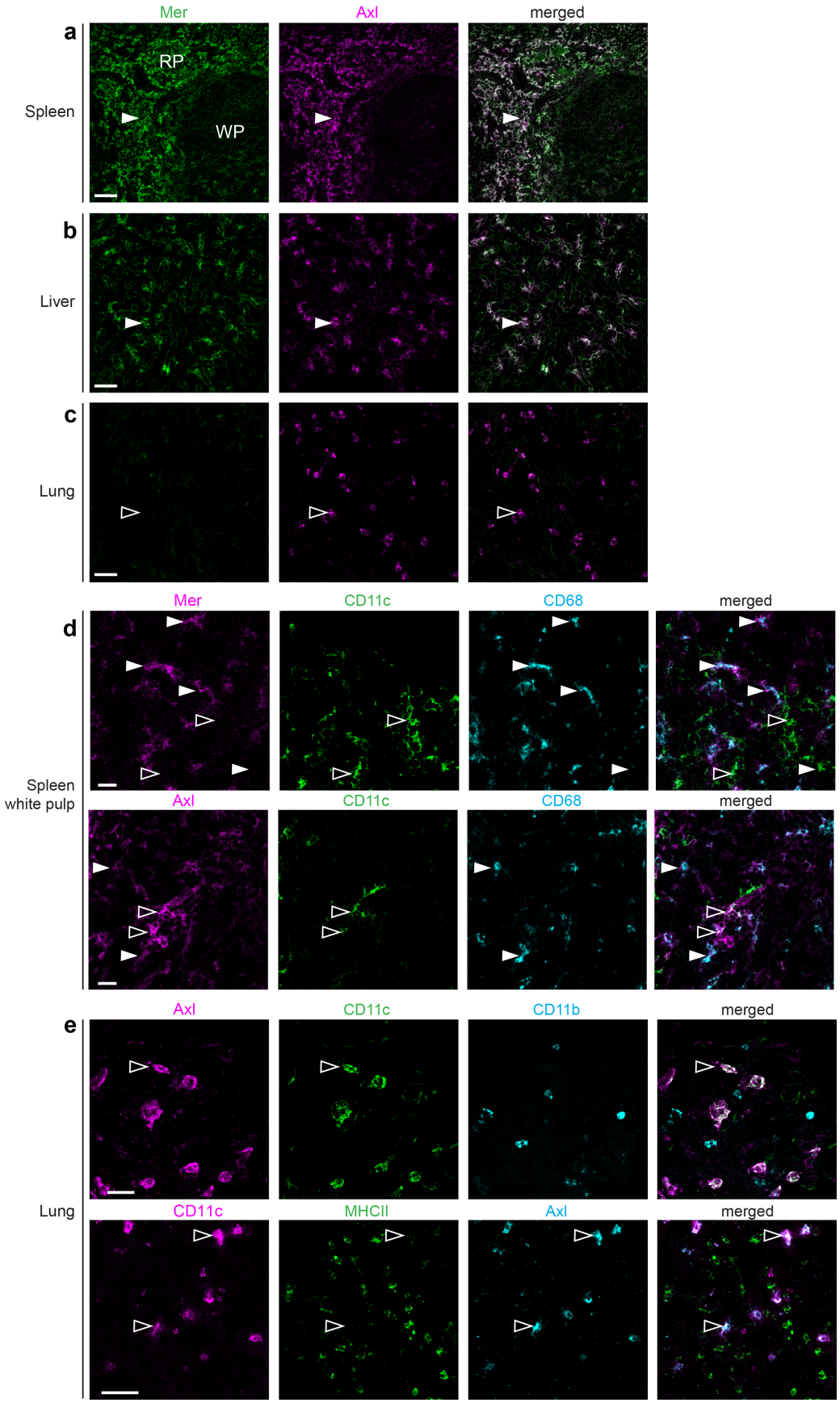
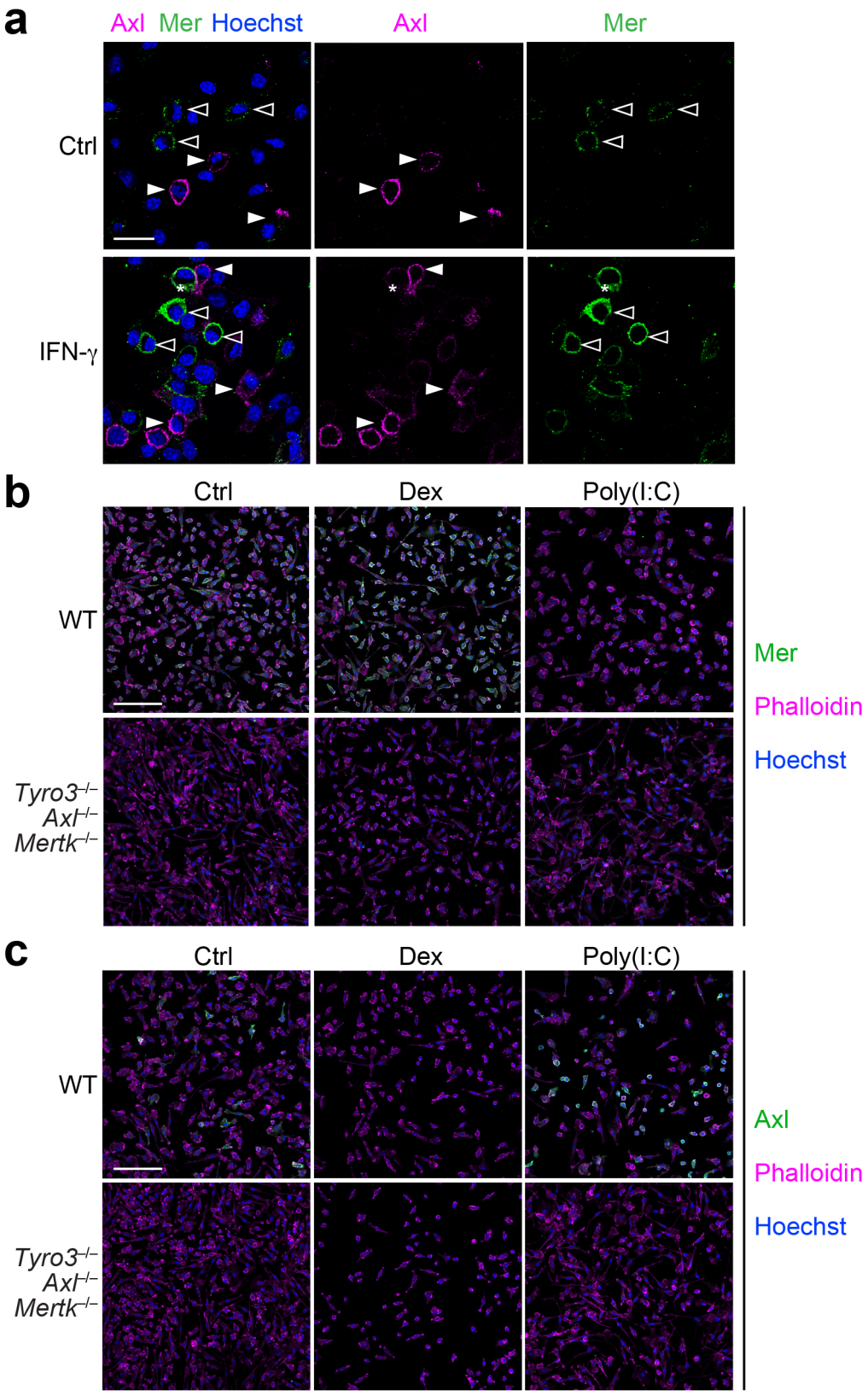

a
Spleen
Mer
Axl
merged
RP
WP
b
Liver
c
Lung
d
Spleen
white pulp
Mer
CD11c
CD68
merged
Axl
CD11c
CD68
merged
e
Lung
Axl
CD11c
CD11b
merged
CD11c
MHCII
Axl
merged

Supplementary Figure 1 (previous page)

Axl and Mer expression on immune cell populations *in vivo*.

Immunohistochemistry of Axl and Mer in spleen (**a**), liver (**b**), and lung (**c**). Closed arrowheads: cells co-expressing Axl and Mer; open arrowheads: cells expressing mostly Axl. In the spleen, the principal Axl-Mer co-expressing cells are $F4/80^{+}$ red pulp (RP) macrophages. In the splenic white pulp (WP) (**d**), Tingible body macrophages ($F4/80^{-}CD68^{+}$) express Mer and low levels of Axl (closed arrowheads), whereas a subpopulation of splenic $CD11c^{+}$ DCs express only Axl (open arrowheads). In the lung (**c** and **e**), alveolar macrophages ($CD11c^{+}CD11b^{-}MHCII^{-}F4/80^{lo}$) are only Axl-positive (open arrowheads). In the liver, $F4/80^{+}$ Kupffer cells are both Axl and Mer positive (**b**, closed arrowheads). Bars, 50 μm (**a-c**); 10 μm (**d**); and 20 μm (**e**). Representative images from n=3 mice.

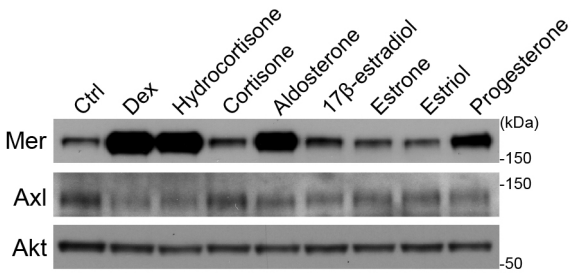

a
Axl Mer Hoechst
Axl
Mer
Ctrl
IFN-γ
b
Ctrl
Dex
Poly(I:C)
WT
Tyro3−/−
Axl−/−
Mertk−/−
Mer
Phalloidin
Hoechst
c
Ctrl
Dex
Poly(I:C)
WT
Tyro3−/−
Axl−/−
Mertk−/−
Axl
Phalloidin
Hoechst

Supplementary Figure 2 (previous page)

Discrete Axl^{+} and Mer^{+} cell populations *in vitro*.

(**a**) Unstimulated (Ctrl) or IFN-γ (250 U/ml, 18 h) treated BMDM cultures were stained live with Mer (green) and Axl (magenta) antibodies. Closed arrows: cells expressing mostly Axl; open arrows- cells expressing mostly Mer. The asterisk marks a single cell that is weakly positive for both Axl and Mer. Bar, 20 μm. Representative images of three independent experiments.

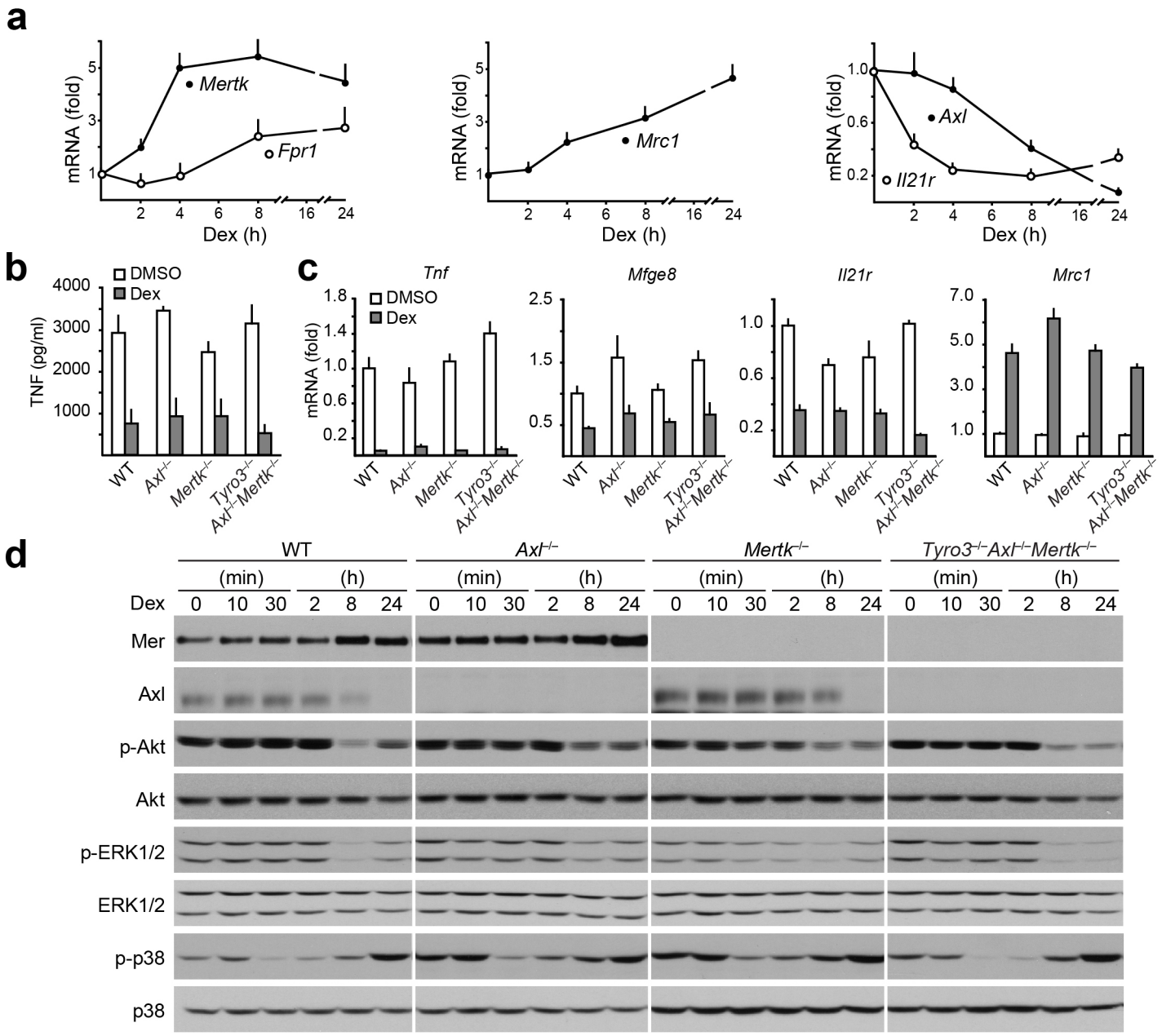
(**b,c**) BMDM cultures were stimulated with 0.1 μM Dex or 10 μg/ml poly(I:C) for 24 h, fixed and stained with Mer (**b**) or Axl (**c**) antibodies and counterstained with Phalloidin-TRITC. Bar, 100 μm. Representative images of three independent experiments.



Supplementary Figure 3

Axl and Mer regulation by steroid hormones.

BMDM cultures were stimulated with 1 μM Dex, hydrocortisone, cortisone, aldosterone, 17β-estradiol, estrone, estriol or progesterone for 24 h. Axl and Mer expression was assayed by immunoblotting. Representative of two independent experiments.



Supplementary Figure 4

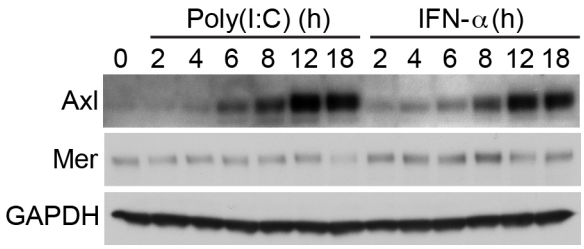
Anti-inflammatory effects of Dex are Mer- and Axl-independent.

(**a**) RT-qPCR showing the kinetics of *Mertk*, *Fpr1*, *Mrc1* mRNA induction and *Axl* and *Il21r* mRNA inhibition in BMDMs in response to 0.1 μM Dex.

(**b**) BMDMs from indicated mice were treated with 100 ng/ml LPS with or without 0.1 μM Dex. TNF secretion was measured 24 h later by ELISA in culture supernatants. Representative of three independent experiments.

(**c**) RT-qPCR showing changes in *Tnf*, *Mfge8*, *Il21r* and *Mrc1* mRNAs in BMDMs after 24 h incubation with 0.1 μM Dex. Data in (**a**) and (**c**) are presented as fold of change normalized to *Hprt* mRNA. Average of two independent experiments, each done in technical duplicate, graphed as mean ± s.d.

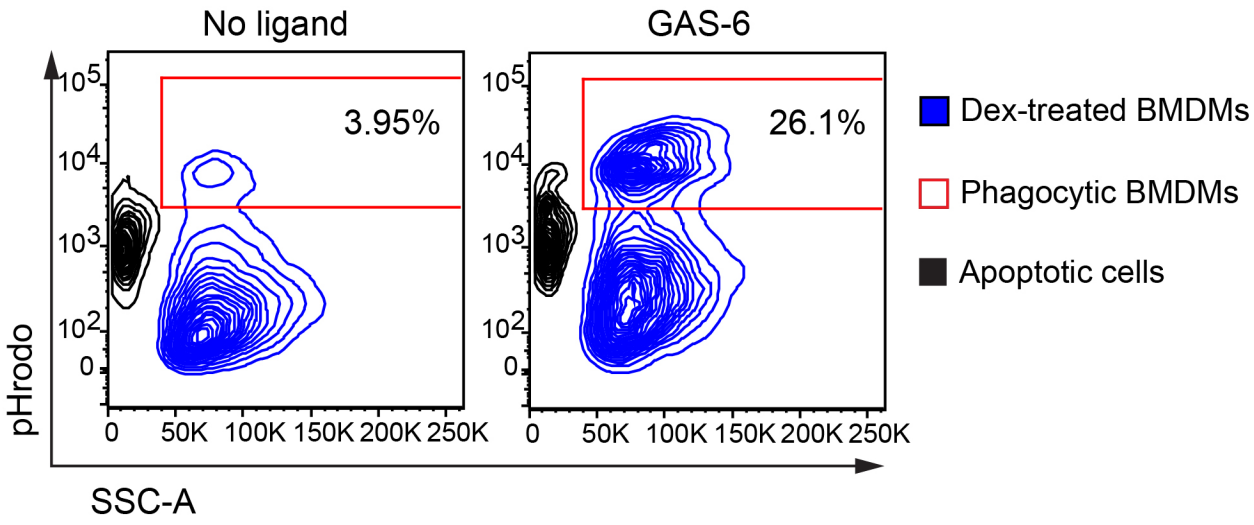
(**d**) Immunoblot showing changes in activity of Akt, ERK1/2 and p38 signaling pathways in response to 0.1 μM Dex treatment of BMDMs derived from indicated knock-out mice. Representative of two independent experiments.



Supplementary Figure 5

Axl induction by poly(I:C) and IFN-α in BMDMs.

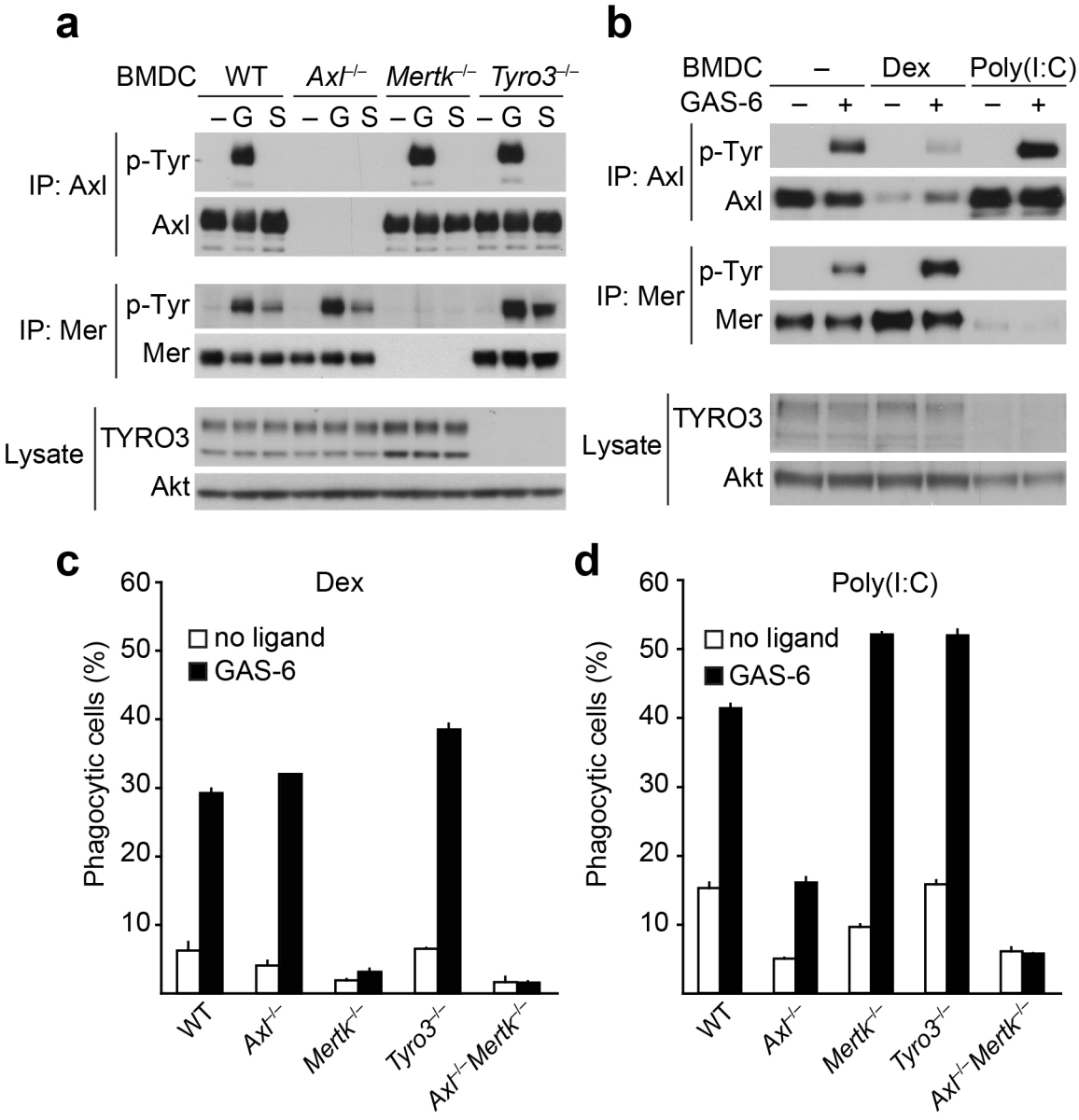
Cells were incubated with either poly(I:C) (1 μg/ml) or IFN-α (250 U/ml) for the indicated times in hours (h), and then blotted for total Axl (top), Mer (middle), or GAPDH (bottom). Representative of two independent experiments.



Supplementary Figure 6

Flow cytometry based phagocytosis assay.

Apoptotic cells are labeled with pH-sensitive dye, pHrodo. Once engulfed into the acidic environment of phagosomes, pHrodo fluorescence is enhanced and phagocytic macrophages are distinguished based on their side scatter (SSC-A) and pHrodo fluorescence intensity using flow cytometry. In this experiment, the percent cells in the phagocytic gate is quantified in an 1-hour assay, in absence or presence of 10 nM GAS-6. Representative plot of 6 independent experiments.



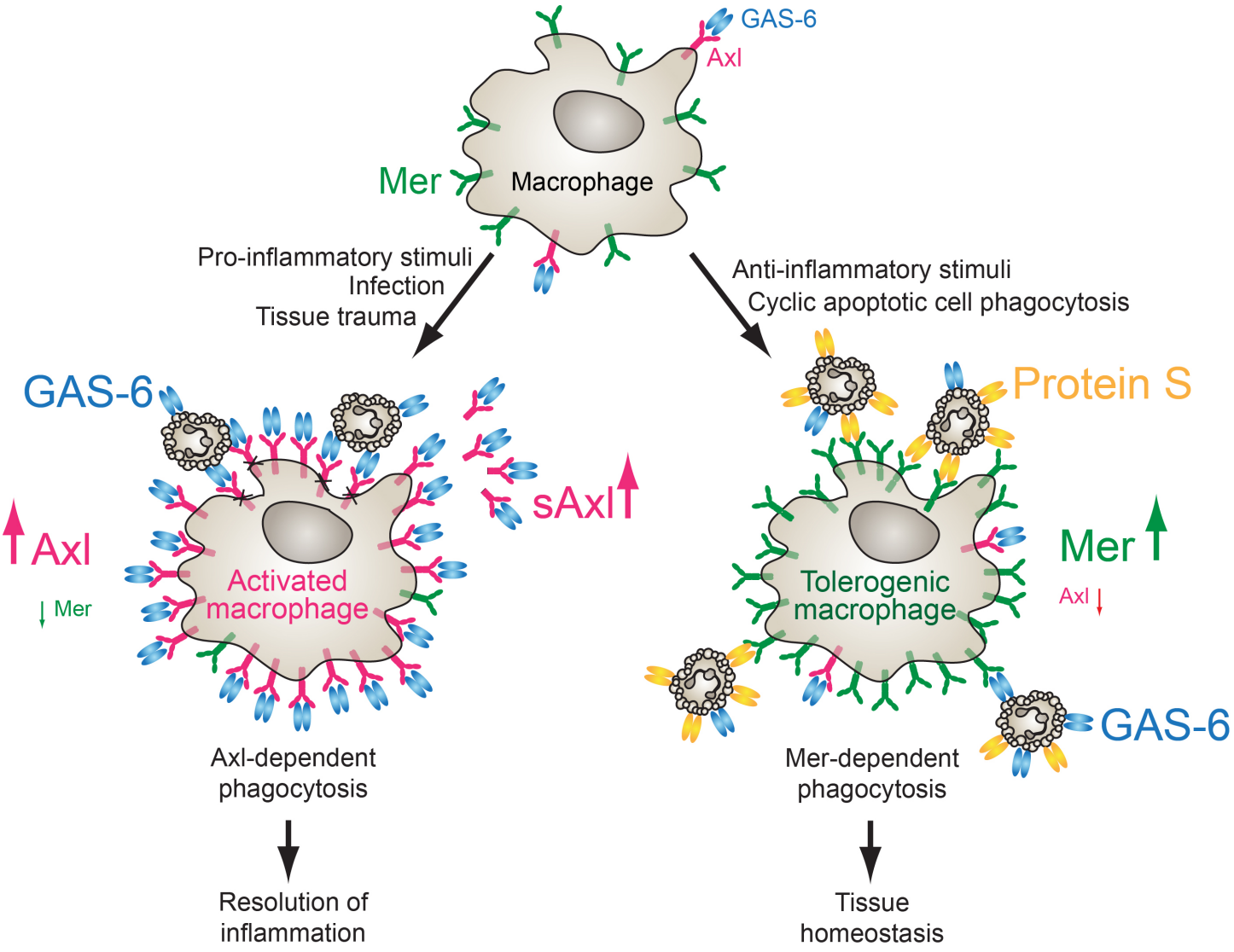
Supplementary Figure 7

Regulation of Axl and Mer expression and phagocytosis in BMDCs.

(**a**) BMDC cultures from the indicated mice were stimulated for 10 min with 10 nM GAS-6 (G) or 25 nM Protein S (S). Receptor activation was assayed by immunoprecipitation and immunoblotting. Representative of two independent experiments.

(**b**) BMDCs were cultured for 18 h in the presence of 0.1 μM Dex or 10 μg/ml poly(I:C) and then stimulated for 10 min with 10 nM GAS-6. Receptor activation was assayed by immunoprecipitation and immunoblotting. Representative of two independent experiments.

(**c, d**) BMDCs from mice of the indicated genotypes were cultured for 24 h in the presence of 0.1 μM Dex (**c**) or 10 μg/ml poly(I:C) (**d**) and then incubated for 1 h with pHrodo stained ACs with or without 10 nM GAS-6. Percent of phagocytosis was measured using flow cytometry. Data are presented as mean ± s.d. from two independent experiments, each done for duplicate cultures for each condition.



Supplementary Figure 8

Model for the differential regulation and action of Axl and Mer in inflammatory and tolerogenic environments.

See text for details.