**Supplemental Materials**

## Supplemental Materials and Methods

### RT-PCR

Total RNAs were isolated from cultured neurons or glia cells using RNeasy Plus mini Kit (Qiagen). First strand cDNAs were synthesized using random primers and SuperScriptII Reverse Transcriptase (Invitrogen). The PCR reactions were performed under the following conditions: 94°C 2 min, 34 cycles for 94°C 30s, 60°C (for mTLR4) or 65°C (for mRAGE) 30s, and 72°C 30s. Primers used for RT-PCR were as follows: 5'-GGACCCTTAGCTGGCACTTAGA-3' (forward) and 5' GAGTCCCGTCTCAGGGTGTCT-3' (reverse) for mRAGE; and 5' GCTTTCACCTCTGCCTTCAC-3' (forward) and 5'-AGGCGATACAATTCCACCTG-3' (reverse) for mTLR4.

### Intracellular staining of TNFα

Peripheral blood was collected from wild-type C57BL/6 mice into sodium-heparine-coated tubes (BD Biosciences) and incubated with 10 μg/ml of BFA (Sigma-Aldrich) (Unstimulated), 10 μg/ml of BFA and 1 μg/ml of LPS (Sigma-Aldrich) (LPS), 10 μg/ml of BFA and protein cleavage buffer (Vehicle), or 10 μg/ml of BFA and 50 ng/ml of S100A8/A9 (S100A8/A9) at 37°C for 4 hours. Blood cells were fixed and permeablized using Fixation Medium and Permeablization Medium (Invitrogen) as per the manufacturer's recommendation. Cells were simultaneously stained with FITC-Mac1, PE-Gr1, and APC-TNFα (BD Biosciences). Flow data were collected using a FACS Calibur (BD Biosciences) and analyzed by the FlowJo software (v9.0.2, TreeStar).

## Supplemental figure legends

**Figure S1. GFAP expression in different brain regions.** (A) Expression levels of GFAP were analyzed by Western blot using protein extracts from forebrain (F), midbrain (M), and hindbrain(H) of 8-weeks old control and KrasG12D/+ mice. (B) GFAP levels relative to β-actin levels in Kras G12D/+ brain regions were quantified and compared to those in control, which are arbitrarily set at 1.

**Figure S2. Microglia accumulate in Kras G12D cortex.**
Representative images of immunohistochemical staining of Iba1, a microglial cell marker, in cerebral cortex of 14-weeks old control or CamKII-Cre; LSL Kras G12D/+ (KrasG12D/+) mice (n=3). (c-d) High magnification views of the boxed area in (a, b). The insets in (c, d) show high magnification views of Iba1-positive microglial cells. Ctx: cerebral cortex.

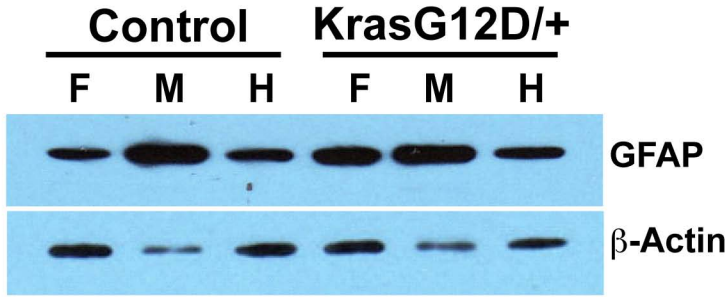
**Figure S3. Primary astrocytes of control and KrasG12D/+ mice express RAGE and TLR4, receptors for S100A8/A9 complex.**
Cortices were isolated from control or CamKII-Cre; LSL Kras G12D/+ (KrasG12D/+) P1 neonates and dissociated. Total RNAs were isolated from cultured astrocytes. RT-PCR analysis of RAGE (A) and TLR4 (B) using primer pairs flanking intron regions. N.C. indicates negative control using PCR mixture without cDNA template.

**Figure S4. S100A8/A9 does not directly activate peripheral blood monocytes/macrophages.** Peripheral blood was collected from wild-type C57BL/6 mice and incubated with 10 μg/ml of BFA (Unstimulated), 10 μg/ml of BFA and 1 μg/ml of LPS (LPS), 10 μg/ml of BFA and protein cleavage buffer (Vehicle), or 10 μg/ml of BFA and 50 ng/ml of S100A8/A9 (S100A8/A9) at 37°C for 4 hours. Monocytes/macrophages are defined as $Mac1^{+}$ $Gr1^{-}$ cells as indicated in the top panels. TNFα expression level is analyzed in the defined monocytes/macrophages (bottom panels). Percentages of $TNF\alpha^{+}$ cells are indicated on histograms.

**Figure S5. A model for Kras G12D/+ neurons-induced astrogliosis.**

Fig. S1

A


Control
KrasG12D/+
F
M
H
F
M
H
GFAP
β-Actin

B

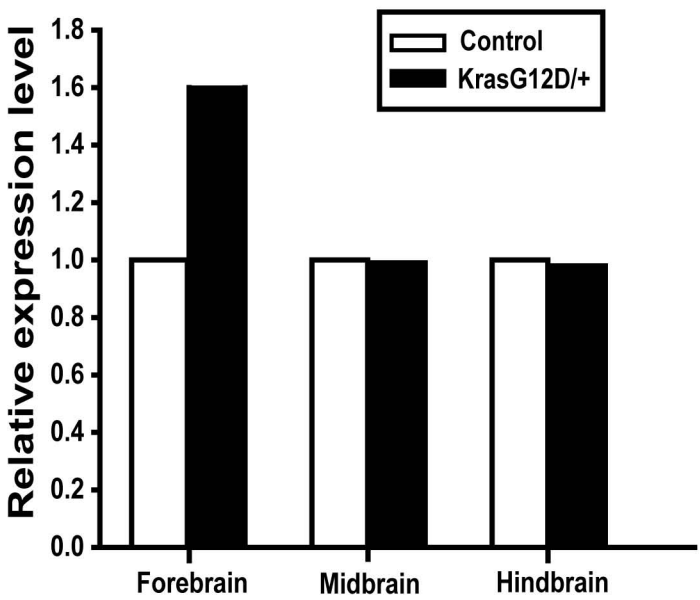

Relative expression level
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
KrasG12D/+
Forebrain
Midbrain
Hindbrain

Fig. S2

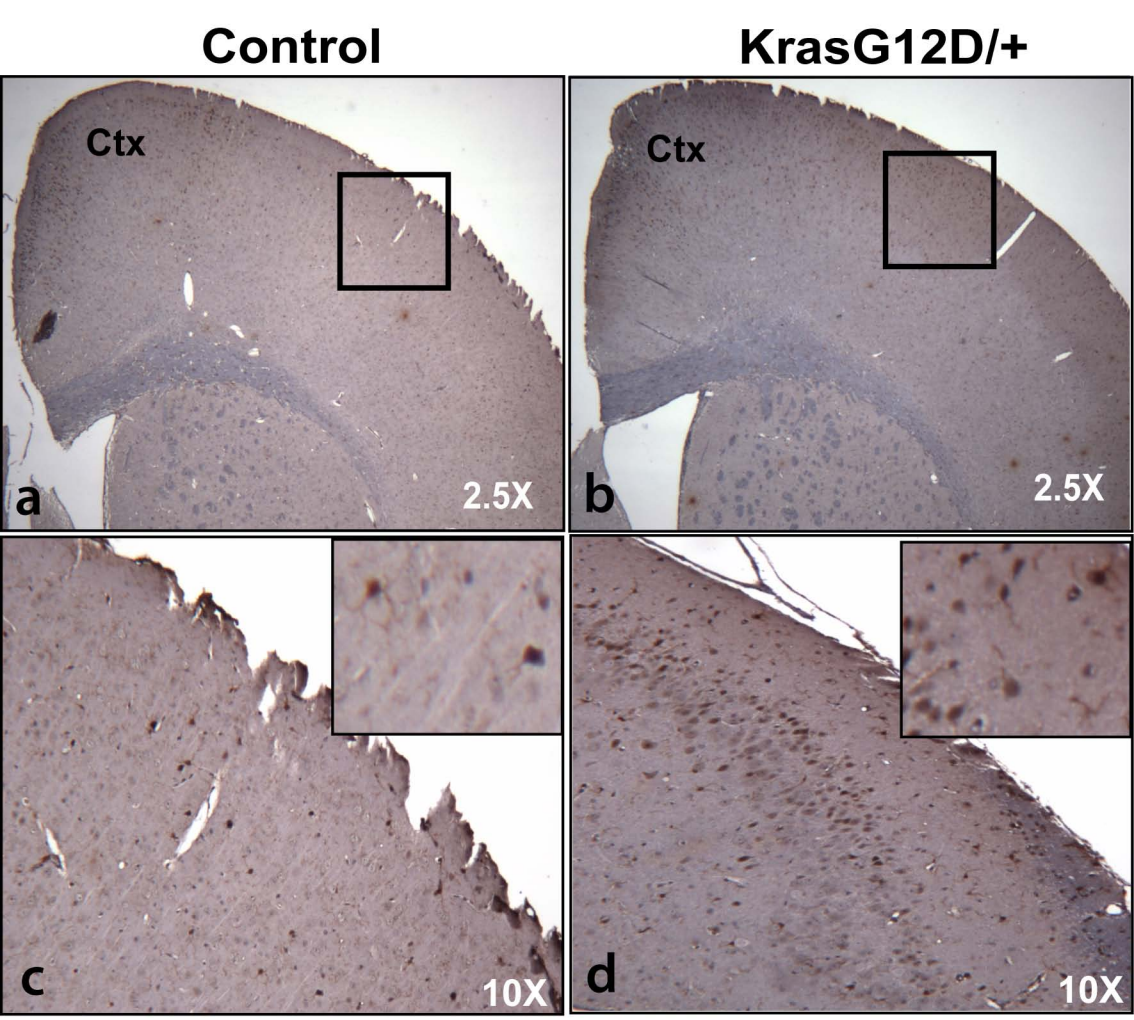


Fig. S3

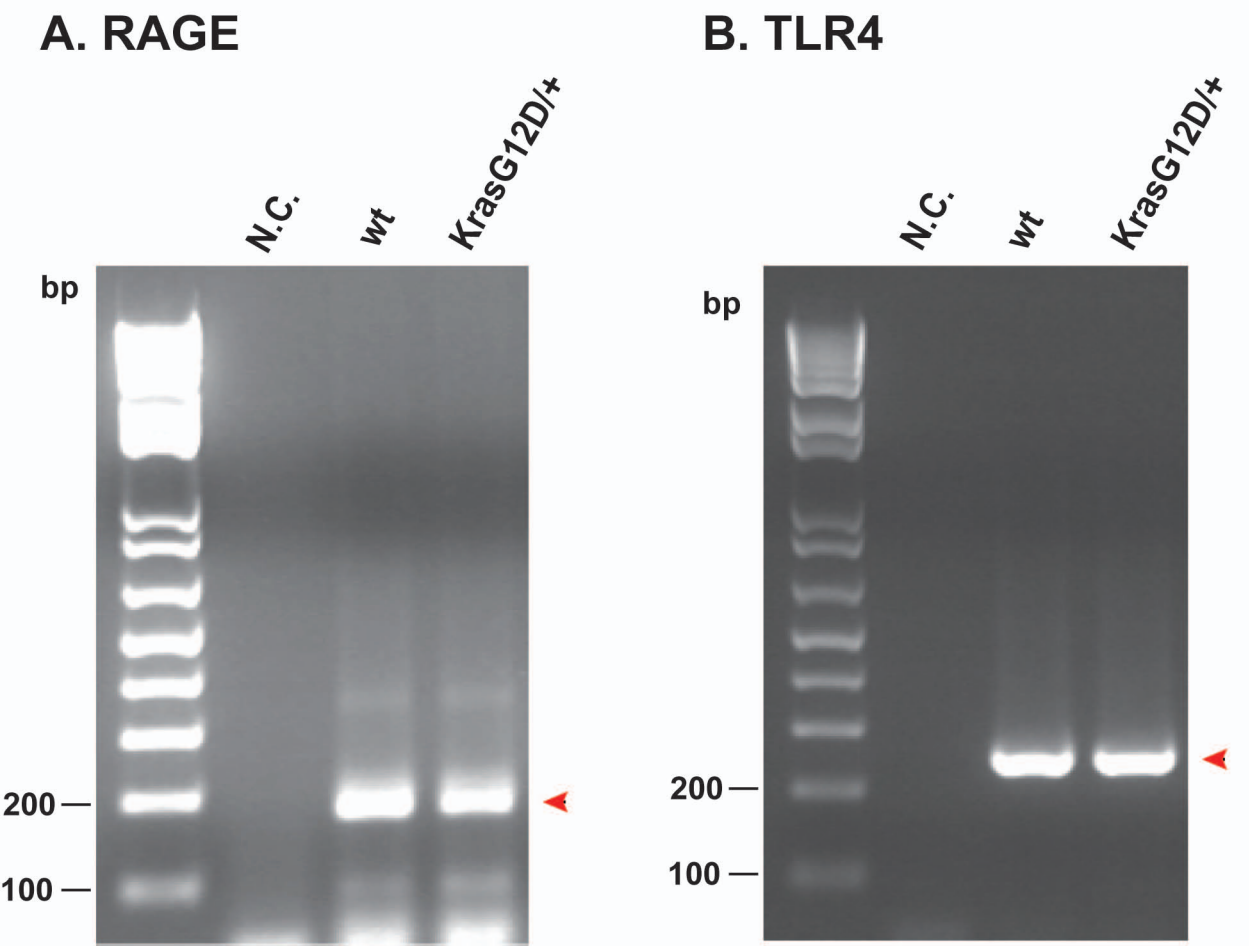

A. RAGE
N.C.
wt
KrasG12D/+
bp
200
100
B. TLR4
N.C.
wt
KrasG12D/+
bp
200
100

Fig. S4

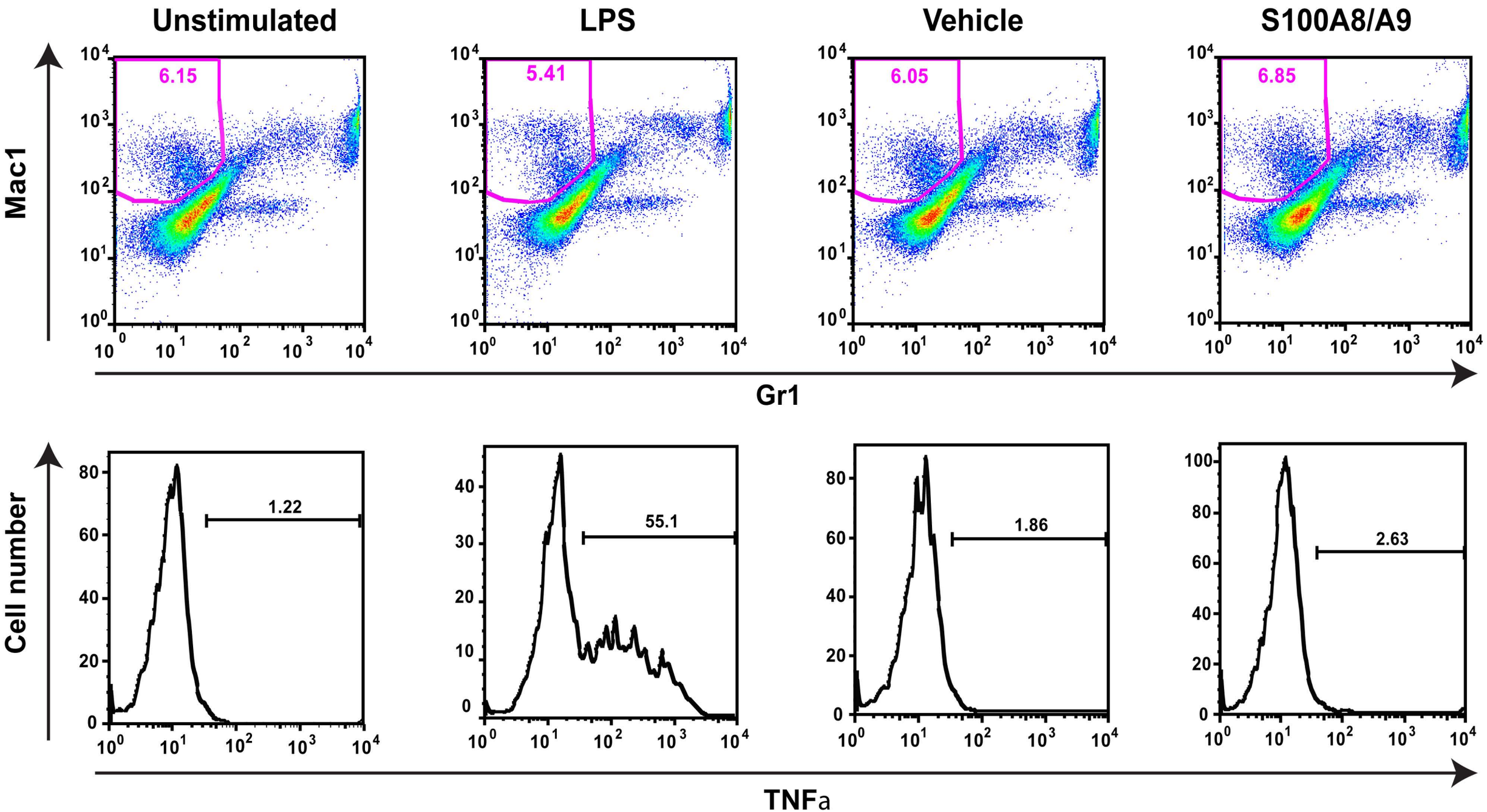
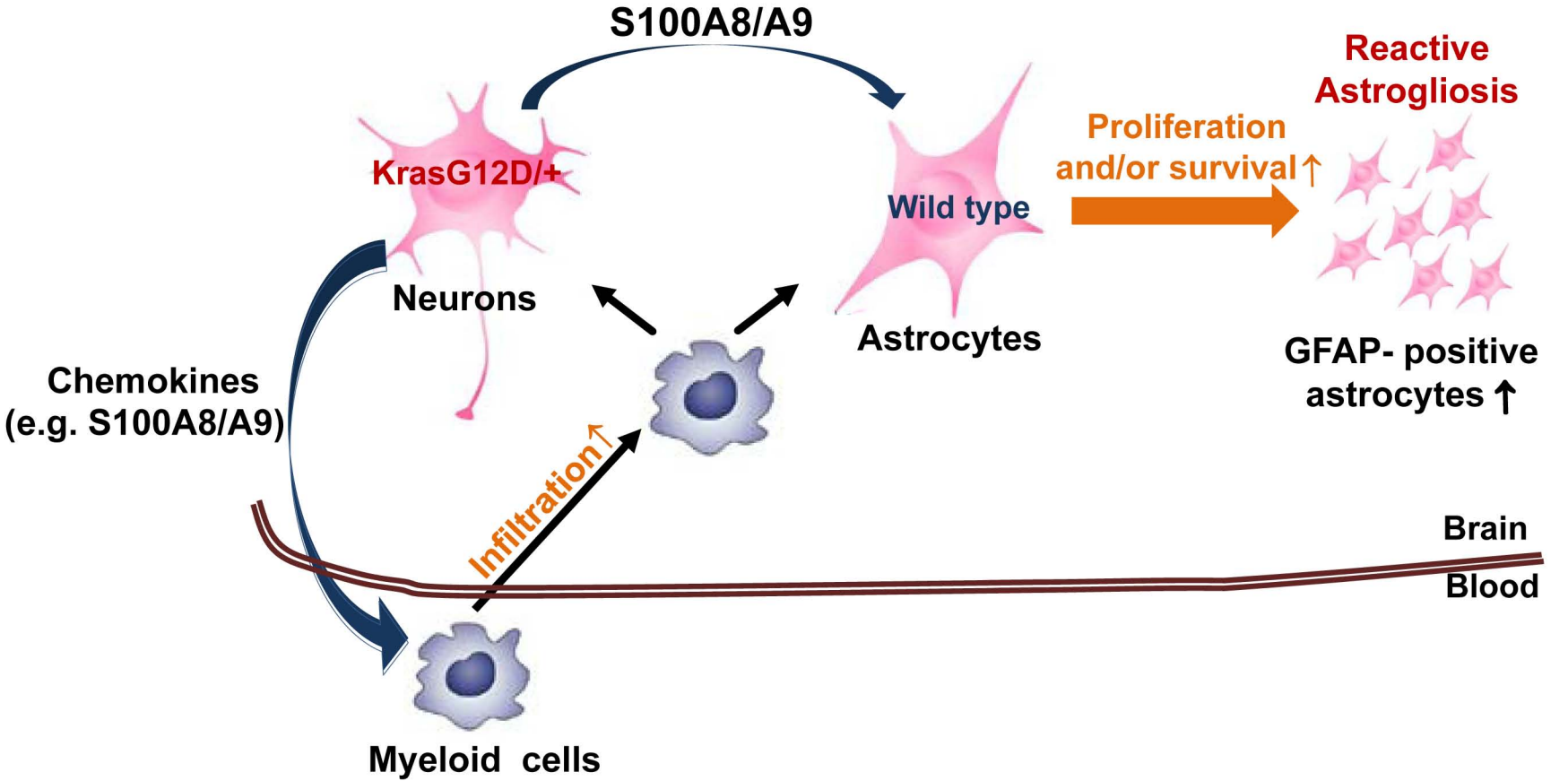

Unstimulated
LPS
Vehicle
S100A8/A9
Mac1
Gr1
6.15
5.41
6.05
6.85
Cell number
TNFa
1.22
55.1
1.86
2.63

Fig. S5


S100A8/A9
KrasG12D/+
Neurons
Wild type
Astrocytes
Proliferation
and/or survival↑
Reactive
Astrogliosis
GFAP- positive
astrocytes ↑
Chemokines
(e.g. S100A8/A9)
Infiltration↑
Brain
Blood
Myeloid cells