Supplementary information

HDAC-mediated Deacetylation of NF-κB is Critical for Schwann cell Myelination

Ying Chen[1,2], Haibo Wang[1], Sung Ok Yoon[3], Xiaomei Xu[1], Michael Hottiger[4], John Svaren[5], Klaus A. Nave[6], Haesun A. Kim[7], Eric N. Olson[2], and Q. Richard Lu[1,2]

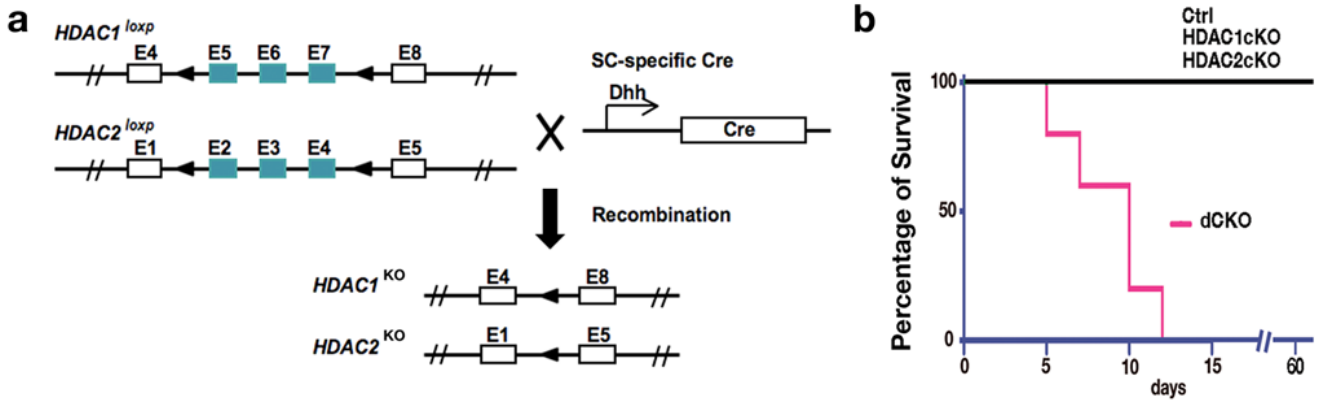
Departments of [1]Developmental Biology and Kent Waldrep Foundation Center for Basic Neuroscience Research on Nerve Growth and Regeneration, [2]Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. [3]Department of Molecular and Cellular Biochemistry, Center for Molecular Neurobiology, The Ohio State University, Columbus, Ohio 43210, USA. [4]Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich, 8057 Zurich, Switzerland. [5]Department of Comparative Biosciences, and the Waisman Center, University of Wisconsin, Madison, Wisconsin 53705. [6]Max-Planck-Institute of Experimental Medicine, Department of Neurogenetics, D-37075 Goettingen, Germany. [7]Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA.

Corresponding author:

Email: qrichard.lu@utsouthwestern.edu

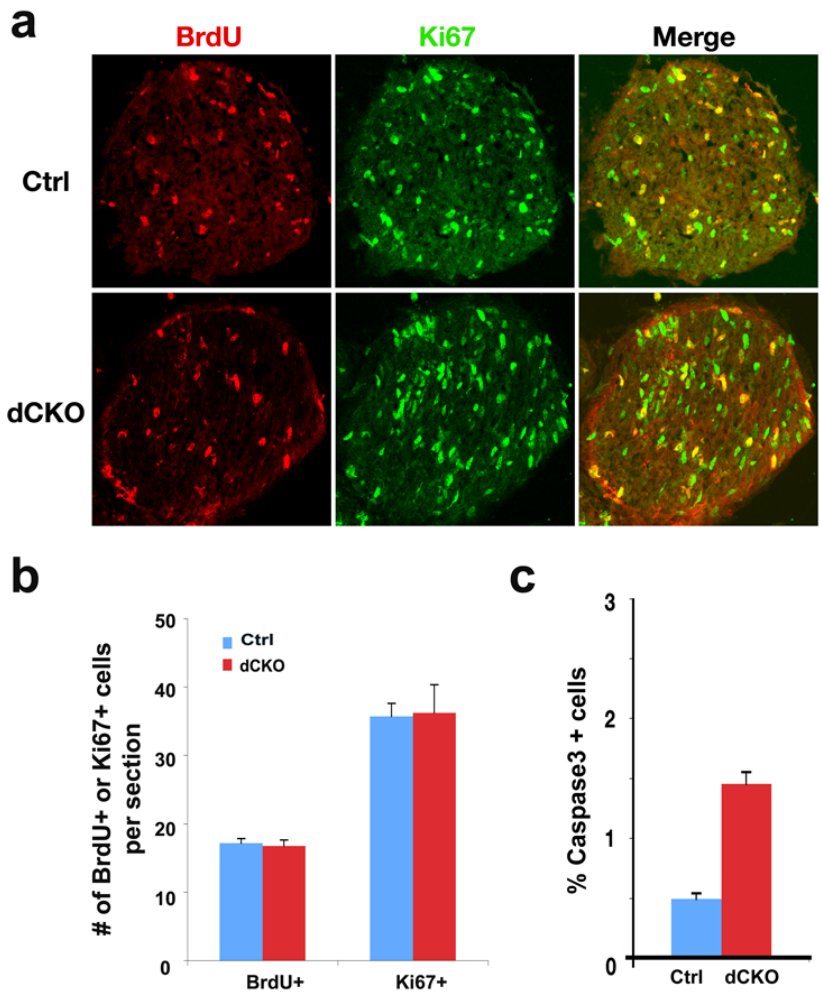
Tel: 214-648-7410; Fax: 214-648-1960

Supplementary Figures:



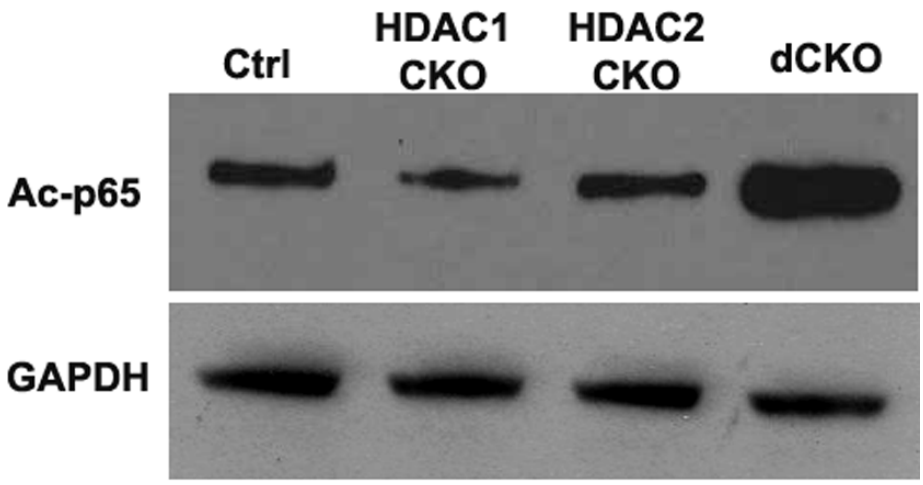
Supplementary Figure 1. Lifespan of mice lacking HDAC1/2 in the Schwann cell lineage.

(**a**) Schematic diagram of *HDAC1* and *HDAC2* conditional knockout mediated by a Schwann cell lineage expressing Dhh-Cre line. (**b**) Survival curve of control (Ctrl) and HDAC1cKO, HDAC2cKO and double mutant (dCKO) mice (> 40 mice tested for each line).



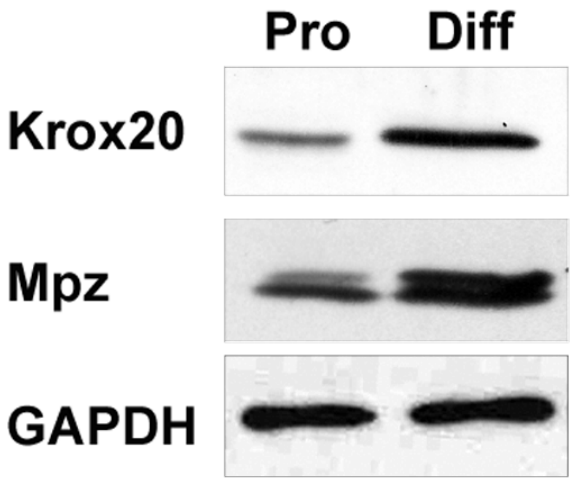
Supplementary Figure 2. Normal formation and proliferation of immature Schwann cells in dCKO mice

(**a)** Sciatic nerves of control (Ctrl) and dCKO mice at P4 after 2 hr BrdU pulse were immunostained with antibodies to proliferative markers BrdU and Ki67. Cell nuclei were counterstained with Topro3. Note: only the cells in the S-phase of the cell cycle were co-labeled with BrdU and Ki67. (**b)** Quantification of the number of BrdU and Ki67-positive cells per cross-section of above sciatic nerves (n = 3). (**c**) Sciatic nerves of control and dCKO mice at P4 were immunostained with anti-active Caspase3. The percentage of Caspase3-positive Schwann cell nuclei per cross-section was quantified.



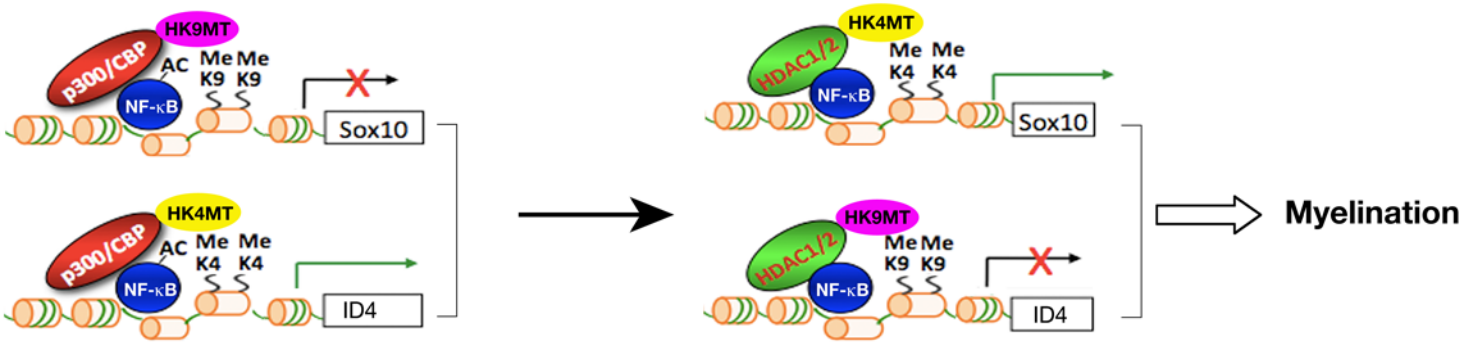
Supplementary Figure 3. The level of acetyl p65 expression is not altered in HDAC1 or HDAC2 single mutants

Lysates of sciatic nerves from control (Ctrl), HDAC1cKO, HDAC2cKO and dCKO at P4 were analyzed by Western blot with an antibody to acetyl-p65. GAPDH as a loading control. Note: expression of either HDAC1 or HDAC2 is capable for maintaining the deacetylation state of NF-κB p65.

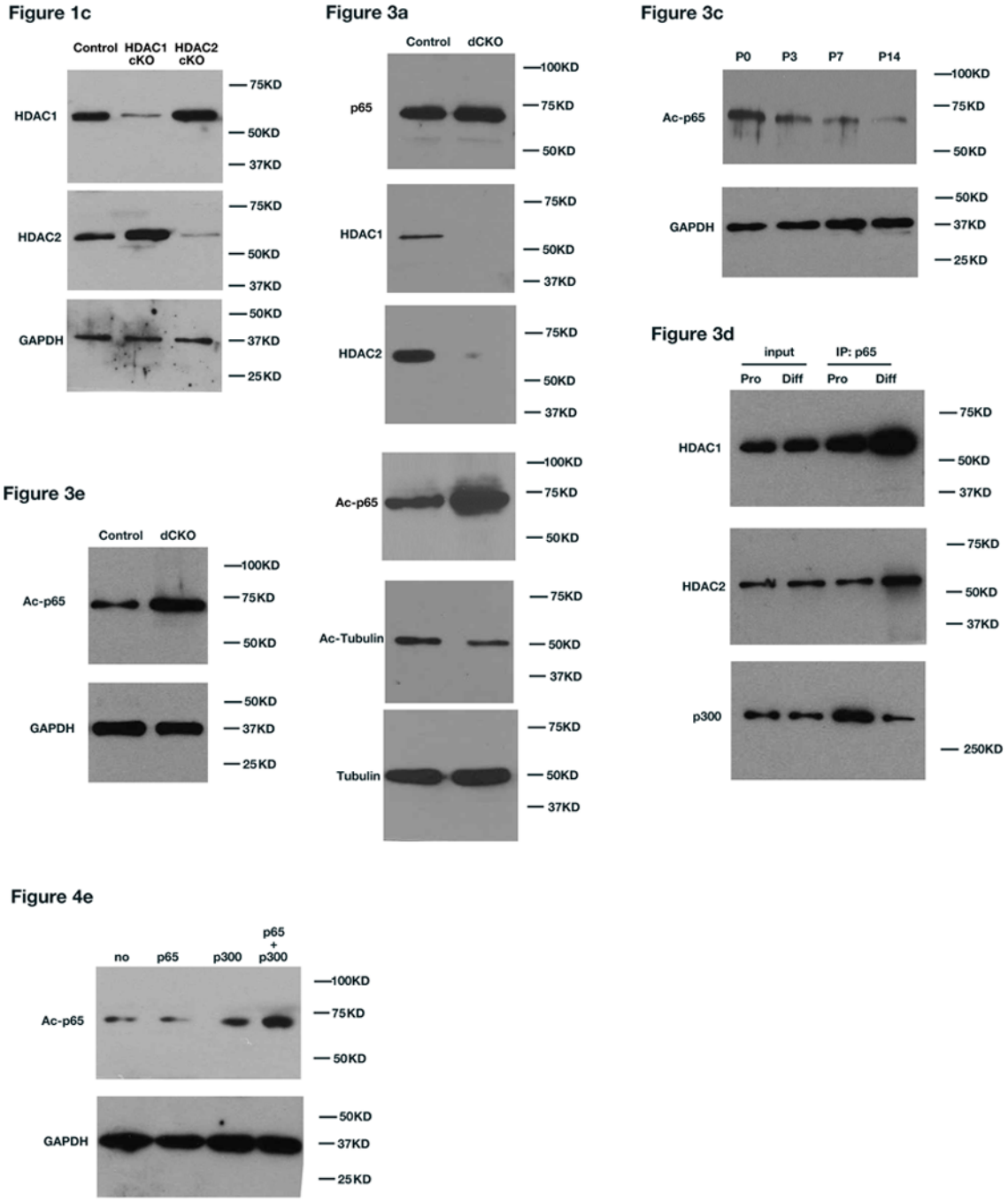


Supplementary Figure 4. Expression of Schwann cell differentiation-associated genes under the differentiation condition

Lysates from rat primary Schwann cells cultured in the proliferation medium (Pro) and the differentiation medium (Diff) for 4 days were immunoblotted with antibodies to differentiation markers Krox20 and Mpz. GAPDH is a loading control. Expression of Krox20 and Mpz increased under the differentiation condition.



Supplementary Figure 5. **Schematic diagram showing a developmental switch of the NF-κB protein complex during Schwann cell differentiation.** Left panel: in immature Schwann cells or Schwann cells in the absence of HDAC1/2, p300/CBP and acetylated NF-κB form a complex and induce chromatin modifications, e.g. by recruiting histone3 K4 or K9 methyltransferases (HK4MT or HK9MT). The NF-κB/p300 complex represses expression of differentiation activators such as Sox10, while activating differentiation inhibitors such as ID4, thereby inhibiting Schwann cell differentiation. Right panel: as Schwann cells differentiate, NF-κB forms a complex with HDAC1/2 to induce specific chromatin configuration changes on the promoters of regulatory genes and promote the Schwann cell myelination program. The protein complex switch of NF-κB p65/p300 over to NF-κB p65/HDAC1/2 promotes Schwann cell myelination.



Supplementary Figure 6. Full-length blots/gels that were presented in **Fig. 1, Fig. 3** and **Fig. 4.**