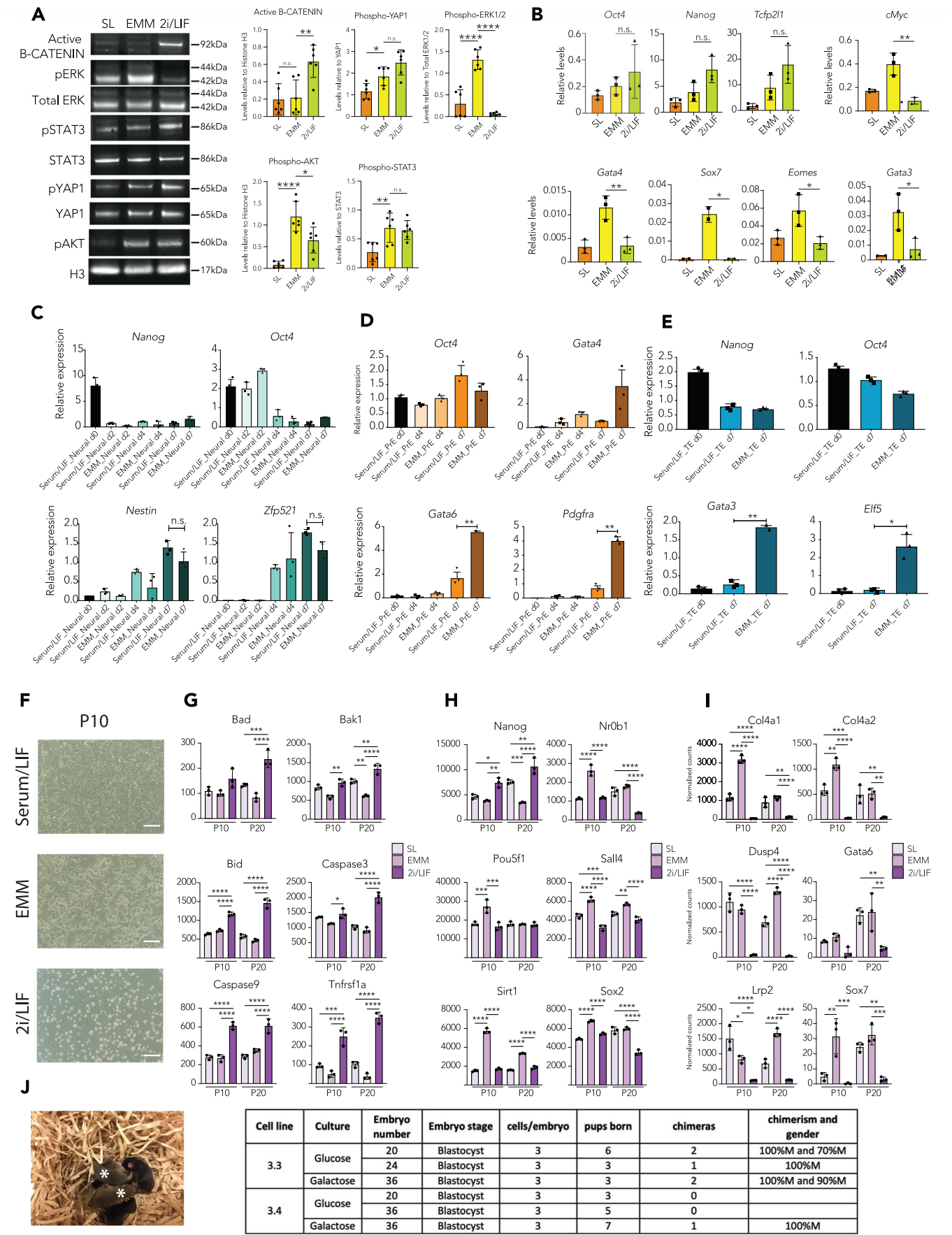
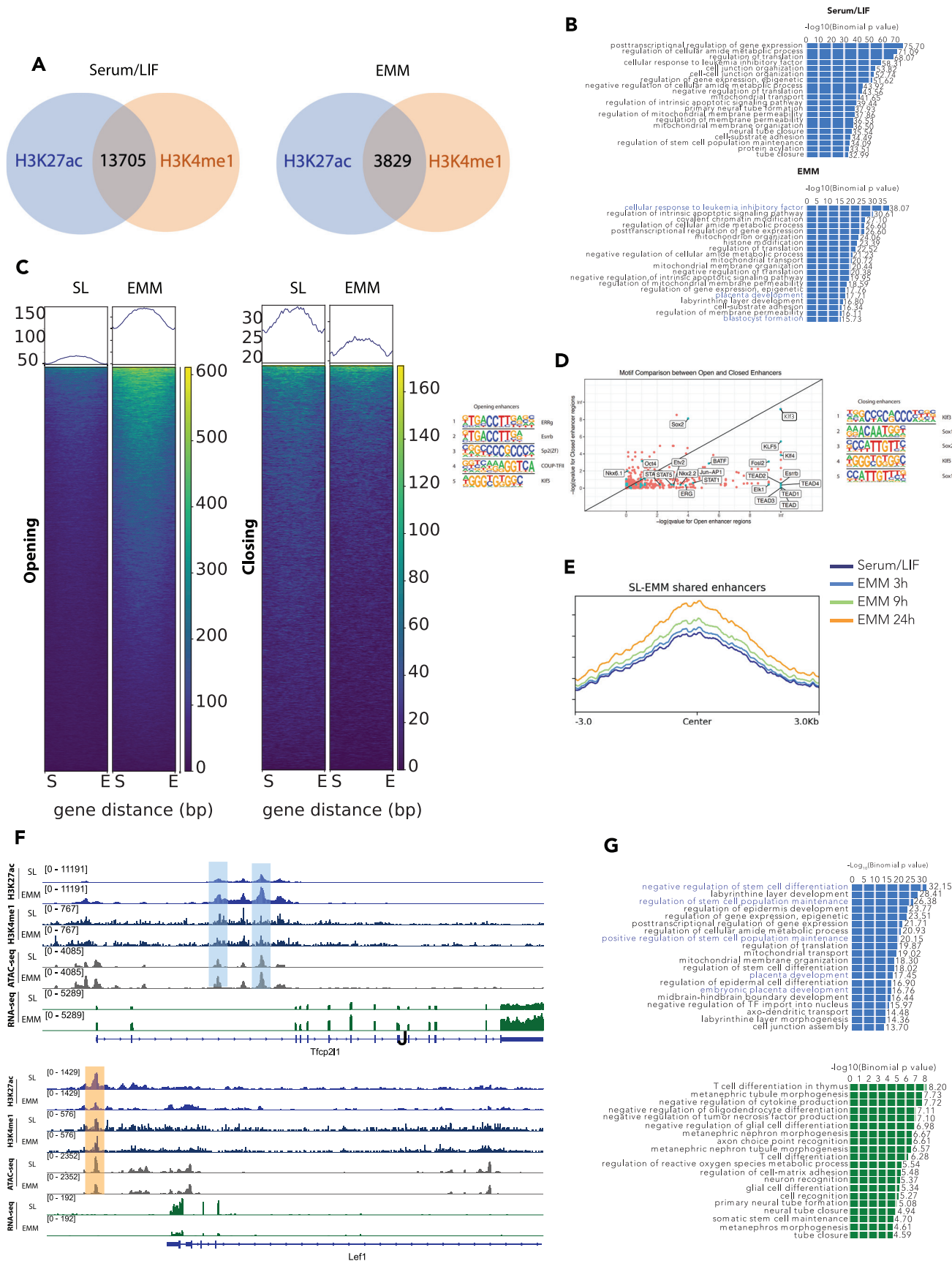


Expanded View Figures

Figure EV1. Differentiation potential characteristics of EMESCs and effects of galactose on pre-implantation development.

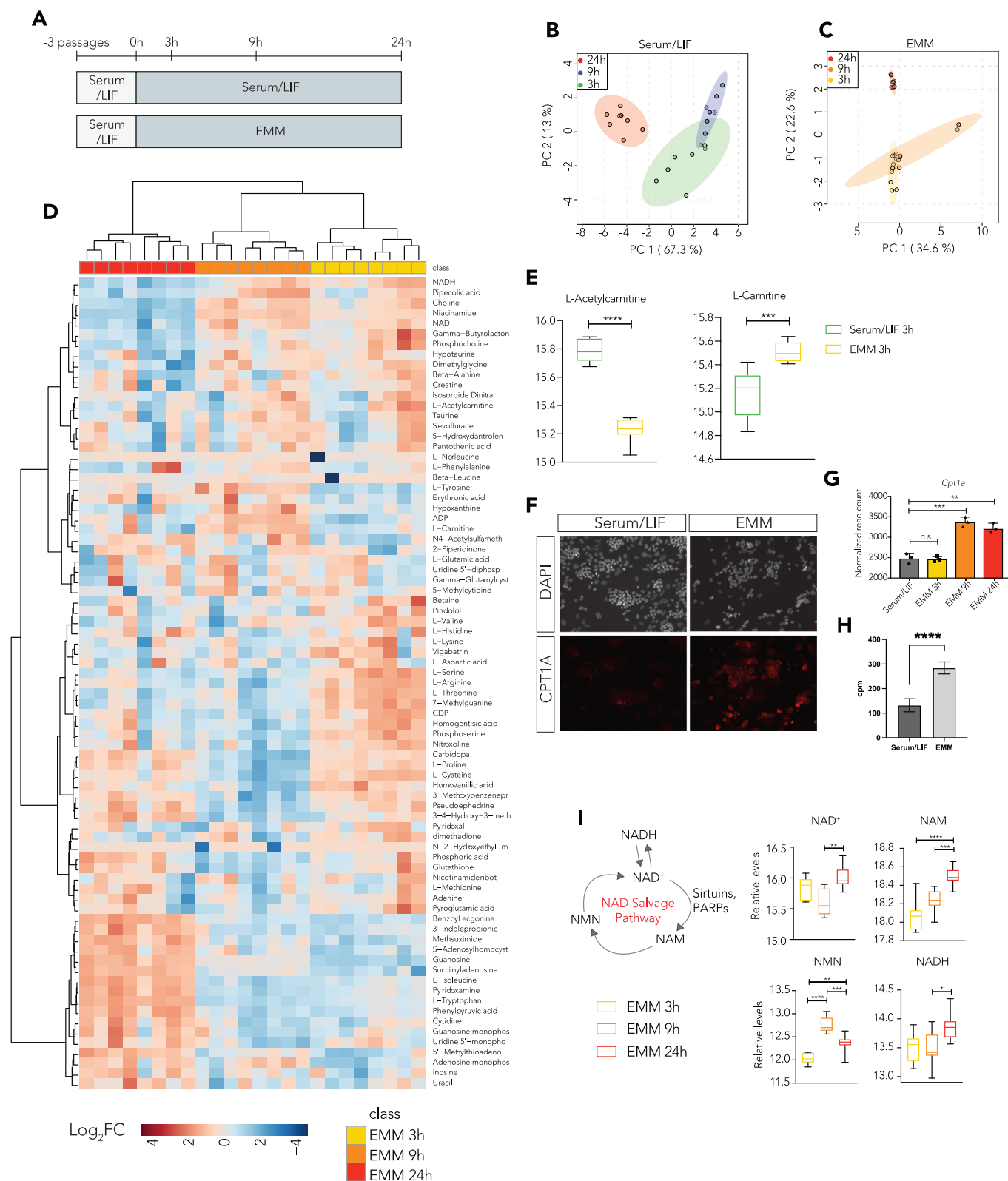
(A) Western blot analysis of SL-, EMM- and 2i/LIF-cultured mESCs. $n = 6$ biologically independent samples. Data are mean + s.d., unpaired two-tailed t test. P values: Active B-CATENIN SL vs EMM n.s.=0.8464, EMM vs 2i/LIF $^{**}P = 0.0040$; pYAP1 SL vs EMM $^{*}P = 0.0124$, EMM vs 2i/LIF n.s.=0.0559; pERK SL vs EMM $^{****}P < 0.0001$, EMM vs 2i/LIF $^{****}P < 0.0001$; pAKT SL vs EMM $^{****}P < 0.0001$, EMM vs 2i/LIF $^{*}P = 0.0148$; pSTAT3 SL vs EMM $^{**}P = 0.0069$, EMM vs 2i/LIF n.s. $P = 0.7369$. (B) RT-qPCR analysis of pluripotency genes *Oct4*, *Nanog* and *Tcfp2l1*; PrE genes *Gata4*, *Sox7* and *cMyc*; and TE genes *Gata3* and *Eomes*. $n = 3$ biologically independent samples. Data are mean + s.d., unpaired two-tailed t test. P values: *Oct4* n.s.=0.4303; *Nanog* n.s.= 0.0729; *Tcfp2l1* n.s.= 0.1515; *cMyc* $^{**}P = 0.0053$; *Gata4* $^{**}P = 0.0097$; *Sox7* $^{*}P = 0.0159$; *Eomes* $^{*}P = 0.0297$; *Gata3* $^{*}P = 0.0380$. (C) RT-qPCR analysis of pluripotency genes *Nanog* and *Oct4*, and neural genes *Nestin* and *Zfp521* at d0 and d7 of neural differentiation. ESCs and EMESCs were cultured in Serum/LIF or EMM respectively for 2 passages prior to d0. Data are mean + s.d., $n = 3$ biologically independent samples, unpaired two-tailed t test. P values: *Nestin* n.s.=0.2267; *Zfp521* n.s.= 0.1027. (D) RT-qPCR analysis of pluripotency gene *Oct4* and PrE genes *Gata4*, *Gata6* and *Pdgfra* at d0, d4 and d7 of PrE differentiation. ESCs and EMESCs were cultured in Serum/LIF or EMM respectively for 2 passages prior to d0. Data are mean + s.d., $n = 3$ biologically independent samples, unpaired two-tailed t test. P values: *Gata6* $^{**}P = 0.0084$; *Pdgfra* $^{**}P = 0.0050$. (E) RT-qPCR analysis of pluripotency genes *Nanog* and *Oct4*, and TSC genes *Gata3* and *Elf5* at d0 and d7 of TSC differentiation. ESCs and EMESCs were cultured in Serum/LIF or EMM respectively for 2 passages prior to d0. Data are mean + s.d., $n = 3$ biologically independent samples, unpaired two-tailed t test. *Gata3* $^{**}P = 0.0011$; *Elf5* $^{*}P = 0.0389$. (F) Brightfield images of mESCs after 10 passages cultured in Serum/LIF, EMM, and 2i/LIF. Scale bar = 100 μ m. (G-I) Comparative expression of apoptosis (G), embryonic (H) and extra-embryonic (I) genes from transcriptomic data in mESCs cultured for 10 and 20 passages in Serum/LIF, EMM, and 2i/LIF. $n = 3$ biologically independent samples. Data are mean + s.d., unpaired two-tailed t test. P values: *Bad* SL vs 2i/LIF P20 $^{**}P = 0.0245$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Bak1* EMM vs 2i/LIF P10 $^{**}P = 0.0012$, SL vs EMM P20 $^{**}P = 0.0012$, SL vs 2i/LIF P20 $^{**}P = 0.0039$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Bid* SL vs 2i/LIF P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs EMM P20 $^{****}P < 0.0001$, SL vs 2i/LIF P20 $^{****}P < 0.0001$; *Caspase3* EMM vs 2i/LIF P10 $^{*}P = 0.03913$, SL vs 2i/LIF P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Caspase9* SL vs 2i/LIF P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs 2i/LIF P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Tnfrsf1a* SL vs 2i/LIF P10 $^{***}P = 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs 2i/LIF P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Nanog* SL vs 2i/LIF P10 $^{*}P = 0.0180$, EMM vs 2i/LIF P10 $^{**}P = 0.0022$, SL vs EMM P20 $^{***}P = 0.0007$, SL vs 2i/LIF P20 $^{**}P = 0.0086$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Nr0b1* SL vs EMM P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs 2i/LIF P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Pou5f1* SL vs EMM P10 $^{***}P = 0.0009$, EMM vs 2i/LIF P10 $^{***}P = 0.0002$; *Sall4*, SL vs EMM P10 $^{****}P < 0.0001$, SL vs 2i/LIF P10 $^{***}P = 0.0007$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs EMM P20 $^{**}P = 0.0050$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Sirt1* SL vs EMM P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs EMM P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Sox2* SL vs EMM P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs EMM P20 $^{****}P = 0.0001$, EMM vs 2i/LIF P20 $^{****}P = 0.0001$; *Col4a1*, SL vs EMM P10 $^{****}P < 0.0001$, SL vs 2i/LIF P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs EMM P20 $^{**}P = 0.0013$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Col4a2*, SL vs EMM P10 $^{**}P = 0.0013$, SL vs 2i/LIF P10 $^{***}P = 0.0006$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs 2i/LIF P20 $^{**}P = 0.0037$, EMM vs 2i/LIF P20 $^{**}P = 0.0024$; *Dusp4*, SL vs 2i/LIF P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{****}P < 0.0001$, SL vs EMM P20 $^{****}P < 0.0001$, SL vs 2i/LIF P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Gata6*, SL vs 2i/LIF P20 $^{****}P = 0.0081$, EMM vs 2i/LIF P20 $^{****}P = 0.0037$; *Lrp2*, SL vs EMM P10 $^{*}P = 0.0131$, SL vs 2i/LIF P10 $^{****}P < 0.0001$, EMM vs 2i/LIF P10 $^{*}P = 0.0113$, SL vs EMM P20 $^{****}P < 0.0001$, EMM vs 2i/LIF P20 $^{****}P < 0.0001$; *Sox7*, SL vs EMM P10 $^{**}P = 0.0015$, EMM vs 2i/LIF P10 $^{***}P = 0.0003$, SL vs 2i/LIF P20 $^{**}P = 0.0099$, EMM vs 2i/LIF P20 $^{***}P = 0.0006$. (J) Representative image of mouse litter, star indicates mice with contribution (left); Table depicting contribution efficiency of mESCs (two different cell lines) cultured in SL or EMM in generating chimeric mice from injected blastocysts (right).





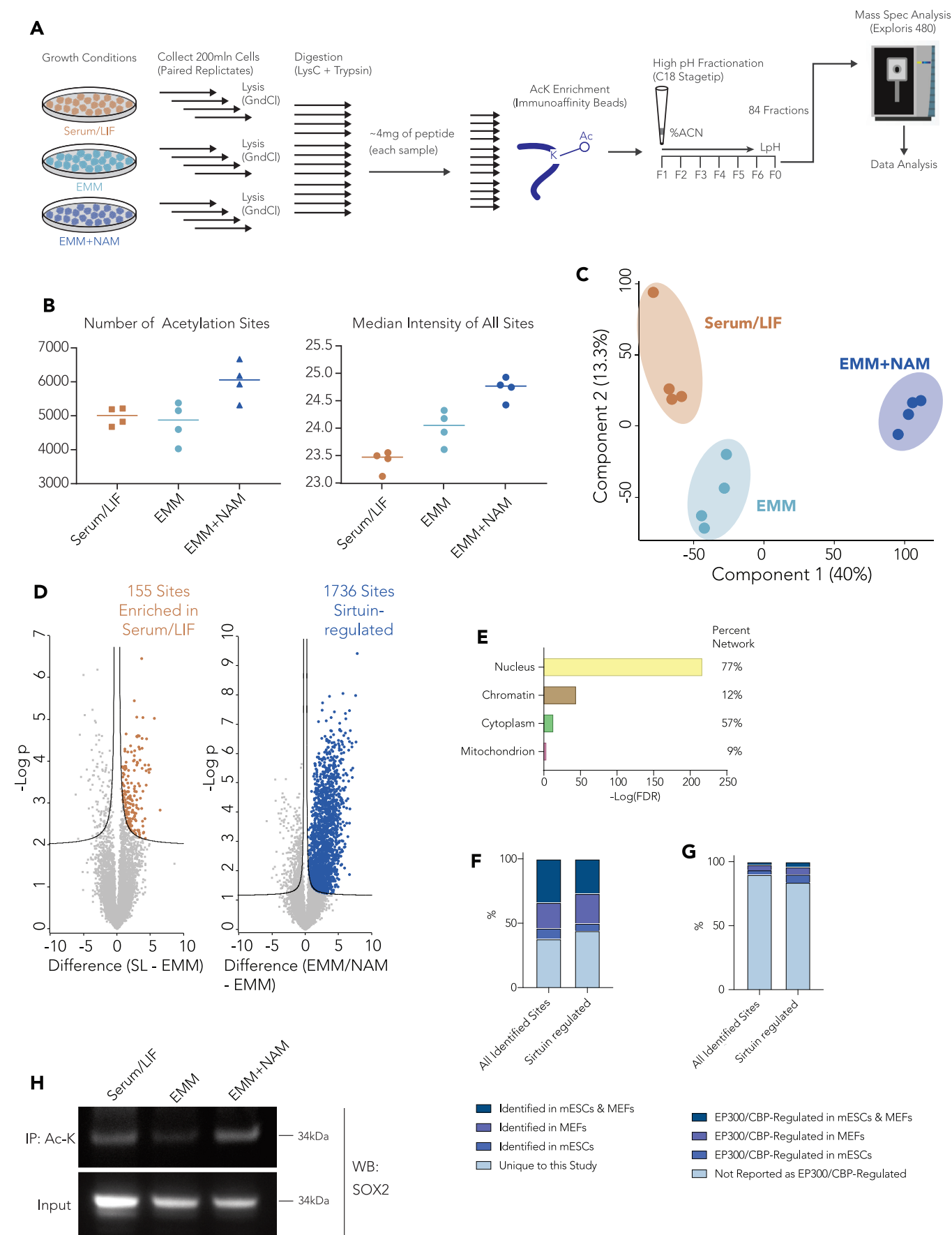
◀ **Figure EV2. Further characterization of the enhancer landscape of EMM-cultured ESCs.**

(A) Overlap of H3K27ac and H3K4me1 CUT&Tag peaks, defining enhancers in Serum/LIF and EMM. (B) GO analysis for Biological Processes of genes associated with enhancers defined by overlapping peaks for H3K27ac and H3K4me1 in ESCs cultured in Serum/LIF (top) and EMM (bottom). Discussed GO terms highlighted in blue. (C) Heatmaps and profiles depicting H3K27ac intensity in both Serum/LIF and EMM conditions at opening and closing enhancers. (D) Motif enrichment analysis of Opening versus Closing enhancers. (E) ATAC-seq time course metaprofiles of SL-EMM shared enhancers. (F) Genome browser tracks (IGV 2.14.0 software) of CUT&Tag for H3K27ac and H3K4me1, ATAC-seq and RNA-seq at the *Tfcp2l1* locus (top) and *Lef1* locus (bottom) at 0 h and 24 h of EMM treatment. Blue boxes depict opening enhancers, orange box depicts a closing enhancer. (G) GO analysis for Biological Processes of genes associated with ATAC-upregulated (top) and -downregulated (bottom) TE regions after 24 h EMM culture (noted processes in blue font).



◀ **Figure EV3. Downstream responses to EMM metabolic phenotypes and the enzymes propagating them.**

(A) Experimental outline for Serum/LIF and EMM time course used for metabolomic analysis. (B) PC1 vs 2 for Serum/LIF-cultured ESCs at 3 h, 9 h and 24 h in culture. (C) PC1 vs 2 for EMM-cultured ESCs at 3 h, 9 h and 24 h in culture. (D) Heatmap of enriched metabolites ($\text{Log}_2\text{FC} < 1$, $P_{\text{adj}} < 0.05$) in Serum/LIF-cultured ESCs versus all time points during EMM culture, $n = 8$ technical replicates per sample. (E) Levels of L-Acetylcarnitine (left) and L-Carnitine (right) in Serum/LIF and EMM-cultured ESCs after 3 h culture. P values: L-Acetylcarnitine **** $P < 0.0001$; L-Carnitine *** $P = 0.0004$, unpaired two-tailed t test, $n = 8$ technical replicates per sample. (F) Immunostaining for CPT1A in Serum/LIF and EMM-cultured ESCs after 24 h. Data are representative of 3 biological replicates. Scale bar = 40 μm . (G) Normalized counts from RNA-seq for *Cpt1a* in Serum/LIF and EMM-cultured ESCs after 3 h, 9 h and 24 h in the indicated condition. P values: n.s.=0.846 *** $P = 0.0008$, ** $P = 0.0015$, unpaired two-tailed t test, $n = 3$ biologically independent samples. (H) Fatty acid oxidation assay, measuring metabolism of C^{14} -labeled palmitic acid in SL- and EMM-cultured ESCs after 24 h, representative of 3 independent experiments. Data was normalized to protein levels. **** $P < 0.0001$, unpaired two-tailed t test. (I) Schematic for NAD^+ salvage pathway with individual plots for the depicted metabolites after 3 h, 9 h, 24 h in EMM culture. P values: NAD^+ 9 h vs 24 h ** $P = 0.001$; NAM 3 h vs 24 h **** $P < 0.0001$, 9 h vs 24 h *** $P = 0.0003$; NMN 3 h vs 9 h **** $P < 0.001$, 3 h vs 24 h ** $P = 0.0012$, 9 h vs 24 h *** $P = 0.0009$; NADH 9 h vs 24 h * $P = 0.0145$; unpaired two-tailed t test, $n = 8$ technical replicates per sample.



◀ **Figure EV4. Further characterization of acetylome of EMM-cultured ESCs.**

(A) The experimental setup to determine differential acetylation in ESCs cultured in Serum/LIF, EMM and EMM + NAM for 24 h. $n = 4$ experimentally independent samples. (B) Total number of acetylated sites detected in samples (left); Median intensity of all acetylated sites detected in samples (right). (C) PCA plot of acetylated sites in different samples. (D) Volcano plots depicting significantly differentially acetylated sites (FDR 5%) between EMM and Serum/LIF (left) and between EMM and EMM + NAM (right). (E) GO Cellular Component Enrichment table for sites deacetylated in EMM, with percent network. (F) Bar chart depicting percentage of all identified sites (6733) and that are also identified in datasets from (Weinert et al, 2018; Narita et al, 2021). (G) Bar chart depicting percentage of all identified sites (6733) and the overlap between the Sirtuin-regulated sites we defined (1731) and those regulated by EP300/CBP in datasets from (Weinert et al, 2018; Narita et al, 2021). (H) IP for Acetylated lysine and Western blot analysis of SOX2 in ESCs cultured for 24 h in Serum/LIF and EMM, +/- NAM. Data are representative of 3 biologically independent samples.

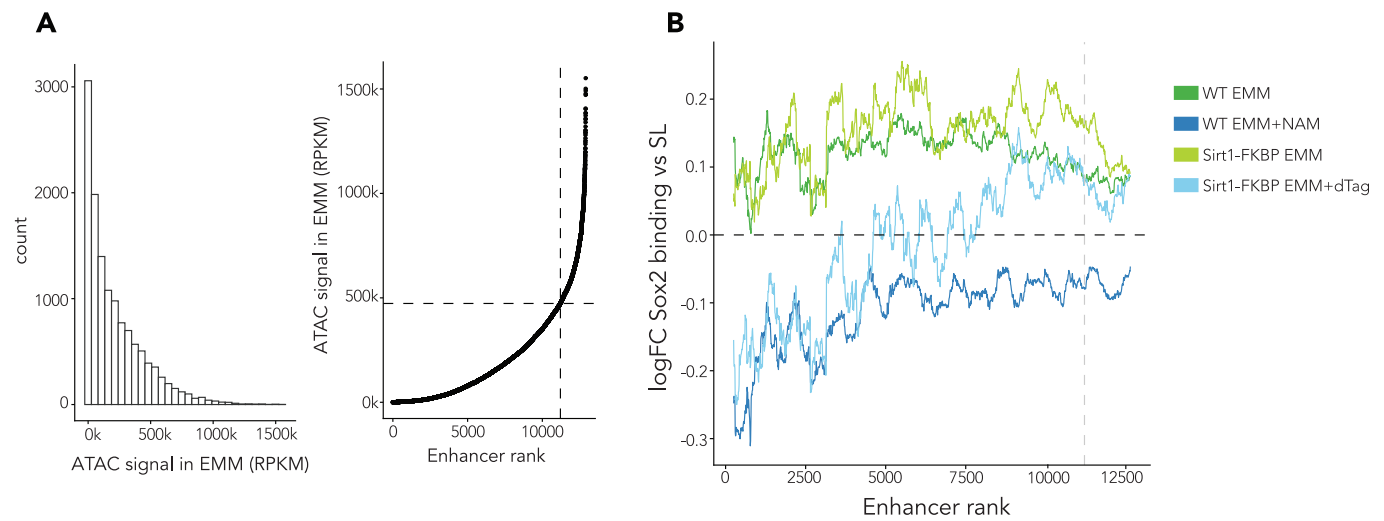


Figure EV5. Extra analysis of SIRT1 deacetylation of SOX2, and of SOX2 CUT&Tag in Sirt1-FKBP cell lines.

(A) Histogram ATAC signal count showing the number of SL-EMM merged enhancers plotted against ATAC signal in EMM (left), and enhancers ranked by ATAC signal (right). (B) Sliding window analysis of SOX2 signal pileup at enhancers ranked by ATAC signal in EMM, with the signal normalized to SL (for WT samples) and SL GFP+ (Sirt1-FKBP samples).