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## Supplemental Information

# Activation of NF-κB-Inducing Kinase in Islet β Cells Causes β Cell Failure and Diabetes

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**Supplemental Information**

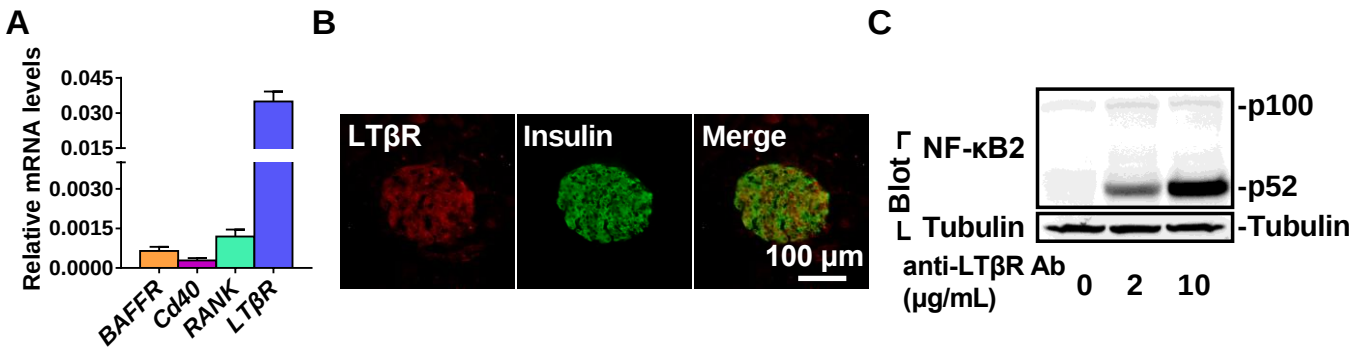


**Figure S1. LTβR/noncanonical NF-κB2 signaling pathway is present in islet β cells.**

(A) The relative expression of *BAFFR*, *Cd40*, *RANK*, and *LTβR* were measured by qPCR in MIN6 cells (n=5 for each group).

(B) Immunostaining of LTβR and insulin in the section of pancreas. The scale bar represents 100 μm.

(C) INS-1 832/13 cells were treated with anti-LTβR antibody at different concentrations (0, 2, 10 ug/ml) for 2 h, and p52 protein levels were measured by immunoblotting. Data represent the mean ± SEM.

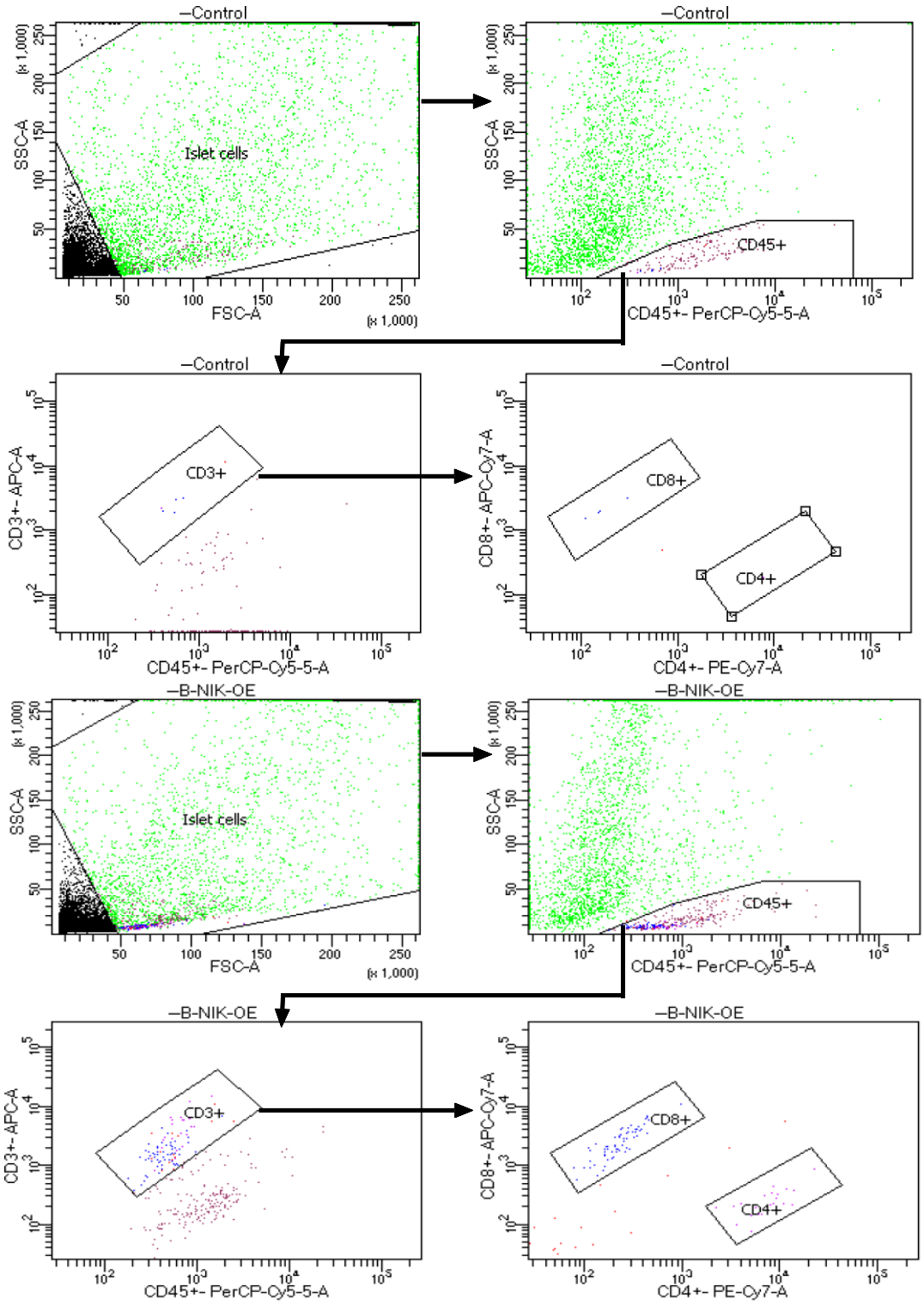


**Figure S2. β-NIK-OE mice display lymphocyte infiltration in islets.**

Islets were isolated from β-NIK-OE mice and control mice at 6 weeks of age. Single cells were dispersed and subjected to FACS analysis. Representative FACS plots of staining for total T ($CD45.2^+CD3^+$) cells, CD4 T ($CD45.2^+CD3^+CD4^+$) cells, and CD8 T ($CD45.2^+CD3^+CD8^+$) cells were presented.

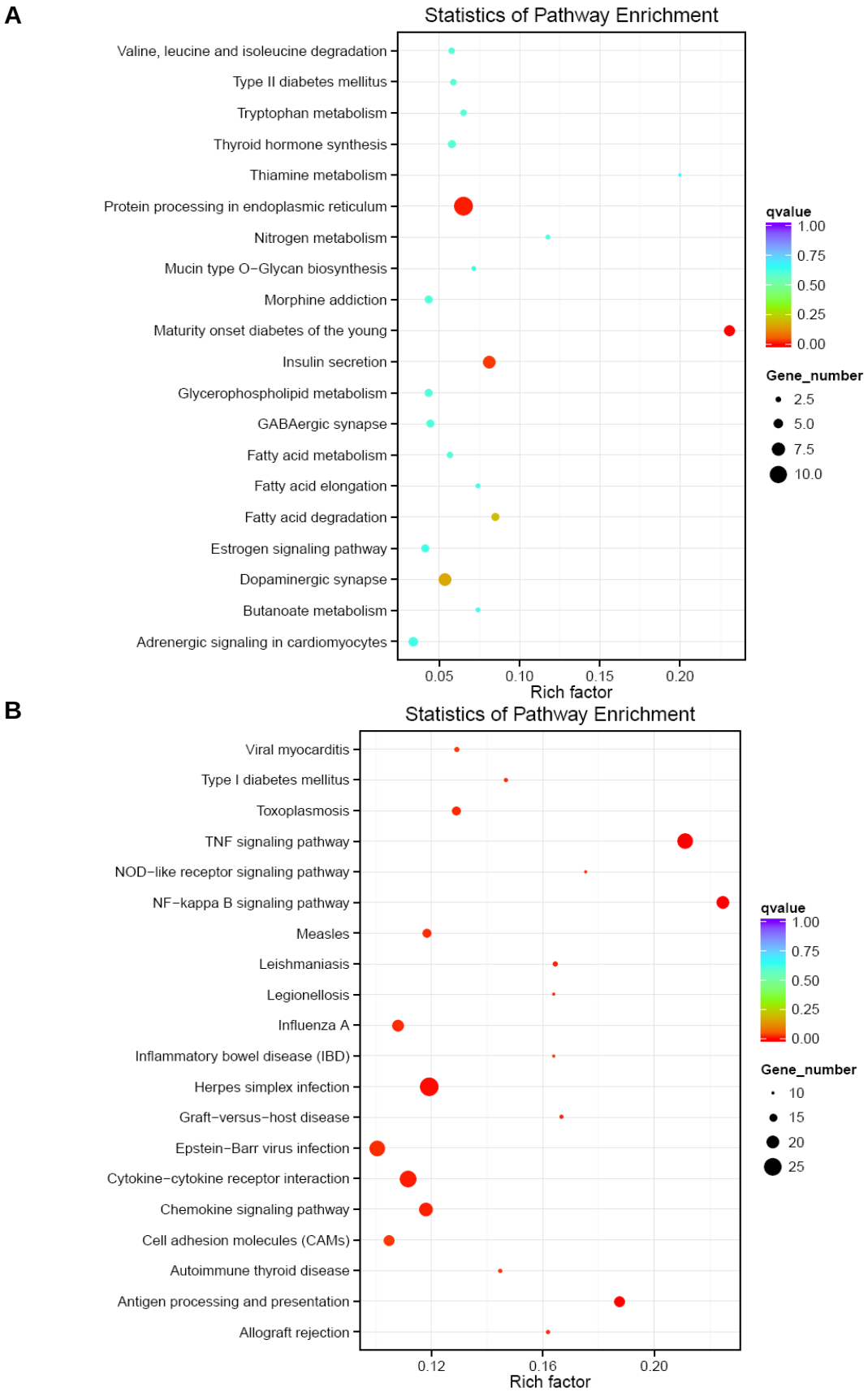


**Figure S3. NIK overexpression decreases signaling pathways associated with insulin secretion while activating immune signaling pathways in INS-1 832/13 cells.**

(A) KEGG pathway enrichment analysis of downregulated genes in NIK-overexpressing INS-1 832/13 cells compared with βGal control showed that signaling pathways associated with insulin secretion, protein processing in endoplasmic reticulum, and maturity onset diabetes of the young were significantly decreased. (B) KEGG pathway enrichment analysis of upregulated genes in NIK-overexpressing INS-1 832/13 cells showed that signaling pathways related to immune response were significantly increased.

**Table S1 List of reagents.**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Antibodies** | | |
| β-actin | proteintech | Cat#60008-1-lg |
| Insulin | Dako | Cat#A0564 |
| Glucagon | Sigma | Cat#G2654 |
| α Tubulin Antibody (B-7) | Santa cruz | Cat#sc-5286 |
| NF-κB2 | CST | Cat #4882 |
| Flag | SIGMA | Cat# F1804 |
| IκBα | proteintech | Cat# 10268-1-AP |
| CD4 Monoclonal Antibody (GK1.5), PE-Cyanine7 | eBioscience™ | Cat#25-0041-82 |
| APC/Cy7 anti-mouse CD8b Antibody | BioLegend | Cat#126620 |
| PE anti-mouse F4/80 Antibody | BioLegend | Cat#123110 |
| PerCP anti-mouse CD45.2 Antibody | BioLegend | Cat#109826 |
| APC anti-mouse CD3 Antibody | BioLegend | Cat#100236 |
| PE/Cy7anti-mouse/human CD45R/B220 Antibody | BioLegend | Cat#103222 |
| **Chemicals** | | |
| RIPA Buffer | Solarbio | Cat#R0020 |
| MTT | Sigma | Cat#M2128 |
| DMEM | Gibco | Cat#12100046 |
| Blue Plus II Protein Marker | Transgen Biotech | Cat#L50824 |
| High Pure dNTPs | Transgen Biotech | Cat#20201025 |
| GoTaq Green Master Mix | Promega | Cat#M7122 |
| M-MLV reverse transcriptase | Promega | Cat#M1701 |
| Random Primers | Promega | Cat#C1181 |
| FBS | Gibco | Cat#16000-044 |
| SYBR™ Green Mix | Roche | Cat#4913914001 |
| TriPure Isolation Reagent | Roche | Cat#94015120 |
| Blood glucose meter | Johnson & Johnson | ONETOUCH UltraEasy |
| **Assay Kit** | | |
| Ultrasensitive Mouse Insulin immunoassay kit | EZassay | Cat#MS100 |
| β- Hydroxybutyrate Assay Kit | NanjingJianchengBioengineering institute | Cat#E030-1-1 |
| Mouse C-Peptide ELISA Kit | ALPCO | Cat#80-CPTMS-E01 |
| TUNEL assay kit | Roche Applied Science | Cat#11684795910 |
| TUNEL Dilution Buffer | Roche Applied Science | Cat#11966006001 |
| Glucagon ELISA Kit | R&D Systems | Cat#DGCG0 |
| **Software** | | |
| Graphpad | Graphpadsoftwave | Graphpad.com |

**Table S2 Primers for qPCR**

| Genes | Forward | Reverse |
|---|---|---|
| *NIK* | 5'-CATCTTCTCAAAATTCGAGTGACAA-3' | 5'-TGGGAGTAGACAAGGTACAACCC-3' |
| *Insulin1/2* | 5-GTCATTGTTTCAACATGGCCCTGT-3′ | 5-TGCAGTAGTTCTCCAGCTGGTA-3′ |
| *Glut2* | 5'-CGGAACCTTGGCTTTCACTGTCTT-3' | 5'-GGTGCATTGATCACACCGATGTCA-3' |
| *Gck* | 5'-AAGCCGCAGTGAGGACGTGATG-3' | 5'-AGGTGATTTCGCAGTTGGGTGTCA-3' |
| *Pdx1* | 5'-CCTTTCCCGTGGATGAAAT-3' | 5'-TGTAGGCAGTACGGGTCCTC-3' |
| *Hnf1a* | 5'-GGAAGACTTCGCGCCACCCATTCT-3' | 5'-AGTGACTCCACCACGGCTTTCTGG-3' |
| *Hnf1b* | 5'-CGGATCTCGACACCAAGCCGGTTT-3' | 5'-TGTCATAGTCGTCGCCGTCCTCTG-3' |
| *Hnf4a* | 5'-GCCCTCTCACCTCAGCAATGGACA-3' | 5'-TGACGATGGTGGTGATGGCTCCTG-3' |
| *Neurod1* | 5'-TGTCCCGAGGCTCCAGGGTTATGA-3' | 5'-GCCCGCTCTCGCTGTATGATTTGG-3' |
| *Ccl2* | 5'-ACTGAAGCCAGCTCTCTCTTCCTC-3' | 5'-TTCCTTCTTGGGGTCAGCACAGAC-3' |
| *Ccl5* | 5'-CCACTTCTTCTCTGGGTTGG-3' | 5'-GTGCCCACGTCAAGGAGTAT-3' |
| *Ccl11* | 5'-GATCTGTGCTGACCCCAAGAA-3' | 5'-CTTAGGCTCTGGGTTAGTGTCA-3' |
| *Cxcl1* | 5'-GGCGCCTATCGCCAATGA-3' | 5'-GACTTCGGTTTGGGTGCAGT-3' |
| *Cxcl10* | 5'-CCAAGTGCTGCCGTCATTTT-3' | 5'-CTCAACACGTGGGCAGGATA-3' |
| *Cxcl11* | 5'-ATTTACCCGAGTAACGGCTG-3' | 5'-GAGGCGAGCTTGCTTGGAT-3' |
| *Il1* | 5'-GCCTTGGGCCTCAAAGGAAAGAATC-3' | 5'-GGAAGACACAGATTCCATGGTGAAG-3' |
| *Il6* | 5'-AGCCAGAGTCCTTCAGA-3' | 5'-GGTCCTTAGCCACTCCT-3' |
| *Tnfα* | 5'-CATCTTCTCAAAATTCGAGTGACAA-3' | 5'-TGGGAGTAGACAAGGTACAACCC-3' |
| *Cd3e* | 5'-AGGTGGACCTGACAGCAGTA-3' | 5'-GGCTCATAGTCTGGGTTGGG-3' |
| *Cd19* | 5'-AGGCAATGTTGTGCTGCCA-3' | 5'-AATCACTAGCAAGATGCCCAGG-3' |
| *Cd74* | 5'-CAAGTGCGACGAGAACGGTA-3' | 5'-CAGGGTGACTTGACCCAGTTC-3' |
| *RT1-Ba* | 5'-CAAGACGACATTGAGGCCGA-3' | 5'-CAGTTGTCCAAACTCGGGGA-3' |
| *RT1-CE4* | 5'-TTGGAGCTGTGGCCATGATT-3' | 5'-TCACAATCTGCGAGGGACAC-3' |
| *RT1-CE7* | 5'-GAACCTTCCAGAAGTGGGCA-3' | 5'-CACAGCAGAGATGGCTCCAA-3' |
| *RT1-Da* | 5'-TGAGGTGGATCACTGGGGTC-3' | 5'-CACTGCGTTGCGTTTCCG-3' |
| *RT1-M2* | 5'-CTGCTGAGCGCTTCTTTGG-3' | 5'-AACCTCGGATTCACTGCCTC-3' |
| *RT1-M3* | 5'-TCGTCTCAGTCCTTTGTCGTC-3' | 5'-AACAGACTCAGTCCCACACAC-3' |
| *B2M* | 5'-GGGAAGCCCAACTTCCTCAA-3' | 5'-ATACATCGGTCTCGGTGGGT-3' |
| *BAFFR* | 5'- CCAGCAAGAGTCCCTGGAAAAT-3' | 5'-CTCCACTGCTGCTATTGCTCT -3' |
| *Cd40* | 5'- TTGTTGACAGCGGTCCATCT-3' | 5'- GCGAATCTCCCTGTTCCACT-3' |
| *RANK* | 5'- CCTTCGACTGGTTCACTGCT-3' | 5'-GGACACGGGCATAGAGTCAG -3' |
| *LTβR* | 5'- CAGCTGGTGCCCCCTTATC-3' | 5'-AAGACAAACTCGCCTGGGG -3' |