Supplementary Information

Peli1 promotes microglia-mediated CNS inflammation by regulating Traf3 degradation

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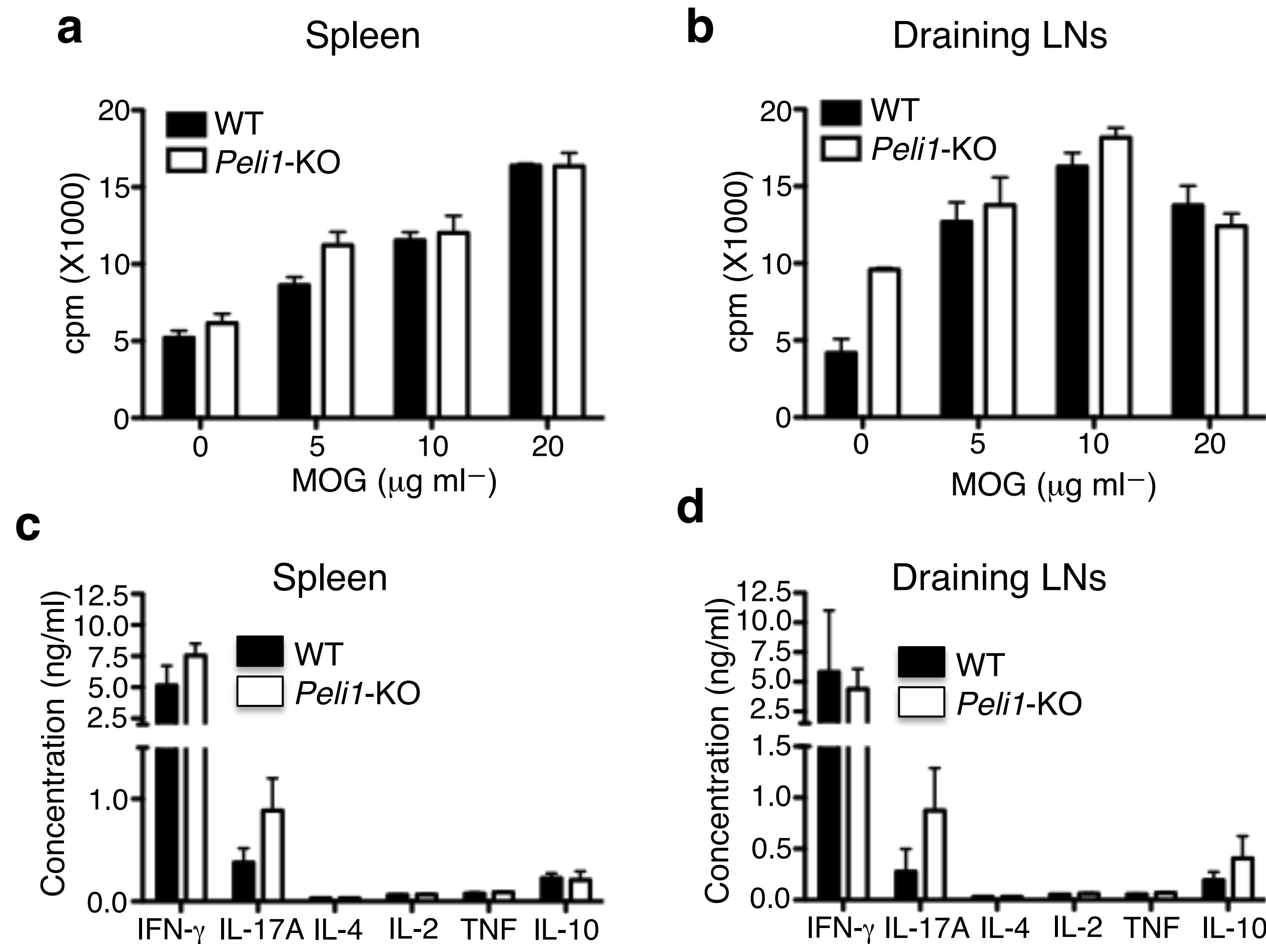
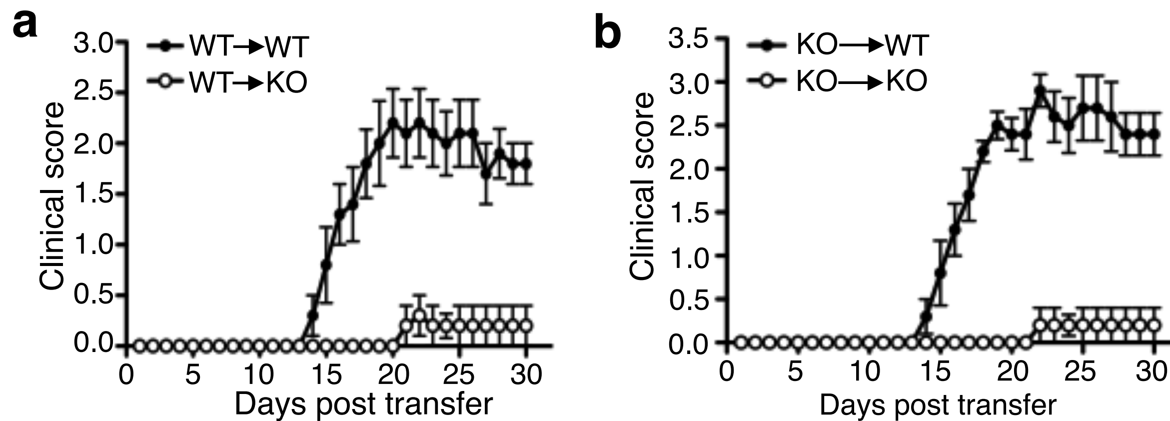
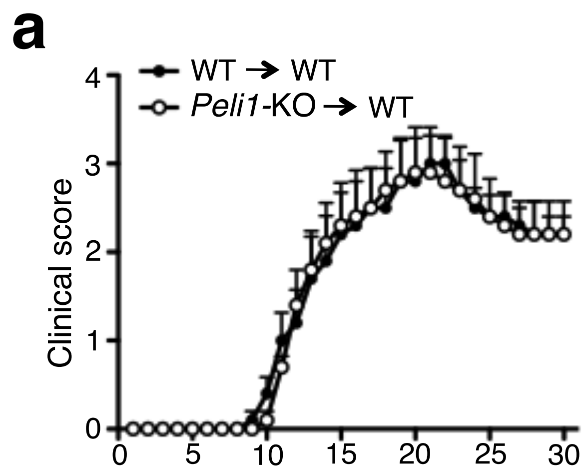
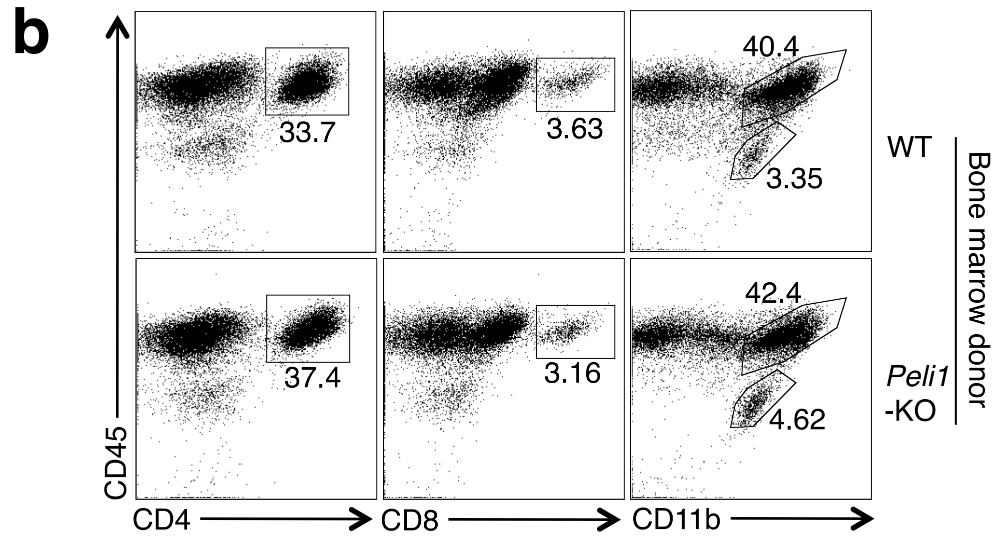


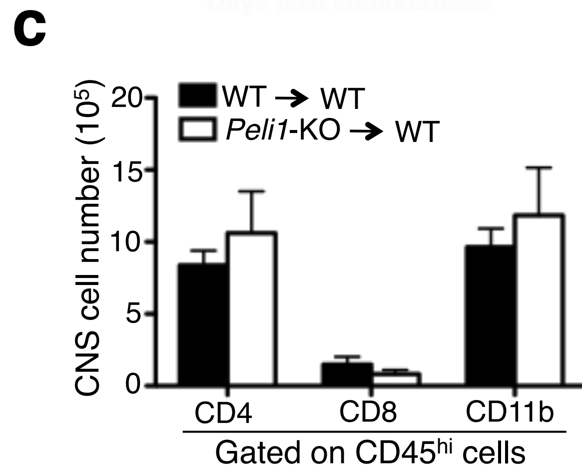
Supplementary Fig. 1. *Peli1*-deficient T cells have normal recall response *in vitro.* **(a,b)** Splenocytes or draining LNs cells isolated from $MOG_{35–55}$-immunized WT and *Peli1*-KO mice were cultured in triplicate in complete medium at a density of 2×10^5 per well in 96-well plates in the presence or absence of MOG peptide at the indicated concentration. Cell proliferation was measured after 72 h based on ^{3}H-thymidine incorporation. (**c,d**) ELISA of the indicated cytokines using supernatants collected from the splenocyte (**c**) and draining lymph node cell (**d**) cultures after 48 h of antigen stimulation.



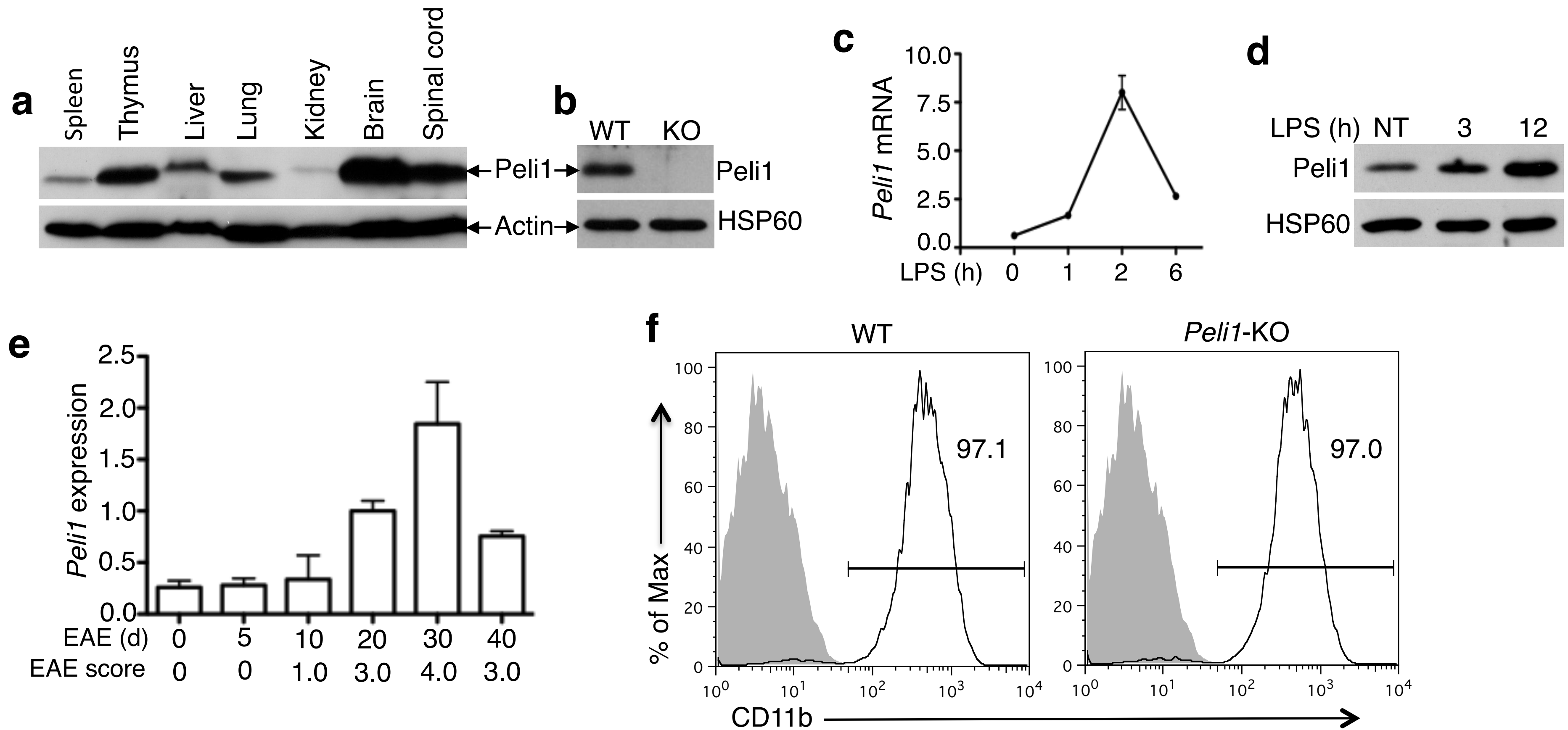
Supplementary Fig. 2. *Peli1*-deficient T cells are competent for inducing EAE. Splenocytes and draining lymph node cells were prepared from $MOG_{35\text{-}55}$-immunized WT (**a**) or *Peli1*-deficient (**b**) mice (at day 10 post immunization) and restimulated *in vitro* for 5 days with $MOG_{35\text{-}55}$. The MOG-specific T cells were transferred into sublethally irradiated WT or *Peli1* KO mice for passive EAE induction. Mean clinical scores were calculated based on multiple recipient mice (n=5/group).



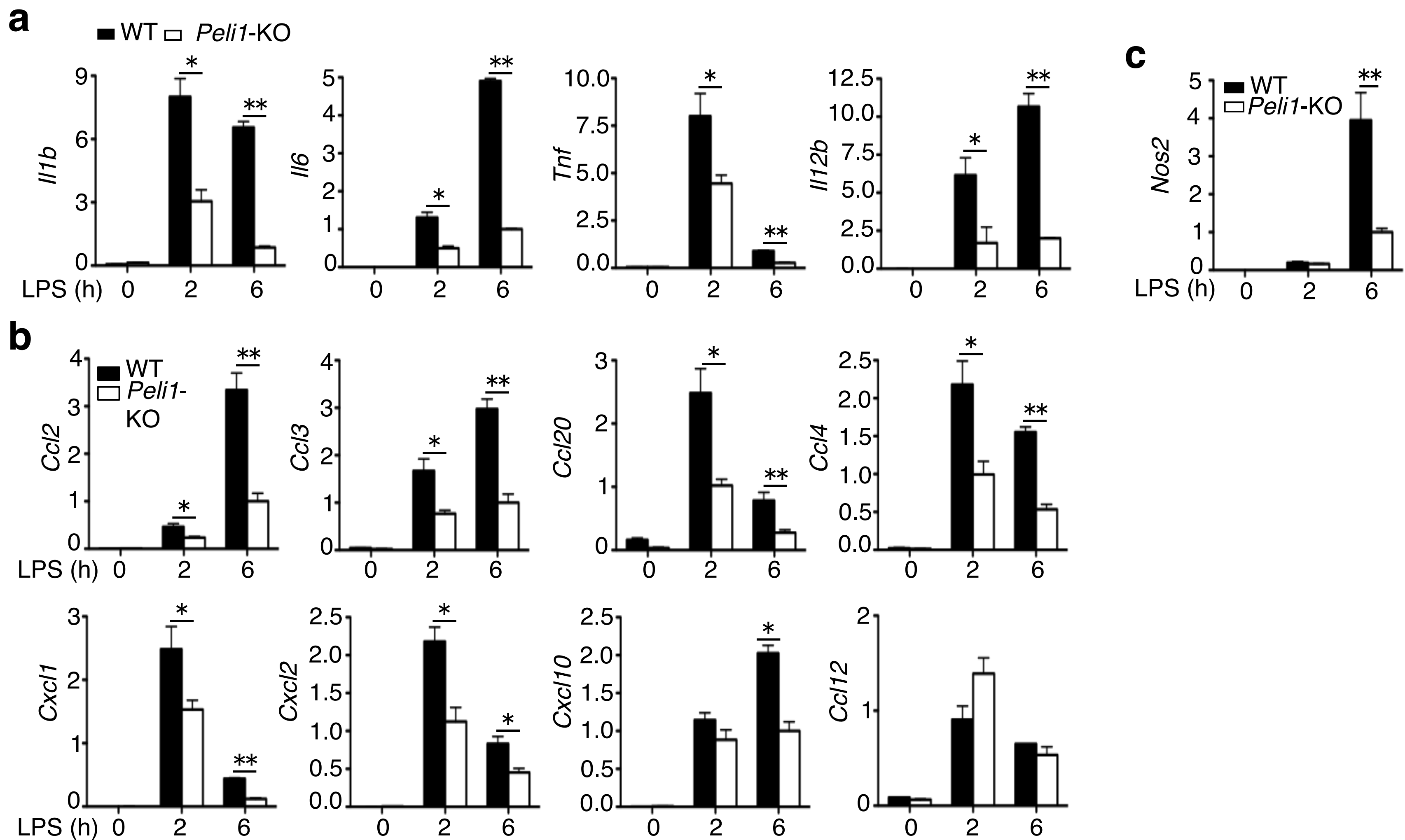




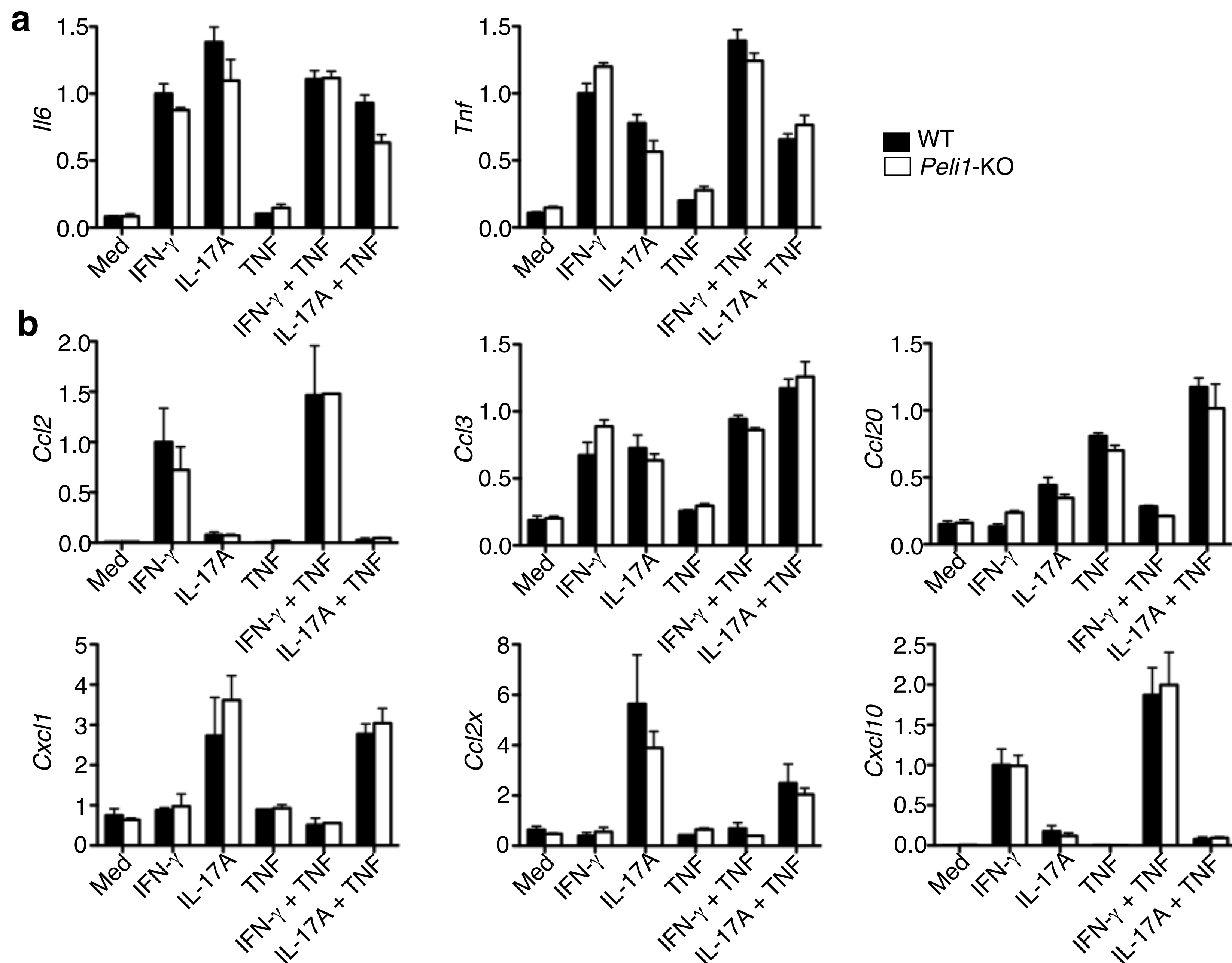
Supplementary Fig. 3. Peli1 is dispensable in BM cells for EAE pathogenesis. (**a**) EAE induction in lethally irradiated WT recipient mice adoptively transferred with WT or *Peli1*-KO BM cells. (**b, c**) Flow cytometry analysis of CNS-infiltrating mononuclear cells of $MOG_{35\text{-}55}$-immunized chimeric mice described in **a**, showing a representative plot (**b**) and a summary graph (**c**).



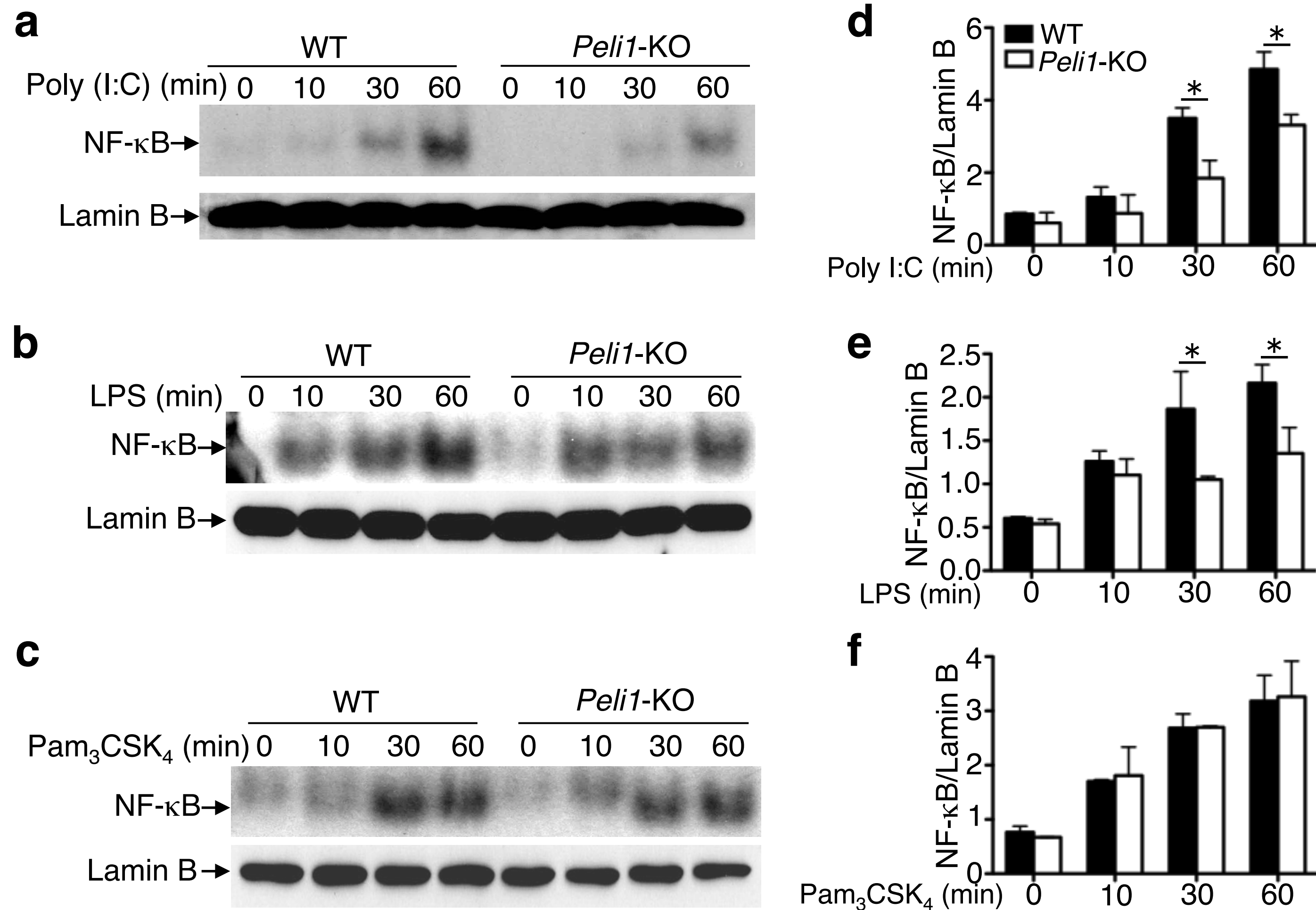
Supplementary Fig. 4. Peli1 is a highly expressed and predominant member of Peli family in microglia. (**a**,**b**) Immunoblot analysis of Peli1 expression in different tissues (**a**) and in WT and *Peli1*-KO microglia (**b**). (**c**,**d**) Real-time PCR (**c**) and immunoblot (**d**) analyses of LPS-stimulated Peli1 expression in WT mouse microglia. (**e**) Real-time PCR analysis of *Peli1* mRNA in spinal cords from EAE-induced mice at the indicated days. The EAE scores of the mice were indicated. (**f**) Flow cytometry analysis of cultured primary microglia derived from WT and *Peli1*-KO newborn mice. Isotype control (grey).



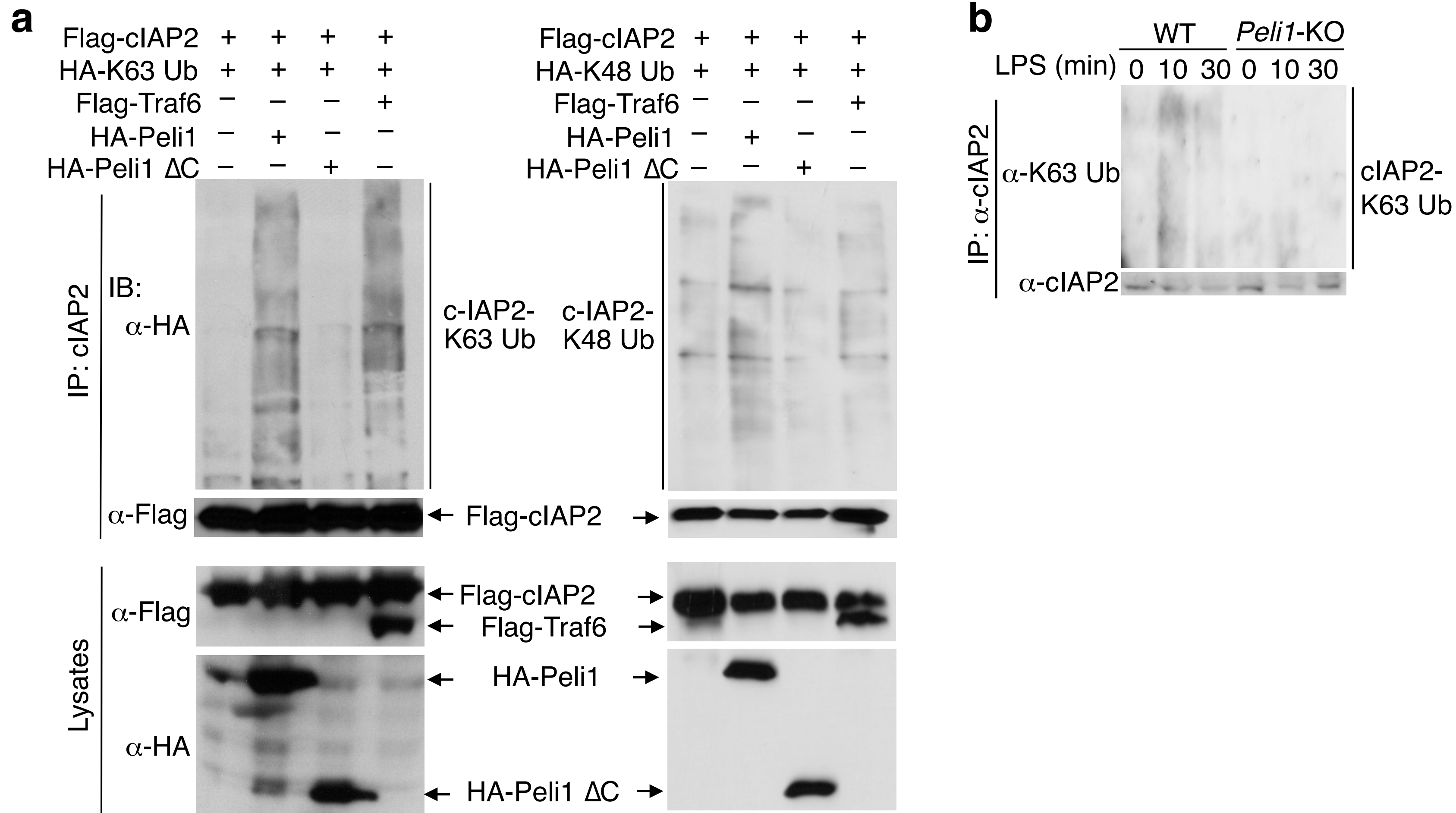
Supplementary Fig. 5. Peli1 mediates induction of proinflammatory cytokine and chemokine genes by LPS. Real-time PCR analysis of relative mRNA induction for the indicated proinflammatory cytokine genes (**a**), chemokine genes (**b**), and *Nos2* gene (**c**) in LPS-stimulated WT and *Peli1*-KO primary microglia.



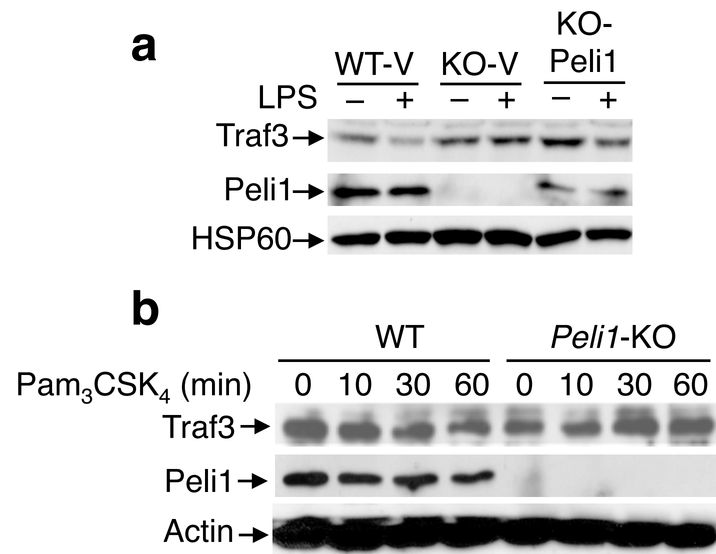
Supplementary Fig. 6. Peli1 is dispensable for the inflammatory response in astrocytes. (**a**,**b**) Real-time PCR analysis of gene induction of the proinflammatory cytokines (**a**) and chemokines (**b**) in WT and *Peli1*-KO astrocytes in response to IFN-γ, IL-17A, TNF, or in combinations as indicated.



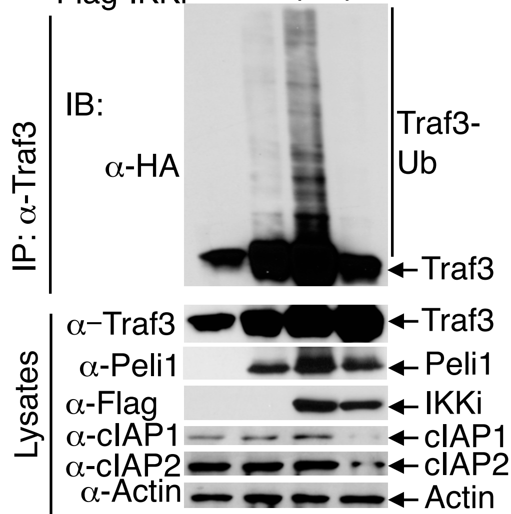
Supplementary Fig. 7. Peli1 regulates NF-κB activation by TRIF-dependent TLRs in microglia. WT and *Peli1*-KO microglia were stimulated with poly(I:C) (10 mg ml⁻) (**a, d**), LPS (100 ng/ml) (**b, e**), or Pam_3CSK_4 (1 μg ml⁻) (**c, f**) for the indicated time periods, and nuclear extracts were subjected to NF-κB EMSAs. Lamin B immunoblots were used as loading controls (**a, b, c**). The NF-κB bands were quantified using ImageJ and presented as fold relative to an internal control Lamin B (**d, e, f**). Data are mean±S.D. values, based on three independent experiments. *$P<0.05$.



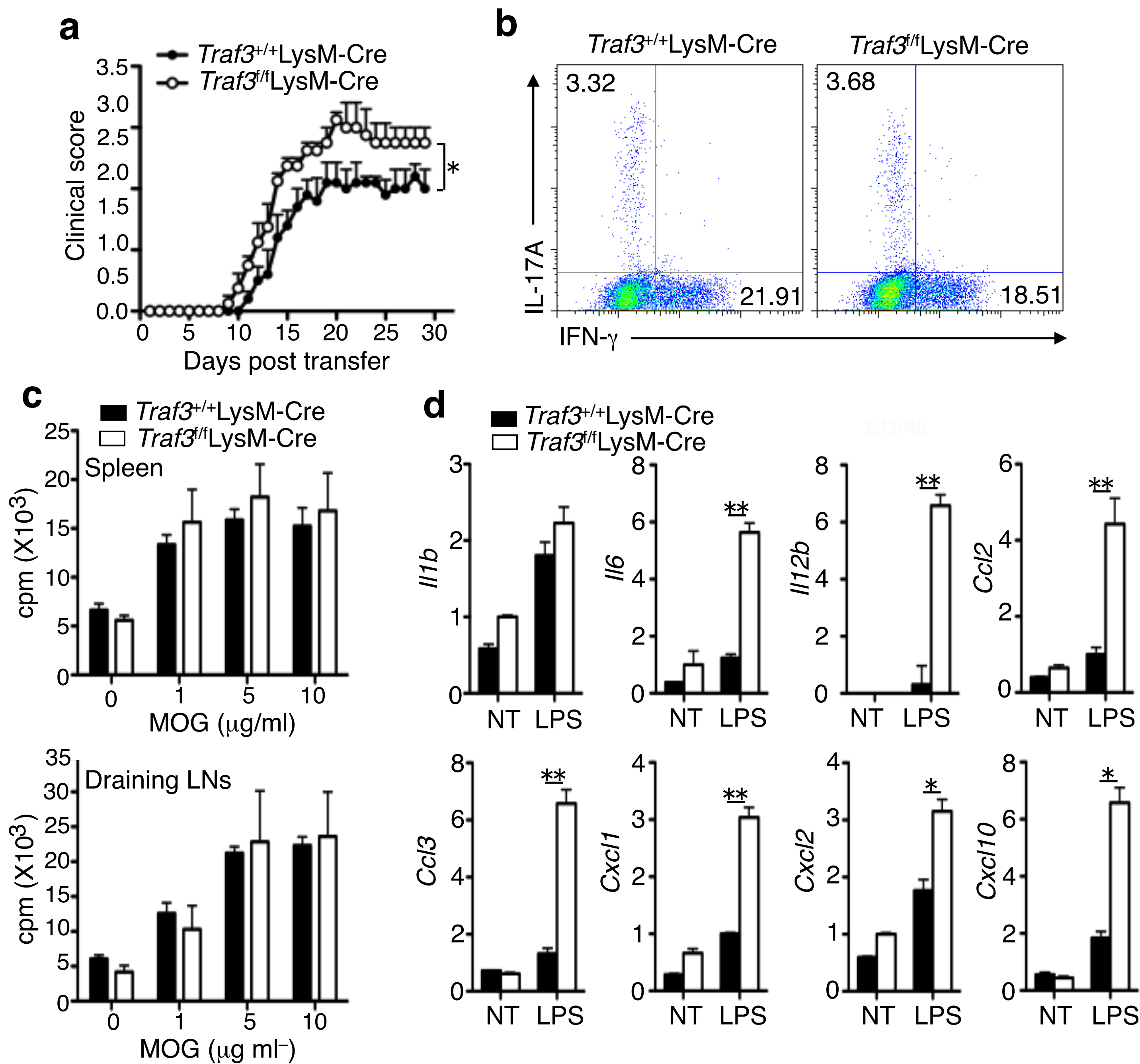
Supplementary Fig. 8. Peli1 mediates K63 ubiquitination of c-IAP2 under both transfected and endogenous conditions. (a) c-IAP2 was isolated by immunoprecipitation from lysates of HEK293 cells tranfected with (+) or without (–) the indicated expression vectors, followed by immunoblot analysis of the K63 and K48 ubiquitinated c-IAP2 and total c-IAP2. Cell lysates were also subjected to direct immunoblot assays to examine protein expression level. Peli1ΔC lacks the C-terminal RING domain. (**b**) c-IAP2 was isolated by IP from the lysates of LPS-stimulated WT or *Peli1*-KO microglia. The K63-ubiquitinated and total c-IAP2 were detected by IB using the indicated antibodies.



Supplementary Fig. 9. Peli1 mediates TLR4- and TLR2-stimulated Traf3 degradation. (**a**) WT and *Peli1*-KO microglia were infected with pRV-GFP empty vector (WT-V and KO-V) or the same vector encoding Peli1 (KO-Peli1). The cells were incubated with (+) or without (–) LPS for 30 min and subjected to IB to detect the indicated proteins. (**b**) IB analysis of Traf3, Peli1, and actin in whole-cell lysates of Pam3CSK4-stimulated WT and *Peli1*-KO microglia.



Supplementary Fig. 10. Peli1-induced Traf3 ubiquitination requires c-IAPs. HEK293 cells were transfected with pLKO.1 empty vector or pLKO.1-based shRNAs for c-IAP1 and c-IAP2. The cells were then transfected with the indicated expression vectors. The cell lysates were subjected to Traf3 ubiquitination (top panels) and direct IB (lower panels) assays.



Supplementary Fig. 11. Traf3 deficiency in myeloid cells promotes EAE severity and microglial activation without altering peripheral T cell response. (**a**) Mean clinical scores of EAE in MOG_{35-55}-immunized Traf3$^{+/+}$LysM-Cre and Traf3$^{f/f}$LysM-Cre mice (n=5/group). (**b**) Flow cytometry analysis of intracelluarly stained IL-17A-secreting or IFN-g-secreting CD4^{+} T cells in draining LNs from MOG_{35-55}-immunized Traf3$^{+/+}$LysM-Cre and Traf3$^{f/f}$LysM-Cre mice. (**c**) Proliferation assay of the splenocytes (upper) and draining LN cells (lower) from MOG_{35-55}-immunized Traf3$^{+/+}$LysM-Cre and Traf3$^{f/f}$LysM-Cre mice. (**d**) Real-time PCR analysis to measure the relative expression of the indicated genes in non-treated (NT) or LPS-stimulated Traf3-KO and WT microglia.
*P<0.05 and **P<0.01

Supplementary Fig. 12. Peli1 expression in grey matters of brains derived from MS and non-MS controls. Total RNA was isolated from normal grey substance or grey substance lesion of different MS patients or the grey substances of non-MS controls. Microarray was performed, and the individual samples are placed along the x-axis, whereas the y-axis displays the normalized hybridization signal obtained from the microarrays.

Y-axis: Normalised hybridisation signal

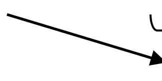
x 10³

60.0
50.0
40.0
30.0
20.0
10.0
0.0

Normal grey substance
Grey substance lesion
Controls

2B 3B 6B 9A 10A 11A 12A 13A 14A 12Bii 17A 18A 20c 21c 22c 23c 24c 25c

Individuals with samples from both normal appearing grey substance and grey substance lesions



1 2 3 4

MS-samples

Controls

	Age (yr)	Sex	Post-mortem interval (h)	Type of MS	Disease duration (yr)	Cause of death	Patient ID
MS	52	F	8.3	SP	>12	Respiratory failure	2B/3B
MS	41	F	8.3	SP	>11	Multiple sclerosis	6
MS	48	F	8.1	SP	8	Euthanasia	9/10
MS	43	M	8.3	SP	11	Pneumonia	11/12
MS	53	F	10.5	SP	27	Euthanasia	13/14
MS	58	F	8.1	NA	NA	Unknown	12bII
C	81	F	6.4	-	-	Euthanasia	17A
C	85	M	4.2	-	-	Cardiac failure	18A
C	88	F	6.2	-	-	Unknown	20C
C	65	M	5.0	-	-	Cachexia/dehydration	21C
C	89	F	15.4	-	-	Cachexia/dehydration	22C
C	86	F	6.3	-	-	Cardiac failure	23C
C	87	M	10.2	-	-	Pneumonia	24C
C	74	M	5.0	-	-	Bronchus carcinoma	25C

Supplementary Table 1. Clinical information of patients. The clinical information of the multiple sclerosis (MS) patients and non-MS controls (C) is provided.
SP, secondary progressive.

Gene	Forward primer	Reverse primer
Ccl2	GGGATCATCTTGCTGGTGAA	AGGTCCCTGTCATGCTTCTG
Ccl3	GTGGAATCTTCCGGCTGTAG	ACCATGACACTCTGCAACCA
Ccl20	TGTACGAGAGGCAACAGTCG	TCTGCTCTTCCTTGCTTTGG
Cxcl1	CTTGACCCTGAAGCTCCCTT	AGGTGCCATCAGAGCAGTCT
Cxcl2	AAAGTTTGCCTTGACCCTGA	TCCAGGTCAGTTAGCCTTGC
Cxcl10	CCTATGGCCCTCATTCTCAC	CTCATCCTGCTGGGTCTGAG
Ccl4	GCTCTGTGCAAACCTAACCC	GAAACAGCAGGAAGTGGGAG
Ccl12	TCCTCAGGTATTGGCTGGAC	CGGACGTGAATCTTCTGCTT
Il1b	AAGCCTCGTGCTGTCGGACC	TGAGGCCCAAGGCCACAGGT
Il6	CACAGAGGATACCACTCCCAACA	TCCACGATTTCCCAGAGAACA
Tnf	CATCTTCTCAAAATTCGAGTGACAA	CCAGCTGCTCCTCCACTTG
Il12b	GGAGACACCAGCAAAACGAT	TCCAGATTCAGACTCCAGGG
Nos2	GTGGTGACAAGCACATTTGG	AAGGCCAAACACAGCATACC
Peli1	CCTTGTCCATGTAAGTTTCTC	CAGAGTTCAGAAGTCTGGAACT
Peli2	CACTCACGGTGGGAATTCAGAC	GGAGCTATCACCTATGCTCACC
Peli3	GCATGTGGGACTCTGCCTGCT	GATCAAGATCTCAGTGACCCTC
Arg1	TTTTTCCAGCAGACCAGCTT	AGAGATTATCGGAGCGCCTT
Actb	CGTGAAAAGATGACCCAGATCA	CACAGCCTGGATGGCTACGT

Supplementary Table 2. The gene-specific primers used for real-time RT-PCR for detecting the indicated mouse mRNAs.