Supplementary data

"The role of N-terminus….. *var* gene silencing" by Deshmukh A *et al*. (NAR-02966-V-2011)

Materials and methods

Purification of recombinant proteins

For Ni-nitrilotriaceticacid (Ni-NTA) purification of proteins (PfORC1N$_{1-238}$, PfORC1N$_{1-182}$, PfORC1NL1$_{1-238}$, PfSir2 and PfHP1), the bacterial pellet was lysed in lysis buffer (100mM Na_2HPO_4, 100mM NaH_2PO_4, 50mM Tris-HCl pH8.0, 300mM NaCl, 10mM β-mercaptoehanol and 100μM PMSF) containing 10mM imidazole. The lysate was cleared by centrifugation at 10000xg. The cleared lysate was incubated with Ni-NTA beads for 1 hour at 4^0C. The beads were washed in wash buffer and eluted with lysis buffer containing 500mM imidazole. All the proteins were dialyzed and stored at -80^0C.

For MBP fusion protein purification (C-terminus of ORC1; ORC1C; residues 689-1022 of full length ORC1), the bacterial pellet was lysed in the lysis buffer (20mM Tris-HCl pH7.5, 200mM NaCl, 1mM EDTA, 100μM PMSF). The lysates were cleared by centrifugation at 10000xg. The soluble fraction was incubated with Amylose resin beads at 4^0C for 1 hour. Beads were washed with lysis buffer containing 300mM NaCl and eluted with elution buffer (50mM Tris-HCl pH7.5, 300mM NaCl and 10mM Maltose).

Immunofluorescence assay

The parasites were synchronized using sorbitol and the parasite pellet obtained from respective stage was washed in phosphate buffered saline (PBS). The parasites were smeared on glass slides and fiixation was performed in chilled methanol for 5 minutes. The air-dried glass slides were rehydrated using 1xPBS and incubated with 0.01% saponin and 3% BSA in 1xPBS at room temperature. Parasites were then incubated with primary antibodies diluted in 1%BSA at room temperature for 1 hour. After few washes in 1xPBS, parasites were incubated with secondary antibodies conjugated with fluorochromes (ALEXA 488 or ALEXA 594, Molecular Probes). Slides were then

washed thoroughly with 1xPBS and mounted with antifade reagent with DAPI. Slides were scanned with Carl Zeiss Apotome microscope. Antibody dilutions were used as followed: rabbit Orc1 1:1000, mouse Sir2 1:500 and mouse HP1 1:1000. Secondary antibodies were used at the dilution of 1:1000. Confocal images were collected using an Olympus FV1000 confocal microscope.

Figure legends

Supplementary figure 1

A. Live cell confocal microscopy of PCNA-GFP expressing parasites. PCNA-GFP shows nuclear punctate staining co-localized with DAPI. Scale bar (2 μm) is shown inset. **B.** Confocal microscopy of GFP-fusion proteins in the live parasites shows nuclear punctate staining for PfORC1N$_{1-238}$ and nuclear diffused pattern for PfORC1N$_{1-182}$. Scale bar (2 μm) is shown inset. The schematic diagrams of the above two diagrams are shown on the top.

Supplementary figure 2

A. PCR amplification using primer sets specific for TARE1, TARE2, TARE2-3, TARE3 and TARE6 regions as detailed in primer list shown below. Standard molecular mass markers (kb) are shown on the left**. B.** Relative intensity of PCR products corresponding to different TARE regions following 20, 25 and 30 cycles. The results indicate that the PCR products do not get saturated within this range.

Supplementary figure 3

A. Live cell fluorescence microscopy images of PfORC1N$_{1-238}$-GFP and PfORC1NL1$_{1-238}$-GFP expressing parasites. PfORC1N$_{1-238}$-GFP shows nuclear punctate staining co-localized with DAPI whereas PfORC1NL1$_{1-238}$-GFP shows diffused staining pattern. Scale bar (2 μm) is shown inset. **B.** ChIP-PCR analysis shows binding of PfORC1N$_{1-238}$-GFP to TARE 1, 2,3 and 6 regions *in vivo* whereas PfORC1NL1$_{1-238}$-GFP does not bind to these regions under the same experimental conditions

Supplementary figure 4

A. Immunolocalization of Sir2 in 3D7 and Sir2 KO parasite lines. Glass slides containing the parasite smears from 3D7 wild type or Sir2 KO parasite lines were treated for immunolocalization using antibodies against PfSir2. DAPI shows the nuclei. Sir2 shows nuclear punctate staining in 3D7 wild type parasites whereas no staining can be found in the Sir2 KO parasites under the same experimental conditions. Scale bar (2μm) is shown inset. **B.** Immuno-localization of endogenous ORC1 and Sir2 in 3D7 parasites using antibodies against the respective proteins. 'R' stands for ring stage parasites whereas 'ET' stands for early trophozoite stage parasites. Scale bar (2μm) is shown in the top extreme right panel. Majority of the ORC1 and Sir2 signals co-localize with each other.

Supplementary figure 5

A. Purification profile of MBP-ORC1C (C-terminus of ORC1; residues 689-1022 of full length ORC1) and His_6-Sir2. **B.** ORC1C does not help forming higher order DNA-protein complex of $ORC1N_{1-238}$. EMSA was performed by incubating radio-labeled AT-rich probe with increasing concentrations of His-$PfORC1N_{1-238}$ alone (lanes 2-5) or MBP-PfORC1C alone (lanes 6-7) or fixed amount of $PfORC1N_{1-238}$ with increasing amount of PfORC1C (lanes 8-11) as indicated on the top. Free probe is indicated at the bottom. No supershift of $PfORC1N_{1-238}$ -DNA complex was found in the presence of PfORC1C.

Supplementary figure 6

The model shows sequential binding of various proteins at the *Plasmodium* telomeres/TAREs (1-6). The loading of Sir2 at the TAREs may facilitate the binding of ORC1 to these regions as dimers mediated through the N-terminal leucine heptad repeats. It may help to form higher order nucleoprotein complex that may spread into the *var* gene cluster leading to the silencing of the *var* gene family. HP1 also binds to the TAREs. In the absence of Sir2, ORC1 fails to be loaded at the TAREs that may not allow the formation of higher order nucleoprotein complex. It results in relieving the silencing effect at the *var* gene cluster allowing RNA polymerase to get access to the *var* gene promoter. The binding of HP1 to the TAREs is not affected due to the absence of Sir2.

Table 1. Primer list

qRT-PCR primers

Pf11_0521F	5'CAGCCAAATATGAAGGGGAAT-3'
Pf11_0521R	5'CTACTCCATTCTTTTTTTGGCA-3'
PFA0015cF	5'GTTATGCTGATTACAGTGATATC3'
PFA0015cR	5'TATCATCCCACCATGTTTTACG3'
PFL0030cF	5'GTTAAATCAAAATGTTCTAATGTTA3'
PFL0030cR	5'CTTCGGATATAGCTTCCCACTG3'
PFL1960WF	5'TGGTAGTGAACGTTGTAGTGGG3'
PFL1960R	5'AAACTACAATGTCTGCCACACG3'
GAPDHF	5'AAAGCTGCTGAAGGTCCACTTA3'
GAPDHR	5'AGGCTAAACCAGCTTTCATGTC3'
PfORC1F589	5'CATAACATTTATAATAAATATAACATA3'
PfORC1R765	5'ATTTTGATTATTCTTTATACTATGTG3'
GFPR51	5'TTCAACAAGAACTGGGACCAC3'

EMSA primers

ATF	5'CGGGATCCTTCAAGAGTAATCGTTCAAC3'
ATR	5'TTTTATATCATTTAATTCATATGT 3'
GCF	5'CAAGCCGTCGACACTGGTCCCGCCA3'
GCR	5'CGCGAGGGGAATCCTTGAAGCTG 3'
TelomereF	5'AATCCGTCGAGCACATGTTT 3'
TelomereR	5'CAGAATTCGCTTGGAATTCC 3'

ChIP primers

TARE1F	5'GTGTTGATGTTGTTAGTTGG 3'
TARE1R	5'AAGACTAAGTAGATGATAATAGG 3'
TARE2F	5'GTGTTGATGTTGTTAGTTGG 3'
TARE2R	5'ATGAGTTTATGAAAAAGAGG 3'
TARE2-3F	5'GGAAAAATGGTGTATTGTTAC 3'
TARE2-3R	5'TATGAATGAAAAATGGGTTG 3'
TARE3F	5'ATTCTGGTCACTTACTATATGG 3'
TARE3R	5'CTTCAAGTGCATGTCAATTGTG 3'
TARE6F	5'GGACCAGATCTCCTAACATTAGTAATGTAGGTCTG 3'
TARE6R	5'GGACCCCGCGGTCTATATTTGTTAAATTAAGACCA 3'
upsBF	5'TATTACAGGATATGTCATATATTATAT 3'
upsBR	5'AAATACGAAAATACATACATATAAAA 3'
upsEF	5'GGAACGACATTATTTTGTATAAAA 3'
upsER	5'ATCACCATAATTAATATGAGAATC
upsCF	5'TCCATATGCTATCCAATAAAT 3'
upscR	5'ATATTCGTCAAATACGTACA
HRPF	5'CAATATTAACCATTCTAATATAAA 3'
HRPR	

5'TAATATTATTTTTTTTTTAATTATTTAGTAGTTATGTTTTGTCG 3'

GFP cell line primers

ORC1N$_{1-238}$F	5'CGGGGTACCATGACTCCTAAGAAAAAAATATT 3'
ORC1N$_{1-238}$R	5'TGGACCTAGGTCACATTTTTTGTTGACATGTATTAT 3'
ORC1N$_{1-182}$F	5'CGGGGTACCATGACTCCTAAGAAAAAAATATT 3'
ORC1N$_{1-182}$R	5'TGGACCTAGGTAATATATTTATAATAGTACTATT 3'
ORC1N$_{25-238}$F	5'CGGGTACCATAAAATTAAATGTTAGTAAATTAAAT 3'
ORC1N$_{25-238}$R	5'TGGACCTAGGTCACATTTTTTGTTGACATGTATTAT 3'
ORC1N$_{1-53}$F	5'CGGGGTACCATGACTCCTAAGAAAAAAATATT 3'
ORC1N$_{1-53}$R	5'TGGACCTAGGAAAAAACTAATTATGAATACAAG 3'

Cloning primers

ORC1N$_{1-238}$F	5'CGGGATCCATGACTCCTAAGAAAAAAATATT 3'
ORC1N$_{1-238}$R	5'CCCTCGAGTCACATTTTTTGTTGACATGTATTAT 3'
ORC1N$_{1-182}$F	5'CGGGATCCATGACTCCTAAGAAAAAAATATT 3'
ORC1N$_{1-182}$R	5'CCCTCGAGTAATATATTTATAATAGTACTATT 3'
ORC1NL1F	5'TCCATATCATCATCAGCAACAAATATATCATCA 3'
ORC1NL1R	5'TGATGATATATTTGTTGCTGATGATGATATGGA 3'
HP1F	5'CGGGATCCATGACAGGGTCAGATGAAGA 3'
HP1R	5'CCCTCGAGTTAAGCTGTAACGGTATCTTAG 3'
Sir2F	5'CGGGATCCATGGGTAATTTAATGATTTCCTTTTTG 3'
Sir2R	5'CGGGATCCCTACATTATTTTCTTATTTTTTTCAC 3'
PCNA1F	5'CGGGGTACCATGTTAGAGGCCAAATTAAATAAT 3'
PCNA1R	5'TGGACCTAGGTTAATCTTTATTATCCATATCGTC 3'

Table 2. ChIP primer details

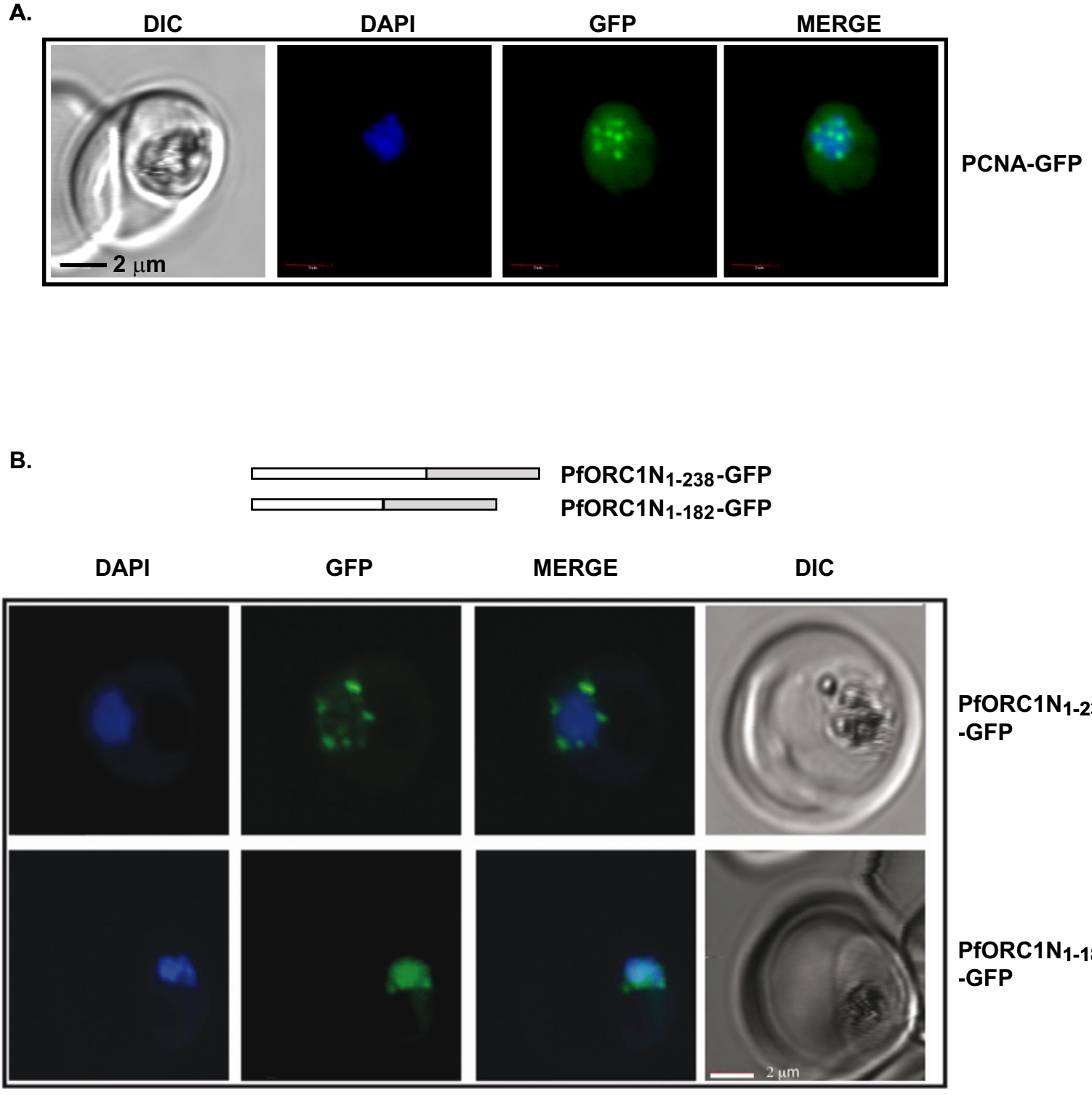
Sr No.	Name of the primer	product size (bp)	Chromosome	Location	Reference
1	TARE1	569	Pf3D7_12	636-1204	Mancio-Silva *et al*., 2008
2	TARE2	617	Pf3D7_12	3987- 4603	Mancio-Silva *et al*., 2008
3	TARE2-3	569	Pf3D7_12	5785- 6353	Mancio-Silva *et al*., 2008
4	TARE3	637	Pf3D7_10	6609-7246	Mancio-Silva *et al*., 2008
5	TARE6	1215	Pf3D7_02	21903-23118	Mancio-Silva *et al*., 2008
6	*upsB*	350	Multiple chromosomes	Multiple chromosomes	Freitas-Junior *et al*., 2005
7	*upsE*	500	Do	Do	Freitas-Junior *et al*., 2005
8	*upsC*	500	Do	Do	Freitas-Junior *et al*., 2005
9	HRP	500	Pf3D7_02	PFB0100c promoter	Freitas-Junior *et al*., 2005

References:

30. Mancio-Silva L, Roias-Meza AP, Vargas M, Scherf A, Hernandez- Rivas R (2008) Differential association of Orc1 and Sir2 proteins to telomeric domains in *Plasmodium falciparum*. *J. Cell. Sci.*, **121**, 2046-2053.

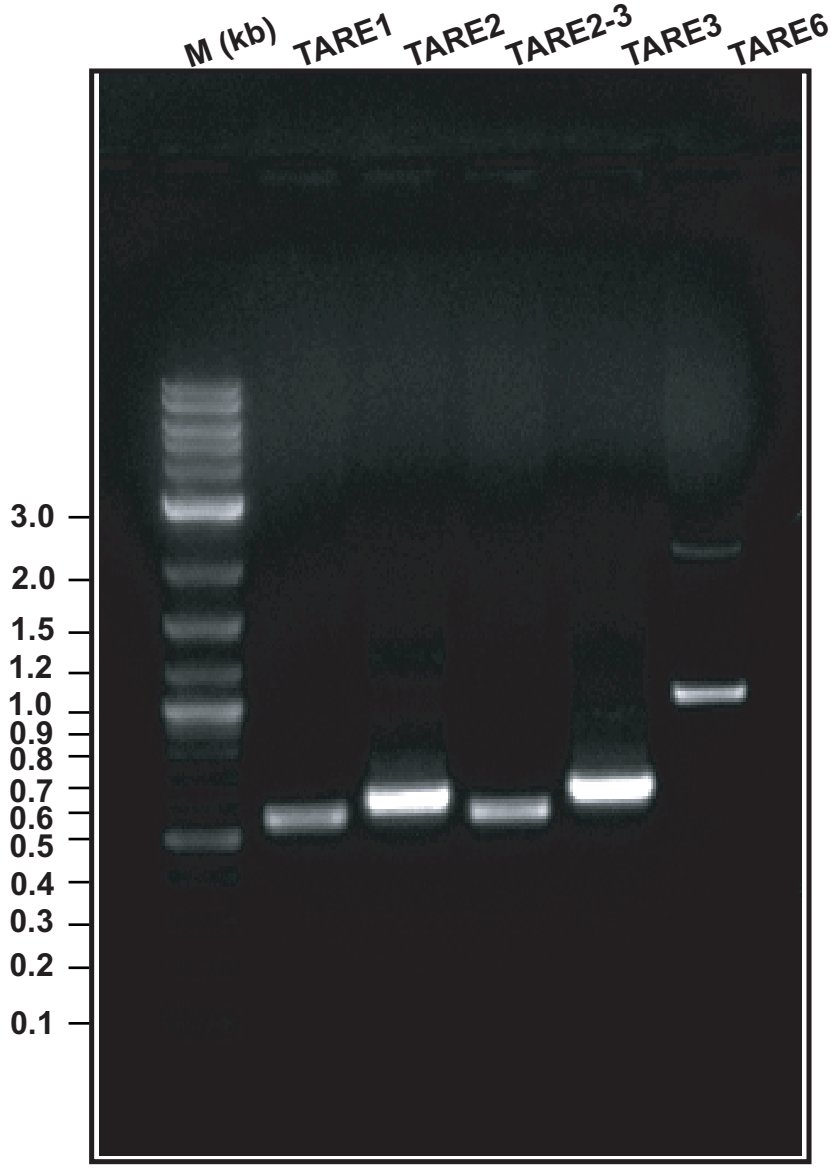
49. Freitas-Junior LH, Hernandez-Rivas R, Ralph SA, Montiel-Condado D, Ruvalcaba-Salazar OK, Rojas-Meza AP, Mâncio-Silva L, Leal-Silvestre RJ, Gontijo AM, Shorte S, Scherf A. (2005) Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. *Cell*. **121**, 25-36.

Supplementary reference numbers are according to the numbers shown in the main text.



Supplementary figure 1

A.



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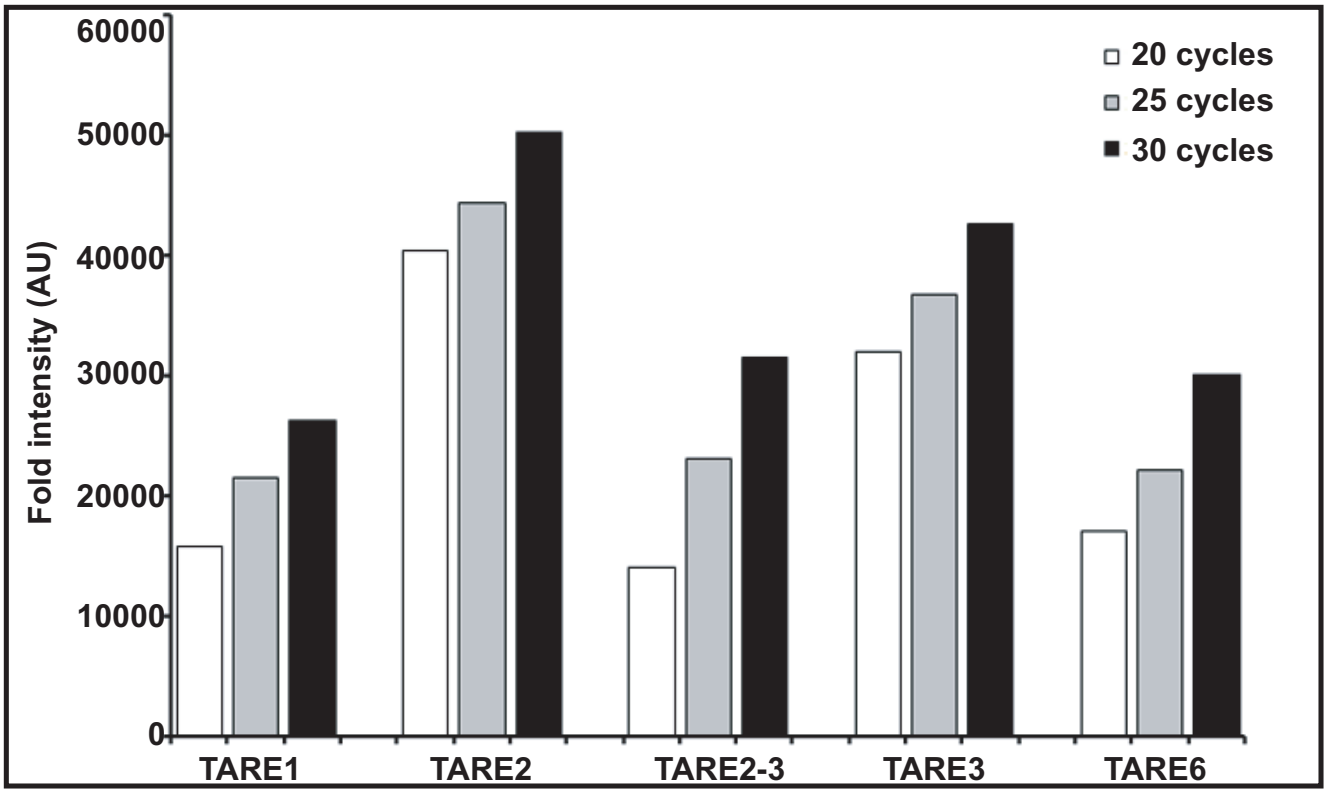

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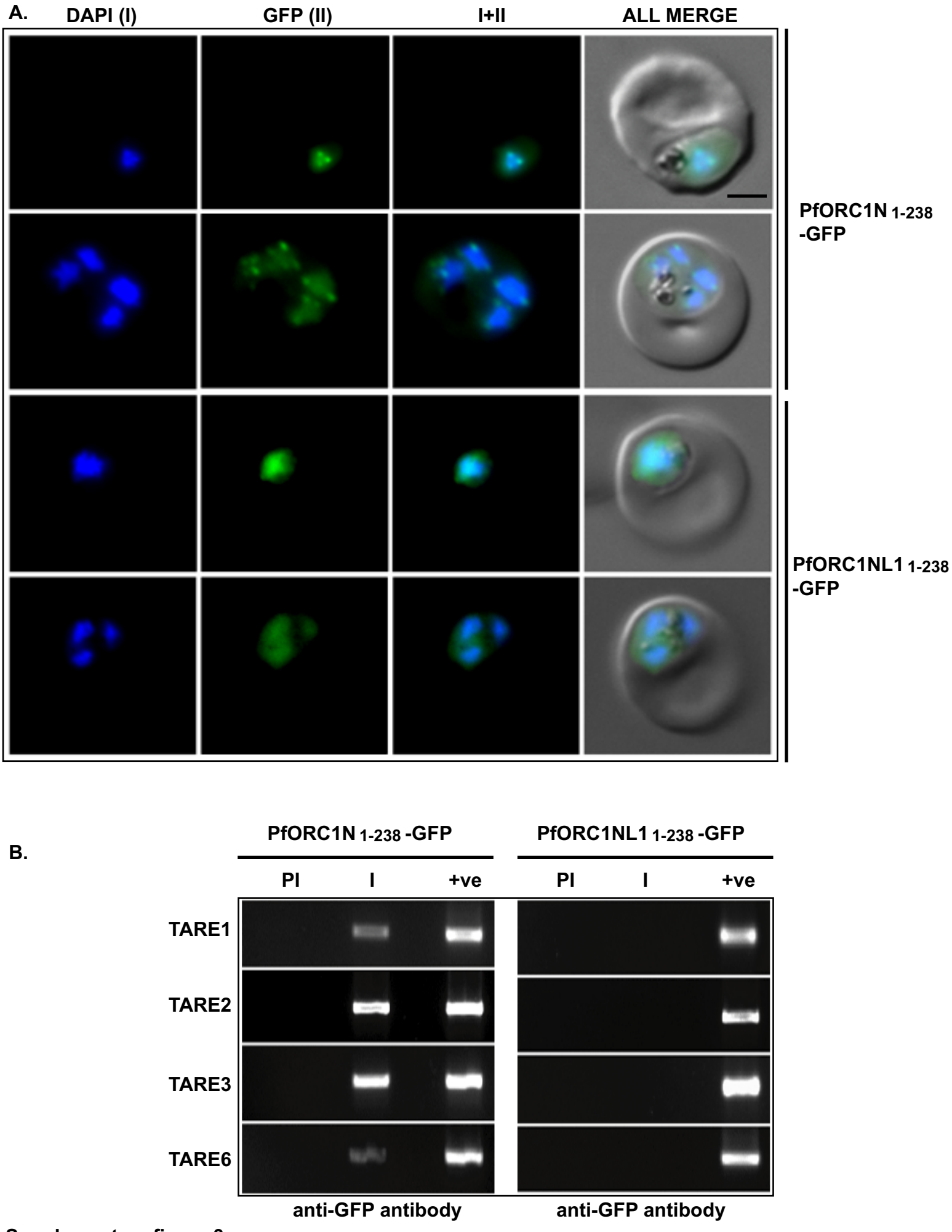

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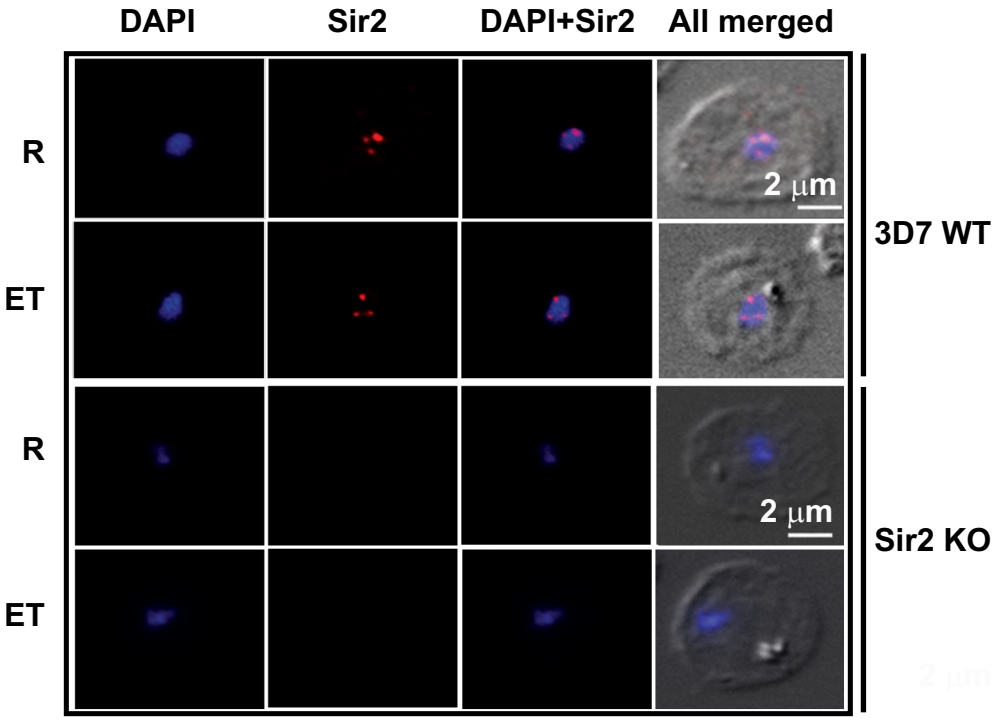
A.



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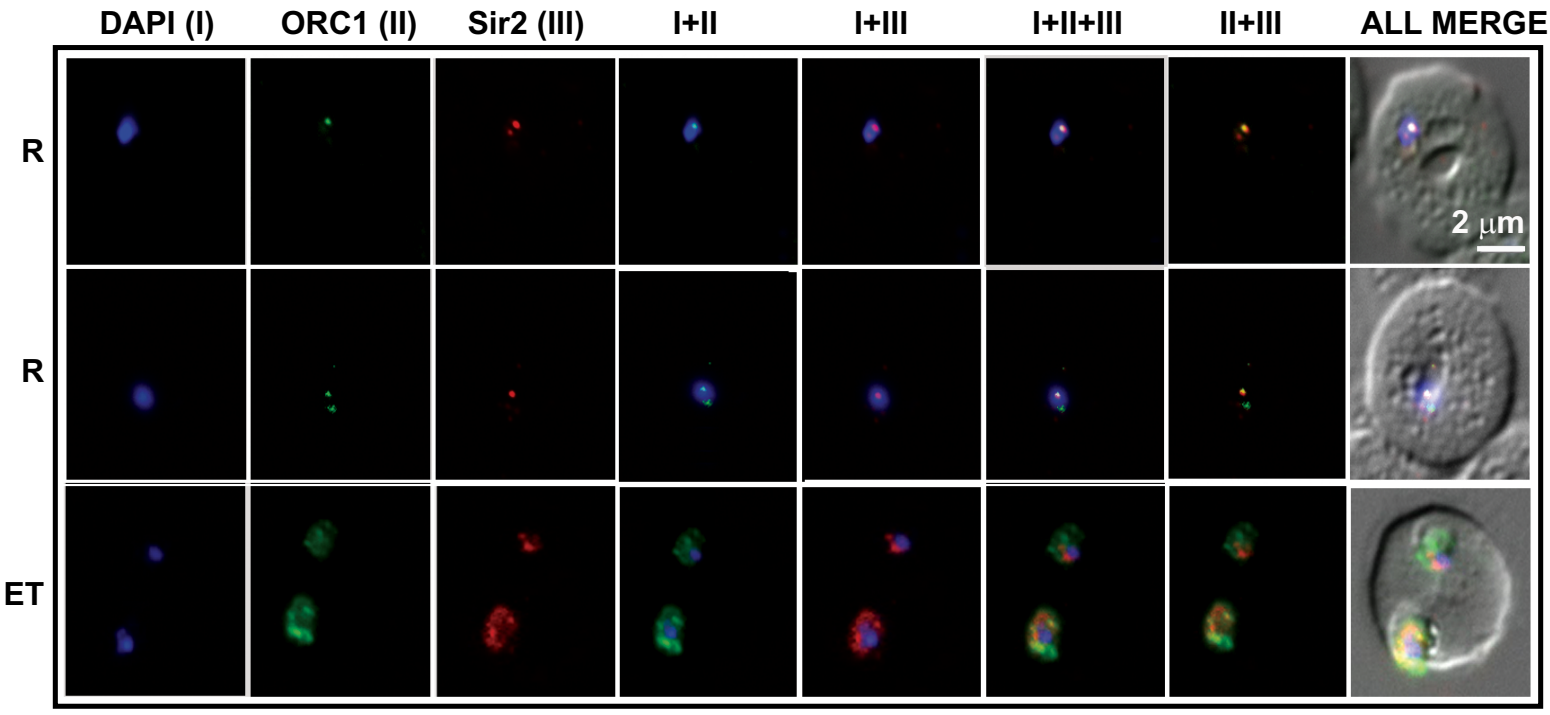

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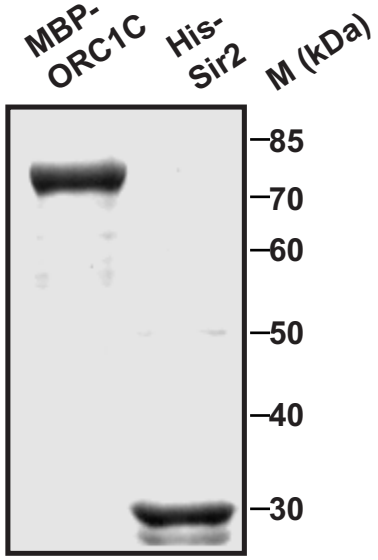
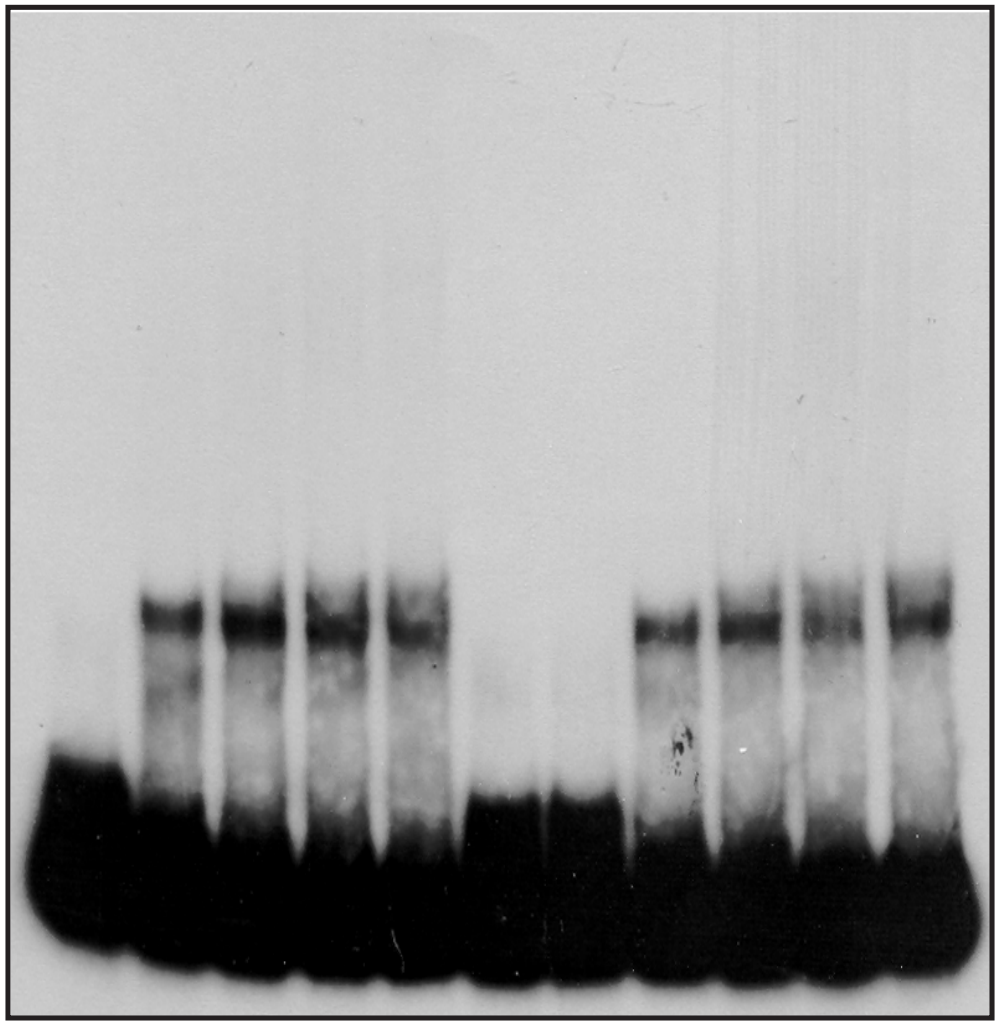


B.

MBPORC1C (ng)	0	0	0	0	0	200	400	100	200	300	400
His-ORC1N1-238 (ng)	0	100	200	300	400	0	0	400	400	400	400



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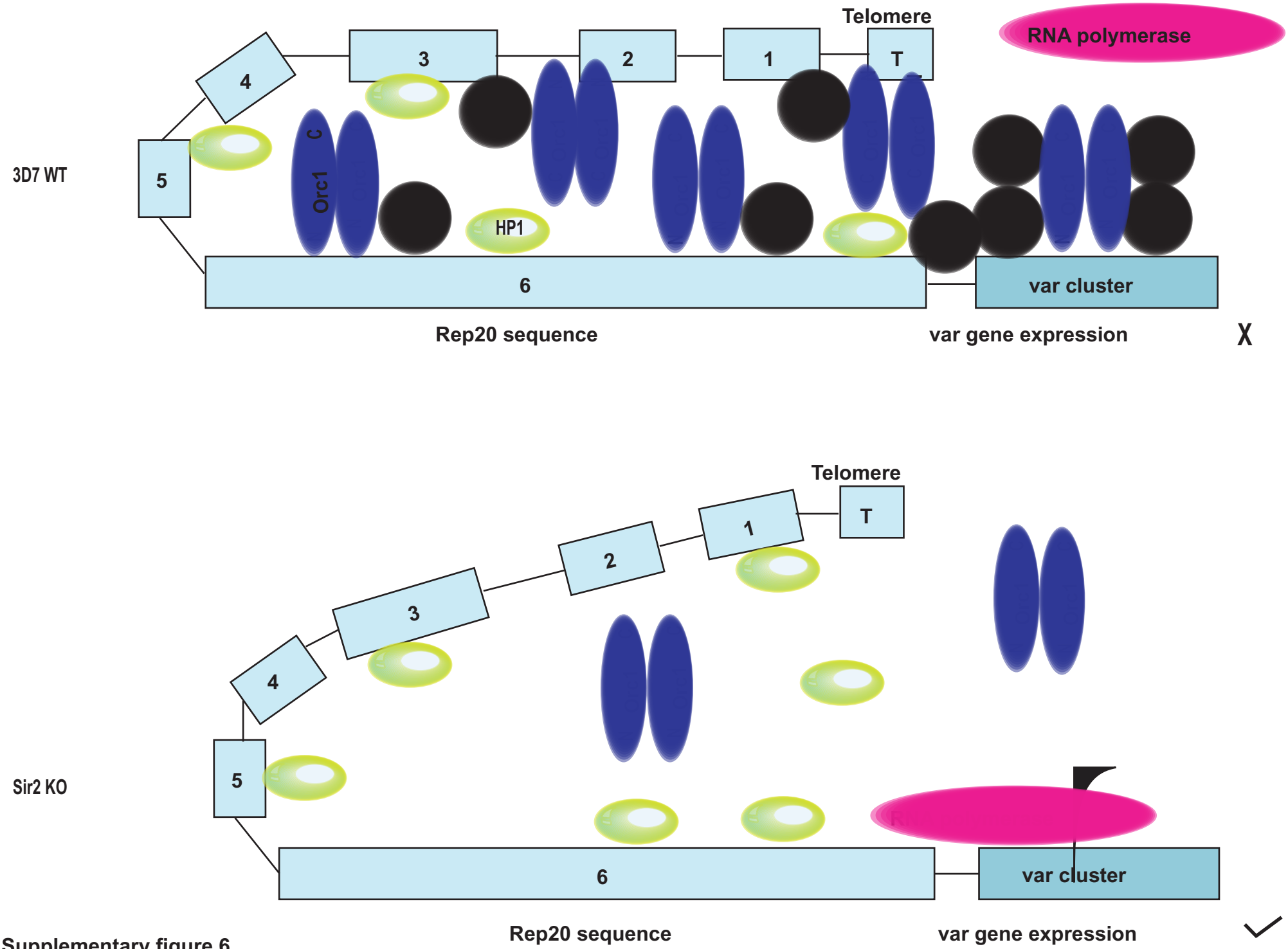

Supplementary figure 6