

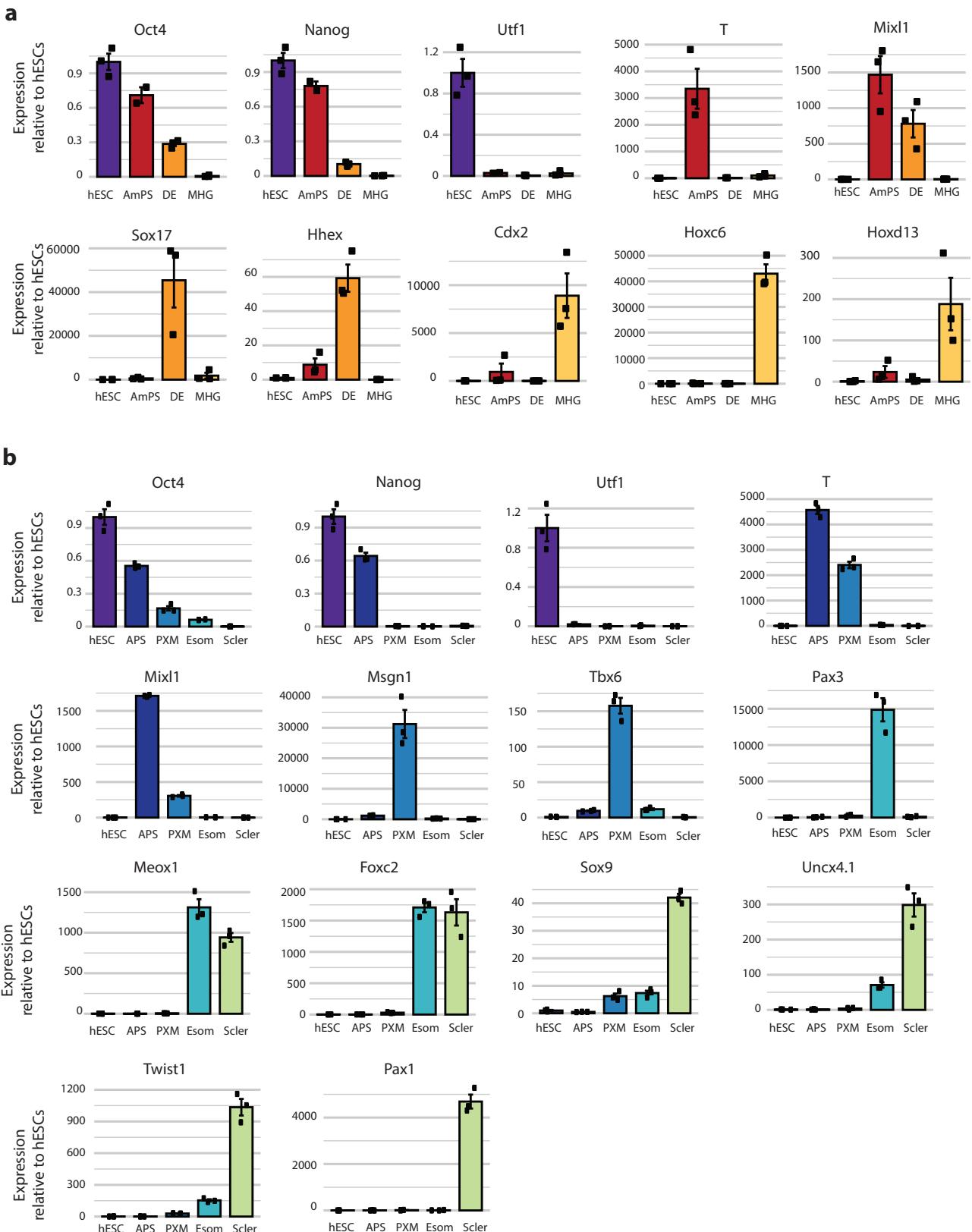
# **A stem cell roadmap of ribosome heterogeneity reveals a function for RPL10A in mesoderm production**

Naomi R. Genuth, Zhen Shi, Koshi Kunimoto, Victoria Hung, Adele F. Xu, Craig H. Kerr,  
Gerald C. Tiu, Juan A. Oses-Prieto, Rachel E.A. Salomon-Shulman, Jeffrey D. Axelrod, Alma L.  
Burlingame, Kyle M. Loh, Maria Barna

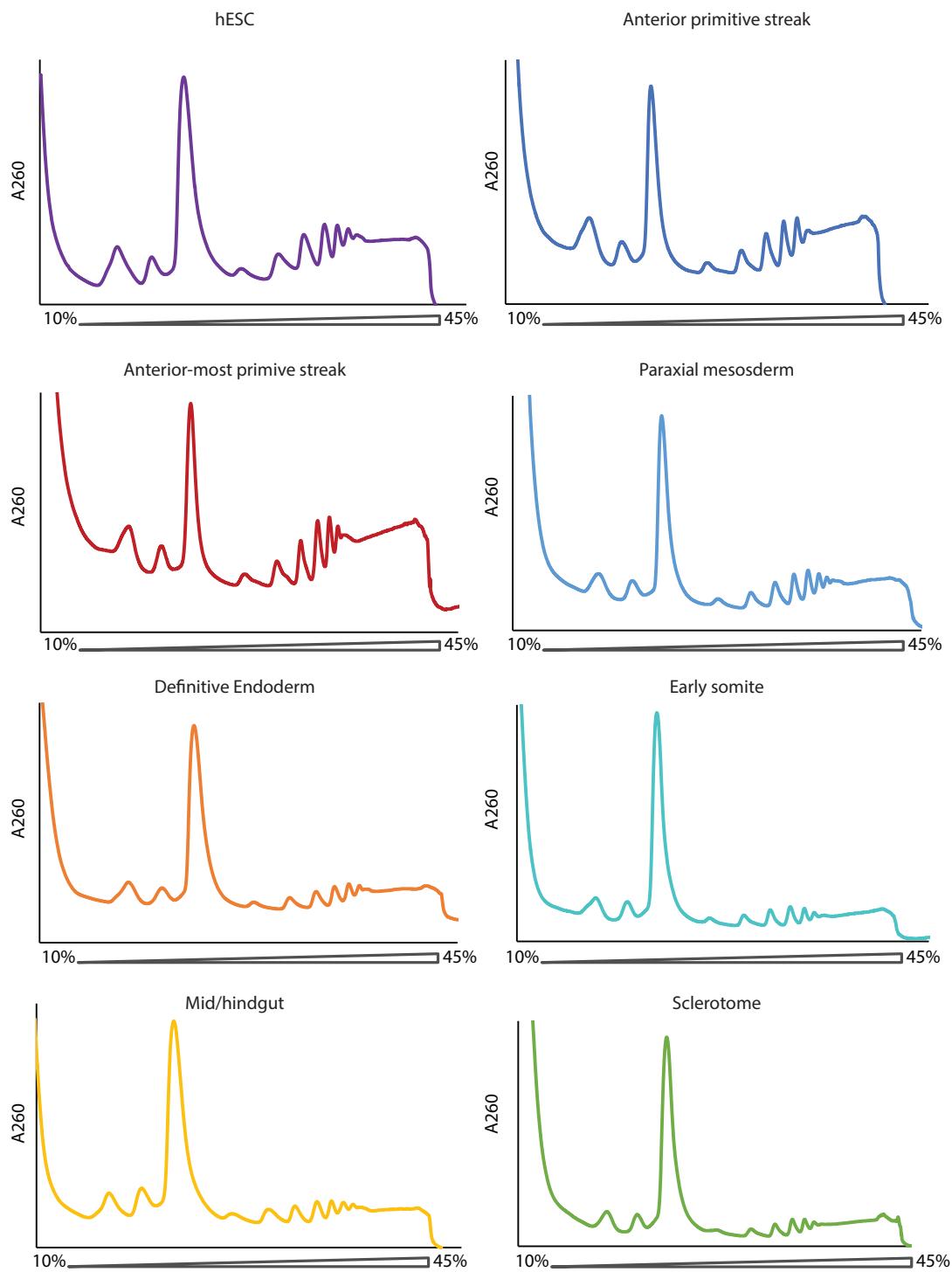
## **Supplementary Information**

Supplementary Figures 1-16

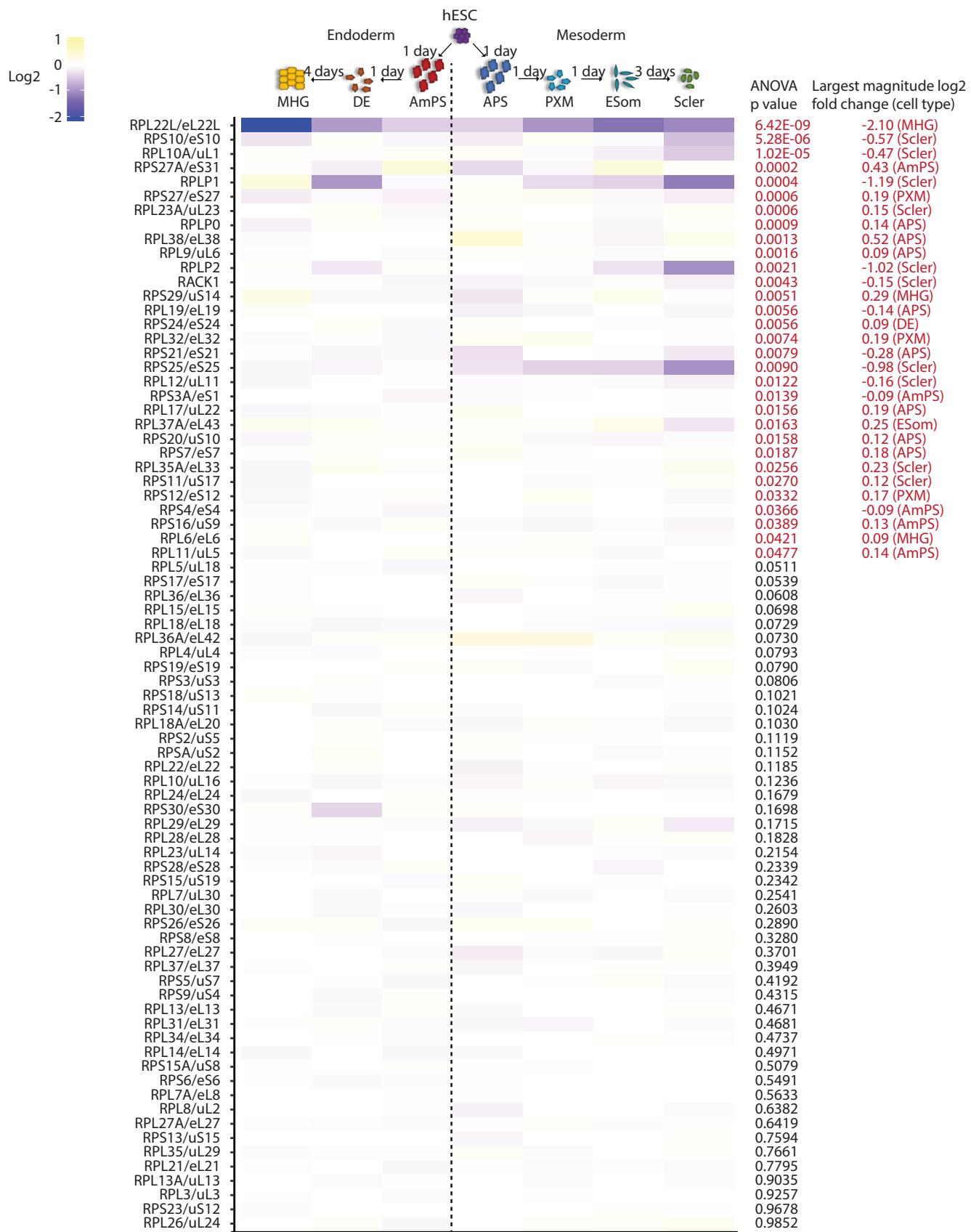
Supplementary Table 1



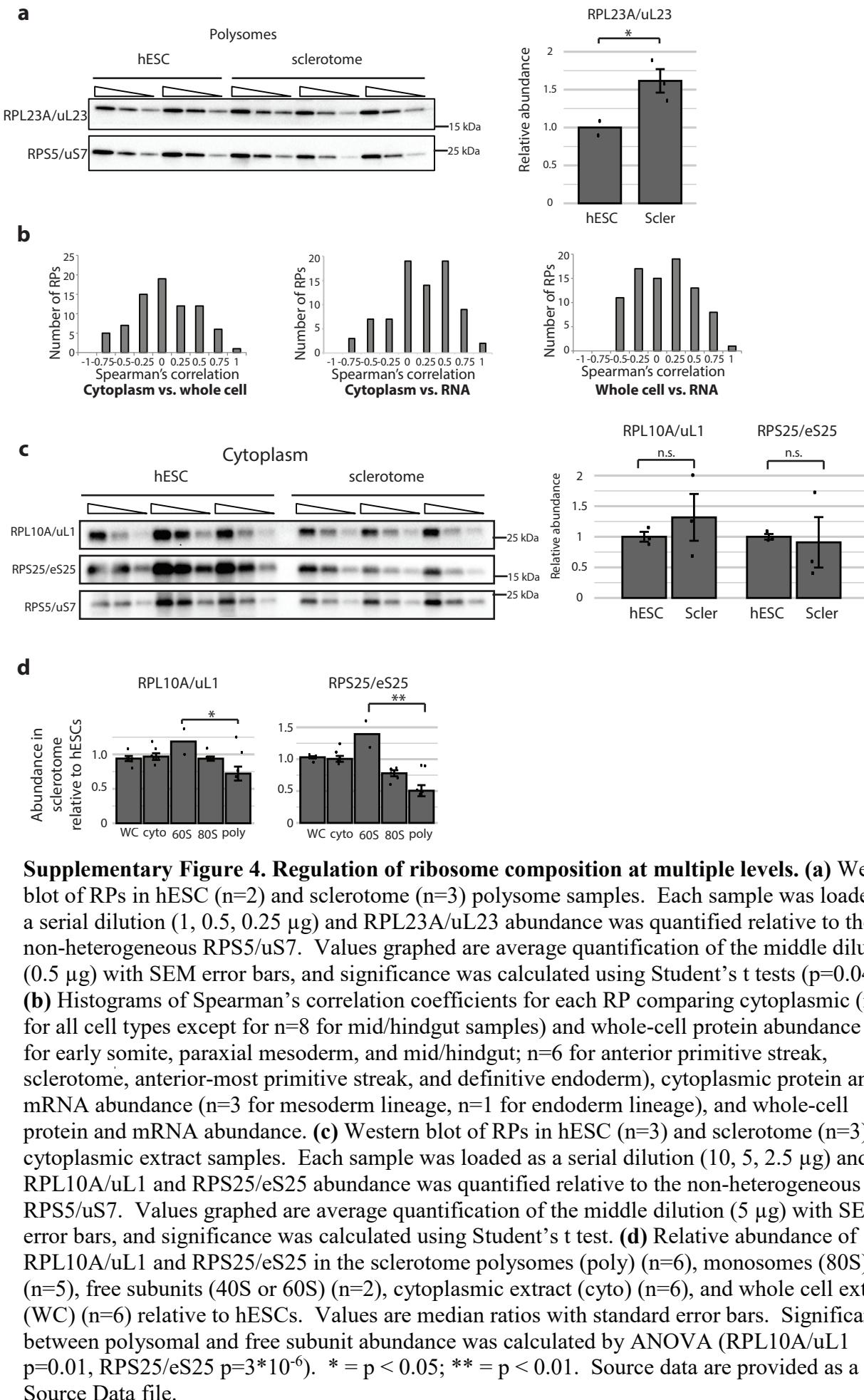
**Supplementary Figure 1. hESC in vitro differentiation marker gene expression.** Analysis of marker gene expression by qPCR for the endoderm (**a**) and mesoderm (**b**) lineages (n=3 for each cell type). Colors match the colors of the cell differentiation schematic in Figure 1. Values for each gene were normalized to the expression of a housekeeping gene (*Nup11*) and presented as mean +/- SEM. All genes show the expected enrichments in the specific cell types. Source data are provided as a Source Data file. AmPS=anterior-most primitive streak; DE=definitive endoderm; MHG=mid/hindgut; APS=anterior primitive streak; PXM=paraxial mesoderm; ESom=early somite; Scler=sclerotome.

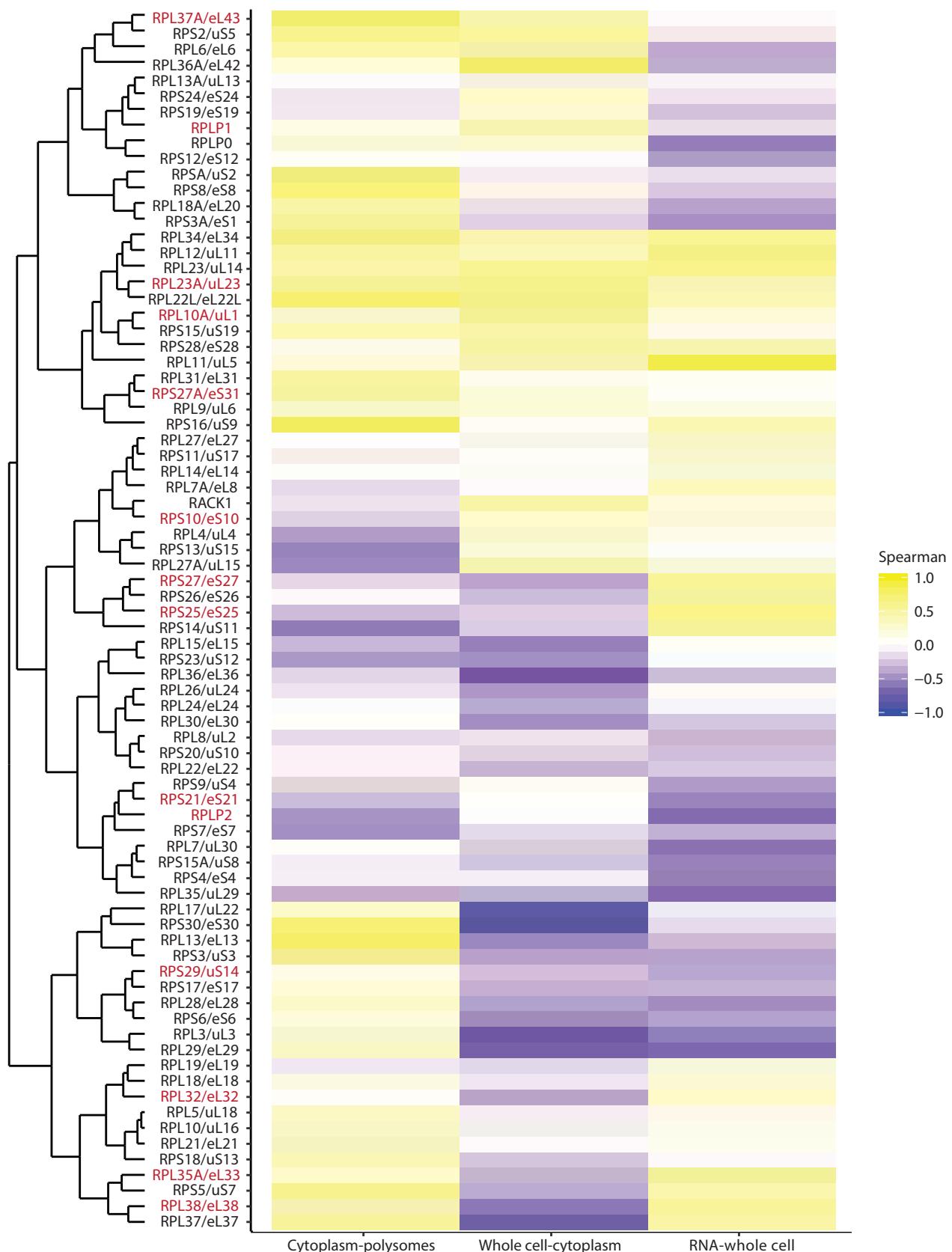


**Supplementary Figure 2. hESC in vitro differentiation sucrose gradient fractionation.**  
Polysome traces from 10-45% sucrose gradients of hESCs and each differentiated cell type used for ribosome mass spectrometry. Colors match cell differentiation schematic in Figure 1.

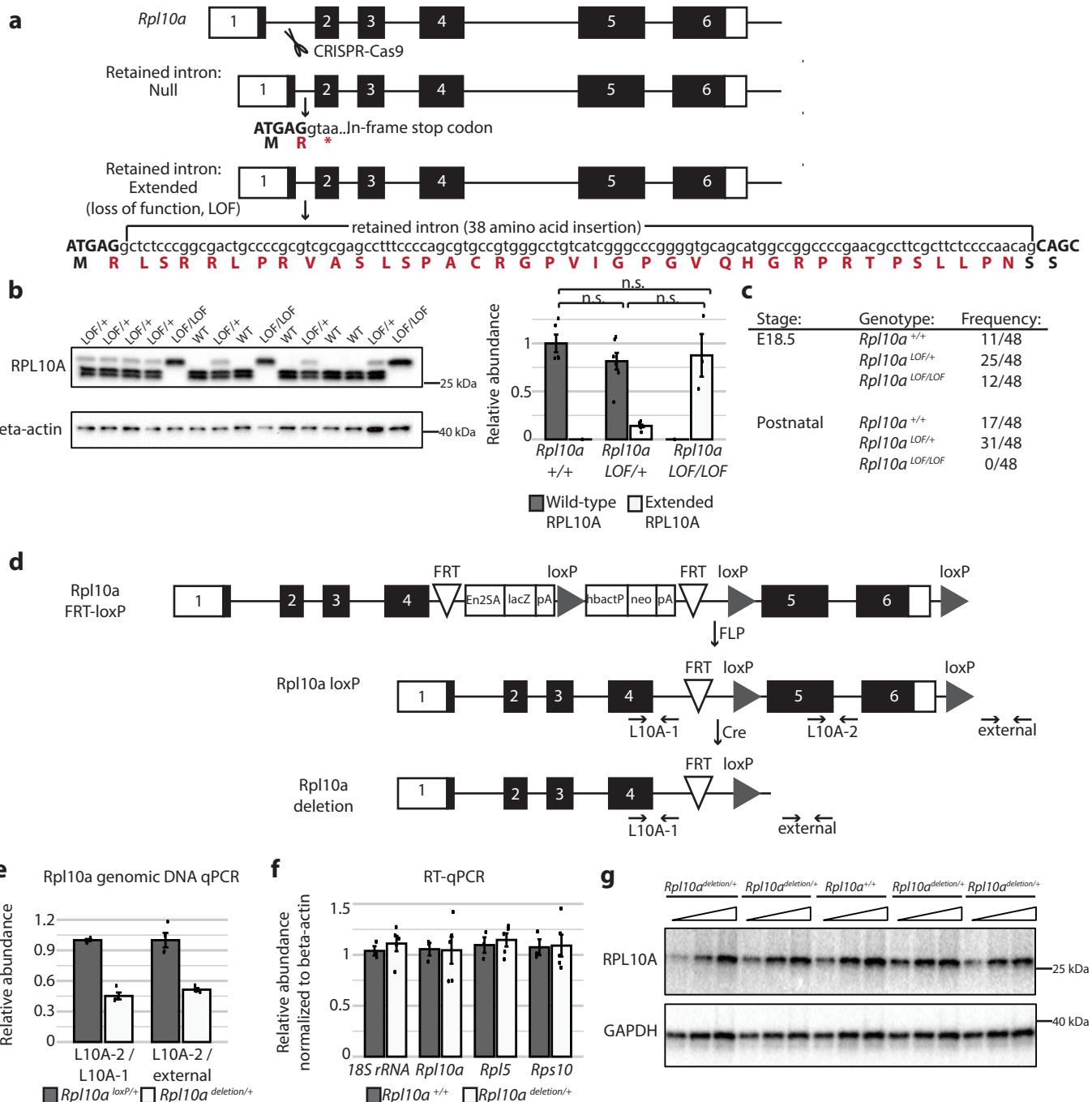


**Supplementary Figure 3. Relative quantification mass spectrometry of polysome ribosome composition during hESC differentiation.** Complete heat map of polysome relative abundance TMT mass spectrometry results. Values are the median ratios of polysome abundance in each differentiated cell relative to hESCs in log2 scale, n=6 for each differentiated cell type except for APS and MHG (n=7 each), and p values were calculated by ANOVA. The p values less than 0.05 are marked in red. For each significantly changing RP, the cell type where the RP shows the greatest magnitude of change is listed, along with the relative polysomal abundance of that RP in that cell type (values in log2 scale). AmPS=anterior-most primitive streak; DE=definitive endoderm; MHG=mid/hindgut; APS=anterior primitive streak; PXM=paraxial mesoderm; ESom=early somite; Scler=sclerotome.

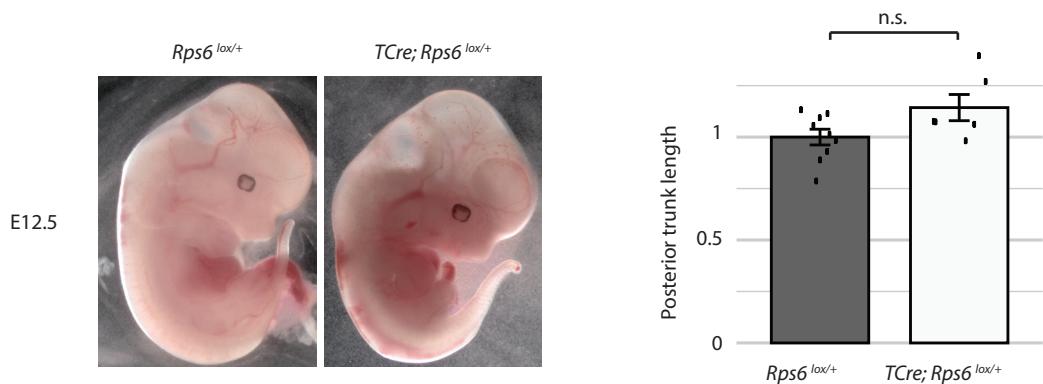




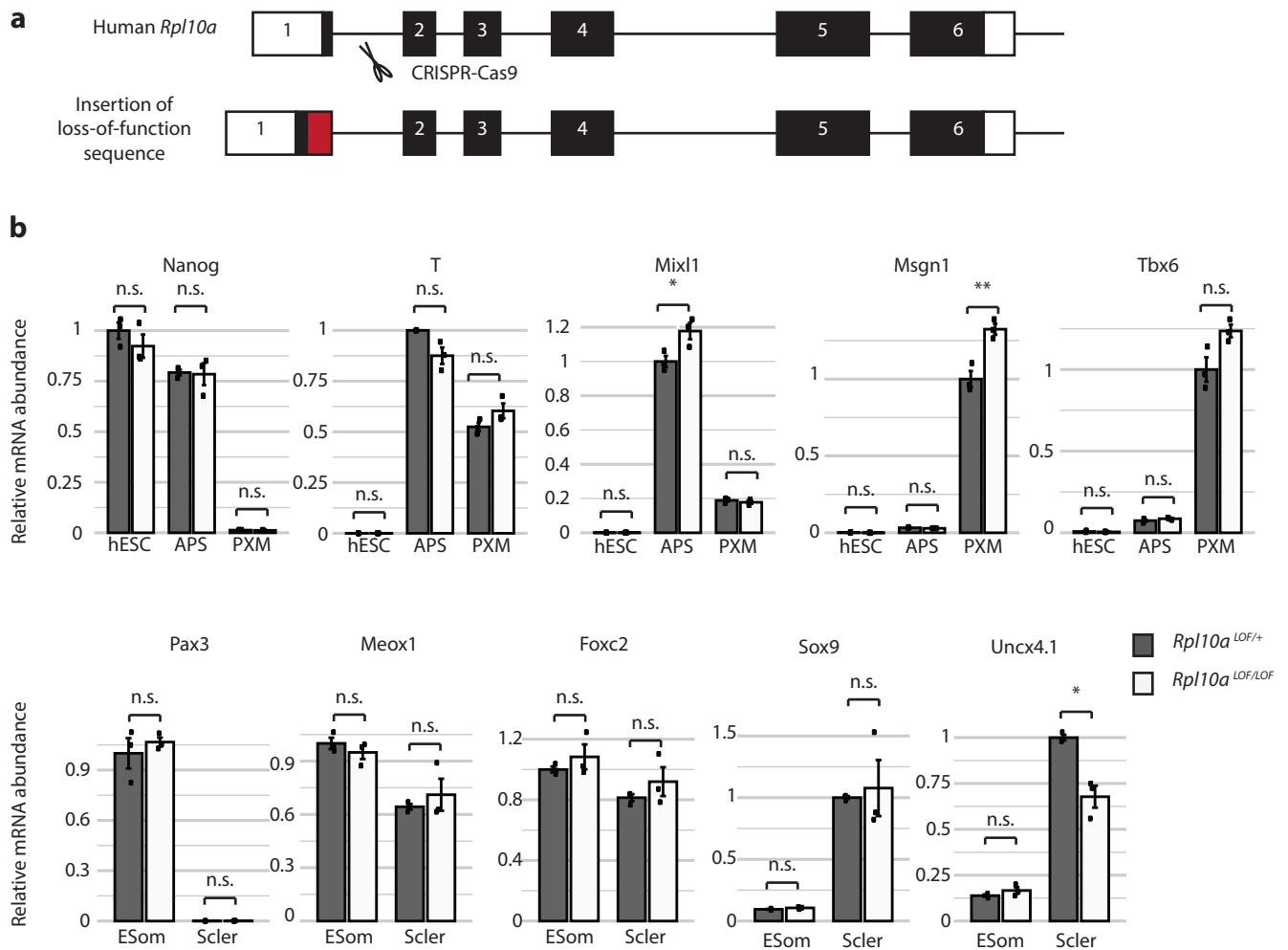
**Supplementary Figure 5. Correlation of RP abundance changes across cellular compartments.** Heatmap of Spearman's correlation coefficients for each RP comparing mRNA and whole-cell protein abundance, whole-cell and cytoplasmic protein abundance, and cytoplasmic and polysomal protein abundance. The RPs that are depicted in Figure 1C (changed significantly in the polysomes and change by at least 10% in at least 2 differentiated cell types relative to hESCs) are marked in red.



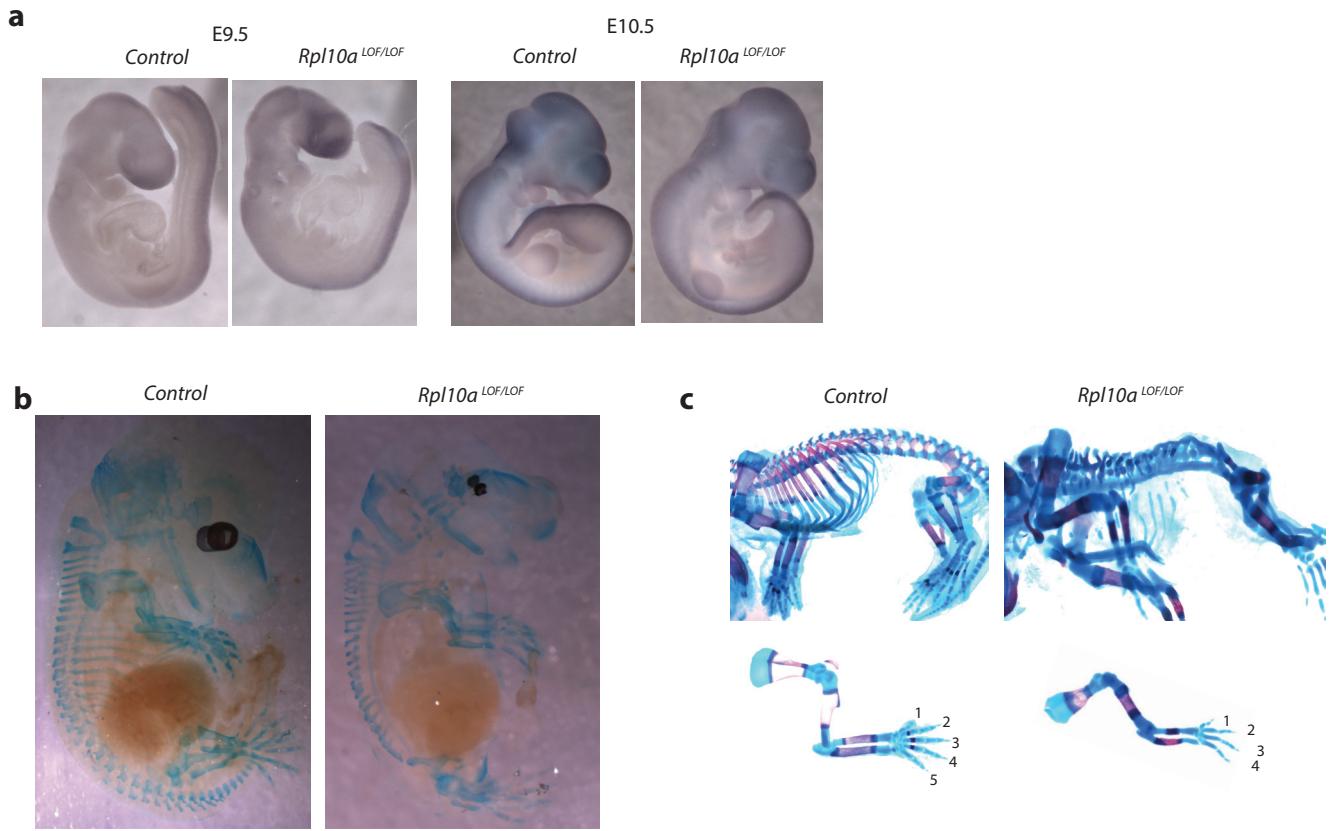
**Supplementary Figure 6. Creation of *Rpl10a* mouse models.** **(a)** Schematics of the wild-type *Rpl10a* mouse locus, the extended and null alleles created by CRISPR-mediated deletion of part of the intron between exons 1 and 2. **(b)** Western blot of RPL10A/uL1 and β-actin in wild-type, *Rpl10a*<sup>LOF/+</sup>, and *Rpl10a*<sup>LOF/LOF</sup> embryo E18.5 whole cell lysates. Abundance of wild-type and extended RPL10A/uL1 protein was quantified for each genotype (n=5 for *Rpl10a*<sup>+/+</sup>, n=7 for *Rpl10a*<sup>LOF/+</sup>, n=3 for *Rpl10a*<sup>LOF/LOF</sup>) and graphed as the average with SEM error bars, and significance was calculated using Student's t test. **(c)** Frequency of recovery of wild-type, *Rpl10a*<sup>LOF/+</sup>, and *Rpl10a*<sup>LOF/LOF</sup> embryos from crossing *Rpl10a*<sup>LOF/+</sup> heterozygotes. At E18.5 the genotypes are present at the expected Mendelian ratios; postnatally (P1-P7), no *Rpl10a*<sup>LOF/LOF</sup> mice were recovered. **(d)** Schematic of the conditional allele used to generate an *Rpl10a* deletion allele. **(e)** Genomic DNA qPCR to confirm Cre excision of *Rpl10a*. SEM error bars, n=3 each for *Rpl10a*<sup>loxP/+</sup> and *Rpl10a*<sup>deletion/+</sup>. **(f)** Reverse transcription qPCR of *Rpl10a* mRNA and controls to evaluate *Rpl10a* expression. Values are normalized by the abundance of β-actin mRNA. SEM error bars, n=3 for control and n=5 for *Rpl10a*<sup>deletion/+</sup>. **(g)** Western blot to detect RPL10A/uL1 and GAPDH in wild-type and *Rpl10a*<sup>deletion/+</sup> E10.5 embryos. Source data are provided as a Source Data file.



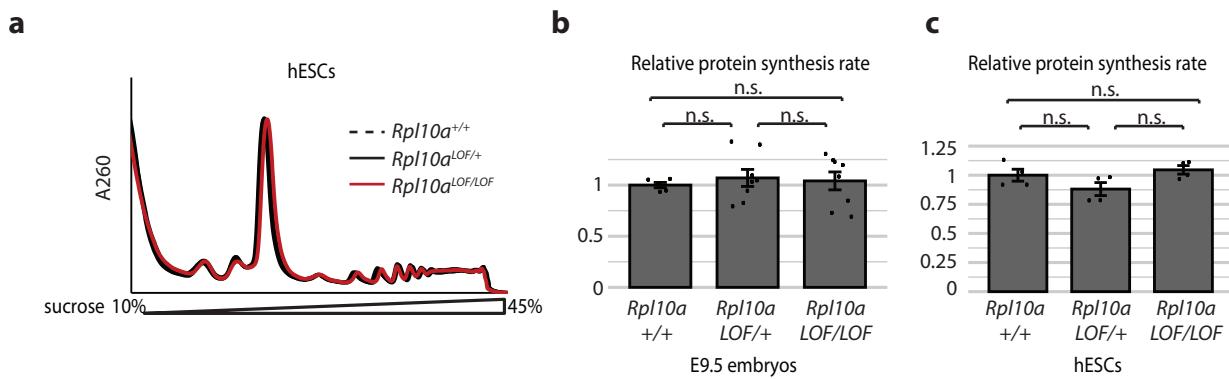
**Supplementary Figure 7. Posterior trunk length is unaffected in *RPS6* haploinsufficient mice.** Lateral views of E12.5 *RPS6*<sup>lox/+</sup> and *TCre; RPS6*<sup>lox/+</sup> embryos. Quantification of posterior trunk length indicates that *RPS6* conditional heterozygosity does not cause posterior trunk truncations. Values graphed are average trunk length with SEM error bars, and significance was calculated using Student's t tests. *RPS6*<sup>lox/+</sup> embryos n=9, and *TCre; RPS6*<sup>lox/+</sup> embryos n=6. Source data are provided as a Source Data file.



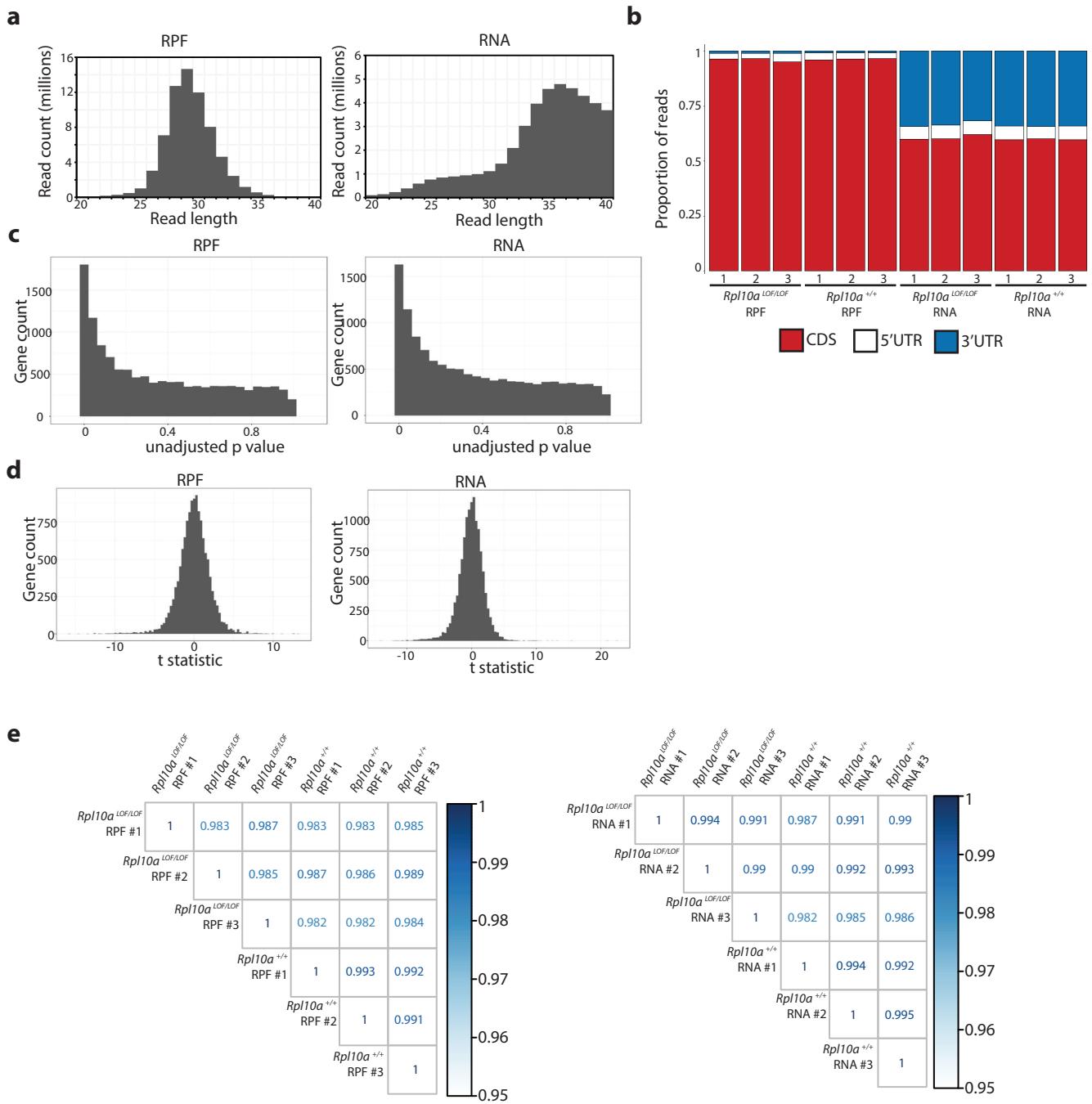
**Supplementary Figure 8. Creation of *Rpl10a* loss-of-function hESC line. (a)** Schematic of CRISPR to introduce LOF insertion into the *Rpl10a* locus in hESCs. **(b)** RT-qPCR of lineage markers in *Rpl10a*<sup>LOF/+</sup> and *Rpl10a*<sup>LOF/LOF</sup> hESCs and *in vitro* differentiated anterior primitive streak (APS), paraxial mesoderm (PXM), early somites (ESom), and sclerotome (Scler) (n=3 for each cell type). Values are normalized to control gene *Nuprl1* and the average with SEM error bars shown, and significance was calculated using Student's t test. P values: APS *Mixl1* p=0.04; PXM *Msgr1* p=0.009; Scler *Uncx4.1* p=0.03. \* = p value < 0.05; \*\* = p value < 0.01. Source data are provided as a Source Data file.



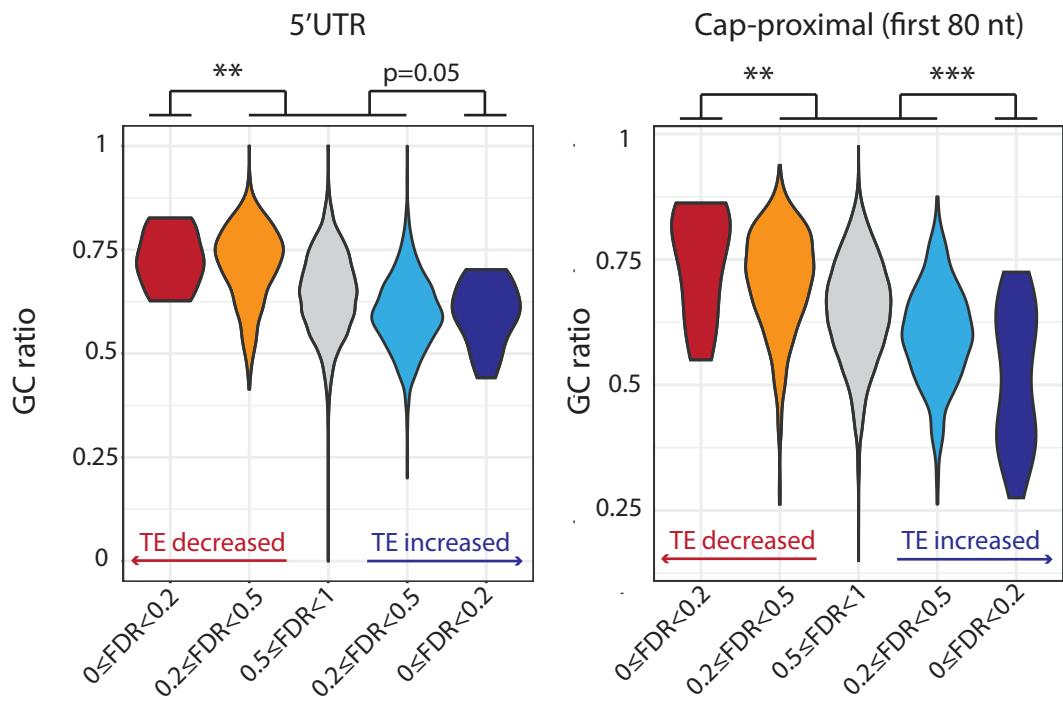
**Supplementary Figure 9. *Rpl10a<sup>LOF/LOF</sup>* embryo neural tube, cartilage, and bone formation.** **(a)** Whole-mount *in situ* hybridizations in control and *Rpl10a<sup>LOF/LOF</sup>* embryos at E9.5 and E10.5 for *Sox2*. **(b)** Cartilage staining (blue) on E14.5 *Rpl10a<sup>LOF/LOF</sup>* and control embryos. **(c)** Bone (red) and cartilage (blue) staining on E17.5 *Rpl10a<sup>LOF/LOF</sup>* and control embryos.



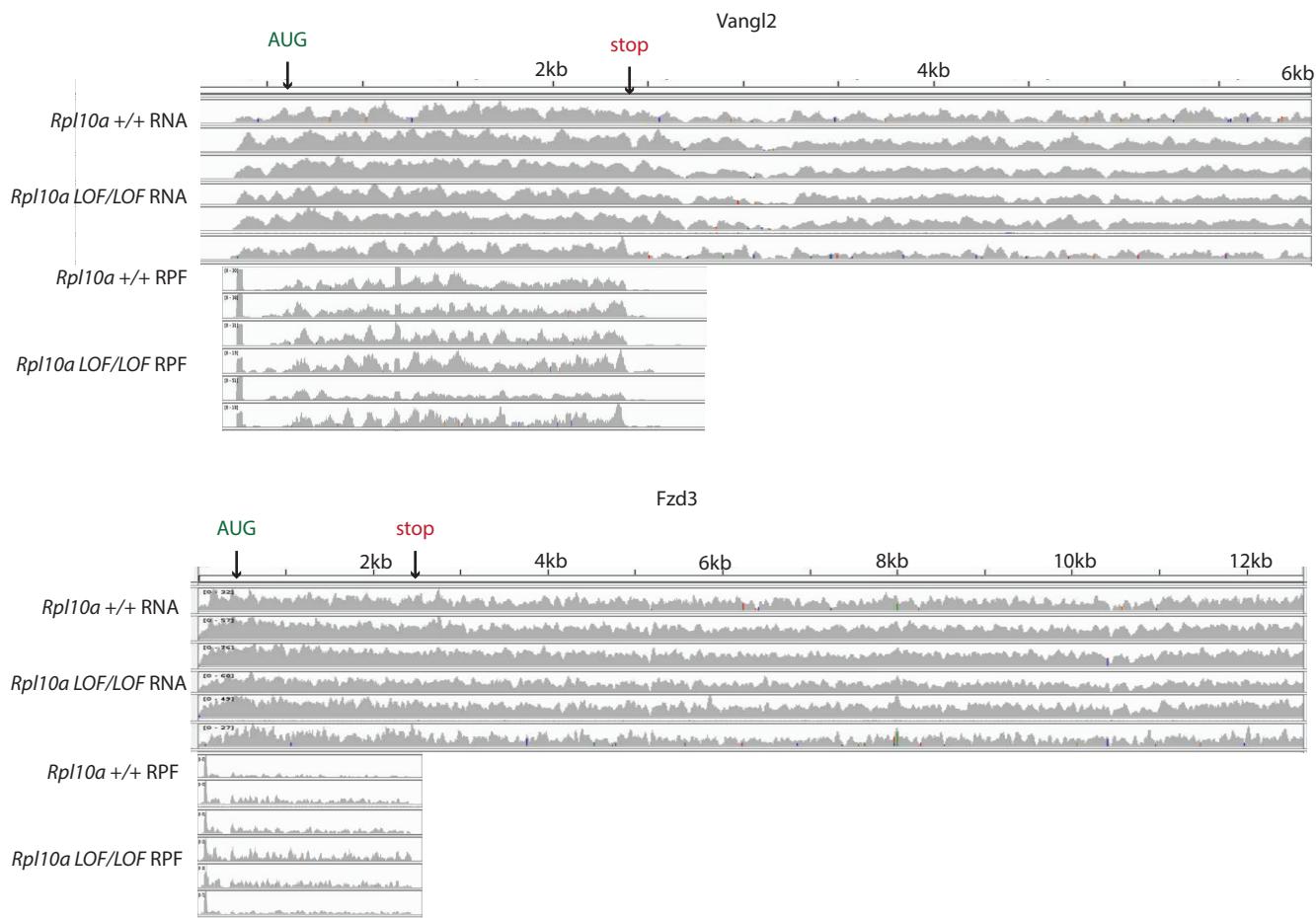
**Supplementary Figure 10. Global protein synthesis is unchanged in *Rpl10a*<sup>LOF/LOF</sup> embryos and hESCs.** (a) Overlay of polysome traces of 10–45% sucrose gradients of wild-type, *Rpl10a*<sup>LOF/+</sup>, and *Rpl10a*<sup>LOF/LOF</sup> hESCs. (b) OPP incorporation rates in E9.5 wild-type, *Rpl10a*<sup>LOF/+</sup>, and *Rpl10a*<sup>LOF/LOF</sup> embryos (n=5 for wild-type, n=8 each for *Rpl10a*<sup>LOF/+</sup> and *Rpl10a*<sup>LOF/LOF</sup>). Values graphed are averages with SEM error bars and significance was calculated using Student's t test. (c) OPP incorporation rates in wild-type, *Rpl10a*<sup>LOF/+</sup>, and *Rpl10a*<sup>LOF/LOF</sup> hESCs (n=4 each). Values graphed are averages with SEM error bars and significance was calculated using Student's t test. Source data are provided as a Source Data file.



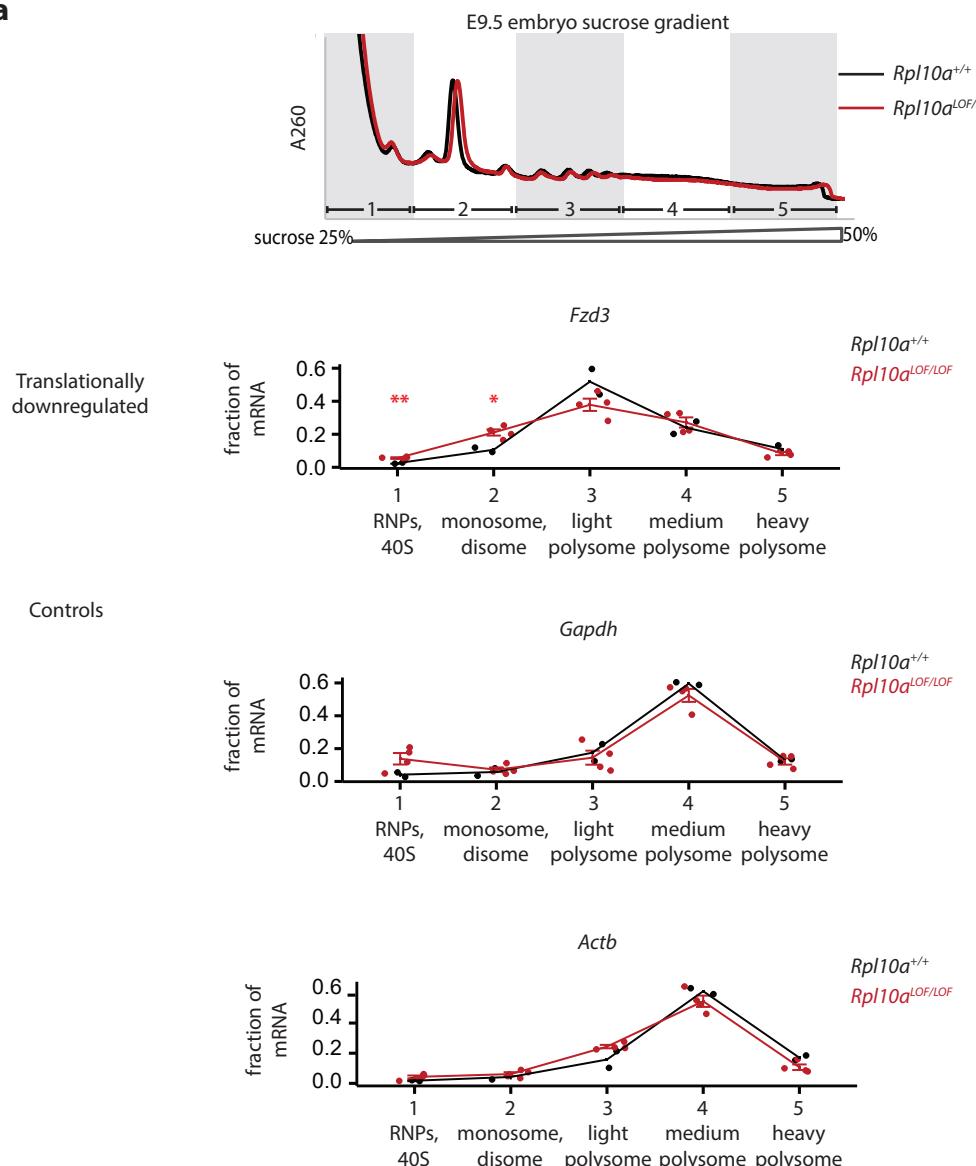
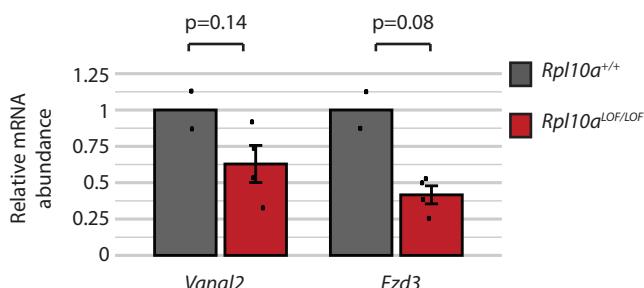
**Supplementary Figure 11. *Rpl10a*<sup>LOF/LOF</sup> embryo ribosome profiling analysis.** (a) Histogram of read lengths in ribosome profiling (RPF) and RNA-seq (RNA) libraries. (b) Distribution of reads across the 5' untranslated region (5'UTR), coding sequence (CDS), and 3' untranslated region (3'UTR) in each wild-type and *Rpl10a*<sup>LOF/LOF</sup> RNA-seq (RNA) and ribosome profiling (RPF) replicate. (c) Histogram of unadjusted p values for ribosome profiling (RPF) and RNA-seq (RNA) libraries. (d) Histogram of t statistics for ribosome profiling (RPF) and RNA-seq (RNA) libraries. (e) Correlation plots of each wild-type and *Rpl10a*<sup>LOF/LOF</sup> ribosome profiling (RPF) and RNA-seq (RNA) replicate.



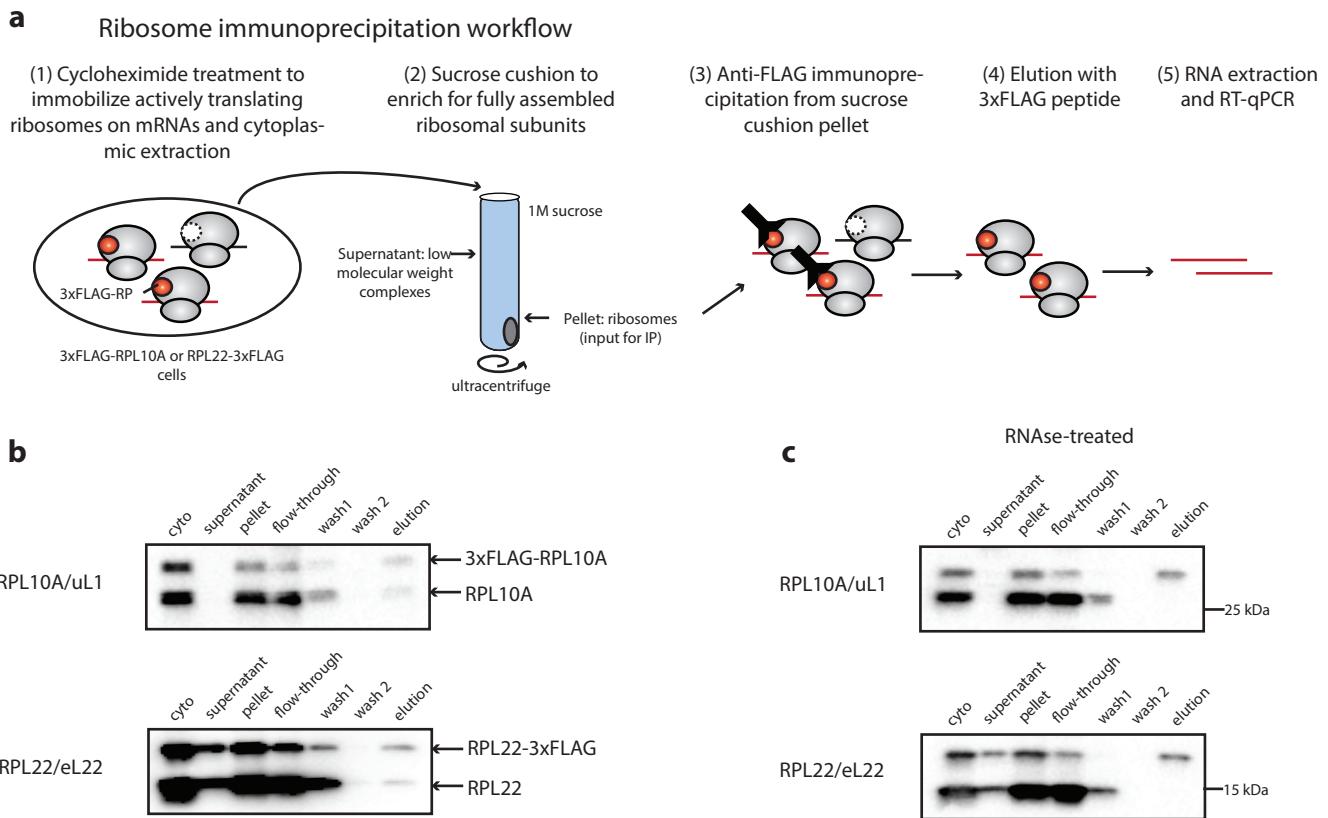
**Supplementary Figure 12. Transcript features associated with altered translation in *Rpl10a* *LOF/LOF* embryos.** Violin plots quantifying GC content of 5'UTRs (left) or cap-proximal regions (first 80 nucleotides, right). Transcripts are grouped by direction of change in translation efficiency (decreased translation in *Rpl10a* *LOF/LOF* embryos in red or orange, no change in gray, increased translation in blue) and by FDR. Significance was calculated using the Mann-Whitney test. P values: 5'UTR, TE decreased p=0.008; cap-proximal, TE decreased p=0.009; cap-proximal, TE increased p=1.7\*10<sup>-5</sup>. \*\* = p value < 0.01; \*\*\* = p value < 0.001.



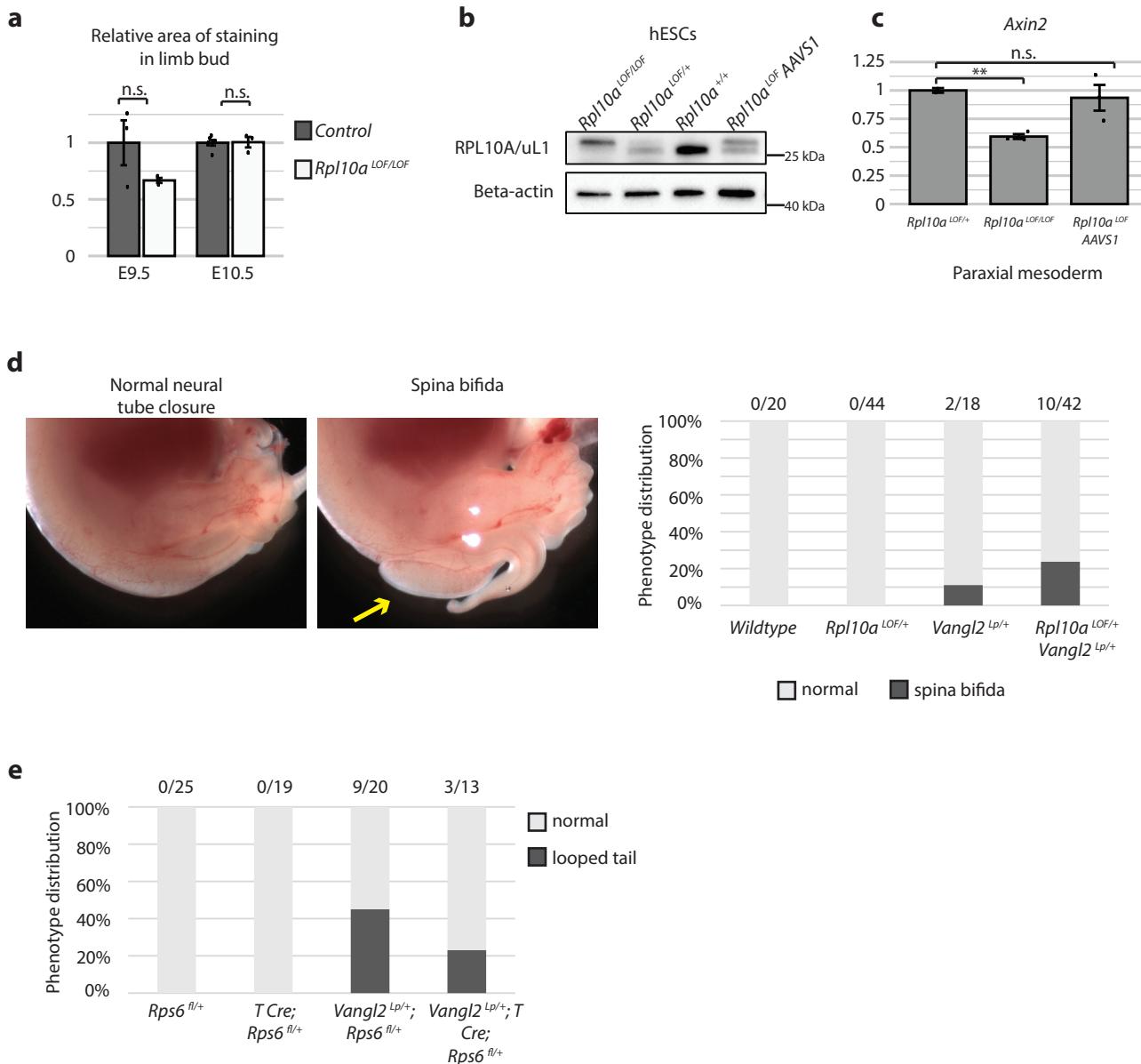
**Supplementary Figure 13. Coverage of *Vangl2* and *Fzd3* in *Rpl10a* *LOF/LOF* embryo ribosome profiling.** IGV views showing coverage of the *Vangl2* and *Fzd3* loci in each RNA-Seq and ribosome profiling replicate. Start and stop codons are marked as AUG and stop respectively.

**a****b**

**Supplementary Figure 14. *Rpl10a*<sup>LOF/LOF</sup> embryo gradient qPCR reveals decreased translation of *Fzd3*.** (a) E9.5 wild-type (n=2) and *Rpl10a*<sup>LOF/LOF</sup> (n=4) polysome gradient RT-qPCR results for the translationally downregulated gene *Fzd3* and the control genes *Gapdh* and *Actb*. *Fzd3* is significantly increased in the *Rpl10a*<sup>LOF/LOF</sup> earlier fractions relative to wild-type (Student's t test p value = 0.007 for fraction 1, p=0.01 for fraction 2). Error bars are SEM. (b) Abundance of *Vangl2* and *Fzd3* mRNAs relative to *Actb* and *Gapdh* in the E9.5 cytoplasmic extract inputs for polysome gradients. Wild-type (n=2) and *Rpl10a*<sup>LOF/LOF</sup> (n=4), error bars are SEM, significance calculated using Student's t test. \* = p value < 0.05; \*\* = p value < 0.01. Source data are provided as a Source Data file.



**Supplementary Figure 15. 3xFLAG-RPL10A/uL1 and RPL22/eL22-3xFLAG ribosome immunoprecipitation.** (a) Schematic of ribosome immunoprecipitation workflow. (b) Western of immunoprecipitation of 3xFLAG-RPL10A/uL1 or RPL22/eL22-3xFLAG ribosomes loading 2% of the volume of each sample. The untagged RPL10A/uL1 and RPL22/eL22 that appears in the elutions are due to untagged and tagged ribosomes forming a polysome together bound to the same mRNA molecule. Equivalent results were obtained from multiple independent experiments (n=4 each for 3xFLAG-RPL10A/uL1 and RPL22/eL22-3xFLAG) (c) Western of immunoprecipitation of 3xFLAG-RPL10A/uL1 or RPL22/eL22-3xFLAG ribosomes after treatment with RNase A/T1 to cut unprotected mRNA, thereby cleaving polysomes into single monosomes. 2% of the volume of each sample was loaded. No untagged RPL10A/uL1 or RPL22/eL22 appears in these elutions because under these conditions only one ribosome can be present on a single mRNA. Equivalent results were obtained from multiple independent experiments (n=2 each for 3xFLAG-RPL10A/uL1 and RPL22/eL22-3xFLAG). Source data are provided as a Source Data file.



**Supplementary Figure 16. Decreased canonical and non-canonical Wnt signaling in *Rpl10a* *LOF/LOF* embryos and *in vitro* differentiated paraxial mesoderm.** (a) Quantification of area of X-gal staining in limb bud relative to overall limb bud area. E9.5 n=4, E10.5 n=3 for *Rpl10a* *LOF/LOF* and n=6 for control. Values shown are averages with SEM error bars, significance was calculated using Student's t test. (b) Western blot of RPL10A/uL1 and β-actin in whole cell lysates from wild-type hESCs, *Rpl10a* *LOF/+* hESCs, *Rpl10a* *LOF/LOF* hESCs, and hESCs with a *LOF-Rpl10a* transgene at the AAVS1 locus (*LOF AAVS1*). (c) Abundance of *Axin2* mRNA relative to *Pbgd* control transcript as measured by RT-qPCR in *in vitro* differentiated paraxial mesoderm derived from *Rpl10a* *LOF/+* hESCs, *Rpl10a* *LOF/LOF* hESCs, and hESCs with a *LOF-Rpl10a* transgene at the AAVS1 locus (*LOF AAVS1*) (n=3 each for *Rpl10a* *LOF/LOF* and *LOF-Rpl10a AAVS1*, n=2 for *Rpl10a* *LOF/+*). Values shown are averages with SEM error bars and significance was calculated using Student's t test (p values 0.001 (*Rpl10a* *LOF/LOF*)), 0.63 (*LOF-Rpl10a AAVS1*)). (d) Frequency of spina bifida in wild-type, *Rpl10a* *LOF/+*, *Vangl2* *Lp/+*, and *Rpl10a* *LOF/+* *Vangl2* *Lp/+* embryos. Spina bifida occurs more frequently in *Rpl10a* *LOF/+* *Vangl2* *Lp/+* compound heterozygotes compared to *Vangl2* *Lp/+* embryos (Fisher's exact test p value = 0.3). (e) Frequency of looped tail phenotype in *Rps6* *lox/+*, *T Cre; Rps6* *lox/+*, *Vangl2* *Lp/+*; *Rps6* *lox/+*, and *Vangl2* *Lp/+*; *T Cre; Rps6* *lox/+* embryos. The looped tail phenotype does not differ significantly between *Vangl2* *Lp/+*; *Rps6* *lox/+*, and *Vangl2* *Lp/+*; *T Cre; Rps6* *lox/+* embryos (Fisher's exact test p value = 0.3). \*\* = p value < 0.01. Source data are provided as a Source Data file.

### Supplementary Table 1: Primers and oligos

|                   |                            |                                   |
|-------------------|----------------------------|-----------------------------------|
| Twist1 qPCR F     | ctgcagcaccggcaccgtt        | qPCR                              |
| Twist1 qPCR R     | cccaacggctggacgcacac       | qPCR                              |
| Pax1 qPCR F       | cgcctatggaggcagacgtatggcga | qPCR                              |
| Pax1 qPCR R       | aatgcgcaaggcgatggcgttg     | qPCR                              |
| L10A-1 F          | gaccctcagaaggacaacg        | Rpl10a gDNA and transcript qPCR   |
| L10A-1 R          | agaacgcacaccgagaactt       | Rpl10a gDNA and transcript qPCR   |
| L10A-2 F          | gaaaacatggggccaaagt        | Rpl10a gDNA qPCR                  |
| L10A-2 R          | ctaatacagacgctggggct       | Rpl10a gDNA qPCR                  |
| Ptms qPCR F       | ccggaaagaacgaaagaaag       | Rpl10a gDNA qPCR external control |
| Ptms qPCR R       | cctctccatectctgcagtt       | Rpl10a gDNA qPCR external control |
| 18S rRNA qPCR F   | acatccaaggaaaggcagcag      | qPCR                              |
| 18S rRNA qPCR R   | cattccaattacaggcctc        | qPCR                              |
| Rpl5 qPCR F       | cccgaactacaactggcaat       | qPCR                              |
| Rpl5 qPCR R       | cattctgacccatgtatgtc       | qPCR                              |
| Rps10 qPCR F      | tttttaaggaggggcggtatg      | qPCR                              |
| Rps10 qPCR R      | atgccctcgatcgtaaggta       | qPCR                              |
| Rluc F            | tggagaataacttctcgatgg      | qPCR                              |
| Rluc R            | ttggacgacgaaacttcacc       | qPCR                              |
| Mouse Actb qPCR F | gccaaccgtgaaaagatgac       | qPCR                              |
| Mouse Actb qPCR R | catcacaatgcctgtgtac        | qPCR                              |
| Gapdh qPCR F      | acagtccatgccatactgccc      | qPCR                              |
| Gapdh qPCR R      | gcctgcitaccacccttcgt       | qPCR                              |
| Vangl2 qPCR F     | atctttgcatccatggctcg       | qPCR                              |
| Vangl2 qPCR R     | gccaatatcgctccaggaaag      | qPCR                              |
| Fzd3 qPCR F       | agatgttgtgtcccgatgg        | qPCR                              |
| Fzd3 qPCR R       | cacaagtggggatatggct        | qPCR                              |
| Human Actb qPCR F | gccaaccgtgagaagatgac       | qPCR                              |
| Human Actb qPCR R | catcacatgcctgtgtac         | qPCR                              |
| Fgfr1 qPCR F      | gagtgacttccacagccaga       | qPCR                              |
| Fgfr1 qPCR R      | tggagtcaacgttgcactgtt      | qPCR                              |
| Smad4 qPCR F      | ccagctctgttagccccatc       | qPCR                              |
| Smad4 qPCR R      | tactggcaggctgacttgc        | qPCR                              |
| Tgfbr1 qPCR F     | aaccgcactgtcattccca        | qPCR                              |
| Tgfbr1 qPCR R     | agcaatggtaaacctgagcca      | qPCR                              |
| Dhcr24 qPCR F     | tccaacacatctgcactgt        | qPCR                              |
| Dhcr24 qPCR R     | ggctcgatgtttcgacgg         | qPCR                              |
| Rac1 qPCR F       | tacggcccttatcctatccg       | qPCR                              |
| Rac1 qPCR R       | caatcggttgcatttgc          | qPCR                              |
| Sfrp2 qPCR F      | gtttcccccaggacaacgc        | qPCR                              |
| Sfrp2 qPCR R      | gcaggcgttccatcaccttgg      | qPCR                              |

|   |   |  |
|---|---|--|
| Axin2 qPCR F                                | gagagttagcgccaggc   | qPCR   |
| Axin2 qPCR R                                | cggctgactcggttcct   | qPCR   |
| Universal miRNA Cloning Linker (NEB S1315S) | 5'- rAppCTGTAGGCACCATCAAT-NH2-3'  | Ribosome Profiling                               |
| Reverse Transcription Primer1_ATCACG        | /5phos/DDDNNCGTGATNNNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC  | Ribosome Profiling                               |
| Reverse Transcription Primer2_CGATGT        | /5phos/DDDNNACATCGNNNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC  | Ribosome Profiling                               |
| Reverse Transcription Primer3_TCTGAC        | /5phos/DDDNNNGTCAGANNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC  | Ribosome Profiling                               |
| Reverse Transcription Primer4_GAGCTA        | /5phos/DDDNNTAGCTCENNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC  | Ribosome Profiling                               |
| Reverse Transcription Primer5_AGTCCT        | /5phos/DDDNNAAGGACTNNNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC | Ribosome Profiling                               |
| Reverse Transcription Primer7_TACGGA        | /5phos/DDDNNTCCGTANNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC   | Ribosome Profiling                               |
| Reverse Transcription Primer8_CTGTAC        | /5phos/DDDNNGTACAGNNNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC  | Ribosome Profiling                               |
| Reverse Transcription Primer11_TCACTG       | /5phos/DDDNNCAGTGANNNTACCCCTTC<br>GCTTCACACACAAG/iSp18/GGATCC/iSp18<br>/TACCTGATTGATGGTGC   | Ribosome Profiling                               |
| PCR Primer P6Solexa_F                       | aatgatacgccgaccaccgagatctacactttcccttgtgt<br>gaaggcaagggtta                                 | Ribosome Profiling                               |
| PCR Primer P3Solexa_R                       | caagcagaagccgcatacgagatcggtctcggeattcctgattt<br>atggtgcctacag                               | Ribosome Profiling                               |
| Sequencing Primer P6_custom_SeqPrimer       | cactttcccttgtgtgaagcgaagggtta   | Ribosome Profiling                               |
| oNTI199                                     | auguacacggagucgaccgcacgcga  | Ribosome Profiling                               |
| oNTI265                                     | auguacacggagucgagcuacccgcacgcga   | Ribosome Profiling                               |
| OS57  | aaaccgctcacccgcgttgc  | Rpl10a LOF and null mouse genotyping             |
| OS60  | agcaggggagaaatccatcc  | Rpl10a LOF and null mouse genotyping             |
| OS127                                       | caagaaacagctacagtggctt  | Rpl10a conditional and deletion mouse genotyping |
| OS349                                       | cataacgataccacgatatcaaca  | Rpl10a conditional and deletion mouse genotyping |
| FloxedS6 WT F                               | gcttctacttctaagtctgatccagtc   | Rps6 conditional mouse genotyping                |
| FloxedS6 Mut F                              | tccgccggagaaagtatccatcatgt  | Rps6 conditional mouse genotyping                |
| FloxedS6 WT Mut2 R                          | ctgcagccctttcttttagcataacctg  | Rps6 conditional mouse genotyping                |
| WUSTL Cre F                                 | gcattaccggcgtgatgcaacgagtatgag  | T Cre mouse genotyping                           |
| WUSTL Cre R                                 | gagtgaacgaaacctgtcgaaatcgtgcg   | T Cre mouse genotyping                           |
| Fabpi-200 F                                 | tggacaggactggaccctctgcatttctaga   | T Cre mouse genotyping                           |

|                 |                              |                             |
|-----------------|------------------------------|-----------------------------|
| Fabpi-200 R     | tagagcttgcacatcacaggtcattcag | T Cre mouse genotyping      |
| meox1creF1      | ctccgcaaaacccaaatggc         | Meox1 Cre mouse genotyping  |
| En2SArevsor     | ctccaacctccgcaaactcc         | Meox1 Cre mouse genotyping  |
| oIMR9020        | aagggagctgcagtggagta         | Ai9 mouse genotyping        |
| oIMR9021        | ccgaaaatctgtggaaagt          | Ai9 mouse genotyping        |
| oIMR9103        | ggcattaaaggcagcgatcc         | Ai9 mouse genotyping        |
| oIMR9105        | ctgttcctgtacggcatgg          | Ai9 mouse genotyping        |
| Axin2-LacZ 9391 | aagctgcgtcgatacttgaga        | Axin2 LacZ mouse genotyping |
| Axin2-LacZ 9392 | agtccatcttcattccgcctagc      | Axin2 LacZ mouse genotyping |
| Axin2-LacZ 9393 | tggtaatgcgtcgactggcttg       | Axin2 LacZ mouse genotyping |
| Lp-48765 F      | tggctgtctctgcactcac          | Vangl2 Lp mouse genotyping  |
| Lp-48766 R      | gcacccctttgggtcact           | Vangl2 Lp mouse genotyping  |