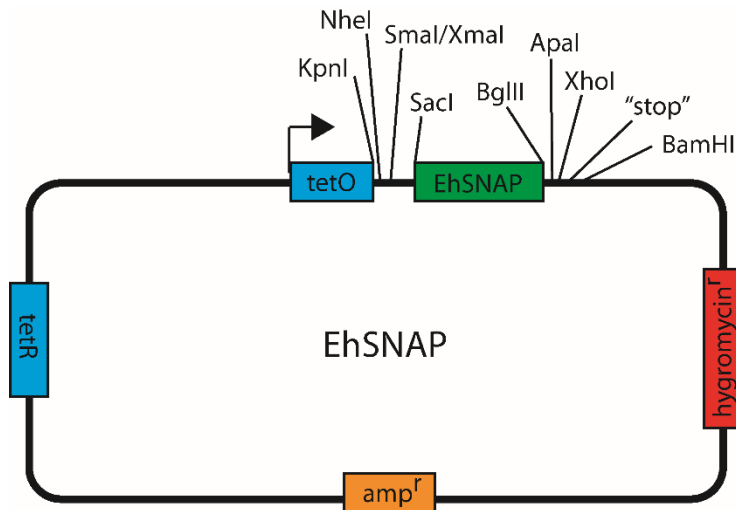
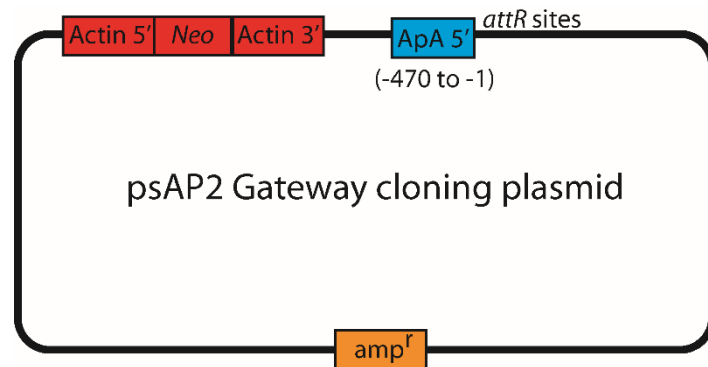
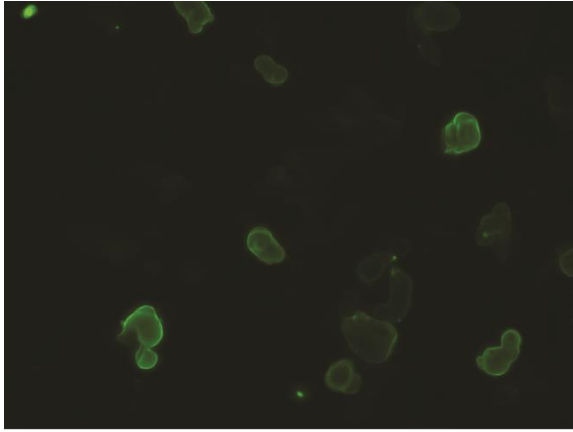


Supplemental Figure 1: Gateway cloning gene silencing and tetracycline-regulated EhSNAP expression plasmids. The Gateway cloning plasmid for G3 strain *E. histolytica* gene silencing was derived from the previously reported psAP-2 plasmid (1). The EhSNAP expression plasmid was constructed from the tetracycline-regulated plasmid EhSNAPspFLAG (2), which includes the SNAP gene with codon usage optimized for expression in *E. histolytica*.



Supplementary Figure 2: Processing of cell monolayer adherence images.
Images from adherence assay, pre (left) and post (right) NIH Image J batch processing.

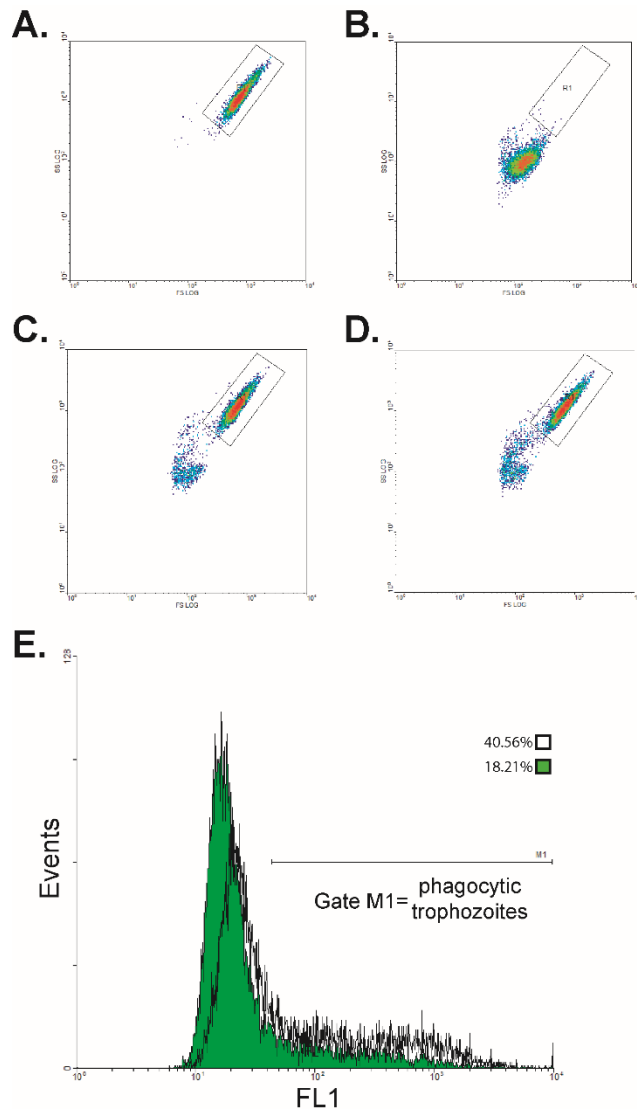


Monolayer adherence image pre-ImageJ

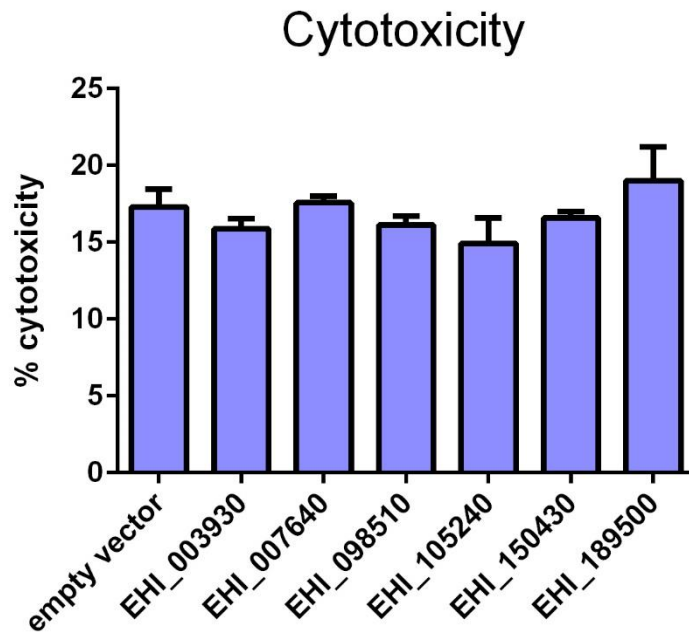


Monolayer adherence image post-ImageJ

Supplementary Figure 3: Gating scheme for flow cytometry phagocytosis assay. Data from a representative experiment are shown. (A) Forward and side scatter plot for amebic trophozoites with gating window set on them. (B) Forward and side scatter plot for fluorescently-labeled Jurkat lymphocytes, showing exclusion by the ameba specific gate. (C and D) Forward and side scatter plots for mixed *E. histolytica* and apoptotic (C) or previously healthy (D) Jurkat lymphocytes. (E) Representative fluorescence histograms for gated *E. histolytica* trophozoites after incubation with healthy cells (green) or cells previously killed by apoptosis (white). The M1 gate shows trophozoites with fluorescence above background, which are scored as phagocytic.



Supplementary Figure 4: Effects of gene silencing on *E. histolytica* killing of host cells. Jurkat T lymphocytes loaded with the fluorescent dye BCECF were incubated with G3 strain *E. histolytica* trophozoites silenced for expression of the indicated gene or cells transfected with the empty vector control plasmid(30 min, 37°C). Specific cytotoxicity was calculated as % cytotoxicity = $(\text{fluorescence}_{\text{experimental}} - \text{fluorescence}_{\text{spontaneous}}) / (\text{fluorescence}_{\text{detergent lysed}} - \text{fluorescence}_{\text{spontaneous}}) \times 100$. The graph shows the mean and SEM for six biological replicates.



Supplementary Table 1: Genes targeted for silencing and preliminary outcome.

Gene	Outcome of silencing	Preliminary phagocytosis result	Preliminary adherence result
EHI_003930	Silenced	Reduced	Increased
EHI_007320	Not viable (2 attempts)	N/A	N/A
EHI_007480	Silenced	No effect	No effect
EHI_007640	Silenced	Reduced	No effect
EHI_009570	Not viable (2 attempts)	N/A	N/A
EHI_021260	Silenced	No effect	No effect
EHI_021270	Not silenced	N/A	N/A
EHI_029600	Silenced	No effect	No effect
EHI_045540	Silenced	No effect	No effect
EHI_098510	Silenced	Reduced	No effect
EHI_105240	Silenced	Reduced	No effect
EHI_122800	Silenced	No effect	No effect
EHI_148910	Silenced	No effect	No effect
EHI_150430	Silenced	Reduced	No effect
EHI_167130	Not viable (2 attempts)	N/A	N/A
EHI_167940	Silenced	No effect	No effect
EHI_189500	Silenced	Possibly reduced	Increased
EHI_197040	Silenced	No effect	No effect
EHI_197120	Silenced	No effect	No effect

Supplementary Table 2: Raw (non-normalized) and normalized flow cytometry phagocytosis assay data from Figure 1. Phagocytic trophozoites were determined to be trophozoites with fluorescence above unlabeled control, and gated mean represents the mean fluorescence of phagocytic trophozoites. Phagocytic index is calculated as phagocytic trophozoites (or % gated) multiplied by the gated mean. Gated mean and phagocytic index has been normalized to the average of the empty vector strain.

Phagocytosis of Apoptotic Cells (10 minute incubation)			
Strain	% Phagocytic Trophozoites	% Gated Mean	% Phagocytic Index
empty vector	45.33±5.4	100±3.7	100±6.2
EHI_003930	37.87±5.9	70.68±4.6	58.99±7.5
EHI_007640	36.49±5.6	80.23±5.0	64.63±9.2
EHI_098510	41.94±4.3	81.07±2.4	75.09±4.0
EHI_105240	35.16±11	74.34±6.4	57.65±16.9
EHI_150430	34.84±6.6	86.35±6.4	49.25±26.4
EHI_189500	44.30±4.7	105.15±3.5	103.03±8.6

Phagocytosis of Live Cells (25 minute incubation)			
Strain	% Phagocytic Trophozoites	% Gated Mean	% Phagocytic Index
empty vector	70.30±6.2	100±4.8	100±3.7
EHI_003930	59.65±8.0	82.03±16.6	70.05±17.3
EHI_007640	68.06±6.7	80.98±14.7	77.55±7.1
EHI_098510	70.25±8.5	87.47±21.1	85.32±6.8
EHI_105240	65.66±9	78.40±19.1	73.72±21.4
EHI_150430	67.34±6.1	81.94±23.5	78.76±23.5
EHI_189500	74.75±8.4	98.37±20.75	103.29±13.5

Supplementary Table 3: Predicted conserved protein domains and corresponding E-values.

Protein	Predicted domain (E-value)
EHI_003930	ANTH (E=2.2x10 ⁻²⁶); I/LWEQ (E=2.4x10 ⁻⁴⁷)
EHI_007640	Calponin homology (CH)(E=8.7x10 ⁻¹⁹); LIM (E=2.5x10 ⁻¹⁵); C2 (E=8.3x10 ⁻⁷); Pleckstrin homology (PH)(E=5.2x10 ⁻⁷)
EHI_098510	Signal Peptide (SP-HMM algorithm; probability=0.999); Transmembrane Domain (TMHMM algorithm; cumulative probability=1.21)
EHI_105240	BAR (E=1.3x10 ⁻⁴); SH3 (E=1.1x10 ⁻²⁰)
EHI_150430	3 LRR domains (E≤1.7x10 ⁻³); Gelsolin homology (GH); (E≤8.2x10 ⁻⁶); Villidin headpiece (E=2.2x10 ⁻⁸)
EHI_189500	Calponin homology (CH)(E=6.2x10 ⁻⁶)

Supplemental Information 1: PCR primers.

Primers for Gateway cloning of gene silencing constructs:

EHI_003930:

(forward) 5'-ATGGAGAAAACCTATTAATAAAGCAACTGA-3'

(reverse) 5'-TTAATTGTTTTGTCTTTCATAACGTTGT-3'

EHI_007320:

(forward) 5'-ATGAGCCAATTCTCTAAAAAACCA-3'

(reverse) 5'-TTACTTTTTGCCTTACTTTTTCTTCTCT-3'

EHI_007480:

(forward) 5'-ATGAGTGATTAACTGATTTGATGAAAA-3'

(reverse) 5'-TTAGAATAATTTAGCTTTTCTTCGTTTCAG-3'

EHI_007640:

(forward) 5'-ATGCAAGGGACACAAGCTTTAC-3'

(reverse) 5'-TTAGATAAGCACTATATTGTCAATAGCTTCTT-3'

EHI_009570:

(forward) 5'-ATGTCAGAAGAGGAAGGAAAAAAA-3'

(reverse) 5'-TTATTTATCGTGTTCTAAATCAGGCC-3'

EHI_021260:

(forward) 5'-ATGTCAGACGAAGAGATTAGGAAAC-3'

(reverse) 5'-TTAGAACAATTTATGTTGTTTTTTCAATT-3'

EHI_021270:

(forward) 5'-ATGGGGGAACAACAATCTATACC-3'

(reverse) 5'-TTAATAAAGCTTCTTTGCTTTTTTTTAGG-3'

EHI_029600:

(forward) 5'-ATGAGTGATTGTGTTGGTATTGATAAC-3'

(reverse) 5'-TTATCCTCTACCTCTTCTACTGAATGAAT-3'

EHI_045540:

(forward) 5'-ATGAGTCAAATGATTGTAAAGGCTAA-3'

(reverse) 5'-TCATTTTGTTTCTATGGATTTGATAAG-3'

EHI_098510:

(forward) 5'-ATGTTAATCATTTTCTTATTTACATTATTTACTCT-3'

(reverse) 5'-TTACTCGCTTGCAGAACCTG-3'

EHI_105240:

(forward) 5'-ATGTCTATCAGAGATGCATTGAGC-3'

(reverse) 5'-TTAAAGTGGTTGTAAAAAGTTGGATG-3'

EHI_122800:

(forward) 5'-ATGAGTGTTTCAAGAAGAACATTGTC-3'

(reverse) 5'-TTAATAGAGATAAACTGAGTGTTTAACTTATCTC-3'

EHI_148910:

(forward) 5'-ATGGAGGAAATTACTAAGGAAAGTATTATTC-3'

(reverse) 5'-TTAGTGAAACGAATGGTGGTAAGTT-3'

EHI_150430:

(forward) 5'-ATGGGACAAGAGAACTCAAACA-3'

(reverse) 5'-TTAATAAAGTTTTAGTGCATGTTTTTGC-3'

EHI_167130:

(forward) 5'-ATGACACAAGTAATTCTTCGTATACATTTT-3'
(reverse) 5'-TTATTCCTTTTGTCTTCTCTTAGCCA-3'

EHI 167940:

(forward) 5'-ATGGAAGTTATTGGATATGCACC-3'
(reverse) 5'-TACTTAGTTGAAAGAAGTGATAATTTTCTTC-3'

EHI 189500:

(forward) 5'-ATGGTTAAATGGCTTGAATTTGTT-3'
(reverse) 5'-TTAATTCATTGAGATATTATCATTCTGC-3'

EHI 197040:

(forward) 5'-ATGAGCGTTAAGGAAAACCAAG-3'
(reverse) 5'-TTAATGTATACTTTCTCGTGAATCACTTC-3'

EHI 197120:

(forward) 5'-ATGTCTGAGGGAACAAAACCTCAG-3'
(reverse) 5'-TTAAAGGATCTTAACGACAATGACAG-3'

Primers for qRT-PCR:

EHI 003930:

(forward) 5'-ATGGAGAAAACCTATTAATAAAGCAACTGA-3'
(reverse) 5'-AATCTATCTAATTCTGGAATCCATCCT-3'

EHI 007320:

(forward) 5'-ATACAAATACCACATCAAATTGACTCC-3'
(reverse) 5'-TACTTTTTGCCTTTACTTTTTCTTCTCT-3'

EHI 007480:

(forward) 5'-TTCGTTTTAGATTACAGTTCAAAGGAT-3'
(reverse) 5'-TTAGAATAATTTAGCTTTTCTTCGTTTCAG-3'

EHI 007640:

(forward) 5'-TTCGTTTTAGATTACAGTTCAAAGGAT-3'
(reverse) 5'-TTAGATAAGCACTATATTGTCAATAGCTTCTT-3'

EHI 009570:

(forward) 5'-GCAGTGGACAAGAAGAACACC-3'
(reverse) 5'-TTATTTATCGTGTTCTAAATCAGGCC-3'

EHI 021260:

(forward) 5'-ATGTCAGACGAAGAGATTAGGAAAC-3'
(reverse) 5'-GGAGTTGTTCTGCTTCTAACCTAACT-3'

EHI 021270:

(forward) 5'-ATGGGGGAACAACAATCTATACC-3'
(reverse) 5'-ACACGAAGTTGATTACTTTCTACCTGT-3'

EHI 029600:

(forward) 5'-GTAGACGTTTCATCAAGTATTTCAAGTG-3'
(reverse) 5'-TTATCCTCTACCTCTTCTACTGAATGAAT-3'

EHI 045540:

(forward) 5'-ATGAGTCAAATGATTGTTAAGGCTAA-3'
(reverse) 5'-TAAAACCTTCTCCTGCTTTAGTTTTTCAT-3'

EHI 098510:

(forward) 5'-CTCTTAGTTGCTTTCATTCTTTATGCT-3'
(reverse) 5'-TACTCGCTTGCAGAACCTG-3'

EHI 105240:

(forward) 5'-GAATGTGATGAAGATACTGAAGAA-3'
(reverse) 5'-TTAAAGTGGTTGTAAAAAGTTGGATG-3'

EHI 122800:

(forward) 5'-ATGAGTGTTC AAGAAGAACATTGTC-3'
(reverse) 5'-TGGTTTATCACTTATTCTTTCTGCTTT-3'

EHI 148910:

(forward) 5'-ATTTGGGGATACATTAACAACAAAA-3'
(reverse) 5'-TTAGTGAAACGAATGGTGGTAAGTT-3'

EHI 150430:

(forward) 5'-ATGGGACAAGAGAACTCAAACA-3'
(reverse) 5'-TTCAGCTAAAACACATAATAATGATGAA-3'

EHI 167130:

(forward) 5'-ATGACACAAGTAATTCTTCGTATACATTTT-3'
(reverse) 5'-ATGGTCTAATATCAAGTGTCTCGGTAG-3'

EHI 167940:

(forward) 5'-CAACAGGTGATAGTATTTCACAAAGT-3'
(reverse) 5'-TACTTAGTTGAAAGAAGTGATAATTTTCTTC-3'

EHI 189500:

(forward) 5'-ATGGTTAAATGGCTTGAATTTGTT-3'
(reverse) 5'-TTTTTGAGCAATAATAGTAAAGAATGC-3'

EHI 197040:

(forward) 5'-ATAATTGTGTTAGCTTAAATTGGCTTG-3'
(reverse) 5'-TTAATGTATACTTTCTCGTGAATCACTTC-3'

EHI 197120:

(forward) 5'-ATGTCTGAGGGAACAAAACCTCAG-3'
(reverse) 5'-ATTTATATTCCTTCTCACTCCCTTCTT-3'

Primers for cloning EhSNAP expression vectors:

EHI_003930 (EhILWEQ):

BglII-EHI_003930 (forward) 5'-AGATCTATGGAGAAAAC TATT
AATAAGCAACTGA-3'

XhoI-EHI_003930 (reverse) 5'-CTCGAGTTAATTGTTTTGTCT
TTCATAACGTTG-3'

EHI_105240 (EhBAR):

BglII-EHI_105240 (forward) 5'-AGATCTATGTCTATCAGAGAT
GCAT TGAGC-3'

XhoI-EHI_105240 (reverse) 5'-CTCGAGTTAAAGTGGTTGTAA
AAAGTTGGATG-3'

Supplemental reference list:

1. **Bracha R, Nuchamowitz Y, Mirelman D.** 2003. Transcriptional silencing of an amoebapore gene in *Entamoeba histolytica*: molecular analysis and effect on pathogenicity. *Eukaryot Cell* **2**:295-305.
2. **Sateriale A, Roy NH, Huston CD.** 2013. SNAP-tag technology optimized for use in *Entamoeba histolytica*. *PLoS One* **8**:e83997.