



Supplemental Material to:

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Two splicing factors carrying serine-arginine motifs, TSR1
and TSR1IP, regulate splicing, mRNA stability, and rRNA
processing in *Trypanosoma brucei*

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Supplemental Material

1. 2013RNABIOL0108R2-S2
2. 2013RNABIOL0108R2-S3
3. 2013RNABIOL0108R2-S5

S-1. List of oligonucleotides

Oligonucleotides for RT-PCR

Sense Tb11.02.1100: 5'-ATGGCTCTTGGATTTTCCTC-3', from position 1-20.

Anti Tb11.02.1100: 5'-ATGGTATGATTTCCCAATAC-3', complementary to position 400-420.

Sense pre Tb11.02.1100: 5'-AAACTGTAAGCTTAGTCACT-3', from position -186 to -165 of the ATG.

SenseTb11.01.6410: 5'-ATGTCGAAGTTAATTAAATT-3', from position 1-20.

AntiTb11.01.6410: 5'-TGCTTCGAAAGGCGTCAGTG-3', complementary to position 400-420.

Sense pre Tb11.01.6410: 5'-TTCATTCATTTAATCATTTA-3' from position -75 to -56 of the ATG.

SenseTb927.2.3460 : 5'-ATGGAAAGAGAGCCAGCGGT-3', from position 1-20.

AntiTb927.2.3460 : 5'-TTCCACGCATTGCCACTTTA-3', complementary to position 400-420.

Sense pre Tb927.2.3460 : 5'-CTCTTGTGCCTCAGTCACAC-3', from position -396 to -375 of the ATG.

Sense Tb927.10.1210: 5'-ATGGACGACGACGTGGGGCT-3', from position 1-20.

Anti Tb927.10.1210: 5'-AGCATAGTGGACTTCTTCAC-3', complementary to position 400-420.

Sense pre Tb927.10.1210: 5'-AGTTTTAAAGAGTTCCATTT-3', from position -172 to -153 of the ATG.

SenseTb927.3.3360: 5'-ATGGACGCCGGTAAACTTTT-3', from position 1-20.

AntiTb927.3.3360: 5'-CATCCGCGGACGAACATAAAA-3', complementary to position 400-420.

Sense pre Tb927.3.3360: 5'-GGGTCGTACCTGCTTCTTTA-3', from position -95 to -76 of the ATG.

SenseTb927.6.3230: 5'-ATGGTACGCAGCATTCGCTA-3', from position 1-20.

AntiTb927.6.3230: 5'-ATTTGCCGTCTCCCATGTGA-3', complementary to position 400-420.

Sense pre Tb927.6.3230: 5'-AATAACGATGATGATATTGA-3', from position -80 to -61 of the ATG.

Sense Tb927.1.2260: 5'-ATGGGTTGCGGTGGCTCAAC-3', from position 1-20.

Anti Tb927.1.2260: 5'-TTATTTCTGCCAGATCCCC-3', complementary to position 371-390.

Sense Tb927.10.5840: 5'-ATGTCATCGCTAAAGGAAAT-3', from position 1-20.

Anti Tb927.10.5840: 5'-GTCACCCACAGTAGACCAT-3', complementary to position 431-450.

Sense pre Tb927.10.5840: 5'- CCATATTTGCATGCCTAGCG -3', from position -140 to -131 of the ATG.

Sense Tb11.02.4220 (Tb 927.11.6360): 5'-ATGAGGATTGAGCAGTGTTC-3', from position 1-20.

Anti Tb11.02.4220 (Tb 927.11.6360): 5'-CACCGCCACAAGATCATCAC-3', complementary to position 431-450.

Sense pre Tb11.02.4220 (Tb 927.11.6360): 5'- CTCCTTTACTTTTATCCCC -3', from position -120 to -101 of the ATG.

Sense Tb927.10.13730: 5'-ATGGGTAAAAACCCTCCTAA-3', from position 1-20.

Anti Tb927.10.13730: 5'-GGTGAAGGGTTCAAACATA-3', complementary to position 431-450.

Sense pre Tb927.10.13730: 5'- ATTTTCCATCTAAACTTCT -3', from position -180 to -161 of the ATG.

Sense Tb927.2.4710: 5'-ATGCAACAATATACCCTTCG-3', from position 1-20.

Anti Tb927.2.4710: 5'-AGTACCTGCGCTTTGAAACA-3', complementary to position 431-450.

Sense pre Tb927.2.4710: 5'- ATATATATATATATATATA -3', from position -559 to -540 of the ATG.

Sense Tb927.10.10010: 5'-ATGCCAAAATCGAAGCGAGC-3', from position 1-20.

Anti Tb927.10.10010: 5'-CATGCTGTGTGGAAACTGTG-3', complementary to position 431-450.

Sense pre Tb927.10.10010: 5'- ATGAAGTTCATTTTCTTTA -3', from position -120 to -101 of the ATG.

Sense Tb927.8.2000: 5'-ATGCTAAAGAGCCCGCAAAA-3', from position 1-20.

Anti Tb927.8.2000: 5'-ACGAAACGTTGTGCCGTATA-3', complementary to position 431-450.

Sense Tb927.5.4420: 5'-ATGTCTTCCAGAGCTGAGGA-3', from position 1-20.

Anti Tb927.5.4420: 5'-TGCAGCAACCACGAGGTCCC-3', complementary to position 431-450.

Sense pre Tb927.5.4420: 5'- ACTGTTTTGACTGATTGCTC -3', from position -286 to -265 of the ATG.

Sense Tb927.7.270: 5'-ATGTCTAGCGTCGCGCTGCT-3', from position 1-20.

Anti Tb927.7.270: 5'-GAGGAACTCCAGGTGCTCGC-3', complementary to position 431-450.

Sense Tb927.5.1150: 5'-ATGCTGAGTAAAAGGAACAG-3', from position 1-20.

Anti Tb927.5.1150: 5'-GGGATCACCCATGGCCTCG-3', complementary to position 426-444.

Sense Tb927.10.8450 : 5'-ATGACTGAGCGTCGTGATAA-3', from position 1-20.

Sense pre Tb927.10.8450: 5'- CGTCTCCCTTCCATATTGTT -3', from position -80 to -61 of the ATG.

Sense Tb927.2.1180 : 5'-ATGAATCAACAGGTTTCTAT-3', from to position 1-20.

Sense pre Tb927.2.1180: 5'- TTGGTTTAGAAATGTATCCA -3', from position -120 to -101 of the ATG.

Sense Tb927.7.4570: 5'-ATGGTTCATCGAAAATTAT-3', from position 1-20.

Sense Tb09.160.5480: 5'-ATGCTCGGGTTTGAGTCGTT-3', from position 1-20.

Sense pre Tb09.160.5480: 5'- TCACACTCTTGTTGCCTAT -3', from position -139 to -120 of the ATG.

Sense Tb11.01.5500: 5'- AAGCGCAGCAAATTCACACC-3' from position 94 - 113.

Anti Tb11.01.5500 : 5'-CCACGTGACGTAATCCTCCAAG-3' complementary to position 594 - 615.

Sense pre Tb11.01.5500: 5'- ATATTGTCACCATTTCTACA -3', from position -60 to -41 of the ATG.

Sense Tb11.01.1370 : 5'-TCCCACGACGACAATCTGTTG-3' from position 307 - 327.

Anti Tb11.01.1370 : 5'-CCCTGTGTTGTCACATCCTGC-3' complementary to position 786 - 806.

Sense pre Tb11.01.1370 : 5'- ATGTGGGGAAAACCACCATC -3', from position -240 to -221 of the ATG.

Sense Tb927.7.5940 : 5'-ATGAGCGCACCCGTCGACAA-3' from position 1-20.

Anti Tb927.7.5940 : 5'-ACCCAAGTCAAACAACATAC-3' complementary to position 401-420.

Sense Tb927.3.3160 (PAP): 5'-CCCAACGAAACCACTAGAGG-3' from position 18-37.

Anti Tb927.3.3160 (PAP): 5'-ATCGACAGCTGTGCCTCTG-3' complementary to position 1037-1055.

Sense Tb09.211.3210: 5'-ATGCTCAACAGGGACGGTAC-3' from position 1-20.

Anti Tb09.211.3210: 5'-GCGTCAAACCCAACAGCCCT-3' complementary to position 481-500.

Sense Pre Tb09.211.3210: 5'- TTCCTCCCTTTTTTTTTTCGA-3' from position -290 to -271 of ATG.

Sense Tb927.10.2020: 5'-ATGTCTAGACGCCTAAACAA-3' from position 1-20.

Anti Tb927.10.2020: 5'-GTCTGCTCCACGGGGAAACT-3' complementary to position 481-500.

Sense Pre Tb927.10.2020 : 5'-ACCGGTGCAATACTGCCGTG-3' from position -180 to -161 of ATG.

Sense Tb927.4.3930: 5'-ATGACGGAAGGACTAGGCGA-3' from position 1-20.

Anti Tb927.4.3930: 5'-GTAGCGTCGGAGCTTTTCAC-3' complementary to position 481-500.

Sense Pre Tb927.4.3930 : 5'-TCATTTTTTGCGGTCACCAT-3' from position -375 to -356 of ATG.

Sense Tb927.1.2230: 5'-ATGGGTTGTGGTGGCTCAAA-3' complementary to position 1-20.

Anti Tb927.1.2230: 5'-CTATTGCCTGTCTCCATCAG-3' complementary to position 481-500.

Sense Pre Tb927.1.2230: 5'-CACGCCTGCCTGGTCCCGTC-3' from position -433 to -414 of ATG.

Sense Tb927.7.1910: 5'-ATGTATCGCAGTGCGTTTCG-3', from position 1-20.

Anti Tb927.7.1910: 5'-GCCTCAGCACAAGCGGTTGC-3', from position 481-500.

Sense Pre Tb927.7.1910: 5'-TGCCATCGTTGGGGAAAATA-3' from position -108 to -89 of ATG.

Sense Tb927.10.15410: 5'-ATGGTCAGCGTTGCCGTAAT-3', from position 1-20.

Anti Tb927.10.15410: 5'-ATGAAACGGGTAGCACGGAG-3', from position 481-500.

Sense Tb09.160.2600 (Tb927.9.4040): 5'-ATGTCTACATCCCGCATTAC-3', from position 1-20.

Anti Tb09.160.2600 (Tb927.9.4040): 5'-ACAACGGTAAATTCCTCTTG-3', from position 481-500.

Sense Pre Tb09.160.2600 (Tb927.9.4040): 5'-TACTTCAATTTTTTTGTACC-3' from position -211 to -192 of ATG.

Tubulin sense: 5'- TGCGTGAGGCTATCTGCATC -3', sense, complementary to position 2- 21 of Tb927.1.2380 (α Tubulin).

Tubulin antisense: 5'-CCCTGAAGACCAGTGCAGTTG -3', antisense, complementary to position 381-401 of Tb927.1.2380 (α Tubulin).

7SL sense: 5'- CCGCTCGAGAGCCGGAGCGCATTGCTCTG from position 1-21 of 7SL, Tb927.8.2861.

7SL antisense: 5'- CCCAAGCTTCCGCCTCGCGACGACACTTG from position 255-274 of 7SL, Tb927.8.2861.

Oligonucleotides for T7 Transcribed RNA probes for Northern:

Anti T7 Tb09.160.5480:

5'-TTAATACGACTCACTATAGGGAGAGCGTTGTAGTACGTGAGTAT-3', complementary to position 181-200.

Anti T7 Tb927.7.4570:

5'-TTAATACGACTCACTATAGGGAGAGCCCCACGAAAAACGGTAT-3', complementary to position 181-200.

Anti T7 Tb927.2.1180:

5'-TTAATACGACTCACTATAGGGAGAAACATCATATTTCTAGGATG-3',
complementary to position 181-200.

Anti T7 927.10.8450:

5'-TTAATACGACTCACTATAGGGAGAGCCGTCAGGTACAGCAGCAA-3',
complementary to position 181-200.

Anti T7 Tb927.5.1150:

5'-TTAATACGACTCACTATAGGGAGACTCCAGCACAAATTGCGGTA-3',
antisense complementary to position 221-240.

Anti T7 Tb927.10.10010 :

5'-TTAATACGACTCACTATAGGGAGATCGGCCAAGAGCGATCATAA-3',
antisense complementary to position 221-240.

Anti T7 Tb11.02.4220 (Tb 927.11.6360):

5'-TTAATACGACTCACTATAGGGAGACTTAACGGGGACGTGGCGAC-3',
antisense complementary to position 221-240.

Anti T7 Tb927.10.13730:

5'- TTAATACGACTCACTATAGGGAGACCCTTGCTTTTCCCTCAGTT-3',
antisense complementary to position 221-240.

Anti T7 09.211.3210:

5'-TTAATACGACTCACTATAGGGAGAAACTTGGCACCAACAATTGC -3',
from position 181-200.

Anti T7 Tb927.10.2020:

5'-TTAATACGACTCACTATAGGGAGAACGGTGCTTTCACGACCCTC-3',
from position 181-200.

Anti T7 927.4.3930:

5'-TTAATACGACTCACTATAGGGAGAGTGCTTGCGAGATTGAAAGA-3',
from position 181-200.

Anti T7 09.160.2600:

5'-TTAATACGACTCACTATAGGGAGAAATGTGTCCAGGAGGGTGCCA-3',
from position 181-200.

Anti T7 927.1.2230:

5'-TTAATACGACTCACTATAGGGAGATCCTCGCTAAAGGTAACTTT-3',

from position 181-200.

Anti T7 927.7.1910:

5'-TTAATACGACTCACTATAGGGAGATTCATAGGTGGTCGCATAAG-3',

from position 181-200.

Sr1 sense (160) (+10): 5'- AGTTCTAACTAAAATGCAGCTCACCTACG-3', sense, specific to *T. brucei* srRNA1, from position 161-179; unpaired nucleotides are highlighted in gray, used for synthesis of ITS4 PCR product for Northern analysis.

LSUB AS T7 1-20 (+5): 5'- TTAATACGACTCACTATAGGGAGAACTACAGTA
CGGTCTGCAGTTGGG -3', antisense, specific to *T. brucei* LSU 3' rRNA, from position 1-19, carrying T7 promoter sequence (underlined); unpaired nucleotides are highlighted in gray, used for synthesis of ITS4 PCR product for Northern analysis.

Oligonucleotides for Silencing Constructs:

TSR1: Tb927.8.900

Sense TSR1 Xba I: 5'- GCTCTAGAGGAATGTGCCTTTGTCG-3' from position 513-529.

Anti TSR1 Mlu: 5'- CGACGCGTCTAATCTCGGCGTTTGC-3' complementary to position 971-987.

Anti TSR1 Hind III: 5'- CCCAAGCTTCTAATCTCGGCGTTTGC-3' complementary to position 971-987.

TSR1 IP: Tb927.10.2910

Sense TSR1 IP Xba I: 5'- GCTCTAGAATCTGAACATCCAGAGCAG-3' from position 102-120.

Anti TSR1 IP Mlu: 5'- CGACGCGTTATGACGATGCCTGTGAC-3' complementary to 587-603.

Anti TSR1 IP Hind III: 5'- CCCAAGCTTTATCACGATGCCTGTGAC-3' complementary to position 587-603.

Oligonucleotides for PTP tagging in *T brucei* :

TSR1 : linerised at position 471 with Aval

Sense Tsr1 apa1 tap: 5'-AAAGGGCCCGTAGTTCAACATAGTCGTGG -3' from position 241-260.

Anti Tsr1 not1 tap: 5'-AAAGCGGCCGCGCCATCTCGGCGTTTGCGGTAAT -3' from position 965 -984.

TSR1 IP : linerised at position 415 with NsiI

Sense Tsr1 IP apa1 tap: 5'-AAAGGGCCCCCTATTTTAGGTAATCAGCG -3' from position 241-260.

Anti Tsr1 IP not1 tap: 5'-AAAGCGGCCGCGCCCTCCTGTATCGCCCTTCAT -3' from position 1115 -1134.

Oligonucleotides for TAP tagging in *L. tarentolae*:

Tsr1: LmjF.07.0870

Sense BamHI Tsr1 tap : 5'-AAAGGATCCATGTCACAGTCGCTCAGT-3' from position 1-18

Anti XbaI Tsr1 tap: 5'-GGGTCTAGAACGACGTGTGCGGCGCTCG-3' complementary to position 1098-1116.

Tsr1IP: LmjF.34.0495

Sense BamHI Tsr1 IP tap : 5'-AAAGGATCCATGGATGTGATTCCGCCCGA-3' from position 1-20.

Anti XbaI Tsr1IP tap: 5'-GGGTCTAGAGCGCCTGTATGTGTTATTGC-3' complementary to position 752- 771.

Oligonucleotides for Primer Extensions:

U3 : 5'-TGCCGTTTCATCGAAC-3', antisense, complementary to positions 107–121 of U3 snRNA.

SL EXON : 5'- GGGAGCTTCTCATAC-3', Antisense complementary to positions 40–55 of SL RNA (exon).

SL INTRON : 5'- TGCGTCTGTTGGCCC-3', Antisense complementary to positions 65 to 79 of SL RNA(intron).

Oligonucleotides for Northern blots for sucrose gradient:

U2 for L.t. - TTCTATTCCTTTGCCCCGA

SL for L.t. - TTGCCGGAAGACGGGTTCGGA

S-4: Genes affected by SR proteins and their splicing and poly (A) signals. We used the splice sites and poly (A) sites published in (1) to compare splicing and poly (A) signals between regulated and non-regulated genes. Each row corresponds to one property of the signals, with the “regulated genes (mean)” column presenting the mean value of the property over all genes regulated in a given experiment and in a given direction (e.g., TSR1 up), and with the “other genes (mean)” column presenting the mean value of the statistic over all other *T. brucei* genes. We then used the Mann-Whitney U (MWU) to compute the significance of the difference. Rows with $P < 0.001$ are highlighted in bold, and rows with $P < 0.05$ appear in italics. The SL/Poly (A) diversity is a measure of the heterogeneity of splicing/poly (A) site selection, which is large when splicing is equally likely to take place in any number of sites and is approaching zero when there is only a single site (2). PPT length and PPT-AG distance are based on a PPT computationally predicted as the longest stretch of pyrimidines (separated by at most one purine) in a window of 120 nts upstream of the major splice site. The pyrimidine fraction upstream of splice site is defined in a window of 120 nts upstream of the major splice site, and the purine fraction downstream of splice site is similarly defined in a window of 120 nts downstream of the major splice site.

Property	TSR1 up-regulated genes			TSR1 down-regulated genes		
	Regulated genes (mean)	Other genes (mean)	P-value (MWU)	Regulated genes (mean)	Other genes (mean)	P-value (MWU)
SL diversity	0.601698	0.588656	0.547874	0.55519	0.589257	0.262472
5'UTR	<i>198.693</i>	<i>144.669</i>	<i>0.0471325</i>	<i>112.025</i>	<i>145.747</i>	<i>0.0131667</i>
PPT-AG distance	42.2353	37.482	0.291205	38.1348	37.5329	0.985453
PPT length	18.8675	18.2912	0.332216	18	18.3022	0.613904
Pyrimidine fraction upstream of splice site	0.633333	0.636977	0.42491	<i>0.618539</i>	<i>0.637172</i>	<i>0.0126593</i>
Purine fraction downstream of splice site	0.551569	0.54691	0.356263	<i>0.53427</i>	<i>0.547134</i>	<i>0.0331493</i>
PolyA diversity	<i>2.30632</i>	<i>1.94459</i>	<i>0.030022</i>	2.17328	1.94897	0.364185
3'UTR	660.842	650.248	0.289708	703.852	649.643	0.57085
Distance from polyA site to downstream splice site	340.871	258.61	0.981323	349.75	259.092	0.97277
	TSR1IP up-regulated genes			TSR1IP down-regulated genes		

SL diversity	0.506502	0.590201	0.119977	0.658658	0.587337	0.280052
5'UTR	122.12	145.693	0.303528	95.8462	146.259	0.000116102
PPT-AG distance	37.0088	37.5496	0.743054	38	37.5309	0.674964
PPT length	17.8584	18.3057	0.690853	16.3571	18.3392	9.15E-05
Pyrimidine fraction upstream of splice site	0.630263	0.637044	0.347617	0.618648	0.637319	0.000649488
Purine fraction downstream of splice site	0.559576	0.546755	0.149995	<i>0.53345</i>	<i>0.547254</i>	<i>0.0361446</i>
PolyA diversity	<i>2.18869</i>	<i>1.94348</i>	<i>0.0321258</i>	2.18273	1.94746	0.137246
3'UTR	<i>399.156</i>	<i>659.989</i>	<i>0.0311207</i>	741.243	648.522	0.142036
Distance from polyA site to downstream splice site	236.217	261.259	0.245146	187.033	261.742	0.475672

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2. Gupta, S. K., Carmi, S., Waldman Ben-Asher, H., Tkacz, I. D., Naboishchikov, I., and Michaeli, S. (2013) Basal Splicing Factors Regulate the Stability of Mature mRNAs in Trypanosomes. *J Biol Chem* **288**, 4991-5006