

Supplemental Figure Legends

Supplemental Figure 1. **KLHL7 deletion mutants do not form punctate structures in the cell.** HeLa cells cultured on coverslips were transfected with FLAG-KLHL7 wild-type or deletion mutants. The cells were fixed and stained with anti-FLAG antibody and Hoechst33342. Scale bars, 20 μ M.

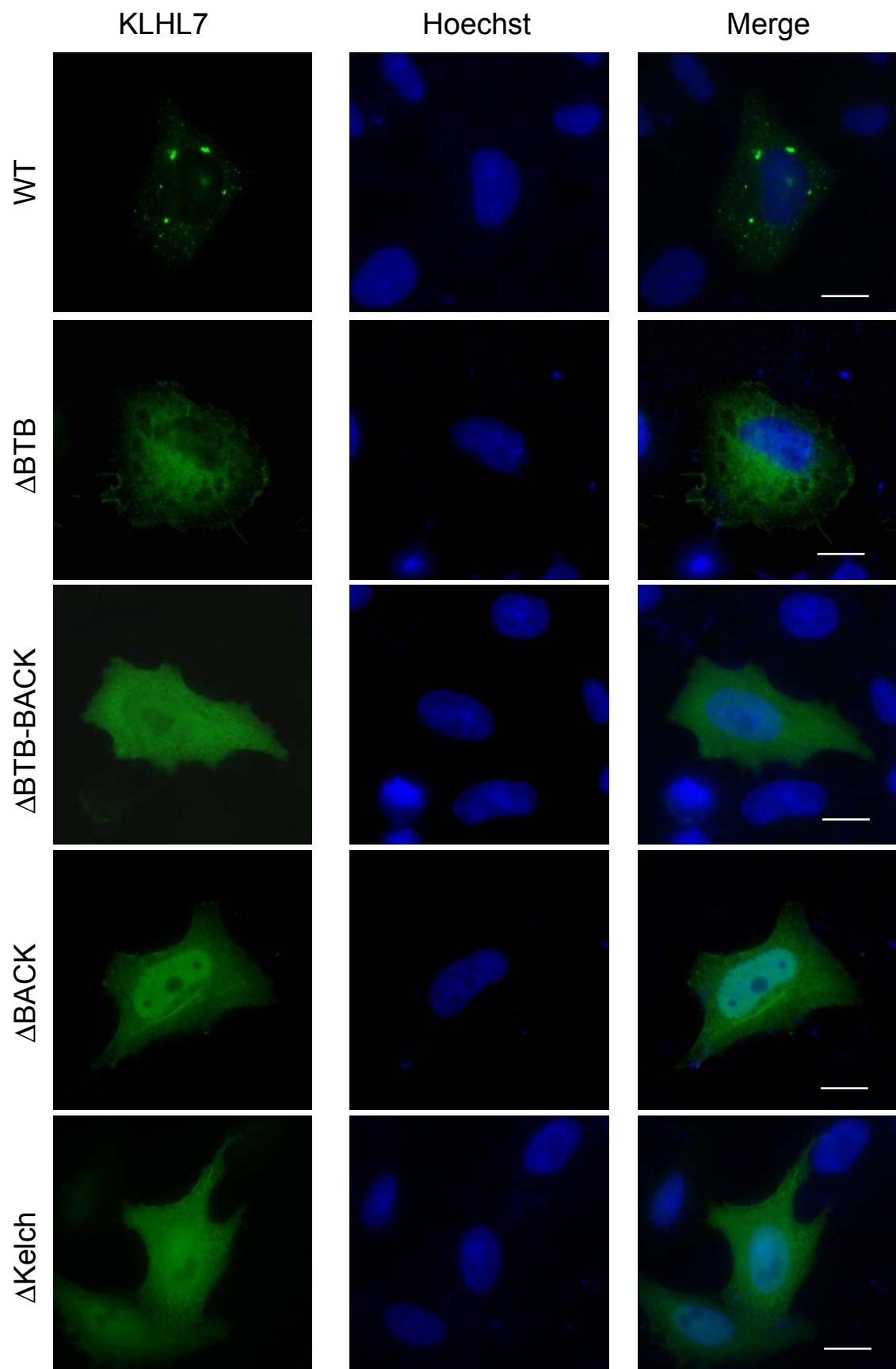
Supplemental Figure 2. **KLHL7 co-localization with GFP-LC3 does not increase with proteasome or lysosome inhibition.** ATG5 $^{+/+}$;GFP-LC3 and ATG5 $^{-/-}$;GFP-LC3 MEFs expressing FLAG-KLHL7 were subjected to proteasome inhibition with MG132 or lysosome inhibition with NH₄Cl and stained with anti-FLAG and anti-GFP antibodies. The nucleus was stained with Hoechst 33342. Scale bars, 20 μ M.

Supplemental Figure 3. **Alignment of KLHL7.** *A*, Alignment of several vertebrate KLHL7 BTB-BACK domains. Homo, Homo sapiens; Pan, Pan troglodytes; Canis, canis lupus familiaris; Bos, Bos taurus; Mus, Mus musculus; Rattus, Rattus norvegicus; Gallus, Gallus gallus. The numbers denote the amino acid position. *B*, Multiple sequence alignment of the ‘3-box’ region of human BTB proteins. The S150 and A153 are highlighted with blue and red boxes.

Supplemental Figure 4. **KLHL7 S150N co-localizes with Cul3, but A153T does not.** *A*. HeLa cells on coverslips transfected with FLAG-KLHL7 S150N or A153V with GFP-Cul3, were fixed and stained with anti-FLAG and anti-GFP antibodies. The nucleus was stained with Hoechst 33342. Scale bars, 20 μ M.

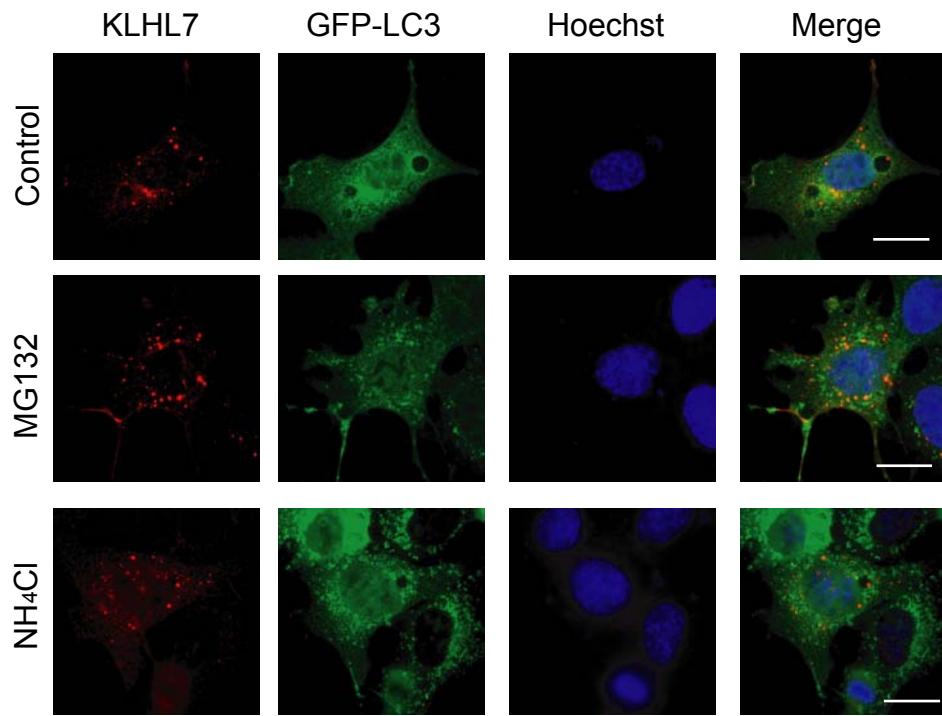
Supplemental Figure 5. **KLHL7 S150N and A153T hetero dimerize with Cul3, and A153T associate with Cul3 through the hetero dimer.** *A*. HCT116 cells were transfected with FLAG-KLHL7 S150N or A153T with Myc-KLHL7 wild-type and the cell lysates were immunoprecipitated with anti-FLAG antibody. The immunoprecipitates were immunoblotted with anti-Myc and anti-FLAG antibodies. *B*. HCT116 cells were transfected with FLAG-KLHL7 A153T and Myc-KLHL7, as indicated. The cell lysates were immunoprecipitated with anti-FLAG antibody and immunoblotted with anti-Cul3, anti-Myc and anti-FLAG antibodies.

Supplemental Figure 1

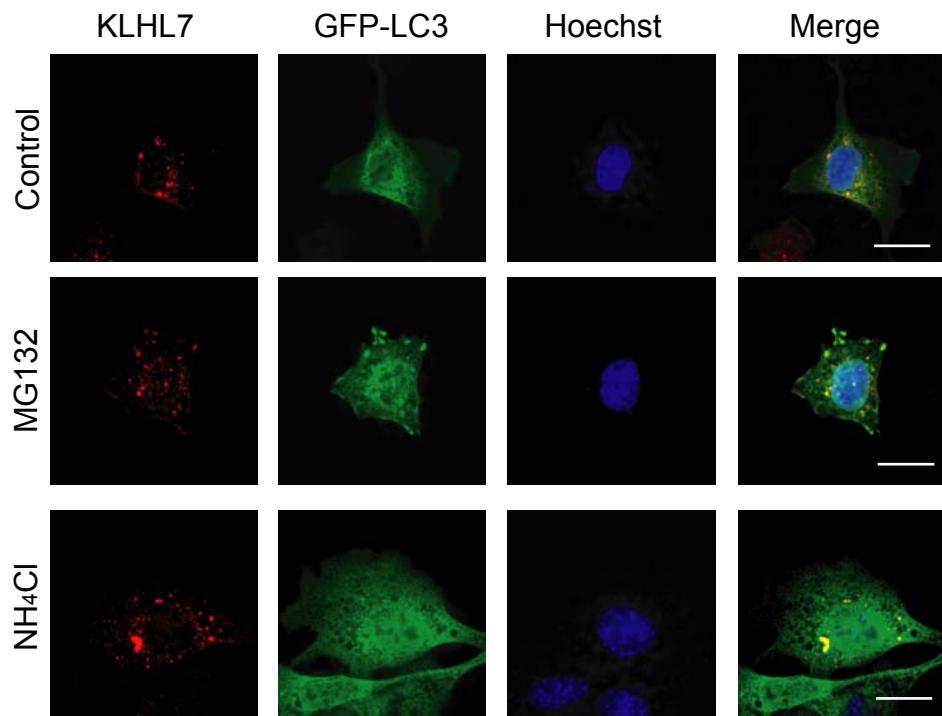


Supplemental Figure 2

ATG5^{+/+}: GFP-LC3



ATG5^{-/-}: GFP-LC3



Supplemental Figure 3

A

Homo	34	MNNMRKQKTLCDVILMVQERKI PAHRVVLAASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	140
Pan	34	MNNMRKQKTLCDVILMVQERKI PAHRVVLAASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	140
Canis	- - - - -	MVQERKI PAHRVVLAASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	92
Bos	34	MNNMRKQKTLCDVILMVQERKI PAHRVVLAASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	140
Mus	34	MNNMRKQRTLCDVILTVQERKI PAHRVVLAASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	140
Rattus	34	MNNMRKQRTLCDVILMVQERKI PAHRVVLAASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	140
Gallus	34	MNSMRKQRTLCDVILMVQERRI PAHRVVLASASHFFNLMFTTNMLESKSFEVELKDAEPDIIEQLVEFAYTARISVNSNNVQSLDAANQYQIEPVKKMCVDFLKEQ	140

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Homo	VDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKTDEFLQLDVKRVTLLNQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	246
Pan	VDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKTDEFLQLDVKRVTLLNQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	246
Canis	VDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKTDEFLQLDVKRVTLLNQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	198
Bos	VDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKTDEFLQLDVKRVTLLNQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	246
Mus	VDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKTDEFLQLDVKRVTLLNQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	246
Rattus	VDASNCLGI SVIAECLDCPELKSTADDFIHQHFTEVYKTDEFLQLDVKRVTLLSQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	246
Gallus	VDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKTDEFLQLDVKRVTLLNQDTLTVRAEDQVYDAAVRWLKYDEPNRQPFMVDILAKVRFPLISKNFLSKT	246

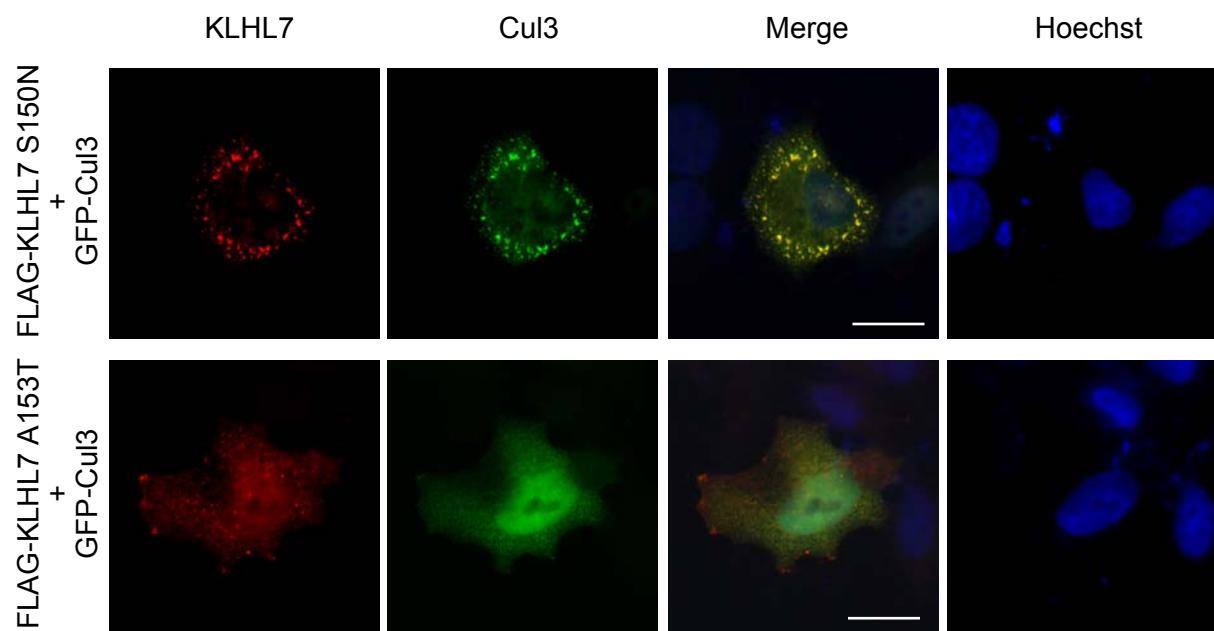
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B

KLHL7	AANQYQIEPVKKMCVDFLKEQVDASNCLGI SVIAECLDCPELKATADDFIHQHFTEVYKT	179
KLHL11	LADRFLLIRLKEFCGEFLKKLHLSCVAIHSLAHMYTLSQLALKADMIRRNPFHKVIQD	238
KLHL9	AASFHQILPVLDFCKVFLISGVSLDNCEVGRIANTYNLIEVDKYVNNFILKNFPALLST	187
KEAP1	GAVMYQIDSVVVRACSDFLVQQLDPSNAIGIANFAEQIGCVELHQRAREYIYMHGEVAKQ	217
gigaxonin	AADLLLLTDLTKLCCEFLEGCTAAENCIGIRDHALHYCLHHVHYLATELETHFRDVSS	167

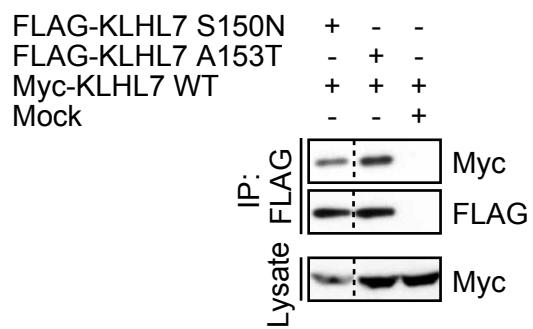
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Supplemental Figure 4



Supplemental Figure 5

A



B

