Supporting Material

Novel glycoprotein SBSPON suppressed bladder cancer through the AKT signal pathway by inhibiting HSPA5 membrane translocation

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A	HUMAN Chimpanzee Dog Cattle Mouse Rat	ATGAGGACCTETGGATGGCCTETGGCGCCTGGCGCGGGCGCTGGCCGGGGCCCAGGCCGGGCCCCGGGCCGGGCCCGGCCGGCGCCGGCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCGGCCCC				
	HUMAN Chimpanzee Dog Cattle Mouse Rat	TGEAGECTGEACAGEGICIACGEGACGIGITICIGCEACCAAGCCIGICGCTICACCBEGEACTGCICICGATIAGEACBGGGGGGCIGCCCAGCIGGCCGGCIGIGGGGAATGE 240 TGEAGECTGEACAGEGICIACGEGACGIGITICIGCEACCAAGCCIGICGCCIACGCEGEGGGGAATGE 240 GGEAGETGEACAGEGICIACGEGACGIGCITICIGCEACCAAGCCIGCCCCCACGEGGGGCACTGCIGCIGCCGGGGGAATGE 240 TGEAGECTGEACAGEGICIACGEGACGIGCITICIGCEACCAAGCCIGCCCGCCCCACGEGGGCAATGE 240 GGEAGETGEACAGEGICIACGEGACGIGCITICIGCEACCAAGCCIGCCGCCCACGEGGGCAATGE 240 TGEAGECTGEACAGEGICIACGEGACGIGCITICIGCEACCAAGCCIGCCGCCCCCGCGGGGGCAATGE 240 GGEAGECGGACGAGEGICIACGEGACGIACCIGCCGCCCCCCCCGGGGGCGCCCCGCCC				
	HUMAN Chimpanzee Dog Cattle Mouse Rat	SECCETGBARTEGETERCARACCASTECARACCTACAACCESTESEGBARGEGETGGTECACKGBRACCTCBARACGGBGGGGGGGGCCCTCCCCCCCCCCCCCCCCCCC SECCETGBASTGTTETECABACCASTECAACCTACAACCESTESCBASGCGTCGGTECAACAGBAACCTACBAACGGGGGGGGCCCCTGCCACCCCTGGAAGBAAGACTGG SECCETGBAGTGGTEGGGACGASTCAACCGCCCCGGTGGGGGGGGCGCGGGGGGGGGG				
	HUMAN Chimpanzee Dog Cattle Mouse Rat	<pre>IdeCT66AGTACTCCCCCCCGCAG6GCCAGGACT6CG6GCACACCTAGTTCCTGCCTTATAACTACCTGCATTCAACAAGBAGBACACBACABGCAGGCAGGCAGGCT6CG6GCCAACCTAGTTCCTGCCTTATAACTACCTGCCTTCCACAAGBAGBAGBACGACGAAGAAGCTACGTCCAACCTGGCTAGGCAGGCCAGGCCAGGCTGCGGGCCAGGCTGGGGCCACGCCTAGTTCGCCCGCC</pre>				
	HUMAN Chimpanzee Dog Cattle Mouse Rat	ACACACACABAGATCTGGATACTETATSGASTTTAAGACAGASTCCTTGATCTCTCACIGTGCTCTGGAAAACTGGCCCTTGATAGAGAGAGAGAGAGA				
	HUMAN Chimpanzee Dog Cattle Mouse Rat	etetetetesantetchacciccacchachactictetesaccicceticetetesacatescicceantesantcacciccicantesciantesistes etetetetesacciccaccintesacciccesaccintesacciccestesacciccestesacciccestesacciccestesaccintesciancestesistes etetetetetesacciccaccintesacciccestesacci				
	HUMAN Chimpanzee Dog Cattle Mouse Rat	CAAGGAACTTGGAAAAAAGTTCGGCGAGTAGACCAGTGTICITGICCAGCTGTTCACAGTITTAITTIATATAG 795 CAAGGAACTTGGAAAAAAGTTCGGCGAGTAGACCAGTGTICTIGTCCAGCTGTTCACAGTITTAITTITAITATAG 795 CAAGGAACTTGGAAAAAAGTTCGGCGGGTAGATCAGTTCTIGTICTCAGTTGTICTCAGTITTAITTITTAITTAG 795 CAAGGAACTTGGAAAAAAGTTCGGCGGGTAGAACAGTGTCTIGTICTCAGTGTGTCACGTTTIAITTITAITTAG 795 CAAGGAACTTGGAAAAAAGTCGGCGGGTAGAGACAGTGTCCIGTGTCCAGGTGTCACGGTTGTCACGTTTAITTITAITTAG 795 CAAGGAACTTGGAAAAAAGTCGGCGGGTAGAGACGGTGCCCGCTGTCCAGGTGTGCACGCTGTCAITTITAITTAGTG 795				
В						
	CLUSTAL	2.1 multiple sequence alignment				
	HUMAN Chimpanza Mouse Rat Dog Cattle	MRTLWMALCALSRLWFGAQAGCAEAGRCCFGRDPACFARGWRLDRVYGTCFCDQACRFTGDCCFDYDRACPARFCFVGEWSFWSGCAD MRTLWMTLCALSRLWFGALAGCAEAGRCCFGRDPACFARGWRLDRVYGTCFCDQACRLTGDCCFDYDRACPARFCFVGEWSFWSGCAG MRTLWMVLCALARLWFGALAGCAEAGRCCFGRDPACFARGWRLDRVYGTCFCDQACRLTGDCCFDYDRACPARFCFVGEWSFWSGCAG MRTLWMLCALARLWFGALAGCAEAGRCCFGRDPACFARGWRLDRVYGTCYCDQACRLTGDCCFDYDRACPARFCFVGEWSFWSGCAG MRTLWMLCALARLWFGALAGCAEAGRCCFGRDPACFARGWRLDRVYGTCYCDQACRLTGDCCFDYDRACPARFCFVGEWSFWSGCAG MRTLWMLCALARLWFGALAGCAEAGRCCFGRDPACFASGWRQDRVGTCYCDQACRLTGDCCFDYARACPARFCTVGEWSFWSGCAG MRTLWMLCALARLWFGALAGCAEAGRCCFGRDPACFASGWRQDRVGTCYCDQACRLTGDCCFDYARACPARFCTVGEWSFWSGCAS				
	HUMAN Chimpanze Mouse Rat Dog Cattle	QCKPTTRVRRRSVQQEPQNGGAPCPPLEERAGCLEYSTPQGQDCGHTYVPAFITTSAFNKERTRQATSPHWSTHTEDAGYCMEFKTES e QCKPTTRVRRRSVQQEPQNGGAPCPPLEERAGCLEYSTPQGQDCGHTYVPAFITTSAFNKERTRQATSPHWSTHTEDAGYCMEFKTES QCQPTTRVRRSVRQEPLNGGAPCPPLEERAGCLEYSSQSQDCGHSFVPAFITSSVFNKKRIIQAVSPQWSTHTEDAGYCMEFKTES QCQPTMRVRRRSVRQEPLNGGAPCPPLEERAGCLEYSSQSQDCGHSFVPAFITSSAFNKKRIVQAVSPQWSTHTEDAGYCMEFKTES QCRPTARVRRRPVQQEPRNGGAPCPPLEERAGCLEYSTPRGQDCGRAFVPAFITSSAFNKKRIVQAVSPQWSTHTEDAGYCMEFKTES QCRPTARVRRRPVQQEPCNGGAPCPPLEERAGCLEYSTPRGQDCGRAFVPAFITSSAFNKKRIVGAVSPGWSTDTEDAGYCMEFKTES QCRPTARVRRRPVQQEPQNGGPCPLEERAGCLEYSTPGGEDCGRAFVPAFITSSAFNKKRIVGAVSPGWSTDTEDAGYCMEFKTES XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX				
	HUMAN Chimpanzee Mouse Rat Dog Cattle	LTPHCALENNPLIRNMQYLREGYTVCVDCQPPAMNSVSLRCSGDGLDSDGNQTLHWQAIGNPRCQGTWK4VRRVDQCSCPAVHSFIFI264 LTPHCALENRPLTRNMQYLREGYTVCVDCQPPAMNSVSLHCSGDGLDSDGNQTLHWQAIGNPRCQGTWK4VRRVDQCSCPAVHSFIFI264 LTPHCALVNSPLTRNMQYLREGYTVCVDCQPPAMNSVSLRCSGDGLDSDGNTLHWQAIGNPRCQGTWK4VRRVEQCSCPDVHRFIFI264 LTHHCALENRPLTRNMQYLREGYTVCVDCQPPAMNSVSLRCSGDGLDSDGNTLHWQAIGNPRCQGTWK4VRRVEQCSCPDVHRFIFI264 LTHHCALENRPLTRNMQYLREGYTVCVDCQPPAMNSVSLRCSGDGLDSDGNTLHWQAIGNPRCQGTWK4VRRVEQCSCPDVHRFIFI264 LTHHCALENRPLTRNMQYLREGYTVCVDCQPPAMNSVSLRCSGDGLDSDGNTLHWQAIGNPRCQGTWK4VRRVEQCSCPAVHSFIFI264				

Fig. S1. Sequence alignment of SBSPON orthologs and encoded proteins from human, chimpanzee, dog, cattle, mouse and rat.

(A, B) Sequence alignment of SBSPON orthologs and encoded proteins from human, chimpanzee, dog, cattle, mouse and rat. The gene and protein sequence alignments were initially performed with LaserGene and ClustalW, respectively, and were then manually modified. The conserved (100%) residues are labeled with a star below the residues.



Fig. S2. The profiles and effects of SBSPON on different tissues and cells.

(A) SBSPON mRNA was widely expressed in majority of the normal human tissues

assessed.

(B)Western blot analysis was performed to assess the expression of SBSPON in human tumor cell lines.

(C) The influence of SBSPON on the cell cycle was analyzed using flow cytometry.

- (D) The impact of SBSPON on the apoptosis was evaluated using flow cytometry.
- (E) The expression levels of P21 and P27 were assessed using western blot analysis.
- β -Tubulin was utilized as a loading control.



Fig. S3. The effects of SBSPON overexpression on the proliferation and migration were accessed in SW780 cells.

(A) SBSPON expression was determined by western blot analyses following transduction using lentiviral construct. β-Tubulin was utilized as a loading control.(B) The effect of SBSPON on the proliferation of bladder cancer cells was evaluated using CCK-8 assays.

(C) The impact of SBSPON on the cell migration was accessed by transwell assays.

P* < 0.01, *P* < 0.001.



Fig. S4. The phenotypic differences of Sbspon knockout mice and their wild-type counterparts. Sbspon^{-/-} male mice exhibited increased grip strength, higher alanine aminotransferase level, and lower total bilirubin level comparison to wild-type mice. *P < 0.05, **P < 0.01.



Fig. S5. Hydrogen bonding interactions between SBSPON (cyan) and HSPA5 (green)(Detail).



Fig. S6. The expression levels of SBSPON and HSPA5 proteins in bladder cancer cells were assessed under different treatment conditions.

(A) Western blot analysis was performed to assess the expression of HSPA5 after TM treatment.

(B) 5637 cells were treated with CHX, and the SBSPON and HSPA5 protein level was assessed by western blot analysis.

(C) Western blot analysis was performed to assess the levels of HSPA5 in the SBSPON-overexpressing cells. β -Tubulin was utilized as a loading control.



Fig. S7. The functional of SBSPON-overexpression and SBSPON/HSPA5overexpression cells.

(A) The viability and the apoptosis rate of SBSPON-overexpression and SBSPON/HSPA5-overexpression cells following exposure to DDP or TM.

(B) TUNEL staining was carried out to analyze SBSPON-overexpression and SBSPON/HSPA5-overexpression cells apoptosis.

(C) Western blot analysis was performed to examine the expression of HSPA5, PERK,

CHOP and ATF4 in the SBSPON-overexpression cells following exposure to DDP

and TM. β -Tubulin was utilized as a loading control.

N	PepCount	UniquePepCount	Number of	Mol.weight
Name			proteins	[kDa]
HSPA5	13	12	1	72.332
JUP	8	8	5	81.744
XRCC5	13	13	3	82.704
DNAJC10	20	20	6	91.079
DSC1	5	5	2	93.834

Table S1 A list of potential SBSPON-interacting protein candidates based onimmunoprecipitation assays and mass spectrometry analysis