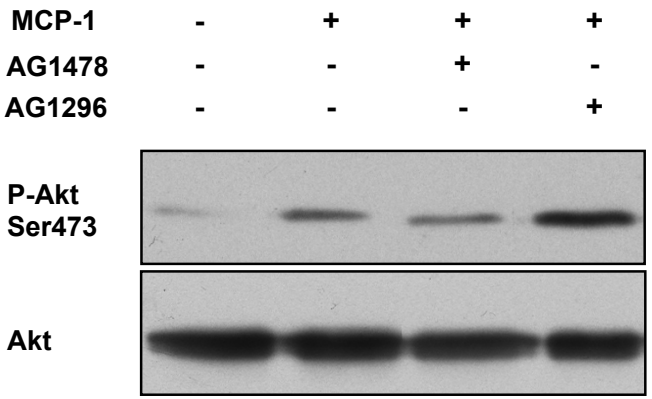
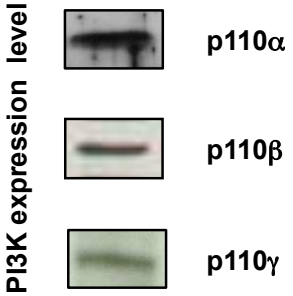


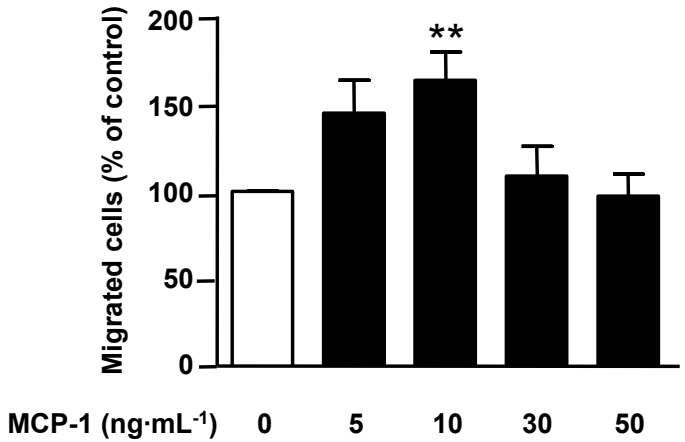
Supplementary figure 1. Akt phosphorylation induced by MCP-1 in human aortic SMC does not require tyrosine kinase receptor transactivation.
Human aortic SMC were pre-treated for 30 min with EGFR or PDGFR inhibitors (AG1478 : 300 nM, AG1296 : 10 µM) and stimulated with MCP-1 (10 $ng \cdot mL^{-1}$, 5 min). Akt phosphorylation and expression were analyzed with antibodies against phosphorylated Akt (serine 473) and total Akt.

- Supplementary Figure 1-



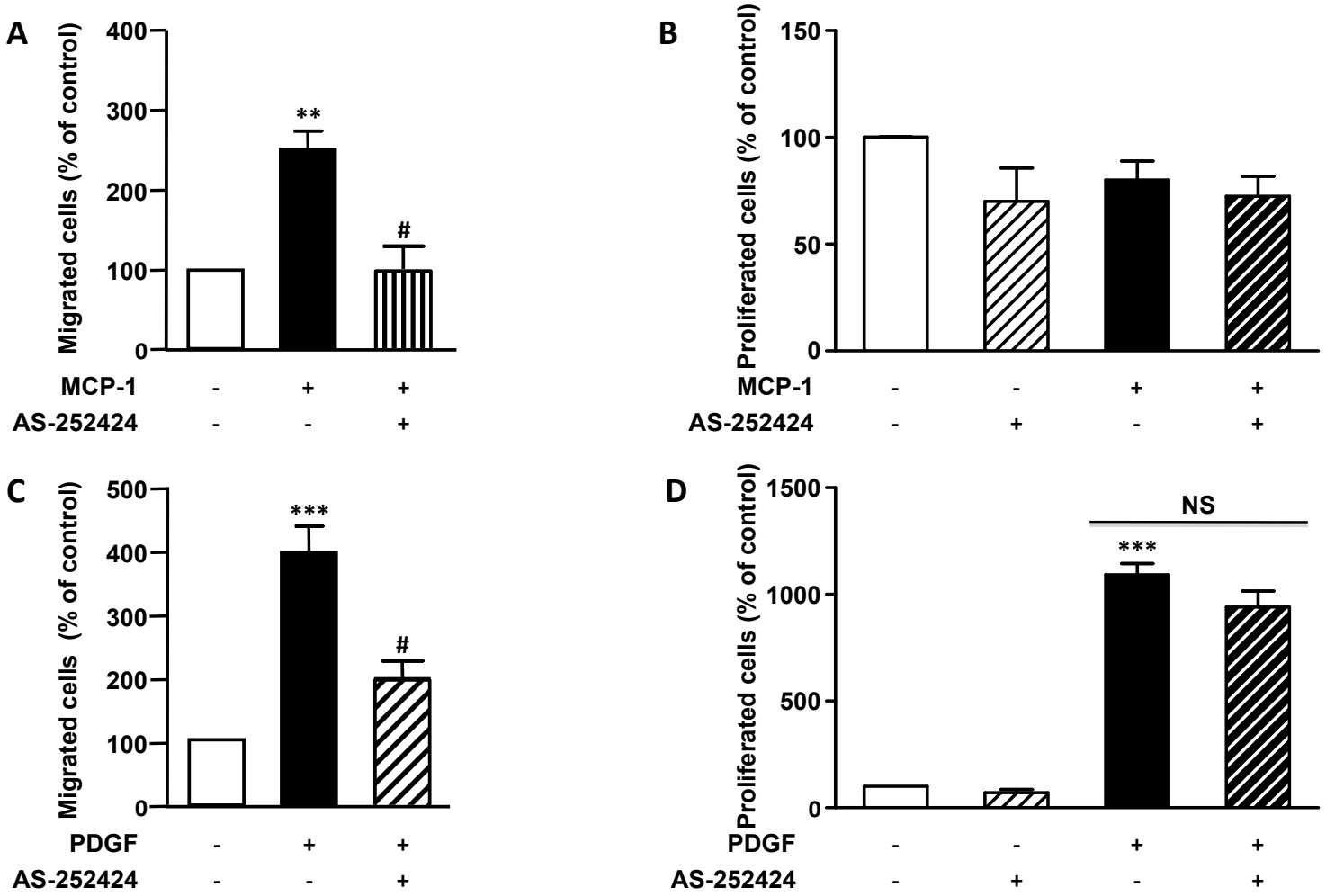
Supplementary figure 2. PI3K isoforms are expressed in pig aortic SMC. PI3K isoforms expression was analyzed with specific antibodies against p110α, p110β, p110γ in pig aortic SMC.

- Supplementary Figure 2-



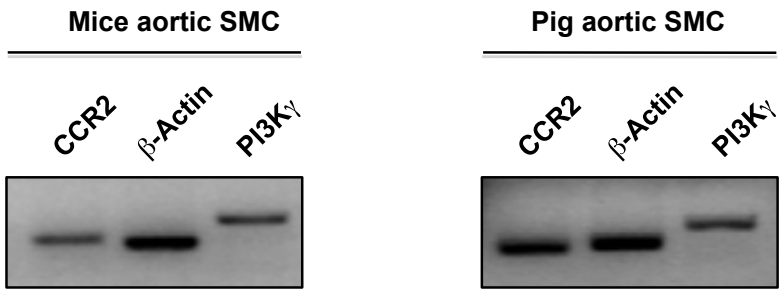
Supplementary figure 3. Dose-response of MCP-1 induced aortic SMC migration.
Pig aortic SMC migration was quantified with a wound-healing assay using the Oris™ cell migration kit. Confluent SMC were treated with the indicated concentrations of MCP-1 in serum-free medium and allowed to migrate in the wound surface for 48 h. Migrated cells were stained with DAPI and counted under a fluorescence microscope. Results are expressed as a percentage of control.
***P* < 0.01, compared to control.

- Supplementary Figure 3-



Supplementary Figure 4. PI3Kγ is essential to MCP-1 and PDGF induced aortic SMC migration and not proliferation.
A, C, Pig aortic SMC migration was quantified with a chemotaxis assay using the Boyden chamber cell migration system. SMC were treated with MCP-1 (10 ng·mL^{-1}) only or together with a specific PI3Kγ inhibitor (AS-252424 : 100 nM) (A) and with PDGF (10 ng·mL^{-1}) only or together with AS-252424 (100 nM) (C).
$^{**}P < 0.01$, $^{***}P < 0.001$ compared to control, $^{\#}P < 0.05$ compared to MCP-1 or PDGF stimulation.
B, D, Pig aortic SMC proliferation was quantified with BrdU staining. SMC were treated with MCP-1 (ng·mL^{-1}) only or together with AS-252424 (100nM) (B), and with PDGF (10 ng·mL^{-1}l) only or together with the same specific PI3Kγ inhibitor (D). $^{***}P < 0.001$ compared to control.

- Supplementary Figure 4-



Supplementary figure 5. PI3Kγ and CCR2 are expressed in mouse and pig aortic SMC.
Reverse transcription PCR products of CCR2 and PI3Kγ are analyzed in mouse and pig aortic SMC. β-Actin product was used as a positive control.

- Supplementary Figure 5-

Supplementary Methods

Boyden chamber migration assay

Cell migration was assessed by using a modified method of Boyden chamber assay. This method used a fluorescence blocking membrane that blocked wavelengths between 490 and 700 nm. Membrane inserts were coated with collagen (50 µg·mL^{-1}) overnight at 4 °C. Then, nonspecific adherence was blocked by incubation with 0.5 % BSA in PBS for 1 h at 37 °C. SMC were labeled with a lipophilic carbocyanine, CM-DiI (10 µM), in DMEM containing 0.2 % BSA 24 h before carrying out the migration assay. Labeled VSMC (5×10^4) suspended in migration medium (DMEM without phenol red containing 0.2 % BSA) were incubated for 30 min at 37 °C with or without PI3Kγ inhibitor AS-252424 (100 nM) and then transferred to the upper chamber. MCP-1 (10 ng·mL^{-1}) or PDGF (10 ng·mL^{-1}) was added to the lower chamber. Fluorescence of migrating cells was measured at 6 h on a plate reader at excitation/emission wavelengths of 544/590 nm. We checked that fluorescence was linearly related to cell number in the range used for these experiments.

BrdU incorporation assay

VSMC were seeded in 6 well plates until attachment. Cells were incubated for 48 h with growth medium (DMEM containing 10 % FCS), or blocking medium (DMEM containing 0,2 % BSA) supplemented or not with agonists (MCP-1, 10 ng·mL^{-1} or PDGF, 10 ng·mL^{-1}) and PI3Kγ inhibitor AS-252424 (100 nM). BrdU (10 µM), a thymidine analog, was added during 24 h into the wells. For anti-BrdU immunostaining, cells were washed two times with PBS, fixed with ice-cold 80 % ethanol for 20 min, then 100 % ethanol for 20 min. Cells were permeabilized with 0,25 % Triton X-100 in PBS for 20 min. To allow access for BrdU antibodies, DNA was denatured through the action of 4 M HCl for 20 min. HCl was neutralized by carefully washing three times with PBS. Non specific sites were saturated with PBS containing 0,5 % BSA and 0,1 % Tween-20 for 20 min before incubation with FITC-conjugated anti-BrdU antibody (eBioscience) overnight at 4 °C. Cells stained with anti-BrdU antibody were counted using fluorescence microscope (ApoTome, Zeiss).

Reverse transcription PCR and PCR

Total RNA from pig or mouse SMC was extracted with Trizol reagent (Invitrogen). Phase separation was performed with chloroform and RNA precipitation with isopropanol. RNA

concentrations were determined using a spectrophotometer. One microgram of total RNA was used for cDNA synthesis with hexameres. The cDNA was amplified by PCR with Taq platinum (Invitrogen). The primers to amplify CCR2 RNA, β-Actin RNA and PI3Kγ RNA were designed using the Primer Express software. The primers sequences used are summarized in the following table:

	Sus scrofa Primers	*Mus musculus* Primers
CCR2	5'-TTGTGTGACCCAAGAGAGACTTACG-3' (F) 5'-GTTACAGCCAAACCATCCTAAAGC-3' (R)	5'-AAGGGGCCACCACACCGTATGA-3' (F) 5'-GCAGCATAGTGAGCCCAGAATGGT-3' (R)
PI3Kγ	5'-CCCACTCACAGCTGAAGACA-3' (F) 5'-CAGCAGAAATCTGGCTAGGG-3' (R)	5'-GGCCCAAAGCAATGTAGTGT-3' (F) 5'-ACATGGTGGACACACGAGAA-3' (R)
β-actine	5'-AGAGCGCAAGTACTCCGTGT-3' (F) 5'-AAAGCCATGCCAATCTCATC-3' (R)	5'-AGCCATGTACGTAGCCATCC-3' (F) 5'-CTCTCAGCTGTGGTGGTGAA-3' (R)

After amplification, the samples were separated on an agarose gel and RT-PCR products for CCR2, PI3Kγ, and β-actine were visualized by ethidium bromide fluorescence.