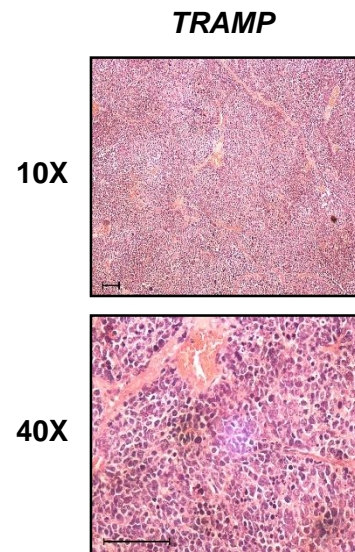
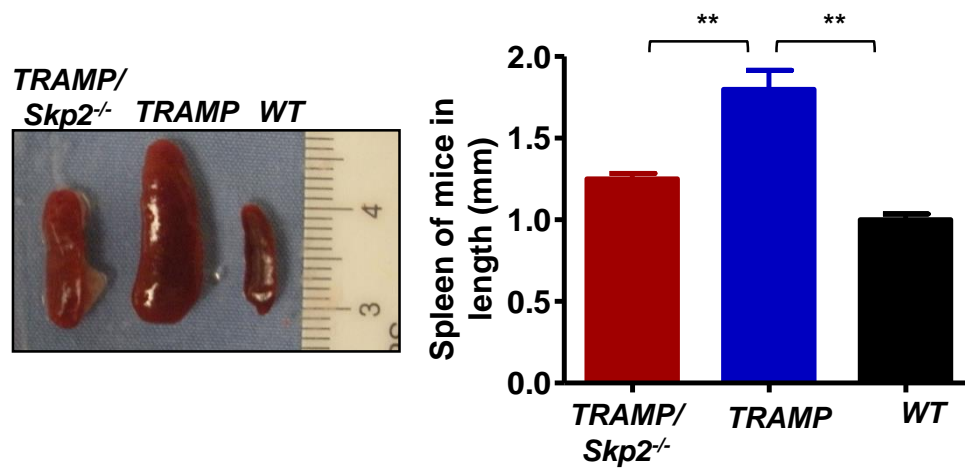
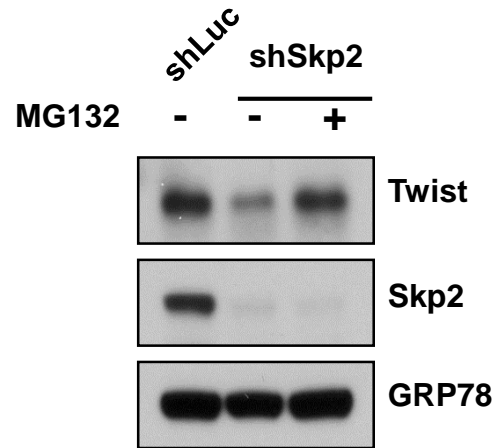


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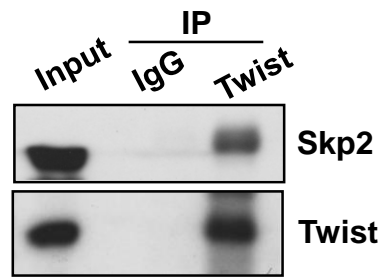
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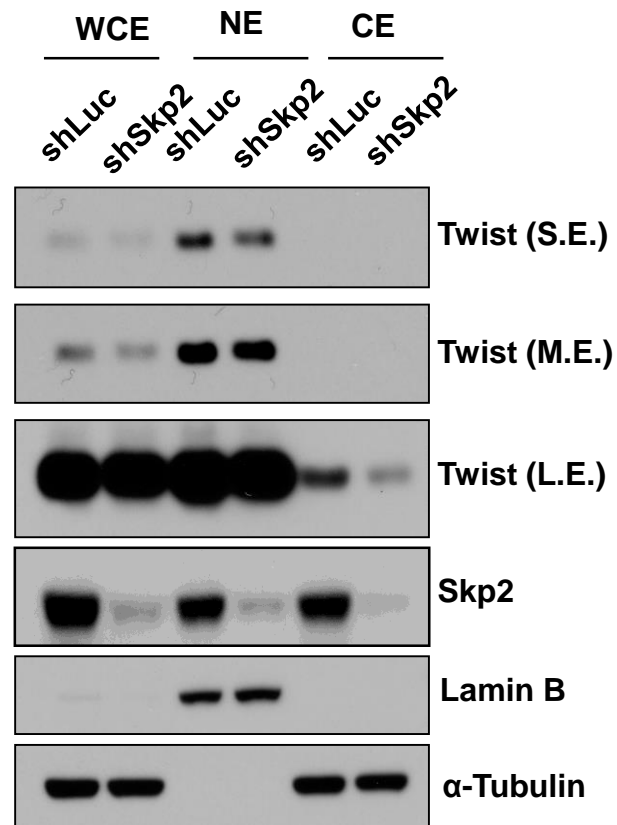


Supplementary Figure S2.

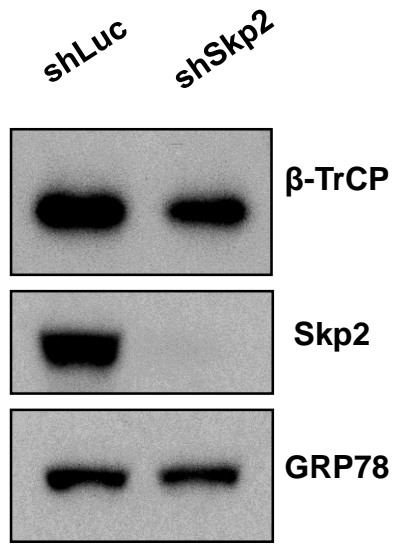
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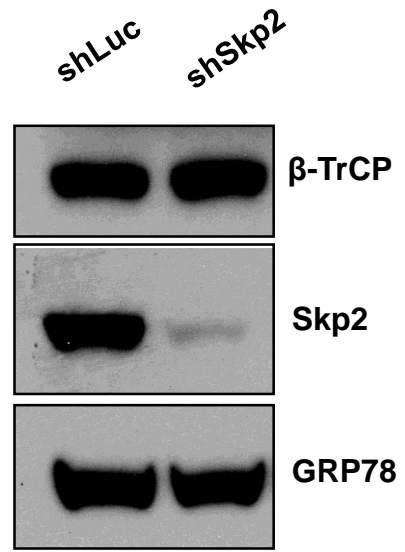
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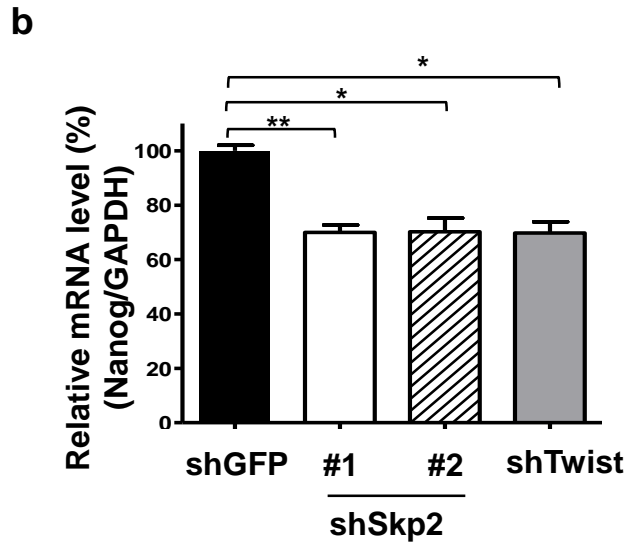
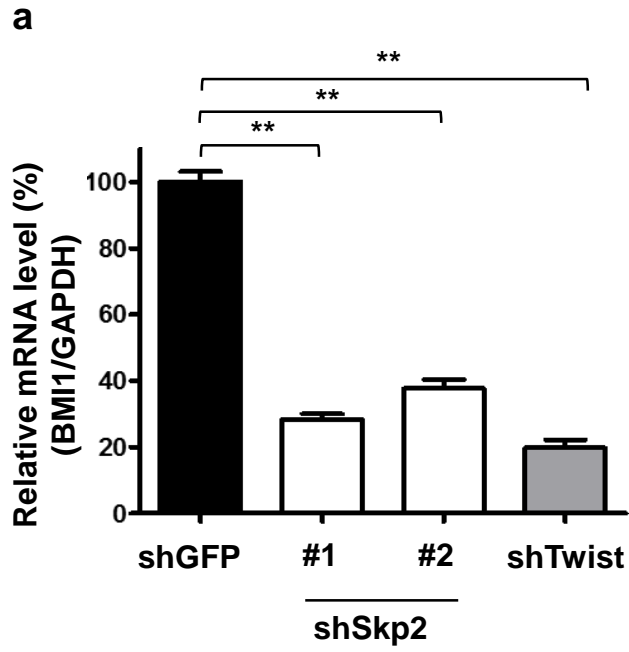


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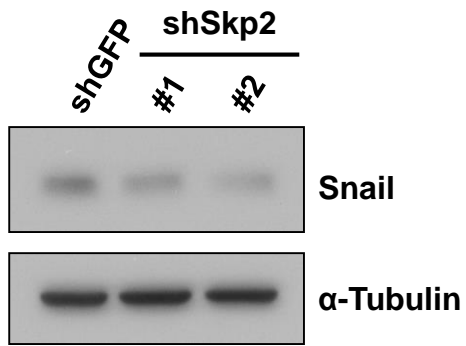


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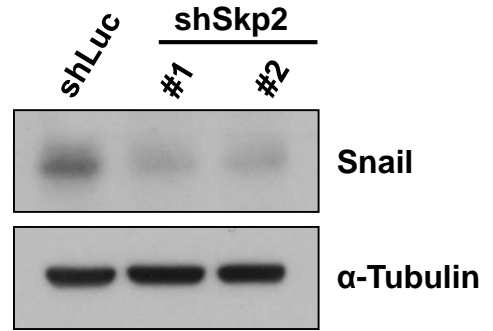




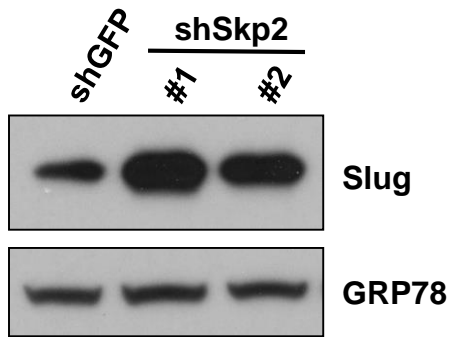
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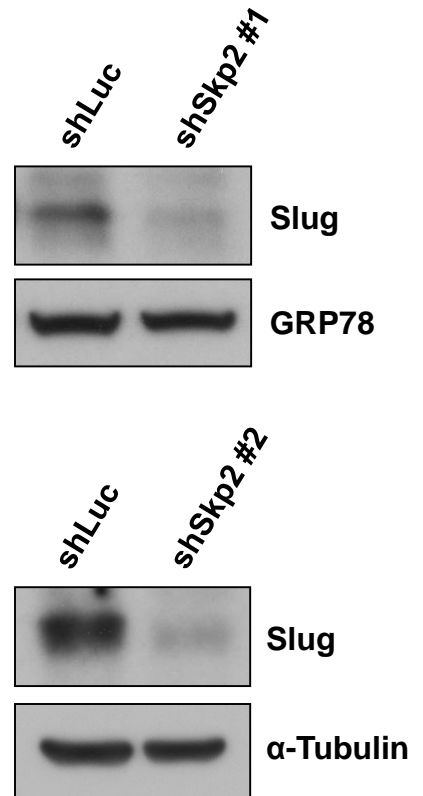
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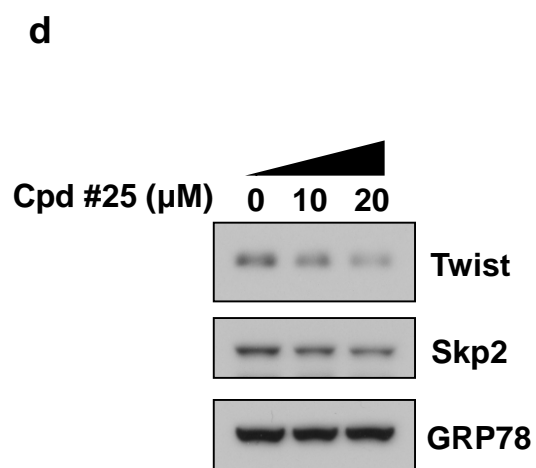
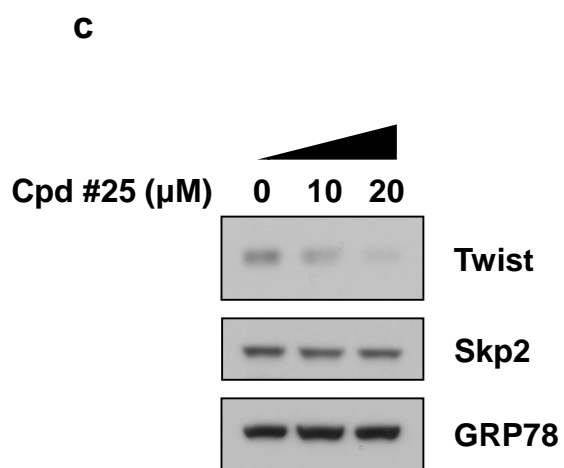
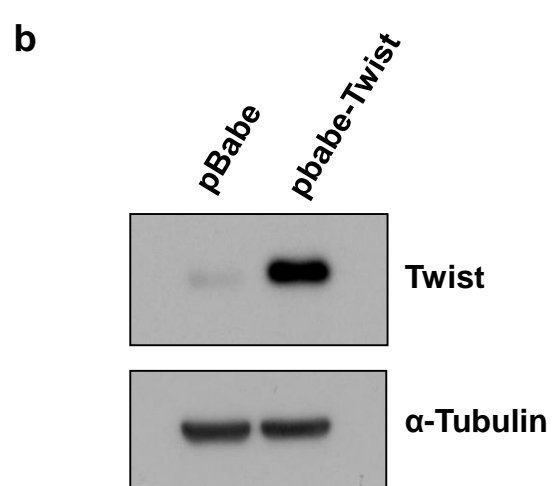
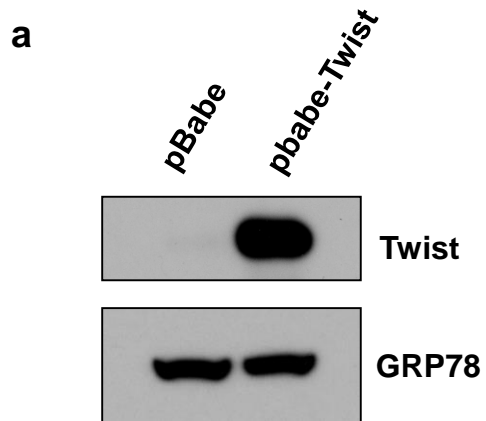


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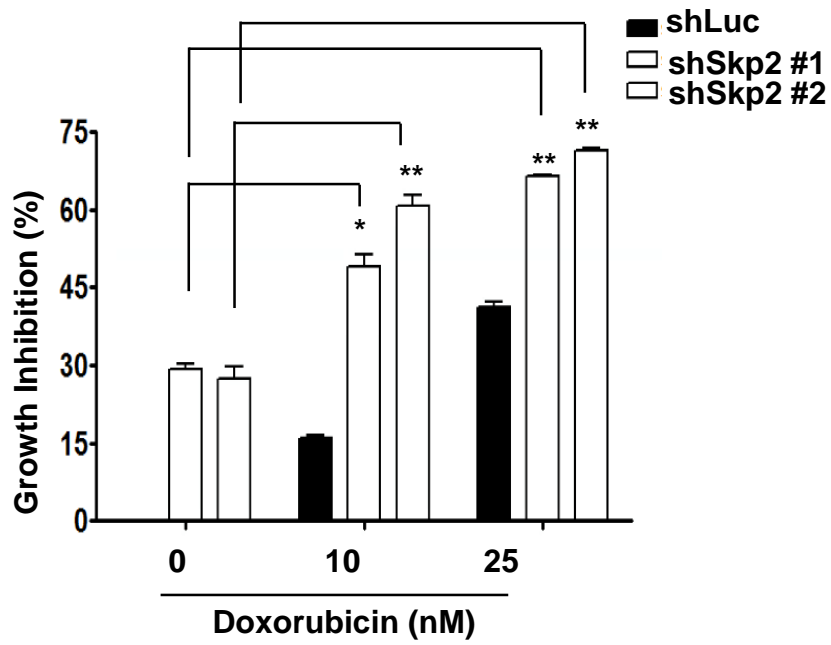


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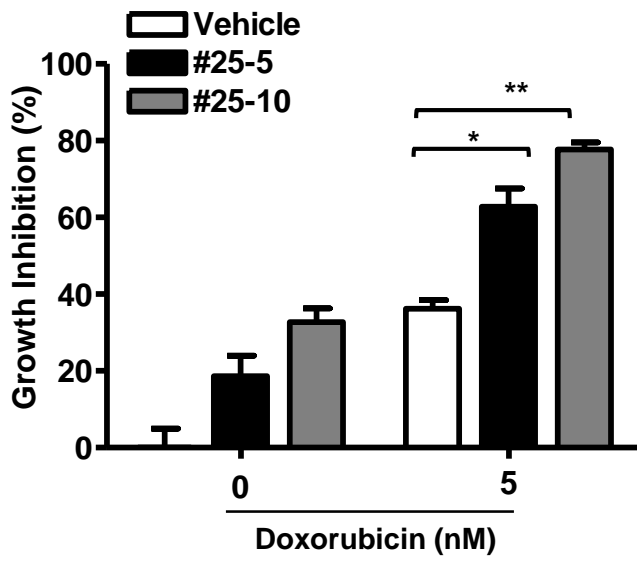


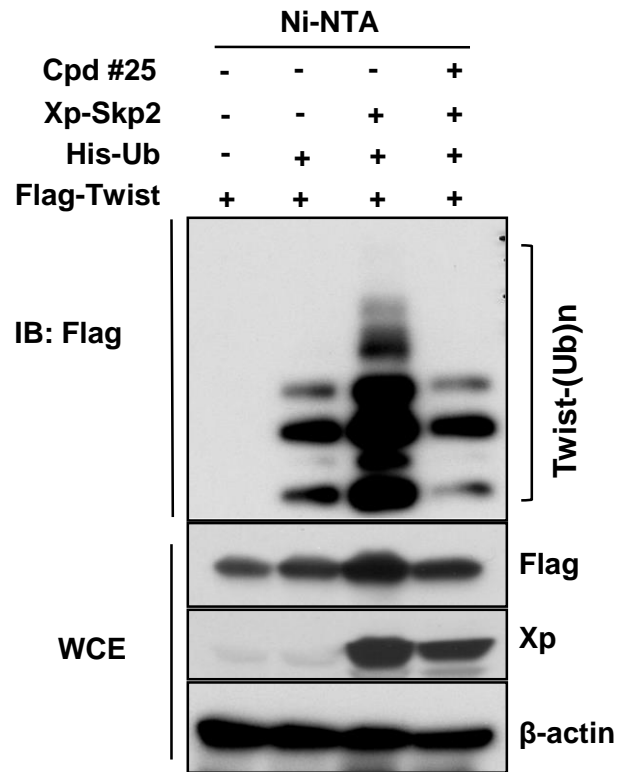


a



b





Supplementary Figure S9.

Supplementary Information

Supplementary Figure S1. Neuroendocrine differentiated prostate adenocarcinoma in TRAMP mice. (a) Histopathological image of TRAMP mice with neuroendocrine differentiated prostate adenocarcinoma was shown with magnification of 10X and 40X. (b) The length of the spleen was measured from age-matched TRAMP/Skp2^{-/-} n=10, TRAMP n=11 and WT mice n=10. Quantitative results are presented as means ± SD; **, $p < 0.01$.

Supplementary Figure S2. Skp2 regulates proteasomal degradation of Twist.

22Rv1 cells with luciferase or Skp2 knockdown were treated with MG132 and subjected to western blot analysis.

Supplementary Figure S3. Skp2 deficiency does not affect Twist nuclear localization.

(a) 293T cells were used for immunoprecipitation (IP) with Twist antibody followed by Western blot analysis. (b) PC3 cells with luciferase or Skp2 deficiency were subjected to biochemical fractionation followed by Western blot analysis.

Supplementary Figure S4. Skp2 knockdown does not result in β -TrCP accumulation.

(a, b) PC3 (a) and 22Rv1 (b) cells with luciferase or Skp2 knockdown were subjected to Western blot analysis to examine β -TrCP levels.

Supplementary Figure S5. Skp2 deficiency reduces transcription of stemness markers to a similar extent as Twist knockdown in CRPC cells.

(a, b) Real-time (RT)-PCR analysis of stemness markers BMI1 **(a)** and Nanog **(b)** in PC3 cells with luciferase, Skp2, or Twist knockdown. Quantified results are presented as means \pm SD; *, $p < 0.05$ and **, $p < 0.01$.

Supplementary Figure S6. Skp2 deficiency reduces protein expression of Snail.

(a-d) Western blot analysis of Snail and Slug protein expression in PC3 **(a, c)** and 22Rv1 **(b, d)** cells with or without Skp2 knockdown.

Supplementary Figure S7. Twist overexpression in CRPC cells enhances CSC self-renewal.

(a, b) Western blot analysis of Twist protein expression in PC3 **(a)** and 22Rv1 **(b)** cells overexpressed with Twist. **(c, d)** Western blot analysis of Twist protein expression in PC3 **(c)** and 22Rv1 **(d)** cells treated with various concentrations of compound #25.

Supplementary Figure S8. Skp2 targeting inhibits cell growth in CRPC cells.

(a) PC3 cells with luciferase or Skp2 deficiency were seeded for cell growth inhibition assay followed by treatment of doxorubicin. **(b)** PC3 cells were seeded for cell growth inhibition assay followed by treatment of a combination of doxorubicin and compound #25 (Skp2 inhibitor). Quantified results are presented as means \pm SD; *, $p < 0.05$ and **, $p < 0.01$.

Supplementary Figure S9. Skp2 inhibitor reduces Skp2 ubiquitination on Twist.

In vivo ubiquitination assay of 293T cells transfected with various plasmids with or without treatment by compound (cpd) #25.