

SIVA1 REGULATES THE STABILITY OF SINGLE-STRANDED DNA-BINDING PROTEIN 3 ISOFORMS

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SUPPLEMENTARY MATERIALS

Siva regulates the stability of Single-stranded DNA-binding proteins 3

This file includes:

Figures. S1

Figures. S2

Table. S1-S2

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ha MFAKKGSAVPSDQAREKLALYVYEYLLHVGAQKSAQTFLSEIRWEKNI TLGEPPG
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hc FLHSWWCVFWDLYCAAPERDTCESSEAKAFHDYSAAAAPSPVLGNI PPNDGMPGG
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mb FLHSWWCVFWDLYCAAPERDTCESSEAKAFHDYSAAAAPSPVLGNI PPNDGMPGG
co *****

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mb PIPPGFFQ-----PFMSPRYAGGPRPPIRMGNQPP
co *****

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co *****
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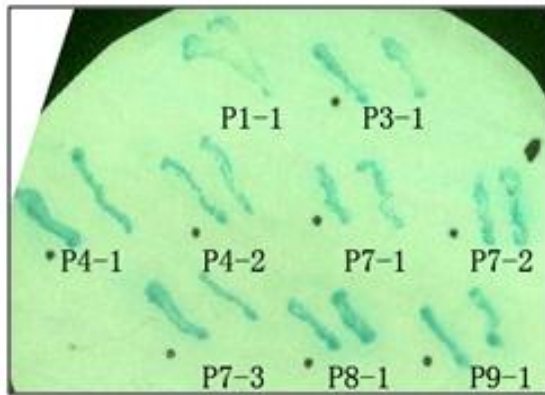
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hc DSTNSSDNIYTMINPVPPGGSRSNFPMGPGSDGPMGGMGGMEPHHMNGSLGSGDIDG
ma DSTNSSDNIYTMINPVPPGGSRSNFPMGPGSDGPMGGMGGMEPHHMNGSLGSGDIDG
mb DSTNSSDNIYTMINPVPPGGSRSNFPMGPGSDGPMGGMGGMEPHHMNGSLGSGDIDG
co *****

ha LPKNSPNNISGISNPPGTPRDDGELGGNFLHSFQNDNYSPSMTMSV
hb LPKNSPNNISGISNPPGTPRDDGELGGNFLHSFQNDNYSPSMTMSV
hc LPKNSPNNISGISNPPGTPRDDGELGGNFLHSFQNDNYSPSMTMSV
ma LPKNSPNNISGISNPPGTPRDDGELGGNFLHSFQNDNYSPSMTMSV
mb LPKNSPNNISGISNPPGTPRDDGELGGNFLHSFQNDNYSPSMTMSV
co *****

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Figure S1. Sequence alignment of SSBP3 proteins. Sequence alignment of human and mouse SSBP3 protein isoforms. Human isoforms: hSSBP3a (ha), hSSBP3b (hb), and hSSBP3c (hc). Mouse isoforms: mSSBP3a (ma) and mSSBP3b (mb). For gene and protein accession numbers see Table S2. Identities was calculated by T-Coffee (%): mouse SSBP3a to human SSBP3a (100%), mouse SSBP3b to human SSBP3c (100%) , and to human SSBP3b (98%). (co) conserved, asterisk shows highly conserved amino acid.

(a)



(b)



Figure S2. yeast-two hybrid screening. 11 positive clones were produced, plasmids were extracted by EZNA yeast plasmids kit (Omega Bio-tek), electric transformed to DH5 α E. coli competent cells. The rescued plasmids were co-transformed to AH109 yeast with pGBKT7-SSBP3-pra, and plated on SD/-Ade-His-Leu-Trp media.

(a) Colony-lift filter assay. 9 clones were positive, all these clones are positive in colony-lift filter assay. After sequencing, two of these clones (p7-1 and p3-1) were revealed to be Siva1 gene with full length cDNA fused in frame with Gal4 AD domain, but the first codon of Siva1 gene was deleted. Clone p1-1 was LDB3 gene in frame fused to Gal4 AD domain., One clone was revealed be STAT6, 3 clones are not inframe fused to GAL4 AD domain, and 2 clones are unknown genes.

(b) Co-transformation assay. pACT2-Siva1 and pGBKT7-SSBP3-pra or pGBKT7-SSBP3-prb along with known positive and negative controls to SD- SD/-Ade-His-Leu-Trp+X- α -gal media, both long and short proline rich domain of SSBP3 can interact with Siva1.1: pGBKT7-ssbp3-proline-rich-domain-A + pACT2-siva1; 2: pGBKT7-ssbp3-proline-rich-domain-B + pACT2-siva1; 3: pACT2-Siva1; 4 : pGBKT7-ssbp3-proline-rich-domain-A + pGADT7-lam; 5: pGBKT7-p53 + pGADT7-SV40T1; 6: pGBKT7-p53 + pGADT7-lam;

Table S1. PCR Primers

Transcript	Forward	Reverse
CDS-SSBP3	5' ATGTTTGCCAAAGGCAAAGGC 3'	5' CAGCTCCTGGATAATGACACCTCA 3'
RT-SSBP3	5' GCCCTGTGCTTGGCAACATT 3'	5' CTGGAGGCTGGTTTCCCATTTC 3'
RT-GAPDH	5' CAAGGTCATCCATGACAACCTTTG 3'	5' GTCCACCACCCTGTTGCTGTAG 3'
Y-SSBP3:	5'TCCCGCCAGGTTTCTTTCAG 3'	5'CCAGGTGATGAGGAGGAGTATGG3'

Table S2. Accession numbers of SSBP3 transcripts and protein isoforms

Transcripts	Accession number
human SSBP3a	NM_145716.3
human SSBP3b	NM_018070.4
human SSBP3c	NM_001009955.3
mouse Ssbp3a	NM_023672.2
mouse Ssbp3b	NM_198438.1
Isoforms	Accession number
human SSBP3a	NP_663768.1
human SSBP3b	NP_060540.2
human SSBP3c	NP_001009955.1
mouse SSBP3a	NP_076161.2
mouse SSBP3b	NP_940840.1