

## Supporting Information

### *Methylation-Directed O-Glycosylation of Chromatin Factors Represses Retrotransposon Promoters*

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**Supplementary Figure S1:** The protein TRIM28 is necessary to repress transcription of methylated retrotransposons.

**Supplementary Figure S2:** Loss of imprinted expression in Trim28C/C embryos occurs despite normal DNA methylation at differentially methylated regions (DMRs).

**Supplementary Figure S3:** Demethylation does not cause dissociation of TRIM28 from IAP retrotransposon sequences.

**Supplementary Figure S4:** O-GlcNAcylation of proteins associated with TRIM28.

**Supplementary Figure S5:** Specific reactivation of IAPEz transposons upon targeting of dCas9-OGA to their LTRs.

**Supplementary Figure S6:** Predominance of proteins with KRAB and Zinc finger domains among the DNA binding proteins found to Co-IP with TRIM28 by mass spectrometry.

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# SUPPLEMENTARY TABLES

Table S1: Mass spectrometric identification of proteins co-IP with TRIM28 (native IP, ES cells, Anti-body MAB362)

WT				Dmrt1-null				WT				Dmrt1-null				WT				Dmrt1-null								
Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	Unq. Total reference	Gene Syc AVG	
100	1701RPPB_MOUSE	Prrab2	3.22	86	1502RPPB_MOUSE	Prrab2	3.26	12	15	CXCR2_MOUSE	Cxcr2	3.7	7	CXCR2_MOUSE	Cxcr2	3.7	4	4	BRP9_MOUSE	Brp9	3.62	5	6	BRP9_MOUSE	Brp9	2.99	2	
101	1074RPPB_MOUSE	Sprnd2	3.24	86	1067RPPB_MOUSE	Sprnd2	3.24	10	10	ITIH5_MOUSE	Itih5	3.57	13	ITIH5_MOUSE	Itih5	3.57	13	10	ITIH5_MOUSE	Itih5	3.57	13	10	ITIH5_MOUSE	Itih5	3.57	13	10
102	1209RPPB_MOUSE	Slit2	3.25	87	1117RPPB_MOUSE	Slit2	3.25	14	14	CXCR4_MOUSE	Cxcr4	3.31	11	CXCR4_MOUSE	Cxcr4	3.31	11	14	CXCR4_MOUSE	Cxcr4	3.31	11	14	CXCR4_MOUSE	Cxcr4	3.31	11	14
103	1614RPPB_MOUSE	Rpl1	3.27	61	1117RPPB_MOUSE	Rpl1	3.27	14	14	LRWD4_MOUSE	Lrwd4	3.40	13	LRWD4_MOUSE	Lrwd4	3.40	13	14	LRWD4_MOUSE	Lrwd4	3.40	13	14	LRWD4_MOUSE	Lrwd4	3.40	13	14
64	1201TOPA_MOUSE	Toga2a	3.45	67	1381TOPA_MOUSE	Toga2a	3.26	14	14	USO1_MOUSE	Uso1	3.24	12	USO1_MOUSE	Uso1	3.24	12	14	USO1_MOUSE	Uso1	3.24	12	14	USO1_MOUSE	Uso1	3.24	12	14
104	89R02B_MOUSE	Olf4	3.46	41	1780R02B_MOUSE	Olf4	3.46	15	15	HAO1_MOUSE	hao1	3.12	12	HAO1_MOUSE	hao1	3.12	12	15	HAO1_MOUSE	hao1	3.12	12	15	HAO1_MOUSE	hao1	3.12	12	15
57	96RFB1_MOUSE	Pa2a3	3.46	61	1081RFB1_MOUSE	Pa2a3	3.42	14	14	22M2M_MOUSE	Zm2m	3.13	12	22M2M_MOUSE	Zm2m	3.13	12	14	22M2M_MOUSE	Zm2m	3.13	12	14	22M2M_MOUSE	Zm2m	3.13	12	14
52	91MBR1A_MOUSE	Mbr1a3	3.48	38	101MBR1A_MOUSE	Mbr1a3	3.47	14	14	CD3ZM2_MOUSE	Cd3z	3.19	8	CD3ZM2_MOUSE	Cd3z	3.19	8	14	CD3ZM2_MOUSE	Cd3z	3.19	8	14	CD3ZM2_MOUSE	Cd3z	3.19	8	14
51	169C04_MOUSE	C04a	3.49	26	35C04_MOUSE	C04a	3.49	15	15	FBP1_MOUSE	Fbp1	3.31	18	FBP1_MOUSE	Fbp1	3.31	18	15	FBP1_MOUSE	Fbp1	3.31	18	15	FBP1_MOUSE	Fbp1	3.31	18	15
49	77SP7H_MOUSE	Su7b6	3.58	49	89SP7H_MOUSE	Su7b6	3.61	18	18	FNBP4_MOUSE	Fbnp4	3.4	11	FNBP4_MOUSE	Fbnp4	3.4	11	18	FNBP4_MOUSE	Fbnp4	3.4	11	18	FNBP4_MOUSE	Fbnp4	3.4	11	18
47	162D9H_MOUSE	D9h3	3.59	52	152D9H_MOUSE	D9h3	3.58	12	12	FNUG13_MOUSE	Fnu13	3.65	11	FNUG13_MOUSE	Fnu13	3.65	11	12	FNUG13_MOUSE	Fnu13	3.65	11	12	FNUG13_MOUSE	Fnu13	3.65	11	12
44	82SR91_MOUSE	Srb1	3.64	41	83SR91_MOUSE	Srb1	3.64	13	13	NPM1_MOUSE	Npm1	3.47	3	NPM1_MOUSE	Npm1	3.47	3	13	NPM1_MOUSE	Npm1	3.47	3	13	NPM1_MOUSE	Npm1	3.47	3	13
44	89SR3_MOUSE	Srb3a	3.64	41	110SR3_MOUSE	Srb3a	3.54	11	11	26NPM_MOUSE	Npm	4.2	18	26NPM_MOUSE	Npm	4.2	18	11	26NPM_MOUSE	Npm	4.2	18	11	26NPM_MOUSE	Npm	4.2	18	11
41	47SP4M_MOUSE	Smz2	3.34	28	48SP4M_MOUSE	Smz2	3.33	13	13	19HNRK_MOUSE	Hnrk	3.48	9	19HNRK_MOUSE	Hnrk	3.48	9	13	19HNRK_MOUSE	Hnrk	3.48	9	13	19HNRK_MOUSE	Hnrk	3.48	9	13
40	45C9AP_MOUSE	C9ap	3.37	41	45C9AP_MOUSE	C9ap	3.37	13	13	16SRP9_MOUSE	Srp9	4.03	8	16SRP9_MOUSE	Srp9	4.03	8	13	16SRP9_MOUSE	Srp9	4.03	8	13	16SRP9_MOUSE	Srp9	4.03	8	13
38	2351TF1B_MOUSE	Trf1b3	3.48	43	1921TF1B_MOUSE	Trf1b3	3.75	11	11	15OBRV_MOUSE	Obv1	3.40	13	15OBRV_MOUSE	Obv1	3.40	13	11	15OBRV_MOUSE	Obv1	3.40	13	11	15OBRV_MOUSE	Obv1	3.40	13	11
37	43LTP2D_MOUSE	Ltp2d	3.54	38	44LTP2D_MOUSE	Ltp2d	3.52	13	13	15ARIB_MOUSE	Arb1	3.08	11	15ARIB_MOUSE	Arb1	3.08	11	13	15ARIB_MOUSE	Arb1	3.08	11	13	15ARIB_MOUSE	Arb1	3.08	11	13
36	58G02_MOUSE	G02a	3.49	49	61G02_MOUSE	G02a	3.49	15	15	13DNK3_MOUSE	Dnk3	3.28	14	13DNK3_MOUSE	Dnk3	3.28	14	15	13DNK3_MOUSE	Dnk3	3.28	14	15	13DNK3_MOUSE	Dnk3	3.28	14	15
36	58G03_MOUSE	G03a	3.49	49	61G03_MOUSE	G03a	3.49	15	15	14NCP1_MOUSE	Ncp1	3.42	11	14NCP1_MOUSE	Ncp1	3.42	11	15	14NCP1_MOUSE	Ncp1	3.42	11	15	14NCP1_MOUSE	Ncp1	3.42	11	15
34	103LTD1_MOUSE	Ltd1	3.37	10	103LTD1_MOUSE	Ltd1	3.32	11	11	12CZD2_MOUSE	Czd2	3.37	9	12CZD2_MOUSE	Czd2	3.37	9	11	12CZD2_MOUSE	Czd2	3.37	9	11	12CZD2_MOUSE	Czd2	3.37	9	11
33	103MYH1_MOUSE	Myh1	3.33	23	103MYH1_MOUSE	Myh1	3.26	11	11	10C2D3_MOUSE	C2d3	3.27	9	10C2D3_MOUSE	C2d3	3.27	9	11	10C2D3_MOUSE	C2d3	3.27	9	11	10C2D3_MOUSE	C2d3	3.27	9	11
33	77CCDL_MOUSE	Ccdl	3.61	30	54CCDL_MOUSE	Ccdl	3.61	10	10	12RAGP1_MOUSE	Ragp1	3.31	14	12RAGP1_MOUSE	Ragp1	3.31	14	10	12RAGP1_MOUSE	Ragp1	3.31	14	10	12RAGP1_MOUSE	Ragp1	3.31	14	10
33	57R2C_MOUSE	R2c2	3.56	36	36R2C_MOUSE	R2c2	3.63	13	13	12CCK1_MOUSE	Cck1	3.37	9	12CCK1_MOUSE	Cck1	3.37	9	13	12CCK1_MOUSE	Cck1	3.37	9	13	12CCK1_MOUSE	Cck1	3.37	9	13
32	33MYH1_MOUSE	Myh1	3.33	23	33MYH1_MOUSE	Myh1	3.26	11	11	10C2D3_MOUSE	C2d3	3.27	9	10C2D3_MOUSE	C2d3	3.27	9	11	10C2D3_MOUSE	C2d3	3.27	9	11	10C2D3_MOUSE	C2d3	3.27	9	11
32	37ZAMA_MOUSE	Zam1	3.19	30	37ZAMA_MOUSE	Zam1	3.07	10	10	11EBC3_MOUSE	Ebc3	2.94	9	11EBC3_MOUSE	Ebc3	2.94	9	10	11EBC3_MOUSE	Ebc3	2.94	9	10	11EBC3_MOUSE	Ebc3	2.94	9	10
31	49CUBJ_MOUSE	Cubj2	3.64	38	40CUBJ_MOUSE	Cubj2	3.67	11	11	11RBC4_MOUSE	Rbc4	2.71	11	11RBC4_MOUSE	Rbc4	2.79	11	11	11RBC4_MOUSE	Rbc4	2.71	11	11	11RBC4_MOUSE	Rbc4	2.71	11	11
31	33B0C1_MOUSE	B0c1	3.32	29	33B0C1_MOUSE	B0c1	3.32	10	10	11RBC4_MOUSE	Rbc4	2.71	11	11RBC4_MOUSE	Rbc4	2.79	11	10	11RBC4_MOUSE	Rbc4	2.71	11	11	11RBC4_MOUSE	Rbc4	2.71	11	11
31	37ACNU_MOUSE	Acnu1	3.14	24	30ACNU_MOUSE	Acnu1	3.1	10	10	11FNCI_MOUSE	Fnci	3.33	8	11FNCI_MOUSE	Fnci	3.33	8	10	11FNCI_MOUSE	Fnci	3.33	8	10	11FNCI_MOUSE	Fnci	3.33	8	10
31	33X93B_MOUSE	X93b1	3.11	29	33X93B_MOUSE	X93b1	3.08	10	10	11EAMY_MOUSE	Eam1	2.88	4	11EAMY_MOUSE	Eam1	2.88	4	10	11EAMY_MOUSE	Eam1	2.88	4	10	11EAMY_MOUSE	Eam1	2.88	4	10
31	33HNR1_MOUSE	Hnr1	3.46	28	33HNR1_MOUSE	Hnr1	3.38	10	10	11RAN1_MOUSE	Ran1	3.21	10	11RAN1_MOUSE	Ran1	3.21	10	10	11RAN1_MOUSE	Ran1	3.21	10	10	11RAN1_MOUSE	Ran1	3.21	10	10
30	44PINN_MOUSE	Pinn	3.27	25	44PINN_MOUSE	Pinn	3.26	10	10	20HD4C1_MOUSE	Hd4c1	3.87	10	20HD4C1_MOUSE	Hd4c1	3.87	10	10	20HD4C1_MOUSE	Hd4c1	3.87	10	10	20HD4C1_MOUSE	Hd4c1	3.87	10	10
30	39RPS_MOUSE	Rps11	3.33	23	29RPS_MOUSE	Rps11	3.27	10	10	16G39T1_MOUSE	G39t1	3.75	6	16G39T1_MOUSE	G39t1	3.75	6	10	16G39T1_MOUSE	G39t1	3.75	6	10	16G39T1_MOUSE	G39t1	3.75	6	10
29	36DQ2_MOUSE	Dq2	3.31	29	36DQ2_MOUSE	Dq2	3.21	10	10	15HNRP1_MOUSE	Hnrp1	3.28	11	15HNRP1_MOUSE	Hnrp1	3.28	11	10	15HNRP1_MOUSE	Hnrp1	3.28	11	10	15HNRP1_MOUSE	Hnrp1	3.28	11	10
29	36DQ3_MOUSE	Dq3	3.31	29	36DQ3_MOUSE	Dq3	3.21	10	10	15HNRP1_MOUSE	Hnrp1	3.28	11	15HNRP1_MOUSE	Hnrp1	3.28	11	10	15HNRP1_MOUSE	Hnrp1	3.28	11	10	15HNRP1_MOUSE	Hnrp1	3.28	11	10
29	37EPW9_MOUSE	Ew9	3.32	31	37EPW9_MOUSE	Ew9	3.48	10	10	15KHRI_MOUSE	Khr1	2.83	9	15KHRI_MOUSE	Khr1	2.83	9	10	15KHRI_MOUSE	Khr1	2.83	9	10	15KHRI_MOUSE	Khr1	2.83	9	10
29	37M1_MOUSE	M1	3.46	18	18M1_MOUSE	M1	3.17	10	10	15M1_MOUSE	M1	3.25	12	15M1_MOUSE	M1	3.25	12	10	15M1_MOUSE	M1	3.25	12	10	15M1_MOUSE	M1	3.25	12	10
28	81TE1X_MOUSE	Te1x1	3.11	61	81TE1X_MOUSE	Te1x1	3.10	14	14	15THOS_MOUSE	Thos	3.32	12	15THOS_MOUSE	Thos	3.32	12	14	15THOS_MOUSE	Thos	3.32	12	14	15THOS_MOUSE	Thos	3.32	12	14
28	43D3ZM2_MOUSE	D3zm2	3.09	12	43D3ZM2_MOUSE	D3zm2	3.05	12	12	15W1_MOUSE	W1	3.46	8	15W1_MOUSE	W1	3.46	8	12	15W1_MOUSE	W1	3.46	8	12	15W1_MOUSE	W1	3.46	8	12
28	43DYW4_MOUSE	Dyw4	3.66	25	35DYW4_MOUSE	Dyw4	3.66	10	10	14DSD2_MOUSE	Dsd2	3.52	15	14DSD2_MOUSE	Dsd2	3.52	15	10	14DSD2_MOUSE	Dsd2	3.52	15	10	14DSD2_MOUSE	Dsd2	3.52	15	10
28	12ZN6B_MOUSE	Zn6b	3.48	11	12ZN6B_MOUSE	Zn6b	3.48	10	10	14DSD2_MOUSE	Dsd2	3.52	15	14DSD2_MOUSE	Dsd2	3.52	15	10	14DSD2_MOUSE	Dsd2	3.52	15	10	14DSD2_MOUSE	Dsd2	3.52	15	10
28	38SOGA2_MOUSE	Soga2	3.29	17	18SOGA2_MOUSE	Soga2	3.25	10	10	13P68B_MOUSE	P68b	3.84	8	13P68B_MOUSE	P68b	3.84	8	10	13P68B_MOUSE	P68b	3.84	8	10	13P68B_MOUSE	P68b	3.84	8	10
28	43CPS1_MOUSE	Cps1	3.28	22	43CPS1_MOUSE	Cps1	3.32	10	10	13CTE1_MOUSE	Cte1	3.13	15	13CTE1_MOUSE	Cte1	3.13	15	10	13CTE1_MOUSE	Cte1	3.13	15	10	13CTE1_MOUSE	Cte1	3.13	15	10
28	33MYH1_MOUSE	Myh1	3.33	23	33MYH1_MOUSE	Myh1	3.26	11	11	13MYH1_MOUSE	Myh1	3.33	15	13MYH1_MOUSE	Myh1	3.33	15	11	13MYH1_MOUSE	Myh1	3.33	15	11	13MYH1_MOUSE	Myh1	3.33	15	11
27	27MGAP_MOUSE	Mga	3.15	33	43MGAP_MOUSE	Mga	3.26	10	10	13M7_MOUSE	M7	3.44	15	13M7_MOUSE	M7	3.44	15	10	13M7_MOUSE	M7	3.44	15	10	13M7_MOUSE	M7	3.44	15	10
26	28TRPC_MOUSE	Trp2	3.85	38	30TRPC_MOUSE	Trp2	3.46	10	10	13SCAP_MOUSE	Scap	3.88	18	13SCAP_MOUSE	Scap	3.88	18	10	13SCAP_MOUSE	Scap	3.88	18	10	13SCAP_MOUSE	Scap	3.88	18	10
26	40SRBP_MOUSE	Srbp	3.41	29	40SRBP_MOUSE	Srbp	3.42	11	11	13SRP1_MOUSE	Srp1	3.82	18	13SRP1_MOUSE	Srp1	3.82	18	11	13SRP1_MOUSE	Srp1	3.82	18	11	13SRP1_MOUSE	Srp1	3.82	18	11
26	38ACR_MOUSE	Ac1	3.67	30	37ACR_MOUSE	Ac1	3.67	10	10	13M4_MOUSE	M4	2.84	6	13M4_MOUSE	M4	2.84	6	10	13M4_MOUSE	M4	2.84	6	10	13M4_MOUSE	M4	2.84	6	10
25	31F2B8_MOUSE	F2b8	3.26	31	31F2B8_MOUSE	F2b8	3.26	3	3	12M1_MOUSE	M1	3.14	10	12M1_MOUSE	M1	3.14	10	3	12M1_MOUSE	M1	3.14	10	3	12M1_MOUSE	M1	3.14	10	3
25	42NPCR_MOUSE	Npcr	3.44	29	42NPCR_MOUSE	Npcr	3.48	10	10	12R2_MOUSE	R2	3.19	10	12R2_MOUSE	R2													

**Table S2: O-GlcNAcylated proteins associated with TRIM28 in wild type and *Dnmt1*<sup>-/-</sup> ES cells.**

Gene Symbol	Tandem IP : TRIM28 followed by WGA				Proteins depleted by more than 2 folds in <i>Dnmt1</i> <sup>-/-</sup> WT after tandem IP (Unique WT >= 6)	Function	Other name	Protein complex
	Wild type		<i>Dnmt1</i> <sup>-/-</sup>					
	Unique	Total	Unique	Total				
Rif1	13	13	9	9		Telomere-associated protein		
Snmp200	8	8	7	7		RNA helicase		
<b>Chd4</b>	<b>7</b>	<b>8</b>	<b>0</b>	<b>0</b>	<b>CHD4/MI2β</b>	<b>Transcriptional regulator</b>	<b>MI-2β</b>	<b>NURD</b>
Hdc1	10	10	15	17		Interacts with OGT		OGT
Nup98	11	13	13	16		Nuclear pore complex		
<b>Cul7</b>	<b>16</b>	<b>17</b>	<b>1</b>	<b>1</b>	<b>CUL7</b>	<b>Regulates the imprinted cluster <i>H19-Igf2</i></b>	<b>p185</b>	<b>Cul7-RING</b>
Oser1	1	1	18	18		In complex with RNA PolII		RNA PolII
Smarca4	5	5	2	2		Transcriptional regulator	Big1	SNF2/SMARCS
Top2a	42	49	34	36		DNA topoisomerase II		
<b>Zmy2</b>	<b>16</b>	<b>16</b>	<b>6</b>	<b>6</b>	<b>ZMY2/ZFP198</b>	<b>In complex with HDAC1 and LSD1</b>	<b>Zfp198</b>	<b>LSD1</b>
Mybbp1a	17	17	25	26		Transcriptional regulator		SNF2/SMARCS
Msi6	13	13	18	18		DNA mismatch repair		
Dhx9	30	34	23	23		RNA helicase		
<b>Sf3b1</b>	<b>12</b>	<b>12</b>	<b>6</b>	<b>6</b>	<b>SF3B1</b>	<b>Essential for Polycomb silencing in mouse</b>		<b>SNF2/SMARCS</b>
Bms1	11	11	17	18		Ribosome biogenesis		
Sf3b3	27	28	22	22		Splicing factor		
Emy1	10	11	13	13		Transcriptional repressor		HDAC1
Tcf11	10	10	8	8		Ribosome biogenesis		
Rfc1	7	7	11	11		DNA replication		
Tocg1	10	10	16	16		Interacts with RNA PolII		RNA PolII
Smarca1	9	10	6	6		Nucleosome remodeling	Snf2l	NURF
<b>Smarca5</b>	<b>19</b>	<b>20</b>	<b>4</b>	<b>4</b>	<b>SMARCA5/SNF2H</b>	<b>Transcriptional repressor</b>	<b>Snf2h</b>	<b>SNF2/SMARCS</b>
Supt16h	18	18	13	13		Nucleosome remodeling		FACT
U2urf	0	0	14	14		U2 snRNP-associated SURF motif-containing protein		
Zf	9	9	16	19		Zinc-finger protein		
Smarca2	13	16	18	22		Transcriptional regulator - interacts with TRIM28		SNF2/SMARCS
<b>Mov10</b>	<b>13</b>	<b>13</b>	<b>1</b>	<b>2</b>	<b>MOV10</b>	<b>Inhibits retrotransposition</b>		<b>RISC</b>
Pap1	12	12	10	10		Chromatin compaction		
Etfud2	25	26	23	25		U5 small nuclear ribonucleoprotein		
Pip40a	12	13	12	12		Pre-mRNA-processing		
Thrap3	9	11	8	9		Pre-mRNA-processing		
Mei2	8	8	12	12		Transcriptional regulator		Smrad11/SNF2
Zfp715	7	8	5	5		Transcriptional regulator		
Dnmt3l	7	7	6	6		Chromatin histone binding		
Wdr36	7	7	9	9		rRNA processing		
Rbm25	9	9	11	11		rRNA binding		
Scn2l	0	0	17	21		Regulate ubiquitination of H2A		RNF2-RING1B
Sf3b2	8	8	20	21		Splicing factor		
IFI	24	25	22	26		RNA binding		PRMT1
Ddx23	18	18	20	23		RNA helicase		
Hells	8	8	5	5		DNA helicase	LSH	
Ddx21	22	33	23	25		Transcriptional regulator		SNF2/SMARCS
<b>Kdm1a</b>	<b>13</b>	<b>14</b>	<b>3</b>	<b>3</b>	<b>LSD1/KDM1A</b>	<b>Transposon silencing</b>	<b>KDM1A</b>	<b>LSD1</b>
Wdr75	8	8	5	5		Transcriptional regulator		
Trim71	9	9	5	5		RNA binding	mLin41	
Sart1	2	2	16	17		RNA binding		
Top1	17	19	19	23		DNA topoisomerase		
Ubf1	16	16	18	19		Transcriptional regulator of rDNA		
Las11	6	7	15	15		Transcriptional regulator		PRMT1
Trim28	25	46	30	102		Transcriptional repressor	KAP1, TIF1β	TRIM28
Cdc5l	12	13	19	19		Cell division cycle 5-like protein		
Tad3	6	6	6	6		mRNA processing		
L1td1	17	17	19	29		Inhibits retrotransposition	Ecat 11	
Hmnp1	13	13	10	10		Pre-mRNA-processing		
Utp14a	1	1	13	13		U5 small nucleolar RNA-associated protein 14		
Nop2	14	14	12	12		RNA methyltransferase		
Ddx27	8	8	11	11		RNA helicase		
Gnt2	5	5	14	15		RNA binding		
Snp1	12	13	15	17		Nucleosome remodeling		FACT
Mphosph10	8	8	8	8		Pre-mRNA-processing		
Hmnp1	12	14	11	13		Pre-mRNA-processing		
Pip3	8	8	14	14		U4/U6 small nuclear ribonucleoprotein		
Zfp568	12	20	11	17		In complex with TRIM28	CHATO	
Ncl	13	13	18	18		rRNA processing		Nucleolin
Ddx8	7	7	12	12		RNA helicase		
Ctbp4	12	13	17	20		Nucleolar GTP-binding protein 1		
Klba0020	6	6	7	7		PARP1 inhibitor	Pum3	
Ddx17	16	17	16	16		Interacts with HDAC1		HDAC1
Ddx5	16	18	19	24		Transcriptional repressor, interacts with HDAC1		HDAC1
<b>Fbxw8</b>	<b>12</b>	<b>12</b>	<b>3</b>	<b>3</b>	<b>FBXW8</b>	<b>Cul7-RING ubiquitin-protein ligase complex</b>		<b>Cul7-RING</b>
Ddx52	6	6	5	5		RNA helicase		
Dmtra2	6	6	7	7		Nuclear hormone receptor	ER-alpha	
Lmbd1	16	17	20	20		Lamin-B1		
Nop56	22	22	21	21		Ribosome biogenesis		
Senp3	7	8	8	10		SUMO-1-specific protease 3		
Zfp472	7	7	4	4		Transcriptional regulator		
Nop58	11	13	8	10		Ribosome biogenesis		
Rbm39	8	9	8	9		Transcriptional regulator		
Snr1	10	12	9	11		Pre-mRNA-processing		
Nacc1	9	9	5	5		Transcriptional regulator		
Pip19	10	11	6	6		Ubiquitin-protein ligase		
<b>Hdac1</b>	<b>6</b>	<b>6</b>	<b>3</b>	<b>3</b>	<b>HDAC1</b>	<b>Transcriptional repressor</b>		<b>HDAC1</b>
D1Pas1	8	10	10	10		RNA helicase		
Vim	6	6	13	14		Vimentin		
U2af2	8	9	6	9		Pre-mRNA-processing		
Rest	8	8	8	9		Transcriptional repressor		LSD1
Rai1d1	11	11	11	11		Ribosomal protein		
Tuba1a	9	9	9	13		Alpha-tubulin 1		
Ddx39a	8	9	8	9		RNA helicase		
Rpl4	13	14	21	26		Ribosomal protein		
Ef4a3	12	14	9	9		RNA helicase		
Dak	11	13	13	20		Chromatin DNA binding		SNF2/SMARCS
Rbm7	7	8	8	10		RNA binding		
Snmp40	7	7	6	6		Pre-mRNA-processing		
Gnb2h1	11	11	10	11		40S ribosomal subunit	Rack1	
Hmmpc	13	14	11	12		Pre-mRNA-processing		
Fbl	12	14	11	18		Fibrillin		
Trab2	8	12	7	7		RNA binding		
Raly	8	8	10	11		RNA binding	Merc	
Rpl7	17	19	20	29		Ribosomal protein		
Rpl7a	9	10	10	14		Ribosomal protein		
Rps3a	7	8	11	15		Ribosomal protein		
Rps4x	15	21	16	21		Ribosomal protein		
Rps6	8	8	9	9		Ribosomal protein		
Rps8	9	9	7	7		Ribosomal protein		
Sra1	10	14	11	12		Ribosomal protein		
Rps3	15	18	13	18		Ribosomal protein		
Rpl13	7	8	7	9		Ribosomal protein		
Rps15a	7	7	8	8		Ribosomal protein		
Rps9	12	12	14	16		Ribosomal protein		
HistH10	6	7	7	7		Histone	Histone H1	
HistH1a	7	8	5	5		Histone	Histone H1	
Rpl18	6	7	8	18		Ribosomal protein		
Rpl23a	9	9	14	17		Ribosomal protein		
Rps13	8	8	8	8		Ribosomal protein		
HistH4a	8	20	7	12		Histone	Histone H4	

**Table S3: KRAB-C2H2-Zinc fingers TFs associated with TRIM28 in ES cells**

Gene Symbol	Ensembl ID	Mass spec after TRIM28 IP (peptide counts)			
		WT		D1-/-	
		Unique	Total	Unique	Total
Zfp568	ENSMUSG00000074221	12	14	11	13
Rbak	ENSMUSG00000061898	11	11	11	12
Zfp715	ENSMUSG00000012640	10	16	6	7
Zfp600	ENSMUSG00000058186	10	32	11	33
Zfp719	ENSMUSG00000030469	9	12	6	6
Znf569	ENSMUSG00000059975	8	14	7	11
Zfp101	ENSMUSG00000055240	7	10	4	6
Zfp84	ENSMUSG00000046185	7	11	5	6
Zfp869	ENSMUSG00000054648	7	8	4	4
Zfp986	ENSMUSG00000078500	7	8	4	4
AW146154	ENSMUSG00000074166	7	7	4	4
Gm15446	ENSMUSG00000090015	6	7	5	5
Zfp655	ENSMUSG00000007812	6	8	6	9
Zfp263	ENSMUSG00000022529	6	8	7	10
Zfp26	ENSMUSG00000063108	5	5	2	2
Zfp808	ENSMUSG00000074867	5	7	7	9
Zfp60	ENSMUSG00000037640	5	7	7	8
Zfp7	ENSMUSG00000033669	5	5	8	9
Zfp141	ENSMUSG00000092416	5	5	1	1
Zfp799	ENSMUSG00000095253	5	7	5	5
Zfp955a	ENSMUSG00000094441	5	6	6	6
Zfp948	ENSMUSG00000067931	4	4	5	5
Zfp418	ENSMUSG00000034538	4	5	2	2
Zfp472	ENSMUSG00000053600	4	7	4	5
Zfp981	ENSMUSG00000056300	4	6	6	14
Zfp317	ENSMUSG00000057551	3	3	2	2
Zfp266	ENSMUSG00000060510	3	3	0	0
Zfp617	ENSMUSG00000066880	3	3	4	4
Zfp811	ENSMUSG00000055202	3	3	0	0
Zfp119b	ENSMUSG00000062101	3	5	1	2
Znf250	ENSMUSG00000054967	3	3	3	4
Znf354c	ENSMUSG00000044807	3	3	3	3
Zfp612	ENSMUSG00000044676	3	4	1	1
Zfp558	ENSMUSG00000074500	3	6	3	6
4930522L14Rik	ENSMUSG00000072762	3	3	4	4
9830147E19Rik	ENSMUSG00000074158	3	4	2	3
Zfp61	ENSMUSG00000066880	3	3	4	4
Zfp51	ENSMUSG00000023892	2	3	1	1
Zfp120	ENSMUSG00000068134	2	3	0	0
Zfp600	ENSMUSG00000066007	2	2	0	0
Zfp871	ENSMUSG00000024298	2	2	5	6
Zfp169	ENSMUSG00000050954	2	3	3	4
Zfp128	ENSMUSG00000060397	2	2	3	3
Zfp870	ENSMUSG00000095325	2	2	0	0
5730507C01Rik	ENSMUSG00000073197	2	3	0	0
Zfp989	ENSMUSG00000086147	2	3	2	4
Zfp978	ENSMUSG00000078497	2	2	4	7
Gm14443	ENSMUSG00000078902	2	2	4	5
Zfp640	ENSMUSG00000074830	2	2	2	2
Rex2	ENSMUSG00000067919	2	10	2	8
Znf12	ENSMUSG00000029587	1	1	2	2
Zfp938	ENSMUSG00000062931	1	1	1	3
Zfp819	ENSMUSG00000055102	1	1	0	0
Zfp90	ENSMUSG00000031907	1	1	0	0
Zfp37	ENSMUSG00000028389	1	1	0	0
Znf182	ENSMUSG00000054737	1	1	0	0
Zfp931	ENSMUSG00000078861	1	1	0	0
Gm13212	ENSMUSG00000078502	1	4	0	0
Zfp985	ENSMUSG00000069727	1	1	1	1
Zfp975	ENSMUSG00000078498	1	1	1	1
Zfp988	ENSMUSG00000096916	1	1	0	0
Zfp975	ENSMUSG00000079008	1	1	0	0
Zfp977	ENSMUSG00000092335	1	1	0	0
Gm17067	ENSMUSG00000091594	1	1	0	0
Zfp759	ENSMUSG00000057396	1	1	1	1
Zfp868	ENSMUSG00000060427	1	5	1	2
Zfp758	ENSMUSG00000044501	1	2	1	2
Zfp599	ENSMUSG00000062794	1	1	0	0
Zfp53	ENSMUSG00000057409	1	1	1	1
Gm14124	ENSMUSG00000079008	1	1	0	0
Zfp937	ENSMUSG00000060336	1	1	0	0
Zfp160	ENSMUSG00000067942	1	1	0	0
Zfp873	ENSMUSG00000061371	1	1	1	1
9130019O22Rik	ENSMUSG00000030823	1	1	2	2
Zfp597	ENSMUSG00000039789	1	1	0	0
Zfp712	ENSMUSG00000090641	1	1	0	0
Zfp763	ENSMUSG00000067430	1	1	0	0
Zfp820	ENSMUSG00000069743	1	1	0	0
Zfp934	ENSMUSG00000091183	1	1	1	1
Zfp949	ENSMUSG00000032425	1	1	0	0
Zfp952	ENSMUSG00000053390	1	1	0	0
Zfp97	ENSMUSG00000095990	1	1	0	0
Zfp59	ENSMUSG00000078779	0	0	5	6
Zfp57	ENSMUSG00000036036	0	0	1	1
Zfp457	ENSMUSG00000055341	0	0	1	1
Zfp985	ENSMUSG00000065999	0	0	1	2
Zfp951	ENSMUSG00000072774	0	0	1	1
A1987944	ENSMUSG00000056383	0	0	2	2
Zfp235	ENSMUSG00000047603	0	0	1	1
Zfp747	ENSMUSG00000054381	0	0	1	1
Zfp809	ENSMUSG00000057982	0	0	1	1

**Table S4: Reagents and Sequences**

**Primers Bisulfite sequencing**

	Primer Name	Forward Primer	Reverse Primer	Position (Assembly NCBI37/mm9)	Primer Source
Gnas	Gnas-XL_BS1	TAGTTAGTTTTTTTGTAAAGTTTATGATTTG	CTTAAACAACAAAACTAATTAACATCC	Chr 2: 174123938; 174124546	
	Gnas-XL_BS2	GGAAGTATTGGTTTTAGAGTTTTTTT	TCCATTACTTCAAACATAAATAAATCC		
Snrpn	Snrpn_BS1	TATGTAATATGATATAGTTAGAAAATTAG	AAATAACCCAACTAAAATATTTTAATC	Chr 7: 67149878; 67150301	
	Snrpn_BS1	AATTTGTGTGATGTTGTAATTTATTTGG	ATAAAATACACTTTCAGTACTAAAATCC		
H19	H19_BS1	GAGTATTTAGGAGGTATAAGAATT	ATCAAAAACCTAACATAAACCCCT	Chr 7: 149767598; 149768017	Hiura et al. 2006
	H19_BS2	GTAAGGAGATTATGTTTATTTTGG	CCTCATTATCCATAACTAT		
Kcnq1ot1	Kv-DMR_BS1	TATAAGGAAGGTTAAGAATTTATTTGAATTTG	AAATTTCTCTCTAAATCAACACRACACAAA	Chr 7: 150480959; 150481648	Tremblay et al. 199:
	Kv-DMR_BS2	GTTTTTTTTAAAGTTTTAATTTTTATATTTGAATTT	ACAAACACTCACRACAAAACAAAATAAT		
Gtl2	IG-DMR_BS1	AGATGTGTTGTGGATTTAGTTGTAG	CTAAACTCAATCTATATAATCAACACAC	Chr 12: 110766408; 110766806	
	IG-DMR_BS2	AAGTGTGTTGTGTTTATGGGTAAG	CCATCCCAATCTATAAAAATATTTTAACC		

**Primers Northern-blot probe**

	Primer Name	Forward Primer	Reverse Primer
GAPDH	GAPDH	GCACAGTCAAGGCCGAGAATGG	TGGGGCCGAGTTGGGATAGG

**Primers ChIP**

	Primer Name	Forward Primer	Reverse Primer	Position*	Primer Source
Negative	TRIM28_negative_ChIP	CTAGTCTCCTGTGCGTATGTGG	CTCAAGAGAGCCAGCCATTAG	Chr 5: 135766812; 135767045	
IAP U3	U3_ChIP	CGAGGGTGGTCTCTACTCCAT	GACGTGTGCTCCTGATTTGG	5' LTR:87 - 174 ; 3' LTR : 6817 - 6904	Rowe et al. 2010
IAP 5' LTR	IG_LTR_ChIP	TGGGGAACGAGATACCAGT	GGAACAAAAGGGCTTCTAACAC	509 - 653	
IAP pool	pool_ChIP	TGTGCCAAAAGGTAGAGATA	CCTTAATCAAGCGAAGGAAT	3755 - 3875	

\* reference IAP sequence RP23-92L23

**Sequence of the sgRNAs targeting the U3 region of the IAP transposons LTRs**

sgRNA	Sequence	5' LTR	3' LTR
sgRNA_IAP_U3_#1	CCATCTGTAAACGGCGAATG	19 - 38	6749 - 6768
sgRNA_IAP_U3_#2	ATAATCTGGCGCATGTGCCGA	70 - 89	6800 - 6819
sgRNA_IAP_U3_#3	GTGACGTCAACTCGGCCGAT	125 - 144	6855 - 6874
sgRNA_IAP_U3_#4	AATTCCTTTAATAGGGACG	188 - 207	6918 - 6937

\* reference IAP sequence RP23-92L23

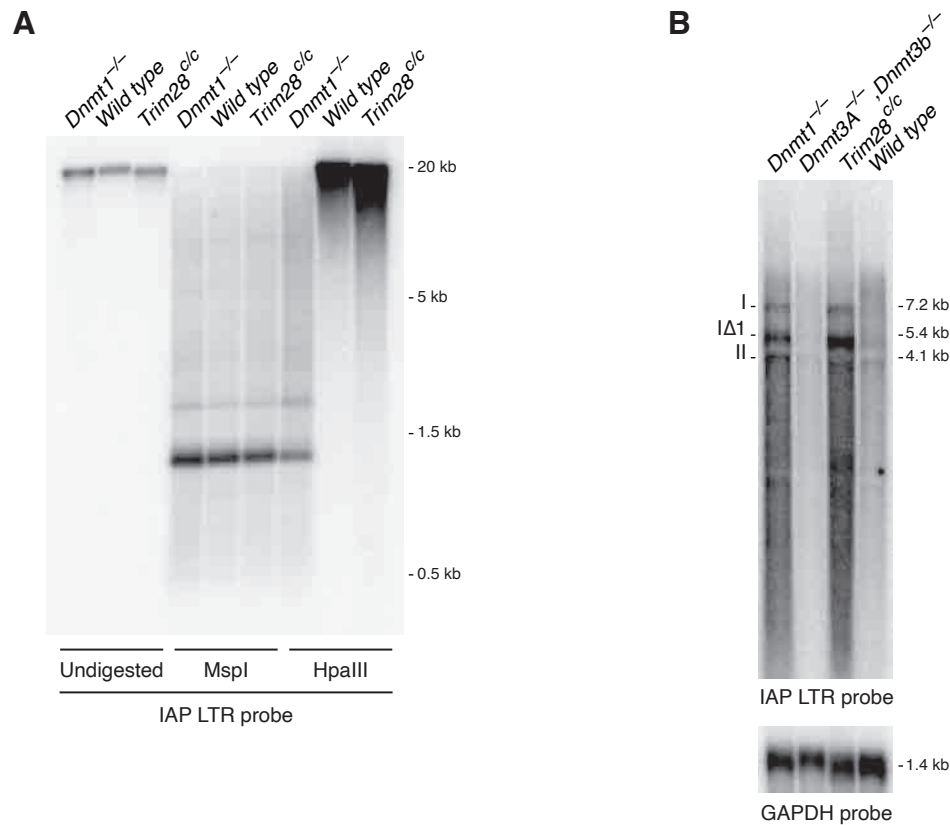
Antibody	Source	Identifier	Application
TRIM28	EMD Millipore	MAB3662	IP - ChIP
O-GlcNAc	Thermo Fisher Scientific	HGAC85	ChIP-seq
TRIM28	Abcam	ab10483	WB
OGT	Santa Cruz	SC-92921	WB
O-GlcNAc	Santa Cruz	SC-59623	WB
TUBULIN	Sigma	T6199	WB
FLAG	Sigma	F3165	WB
DNMT1	Santa Cruz	SC-20701	WB
DNMT3A	Bethyl Laboratories	A-304-278A	WB
DNMT3B	Novus	52A1018	WB
CHD3	Cell Signaling	4241	WB
CHD4	Sigma	SAB4200107	WB
BRG1	Cell Signaling	D1Q7F	WB
ZNF198	Bethyl Laboratories	A301-711A	WB
SMARCA5	EMD Millipore	ABE1026	WB
SF3B1	Thermo Fisher Scientific	PA5-19679	WB
SIN3A	EMD Millipore	MABE607	WB
SETDB1	Proteintech	11231-1-AP	WB
G9A	Cell Signaling	3306	WB
PARP1	Cell Signaling	9532	WB
LSD1	Abcam	Ab17721	WB
CTCF	Cell Signaling	3418	WB
SUV39H1	Cell Signaling	8729	WB
HDAC2	Cell Signaling	5113	WB
HDAC1	Cell Signaling	5356	WB
HP1α	EMD Millipore	05-669	WB
Histone H3.3	EMD Millipore	09-638	WB
Histone H2A.Z	Gift from Stefan Dimitrov		WB
Histone H2A.X	Cell Signaling	7631	WB
Histone H3	Abcam	Ab1791	WB
Histone H2A	EMD Millipore	ABE327	WB
Histone H2B	EMD Millipore	07-371	WB
Histone H4	Thermo Fisher Scientific	MA5-14816	WB
NANOG	Abcam	Ab80892	WB

**Bisulfite sequencing: SNPs positions**

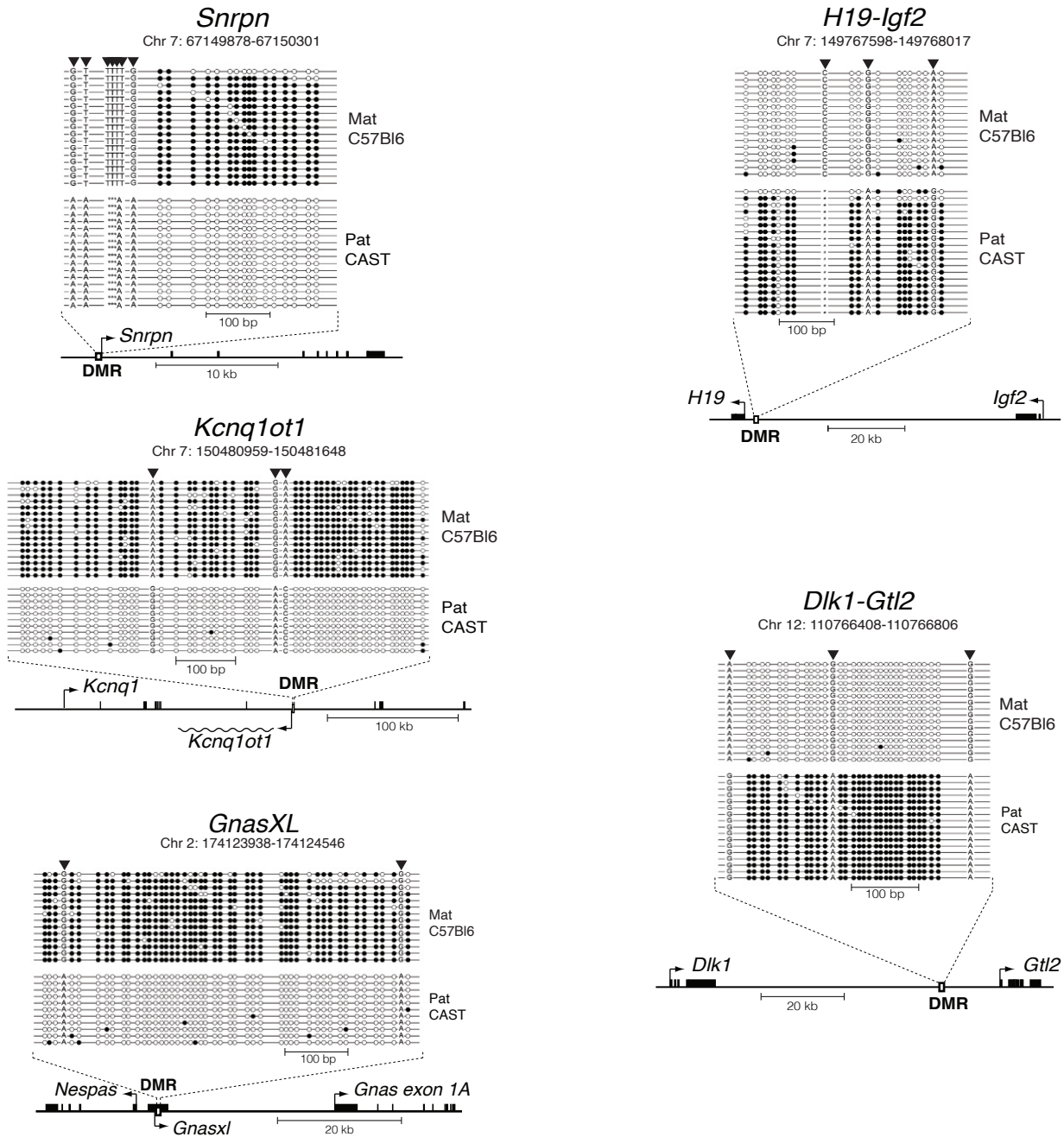
ICR	SNP Position (Assembly NCBI37/mm9) BIG	CAST
Gnas	Chr 2:174,123,975	G
	Chr 2:174,124,504	G
	Chr 7:67,150,273	G
	Chr 7:67,150,263	T
Snrpn	Chr 7:67,150,203	G
	Chr 7:67,150,193	TTT
	Chr 7:67,150,183	G
	Chr 7:67,150,103	G
H19	Chr 7:149,767,840	G
	Chr 7:149,767,759	G
	Chr 7:149,767,672	A
Kv-DMR	Chr 7:150,481,047	A
	Chr 7:150,481,242	GGA
IG-DMR	Chr 12:110,766,358	A
	Chr 12:110,766,469	G
	Chr 12:110,766,665	G

\* deletion

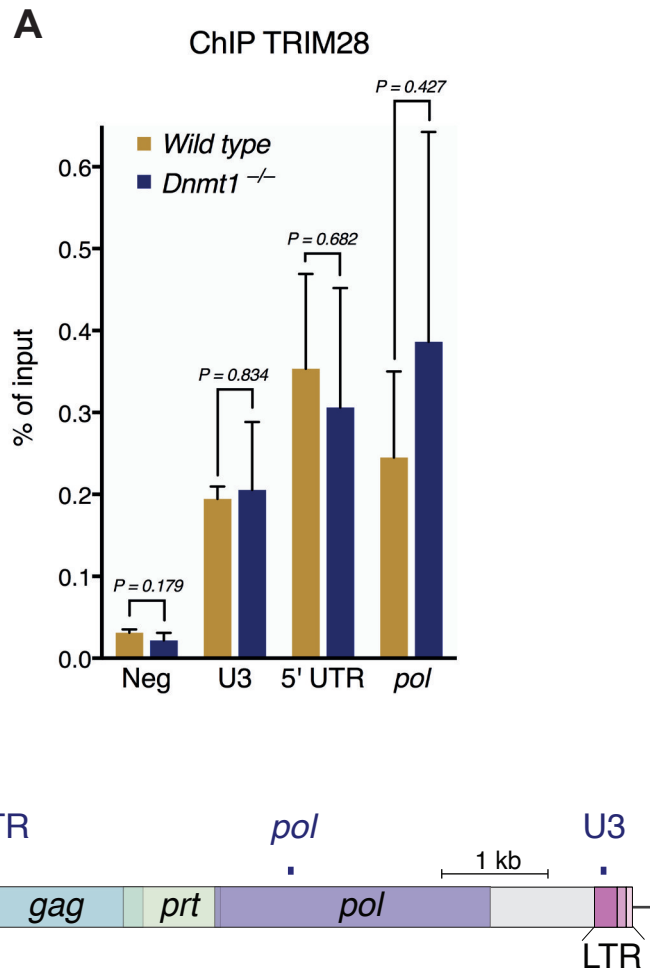
## SUPPLEMENTARY FIGURES



**Fig. S1. The protein TRIM28 is necessary to repress transcription of methylated retrotransposons.** The *Chatwo* or *C* allele of *Trim28* was ENU-induced and bears two adjacent non-conservative amino acid substitutions and has a similar but slightly less severe phenotype than the null allele (1, 2). (A) Methyl-sensitive southern-blot showing dense cytosine methylation at IAP (intracisternal A-type particles) LTR (long terminal repeats) in embryos bearing the homozygous mutation *Chatwo* (*Trim28<sup>C/C</sup>*). Genomic DNA extracted from wild type embryos is digested by the methylation-insensitive restriction enzyme MspI, but is not digested by its methylation sensitive isoschizomer HpaIII. Genomic DNA isolated from *Dnmt1<sup>-/-</sup>* ES cells is fully digested by HpaIII, reflecting global DNA demethylation. In contrast, DNA extracted from *Trim28<sup>C/C</sup>* embryos is refractory to cleavage by HpaIII, showing that IAP LTRs are densely methylated in *Trim28<sup>C/C</sup>* embryos. (B) Transcriptional activation of IAP retrotransposons in *Trim28<sup>C/C</sup>* embryos. RNA blot hybridized with IAP LTR probes showing that IAPs are expressed at the same high levels in *Trim28<sup>C/C</sup>* embryos and *Dnmt1<sup>-/-</sup>* cells; the spectrum of IAP subtypes reactivated is very similar. In contrast, IAP retrotransposons are repressed in wild type littermate embryos and early passage *Dnmt3a<sup>-/-</sup>*, *Dnmt3b<sup>-/-</sup>* ES cells (normally methylated at IAP). Expression of methylated IAP retrotransposons in *Trim28<sup>C/C</sup>* embryos indicates that DNA methylation requires TRIM28 protein to repress transcription.

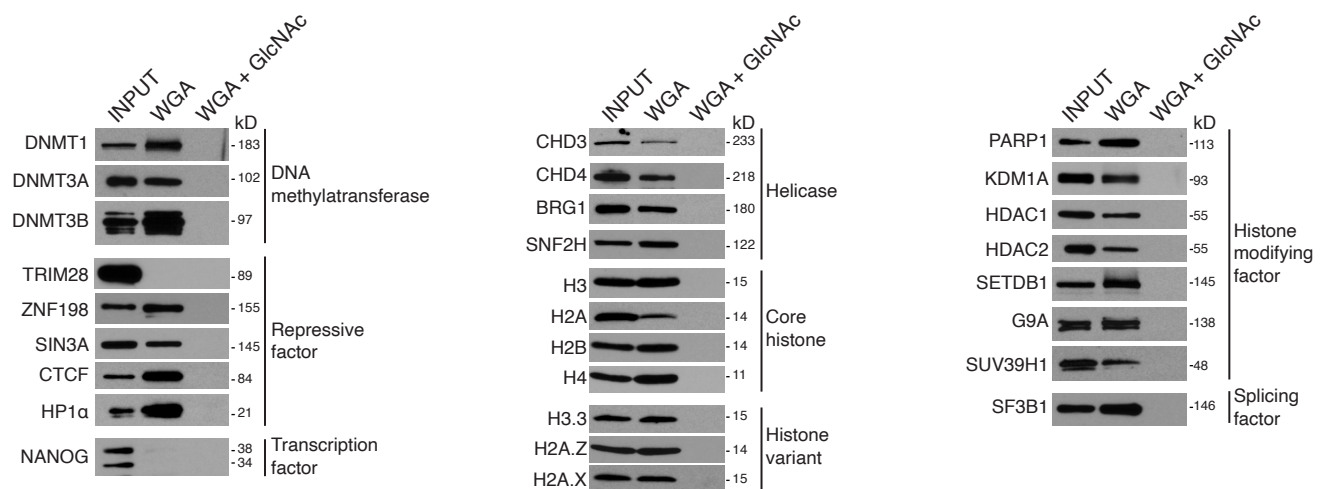


**Fig. S2. Loss of imprinted expression in *Trim28<sup>C/C</sup>* embryos occurs despite normal methylation at differentially methylated regions (DMRs).** Normal mono-allelic methylation at imprinting control regions DMRs in *Trim28<sup>C</sup>* homozygous embryos that show bi-allelic expression of imprinted genes and derepression of methylated IAP retrotransposon. The DMRs of the *H19-Igf2* and *Dlk1-Gtl2* reciprocally imprinted gene pairs are paternally (Pat) methylated; *Snrpn*, *Kcnq1ot1* and *GnasXL* are maternally (Mat) methylated. The methylation status of each CpG site within the five analyzed DMRs was determined by bisulfite sequencing from single *Trim28<sup>C/C</sup>* embryos. The parent of origin of the alleles was determined using single nucleotide polymorphisms in C57Bl6J x M. m. castaneus (CAST) F1 hybrids as indicated by arrows. Open and filled circles respectively represent unmethylated and methylated CpG sites. The previously reported bi-allelic expression of *H19*, *Gtl2*, *Snrpn*, *Kcnq1ot1* and *GnasXL* in *Trim28<sup>C/C</sup>* homozygous embryos (2) indicates that TRIM28 protein is crucial for the monoallelic expression of imprinted genes.

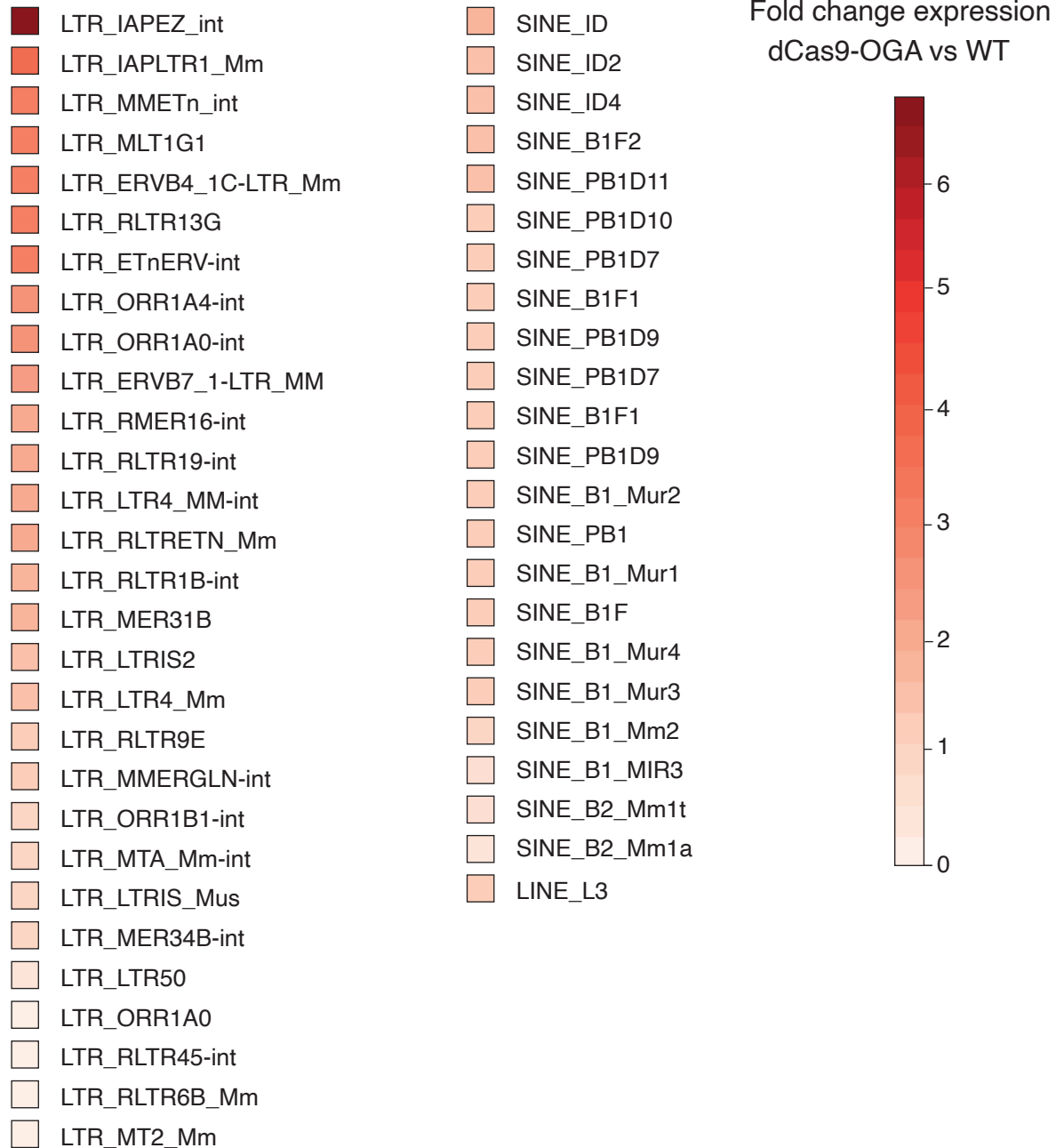


**Fig. S3. Demethylation does not cause dissociation of TRIM28 from IAP retrotransposon sequences.** (A) ChIP-qPCR showing high occupancy of TRIM28 at IAP LTRs in both wild type (normally methylated) and *Dnmt1*<sup>-/-</sup> ES cells (demethylated). The binding of TRIM28 to unmethylated IAP sequences indicates that its recruitment to retrotransposons is independent of DNA methylation (see Fig. S7). This is comparable to the case in *Drosophila* where *Hox* genes are derepressed in the absence of OGT/Scx despite normal binding of Polycomb factors (3). (B) The positions of the primer sets used for real-time PCR quantification of DNA binding are indicated as black squares on the schematic representation of an IAP element. Sequence coordinates are in Table S4. Results are presented as a relative enrichment as compared to an arbitrarily chosen genomic locus (Chr5: 135766812-135767045 in mm10). Bar represents means and standard deviations of three biological replicates. P values were calculated using an unpaired *t*-test.

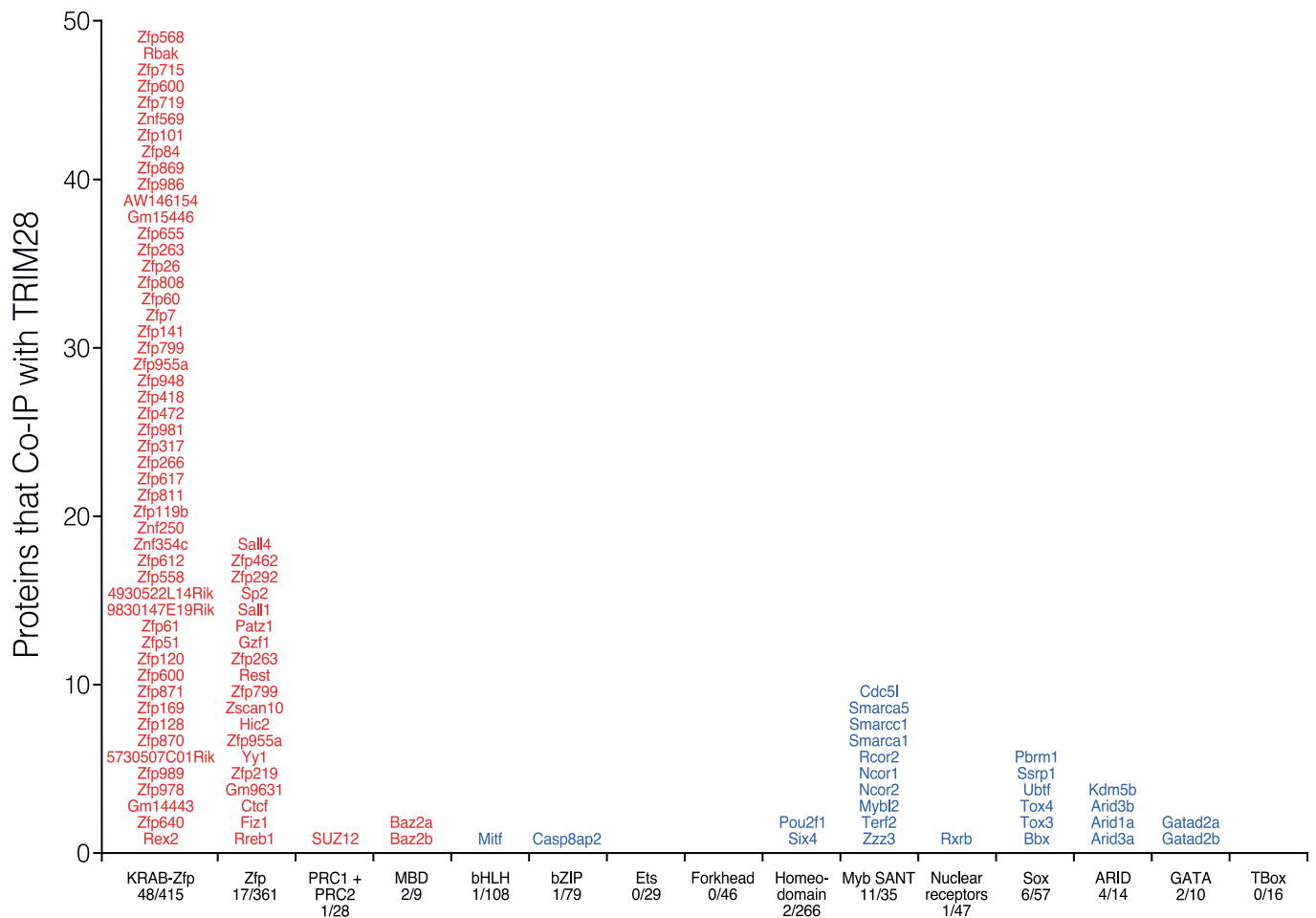




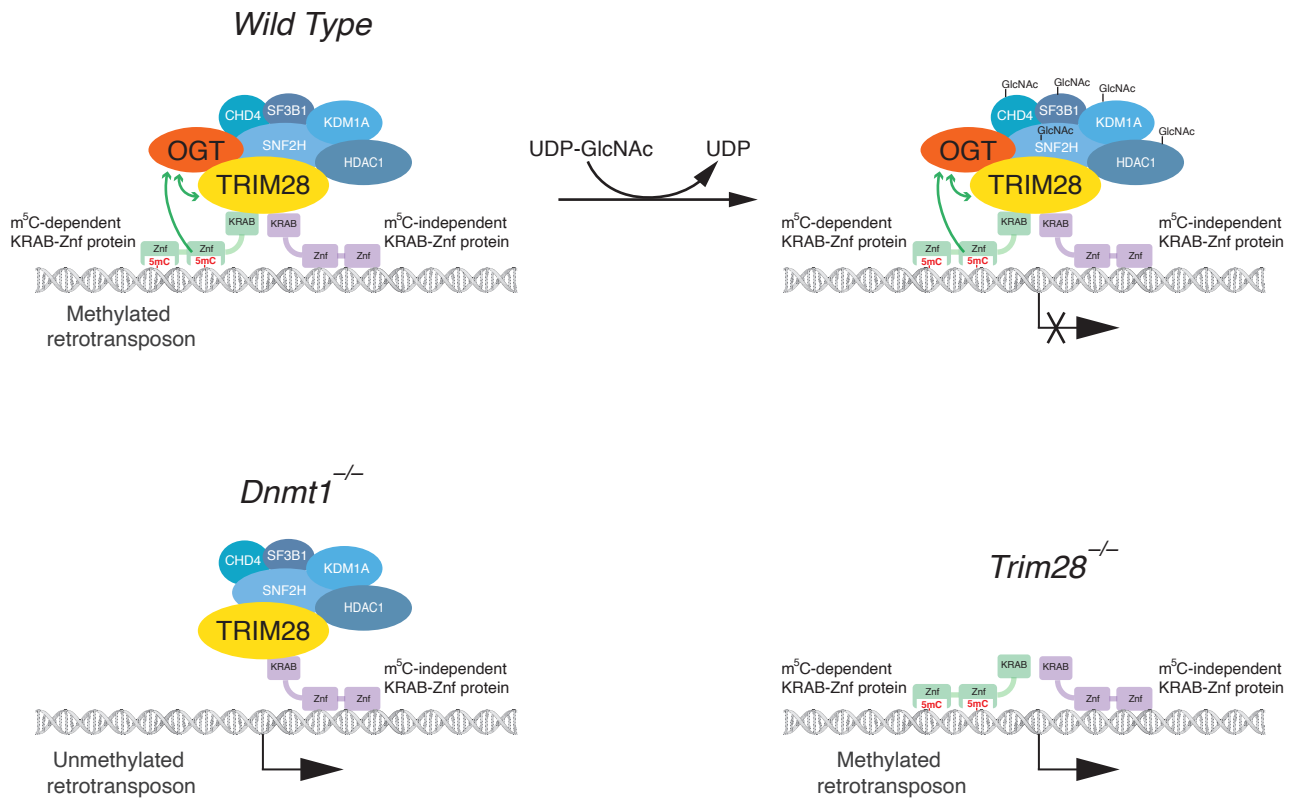
**Fig. S4. O-GlcNAcylation of proteins associated with TRIM28.** Nearly all chromatin-associated proteins tested were O-GlcNAcylated in wild type ES cells, as assessed by binding to the O-GlcNAc specific lectin wheat germ agglutinin (WGA); a notable exception is TRIM28 itself. NANOG had been previously reported to lack O-GlcNAcylation (4), as confirmed here. Specificity of binding of WGA to O-GlcNAcylated proteins was evaluated by measuring inhibition of binding by free N-Acetylglucosamine (GlcNAc). Gel loading from left to right, first lane: input, second lane: WGA purification, third lane: WGA purification in presence of free GlcNAc.



**Fig. S5. Specific reactivation of IAPEz transposons upon targeting of dCas9-OGA to their LTRs.** Heat map showing the expression fold change of all families of transposons in ES cells expressing dCas9-OGA and four sgRNA against IAPEz LTRs. Transposons families with fold change equal to zero are not shown. IAPEz is the only type of transposons that substantially reactivates after targeted de-GlcNAcylation of proteins bound to their LTRs.



**Fig. S6. Predominance of proteins with KRAB and Zinc finger domains among the DNA binding proteins found to Co-IP with TRIM28 by mass spectrometry.** TRIM28 primarily associates with KRAB and Zinc finger domains proteins. Above each category are the names of the TRIM28-associated transcription factors.



**Fig. S7. Model.** Top: Schematic representation of the interactions between methylated LTRs and the TRIM28-OGT repressive complex. TRIM28 and local O-GlcNAcylation are both required for stable silencing of methylated retrotransposons. OGT catalyzes the linkage of O-GlcNAc on serine and threonine residues of several repressors complexed with TRIM28/OGT, including CHD4, SF3B1, KDM1A, SNF2H and HDAC1. The binding of TRIM28 to retrotransposons' LTRs occurs independently of their methylation state while OGT's recruitment to the TRIM28 complex and subsequent O-GlcNAcylation of associated proteins depend both on cytosine methylation. The identity of the transcription factor(s) that recruit TRIM28 and OGT to methylated transposons is unknown. TRIM28 is complexed with a plethora of KRAB-Znfs, the largest family of transcription factors. Most of KRAB-Znfs have unknown binding motif but structure studies showed that  $\sim 1/3$  have features compatible with recognition of 5-methyl cytosines (5). Based on this evidence, we propose that one or several methylation-independent KRAB-Znfs recruit TRIM28 while other(s) methylation-dependent KRAB-Znfs are responsible for OGT recruitment to the repressive complex. Note that the protein interactions depicted in the scheme are not necessarily direct. Bottom: Schematic representation of possible disruptions of the repressive complex in *Dnmt1*<sup>-/-</sup> and *Trim28*<sup>-/-</sup> cells. Both mutations cause a pervasive reactivation of transposons and bi-allelic expression of imprinted genes. Local de-O-GlcNAcylation of chromatin at LTRs phenocopies *Trim28*<sup>-/-</sup>: retrotransposons undergo release from silencing without loss of DNA methylation.

## SUPPLEMENTARY METHODS

### *DNA methylation analysis by methylation-sensitive restriction enzyme*

Genomic DNA was isolated from a pool of three E8.5 dpc *Trim28<sup>C/C</sup>* embryos or from  $1 \times 10^6$  ES cells and quantified using Qubit Fluorometric Quantitation (Thermo Fisher Scientific). Genomic DNA was digested with either the methylation-sensitive endonuclease HpaII or its methylation insensitive isoschizomer MspI (New England Biolabs). The southern blot protocol and the probes were previously described<sup>6</sup>. The ENU-induced *Trim28<sup>C</sup>* mutation was previously described<sup>1,2</sup>.

### *DNA methylation analysis by bisulfite sequencing*

Genomic DNA extracted from a single E8.5 embryo homozygous for *Trim28<sup>C</sup>* and heterozygous for C57Bl6 and M.m castaneus SNPs at imprinting control regions (ICRs) was bisulfite converted using the EZ DNA Methylation Kit (Zymo Research). ICRs were amplified with 2 rounds of nested PCR (primers sequences SI Appendix table S4). The PCR product resulting from the second round of PCR was cloned into the pJET vector (Thermo Fisher Scientific). Approximately 40 positive clones for each ICR were sequenced and all sequences had a conversion rate >95%. The parental origin of each ICR was determined on the basis of its strain-specific SNP content according to <http://www.sanger.ac.uk/science/data/mousegenomes-project>. Sequences were analyzed with Quma online software<sup>7</sup>.

### *Chromatin Immunoprecipitation followed by QPCR*

ChIP-QPCR of TRIM28 was performed on formaldehyde cross-linked chromatin.  $10^8$  ES cells were fixed for 10 min at room temperature with 1.1% formaldehyde and quenched with 125 mM glycine. Soluble chromatin isolated as previously described<sup>8</sup> was sheared by sonication to an average size of 500-1,000 bp (Branson sonicator). The chromatin was then diluted to a concentration of 1 mg/ml. Immunoprecipitation was carried out overnight at 4°C with 3 µg of monoclonal antibody to TRIM28 (EMD Millipore, MAB3662) bound to 10 µl Dynabeads conjugated with protein G (Life Technologies). Beads were washed and chromatin eluted as described previously<sup>8</sup>. Enrichment compared to input was analyzed by qPCR using the primers listed in SI Appendix Table. S4. The experiment was performed in biological triplicate and p-values were calculated with the unpaired *t*-test.

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