Expanded View Figures

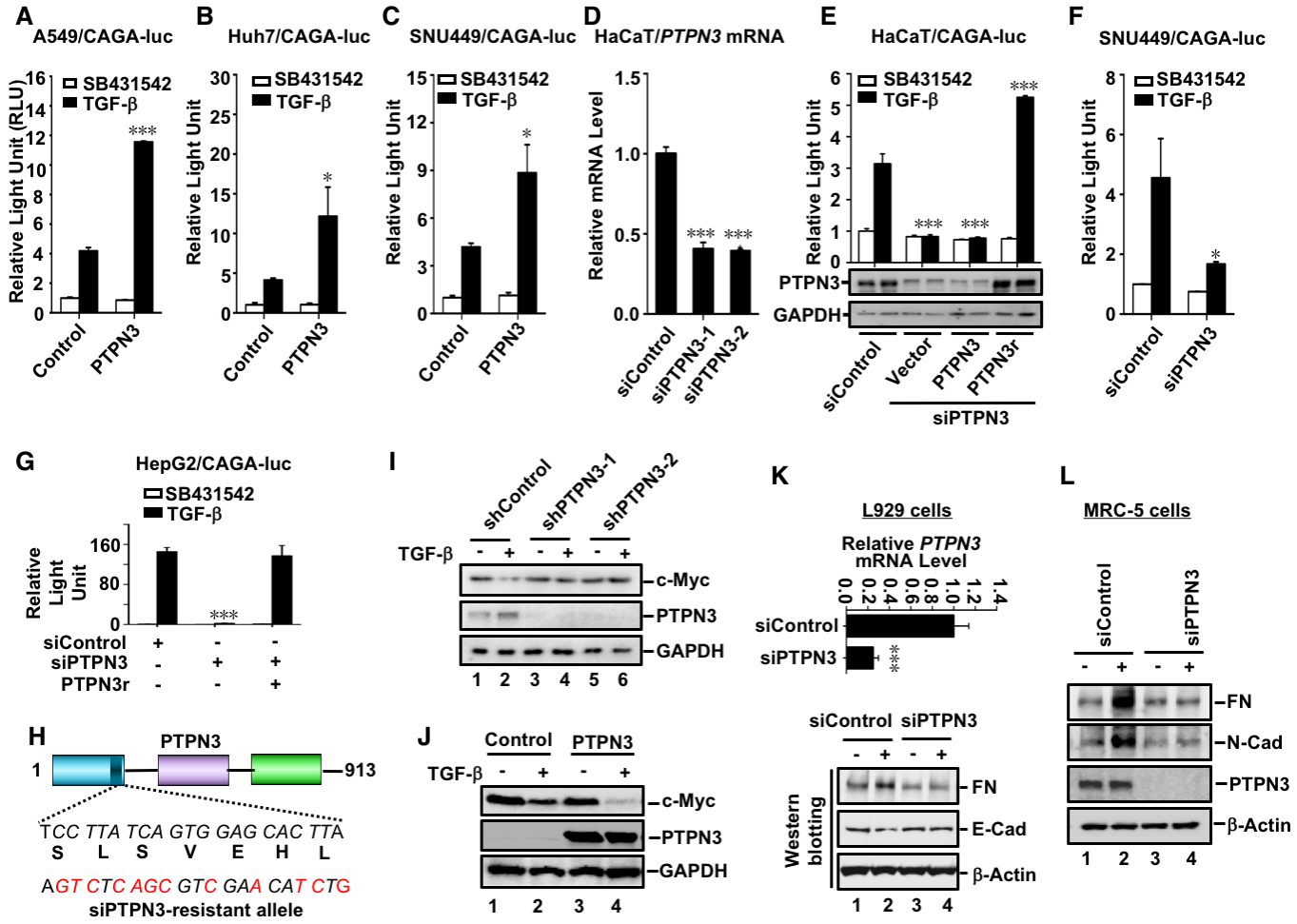

Figure EV1.

Figure EV1. PTPN3 enhances TGF-β-induced transcriptional responses.

A PTPN3 promotes TGF-β-induced CAGA-luc reporter activity. A549 cells were transfected with expression plasmids for PTPN3, CAGA-luc reporter, and Renilla-luc reporter as indicated and treated with TGF-β (2 ng/ml, 8 h). Relative luciferase activity was measured as described in the text. Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

B PTPN3 augments TGF-β-induced CAGA-luc reporter activity in Huh7 cells. Cell transfection, TGF-β treatment, and luciferase assays were similarly done as in (A). Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. *$P < 0.05$.

C PTPN3 facilitates TGF-β-induced CAGA-luc reporter activity in SNU449 cells. Cell transfection, TGF-β treatment, and luciferase assays were similarly done as in (A). Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. *$P < 0.05$.

D PTPN3 is efficiently knocked down by siRNA. Two independent siRNAs against *PTPN3* were transfected into HaCaT cells. PTPN3 mRNA level was detected by using qRT–PCR. Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

E Knockdown of PTPN3 decreases TGF-β-induced CAGA-luc reporter activity, and siRNA-resistant variant of PTPN3 rescues the siPTPN3 effect. HaCaT cells were transfected with siPTPN3, and expression plasmids for PTPN3 or RNAi-resistant variant of PTPN3 (PTPN3r), CAGA-luc reporter, and Renilla-luc reporter as indicated and treated with TGF-β (2 ng/ml, 8 h). Twenty-four hours later, cells were harvested for luciferase analysis. Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

F Knockdown of PTPN3 decreases TGF-β-induced CAGA-luc reporter activity in SNU449 cells. Cells were transfected with siRNA against PTPN3 or control siRNA; 24 h post-transfection, expression plasmids for CAGA-luc reporter and Renilla-luc reporter were transfected as indicated. Cells were harvested for relative luciferase assay after another 24 h with TGF-β treatment (2 ng/ml, 8 h). Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. *$P < 0.05$.

G Knockdown of PTPN3 decreases TGF-β-induced CAGA-luc reporter activity, and siRNA-resistant variant of PTPN3 rescues the siPTPN3 effect in HepG2 cells. Cells were transfected with siControl, siPTPN3, expression plasmids for RNAi-resistant variant of PTPN3 (PTPN3r), CAGA-luc reporter, and Renilla-luc reporter as indicated and treated with TGF-β (2 ng/ml, 8 h). Twenty-four hours later, cells were harvested for luciferase analysis. Data are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

H Schematic representation of the RNAi-resistant PTPN3 variant. The siPTPN3 target sequence (corresponding to amino acids 214–220) is indicated. Silent mutations introduced are shown by italic/red letters.

I Depletion of PTPN3 abolishes TGF-β-induced target protein expression. HaCaT cells with stable knockdown of PTPN3 were treated with TGF-β (2 ng/ml) for 6 h before harvested. Cells were harvested for Western blotting with appropriate antibodies as indicated.

J PTPN3 facilitates TGF-β-induced target protein expression. HaCaT cells stably expressing HA-PTPN3 were stimulated with 2 ng/ml of TGF-β for 6 h and then harvested for cell lysates. Protein levels were examined by Western blotting with anti-c-myc, anti-PTPN3, and anti-GAPDH antibodies.

K Knocking down efficiency of siPTPN3 in L929 cells. L929 cells were transfected with siControl or siPTPN3 with RNAiMax. Forty-eight hours after transfection, cells were harvested for qRT–PCR analysis as indicated. Data in the upper panel are shown as mean ± SEM; $n = 3$. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$. Depletion of PTPN3 attenuates TGF-β-induced EMT. L929 cells were transfected with siControl or siPTPN3 with RNAiMax. Forty-eight hours later, cells were harvested for Western blotting analysis with antibodies as indicated.

L Depletion of PTPN3 attenuates TGF-β-induced EMT. MRC-5 cells were transfected with siControl or siPTPN3 with RNAiMax. Forty-eight hours later, cells were harvested for Western blotting analysis with antibodies as indicated.

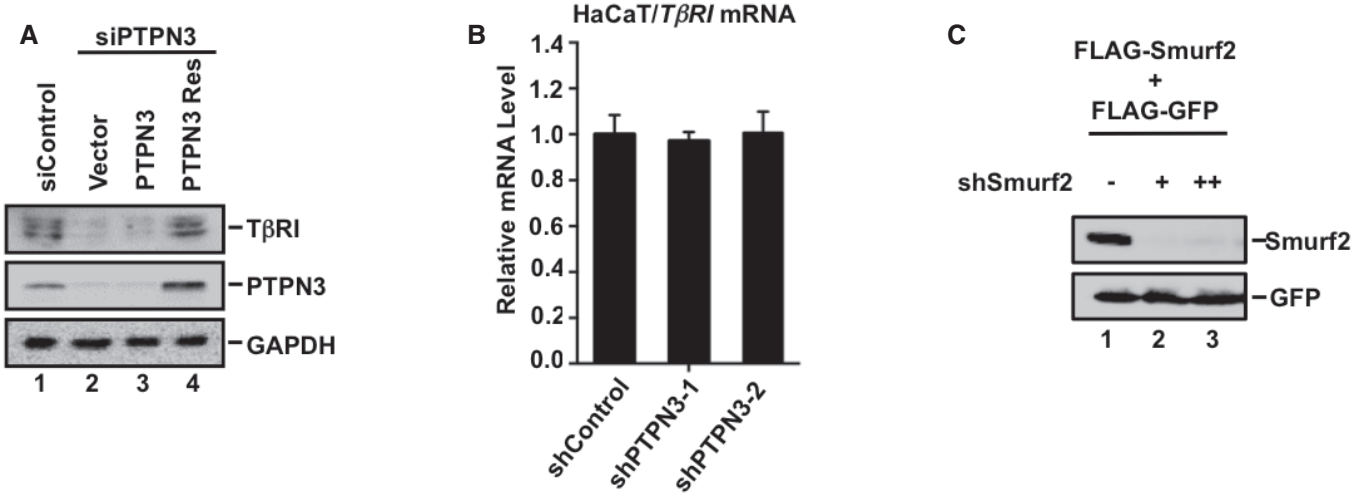

Figure EV2. Assessment of PTPN3 mutants and RNAi knockdown efficiency.

A Knockdown of PTPN3 decreases the TβRI protein level and RNAi-resistant variant of PTPN3 rescues the siPTPN3 effect. HaCaT cells were transfected with siPTPN3, together with PTPN3 and RNAi-resistant variant of PTPN3. Twenty-four hours later, cells were harvested for Western blotting analysis.

B Depletion of PTPN3 has no effect on the mRNA level of *TβRI*. qRT–PCR was used to examine the mRNA level of *TβRI* in HaCaT cells expressing shRNA Control, shPTPN3-1, and shPTPN3-2. Data are shown as mean ± SEM; $n = 3$.

C shSmurf2 efficiently knocks down Smurf2. HEK293T cells were co-transfected with FLAG-Smurf2, FLAG-GFP, and increasing amounts of pSRGshSmurf2 as indicated. Cells were harvested for Western blotting analysis with anti-FLAG antibodies.

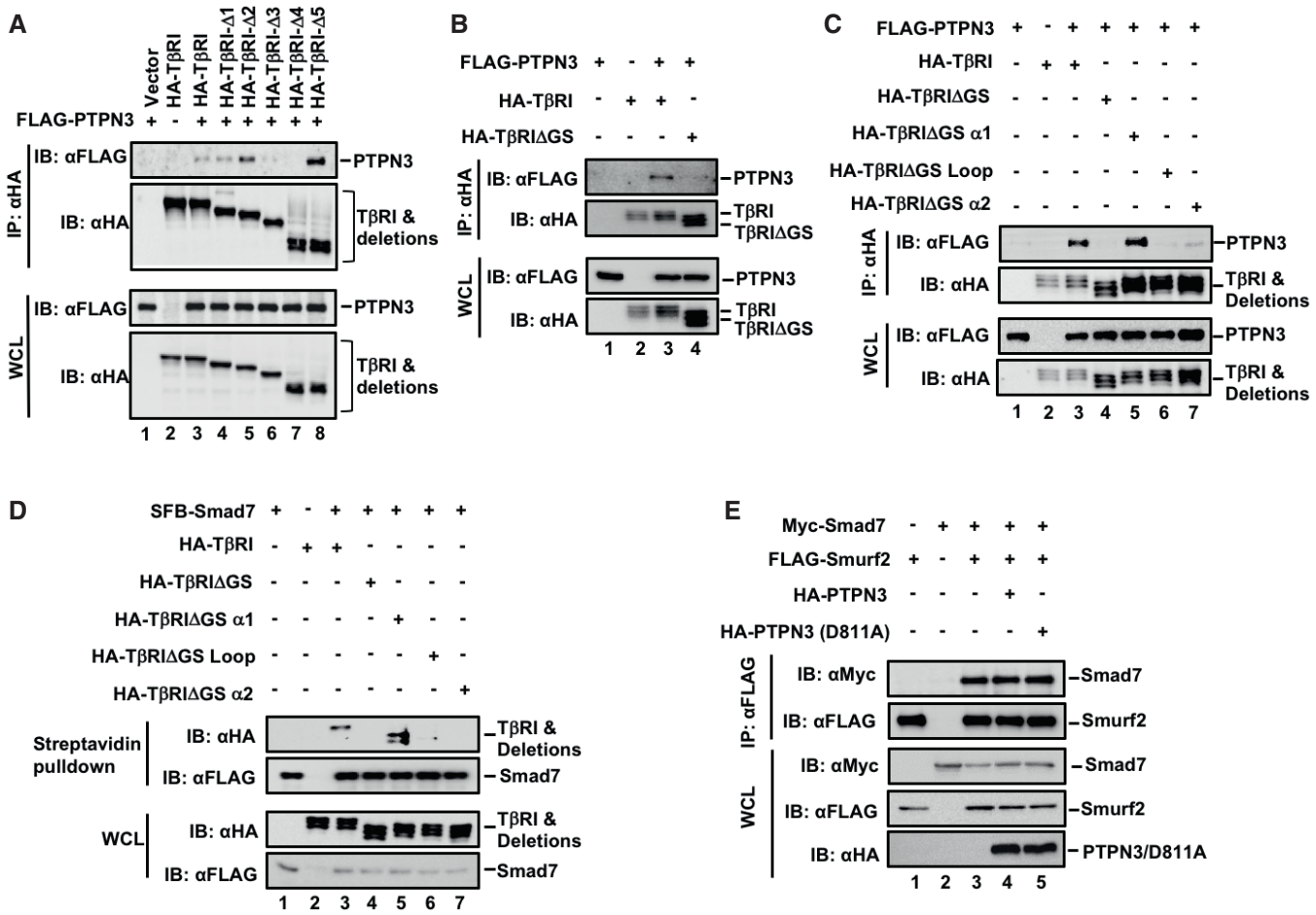

Figure EV3. PTPN3 binds to TβRI and disrupts its interaction with the Smad7-Smurf2 E3 ligase.

A Deletion of the GS region of TβRI disabled its interaction with PTPN3. HEK293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, immunoprecipitation and immunoblotting analysis were performed with the anti-HA antibody. Levels of indicated proteins were examined by Western blotting with the indicated antibodies.

B GS region of TβRI is necessary for its binding to PTPN3. FLAG-PTPN3, HA-TβRI, and HA-TβRI truncations were transfected into HEK293T cells. Cells were harvested after 24 h and immunoprecipitated by HA antibody. PTPN3, TβRI, and TβRI truncations were detected by Western blotting with anti-Flag and anti-HA antibodies.

C Deletion of the GS or GS-loop or GS-α2 region of TβRI disabled its interaction with PTPN3. HEK293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, immunoprecipitation and immunoblotting analyses were performed with the anti-HA antibody. Levels of indicated proteins were examined by Western blotting with the indicated antibodies.

D Deletion of the GS or GS-loop or GS-α2 region of TβRI disabled its interaction with Smad7. HEK293T cells were transfected with the indicated plasmids. Cells were treated with MG132 (20 μM, 4 h) before harvested. Then, immunoprecipitation was performed with the streptavidin. Levels of indicated proteins were examined by Western blotting with the indicated antibodies.

E PTPN3 wild-type or the D811A mutant did not affect the Smurf-Smad7 interaction. HEK293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, immunoprecipitation and immunoblotting analyses were performed with the anti-HA antibody. Levels of indicated proteins were examined by Western blotting with the indicated antibodies.

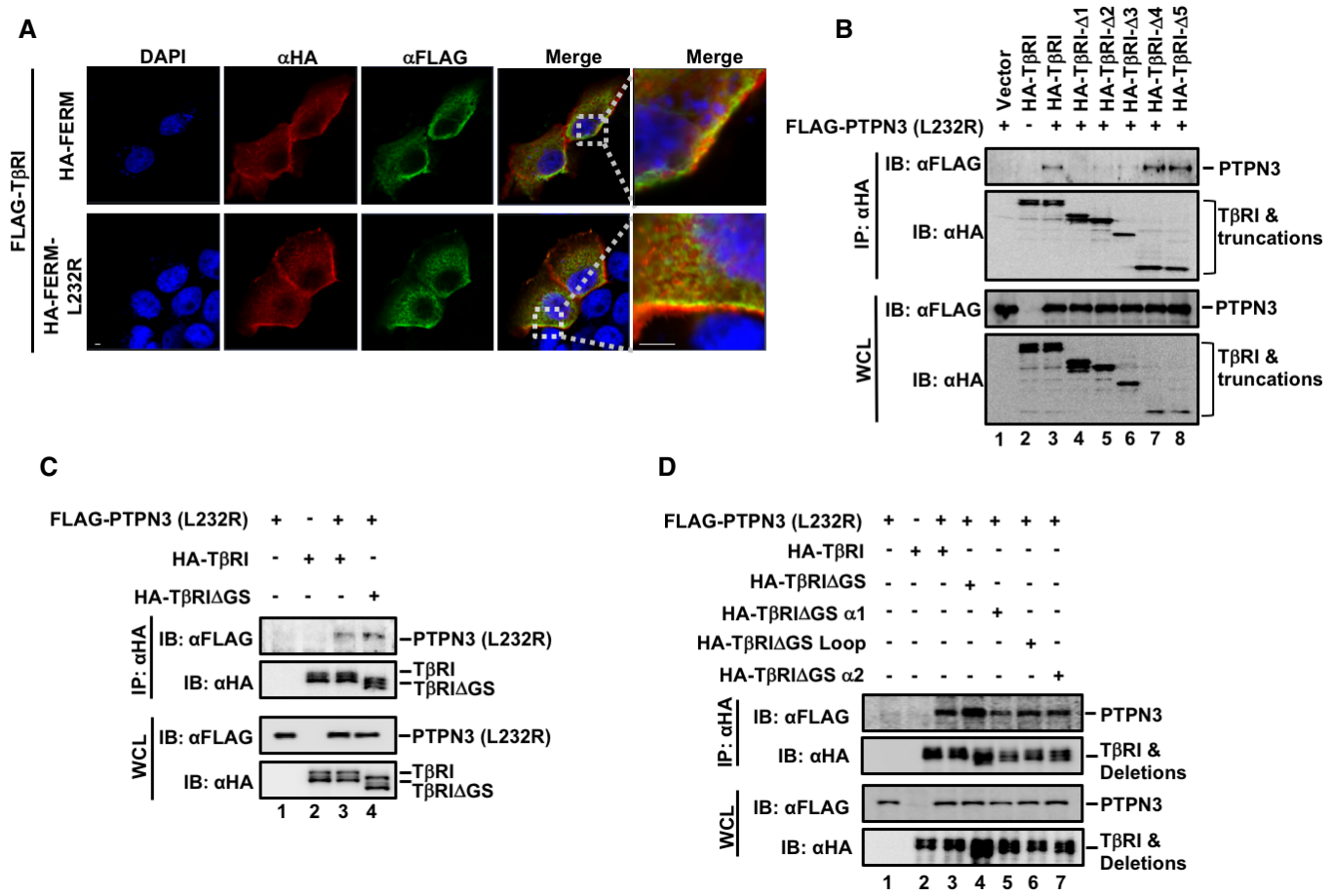

Figure EV4. Subcellular localization and TβRI-binding feature of PTPN3 L232R.

A PTPN3-FERM and PTPN3-FERM (L232R) share similar patterns of subcellular localization and co-localization with TβRI. HaCaT cells were transfected with FLAG-TβRI, HA-PTPN3-FERM, and HA-PTPN3-FERM (L232R). Twenty-four hours later, cells were harvested for immunofluorescence with the indicated antibodies. Scale bar = 10 μm (except scale bar = 5 μm in the last column).

B The interactions between PTPN3 (L232R) and TβRI truncations. HEK293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, immunoprecipitation and immunoblotting analysis were performed with the anti-HA antibody. Levels of indicated proteins were examined by Western blotting with the indicated antibodies.

C Deletion of the GS region of TβRI had no effect on its interaction with PTPN3 (L232R). HEK293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, immunoprecipitation and immunoblotting analyses were performed with the anti-HA antibody. Levels of indicated proteins were examined by Western blotting with the indicated antibodies.

D Deletion of the GS, GS-loop, GS-α1, or GS-α2 region of TβRI had no effect on its interaction with PTPN3 (L232R). HEK293T cells were transfected with the indicated plasmids. Twenty hours later, cells were treated with MG132 (20 μM) for 4 h, followed by Western blotting with the indicated antibodies.

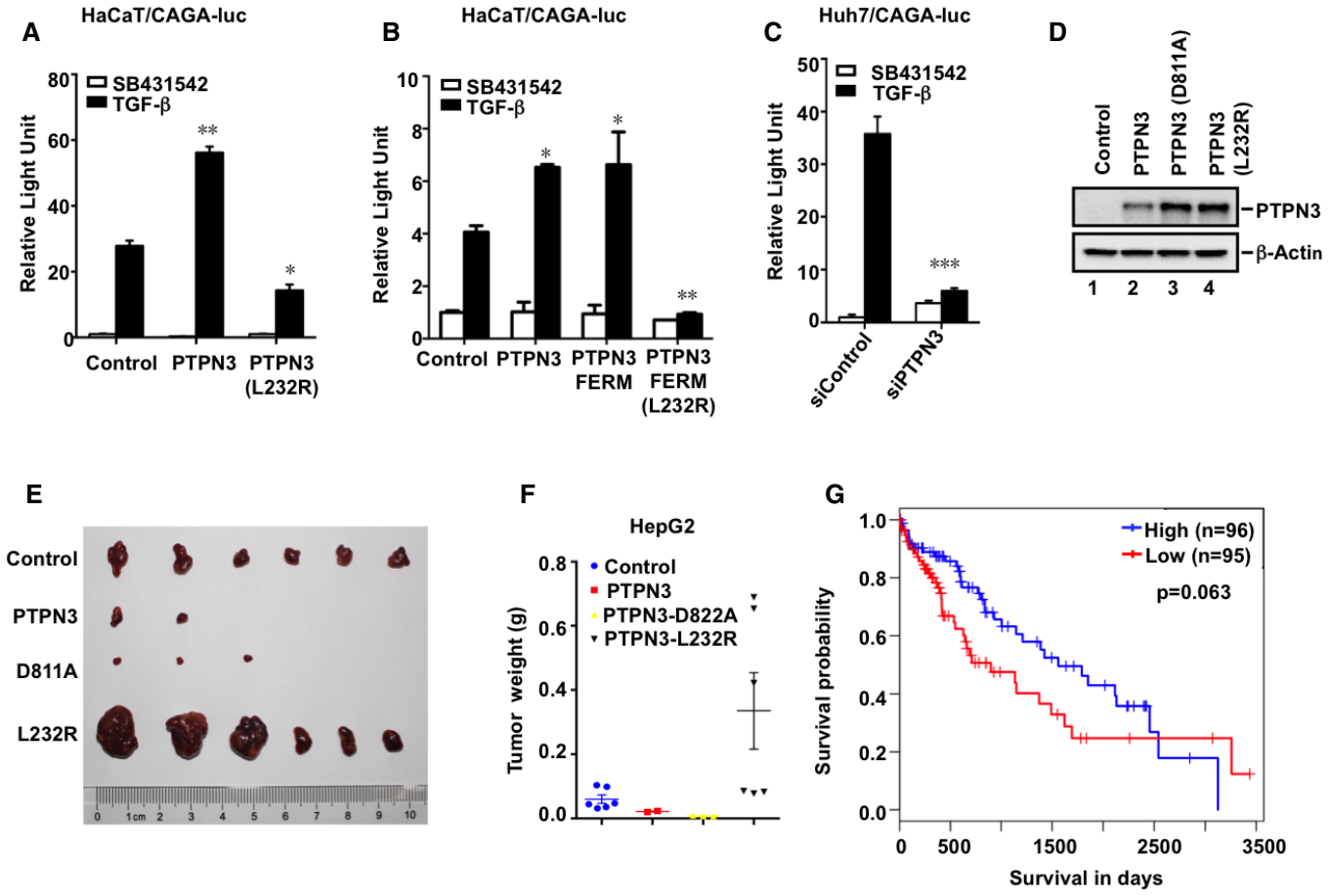

Figure EV5. Role of PTPN3 in tumorigenesis.

A PTPN3 (L232R) inhibits TGF-β-induced reporter gene activity. Transfection of HaCaT cells, TGF-β treatment, and relative luciferase assays were similarly done as in Fig EV1A. Data are shown as mean ± SEM; n = 3. Statistical analysis was performed using two-tailed Student's t-test. *P < 0.05, **P < 0.01.

B PTPN3-FERM (L232R) inhibits TGF-β-induced reporter gene activity. Experiments were done as in (A). Data are shown as mean ± SEM; n = 3. Statistical analysis was performed using two-tailed Student's t-test. *P < 0.05, **P < 0.01.

C Knockdown of PTPN3 decreases TGF-β-induced CAGA-luc reporter activity in Huh7 cells. Transfection of Huh7 cells, TGF-β treatment, and luciferase assays were similarly done as in (A). Data are shown as mean ± SEM; n = 3. Statistical analysis was performed using two-tailed Student's t-test. ***P < 0.001.

D Expression levels of PTPN3, PTPN3 (D811A), and PTPN3 (L232R) of HepG2 stable cells were analyzed by Western blotting.

E PTPN3 attenuates tumorigenesis in mouse models, while PTPN3 (L232R) exhibited the blocking effect. HepG2 cells stably expressing PTPN3, PTPN3 (D811A), and PTPN3 (L232R) were subcutaneously injected into nude mice. Fifty days after cell implantation, tumors were photographed.

F Weight from all tumors in (E) was recorded from Control, PTPN3, PTPN3 (D811A), and PTPN3 (L232R) groups. Data are shown as mean ± SEM; n = 6 tumors for each group.

G Higher PTPN3 expression is corrected with better survival in LIHC patient. A total of 191 samples from the TCGA LIHC database were analyzed. The log-rank test was used to test whether gene levels were significantly associated with overall patient survival. Kaplan–Meier curve shows that LIHC patients with lower PTPN3 expression had a significantly high risk of recurrence than those with higher PTPN3 expression.