Supplementary information

Supplementary Table 1. Collection sites for individuals of northern European descent and *RPGRIP1L* A229T frequencies for each group.

Clinical Diagnosis	Ascertainment Location	n=	Genotype Counts			Genotype Frequency			Allele Counts		Allele Frequency	
			GG	GA	AA	GG	GA	AA	G	A	G	A
Controls	North America[1,2]	999	935	63	1	0.936	0.063	0.001	1933	65	0.967	0.033
Controls	UK[3]	509	490	19	0	0.963	0.037	0	999	19	0.981	0.019
Total		**1508**	**1425**	**82**	**1**	**0.945**	**0.054**	**0.001**	**2932**	**84**	**0.972**	**0.028**
Ciliopathy Non-RP	North America[4]	66	66	0	0	1	0	0	132	0	1	0
Ciliopathy Non-RP	UK[3]	20	20	0	0	1	0	0	40	0	1	0
Ciliopathy Non-RP	France[5]	22	22	0	0	1	0	0	44	0	1	0
Ciliopathy Non-RP	Germany[6]	7	7	0	0	1	0	0	14	0	1	0
Total		**115**	**115**	**0**	**0**	**1**	**0**	**0**	**230**	**0**	**1**	**0**
Ciliopathy RP	North America[2,4,8,9]	373	340	32	1	0.912	0.086	0.003	712	34	0.954	0.046
Ciliopathy RP	UK[3,10]	67	61	6	0	0.910	0.090	0	128	6	0.955	0.045
Ciliopathy RP	Germany[6]	47	43	4	0	0.915	0.085	0	90	4	0.957	0.043
Total		**487**	**444**	**42**	**1**	**0.912**	**0.086**	**0.002**	**930**	**44**	**0.955**	**0.045**

[1]Johns Hopkins University, Baltimore, MD, USA; [2]Baylor College of Medicine, Houston, TX, USA; [3]St. James University Hospital, Leeds, UK; [4]University of Michigan, Ann Arbor, MI, USA; [5]Hôpital Necker-Enfants Malades, Paris, France; [6]RWTH University of Aachen, Aachen, Germany; [8]McGill University Health Centre, Montreal, Quebec, Canada; [9]University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [10]University College London, London, UK.

Supplementary Table 2. ***In vivo*** **assessment of** ***RPGRIP1L*** **variants in zebrafish**

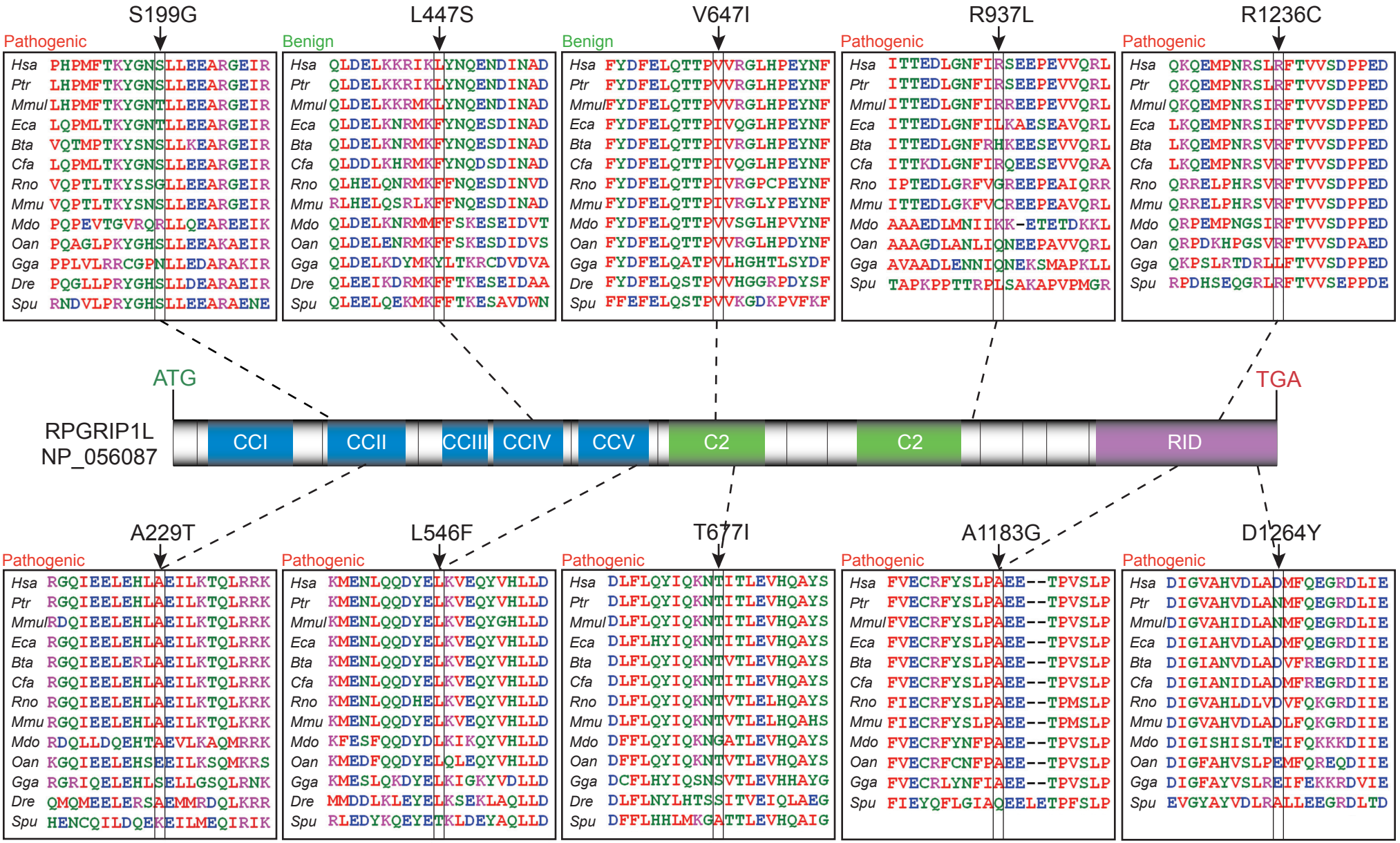
Injection	Embryo counts				Statistical assessment			
	total n=	Normal	Class I	Class II	χ^2	p-value*	χ^2	p-value*
					vs. wt Controls		vs. MO	
Controls	99	96	3	1				
MO	201	104	80	17	791.876	<0.0001		
MO + wt mRNA	116	104	11	1	25.005	<0.0001	171.822	<0.0001
					vs. wt rescue		vs. MO	
MO + S199G mRNA	124	102	21	1	7.822	0.02	36.658	<0.0001
MO + A229T mRNA	88	64	16	8	76.211	<0.0001	20.706	<0.0001
MO + L447S mRNA	119	104	12	3	4.211	0.1218	49.183	<0.0001
MO + L546F mRNA	115	71	37	7	92.489	<0.0001	4.023	0.1338
MO + T615P mRNA	85	60	16	9	113.972	<0.0001	18.381	<0.0001
MO + V647I mRNA	95	73	12	10	102.619	<0.0001	30.427	<0.0001
MO + T677I mRNA	95	77	8	10	101.011	<0.0001	42.898	<0.0001
MO + A695P mRNA	115	67	38	10	139.378	<0.0001	2.042	0.3602
MO + R937L mRNA	88	51	28	9	151.156	<0.0001	2.792	0.2475
MO + G1025S mRNA	99	88	11	0	0.540	0.7634	51.210	<0.0001
MO + A1183G mRNA	118	88	28	2	28.208	<0.0001	20.855	<0.0001
MO + R1236C mRNA	110	92	15	3	7.097	0.0288	39.314	<0.0001
MO + D1264Y mRNA	83	47	25	11	205.1	<0.0001	6.106	0.0472

* χ^2 with two degrees of freedom

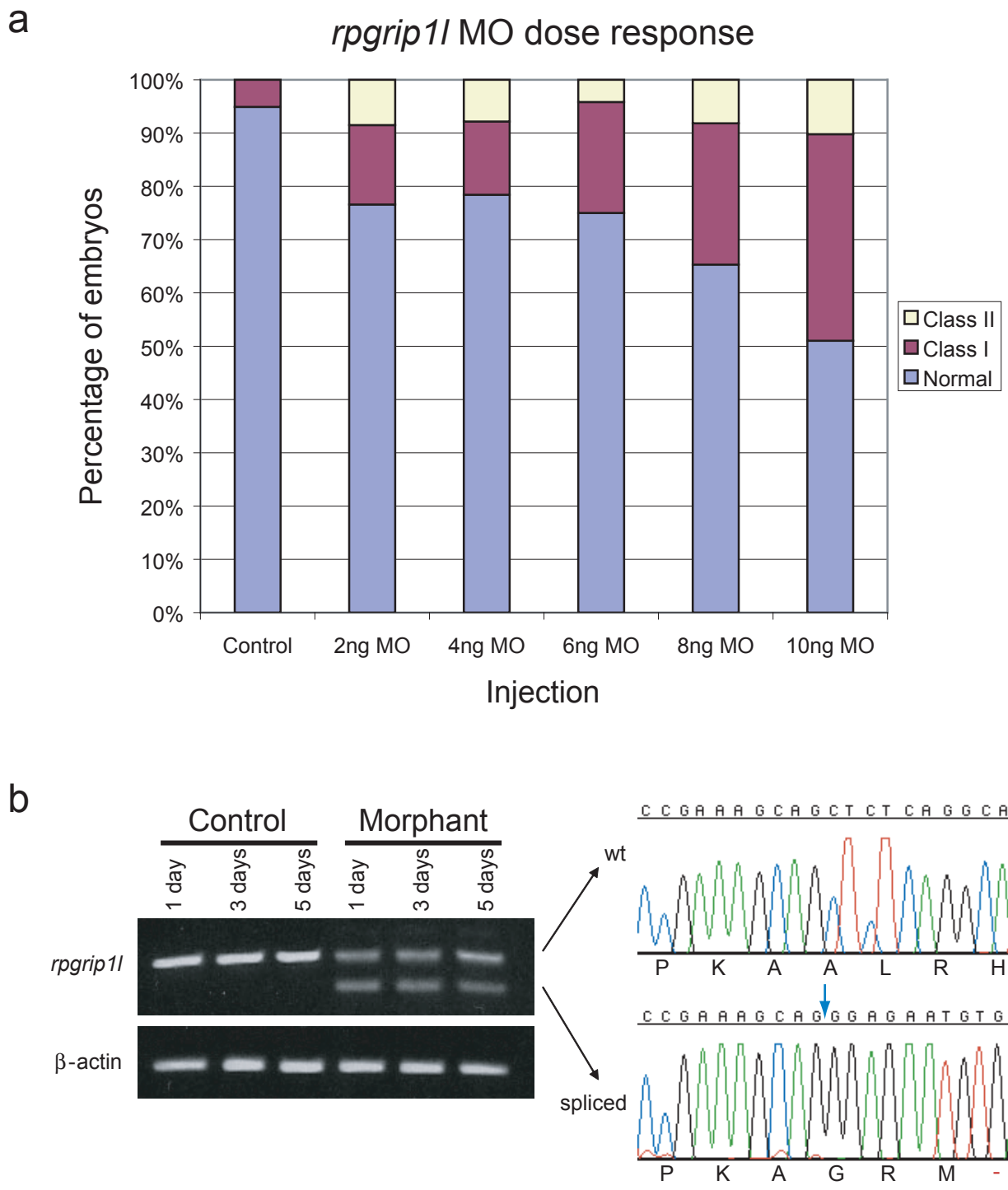
Supplementary Table 3. Morphological assessment of *RPGRIP1L* variants in zebrafish.

Injection	Embryo measurements			Two-tailed student's t-test	
	total n=	mean w/l ratio*	standard dev.	p-value	p-value
				vs. Controls	vs. MO
Controls	11	0.1868	0.0129		
MO	11	0.2158	0.0375	0.0248	
MO + wt mRNA	8	0.1765	0.0144	0.1174	0.0122
				vs. wt rescue	vs. MO
MO + S199G mRNA	18	0.2060	0.0373	0.0422	0.4968
MO + A229T mRNA	20	0.2278	0.0266	<0.0001	0.3084
MO + L447S mRNA	15	0.1819	0.0221	0.5367	0.0080
MO + L546F mRNA	19	0.2104	0.0307	0.0065	0.6739
MO + T615P mRNA	19	0.2201	0.0219	<0.0001	0.6904
MO + V647I mRNA	20	0.2050	0.0409	0.0671	0.4742
MO + T677I mRNA	20	0.2114	0.0199	<0.0001	0.6732
MO + A695P mRNA	18	0.2324	0.0421	0.0013	0.2926
MO + R937L mRNA	9	0.2435	0.0677	0.0152	0.2603
MO + G1025S mRNA	18	0.1812	0.0100	0.3431	0.0009
MO + A1183G mRNA	15	0.2377	0.0219	<0.0001	0.0734
MO + R1236C mRNA	10	0.2098	0.0229	0.0025	0.6657
MO + D1264Y mRNA	13	0.2428	0.0214	<0.0001	0.0381

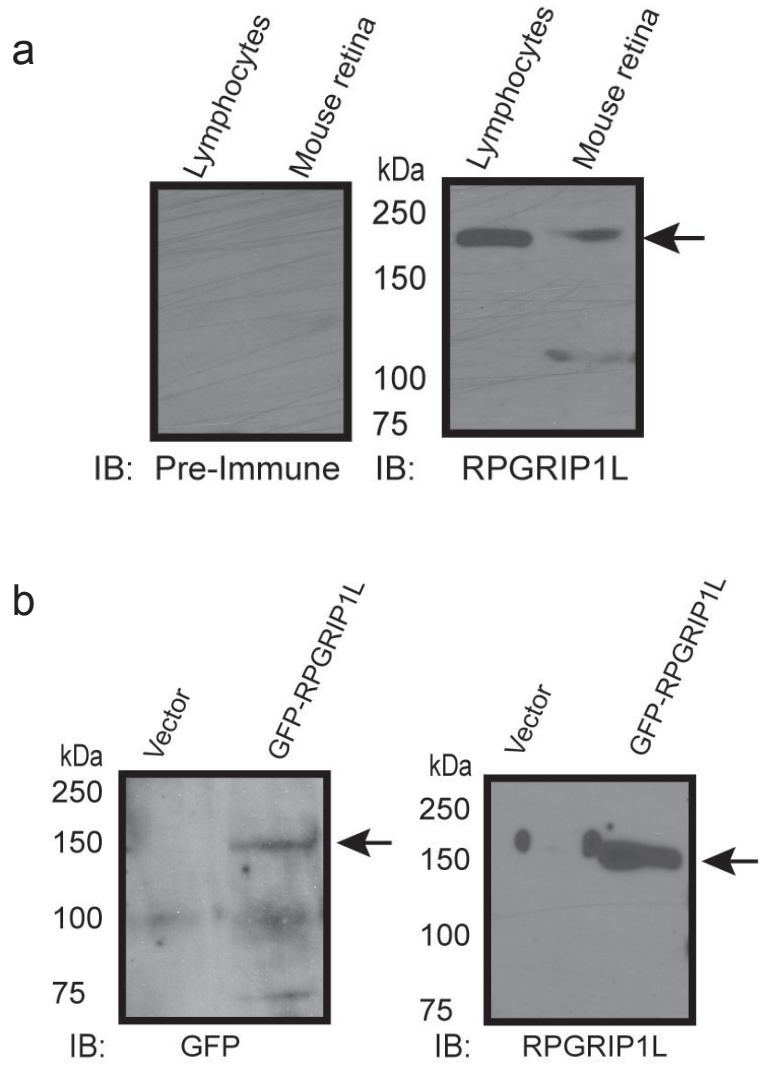
*(w) width spanning the 5th somites from the anterior end/ (l) length of the notochord as defined by *myoD*



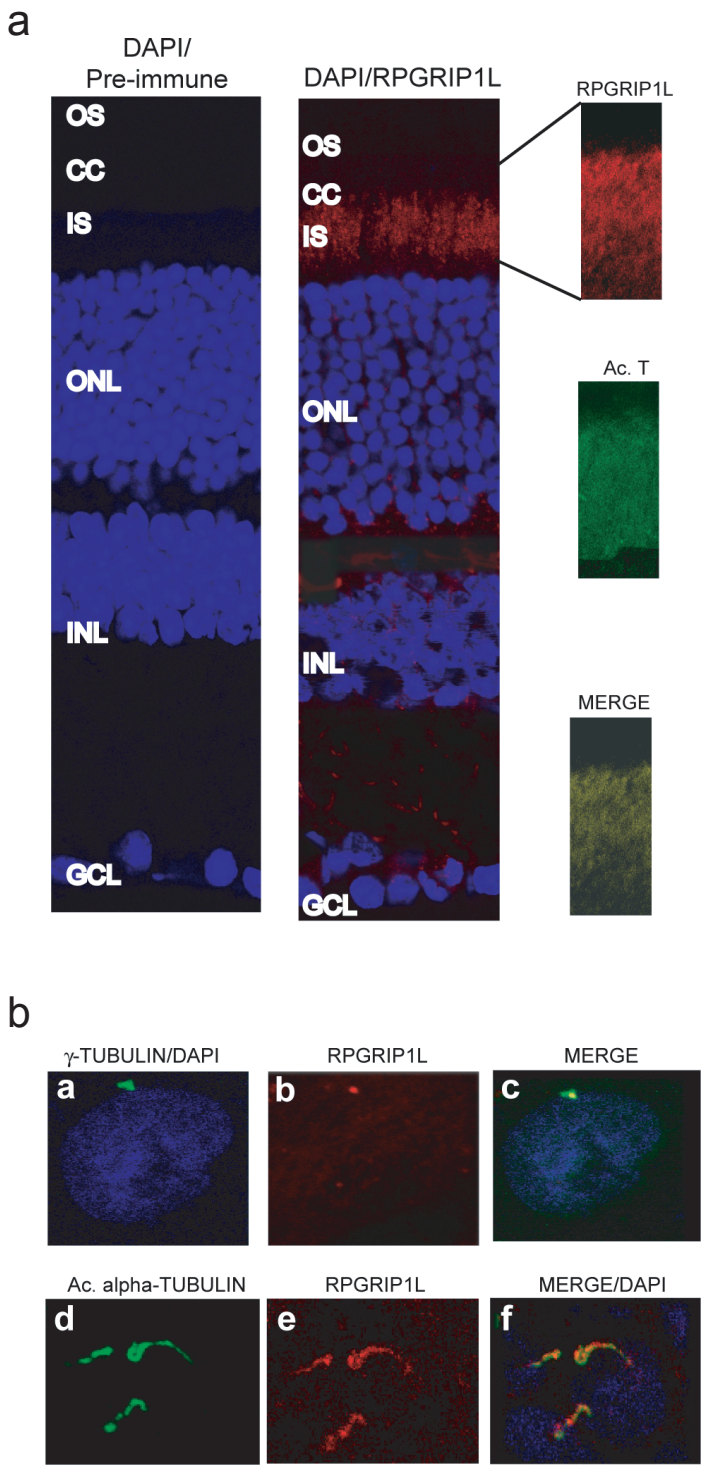
Supplementary Figure 1. Schematic of RPGRIP1L and amino acid conservation of variants in vertebrate species. RPGRIP1L (NP_056087) consists of 1315 amino acids and contains protein motifs including five coiled-coil domains (CCI, CCII, CCIII, CCIV, and CCV; shown in blue), two protein kinase C (PKC) conserved region 2 motifs (C2; shown in green); and a domain with homology to the RPGR-interacting domain of RPGRIP1 (RID; shown in purple). Nine of ten missense *RPGRIP1L* missense variants are located within the known protein domains and all are depicted with multiple sequence alignments from 13 different organisms in which we could identify a reciprocal ortholog. *Hsa- H. sapiens*; *Ptr- Pan troglodytes*; *Mmul- M. mulatta*; *Eca- E. caballus*; *Bta- B. taurus*; *Cfa- C. familiaris*; *Rno- R. norvegicus*; *Mmu- M. musculus*; *Mdo- M. domestica*; *Oan- O. anatinus*; *Gga- G. gallus*; *Dre- D. rerio*; *Spu- S. purpuratus*. Alignments were carried out with ClustalW and amino acid residues are given as single letter code with the following coloring for residues: Red- hydrophobic or aromatic (AVFPMILWY); blue- acidic (DE); magenta- basic (RHK); green- hydroxyl, amine, basic and glutamine (STYHCNGQ). Pathogenicity as determined by *in vivo* analysis in zebrafish is indicated by benign, or pathogenic.



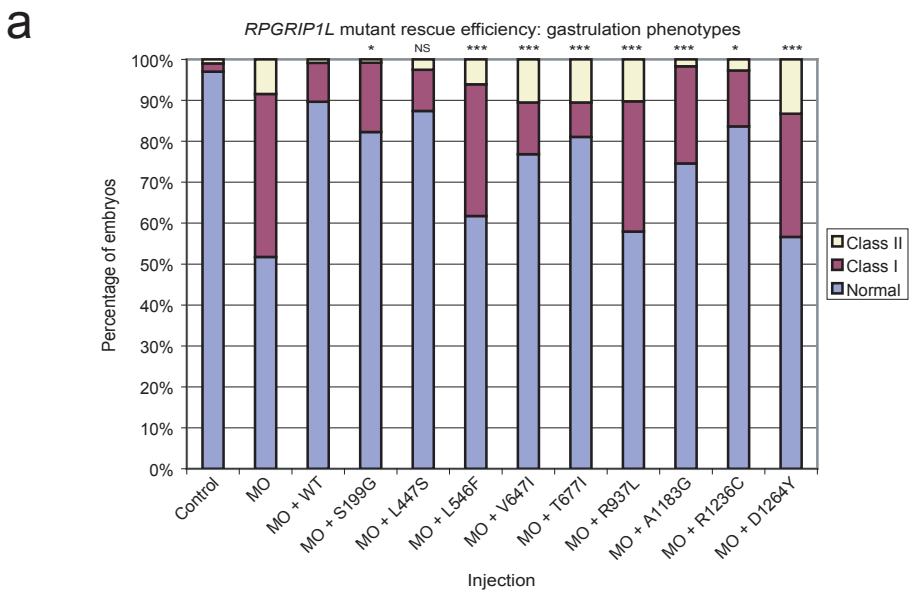
Supplementary Figure 2. Characterization of the *rpgrip1l* morpholino.
a. Dose-response curve of *rpgrip1l* MO at progressively increasing concentrations. We designed a splice donor-targeting MO against the single *D. rerio* ortholog of human RPGRIP1L and injected embryos with increasing amounts of MO at the two-cell stage, scored them for gastrulation defects at the eight-nine somite stage and categorized embryos within groups of 50-100 as Class I or Class II (see Figure 1 legend for explanation). An increased incidence of deleterious phenotypes corresponds with an increased dose.
b. Assessment of *rpgrip1l* MO efficiency by RT-PCR and sequencing. cDNA was produced from total RNA isolated from zebrafish embryos injected with 10ng MO, and amplified by limited-cycle PCR. Agarose gel electrophoresis of the PCR product indicates that the MO blocks ~50% of *rpgrip1l* message (left); direct sequencing shows that aberrant splicing introduces a premature stop codon (right). β-actin was used as a control for RNA quantity and integrity.

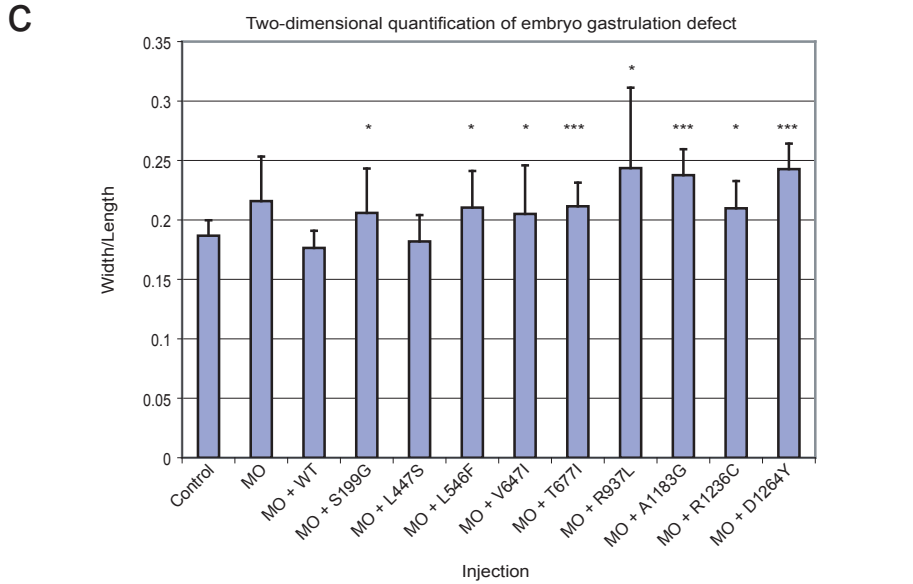


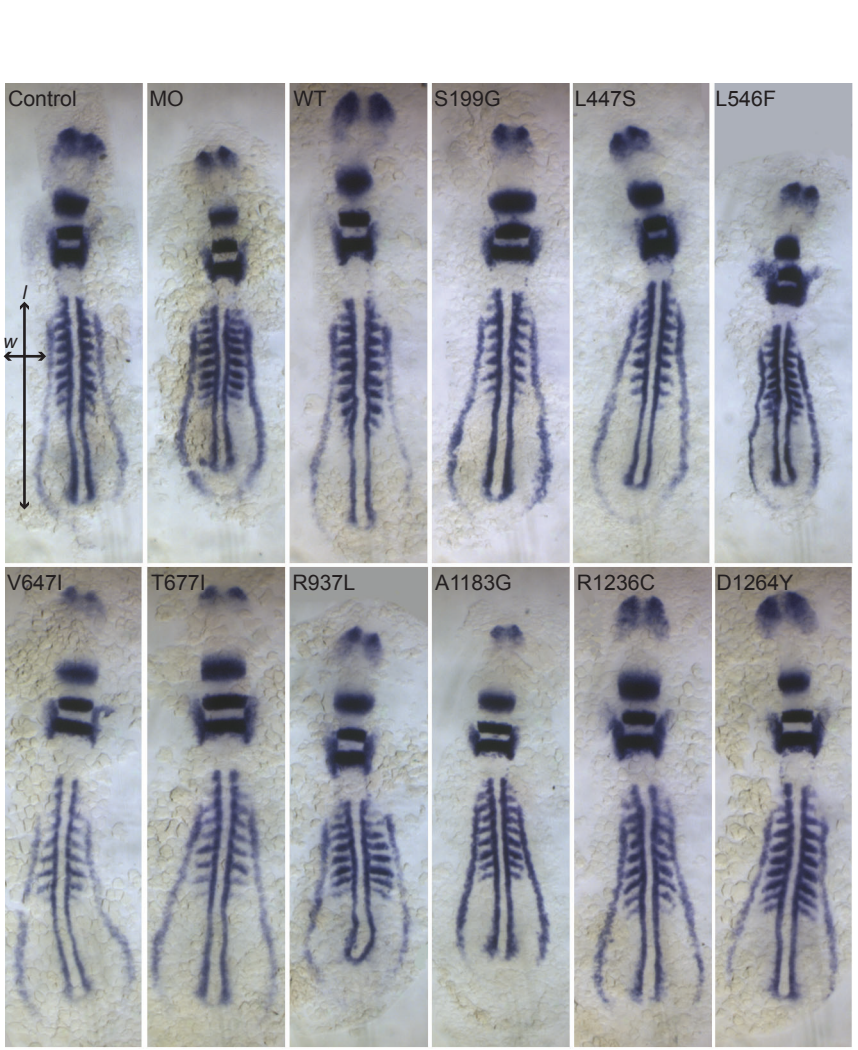
Supplementary Figure 3. Target specificity of the anti-human RPGRIP1L antibody.
a. Characterization of the RPGRIP1L antibody. Mouse retinal extract and human lymphocyte protein extracts were separated by SDS-PAGE followed by immunoblotting using pre-immune serum or the anti-RPGRIP1L antibody. Arrow indicates the antibody-reactive RPGRIP1L band, which is not detected by the pre-immune serum.
b. Confirmation of the anti-RPGRIP1L antibody. COS-1 cells were transiently transfected with constructs encoding GFP or GFP-RPGRIP1L fusion protein. Cell extracts were analyzed by SDS-PAGE and immunoblotting using the anti-GFP (left) or anti-RPGRIP1L (right) antibody. Arrows indicate the specific RPGRIP1L-specific bands. Molecular mass markers are shown in kDa.



Supplementary Figure 4. RPGRIP1L localizes to cilia in mammalian cells.
a. Localization of RPGRIP1L in the retina. Adult mouse retinal cryosections were subjected to immunohistochemical analysis using anti-RPGRIP1L (red) and anti-acetylated-α tubulin (Ac. T; green) antibodies. Inset shows co-localization of RPGRIP1L and Ac. T at the photoreceptor connecting cilium (Merge; yellow). Nuclei were stained with DAPI (blue).
b. Ciliated or non-ciliated mIMCD-3 cells were stained with antibodies against γ-tubulin (centrosome marker; green; 'a'), acetylated α-tubulin (ciliary marker; green; 'b') and RPGRIP1L (red; 'b' and 'e'). Merged image shows co-localization of RPGRIP1L at centrosomes ('c') and primary cilia ('f') (yellow). Nuclei were stained with DAPI.







Supplementary Figure 5. Functional assessment of *RPGRIP1L* variants ***in vivo*****.**

a. Live scoring of embryos co-injected with MO and human *RPGRIP1L* mRNA indicates that most *RPGRIP1L* variants are pathogenic in our assay as indicated by the comparison of WT rescue efficiency to mutant rescue efficiency. MO, morpholino alone; WT, wild-type human *RPGRIP1L* mRNA. Statistical significance (mutant vs. WT rescue) is depicted as (*), p<0.05 and (***), p<0.0001 (χ^2); see Suppl Table 2 for χ^2 and p-values.

b. Representative examples of whole-mount embryos hybridized *in situ* with *pax2*, *krox20*, and *myoD* riboprobes.

c. Two-dimensional morphometric quantification of the efficiency of human *RPGRIP1L* mRNA to rescue *rpgrip1l* MO phenotypes. Whole embryos with eight-nine appreciable somites (n=8-20/injection) labeled with *pax2*, *krox20*, and *myoD* riboprobes were flat-mounted, photographed and measured in two dimensions. The width/length ratio was calculated as the width (w) spanning between the distal ends of the 5th anterior somites (horizontal arrow in panel c) vs. the length (l) of the notochord as indicated by the staining of adaxial cells (vertical arrow in panel b). Statistical significance (mutant vs. WT rescue) is depicted as (*), p<0.05 and (***), p<0.0001 (two-tailed student's t-test); see Supplementary Table 3 for p-values.