Supplementary figure 1 – Sequencing statistics

Table listing information regarding the sequencing statistics and prioritization of variants.

	2	3
Proportion of Exome covered at least 10X	95,60%	96,11%
Average Coverage	111.3 X	119.8 X
Rare variants (n=)[1]	1641	2137
Rare Non-Synonymous variants (n=)[2]	298	300
Rare Non-Synonymous Compound Heterozygous (n=)	34	12
Rare Non-Synonymous Compound Heterozygous, shared (n=) (*Genes*)	4 (IGSF3, CTDSP2)	
Rare Non-Synonymous Homozygous (n=2)	7	9
Rare Non-Synonymous Homozygous, shared (n=) (*Genes*)	2 (STXBP1 , ZNF684)	

[1] = Frequency of minor allele (MAF) ≤ 0.001

[2] = include splice variants affecting intronic sequences within +/- 2bp from exon boundary

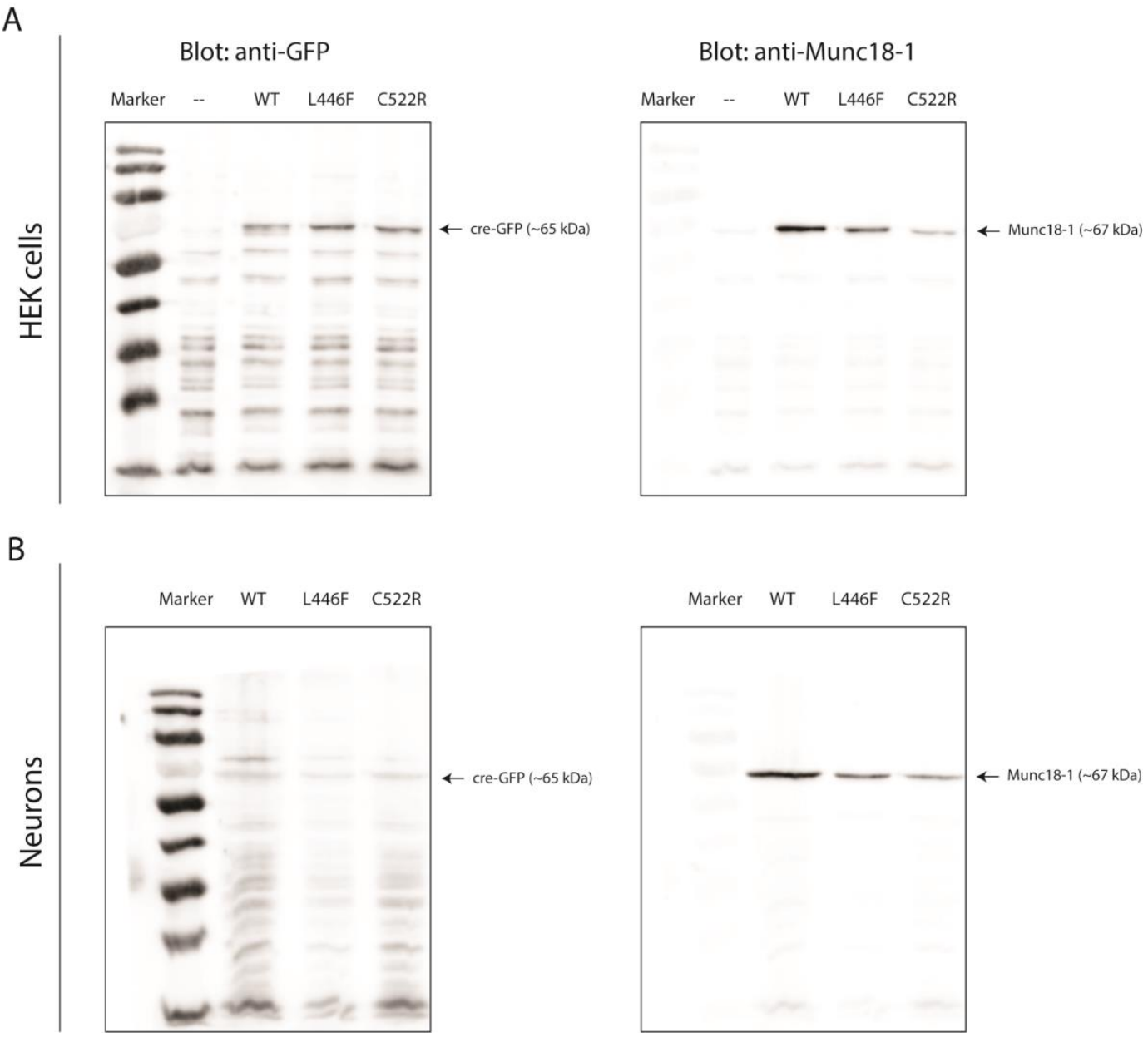
Location	Ref Allele	Variant type	Gene	CDS position	Codons	Protein position	Amino acids	SNP ID	MAF (gnomAD)	Predictions			
										SIFT	PolyPhen	CADD PHRED	GERP+ + NR
Compound Heterozygous													
1:117146504	G	missense	IGSF3	1426	CGC/TGC	476	R/C	rs61786577	0,000008	Deleterious (0.01)	Probably Damaging (0.991)	34	5.27
1:117156459	C	missense	IGSF3	760	GAT/AAT	254	D/N	-	-	Deleterious (0.02)	Probably Damaging (1)	32	4.77
12:58220816	A	missense	CTDSP2	317	ATT/ACT	106	I/T	rs76940645	0,000008	Tolerated (0.08)	Possibly Damaging (0.715)	25.3	4.92
12:58220844	C	missense	CTDSP2	289	GAA/AAA	97	E/K	rs75591888	0,000004	Tolerated (0.56)	Benign (0)	19.82	4.92
Homozygous													
9:130439009	C	missense	STXBP1	1336	CTC/TTC	446	L/F	-	-	Deleterious (0.03)	Probably Damaging (0.983)	23.9	5.2
1:41012540	C	missense	ZNF684	545	ACA/AGA	182	T/R	rs959941154	0,000008	Deleterious (0.03)	Benign (0.041)	23.4	4.04

Gene	Mutation	Gene description	Clinical significance	Mouse models	Segregation analysis	Variant Effect Predictor (VEP)[3]
IGSF3	c.1426 C>T (p.R476C) heterozygous c.760 G>A (p.D254N) heterozygous	IGSF3 is an immunoglobulin (Ig)-like membrane protein showing high expression in placenta, kidney, and lung (Saupe et al., 1998). Functional studies suggested a role of IGSF3 in neuronal morphogenesis in the cerebellum through the interaction with TSPAN7 and in cell adhesion and motility in the lung (Usardi et al., 2017; Schweitzer et al., 2018).	Homozygous 1-bp frameshift deletion (c.2995delC, Arg999AlafsTer15) in the IGSF3 gene was found in four brothers affected by bilateral nasolacrimal duct obstruction (LCDD; Foster et al., 2014).	N/A	Mother: heterozygous for c.1426 C>T (p.R476C). Father: heterozygous for c.760 G>A (p.D254N) variant. Unaffected sibling is compound heterozygous.	The identified variants are evolutionary conserved and may be functionally significant (Mc Laren et al., 2016).
CTDSP2	c.317 T>C (p.I106T) heterozygous c.289 G>A (p.E97K) heterozygous	CTDSP2 encodes for a ubiquitously expressed protein with highest expression in pancreas and lowest expression in brain, lung, and liver (Su et al., 1997). CTDSP2 belongs to of class-C RNA polymerase II (RNAPII) carboxyl-terminal domain (CTD) phosphatases and was shown to be involved in the silencing neuronal gene expression in non-neuronal cells (Yeo et al. 2003; Yeo et al., 2005). CTDSP2 is specifically involved in promoter clearance during steroid-activated transcription and also regulates cell cycle progression through Ras and p21 (Thompson et al., 2006; Kloet et al., 2015).	N/A	N/A	Mother: heterozygous for c.289 G>A (p.E97K) variant. Father: heterozygous for c.317 T>C (p.I106T). Unaffected sibling is heterozygous for c.317 T>C (p.I106T).	The identified CTDSP2 variants are not highly conserved and evidence for a functional role is weak.
ZNF684	c.545 C>G (p.T182R) homozygous	ZNF684 encodes a ubiquitously expressed putative transcription factor of unknown function.	N/A	N/A	The parents and unaffected sibling:heterozygous carriers.	The identified variant is not evolutionary conserved and is unlikely to be functionally significant.

[3] = McLaren et al., 2016

Supplementary figure 2 – Unmodified full-length western blots

Full-length blots of typical examples displayed in Fig. 2A and B.

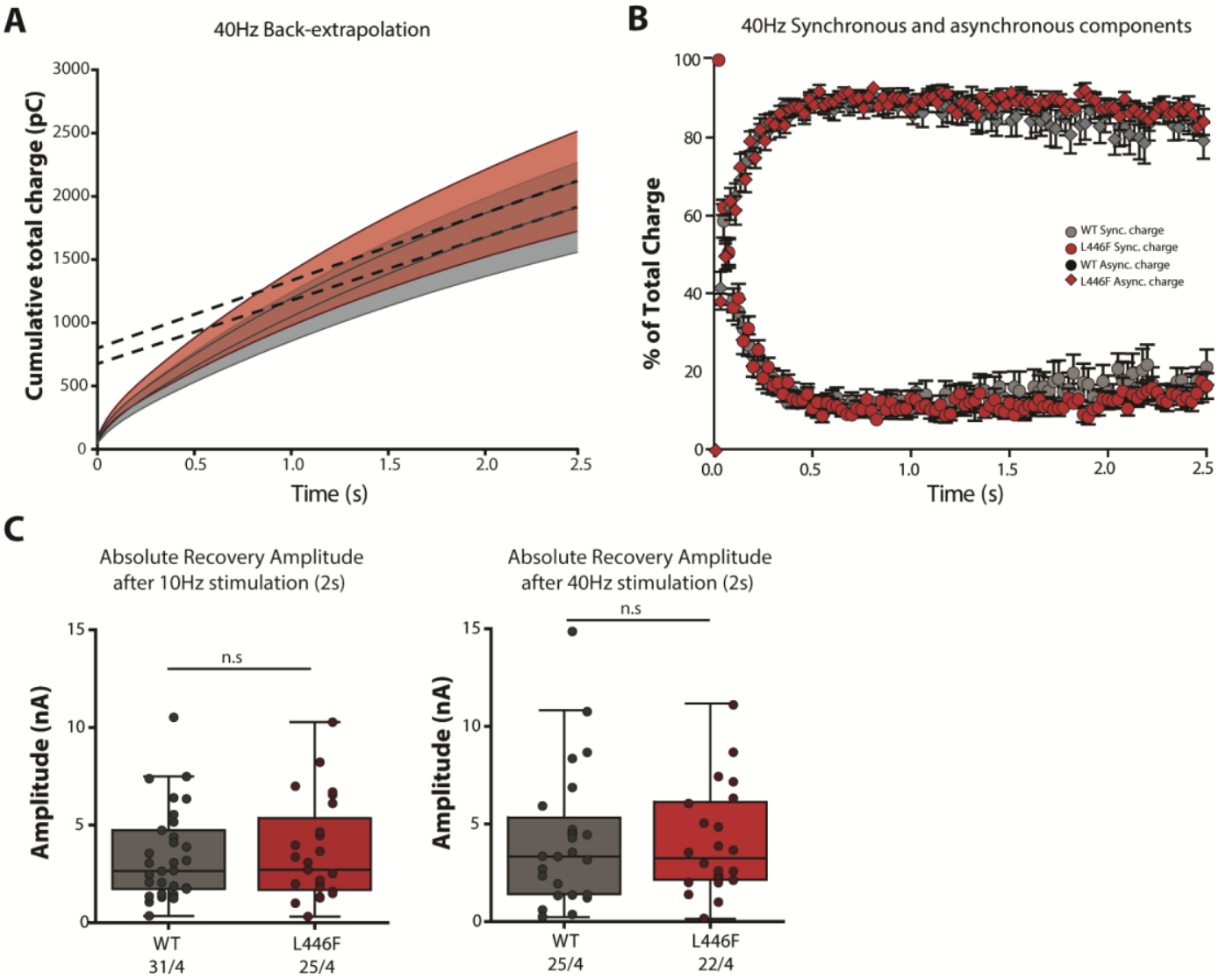


Supplementary figure 3 - Patch-clamp electrophysiological characterization of Munc18^{L446F} neurons– additional information

(A) Schematic representation of the back-extrapolation procedure. Cumulative total charge transferred during the 40Hz train (100 action potentials) is plotted and a line is back-extrapolated on the last 30 pulses of the train. Y-intercept represents an estimate of the readily releasable pool (in pC), the slope of the line represents the recruitment rate of vesicles at steady state during stimulation.

(B) There is no difference between the relative contribution of synchronous (circles) and asynchronous (squares) charge components during the 40Hz train between Munc18^{WT} or M18$_{L446F.}$

(C) No significant difference is observed in absolute EPSC amplitude in response to the single 'recovery' stimulations, delivered 2 seconds following the 10Hz (left; Munc18^{WT} median=2652pA, IQR=4737-1737, Munc18^{L446F} median=2714pA, IQR=5359-1684, p = 0.856, Mann-Whitney U test) or 40Hz (right; Munc18^{WT} median=3330pA, IQR=5330-1420, Munc18^{L446F} median=3252pA, IQR=6126-2149, p = 0.823, Man-Whitney U test) train stimulation.



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Full blots for Fig 2 A and B.
Note that not all lanes on these blots were part of this experiment: for Fig. 2A, the first four lanes after the marker are part of this experiment, for Fig. 2B, the first three lanes after the marker. For both blots, the green signal is Munc18-1, red is GFP.

Fig 2A

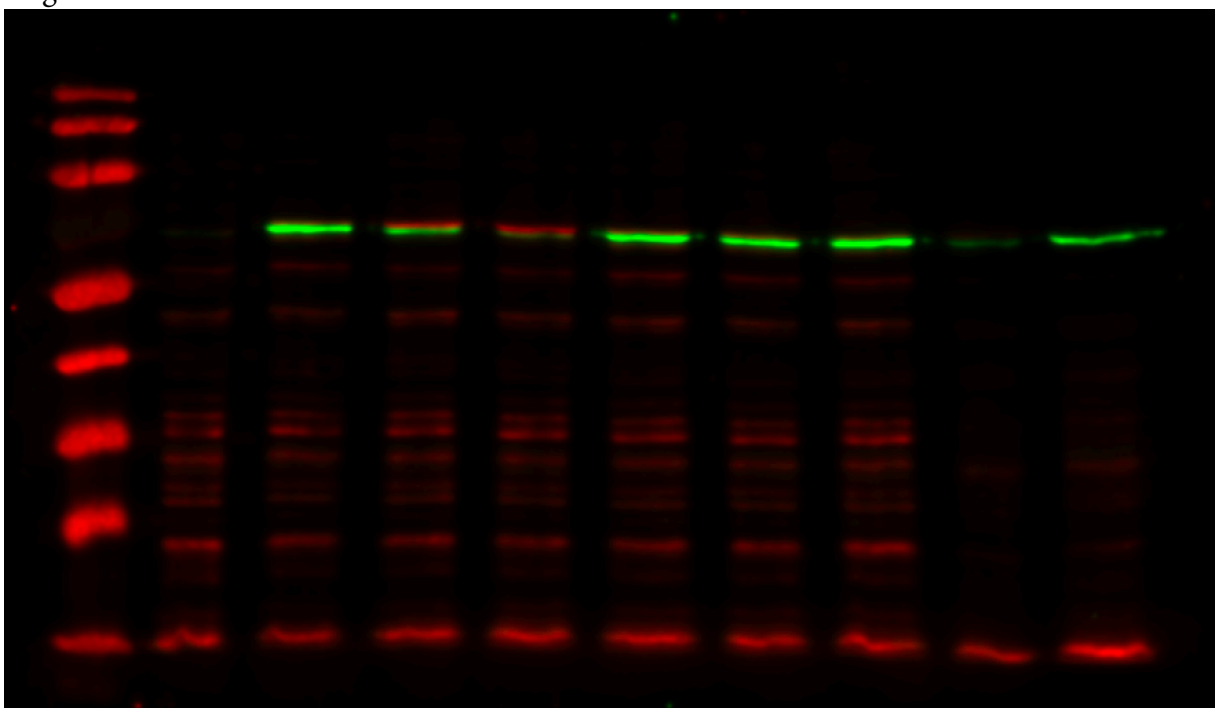

Fig 2B