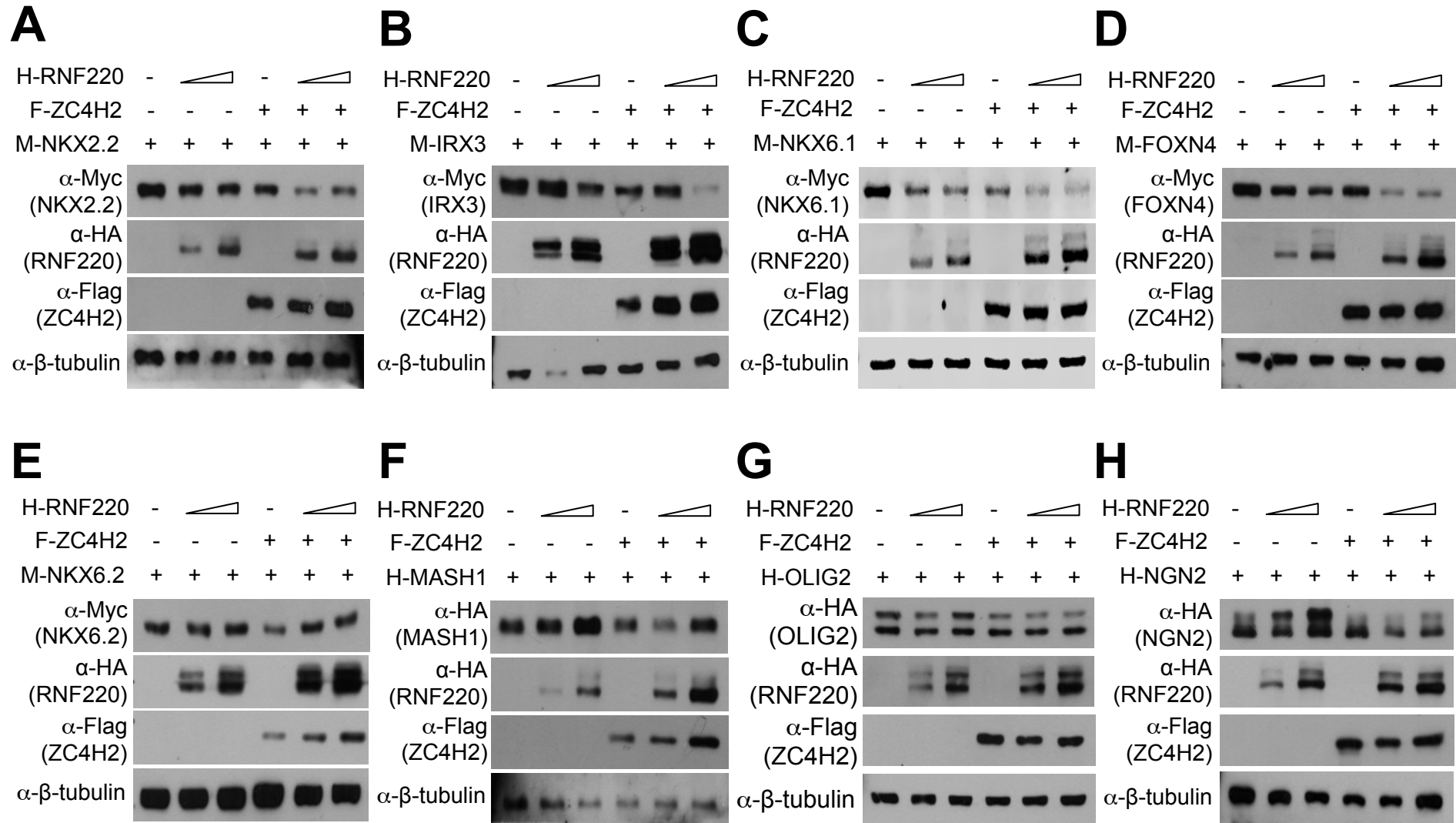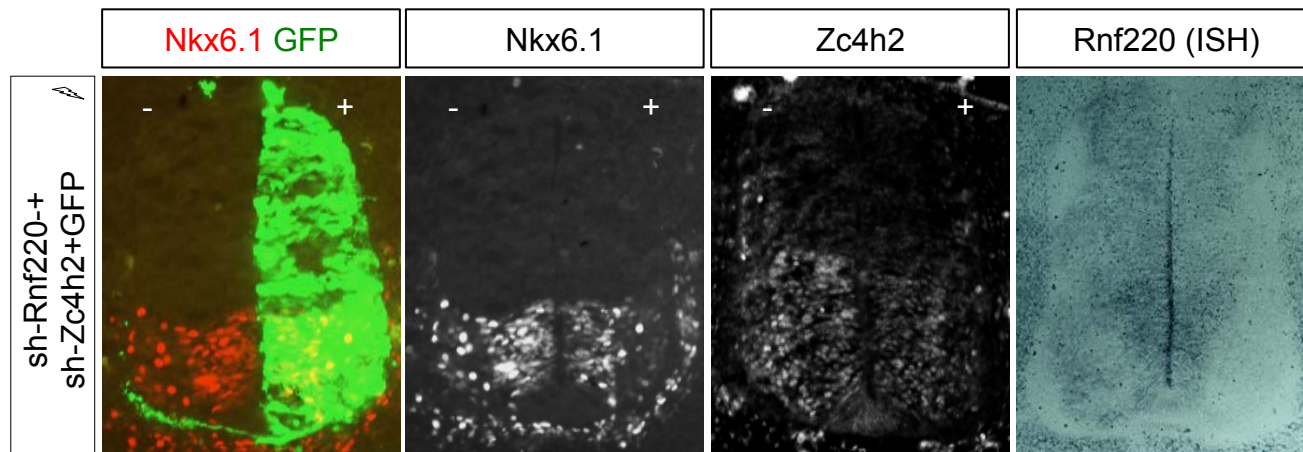**Supplementary Fig. 1: Identification of RNF220 as an interactor of ZC4H2.** Interacting proteins with Gal4 fusion to ZC4H2 from our yeast two hybrid screening includes the RNF220.

Supple Fig 2

Supplementary Fig. 2: NKX2.2, IRX3, NKX6.1 and FOXN4 are degraded by RNF220 in the presence of ZC4H2. The protein levels of NKX2.2, IRX3, NKX6.1 and FOXN4 were reduced in the presence of RNF220/ZC4H2 in a dose-dependent manner but the levels of NKX6.2, MASH1, OLIG2 and NGN2 were not reduced by the presence of RNF220/ZC4H2.

Supple Fig 3
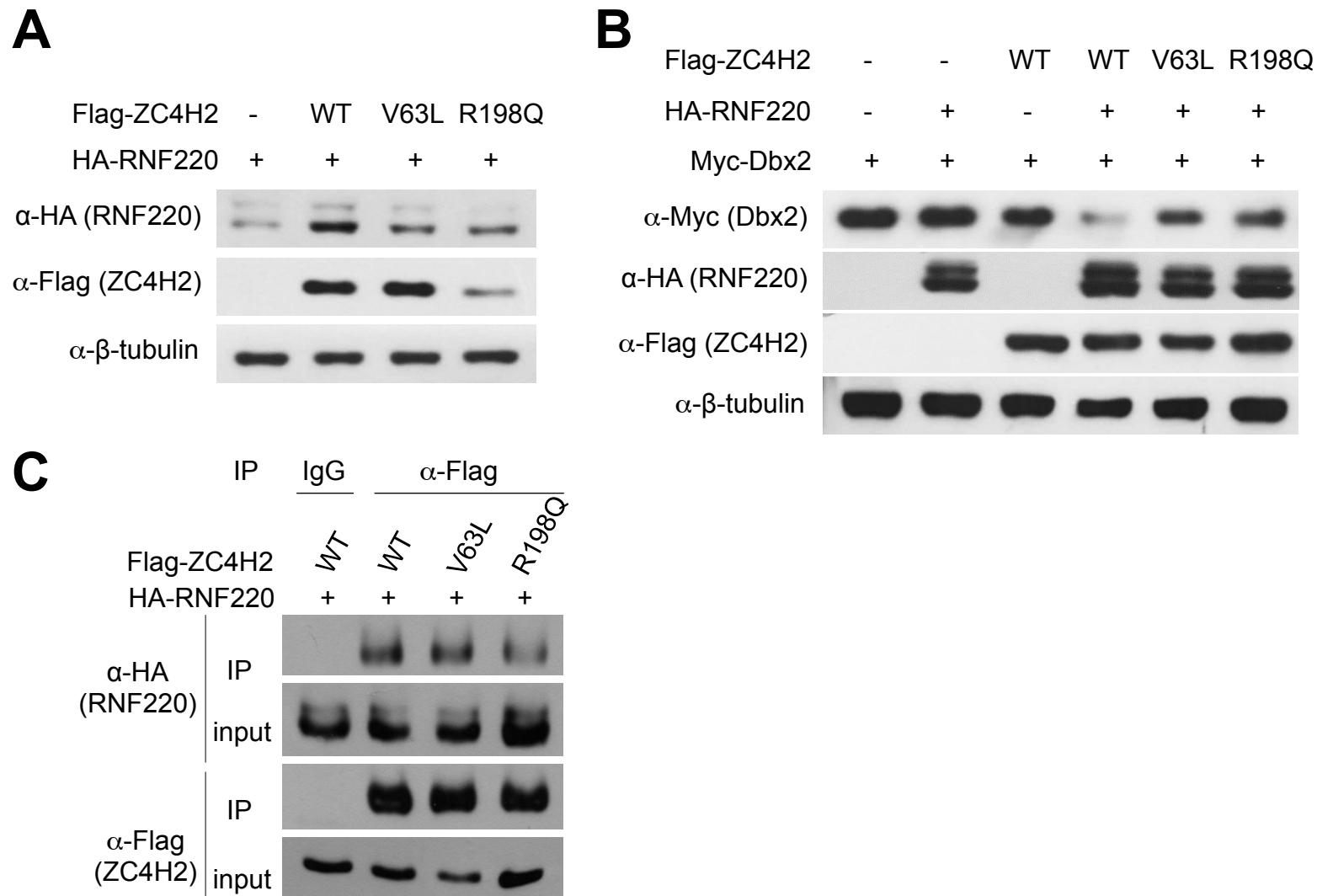
Supplementary Fig. 3: ZC4H2 point mutants lose the ability to stabilize RNF220 and to degrade Dbx2 protein. (A) ZC4H2 V63L and R198Q mutants found in WRWF patients failed to stabilize RNF220 proteins, while ZC4H2 WT increased the protein level of RNF220. (B) ZC4H2 V63L and R198Q mutants were less effective in degrading Dbx2 protein compared to the WT ZC4H2 in the presence of RNF220. (C) The interaction between RNF220 and ZC4H2 R198Q mutant was reduced compared to ZC4H2 WT.

Supplementary Fig. 4: Reduced expression of Rnf220, Zc4h2 and Nkx6.1 in chick neural tube electroporated with sh-Rnf220 and sh-Zc4h2. GFP expression indicates the efficiency of the electroporation. In the knockdown constructs electroporated side (+), Zc4h2 and Rnf220 level was reduced and Nkx6.1 expression was also reduced marginally.

Supple Fig 5
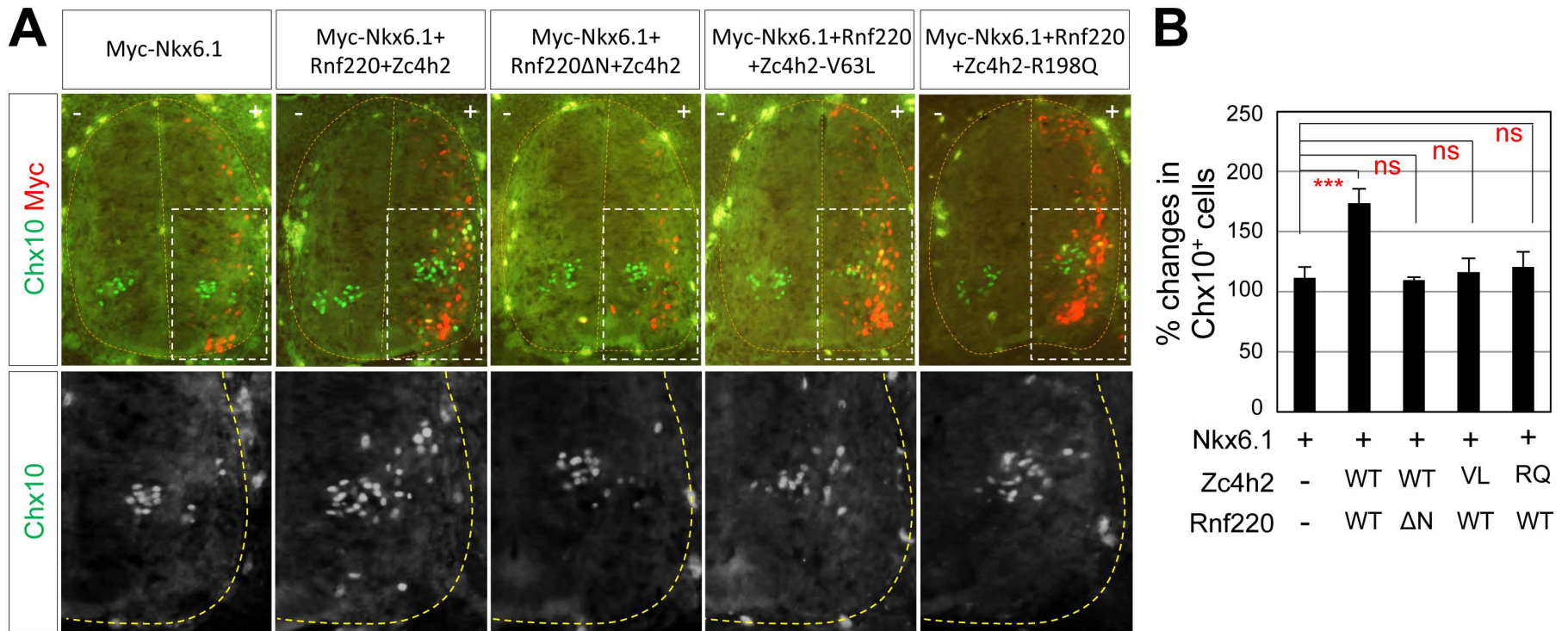
Supplementary Fig. 5: RNF220 mt (ΔN) and Zc4h2 mt (V63L, R198Q) lost the ability to promote Nkx6.1 to induce ectopic Chx10 in chick neural tube. (A) Immunostaining analyses with Chx10 and Myc antibodies for chick embryos electroporated (+ side) with Myc-Nkx6.1+LacZ, Myc-Nkx6.1+Rnf220+Zc4h2, Myc-Nkx6.1+Rnf220-ΔN+Zc4h2, Myc-Nkx6.1+Rnf220+Zc4h2-V63L or R198Q (thoracic level). (B) Quantification of Chx10+ neurons in the electroporated side (+) relative to those in the unelectroporated side (−) of chick neural tube. Values are means ± s.d. *** p<0.001, 'ns' indicates not significant.