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Supplemental information

CD46 splice variant enhances translation of specific mRNAs linked to an aggressive tumor cell phenotype in bladder cancer

Jin Zeng, Hua Xu, Chunhua Huang, Yi Sun, Haibing Xiao, Gan Yu, Hui Zhou, Yangjun Zhang, Weimin Yao, Wei Xiao, Junhui Hu, Lily Wu, Jinchun Xing, Tao Wang, Zhiqiang Chen, Zhangqun Ye, and Ke Chen

Supplementary Figures

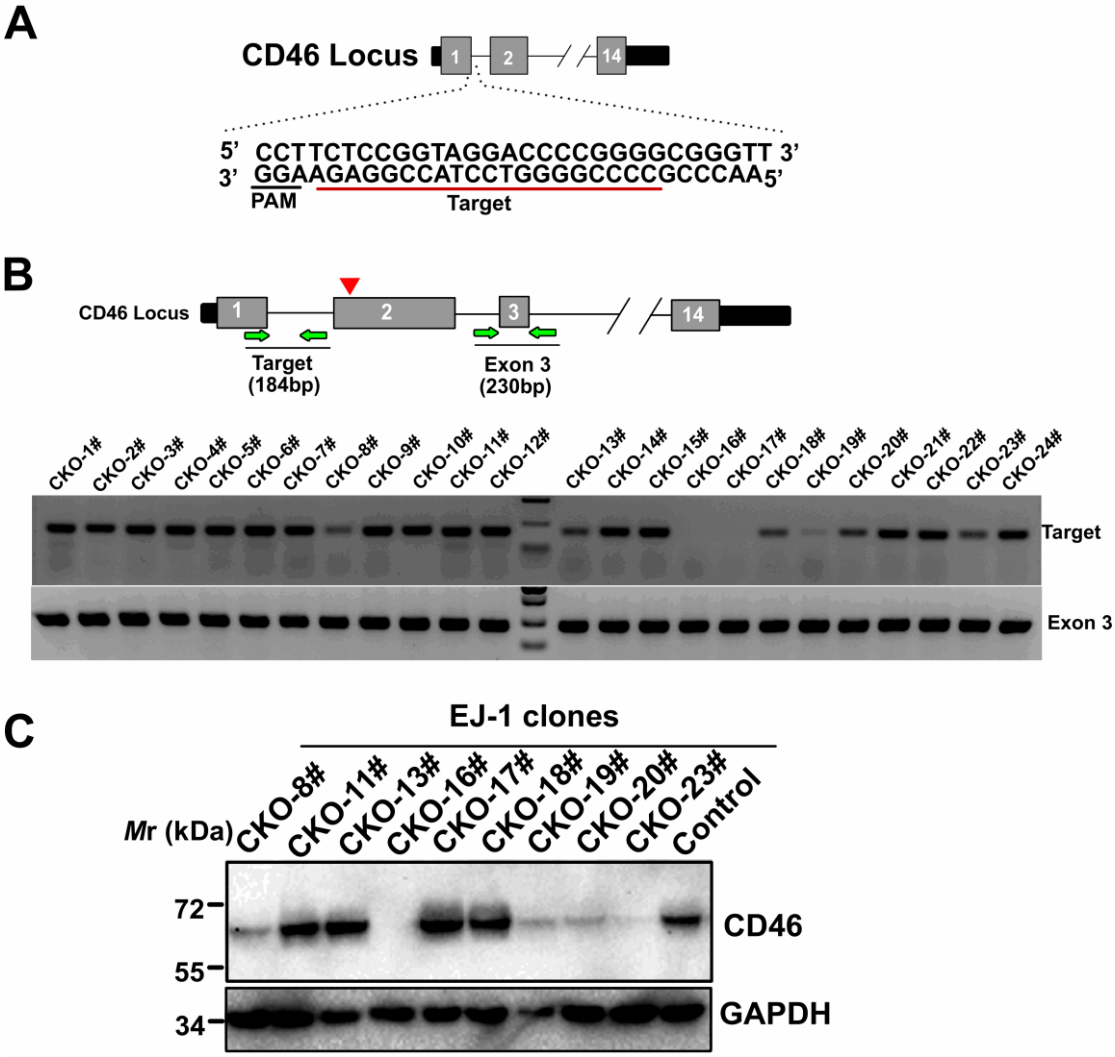

Figure S1. Knockout of CD46 in EJ-1 cells using CRISPR/Cas systems. (**A**) sgRNA design for targeting the human CD46 locus. (**B**) PCR analysis of different CD46-targeted clones. Genomic DNA was extracted, and then PCR was performed using primers on the edges of the sgRNA-site. A PCR product of Exon 3 of CD46 gene was used as control DNA. (**C**) Western blot analysis validated the depletion of CD46 protein in different CD46-targeted EJ-1 cell lines.

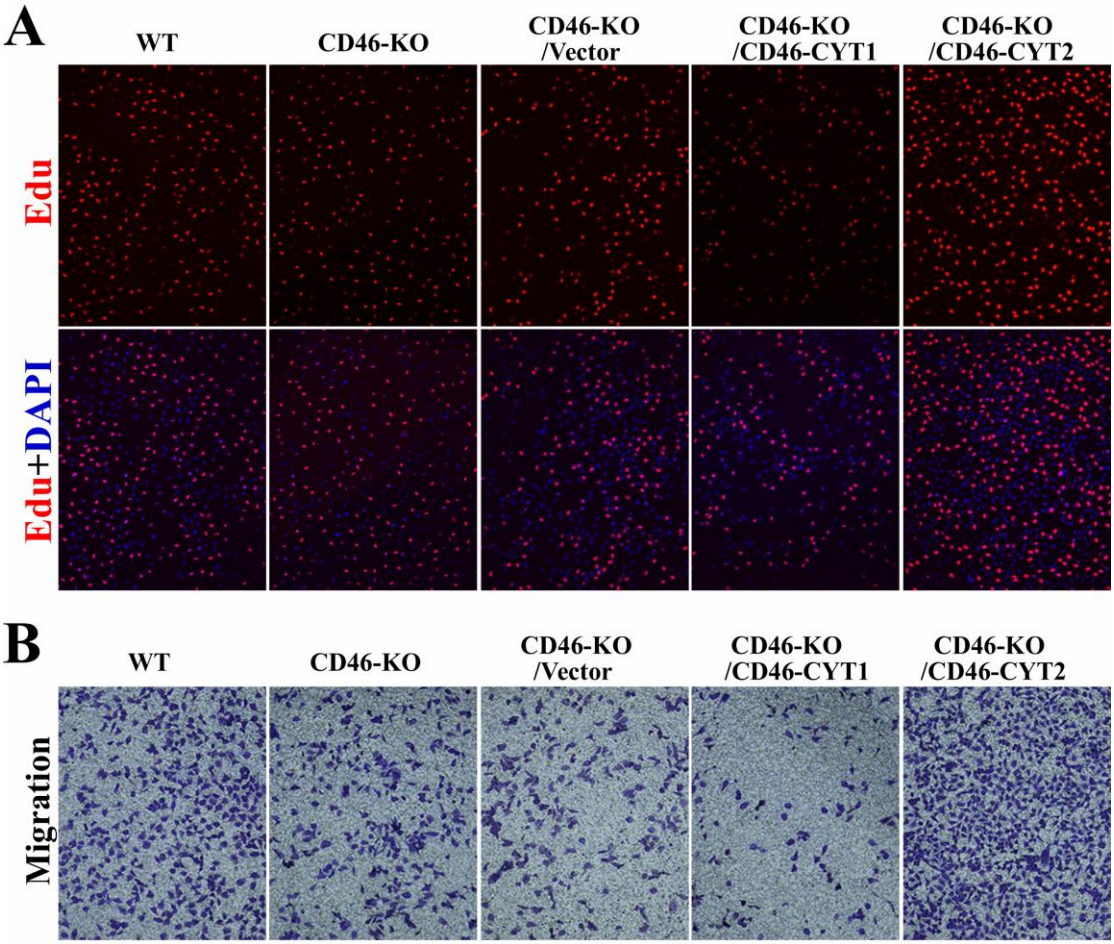

Figure S2. CD46-CYT1 and -CYT2 have opposite roles in bladder cancer development. (**A**) Representative micrographs of EdU incorporated-cells in indicated engineered cell lines. (**B**) Transwell cell migration assay for EJ-1 cells. Representative photographs were taken at × 200 magnification. Numbers of migrated cells were quantified in 5 random images from each treatment group which showed in Fig. 2E.

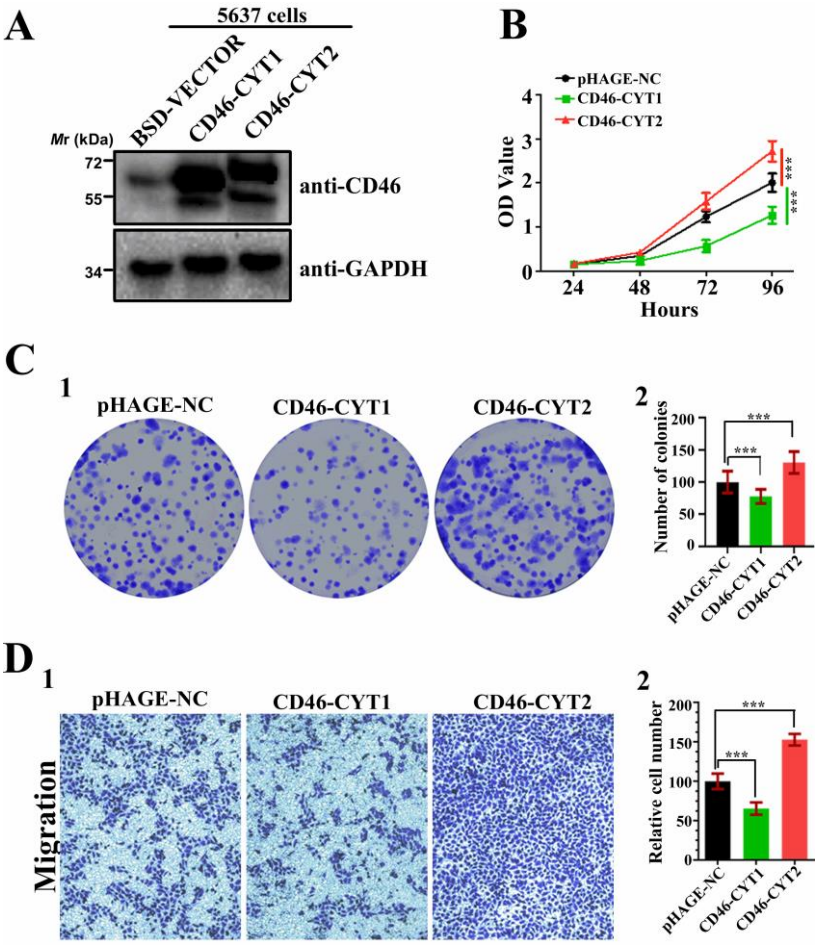

Figure S3. CD46-CYT1 and -CYT2 have opposite roles in 5637 cells.

(**A**) Generation of CD46-CYT1 and CD46-CYT2 stably overexpressing 5637 cells. Immunoblotting was performed to evaluate the expression of CD46. GAPDH is an internal control. (**B**) The cell viability was determined by CCK8 assays at indicated time points. The data represent mean ± SD and were analysed by Two-way ANOVA (n=3). ***P < 0.001. (**C**) Colony formation assay and quantification was performed with 5637 cells expressing CD46-CYT1 or CD46-CYT2. The data represent mean ± SD and were analysed by unpaired two-tailed Student's *t test* (n = 3). ***p<0.001. (**D**) Transwell cell migration assay for 5637 cells. Numbers of migrated cells were quantified in 5 random images from each treatment group. The data represent mean ± SD and were analysed by unpaired two-tailed Student's *t test* (n = 5). ***p < 0.001.

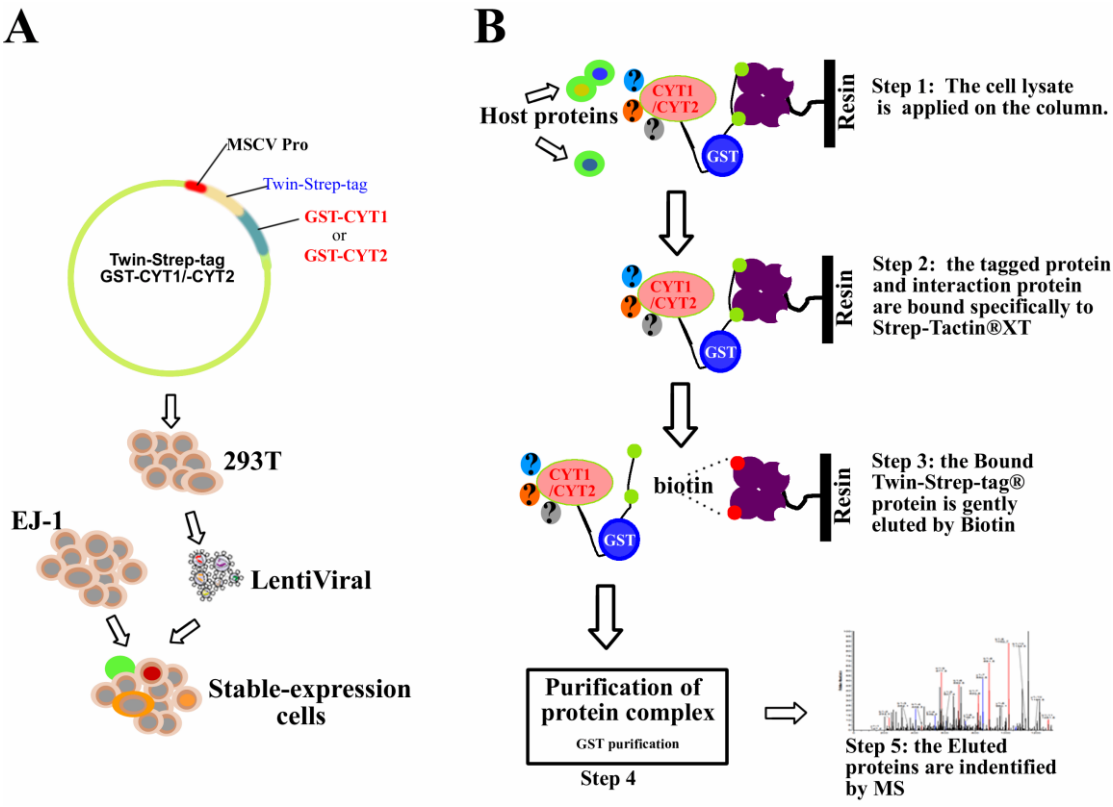

Figure S4. Flow-chart of tandem affinity purification (TAP) and mass spectrometry (MS) analysis. (**A**) The scheme for generation of StrepII-GST, StrepII-GST-CYT1 and StrepII-GST-CYT2 stably overexpressing EJ cells. (**B**) Purification scheme and analysis of CYT1 and CYT2 complexes tandem affinity purification (TAP) and mass spectrometry (MS) analysis.

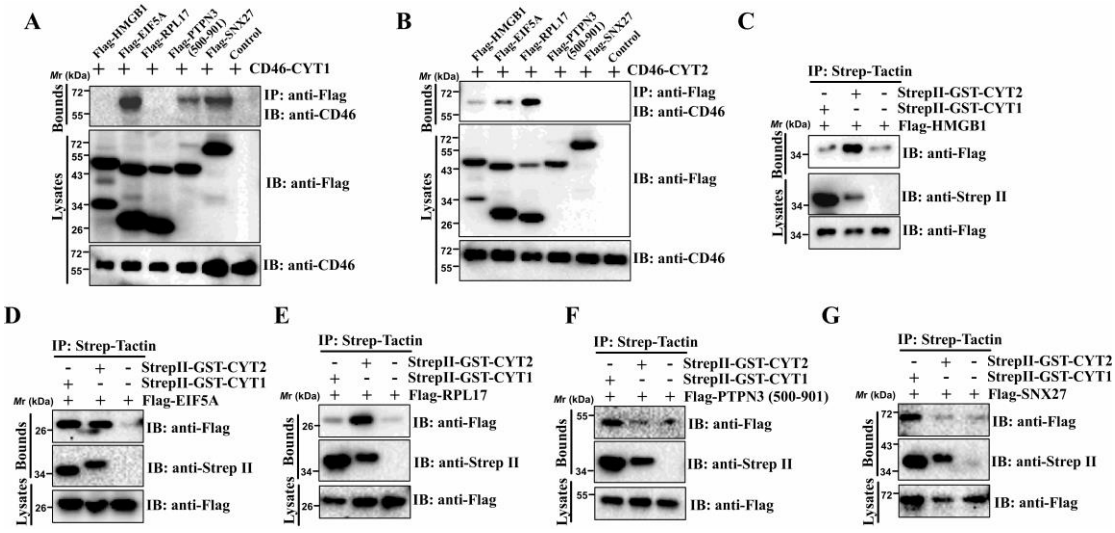

Figure S5. Co-Immunoprecipitation validation of TAP-MS data. (**A, B**) Cotransfection of CD46-CYT1 (A) or CD46-CYT2 (B) into CD46-KO EJ-1 cells was performed, together with Flag-HMGB1, Flag-EIF5A, Flag-RPL17, Flag-PTPN3 (500-901aa), Flag-SNX27, or an empty control plasmid psi-Flag, respectively. At 48h after transfection, the whole cell lysate was extracted for coimmunoprecipitation with anti-Flag, followed by probing with anti-CD46. (**C-G**) 293T cells were cotransfected with Flag-HMGB1 (C), Flag-EIF5A (D), Flag-RPL17 (E), Flag-PTPN3 (500-901aa) (F), or Flag-SNX27 (G) together with StrepII-GST-CYT1, or StrepII-GST-CYT2, respectively.

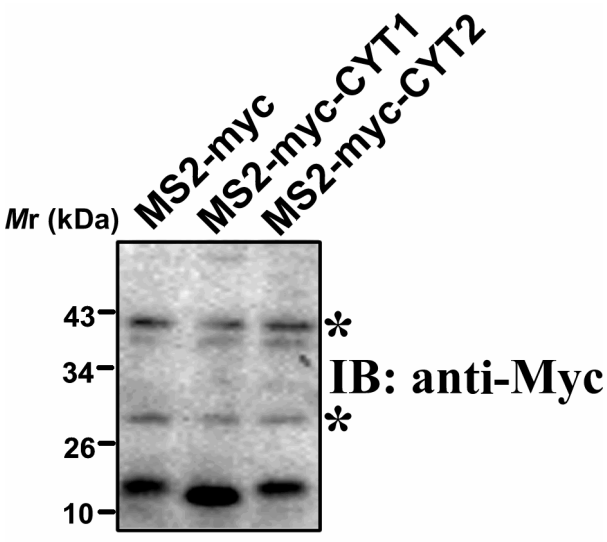

Figure S6. The expression of MS2-myc, MS2-myc-CYT1, and MS2-myc-CYT2 confirmed by Western blot. The EJ-1 cells transfected with MS2-myc (control), MS2-myc-CYT1 or MS2-myc-CYT2 with the indicated tethering reporter plasmid. Immunoblotting was performed to evaluate the expression of transfected plasmids. GAPDH is an internal control.

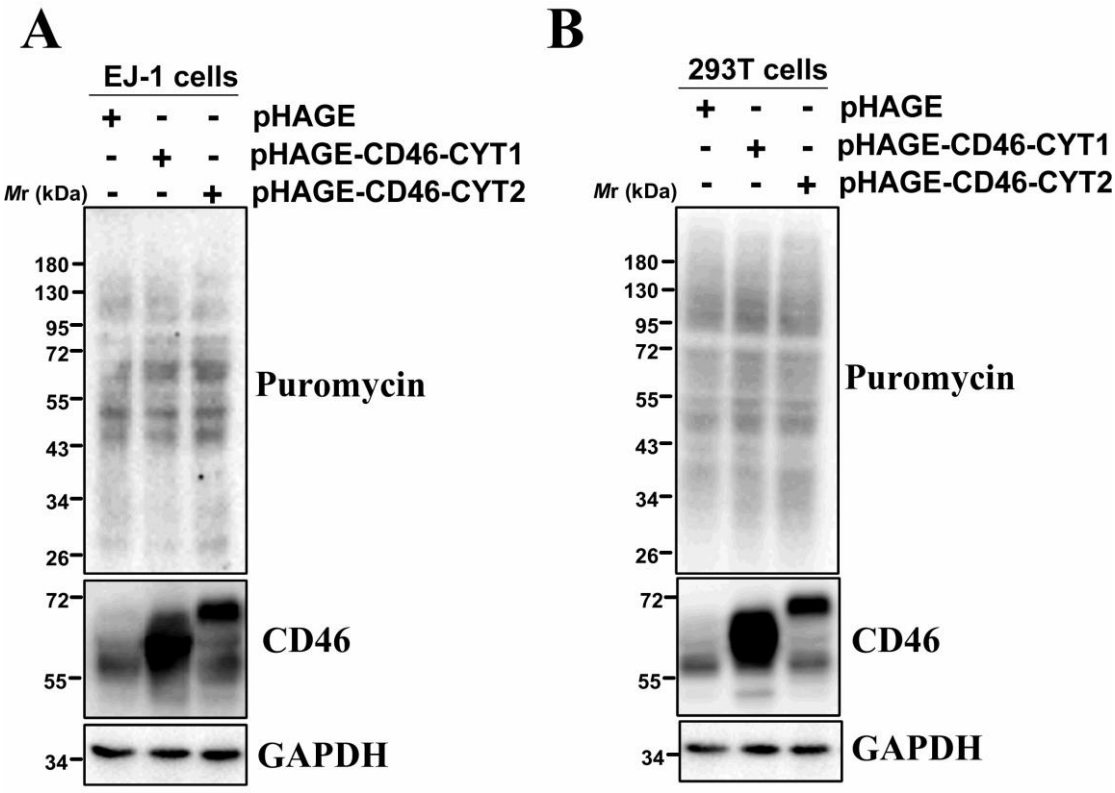

Figure S7. CD46 has no effect on global translation. (**A**, **B**) EJ-1 and 293T cells stably expressing pHAGE (vector control, pHAGE-CD46-CYT1 or -CYT2) were pre-treated with cycloheximide (CHX, 50 μg/ml) for 4 h to inhibit protein translation, followed by a pulse treatment with puromycin (0.5 μM) for 15 min and then harvested. The puromycin incorporated peptides were detected by Western blot analysis with puromycin antibody.

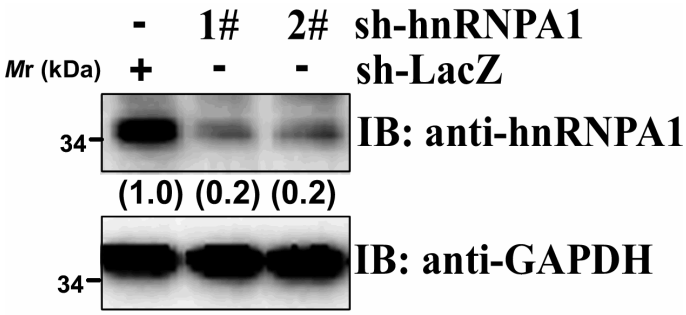

Figure S8. Generation of hnRNPA1 knockdown cells. EJ-1 cells were infected with lentiviruses expressing shRNA against hnRNPA1 or LacZ. Immunoblotting was performed to evaluate the expression of hnRNPA1. GAPDH is an internal control. Protein levels of hnRNPA1 are normalized against GAPDH and expressed as fold change relative to base expression determined using control sh-LacZ.

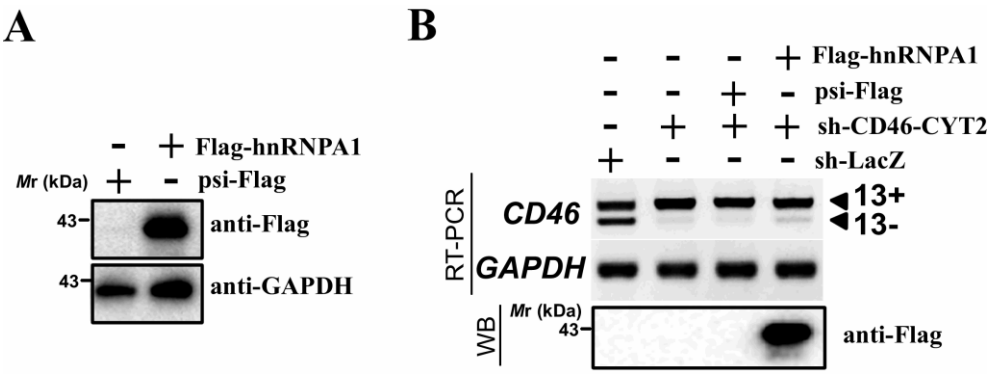

Figure S9. Generation of stably hnRNPA1 overexpressing and/or CD46-CYT2 knockdown cells. (**A**) Generation of hnRNPA1-overexpression EJ-1 cells. EJ-1 cells were infected with lentiviruses expressing Flag-hnRNPA1 or psi-Flag (control). Immunoblotting was performed to evaluate the expression of Flag-hnRNPA1. GAPDH is an internal control. (**B**) Generation of sh-CD46-CYT2 and sh-CD46-CYT2/hnRNPA1 EJ-1 cell lines. Semiquantitative RT-PCR analysis of *CD46* and *GAPDH* (control) were performed to detect the specific knockdown of *CD46-CYT2*. Immunoblotting was performed to evaluate the expression of Flag-hnRNPA1.

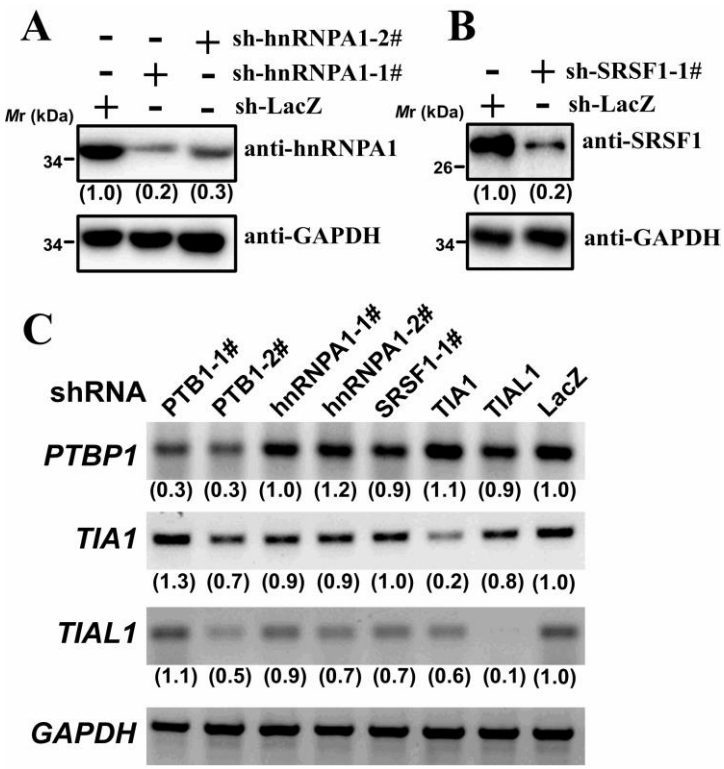

Figure S10. The knockdown efficiency of shRNAs of hnRNPA1, PTBP1, SRSF1, TIA1 and TIAL1. (A, B) EJ-1 cells were infected with lentivirus expressing several indicated shRNAs to establish stably expressing cell lines. Immunoblotting was performed to evaluate the expression of hnRNPA1 (A) and SRSF1 (B). GAPDH is an internal control. Levels of hnRNPA1 and SRSF1 are normalized against GAPDH and expressed as fold change relative to base expression determined using control sh-LacZ. (**C**) Semiquantitative RT-PCR analysis of *PTBP1*, *TIA1*, *TIAL1* and *GAPDH* (control) were performed to confirm the knockdown efficiency of the shRNAs. Levels of PTBP1, TIA1 and TIAL1 are normalized against input and expressed as fold change relative to base expression determined using control sh-LacZ.

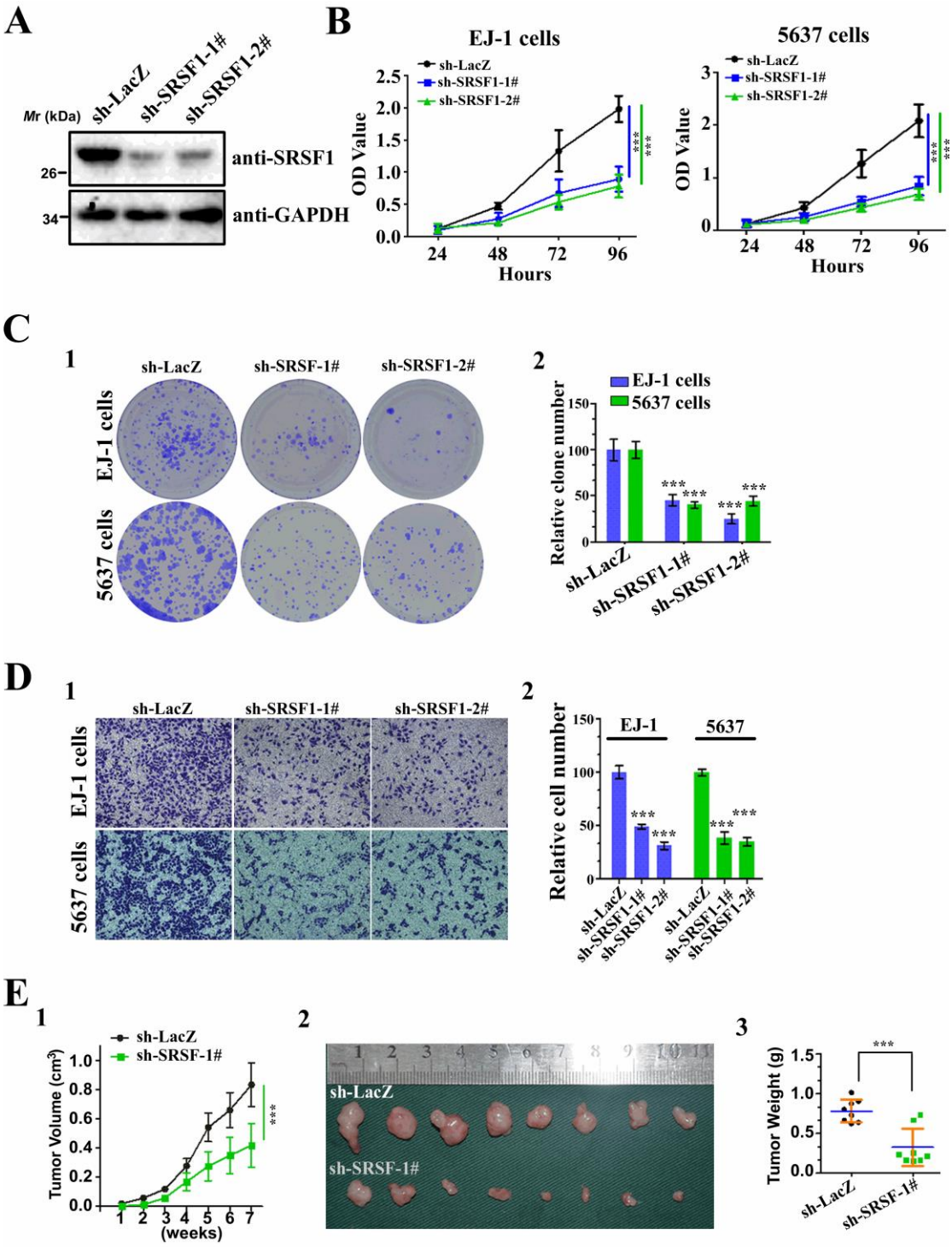

Figure S11. SRSF1 is required for the tumorigenesis of bladder cancer cells. (**A**) Western blot analysis of SRSF1 protein showed efficient SRSF1 knockdown by shRNA expression. (**B**) CCK-8 kit was utilized to quantify cell viability at each time point. Data are plotted as the mean ± SD of 3 independent experiments and were

analyzed by Two-way ANOVA. ***P < 0.001. (**C**) 1. Representative photographs of cell culture plates following staining for colony formation of EJ-1 and 5637 cells. 2. Number of colonies was quantified. (**D**) 1. Migration assay for the indicated cell lines. 2. Number of migrated cells was quantified in 5 random images from each treatment group. The data represent mean ± SD and were analyzed by unpaired two-tailed Student's *t test* (n=5). ***, P < 0.001 versus control. (**E**) 1. Mean tumor volume of sh-SRSF1 or sh-LacZ–treated EJ-1 cells measured by caliper on the indicated weeks. The data represent mean ± SD and were analyzed by Two-way ANOVA (n=8). ***P < 0.001. 2. Photographs of tumors excised 7 weeks after inoculation of stably transfected EJ-1 cells into nude mice. 3. The tumor weight of sh-SRSF1 or sh-LacZ–treated EJ-1 cells in nude mice at the end of 7 weeks after transplantation. The data represent mean ± SD and were analyzed by unpaired two-tailed Student's *t test* (n=8). **P < 0.01.

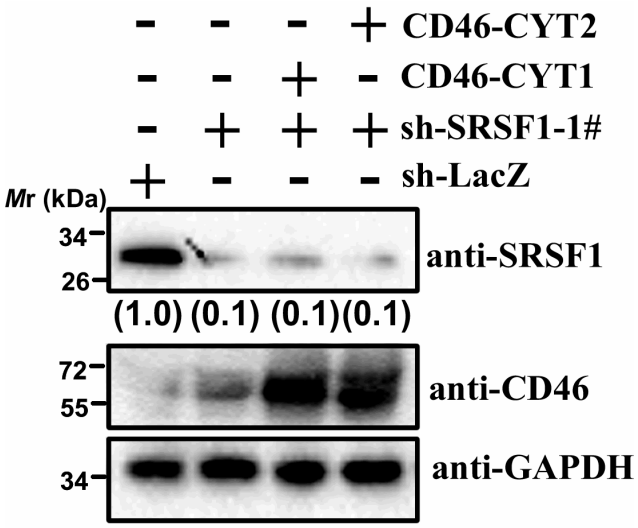

Figure S12. Generation of sh-SRSF1, sh-SRSF1/CD46-CYT1, and sh-SRSF1/CD46-CYT2 EJ-1 cell lines. Western blotting analysis of the whole-cell lysates from selected clones was performed to evaluate the expression of CD46 and SRSF1. GAPDH is an internal control. Protein levels of SRSF1 are normalized against GAPDH and expressed as fold change relative to base expression determined using control sh-LacZ.

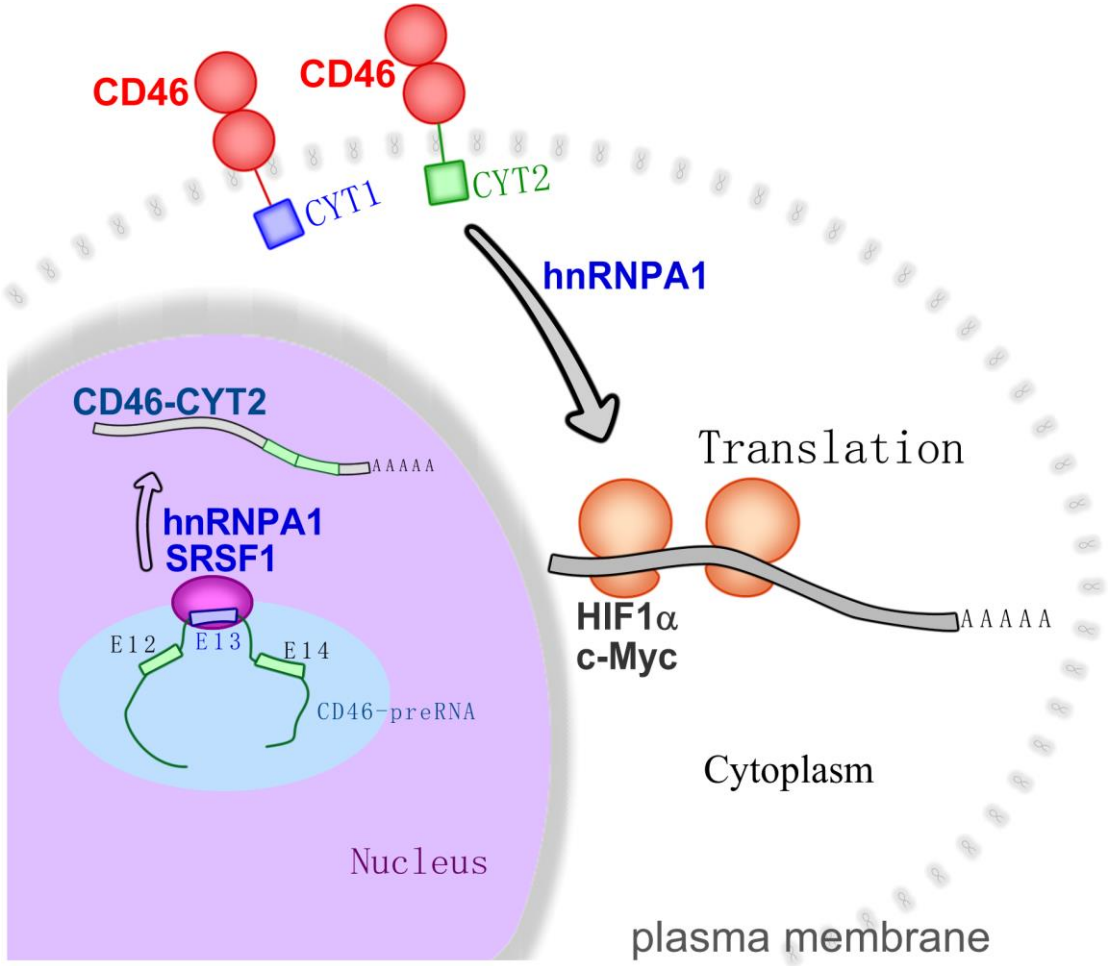

Figure S13. Model of crosstalk between the splicing regulation of CD46 exon 13 and translational regulation. The splicing factor SRSF1 and hnRNPA1 induce CD46 exon13 exclusion, and thus promote CYT1-to-CYT2 splice switch. CD46-CYT2 promote hnRNPA1-mediated IRES dependent translation of a subset genes, including HIF1a and c-Myc.

Supplementary Table S1. Dysregulated alternative splicing events, from RNA-Seq in bladder cancer and matched normal bladder tissues.

Gene Symbol	RPKM Normal	RPKM Tumor	Splice Type	Splice Exons	Upstream- Downstream Exons isoforms	Expression	
						Tumor	Normal
APP	210.277	287.971	ES	10	-9……11-	60%	27%
					-9…10…11-	30%	62%
ATP5C1	241.064	84.4433	ES	9	-8……10-	4%	35%
					-8…9…10-	93%	64%
BCAP31	140.443	139.317	AP	1.1	3-	97%	89%
					2…3-	3%	11%
BOLA3	90.0887	10.6855	ES	3	-2…3…4-	90%	74%
					-2……4-	10%	26%
CD44	134.652	173.299	ES	12-14	-5…12…13…14…15-	76%	1%
					-5……15-	5%	92%
CD46	108.891	36.031	ME	13	-12……14-	82%	54%
					-12…13…14-	5%	31%
CD74	97.0565	1476.03	ES	8	-7……9-	91%	78%
					-7…8…9-	5%	20%
CTNND1	98.8693	24.4781	ES	5	-4……6-	84%	16%
					-4…5…6-	3%	52%
EDF	168.186	172.503	AT	4.4	-4.1…4.4	90%	78%
					-4.1…4.3	7%	15%
					-4.1…4.2	4%	7%
EIF4A2	207.464	113.477	ES	11	-10……12-	62%	46%
					-10…11…12-	13%	28%
GABARAP	161.977	234.599	AP	1.1	1…2…3-	73%	94%
					2.1…3-	26%	4%
GSN	17.7845	168.795	AP	1	1.6-	66%	90%
					1.4…1.6-	32%	2%
HNRNPA2B1	295.846	273.388	ES	2	1……3-	94%	82%
					1…2…3-	5%	17%
LAMP2	106.898	56.058	AT	10	-9…11	55%	73%
					-9…10	38%	25%
MRPL33	142.082	42.8772	ES	3	-2……4-	80%	58%
					-2…3…4-	16%	42%
NDUFB8	100.019	49.892	AD	1.2	2.1…3-	88%	58%
					1.2…2.1…3-	11%	34%
NPM1	284.979	230.147	AT	11	-10…12…13	54%	83%
					-10…11	44%	15%
PFN2	127.991	10.4649	AT	6.4	-5…6.1	82%	14%

					-5…6.2…6.3	13%	83%
PSAP	235.356	1390.49	AA	8.1	-7…8.2-	67%	98%
					-7…8.1…8.2-	33%	2%
PSMA 4	165.307	75.7989	AP	1.2	3…5…6-	81%	88%
					6-	17%	5%
PTPRF	117.296	5.82104	ES	14	-13……15-	68%	100%
					-13…14…15-	31%	0%
RAC1	152.315	117.508	ES	4	-3……5-	88%	98%
					-3…4…5-	12%	2%
RHEB	127.371	39.9315	AP	1.2	-6…7-	84%	97%
					7-	16%	3%
RPS24	1344.29	459.467	AA	5	-4…5	83%	7%
					-4	11%	73%
RTN4	393.185	104.392	AP	7	-7…8-	95%	3%
					-5…8-	4%	95%
SHC1	20.7383	108.115	AP	2.1	2…3-	55%	8%
					1…3-	45%	91%
TMEM 106C	179.022	11.8754	AD	5.2	-5.1…5.2..6-	71%	59%
					-5.1…6-	28%	40%
TPD52	110.779	16.3849	AP	1	4…5-	61%	1%
					1…5-	36%	91%
TPM1	62.0153	136.03	AP	1	4… 5.2-	45%	2%
					1…3…5.2-	21%	73%
TPM4	111.761	571.307	AP	1	1…2…4-	87%	23%
					3…4-	8%	77%
TUSC3	170.358	12.0225	ES	11	-10……12-	89%	61%
					-10…11…12-	8%	36%
PSMB 7	97.9895	47.9963	AT	8	-7…8	93%	70%
					-7	7%	30%
ELOV L5	161.005	43.1037	ES	9	-8…9…10-	92%	83%
					-8……10-	0%	12%

Supplementary Table S2. Proteomic profile ofthe C-terminal cytosolic tail of CD46 interacting proteins.

Supplementary Table S3. Function groups of the proteins identified as CYT1 domain partners.

Supplementary Table S4. Function groups of the proteins identified as CYT2 domain partners.

Supplementary Table S5. The primers used in the study.