

Expanded View Figures

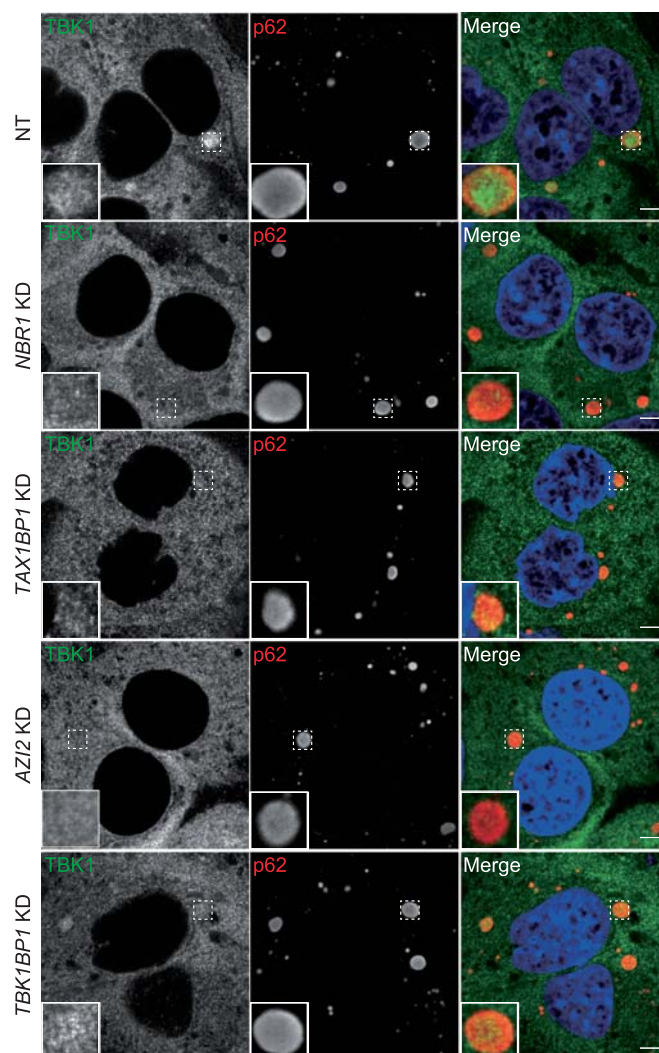


Figure EV1. TBK1 localizes to p62 bodies.

Immunofluorescence microscopy. Huh-1 cells transfected with siRNAs targeting *NBR1*, *TAX1BP1*, *AZI2*, or *TBK1BP1* were stained with antibodies against p62 and TBK1. Scale bars, 5 μm. Source data are available online for this figure.

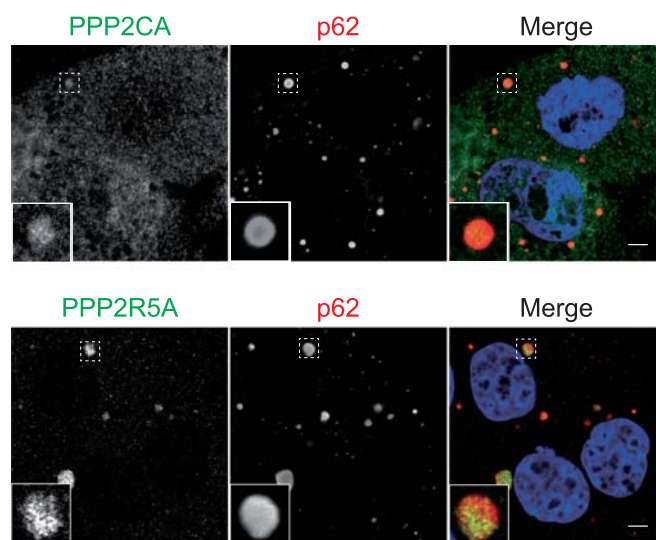


Figure EV2. PPP2CA and PPP2R5A colocalize with p62 bodies.

Immunofluorescence microscopy of Huh-1 cells stained for PPP2CA and p62 (upper) or PPP2R5A and p62 (bottom). Scale bars, 5 μ m. Source data are available online for this figure.

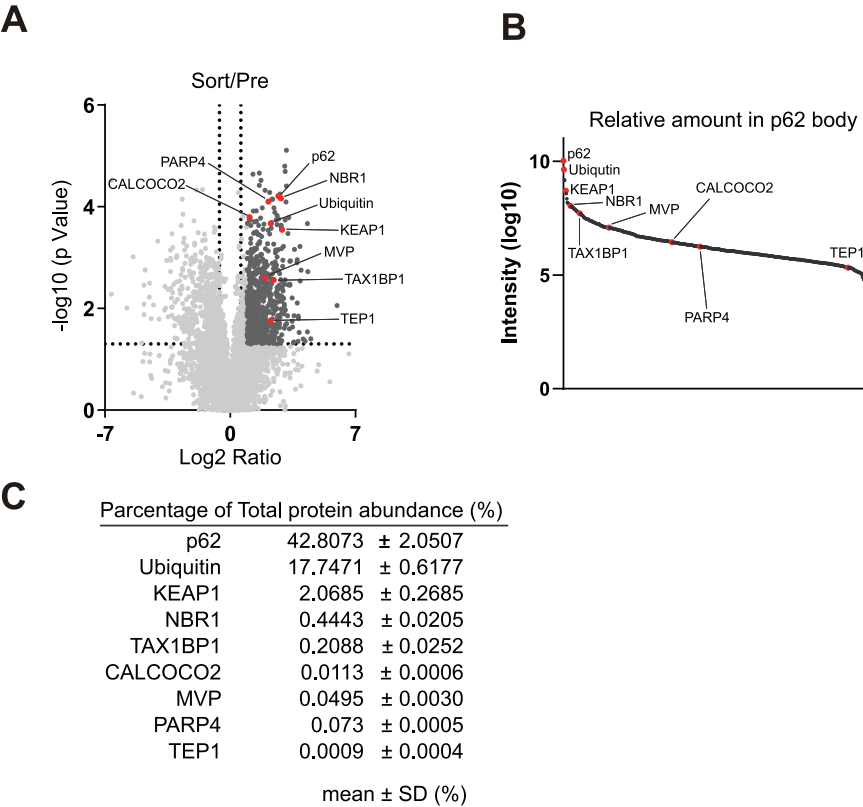


Figure EV3. Proteomic analysis of p62 bodies.

(A) Proteomic profiles of pre- and post-sorted p62 bodies isolated from mEGFP-p62-expressing Huh-1 cells ($n = 3$). Statistics: two-tailed unpaired t test. Selective autophagy receptors (NBR1, TAX1BP1, and CALCOCO2) and known client proteins (Ubiquitin, KEAP1, PARP4, and TEP1) are highlighted in red. (B) Relative abundance of each protein in p62 bodies was calculated based on label-free quantification (LFQ) intensity. Data are presented as the mean from three independent experiments. (C) The abundance ratio of each protein was calculated from the relative abundance data shown in (B). The total amount was defined as the sum of abundances of all p62 body-enriched proteins. Source data are available online for this figure.

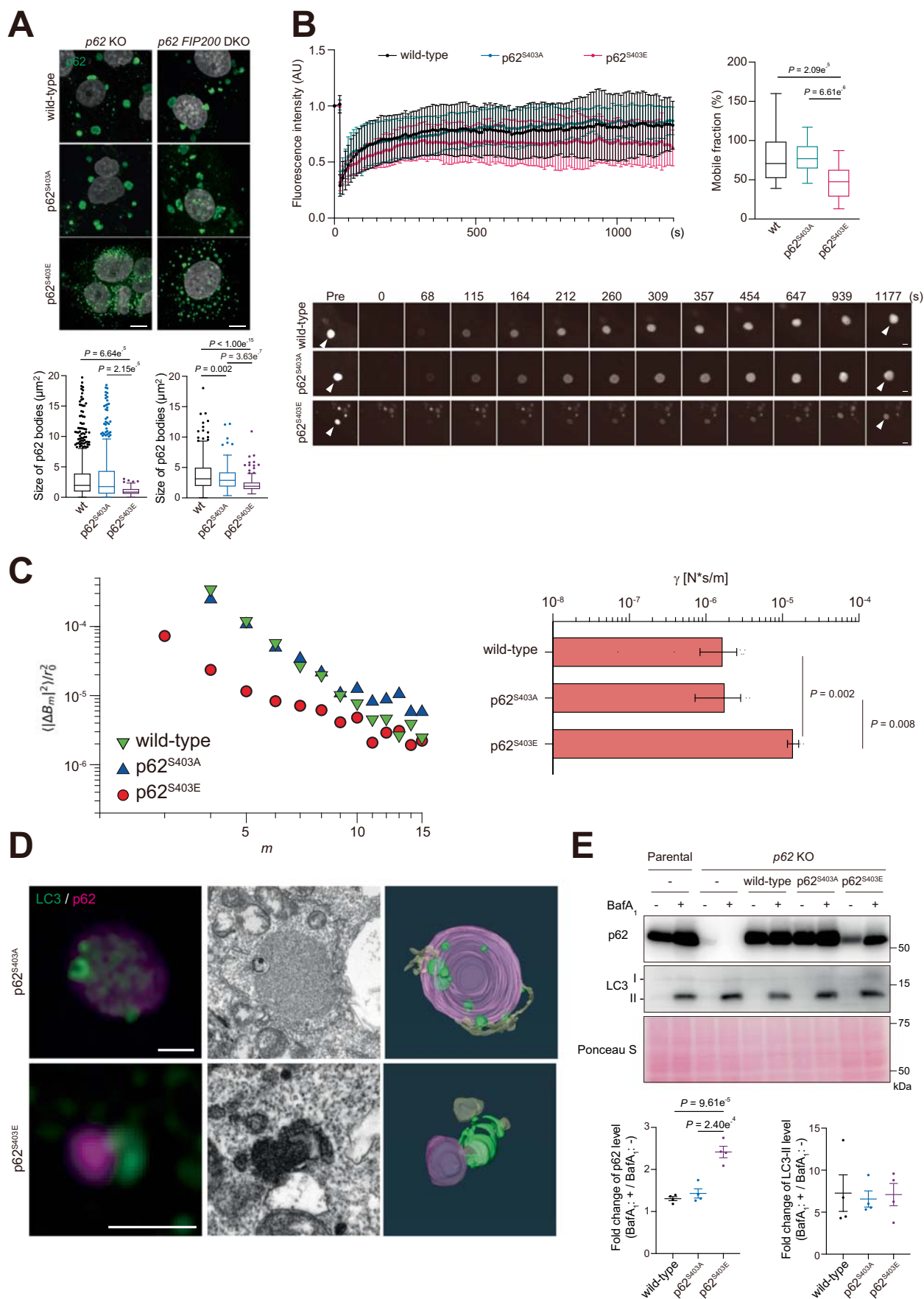



Figure EV4. Phospho-mimetic p62 enhances p62 body function.

(A) Immunofluorescence of *p62*-knock-out (KO) and *p62/FIP200* Huh-1 cells re-expressing wild-type (WT) p62, the phospho-defective mutant p62^{S403A}, or the phospho-mimetic mutant p62^{S403E}. Diameters of individual p62 bodies were quantified in p62-KO cells expressing WT p62 ($n = 1154$), p62^{S403A} ($n = 529$) or p62^{S403E} ($n = 81$), and in *p62/FIP200* double-KO cells expressing WT p62 ($n = 349$), p62^{S403A} ($n = 187$) or p62^{S403E} ($n = 410$). Statistics: one-way ANOVA with Tukey's multiple-comparison test. Exact P values are indicated; values below the detection limit are reported as $P < 1 \times 10^{-15}$. Horizontal bars indicate medians; boxes indicate the interquartile range (25th–75th percentiles); whiskers extend to $1.5 \times$ the interquartile range. Individual data points outside this range are plotted as outliers. Scale bar, 10 μm . (B) FRAP analysis of GFP-tagged p62 variants. FRAP curve is shown as mean $\pm$ s.d. Mobile fractions were determined for cells expressing WT p62 ($n = 26$), p62^{S403A} ($n = 23$) or p62^{S403E} ($n = 31$). One-way ANOVA with Tukey's test. Scale bar, 1 μm . (C) Surface tension analysis of p62 condensates. Fourier spectra of condensates in p62-KO Huh-1 cells expressing WT p62, p62^{S403A} or p62^{S403E}. The spectra were obtained by decomposing radial fluctuations into azimuthal modes (m) and fitted to a theoretical model of thermally driven surface fluctuations to estimate effective surface tension (γ). (Bottom) Quantification of γ (N·s/m) obtained from the fitting analysis in p62-KO Huh-1 cells expressing p62 wild-type ($n = 4$), p62^{S403A} ($n = 3$) or p62^{S403E} ($n = 3$). Data are presented as mean $\pm$ s.e.m. Statistics: one-way ANOVA followed by Tukey's multiple-comparison test. (D) Three-dimensional CLEM showing LC3 colocalization with p62 bodies in cells expressing p62^{S403A} or p62^{S403E}. LC3 (green), p62 (magenta), and the ER (yellow). Scale bar, 1 μm . (E) Immunoblot analysis of autophagic flux in parental and *p62*-null cells reconstituted with WT or mutant p62 in the absence or presence of bafilomycin A₁ (BafA₁). Band intensities for p62 and LC3-II were normalised to total protein ($n = 3$). Data are mean $\pm$ s.e.m. Statistics: one-way ANOVA with Tukey's multiple-comparison test. Source data are available online for this figure.

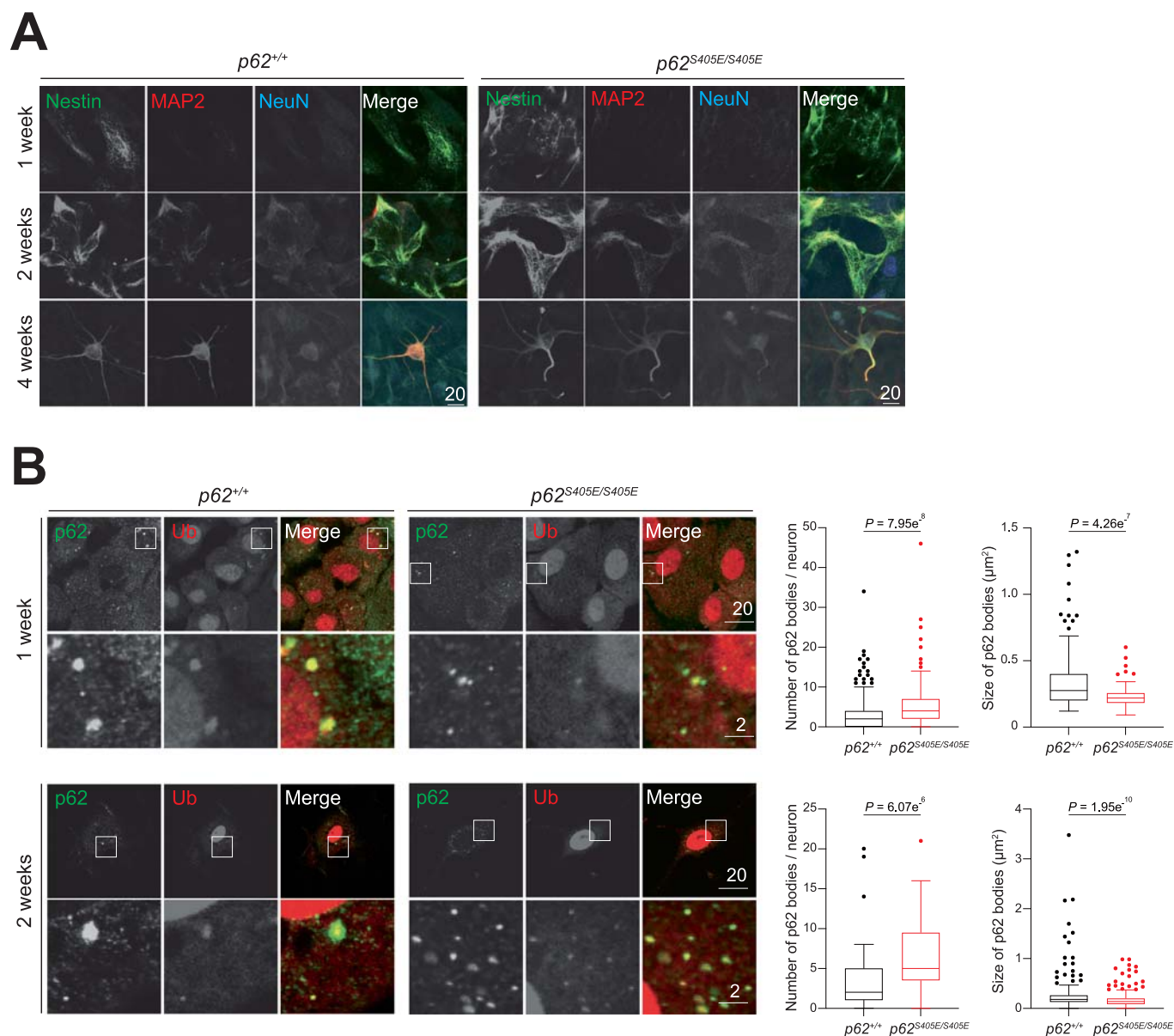


Figure EV5. p62 body size and number in wild-type and *p62^{S405E/S405E}* ES-derived neurons.

(A) Immunofluorescence analysis of neurons differentiated from parental and *p62^{S405E/S405E}* ES cells after 1, 2, and 4 weeks of culture. Both genotypes successfully differentiated into neurons, as shown by staining for neuronal markers. Scale bars, 20 μm . (B) Immunostaining for p62 and ubiquitin in neurons from both genotypes. The numbers and diameters of p62 bodies in wild-type neurons ($n = 247$, $n = 147$) and *p62^{S405E/S405E}* neurons ($n = 141$, $n = 129$) at 1 week, and in wild-type neurons ($n = 75$, $n = 241$) and *p62^{S405E/S405E}* neurons ($n = 45$, $n = 298$) at 2 weeks were quantified. Statistical analysis was performed using the Mann-Whitney *U* test. Horizontal bars indicate medians; boxes indicate the interquartile range (25th–75th percentiles); whiskers extend to 1.5 $\times$ the interquartile range. Individual data points outside this range are plotted as outliers. Scale bars, 20 μm ; enlarged views, 2 μm . Source data are available online for this figure.