Kolle et al., Supplementary Figure Legends

Figure S1. Whole transcriptome sequencing by Solid (WTSSolid). Input RNA was fragmented by enzymatic digestion with RNAse III. Double stranded adapters with random nucleotide overhangs were hybridized to RNA fragments, ligated and reverse transcribed into cDNA. The sample was treated with RNAseH and adapter specific primers were then used to PCR amplify the cDNA fragments (incorporating barcodes for multiplex sequencing). PCR products were gel purified to the appropriate size range and sequenced on the SOLiD analyzer. B. Membrane polysome fractionation and sample collection. A. Flowchart of sample preparation, membrane fractionation and RNA collection. B. A260 as a measure of RNA concentration against fraction number for sucrose gradient separation of cytoplasmic RNA into CPR (fractions 1-13) and MPR (fractions 27-37).

Figure S2. Size fractionation of hESC mRNA. a. Flowchart of sample preparation and extraction. b. Bioanalyser composite of Fluorescent Units (FU) (as a measure of RNA amount) against time (as a measure of size), showing clear size separations between each of the fractionated samples.

Figure S3. No correlation between sense and antisense signal from WTSSolid sequencing. A. Scatter plot of Antisense (log of gene RPKM) against Sense (log of gene RPKM) for the highest expressed 5,000 genes in the mRNA sample. Cor = Pearson correlation in the pairwise comparison. B. Enrichement ratio (Sense tags/antisense tags) for each sample type and for tags mapping with known exon boundaries (exons) or across exon-exon junctions (junctions) for the

top 5,000 highest expressed Refseq genes. We therefore estimate the strand-specificity of the method to be > 10,000 fold.

Figure S4. Comparison between SOLiD sequencing and MPSS. A. Scatter plot and pearson correlation coefficient for pairwise comparisons of ESC SOLiD mRNA, ESC MPSS and Testis MPSS samples. B. Overlap to recent strand non-specific sequencing of hESC and N3 (hESC derived) neural cells performed by Wu et al., 2010.

Figure S5. Strong correlation of MPR/CPR enrichment levels as measured by WTSSolid sequencing and Illumina microarray. Scatter plot shows the log2 (MPR/CPR) enrichment ratio as determined by Illumina microarray (Kolle et al., 2009) and sequencing in this study for 9,481 genes for which there was above background expression values in both platforms.

Figure S6. Real time PCR validation of MPR-enriched alternative splicing usage. Graph of cycle difference (Cycle difference (CPR-MPR), which is approximately equivalent the log2 (MPR/CPR) ratio) for each variant. In each case real time PCR was performed by isoform specific real time PCR, with variant A and B as described in Table S14. Top panel shows events based on alternative splicing junctions, bottom panel shows events where there is clear expression of one of more last exons based on unique 3'UTR sequence usage. Controls are the C/N genes *HPRT1* and *ACTB* and the M/S gene *CXCL12*.

Figure S7. Consistent MPR/CPR ratio across the length of the long 3'UTR. Real time PCR showing the MPR enrichment of both the coding region and the Long 3UTR region (calculated as

the cycle difference: CPR minus MPR) for the M/S genes: *CDS2, GRPR, MGAT5, ROR1, PCDH1* and *UGGT1* and the CPR enrichment for the C/N genes: *KCNG3* and *TRIM71*. Gene specific primer information as described in Table S15.

Figure S8. Plurinet refined with transcriptional complexity. Nodes are coloured according to mRNA expression levels (RPKM). Node shape denotes the presence of experimentally determined transcription factor binding sites in the promoter region (see legend) based on combined evidence from Chip-chip (Boyer et al. 2005) and Chip-seq (Kunarso et al. 2010) studies. Node outline colour denotes the presence of at least two alternatively spliced variants (red), novel sequence feature(s) (pink) or both (green) for the gene. Interactions between genes (edges) are displayed for ligand-receptor (directed arrow), inhibitory action (directed inhibitory line), gene family member (dotted line) or non-directional interaction (solid line).

Figure S9. Models of cell-cell interactions and cell communication.