Supporting Online Material for

Betatrophin: a hormone that controls pancreatic β cell proliferation

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This PDF file includes:

Figure S1-6

Supplemental Figure Legends

Figure S1.Time course of pancreatic β cell proliferation following S961 treatment, related to Figure 2. The pancreatic β cell replication rate starts to increase at day 1 (**A**) and reaches a peak and remains high from day 2 on (**B-E**). The rate drops to normal 4 days after S961 treatment is terminated (**F**). 10nMol/week S961 was used to treat the mice. White arrows point to insulin^{+} (β) cells that co-stain for the division marker, Ki67.

Figure S2. Infusion of the insulin receptor antagonist S961 or injection of betatrophin induce pancreatic β cell proliferation, related to Figure 2 and Figure 5. S961(10nMol/week) induced pancreatic β cell proliferation compared to vehicle shown by co-staining of the β-cell specific nuclear marker Nkx6.1 and Ki67 (**A**) or co-staining of insulin and PCNA (**B**).Expression of betatrophin in liver induces a dramatic pancreatic β cell proliferation increase compared to GFP control animals, shown by co-staining of the β-cell specific nuclear marker Nkx6.1 and Ki-67 (**C**) or co-staining of insulin and PCNA (**D**).

Figure S3. Analysis of β cell proliferation and β cell mass in vehicle or S961 treated mice and GFP or betatrophin injected mice, related to Figure 2 and Figure 5. (**A**) The expression level of various cell cycle genes in pancreatic islets after vehicle or S961 (10nMol/week) treatment. (**B**) The average β cell replication rate per islet, in each individual mouse, treated with either vehicle or S961 (2.5-20nMol/week). (**C**) Average islet size of S961 treated mcie is shown as the fold increase over the control (vehicle alone). (**D**) Average β cell size of S961 treated mice is shown as a fold increase over the vehicle alone control. No significant differences are found. (**E**) The expression level of various cell cycle genes in pancreatic islets of GFP or betatrophin injected mice. (**F**) The average β cell replication rate per islet for each individual mouse injected with either GFP or betatrophin. (n=5 for GFP group and n=7 for betatrophin group.) (**G**) Average islet size of betatrophin injected mice is shown as the fold increase over the control (GFP injected mice). (* indicates that $p<0.05$ compared to vehicle treated or control injected mice). Data are represented as mean +/- SEM.

Figure S4. Gene structure of betatrophinand alignment of betatrophin protein sequence between species, related to Figure 3. (**A**) Gene structure of mouse (mbetatrophin) and human (hbetatrophin). Both mbetatrophin and hbetatrophin lie within the intron of another gene, Dock6, but on the opposite

strand. (**B**) Protein sequence alignments show a high level of homology between mouse, rat, chimpanzee and human. The putative signal peptide is noted.

Figure S5.Expression of mbetatrophin-Myc, hbetatrophin-Myc or GFP in liver by hydrodynamic tail vein injection, related to Figure 5. Expression plasmids encoding GFP (**A**) mbetatrophin-Myc (**B**) or hbetatrophin-Myc (**C**) were expressed in liver by tail vein injection (3 days after injection). Immunofluorescence for the relevant proteins is shown in color (green or red) with nuclei stained with DAPI (blue). About 5-10% of the liver cells express the injected genes.

Figure S6. *In vitro* GSIS analysis of the islets from GFP or betatrophin injected mice, related to Figure 6. The insulin secretion is evaluated in low glucose (2.5mM), high glucose (15mM) and KCl (30mM) buffers. The insulin concentration is normalized to total genome DNA from all islets. There is no significant difference between GFP and betatrophin injected animals. Data are represented as mean +/- SEM.

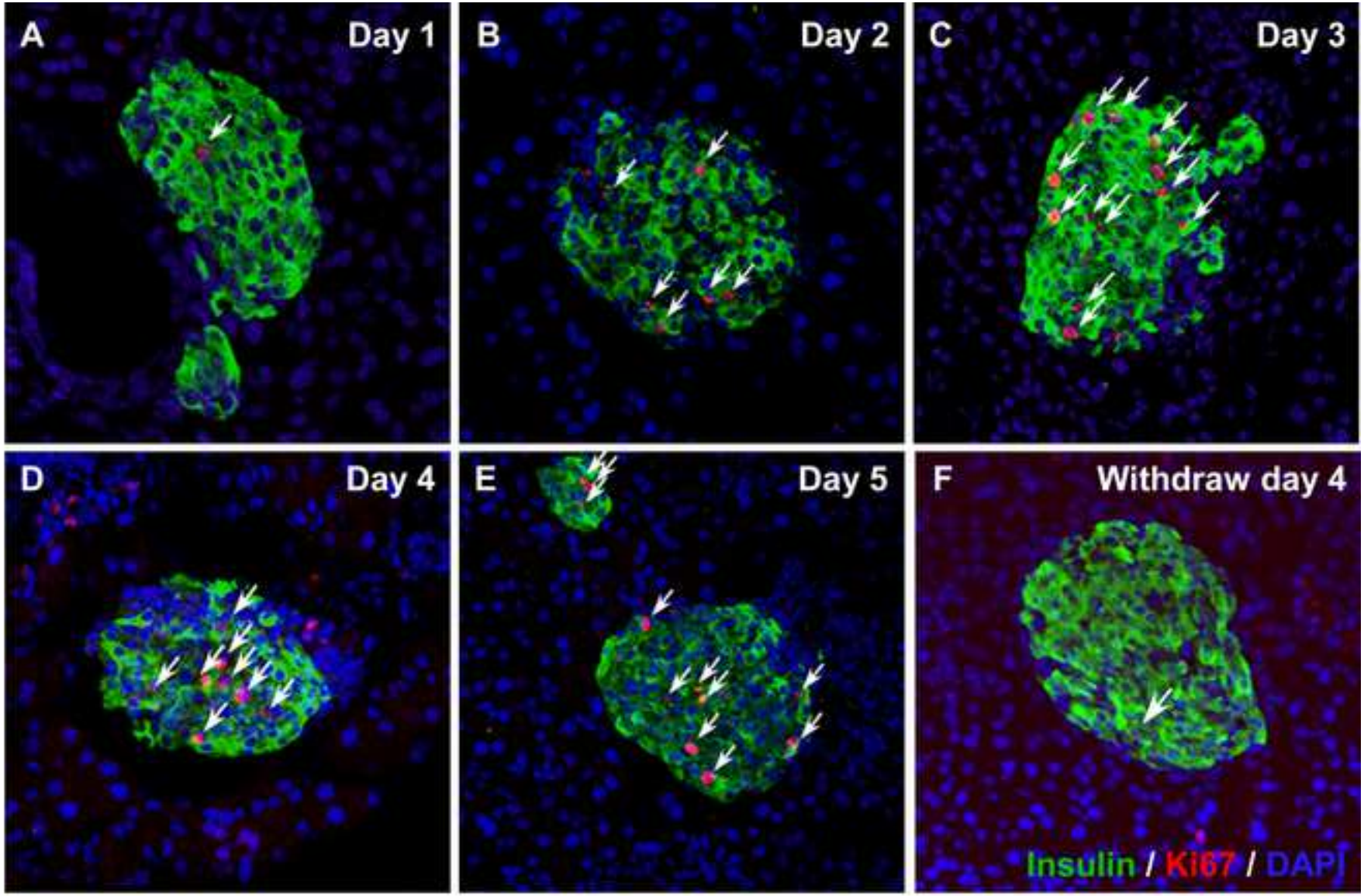

Figure S1

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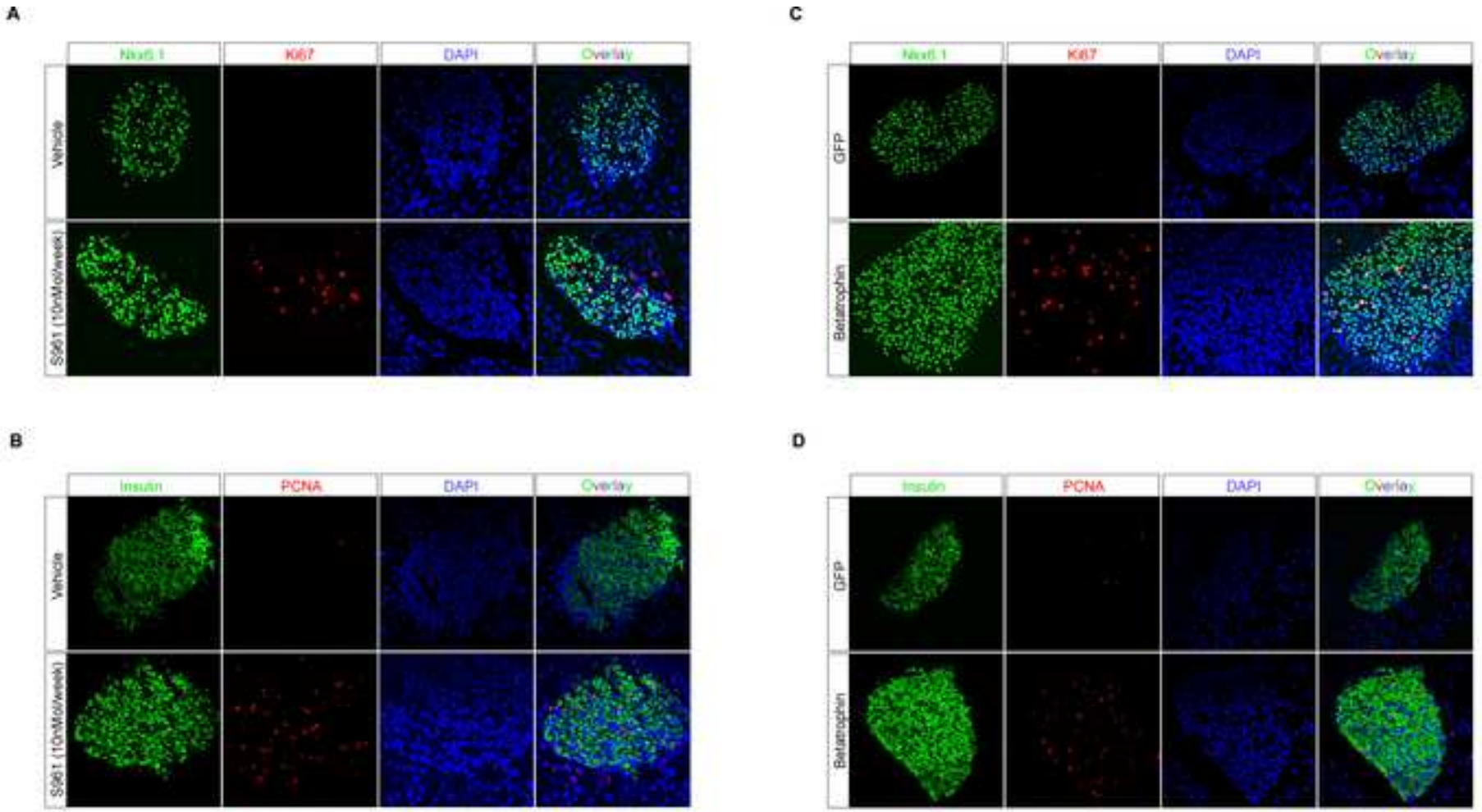

Figure S2
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Supplemental Figure 3

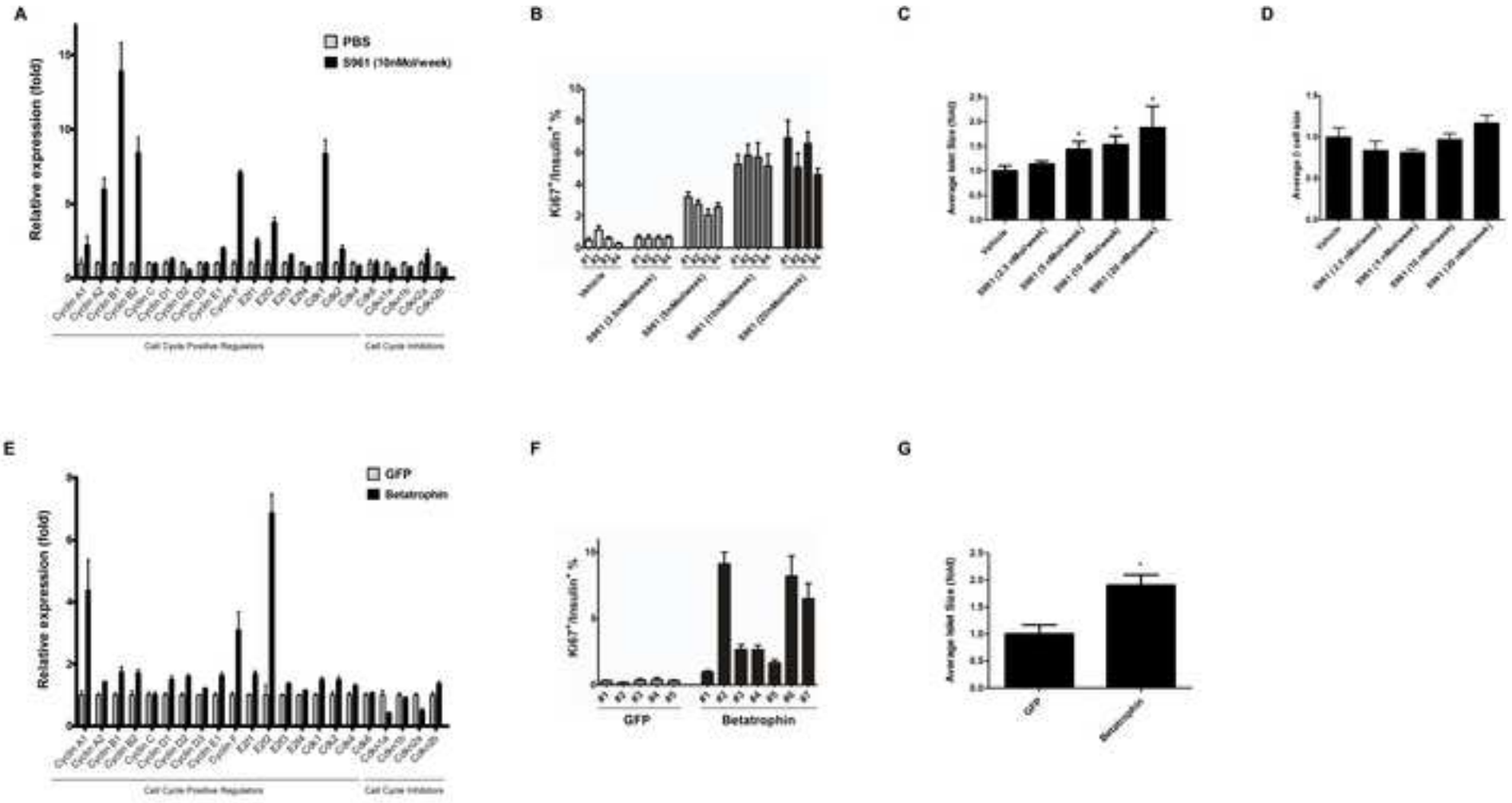

Figure S3
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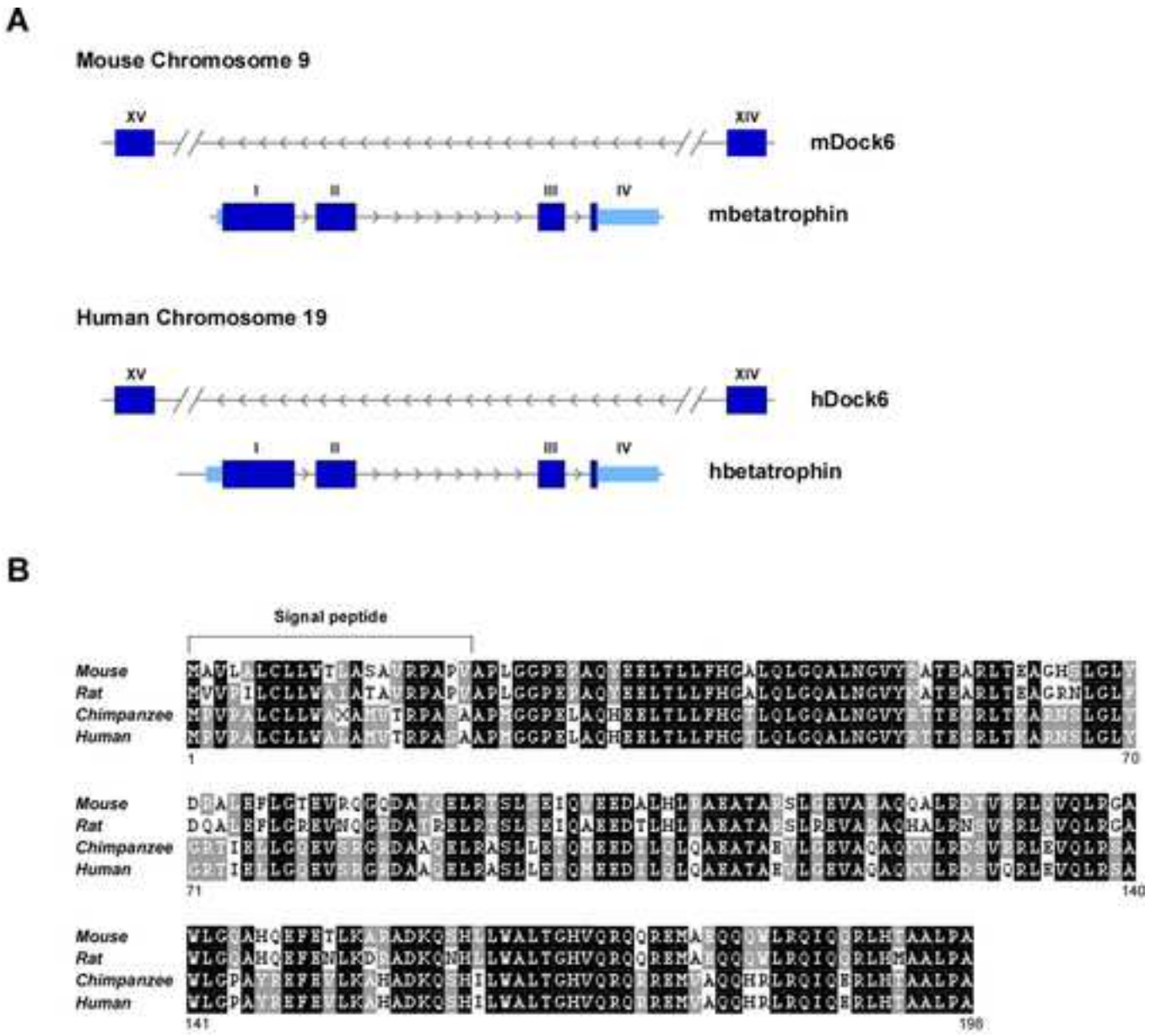

Figure S4

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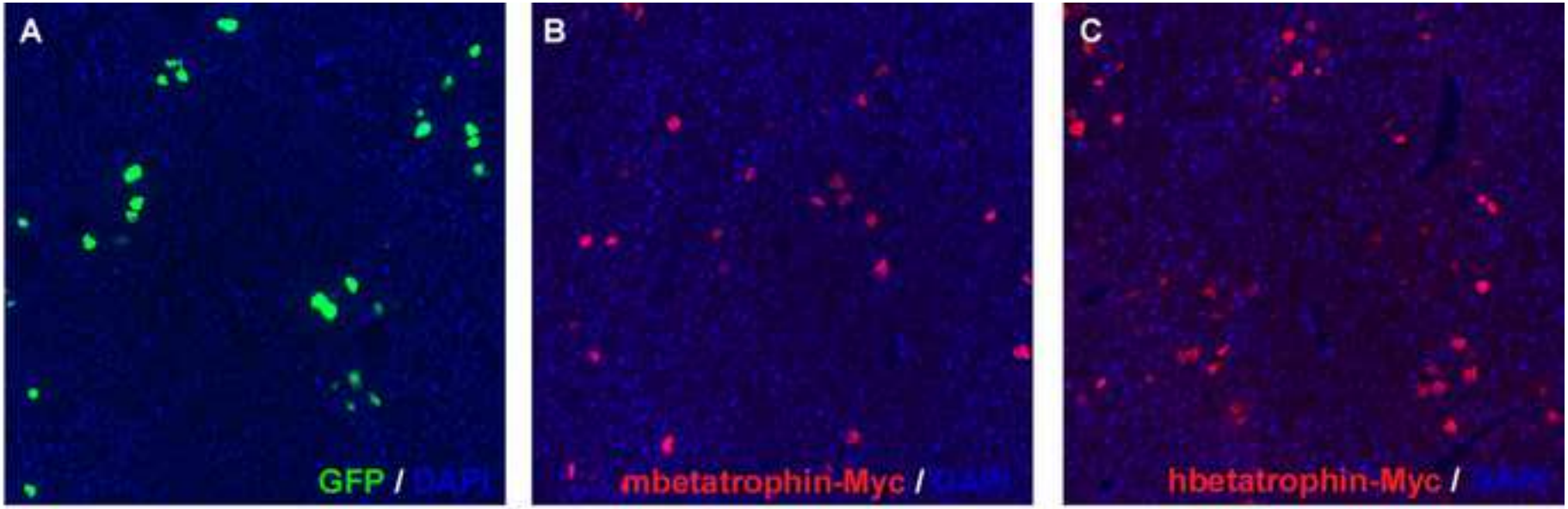

Figure S5
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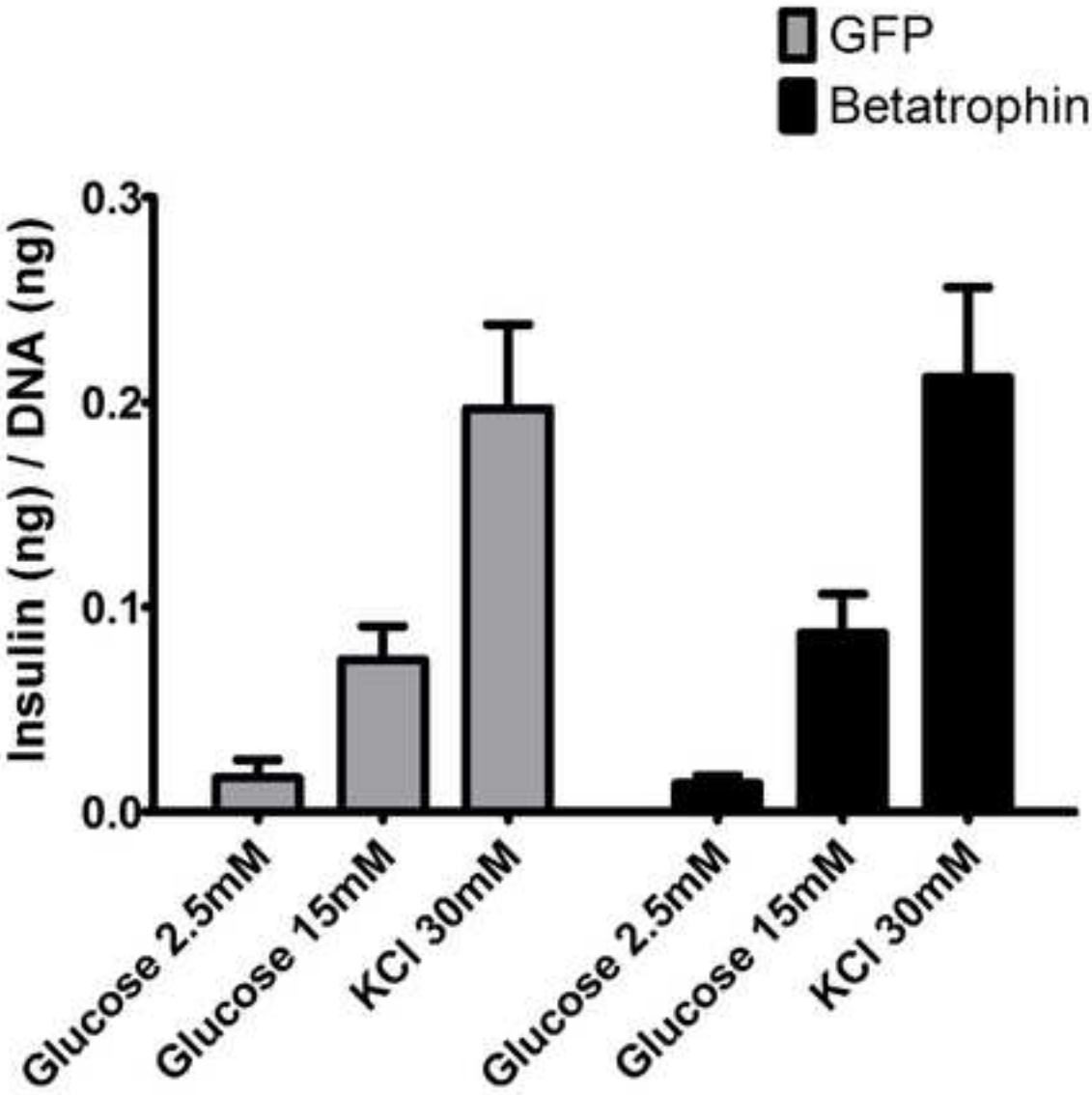

Figure S6

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