SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table. 1 Additional SNPs identified in *ADA2* via targeted Sanger sequencing. Clinical significance were update from gnomAD v4.1.0 (1). MAF, mean allele frequency

Patient	Variant	Clinical significance	MAF
P1	c.159C>T, p.(Asn53=)	Benign	0.495
	c.213G>A, p.(Met71Ile)	/	0.00000681
	c.1359T>C, p.(Tyr453=)	Benign	0.307
	c.1386T>C, p.(Ile462=)	Likely Benign	0.000102
P2	c.159C>T, p.(Asn53=)	Benign	0.495
	c.213G>A, p.(Met71Ile)	/	0.00000681
	c.1386T>C, p.(Ile462=)	Likely Benign	0.000102
P3	c.159C>T, p.(Asn53=)	Benign	0.495
	c.1359T>C, p.(Tyr453=)	Benign	0.307
P5	c.159C>T, p.(Asn53=)	Benign	0.495
P7	c.159C>T, p.(Asn53=)	Benign	0.495
P8	c.159C>T, p.(Asn53=)	Benign	0.495

Supplemental Table. 2 Overview of site-directed mutagenesis primers

Variant	Forward primer	Reverse primer
G47A	ATGCGGCTGGCGGGGCGGCTG	CATCTTTTCTTTCAACAACAGATGCGC
G47R	GATGCGGCTGAGGGGGCGGCT	ATCTTTTCTTTCAACAACAGATGCGCCCGTG
G47V	ATGCGGCTGGTGGGGCGGCTG	CATCTTTTCTTTCAACAACAGATGCGCCCGTG
G47W	GATGCGGCTGTGGGGGCGGCT	ATCTTTTCTTTCAACAACAGATGCGCCCGTG
R169Q	GAGGATTATCAGAAGCGGGTG	CAGCAGAATCCACTTGGAAC
E328K	GGTGGGGCATAAGGACACTGG	AGGTCAAACCCTGCCACC
F355L	CTTACTTCTTACACGCCGGAG	GCAGCTTAACGCCATCCT
T360A	CGCCGGAGAAGCAGACTGGCA	TGGAAGAAGTAAGGCAGCTTAAC
N370K	TAGACAGGAAAATTCTGGATGCTC	TGGAAGTACCCTGCCAGT
H424N	CTTGAGGAACAACCCTTAGC	TCAGACACCAGTTTCAGC
Y453C	GGCTTGTCCTGTGATTTCTATG	TTTGGCACCAAACATAGC

10 Supplemental Table. 3 Overview of ICD-10 codes

	ICD-10 code	Name
Liver	K70.1	Hepatitis (chronic): alcoholic
	K71.-	Hepatitis (chronic): drug-induced
	K75.3	Hepatitis (chronic): granulomatous NEC
	K75.2	Hepatitis (chronic): reactive, non-specific
	B15 - B19	Hepatitis (chronic): viral
	K73.0	Chronic persistent hepatitis, not elsewhere classified
	K73.1	Chronic lobular hepatitis, not elsewhere classified
	K73.2	Chronic active hepatitis, not elsewhere classified
	K73.8	Other chronic hepatitis, not elsewhere classified
	K73.9	Chronic hepatitis, unspecified
	K74.6	Other and unspecified cirrhosis of liver
	K76.6	Portal hypertension
	K76.8	Other specified diseases of liver
	K76.9	Liver disease, unspecified
Stroke	G46.4	Cerebellar stroke syndrome
	G46.5	Pure motor lacunar syndrome
	G46.6	Pure sensory lacunar syndrome
	G46.7	Other lacunar syndromes
	G46.3	Brain stem stroke syndrome
	G45	Transient cerebral ischaemic attacks and related syndromes
	G45.8	Other transient cerebral ischaemic attacks and related syndromes
	G45.9	Transient cerebral ischaemic attack, unspecified
Other	D83	Common variable immunodeficiency
	M32	SLE
	L95.0	Vasculitis
	D60	Acquired pure red cell aplasia

	D60.1	Transient acquired pure red cell aplasia
	D60.8	Other acquired pure red cell aplasias
	D60.9	Acquired pure red cell aplasia, unspecified
	D61.0	Constitutional aplastic anaemia
	D61.3	Idiopathic aplastic anaemia
	D61.8	Other specific aplastic anaemia
	D61.9	Aplastic anaemia, unspecified
	D70	Agranulocytosis
	D69.3	Idiopathic thrombocytopenic purpura
	D69.4	Other primary thrombocytopenia
	D69.5	Secondary thrombocytopenia
	D69.6	Thrombocytopenia, unspecified

11

12

Supplemental Table. 4 Clinical manifestations of the ten DADA2 carriers from seven unrelated kindreds. *: recurrent verruca vulgaris; **: upper respiratory tract infections necessitating frequent antibiotic therapy. ***: retinal vasculitis, uveitis and vitritis. ****: termed as erythromelalgia.

Patient no. (Kindred no.)	**P1(F1)**	**P2(F1)**	**P3(F2)**	**P4(F2)**	**P5(F3)**	**P6(F4)**	**P7(F5)**	**P8(F5)**	**P9(F6)**	**P10(F7)**	Cumulative N° of patient
***ADA2* mutation**	p.H424N/WT		p.G47V/WT			p.R169Q/WT		p.G47V/WT	p.R169Q/WT	p.G47R/WT	
Manifestations											
(Muco)cutaneous		**+**		**+**	**+**			**+**		**+**	**5/10**
Livedo		+						+			2/10
Raynaud phenomenon				+							1/10
Non-specific cutaneous vasculopathic lesions, including chilblain-like lesions					+****					+	2/10
Neurological	**+**					**+**			**+**		**3/10**
Ischemic stroke	+								+		2/10
White matter lesions						+					1/10
Immunological/ hematological			**+**	**+**	**+**	**+**					**4/10**
Hypogammaglobulinemia			+		+	+					3/10
Insufficient pneumococcal antibody response			+			+					2/10
Neutropenia			+								1/10
Thrombocytopenia			+								1/10
Deep venous thrombosis/ pulmonary embolism				+/+							1/10
Infections			**+**	**+**	**+**						**3/10**
Viral			+		+*						2/10
Bacterial			+		+**						2/10
Gastro-intestinal			**+**						**+**	**+**	**3/10**
Abdominal pain			+						+		2/10

Chronic dyspepsia			+								1/10
Nodular regenerative hyperplasia			+								1/10
Portal hypertension			+								1/10
Hematemesis										+	1/10
Muscoloskeletal							**+**			**+**	**2/10**
Arthritis							+			+	2/10
Tenditinis										+	1/10
Cardiovascular										**+**	**1/10**
Pericarditis										+	1/10
Ocular						**+*** **					**1/10**
Treatment							**+**				**1/10**
TNF-inhibitor							+				1/10

15

Supplemental Table. 5 Immunological blood results including immunological phenotype, immunoglobulin levels and auto-antibodies of patient 3 (P3), patient 5 (P5) and patient 6 (P6). -: negative; /: not applicable; *, prior to IVIG treatment; **, homogenous nuclear pattern, titer 1:80; ***, p-ANCA titer 1:80.

		P1	P3		P5	P6	P7	P9
	Ref. value							
Hemoglobin	12.0-16.0 g/L	13.4	14.4	14.0	13.3	15.8	15	/
Platelets	150 000-450 000/L	297 000	159 000	163 000	265 000	259 000	313000	/
White blood count	4 500-13 000/µL	8 820	5 850	5 760	4 890	10 650	9 670	6 970
Neutrophils	1 800-8 000/µL	4 730	3 600	3 600	2 700	5 500	6 700	4 070
Monocytes	600/µL	600	400	300	300	400	500	690
Lymphocytes	1 000-5 300/µL	3 290	1 802	1 800	1 700	4 500	2 100	1 920
T cells (CD3+)	800-3 500/µL	2 740	1 297	1 466	1 404	3 412	1 592	1 425
CD4+	400-2 100/µL	1 722	749	908	891	2 611	951	943
CD8+	200-1 200/µL	822	429	457	434	836	620	362
CD56+	4.3–16.2% of CD3+	3.2%	12.7%	10.4%	83%	0.3%	3.4%	4.5%
HLA-DR+	2.3-8.6% of CD3+	12.3%	6.4%	7.5%	4%	11.1%	15.0%	12.8%
CD27+ CD45RA+	40.9-65.7% of CD3+	72.5%	49%	46.5%	66.8%	56.5%	36.1%	42.1%
CD4+/CD25+CD127 low	5.0-12.0% of CD4+	/	5.6%	8.9%	7.2%	6%	/	/
T-cell receptor								
αβ TcR	87-99.3% of CD3+	94.6%	88.10%	90.26%	94.3%	98.53%	99.46%	92.94%
γδ TcR	3.3-10% of CD3+	5.5%	10.5%	10.0%	5.2%	1.4%	0.3%	6.3%
CD3+/CD4-CD8-	4.3–10.7% of CD3+	5.2%	9.7%	9.0%	5.6%	1%	1.5%	7.0%
B cells (CD19+)	200-600/µL	394	251	222	209	956	252	209
CD27+ IgM+ IgD+	2.6-13.4% of CD19+	6.4%	9.9%	22.4%	12%	9.4%	25.6%	7.8%
CD27+ IgM- IgD-	4.0-21.2% of CD19+	5.4%	5.9%	10.6%	8.4%	4.3%	14.5%	16.5%
CD27- IgM+ IgD+	61.6-87.4% of CD19+	84.7%	78.5%	61.7%	72.1%	84.8%	53.7%	70.1%
NK cells (CD3-/CD56 and/or CD16+)	70-1200/µL	145	191	86	105	100	268	293
IgG	5.76-12.65 g/L	11.90	5.03*	12.5	10.7	4.43*	17.10	/

IgG2	1.06-6.10 g/L	1.53	1.05*	4.10	3.03	0.77*	5.43	
IgG3	0.18-1.63 g/L	0.78	0.33*	0.33	0.27	0.18*	0.36	
IgA	0.81-2.32 g/L	2.70	0.44*	0.84	0.68	1.37*	1.56	
IgM	0.30-1.59 g/L	0.90	1.29*	1.22	0.66	0.75*	1.69	
Lymphocyte stimulation test								
Candida-index	≥ 5.00	/	43.17	/	/	/	/	/
Tetanus toxoid-index	≥ 5.00		5.53		/	132.27		/
PHA-index	≥ 5.00		31.04		25.08	247.82		44.55
Pneumococcal antibody response	Prior to vaccination							
Pn type 8	0.5 mg/L	/	2.0 mg/L	/		1.4 mg/L	/	/
Pn type 9N	0.7-0.9 mg/L		2.3 mg/L		/	0.8 mg/L		
Pn type 15B	1.2-1.9 mg/L		4.6 mg/L			1.7 mg/L		
Auto-antibodies								
ANA			-	-	-	-	positive**	
ANCA			-	-	-	-	positive***	
Anti-parietal cell antibody - IIF			-					
Intrinsic factor antibody			-					
Smooth muscle			-					
Mitochondria – IIF			-					
Cardiac muscle			-					
Skeletal muscle			-					
Pancreas			-					
Adrenal gland		/	-					/
Glutamic acid decarboxylase 65kDa	< 0.9 kU/L		< 0.1 kU/L					
Insulin	≤ 5%		1%					
Skin			-					
Salivary gland			-					
Deamidated gliadin IgG	≤ 20.0 CU		< 2.8 CU					
TPO	≤ 34 IU/mL		9 IU/mL					
TSH-receptor antibodies	≤ 1.0 IU/L		< 1.0 IU/L					
Liver-kidney-microsome – IIF			-					

Supplemental Table. 6 Genetic characteristics and in silico prediction of pathogenicity of mutations in *ADA2* identified by whole exome sequencing. Genomic position according to the hg18 (GRCh38) physical position. NM_001282225.1 was used as reference transcript. CADD: Combined Annotation-Dependent Depletion; Polyphen: Polymorphism Phenotyping v2; AF: Allele Frequency, AF was derived using the gnomAD browser; SIFT: Sorting Intolerant From Tolerant (1).

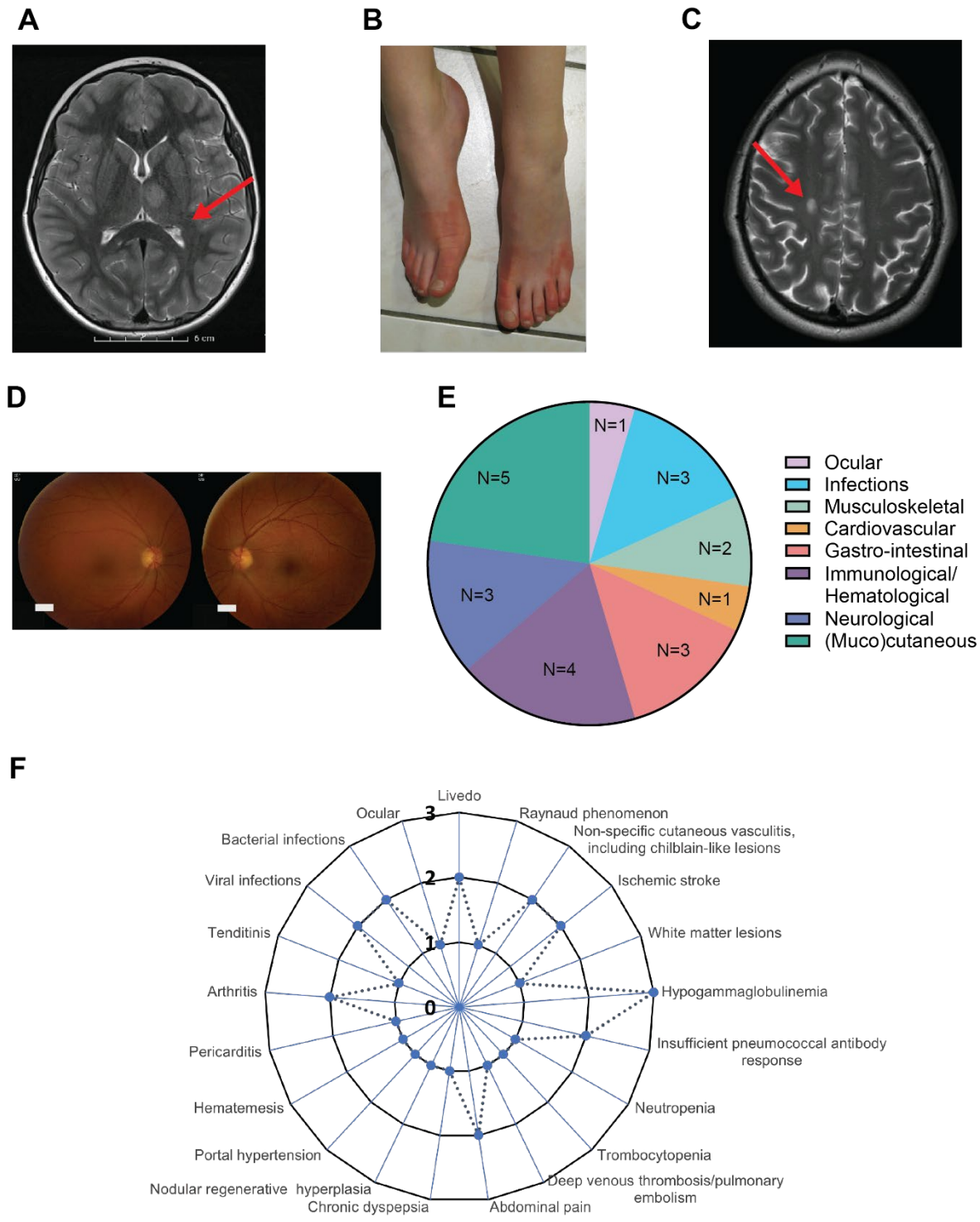
Patient	**P1-2**	**P3-5, P8**	**P6-7, P9**	**P10**
Chromosome	22			
Genomic position (substitution)	g.17181992G>T	g.17209538C>A	g.17207107C>T	g.17209539C>T
Ref SNP cluster ID	rs1416783635	rs200930463	rs77563738	rs202134424
cDNA position (substitution)	c.1270C>A	c.140G>T	c.506G>A	c.139G>A
Protein position substitution	p.H424N	p.G47V	p.R169Q	p.G47R
Zygosity	Heterozygous			
AF	0.0000006196	0.000048	0.00047	0.00006692
CADD	24.8	21.5	21.4	22.9
MSC	3.2			
Polyphen-2	Probably damaging			
SIFT	Pathogenic supporting			

Supplemental Table. 7 Genetic intolerance scores for ADA2. f parameter: frequency parameter; lofTool: loss-of-function Tool; SIS: Selection Intensity Score; evoTol: Evolutionary Tolerance Score; RVIS: Residual Variation Intolerance Score; pLI: Probability of Loss-of-Function Intolerance; LOEUF: Loss-of-Function Observed/Expected Upper Fraction; CoNeS: Combined Network Score; IEI classification: Inborn Errors of Immunity Classification; IEND classification: Inborn Errors of Neurodevelopment classification; hOMIM classification: Human Online Mendelian Inheritance in Man Classification; IEI mode: Mode of Inheritance for Inborn Errors of Immunity; IEND mode of dominance: Mode of Dominance for Inborn Errors of Neurodevelopment; EOHP/LOIP: Early-Onset/ Late-Onset Immunodeficiency Predisposition; DOMINO: Dominance Inference for Inherited Disease Genes; p(HI): Probability of Haploinsufficiency; SCoNeS: Single Cell Network Score; SCoNeS in leave-one-out: Single Cell Network Score in Leave-One-Out Analysis.

Gene	***ADA2***
f parameter	0,56162038
lofTool	0,161
SIS	0,41382086
evoTol	4,7380157
RVIS	-0,10570191
pLI	8,1396E-08
LOEUF	0,68448
CoNeS	-0,115379718
IEI classification	IEI AR
IEND classification	IEI
hOMIM classification	IEI
IEI mode	NA
IEND mode of dominance	NA
EOHP/LOIP	LOIP
DOMINO	0,069576
p(HI)	0,37
SCoNeS	0,994
SCoNeS in leave-one-out	0,992

Supplemental Table. 8 Frequency of ADA2 dominant negative variants in the general population from gnomAD v4.1.0 (1) * ENST00000399837.8, ** There are 0 homozygotes in gnomAD for any of these variants

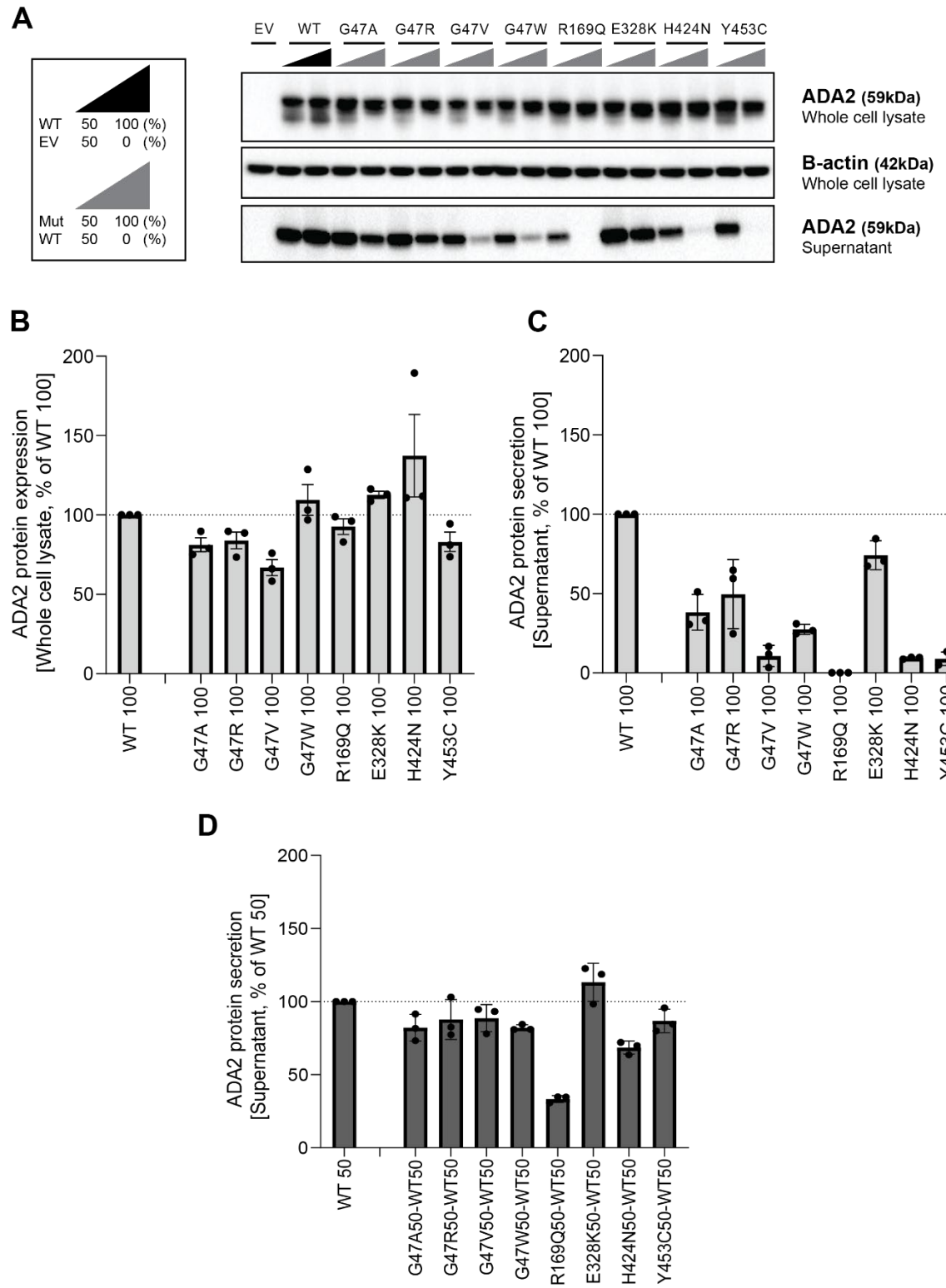
Variant MANE transcript* protein impact	GRCh38 coordinates	Allele count** in gnomAD v4.1.0	Allele Number in gnomAD v4.1.0	Pop max in gnomAD	AF Pop max in gnomAD
p.G47V	22-17209538-C-A	48	1,613,586	Middle Eastern	0.00033
p.G47A	22-17209538-C-G	78	1,613,704	Admixed American	0.000083
p.G47R	22-17209539-C-T	108	1,613,980	Middle Eastern	0.00082
p.G47R	22-17209539-C-G	34	1,613,982	South Asian	0.00011
p.R169Q	22-17207107-C-T	810	1,614,176	European (Finnish)	0.0018
p.E328K	Not present	Not present	NA	NA	NA
p.H424N	22-17181992-G-T	1	1,613,878	NA	NA
p.Y453C	22-17181904-T-C	183	1,614,136	European (non-Finnish)	0.00014



Supplemental Figure 1. Clinical and radiographic findings of the 10 DADA2 carriers.

A. Brain MRI of P1 revealing a diffusion restrictive T2-weighted hyperintense lesion with focus anteromedially in the left thalamus, indicating a recent ischemic infarct. **B**. Clinical image of P5 displaying painful purple to red skin discoloration and swelling of the feet. **C.** Brain MRI of P6 showing an oval lesion in the right centrum semiovale, hyperintense on T2 and FLAIR, hypointense on T1, with restricted diffusion and a maximum diameter of 11 mm. **D**. Fundoscopy of P6. The right eye (OD, oculus

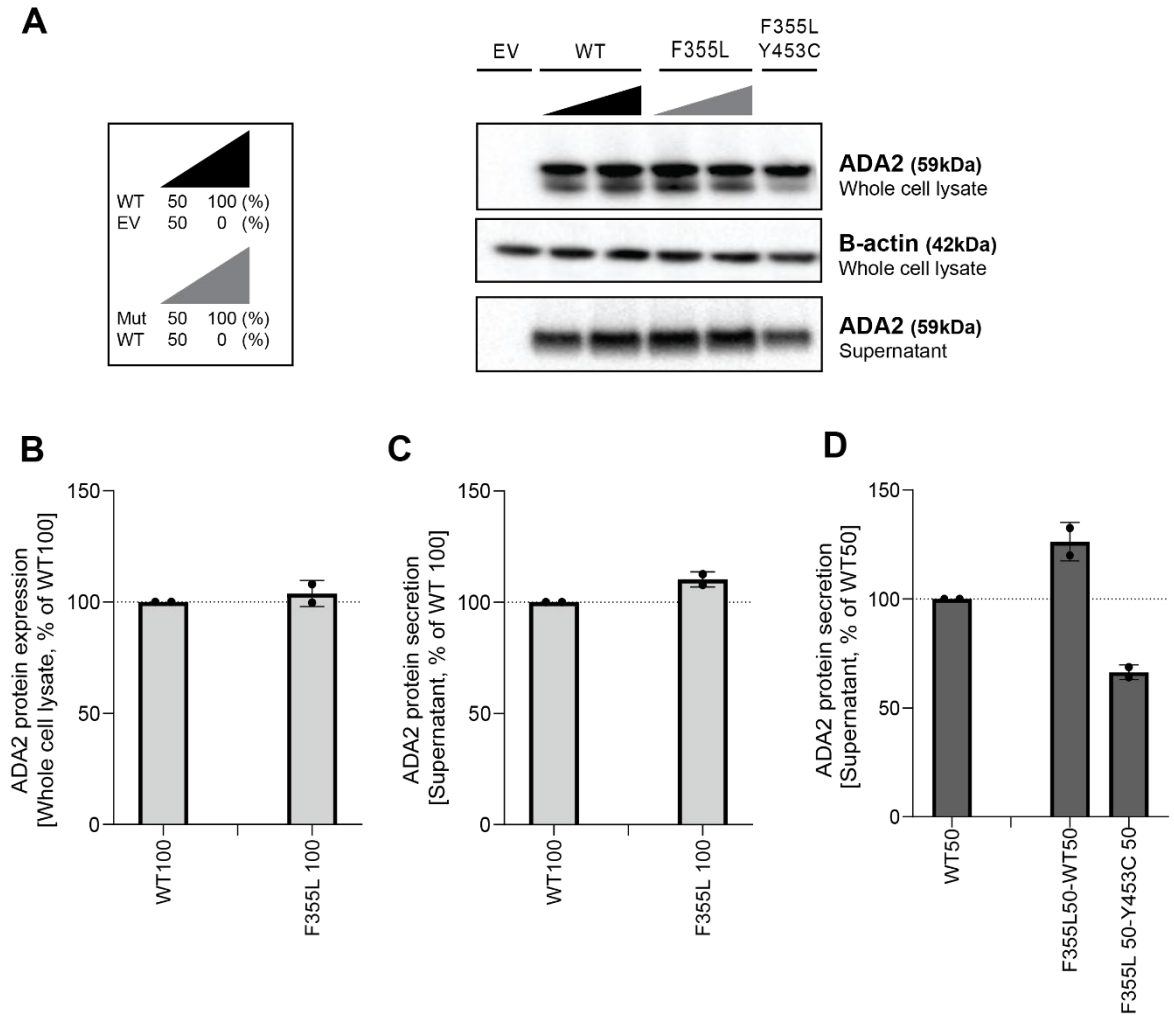
dexter) shows retinal vasculitis and retinitis with inferiorly located snow ball opacities; left eye (OS, oculus sinister) shows retinitis and vitritis. **E.** Circle diagram illustrating the phenotype distribution by absolute number of affected patients**. F.** Radar graph representing the number of patients affected by various clinical manifestations.



Supplemental Figure 2. ADA2 protein expression and secretion in homogenous and heterozygous state on denaturing gel.

A. Immunoblot of whole cell lysate and supernatants of HEK293T cells transfected with different ADA2 variants in homozygous state or together with WT ADA2 (heterozygous state). Cells and supernatant were collected 48h after transfection. Image shown represents 3 independent experiments. Loading control: B-actin. **B.** Quantification of ADA2 protein expression in whole cell lysate of transfected

HEK293T cells with wild-type ADA2 or ADA2 variants in homozygous conditions. Bar graphs represent percentage of ADA2 protein expression relative to wild-type ADA2 100%. **C.** Quantification of ADA2 secretion in supernatant of transfected HEK293T cells with wild-type ADA2 or ADA2 variants in homozygous conditions. Bar graphs represent percentage of ADA2 protein expression relative to wild-type ADA2 100%. **D.** Quantification of ADA2 secretion in supernatant co-transfected HEK293T cells of ADA2 variants together with wild-type in heterozygous conditions. Bar graphs represent percentage of ADA2 secretion relative to wild-type ADA2 50%. **A-D.** Each bar represents mean ± SD from 3 independent experiments.

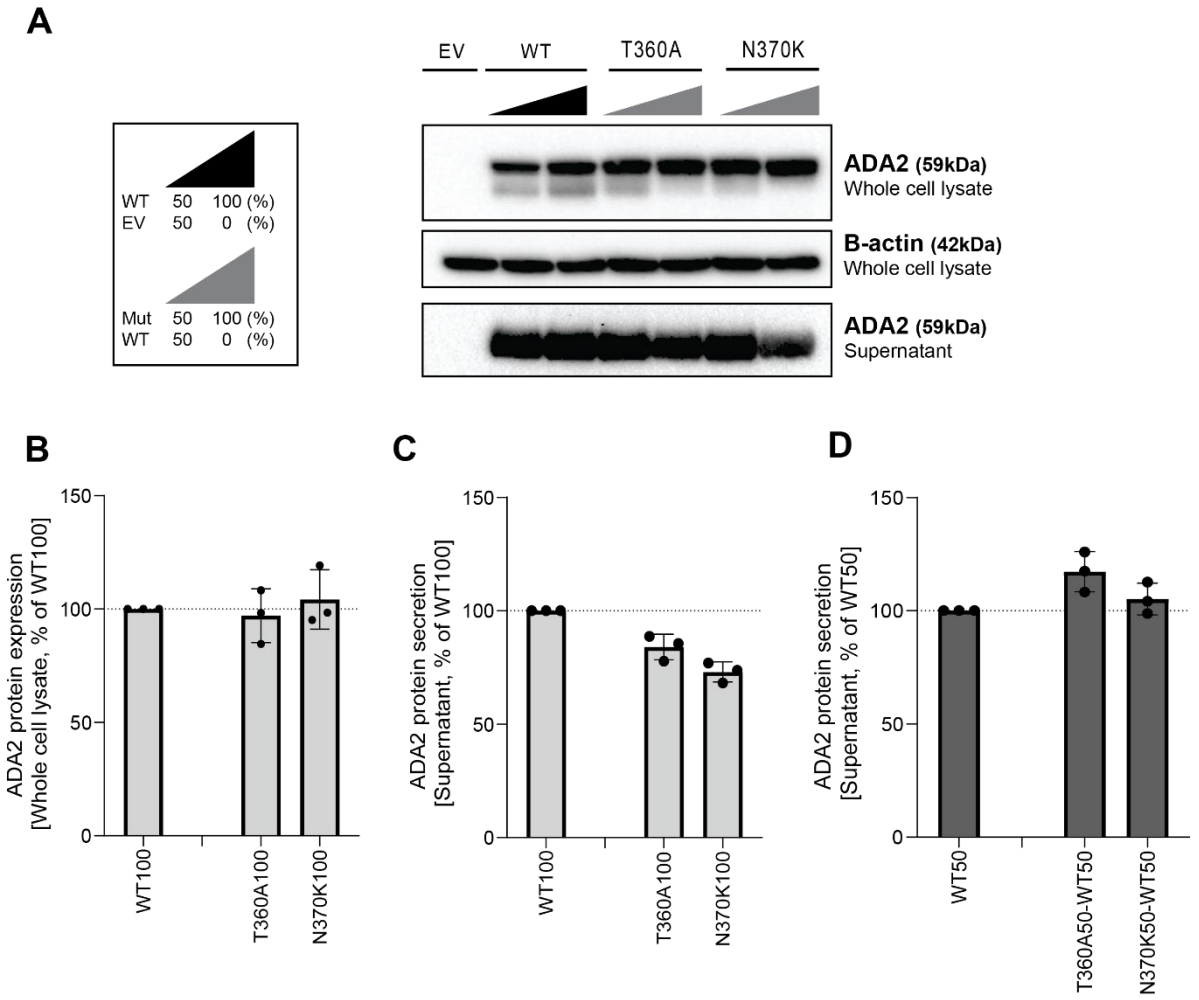


78

79 Supplemental Figure 3. ADA2 protein expression and secretion in homogenous and heterozygous state
80 of variant F355L on denaturing gel.

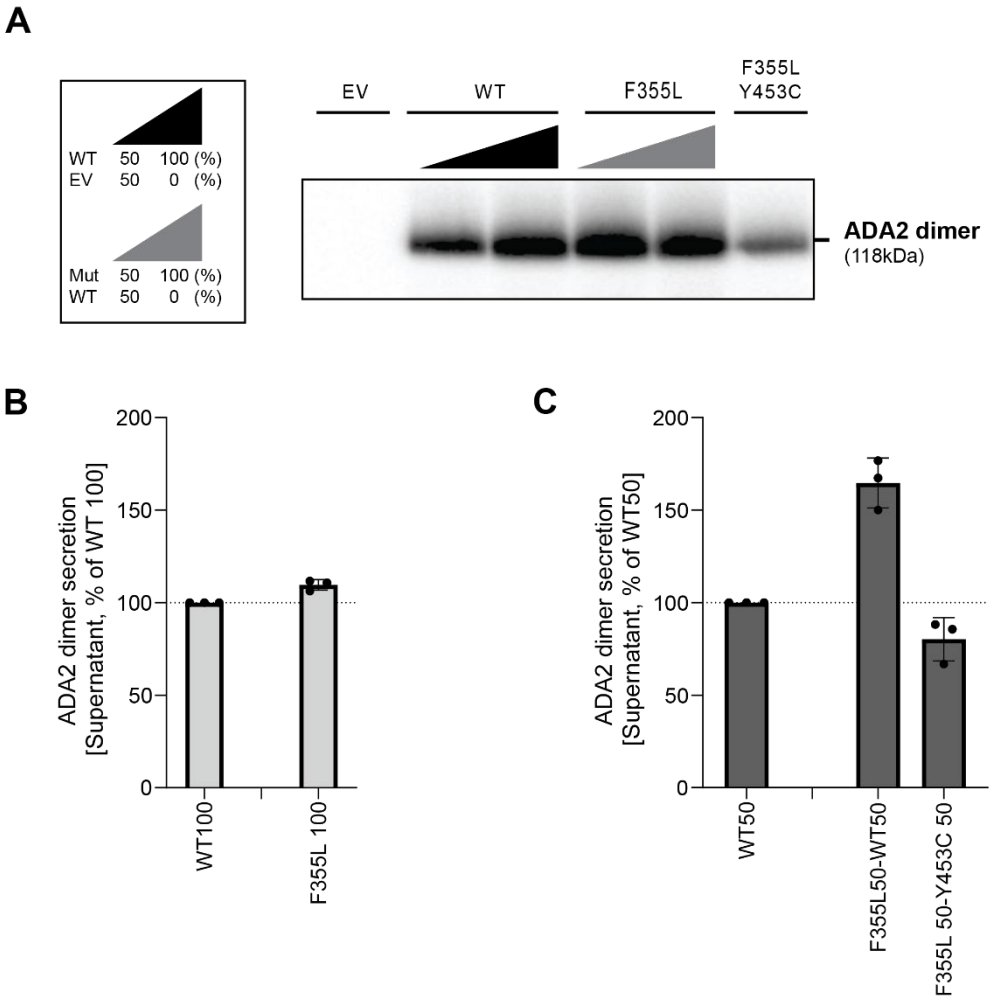
81 **A.** Immunoblot of whole cell lysate and supernatants of HEK293T cells transfected with ADA2 variant
82 F355L in homozygous state, together with WT ADA2 or with ADA2 variant Y453C in carrier state. Cells
83 and supernatant were collected 48h after transfection. Image shown represents 2 independent
84 experiments. Loading control: B-actin. **B.** Quantification of ADA2 protein expression in whole cell lysate
85 of transfected HEK293T cells with wild-type ADA2 or ADA2 variant F355L in homozygous conditions.
86 Bar graphs represent percentage of ADA2 protein expression relative to wild-type ADA2 100%. **C.**
87 Quantification of ADA2 secretion in supernatant of transfected HEK293T cells with wild-type ADA2 or
88 ADA2 variant F355L in homozygous conditions. Bar graphs represent percentage of ADA2 protein
89 expression relative to wild-type ADA2 100%. **D.** Quantification of ADA2 secretion of co-transfected
90 HEK293T cells of ADA2 variant F355L together with wild-type in heterozygous conditions. Bar graphs
91 represent percentage of ADA2 secretion relative to wild-type ADA2 50%. . **A-D.** Each bar represents
92 mean ± SD from 2 independent experiments.

93



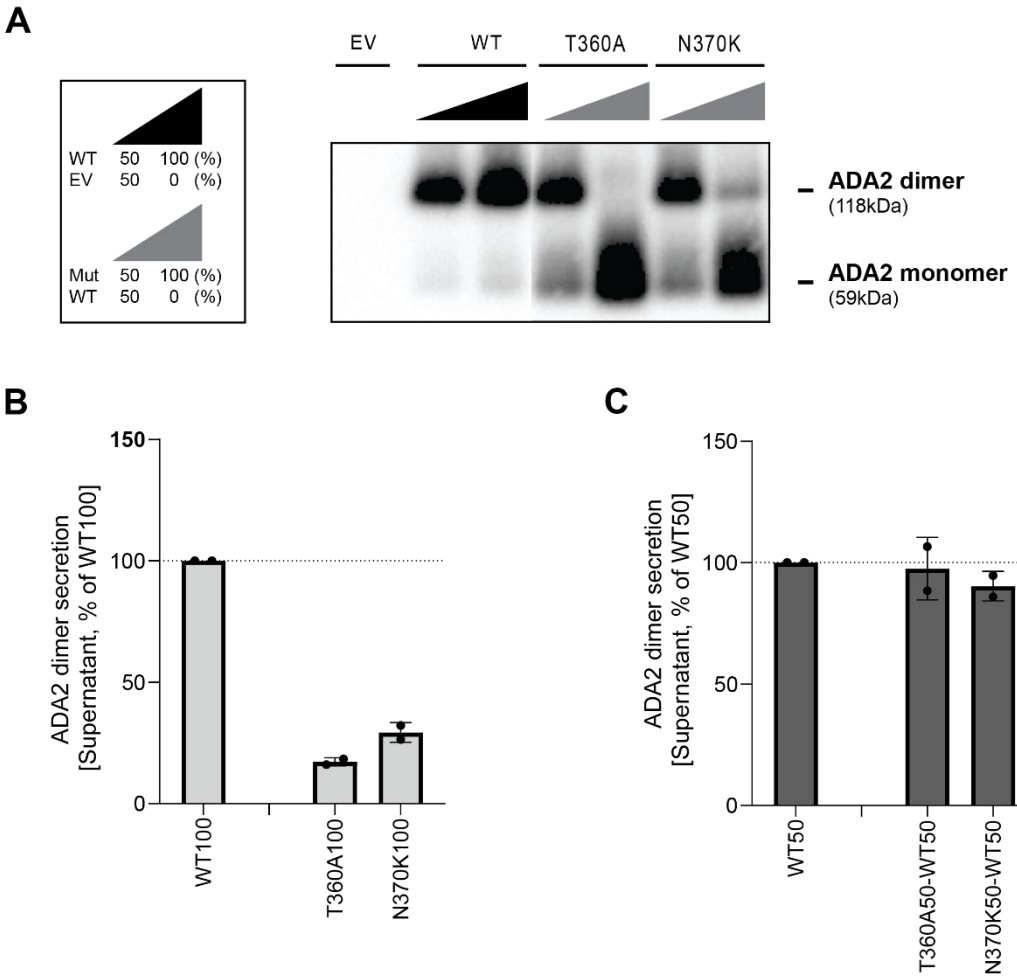
Supplemental Figure 4. ADA2 protein expression and secretion in homogenous and heterozygous state of variants T360A and N370K on denaturing gel.

A. Immunoblot of whole cell lysate and supernatants of HEK293T cells transfected with ADA2 variants T360A and N370K in homozygous state or together with WT ADA2. Cells and supernatant were collected 48h after transfection. Image shown represents 3 independent experiments. Loading control: B-actin. **B.** Quantification of ADA2 protein expression in whole cell lysate of transfected HEK293T cells with wild-type ADA2 or ADA2 variants T360A and N370K in homozygous conditions. Bar graphs represent percentage of ADA2 protein expression relative to wild-type ADA2 100%. **C.** Quantification of ADA2 secretion in supernatant of transfected HEK293T cells with wild-type ADA2 or ADA2 variants T360A and N370K in homozygous conditions. Bar graphs represent percentage of ADA2 protein expression relative to wild-type ADA2 100%. **D.** Quantification of ADA2 secretion of co-transfected HEK293T cells of ADA2 variants T360A and N370K together with wild-type in heterozygous conditions Bar graphs represent percentage of ADA2 secretion relative to wild-type ADA2 50%. experiments. **A-D.** Each bar represents mean ± SD from 3 independent experiments.



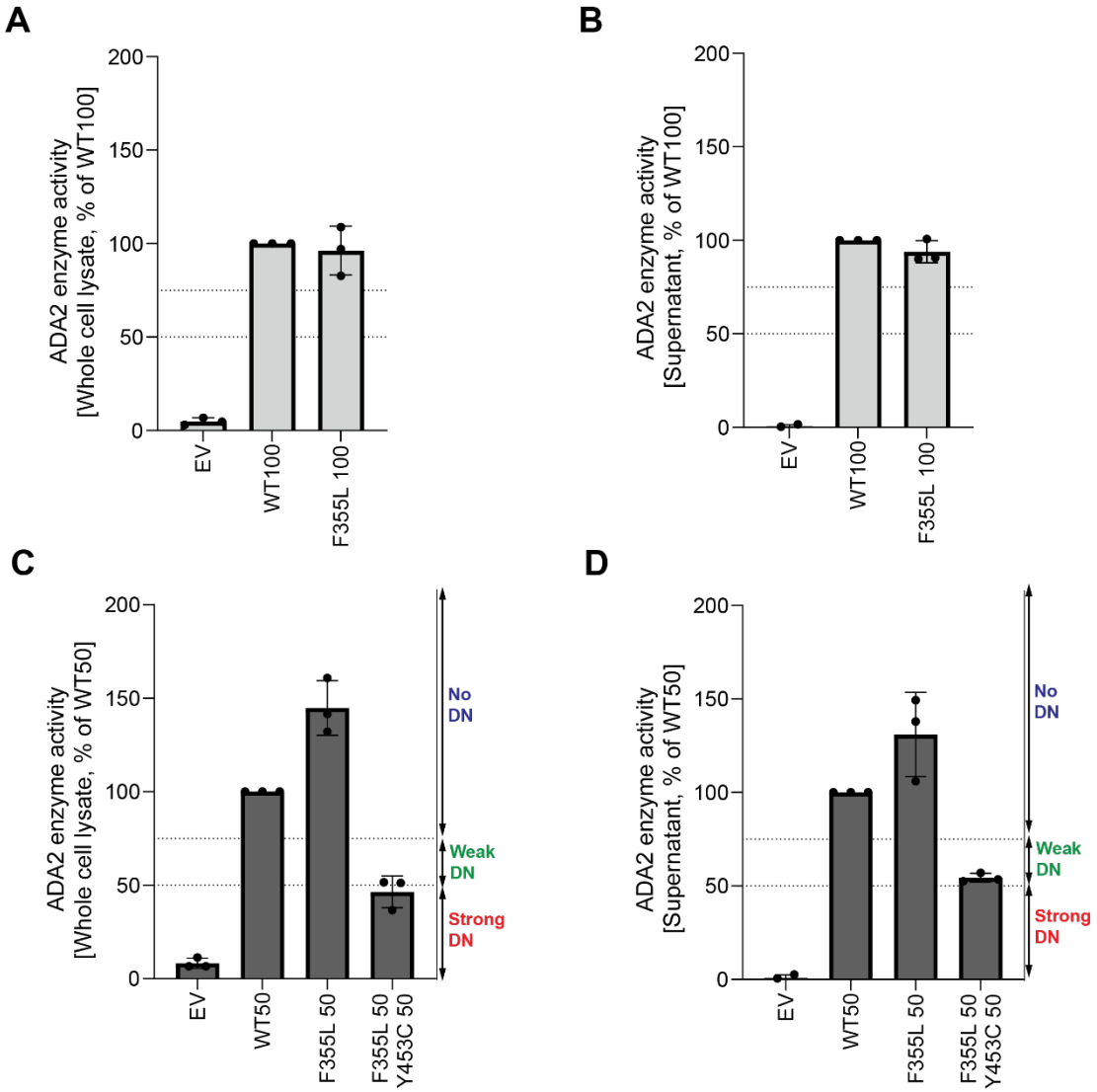
Supplemental Figure 5. Secretion of ADA2 dimers in homozygous or heterozygous state of variant F355L on non-denaturating gel.

A. ADA2 dimer secretion of HEK293T cells transfected with WT and/or ADA2 variant F355L. Cells and supernatant were collected 48h after transfection. Image shown represents 3 independent experiments. **B.** Quantification of ADA2 secretion in supernatant of transfected HEK293T cells with wild-type ADA2 or ADA2 variant F355L in homozygous conditions. Bar graphs represent percentage of ADA2 protein secretion relative to wild-type ADA2 100%. **C.** Quantification of ADA2 secretion of co-transfected HEK293T cells of ADA2 variant F355L together with wild-type ADA2 in heterozygous conditions. Bar graphs represent percentage of ADA2 secretion relative to wild-type ADA2 50%. **B-C.** Each bar represents mean ± SD from 3 independent experiments.



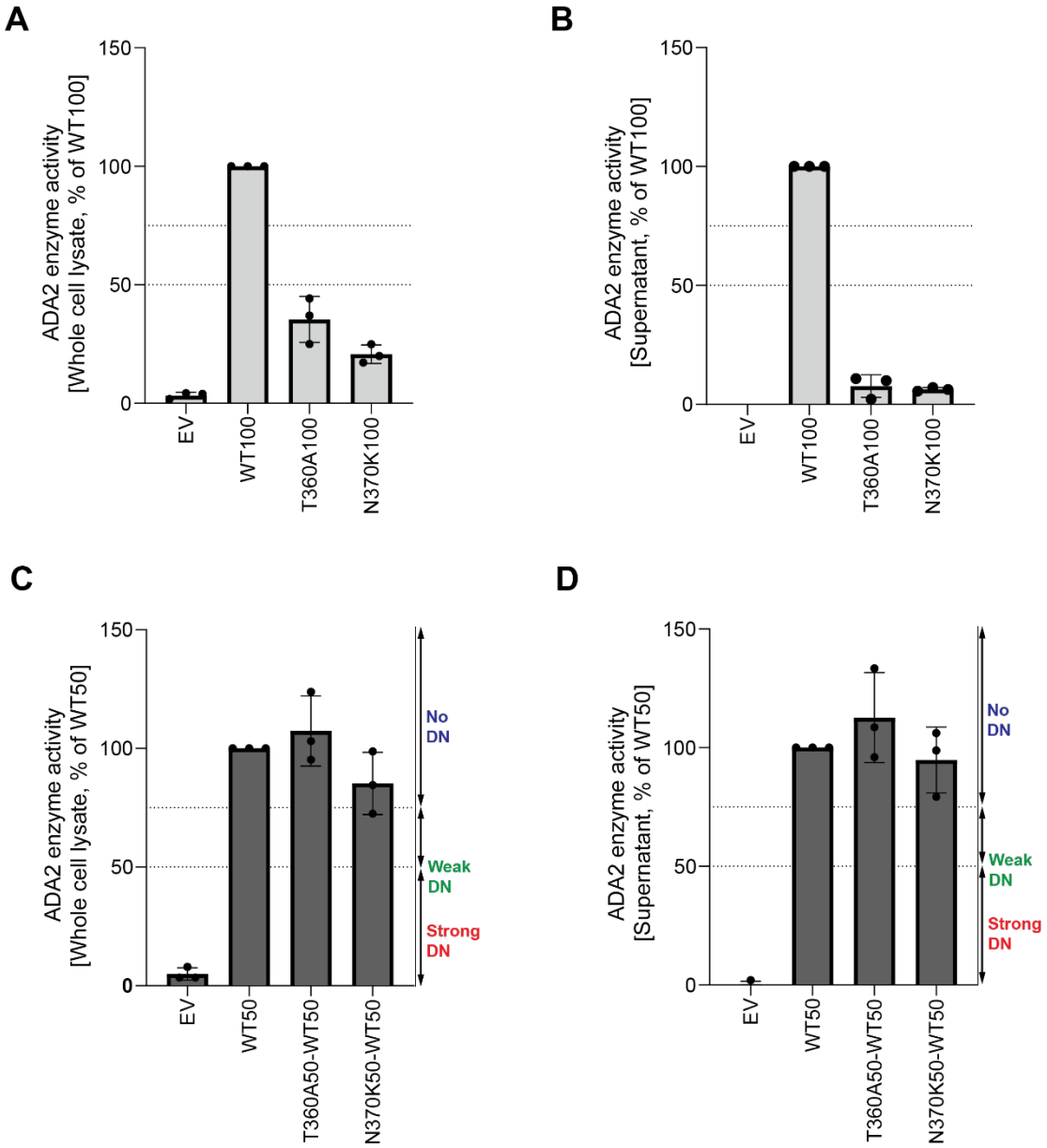
Supplemental Figure 6. Secretion of ADA2 dimers in homozygous or heterozygous state of ADA2 variants T360A and N370K on non-denaturating gel.

A. ADA2 dimer secretion of HEK293T cells transfected with WT and/or ADA2 variants T360A and N370K. Cells and supernatant were collected 48h after transfection. Image shown represents 2 independent experiments. **B.** Quantification of ADA2 secretion in supernatant of transfected HEK293T cells with wild-type ADA2 or ADA2 variants T360A and N370K in homozygous conditions. Bar graphs represent percentage of ADA2 protein secretion relative to wild-type ADA2 100%. **C.** Quantification of ADA2 secretion of co-transfected HEK293T cells of ADA2 variants T360A and N370K together with wild-type ADA2 in heterozygous conditions. Bar graphs represent percentage of ADA2 secretion relative to wild-type ADA2 50%. **B-C.** Each bar represents mean ± SD from 3 independent experiments.



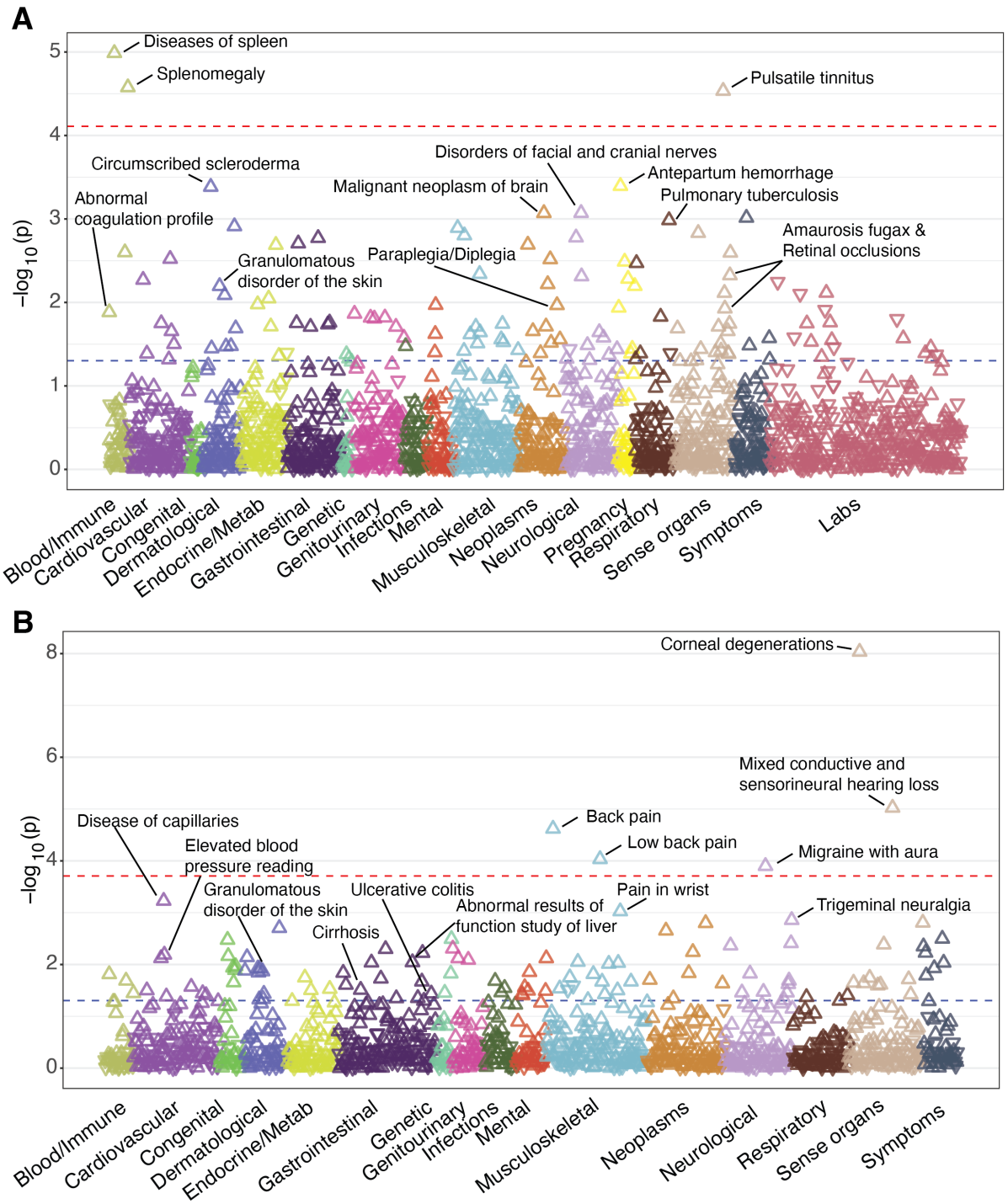
Supplemental Figure 7. Adenosine deaminase activity of variant F355L in ADA2 in homozygous or heterozygous state.

A. Adenosine deaminase activity in whole cell lysate of HEK293T transfected cells with WT and ADA2 variant F355L in homozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 100%. **B.** Adenosine deaminase activity in supernatant of HEK293T transfected cells with WT and ADA2 variant F355L in homozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 100%. **C.** Adenosine deaminase activity in whole cell lysate of HEK293T transfected cells with WT and/or ADA2 variant F355L in heterozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 50%.. **D.** Adenosine deaminase activity in supernatant of HEK293T transfected cells with WT and/or ADA2 variant F355L in heterozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 50%. **A-D.** Data represents mean ± SD from 3 independent experiments.



Supplemental Figure 8. Adenosine deaminase activity of ADA2 variants T360A and N370K in homozygous or heterozygous state.

A. Adenosine deaminase activity in whole cell lysate of HEK293T transfected cells with WT and ADA2 variants T360A and N370K in homozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 100%. **B.** Adenosine deaminase activity in supernatant of HEK293T transfected cells with WT and ADA2 variants T360A and N370K in homozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 100%. **C.** Adenosine deaminase activity whole cell lysate of HEK293T transfected cells with WT and/or ADA2 variants T360A and N370K in heterozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 50%. **D.** Adenosine deaminase activity in supernatant of HEK293T transfected cells with WT and/or ADA2 variants T360A and N370K in heterozygous conditions. Bar graphs represent the percentage of enzymatic activity relative to wild-type ADA2 50%. **A-D.** Data represents mean ± SD from 3 independent experiments.



Supplemental Figure 9. PheWAS of *ADA2* pLOFs in the Bio*Me* BioBank and the UK Biobank.

A. Gene-based PheWAS results in the Bio*Me* BioBank (*n* = 27,742). **B.** Gene-based PheWAS results in the UK Biobank (*n* = 189,440). The direction of the triangles indicates the direction of effect (up: increased risk, down: decreased risk). The red dashed line represents false discovery rate (FDR)-adjusted *P* value threshold, whereas the blue dashed line indicates the nominal significance level (*P* = 0.05).

References:

1. Karczewski KJ, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434–443.