Supplementary data

Clinical and functional analyses of *AIPL1* variants reveal mechanisms of pathogenicity linked to different forms of retinal degeneration

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Supplementary Table 1: Clinical information of patients with *AIPL1* variations

Case	Allele 1	Allele 2	Visual Acuity R - L	OCT	Posterior Pole Findings	ERG	Clinical Diagnosis	Reference
Patients with *AIPL1* variations identified on both alleles								
P1	c.116C>A; **p.T39N**	c.116C>A; **p.T39N**	N/A	N/A	Macular degeneration, diffuse pigmentary retinopathy	N/A	LCA	46
P2	c.214T>C; **p.W72R**	c.265T>C; p.C89R	PL - PL	Abnormal lamination, foveal depression, reduced foveal retinal thickness, foveal ONL barely discernible or not detectable	N/A	Undetectable	LCA	25
P3	c.266G>A; **p.C89Y**	c.266G>A; **p.C89Y**	N/A	N/A	N/A	Markedly diminished	LCA	47
P4	c.364G>A; **p.G122R**	c.834G>A; p.W278X	HM - HM	N/A	Macular dystrophy	Undetectable	LCA	32
P5	c.364G>A; **p.G122R**	c.364G>A; **p.G122R**	20/50 – 20/100	N/A	N/A	Discernible rod and cone responses	RP	This study
P6	c.364G>C; **p.G122R**	c.834G>A; p.W278X	20/60 - 20/400	Deep foveal depression, loss of foveal ONL, signs of retinal remodeling in the macular region	Bull's-eye – like lesion in the foveal region, pigmentary retinal degeneration with atrophy extending from the arcades into the midperipheral retina and attenuated retinal vessels	Undetectable	RP/ Late onset retinal degeneration	25
P7	c.364G>C; **p.G122R**	c.834G>A; p.W278X	20/20 - 20/20	Extensive loss of the photoreceptor-RPE complex and the EZ, EZ and IZ are only visible in the foveal zone	N/A	Dark-adapted and light adapted full-field ERG responses are reduced within 50% and 25% respectively of the normal range	Mild RP	This study
P8	c.364G>C; **p.G122R**	c.834G>A; p.W278X	20/20 - 20/20	The photoreceptor-RPE complex and the EZ are only visible in the foveal zone	N/A	N/A	Mild RP	This study

P9	c.582C>G; **p.Y194X**	c.834G>A; p.W278X	PL - PL	N/A	Pigmentary changes	N/A	LCA	48
P10*	c.666G>A; **p.W222X***	c.834G>A; p.W278X	HM/30cm - HM/30cm	Loss of outer retinal structure	N/A	N/A	LCA	This study
P11	c.733G>T; **p.E245X**	c.834G>A; p.W278X	6/240 - 6/240	N/A	Pigmentary changes	N/A	LCA	48
P12	c.809G>A; **p.R270H**	c.834G>A; p.W278X	HM - HM	N/A	Macular atrophy	Undetectable	LCA	49
P13	c.809G>A; **p.R270H**	c.834G>A; p.W278X	20/1200 – 20/1000	101/111, PSJ not preserved	Macular dystrophy	Undetectable	LCA	32
P14	c.809G>A; **p.R270H**	c.834G>A; p.W278X	N/A	N/A	N/A	Markedly diminished	LCA	47
P15	c.926_927ins CCTGAACCGCAGGGAGCT; **p.E309DinsLNRREL**	c.926_927ins CCTGAACCGCAGGGAGCT; **p.E309DInsLNRREL**	HM/50cm - HM/45cm	N/A	Attenuated vessels, carpet like retinal degeneration	Undetectable	LCA	36
P16	c.1126C>T; **p.P376S**	c.341C>T; p.T114I	N/A	N/A	N/A	N/A	LCA	26
P17	c.1126C>T; **p.P376S**	c.341C>T; p.T114I	20/400	N/A	Peripheral retinal mottling, severe maculopathy, moderate pigmentary retinopathy	N/A	LCA	23
P18	c.1126C>T; **p.P376S**	c.341C>T; p.T114I	N/A	N/A	Severe pigmentary retinopathy	N/A	LCA	23
P19	c.1126C>T; **p.P376S**	c.341C>T; p.T114I	N/A	N/A	N/A	N/A	EOSRD LCA	50
P20	c.1126C>T; **p.P376S**	c.341C>T; p.T114I	6/19 - 6/48	Reduced retinal and foveal thickness	Moderate pigmentary changes. Bone spicule retinopathy, no obvious macular atrophy but some pigmentary changes seen in the macula	Full field and rod ERG responses reduced in R and L, subnormal and delayed cone 30 Hz flicker ERG in R and L	ESORD	35 This study
P21	c.1126C>T; **p.P376S**	c.1126C>T**; p.P376S**	6/60 - PL	N/A	Pigmentary retinopathy with severe clumping, severe macular atrophy	Reduced responses in R, undetectable in L	EOSRD	35 This study

Supplementary Table 1: Clinical information of patients (unrelated probands) with *AIPL1* variations - Continue

Case	Allele 1	Allele 2	Visual Acuity R - L	OCT	Posterior Pole Findings	ERG	Clinical Diagnosis	Reference
Patients with a single *AIPL1* variation identified on one allele								
P22	c.157C>T; **p.R53W**	N/A	N/A	N/A	N/A	N/A	LCA	51
P23	c.214T>C; **p.W72R**	N/A	N/A	N/A	N/A	N/A	LCA	33
P24	c.390C>A; **p.H130Q**	N/A	N/A	N/A	N/A	N/A	EOSRD LCA	35
P25	c.593C>T; **p.S198F**	N/A	N/A	N/A	N/A	N/A	EOSRD LCA	35
P26	c.617T>A; **p.I206N**	N/A	N/A	N/A	N/A	N/A	LCA	51
P27	c.878T>C, **p.L293P**	N/A	N/A	N/A	N/A	N/A	LCA	33
P28	c.894G>C; **p.Q298H**	N/A	N/A	N/A	N/A	N/A	EOSRD LCA	35
P29	c.1053_1064del TGCAGAGCCACC; **p.A352_P355del**	N/A	N/A	N/A	N/A	N/A	adCORD, juvenile RP	26
P30	c.1091C>G; **p.A364G**	N/A	N/A	N/A	N/A	N/A	EOSRD LCA	35
P31	c.1097C>G; **p.P366R**	N/A	N/A	N/A	N/A	N/A	EOSRD LCA	35
P32	c.1103_1114dup; **p.E369_T372dup**	N/A	N/A	N/A	N/A	N/A	LCA	33
P33	c.1111_1122dup; **p.A371_P374dup**	N/A	20/200 (better eye)	N/A	Primarily peripapillary hypopigmentation with patchy pigment clumping as well as drusen like deposits and macular hypopigmentary changes	Undetectable/ Severely reduced	LCA	52
P34	c.1126C>T; **p.P376S**	N/A	N/A	N/A	N/A	N/A	LCA†	33
P35	c.1126C>T; **p.P376S** c.341C>T; p.T114I *Cis*-allelic inheritance from mother	N/A	N/A	N/A	N/A	N/A	LCA	34
P36	c.1126C>T; **p.P376S**	N/A	6/30 – 6/38	N/A	Moderate pigmentary changes, clumping Macular pigmentation and atrophy	Absent rod and cone ERG responses, delayed 30 Hz flicker cone ERG of low amplitude.	EOSRD	35 This study
P37	c.1126C>T; **p.P376S**	N/A	3/60 - 3/60	N/A	Severe pigmentary retinopathy, no maculopathy	Reduced in R and L	RP EOSRD	35 This study

R, right eye; L, left eye; HM, hand motion; PL, perception of light; RPE, retinal pigment epithelium; LCA, Leber congenital amaurosis; EOSRD, early-onset severe retinal dystrophy; RP, retinitis pigmentosa; adCORD; autosomal dominant cone-rod dystrophy; ONL, outer nuclear layer; N/A, not available; *, novel variation; †, did not segregate with disease. The AIPL1 variants investigated in this study are shown in **BOLD**.

Supplementary Figures

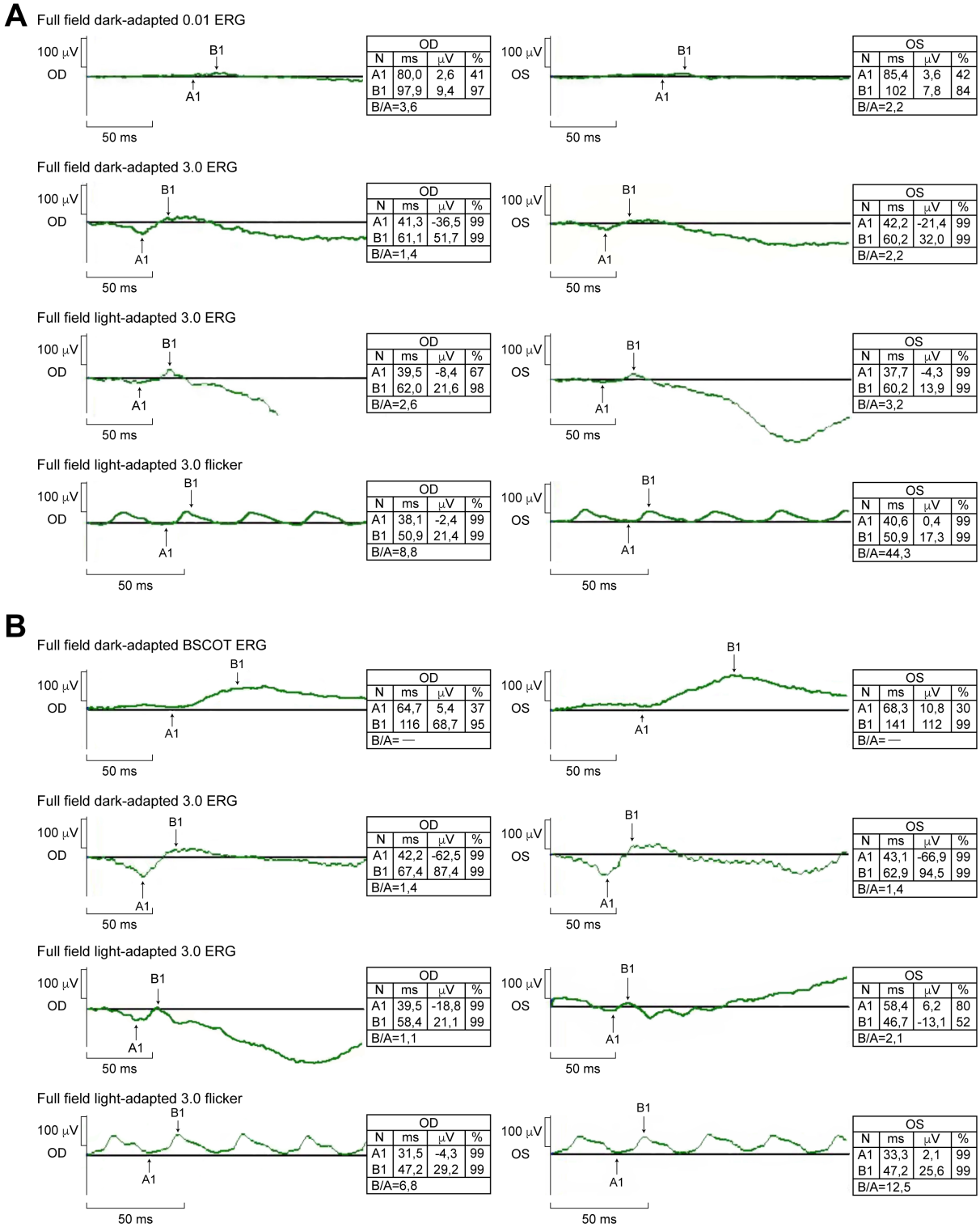
Figure Legends:

Supplementary Figure 1: (A) Full-field ERG from a 34-year-old man with a retinitis pigmentosa phenotype carrying a homozygous *AIPL1* p.G122R variant. Dark-adapted (rod responses) and light-adapted (cone responses) are reduced within 20% of the normal range. (B) Full-field ERG from an 18-year-old woman with a retinitis pigmentosa phenotype with *AIPL1* c.364G>C, p.G122R/c.834G>A, p.W278X variants. Dark-adapted (rod responses) are reduced within 50% and light-adapted (cone responses) within 25% of the normal range.

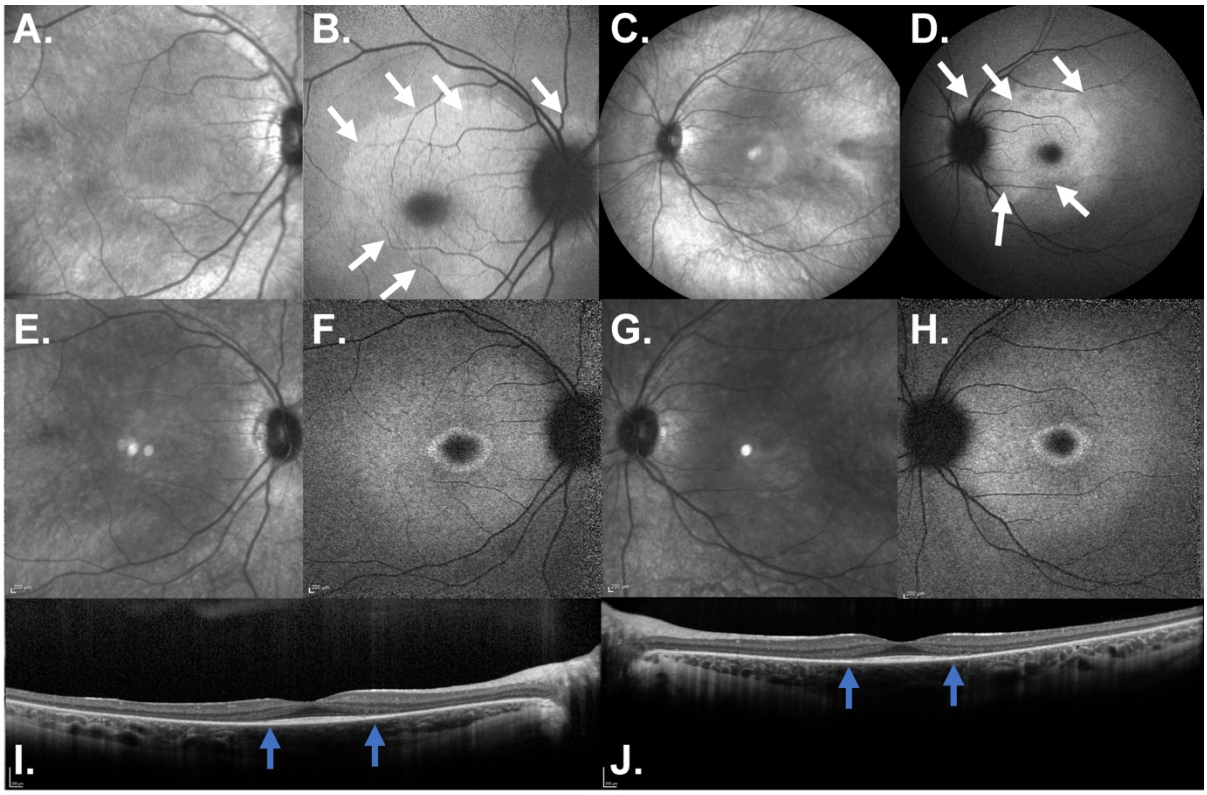
Supplementary Figure 2: Longitudinal infrared and autofluorescence imaging of the brother of the woman of the figure 2. (A-D) First examination at the age of 7 with nyctalopia noted at the age of 6. Visual acuity is 20/20 in both eyes. On infrared reflectance imaging, the vessels are preserved (A 30° field of view, C 50° field of view). On FAF imaging (B, D), there is a change of the autofluorescence of the macula and the nasal part of the optic nerve head with a hyperautofluorescence pattern in both eyes (white arrows). (E-J) Multimodal imaging of the brother at the age of 14: (E, G) infrared reflectance imaging, (F, H) FAF imaging, (I, J), SD OCT imaging. Visual acuity is 20/20. FAF imaging (F and H) discloses a macular hyperautofluorescent ring in both eyes. On SD-OCT (I and J), the PR+RPE complex and the EZ are only visible in the foveal zone (between blue arrows) in accordance with the visual acuity of 20/20.

Supplementary Figure 3: Full blots: Expression of AIPL1 variants. Western blotting analysis (different exposures) of wild-type (w/t) AIPL1 and the indicated AIPL1 variants. The proteins were resolved by SDS-PAGE on a 12% gel. (A) The anti-myc antibody recognizes the myc tag fused to the N-terminus of AIPL1. (B) The anti-AIPL1 antibody is directed against a C-terminal AIPL1 epitope [32]. GAPDH was detected as a loading control. * demarcates an *in silico* designed AIPL1 variant not found in patients and not associated with disease. (C) Relative quantitation of AIPL1 levels with the anti-myc antibody (upper panel) and the anti-AIPL1 antibody (lower panel).

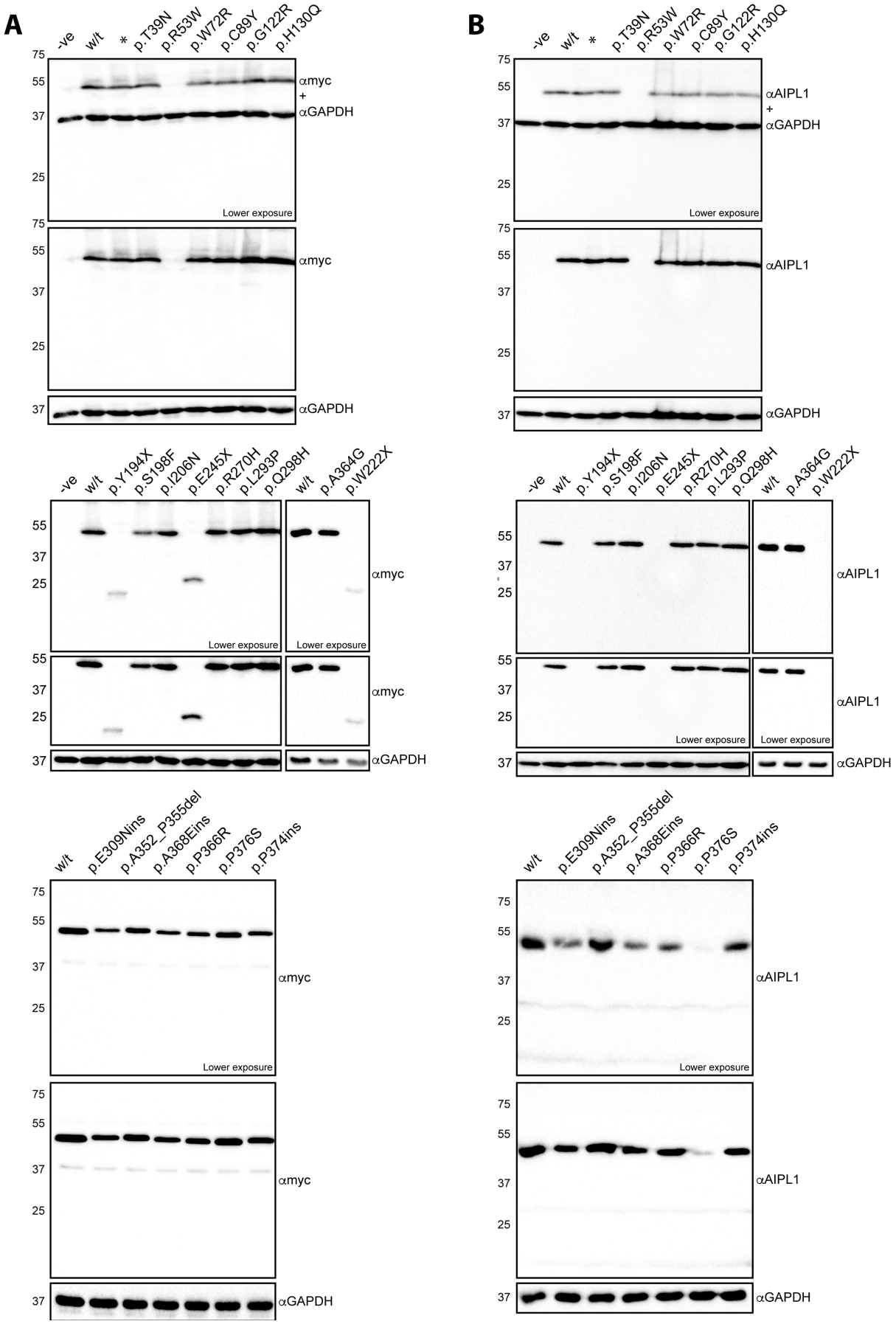
Supplementary Figure 1

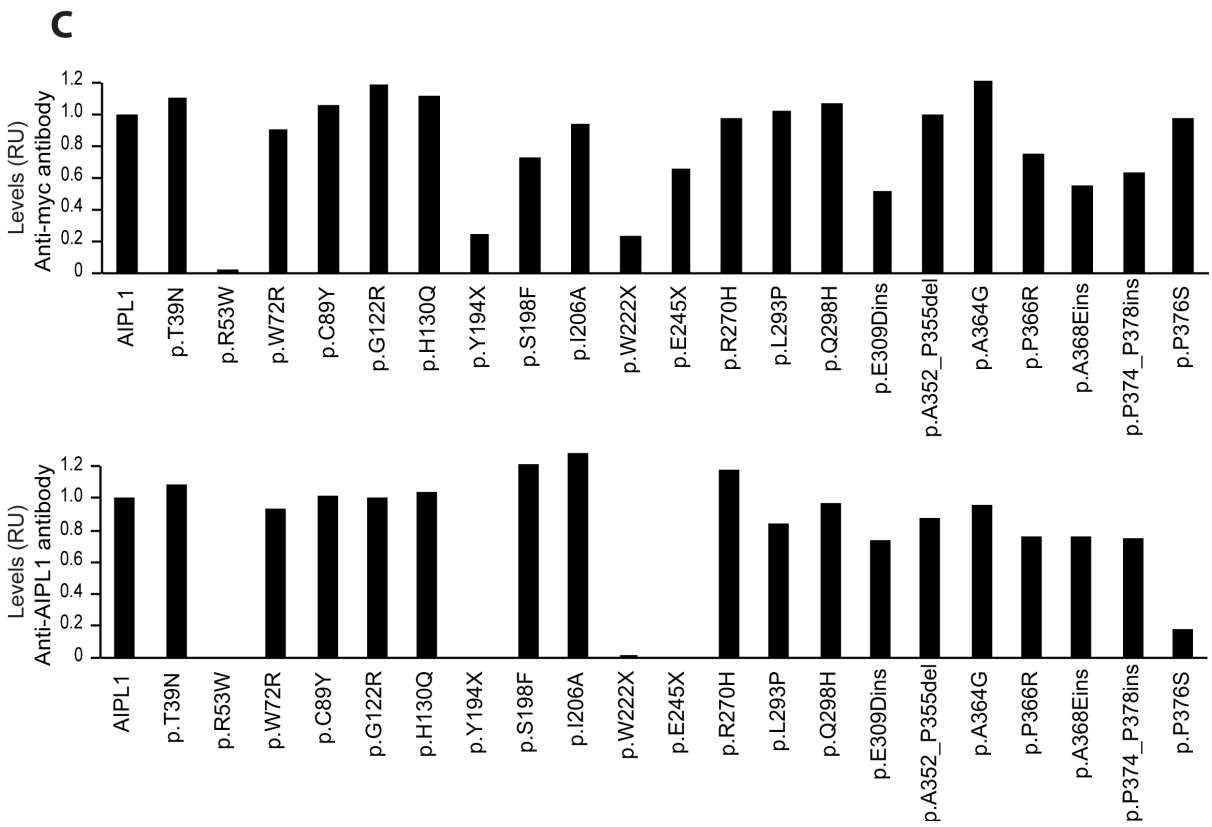


Supplementary Figure 2



Supplementary Figure 3:




C
Levels (RU)
Anti-myc antibody
1.2
1.0
0.8
0.6
0.4
0.2
0
AIPL1
p.T39N
p.R53W
p.W72R
p.C89Y
p.G122R
p.H130Q
p.Y194X
p.S198F
p.I206A
p.W222X
p.E245X
p.R270H
p.L293P
p.Q298H
p.E309Dins
p.A352_P355del
p.A364G
p.P366R
p.A368Eins
p.P374_P378ins
p.P376S
Levels (RU)
Anti-AIPL1 antibody
1.2
1.0
0.8
0.6
0.4
0.2
0
AIPL1
p.T39N
p.R53W
p.W72R
p.C89Y
p.G122R
p.H130Q
p.Y194X
p.S198F
p.I206A
p.W222X
p.E245X
p.R270H
p.L293P
p.Q298H
p.E309Dins
p.A352_P355del
p.A364G
p.P366R
p.A368Eins
p.P374_P378ins
p.P376S