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Supplementary Information for

Oct4 induces embryonic pluripotency via Stat3 signalling and metabolic mechanisms

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Supplementary Information Text

Fig. S1. (A) t-SNE plot for early blastocyst cells. Sample colors represent Pou5f1 log2 expression. (B) Heatmap showing the top co-regulated genes between E3.5 MUT and E3.5 WT/HET (> 5 interaction; n=346 MUT genes and n=398 WT/HET genes). (C) Boxplots for Nanog, Klf4 and Esrrb expression. (D) FPKM expression of genes associated with pluripotency and mean ± sd for WT/HET and MUT cells (Padj Nanog:0.3, Sox2:0.74, Esrrb:0.75, Utf1:2.32e-10). (E) Confocal images and normalized expression of OCT4 HET and MUT embryos stained for SOX2 and ESRRB (dotted lines represent ICM). (F) FPKM expression of Stat3 in WT, HET and MUT cells. (G) PCA computed with genes in KEGG JAK/STAT signalling pathway. Each dot represents a cell. (H) Cumulative sum of relative percentages between the expression of genes in STAT3 pathway (KEGG); student's t-test p.val < 0.05. (I) Total Oct4 level in iOct4 ESCs (J) Western blot for pSTAT3 after induction of Oct4.

Fig. S2. (A) Diffusion plot of early and late blastocyst cells; color represents Pou5f1 expression. (B) Barplot shows FPKM expression of Pou5f1 for each single cell at E4.5 stage. (C) One-way hierarchical cluster of eigengenes value computed from WGCNA (power 8; dist=0.35, size =30). (D) Density distribution of log2FPKM in late blastocyst PrE and MUT cells of 667 genes and late blastocyst EPI and MUT cells of 517 genes. (E) Scatter plot of Nanog and Gata6 FPKM expression values for E4.5 EPI (WT/HET), E4.5 PrE (WT/HET) and E4.5 MUT cells. (F) log2FPKM expression of Fgf4, Fgfr1 and Fgfr2 in WT/HET EPI/PrE and MUT cells. (G) log2FPKM scatter plot of MAPK signalling genes between late PrE and MUT blastocyst cells. (H)Ternary plot of early WT/HET blastocyst cells, WT/HET EPI and TE cells. Axes show the density of the relative fraction of expression.

Fig. S3. (A) GSEA analysis computed with log2FC between E4.5 EPI-WT.HET/E4.0 TE and (B) E4.5 MUT/E4.0 TE. (C) Distribution of expression values in WT and MUT Oct4 ESC for Fabp3, Cldn4 and (D) Lats2 and Amotl2. (E) Barplot of OCT4 CHIP targets in ESC. (F) Pathways enriched for the top1000 OCT4 CHIP targets in ESC.

Fig. S4. (A)TCA associated enzymes (arrows) colored by the ratio between E4.5 WT and MUT cells and heatmap of the associated enzymes. (B) Volcano plot showing the contribution on principal component 1 (Fig.4A) and OCT4 ChIP-seq score in ESCs. (C) Venn diagram of KEGG pathways enriched for variable genes in E4.5 EPI/PrE vs MUT and between OCT4 depleted ESC vs control. (D) Scatter plot of autophagy related genes (Atgs) between E4.5 WT/HET ICM and E4.5 MUT. (E) Enzymes in fatty acid oxidation and synthetase; color represents the ratio between E4.5 WT/HET and MUT cells. (F) RT-qPCR analysis of gene expression in a time-course following OCT4 degradation in ESCs (days following addition of IAA in 2iL), normalized to the highest value for each gene. Bars represent the mean of three technical replicates, error bars indicate standard deviation. (G) TFEB localization in OCT4+/+ and OCT4-/- ESCs cultured in CHIR+LIF

Supplementary Tables

Table S1. Table_S1. Number of cells and embryo of origin used in this study.

Table S2. Table_S2. Genes co-regulated identified with WGCNA

Table S3. Table_S3. Average score of OCT4 CHIP target in ESC.

Table S4. Table_S4. Genes co-regulated identified with WGCNA

Table S5. Table_S5. Average gene expression table containing all samples analyzed in this study.

Table S6. Table_S6. Scheme of the antibodies used for IF.

Table S7. Table_S7. Significance and mean fluorescence for IF data.