Supplemental Experimental Procedures

Collagenase-purified islet secretome

Mice were sacrificed, and the abdomen was opened. The bile duct was clamped upstream of Oddi's sphincter, and 3 ml of collagenase P (1 mg/ml) (Roche) was injected through the superior bile duct into the pancreas. The inflated pancreas was removed and placed into a 50-ml conical tube on ice, then for 17 minutes in 37^{o}C water bath. Then, 20 ml of ice-cold M199 (Invitrogen) supplemented supplemented with 10% newborn calf serum (NBCS) were added. The tube was shaken manually for 10 seconds to break up the tissue. The following procedures were carried out on ice or in a 4^{o}C centrifuge. The sample was pelleted at 1,100 rpm for 2 min, re-suspended, and washed three times with 15 ml of M199 +10% NBCS. The sample was filtered though a sterile 400μm wire mesh disc, and spun again. The pellet was re-suspended in 5 ml of Histopaque (Histopaque-1077, Sigma), overlaid with 10 ml of M199, and centrifuged at 2,800 rpm for 20 min. Thereafter, islets were collected, washed three times with 30 ml of M199 +10% NBCS followed by centrifugation at 1,100 rpm for 2 min, and further washed at least three times followed by gravity sedimentation. After the last sedimentation, islets were re-suspended in RPMI (1640, Invitrogen) supplemented with 15% NBCS, seeded on a petri dish, handpicked onto another plate with fresh RPMI+ 15% NBCS, followed by overnight recovery in a 37^{o}C cell culture incubator.

The next day, size-matched islets (~200 micron) from WT and TKO mice were handpicked, placed into microfuge tubes, washed twice with 1 ml PBS, centrifuged at 4^{o}C, 1,000 rpm for 1 min, and washed once with 1 ml of freshly made and filtered Kreb's buffer pH 7.4 (118.5 mM NaCl, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, 10 mM HEPES, 2% BSA, and 2.54 mM $CaCl_2$ added last) supplemented with 2.8mM glucose. Islets were allowed to recover in 1-ml Kreb's buffer+ 2.8mM glucose for 1 h at 37^{o}C, and incubated in 100 μl of fresh Kreb's buffer+ 2.8mM glucose for 1 h at 37^{o}C. This 100-μl sample was collected to determine pre-challenge protein patterns. Islets were incubated in 100 μl of Kreb's buffer+ 16.8 mM glucose+ 10 mM L-arginine for 30 min at 37^{o}C. This volume was collected to determine the post-challenge protein pattern. Insulin content of both sets of samples was determined by ELISA. Islets

were washed three times with PBS, lysed in RIPA buffer, and subjected to total protein (Pierce #23225) and insulin measurement. These ex vivo secretome samples were subjected to BSA/IgG depletion, in-gel trypsin digestion and extraction, nanoLC-MS/MS, and MaxQuant-based label free quantification and protein identification.

Sample processing for mass spectrometry (MS) analysis

I. *In vivo* secretome. For the 12-week-old group, we used WT mice (n=2) and TKO mice (n=2), with two separate LC-MS/MS and data analysis. For 30-week-old group, we used WT mice (n=3) and TKO mice (n=3), with three separate LC-MS/MS and data analysis.

I-a. Affinity depletion of highly abundant proteins. 20-µl plasma were subjected to albumin and IgG were removed using ProteoPrep Immunoaffinity Albumin and IgG depletion kit (Sigma #PROTIA).

I-b. Protein reduction and digestion. 50 µg of each sample (in 100 mM TEAB+ 150 mM NaCl+ 0.1% SDS) was reduced with 5 mM tris-(2-carboxylethyl) phosphine at 60ºC for 1 h, and cysteine residues were blocked with 8 mM methyl methanethiosulfonate at room temperature for 10 min. Then, sample was digested with 5 µg of sequencing-grade-modified trypsin at 37 ºC for 16 h.

I-c. iTRAQ labeling. Digested and reduced peptides were labeled with iTRAQ (**i**sobaric **T**ag for **R**elative and **A**bsolute **Q**uantification, AB Sciex) reagent following manufacturer's instructions (1). After labeling with iTRAQ reagent, 1µl-aliquot from each sample was pooled and desalted with SCX ZipTip (Millipore), and analyzed by 4000 Q Trap (AB Sciex) for iTRAQ incorporation. After validation, the four samples were pooled, dried, and reconstituted in 3 ml of loading buffer (10 mM potassium phosphate at pH 3.0+ 25% acetonitrile). The pH was adjusted to 3.0 with formic acid, prior to cation exchange chromatography with PolyLC SCX (**S**trong **C**ation E**x**change) cartridge (10 mm ID x 14 mm, PolyLC Inc.). After washing the SCX cartridge with loading buffer, the 4plex sample was loaded and then washed

with 2 ml of loading buffer. The peptides were eluted with 1-ml loading buffer supplemented with 500 mM KCl. Desalting of SCX fractions was performed with solid phase extraction (SPE) on Sep-Pak cartridges (Waters).

I-d. High pH reverse phase fractionation

A Dionex Ultimate 3000 standard HPLC system (Thermo Scientific), with built-in micro fraction collection option in its autosampler and UV detection, was used to perform high pH reverse phase fractionation (2). The 4plex sample was reconstituted in Buffer A (20 mM ammonium formate at pH 9.5 in H_2O) and loaded onto a hybrid silica column XTerra MS C18 (2.1 mm × 150 mm, Waters). A gradient of Buffer B (20 mM ammonium formate at pH 9.5 with 90% acetonitrile in H_2O) was applied. The high pH RP chromatography separation was carried at a flow rate of 200 µl/min using 5% Buffer B for 3 min, 5-45% Buffer B in 30 min, and up to 90% Buffer B in 5 min. During gradient elution, 48 fractions were collected at 1 min per fraction with UV trace at 214 nm, and pooled into 8 fractions based on the UV absorbance at 214 nm along with multiple fraction concatenation strategy (3). These fractions were dried and reconstituted in 100 µl of 2% acetonitrile and 0.5% FA for nanoLC-MS/MS analysis.

I-e. Nano-scale reverse phase chromatography and tandem mass spectrometry (nanoLC-MS/MS)

This analysis was performed with Orbitrap Elite mass spectrometer (Thermo Scientific) equipped with nano ion source using high-energy collision dissociation (HCD). The Orbitrap is equipped with CorConneX nano ion source (CorSolutions LLC) coupled with UltiMate 3000 RSLCnano (Thermo Scientific). 5µl of sample was injected onto a pepMap100 C18 RP nano trap column (5 µm, 100 µm x 20mm, Thermo Scientific) with nano Viper fittings at 20 µl/min flow rate, separated on a PepMap C18 RP nano column (3 µm, 75 µm x 15 cm), and eluted in a 120-min gradient of 5% to 38% acetonitrile in 0.1% formic acid at 300 nl/min, followed by a 5-min ramping to 95% acetonitrile-0.1% FA and a 5-min hold at 95% acetonitrile-0.1% FA. The column was re-equilibrated with 2% acetonitrile-0.1% FA for 20 min prior to the next run. The eluted peptides were detected by Orbitrap through "Plug and Play" nano

ion source with a 10-µm analyte emitter (New Objective). Three repeat injections on nanoLC-MS/MS analysis for each set of samples were performed.

The Orbitrap Elite is operated in positive ion mode with nano spray voltage set at 1.6 kV and source temperature at 250 °C. The instrument was operated in data-dependent acquisition (DDA) mode using FT mass analyzer for one survey MS scan for selecting top 15 precursor ions, followed by data-dependent HCD-MS/MS scans on the precursor peptides with multiple charged ions above a threshold ion count of 8000 with normalized collision energy of 37.5%. MS survey scans at a resolution of 60,000 (FWHM at m/z 400) for the mass range of m/z 375-1600, and MS/MS scans at 15,000 resolution for the mass range of m/z 100-2000. Dynamic exclusion parameters were set at repeat count 1 with a 30 s repeat duration, an exclusion list size of 500, 60s exclusion duration with ±10 ppm exclusion mass width. The activation time was 0.1 ms for HCD analysis. All data were acquired from Xcalibur 2.2 operation software (Thermo).

I-f. Protein identification and data analysis

All MS and MS/MS raw spectra were processed using Proteome Discoverer 1.4 (PD1.4, Thermo Scientific), and the spectra from each DDA file were plotted as an MGF file for subsequent dataset search using licensed Mascot Daemon (version 2.3.02, Matrix Science). The mouse RefSeq sequence database (77,949 sequence entries) was downloaded on August 2, 2014 from NCBI nr database. The search settings used for 4plex iTRAQ quantitative processing and protein identification in Mascot server were the following: two mis-cleavage for full trypsin with fixed MMTS modification of cysteine, fixed 4plex iTRAQ modifications on lysine and N-terminal amines and variable modifications of methionine oxidation, and 4-plex iTRAQ on tyrosine. The peptide mass tolerance and fragment mass tolerance values were 10 ppm and 50 mDa, respectively. To estimate the false discovery rate (FDR) for a measure of identification certainty in each replicate set, an automatic decoy database search was performed in which a random sequence of the database is generated and tested for raw spectra along with the real database. To reduce the probability of false peptide identification, we set a 95% confidence interval for peptides

defined by a Mascot probability analysis greater than "identity", along with ~0.5% FDR, and $p < 0.05$ (expectation value) as filter. Intensities of the reporter ions from iTRAQ tags upon fragmentation were used for quantification, and the relative quantitation ratios were normalized at median ratio for the 4plex. The comparison between groups was carried in Excel. Raw data are available upon request.

II. *ex vivo* secretome. Experiment used WT mice (n=2) and TKO mice (n=2), with two separate LC-MS/MS and data analysis.

II-a. Affinity depletion of highly abundant proteins. 100-µl sample was subjected to Amicon centrifugal filter unit (Ultra-15, MWCO 10 kDa) for buffer exchange to 25 mM Tris at pH 7.4, followed by ProteoPrep Immunoaffinity Albumin and IgG depletion kit. Then, the sample buffer was exchanged to 100 mM TEAB+ 150 mM NaCl+ 0.1% SDS. Protein concentration was determined by Bradford assay, and confirmed by SDS-PAGE (Novex 12% precast Tris/Glycine minigel, Invitrogen) with a series of E. coli lysates (2, 5, 10, 20 µg/lane) as standards. SDS-PAGE gel was stained with Colloidal blue (Invitrogen), imaged with Typhoon 9400 scanner, and analyzed with ImageQuant software version TL 8.1 (GE Healthcare).

II-b. In-gel trypsin digestion. Protein bands from an SDS-PAGE gel were cut into ~1mm cubes, and subjected to in gel digestion, followed by tryptic peptide extraction (4). We excluded the BSA gel slice (55 kDa region) to avoid interference in subsequent analysis. We also discarded proteins > 110kDa, as they are less likely to be secreted (5). The rest of the excised gel pieces were washed consecutively in 200 µl distilled water, 100 mM ammonium bicarbonate (Ambic)/ACN (1:1), and ACN. Then, they were reduced with 70 µl of 10 mM DTT in 100 mM Ambic for 1 h at 56°C, alkylated with 100 µl of 55 mM Iodoacetamide in 100 mM Ambic at room temperature in the dark for 60 min. These gel slices were dried and rehydrated with 50 µl trypsin in 50 mM Ambic, 10% ACN (20 ng/µl) at 37°C for 16 h. The digested

peptides were extracted twice with 70 µl of 50% ACN and 5% FA, then once with 70 µl of 90% ACN and 5% FA. Extracts from each sample were combined and lyophilized.

II-c. High pH reverse phase fractionation. As described in I-d above.

II-d. Nano-scale reverse phase chromatography and tandem mass spectrometry (nanoLC-MS/MS). As described in I-e above, with the following modifications. The instrument was operated in parallel with data-dependent acquisition (DDA) under FT-IT mode using FT mass analyzer for one MS survey scan from m/z 375 to 1800 with a resolution of 120,000 (FWHM at m/z 400), followed by MS/MS scans for top 15 most intensive peaks with multiple charged ions above a threshold ion count of 10,000 in LTQ mass analyzer. The LTQ parameters were set at the following values: isolation width 2.0 m/z, normalized collision energy 35%, activation Q at 0.25, and activation time 10 ms.

II-e. Protein identification and data analysis. The MS and MS/MS raw files were analyzed using MaxQuant version 1.5.1.2 (6,7). Proteins were identified by searching against the mouse RefSeq sequence database containing 77,949 sequence entries, which was downloaded on August 2, 2014 from NCBI nr. The minimal peptide length was set to be 7 amino acids. The initial peptide mass tolerance in MS mode was set to 10 ppm. The MS/MS tolerance was set to 0.5 Da. The FDR for identification was set to < 1.0%. Up to two missing cleavage points were allowed. Fixed modifications were for Carbamidomethyl (C), variable modifications were Oxidation M and deamidation of N/Q. Protein quantitation was performed using unique peptides and razor peptides. The LFQ intensity for each identified protein in each sample was used for determining the relative quantitation of the identified proteins among the different samples. Raw data are available upon request.

Supplemental References

1. Ross, P. L., Huang, Y. N., Marchese, J. N., Williamson, B., Parker, K., Hattan, S., Khainovski, N., Pillai, S., Dey, S., Daniels, S., Purkayastha, S., Juhasz, P., Martin, S., Bartlet-Jones, M., He, F., Jacobson, A., and Pappin, D. J. (2004) Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Molecular & cellular proteomics : MCP* **3**, 1154-1169

2. Yang, Y., Qiang, X., Owsiany, K., Zhang, S., Thannhauser, T. W., and Li, L. (2011) Evaluation of different multidimensional LC-MS/MS pipelines for isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomic analysis of potato tubers in response to cold storage. *Journal of proteome research* **10**, 4647-4660

3. Wang, Y., Yang, F., Gritsenko, M. A., Wang, Y., Clauss, T., Liu, T., Shen, Y., Monroe, M. E., Lopez-Ferrer, D., Reno, T., Moore, R. J., Klemke, R. L., Camp, D. G., 2nd, and Smith, R. D. (2011) Reversed-phase chromatography with multiple fraction concatenation strategy for proteome profiling of human MCF10A cells. *Proteomics* **11**, 2019-2026

4. Yang, Y., Thannhauser, T. W., Li, L., and Zhang, S. (2007) Development of an integrated approach for evaluation of 2-D gel image analysis: impact of multiple proteins in single spots on comparative proteomics in conventional 2-D gel/MALDI workflow. *Electrophoresis* **28**, 2080-2094

5. Grimmond, S. M., Miranda, K. C., Yuan, Z., Davis, M. J., Hume, D. A., Yagi, K., Tominaga, N., Bono, H., Hayashizaki, Y., Okazaki, Y., Teasdale, R. D., Group, R. G., and Members, G. S. L. (2003) The mouse secretome: functional classification of the proteins secreted into the extracellular environment. *Genome research* **13**, 1350-1359

6. Cox, J., and Mann, M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature biotechnology* **26**, 1367-1372

7. Luber, C. A., Cox, J., Lauterbach, H., Fancke, B., Selbach, M., Tschopp, J., Akira, S., Wiegand, M., Hochrein, H., O'Keeffe, M., and Mann, M. (2010) Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* **32**, 279-289