Supplemental materials

Methods

Real-time RT-PCR

The primers for real-time RT-PCR are as follows. MMP-2: sense 5′-CGCTCAGATCCGTG GTGA-3′ and antisense 5′-CGCCAAATAAACCGGTCCTT-3′; TIMP-1: sense, 5'-TATCCGGTACGCCTACACCC-3' and antisense, 5'-TGGGCATATCCACAGAGGCT-3'; CB2R: sense, 5'- GTGATCTTCGCCTGCAACTTT -3' and antisense, 5'- GGAGTCGACCCCGTGGA -3'.

Chemotaxis assay

Chemotaxis assay was performed in Transwell inserts (6.5 mm) fitted with polycarbonate filters (8-μm pore size; Corning, Acton, MA). DC cultured with TNFα, IL-1β, IL-6, and PGE2 with or without GP1a (5 or 1 μM) for indicated timepoints were tested for migration to either serum-free medium or CCL19 (100 ng/mL) placed in the lower chambers. DC (1×10^5 cells in 0.1 mL) were added to the upper Transwell chambers and allowed to migrate for 2h at 37°C. Migrated DC harvested from the lower chambers were counted by FACS (60-second counts).

Figure Legends

Figure S1. GP1a neither modulates expression of CCR7 nor affects chemotaxis of mature DC to CCL19.

DC were treated with cytokine cocktail + PGE2 (CCP) with or without different concentrations of GP1a for 24 and 48h. Controls consisted of DC cultured in medium. (**A**) RNA was extracted and subjected to qRT-PCR for CCR7. (**B**) DC ($1x10^5$ cells) were placed in the upper chambers of a Transwell plate and the bottom chambers were filled with serum free medium with or without CCL19. 2h later the migrating cells were collected from the lower chambers and counted by

FACS. *** p<0.001 compared with medium. Data are representative of 2 independent experiments.

Figure S2. Effect of GP1a on MMP-9, MMP-2 and TIMP-1 expression in CCP-activated DC.

DC were treated with CCP with or without GP1a (5 μM) for 24h. RNA was extracted and subjected to qRT-PCR for (**A**) MMP-9, (**B**) MMP-2, and (**C**) TIMP-1.

Figure S3. Inhibition of MMP-9 production by GP1a is mediated through the CB2R.

(**A**) RNA was extracted from purified CD11c+ BMDC from CB2R+/+ or CB2R-/- mice and subjected to qRT-PCR for CB2R. Control samples (no RT) IS included for BMDC. Data are represented as ratio of CB2R to β-actin. (**B**) BMDC from CB2R+/+ or CB2R-/- mice were treated with CCP with or without various concentrations of GP1a for 24 and 48h. Culture supernatants were subjected to MMP-9 ELISA. *p<0.05, **p<0.01, *** p<0.001 compared with CCP. Data are representative of 2 (A) and 3 (B) independent experiments.

Figure S4. GP1a reduces the in vivo migration of CB2R+/+ but not of CB2R-/- DC.

DC generated from CB2R+/+ (wt) or CB2R-/- (KO) littermates were treated with CCP with or without GP1a (5 μM) for 48h, followed by labeling with the fluorescent dye PKH-26. $1X10^6$ labeled DC were injected s.c in the footpads of wild-type mice pre-injected 24h earlier with 40 ng TNFα s.c in the each of the hind footpads. Recipient mice received CCP-treated DC (control) in the right footpad and CCP+Gp1a-treated DC in the left footpad. 48h later, cells were collected from popliteal lymph nodes and PKH-26-labeled cells were analyzed by FACS. Representative FACS plots are shown. No cells control indicates cells from popliteal lymph nodes of mice that were injected with TNFα but received no DC.

Fig. S1

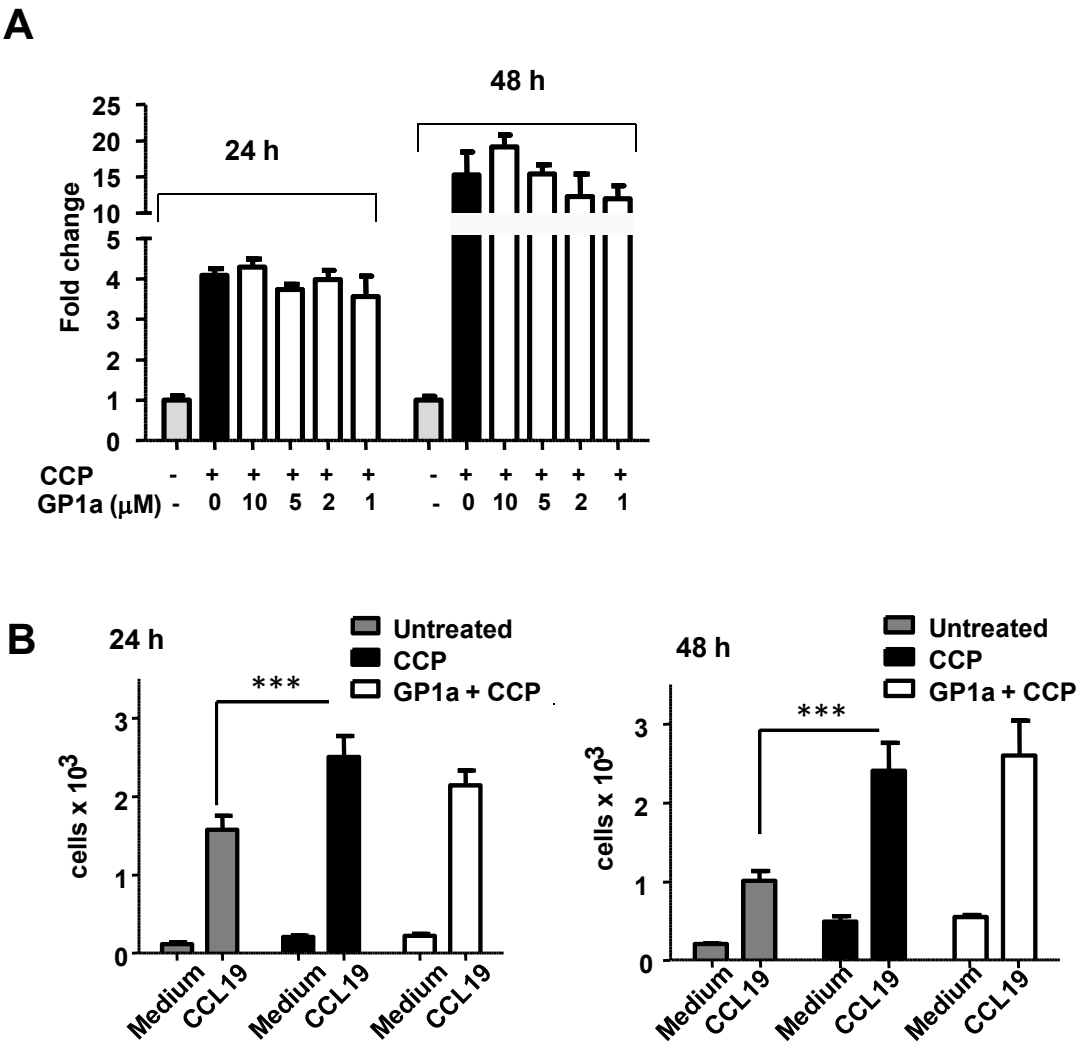

A
48 h
24 h
Fold change
25
20
15
10
5
4
3
2
1
0
CCP - + + + + + - + + + + +
GP1a (μM) - 0 10 5 2 1 - 0 10 5 2 1
B
24 h
48 h
Untreated
CCP
GP1a + CCP
***
cells x 10^3
3
2
1
0
Medium
CCL19

Fig. S2

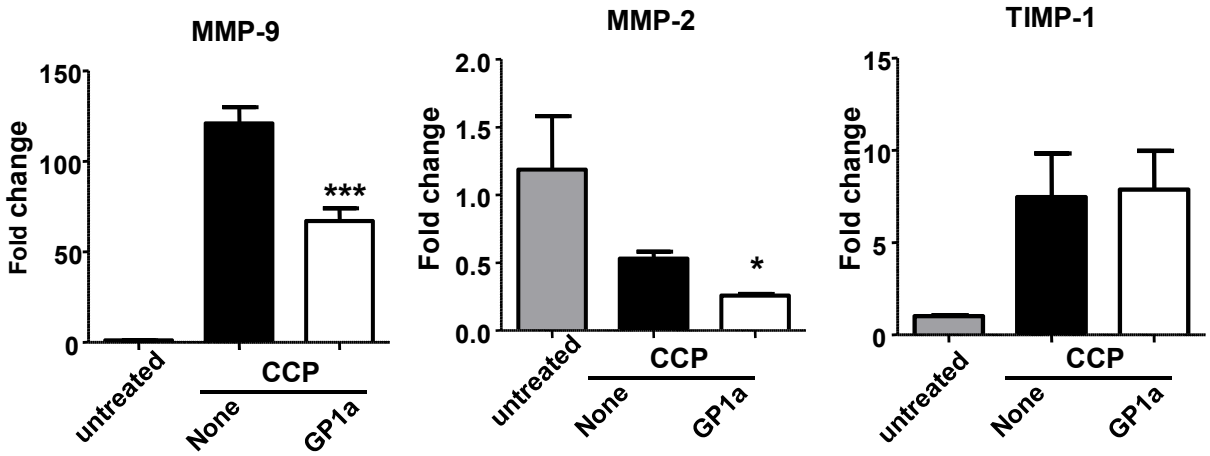

MMP-9
Fold change
150
100
50
0
***
untreated
CCP
None
GP1a
MMP-2
Fold change
2.0
1.5
1.0
0.5
0.0
*
untreated
CCP
None
GP1a
TIMP-1
Fold change
15
10
5
0
untreated
CCP
None
GP1a

Fig. S3

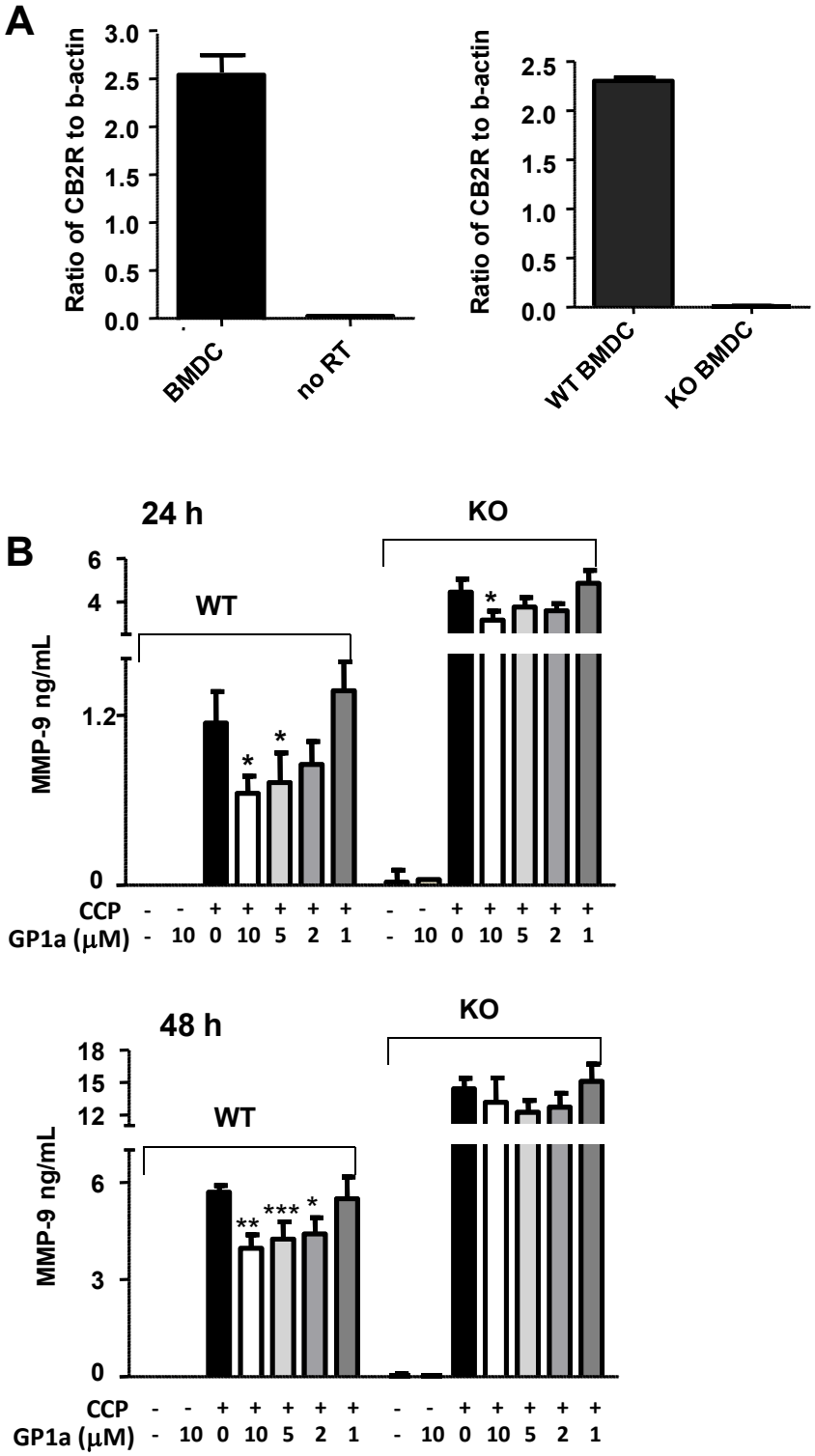
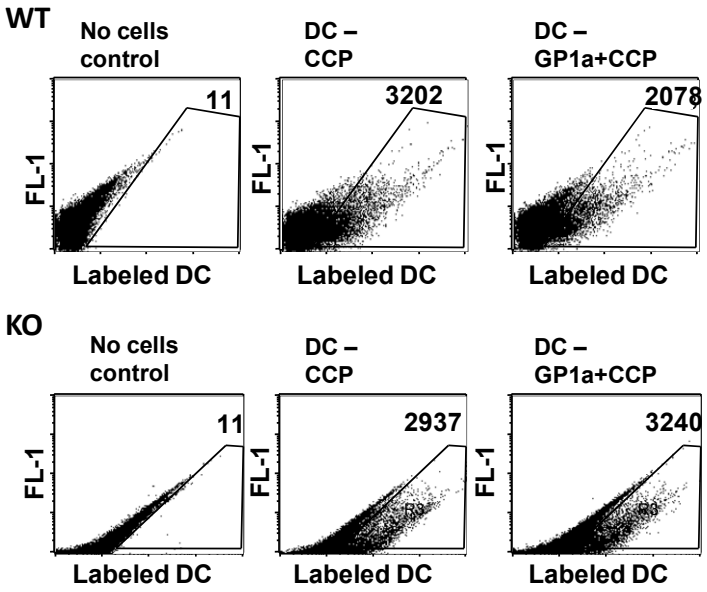

A
Ratio of CB2R to b-actin
3.0
2.5
2.0
1.5
1.0
0.5
0.0
BMDC
no RT
Ratio of CB2R to b-actin
2.5
2.0
1.5
1.0
0.5
0.0
WT BMDC
KO BMDC
B
24 h
KO
WT
MMP-9 ng/mL
6
4
1.2
0
CCP - - + + + + + - - + + + + +
GP1a (μM) - 10 0 10 5 2 1 - 10 0 10 5 2 1
48 h
KO
WT
MMP-9 ng/mL
18
15
12
6
3
0
CCP - - + + + + + - - + + + + +
GP1a (μM) - 10 0 10 5 2 1 - 10 0 10 5 2 1

Fig. S4


WT
No cells control
DC – CCP
DC – GP1a+CCP
11
3202
2078
FL-1
Labeled DC
KO
No cells control
DC – CCP
DC – GP1a+CCP
11
2937
3240
R3
FL-1
Labeled DC