

Supplemental Information

GCN5 Regulates FGF Signaling and Activates Selective MYC Target Genes during Early Embryoid Body Differentiation

L. Wang, E. A. Kottmann, C. A. Ho, R. A. M. Cao, A. O. S. Kottmann, L. Y. Li, C. A. Kottmann, J. A. S. M. C. Bao, and S. A. O. Y. R. D.

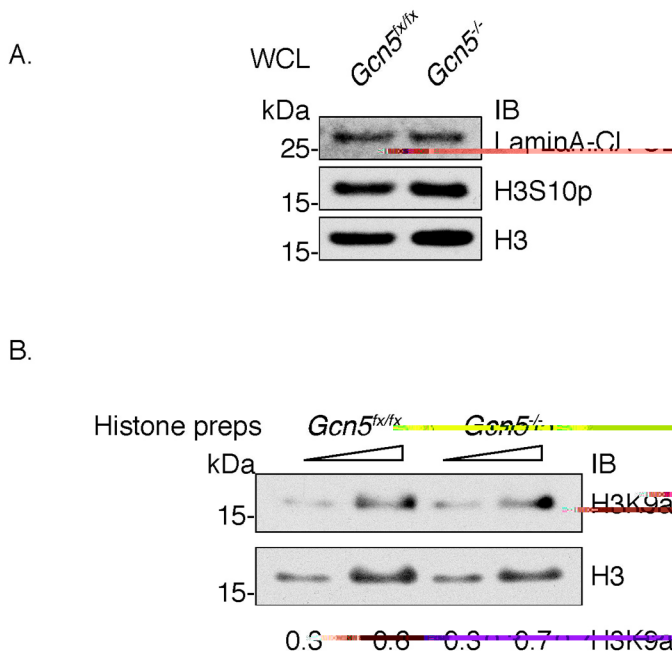
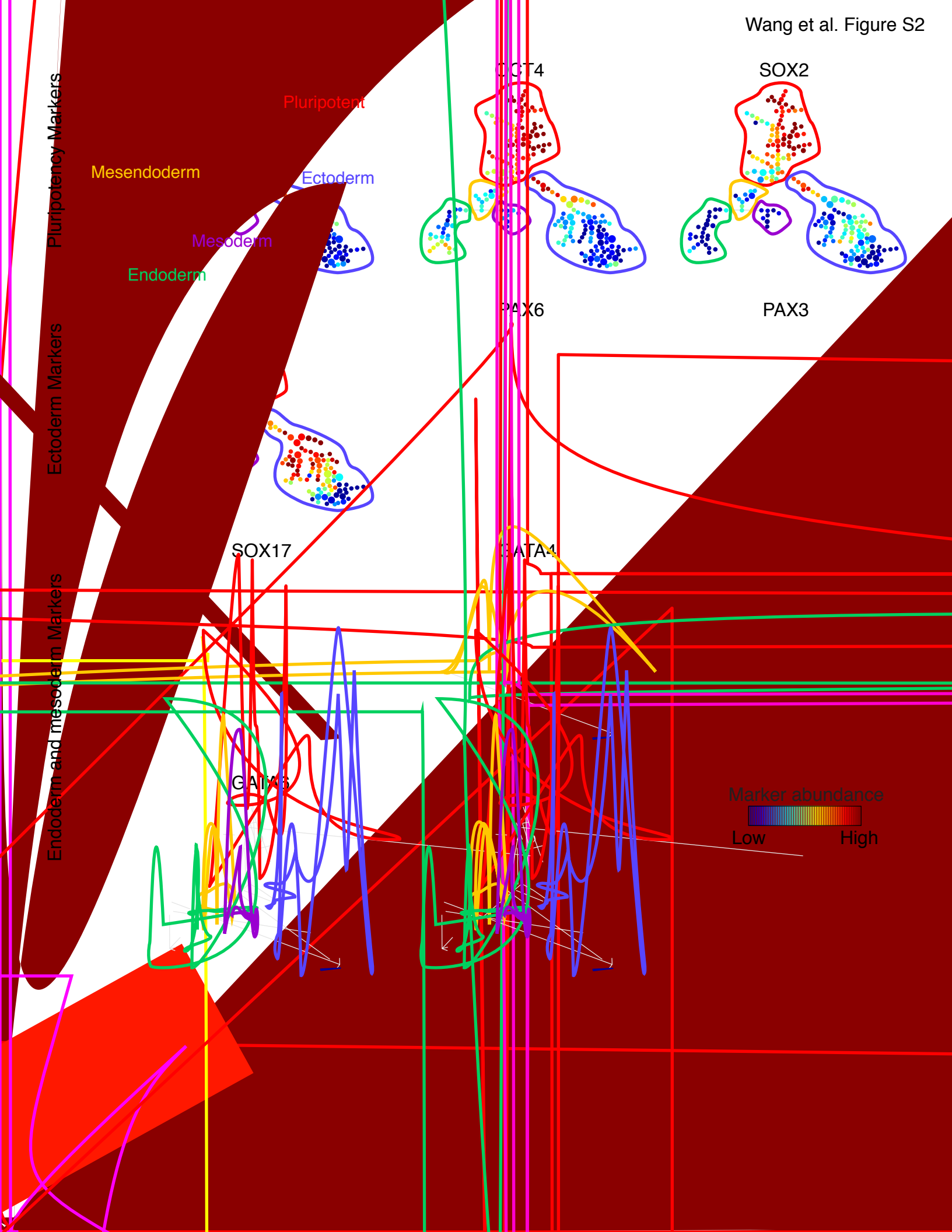


Figure S1 Loss of *Gcn5* does not cause over-representation of proliferating EBs at day 5.

(A) Immunoblots of mitotic marker (H3S10p) and apoptosis marker (cleaved LaminA/C) showing no differences between *Gcn5^{flx/flx}* and *Gcn5^{-/-}* EBs at day 5. WCL, whole cell lysates.

(B) Immunoblot of H3K9ac in day 5 EBs demonstrated no global changes upon loss of *Gcn5* at early stage of differentiation.



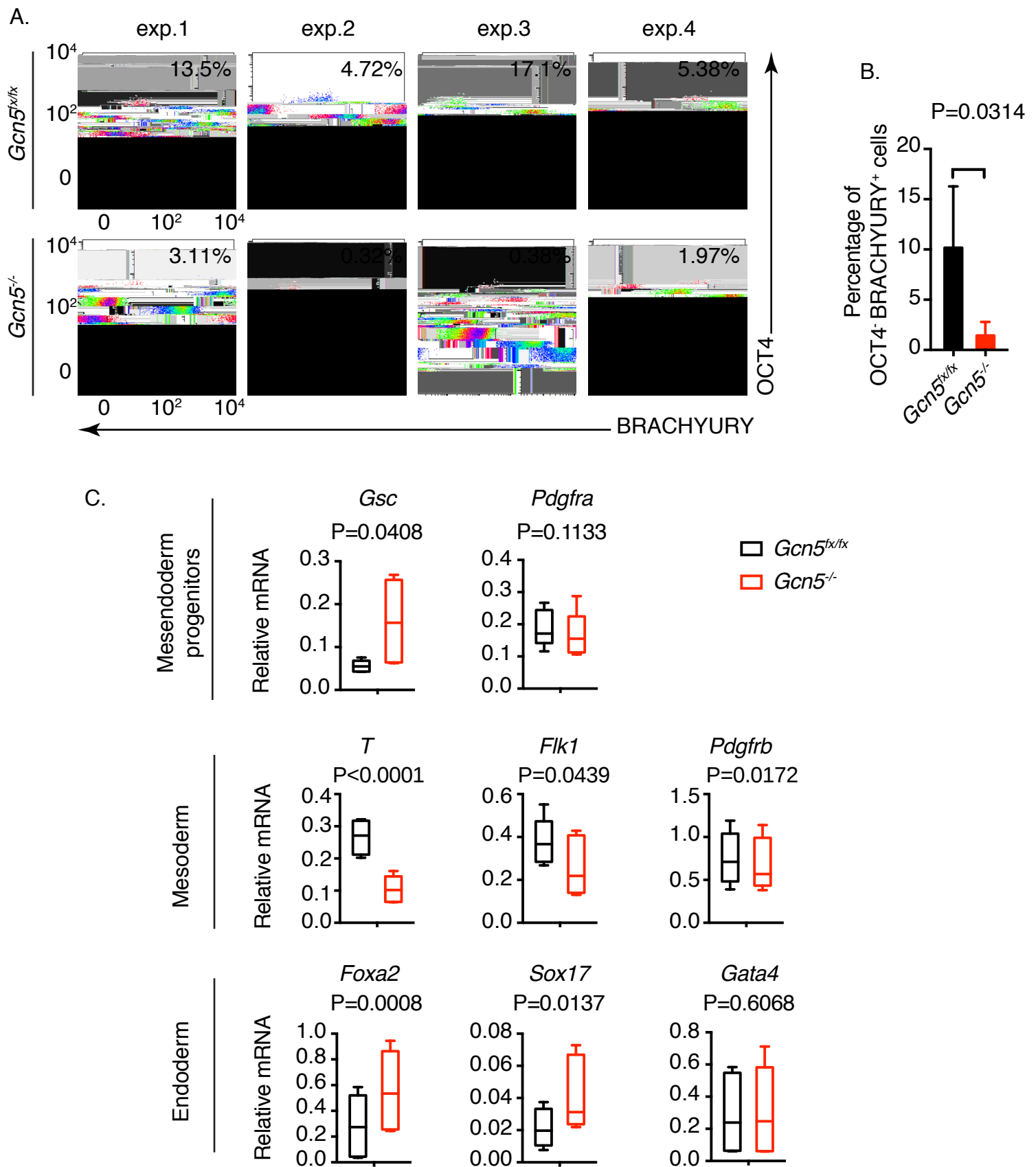


Figure S3 Loss of *Gcn5* impedes mesoderm differentiation. Related to Figure 2

(A) Gated mesoderm population of day 5 EBs from 4 independent mass cytometry experiments.

(B) Quantification of (A).

(C) qRT-PCR analysis of marker genes for indicated populations derived from monolayer mesoderm/endoderm differentiation of control and *Gcn5*^{-/-} ES cells (n=3).

Data are presented as Mean $\pm$ SD, and student t-test was used for pair-wise comparisons.

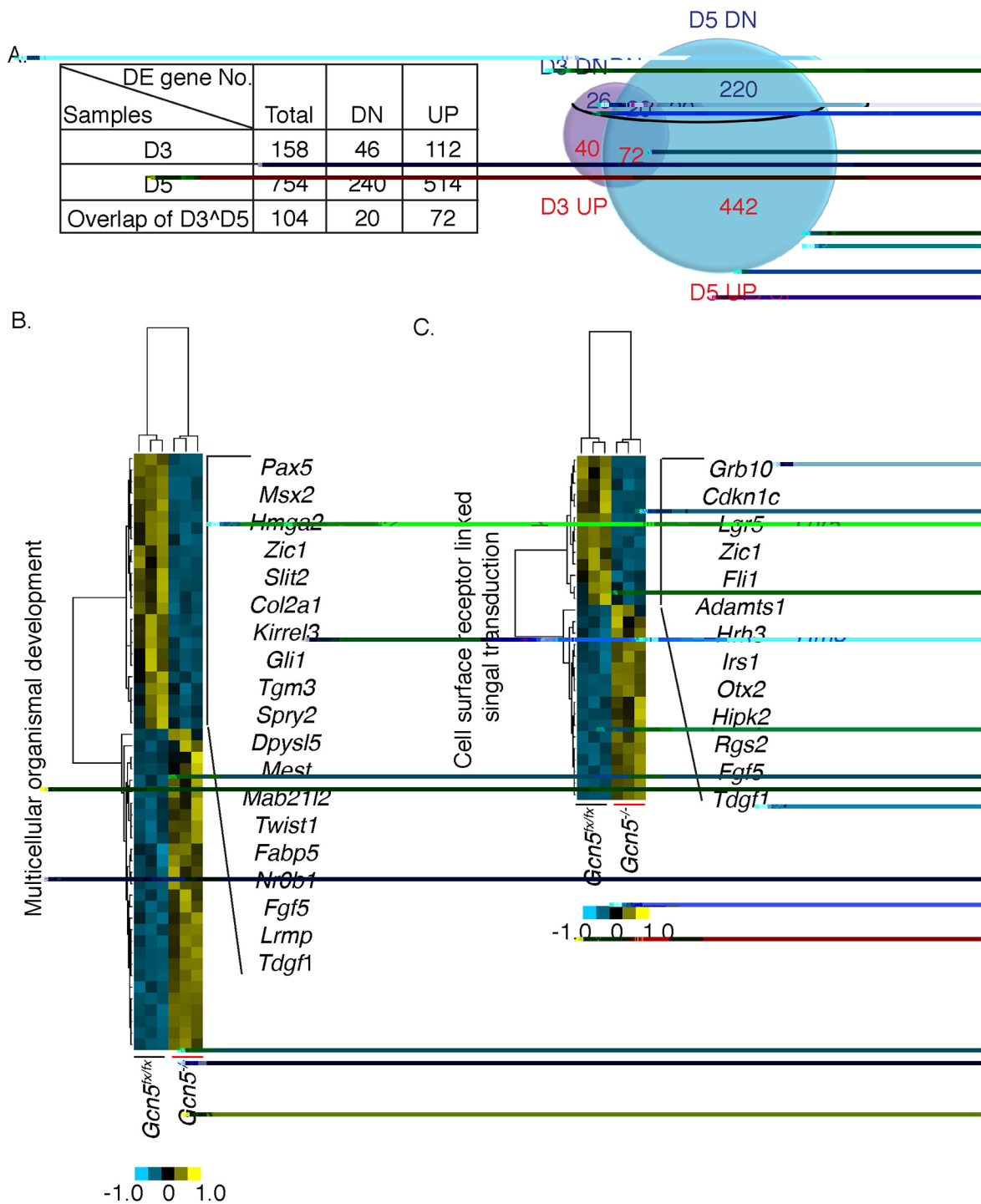


Figure S4 Loss of *Gcn5* impacted genes critical for development and signaling. Related to Figure 3

(A) Break down of the numbers of genes altered upon *Gcn5* loss in day 5 EBs.

(B and C) Heatmaps showing the top enriched genes in MOD (B) and CSRI ST (C).

Color bars, normalized RPKM counts (False discovery rate 0.05, Fold change ≥ 2)

Table S1 Lineage markers used for mass cytometry. Related to Figure 2

Antibodies	Expression	Isotope Label
A - A G		163D
A - C 4		146
A - 2		147
A - 1	E	176
A - A 3	E	170E
A - A 6	E	153E
A -F A2	E	150
A -GA A6	E	142
A -GA A4	E	171
A - 17	E	175
A -B ()		156G
A - A D1		158G

Table S2 List of genes enriched in the Multicellular Organismal Development category identified by GSEA. Related to Figure 3

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
_0	PAX5	PAX5	Paired box gene 5 (Drosophila cell lineage specific factor)	2	3.143970966	0.026952207	Ye
_1	MSX2	MSX2	Homeobox protein 2 (Drosophila)	6	2.793720722	0.048327506	Ye
_2	HMGA2	HMGA2	High mobility group AT-hook 2	10	2.60679245	0.067849986	Ye
_3	ZIC1	ZIC1	Zinc finger protein 1 (Drosophila)	13	2.445233583	0.08787638	Ye
_4	SLIT2	SLIT2	Lilioid homeobox 2 (Drosophila)	14	2.438150406	0.11204308	Ye
_5	COL2A1	COL2A1	Collagen, type II, alpha 1 (chondrocyte, dermal, lens capsule)	20	2.313369513	0.12444666	Ye
_6	KIRREL3	KIRREL3	Kirre-like 3 (Drosophila)	23	2.268698215	0.14272325	Ye
_7	GLI1	GLI1	Glioma associated oncogene 1 (chondrocyte)	25	2.187872887	0.16230397	Ye
_8	TGM3	TGM3	Transglutaminase 3 (endothelial, epidermal, placental)	27	2.169377327	0.18170136	Ye
_9	SPRY2	SPRY2	Spry domain 2 (Drosophila)	31	2.100628138	0.1962068	Ye
_10	DPYSL5	DPYSL5	Drosophila yolk-like 5	33	2.077706337	0.21469556	Ye
_11	MEST	MEST	Mesoderm specific factor 1 (chondrocyte)	44	1.956736565	0.21303791	Ye
_12	MAB21L2	MAB21L2	Mab-21-like 2 (C. elegans)	54	1.908952236	0.21301188	Ye
_13	TWIST1	TWIST1	Twist 1 (chondrocyte, dermal, Saehe-Chondrocyte) (Drosophila)	55	1.892674208	0.23177189	Ye
_14	FABP5	FABP5	Fatty acid binding protein 5 (intestinal, cardiac)	57	1.876633048	0.24826762	Ye
_15	OTX2	OTX2	Orthodenticle 2 (Drosophila)	74	1.794868827	0.23237398	N
_16	PTCH2	PTCH2	Patched 2 (Drosophila)	81	1.774591208	0.23733197	N
_17	NDP	NDP	Niederlede (endoderm)	101	1.670649171	0.21389128	N
_18	FGF17	FGF17	Fibroblast growth factor 17	117	1.568956375	0.19786368	N
_19	EPHA2	EPHA2	EPH receptor A2	127	1.409094691	0.19288312	N
_20	NR0B1	NR0B1	Nuclear receptor subfamily B, group 0, member 1	134	1.361806631	0.19374964	N
_21	FGF5	FGF5	Fibroblast growth factor 5	160	1.005194783	0.15108146	N
_22	LRMP	LRMP	Laminin receptor 1 (chondrocyte)	162	1.003890395	0.15892665	N
_23	TDGF1	TDGF1	Transforming growth factor 1	166	0.946586847	0.16199334	N
_24	ALOX12B	ALOX12B	Arachidonic acid 12-lipoxygenase, 12R	174	-1.041075826	0.15757555	N
_25	TRIM14	TRIM14	Tripartite motif 14	181	-1.18687892	0.1567082	N
_26	ANXA2	ANXA2	Annexin A2	198	-1.373691678	0.13663988	N
_27	SGCD	SGCD	Serpin, class C, member 1 (35kDa, dermal, chondrocyte)	201	-1.397668123	0.1462829	N
_28	ETS1	ETS1	Ets transcription factor 1 (endothelial)	217	-1.476131439	0.12933522	N
_29	ERG	ERG	Ets transcription factor 1 (endothelial)	222	-1.50989151	0.13588007	N
_30	RAPGEFL1	RAPGEFL1	Ras guanine nucleotide exchange factor (GEF)-like 1	227	-1.535297155	0.14267674	N
_31	IGFBP3	IGFBP3	Insulin-like growth factor binding protein 3	249	-1.666120291	0.11498063	N
_32	SGCE	SGCE	Serpin, class C, member 1	251	-1.675953865	0.12948726	N

_33	JAG2	JAG2	jagged 2	253	-1.683297992	0.14406668	N
_34	SGCG	SGCG	glial cell-specific (35kDa) (hi -a -cia ed gl c ei)	282	-1.763670683	0.10260065	N
_35	MYH3	MYH3	myosin heavy chain 3, skeletal muscle, cardiac	290	-1.779546738	0.10550249	N
_36	IGFBP4	IGFBP4	insulin-like growth factor binding protein 4	301	-1.796701074	0.10225859	N
_37	CACNA1H	CACNA1H	calcium channel voltage-dependent, alpha 1H subunit	311	-1.824573159	0.10139622	N
_38	SHOX2	SHOX2	short stature homeobox 2	315	-1.830768824	0.11322682	N
_39	AEBP1	AEBP1	AE binding protein 1	340	-1.907915235	0.08161158	N
_40	RASGRP4	RASGRP4	RAS guanine nucleotide exchange factor 4	376	-2.075456142	0.028499093	N
_41	NEUROG3	NEUROG3	neurogenin 3	385	-2.098732948	0.032459423	N
_42	FGF11	FGF11	fibroblast growth factor 11	389	-2.107631922	0.04703428	N
_43	ALDH3A2	ALDH3A2	aldehyde dehydrogenase 3 family class A2	397	-2.13826108	0.05349167	N
_44	MEF2C	MEF2C	MADS box domain containing 2, muscle-specific C-terminal domain containing 2C	398	-2.148215771	0.07478458	N
_45	STAT3	STAT3	signal transducer and activator of transcription 3 (acute-phase reactant)	401	-2.162971735	0.09201321	N
_46	CRIM1	CRIM1	connective tissue growth factor BMP signaling inhibitor (chordin-like)	429	-2.307478189	0.05804261	N
_47	NMUR2	NMUR2	neuropilin-2	462	-2.529047012	0.015741859	N
_48	DMRT1	DMRT1	doublesex domain-3 related transcription factor 1	474	-2.65857625	0.01893551	N
_49	EVPL	EVPL	evlakin	485	-2.816974878	0.02580446	N
_50	GFRA3	GFRA3	GDNF family receptor class 3	486	-2.819857836	0.053754613	N
_51	SNAI2	SNAI2	snail homolog 2 (Drosophila)	492	-2.860271454	0.07157903	N

T S3 L

C S R L S T

identified GSEA. R

F 3.

NAME	PROBE	GENE S MBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	GRB10	GRB10	growth factor receptor-bound protein 10	0	4.903269768	0.07419716	Yes
row_1	CDKN1C	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	1	3.456902027	0.12650761	Yes
row_2	LGR5	LGR5	leucine-rich repeat-containing G protein-coupled receptor 5	11	2.555413246	0.14706793	Yes
row_3	ZIC1	ZIC1	Zic family member 1 (odd-paired homolog, Drosophila)	13	2.445233583	0.18205757	Yes
row_4	GLI1	GLI1	glioma-associated oncogene homolog 1 (zinc finger protein)	25	2.187872887	0.19303207	Yes
row_5	ADAMTS1	ADAMTS1	ADAM metalloproteinase with thrombospondin type 1 motif, 1	36	2.065867424	0.20417242	Yes
row_6	HRH3	HRH3	histamine receptor H3	37	2.059562922	0.23533809	Yes
row_7	IRS1	IRS1	insulin receptor substrate 1	53	1.913015485	0.2341051	No
row_8	OTX2	OTX2	orthodenticle homolog 2 (Drosophila)	74	1.794868827	0.22102393	No
row_9	HIPK2	HIPK2	homeodomain interacting protein kinase 2	106	1.659950376	0.18376835	No
row_10	RGS2	RGS2	regulator of G-protein signalling 2, 24kDa	115	1.601378202	0.19190411	No
row_11	FGF5	FGF5	fibroblast growth factor 5	160	1.005194783	0.11858372	No
row_12	TDGF1	TDGF1	teratocarcinoma-derived growth factor 1	166	0.946586847	0.12284728	No
row_13	STC1	STC1	stanniocalcin 1	186	-1.224491358	0.10314713	No
row_14	FLT4	FLT4	fms-related tyrosine kinase 4	191	-1.312462449	0.114959255	No
row_15	RAPGEFL1	RAPGEFL1	Rap guanine nucleotide exchange factor (GEF)-like 1	227	-1.535297155	0.06776911	No
row_16	TBXA2R	TBXA2R	thromboxane A2 receptor	256	-1.689142942	0.0369915	No
row_17	RAMP1	RAMP1	receptor (calcitonin) activity modifying protein 1	285	-1.764481425	0.007353922	No
row_18	GPR20	GPR20	G protein-coupled receptor 20	295	-1.789713264	0.016327534	No
row_19	IL13RA1	IL13RA1	interleukin 13 receptor, alpha 1	356	-1.987249494	-0.07432539	No
row_20	BDKRB2	BDKRB2	bradykinin receptor B2	360	-2.004709721	-0.050025985	No
row_21	SOCS1	SOCS1	suppressor of cytokine signaling 1	381	-2.084973097	-0.058717243	No
row_22	OPRD1	OPRD1	opioid receptor, delta 1	394	-2.121104479	-0.05076518	No
row_23	RASD1	RASD1	RAS, dexamethasone-induced 1	439	-2.354247332	-0.10367147	No
row_24	GABRA4	GABRA4	gamma-aminobutyric acid (GABA) A receptor, alpha 4	442	-2.365311384	-0.071903296	No
row_25	NMUR2	NMUR2	neuromedin U receptor 2	462	-2.529047012	-0.07186268	No
row_26	CD274	CD274	CD274 molecule	463	-2.539172888	-0.03343946	No
row_27	LAT	LAT	linker for activation of T cells	500	-2.93972373	-0.06138964	No
row_28	IL12RB1	IL12RB1	interleukin 12 receptor, beta 1	521	-3.552495718	-0.04787409	No
row_29	CLEC1A	CLEC1A	C-type lectin domain family 1, member A	524	-3.695590973	0.004024112	No

Supplemental Experimental Procedures

Chromatin immunoprecipitation

28906) 10 5 - 1% (, 5 ,
125 (, 8340),
8.0, 85 1% 40) 20
(50 - 8.0, 10
A 1%

RNAseq data analysis

_____ : (10) (2.0.10) (_____, 2013)
 84-94%. 72-91%
 _____ :
 (_____, 2015) - (0.6.0)
 (A_____, 2015). 10
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Monolayer differentiation of mESCs

_____, 2013
 (_____, 2014)
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 2 (- , 3002201)
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 30010), 0.1 - () (, 03446 -100), 1 (,
 11360070)) 24 , A A (50 /)

Antibodies used in this study

Antibodies	Manufacturers	Catalog No.	Applications
A - -		4370	
A -		4695	
A - -A		4060	
A -A		4691	
A - - 38		4511	
A - 38		9212	
A - - - A (259)		9421	
A - - A		53745	
A - 1		9740	
A -		9402	/
A - 3	A	1791	
A - 3 9		07-352	
A -		12-370	
A - 1		560749	/
A - A A4	A	84593	
A -		A 2034	
A -	A	92547	
A 568		A12380	
- A 488		A21206	
- A 555		A31572	
- A 647		A31573	
- A 488		A21202	
A - A 568		A11004	
- A 647		A31571	
A - A		3580	
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A - A A6	&	A 1700	
A - A 3	&	A 2457	
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A - A	&	A 2085	
A - A 1	&	A 3168	
A - A2		561580	
A - A A4		560327	
A - 17		561590	

Primers used in this study

Oligo name	Sequences (5'-3')	Source
qRT-PCR (RNA analysis)		
2	A A A A	(_____, 2011)
2	A A A A	
3	A A AA A A A	
3	A AA AA A A A	
4	A A A A	
4	A A A	
5	A A A AA AAAA	(_____, 2011)
5	A AA A	
2	A A AA A A A A	
2	A A A	
6	AAAA AA	(_____, 2011)
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10	A A	
10	A A	
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2	A A	
	A A AA A	
	A A AA	
	AA AA A A	
	A A A A	
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4	A AA A AA	
4	A A A A A	
3	A A	
3	A A A A	
	A A	(_____, 2011)
	A A A A AA	
ChIP-qPCR		
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1	A A	
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6 2	AA A	
2	AA A A	
2	A AA A A	
	AA AAAAA	
	A A A A A A	

A (2015). --
31, 166-169.

A , A. . (2013). :
29, 1922-1924.

57-74. . . . (2012). A A . 489,

20, 1453-1454. . . . (2004). .

470, 503-509. . . . , et al. (2011). - .

12, 996-1006. , A. . . . (2002).

2: . 14,

36. . . . (2009). -
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57 6/
26, 366-378.

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26, 139-140.

3246-3248. , A. . (2004). -- . 20,

102, 15545-15550.

et al. (2005). : -
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et al. (2008). - (A). 9, 137.