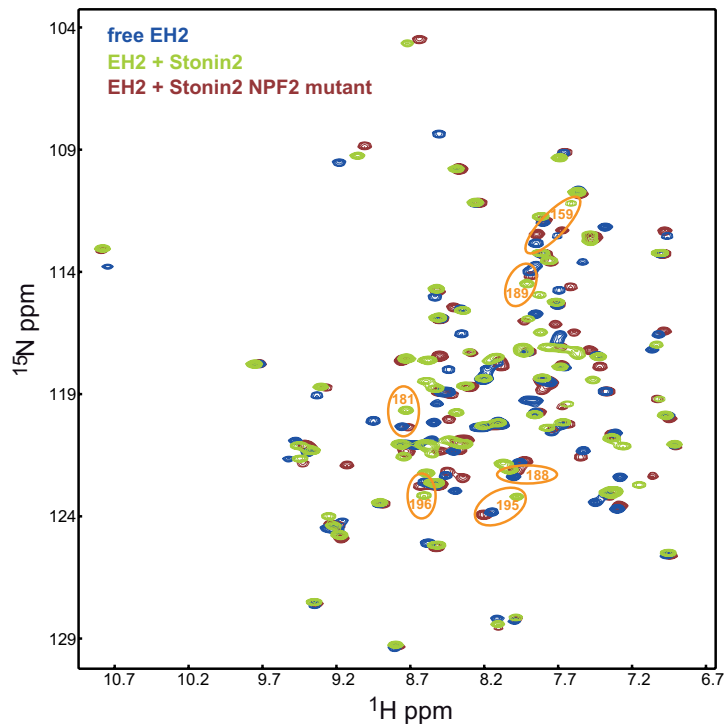


Supplementary Figure 4: Overlay of ^1H , ^{15}N HSQC spectra of free EH2, Stonin2-bound EH2 and EH2 bound to a Stonin2 mutant (NPF2-AAA).



Overlay of ^1H , ^{15}N HSQC spectra of free Eps15-EH2 domain (blue), EH2 bound to a stonin2 mutant (NPF2-AAA) (red) or to stonin2 wt (green). Despite the relatively low affinity of the mutant (K_d of $7.5 \mu\text{M}$), chemical shift perturbations are observed for residues of the EH domain in the first and second binding site. Peaks belonging to residues of the second binding site are highlighted by orange ellipses with residue numbers indicated. This demonstrates that even in the absence of the second NPF motif the flanking regions of NPF2 are still capable of binding to the second binding site of the EH domain.