Supplementary information for

The isoenzyme of Glutaminyl Cyclase is an important regulator of monocyte infiltration under inflammatory conditions

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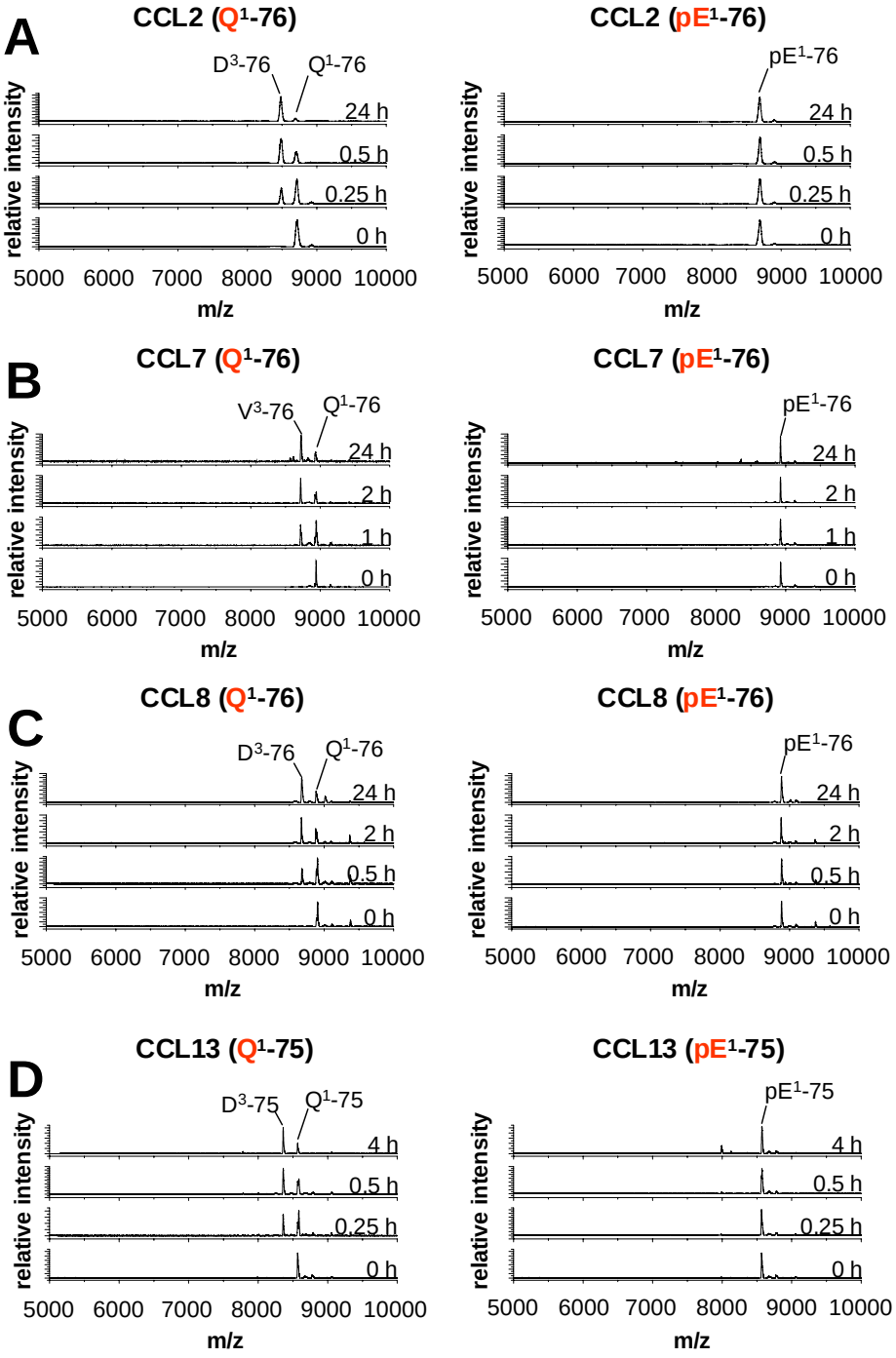
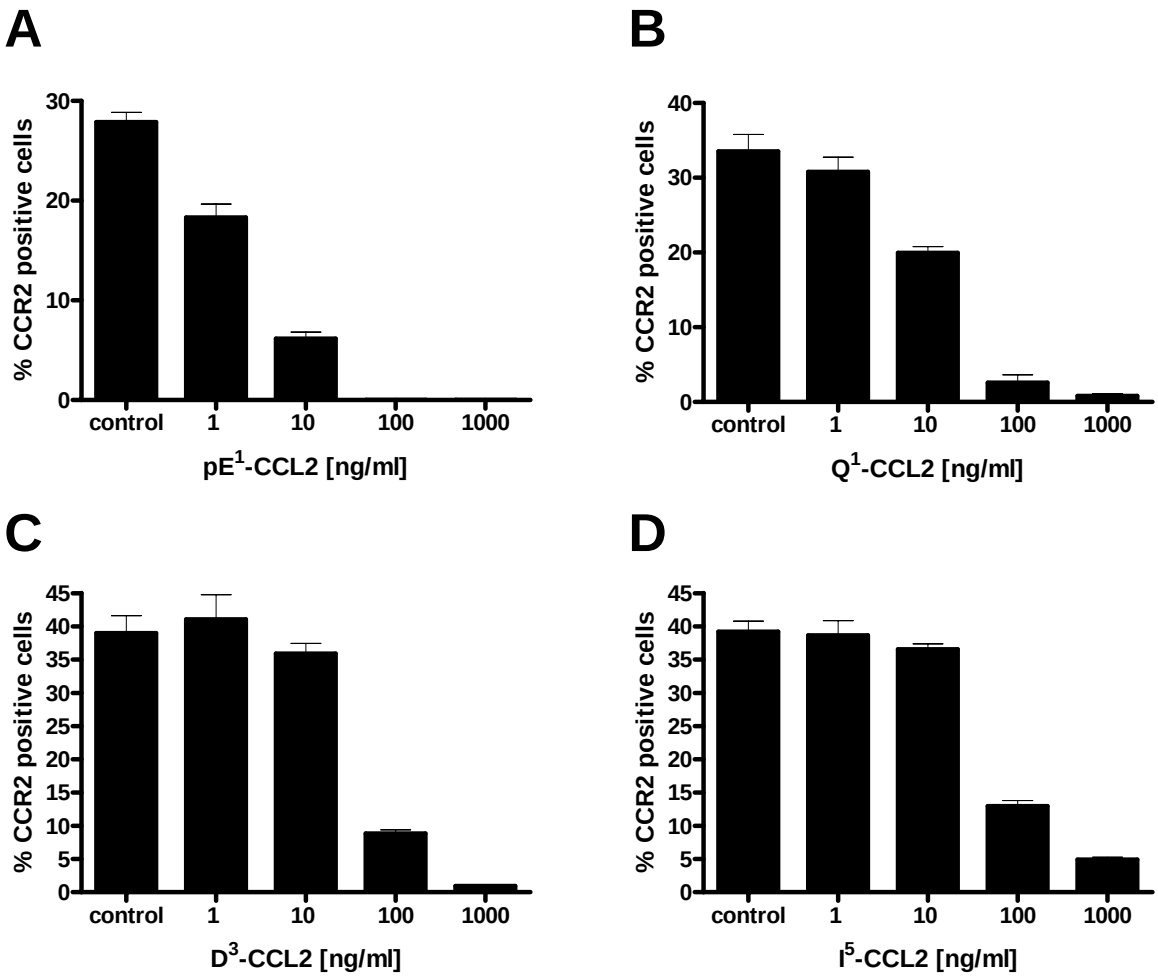
Supplemental Fig 1.



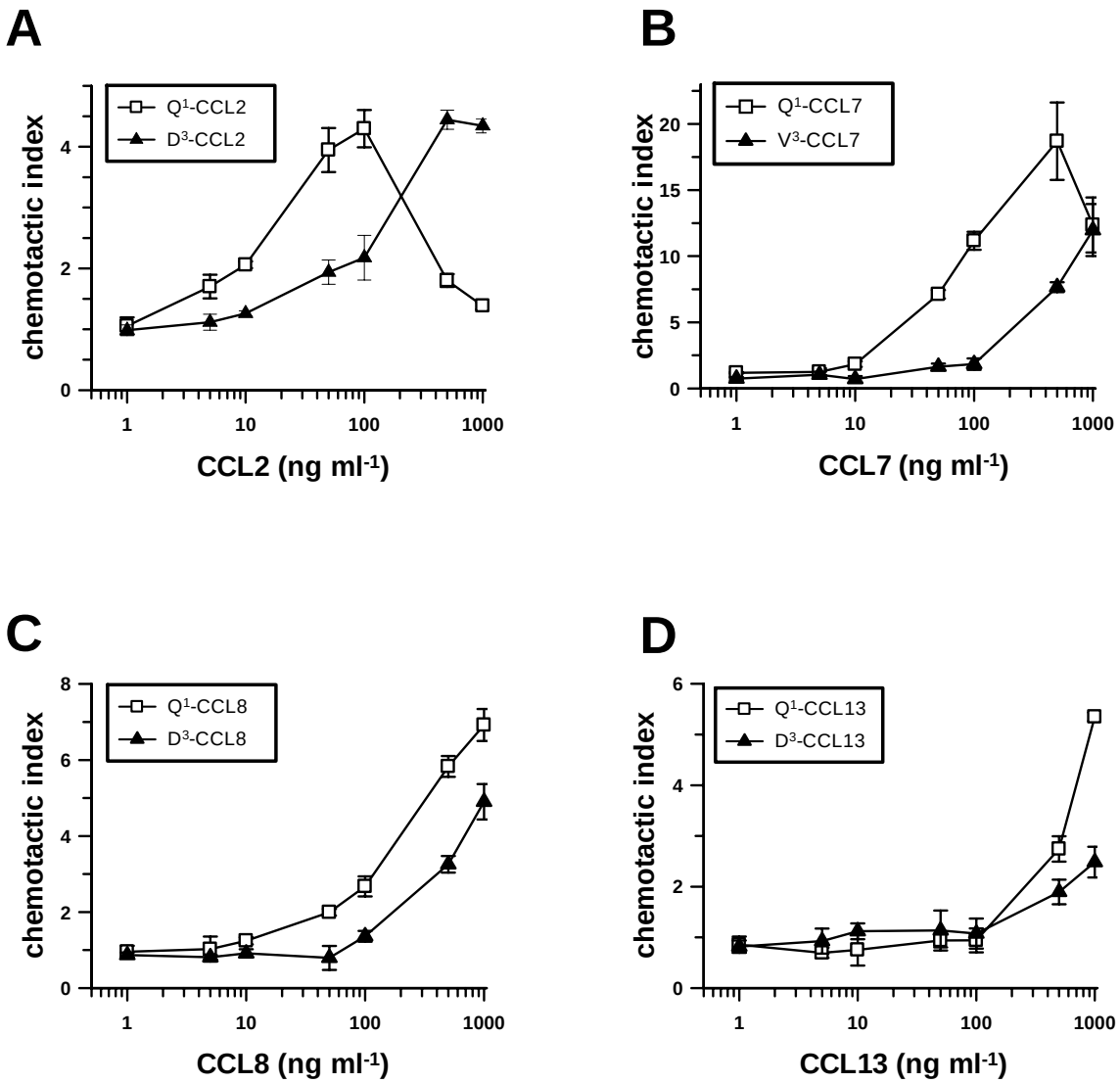
Role of N-terminal pE-residue for stability of human CCL2, CCL7, CCL8 and CCL13. Mass spectra for the time course of the degradation of human (**A**) CCL2, (**B**) CCL7, (**C**) CCL8 and (**D**) CCL13 by DP4 in absence and presence of the N-terminal pE-residue. Only peptides with an N-terminal glutaminyl residue are accessible for cleavage by DP4.

Supplemental Fig 2.



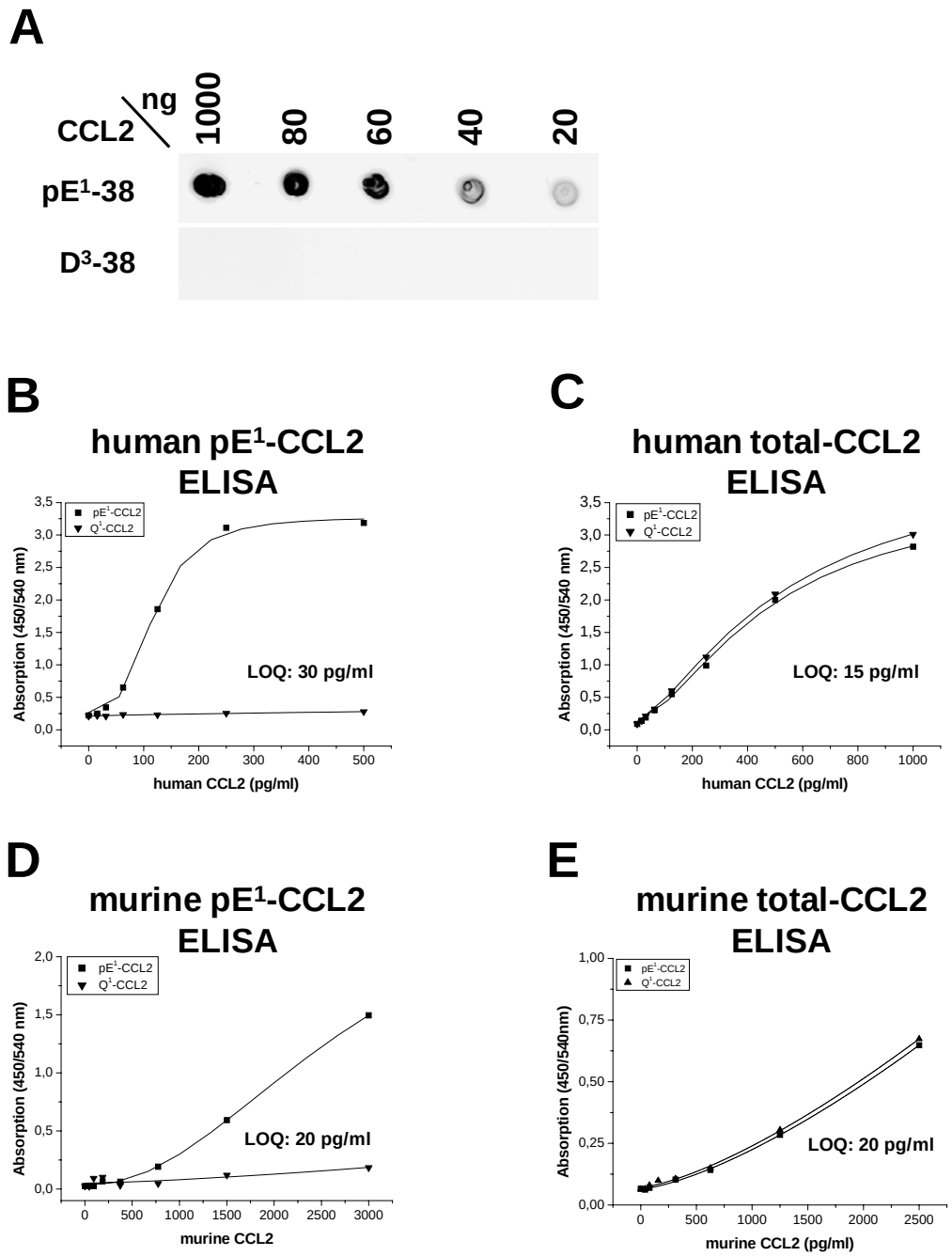
Receptor internalization by different human CCL2-variants. Analysis of CCR2 receptor activation/internalization using human THP-1 monocytes. THP-1 monocytes were incubated with human (**A**) pE^1-CCL2, (**B**) Q^1-CCL2, (**C**) D^3-CCL2 and (**D**) I^5-CCL2. Internalization was measured by FACS analysis (n=4).

Supplemental Fig 3.



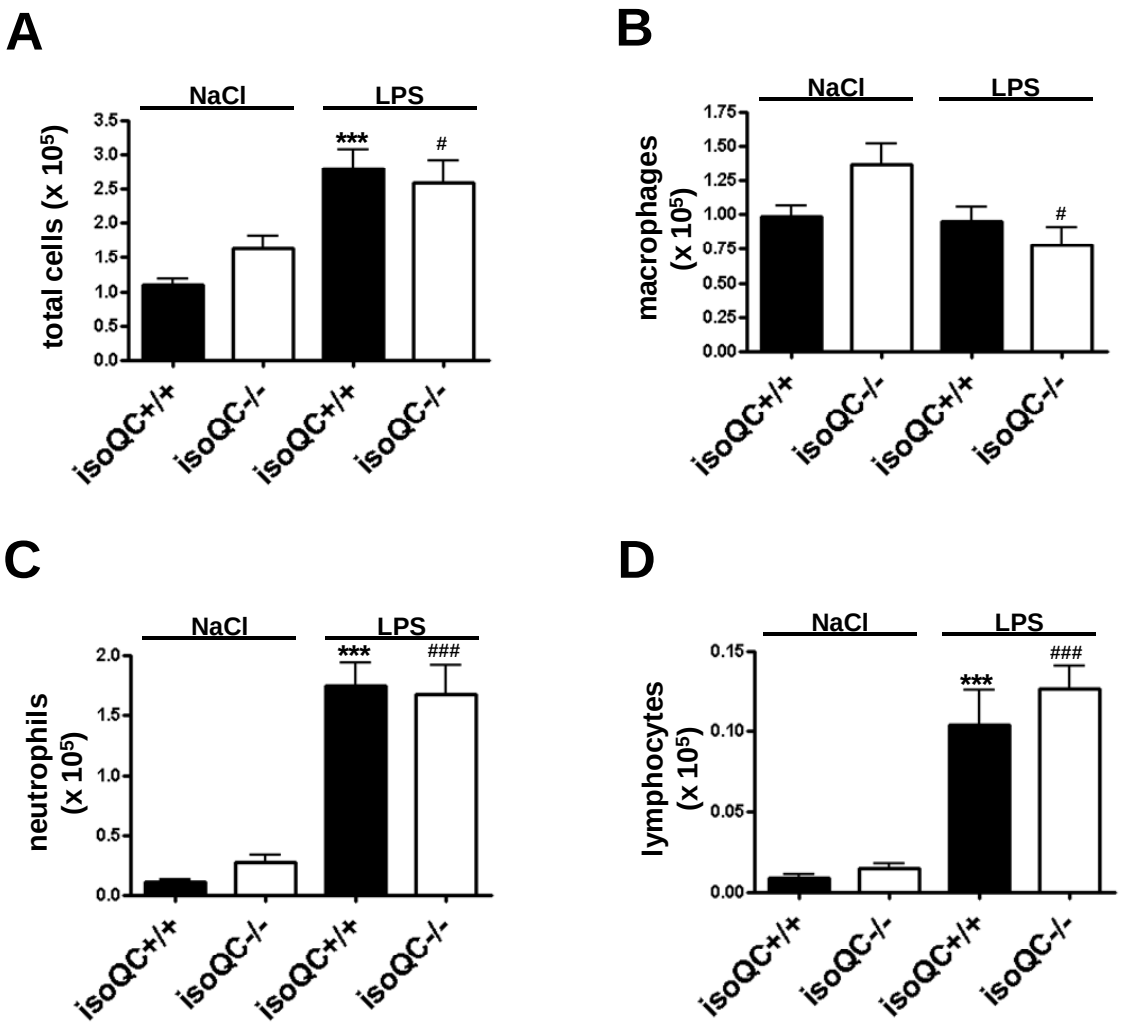
Chemotactic potential of N-terminally truncated MCPs. Comparison of the ability of (**A**) CCL2, (**B**) CCL7, (**C**) CCL8 and (**D**) CCL13 starting with N-terminal amino acid Q^1 or D^3 to attract THP-1 monocytes.

Supplemental Fig 4.



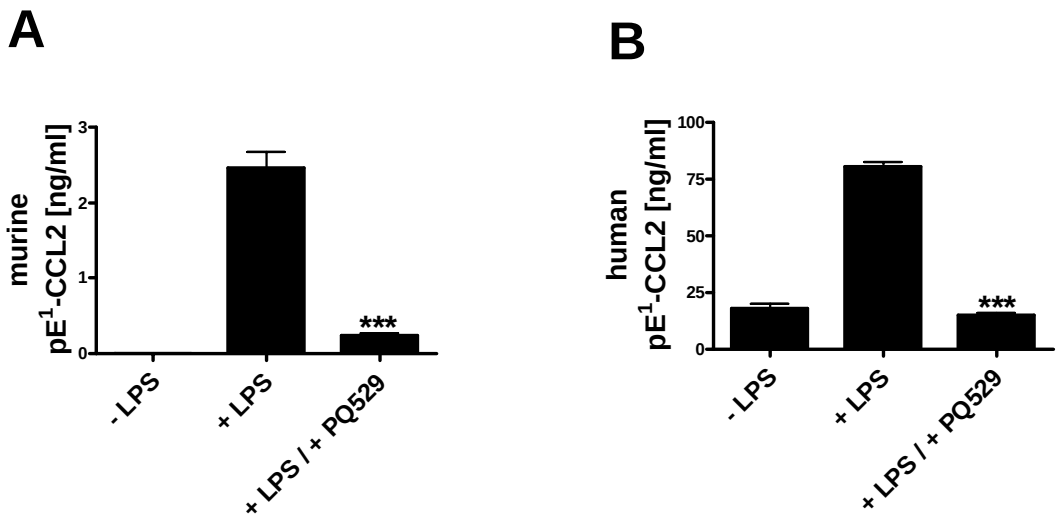
(**A**) Dot Blot analysis to assess the specificity of antibody 2C9 for human pE^{1}-CCL2 and truncated D^{3}-CCL2. 2C9 does not cross-react with truncated forms. In addition, representative standard curves and limit of quantification (LOQ) for pE^{1}-CCL2 and Q^{1}-CCL2 applied on the newly developed (**B**) human pE^{1}-CCL2 ELISA, (**C**) human total-CCL2 ELISA, (**D**) murine pE^{1}-CCL2 ELISA and (**E**) murine total-CCL2 ELISA are shown.

Supplemental Fig 5.



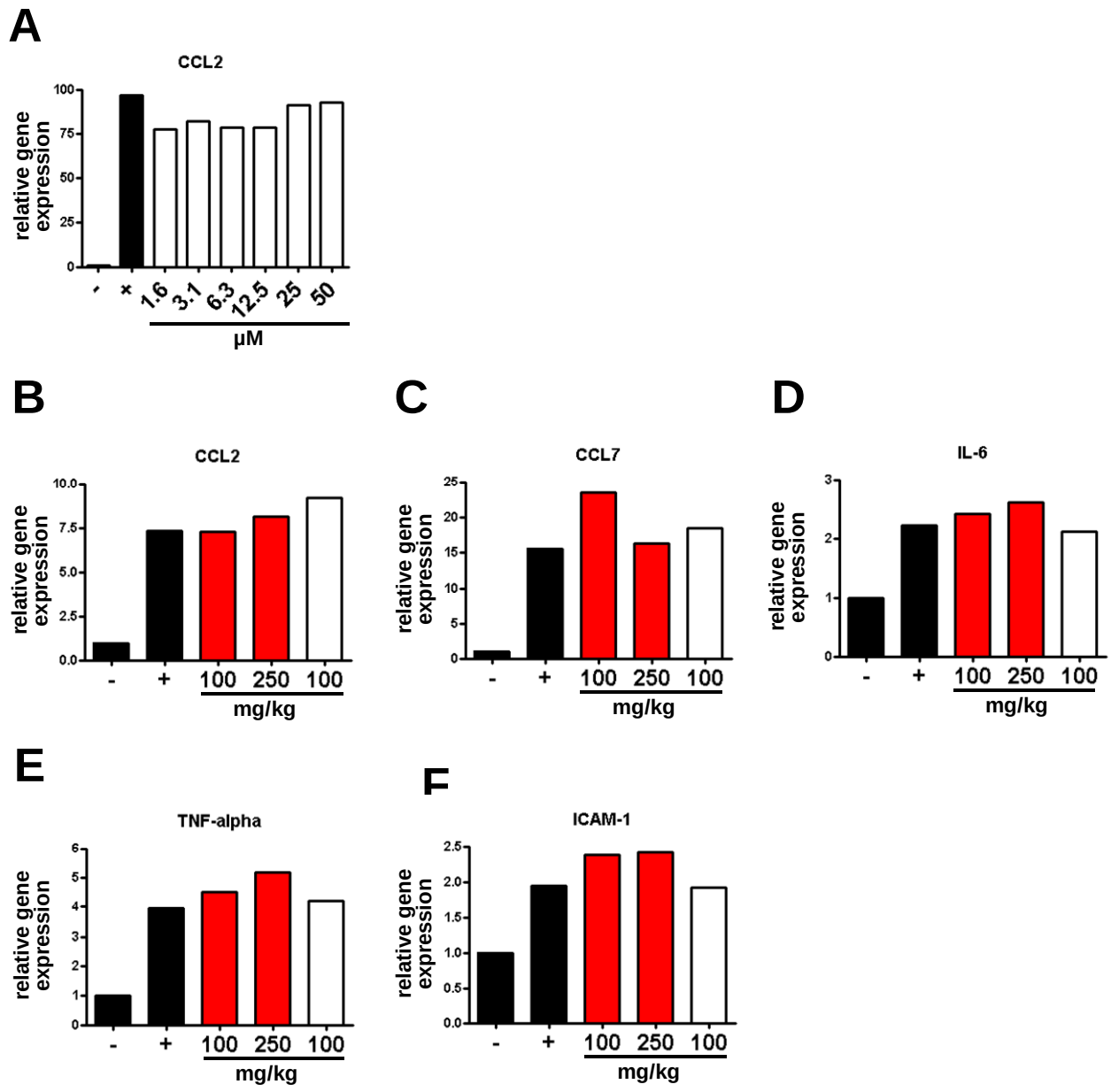
Total (**A**) and differential (**B**-**D**) cell count in the bronchoalveolar fluid of isoQC knockout mice (isoQC$^{-/-}$) compared to wild type littermates (isoQC$^{+/+}$) showing the presence of (**B**) macrophages, (**C**) neutrophils and (**D**) lymphocytes (***, P<0.001 vs. isoQC$^{+/+}$, $^{###}$, P<0.001, $^{#}$, P<0.05 vs. isoQC$^{-/-}$, Student's t-test, n=7-8, mean ± SEM)

Supplemental Fig 6.



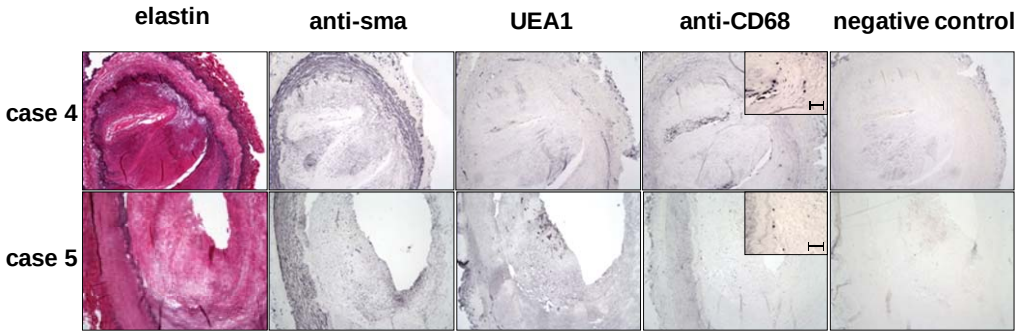
(**A**) Inhibition of pE-formation by PQ529 in primary macrophages isolated from C57BL/6J wildtype mice (***, P<0.001 vs. LPS, ANOVA followed by Tukey post-hoc test, n=3, mean ± SEM). (**B)** Inhibition of pE-formation by PQ529 in primary macrophages isolated from human blood. (***, P<0.001 vs. LPS, ANOVA followed by Tukey post-hoc test, n=3, mean ± SEM).

Supplemental Fig 7.



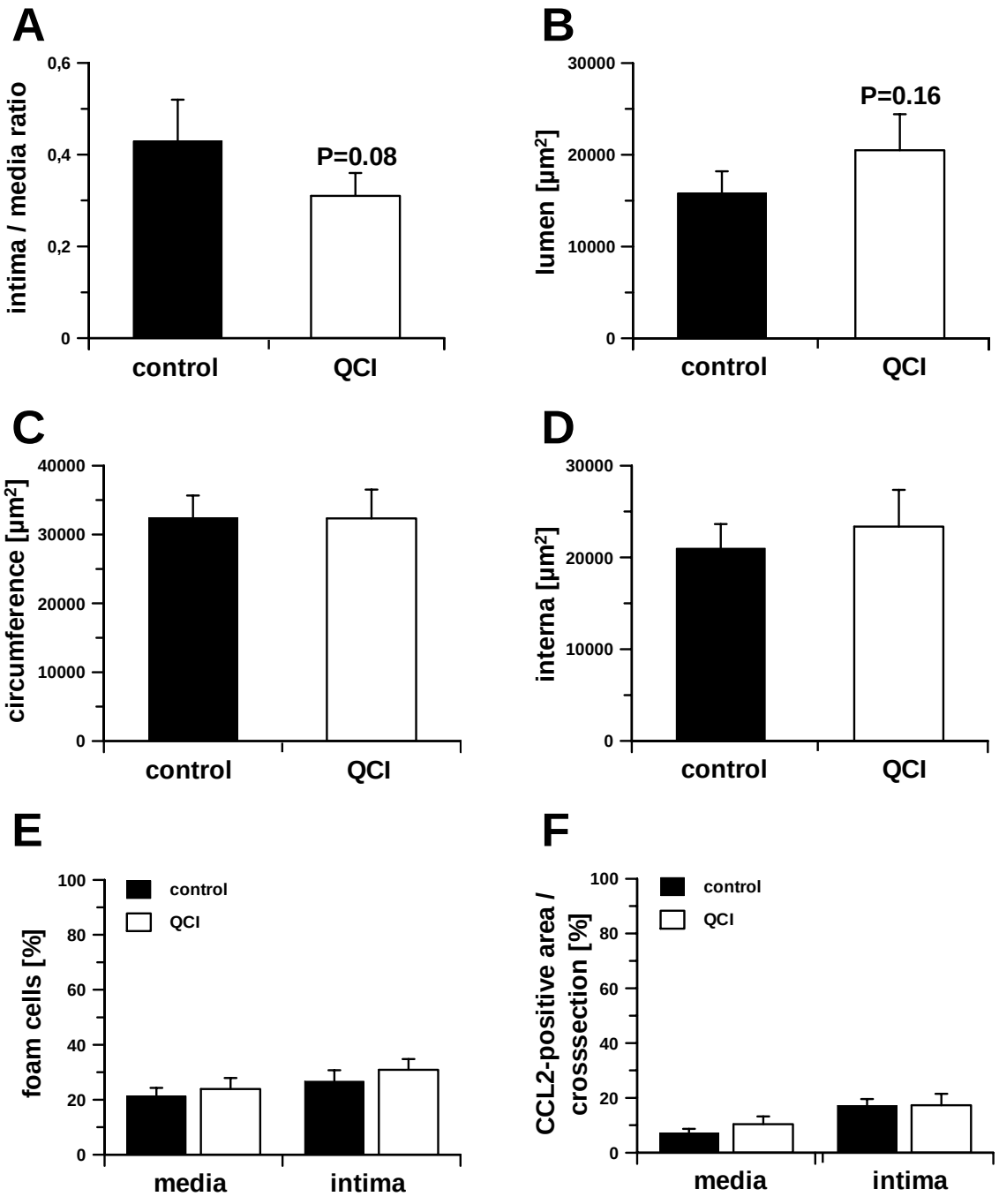
(**A**) Analysis of CCL2 expression in THP-1 cells stimulated with LPS and treated with increasing concentrations of PQ529. In addition, RT-PCR was performed from peritoneal lavage cells isolated from the experiment depicted in Figure 4E. The cells of individual animals were pooled and average gene expression of (**B**) CCL2, (**C**) CCL7, (**D**) IL-6, (**E**) TNF-alpha and (**F**) ICAM-1 was analyzed.

Supplemental Fig 8.



Analysis of human atherosclerotic arteries. Representative images of the histopathological analysis of atherosclerotic vessel segments of case 4 and 5 are exemplarily shown. Stainings include elastin, smooth muscle cell actin (anti-sma), endothelial cells (UEA1) and CD68-positive macrophages (anti-CD68) compared to a negative control.

Supplemental Fig 9.



Additional histomorphometric analysis of cuffed vessel segments. Morphometric quantification of (**A**) the intima /media ratio, (**B**) remaining lumen, (**C**) the circumference of the cuffed vessel segments, (**D**) the area of the interna, (**E**) foam cell content and (**F**) CCL2-immunoreactivity (control vs. QCI, Student's t-test, n=10).

Supplemental Tables

Supplemental Table 1.

Determination of cross reactivity of pE^1-CCL2 specific monoclonal antibody 2C9 to different pE- peptides

pE^1-peptides	**% cross reactivity**
CCL2(pE^1-38)	100
CCL2(P^2-38)	< 1
CCL2(D^3-38)	< 1
CCL8(pE^1-11)	< 1
Abeta(pE^3-40)	< 1
Big Gastrin	< 1
GnRH	< 1
Neurotensin	< 1
Orexin A	< 1
Fibronectin	< 1
Collagen 1	< 1
TRH	< 1

Supplemental Table 2.

Primer sequences of primers used for real-time PCR.

Name	Forward	Reverse
CCL2 (human)	GCCTCCAGCATGAAAGTCTC	CAGATCTCCTTGGCCACAAT
GAPDH (human)	ACCCAGAAGACTGTGGATGG	TTCTAGACGGCAGGTCAGGT
RPII (human)	CGCTTAAGCCTTCCAACAAG	GAGGACGACCTTGCTGTCTC
YWHAZ (human)	AGCAGGCTGAGCGATATGAT	TCTCAGCACCTTCCGTCTTT
CCL2 (mouse)	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCCTTCTTG
CCL7 (mouse)	AAT GCA TCC ACA TGC TGC TA	CTT TGG AGT TGG GGT TTT CA
ICAM-1 (mouse)	AGGGCTGGCATTGTTCTCTA	CTTCAGAGGCAGGAAACAGG
IL-6 (mouse)	CCGGAGAGGAGACTTCACAG	TTCTGCAAGTGCATCATCGT
PHKA1 (mouse)	CAGGTTCCTTCCGTTCATGT	GTGCCGAATTTCACCAACTT
TNF-alpha (mouse)	GAACTGGCAGAAGAGGCACT	AGGGTCTGGGCCATAGAACT
YWHAZ (mouse)	TGAAGCCATTGCTGAACTTG	GTTGGAAGGCCGGTTAATTT