

Supplemental Materials

Molecular Biology of the Cell

Meredith et al.

Table S1

Gsk-3, DNA Methylation and Imprinting

Sample	Total Reads	All mapped (%)	# Uniquely Mapped	% Uniquely Mapped	# Unique Starts	% Genome >= 1X Unique Starts	# CpG Islands covered (of 16,026)	% CpG Islands covered
WT_Input	80,620,652	64.23%	40,441,038	50.16%	33,872,997	43.43%	11,820	73.8%
DKO_Input	83,792,353	66.57%	43,386,204	51.78%	38,628,827	47.66%	14,498	90.5%
WT_600mM	83,005,355	58.45%	32,715,506	39.41%	25,419,053	20.90%	6,581	41.1%
DKO_600mM	81,626,406	51.39%	27,519,004	33.71%	20,050,704	19.45%	2,148	13.4%
WT_2M	86,206,306	45.77%	12,379,948	14.36%	6,812,074	4.73%		
WT_2M_repl	83,373,887	53.12%	14,120,501	16.94%	8,034,671	5.05%		
WT_2M_pooled					13,147,662		4,760	29.7%
DKO_2M	86,443,789	49.53%	15,077,254	17.44%	7,972,570	4.66%		
DKO_2M_repl	54,240,005	54.43%	10,119,643	18.66%	6,008,450	4.04%		
DKO_2M_pooled					11,236,322		3,940	24.6%

Table S1. MBD-seq sequencing mapping and coverage statistics.

A. Hypomethylated DMRs in **2M fraction**, where DKO samples have fewer reads than WT:

Number of DMRs in set: **29,029**. Total size: **12.7 Mb** (0.50% of genome)

Mean region size: **439 bp**.

Mean score: **128**; SD=**121**

- DMRs scoring 1 standard deviations or higher than the mean (1SD), i.e. scoring > 249
Number = **2,545**. Mean region size = **609 bp** (SD = 318). Total size = **1.6 Mb**
- DMRs scoring 2 standard deviations or higher than the mean (2SD), i.e. scoring > 370
Number = **993**. Mean region size = **688 bp** (SD = 377). Total size = **683 Kb**

B. Hypomethylated DMRs in **600mM fraction**, where DKO samples have fewer reads than WT:

Number of DMRs in set: **40,404**. Total size: **30.5 Mb** (1.19% of genome)

Mean region size: **756 bp**.

Mean score: **84**; SD=**48**

- DMRs scoring 1 standard deviations or higher than the mean (1SD), i.e. scoring > 132
Number = **3,929**. Mean region size = **1,218 bp** (SD = 716). Total size = **4.79 Mb**
- DMRs scoring 2 standard deviations or higher than the mean (2SD), i.e. scoring > 180
Number = **1,540**. Mean region size = **1,469 bp** (SD = 848). Total size = **2.26 Mb**

Table S2. DMR identification in *Gsk-3* DKO ESCs. This table describes the process used to select top scoring hypomethylated DMR candidate regions for further study and evaluation. We used peak-finding software (MACS) to obtain regions ("peaks") with significant differences in read pile-ups between WT and DKO samples as candidate DMR regions. We ranked these regions using significance scores returned by MACS (based on p-values), and obtained sets of regions scoring one or two standard deviations (SD) higher than the mean score of all regions.

Table S3

Gsk-3 Regulates DNA Methylation at Imprinted Loci

Locus	DMR Location	Locus	DMR Location
Gpr1	chr1:63,298,864-63,300,102	Th	chr7:149,975,435-149,976,040
Zdbf2	chr1:63,298,864-63,300,102	Ascl2	chr7:150,195,385-150,195,826
Sfmbt2	chr2:10,622,899-10,623,741	Tspan32	chr7:150,195,385-150,195,826
Wt1	chr2:104,968,308-104,969,763	Cd81	chr7:150,246,982-150,247,324
H13	chr2:152,512,655-152,513,514	Tssc4	chr7:150,259,758-150,260,051
Mcts2	chr2:152,512,655-152,513,514	Kcnq1	chr7:150,480,885-150,482,591
Nnat	chr2:157,385,135-157,386,439		chr7:150,560,917-150,562,567
Blcap	chr2:157,385,135-157,386,439	Kcnq1ot1	chr7:150,480,885-150,482,591;
Gnas	chr2:174,118,256-174,126,849;	Cdkn1c	chr7:150,699,793-150,701,044
	chr2:174,152,116-174,152,972	AF313042	chr7:150,699,793-150,701,044
Nespas	chr2:174,118,256-174,126,849;	Slc22a18	chr7:150,699,793-150,701,044
	chr2:174,152,116-174,152,972	Phlda2	chr7:150,699,793-150,701,044
Casd1	chr6:4,608,663-4,609,634	Nap114	chr7:150,699,793-150,701,044
Sgce	chr6:4,608,663-4,609,634	Tnfrsf23	chr7:150,941,307-150,941,730
	chr6:4,695,944-4,698,983	Osbp15	chr7:150,941,307-150,941,730
Peg10	chr6:4,695,944-4,698,983	Dhcr7	chr7:150,966,523-150,968,091
Ppp1r9a	chr6:4,958,718-4,958,993	Rasgrf1	chr9:89,776,508-89,776,680
Pon3	chr6:5,353,264-5,353,686	Mst1r	chr9:107,848,431-107,850,404
Pon2	chr6:5,353,264-5,353,686	Plagl1	chr10:12,809,612-12,811,939;
Asb4	chr6:5,353,264-5,353,686		chr10:12,831,574-12,832,237;
Dlx5	chr6:6,817,030-6,817,429		chr10:12,846,884-12,847,847
Mest	chr6:30,685,029-30,689,099	Ddc	chr11:11,789,864-11,790,618
Copg2	chr6:30,685,029-30,689,099	Grb10	chr11:11,832,325-11,833,753;
Klf14	chr6:30,907,440-30,908,266		chr11:11,925,311-11,926,083
Nap115	chr6:58,856,407-58,857,273	Cobl	chr11:11,925,311-11,926,083
Copg2as1	chr6:58,856,407-58,857,273	Zrsr1	chr11:22,871,632-22,874,229
Zim1	chr7:6,680,992-6,682,247	Commd1	chr11:22,871,632-22,874,229
Peg3	chr7:6,680,992-6,682,247	Begain	chr12:110,292,235-110,293,319;
Apeg3	chr7:6,680,992-6,682,247		chr12:110,311,322-110,311,926
Zim2	chr7:6,680,992-6,682,247	DIK1	chr12:110,665,993-110,666,474;
Usp29	chr7:6,680,992-6,682,247		chr12:110,674,189-110,676,117
Zim3	chr7:6,680,992-6,682,247	Meg3	chr12:110,665,993-110,666,474;
Zfp264	chr7:6,680,992-6,682,247		chr12:110,674,189-110,676,117
Ndn	chr7:69,493,426-69,494,341;	Rtl1	chr12:110,665,993-110,666,474;
	chr7:69,522,555-69,525,773		chr12:110,674,189-110,676,117
Magel2	chr7:69,493,426-69,494,341;	Rian	chr12:110,665,993-110,666,474;
	chr7:69,522,555-69,525,773		chr12:110,674,189-110,676,117
Mkrn3	chr7:69,493,426-69,494,341;	Dio3	chr12:111,517,494-111,518,439
	chr7:69,522,555-69,525,773		chr12:111,519,395-111,520,241
Peg12	chr7:69,493,426-69,494,341;	Kcnk9	chr15:72,375,760-72,376,828
	chr7:69,522,555-69,525,773	Trappc9	chr15:72,640,285-72,641,813
Art5	chr7:109,250,791-109,251,094	Peg13	chr15:72,640,285-72,641,813
Ampd3	chr7:117,920,949-117,921,556	Slc38a4	chr15:96,858,968-96,859,582;
Inpp5f	chr7:135,673,289-135,675,023		chr15:96,883,391-96,883,715
H19	chr7:149,765,531-149,768,335	Fbxo40	chr16:36,989,502-36,990,653
Igf2as	chr7:149,825,743-149,826,030	Igf2r	chr17:12,934,068-12,936,251
Igf2	chr7:149,825,743-149,826,030	Airn	chr17:12,934,068-12,936,251
Ins2	chr7:149,975,435-149,976,040	Impact	chr18:13,131,195-13,132,471

Table S3. Gsk-3-dependent DMRs at imprinted loci. Imprinted genes in the mouse are shown, along with the chromosomal coordinates for Gsk-3-dependent DMRs that are located within 250 kb of a given imprinted locus. Maternally imprinted genes are in **bold**, while paternally imprinted genes are not.

Table S4

Gsk-3, DNA Methylation and Imprinting

	<u>ID</u>	<u>p-value</u>
<u>GO: Molecular Function</u>		
Sequence-specific DNA binding	GO:0043565	4.29x10 ⁻¹⁷
Nucleic acid binding transcription factor activity	GO:0001071	1.11x10 ⁻¹⁶
Sequence-specific DNA binding transcription factor activity	GO:0003700	1.34x10 ⁻¹⁶
Regulatory region DNA binding	GO:0000975	8.07x10 ⁻¹⁴
<u>GO: Biological Process</u>		
Cell development	GO:0048468	1.82x10 ⁻²⁷
Regulation of developmental process	GO:0050793	4.96x10 ⁻²⁶
Generation of neurons	GO:0048699	6.98x10 ⁻²⁴
Neuron differentiation	GO:0030182	1.08x10 ⁻²²
<u>GO: Cellular Component</u>		
Cell junction	GO:0030054	1.51x10 ⁻²⁰
Cell projection	GO:0042995	8.47x10 ⁻¹⁹
Neuron projection	GO:0043005	2.15x10 ⁻¹³
Synapse	GO:0045202	2.90x10 ⁻¹³
<u>Mouse Phenotype</u>		
Perinatal lethality	MP:0002081	1.30x10 ⁻⁹
Neonatal lethality	MP:0002058	5.60x10 ⁻⁸
<u>Domain</u>		
PH_type	IPR011993	1.59x10 ⁻⁶
Homeodomain-like	IPR009057	5.70x10 ⁻⁶
Homeobox	IPR001356	1.00x10 ⁻⁵
Prot_kinase_cat_dom	IPR000719	7.38x10 ⁻⁵
<u>Interaction</u>		
MAPK1 interactions	int:MAPK1	3.83x10 ⁻⁸
SMAD3 interactions	int:SMAD3	1.19x10 ⁻⁷
SMAD2 interactions	int:SMAD2	1.24x10 ⁻⁵
<u>Transcription Factor Binding Site</u>		
V\$SREBP1_Q6		2.98x10 ⁻⁹
GGGTGGRR_V\$PAX4_03		4.59x10 ⁻⁷
V\$MYOD_Q6_01		1.86x10 ⁻⁶
<u>Gene Family</u>		
Forkhead box genes	FOX	6.66x10 ⁻¹²
Calcium channels	CACN	8.89x10 ⁻¹⁰
CD molecules	CD	2.30x10 ⁻⁹
Potassium channels	KCN	1.21x10 ⁻⁷
T-box gene family	TBX	3.17x10 ⁻⁵
<u>Drug</u>		
2-(1'H-indolo-3'-carbonyl)thiazole-4-carboxylic acid methyl ester	C548651	1.84x10 ⁻¹⁴
Raloxifene	D020849	4.58x10 ⁻¹¹
Mustard Gas	D009151	6.78x10 ⁻¹¹

Table S4. Summary of ToppFun functional enrichment analysis/600mM fraction. Represents the enrichment analysis of 4085/4566 genes located near a *Gsk-3*-dependent DMR from the 600mM fraction.

Table S5

Gsk-3, DNA Methylation and Imprinting

Maternal DMRs

Uniparental DMR coordinates	Gsk-3 DMR Peak #	Gene
chr1:5905383-5908212	22	Npbwr1
chr1:13114802-13119362	52	Prdm14
chr1:36827335-36829099	421	Zap70
chr1:82282756-82286767	761, 1117	Irs1
chr1:91828501-91832261	891	Gbx2
chr1:122496649-122502023	1089, 1091, 1093, 1875	En1§
chr1:122509797-122511972	1093	En1
chr1:123423867-123425684	1889	Htr5b
chr2:24949781-24951802	29864	Noxa1
chr2:25431496-25436082	29933, 29934	Gm996
chr2:61641254-61645892	19385	Tbr1
chr2:70404282-70406773	19463	Gad1
chr2:105499606-105502745	31982	Pax6os1
chr2:130401438-130403410	32467	Oxt
chr2:152511536-152513972	20342	H13*
chr2:157384621-157386876	20482	Blcap*
chr2:170352524-170354950	21040, 21041	Pfdn4
chr2:174111275-174114120	21206	Nespas/Gnas*§
chr2:174119780-174123613	21207, 21208	Nespas/Gnas*§
chr2:174151537-174153945	34326	Nespas/Gnas*
chr2:181404926-181407123	34587	Sox18
chr3:34538842-34542342	21497	Sox2ot
chr3:55583532-55588393	35134	Nbea/Mab211
chr4:44722763-44724578	23408, 37307	Pax5
chr5:72090742-72092688	42818	Gabrb1
chr5:77284231-77288593	42915	C530008M17Rik
chr5:102075504-102078013	26696	Nkx6-1
chr5:108116041-108118198	26832	Rpap2/Gf11
chr5:120110935-120114064	27442, 44255	Tbx3
chr5:127722742-127724470	27777, 44904	Tmem132c
chr5:135725651-135726630	45256	Fzd9
chr6:4695855-4698573	28734, 28736	Sgce/Peg10*
chr6:7503951-7507696	28785	Tac1
chr6:17979126-17983000	46542	Wnt2
chr6:21935187-21937333	46554	A430107O13Rik
chr6:30682455-30689986	28961-28963, 46714	Mest/Copg2*§
chr6:36337103-36339692	46856	9330158H04Rik/Chrm2
chr6:58856078-58857635	29381	Herc3
chr6:97008931-97010684	48139	Fam19a4
chr7:6680830-6684822	49555	Peg3/Usp29*§
chr7:50861975-50863601	31333	Zfp819
chr7:117769427-117773742	32130, 52490	Adm
chr7:129814087-129815764	52845	Cacng3
chr7:146768977-146771866	32592, 32594, 53442, 53443	Nkx6-2
chr7:148477303-148479376	53545	Drd4
chr7:150480167-150483190	32676	Kcnq1*§
chr9:37234527-37238049	58061, 58064	Robo3
chr9:50558533-50560579	35373	Hspb2
chr10:12809265-12811775	2329, 2330	Plagl1*
chr10:22535410-22540678	2450, 3828, 3829	Tcf21
chr10:126614413-1266181164	4277, 6229, 6230	Slc26a10
chr11:11925468-11927317	4504	Grb10*

Table S5

Gsk-3, DNA Methylation and Imprinting

chr11:22872001-22874562	4594	Commd1/Zrsr1*
chr11:53275894-53280607	7512	Shroom1
chr11:53840838-53843040	7558	Slc22a4
chr11:57640106-57645991	7652	Hand1
chr11:72174904-72176140	5338	Tekt1
chr11:85700158-85703236	9116	Tbx4
chr11:102006426-102009769	10176	Nags
chr12:106460263-106461618	8296	Tcl1
chr12:112509517-112511274	13745, 13746	Amn
chr13:31705647-31708761	14384	Foxf2
chr14:41706520-41708821	10472	Sh2d4b
chr14:109309909-109313898	11371, 18081	Slitrk1
chr15:61868846-61869996	12080	Pvt1
chr15:72375688-72376760	19053	Kcnk9*
chr15:72639497-72641974	12892	Slc38a4*
chr16:91219522-91221287	22818	Olig2
chr16:91223697-91227175	14293, 14294, 22818	Olig2
chr16:92694160-92697032	14367	Runx1
chr17:12933946-12936040	14675, 23317	Igf2r/Airn*
chr17:46865300-46867019	25072	Gnmt
chr17:48231878-48234471	25213	1700067P10Rik
chr18:13130142-13133748	16273, 16274	Impact*
chr18:37105549-37108384	26654	Pcdha1/Pcdha3
chr18:37119559-37123000	16543, 16545, 26655	Pcdha1
chr18:37965297-37968313	16595	Pcdha4-g
chr18:66616506-66621075	17024, 17026	Pmaip1
chr18:72508900-72511335	17125, 17126, 27241	Dcc
chr19:37770240-37774549	28750	Cyp26a1
chr19:38598415-38600381	18019	Plce1
chr19:59541485-59544578	18641	Emx2

Paternal DMRs

chr1:75443155-75446409	1027, 1028 1029	Accn4
chr2:157800398-157801233	20492	Tti1
chr3:101887060-101888794	36010	Casq2
chr6:52129838-52131977	29270, 47165	Hoxa3
chr7:139999997-140001706	53273	Fam53b
chr8:123598383-123603913	56848, 56853	Foxf1a
chr8:123643112-123647535	56862	Foxc2
chr11:84341550-84343316	9054	1500016L03Rik
chr12:9582586-9583557	7191, 11923	Osr1
chr12:74151214-74153184	12569	Six1
chr13:53328329-53332024	14879, 14881	Ror2
chr17:46755575-46756422	25050	Ptk7
chr19:57192913-57193457	18568, 29454	Ablim1

Table S5. List of DMRs in common between *Gsk-3* DKO ESCs and uniparental ESCs. Genes are listed according to whether the differential methylation was maternal or paternal in the uniparental ESCs. The genomic coordinates of the uniparental DMRs are shown, along with the peak numbers assigned to the DMRs from *Gsk-3* DKO ESCs. The corresponding gene is listed on the right. (*) denotes known imprinted genes. (§) denotes DMRs validated by bisulfite sequencing in this study.

Table S6

Gsk-3, DNA Methylation and Imprinting

<u>Gene</u>	<u>Fold change</u>	<u>DMR location</u>
Art5	0.339	chr7:109,250,791-109,251,094
Begain	0.173	chr12:110,292,235-110,293,319; chr12:110,311,322-110,311,926
Casd1	0.421	chr6:4,608,663-4,609,634
Cdkn1c	0.039	chr7:150,699,793-150,701,044
Cobl	0.428	chr11:11,925,311-11,926,083
Dhcr7	2.692	chr7:150,966,523-150,968,091
Dio3	0.296	chr12:111,517,494-111,518,439 chr12:111,519,395-111,520,241
Dlx5	0.083	chr6:6,817,030-6,817,429
Gpr1	0.268	chr1:63,298,864-63,300,102
Grb10	0.015	chr11:11,832,325-11,833,753; chr11:11,925,311-11,926,083
Igf2as	0.193	chr7:149,825,743-149,826,030
Ins2	0.239	chr7:149,975,435-149,976,040
Kncq1	0.009	chr7:150,480,885-150,482,591 chr7:150,560,917-150,562,567
Klf4	0.446	chr6:30,907,440-30,908,266
Magel2	0.257	chr7:69,493,426-69,494,341; chr7:69,522,555-69,525,773
Nespas	3.572	chr2:174,118,256-174,126,849; chr2:174,152,116-174,152,972
Peg10	2.158	chr6:4,695,944-4,698,983
Peg3	0.220	chr7:6,680,992-6,682,247
Phlda2	0.487	chr7:150,699,793-150,701,044
Plagl1	6.131	chr10:12,809,612-12,811,939; chr10:12,831,574-12,832,237; chr10:12,846,884-12,847,847
Rasgrf1	0.215	chr9:89,776,508-89,776,680
Th	0.173	chr7:149,975,435-149,976,040
Tnfrsf23	0.179	chr7:150,941,307-150,941,730
Tspan32	0.222	chr7:150,195,385-150,195,826
Wt1	0.082	chr2:104,968,308-104,969,763
Zfp264	0.319	chr7:6,680,992-6,682,247
Zim2	0.128	chr7:6,680,992-6,682,247
Zim3	0.291	chr7:6,680,992-6,682,247

Table S6. Summary of integrated TaqMan qPCR data and MBD-Seq data for imprinted loci. Shown are the imprinted genes whose expression is changed 2-fold or more in *Gsk-3* DKO ESCs (from Table 8), and the genomic coordinates of nearby *Gsk-3*-dependent DMRs.

Table S7

Gsk-3, DNA Methylation and Imprinting

Locus	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Expected Amplicon Size
Pax3	TTTYGGGTTTATTTTGGGTTTGT	CTTTTTACTACTTTCTATTCTTCC	316 bp
En1	GTTTTGTTAGTGTTATGTGATAG	TCTCCAACCTTTCTAAAACCTT	440 bp
Blcap/Nnat	AGTAGGAATGTAGAAAAAAG	CCTTAAATACCCTCTTACCA	400 bp
Gnas	TTAGTGGTTGAGATTTAGTT	TCTAAAATCTAACATATCCCTCT	304 bp
Gnas	TGGGGTGATAAAGTTTTTTTGTGA	CTAATAAAATCTATCACCTTCCT	499 bp
Gnas	GGAGATTATTAGATTTTGTGTTGAG	AAAAAACTTTTCCTTCCAC	228 bp
Gnas	TTTATYGATAAGTAATTGGAGGAG	CCACCCCAAACAAAATTAATAAA	231 bp
Adar	GAGTGAAGATTTAGAAAAGT	CCCAAATAAAAACACCTAAA	463 bp
Klf4	TTTTTTATGTGTAAGGTAAGGTG	TTCCTTCTAACAATAACTTC	213 bp
Cdc42	GTTYGTTGGTTGTTATGATTTTTG	CTAAATTTTCTTATCTCTACCTC	428 bp
Fkbp6	ATTGTGTAAGGATGGAAAA	CATACRACCAAAAAACAAAACCTC	493 bp
Mest/Copg2	TAATATGGGAGTTTTAAGGGTAA	CAAATAAACCTATCCTACA	453 bp
Mest/Copg2	AAATGGGTTAGAAAATAGAAAGG	CACTACTAAAACCAACTAAAAC	465 bp
Peg3	GATGGGTTGTTATTTAAGATTGT	CAAACCTTATTCACCTTTACT	398 bp
Kcnq1	TGTTTTAGGTTATTTATTTTGGG	TTCTCTCAATTTTCTTCAACAC	484 bp
Kcnq1	AAGGTAGTAGGGAATTTAAT	RTAACTAAAACAAACAAACCCC	446 bp
Kcnq1	GAGAGATTTTAAGATATGGA	CTTTACACAATCCCCATTAAAAA	456 bp
Mycn	TTAAAATGTGTAAAGTGGTAGTG	CCCCCTCAAAAATATTATTC	368 bp
Begain	GTGGAGTTTTTTTTTTTAGA	TCAAACTTATCACATTACACT	241 bp

Table S7. Primer sequences for amplification of bisulfite-converted DNA. Expected amplicon size for each region is shown.

Table S8

Gsk-3, DNA Methylation and Imprinting

Gene	TaqMan ID	Gene	TaqMan ID	Gene	TaqMan ID
Ampd3	Mm00477495_m1	Hoxa6	Mm00550244_m1	Peg3	Mm00493299_s1
Art5	Mm00464364_m1	Hoxa7	Mm00657963_m1	Phlda2	Mm00493899_g1
Asb4	Mm00480830_m1	Hoxa9	Mm00439364_m1	Plagl1	Mm00494250_m1
Ascl2	Mm01268891_g1	Hoxa10	Mm00433966_m1	Pon2	Mm00447159_m1
Atp10a	Mm00437724_m1	Hoxa11	Mm00439360_m1	Pon3	Mm00447161_m1
Begain	Mm01327850_m1	Hoxa13	Mm00433967_m1	Ppp1r9a	Mm00725102_m1
Bicap	Mm00727119_s1	Htr2a	Mm00555764_m1	Rasgrf1	Mm00441097_m1
Calcr	Mm00432271_m1	Igf2	Mm00580426_m1	Rian	Mm01325843_m1
Casd1	Mm00520462_m1	Igf2as	Mm03455591_m1	Scin	Mm00485972_m1
Cd81	Mm00504869_m1	Igf2r	Mm00439576_m1	Sfmbt2	Mm00616783_m1
Cdkn1c	Mm00438170_m1	Impact	Mm00492647_m1	Sgce	Mm00448714_m1
Cmah	Mm00483341_m1	Inpp5f	Mm00724391_m1	Slc22a18	Mm00485426_m1
Cntn3	Mm00500927_m1	Ins1	Mm01259683_g1	Slc22a2	Mm00457295_m1
Cobl	Mm00552805_m1	Ins2	Mm00731595_gH	Slc22a3	Mm00488294_m1
Commd1	Mm01239669_m1	lpw	Mm003456284_m1	Slc38a4	Mm00459056_m1
Copg2	Mm00444398_m1	Kcnq1	Mm00434641_m1	Snrpn	Mm01310473_g1
Dcn	Mm00514535_m1	Kcnq1ot1	Mm03959195_s1	Snurf	Mm01310473_g1
Ddc	Mm00516688_m1	Klf14	Mm03646643_s1	Tbc1d12	Mm00461246_m1
Dhcr7	Mm00514571_m1	Klrb1f	Mm00467635_m1	Tfpi2	Mm00436948_m1
Dio3	Mm00548953_s1	Magel2	Mm00844026_s1	Th	Mm00447546_m1
Dlk1	Mm00494477_m1	Mcts2	Mm00481540_s1	Tnfrsf23	Mm00656375_m1
Dlx5	Mm00438430_m1	Meg3	Mm00522599_m1	Tspan32	Mm00451458_m1
Drd1a	Mm01353211_m1	Mest	Mm00484993_m1	Tssc4	Mm00502351_g1
Gapdh	Mm99999915_g1	Mkfn3	Mm00844003_s1	Ube3a	Mm00839910_m1
Gatm	Mm00491879_m1	Mst1r	Mm00436365_m1	Usp29	Mm00498669_m1
Gnas	Mm01242435_m1	Nap114	Mm00500720_m1	Wt1	Mm00460570_m1
Gpr1	Mm00461557_m1	Nap115	Mm02526917_s1	Xlr3b	Mm00496001_m1
Grb10	Mm01180443_m1	Ndn	Mm02524479_s1	Xlr4b	Mm00786658_s1
H13	Mm00468792_m1	Nespas	Mm03455705_m1	Zdbf2	Mm01254507_m1
H19	Mm00469706_g1	Nnat	Mm00440480_m1	Zfp264	Mm01325533_m1
Hoxa1	Mm00439359_m1	Osbpl5	Mm00600357_m1	Zim1	Mm00496103_m1
Hoxa2	Mm00439361_m1	Pde4d	Mm00456879_m1	Zim2	Mm01335522_m1
Hoxa3	Mm01326402_m1	Peg10	Mm01167724_m1	Zim3	Mm01335891_g1
Hoxa4	Mm01335255_g1	Peg12	Mm00844053_s1	Zrsr1	Mm00495837_s1
Hoxa5	Mm00439362_m1	Peg13	Mm03456028_s1		

Table S8. TaqMan probes used in this study.

Figure S1.

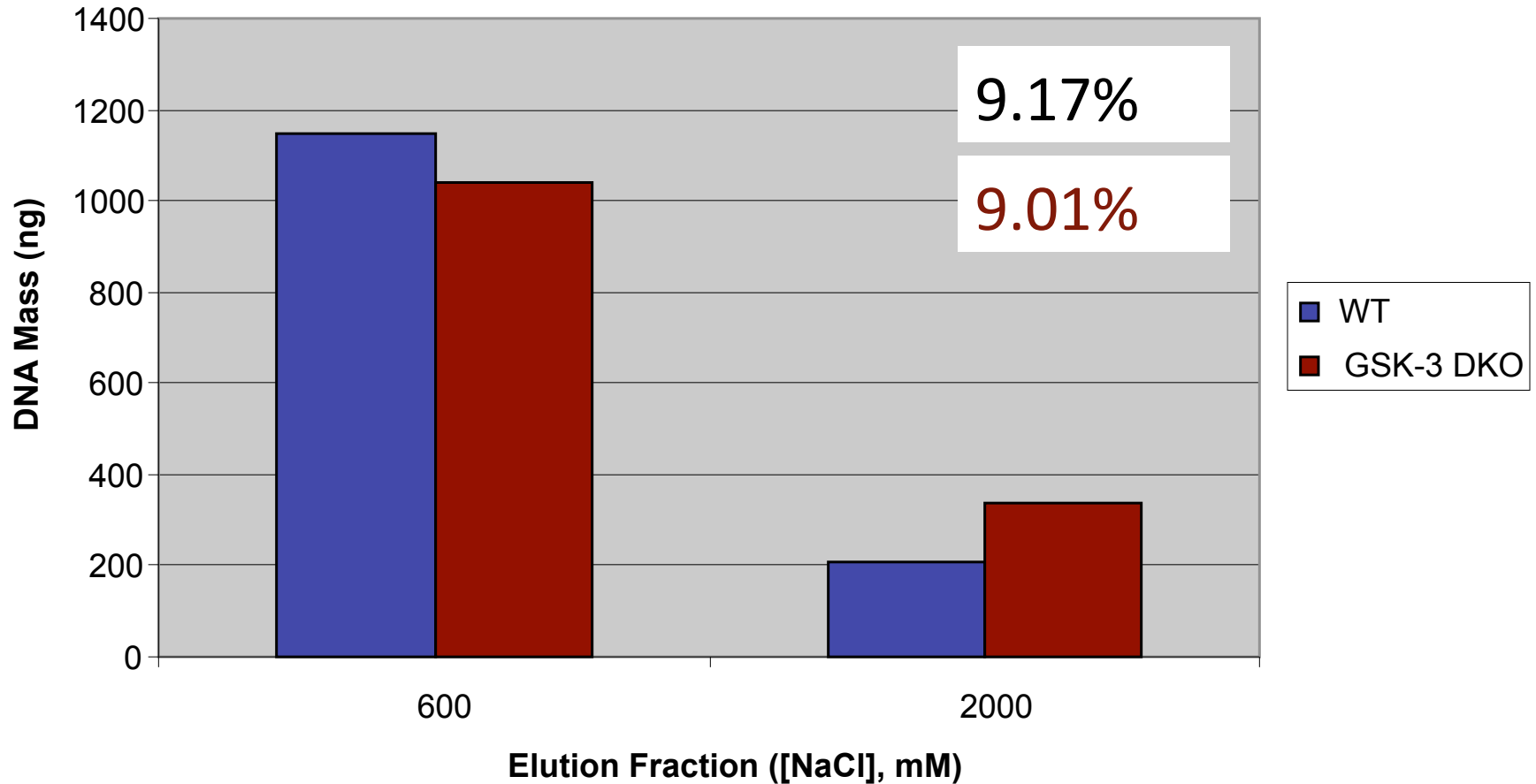


Figure S1. MethylMiner elution data. Graph showing the amount of DNA eluted from MBD2-streptavidin beads with either 600mM NaCl or 2M NaCl. DNA from WT ESCs is shown in blue, while Gsk-3 DKO ESCs is shown in red. The percentage of eluted DNA relative to total input DNA is shown.

Figure S2.

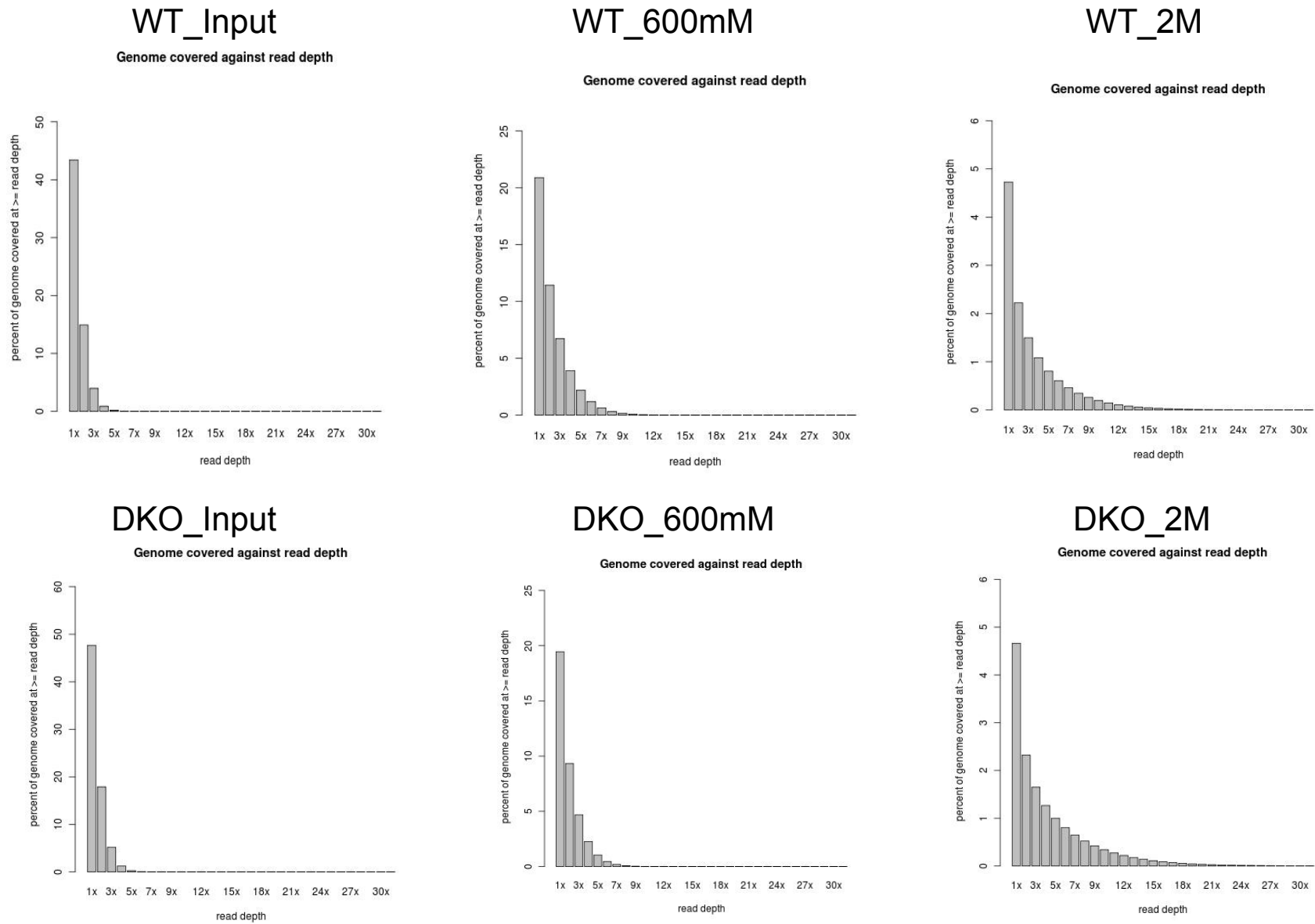


Figure S2. Graphical representation of percent of the mouse genome covered in MBD-seq, and the depth of sequencing reads.

Figure S3.

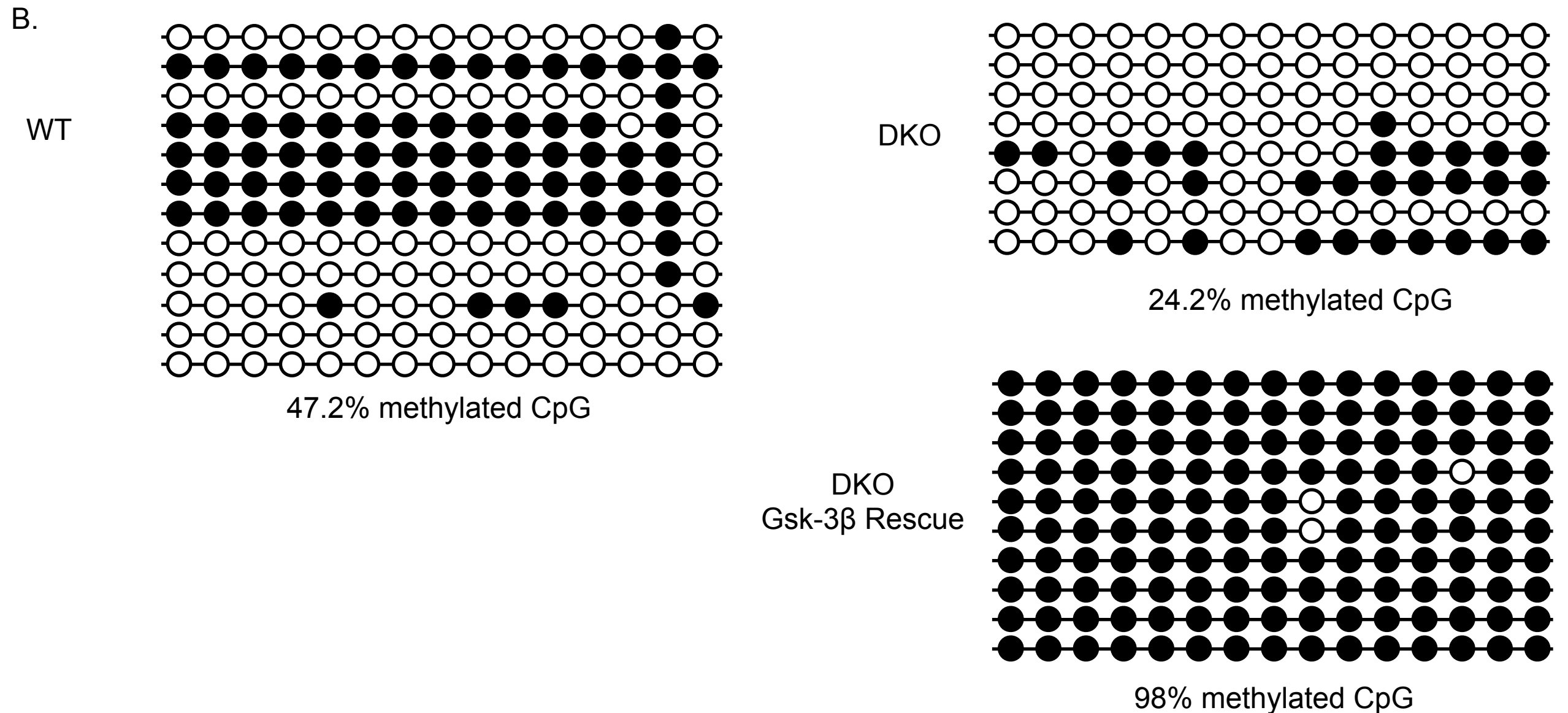
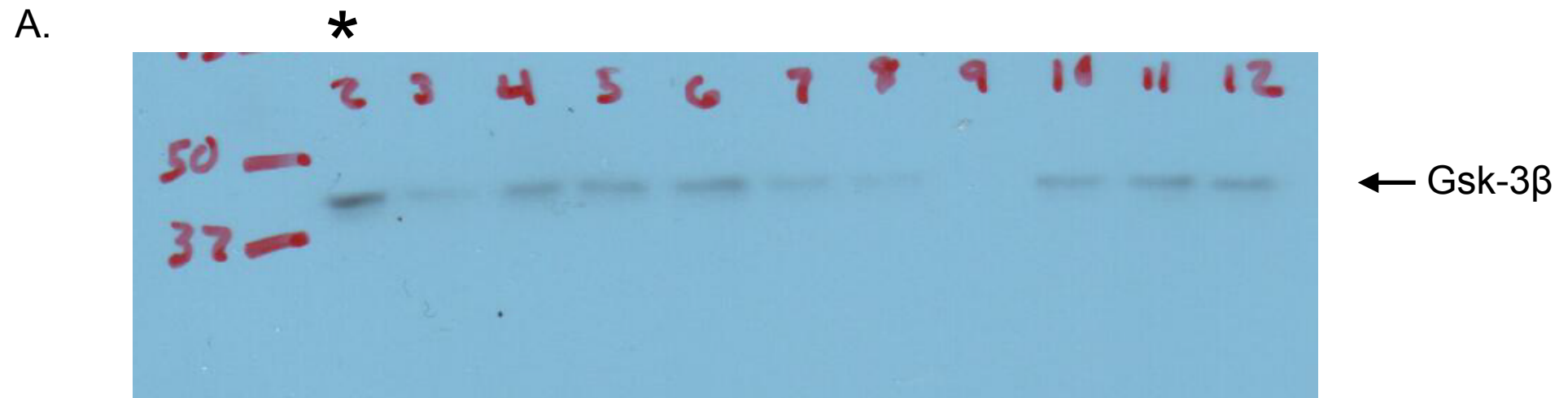


Figure S3. Gsk-3 DKO ESCs were stably transfected with human Gsk-3β. (A) Gsk-3α/β western blot for various colonies re-expressing Gsk-3β. We selected clone #2 (denoted by *) for analyzing DNA methylation. (B) Bisulfite sequencing of the *H19/Igf2* locus in WT, Gsk-3 DKO, and Gsk-3 DKO-Gsk-3β rescue ESCs. Re-expression of Gsk-3β results in hypermethylation of DNA at the *H19/Igf2* locus. Each circle represents a CpG dinucleotide; horizontal rows represent individual clones, while vertical columns show the position of each CpG. Filled in circles represent methylated cytosines, while open circles represent unmethylated cytosines.

Figure S4.

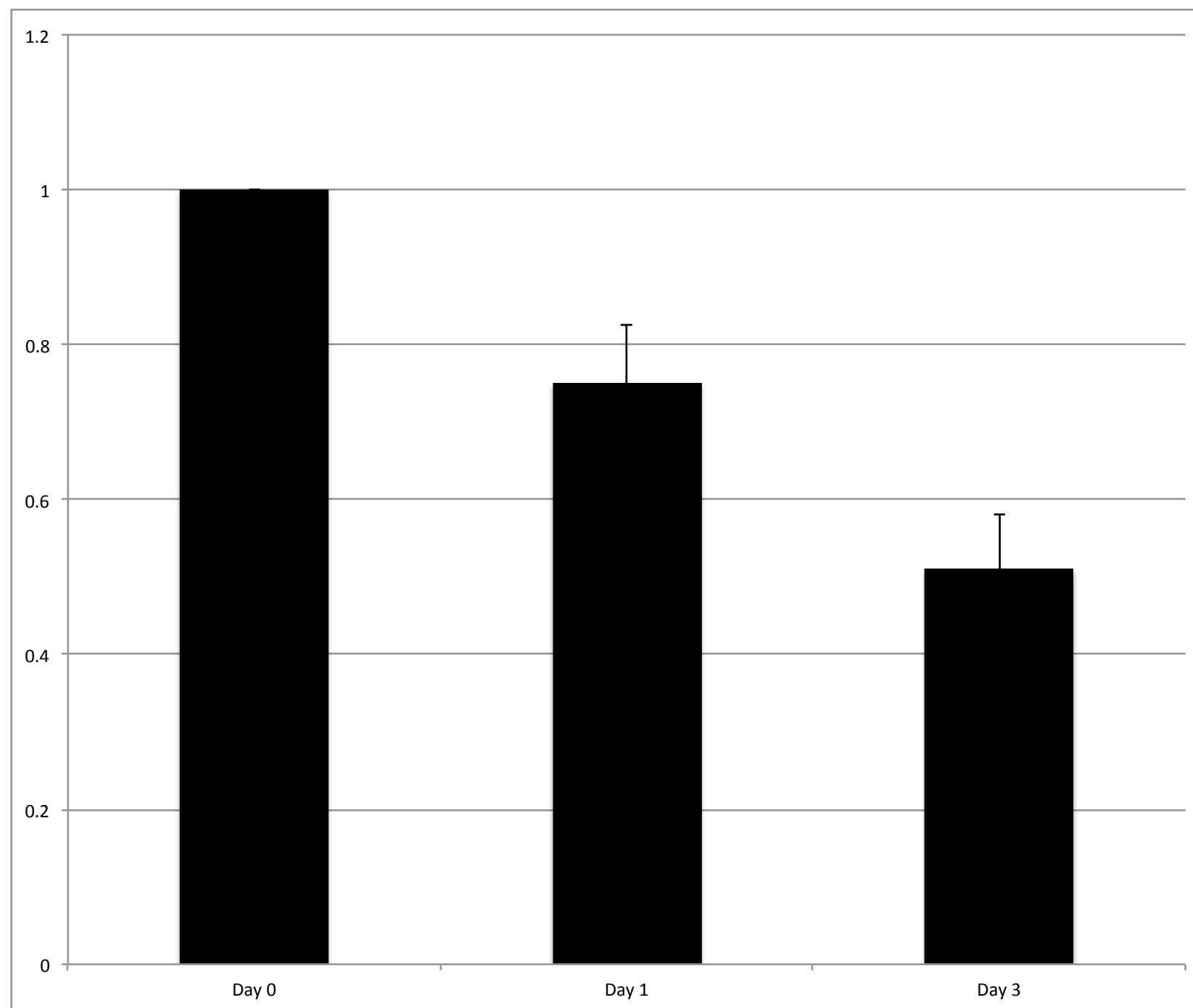


Figure S4. WT ESCs were stably transfected with a Dnmt3a2-luciferase reporter construct. Equal cell numbers were plated in a 24-well plate, then cells were treated with 10 μ M SB-415,286. Cell lysates were collected on the days indicated above, and luciferase assays performed. Each condition was assayed in triplicate, and expression levels normalized to untreated cells (Day 0). Error bars represent SEM.

Figure S5.

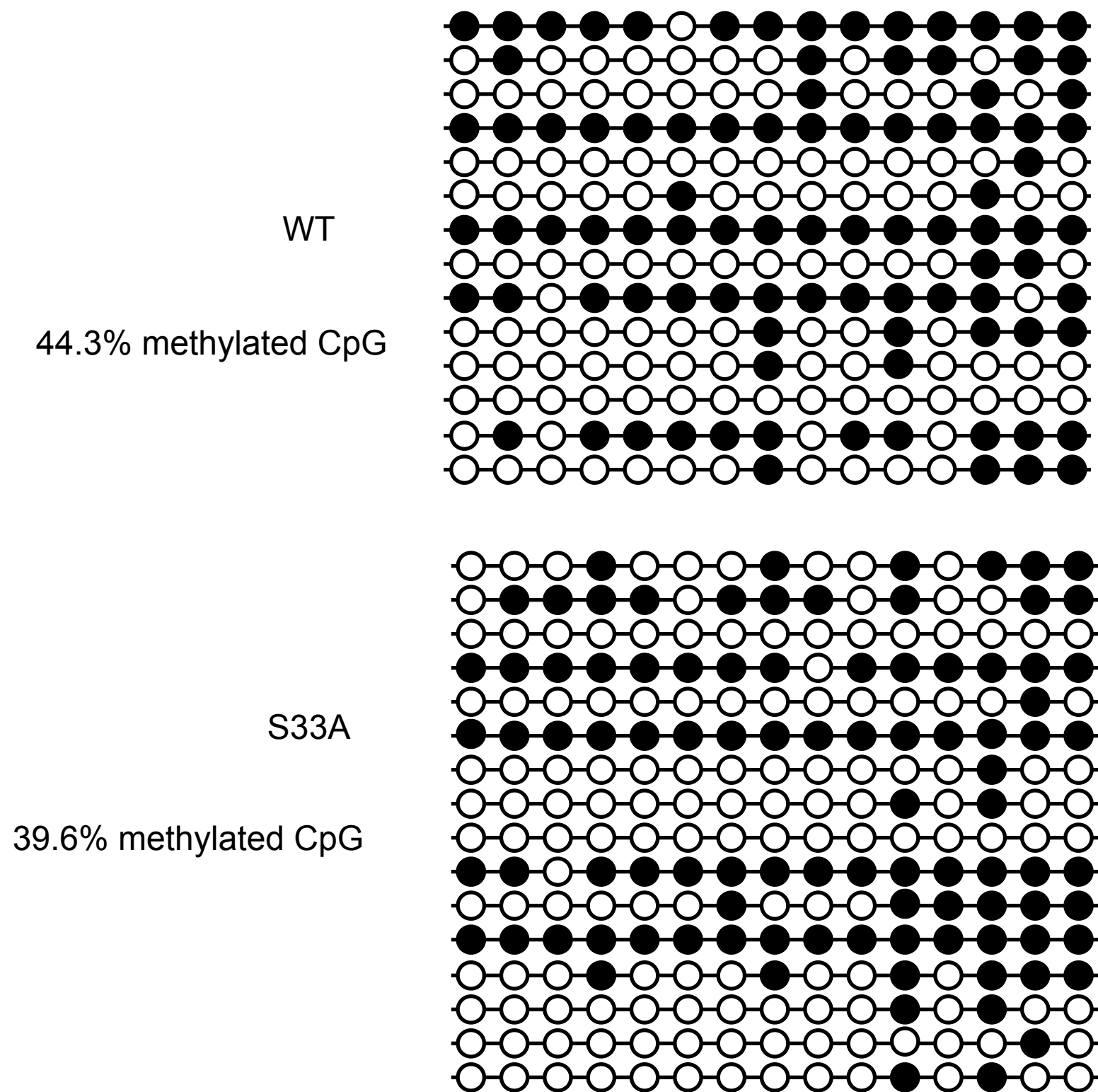


Figure S5. Bisulfite sequencing was performed on WT and β -catenin S33A stable cells. The DNA methylation status of the *H19/Igf2* DMR was examined. No significant difference in DNA methylation is seen in the β -catenin S33A expressing ESCs compared to WT ESCs. Each circle represents a CpG dinucleotide; horizontal rows represent individual clones, while vertical columns show the position of each CpG. Filled in circles represent methylated cytosines, while open circles represent unmethylated cytosines.