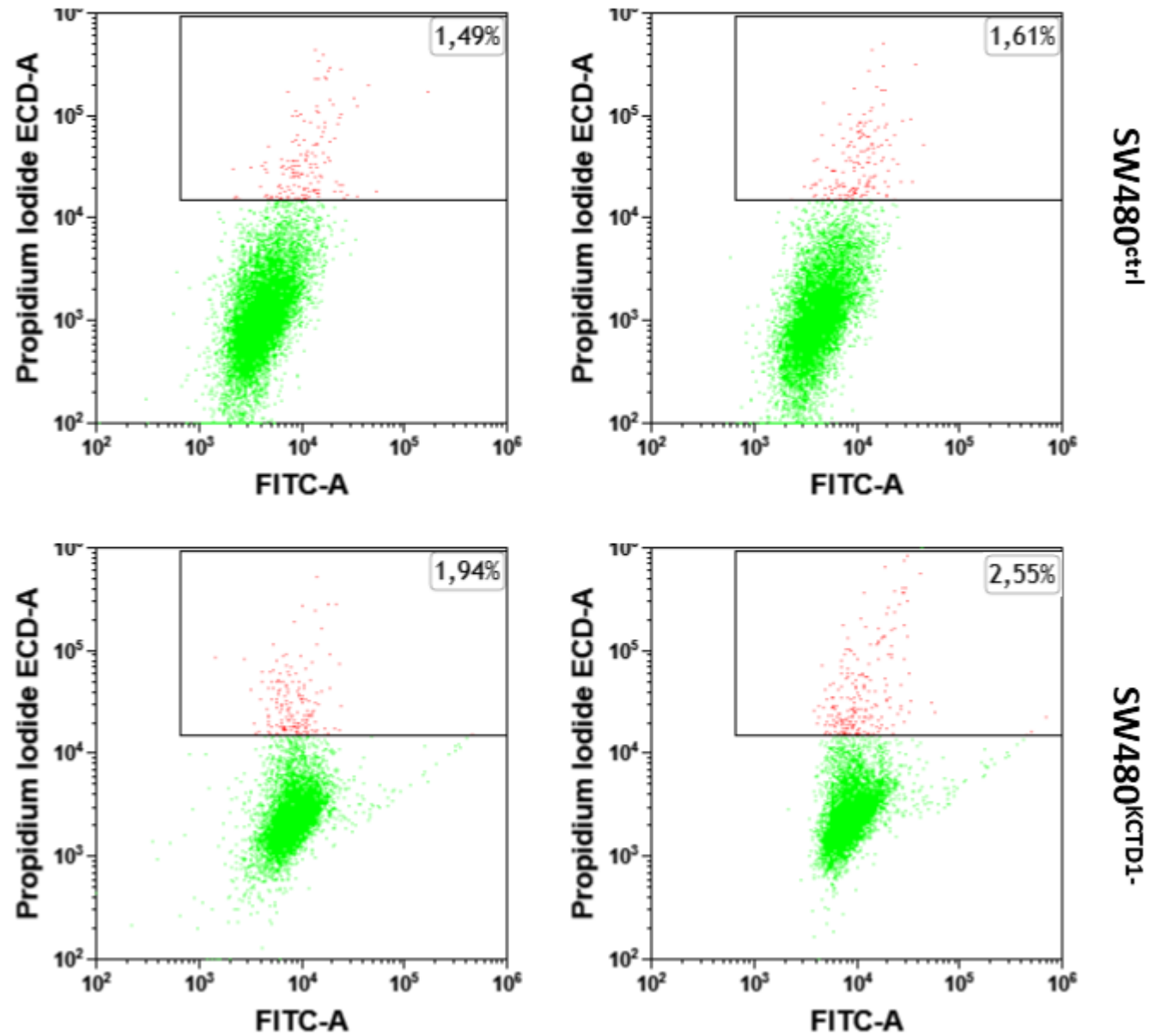
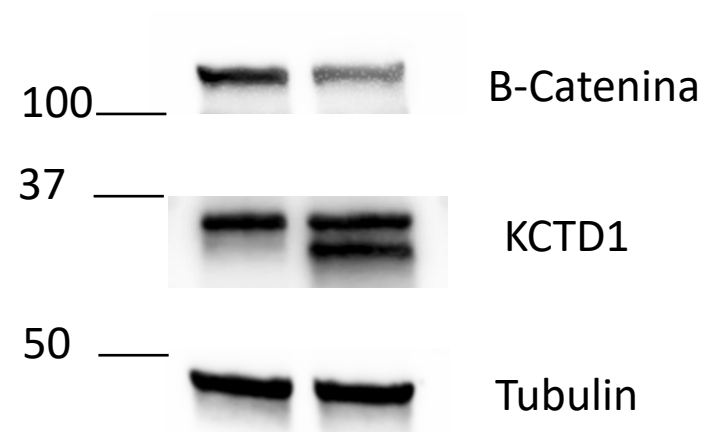
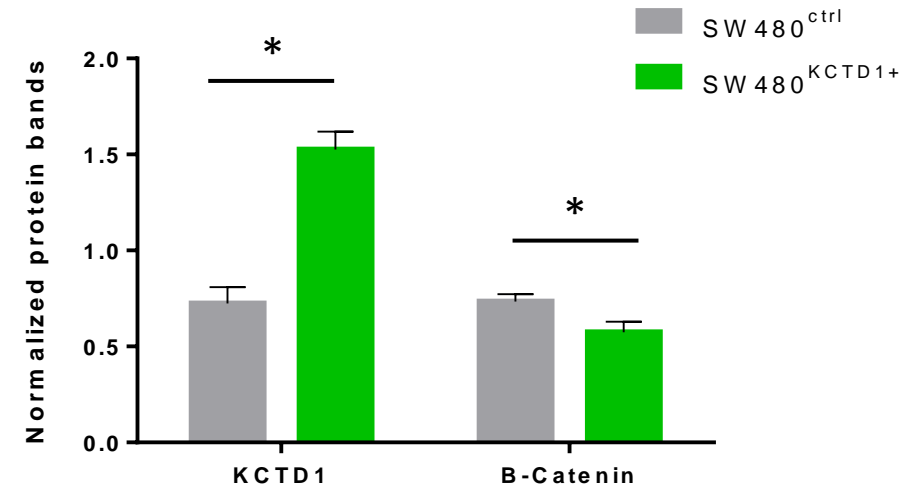


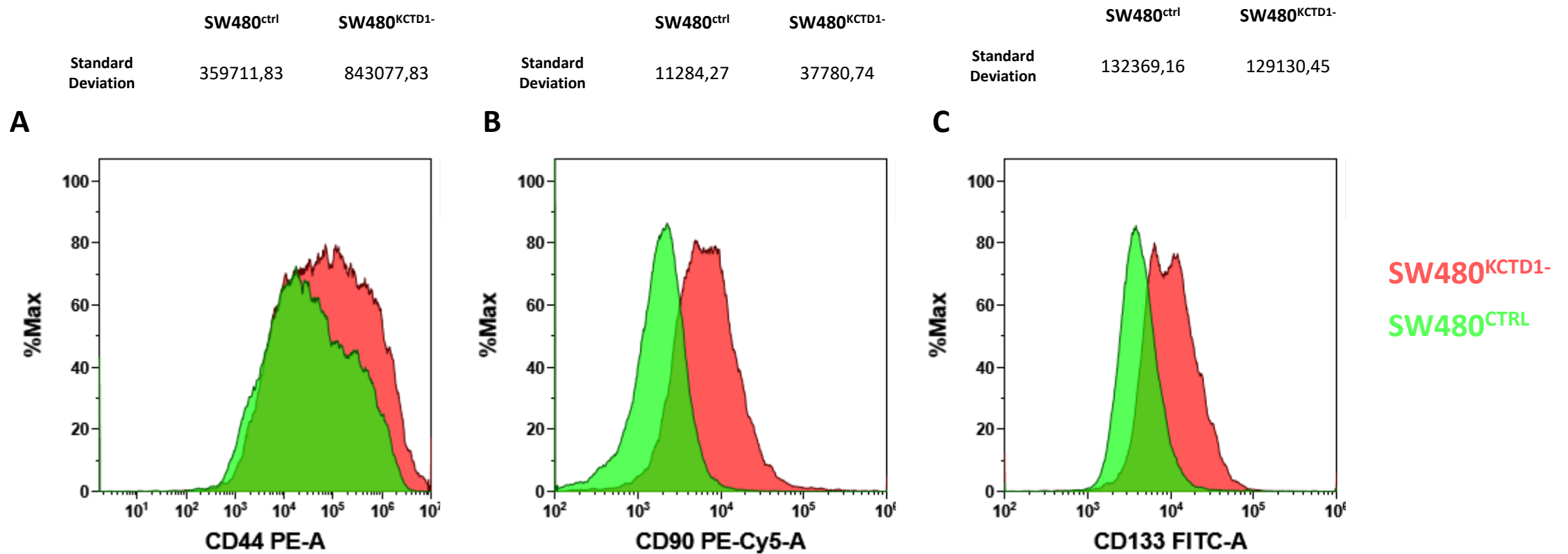
REPLICATES



Supplementary Figure S1. Replicates of FACS analysis of the Figure 1B. Propidium Iodide VS FITC Dot plots analysis of SW480^{ctrl} and SW480^{KCTD1-} cells. Numbers report the Propidium Iodide positive cells.

A**B**

Supplementary Figure S2. Western blot analyses of the indicated protein (A) and protein band quantifications (B) were performed on SW480^{ctrl} (grey bars) and SW480^{KCTD1+} (green bars) after 24 h of over-expression. Numbers represent molecular weight of proteins expressed in kDa. ns = not significant; * = p-value < 0.05.



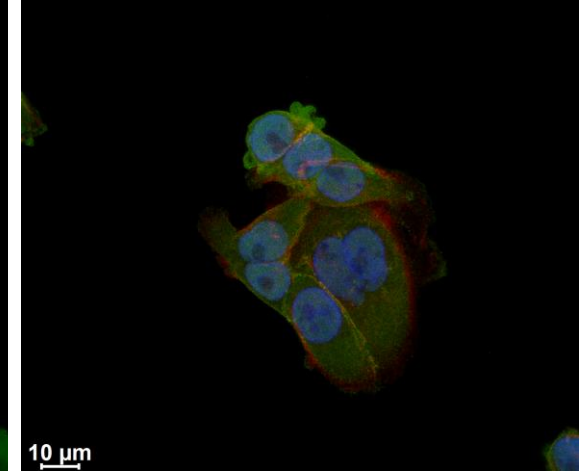
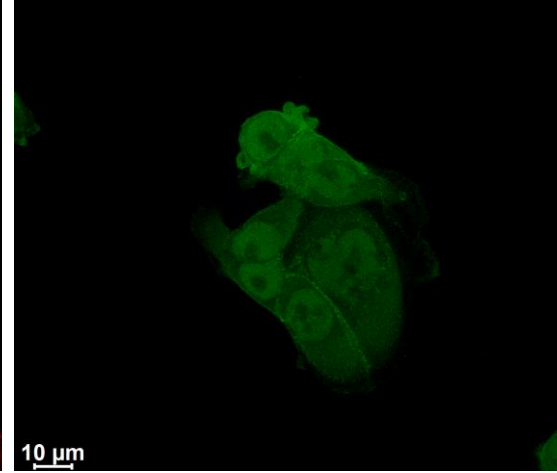
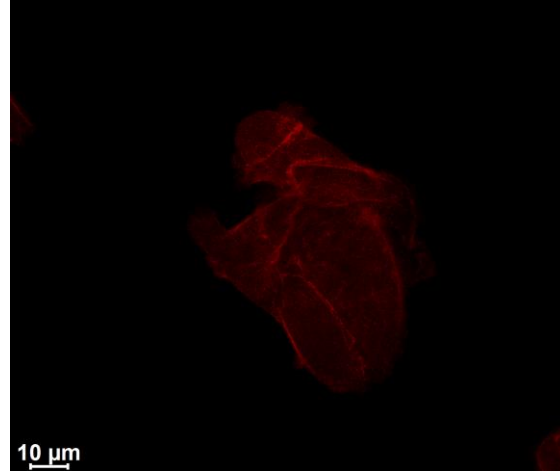
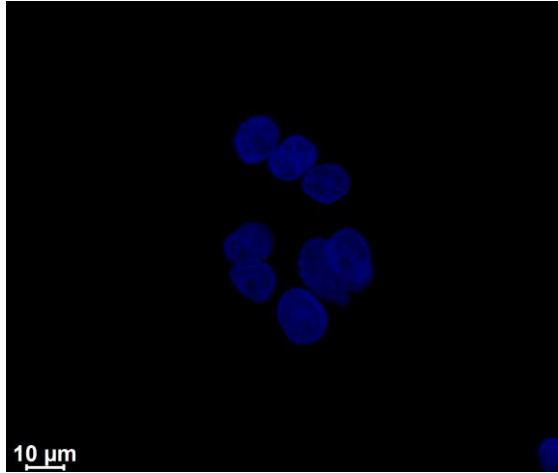
Supplementary Figure S3. Cytofluorimetric analysis of CD44 (A), CD90 (B) and CD133 (C) membrane expression in SW480^{KCTD1-} and SW480^{ctrl} cells

I) DAPI

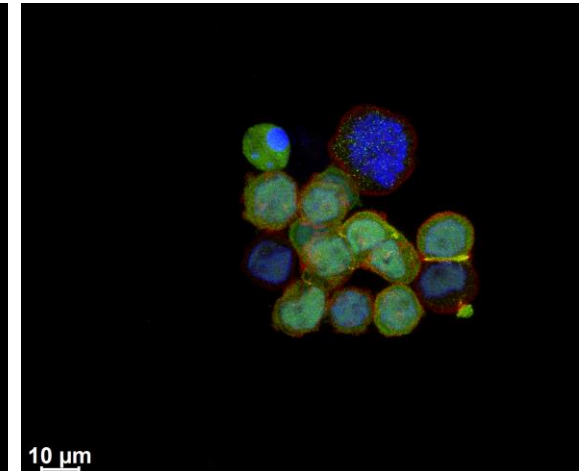
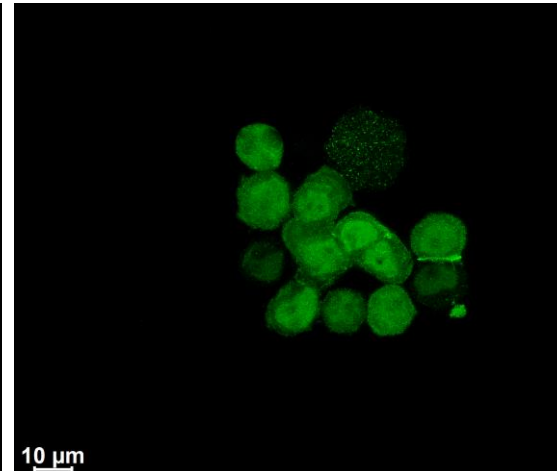
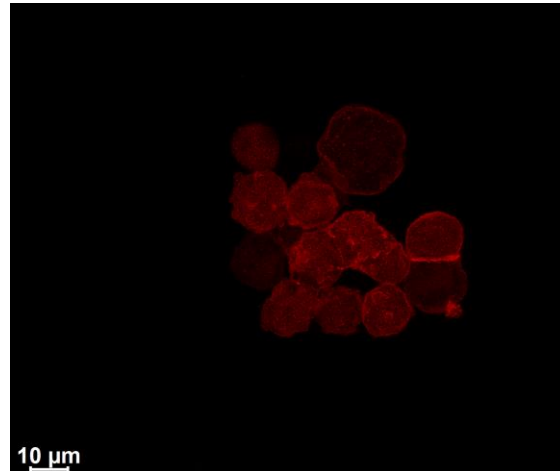
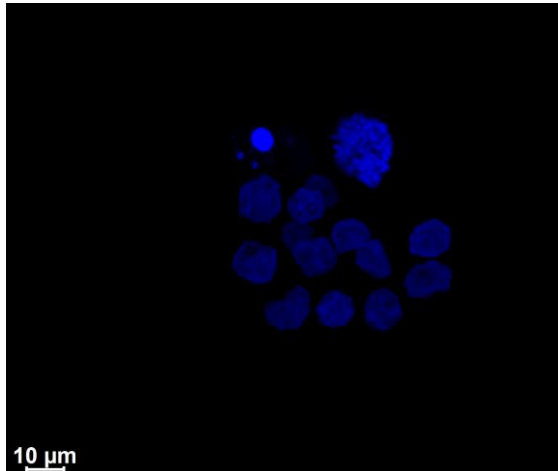
II) Phalloidin

III) β -Catenin

IV) Merge

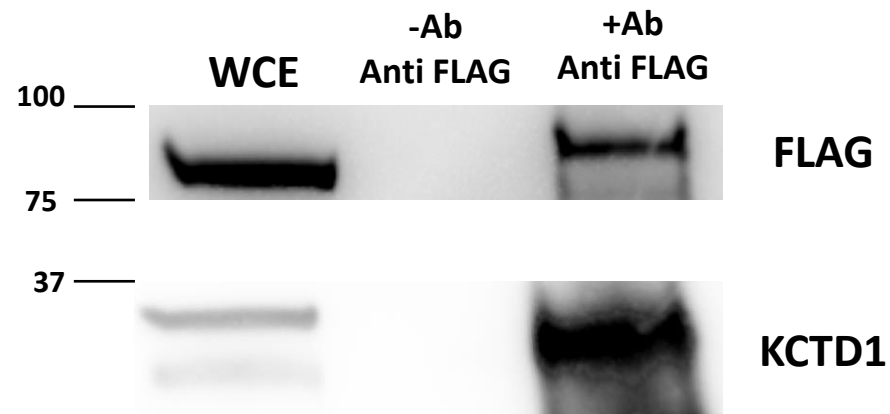
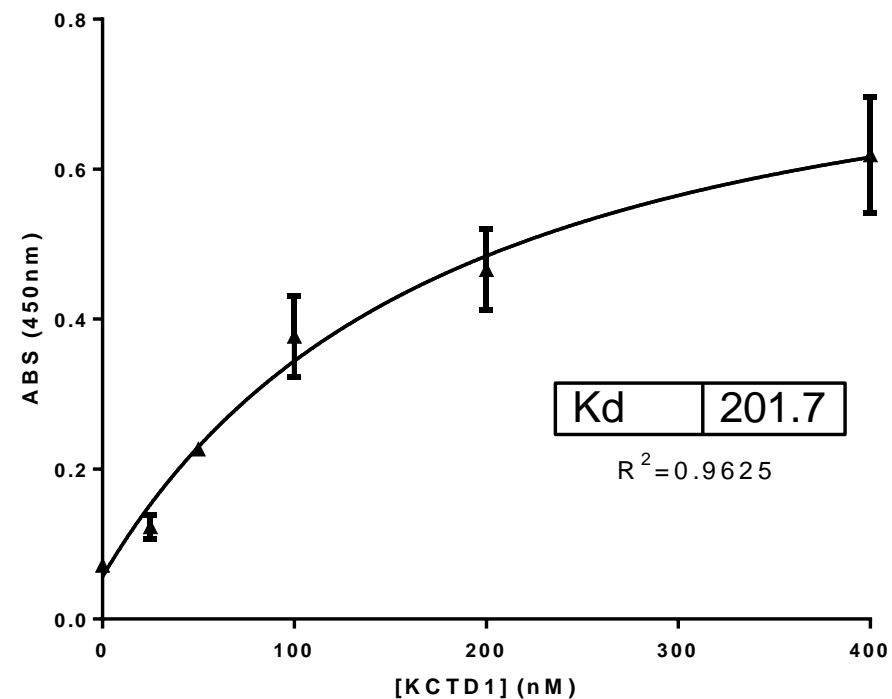


SW480^{ctrl}

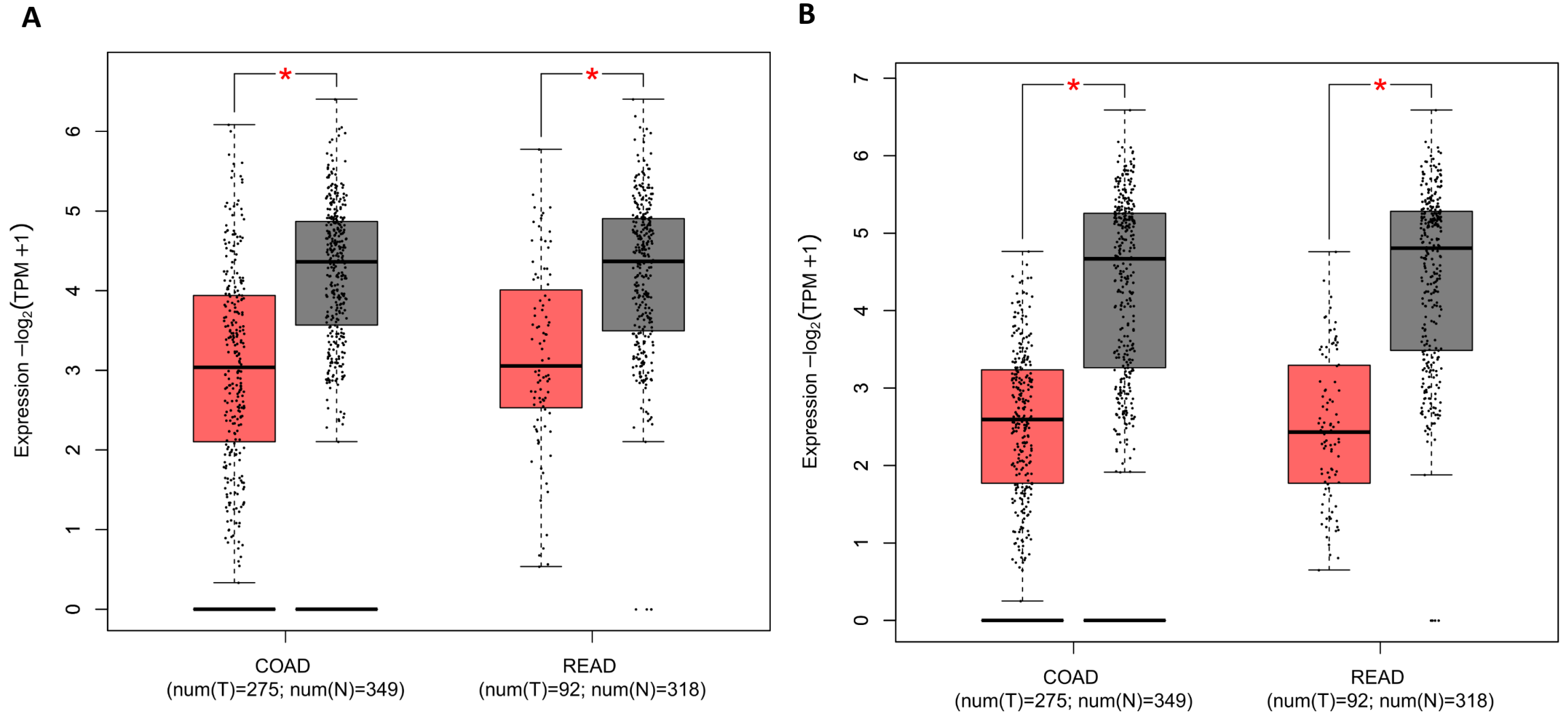


SW480^{KCTD1-}

Supplementary Figure S4. Immunofluorescence analyses of SW480ctrl (upper panels) and SW480KCTD1- (lower panels). I) Nuclei staining with DAPI (blue). II) Phalloidin-Dye light 554. III) β -catenin staining with Alexa588-conjugated secondary antibody. IV) Merge of DAPI, Dye light 554 and Alexa 488. Magnification 63 \times . Scale bars 10 μ m.

A**B**

Supplementary Figure S5. A) SW480 over-expressing FLAG-IKK- β and KCTD1 cell extracts were incubated with or without anti-FLAG antibody, precipitated by Protein A/G beads, and detected by Western blots using the reported antibody. (+ Ab Anti FLAG) = elution step in the presence of Anti Flag antibody. (- Ab Anti FLAG) = elution without antibody. WCE whole cell extract. The numbers represent the molecular weight of the protein marker expressed in kDa. B) Binding of recombinant KCTD1 with IKK-B kinase by ELISA. Different KCTD1 concentration are tested (from 400nM to 25 nM). K_d was calculated using One-site Total equation.



Supplementary Figure S6. KCTD12 (A) and KCTD15 (B) mRNA expression in Colon Adenocarcinoma (COAD) and Rectum Adenocarcinoma (READ) and normal tissues (GEPIA). Box plots show median mRNA expression of KCTDs in tumor tissues (red plots) and the corresponding normal tissues (gray plots). Axis units are $\text{Log}_2(\text{TPM} + 1)$. * red = p-value < 0.01. T = tumor tissues. N = normal tissues.

**Supplementary Figure S7:
Uncropped Western Blot Images**

Figure 1A's uncropped Western blot image

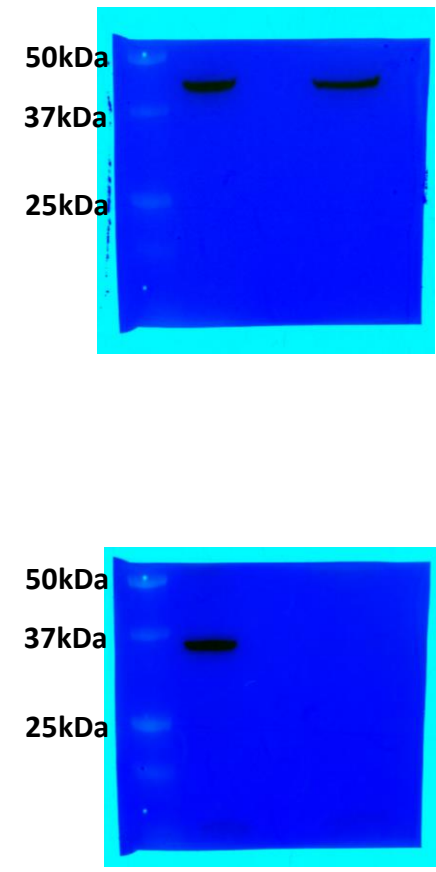
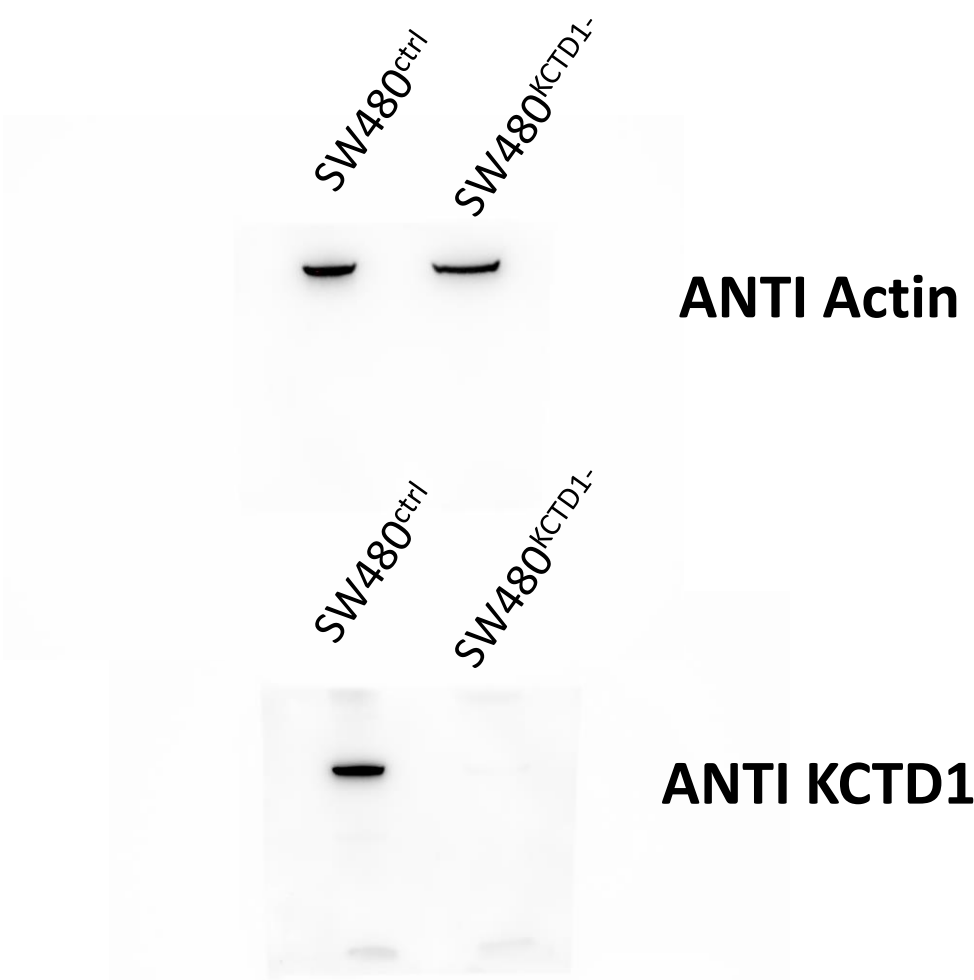
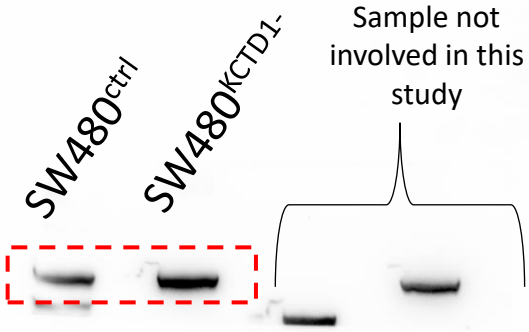
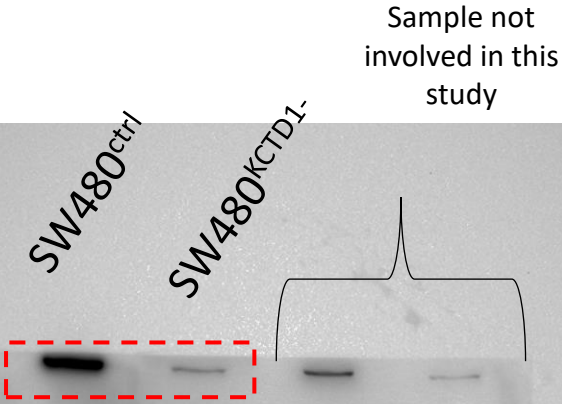
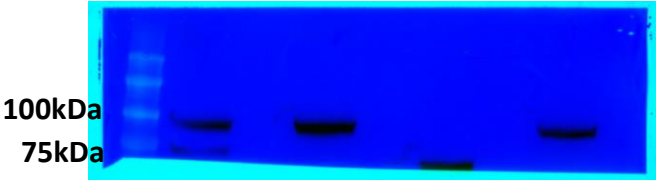


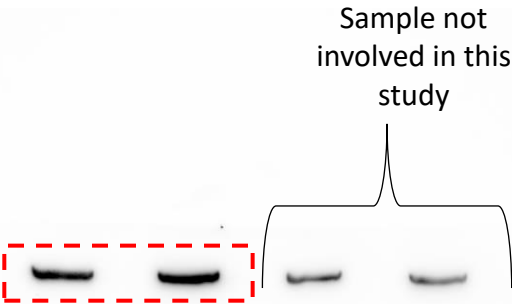
Figure 1D's uncropped Western blot image



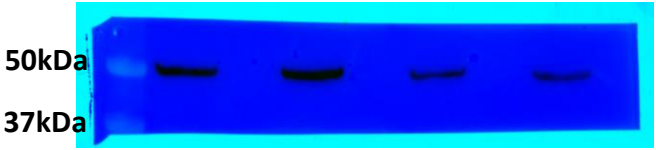
ANTI B-Catenin



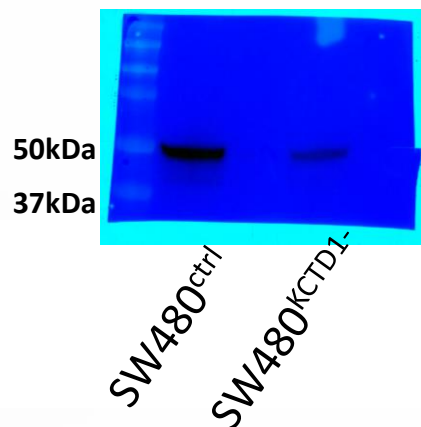
ANTI KCTD1



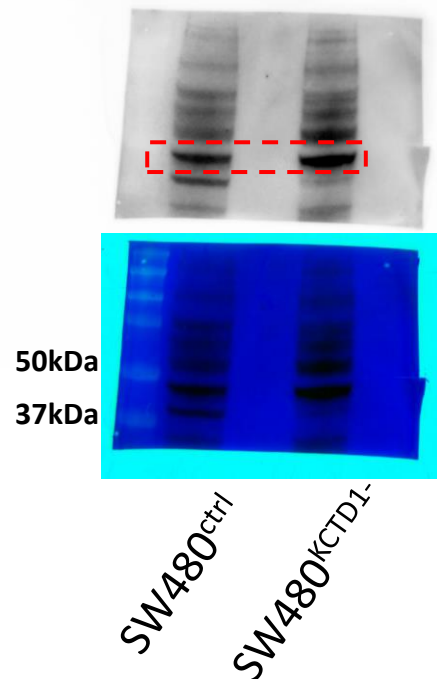
ANTI Tubulin



ANTI p53



ANTI CDK2



ANTI p18

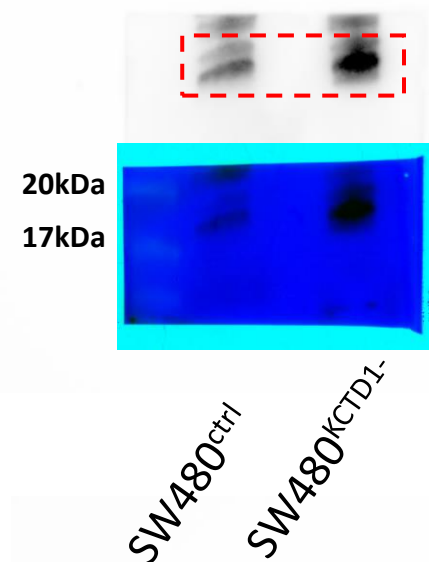
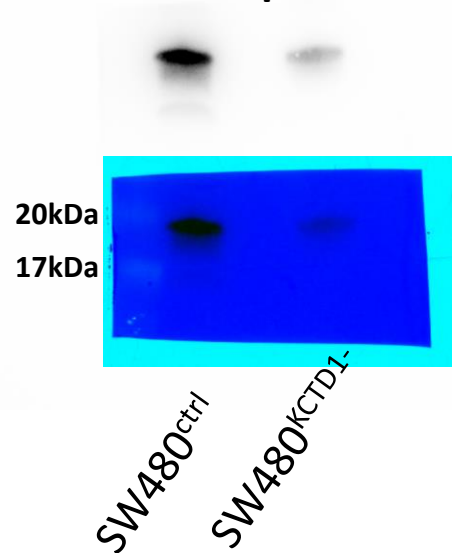
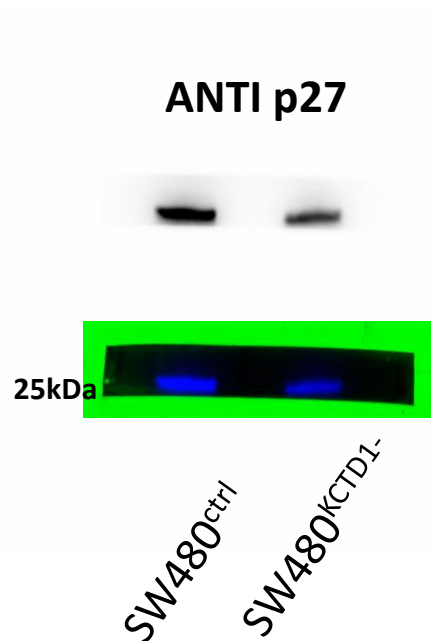


Figure 5C's
uncropped
Western blot
image

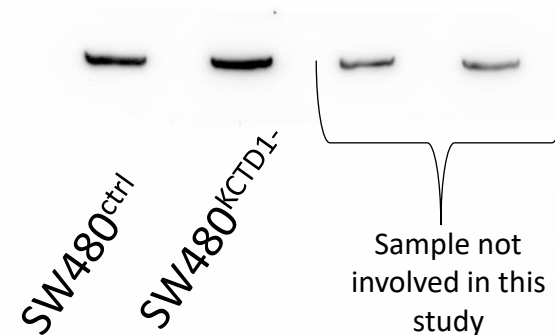
ANTI p21



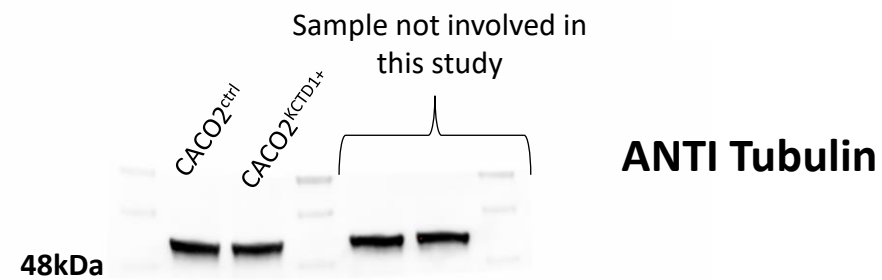
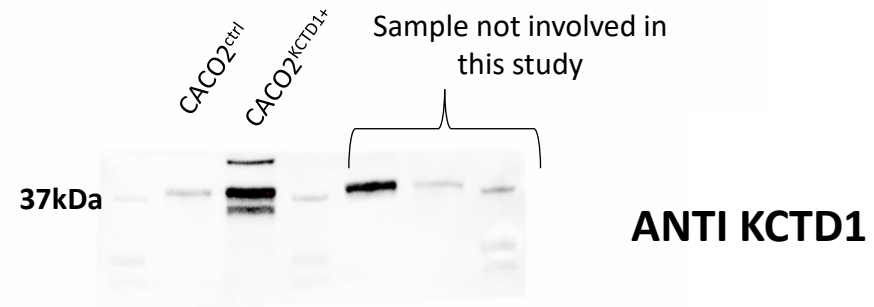
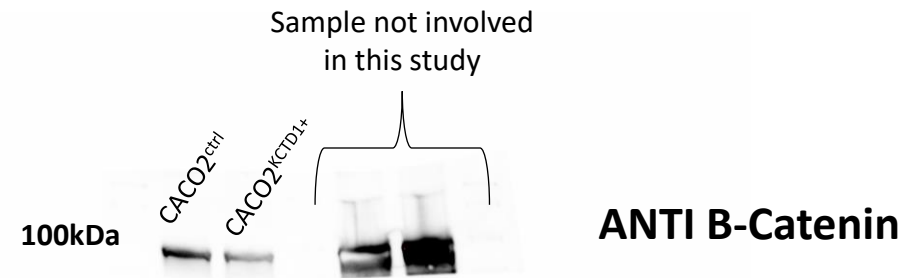
ANTI p27



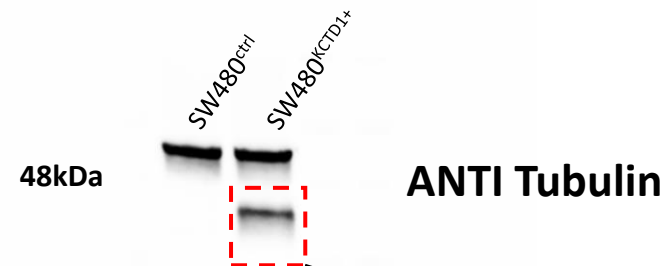
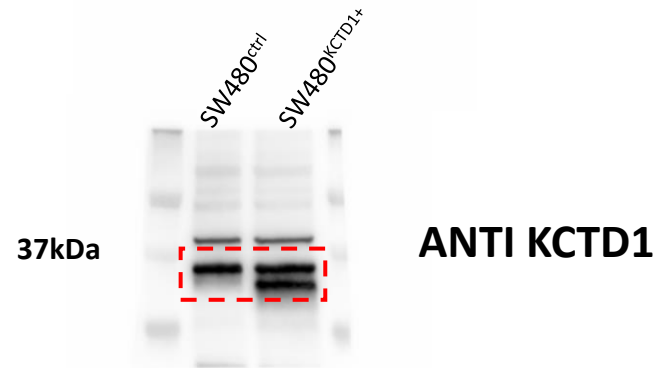
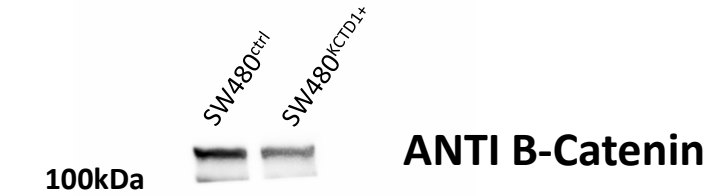
ANTI Tubulin



**Figure 7A's uncropped
Western blot image**



Supplementary Figure S5A's uncropped Western blot image



Anti FLAG antibody (to detect
the over-expression of FLAG-
KCTD1 protein)