

Supplementary Materials: The Ginsenoside Compound K Suppresses Stem-Cell-like Properties and Colorectal Cancer Metastasis by Targeting Hypoxia-Driven Nur77-Akt Feed-Forward Signaling

Minda Zhang ^{1,†}, Zeyu Shi ^{2,†}, Shuaishuai Zhang ¹, Xudan Li ¹, Sally Kit Yan To ², Yijia Peng ¹, Jie Liu ¹, Siming Chen ¹, Hongyu Hu ³, Alice Sze Tsai Wong ^{2,*} and Jin-Zhang Zeng ^{1,*}

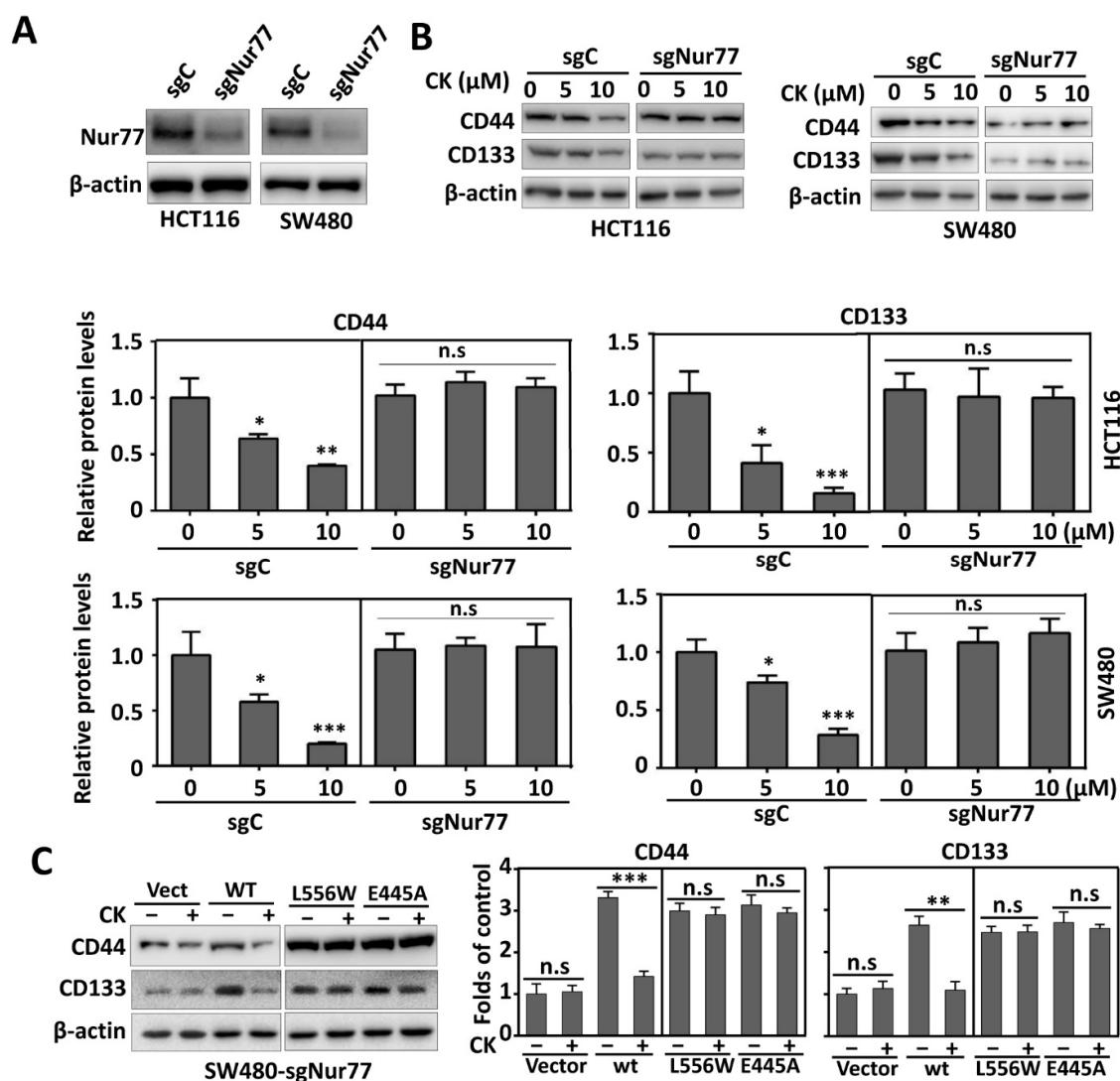


Figure S1. The supplementary figures for Figure 2. (A) Nur77 was stably knocked out in HCT116 and SW480 cells through CRISPR-Cas9. The efficiency of Nur77 knockout was tested by Western blotting. (B) Nur77/WT and Nur77/KO cells were treated with vehicle, 5 μ M or 10 μ M of CK under hypoxic condition for 24 h. The protein levels of CD44 and CD133 were examined by Western blotting. (C) Nur77/KO cells were transfected with indicated Nur77 mutants for 24 h and treated with or without 10 μ M CK for 24 h under hypoxic condition. The protein levels of CD44 and CD133 were examined by Western blotting. * p < 0.05; ** p < 0.01; *** p < 0.001 vs respective control. Molecular weights for proteins were indicated in the full un-cropped annotated Western blot images (Figure S10).

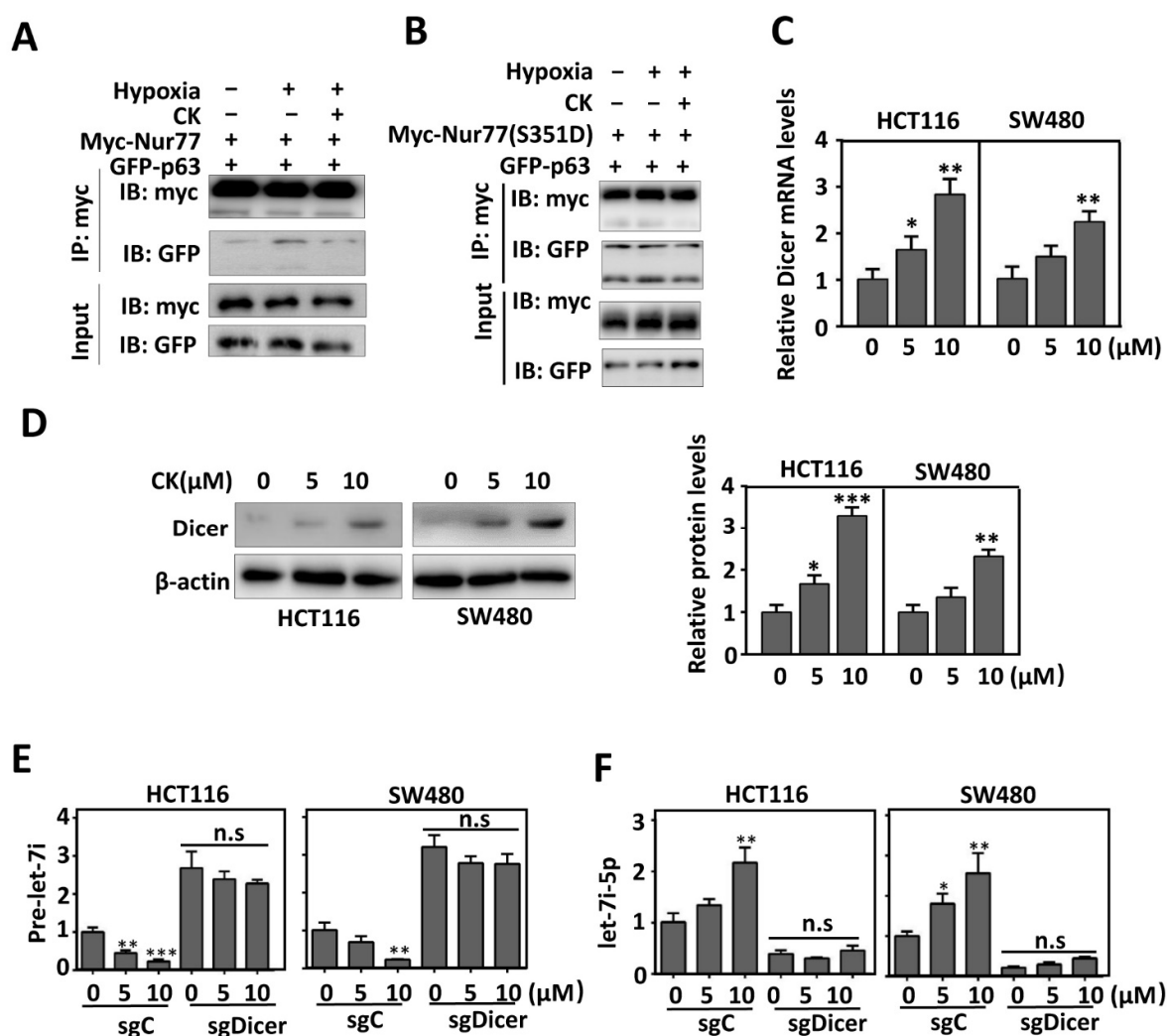


Figure S2. The supplementary figures for Figure 5 and 6. (A) HEK-293T cells were co-transfected with Myc-Nur77 and GFP-p63 for 24 h and treated with or without 10 μM CK under hypoxic condition for 4 h. Co-IP was performed with anti-Myc antibodies and blotted with anti-Myc and anti-GFP antibodies. (B) HEK-293T cells transfected with GFP-p63 and Myc-Nur77-S351D were treated with or without 10 μM CK for 4 h under hypoxia. Co-IP was performed with anti-Myc antibodies and blotted with anti-Myc and anti-GFP antibodies. (C) qPCR analysis of Dicer transcripts in CRC cells treated with vehicle or CK (5 μM or 10 μM) under hypoxic condition for 24 h. (D) The protein levels of Dicer were determined by Western blotting in CRC cells similarly treated as described in C. β-actin was served as an internal control. (E–F) Dicer/WT and Dicer/KO cells were treated with vehicle, 5 μM or 10 μM CK for 24 h under hypoxic condition. Levels of pre-let-7i and let-7i-5p were examined by qRT-PCR. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. respective control. Molecular weights for proteins were indicated in the full un-cropped annotated Western blot images (Figure S11).

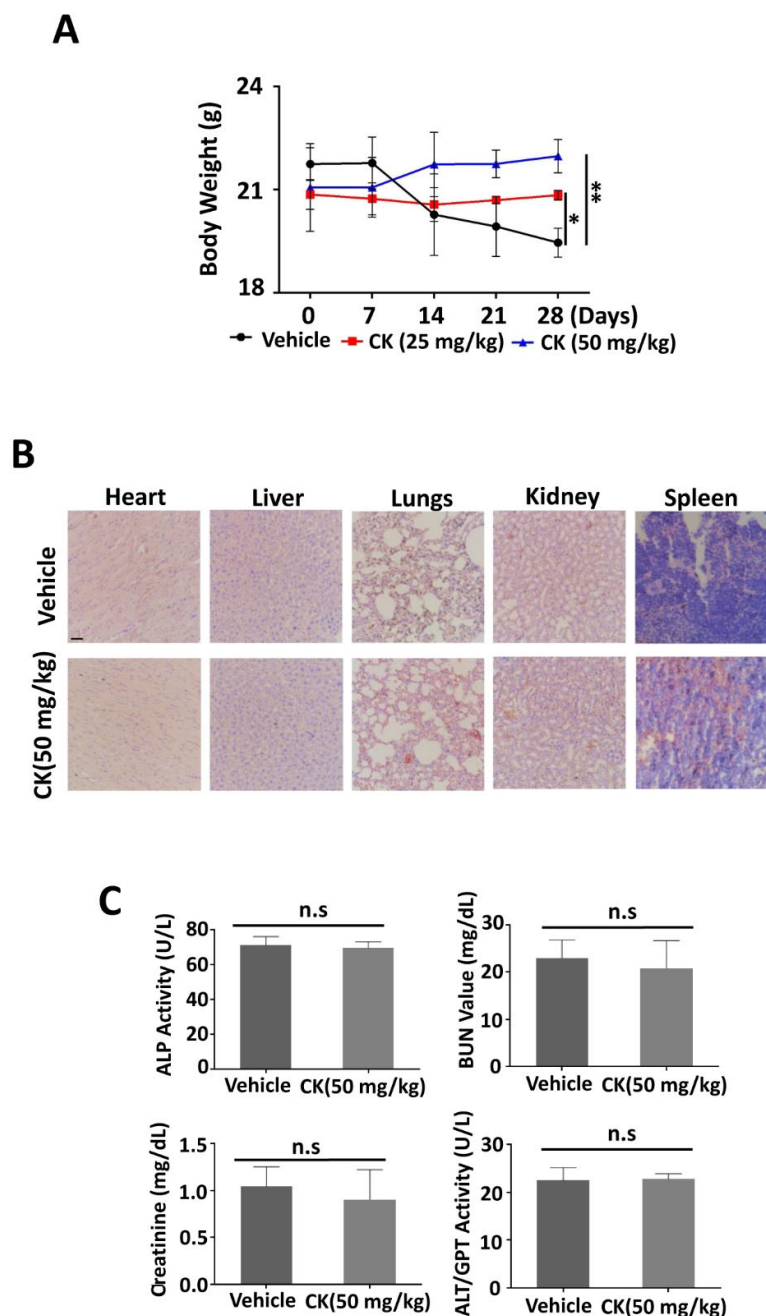


Figure S3. The supplementary figures for Figure 7. (A) The body weight of mice in each group shown. (B) Representative H&E staining images of heart, lungs, liver, spleen and kidney tissues from mice treated with 50 mg/kg CK. Scale bars: 100 μ m in 10 \times . (C) Peripheral blood was collected from heart at sacrifice and alkaline phosphatase (ALP), alanine transaminase (ALT), creatine kinase, blood urea nitrogen (BUN) and creatine were measured and compared to vehicle injected mice. Data are shown as mean \pm SD. * p < 0.05; ** p < 0.01 vs. respective control.

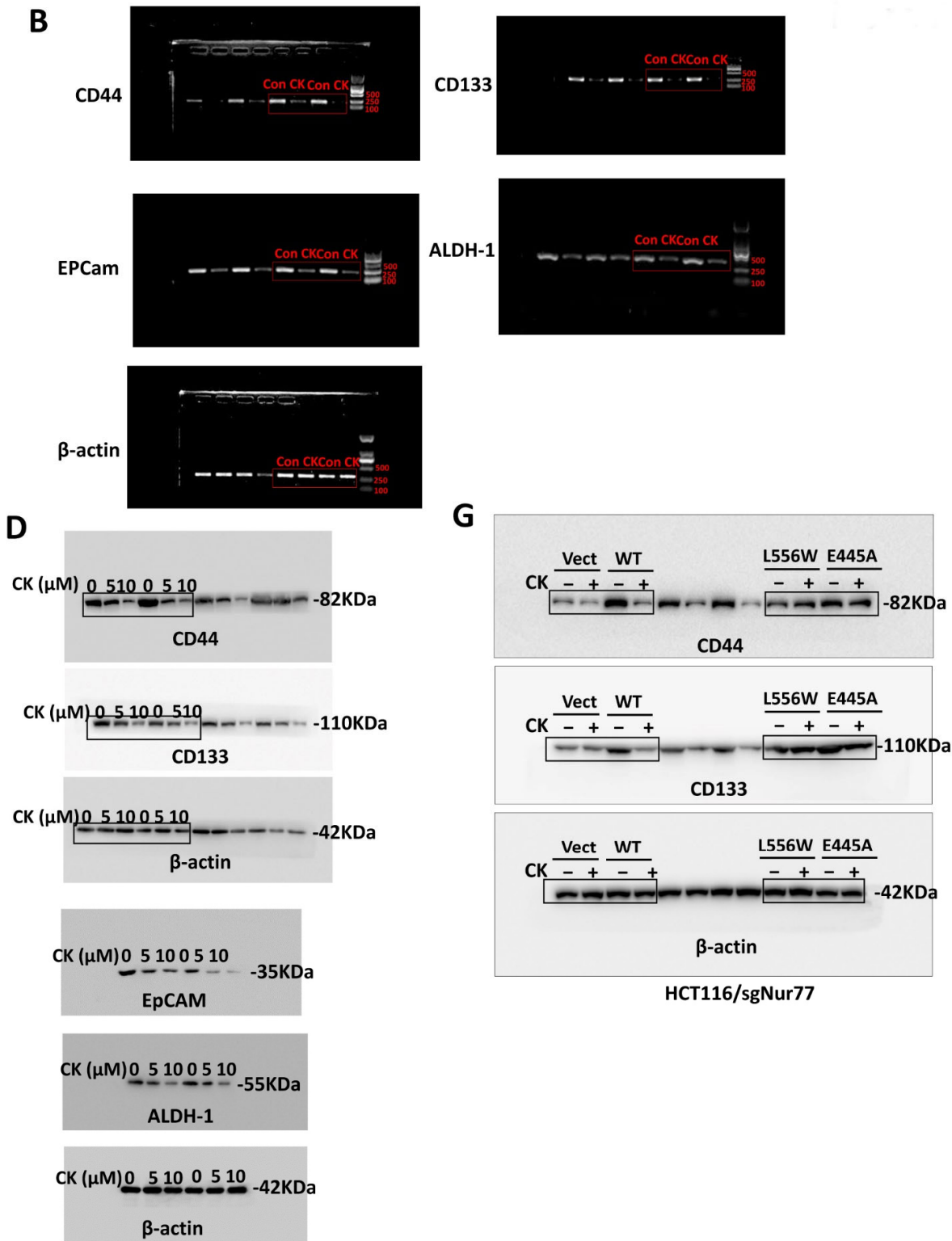


Figure S4. Uncropped Western Blot image for Figure 2.

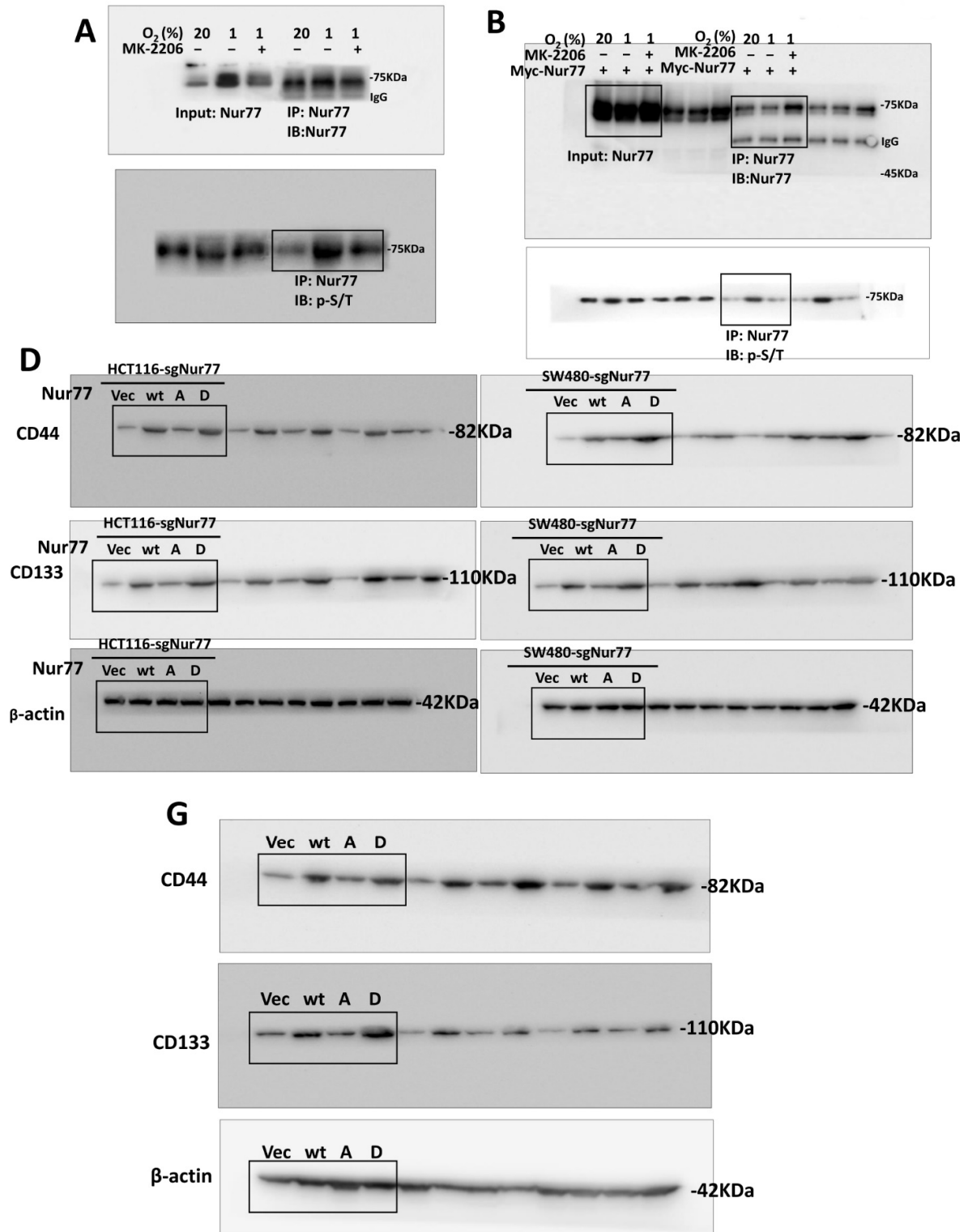


Figure S5. Uncropped Western Blot image for Figure 3.

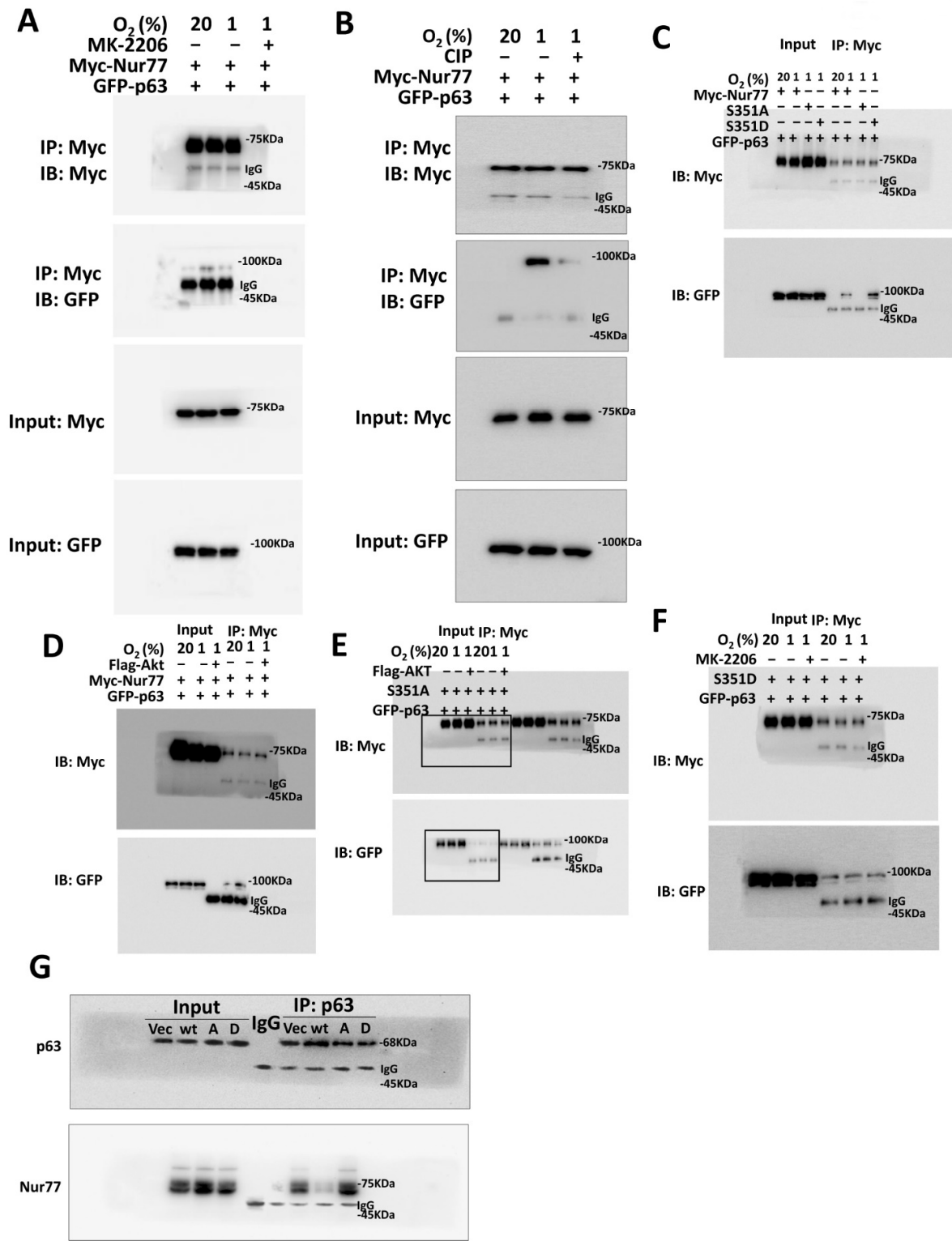
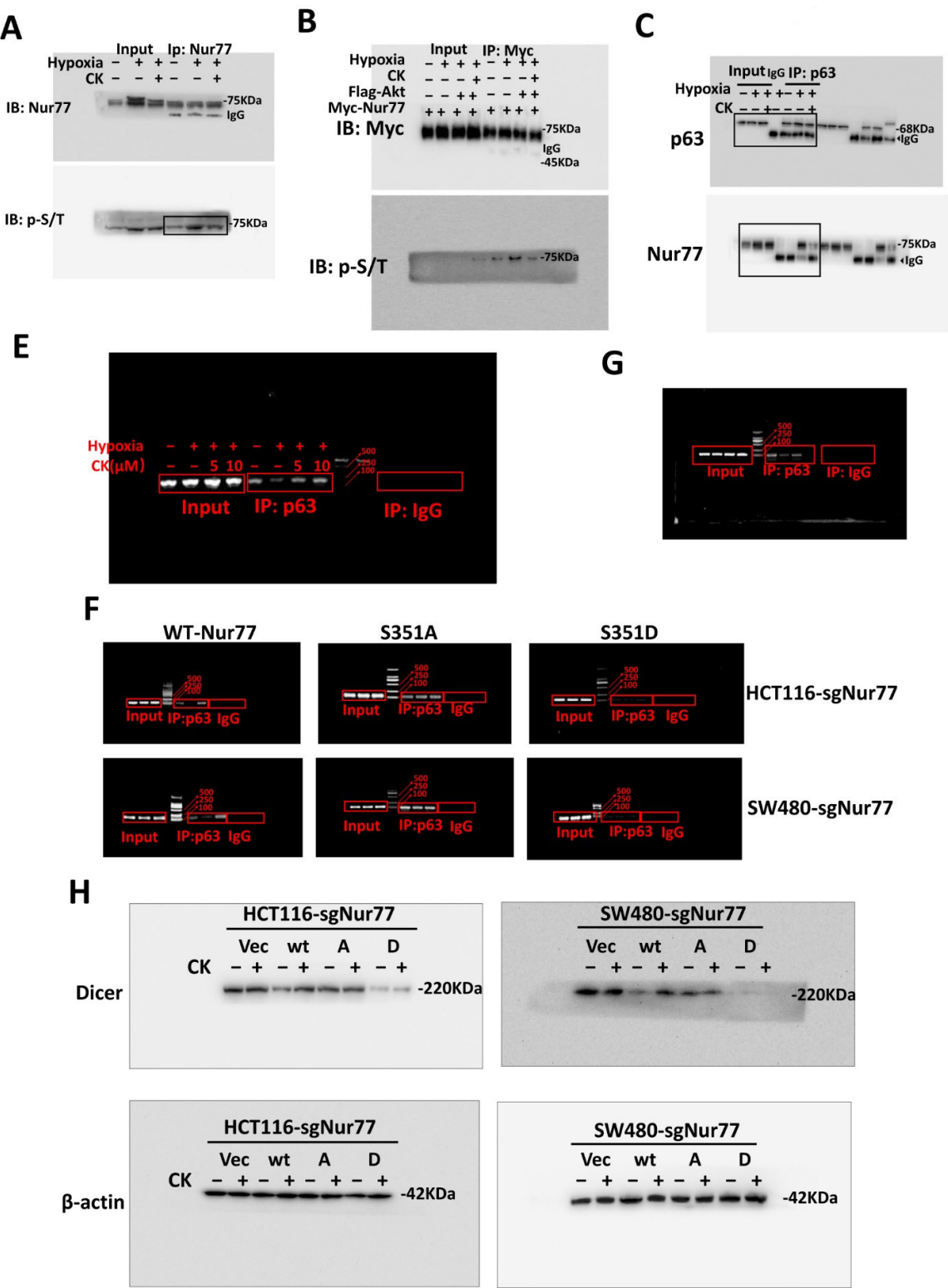


Figure S6. Uncropped Western Blot image for Figure 4.



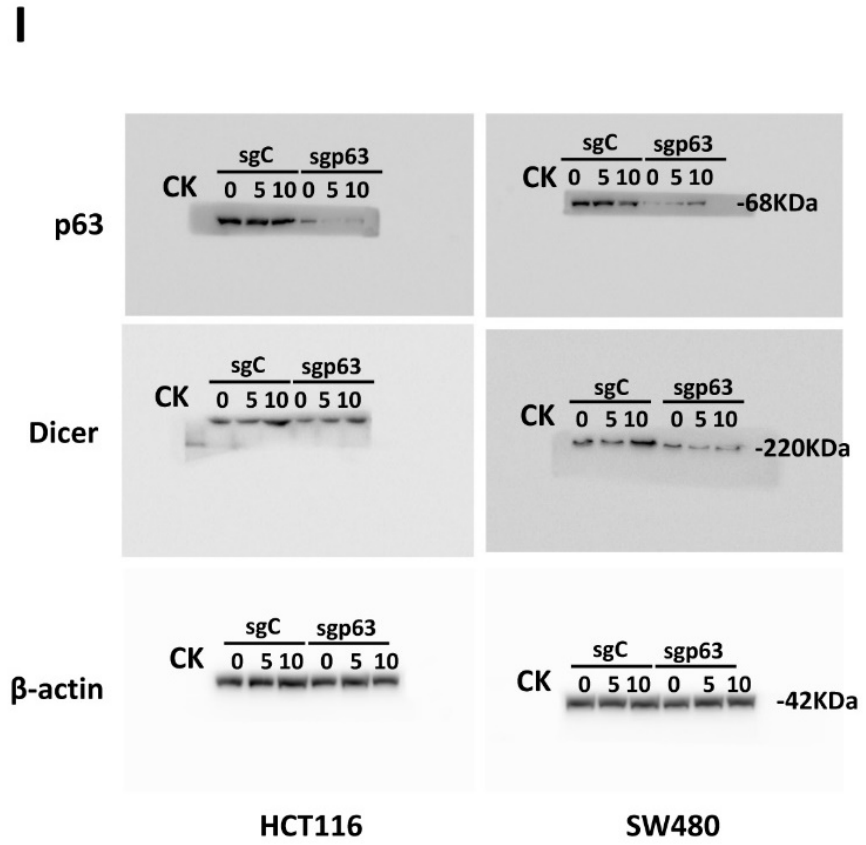
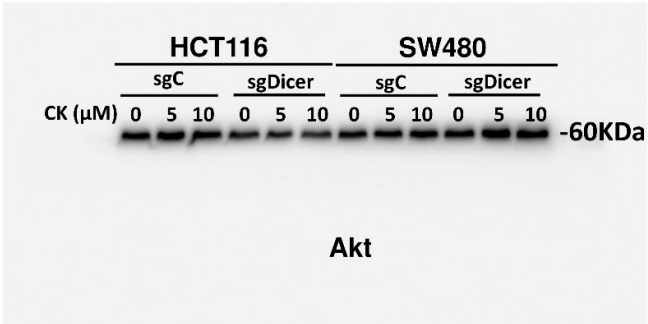
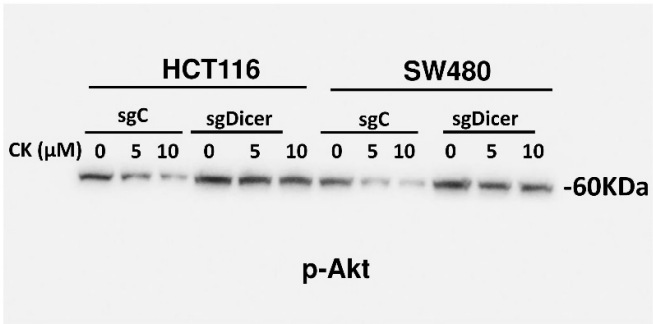
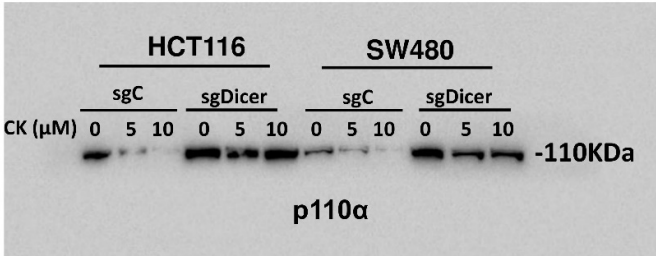


Figure S7. Uncropped Western Blot image for Figure 5.

B



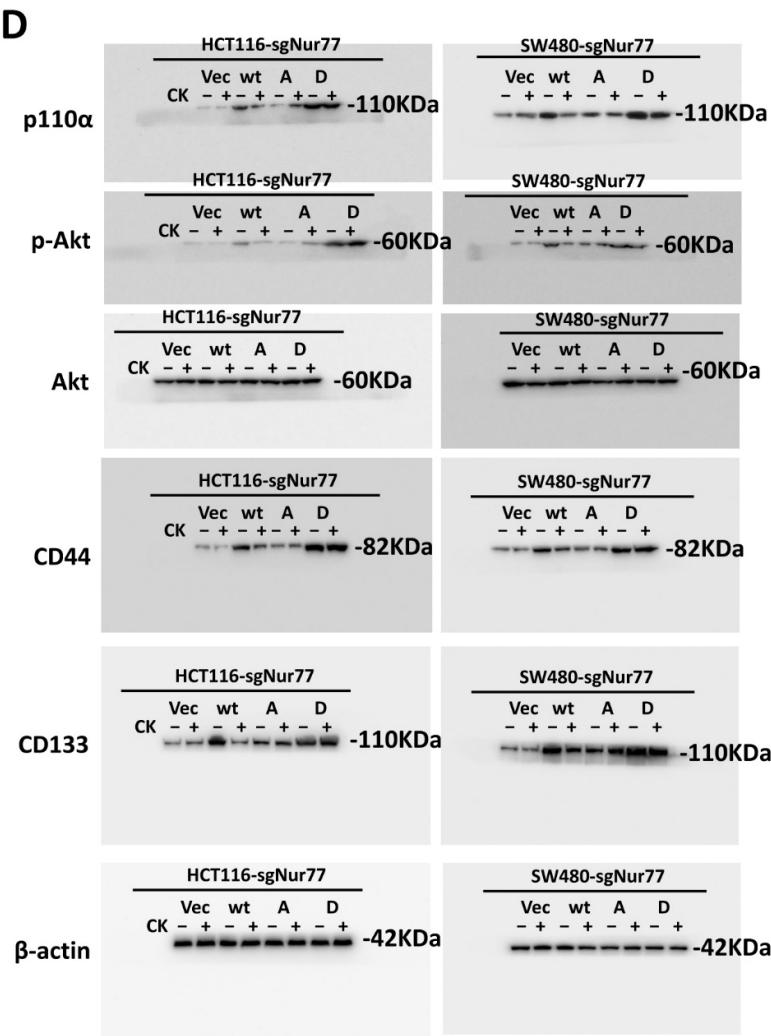


Figure S8. Uncropped Western Blot image for Figure 6.

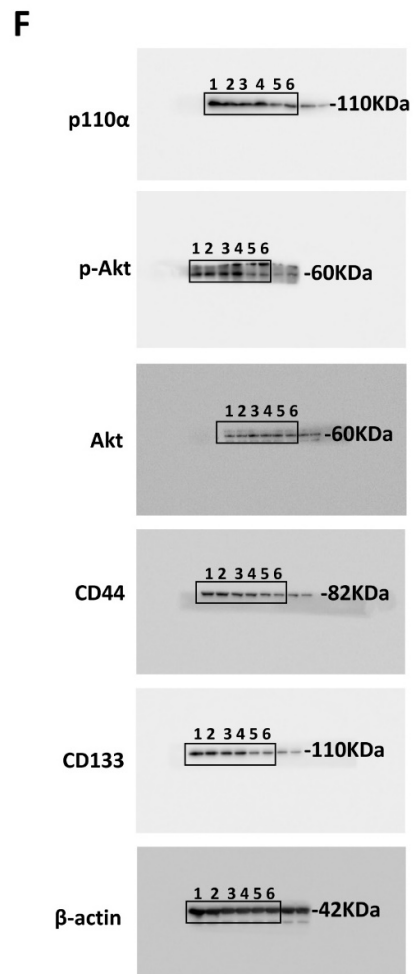


Figure S9. Uncropped Western Blot image for Figure 7.

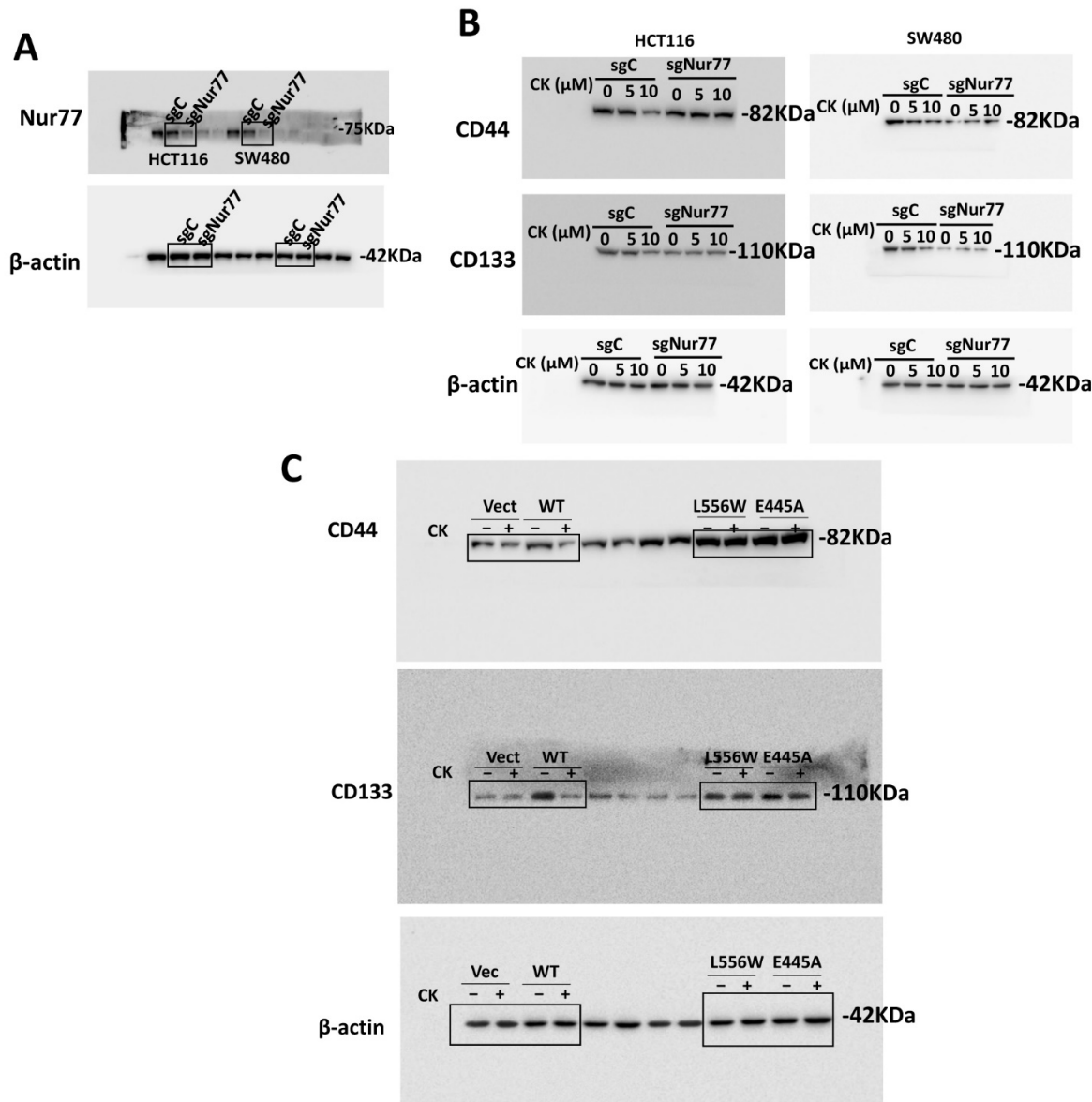


Figure S10. Uncropped Western Blot image for Figure S1.

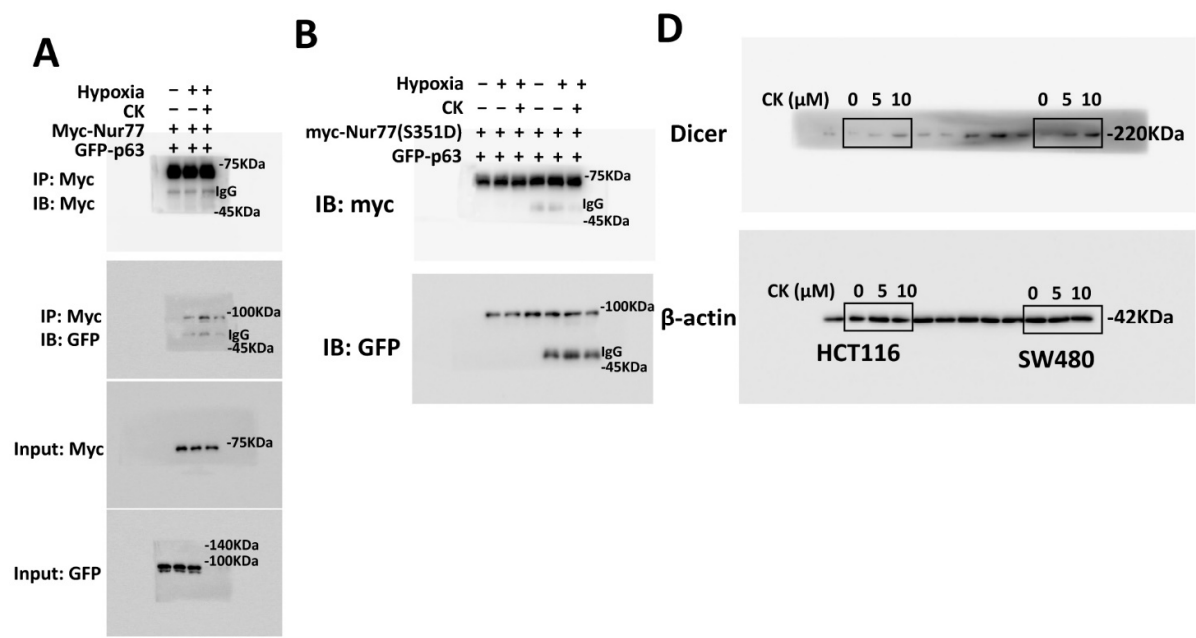


Figure S11. Uncropped Western Blot image for Figure S2.