Supplemental Information

Figure S1. Time profile of monokine secretion. Secreted IL-6 (left) and MCP-1 (right) concentrations were measured after 4 h culture of aortic explants from sham- (n=6) or Ang II-infused mice after 6 d or 10 d (n=7 in each group). Data are presented as mean ± SEM. The differences in IL-6 for sham versus Ang-II at 6 days and at 10 days are significant at $p<0.001$. The differences in MCP-1 for sham versus Ang-II at 6 days and at 10 days are also significant at $p<0.01$. In neither case is the difference between 6 d and 10 d significant. Data were analysed using one-way analysis of variance (3 groups), followed by a Tukey's HSD post-hoc test for significance.

Figure S2. Time profile of aortic digestion. Dissected aortae were subjected to collagenase- elastase digestion for the indicated times (Methods). At each time point, the weight of undigested tissue and dissociated cells was determined and plotted. Greater than 50 % digestion is seen within 1 h of collagenase incubation.

Figure S3. β-actin staining in undigested tissue. Dissected aortae were subjected to collagenase- elastase digestion for the indicated times (Methods). At each time point, undigested aortic tissue was extracted in SDS-PAGE buffer, sonicated, and fractionated by SDS-PAGE for Western immunoblot analysis. Top panel, Western immunoblot showing specific β-actin signal. Bottom panel, quantification of results.

Figure S4. Viability of CD14 $^{+}$ cell population. Collagenase-dissociated cells from unstimulated aortae (digested for 2 h, room temperature) were incubated with 8 μM ethidium homodimer solution in PBS for 15-20 min at room temperature. The cells were washed, and subjected to flow cytometric analysis. Left panel, gate containing $CD14^{+}$ cells are indicated by the circle in the SSC-A vs FSC-H dot blot. Right panel, measurement of cell viability. X axis, ethidium bromide staining intensity (emission at 585 nm). Y axis, number of cell events. 13 % of the cells in this gate were positive for ethidium staining (nonviable); 87 % were negative (viable).

Figure S5. Enlarged Figure 1B.

Figure S6. CCR2 Ab staining specificity. Immunohistochemical staining of anti-CCR2 Ab in Ang II-treated WT and $CCR2^{-/-}$ mice. CCR2 staining is red. Autofluorescence from elastin lamella is green. Secondary antibody (2^{nd} only) is negative control. Note the specific red staining only in the Ang II stimulated WT sections.

Figure S7. Enlarged Figure 3D.

Figure S1

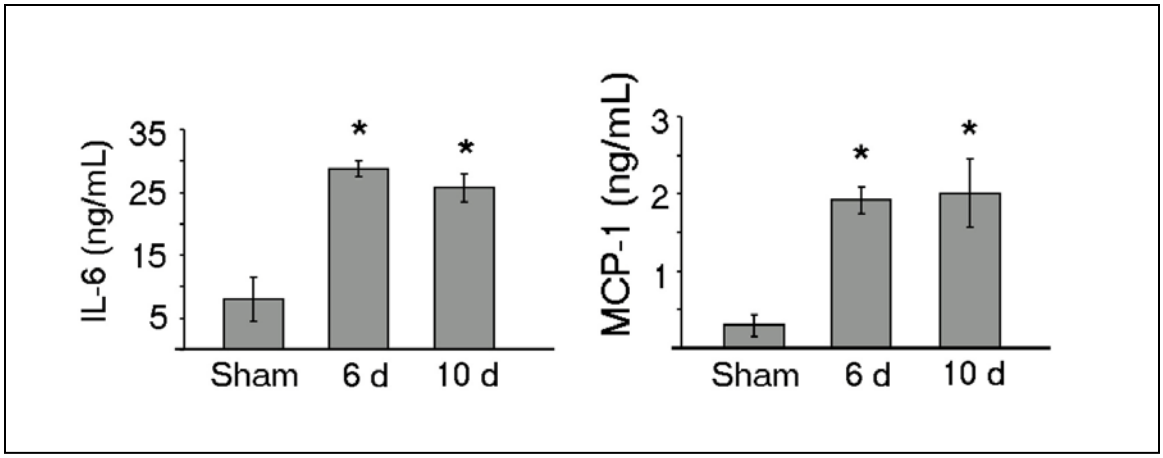

Figure S2.

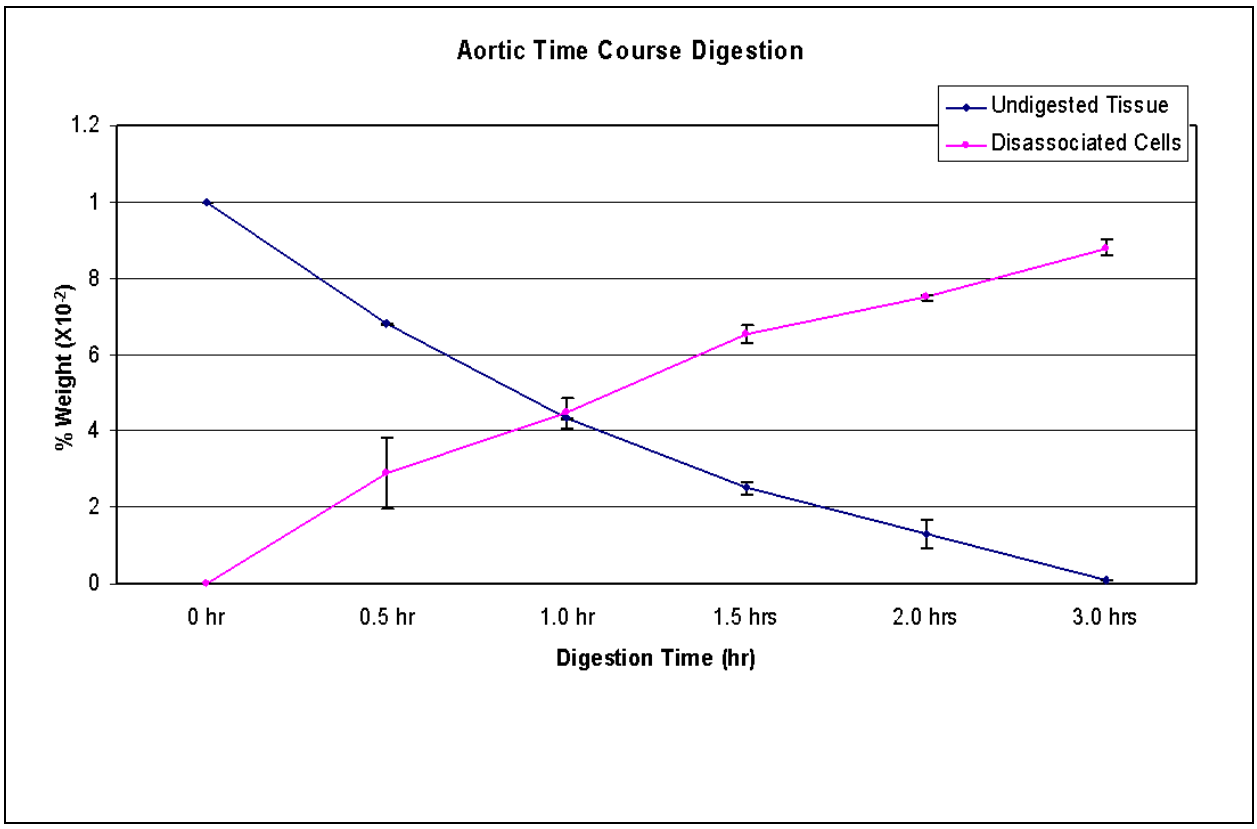
Aortic Time Course Digestion
Undigested Tissue
Disassociated Cells
% Weight (X10-2)
1.2
1
8
6
4
2
0
0 hr
0.5 hr
1.0 hr
1.5 hrs
2.0 hrs
3.0 hrs
Digestion Time (hr)

Figure S3.

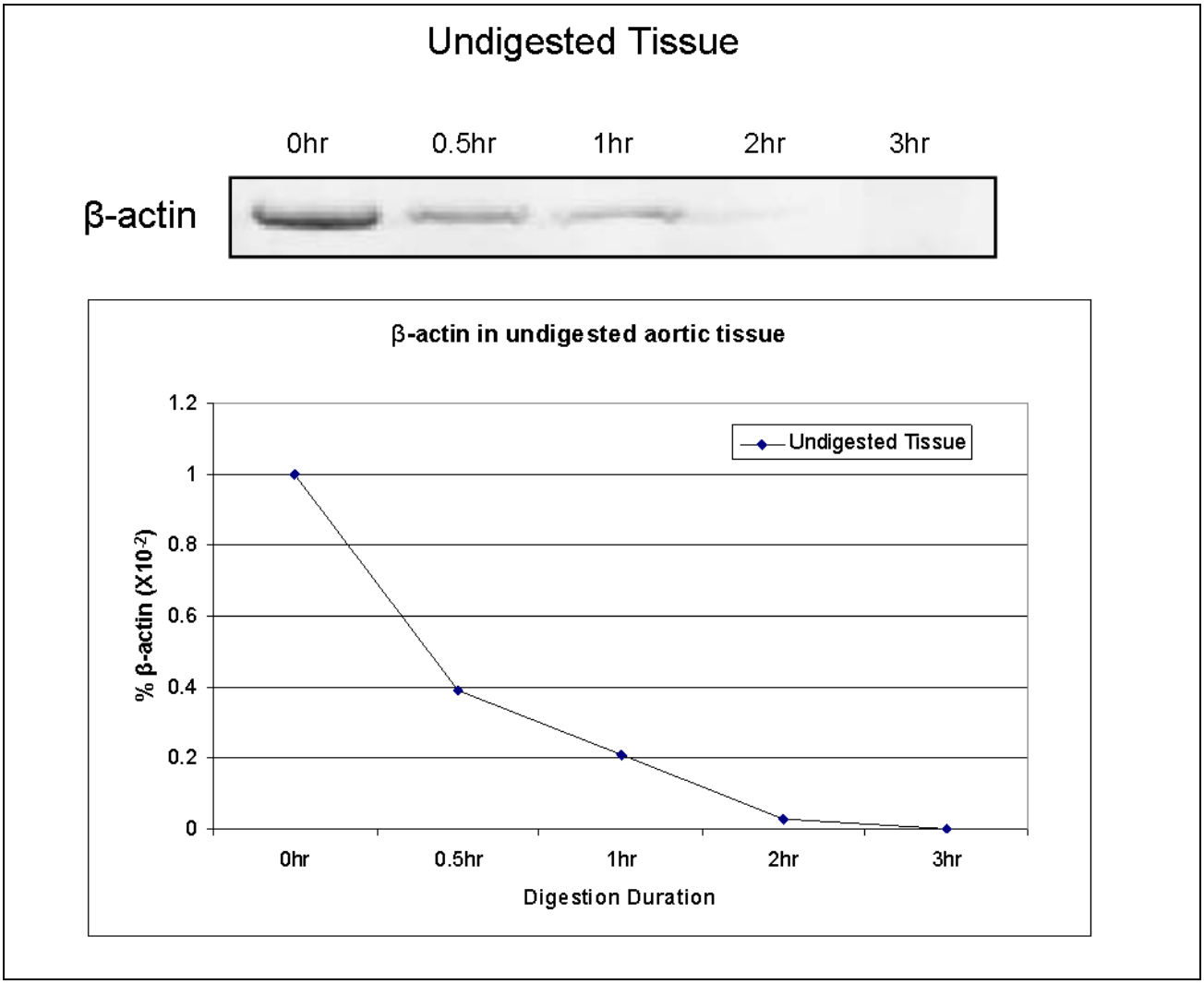
Undigested Tissue
0hr
0.5hr
1hr
2hr
3hr
β-actin
β-actin in undigested aortic tissue
Undigested Tissue
% β-actin (X10-2)
1.2
1
0.8
0.6
0.4
0.2
0
0hr
0.5hr
1hr
2hr
3hr
Digestion Duration

Figure S4.

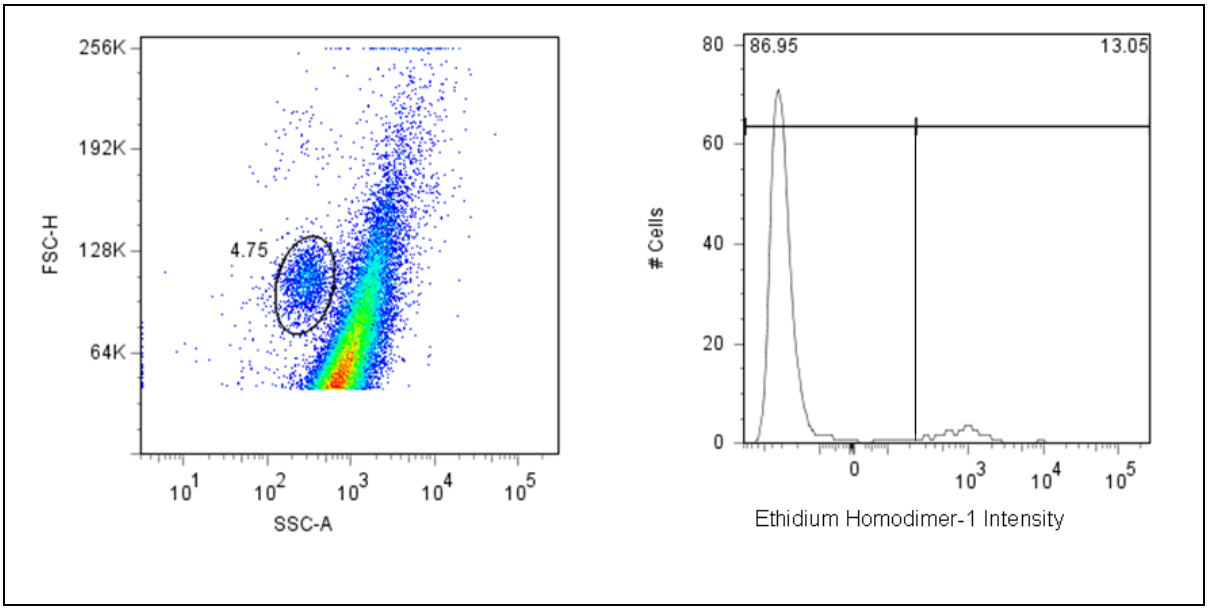
256K
192K
128K
64K
FSC-H
4.75
10^1
10^2
10^3
10^4
10^5
SSC-A
80
60
40
20
0
Cells
86.95
13.05
0
10^3
10^4
10^5
Ethidium Homodimer-1 Intensity

Figure S5.

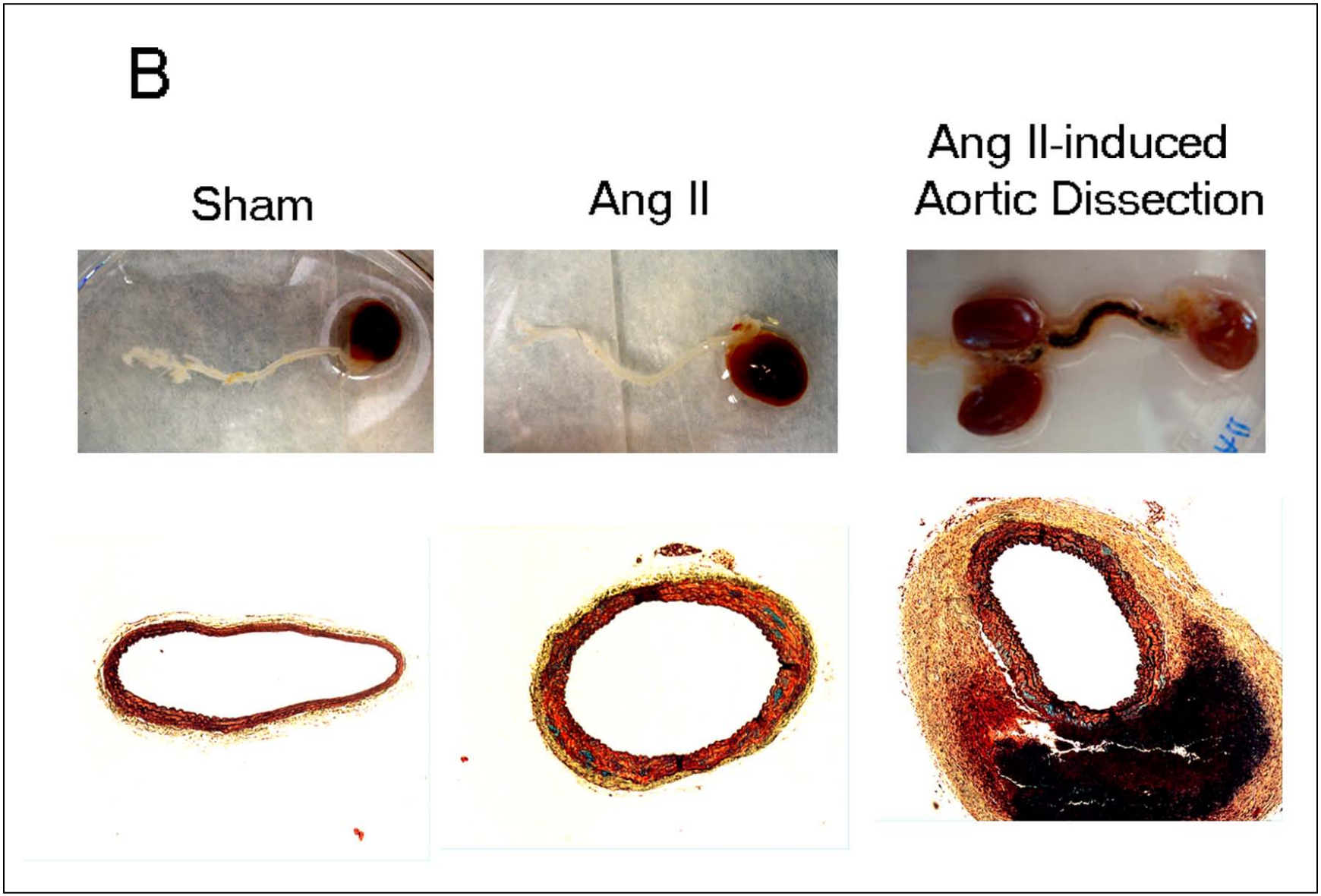

Figure S6.

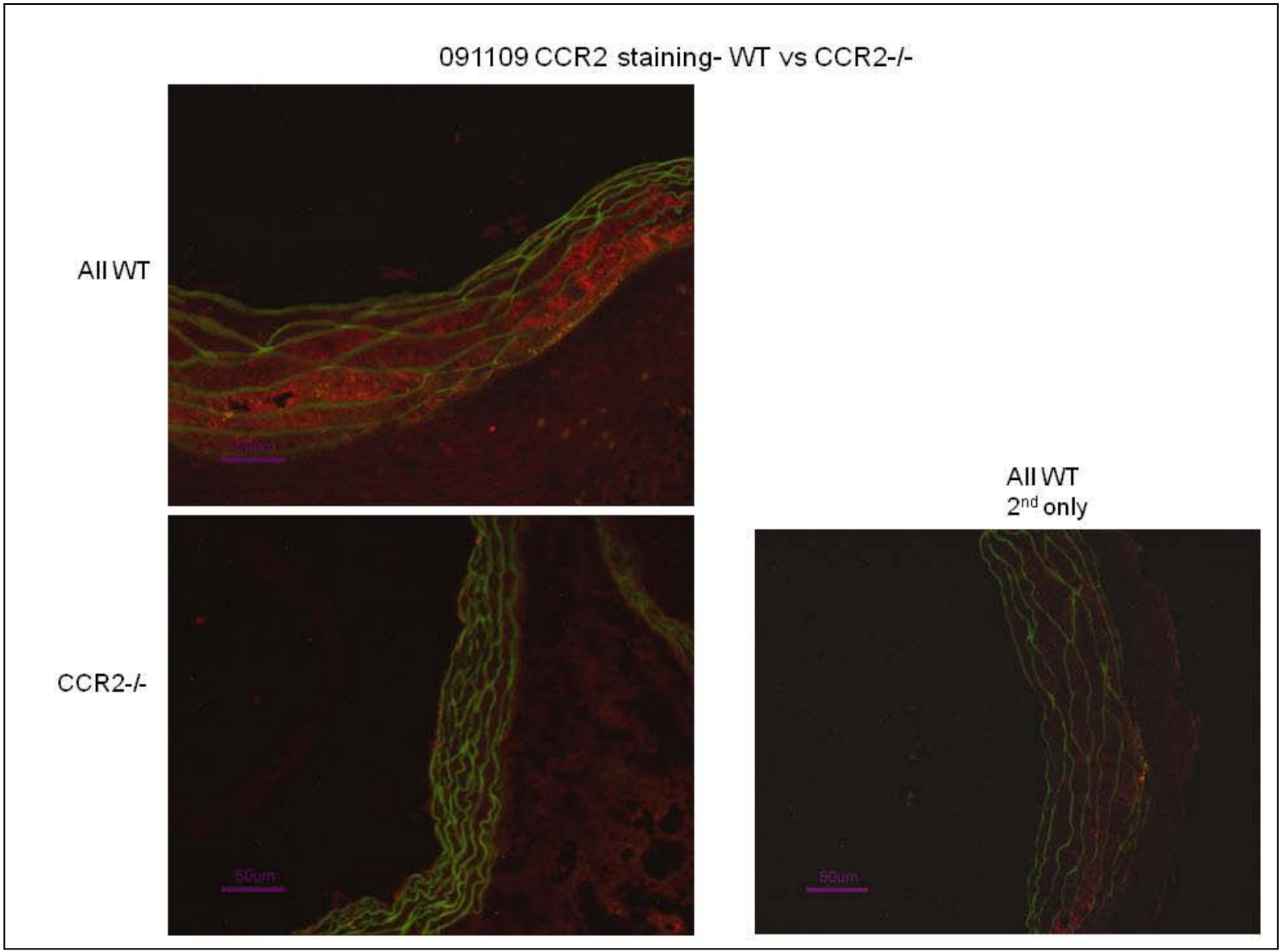
091109 CCR2 staining- WT vs CCR2-/-
All WT
CCR2-/-
All WT
2nd only
50um
50um
50um

Figure S7.