

SUPPLEMENTARY INFORMATION

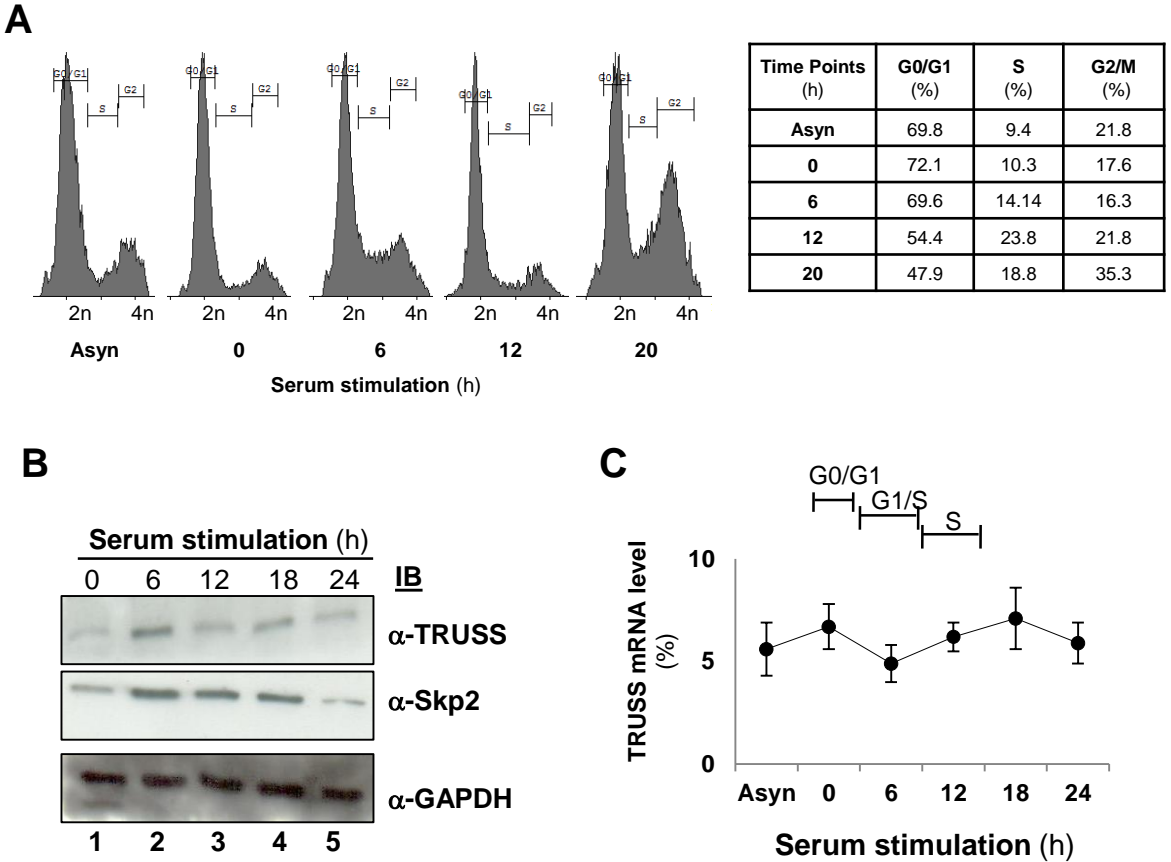


Fig. S1. Cell cycle analysis by Florescence activated cell sorting. Un-transfected Huh-7 cells were synchronized by serum starvation for 72 h. After serum stimulation for indicated time periods, cells were harvested and subjected to different analysis. (A) Cell cycle analysis of the untransfected cells was performed by florescence-activated cell sorting, (B) western blot analysis of endogenous TRUSS and Skp2. GAPDH used as a loading control, (C) RT-qPCR analysis of the TRUSS mRNA expression in un-transfected cells. Data is shown as mean \pm SD of three independent experiments.

A

shTRUSS (S) 5' GCCTCCACAGTGACTTT
GTTTTCAAGAGAAACAAAGTCACTGTGG
AGGCTTTTTT 3'
shTRUSS (As) 5' AATTAACAAACCTCCAC
AGTGACTTTGTTTCTCTTGAAAACAAAGT
CACTGTGGAGGCGGCC 3'



Cloned in pSilencer 1.0 vector

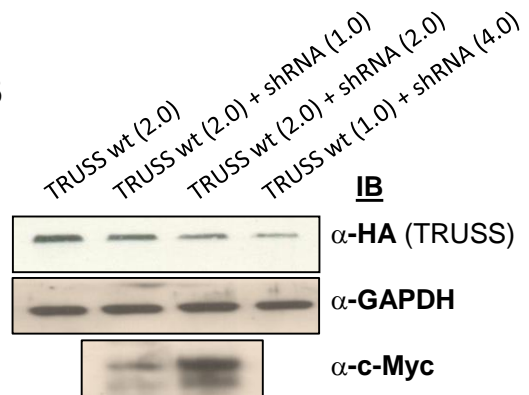
B

Fig. S2. Development of TRUSS shRNA and its validation. (A) The sequence of oligos designed to clone in pSilencer vector to suppress endogenous TRUSS protein (B) Huh7 cells were transiently transfected with expression vectors for TRUSS alone or with shRNA construct in different concentrations and the expression of TRUSS and GAPDH was monitored by western blot.

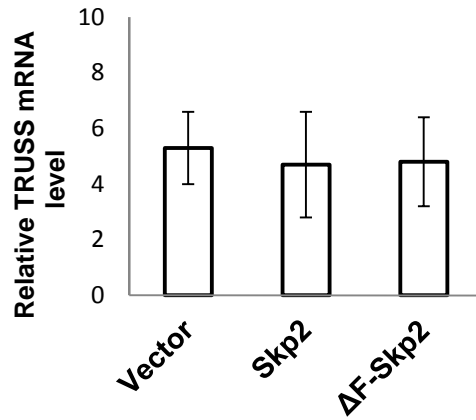


Fig. S3. Expression of TRUSS mRNA in the presence of Skp2. Huh-7 cells were transfected with WT-Skp2 and Flag-ΔF-Skp2 and the TRUSS mRNA level was measured by RT-qPCR. Data is represented as mean \pm SD of three independent experiments.

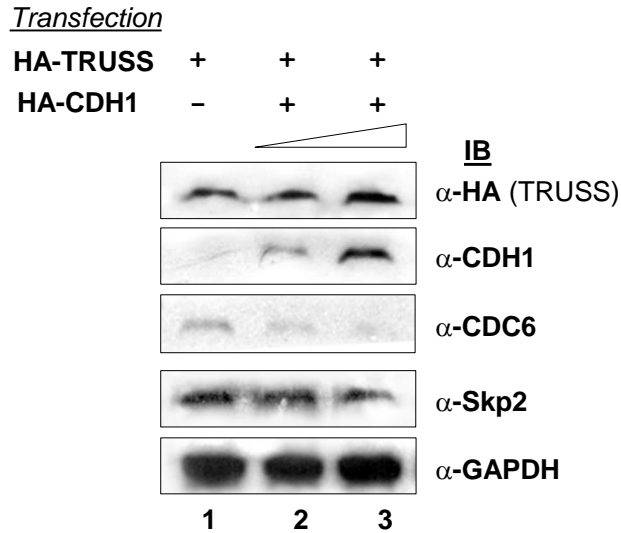


Fig. S4. TRUSS protein levels after CDH1 overexpression. Huh-7 cells were transfected with expression vectors of HA-TRUSS (1 μ g) along with 1 and 2 μ g HA-CDH1. Cell lysates were western-blotted for recombinant TRUSS, Skp2, CDH1 and CDC6. GAPDH served as an input control.

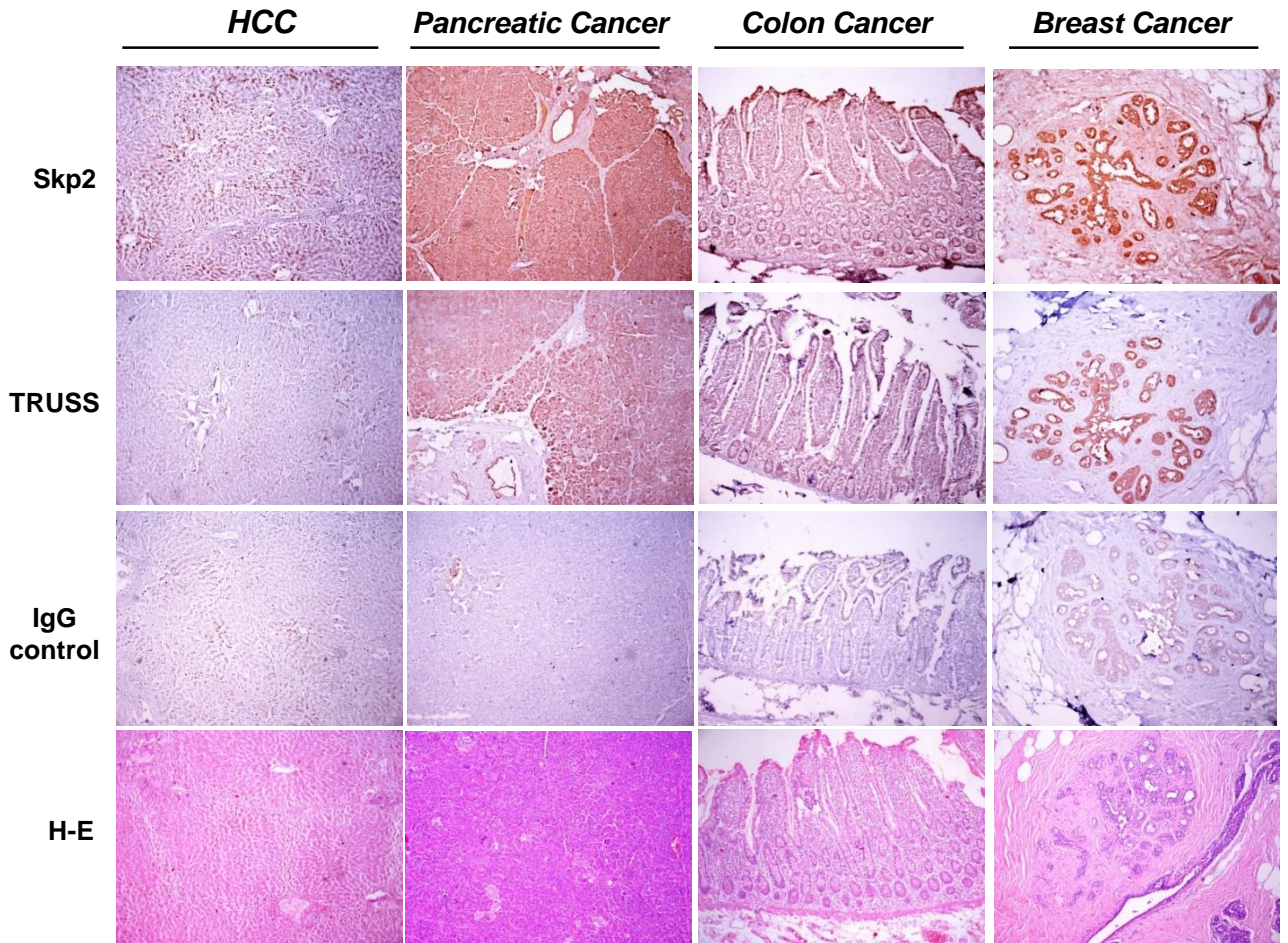


Fig. S5. TRUSS and Skp2 protein expression in Adjacent Non-cancerous Tissue (ANCT).

Serial tissue sections of ANCT specimens of HCC, pancreas colon and breast tumors were analyzed for the expression of TRUSS and Skp2 using specific antibodies. Incubation of samples with IgG was used as negative control. The hematoxylin and eosin (H-E)-stained sections of ANCT are also shown . All images, original magnification, x 100.

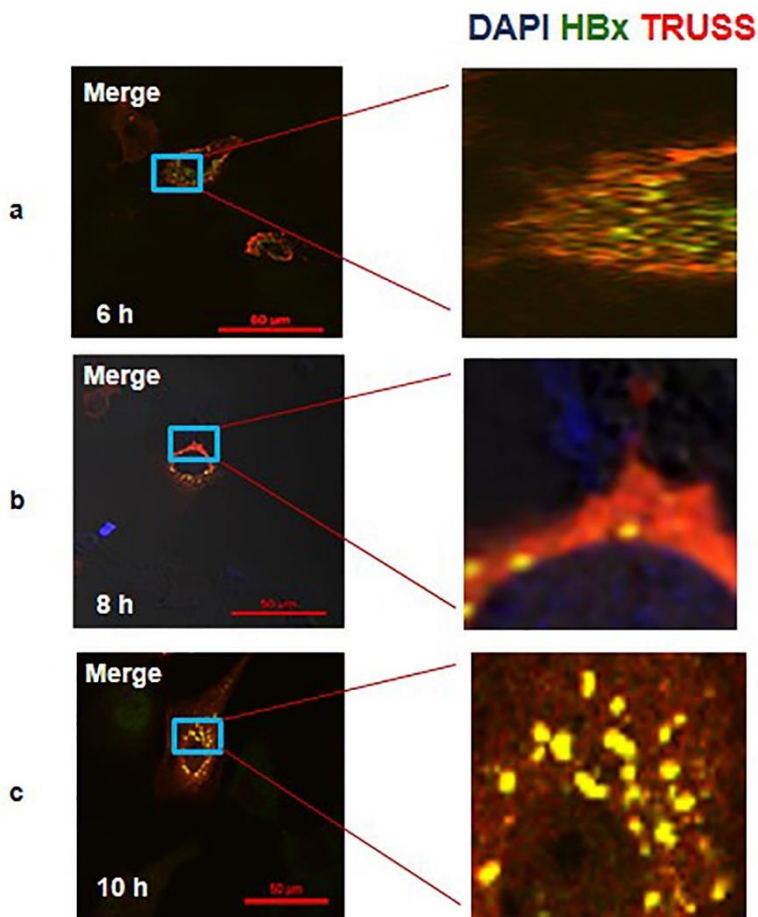


Fig. S6. Intracellular localization of TRUSS in the presence of HBx. Huh7 cells seeded on coverslip in 12 well plate and were transiently transfected after 16 h of seeding with expression vectors for TRUSS and HBx alone or in combination for 36 h and serum starved for the next 72 h. Cells were released at indicated time points and the expression of TRUSS and HBx was monitored by confocal microscopy. Nuclear staining of cells was done with DAPI. (a) Co-localization of HA-TRUSS (red) and HBx (green) in transfected cells after 6 h (b) after 8 h and (c) after 10 h of release in the perinuclear space, shown by yellow dots in the merge panel.