

An allosteric inhibitor of RhoGAP class-IX myosins suppresses the metastatic features of cancer cells

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DESCRIPTION TO SUPPLEMENTARY DATA

- Supplementary movie legends
- Description of Supplementary Data 1

SUPPLEMENTARY MOVIE LEGENDS

Supplementary Movie 1. Adhibin causes the detachment of A459 and MLE-12 cells. A549 and MLE-12 cells treated with 1% DMSO or 25 μ M adhibin and imaged on glass-bottom dishes using brightfield microscopy and a 60x objective. Time is indicated in hr:min. Scale bar: 20 μ m.

Supplementary Movie 2. Adhibin impairs B16-F1 spreading. Untreated control B16-F1 cells and 1% DMSO or 5 μ M adhibin treated B16-F1 cells, were imaged in phase contrast to monitor spreading on laminin-coated glass-bottom dishes using a 10x objective. Time is indicated in min:sec. Scale bar is 100 μ m.

Supplementary Movie 3. Adhibin impairs B16-F1 random migration. Untreated control B16-F1 cells and 1% DMSO, 5 μ M, or 10 μ M adhibin treated B16-F1 cells, were imaged in DIC to monitor random migration on laminin-coated glass-bottom dishes using a 10x objective. Time is indicated in hr:min. Scale bar is 100 μ m.

Supplementary Movie 4. Adhibin impairs B16-F1 lamellipodia architecture and dynamics. 1% DMSO or 5 μ M adhibin treated B16-F1 cells, were imaged with bright field microscopy to closely resolve the lamellipodium on laminin-coated glass-bottom dishes using a 60x objective. B16-F1 cells were also transfected with mscarlet-LifeAct, treated with 1% DMSO or 5 μ M adhibin and imaged using TIRF-microscopy on laminin-coated glass-bottom dishes using a 60x objective. For FRAP experiments, B16-F1 cells were transfected with EGPF- β -actin, treated with 1% DMSO or 5 μ M adhibin and imaged using confocal microscopy on laminin-coated glass-bottom dishes using a 63x objective. The area was bleached for 0.5ms. Time is indicated in min:sec. Scale bar is 5 μ m. Time is indicated in min:sec. Scale bar: 20 μ m.

Supplementary Movie 5. Adhibin affects Rho localization in B16-F1 cells. B16-F1 cells were transfected with dtomato-rhotekin, treated with 1% DMSO or 5 μ M adhibin and imaged using TIRF-microscopy on laminin-coated glass-bottom dishes using a 60x objective. Time is indicated in min:sec. Scale bar: 20 μ m.

Supplementary Movie 6. Adhibin impairs cell division. B16-F1 cells were treated with 1% DMSO or 5 μ M adhibin and imaged on laminin-coated glass-bottom dishes with DIC microscopy using a 10x objective and with brightfield microscopy using a 60x objective. Time is indicated in hours:min:sec. Scale bar: 20 μ m.

DESCRIPTION OF SUPPLEMENTARY DATA 1 HOMOLOGY MODELS

Homology Model 1. Homology model of *RnMyo9a*.

Homology Model 2. Homology model of *RnMyo9a* in complex with adhibin.

Homology Model 3. Homology model of *RnMyo9b*.

Homology Model 4. Homology model of *RnMyo9b* in complex with adhibin.