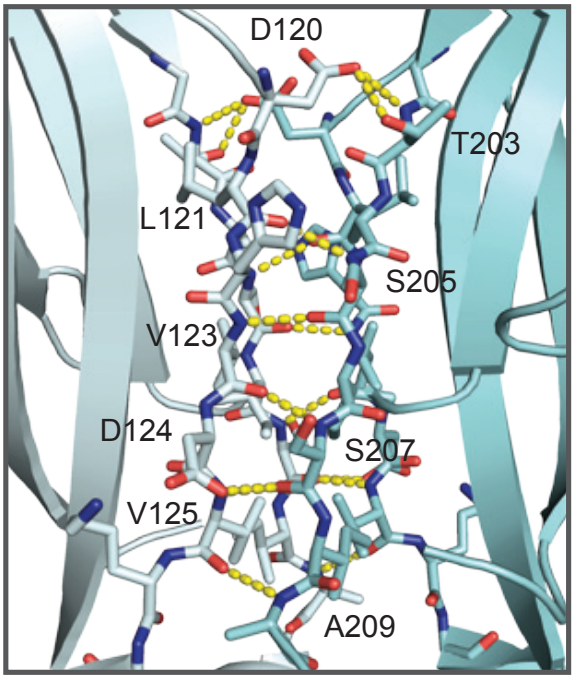
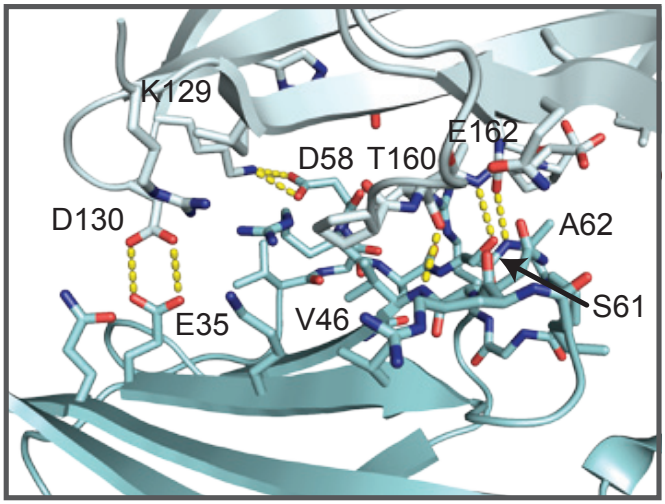
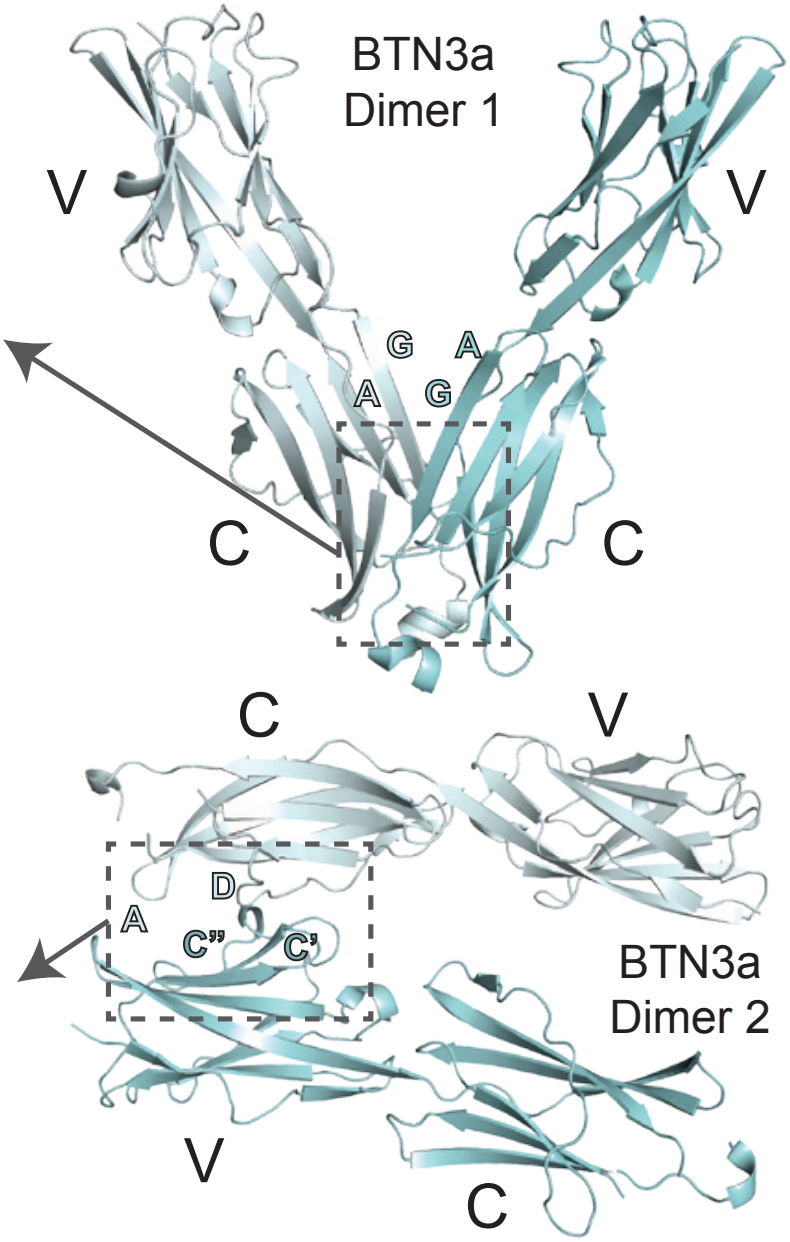
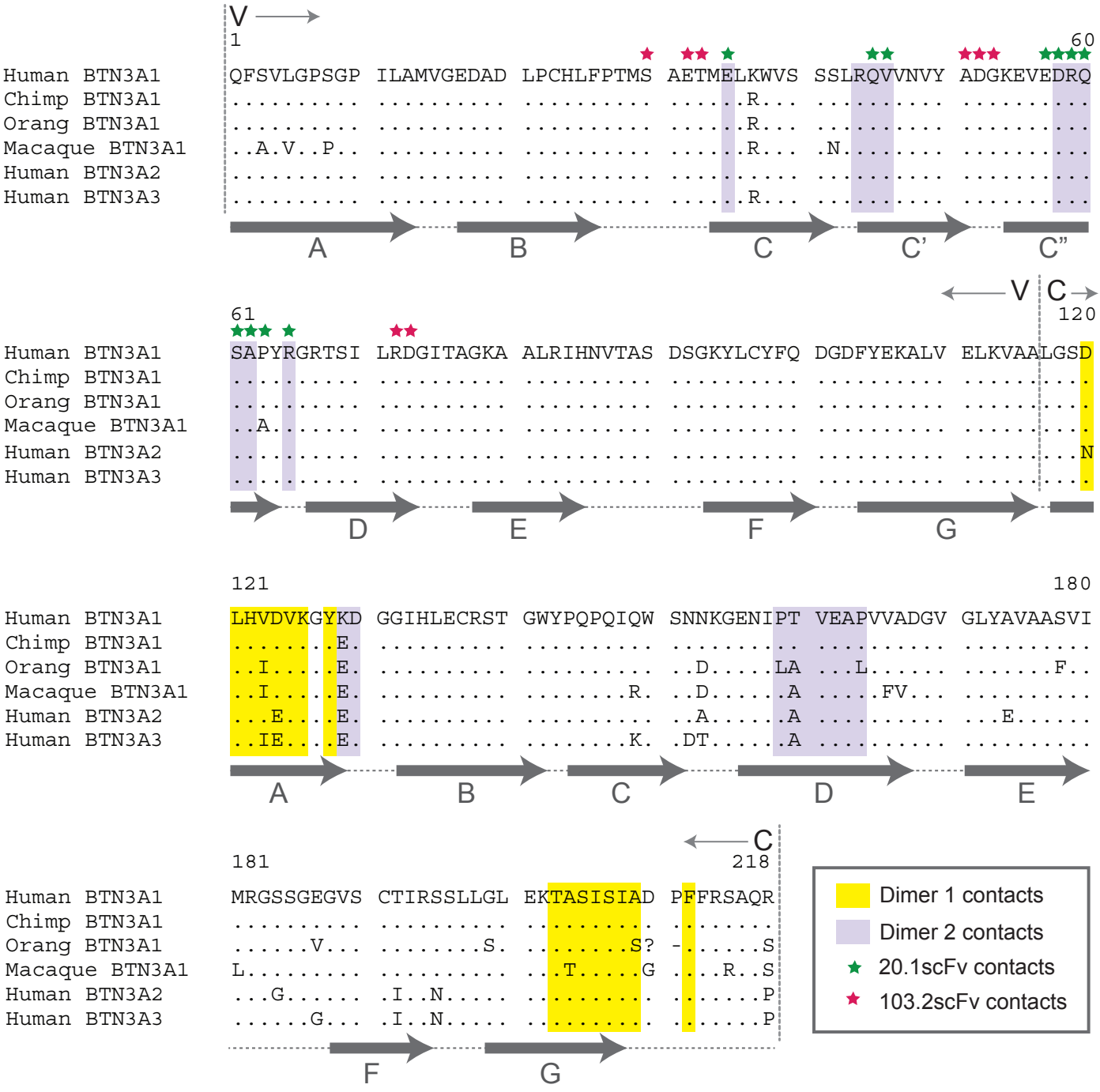
Supplemental Figure 1



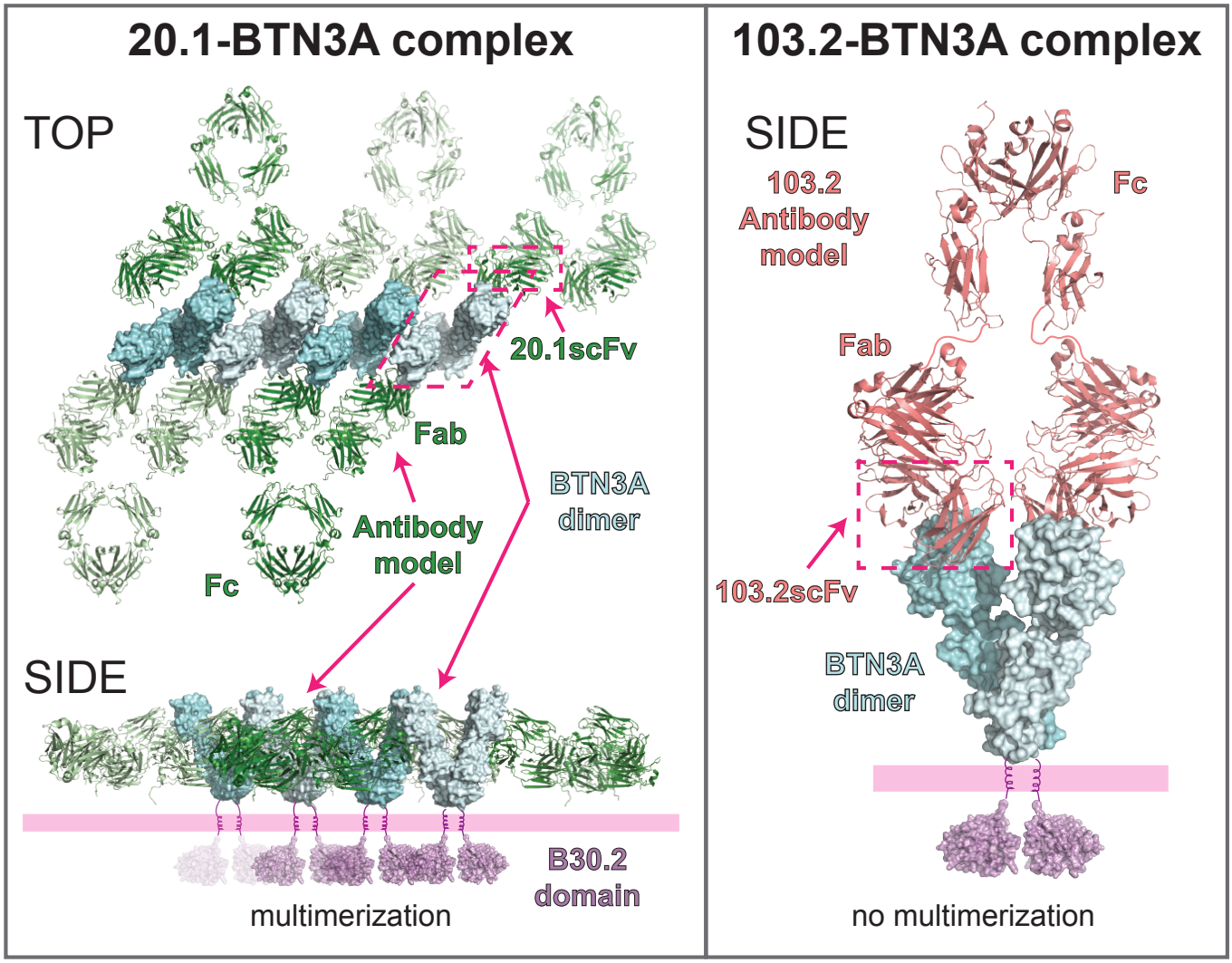




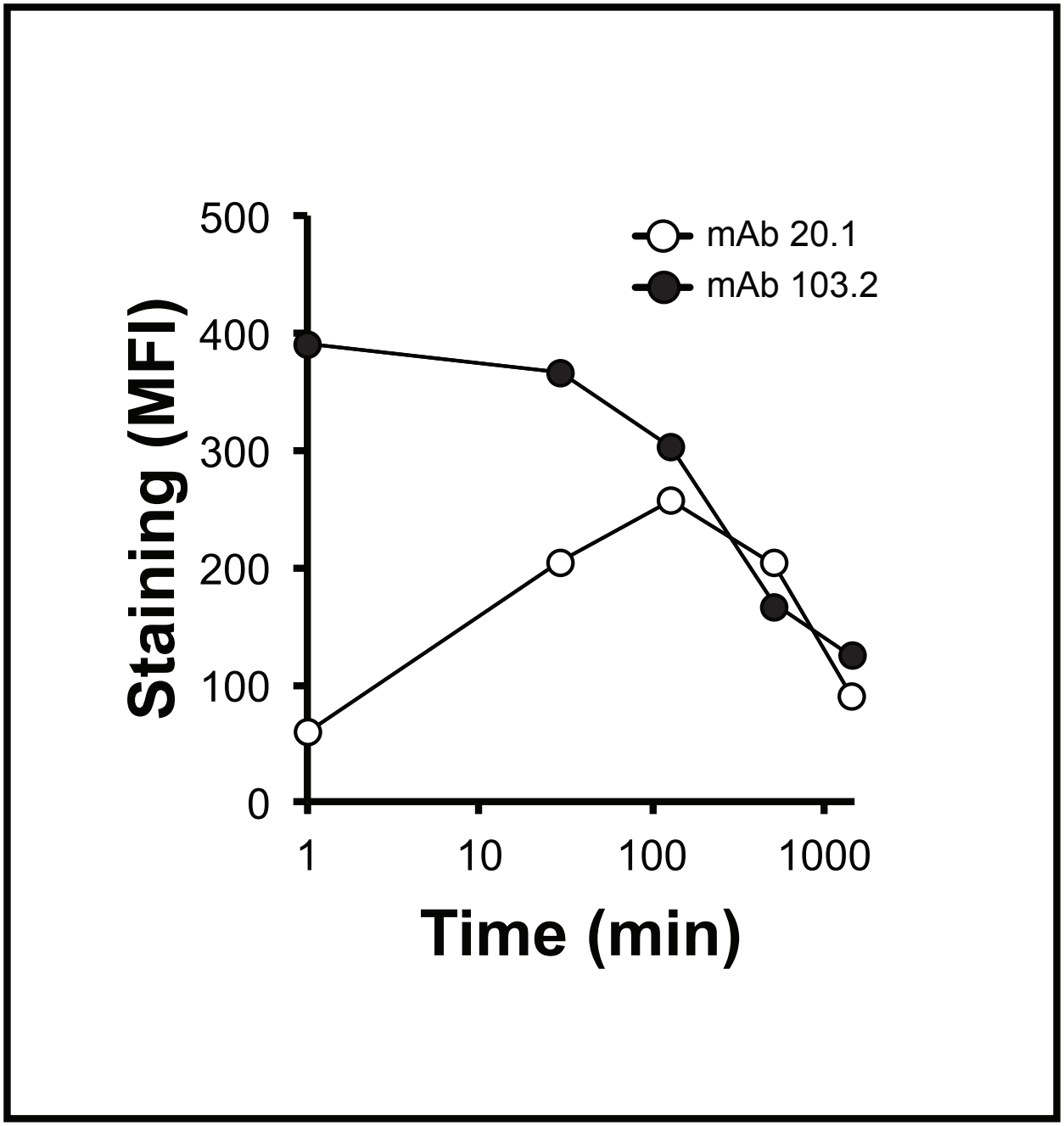
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplementary Figure Legends:

Supplementary Figure 1. Dimerization interfaces of the two BTN3A dimers. The two dimer forms of BTN3A molecules are shown in ribbon format colored cyan and light cyan. Dimer 1 is shown at top and Dimer 2 at bottom; to the left are close-ups of the interface showing the residues involved in the interface. Hydrogen bonds (distance < 3.3Å) are shown as yellow dashed lines. The b-strands involved in the interface are labeled as shown.

Supplementary Figure 2. Amino acid alignment of the extracellular domains of the three human BTN3A isoforms and the related BTN3A molecules from chimpanzee (*Pan troglodytes*), organutan (*Pongo albei*), and macaque (*Macaca mulatta*). Amino acid identity is shown as a "." and differences from the concensus (human BTN3A1) are shown as capital letters. Residues contributing to Dimer 1 interface are colored yellow, those to Dimer 2 are colored lavender. The BTN3A epitope for the 20.1scFv and 103.2 scFv are designated with green and red stars, respectively. Grey arrows beneath the aligned sequences show the b-strands secondary structure and are labeled according to the conventional Ig-V and Ig-C designations.

Supplementary Figure 3. Model of full-length 20.1 and 103.2 antibody binding to BTN3A. Left panel shows our model of the full length 20.1 antibody binding to BTN3A based on our BTN3A-20.1 scFv complex crystal structure. Full length antibodies are shown as ribbon format in green, BTN3A dimers are shown as surface representation in cyan and light cyan. Fabs were superimposed on the scFvs and the Fc portions modeled based on the estimated linker distances between Fab and Fc. Dimer 1-20.1 model is shown at left: The 20.1 antibody binds to two BTN3A dimers due to the orientation of the scFv on BTN3A. This cross-linking of BTN3A dimers results in oligomerization of these molecules on the cell surface, with antibodies laying parallel to the cell surface. A model of the B30.2 intracellular domain is shown in light purple connected to the BTN3A extracellular domains via a single pass transmembrane helix. Right Panel shows the 103.2 full length antibody (shown in salmon) modeled onto a BTN3A Dimer 1 (cyan). Our model suggests a 1:1 binding stoichiometry of the 103.2 antibody to BTN3A dimer.

Supplementary Figure 4. Kinetics of full-length 20.1 and 103.2 antibody staining on human T cells. Human $CD8^+$ ab T cells were incubated with either 20.1 (open circles) or 103.2 (filled circles) mAbs (10 mg/ml) and incubated at 37°C for the indicated time points. Stainings were performed by using fluorochrome-conjugated secondary mAbs and analyzed by flow cytometry. MFI, geometric mean of fluorescence intensity. Data are representative of more than three experiments performed on various human cell lines.

Supplementary Table 1. BTN3A1 dimer interface contacts.

Dimer 1 (symmetric)

BTN3A1 (A)	Strand	BTN3A1 (B)	Strand	Contact
Asp120^{Oδ2}	A^{C}	Thr203^{N}	G^{C}	H-bond
Asp120^{Oδ2}	A^{C}	Thr203^{Oγ1}	G^{C}	H-bond
Asp120	A^{C}	Thr203	G^{C}	VDW
Leu121^{O}	A^{C}	Ser205^{N}	G^{C}	H-bond
Leu121	A^{C}	Ser205	G^{C}	VDW
Leu121	A^{C}	Ala204	G^{C}	VDW
His122	A^{C}	Ser205	G^{C}	VDW
Val123^{N}	A^{C}	Ser205^{O}	G^{C}	H-bond
Val123	A^{C}	Ser205	G^{C}	VDW
Val123	A^{C}	Ile206	G^{C}	VDW
Val123^{O}	A^{C}	Ser207^{N}	G^{C}	H-bond
Val123	A^{C}	Ser207	G^{C}	VDW
Asp124	A^{C}	Ser207	G^{C}	VDW
Asp124^{Oδ1}	A^{C}	Ser207^{O}	G^{C}	H-bond
Val125^{N}	A^{C}	Ser207^{O}	G^{C}	H-bond
Val125	A^{C}	Ser207	G^{C}	VDW
Val125	A^{C}	Ile208	G^{C}	VDW
Val125^{O}	A^{C}	Ala209^{N}	G^{C}	H-bond
Val125	A^{C}	Ala209	G^{C}	VDW
Val125	A^{C}	Phe212	G^{C}	VDW
Lys126	A^{C}	Phe212	G^{C}	VDW
Tyr128	A^{C}	Phe212	G^{C}	VDW
Thr203^{N}	G^{C}	Asp120^{Oδ2}	A^{C}	H-bond
Thr203^{Oγ1}	G^{C}	Asp120^{Oδ2}	A^{C}	H-bond
Thr203	G^{C}	Asp120	A^{C}	VDW
Ala204	G^{C}	Leu121	A^{C}	VDW
Ser205^{N}	G^{C}	Leu121^{O}	A^{C}	H-bond
Ser205	G^{C}	Leu121	A^{C}	VDW
Ser205	G^{C}	His122	A^{C}	VDW
Ser205^{O}	G^{C}	Val123^{N}	A^{C}	H-bond
Ser205	G^{C}	Val123	A^{C}	VDW
Ile206	G^{C}	Val123	A^{C}	VDW
Ser207^{N}	G^{C}	Val123^{O}	A^{C}	H-bond
Ser207	G^{C}	Val123	A^{C}	VDW
Ser207^{O}	G^{C}	Asp124^{Oδ1}	A^{C}	H-bond
Ser207	G^{C}	Asp124	A^{C}	VDW
Ser207^{O}	G^{C}	Val125^{N}	A^{C}	H-bond
Ile208	G^{C}	Val125	A^{C}	VDW
Ala209^{N}	G^{C}	Val125^{O}	A^{C}	H-bond
Ala209	G^{C}	Val125	A^{C}	VDW
Phe212	G^{C}	Val125	A^{C}	VDW
Phe212	G^{C}	Lys126	A^{C}	VDW
Phe212	G^{C}	Tyr128	A^{C}	VDW

Dimer 2 (asymmetric)

BTN3A1 (A)	Strand	BTN3A1 (B)	Strand	Contact
Glu35^{Oε1}	C^{V}	Asp130^{Oδ1}	A^{C}	H-bond
Glu35^{Oε2}	C^{V}	Asp130^{Oδ2}	A^{C}	H-bond
Glu35	C^{V}	Asp130	A^{C}	VDW
Arg44	C$'^{V}$	Thr160	D^{C}	VDW
Gln45	C$'^{V}$	Thr160	D^{C}	VDW
Gln45	C$'^{V}$	Glu162	D^{C}	VDW
Val46	C$'^{V}$	Pro159	D^{C}	VDW
Val46^{N}	C$'^{V}$	Thr160^{O}	D^{C}	H-bond
Val46	C$'^{V}$	Thr160	D^{C}	VDW
Val46	C$'^{V}$	Val161	D^{C}	VDW
Val46	C$'^{V}$	Lys129	A^{C}	VDW
Asp58^{Oδ1}	C$''^{V}$	Lys129^{Nζ}	A^{C}	H-bond
Asp58^{Oδ2}	C$''^{V}$	Lys129^{Nζ}	A^{C}	H-bond
Asp58^{Oδ1,Oδ2}	C$''^{V}$	Lys129^{Nζ}	A^{C}	Salt-Bridge
Asp58	C$''^{V}$	Lys129	A^{C}	VDW
Asp58	C$''^{V}$	Val161	D^{C}	VDW
Arg59^{Nη1,Nη2}	C$''^{V}$	Asp130^{Oδ1,Oδ2}	A^{C}	Salt-Bridge
Arg59	C$''^{V}$	Asp130	A^{C}	VDW
Arg59	C$''^{V}$	Val161	D^{C}	VDW
Gln60	C$''^{V}$	Val161	D^{C}	VDW
Ser61	C$''^{V}$	Thr160	D^{C}	VDW
Ser61	C$''^{V}$	Val161	D^{C}	VDW
Ser61^{Oγ}	C$''^{V}$	Glu162^{N}	D^{C}	H-bond
Ser61	C$''^{V}$	Glu162	D^{C}	VDW
Ala62^{N}	C$''^{V}$	Glu162^{O}	D^{C}	H-bond
Ala62	C$''^{V}$	Glu162	D^{C}	VDW
Ala62	C$''^{V}$	Ala163	D^{C}	VDW
Ala62	C$''^{V}$	Pro164	D^{C}	VDW
Arg65	C$''^{V}$	Val161	D^{C}	VDW
Arg65	C$''^{V}$	Pro164	D^{C}	VDW

Supplementary Table 2: Interface Contacts of BTN3A1/20.1 complex

Light Chain

CDR1	BTN3A1	Contact
Trp32	Arg59§	VDW
CDR3	**BTN3A1**	**Contact**
Gly91	Arg59§	VDW
$Gly91^{O}$	$Arg59^{N\eta1}$§	H-bond
His92	Asp58§	VDW
Ser93	Asp58§	VDW
Tyr94	Asp58§	VDW
Tyr94	Arg65§	VDW
$Tyr94^{O\eta}$	$Arg65^{N\eta2}$§	H-bond

Heavy Chain

CDR1	BTN3A1	Contact
Thr161	Ala62§	VDW
Thr161	Pro63§	VDW
$Arg162^{O}$	$Gln45^{N\varepsilon2}$§	H-bond
Tyr164	Gln60§	VDW
$Tyr164^{O\eta}$	$Gln60^{O}$§	H-bond
Tyr164	Ser61§	VDW
Tyr164	Ala62§	VDW
$Tyr164^{O\eta}$	$Ala62^{N}$§	H-bond
Tyr164	Arg65§	VDW
$Tyr164^{O\eta}$	$Arg65^{N\varepsilon,N\eta1}$§	H-bond
CDR2	**BTN3A1**	**Contact**
Glu181	Arg65§	VDW
$Glu181^{O\varepsilon2}$	$Arg65^{N\eta1}$§	H-bond
$Glu181^{O\varepsilon2}$	$Arg65^{N\eta1,N\eta2}$§	Salt-bridge
Asn183	Ala62§	VDW
$Asn183^{N\delta2}$	$Ala62^{O}$§	H-bond
Asn185	Ala62§	VDW
Asn185	Pro63	VDW
Lys190	Glu57	VDW
$Lys190^{N\zeta}$	$Glu57^{O\varepsilon2}$	H-bond
$Lys190^{N\zeta}$	$Glu57^{O\varepsilon1,O\varepsilon2}$	Salt-bridge
CDR3	**BTN3A1**	**Contact**
Gly235	Gln45§	VDW
Gly235	Ser61§	VDW
$Gly235^{O}$	$Ser61^{O\gamma}$§	H-bond
Thr236	Val46§	VDW
Thr236	Arg59§	VDW
$Thr236^{O\gamma1}$	$Arg59^{N\eta1}$§	H-bond
Pro237	Asp58§	VDW
Pro237	Arg59§	VDW
$Pro237^{O}$	$Arg59^{N\eta1,N\eta2}$§	H-bond

§ interface residues for BTN3A dimer 2.