Supplemental Table 1 (Relevant to Figure 2):

				Methylation percentages		
CpG number	**Context**	**chr**	**position**	**Control**	**WNT3A**	**WNT3A + FGF2**
1	CpG	chr18	9068437	2.21	1.32	4.03
2	CpG	chr18	9068467	4.48	7.85	3.18
3	CpG	chr18	9068478	4.82	11.66	6.62
4	CpG	chr18	9068482	1.67	1.59	1.21
5	CpG	chr18	9068501	9.94	11.12	8.36
6	CpG	chr18	9068530	14.73	21.09	16.17
7	CpG	chr18	9068538	8.12	12.56	9.80
	ATG	chr18	9068541			
8	CpG	chr18	9068597	3.53	6.17	4.57
9	CpG	chr18	9068600	1.50	1.63	2.03
10	CpG	chr18	9068620	7.50	16.03	12.89
11	CpG	chr18	9068630	15.18	32.58	20.05
12	CpG	chr18	9068633	3.52	3.95	5.78
13	CpG	chr18	9068651	10.83	14.51	13.41
14	CpG	chr18	9068657	17.57	30.71	16.51
15	CpG	chr18	9068662	11.08	25.73	13.25
16	CpG	chr18	9068668	8.27	13.93	10.97
17	CpG	chr18	9068679	8.46	15.67	7.30
18	CpG	chr18	9068708	20.38	29.37	20.69
19	CpG	chr18	9068713	10.04	23.26	15.81
20	CpG	chr18	9068732	29.54	43.92	35.26
21	CpG	chr18	9068735	14.87	31.29	19.12
22	CpG	chr18	9068750	8.28	11.82	7.74
23	CpG	chr18	9068760	18.96	30.95	24.41
24	CpG	chr18	9068762	5.35	8.77	6.43
25	CpG	chr18	9068768	9.15	20.51	14.83
26	CpG	chr18	9068792	9.95	23.28	14.12
27	CpG	chr18	9068816	12.24	20.90	15.51
28	CpG	chr18	9068820	24.49	39.10	30.46
29	CpG	chr18	9068828	30.14	48.52	34.79
30	CpG	chr18	9068852	13.14	25.33	16.52
31	CpG	chr18	9068859	43.82	62.49	50.24
32	CpG	chr18	9068870	20.92	35.32	24.15
33	CpG	chr18	9068893	11.84	23.14	18.16
34	CpG	chr18	9068898	8.08	18.95	10.78
35	CpG	chr18	9068923	17.21	32.99	16.20
36	CpG	chr18	9068936	4.74	8.61	4.88
37	CpG	chr18	9068939	5.92	19.64	10.11
38	CpG	chr18	9068953	6.80	20.89	12.68
39	CpG	chr18	9068977	16.80	45.67	28.95
40	CpG	chr18	9068990	13.14	32.02	23.01

41	CpG	chr18	9068995	18.95	28.97	21.90
42	CpG	chr18	9069007	14.27	34.79	19.49
43	CpG	chr18	9069015	16.81	37.60	21.58
44	CpG	chr18	9069036	11.92	34.07	18.55
45	CpG	chr18	9069045	12.74	43.93	24.56
46	CpG	chr18	9069048	16.66	41.39	24.46
47	CpG	chr18	9069050	11.70	31.35	20.09
48	CpG	chr18	9069105	11.25	19.94	14.34
49	CpG	chr18	9069121	32.94	53.42	38.83
50	CpG	chr18	9069137	10.01	21.60	13.05
51	CpG	chr18	9069140	30.90	62.31	38.53

Supplemental Table 1 Legend.

The percent methylation of CpG residues (determined by bisulfite sequencing) located in the second Sox9 CpG island (CpG2) in genomic DNA isolated from LBMCs that had been cultured for 4 days in either control medium, medium containing soluble Wnt3A, or medium containing both Wnt3A and FGF2 is displayed. The genomic location of either the relevant CpG residue or the ATG translation initiation site of Sox9 are indicated. The relative levels of CpG methylation are indicated by a gradient of colors: dark blue (lowest levels of CpG methylation); light blue; white; pink; dark red (highest levels of CpG methylation). Consistent with our methyl-DNA IP results, we found that Wnt3A administration increased cytosine methylation of CpG residues located throughout the Sox9 CpG2, with the greatest effect occurring in the 3' region of this CpG island. Most notably, concurrent administration of FGF2 (together with Wnt3A) decreased cytosine methylation of CpG residues throughout the Sox9 CpG2.

Supplemental Experimental Procedures

ERK2 Kinase assay. 20 pmol of either GST-hDNMT3A-WT, GST-hDNMT3A-(L373,637A) or GST-hDNMT3A-(S255A) or bacterially produced and purified fragments of mDNMT3A (Jia et al., 2007) were incubated with 50 μM ATP, 50 μM [γ-^{32}P] ATP (10 Ci/mmol), and 2 U ERK2 (NEB) in kinase buffer at 30°C for 30 min. The reaction was terminated by the addition of Laemmli sample buffer. The phosphorylation of substrate proteins was examined after SDS-PAGE by autoradiography.

Alcian Blue staining. The micromass cultures were stained with alcian blue as previously described (Kumar and Lassar, 2009).

Immunocytochemistry. Limb buds were isolated from 4 day chicken embryos (H. H. stage 22-24). After overnight fixation in 4% paraformaldehyde at 4°C, they were subjected to a series of sucrose solutions (5% sucrose for 2 hrs, 10% sucrose overnight at 4°C, 20% sucrose for 2hrs at room temperature, 50:50 solution of 20% sucrose and OCT medium for 2 hrs at room temperature) prior to putting them in OCT medium and freezing. For immunofluorescence staining, 10 micron thick sections were permeabilized using 0.25% triton X-100 for 15 minutes followed by blocking in 5% BSA for 1 hr. The sections were incubated with either rabbit anti-phospho-S255 hDNMT3A (1 mg/ml in blocking buffer) or mouse anti-DNMT3A (4 mg/ml in blocking buffer; Abcam ab13888) at 4°C overnight. During incubation with primary antibody, in some cases we added 10 mg/ml of competitor non-phosphorylated or phosphorylated DNMT3A peptide (246a.a.-AVQQPTDPApSPTVATTC-262a.a.). After three washings, the sections were

incubated with secondary antibodies: anti-mouse IgG1 conjugated to Alexa Fluor 594 (Invitrogen) or anti-rabbit IgG conjugated to Alexa Fluor 488 (Invitrogen) for 1 hr. After three washes, the samples were stained with DAPI for 5 minute and then covered with a coverslip using mounting media (Vector Laboratories). Images were obtained using Nikon 80i Upright Microscope with 10x objective.

Methyl-DNA immunoprecipitation. For Methyl-DIP analysis we analyzed genomic DNA (approximately 10 micrograms) prepared from a minimum of 20 micromass spots per condition. In most cases, independent Methyl-DIP analyses were performed on 2-3 biological replicates per condition; with three technical repeats of each PCR assay. Significance was calculated using Student's *t*-test. Micromass cultures were washed with PBS and treated with 120 mg/ml trypsin (Sigma) and 120 mg/ml collagenase (Sigma) to prepare a single cell suspension. The cells were harvested after washing with PBS and genomic DNA was isolated using a DNeasy Blood and Tissue kit (Qiagen) and sheared by sonication using Misonix 3000 (12 minutes sonication time; 10s on, 20s off) to obtain approximately 500 bp DNA fragments. Ten microgram of sheared DNA was heated at 95^{o}C for 10 min and snap-chilled before mixing with 10 ml of freshly prepared protein A and protein G Dynal magnetic beads (Invitrogen) that were pre-incubated with 5 mg of either anti-5-methyl-cytidine antibody (ab10805, Abcam), or mouse IgG control antibody (ab18413, Abcam) for a minimum of 4 hr and washed three times with ice-cold PBS plus 5% BSA. The mixture was then incubated overnight in buffer (20 mM Tris-HCl [pH 8.1], 150 mM NaCl, 2 mM EDTA, 1% Triton X-100). The bead-DNA complexes were then washed three times in RIPA buffer (50 mM HEPES

[pH 7.6], 1 mM EDTA, 0.7% Na deoxycholate, 1% NP-40, 0.5 M LiCl) followed by three times with TE buffer (10 mM Tris HCl [pH 8.0], 1 mM EDTA). Immunoprecipitated DNA was then extracted by heating at 65^{o}C in 1% SDS, 0.1 M $NaHCO_3$ for at least 6 hr. DNA fragments were purified with a QIAquick DNA purification Kit (Qiagen) and analyzed using Sybr-green real time PCR. In all Methyl-DIP analyses, the relative amount of qPCR product generated from immunoprecitated DNA was normalized to that generated by input DNA. The following primer pairs were used for qPCR: Sox9(CpG1)-FP: 5'-CCTCTCCGCCGCCCTTCTGA-3', Sox9(CpG1)-RP: 5'-CCGGCTGCGAGAGGAGGGAA-3', Sox9(CpG2)-FP: 5'-TTCCCCAAAGGCGACCCGGA-3', and Sox9(CpG2)-FP: 5'-AGGCGTTCATGGGGCGCTTC-3'.

Chromatin immunoprecipitation. For ChIP analysis we analyzed pooled chromatin isolated from a minimum of 30 micromass spots per condition. In most cases, independent ChIP analyses were performed on 2-3 biological replicates per condition; with three technical repeats of each PCR assay. Significance was calculated using Student's *t*-test. Micromass cultures were washed with PBS and treated with 120 mg/ml trypsin (Sigma) and 120 mg/ml collagenase (Sigma) to prepare a single cell suspension. The cells were harvested after washing with PBS and cross-linked with 1% formaldehyde 15 minutes at 37°C followed by lysis with 0.35 ml of buffer (50 mM Tris-HCl [pH 8.1], 1% SDS, 10 mM EDTA, 1x protease inhibitor cocktail (Roche). Chromatin was sheared by sonication using Misonix 3000 (12 minutes sonication time; 10s on, 20s off) and supernatants were collected after centrifugation and diluted in buffer (20 mM

Tris-HCl [pH 8.1], 150 mM NaCl, 2 mM EDTA, 1% Triton X-100). Five micrograms of antibody was prebound for a minimum of 4 hr to protein A and protein G Dynal magnetic beads (Invitrogen) and washed three times with ice-cold PBS plus 5% BSA and then added to the diluted chromatin and immunoprecipitated overnight. The bead-chromatin complexes were then washed three times in RIPA buffer (50 mM HEPES [pH 7.6], 1 mM EDTA, 0.7% sodium deoxycholate, 1% NP-40, 0.5 M LiCl) followed by three times with TE buffer (10 mM Tris HCl [pH 8.0], 1 mM EDTA). Immunoprecipitated chromatin was then extracted by heating at 65^{o}C in 1% SDS, 0.1 M $NaHCO_3$ for at least 6 hr. DNA fragments were purified with a QIAquick DNA purification Kit (Qiagen) and analyzed using Sybr-green real time PCR. In all ChIP analyses, the relative amount of qPCR product generated from immunoprecitated DNA was normalized to that generated by input DNA. The following antibodies were used for ChIP: Anti-H3K4me3 (ab8580, Abcam), Anti-H3K27me3 (ab6002, Abcam), Anti-H3K9Ac (ab10812, Abcam), Anti-HA (sc-805; Santacruz), Rabbit IgG control (ab46540, Abcam), Mouse IgG control (ab18413, Abcam). The following primer pairs were used for qPCR: Sox9(promoter)-FP: 5'-AACACTGAATTGGGGGATTAAA-3', and Sox9(promoter)-RP: 5'-GCCCGGAATCGTCTTTTT-3'; Sox9(-350 kb)-FP: 5'-AGCAGAAACAAAGCCCAAGA-3', and Sox9(-350 kb)-RP: 5'-AGTTGCAGGCTAAGCCAAAC-3'; TBP(promoter)-FP: 5'-GCATGTGATGGTGAGCATGCAGGA-3', TBP(promoter)-RP: 5'-TGTGAGGACACTCATACGCACGCA-3'.

RT-qPCR. Total RNA was harvested using a RNeasy mini kit (Qiagen). Reverse transcription and qPCR was carried out as previously described (Kumar et al., 2009)

using SYBR Premix Ex Taq kit (Takara). For RT-PCR analysis we analyzed pooled RNA isolated from 3 micromass spots per condition. In most cases, independent RT-qPCR analyses were performed on 2-3 biological replicates per condition; with three technical repeats of each PCR assay. Significance was calculated using Student's *t*-test. In all RT-qPCR analyses, the relative amount of qPCR product generated by various primer sets was normalized to that generated by GAPDH primers. The following primer pairs were used for qPCR: GAPDH-FP: 5'-AGTCATCCCTGAGCTGAATG-3', GAPDH-RP: 5'-AGGATCAAGTCCACAACACG-3', Sox9-FP: 5'-AGGAAGCTGGCTGACCAGTA-3', Sox9-RP: 5'-CGTTCTTCACCGACTTCCTC-3', Collagen II-FP: 5'-ATGCCACCCTCAAATCCCTC-3', Collagen II-RP: 5'-AATCTCCGCTCTTCCACTCG-3', Aggrecan-FP: 5'-CCTGCCTGACCTCTTTGC-3', Aggrecan-RP: 5'-TGGGGAGGAGGGCAACAT-3', Twist2-FP: 5'-TTTCCCCTGTGGATAGCTTG-3', and Twist2-RP: 5'-CATAAGACTGGGAGCTGGGA-3'. FH-DNMT1-FP: 5'- GCTCATGCTTACAACCGGGA-3', FH-DNMT1-RP: 5'-TGATGGTGGTTTGCCTGGTG-3', FH-DNMT3A-FP: 5'-TGTTCCAGATTACGCTGCTCGAGGT-3', FH-DNMT3A-RP: 5'-GCCATGGATGGGGACTTGGAGATCA-3', FH-DNMT3B-FP: 5'-ACACCCCAGTCATGCCAAAG-3', and FH-DNMT3B-RP: 5'-GGGACTCGTCCACATGGTTG.

Bisulfite sequencing. Micromass cultures were harvested after 4 days of incubation and genomic DNA was prepared using Qiagen DNeasy Blood and Tissue kit. Bisulfite conversion, PCR amplification, DNA sequencing and sequence analysis was carried out

by Active Motif (Carlsbad, CA). Briefly, PCR primers to the target region were designed with the MethPrimer software (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi). All primers were designed to the plus strand. Genomic DNA was bisulfite-converted using MethylDetector (Active Motif). PCR reactions (40 cycles) were performed using Invitrogen's Platinum PCR supermix. For each sample, about equal amounts of the 4 PCR products were pooled and treated with T4 DNA polymerase, Klenow large fragment, and T4 polynucleotide kinase to generate 5'-phophorylated blunt ends. After concatemerization with T4 DNA ligase the sample was sonicated to an average fragment length of 150-300 bp using a Misonix cuphorn sonicator 3000. Libraries were generated from these sonicated DNA samples using the standard Illumina protocol. The 4 samples were indexed with 6-bp barcodes (independent Illumina index read). Sequencing on Hi-Seq generated 3.5-4.5 million reads per sample. Reads were aligned to the chicken chr18 reference sequence (galGal4 assembly) using the bismark software (version 0.7.7; (Krueger and Andrews, 2011)). Alignment and methylation information was captured in BAM files, and percentage methylation and read coverage at each CpG site was determined by running the appropriate bismark scripts. Alignments to the strands and genomic locations not expected to be present in the PCR products were filtered out using a combination of Samtools (Li et al., 2009) and standard UNIX commands.

BrdU incorporation. Twenty four hours before harvesting, micromass cultures were washed with PBS and treated with fresh media containing 10μM BrdU. The cultures

were processed and BrdU incorporation was measured by BrdU labeling and detection kit III (Roche) as per manufacturer's instruction.

Supplemental References

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