SUPPLEMENTAL MATERIAL

Supplemental Tables

Table S1. Clinical characteristics of the proband.

Supplemental Table 1. Proband Characteristics
Autoimmune and inflammatory conditions (Age of onset) • Type 1 diabetes (14 yrs) • Autoimmune hypothyroidism (15 yrs) • Hemolytic anemia (15 yrs) • Undifferentiated connective tissue disease (16 yrs) • Raynaud's syndrome (14 years) • Eczema (2 years) • Asthma (1 yr) • Multiple severe food allergies (1 yr) - egg, milk, peanut, tree nut, shellfish
Autoantibodies[A] and genetics • Pancreas (GAD+, IA2+) • Thyroid (Tg+, TPO+) • Other (ANA+, SCL-70+) • T1D-GRS[B] (25^{th}-50^{th}) • HLA[C] (DR3+, DR4-)
Medications • Hydroxychloroquine • Insulin • Levothyroxine

[A] GAD=Glutamic Acid Decarboxylse, IA2=Islet Antigen 2, Tg=Thyroglobulin, TPO=Thyroid peroxidase, ANA=Anti-nuclear antibody, SCL-70=Topoisomerase 1; [B]T1D-GRS=Type 1 Diabetes Genetic Risk Score; [C]HLA=Human Leukocyte Antigen.

Table S2. Proband Complete Blood Count at T1D onset.

Supplemental Table 2. Complete Blood Count		
Variable	**On Presentation (Age 14)**	**Reference Range**
White Blood Cell Count (K/μL)	3.7	3.5-11
Red Blood Cell Count (M/μL)	2.62*	3.88-5.26
Hemoglobin (g/dL)	8.7*	11.5-15.5
Hematocrit (%)	26.5*	36-47
Mean Corpuscular Volume (fL)	101.1*	81-99
Mean Corpuscular Hemoglobin (pg)	33.2*	26-33
Mean Corpuscular Hemoglobin Concentration (g/dL)	32.8	32-35
Red Blood Cell Distribution Width (%)	14	<15
Platelet Count (K/μL)	201	150-450

Table S3. SKAP2 variant information. The Genome Aggregation Database (gnomAD) database is a highly annotated, searchable database of 141,456 human exome and genome sequences (1). The SKAP2 variant was not found in this database, as indicated by “-“. The Combined Annotation-Dependent Depletion (CADD) score is an integrative annotation built from more than 60 genomic features, and variants with a scaled CADD score of >20 are predicted to be in the top 1% of deleterious variants in the human genome (2).

Supplemental Table 3. SKAP2 mutation	
Genomic position	chr7:26778426
Zygosity	heterozygous
Transcript variant	c.457G>A
Protein variant	p.G153R
CADD score	30
gnomAD allele frequency	-

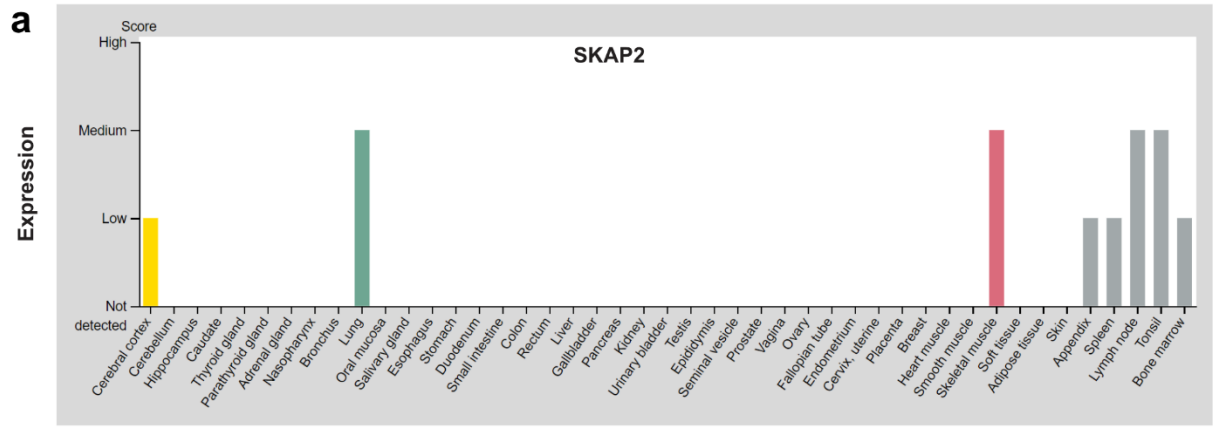
Table S4

Antigen	Reference
ERK 2	Santa Cruz Biotechnology Cat# sc-1647, RRID:AB_627547
p-ERK1/2	Cell Signaling Technology Cat# 9101, RRID:AB_331646
GAPDH	Thermo Fisher Scientific Cat# AM4300, RRID:AB_2536381
SKAP2	Proteintech, Cat# 12926-1-AP, RRID:AB_2189317

Table S5

Antibody	Reference
IRDye 800CW Donkey anti-rabbit IgG	LI-COR Biosciences Cat# 926-32213, RRID:AB_621848
IRDye 680RD Donkey anti-Mouse IgG antibody	LI-COR Biosciences Cat# 925-68072, RRID:AB_2814912

Supplemental Figures



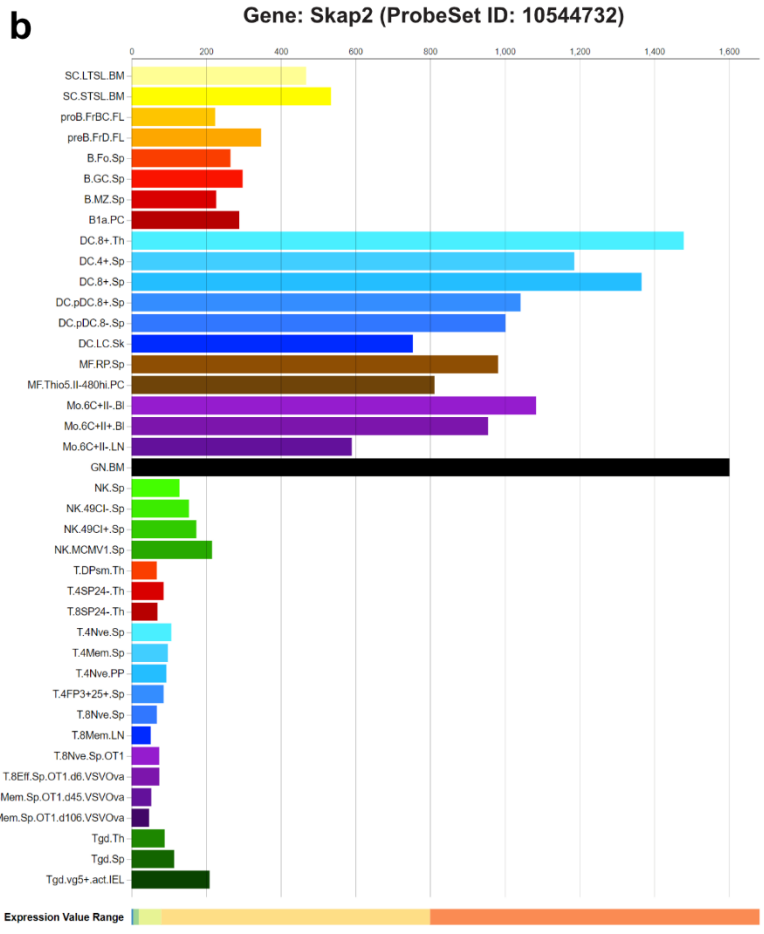


Figure S1. SKAP2 expression.
a, Distribution of SKAP2 protein expression in human tissues(3). **b,** Distribution of Skap2 gene expression in mouse immune cells(4).

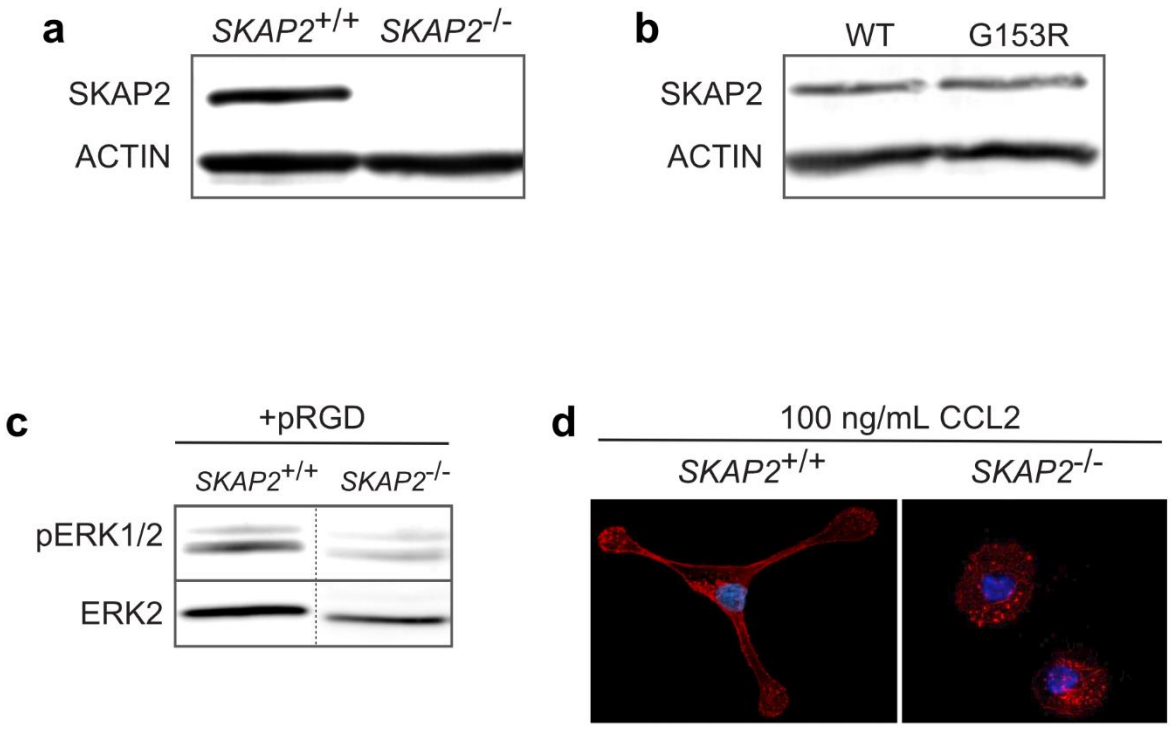


Figure S2. *SKAP2*$^{-/-}$ THP-1 macrophages.
a, Western blot analysis of SKAP2 protein in *SKAP2*$^{+/+}$ and *SKAP2*$^{-/-}$ THP-1 macrophages. **b,** Western blot analysis of SKAP2 protein in *SKAP2*$^{-/-}$ THP-1 macrophages retrovirally expressing either wild-type or p.Gly153Arg SKAP2. **c,** Western blot analysis of ERK phosphorylation in polyRGD plated *SKAP2*$^{+/+}$ and *SKAP2*$^{-/-}$ THP-1 macrophages. **d,** CCL2 treated *SKAP2*$^{+/+}$ and *SKAP2*$^{-/-}$ macrophages stained and imaged for phalloidin actin (red) and the DNA marker DAPI (blue).

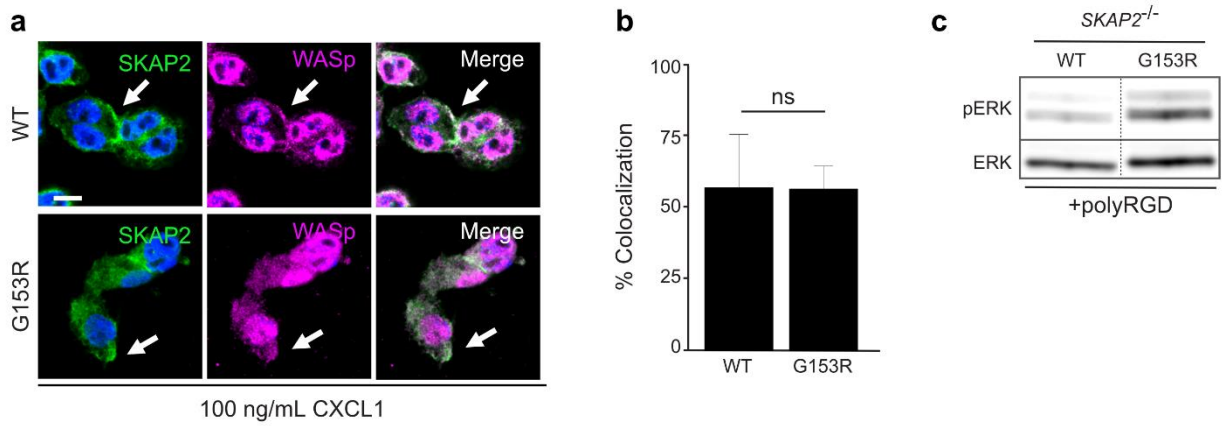


Figure S3. Localization of SKAP2 and WASp in CCL2 treated THP-1 macrophages. **a,** Immunostaining for SKAP2 (green) and WASp (purple) in CCL2 treated *SKAP2*$^{-/-}$ THP-1 macrophages expressing either wild-type (WT) or p.Gly153Arg SKAP2 by retroviral transduction. DNA marker DAPI is in blue. **b,** Percent colocalization of SKAP2 and WASp (n=3, ns=not significant). **c**, Western blot of ERK phosphorylation in pRGD plated THP-1 macrophages. Scale bar=15μm.

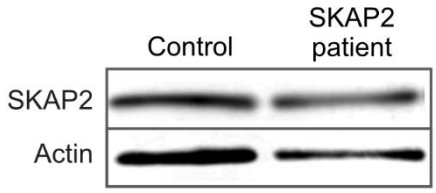


Figure S4. SKAP2 expression in monocyte-derived patient and control macrophages.
SKAP2 immunoblot of macrophages differentiated *ex vivo* from monocytes derived from the SKAP2 patient (II-1) or unaffected control (I-2).

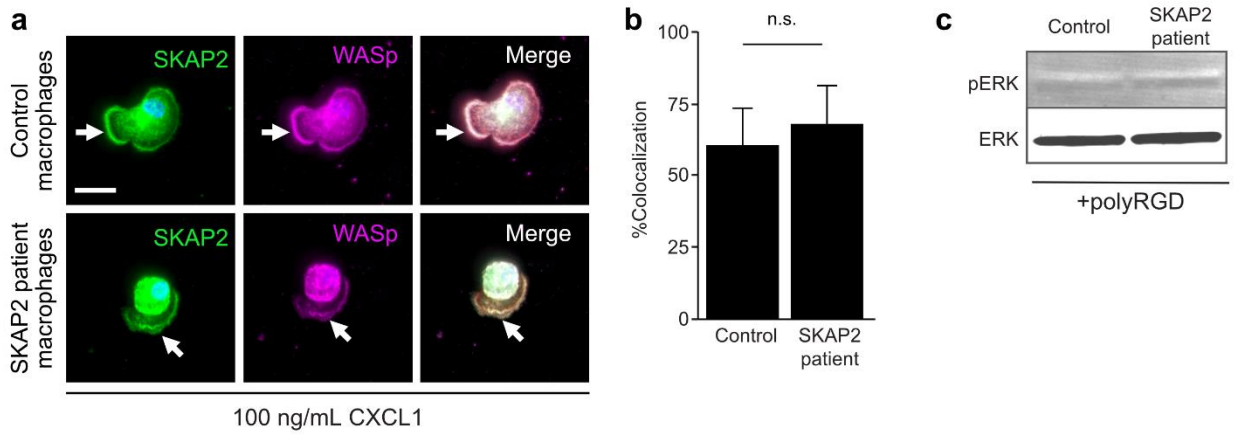


Figure S5. Localization of SKAP2 and WASp in CCL2 treated macrophages.
a, Immunostaining for SKAP2 (green) and WASp (purple) in SKAP2-patient and control macrophages treated with CCL2. DNA marker DAPI is in blue. **b,** Percent patient and control macrophages with colocalized SKAP2 and WASp (n=3, ns=not significant). c, Western blot of ERK phosphorylation in pRGD plated control and SKAP2 patient macrophages. Scale bar=15μm.

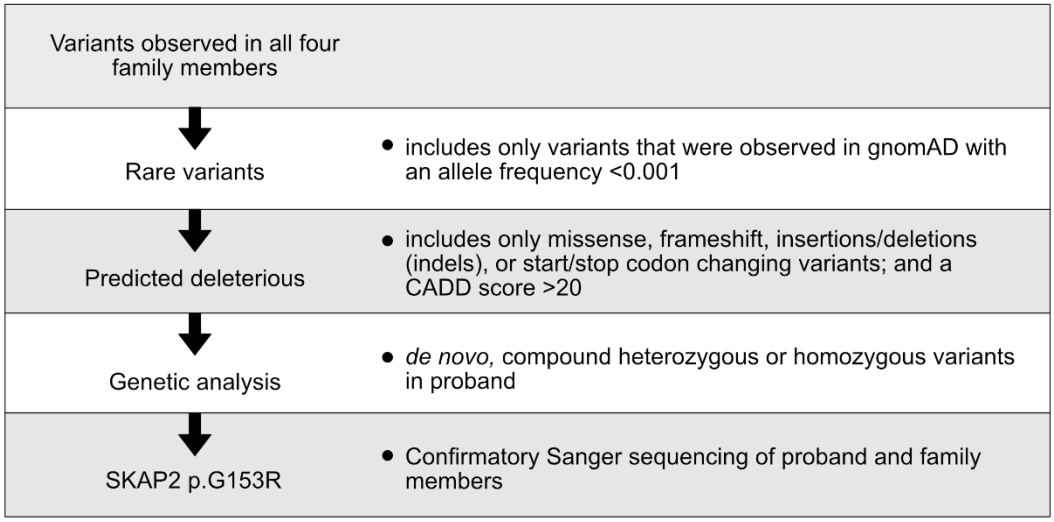


Figure S6. Flow chart for whole exome sequencing and analysis. Variants identified by whole exome sequencing were filtered using three criteria. First, only variants with an allele frequency <0.001% within the Genome Aggregation Database (gnomAD) database were kept. gnomAD is a highly annotated, searchable database of 141,456 human exome and genome sequences(1). Second, only variants with a scaled Combined Annotation-Dependent Depletion (CADD) score >20 were selected. The scaled CADD score is an integrative annotation built from more than 60 genomic features, and variants with a scaled CADD score of >20 are predicted to be in the top 1% of deleterious variants in the human genome (2). Third, only variants that were present in the proband and absent in unaffected family members were kept. Confirmation of SKAP2 variant was done by Sanger sequencing.

Figure S7. ImageJ macro used for shape analysis.

ImageJ Cell Shape Macro

```
(Language = IJ1 Macro)
setSlice(1);
id = getImageID ();
dir = getDirectory ("image");
name = getTitle;
name = "Intesity" + name + ".csv";
run("Duplicate...", " ");
setAutoThreshold("Triangle");
setOption("BlackBackground", false);
run("Convert to Mask");
run("Invert");
run("Fill Holes");
run ("Watershed");
run("Set Measurements...", "area mean min shape redirect=None decimal=3");
run("Analyze Particles...", "size=500-Infinity pixel show=Overlay display exclude clear
include summarize add");
selectImage (id);
run("Duplicate...", "use");
run("From ROI Manager");
run("ROI Manager...");
roiManager("Measure");
selectWindow ("Results");
saveAs ("Results", dir+name);
        close();
```

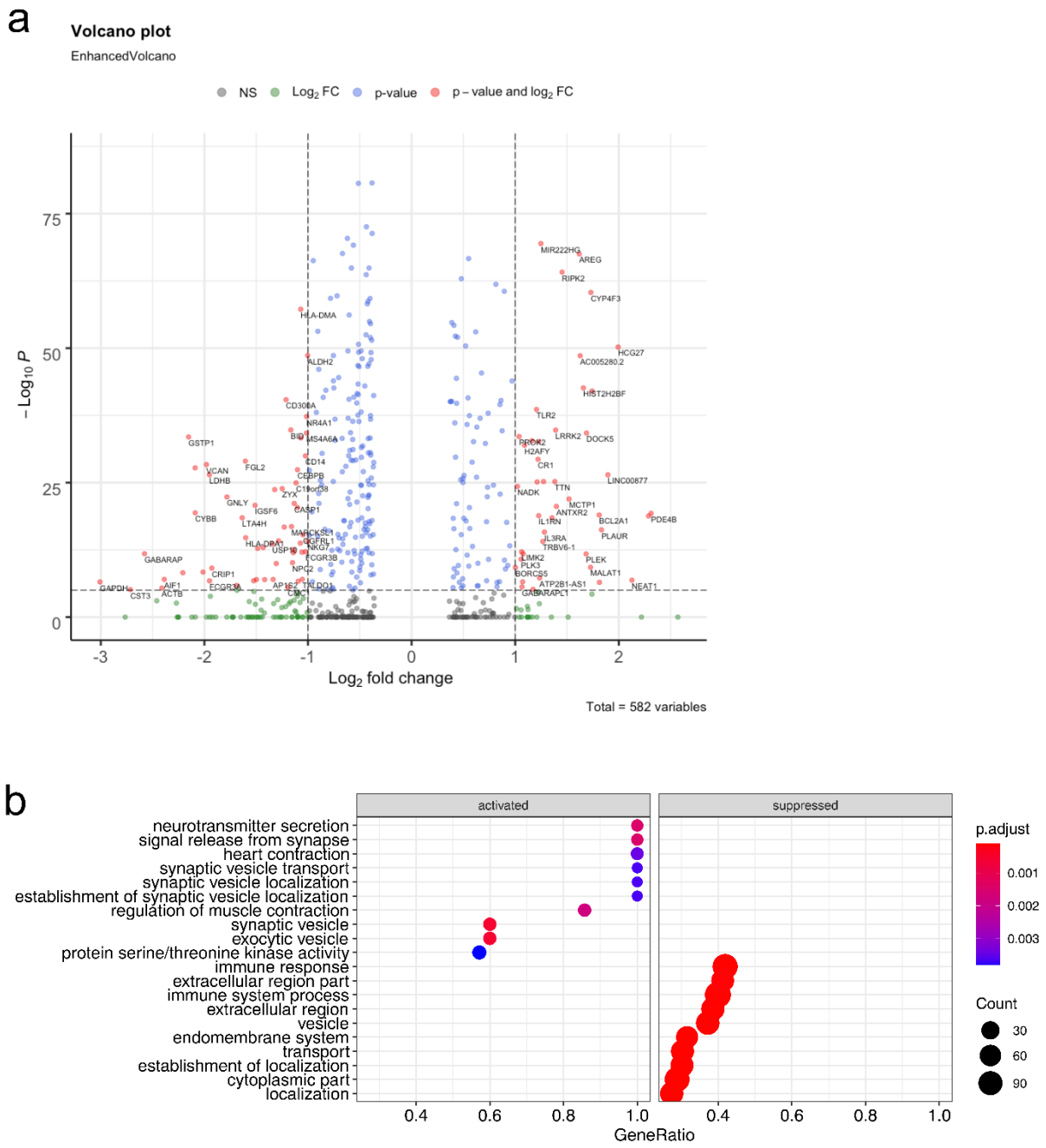


Figure S8: Single cell RNA sequencing of patient and control neutrophils

a, Volcano Plot showing the differential gene expression in the neutrophil cluster of the SKAP2 p.Gly153Arg patient against the control's neutrophil cluster. b, Pathway analysis of the SKAP2 patient's neutrophil cluster against the control.

1. Carrero JA, McCarthy DP, Ferris ST, Wan X, Hu H, Zinselmeyer BH, et al. Resident macrophages of pancreatic islets have a seminal role in the initiation of autoimmune diabetes of NOD mice. *Proc Natl Acad Sci U S A.* 2017;114(48):E10418-E27.
2. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, and Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet.* 2014;46(3):310-5.
3. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. *Science.* 2015;347(6220):1260419.
4. Heng TS, Painter MW, and Immunological Genome Project C. The Immunological Genome Project: networks of gene expression in immune cells. *Nat Immunol.* 2008;9(10):1091-4.