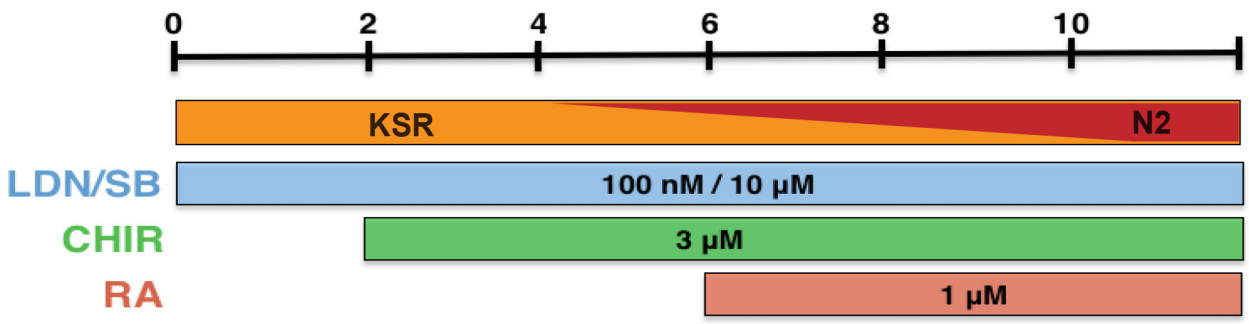
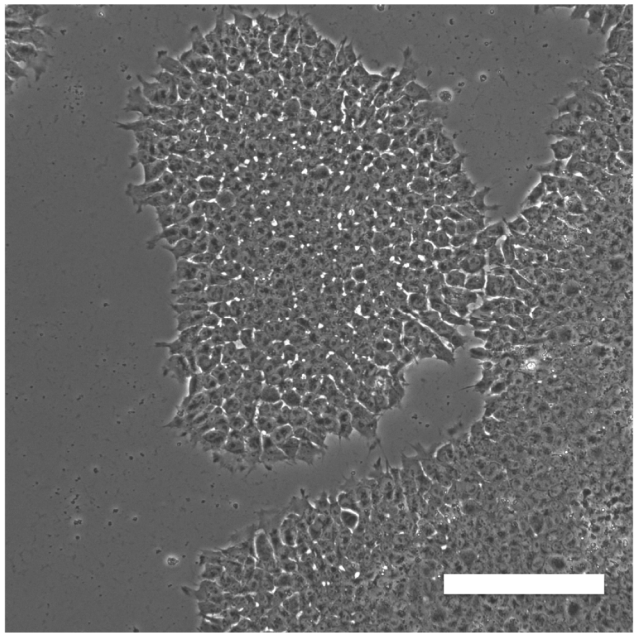


Supplementary Figure 1

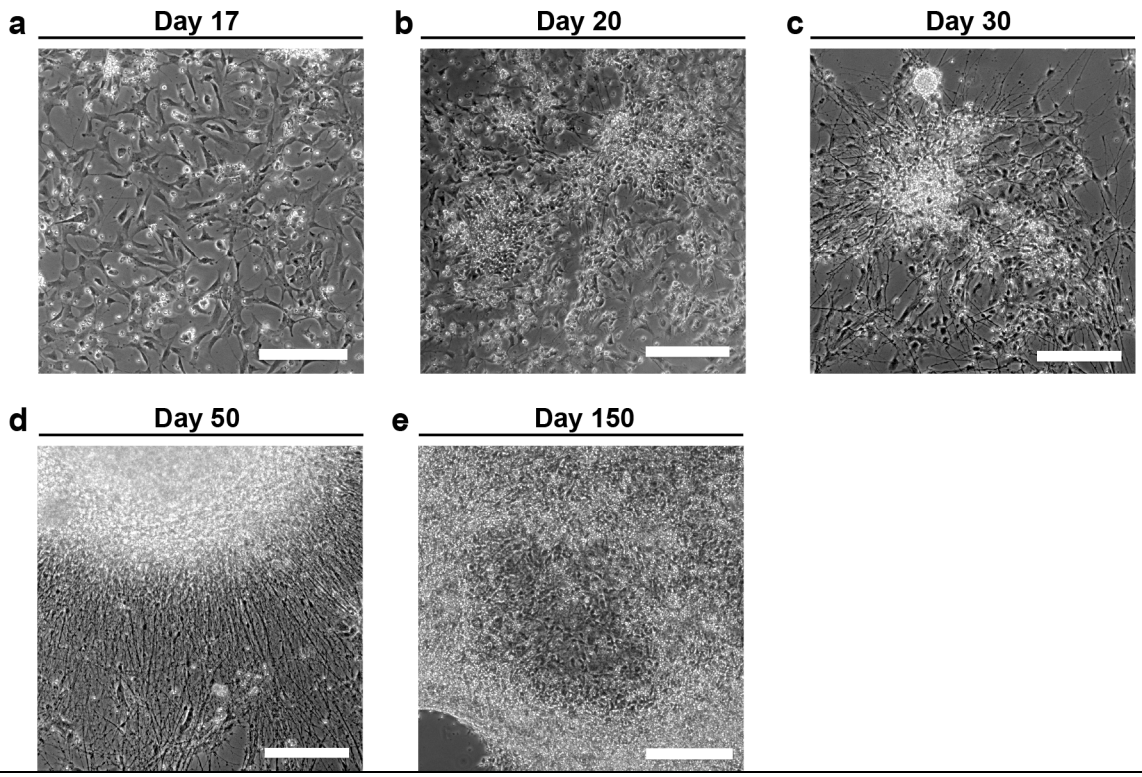
Protocol (days 0-12) for ENC induction using option A.

KSR, knockout serum replacement differentiation medium; LDN, LDN-193189, SB, SB431542, CHIR, CHIR 99021; RA, Retinoic Acid; SB, SB431542.



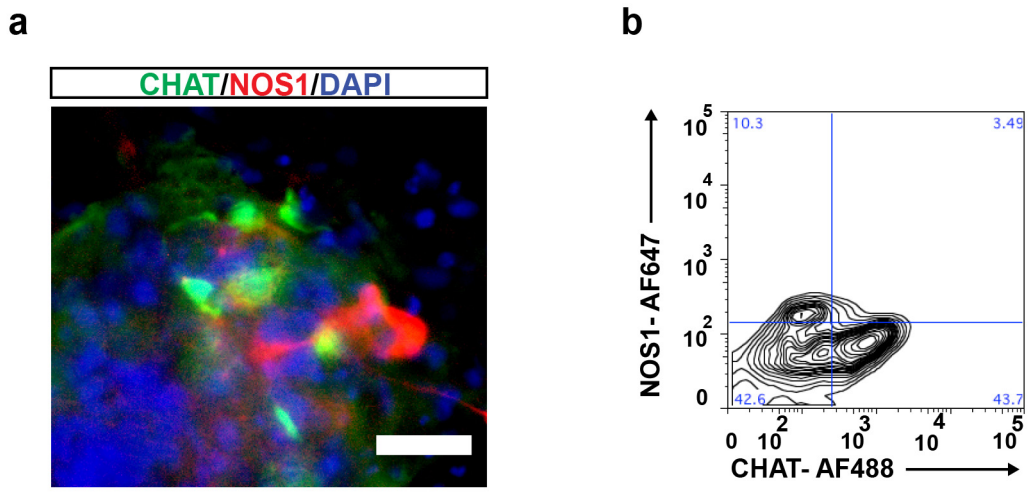
Supplementary Figure 2

Representative phase contrast image of WA09 embryonic stem cells cultured in E8 medium.
Scale bar = 100 μm.



Supplementary Figure 3

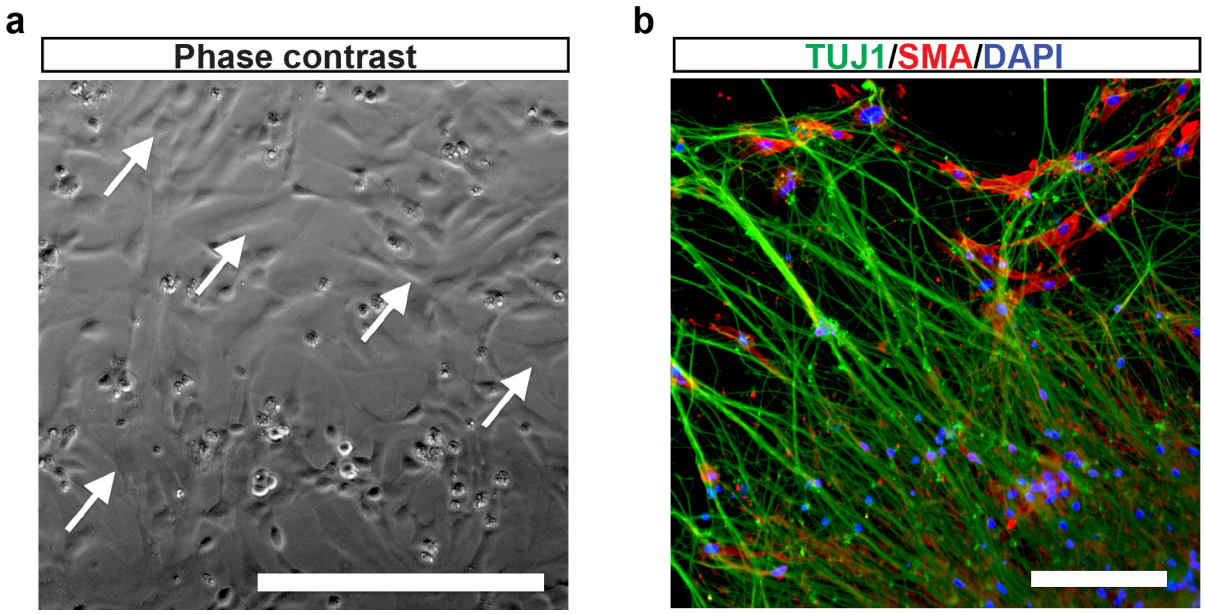
Representative phase contrast images of differentiating cells at different time points of EN induction. Scale bar = 200 μm.



Supplementary Figure 4

Distinct populations of NOS1+ and CHAT+ cells in hESC-derived EN cultures.

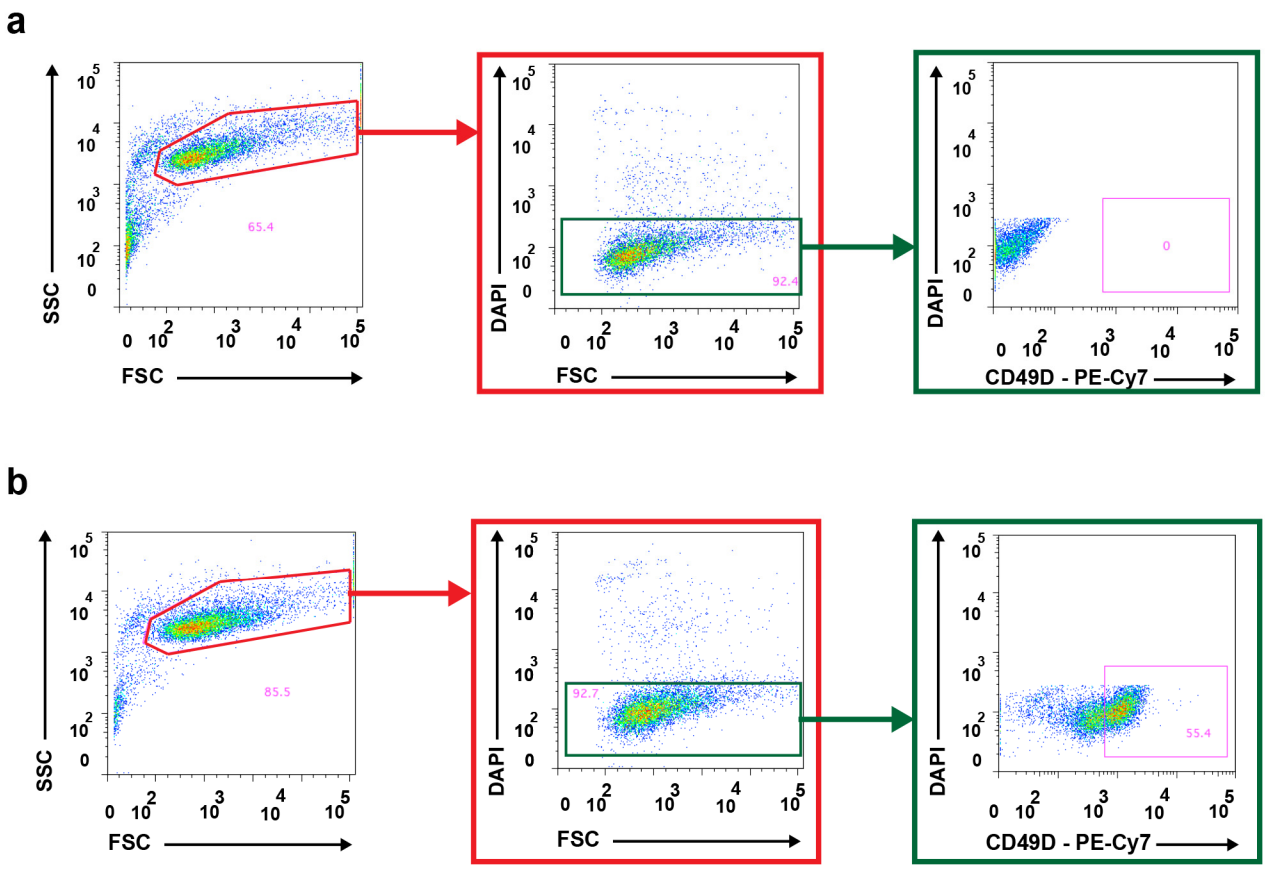
a) Immunofluorescence staining of NOS1 and CHAT on day 75 of EN induction. b) Flow cytometry analysis of NOS1 and CHAT expression on day 75 on EN induction. AF647, Alexa Fluor™ 647; AF488, Alexa Fluor™ 488. Scale bar = 20 μm.



Supplementary Figure 5

Characterization of contaminating cells in hESC-derived EN cultures.

a) Phase contrast image of low density regions of culture plates on day 75 of differentiation. Arrows point to flat non-neuronal contaminating cells. b) Immunofluorescence staining of EN cultures with SMA and TUJ1 on day 75 of differentiation. Scale bar = 100 μm in a and 200 μm in b.



Supplementary Figure 6

Example of FACS gating strategy for purification of CD49D+ ENCs on day 12 of differentiation.

a) Unstained control sample. b) Sample stained with CD49D.