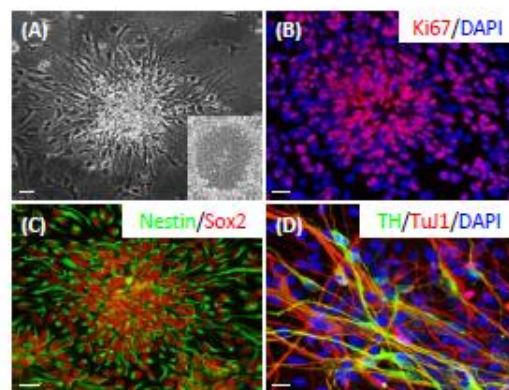Supplemental Figures – Park et al.

Fig. S1. Characterization of H9 hESC-derived human neural stem cells (hNSCs). (A) Phase contrast image of neural rosette formation (inset) and neural clusters. (B, C) The majority of cells in the clusters were positive for the proliferation marker Ki67 (red) (B) and the NSC marker nestin (green), and showed abundant expression of neural lineage markers such as SOX2 (C). (D) Immunofluorescent staining of hNSCs with anti-TuJ1 (red) and anti-TH (green) antibodies at 5 days after differentiation (magnification ×400).

(A) H9-NSC

| | Insulin | - | + | + | 1(μM) |
| | LY294002 | - | - | + | 10(μM) |

(B) CHA 13-NSC

| | Insulin | - | + | + | 1(μM) |
| | LY294002 | - | - | + | 10(μM) |

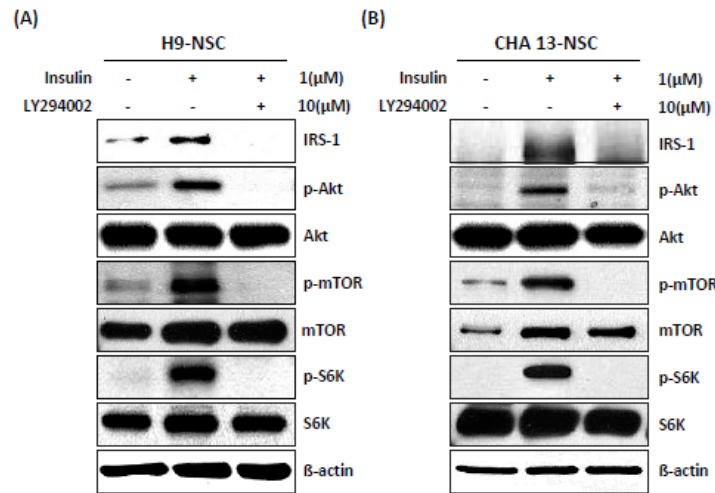IRS-1
p-Akt
Akt
p-mTOR
mTOR
p-S6K
S6K
ß-actin

Fig. S2. Insulin stimulates Akt and mTOR/S6K phosphorylation in cultured hNSCs. Monolayers of cultured H9 hESC-derived or CHA13 hESC-derived hNSCs were starved for 24 h before treatment with 1 μM insulin. After lysing the cells, proteins were analyzed by immunoblotting with specific antibodies against p-Akt, p-mTOR, p-S6K, and β-actin. Insulin induced phosphorylation of Akt, mTOR, and S6K. The optical density of specific bands on immunoblotted membranes was measured by densitometry. (A) Cells were treated with LY294001 (10 μM) for 1 h, and then exposed to insulin for 1 h. Pretreatment with LY294002 inhibited the activation of Akt after insulin treatment and abrogated the expression of mTOR and S6K. (B) CHA13 hESC-derived hNSCs were treated with LY294001 (10 μM) for 1 h, and then exposed to insulin for 1 h. Pretreatment with LY294002 inhibited activation of Akt after insulin treatment and abrogated the expression of mTOR and S6K. All experiments were repeated three times and a representative result is shown.