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Supplemental information

Variants in the degron of AFF3 are associated with intellectual disability, mesomelic dysplasia, horseshoe kidney, and epileptic encephalopathy

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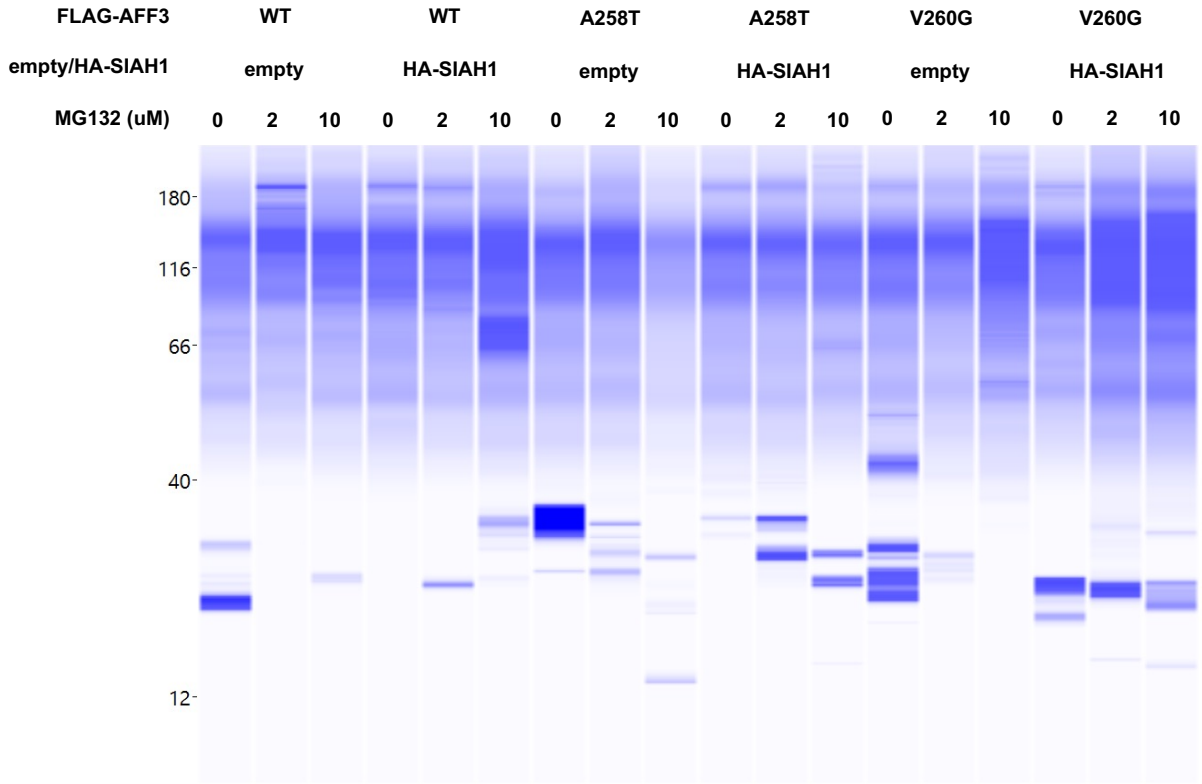
Figure S1

A



B

Sample	Primary	PN (%)
AFF3 WT + empty	Flag M2- SLCD3990	100
AFF3 WT +empty + MG132 -2	Flag M2- SLCD3990	103
AFF3 WT +empty + MG132 -10	Flag M2- SLCD3990	104
AFF3 WT +SIAH	Flag M2- SLCD3990	107
AFF3 WT +SIAH + MG132-2	Flag M2- SLCD3990	103
AFF3 WT +SIAH + MG132-10	Flag M2- SLCD3990	117
AFF3 A258T +empty	Flag M2- SLCD3990	104
AFF3 A258T +empty + MG132 -2	Flag M2- SLCD3990	104
AFF3 A258T +empty + MG132 -10	Flag M2- SLCD3990	84
AFF3 A258T +SIAH	Flag M2- SLCD3990	102
AFF3 A258T +SIAH + MG132-2	Flag M2- SLCD3990	101
AFF3 A258T +SIAH + MG132-10	Flag M2- SLCD3990	99
AFF3 V260G +empty	Flag M2- SLCD3990	110
AFF3 V260G +empty + MG132 -2	Flag M2- SLCD3990	100
AFF3 V260G +empty + MG132 -10	Flag M2- SLCD3990	114
AFF3 V260G +SIAH	Flag M2- SLCD3990	103
AFF3 V260G +SIAH + MG132 -2	Flag M2- SLCD3990	122
AFF3 V260G +SIAH + MG132 -10	Flag M2- SLCD3990	124

Figure S1: Loading control and normalization of the FLAG immuno-assays presented in Figure 2A. Protein extracts were separated by capillarity on a Jess system and immuno-assayed with an anti-FLAG antibody. (A) Fluorochrome fixation for normalisation of protein extracts of the Jess run. (B) Normalisation values of all samples in percent (PN) using as reference the protein extract FLAG-AFF3 WT and empty vector.

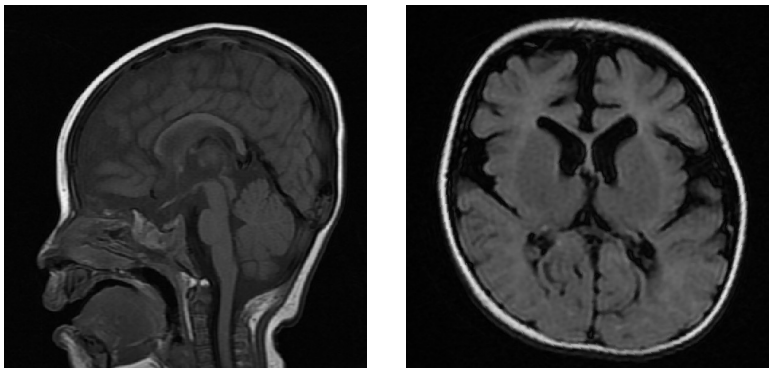


Figure S2: Brain MRI of proband 11 carrying a *de novo* variant in *AFF3.* FLAIR (right) and T1 (left) at 9 months old are shown.

Table S2: Phenotypes of Aff3del/del homozygous in-frame truncation (chimeric mice from diploid aggregation with homozygous ES cells

	E18.5		E14.5		E14.5/E18.5 combined	
phenotype	numbers	pct[%]	numbers	pct[%]	numbers	pct[%]
polydactyly	5	42	1	25	6	38
mesomelic dyplasia	12	100	4	100	16	100
triangular tibia	12	100	4	100	16	100
hypoplastic fibula	12	100	4	100	16	100
hypoplastic pelvis	12	100	NA		NA	
craniofacial dysmorphism	12	100	4	100	16	100
delayed ossification (skull)	12	100	NA		NA	
intestinal prolapse	10	83	NA		NA	
lack of motoric reflexes /neurological dysfunction	12	100	NA		NA	
reduces body size	12	100	4	100	16	100
abort	1	8	0	0	1	6
total numbers of fetuses (abort excluded)	12	100	4	100	16	100
total numbers of litters	3		1		4	

motoric reflex = touch triggers contraction in E18.5
NA = not available, not investigated, in case of motoric reflexes not applicable