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Supplemental Material to:

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Notch Epigenetics: DNA hypomethylation and 5-hydroxymethylcytosine

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Table S1 and S2

Figures S1 - S3

Table S1. RNA-seq analysis of expression of some Notch signaling genes

A RNA-seq data for Mb and nonmuscle cell cultures at Notch signaling genes

Gene name	RNA isoform	Length	Mb FPKM	HUVEC FPKM	NHEK FPKM	NHLF FPKM	LCL GM 12878 FPKM	H1 ESC FPKM
NOTCH1	NM_017617	9371	6.2	30.4	35.9	6.5	8.2	14.2
NOTCH2	NM_024408	11389	48.8	22.0	29.6	41.7	2.8	15.2
DLL1	NM_005618	3906	3.3	0.6	2.2	0.3	0.1	0.7
DLL3	NM_016941	2380	0.1	0.3	0.1	0.1	0.3	1.2
DLL3	NM_203486	2043	0.7	0.9	1.0	0.2	0.6	3.8
DLL4	NM_019074	3380	0.1	3.0	0.1	0.6	0.2	0.2
JAG1	NM_000214	5901	6.5	18.0	44.2	8.4	2.6	9.4
JAG2	NM_145159	5720	2.6	6.7	8.9	0.0	0.0	0.1
LFNG	NM_002304	2058	0.7	0.0	0.0	0.0	0.0	0.0

[1] We determined the FPKM (Fragments Per Kilobase of transcript per Million mapped reads) from ENCODE data (http://genome.ucsc.edu; non-strand-specific RNA-seq, poly(A)$^+$ RNA, B. Wold, CalTech) using Cuffdiff as described in Methods. The observed transcripts were originally mapped to ENSEMBL transcripts, whose length are given. For comparison to (B), the closest RefSeq transcript is given. Mt are not yet publicly available in the ENCODE data base. *NOTCH3* and *NOTCH4* data are not given because they were unreliable.

B RNA-seq data for comparison of Mb and Mt at Notch signaling genes[1]

Gene name	RNA isoform	Length	Mb4	Mt4	Mb13	Mt13
OBSCN (non-Notch)	NM_052843	20402	0.8	25.2	0.2	4.1
OBSCN (non-Notch)	NM_001098623	24030	0.2	7.7	0.0	1.2
NOTCH1	NM_017617	9295	0.7	4.5	1.3	1.2
NOTCH2	NM_024408	11466	38.6	24.5	32.8	26.1
NOTCH3	NM_000435	8071	3.2	20.6	26.7	19.4
NOTCH4	NM_004557	6745	0.0	0.0	0.2	0.1
DLL1	NM_005618	3310	3.6	8.8	0.9	6.6
DLL3	NM_016941	2383	0.0	0.0	0.1	0.1
DLL3	NM_203486	2046	0.2	0.2	0.3	0.2
DLL4	NM_019074	3420	0.0	0.1	0.0	0.0
JAG1	NM_000214	5987	7.1	7.6	5.8	14.9
JAG2	NM_145159	4963	0.2	2.6	0.1	0.4
JAG2	NM_002226	5077	0.8	8.1	0.3	2.5
LFNG	NM_002304	2058	0.7	2.5	7.8	2.1

[1] We determined FPKM from previously unpublished RNA-seq data on poly(A)$^+$ RNA (Crawford/Ehrlich laboratories) using Cuffdiff. *OBSCN*, which encodes a structural protein of skeletal muscle, is included in the table to show the expected upregulation of its RNA from Mb to Mt. Observed transcripts were mapped to RefSeq transcripts.

Table S2. Primers for quantification of 5hmC and 5mC at selected CCGG sites

ID of studied CCGG site	Coordinates of CCGG site (hg19)	Gene subregion	Forward primer for Epimark[1] 5hmC/5mC analysis	Reverse primer for Epimark 5hmC/5mC analysis	Size of amplicon for Epimark analysis (bp)
NOTCH1-19938	chr9:139,419,938	*NOTCH1* intron 2	TCTGAAGCAGAACATGCCAC	TCCCTCTGTACATCCCATCTG	115
NOTCH1-ex20	chr9:139,402,737	*NOTCH1* exon 20	ACAGCAGGTTACCTTGTCGC	AACCTTGTGCACTGGTGTGA	166
NOTCH1-ex32	chr9:139,393,700	*NOTCH1* exon 32	GGCTCACCCAGGTCATCTAC	AGGCATCGGTGTACGTCTG	209
NOTCH2-DMR	chr1:120,469,121	*NOTCH2* exon 24	ATGGATTGGGAAGCTCACTG	GACTTGTGCTGTGGCCAGTA	294
DLL1-93978	chr6:170,493,978	97 kb dnstrm of *DLL1*	AGACAGGGTGCCTTCGTCTA	GGAGGAGGCGCTCATATTAC	268
DLL1-35577	chr6:170,535,577	56 kb dnstrm of *DLL1*	TTCTCCCAACCAGCCATTGT	TGGATATTATGCTGCGCTGTG	74
JAG2-19503	chr14:105,619,503	*JAG2* intron 5	GGAATGTCATCCAGGCCA	AGCTGAACTGGGCTGATAGG	139

[1] The Epimark kit from New England Biolabs, Ipswitch, MA.

Figure S1. The *NOTCH1* intron 2 region displays differential epigenetics in myoblasts (Mb), myotubes (Mt), and skeletal muscle vs. other samples. a) Significantly hypomethylated (green) or hypermethylated (red) DMRs or individual DM sites in the skeletal muscle lineage. (b)Predicted chromatin structures of subregions [1]. (c) MyoD binding from C2C12 ChIP-seq [2] and identification of orthologous human sequences. Sites shown in blue overlapped the MyoD-like recognition sequences. (d) DNaseI-seq [3]. (e) RNA-seq data from the minus-strand; vertical viewing range, 1-to-30). (f) Examples of RRBS data. Chr9:139,417,716-139,422,160 is shown (hg19, http://genome.ucsc.edu). Heart muscle is shown but was not included in the statistical determination of myogenic hypomethylation. Overlapping epigenetic features mentioned in the main text are boxed. Pairs of samples labeled 1 and 2 are technical duplicates but the LCLs and skin fibroblasts are separately derived cell cultures. The other skeletal muscle sample is not included because for this gene it had too low RRBS signals. See legends to Figures 1 and 2 and Tsumagari et al. [3] for details about samples and tracks.

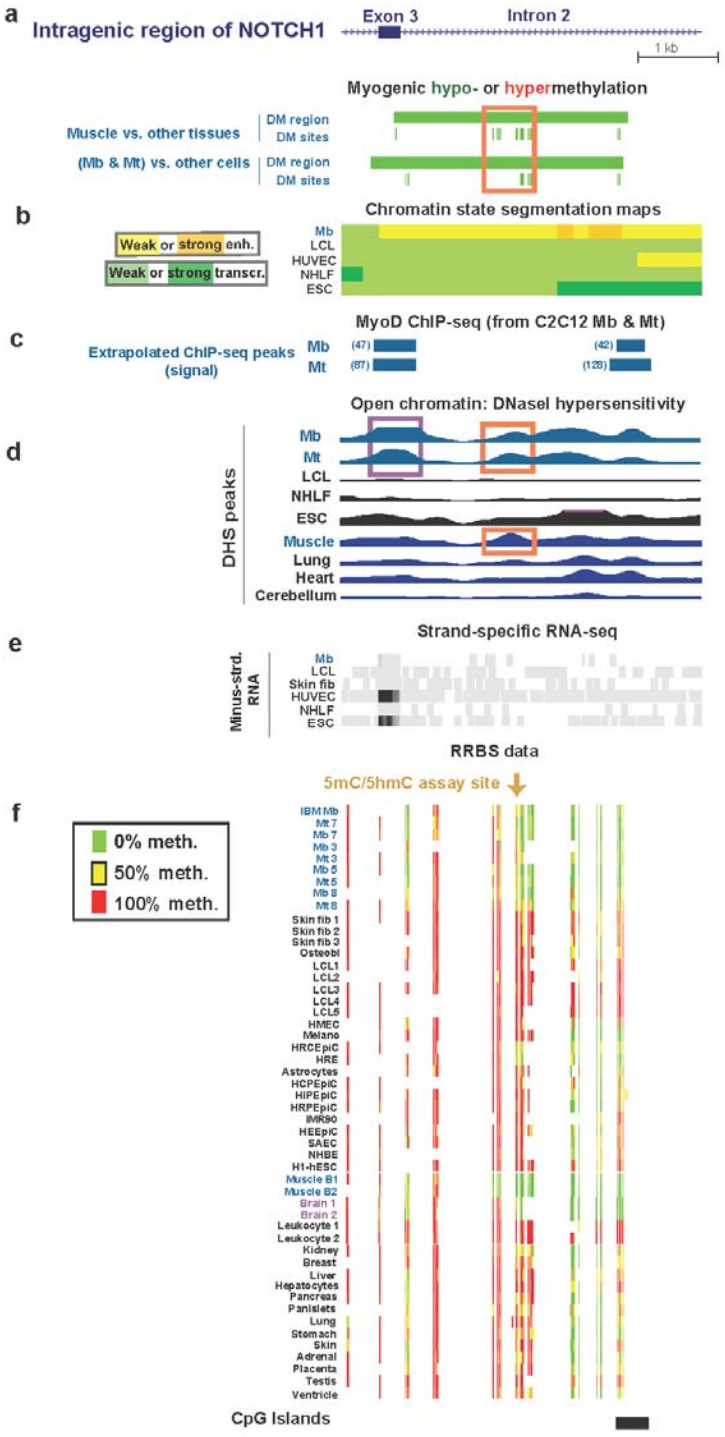


Figure S2. Myogenic hypomethylated DMRs are present far upstream of *NOTCH1* as well as in the body of the gene. (a) Significant myogenic hypo- or hyper-methylated DMRs. (b). MyoD binding from C2C12 ChIP-seq as in Figure S1 except that only the signal strengths ≥40 are indicated. (c) RNA-seq data as in the main Figure 1 except that the vertical viewing range was 1-to-100. (d) Examples of RRBS data. The region shown is chr9:139,339,111-139,592,061. The thick orange boxes indicate DMRs that overlap DNaseI-hypersensitivity sites (DHS) and enhancer-type chromatin [1] in Mb and Mt but not in other tested cell types (left box) or in Mb and several other tested cell types (middle box; data not shown). The MbMt-hypomethylated DMR with a thin-lined orange box around it overlapped a Mb- and Mt-associated DHS but not enhancer-type chromatin in Mb or Mt. The black boxes in panel d indicate some regions with differential methylation that can be seen at this magnification. Upon zooming in, the hypomethylation of LCLs vs. other cell types can be seen at the intragenic *NOTCH1* MbMt-hypermethylated DMRs of all but one of the sites between *NOTCH1* and *EGFL7*.

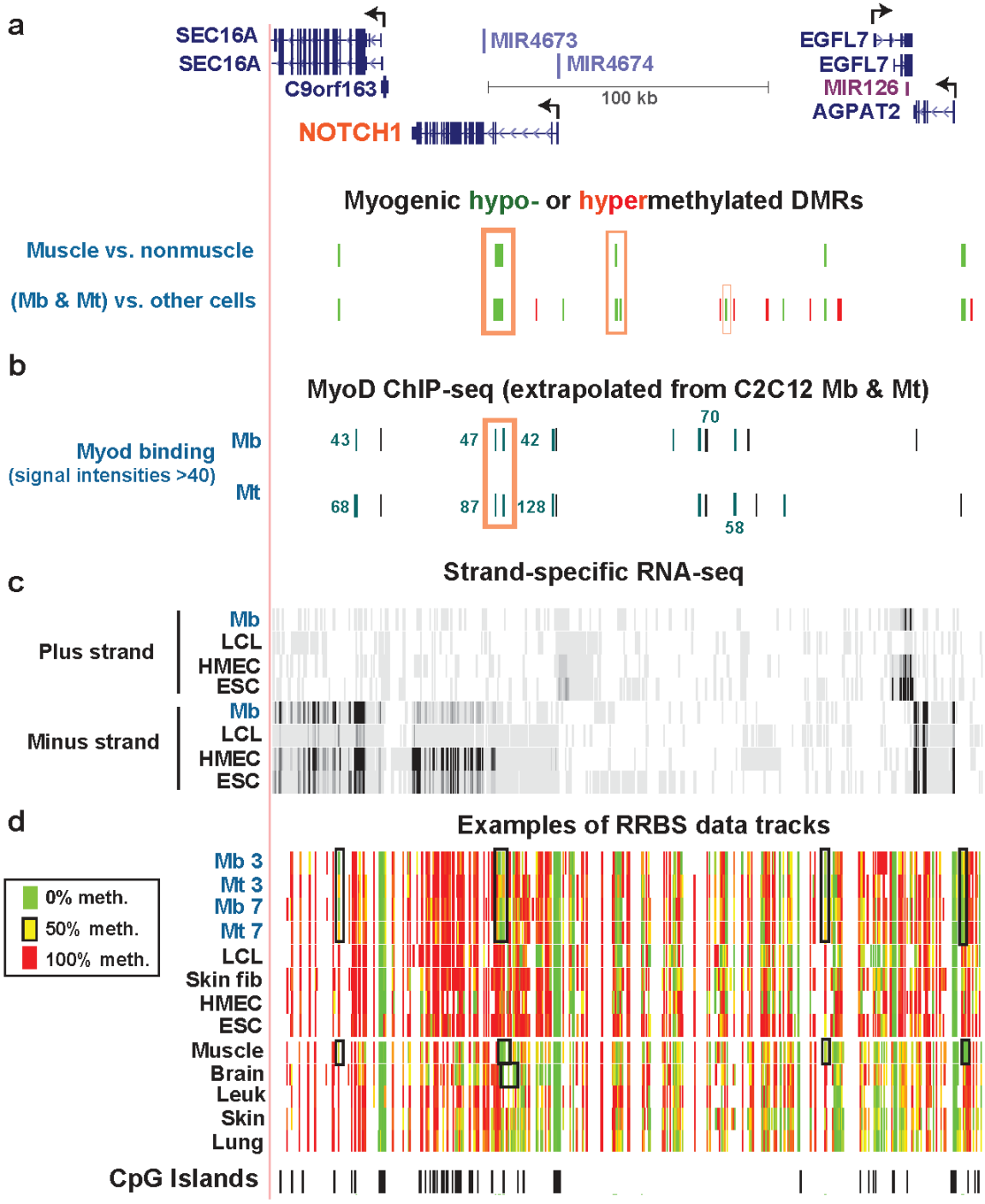
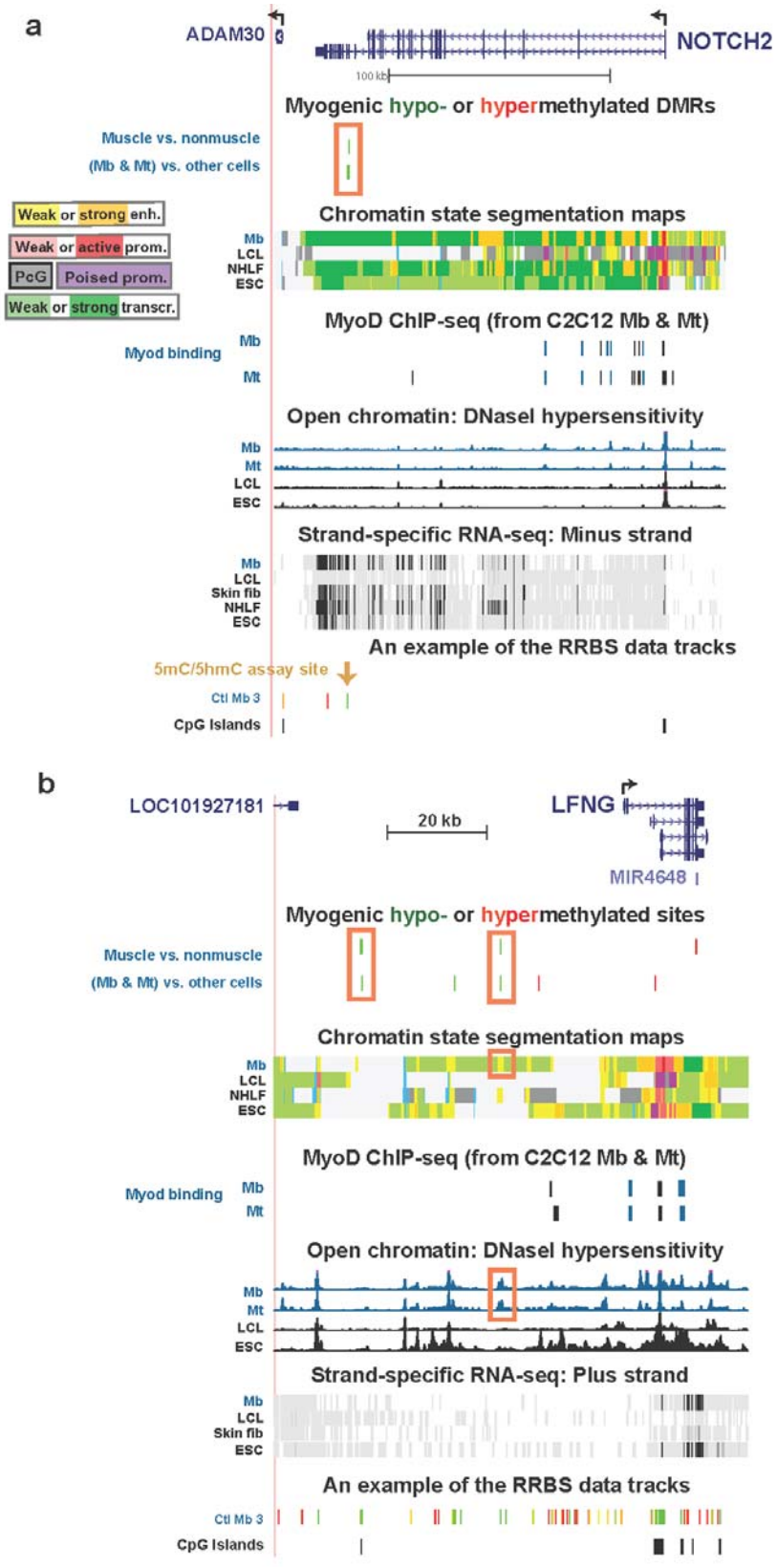


Figure S3. Myogenic hypomethylated DMRs associated with *NOTCH2* and *LFNG*. (a) and (b) for the *NOTCH2* and *LFNG* regions, respectively, we display the following tracks: significant myogenic hypo- or hyper-methylated DMRs, chromatin state segmentation, MyoD ChIP-seq, DNaseI-seq, RNA-seq, and one RRBS data track (which indicates the RRBS coverage in these regions). The regions shown are chr1:120,434,525-120,639,399 and chr7:2,482,696-2,576,953 with RNA-seq vertical viewing range at 1-to-200 and 1-to-100, respectively.



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