

Figure S1: Calibration curve for the estimation of molecular weight of BTNL2 and its tetramer as eluted from size exclusion chromatography

Comparison of BTNL2 and tetrameric BTNL2 elution volumes to the SEC calibration curve resulted in an estimated molecular mass of 15.4 kDa and 109kDa respectively, which is in good agreement with the expected molecular mass of 13.3 kDa and 112 kDa respectively.

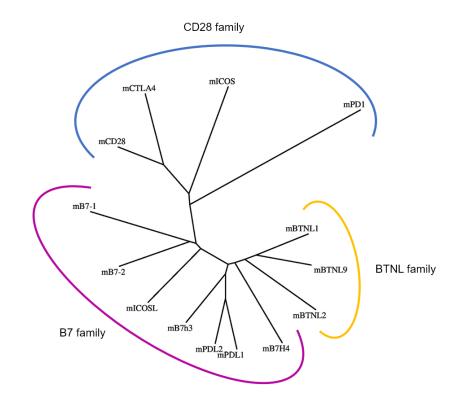


Figure S2: Phylogenetic relationship of BTNL2 with key T cell costimulatory receptors and ligands

The N-terminal IgV domain sequences were aligned, followed by the construction of an unrooted phylogenetic tree. From this analysis it is clear that BTNL2 clusters well with the B7 family of costimulatory ligands.

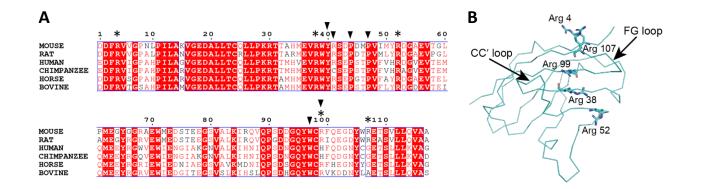


Figure S3: Sequence alignment of N-terminal IgV domain of BTNL2 across six mammalian species

A. The black triangles indicate the conservation of residues involved in the stability of the non-canonical conformation of CC' loop of BTNL2 (as shown in figure 6). The black asterisk indicates the conservation of arginine residues which form an unique stretch of positively charged residues across the front face of BTNL2. **B.** Ribbon representation of BTNL2 showing the arginine residues (sticks) which contribute in forming the unique positively charged stretch across the front face of this molecule.