Table S1. Overview of siRNA screen results

The effect of siRNA depletion of proteins on focal adhesion (FA) formation and spreading under basal conditions, or when the Epac1-Rap signal transduction pathway had been activated, was determined by visual inspection of replated cells.

Protein	Spreading and focal adhesion (FA) induction	Actin phenotype
α-catenin	Normal	Normal
AF6	Normal. Following 007, many cells showed increased spreading with more FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Normal
AF6-L/Radil	Partial defect in the 007-induced spreading and FA formation.	Mixed
αPKC	Normal	Normal
Arap1	Normal	Normal
Arap3	Small defect in basal adhesion and spreading, but a normal response to 007.	Normal
ARHGAP20	Small defect in basal adhesion and spreading, but a normal response to 007.	Normal
β-catenin	Unstimulated cells were elongated and displayed an increase in cell protrusions. Spreading and FAs were stimulated by 007, but the cells remained elongated.	Increase in thin actin fibers.
C3G	Cells displayed a defect in basal spreading, which was rescued and further induced by 007.	Normal
CCM1	Normal	Normal
CCM2	Many cells showed increased FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Increase in stress fibers.
CCM3	Unstimulated knockdown cells were more spread than control cells, and showed many cellular protrusions. Cells still spread and formed FAs with 007.	Increase in stress fibers.
CDC42	Normal	Normal
DGKQ	Normal	Normal
DLG5	Normal	Normal
EBP50	Normal	Normal

E-cadherin	Normal	Normal
Epac1	Cells did not spread or form FAs with 007.	Normal
Epac2	Normal	Normal
Exoc2	Normal	Normal
Exoc8	Unstimulated cells were already spread, with small FAs. 007 still induced spreading but FAs remained small.	Increase in actin protrusions/filopodia in basal conditions. 007 induced small stress fibers throughout cells.
Ezrin	Cells displayed a defect in spreading and FA induction in response to 007.	Mixed
FRMPD1	Normal	Normal
ICAP	Normal	Normal
IQGAP	Normal	Normal
Lamellipodin	Normal	Thin unorganized actin fibers throughout cells.
LIMK	Normal	Normal
Moesin	Normal	Normal
Occludin	Normal	Normal
p120-catenin	Normal	Normal
Par3	Normal	Normal
Par6	Normal	Normal
PDZGEF1	Mixed effect. Some unstimulated cells showed the 007-phenotype, although this was not reproducible. The response to 007 was normal.	Some unstimulated cells showed the 007-phenotype, although this was not reproducible.
PDZGEF2	Normal. Following 007, some cells showed an increase in FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Increase in small stress fibers throughout cells.
PDZK10	Normal	Normal
Rac1	Normal	Normal
Radixin	Normal	Normal
RalA	Normal	Normal
RalB	Normal	Normal
RalGDS	Normal	Normal
Rap1A	Cells displayed a defect in basal spreading. There was no spreading or FA induction upon 007.	Normal
Rap1B	Normal	Normal

Rap1GAP1	Normal. Following 007, some cells showed an increase in FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Increase in small stress fibers throughout cells.
Rap1GAP2	Normal	Normal
Rap2A	Normal	Normal
Rap2B	Normal	Normal
Rap2C	Normal	Normal
RapL	Normal	Increase in small stress fibers throughout cells.
RasGRP1	Normal. Following 007, some cells showed an increase in FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Normal
RasGRP2	Normal	Normal
RasGRP3	Normal	Normal
Rgl1	Normal. Following 007, some cells showed an increase in FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Normal
RhoA	Normal	Normal
Riam	Normal	Increase in small stress fibers throughout cells.
Rin1	Normal. Following 007, some cells showed an increase in FAs. Focal adhesions were no longer restricted to the periphery of the cell.	Normal
RockI	Normal	Normal
RockII	Basal adhesion of cells was decreased. The spreading and FA induction defect in response to 007 was inhibited.	Mixed
R-Ras	Normal	Normal
Sec15L1	Normal	Normal
Sec15L2	Normal	Normal
Sec8	Normal	Normal
SHIP2	Normal	Normal
Spa1	Normal	Normal
Talin1	Cells showed a defect in basal adhesion and spreading, which was not rescued by 007.	Normal actin phenotype, although the cells did not spread well
Talin2	Normal	Normal
Tiam1	Normal	Normal
Vasp	Normal	Normal
Vav1	Normal	Normal

Vav2	Normal. Cells did not become very round with 007.	Normal
Vav3	Normal	Normal
ZAK	Normal	Increase in small actin fibers.