Supplemental Figures

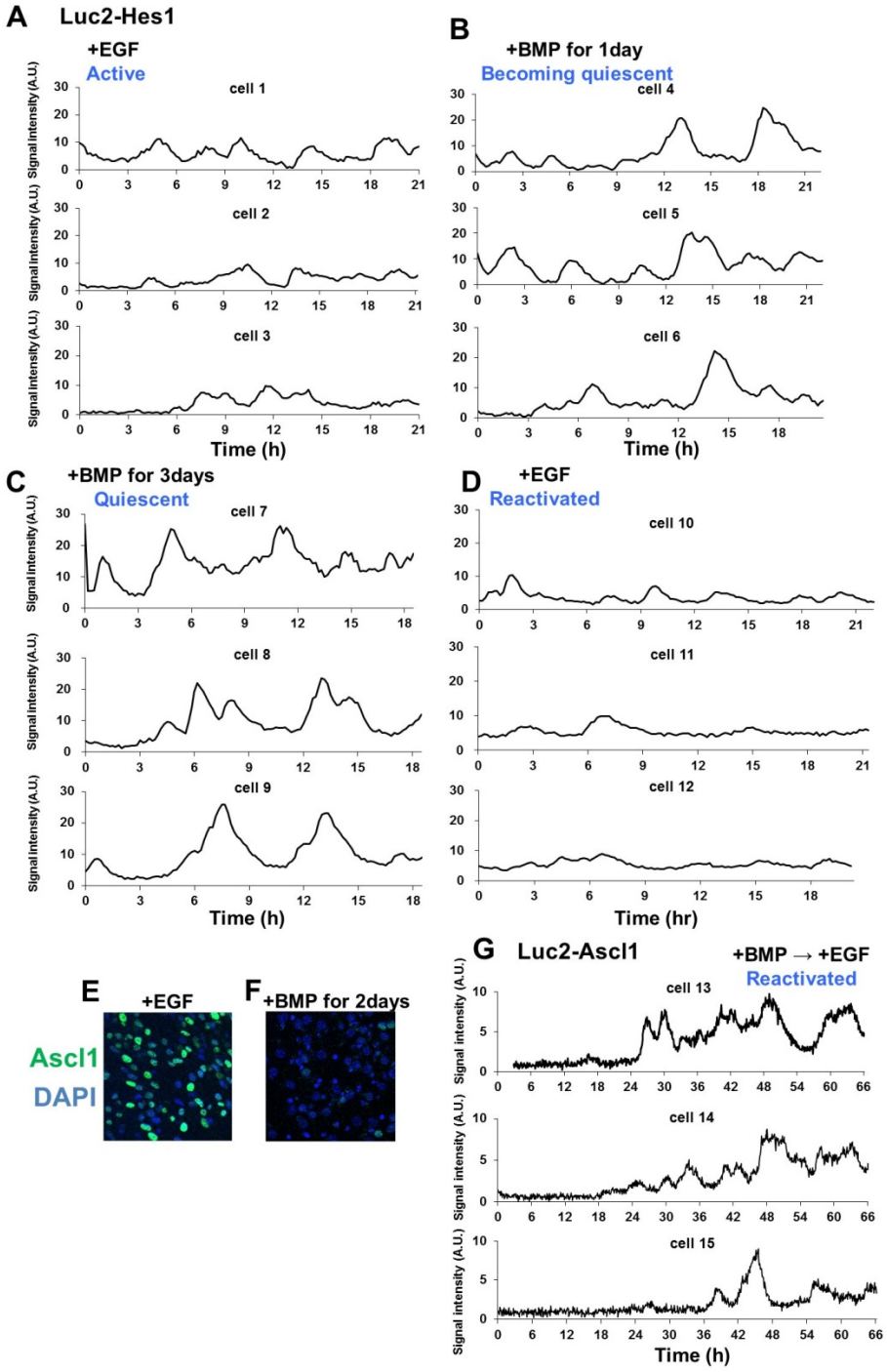

Figure S1, Related to Figure 1. Variable levels of Hes1 and Ascl1 in cultured NS cells. (A-D) Quantification of bioluminescence imaging of the Luc2-Hes1 reporter in cultured NS cells. NS cells carrying the Luc2-Hes1 reporter were cultured in the presence of EGF (A,D) or BMP (B,C) in addition to bFGF. (E,F) Immunostaining of Ascl1 in NS cells cultured in the presence of EGF (E) or BMP (F) in addition to bFGF. (G) Quantification of bioluminescence imaging of the Luc2-Ascl1 reporter in cultured NS cells. BMP medium was switched to EGF medium. Note that the level of the Luc2-Ascl1 reporter was initially low but then increased and started to oscillate.

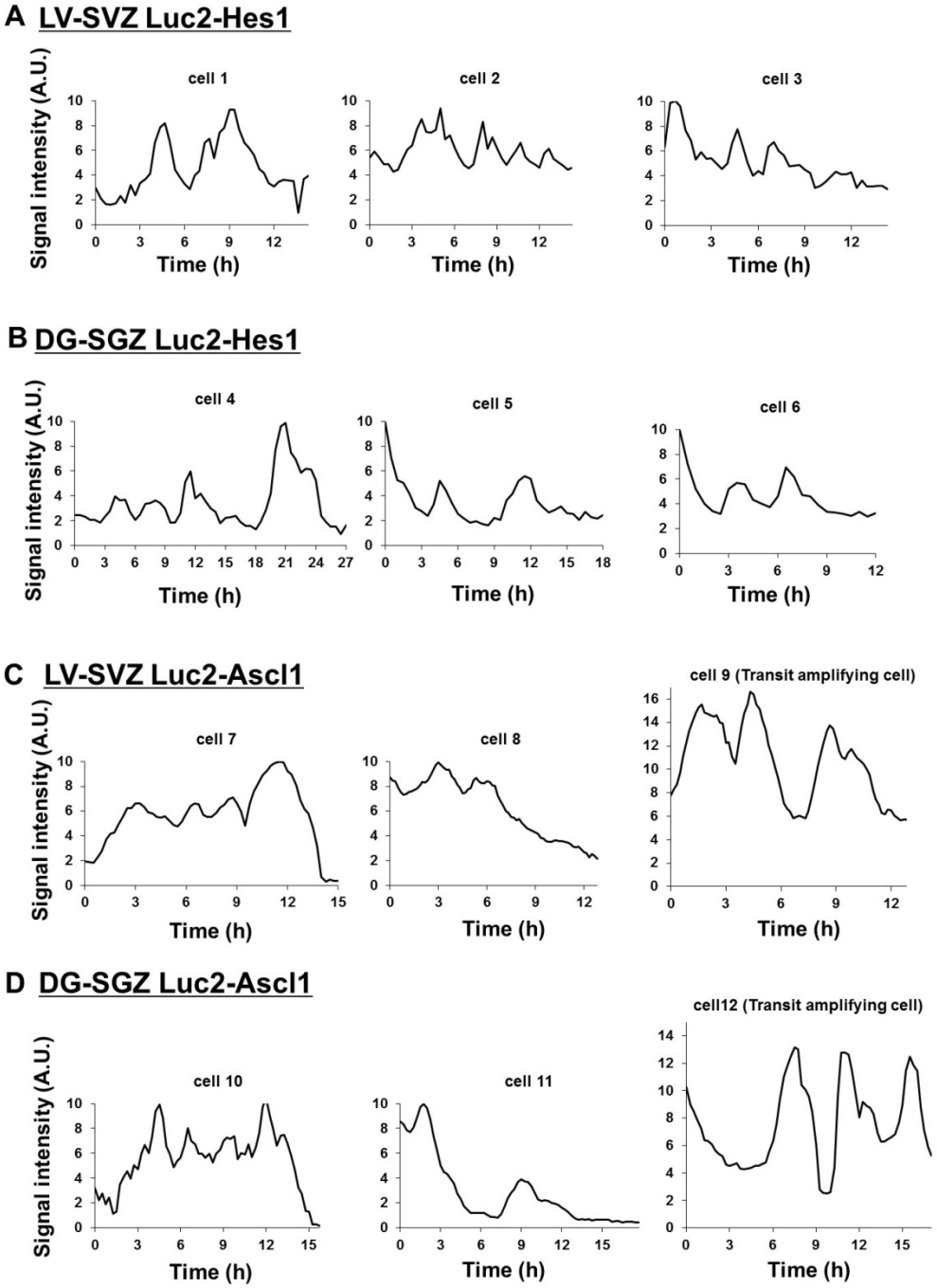

Figure S2, Related to Figure 1. Variable levels of Hes1 and Ascl1 in neural stem cells of the adult mouse brain. (A,B) Quantification of bioluminescence imaging of the Luc2-Hes1 reporter in slice cultures of the SVZ (A) and SGZ (B). (C,D) Quantification of bioluminescence imaging of the Luc2-Ascl1 reporter in slice cultures of the SVZ (C) and SGZ (D).

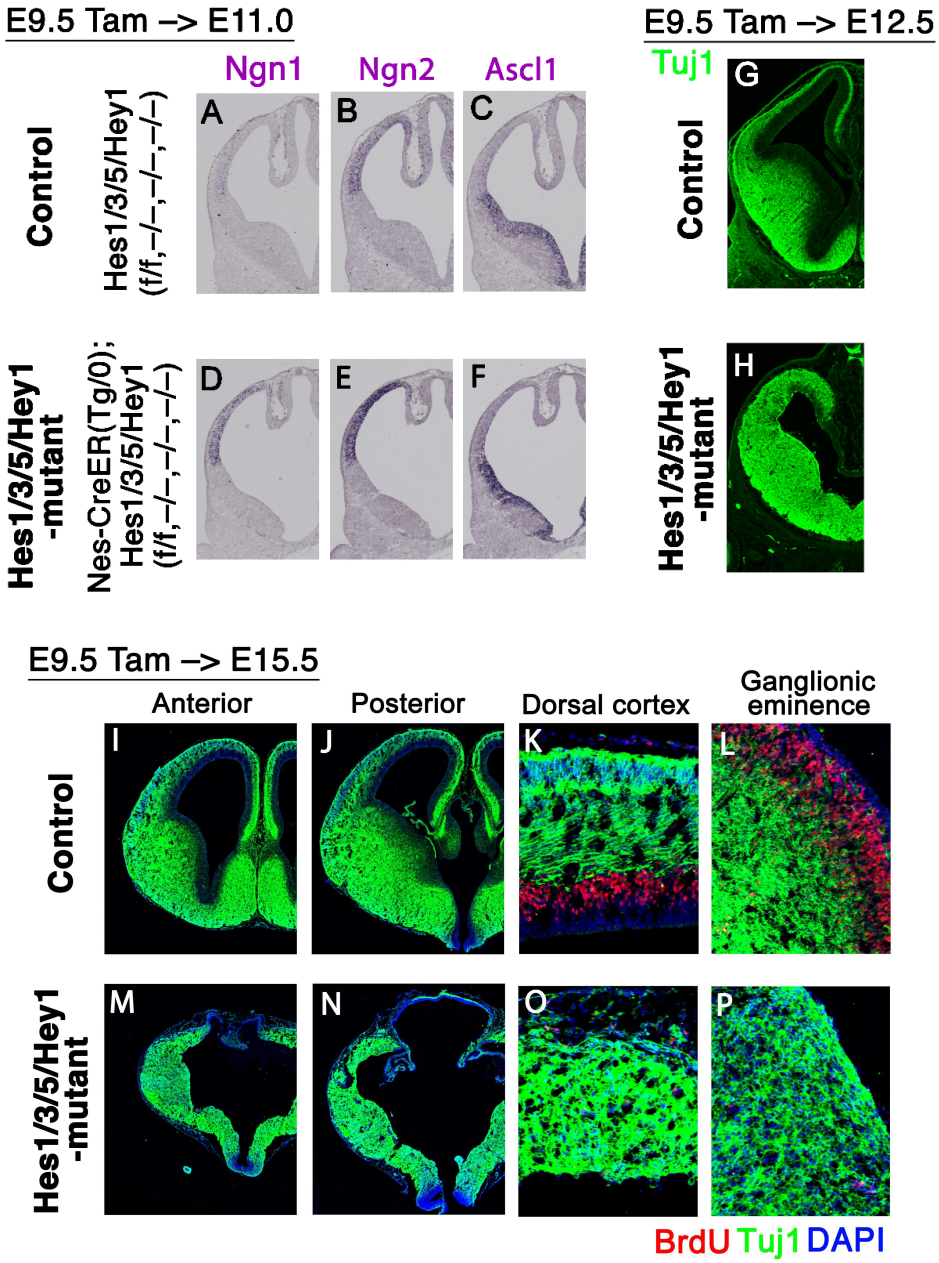

Figure S3, Related to Figure 2. Up-regulation of proneural gene expression and premature loss of neural stem cells in *Hes1/3/5/Hey1*-mutant mouse embryos. (A-F) The expressions of proneural genes were analyzed in control (A-C) and *Hes1/3/5/Hey1*-mutant mice (D-F) at E11.0. Tamoxifen was administered to pregnant mothers at E9.5 to induce CreER^{T2} recombinase activity. Levels of Neurog1, Neurog2, and Ascl11 were greater in *Hes1/3/5/Hey1*-mutants than in the control. (G,H) Staining for Neuron-specific class III β–tubulin (Tuj1)-positive neurons was also higher in *Hes1/3/5/Hey1*-mutant mouse embryos at E12.5 (H), than in the control (G). (I-P) Immunohistological analysis of control (I-L) and *Hes1/3/5/Hey1*-mutant mice (M-P) at E15.5. Tamoxifen was administered to pregnant mothers at E9.5. In control embryos, Tuj1^{+} neurons (green) reside in the outer layers and BrdU-incorporating dividing neural stem/progenitor cells (red) are located in the ventricular zone (I-L). In *Hes1/3/5/Hey1*-mutant embryos, the number of BrdU-incorporating neural stem/progenitor cells was dramatically reduced (O,P).

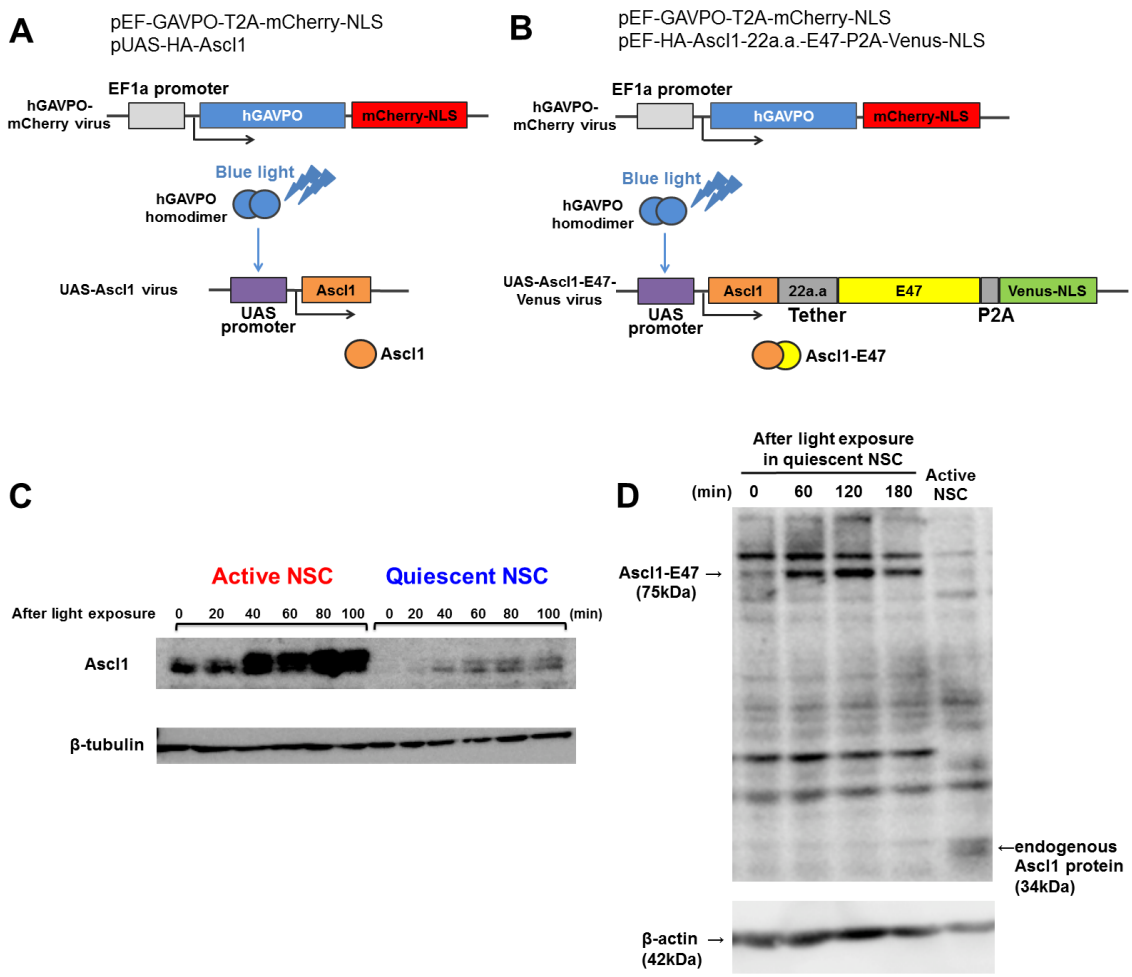

Figure S4, Related to Figure 5. Optogenetic induction of Ascl1 and Ascl1-E47 in cultured NS cells. (A,B) The hGAVPO optogenetic system to induce Ascl1 (A) and Ascl1-E47 (B). Blue light illumination activates hGAVPO, which forms a dimer and induces gene expression by binding to the UAS promoter. (C) Optogenetic induction (2-min blue light) of Ascl1 protein expression with the hGAVPO system in cultured NS cells was examined by western blotting. Note that Ascl1 expression was more efficiently induced in active NS cells (EGF medium) than in quiescent NS cells (BMP medium). (D) Optogenetic induction (2-min blue light) of the Ascl1-E47 fusion protein with the hGAVPO system in cultured quiescent NS cells (BMP medium) was examined by western blotting. Note that Ascl1-E47 expression was efficiently induced in quiescent NS cells, reaching levels similar to or higher than those of endogenous Ascl1 in active NS cells (EGF medium).

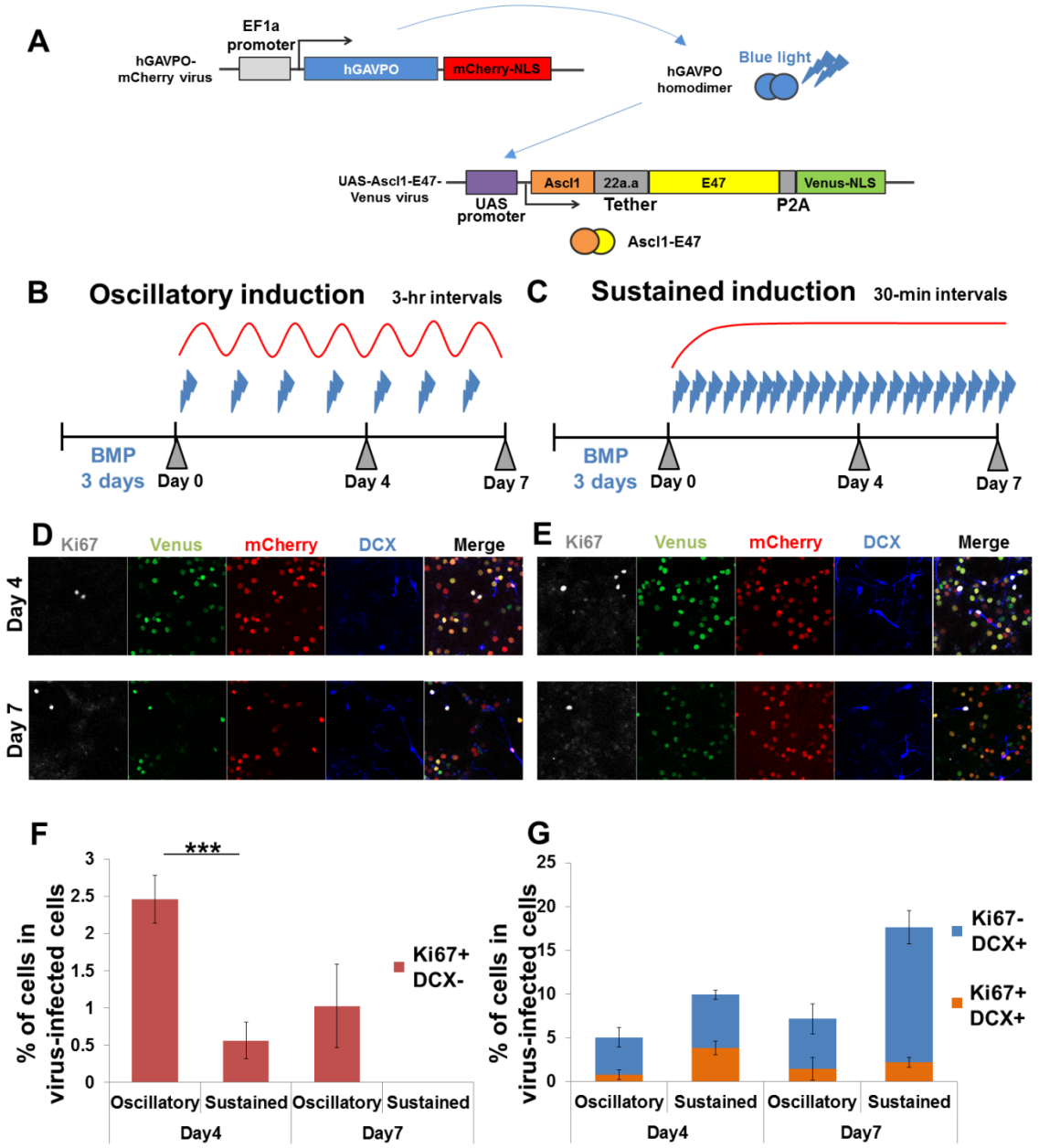

Figure S5, Related to Figure 5. Optogenetic induction of Ascl1-E47 expression in cultured quiescent NS cells. (A) Strategy for optogenetic induction of Ascl1-E47 expression. (B,C) Time schedule of blue light illumination for oscillatory (B) or sustained expression (C). (D,E) Optogenetic induction of oscillatory (D) or sustained (E) expression of Ascl1-E47 in cultured quiescent NS cells. After 4 or 7 days of blue light illumination, cells were examined by immunostaining. (F) Quantification of $Ki67^{+}$;DCX^{-} cells. (G) Quantification of $Ki67^{+}$;DCX^{+} and $Ki67^{-}$;DCX^{+} cells.

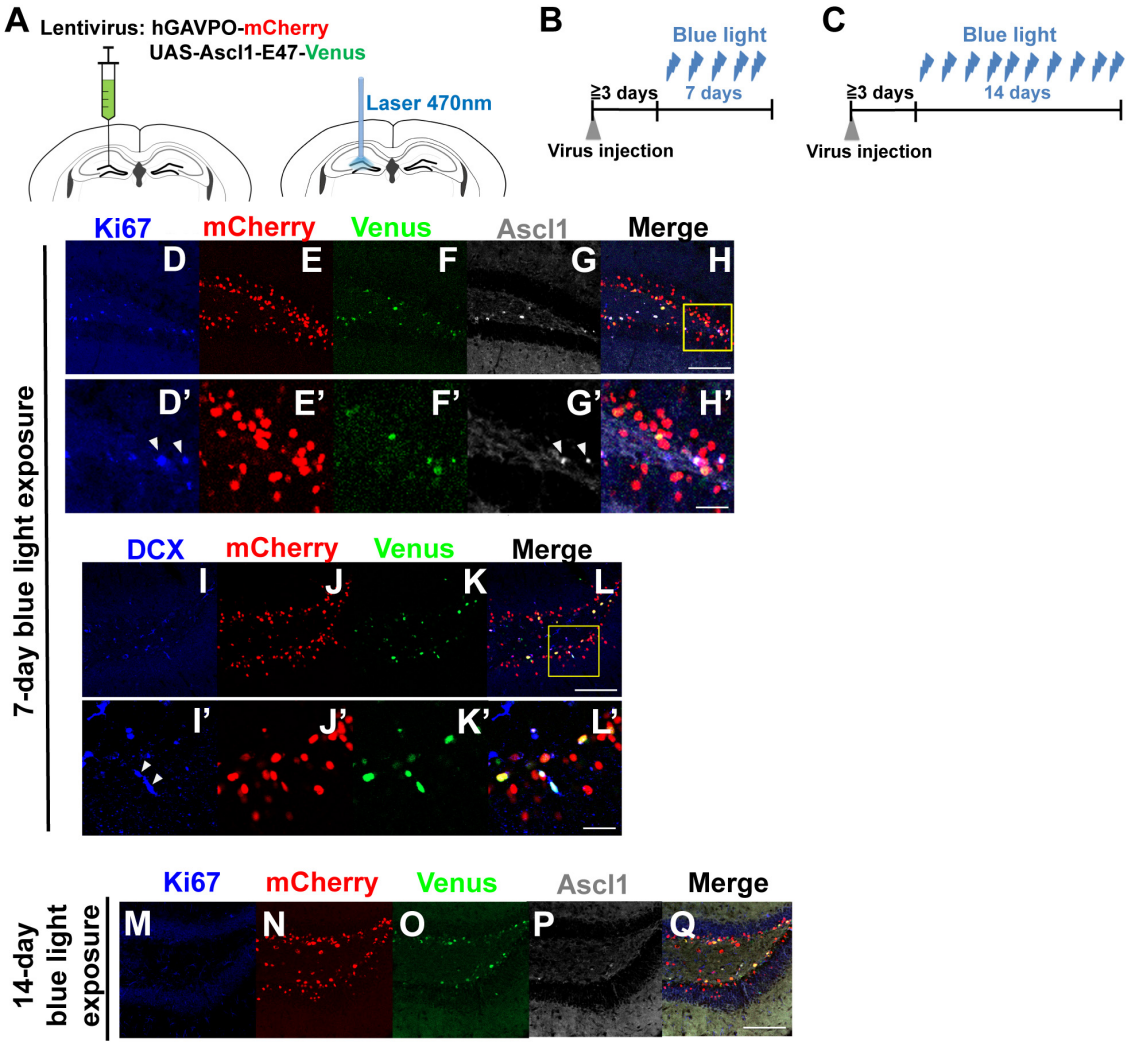

Figure S6, Related to Figure 5. Optogenetic induction of Ascl1 oscillations in neural stem cells in the adult mouse brain (13-14 months old). (A-C) Experimental design. After virus injection (A, left), optic fiber connected to Optoflash was implanted to illuminate with blue light (A, right). Ascl1 oscillations with 2.5-h periodicity was induced, and brain sections were examined after 7-day (B) or 14-day (C) illumination. (D-Q) Immunohistological analysis of cells in the adult mouse hippocampal dentate gyrus infected with hGAVPO-mCherry virus and UAS-Ascl1-E47-Venus virus. Sections were examined after 7-day (D-L) or 14-day (M-Q) light stimulation with 2.5-h periodicity. Boxed regions in H and L are enlarged in (D'-H') and (I'-L'), respectively. Size bars: D-Q, 100 μm; D'-L', 30 μm.

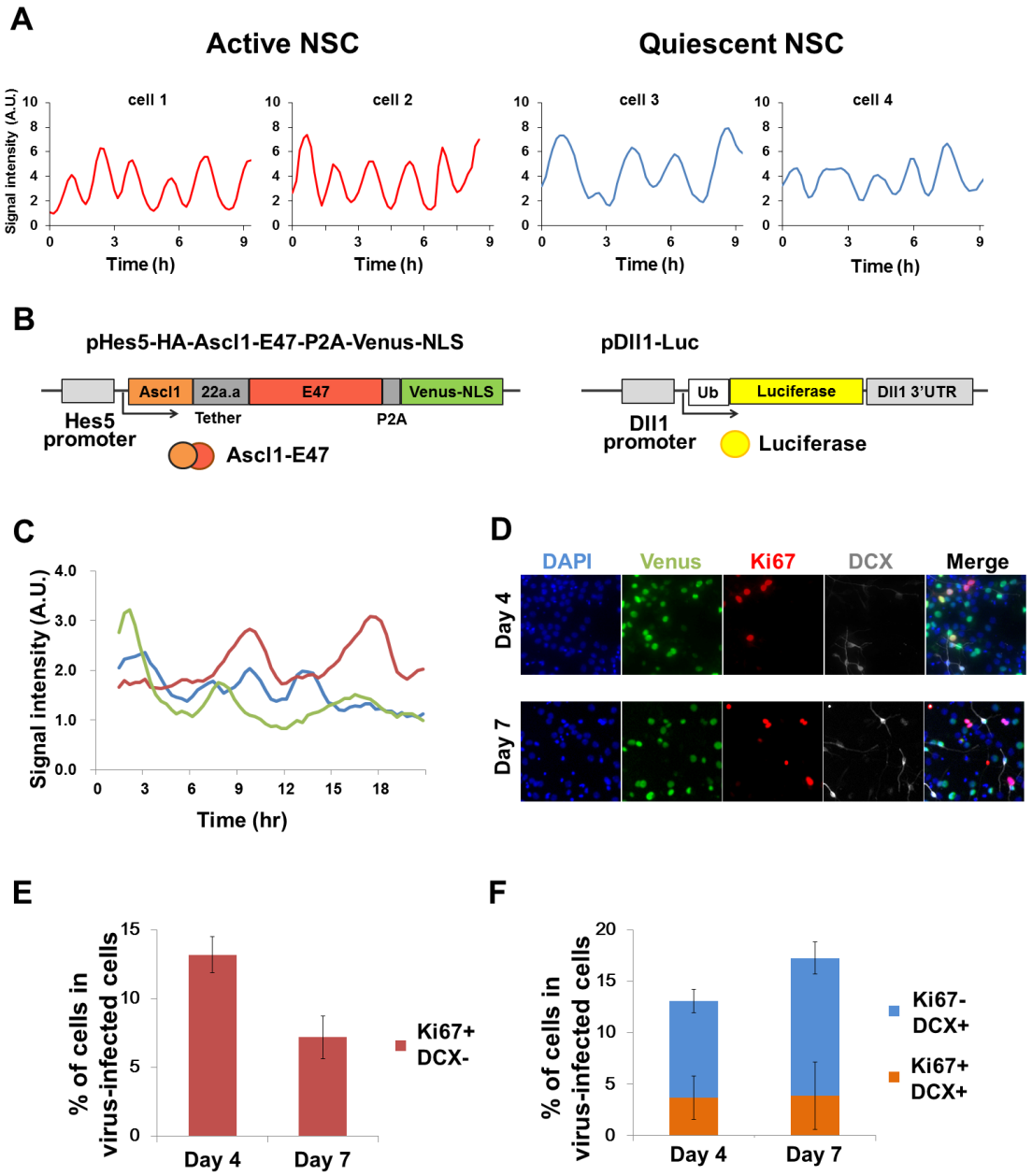

Figure S7, Related to Figure 6. (A) *Hes5* promoter-driven destabilized luciferase reporter expression in active and quiescent NS cells (NSC). (B,C) Induction of Ascl1-E47 expression from the *Hes5* promoter in cultured quiescent NS cells. NS cells carrying the Dll1 reporter (pDll1-Luc) were cultured in BMP medium. After lentivirus infection, Dll1 reporter expression in virus-infected cells ($Venus^+$) was quantified (C). The patterns of three representative cells are shown. (D) Cells infected with lentivirus directing Ascl1-E47 expression from the *Hes5* promoter were examined by immunostaining after 4 or 7 days. (E) Quantification of $Ki67^+$;DCX^- cells. (F) Quantification of $Ki67^+$;DCX^+ and $Ki67^-$;DCX^+ cells.