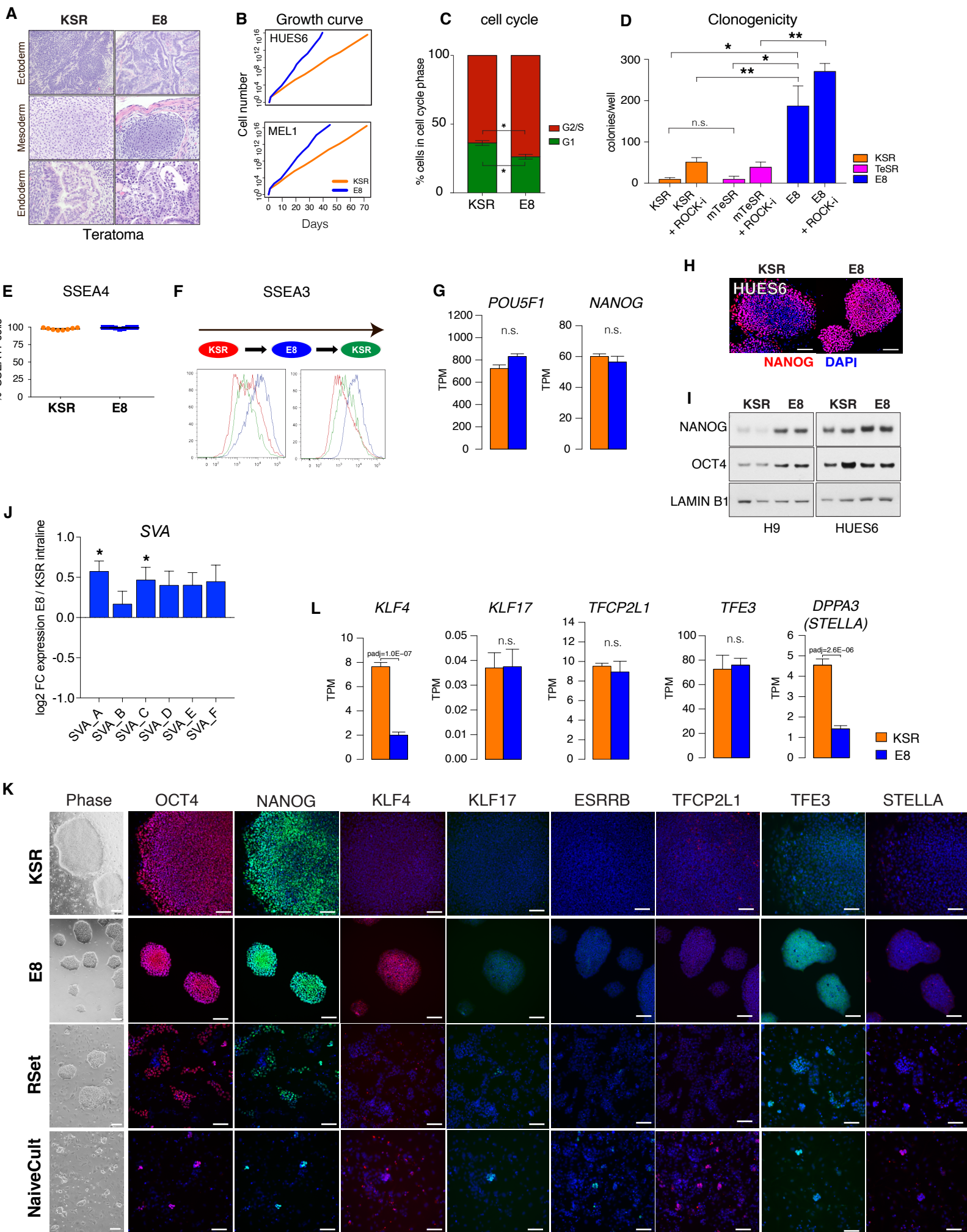


## Figure S1, Related to Figure 1

### Germlayer differentiation propensity of hPSC in KSR, E8 and mTeSR medium. Validation of newly derived hiPSC.

**A.** Immunofluorescence of neuroectodermal (NE) precursor markers SOX1 and PAX6 at day 10 of neural induction in HUES6 and MEL1 hESC in KSR or E8. **B.** Immunofluorescence of OCT4 and NANOG pluripotency markers in newly reprogrammed iPSC lines 706 and 731. **C.** Immunofluorescence of neuroectodermal (NE) precursor markers SOX1 (red) and PAX6 (green) at day 10 of neural induction in 706 and 731 hiPSC lines in KSR or E8. **D.** Immunofluorescence of PAX6 neuroectodermal marker in 3 independent hPSC lines (H9, SeV, TLR3) grown KSR and differentiated in E6 medium or grown in E8 and differentiated in KSR medium at day 10 of neural induction. **E.** Immunofluorescence of SOX1 and PAX6 at day 10 of neural induction in H9, HUES6, MEL1 hESC grown in mTeSR and differentiated in either KSR or E6-based medium. **F.** Percentage of cells positive for endoderm (ENDO) marker SOX17 and mesoderm (MESO) marker T at day 10 of directed differentiation towards ENDO (top) or MESO (bottom), respectively of KSR versus E8 hESC (left).  $n \geq 7$  wells per each line (H9, HUES6, MEL1) were quantified. Relative expression of SOX17 and T in day 7 embryoid bodies (EB) generated from KSR or E8 hPSC (middle)  $n=3$  replicates per each hESC line (H9, HUES6, MEL1). Relative expression of SOX17 and T in undifferentiated cells (right).  $n=3$  replicates per each hESC line (H9, HUES6, MEL1). Mean values and SEM of three independent hESC lines H9, Hues6, MEL1 are shown. **A.-E.** Scale bars 100  $\mu\text{m}$ , p-values (two tailed t-test): \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, \*\*\*\* < 0.0001



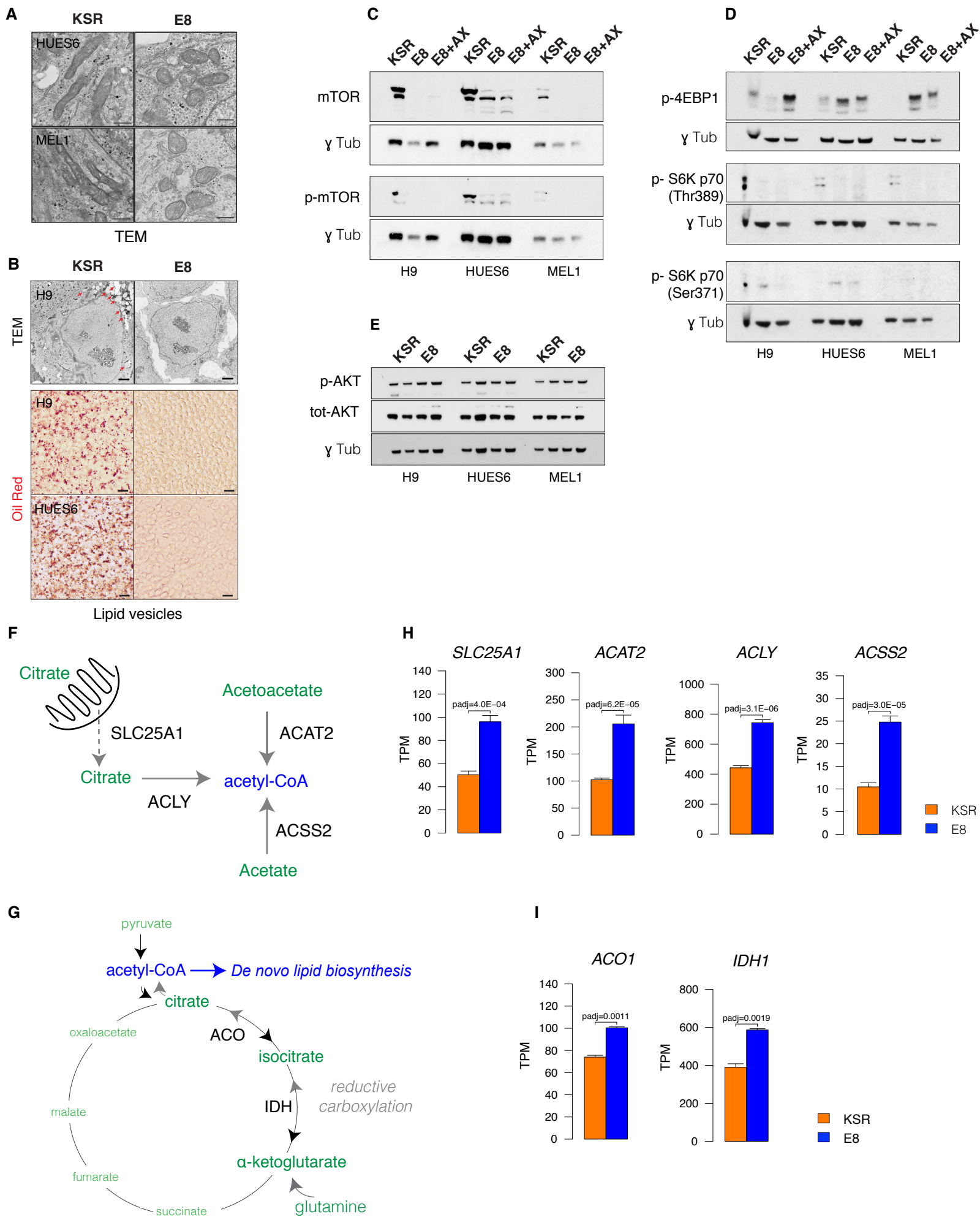


## Figure S2, Related to Figure 2

### General and naïve-associated pluripotency features in KSR and E8 hPSC.

**A.** Representative images of teratoma assay from KSR or E8 hESC (HUES6). **B.** Growth curve of hESC (HUES6, MEL1) grown in KSR or E8. **C.** Flow cytometry analysis of cell cycle distribution of KSR or E8 hPSC. n=9 **D.** Quantification of colony formation after single cell passaging of KSR, mTeSR or E8 hPSC without or with Rock-inhibitor drug (+ROCK-i). Mean values and SEM of 3 technical replicates per hESC line H9, HUES6, MEL1 are shown. KSR and E8 values are same as shown in Figure 2D. **E.** Flow cytometry quantification of SSEA4 positive cells in KSR and E8 hPSC. Single dots represent technical replicates from H9, HUES6, MEL1 hESC. **F.** Flow cytometry histogram of SSEA3 intensity in hESC grown in KSR (red), adapted to E8 (blue) and re-adapted to KSR (green). **G.** Expression level (RNA-seq) of OCT4 (POU5F1), NANOG; **H.** Immunofluorescence staining of NANOG in KSR or E8 hPSC (HUES6) **I.** Western blot of NANOG, OCT4 and loading control LAMIN B1 in KSR and E8 hPSC (H9, HUES6) **J.** Log2 fold change expression E8/KSR within each cell line of SVA transposable elements. P-value was calculated on triplicate values from all 3 cell lines (H9, HUES6, MEL1) in E8 vs. KSR. **K.** Immunofluorescence of core pluripotency and naïve markers in KSR, E8, RSet and NaiveCult H9 hESC. Scale bar bright field images 50  $\mu$ m. Scale bars fluorescence images 100  $\mu$ m L. Expression level (RNA-seq) of KLF4, KLF17, TFCEP2L1, TFE3, DPPA3 (STELLA) (padj determined by DESeq2). p-values (two tailed t-test): \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, \*\*\*\* < 0.0001. tailed t-test): \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, \*\*\*\* < 0.0001



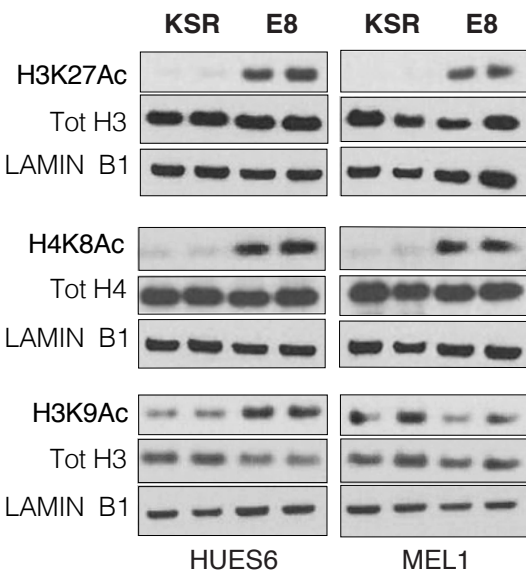


### Figure S3, Related to Figure 4

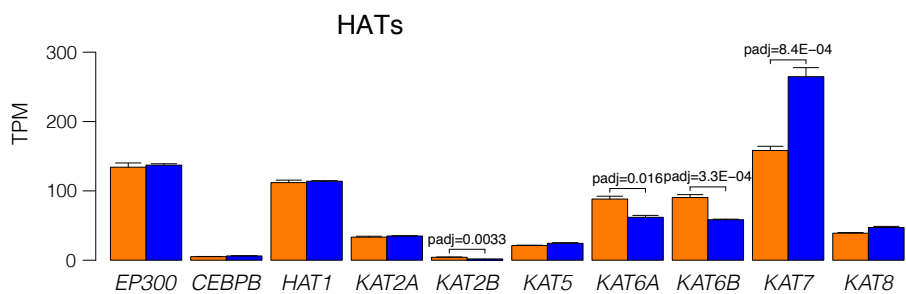
#### **E8 vs. KSR hPSC comparison of mitochondrial morphology, intracellular lipid storage, mTOR activation state and expression levels of genes involved in the generation of lipogenic precursor acetyl-coA.**

**A.** Transmission electron microscopy (TEM) images of mitochondrial morphology in KSR and E8 hESC (HUES6, MEL1). Scale bar 0.5  $\mu\text{m}$ . **B.** TEM images of KSR and E8 hESC (H9). Red arrows indicate fat droplets (top). Scale bar 2  $\mu\text{m}$ . Oil Red staining of intracellular lipids (bottom) in KSR and E8 hESC (H9, HUES6). Scale bar 20  $\mu\text{m}$  **C, D.** Western blot of mTOR signaling (total mTOR, activated p(phosphor)-mTOR) and downstream effectors p(hospho)-4EBP1 and p(hospho)-S6K p70 (Thr389/Ser371) in KSR, E8 and E8 + Albumax hESC. **E.** Western blot of AKT signaling (total AKT, activated p(hospho)-AKT in E8 and KSR hESC. **F.** Scheme of enzymatic pathways of acetyl-coA generation. **G.** Scheme of acetyl-coA generation through canonical pathways (pyruvate) or via reductive carboxylation (glutamine) **H, I.** Expression level (RNA-seq) of enzymes involved in canonical (H) or reductive carboxylation-mediated (I) acetyl-CoA production SLC25A1, ACLY, ACAT2, ACSS2 (H) and ACO1, IDH1 (I) (p<sub>adj</sub> determined by DESeq2).

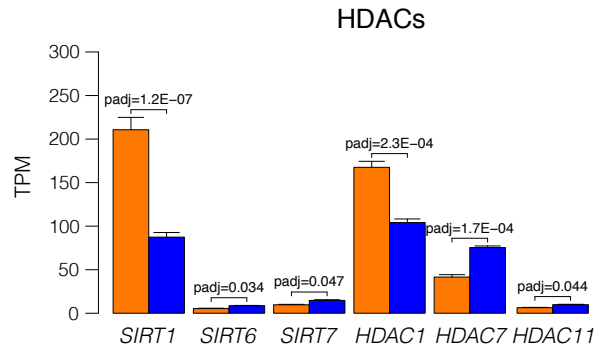
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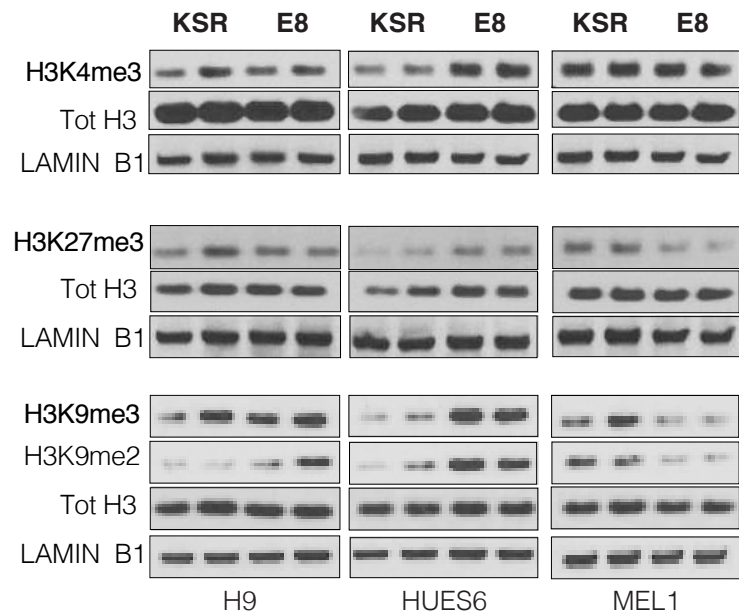
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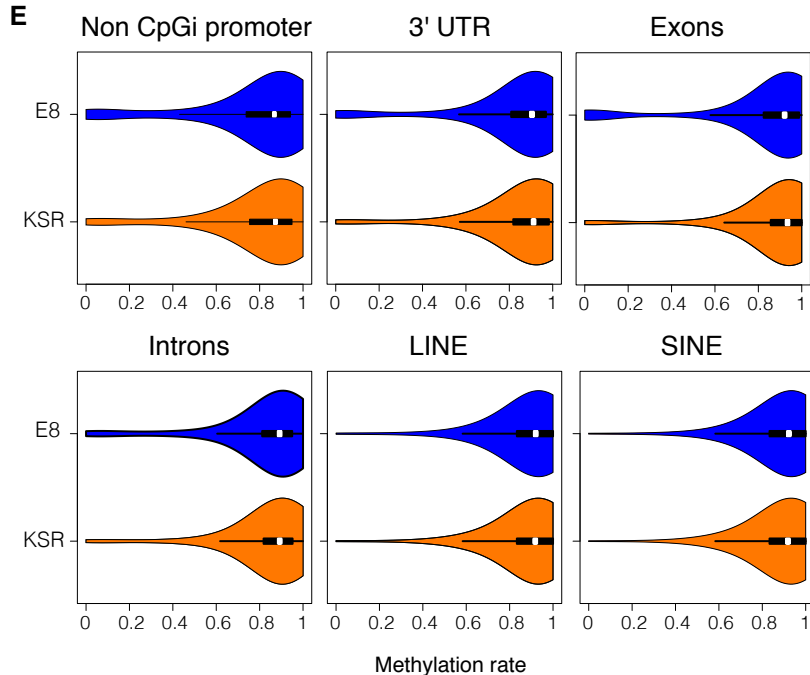
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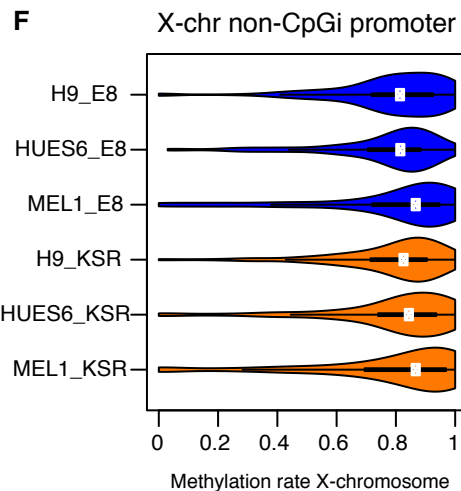
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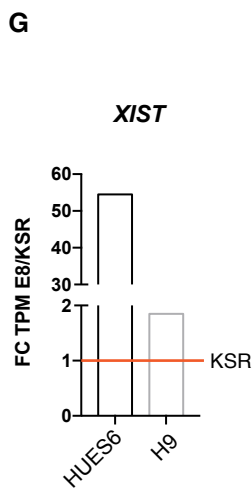
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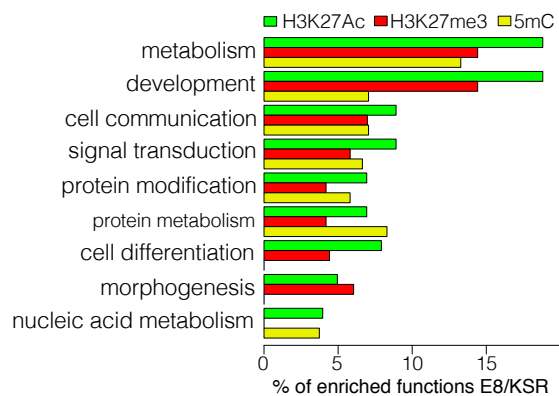
**F**



**G**



**H**





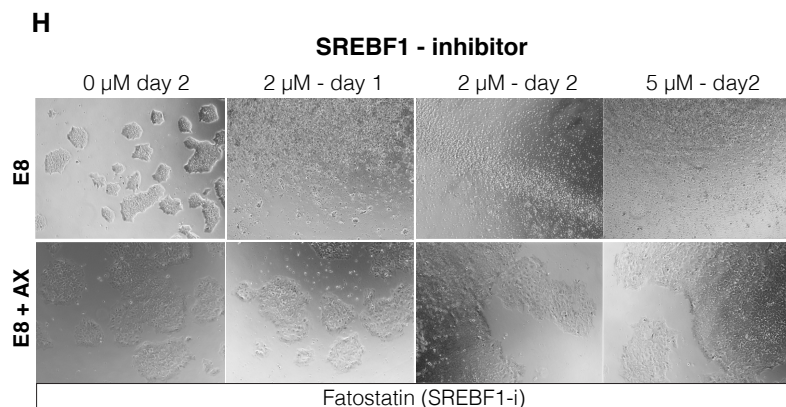
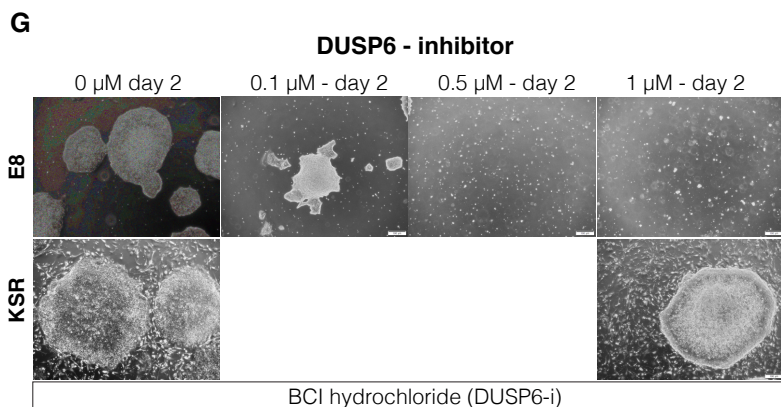
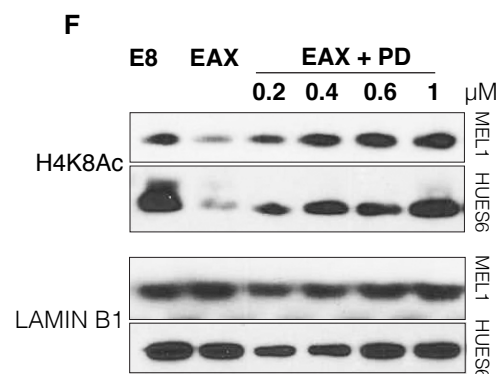
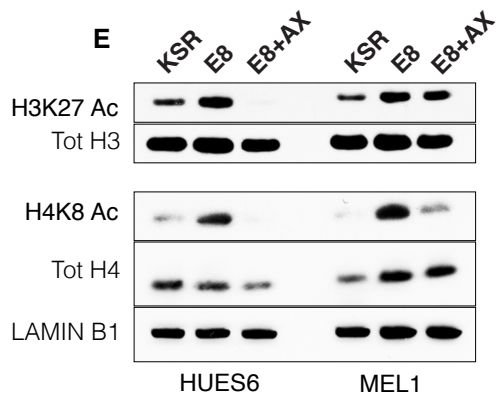
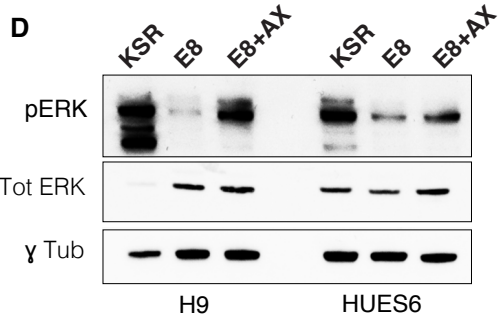
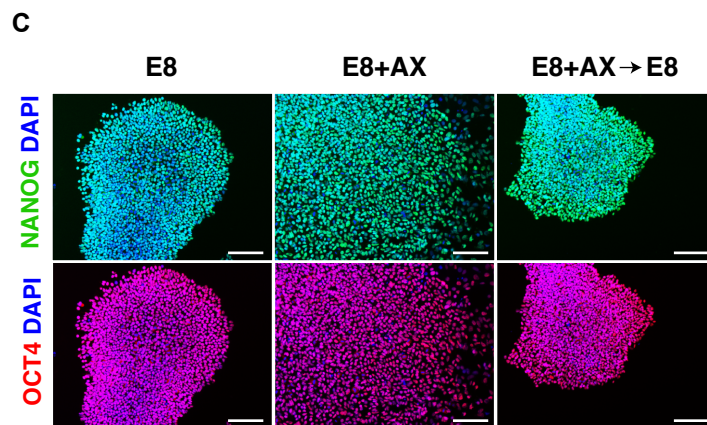
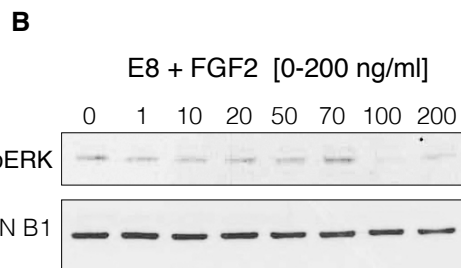
## Figure S4, Related to Figure 5

### Epigenomic characterization of E8 vs. KSR hPSC.

**A.** Western blot of H3K27Ac, H4K8Ac, H3K9Ac levels in KSR and E8 hESC (HUES6, MEL1). **B., C.** Expression level (RNA-seq) of histone acetyl transferase (HAT, (B)) and histone deacetylase (HDAC; (C)) genes in KSR and E8 hESC. Mean values and SEM of hESC H9, HUES6, MEL1 are shown. **D.** Western blot of H3K4me3, H3K27me3, H3K9me3 levels in KSR and E8 hESC. **E.** Violin plots of 5mC rate distribution at different genomic compartments in KSR and E8 hESC. (padj determined by DESeq2). **F.** Methylation rate distribution at non-CpGi promoters on X-chromosome in each analyzed cell line, E8 and KSR (XX indicates female and XY indicates male line). **G.** Fold change expression of XIST in E8 vs. KSR in two female lines H9 and HUES6. **H.** Top enriched functional groups (GO) of H3K27Ac, H3K27me3 and 5mC differentially enriched gene promoters between KSR and E8.

**A**

	KSR medium	E8 medium
Base medium	DMEM-F12	DMEM-F12
Core Supplement	KSR supplement: - ALBUMAX - aa* - trace elements - Vitamins: Thiamine* reduced glutathione ascorbic acid - Proteins: transferrin insulin	E8 supplement: Selenium - Vitamins: ascorbic acid - Proteins: transferrin insulin
additional supplements	FGF2 (10 ng/ml) β-ME L-Glutamine* NE-aa*	FGF2 (100 ng/ml) TGFβ
substrate	iMEF	Vitronectin
* contained in DMEM F12		

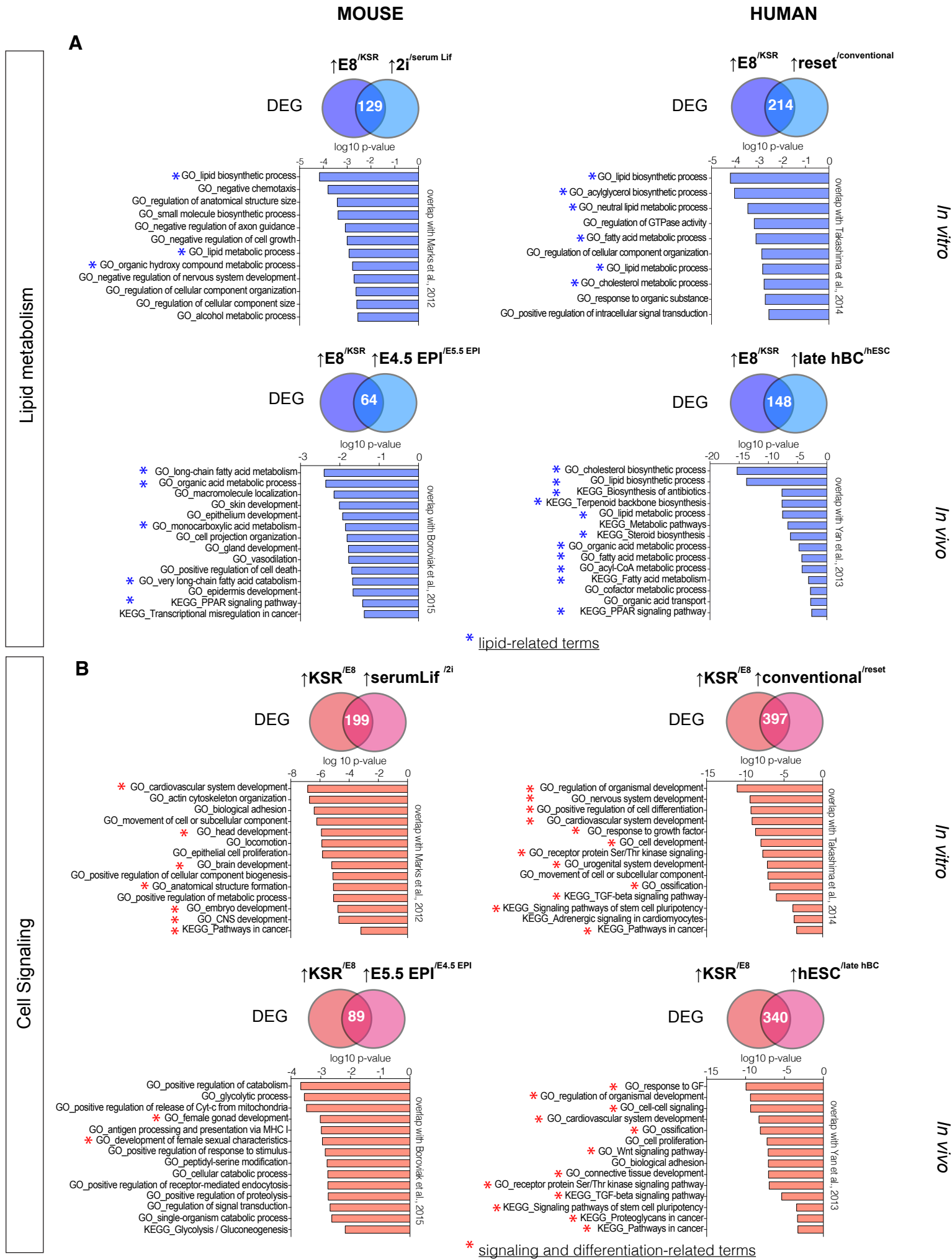


**Figure S5, Related to Figure 6**

**KSR vs. E8 medium composition, FGF2 concentration response and effect of Albumax treatment on ERK signaling and epigenetic marks; tolerance to DUSP6 and SREBF1 inhibition.**

**A.** KSR and E8 medium composition **B.** Western blot of phospho-ERK levels in E8 containing different concentrations of FGF2 (0, 1, 10, 20, 50, 100, 200 ng/ml). **C.** Immunofluorescence staining of NANOG and OCT4 in E8, E8+Albumax (E8+AX) and after Albumax withdrawal (E8+AX -> E8). Scale bar 200  $\mu$ m. **D.** Western blot of phospho-ERK levels in KSR, E8 and E8 + Albumax (E8 + AX) hESC (H9, HUES6). Loading controls ERK,  $\gamma$  Tubulin. **E.** Western blot of H3K27Ac and H4K8Ac levels in KSR, E8 and E8+AX hESC (H9). Loading controls: Total H3, H4 and LAMIN B1. **F.** Western blot of H4K8Ac levels in E8, E8+Albumax (EAX) and EAX + 0.2, 0.4, 0.6 or 1  $\mu$ M PD0325901. Loading control LAMIN B1. **G.** Bright field images of E8 and KSR hESC (H9) after a 2-day treatment with 0  $\mu$ M, 0.1  $\mu$ M, 0.5  $\mu$ M BCI hydrochloride (DUSP6 inhibitor) **H.** Bright field images of E8 and E8+Albumax (E8+AX) hESC (H9) after a treatment with 0  $\mu$ M, 2  $\mu$ M or 5  $\mu$ M Fatostatin (SREBF1 inhibitor) for 1 or 2 days. **C-F.** Length of treatment/withdrawal from treatment: 3 days.

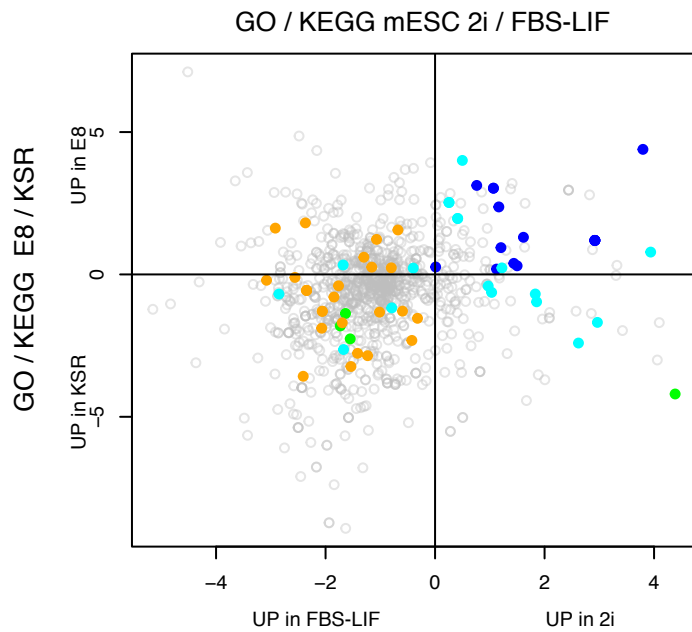




**Figure S6, Related to Figure 7**

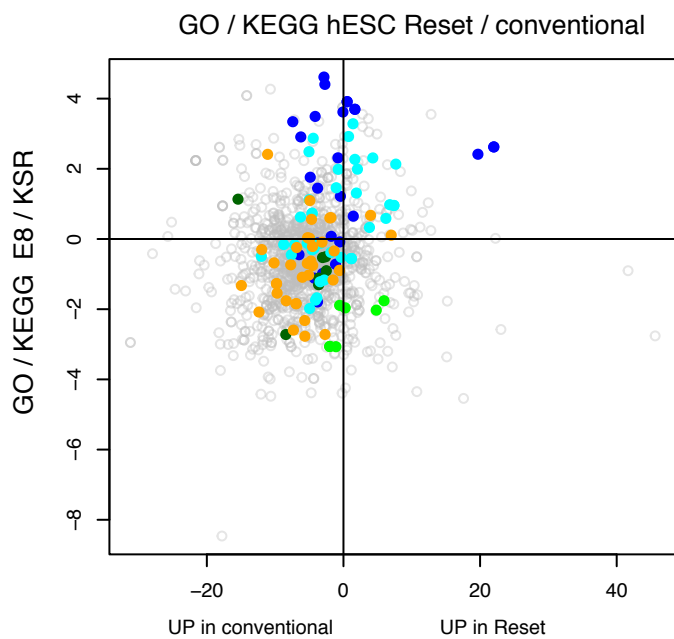
**Functional enrichment of shared upregulated genes between E8 vs. naïve and KSR vs. primed datasets.**

**A.** Top enriched functional categories (GO/KEGG) of common upregulated genes in E8/KSR and each naïve/primed datasets (Boroviak et al., 2015; Marks et al., 2012; Takashima et al., 2014; Yan et al., 2013). Blue asterisks indicate lipid-related categories. **B.** Top enriched functional categories (GO/KEGG) of common downregulated genes in E8/KSR and each naïve/primed datasets (Boroviak et al., 2015; Marks et al., 2012; Takashima et al., 2014; Yan et al., 2013). Red asterisks indicate signaling-related categories.

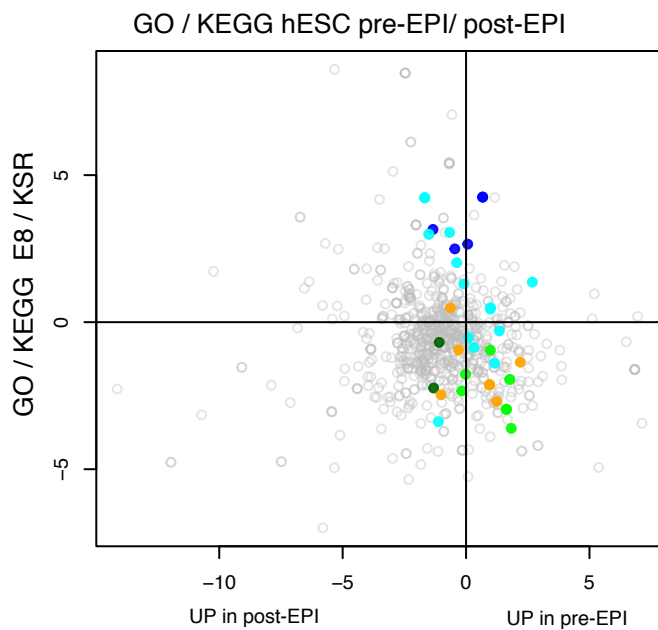


Marks et al., 2012

Lipid biosynthesis  
Lipid metabolism  
Lipid catabolism  
Response to lipid  
MAPK/TGFbeta signaling



Takashima et al., 2014



Boroviak et al., 2015



**Figure S7, Related to Figure 7**

**Gene function correlation plot of E8/KSR vs mouse naïve/primed datasets and E8/KSR vs. Reset/conventional hESC.**

2D-plot of lipid and signaling pathway regulation in E8/KSR and different naïve/primed datasets (Boroviak et al., 2015; Marks et al., 2012; Takashima et al., 2014)

## LIPID-RELATED GENE FUNCTIONS IN NAIVE AND PRIMED DATASETS

		<b>UP in E8</b>		<b>Tot genes</b>	
<b>Category</b>	<b>Term</b>	<b>nr. of genes</b>	<b>PValue</b>	<b>in category</b>	
KEGG_PATHWAY	Steroid biosynthesis	10	1.51E-05	20	
KEGG_PATHWAY	Biosynthesis of antibiotics	33	8.54E-04	212	
KEGG_PATHWAY	Fatty acid metabolism	11	0.006	48	
KEGG_PATHWAY	Terpenoid backbone biosynthesis	7	0.008	22	
KEGG_PATHWAY	Fatty acid biosynthesis	5	0.020	13	
KEGG_PATHWAY	Glycerolipid metabolism	11	0.022	58	
KEGG_PATHWAY	PPAR signaling pathway	12	0.024	67	
KEGG_PATHWAY	Glycerophospholipid metabolism	15	0.028	95	
KEGG_PATHWAY	Biosynthesis of unsaturated fatty acids	6	0.040	23	
GOTERM_BP_FAT	cholesterol biosynthetic process	21	2.04E-10	48	
GOTERM_BP_FAT	organic hydroxy compound metabolic process	60	7.99E-06	410	
GOTERM_BP_FAT	steroid metabolic process	44	1.97E-05	276	
GOTERM_BP_FAT	lipid biosynthetic process	77	1.61E-04	624	
GOTERM_BP_FAT	long-chain fatty-acyl-CoA biosynthetic process	12	3.59E-04	42	
GOTERM_BP_FAT	lipid metabolic process	139	8.81E-04	1330	
GOTERM_BP_FAT	acylglycerol metabolic process	20	0.003	118	
GOTERM_BP_FAT	neutral lipid metabolic process	20	0.003	119	
GOTERM_BP_FAT	low-density lipoprotein particle clearance	6	0.004	14	
GOTERM_BP_FAT	glycerolipid metabolic process	45	0.008	379	
GOTERM_BP_FAT	long-chain fatty acid metabolic process	15	0.012	90	
GOTERM_BP_FAT	regulation of plasma lipoprotein particle levels	11	0.013	56	

		<b>UP in 2i</b>		<b>Tot genes</b>	
<b>Category</b>	<b>Term</b>	<b>nr. of genes</b>	<b>PValue</b>	<b>in category</b>	
KEGG_PATHWAY	Glycosphingolipid biosynthesis - globo	8	5.87E-05	15	
KEGG_PATHWAY	Biosynthesis of antibiotics	33	2.04E-04	214	
KEGG_PATHWAY	Fatty acid metabolism	11	0.005	51	
KEGG_PATHWAY	Glycosphingolipid biosynthesis - lacto and neolacto	7	0.013	26	
KEGG_PATHWAY	Glycosphingolipid biosynthesis - ganglio	5	0.024	15	
KEGG_PATHWAY	Glycerophospholipid metabolism	14	0.028	94	
KEGG_PATHWAY	Fatty acid degradation	9	0.032	49	
GOTERM_BP_FAT	glycolipid metabolic process	24	7.94E-07	95	
GOTERM_BP_FAT	liposaccharide metabolic process	24	9.67E-07	96	
GOTERM_BP_FAT	lipid metabolic process	135	2.62E-05	1217	
GOTERM_BP_FAT	glycerophospholipid metabolic process	32	3.84E-05	184	
GOTERM_BP_FAT	glycerolipid metabolic process	43	6.49E-05	288	
GOTERM_BP_FAT	membrane lipid metabolic process	30	9.14E-05	175	
GOTERM_BP_FAT	lipid modification	32	1.07E-04	194	
GOTERM_BP_FAT	lipid biosynthetic process	65	6.88E-04	545	
GOTERM_BP_FAT	phospholipid dephosphorylation	10	9.32E-04	34	
GOTERM_BP_FAT	cellular lipid catabolic process	28	0.001	183	
GOTERM_BP_FAT	plasma lipoprotein particle organization	8	0.017	35	
GOTERM_BP_FAT	response to low-density lipoprotein particle	5	0.025	15	
GOTERM_BP_FAT	regulation of plasma lipoprotein particle levels	10	0.032	57	
GOTERM_BP_FAT	cholesterol efflux	8	0.048	43	
GOTERM_BP_FAT	regulation of phospholipid biosynthetic process	4	0.049	11	

		<b>UP in KSR</b>		<b>Tot genes</b>	
<b>Category</b>	<b>Term</b>	<b>nr. of genes</b>	<b>PValue</b>	<b>in category</b>	
GOTERM_BP_FAT	lipid metabolic process	154	5.64E-06	1330	
GOTERM_BP_FAT	lipid biosynthetic process	78	1.57E-04	624	
GOTERM_BP_FAT	lipid modification	40	1.75E-04	262	
GOTERM_BP_FAT	cellular response to lipid	65	3.25E-04	509	
GOTERM_BP_FAT	membrane lipid metabolic process	32	5.43E-04	204	
GOTERM_BP_FAT	fatty acid oxidation	19	5.92E-04	95	

		<b>UP in serum Lif</b>		<b>Tot genes</b>	
<b>Category</b>	<b>Term</b>	<b>nr. of genes</b>	<b>PValue</b>	<b>in category</b>	
KEGG_PATHWAY	Fatty acid biosynthesis	5	0.039	14	
KEGG_PATHWAY	Regulation of lipolysis in adipocytes	11	0.043	57	
KEGG_PATHWAY	Glycerolipid metabolism	11	0.047	58	

## UP in reset

Category	Term	nr. of genes	PValue	Tot genes in category
KEGG_PATHWAY	Biosynthesis of antibiotics	36	6.98E-03	212
GOTERM_BP_FAT	lipid metabolic process	173	7.59E-05	1330
GOTERM_BP_FAT	cellular lipid catabolic process	35	1.78E-04	182
GOTERM_BP_FAT	regulation of lipid metabolic process	47	5.98E-04	287
GOTERM_BP_FAT	regulation of plasma lipoprotein particle levels	15	8.12E-04	56
GOTERM_BP_FAT	acylglycerol metabolic process	24	0.001	118
GOTERM_BP_FAT	neutral lipid metabolic process	24	0.001	119
GOTERM_BP_FAT	glycerolipid metabolic process	57	0.001	379
GOTERM_BP_FAT	lipid catabolic process	46	0.002	294
GOTERM_BP_FAT	lipid biosynthetic process	84	0.003	624
GOTERM_BP_FAT	positive regulation of lipid metabolic process	23	0.005	126
GOTERM_BP_FAT	regulation of cholesterol homeostasis	6	0.006	13
GOTERM_BP_FAT	positive regulation of cholesterol efflux	6	0.009	14
GOTERM_BP_FAT	lipid homeostasis	22	0.009	124

## UP in late BC

Category	Term	nr. of genes	PValue	Tot genes in category
KEGG_PATHWAY	Terpenoid backbone biosynthesis	12	8.34E-07	22
KEGG_PATHWAY	Biosynthesis of antibiotics	41	4.46E-06	212
KEGG_PATHWAY	Steroid biosynthesis	8	1.29E-03	20
KEGG_PATHWAY	Fatty acid metabolism	12	3.03E-03	48
KEGG_PATHWAY	PPAR signaling pathway	13	1.56E-02	67
KEGG_PATHWAY	Sphingolipid metabolism	10	2.28E-02	47
GOTERM_BP_FAT	lipid biosynthetic process	92	8.52E-09	624
GOTERM_BP_FAT	lipid metabolic process	156	4.44E-07	1330
GOTERM_BP_FAT	sphingolipid metabolic process	28	8.33E-05	155
GOTERM_BP_FAT	phospholipid metabolic process	54	9.77E-05	393
GOTERM_BP_FAT	cellular lipid catabolic process	31	9.88E-05	182
GOTERM_BP_FAT	very-low-density lipoprotein particle assembly	6	1.43E-04	8
GOTERM_BP_FAT	lipid catabolic process	43	1.43E-04	294
GOTERM_BP_FAT	membrane lipid metabolic process	33	1.54E-04	204
GOTERM_BP_FAT	phospholipid biosynthetic process	34	2.87E-04	220
GOTERM_BP_FAT	fatty acid metabolic process	46	0.002	366
GOTERM_BP_FAT	response to lipoprotein particle	6	0.0111	18
GOTERM_BP_FAT	long-chain fatty acid import	4	0.014	7
GOTERM_BP_FAT	liposaccharide metabolic process	18	0.015	121
GOTERM_BP_FAT	lipid transport	37	0.015	312
GOTERM_BP_FAT	acyl-CoA metabolic process	15	0.019	96

## UP in conventional

Category	Term	nr. of genes	PValue	Tot genes in category
KEGG_PATHWAY	Regulation of lipolysis in adipocytes	16	3.27E-04	56
KEGG_PATHWAY	Sphingolipid signaling pathway	21	0.017	120

## UP in hESC

Category	Term	nr. of genes	PValue	Tot genes in category
KEGG_PATHWAY	Regulation of lipolysis in adipocytes	10	0.046	56



## UP in 4.5 EPI

Category	Term	nr. of genes	PValue	Tot genes in category
KEGG_PATHWAY	Biosynthesis of antibiotics	21	6.18E-04	214
KEGG_PATHWAY	Fatty acid metabolism	9	0.0011	51
KEGG_PATHWAY	Fatty acid degradation	7	0.016	49
KEGG_PATHWAY	PPAR signaling pathway	9	0.018	80
KEGG_PATHWAY	Sphingolipid metabolism	6	0.049	48
GOTERM_BP_FAT	cellular lipid catabolic process	24	3.08E-07	183
GOTERM_BP_FAT	lipid modification	23	3.18E-06	194
GOTERM_BP_FAT	lipid metabolic process	77	4.77E-06	1217
GOTERM_BP_FAT	lipid oxidation	16	5.04E-06	102
GOTERM_BP_FAT	glycerolipid metabolic process	25	1.90E-04	288
GOTERM_BP_FAT	fatty acid metabolic process	29	3.05E-04	371
GOTERM_BP_FAT	cholesterol efflux	8	9.31E-04	43
GOTERM_BP_FAT	very-low-density lipoprotein particle clearance	4	1.58E-03	7
GOTERM_BP_FAT	lipid biosynthetic process	34	3.97E-03	545
GOTERM_BP_FAT	regulation of plasma lipoprotein particle levels	8	4.89E-03	57
GOTERM_BP_FAT	regulation of phospholipid biosynthetic process	4	6.67E-03	11
GOTERM_BP_FAT	regulation of lipid metabolic process	22	7.26E-03	316
GOTERM_BP_FAT	acylglycerol metabolic process	11	7.64E-03	110
GOTERM_BP_FAT	neutral lipid metabolic process	11	8.64E-03	112
GOTERM_BP_FAT	plasma lipoprotein particle organization	6	8.86E-03	35
GOTERM_BP_FAT	lipoprotein metabolic process	12	8.94E-03	130
GOTERM_BP_FAT	protein-lipid complex subunit organization	6	1.25E-02	38
GOTERM_BP_FAT	negative regulation of fatty acid biosynthetic process	4	1.35E-02	14
GOTERM_BP_FAT	response to lipid	51	1.62E-02	991
GOTERM_BP_FAT	lipid homeostasis	11	1.87E-02	126

## UP in 5.5 EPI

Category	Term	nr. of genes	PValue	Tot genes in category
KEGG_PATHWAY	Biosynthesis of antibiotics	17	0.014	214
GOTERM_BP_FAT	positive regulation of lipid biosynthetic process	11	3.54E-03	79

Table S7, Related to Figure 7

Lipid-related gene functions upregulated in either naïve or primed datasets.