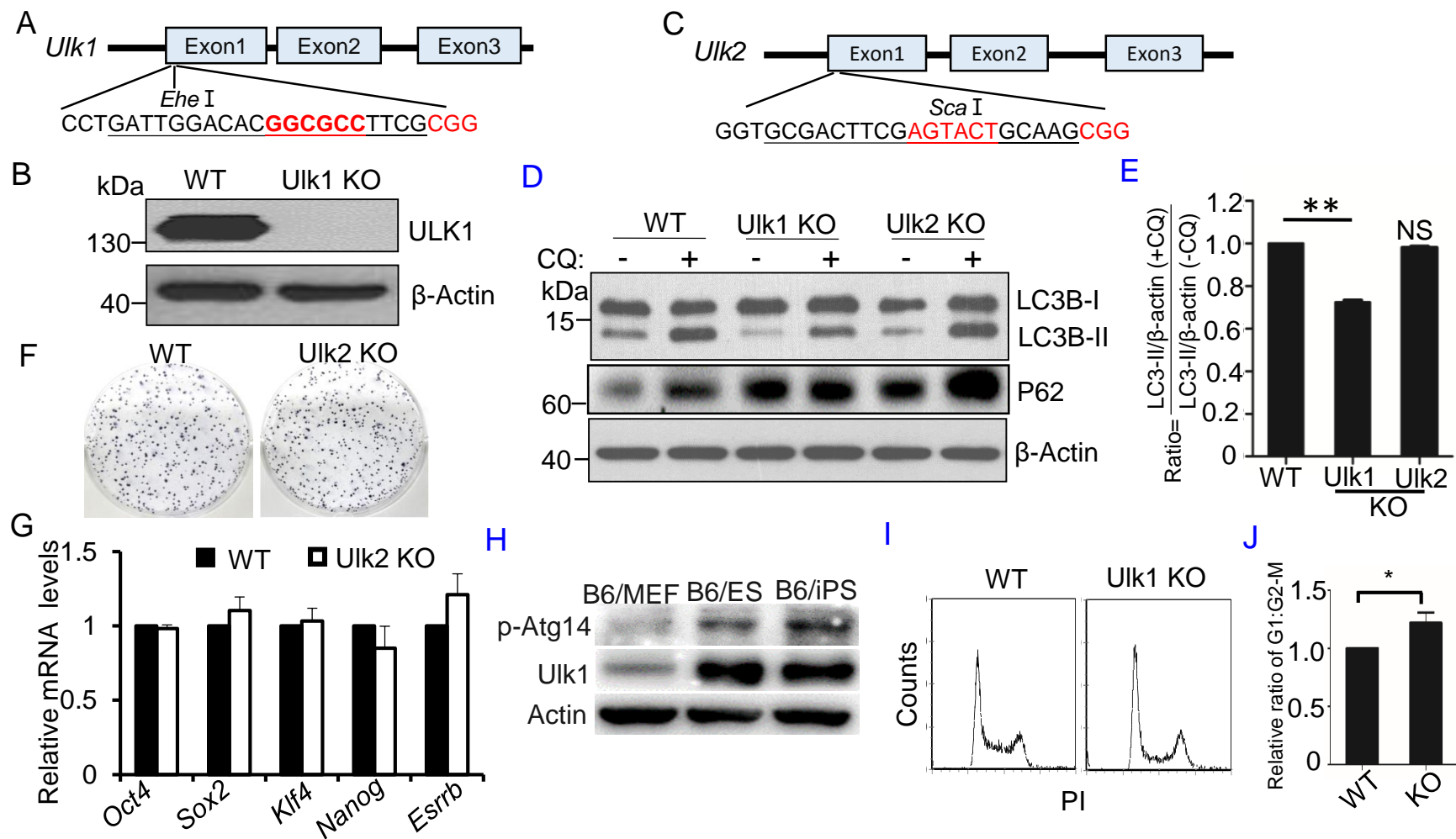
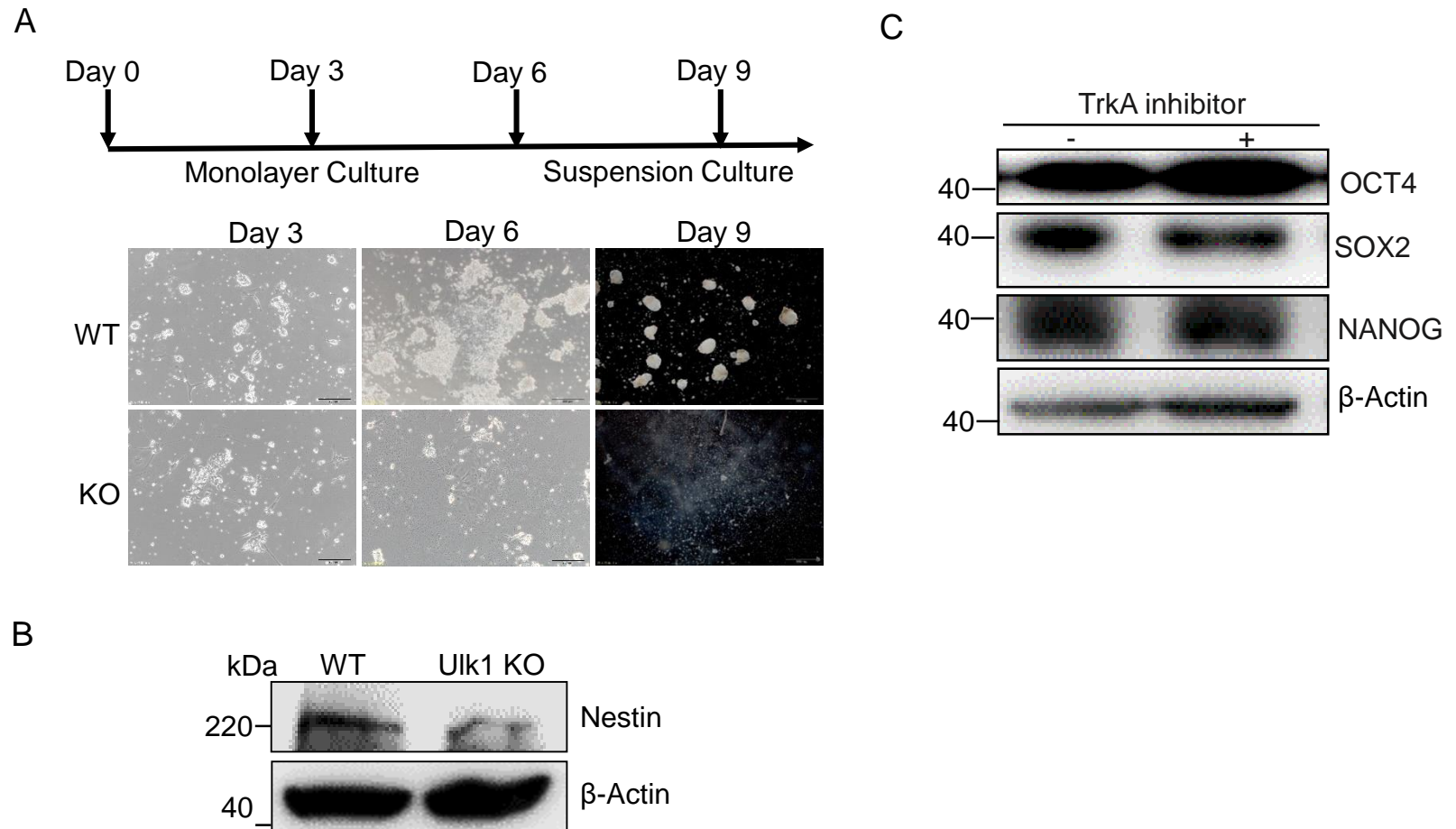


Supplementary Figure 1. Ulk1 but not Ulk2 silence impaired ESC self-renewal and pluripotency



Supplement Figure 1. Ulk2 silence did not affect ESC self-renewal and pluripotency. **(A)** sgRNA designed for targeting the first exon of *Ulk1*. **(B)** Western blot analysis of whole cell extracts from *Ulk1*^{+/+} and *Ulk1*^{-/-}, β -Actin served as a loading control. **(C)** sgRNA designed for targeting the first exon of *Ulk2*. **(D)** The autophagic flux was impaired in *Ulk1*^{-/-} but not in *Ulk2*^{-/-}. Western blotting was performed in *Ulk1*^{+/+}, *Ulk1*^{-/-} and *Ulk2*^{-/-} ESC lines using anti-LC3 and anti-P62 antibodies. Samples were treated with or without Chloroquine (100 μ M) for 4 h. β -Actin served as the loading control. **(E)** Quantification of the LC3 turnover ratio by calculating the ratio of LC3-II with or without CQ treatment in **(D)**. Data normalized to WT ESCs and shown as mean \pm SD, n=3; **, P<0.01, Student's t-test. **(F)** *Ulk2* deletion did not affect ESC colony formation ability. **(G)** *Ulk2* deletion did not affect expression of pluripotent genes. **(H)** The phosphorylated Atg14 (p-Atg14) is highly expressed in ESCs than in MEFs. Whole cell extracts from MEFs, ESCs and iPSCs were harvested for western blot using anti-p-Atg14, anti-*Ulk1* and anti- β -Actin antibodies. **(I)** PI staining of DNA contents of both WT and *Ulk1* KO ESCs. **(J)** *Ulk1* deletion enhanced G1/G2-M ratio.

Supplementary Figure 2. Ulk1 silence impaired the NSC differentiation from ESC



Supplement Figure 2. Ulk1 silence impaired the neural stem cell differentiation of mESCs in vitro. **(A)** Differentiation of mESCs into neural stem cell. Images were captured on days 3, 6 and day 9. **(B)** Western blotting for the Nestin between WT and Ulk1 KO ESC-derived NSCs. **(C)** The pluripotent gene expression was not affected upon TrkA inhibitor treatment (2 μ M, GW441756, Selleck) for 12h. Western blotting was performed in WT and Ulk1^{-/-} ES lines using anti-OCT4, anti-SOX2 and anti-NANOG antibodies.