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Supplementary Materials for

A C9ORF72/SMCR8-containing complex regulates ULK1 and plays a dual role in autophagy

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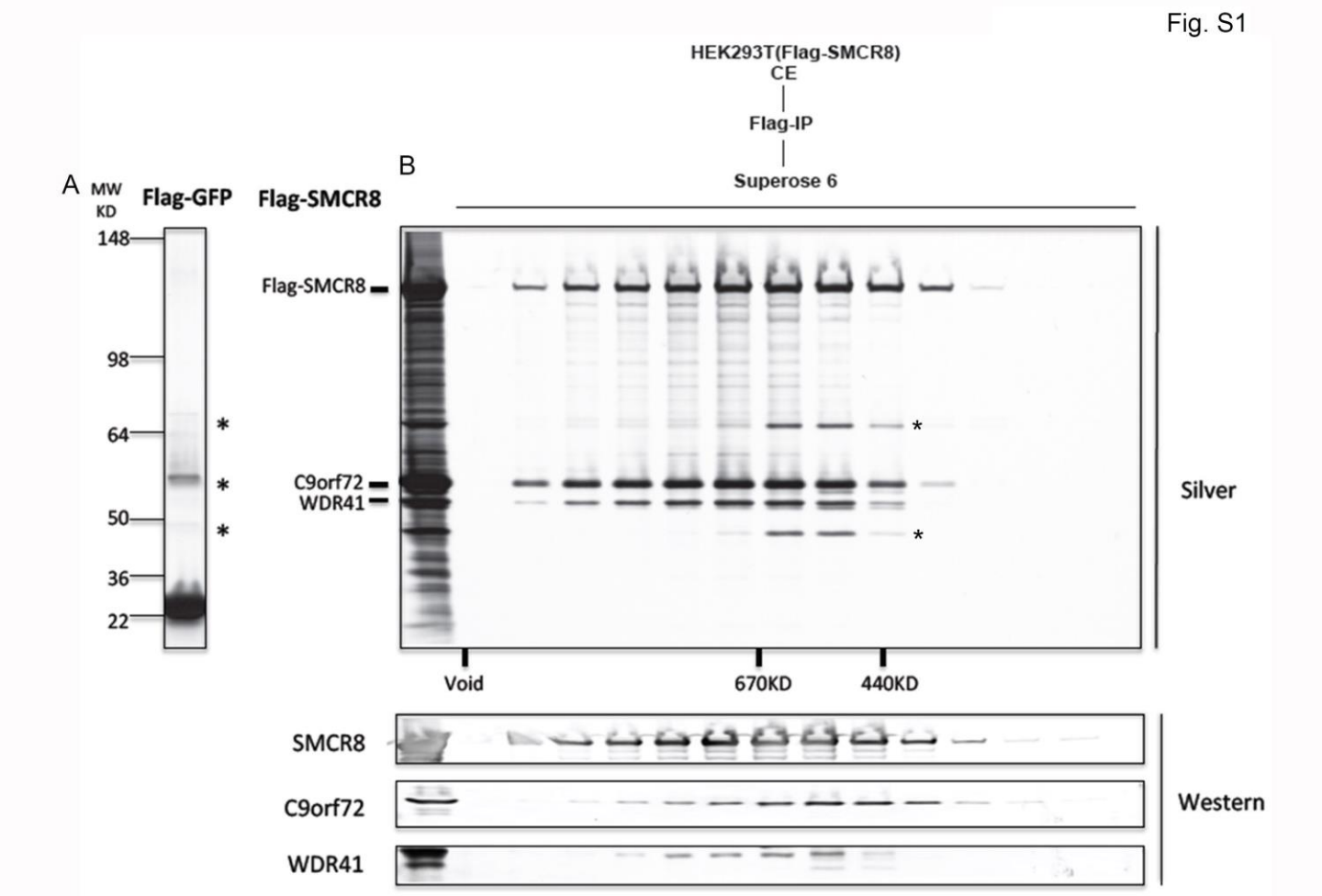

fig. S1. Isolation of SMCR8-associated proteins. (**A** and **B**) Silver staining analysis of Flag affinity purified fractions from cytoplasmic extracts of Flag-GFP and Flag-SMCR8 HEK293 cell lines. Asterisks indicate common contaminants of Flag purification (SKB1, α-tubulin and MEP50). Flag-SMCR8 associated proteins as identified by mass spectrometry are indicated. (B) Superose 6 gel filtration fractions from SMCR8 cytoplasmic flag affinity purification. Fractions were resolved by 4-12% SDS-PAGE and analyzed by silver staining (Top) and western blot with corresponding antibodies (Bottom).

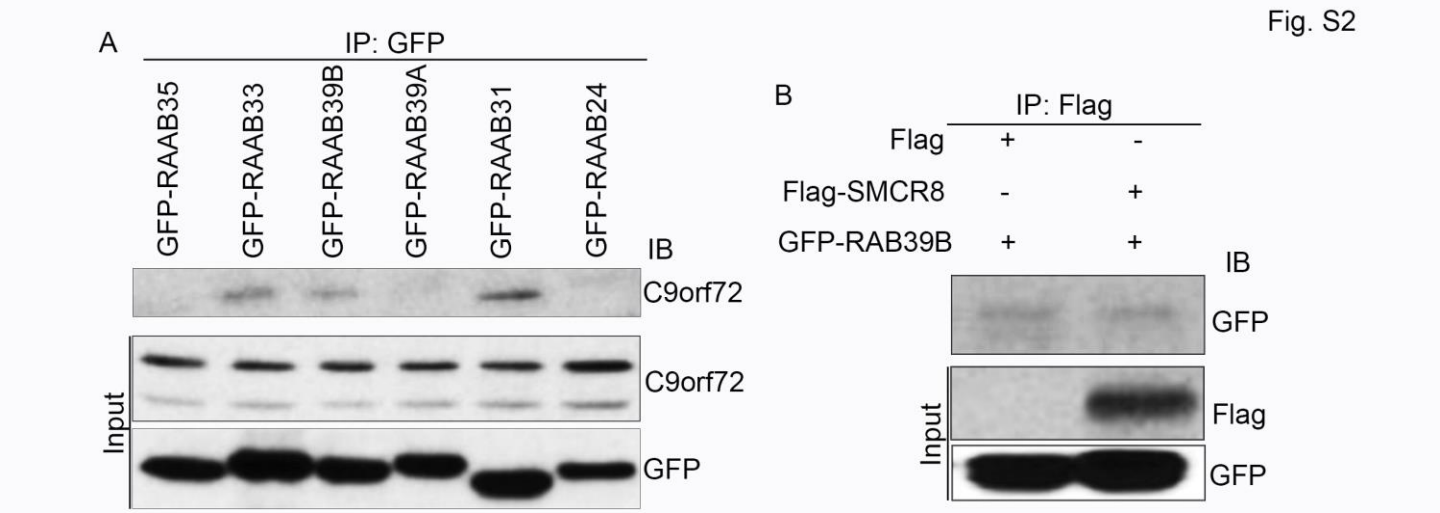

fig. S2. Identify C9orf72- or SMCR8-associated Rab GTPases. (**A**) Individual GFP-tagged Rab GTPase constructs were transfected into N2A cells. GTPase proteins were immunoprecipitated (IP) with GFP beads followed by western blot analyses using antibodies against C9orf72. (**B**) Flag-tagged *SMCR8* and GFP-tagged *RAB39B* were transfected into N2A cells. SMCR8 proteins were immunoprecipitated (IP) with m2 beads (anti-Flag) followed by western blot analyses using antibodies against GFP.

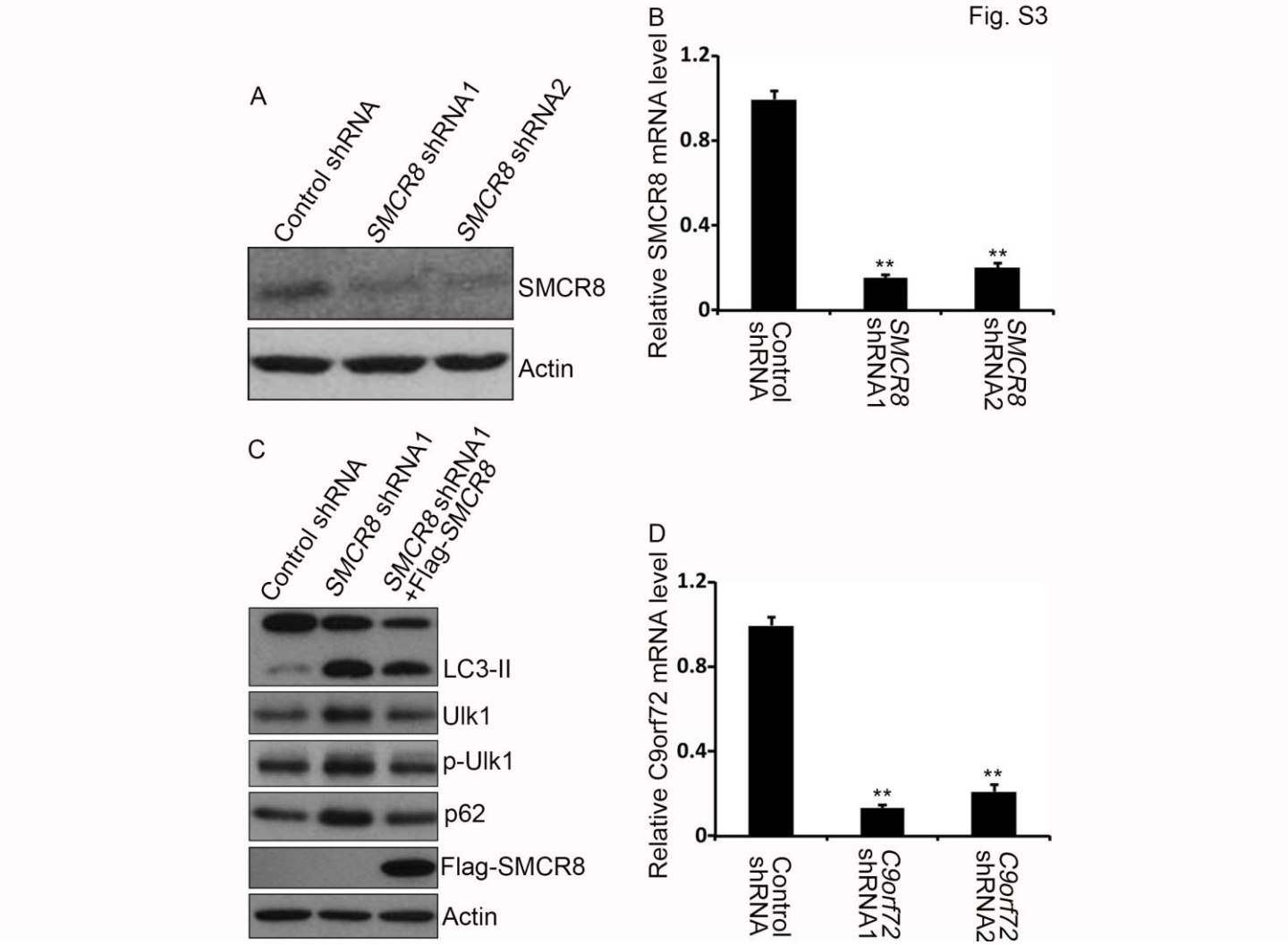

fig. S3. Characterization of *SMCR8* and *C9orf72* shRNA constructs. (**A**) Western blot analysis of SMCR8 expression in HEK293 cells transfected with control, *SMCR8* shRNA1, or *SMCR8* shRNA2 constructs. β-actin serves as the loading control. (**B**) RT-PCR analysis of *SMCR8* mRNA in HEK293 cells transfected with control, *SMCR8* shRNA1, or *SMCR8* shRNA2 constructs. (**C**) HEK293 cells transfected with control, *SMCR8* shRNA1, or *SMCR8* shRNA1 plus *Flag-SMCR8* DNA followed by western blot analyses using antibodies as indicated. β-actin serves as the loading control. (**D**) RT-PCR analysis of *C9orf72* mRNA in N2A cells infected with lentiviruses expressing control, *C9orf72* shRNA1, or *C9orf72* shRNA2.

table S1. Polypeptide composition through Flag-C9ORF72 immunopurification by mass spectrometry.

Supplementary Table 1

ID	Description	Calculated MW	Coverage	Identified Unique Peptides
UR1H_Q8TEV9	__HOMO Smith-Magenis syndrome chromosomal region candidate gene 8 protein	105,022	81.1	399
UR1H_Q96LT7	__HOMO Uncharacterized protein C9orf72	54,328	74.6	259
UR1H_Q9BSB4	__HOMO Autophagy-related protein 101	25,003	63.8	21
UR1H_Q9HAD4	__HOMO WD repeat-containing protein 41	51,728	63.2	246
UR1H_O75385	__HOMO Serine/threonine-protein kinase ULK1	112,631	32.4	49
UR1H_E9PQZ8	__HOMO Autophagy-related protein 13	60,155	26.7	19