SUPPLEMENTARY DATA

Purification of MHC peptide complexes

Cell pellets or tissues were ground in a Retsch Mixer Mill MM 400 under cryogenic conditions, resuspended in 0.5 % IGEPAL, 50 mM Tris pH 8, 150 mM NaCl and protease inhibitors (Complete Protease Inhibitor Cocktail Tablet; Roche Molecular Biochemicals) at a density of 5 x 10^7 cells/ml and incubated for 1 hr at 4 °C. Lysates were cleared by ultracentrifugation (200,000 x g) and MHC-peptide complexes immunoaffinity purified using solid phase bound monoclonal antibodies SF1.1.10 (anti-K^d) and 28.8.6s (anti-D^bK^b) as described [1]. Bound complexes were eluted with 10 % acetic acid. The mixture of peptides, class I heavy chain and β-2 microglobulin was fractionated on a 4.6 mm internal diameter x 50 mm long reversed-phase C18 HPLC column (Chromolith Speed Rod, Merck) using an ÄKTAmicro HPLC (GE Healthcare) running a mobile phase buffer A of 0.1 % trifluoroacetic acid (TFA) and buffer B of 80 % acetonitrile/0.1 % TFA. The MHC-peptide mixtures were loaded onto the column run at a flow rate of 1 ml/min with separation based on a gradient of 2 to 40 % B for 4 min, 40 to 45 % for another 4 min, and a rapid 2 min increase to 100 % B. Fractions (500 µl) were collected, vacuum concentrated to 10 µl and diluted in 0.1 % formic acid to reduce the concentration of acetonitrile. Peptide containing fractions were determined empirically by LC-MS/MS. β-2 microglobulin and class I heavy chain containing fractions were confirmed by LC-MS and western blot analysis.

Identification of MHC-bound peptides using LC-MS/MS

Concentrated peptide fractions were loaded onto a microfluidic trap column packed with ChromXP C18-CL 3 □m particles (300 Å nominal pore size; equilibrated in 0.1 % formic acid/5 % acetonitrile) at 5 µl/min using an Eksigent NanoUltra cHiPLC system. An analytical (15 cm x 75 □m ChromXP C18-CL 3) microfluidic column was then switched in line and peptides separated using linear gradient elution of 0-80 % acetonitrile over 90 min (300 nl/min). Separated peptides were analysed using an AB SCIEX 5600 TripleTOF mass spectrometer equipped with a Nanospray III ion source and accumulating up to 30 MS/MS spectra per second. The following experimental parameters were used; ion spray voltage (IS) was set at 2400 V, curtain gas at 22 L/min, ion source gas at 8 L/min and an interface heater temperature setting of 150 ºC. MS/MS switch criteria included ions of m/z >200amu, charge state +2 to +5, intensity >40 cps and the top 30 ions meeting this criteria were selected for MS/MS per cycle. The instrument was calibrated every three LC runs using glu-fibrinopeptide standard as per manufacturer's instructions. Data was analysed with ProteinPilot™ software [2] and peptide identities determined subject to strict bioinformatic criteria that included the use of a decoy database to calculate the false discovery rate (FDR). A FDR cut-off of 10 % was applied and the filtered dataset was further analysed manually to exclude redundant peptides and known contaminants. Contaminants were determined by affinity purification from tissue culture media alone together with comparison against multiple independent datasets obtained from murine and human cell lines. The following protein pilot search parameters were used; no cysteine alkylation, no enzyme digestion (considers all peptide bond cleavages), instrument specific settings for 5600 TripleTOF (MS tolerance 0.05 Da, MS/MS tolerance 0.1 Da, charge state +2-5), species *Mus musculus*, biological modification probabilistic features on, Uniprot database (version 32.0), thorough ID algorithm, detected protein threshold 0.05. Cellular localisation of source proteins was analysed using Software Tool for Researching Annotations of Proteins (STRAP) [3] and pathway analysis performed though the use of IPA (Ingenuity® Systems, www.ingenuity.com).

SUPPLEMENTARY DATA

Validation and quantitation of peptide epitopes by MRM

a) Selective validation of global LC-MS/MS analysis by MRM.

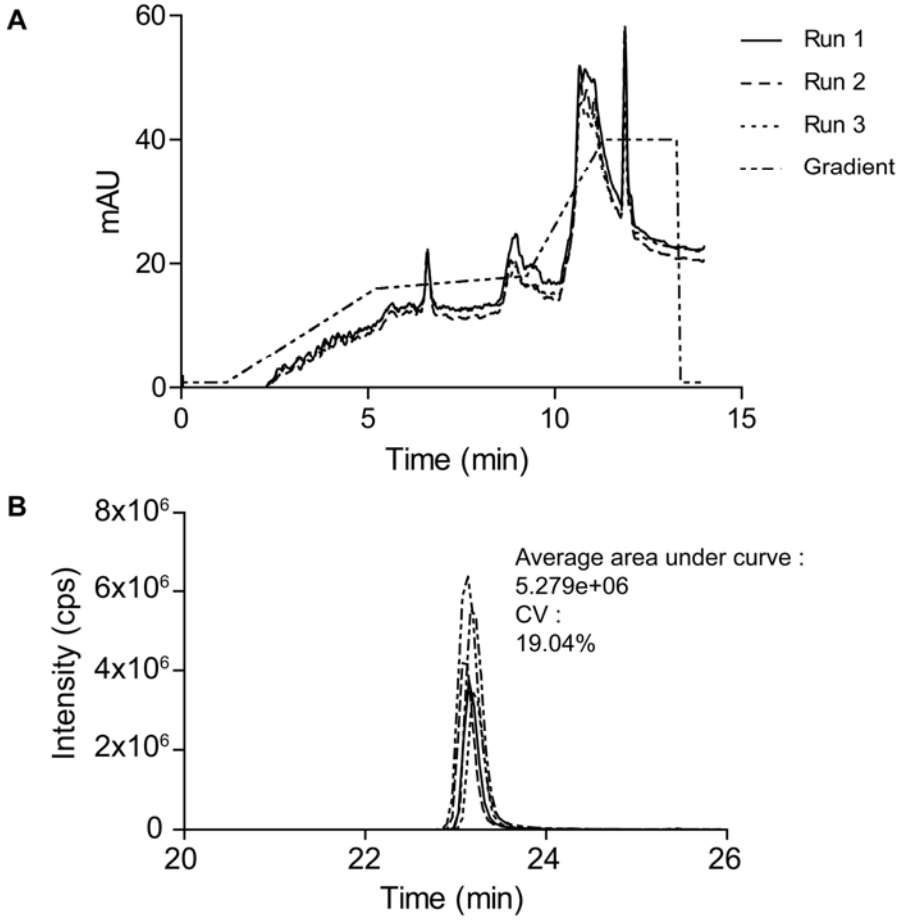
To determine the validity of the LC-MS/MS dataset from generated from the 5600 TripleTOF experiments, 97 peptides from the K^d dataset and 96 peptides from the D^b dataset were selected for MRM detection. For the D^b set, two known sub-dominant peptides from IGRP were also included ($IGRP_{225\text{-}233;}$ LRLFGIDLL and $IGRP_{241\text{-}249;}$ KWCANPDWI). MRM transitions for these peptides were performed *in silico* using the open source software, Skyline v1.1 (University of Washington, Seattle, WA). Peptide eluates were prepared from $1x10^8$ IFNγ treated NIT-1 cells in duplicate. Peptide containing fractions were concentrated by vacuum centrifugation to a volume of 20 µl before being analysed using a Tempo nanoLC-1Dplus in combination with a cHiPLC™-nanoflex system (Eksigent) coupled to a 5500 QTRAP® mass spectrometer (AB SCIEX) using experimental conditions described in supplemental methods. Two transitions with a dwell time of 10 ms were assigned per peptide and an IDA of a full mass MS/MS spectrum in EPI mode triggered by MRM transitions that exceed 200 counts. The IS voltage was set at 2200 V, curtain gas at 22 L/min, ion source gas at 25 L/min, high collision gas (CAD), and an interface heater temperature setting of 90 ºC. For the MRM transitions, the dwell time for each transition was fixed at 40 ms with the resolution of the first (Q1) and third (Q3) quadrupole operating at unit resolution. A scan rate of 1000 Da/s was used for the EPI scan with a scanning m/z range of 70 to 1000 Da, a fixed LIT fill time of 80 ms, and the Q1 resolution set to unit.

b) Quantitation of the immunodominant islet specific epitope $IGRP_{206\text{-}214}$ by MRM.

Transitions for MRM experiments were designed following inspection of experimental MS/MS data. Three transitions were designed per peptide to achieve maximum signal to noise. In general larger b or y ions were chosen to pair with unique parental masses to reduce non-specific signals. MRM transitions for each peptide were then validated through a series of experiments to investigate the reproducibility and background (Supplemental Figures 1-4). Peptide fractions were concentrated and analysed with a Tempo nanoLC-1Dplus system coupled to a 5500 QTRAP mass spectrometer with instrument settings as listed for the MRM validation experiments. The amount of JAK-$1_{355\text{-}363}$ and $IGRP_{206\text{-}214}$ peptide present in each HPLC fraction was quantitated by examining the area under each MRM transition peak relative to an internal standard AQUA peptide as described [4]. The addition of the AQUA peptide occurred immediately after immunoaffinity chromatography, since this is the first practical point for addition. This provides accurate quantitation of the peptide isolated from the immunoaffinity purified MHC complexes and allows an estimation of the number of complexes per cell [4].

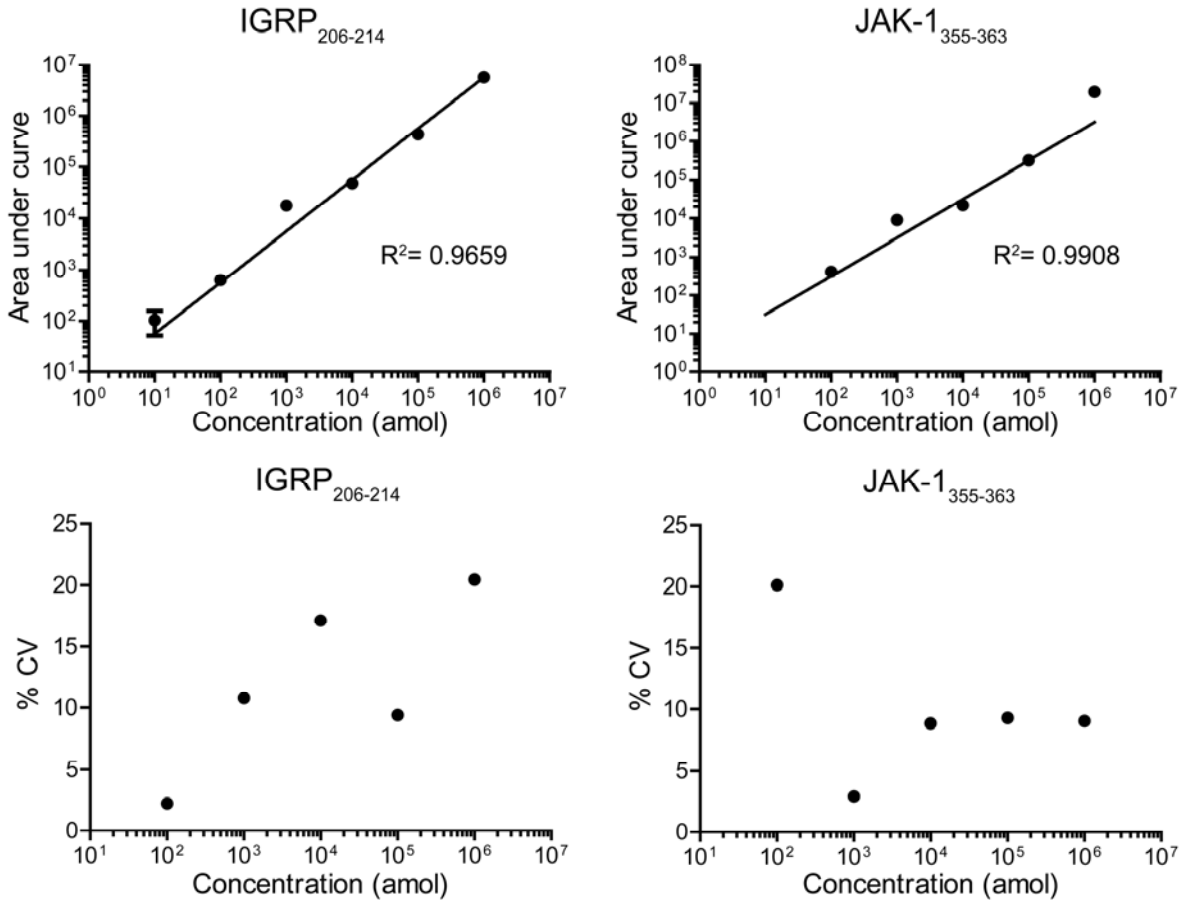
SUPPLEMENTARY DATA

Supplementary Figure 1. Determination of MRM quantitation workflow reproducibility. (*A*) Overlay of the UV trace (at 215 nm) of three separate MHC immunoaffinity sample fractionation runs. After acid elution of affinity captured MHC-peptide complexes, samples were further separated by RP-HPLC on a AKTA Ettan micropreparative HPLC system and peptides separated using a C18 Chromolith Speed Rod column running on a mobile phase buffer A of 0.1 % trifluoroacetic acid (TFA) and buffer B of 80 % acetonitrile/0.1 % TFA. The MHC-peptide mixtures were loaded onto the column at a flow rate of 1 ml/min with separation based on a gradient of 2 to 40 % B for 4 min, 40 to 45 % for another 4 min, and a rapid 2 minute increase to 100 % B. Fractions (500 µl) were collected, diluted 10-fold in 0.1 % formic acid to reduce the concentration of acetonitrile. (*B*) Comparison of MRM spectrum of JAK-$1_{355\text{-}363}$ AQUA peptide from five separate MHC immunoaffinity sample preparations that were spiked with AQUA peptide prior to step (*A*) above.



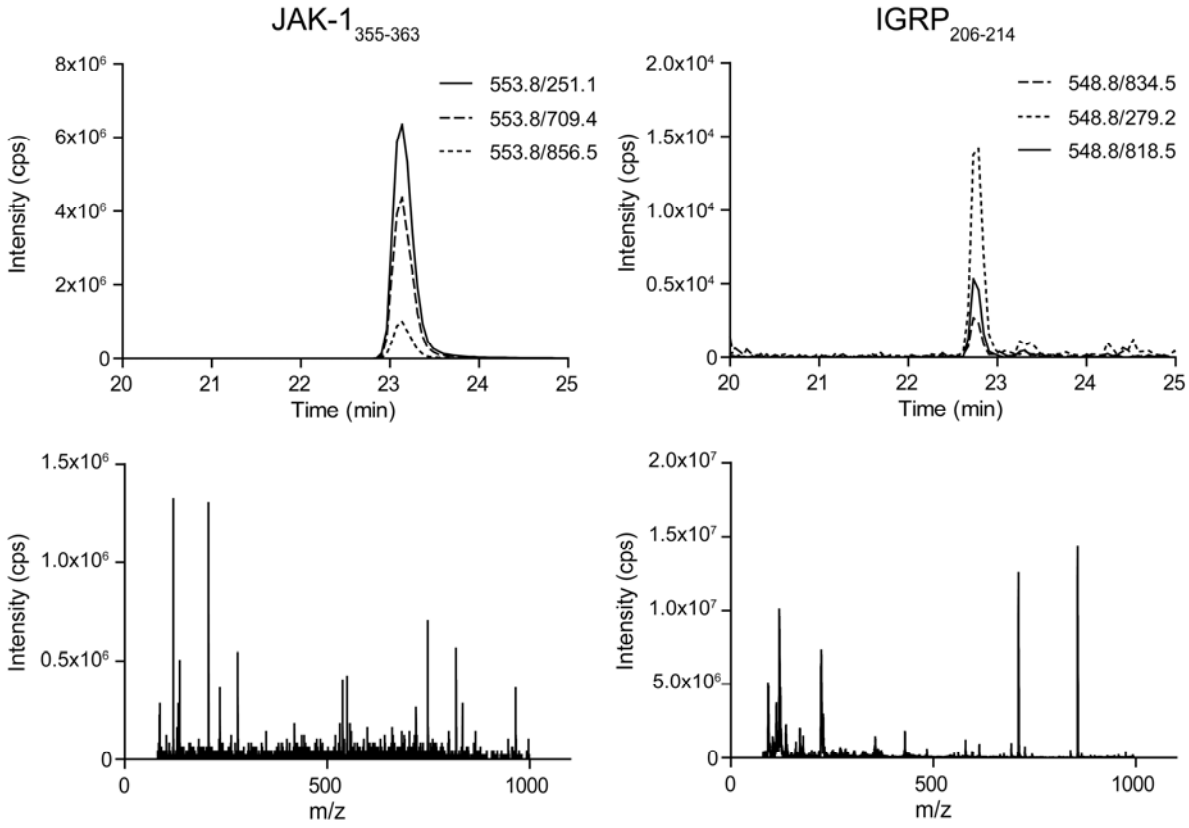
SUPPLEMENTARY DATA

Supplementary Figure 2. Standard curve and plot of coefficient of variation (CV) of AQUA peptide titration. Varying concentrations of $IGRP_{206-214}$ and $JAK\text{-}1_{355-363}$ AQUA peptide was loaded onto the AKTA Ettan HPLC system (GE) and separated into fractions via a C18 Chromolith Speed Rod (Merck). The fractions were then concentrated via vacuum centrifugation and the peptides were quantitated using a 5500 Q-Trap hybrid triple quadrupole-linear ion trap (LIT) mass spectrometer (AB SCIEX) operating in MRM positive ion scanning mode. Area under curve of each peptide peak was calculated using Multiquant 2.0 analysis software (AB SCIEX).



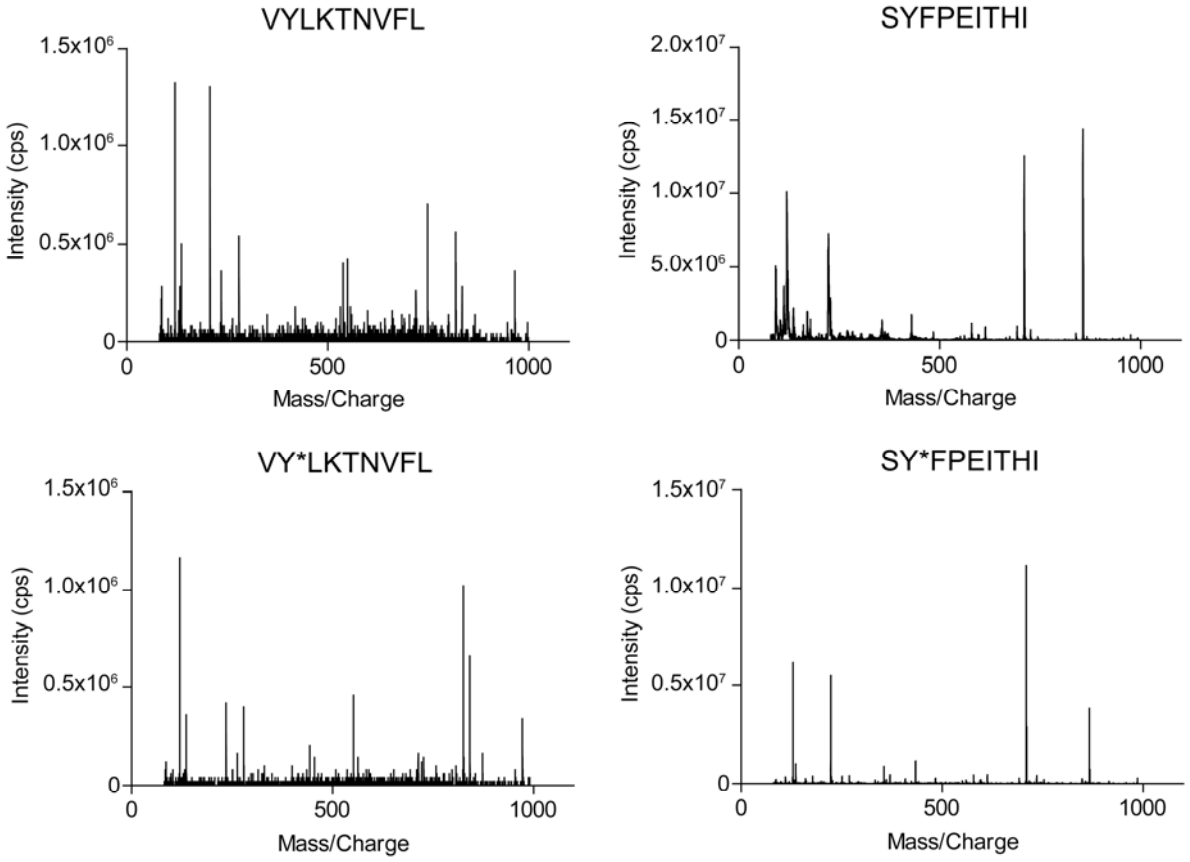
SUPPLEMENTARY DATA

Supplementary Figure 3. MRM chromatograms for quantitative studies. Representative extracted ion chromatograms (XIC, top panels) and product ions chromatograms (ms/ms, bottom panels) for $IGRP_{206-214}$ and $JAK\text{-}1_{355-363}$ presented by NIT-1 β cells. Peptides were targeted by MRM scanning acquisition and ions with intensity exceeding 500 counts per second (cps) were further subjected to a product ion scan to confirm the identity of the peptides via its fragmentation pattern



SUPPLEMENTARY DATA

Supplementary Figure 4. Product ion scan evidence of $IGRP_{206-214}$ and $JAK\text{-}1_{355-363}$ AQUA peptide and $IGRP_{206-214}$ and $JAK\text{-}1_{355-363}$ detected isolated from NIT-1 β cells. NIT-1 cells ($5x10^7$) were treated with IFNγ for 0-72 hrs and K^d peptide complexes immunoaffinity purified at each time point. Samples were spiked with AQUA peptides prior to HPLC separation and fractions subjected to LC-MRM analysis. The MRM acquisition method was set up to trigger a product ion scan if the targeted MRM ion exceeds 500 counts per second (cps).



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