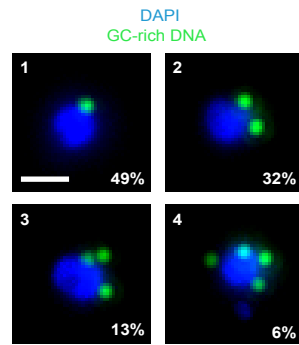
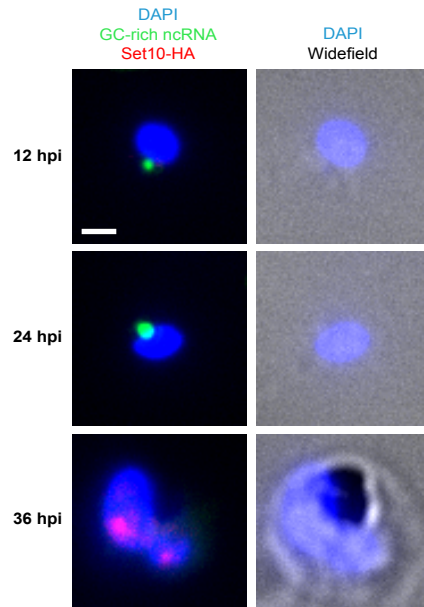


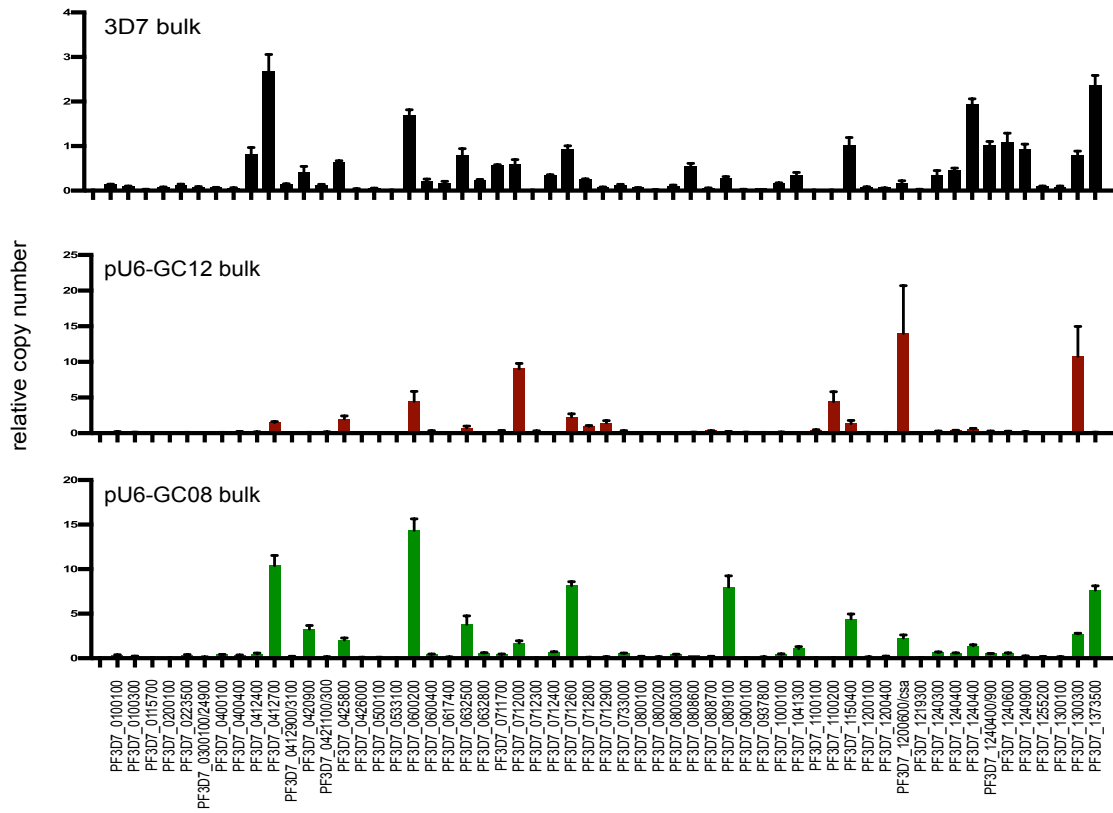
Supplementary Figure 1. Genomic organization and sequence alignment of GC-rich ncRNA gene family. **(A)** Genomic organization of GC-rich elements and shows proximity to central var genes. Top panel displays two occurring genomic arrangements for GC-rich elements. In most cases they are in a tail to tail conformation with the closest var gene (9/15) while sometimes (5/15) a rifin gene is intercalated between them. Schematics below show chromosomal location of var genes (black arrowheads), GC-rich ncRNA elements (red arrowheads) and T-serine ligase gene (green arrowhead) within the 3D7 genome (modified from Fastman et al., 2012). Only five last digits of gene numbers are displayed (PF3D7_chr#xxxxx). Direction of the arrowhead indicates orientation of gene. Drawing is not to scale and chromosome 14 is omitted since it does not contain any var genes or GC-rich elements. Promoter subtype for each var genes is shown (ups A, A/B, B, B/C, C, E). **(B)** Multiple sequence alignment of highly conserved GC-rich non-coding RNA elements. 15 members of RUF-6 gene family aligned by Clustal Omega (<http://www.ebi.ac.uk>) and presented in Jalview (<http://www.jalview.org>). Degree of conservation per base and consensus sequence are displayed below. Black lines show position of potential A- and B-box consensus motifs (as assessed in Dieci et al., 2013).



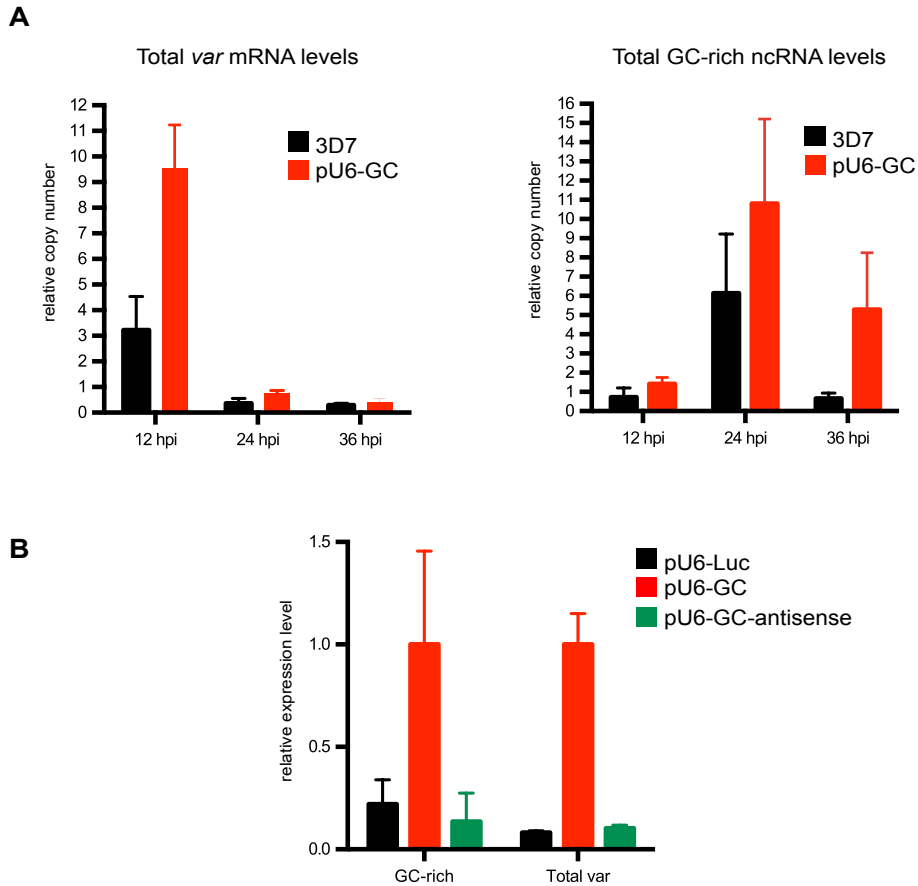
Supplementary Figure 2. Perinuclear clustering of GC-rich ncRNA gene loci. Fluorescent microscopy images of DNA-FISH labeling of four different ring stage parasites using a probe targeting all GC-rich elements (green). Nucleus is stained with DAPI (blue). Scale bar, 1 μ m. Percentages indicate occurrence of cells with 1,2,3, or 4 foci within the imaged population.



Supplementary Figure 3. Differential temporal expression of PfSet10 and GC-rich ncRNA. Fluorescence images show combination of RNA-FISH using probe against all GC-rich ncRNA transcripts (red) with immunolabeling of endogenously tagged PfSet10-HA using anti-HA antibody (red) for three stages of the blood stage cycle. Nucleus is stained with DAPI (blue) and overlaid with widefield on the right. Scale bar, 1 μ m. We used identical acquisition parameters and contrast settings for all images.



Supplementary Figure 4. *Var* gene mRNA profile in GC-rich ncRNA overexpressing bulk cultures and control strains. Individual *var* gene mRNA levels are quantified by real time PCR as relative copy numbers after normalization to T-Serine Ligase levels. Error bars shown standard deviation within triplicates. Limit of detection (LOD) for this data set is 0.0114 (relative copy number).



Supplementary Figure 5. Temporal regulation of *var* and GC-rich ncRNA gene expression is not affected by overexpression of GC-rich ncRNA or GC-rich ncRNA antisense. **(A)** Total *var* gene mRNA levels are quantified by real time PCR using ups subtype specific primers covering all genes at three timepoints of the blood stage development. *Var* mRNA levels of 3D7 wildtype (black) and GC-rich ncRNA overexpressing (orange) parasites peak at 12 hpi. GC-rich ncRNA levels are also quantified for both parasite lines and peak at 24 hpi. Limit of detection (LOD) for this data set is 0.0114 (relative copy number). **(B)** Total *var* gene mRNA and GC-rich ncRNA levels are quantified by real time PCR in three different parasite lines at 12 hpi. Control line pU6-Luc expresses luciferase fragment (black), pU6-GC line overexpresses GC1241000 ncRNA (red), and pU6-GC-antisense GC1241000 antisense transcript (green). No significant difference between pU6-Luc and pU6-GC-antisense can be detected. Graph shows relative expression levels. Limit of detection (LOD) for this graph is 0.0019 (relative expression level).

Supplementary Table 1. Primer pairs for FISH probes

Target	Forward primer	Reverse primer
Seryl (0717700)	AGGAGGGAATCCTGACAAGA	AAGAAGACAACATAAGAATC
FBA (1444800)	ATTAGCAACCACCGCCAAA	CCTGCATTTTCACCACCTGC
GC-rich-ncRNAs	CTGCCTCAGTAGCCAGTCG	CGGGTAACTCAGCAGCTCGAT
var2csa (1200600)	AGCTGATCCTAGTGAAGTG	TGAAGTATCTTGTTCCAGCAG
upsA-subtype var	ATTCCATACATCCGATATAGG	CCGAAATCACCTGTTGACCTC
central var (0412700)	CTA GTG GAT GAG GAT TGG GTG	CTA GTG GAT GAG GAT TGG GTG
GC-rich 5'UTR F1	GAATTTGGTCTAGTTTGTCTA	GTCGAACCAACGTTCTCA
GC-rich 5'UTR F2	GAATTTGGTCTGGTTTGTCTAT	GTCGAACCAACGTTCTCA
upsA var (1300300)	AAGTCACAACAGTGCAAGGG	ACAATCGTAACCCTCACGAC
upsC var (0712000)	CGCCTACAAGTGATGGTGGT	TACTTGCATCAGTTCCGCGA
upsA var (0600200)	GCAACGTGCCGAAGATTCTAA	TGCATTTATTCGAACACGGAGT
upsC var (0808600)	GGTTGGTGGTAGTCCACAGG	TCCACGACATTTTGTATCGCA

Supplementary Table 2. Real time PCR primer pairs

Target	Forward primer	Reverse primer
Seryl (0717700)	AAGTAGCAGGTCATCGTGGTT	TTCGGCACATTCTTCCATAA
FBA(1444800)	TGTACCACCAGCCTTACCAG	TTCCTTGCCATGTGTTCAAT
GC-rich-ncRNA-A	AAGCTGCCTCAGTAGCCCA	AAAAATTGCGCCACCCCC
GC-rich-ncRNA-B	AAGCTGCCCCAGTAGCCCA	AAAAATTGCGCCGCCCC
Var genes		
PF3D7_1240400/900	AAAGCCACTAGCGAGGGTAA	TGTTTTGCCACTCCTGTA
PF3D7_1240600	CATCCATTACGCAGGATACG	AAATAGGGTGGGCGTAACAC
PF3D7_1300300	CACAGGTATGGGAAGCAATG	CCATACAGCCGTGACTGTTC
PF3D7_0412700	TAAAGACGCCAACAGATGC	TCATCGTCTTCGTCTTCGTC
PF3D7_0412900/3100	ACTTTCTGGTGGGAATCAG	TTCACCGCCACTTCATTAG
PF3D7_0425800	AAACACGTTGAATGGCGATA	GACGCCGAGGAGGTAAATAG
PF3D7_0500100	GAAGCTGGTGGTACTGACGA	TATTTTCCACCAGGAGGAG
PF3D7_0632500	ATGTGTGCGAGATGAAG	TGCCTTCTAGGTGGCATAACA
PF3D7_0421100/300	ACCAAGTGGTGACAAAGCAG	GGGTGGCACACAAACTACTAC
PF3D7_0617400	ATTTGTGCGACATGAAGGAA	AACTTCGTGCCAATGCTGTA
PF3D7_0412400	ACCGCCCCATCTAGTGATAG	CACCTTGGTATGTGGTGTCA
PF3D7_0420900	AGAGGGTTATGGGAATGCAG	GCATTCTTTGGCAATTCCTT
PF3D7_0533100	AAGAAAGTGCCACAACATGC	GTTTCGTACGCCTGTCTGTTTA
PF3D7_0937800	CACACGTGGACCTCAAGAAC	AAAACCGATGCCAATACTCC
PF3D7_0600200	TGGAAGAAGCATGGACCTGA	TTCCTCGAGGGAAGAATCAC
PF3D7_1200600	TGGTGATGGTACTGCTGGAT	TTTATTTTCGGCAGCATTTG
PF3D7_0712800	ACGTGGTGGAGACGTAACA	CCTTTGTTGTTGCCACTTTG
PF3D7_0712400	GCGACGCTCAAAAACATTTA	TCATCCAACGCAATCTTTGT
PF3D7_1200400	TCGATTATGTGCCGAGTAT	TTCCCGTACAATCGTATCCA
PF3D7_0600200	TGGAAGAAGCATGGACCTGA	TTCCTCGAGGGAAGAATCAC
PF3D7_0100300	TCATTATGGGAAGCACGATT	TGATTTCTACCATCGCAAGG
PF3D7_0712000	GTTGAGTCTGCGGCAATAGA	CTGGGGTTTTGTCAACTCTG
PF3D7_0712600	CGTGGTAGTGAAGCACCATC	CCCACCTTCTGTGGTTTTCT
PF3D7_0711700	CAATTTTTCCGACGCTTGTA	CACATATAGCGCCGTCCTTA
PF3D7_0712900	CACACATGTCCACCACAAGA	ACCCTTCTGTGGTGTCTTCC
PF3D7_0808600	CCTAAAAAGACGCAGAAGG	CCAGCAACACTACCACCAGT
PF3D7_0712300	GGTGGAGGTAGTCCACAGGA	CAGCTATTTCCACCAGAA
PF3D7_0809100	TGCAAGGGTGCTAATGGTAA	CCTGCATTTTGACATTCGTC
PF3D7_0808700	TTTGTCCGGAAGACGATACA	ATCTGGGGCAGAATTACCAC
PF3D7_1240300	AGCAAAATCCGAAGCAGAAT	CCCACAGATCTTTCTCGT
PF3D7_0800200	GGTGTCAAGGCAGCTAATGA	TATGTCCTGCGCTATTTTGC
PF3D7_0400400	ATATGGGAAGGGATGCTCTG	TGAACCATCGAAGGAATTGA
PF3D7_1100200	GACGGCTACCACAGAGACAA	CGTCATCATCGTCTTCGTTT
PF3D7_0600400	CGTAAAACATGGTGGGATGA	GGCCATTAGTTAACCATC
PF3D7_1150400	TGCTGAAGACCAATTGAGC	TTGTTGTGGTGGTGTGTTG
PF3D7_0632800	GACAAATACGGCGACTACGA	TGTTTCACCCATTCTTCAA
PF3D7_0733000	TGACGACGATAAATGGGAAA	TTCTTTGGAGCAGGGAGTT
PF3D7_0800100	GTCGTGGAAAAACGAAAGGT	TATCTATCCAGGGCCCAAAG
PF3D7_1000100	GACGAGGAGTCGAAAAGAC	TGGACAGGCTTGTGAGAG
PF3D7_1041300	GTGCACCAAAAAGACTCAA	ACAAAACCTCTGCCATT
PF3D7_1100100	GAGGCTTATGGGAAACCAGA	AGGCAGTCTTTGGCATCTTT
PF3D7_1300100	ACAAAGGAACGTCCATCTCC	GCCAATACTCCACATGATCG
PF3D7_1373500	CGGAATTAGTTGCCCTTACA	CATTGGCCACCAAGTGATC
PF3D7_0100100	TGCGCTGATAACTACAACA	AGGGGTTTCATCGTCATCTTC
PF3D7_0115700	AACCCCAATACCATTACGA	TTCCCACTCATGTAACCAA
PF3D7_0200100	ATGTGCGCTACAAGAAGCTG	TTGATCTCCCATTCAGTCA
PF3D7_0223500	CAATTTTGGGTGTGGAATCA	CACTGGCCACCAAGTGATC

PF3D7_0300100/24900	CAATCTGCGGCAATAGAGAC	CCACTGTTGAGGGGTTTTCT
PF3D7_0400100	GACGACGATGAAGACGAAGA	AGATCTCCGCATTTCCAATC
PF3D7_0426000	TGACGACTCCTCAGACGAAG	CTCCACTGACGGATCTGTTG
PF3D7_0900100	TGCAAACCACCAGAAGAAAG	GTTCTCCGTGTTGTCCTCCT
PF3D7_1200100	CGGAGGAGGAAAAACAAGAG	TGCCGTATTTGAGACCACAT
PF3D7_1219300	GACGCCTGCACTCTCAAATA	TTGGAGAGCACCACCATTTA
PF3D7_1255200	GGCACGAAGTTTTGCAGATA	TTTGTGCGTCTTTCTTCGTC
PF3D7_0800300	TTTGGGATGACACCAAGAAA	GTCGCTTGATGAAGGAGTCA
PF3D7_1240400	TGCCGACAAGCCAAATACCG	CAAAAGCTTCTGCCCATTCCTC
PF3D7_1240400	ACCGCCTTTGAAGAGGACGATG	TTGGTGCTGGTGTGCTGGAC
PF3D7_1240900	CAAAATGGTAGTGATGGTGGTCG	CCCCCTGCTTATTATCTTTTCGTC

Var ups subtypes

upsA-1	TTGGGRAATBTGTTAGTTAYRGCAA	CTGCAAACTKCGWGCAAG
upsA-2	AACCCATCTGRRATGATATACCTATGGA	GTTCCAASGATCCATTRGATGTATTA
upsB-1	CATCCGCCATGCAAGTATAA	CGTGCACGATTCGATTTTTTG
upsB-2	ATCAAGGTAATTCATACATATGTGATA	GTCCGTGCACGATTCGATTTT
upsBC-1	AATGATCGGTGTAACCACTATC	GACAAAACTTTCACCCAATAGA
upsBC-2	CATCTGTTGCAAATTTATTCCAAATAC	TCAGTAGTATCAGACATAAATGCATA
upsC-1	CACATCGATTACATTTTAGCGTTT	TGTGGTAATATCATGTAATGG
upsC-2	CATTGTAAACATAGTCTACCATTA	GTAGCGACAACCACGRYATCATGG