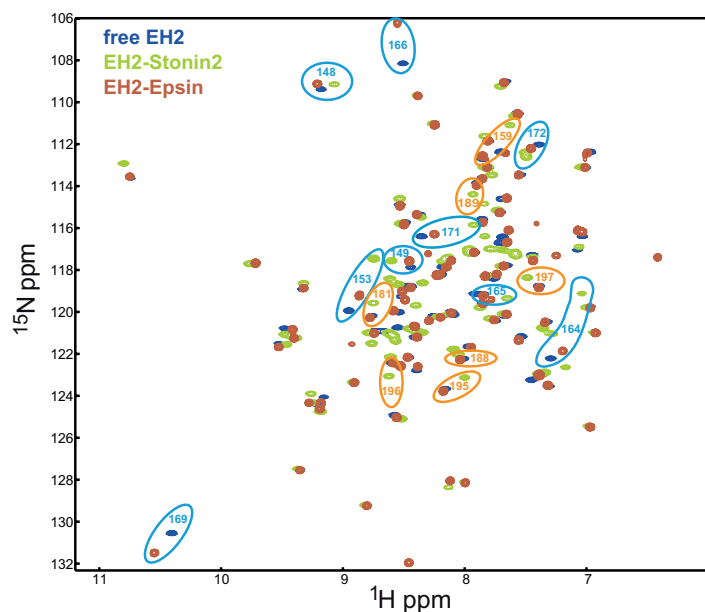


Supplementary Figure 5: Overlay of  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of Eps15-EH2 free or bound to Epsin and Stonin2 NPF peptides.



Overlay of  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of free Eps15-EH2 domain (blue) and EH2 bound to epsin (light red) or stonin2 (green) at 20°C and pH 7. Peaks corresponding to residues of the first binding site are highlighted by cyan ellipses with respective residue numbers indicated. Shifts observed for residues of the first binding site are similar for epsin- and stonin2-bound EH2, but binding of stonin2 results in more pronounced shifts. Peaks of residues belonging to the second binding site are highlighted by orange ellipses, with residue numbers indicated. These peaks shift when stonin2, but not epsin, is titrated to EH2.