

# Supporting Information

Kobayashi et al. 10.1073/pnas.1222093110

## SI Text

The sequences of PCR primers:

TNF- $\alpha$ : forward primer: 5'-ATGGCCTCCCTCTCATCAGT-3'; reverse primer: 5'-GCTCCTCCACTTGGTGGTTT-3'.

IL-12a: forward primer: 5'-TGGCTACTAGAGAGACTTCT-TCCACAA-3'; reverse primer: 5'-GCACAGGGTCATCAT-CAAAGAC-3'.

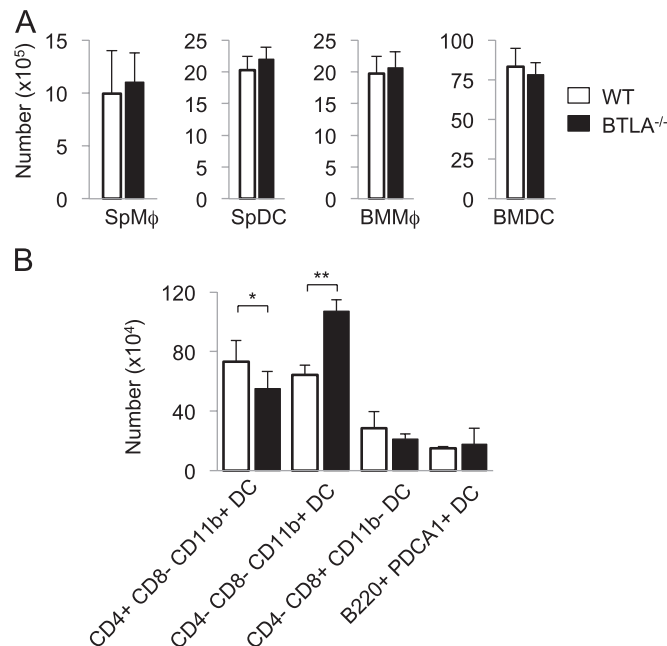
IL-12b: forward primer: 5'-TGGTTTGCCATCGTTTTGCTG-3'; reverse primer: 5'-ACAGGTGAGGTTCACTGTTTCT-3'.

IL-6: forward primer: 5'-GAGGATACCACTCCCAACAGACC-3'; reverse primer: 5'-AAGTGCATCATCGTTGTTC-ATACA-3'.

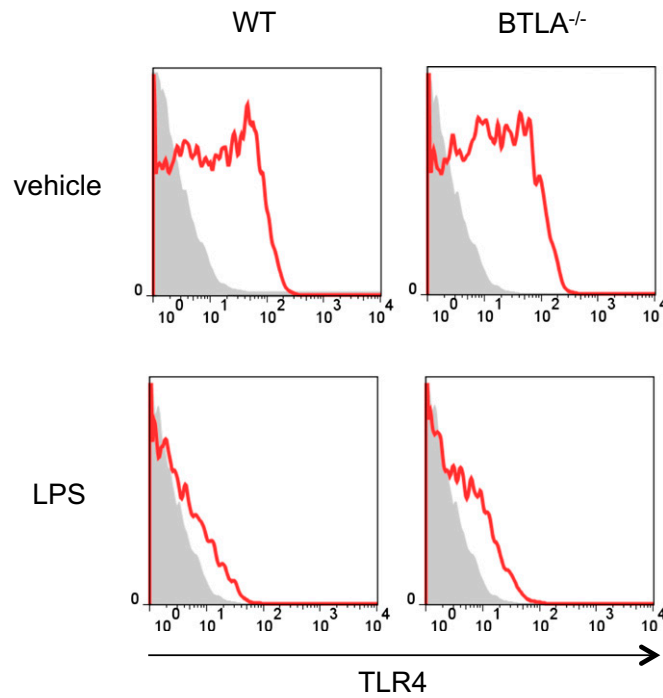
IFN- $\beta$ : forward primer: 5'-CCCAGTGCTGGAGAAATTGT-3'; reverse primer: 5'-CCCTATGGAGATGACGGAGA-3'.

B and T lymphocyte attenuator: forward primer: 5'-GCTGCTGCTCTGCTTTCT-3'; reverse primer: 5'-ATGGCAGTGCTTGGTGATTT-3'.

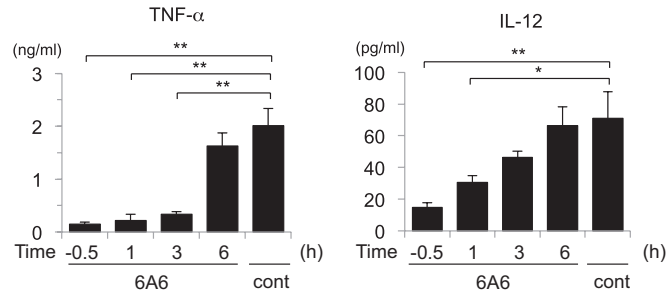
$\beta$ -actin: forward primer: 5'-GCTCTGGCTCCTAGCACCAT-3'; reverse primer: 5'-GCCACCGATCCACACAGAGT-3'.



**Fig. S1.** The development of dendritic cells (DCs) and macrophages (Mφs) in B and T lymphocyte attenuator-deficient (BTLA<sup>-/-</sup>) mice. Splenocytes from BTLA<sup>-/-</sup> mice and littermate WT mice were analyzed by flow cytometry. (A) Data are mean  $\pm$  SD of the number of DCs (CD11c<sup>+</sup> MHC II<sup>+</sup> cells) and Mφs (F4/80<sup>+</sup> CD11b<sup>+</sup> cells;  $n = 8$ ). Mean  $\pm$  SD of the numbers of bone marrow-derived Mφs (BMMφs) and bone marrow DCs (BMDCs) recovered from the culture is also shown ( $n = 8$ ). (B) Data are mean  $\pm$  SD of the number of subpopulation of splenic DCs ( $n = 8$ ). \* $P < 0.05$ , \*\* $P < 0.01$ .



**Fig. S2.** The expression of Toll-like receptor 4 (TLR4) in  $BTLA^{-/-}$  DCs.  $BTLA^{-/-}$  BMDCs and WT BMDCs were stimulated in the presence or absence of LPS (100 ng/mL) for 12 h. The expression of TLR4 was analyzed by flow cytometry. Shown are representative histograms of TLR4 staining with control staining (gray histograms) of three independent experiments.



**Fig. S3.** The expression of BTLA is enhanced by LPS stimulation. WT BMDCs were stimulated with LPS (100 ng/mL) for 12 h. Where indicated, 6A6 was added to the culture at  $-0.5$ ,  $+1$ ,  $+3$ , and  $+6$  h from the timing of LPS administration. The levels of TNF- $\alpha$  and IL-12 in the supernatants were measured by ELISA. Data are mean  $\pm$  SD,  $n = 4$ . \* $P < 0.05$ , \*\* $P < 0.01$ .