TcUBP1 induces up-regulation of a surface glycoprotein RNA regulon and promotes infectivity of the human parasite *Trypanosoma cruzi*

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Running title: *Coordinated regulation of multiple mRNAs in T. cruzi*

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SUPPORTING INFORMATION

Material included:

- Figure S1
- Figure S2
- Figure S3
- Figure S4
- Figure S5
- Figure S6
- Table S1
- Table S2
- Supplemental File 1
- Supplemental File 2

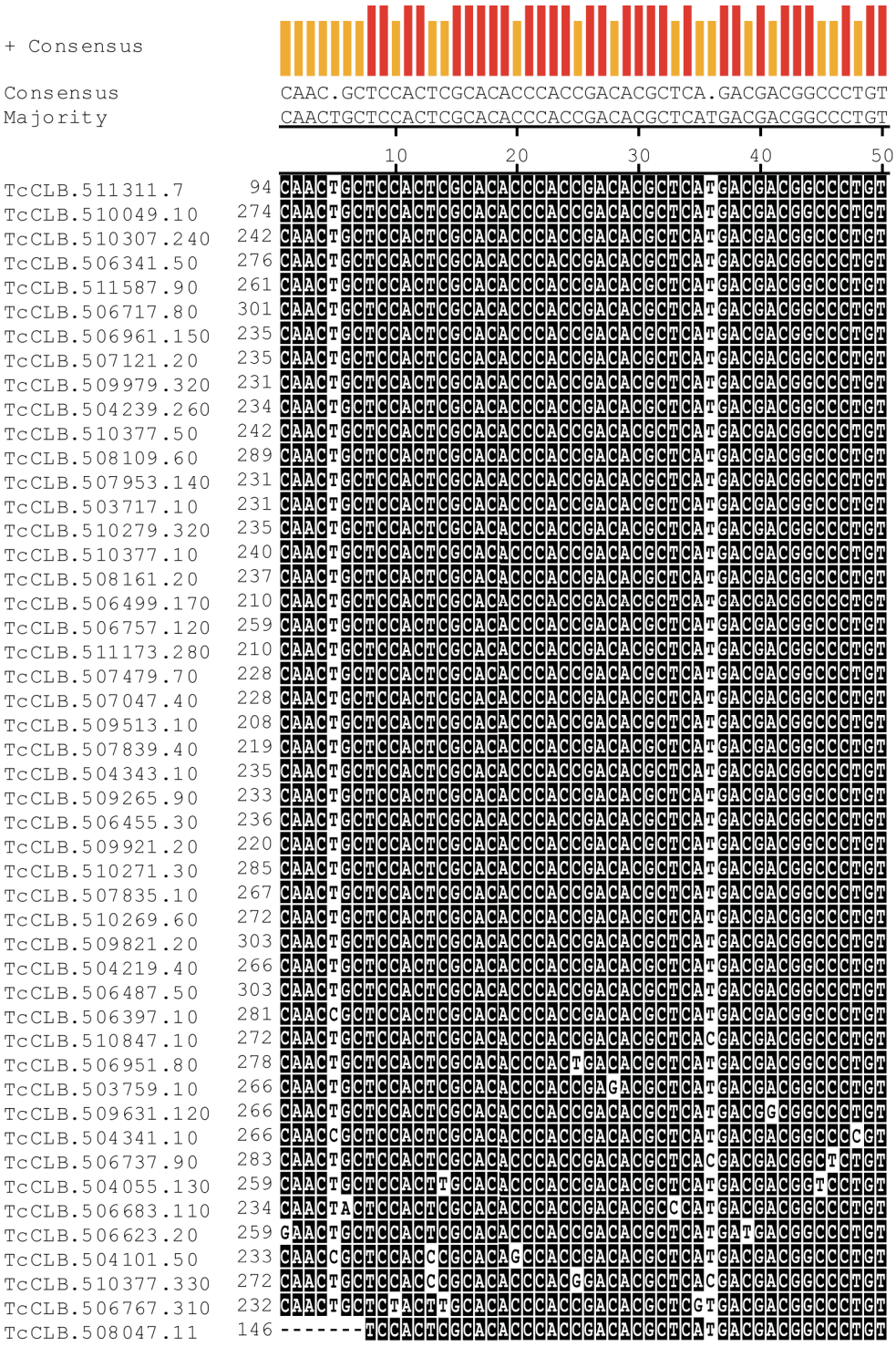
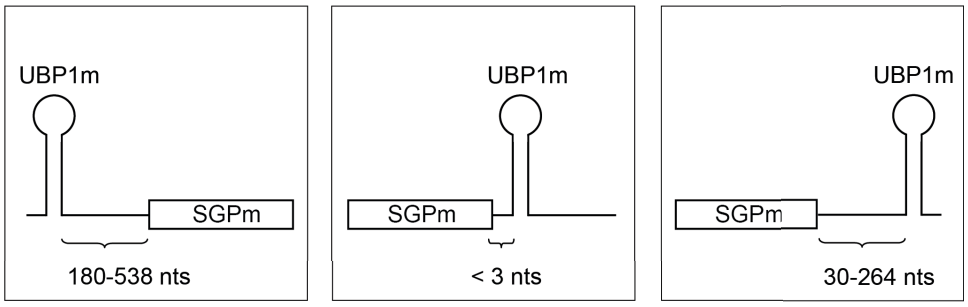


Figure S1. **Multiple sequence alignment of SGPm.** Comparison of 48 short sequence motifs obtained from BLASTN searches likely to be present at the 3′-UTR of some large gene family members. Systematic gene names are shown at the left. The numbers in the sequences indicate the position of SGPm within the 3′-UTR (distances from stop codon). Shade with solid black indicates residues that match the consensus exactly.



Configuration	a	b	c
No. of seqs.	9	266	12
Percentaje	3.1%	92.7%	4.2%

Figure S2. **Distribution of *cis*-elements.** Three alternative configurations (a, b and c) of distribution of UBP1m and SGPm within 3′-UTRs.

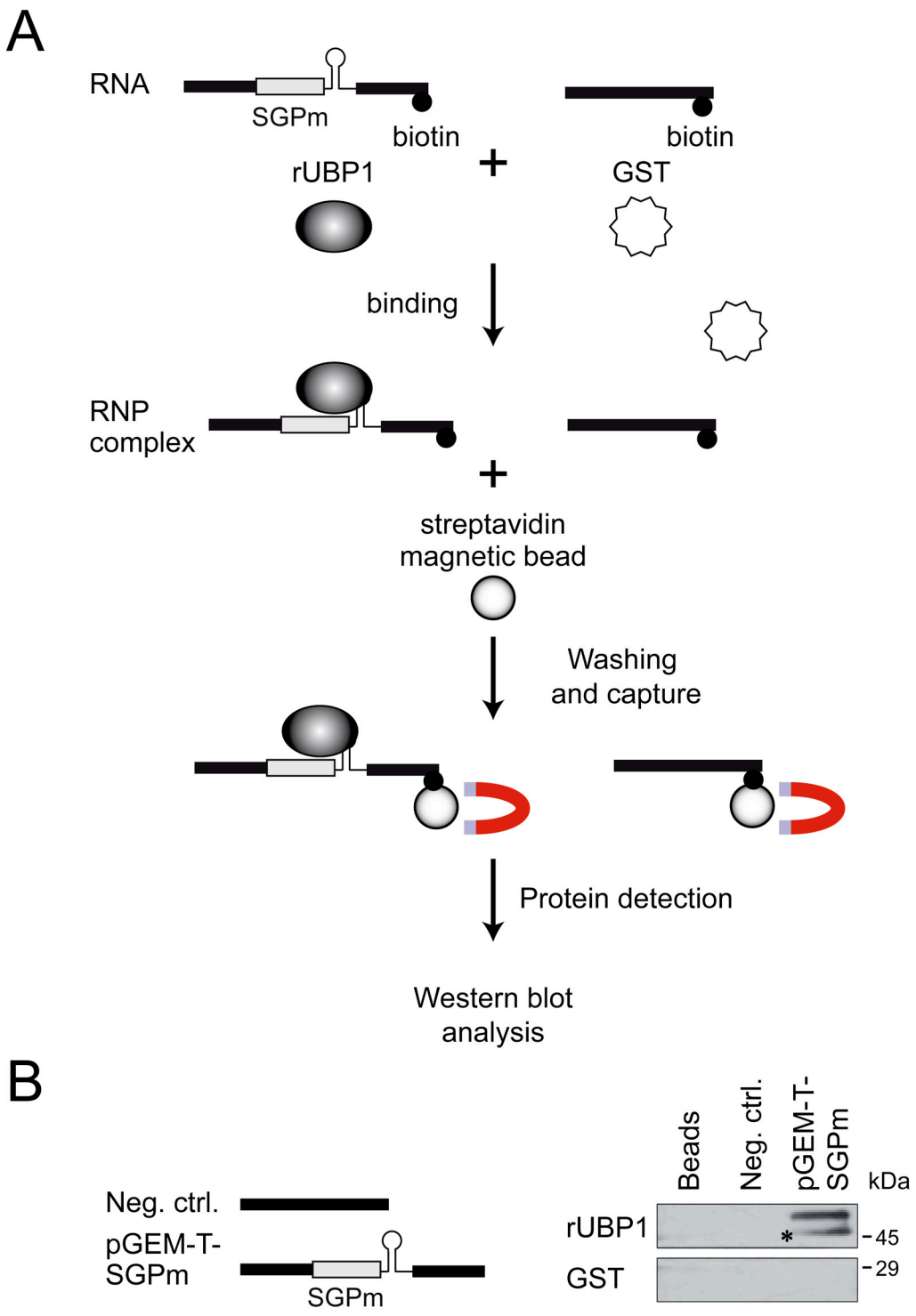


Figure S3. ***In vitro* RNA-binding assay.** *A*, Biotinylated transcripts containing (pGEM-T SGPm) or lacking SGPm (Neg. ctrl.) were incubated with recombinant proteins. The formed mRNP complex were pulled down using streptavidin-conjugated beads and the presence of the proteins detected by Western blot. *B*, Interaction of the GST-TcUBP1 (rUBP1) with a transcript bearing SGPm. The element SGP present in TSA-1 transcript was inserted into pGEM-T polylinker and transcribed *in vitro* using CTP-biotin (Invitrogen). As a control, the same construct lacking SGPm was used (Neg. ctrl.). GST or rUBP1 (25nM) were incubated with biotinylated transcripts and the presence of proteins in the recovered sample was revealed by Western blot. *, degradation product of recombinant GST-TcUBP1.

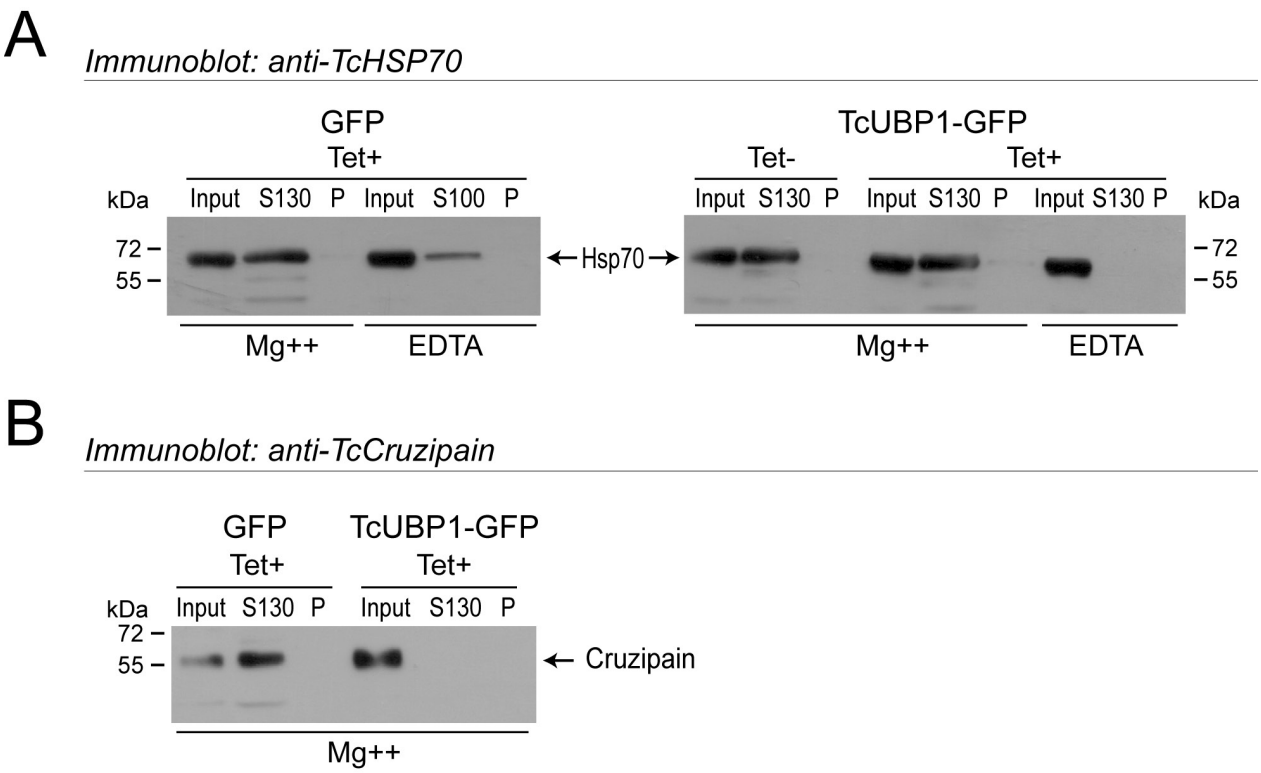


Figure S4. **Polysome purification (experiment controls).** Western blots analysis of cell extracts from GFP and TcUBP1-GFP samples, using induced (Tet+) control cultures. A clarified (S10) cytoplasmic extract from log-growth epimastigote *T. cruzi* culture, in the presence of magnesium (Mg++) or EDTA, was pelleted through a 30% sucrose cushion to separate prepolysome supernatant (S130) and polysomal pellet (P) fractions. Aliquots of S10, S130 and fivefold-concentrated aliquots of the P fraction were probed with serum. The position of the identified bands corresponding to TcHSP70 (*A*) and TcCruzipain (*B*) are noted by arrows. For TcUBP1-GFP, a non-induced (Tet-) sample in the presence of magnesium (Mg++) was also shown for the filter probed with anti-TcHSP70 antibody.

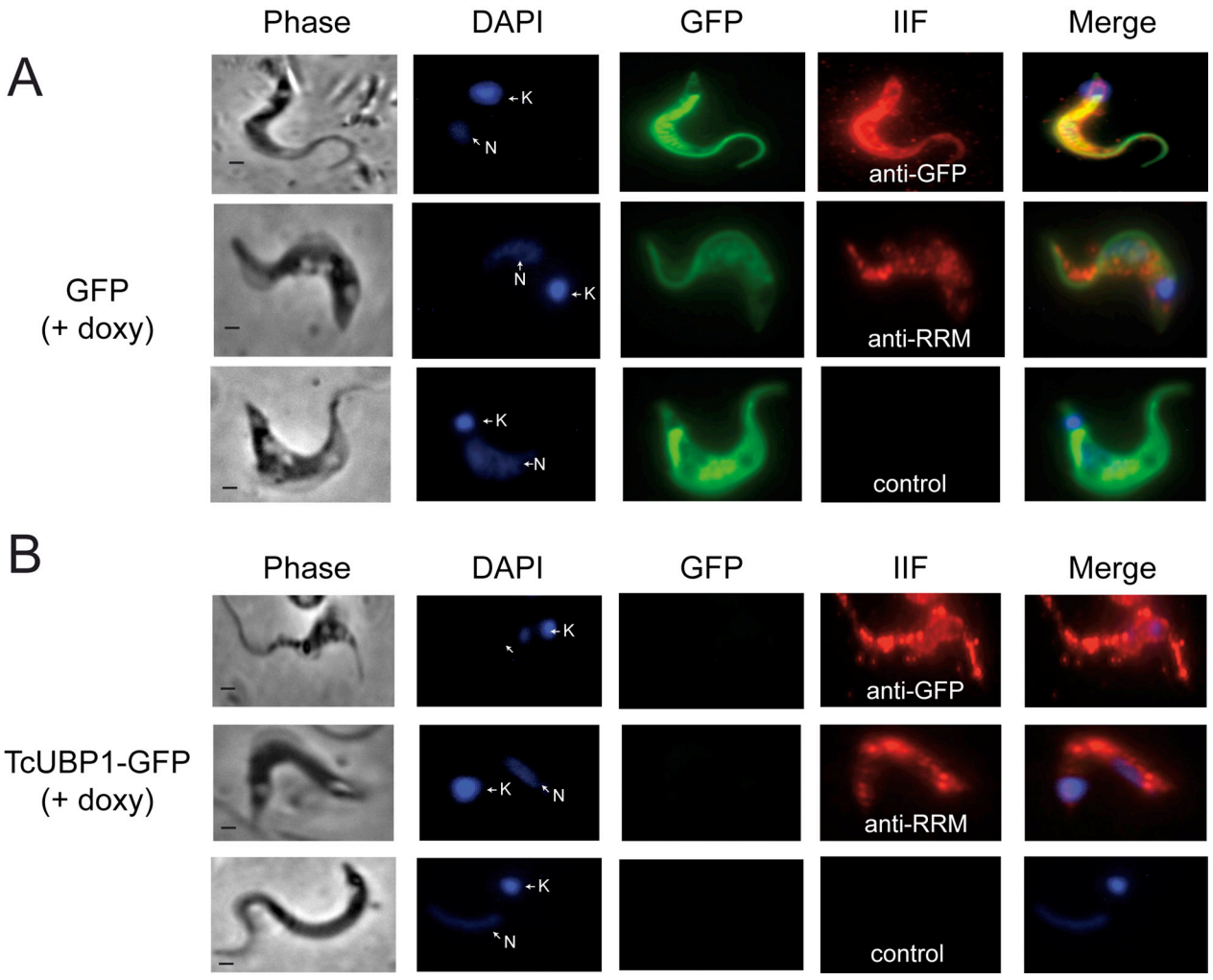


Figure S5. **Protein expression in transgenic trypomastigotes (induced samples).** Representative photographs of individual transgenic trypomastigotes with pTcINDEX-TcUBP1-GFP or pTcINDEX-GFP (induced with doxycycline), showing the fluorescence signals for TcUBP1 (anti-RRM) or GFP expression. DAPI staining of nuclear (N) and kinetoplast (K) DNA, with phase and merged images are also shown. Scale bars, 1 μm.

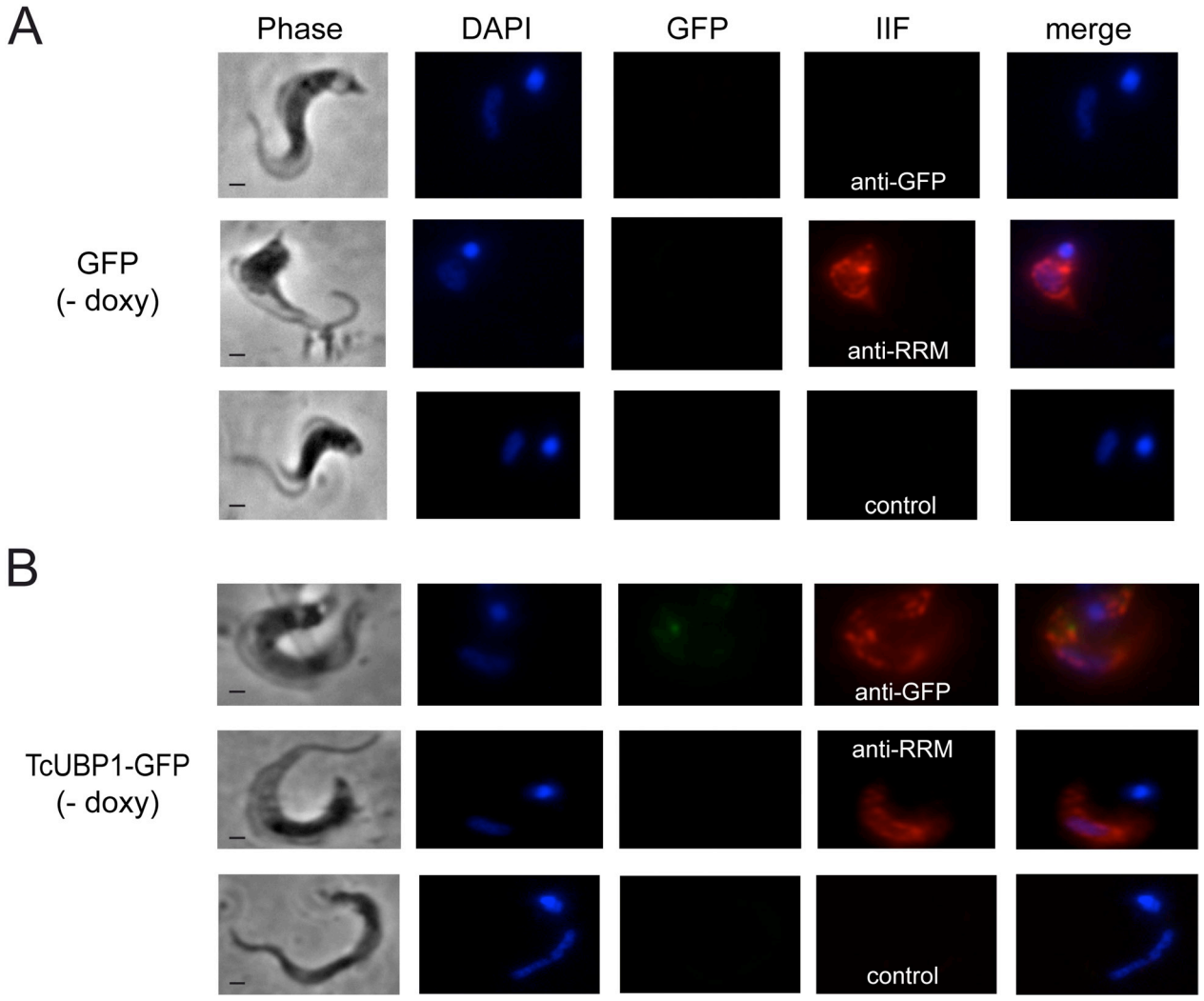


Figure S6. **Protein expression in transgenic trypomastigotes (non-induced controls).** Representative photographs of individual transgenic trypomastigotes with pTcINDEX-TcUBP1-GFP or pTcINDEX-GFP (without induction with doxycycline), showing the basal fluorescence signals for TcUBP1 (anti-RRM) or GFP expression. DAPI staining of nucleous (N) and kinetoplast (K), with phase and merged images are also shown. Scale bars, 1 μm.

SUPPLEMENTAL TABLES

Supplemental Table S1

Number of SGP-containing genes clustered in the *T. cruzi* genome.

Distance in a given chromosome (less than):	No. of Clusters	Total genes
1 Kb	1	2
2 Kb	9	16
10 Kb	32	~60
100 Kb	145	<290
1 Mb	191	<380

Supplemental Table S2
List of oligonucleotides used in this work.

Experiment	Target	GeneID	Name	Sequence (from 5′ to 3′)[a]
PCR	TSA-1	TcCLB.506471.120	Fwd	aagcttGGGGCAACCACTATGAACT
			Rev	ctcgagCTTTCAACCGTCTGACCCTCC
	SGPm	-	Fwd	aagcttTCCCGCCCAACTGCTCCACTC
			Rev	gagctcAAAAACACGGCGGCAGGGTG
qPCR	TSA-1	TcCLB.506471.120	Fwd	TGGAACAGTAATAGAGGACAACCTTTT
			Rev	CAATTCCCTTTCATCATATATTAACCC
	C71	TcCLB.510163.60	Fwd	TCTGCGTGGAGCACCTCAG
			Rev	CGCATAGTAATCCACTACCAGGAGT
	SA85	TcCLB.508285.60	Fwd	TTTGCGCCCTCTTCCACA
			Rev	GCAAGGTGAACTTTGTTCCGA
	GP85	TcCLB.506455.30	Fwd	GGAGTGTGCGGCCTCAGT
			Rev	TTCCTAGCGGTGTGTGTGTTGT
	SGPm	-	Fwd	AACTGCTCCACTCGCACAC
			Rev	CAGGGCCGTCGTCATGAG
	TcSMUGL	TcCLB.504539.20	Fwd	TGCAGCGGTGTGGACGTATAT
			Rev	CGCAGAGAATGCACGGG
	RpL6	TcCLB. 507709.50	Fwd	GCACCCACCCACCAAAA
			Rev	TCATGCGTTGCGTGTGTGT
	18S rRNA (small subunit)	TcCLB.508653.20	Fwd	CGGAATGGCACCACAAGAC
			Rev	TGGTAAAGTTCCCGTGTTGA
	28S rRNA (large subunit)	TcCLB.504153.50	Fwd	GTAGTATAGGTGGAAGCGCAAG
			Rev	CCAGCTCACGTTCCCTGTCA
	Luciferase	-	Fwd	CGGATTACCAGGGATTTCAG
			Rev	TCACGATCAAAGGACTCTGG
FISH	SGPm	-	5′Cy3	ACAGGGCCGTCGTCATGAGCGTGTCGG
				TGGGTGTGCGAGTGGAGCAGTTG

[a]The restriction sites used for molecular cloning are indicated underlined in lowercase.

SUPPLEMENTAL FILES

Supplemental File 1. Genes containing the 50-nt SGPm obtained from BLASTN searches.

Supplemental File 2. Genes containing both the SGPm and TcUBP1m.