

SUPPLEMENTAL MATERIAL

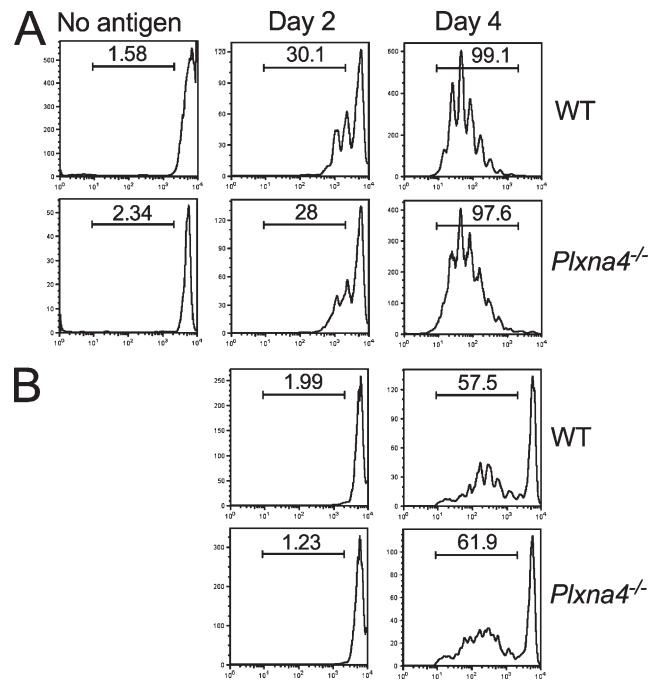
Wen et al., <http://www.jem.org/cgi/content/full/jem.20101138/DC1>

Figure S1. Plexin-A4 does not modulate the presentation of OVA antigen by BMDCs. (A and B) BMDCs generated from WT or *Plxna4*^{-/-} mice were pulsed with either 1 µg/ml OVA₃₂₃₋₃₃₉ peptide (A) or 50 µg/ml OVA whole protein (B) and co-cultured with CFSE-labeled splenic CD4⁺ T cells isolated from naive TCR transgenic OTII mice. 2 or 4 d later, T cells proliferation was analyzed by the dilution of CFSE fluorescence. The numbers above the horizontal black lines indicate the percentage of CD4⁺ T cells that exhibited diluted CFSE staining. The results shown in A and B are representative of two independent experiments.

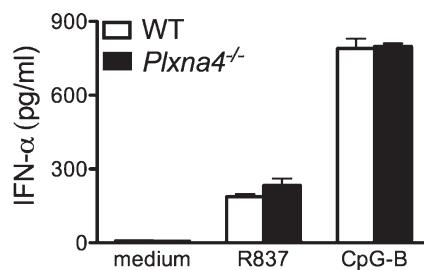


Figure S2. Plexin-A4 does not affect TLR-induced IFN- α production by pDCs. pDCs were isolated from the BMs of WT and *Plxna4*^{-/-} mice by FACS sorting. Cells were left unstimulated (medium) or stimulated with 10 µg/ml R837 (TLR7) or 4 µg/ml CpG-B (TLR9) for 16 h. IFN- α in the supernatants was determined by ELISA. The results are representative of two independent experiments and are expressed as mean \pm SD.

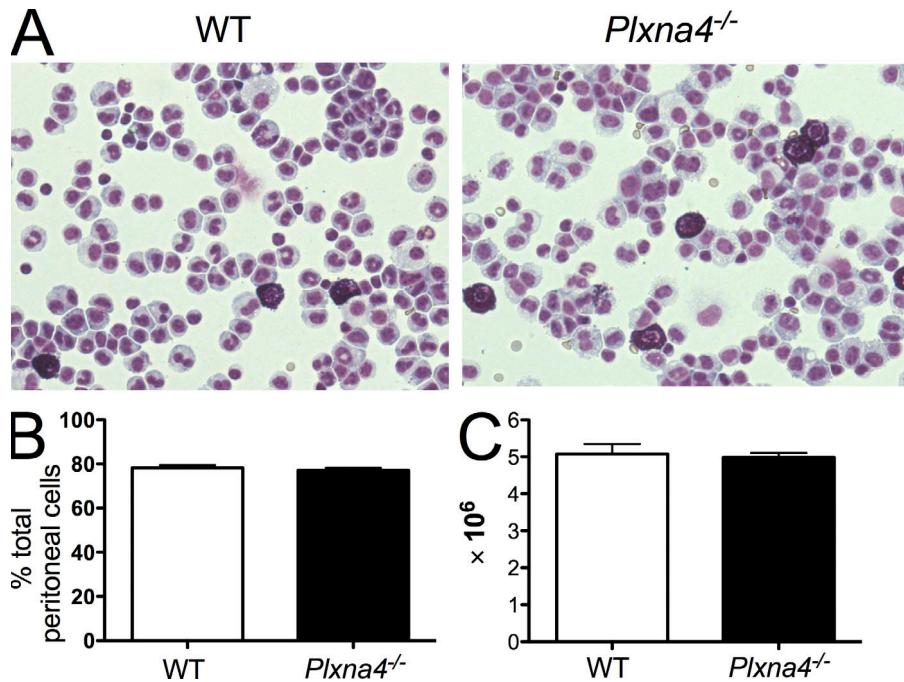


Figure S3. Plexin-A4 does not affect the number of peritoneal macrophages. Total peritoneal cells were harvested from WT and *Plxna4*^{-/-} mice by peritoneal lavage. (A) Cytospins were prepared and stained by hematoxylin and eosin. (B and C) The percentage of peritoneal macrophages from WT and *Plxna4*^{-/-} mice (B) was multiplied by the total cell count to determine the absolute numbers (C). The results are representative of two independent experiments and are expressed as mean \pm SD.

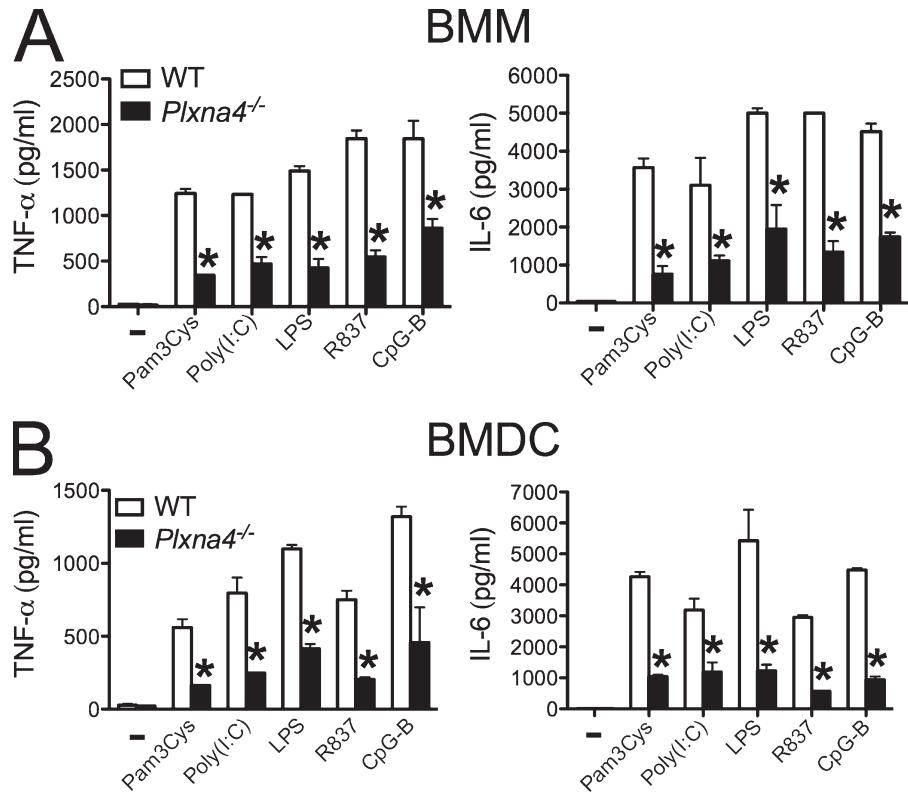


Figure S4. Plexin-A4 is required for TLR-induced cytokine production in BMMs and BMDCs. (A and B) BMMs (A) and BMDCs (B) were generated from the BM cells of WT and $Plxna4^{-/-}$ mice. Cells were left unstimulated or stimulated with various TLR agonists for 4 h. TNF and IL-6 in the supernatant were determined by ELISA. The results shown are representative of two independent experiments and are expressed as mean \pm SD. *, P < 0.05 compared with WT cells.

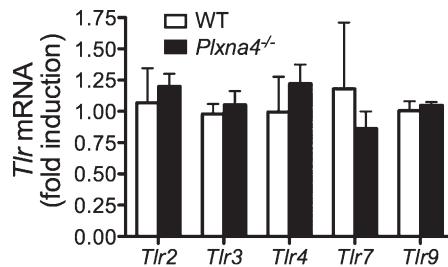


Figure S5. Plexin-A4 does not affect TLR mRNA expression in peritoneal macrophages. mRNA levels of *Tlr2*, *Tlr3*, *Tlr4*, *Tlr7*, and *Tlr9* in WT and $Plxna4^{-/-}$ peritoneal macrophages were analyzed by RT-PCR using different primer sets, as described in Table S2. The results shown are representative of two independent experiments and are expressed as mean \pm SD.

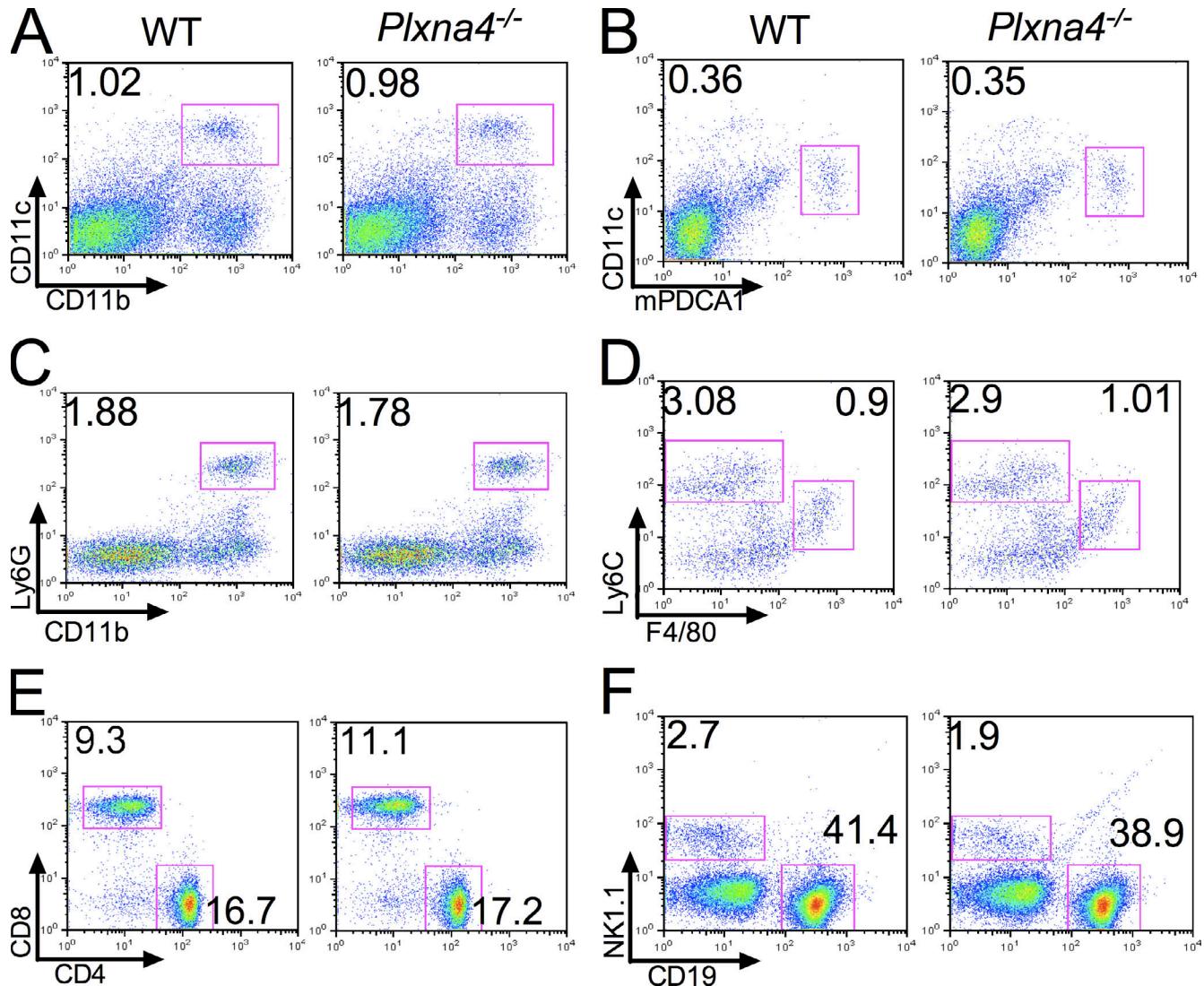


Figure S6. Plexin-A4 does not affect immune cell composition in the spleen. (A-F) Cells of myeloid or lymphoid lineage in the spleen were analyzed by FACS, including CD11b⁺CD11c⁺ mDCs (A), B220⁺mPDCA1⁺CD11c^{low} pDCs (B), CD11b⁺Ly6G⁺ neutrophils (C), CD11b⁺Ly6c⁺ monocytes and CD11b⁺F4/80⁺ macrophages (D), CD3⁺CD4⁺ T cells and CD3⁺CD8⁺ T cells (E), and CD19⁺ B cells and NK1.1⁺ NK cells (F). Cells of different lineages are defined by the staining of specific surface markers and are indicated by gray boxes. The large numbers indicate the percentage of selected cells of the total splenocytes. The results shown are from one experiment.

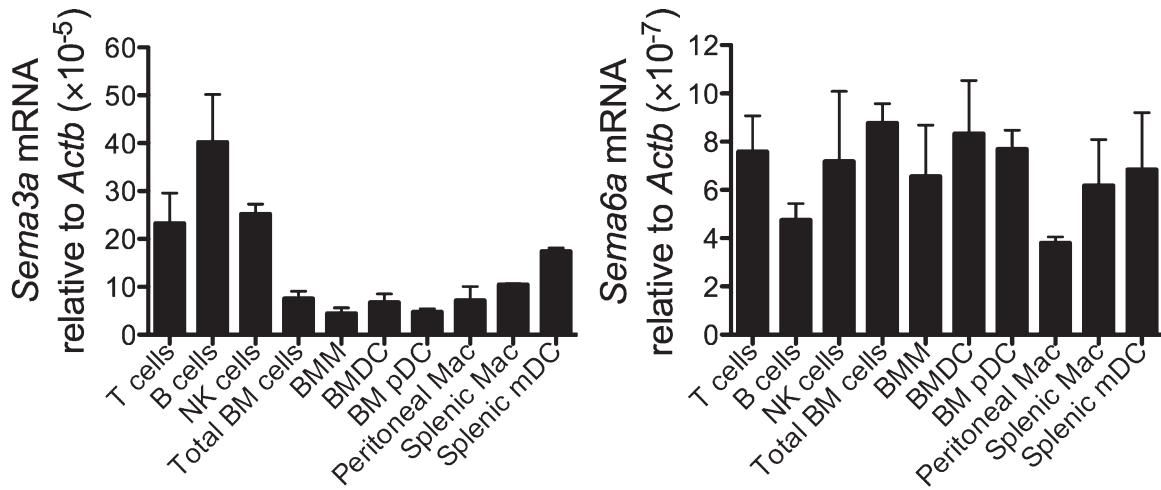


Figure S7. Expression of *Sema3a* and *Sema6a* mRNA in different immune cells. mRNA levels of *Sema3a* and *Sema6a* in different immune subpopulations were analyzed by RT-PCR and normalized to *Actb* mRNA level. The preparation of different immune cells has been described in Fig. 1 A. The results shown are representative of two independent experiments and are expressed as mean \pm SD.

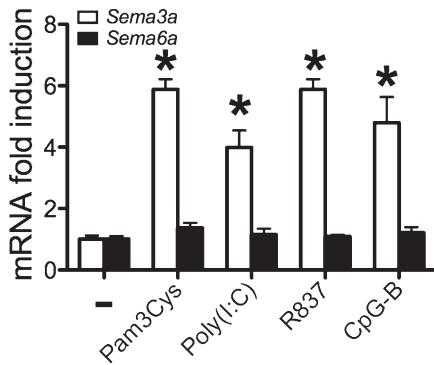


Figure S8. TLR agonists induce an increase in *Sema3a* mRNA level in peritoneal macrophages. WT peritoneal macrophages were left unstimulated (−) or stimulated with 5 μ g/ml Pam3Cys, 10 μ g/ml poly(I:C), 10 μ g/ml R837, and 4 μ g/ml CpG-B for 2 h. mRNA levels of *Sema3a* and *Sema6a* were measured by RT-PCR and normalized to *Actb* mRNA level. The results shown are representative of two independent experiments and are expressed as mean \pm SD. *, P < 0.05 compared with untreated controls.

Table S1. Splenic leukocyte subsets in WT and *Plxna4*^{−/−} naive mice

Group	WT	<i>Plxna4</i> ^{−/−}
Total cells	793.5 ± 66	805.5 ± 78.2
CD11b ⁺ CD11c ⁺ mDC	9.4 ± 2.4	9.0 ± 1.9
B220 ⁺ mPDCA1 ⁺ CD11c ^{low} pDC	3.6 ± 0.8	3.8 ± 0.9
CD11b ⁺ Ly6G ⁺ neutrophil	19.4 ± 2.4	17.0 ± 2.9
CD11b ⁺ Ly6C ⁺ monocyte	25.4 ± 3.6	23.6 ± 3.8
CD11b ⁺ F4/80 ⁺ macrophage	10.4 ± 0.8	13.6 ± 1.2
CD3 ⁺ CD4 ⁺ T cell	167.4 ± 12.6	181.5 ± 15.3
CD3 ⁺ CD8 ⁺ T cell	77.3 ± 8.4	82.7 ± 9.4
NK1.1 ⁺ NK cell	18.1 ± 1.4	16.7 ± 1.1
CD19 ⁺ B cell	345.5 ± 37.6	321.6 ± 35.4

All values are $\times 10^5$ per mouse. For WT and *Plxna4*^{−/−}, n = 7 and n = 6, respectively.

Table S2. Sequences of RT-PCR primers

Mouse genes	Forward	Reverse
<i>Sema3a</i>	5'-GCCTGCAGAAGAAGGATTCA-3'	5'-TCAGGTTGGGTGGTAATG-3'
<i>Sema6a</i>	5'-AATGGCCAGATGCCCTTATG-3'	5'-CCGAGTAGAGTTCCATTGCA-3'
<i>Tlr2</i>	5'-CTCCTGAAGCTGTTGCGTTAC-3'	5'-TACTTACCCAGCTCGCTCACTAC-3'
<i>Tlr3</i>	5'-TCTTCTTACGAAAGTTGGACTTGC-3'	5'-TTGCCAATTGTCTGGAACACC-3'
<i>Tlr4</i>	5'-ATGGCATGGCTTACACCAACC-3'	5'-GAGGCCAATTGTCTCCACA-3'
<i>Tlr7</i>	5'-CTGGAGTTCAAGAGGCAACCATT-3'	5'-GTTATCACCGGCTCTCCATAGAA-3'
<i>Tlr9</i>	5'-AGCTAACATGAACGGCATCT-3'	5'-TGAGCGTGTACTTGTGAGCG-3'

Table S3. Sequences of primers for the ChIP assay

Mouse genes	Forward	Reverse
<i>Tnfa</i> promoter κB binding site	5'-TGAAAGGAGAAGGCTGTGA-3'	5'-TAATGGGATGAGTATGGGCA-3'
<i>Tnfa</i> promoter c-Jun binding site	5'-CCCAACTTCCAAACCCCTCT-3'	5'-ACCATGATCTCATGTGGAGGA-3'