

SUPPLEMENTARY FIGURE LEGENDS:

Supplementary Fig. 1. **A)** Western blot analysis of cleaved-PARP-1, p53 and γ -H2AX protein levels in U2OS cells in untreated condition and after 1, 3, 6, 18 and 24 hrs of etoposide. **B)** Scatter plot shows the mean intensity of TSPYL2 in U2OS nuclei in unstressed condition and after etoposide treatment. Average \pm S.E.M are indicated in red. P-value was calculated using Student's t-test. **C)** Proliferation rate analyses of the indicated cancer cell lines. No statistically significant difference among the cell lines was found according to ANOVA test. **D)** Cell cycle profile analyses by flow cytometry of the indicated cancer cell lines. **E)** Western blot analysis of TSPYL2 levels in different human cancer cell lines in untreated conditions. **F)** Examples of EdU and TSPYL2 staining in untreated and etoposide treated cells. **G)** Chart representing the percentage of EdU positive and negative cells with accumulated TSPYL2 after etoposide treatment. **H)** Immunofluorescence staining of TSPYL2 and γ -H2AX in untreated and etoposide treated U2OS cells.

Supplementary Fig. 2. **A)** The MA0024.3 matrix of the JASPAR CORE database (left) and visualization of E2F1 binding site position between -164bp and -176bp from the TSPYL2 ATG start site (right). **B)** E2F1 binding site on TSPYL2 promoter is conserved among species. **C)** Western blot analysis of ectopic E2F1 levels upon etoposide treatment in U2OS cells transfected with MOCK and FLAG-E2F1 expression vectors, used for luciferase assays.

Supplementary Fig. 3. Western blot analysis of TSPYL2 levels in MG-63 (left) and A549 (right) cells treated with MG-132 for 8 hrs, in untreated and etoposide-treated conditions.

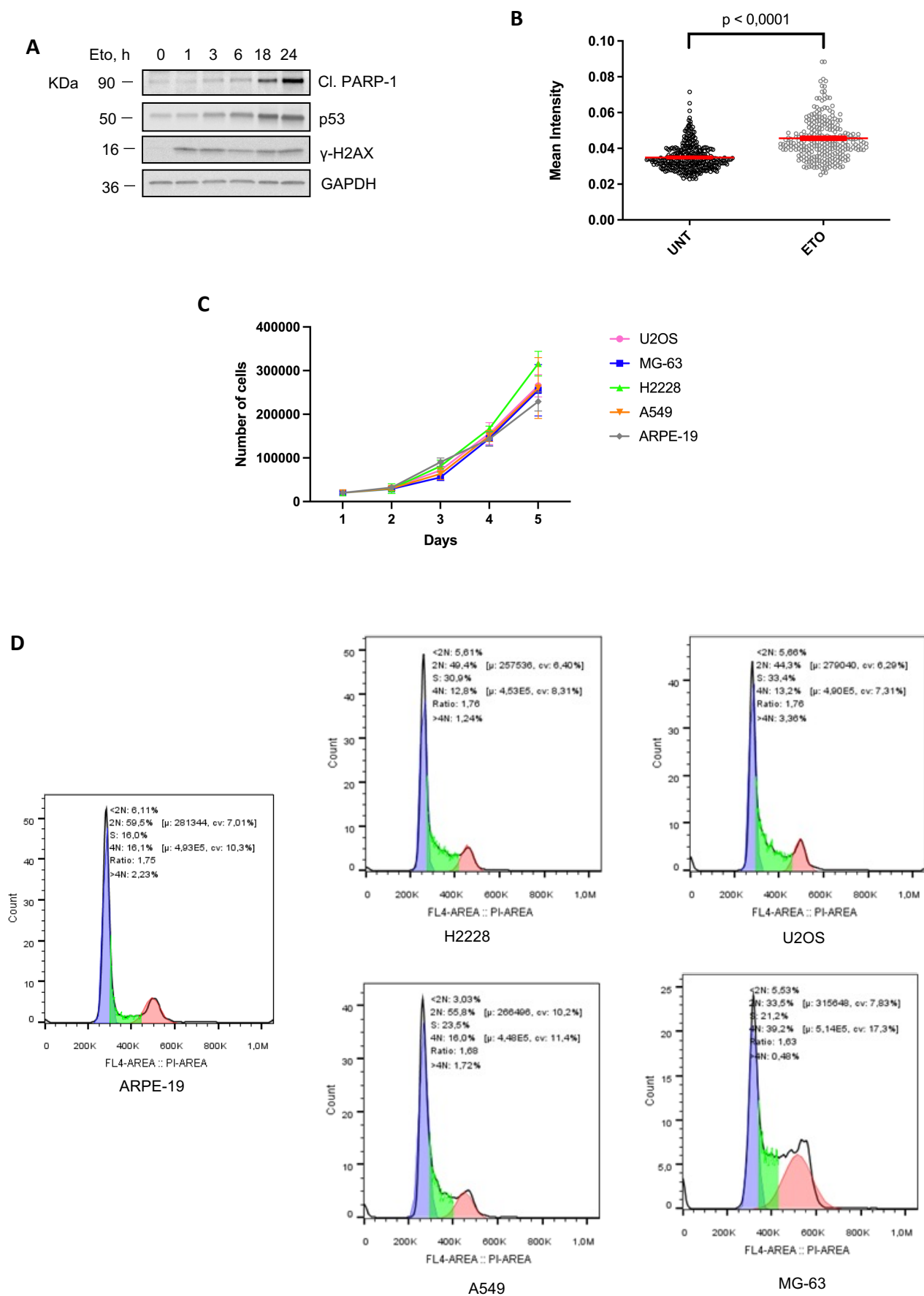
Supplementary Fig. 4. **A)** Western blot analysis of cleaved PARP-1 and acetylated-p53 levels after etoposide treatment for 24 and 48 hrs, in TSPYL2 depleted U2OS and **B)** MG-63 cells.

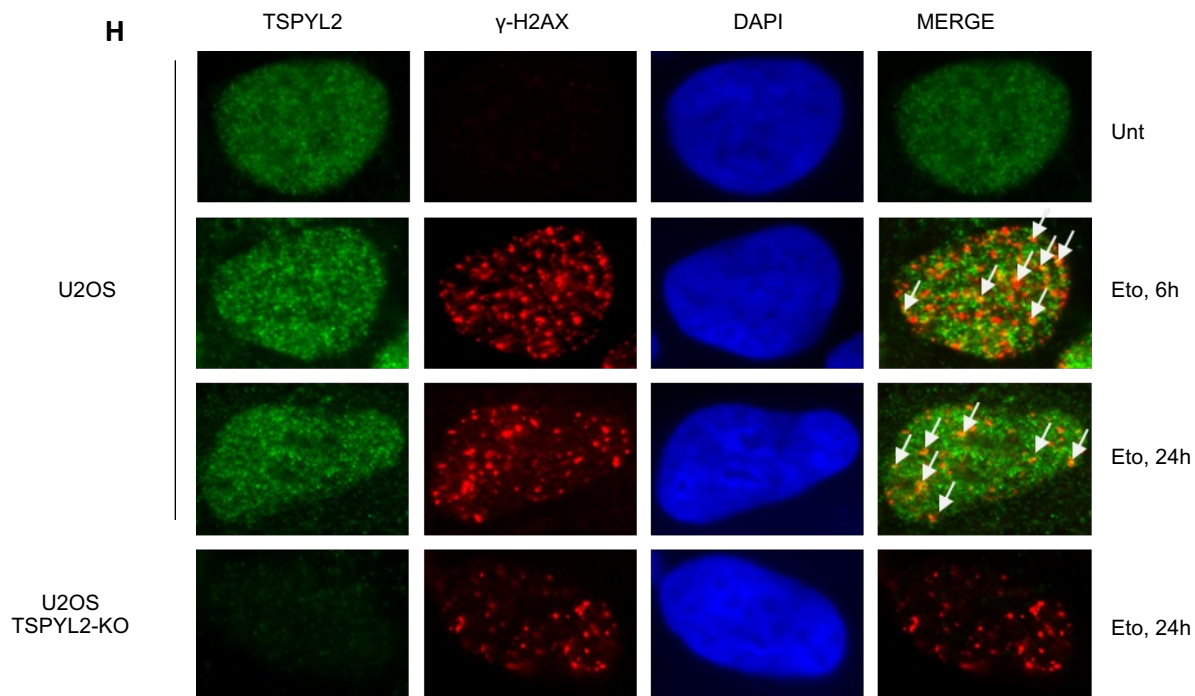
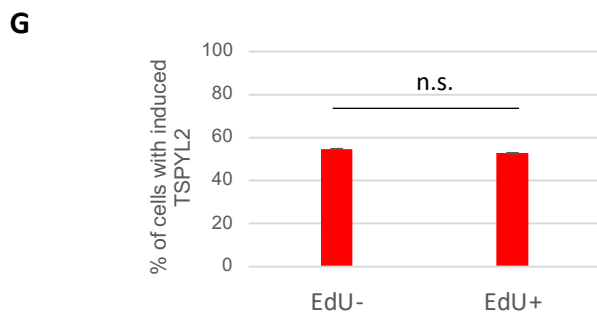
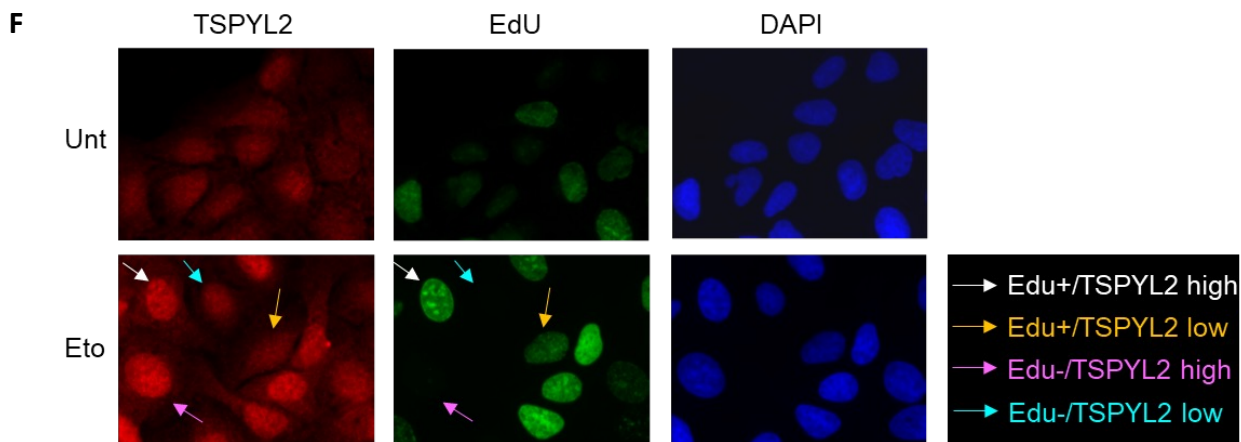
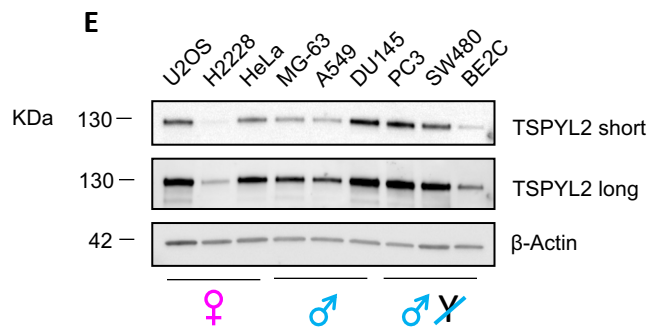
Supplementary Fig. 5. **A)** TCGA data available for TSPYL2 gene according to the sex in different cancer types. **B)** Types and distribution of TSPYL2 mutations in the different cancer cases of the TCGA database. **C)** Mutations localization along TSPYL2 protein in all, sex-related and somatic tumors. **D)** Kaplan-Meier plots showing the percentage of patients who are alive at a time point in different sex specific tumors according to TSPYL2 expression level (high in red and low in blue). **E)** Kaplan-Meier plots, obtained from “The Human Protein Atlas” web site, summarizing the prognostic role of TSPYL2.

Supplementary Table I

List of the tumor types investigated during TCGA data analyses aimed at the identification of TSPYL2 mutations.

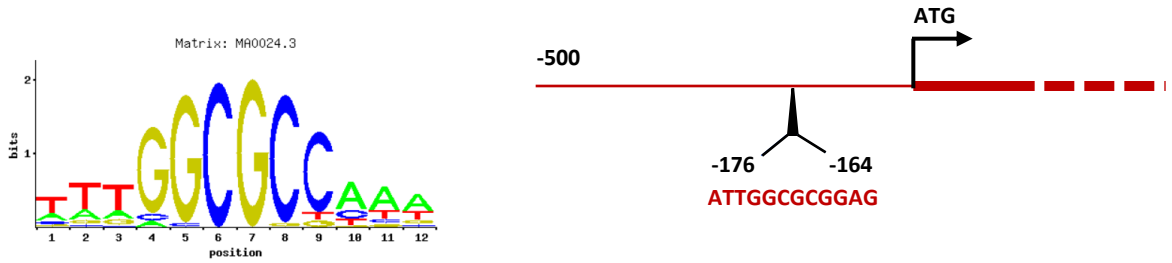
Supplementary Figure 1





Supplementary Figure 2

A E2F1 JASPAR_CORE_2016,MA0024.3,MA0024.3



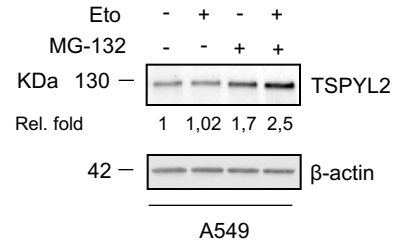
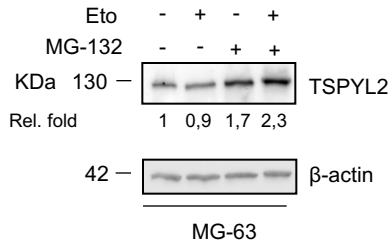
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human/1-35	1 aggagtgggcggggccttcggcg	a-ttggcgcggg	-ag-g	35
orangutan/1-35	1 aggagtgggcggggccttcggcg	a-ttggcgcggg	-ag-g	35
rhesus/1-35	1 aggagcgggcggggccttcggcg	a-ttggcgcggg	-ag-g	35
baboon/1-35	1 aggagcgggcggggccttcggcg	a-ttggcgcggg	-ag-g	35
marmoset/1-35	1 aggagggggcggggccttcagcg	a-ttggcgcggg	-ag-g	35
bushbaby/1-30	1 agaag-gggcggggccttcggcta	- - - - agcca-ca-g		30
treeshrew/1-36	1 aggagtgggcggggccttcggcgg	-attggcgcggg	-agag	36
mouse/1-35	1 gggagtgggcggggccttcggcga	-taggcctg-tg-g		35
guinea-pig/1-35	1 aagagtgggcggggccttcggcaa	-ttggcgcggg	-ag-g	35
kangaroo-rat/1-35	1 aggagtgggcggggccttcagagg	-ctggcgcggg	-ag-g	35
pika/1-35	1 acgagtgggcgggatctctacga	-ttggcgcggg	-ag-g	35
rabbit/1-35	1 aggagtgggcggggacctcgacga	-ttggcgcggg	-ag-g	35
alpaca/1-33	1 -gcagggggcgggtgccca-gg	cg-ttggcgcggg	-ag-g	33
cow/1-33	1 -tgagggggcggggcct-gacga	-ttggcgcggg	-ag-g	33
dolphin/1-34	1 gcgagggggcggggctt-ggcgc	-ttggcgcggg	-ag-g	34
cat/1-35	1 cggagagggcggggccttcgacgc	-ttggcgcggg	-ag-g	35
dog/1-35	1 gggagggggcgggtcctgggcgc	-ttggcgcggg	-ag-g	35
horse/1-33	1 gggagagggaggg-ccgggcgc	-ttggcgcggg	-ag-g	33
microbat/1-34	1 gcg-aggggcgggaccgagcag	-ttggcgcggg	-ag-g	34
megabat/1-31	1 gggaaagggacgggacctgggc-	-tcggcgcg--g-g		31
hedgehog/1-35	1 gaggaagggaggggccggcgcct	-ttggcgcggg	-ag-g	35
elephant/1-35	1 gggtgggggcgggacattggcgcg	-ttggcgcggg	-ag-g	35
rock-hyrax/1-35	1 gggagggggcgggacgttggcgg	-ttggcgcggg	-ag-g	35
armadillo/1-34	1 - - - agagggcgggaaaccttcgcgg	-attggcgcggg	-ag-g	34

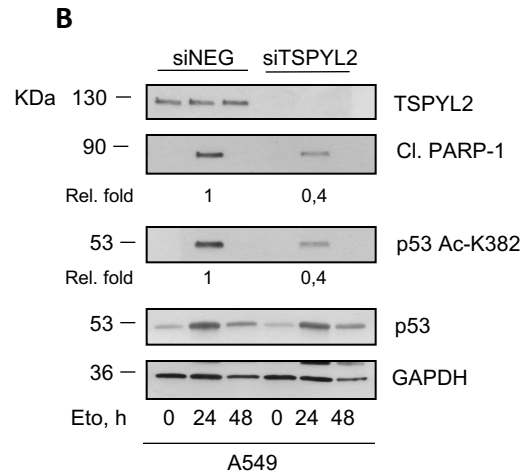
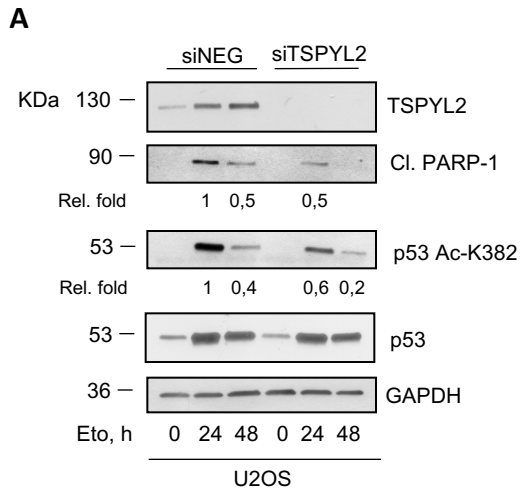
C

MOCK	+	-	+	-	
FLAG-E2F1	-	+	-	+	
KDa	60	-			FLAG
	42	-			β-actin
Eto	-	-	+	+	

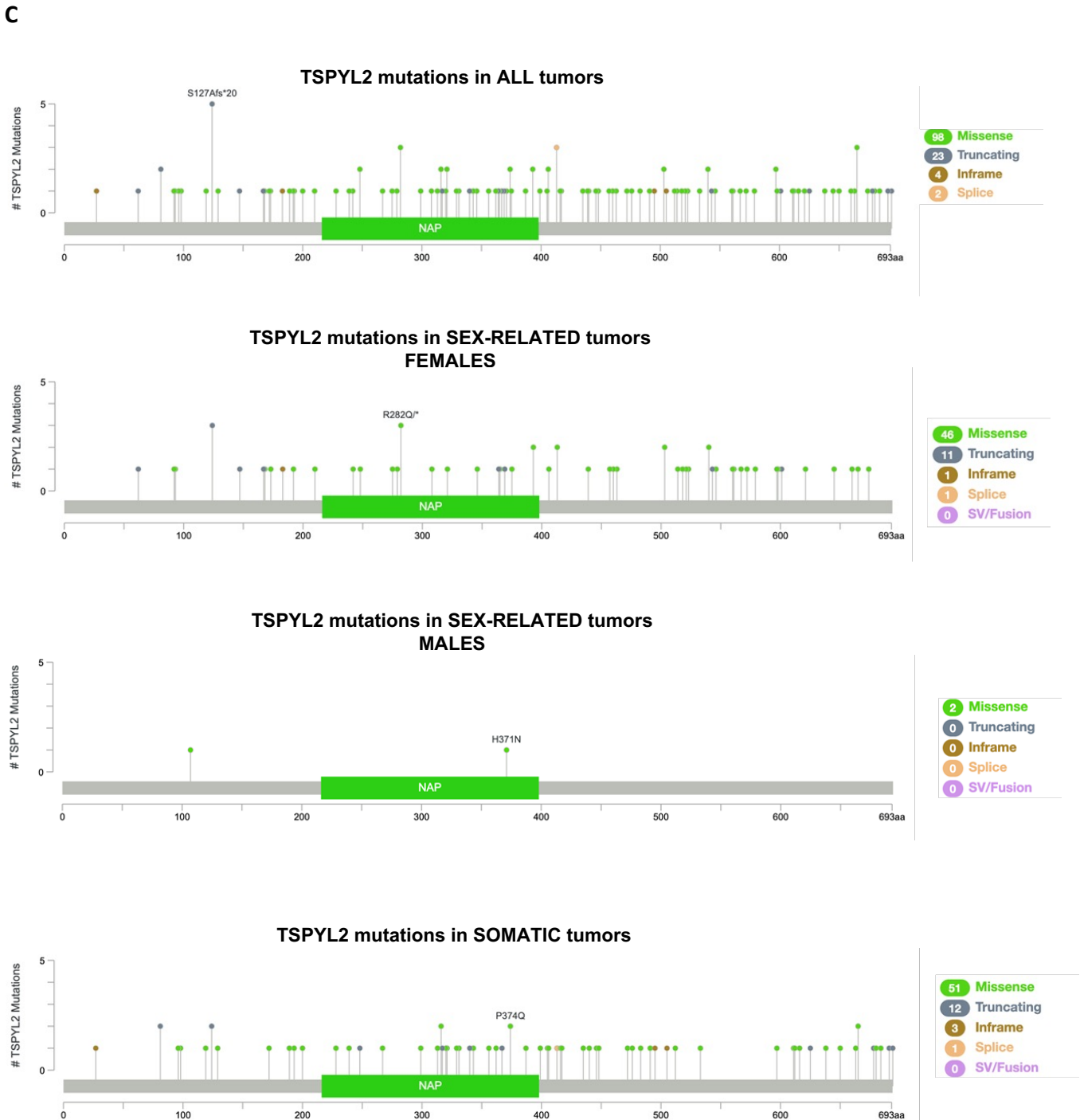
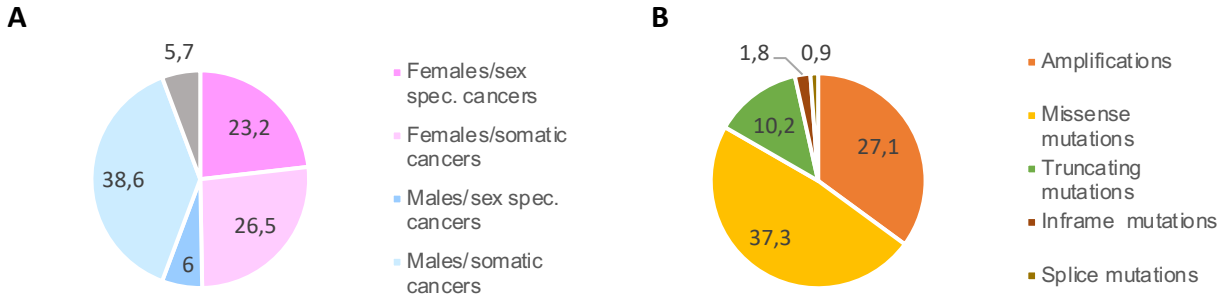
Supplementary Figure 3

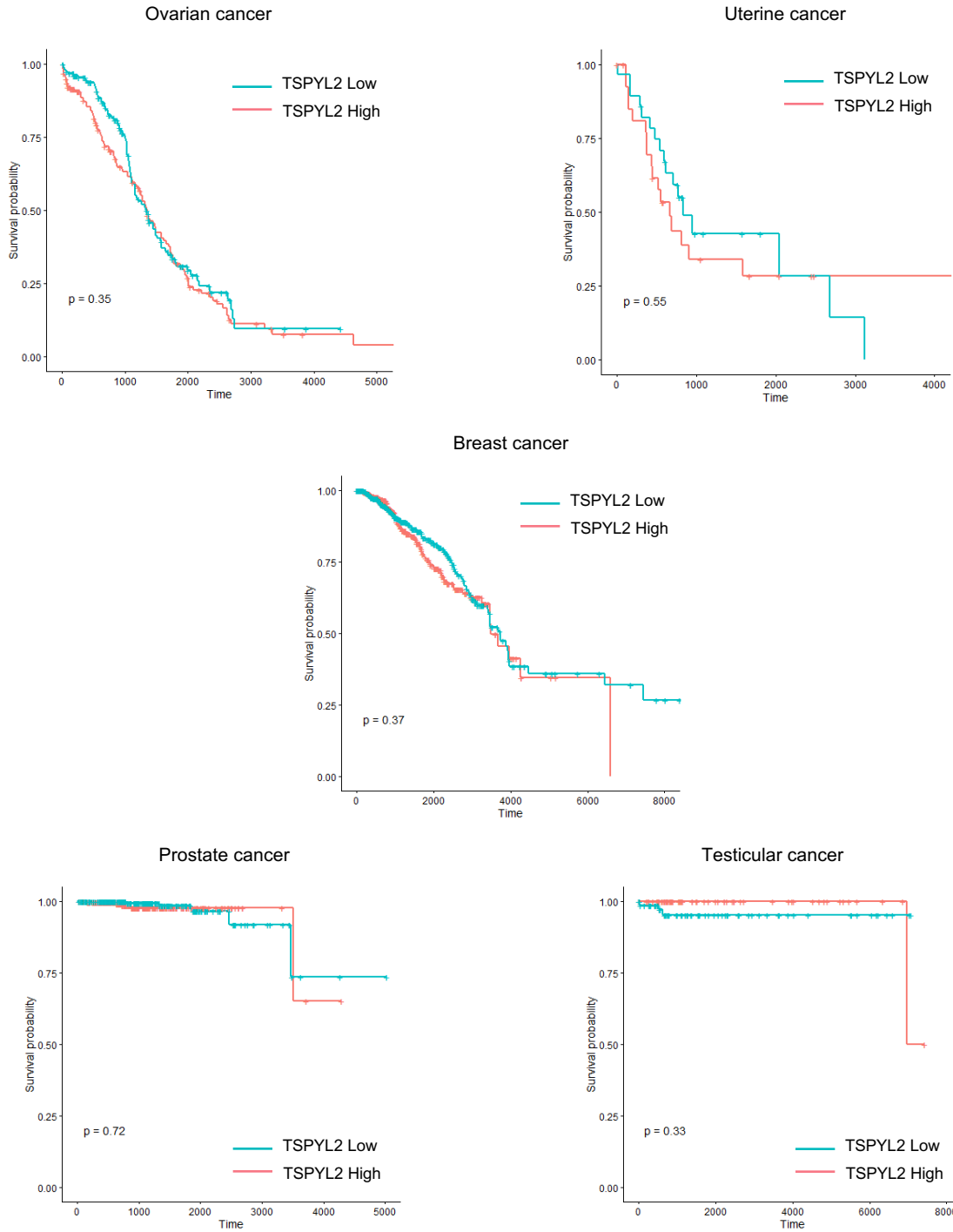
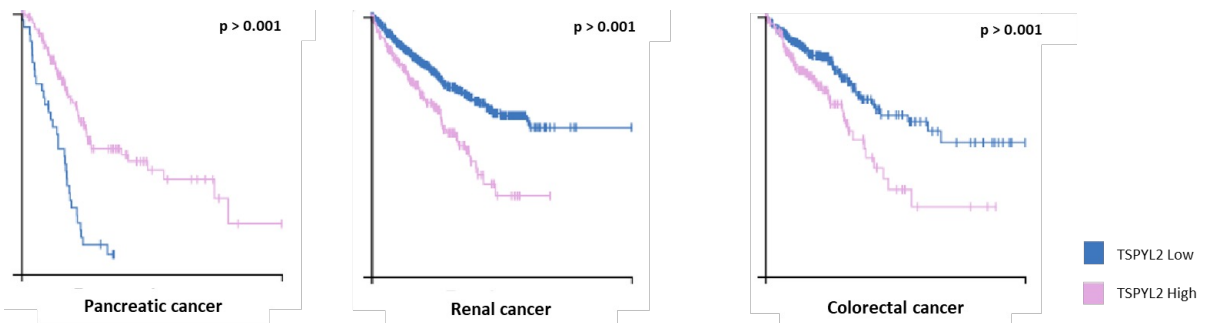


Supplementary Figure 4



Supplementary Figure 5



D**E**

Supplementary Table I

SEX-RELATED TUMORS		SOMATIC TUMORS
FEMALE	MALE	
Cervical Squamous Cell Carcinoma	Prostate Adenocarcinoma	Acute Myeloid Leukemia
Ovarian Serous Cystadenocarcinoma	Testicular Germ Cell Tumors	Adrenocortical Carcinoma
Uterine Carcinoma		Bladder Urothelial Carcinoma
Breast Invasive Carcinoma		Brain Lower Grade Glioma
		Cholangiocarcinoma
		Colorectal Adenocarcinoma
		Diffuse Large B-Cell Lymphoma
		Esophageal Carcinoma
		Glioblastoma Multiforme
		Head and Neck Squamous Cell Carcinoma
		Kidney Carcinoma
		Liver Carcinoma
		Lung Carcinoma
		Mesothelioma
		Pancreatic Adenocarcinoma
		Pheochromocytoma and Paranglioma
		Sarcoma
		Melanoma
		Stomach Adenocarcinoma
		Thymoma
		Thyroid Carcinoma