#### **Supplementary Information**

# An alternative N-terminal fold of the intestine-specific annexin A13a induces dimerization and regulates membrane-binding

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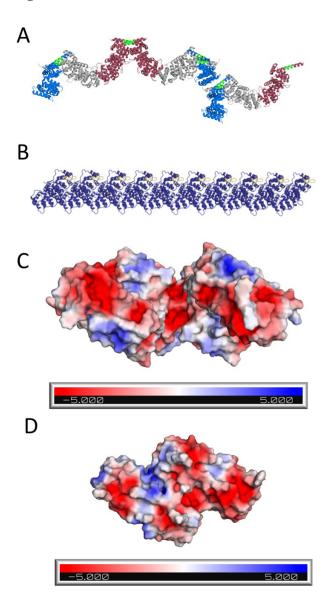
Running title: Annexin A13a dimerization regulates membrane-binding

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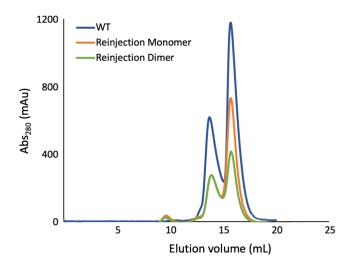
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### SI Figure 1.



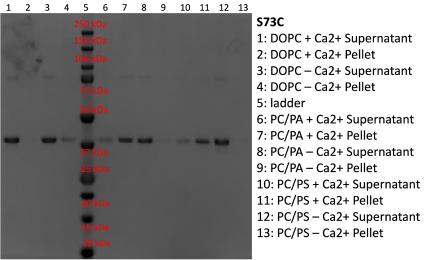
SI Figure 1. Crystal packing and electrostatic surface potential analysis of Anx A13a and Anx A5 (PDB 1A8A). A. Crystal packing of Anx A13a shows that the 25-34 region (highlighted in green) assists in dimer formation but does not make additional contacts within the lattice. B. Crystal packing of Anx A5 (PDB entry 1A8A). C. Electrostatic surface potential representation of the Anx A13a dimer. The view is of the putative Ca<sup>2+</sup> binding surface and is 180° rotated from the view in Fig. 2B. Bound Ca<sup>2+</sup> is anticipated to neutralize the negative charge and bind to membrane phospholipids. D. Electrostatic surface potential of the membrane binding surface of the Anx A5 monomer shows a similar charge distribution.

## SI Figure 2.



**SI Figure 2. Dynamic monomer-dimer equilibrium of Anx A13a.** Size-exclusion chromatogram of wild-type Anx A13a injected at a concentration of 1 mg/mL (blue trace). Fractions from the dimer peak (elution volume ~13.6 mL) and monomer peak (elution volume ~15.3 mL) were collected and subsequently reinjected onto the same column. Both reinjection traces of the dimer (green) and monomer (orange) show the redistribution into the dimer and monomer peaks.

#### SI Figure 3. 1 2 3 4 5 6 7 8 9 10 11 12 13 WT Annexin 1: ladder 250 kDa 2: DOPC + Ca2+ Supernatant 150 kDa 100 kDa 3: DOPC + Ca2+ Pellet 75 kDa 4: DOPC – Ca2+ Supernatant 5: DOPC - Ca2+ Pellet 50 kDa 6: PC/PA + Ca2+ Supernatant 7: PC/PA + Ca2+ Pellet 37 kDa 8: PC/PA - Ca2+ Supernatant 9: PC/PA - Ca2+ Pellet 25 kDa 10: PC/PS + Ca2+ Supernatant 20 kDa 11: PC/PS + Ca2+ Pellet 15 kDa 12: PC/PS - Ca2+ Supernatant 13: PC/PS - Ca2+ Pellet 10 kDa В 4 5 6 7 8 9 10 11 12 13 T32P/N33G/E34P 250 kDa 1: ladder 150 kDa 2: DOPC + Ca2+ Supernatant 100 kDa 3: DOPC + Ca2+ Pellet 75 kDa 4: DOPC - Ca2+ Supernatant 5: DOPC - Ca2+ Pellet 50 kDa 6: PC/PA + Ca2+ Supernatant 37 kDa 7: PC/PA + Ca2+ Pellet 8: PC/PA - Ca2+ Supernatant 25 kDa 9: PC/PA - Ca2+ Pellet 20 kDa 10: PC/PS + Ca2+ Supernatant 11: PC/PS + Ca2+ Pellet 15 kDa 12: PC/PS – Ca2+ Supernatant 13: PC/PS – Ca2+ Pellet C 8 9 10 11 12 13 6 7 **S73C** 1: DOPC + Ca2+ Supernatant



**SI Figure 3. Full SDS-PAGE gels of representative liposome spin assays.** Full SDS-PAGE gels for Anx A13a-liposome spin assays performed in **Figure 6** for A. Wild-type Annexin A13a, B. Constitutive monomer, and C. Enhanced dimer.