Supplemental Information

Title

Changes in the enhancer landscape during early placental development uncover a trophoblast invasion gene-enhancer network.

Authors

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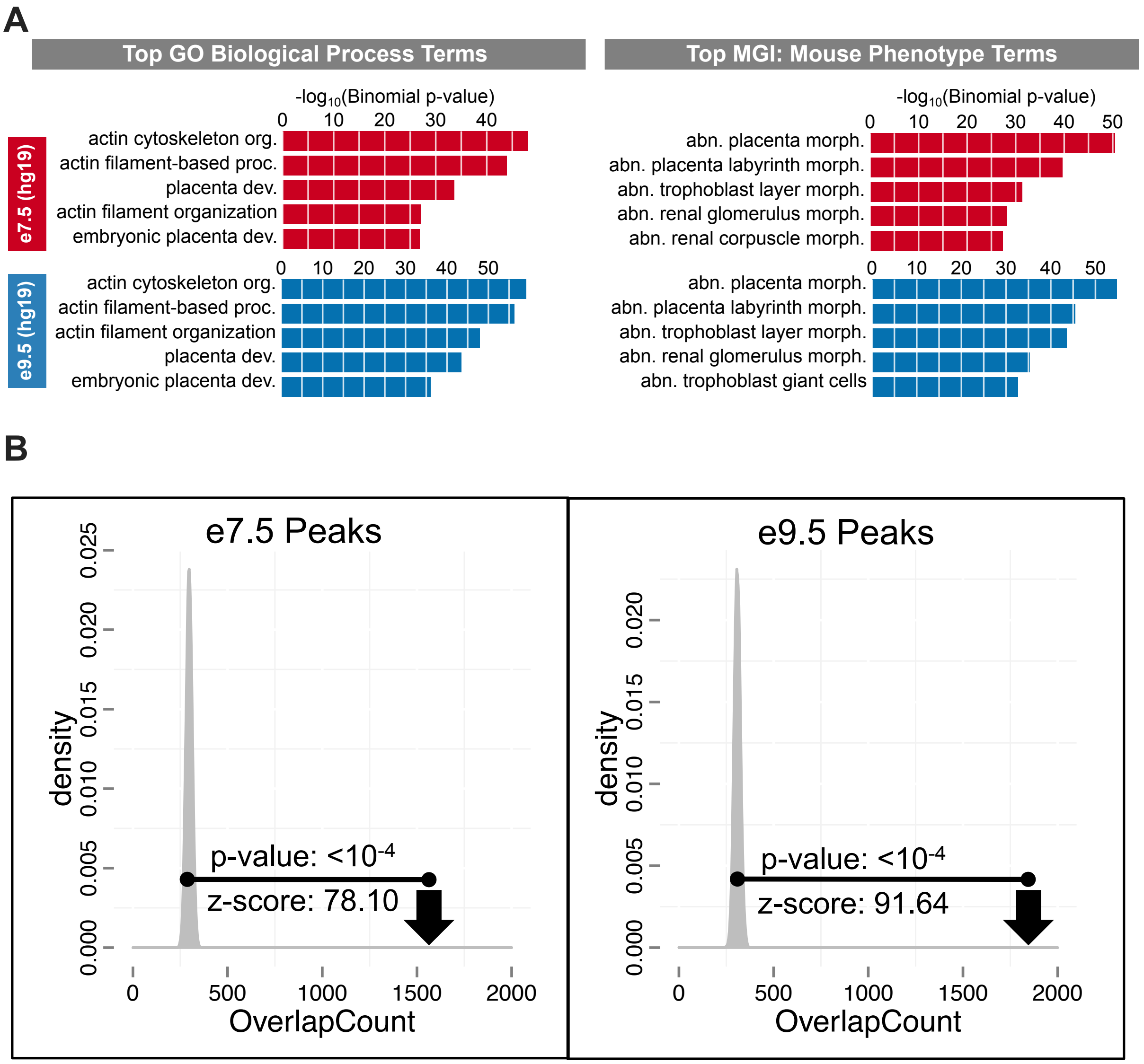


Figure S1. Mouse E7.5 and e9.5 placenta H3k27Ac peaks are relevant to human. (A) Enrichment analysis using GREAT for human (hg19) after mapping mouse peaks to orthologous human coordinates shows peaks are still associated with placental genes. (B) e7.5 and e9.5 peaks have significant overlap with human placenta (85-113 days) DNAse-Seq data.

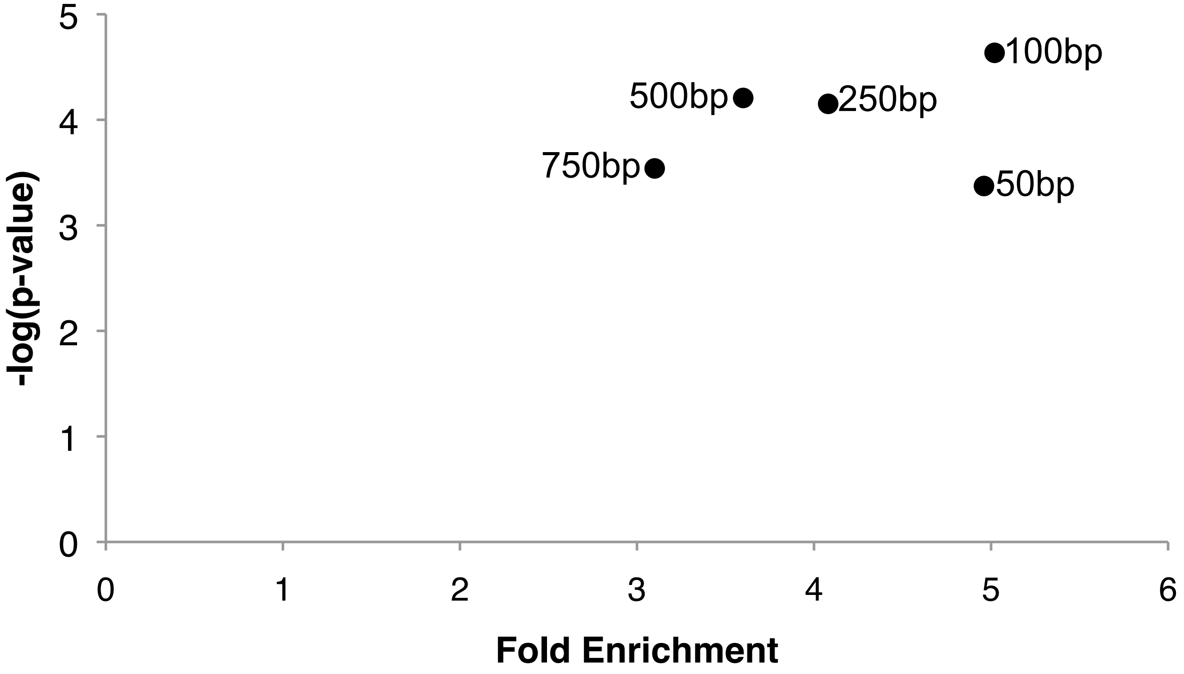


Figure S2. AP1 and Ets motif within 100bp of one other are most enriched for cell migration terms.

Shown are the –log(p-value) vs. Fold Enrichment for the term “regulation of cell motility” for sets of e7.5-specific enhancers with AP1 and Ets motifs separated by 50bp, 100bp, 250bp, 500bp, or 750bp. Enhancers with the motifs within 100bp of each other show the most significant enrichment for this term.

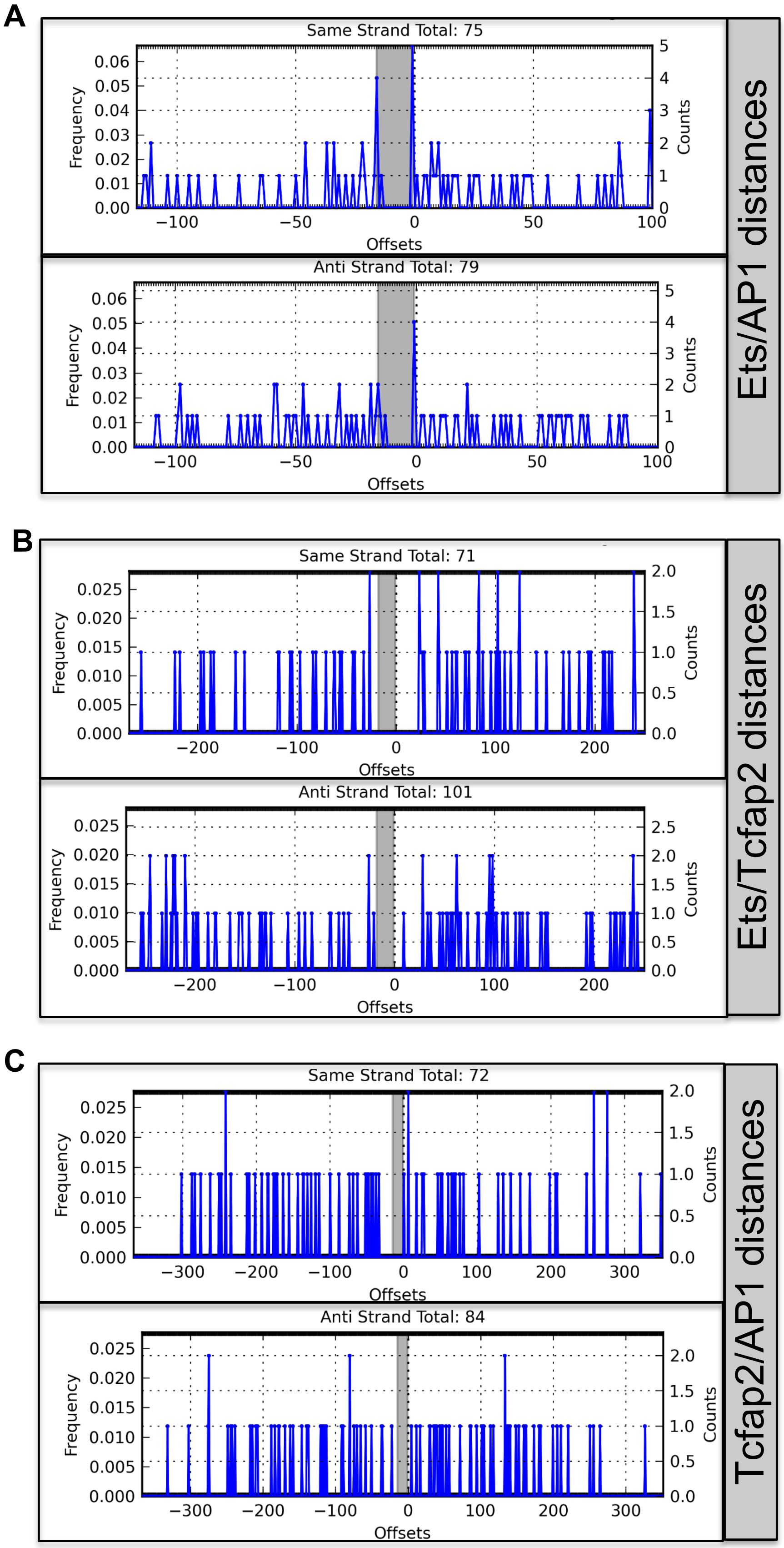
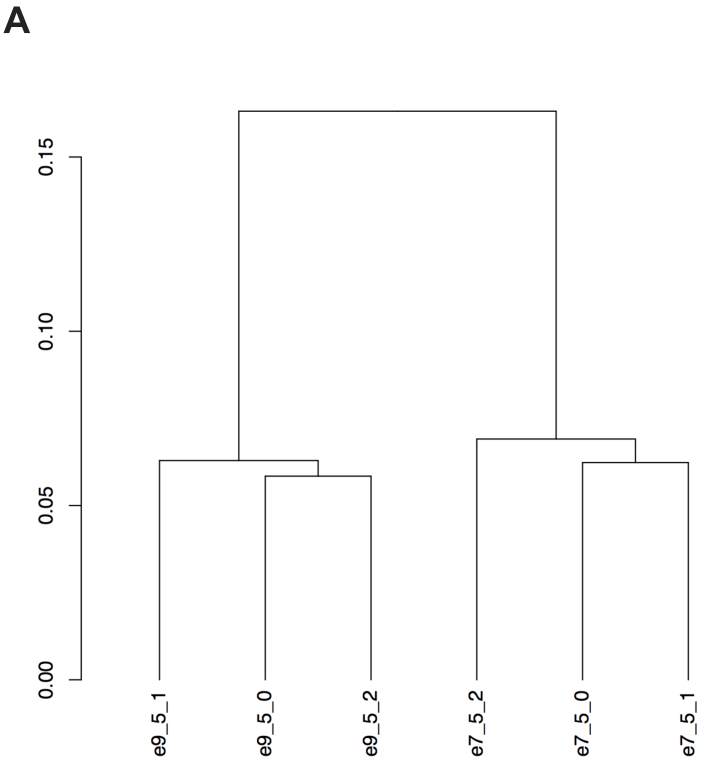

A
Same Strand Total: 75
Frequency
Counts
Offsets
Anti Strand Total: 79
Frequency
Counts
Offsets
Ets/AP1 distances
B
Same Strand Total: 71
Frequency
Counts
Offsets
Anti Strand Total: 101
Frequency
Counts
Offsets
Ets/Tcfap2 distances
C
Same Strand Total: 72
Frequency
Counts
Offsets
Anti Strand Total: 84
Frequency
Counts
Offsets
Tcfap2/AP1 distances

Figure S3. Distance between each pair of AP1, Ets, and Tcfap2 motifs.
Distances between Ets and AP1 motifs (A), Ets and Tcfap2 motifs (B), and Tcfap2 and AP1 motifs (C) in the set of 108 enhancers from Figure 3C are shown. Motifs do not prefer specific spacing or orientation relative to one another.


A
0.15
0.10
0.05
0.00
e9_5_1
e9_5_0
e9_5_2
e7_5_2
e7_5_0
e7_5_1

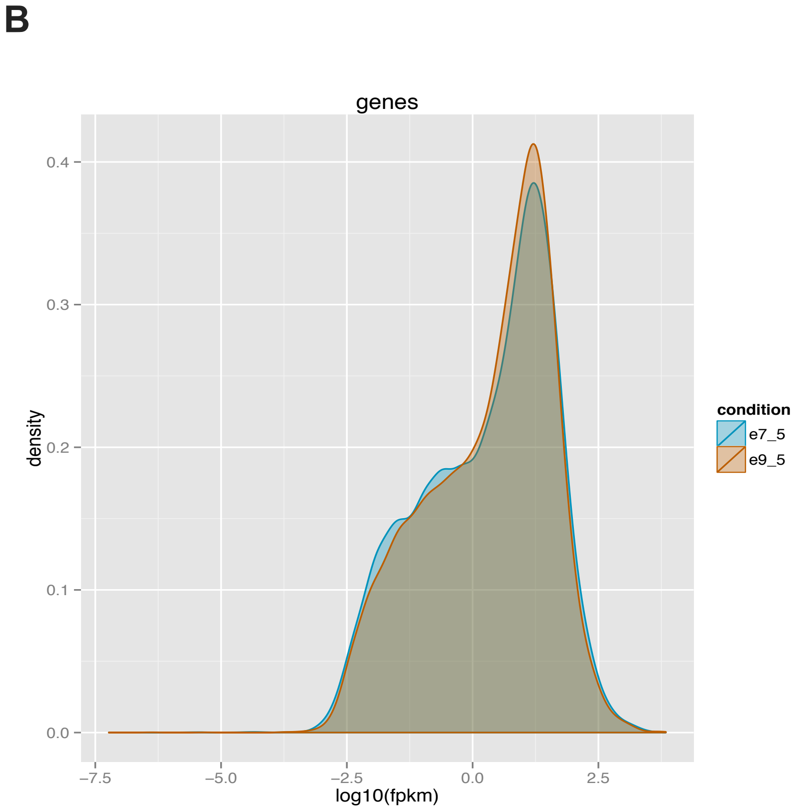

B
genes
0.4
0.3
0.2
0.1
0.0
density
−7.5
−5.0
−2.5
0.0
2.5
log10(fpkm)
condition
e7_5
e9_5

Figure S4. RNA-Seq data quality control.
(A) E7.5 RNA-Seq data replicates cluster with one another, as do e9.5 RNA-Seq data replicates. Clustering is based on gene FPKM values. (B) FPKM distributions for e7.5 and e9.5 are similar.

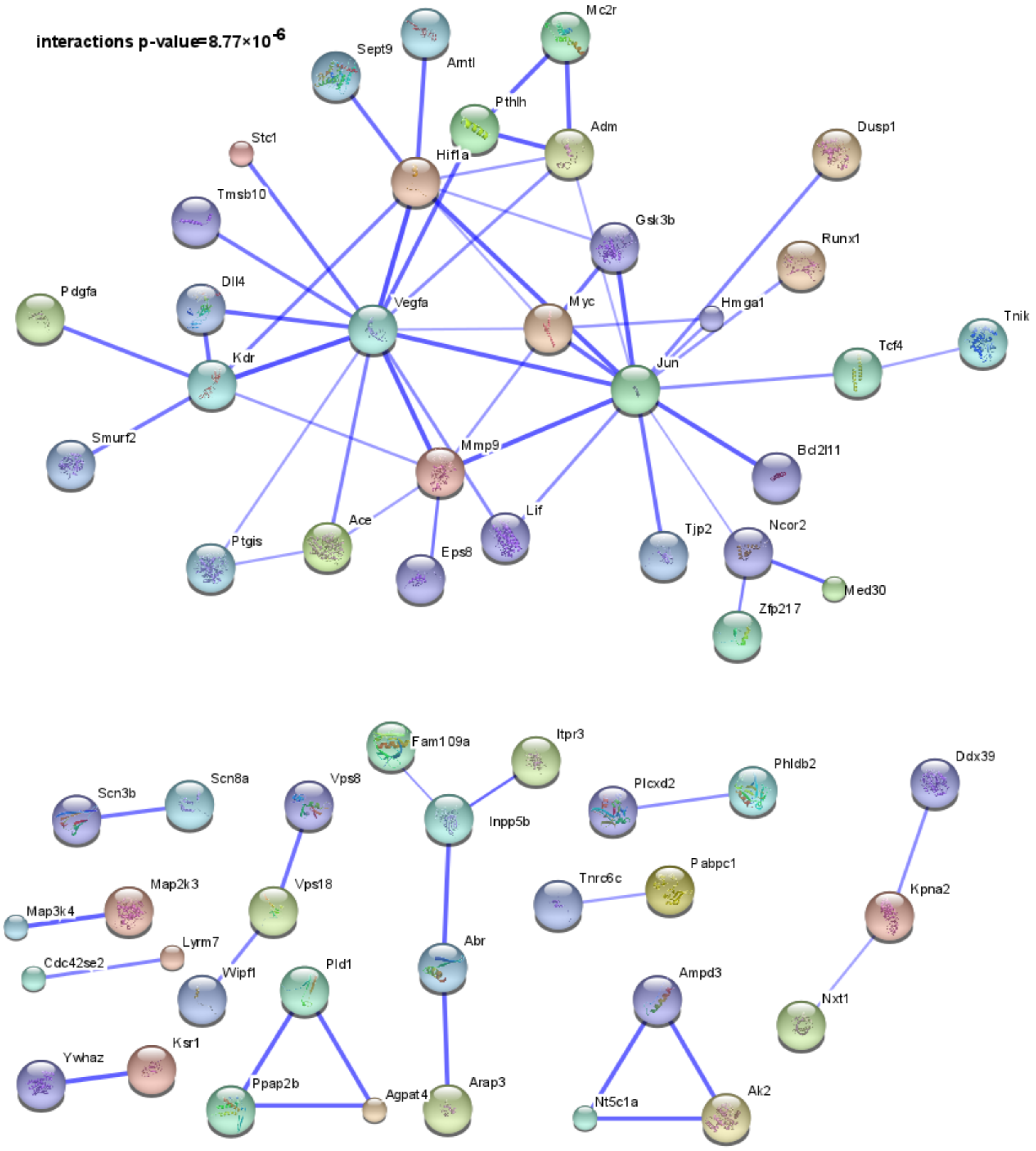


Figure S5. Genes with AP1/Ets/Tcfap2 enhancers are enriched for interactions.
String-db network showing known/predicted interactions between genes with AP1/Ets/Tcfap2 enhancers. Interactions p-value=8.77×10^{-6}.

Table S1. Number of reads that uniquely align to mm9 with up to 2 mismatches.

Time point	Replicate 1 (H3k27Ac)	Replicate 2 (H3k27Ac)	Replicate 1 (Input)	Replicate 2 (Input)
e7.5	29,526,188	27,274,271	26,997,654	25,226,807
e9.5	26,527,620	29,376,051	24,541,293	15,296,868

Table S2. List of primers used to clone candidate regions in Figure 1B. Sequences listed are for mm9.

Element name	Forward	Reverse
mPL-II	CTGGAGGCAAATGGTAAAGG	CCAAGGATGATCCAGCAAAT
Agps	AAAGTGAAGAACGATTGAGACCA	TGCCACGTGTGTGCTGA
Cbx7	GGGACTCCCAGCACACG	CAGCCGCCTACATTCCTG
Ets2	TCCCCACTTCCATCCTCA	GCTGCAGCCTGCTCTGA
Fosb	AGGGCGGGGCTTGTT	CACCACATCTGGCTTCAATC
Klf7	CCCCCAAACACACACACA	GGCTCGCTGCCTTGC
Mmp9	AGGGGGTCAGGTTTTTGC	CAGTGGGTAAGCAGTGGGTA
Mrpl14	GGGGCAGTTGGGAGGA	GCTTGGGCCTGTGGTG
Mtss1	CCCAAACAGCACCACCA	CAACAGTGGCCCAGAGC
Ndufa13	TGTGTGTTTTCAACATAGACAGG	CCAGGTCCACTGACTGTGC
Pias1	GGCTAGGTACCCCCAATCA	GCTGCCTTTTGCTTGCTC
Ppard	GCCAGGCCACTCTGCT	CCCCAGCCTGCACAGA
Prkch	TCCCTAAGGACCCTTCCA	TGAGGGGCTCGTGTTGA
Rcan1	CCTGGCACGCCTGGT	TGCAGCACAGTTCCTTGG
Tcf7l2	TGGCTGTGTCCTGGTGAG	GGAAGCCCCTCTTATCTGC
Tha1	GGGGACTTGGAAAGCCTA	GGGAATCCTGGAGGACTACTG

Table S3. Genes associated with enhancers tested in Figure 1B are expressed in trophoblast giant cells. The gene expression data is from Hannibal et al, 2014.

Gene name	Normalized expression value	Percentile of expression
Agps	124.24	26.3
Cbx7	1880.30	5.4
Ets2	170.02	23.3
Fosb	553.54	13.7
Klf7	44.59	38.9
Mmp9	1323.31	7.4
Mrpl14	5662.77	1.3
Mtss1	105.05	28.0
Ndufa13	4330.33	1.8
Pias1	233.92	20.0
Ppard	1114.58	8.5

Prkch	47.81	37.9
Rcan1	641.27	12.7
Tcf7l2	25.36	47.2
Tha1	N/A	N/A

Table S4. E7.5-specific peaks are significantly associated with genes up-regulated at e7.5 but not e9.5. Genes are considered up-regulated if the FPKM>1 and FPKM fold between the two time points is ≥1.5.

Gene Set (# genes)	p-value	Fold enrichment	# peaks assoc. with gene set
Up-regulated at e7.5 (1,681)	5.41×10^{-220}	3.15	841
Up-regulated at e9.5 (2,432)	0.617	0.99	287