Supplemental Materials

Molecular Biology of the Cell

Amick et al.

Supplemental Data for:

WDR41 supports lysosomal response to changes in amino acid availability

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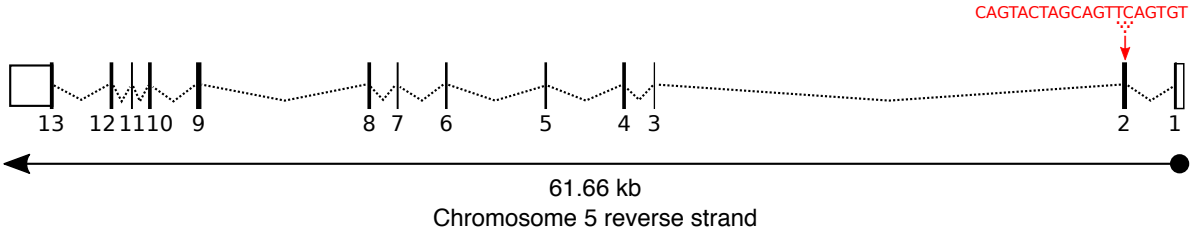
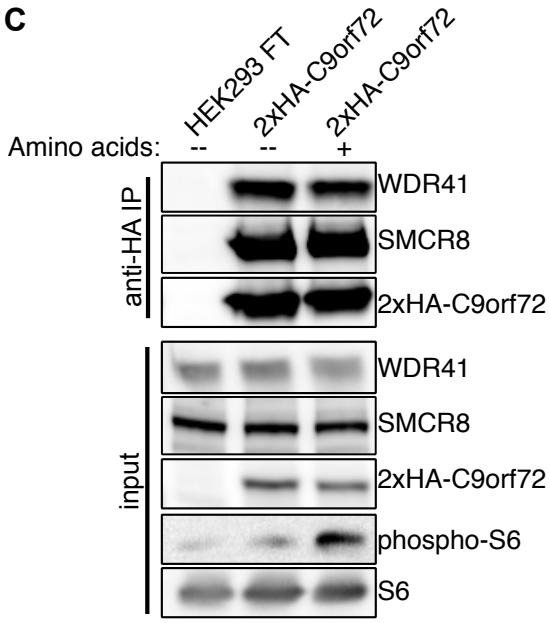
A



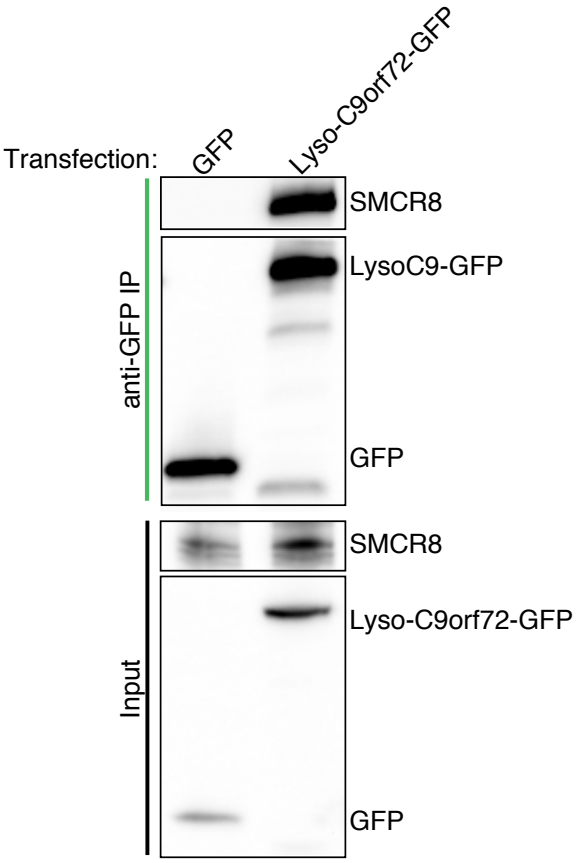
B

Cell line	*Mutation*
WDR41 KO 2xHA-C9orf72 HEK293 FT	2 base pair deletion 31 base pair insertion 4 base pair deletion
WDR41 KO HeLa	2 base pair deletion (CT) 2 base pair deletion (AC) 13 base pair deletion 10 base pair deletion

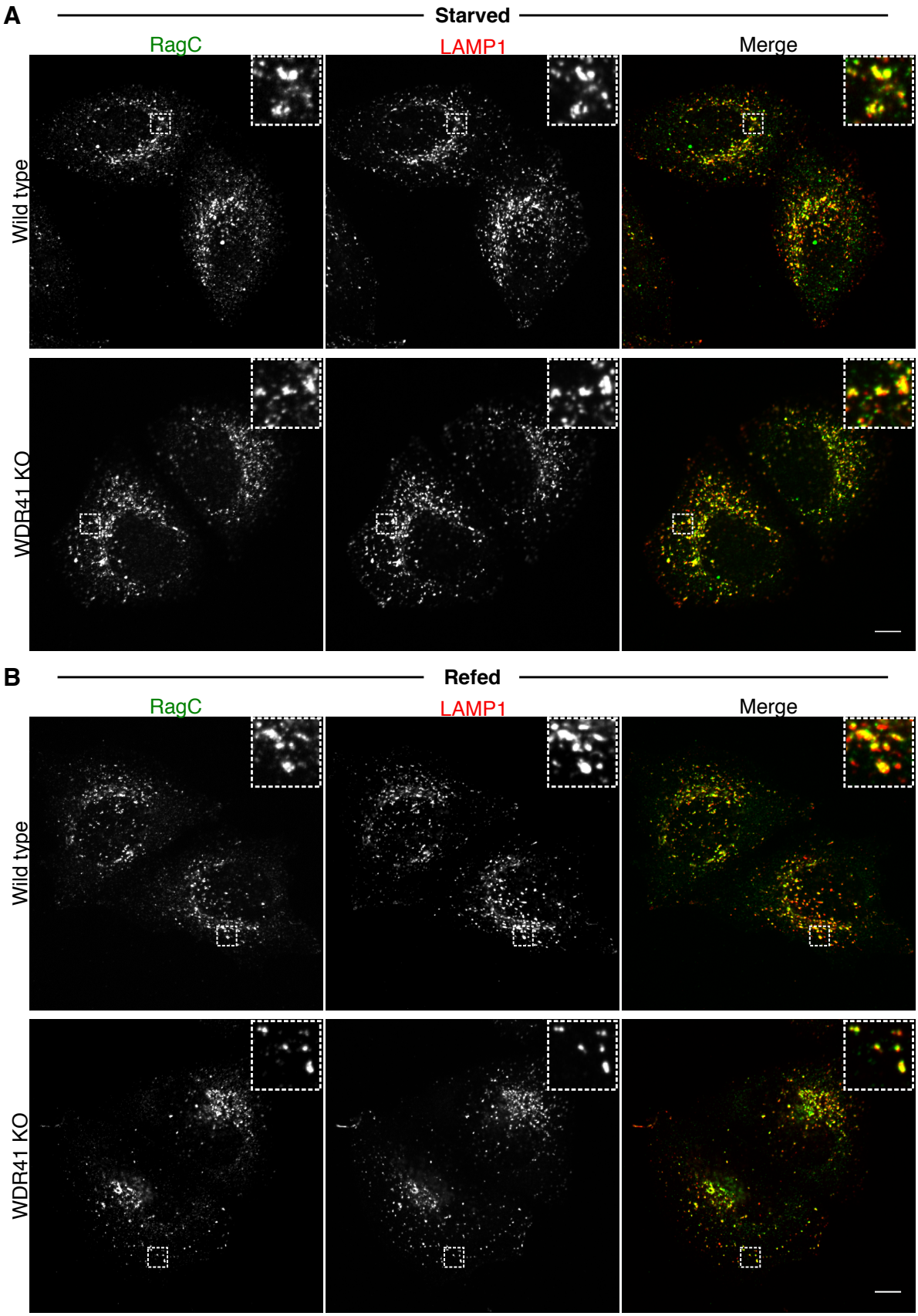
C



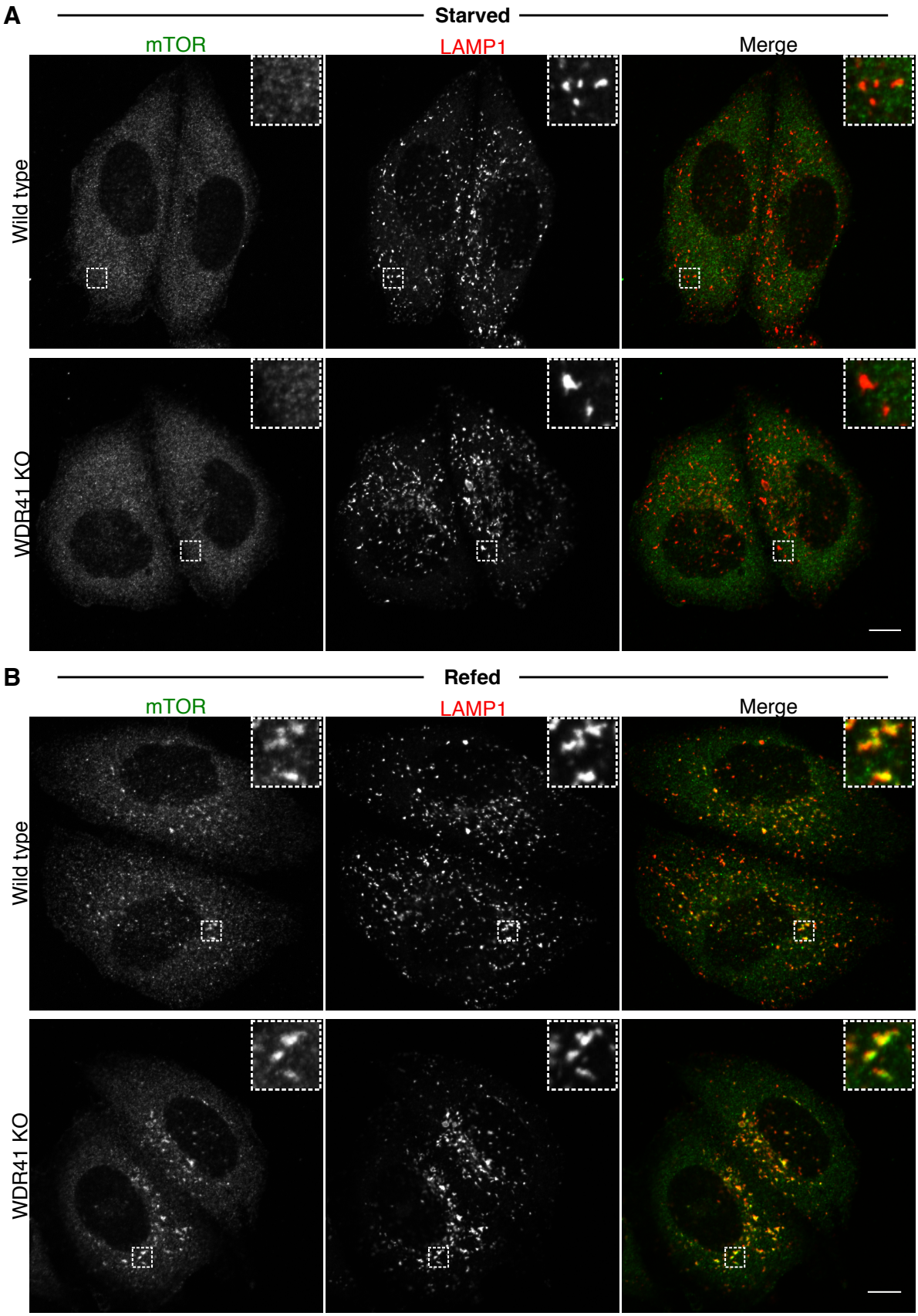
Supplemental Figure 1. Characterization of WDR41 knockout cell lines (A) Diagram of the WDR41 locus indicating the target site in exon 2 and sequence of the Cas9 guide RNA used to target WDR41 in red. **(B)** Summary of the insertions and deletions (indels) that were identified by sequencing of the targeted locus in the WDR41 KO cell lines. Consistent with loss of the WDR41 protein, all of the indels detected in our KO cell lines yield to frameshift mutations and premature stop codons. **(C)** Anti-HA immunoprecipitations from 2xHA-C9orf72 cells harvested under starvation and amino acid-fed conditions revealed similar levels of WDR41 and SMCR8 interactions.



Supplemental Figure 2. Lyso-C9orf72-GFP interacts with SMCR8. Lysates from WDR41 knockout HeLa cells stably expressing Lyso-C9orf72-GFP were anti-GFP immunoprecipitated and subjected to immunoblotting for GFP and SMCR8. Cells transfected with GFP served as a negative control.



Supplemental Figure 3. Lysosomal localization of RagC does not require WDR41. Immunfluorescence analysis of RagC and LAMP1 localization in wild type and WDR41 knockout HeLa cells under **(A)** starved conditions (1.5 h) and **(B)** amino acid refed conditions (15 min). Scale bar, 10μm.



Supplemental Figure 4. mTOR subcellular localization shows normal responses to starvation and amino acid re-feeding in WDR41 knockout cells. Immunofluorescence analysis of mTOR and LAMP1 localization in wild type and WDR41 knockout HeLa cells under starved conditions (1.5 h) **(A)** and amino acid refed conditions (15 min) **(B)**. Scale bar, 10μm.

Supplemental Table 1: PCR primers and other oligo sequences

Target	Sequence	Notes
WDR41 forward	5' - gtcgacggtaccgcgggcccACCATGGGTTGAACCATGTTGCGATGGCTGATC - 3'	WDR41 forward primer with uORF for insertion into pLVX puro vector
WDR41 reverse	5' - atctagagtcgcgggatcccTTAGACAGCAAGGTATAAGTCACC - 3'	Reverse primer for insertion of untagged WDR41 into pLVX puro vector
WDR41-HA reverse	5' - atctagagtcgcgggatcccTTAGACAGCAAGGTATAAGTCACC - 3'	Reverse primer for insertion of WDR41-HA into pLVX puro
Lyso-C9orf72-GFP forward	5’ – gtcgacggtaccgcgggcccACCATGGGGTGCTGCTAC – 3’	forward primer for insertion of LAMTOR1(1-39)-C9orf72-GFP fusion into pLVX puro vector
Lyso-C9orf72-GFP reverse	5' - atctagagtcgcgggatcccTTACTTGTACAGCTCGTCCATG - 3'	reverse primer for insertion of LAMTOR1(1-39)-C9orf72-GFP fusion into pLVX puro vector
WDR41 genomic sense #1	5' - atcatctcattattttgtgg - 3'	sequencing genomic DNA of WDR41-2xHA CRISPR knockin
WDR41 genomic antisense #1	5'-TCAAAGTCAAATGGAAAAAC-3'	sequencing genomic DNA of WDR41-2xHA CRISPR knockin
WDR41 genomic sense #2	5'-AACTTGCAGTTATTTAGTTG-3'	sequencing genomic DNA of WDR41 knockout cells
WDR41 genomic antisense #2	5' - CGAGATGCAGAGGCCAATTT - 3'	sequencing genomic DNA of WDR41 knockout cells
WDR41 gRNA sense	5' - caccgCAGTACTAGCAGTTCAGTGT - 3'	cloning of guide RNA into px459 vector
WDR41 gRNA antisense	5' - aaacACACTGAACTGCTAGTACTGc - 3'	cloning of guide RNA into px459 vector
RB1CC1 gRNA #1 sense	5' - caccgTATGTATTTCTGGTTAACAC - 3'	cloning of guide RNA into px459 vector
RB1CC1 gRNA #1 antisense	5' - aaacGTGTTAACCAGAAATACATAc - 3'	cloning of guide RNA into px459 vector
RB1CC1 gRNA #2 sense	5' - caccgCAAGATTGCTATTCAACACC - 3'	cloning of guide RNA into px459 vector
RB1CC1 gRNA #2 antisense	5' - aaacGGTGTTGAATAGCAATCTTGc - 3'	cloning of guide RNA into px459 vector

Supplemental Table 2: Sequences used for CRISPR knockin of 2xHA epitope tag into WDR41 locus

Gene	Sequence	Note
WDR41	AUUCCUUAAACUAGACAGCA	guide RNA for knockin of tag
WDR41	ATTATTTTGTGGAAAAATGGAGAGCGAG AATCTGGATTGCGCAGTTTAAGATTATT TCAAAAATTAGAGGAGAATGGTGACTTA TATCTTGCTGTCtacccatacgatgttccagattac gcttatccctatgacgtcccggactatgcaTAGTTTAA GGAATTAAAAATACACATGCATG	ssDNA repair template for knockin of 2xHA epitope tag

Supplemental Table 3: Summary of antibodies used in this study

Antibody	Source	Catalog/clone number
Actin	Abcam	ab8224
C9orf72	Proteintech	22637-1-AP
Cathepsin D	R&D Systems	AF1014
Calnexin	Cell Signaling Technology	2679 (C5C9)
Donkey anti-Goat, Alexa Fluor 488	Thermo Fisher Scientific	A-11058
Donkey anti-Rabbit, Alexa Fluor 594	Thermo Fisher Scientific	A-21206
EEA1	Thermo Fisher Scientific	PA1-063A
FLCN	Cell Signaling Technology	3697
FNIP1	Epitomics	EPNCIR107
GM130	BD Biosciences	610822
GFP-HRP	Rockland	600-103-215
Goat Anti-Mouse IgG	Jackson ImmunoResearch	115-005-146
HA	Cell Signaling Technology	2367
HA-Peroxidase (3F10)	Roche	12 013 819 001
LAMP1	Cell Signaling Technology	9091
LAMP1	University of Iowa DSHB	H4A3
LAMTOR1	Cell Signaling Technology	8975 (D11H6)
Lamin A/C	Santa Cruz	SC-20681 (H-110)
LC3	MBL International	PM036
phospho-p70 S6 Kinase (Thr389)	Cell Signaling Technology	9234
phospho-S6 Ribosomal protein (Ser 235/236)	Cell Signaling Technology	4858
p70 S6 Kinase	Cell Signaling Technology	9202
RB1CC1	Proteintech	10043-2-AP
Rab7A	Sigma-Aldrich	HPA006964
SMCR8	Bethyl Laboratories	A304-694A
S6	Cell Signaling Technology	2217
Tubulin	Sigma-Aldrich	T5168
WDR41	Novus Biologicals	NBP1-83812