Supplementary Material for:

An endonuclease-generated DNA break induces antigenic switching in *Trypanosoma brucei*

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Supplementary Figure Legends

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Supplementary Figure S1: Processing of the I-*Sce*I-induced DSB. a, Sequences of the 70-bp repeat regions from the 70.II (*VSG* 221 ES) cell line (top, black) and *VSG* 224 ES (bottom, red). The I-*Sce*I RS is underlined. Regions homologous to the primer used for sequencing (in b) are indicated in bold. Sequencing results from the five clones that switched to *VSG* 224 (shown in b) indicate loss of the I-*Sce*I RS, exonucleolytic degradation of ~500 bp of the *VSG* 221 ES (recipient, to the region highlighted in blue), and invasion of the first homologous region in the *VSG* 224 ES proximal to the *VSG* (donor, region highlighted in gray). **b**, Sequencing results of the five clones that switched to *VSG* 224. Sequences coming from the *VSG* 221 ES and *VSG* 224 ES are shown in black and red, respectively. There are several regions with polymorphisms (blue), such as the one shown to the right.

Supplementary Figure S2: Loss of the *VSG* 221 sub-telomeric region in the switched clones. The sub-telomeric region from the switched clones was amplified with primers specific to the *VSG* 221 sub-telomeric region (shown in Fig. 3a). Only the parental line (PA) retains this region. Amplification of *tubulin* is shown as a control.

Supplementary Figure S3: Correct integration of the I-*Sce*I RS. a, Schematic representation of restriction sites and expected fragments used to verify integration of the I-*Sce*I RS. **b**, Southern blot analyses showing clones in which the integration of the I-*Sce*I RS was verified by digestion with *Xho*I and I-*Sce*I and probing with Puromycin (circled bands). Up to 21 clones were screened for each cell line.

Supplementary Table S1: Summary table of *VSG* donors in 18 independent switching events. The *VSG* genes expressed in the switched clones were duplicated from silent ESs and mini-chromosomes. Like-colored boxes represent clones expressing the same *VSG*s.

Supplementary Figure S1

a **Primer:** **ggagagtgttgtgagtgtg**

70.II (*VSG* 221 ES)

ggagagtgttgtgagtgtgtgtatatacgaatattataataagagcagtaataataataatgataatgataatagaagagtgttgttagtgtgtatatacgaatattataa
taagagcagtaataatgataatgataatatcaggagagtgttgtgagtgtgtatatacgaatataataataaaagcagtaataataataataataatgataatgataatgata**ggag**
agtgttgtgagtgtgtgtatatacgaatattataataacaccagtaatagtaataacaataagaatcataatgataataggagagtgttgtgagtgtatatatacaaatattat
aataagagcagtactaatgataataatatta**ggaggagtgttgtgagtgtg**tgtatatacgaatattatactaagagcagtaataagtataataataat**ggagagtgttgtgagt**
gtgtgcttaccaatattataataatgatagcaacgaccaa*tagggataacagggtaat*tcgagggtgctcaagct**[..][*VSG*221]**

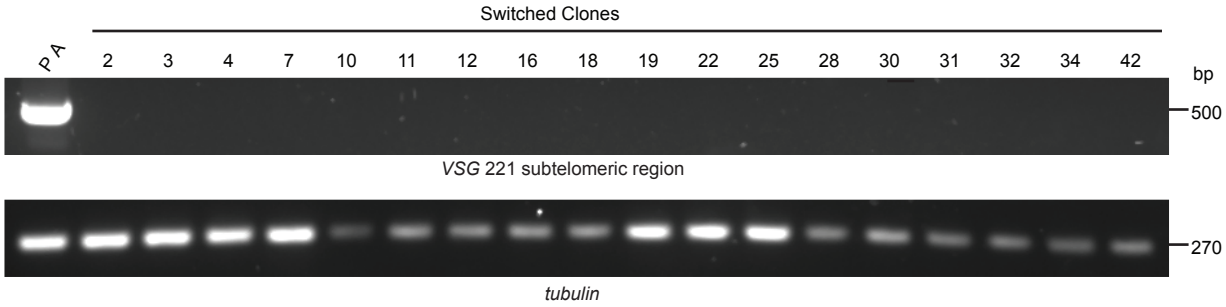
***VSG* 224 ES**

ggagagtgttgtgagtgtgtgtatatacgaatattataataaaagcagtaataataataataatgataataatgata**ggagagtgttgtgagtgtg**tatatacgaatattataa
taagagcagtaataatataataataatgataatgataaaaatagggagagtgttgtaagtgtgtgtatatacaaatattatagtaagagcagtaataataatgataataataat
aggagagtgttgtaa**gtgtgtgtatatacgaatattataataa**gtaatgatagtaatagtaaaaatat**[..][ΨESAG3][*VSG*224]**

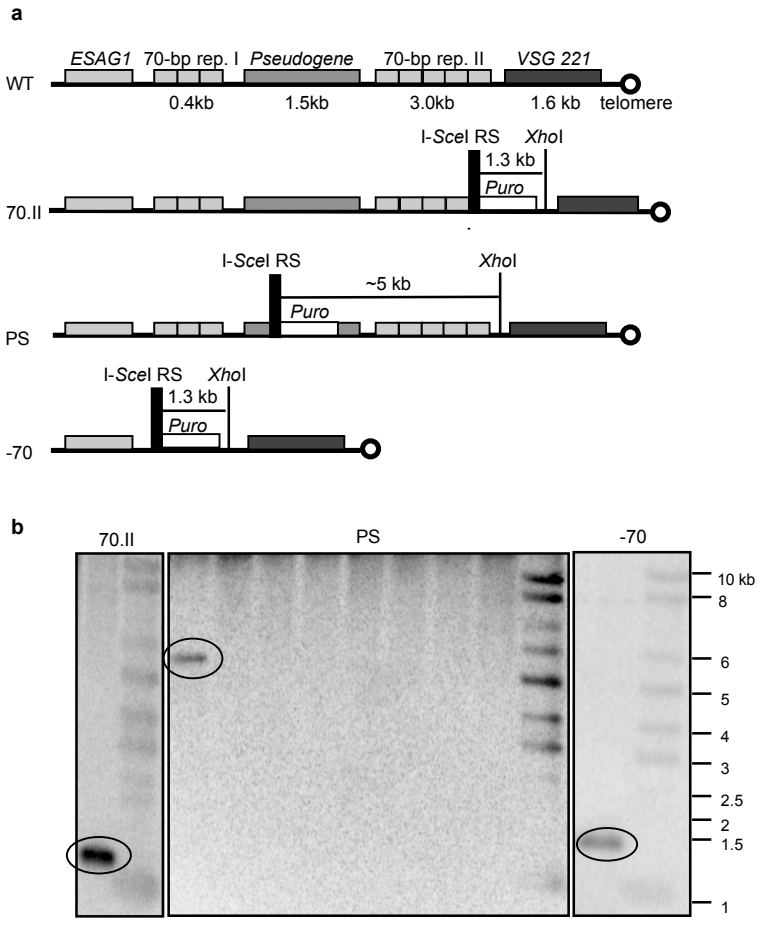
b

***VSG* 221**	TGTTGTGA**GTGTGTGTATATACGAATATTATAATAA**gagcagtaataataataataatga	[..]	
***VSG* 224**	tgttgtaa**gtgtgtgtatatacgaatattataataa**GTAATGATAGTAATAGTAAAAATA	[..]	AAATAACACACCA
Cl. 2	TGTTGGTGAGTGTGTGTATATACGAATATTATAATAAGAGCAGTAATGATAGTAATAGTAAAAA	[..]	AAATAACACACCA
Cl. 3	TGTTGTGAGTGTGTGTATATACGAATATTATAATAAGTAATGATAGTAATAGTAAAAATA	[..]	AAAATACACACCA
Cl. 4	TGTTGTGAGTGTGTGTATATACGAATATTATAATAAGAGCAGTAATGATAGTAATAGTAAAAATA	[..]	AAATAACACACCA
Cl. 22	TGTTGTGAGTGTGTGTATATACGAATATTATAATAAGTAATGATAGTAATAGTAAAAATA	[..]	AAA-TACCCACCA
Cl. 25	TGTTGTGAGTGTGTGTATATACGAATATTATAATAAGTAATGATAGTAATAGTAAAAATA	[..]	AAAATAACACACCA

Supplementary Figure S2



Supplementary Figure S3



Clone #	Donor VSG				
	MITat	Hertz-Fowler[6]	Lab-Specific Name	Location	GenBank Number
2	1.3	427-3	224	BES7/TAR153	AY935575
3	1.3	427-3	224	BES7/TAR153	AY935575
4	1.3	427-3	224	BES7/TAR153	AY935575
7	1.8	427-8	1.8	BES12/TAR29	AY935574
10	1.1	427-1	060	Chr.5	X56761
11	1.9	427-9	V02	BES2/TAR129	AY935573
12	1.6	427-6	121	BES3/TAR15	X56764
16				Chr.11	XM_824682
18				Chr.11	XM_824682
19	1.11	427-11	bR-2	BES15/TAR126	AY935571
22	1.3	427-3	224	BES7/TAR153	AY935575
25	1.3	427-3	224	BES7/TAR153	AY935575
28		427-23		Mini-chromosome	FJ98214
30	1.9	427-9	V02	BES2/TAR129	AY935573
31		427-24		Mini-chromosome	FJ98212
32	1.8	427-8	1.8	BES12/TAR29	AY935574
34	1.1	427-1	060	Chr.5	X56761
42		427-25		Mini-chromosome	FJ98213