Supplemental Materials

Supplemental Methods

Quantitative RT-PCR. Total RNA was extracted and purified from wild-type and S100A4$^{-/-}$ BMMs using the Qiagen RNAeasy kit (Qiagen). Total RNA was reverse transcribed and qRT-PCR analysis was performed using the SsoFast EvaGreen Supermix (BioRad) as per the manufacturer's instructions. The PCR was performed on a Bio-Rad CFx96 Real-Time PCR System with a C1000 Thermal Cycler. cDNA levels during the linear phase of amplification were normalized against HPRT controls and quantified by the method of Livak and Schmittgen (Livak and Schmittgen, 2001).

The expression of S100A1, S100A8, S100A9, S100A10 and S100A11 was examined as these S100 proteins were reported previously to be expressed in BMMs (Sasmono *et al.*, 2007). The following primers were used: mouse β-actin (NM_007393) forward primer ACCTTCTACAATGAGCTGCG, reverse primer CTGGATGGCTACGTACATGG; mouse HPRT forward primer TCCTCCTCAGACCGCTTT, reverse primer TTTTCCAAATCCTCGGCATAATG; mouse S100A1 (NM_011309) forward primer CCCTTCTGTCGAGAATCTGTTC, reverse primer TCAGCTTATATTTGTCCCCTTCC; mouse S100A4 (NM_011311) forward primer CAGCAACAGGGACAATGAAG, reverse primer ACTACACCCCAACACTTCATC; mouse S100A8 (NM_013650) forward primer AGTGTCCTCAGTTTGTGCAG, reverse primer ACTCCTTGTGGCTGTCTTTG; mouse S100A9 (NM_009114) forward primer GTTGATCTTTGCCTGTCATGAG, reverse primer AGCCATTCCCTTTAGACTTGG; mouse S100A10 (NM_009112) forward primer CCAGGTTTCGACAGACTCTTC; reverse primer CCGTTCCATGAGCACTCTC; mouse S100A11 (NM_016740) forward primer AAGCTGGACCTCAACTGTG, reverse primer GTGGTTGGATGGGAACTAAGAG.

Mouse spleen was washed with PBS and snap frozen in liquid nitrogen. To prepare spleen extracts, mouse spleen was homogenized in modified RIPA buffer (300 mM NaCl, 50

mM Tris-HCl pH 8.0, 5 mM EDTA, 1 mM PMSF, 1% NP-40, 1% sodium deoxycholic acid, 0.1% SDS, and 5 μg/ml each of chymostatin, leupeptin, pepstatin). Tissue and cell debris were removed by centrifugation. Spleen and BMM lysates were boiled for 5 min in 1X SDS sample buffer, separated on a 12% Tricine SDS-polyacrylamide gel and transferred to a PVDF membrane. The monoclonal anti-mouse S100A8 antibody was from R&D Systems.

Cell attachment assay. Estimation of adherent ability of wild-type and S100A4-deficient macrophages was assessed on tissue culture plastic. Approximately 5 x 10^4 BMMs were added in triplicate to each well of flat-bottom 96-well polystyrene plates and incubated at 37 °C for up to 30 min. At different time points, unbound cells were removed by gentle aspiration and wells were rinsed with cold PBS. Adherent cells were fixed for 15 min with 4% formaldehyde and stained with 0.1% crystal violet (Eastman Organic Chemicals). After washing with PBS, the stain was eluted with 33% acetic acid, and the absorbance was determined at 595 nm using a SpectraMax M5 microplate reader (Molecular Devices).

Supplemental Figures

Figure S1. Control FACS data for wild-type BMMs showing unstained cells, cells stained for CD11b or F4/80.

Figure S2. (A) Representative gel showing RT-PCR of S100 proteins in wild-type and S100A4$^{-/-}$ BMMs. 1: S100A1, 2: S100A4, 3: S100A8, 4: S100A9, 5: S100A10, 6: S100A11, 7: HPRT and 8: β-actin. (B) QRT-PCR analysis of S100 proteins in wild-type and S100A4$^{-/-}$ BMMs. The data represent the mean ± sd from three independent assays performed in triplicate. (C) Immunoblot analysis of S100A8 expression in wild-type and S100A4$^{-/-}$ BMMs. Expression in spleen was used as a positive control (Iismaa *et al.*, 1994). Right: Coomassie-stained gel; Left: autoradiogram.

Figure S3. Flow cytometric analysis of peritoneal cells from unstimulated mice, and 72 hours after thioglycollate injection. Left panels: Representative flow cytometric analysis of F4/80 fluorescence and side scatter (measuring granularity) to distinguish resident peritoneal macrophages. Resident macrophages (boxed region marked A) are characterized by medium granularity and positive staining for F4/80. Center panels: Representative flow cytometric analysis of GR-1 and CD11b fluorescence. Three regions are defined; cells in R1 are macrophages, cells in R2 are neutrophils and cells in R3 are eosinophils. Right panels: Representative flow cytometric analysis of F4/80 and CD11b fluorescence. Regions are defined as for the center panels. Macrophages are characterized by $F4/80^{+}$, $CD11b^{hi}$ and $GR\text{-}1^{lo}$, neutrophils are characterized by $F4/80^{lo}$, $CD11b^{hi}$ and $GR\text{-}1^{hi}$, and eosinophils by $F4/80^{+}$, $CD11b^{lo}$ and $GR\text{-}1^{lo}$.

Figure S4. The loss of S100A4 does not affect protrusion dynamics in randomly migrating cells. BMMs were plated on 13.2 μg/ml fibronectin. Quantification of protrusion (A) persistence, (B) velocity, (C) frequency and (D) distance. Values represent the mean ± sem for 18 wild-type and 17 $S100A4^{-/-}$ cells.

Figure S5. $S100A4^{-/-}$ BMMs exhibit defects during early attachment times to tissue culture plastic. Dark gray bars: wild-type BMMs; light gray bars: $S100A4^{-/-}$ BMMs. Values represent the mean ± sem for three to five independent experiments performed in triplicate.

Figure S6. Immunoblot analysis of S100A4 and β-actin expression in BMMs and MEFs isolated from wild-type and $S100A4^{-/-}$ mice.

Supplemental Video 1. Phase-contrast microscopy of CSF-1-stimulated wild-type or S100A4$^{-/-}$ BMMs. A micropipette, containing 120 ng/ml CSF-1, was placed ~60 μm away from the cell, and the cell response was monitored over a 20 min period with images collected every 20 sec. Cells were plated on 13.2 μg/ml fibronectin.

Supplemental Video 2. Phase-contrast microscopy of CSF-1-stimulated S100A4$^{-/-}$ BMMs treated with 5 μM blebbistatin. A micropipette, containing 120 ng/ml CSF-1, was placed ~60 μm away from the cell, and the cell response was monitored over a 15 min period with images collected every 20 sec. Cells were plated on 13.2 μg/ml fibronectin.

References

Iismaa, S.E., Hu, S., Kocher, M., Lackmann, M., Harrison, C.A., Thliveris, S., and Geczy, C.L. (1994). Recombinant and cellular expression of the murine chemotactic protein, CP-10. DNA Cell Biol *13*, 183-192.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*, 402-408.

Sasmono, R.T., Ehrnsperger, A., Cronau, S.L., Ravasi, T., Kandane, R., Hickey, M.J., Cook, A.D., Himes, S.R., Hamilton, J.A., and Hume, D.A. (2007). Mouse neutrophilic granulocytes express mRNA encoding the macrophage colony-stimulating factor receptor (CSF-1R) as well as many other macrophage-specific transcripts and can transdifferentiate into macrophages in vitro in response to CSF-1. J Leukoc Biol *82*, 111-123.

Figure S1

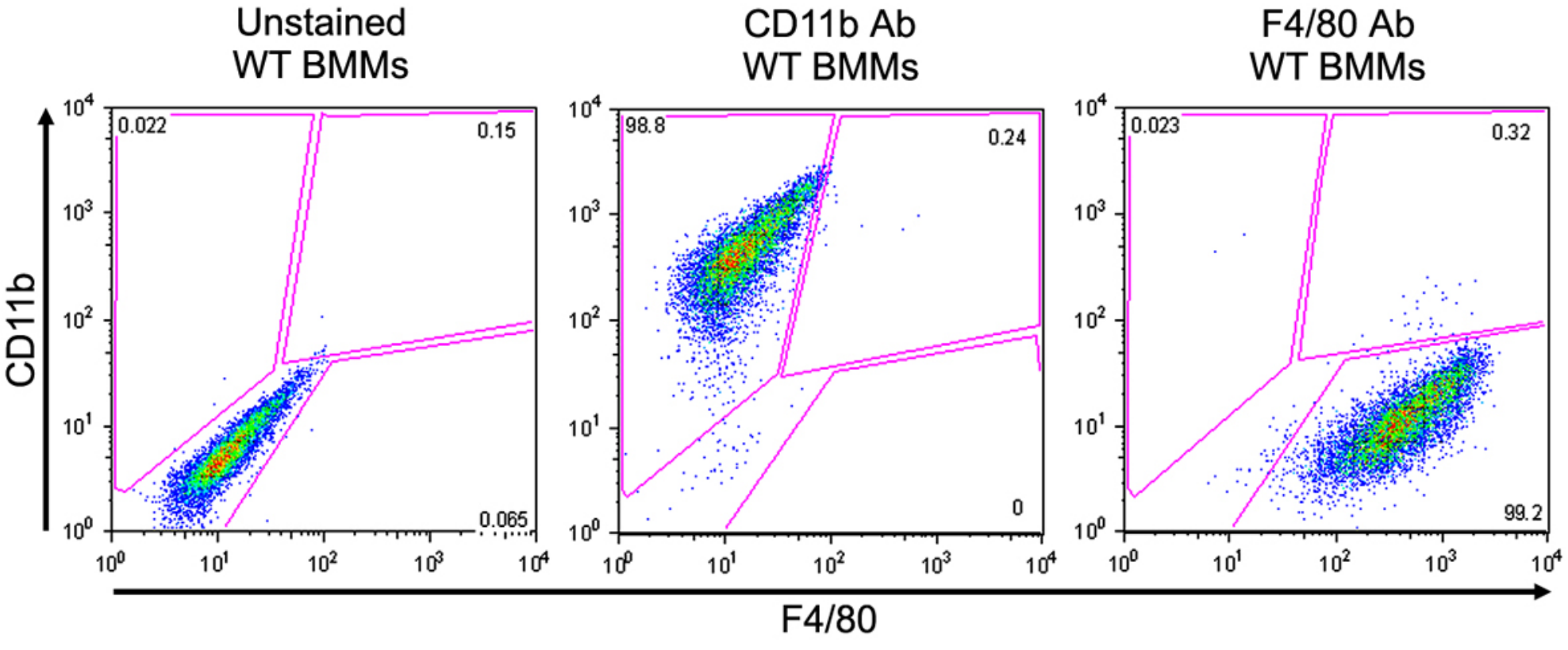
Unstained
WT BMMs
CD11b Ab
WT BMMs
F4/80 Ab
WT BMMs
CD11b
F4/80
0.022
0.15
0.065
98.8
0.24
0
0.023
0.32
99.2

Figure 2S

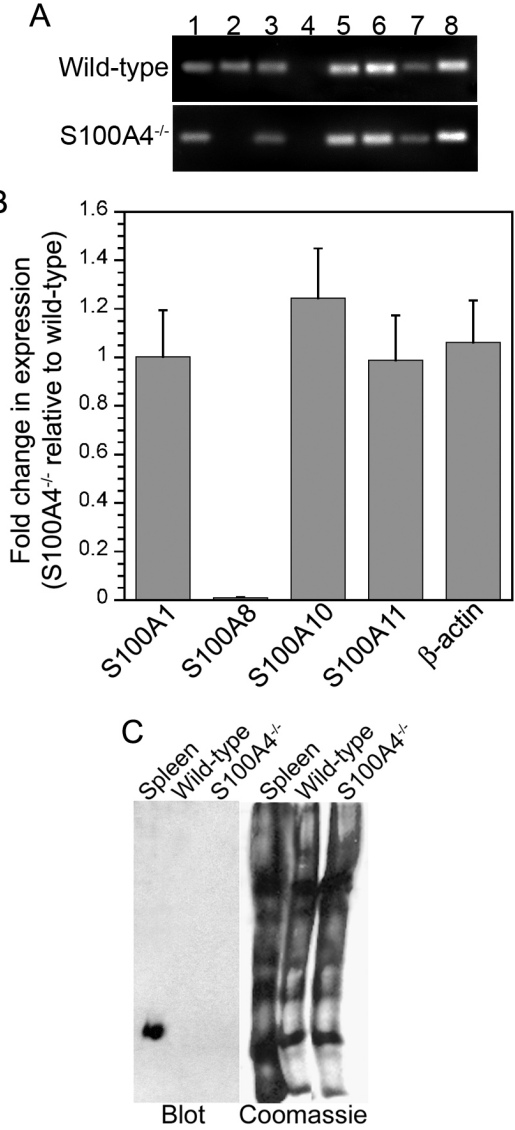
A
1 2 3 4 5 6 7 8
Wild-type
S100A4-/-
B
Fold change in expression (S100A4-/- relative to wild-type)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
S100A1
S100A8
S100A10
S100A11
β-actin
C
Spleen
Wild-type
S100A4-/-
Spleen
Wild-type
S100A4-/-
Blot
Coomassie

Figure S3

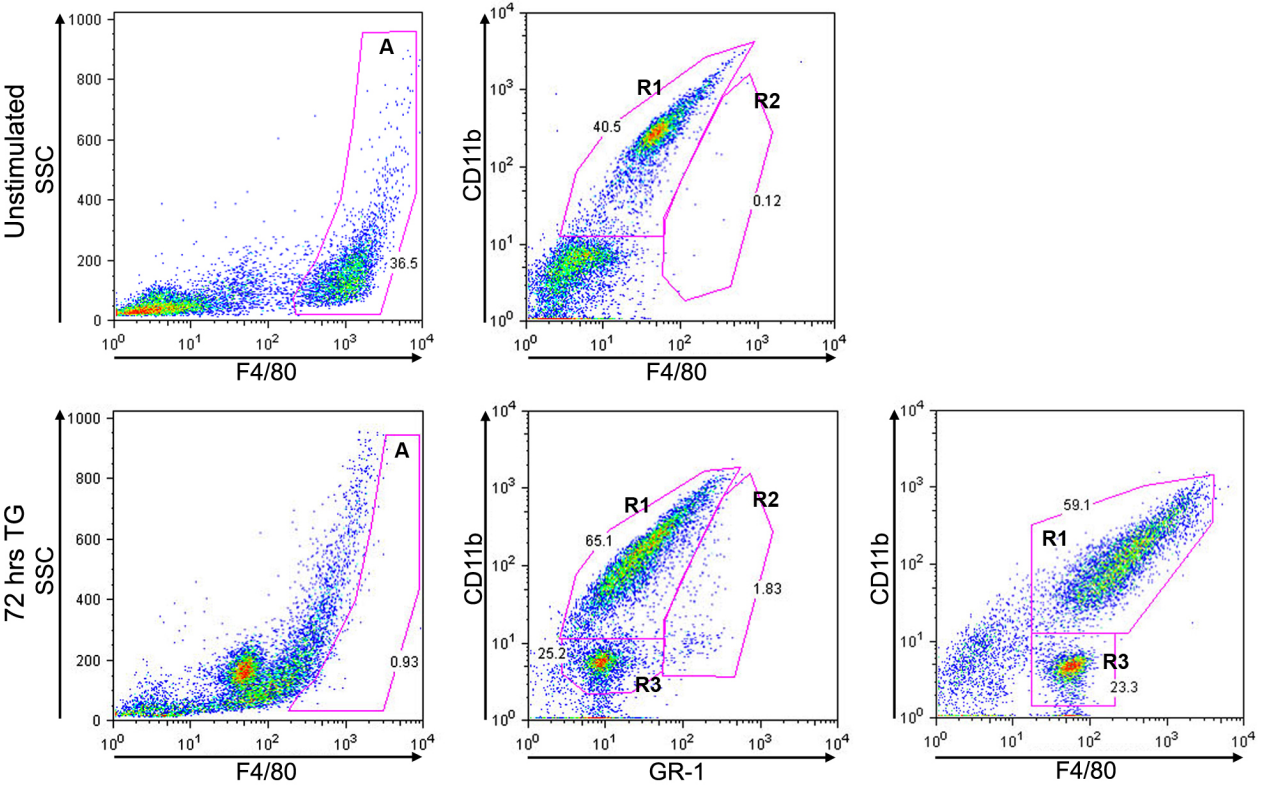
Unstimulated
SSC
A
36.5
F4/80
CD11b
R1
R2
40.5
0.12
F4/80
72 hrs TG
SSC
A
0.93
F4/80
CD11b
R1
R2
65.1
1.83
25.2
R3
GR-1
CD11b
59.1
R1
R3
23.3
F4/80

Figure S4

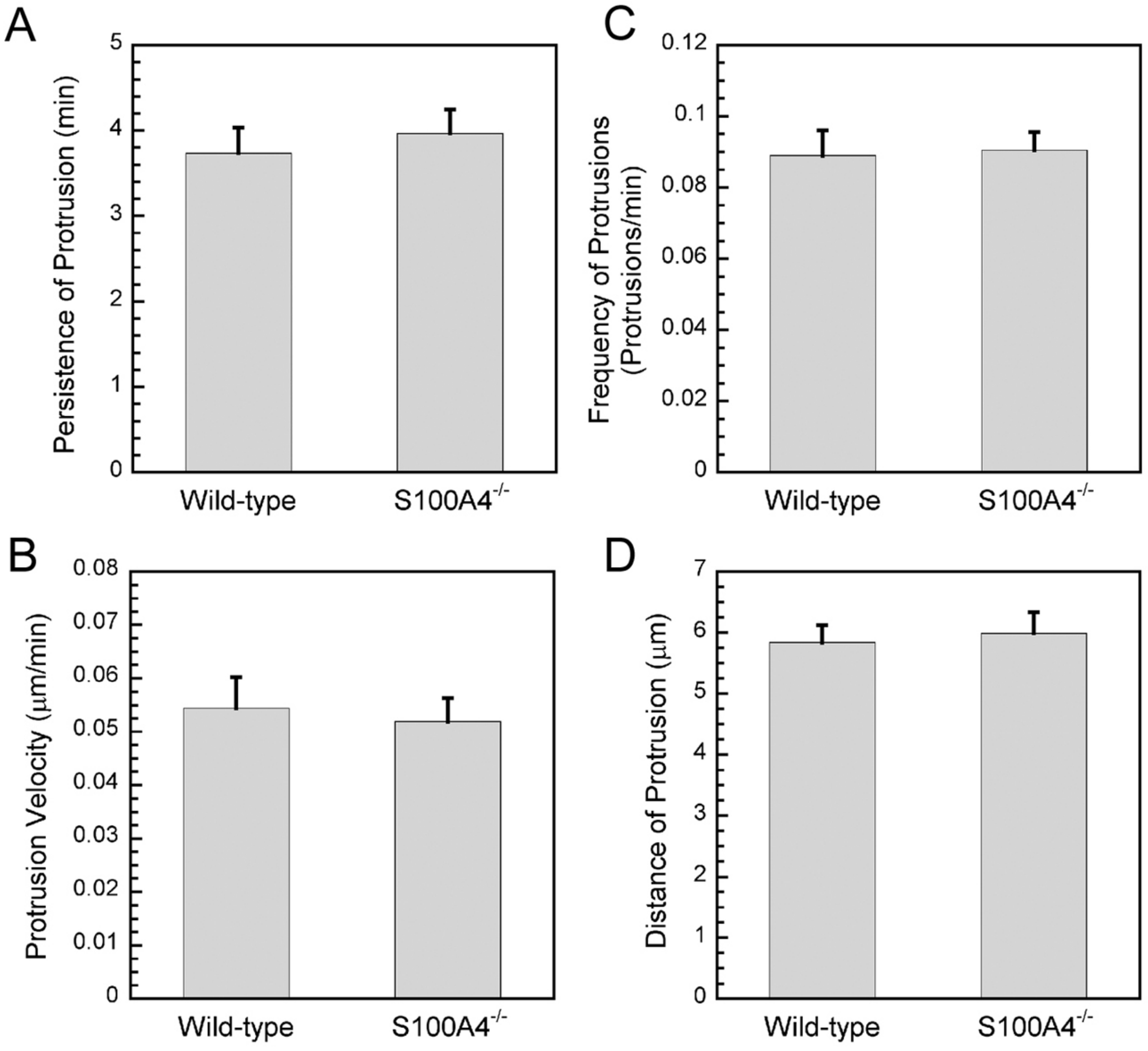
A
Persistence of Protrusion (min)
0
1
2
3
4
5
Wild-type
S100A4-/-
C
Frequency of Protrusions (Protrusions/min)
0
0.02
0.04
0.06
0.08
0.1
0.12
Wild-type
S100A4-/-
B
Protrusion Velocity (μm/min)
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Wild-type
S100A4-/-
D
Distance of Protrusion (μm)
0
1
2
3
4
5
6
7
Wild-type
S100A4-/-

Figure S5

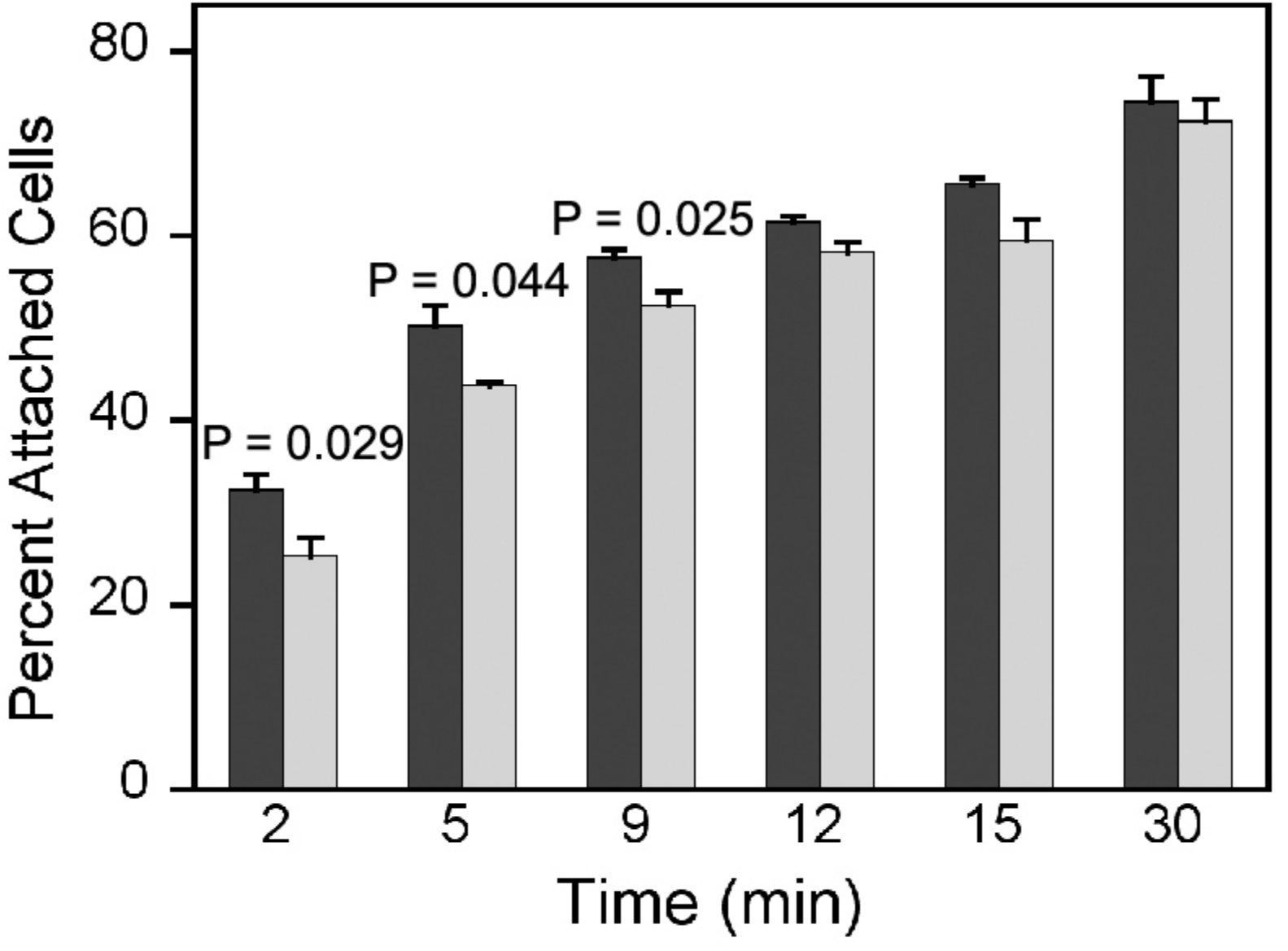
Percent Attached Cells
80
60
40
20
0
P = 0.029
P = 0.044
P = 0.025
2
5
9
12
15
30
Time (min)

Figure S6