nature cell biology

DOI: 10.1038/ncb2735

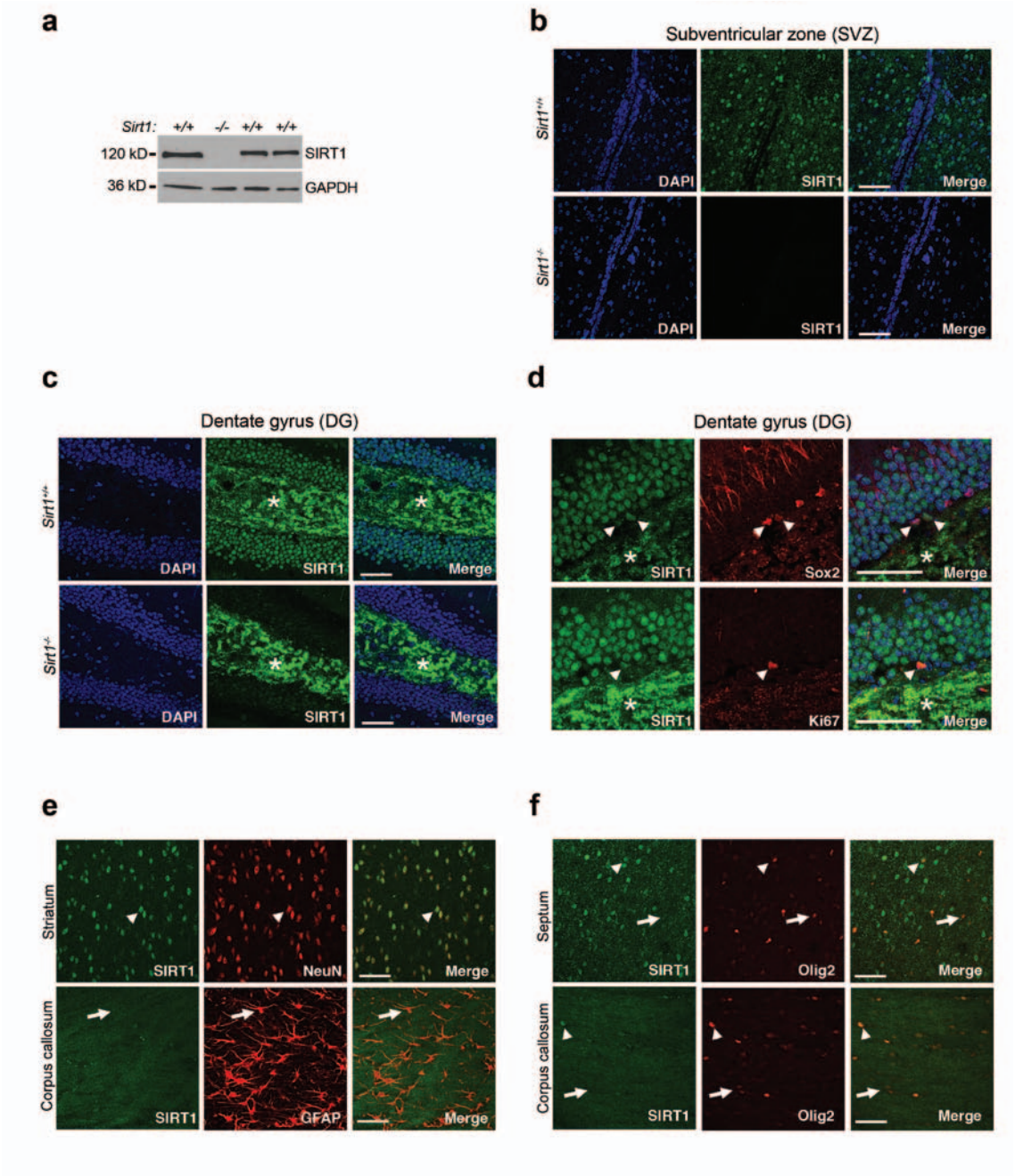

Figure S1 SIRT1 expression in the adult brain. **a,** Immunoblots of protein lysates of *Sirt1*$^{+/+}$ and *Sirt1*$^{-/-}$ postnatal NSCs/neural progenitors growing in self-renewal/proliferation conditions using antibodies to SIRT1 and the loading control GAPDH. **b,** SIRT1 antibody is specific to SIRT1 in the subventricular zone (SVZ). Immunohistochemistry on brain sections of 8-week-old *Sirt1*$^{+/+}$ and *Sirt1*$^{-/-}$ mice using antibodies to SIRT1 (green). DAPI staining (blue) marks the nuclei. Scale bars: 50 μm. **c,** SIRT1 antibody is specific to SIRT1 in the dentate gyrus (DG). Immunohistochemistry on brain sections of 8-week-old *Sirt1*$^{+/+}$ and *Sirt1*$^{-/-}$ mice using antibodies to SIRT1 (green). Asterisks indicate non-specific staining in the hilus as observed in a previous study[31]. DAPI staining marks the nuclei. Scale bars: 50 μm. **d,** SIRT1 is expressed in NSCs/neural progenitors in the adult DG. Immunohistochemistry on brain sections of 8-week-old mice using antibodies to SIRT1 (green) and Sox2 (red, upper panel) or Ki67 (red, lower panel). White arrowheads show examples of double-stained cells. Asterisks indicate non-specific staining in the hilus that is also observed in *Sirt1*$^{-/-}$ brains (see Supplementary Fig. 1c). Merged images include DAPI to illustrate nuclei. Scale bars: 50 μm. **e,** SIRT1 is expressed in neurons but not in non-NSC astrocytes. Immunohistochemistry on brain sections of 8-week-old mice using antibodies to SIRT1 (green) and the mature neuron marker NeuN (red, upper panel) or the astrocyte marker GFAP (red, lower panel). White arrowheads show an example of a NeuN-positive cell that expresses SIRT1. White arrows show an example of a GFAP-positive cell that does not express SIRT1. Scale bars: 50 μm. **f,** SIRT1 is expressed in some oligodendrocyte lineage cells. Immunohistochemistry on brain sections of 8-week old mice using antibodies to SIRT1 (green) and the oligodendrocyte lineage marker Olig2 (red). White arrowheads show examples of lineage-positive cells that express SIRT1. White arrows show examples of lineage-negative cells that do not express SIRT1. Scale bars: 50 μm.

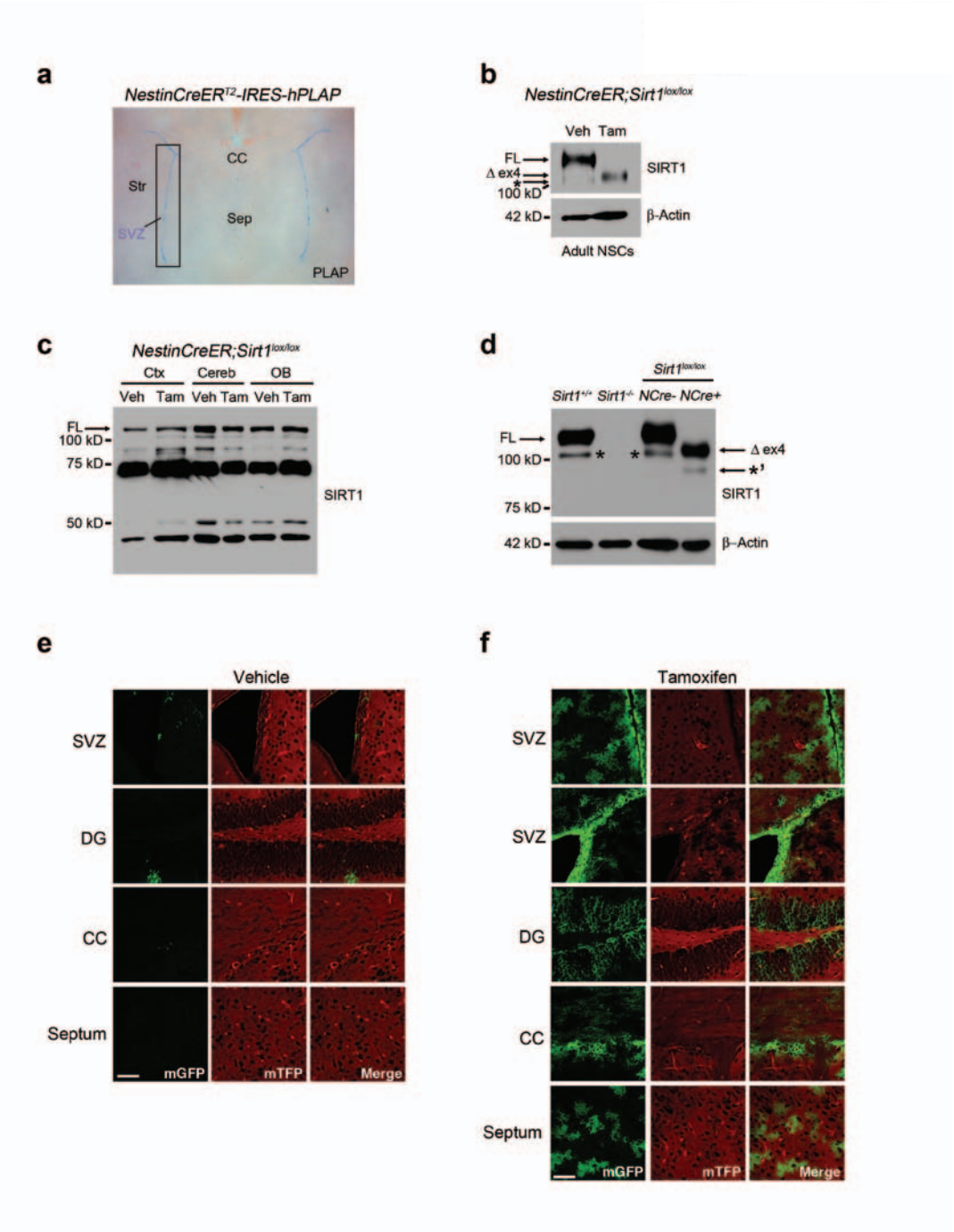

Figure S2 Characterization of SIRT1 inactivation in adult NSCs/neural progenitors. **a,** *Placental alkaline phosphatase (PLAP) is specifically expressed in the SVZ of NestinCreER^{T2}–IRES–hPLAP;Sirt1$^{lox/lox}$ (NestinCreER;Sirt1$^{lox/lox}$) mice. Bright-field image of a coronal brain section stained for PLAP activity (blue).* SVZ: subventricular zone, *Str: striatum, Sep: septum,* CC: corpus callosum. **b,** *Full-length SIRT1 is deleted in* adult NSCs/neural progenitors of tamoxifen-injected *NestinCreER;Sirt1$^{lox/lox}$* mice. Immunoblot of lysates from adult NSCs/neural progenitors isolated from *NestinCreER;Sirt1$^{lox/lox}$ mice injected with tamoxifen (Tam) or vehicle (Veh). See Supplementary Fig. 2d.* **c,** *Full-length SIRT1 is not deleted in brain tissues of tamoxifen-injected NestinCreER;Sirt1$^{lox/lox}$ mice. Immunoblot of brain tissues from adult NestinCreER;Sirt1$^{lox/lox}$ mice injected with vehicle or tamoxifen. Ctx: cortex, Cereb: cerebellum, OB: olfactory bulb.* **d,** SIRT1 truncated protein and SIRT1 splice variant have different molecular weights. Immunoblots of lysates from adult NSCs/neural progenitors. The truncated SIRT1 protein obtained by Cre recombination of *Sirt1* exon 4 has a slightly greater molecular weight compared to a second SIRT1 protein product (*) in wild-type cells, presumably formed from a splice variant that has previously been observed[19] and that is no longer present in *Sirt1$^{-/-}$* cells. NCre: NestinCre, FL: full-length SIRT1, Δex4: truncated SIRT1 protein product from the *Sirt1* gene lacking exon 4, *: second SIRT1 protein product detected by SIRT1 antibody, *': splice variant of the truncated SIRT1 protein product from the *Sirt1* gene lacking exon 4 detected by SIRT1 antibody. **e,** Neglible expression of membrane-targeted green fluorescent protein (mGFP) in the NSC niche and surrounding areas after corn oil/ethanol vehicle injection of *NestinCreER;mT/mG;Sirt1$^{lox/lox}$* mice. A rare mGFP-positive cell was observed in brains of vehicle-injected mice 10 days after injection. Scale bar: 50 μm. **f,** Expression of mGFP in the NSC niche and surrounding areas after tamoxifen injection of *NestinCreER;mT/mG;Sirt1$^{lox/lox}$* mice. DG: dentate gyrus, mTFP: membrane-targeted tomato red fluorescent protein. *Scale* bar: 50 μm.

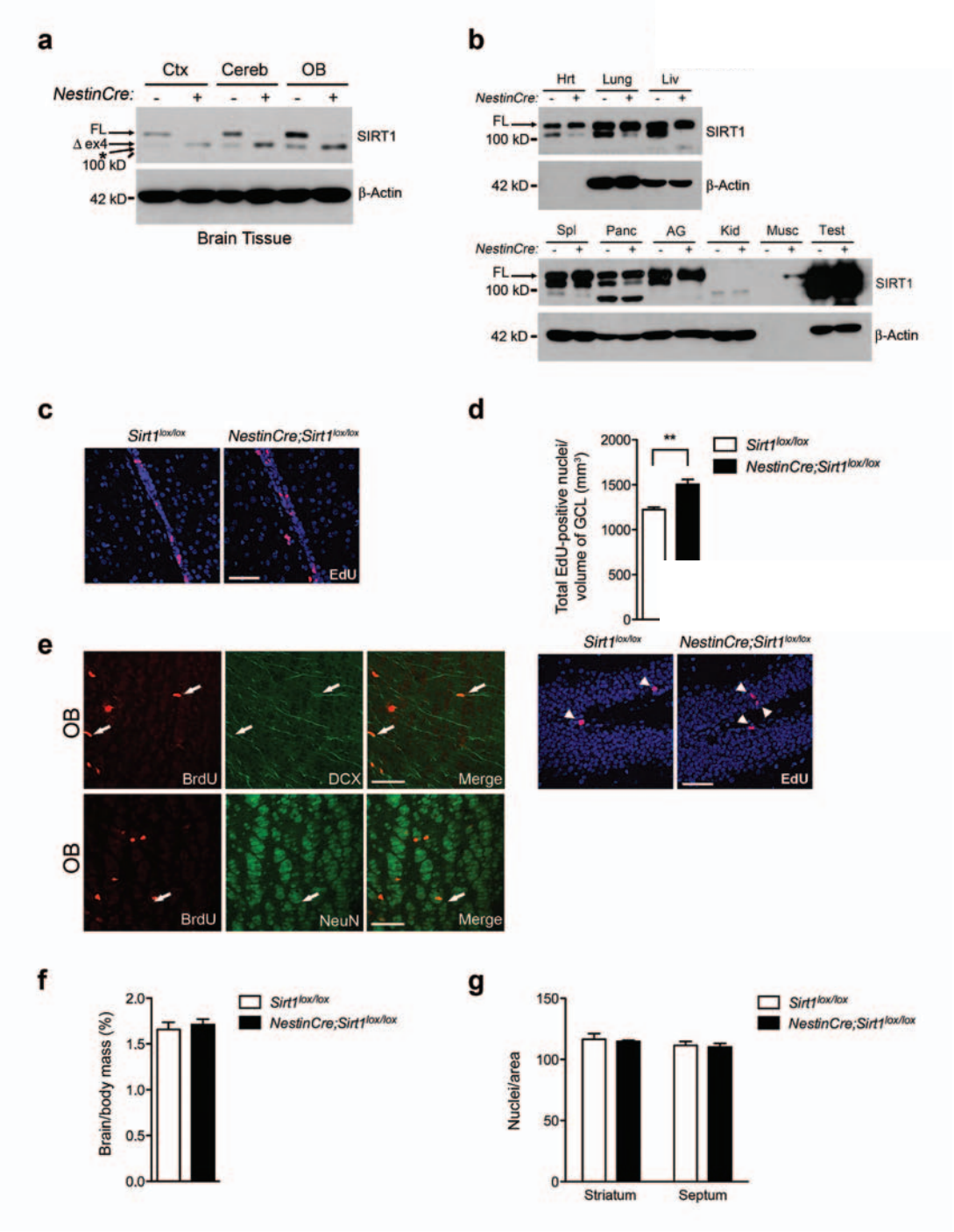

Figure S3 Chronic inactivation of SIRT1 in the nervous system leads to increased NSC proliferation and impaired neuronal maturation. **a,** Deletion of full-length SIRT1 protein was observed in neural tissues of 8-week-old *NestinCre;Sirt1*$^{lox/lox}$ mice by immunoblots using antibodies to SIRT1 and b-Actin (loading control). Ctx: cortex, Cereb: cerebellum, OB: olfactory bulb, FL: full-length SIRT1; *Δex4: truncated SIRT1 protein generated from the Sirt1 gene that lacks exon 4. *: protein that is distinct from the truncated SIRT1 protein and likely represents a splice variant of SIRT1 (see Supplementary Fig. 2d).* **b,** No deletion of full-length SIRT1 protein in non-neural tissues of *NestinCre;Sirt1*$^{lox/lox}$ mice. b-Actin is not expressed in heart or muscle tissues. Hrt: heart, Liv: liver, Spl: spleen, Panc: pancreas, AG: adrenal gland, Kid: kidney, Musc: muscle, Test: testes. **c,** Images of EdU-positive nuclei in the SVZ of *NestinCre;Sirt1*$^{lox/lox}$ and *Sirt1*$^{lox/lox}$ mice injected with EdU for 2 hrs. Ethynyl deoxyuridine: EdU. Scale bar: 50 μm. **d,** Adult mice lacking SIRT1 in the brain have increased numbers of proliferating cells in the dentate gyrus (DG). Mean +/- SEM of 3 mice per genotype. Two-tailed, unpaired Student's t-test, ** p = 0.0084. Scale bar: 50 μm. **e,** Images of BrdU-positive cells expressing the neuroblast marker DCX or the mature neuron marker NeuN in the OB granule cell layer. Scale bars: 50 μm. **f,** The brain to body mass ratio of *NestinCre:Sirt1*$^{lox/lox}$ mice does not differ from control *Sirt1*$^{lox/lox}$ mice. Male mice were weighed at 4.5 months of age. Mean +/- SEM of n = 4 *Sirt1*$^{lox/lox}$ and n = 5 *NestinCre;Sirt1*$^{lox/lox}$ mice. No significant difference. **g,** Cell density is not affected by loss of active SIRT1 in the brain. The number of DAPI-positive nuclei in the striatum and septum was counted and normalized to the area under analysis. Mean +/- SEM of 3 mice per genotype. No significant difference.

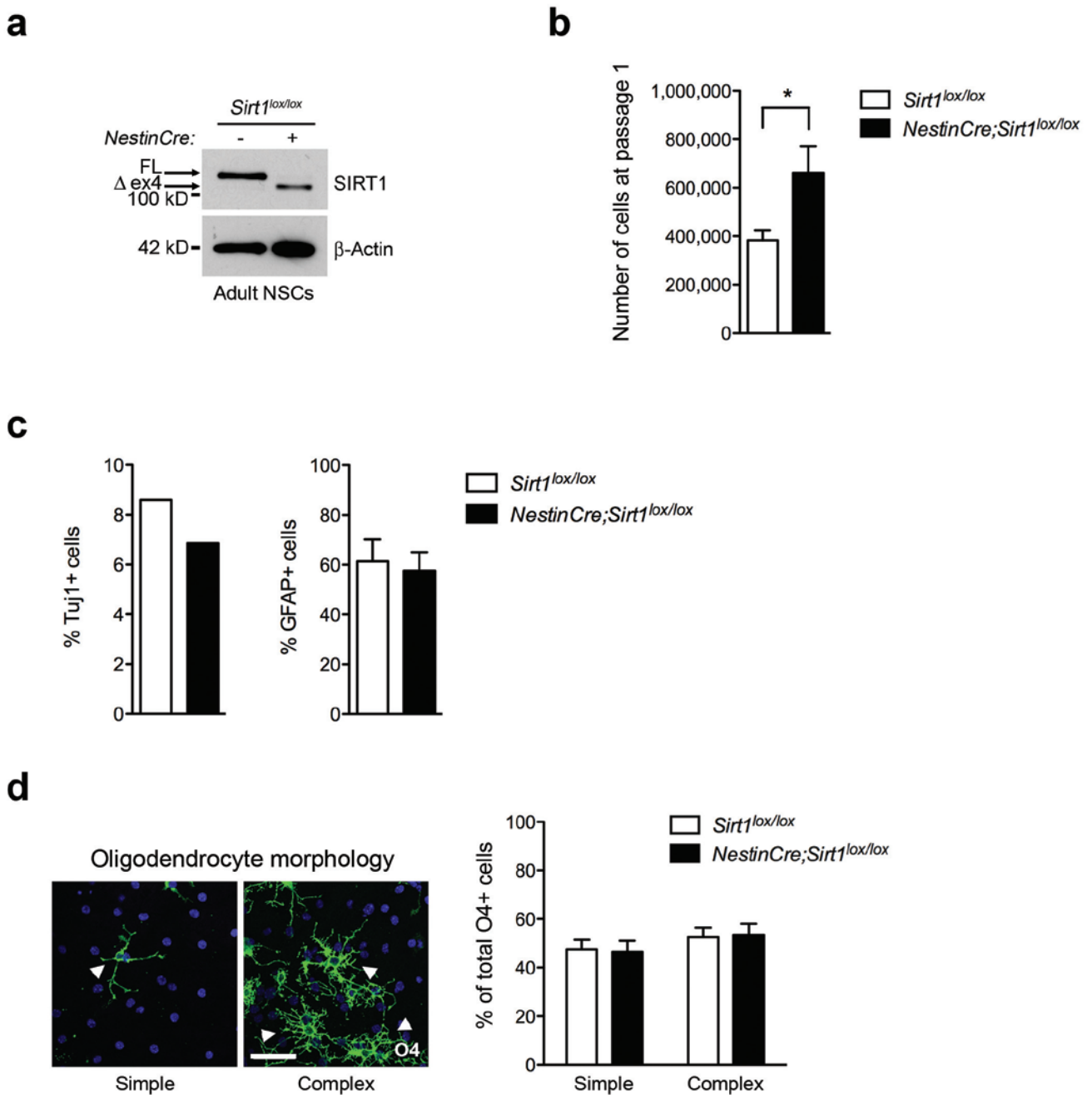

Figure S4 SIRT1 inactivation leads to NSC/neural progenitor expansion in vitro and has no effect on neuron/astrocyte differentiation or oligodendrocyte morphological complexity. **a,** SIRT1 inactivation in NSC/neural progenitor cultures from adult mice. Immunoblot of lysates from primary NSCs/neural progenitors cultured from *NestinCre;Sirt1*$^{lox/lox}$ and control *Sirt1*$^{lox/lox}$ 8-week-old mice. b-Actin serves as loading control. FL: full-length SIRT1, *Δex4: truncated SIRT1 protein generated from the Sirt1 gene that lacks exon 4*. **b,** SIRT1 inactivation results in increased cell numbers in primary adult NSC/neural progenitor cultures. Mean +/- SEM of 4 independent experiments using 2 mice per genotype in each experiment. Two-tailed, unpaired Student's t-test, * p = 0.0542. **c,** SIRT1 does not significantly affect the differentiation of neurons or astrocytes in vitro. NSCs/neural progenitors from adult *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ mice were differentiated for 7 days in the absence of growth factors and in the presence 1% FBS. For Tuj1, mean of 2 independent experiments; for GFAP, mean +/- SEM of 3 independent experiments. No significant difference. **d,** SIRT1 does not influence the morphology of O4-positive cells. NSCs/neural progenitors from adult *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ mice were differentiated for 7 days in the absence of growth factors and in the presence 1% FBS. The percentage of O4-positive cells with simple and complex morphologies was counted. Arrowheads indicate O4-positive cells. Mean +/- SEM of 4 independent experiments. Scale bar: 50 μm.

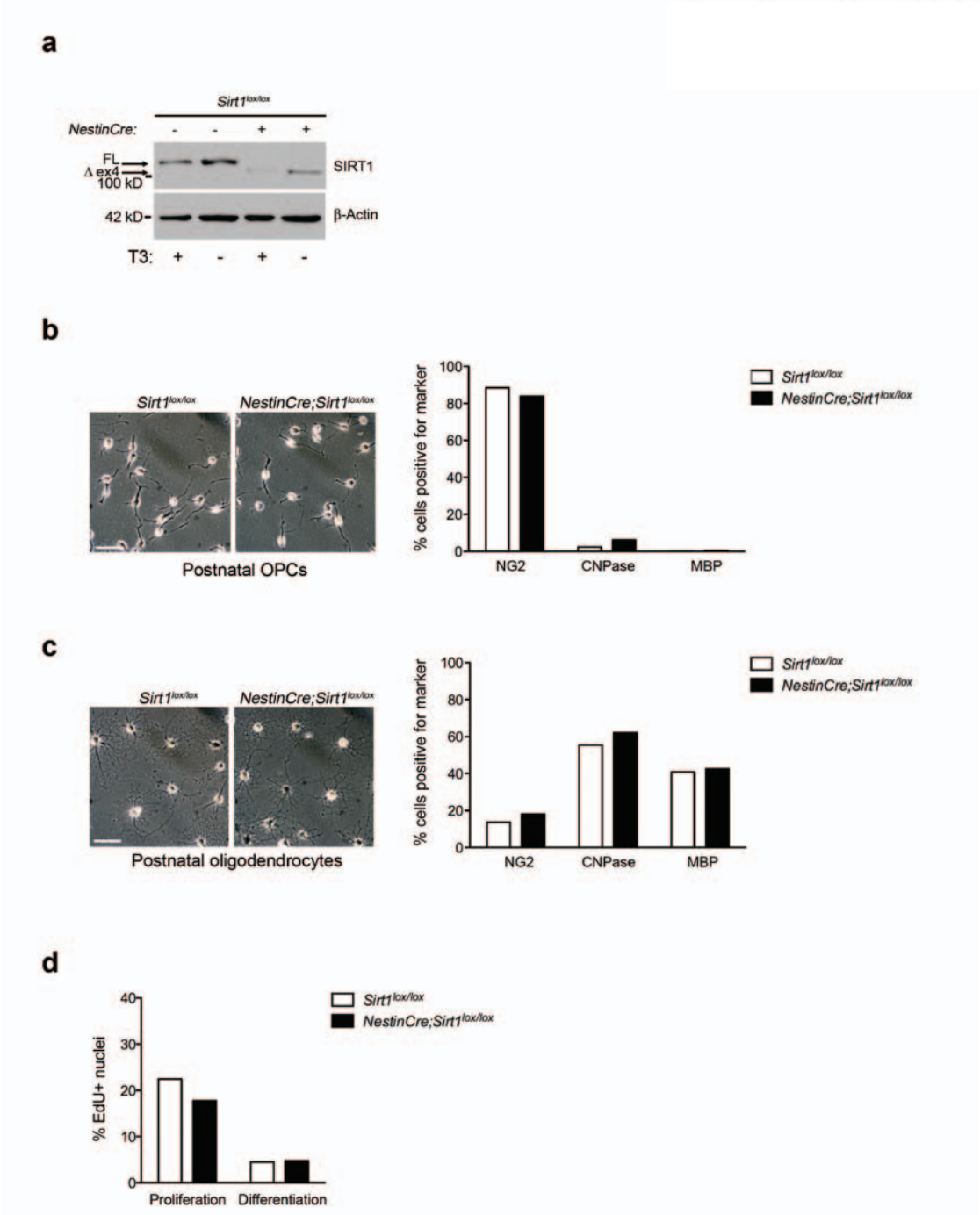

Figure S5 SIRT1 does not affect postnatal OPC identity and proliferation. **a,** Deletion of full-length SIRT1 in OPCs isolated from postnatal *NestinCre;Sirt1*$^{lox/lox}$ mice. Immunoblot of lysates from primary OPCs purified from *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ 7-8 day old pups mice grown in proliferation media (+PDGF -T3) or differentiation media (-PDGF +T3). The truncated SIRT1 protein indicated by the arrow migrates faster than the full-length SIRT1 protein. b-Actin serves as loading control. T3: thyroid hormone triiodothyronine, FL: full-length SIRT1; *Δex4: truncated SIRT1 protein generated from the Sirt1 gene that lacks exon 4.* **b,** Maintenance of undifferentiated OPC state is not affected by loss of SIRT1. Proliferating postnatal OPCs from *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ 7-8 day old pups were immunostained for the OPC marker NG2 and two markers of mature oligodendrocytes, CNPase and MBP. Brightfield images demonstrate the gross morphology of the proliferating OPCs. Mean of 2 independent experiments. Scale bar: 50 μm. **c,** Gross morphology and marker expression of cells differentiated from postnatal OPCs are not affected by the loss of active SIRT1. Postnatal OPCs (grown in media without PDGF and with T3) from *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ 7-8 day old pups were differentiated from three days and immunostained for the OPC maker NG2 and two markers of mature oligodendrocytes, CNPase and MBP. Brightfield images demonstrate the gross morphology of the differentiating oligodendrocytes. Mean of 2 independent experiments. Scale bar: 50 μm. **d,** Proliferation of postnatal OPCs is not affected by loss of SIRT1. Proliferating or differentiating postnatal OPCs from *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ 7-8 day old pups were incubated with EdU for 4 and 24 hours, respectively and EdU incorporation was quantified. Ethynyl deoxyuridine: EdU. Mean of 2 independent experiments.

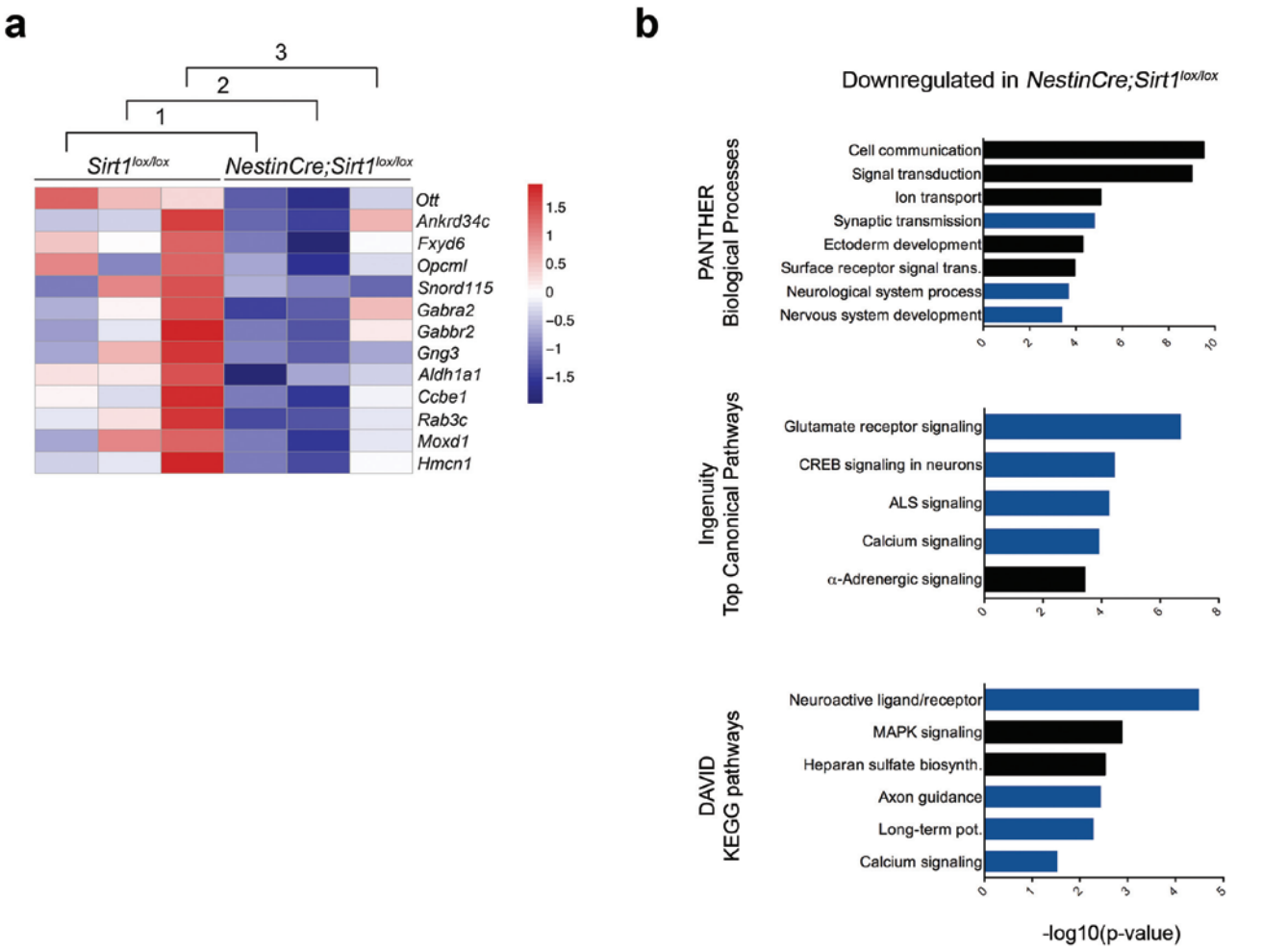

Figure S6 Genome-wide analysis of genes downregulated in adult NSCs/neural progenitors lacking active SIRT1. **a,** Genes downregulated in *NestinCre;Sirt1*$^{lox/lox}$ NSCs/neural progenitors compared to *Sirt1*$^{lox/lox}$ control NSCs/neural progenitors. Whole-genome microarray data were obtained from three independent biological replicates of RNA from proliferating *NestinCre;Sirt1*$^{lox/lox}$ and *Sirt1*$^{lox/lox}$ NSCs/neural progenitors isolated from 8-week-old mice. Heat-map represents a subset of genes downregulated in the absence of active SIRT1 that was selected based on a false discovery rate of less than 0.001%. Numbers 1-3 designate independent biological experiments. Colors represent the Z score of the RMA-normalized expression level for each gene (red is high expression, blue is low expression). **b,** Genes downregulated in the absence of active SIRT1 are significantly over-represented in biological processes related to nervous system development and neuronal function. Genes differentially regulated by SIRT1 were selected based on false discovery rate of less than 1% and analyzed using PANTHER, Ingenuity Pathway Analysis (IPA), and DAVID Bioinformatics Database for significantly over-represented functional categories. Blue color highlights categories of genes related to neural processes.

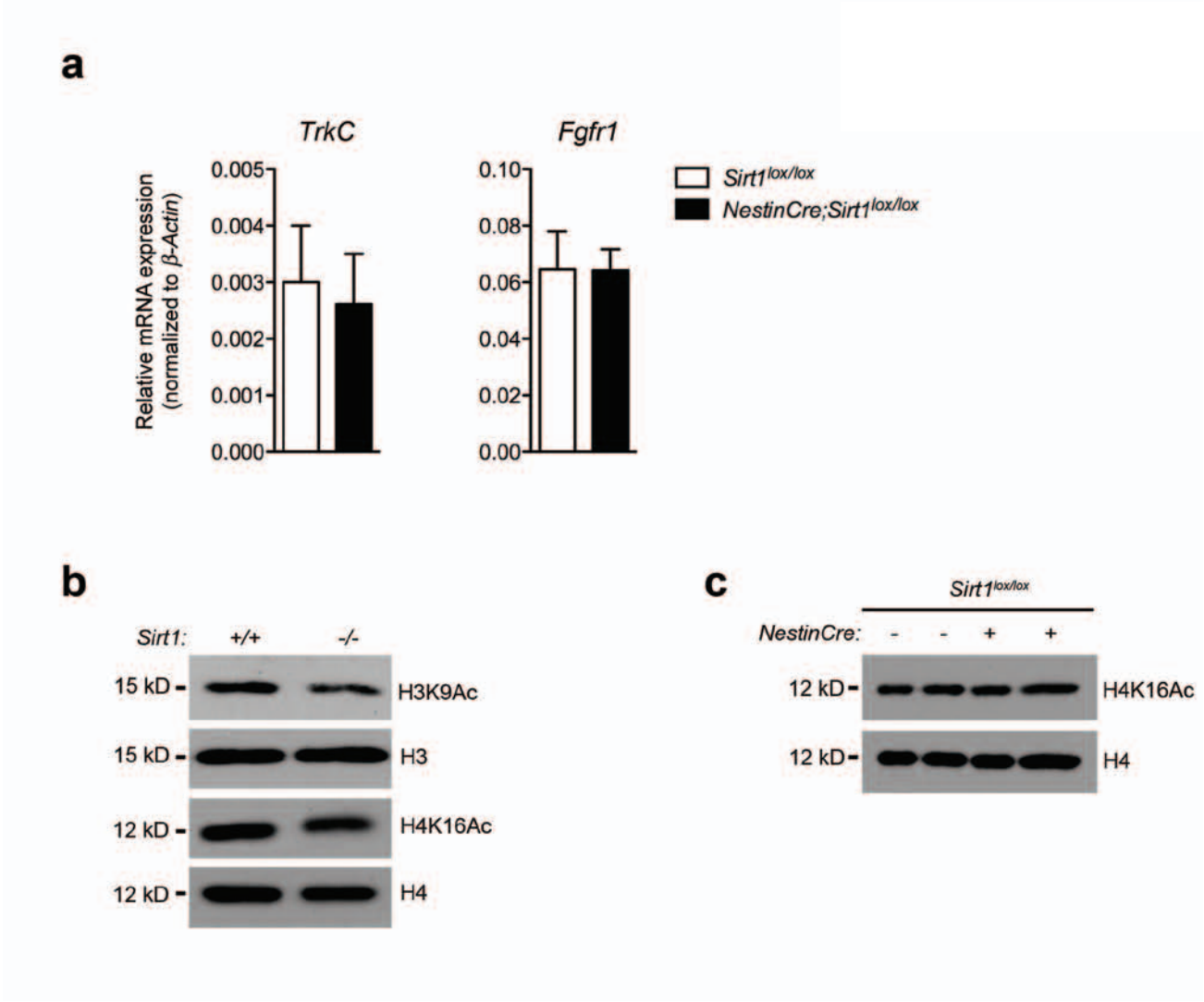

Figure S7 SIRT1 inactivation in NSCs/neural progenitors does not affect *TrkC* and *Fgfr1* levels and does not impact global H3K9 and H4K16 acetylation. **a,** Expression of growth factor receptors *TrkC* and *Fgfr1* in adult NSCs/neural progenitors lacking active SIRT1. mRNA levels were normalized to *b-Actin* mRNA. Mean +/- SEM of 5 independent experiments. **b,** Global levels of H3K9 acetylation and H4K16 acetylation are not increased in *Sirt1*$^{-/-}$ NSCs/neural progenitors compared to control cells. Chromatin was isolated from *Sirt1*$^{+/+}$ and *Sirt1*$^{-/-}$ adult NSCs/neural progenitors and immunoblotted with antibodies specific to H3K9Ac, H4K16Ac, total H3, and total H4. **c,** Global levels of H4K16 acetylation are not increased in *NestinCre;Sirt1*$^{lox/lox}$ NSCs/neural progenitors compared to control cells. Histones were extracted from *Sirt1*$^{lox/lox}$ and *NestinCre;Sirt1*$^{lox/lox}$ adult NSCs/neural progenitors and immunoblotted with antibodies specific to H4K16Ac and total H4.

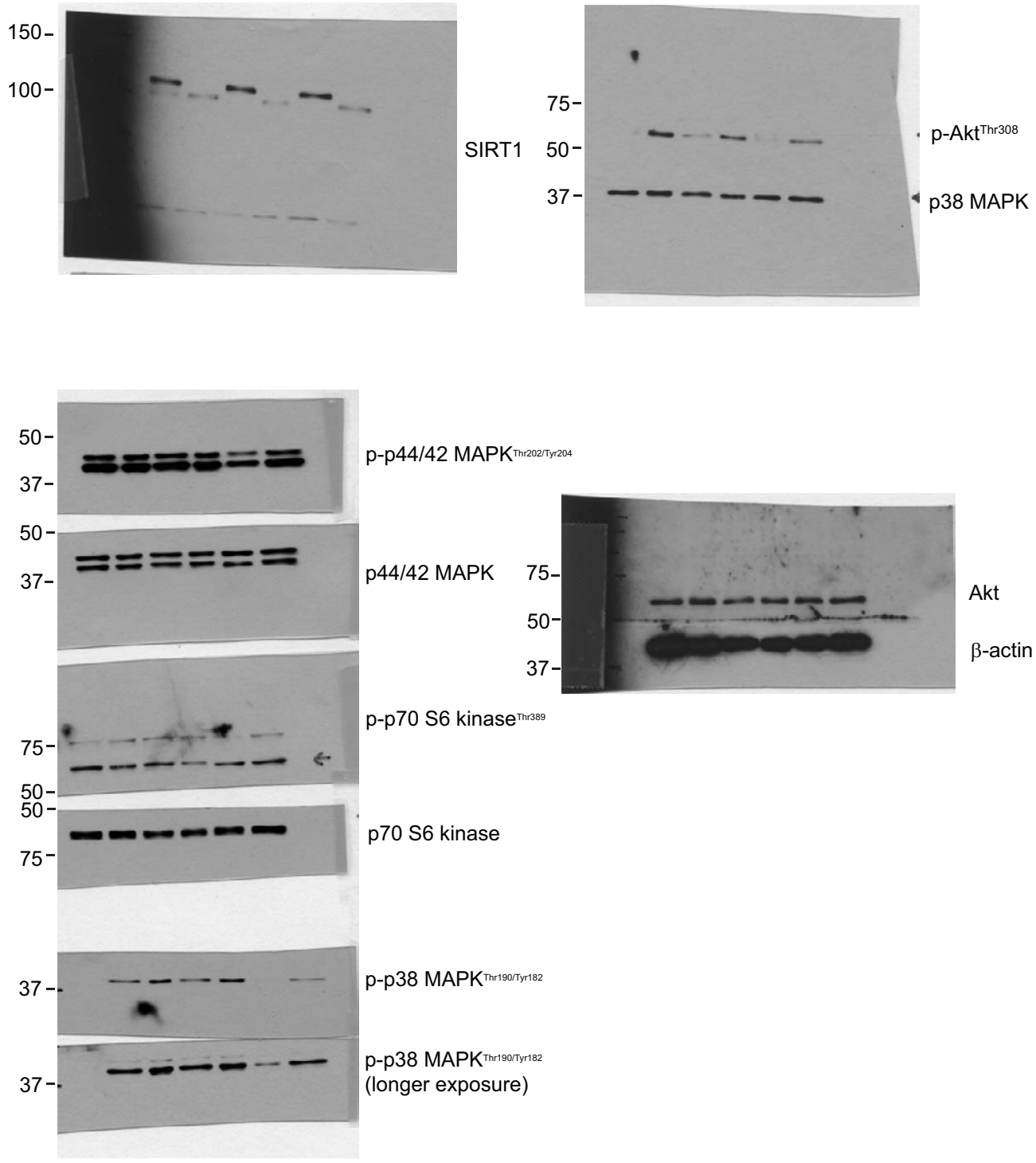

Figure S8 Full size immunoblots presented in Fig. 8a. Molecular weight markers in kilodaltons are indicated on the left sides of the immunoblots.

Supplementary Table 1 Genes differentially expressed in adult *NestinCre;Sirt1*$^{lox/lox}$ **and** *Sirt1*$^{lox/lox}$ NSC/neural progenitor cultures**.** List of genes significantly upregulated and downregulated in the absence of active SIRT1 obtained by Rank Products analysis of microarray data, broken down by false discovery rates ranging from <1% and <0.001%.