Targeted Deep Sequencing in Multiple-Affected Sibships of European Ancestry Identifies Rare Deleterious Variants in \textit{PTPN22} that Confer Risk for Type 1 Diabetes

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 Table 1 lists the primers used in the quantitative real-time PCR (Fig. 1 and 2). Supplementary Table 2 lists the protein-coding genes that were captured and deeply sequenced in this study. Supplementary Table 3 shows the distributions of HLA haplotypes in the 70 T1D cases sequenced in this study and in the initial T1DGC population from which the 70 cases were selected. Supplementary Figures 1 and 2 show intracellular calcium flux profiles in CD4$^+$ T cells from subjects without autoimmune diseases and in CD19$^+$ B cells, respectively.
**Supplementary Table 1.** Primers used for quantitative RT-PCR

| Transcript | Forward primer (5’ to 3’) | Reverse primer (5’ to 3’) |
|------------|---------------------------|--------------------------|
| PTPN22 transcript 1 | TCCTGACACCATGGAAAAATTCA | GGTGGATTCCTTGGTCCTTTG |
| PTPN22 transcript 2 | GTGAAAAACTCCGAAGTCCTAAA | CCCCATTTCAGAAAAATGAGCCT |
| PTPN22 transcript 3 | TCAG | AA | GGA |
| GAPDH | CAGCCGAGCCACATCGC | CATGGGTGGAATCATATTGG | AACA |

*PTPN22* transcript 1 is the full-length *PTPN22* transcript, *PTPN22* transcript 2 the intron-18-run-on transcript, and *PTPN22* transcript 3 the exon-18-skipping transcript (see Fig. 1A).
**Supplementary Table 2.** Known protein-coding genes in T1D-associated regions targeted in deep sequencing

| T1D-associated regions | Protein-coding genes                                                                 |
|------------------------|--------------------------------------------------------------------------------------|
| 1p13.2                 | *OLFML3, DCLRE1B, AP4B1, BCL2L15, PTPN22, HIPK1, RSBN1, PHTF1, MAGI3, SYT6          |
| 1q31.2                 | *RGS1                                                                               |
| 1q32.1                 | *IL20, IL19, IL10, MAPKAPK2, DYRK3                                                  |
| 2q11.2                 | *CHST10, LONRF2, AFF3                                                               |
| 2q12.1                 | *SLC9A4, IL18RAP, IL18R1, IL1RL1, IL1RL2                                             |
| 2q24.2                 | *GCA, IFIH1, FAP, KCNH7, GCG                                                        |
| 2q32.2                 | *STAT4                                                                              |
| 2q33.2                 | *ICOS, CTLA4                                                                        |
| 3p21.31                | *TDGF1, LRRC2, RTP3, LTF, CCRL2, CCR5, CCR3, CCR2, CCR1, XCR1, CXCR6, FYCO1         |
| 4p15.2                 | -                                                                                    |
| 4q27                   | *IL21, ADD1, IL2, KIAA1109                                                          |
| 5p13.2                 | *UGT3A2, UGT3A1, CAPSL, IL7R, SPEF2                                                  |
| 6q15                   | *BACH2                                                                              |
| 6q22.32                | *CENPW                                                                              |
| 6q23.3                 | *TNFAIP3                                                                            |
| 6q25.3                 | *TAGAP, RSPH3, C6orf99                                                             |
SUPPLEMENTARY DATA

7p15.2  
HOXA7, HOXA6, HOXA5, HOXA4, HOXA3, HOXA2, HOXA1, SKAP2, C7orf71, HOXA9

7p12.1  
COBL

9p24.2  
GLIS3

10p15.1  
PFKFB3, RBM17, IL2RA

10p15.1  
PRKCQ

10q22.3  
ZMIZ1

10q23.31  — RNLS

11p15.5  
ASCL2, TH, INS, IGF2-AS, IGF2

12p13.31  
CD69, CLEC11, CLEC2D, KLRB1

12q13.2  
APOF, STAT2, IL23A, PAN2, CNPY2, CS, COQ10A, ANKRD52, SLC39A5, NABP2, RNF41, SMARCC2, MYL6, MYL6B, ZC3H10, ESYT1, PA2G4P4, IKZF4, RPS26P53, ERBB3, SUOX, RAB5B, CDK2, PMEL

12q13.3  
CTDSP2, AVIL, TSFM, METTL21B, METTL1, MARCH9, CYP27B1, CDK4, AGAP2, TSPAN31, OS9, B4GALNT1, ARHGEF25, DTX3, PIP4K2C, KIF5A, DCTN2, MBD6, MARS, DDIT3, ARHGAP9, GLI1, INHBE, INHBC, R3HDM2, STAC3, NDUFA4L2, SHMT2, NXPH4, LRP1, NAB2, STAT6, TMEM194A, MYO1A, TAC3, ZBTB39, GPR182, RDH16, SDR9C7, HSD17B6, PRIM1, NACA, PTGES3, ATP5B, BAZ2A, RBMS2P1, XRCC6BP1

12q24.12  
PTPN11, RPL6P27, HECTD4, TRAFD1, NAA25, ERP29,
| Chromosome | Gene(s) |
|------------|---------|
| 14q24.1    | ZFP36L1, C14orf181* |
| 14q32.2    | - |
| 15q14      | RASGRP1, C15orf53 |
| 15q25.1    | CTSH, RASGRF1, MORF4L1, ADAMTS7 |
| 16p13.13   | RMI2, PRM1, PRM2, PRM3, TNP2, SOCS1, CLEC16A, DEXI, CIITA, LITAF |
| 16p11.2    | NFATC2IP, SPNS1, CD19, RABEP2, ATP2A1, SH2B1, ATXN2L, TUFM, EIF3C, SULT1A2, SULT1A1, CCDC101, IL27, APOBR, CLN3, EIF3CL, SBK1, LAT |
| 16q23.1    | CHST6, TMEM170A, CFDP1, BCAR1, CTRB1, CTRB2, ZFP1 |
| 17q12      | THRA, MED24, CSF3, GSDMA, PSMD3, ORMDL3, GSDMB, ZPBP2, IKZF3, GRB7, MIEN1, ERBB2, PGAP3, PNMT, TCAP, PPP1R1B, STARD3, NEUROD2, CDK12, MED1, FBXL20, STAC2, NR1D1 |
| 17q21.2    | KRT24, KRT222, SMARCE1 |
| 18p11.21   | PTPN2 |
| 18q22.2    | CD226, DOK6 |
| 19p13.2    | S1PR5, KEAP1, PDE4A, CDC37, TYK2, ICAM3, RAVER1, FDX1L, ZGLP1, ICAM5, ICAM4, ICAM1 |
| 19q13.32   | FKRP, STRN4, PRKD2, DACT3, SLC1A5 |
SUPPLEMENTARY DATA

20p13  SIRPG, SIRPB1, SIRPD
21q22.3  TMPRSS3, UBASH3A
22q12.2  LIF, HORMAD2, MTMR3, ASCC2, UQCR10, ZMAT5, CABP7,
         NF2, THOC5, NIPSNAP1, OSM, NEFH, RFPL1
22q12.3  IL2RB
22q13.1  RAC2, SSTR3, C1QTNF6
Xp22.2  TMSB4X, TLR8
Xq28  BRCC3, CMC4, MTCP1, FUNDC2, H2AFB1, F8A1, F8,
      MPP1, DKC1, GAB3, CTAG2, CTAG1B, VBPI

*C14orf181 was withdrawn from the online repository of HGNC-approved genes. (http://www.genenames.org/ accessed on April 19, 2013).
## Supplementary Table 3. Distribution of HLA DR-DQ haplotypes associated with type 1 Diabetes

| HLA*                      | DRB1   | DQA1   | DQB1   | Exon Seq Freq. † | T1DGC EUR Freq.* | Chi-square P value |
|---------------------------|--------|--------|--------|------------------|-------------------|-------------------|
| Risk Haplotype 1 (S1)     | 04:05  | 03:01  | 03:02  | 0.021            | 0.025             | 1.00              |
| Risk Haplotype 2 (S2)     | 04:01  | 03:01  | 03:02  | 0.264            | 0.281             | 0.74              |
| Risk Haplotype 3 (S3)     | 03:01  | 05:01  | 02:01  | 0.307            | 0.341             | 0.48              |
| Risk Haplotype 4 (S4)     | 04:02  | 03:01  | 03:02  | 0.043            | 0.035             | 0.80              |
| Risk Haplotype 5 (S5)     | 04:04  | 03:01  | 03:02  | 0.064            | 0.050             | 0.61              |
| Protective Haplotype 1 (P1)| 07:01  | 02:01  | 03:03  | 0                | 0.001             | 1.00              |
| Protective Haplotype 2 (P2)| 14:01  | 01:01  | 05:03  | 0                | 0                 | /                 |
| Protective Haplotype 3 (P3)| 15:01  | 01:02  | 06:02  | 0                | 0.004             | 0.98              |
| Protective Haplotype 4 (P4)| 11:04  | 05:01  | 03:01  | 0                | 0.002             | 1.00              |
| Protective Haplotype 5 (P5)| 13:03  | 05:01  | 03:01  | 0                | 0.001             | 1.00              |
| Risk Haplotype 1-5        |        |        |        | 0.700            | 0.732             | 0.42              |
| Protective Haplotype 1-5  |        |        |        | 0                | 0.008             | 0.58              |
| DR3/DR4 Diplotype         |        |        |        | 0.400            | 0.394             | 1.00              |

*Information on the risk DR-DQ haplotypes (S1-S5), the protective DR-DQ haplotypes (P1-P5), and the haplotype/diplotype frequencies in 606 probands of T1D families of European ancestry ascertained by the Type 1 Diabetes Genetics Consortium was obtained from Reference (1).
†Frequencies in the 70 T1D cases that were deeply sequenced in this study.
Supplementary Figure 1. The risk allele of rs56048322 does not impair T cell receptor-mediated intracellular calcium flux in CD4+ T cells from subjects without autoimmune diseases. Previously frozen peripheral blood mononuclear cells were loaded with cellpermeant indo-1 AM dye and stained with antibodies against T cell surface markers. Shown are kinetics profiles depicting the mean indo-1 ratio (violet/blue) as a function of time in gated total CD4+ T cells before and after stimulation with 10 μg/ml of anti-CD3 antibody. Percent maximal calcium flux is expressed as [(peak anti-CD3 flux – mean baseline)/(peak ionomycin flux – mean baseline)] x100. All the subjects are homozygous for the major, non-risk allele of rs2476601 in PTPN22.
Supplementary Figure 2. The risk allele of rs56048322 does not alter B cell receptor mediated intracellular calcium flux in human primary CD19⁺ B cells. Previously frozen peripheral blood mononuclear cells were loaded with cell-permeant indo-1 AM dye and surface-stained with anti-CD19. Shown are kinetics profiles depicting the mean indo-1 ratio (violet/blue) as a function of time in gated total CD19⁺ B cells before and after stimulation with 20 μg/ml anti-κ + anti-λ F(ab’2). Percent maximal calcium flux is expressed as [(peak anti-κ/λ flux – mean baseline)/(peak ionomycin flux – mean baseline)] x100. All the subjects are homozygous for the major, non-risk allele of rs2476601 in PTPN22. T1D, type 1 diabetes.
SUPPLEMENTARY DATA

References:
1. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DRDQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008 Apr;57(4):1084–92.