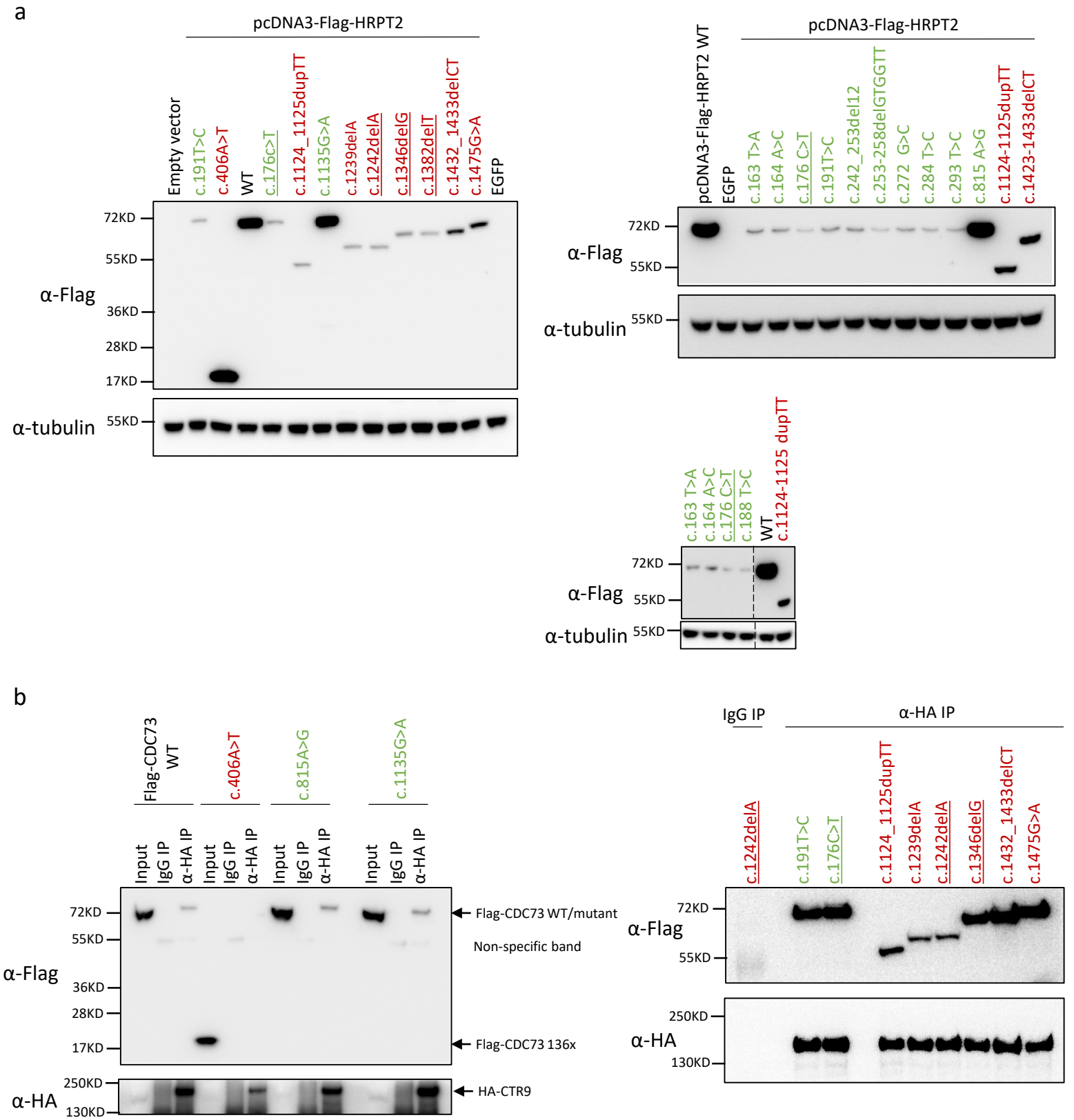

Supplementary Figure 4. Impact of the mutations on expression of parafibromin and binding to CTR9 (a key component of PAF1 complex). a) Various Flag-tagged CDC73 (HRPT2) mutants and WT were transiently expressed in HEK 293 cells and expression of parafibromin was examined by western blot using an anti-Flag antibody at 48 hours after transfection. b) Co-immunoprecipitation of HA-tagged CTR9 and Flag-tagged CDC73 WT/mutants using anti-HA antibody. Rabbit IgG isotype control (Cell Signaling) was used as a negative control for IP (IgG IP). The presence of Flag-tagged CDC73 WT/mutants and HA-tagged CTR9 in the IP eluates were detected by western blot using anti-Flag HRP-conjugated antibody and anti-HA HRP-conjugated antibody, respectively. WT CDC73 was used as a positive control and the c.406A>T (p.136X) mutant, which lacks the central CTR9 binding sequence (aa. 226-413), was used as a negative control. The low-impact mutations are highlighted in light green color and high-impact mutations are in magenta color; the parathyroid carcinoma-associated mutations are underlined. The experiments were performed at least twice.