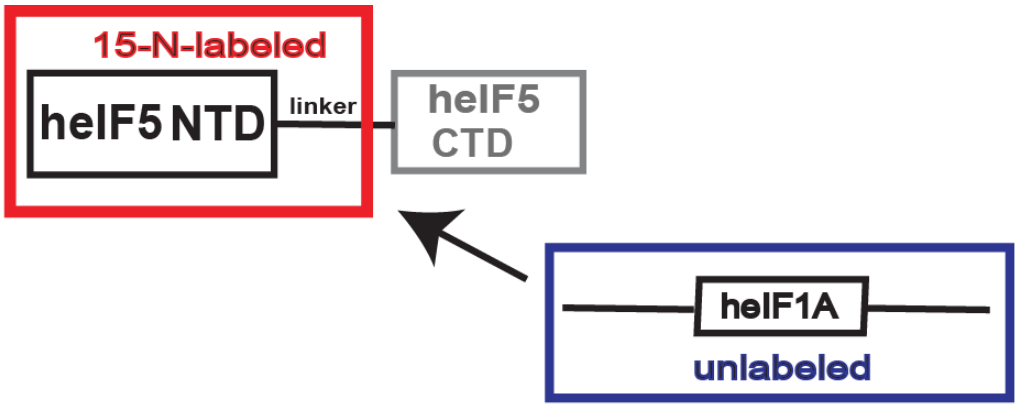
A.


15-N-labeled
heIF5 NTD
linker
heIF5
CTD
heIF1A
unlabeled

B.

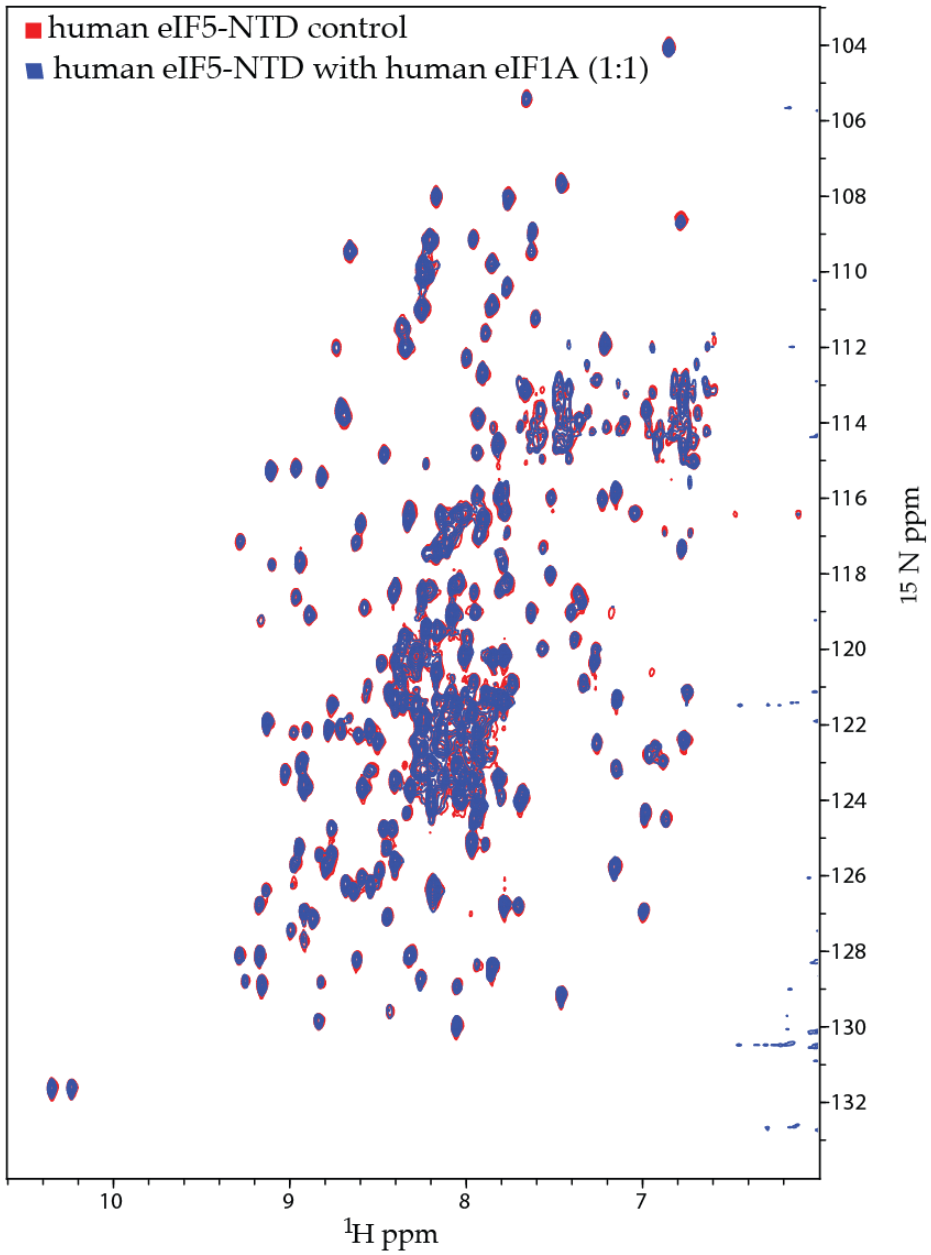
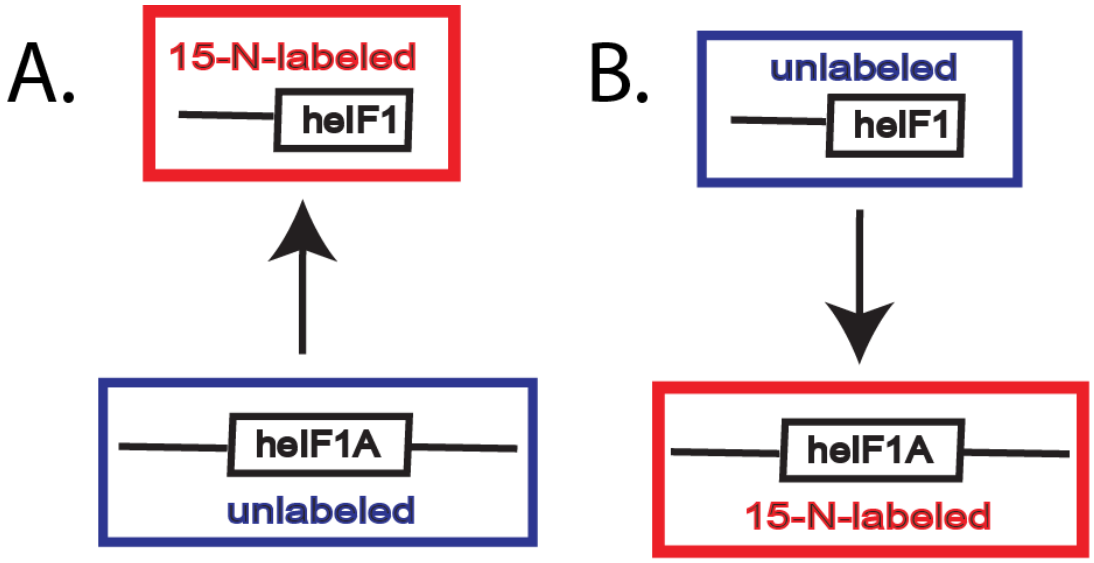

human eIF5-NTD control
human eIF5-NTD with human eIF1A (1:1)
104
106
108
110
112
114
116
118
120
122
124
126
128
130
132
15 N ppm
10
9
8
7
1H ppm

Fig. S1

Figure S1. The eIF5-NTD does not interact with eIF1A.

(A) Schematic representation of the labeling scheme used in the NMR spectra, i.e. ^{15}N-isotopically labeled human eIF5-NTD alone (circumscribed in a red box) and in the presence of unlabeled human eIF1A domain (circumscribed in a blue box). (B) Overlay of ^{1}H-^{15}N HSQC spectra of 0.2 mM ^{15}N-labeled eIF5-NTD alone (red) and in the presence of 0.2 mM (blue) unlabeled wild-type eIF1A.


A.
15-N-labeled
heIF1
heIF1A
unlabeled
B.
unlabeled
heIF1
heIF1A
15-N-labeled

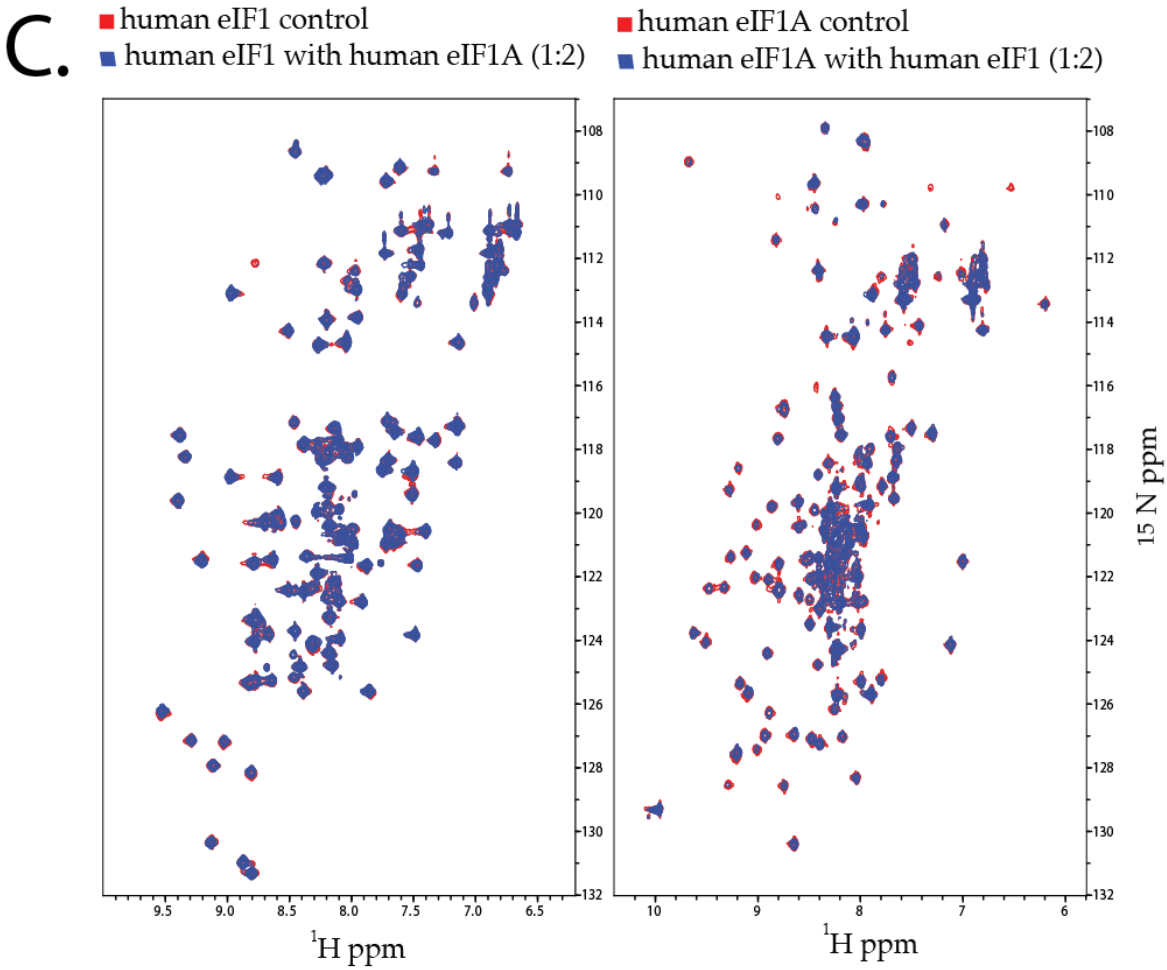

C.
human eIF1 control
human eIF1 with human eIF1A (1:2)
human eIF1A control
human eIF1A with human eIF1 (1:2)
15 N ppm
1H ppm
1H ppm

Fig. S2

Figure S2. Human eIF1A and eIF1 do not interact in solution. (A) Schematic representation of the labeling scheme used in the NMR spectra, i.e. ^{15}N-isotopically labeled eIF1 alone (circumscribed in a red box) and in the presence of unlabeled eIF1A (circumscribed in a blue box). (B) Schematic representation of the labeling scheme used in the NMR spectra, i.e. ^{15}N-isotopically labeled eIF1A alone (circumscribed in a red box) and in the presence of unlabeled eIF1 (circumscribed in a blue box). (C) Left Panel: Overlay of ^{1}H-^{15}N HSQC spectra of 0.2 mM ^{15}N-labeled eIF1 alone (red) and in the presence of 0.4 mM (blue) unlabeled wild-type eIF1A. Right Panel: Overlay of ^{1}H-^{15}N HSQC spectra of 0.2 mM ^{15}N-labeled eIF1A alone (red) and in the presence of 0.4 mM (blue) unlabeled eIF1.

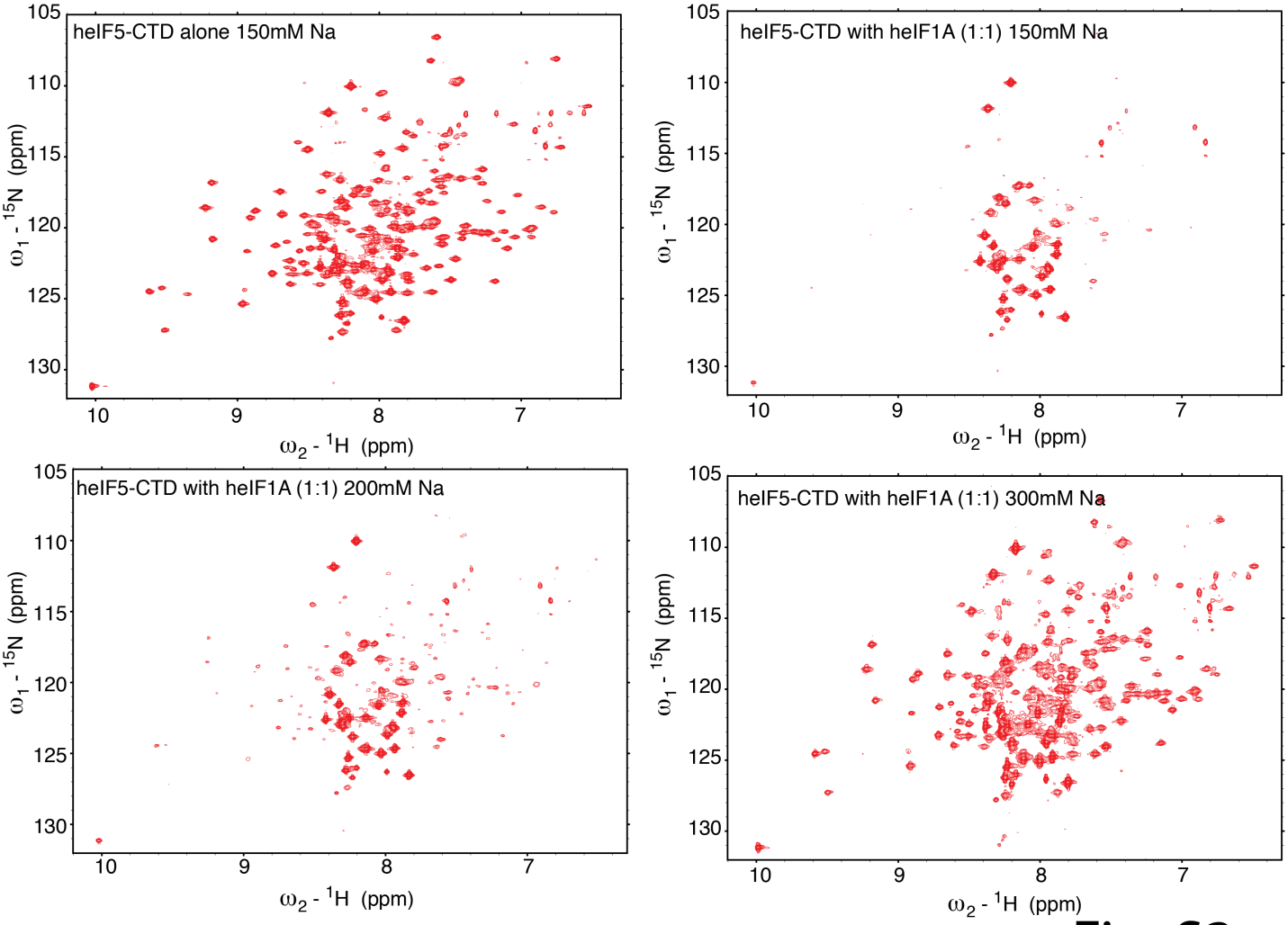


Fig. S3
Luna *et. al.*

Figure S3. Human eIF5-CTD binds to eIF1A in a salt-dependent manner.

Top left; ^{15}N-isotopically labeled eIF5-CTD alone in 150mM NaCl. Top right; ^{15}N-isotopically labeled eIF5-CTD with equimolar concentration of heIF1A in 150mM NaCl. Bottom left; ^{15}N-isotopically labeled eIF5-CTD with equimolar concentration of heIF1A in 200mM NaCl. Bottom right; ^{15}N-isotopically labeled eIF5-CTD with equimolar concentration of heIF1A in 300mM NaCl.

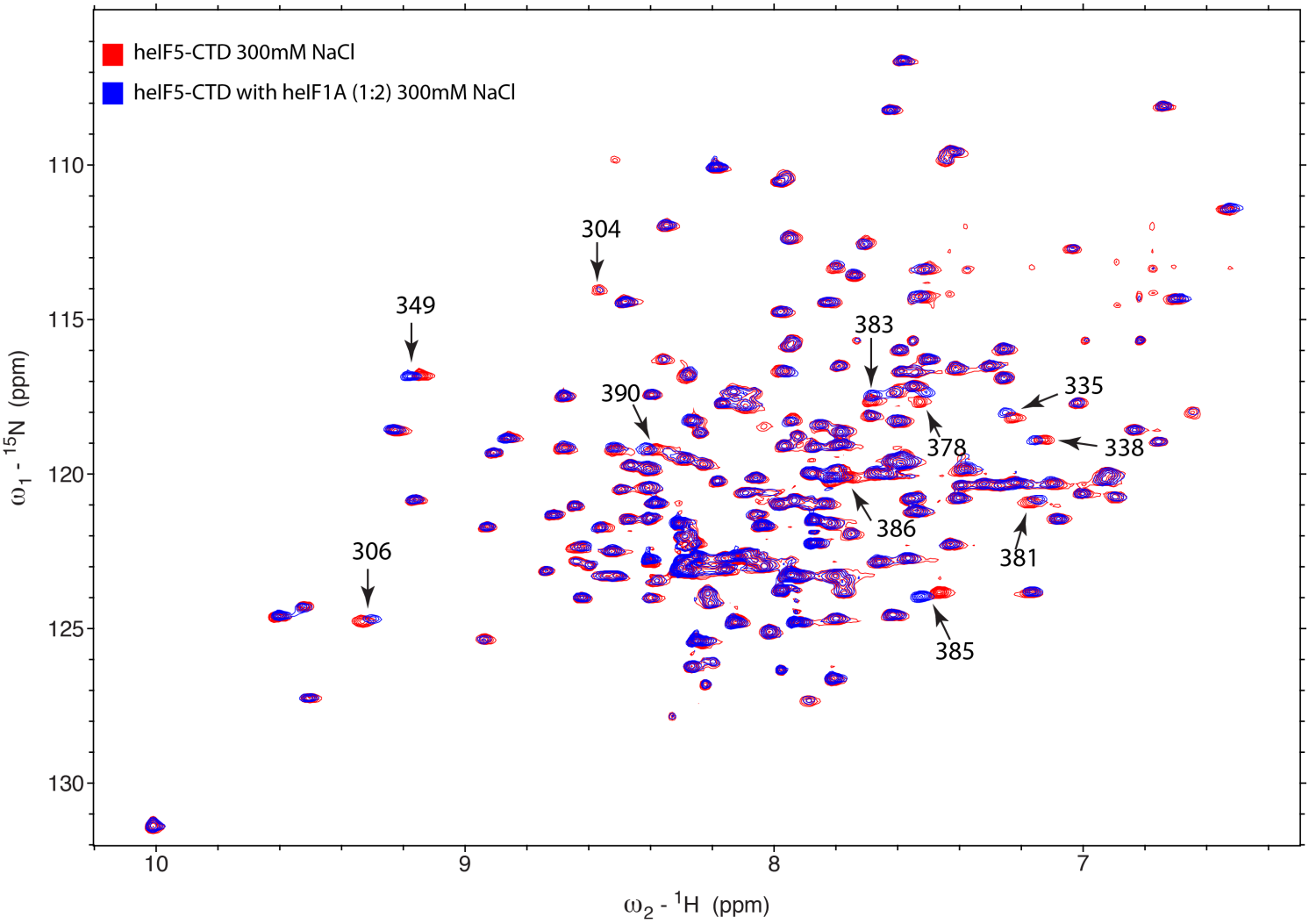


Fig. S4
Luna *et. al.*

Figure S4. heIF5-CTD resonances experiencing chemical shift perturbations from heIF1A.

Overlay of ^{1}H-^{15}N HSQC spectra is shown, wherein ^{15}N-labeled heIF15-CTD alone (red) and in the presence of eIF1A (blue) at concentration ratio of 1:2.

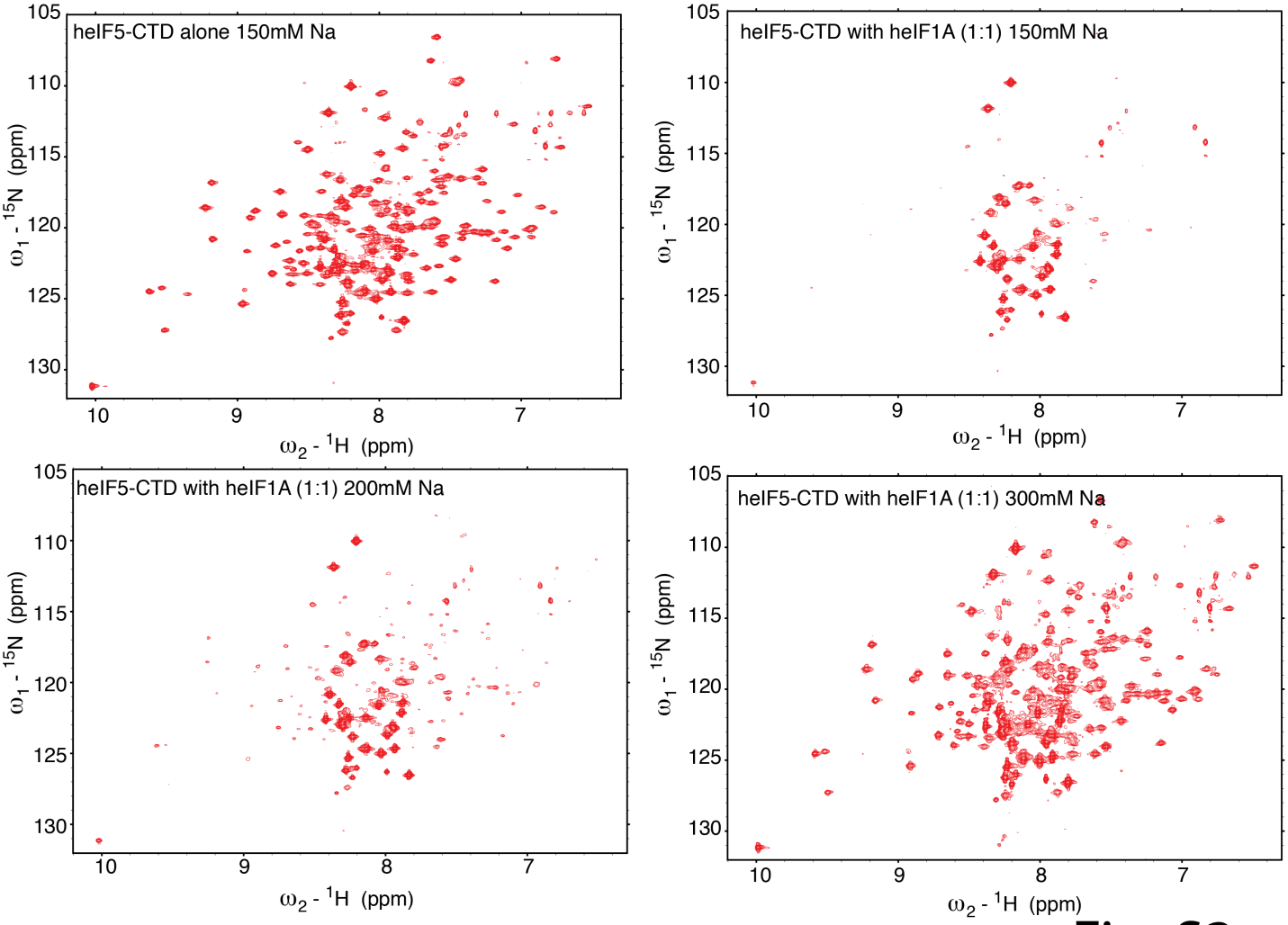


Fig. S3
Luna *et. al.*

Figure S3. Human eIF5-CTD binds to eIF1A in a salt-dependent manner.

Top left; ^{15}N-isotopically labeled eIF5-CTD alone in 150mM NaCl. Top right; ^{15}N-isotopically labeled eIF5-CTD with equimolar concentration of heIF1A in 150mM NaCl. Bottom left; ^{15}N-isotopically labeled eIF5-CTD with equimolar concentration of heIF1A in 200mM NaCl. Bottom right; ^{15}N-isotopically labeled eIF5-CTD with equimolar concentration of heIF1A in 300mM NaCl.

Figure S5. Summary of mapped interactions on the surface of eIF5-CTD affected by eIF1A, eIF1 and eIF2β.

(A-E) Comparison of mapped interactions on the surface of the eIF5-CTD, shown in the same orientation. The effects of the following proteins on heIF5-CTD: (A) heIF1A and (B-E) The following mapped interactions on heIF5-CTD were adapted from a previously published study (Luna et al., 2012). (B) heIF1 (colored coded residues are the same as Figure 1D), (C) N-terminally deleted heIF1 (ΔNTT-eIF1; deleted amino acid residues 1-28) (Luna et al., 2012) and (D) heIF2β-K2K3, wherein the red colored residues experience CSPs and the residues painted yellow are residues that correspond to resonances that are broadened, while orange colored residues experience both effects (Luna et al., 2012). (E) The location of point mutations on the surface of the heIF5-CTD-Quad mutant [(H305D and N306D) are painted cyan and (E347K and E347K) are painted magenta]. The heIF5-CTD-Quad mutant disrupts the interactions with heIF1A, heIF1 and heIF2β (Luna et al., 2012).