

Supplementary Information

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Supplemental data

Fig. S1

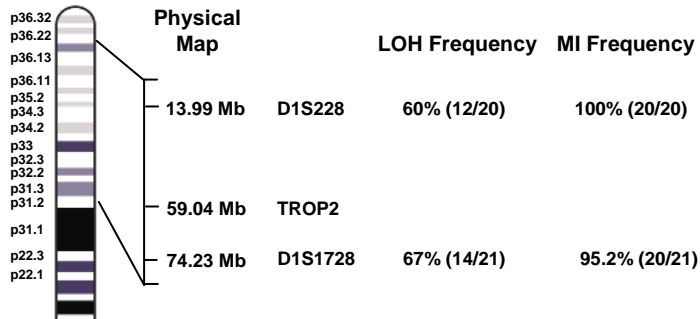


Figure S1. Loss of Heterozygosity (LOH) of *TROP2* gene

TROP2 is located at position of 59.04 Mb on chromosome 1p32. The two surrounding LOH markers in our LOH data are D1S228 (13.99Mb)-*TROP2* (59.04)-D1S1665 (74.23). *TROP2* is located between markers D1S228 (60%) and D1S1728 (67%), which show frequent LOH in 48 paired lung adenocarcinoma samples.

Fig. S2

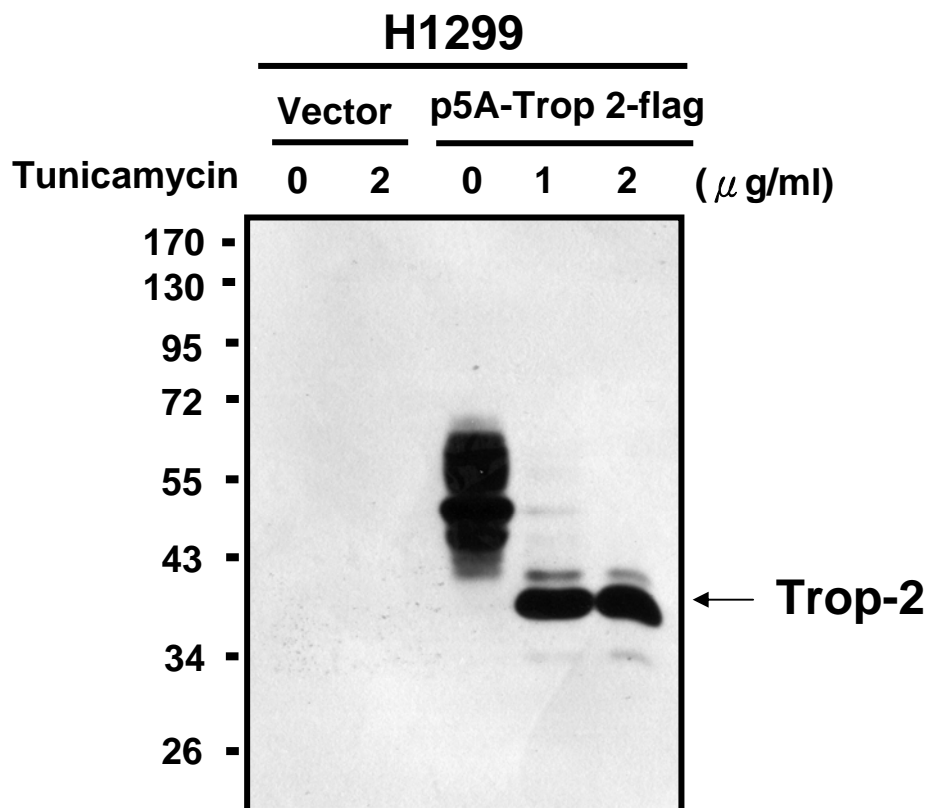
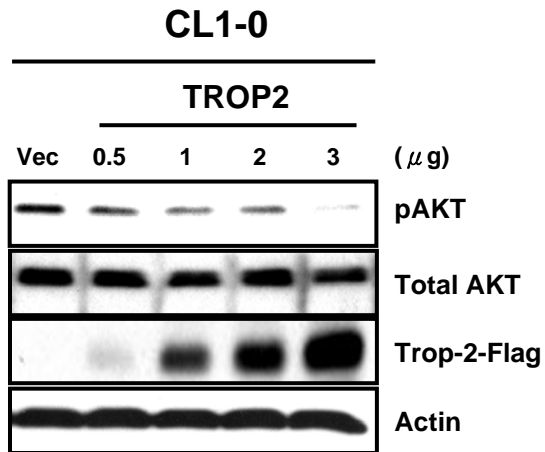


Figure S2. N-glycosylation of Trop-2 protein

H1299 cells were transfected with the Trop-2-flag and then treated with an N-linked glycosylation inhibitor, tunicamycin for 24 h at the indicated concentrations. The results show that multiple bands prior to tunicamycin treatment become a major single band near the predicted molecular weight, 35 kD in cells after the treatment, suggesting that the multiple bands in Western blots are related to different glycosylation.

Fig. S3

A.



B.

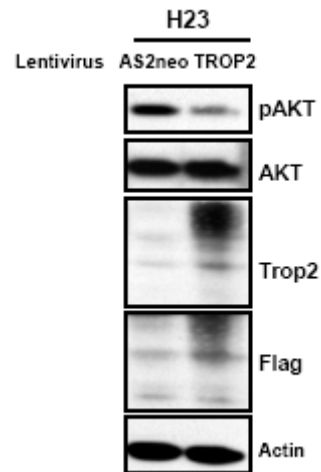


Figure S3. Trop-2 expression suppresses the activation of AKT

Trop-2 was overexpressed in CL1-0 (A) and H23 (B) by either transfection or lentiviral transduction as indicated. The cells were harvested and separated by SDS-PAGE. The activity of AKT was determined by Western blotting with anti-pAKT.

Fig. S4

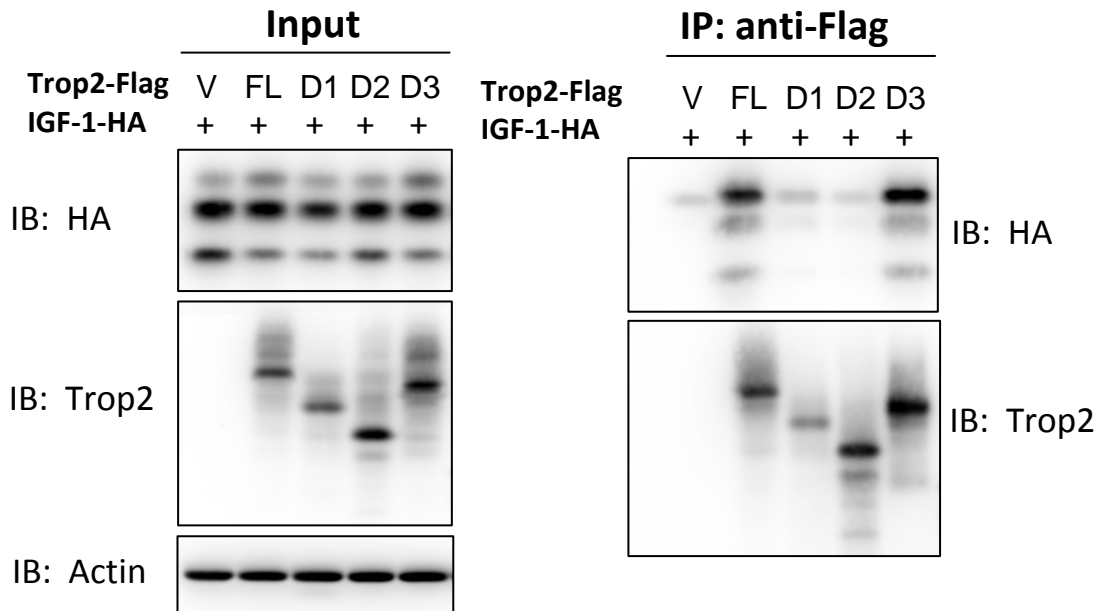
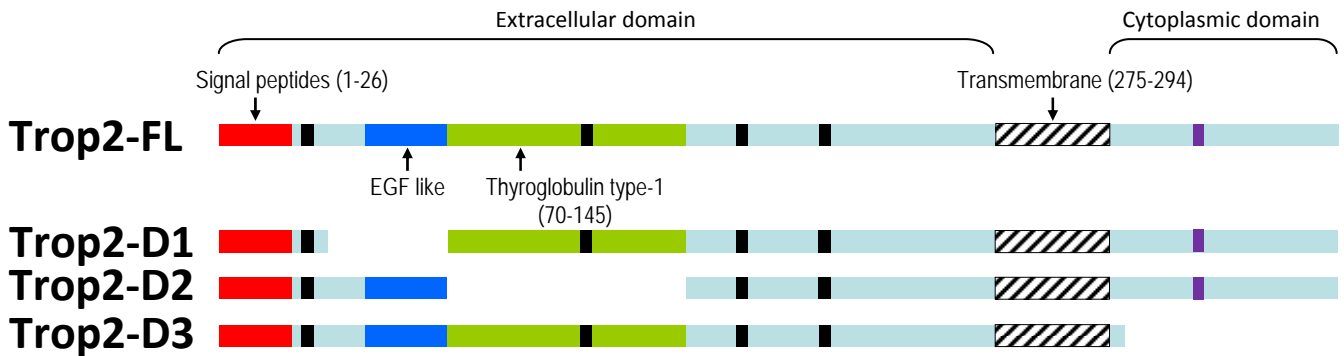


Figure S4. Deletion Mapping of Trop-2 binding domain to mIGF-1

Several deletion constructs of Trop-2-expressing plasmids as indicated were made. The cells were co-transfected with Trop-2-expressing and mIGF1-expressing plasmid. The lysate was incubated with anti-flag antibody and extracted by protein A Sepharose (Sigma). The protein samples were separated by SDS-PAGE, transferred to a PVDF membrane, immunoblotted with the indicated antibodies, and visualized by chemiluminescence. The results showed that loss of either EGF-like or Thyroglobulin type-1 domains would abolish the interactions between Trop-2 and mIGF-1. This result indicated that the interaction domain located on the region including EGF-like and Thyroglobulin type-1 domains. WB: western blot. IP: immunoprecipitation.

Fig. S5

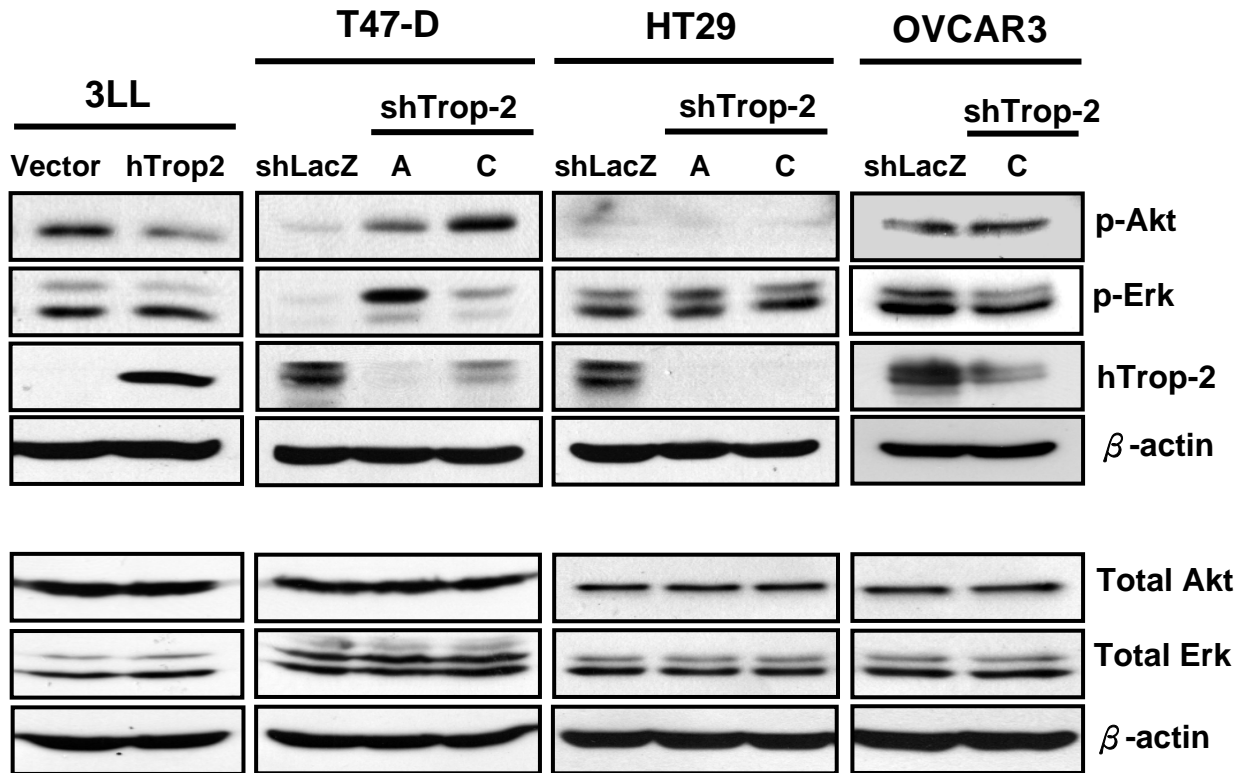


Fig. S5 Trop-2 protein modulates the activities of AKT and ERK

Trop-2 was overexpressed in mouse Lewis Lung Cancer Cells (3LL) which had low Trop-2 expression and Trop-2 was knock down in high Trop-2 expression cells including T47-D (mammary), HT29 (colon) and OVCAR3 (ovary) cancer cells by using lentiviral transduction as indicated. The soluble protein samples were separated by SDS-PAGE and immunoblotted with the indicated antibodies. The upper and lower panels were performed by two separate blot membranes with the same experimental condition. The results showed that Trop-2 overexpression could suppress the phosphorylation of AKT and ERK (slightly). Conversely, knockdown of Trop-2 in human breast cancer (T47-D) cells can promote the activation of AKT and ERK but there was no obvious effect on human colon cancer (HT29) and ovary cancer (OVCAR3) cells.