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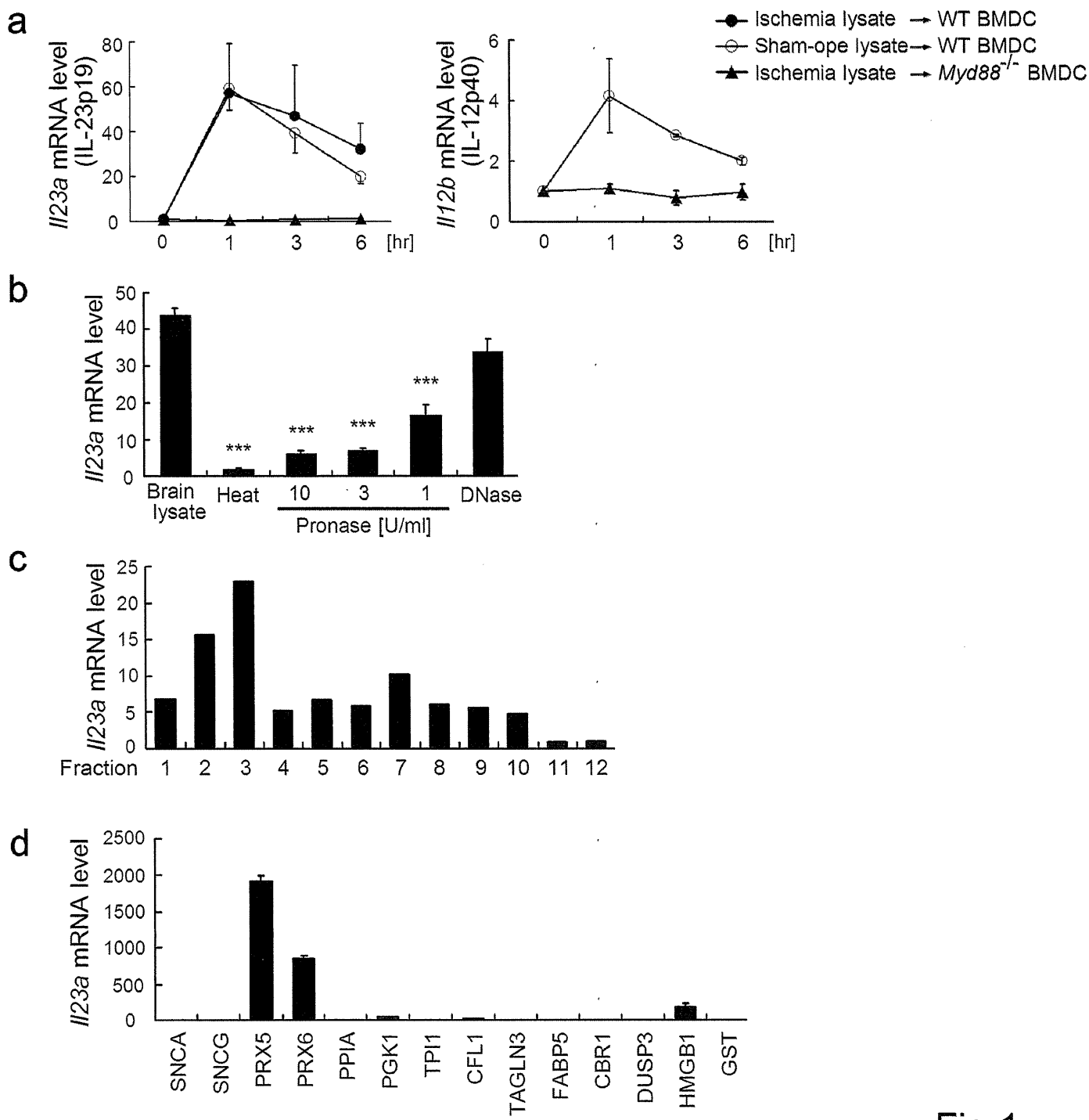

a
Ischemia lysate → WT BMDC
Sham-ope lysate → WT BMDC
Ischemia lysate → Myd88-/- BMDC
Il23a mRNA level (IL-23p19)
Il12b mRNA level (IL-12p40)
[hr]
b
Il23a mRNA level
Brain lysate
Heat
Pronase [U/ml]
DNase
c
Il23a mRNA level
Fraction
d
Il23a mRNA level
SNCA
SNCG
PRX5
PRX6
PPIA
PGK1
TPI1
CFL1
TAGLN3
FABP5
CBR1
DUSP3
HMGB1
GST

Fig.1

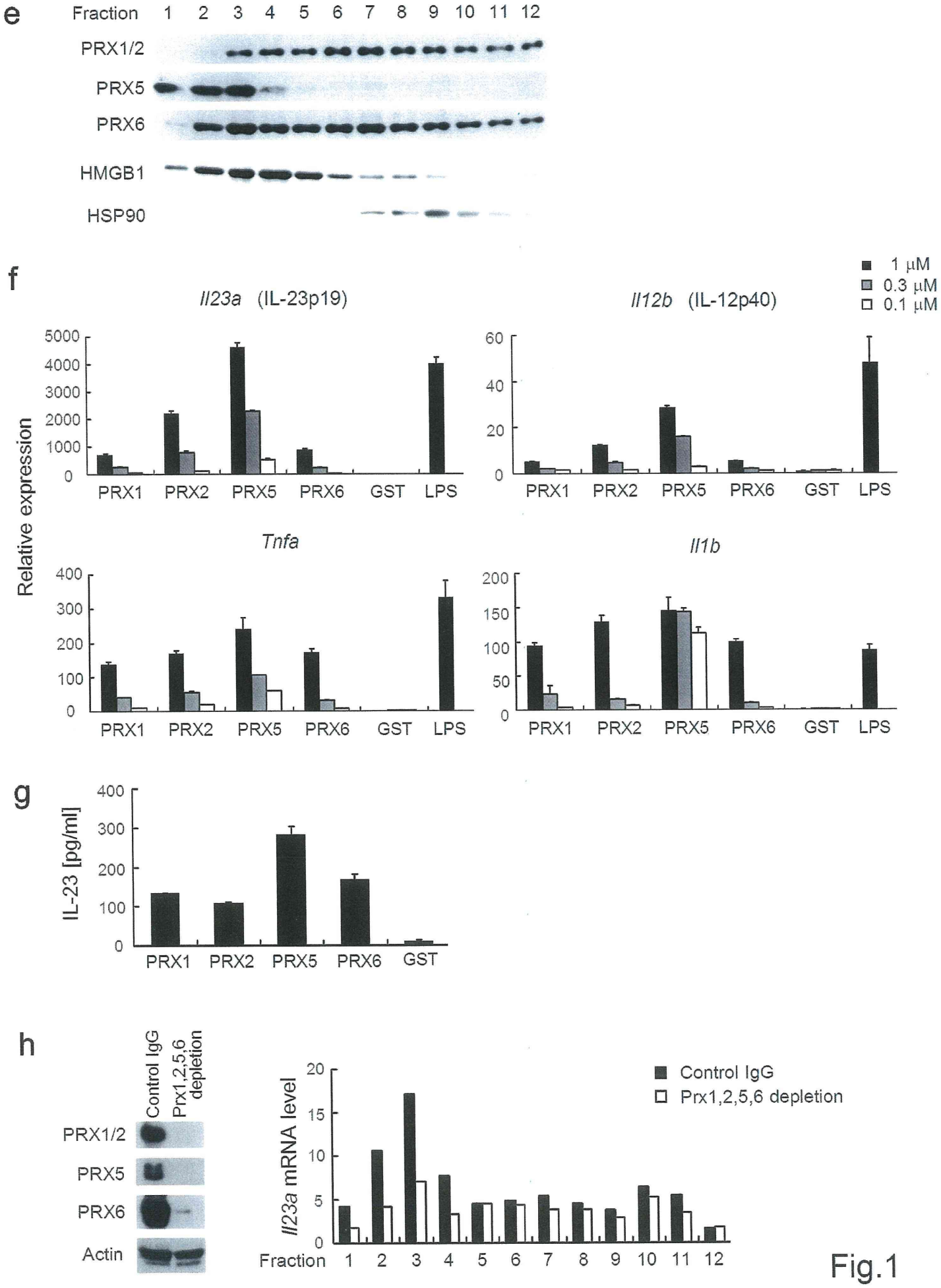

e
Fraction 1 2 3 4 5 6 7 8 9 10 11 12
PRX1/2
PRX5
PRX6
HMGB1
HSP90
f
1 μM
0.3 μM
0.1 μM
Il23a (IL-23p19)
Il12b (IL-12p40)
Relative expression
5000
4000
3000
2000
1000
0
60
40
20
PRX1 PRX2 PRX5 PRX6 GST LPS
Tnfa
Il1b
400
300
200
100
0
150
50
g
IL-23 [pg/ml]
h
Control IgG
Prx1,2,5,6 depletion
PRX1/2
PRX5
PRX6
Actin
Il23a mRNA level
20
15
10
5
Fraction 1 2 3 4 5 6 7 8 9 10 11 12

Fig.1

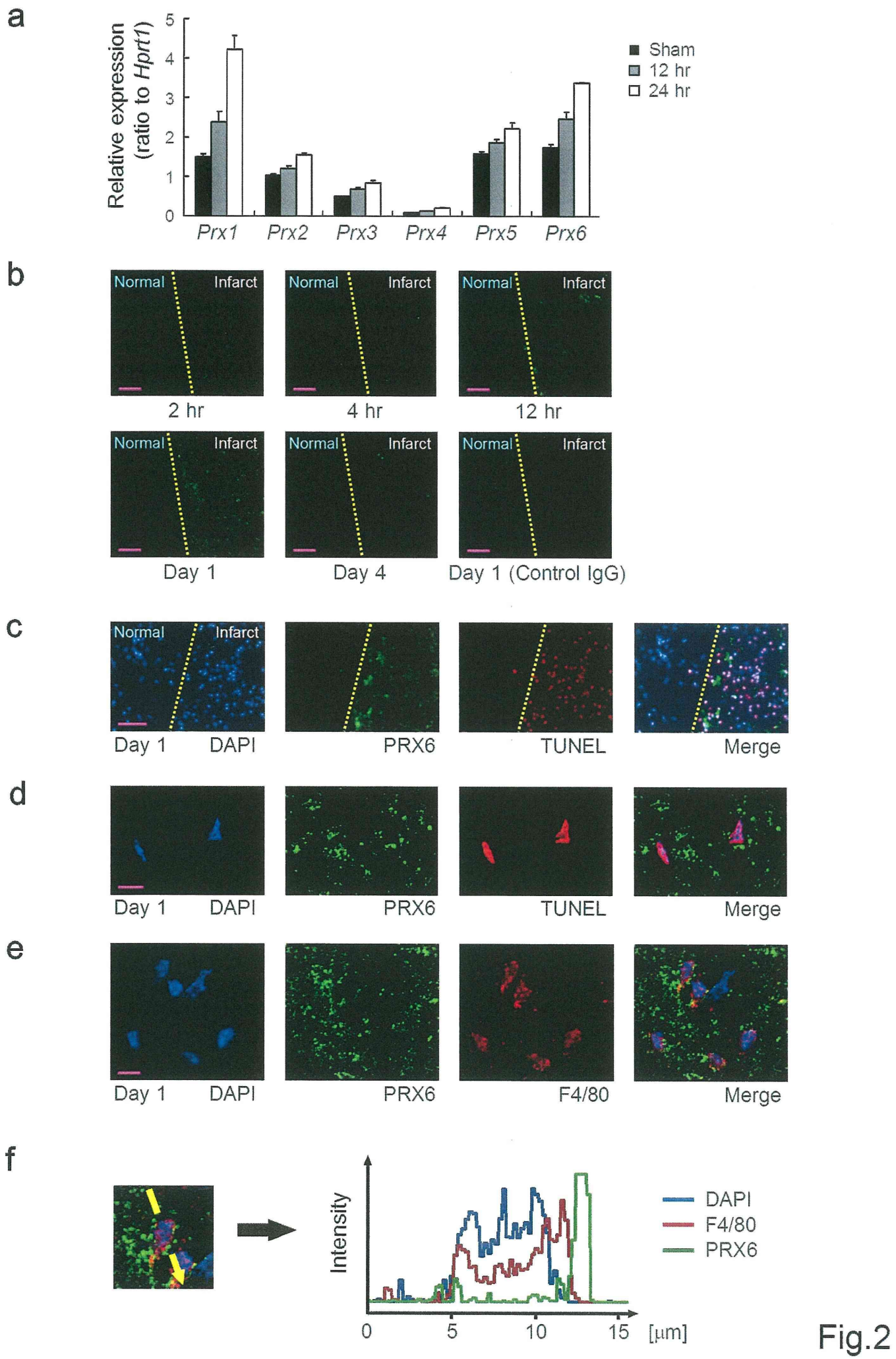

a
Relative expression (ratio to Hprt1)
0
1
2
3
4
5
Prx1
Prx2
Prx3
Prx4
Prx5
Prx6
Sham
12 hr
24 hr
b
Normal
Infarct
2 hr
4 hr
12 hr
Day 1
Day 4
Day 1 (Control IgG)
c
Day 1
DAPI
PRX6
TUNEL
Merge
d
Day 1
DAPI
PRX6
TUNEL
Merge
e
Day 1
DAPI
PRX6
F4/80
Merge
f
Intensity
0
5
10
15
[μm]
DAPI
F4/80
PRX6

Fig.2

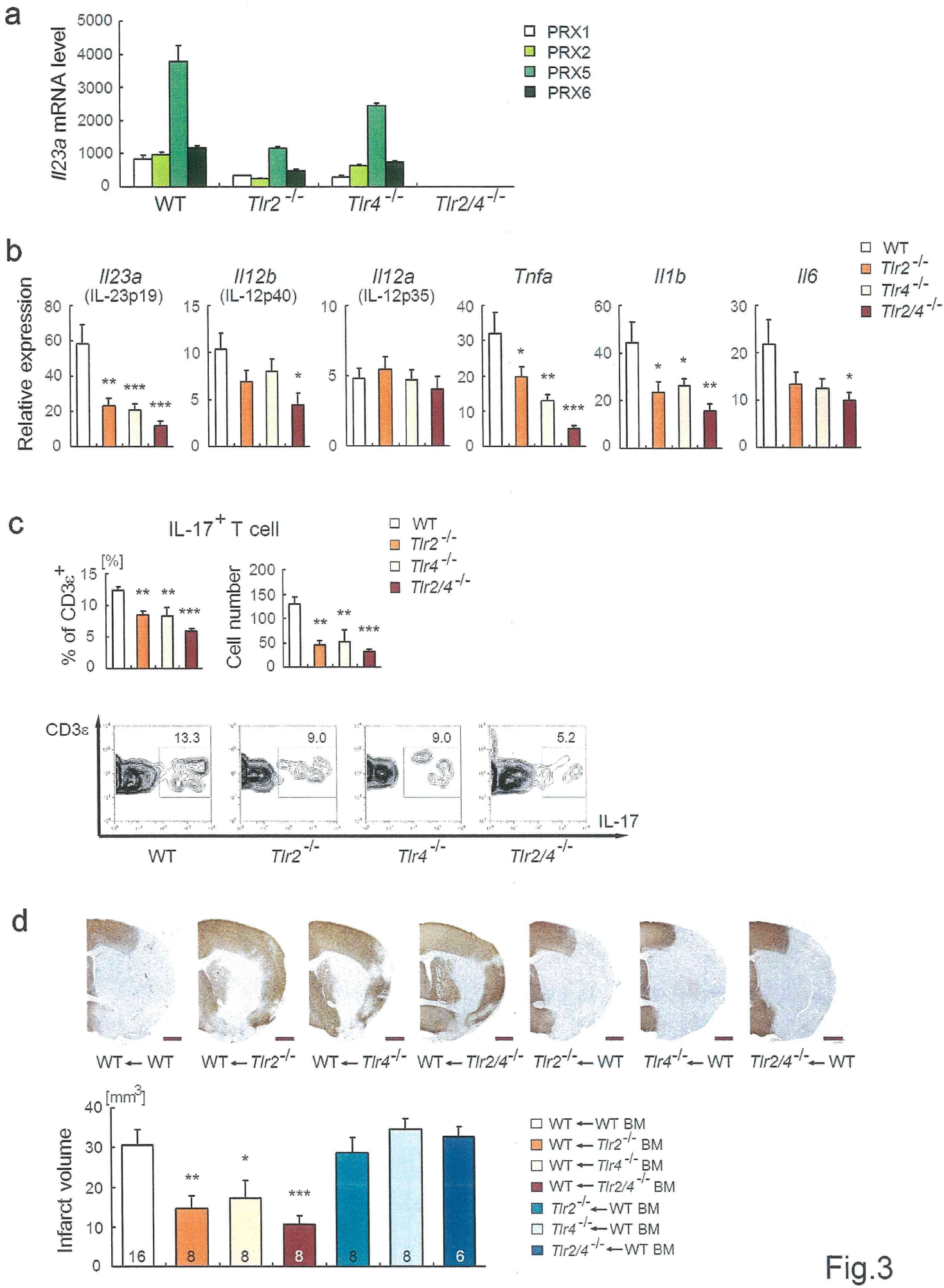

a
Il23a mRNA level
5000
4000
3000
2000
1000
0
PRX1
PRX2
PRX5
PRX6
WT
Tlr2 -/-
Tlr4 -/-
Tlr2/4 -/-
b
Relative expression
Il23a (IL-23p19)
Il12b (IL-12p40)
Il12a (IL-12p35)
Tnfa
Il1b
Il6
WT
Tlr2 -/-
Tlr4 -/-
Tlr2/4 -/-
c
IL-17+ T cell
% of CD3ε+ [%]
Cell number
CD3ε
IL-17
13.3
9.0
9.0
5.2
d
WT ← WT
WT ← Tlr2-/-
WT ← Tlr4-/-
WT ← Tlr2/4-/-
Tlr2-/- ← WT
Tlr4-/- ← WT
Tlr2/4-/- ← WT
Infarct volume [mm3]
16
8
8
8
8
8
6
WT ← WT BM
WT ← Tlr2-/- BM
WT ← Tlr4-/- BM
WT ← Tlr2/4-/- BM
Tlr2-/- ← WT BM
Tlr4-/- ← WT BM
Tlr2/4-/- ← WT BM

Fig.3

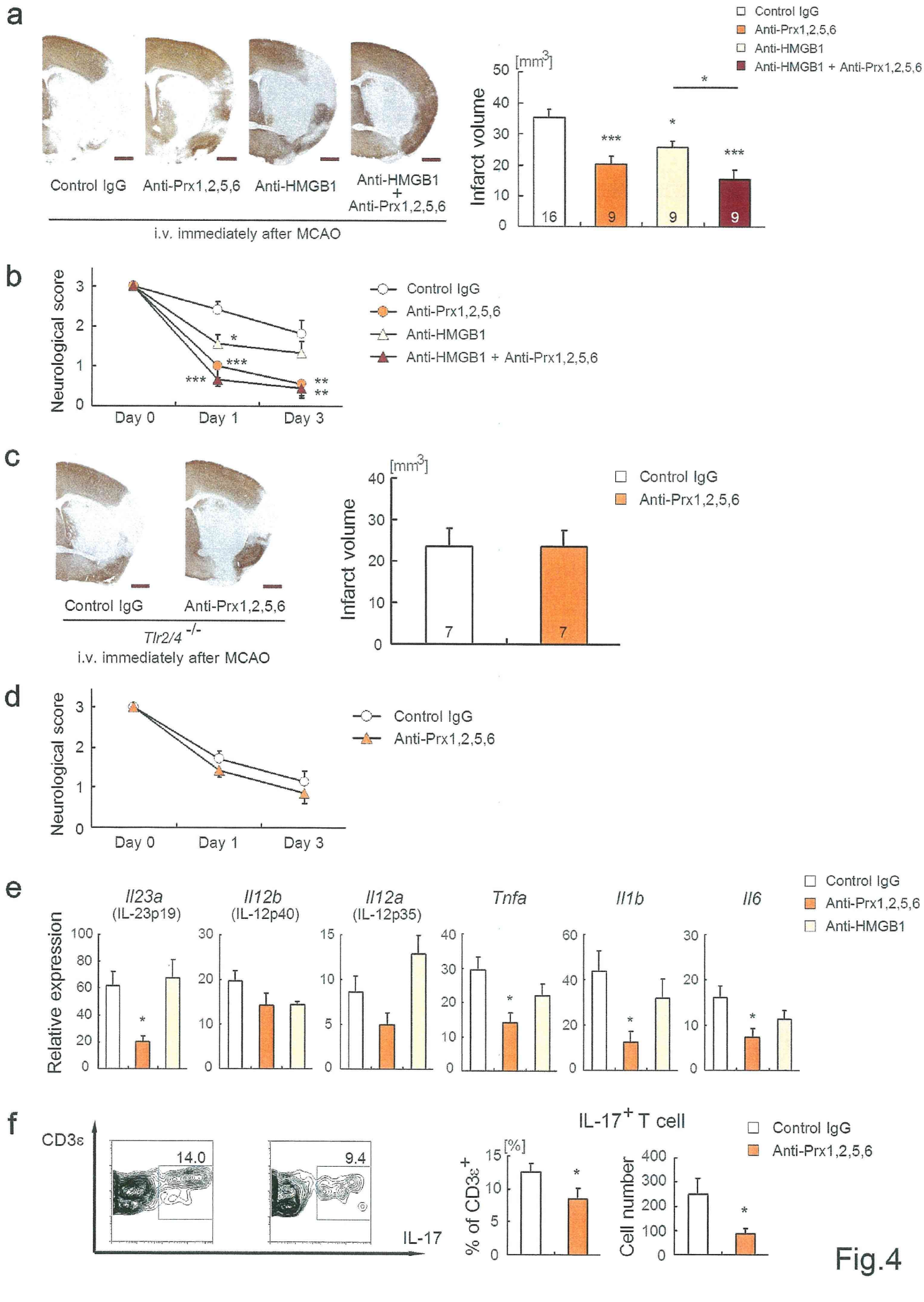
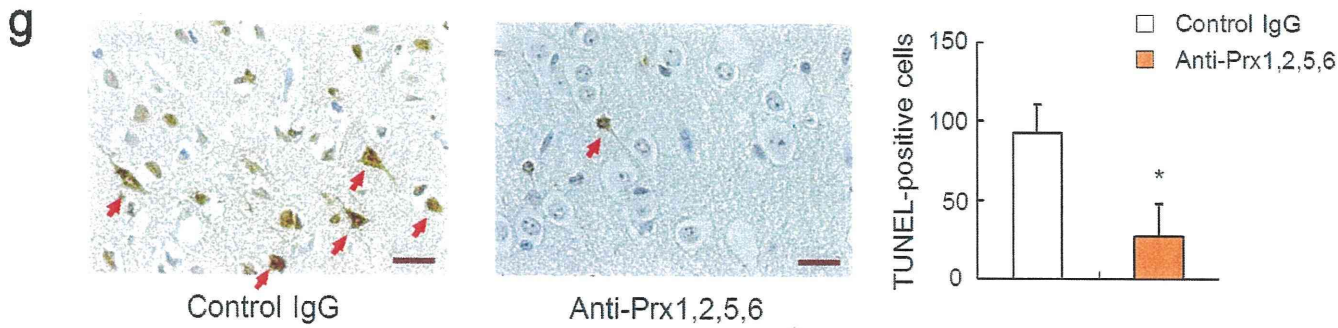
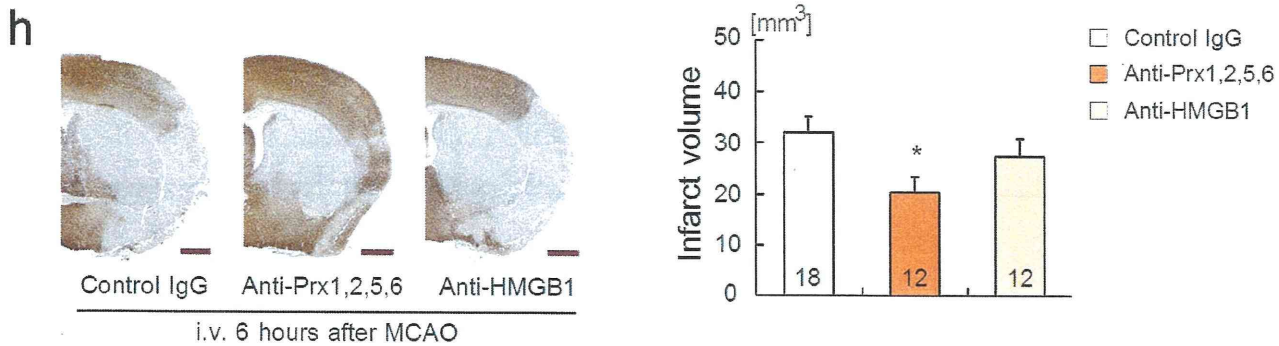
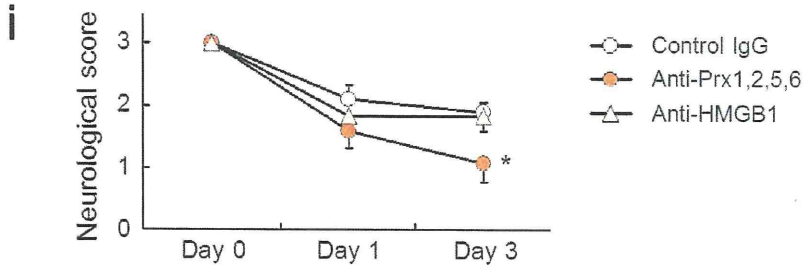
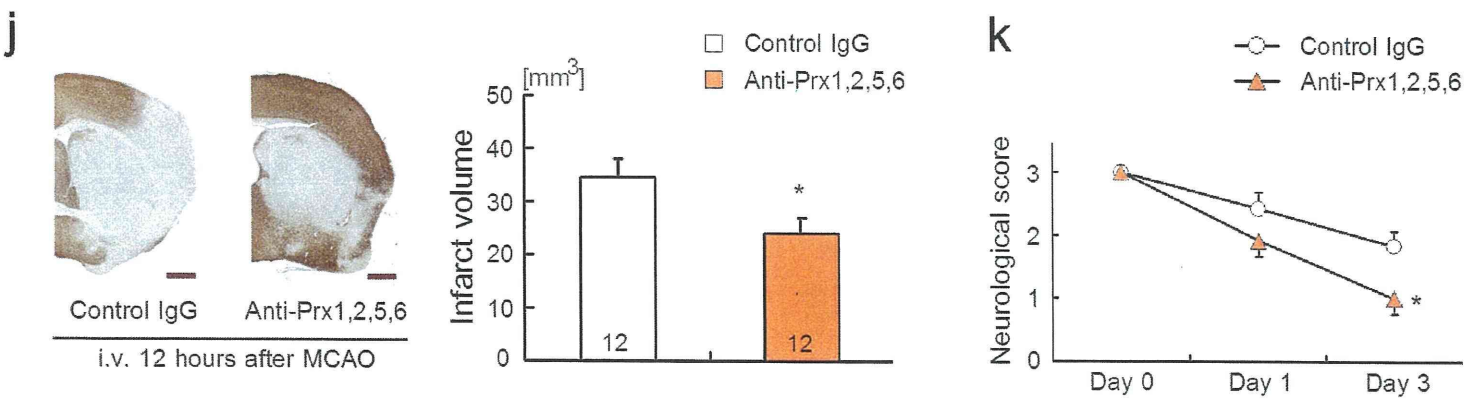

a
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1
Anti-HMGB1 + Anti-Prx1,2,5,6
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1
Anti-HMGB1 + Anti-Prx1,2,5,6
i.v. immediately after MCAO
Infarct volume
[mm3]
16
9
9
9
b
Neurological score
Day 0
Day 1
Day 3
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1
Anti-HMGB1 + Anti-Prx1,2,5,6
c
Control IgG
Anti-Prx1,2,5,6
Tlr2/4 -/-
i.v. immediately after MCAO
Infarct volume
[mm3]
7
7
d
Neurological score
Day 0
Day 1
Day 3
Control IgG
Anti-Prx1,2,5,6
e
Il23a (IL-23p19)
Il12b (IL-12p40)
Il12a (IL-12p35)
Tnfa
Il1b
Il6
Relative expression
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1
f
CD3ε
14.0
9.4
IL-17
IL-17+ T cell
% of CD3ε+
[%]
Cell number
Control IgG
Anti-Prx1,2,5,6

Fig.4


g
Control IgG
Anti-Prx1,2,5,6
TUNEL-positive cells
150
100
50
0
Control IgG
Anti-Prx1,2,5,6
*


h
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1
i.v. 6 hours after MCAO
Infarct volume
[mm3]
50
40
30
20
10
0
18
12
12
*
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1


i
Neurological score
3
2
1
0
Day 0
Day 1
Day 3
*
Control IgG
Anti-Prx1,2,5,6
Anti-HMGB1


j
Control IgG
Anti-Prx1,2,5,6
i.v. 12 hours after MCAO
Infarct volume
[mm3]
50
40
30
20
10
0
12
12
*
Control IgG
Anti-Prx1,2,5,6
k
Control IgG
Anti-Prx1,2,5,6
Neurological score
3
2
1
0
Day 0
Day 1
Day 3
*

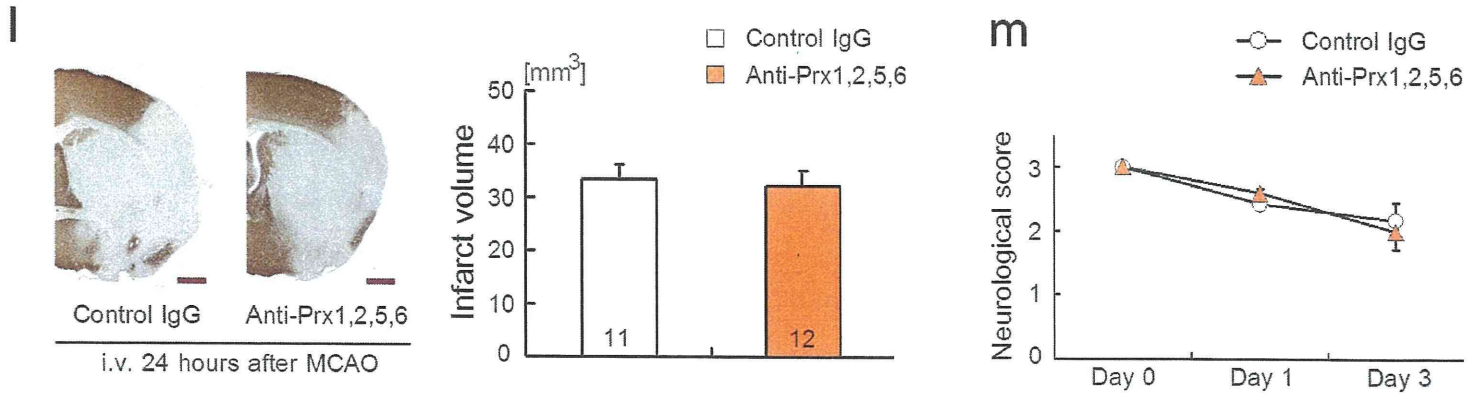

l
Control IgG
Anti-Prx1,2,5,6
i.v. 24 hours after MCAO
Infarct volume
[mm3]
50
40
30
20
10
0
11
12
Control IgG
Anti-Prx1,2,5,6
m
Control IgG
Anti-Prx1,2,5,6
Neurological score
3
2
1
0
Day 0
Day 1
Day 3

Fig.4

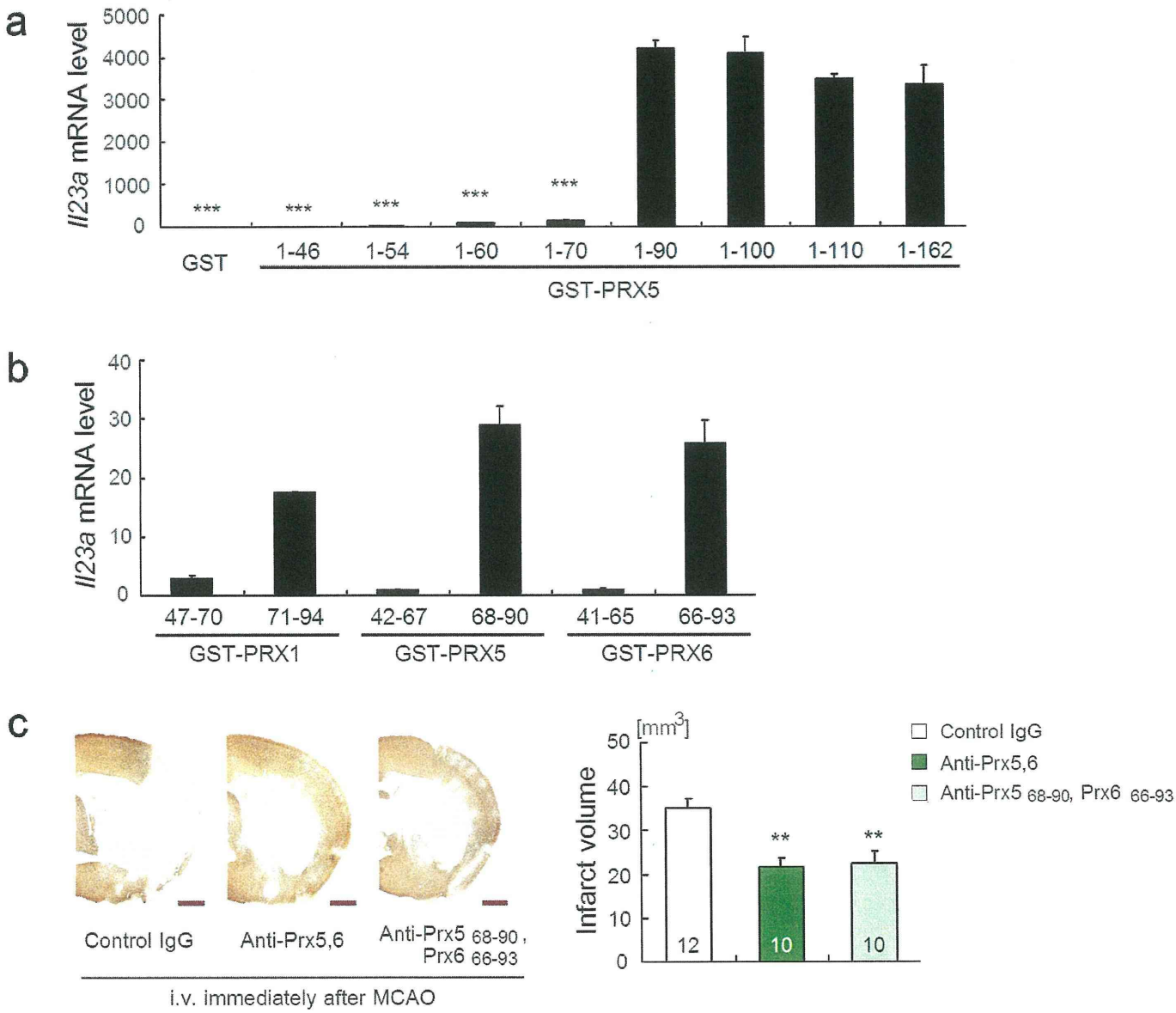
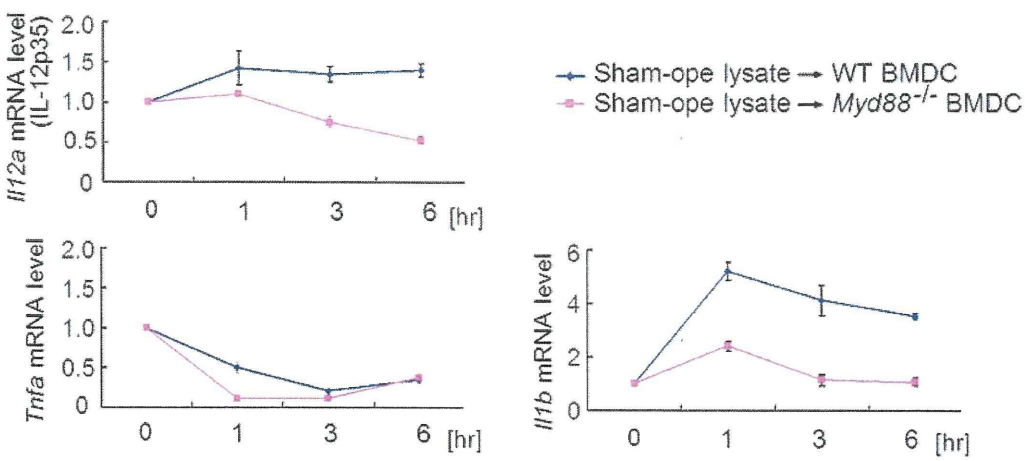

a
Il23a mRNA level
5000
4000
3000
2000
1000
0
***
GST
1-46
1-54
1-60
1-70
1-90
1-100
1-110
1-162
GST-PRX5
b
Il23a mRNA level
40
30
20
10
0
47-70
71-94
42-67
68-90
41-65
66-93
GST-PRX1
GST-PRX5
GST-PRX6
c
Control IgG
Anti-Prx5,6
Anti-Prx5 68-90, Prx6 66-93
i.v. immediately after MCAO
Infarct volume
[mm3]
50
40
30
20
10
0
12
10
10
**
Control IgG
Anti-Prx5,6
Anti-Prx5 68-90, Prx6 66-93
d
Neurological score
3
2
1
0
Day 0
Day 1
Day 3
*
**
***
Control IgG
Anti-Prx5,6
Anti-Prx5 68-90, Prx6 66-93

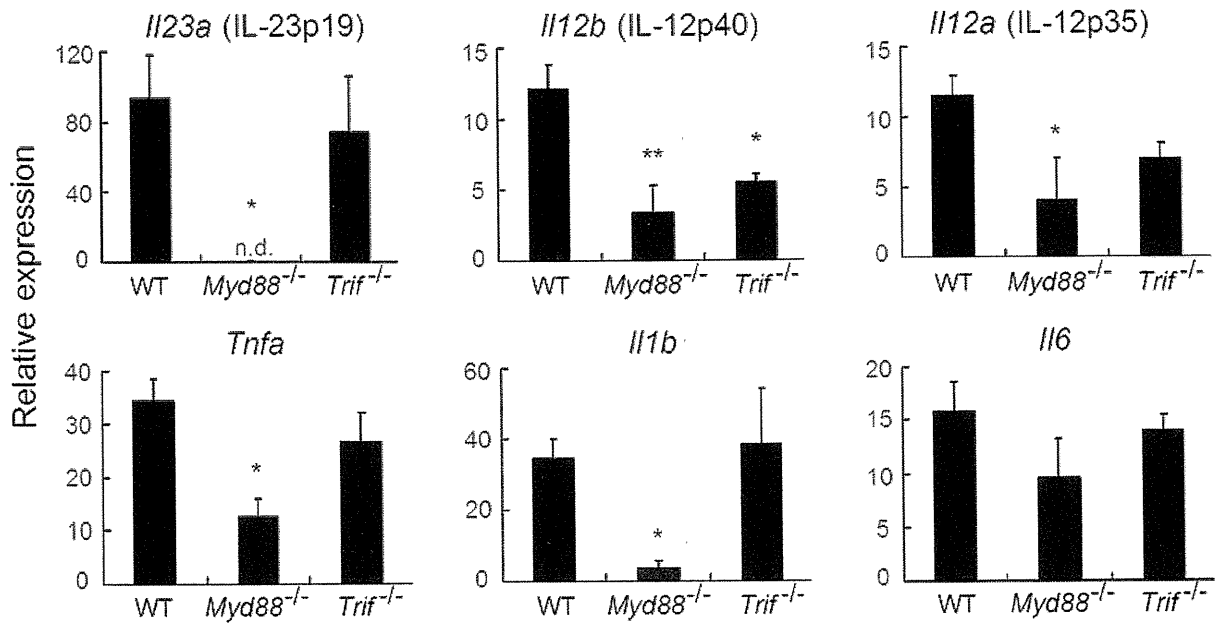
Fig.5

Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain

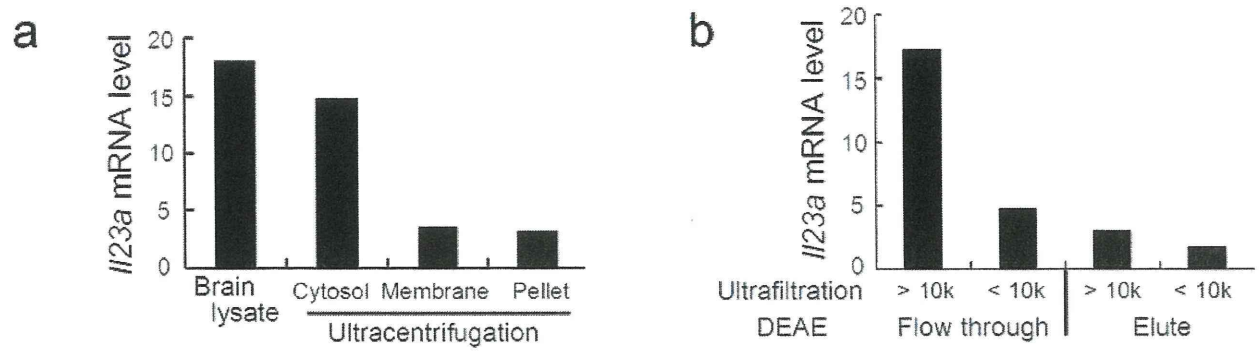
Takashi Shichita, Eiichi Hasegawa, Akihiro Kimura, Rimpei Morita, Ichiro Takada, Takashi Sekiya, Hiroaki Ooboshi, Takanari Kitazono, Toru Yanagawa, Tetsuro Ishii, Hideo Takahashi, Shuji Mori, Masahiro Nishibori, Kazumichi Kuroda, Kensuke Miyake, Shizuo Akira, and Akihiko Yoshimura



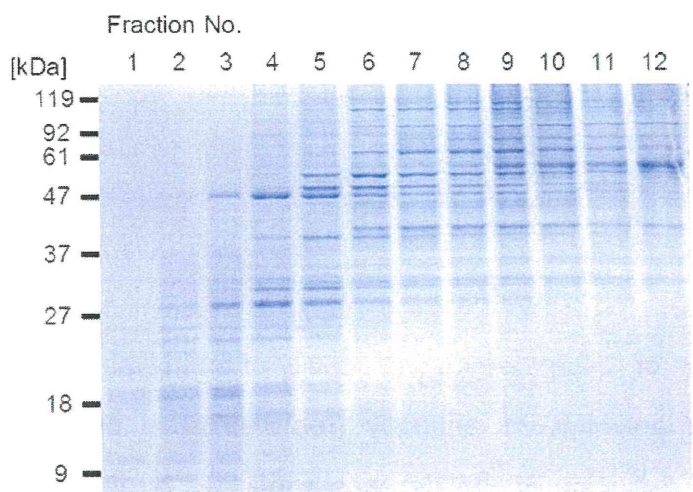
Supplementary Figure 1. The time course of IL-12p35, TNF-α, and IL-1β mRNA expression levels in WT and *Myd88*$^{-/-}$ BMDC induced by the addition of sham-operated brain lysate (n = 3, each).



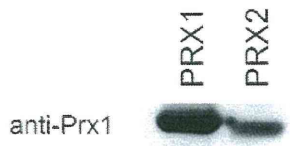
Supplementary Figure 2. The mRNA expression levels of inflammatory cytokines in the infiltrating immune cell in the ischemic brain on day 1. WT, $n = 5$; *Myd88*$^{-/-}$ and *Trif*$^{-/-}$, $n = 3$. *$p < 0.05$, **$p < 0.01$ vs. wild-type [one-way ANOVA with Dunnett's correction; error bars represent s.e.]. n.d.: not detected.



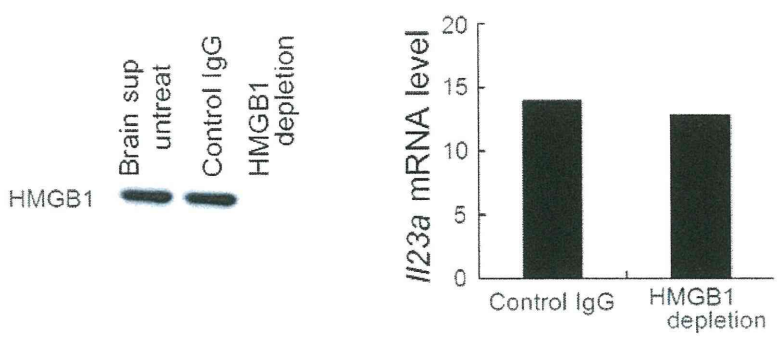
Supplementary Figure 3. (**a**) The IL-23p19 mRNA expression levels in BMDC 1 hour after the stimulation by each fraction of ultracentrifugation. (**b**) The IL-23p19-inducing activity of each fraction by ultrafiltration (10 kDa cut-off) and DEAE Sepharose (representative data of three independent experiments).



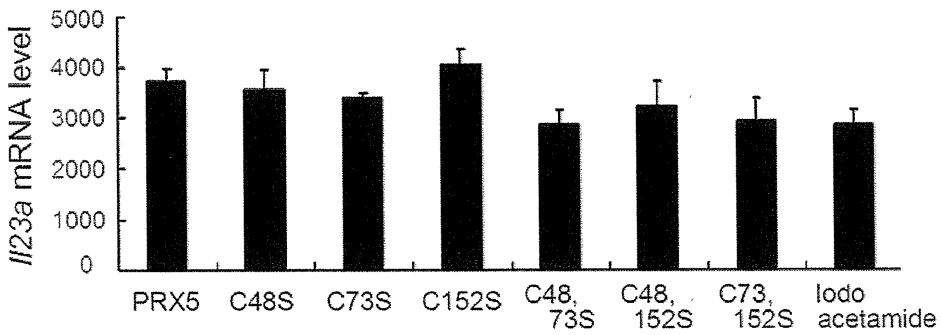
Supplementary Figure 4. SDS-PAGE analysis with CBB staining of each sucrose gradient fraction of the brain lysate (representative data of five independent experiments).



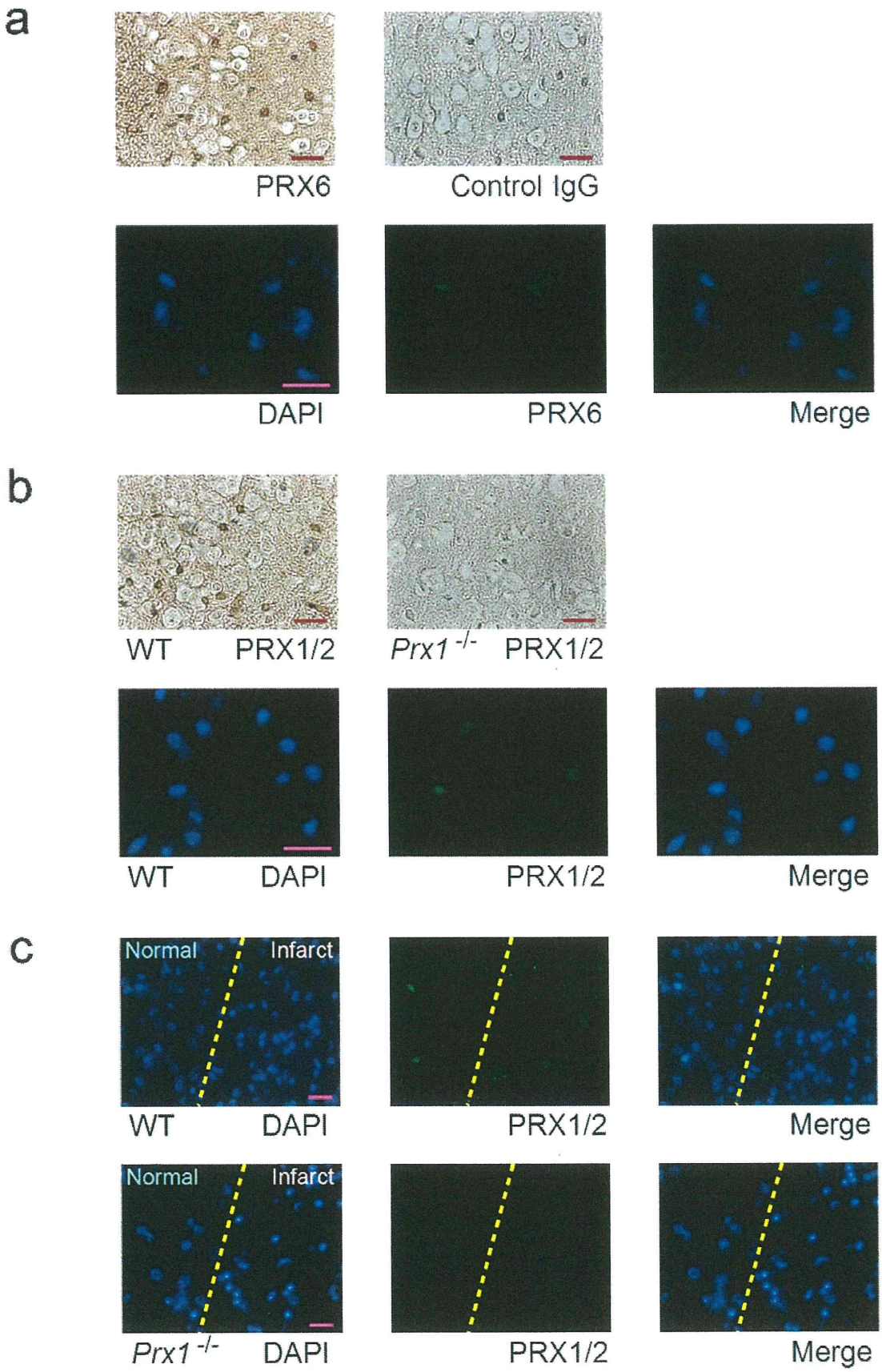
Supplementary Figure 5. Western blot analysis using rabbit polyclonal anti-Prx1 antibody. Due to the high homology between Prx1 and Prx2, anti-Prx1 antibody could also detect Prx2.



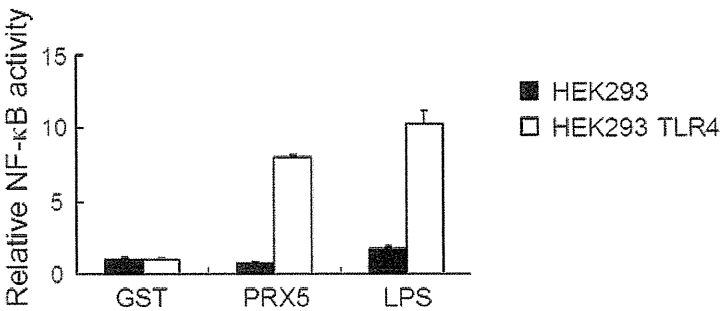
Supplementary Figure 6. The comparison of IL-23p19-inducing activity of brain lysate after the immunoprecipitation with control IgG or anti-HMGB1 antibody (representative data of two independent experiments). The complete depletion of HMGB1 was confirmed by Western blot analysis.



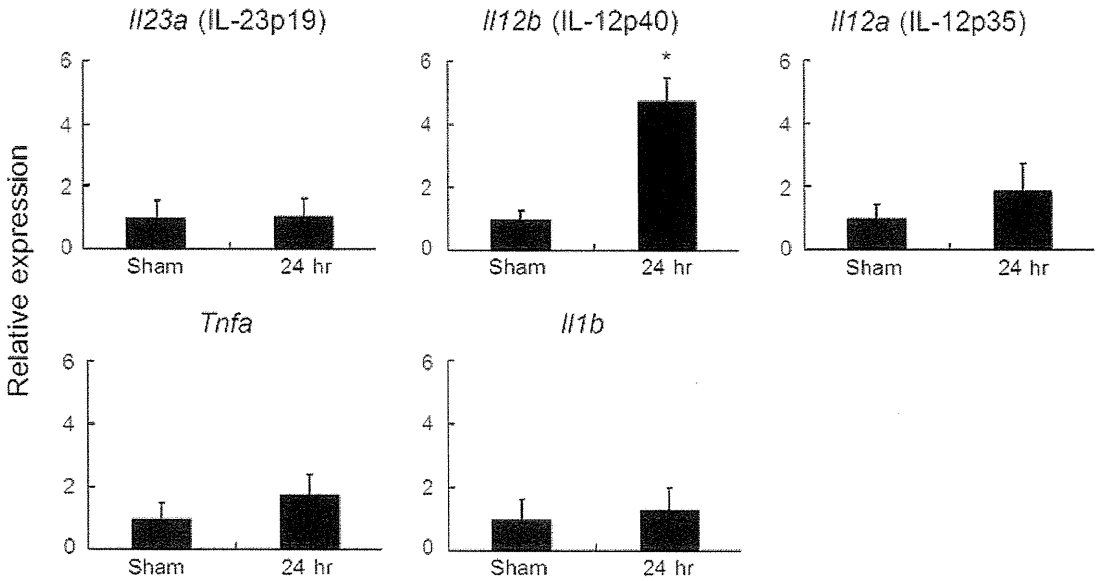
Supplementary Figure 7. The IL-23p19-inducing activity of point-mutated PRX5 protein and alkylated PRX5 protein by iodoacetamide in BMDC (n = 3, each).


a
PRX6
Control IgG
DAPI
PRX6
Merge
b
WT
PRX1/2
Prx1 -/-
PRX1/2
WT
DAPI
PRX1/2
Merge
c
Normal
Infarct
WT
DAPI
PRX1/2
Merge
Normal
Infarct
Prx1 -/-
DAPI
PRX1/2
Merge

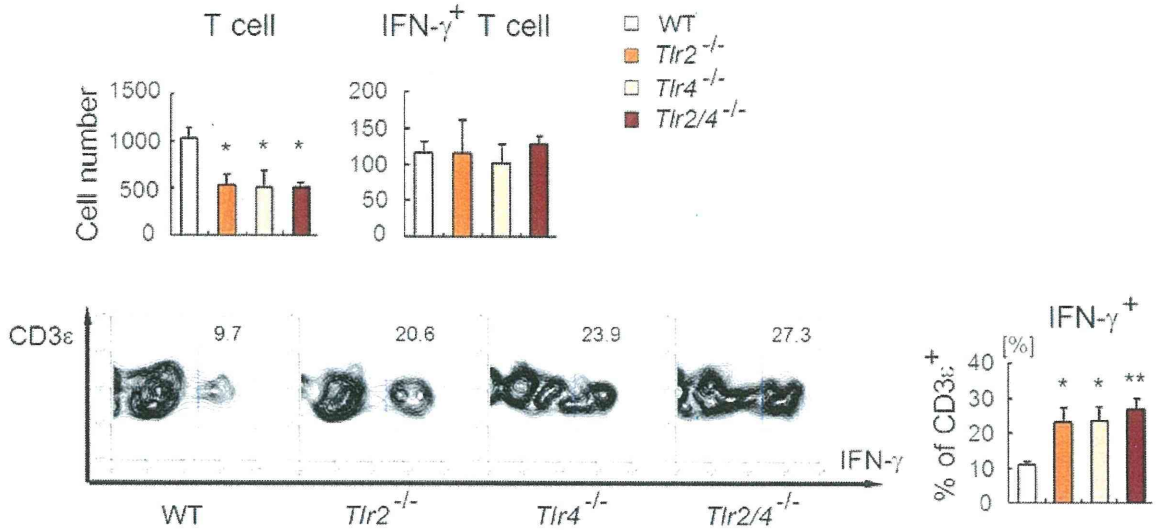
Supplementary Figure 8. (**a**) Anti-Prx6 immunohistostaining of normal brain tissue detected by DAB (upper panel) or Alexa Fluor 488 (lower panel). Control IgG was used for negative control. The pattern of anti-Prx6 immunostaining was similar to previous report[1]. We could find anti-Prx6 positive cells by high sensitivity imaging (lower panel), although this fluorescence intensity was weak compared to Prx6-positive debris (**Fig.2b**) (bar: 20 μm). (**b**) Anti-Prx1/2 immunohistostaining of normal brain tissue detected by DAB (upper panel) or Alexa Fluor 488 (lower panel). (bar: 20 μm) (**c**) Anti-Prx1/2 immunohistostaining of day 1 ischemic brain tissue detected by Alexa Fluor 488. (bar: 20 μm)



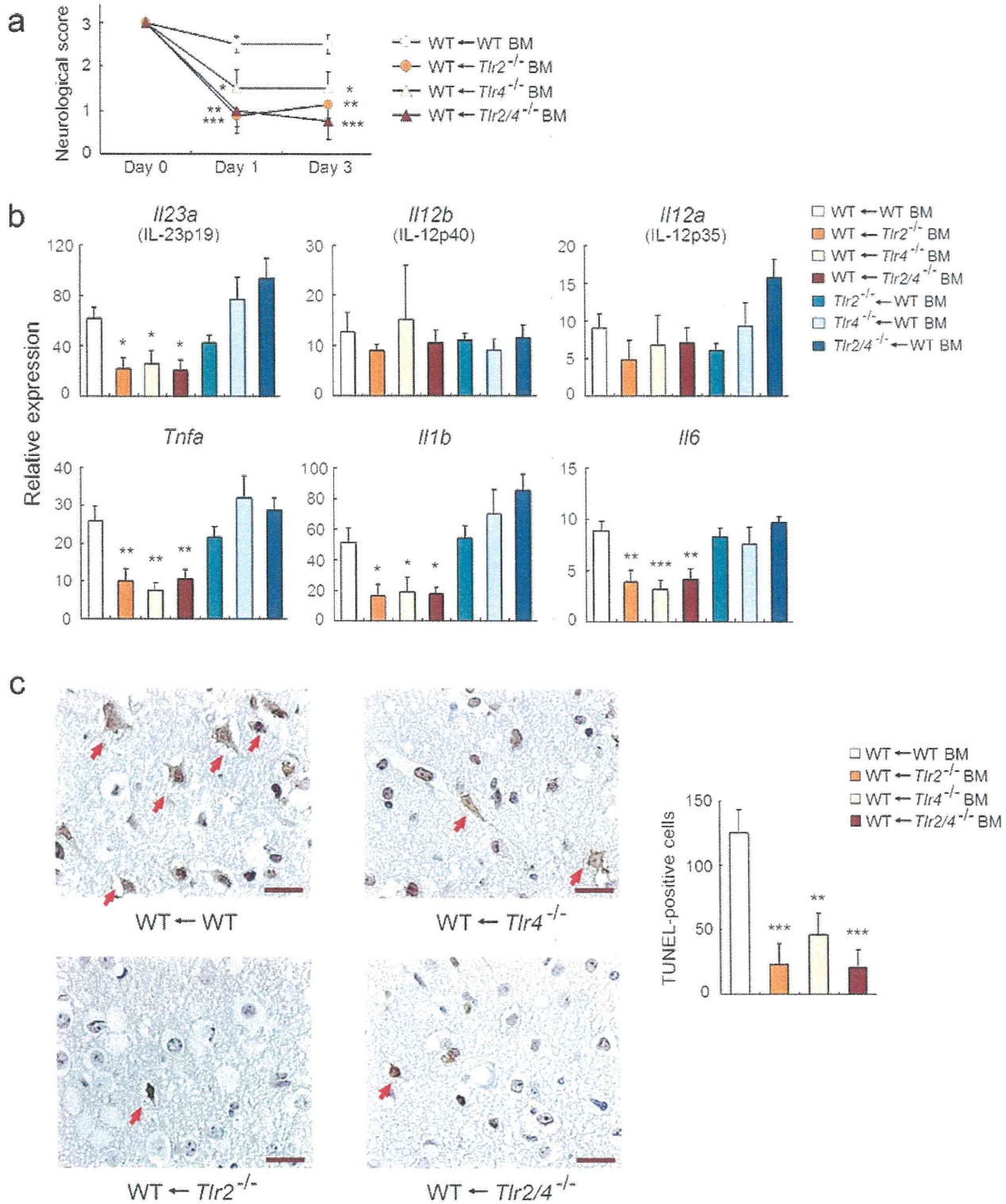
Supplementary Figure 9. The relative NF-κB activity in HEK293 or HEK293 TLR4 cell line by NF-κB luciferase assay 1 hour after stimulation by GST or PRX5 protein (1 μM) or LPS (100 ng ml^{-1}).



Supplementary Figure 10. The mRNA expression levels of inflammatory cytokines in peripheral blood monocytes of sham-operated mice or mice 24 hours after the induction of brain ischemia (n = 3 for each). *p < 0.05 vs. Sham [two-sided Student's t-test; error bars represent s.e.].



Supplementary Figure 11. The absolute number and the ratio of IFN-γ^+ T cell on day 3 (n = 8 for WT and n = 6 for other samples). TLR2 and/or TLR4 deficiency significantly increased the ratio of IFN-γ^+ T cells, because the number of IFN-γ^+ T cells was kept in spite of decreased number of total infiltrating T cells. *p < 0.05, **p < 0.01 vs WT (one-way ANOVA with Dunnett's correction; the error bars represent s.e.).



Supplementary Figure 12. (**a**) Neurological scores of WT or TLR-KO BM chimeric mice on days 1 and 3. (**b**) The mRNA expression levels of inflammatory cytokines in the infiltrating immune cells of WT or TLR-KO BM chimeric mice on day 1. (**c**) The results of TUNEL staining of the