Supplementary Information

ZZ-Dependent regulation of p62/SQSTM1 in autophagy

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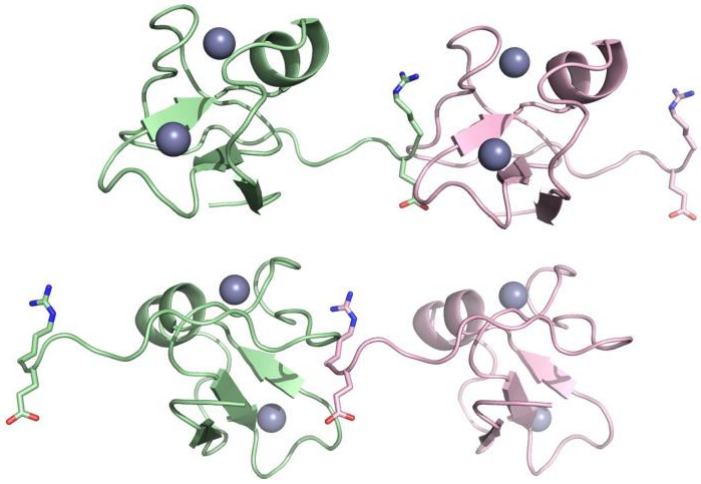
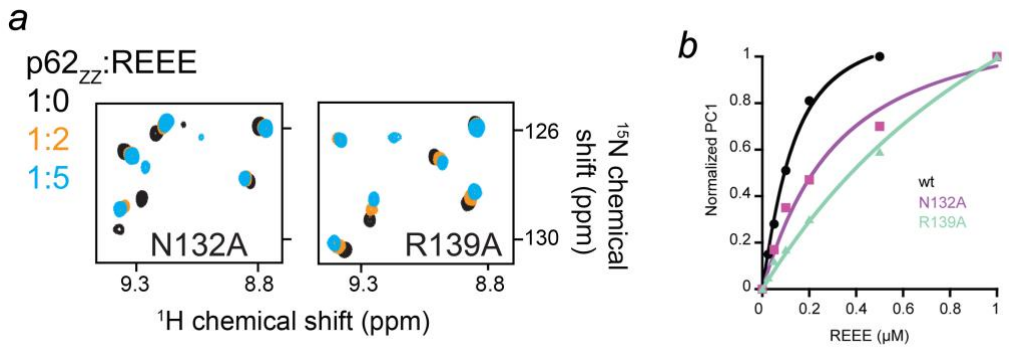
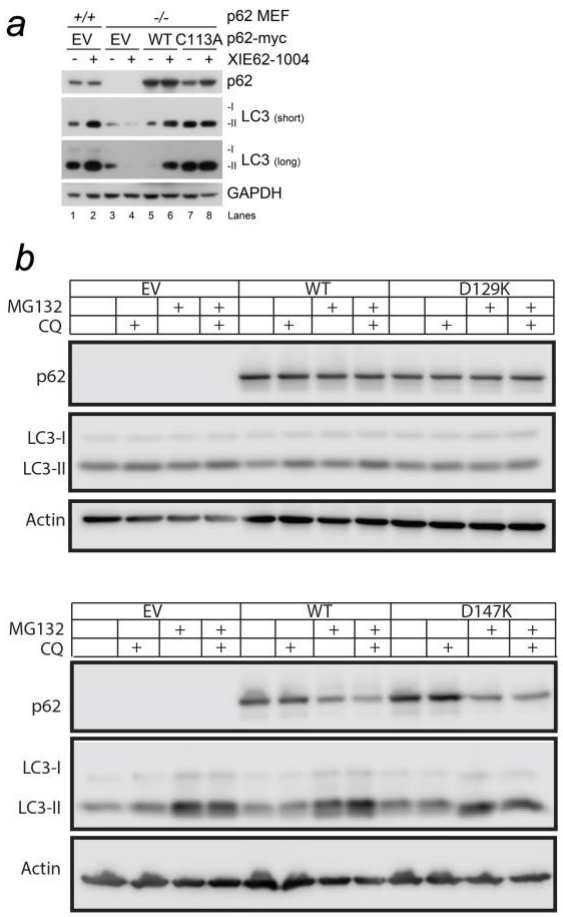


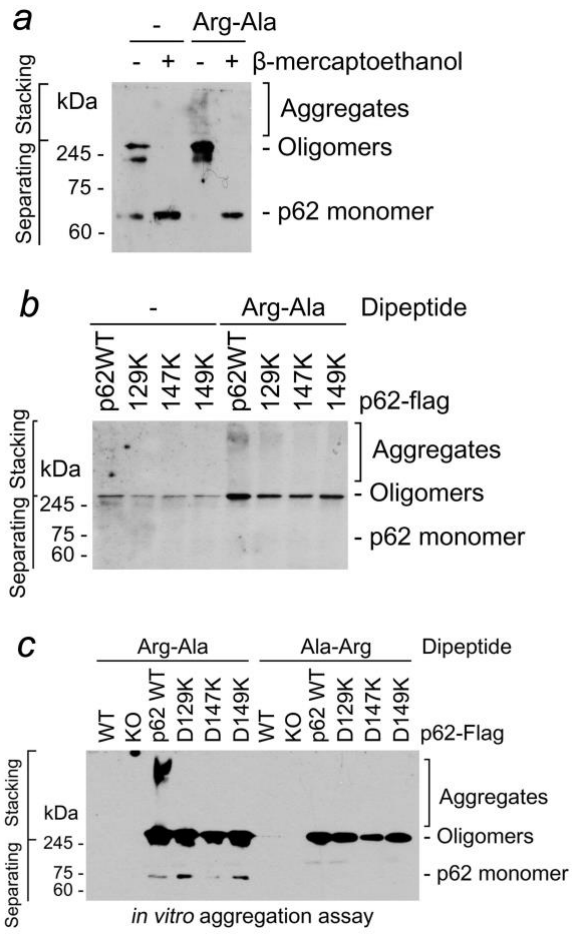
Supplementary Figure 1. The crystal structure of the RELGS-ZZ construct.



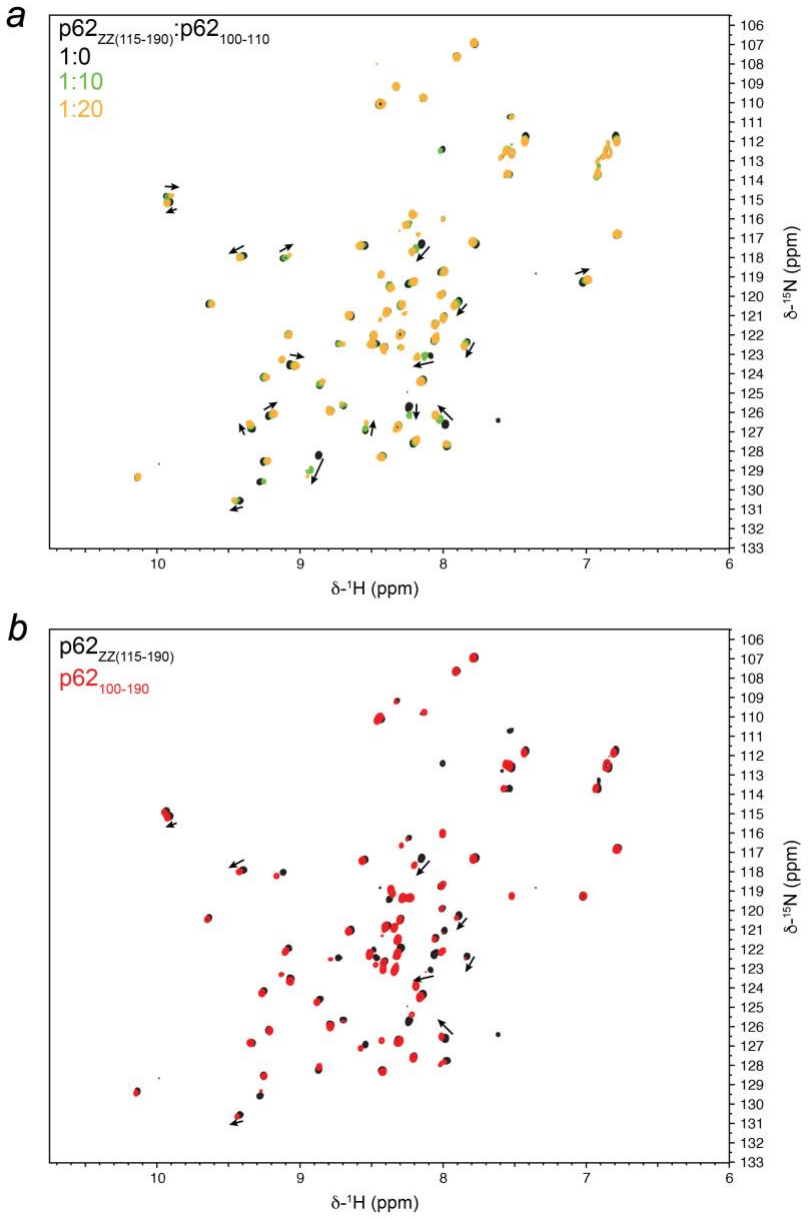
Supplementary Figure 2. (a) Superimposed $^{1}H,^{15}N$ HSQC spectra of the indicated mutants of $p62_{ZZ\ (115\text{-}190)}$ collected upon titration with the REEE peptide. Spectra are color coded according to the protein:peptide molar ratio. (b) Binding isotherms for the interaction of WT $p62_{ZZ\ (115\text{-}190)}$ and N132A and R139A mutants with the REEE peptide obtained using NMR principal component analysis (PCA) implemented with TREND software[1].



Supplementary Figure 3. (a) p62 aggregation via Cys113 is also essential for induction of autophagy with XIE62-1004. Treatment of p62 +/+ cells with XIE62-1004 induced LC3-II lipidation, whereas p62 -/- cells were non-responsive. As expected, transient expression of recombinant p62-myc in p62 -/- MEFs rescued their ability to induce LC3-II lipidation upon XIE62-1004 treatment. Importantly, p62 C113A mutant failed to induce autophagy upon XIE62-1004 treatment. Intriguingly, the mere expression of the p62 C113A mutant was sufficient to increase the level of LC3-II. (b) Western blot for LC3 I/II, p62 and β-Actin on whole cell lysates harvested from p62-/- MEFs with stable expression of an empty vector, WT p62, D129K p62 (top) or D147K (bottom) p62 after treatment with MG132 (500nM) or CQ for 24hrs.



Supplementary Figure 4. (a) *In vitro* aggregation assays of wild-type p62 stably expressed in p62 f/f MEFs with or without β-mercaptoethanol. (b) *In vitro* aggregation assays of wild-type or mutated p62 stably expressed in p62 f/f MEFs treated with or without Arg-Ala. (c) *In vitro* aggregation assays of wild-type or mutated p62 stably expressed in p62 f/f MEFs treated with Arg-Ala or Ala-Arg.



Supplementary Figure 5. (a) Superimposed $^{1}H,^{15}N$ HSQC spectra of $p62_{ZZ\ (115\text{-}190)}$ collected upon titration with the $p62_{ZZ\ (100\text{-}110)}$ peptide. Spectra are color coded according to the protein:peptide molar ratio. (b) Superimposed $^{1}H,^{15}N$ HSQC spectra of $p62_{ZZ\ (115\text{-}190)}$ and $p62_{RL\text{-}ZZ\ (100\text{-}190)}$ in apo-states.

Primer Sequences

p62 D129K
5'-ctgcaaaggctgcaatgggcctgtggtaggaac-3'
5'-aacatggtgcaccccaatgtgatctgcaaaggc-3'

p62 D147K
5'-gcccaaaatacgacttgtgtagcgtctgcgaggg-3'
5'-cccgctacaagtgcagcgtctgcccaaaatac-3'

p62 D149K
5'-ctacaaattgtgtagcgtctgcgagggaaagggc-3'
5'-cccgctacaagtgcagcgtctgcccagactacaaattg-3'

p62 115-190
5'-ccctgggatcccaggaggcgccccgcaacatggtg-3'
5'-gttctgttccaggggcccctgggatcccaggaggcg-3'

p62 120-190
5'-ccctgggatccaacatggtgcaccccaatgtgatctgcgatggc-3'
5'-gttctgttccaggggcccctgggatccaacatggtgcacc-3'

NdeI_pCIOX_F
5'-AGATATACATATGGGCAGCAG-3'

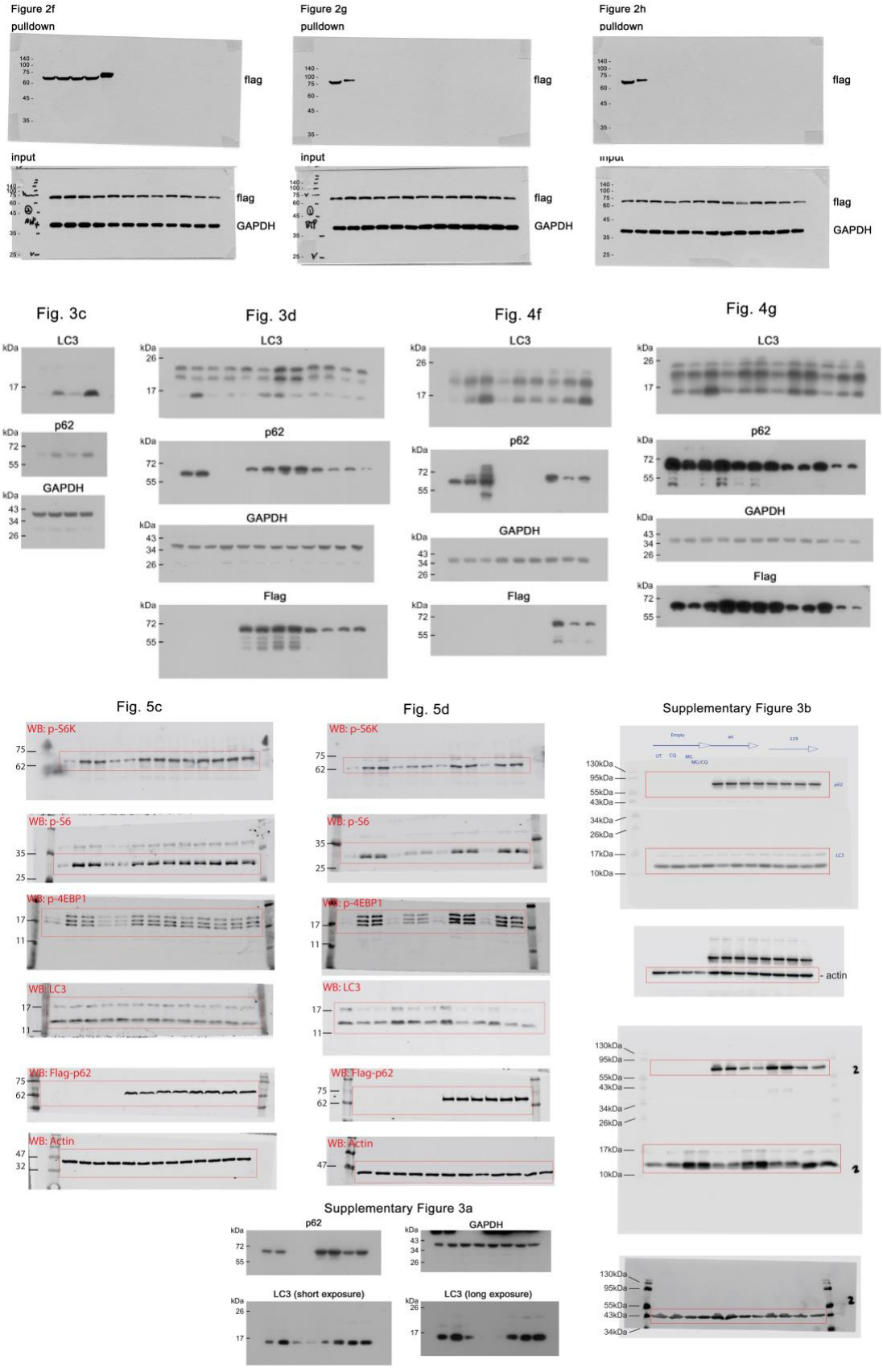
p62_171_EcoRI_R
5'-GAGCTCGAATTCttaGGGGCTGGGGAATGCG-3'

p62_190_EcoRI_R
5'-GAGCTCGAATTCttaGTGTTTCACCTTCCGGAG-3'

Sumo_LGS_p62_120
5'-GAGAACAGATTGGTGGTctgggtagcAACATGGTGCACCCC-3'
5'-GGGGTGCACCATGTTgctacccagACCACCAATCTGTTCTC-3'

Sumo_RELGS_p62_120
5'-GAGAACAGATTGGTGGTcgtgagctgggtagcAACATGGTGCACCCC-3'
5'-GGGGTGCACCATGTTgctacccagctcacgACCACCAATCTGTTCTC-3'

Supplementary Figure 6. List of primers.



Supplementary Figure 7. Uncropped Western blots.

Supplementary Table 1. Data collection and refinement statistics for the $p62_{ZZ}$ complexes

	RE-ZZ	ZZ/Arginine
Data collection		
Wavelength	1.5418 Å	1.2782 Å
Space group	P1	C2
Resolution (Å)	25.31–1.90	50.00-1.41
Cell dimensions (Å)		
	a=26.99	a=44.97
	b=27.86	b=32.27
	c=38.99	c=34.94
	α=90.06°	**α**=**γ**=90.00°
	β=90.45°	**β**=122.16°
	γ=110.3°	
Number of measured reflections	51427	107631
Number of unique reflections	8404	8142
Completeness(%)	99.5 (93.1)	98.4 (78.4)
Redundancy	6.1 (3.0)	13.2 (9.3)
I/sigma (I)	27.8 (5.1)	83.2 (45.5)
Rmerge(%)	6.4 (19.9)	6.3 (16.5)
Refinement		
Resolution (Å)	25.31-1.90	29.58-1.41
Number of reflections	14195	8142
R-factor (%)	0.1546	0.1356
R-free (%)	0.2123	0.1646
Number of protein atoms	888	422
Number of Arginine	-	12
Number of Zinc	4	2
Number of 1,4-Dioxane	-	6
Number of water molecules	133	107
R.m.s.d. from ideal values		
Bond lengths (Å)	0.007	0.005
Bond angles (°)	1.080	0.82
Average B-values ($Å^2$)	25.8	13.3
Protein chain A	20.2	9.9
Protein chain B	19.2	
Water	31.2	26.9
Zinc	19.3	8.8
Arginine	-	15.4
1,4-Dioxane	-	12.7
Ramachandran Plot analysis		
Favored	95.6%	96.2%
Allowed	4.4%	3.9%
Outliers	0%	0%

* Values in parentheses refer to data in the highest resolution shell. Datasets collected from a single crystal.

Supplementary References:

1. Xu, J. & Van Doren, S.R. Tracking Equilibrium and Nonequilibrium Shifts in Data with TREND. *Biophys J* **112**, 224-233 (2017).

Supplementary Data 1. List of antibodies.