Supplementary information

Fig.S1

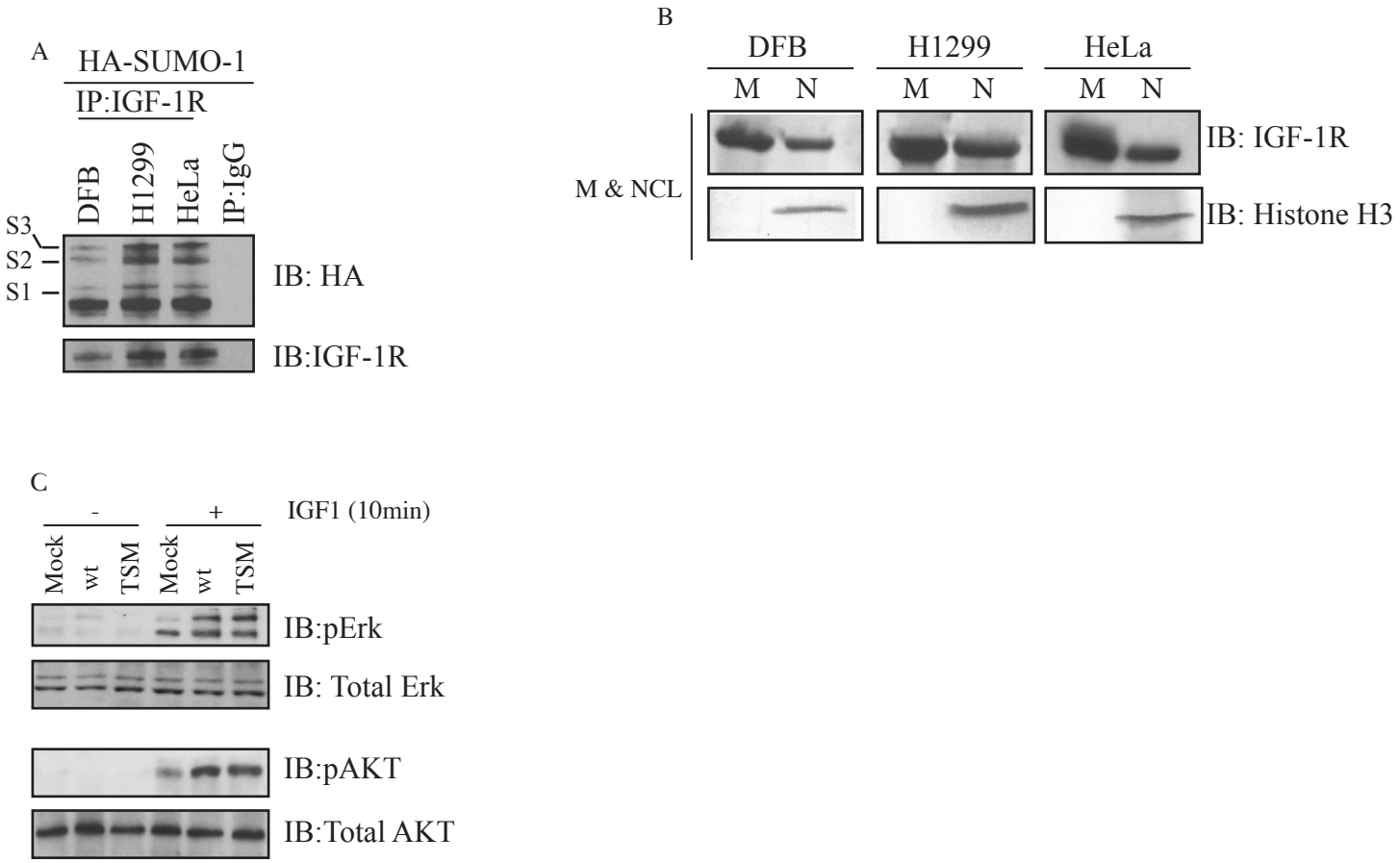


Figure legend

Fig.S1 (A) The SUMOylation profile of IGF-1R was investigated by transiently transfecting DFB, H1299 and HeLa cells with HA-SUMO-1. Total cell lysate of these cells were immunoprecipitated (IPd) with anti-IGF1R and was analyzed by immunoblotting (IB) with anti-HA. Mono-, di-, and tri-SUMOylated IGF-1R are indicated by S1, S2, and S3, respectively.
(B) Membrane and nuclear cell lysates (M- & NCL) of cells described in (A) were analyzed by IB with anti-IGF1R. Histine H3 was used as nuclear marker. (C) Lysates from serum-starved DFB cells, transfected with empty vector (Mock), plasmids encoding wt- and TSM-IG1R were stimulated with IGF-1 for 10 min and analyzed by IB for the presence of phosphorylated ERK (pERK) and phosphorylated Akt (pAkt). The blots were then stripped and incubated with antibodies against Akt or ERK to demonstrate equal loading of lanes. The experiments were repeated three times with similar results.

## METHODS

***Reagents***— Monoclonal β-catenin antibody was obtained from BD Biosciences. Polyclonal IGF1Rβ, histone H3, Myc-tag, Na-K-ATPase, IRS1, LEF and cyclin D1 antibodies were from Cell Signaling Technology. Polyclonal GAPDH antibody was from Santa Cruz Biotechnology. All other reagents unless stated otherwise came from Sigma-Aldrich.

***Cell Cultures***—HeLa (human cervical carcinoma) and SKUT-1 (human leiomyosarcoma) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen), H1299 cells (human non-small cell lung carcinoma) and the human melanoma cell line DFB were maintained in RPMI-1640 (Invitrogen). R- cells (mouse embryo 3T3-like fibroblasts with targeted disruption of the IGF1R gene) and R- stably transfected with either human wild type (wt) IGF1R (R+) or IGF1R mutant lacking the IRS1 binding site (R-Δ-IRS1) were kind gifts from Dr Renato Baserga (Thomas Jefferson University, USA). R+, R- and R-Δ-IRS1 were maintained in DMEM supplemented with 100 μg/ml hygromycin, 100 μg/ml G418 and 250 μg/ml G418 respectively. All cell media were supplemented with 10 % (v/v) fetal bovine serum and 5 mg/ml penicillin/streptomycin.

***Plasmids*** — The plasmid containing wt human IGF1R cDNA was a kind gift from Dr. Renato Baserga (Thomas Jefferson University, USA). The triple SUMO mutant (TSM) IGF1R was constructed using QuickChange site-directed mutagenesis kit (Stratagene) to introduce three point mutations at K1025, K1100R, and K1120R into the wt-IGF1R

plasmid causing inhibition of SUMOylation and nuclear translocation of the receptor. TOPflash/FOPflash plasmids were purchased from Upstate Biotechnology. Human wt and Δβ–cat LEF1 plasmid, which lacks the N-terminal β–catenin binding domain, was subcloned from a LEF1 cDNA clone (Addgene plasmid 16709) and inserted into a EcoRI and BamHI digested pcDNA3 expression vector with an N-terminal 3xMyc tag. Primer sequences were; forward wt 5'- GGAAGATCTGGAATTCATGCCCCAACTTTCCGG-'3, forward Δβ–cat 5'-GGAAGATCTGGAATTCAGCAACGGACACGAGGTG-'3 and reverse 5'-GCGGATATCAGGATCCTCAGATGTAGGCAGCTGTCATTC-'3. Correct sequences were confirmed by sequencing. Renilla luciferase vector (pGL4.70[*hRluc*]) was purchased from Promega. Cyclin D1 promotor plasmid was a kind gift from Dr Dan Grandér (Karolinska Institute, Sweden) and Axin2 promotor plasmid was purchased from Addgene (Addgene plasmid 25701).

***Co-Immunoprecipitation/Western blot***—Cells were lysed in 50mM Tris-HCl (pH7.5), 150mM NaCl, 5mM EDTA, supplemented with complete protease and phosphatase inhibitor cocktails. After pre-clearing (10 µl of Dynabeads Protein G (Invitrogen) for 1h at 4$^{o}$C) the lysates were incubated with 30 µl of Dynabeads coupled with 1 µg of antibody overnight. The immuno-complexes were washed three times with lysis buffer and eluted by boiling in SDS sample buffer. Qproteome Cell Compartment kit (Qiagen) was used according to the manufacturer's protocol for subcellular fractionations. Each fraction was diluted 1:1 with lysis buffer followed by co-immunoprecipitation. Protein lysates were separated by SDS-PAGE and transferred onto a nitrocellulose membrane (Amersham) and detected by Western blotting using primary antibodies overnight

followed by incubation with horseradish peroxidase conjugated secondary antibody and visualized with ECL detection reagents (GE Healthcare).

***Dual luficerase assay***— TCF/LEF activity was measured using the TOPflash/FOPflash Firefly luciferase assay system (Upstate). Cells were co-transfected with wt-IGF1R, TSM-IGF1R or mock for 48h before the assay was performed. The results are expressed as the ratio of TOPflash/FOPflash luciferase activity after normalization with *Renilla* luciferase. Cyclin D1 and Axin2 promoter analysis were conducted by co-transfecting SKUT-1 cells with the cylin D1 or Axin2 promotor Fire Fly luciferase construct, *Renilla* luciferase, and wt-IGF1R, TSM-IGF1R or mock for 48h. Both TCF/LEF activity and cyclin D1 or Axin2-dependent promotor activity were determined with Dual Luciferase Assay System (Promega).

***Protein quantification***–Protein quantification from Western blots was analysed with ImageJ. To determine that R-ΔIRS1 has relatively equal amount of nuclear IGF1R as R+ the IGF1R expression was normalized against its fractionation controls, and the nuclear ratios were calculated for the both cell lines. Co-IP between LEF1 and IGF1R was first normalized to LEF1. Total levels of IGF1R of the two cell lines were normalized to GAPDH. The ratios between IGF1R in total cell lysate and LEF1-IGF1R complex were compared between R+ and R-ΔIRS1.

***Statistical analysis***-Using normalized raw data from at least three independent experiments, ANOVA with a 95 % confidence interval were conducted for the Axin2 and cyclin D1 promotor activity and protein levels.