cancers

Supplementary Materials: TSPAN1: A Novel Protein Involved in Head and Neck Squamous Cell Carcinoma Chemoresistance

Yoelsis Garcia-Mayea, Cristina Mir, Laia Carballo, Josep Castellvi, Jordi Temprana-Salvador, Juan Lorente, Sergi Benavente, Juana M. García-Pedrero, Eva Allonca, Juan P. Rodrigo and Matilde E. LLeonart

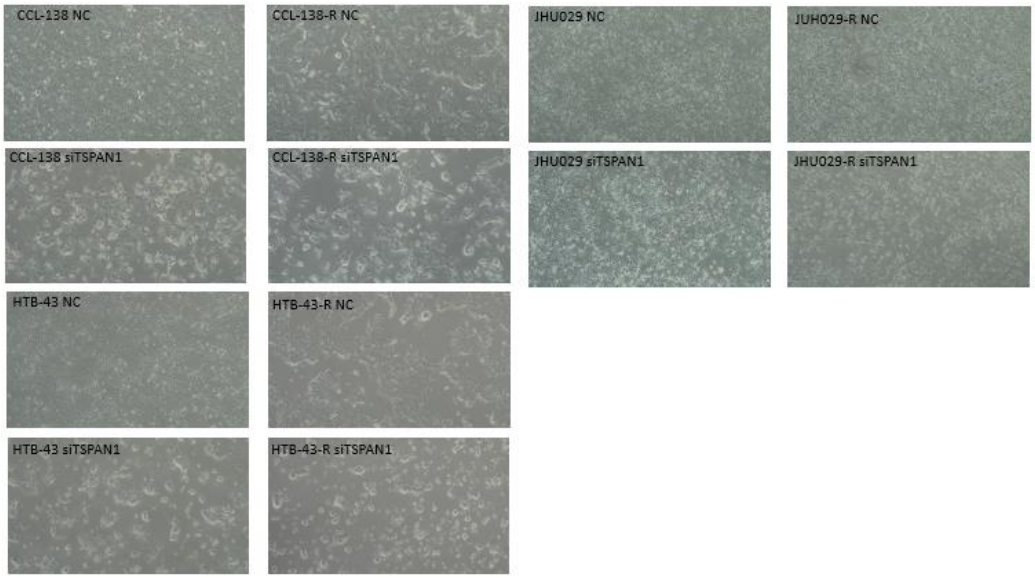
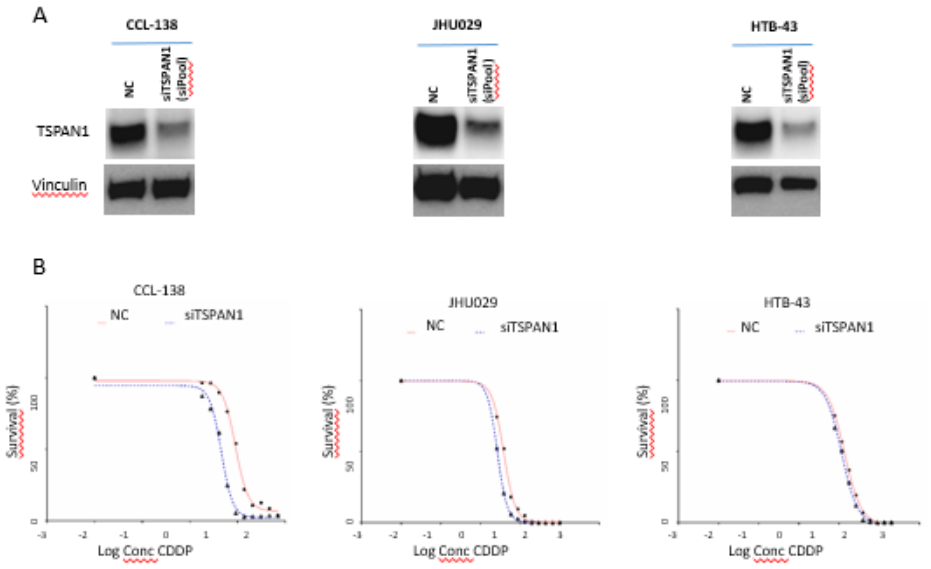


Figure S1. TSPAN1 depletion inhibits cell proliferation. Pictures show the decrease in cell number in conditions where TSPAN1 was depleted (pictures were taken 72 h after transfections, 4X magnification).

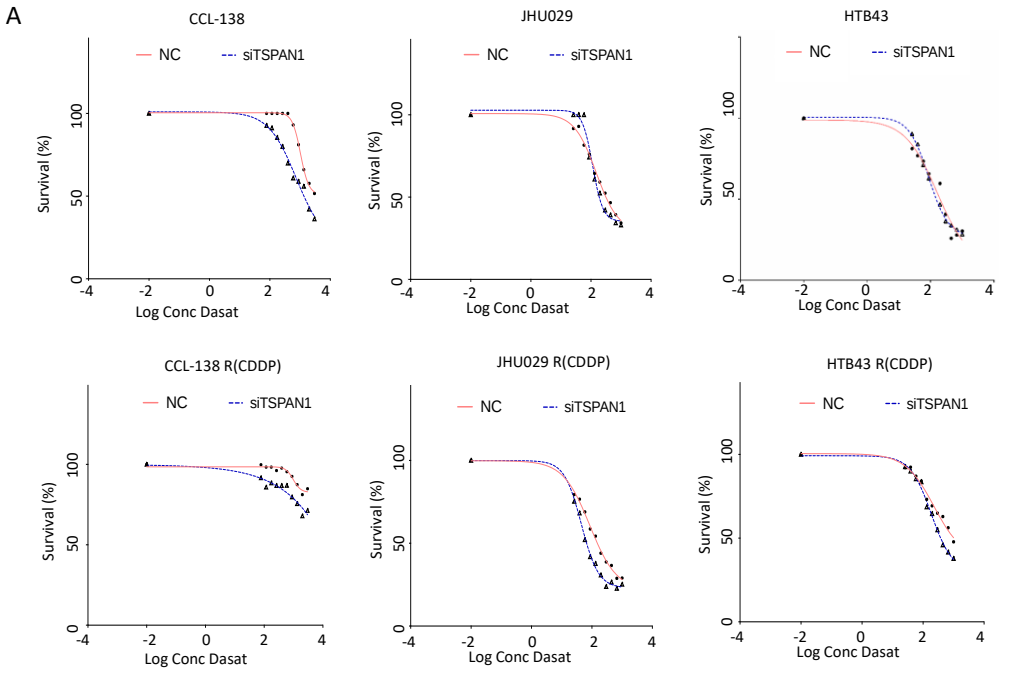


C

CCL-138		JHU029		HTB-43	
NC	siTSPAN1	NC	siTSPAN1	NC	siTSPAN1
9.0	4,6	3.8	2.7	13.6	11.5

Figure S2. TSPAN1 inhibition under an alternative specific mix of 30 siRNA (siPool) sensitizes HNSCC cells to the effects of CDDP. (**A**) Western blot in CCL-138, JHU029 and HTB-43 cell lines

under TSPAN1 inhibition with the siRNA again TSPAN1 (siPool). (**B**) siPool against TSPAN1 induces CDDP sensibilization in HNSCC cell lines. (**C**) IC50 values to CDDP (μM) from panel B.



B

	CCL-138		JHU029		HTB-43	
	NC	**siTSPAN1**	**NC**	**siTSPAN1**	**NC**	**siTSPAN1**
Parentals	1035	748.0	150.1	114.3	159.9	86.6
R(CDDP)	982.0	----	85.33	44.85	230.2	185.4

Figure S3. TSPAN1 inhibition sensitizes HNSCC cells to the effects of dasatinib. (**A**) Plots indicating the effect of dasatinib in cells inhibited for TSPAN1 versus control cells. (**B**) IC50 values to dasatinib (nM) of the indicated cell lines.

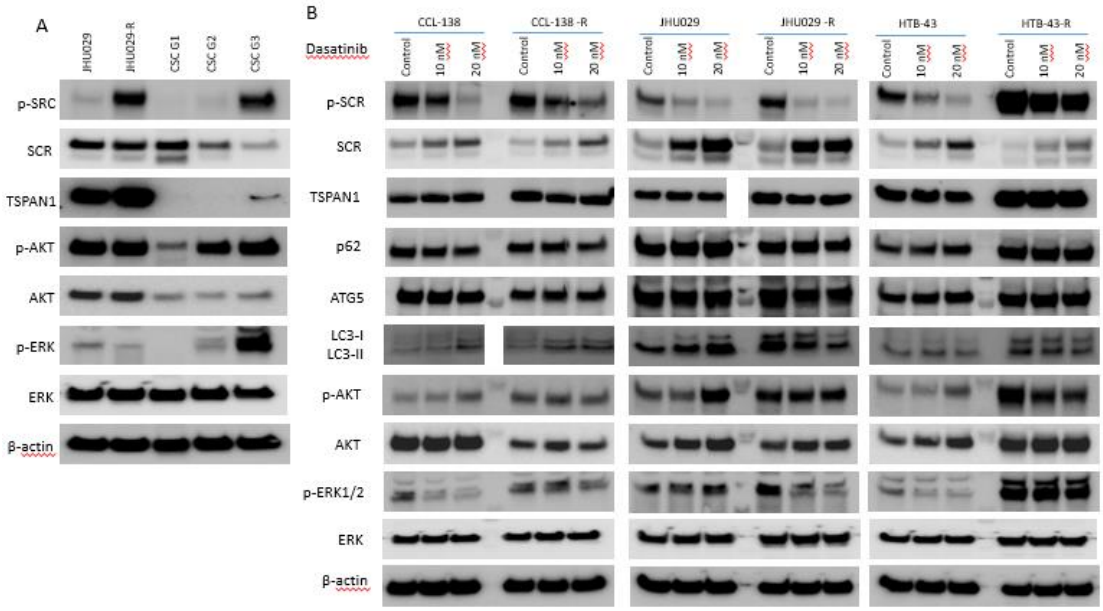


Figure S4. inhibition of SRC activation (p-SRC) through dasatinib does not affect TSPAN1 expression either TSPAN1 downstream pathway. (**A**) p-SRC, TSPAN1, p-AKT and p-ERK expression in JHU029, JHU029-R cells and different generations of CSCs (G1, G2 and G3). Note that G1 derives directly from parental cells growing in non-adherent conditions and G3 is the most enriched CSCs population. (**B**)

The inhibition of p-SRC through dasatinib does not affect TSPAN1 expression or its downstream targets.

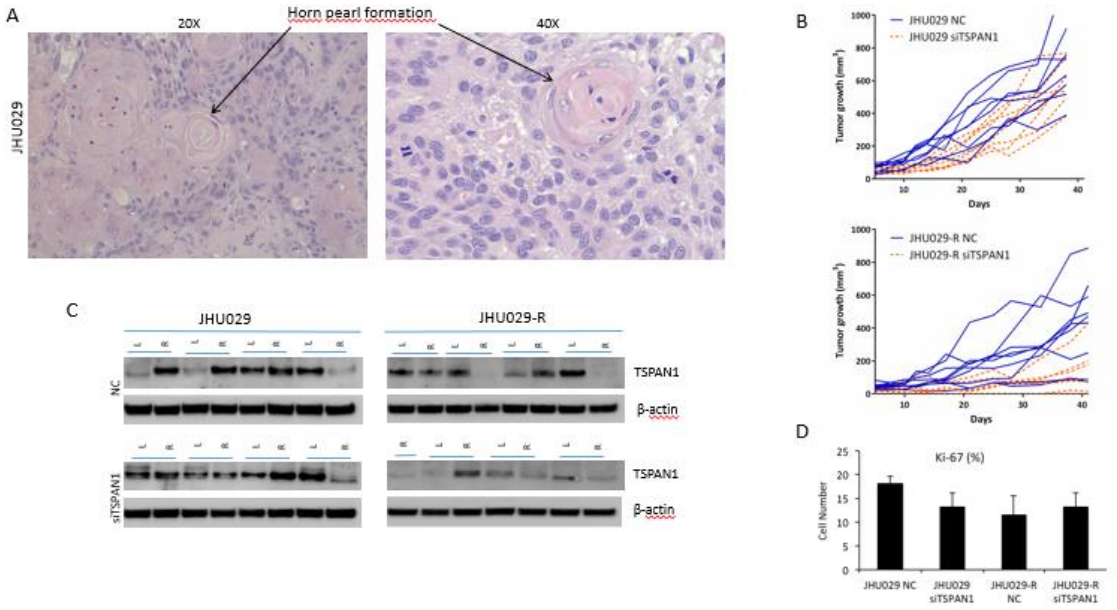


Figure S5. Phenotypic characteristics, TSPAN1 levels, and proliferative potential of tumors formed in mice. (**A**) Formation of laryngeal tumors by injection of JHU029 cells mimicking the horn pearls characteristic of human HNSCC. (**B**) Plots indicating the tumor growth (JHU029 and JHU029-R tumors) in each individual mouse. (**C**) TSPAN1 levels at the endpoint in the tumors formed in mice. (**D**) Percentage of Ki-67 positive cells in the indicated tumor types. Note the presence of proliferative cells given by the positivity of the proliferative marker Ki-67.

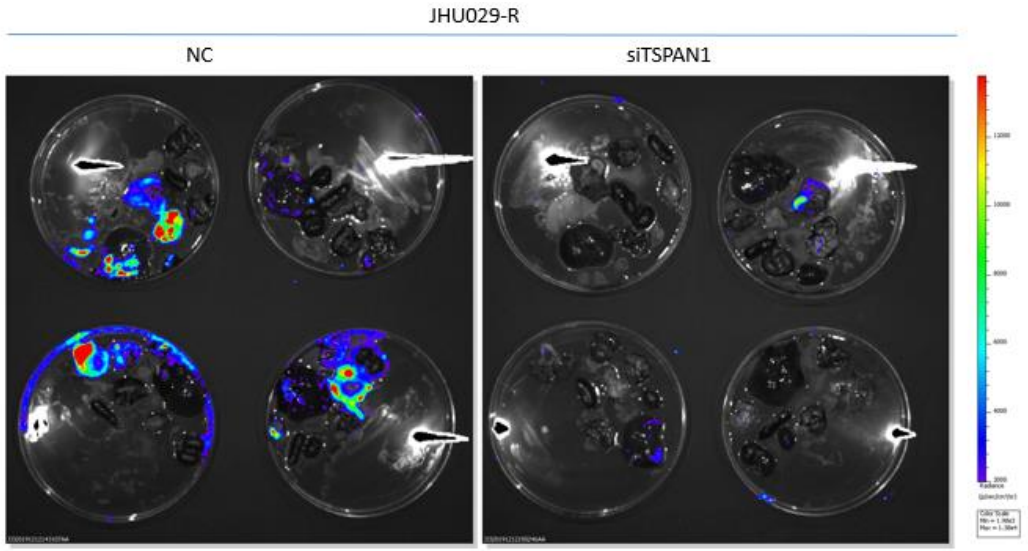


Figure S6. Ex vivo analysis of micrometastases under TSPAN1 depletion. Pictures of the presence of luminescent tumoral cells detected by IVIS showing the presence of micrometastases in several organs including liver, lung and bones.

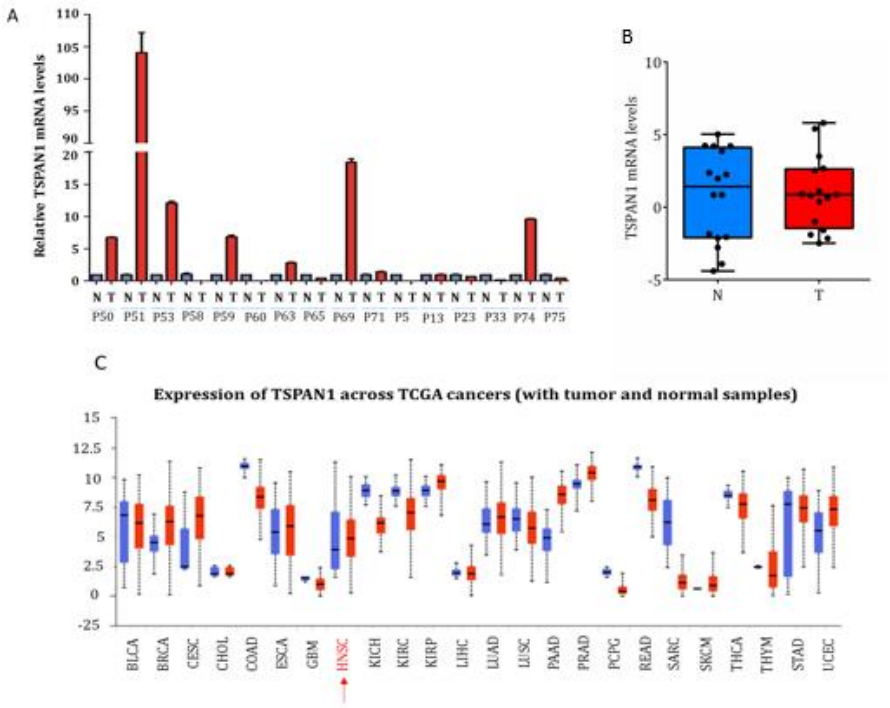


Figure S7. TSPAN1 expression at mRNA level. (**A**) mRNA TSPAN1 expression levels in normal (N) and tumor tissue (T) in fresh biopsies from 16 patients with HNSCC. (**B**) mRNA TSPAN1 expression levels considering the 16 patients included in panel A, non-significant difference were found between both groups (Wilcoxon test, $p>0.05$) (**C**) mRNA levels of TSPAN1 in different tumor types according to the TCGA databases. Normal (blue) and tumor tissue (red) are indicated for each tumor type. Note that in pancreatic adenoma, prostate adenocarcinoma and cervical squamous cell carcinoma show the most increment in tumor tissue versus normal tissue. BLCA, Bladder Urothelial Carcinoma; BRCA, Breast Carcinoma; CESC, Cervical Squamous Cell Carcinoma; CHOL, Cholangiocarcinoma; COAD, Colon Adenocarcinoma; ESCA, Esophageal Carcinoma; GBM, Glioblastoma Multiforme; HNSC, Head and Neck Squamous Cell Carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney Renal Clear Cell Carcinoma; KIRP, Kidney Renal Papillary Cell Carcinoma; LIHC, Liver Hepatocellular Carcinoma; LUAD, Lung Adenocarcinoma; LUSC, Lung Squamous Cell Carcinoma; PAAD, Pancreatic Adenocarcinoma; PRAD, Prostate Adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; READ, Rectum Adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; THCA, Thyroid Carcinoma; THYM, Thymoma; STAD, Stomach Adenocarcinoma; UCEC, Uterine Corpus Endometrial Carcinoma.

Table S1. General characteristics of the 106 patients analyzed in this study. The number of patients corresponding to each feature is shown. No, number of patients.

Characteristic	No.
- pT classification	
T1-T2	23
T3-T4	83
- pN classification	
N0	31
N1-3	75
- Disease stage	
I-II	8
III-IV	98
- Pathological grade	
Well differentiated	26
Moderately differentiated	50
Poorly differentiated	30
- Site	
Pharynx	53
Larynx	53
- Tumor Recurrence	
No	40
Yes	66
Total Cases	106