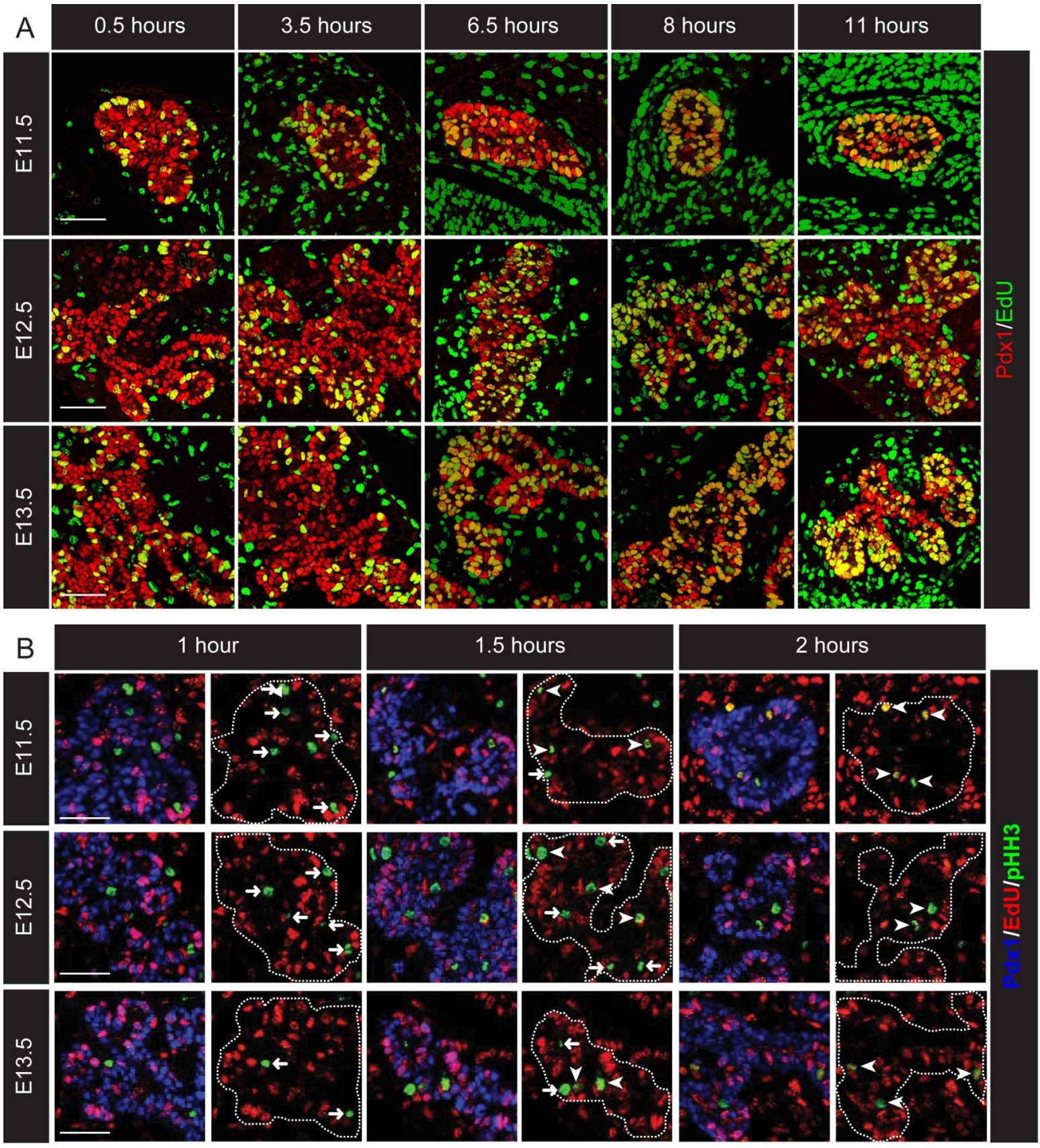

Figure S1: Cumulative EdU labeling demonstrated cell cycle length increases during development, related to Figure 1.

(A) Immunofluorescence confocal images of time dependent incorporation of EdU into pancreatic progenitors (PDX1; red & EdU; green) at E11.5, E12.5 and E13.5 after 0.5, 3.5, 6.5, 8 and 11 hours of EdU labeling.

(B) Immunofluorescent staining for PDX1 (blue), EdU (red), and pHH3 (green) in E11.5, E12.5 and E13.5 embryos sacrificed at 1, 1.5 and 2 hours following a single injection of EdU; arrowheads: pHH3+EdU+ and arrows: pHH3+EdU-, pancreatic epithelium outlined (--).

Scale bars = 50 μm.

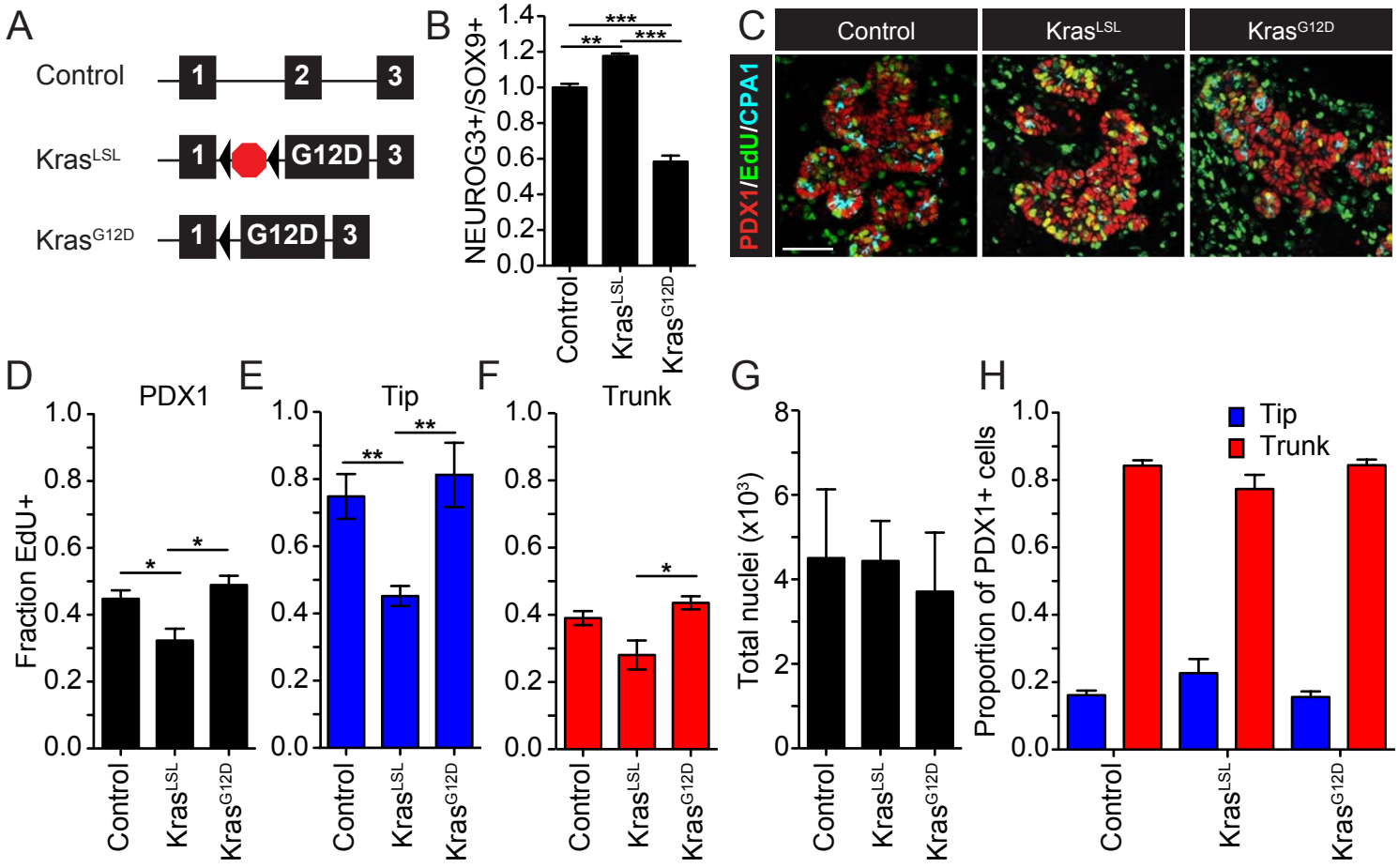

Figure S2: Kras$^{LSL\text{-}G12D}$ mutations lead to altered cell cycle length during mouse pancreatic development, related to Figure 2.

(A) Schematic outlining the alleles present in the three different genotypes.

(B) Quantification of the number of NEUROG3+ cells relative to SOX9+ trunk cells in Control, Kras^{LSL} and Kras^{G12D} embryos at E13.5. n = 3. **p < 0.01, ***p < 0.001 by one-way ANOVA and Tukey post test.

(C) PDX1 (red), CPA1 (cyan) and EdU (green) immunostaining in Control, Kras^{LSL} and Kras^{G12D} embryos at E12.5 following 3.5 hours of EdU exposure. Scale bar = 50 μm.

(D-F) Kras^{LSL} embryos had reduced EdU-labeled PDX1+ pancreatic progenitor cells and (E) reduced EdU+ tip cells (PDX1+CPA1+) but (F) no significant changes in EdU+ trunk cells (PDX1+CPA1-) at E12.5. n = 3 *p < 0.05, **p < 0.01 by one-way ANOVA and Tukey post test.

(G) The total number of E12.5 pancreatic cells did not change with Kras expression level. n = 3.

(H) The relative proportion of tip and trunk cells in Control, Kras^{LSL} and Kras^{G12D} embryos at E12.5 remained constant. n = 3.

Data are presented as mean ± SEM.

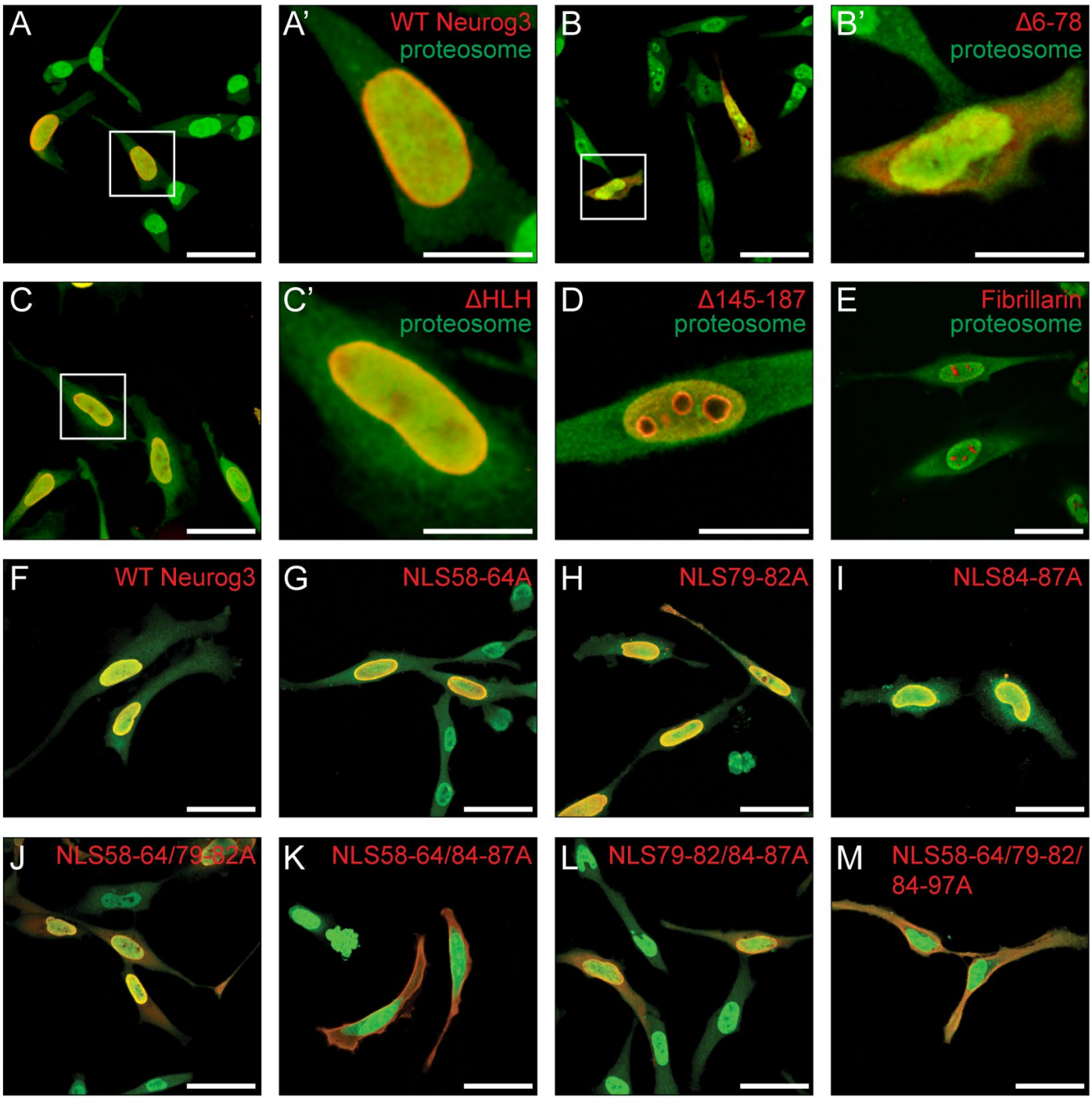

Figure S3. NEUROG3 requires its N-terminal region for efficient nuclear localization and nuclear import requires at least one of its two NLSs, related to Figures 3 and 4.

(A-C) HeLa cells expressing FLAG-tagged WT NEUROG3 (A), NEUROG3$_{\Delta 6\text{-}78}$ (B), NEUROG3$_{\Delta HLH}$ (C) or NEUROG3$_{\Delta 145\text{-}187}$ (D) were fixed and stained with anti-FLAG antibody (red) and anti-proteasome 20S α/β antibody (green). Scale bars = 50 μm.

(A'-C') Higher magnifications of the indicated areas. Scale bars = 20 μm.

(E) HeLa cells expressing FLAG-tagged NEUROG3$_{\Delta 145\text{-}187}$ stained for fibrillarin (red) and proteasome (green).

(F-M) HeLa cells expressing FLAG-tagged WT NEUROG3 (F) or mutants with the arginine and lysine residues in their putative NLSs mutated to alanine (G-M) were stained with anti-FLAG antibody (red) and anti-proteasome 20S α/β antibody (green).

Representative of n = 3.

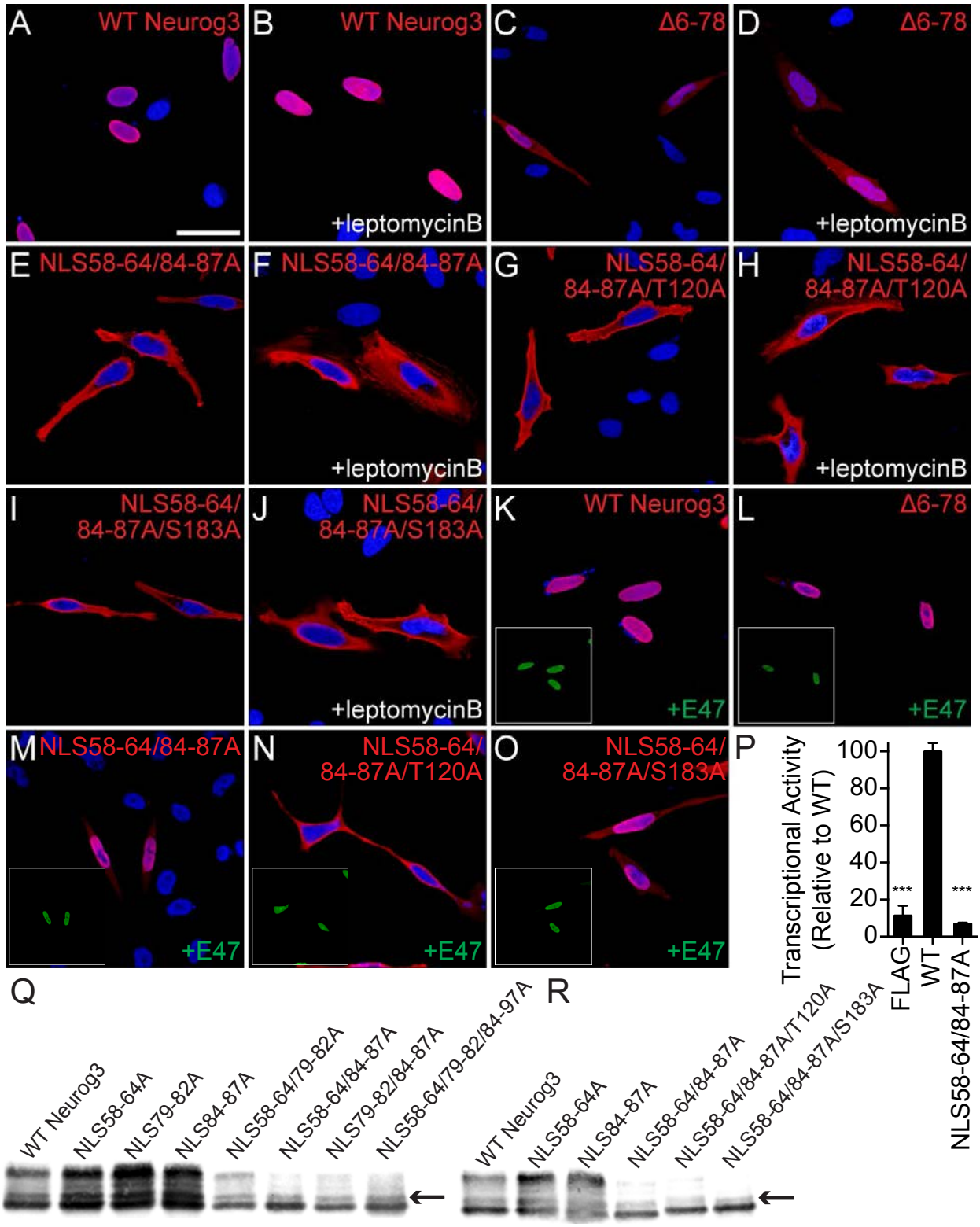

Figure S4: Nuclear import of NEUROG3 precedes hyperphosphorylation, requires at least one of its two NLSs or E47, and involves T120, related to Figures 3 and 4.

(A-O) HeLa cells expressing FLAG-tagged NEUROG3 constructs were incubated with leptomycin B, to inhibit nuclear export, or cotransfected with 6×His-tagged E47 as indicated. Cells were stained with anti-FLAG (red), anti-6xHis antibodies (green) and DAPI (blue). Scale bars = 50 μm. n = 3.

(P) Relative luciferase levels were measured for HeLa cells transfected with the Pax4 promoter driving Luciferase reporter, a Hnf1α-expression construct and indicated empty or FLAG-tagged NEUROG3 expression construct. ***p < 0.0005. n = 3

(Q&R) Western blots probed with anti-FLAG antibody with lysates of HeLa cells expressing FLAG-tagged WT NEUROG3 (lane 1) or the NLS mutants as indicated. Arrows indicate a slower migrating band representing a form that is likely phosphorylated outside the nucleus. n = 3

Data are presented as mean ± SEM.

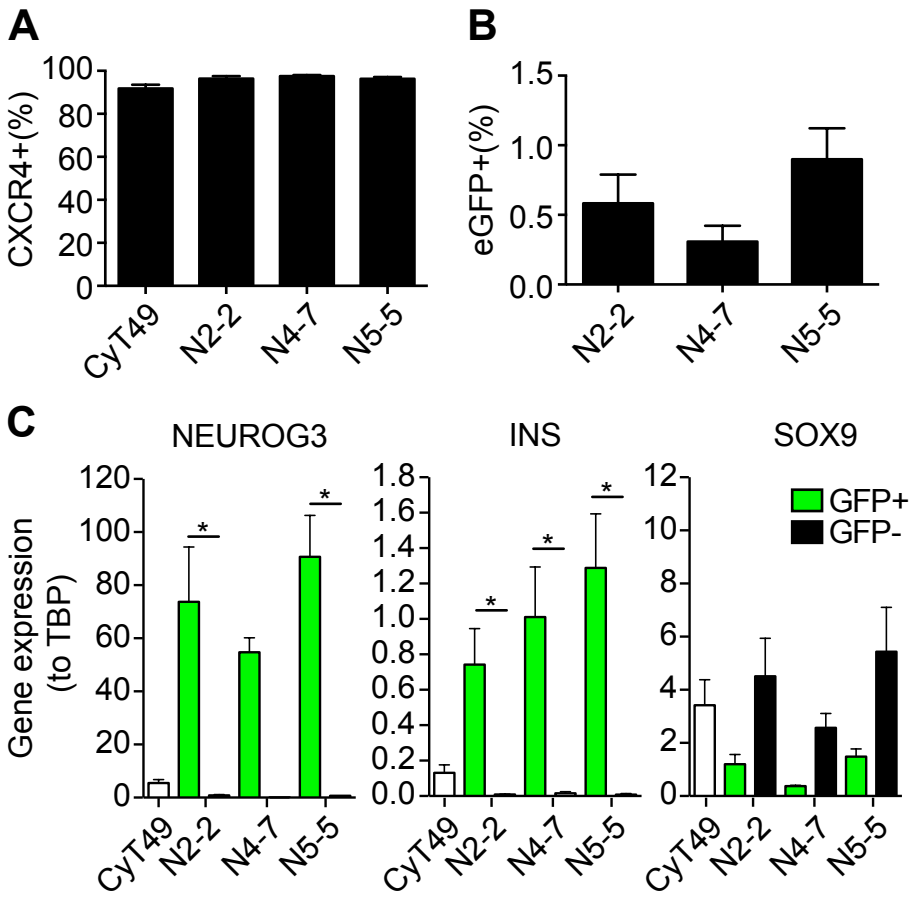

Figure S5: Validation of Neurog3-2A-eGFP CyT49 hESC reporter lines, related to Figure 6.

(A) Three clonally-derived CyT49 reporter lines were differentiated using Rezania et al., 2014 protocol to definitive endoderm (S2D1), fixed and stained for CXCR4 and the number of CXCR4+ cells was quantified using flow cytometry. n = 7 from two differentiations.

(B) Reporter hESC lines were differentiated to stage 6 and the number of GFP+ (NEUROG3+) cells were quantified using flow cytometry. n = 7 from two differentiations.

(C) NEUROG3 and INSULIN transcription was increased in GFP+ cells compared to GFP- cells in Stage 6 endocrine progenitors in N2-2, N4-7 and N5-5 hESC lines. Conversely, SOX9 expression was higher in GFP- cells compared to GFP+ cells. *p < 0.05 by one-way ANOVA and Tukey post test. n = 3 from two differentiations Data are presented as mean ± SEM.