Expanded View Figures

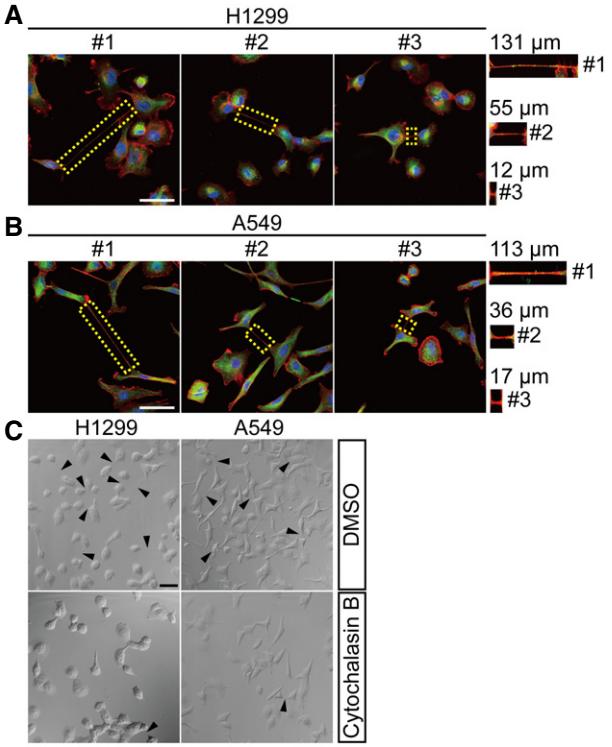

Figure EV1. TNTs of different lengths are formed between lung cancer cells.

A, B H1299 (A) and A549 (B) cells were stained with anti-β-tubulin (green) antibody and phalloidin (red) followed by fluorescent confocal microscopy. The dotted area marked TNTs with their lengths measured. Scale bar: 50 μm.

C H1299 and A549 cells were treated by Cytochalasin B (500 nM) for 24 h. TNT formation was observed by confocal microscopy. Black triangles marked TNTs. Scale bar: 50 μm.

Data information: In (A, B), nuclei were stained with Hoechst 33342 (blue).
Related to Fig 1.

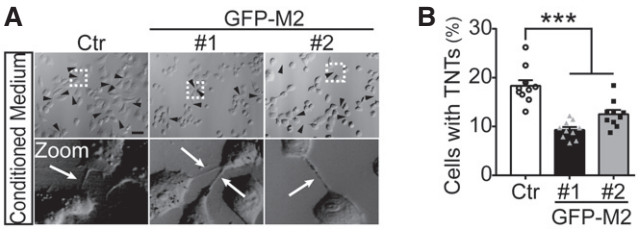

Figure EV2. MICAL2PV expression suppresses TNT formation.

A, B H1299 cells stably expressing the Ctr or GFP-M2 were treated by cultured in conditioned media for 24 h to induce TNT formation (see Methods). TNTs were imaged by confocal microscopy (A), and the percentage of cells forming TNTs was quantified (B). Ten images were captured from Ctr, GFP-M2#1, and GFP-M2#2, respectively, for quantification. Three independent experiments were performed. Scale bar: 60 μm.

Data information: In (A), the black arrowheads (or white arrows) marked TNTs. In (B), data were analyzed using one-way ANOVA with Bonferroni post-test and shown as mean ± SEM; ***$P < 0.001$.
Related to Fig 2.

Source data are available online for this figure.

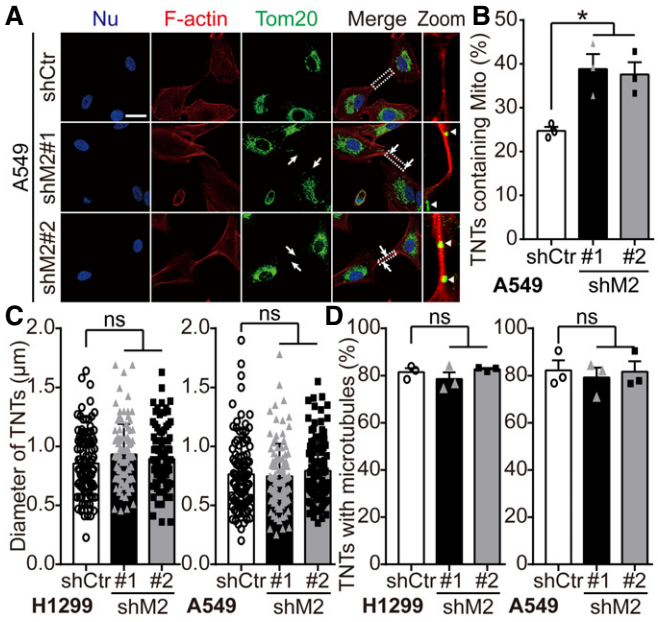

Figure EV3. Down-regulating MICAL2PV increases mitochondria-containing TNTs without affecting the diameter of TNTs nor the percentage of TNTs containing microtubules.

A Control and shM2 A549 cells were stained with anti-Tom20 antibody (green) and phalloidin (red). The white arrowheads marked mitochondria in TNTs. White triangles marked mitochondria in TNTs in zoomed images. Scale bar: 25 μm.

B The percentage of TNTs containing mitochondria was quantified. n = 162, 183, and 177 TNTs in the shCtr, shM2#1, and shM2#2 groups, respectively, from three independent experiments.

C Quantification of diameter of TNTs. One hundred of TNTs in cells stably expressing shCtr or shM2 were measured by ImageJ from three independent experiments.

D More than 50 TNTs in each group were imaged by confocal microscopy with the percentage of TNTs containing microtubules quantified. For shCtr and shM2 H1299 cells, n = 209, 201, and 185 TNTs in the shCtr, shM2#1, and shM2#2 cells, respectively, from three independent experiments. For shCtr and shM2 A549 cells, n = 162, 183, and 177 TNTs in the shCtr, shM2#1, and shM2#2 cells, respectively, from three independent experiments.

Data information: In (A), nuclei were stained with Hoechst 33342 (blue). In (B-D), data were analyzed using one-way ANOVA with Bonferroni post-test and shown as mean ± SEM; *P < 0.05, ns, not significant.
Related to Fig 3.

Source data are available online for this figure.

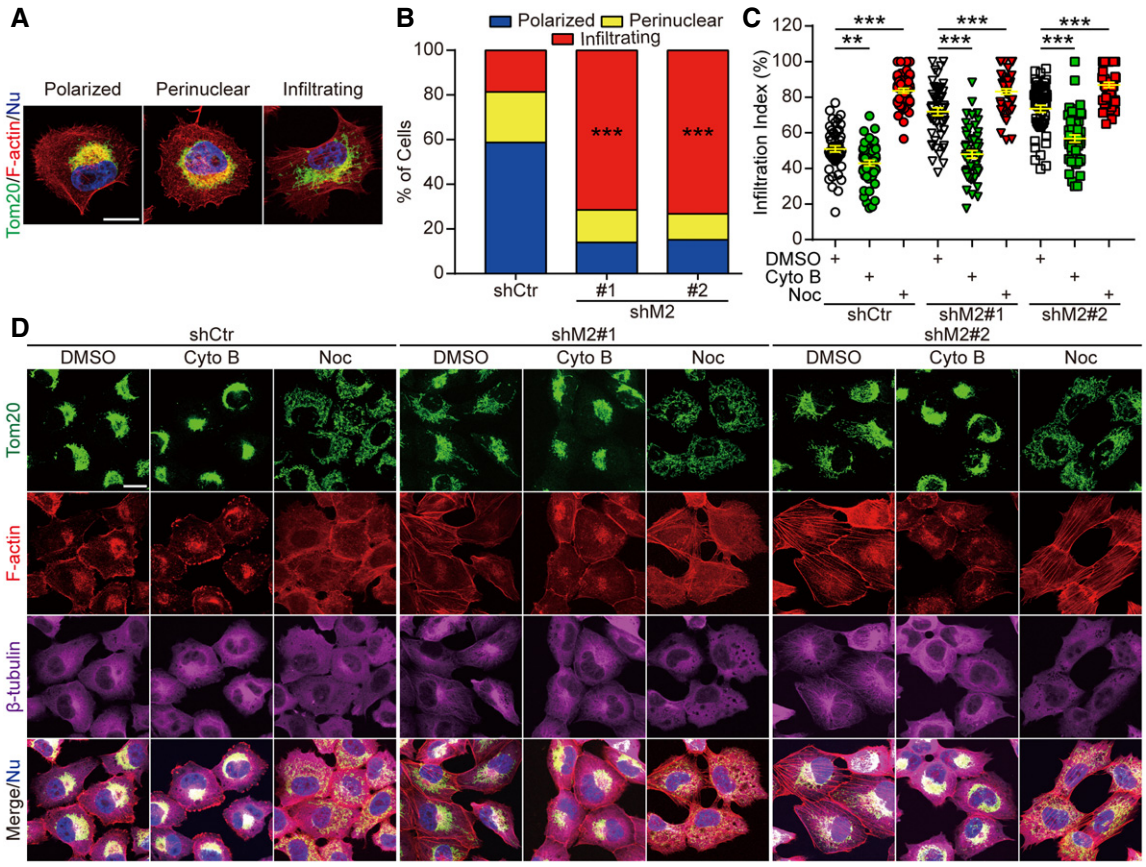

Figure EV4. Down-regulating MICAL2PV enhances mitochondrial infiltration into the cortical cytoskeleton; and F-actin contributes to mitochondrial infiltration.

A Three types of mitochondrial distribution patterns as detected by confocal microscopy. H1299 cells were subjected to immunofluorescent staining using anti-Tom20 antibody (green) and phalloidin (red). Images of different patterns of mitochondrial distribution (polarized, perinuclear, or infiltrating) were shown. Scale bar: 20 μm.

B Down-regulating MICAL2PV significantly increases the proportion of cells with infiltrating mitochondria. Control and shM2 H1299 cells were stained with anti-Tom20 antibody (green) and phalloidin (red). The proportion of cells with different mitochondrial distribution patterns (polarized, perinuclear, or infiltrating) was quantified. n = 437, 337, and 372 cells in shCtr, shM2#1, and shM2#2, respectively, from three independent experiments.

C, D Depolymerizing F-actin inhibits mitochondrial infiltration. Control and shM2 H1299 cells were treated with Cytochalasin B (5 μM, 4 h), Nocodazole (Noc) (5 μM, 1 h), or DMSO and subjected to immunofluorescence with anti-Tom20 antibody (green), anti-β-tubulin antibody (magenta), and phalloidin (red). Images were taken by confocal microscopy (D). Mitochondrial infiltration of index was quantified in each group, and 50 cells were quantified for each group from three independent experiments (C). Scale bar: 20 μm.

Data information: In (A, D), nuclei were stained with Hoechst 33342 (blue). In (B, C), data were analyzed using one-way ANOVA with Bonferroni post-test and shown as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001.
Related to Fig 4.

Source data are available online for this figure.

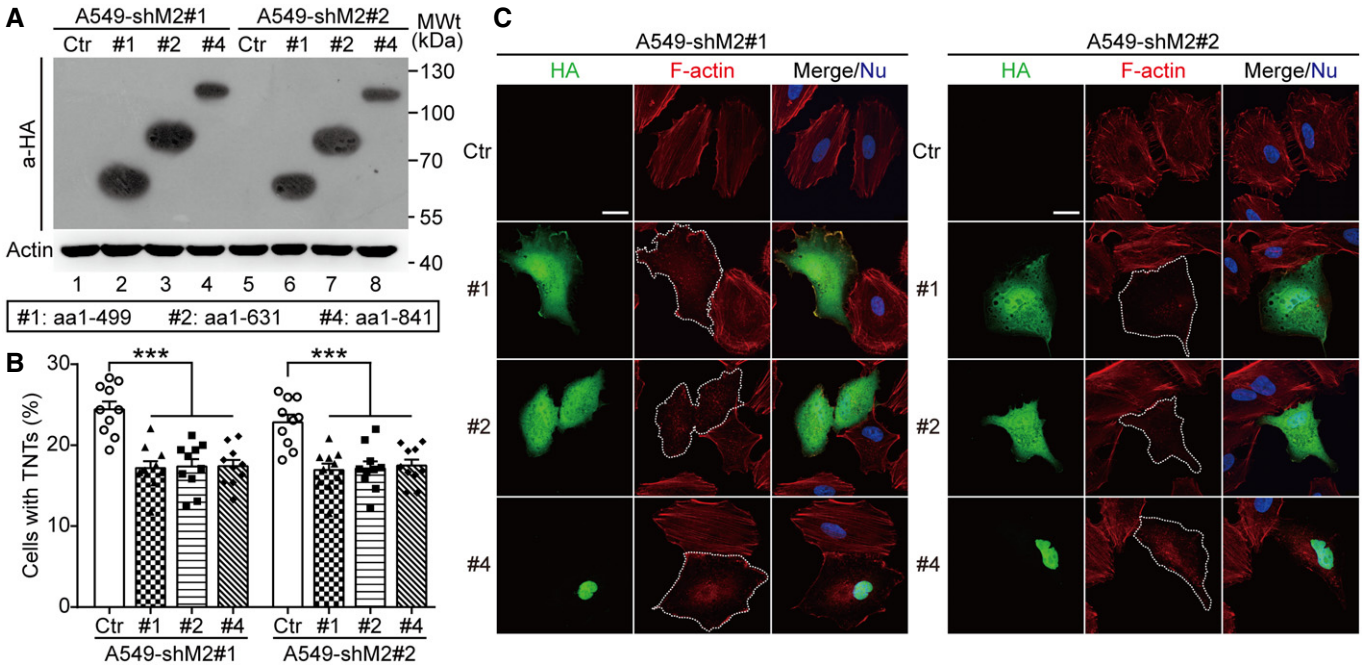

Figure EV5. The monooxygenase (MO) domain of MICAL2PV is required for its activity in actin depolymerization and suppression of TNT formation.

A shM2 A549 cells were transfected with the vector control plasmid (Ctr) or plasmids encoding different MICAL2PV mutants tagged with a hemagglutinin (HA) tag; and expression of HA-tagged MICAL2PV proteins were detected by Western blotting using anti-HA antibody.

B TNT formation were imaged by confocal microscopy in the control cells and cells expressing different MICAL2PV mutants. The percentage of cells forming TNTs was quantified. Ten images were captured for quantification in each group cells. Three independent experiments were conducted.

C MICAL2PV mutants containing MO domain were capable of depolymerizing F-actin. shM2 A549 cells transfected with different MICAL2PV mutants were stained with phalloidin (red) and anti-HA antibody (green) followed by confocal microscopy (dotted lines depicting transfected cells). Scale bar: 20 μm.

Data information: In (C), nuclei were stained with Hoechst 33342 (blue). In (B), data were analyzed using one-way ANOVA with Bonferroni post-test and shown as mean ± SEM; ***$P < 0.001$.

Related to Fig 8.

Source data are available online for this figure.