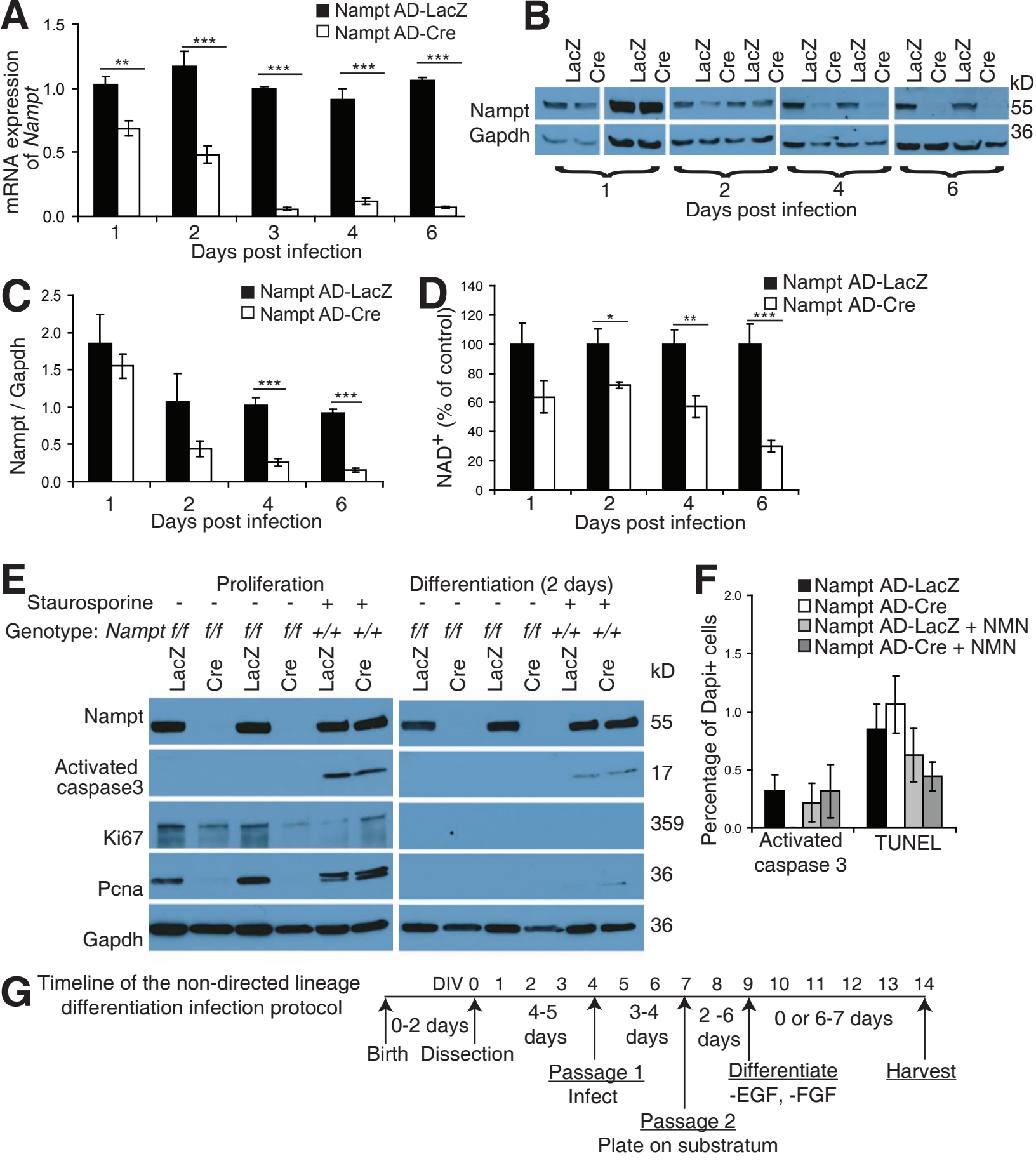

SUPPLEMENTARY FIGURE 4

Supplementary Figure S4. Genetic ablation of *Nampt* in NSPCs *in vitro* impairs NAD^+ biosynthesis, proliferation, and differentiation.

A-G) Neurospheres were isolated from *Nampt*$^{flox/flox}$ (*f/f*) mice and infected with a Cre-recombinase expressing adenovirus (Nampt AD-Cre) or a control adenovirus expressing LacZ (Nampt AD-LacZ). Analyses were conducted after passage 2, at 6 or more days post infection. **A)** Quantitative RT-PCR results for mRNA expression of Nampt in AD-LacZ and Nampt Ad-Cre infected neurospheres (n=3-33). **B)** Representative immunoblots for Nampt and Gapdh. **C)** Quantification of immunoblots for Nampt in neurospheres normalized by Gapdh (n=4-13). **D)** HPLC analysis of NAD^+ levels. NAD^+ levels in Nampt Ad-Cre infected neurospheres were normalized by NAD^+ levels in Nampt Ad-LacZ infected neurospheres (n=4-9). **E)** Representative immunoblots of Nampt Ad-Cre or Nampt AD-LacZ infected neurospheres 8 days post infection for markers of cell death (activated caspase 3) and proliferation (Ki67, Pcna). Neurospheres were grown under proliferation conditions (left blot) or differentiated for 2 days (right blot). As a positive control for activated caspase 3 immunoreactivity, indicated samples isolated from *Nampt*$^{+/+}$ mice were treated with stauroporine (1 mM) (n=6). **F)** Immunoflorescence analysis of dissociated neurospheres cultured in proliferation media. Histogram shows the percentages of activated caspase 3+ (n=3 independent samples, 6 fields of view) or TUNEL+ cells (n=9 independent samples, 14-21 fields of view) relative to the total number of Dapi+ cells. **G)** A scheme for the non-directed lineage differentiation protocol used. Data are presented as mean ± s.e.m. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.