SUPPLEMENTARY DATA

UBASH3A Mediates Risk for Type 1 Diabetes through Inhibition of T-Cell Receptor-Induced NF-κB Signaling

Justification on the necessity of the online supplement

The supplementary tables and figures do not warrant inclusion in the main text, but they provide additional information related to the methods and data of the main text.

Supplementary Figure 1 shows ubiquitination of NEMO and TRAF6 upon stimulation in Jurkat cells, UBASH3A−/− 2.1F7 cells, and UBASH3A-overexpressing 2F5 cells. This figure is related to Figures 3 and 4 of the main text.

Supplementary Figure 2 shows the quality of the GST fusion proteins used in the GST pull-down assay, and it is related to Figure 5C of the main text.

Supplementary Figure 3 shows the result of an allele-specific expression analysis for rs11203203 in human primary CD4+ T cells, which complements the quantitative PCR findings shown in Figure 6.

Supplementary Table 1 lists antibodies used for co-immunoprecipitation and immunoblotting.

Supplementary Table 2 lists the primers used in the quantitative real-time PCR.

Supplementary Table 3 shows densitometry and statistical test results for all the protein experiments of Figure 3.
Supplementary Figure 1. Ubiquitination of NEMO and TRAF6 in Jurkat and Jurkat-derived clones. (A-B) Jurkat, \textit{UBASH3A}\textsuperscript{-/-} 2.1F7, and \textit{UBASH3A}-overexpressing 2F5 cells were mock stimulated or stimulated with anti-CD3 plus anti-CD28 for 10 min. Whole-cell lysates from the cells were extracted with lysis buffer containing 5 mM NEM and immunoprecipitated with the indicated antibodies. The immunoprecipitates were subjected to immunoblotting with anti-ubiquitin. The blots in (A-B) are representative of two independent experiments.
SUPPLEMENTARY DATA

Supplementary Figure 2. SDS-PAGE analysis of the purified GST-tagged domains of UBASH3A. Purified GST-fusion proteins and GST were subjected to SDS-PAGE analysis. The gel was stained with Bio-Safe colloidal Coomassie Blue G-250 (Bio-Rad) following the manufacturer’s protocol. The target GST-fusion proteins are indicated by arrows.
**Supplementary Figure 3. Allele-specific expression analysis in human primary CD4+ cells.** Allele-specific expression analysis was performed using mRNA-seq read counts from CD4+ cells from 38 T1D subjects of European ancestry who were heterozygous at the coding SNP rs2277798 (G>A) in UBASH3A, and either homozygous for the major allele of rs11203203 (G>A) (n=16), or heterozygous at rs11203203 (n=22). For mRNA-seq, CD4+ T cells were purified from PBMCs by positive selection (Miltenyi), and RNA was extracted and sequenced (50 million reads/sample) using the Illumina HiSeq 2000 platform. Paired-end 50-bp sequencing reads were aligned to the complete human genome (GRCh37/hg19 version) using the BWAMEM algorithm. Reads aligning to the UBASH3A locus (± 1 kb) on chromosome 21 were extracted and aligned to allele-specific 74-bp sequences flanking rs2277798 using the Bowtie1 algorithm with no mismatches or indels allowed (i.e., reads that mapped exactly to either allelespecific reference sequence). Allele-specific sequences were then quantified and summed across technical replicates for each subject. The proportion of sequencing reads carrying the G allele rs2277798 was calculated and is shown. The blue dashed line represents the proportion of 0.5 expected for equal expression of each allele. The minor, risk allele of rs11203203 is highlighted in bold and underscored. Each data point represents one subject. The mean and SEM values are indicated by black solid lines and error bars, respectively. Unpaired two-tailed Student’s t-test was performed to compare the two rs11203203 genotype groups, and the resulting p-value shown in black. One-sample two-tailed Student’s t-tests were performed to compare the proportions of sequencing reads carrying the G allele at rs2277798 in each rs11203203 genotype group to the null hypothesis where the true mean proportion is 0.5, and the resulting p-values are shown in blue.

©2017 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-1023/-/DC1
Supplementary Table 1. Antibodies used for co-immunoprecipitation and immunoblotting

| Antibody name       | Company and catalog number                  |
|---------------------|---------------------------------------------|
| anti-Fibrillarin    | Cell Signaling, #2639                      |
| anti-goat IgG       | SouthernBiotech, #6425-05                   |
| anti-HA             | Santa Cruz, #sc-805                         |
| anti-IκBα           | Santa Cruz, #sc-371                         |
| anti-IKKα/β         | Santa Cruz, #sc-7607                        |
| anti-Mouse Ig       | BD Phamingen, #554002                       |
| anti-NEMO           | Santa Cruz, #sc-8330; Cell Signaling, #2695|
| anti-NF-κB p65      | Santa Cruz, #sc-372                         |
| anti-phospho-IKKα/β | Cell Signaling, #2697                       |
| anti-phospho-PKCθ   | Cell Signaling, #9377                       |
| anti-Phosphorytrosine | MilliporeSigma, #05-321                   |
| anti-Rabbit IgG     | BD Phamingen, #554021                       |
| anti-Sheep IgG      | R&D Systems, #HAF016                        |
| anti-TAK1           | Santa Cruz, #sc-1839                        |
| anti-TRAF6          | Santa Cruz, #sc-7221                        |
| anti-γ-Tubulin      | Sigma-Aldrich, #T6557                       |
| anti-UBASH3A        | Sigma, SAB1410972; Proteintech, #15823-1-AP|
| anti-UBASH3B        | R&D Systems, #AF6696                        |
| anti-Ubiquitin      | Santa Cruz, #sc-8017 and #sc-9133           |
| anti-V5             | Thermo Fisher Scientific, #R960-25          |
Supplementary Table 2. Primers for quantitative PCR

| Transcript | Forward primer       | Reverse primer                                      |
|------------|----------------------|----------------------------------------------------|
| UBAH3A     | CTTGCAGGCTACCGTTGC   | CAGGGGAAATTCAGGTCTGGC                              |
| IL2        | AACTCACCAGGATGCTCACA | AGTCCCTGGGGTCTTAAGTGA                              |
### Supplementary Table 3. Relative amounts of the proteins shown in Figure 3

| Protein name | Cell line | Stimulation          | Mean relative amount (n=3) | P-value (<0.05)                      |
|--------------|-----------|----------------------|----------------------------|-------------------------------------|
| Nuclear NF-κB p65 | Jurkat    | Mock stimulation     | 1.00                       |                                     |
|              | 2.1F7     | Mock stimulation     | 1.10                       |                                     |
|              | 2F5       | Mock stimulation     | 0.84                       |                                     |
|              | Jurkat    | 6-h stimulation      | 1.53                       |                                     |
|              | 2.1F7     | 6-h stimulation      | 3.01                       | 0.015 (vs. Jurkat 6-h stimulation)  |
|              | 2F5       | 6-h stimulation      | 1.07                       |                                     |
| Phosphorylated IKKα/β | Jurkat | 15-min stimulation | 1.00                       |                                     |
|              | 2.1F7     | 15-min stimulation   | 2.18                       | 0.045 (vs. Jurkat 15-min stimulation) |
| IKKα/β       | Jurkat    | 15-min stimulation   | 1.00                       |                                     |
|              | 2.1F7     | 15-min stimulation   | 1.00                       |                                     |
| IκBα         | Jurkat    | 0-min stimulation    | 1.00                       |                                     |
|              | 2.1F7     | 0-min stimulation    | 0.70                       | 0.023 (vs. Jurkat 0-min stimulation) |
|              | Jurkat    | 15-min stimulation   | 0.42                       |                                     |
|              | 2.1F7     | 15-min stimulation   | 0.16                       | 0.021 (vs. Jurkat 15-min stimulation) |
| IκBα         | Jurkat    | 0-h stimulation      | 1.00                       |                                     |
|              | 2.1F7     | 0-h stimulation      | 0.74                       | 0.011 (vs. Jurkat 0-h stimulation)  |
|              | Jurkat    | 2-h stimulation      | 1.55                       |                                     |
|              | 2.1F7     | 2-h stimulation      | 0.71                       | 0.0013 (vs. Jurkat 2-h stimulation) |
|              | Jurkat    | 6-h stimulation      | 1.61                       |                                     |
|              | 2.1F7     | 6-h stimulation      | 1.01                       | 0.012 (vs. Jurkat 6-h stimulation)  |
|              | Jurkat    | 24-h stimulation     | 1.76                       |                                     |
|              | 2.1F7     | 24-h stimulation     | 1.31                       |                                     |
SUPPLEMENTARY DATA

|                | Jurkat | 15-min stimulation | 1.00 |
|----------------|--------|--------------------|------|
| Phosphorylated PKCθ |        |                    |      |
| 2.1F7          |        | 15-min stimulation | 1.78 |
| Phosphorylated TAK1 | Jurkat | 10-min stimulation | 1.00 |
| 2.1F7          |        | 10-min stimulation | 1.21 |
| TAK1           | Jurkat | 10-min stimulation | 1.00 |
| 2.1F7          |        | 10-min stimulation | 0.94 |

Each experiment shown in Figure 3 was performed three times. Films of Western blots from each of the 3 independent replicate experiments were scanned, and the protein bands were quantified using the Image Studio Lite software (LI-COR). The relative amounts of proteins other than phosphorylated TAK1 were calculated for each experiment using the following formula where X denotes the protein of interest and Y the cells of interest:

\[
\frac{\text{Intensity of } X \text{ in } Y \text{ cells}}{\text{Intensity of the corresponding loading control for } X \text{ in } Y \text{ cells}} \div \frac{\text{Intensity of } X \text{ in Jurkat cells}}{\text{Intensity of the corresponding loading control for } X \text{ in Jurkat cells}}
\]

For phosphorylated TAK1 shown in the top blot of Figure 3E, the following formula was used to calculate its relative amount:

\[
\frac{\text{Intensity of pTAK1 in stimulated 2.1F7 KO cells}}{\text{Intensity of pTAK1 in stimulated Jurkat cells}}
\]

Unpaired two-tailed Student’s t-tests were performed using the relative protein amounts from 3 replicate experiments, and p-values less than 0.05 are shown.