SUPPLEMENTAL TABLE LEGENDS

TABLE S-I Oligonucleotide probes used for Northern blot analysis for detecting sRNAs in G3 and G3-based gene silencing transfectants.

TABLE S-II Primers used for plasmid construction for G3:5′STIRP and G3:3′STIRP.

TABLE S-III Primers used for generation of PCR probes for Northern blots.

TABLE S-IV IDT probes used for FISH experiments.

TABLE S-V PCR primers for CHIP analysis.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1A: G3 strain is silenced for Ap-A, AP-B and SAPLIP 1 genes. Northern blot analysis detects expression of all three genes in HM-1:IMSS but not in G3 parasites. The blot was loaded with 15μg total RNA sample from *E. histolytica* trophozoites of HM-1:IMSS or G3 strain and probed with each specific oligonucleotide probe as shown under each blot. Methylene blue staining is shown as a loading control.

Fig. S1B: Northern blot analysis detects antisense sRNAs for Ap-B and SAPLIP-1 in G3 parasites. The Northern blot (same as in Fig. 1B) is probed for oligonucleotide probes specific to Ap-B and SAPLIP 1 as shown under each blot.

Fig. S1C: Equal loading for three parallel blots. As in Fig. 1B, the three identical parallel blots were made with each blot loaded with 20μg sRNA enriched sample from *E. histolytica* trophozoites of HM-1:IMSS or G3 strain. These three blots were probed for a previously identified endogenous small RNA using probe EHS-D-A16-1, which showed equal signal for HM-1:IMSS and G3 in all three blots. Methylene blue staining is shown as a loading control.

Fig. S1D: Sequence alignment between Ap-A and Ap-B. The oligonucleotide probe AP-B sense probe 1 was derived from a region divergent between the two genes, indicated in blue.

Fig. S1E: Sequence alignment between Ap-A and SAPLIP-1. The oligonucleotide probe SAPLIP 1 sense probe 1 was derived from a region divergent between the two genes, indicated in pink.

Fig. S2: Antisense sRNAs to the silenced EhROM1, and EhSTIRP genes in G3 parasites have 5′-polyphosphate termini. Biochemical analysis of the 5′ termini of antisense sRNAs to the silenced EhROM1, and EhSTIRP genes shows that they are resistant to treatment with Terminator enzyme and can be capped with a single G, and hence likely have 5′ polyphosphate termini. The control probe was susceptible to Terminator enzyme and did not cap with capping enzyme, confirming its 5′-monophosphate structure.

Fig. S3: Western blot detects EhAGO2-2 using an anti-EhAGO2-2 antibody. Custom anti-EhAGO2-2 antibody recognizes a band of 110 kDa in lysate from both untransfected HM-1:IMSS and HM-1:IMSS/pKT3M-MycAgo2-2. Anti-Myc antibody only recognizes a band of 110 kDa in HM-1:IMSS/pKT3M-MycAgo2-2 but not in the non-transfected HM-1:IMSS parasites.

Fig. S4: Argonaute protein EhAGO2-2 is primarily localized in the parasite nucleus. IFAs using an anti-Myc antibody showed no signal for the control parasites HM-1:IMSS but a clear nuclear signal for the stable transfectants with Myc tagged EhAGO2-2 (pKT3M-MycAGO2-2).

Fig. S5: Quantitation of enrichment after CHIP with anti-Histone H3 and anti-EhAGO2-2 at the silenced gene loci in G3:ROM(KD) and G3:5′STIRP by CHIP analysis. The enrichment for each gene primer pair was calculated relative to the control gene primer pairs in whole cell extract (WCE). For G3:ROM(KD), STIRP is normalized to one. For G3:5′STIRP, EhROM1 is normalized to one. EhRpl21 has been used as a control for both samples.

TABLE S-I Oligos used for Northern blot analysis for detecting sRNAs in G3 and G3-based gene silencing transfectants.

Oligo name	Targeting gene	Orientation	Sequence
Ap-A			
S1	Ap-A	Sense	ATGAAAGCCATCGTCTTTGTTTTAATCTTCGCTGTTGCTT
S3	Ap-A	Sense	CCTTTGCACTGGACTTATTAACACACTTGAAAACCTCCTT
S5	Ap-A	Sense	GCCTTTGCAATAAAGCCTCAGGATTCATTGCTACTCTTTG
S7	Ap-A	Sense	CTTATTGAAGACAAAGTTGATGCCAATGCTATTTGTGCTA
AS1	Ap-A	Antisense	AAGCAACAGCGAAGATTAAAACAAAGACGATGGCTTTCAT
AS3	Ap-A	Antisense	AAGGAGGTTTTCAAGTGTGTTAATAAGTCCAGTGCAAAGG
US1S	Ap-A	Sense	ATCACCGTGAATTATTTAAAGAGTAGGTTGAAAACTACAA
US1AS	Ap-A	Sense	TTGTAGTTTTCAACCTACTCTTTAAATAATTCACGGTGAT
SINE1-AS	SINE1	Antisense	CAGATAATAACTGGGAGAGTCGAAGTATGAATTTGTACTAG
SINE1-S	SINE1	Sense	CTAGTACAAATTCATACTTCGACTCTCCCAGTTATTATCTG
5UTRS	Ap-A	Sense	AAACATATCTTACAAACAATC
5UTRAS	Ap-A	Antisense	GATTGTTTGTAAGATATGTTT
3UTRS	Ap-A	Sense	TTTAAGCTACTCAATTGAGTAAATTTTCATAC
DS1	Ap-A	Sense	CTTTCTTTAAATAAAGAAAAGATATAAAATATGAAATAAA
EhROM1			
RS-86	EhROM1	Sense	ATTCTCCACCACATAACAATATACATACATTT
RS1	EhROM1	Sense	ATGGATGATGATAGAGAACCAACACCTACTTCAACAGCTAAA
RS168	EhROM1	Sense	TCACATAGGTATTGTTTAGCACTATTTTCAGCAAGTTTTATG
RS471	EhROM1	Sense	TTCAGATTTTTGATTGTTTATTTTGTT
EhSTIRP			
SS1	EhSTIRP1	Sense	ATGATTATTATTAATCTACTCTTTCTTTTT
SS624	EhSTIRP1	Sense	ACAACAAAAAGATGTTGTTGTAACTGT
SS1028	EhSTIRP1	Sense	ATATACCCTTGGAGAATCTTTA
SS2520	EhSTIRP1	Sense	AATGGAGGATCTACTATTCATCTTA
SS6188	EhSTIRP1	Sense	ATGAGAATCACAGGAGTGACGTTAAAT
SS6342	EhSTIRP1	Sense	AGCCATTGGTTGAAGTAGAAGGAAA
SS6501	EhSTIRP1	Sense	AGAAATAGAAGGAAATAGGAGTGATGAAGA
SS6801	EhSTIRP1	Sense	AAAAACATGTCCATGTTCAGGAGATAAGTG
Ap-B			
Ap-B S	Ap-B	Sense	TGTTGATGGTGCTCAAGCTGTTAGACAA
Ap-B AS	Ap-B	Antisense	TTGTCTAACAGCTTGAGCACCATCAACA
SAPLIP 1			
SAPLIP 1 S	SAPLIP 1	Sense	TGGCCTTATCGTAAATAAAGGT
SAPLIP 1 AS	SAPLIP 1	Antisense	ACCTTTATTTACGATAAGGCCA
tRNA probe	tRNA-encoding array unit G^GCC	Antisense	TGGTGCACTTGCCGGGAAACGAACCCGG
U5 snRNA	U5 snRNA gene	Antisense	GGCGAAAGATAAGATCGATTTTGTTAG

TABLE S-II Primers used for plasmid construction for G3:5′STIRP and G3:3′STIRP.

Construct	Primer	Sequence
G3:5′STIRP	F	AGGCCTATGATTATTATTAATCTACTCTTTCTTTTT
	R	GAGCTCTGACTGTTGACAATCAGGTTCT
G3:3′STIRP	F	AGGCCTTGAGAATCACAGGAGTGACGTT
	R	GAGCTCTGTTCCTGAAACCTCTACTCGAC

TABLE S-III Primers used for generation of PCR probes for Northern blot.

Gene	F/R	Sequence
STIRP	F	GCCATTGGTTGAAGTAGAAGG
	R	GAACATTTGCTTCAACTCCTTTT
Ap-A	F	TTATTTGTATGAAGTTATTTG
	R	TTAAACAAAACAAATAGTTG
Actin	F	GGGAGACGAAGAAGTTCAAGC
	R	GGCAAGAATTGATCCTCCAA

TABLE S-IV IDT probes used for FISH experiments.

Gene	Orientation	Sequence
Ap-A FAM	Sense	/56-FAM/ATGAAAGCCATCGTCTTTGTTTTAATCTTCGCTGTTGCTT
EhROM1 FAM	Sense	/56-FAM/CTCCACCACATAACAATATACATACATTTGATTGGAGTAA

TABLE S-V PCR primers for CHIP analysis.

gene	F/R	Sequence
Ap-A	F	AAACAATCATGAAAGCCATCG
	R	GCTTTATTGCAAAGGCTGC
Ap-B	F	TTGCTGCAACAAGAGAAGGA
	R	TGTCTAACAGCTTGAGCACCA
SAPLIP 1	F	TTTCTTTTGCAGTTGCTGGA
	R	CCATCAGCCTTACCACAAAGA
EhROM1	F	GGGAGACATGAGGTTCGAGT
	R	GGTGAACATGGTGGTGGAAT
EhRPL21	F	CTAACGGTAGAAATAGAAGAACCAG
	R	TCCAAACACGTCCAGTCTTTC
5′STIRP	F	TGGTGAAATTAAAGTTAAGCTTGG
	R	TGTGAATCAATAATCAGTGGACAA

Fig. S1A

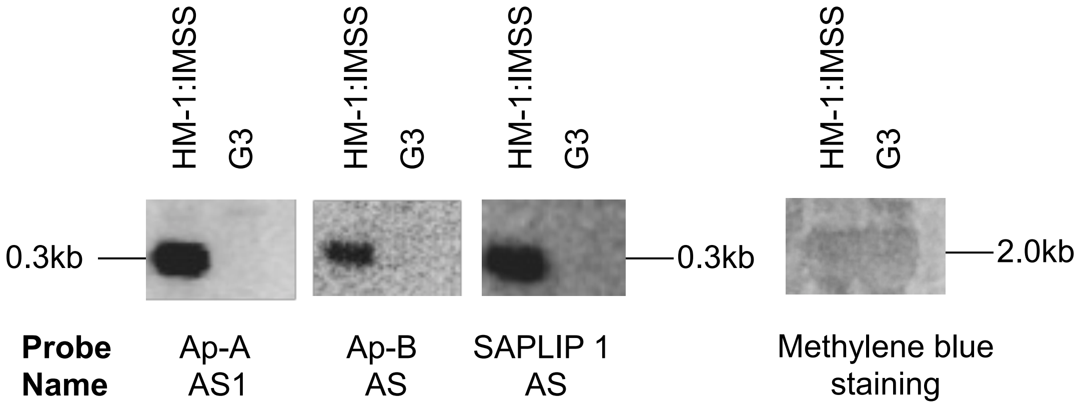
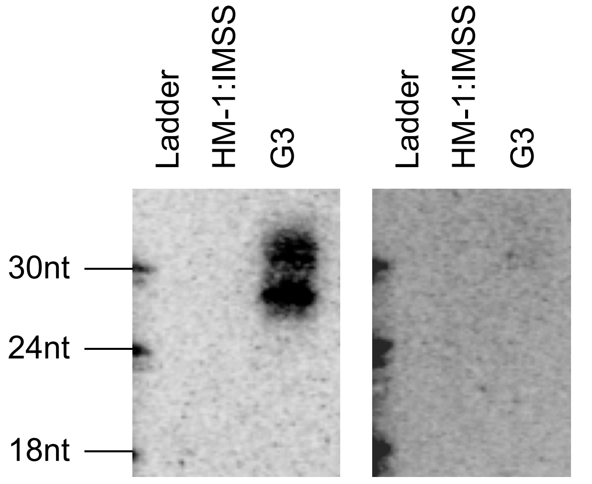

HM-1:IMSS
G3
HM-1:IMSS
G3
HM-1:IMSS
G3
HM-1:IMSS
G3
0.3kb
0.3kb
2.0kb
Probe Name
Ap-A AS1
Ap-B AS
SAPLIP 1 AS
Methylene blue staining

Fig. S1B



Ap-B specific
sense probe

SAPLIP 1 specific
sense probe

Fig. S1C

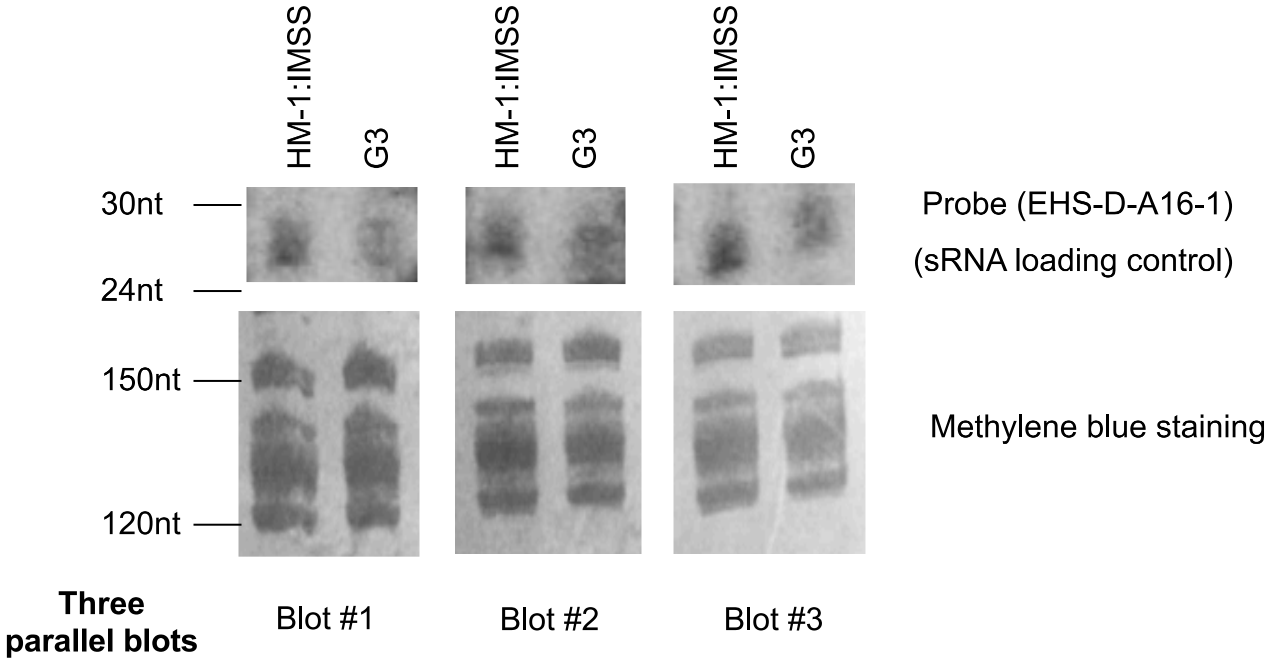

HM-1:IMSS
G3
HM-1:IMSS
G3
HM-1:IMSS
G3
30nt
24nt
150nt
120nt
Probe (EHS-D-A16-1)
(sRNA loading control)
Methylene blue staining
Three parallel blots
Blot #1
Blot #2
Blot #3

Fig. S1D

Sequence alignment for Ap-A and Ap-B

```
CLUSTAL 2.0.12 multiple sequence alignment

Ap-A            ATCACCGTGAATTATTTAAAG-AGTAGGTTGAAAACTACAAAAACATATCTTA-CAAACA 58
Ap-B            -------GATGGTATTTAAACTAATAAAATGAACTAAATTATCAATTGTTTAATCAAATT 53
                            ********  * **   ****    *  *  *  * * * * ****

Ap-A            ATCATGAAAGCCATCGTCTTTGTTTTAATCTTCGCTGTTGCTTTTGCAGTCACTGCTACT 118
Ap-B            ATTATGAGAGCTATTATCTTTGTTTTAATCTTTGCTATTGCCTTTGC------TGCAACA 107
                ** **** *** **  **************** *** **** *****      *** **

Ap-A            CACCAAGGAGAAATCCTCTGCAACCTTTGCACTGGACTTATTAACACACTTGAAAACCTC 178
Ap-B            AGAGAAGGAGCTATTCTTTGCAATCTTTGTAAAGATACAGTTAAACTTGTTGAAAACCTT 167
                    ******  ** ** ***** ***** *  *      ****     **********

Ap-A            CTTACCACTAAGGGAGCTGATAAAGTAAAAGATTACATTAGCAGCCTTTGCAATAAAGCC 238
Ap-B            TTAACTGTTGATGGTGCTCAAGCTGTTAGACAATATATCGACAACCTTTGTGGTAAAGCT 227
                 * **   * * ** *** *    ** * * * ** **   ** ******   ******

Ap-A            TCAGGATTCATTGCTACTCTTTGCACTAAAGTTCTTGATTTTGGAATTGATAAACTCATC 298
Ap-B            AGTGGATTCCTTGGAACTCTTTGTGAAAAAATCCTTTCATTTGGTGTTGATGAACTTGTC 287
                   ****** ***  ********    *** * ***   *****  ***** ****  **

Ap-A            CAACTTATTGAAGACAAAGTTGATGCCAATGCTATTTGTGCTAAGATTCATGCTTGCTAA 358
Ap-B            AAGCTTATTGAAAATCATGTTGACCCAGTCGTTGTTTGCGAAAAGATTCACGCTTGTTAA 347
                 * ********* *  * *****  *    * * **** *  ******** ***** ***

Ap-A            GTT---TTAAGCTACTCAATTGAGTAAAT-TTTCATACTTTCTTTATGTTTTTTTTATTC 414
Ap-B            GTGAAGTCAAAATGATTAATTCAATCATTGTTTTATTCTTTTTTTCTATTAAATATTTTT 407
                **    * **  *  * **** * * * * *** ** **** *** * **   * * **

Ap-A            TCTTTC------- 420
Ap-B            ATTTTTATAAATA 420
                  ***
```

Ap-B specific sense probe 1

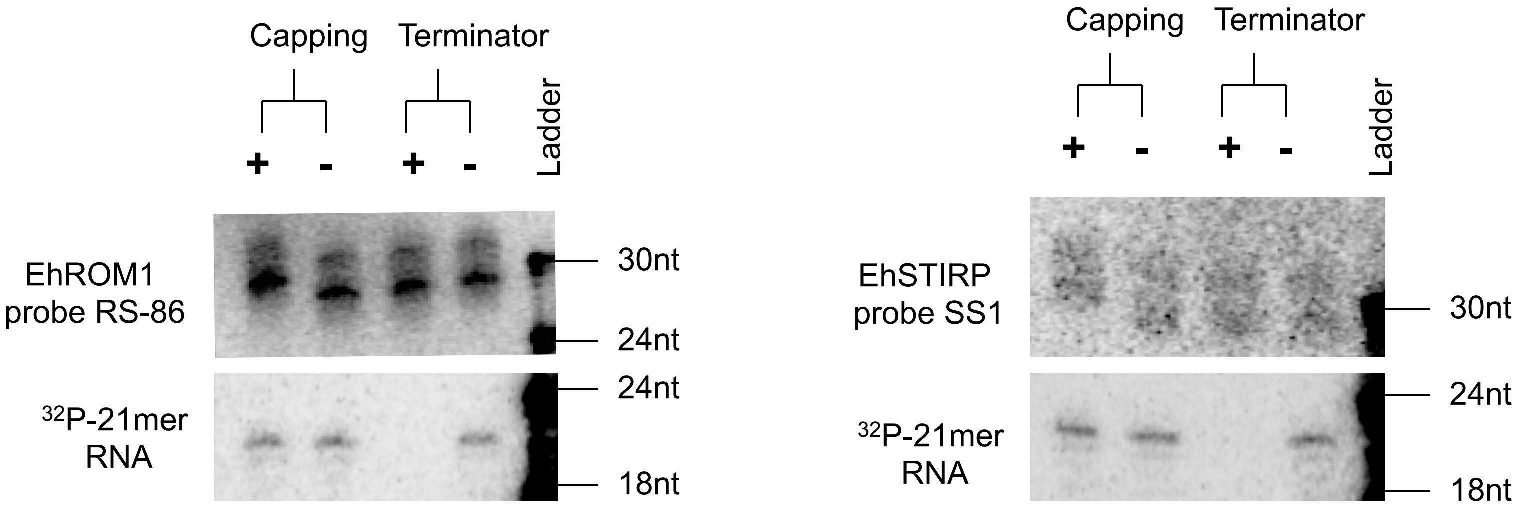
Fig. S1E

Sequence alignment for Ap-A and SAPLIP 1

```
CLUSTAL 2.0.12 multiple sequence alignment

Ap-A            ATCACCGTGAATTATTTAAAGAGTAGGTTGAAAACTACAAAAACATATCTTACAAACAAT 60
SAPLIP-1        ------------------------------------------------------------


Ap-A            CATGAAAGCCATCGTCTTTGTTTTAATCTTCGCTGTTGCTTTTGCAGTCACTGCTACTCA 120
SAPLIP-1        -ATGAGAGCATTTCTTATTGTCTTATTA------ATTTCTTTTGCAGTTGCTG------- 46
                 **** ***  *  *  **** *** *        ** **********  ***

Ap-A            CCAAGGAGAAATCCTCTGCAACCTTTGCACTGGACTTATTAACACACTTGAAAACCTCCT 180
SAPLIP-1        -----GACCAATTGTTTGCAACCTTTGTATTGGACTTGTTAATACCCTTGATGGCCTTAT 101
                     **  ***  * *********** * ******* **** ** *****   ***  *

Ap-A            TACCACTAAGGGAGCTGATAAAGTAAAAGATTACATTAGCAGCCTTTGCAATAAAGCCTC 240
SAPLIP-1        CGTAAATAAAGGTGCTGATAGAGTAAAAAATTATATTGATGATCTTTGTGGTAAGGCTGA 161
                    * *** ** ******* ******* **** ***      *****   *** **

Ap-A            AGGATTCATTGCTACTCTTTGCACTAAAGTTCTTGATTTTGGAATTGATAAACTCATCCA 300
SAPLIP-1        TGGATTTATTGCTCAGCTCTGTGAAAAACTTCTTTCATTTGGACTTGATAAACTTATTGA 221
                 ***** ******   ** **    *** *****   ****** ********** **  *

Ap-A            ACTTATTGAAGACAAAGTTGATGCCAATGCTATTTGTGCTAAGATTCATGCTTGCTAAGT 360
SAPLIP-1        TCTTATTCAAAGTAAAGTAGATCCTAATGCAATTTGTGCTCAAATGAATGCTTGCTAA-- 279
                 ****** **   ***** *** * ***** ********* * **  ***********

Ap-A            TTTAAGCTACTCAATTGAGTAAATTTTCATACTTTCTTTATGTTTTTTTTATTCTCTTTC 420
SAPLIP-1        ------------------------------------------------------------
```

SAPLIP 1 specific sense probe 1

Fig. S2



G3:ROM(KD) RNA

G3:3′STIRP RNA

Fig. S3

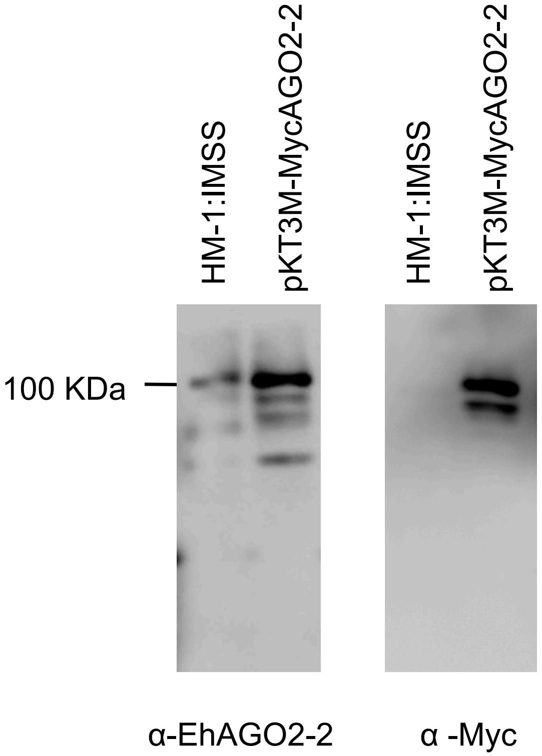

HM-1:IMSS
pKT3M-MycAGO2-2
HM-1:IMSS
pKT3M-MycAGO2-2
100 KDa
α-EhAGO2-2
α -Myc

Fig. S4

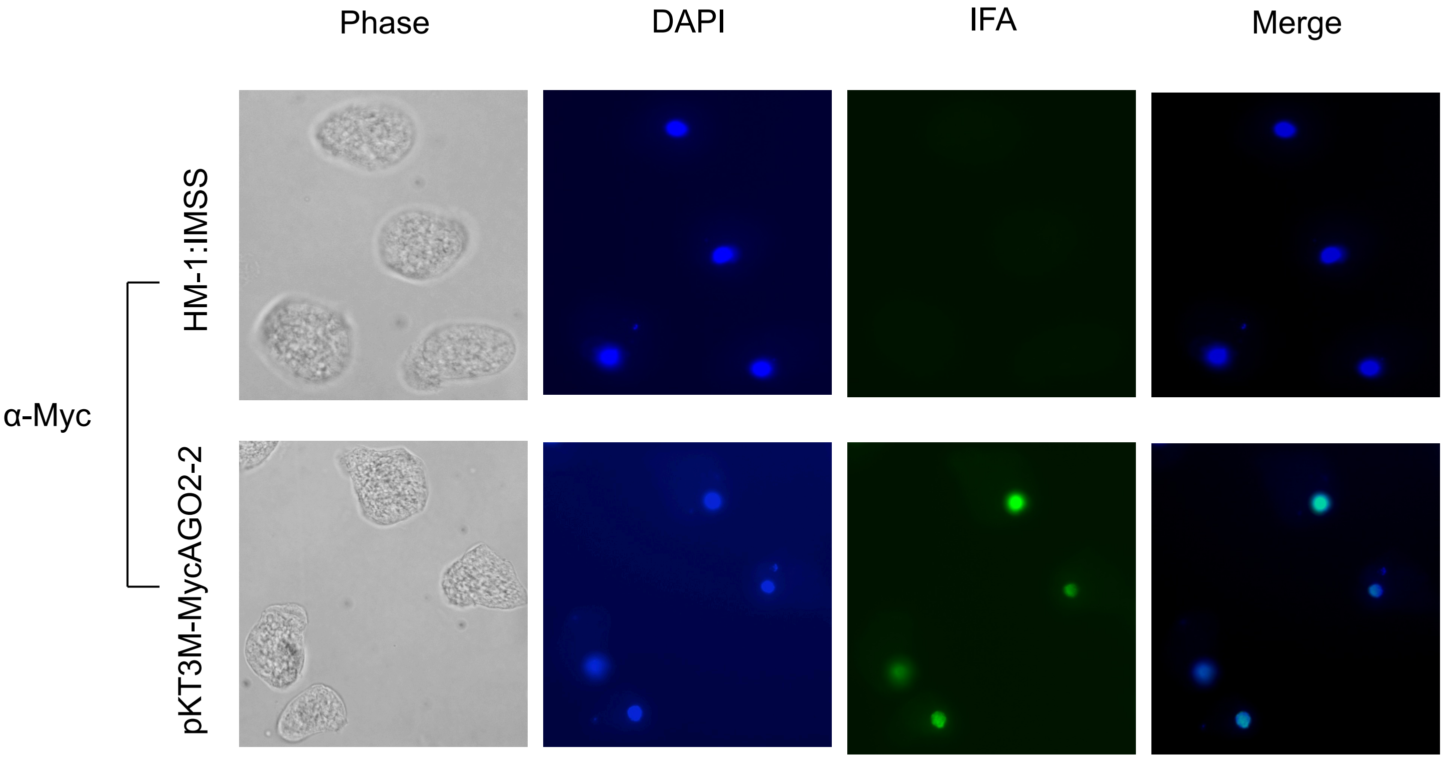
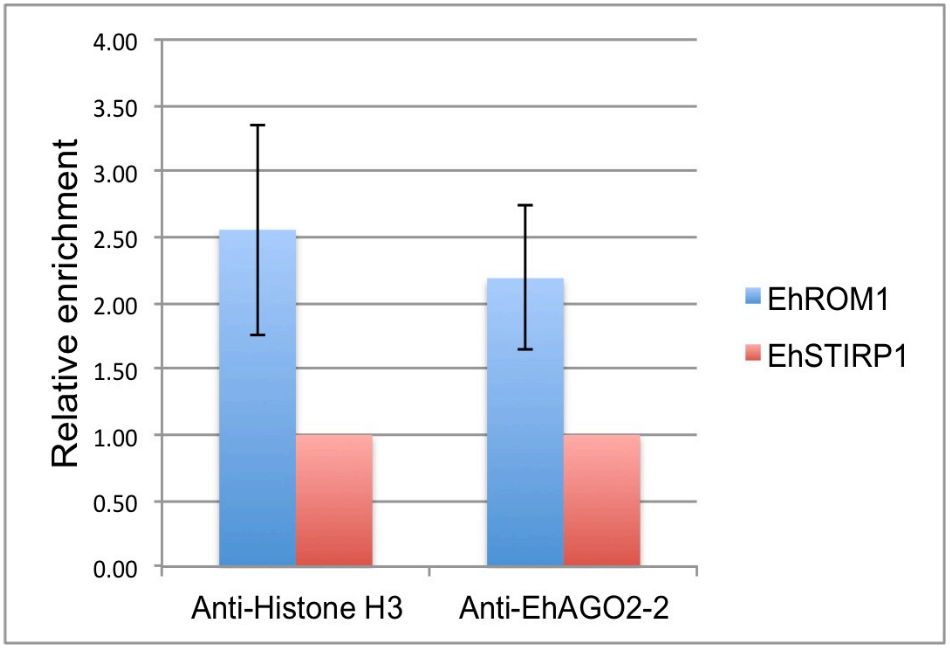
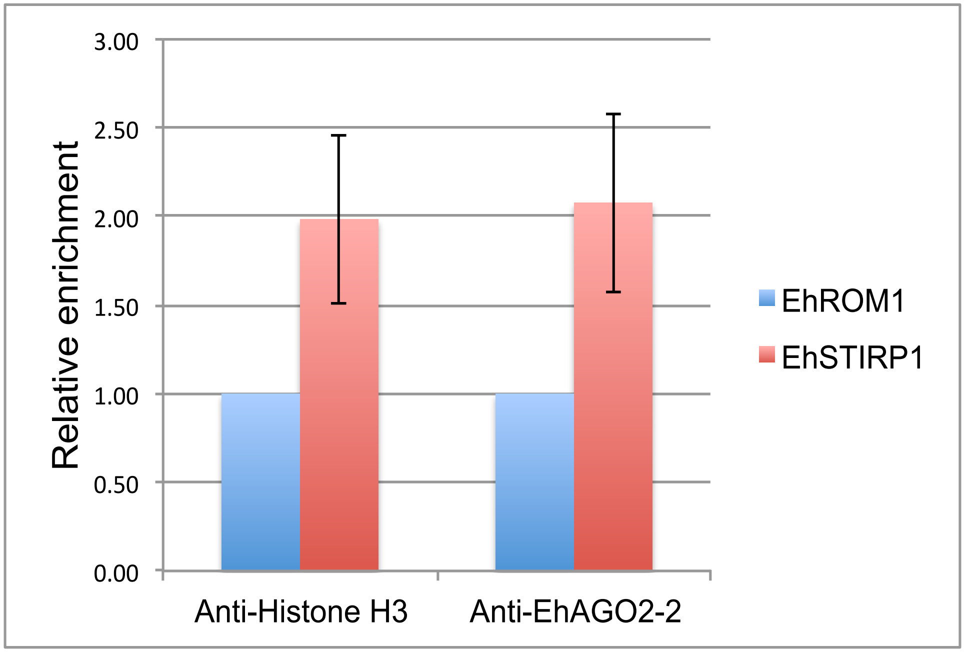


Fig. S5



G3:ROM(KD) CHIP



G3:5′STIRP CHIP