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Supplementary Materials for

A p62-dependent rheostat dictates micronuclei catastrophe and chromosome rearrangements

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The PDF file includes:

Figs. S1 to S7

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist
Movies S1 and S2

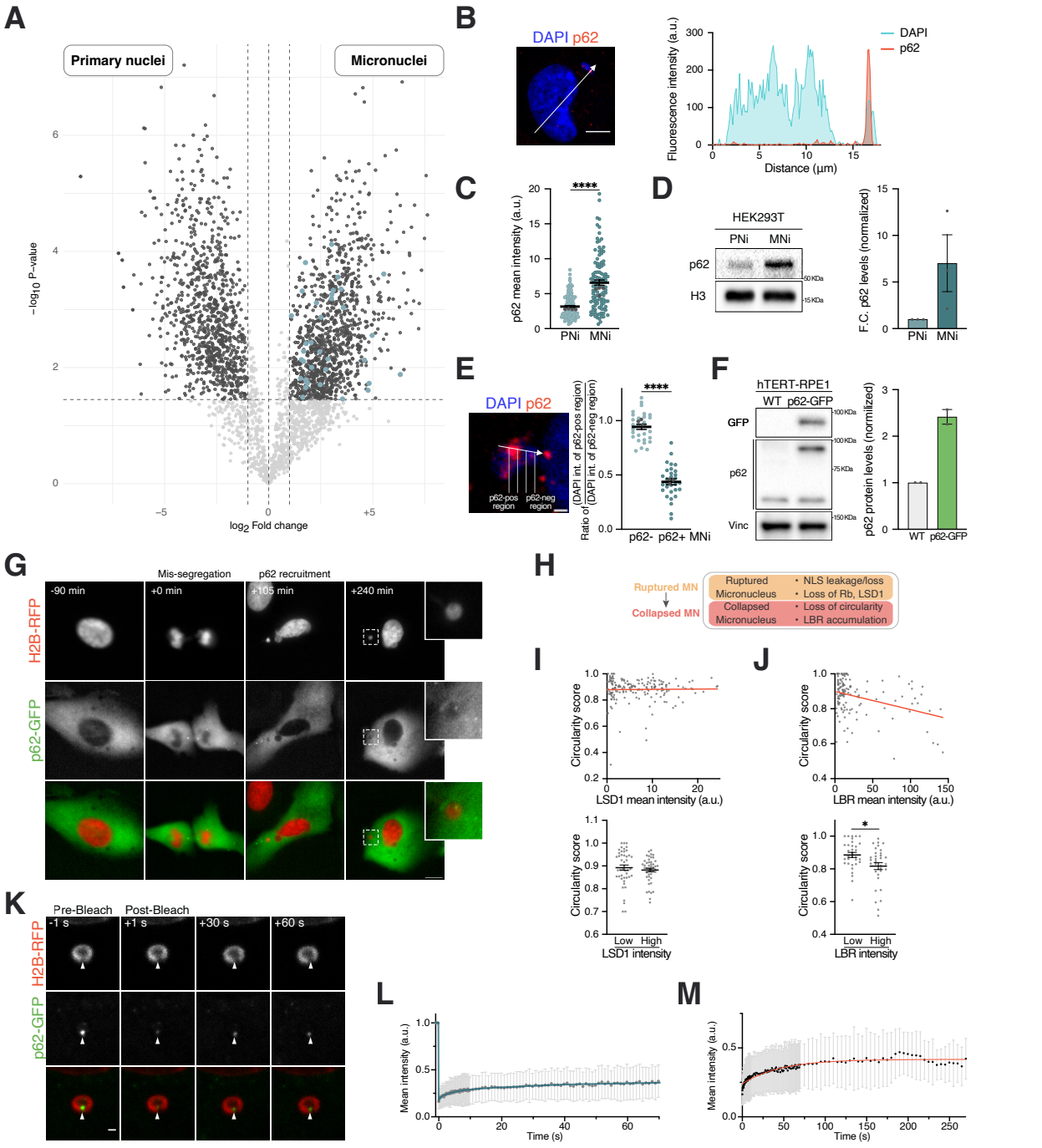

fig. S1. (A) Volcano plot showing significantly up- and down-regulated proteins by comparing primary nuclei (left) and micronuclei (right). Differential protein expression cut off: FDR < 0.05, Fold Change > 1. Proteins belonging to "ubiquitin ligand binding" GO molecular function term are colored in light blue. **(B)** Representative confocal image of a cell harboring a p62-positive micronucleus in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and p62; scale bar 5 μm. Line scan graph of DAPI (micronuclear DNA) and p62 fluorescence intensities (a.u. arbitrary unit), with respect to primary nucleus and micronucleus. The arrow on the confocal image represents the directionality of the X axis of the graph. **(C)** Quantification via immunofluorescence

of p62 levels (a.u. arbitrary unit) in primary nuclei (PNi) and micronuclei (MNi) (identified using DAPI masking) in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and p62. From at least 4 biological replicates. Each colored data point indicates the mean of a biological replicate. Mean ± SEM. Mann-Whitney test performed: p = <0.0001. **(D)** Western blot analysis and relative quantification of p62 levels in primary nuclei (PNi) and micronuclei (MNi) upon cellular fractionation of HEK293T cells treated with Mps1i, immunolabeled with p62. H3 was used as loading control. From 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. **(E)** Representative confocal image of a p62-positive micronucleus in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and p62; scale bar 1 μm. Line scan quantification via immunofluorescence of p62 localization within micronuclear cavities: DAPI fluorescence intensity of a p62-positive region in a p62-positive micronucleus was divided by the DAPI fluorescence intensity of a p62-negative region in proximity; as a control, DAPI fluorescence intensity of a randomly selected micronuclear region in a p62-negative micronucleus was divided by the DAPI fluorescence intensity of a region in proximity. In case of p62 localization occurring outside micronuclear cavities the expected ratio value is closer to 1 (as a consequence of having similar DAPI intensities in the two different regions analyzed, as it is observed in the negative control), in case of p62 localization occurring within cavities the expected ratio value is below 1 (as a consequence of having a lower DAPI intensity in the p62-positive region - and thus a cavity - compared to the negative one). From 3 biological replicates. Each colored data point indicates the mean of a biological replicate. Mean ± SEM. Unpaired t test performed: p = <0.0001. **(F)** Western blot analysis and relative quantification of p62 levels in hTERT-RPE1 cells WT or stably expressing p62-GFP, immunolabeled with GFP and p62. Vinculin was used as loading control. From 2 biological replicates. Each data point indicates a biological replicate. Mean ± SD. **(G)** Representative images of a live cell imaging experiment aimed to visualize p62 recruitment to micronuclei in hTERT-RPE1 cells stably expressing H2B-RFP and p62-GFP, filmed before (-90 min) and during mitosis in presence of Mps1i, during micronuclei formation (0 min), p62 recruitment to micronuclei (+105 min) and p62 lasting localization to the micronucleus (+240 min). Still images taken from Movie S1. Scale bar 10 μm. **(H)** Representative scheme of the markers of micronuclear rupture and collapse, visualized by immunofluorescence. **(I)** Top: correlative analysis of circularity score and LSD1 fluorescent intensity (a.u. arbitrary unit) of micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and LSD1. From 3 biological replicates. The red line represents the simple linear regression line: R-Square = 0.0002315. Bottom: quantification of the circularity score of micronuclei characterized by low and high levels of LSD1 (bottom and top quartile respectively) represented in the top graph. From 3 biological replicates. Mean ± SEM. Two-sided Mann-Whitney test performed. **(J)** Top: correlative analysis of circularity score and LBR fluorescent intensity (a.u. arbitrary unit) of micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and LBR. From 3 biological replicates. The red line represents the simple linear regression line: R-Square = 0.1360, p = <0.0001. Bottom: quantification of the circularity score of micronuclei characterized by low and high levels of LBR (bottom and top quartile respectively) represented in the top graph. From 3 biological replicates. Mean ± SEM. Two-sided Mann-Whitney test performed: p = 0.0165. **(K)** Representative images of the FRAP experiment of hTERT-RPE1 cells expressing H2B-RFP and p62-GFP representing p62-GFP on the micronucleus before (-1 s) and immediately after photo-bleaching (+1 s), and after recovery (+30 s, +60 s); scale bar 1 μm. **(L)** Quantification of fluorescence intensity (a.u. arbitrary unit) from -1 s to 60 s at the bleached region from the FRAP experiment shown in panel K. Time = 0 s refers to the bleaching event. The blue line was added

to facilitate the visualization of fluorescence intensity changes. 20 cells analyzed from 2 biological replicates. Mean ± SD. **(M)** Quantification of fluorescence intensity (a.u. arbitrary unit) from 0 s to 300 s at the bleached region after photo-bleaching from the FRAP experiment shown in panel K. The red line represents the nonlinear fitting curve calculated by one-phase association: plateau = 0.4172, half-time = 31.49 s, Tau = 45.42. 20 cells analyzed from 2 biological replicates. Mean ± SD.

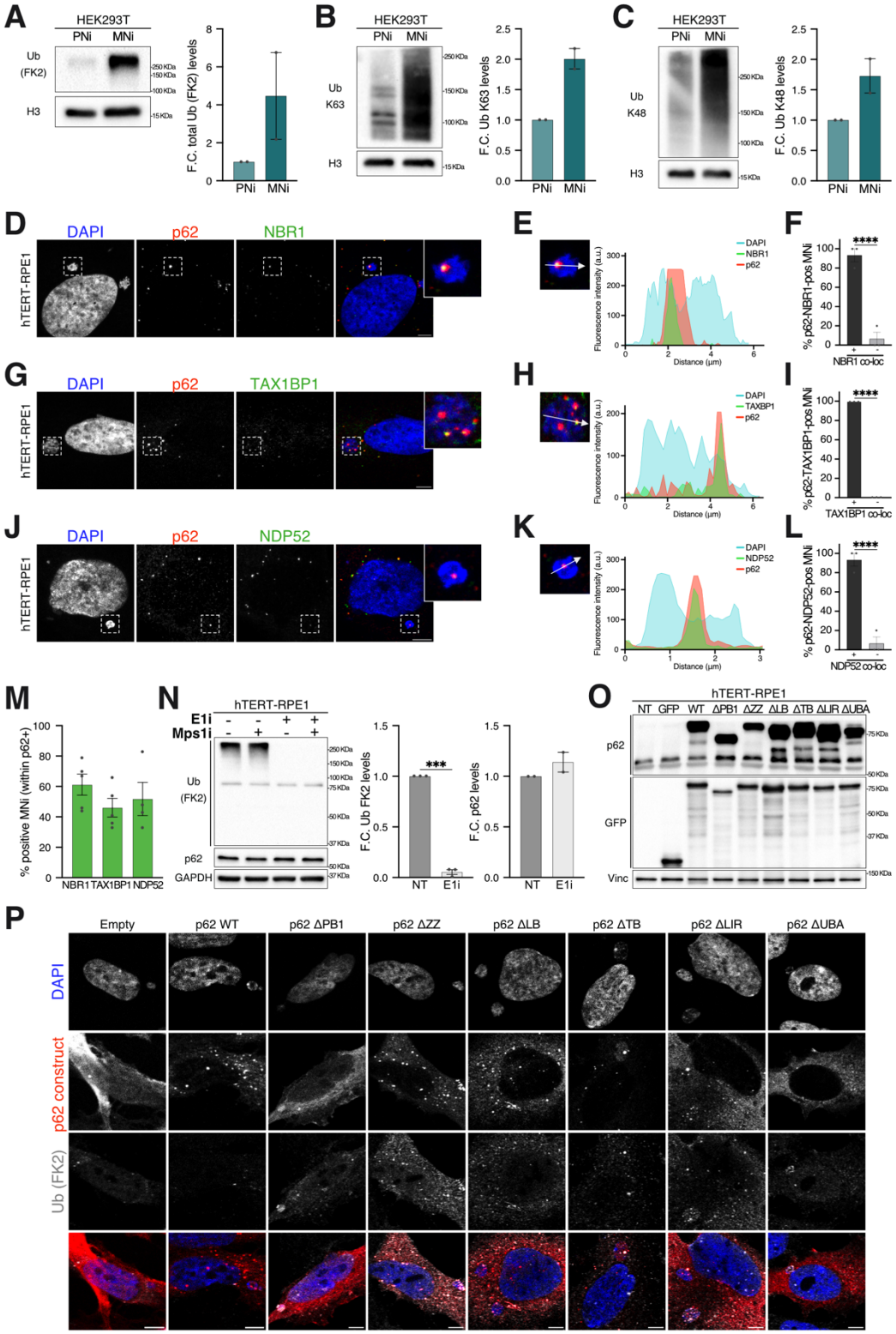

fig. S2. (A) Western blot analysis and relative quantification of pan-ubiquitin (FK2) levels in primary nuclei (PNi) and micronuclei (MNi) upon cellular fractionation of HEK293T cells treated with Mps1i, immunolabeled with pan-ubiquitin (FK2). H3 was used as loading control. From 2 biological replicates. Each data point indicates a biological replicate. Mean ± SD. **(B)**

Western blot analysis and relative quantification of ubiquitin poly-Lys K^{63} (Ub K63) levels in primary nuclei (PNi) and micronuclei (MNi) upon cellular fractionation of HEK293T cells treated with Mps1i, immunolabeled with ubiquitin poly-Lys K^{63}. H3 was used as loading control. From 2 biological replicates. Each data point indicates a biological replicate. Mean ± SD. **(C)** Western blot analysis and relative quantification of ubiquitin poly-Lys K^{48} (Ub K48) levels in primary nuclei (PNi) and micronuclei (MNi) upon cellular fractionation of HEK293T cells treated with Mps1i, immunolabeled with ubiquitin poly-Lys K^{48}. H3 was used as loading control. From 2 biological replicates. Each data point indicates a biological replicate. Mean ± SD. **(D)** Representative confocal images of a p62-positive micronucleus showing the localization of autophagic receptor NBR1, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and NBR1. Scale bar 5 μm. **(E)** Line scan graph of DAPI (micronuclear DNA), p62 and NBR1 fluorescence intensities (a.u. arbitrary unit), respective to the micronucleus represented in fig. S2D. The arrow on the confocal image represents the directionality of the X axis of the graph. **(F)** Quantification of NBR1 signal -positive and -negative for co-localization with p62 signal within p62-positive NBR1-positive micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and NBR1. 30 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Chi-Squared test performed: p = <0.0001. **(G)** Representative confocal images of a p62-positive micronucleus showing the localization of autophagic receptor TAX1BP1, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and TAX1BP1. Scale bar 5 μm. **(H)** Line scan graph of DAPI (micronuclear DNA), p62 and TAX1BP1 fluorescence intensities (a.u. arbitrary unit), respective to the micronucleus represented in fig. S2G. The arrow on the confocal image represents the directionality of the X axis of the graph. **(I)** Quantification of TAX1BP1 signal -positive and -negative for co-localization with p62 signal within p62-positive TAX1BP1-positive micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and TAX1BP1. 30 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Chi-Squared test performed: p = <0.0001. **(J)** Representative confocal images of a p62-positive micronucleus showing the localization of autophagic receptor NDP52, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and NDP52. Scale bar 5 μm. **(K)** Line scan graph of DAPI (micronuclear DNA), p62 and NDP52 fluorescence intensities (a.u. arbitrary unit), respective to the micronucleus represented in fig. S2J. The arrow on the confocal image represents the directionality of the X axis of the graph. **(L)** Quantification of NDP52 signal -positive and -negative for co-localization with p62 signal within p62-positive NDP52-positive micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and NDP52. 30 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Chi-Squared test performed: p = <0.0001. **(M)** Quantification of NBR1-, TAX1BP1-, NDP52- positive micronuclei within p62-positive ones in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and NBR1 or TAX1BP1 or NDP52. At least 140 micronuclei analyzed from at least 4 biological replicates (N = 5, 5, 4). Each data point indicates a biological replicate. Mean ± SEM. **(N)** Western blot analysis and relative quantification of pan-ubiquitin (FK2) and p62 levels in hTERT-RPE1 cells with or without micronuclei (± Mps1i), upon E1i treatment (± E1i; NT not treated), immunolabeled with pan-ubiquitin (FK2) and p62. GAPDH was used as loading control. From at least 2 biological replicates (N = 3, 2). Each data point indicates a biological replicate. Mean ± SEM (pan-ubiquitin), mean ± SD (p62). One sample t test followed by Wilcoxon test performed: Ub (FK2) p = 0.0006. **(O)** Western blot analysis of expression levels of endogenous p62 and p62-

overexpressing constructs in hTERT-RPE1 WT cells, immunolabeled with GFP and p62. Vinculin was used as loading control. Images are representative of at least 3 biological replicates. **(P)** Representative confocal images of p62-GFP constructs and their localization to ubiquitin-positive micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and ubiquitin (FK2); scale bar 5 μm.

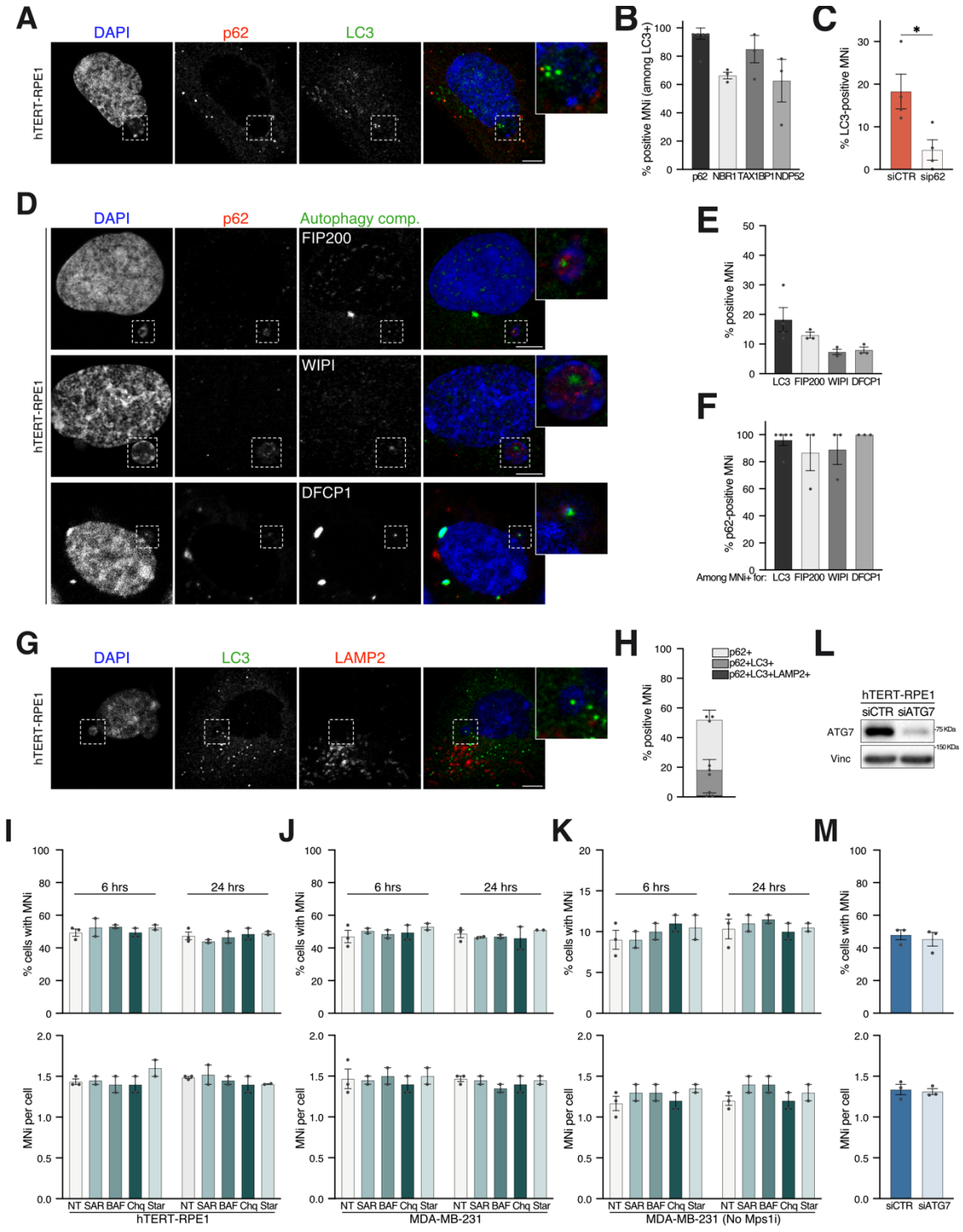

fig. S3. (A) Representative confocal images of a p62-positive micronucleus showing the localization of autophagic component LC3, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and LC3. Scale bar 5 µm. **(B)** Quantification of p62-, NBR1-, TAX1BP1-, NDP52- positive micronuclei within LC3-positive ones in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, LC3 and p62 or NBR1 or TAX1BP1 or NDP52. From at least 100 micronuclei analyzed from at least 3 biological replicates (N = 5, 3, 3, 3). Each data

point indicates a biological replicate. Mean ± SEM. **(C)** Quantification of LC3-positive micronuclei in fixed hTERT-RPE1 cells treated with Mps1i upon ± sip62. From at least 140 micronuclei analyzed from 4 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0266. **(D)** Representative confocal images of a p62-positive micronucleus showing the localization of autophagic components FIP200 (top), WIPI (center), DFCP1 (bottom), in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and FIP200 or WIPI or overexpressing DFCP1. Scale bar 5 μm. **(E)** Quantification of LC3-, FIP200-, WIPI-, DFCP1- positive micronuclei in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, LC3 or FIP200 or WIPI or overexpressing DFCP1. From at least 100 micronuclei analyzed from at least 3 biological replicates (N = 4, 3, 3, 3). Each data point indicates a biological replicate. Mean ± SEM. **(F)** Quantification of p62-positive micronuclei among LC3-, FIP200-, WIPI-, DFCP1- positive ones in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and LC3 or FIP200 or WIPI or overexpressing DFCP1. From at least 100 micronuclei analyzed from at least 3 biological replicates (N = 5, 3, 3, 3). Each data point indicates a biological replicate. Mean ± SEM. **(G)** Representative confocal images of a LC3-positive micronucleus showing no localization of the autolysosome component LAMP2, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, LC3 and LAMP2. Scale bar 5 μm. **(H)** Quantification of micronuclei positive for p62, double positive for p62 and LC3, or triple positive for p62, LC3 and LAMP2 in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62, LC3 and LAMP2. From at least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. **(I)** Quantification of cells with micronuclei and micronuclei per cell in fixed hTERT-RPE1 cells treated with Mps1i and with autophagic treatments (NT = untreated, SAR = SAR405, BAF = Bafilomycin A1 – Baf-A1,Chq = Chloroquine, Star = starvation), labeled with DAPI. From at least 100 micronuclei analyzed from at least 2 biological replicates (N = 3, 2, 2, 2, 2, 3, 2, 2, 2, 2). Each data point indicates a biological replicate. Mean ± SD. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test. **(J)** Quantification of cells with micronuclei and micronuclei per cell in fixed MDA-MB-231 cells treated with Mps1i and with autophagic treatments (NT = untreated, SAR = SAR405, BAF = Bafilomycin A1 – Baf-A1, Chq = Chloroquine, Star = starvation), labeled with DAPI. From at least 100 micronuclei analyzed from at least 2 biological replicates (N = 3, 2, 2, 2, 2, 3, 2, 2, 2, 2). Each data point indicates a biological replicate. Mean ± SD. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test. **(K)** Quantification of cells with micronuclei and micronuclei per cell in fixed MDA-MB-231 cells treated with autophagic treatments (NT = untreated, SAR = SAR405, BAF = Bafilomycin A1 – Baf-A1, Chq = Chloroquine, Star = starvation), labeled with DAPI. From at least 100 micronuclei analyzed from at least 2 biological replicates (N = 3, 2, 2, 2, 2, 3, 2, 2, 2, 2). Each data point indicates a biological replicate. Mean ± SD. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test. **(L)** Western blot analysis of ATG7 levels in hTERT-RPE1 cells ± siATG7, immunolabeled with ATG7. Vinculin was used as loading control. Images are representative of at least 3 biological replicates. **(M)** Quantification of cells with micronuclei and micronuclei per cell in fixed hTERT-RPE1 cells treated with Mps1i and ± siATG7, labeled with DAPI. From at least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed.

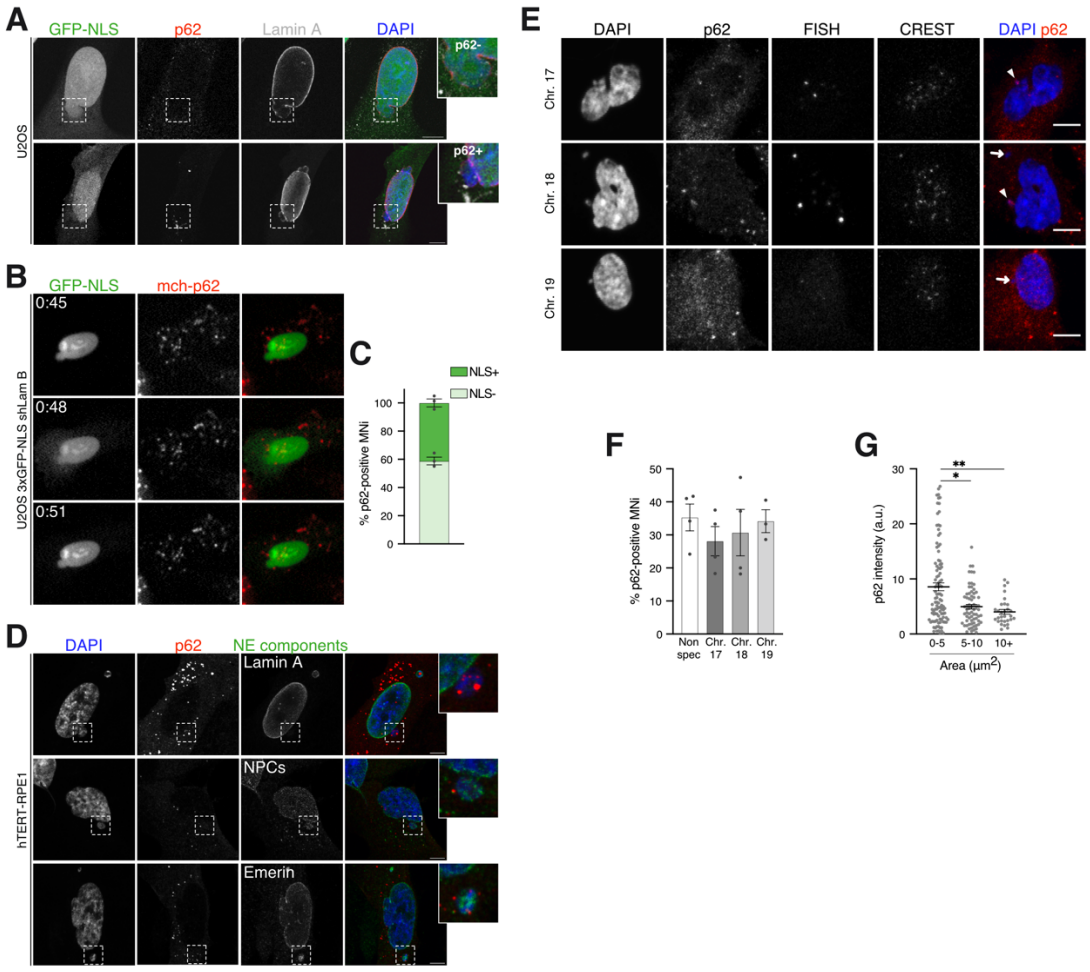

fig. S4. (A) Representative confocal images of p62- negative (top) (also shown in Fig. 3C) and positive (bottom) rupture sites in primary nuclei of S-phase arrested shRNA-lamin B1 NLS-GFP U2OS cells, labeled with DAPI, p62 and Lamin A. Scale bar 10 μm. **(B)** Representative images of a live cell imaging experiment showing p62 does not specifically associate with the rupture site of primary nuclei in U2OS 3xGFP-NLS shRNA-LMNB1 cells transiently expressing mCherry-p62 arrested in S phase 24 hours prior to imaging. **(C)** Quantification of NLS -positive and -negative micronuclei within p62-positive ones in fixed U2OS 3xGFP-NLS shRNA-LMNB1 cells treated with Mps1i. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Chi-Squared test performed. **(D)** Representative confocal images of a p62-positive micronuclei characterized by absence of Lamin A (top), presence of nuclear pore complexes (NPCs) (middle), presence of emerin (bottom), in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62 and Lamin A/NPCs/Emerin. Scale bar 5 μm. **(E)** Representative confocal images of micronuclei containing a single focus of CREST (anti-centromeric antibody) and the indicated FISH probe in fixed hTERT-RPE1 cells synchronized and treated with Mps1i, labeled with DAPI, p62 and CREST. p62-positive micronuclei are indicated by arrows, p62-negative micronuclei are indicated by arrowheads. Scale bar 10 μm. **(F)** Proportion of p62-positive micronuclei within the nonspecific (Non spec) population or within chromosomes 17, 18 or 19, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI, p62, ACA and hybridized with HSA FISH probes. At least 100 micronuclei analyzed from at least 3 biological replicates (N = 4, 4, 4, 3). Each data point

indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test. **(G)** Quantification of p62 fluorescence intensity (a.u. arbitrary unit) in micronuclei (identified by DAPI masking) characterized by different sizes, in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and p62. From 3 biological replicates. Mean ± SEM. Kruskal-Wallis test performed followed by Dunn's multiple comparison test: 0-5 vs. 5-10 $p = 0.0138$, 0-5 vs. 10+ $p = 0.0066$.

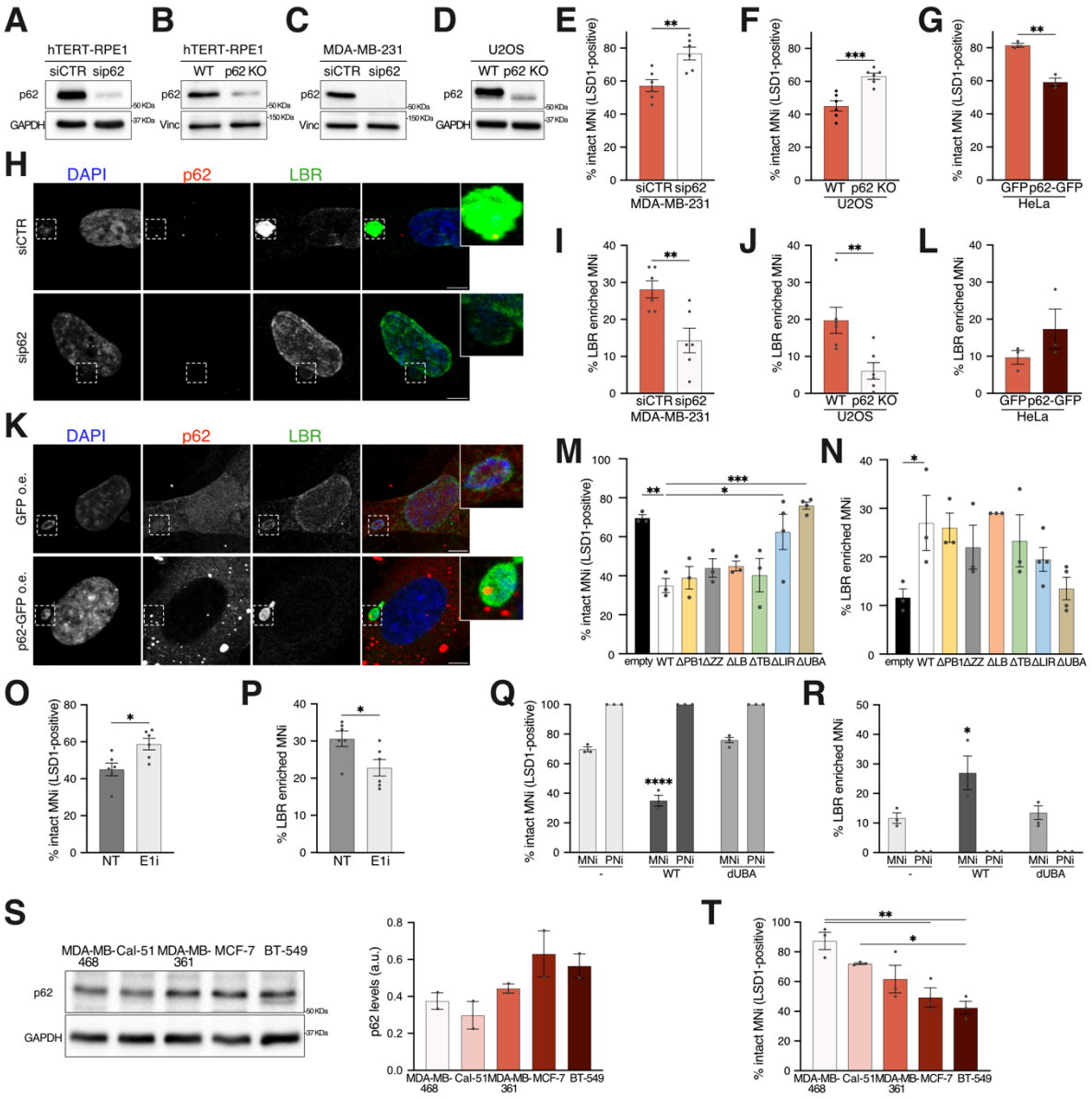

fig. S5. (A) Western blot analysis of p62 protein levels to demonstrate p62 KD efficiency in hTERT-RPE1 cells. GAPDH was used as loading control. Images are representative of 3 biological replicates. **(B)** Western blot analysis of p62 protein levels in hTERT-RPE1 WT and p62 KO cell lines to demonstrate the efficiency of sgRNAs. hTERT RPE1 p62 KO#2 cells were selected for further analysis. GAPDH was used as loading control. Images are representative of 3 biological replicates. **(C)** Western blot analysis of p62 protein levels to demonstrate p62 KD efficiency in MDA-MB-231. Vinculin was used as loading control. Images are representative of 3 biological replicates. **(D)** Western blot analysis of p62 protein levels in U2OS WT and p62 KO cells to demonstrate the efficiency of the sgRNA. Vinculin was used as loading control. Images are representative of 3 biological replicates. **(E)** Quantification of intact micronuclei (LSD1-positive) in fixed MDA-MB-231 cells treated with Mps1i upon ± sip62, labeled with DAPI, p62 and LSD1. At least 210 micronuclei analyzed from 6 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0045. **(F)** Quantification of intact micronuclei (LSD1-positive) in fixed U2OS cells treated with Mps1i

upon ± p62 KO, labeled with DAPI, p62 and LSD1. At least 210 micronuclei analyzed from 6 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0006. **(G)** Quantification of intact micronuclei (LSD1-positive) in fixed HeLa cells treated with Mps1i upon ± p62 o.e., labeled with DAPI and LSD1. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0011. **(H)** Representative confocal images showing p62 on a collapsed (LBR enriched) micronucleus upon siCTR and an intact (LBR not enriched) micronucleus upon sip62 in fixed hTERT-RPE1 cells treated with Mps1i and ± sip62, labeled with DAPI, p62 and LBR; scale bar 5 μm. **(I)** Quantification of collapsed micronuclei (LBR enriched) in fixed MDA-MB-231 cells treated with Mps1i upon ± sip62, labeled with DAPI, p62 and LBR. At least 200 micronuclei analyzed from 6 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0067. **(J)** Quantification of collapsed micronuclei (LBR enriched) in fixed U2OS cells treated with Mps1i upon ± p62 KO, labeled with DAPI, p62 and LBR. At least 200 micronuclei analyzed from 6 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0086. **(K)** Representative confocal images showing an intact (LBR not enriched) micronucleus upon overexpression (o.e.) of GFP and a collapsed (LBR enriched) micronucleus upon overexpression of p62-GFP in fixed hTERT-RPE1 cells treated with Mps1i, labeled with DAPI and LBR; scale bar 5 μm. **(L)** Quantification of collapsed micronuclei (LBR enriched) in fixed HeLa cells treated with Mps1i upon ± p62 o.e., labeled with DAPI and LBR. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. **(M)** Quantification of intact micronuclei (LSD1-positive) in fixed hTERT-RPE1 p62 KO cells treated with Mps1i after overexpression of GFP-empty or p62-GFP deleted-constructs, labeled with DAPI and LSD1. At least 100 micronuclei analyzed from at least 3 biological replicates (N = 3, 3, 3, 3, 3, 3, 4, 4). Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test (WT vs. all): WT vs. empty p = 0.0038, WT vs. ΔLIR p = 0.0144, WT vs. ΔUBA p = 0.0004. **(N)** Quantification of collapsed micronuclei (LBR enriched) in fixed hTERT-RPE1p62 KO cells treated with Mps1i after overexpression of GFP-empty or p62-GFP deleted-constructs, labeled with DAPI and LBR. At least 100 micronuclei analyzed from at least 3 biological replicates (N = 3, 3, 3, 3, 3, 3, 4, 4). Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test (WT vs. all): WT vs. empty p = 0.0380. **(O)** Quantification of intact micronuclei (LSD1-positive) in hTERT-RPE1 cells treated with Mps1i upon ± E1i (NT not treated), labeled with DAPI, p62 and LSD1. At least 200 micronuclei analyzed from 6 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0147. **(P)** Quantification of collapsed micronuclei (LBR enriched) in fixed hTERT-RPE1 cells treated with Mps1i upon ± E1i (NT not treated), labeled with DAPI, p62 and LBR. At least 200 micronuclei analyzed from 6 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p = 0.0271. **(Q)** Quantification of intact (LSD1-positive) micronuclei (MNi) and primary nuclei (PNi) in fixed hTERT-RPE1 p62 KO cells treated with Mps1i after overexpression of GFP-empty or p62-GFP WT or p62-GFP ΔUBA, labeled with DAPI and LSD1. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test (MNi: GFP-empty vs. all; PNi: GFP-empty vs. all): MNi

empty vs. WT p = <0.0001. **(R)** Quantification of collapsed (LBR enriched) micronuclei (MNi) and primary nuclei (PNi) in fixed hTERT-RPE1 p62 KO cells treated with Mps1i after overexpression of GFP-empty or p62-GFP WT or p62-GFP ΔUBA, labeled with DAPI and LSD1. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test (MNi: GFP-empty vs. all; PNi: GFP-empty vs. all): MNi empty vs. WT p = 0.0134. **(S)** Western blot analysis and relative quantification of p62 protein levels of the breast cancer cell lines chosen for microscopy analysis. GAPDH was used as loading control. From 2 biological replicates. Each data point indicates a biological replicate. Mean ± SD. **(T)** Quantification of intact micronuclei (LSD1-positive) spontaneously forming in breast cancer cell lines, labeled with DAPI and LSD1. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test: MDA-MB-468 vs. MCF7 p = 0.0081, MDA-MB-468 vs. BT549 p = 0.0025, Cal-51 vs. BT549 p = 0.0364.

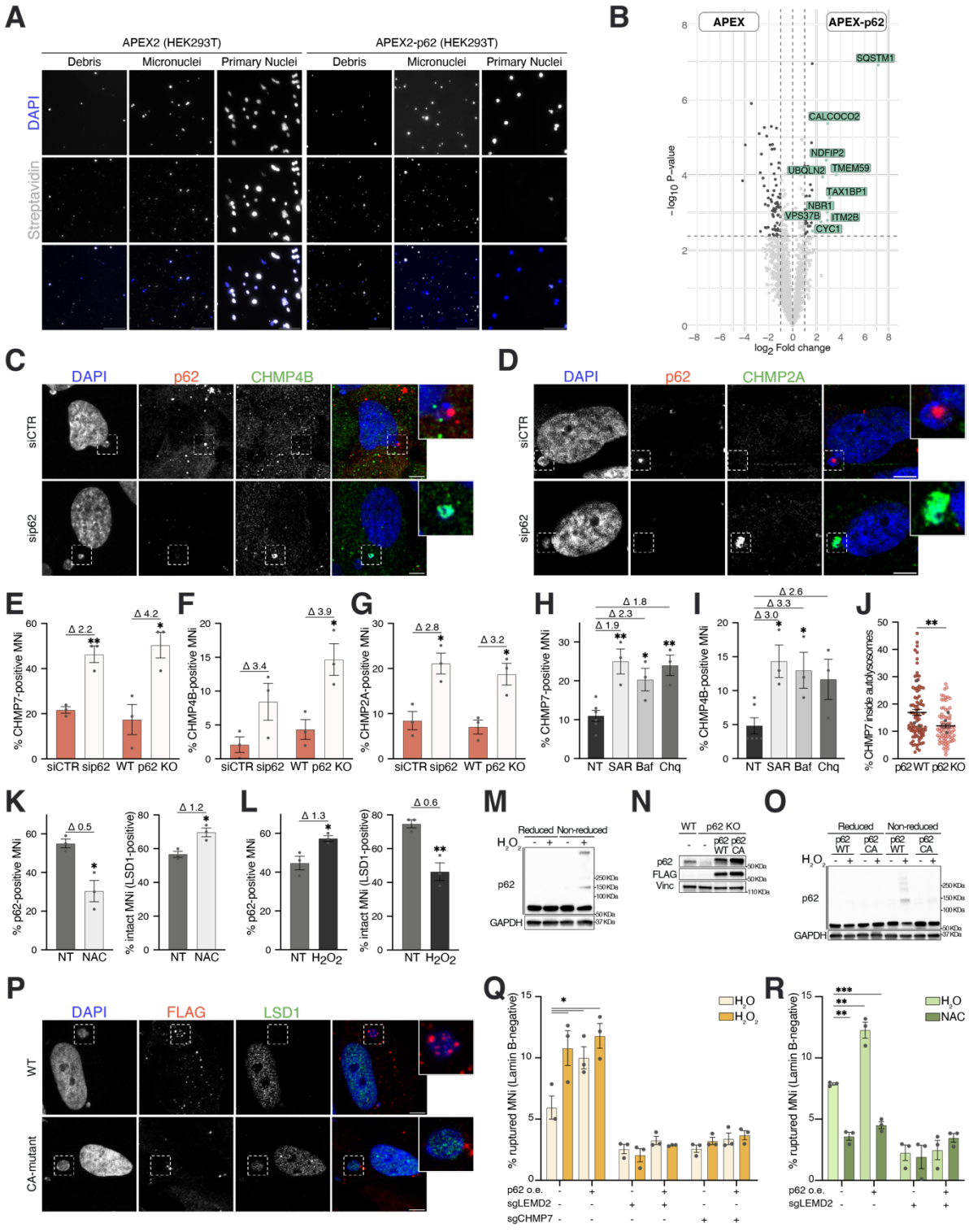

fig. S6. (A) Representative confocal images of cellular fractions after biotinylation and purification in HEK293T APEX2 and APEX2-p62, labeled with DAPI and Streptavidin. Scale bar 50 µm. **(B)** Volcano plot showing significantly enriched biotinylated proteins in APEX2 (control) and APEX2-p62 micronuclei. The top 10 hits identified in APEX2-p62 micronuclei are

labeled in green. **(C)** Representative confocal images showing a CHMP4B-negative p62-positive micronucleus upon siCTR and a CHMP4B-positive micronucleus upon sip62 in fixed hTERT-RPE1 cells treated with Mps1i and ± sip62, labeled with DAPI, p62 and CHMP4B; scale bar 5 µm. **(D)** Representative confocal images showing a CHMP2A-negative p62-positive micronucleus upon siCTR and a CHMP2A-positive micronucleus upon sip62 in fixed hTERT-RPE1 cells treated with Mps1i and ± sip62, labeled with DAPI, p62 and CHMP2A; scale bar 5 µm. **(E)** Quantification of CHMP7-positive micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon ± sip62 or ± p62 KO, labeled with DAPI, p62 and CHMP7. Fold changes upon normalization to the relative controls are displayed above. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: siCTR vs. sip62 p = 0.0033, WT vs. p62 KO p = 0.0197. **(F)** Quantification of CHMP4B-positive micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon ± sip62 or ± p62 KO, labeled with DAPI, p62 and CHMP4B. Fold changes upon normalization to the relative controls are displayed above. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: WT vs. p62 KO p = 0.0198. **(G)** Quantification of CHMP2A-positive micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon ± sip62 or ± p62 KO, labeled with DAPI, p62 and CHMP2A. Fold changes upon normalization to the relative controls are displayed above. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: siCTR vs. sip62 p = 0.0146, WT vs. p62 KO p = 0.0149. **(H)** Quantification of CHMP7-positive micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon treatment with SAR405 (SAR) or Baf-A1 (Baf) or Chloroquine (Chlq) (NT not treated), labeled with DAPI, p62 and CHMP7. Fold changes upon normalization to the relative controls are displayed above. At least 100 micronuclei analyzed from at least 3 biological replicates (N = 6, 3, 3, 3). Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test (all vs. NT): NT vs. SAR405 p = 0.0026, NT vs. Baf-A1 p = 0.0334, NT vs. Chloroquine p = 0.0044. **(I)** Quantification of CHMP4B-positive micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon treatment with SAR405 (SAR) or Baf-A1 (Baf) or Chloroquine (Chlq) (NT not treated), labeled with DAPI, p62 and CHMP4B. Fold changes upon normalization to the relative controls are displayed above. At least 100 micronuclei analyzed from at least 3 biological replicates (N = 6, 3, 3, 3). Each data point indicates a biological replicate. Mean ± SEM. Ordinary one-way ANOVA test performed followed by Tukey's multiple comparison test (all vs. NT): NT vs. SAR405 p = 0.0157, NT vs. Baf-A1 p = 0.0363. **(J)** Quantification via immunofluorescence of CHMP7 contained within autolysosomes in fixed hTERT-RPE1 cells treated with Mps1i and Baf-A1 upon ± p62 KO, labeled with DAPI, CHMP7 and LAMP2. From 3 biological replicates. Each colored data point indicates the mean of a biological replicate. Mean ± SEM. Mann-Whitney test performed: p = 0.0020. **(K)** Quantification of p62-positive micronuclei and intact (LSD1-positive) micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon ± NAC treatment (NT not treated), labeled with DAPI, p62 and LSD1. Fold changes upon normalization to the relative controls are displayed above. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p62-pos p = 0.0143, LSD1-pos p = 0.0144. **(L)** Quantification of p62-positive micronuclei and intact (LSD1-positive) micronuclei in fixed MDA-MB-231 cells treated with Mps1i upon ± H_2O_2 treatment (NT not treated), labeled with DAPI, p62 and LSD1. Fold changes upon normalization to the relative controls are

displayed above. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: p62-pos p = 0.0294, LSD1-pos p = 0.0078. **(M)** Reduced and non-reduced western blot analysis of p62 homo-oligomerization in hTERT-RPE1 cells upon ± H_2O_2 treatment, immunolabeled with p62. GAPDH was used as loading control. Images are representative of 3 biological replicates. **(N)** Western blot analysis of expression levels of p62 in wild type (first lane) and in p62 KO hTERT-RPE1 cells (second lane) stably expressing p62 constructs (FLAG-p62-WT and FLAG-p62-CA - third and fourth lane, respectively), immunolabeled with p62 and FLAG. Vinculin was used as loading control. Images are representative of 2 biological replicates. **(O)** Reduced and non-reduced western blot analysis of oxidation-driven p62 homo-oligomerization in hTERT-RPE1 p62 KO cells stably expressing FLAG-p62-WT or FLAG-p62-CA, upon ± H_2O_2 treatment, immunolabeled with p62. GAPDH was used as loading control. Images are representative of 3 biological replicates. **(P)** Representative confocal images showing p62-WT localization on a ruptured (LSD1-negative) micronucleus and p62-CA mutant not localizing to an intact (LSD1-positive) micronucleus in fixed hTERT-RPE1 p62 KO cells treated with Mps1i and stably expressing FLAG-tagged p62-WT or p62-CA mutant, labeled with DAPI, FLAG and LSD1; scale bar 5 μm. **(Q)** Quantification of ruptured (Lamin B-negative) micronuclei spontaneously forming in WT, CHMP7 KO and LEMD2 KO HeLa cells upon ± p62 o.e. and ± H_2O_2 treatment, labeled with DAPI and Lamin B. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: WT H_2O vs. WT H_2O_2 p = 0.0469, WT H_2O vs. WT p62 o.e. H_2O p = 0.0358, WT H_2O vs. WT p62 o.e. H_2O_2 p = 0.0134. **(R)** Quantification of ruptured (Lamin B-negative) micronuclei spontaneously forming in WT and LEMD2 KO HeLa cells upon ± p62 o.e. and ± NAC treatment, labeled with DAPI and Lamin B. At least 100 micronuclei analyzed from 3 biological replicates. Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: WT H_2O vs. WT NAC p = 0.002, WT H_2O vs. WT p62 o.e. H_2O p = 0.0027, WT H_2O vs. WT p62 o.e. NAC p = 0.0004.

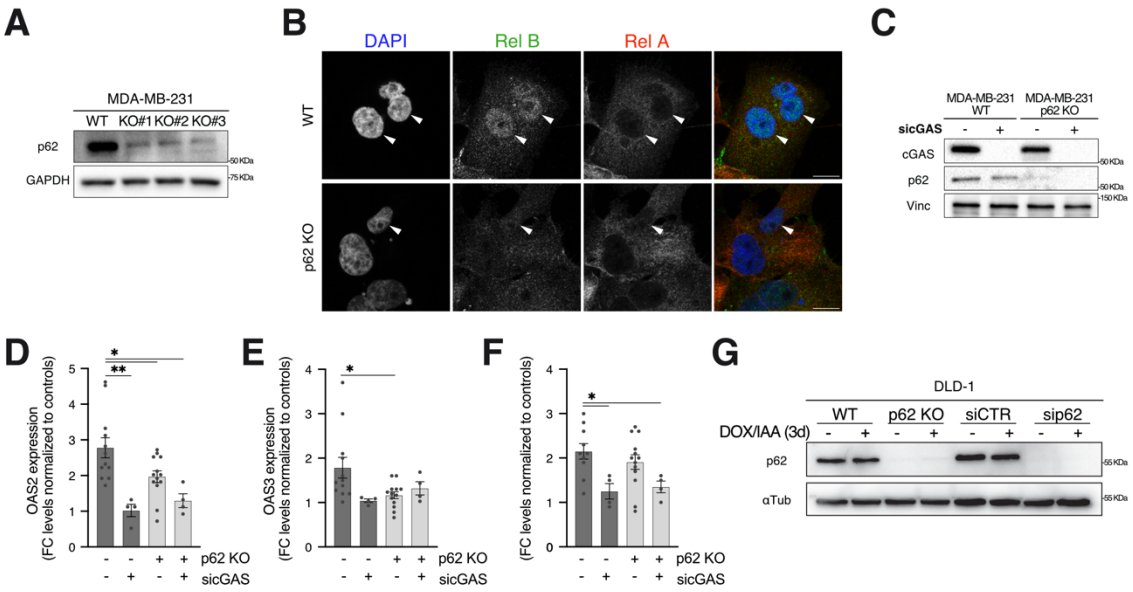

fig. S7. (A) Western blot analysis of p62 protein levels in MDA-MB-231 WT and p62 KO cell lines to demonstrate the efficiency of the sgRNA. MDA-MB-231 p62 KO #2 and #3 cells were selected for further analysis. GAPDH was used as loading control. Images are representative of 3 biological replicates. **(B)** Representative confocal images showing elevated RelB translocation into primary nuclei in WT cells or limited RelB translocation into primary nuclei in p62 KO cells, in fixed MDA-MB-231 cells treated with Mps1i upon ± p62 KO, labeled with DAPI, RelA and RelB. Arrowheads indicate RelB-positive primary nuclei. Scale bar 10 µm. **(C)** Western blot analysis of p62 and cGAS protein levels in MDA-MB-231 WT and p62 KO cell lines showing the efficiency of the siRNA and sgRNA. Vinculin was used as loading control. Images are representative of 3 biological replicates. **(D)** Quantification of *OAS2* gene expression levels in MDA-MB-231 cells treated with Mps1i, ± sicGAS and ± p62 KO (also shown in Fig. 4D), normalized on the respective untreated controls (DMSO). *GAPDH* was used as loading control. From at least 3 biological replicates (N = 12, 4, 13, 4). Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: WT siCTR vs. WT sicGAS p = 0.0036, WT siCTR vs. p62 KO siCTR p = 0.0176, WT siCTR vs. p62 KO sicGAS p = 0.0109. **(E)** Quantification of *OAS3* gene expression levels in MDA-MB-231 cells treated with Mps1i, ± sicGAS and ± p62 KO (also shown in Fig. 4D), normalized on the respective untreated controls (DMSO). *GAPDH* was used as loading control. From at least 3 biological replicates (N = 10, 4, 13, 4). Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: WT siCTR vs. p62 KO siCTR p = 0.0160. **(F)** Quantification of *MX1* gene expression levels in MDA-MB-231 cells treated with Mps1i, ± sicGAS and ± p62 KO (also shown in Fig. 4D), normalized on the respective untreated controls (DMSO). *GAPDH* was used as loading control. From at least 3 biological replicates (N = 10, 4, 13, 4). Each data point indicates a biological replicate. Mean ± SEM. Unpaired t test performed: WT siCTR vs. WT sicGAS p = 0.0118, WT siCTR vs. p62 KO sicGAS p = 0.0191. **(G)** Western blot analysis of p62 protein levels in DLD-1 cells lines to confirm p62 downregulation and depletion via small RNA interference (± sip62) and CRISPR/Cas9-edited p62 knockout (± p62 KO). α-tubulin was used as loading control. Images are representative of 1 biological replicate.

Movie S1. Live cell imaging of p62 recruitment to micronuclei in hTERT-RPE1 cells stably expressing H2B-RFP and p62-GFP. Top: H2B-RFP channel. Middle: p62-GFP channel. Bottom: merge of H2B-RFP signal (red) and p62-GFP signal (green). Still images shown in Fig. S1G. Scale bar 10 μm. Images collected every 15 minutes.

Movie S2. 3D reconstruction of electron tomography analysis of micronucleus-mitochondrion proximity. 3D view of reconstructed tomograms shown in Fig. 6A. 131 images acquired (from −65° to +65°, acquisition every 1°). Mitochondria yellow, NE cyan, ER green.