Supplemental Information

Deficiency of chemokine receptor CCR1 causes osteopenia due to impaired functions of osteoclasts and osteoblasts

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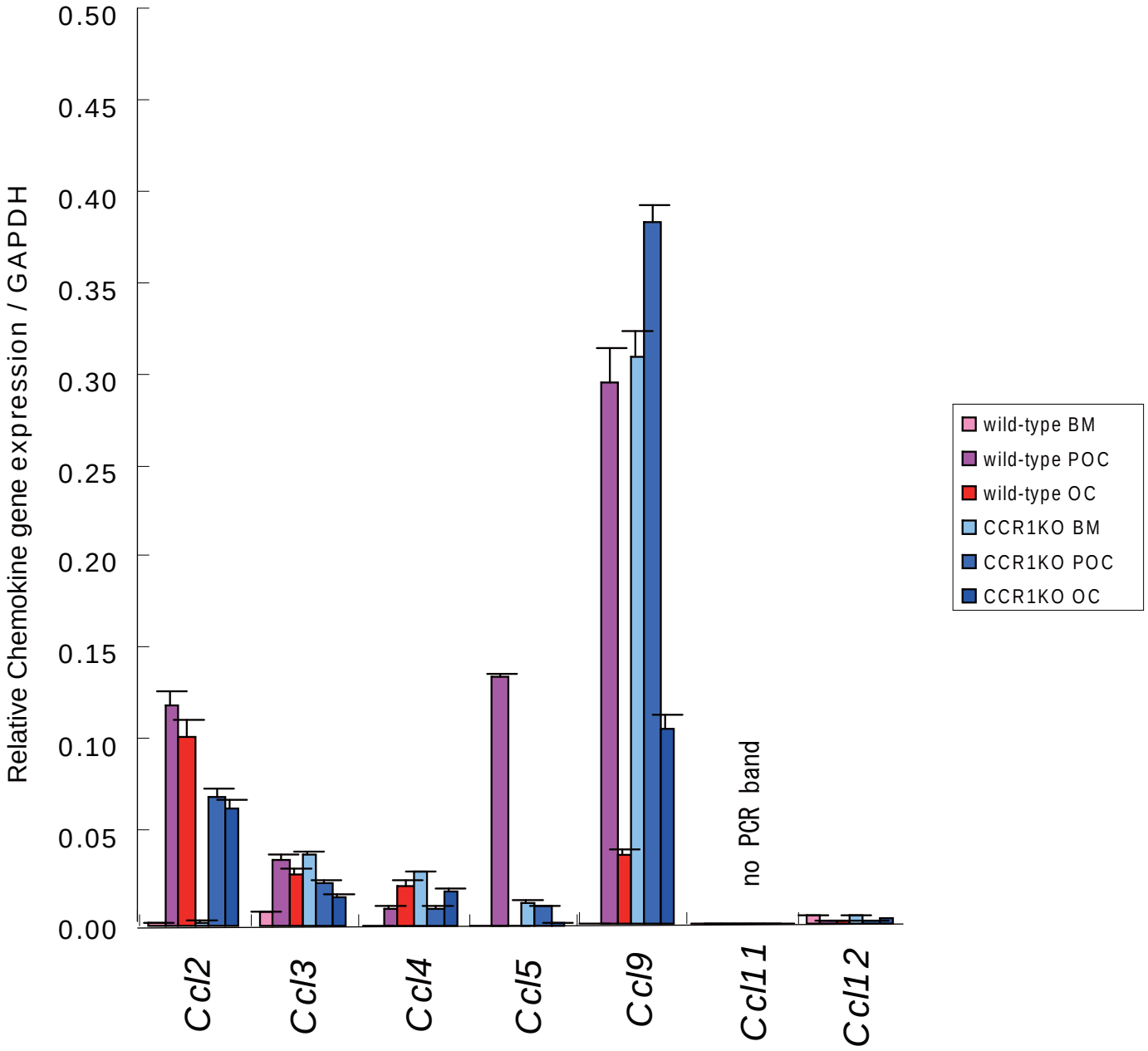
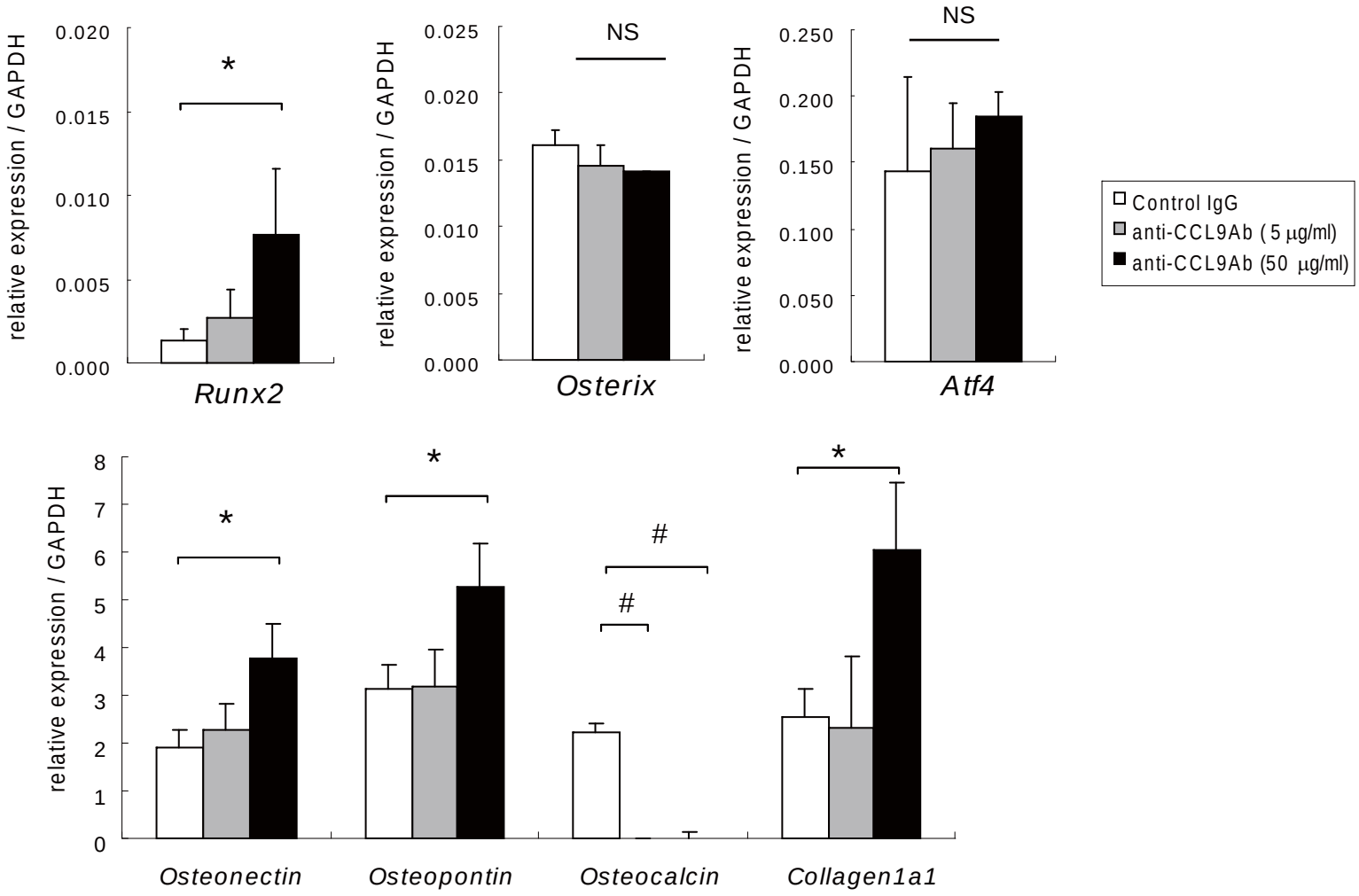
Supplemental Figure Legends

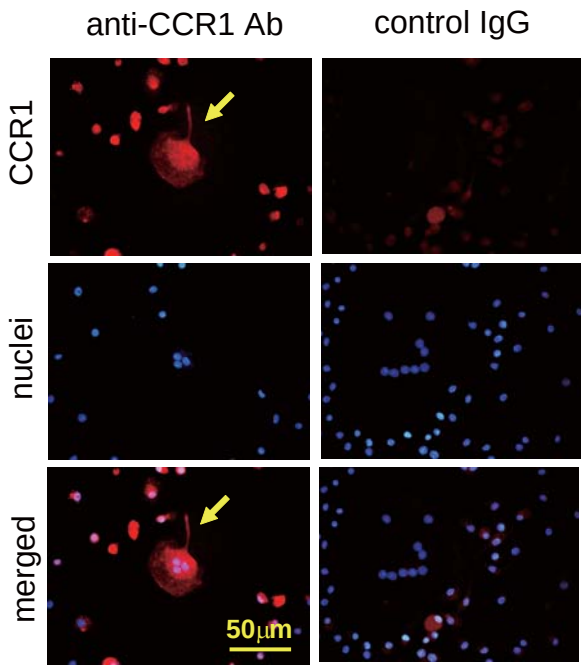
.Supplemental Figure 1. Expression of CCR1-related chemokine ligands by bone marrow macrophages, preosteoclasts and mature osteoclasts by wild-type and CCR1$^{-/-}$ mice. *The panel* shows the relative expression levels of CCR1-related chemokines (*Ccl3*, *Ccl4*, *Ccl5*, *Ccl9*, and *Ccl11*) and CCR2-related chemokines (*Ccl2* and *Ccl12*) by *Ccr1*$^{-/-}$ osteoblasts as measured by realtime Q-PCR (mean ± SEM, n=3).

Supplemental Figure 2. The effect of chemokine neutralization during osteoblastogenesis. Total RNAs were isolated from osteoblastic cells after treatment for 21 days at the indicated concentrations of anti-CCL9 neutralizing antibodies. Realtime Q-PCR quantified the relative expression levels of osteoblast-related transcriptional factor mRNAs (*Runx-2*, *Osterix, Atf4)* and osteoblast-related marker mRNAs (*Osteonectin, Osteopontin, Osteocalcin*, and *Collagen1α1*). Data are expressed as the copy numbers of these markers as normalized to *Gapdh* expression (mean ± SEM, n=4).

Supplemental Figure 3. immunostaining of CCR1 by osteoclasts. *The panel* shows the expression of CCR1 protein in mature osteoclasts as detected by immunostaining (shown in red). Nuclei were counterstained with by hoechst33258 dye (shown in blue) (magnification ×400)**.** The arrow shows the localization of CCR1 in the pseudopod-like structure.


Relative Chemokine gene expression / GAPDH
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
wild-type BM
wild-type POC
wild-type OC
CCR1KO BM
CCR1KO POC
CCR1KO OC
no PCR band
Ccl2
Ccl3
Ccl4
Ccl5
Ccl9
Ccl11
Ccl12


relative expression / GAPDH
0.020
0.015
0.010
0.005
0.000
*
Runx2
0.025
0.020
0.015
0.010
0.005
0.000
NS
Osterix
0.250
0.200
0.150
0.100
0.050
0.000
NS
Atf4
Control IgG
anti-CCL9Ab (5 μg/ml)
anti-CCL9Ab (50 μg/ml)
8
7
6
5
4
3
2
1
0
*
*
#
#
*
Osteonectin
Osteopontin
Osteocalcin
Collagen1a1


anti-CCR1 Ab
control IgG
CCR1
nuclei
merged
50μm