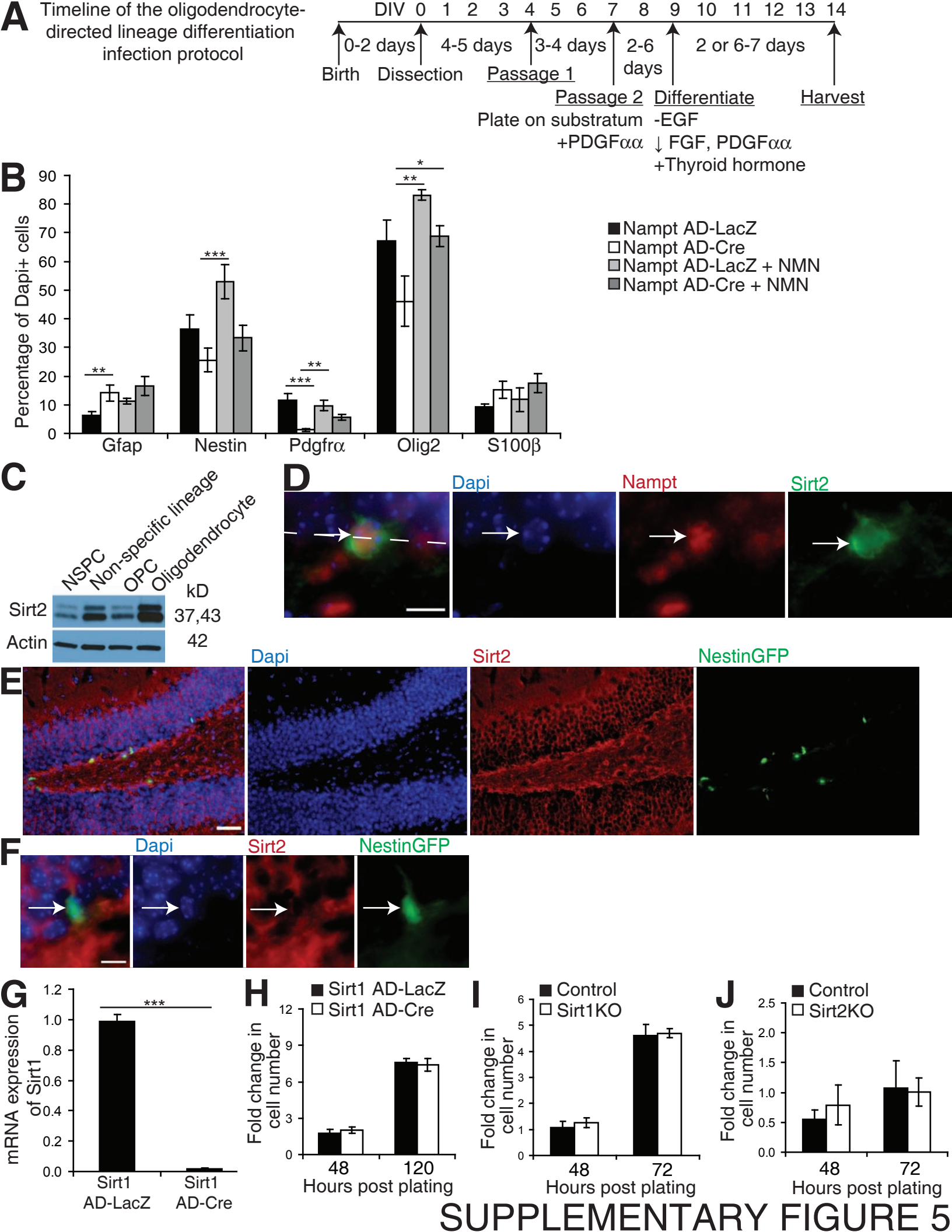

SUPPLEMENTARY FIGURE 5

Supplementary Figure S5. Genetic ablation of *Nampt in vitro* impairs OPC formation.

A) A scheme for the oligodendrocytic lineage differentiation protocol used. Neurospheres were isolated from *Nampt*$^{flox/flox}$ mice and infected with a Cre-recombinase expressing adenovirus (Nampt AD-Cre) or a control adenovirus expressing LacZ (Nampt AD-LacZ). **B)** Dissociated neurospheres were cultured in proliferation media containing PDGFαα and assessed by immunoflorescence. Histogram shows the percentages of Dapi+ cells that express markers of NSPCs (Gfap, Nestin), OPCs (Pdgfrα+, Olig2+), and astrocytes (S100β) (n=3-12 independent samples, 6-30 fields of view). **C)** A representative immunoblot for Sirt2 in neurospheres cultured as NSPCs (with EGF, FGF) or OPCs (with EGF, FGF, PDGFαα) before and after differentiation. **D)** Immunoflorescence for Dapi (blue), Nampt (red), and Sirt2 (green) along the SGZ. Dotted lines denote the SGZ. Single arrowheads indicate examples of colocalization of cell immunoreactivity. Scale bar denotes 10 μm. **E-F)** Immunoflorescence for Dapi (blue), Sirt2 (red), and NestinGFP (green, 3 days post TAM) along the SGZ. Dotted lines denote the SGZ. **E)** Scale bar denotes 50 μm. **F)** Scale bar denotes 20 μm. **G-H)** Neurospheres were isolated from *Sirt1*$^{flox/flox}$ mice and infected with a Cre recombinase-expressing adenovirus (Sirt1 AD-Cre) or a control adenovirus expressing LacZ (Sirt1 AD-LacZ). **G)** Quantitative RT-PCR results for mRNA expression of Sirt1 (n=17-24). **H-J)** Quantification of the fold increase in cell number (n=5-20). Neurospheres were derived from full body Sirt1 KO mice (**I**), Sirt2 KO (**J**) mice, and their respective littermate controls. Data are presented as mean ± s.e.m. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.