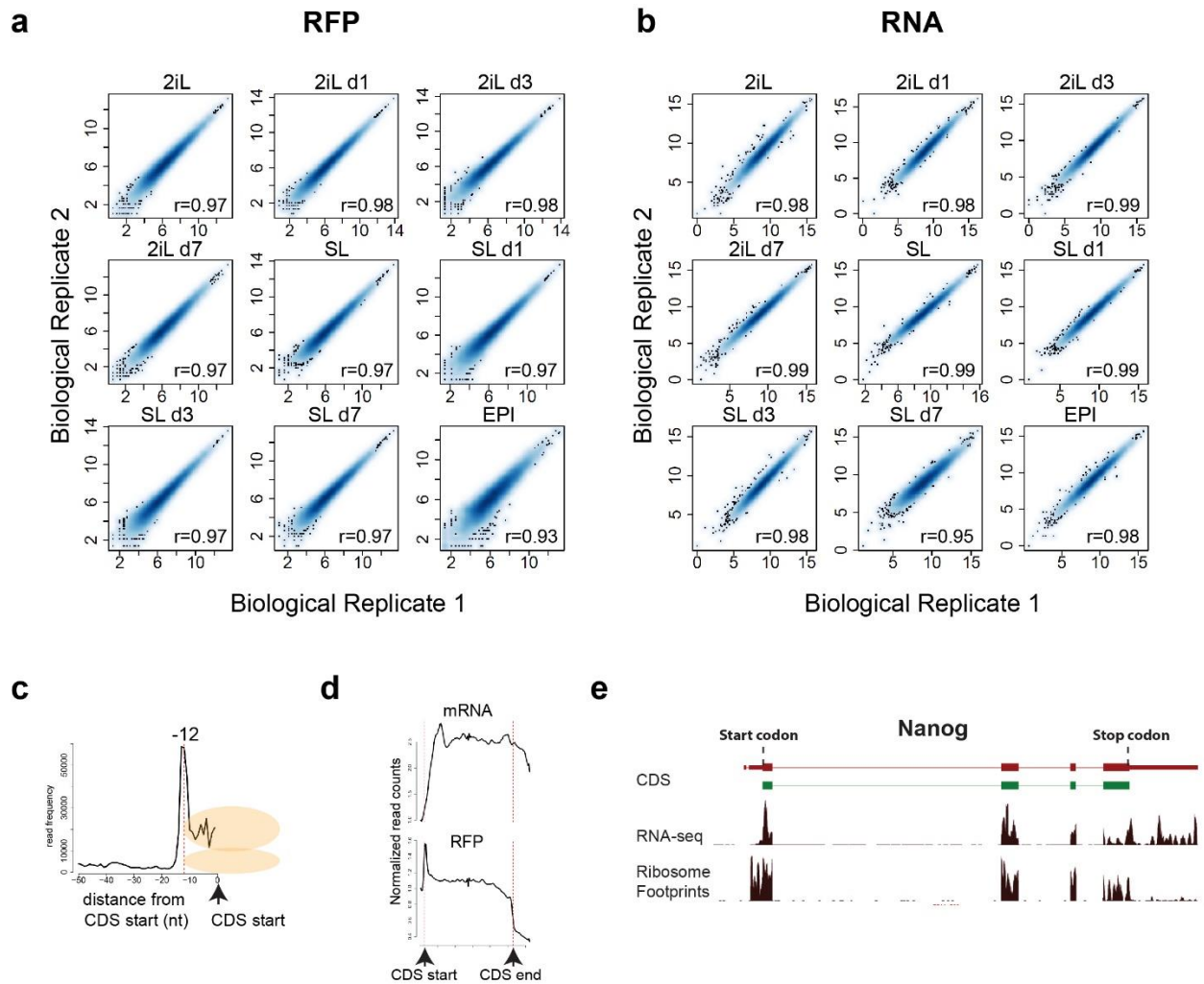Supplementary Information

The translational landscape of ground state pluripotency

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Supplementary Figure 1: Quality control of Ribo-Seq and RNA-seq data

a) Scatter plots showing the correlation between individuals replicates in RFP libraries. r-values represent Pearson correlation coefficient. n=8,546 RFP IDs

b) Scatter plots showing the correlation between individuals replicates in RNA-seq libraries. r-values represent Pearson correlation coefficient. n=11,538 RNA IDs

c) Graph showing the read distribution of the 5'-end of reads in mRNA-Seq and RFP-libraries . For RFP libraries a 12-nt offset was employed to accurately represent the codon at the p-site of the translating ribosomes. Graph was generated using pooled RFP libraries.

d) Graph showing the read coverage at 5´ UTR, coding sequence and 3´ UTR regions in a pool of all prepared RNA-seq and RFP libraries.

e) Genome-browser view of *Nanog*, as a representative example, in RFP and RNA-seq libraries prepared from 2i-ESCs. CDS: coding sequence.
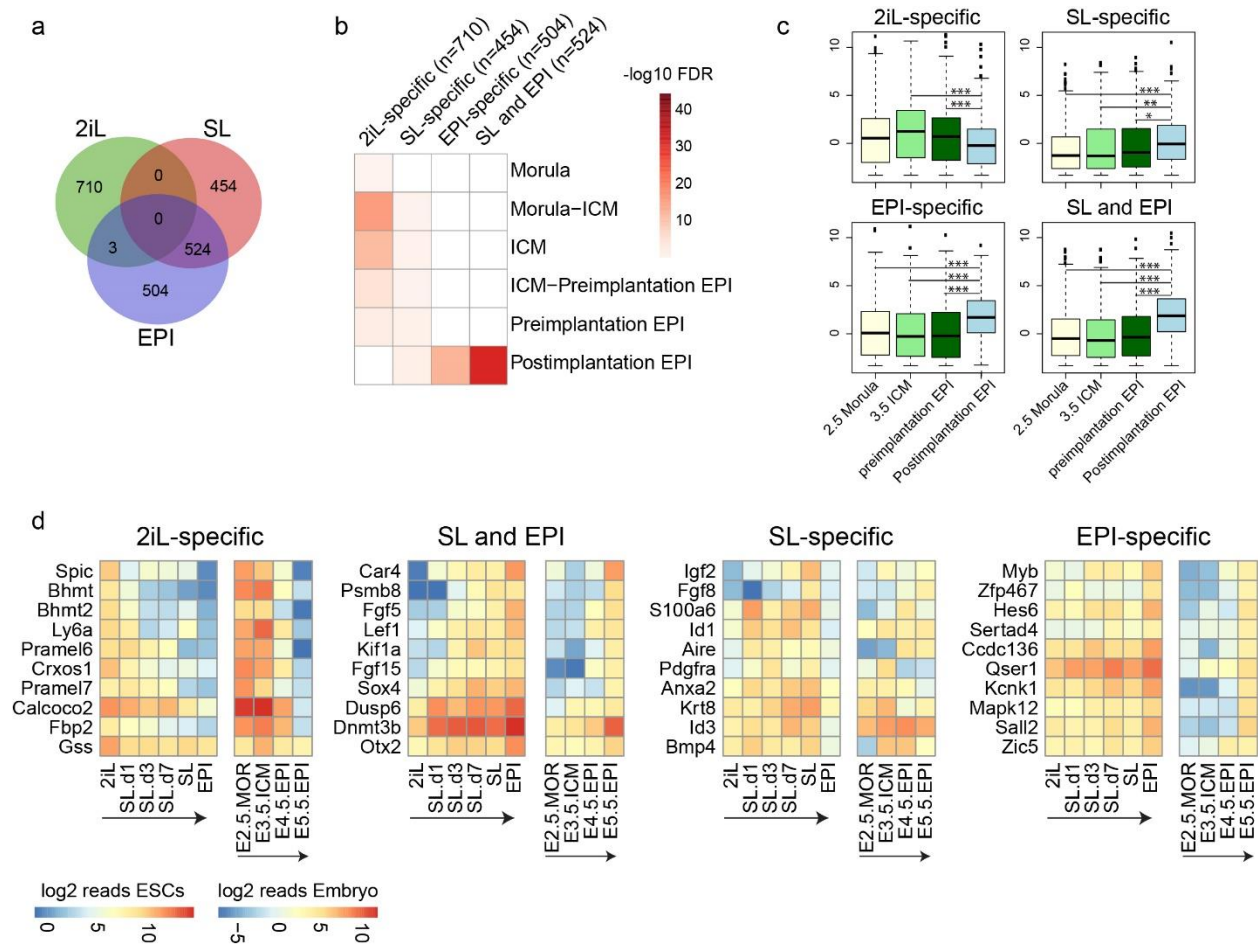
Supplementary Figure 2: Comparison with early embryonic profiles

a) Venn diagram showing the overlap of differentially expressed genes (FC>=3 and DEseq, Benjamini-Hochberg corrected and two-sided P-value <0.05) in 2iL, SL and EPI. N numbers represent mRNAs and are stated in the figure.

b) Heatmap showing enrichment of genes from panel 'a' in different stages of embryonic development. N number indicated in the panel represents genes. Two-sided P-values were computed using Hypergeometric tests with Benjamini-Hochberg correction.

c) Box plots showing the expression levels of genes shown in panel 'b'. Box=25-75th percentile; bar=median; whiskers=5-95th percentile. N numbers represent genes and are stated in the panel 'b'. Significant difference between groups are marked with asterisks (* p=8.894e-05, ** p=8.999e-07, *** p =4.2e-10); Wilcox test was used to calculate the two-sided P-values.

d) Heat-map showing the DESEQ-normalized mRNA expression values for selected genes during 2iL-to-EPI transition and at different stages of embryonic development.
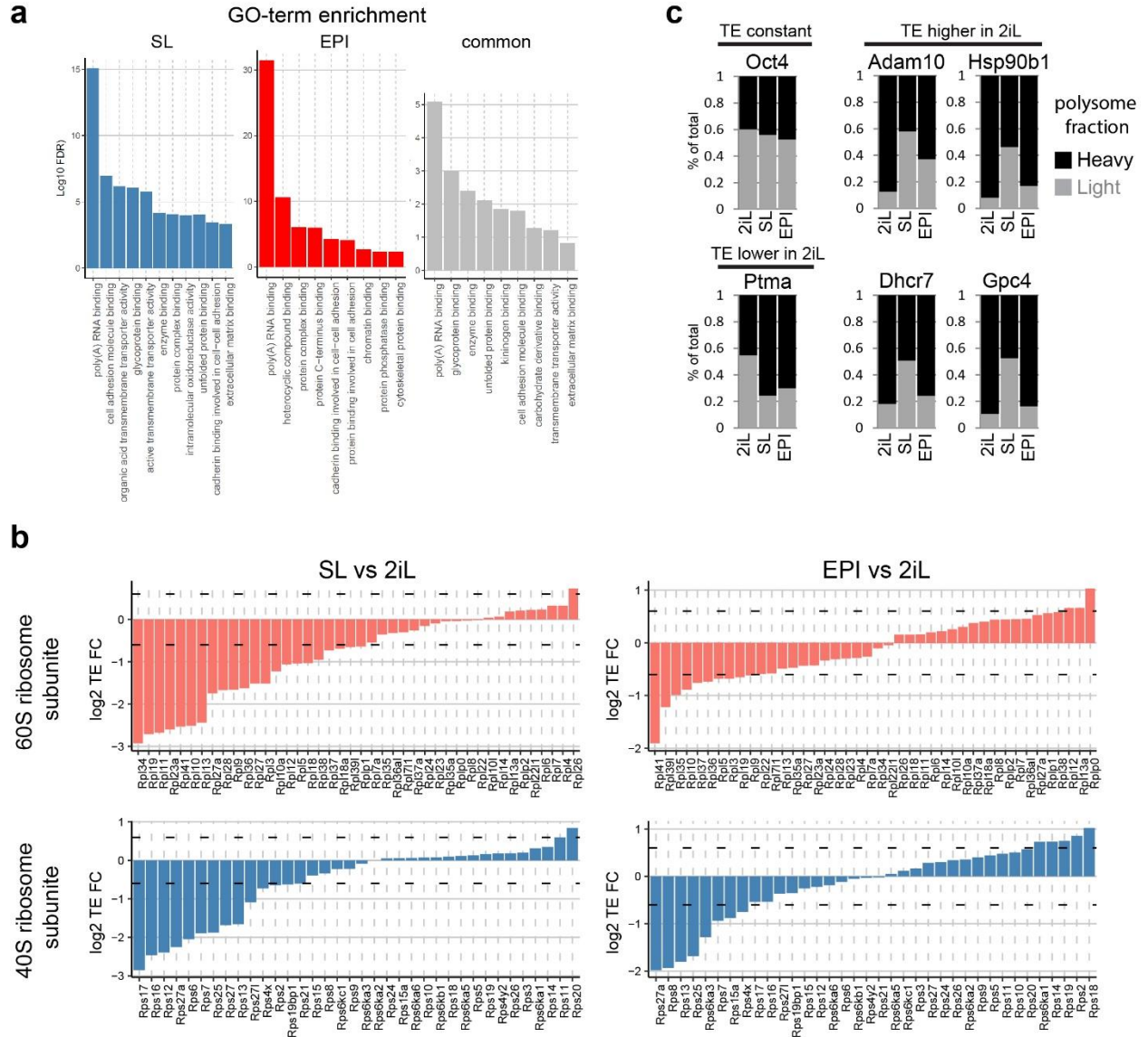
Supplementary Figure 3: GO-term enrichment and selected examples of differentially translated mRNAs

a) GO-term enrichment analysis for SL- , 2iL- or 'common'-genes. Two-sided P-values were adjusted for multiple testing using Benjamin-Hochberg correction.

b) Graph showing the distribution of selected mRNAs in low- and high-density polysome fractions and in different states of ESCs. The qRT-PCR values represent the mean of two biological replicates.

c) Bar plots showing the TE fold-change of genes encoding the ribosomal proteins in SL or EPI when compared to 2iL. Genes with sufficient number of reads in RNA-seq and RFP-seq (see methods) are shown. Genes were separated based on their distribution in the small or the large ribosomal subunits.

Supplementary Figure 4: Structural features and protein-levels of differentially translated mRNAs

a) Volcano plots showing the total proteome quantifications during 2iL-to-SL or SL-to-2iL transition. Values represent the mean of three biological replicates. Differential expression was calculated using a two-sided t-test (FDR <0.05 and FC≥3).

b) Bar plots showing the fold change in RNA, RFP, or protein-levels for 2 representative genes with increased or decreased TE in 2iL ESCs. Changes are shown during 2iL-to-SL and EPI transition.

c) Graphs showing the sequence and structural features of 'common'-mRNAs that have increased or decreased TEs in 2iL ESCs. Similar number of randomly selected mRNAs were used as control (blue). Wilcox test was used to calculate the P Values.

d) Motif enrichment for RNA-binding protein at the 3′ UTR of 'common'-genes with increased TE in 2iL ESCs. Two-sided P-values are derived from the hypergeometric tests associated with each motif and were adjusted for multiple testing using Benjamin-Hochberg correction.

e) Box plots showing the RNA, RFP and protein levels for genes that undergo specific translational regulation. Box=25-75th percentile; bar=median; whiskers=5-95th percentile. N numbers represent genes and are stated in the figure.