Supplementary Figure 1

### Tandem HIPS 1

| InsC - ChgA | InsC - Amylin | InsC - IAPP2 | InsC - IAPP1 |
|-------------|---------------|--------------|-------------|
| GDLQTL     | GDLQTLWSRM    | GDLQTLKCNTA  | GDLQTLNAARD | GDLQTLTPVRS |
| DLQTLA     | DLQTLAWSRM    | DLQTLAKCNTA  | DLQTLNAARD  | DLQTLTPVRS  |
| LQTLAL     | LQTLALWSRM    | LQTLALKCNTA  | LQTLALNAARD | LQTLALTPVRS |
| QTLALE     | QTLALEWSRM    | QTLALEKCNTA  | QTLALENAARD | QTLALETPVRS |
| TLALEV     | TLALEVWSRM    | TLALEVKCNTA  | TLALEVNAARD | TLALEVTPVRS |
| HLVEAL     | HLVEALWSRM    | HLVEALKCNTA  | HLVEALNAARD | HLVEALTPVRS |
| LVEALY     | LVEALYWSRM    | LVEALYKCNTA  | LVEALYNAARD | LVEALYTPVRS |
| VEALYL     | VEALYLSRM     | VEALYLKCNTA  | VEALYNAARD  | VEALYTPVRS  |

### Tandem HIPS 2

| InsB / C/A-IAPP1 | InsB / C/A-Amylin | InsB / C/A-IAPP2 | 2.5mi |
|------------------|-------------------|------------------|-------|
|                  |                   |                  |       |
| Ins B-chain      | Ins C-pep         | Ins A-chain      |       |
|                  | QLELGGS           | QLELGGS          |       |
|                  | GDLQT             | SICSLY           |       |
|                  | HLVEAL            | TSICSL           |       |
|                  | LVEALY            | SICSLYTPVRS    |       |
|                  | LVEALYL           | SICSLYPVRS    |       |
|                  | QLELGGS           | QLELGGS          |       |
|                  | GDLQT             | SICSLYPVRS    |       |
|                  | HLVEAL            | TSICSL          |       |
|                  | LVEALY            | SICSLYPVRS    |       |
|                  | LVEALYL           | SICSLYPVRS    |       |

### Tandem HIPS 3

| InsB / C/A-NPY  | InsB / C/A-Sg1  | InsB / C/A-Sg2  | 2.5mi |
|-----------------|-----------------|-----------------|-------|
|                 |                 |                 |       |
| Ins B-chain     | Ins C-pep       | Ins A-chain     |       |
|                 | QLELGGS         | QLELGGS         |       |
|                 | GDLQT           | SICSL           |       |
|                 | HLVEAL          | TSICSL          |       |
|                 | LVEALY          | SICSL           |       |
|                 | LVEALYL         | SICSL           |       |
|                 | QLELGGS         | QLELGGS         |       |
|                 | GDLQT           | SICSL           |       |
|                 | HLVEAL          | TSICSL          |       |
|                 | LVEALY          | SICSL           |       |
|                 | LVEALYL         | SICSL           |       |
Supplementary Figure 1. Expression vectors encoding for tandem sequences of HIPs. The sequence encoding for Ii1-80 was C-terminal fused with a tandem sequence HIPs plus the sequence of the 2.5mi epitope and cloned in a pCMV expression vector. Following this approach, we generated 3 constructs (Tandem HIPs 1, 2 and 3) that were used to determine 4.1-TCR reactivity. Notice that the Tandem-HIPs 1 does not include the C-terminal 2.5mi sequence, as the BDC2.5-TCR natural agonistic HIP sequence (encoding for LQTLALWSRMD) which is the result of combining left InsC peptide (LQTLAL) with the right ChrA peptide (WSRMD) is included in the pool of tandem peptides.
### Supplementary Table 1. Peptides eluted from I-A<sup>g7</sup> molecules from NIT-1 cells that were tested for 4.1-TCR activation.

| I-A<sup>g7</sup> eluted peptide from NIT-1 | Sequence                  |
|-------------------------------------------|---------------------------|
| Synaptotagmin 11 (174-191)                | VTIQEAHGLPVMDQTQ          |
| teneurin transmembrane protein 1 (835-849) | LQTPSQQAASKFYDR          |
| Neuromodulin (184-201)                    | QPPTETAESSQAEKDA         |
| Synapse associated protein (262-279)      | TPPVVIKSQLKSEQEDEEE       |
| NCAM (189-204)                            | SAPKVAPLVDLSDT           |
| Secretogranin III (229-244)              | IPEKVTVPVAVQDGF          |
| Axonal Transporter of synaptic vesicles (885-898) | VAVQAIADDEEAPD         |
| Beta-siete APP-Cleaving Enzyme (109-127)  | SSNFAVAGAPHSYIDTYFD      |
| Synaptic cell adhesion molecule (203-218) | TVTSQMLKLKHEDDCG        |
| Secretogranin II (234-248)               | DVYKTNNIAYEDV           |
| Secretogranin II (420-434)               | APGRGMVEALPDGLS        |
| Chromogranin A (407-423)                 | RPSSREDVESRDFE           |
| NMDA 2A (36-46)                          | IAVLLGHSHDV            |
| Gamma-aminobutyric acid receptor-associated protein (29-45) | VPVIVEKAPKARIAGLD |  
| Carboxipeptidase H (348-363)             | KFPPEETLKSYEDKNK         |
| Lisch 7 (491-509)                        | SGRPRARSVDALDDINRP       |
| Amyloid beta A4 (237-249)                | KSEFPTEADLDF            |
| Amyloid beta A4 (475-489)                | NVPAAEEIQDEVDE          |
| Amyloid beta A4 (524-539)                | ETKTTVELPPVNGES         |
| Solute carrier family 12 member 7 (776-18) | TVVPVEARADGAG          |
| Reticulon 4 receptor-like 1 (366-380)    | RNQISKVSSGKELTE         |
## Supplementary Table 2. Proteolytic products contributing to the generation of HIP sequences sharing the 4.1-TCR activation motif.

| HIP ID | Left side donor | Left fragment HIP | Location | HIP sequence | Right fragment HIP | Right side donor | Location |
|--------|-----------------|------------------|----------|--------------|-------------------|-----------------|----------|
| HIP 15 | Ins1C (57–79)   | Ins1C (57–79)    | Islet as Ins2C(61-81) (MHCII peptidome), pLN (MHCII peptidome), spleen (MHCII peptidome), DCGs, secretome, crinosomes (23), beta cell extracts (5) | LOTLAE–LEGEDDP | ChgA(374–381) | ChgA(374–402) | DCGs, secretome, crinosomes (23) |
| HIP 18 | Ins1C (57–82)   | Ins1C (57–82)    | Secretome, crinosomes (23) | LOTLAE–EEEGS | ChgA(426–430) | ChgA(426–460) | crinosomes (23) |
| HIP 30 | Ins1C (57–80)   | Ins1C (57–80)    | Islet (MHCII peptidome), pLN (MHCII peptidome), spleen (MHCII peptidome), DCGs, secretome, crinosomes (23), beta cell extracts (5), peptide forming HIP (24) | LQTLAL–EVEDPQV | Ins(57–63) | Partial fragments from Ins1C (57–85) Ins2C (57–87) | beta cell extracts (5), peptide forming HIP (24) |
| HIP 30 | Q15E            | Ins1C (57–80)    | Secretome, crinosomes (23) | LQTLALE–EVEDPEV | Ins(57–63) | Partial right part post-translational modified [PqVEQ -> PeVEQ] found in pLN (MHCII peptidome) (23) |
| HIP 32 | Ins1C (57–81)   | Ins1C (57–81)    | DCGs, secretome, crinosomes (23) | LOTLAE–AEDQEL | ChgA(435–440) | ChgA(435–460) | DCGs, crinosomes (23) |
| HIP 32 | Q15E            | Ins1C (57–81)    | DCGs, secretome, crinosomes (23) | LOTLAE–AEDQEL | ChgA(435–440) | ChgA(435–460) | Partial right part post-translational modified [AEDqEL -> AEdqEL] in crinosomes (23) |
| HIP 39 | Ins1C (57–82)   | Ins1C (57–82)    | Secretome, crinosomes (23) | LOTLAE–EVEDPQRSM | ChgA(377–384) | ChgA(377–388) | crinosomes (23) |
| HIP 40 | Ins1C (57–82)   | Ins1C (57–82)    | Secretome, crinosomes (23) | LOTLAE–AEDQEL | ChgA(435–440) | ChgA(435–460) | DCGs, crinosomes (23) |
| HIP 43 | InsC (57–69)    | InsC (57–69)     | pLN (MHC II peptidome), DCGs, secretome, crinosomes (23), beta cell extracts (5) | QLELGA–LEGEDDP | ChgA(374–384) | ChgA(374–402) | DCGs, secretome, crinosomes (23) |
| HPS55 | InsC (57–70)    | InsC (57–70)     | pLN (MHCII peptide) as Ins1C (61-70) and Ins2C (61-70), DCGs, secretome, crinosomes (23) | QLELGO–EVEDPQRSM | Ins(57–63) | Partial fragments from Ins1C (57–85) Ins2C (57–87) | beta cell extracts (5), peptide forming HIP (24) |
| HIP D1 | Ins1C (57–83)   | Ins1C (57–83)    | Islet (MHCII peptidome), DCGs, secretome, crinosomes (23), beta cell extracts (5) | LOTLAE–EEEGS | ChgA(426–430) | ChgA(426–460) | crinosomes (23) |
| HIP D2 | Ins2C (57–70)   | Ins2C (57–70)    | pLN (MHCII peptide) as Ins1C (61-70) and Ins2C (61-70), DCGs, Secretome, Crinosomes (23) | VAQLELGO–LEGEDDP | ChgA(374–380) | ChgA(374–402) | DCGs, secretome, crinosomes (23) |

Orange: Insulin C  Blue: Chromogranin A