Supplements

Supplemental Method Description

Antibodies, chemokines, inhibitors

Antibodies are listed according to the supplying company. R&D System (Wiesbaden, Germany): mouse monoclonal antibody against human ADAM10 (clone 163003), human ADAM17 (clone 111633), human β1-integrin/CD29 (clone 4B7R), human β2-integrin/CD18 (clone 212701), human α1-integrin/CD11a (clone 345913), human α5-integrin/CD49e (clone 230307), mouse IgG2B isotype control (PE-labeled), mouse IgG1 isotype control, rat monoclonal against murine ADAM10 (PE-labeled, clone 139712); Jackson ImmunoResearch Laboratories, Inc (West Grove, USA): allophycocyanin (APC)-conjugated goat anti-mouse antibody; Miltenyi (Bergisch Gladbach, Germany): rat APC-labeled monoclonal antibody against murine Ly6G (APC-anti-mLy6G, clone 1A8); AbD Serotec (Duesseldorf, Germany): rat monoclonal fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled anti-mF4/80 (clone Cl:A3-1); BD Pharmingen (Heidelberg, Germany): rat monoclonals Cy™7-conjugated APC (APC-Cy7)-anti-m-CD4 (clone GK1.5), APC-Cy7-anti-m-CD11b (clone M1/70), PacificBlue-anti-m-CD8a (clone 53-6.7); SantaCruz (Dallas, TA, USA): polyclonal rabbit anti-h-ERK1 also binding ERK2 to a lesser degree, monoclonal mouse anti-h-phospho-ERK (clone 12D4); Cell Signaling (Danvers, MA, USA): polyclonal rabbit anti-h-p38α and monoclonal mouse anti-h-phospho-p38; Antikoerper Online (Aachen, Germany): rat monoclonal against mouse α5β1-integrin (clone BMC5), rat monoclonal against mouse αM-integrin (clone ICRF44); Millipore (Temecula, USA): rat monoclonal against activated human β1-integrin (clone HUTS-4). Lipopolysaccharide (LPS) from E. coli 0127:B8 was from Sigma-Aldrich (Munich, Germany). Human CXCL8 and CCL2 as well as murine CXCL1 and CCL2 were from Peprotech (Rocky Hill, USA). The metalloproteinase inhibitor GI254023X was synthesized and assayed for inhibition of human and mouse ADAM10 as described.1,2 TAPI-1 was from Calbiochem. The protease inhibitor mix was from Roche (Complete, Munich, Germany). The p38 inhibitor SB203580, the ERK inhibitor U0126, the EGFR kinase inhibitor PD168393, and the EGFR neutralizing monoclonal antibody cetuximab (Erbitux™) were from Merck Millipore (Darmstadt, Germany). Cell culture media and FBS were from PAA/GB Healthcare (Pasching, Austria).
Mice

Animal experiments were approved by the local authorities and performed with 6 to 8 week old female mice on C57BL/6 background (82-02.04.2011.A335 and 87-51.04.2010.A026, LANUV NRW, Germany). Vav-Adam10/− and Vav-Adam17/− mice expressed Cre recombinase under control of the Vav-promotor and were homozygous for floxed Adam10 or floxed Adam17, respectively.3,4 LysM-Adam10/− mice expressed Cre recombinase under control of the LysM-promotor and were homozygous for floxed Adam10. Mice were compared to the appropriate litter controls expressing the non-floxed ADAMs and Cre recombinase. Animals were hosted in a pathogen free environment and were transferred to IVC conditions for experiments.

Cell culture and cell preparation

THP-1 cells (human acute monocytic leukemia, DSMZ, Braunschweig, Germany) were cultured in RPMI1640 supplemented with 10% FBS, 100U/ml penicillin, 100μg/ml streptomycin (F/P/S) by seeding at 2.5 x 10^5 cells/ml and subculture at 1 x 10^6 cells/ml.

Human neutrophils from citrated (0.38%) peripheral blood of healthy volunteers were isolated by sedimentation on ficoll hypaque (Amersham, Freiburg, GER) and subsequent incubation for 5min in red blood cell lysis buffer (155mM NH₄Cl, 10mM NaHCO₃, 5mM EDTA, pH7.4) as described.5 Human epithelial bladder carcinoma ECV304 cells (DSMZ) were cultured in M199 plus F/P/S.

Human microvascular endothelial cells of the lung (HMVEC-L) (Lonza, Belgium) were cultured in EGM-2 MV medium (Lonza) and subcultured following the manufacturer’s protocol.

Bone-marrow-derived macrophages (BMDM) were isolated out of femur and tibia of hind limbs of 8 to 10 week old mice. The muscle tissue was removed by cutting and using paper tissue, storing the bones in cold PBS throughout the isolation procedure. Femur and tibia were cut at both ends with sterile scalpels and flushed with ice-cold RPMI1640 supplemented with F/P/S using a syringe with an 18G needle. Cells were centrifuged at 300g for 5min at 4°C and resuspended in culture medium (RPMI1640 supplemented with F/P/S, and 20% L929-conditioned medium) using a syringe with 26G needle. Cells were plated on two 15cm cell culture dishes per mouse within 20ml culture medium. After 3d, 10ml culture medium were added, followed by a culture medium change at day 6. At day 9, BMBM were subcultured on 10cm dishes in culture medium for at least 2d. Prior to experiments, cells were cultured in the absence of L929-conditioned medium for 24h. At the end of differentiation
BMDM of the different genotypes (Adam10<sup>−−</sup>, Adam17<sup>−−</sup> and litter controls) were almost all (more than 95%) F4/80-positive as determined by flow cytometry (supplemental Figure 3E) and did not differ in iNOS, CD206 and ICAM-1 expression suggesting no differences in M1/M2 polarization (data not shown). There was no altered mRNA expression of Notch1, Notch2, Hes1, Hey1, and Hey2 among the BMDM genotypes as confirmed by RT-qPCR (data not shown).

Citrate-treated (0.38%) murine blood was collected from vena cava and subjected to erythrocyte lysis twice (see above). Blood leukocytes were cultured for 24h in RPMI1640 supplemented with F/P/S before migration experiments.

**Lentiviral transduction**

Short hairpin RNA (shRNA) targeting ADAM10 and ADAM17 was inserted into the lentiviral expression vector pLVTHM (Addgene plasmid 12247) as described. The targeting sequences were gacatttcaacctacgaat (ADAM10-650) and acagtgcagtccaagtcaa (ADAM10-1947) for ADAM10 mRNA and aggaaagcctgtacagta (ADAM17-2061) and gaaacagagtgctaattta (ADAM17-2646) for ADAM17 mRNA. A sequence of ccgtcacatcaattgccgt served as scramble control (scr).

For production of recombinant lentiviral particles, subconfluent HEK293T cells were cotransfected with 10 µg of the specific pLVTHM-plasmid, 7.4 µg of psPAX2 (plasmid 12260, Addgene) and 2.6 µg of pMD2G (plasmid 12259, Addgene) using 40 µl polybrene (Polyplus, Illkirch, France) as transfectant. The medium was changed after 24h, and the lentivirus containing supernatants were harvested after another 48h of incubation. Lentiviral particles were concentrated 500 times by ultracentrifugation at 50,000g for 2h and resuspension in PBS. To transduce THP-1 cells, 2 µl concentrated lentivirus was added to 2x10<sup>5</sup> cells in 500 µl cell culture medium supplemented with polybrene (4 µg/ml, Sigma, Munich, Germany). If necessary a second transduction was carried out after 3d. Successful and efficient transduction was controlled by GFP expression encoded by pLVTHM.

**RT-qPCR**

The mRNA levels for ADAM10 and ADAM17 in BMDM and THP-1 cells were quantified by RT-qPCR analysis and normalized to the mRNA level of murine RPS29 or human GAPDH. RNA was extracted using RNeasy Kit (Qiagen, Hilden, Germany) and quantified by spectrophotometry (NanoDrop, Pehlab, Germany). RNA (equal amounts within each data set) was reverse transcribed using RevertAid First Strand cDNA Synthesis Kit (Fermentas, St.
Leon-Rot, Germany) according to the manufacturer’s protocol. PCR reactions were performed using LightCycler®480 SYBR Green I Master Mix (Roche) according to the manufacturer’s protocol. Following primers were used with the specific primer annealing temperature GI254023Xven in brackets: mAdam10 forward, agcaacatctggggacaaac, mAdam10 reverse, tggccagattcaacaaaaac (57°C); mAdam17 forward, aaaccagaacagacccaaacg, mAdam17 reverse, gtaagctgatgacagcagaaaa (57°C); mNotch1 forward, tggagctacctgcactgactatc, mNotch1 reverse, ttcctcgagcagcttagacc (62°C); mNotch2 forward, caaagaagaagccagactgag, mNotch2 reverse, gttgtcctgagagcaggtg (59°C); mHes1 forward, acacccgagaaaaaagac, mHes1 reverse, acccaaactcgagatgctc (59°C); mHey1 forward, tggaactagagctggtac, mHey1 reverse, acacccgagaaaaaagac, mHey2 reverse, tggcatcagattagcttta (57°C); mRps29 forward, gacgcagccgggca, mRps29 reverse, cctttctctctgggggc (61°C); hAdam17 forward, gaagttgcagaggcagatta, hAdam17 reverse, cgggacactctgcttattacc (55°C); hAdam10 forward, ggattgtgcctgtggtgcgca, hAdam10 reverse, actctctgagggcgcggtc (61°C); hGapdh forward, cggggctctccagcaatctcc, hGapdh reverse, cagcccccagctcagcttg (66°C). All PCR reactions were run on a LightCycler® 480 System (Roche) with the following protocol: 40 cycles of 10s denaturation at 95°C, followed by 10s annealing at the indicated temperature and 15s amplification at 72°C. Standard curves were determined by a serial dilution of a defined cDNA standard within each data set. Data were obtained as cycle crossing point (CP) values and calculated as delta CP values using the LightCycler®480 software and used for statistic analysis.

Chemotaxis and transmigration assays

For THP-1 or neutrophil chemotaxis assays blind 48-well chemotaxis chambers with polycarbonate membranes of 8 or 5μm pores, respectively, were used (Neuroprobe, Gaithersburg, MD). Lower wells were filled with stimulus solution (CCL2 or CXCL8, respectively, at the indicated concentrations in assay buffer (RPMI/0.2% BSA). 2x10^5 THP-1 cells or neutrophils were added to the upper wells. After incubation for 2h or 45min, respectively, migrated cells were quantified in the lower well. In some experiments non-migrated cells were scraped off from the upper side of the membrane and cells remaining within the pores or below the membranes were stained with Diff Quick. Cells were quantified in the cell membrane using ImageJ (Rasband, NIH, Bethesda, Maryland, USA).

For chemotaxis experiments with murine cells, transwells were filled with 100μl of cell suspension (1x10^6 BMDM or murine blood cells in 100μl DMEM/0.2% BSA). Lower wells contained 600μl RPMI/0.2% BSA with the indicated concentrations of murine CCL2
for BMDM migration or 2% non-heat-inactivated (nhi) FBS for blood leukocyte migration. After incubation for 2h, transmigrated cells were quantified.

Migrated THP-1 cells, neutrophils or BMDM were quantified by measurement of endogenous glucuronidase activity as described. Transendothelial migration of transduced THP-1 cells expressing GFP was analyzed by flow cytometry. Migrated murine blood cells were further analyzed by flow cytometry using gates for leukocyte subpopulations.

For transendothelial migration assays transwell chambers (Corning, Amsterdam, Netherlands) were used. HMVEC-L (5,000 cells) or ECV304 cells (10,000 cells) were seeded on transwell inserts with a pore size of 8 \( \mu \)m and grown to confluency for 4d. Wells were filled with 600\( \mu \)l assay buffer with or without human CCL2 (3nM). \( 2 \times 10^5 \) THP-1 cells were added to each transwell insert and migrated cells in the lower well were analyzed after 2h.

For microscopic analysis THP-1 cells and ECV304 cells were transduced to express GFP or DsRed, respectively, prior to the transmigration experiment. After washing of non migrated cells and subsequent fixation with 1% PFA for 20min, GFP-expressing THP-1 cells that transmigrated through the dsRed-expressing ECV304 cell layer were visualized by confocal laser scanning microscopy (LSM 7 Duo Microscope, Zeiss, Goettingen, Germany). THP-1 cells were quantified within and below the ECV304 cell layer using ImageJ.

**Adhesion assay**

48-well-plates (Nunclon) were coated with 125 \( \mu \)l soluble ICAM-1 (1.5 \( \mu \) g/ml) or fibronectin (6 \( \mu \) g/ml) for 1h at RT and then blocked with 200 \( \mu \)l 0.5% BSA. THP-1 cells, neutrophils, or BMDM (1x10^5 cells/ml HBSS) were incubated in absence or presence of 3nM human or murine CCL2 or 10nM CXCL8/CXCL1 for 15min at 37°C if not otherwise indicated. 200\( \mu \)l cell suspension was added per well and sedimented for 30s at 300g. After 15min (neutrophils) or 30min (monocytic cells) incubation at 37°C, the wells were washed four times. Cells were fixated with 0.2% PFA for 5min, followed by nuclei staining with Hoechst 33342. HBSS was used as assay buffer in all steps. Adhesive cells were counted in 4 representative images of each well using ImageJ.

**Calcium assay**

THP-1 cells were harvested and resuspended in \( \text{Ca}^{2+} \)-staining buffer (HBSS, 10mM HEPES, 720mg/l Probenicid, 2.65mM Fluo-3-AM, 32nM Pluronic F-127, 1% FBS) at a density of 2x10^6 cells/ml. After an incubation for 30min at 37°C, cells were washed twice with \( \text{Ca}^{2+} \)-assay buffer (HBSS, 10mM HEPES, 720mg/l Probenicid). The cells were resuspended and
100 μl of the cell suspension containing 4x10^5 cells was added to a black 96-well-plate with a transparent bottom (Greiner, Frickenhausen, Germany). The calcium influx in response to stimulation with 3nM CCL2 was then measured at a constant temperature of 37°C in a FLUOstar OPTIMA plate-reader (BMG-Labtech, Ortenberg, Germany). After recording the fluorescent baseline for 10s, CCL2 was injected to a final concentration of 3nM. As control, the same volume of calcium assay buffer without CCL2 was injected. Fluorescence was constantly measured at an excitation wavelength of 488nm and an emission wavelength of 526nm. At the end of each measurement, maximal and minimal fluorescence was determined by cell lysis with digitonin (125 μg/ml final) followed by addition of EGTA (15mM final).

**Flow cytometric analysis**
PBS supplemented with 0.2% BSA (human cells) or 1% FBS and 5mM EDTA (murine cells) was used as assay buffer, and all steps of the staining process were performed at 4°C. THP-1 were analyzed for expression of ADAM10, ADAM17, or integrins by incubation with mouse monoclonal antibodies against ADAM proteins (5μg/ml) or integrins (2.5μg/ml) followed by incubation with APC-conjugated anti-mouse antibody (1:100) as described. Isotype controls were used in parallel. For analysis of murine BAL fluid cells and blood leukocytes, the neutrophil, macrophage, and lymphocyte gates, were defined by staining for CD11b, CD4, CD8e, Ly6G, and F4/80 and cross-checked with the appropriate isotype controls following the manufacturer’s protocols as described. The detailed gating strategy is exemplary shown in supplemental Figure 3J. Additionally, autofluorescence was used to further distinguish the dendritic cell and macrophage gate. The total number of leukocytic cells within BAL fluid and blood samples was determined using CountBright™ absolute counting beads (Invitrogen, Darmstadt, Germany) following the manufacturer’s protocol. The fluorescence signal was detected by flow cytometry (LRS Fortessa, BD Biosciences) and analyzed with FlowJo 8.7.3 software (Tree Star, Inc., Ashland, USA).

**ERK1/2 and p38 activation**
THP-1 cells were serum starved for 24h and 1.5x10^6 cells were stimulated in 1ml RPMI with 3nM CCL2. Subsequently, cells were cooled down on ice, sedimented by centrifugation and resuspended in lysis buffer containing 20mM Tris, 150mM NaCl, 5mM EDTA, 30mM NaF, 5mM DTT, 1mM PMSF, 10mM pNPP, 1mM benzamidine, 10mM glycerophosphate, 1mM Na3PO4, 1% Triton X-100, and Complete protease inhibitor (Roche Diagnostics). Lysates
were then subjected to SDS-PAGE and Western blotting using antibodies against phosphorylated and non-phosphorylated forms of ERK1/2 and p38.

**Phalloidin stain**

THP-1 cells or BMDM (1x10^6 cells/ml in HBSS) were stimulated with 3nM human or murine CCL2 for the indicated time at 37°C and directly fixated using PFA (2% end concentration for 5min). After 5min cell permeabilization in PBS with 0.5% Triton, cells were stained with 0.2 U AlexaFluor555-phalloidin (Life Technologies, Darmstadt, Germany) in PBS with 0.2% BSA. After 2 wash steps, cells were subjected to flow cytometry.

**Rho GTPase activation assay**

THP-1 cells (2x10^6 cells/ml in HBSS) were incubated for 15min in the absence or presence of 3nM CCL2. Subsequently, cells were cooled down on ice and sedimented by centrifugation. Cell pellets were analyzed using a commercial Rho activation kit (Enzo Life Sciences, Plymouth, USA) according to the manufacturer’s protocol. To determine Rho total and Rho active content, 100µg total protein was subjected to SDS-PAGE and Western blotting.

**LPS-induced acute pulmonary inflammation**

The model of LPS-induced acute pulmonary inflammation has been described in detail before. In brief, pulmonary inflammation was induced by intranasal application of 400µg/kg LPS. Instillation of PBS alone served as control. After 24h, the right lobes of the lungs were lavaged with 0.5 ml ice-cold PBS for collection of BAL cells and fluid. Wet-dry-ratio was determined for non-lavaged and non-perfused lung tissue (post-caval lobe). For histological examination, the right lobe of the lungs was fixed by intratracheal instillation of Roti-Fix® (Roth, Germany) followed by bronchial ligation after 5min. After 48h of fixation, the tissue was dehydrated, embedded in paraffin and cut in 3µm slices. Hematoxylin-eosin staining was performed using standard protocols. Images were taken with a Zeiss microscope (AxioLab.A1, Carl Zeiss MicroImaging GmbH, Germany) and analyzed for influx of monocytes and neutrophils using the AxioVision software (Carl Zeiss MicroImaging GmbH). The leukocyte composition of BAL cells was determined by flow cytometry, and the release of cytokines into the BAL fluid was measured by ELISA as described. The leukocyte composition of the blood was measured in heparin-treated blood using the Vet abc system (LabTechnologies Medizintechnik, Wien, Austria).
The protein content of BAL fluid (each in equal volumes of recovery), and cell lysates was determined using a commercial bicinchoninic acid assay kit (Interchim, Mannheim Germany) following the manufacturer’s protocol and by running a bovine serum albumin standard (0 to 3000 ng/ml) in parallel.

**ELISA measurement**

Release of mTNF, mCXCL1 (KC), mCCL2, and mIL-6R to BAL fluid was analyzed using commercial ELISA Kits (R&D Systems, DuoSet) according to the manufacturer’s protocols.
Supplemental references


Supplemental figure legends

Suppl. Figure 1
A, B) THP-1 cells were treated with 10µM GI254023X or DMSO for 15min and subsequently investigated for random cell migration in the absence of CCL2 and chemotactic migration in the presence of CCL2 (3nM) over a period of 2h in A or for CCL2-induced migration over the indicated periods of time in B. (C/D) Two shRNA sequences were used for downregulation of ADAM10 (A10-650 and A10-1947) and ADAM17 (A17-2061 and A17-2646) on THP-1 cells and compared to a scramble control shRNA sequence (scr). Downregulation was demonstrated by analysis of ADAM10 and ADAM17 mRNA expression in relation to GAPDH mRNA using RT-qPCR in C or by flow cytometry surface expression analysis in D. (E) THP-1 cells were transduced with lentivirus for simultaneous expression of shRNA and GFP and then tested for CCL2 (3nM)-induced and random transmigration through HMVEC-L cells cultured on 8µm pore transwell inserts. GFP-positive cells were quantified by flow cytometry and migrated cells were expressed as percentage of total cells before migration. (F) THP-1 cells were transduced to express the indicated shRNA and assayed for cell migration in response to 3nM CCL2. After removing cells from the top of the chemotaxis membrane, cells were stained in the chemotaxis membrane. THP-1 cells were counted in three representative images of each experiment. Data represent means ± SEM of three independent experiments. Significance was calculated using one-way ANOVA and Bonferroni post-test and is indicated by crosses. Asterisks without line indicate significant differences to the non-treated control analyzed by one-sample t-test.

Suppl. Figure 2
(A-D) THP1 cells were loaded with the calcium indicator Fluo3, treated for 15min with 10µM GI254023X or DMSO (0.1%) and immediately investigated for intracellular free calcium transients in response to CCL2 (3nM) over time. A representative recording and a summary of three independent experiments is shown in A and B, respectively. THP1 cells were silenced for ADAM10 or ADAM17 expression via shRNA and assayed for CCL2-induced calcium transients in C or D, respectively. (E-H) THP-1 cells were pretreated with 10µM ERK1/2 inhibitor SB203580 (SB), 10µM p38 inhibitor U0126 (U), 10µM EGFR inhibitor PD168393 (PD), 100 ng/ml EGFR neutralizing antibody cetuximab (Cet) or DMSO for 15min and subsequently investigated for chemotaxis in E and F and cell adhesion to coated fibronectin in...
G and H in response to CCL2 (3nM). (I-M) THP-1 cells were pretreated with 10µM GI254023X or DMSO control for 15min, stimulated with CCL2 (3nM) or left unstimulated for 15min and subsequently investigated for cell adhesion to coated ICAM-1 in I, upregulation of β2-integrin in J, upregulation of α5-integrin in K, upregulation of αM-integrin in L and surface expression of activated β1-integrin in M by flow cytometry. (N, O) THP-1 cells were pretreated with DMSO, 10µM SB203580 or 10µM U0126 in N, or 10µM PD168393 or 100 ng/ml cetuximab in O for 15min and subsequently investigated for upregulation of α5-integrin by CCL2 (3nM). Data represent means ± SEM of three independent experiments. Crosses indicate significance among treated cells calculated using one-way ANOVA and Bonferroni post-test. Asterisks without line indicate significant differences to the non-treated control analyzed by one-sample t-test.

Suppl. Figure 3

(A, C) Human neutrophils were pretreated with the indicated concentrations of GI254023X in A and B or TAPI-1 in C for 15min and investigated for chemotaxis induced by CXCL8 (10nM). DMSO (0.1%) was used as control. Cells were quantified in the lower chemotaxis chamber in A and C or stained and quantified in the chemotaxis membrane after removing non-migrating cells from the top of the filter in B. (D) Human neutrophils were pretreated with 10µM GI254023X or DMSO (0.1%) for 15min, stimulated with CXCL8 (10nM) and investigated for adhesion to coated ICAM-1. (E) BMDM were generated from Vav-Adam10−/−, Vav-Adam17−/− and litter control mice, and differentiation into macrophages was controlled by F4/80 surface staining and flow cytometry. (F) Surface expression of ADAM10 on BMDM was determined by flow cytometry using an antibody directed against murine ADAM10. (G) Expression of ADAM10 and ADAM17 mRNA in BMDM was determined by RT-qPCR and expressed as arbitrary units in relation to Rps29 reference gene. (H) BMDM from Vav-Adam10−/−, Vav-Adam17−/− and litter control mice were pretreated with or without 10µM GI254023X for 15min followed by stimulation with murine CCL2 (3nM) for 15min and investigated for adhesion to coated ICAM-1. (I) BMDM from Vav-Adam10−/− (n=3) and litter control mice (n=2) were stimulated with CCL-2 for 15 min and subsequently assayed for Rho GTPase activation. Data are shown as representative Western blots and summarized as percent increase in relation to the unstimulated cells. (J,K) Murine blood leukocytes from Vav-Adam10−/− and Vav-Adam17−/− mice and litter controls were pretreated with or without 10µM GI254023X. After 15min, chemotaxis was analyzed using the transwell system and 2% non-heat-inactivated FBS as chemotactic stimulus. Migrated cells were examined by flow
cytometry and the gating strategy is shown in J. Quantitative data represent means ± SEM of three independent experiments and cell preparations. Crosses indicate significance among treated cells calculated using one-way ANOVA and Bonferroni post-test. Asterisks without line indicate significant differences to the non-treated control analyzed by one-sample t-test.

Suppl. Figure 4

(A, B) Vav-Adam10−/−, Vav-Adam17−/− and respective litter control mice were intranasally treated with 400 µg/kg LPS or vehicle (PBS). The number of blood lymphocytes, monocytes and neutrophils was determined in heparin-treated blood using the Vet abc system 24h after LPS-instillation. Data represent means ± SEM (n=5). Significance was calculated using one-way ANOVA and Bonferroni post-test and is indicated by crosses. Crosses without line indicate significance to the appropriate PBS-treated control.

Suppl. Figure 5

(A-D) Vav-Adam10−/−, Vav-Adam17−/− and respective litter control mice were intranasally treated with 400 µg/kg LPS or vehicle (PBS) for 24h. BAL fluid was precleared by centrifugation and investigated for TNF in A, CCL2 in B, CXCL-1 in C, and sIL6R in D by ELISA. Data represent means ± SEM (n=5). Significance was calculated using one-way ANOVA and Bonferroni post-test and is indicated by crosses. Crosses without line indicate significance to the appropriate PBS-treated control. Student’s t-test was additionally performed for nearly significant differences.

Suppl. Figure 6

(A-B) Vav-Adam10−/−, Vav-Adam17−/− and litter control mice were intranasally treated with 400 µg/kg LPS or vehicle (PBS) for 72h and the number of blood lymphocytes, monocytes, and granulocytes was determined in heparin-treated blood using the Vet abc system. (C-D) BMDM from LysM-Adam10−/− and litter control mice were analyzed for ADAM10 mRNA expression by RT-qPCR or ADAM10 surface expression by flow cytometry. (E) LysM-Adam10−/− and litter control mice were intranasally treated with 400 µg/kg LPS or vehicle (PBS) for 72h, and blood lymphocytes, monocytes and granulocytes were counted using the Vet abc system.
Supplemental Figure 1

A

migration index

DMSO
GI (10µM)

[Graph showing migration index with DMSO and GI (10µM) treatments.]

B

migration index

0 2 4 6

[Graph showing migration index over time with GI (10µM) /CCL2 (3nM) and DMSO/CCL2 (3 nM) treatments.]

C

A10 mRNA expression [a.u.]

0.0 0.5 1.0

[Bar graph showing A10 mRNA expression with scr, A10-650, A10-1947.]

A17 mRNA expression [a.u.]

0.0 0.3 0.6

[Bar graph showing A17 mRNA expression with scr, A17-2061, A17-2646.]

D

expression:

ADAM10

shRNA:

A10-650
A10-1947

relative cell number

fluorescence intensity

[Histograms showing relative cell number and fluorescence intensity for ADAM10 shRNA.]

ADAM17

shRNA:

A17-2061
A17-2646

[Histograms showing relative cell number and fluorescence intensity for ADAM17 shRNA.]

E

% transmigrated cells

0 20 40

[Bar graph showing % transmigrated cells with scr, A10-650, A10-1947.]

% transmigrated cells

0 20 40

[Bar graph showing % transmigrated cells with scr, A17-2061, A17-2646.]

F

[Images of cells with annotations showing % transmigrated cells for scr, A10-650, A17-2061.]

[Graph showing cells per image with scr, A10-650, A17-2061.]
Supplemental Figure 3

A. Migration index

B. Fluorescence intensity isotype, Vav-Adam10-/- isotype, litter control

C. Migration index

D. Adhesion to ICAM-1 [% of control]

E. % F4/80 positive cells in cultured BMDM

F. Relative cell number

G. A10 mRNA [a.u.]

H. % F4/80 positive cells in cultured BMDM

I. Increase in Rho activity [% of control]

J. Migration index

K. Citrated blood

L. Monocyte-neutrophil gate

M. Cell gate

N. SSC

O. FSC

P. F4/80
Supplemental Figure 4

A

B

litter control
Vav-Adam10−/−

blood lymphocytes [10^6/ml]

blood monocytes [10^5/ml]

blood granulocytes [10^6/ml]

PBS
LPS

PBS
LPS

PBS
LPS

PBS
LPS

litter control
Vav-Adam17−/−

blood lymphocytes [10^6/ml]

blood monocytes [10^5/ml]

blood granulocytes [10^6/ml]

PBS
LPS

PBS
LPS

PBS
LPS

PBS
LPS

+ + +

+ +

++

+ +
Supplemental Figure 5

A

TNF in BAL fluid [ng/ml]

B

CCL2 in BAL fluid [10^{-1} ng/ml]

C

CXCL1 in BAL fluid [ng/ml]

D

sIL-6R in BAL fluid [pg/ml]

- PBS
- LPS

litter control
Vav-Adam10^{-/-}
Vav-Adam17^{-/-}

p = 0.0644
Supplemental Figure 6

A

![Graph A]

B

![Graph B]

C

![Graph C]

D

![Graph D]

E

![Graph E]