Supplementary information

Loss of myosin light chain kinase induces the cellular senescence associated secretory phenotype to promote breast epithelial cell migration

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Supplementary Figure 1. Downregulation of MLCK or H_2O_2 treatment induces cellular senescence in MCF10A cells.

Supplementary Figure 2. Downregulation of MLCK decreases cell proliferation and promotes cell migration in HER2-positive breast cancer cells.

Supplementary Figure 3. Downregulation of MLCK increases p53-dependent p21 expression.

Supplementary Figure 4. Source data for Figure 2a and Figure 4c-e

Supplementary Figure 5. Source data for Supplementary Figure 1a and 2a

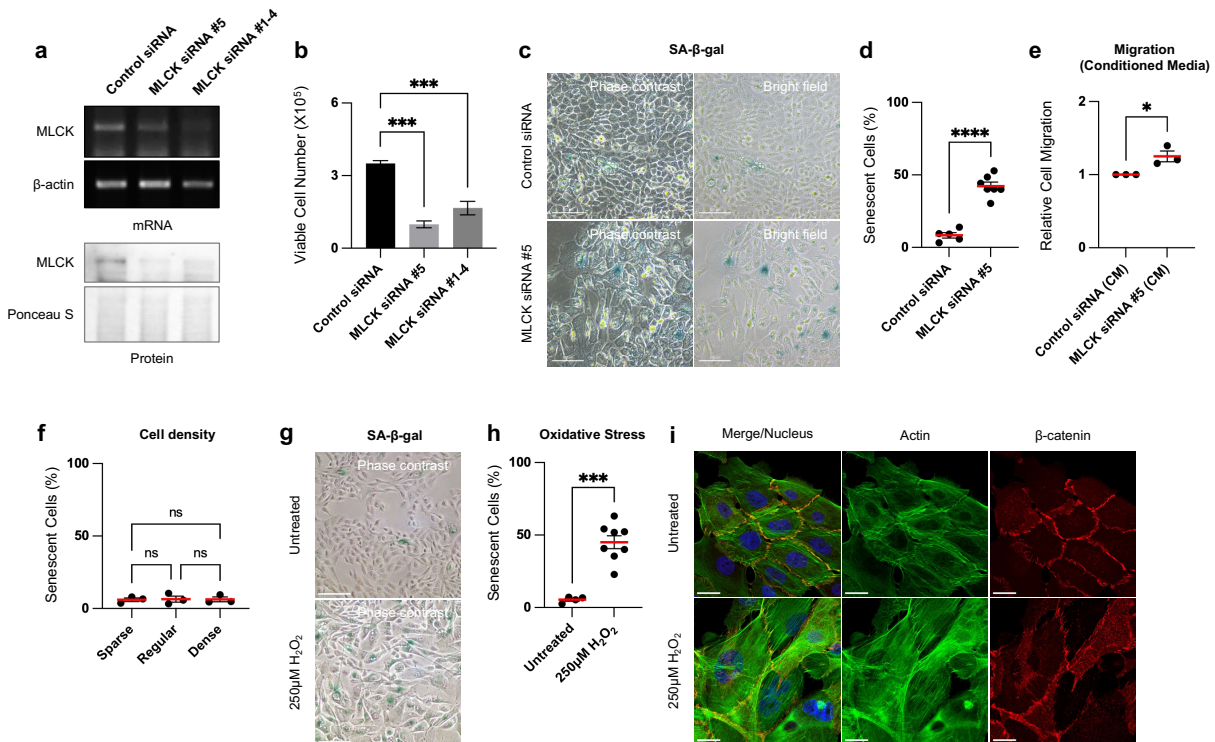**Supplementary Figure 6.** Source data for Supplementary Figure 3

Supplementary Video 1. Scratch wound migration with control siRNA-treated conditioned media.

Supplementary Video 2. Scratch wound migration with MLCK siRNA-treated conditioned media.

Supplementary Table 1. Reverse-phase protein array results comparing control and MLCK-depleted cells.

Supplementary Figure 1

Supplementary Figure 1. Downregulation of MLCK or H_2O_2 treatment induces cellular senescence in MCF10A cells. (a) MLCK mRNA transcript and protein levels 72 hours after treatment with control, single MLCK siRNA (MLCK siRNA #5), or a mixture of four MLCK siRNAs (MLCK siRNA #1-4). β-actin or Ponceau S is shown as a loading control. Original gels/blots are presented in Supplementary Figure 5. (b) MCF10A cells were seeded at identical quantities and counted after 72 hours. Dead cells were excluded using trypan blue staining. n=3 independent experiments. Ordinary one-way ANOVA with Dunnett's multiple comparisons test. (c-d) Representative images and quantification of SA-β-gal staining. Scale bars, 100 μm. n=5-7 fields, 2 independent experiments. Paired *t*-test. (e) Scratch wound migration assays were performed under conditioned media from control or MLCK siRNA #5-treated cells. n=3 independent experiments. Paired *t*-test. (f) Quantification of SA-β-gal positive cells in MCF10A cells at different cell densities. n=3 independent experiments. Ordinary one-way ANOVA with Tukey's multiple comparisons test. (g-h) Representativ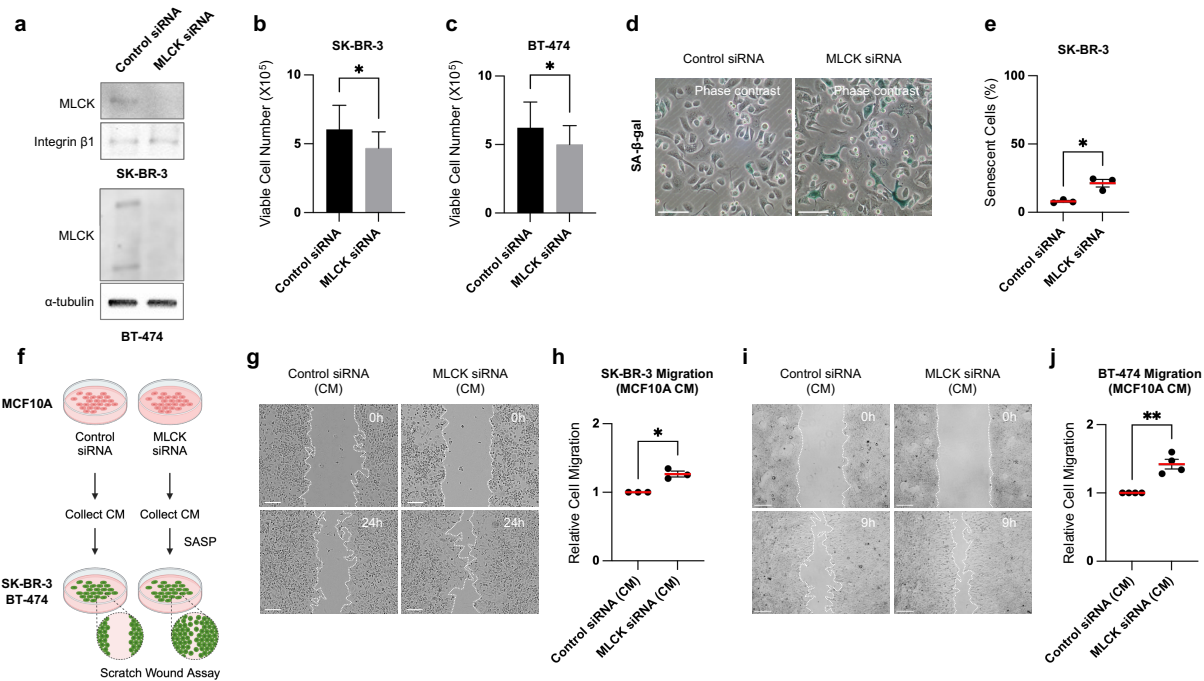e images and quantification of SA-β-gal positive cells after H_2O_2 treatment in MCF10A cells. Scale bars, 100 μm. n=4-10 fields, 2 independent experiments. Unpaired *t*-test. (i) Representative immunofluorescence max projection images showing actin (green), β-catenin (red) and nucleus (blue). Scale bars, 20 μm. mean±s.e.m. *$P<0.05$, ***$P<0.001$, ****$P<0.0001$, ns = not significant.
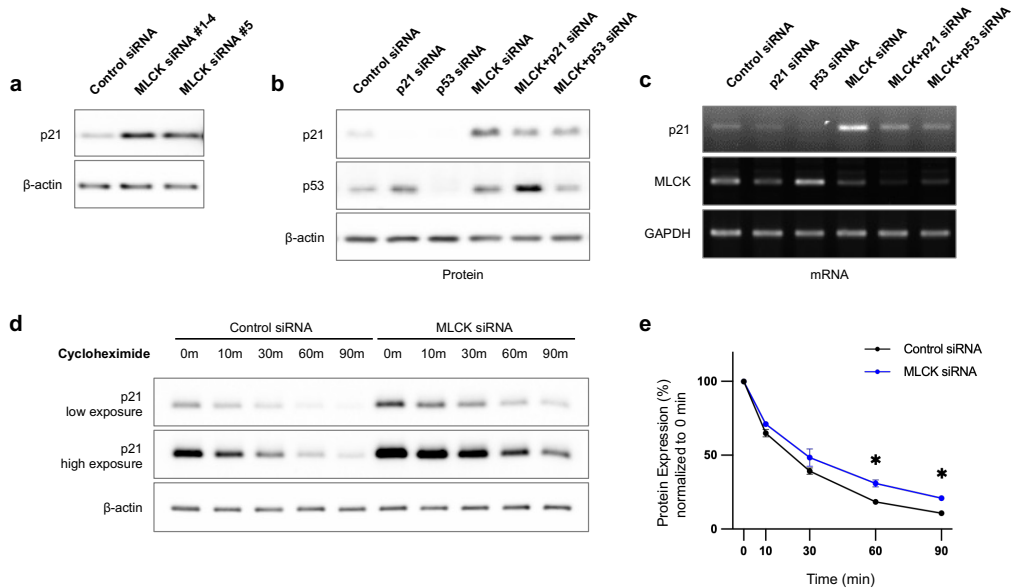
Supplementary Figure 2

Supplementary Figure 2. Downregulation of MLCK decreases cell proliferation and promotes cell migration in HER2-positive breast cancer cells. (a) Western blot analysis for MLCK siRNA expressing SK-BR-3 and BT-474 cells. Integrin β1 or α-tubulin is shown as a loading control. Original blots are presented in Supplementary Figure 5. (b-c) SK-BR-3 and BT-474 cells were seeded at an identical quantity and counted after 72 hr. Dead cells were excluded using trypan blue staining. n=3 independent experiments. Ratio Paired *t*-test. (d-e) Representative images and quantification of SA-β-gal staining in SK-BR-3 cells. Scale bars, 100 μm. n=3 independent experiments. Paired *t*-test. (f-j) Scratch wound migration assays were performed in SK-BR-3 (g, h) and BT-474 (i, j) cells using conditioned media from control or MLCK siRNA-treated MCF10A cells. Scale bars, 200 μm. n=3-4 independent experiments. Paired *t*-test. mean±s.e.m. *P<0.05, **P<0.01

Supplementary Figure 3

Supplementary Figure 3. Downregulation of MLCK increases p53-dependent p21 expression. (a) Western blot analysis of control and MLCK siRNA-treated cells. β-actin is shown as a loading control. (b-c) The level of p21 protein and mRNA transcripts are downregulated after silencing of p53 in MLCK-depleted cells. β-actin or GAPDH is shown as a loading control. (d-e) The 20 µg/ml cycloheximide was used to analyze p21 protein degradation in both control and MLCK-depleted cells, with subsequent quantification of p21 protein expression levels following cycloheximide treatment. β-actin is shown as a loading control. Original gels/blots are presented in Supplementary Figure 6. n=2 independent experiments. Two-way ANOVA with Sidak's multiple comparisons test. mean±s.e.m. *P<0.05
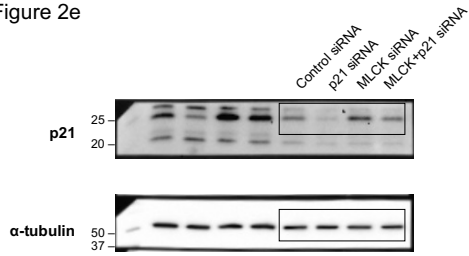
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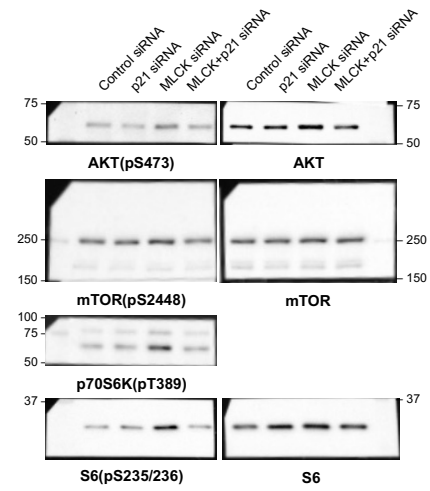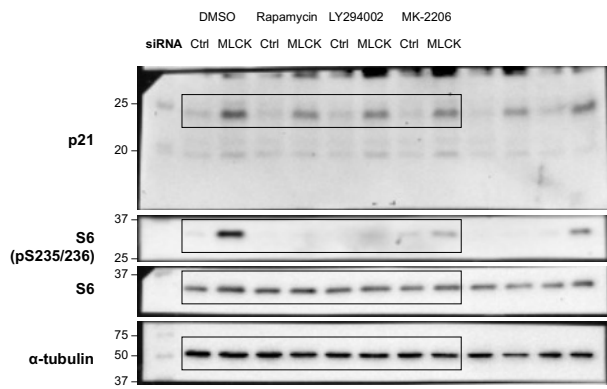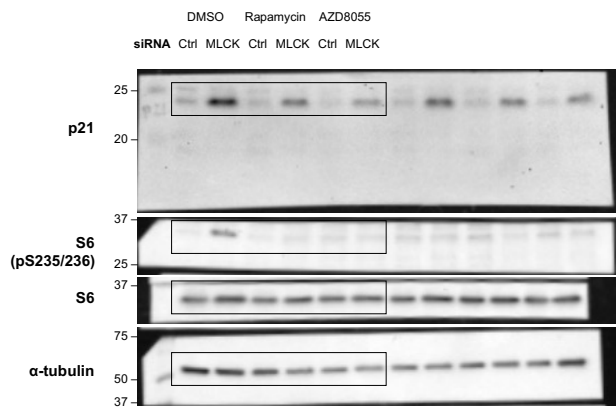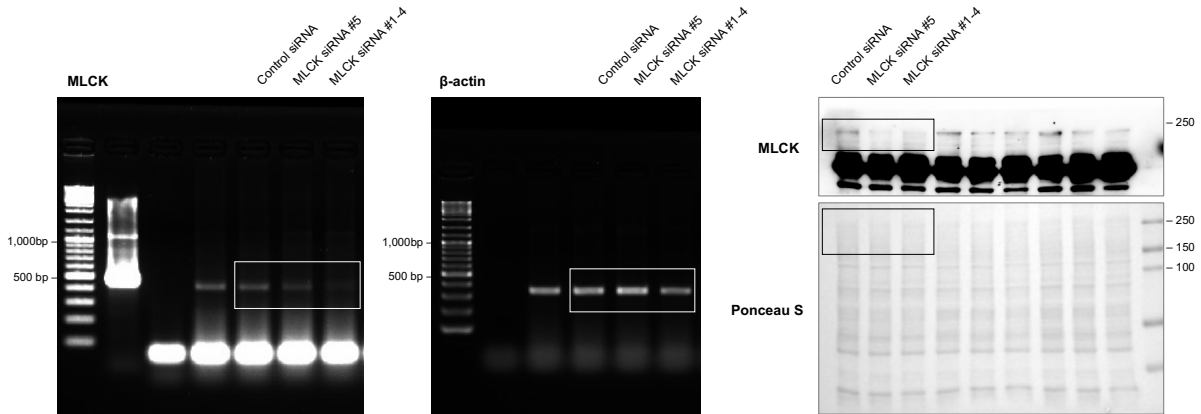
Figure 2e

Figure 4c

Figure 4d

Figure 4e
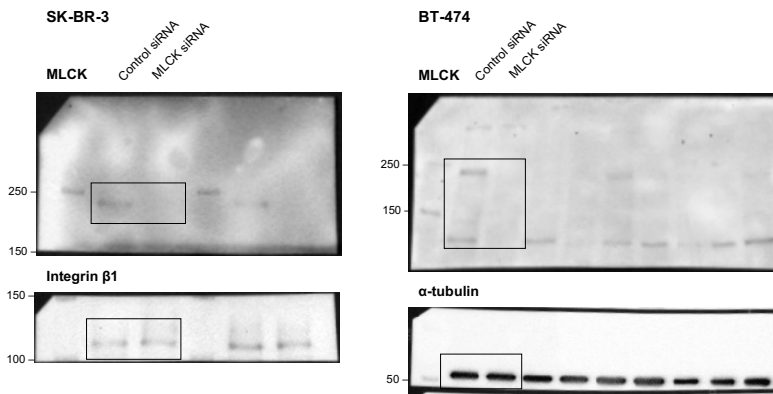
Supplementary Figure 4. Source data for Figure 2a and Figure 4c-e

73 **Supplementary Figure 5**

Supplementary Figure 1a
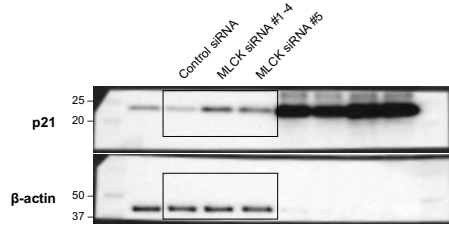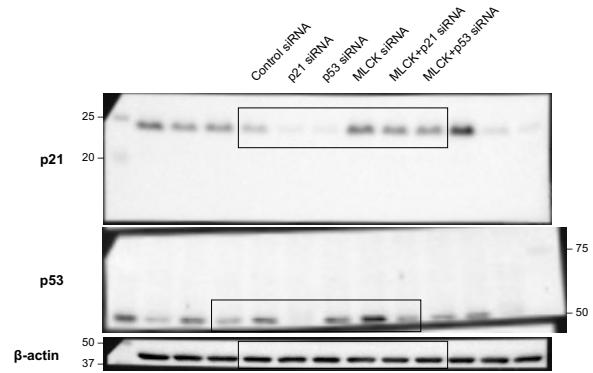

Supplementary Figure 2a

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75 **Supplementary Figure 5. Source data for Supplementary Figure 1a and 2a**
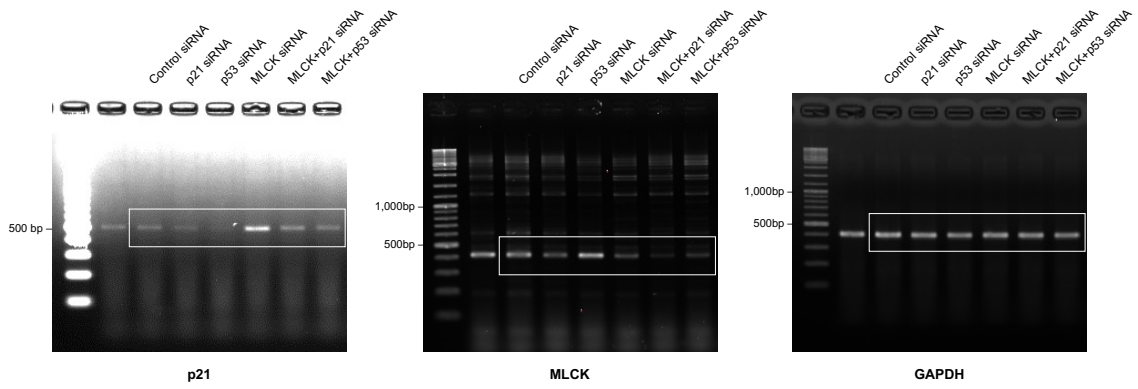
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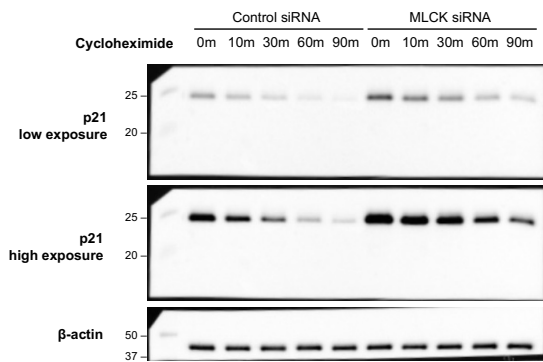
Supplementary Figure 3a

Supplementary Figure 3b

Supplementary Figure 3c

Supplementary Figure 3d

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78   **Supplementary Figure 6. Source data for Supplementary Figure 3**

79   **Supplementary Video 1. Scratch wound migration with control siRNA-treated conditioned**

80   **media.** MCF10A cells were incubated with CM from control siRNA-treated cells. Cells were

81   imaged every 10 minutes for 16 hours. Scale bars, 100 µm.

82

83   **Supplementary Video 2. Scratch wound migration with MLCK siRNA-treated conditioned**

84   **media.** MCF10A cells were incubated with CM from MLCK siRNA-treated cells. Cells were

85   imaged every 10 minutes for 16 hours. Scale bars, 100 µm.

86

87   **Supplementary Table 1. Reverse-phase protein array results comparing control and**
88   **MLCK-depleted MCF10A cells.**