

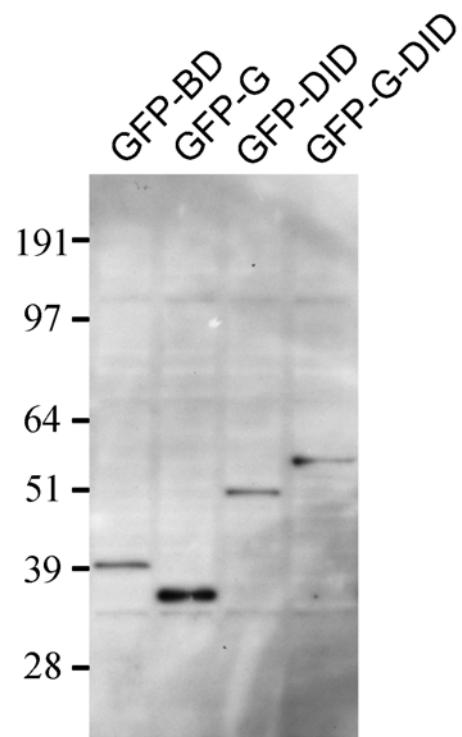
Supplemental Figure 1

| | | | |
|-------|---|-----|-----|
| mDia2 | MERHRARALGRDSKSSRRKGLQSAPPAGPYEPGEKRPKLHLNIRTLTDDMLDKFASIRIP | 60 | |
| mDia1 | MEPSGG-GLG-PGRGTRDKKGRSPDELPAATGGDGGK-----HKKFLERFTSMRIK | 49 | |
| | ** . . ** . : * * : * * : * * : * * : * : * : * : * : * : | | |
| mDia2 | GSKKERPPLPHLKTVSGISDSSSLSSETMENNPKALPESEVLKLFEKMMEDMLNEDKKA | 120 | G |
| mDia1 | -KEKEKPNSAHRN-----SSASYGDDPTAQSLQDISDEQLVLFEQMLVDMNLNEEKQQ | 102 | |
| | . : * : * . * : . * : * . . : . : . : . : * : * : * : * : * : * : * : | | |
| mDia2 | PLREKDFGIKKEMVMQYINTASKTGSRLSSRQISPQEFLHELKMGYTDERLFTYLESLRV | 180 | DID |
| mDia1 | PLREKDIVIKREMVSQYLHTS-KAGMNQKESSRSAMMYIQELRSGLRDMHLLSCLESLRV | 161 | |
| | *****: * : * : * : * : * : * : . . * . : : * : * * : * : * : * : * : * : | | |
| mDia2 | SLTSHPVSVQSFHGEGLLLDILEKLING--QIQEKKVKTQHKVIQCLRALMNTQYG | 238 | |
| mDia1 | SLNNNPVSWVQTFGAEGLASLLDILKRLHDEKEETSGNYDSRNQHEIIIRCLKAFMNNKFG | 221 | |
| | * * . : * * : * * . * * : * : * : . : . : . : * : * : * : * : * : * : * : | | |
| mDia2 | LERIMSDKRSLSLAKAMDPRQPAMMADVVKLLSAVCIVGE-ESILEEVLEALTSAGEER | 297 | |
| mDia1 | IKTMLETEEGILLVRAMDPAVPNMMDAAKLLSALCILPQPEDMNERVLEAMTERAEMD | 281 | |
| | : : : . . : . : * : * : * : * : * : . . * : * : * : * : * : * : * : * . * | | |
| mDia2 | KIDRFFSIVEGLRHNVSVNLQVACMQLINALVTSPDDLFRLHLRNEFMRCGLKEILPNL | 356 | |
| mDia1 | EVERFQPLLDGLKSGTSIALKGCLQLINALITPAEELDFRVHIRSELMRGLHQVLQEL | 341 | |
| | : : : * . : : * : . * : * : * : * : * : * : * : * : * : * : * : * : * : * : | | |
| mDia2 | KGIKNDGLDIQLKVFDEHKEEDLSEFFHRLEDIRAELEASDVYSMLWDTVKETRAEGHF | 416 | DD |
| mDia1 | REIENEDMKVQLCVFDEQGDEDFFDLKGRLDIRMEMDDFGEVFQIIILNTVKDKSAEPHF | 401 | |
| | : * : * . : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : | | |
| mDia2 | LSILQHLLLIRNDRFIREQYFKLIDECVSQIVLHRDGTDPDFTYRKRLDLDLSQFVDVC1 | 476 | CC |
| mDia1 | LSILQHLLLVRNDYEARPQYYKLIIECVSQIVLHKNGTDPDFKCR-HLQIDIERLVDQMI | 460 | |
| | * * * * * : * * : * * : * * : * * : * : * : * : * : * : * : * : * : * : | | |
| mDia2 | DQAKLDEEEKASEHCKFEKECTDHQETQAQLQKRE---AKINELQAEQAFKSQFG- | 531 | FH1 |
| mDia1 | DKTKVEKSEAKATELEKLDSELTARHELQVEMKKMENDFEQKLQDQGEKDALDSEKQQ | 520 | |
| | * : : * : : * * : * : * : * : * : * : * : * : * : * : * : * : * : * : | | |
| mDia2 | -----ITAQKQDLEAEVSKLTGEVAKLSKELEDAKNEMASLSAVVVAPSVSSAAVPPAPPLPGD | 580 | |
| mDia1 | | | |
| mDia2 | -----ALPPGTKIPLQPSVEG-----EAGPSALPPAP- | 558 | |
| mDia1 | SGTVI PPPPPPPLPGGVVPPSPPLPGTCI PPPPLPGGACI PPPPLPGSAAI PPPPP | 640 | |
| | . * * * * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : | | |
| mDia2 | -----PALSGG--VPPPPPPP-----PPPPPLP-----GMPMP- | 585 | |
| mDia1 | LPGVASI PPPPLPGATAI PPPPLPGATAI PPPPLPGGTGIPPPPPLPGSGVGVPPP | 700 | |
| | * . * . : * : * * * * : * : * : * : * : * : * : * : * : * : * : | | |
| mDia2 | --FGGPVPPPPPLGFLGGQSSIPLN-----LPFGLKEKKEFKPEI | 623 | FH2 |
| mDia1 | PLPGGPGLPPPPPFPGAPGIPPPPPGMGVPPPPFGFGVPAAPVLPFGLTPKKVYKPEV | 760 | |
| | *** * * * * * : * * : * : * : * : * : * : * : * : * : * : * : * : | | |

| | | |
|-------|---|----------|
| mDia2 | SMRRLNWLKIGPNESENCFWIKVNENKYENRDLLCKLENTFCCQEKEKRNTNDFD----- | 679 |
| mDia1 | QLRRPNWSKFVAEDLSQDCFWTKVKEFRFENNELFAKLTAFSAQTKTSKAKKDQEGGEE | 820 |
| | ..:*** * * : .::: * : *** * * : * : * : * .: .: * : | |
| mDia2 | EKKVIKKRMKELKFLDPKIAQNLSIFLSSFRVPYEKIRTMILEVDETQLSESMIQNLIKH | 739 |
| mDia1 | KKSVQKKVKELKVLDKTAQNLSIFLGSFRMPYQEIKNVILEVNEAVLTESMIQNLIKQ | 880 |
| | :* . * * : * * * . * . * * * * * * : * : * : * : * : * : * : * : | |
| mDia2 | LPDDEQLKSLSQFRSDYNLSCEPEQFAVVMSNVKRLRPLSAILFKLQFEEQVNNIKPDI | 799 |
| mDia1 | MPEPEQLKMLSELKEYYDDLAESEQFGVVMGTVPRLRPLRNAILFKLQFSEQVENIKPEI | 940 |
| | :* : * * * * : * : * : * . * . * * . * * * * * * * * : * : * : | |
| mDia2 | MAVSTACEEIKKSKGFSKLLELVLMGNYMAGSRNAQTFGFDLSSLCKLKDTKSADQKT | 859 |
| mDia1 | VSVTAACEELRKSENFSLLLETLVGNYMAGSRNAGAFGNISFLCKLRDTKSADQKM | 1000 |
| | : : * : * * * : * : * . * * . * : * : * : * : * : * : * : * : | |
| mDia2 | TLLHFLVDVCEEKHADILHFVDDLALDKASRVSVEMLEKNVKQMGRLQQLEKNLETFP | 919 |
| mDia1 | TLLHFLAELCENDHPEVLKFDPDELAHVEKASRVSAENLQSKLDQMKKQIADVERDVQNFP | 1060 |
| | * * * * . : * : * . : * : * : * : * : * : * : * : * : * : * : * : | |
| mDia2 | PPEDLHDKFVIMKSSFVISANEQYEKLSTLLGSMTQLYQSIMGYYAVDMKKVSVEEFFND | 979 |
| mDia1 | AATDEKDKFVEKMTSFVKDAQEYQYNKLRRMMHSNMETLYKELGDYFVFDPKLVEEFFMD | 1120 |
| | . . * : * * * * : * : * : * : * : * : * : * : * : * : * : * : * : | |
| mDia2 | LNNFRTSFMLALKENIKKREAAEKEKRARIAKERLERQQEKKRLLEMKTEGDETG | 1039 DAD |
| mDia1 | LHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKAERLEKQQKREQLIDMNAEGDETG | 1180 |
| | * : * * . * : * : * * : * : * : * : * : * : * : * : * : * : * : * : | |
| mDia2 | VMDSLLEALQSGAAFRDRRKRTPKLDIRQSLSPLMSQRPVLKVCNHENQKMQLTEGSRPH | 1099 |
| mDia1 | VMDSLLEALQSGAAFR-RKRGPRQVNRKAGCAVTS----- | 1214 |
| | * : * * * * * * * * * : * : * : * : * : * : * : | |
| mDia2 | HSINCNSTRTPVAKELNYNLDTHASTGRIKAVEKEACNAESNKKEMELLGSVAKSESVP | 1159 |
| mDia1 | -----LLASELTK--DDAMAPGPVKVPKKSEG-VPTILEEAKELVGGRAS----- | 1255 |
| | : * . * . * : * : * : * . : * . : * : * : * : * : * : * : | |
| mDia2 | EVEALLARLRAL 1171 | |
| mDia1 | ----- | |

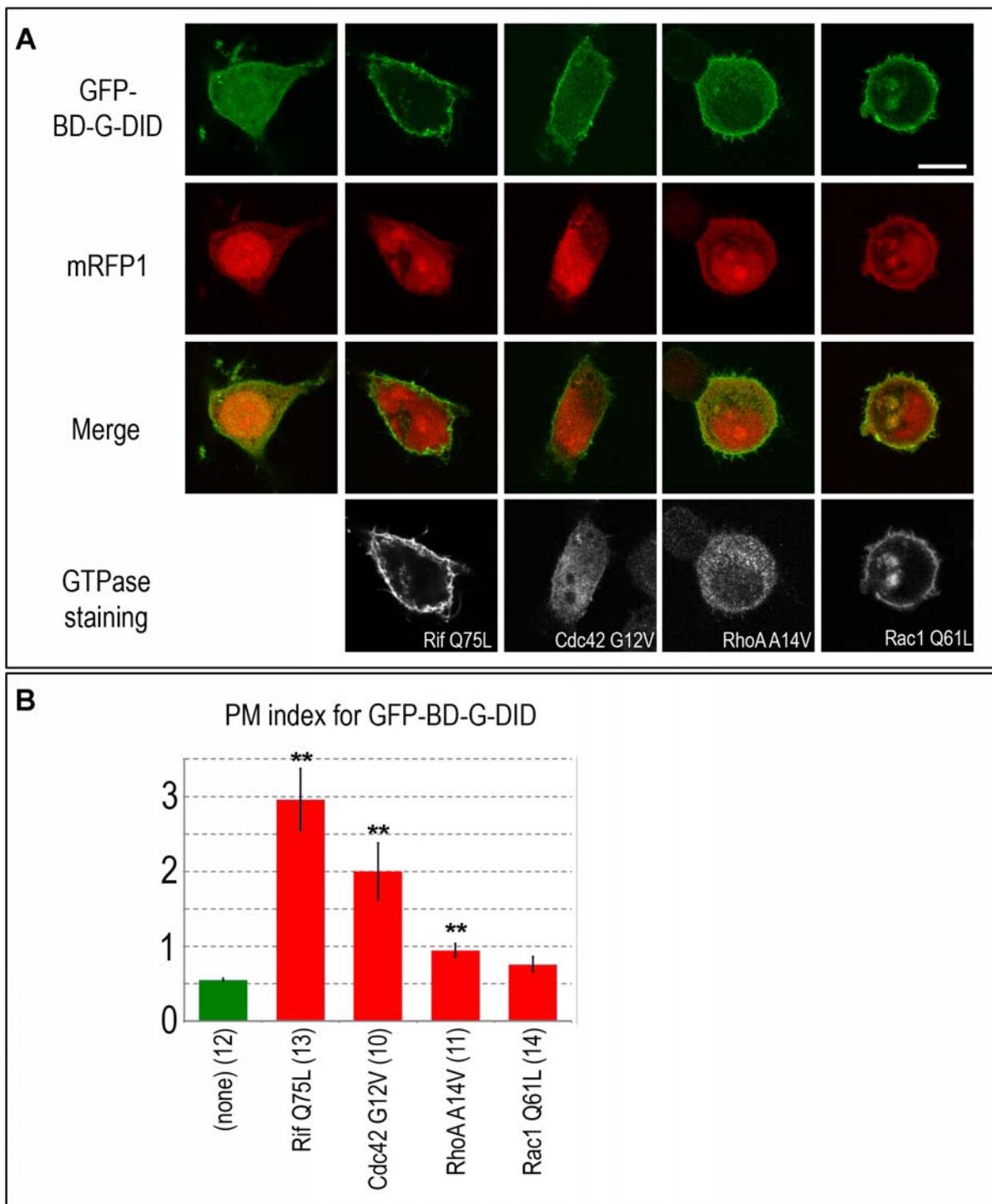
Supplemental Figure 1. Sequence alignment of mDia2 and mDia1 generated with Clustal W (1.83). The basic domain (BD) sequences at the N-terminus display colored font to differentiate between polar (green), non-polar (red), basic (blue), and acidic (magenta) amino acids. The subsequent domains are G (highlighted in cyan), DID (green), DD (yellow), CC (red), FH1 (magenta), FH2 (brown), DAD (violet).

Supplemental Figure 2



Supplemental Figure 2. Cell lysates of HeLa cells expressing indicated GFP-mDia2 fusion proteins are separated by SDS-PAGE and probed with GFP antibody. No degradation is observed. Molecular weight ladder in kDa is shown to the left of the blot.

Supplemental Figure 3



Supplemental Figure 3. Effect of indicated constitutively active GTPases on plasma membrane targeting of mDia2 construct GFP-BD-G-DID. Rif causes the greatest increase in PM index (A) Confocal microscopy of HeLa cells co-expressing GFP-BD-G-DID (top row), mRFP1 (second row) and indicated Myc or HA-tagged constitutively active GTPases immunostained with respective tag antibodies (bottom row). Merged GFP-BD-G-DID/mRFP1 images are shown in the third row. Scale bar, 10 μ m. (B) Quantification of PM index of GFP-BD-G-DID in control HeLa cells (green) and in HeLa cells co-expressing indicated constitutively active GTPases (red). Statistically significant difference with p-value < 0.01 as compared to control cells is marked by two asterisks. P-values in the 0.01-0.05 range are marked with one asterisk.