SUPPLEMENTAL DATA

Functional fibroblast growth factor receptor-1 is expressed in murine βTC3 cells. FGFR1 expression has been observed previously in adult mouse islets (1, 2). To elucidate endogenous islet β-cell FGFR1 expression and activity, we examined the murine insulin-secreting βTC3 cell line (Fig. S1). By RT-PCR, we confirmed expression of the FGFR1IIIc mRNA transcript that was not detectable in the αTC6 cell line (Fig. S1A). FGFR1IIIc plasmid DNA was used as a positive control. Full-length endogenous receptor was also identified by Western immunoblotting as a protein band with a molecular mass of ~150 kDa (Fig. S1B, *top*). This band exhibited slight retardation in protein mobility with FGF-1 stimulation, consistent with tyrosine phosphorylation of activated receptor. Phosphorylation of the FRS2 docking protein (~90 kDa molecular mass) was also enhanced with FGF stimulation confirming activation of receptor (Fig. S1B; *middle*). Taken together, these data are consistent with endogenous FGFR1 expression and activity in the βTC3 cell line, providing a translational model system for islet studies.

Generation of functional fluorescent protein-tagged FGFR1 isoforms. To measure FGFR1-dependent ERK1/2 activity in islet β-cells and to generate tools for examining the molecular mechanisms of FGFR activation, we created Venus fluorescent protein-tagged constructs of full-length (R1βv) and kinase-deficient (KDR1βv) FGFR1c. Construct expression was driven by the rat insulin promoter (3). By using lentiviral infection (MOI ~5), we achieved 80-90% expression efficiency in βTC3 cells (Fig. S2A). Western immunoblotting of transfected cell lysates was performed using antibodies directed against Venus fluorescent protein (αGFP) and the C-terminus of FGFR1 (Flg (C-15)) to confirm and characterize construct expression (Fig. S2B). The full length receptor isoform R1βv was identified as a protein band at the expected molecular mass of approximately 150 kDa by both antibodies (Fig. S2B; *solid* arrow). KDR1v was detected as a protein band at the expected molecular mass of 80-90 kDa by only the αGFP antibody since this isoform is deficient for the C-terminal tail recognized by the Flg receptor antibody (Fig. S2B; *asterisk*). Protein bands of approximately 28-30 kDa in lysates of all transfected cells reflect expression of Venus indicating some enzymatic cleavage of the fluorescent protein in samples expressing receptor constructs (Fig. S2B; *arrowhead*). To determine membrane association of the receptor constructs, we subsequently measured fluorescent protein diffusion using live cell fluorescence recovery after photobleaching (FRAP) (Fig. S2C). Significantly slower rates of recovery were observed for the FGFR1 constructs ($t_{1/2}$ ~12 s for R1βv and KDR1βv) than the Venus construct ($t_{1/2}$ ~0.7 s), consistent with association at the plasma membrane. These data indicate that the fluorescent protein-tagged receptor isoforms are expressed appropriately in βTC3 cells and translocate to the plasma membrane.

Rat insulin promoter driven expression of R1βv, KDR1βv and Venus. Although islets are comprised of a majority of β-cells (~85 %), a number of other endocrine cells are present including α-, δ-, pancreatic polypeptide-, and ghrelin-cells. To confirm that the constructs driven by the rat insulin promoter were expressed exclusively in β-cells, we examined fluorescence protein expression in islets immunostained for insulin (Fig. S3A) and glucagon/somatostatin (non-β cell; Fig. S3B). By examining co-localization of Venus fluorescent protein and insulin, it was evident that the constructs were expressed in insulin positive β-cells only (Fig. S3A). However, while all R1βv-, KDR1βv- and Venus-infected cells were positive for insulin, not all insulin-positive cells were positive for Venus fluorescent protein suggesting <100% transfection efficiency. By examining Venus fluorescence in association with glucagon- and somatostatin-associated immunofluorescence, we confirmed that the Venus-tagged constructs were not expressed in non-β-type cells. These data demonstrate that the rat insulin promoter confines FGFR construct expression exclusively in β-cells in intact islets.

SUPPLEMENTAL FIGURE CAPTIONS

Figure S1. Murine βTC3 cells express functional FGFR1. (A) RT-PCR of total RNA using primers designed to amplify FGFR1IIIc (*top*) and GAPDH (*bottom*) in βTC3 cells (β), αTC6 cells (α), water negative control (-), and R1βv plasmid DNA positive control (+). Sense (5'-CAA CCT GCC TTA TGT CCA GA-3') and antisense (5'-GCT TCC AGA ACG GTC AAC CA-3') primers were used [35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min]. The housekeeping transcript glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was a positive control of RNA expression and was amplified over 35 cycles using sense (5'-ATC GAG CTC ATC CCA TCA CCA TCT TCC AGG-3') and antisense (5'-ACA TCT AGA GCC ATC ACG CCA CAG TTT CCC-3') primers. (B) Western immunoblotting of whole cell lysate (10 μg/lane) from cultures stimulated with FGF-1 (10 ng/ml; times as indicated) using FGFR1 antibodies recognizing the N-terminus (*top*), phospho-FRS2 (*middle*), and β-actin (loading control, *bottom*).

Figure S2. Expression and membrane association of Venus-tagged full-length (R1βv) and kinase-deficient (KDR1βv) FGFR1c in βTC3 cells. (A) Live cell images of Venus-, R1βv-, and KDR1βv-associated fluorescence in βTC3 cells. Receptor isoforms are excluded from the nucleus compared to Venus control protein. (B) Western immunoblotting of whole cell lysates (10 μg/lane) from cells expressing Venus, R1βv, KDR1βv or wild type βTC3 (WT) using antibodies recognizing either Venus (left panel) or the C-terminus of FGFR1 (Flg, C-15) (C) Fluorescence recovery after photobleaching (FRAP) curves revealing protein diffusion rates in cells expressing Venus (-■-), R1βv (-○-), and KDR1βv (-▲-). Data are expressed as the average fluorescence recovery ($\Delta F/F_0$) ± standard error of the mean for three independent experiments.

Figure S3. Insulin-positive islet β-cells express fluorescent protein constructs driven by the rat insulin promoter. (A) Islets were infected with a low MOI of Venus, full-length R1 (R1βv), and kinase-deficient R1 (KDR1βv) and immunostained with anti-insulin antibody. Fluorescence images for Venus fluorescence protein (FP) and insulin were acquired and overlaid (FP/insulin). (B) Non-insulin producing cells were identified in a control islet expressing Venus protein by co-labeling for non-β-cells (glucagon and somatostatin). The overlay image (Venus/Non-β-cell) demonstrates that Venus-expressing cells do not express glucagon or somatostatin.

REFERENCES

1. **Dichmann DS, Rescan C, Frandsen U, Serup P** 2003 Unspecific labeling of pancreatic islets by antisera against fibroblast growth factors and their receptors. J Histochem Cytochem 51:397-400
2. **Hart AW, Baeza N, Apelqvist A, Edlund H** 2000 Attenuation of FGF signalling in mouse beta-cells leads to diabetes. Nature 408:864-8
3. **Hanahan D** 1985 Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature 315:115-22

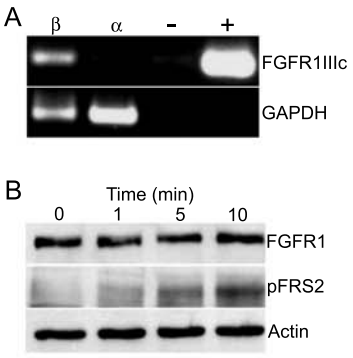

Figure S1.

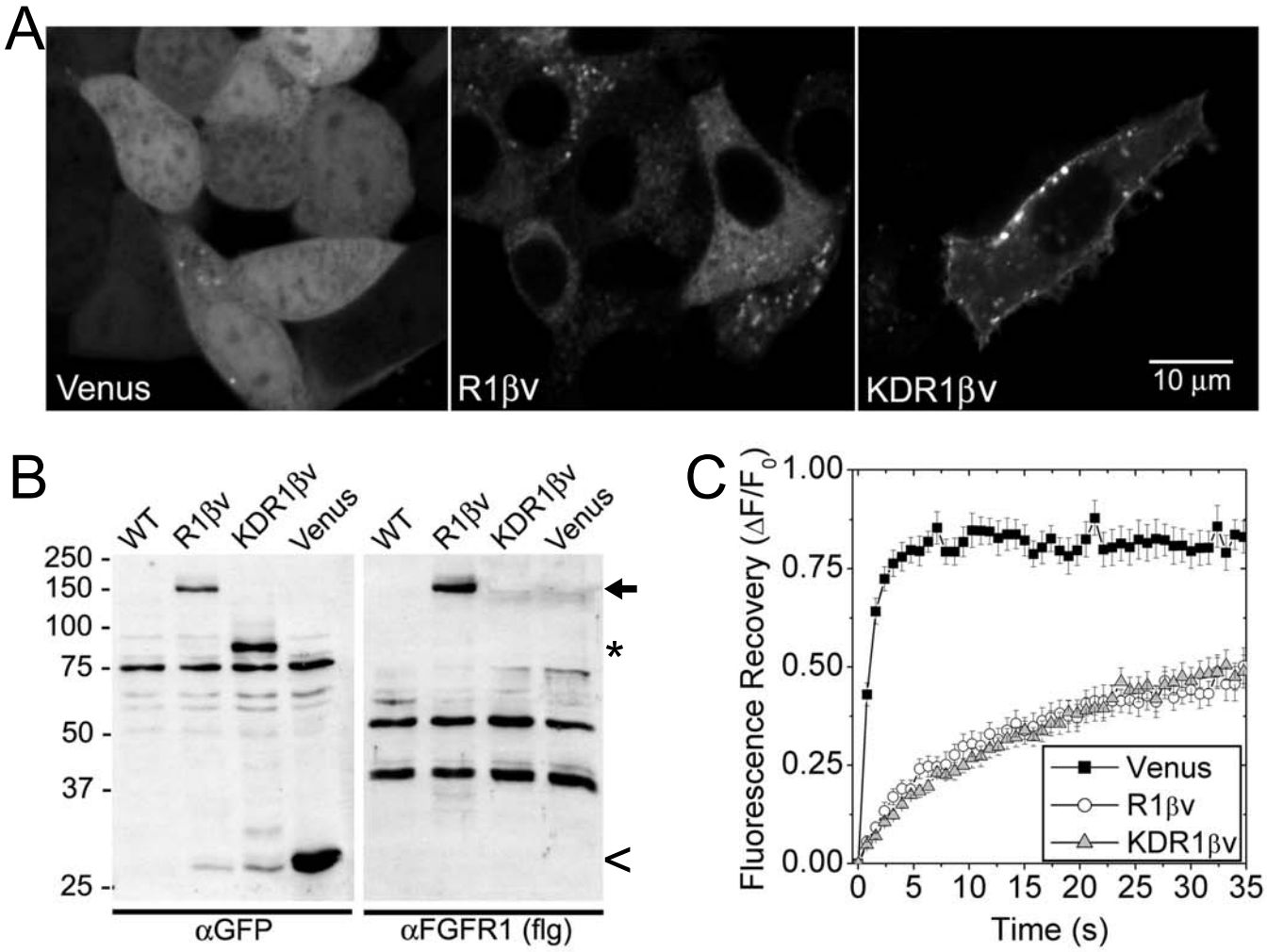
A
Venus
R1βv
KDR1βv
10 μm
B
WT
R1βv
KDR1βv
Venus
250
150
100
75
50
37
25
αGFP
αFGFR1 (flg)
*
C
Fluorescence Recovery (ΔF/F0)
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
30
35
Time (s)
Venus
R1βv
KDR1βv

Figure S2.

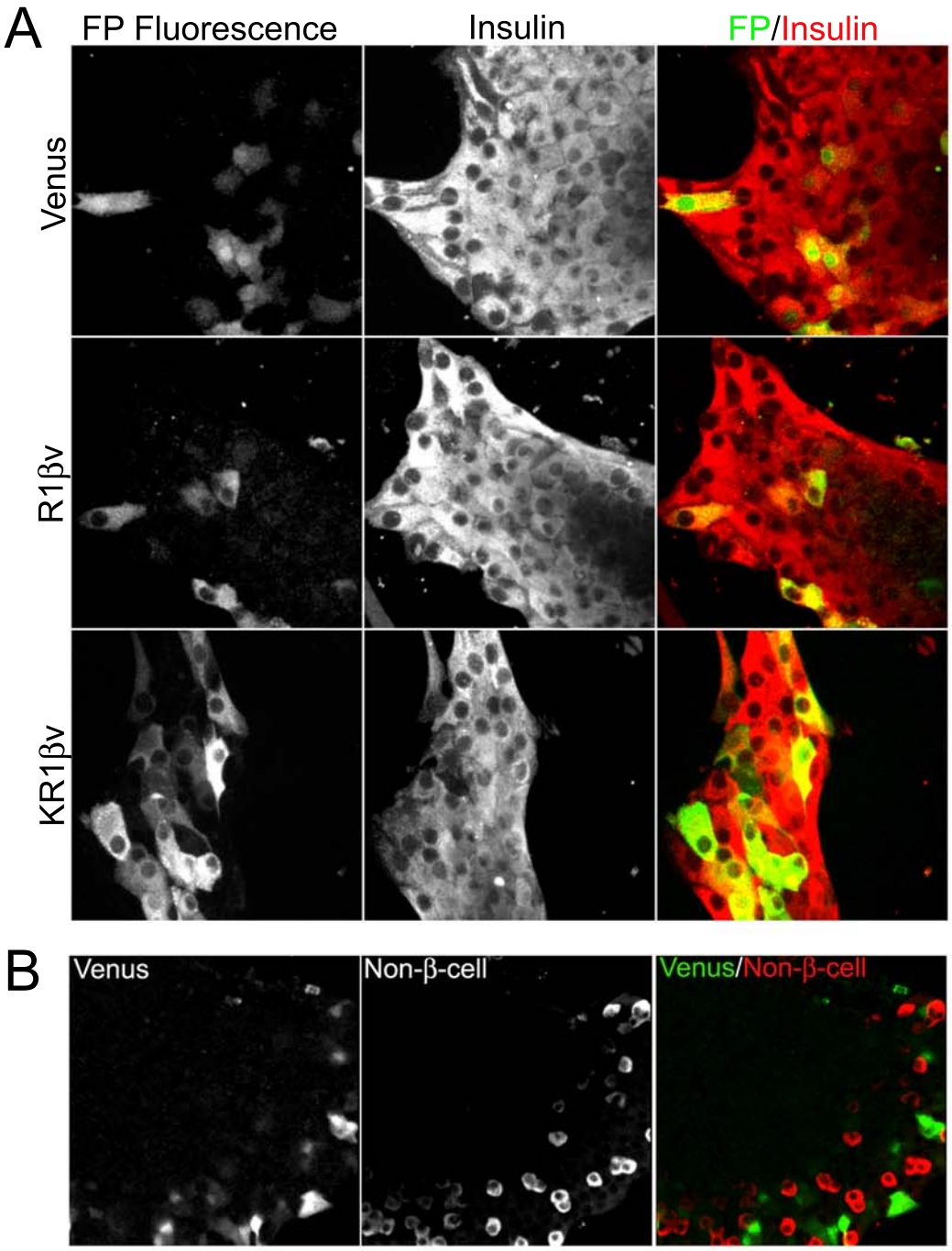
A
FP Fluorescence
Insulin
FP/Insulin
Venus
R1βv
KR1βv
B
Venus
Non-β-cell
Venus/Non-β-cell

Figure S3.