Vaccinia-related kinase 2 mediates accumulation of polyglutamine aggregates via negative regulation of the chaperonin TRiC

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Supplementary Figures

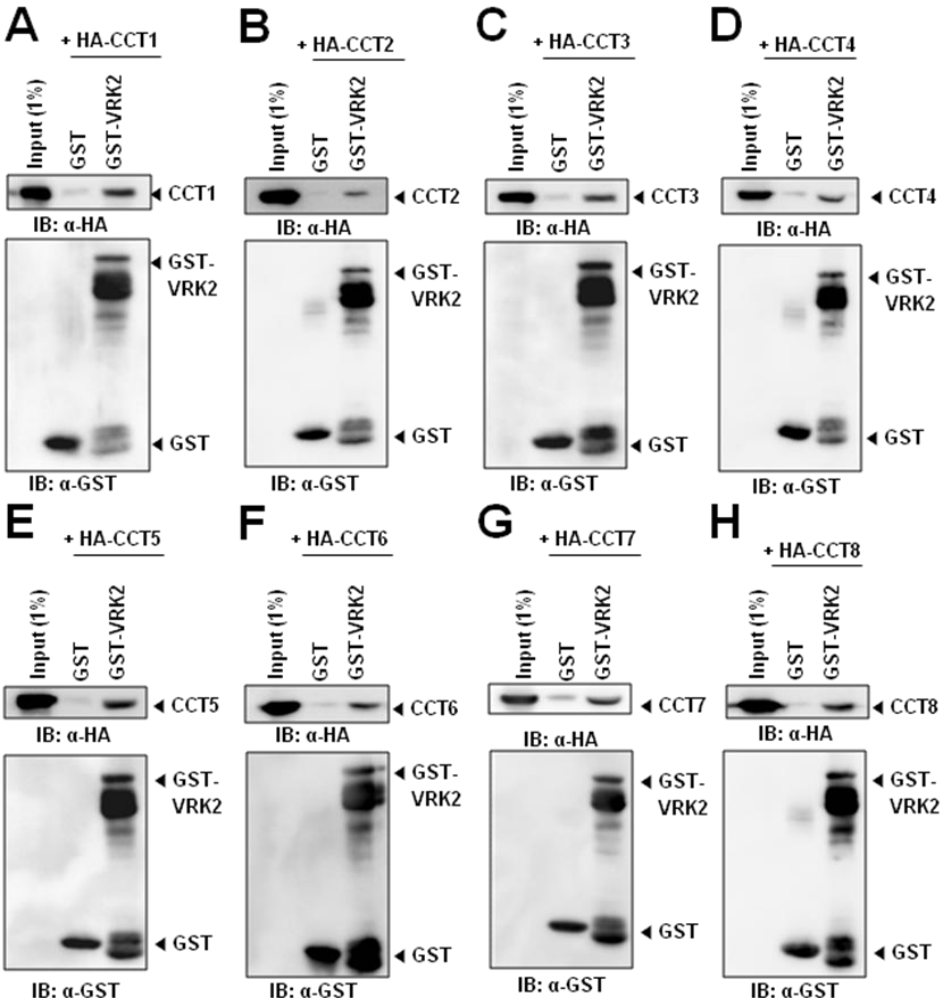

FIG S1 VRK2 could directly or indirectly with all subunit of CCT because all 8 subunits of chaperonin assemble into one complex. (A-H) GST-pull down assay using HA-CCTs from overexpressed cells and purified GST-VRK2. Eight encoded HA-CCTs were pulled down by GST-VRK2.

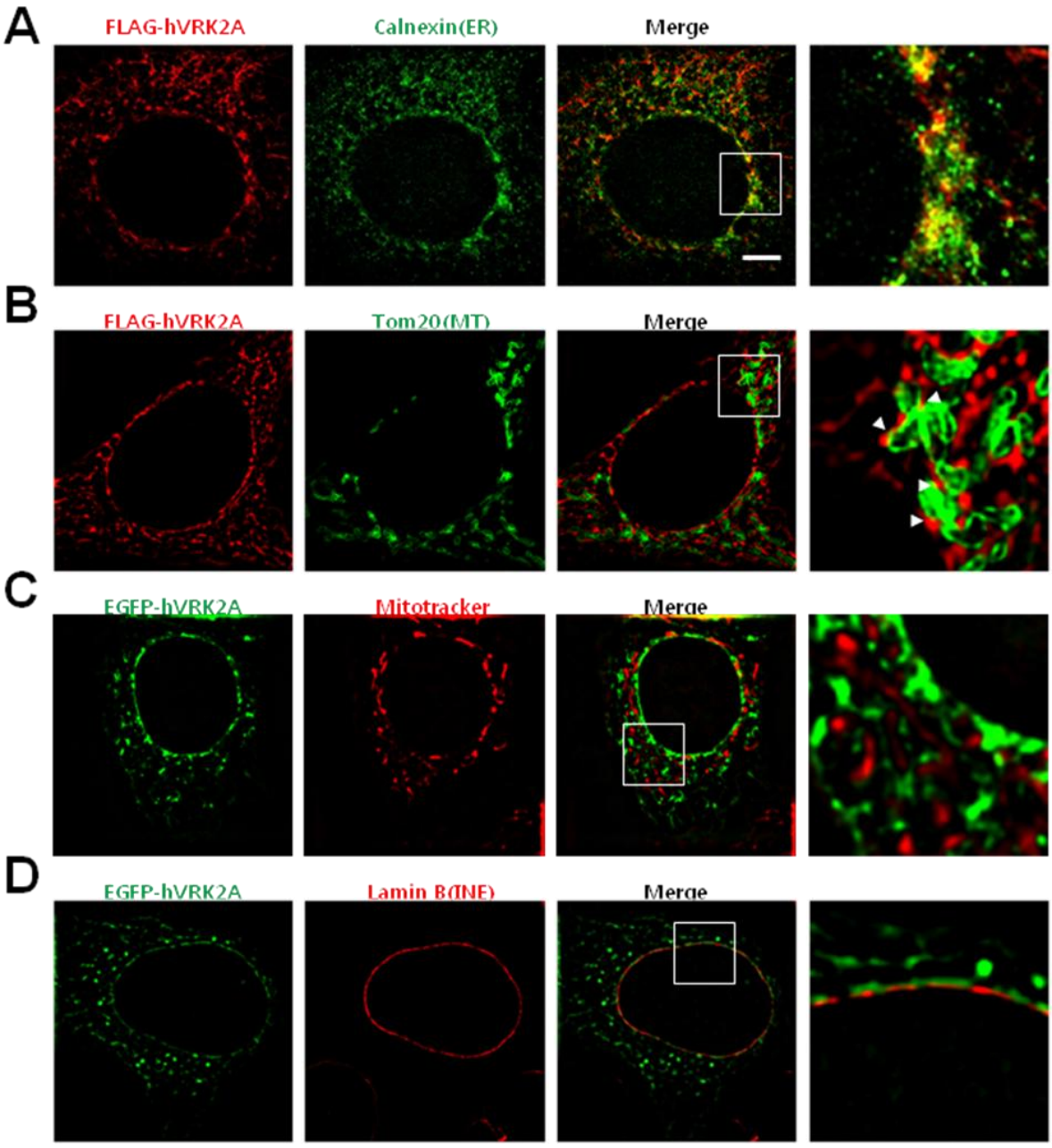

FIG S2 VRK2 mainly localized in endoplasmic reticulum (ER) membrane. (A-C) Cells transfected with pFlag-CMV2-VRK2A or EGFP-VRK2A were visualized, and then ER, mitochondria and inner nuclear envelop were detected using anti-Calnexin (ER marker, A), anti-Tom20 (outer mitochondrial membrane marker, B), Mitotracker (mitochondria marker, C) and anti-Lamin B (inner nuclear envelop, D) antibody, respectively. We used the super-resolution structured illumination microscopy (SIM; Nikon N-SIM). VRK2A colocalized the ER membrane due to its C-terminal transmembrane domain. VRK2A also slightly detected outer mitochondrial membrane, however, we observed different localization pattern between VRK2A and mitochondria. Notably, VRK2 expressed along with outer nuclear membrane known to linked ER membrane, but not inner nuclear envelop. Endopasmic reticulum (ER), Mitochondria (MT), Inner nuclear envelope (INE). Arrow head represents overlapped position of VRK2 and outer mitochondrial membrane. The scale bar represents 5 μm.

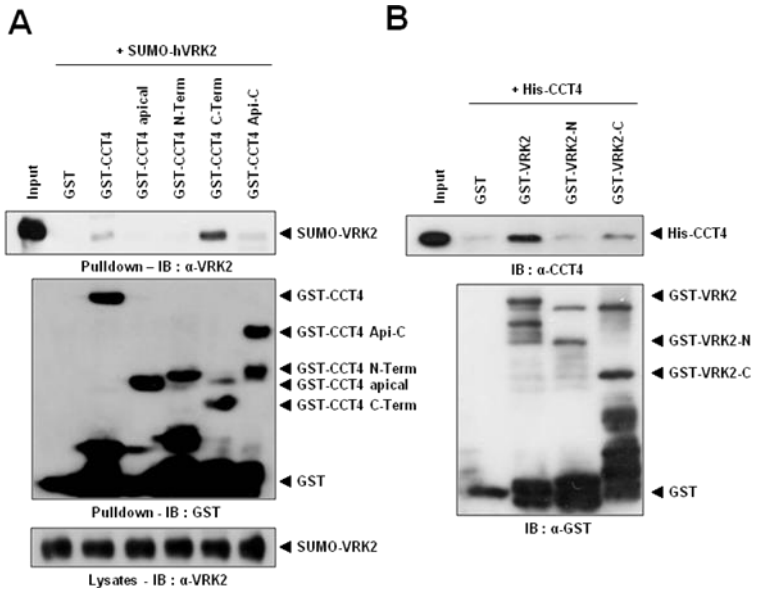

FIG S3 Mapping of the domain for interaction between VRK2 and CCT4. (A) GST-pull down analysis of SUMO-VRK2 and GST-CCT4 fragments derived from *E.coli.* cultures. VRK2 interacts with equatorial domain of CCT4. (B) GST-pull down analysis of His-CCT4 and GST-VRK2 fragments derived from *E.coli*. cultures. CCT4 bounds to VRK2 C-terminal domain which is considered as flexible regions.

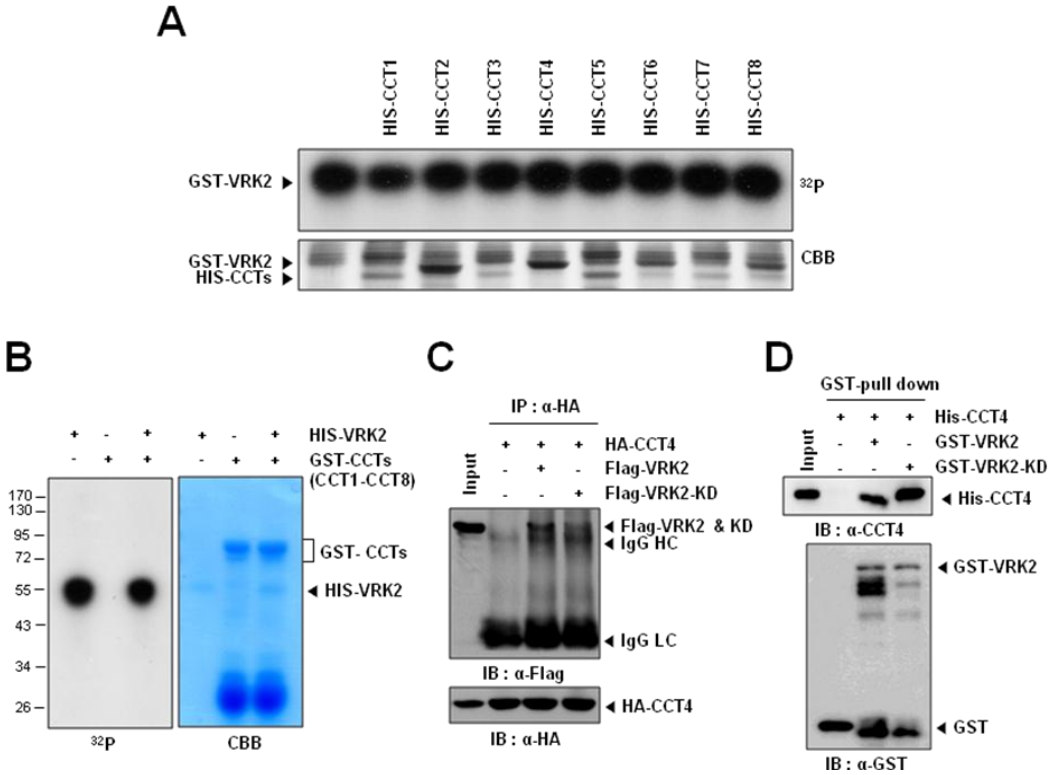

FIG S4 None of Chaperonin subunits was phosphorylated by VRK2. (A) *in vitro* kinase assay with GST-VRK2 and His-CCTx (x = subunit 1-8) derived from *E.coli*. transformed by each vectors. (B) *In vitro* kinase assay with His-VRK2 and GST-tagging eight CCT subunits. (C, D) The interaction of VRK2 with CCT4 does not affect its kinase activity detected by immunoprecipitation assay (C) and GST-pull down (D).

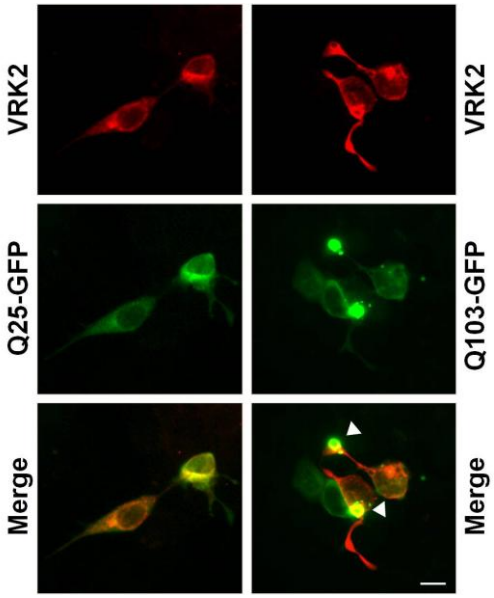

FIG S5 VRK2 has no effect on HttQ25-GFP. Flag-VRK2 with HttQ103-GFP or HttQ25-GFP were co-expressed in HEK293T cells, and then fluorescence microscopic images were analyzed to detect polyQ aggregates. The arrow means the polyQ aggregates. The scale bar represents 20 μm.

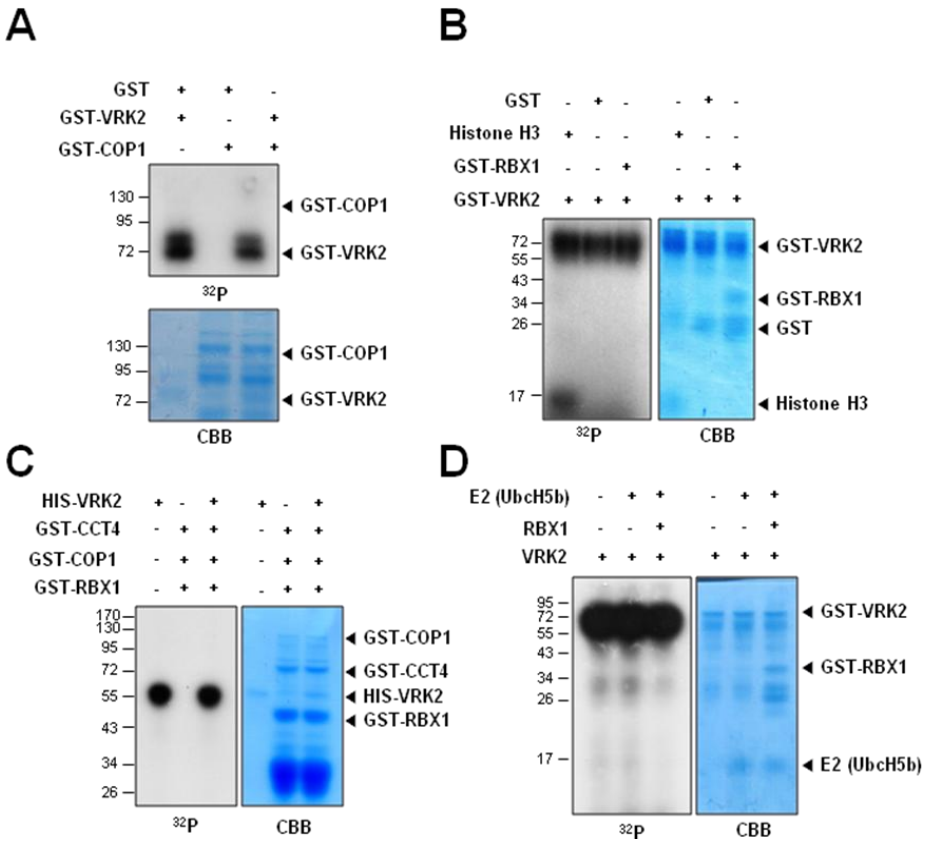

FIG S6 COP1, RBX1 and E2 enzyme were not a substrate of VRK2. (A-B) *In vitro* kinase assay of VRK2 with COP1 (A) or RBX1 (B). (C) *In vitro* kinase assay of VRK2 with COP1, CCT4 and RBX1. (D) *In vitro* kinase assay of VRK2 with RBX1 and E2 enzyme. There is only VRK2 autophosphorylation band. Histone H3 was used to positive control of substrate phosphorylation.