

# Supplementary Figure 1

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mBTN1A1 -----MAVPTNSCLLVCLLTLTVLQLPTLDSAAPFDVTAPQEPVLALVGSDAELTCGFSPNASSEYMELLWFRQTRSTAVLLYRDGQEQEQQMTEYRGRATLATAGLLDGRATLL
mBTNL9  MADFSVFLGFLKQIPRCLSIFFTYLLFLQLWEVNSDK-VWVLGPEESILARVGEAVEFPCLSSYQDAEHMEIRWFRAQVSNVYVLYQEPQGRSSLQMAQFRNRTLFEAYDIAEGSVNLH
mBTNL1  -----MMKGSPVPPAGCLLPLLLLLFTGVSGEVSWFVSKGPAEPI TVLLGTEATLPCQLSPEQSAARMHIRWYRAQTPAVLVFHNGQEQGEVQMPPEYRGRTQMVRQAIDMGVALQ
mB7-1   -----MACNCQLMQDTPLLKFCPCRLILFLVLLIRLSQVSSDVEQLSKSVKDKVLLPCRYNSPHEDESEDRIYQKHDKVLSVIAG-----KLKVWPEYKNRTLVDN-----TTYSLI
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mBTN1A1  IRDVRVSDQGEYRCLFKDND-----DFEEAAVYLKVAAVGSDPQISMVTV-QENGEMELECTSSGWYPEPQVQWRTGNREMLPSTSESKKHNEEGLFTVAVSMMIRDSSIKNMSCGIQNIL
mBTNL9   ILKVLPSDEGRYGCFLSDN-----FSGEATWELEVAGSGSDPHISLQG-FSGEGIQLCSSSGWYPKPKVQWRGHGQCLSPSEAITQNAQGLFSLVSVIRGGAHNSVSCIQNPL
mBTNL1   IQVQASDDGLYHCQFTDGF-----TSQEVSMELRVIGLGSAPLVHMTG-PENDGIRVLCSSSGWFPKPKVQWRDTSGNMLSSSELQTQDREGLFQVEVSLLVTDRAIGNVICSIQNPM
mB7-1    ILLGLVLSDRGTYSVQKKEKRGTYEVKHLALVKLSIKADFSTPNITESGNPSADTKRITCFASGGFPKPRFSWLENGRELPGINTTISQDPESELYTISQLDFNTTRNHTIKCLIKYGD
          *  :  ** * * * . . . . . * : . . . * * : . . . : * : ** : * : * : . * : . . . * : : . . . : . . . * * :

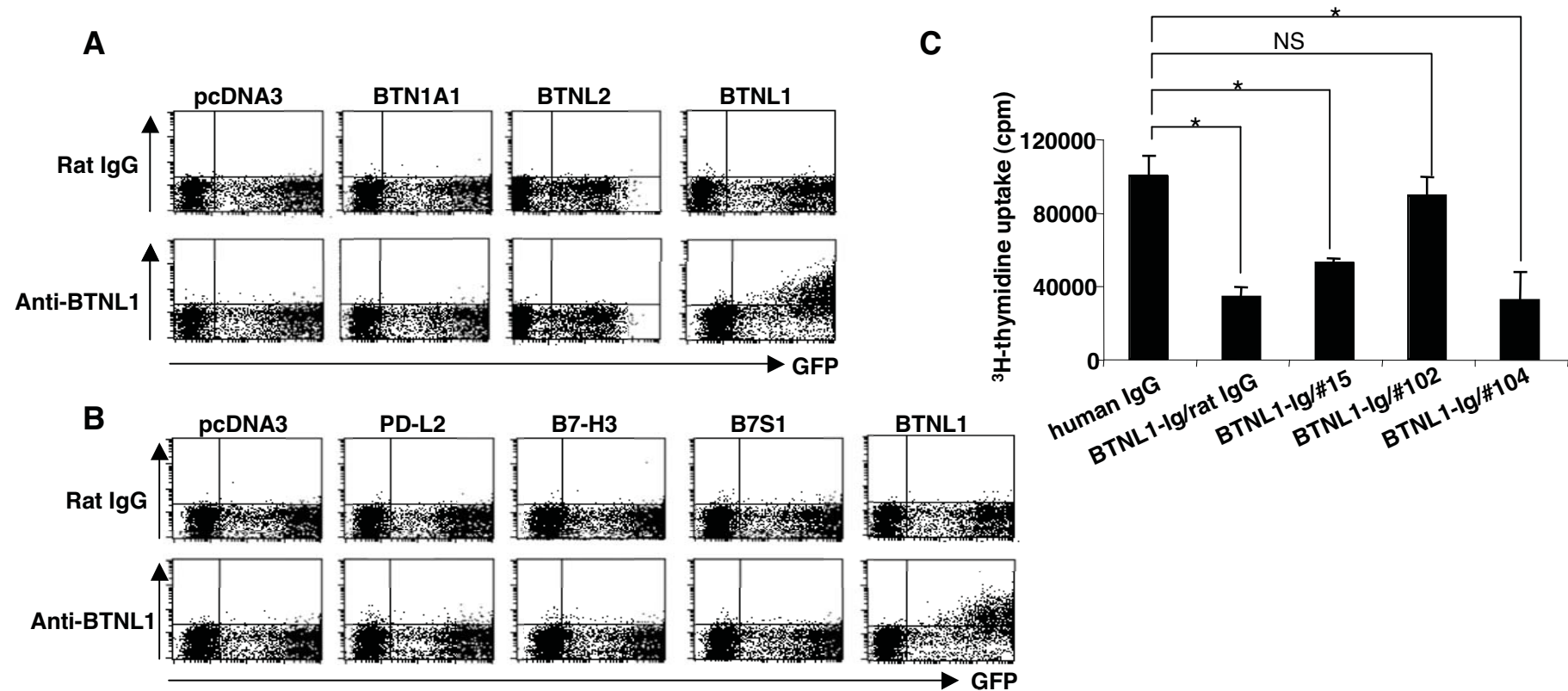
mBTN1A1  LGQGKEVEISLPAPFVPRLTPWIVAVAILLALGFLTIGSIFFTWKLYKERSS-----LRKKEFGSKERLLFELR-----CKKTVLHEVD-VTLDPDTAHPHL
mBTNL9   LPQKKEFVIQIADVFLPRMSPWKKAFVGTLLVPLSLIVLTMALRYFYKLS-----FQEKQVKQGEVREKLTQELDWRRSEGGAEWRAAQYAAD-VTLDPATAHPSL
mBTNL1   YDQEKSKAILLPEFPFKTCPWKVALVCSVLILLVLLGGISLGIWKEHQVKRREIKKWSKEHEEMLLKKGTKSVLKIRDDLQADLDRRKALYKEDWKKALLYPDWRKELFQEA PVRIN Y
mB7-1    AHVSEDFTWEKPPEDPPDSK-NLVLFGAGFGAVITVVVIVVVIKCFCKHRS-----
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mBTN1A1  FLYEDSKSVRLD-SRQILPDRPERFDSWPCVLGREFTTSGRHYWEVEVGDRTDWAIGVCRENVVKKGDFPMPDNGFWAVELYG-NGYWALTPLRT-----SLRLAGPPRRVGVFLDY
mBTNL9   EVSNGKTVSSRLGVPSIAAGDPQRFSEQTCVLSRERFSSGRHYWEVHVGRRSRWFLGACLESVERS GPARLSPAAGYVVMGLWNRCEYFVLDPHRV-----ALALRVPPRRIGVLLDY
mBTNL1   EMPDQDKTDSRTEENRGEETVSSSQVDHNLITLSQEGFMLGRYWEVDVKDTEEWTLGVYELCTQDASLT--DPLRKFRVLEKNG-DGYRALDFCSQINSEELQLKTRPLKIAIFLDQ
mB7-1    ---CFRRNEASRETNNSLTFGPEEALAEQTVFL-----
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mBTN1A1  DAGDISFYNMSGSLIYTFPSISFSGPLRPFCLWSCG----KKPLTICSTAN-GPEKVTVIANVQDDIPLSPLGEGCTSGDKDTLHSKLIPFSPSQAAP
mBTNL9   EAGKLSFFNVSDGSHIFSFTDT-FSGALRAYLRPRAHDGSEHPDPMTCSLPVRGPQVLEENDNDNLQPYEPLDPAWAVNEAVS-----
mBTNL1   EDNDLSFYNMTDETHIFSFAQVPFLGSPYPYFTRNSMG-----LSATAQP-----
mB7-1    -----
  
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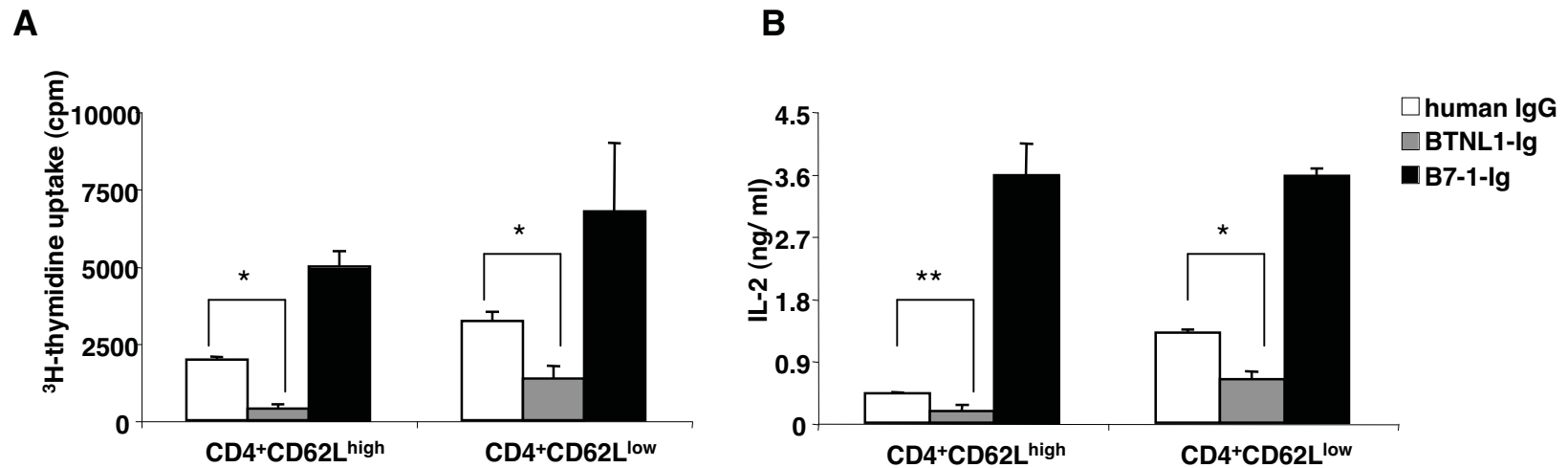
**Supplementary Figure 1. Alignment of amino acid sequence of human and mouse BTNL1.** Predicted amino acid sequence of mouse BTNL1 (TrEMBL; A6X8K2) was compared with mouse BTN1A1 (Swiss-Prot; Q62556), mouse BTNL9 (TrEMBL; Q8BJE2), and mouse B7-1 (Swiss-Prot; Q00609). Predicted leader peptide (thin line), Ig-like regions (bold line), transmembrane region (doublet), and B30.2 region (dashed line) was showed on the alignment. An asterisk (\*) indicates identity, a colon (:) indicates conservation of strong groups, and period (.) indicates conservation of weak groups between mouse and human amino acid sequence of BTNL1.

## Supplementary Figure 2



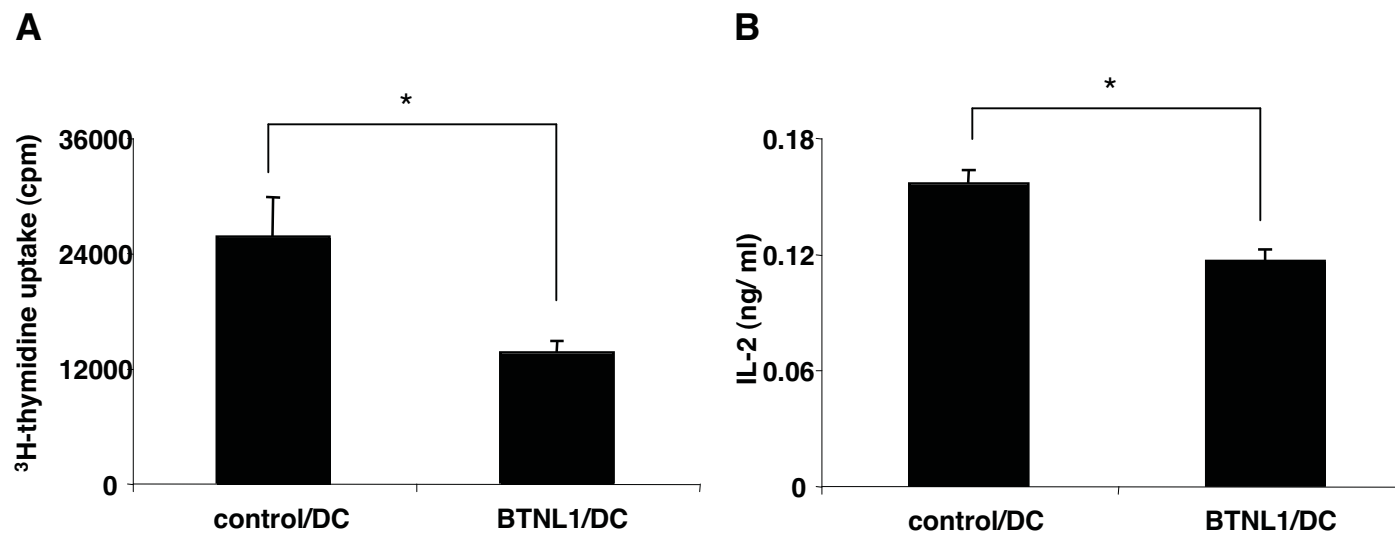
**Supplementary Figure 2. Characterization of rat-derived anti-mouse BTNL1.** **A-B.** Reactivity of anti-BTNL1 to mouse BTN family molecules and B7 family transfectants. 293T-derived BTN (**A**) or B7 (**B**) family molecule transfectants and empty vector transfectants (**A**, **B**) were stained with biotinylated rat IgG or anti-BTNL1 (clone #102) followed by APC-labeled streptavidin. **C.** Blocking activity of anti-BTNL1. Anti-CD3 (2  $\mu$ g/ml)-stimulated CD4<sup>+</sup> T cells were cultured with indicated Igs in the presence of different clones of anti-BTNL1 or rat IgG. Proliferative response was assessed at 72 h by pulsing the culture with [<sup>3</sup>H]thymidine for last 6 h. The data are expressed as the mean  $\pm$  SD of triplicate wells. All data are representative of three independent experiments. \*,  $p < 0.01$ ; \*\*,  $p < 0.05$ ; NS, not significant.

## Supplementary Figure 3



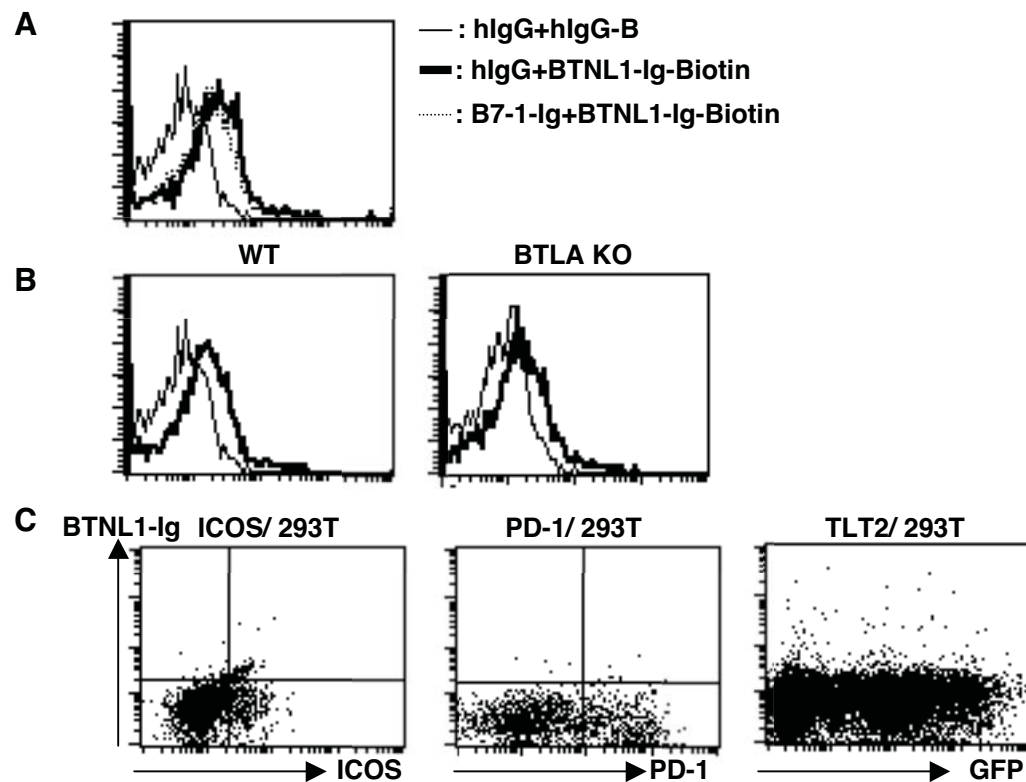
**Supplementary Figure 3. BTNL1 inhibits naive and memory T cell activation.** Naive (CD4<sup>+</sup>CD62L<sup>high</sup>) and memory (CD4<sup>+</sup>CD62L<sup>low</sup>) CD4<sup>+</sup> T cells were sorted by flow cytometry. The cells were stimulated with anti-CD3 (2 μg/ml) in the presence of indicated Igs (10 μg/ml). **A.** Proliferative response was assessed at 72 h by pulsing the culture with [<sup>3</sup>H]thymidine for last 6 h. **B.** Production of IL-2 in the culture supernatants was measured at 24 h of the culture by ELISA. The data are expressed as the mean ± SD of triplicate wells. These data are representative of two independent experiments. \*,  $p < 0.01$ ; \*\*,  $p < 0.05$ .

## Supplementary Figure 4



**Supplementary Figure 4. Overexpressed BTNL1 on BMDC inhibits CD4<sup>+</sup> T cell proliferation and IL-2 production.** Bone-marrow cells were infected *IRES-GFP*-containing bicistronic retrovirus expressing BTNL1 or vector control retrovirus and cultured with GM-CSF. GFP<sup>+</sup>CD11c<sup>+</sup> cells were isolated by FACS-sorting. Purified CD4<sup>+</sup> T cells were cocultured with BTNL1 transduced DC (BTNL1/DC) or control vector transduced DC (control/DC) in the presence of anti-CD3. **A.** Proliferative response was assessed at 72 h by pulsing the culture with [<sup>3</sup>H]thymidine for last 6 h. **B.** Production of IL-2 in the culture supernatants was measured at 24 h of the culture by ELISA. The data are expressed as the mean  $\pm$  SD of triplicate wells. These data are representative of three independent experiments. \*,  $p < 0.01$ .

## Supplementary Figure 5



**Supplementary Figure 5. CD28/CTLA-4 family molecules and TLT2 are not the receptor for BTNL1.** **A.** ConA-activated CD4<sup>+</sup> T cells from C57BL/6 mice were incubated with B7-1-Ig. The cells were stained with biotinylated BTNL1-Ig followed by APC-labeled streptavidin. **B.** CD4<sup>+</sup> T cells from C57BL/6 or BTLA KO mice were activated with ConA and stained with biotinylated BTNL1-Ig or human IgG, followed by APC-labeled streptavidin. The bold histograms indicate staining with BTNL1-Ig and the thin histograms indicate background staining with human IgG. **C.** ICOS, PD-1, or TLT2 transfected 293T cells were stained with biotinylated BTNL1-Ig or human IgG followed by APC-labeled streptavidin. All data are a representative of two independent experiments.