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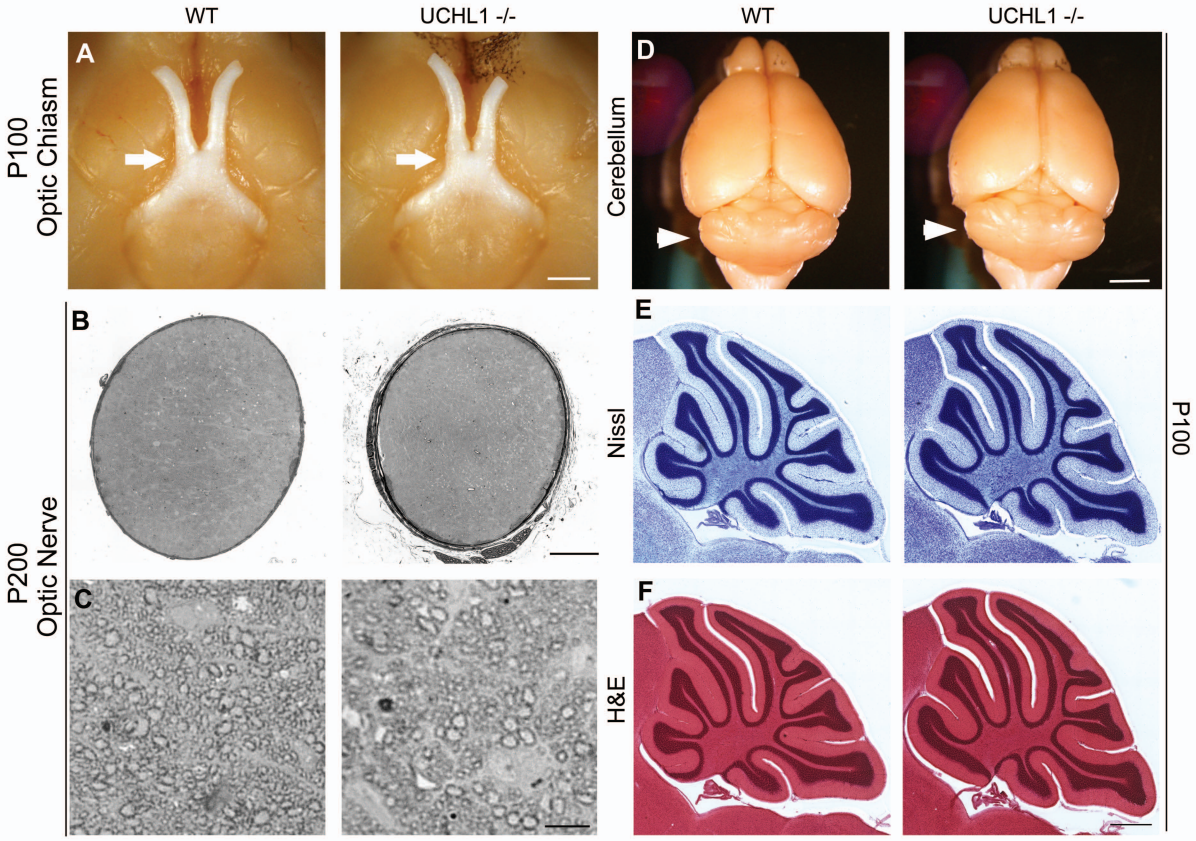

Supplementary Figure 1. UCHL1 -/- mice do not display major optic chiasm and cerebellar defects. *(A)* Optic chiasm in WT and UCHL1 -/- mice are comparable at P100 (arrows) and do not indicate signs of major degeneration. *(B-C)* Toluidine blue staining of optic nerve cross sections at P200 do not reveal any degenerating axons at low *(B)* or high *(C)* magnification. *(D)* Representative images of brains isolated from WT and UCHL1 -/- mice, with a normal-appearing cerebellum (arrowheads). *(E-F)* Sagittal sections of the cerebellum from WT and UCHL1 -/- mice show no pathology by Nissl *(E)* or H&E staining *(F)*. Scale bars, (*A)* 1 mm, (*B)* 200 µm, (*C)* 20 µm, and (*D-F)* 2.5 mm.

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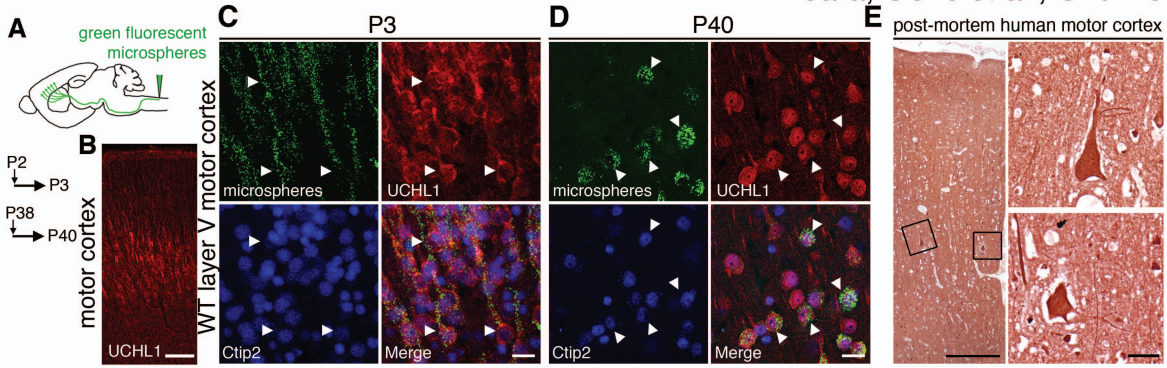

Supplementary Figure 2. **UCHL1 is expressed in CSMN and in Betz cells**. *(A)* CSMN are retrogradely labeled at P3 and P40 by green fluorescent microspheres injection into the corticospinal tract at P2 and at P38, respectively. *(B)* UCHL1 is primarily expressed by large pyramidal neurons in layer V of the motor cortex. Retrogradely labeled CSMN contain green microspheres, and express Ctip2 and UCHL1 at P3 (*C*, arrowheads) and at P40 (*D*, arrowheads). (*E*) UCHL1 immunocytochemistry in post-mortem human brain demonstrates high levels of UCHL1 expression, mainly in Betz cells located in layer V of the motor cortex. Boxed areas are enlarged to the right. Scale bars, (*B)* 100 µm, (*C-D)* 20 µm, (*E)* 500 µm (low magnification) and 50 µm (high magnification).

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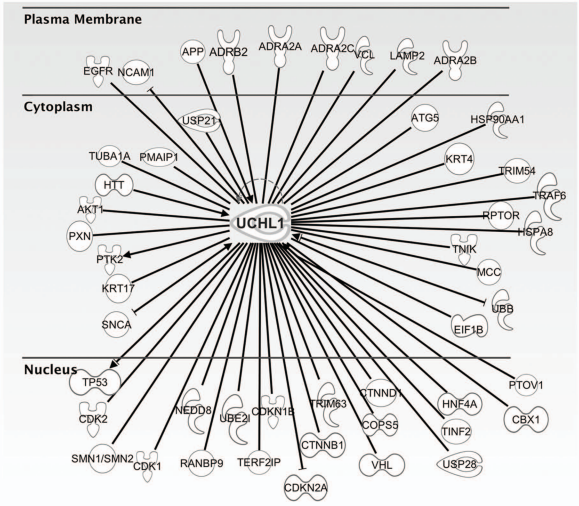
A



B

Organization of cytoskeleton	p= $8.35X10^{-17}$
EGFR, NCAM1, APP, ADRA2a, VCL, HSP90aa1, TRIM54, TNIK, TRAF6, HNF4a, CTNND1, CTNNB1, CDKN1b, UBE2l, TP53, SNCA, KRT17, PTK2, PXN, AKT1, HTT	
Ubiquitination of proteins	p= $8.53X10^{-14}$
ADRB2, ATG3, TRAF6, UBB, VHL, CDKN2a, TRIM63, UBE2l, SNCA, AKT1, HTT, USP21	
Microtubule dynamics	p= $1.04X\ 10^{-13}$
EGFR, NCAM1, APP, VCL, HSP90aa1, TRIM54, TRAF6, HNF4a, CTNND1, CTNNB1, CDKN1b, UBE2l, TP53, SNCA, PTK2, PXN, AKT1, HTT	
Apoptosis	p= $2.26X10^{-12}$
EGFR, NCAM1, APP, ADRB2, VCL, ATG5, HSP90aa1, TRIM54, TRAF6, RPTOR, HSPA8, UBB, HNF4a, COPS5, VHL, CTNNB1, CDKN2a, UBE2l, CDK1, SMN1/SMN2, CDK2, TP53, SNCA, KRT17, PTK2, PXN, AKT1, HTT, PMAIP1	
Movement Disorders	p= $4.66X10^{-11}$
NCAM1, APP, ADRB2, ADRA2a, ADRA2c, ADRA2b, HSP90aa1, TRAF6, HSPa8, UBB, CTNNB1, CDKN1b, SMN1/SMN2, TP53, SNCA, AKT1, HTT, TUBA1a	
Motor Dysfunction	p= $4.63X10^{-10}$
APP, ADRA2a, ADRA2c, ADRA2b, UBB, SMN1/SMN2, SNCA, HTT	
Degradation of proteins	p=$1.33X10^{-10}$
AKT1, HTT, EGFR, APP, LAMP2, VHL, CDKN2a, TRIM63, CDKN1b, UBE2l, NEDD8, SNCA	
Cellular Homeostasis	p= $8.86X10^{-9}$
APP, ADRb2, ADRA2a, LAMP2, ATG5, HSP90aa1, TRAF6, RPTOR, UBB, HNF4a, CDKN2a, CTNNB1, CDKN1b, CDK2, TP53, PTK2, AKT1, HTT, PMAIP1	
Neuromuscular disease	p= $1.35X10^{-9}$
APP, ADRA2b, ADRA2a, ADRA2c, ADRA2b, HSP90aa1, TRAF6, HSPA8, UBB, CTNNB1, TP53, SNCA, AKT1, HTT, TUBA1a	

Supplementary Figure 3. Ingenuity Pathway Analysis (IPA) of UCHL1 interacting proteins suggest key canonical pathways that are possibly affected in the absence of UCHL1 function. (*A)* The schematic drawing of UCHL1 and its interacting proteins inside the cells. (*B)* The table summarizing the molecular network and canonical pathway analysis performed by IPA based on experimental findings and data that confirm direct binding.UCHL1 interacting proteins are suggested to be primarily involved in the organization of cytoskeleton, ubiquitination and degradation of proteins, microtubule dynamics, apoptosis, and cellular homeostasis. The *P* values represent high significance, and no association with randomness.

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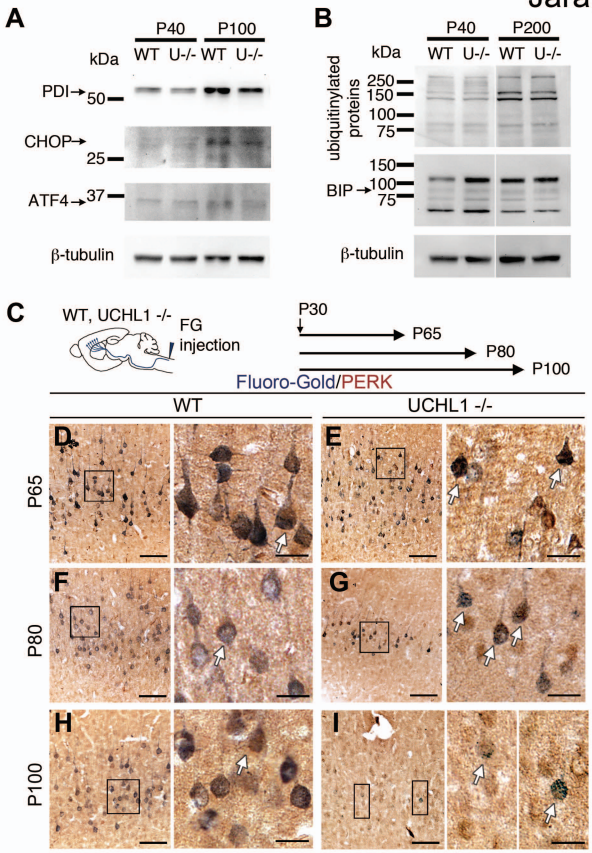

Supplementary Figure 4. Increased ER stress in CSMN of UCHL1 -/- mice. *(A-B)* Western blot analysis of PDI, CHOP, ATF4 ***(A)*** and ubiquitinylated proteins, BIP ***(B)*** expression in the motor cortex extracts of WT and UCHL1 -/- mice at P40, P100, and P200. β−tubulin is used as loading control. Arrows indicate location of the protein of interest. ***(C)*** Experimental design for CSMN retrograde labeling by FG injection in WT and UCHL1 -/- mice. Surgeries were performed at P30, and motor cortex analyzed at P65, P80, and P100. ***(D-I)*** Retrogradely labeled CSMN (blue) did not express PERK (brown) in WT mice but only a few CSMN were positive for PERK (***D***, ***F***, ***H***, arrows). However, even though there was reduction in the FG-labeled CSMN at later ages, remaining CSMN expressed PERK (***G***, ***I***, arrows). Scale bars, 100 μm (low magnification) and 25 μm (high magnification).