Supporting Information

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Supporting Methods

Expression and Purification of Reduced BST2. Human BST2(47–152) was cloned into pET151/D-TOPO (Invitrogen) and expressed in BL21(DE3)RIL cells using the autoinduction technique (1). Ni-NTA affinity chromatography, dialysis against 20 mM Tris, pH 8.0, 100 mM NaCl, 2 mM DTT, and cleavage of the His-tag with TEV protease overnight at room temperature, was followed by Q (Buffer A: 20 mM Tris 8.8, 10 mM NaCl, 1 mM DTT; Buffer B: 20 mM Tris 8.8, 1 M NaCl, 1 mM DTT) and size-exclusion chromatography in 20 mM HEPES pH 7.0, 100 mM NaCl, 2 mM DTT. Protein was concentrated to 9–16 mg/ml for crystallization. Identical procedures were used for BST2(47–154) and selenomethionine-substituted BST2(47–152).

Expression and Purification of Oxidized BST2. Human BST2(51–151) was cloned into a pHLsec vector (a kind gift of Dr. Aricescu, Oxford) between the *AgeI* and *KpnI* cloning sites with a C-terminal $GTKH_6$ tag. After secretion signal cleavage, an EGT tripeptide is left at the N terminus resulting in the final EGT-(BST2 E51–K151)-$GTKH_6$ protein product [BST2(51–151)]. Transient protein expression was performed in HEK293T cells essentially as described (2). To facilitate protein deglycosylation, the *N*-glycosylation inhibitor swainsonine was added at a final concentration of 20 μM during DNA-PEI complex formation (3). Four days after transfection, the supernatant was collected for protein purification under nonreducing conditions. Following overnight binding to a His-Trap column (GE Healthcare), BST2(51–151) was eluted in 50 mM phosphate buffer pH 7.5, 300 mM NaCl with a linear (10–300 mM) imidazole gradient. The protein was then dialyzed against 50 mM Tris-HCl buffer, 50 mM NaCl with pH adjusted to 5.5 with a 1 M sodium citrate solution prior to overnight deglycosylation at 37 °C with Endoglycosidase-H (New England Biosciences) according to the manufacturer's instructions. Deglycosylated BST2(51–151) was further purified by size-exclusion chromatography on a S200 16/60 column (GE Healthcare) in 50 mM Tris-HCl pH 7.5, 100 mM NaCl buffer.

1. Studier FW (2005) Protein production by auto-induction in high density shaking cultures. *Protein Expres Purif* 41(1):207–234.
2. Aricescu AR, Lu W, & Jones EY (2006) A time- and cost-efficient system for high-level protein production in mammalian cells. *Acta Crystallogr D* 62(Pt 10):1243–1250.
3. Chang VT et al. (2007) Glycoprotein structural genomics: Solving the glycosylation problem. *Structure* 15(3):267–273.

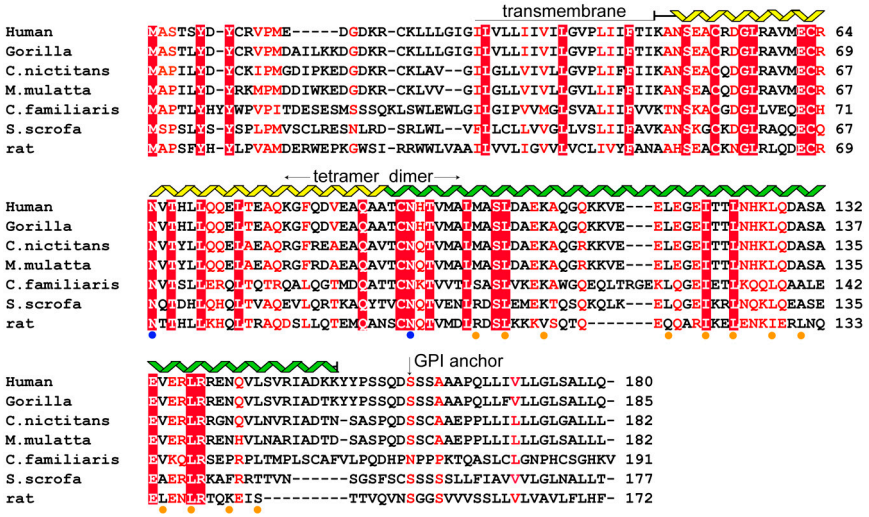

Fig. S1. Sequence alignment with knobs and holes analysis. An alignment of representative sequences with secondary structure observed in the reduced structure shown above. The mature human protein is truncated at Ser160, the site of GPI anchor attachment. The glycosylation sites, Asn65 and Asn92, are indicated with blue dots and are solvent exposed in the crystal structures. Residues that display *a* or *d* knobs into holes packing, as defined by the program SOCKET (1), are indicated with orange dots. Residues that are invariant in this alignment are highlighted in red boxes, and residues that are highly conserved are shown in red.

1. Walshaw J & Woolfson DN (2001) Socket: A program for identifying and analysing coiled-coil motifs within protein structures. *J Mol Biol* 307(5):1427–1450.