Supporting Information

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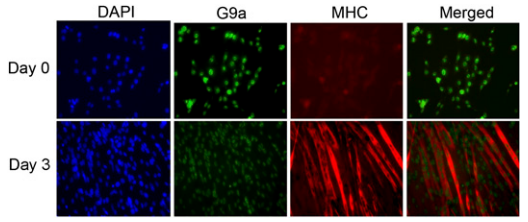

Fig. S1. Subcellular localization of endogenous G9a (green) was determined by immunofluorescence in undifferentiated (D0) and differentiated (D3) cells. Differentiated cells were identified by MHC immunostaining (red), and nuclei were stained with DAPI (blue).

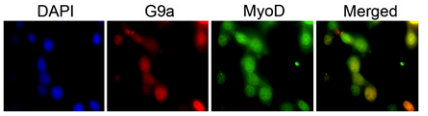

Fig. S2. Colocalization of endogenous G9a (red) and MyoD (green) in myoblasts was detected by immunofluorescence assays. Nuclei were stained with DAPI (blue).

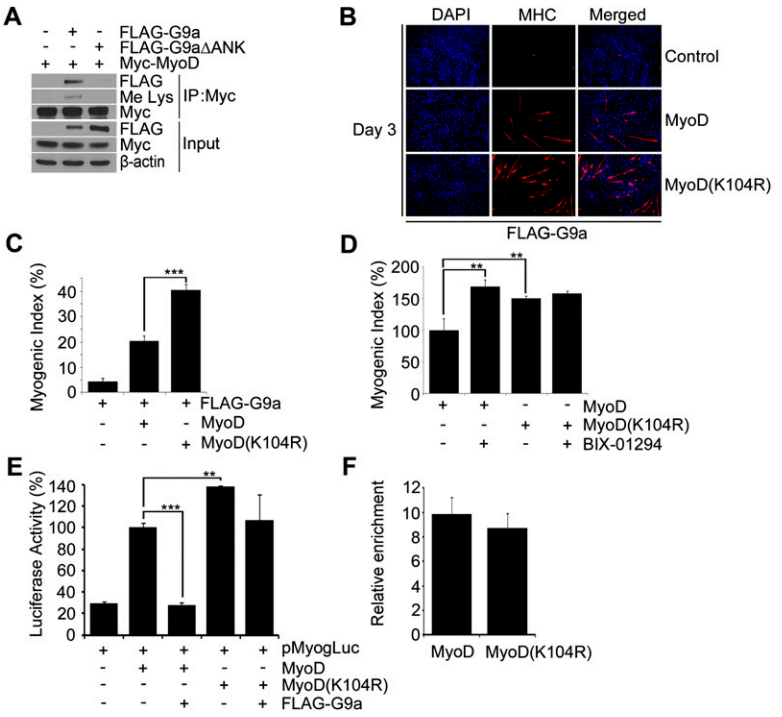

Fig. S3. (*A*) 293 cells were transfected with Myc-MyoD alone, or together with Flag-G9a and Flag-G9aΔANK. Proteins were immunoprecipitated with α-Myc agarose beads and immunoblotted with α-Flag, α-Me Lys, and α-Myc antibodies. Lysates (input) were analyzed for G9a and MyoD expression by Western blot. (*B* and *C*) Flag-G9a–overexpressing cells transfected with equivalent amounts of vector alone (control), MyoD, and MyoD(K104R) (shown in Fig. 4*K*) were differentiated for three days and analyzed for differentiation by α-MHC staining (*B*). Nuclei were stained with DAPI. Quantification of the myogenic index (*C*) revealed significantly enhanced differentiation with MyoD(K104R) compared with MyoD. (*D*) Myogenic index was quantified in C3H10T1/2 cells transfected with MyoD and MyoD(K104R) in the absence and presence of BIX-01294 (shown in Fig. 4*L*). (*E*) C3H10T1/21/2 cells were transfected with 80 ng of pMyogLuc reporter, 50 ng of MyoD or MyoD(K104), 50 ng of G9a, and 5 ng of Renilla luciferase as indicated. Empty vector was added to normalize the DNA in each well. Cells were cultured in differentiation medium for 24 h. Luciferase assays were performed by using the dual luciferase system (Promega). (*F*) G9a–overexpressing cells transfected with MyoD and MyoD(K104R) were analyzed for H3K9me2 on the myogenin promoter 2 d after differentiation by ChIP assays. Error bars indicate mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$.

Table S1. Primers for ChIP assays, Q-PCR, and G9a ankyrin deletion mutant (Δ675–807 aa)

Gene	Forward primer	Reverse primer	Melting temperature, °C
ChIP assays			
Myogenin promoter	TGGCTATATTTATCTCTGGGTTCATG	GCTCCCGCAGCCCCT	60
β-actin	GCTTCTTTGCAGCTCCTTCGTTG	TTTGCACATGCCGGAGCCGTTGT	60
Q-PCR			
G9a	TCGGGCAATCAGTCAGACAG	TGAGGAACCCACACCATTCAC	60
Myogenin	GCAATGCACTGGAGTTCG	ACGATGGACGTAAGGGAGTG	60
GAPDH	AGGAGCGAGACCCCACTAACAT	GTGAAGACACCAGTAGACTCCACG	60
G9a ankyrin deletion mutant (Δ675–807 aa)			
Flag-G9aΔANK	AACAGCATCAGGATCACCTT	GTCAACGGTGTGGATGGGGAGCCCT	—