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Supplemental Data

Mutations in *IMPG1* Cause Vitelliform Macular Dystrophies

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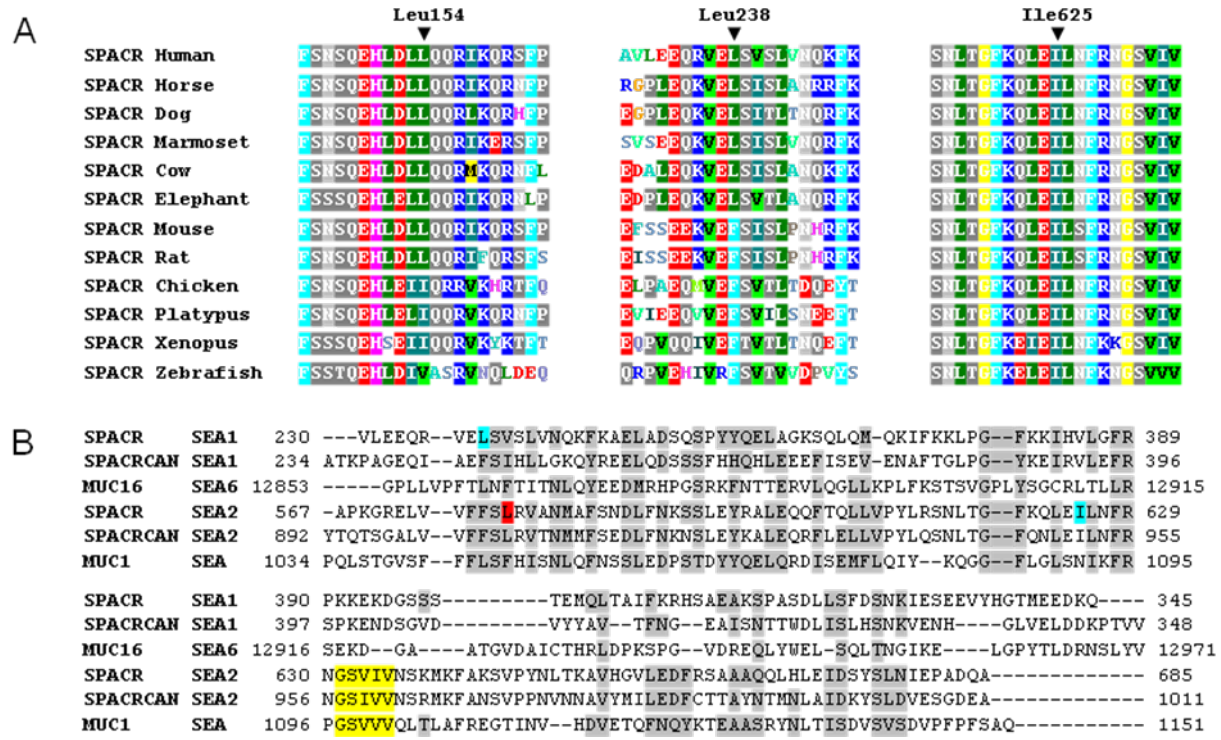
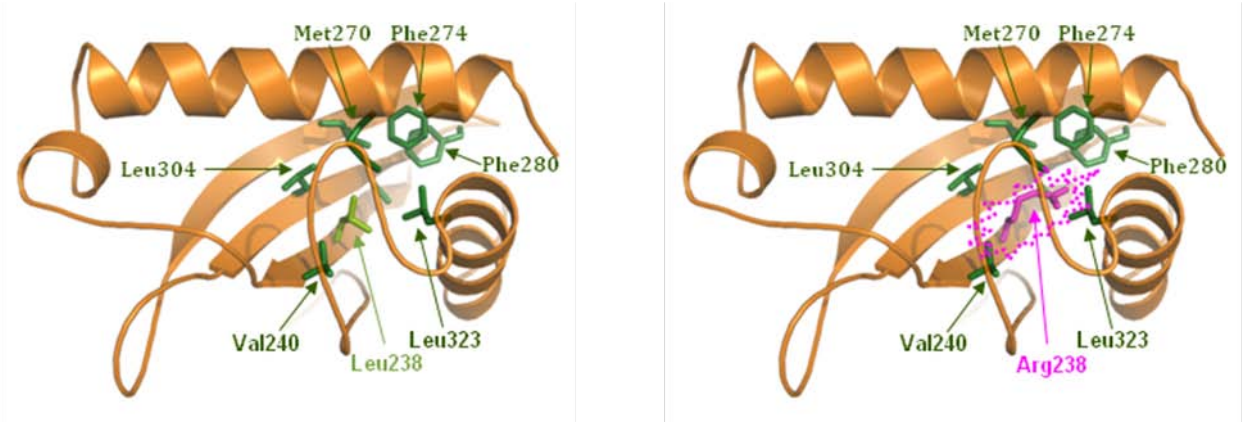


<u>Figure S1</u>: Amino-acid alignments

(**A**) Multiple amino-acid sequence alignment of SPACR for a region surrounding the p.Leu154Pro, p.Leu238Arg and p.Ile625Met missense changes. The site of the mutation is indicated by an arrowhead. (**B**) Multiple amino-acid sequence alignment of SPACR and SPACRCAN SEA1 and SEA2 domains with the non-proteolytic SEA6 domain of Mucin 16 (Muc16) and the proteolytic SEA domain of Mucin 1. Conserved amino-acids are in grey. Two missense *IMPG1* mutations found in this study are in blue and the missense *IMPG1*

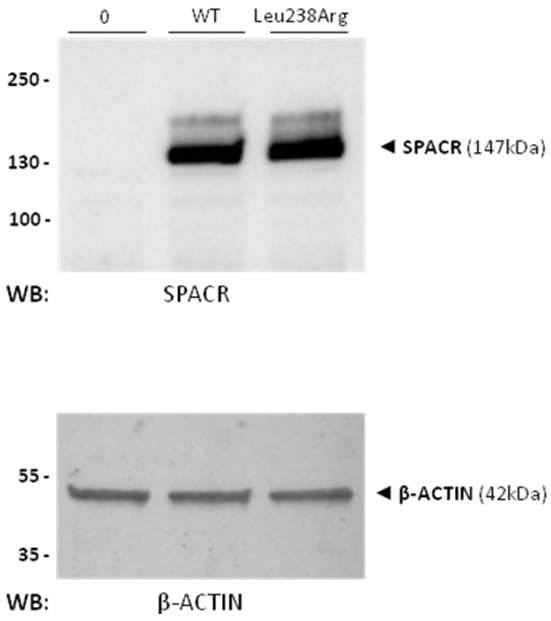
mutation found in BCAMD condition is in red. The consensus sequence for autoproteolysis is in yellow.

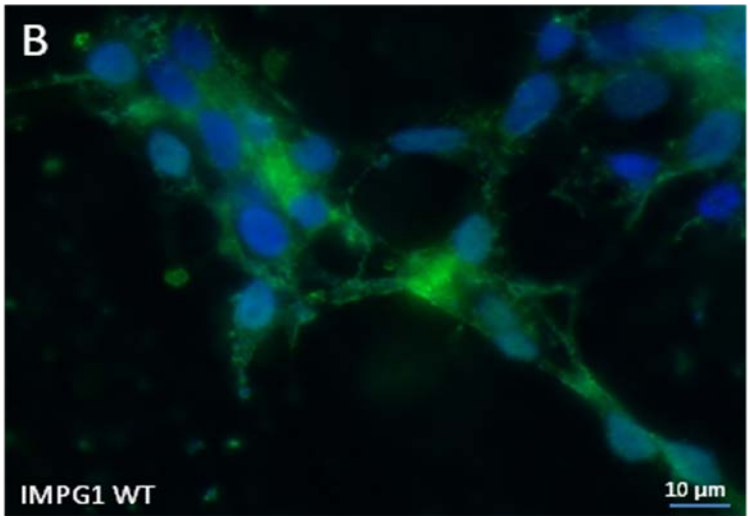


<u>Figure S2</u>: SPACR SEA1 domain structure

Three-dimensional model of the N-terminal SEA1 domain of SPACR using Pymol showing α-helices (helix shape) and β-sheets (ribbon shape). Interactions of wild-type Leu238 (pale green) and mutated Arg238 (flashy pink) with the hydrophobic residues (Val240, Met270, Phe274, Phe280, Leu304, and Leu323) surrounding the position 238 are shown. The highly unfavourable contacts between the polar and charged side-chain of the arginine residue are depicted by the dotted pink line.

A


0
WT
Leu238Arg
250 -
130 -
100 -
◄ SPACR (147kDa)
WB:
SPACR
55 -
35 -
◄ β-ACTIN (42kDa)
WB:
β-ACTIN


B
IMPG1 WT
10 µm

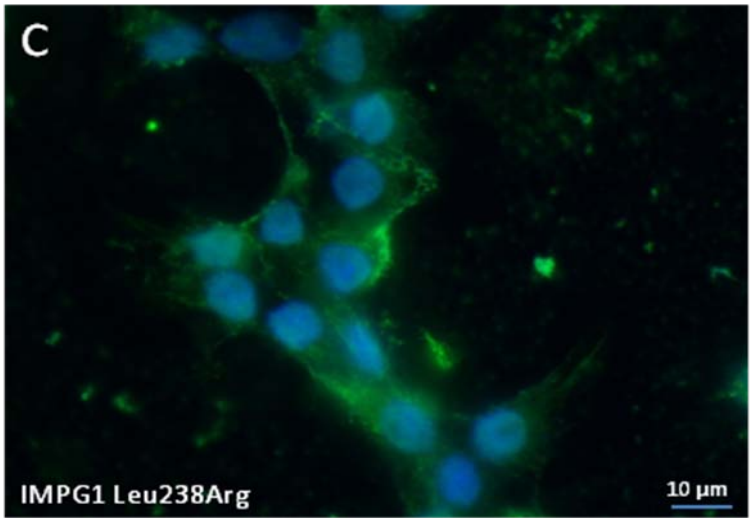

C
IMPG1 Leu238Arg
10 µm

Figure S3: Characterization of wild-type and p.Leu238Arg mutant SPACR proteins by Western blot analysis and immunofluorescence.

Cos7 cells were transiently transfected with pRK5 fused in-frame with wild-type (WT) or p.Leu238Arg mutant IMPG1. (**A**) Protein expression levels of wild-type (WT) and p.Leu238Arg mutant were examined by Western blot. Lane 0, untransfected lysate. SPACR was detected with a rabbit anti-SPACR and an anti-rabbit-HRP antibodies. Beta-Actin was detected with mouse β-Actin and anti-mouse-AP antibodies. Both WT and mutant proteins migrate at the predicted molecular weight of 147 kDa. (**B**, **C**) SPACR is stained in green (rabbit anti-SPACR and donkey anti-rabbit Alexa A488), and nuclei are stained in blue (DAPI). Both wild-type (**B**) and mutant (**C**) proteins localize to the cytoplasm with the same pattern.

Table S1: Heterozygous variants common to affected patients on the mapped locus on chromosome 6p12.1-q24.3

Position	Gene	Type	Exon	Variation	EVS
57 398 187	*PRIM2*	indel	10	-/A	0/13006
71 212 388	*FAM135A*	missense	10	p.Ala308Val	0/13006
76 728 529	*IMPG1*	missense	7	p.Leu238Arg	0/13006
84 798 955	*MRAP2*	missense	4	p.Arg125Cys	6/13006
88 265 144	*RARS2*	missense	5	p.Lys126Arg	0/13006
128 134 516	*THEMIS*	missense	4	p.Ala389Thr	117/13006
148 789 688	*SASH1*	missense	5	p.Leu132Val	12/13006

EVS: Exome Variant Server