## **Supplemental Figure S3**

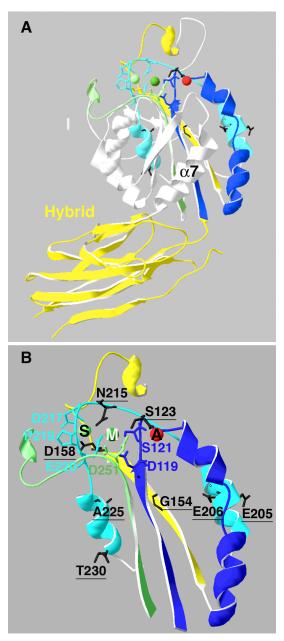


Fig. S3. Mutations reducing ligand binding map to MIDAS and SyMBS domains. A. Ribbon structure [from (28)] illustrating the location of the I and Hybrid (yellow) domains. Loops with residues that contribute to SyMBS, and MIDAS domains and the helices and β-strands flanking them are colored as are metal coordinating side chains. B. Enlarged MIDAS and SyMBS loops. β1-loop- $\alpha$ 1,1' containing the MIDAS DXSXS (**119-123**) is in dark blue.  $\beta$ 4-loop-3<sub>10</sub> helix containing MIDAS **D251** is green. SyMBS coordinating N215, D217, P219, and E220 (also a MIDAS residue) are between  $\alpha 2$  and  $\alpha 3$  (light blue). The loop following  $\beta$ 2 contributes SyMBS **D158** (yellow). Amino acids corresponding to those mutated in Drosophila βPS and analyzed for ligand binding are labeled (black and underlined). E205 corresponds to the position of amino acids (following N204) that are inserted in  $mys^{b69}$ and  $mys^{b50}$  Coloring of divalent cations is; SyMBS (S) Ca<sup>2+</sup>, light green; MIDAS (M) Mg<sup>2+</sup>, green; and ADMIDAS (A) Ca<sup>2+</sup>, red.