# Supplemental Materials
*Molecular Biology of the Cell*

Rao et al.

# SUPPLEMENTARY FIGURE LEGENDS

**A**



**B**



**C**
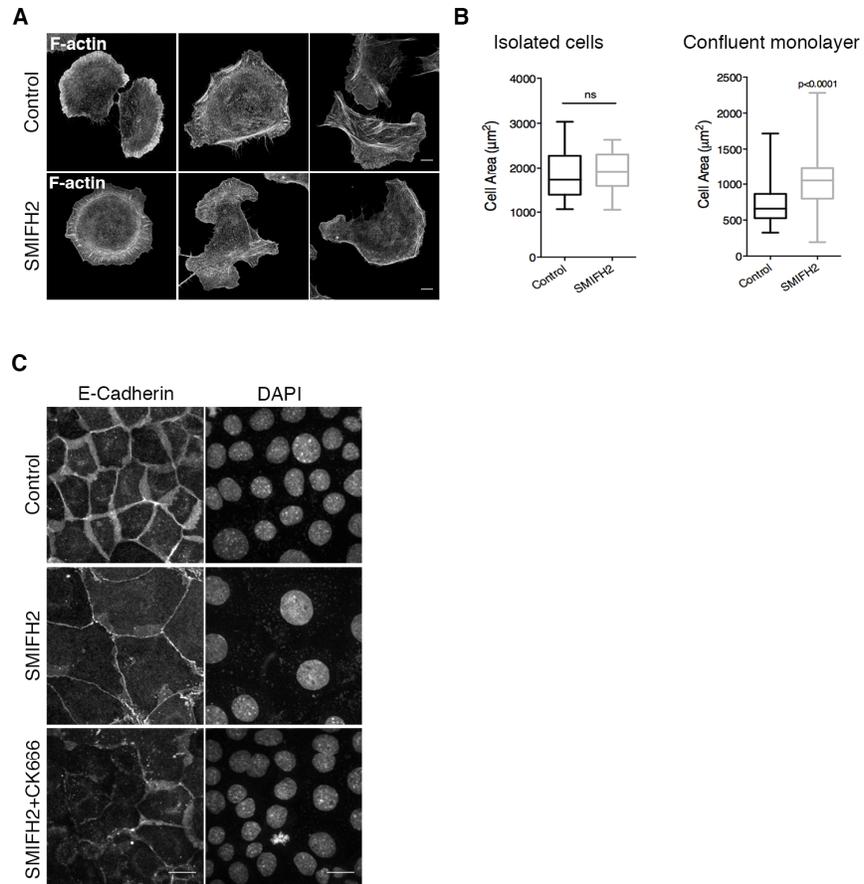
**Supplementary Figure 1**

(**A**) Three examples each of isolated Eph4 cells in control and SMIFH2-treated conditions, labeled to visualize F-actin. (**B**) Quantification of cell area in isolated cells or confluent monolayers post-SMIFH2 treatment. Note that SMIFH2 treatment on isolated cells does not induce excess cell spreading. n=30 isolated cells or 100 cells in a monolayer. (**C**) Double inhibition of formin and Arp2/3 complex activities (SMIFH2+CK666, bottom panel) abrogates cell spreading induced by SMIFH2 (middle panel). Note the reduction in junctional E-cadherin with combined SMIFH2 and CK666 treatment (DAPI labeling shown for reference). Statistical significance assessed using Student's t-test in (**B**). Scale bars, 10μm (**A**); 20μm (**C**).
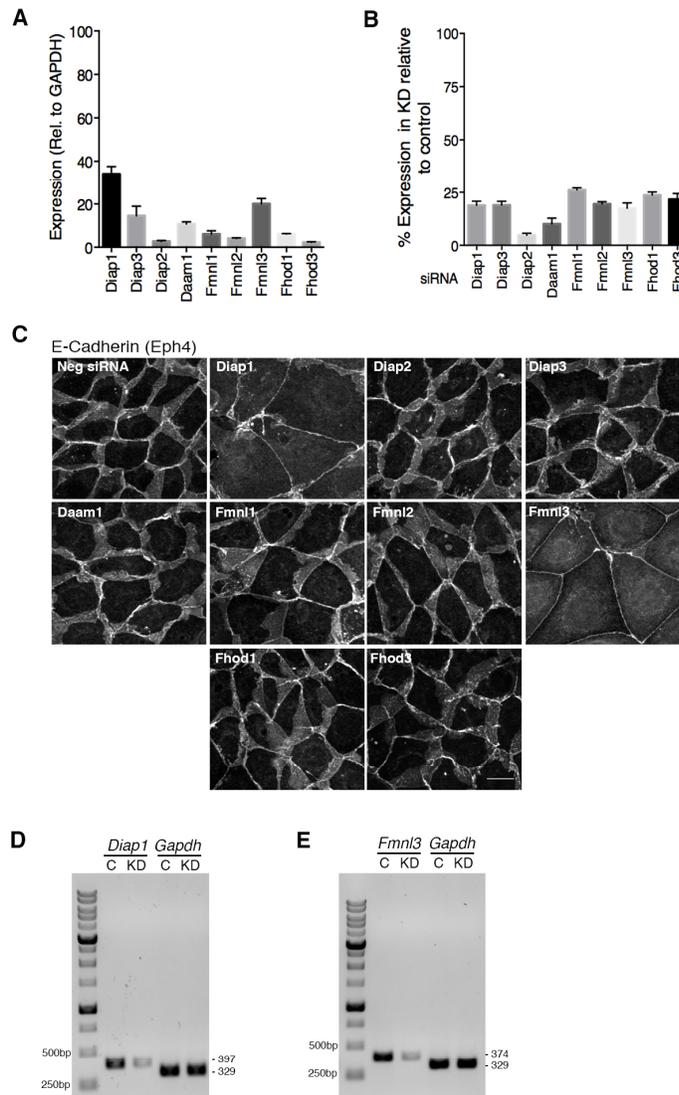
**Supplementary Figure 2**

(**A**) Expression profile of Diaphanous-related family of formins in Eph4 cells. Error bars represent s.e.m., n=2 independent experiments. (**B**) Quantification of KD efficiency for Diaphanous-related family of formins. Error bars represent s.e.m., n=2-3 independent experiments. (**C**) Representative images of AJ morphology visualized by labeling E-cadherin after KD of individual formins as indicated. (**D**) & (**E**)

Representative gel images showing KD efficiency for formins *Diap1* and *Fmnl3* in Eph4, respectively. *Gapdh* was used as a control. C: Non-targeting control siRNA, KD: Knockdown. Scale bar, 20μm (**C**).
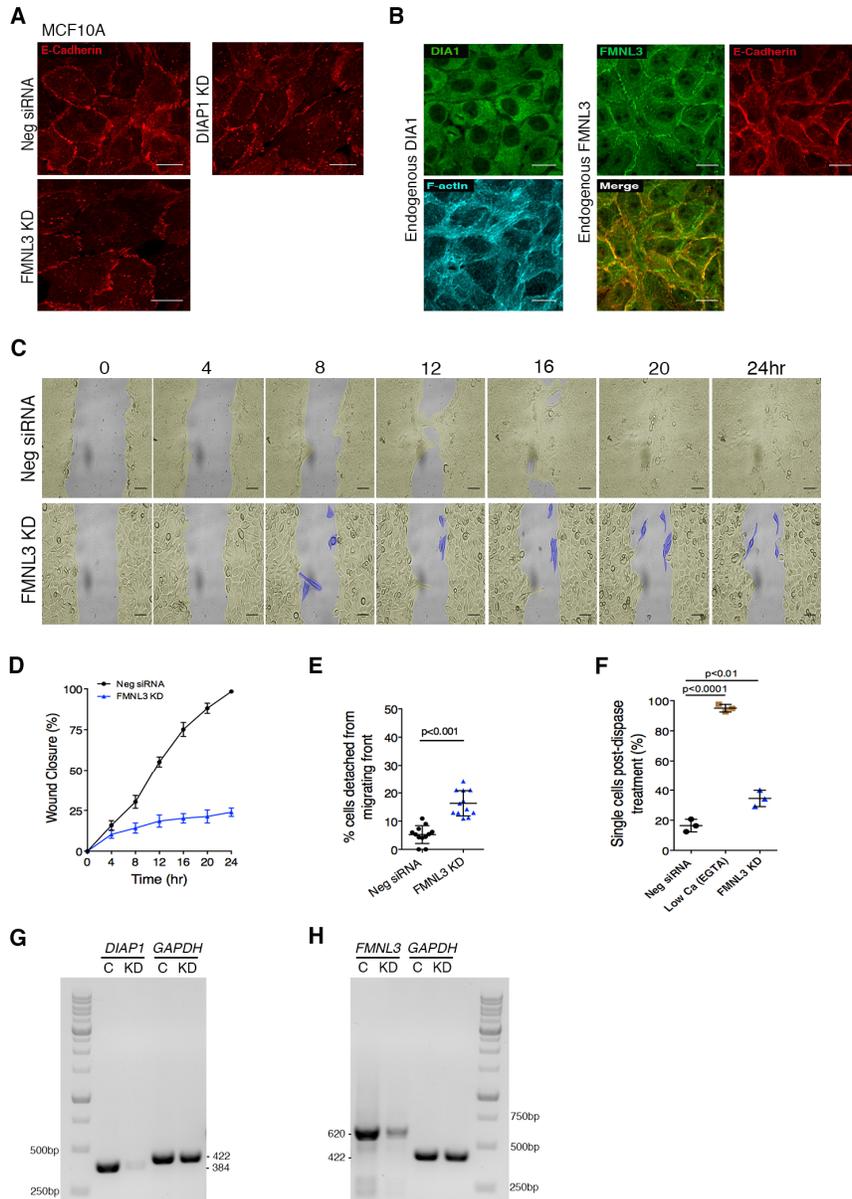
**Supplementary Figure 3**

(**A**) Phenotypes obtained upon siRNA-mediated KD of DIAP1 or FMNL3 in MCF10A monolayers, labeled to visualize E-cadherin; note the reduction in lateral junctions and increased cell area associated with DIAP1 or FMNL3 KD. (**B**) Endogenous DIA1 exhibits diffuse localization in MCF10A cells (left panel), while endogenous FMNL3 localizes at the AJ (right panel), co-localizing with E-cadherin. (**C**) Montage from a movie of an *in vitro* scratch assay in control (Neg siRNA) or FMNL3KDconditions in MCF10A monolayers. Images are pseudo-colored in yellow to indicate cohesive regions of the cell sheet, and in blue (bottom panel) to highlight cells that have detached from the migrating front. Related to Supplementary Movie 5 and 6. (**D**) Quantification of wound closure. n=12 movies for each condition, with mean±s.e.m. (**E**) Quantification of cells detaching from migrating front (%). n=12 movies for each condition, with mean±s.d. (**F**) Dispase assayto determine adhesion strength in MCF10A for conditions tested in (**C**). EGTA treatment served as a low adhesion strength control. Individual dots represent a single experiment (n=3), with mean±s.d. (**G**) & (**H**) Representative gel images showing KD efficiency for formins *DIAP1* and *FMNL3* in MCF10A, respectively. *GAPDH* was used as a control. C: Non-targeting siRNA control, KD: Knockdown. Statistical significance assessed using Student's t-test in (**E**); one-way ANOVA in (**F**). Scale bars, 20μm (**A** and **B**); 50μm (**C**).
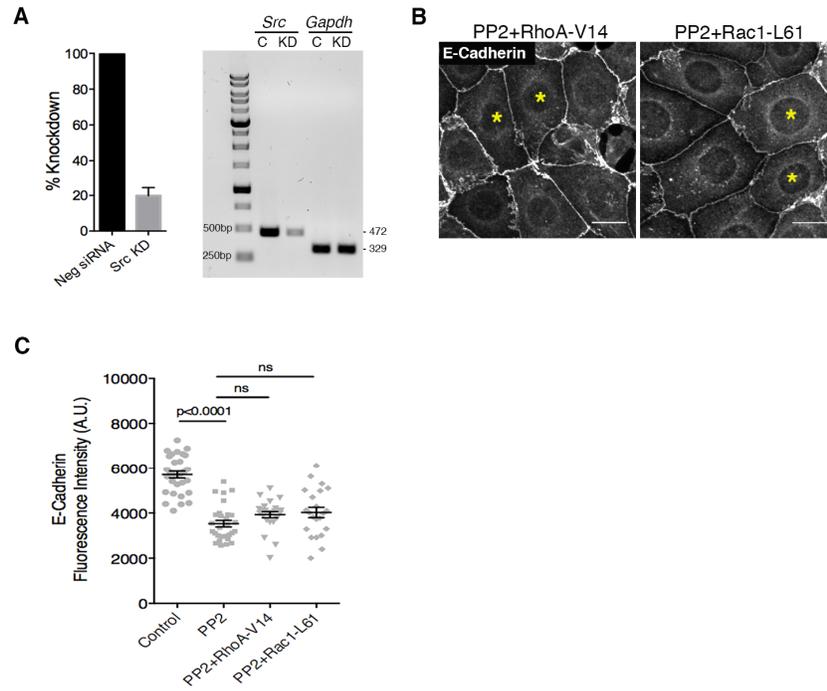
**Supplementary Figure 4**

(**A**) Quantification of KD efficiency for Src kinase. Error bar represents s.e.m., n=3 independent experiments. Representative gel image showing KD of *Src* is provided. *Gapdh* was used as a control. C: Non-targeting siRNA control, KD: Knockdown. (**B**) Expression of constitutively active RhoA-V14 or Rac1-L61 does not rescue Src inhibition phenotype (transfected cells marked with yellow asterisks). (**C**)

Quantification of E-cadherin intensity at the AJ for (**B**). Control cells were transfected with EGFP. n=25-30 cells from 3 experiments. Statistical significance assessed using one-way ANOVA in (**C**). Scale bar, 20μm (**B**).
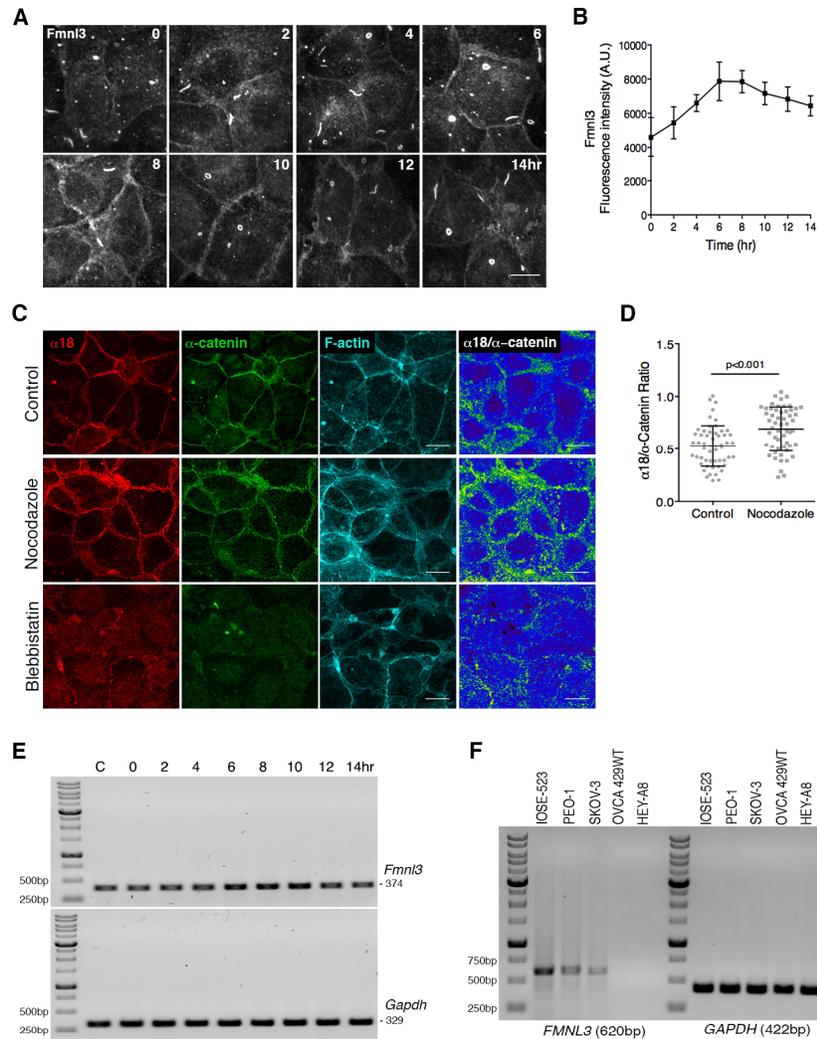


**Supplementary Figure 5**

(**A**) Immuno-labeling for endogenous Fmnl3 during the process of wound closure over 14hr. Cells depicted here were located 4-5 cells rows behind the wound edge. Note the increased localization of Fmnl3 at the AJ between 6hr-10hr time points. (**B**) Quantification of Fmnl3 fluorescence intensity for (**A**). Error bars represent s.e.m., n=2 independent experiments. (**C**) Representative images of Eph4 monolayers labeled to visualize α-catenin α18, α-catenin and F-actin following treatment with nocodazole or blebbistatin. α18/α-catenin ratio images illustrate the increase or decrease in force-dependent stretching of α-catenin at cell-cell junctions. (**D**) Quantification of α18/α-catenin intensity ratio for (**C**). n>50 junctions for each condition tested, with mean±s.e.m. (**E**) Uncropped gel image, related to Figure 6B. (**F**) Uncropped gel image, related to Figure 6F. Statistical significance assessed using Student's t-test in (**D**). Scale bars, 20µm (**A** and **C**).

**Supplementary Table 1**

**ON-TARGET plus siRNA oligonucleotides used for gene knockdown**

| S.No | Gene Symbol | Species | Gene ID | Gene Accession | Sequence |
|------|-------------|---------|---------|----------------|----------|
| 1. | *Diap1* | Mouse | 13367 | NM_007858 | AGGUCGGGCUUGCGGGAUA GGGAGAUGGUGUCGCAAUA UCACACUGCUGGUCGGAAA UGGAUGAGGUCGAACGCUU |
| 2. | *Diap3* | Mouse | 56419 | NM_019670 | CAUAAAUGCUCUCGUUACA GUGCAUUGUCGGCGAGGAA CAGGAUAGCGAAAGAGCGA GUGGAAGGCCUCCGGCAUA |
| 3. | *Diap2* | Mouse | 54004 | NM_017398 | CCGCAUGCCAUACGAGGAA GAUGAGAAAUACCGGGAUA GCAAUAUGUUGAAGCUCUA CCUAGAUGCUUGUGUAAAU |
| 4. | *Daam1* | Mouse | 208846 | NM_172464 | AGCGAAGAGUUGCGGGAUA CAGGAGAGGUGUUCGACAA GAUGAAAUCAAGCGGGCAA GCCCAAAGUAGAAGCGAUU |
| 5. | *Fhod1* | Mouse | 234686 | NM_177699 | CUACAUACCGUGAGCGCAA GUAUCGGACUUGUCGGGAA UCGCAUGAUUACCGAGACA UGAGAGUGCCCUUCGGUUA |
| 6. | *Fhod3* | Mouse | 225288 | NM_175276 | GGAACAAAUUCAACCGGGA GCAGAGGAUAGAACGGGAA GAGCCGAGGCGGAUCAGAA CGGCAAGAGAGAGAGGAAA |
| 7. | *Fmnl1* | Mouse | 57778 | NM_019679 | GGGUUUAGGAGGCGAGUUC UUACACAGGUGCUGCGGGA AGAGAGAGUUUGUGCGGCA CCUACAAGAAAGCGGAACA |
| 8. | *Fmnl2* | Mouse | 71409 | NM_172409.2 | UGUUAAUGGUGCCGAAAUA GGACUUAAAUGUGGACGAA CAAAGUCGACAGACCGAAA GCGGAGAAAAGCAGCGUUU |
| 9. | *Fmnl3* | Mouse | 22379 | NM_011711 | GUAAAGAACUGCAUCGGUU |

| 10. | *Src* | Mouse | 20779 | NM_001025395 | GCACGGGACAGACCGGUUA<br>GGGAGCGGCUGCAGAUUGU<br>UCAGAUCGCUUCAGGCAUG<br>GCUCGUGGCUUACUACUCC |
|---|---|---|---|---|---|
| 11. | *DIAP1* | Human | 1729 | NM_005219.4 | GAAGUGAACUGAUGCGUUU<br>GAAGUUGUCUGUUGAAGAA<br>GAUAUGAGAGUGCAACUAA<br>GCGAGCAAGUGGAGAAUAU |
| 12. | *FMNL3* | Human | 91010 | NM_175736.4 | AAGAACAGCUGGAGCGAUA<br>CCUCAUUACUUACGAGAGA<br>AGGUAAAGCUGCUGCGGCA<br>GCAUGGUGGUCUUGGCUAU |
| 13. | Neg siRNA | Mouse/<br>Human | - | - | UGGUUUACAUGUCGACUAA<br>UGGUUUACAUGUUGUGUGA<br>UGGUUUACAUGUUUUCUGA<br>UGGUUUACAUGUUUUCCUA |

## Supplementary Table 2

## Gene-specific primers for semi-quantitative PCR analysis

| S.No | Gene | Species | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|---|---|
| 1. | *Diap1* | Mouse | GGCCTAAATGGTCAAGGAGATAG | CAGAGGTGACAGCAGTGAAA |
| 2. | *Diap3* | Mouse | GTGGACGATTTGGCACATTTAG | CTCTTTCTCTGCTCGCTCTTT |
| 3. | *Diap2* | Mouse | CGCCATCTGAAGACAGGATAAT | CCGATAGGAGGAAGTGAAGAAAG |
| 4. | *Daam1* | Mouse | CAGGCAGAGAAGATGAGGAAAG | GAACTCCTGCTGTCTTTGGTAG |
| 5. | *Fhod1* | Mouse | TCTCCCTTCCTGTCATCTCTATC | CCTTGGCTCTGGACTCAAATAG |
| 6. | *Fhod3* | Mouse | GTTCCTTCTCACACTCTCTTCC | GCGTCTCTCCATCTGACATAAA |
| 7. | *Fmnl1* | Mouse | CAGCCTATGGTTTCCGACTT | GGGATGTGGTCTTGGGATTT |
| 8. | *Fmnl2* | Mouse | CCGTGTTCTTCCCTGTCTTT | CTCGTCTGTAGGGTTGGTTTC |
| 9. | *Fmnl3* | Mouse | AAATACCCGGAACTGGCTAAC | CGCATTACCTCCTCTTGTTTCT |
| 10. | *Src* | Mouse | CTCGTGGCTTACTACTCCAAAC | CATAGTTCATCCGCTCCACATAG |
| 11. | *Gapdh* | Mouse | AACAGCAACTCCCACTCTTC | TGGGTGCAGCGAACTTTAT |
| 12. | *DIAP1* | Human | CTCTCCTGGCTGTGGTTATTT | CACCTCCCATTTCCTTGTAGAC |
| 13. | *FMNL3* | Human | CAAGAAGCAGGAGGAGGTAATG | CTACAGACCTAGTGCCCATAGT |
| 14. | *GAPDH* | Human | GGTCGGAGTCAACGGATTT | TCTTGAGGCTGTTGTCATACTT |