

Supplementary Method Information:

Immunoblotting and Immunoprecipitation

Cells were washed once with phosphate-buffered saline (PBS) and lysed on ice in RIPA buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate) supplemented with complete inhibitor (Roche Biochemicals). For fractionation of cell membranes and cytosol, probes were preclared by centrifugation at 1000 x g for 15 min at 4°C and separated by ultracentrifugation for 1 h at 100000 x g at 4°C. Same amounts of protein were loaded on 10% SDS-Page gels. The samples were electrotransferred onto polyvinylidene difluoride (pvdf) membranes (Hybond-P; Amersham), which were blocked over night with 3% skim milk in Tris-buffered saline (TBS). After incubation with the indicated antibody in blocking buffer, membranes were washed three times in 0,1% Tween-TBS. Primary antibodies were detected using affinity purified peroxidase-conjugated antibodies as follows: sheep anti-mouse antibody (Dianova), rabbit anti-rat antibody (Sigma), donkey anti-rabbit antibody (Santa Cruz) and rabbit anti-goat antibody (Dianova) for 1h at room temperature. After three washes, detection of secondary antibodies was carried out using the ECL detection system (Amersham). Signals were recorded by a luminescent image analyser (Fujifilm image reader, LAS1000, Tokyo, Japan) and analysed with image analyzer software (Gel-ProAnalyser, Media Cybernetics, USA). For immunoprecipitation, the cell lysate was preclared with sepharose-beads for 1h at 4°C. Afterwards, the appropriate antibody and Protein G-sepharose were added to the lysate and incubated for 2h at 4°C. The beads were then washed three times with lysis buffer and resuspended in 2x sample loading buffer supplemented with 5% β -mercaptoethanol. After incubation at 95°C for 5 minutes, samples were loaded on 10% SDS-PAGE. For reprobing blots, pvdf-membranes were incubated in stripping buffer (100mM 2-Mercaptoethanol, 2%SDS, 62.5mM Tris-Hcl pH 6.7) for 1h at 60°C with occasional shaking. After three washes in large volumes of TBS-

T, membranes were blocked in blocking buffer for 1h at room temperature and immunodetection was repeated. For Western Blot analysis of supernatants, cells were incubated in DMEM over night and supernatants were concentrated (30fold) through centrifugation in Microcon Centrifugal Filter Devices (YM-10, Millipore).

Adhesion assays

For analysing cell-cell adhesion, wildtype and ADAM10-deficient cells were seeded at 5×10^4 cells per well into 96-well dishes (Microlon; Greiner) and cultured to full confluence. Subsequently, the cells were assayed for adhesion of fluorescently labeled wildtype and ADAM10-deficient cells, respectively. For fluorescent labeling, cultured MEFs were suspended at 2×10^6 /ml in PBS/0.1% BSA and incubated with $2.5 \mu\text{M}$ fluorescent dye (calcein AM; Molecular Probes, Leiden, the Netherlands) at 37°C for 30 minutes. Excess dye was removed by washing twice with 15 ml PBS. MEFs were resuspended in growth medium and preincubated with the inhibitory N-cadherin antibody GC4 ($50\mu\text{g}/\text{ml}$) or isotype control ($50\mu\text{g}/\text{ml}$) for 20 min, with EGTA (5mM) for 10 min or left untreated. The labelled MEFs were added to the monolayer of unstained cells at 5×10^4 cells per well in growth medium containing 1mM CaCl_2 plus GC4 ($50\mu\text{g}/\text{ml}$) or isotype control ($50\mu\text{g}/\text{ml}$) or in growth medium containing EGTA. Afterwards the cells were rapidly seeded onto the monolayer by centrifugation for 3 minutes at 100g. The plate was incubated at 37°C for 20 minutes and then washed repeatedly by inversion and by addition of PBS (200 μl per well), followed by inversion. The fluorescence signal from the adherent cells was measured before and after washing using a fluorescence plate reader (Lambda Fluoro 230; MWG Biotech, Munich, Germany) at excitation wavelength of 480 nm and emission wavelength of 530 nm. The differences in fluorescence before and after washing are depicted as percentage of adherent cells. All analyses were performed as triplicates.

To investigate cell substrate adhesion, 96 well plates (Sarstedt Inc., NC, USA) were coated with 10µg/ml recombinant human N-cadherin (R&D Systems, Minneapolis, MN) or 10µg/ml BSA in 100µl PBS at 4°C over night. Calcein labelled MEFs were seeded at a density of 5×10^4 cells/well and incubated for 20 min. The adherent cells were gently washed by rinsing twice with 200 µl PBS followed by inversion of the plate. Fluorescence was determined before and after washing and depicted as percentage of adherent cells. All analyses were performed as triplicates.

For determining the adhesion capacity of ADAM10-deficient and wildtype MEFs, confluent monolayers (10cm dish/Sarstedt) were mechanically dissociated though 30-times pipetting with a 5ml pipette (Sarstedt), photographed and the amount of aggregates was quantified with a Casy TT cell Counter system (Schärfe System, Reutlingen, Germany). The amount of aggregates was calculated determining the ratio of counts (single cells and aggregates) to total cell number. The aggregation of wildtype and ADAM10-deficient cells was only compared when the total cell number was similar.