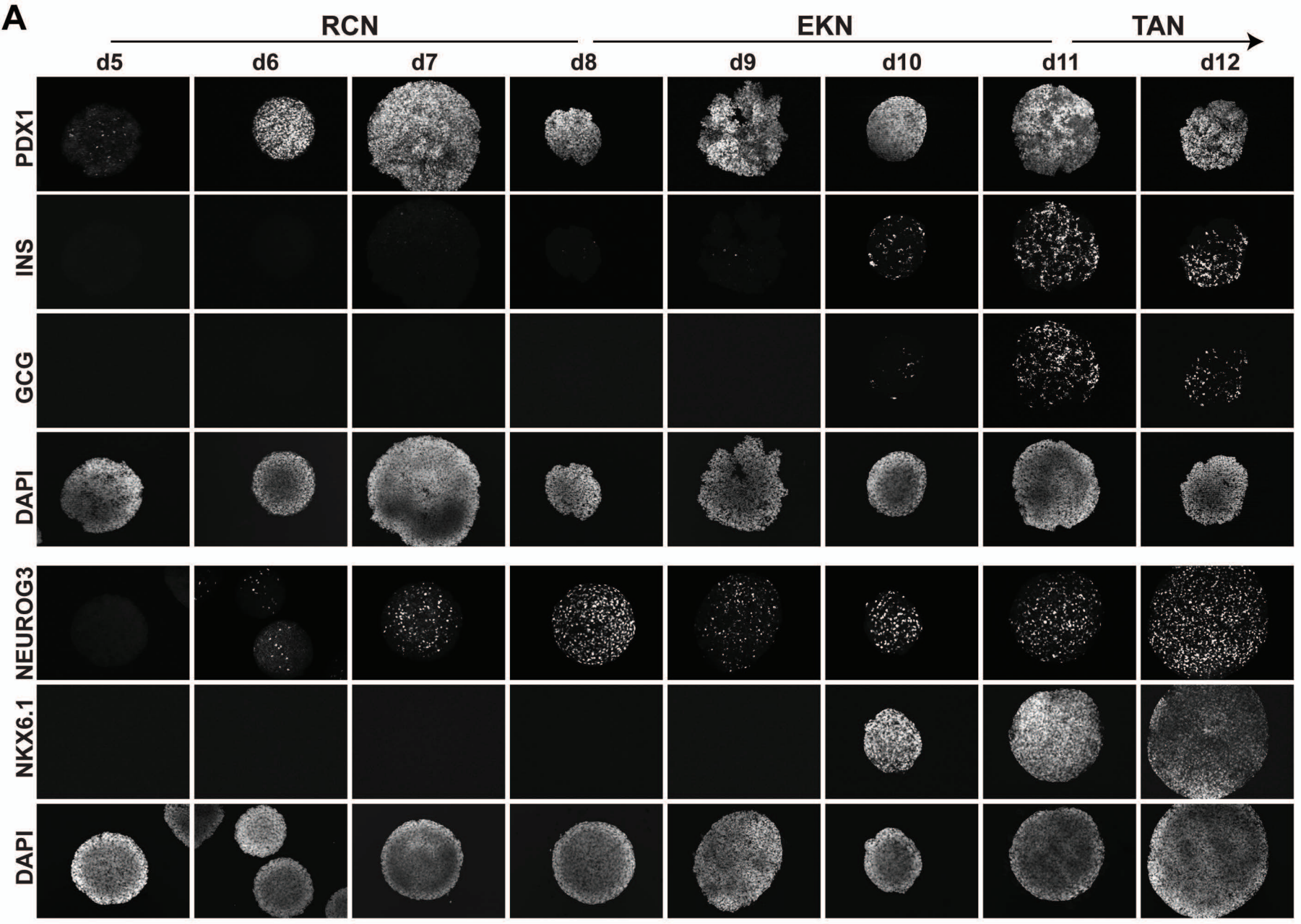
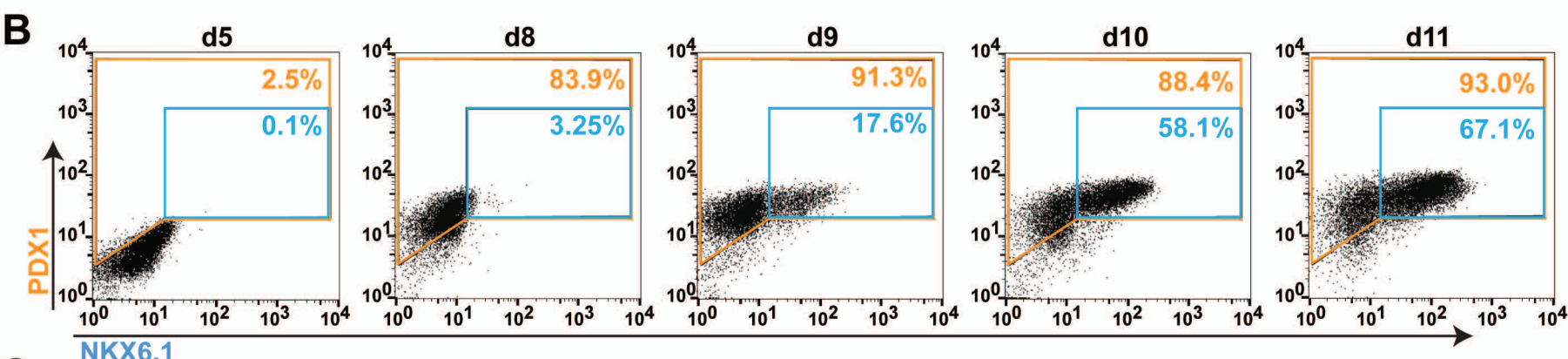
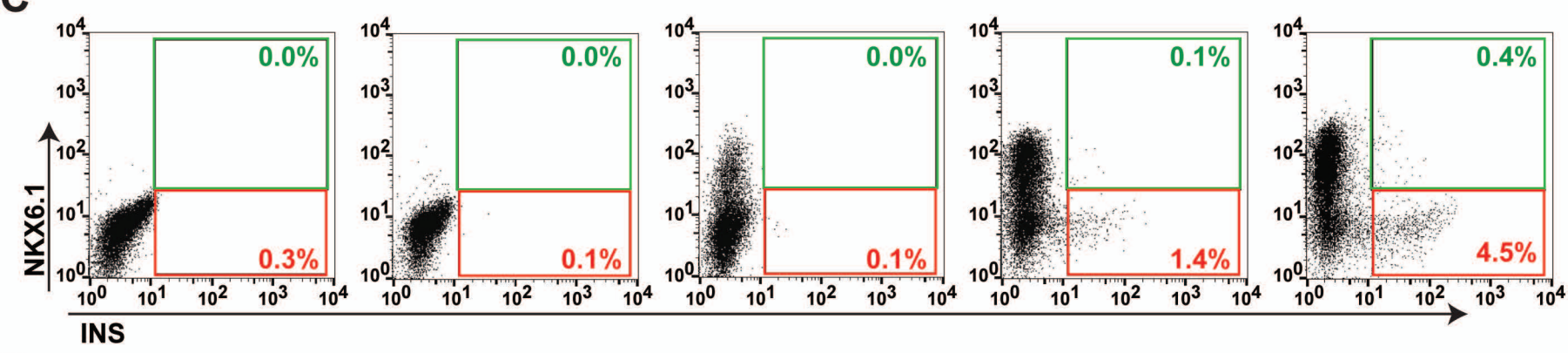
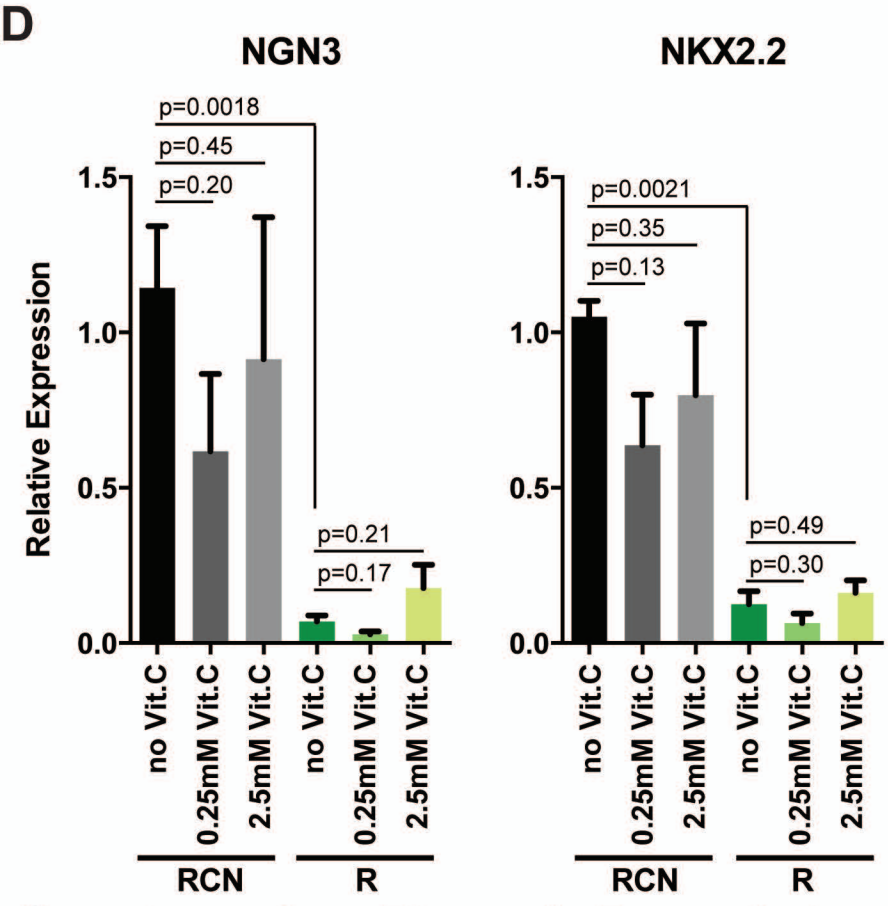
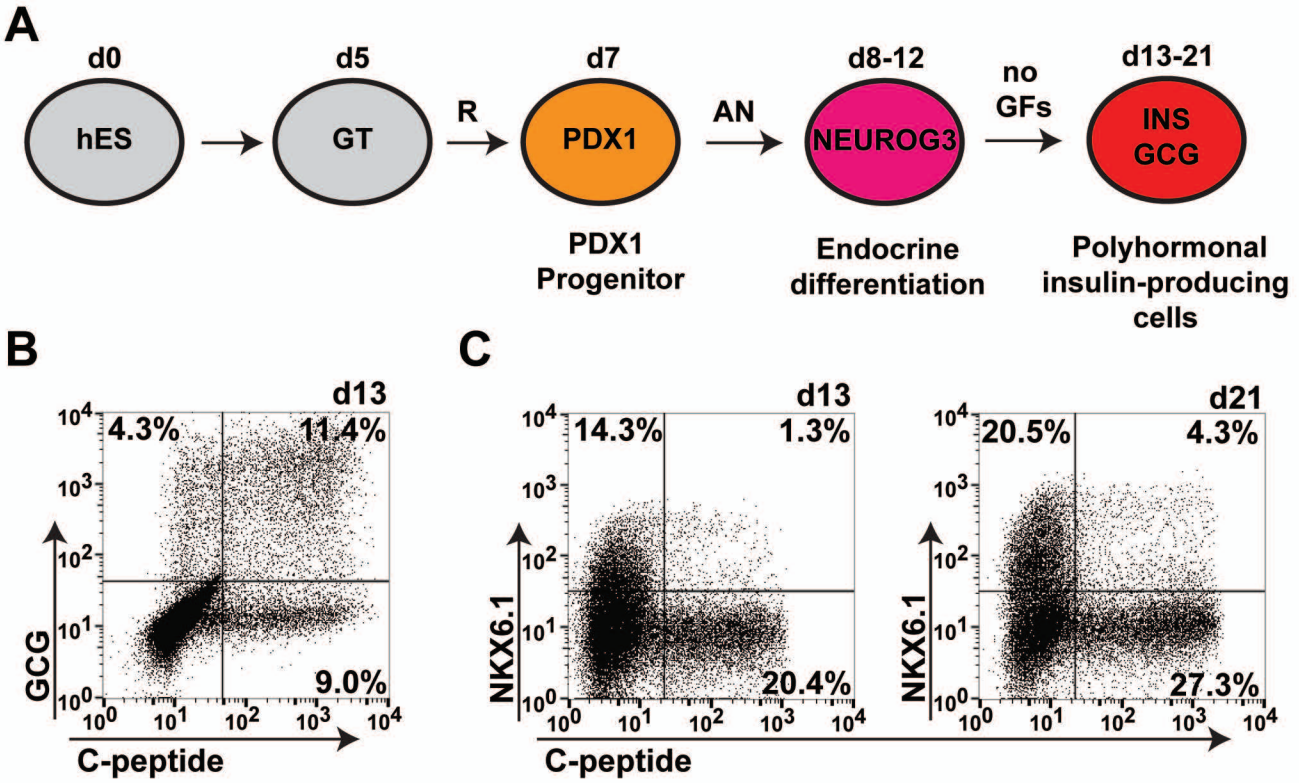




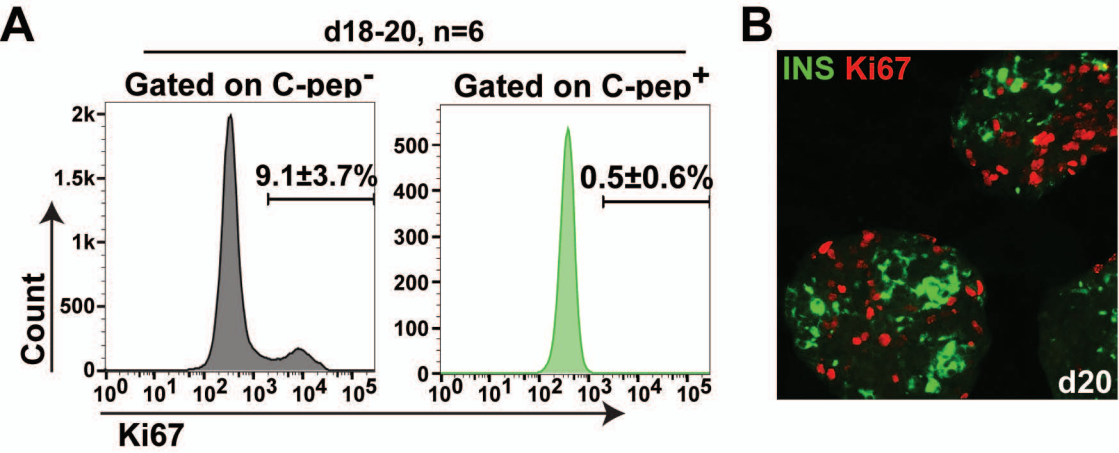




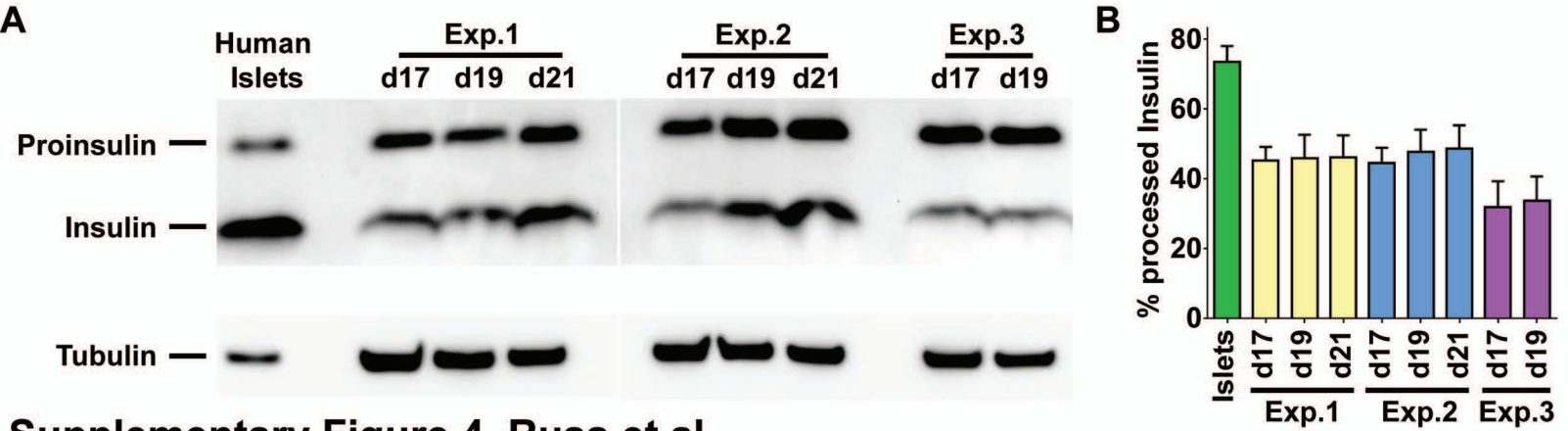
Supplementary Figure 1, Russ et al.



Supplementary Figure 2, Russ et al.



Supplementary Figure 3, Russ et al.



Supplementary Figure 4, Russ et al.

Supplementary Figure 1, related to Figure 2: Published protocols result in precocious endocrine differentiation by NEUROG3 activation.

A-C: Analysis of key pancreatic progenitor markers in clusters differentiated as outlined in Fig.1A. R= Retinoic acid, C=Cyclopamine, N=Noggin, E=Epidermal growth factor, K=Keratinocyte growth factor, T=TBP, and A=ALK inhibitor. Data shown are representative of two independent experiments. **A:** PDX1, INS, GCG, NEUROG3, and NKX6.1 protein expression was assessed by whole mount staining of differentiated clusters at indicated time points. Note precocious expression of the endocrine marker NEUROG3 in the absence of NKX6.1 protein at day 6-9. **B and C:** Flow cytometric quantification of PDX1+ (orange gate), PDX1+/NKX6.1+ (blue gate), INS+/NKX6.1+ (green gate), and INS+/NKX6.1- (red gate) cells at indicated time points. **D**: qPCR analysis of NGN3 and NKX2.2 transcripts at day 8 of differentiation employing RCN (Retinoic acid (R), Cyclopamine (C), and Noggin (N)) or R with two different concentrations of Vitamin C (Vit. C) treatment for 3 days or without. Data are shown as the average ± standard error, relative to RCN and normalized to GAPDH. (n= three independent experiments, technical duplicates)

Supplementary Figure 2, related to Figure 3: Induction of NEUROG3 expression in PDX1+ pancreatic progenitors results in insulin-producing cells that lack NKX6.1 expression.

A: Schematic outlining the differentiation strategy employed. R=Retinoic Acid, N=Noggin, and A=ALK inhibitor. **B and C:** Flow cytometric analysis of human c-peptide (C-PEP), glucagon (GCG), and NKX6.1 expression at the indicated differentiation time points. Data representative of 3 to 4 independent experiments with similar results are shown. **B:** Endocrine differentiation of PDX1+ pancreatic progenitors results in the predominant generation of polyhormonal insulin- and glucagon- producing cells at day 13. **C:** Insulin-producing cells lack expression of the critical beta cell transcription factor NKX6.1 at day 13 and 21. A small population of NKX6.1+/INS- progenitor cells is generated by NEUROG3 inducing treatment with AN.

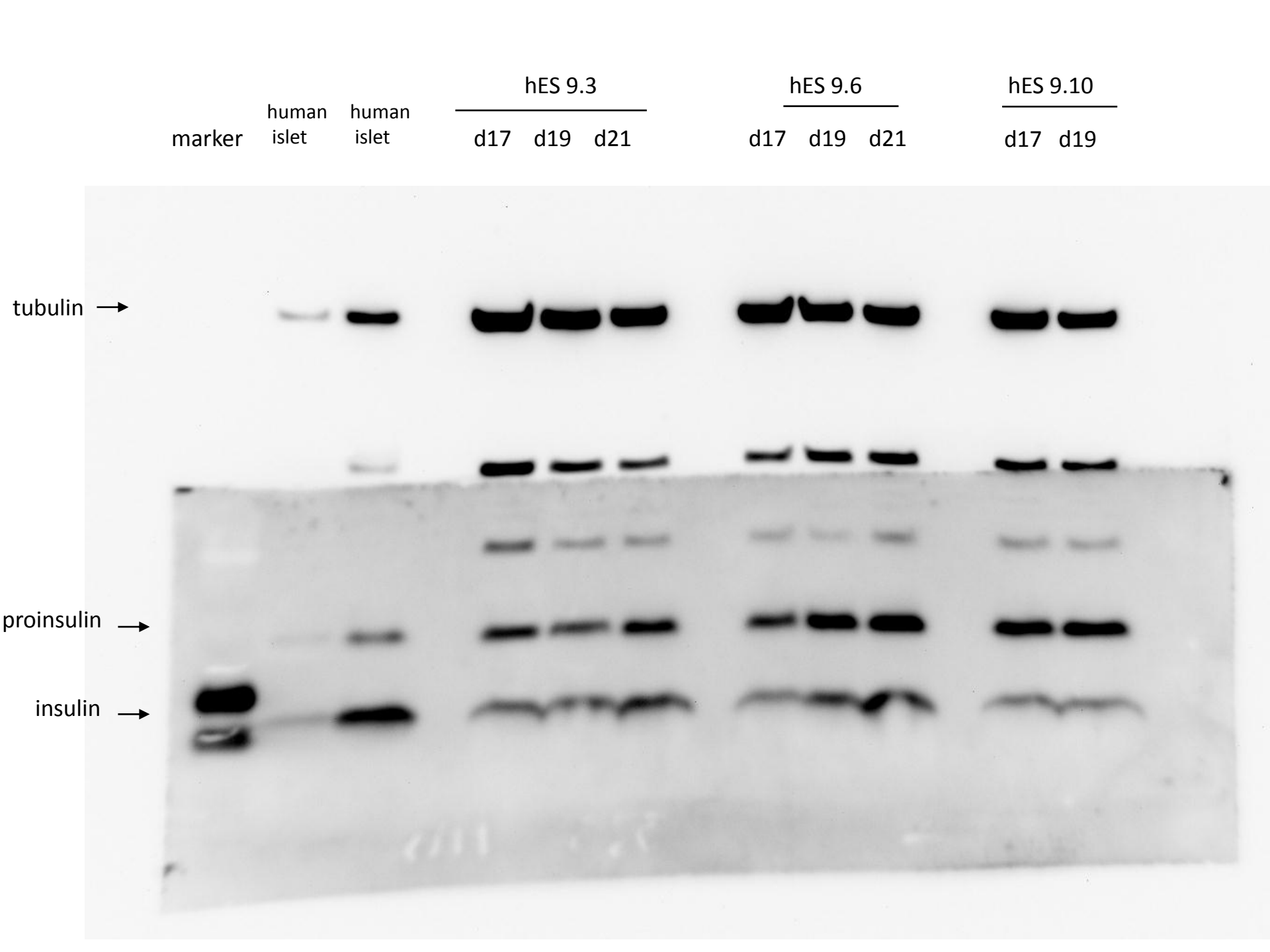
Supplementary Figure 3, related to Figure 4: hESC derived beta-like cells are post-mitotic.

A: Proliferation of C-peptide+ beta-like cells and C-peptide negative cell populations at days 18-20 was determined by co-staining with the proliferation marker Ki67. **B:** Immunofluorescence staining of differentiated clusters at day 20 for the proliferation marker Ki67 and human insulin (INS). Representative data from one of three experiments with similar results are shown.

Supplementary Figure 4, related to Figure 5: Efficient processing of insulin in hESC derived beta-like cells.

A: Western blot analysis of proinsulin processing to insulin in beta-like cells at indicated time points. A human islet preparation is shown for comparison. **B:** Quantification of proinsulin processing in Fig. 5E and panel A. n = 3 for each time point of beta-like cells and n=4 for human islets.

Attachment: Un-cropped Western blot shown in Supp. Fig. 4.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hES 9.6
hES 9.10
marker
human islet
human islet
d17 d19 d21
d17 d19 d21
d17 d19
tubulin
proinsulin
insulin