Supplementary Figures and Tables for *"High resolution temporal transcriptomics of mouse embryoid body development reveals complex expression dynamics of coding and noncoding loci"*

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Supplementary Tables

Supplementary Table S1: Counts of Genes by biotype

Biotype	6 hourly	24 hourly	% increase w 6 hourly	Designation
antisense	148	126	17.5%	noncoding_lncRNA
lincRNA	265	235	12.8%	noncoding_lncRNA
processed_transcript	175	159	10.1%	noncoding_lncRNA
miRNA	17	14	21.4%	noncoding_other
misc_RNA	3	3	0.0%	noncoding_other
Mt_rRNA	2	2	0.0%	noncoding_other
Mt_tRNA	3	3	0.0%	noncoding_other
polymorphic_pseudogene	5	4	25.0%	noncoding_other
pseudogene	292	257	13.6%	noncoding_other
sense_intronic	7	7	0.0%	noncoding_other
sense_overlapping	2	2	0.0%	noncoding_other
snoRNA	40	30	33.3%	noncoding_other
snRNA	1	1	0.0%	noncoding_other
protein_coding	13336	13036	2.3%	protein_coding
Total	**14296**	**13879**		
Novel_MultiExonic_assembled	*1851*	*1174*	*57.7%*	*Novel*

Supplementary Table S2: Periodic Genes

GENCODE_ID	Period	Chr,	Start	End	Strand	Gene Type	Gene Symbol	slRNA
ENSMUSG00000000826.10	17.28571429	chr2	181520485	181555133	+	protein_coding	Dnajc5	TRUE
ENSMUSG00000003847.10	24.2	chr8	107293470	107379517	+	protein_coding	Nfat5	FALSE
ENSMUSG00000004187.10	20.16666667	chr15	76660641	76668196	+	protein_coding	Kifc2	FALSE
ENSMUSG00000005615.8	20.16666667	chr16	32430921	32475070	+	protein_coding	Pcyt1a	FALSE
ENSMUSG00000005682.8	24.2	chr10	128303335	128321358	+	protein_coding	Pan2	FALSE
ENSMUSG00000006463.7	24.2	chr19	4878668	4885397	+	protein_coding	Zdhhc24	FALSE
ENSMUSG00000008200.8	24.2	chr2	90745370	90781021	+	protein_coding	Fnbp4	FALSE
ENSMUSG00000009079.10	24.2	chr11	5069689	5099266	-	protein_coding	Ewsr1	TRUE
ENSMUSG00000013483.8	24.2	chr11	119307768	119345375	+	protein_coding	Card14	FALSE
ENSMUSG00000016427.7	30.25	chrX	37187588	37191163	-	protein_coding	Ndufa1	FALSE
ENSMUSG00000017188.3	24.2	chr11	101277968	101279114	-	protein_coding	Coa3	FALSE
ENSMUSG00000018923.7	24.2	chr11	70451919	70453727	+	protein_coding	Med11	FALSE
ENSMUSG00000019066.7	17.28571429	chr9	21907491	21918192	-	protein_coding	Rab3d	FALSE
ENSMUSG00000020167.8	20.16666667	chr10	80409514	80433647	-	protein_coding	Tcf3	FALSE
ENSMUSG00000020530.8	20.16666667	chr11	84832361	84870817	-	protein_coding	Ggnbp2	TRUE
ENSMUSG00000020843.9	24.2	chr11	76406952	76416292	+	protein_coding	Timm22	FALSE
ENSMUSG00000020894.10	24.2	chr11	69088490	69092384	+	protein_coding	Vamp2	FALSE
ENSMUSG00000021124.7	24.2	chr12	79156017	79172667	-	protein_coding	Vti1b	FALSE
ENSMUSG00000022805.10	17.28571429	chr16	38297754	38342143	-	protein_coding	Maats1	FALSE
ENSMUSG00000024293.9	15.125	chr18	10566602	10610352	-	protein_coding	Esco1	FALSE
ENSMUSG00000024959.7	24.2	chr19	6941861	6951898	+	protein_coding	Bad	FALSE
ENSMUSG00000025486.10	30.25	chr7	140863670	140882309	-	protein_coding	Sirt3	FALSE
ENSMUSG00000025489.4	20.16666667	chr7	140948963	140950239	-	protein_coding	Ifitm5	FALSE
ENSMUSG00000025500.5	20.16666667	chr7	141210043	141214082	-	protein_coding	1600016N20Rik	FALSE
ENSMUSG00000025868.6	30.25	chr13	54590207	54591158	+	protein_coding	Higd2a	FALSE
ENSMUSG00000026034.11	24.2	chr1	58410189	58424066	-	protein_coding	Clk1	TRUE
ENSMUSG00000026181.11	17.28571429	chr16	16896469	16927364	+	protein_coding	Ppm1f	FALSE
ENSMUSG00000026199.11	20.16666667	chr1	75192151	75199387	+	protein_coding	Ankzf1	FALSE

ENSMUSG00000026648.12	24.2	chr2	3424131	3464130	+	protein_coding	Dclre1c	FALSE
ENSMUSG00000026792.10	20.16666667	chr2	32925216	32961614	-	protein_coding	Lrsam1	FALSE
ENSMUSG00000026864.7	24.2	chr2	34771970	34776531	+	protein_coding	Hspa5	FALSE
ENSMUSG00000026977.11	20.16666667	chr2	60209887	60250676	+	protein_coding	March7	FALSE
ENSMUSG00000027170.6	24.2	chr2	104999656	105017080	-	protein_coding	Eif3m	FALSE
ENSMUSG00000027245.5	24.2	chr2	121453290	121458672	+	protein_coding	Hypk	FALSE
ENSMUSG00000027520.9	20.16666667	chr1	63273265	63314576	+	protein_coding	Zdbf2	FALSE
ENSMUSG00000027708.8	30.25	chr3	35892105	35937445	-	protein_coding	Dcun1d1	FALSE
ENSMUSG00000027782.4	20.16666667	chr3	69067149	69127113	-	protein_coding	Kpna4	FALSE
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ENSMUSG00000028053.7	24.2	chr3	88965812	89079373	+	protein_coding	Ash1l	FALSE
ENSMUSG00000028062.9	30.25	chr3	88549819	88553074	-	protein_coding	Lamtor2	FALSE
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ENSMUSG00000028979.11	24.2	chr4	148602554	148615499	+	protein_coding	Masp2	FALSE
ENSMUSG00000028999.9	15.125	chr5	23787711	23820369	+	protein_coding	Rint1	FALSE
ENSMUSG00000029068.10	24.2	chr4	155812489	155824543	+	protein_coding	Ccnl2	FALSE
ENSMUSG00000029238.8	24.2	chr5	76212177	76304548	-	protein_coding	Clock	FALSE
ENSMUSG00000030752.2	20.16666667	chr7	125444676	125462265	+	protein_coding	Kdm8	FALSE
ENSMUSG00000031167.10	24.2	chrX	8138975	8145880	-	protein_coding	Rbm3	FALSE
ENSMUSG00000031380.10	24.2	chrX	164373378	164402650	+	protein_coding	Figf	FALSE
ENSMUSG00000031422.10	24.2	chrX	136732942	136743690	-	protein_coding	Morf4l2	FALSE
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ENSMUSG00000034799.10	24.2	chr8	71624417	71671757	-	protein_coding	Unc13a	FALSE
ENSMUSG00000035458.8	20.16666667	chr7	4518305	4524229	-	protein_coding	Tnni3	FALSE
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ENSMUSG00000087165.1	24.2	chr11	57795499	57801417	-	processed_transcript	2010001A14Rik	FALSE
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ENSMUSG00000089788.1	24.2	chr1	178321108	178324171	-	processed_transcript	Gm16586	TRUE
ENSMUSG00000090086.1	24.2	chr5	123133723	123141666	-	lincRNA	AI480526	TRUE
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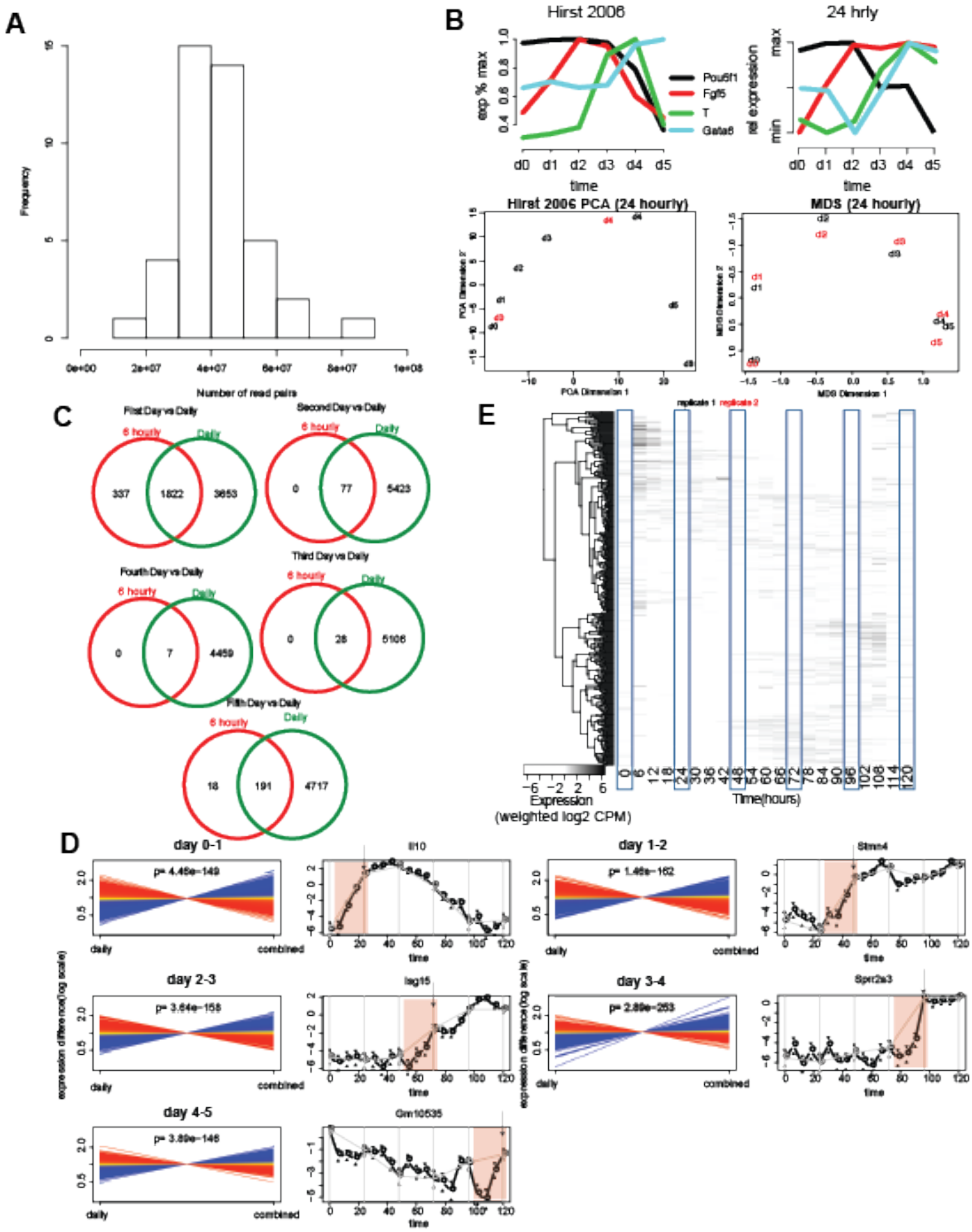
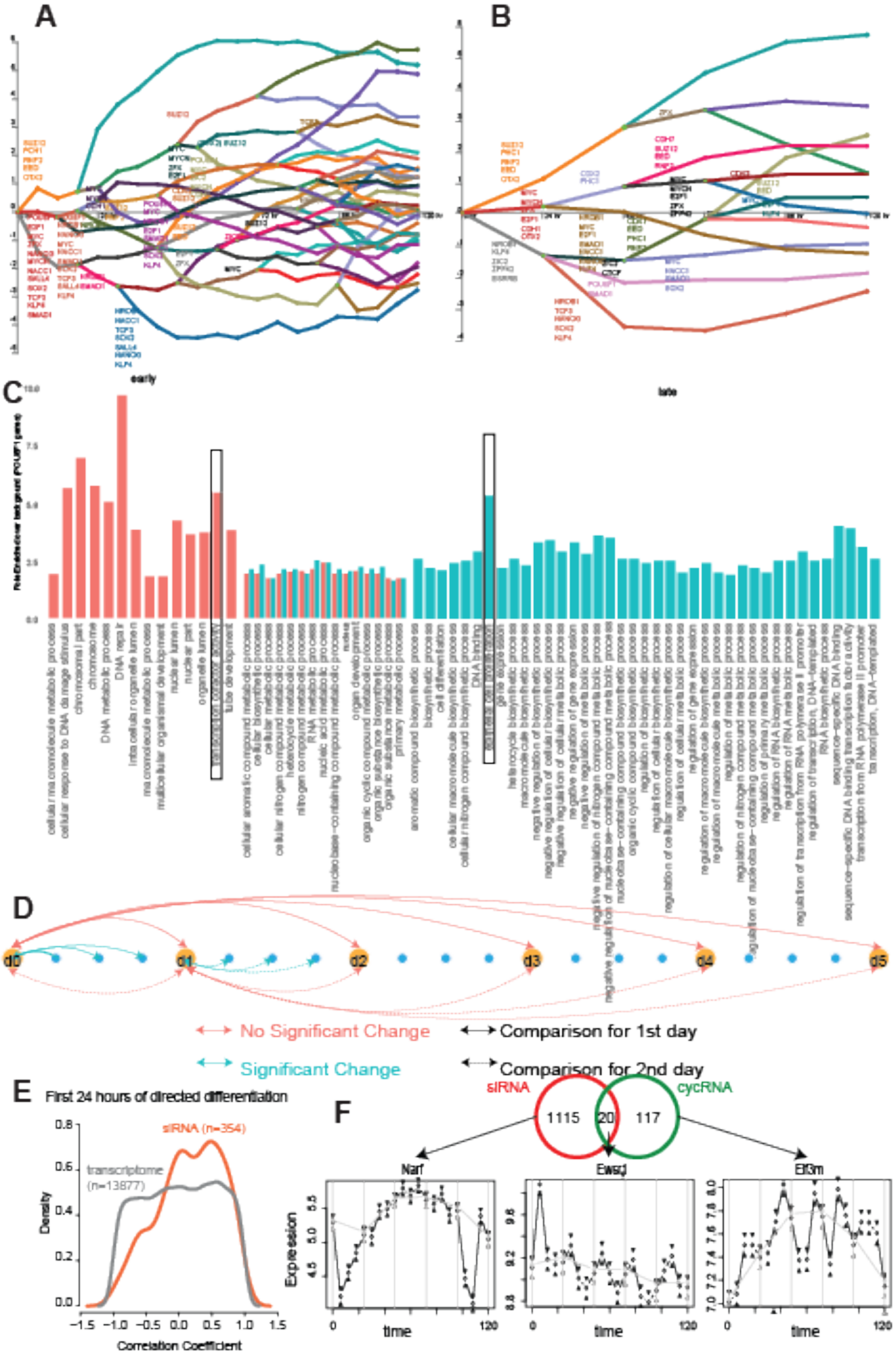
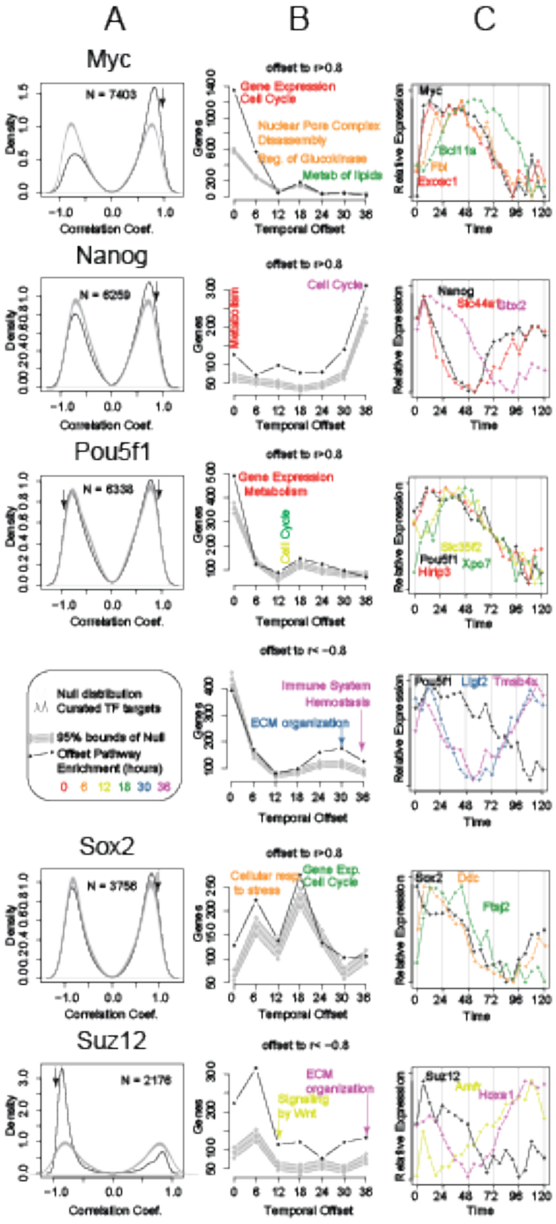
Supplementary Figures and Legends



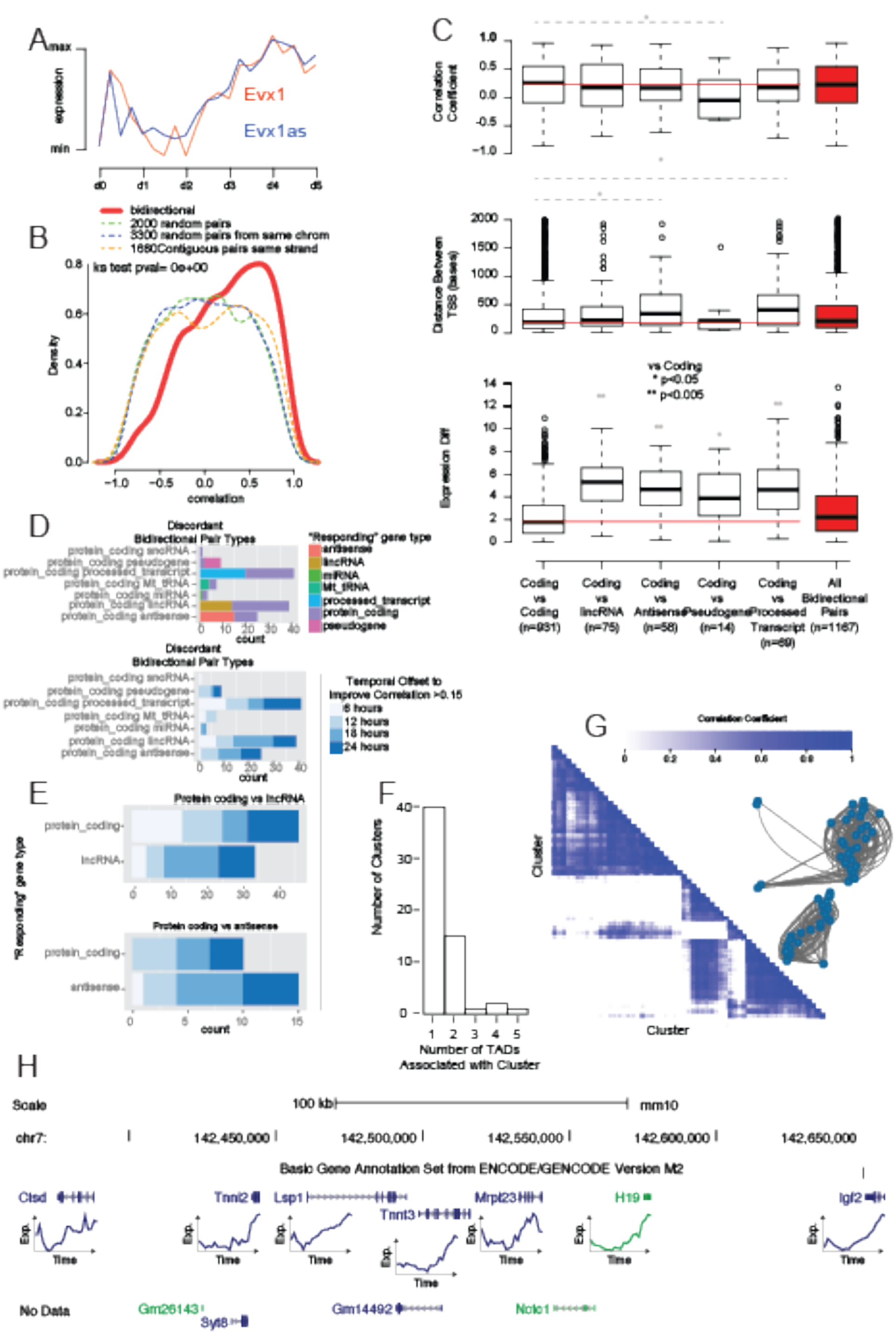
Supplementary Figure S1: Global evaluation of high-resolution transcriptomic data. **(A)** Histogram of mapped read number distribution per sample (pooled from biological replicates). **(B)** Comparison of expression levels and principle components analysis measured 24 hourly between this study and Hirst 2006 **(C)** Evidence of differential expression within one 24 hour period vs. any change across all 24hourly times (p <0.0001). **(D)** Comparing whether the 24 hourly measures "summarize" that 24 hour window by comparing mean expression for that window with the 24 hour time point, a colour gradient was assigned by ranked expression levels in the daily measure. P-value is from a paired Wilcoxon rank-sum test. **(E)** Greyscale heatmap of expression levels for genes only expressed outside of 24 hourly time points, clustered by expression pattern.



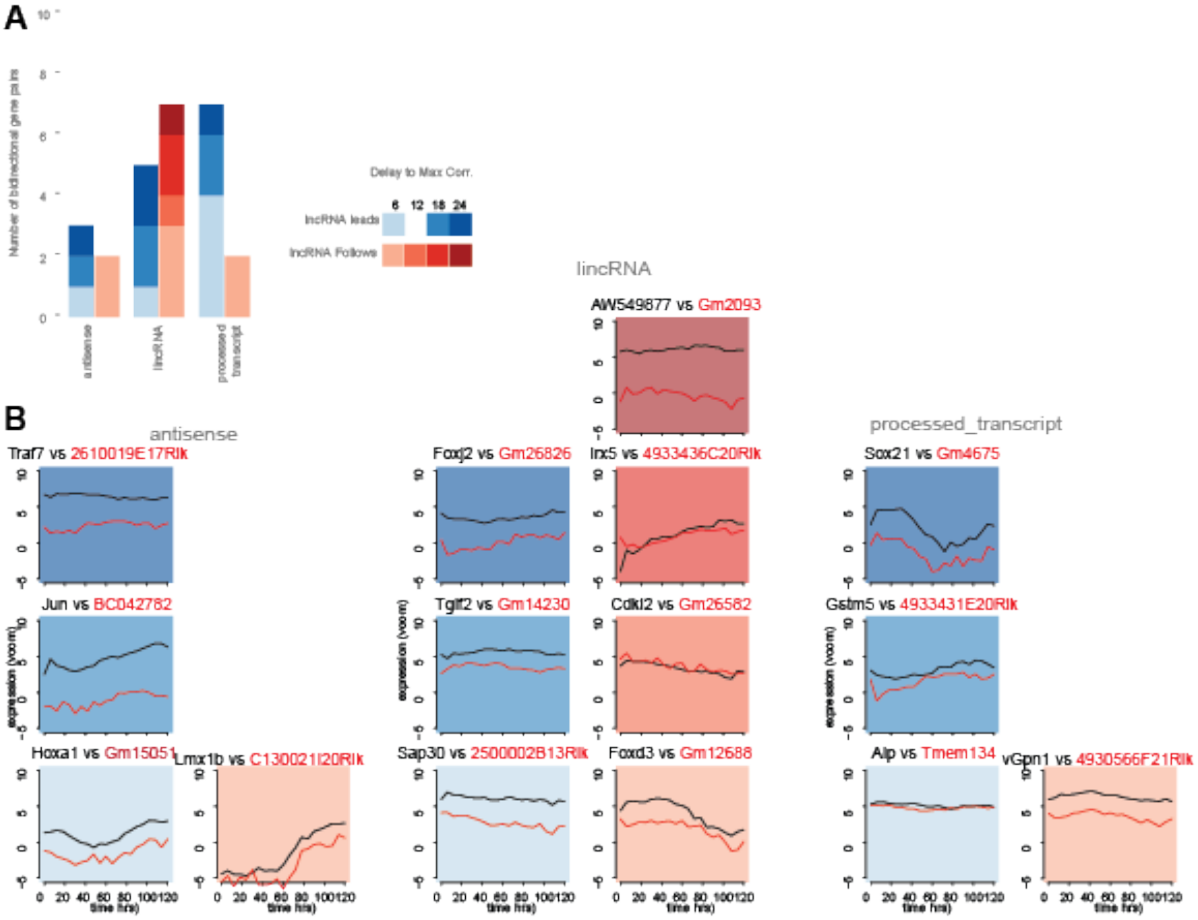
Supplementary Figure S2: Highlighting unique knowledge gained from increased temporal resolution. (A &B) Fully annotated DREM schematic of estimated TF activity of key ESC related TFs at 6hourly (A) vs. 24 hourly (B). Relative circle sizes are proportional to the spread of gene expression levels corresponding to that point. line colors are assigned by branch and are not comparable between panels. Red and blue boxes pertain to branch points of interest **(C)** GO term enrichment (adjusted $p<0.05$) for genes corresponding branch points designated as early (co-observed with change in POU expression) and late (observed after POU5f1 Expression changes) highlighted by the blue boxes in Figure 2A. Black boxes represent similar terms identified in Figure 2D. (**D)** Schematic of differential expression analysis design used to identify slRNAs. (**E)** Correlation of slRNA expression in [21]. **(F)** Comparison of slRNAs and cycRNAs. Venn diagram of the overlap observed and examples from each class.



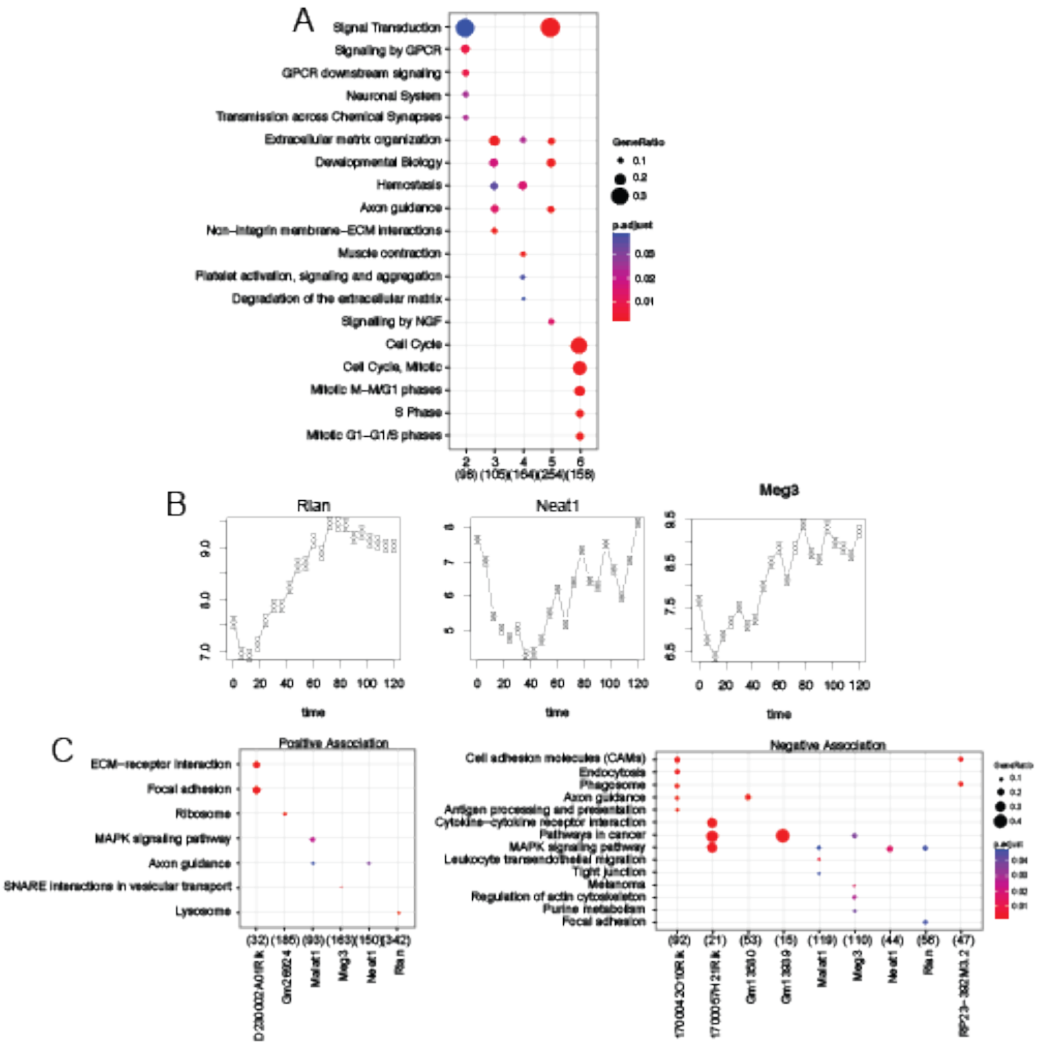
Supplementary Figure S3: Temporal offsets in transcription factor (TF)- target gene expression. (**A)** Curated TF/gene targets were downloaded from chea (http://amp.pharm.mssm.edu/lib/chea.jsp) for Myc, Nanog, Pou5f1, Sox2 and Suz12 (N=number of target genes). Expression of target genes were tested for correlation with their TF at different temporal offsets (0-36 hours) and compared to 500 random selections of the same number of genes (Null). Where absolute correlations of predicted targets exceeded the null distribution (arrow), (**B)** the number of genes achieving a maximal absolute correlation of >0.8 and the offset required to reach these maxima was plotted against the 5th and 95thquantiles of the same results from the null distribution. Where the number of target genes exceeded the null distribution, the lists of genes in each offset were tested for enrichment of Reactome pathways relative to the total predicted target list (enrichment). **(C)** Example expression patterns of genes displaying these attributes were plotted.



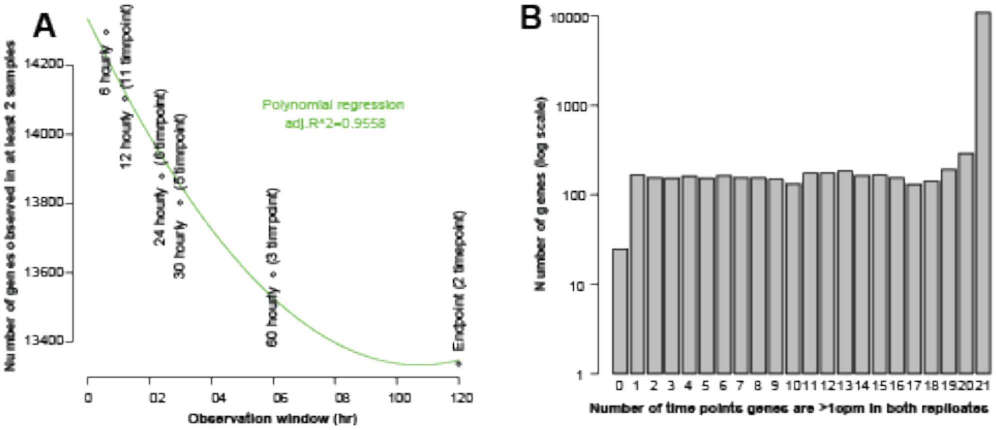
Supplementary Figure S4: Bidirectional and co-expression analysis of mouse ES development. (A) Expression profile of EVX1 and its antisense (and positively correlated) transcript EVX1AS- the peak at 6-18 hours has not been observed previously. **(B)** Distribution of correlation coefficients of bidirectional gene pairs (red) compared to similar numbers of randomly chosen genes pairs, randomly chosen genes from the same chromosome and, randomly selected neighbouring genes (dotted lines). ks=Kolmogorov–Smirnov test (bidirectional vs. random neighbouring gene pairs). **(C)** Characteristics of bidirectional gene pairs (Correlation coefficient, Distance between TSS and Difference of median expression (log scale) based on annotated gene-biotype (p value calculated using the Mann-Whitney test. **(D)** Counts of bidirectional gene pairs of differing biotypes achieving an improved correlation coefficient of >0.15 (to at lease 0.25) over that at time zero, colored by the biotype of the "following" gene or by the temporal offset required to achieve the improvement. **(E)** Comparison of responding gene biotype to the temporal offset for lincRNA and antisense biotypes. **(F)** Number of topological associated domains (TADS, HindIII data mapped to mm10 using liftOver from mm9) associated with each co-expressed gene cluster. **(G)** Clustering of co-regulated gene clusters visualized by network diagram and hierarchical clustering of the correlation matrix showing two distinct groups. **(H)** The imprinted H19/IGF2 cluster identified as a co-expressed gene cluster with gene expression data for measured genes. Some genes did not have expression data (no data).



Supplementary Figure S5: Temporal relationships of highly correlated coding-noncoding bidirectional pairs. (A) Bar chart of the temporal offset required to reach a maximum correlation >0.8 and whether the noncoding gene preceded the protein coding gene or vice versa. **(B)** Example gene expression profiles of bidirectional paired gene over the time course. Gene profiles are arranged and colored as the bar chart.



Supplementary Figure S6: LncRNAs and their role in ES development. (A) Reactome pathway enrichment for 5/6 k-means clusters of time-dependent protein coding genes. (**B)** Expression profiles for characterized lncRNAs described in text. (**C)** Reactome pathway enrichment for putative gene targets positively or negatively associated with candidate lncRNAs (top 4 pathways, enrichment adj. pval. <0.05)



Supplementary Figure S7: (A) The impact of increasing temporal resolution on the number of genes observed to be expressed. **(B)** The number of conditions in which each gene observed is expressed above background in both replicates across the time course. ~150 new genes are observed at a single time point.