SUPPLEMENTARY DATA

**Supplementary Table 1. Primers for RT-PCR**

| | Sequence (5' -> 3') | Tm | Reference | Location | Product size |
|---|---|---|---|---|---|
| BIRC1a (NAIP1) | | | | | |
| Sense | TGCCCAGTATATCCAAGGCTAT | 60.2 | NM_008670 | 708-729 | 116 bp |
| Antisense | AGACGCTGTCGTTGCAGTAAG | 62.6 | | 823-803 | |
| BIRC1b (NAIP2) | | | | | |
| Sense | AGCTTGGTGTCTGTTCTCTGT | 61 | NP_001119654 | 1204-1224 | 180 bp |
| Antisense | GCGGAAAGTAGCTTTGGTGTAG | 61.2 | | 1383-1362 | |
| BIRC2 (c-IAP1) | | | | | |
| Sense | TGTGGCCTGATGTTGGATAAC | 60 | NM_007465 | 256-276 | 164 bp |
| Antisense | GGTGACGAATGTGCAAATCTACT | 60.9 | | 419-397 | |
| BIRC3 (c-IAP2) | | | | | |
| Sense | ACGCAGCAATCGTGCATTTTG | 62.9 | NM_007464 | 1073-1093 | 181 bp |
| Antisense | CCTATAACGAGGTCACTGACGG | 61.6 | | 1253-1232 | |
| BIRC4 (XIAP) | | | | | |
| Sense | CGAGCTGGGTTTCTTTATACCG | 60.7 | NM_009688 | 145-166 | 126 bp |
| Antisense | GCAATTTGGGGATATTCTCCTGT | 60.4 | | 270-248 | |
| BIRC5 (Survivin) | | | | | |
| Sense | GAGGCTGGCTTCATCCACTG | 62.6 | NM_009689 | 118-137 | 250 bp |
| Antisense | CTTTTTGCTTGTTGTTGGTCTCC | 60.7 | | 367-345 | |
| BIRC6 (Apollon) | | | | | |
| Sense | ACAGATTGTCTTACCTCTTGCCC | 61.9 | NM_007566 | 695-717 | 120 bp |
| Antisense | GCCACGAAGTGAAGGTCTCC | 62.5 | | 814-795 | |
| BIRC7 (ml-IAP) | | | | | |
| Sense | AGCCTCCTTCTACGACTGG | 60.1 | NM_001163247 | 291-309 | 245 bp |
| Antisense | GCAAAGGGGTGTAGGTCTGG | 62.2 | | 535-516 | |
| TGF-β1 | | | | | |
| Sense | ACTCCACGTGGAAATCAACGG | 68.1 | NM_011577 | 693-713 | 414 bp |
| Antisense | TAGTAGACGATGGGCAGTGG | 62.7 | | 1106-868 | |
| Podocin | | | | | |
| Sense | AAGCTGAGGCACAAAGACAGG | 65.6 | NM_130456 | 848-868 | 416 bp |
| Antisense | CTATTTGGCAACCAAACAAGTG | 63.0 | | 1263-1242 | |
| GAPDH | | | | | |
| Sense | TGGCCTTCCGTGTTCCTAC | 61.3 | NM_008084 | 686-704 | 178 bp |
| Antisense | GAGTTGCTGTTGAAGTCGCA | 60.9 | | 863-844 | |
| Bcl2 | | | | | |
| Sense | AGCTGCACCTGACGCCCTT | 69.6 | NM_177410 | 344-362 | 192 bp |
| Antisense | GTTCAGGTACTCAGTCATCCAC | 60.1 | | 535-516 | |
| Bax | | | | | |
| Sense | CGGCGAATTGGAGATGAACTG | 68.7 | NM_007527 | 190-210 | 161bp |
| Antisense | GCAAAGTAGAAGAGGGCAACC | 63.8 | | 350-330 | |
| BclxL | | | | | |
| Sense | AGGTTCCTAAGCTTCGCAATTC | 64.4 | NM_001289739 | 128-149 | 248bp |
| Antisense | TGTTTAGCGATTCTCTTCCAGG | 64.2 | | 375-354 | |
| Apaf1 | | | | | |
| Sense | AAGGACAGTGCTGTGTGAA | 59.4 | NM_001042558 | 330-349 | 627bp |
| Antisense | CCTTTGCATTCCTTTATAATAC | 56.1 | | 956-935 | |

SUPPLEMENTARY DATA

BIRC1a,1b, 2, 3, 4, 5, 6, or 7: baculoviral iap repeat-containing 1a, 1b, 2, 3, 4, 5, 6, or 7. NAIP1, or 2: neuronal apoptosis inhibitory protein1, or 2. c-IAP1, or 2: cellular inhibitor of apoptosis protein 1, or 2. x-IAP: x-linked inhibitor of apoptosis proten. ml-IAP: melanoma inhibitor of apoptosis. TGF-β1: transforming growth factor-β1. GAPDH: glyceraldehyde 3-phosphate dehydrogenase. Bcl2: B-cell lymphoma 2. Bax: Bcl-2-associated X protein. BclxL: B-cell lymphoma-extra large. Apaf1: apoptotic protease activating factor 1

SUPPLEMENTARY DATA

**Supplementary Table 2.** Spearman correlation coefficients between clinical variables and plasma levels of total and free protein S and C4BP in type 2 DM patients(n=26).

| Variables | Plasma total protein S | | Plasma free protein S | | Plasma C4BP | |
|---|---|---|---|---|---|---|
| | r values | p values | r values | p values | r values | p values |
| Age | 0.2 | 0.3 | 0.4 | 0.04 | -0.6 | 0.003 |
| Diabetes duration | -0.1 | 0.6 | 0.0 | 0.9 | -0.3 | 0.07 |
| Body mass index | -0.1 | 0.7 | -0.1 | 0.8 | 0.3 | 0.2 |
| Fasting blood glucose | 0.1 | 0.8 | -0.1 | 0.7 | 0.1 | 0.7 |
| Serum Hemoglobin A1c | -0.3 | 0.1 | -0.5 | 0.02 | 0.4 | 0.04 |
| Serum T cholesterol | -0.1 | 0.5 | -0.2 | 0.4 | 0.3 | 0.1 |
| Serum Triglycerides | 0.1 | 0.6 | -0.1 | 0.6 | 0.1 | 0.7 |
| Serum high density lipoproteins | -0.2 | 0.4 | -0.3 | 0.1 | 0.1 | 0.4 |

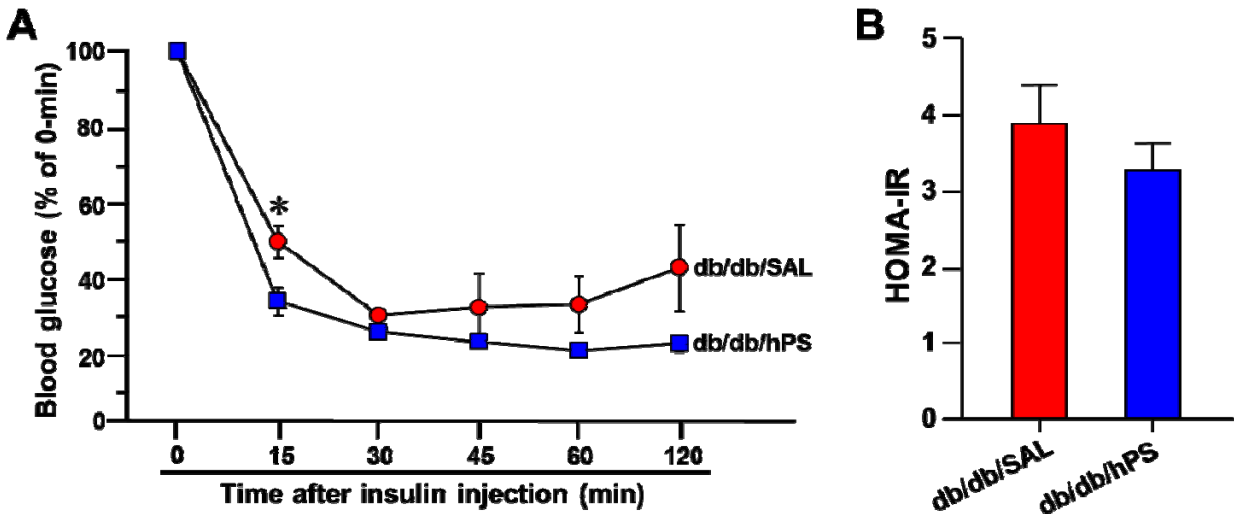
SUPPLEMENTARY DATA

**Supplementary Table 3. Spearman correlation coefficients between clinical variables and plasma levels of total and free protein S and C4BP in type 1 DM patients (n=6).**

| Variables | Plasma total protein S | | Plasma free protein S | | Plasma C4BP | |
|---|---|---|---|---|---|---|
| | r values | p values | r values | p values | r values | p values |
| Age | 0.3 | 0.5 | 0.1 | 0.8 | 0.5 | 0.3 |
| Diabetes duration | 0.6 | 0.2 | 0.5 | 0.3 | 0.1 | 0.9 |
| Body mass index | -0.3 | 0.6 | 0.0 | 0.9 | -0.4 | 0.4 |
| Fasting blood glucose | 0.3 | 0.5 | 0.0 | 0.9 | 0.6 | 0.1 |
| Serum Hemoglobin A1c | -0.5 | 0.2 | -0.4 | 0.4 | -0.0 | 0.8 |
| Serum T cholesterol | -0.7 | 0.1 | -0.8 | 0.06 | 0.1 | 0.8 |
| Serum Triglycerides | -0.1 | 0.9 | 0.1 | 0.8 | -0.6 | 0.1 |
| Serum high density lipoproteins | 0.2 | 0.7 | -0.1 | 0.9 | 0.4 | 0.4 |

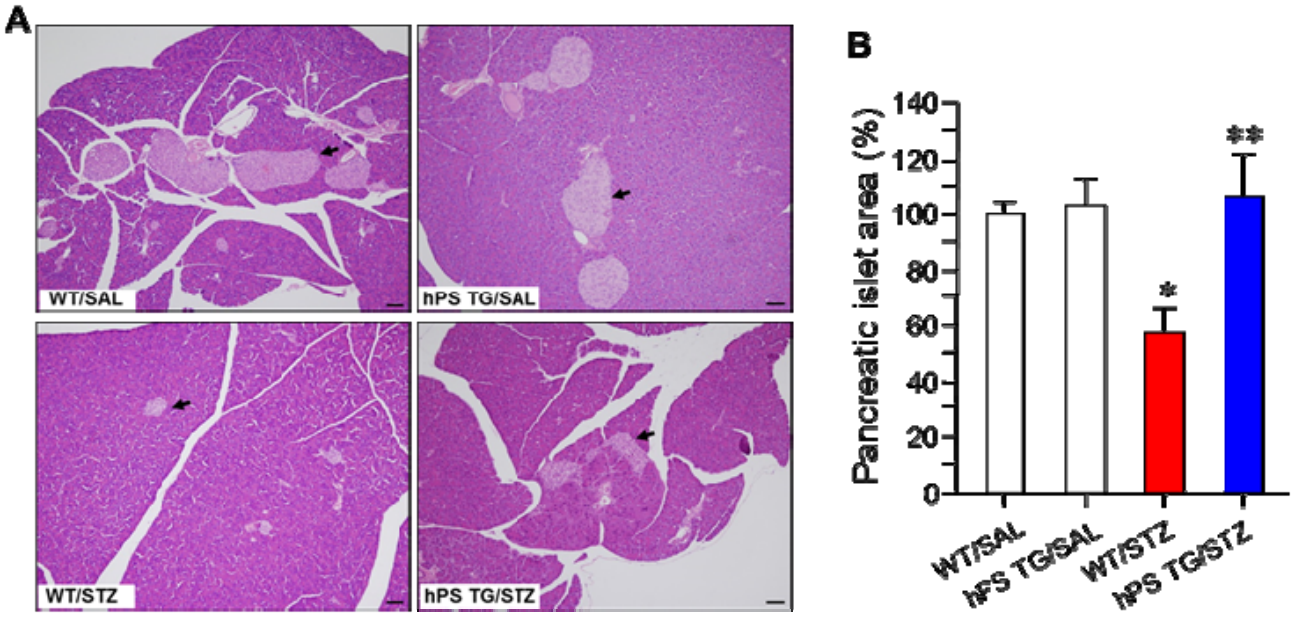
SUPPLEMENTARY DATA

**Supplementary Figure 1. Improvement of insulin sensitivity in db/db mice by hPS treatment.** During fasting db/db mice were subcutaneously treated with hPS (2 mg/kg; n=5) or saline (n=5) at 0, 1, 2, 4 and 6h and then insulin sensitivity test (A) was performed and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated (B). Means of two independent experiments are shown. Data are expressed as mean ± S.E.M. Statistical significance was calculated by Mann-Whitney U test. *p<0.05 vs. *db/db*/hPS



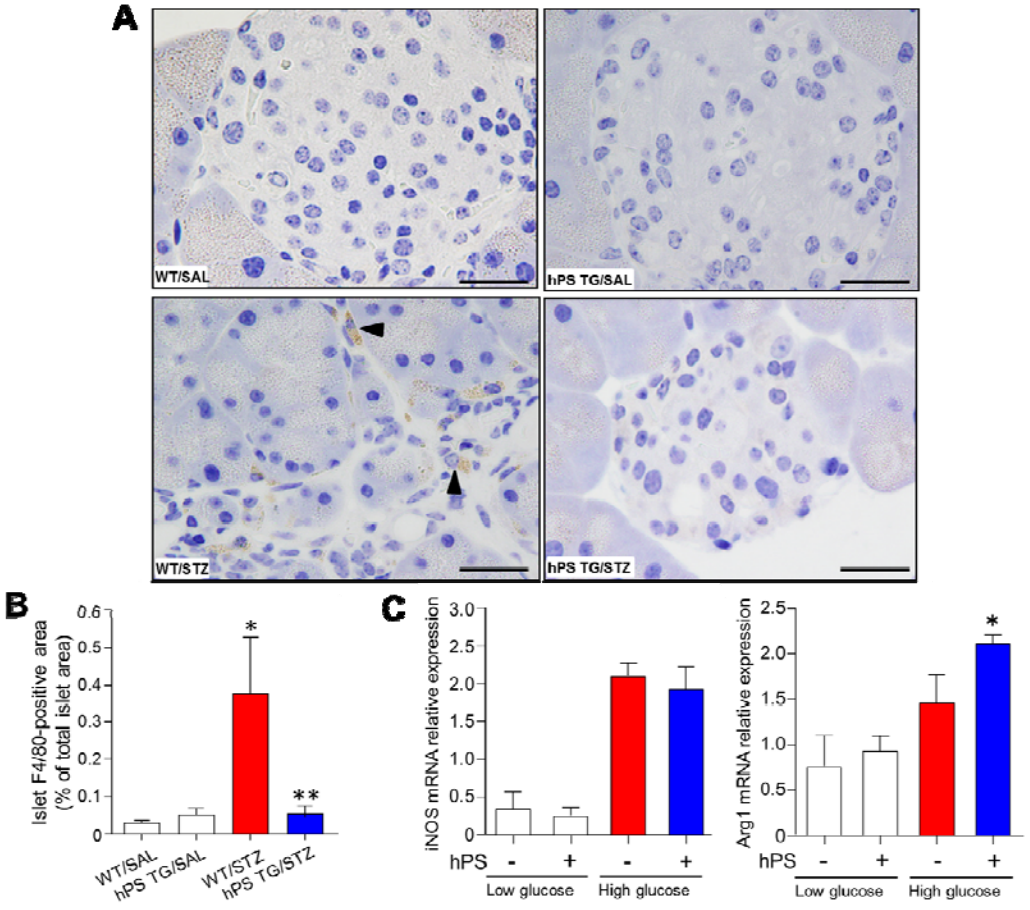
SUPPLEMENTARY DATA

**Supplementary Figure 2. Increased area of pancreatic islets in hPS-TG mice.** (A) Mice were sacrificed on day 28 after STZ or saline intraperitoneal injection. The pancreas was incised, removed and prepared for staining with H&E (WT/SAL n=3; hPS/SAL n=5; WT/STZ n=6; hPS/STZ n=6). (B) Pancreatic islet area was quantified using image software (WinROOF); the mean values of the WT/SAL group were defined as 100%. Data are expressed as mean ± S.E.M. The figure shows a representative section from one of three independent experiments. Scale bars indicate 20 μm. Arrows indicate pancreas islets. WT, wild type; STZ, streptozotocin; hPS, human protein S; SAL, saline. *p<0.05 vs WT/SAL and hPS/SAL groups; **p<0.05 vs WT/STZ group. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.



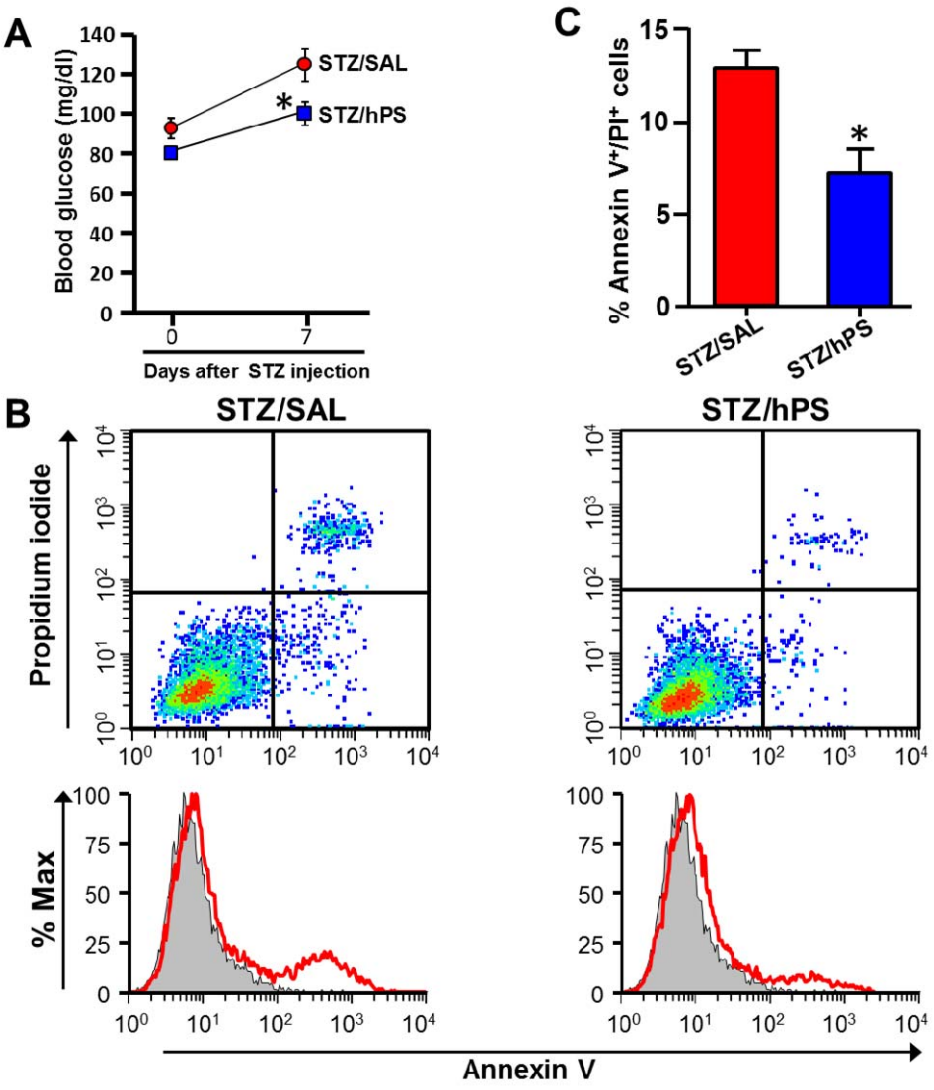
SUPPLEMENTARY DATA

**Supplementary Figure 3. hPS decreases infiltration of macrophages and promotes M2 differentiation.** (A) Mice were sacrificed on day 28 after streptozotocin (STZ) or saline intraperitoneal injection. The pancreas was removed and immunostaining of F4/80 was performed (WT/SAL n=3; hPS/SAL n=5; WT/STZ n=6; hPS/STZ n=6). (B) Positively stained area was quantified using image software (WinROOF). Data are expressed as mean ± S.E.M. The figure shows a representative section from one of three independent experiments. Scale bars indicate 25 μm. Head arrows indicate positively stained macrophages. WT, wild type; hPS, human protein S; SAL, saline. *p<0.05 vs WT/SAL; **p<0.05 vs WT/STZ. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test. (C) RAW264.7 cells were cultured in 12-well microplates in the presence or absence of hPS (20 μg/ml) for 30 min and then high glucose concentration (final concentration, 25 mM) was added to medium the cells and the culture was continued for 24h. Expression of the M1 marker inducible nitric oxide synthase (iNOS) and the M2 marker arginase1 (Arg1) were analyzed by RT-PCR. The figure shows representative result from one of two independent experiments. N=3 mice per group. *p<0.05vs hPS (-), high glucose group. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.



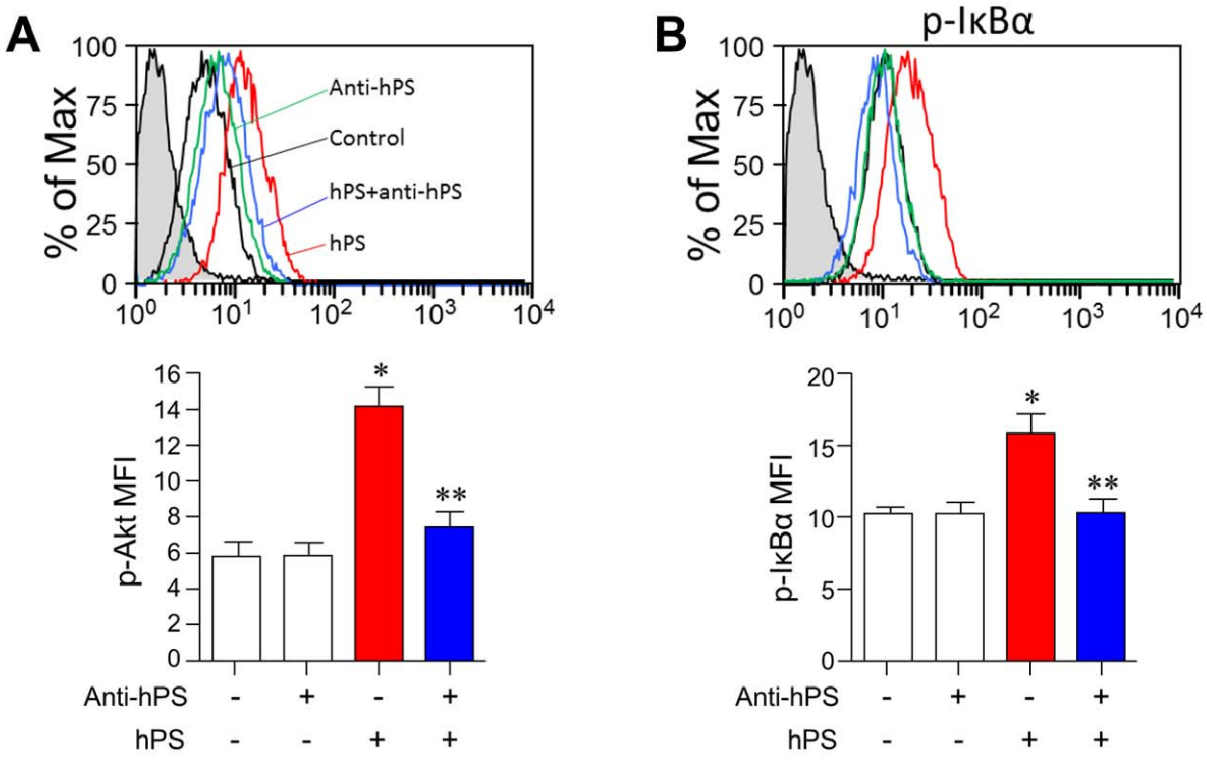
SUPPLEMENTARY DATA

**Supplementary Figure 4. hPS inhibits apoptosis of pancreatic islet β cells *in vivo*.** C57BL/6 WT mice received intraperitoneal injection of streptozotocin (STZ) for 5 days and treated with exogenous hPS (2mg/kg) or saline subcutaneously 1h before each STZ injection and continued for 9 additional days after the last STZ injection. (A) Blood glucose performed on day 7 after STZ and hPS treatment. (B) Mice of each group were then euthanized, islet β cells were isolated and apoptotic cells were assessed by flow cytometry after staining with Annexin-fluorescein isothiocyanate and propidium iodide. (C) Percentage of apoptotic cells in each group. Data are expressed as mean ± S.D. Statistical analysis was done using Mann Whitney U test. *p<0.05 vs STZ/SAL group.



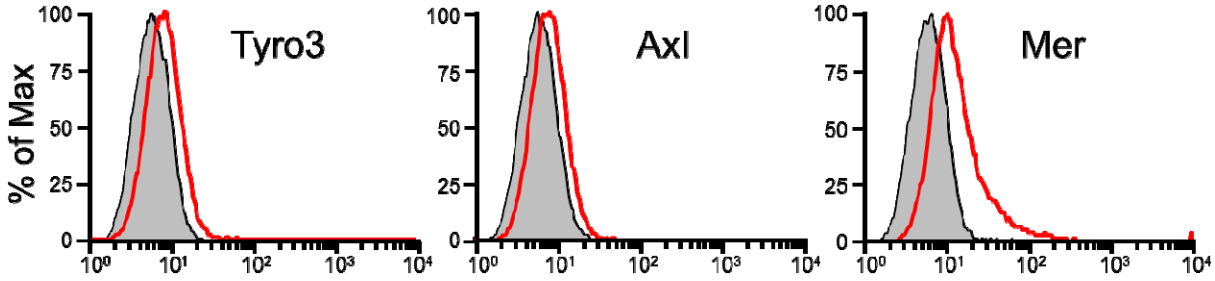
SUPPLEMENTARY DATA

**Supplementary Figure 5. hPS increases phosphrylation of Akt/PKB and iκB in primary islet β cells.** Primary pancreatic islets isolated as described in the method section, and then treated with 20 μg/ml hPS in the presence or absence of 100 μg/ml anti-hPS antibody for 60 min. Islets were dissociated with trypsin/EDTA into a single cell suspension and fixed with 4% paraformaldehyde. After permeabilization with 90 % ice-cold methanol, cells were stained with anti-phospho-Akt (A; Ser473) or anti-phospho-IκBα (B), followed by FITC-conjugated goat anti-rabbit IgG. Black line histogram represents the isotype control (normal rabbit IgG). N=3 mice per group. hPS, human protein S; hPS. *p<0.05 vs anti-hPS(-) hPS(-) group; **p<0.05 vs vs anti-hPS(-) hPS(+) group. Data are expressed as mean ± S.D. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.



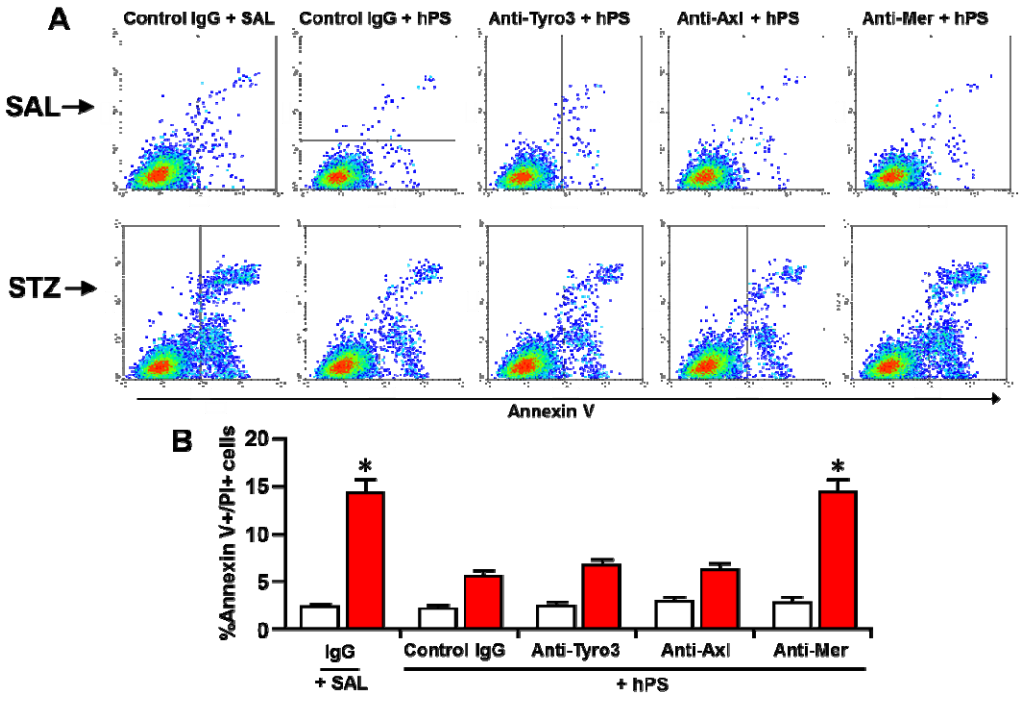
SUPPLEMENTARY DATA

**Supplementary Figure 6. MIN6 cells express the three TAM receptors.** The murine pancreatic β cell line MIN6 was cultured and the surface expression of Tyro3, Axl and Mer receptors was evaluated by flow cytometry using mouse-specific antibodies for the three receptors (red lines). Isotype antibody in grey was used as control.



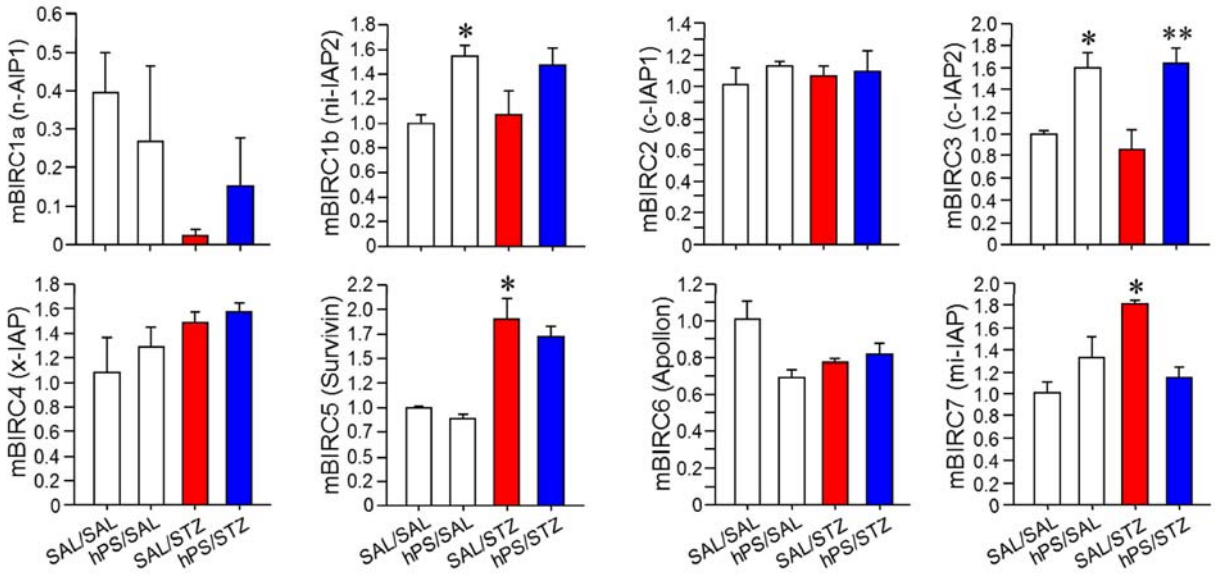
SUPPLEMENTARY DATA

**Supplementary Figure 7. hPS protects β-cells against streptozotocin–induced apoptosis via Mer receptor.** (A) MIN6 cells were pretreated with 20 μg/ml of anti-Tyro3, anti-Axl, anti-Mer or isotype IgG for 30 min, before adding 20 μg/ml hPS. The cells were then treated with 2 mM streptozotocin or saline, cultured for 24h and the number of apoptotic cells was evaluated by flow cytometry after Annexin V-FITC/propidium iodide (PI) double staining. (B) The percentage of apoptotic cells was measured (STZ, solid red bars) or saline (SAL, open white bars). Each bar represents the mean ± S.D. of three independent experiments. *p<0.05 vs. control (IgG+SAL).



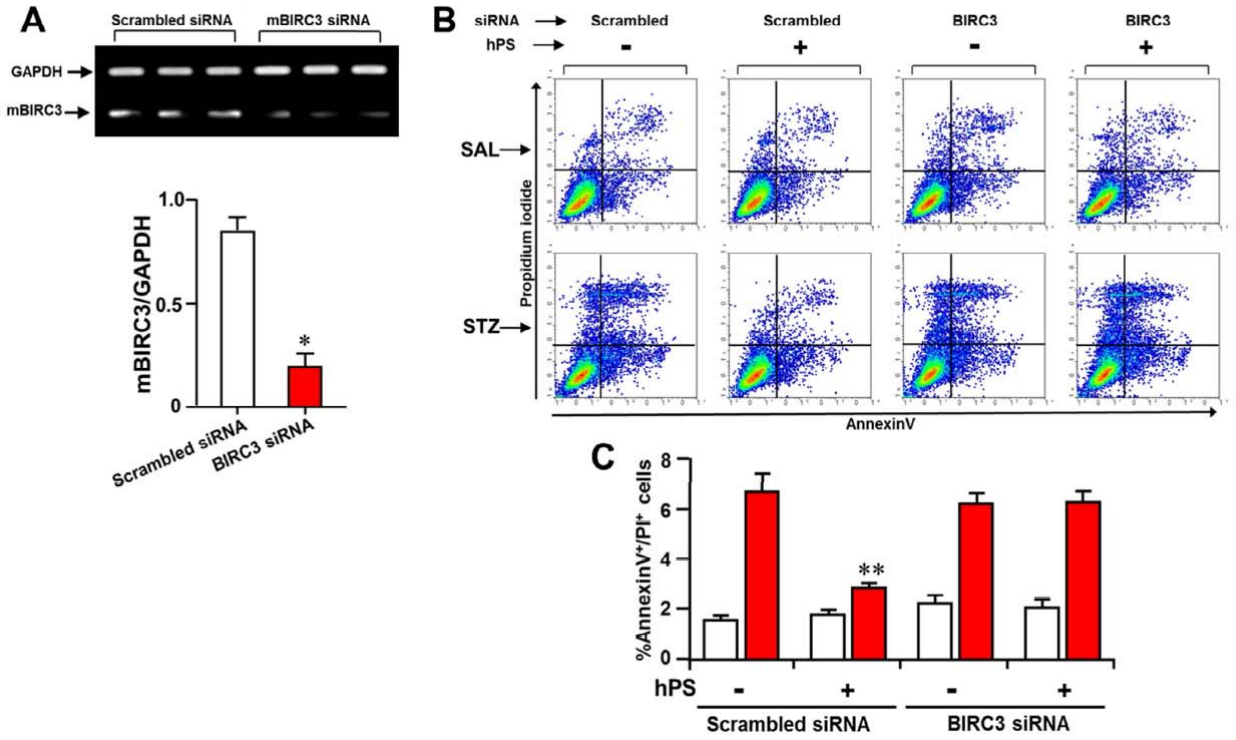
SUPPLEMENTARY DATA

**Supplementary Figure 8. hPS increases the expression of some inhibitors of apoptosis (IAP).** The murine pancreatic β cell line MIN6 was cultured and stimulated as described in the Methods section, RNA isolated and quantitative RT-PCR was performed. Data are expressed as mean ± S.E.M. The figure shows representative result from one of two independent experiments. N=3 mice per group. WT, wild type; STZ, streptozotocin; hPS, human protein S; hPS TG, hPS transgenic mice; SAL, saline. *p<0.05vs SAL/SAL; **p<0.05 vs SAL/STZ. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.



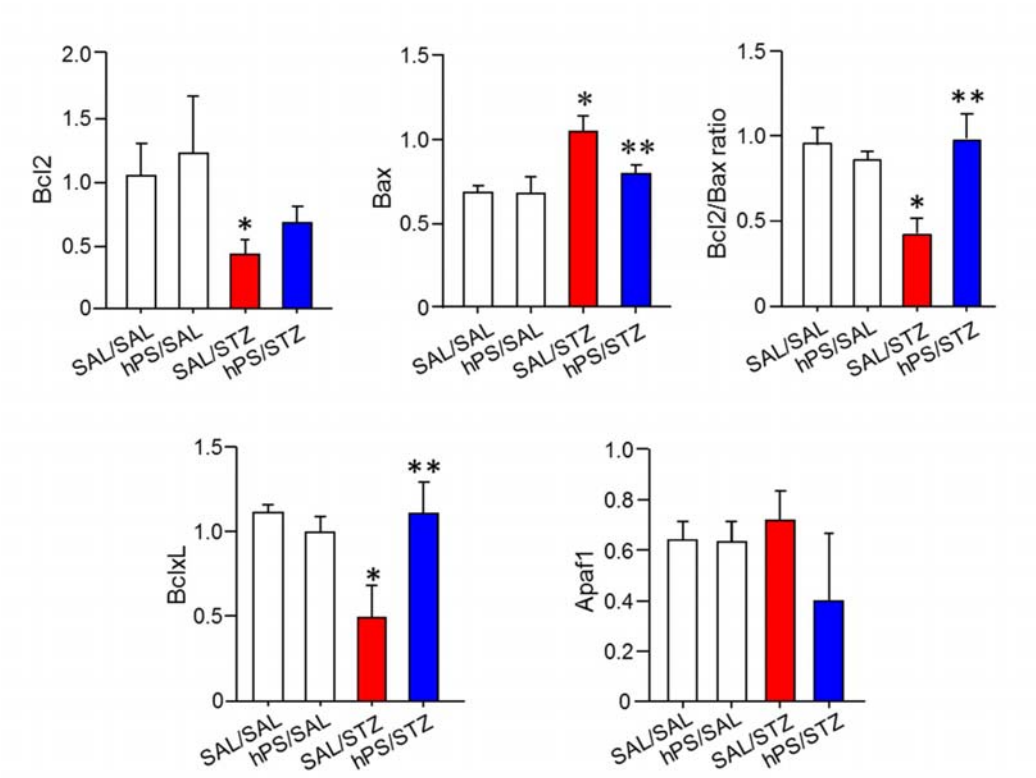
SUPPLEMENTARY DATA

**Supplementary Figure 9. BIRC3 mediates the anti-apoptotic activity of hPS in Min6 β-cell lines.** Min6 cells were transfected with 33 nmol of Birc3 siRNA or scrambled siRNA, cultured in the presence or absence of hPS for 30 min and then treated with or without streptozotocin for 24h. (A) Knock down of mBIRC3 by specific siRNA. (B) Percent of apoptotic cells was assessed by flow cytometry. (C) The percentage of apoptotic cells was measured and compared among groups. Each bar represents the mean ± S.D. of three independent experiments. *p<0.05 vs. scrambled siRNA. **p<0.05 vs hPS (-) /scrambled siRNA



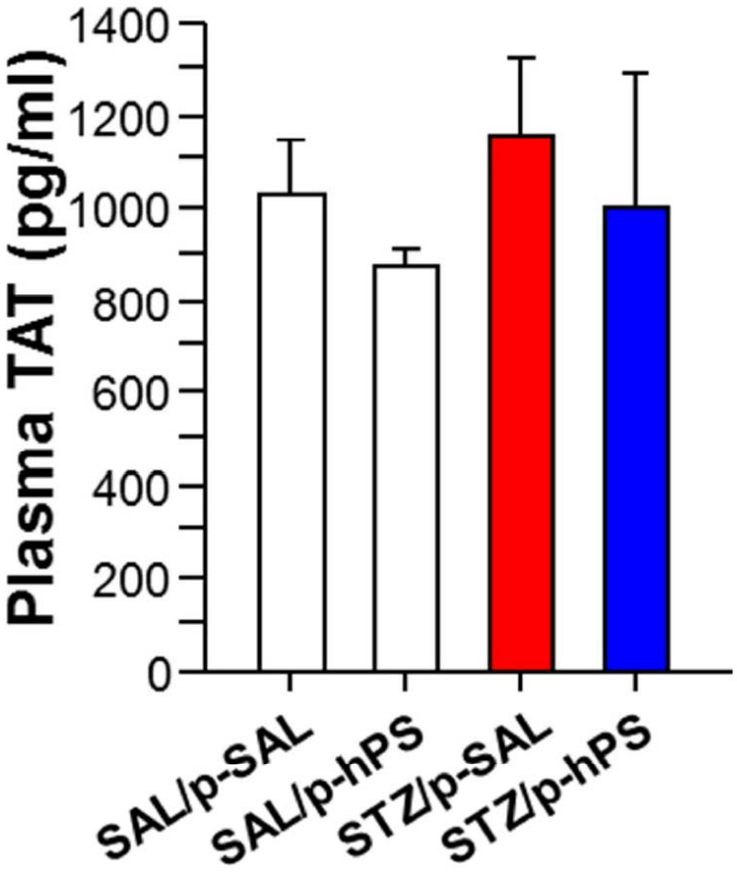
SUPPLEMENTARY DATA

**Supplementary Figure 10. Effect of hPS on the expression of Bcl-2 family proteins and apoptotic protease activating factor 1 (APAF1).** The murine pancreatic β cell line MIN6 was cultured and stimulated as described in the method section and quantitative RT-PCR was performed. Data are expressed as mean ± S.E.M. The figure shows representative results from one of two independent experiments. N=3 mice per group. WT, wild type; STZ, streptozotocin; hPS, human protein S; hPS TG, hPS transgenic mice; SAL, saline. *p<0.05vs SAL/SAL; **p<0.05 vs SAL/STZ. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.



SUPPLEMENTARY DATA

**Supplementary Figure 11. The coagulation system was not affected by exogenous hPS administration in mice with diabetes.** Mice received intraperitoneal injections of STZ (40 mg/kg body weight) after recovery from unilateral nephrectomy and treated with hPS by implanted s.c. pump from the 4th week after STZ injection. Blood was sampled during sacrifice, plasma was separated and thrombin-antithrombin complex (TAT) was measured by enzyme immunoassays. Data are expressed as mean ± S.E.M. The figure shows representative results from one of two independent experiments. N=5 mice per group. WT, wild type; STZ, streptozotocin; p-hPS, human protein S administered by pump; p-SAL, saline administered by pump. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.



SUPPLEMENTARY DATA

**Supplementary Figure 12. Circulating levels of tissue factor (TF), plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) are not significantly affected by hPS overexpression.** Diabetes was induced in WT and hPS-TG mice by intraperitoneal injection of streptozotocin as described under materials and methods and blood samples were drawn after euthanasia. Data are expressed as mean ± S.E.M. The figure shows representative results from one of two independent experiments. N=4 mice per group. WT, wild type; STZ, streptozotocin; hPS, human protein S; SAL, saline. Statistical analysis was done using ANOVA with post hoc analysis by Tukey's test.