Supplementary Information

Requirement for p62 acetylation in the aggregation of ubiquitylated proteins under nutrient stress

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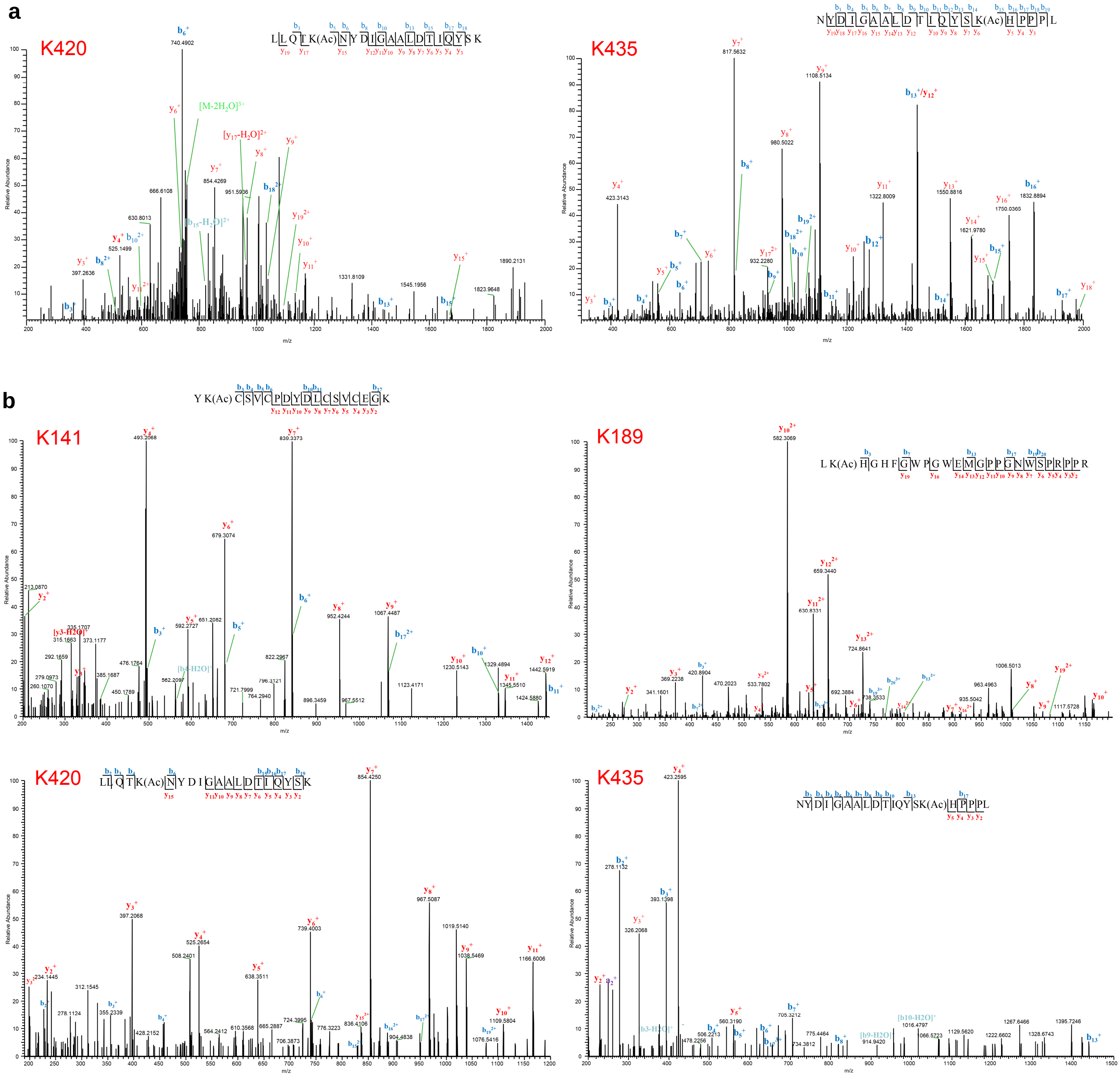
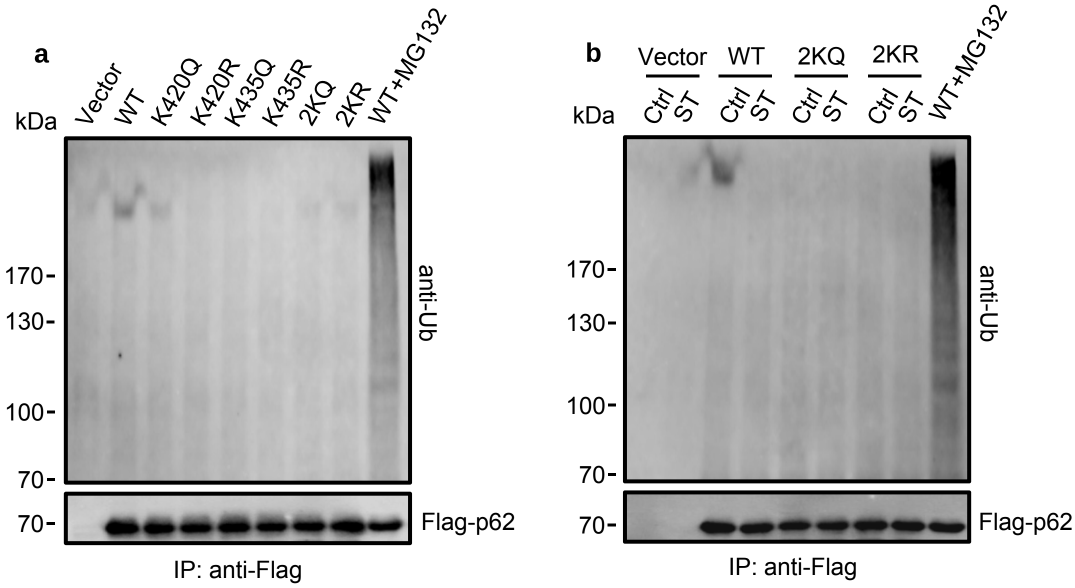
Supplementary Figures



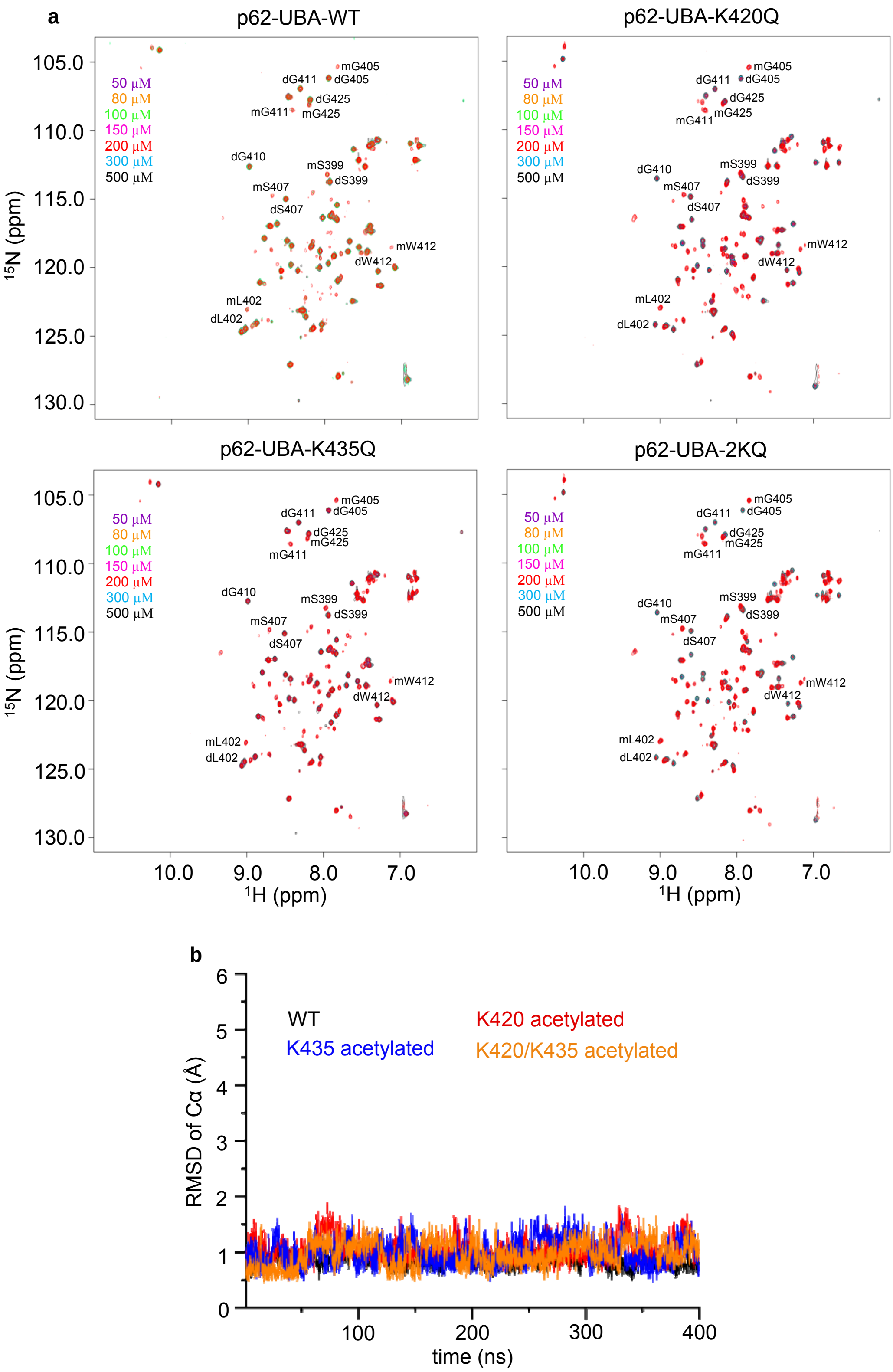
Supplementary Figure 1

MS/MS spectra of tryptic peptides (inset) of purified recombinant p62 protein after in vitro acetylation (**a**) or Flag-p62 immunoprecipitated from TSA-treated Flag-p62-HEK293T cells (**b**) showing a mass shift of 42.01 Da at the lysine residues.



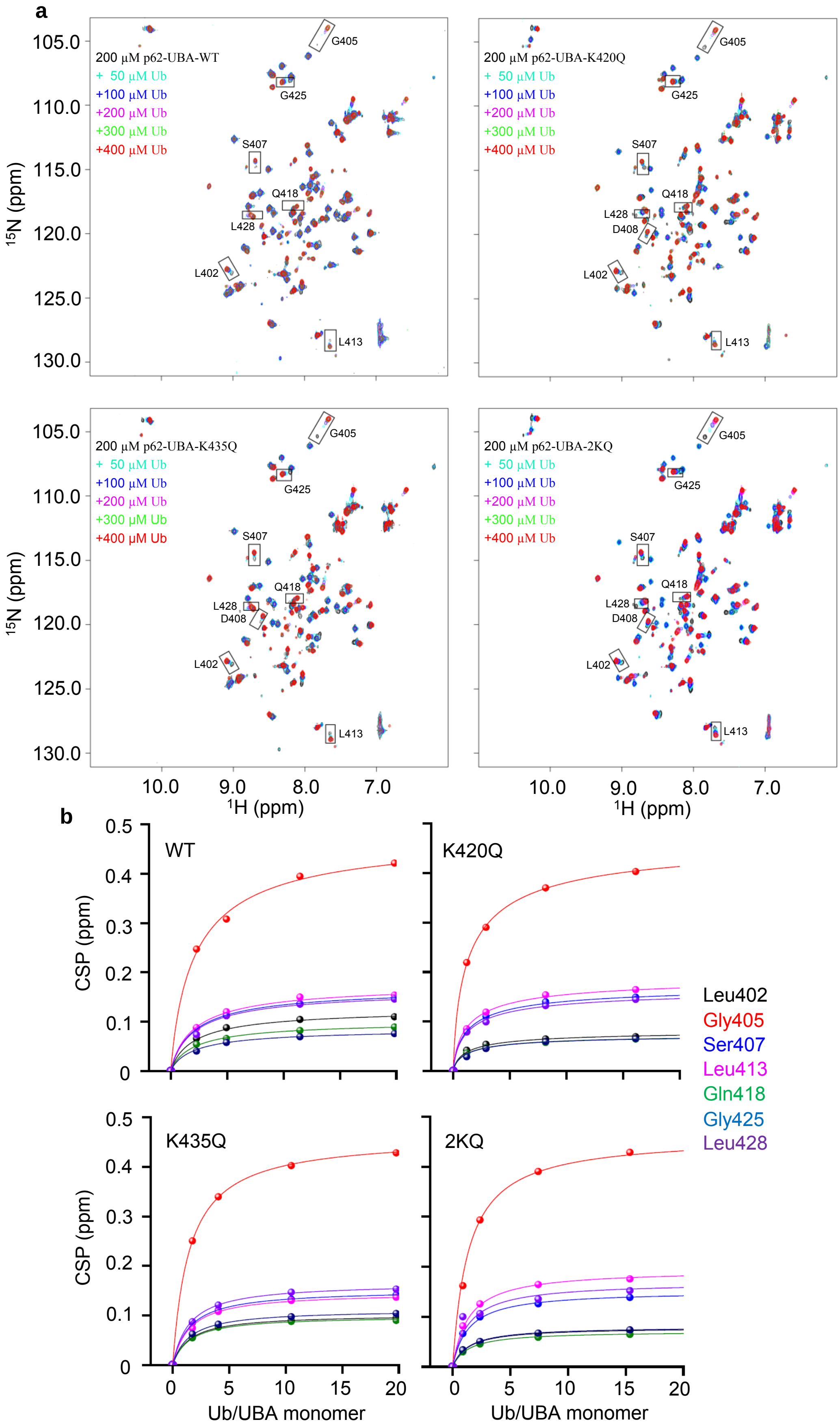
Supplementary Figure 2 p62 acetylation and cell starvation do not cause the ubiquitylation of p62.

(**a**,**b**) *p62*-KO HEK293 cells transiently expressing Flag-tagged WT p62 or each of the indicated p62 mutants were lysed with urea buffer. Then the Flag-p62 proteins were immunoprecipitated with anti-Flag and the precipitates were analyzed using anti-Ub. Source data are provided as a Source Data file.



Supplementary Figure 3 Acetylation at K420 and K435 disrupts UBA dimerization.

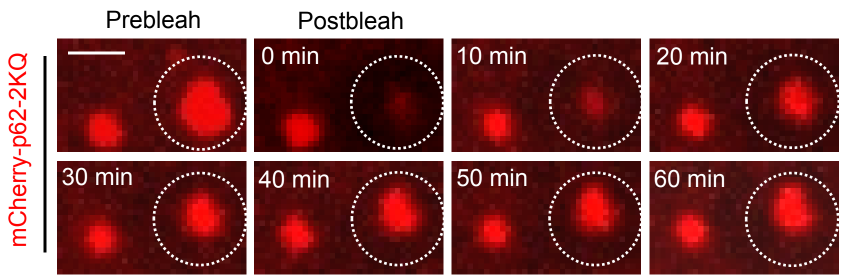
(**a**) ^{1}H-^{15}N correlation spectra of each of the indicated p62-UBA proteins. Amino acid residues used to fit the K_D value are denoted. d: dimer; m: monomer. (**b**) The trajectories for each UBA subunit from the MD simulations for WT, K420 acetylated, K435 acetylated and K420/K435 acetylated p62-UBA dimers. The fluctuations in Cα RMSD are plotted over a time window of 400 ns. Source data are provided as a Source Data file.



a
200 μM p62-UBA-WT
+ 50 μM Ub
+100 μM Ub
+200 μM Ub
+300 μM Ub
+400 μM Ub
200 μM p62-UBA-K420Q
200 μM p62-UBA-K435Q
200 μM p62-UBA-2KQ
G405
G425
S407
Q418
L428
D408
L402
L413
¹⁵N (ppm)
¹H (ppm)
b
WT
K420Q
K435Q
2KQ
CSP (ppm)
Ub/UBA monomer
Leu402
Gly405
Ser407
Leu413
Gln418
Gly425
Leu428

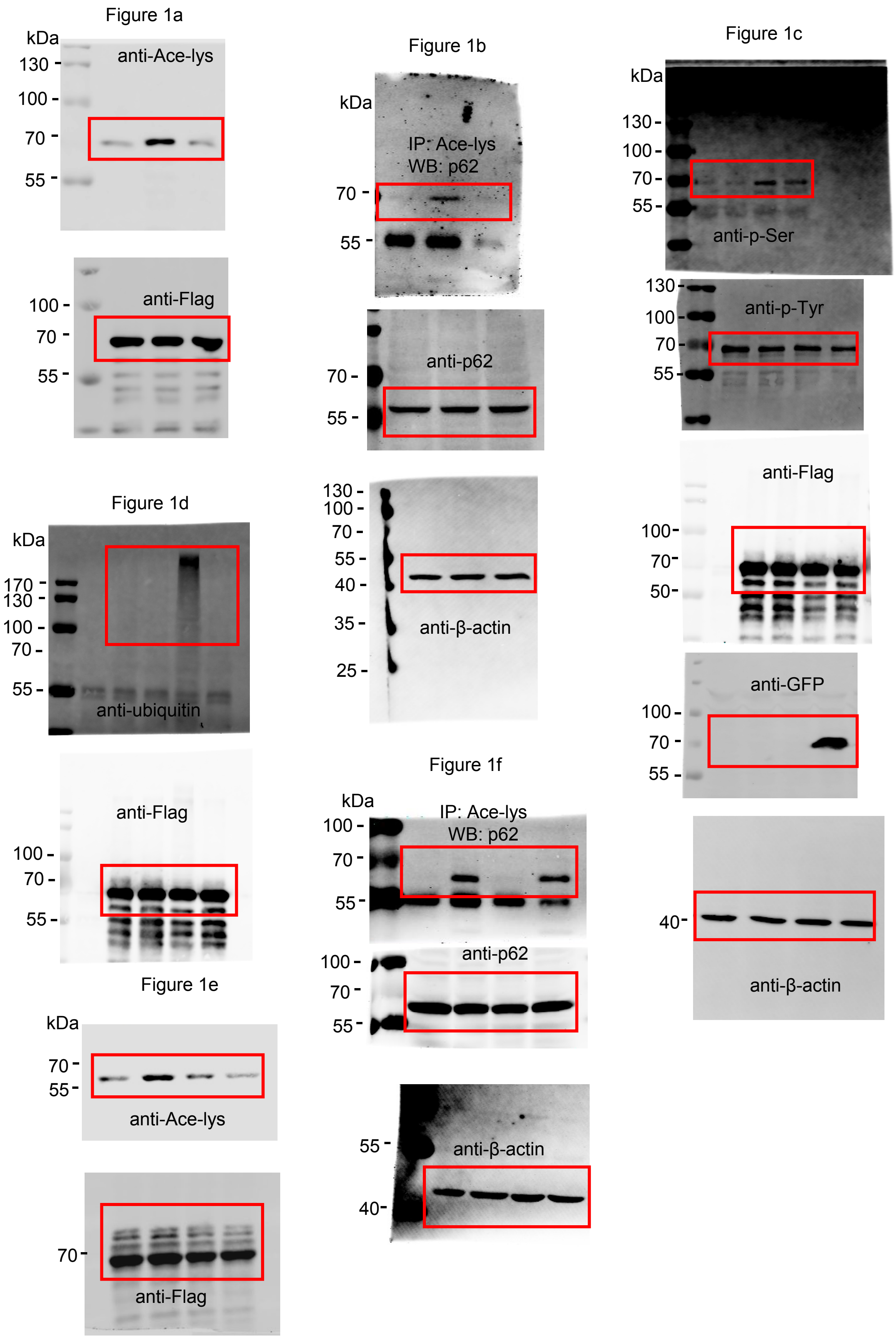
Supplementary Figure 4 K435 acetylation directly enhances the p62-ubiquitin binding.

(**a**) ^{1}H-^{15}N correlation spectra of p62-UBA in the presence of increasing concentrations of ubiquitin. Amino acid residues used to fit the dissociation constant are marked. (**b**) Changes of chemical shift values over ubiquitin concentrations for the binding-perturbed residues were globally fitted to a standard 1:1 binding equation. The chemical shift differences are expressed as $[0.5 \times (\Delta\delta H^2 + 0.2 \times \Delta\delta N^2)]^{0.5}$, in which ΔδH and ΔδN are the chemical shift perturbations (CSP) in ppm unit in the proton and nitrogen dimensions, respectively. Note that the ubiquitin binding equilibrium is affected by the amount of available p62-UBA monomer. Source data are provided as a Source Data file.

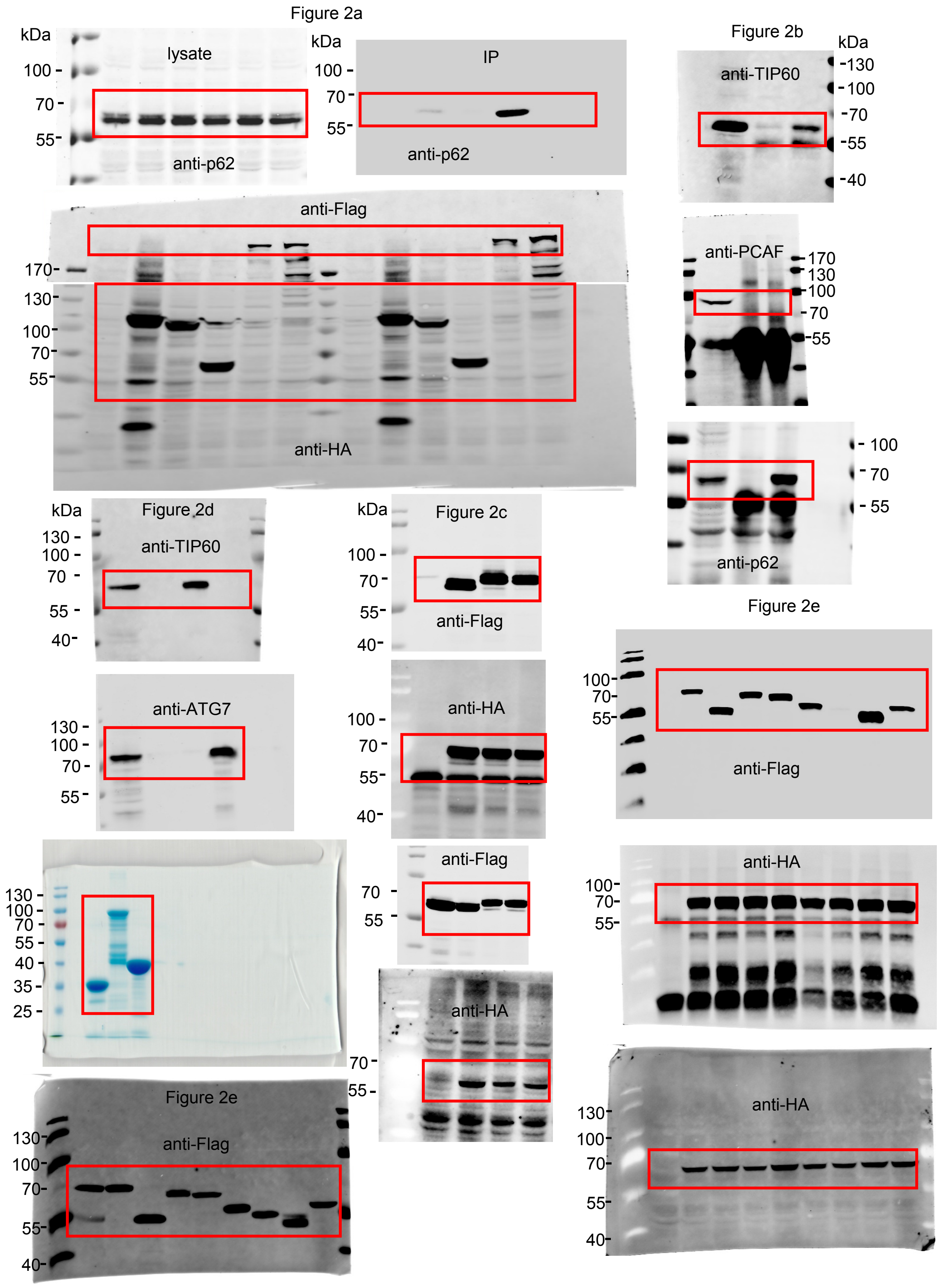


Supplementary Figure 5 Acetylation enhances p62 body formation.

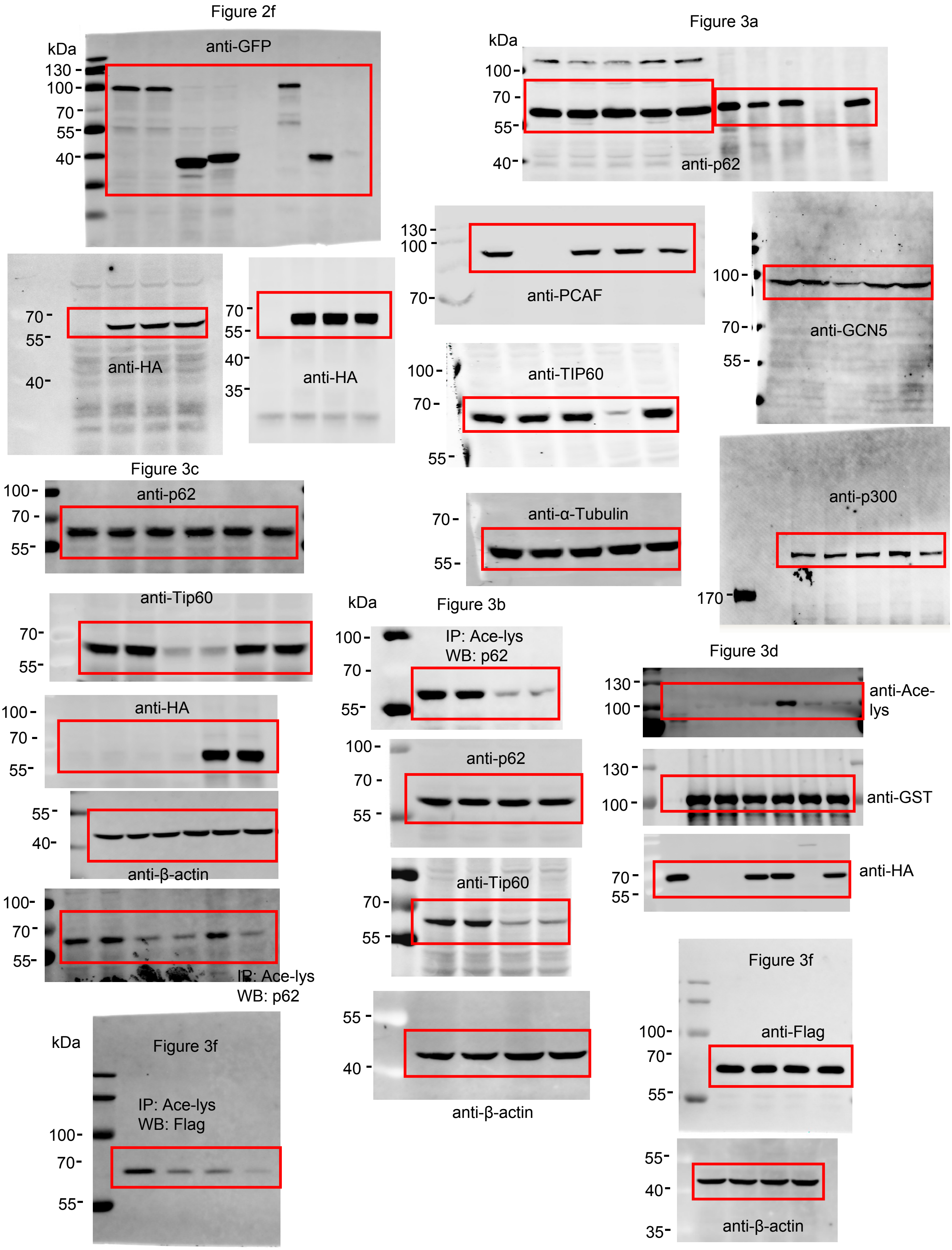
FRAP analysis of mCherry-p62-2KQ clusters. A representative time-course is shown. The experiments were performed 10 min after mixing mCherry-p62-2KQ (5 µM) and 8×ubiquitin (1.5 µM) at RT. Scale bar, 2 µm.



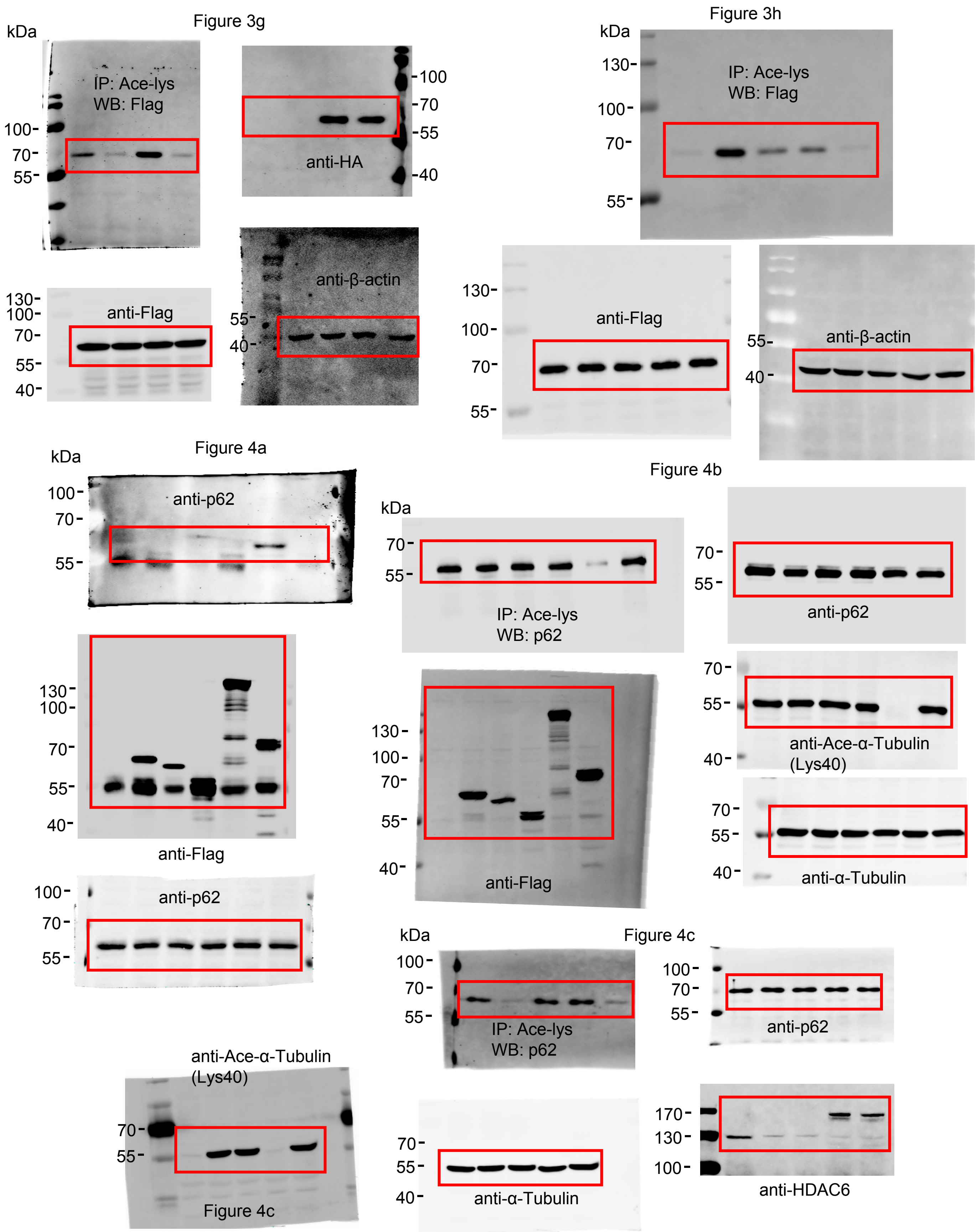
Supplementary Figure 6 Uncropped blots/gels



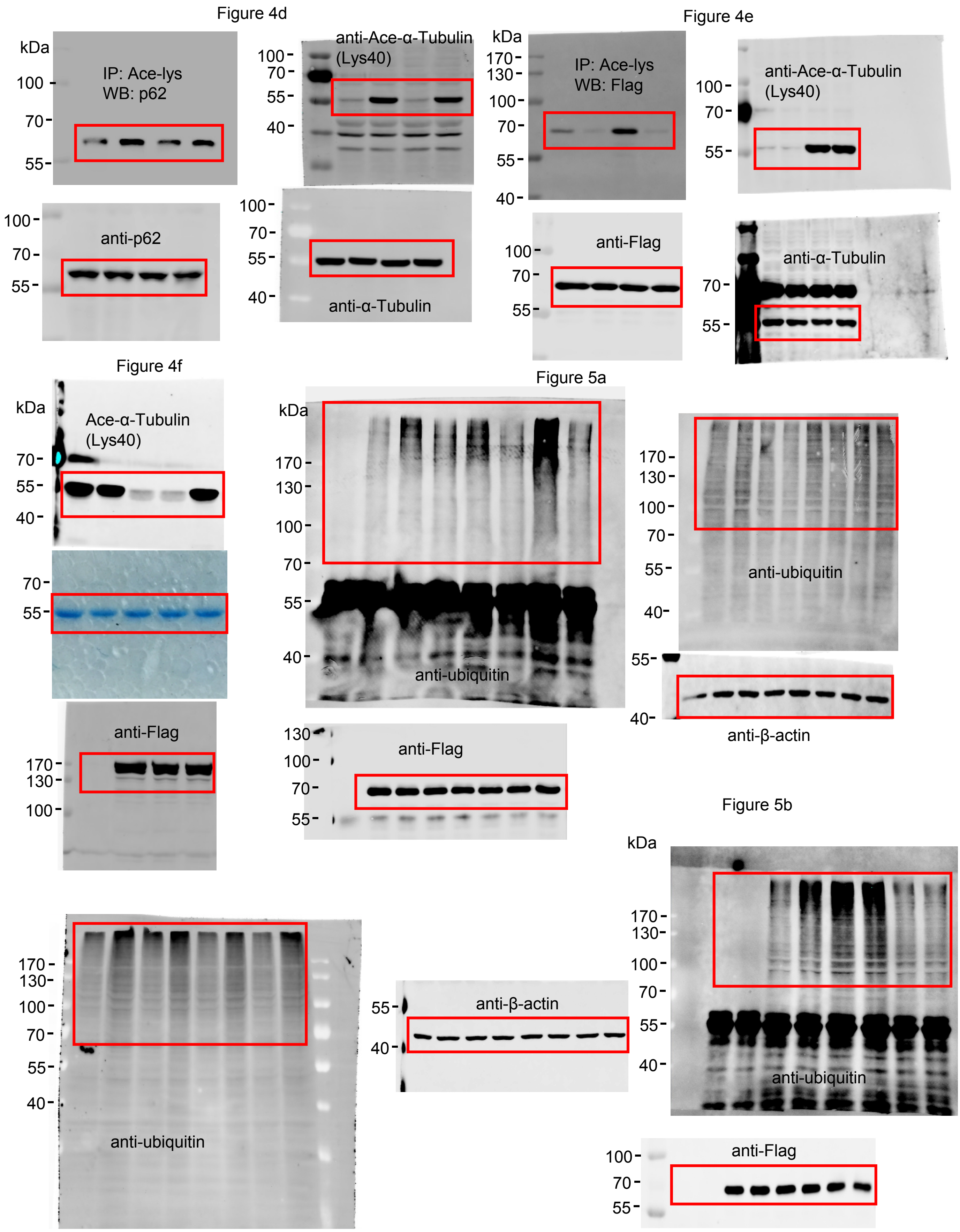
Supplementary Figure 7 Uncropped blots/gels



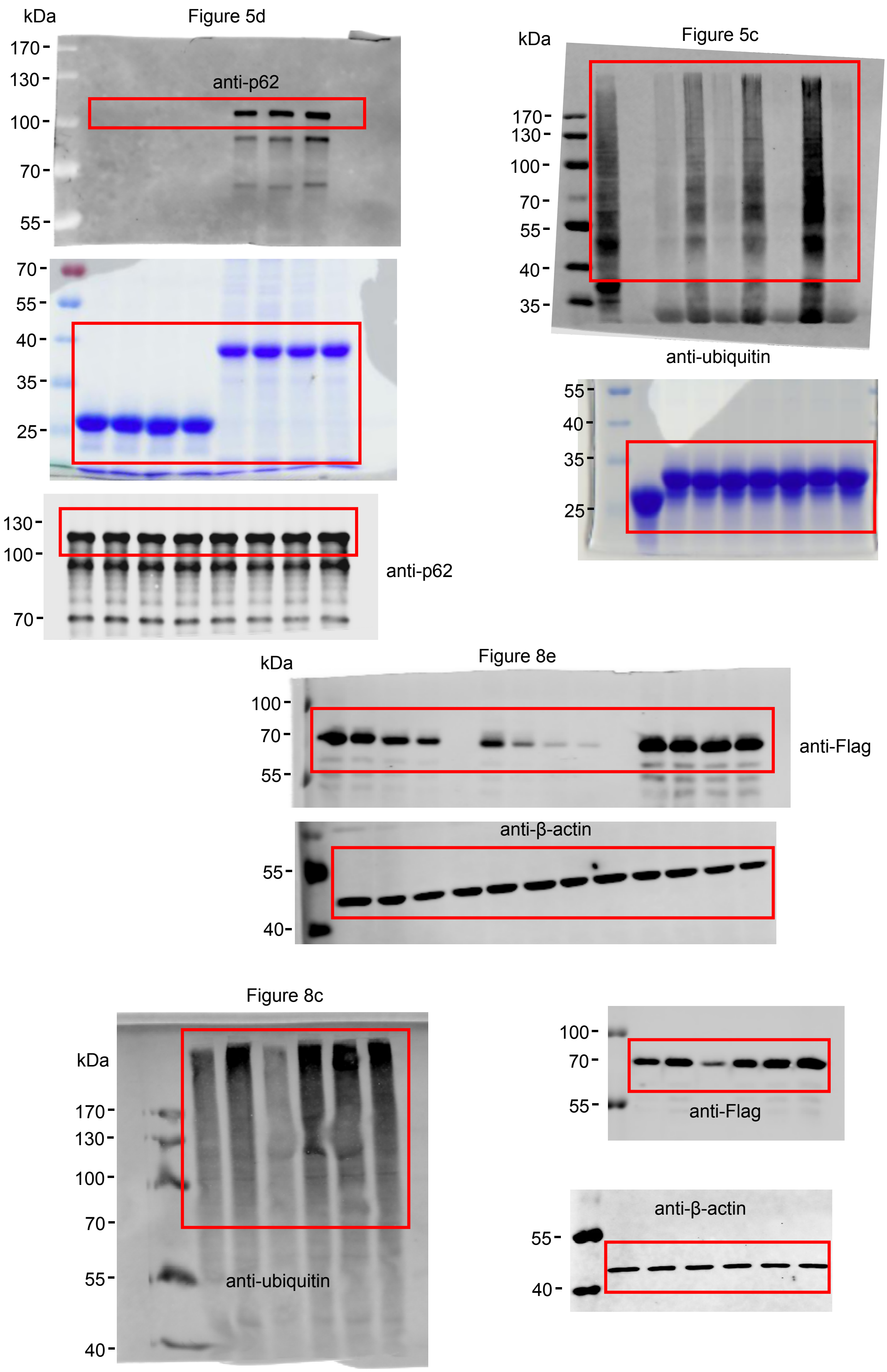
Supplementary Figure 8 Uncropped blots/gels



Supplementary Figure 9 Uncropped blots/gels

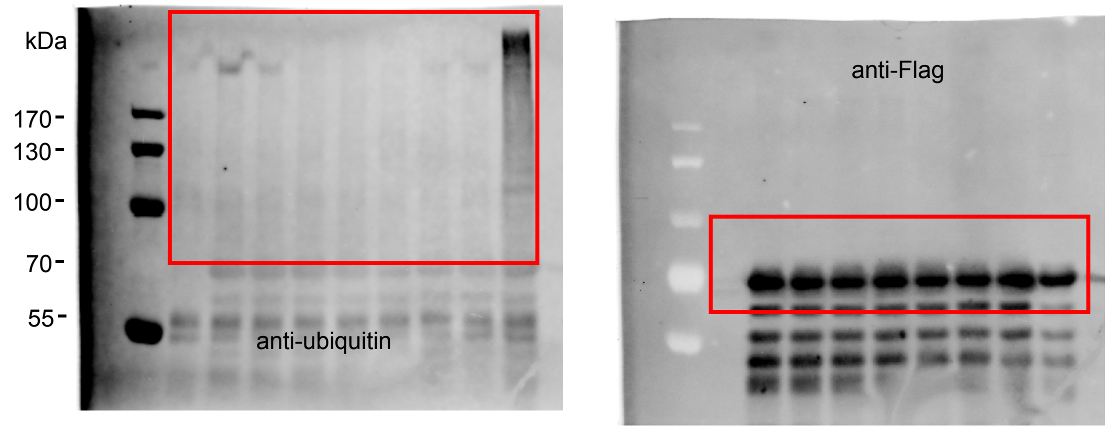


Supplementary Figure 10 Uncropped blots/gels

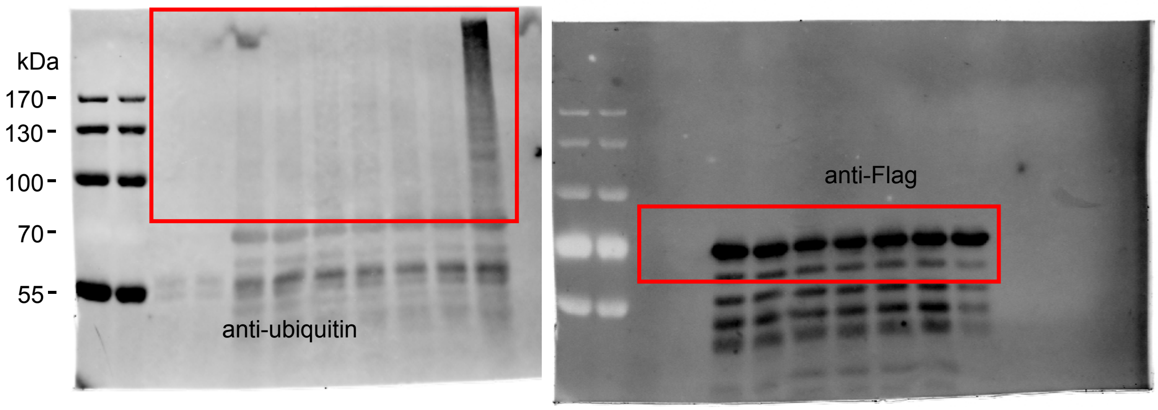


Supplementary Figure 11 Uncropped blots/gels

Supplementary Figure 2a



Supplementary Figure 2b



Supplementary Figure 12 Uncropped blots/gels

Supplementary Tables

Supplementary Table 1 List of primers used in this study.

Primers	Sequences (5'-3')
Flag/GST-p62-F-EcoRI	CCGGAATTCATGGCGTCGTTCACGGTG
Flag/GST-p62-R-XhoI	CCGCTCGAGTCACAATGGTGGAGGGTGC
mCherry-p62-F-XhoI	CCGCTCGAGCTATGGCGTCGTTCACGGTG
mCherry-p62-R-EcoRI	CCGGAATTCTCACAATGGTGGAGGGTGC
GST-mCherry-p62-F-EcoRI	CGGGAATTCATGGTGAGCAAGGGCGAGGAG
GST-mCherry-p62-R-NotI	AAGGAAAAAAGCGGCCGCTCTCACAATGGTGG
GST-8×ubiquitin-F-BamHI	CGCGGATCCATGCAGATCTTCGTG
GST-8×ubiquitin-R-NotI	AAGGAAAAAAGCGGCCGCTTACCCACCTCT
6×His-ubiquitin-F-EcoRI	CCGGAATTCATGCAGATCTTCGTG
6×His-ubiquitin-R-XhoI	CCGCTCGAGTTACCCACCTCTGAG
p62-Δ1-85-F	GCTATGTCCTATGTGAAAG
p62-Δ1-85-R	GAATTCCTTGTCATCGTCATC
p62-Δ86-121-F	GTGCACCCCAATGTGATCTG
p62-Δ86-121-R	CATTGTCAGCTCCTCATCAC
p62-Δ122-168-F	TTTCCCAACCCCTTTG
p62-Δ122-168-R	CATGTTTCGGGGTGCC
p62-Δ169-253-F	GAGGTTGACATTGATGTGG
p62-Δ169-253-R	GATGAGCTTGCTGTGTTC
p62-Δ254-310-F	GAGCAAATGAAAAAGATAG
p62-Δ254-310-R	AATGCCTAGAGGGCTGAG
p62-Δ311-384-F	GCCCTATACCCACATCTC
p62-Δ311-384-R	TGTCAGAGACTGAGCAGG
p62-Δ385-440-F	TGACTCGAGGGCCGCATAGA
p62-Δ385-440-R	AGCTTCCTTCAGCCCTGTG
p62-254-310-XhoI-F	CCGCTCGAGCTGAGGTTGACATT
p62-254-310-EcoRI-R	CCGGAATTCTCATGTCAGAGACTG
p62-311-384-XhoI-F	CCGCTCGAGCTGAGCAAATGAAAAAG
p62-211-384-EcoRI-R	CCGGAATTCTCAAGCTTCCTTCAG
6×His/GST-p62-UBA-F-EcoRI	CCGGAATTCCTCCCGCCAGAG
6×His/GST-p62-UBA-R-XhoI	CCGCTCGAGTCACAACGGCGG

p62-K420R-F	CTACAGACCAGGAATTACGAC
p62-K420R-R	GTCGTAATTCCTGGTCTGTAG
p62-K420Q-F	CTACAGACCCAGAATTACGAC
p62-K420Q-R	GTCGTAATTCTGGGTCTGTAG
p62-K435R-F	CAGTATTCGAGGCACCCTCCA
p62-K435R-R	TGGAGGGTGCCTCGAATACTG
p62-K435Q-F	CAGTATTCGCAGCACCCTCCA
p62-K435Q-R	TGGAGGGTGCTGCGAATACTG
p62-D69A-F	CACTACCGCGCAGAGGATGGG
p62-D69A-R	CCCATCCTCTGCGCGGTAGTG
p62-K7A-F	TTCACGGTGGCGGCCTATCTT
p62-K7A-F	AAGATAGGCCGCCACCGTGAA
pEP-*p62*-KO-F	ACCGTGAATTTCCTGAAGAACGT
pEP-*p62*-KO-R	AACACGTTCTTCAGGAAATTCAC
HDAC6-H216A-F	ATTAGGCCTCCTGGACATGCAGCCCAGCACAGT
HDAC6-H216A-R	ACTGTGCTGGGCTGCATGTCCAGGAGGCCTAAT
HDAC6-H611A-F	CAGGACACGCAGCAGAGCAGGAT
HDAC6-H611A-R	ATCCTGCTCTGCTGCGTGTCCTG
TIP60-Q377/G380E-F	CTCCCTACGAACGCCGGGAATACCGGAAG
TIP60-Q377/G380E-R	CTTCCGGTATTCCCGGCGTTCGTAGGGAG
p62-mLIR-F	AGACGATGACGCAACACATGCATCTTCAAAAG
p62-mLIR-R	CTTTTGAAGATGCATGTGTTGCGTCATCGTCT

Supplementary Table 2 List of shRNAs used in this study.

shRNAs	Sequences (5'-3')
shNC-F	GATCCGAAGGGATGGCAGAGAAGCTTCAAGAGAGCTTCTCTGCCATCCCTTCTTTTTG
shNC-R	AATTCAAAAAAGAAGGGATGGCAGAGAAGCTCTCTTGAAGCTTCTCTGCCATCCCTTCG
sh*p300*-F	GATCCGCCCGGTGAACTCTCCTATAATTTCAAGAGAATTATAGGAGAGTTCACCGGGCTTTTTG
sh*p300*-R	AATTCAAAAAAGCCCGGTGAACTCTCCTATAATTCTCTTGAAATTATAGGAGAGTTCACCGGGCG
sh*PCAF*-F	GATCCGCAGATACCAAACAAGTTTATTTCAAGAGAATAAACTTGTTTGGTATCTGCTTTTTG
sh*PCAF*-R	AATTCAAAAAAGCAGATACCAAACAAGTTTATTCTCTTGAAATAAACTTGTTTGGTATCTGCG
sh*GCN5*-F	GATCCGCTGAACTTTGTGCAGTACAATTCAAGAGATTGTACTGCACAAAGTTCAGCTTTTTG
sh*GCN5*-R	AATTCAAAAAAGCTGAACTTTGTGCAGTACAATCTCTTGAATTGTACTGCACAAAGTTCAGCG
sh*TIP60*-1-F	GATCCGTCGAATTGTTTGGGCACTGATTTCAAGAGAATCAGTGCCCAAACAATTCGACTTTTTG
sh*TIP60*-1-R	AATTCAAAAAAGTCGAATTGTTTGGGCACTGATTCTCTTGAAATCAGTGCCCAAACAATTCGACG
sh*TIP60*-2-F	GATCCGCCTCAATCTCATCAACTACTATTCAAGAGATAGTAGTTGATGAGATTGAGGCTTTTTG
sh*TIP60*-2-R	AATTCAAAAAAGCCTCAATCTCATCAACTACTATCTCTTGAATAGTAGTTGATGAGATTGAGGCG
sh*TIP60*-3-F	GATCCGCCTCCTATCCTATCGAAGCTATTCAAGAGATAGCTTCGATAGGATAGGAGGCTTTTTG
sh*TIP60*-3-R	AATTCAAAAAAGCCTCCTATCCTATCGAAGCTATCTCTTGAATAGCTTCGATAGGATAGGAGGCG
sh*HDAC6*-F	GATCCGCTGCAAGGGATGGATCTGAACTTCAAGAGAGTTCAGATCCATCCCTTGCAGCTTTTTG
sh*HDAC6*-R	AATTCAAAAAAGCTGCAAGGGATGGATCTGAACTCTCTTGAAGTTCAGATCCATCCCTTGCAGCG