Supplementary figure legend:

Fig. S1: Induction of PfSR1 expression using the DDD system does not affect nuclear localization *in vivo*. Live imaging of PfSR1-GFP-DDD expressing parasites growing on 5 μM TMP showing the cellular localization of the induced PfSR1-GFP throughout the IDC. Nuclei were stained with Hoechst. The size bar represents 5μm. The *in vivo* imaging show that PfSR1 shuttles between the nucleus and cytoplasm during IDC.

Fig. S2: RNA immuno precipitation (RIP) reveals RNA molecules which are specifically bound by PfSR1 *in vivo*. The RIP was performed as described in Fig 2 with additional negative control of an unrelated non-nuclear plasmodium protein (PF3D7_0600400) fused to GFP (mock GFP). **(A)** Western blot of the IP with anti-GFP Abs. Lane 1-3: Pre-IP (1), IP (2) and flow through (3) of NF54 parasites that carry PfSR1-GFP-DDD and grown on TMP media + 2 ug/ml BSD; Lane 4-5: Pre-IP (4), IP (5) of NF54 parasites that carried the mock GFP plasmid and grown on TMP + 2 ug/ml BSD. **(B)** Validation of the RNA-IP of representative genes (PF3D7_1412600, PF3D7_0826100 and Pf3D7_0909700 respectively), using RT-PCR on the PfSR1-GFP-DDD IP and mock GFP IP samples.

Fig. S3: Pie chart showing the distribution of the number of exons in genes that are bound by PfSR1.

Fig S4: PfSR1 binds directly to the specific RNA motif SBM-1 *in vitro.* (A-C) Phosphorimaging quantification of the RNA-EMSA data presented on Fig. 3 (C-E) respectively. The percentage of complex formation measured is presented relative to the value measured in the absence of competitive ligand, which was considered to be 100%.

Fig. S5: Characterization of SBM-2 motif by mutation analysis. Competition RNA EMSA of radiolabeled SBM-2 ligand with various mutated non-labeled SBM-2 ligands used as competitors for PfSR1 binding. **(A)** Competitor ligands were mutated by replacement of bases with uridine bases at different location of the core sequence of SBM-2 (underlined on the left). These competitors were named Mut1U-Mut3U. Additional ligands included replacements of adenine at different locations were named Mut1A-Mut3A. The SBM-2 sequence is marked in bold. **(B)** Competitor ligands were mutated by replacement of bases with cytosine at different location of the core sequence of SBM-2 (underlined on the left). These competitors were named Mut1C-Mut5C. As additional competitors we have included a ligand in which the sequence of SBM-2 flanking region was replaced (A-flanked) and a short probe that include the SBM-2 sequence with no flanking sequences (short probe).

Fig. S6: The influence of ectopic expression of PfSR1 on transcript levels of gametogenesis marker genes. Comparison of transcript levels of genes known as markers for early gametogenesis in parasites that ectopically express PfSR1-myc and mock plasmid (pHBIRH). Transcript levels were measured by RT-qPCR and presented as

relative copy number (RCN) calculated relative to the housekeeping gene arginyl-tRNA synthetase (PFL0900c). All values presented are the average of triplicates. Error bars represent standard errors.

Figure S7

PfSR1 is involved in the regulation of steady state RNA levels of genes containing SBM-1 and SBM-2. Transcript levels of various genes containing SBM-1 and SBM-2 were compared in parasites that ectopically express PfSR1-myc to those transfected with a mock plasmid (pHBIRH). Three of these genes (PF3D7_0909700, PF3D7_1149000 and PF3D7_0826100) were significantly enriched in our PfSR1 RIP-chip analysis. Transcript levels were measures by RT-qPCR from RNA of tightly synchronized late stage parasites (~40h post invasion) and presented as relative copy number (RCN) to the housekeeping genes arginyl-tRNA synthetase (PF3D7_1218600). Values represent the average of three different transfections and error bars represent standard error. Samples differences were tested using Student's t-test. Sample differences in which $P < 0.05$ are marked with asterisks.

Table S1:

List of transcripts which were significantly enriched by RIP-chip of induced PfSR1-GFP-DDD

[Gene ID]	[Previous ID(s)]	[# Exons]	[Transcript Length]	[Product Description]
PF3D7_0531600	MAL5_18S, MAL5_18S:rRNA	1	2092	18S ribosomal RNA
Pfa_vasR_6612				putative ncRNA (>PF3D7_0733000, PfEMP1)
PF3D7_0903700	PFI0180w	3	1362	alpha tubulin 1
Pfa_raR_6328a				putative ncRNA – probe not found in PlasmoDB
PF3D7_1352700	PF13_0273	1	7602	P-loop containing nucleoside triphosphate hydrolase, putative
PF3D7_0508900	MAL5P1.90, PFE0440w	1	9405	conserved Plasmodium protein, unknown function
PF3D7_1412600	PF14_0125	1	1491	deoxyhypusine synthase (DHS)
PF3D7_0112700	MAL1_28s, MAL1_28s:rRNA	1	4104	28S ribosomal RNA
PF3D7_1149000	PF11_0506, PF11_0507	2	18282	antigen 332, DBL-like protein (Pf332)
PF3D7_1247400	2277.t00455, MAL12P1.454, PFL2275c	1	915	FK506-binding protein (FKBP)-type peptidyl-prolyl isomerase (FKBP35)
PF3D7_0826100	MAL8P1.23	1	25776	E3 ubiquitin-protein ligase, putative
PF3D7_1038400	2296.t00404, PF10_0374	2	28692	gametocyte-specific protein (Pf11-1)
PF3D7_0910700	PFI0520w	1	1632	actin-like protein, putative (ALP5a)
PF3D7_0909700	PFI0470w	1	3111	FHA domain protein, putative
PF3D7_0317700	MAL3P6.15, PFC0780w	10	8613	CPSF (cleavage and polyadenylation specific factor), subunit A, putative
PF3D7_0811900	MAL8P1.101	6	3531	RNA binding protein, putative
RNAzID:1678				putative ncRNA- (Plasmodium RNA of unknown function RUF1)
PF3D7_1007700	PF10_0075	1	4794	transcription factor with AP2 domain(s) (ApiAP2)
PF3D7_0819300	PF08_0051	1	3132	conserved Plasmodium protein, unknown function
PF3D7_1240900	2277.t00394, MAL12P1.392, PFL1970w	2	6864	erythrocyte membrane protein 1, PfEMP1 (VAR)
PF3D7_0305500	MAL3P2.18, PFC0245c	1	11802	conserved Plasmodium protein, unknown function
PF3D7_0526600	MAL5P1.265, PFE1325w	1	13575	conserved Plasmodium protein, unknown function
PF3D7_1035200	PF10_0343	1	1758	S-antigen
PF3D7_1200600	2277.t00006, MAL12P1.6, PFL0030c	2	9171	erythrocyte membrane protein 1, PfEMP1 (VAR2CSA)

PF3D7_0630000	2270.t00188, MAL6P1.128, PFF1455c	10	843	CPW-WPC family protein
PF3D7_1150400	PF11_0521	2	9360	erythrocyte membrane protein 1, PfEMP1 (VAR)
PF3D7_1024800	PF10_0242	1	4395	conserved Plasmodium protein, unknown function
PF3D7_0801100	MAL8b_28s, MAL8b_28s:ncRNA	1	6175	28S ribosomal RNA
PF3D7_0303100	MAL3P1.11, PFC0145c	1	4326	conserved Plasmodium protein, unknown function
PF3D7_1132000	PF11_0329	1	2649	ubiquitin-like protein, putative
PF3D7_0204900	PF02_0046, PFB0220w	2	1065	ubiE/COQ5 methyltransferase, putative
PF3D7_1229000	2277.t00281, MAL12P1.281, PFL1405w	1	5505	conserved Plasmodium membrane protein, unknown function
PF3D7_0724900	PF07_0104	1	5694	kinesin-like protein, putative
PF3D7_0504800	MAL5P1.48, PFE0235c, PFE0240c	1	16920	conserved Plasmodium protein, unknown function
PF3D7_0801200	PF08_tmp2, PF08_tmp2:ncRNA	1	162	5.8S ribosomal RNA
PF3D7_0801300	PF08_0136b	1	873	von Willebrand factor A-domain related protein (WARP)
PF3D7_0207500	PF02_0071, PFB0335c	4	3096	serine repeat antigen 6 (SERA6)
PF3D7_0821100	PF08_0044	2	2757	protein kinase 1 (PK1)
PF3D7_1428000	PF14_0258	7	1245	conserved Plasmodium membrane protein, unknown function
PF3D7_1014300	PF10_0140	2	7140	conserved protein, unknown function
PF3D7_1030500	PF10_0298	1	1182	26S proteasome regulatory subunit, putative
PF3D7_1201700	2277.t00017, MAL12P1.17, PFL0085c	1	744	conserved Plasmodium membrane protein, unknown function
PF3D7_1318300	PF13_0101	1	5523	conserved Plasmodium protein, unknown function
PF3D7_0317100	MAL3P6.9, PFC0750w	2	2910	conserved Plasmodium protein, unknown function
PF3D7_0612200	2270.t00016, MAL6P1.303, PFF0595c	8	5595	leucine-rich repeat protein (LRR6)
PF3D7_1037500	PF10_0368	1	2130	dynamin-like protein (DYN2)
PF3D7_1113000	PF11_0528	7	17769	conserved Plasmodium protein, unknown function
PF3D7_1307400	PF13_0038	2	1164	conserved Plasmodium protein, unknown function
PF3D7_0900100	PFI0005w	2	6813	erythrocyte membrane protein 1, PfEMP1 (VAR)
PF3D7_1442600	PF14_0404	2	10632	TRAP-like protein,sporozoite-specific transmembrane protein S6 (TREP)
PF3D7_0804700	PF08_0122	1	5997	conserved Plasmodium protein, unknown function
PF3D7_1209300	2277.t00094,	1	4386	zinc finger transcription factor, putative (KROX1)

	MAL12P1.93, PFL0465c			
PF3D7_0829900	PF08TR005, PF08TR005:ncRNA	1	3171	unspecified product
PF3D7_0723500	PF07_0098	1	681	dynactin subunit 5, putative
PF3D7_0321100	MAL3P7.15, PFC0930c	1	5535	conserved Plasmodium protein, unknown function
PF3D7_1240400	2277.t00391, MAL12P1.389, PFL1955w	2	6864	erythrocyte membrane protein 1, PfEMP1 (VAR)
PF3D7_0707200	MAL7P1.207	1	6390	conserved Plasmodium protein, unknown function
PF3D7_0627100	2270.t00158, MAL6P1.157, PFF1315w	1	5139	ankyrin-repeat protein, putative
PF3D7_1131100	PF11_0321	1	2322	serpentine receptor, putative (SR1)
PF3D7_1432300	PF14_0304	3	4920	conserved Plasmodium protein, unknown function
PF3D7_1342200	PF13_0230	1	2919	conserved Plasmodium membrane protein, unknown function
PF3D7_0104300	MAL1P1.34b, PFA0215w, PFA0220w	3	10500	ubiquitin carboxyl-terminal hydrolase 1, putative (UBP1)
PF3D7_1241200	2277.t00396, MAL12P1.394, PFL1980c	3	6930	conserved Plasmodium protein, unknown function
PF3D7_1432400	PF14_0303, PF14_0305, PF14_0306, PF14_0785	18	4515	leucine-rich repeat protein (LRR5)
PF3D7_0533100	MAL5P1.322, PFE1640w	1	9495	erythrocyte membrane protein 1 (PfEMP1), truncated, pseudogene (VAR1CSA)
PF3D7_1478900	PF14TR008, PF14TR008:ncRNA, PF14TR009, PF14TR010, PF14TR011	1	3988	unspecified product
PF3D7_1100100	PF11_0007	2	6549	erythrocyte membrane protein 1, PfEMP1 (VAR)

Table S2:

Genome wide distribution of PfSR1 binding motifs SBM1 and SBM2 (provided as additional excel file).

Table S3:

List of primers used in this study.

Primer	Sequence
GFP-DDDF-SpeI	GGACTAGTATGAGTAAAGGAGAAGAAC
GFP-DDDR-SacI	CCGAGCTCTCAAGCGTAATCTGGAAC
PF3D7_0826100F	CCGAAAGAAATAGTAACGAA
PF3D7_0826100R	TCCAATCTGGATACAATGTT
PF3D7_1038400F	CTGAAGAAGATGTTGAACAAG
PF3D7_1038400R	GGTATCACCTCTTCTAGAATTT
PF3D7_0909700F	TGCTTTACAGCTAAAAATGT
PF3D7_0909700R	CGTTCATATTATCTTCCATGA
PF3D7_1412600F	ATAAGAAGCGATGATGAATG
PF3D7_1412600R	AAGTTAGTTGCTTGAAATCC
PF3D7_0600400F_0020	GGATCCTTATAAAAATGGGATCAGA
PF3D7_0600400R_0020	CCCGGGTATATTCCATACATCC
PF3D7_0509700FulF	CAAATACATTAAATAACATAGG
PF3D7_0509700FulR	TTTTGCTATATCTTCTTCAA
PF3D7_0509700Ex1Ex3F	GACATCTCAAATAAAAAAAATAG
PF3D7_0509700int3ex4R	CCATTTATATTTTTTGCTCTTAA

Figure S1:

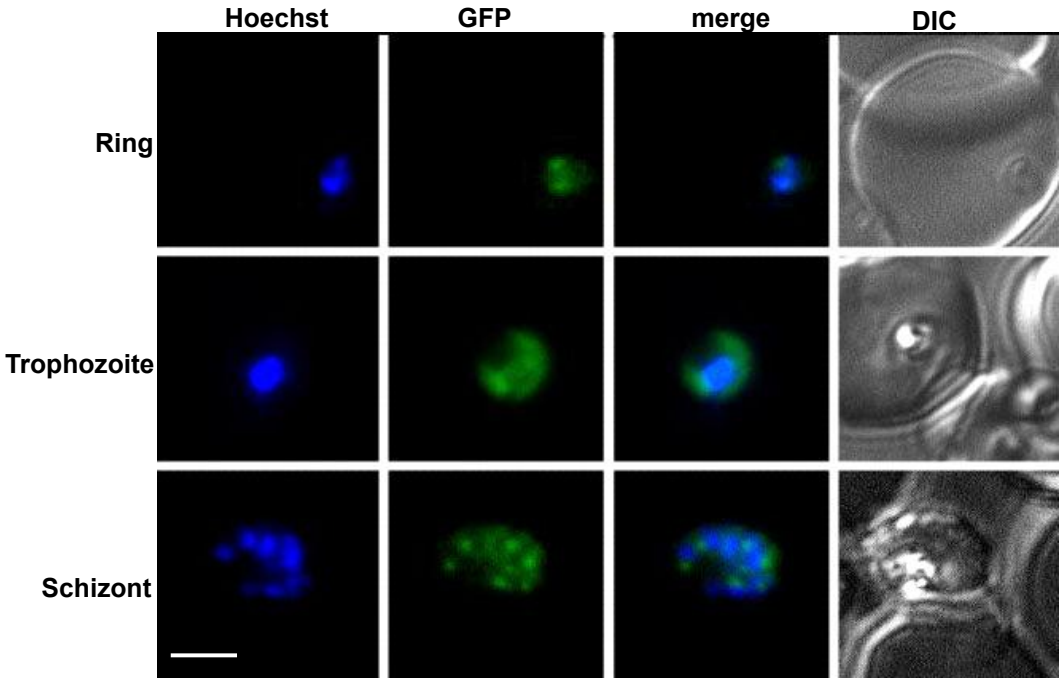
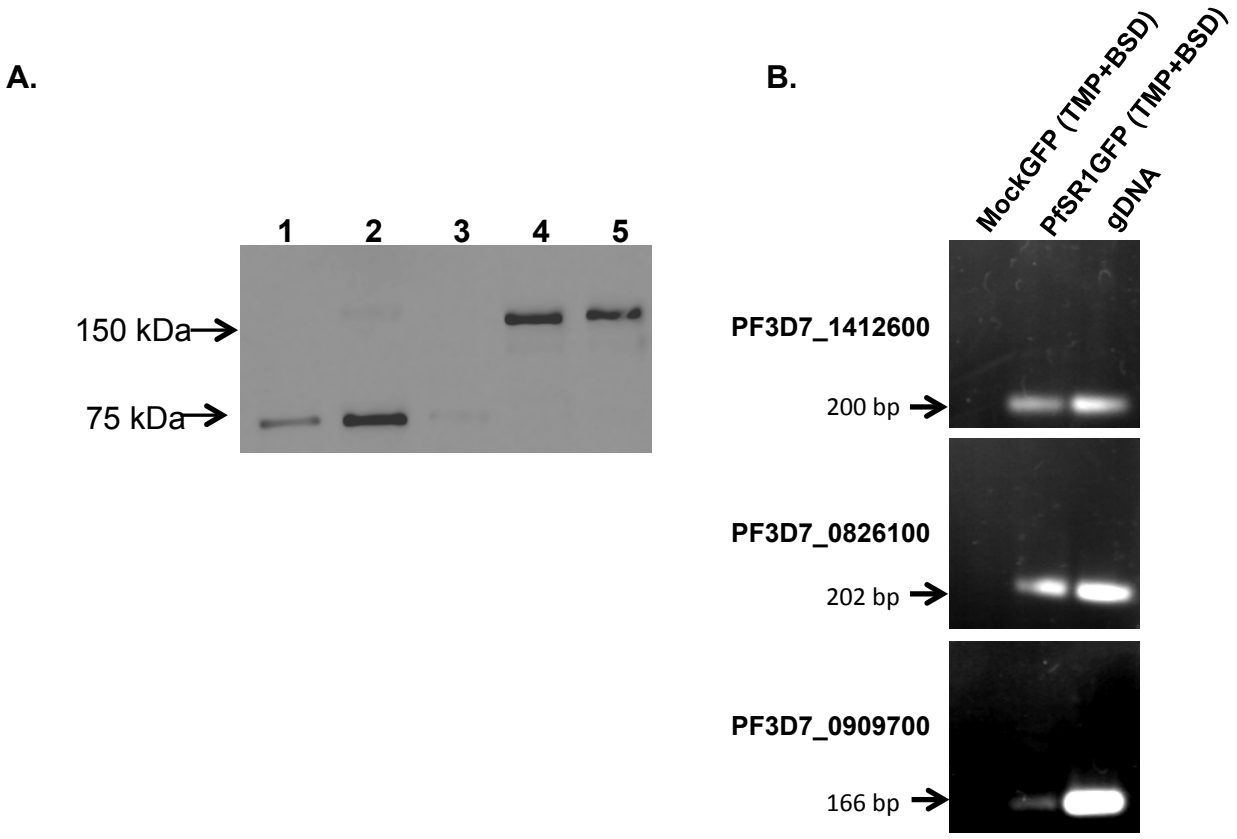


Figure S2: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1
2
3
4
5
150 kDa
75 kDa
B.
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PfSR1GFP (TMP+BSD)
gDNA
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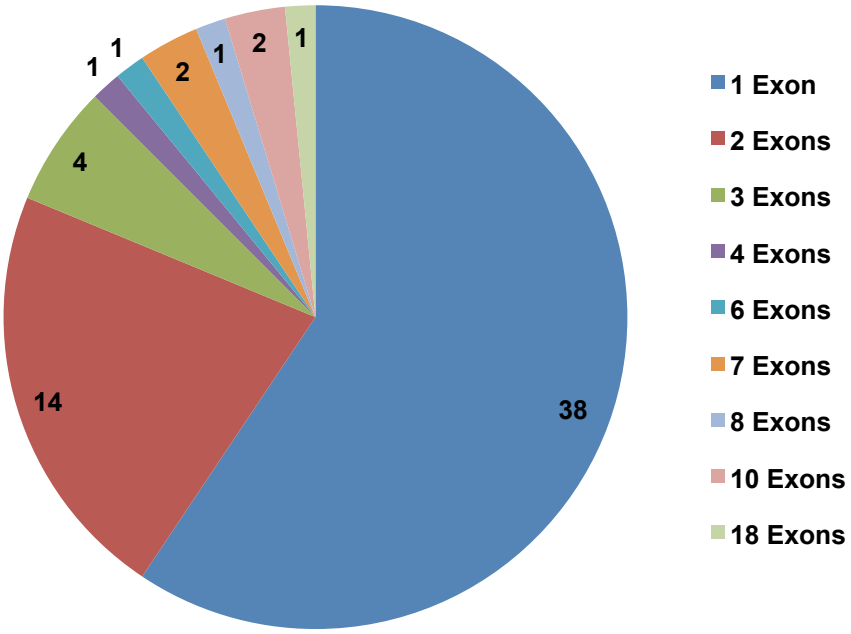
200 bp
PF3D7_0826100
202 bp
PF3D7_0909700
166 bp

Figure S3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4
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1
2
1
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4 Exons
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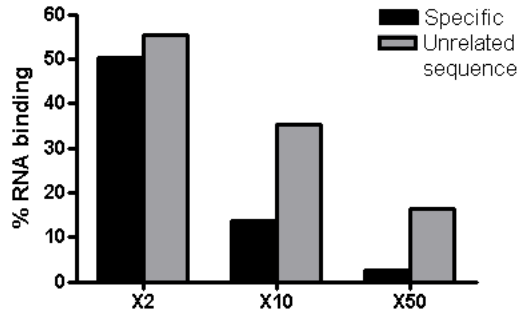
7 Exons
8 Exons
10 Exons
18 Exons

Figure S4:

A.



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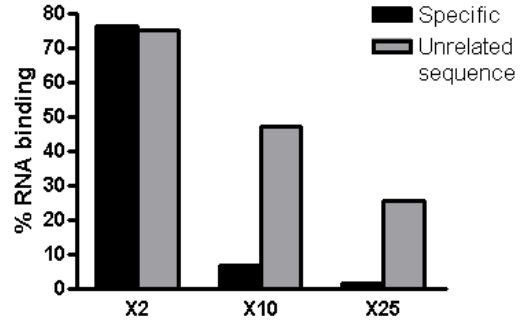
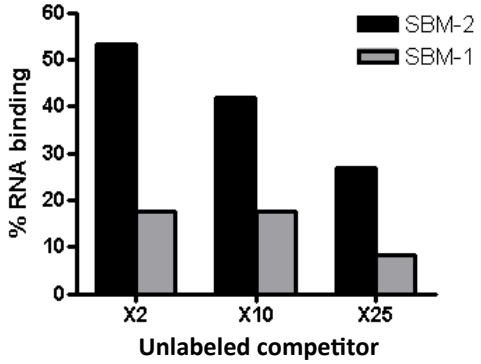


C.



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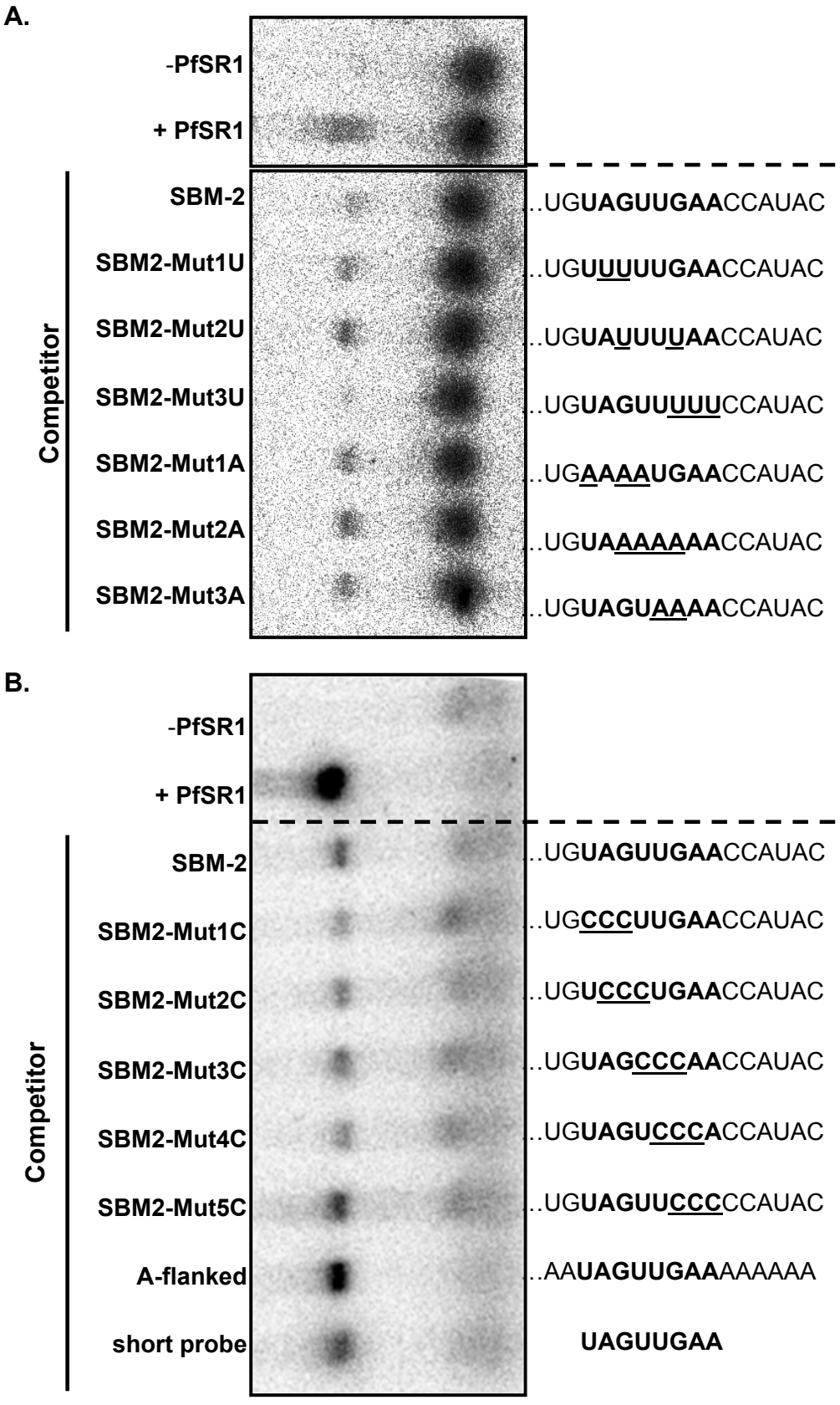
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-PfSR1
+ PfSR1
Competitor
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SBM2-Mut1U
SBM2-Mut2U
SBM2-Mut3U
SBM2-Mut1A
SBM2-Mut2A
SBM2-Mut3A
..UGUAGUUGAACCAUAC
..UGUUUUUGAACCAUAC
..UGUAUUUUAACCAUAC
..UGUAGUUUUCCAUAC
..UGAAAAUGAACCAUAC
..UGUAAAAAAACCAUAC
..UGUAGUAAAACCAUAC
B.
-PfSR1
+ PfSR1
Competitor
SBM-2
SBM2-Mut1C
SBM2-Mut2C
SBM2-Mut3C
SBM2-Mut4C
SBM2-Mut5C
A-flanked
short probe
..UGUAGUUGAACCAUAC
..UGCCCUUGAACCAUAC
..UGUCCCUGAACCAUAC
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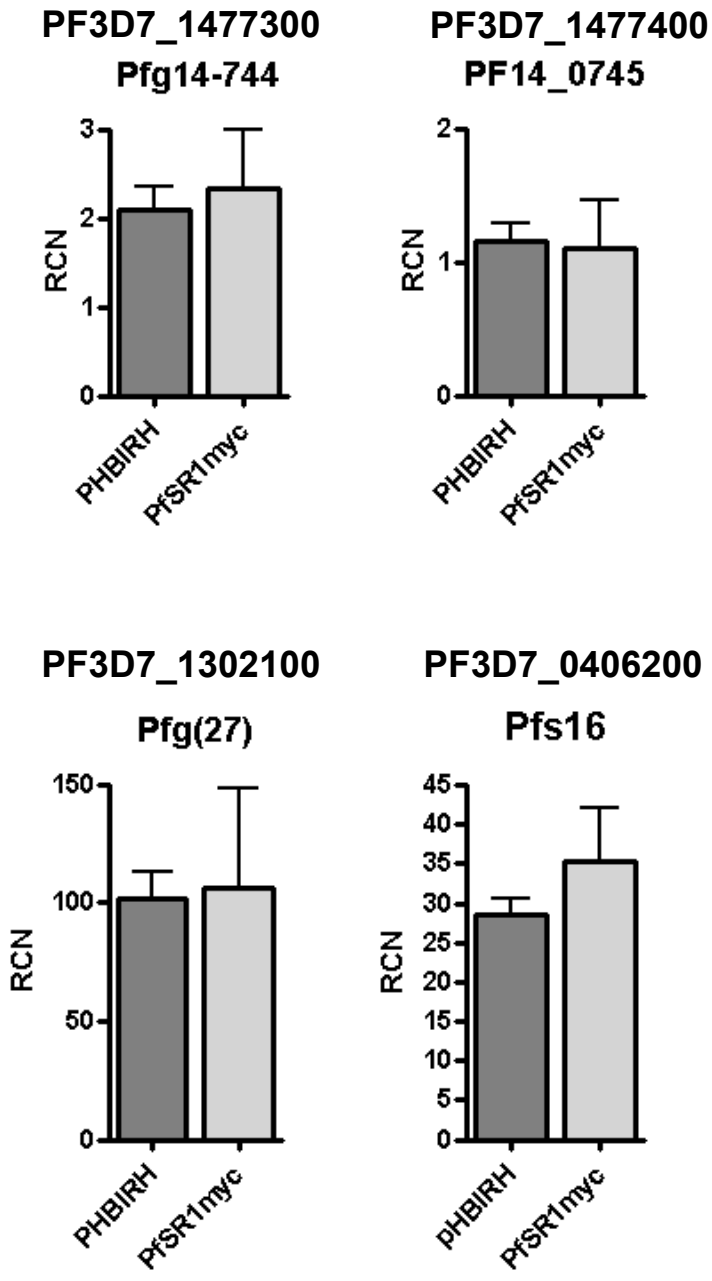
Figure S6: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2
1
0
PHBIRH
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PF3D7_1477400
PF14_0745
RCN
2
1
0
PHBIRH
PfSR1myc
PF3D7_1302100
Pfg(27)
RCN
150
100
50
0
PHBIRH
PfSR1myc
PF3D7_0406200
Pfs16
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35
30
25
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15
10
5
0
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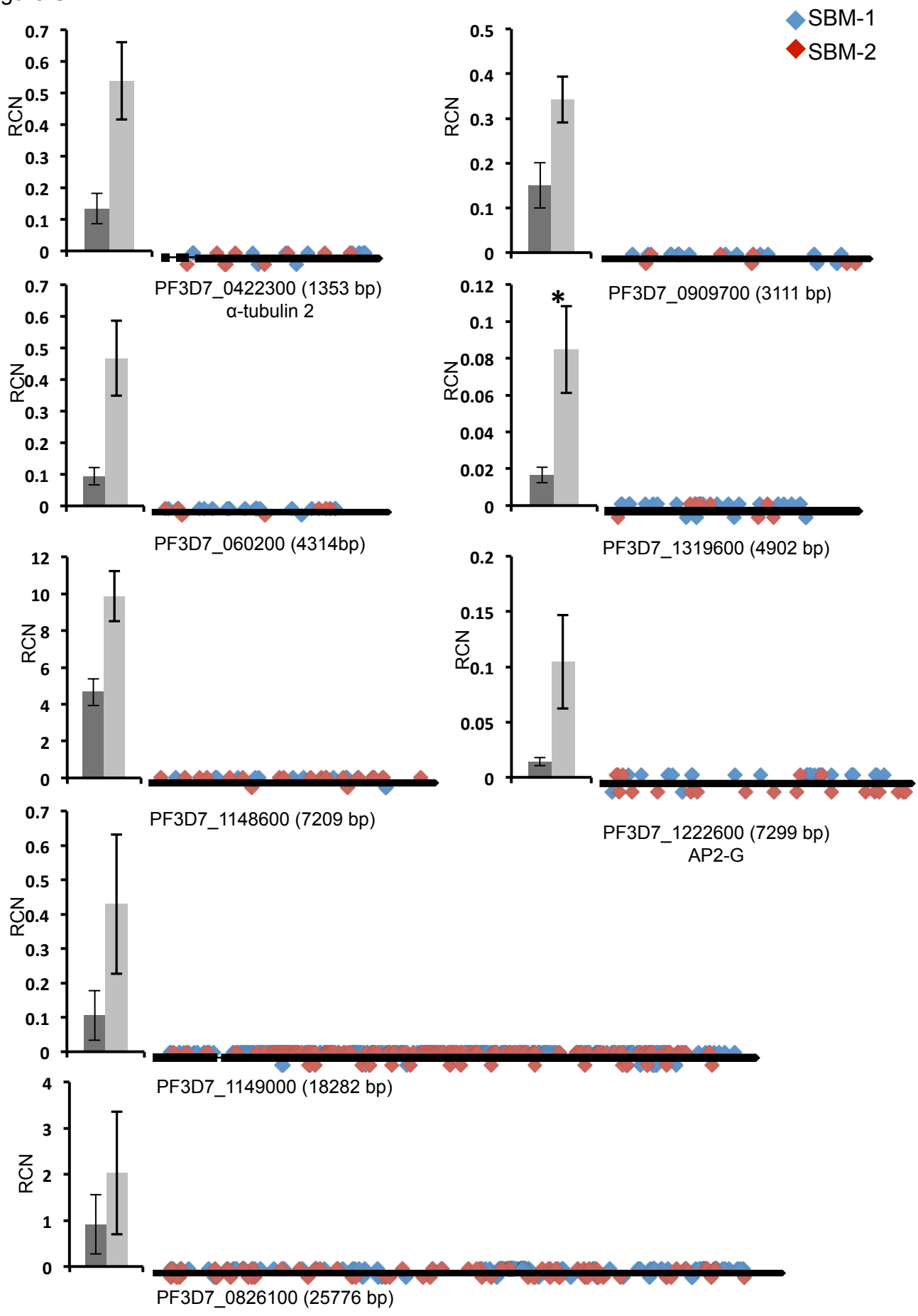
PfSR1myc

Figure S7: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SBM-2
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α-tubulin 2
PF3D7_0909700 (3111 bp)
*
PF3D7_060200 (4314bp)
PF3D7_1319600 (4902 bp)
PF3D7_1148600 (7209 bp)
PF3D7_1222600 (7299 bp)
AP2-G
PF3D7_1149000 (18282 bp)
PF3D7_0826100 (25776 bp)

Figure S7

PfSR1 is involved in the regulation of steady state RNA levels of genes containing SBM-1 and SBM-2. Transcript levels of various genes containing SBM-1 and SBM-2 were compared in parasites that ectopically express PfSR1-myc to those transfected with a mock plasmid (pHBIRH). Three of these genes (PF3D7_0909700, PF3D7_1149000 and PF3D7_0826100) were significantly enriched in our PfSR1 RIP-chip analysis. Transcript levels were measures by RT-qPCR from RNA of tightly synchronized late stage parasites (~40h post invasion) and presented as relative copy number (RCN) to the housekeeping genes arginyl-tRNA synthetase (PF3D7_1218600). Values represent the average of three different transfections and error bars represent standard error. Samples differences were tested using Student's t-test. Sample differences in which $P < 0.05$ are marked with asterisks.