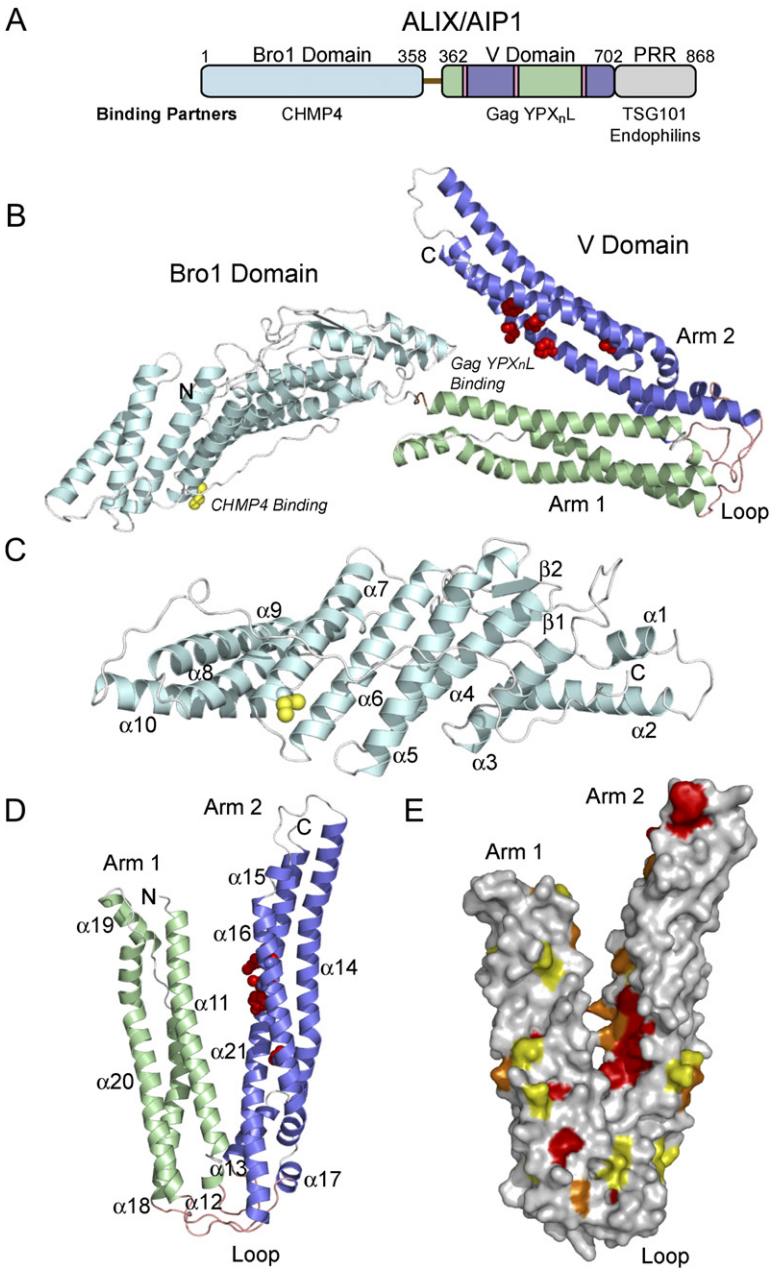

Figure 1. Structure of $ALIX_{Bro1-V}$

(A) shows domain structure of human ALIX. Sequences that compose the different elements are color coded here (and throughout) as follows: Bro1, turquoise; Bro1-V linker, brown; V-domain arm1, green; V-domain loop, salmon; V-domain arm2, blue; and Proline-rich region (PRR), gray.

(B) Ribbon representation of $ALIX_{Bro1-V}$ is shown. Residues implicated in CHMP4 binding (Ile212; yellow) or YPX_nL binding (Phe495, Val498, Ala509, Phe676, Leu680, and Ile683; red) are highlighted. The Bro1 and V domains are connected by a short hydrophobic linker ($_{359}VPV_{361}$). The limited set of interdomain hydrophobic packing interactions is made by Phe24 (α1, Bro1), Val359, Pro360, Val361, Val363 (α11, arm1, and V), and Leu585 (α19, arm1, and V).

(C) This ribbon diagram of $ALIX_{Bro1}$ shows the secondary-structure-labeling scheme. The view is from the underside of the orientation shown in (A).

(D) shows a ribbon diagram of $ALIX_V$ showing the secondary-structure-labeling scheme. As compared to (A), this orientation is rotated clockwise by ~60° relative to a line perpendicular to the plane of the paper.

(E) This surface rendering shows $ALIX_V$ sequence conservation. $ALIX_V$ sequences from seven divergent species were aligned using ESPript (Gouet et al., 1999); residues with scaled similarity scores are color coded as follows: 85–100 are red, 68–84 are orange, and 50–67 are yellow.

electropositive patch at one end of the yeast Bro1 domain is also basic in the human protein, albeit to a lesser extent.

The human and yeast Bro1 domains differ most at one end of the domain (right in Figure 1C), where N- and C-terminal elements differ in secondary structure and packing (Figure S2). Specifically, the C terminus of yeast Bro1p forms three consecutive helical segments that cross the concave side and then turn up into the domain, whereas the equivalent residues in the human protein (342–358) form an extended strand that traverses a similar path but terminates earlier. Thus, the C-terminal helix of the yeast protein packs between α3 and the α1–2 hairpin ($ALIX_{Bro1}$ numbering), whereas the human Bro1 C terminus is shifted by ~10 Å, and the first three helices of human $ALIX_{Bro1}$ collapse into a three-helix bundle (a maximal relative shift of ~12 Å). It appears possible that these distinct C-terminal conformations could alter the relative orientations of the Bro1 and V domains in the different proteins.

The Structure of the ALIX V Domain

Both arms of the ALIX V domain are composed of extended three-helix bundles, although a series of short breaks subdivide the major helices into 11 different segments (Figures 1D, S1, and S3). The topology of the V domain is notable in that the polypeptide chain crosses the arm1/arm2 "loop" region three times in the course of