

Supplementary Information

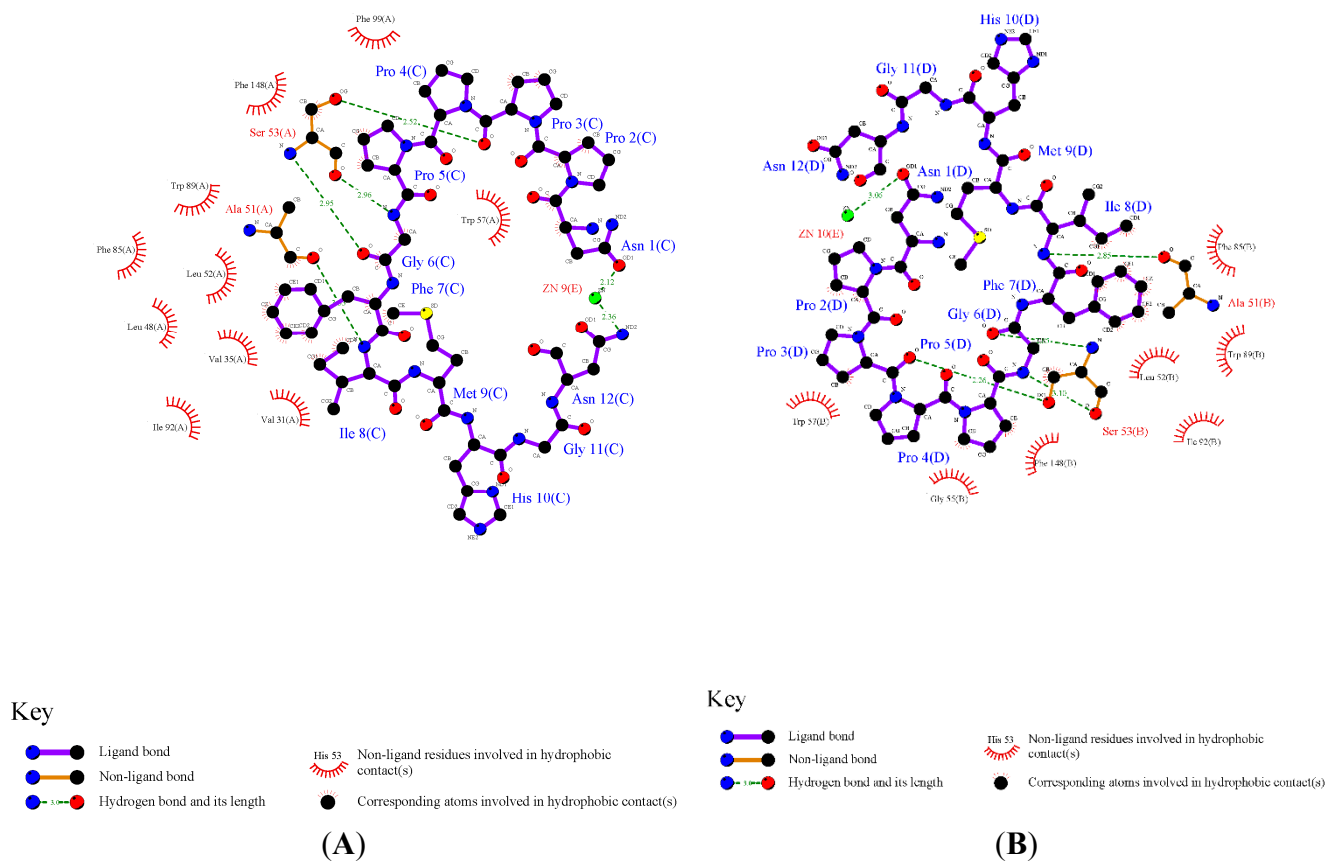


Figure S1. Diagrams of protein-ligand contacts. The LIGPLOT program was used for automatic generation of 2D ligand-protein interaction diagrams of chain A/chain C (**A**) and chain B/chain D (**B**).

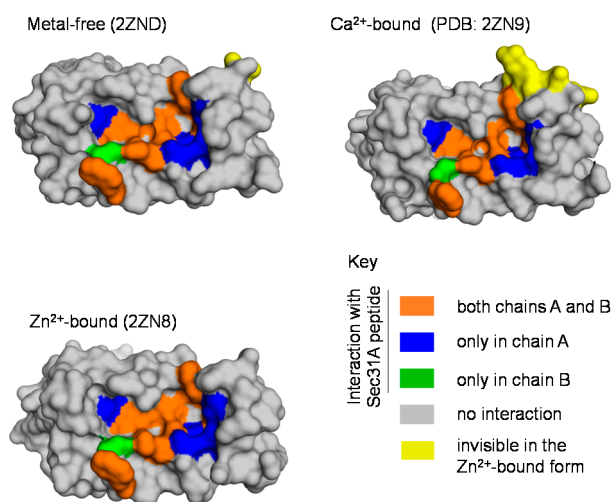


Figure S2. Surface presentations of Pocket 3 areas. Previously resolved structures of ALG-2 in a metal-free form (PDB: 2ZND), a Ca²⁺-bound form (PDB: 2ZN9, chain A) and a Zn²⁺-bound form (PDB: 2ZN8) are compared. Residues that are in contact with the Sec31A peptide in the complex structure (PDB: 3WXA) are colored as indicated. Differences in the N-terminal residues visible in 2ZND (²⁵Q) and 2ZN9 (²¹ALPDQ²⁵), but not in 2ZN8, are colored in yellow.

		ABM-2											
Hs	827	-QTQ	QYY	PHGEN	--	PPPP	GGF	IMHGNVNP	PNAAGQ	-		856	
Mm	826	-QTQ	QYY	PHGEN	--	PPPP	GGF	IMQGNVIP	NPNAAP	-		855	
Gg	833	-SAQ	QYY	QGGDK	--	PPPP	GGF	IMPGAINPS	APPQ	-		862	
Pb	826	-PTP	QYY	QORDN	-	PPPP	GGF	IMQKDMNP	PHMPPH	-		856	
Xt	823	-ISEL	PLL	QRNS	--	ALPL	GFNIP	PGSTYTSG	SLA	-		852	
Dr	871	-LVP	SSDP	QGD	S--	TPPA	FGLQSP	AMASVP	PAS	-		899	
Dm	816	-PPN	QYA	QPSWPAQ	V	PP	LQTSYP	GAQQPQ	APPQ	-		847	
Ce	791	-QPTR	TYQ	QPAP	--	IPPP	NLV	NG	FNPYNPSH	-		818	
Sc	1073	-TTQ	--	PNGSS	--	YPPT	G	PYTNNH	TMTSP	PPV	-	1099	

Figure S3. Alignment of amino acid sequences of Sec31 in the ALG-2-binding motif type 2 (ABM-2) regions. Amino acid sequences of human Sec31A and its orthologous sequences from different organisms were retrieved from UniProt or NCBI and aligned as described in the Experimental Section. Conserved residues in five or more sequences are highlighted in cyan. The ABM-2 sequence is indicated by a red bar. Hs, *Homo sapiens* (human; UniProt, O94979); Mm, *Mus musculus* (mouse; UniProt, Q3UPL0); Gg, *Gallus gallus* (chicken; Q5F3X8); Pb, *Python bivittatus* (snake; NCBI, XP_007428791.1); Xt, *Xenopus tropicalis* (frog; UniProt, F6UZ63); Dr, *Danio rerio* (zebra fish; UniProt, Q7SYD5); Dm, *Drosophila melanogaster* (fly; UniProt, A1Z7J7); Ce, *Caenorhabditis elegans* (nematode; NCBI, NP_502144.1); Sc, *Saccharomyces cerevisiae* (budding yeast; UniProt, P38968).

Table S1. Data collection, processing and refinement statistics.

des3-20ALG-2/Sec31A Peptide Zn ²⁺ -Bound	
PDB Code	3WXA
Data Collection	
Beamline	PF-AR NE3A
Wavelength (Å)	1.2821
Space group	<i>P</i> 6 ₄
a/b/c (Å)	82.0/82.0/103.2
α/β/γ (°)	90.0/90.0/120.0
Resolution (Å)	71.0–2.36 (2.40–2.36)
Measured reflections	166,054
Unique reflections	16,160
Completeness (%)	99.4 (99.3)
<i>R</i> _{merge}	8.0 (25.7)
I/σ	32.2 (10.8)
Refinement	
<i>R</i> _{work} / <i>R</i> _{free} (%)	21.8/29.6
RMSD bond length (Å)	0.014
RMSD bond angle (°)	1.718
Average B-factor (Å ²)	44.0
No. ALG-2 molecule	2
No. Sec31A peptide	2

Values in parentheses are for the highest resolution shell. RMSD, root mean square deviation.