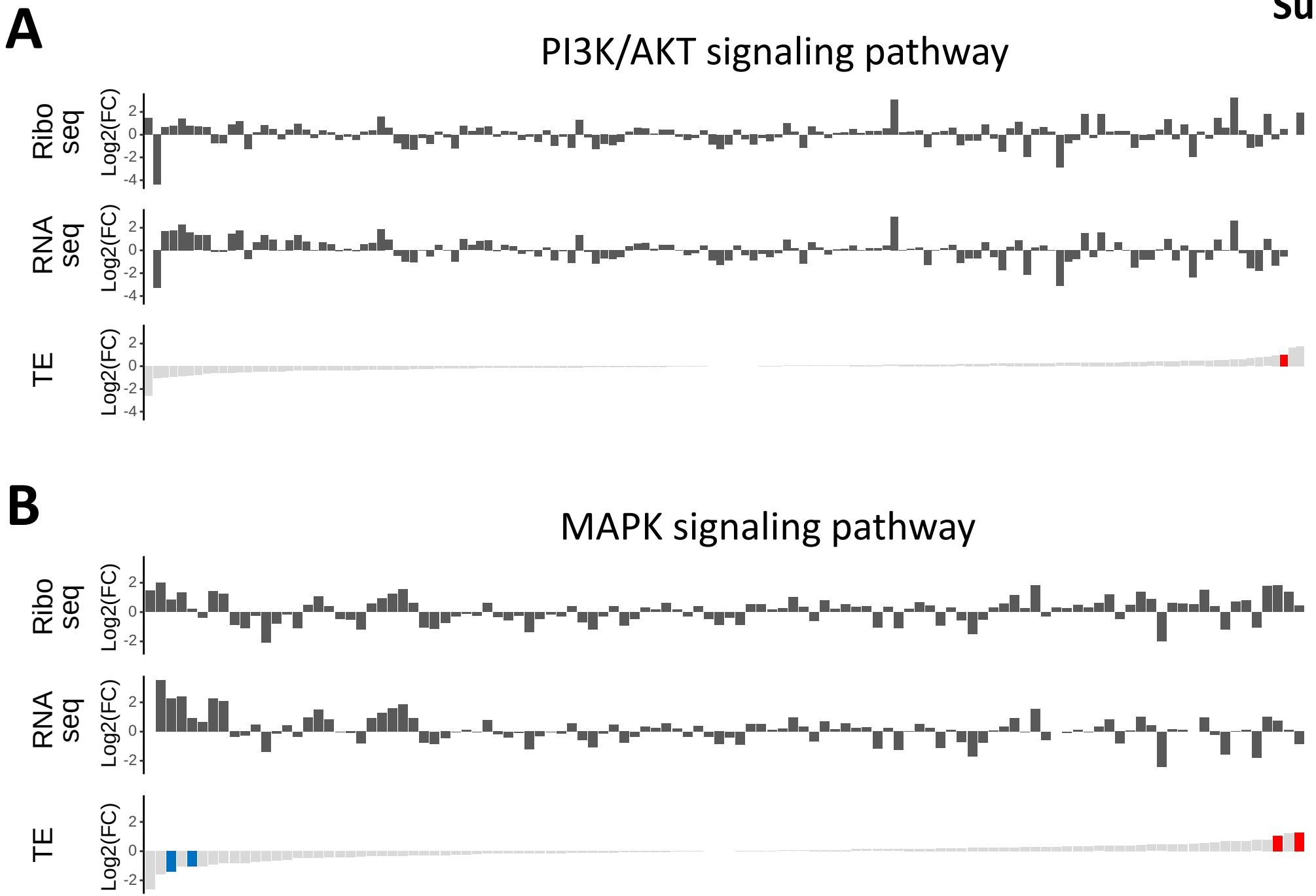
Suppl. Figure 1



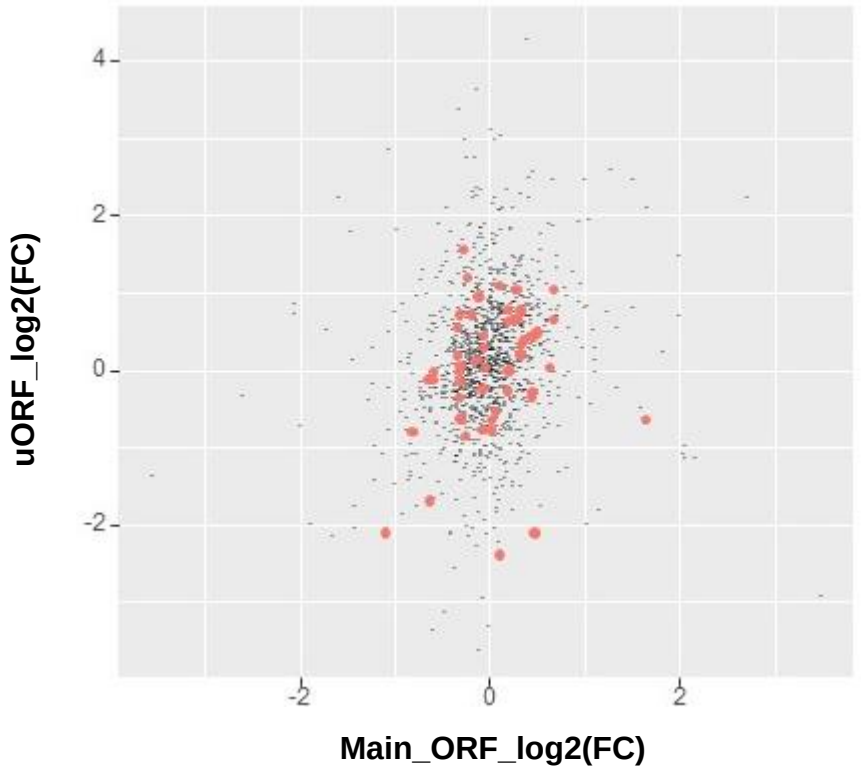
Suppl. Figure 2



Suppl. Figure 3

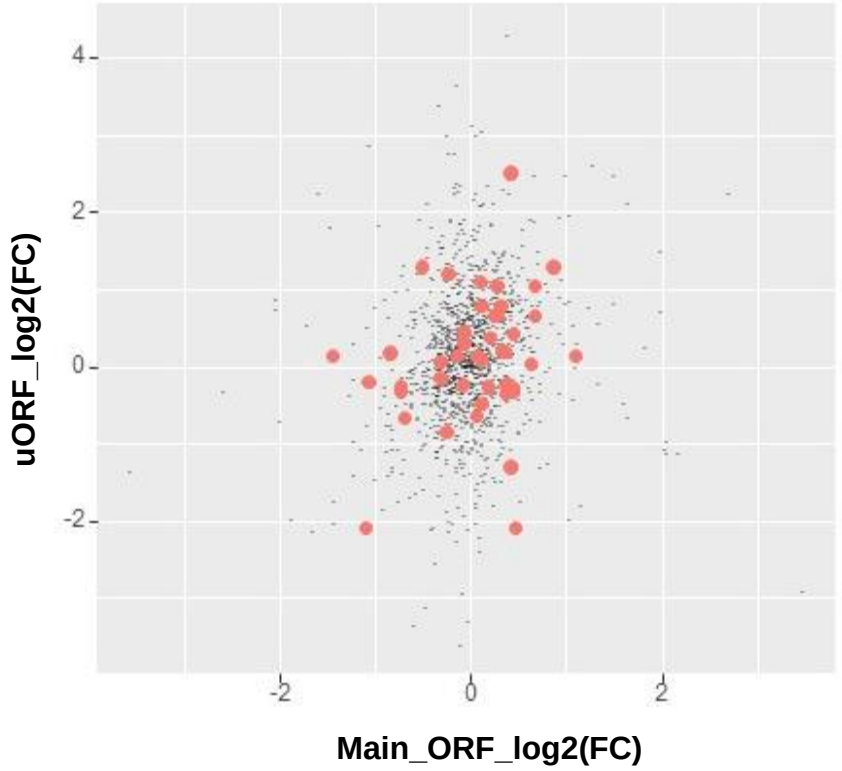
A

PI3K/AKT signaling pathway

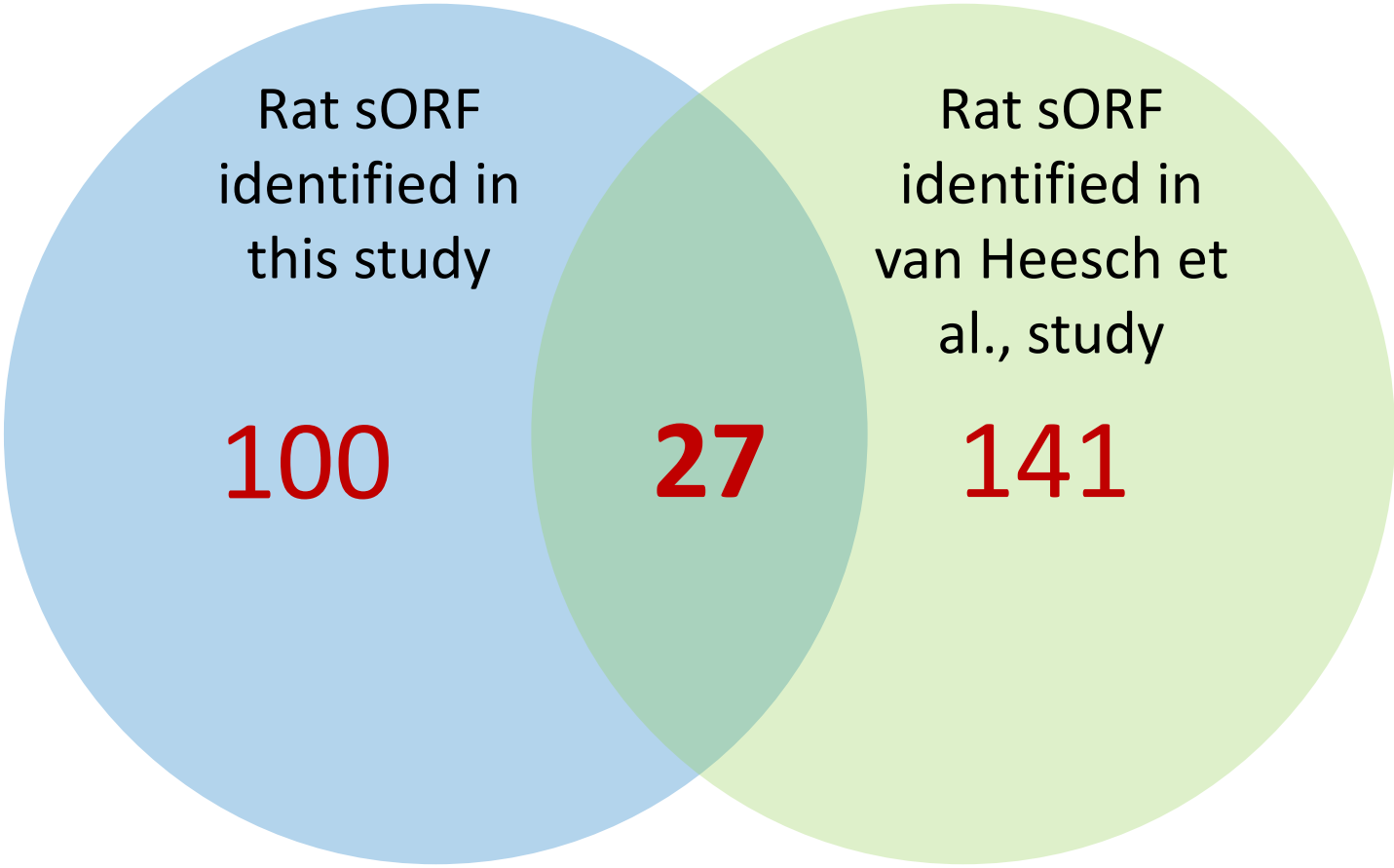


B

MAPK signaling pathway



Suppl. Figure 4



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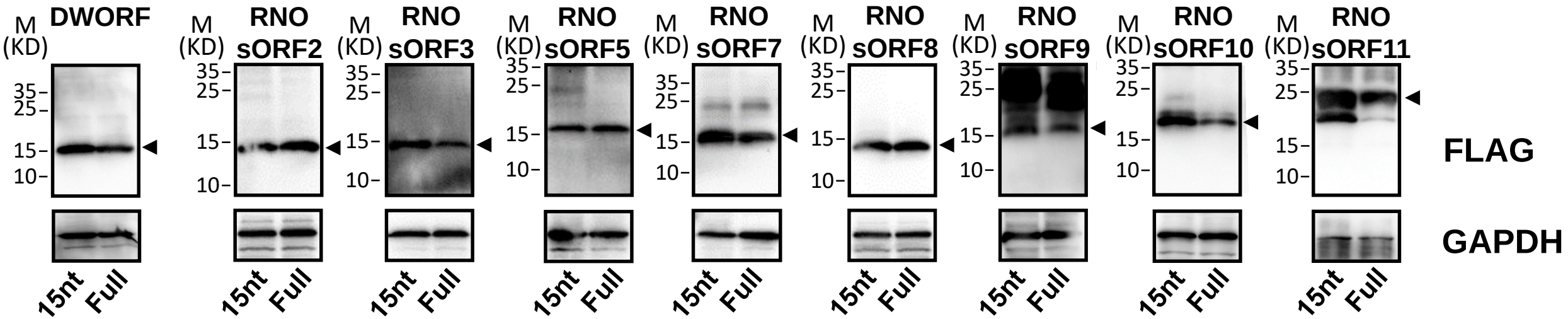
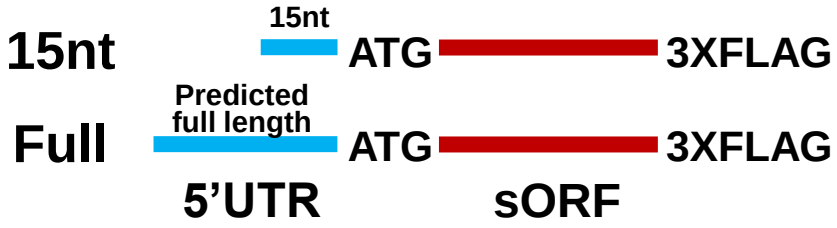
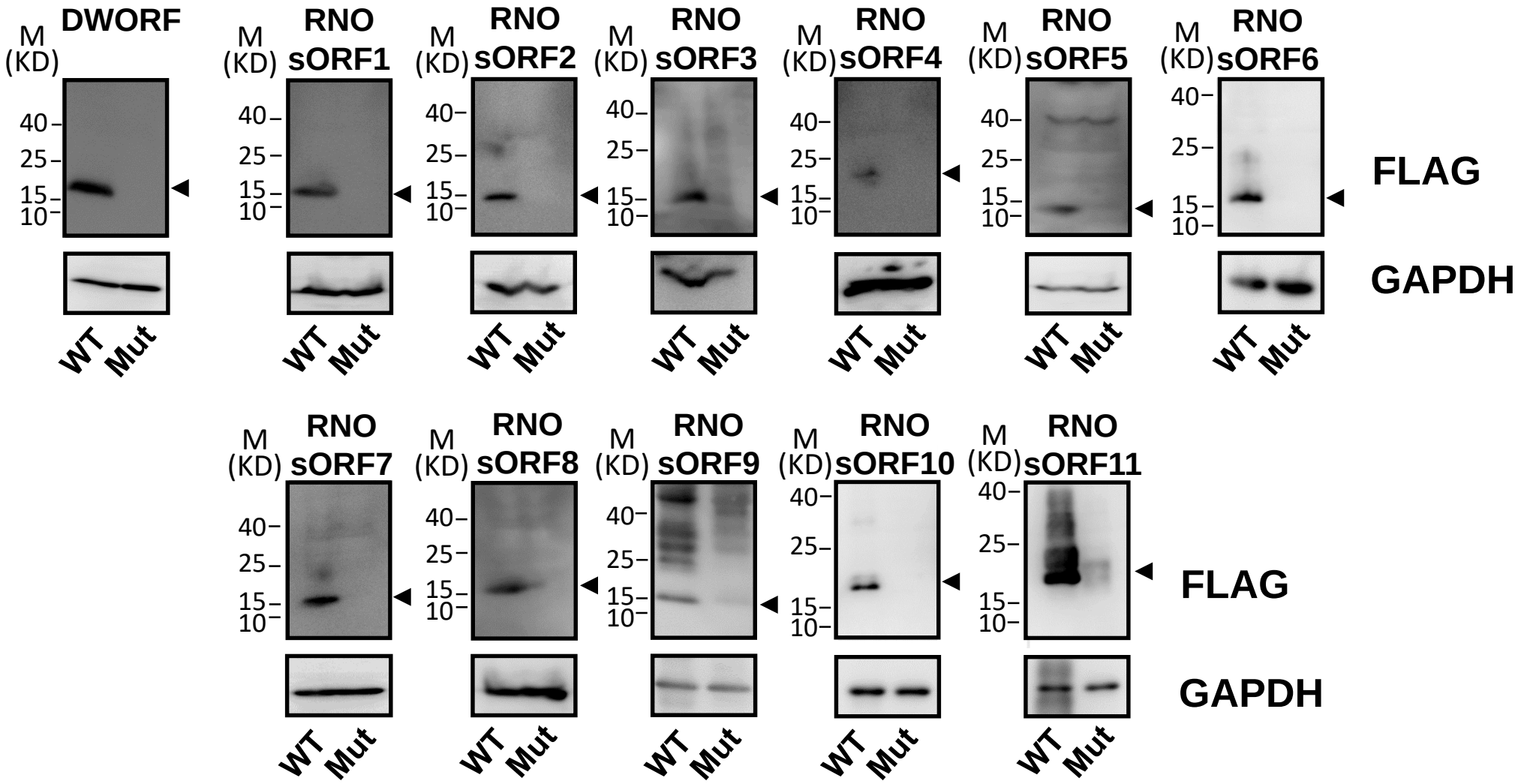
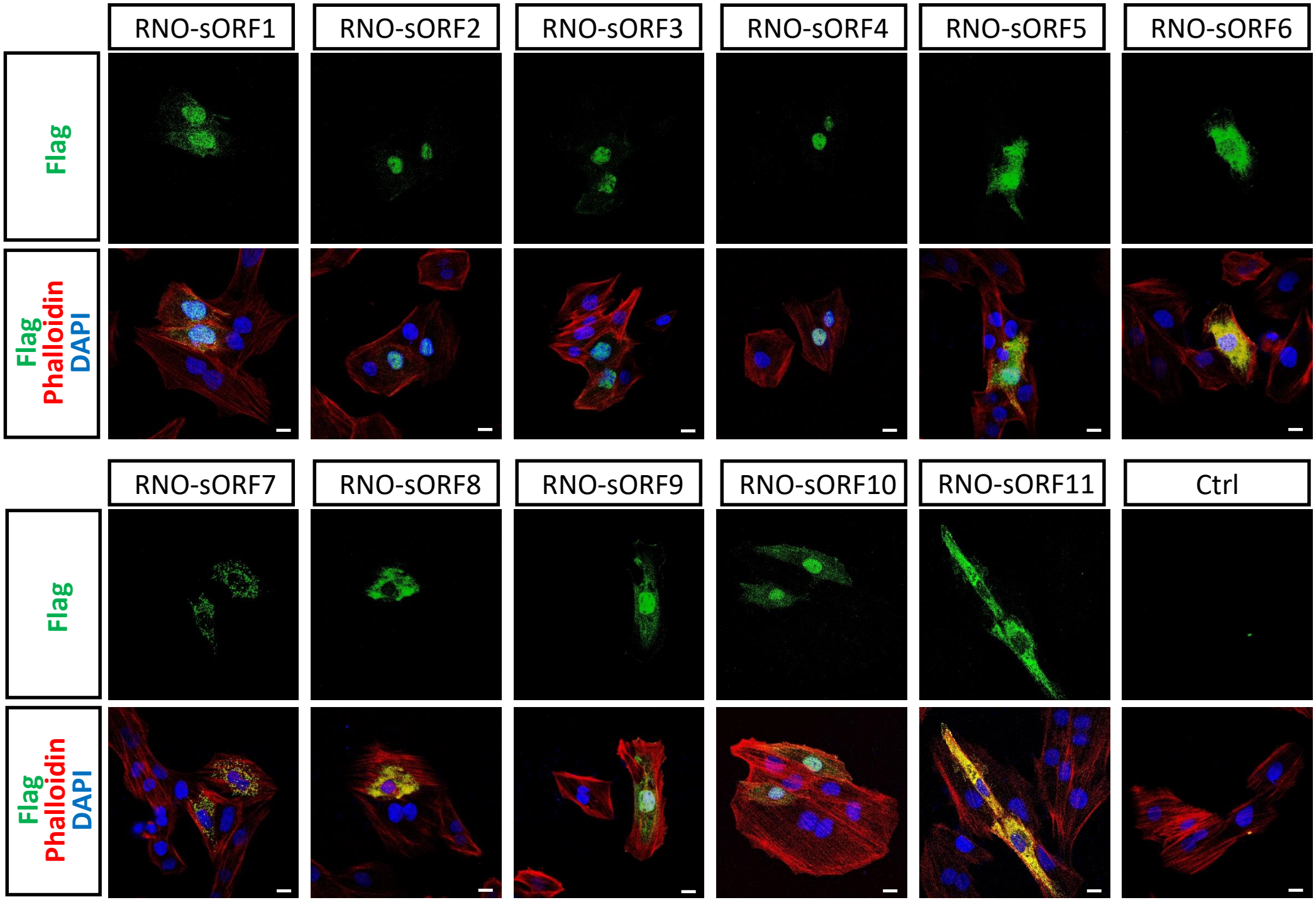




Suppl. Figure 6

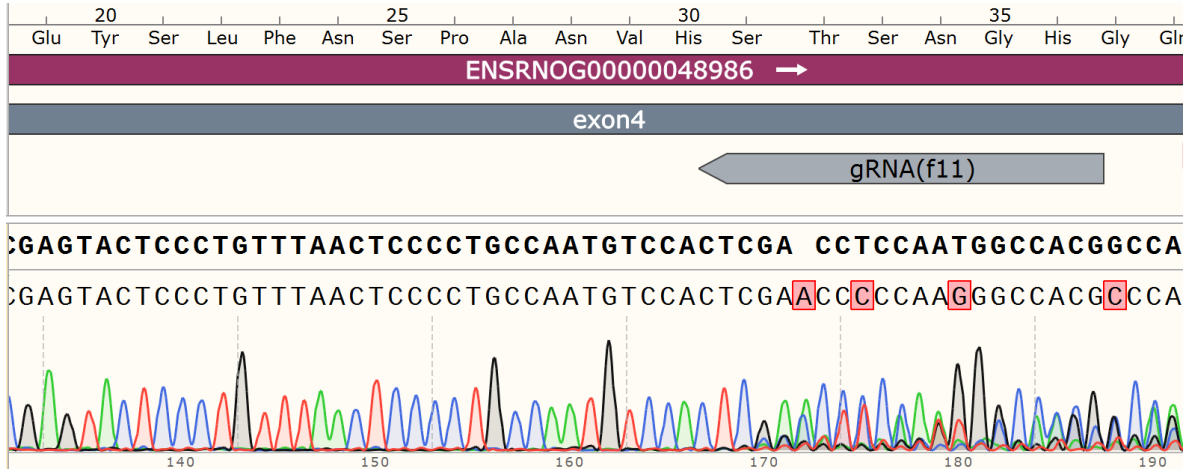




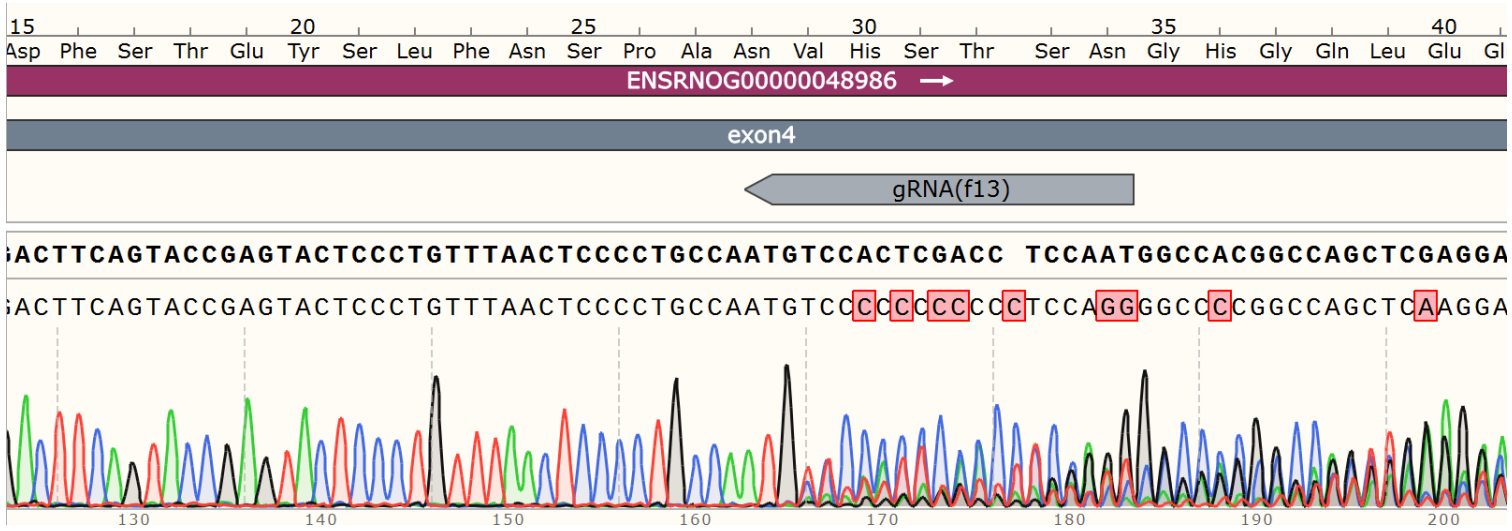
Suppl. Figure 7

Suppl. Figure 8

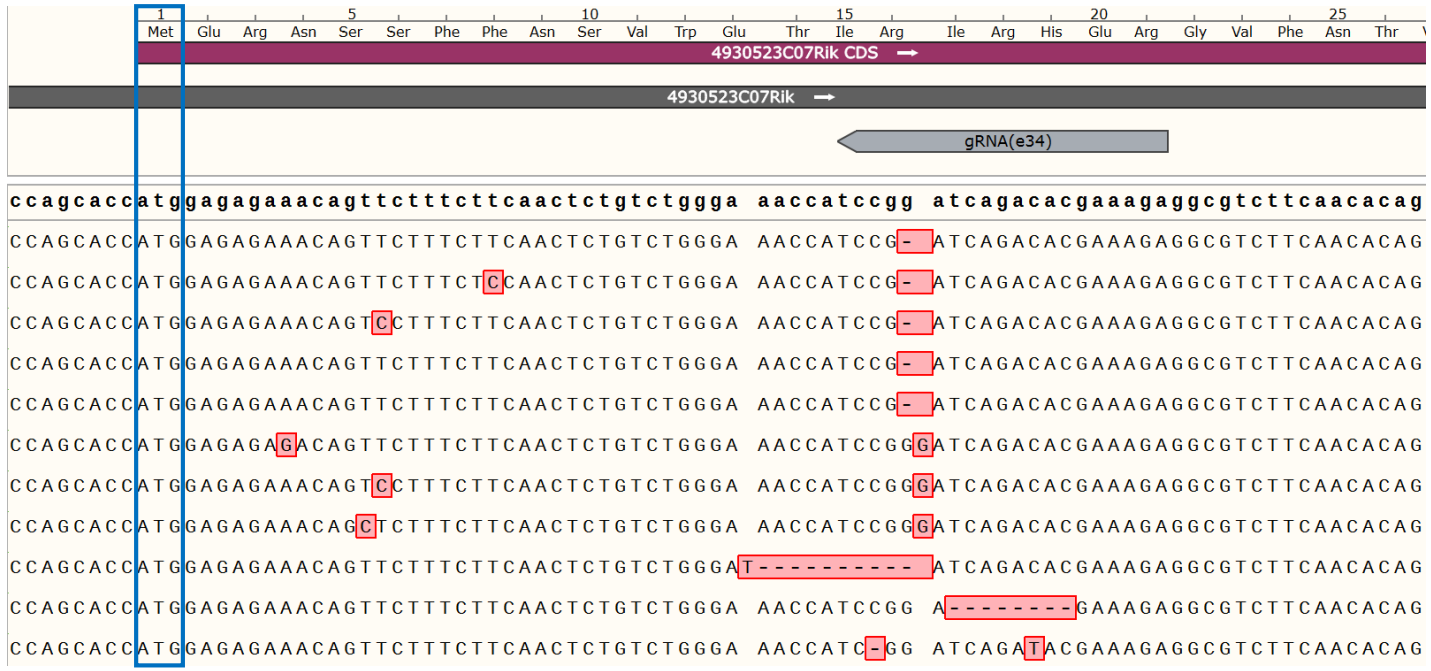
gRNA1



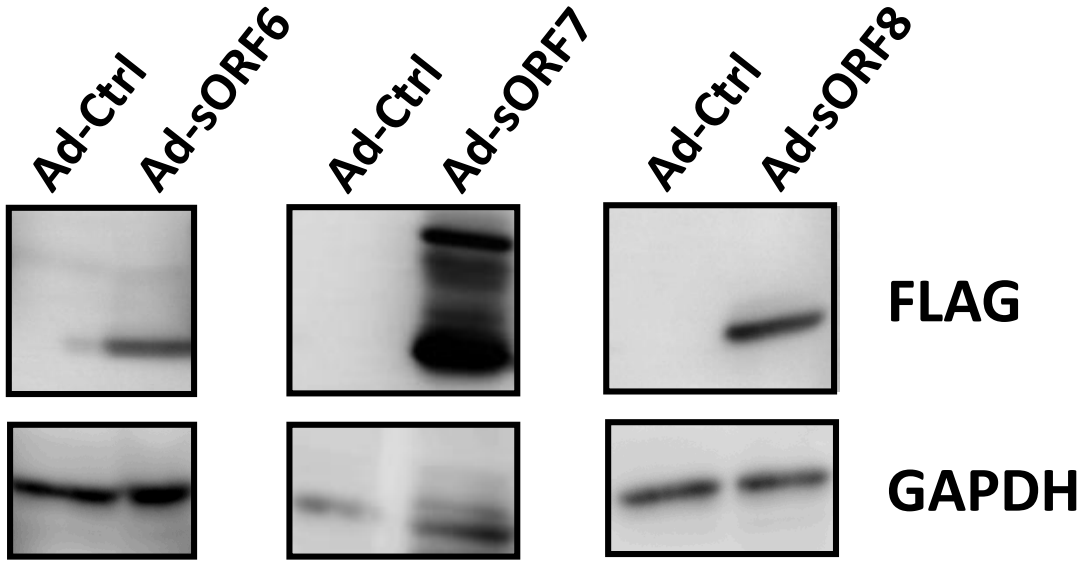
gRNA2



Suppl. Figure 9



Suppl. Figure 10



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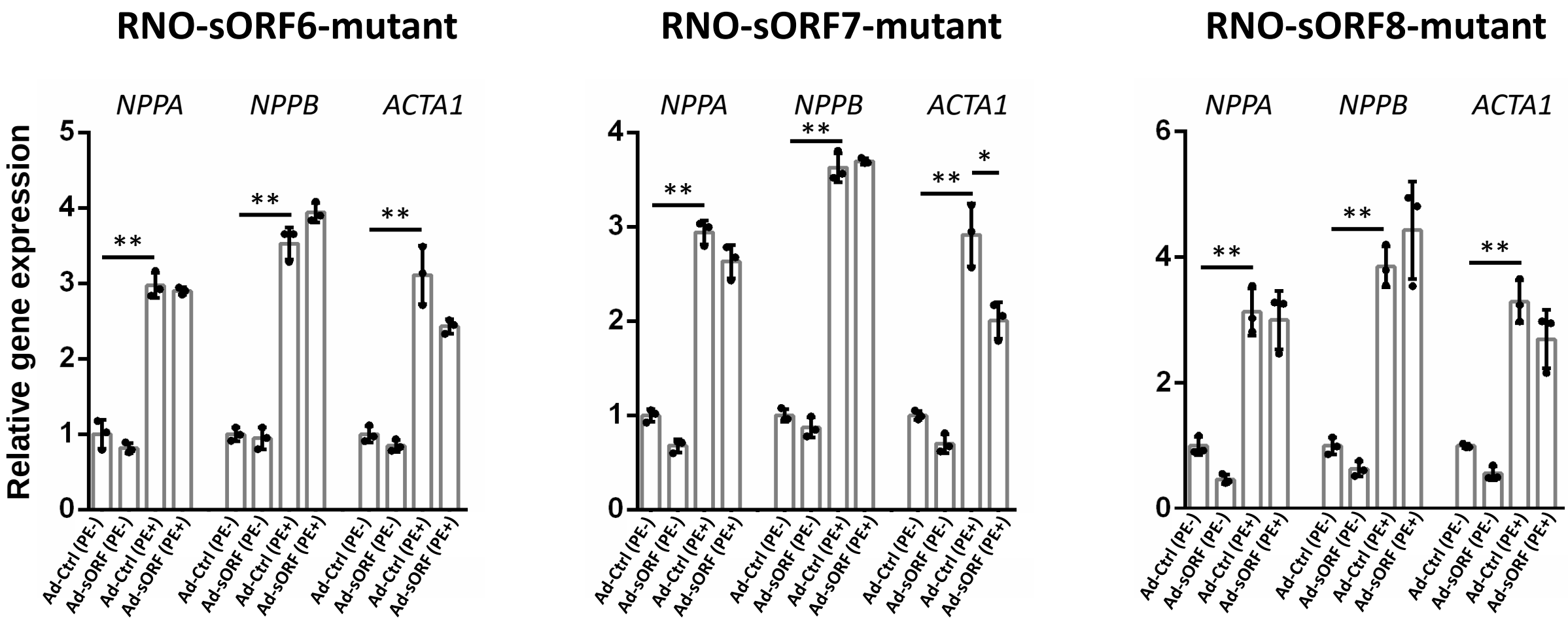

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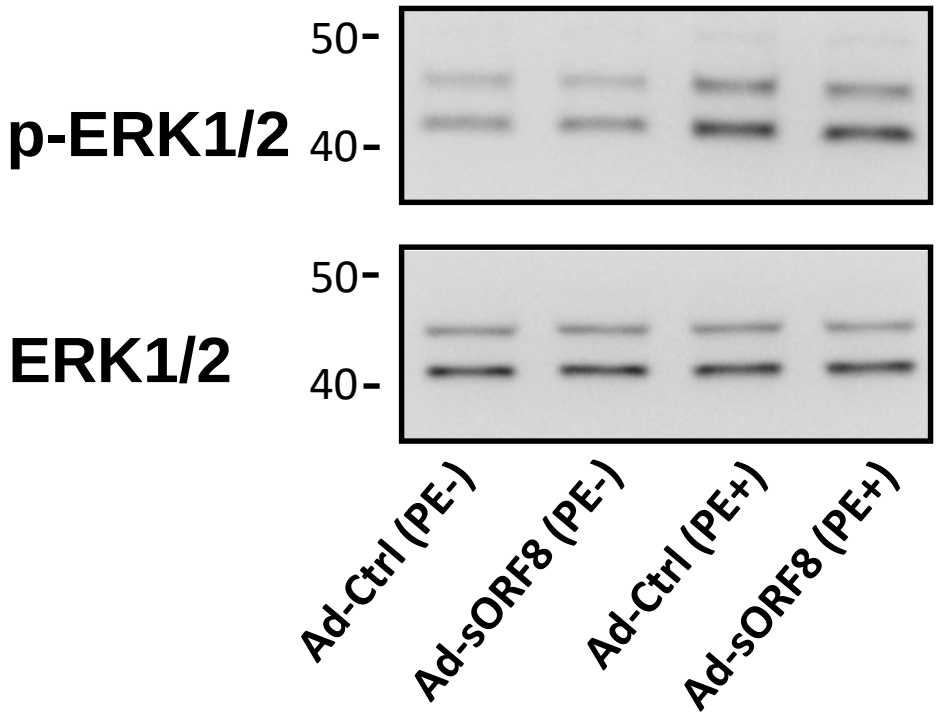
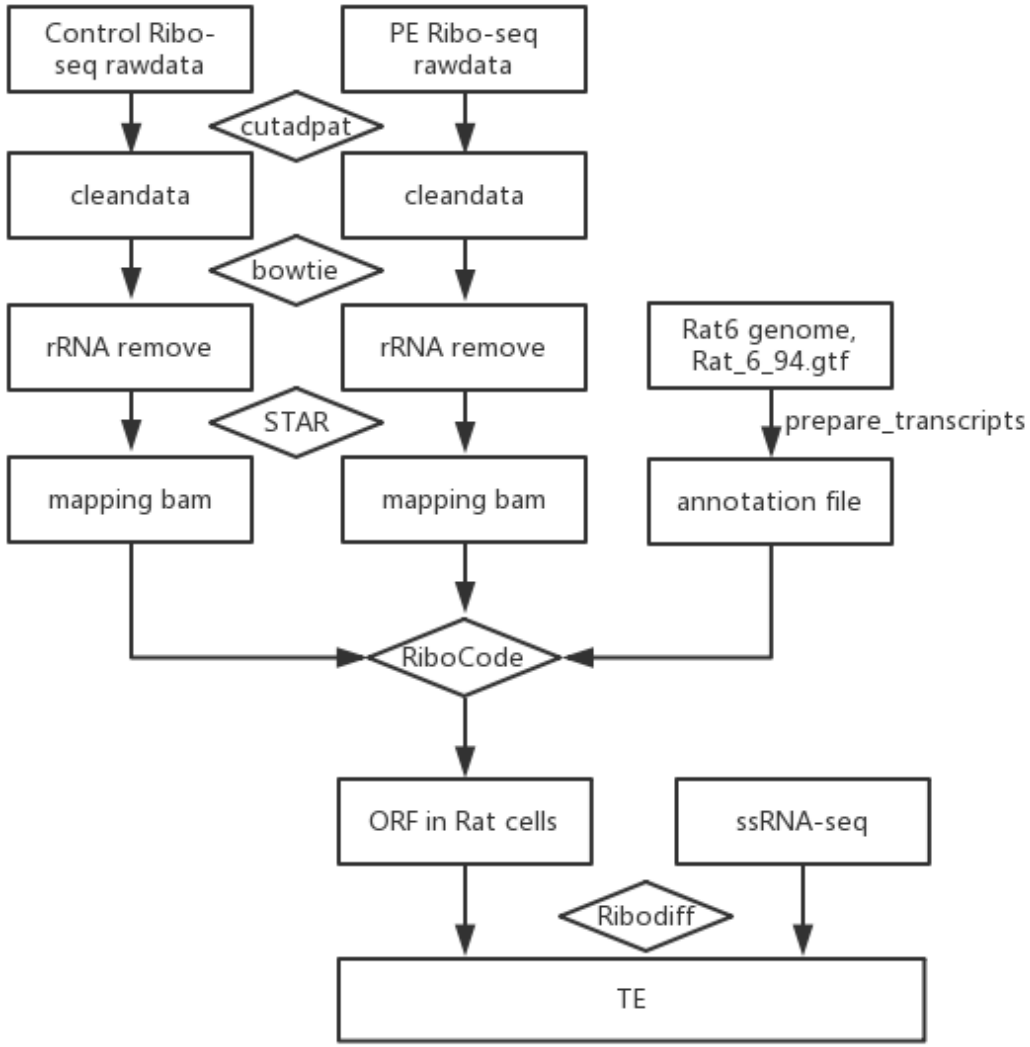


Suppl. Figure 13



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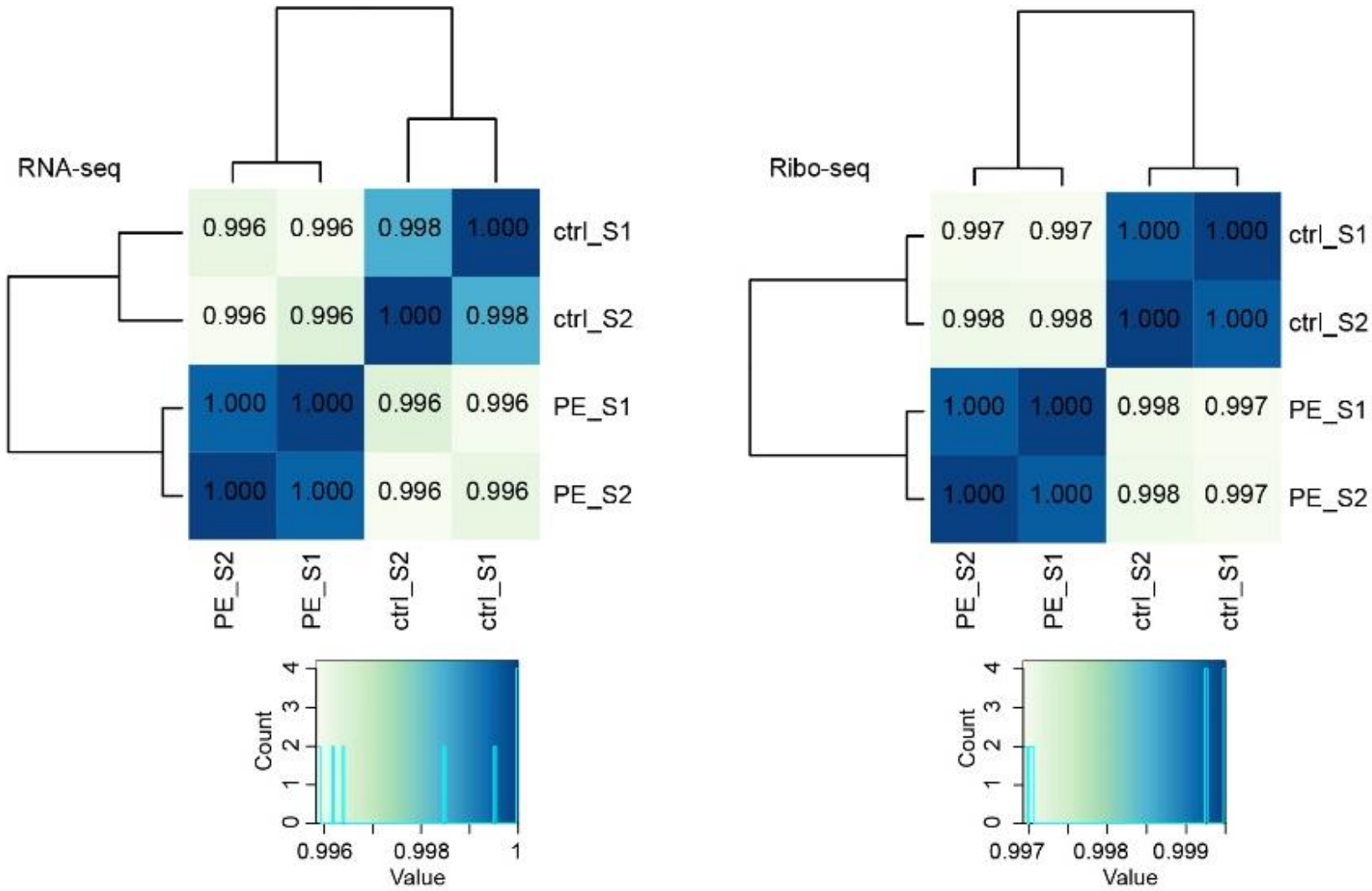

Supplemental Figure legend

Supplemental Figure 1. Examination of PE-induced cardiomyocyte hypertrophy. (A) Immunostaining for α-actinin in primary neonatal rat ventricular cardiomyocytes under phenylephrine treatment or control conditions. Scale bar=100μm; (B) Quantitative analysis of cardiomyocyte cell size. At least 200 cardiomyocytes from 8 staining images were measured for each group (C) Expression of hypertrophic markers, ACTA1 and NPPA, in PE-treated and control cardiomyocytes. N=3 for each group. **P<0.01.

Supplemental Figure 2. Alignments of gene translational efficiency to its expression level in translation and transcription. Genes in PI3K/AKT signaling pathway (A) and MAPK signaling pathway (B) with FDR<0.05 in ribo-seq were aligned. Genes are indicated by bars in each column. Genes are sorted based on their TE. Bars in red and blue indicate TE for these genes significantly increased and decreased, respectively.

Supplemental Figure 3. Plots of ratio of translational efficiency change of uORF and ORF for subsets of uORF-ORF pairs. uORF-ORF pairs (red dots) in PI3K/AKT signaling pathway (A) and MAPK signaling pathway (B) were analyzed. The smaller gray dots are ORF pairs shown in Fig 4D (all uORF-ORF pairs).

Supplemental Figure 4. The overlap of sORFs identified in this study and previous report (*Cell 178, 242-260*)

Supplemental Figure 5. Analyses of the translation of sORFs with different 5'UTR. sORFs with 15nt and predicted full-length 5'UTR were cloned into the 3XFLAG-tagged expressing vector (inlet). Ectopic expression of flag-tagged micropeptides was detected by western blotting with anti-FLAG antibodies. The detected GAPDH expression serves as loading control.

Supplemental Figure 6. A frameshift mutation abolished the expression of micropeptide. Wild-type (WT) and mutant (Mut, one nucleotide insertion after ATG start codon) sORFs were cloned into the 3XFLAG-tagged expressing vector (inlet). Ectopic expression of flag-tagged micropeptides was detected by western blotting with anti-FLAG antibodies. The detected GAPDH expression serves as loading control.

Supplemental Figure 7. Detection of cellular localization of micropeptides. The expression of FLAG-tagged micropeptides mediated by adenovirus is determined by immunostaining in H9C2 cardiomyocytes. Cardiomyocytes were co-stained with Phalloidin. Nuclei are indicated by DAPI. Scale bar=10 μm.

Supplemental Figure 8. Sequence confirmation of CRISPR/Cas9-mediated gene editing in sORF9 in H9C2 cells. PCR amplicons of sORF9 DNA locus from mixed H9C2 cell population after CRISPR/Cas9-mediated gene editing were sequenced. DNA mutations are boxed. Experiments from two independent gRNAs are shown.

Supplemental Figure 9. Sequence confirmation of CRISPR/Cas9-mediated gene editing in sORF11 in NIH-3T3 cells. sORF11 DNA from NIH-3T3 cells after CRISPR/Cas9-mediated gene

editing was cloned. 11 out of 12 clones subjected to sequence showed a frameshift mutation in sORF11. ATG start codon is highlighted. DNA mutations are boxed. gRNAs are indicated.

Supplemental Figure 10. Detection of the overexpression of micropeptides mediated by adenovirus in NRVC. The 3XFLAG-tagged fusion micropeptides and GAPDH (control) were detected with specific antibodies.

Supplemental Figure 11. The regulatory effect of micropeptides on cardiomyocyte hypertrophy is abolished when the translation of sORFs is disrupted by ATG start codon deletion. Expression of hypertrophic markers, NPPA, NPPB and ACTA1 in PE-treated or control cardiomyocytes transduced with control or sORF mutant adenovirus was determined by qRT-PCR. N=3 for each group. **P<0.01, *P<0.05.

Supplemental Figure 12. Detection of the phosphorylation of ERK in sORF8-overexpressing cardiomyocyte during hypertrophy by western blotting. The phosphorylation ratio of ERK did not alter when sORF8 was overexpressed during PE-induced cardiomyocyte hypertrophy compared to PE-treated control cardiomyocyte.

Supplemental Figure 13. The workflow of Ribo-seq analysis

Supplemental Figure 14. Correlation analyses of replicates of RNA-seq and Ribo-seq. Heatmaps of Pearson correlation coefficient of replicates of RNA-seq and Ribo-seq are shown. Pearson correlation coefficient values are indicated and coded by colors illustrated below.

Supplemental Table 1. QC data of Ribo-seq and RNA-seq

Ribo-seq	Control_S1	Control_S2	PE_S1	PE_S2
Raw Reads Number	51990940	63527981	58779595	56823796
Clean Reads Number	42310466 (81.4%)	49317040 (77.6%)	49051163 (83.4%)	46377074 (81.6%)
rRNA Mapping Reads Number	10764584 (20.7%)	12553104 (19.8%)	19127536 (32.5%)	16790553 (29.5%)
Uniquely mapped reads	20645038 (39.7%)	24519478 (38.6%)	24519478 (41.7%)	19192042 (33.8%)

RNA-seq	Control_S1	Control_S2	PE_S1	PE_S2
Raw Reads Number	216147226	201261528	233226640	209601716
Clean Reads Number	208556212 (96.5%)	194411426 (96.6%)	224962006 (96.5%)	201193324 (96.0%)
rRNA Mapping Reads Number	314042 (0.15%)	689082 (0.34%)	379646 (0.16%)	452578 (0.22%)
Uniquely mapped reads	92415284 (42.8%)	85951491 (42.7%)	100585155 (43.1%)	89697979 (42.8%)

Supplemental Table 2. Raw counts of reads mapped to all ORFs and their transcripts.

Supplemental Table 3. Information of differentiated expressed genes in RNA-seq.

Supplemental Table 4. Information of differentiated expressed genes in ribo-seq.

Supplemental Table 5. Information of genes with significant change in translational efficiency.

Supplemental Table 6. Information of uORFs identified from the bioinformatic screening.

Supplemental Table 7. Information of sORFs identified from the bioinformatic screening.

Supplemental Table 8. Expression (RPKM) of RNA transcripts harboring sORFs from RNA-seq

geneID	ctrl_S1	ctrl_S2	PE_S1	PE_S2	ctrl_Mean	PE_Mean
ENSRNOG00000057352	5.611408	5.722743	4.954936	4.592459	5.667076	4.773697
ENSRNOG00000059100	4.141101	5.601615	5.647042	3.9271	4.871358	4.787071
ENSRNOG00000058926	0.41044	0.8171	0.962179	1.044773	0.61377	1.003476
ENSRNOG00000057834	1.579457	1.126796	0.695557	1.069656	1.353126	0.882607
ENSRNOG00000057097	0.712452	0.591175	0.794698	0.651712	0.651813	0.723205
ENSRNOG00000054516	0.003717	0	0.001694	0.01405	0.001859	0.007872
ENSRNOG00000051251	0.375349	0.831518	0.774431	0.810685	0.603433	0.792558
ENSRNOG00000048986	0	0.086951	1.74204	1.650831	0.043475	1.696436
ENSRNOG00000049537	3.925878	3.779405	4.724359	4.41977	3.852642	4.572065

Supplemental Table 9. Information of primers used in this study.

GENE	Forward primer (5'→3')	Reverse primer (5'→3')
GAPDH	ACAACTTTGGCATCGTGGAA	GATGCAGGGATGATGTTCTG
NPPA	CAACACAGATCTGATGGATTTCA	CCTCATCTTCTACCGGCATC
NPPB	GTCAGTCGCTTGGGCTGT	CCAGAGCTGGGGAAAGAAG
ACTA1	AGCTATGAGCTGCCTGACG	GATCCCCGCAGACTCCATA
ACTB	CCCGCGAGTACAACCTTCT	CGTCATCCATGGCGAACT
RN18S	GCCGCTAGAGGTGAAATTCTT	CGTCTTCGAACCTCCGACT
ENSRNOG00000057352	AAACTGAGGCCCGAGGAT	CACTTGCAGAACAGTGAAGCA
ENSRNOG00000059100	CCAGGAAATGGCTATCAATACG	CCTTGGGTCAGTCACTTCATC
ENSRNOG00000058926	TGTGGGCCAAAGAACAGAG	CTTCTTCAGCAATGGGTGGT
ENSRNOG00000057834	AGACGTCCTGTCGCCTTCT	GCCATCGTGTTAATGTATTCCTG
ENSRNOG00000057097	TTGGACAGAGATGCCGAGA	GTTCCCGAGCCTCACACA
ENSRNOG00000054516	CTTGCCAACAGGGAAGTCAG	TGAAAGTGGAGGTGCTGTGA
ENSRNOG00000051251	CTGGCCTGCAAAGGAATCT	GACCGTACCTCAGGCATCTC
ENSRNOG00000048986	CCAATGTCCACTCGACCTCC	AGGCATACACCACCCCTACT
ENSRNOG00000049537	GAACAGATCCAGCGGTCCT	AAGCCACTGGGAGCACCT
CDKN1A	GACATCTCAGGGCCGAAA	GGCGCTTGGAGTGATAGAAA
CDK2	TCCTCTTCCCCTCATCAAGA	CGGTGAGAATGGCAGAATG
CCNE1	CTGAGAGATGAGCACTTTCTGC	TGGAGCTTATAGACTTCACACACC
PCNA	AAATGTGCTGGTAATGAAGACATC	CATAGTCTGAAACTTTCTCTTGATTTG
JUNB	GGGACTGGGAGCTCATACC	AAAGGGTGGTGCATGTGG
BCL3	GCCGGAGGCTCTTTACTACC	GGCCATAGTCGGGGTAGAGTA
TNFAIP3	GCTCAACTGGTGTCGTGAAG	ATGAGGCAGTTTCCATCACC
CEBPB	GCTGAGCGACGAGTACAAGA	CAGCTCCAGCACCTTGTG
CACNA1S	TTCACTGTGGAGATCGTCCTT	TTGAAGTAGTTGCGGCAGAA
FGFR3	CCAGAGCAGCGAGTTGGT	TGCTCCTGCTGGCTAGGT
TGFB3	AGTGGCTGTTGCGGAGAG	GCTGAAAGGTATGACATGGACA
ELK4	CACCAGCACTCTTCTCACAGA	GTGCTCCAAAAGTGGATGCT
MDM2	CAGAAACTTAGTGGTTGTAAGTCAACA	TTCAGGTCACTCCCACCTTC
PMAIP1	GCGAAAGAGCACGATGAGA	GATCACACTCGTCCTTCAGGT
BAX	CGAGCTGATCAGAACCATCA	GGGGTCCCGAAGTAGGAA
PLN	GACGATCACAGAAGCCAAGG	GACAGCAGGCAGCCAAAC
PDGFRA	GCTACACGTTTGAGCTGTCAAC	ATGGTGGTCATCCACAAGC
CALM3	CTGCAGGACATGATCAATGAG	TGGTCAGGAACTCTGGGAAG
IGF2	CGCTTCAGTTTGTCTGTTCG	GCAGCACTCTTCCACGATG
DUSP3	AAGGGCTGCAGACTTCATTG	ACGGCAATGGACAAGCAC
MAP2K1	CAAGATGCCCAAGAAGAAGC	AGGCCTCCAGGTTGGTCT
IL6	CCTGGAGTTTGTGAAGAACAACT	GGAAGTTGGGGTAGGAAGGA
HMOX1	GTCAGGTGTCCAGGGAAGG	CTCTTCCAGGGCCGTATAGA
CYC1	GGCATCTTCCATTACGGACA	CCACAGCTAGCCCTGCAC
UQCRH	GCACGGGATCACTGTGTG	AGCAAGCTGTTCCGATTCC
SDHA	CCCTGAGCATTGCAGAATC	CATTTGCCTTAATCGGAGGA
MDH2	CCTTGACATCGTCAGAGCAA	ACTCGAGCTGGGTCCAAAC
IDH2	TGGCAGTTCATCAAGGAGAA	TTGGTCTGGTCACGGTTTG
ACO2	ACTGGAGCCTCGCCATCT	GCCCTGCTTCTTTAGATTGGT
NUP153	CATTCTGAAAACGCCTGGTT	TGCTGGTCTTGTATAAACTATTGAGC
XPOT	CGAATGCCGATTCAGACTTTA	AGGCATCTGGGGAAATCTTTA
RPP25	GACCCAGGAGGAAACTTGC	TTGGAAGCAATACTCCAGCA
TGFB2	GACATGCCGTCCCACTTC	CACTGAGCCAGAGGATGTTG
RYR2	GGAAGTGAAGCAGCCCAAG	TCATCCATGTGTCCATGTAGC