SUPPLEMENTARY DATA

Anti-aging Gene *Klotho* Attenuates Pancreatic β Cell Apoptosis in Type I Diabetes

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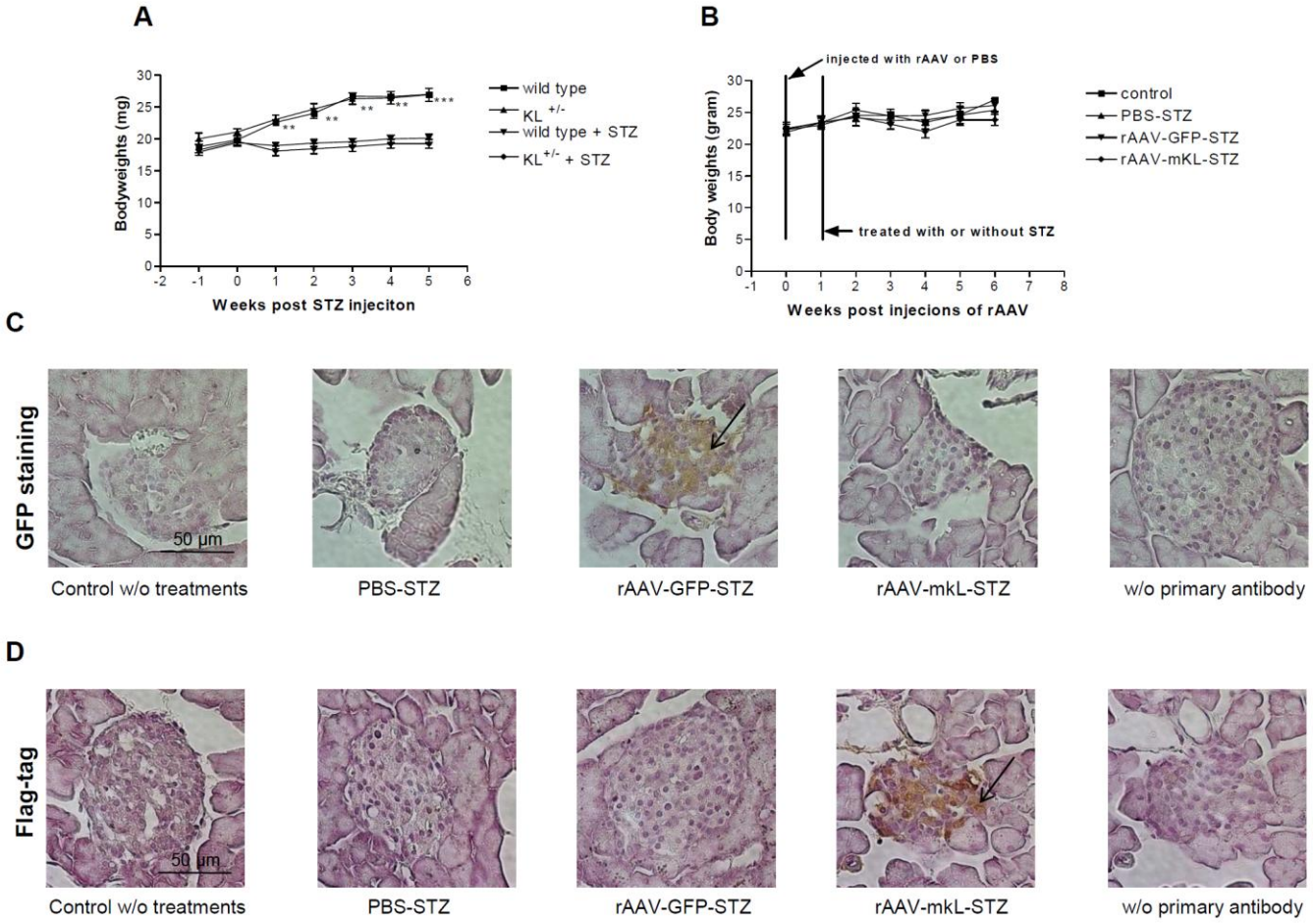
Supplementary Figure S1. Body weights and expressions of GFP or FLAG-tag in pancreatic islets of Langerhans. A: Body weights of 129Sv mice treated with STZ. $KL^{+/-}$ and litter mate wild type male mice were injected with STZ or citrate buffer. **B**: Body weights for 129S1/SvIm mice. 129S1/SvIm male mice were injected with PBS, rAAV-GFP, or rAAV-mKL. One week after gene delivery, these mice were injected with STZ or citrate buffer. **C.** Representative images of GFP staining (brown color) in cross-sections of pancreatic islets. Six weeks after the initial rAAV injections, animals were sacrificed and mouse pancreases were collected. GFP protein was detected using antibody against GFP on cross section of mouse pancreas. **D**. Representative images of FLA-tag staining (brown color) in cross-sections of pancreatic islets. The 3' end of mouse KL gene was coupled with Flag-tag gene in the construct of rAAV-mKL. FLAG-tag was detected using antibody against FLAG-tag on cross section of mouse pancreas.



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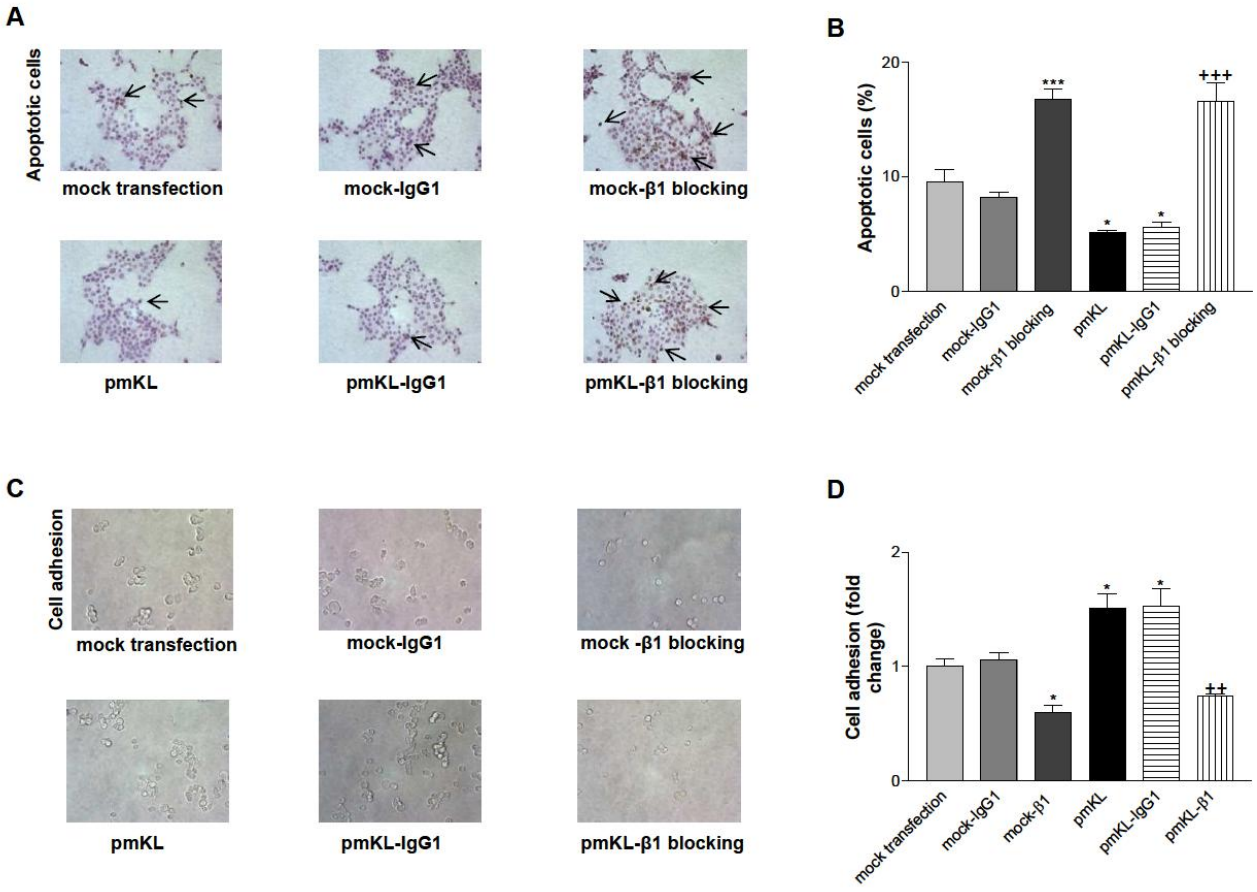
Supplementary Figure S2. Blocking of integrin β1 abolished the protective effect of Klotho on apoptosis and the promoting effect of Klotho on cell adhesion in MIN6 β cells. A. Apoptotic nuclear change (pointed by arrows, brown color) detected by TUNEL staining in MIN6 β cells. Transfected MIN6 cells were seeded on collagen IV-coated 6-well plates and then incubated with or without blocking antibody for 24 hours. **B.** The percentage of apoptotic cells. **C.** Phase contrast images of adherent MIN6 β cells. Transfected cells were preincubated with integrin β1 blocking antibody or isotype control for 1 hour and then seeded on 6-well plates coated with collagen IV for 3 hours. **D:** Quantification of cell adhesion. n = 4. *p < 0.05, ***p < 0.001 *vs* the mock-transfection group; $^{++}$p<0.01, $^{+++}$p < 0.001 *vs* the pmKL group.



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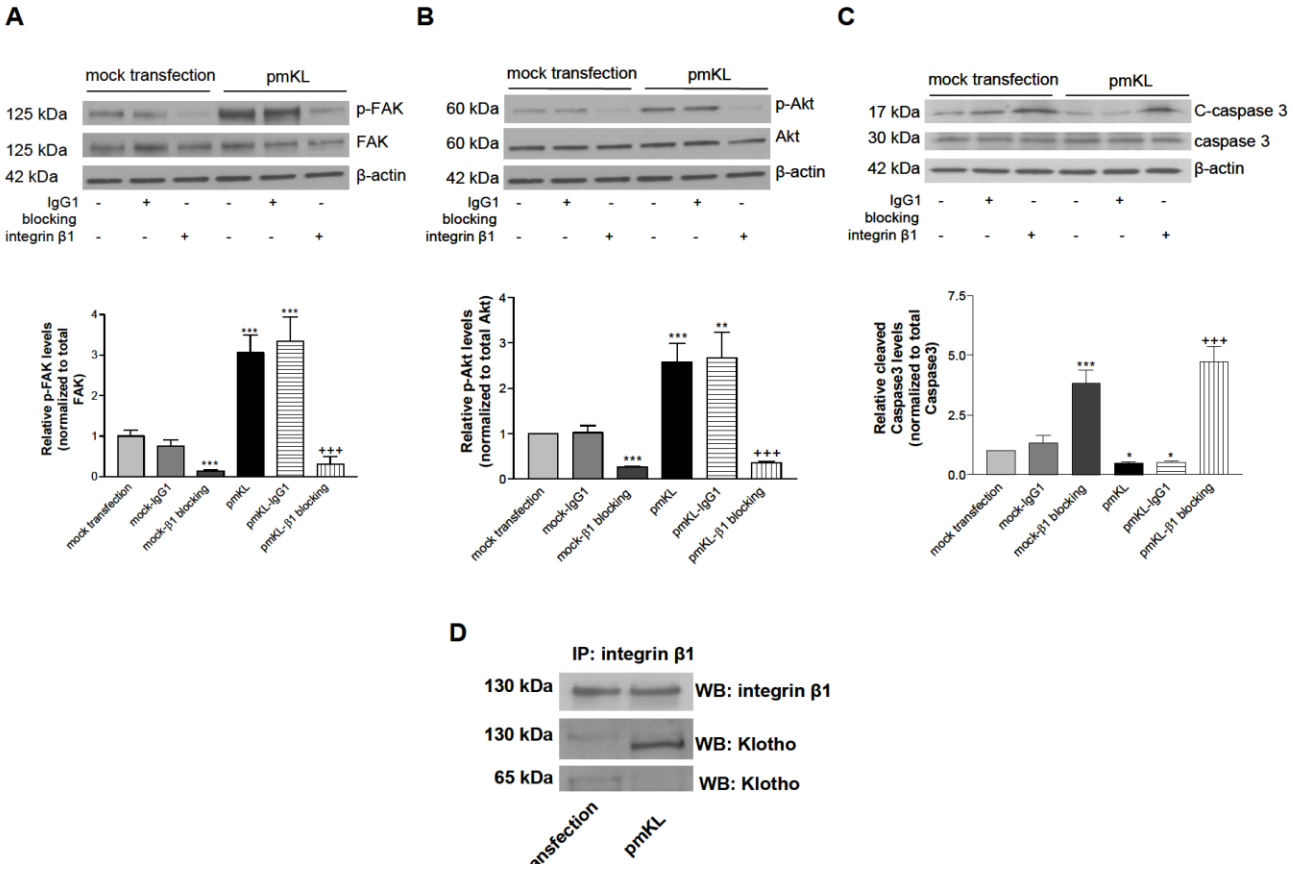
Supplementary Figure S3. Blocking of integrin β1 abolished the promoting effects of Klotho on phosphorylations of FAK and Akt and the inhibiting effects of Klotho on Caspase 3 cleavage in MIN6 β cells. Transfected cells were seeded on collagen IV-coated 6-well plates and then incubated with or without blocking antibody for 24 hours. Cells were lysed with Ripa buffer. **A**. Western blot analysis of phosphorylated FAK (Tyr: 397; upper panel) and FAK protein levels (middle panel) in cell lysates. Quantification of Phosphorylation of FAK (lower figure). Results were standardized to FAK protein level and then expressed as fold changes *vs* the control mock transfection group. **B**. Western blot analysis of phosphorylated Akt (Ser: 473; upper panel) and Akt protein (middle panel) levels**.** Quantification of phosphorylation of Akt (lower figure). Results were standardized to Akt protein level and then expressed as fold changes *vs* the control mock transfection group. **C**. Western blot analysis of cleaved Caspase 3. Quantification of Caspase 3 cleavage (lower figure). Results were standardized to total Caspase and then expressed as fold changes *vs* the control mock transfection group 3. **D**. Co-immunoprecipitation of Klotho with integrin β1. Transfected cells were lysed with Ripa buffer. Integrin β1 was immunoprecipitated with antibody against integrin β1. Klotho in the precipitate was detected with antibody against Klotho (lower two panels). The blot was re-probed with antibody against integrin β1 after stripping (upper panel).



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Supplementary Figure S4. Body weights **(A),** basal blood glucose levels **(B),** the percentage of diabetics at 16 days after gene delivery **(C),** and net changes in blood glucose levels at 16 days after gene delivery (compared to the pre-injection level) **(D)**. Mice with the fasting blood glucose at or over 200 mg/dL were considered as diabetic. n=5.