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Supplemental information

Bi-allelic variants in *NAE1* cause intellectual disability, ischiopubic hypoplasia, stress-mediated lymphopenia and neurodegeneration

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Supplemental Note: Detailed case reports of individuals with *NAE1* genetic variants.

Individual 1:

The first individual is the second child of healthy, Dutch, non-consanguineous parents. During pregnancy, the girl was diagnosed with an aortic coarctation and a ventricular septal defect. After birth, several craniofacial dysmorphias were noted. She has underdeveloped denture and a strikingly asymmetrical palate. During infancy, failure to thrive, generalized hypotonia, mild developmental delay and feeding difficulties were noted. At the age of one she was admitted to the ICU due to a lower respiratory tract infection, which was treated with antibiotics and dexamethasone. This admission was followed by a loss of milestones and a debut of profound therapy resistant epilepsy. Brain MRI, which had been normal, now revealed brain atrophy as evidenced by enlarged ventricles, diminished white mattervolume and a thin corpus callosum. Laboratory tests revealed lymphopenia, leukopenia, hyperglycemia, AST, ALT and AF increase, anemia and decreased immunoglobulin levels. Other laboratory values were within normal range. Currently, her development is still severely delayed. She cannot sit without support; she can produce a few sounds but no words.

Individual 2:

Individual 2, is the first child of healthy, Dutch parents. His parents come from a region with known inbred population and cannot rule out distant relatedness. During pregnancy, he received multiple blood transfusions due to hemolysis as a result of rhesus incompatibility. At birth, craniofacial dysmorphias were noted: widely spaced eyes, a long small face, cheiloschisis, and an asymmetrical palate. His development was normal until the age of 14-months (rolling over at 7 months, sitting at 10 months). At 14 months, he was admitted to the ICU with severe pneumonia, and he was treated with antibiotics and dexamethasone. After ICU admission his development declined, he could not sit without support anymore. After initial regression, he slowly started to regain some of his developmental progress. Eventually, at two and a half years of age he was able to walk. Brain MRI showed diminished white matter volume, and thin corpus callosum. Laboratory tests revealed mild leukopenia and low immunoglobulin levels (G1, G2, G3 and M). He did not develop normal antibody titers after vaccination. During infections, lymphocytopenia and leukopenia were observed. He has recurrent episodes of Impetigo and HSV-1 infection, which can be severe. Currently, he can sit, walk and communicate verbally (produce complete sentences).

Individual 3:

Individual 3 was born as the first child to healthy, consanguineous (first-degree cousins) parents. Pregnancy and birth were unremarkable. As an infant, plagiocephaly, sleep difficulties, hyporeflexia, low muscle tone and spasms were noted. For the spasms, he was first treated with Levetiracetam, but this was ineffective. He was then given

ACTH with resolution of the infantile spasms. He had lost developmental milestones with the spasms but regained some of them after 6-weeks of ACTH treatment. His EEG showed interictal tracing with generalized background slowing and multifocal epileptiform discharges. Brain MRI showed severe, diffuse white matter volume loss. At 14 months of age, his development was still delayed. He could roll, but could not sit unassisted, he could say about 3 words with meaning. At 2.5 years of age, he can roll, sit with support, vocalize, and knows parents from strangers. With vigabatrin (113 mg/kg/day), his epilepsy is treated effectively. On physical examination, three small hyperpigmented lesions (right abdomen and left leg) were noted.

Individual 4:

Individual 4 was born after normal pregnancy to healthy, non- consanguineous parents. After birth, anteverted nares, large appearing ears, epicanthal folds, narrow palpebral fissures were noted, together with limited range of motion at the elbows, knees and feet. As an infant, she developed epilepsy, and it became clear that her development was severely delayed. Seizures and development worsened significantly during and after infections accompanied by fever. She had multiple respiratory infections, urinary tract infections and bacteremia's, for which she was admitted to the hospital repeatedly. Brain MRI performed at the age of 4.5 years showed progressive global volume loss (compared to earlier brain MRI taken at age 2.5), with marked ventriculomegaly, thinning of the white matter and the corpus callosum, and decreased cerebral cortex volume.

Figure S1: Analysis of bulk RNA sequencing data.

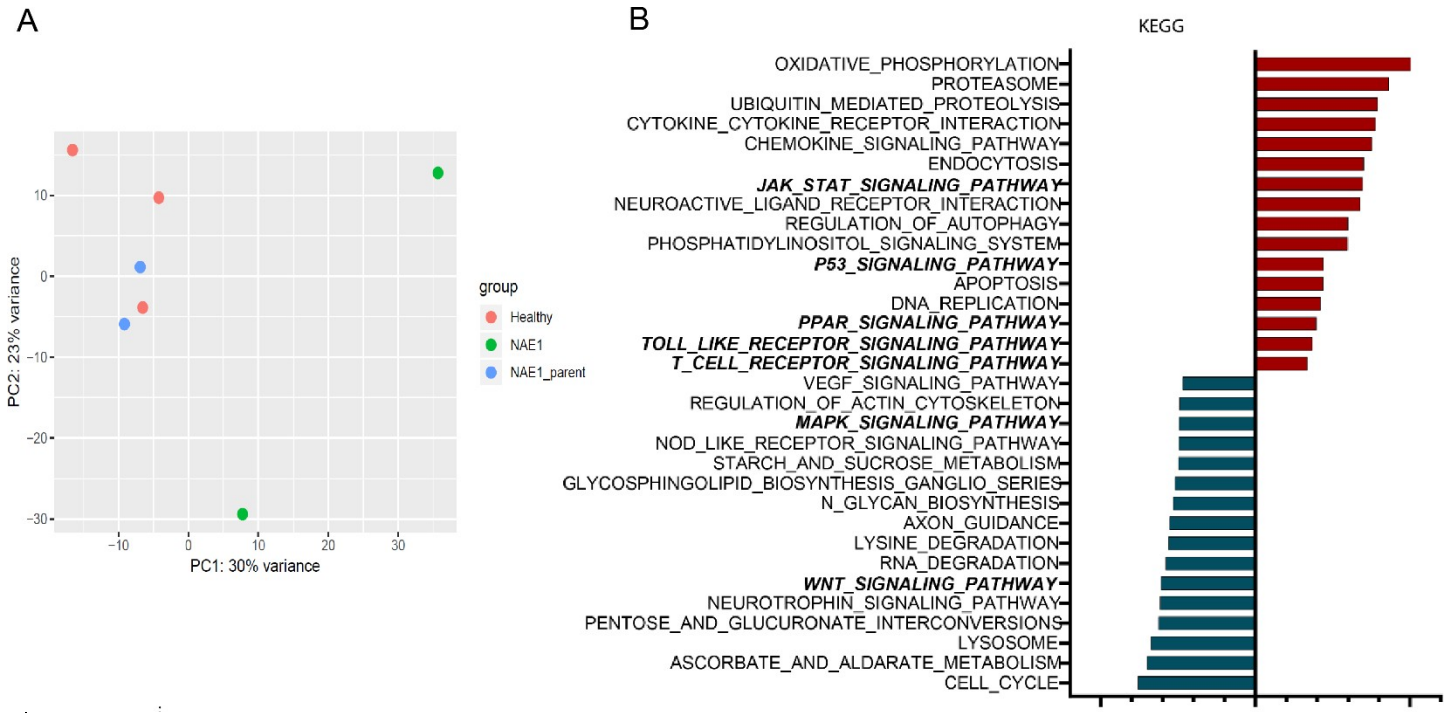


Figure S1: Analysis of bulk RNA sequencing data of individual 1 and individual 2 dermal fibroblasts, parents of individual 1 (heterozygous carriers), and three healthy controls. (A) PCA plot based on the top 2000 differentially expressed genes. (B) Pre-ranked Gene Set Enrichment analysis showing the top 32 significantly enriched (FDR<0.05) pathways (KEGG). Y-axis shows Normalized Enrichment Score (NES).

Figure S2: Gating strategy of flow cytometry experiments.

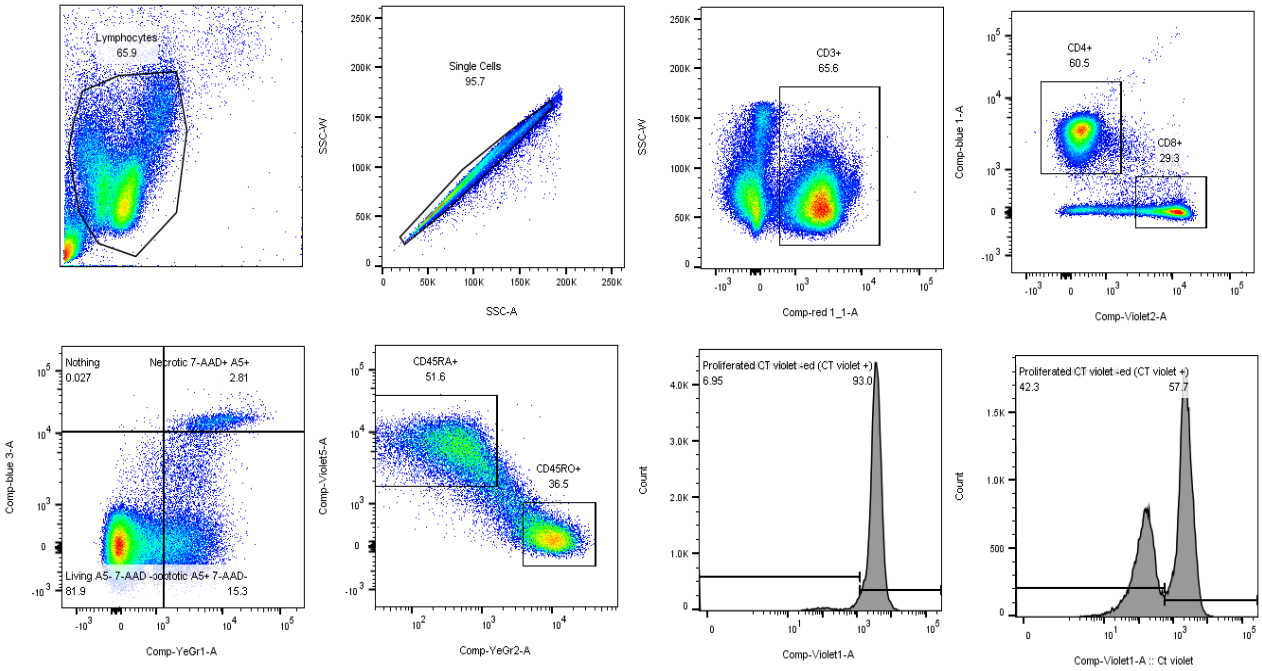


Figure S2: Flow Cytometry Gating Strategy. Lymphocytes were gated using FSC-A and SSC-A. Single cells were gated using SSC-A and SSC-W. Next, CD3, CD4, CD8, CD45RA, CD45RO cells were gated. Living cells were gated using Annexin V and 7-AAD staining.

Figure S3: Imagestream Gating Strategy.

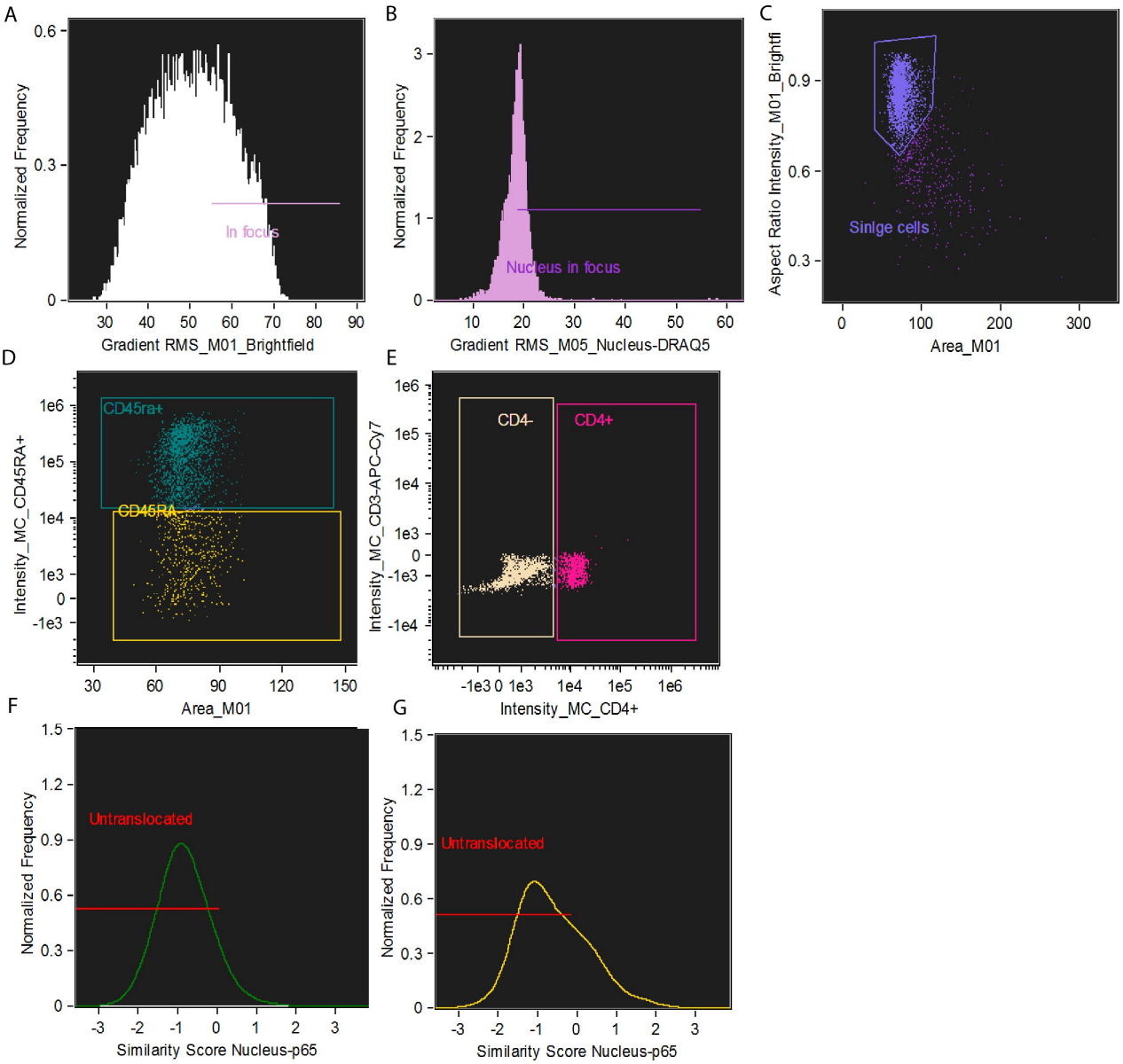
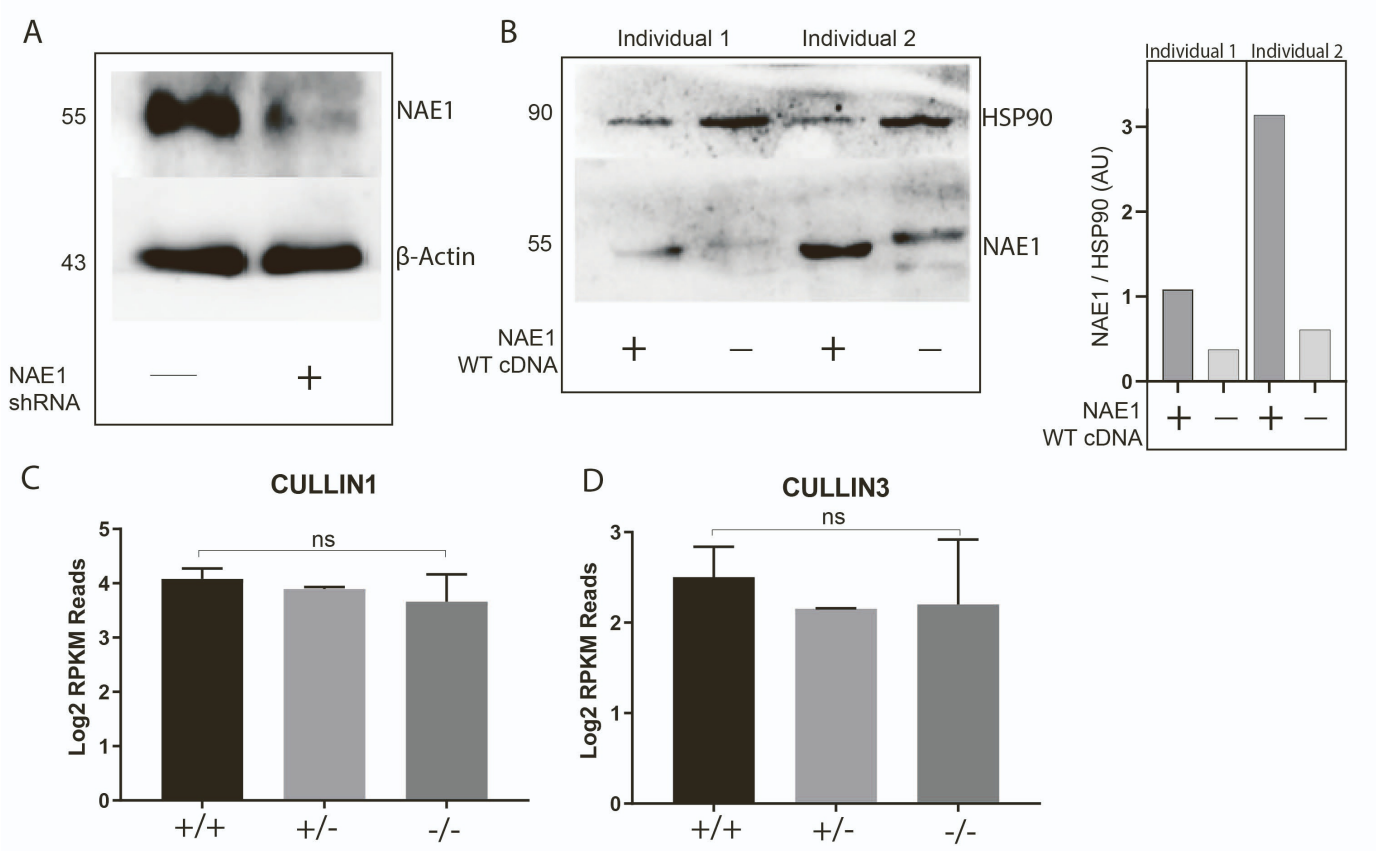


Figure S3: Imagestream Gating Strategy. (A) Using the Brightfield channel, cells out of focus were discarded (Gradient_RMS_M01). (B) Next, cells in focus in the nuclear channel were gated (Gradient_RMS_M05, nuclear channel). (C) Single cells were gated using brightfield Area_M01 and Aspect_Ratio_M01. (D) Next, cells were gated on CD45RA and CD4 expression. (E) The bottom two images show unstimulated CD3 cells (left side, green line). Every similarity score with a value below 0 is gated as untranslocated (red line). (F) The bottom right image shows an example of stimulated CD3+ cells (yellow line).

Figure S4: Western blots and RNA sequencing data of individuals with *NAE1* genetic variants.



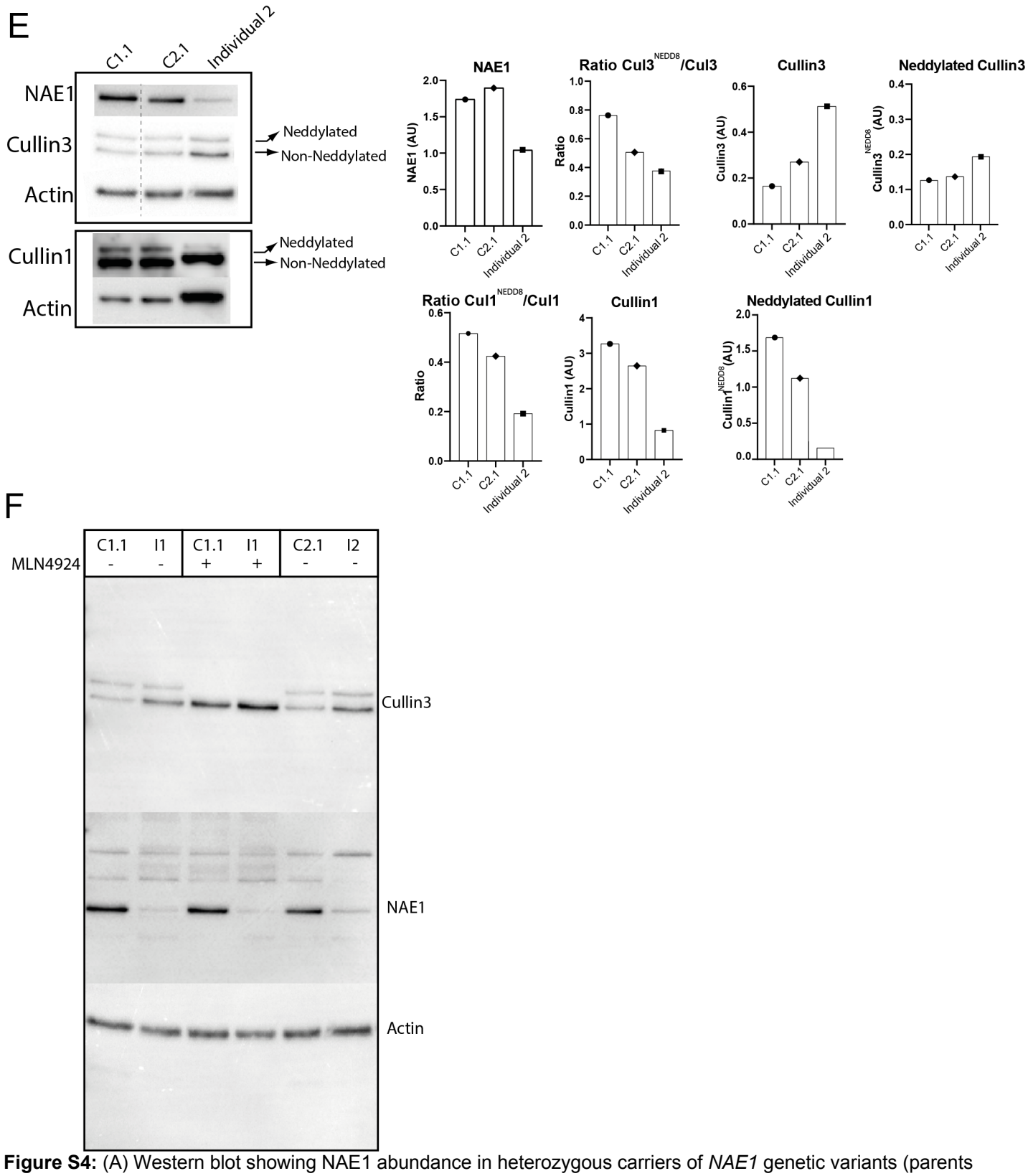


Figure S4: (A) Western blot showing NAE1 abundance in heterozygous carriers of *NAE1* genetic variants (parents of individual 1) with and without addition of NAE1 shRNA. β-actin was used as a housekeeper. (B) Western blot showing NAE1 protein levels in individual 1 and individual 2 with and without vector expressing NAE1 wildtype cDNA('rescue'). HSP90 was used as a housekeeper. The quantification of the blots is shown in bar graphs. Quantification was performed using ImageLab (Biorad). (C, D) CULLIN1 and CULLIN3 log2 RPKM reads derived from fibroblast RNA sequencing data. Bar shows mean log2 RPKM reads of all donors (+/+ N=3, +/- N=2, -/- N=2). Error bars show SD of the log2 RPKM reads. (E) Western blot showing cullin1, cullin3 and NAE1 abundance in two controls (C1.1 and C2.1) and individual 2. The bar graphs show the quantification of the blots, all normalized to actin. Quantification was performed using ImageLab. The dotted line indicates where the blots were cut. Quantification was performed using ImageLab. (F) Uncropped blots used for Figure S4F (NAE1, actin and cullin3).

Figure S5: Conservation of the residues altered in individuals with *NAE1* genetic variants.

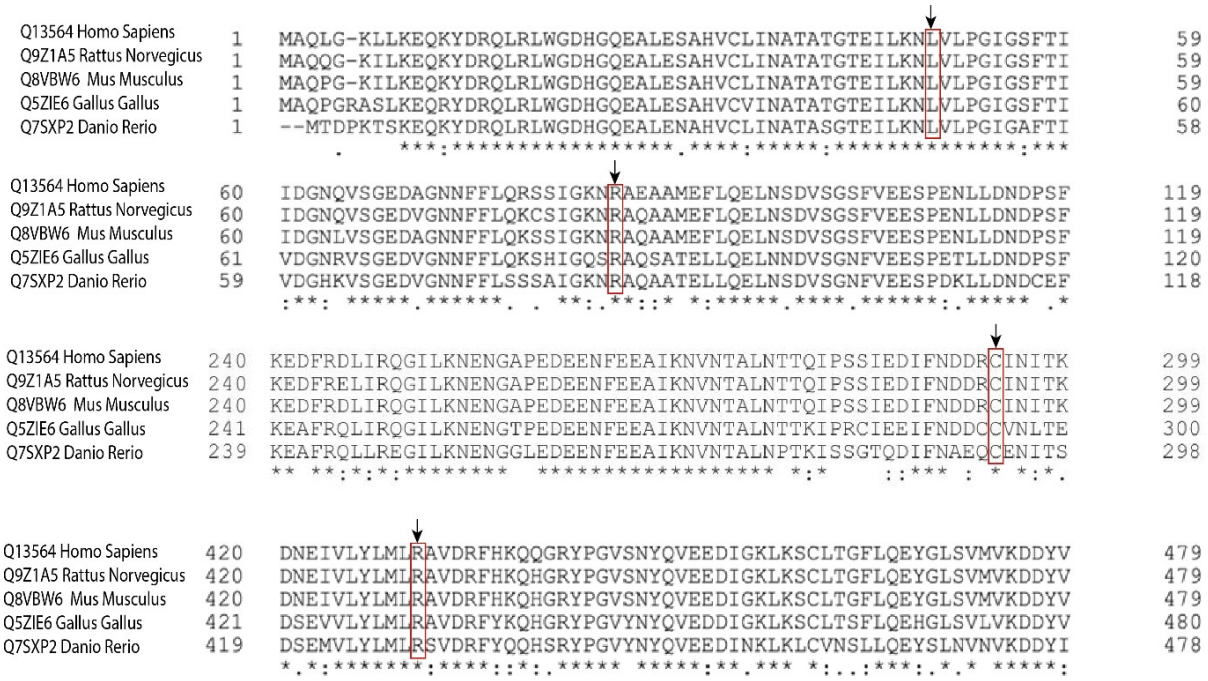
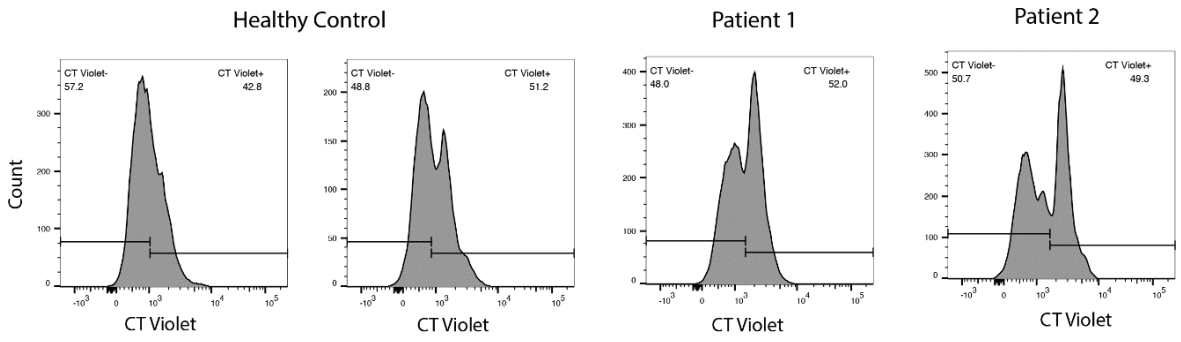


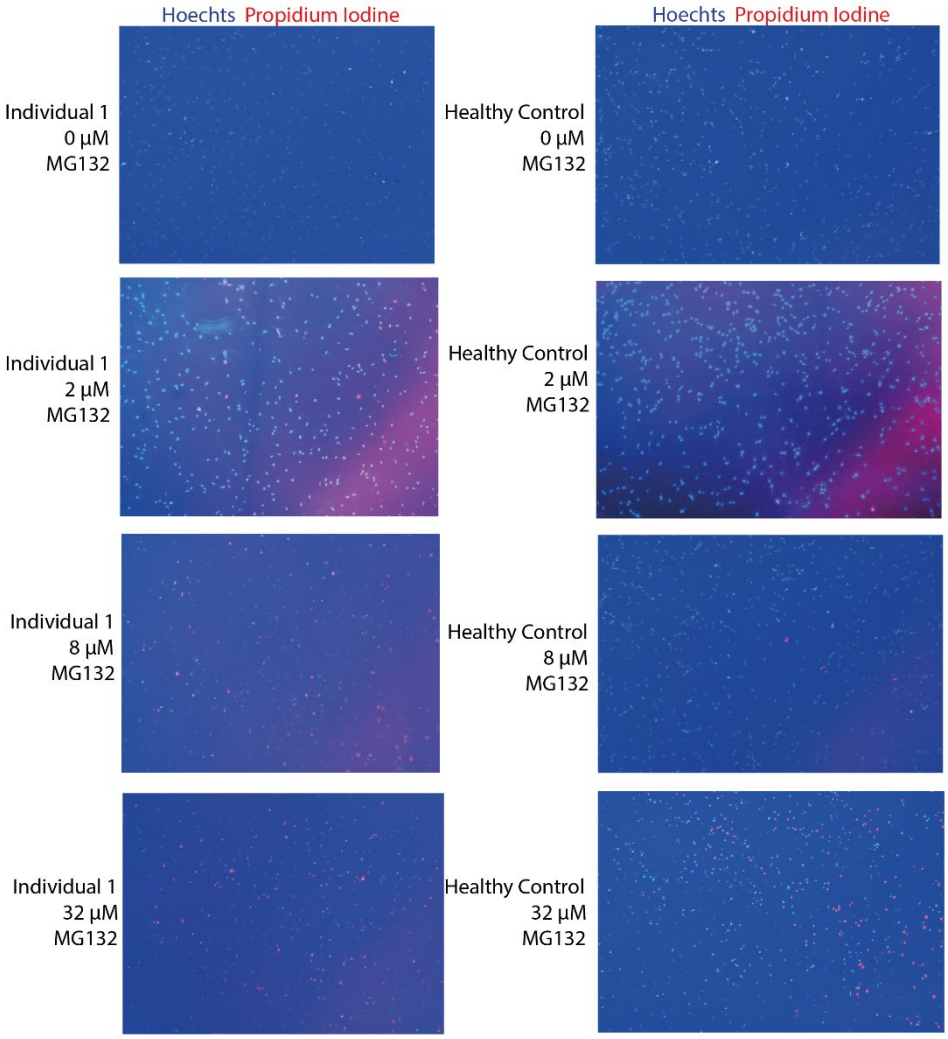
Figure S5: Showing conservation of the residues altered in individuals with *NAE1* genetic variants: Leu49, (top row), Arg85 (second row), Cys294 (third row) and Arg430 (bottom row). All residues are conserved in other species (rat, mouse, chick, zebrafish) that express NAE1 (ULA1).

Figure S6: Proliferation levels are similar between individuals and healthy controls.



Supplementary Figure S6: CD3+ lymphocytes were stained with 2uM CellTrace Violet (Thermofisher) and stimulated with soluble anti-CD3+ (Life technologies, clone OKT3) and anti-CD28+ (Thermofisher) at 1 ug/ml for 3 days. With every cell division, the intensity of CellTrace Violet diminishes. Gating was performed based on the CT violet staining intensity at day zero. Proliferation was similar in healthy controls and individuals with *NAE1* genetic variants (p=0.5).

Supplementary Figure S7: Representative example of microscopic images taken after MG132 treatment in a healthy control and individual 1.



Supplementary Figure S7: Representative example of microscopic images taken after MG132 treatment in a healthy control and individual 1. Staining was performed by incubating fibroblasts with Hoechts (1:2000) and PI (1:20) for 5 minutes. Cell death was calculated by dividing the number of PI positive cells by the total number of cells.

Table S1: Overview of genetic variants identified with WES (after filtering) in individual 1.

Gene	Mode of inheritance	Variant	GnomAD	CADD Score	PolyPhen-2	Mutation Taster	Prediction
NAE1	Compound heterozygous	NM_003905.4: c.147G>C p.Leu49Phe Nonsynonymous	pLI=0, 1x reported in GnomAD.	31.	Probably damaging (0.99).	Disease Causing Might Introduce splice site changes.	Variant of Unknown Significance
NAE1	Compound heterozygous	NM_003905.4: c.254G>A, p.Arg85GlnNonsynonymous	pLI=0, 4x reported in GnomAD.	24	Probably Damaging (0.99).	Disease Causing	Variant of Unknown Significance
TASOR	Compound heterozygous	NM_001112736.2 c.1840T>A, p.Leu614Ile Nonsynonymous	pLI=1 Reported 318x in GnomAD. MAF ≥ 0.001	23.1	Possibly Damaging (0.615).	Disease Causing	Benign (BS1, BS2)
TASOR	Compound heterozygous	NM_001112736.2 c.3094A>G p.Ile1032Val Nonsynonymous	pLI = 1. Reported 36x in GnomAD. MAF <0.001.	16	Benign (0.003).	Polymorphism.	Benign (BS1, BS2)
PIWIL2	De Novo	NM_001135721.2c.1899C>T, p.Gly633=, Synonymous	pLI=0.	10.5	NA	Disease Causing, introduces a cryptic splice site	Likely benign (BP1, BP5).
DPY19L4	De novo	NM_181787.3 c.1338T>C p.Gly446= Synonymous	GnomAD: pLI=0 3x reported: other missense variant at the same AA position	3.5	NA	Disease Causing, Introduces Splice site changes.	Likely benign (BP1, BP5)

Table S1: Overview of genetic variants identified after filtering in individual 1 with Whole Exome Sequencing (WES). Filtering methods can be found in the methods section. The genetic variants are sorted based on Combined Annotation Dependent Depletion (CADD) score (high-low). The 'Prediction' Column represents the likelihood of pathogenicity based on the "Standards and Guidelines for the Interpretation of Sequence Variants" drafted by the American College of Medical Genetics and Genomics (ACMG).(1) Additionally, MutationTaster(2), PolyPhen-2(3), CADD(4) score and GnomAD constraint metrics(5) (Probability of loss of function intolerance, pLI) were used. To indicate the likelihood of pathogenicity, we employed a color scheme. For CADD scores, values 0-10 were colored green, 10-20 were colored yellow and values >20 were colored red. For Polyphen-2, probably damaging was colored red, possible damaging was colored yellow and benign was colored green. For MutationTaster, 'Disease Causing variants' were colored red and 'polymorphisms' were colored green. In the column showing the predictions using the ACMG guidelines, a Variant of Unknown Significance was colored red and likely benign was colored green. Based on these predictions and functional studies, the NAE1 genetic variants were considered likely pathogenic.

Table S2: Comparison of the phenotype of individuals with Dravet syndrome (MIM: 607208) caused by SCN1A mutations to the phenotype of individual 4 from the NAE1 cohort.

Phenotypic Feature	Dravet Syndrome	Individual 4	Cohort of individuals with *NAE1* genetic variants
Short stature	-0.5 SDS	-5.0 SDS	-1, -1.5, -2.3 SDS
Microcephaly	+	-	-
Facial abnormalities	-	+	+
Wide nasal bridge	-	+	+
Long philtrum	-	+	+
Anteverted nares	-	+	+

Large ears	-	+	+
Epicanthal folds	-	+	+
Narrow palpebral fissures	-	+	-
Thick lower lip vermilion	-	+	-
Broad Philtrum	-	+	+
Lymphopenia	-	+	+
Other			
Recurrent infections	-	+	+
Ischiopubic hypoplasia	-	+	+
Neurological Abnormalities	+	+	+
Seizures	+	+	+
Seizure onset in the first year of life	+	NA	+
Seizure triggered by fever	+	+	+
Psychomotor development stagnates.	+	+	+
Mental decline	+	+	+
Behavioral problems	+	-	-
Learning disabilities	+	+	+
Global brain atrophy on MRI	+	+	+
EEG with irregular generalized spike and wave complexes	+	NA	-
Microcephaly	+	-	-
Ataxia	+	NA	-
Limited knee extension	+	+	-
Muscle Weakness	+	NA	+
Dysgenesis of the hippocampus	+	+	+

Table S2: In individual 4, both a heterozygous truncating mutation in the *SCN1A* gene as well as a genetic variant in the *NAE1* gene were identified. To distinguish the clinical phenotype caused by each of the genetic variants, the phenotypic features of Dravet syndrome(6)(MIM: 607208) were compared to all phenotypic features observed in individual 4. Truncating *SCN1A* mutations result mostly in Dravet syndrome. As individual 4 has a truncating mutation in *SCN1A*, features of Dravet syndrome were used for comparison. The characteristic facial features, short stature, lymphopenia, frequent infections and ischipubic hypoplasia have never been identified in Dravet disease, and therefore suggest and additional role for the genetic variants in the *NAE1* gene in disease pathophysiology.

Table S3: List of 24 most specific phenotypes (with the highest occurrence ratio).

HPO code	HPO name	Associated genes	Individual1	Individual2	Individual3	Individual4	Percentage of cohort	Occurrence Ratio
HP:0008822	Hypoplastic ischiopubic rami	1	X	X	X	X	100	100
HP:0006834	Developmental stagnation at onset of seizures	1	X	-	X	X	75	75
HP:0410256	Infection associated lymphopenia	1	X	X	-	X	75	75
HP:0031397	Reduced proportion of naive T cells	1	X	X	NA	NA	50	50
HP:0030887	Increased lymphocyte apoptosis	1	X	X	NA	NA	50	50
HP:0410255	Transient lymphopenia	2	X	X	X	-	75	37.5
HP:0030374	Decreased proportion of memory B cells	2	X	X	X	NA	50	25

HP:0025539	Abnormal B cell subset distribution	2	X	X	NA	NA	50	25
HP:0031869	Recurrent joint dislocation	4	X	X	NA	NA	50	12.5
HP:0007281	Developmental stagnation	12	X	X	X	X	100	8.3
HP:0002265	Large fleshy ears	11	X	X	-	X		6.81
HP:0003173	Hypoplastic pubic bone	15	X	X	X	X	100	6.66
HP:0010975	Abnormal B cell count	9	X	X	NA	NA	50	5.55
HP:0001041	Facial erythema	6	X	-	-	-	25	4.16
HP:0045025	Narrow palpebral fissure	39	X	X	X	X	100	2.56
HP:0007874	Almond-shaped palpebral fissure	41	X	X	X	X	100	2.44
HP:0002344	Progressive neurologic deterioration	43	X	X	X	X	100	2.32
HP:0200005	Abnormal shape of the palpebral fissure	44	X	X	X	X	100	2.27
HP:0002850	Decreased circulating total IgM	26	X	X	NA	NA	50	1.92
HP:0009103	Aplasia/hypoplasia involving the pelvis	57	X	X	X	X	100	1.754
HP:0025540	Abnormal T cell subset distribution	33	X	X	NA	NA	50	1.51
HP:0002180	Neurodegeneration	77	X	X	X	X	100	1.75
HP:0011839	Abnormal T cell count	46	X	X	X	X	50	1.08
HP:0001888	Lymphopenia	107	X	X	NA	NA	50	0.70

Table S3: Overview of the most specific phenotypes as depicted in Figure 3. The first two rows represent the phenotype and the HPO codes attached to the phenotype name. The third column shows the number of genetic diseases associated with the phenotype, as assessed using the Human Phenotype Ontology.(7) Columns four to seven show which individuals display the phenotype, X means the phenotype is present, - means the phenotype is not present, and NA means the phenotype was not assessed. If the phenotype was not assessed, it was considered 'not present' for the calculation of column 8 (percentage). The occurrence ratio is the value of column 8 divided by the value of column 3.

Table S4: Overview of primers used for this study.

Construct	Targeting sequence
NAE1 Tet-PkLo-Puro	5'-CCGGAGTCCTATGATTTGGATCACTCGAGTGATCCAAATCATAGGACTTTTTTG54-3'
pLenti-CMV-Puro NAE1	5'-GAATTCTGCAGTCGACATGGCGCAGCTGGGA-3' 3'-GCTGTCTAGACTCGAGCTACAACTGGAAAGTTGCTGAAGT-5'
NAE1 sequencing primer	5'-GGCTCCAGAAGATGAAGAGAA-3'
Plko-tet-on sequencing Primer	5'-ATTACGCCAAGGTCGACTTAACCCT-3'

Table S5: Overview of significantly up- and downregulated genes identified with RNA sequencing.

Gene	Adjusted p-value	Z-Score HC1	Z-Score HC2	Z-Score HC3	Z-Score Father	Z-Score Mother	Z-Score Individual1	Z-score Individual2	Log2 Fold Change I1	Log2 Fold Change I2
ADA2	0.03585676	2.176	-0.55	0.03	-0	-0.364	-0.65	-0.637	-2.639	-2.542
ADAMTS8	0.02301275	-0.46	2.224	-0.093	-0.34	-0.154	-0.589	-0.589	-5.763	-5.763
ADAMTSL4	1.72E-05	1.018	-0.45	0.607	0.364	0.9949	-1.08	-1.45	-1.852	-3.202
AKAP6	0.02207786	0.955	-0.51	1.393	0.765	-0.873	-1.011	-0.72	-3.498	-1.815
AKR1C2	0.0456592	2.159	0.146	-0.443	-0.04	-0.604	-0.668	-0.555	-5.456	-2.796
AMOT	0.01333326	-0.2	1.246	-0.325	0.07	1.3758	-1.247	-0.922	-2.028	-1.354
AMPH	0.04072475	0.044	1.687	-0.47	1.026	-0.525	-1.041	-0.721	-7.565	-2.098
ARHGAP45	0.00015617	-0.14	-0.45	0.318	1.902	0.3394	-0.985	-0.985	-6.492	-6.492
ATP2A3	0.00727996	0.551	-0.43	1.644	0.781	-0.693	-0.94	-0.917	-6.185	-5.004
AXIN2	7.88E-05	1.456	-0.01	-0.693	1.262	-0.127	-0.995	-0.895	-7.993	-3.713
B4GAT1	0.03114516	0.877	0.481	0.665	-0.57	1.0486	-1.288	-1.215	-1.381	-1.29
BMPER	0.00775421	0.129	1.917	0.289	-0.27	0.0078	-1.041	-1.035	-1.391	-1.38
BOC	0.03355107	0.17	0.501	0.028	-0.14	1.7381	-1.307	-0.984	-1.381	-1.009
BTN3A2	0.04321401	0.16	0.479	-0.101	0.302	1.576	-1.555	-0.861	-6.624	-1.526
C20orf96	0.04361249	0.783	0.161	0.858	0.225	0.7691	-1.21	-1.587	-1.513	-2.238
CAMK2N1	1.10E-07	1.311	0.661	0.231	-0.04	0.4884	-1.469	-1.18	-2.948	-1.971
CASZ1	0.04958066	1.869	0.138	-0.167	0.326	-0.029	-1.253	-0.885	-5.171	-2.054
CD302	0.03355107	0.402	1.123	-0.255	0.533	0.728	-1.763	-0.768	-3.48	-1.032
CD82	0.0109587	-0.48	1.155	0.757	0.38	0.716	-1.309	-1.222	-2.01	-1.815
CDH4	0.00292178	1.496	0.196	0.567	0.445	-0.192	-1.338	-1.173	-1.572	-1.337
CDK18	0.0437208	-0.05	-0.45	2.194	0.018	-0.571	-0.579	-0.567	-7.312	-5.591
CDON	0.03061071	0.047	0.282	-0.07	-0.5	2.0023	-0.66	-1.106	-1.368	-3.08
CELSR1	0.04874031	-0.09	1.598	-0.73	0.048	1.0595	-0.966	-0.92	-4.376	-3.628
CHRD	0.00138544	0.509	0.241	-0.745	1.293	0.9565	-1.127	-1.127	-5.823	-5.823
CILP2	0.00281157	0.251	0.181	1.61	-0.45	0.6964	-1.146	-1.146	-6.088	-6.088
CLEC2A	0.01419896	0.816	-0.13	-0.691	1.902	-0.516	-0.691	-0.691	-8.302	-8.302
CLEC2B	0.00013394	1.331	-0.13	-0.603	1.504	-0.434	-0.892	-0.773	-5.977	-3.167
CLMN	0.01653896	-0.39	0.277	-0.228	2.129	-0.315	-0.737	-0.737	-3.946	-3.946
CNNM1	0.02807154	-0.93	0.906	1.501	0.544	-0.155	-0.932	-0.932	-5.224	-5.224
COL10A1	0.04153762	0.238	-0.02	-0.071	2.058	-0.493	-0.854	-0.854	-5.363	-5.363
COL6A3	0.00051898	0.45	1.023	0.89	-0.25	0.5322	-1.087	-1.559	-1.179	-1.843
COL7A1	0.01159841	1.196	-0.52	-0.273	0.799	1.0304	-0.99	-1.244	-1.426	-1.939
COL8A2	6.74E-05	0.3	1.03	1.232	0.39	-0.592	-1.032	-1.329	-1.895	-3.007
COX7A1	0.00021423	1.263	0.053	-0.003	1.37	-0.609	-1.005	-1.069	-2.699	-3.113
CPXM2	1.53E-06	1.858	-0.14	0.772	-0.34	-0.284	-0.896	-0.964	-3.008	-3.69
CRYBG1	1.25E-05	1.415	0.673	-0.477	0.157	0.6258	-1.331	-1.062	-3.856	-2.269
CSPG4	4.54E-06	1.455	0.557	-0.062	0.748	-0.279	-1.155	-1.264	-1.826	-2.093
CTSK	0.00991428	1.517	0.937	-0.392	-0.09	0.272	-1.313	-0.927	-1.732	-1.138
CYB5R2	0.00018635	0.077	-0.14	0.266	2.004	-0.315	-0.964	-0.927	-3.368	-3.039
DENND2A	1.57E-05	0.022	1.138	-0.357	1.584	-0.46	-0.963	-0.963	-7.599	-7.599
DGCR6	0.01241277	0.649	0.592	-0.447	0.856	0.9454	-1.175	-1.422	-2.489	-4.097
DHCR24	0.0036859	1.391	1.009	-0.86	0.036	0.4522	-0.858	-1.169	-1.653	-2.735
DHRS13	0.00074085	-0.01	-0.08	1.46	0.653	0.4879	-1.405	-1.106	-4.925	-2.439

DNM1	2.43E-05	-0.17	0.635	1.552	-0.45	0.6647	-1.206	-1.031	-3.422	-2.403
DPF3	0.02310761	-0.36	0.612	1.61	-0.6	0.7196	-0.803	-1.172	-1.717	-3.481
DPYSL3	0.00052457	1.565	0.385	0.227	0.638	-0.503	-1.065	-1.247	-1.48	-1.824
ECM1	0.00697725	1.849	0.074	0.096	-0.03	0.234	-0.973	-1.251	-1.14	-1.527
EFNA5	0.00839476	0.083	1.308	-0.642	-0.64	1.482	-0.796	-0.796	-5.849	-5.849
EGR2	0.0332212	-0.53	0.082	2.015	0.489	-0.651	-0.703	-0.703	-5.909	-5.909
ELOVL2	0.01862053	-0.39	1.233	-0.2	-0.32	1.5545	-1.012	-0.861	-6.66	-3.089
ENPP1	0.00292178	-0.66	1.1	-0.081	1.528	0.081	-1.174	-0.792	-4.632	-1.866
EPHB2	0.00697725	-0.47	1.723	0.152	-0.23	0.8284	-1.133	-0.876	-2.866	-1.828
ERMP1	0.00338071	0.918	0.855	-0.356	1.032	0.0769	-1.403	-1.122	-1.87	-1.395
FBLN7	1.66E-05	0.08	-0.29	-0.149	0.739	1.751	-1.047	-1.086	-2.601	-2.806
FGF9	0.00396068	-0.8	1.879	-0.116	0.656	0.0455	-0.834	-0.834	-6.476	-6.476
FHOD3	0.02506544	1.644	-0.43	0.432	0.827	-0.529	-0.785	-1.16	-1.301	-2.215
FMNL1	0.04321401	0.57	0.232	-0.196	1.356	0.6152	-1.434	-1.144	-2.831	-1.898
FST	0.01419896	-0.67	-0.44	0.115	1.445	1.303	-0.984	-0.772	-2.295	-1.599
GDF6	0.01862053	1.94	0.668	-0.122	-0.34	-0.442	-0.94	-0.761	-2.429	-1.739
GJA1	0.00164499	1.305	0.431	0.504	-0.06	0.4501	-1.015	-1.619	-1.103	-1.963
GJD3	0.04361249	0.547	-0.21	0.473	-0.16	1.6578	-1.153	-1.153	-7.004	-7.004
GPR153	0.01121289	0.473	-0.18	0.888	0.145	1.2314	-1.548	-1.012	-1.995	-1.165
GPR3	0.04432331	0.735	0.098	0.455	0.688	0.8556	-1.538	-1.293	-6.739	-3.001
GRIA1	4.54E-06	2.16	-0.35	0.136	-0.08	-0.508	-0.692	-0.674	-7.954	-5.479
HAS2	0.00775421	0.18	0.626	-0.43	1.703	0.0595	-0.749	-1.389	-1.178	-2.855
HECW1	0.0129608	0.61	0.89	-0.267	1.396	-0.293	-1.214	-1.122	-4.566	-3.398
HHIPL1	0.00164664	1.345	0.042	-0.058	0.079	1.0455	-1.428	-1.024	-2.485	-1.509
HLA-B	0.04072475	0.035	-0.91	1.304	-0.12	1.4044	-0.757	-0.952	-1.609	-2.261
HR	1.81E-06	0.705	-0.51	0.843	0.402	1.0836	-1.319	-1.209	-3.397	-2.739
ICAM5	0.01623189	-0.42	-0.47	1.543	-0.24	1.2971	-0.793	-0.926	-2.26	-3.101
IER5	0.00238366	-0.23	0.365	1.121	0.002	1.1973	-1.462	-0.993	-2.165	-1.288
IL15RA	0.0246276	0.462	-0.42	0.334	-0.3	1.8664	-1.098	-0.849	-3.658	-2.099
IL18	0.03631202	-0.18	-0.56	0.023	2.186	-0.167	-0.651	-0.651	-6.24	-6.24
IL1RAP	0.03585676	1.432	0.368	-0.751	0.437	0.706	-1.379	-0.812	-2.255	-1.132
IPMK	0.03013895	0.584	1.489	0.38	-0.19	0.2565	-1.382	-1.133	-1.683	-1.313
IRS2	0.01862053	0.023	-0.52	0.78	0.449	1.5347	-1.144	-1.118	-1.359	-1.323
ISM2	0.0202337	-0.72	-0.54	1.414	1.457	-0.168	-0.722	-0.722	-6.275	-6.275
KCNB1	0.01619268	1.485	0.518	0.644	0.337	-0.925	-1.14	-0.918	-5.846	-2.663
KCNC3	0.00021423	1.386	0.141	1.145	-0.14	-0.21	-1.159	-1.159	-6.319	-6.319
KCNIP3	0.01862053	1.441	0.255	1.003	-0.04	-0.334	-1.044	-1.284	-1.342	-1.745
KCNS1	6.95E-05	0.158	0.62	0.73	1.028	0.2673	-1.402	-1.402	-6.918	-6.918
KIAA1549	0.00991428	0.719	0.825	1.36	0.051	-0.831	-1.014	-1.11	-1.945	-2.252
KIAA1958	0.00309789	0.78	0.89	0.519	0.451	0.1639	-1.112	-1.692	-1.071	-1.766
KRTAP1-1	0.03197017	1.818	0.275	0.597	-0.32	-0.356	-1.073	-0.939	-8.311	-3.408
LAMB1	0.01862053	0.949	1.271	-0.777	0.08	0.6727	-1.167	-1.03	-1.278	-1.113
LAPTM5	0.00641507	-0.13	-0.45	-0.418	2.159	0.1857	-0.673	-0.673	-6.67	-6.67
LGALS9	0.00248602	-0.4	0.746	0.434	1.649	-0.29	-1.071	-1.071	-5.793	-5.793
LIN7A	0.04432331	2.073	-0.44	-0.389	-0.4	0.5102	-0.559	-0.788	-1.895	-3.897
LINC00707	0.00564295	0.873	1.275	-0.915	0.938	-0.256	-0.957	-0.957	-6.862	-6.862

LINC00900	0.04958066	2	0.236	-0.577	0.319	-0.29	-0.844	-0.844	-5.003	-5.003
LINC01139	0.03374528	-0.82	0.779	1.487	0.854	-0.66	-0.82	-0.82	-7.353	-7.353
LOC101927809	0.01513948	-0.1	-0.17	2.032	-0.09	0.2139	-0.943	-0.943	-8.35	-8.35
LOXL4	1.72E-05	-0.01	1.773	0.832	-0.12	-0.555	-0.925	-0.993	-2.493	-2.886
LRIG1	0.00543979	-0.91	0.971	0.164	1.12	0.7954	-1.182	-0.958	-2.612	-1.836
LRP11	0.00157294	0.043	1.164	-0.263	0.679	0.9164	-1.426	-1.113	-1.478	-1.111
LTBP4	1.26E-07	0.49	-0.08	1.188	0.147	0.9016	-1.231	-1.412	-2.246	-2.948
LUM	2.90E-05	0.085	0.692	0.879	0.72	0.4362	-1.204	-1.607	-1.198	-1.703
MAF	0.00011392	2.119	0.027	-0.444	0.2	-0.422	-0.769	-0.711	-7.528	-4.071
MAN1A1	0.01862053	-0.03	1.627	-0.37	-0.05	0.9217	-1.339	-0.754	-2.432	-1.13
MAOA	0.03061071	0.342	0.27	-0.721	2.01	-0.458	-0.721	-0.721	-6.003	-6.003
MARCH9	0.00569747	1.925	-0.69	0.298	-0.13	0.3667	-0.953	-0.819	-7.408	-3.208
MATN2	0.00056662	1.149	0.553	-0.709	0.053	1.1676	-0.942	-1.272	-1.804	-3.108
MC4R	0.01862053	2.244	-0.23	-0.446	-0.12	-0.46	-0.493	-0.493	-9.408	-9.408
MDFI	0.02506544	-0.65	1.288	-0.391	1.575	-0.261	-0.779	-0.779	-6.459	-6.459
MDK	0.03252576	-0.48	0.114	1.55	0.674	0.3615	-0.716	-1.503	-1.135	-3.544
MEIS2	0.00991428	0.349	0.827	-0.374	-0	1.5044	-0.896	-1.41	-1.048	-1.798
MFSD10	0.02858347	1.163	-0.46	0.776	0.853	0.1537	-1.213	-1.268	-1.054	-1.106
MGARP	0.02722563	1.846	-0.25	-0.042	-0.13	0.6218	-1.17	-0.868	-8.546	-2.366
MMP11	0.01544699	-0.35	-0	1.007	0.366	1.3574	-1.356	-1.026	-6.564	-2.409
MOCOS	3.81E-05	-0.01	-0.12	-0.022	2.126	-0.317	-0.81	-0.842	-3.02	-3.352
MPP4	0.00248602	2.104	0.216	-0.557	0.1	-0.446	-0.709	-0.709	-6.468	-6.468
MSL2	0.00874296	0.4	0.877	0.332	0.654	0.6091	-1.567	-1.306	-1.235	-1.014
MSTN	3.01E-06	-0.37	2.117	0.316	-0.27	-0.296	-0.748	-0.748	-9.181	-9.181
MTSS1L	0.00961871	0.4	-0.21	0.528	0.118	1.5675	-0.912	-1.487	-1.041	-1.871
MX1	0.00697725	1.941	-0.53	0.727	-0.08	-0.625	-0.756	-0.679	-6.608	-3.595
MXRA5	7.27E-05	0.767	1.428	-0.459	-0.01	0.6027	-0.964	-1.367	-1.851	-3.772
MYLK	0.02506544	2.085	0.075	0.124	-0.3	-0.279	-0.794	-0.915	-1.383	-1.654
MYRIP	0.01755076	2.242	-0.2	-0.241	-0.23	-0.518	-0.526	-0.526	-7.39	-7.39
NA	9.99E-06	-0.23	-0.27	0.585	1.639	0.5402	-1.107	-1.152	-3.073	-3.415
NA	0.00814197	1.089	-0.13	0.909	-0.44	0.958	-1.147	-1.242	-2.03	-2.318
NA	0.01898174	0.283	0.775	-0.35	0.559	1.2674	-1.451	-1.083	-7.581	-2.379
NA	0.04072475	-0.63	0.814	-0.814	0.676	1.5804	-0.814	-0.814	-5.782	-5.782
NES	4.24E-10	-0.34	1.538	-0.27	0.874	0.4306	-1.131	-1.105	-3.431	-3.215
NFATC2	0.00709268	2.09	-0.12	-0.538	0.381	-0.369	-0.777	-0.667	-6.689	-3.165
NFE2L3	7.12E-05	0.159	-0.6	0.815	1.3	0.6877	-1.212	-1.147	-3.654	-3.117
PBX3	0.02008894	1.62	0.751	-0.547	0.356	-0.076	-1.341	-0.763	-2.646	-1.199
PCOLCE	0.03518198	-0.13	0.027	0.989	-0.21	1.5631	-1.114	-1.131	-1.089	-1.107
PHLDA1	0.01862053	1.608	0.203	-0.472	1.025	-0.394	-0.947	-1.023	-1.391	-1.532
PITPNM3	0.00684464	-0.13	1.515	-0.616	0.098	1.1241	-1.171	-0.816	-2.514	-1.466
PKN3	0.03374528	0.149	-0.17	0.01	1.738	0.5438	-1.295	-0.979	-2.359	-1.557
PLTP	0.04958066	1.006	1.224	0.758	-0.19	-0.52	-1.196	-1.086	-1.469	-1.308
PPP2R2B	0.04762812	1.971	0.008	-0.134	0.484	-0.585	-0.872	-0.872	-4.657	-4.657
PRELP	0.02301275	0.102	-0.58	2.153	-0.19	-0.096	-0.708	-0.689	-3.732	-3.435
PRLR	0.01862053	0.417	1.443	-0.963	1.102	-0.24	-0.851	-0.907	-3.094	-3.71
PSG1	0.0025378	2.022	0.604	-0.233	-0.48	-0.485	-0.678	-0.756	-2.843	-3.842

PSG5	0.0089704	1.78	0.517	-0.756	-0.18	0.4725	-1.082	-0.752	-2.585	-1.488
RALGPS1	0.02881424	-0.52	-0.13	0.339	1.893	0.35	-1.076	-0.855	-5.3	-2.535
RGCC	0.01898174	-0.73	0.378	-0.15	2.055	-0.088	-0.732	-0.732	-8.162	-8.162
RIMS2	0.00540232	-0.02	0.415	-0.808	1.193	1.2763	-1.03	-1.03	-4.609	-4.609
RTN4RL1	0.04432331	2.139	-0.21	0.006	0.073	-0.536	-0.755	-0.722	-6.275	-4.509
RUNX2	0.00224238	1.51	-0.02	-0.014	0.47	0.5453	-1.029	-1.463	-1.625	-2.918
S1PR3	0.00557986	0.366	0.742	-0.706	-0.43	1.7607	-0.989	-0.745	-3.69	-2.023
SALL2	0.0129608	1.177	0.654	-0.15	-0.1	0.9364	-1.482	-1.04	-6.966	-2.129
SAMD14	0.03197017	2.044	-0.1	0.169	0.115	-0.48	-0.793	-0.955	-1.742	-2.327
SDC4	0.02506544	-0.86	0.287	1.428	0.393	0.8342	-0.794	-1.284	-1.188	-2.242
SERPINE2	0.0343186	1.949	0.348	-0.373	0.368	-0.675	-0.659	-0.958	-1.372	-2.326
SFTA1P	0.03252576	-0.5	-0.41	-0.418	2.037	0.6161	-0.665	-0.665	-8.078	-8.078
SHISAL1	0.01120488	0.72	-0.4	0.209	1.843	-0.728	-0.784	-0.856	-2.55	-3.087
SLC29A4	0.00697725	1.676	-0.29	0.073	0.686	0.1235	-1.286	-0.98	-6.802	-2.446
SLC43A1	0.00395203	0.672	0.134	1.079	-0.13	0.8821	-1.536	-1.102	-5.661	-2.147
SLC8A1-AS1	0.01121289	2.209	-0.3	-0.454	-0.01	-0.197	-0.627	-0.627	-5.9	-5.9
SOX9	0.03197017	1.306	-0.29	0.268	-0.58	1.3202	-0.936	-1.087	-1.764	-2.219
SPON2	7.88E-05	-0.53	-0.11	0.185	2.133	-0.202	-0.734	-0.734	-7.151	-7.151
STAP2	0.00874296	0.5	0.842	-0.115	1.295	0.0648	-1.343	-1.242	-6.871	-4.005
STEAP2	0.00243819	0.01	0.809	0.943	0.064	0.8259	-1.681	-0.971	-2.404	-1.154
STMN3	0.0456592	-0.22	-0.45	-0.514	2.164	0.2361	-0.532	-0.676	-2.086	-3.425
STX1B	0.00382025	0.813	-0.05	0.646	-0	1.2161	-1.363	-1.258	-2.409	-2.097
TCTN2	0.02881424	-0.05	0.886	-0.346	0.655	1.2486	-1.548	-0.841	-2.859	-1.189
TGFA	0.00091112	2.206	-0.31	-0.015	-0.25	-0.306	-0.708	-0.618	-6.479	-3.291
TGFBR3L	0.03895544	1.374	0.299	1.175	-0.53	-0.169	-1.174	-0.979	-3.467	-2.315
TLE2	0.01862053	1.331	0.846	0.514	-0.43	0.163	-1.427	-1.002	-7.287	-2.138
TMED1	0.00874296	0.543	0.465	0.036	1.076	0.6302	-1.648	-1.102	-1.818	-1.115
TMEM119	0.00074085	-0.4	0.466	-0.172	0.085	1.9367	-0.916	-0.996	-1.892	-2.158
TMEM25	0.00052457	1.266	-0.12	1.474	-0.17	-0.486	-1.045	-0.925	-4.096	-2.871
TMEM9B-AS1	0.04361249	-0.62	-0.45	0.534	1.565	0.9017	-0.966	-0.966	-7.031	-7.031
TNFRSF21	0.01648881	0.447	0.32	-0.663	-0.32	1.9225	-0.907	-0.797	-2.46	-1.978
TOR4A	0.03751673	0.482	-0.32	1.86	0.403	-0.708	-0.68	-1.038	-1.552	-3.178
TPP1	0.00049575	1.338	0.25	0.725	-0.37	0.5667	-1.306	-1.208	-1.601	-1.45
TRIM14	0.0083662	1.622	0.524	-0.185	0.658	-0.404	-1.184	-1.032	-1.813	-1.508
TRIM16	0.0056343	1.632	-0.24	0.085	0.604	0.2935	-1.238	-1.134	-1.926	-1.701
TRIM55	1.81E-06	1.027	-0.28	1.582	-0.66	0.2603	-0.968	-0.964	-9.965	-8.029
TRIM7	0.00488864	0.882	-0.59	-0.331	1.687	0.2711	-0.986	-0.935	-6.013	-4.26
TSPOAP1	0.00992749	-0.33	-0.11	1.204	0.904	0.7798	-1.226	-1.225	-2.031	-2.028
TYRO3	0.00082187	1.321	0.903	0.13	0.169	0.0306	-1.534	-1.02	-3.034	-1.545
VAT1L	0.03627585	0.487	-0.78	0.933	-0.52	1.5852	-0.705	-1.006	-1.857	-3.878
ZNF385D	0.02443378	1.144	0.729	1.1	-0.15	-0.619	-1.337	-0.87	-3.008	-1.496
ZNF521	0.0202337	1.498	-0.23	-0.91	0.325	1.0924	-0.875	-0.9	-2.01	-2.104
ZNF578	0.02998066	1.278	0.267	-0.343	1.119	0.0903	-1.266	-1.146	-5.181	-3.406
ZNF853	0.0021976	-0.31	0.44	1.33	1.147	-0.381	-1.114	-1.114	-6.393	-6.393

Table S4: Overview of significantly (adjusted p-value <0.05, log2foldchange >2 or <2) up- or downregulated genes identified with RNA sequencing in individuals with *NAE1* genetic variants and healthy control fibroblasts. Heterozygous carriers (parents of individual 1) fibroblasts were also

included. The first column shows the adjusted (Bonferroni) p-value comparing individuals with healthy controls. The 3th to the 9th column show the Z-scores of all donors comparing the RPKM score of the gene to the RPKM of that gene in all other donors. The 10th and 11th columns show the log2foldchange in individual 1 and individual 2.

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