

1 **SUPPLEMENTAL ONLINE MATERIAL**

2 **FIGURE LEGENDS**

3

4 **Figure S1: Mass spectrometry analysis of histones.** Trophozoites were treated with 2 μ M FR235222 for 24 h and
5 histones immunoprecipitated as described in the experimental procedures section. Protein were separated by SDS-
6 PAGE, silver stained (left panel) or probed with anti-acetylated lysine antibody (right panel). Asterisks indicate the
7 bands excised and analysed by LC/MS/MS. TOT, total extract; SN, supernatant after nuclear pellet isolation; M,
8 mock IP; α AcK, IP with anti-acetyl lysine rabbit antiserum.

9 **Figure S2: Time course of FR235222 inhibition of encystation.** The effect of 2 μ M FR235222 on encystation
10 was monitored by adding the inhibitor in pre-encysting medium at 0, 1, 3, 7 h before inducing encystation for 16 h
11 in presence of 2 μ M FR235222. Parasites were stained for CWP1 and analyzed by flow cytometry. Left panel,
12 overlay histogram showing cells unstained (U), solvent treated (Cntl) and pre-treated for 7 h with 2 μ M FR235222
13 (FR). Right panel, the amount of cells with M1 fluorescence is expressed as percentage of total cell number (TOT).

14 **Figure S3: Viability of FR235222 treated intestinal cells co-cultured with *G. lamblia*.** Caco-2 and CCL-6 cells
15 were cultured with or without *G. lamblia* trophozoites of the WBC6 strain (WB) for 24 h in presence of solvent
16 (cntl) or FR235222 at concentrations not affecting the viability of the cells (2 and 0.02 μ M, respectively). Viability
17 was assessed by measuring AlamarBlue reduction (Biosource). Results of a representative experiment are presented
18 as percentage of control treated cells cultured in absence of *G. lamblia* (\pm standard errors, $n = 3$). WB bars, *G.*
19 *lamblia* trophozoites cultured without intestinal cells.

20 **Figure S4: CWP1 expression upon FR235222 treatment.** Trophozoites (T) and encysting cells (E) were treated
21 for 24 h with 2 μ M FR235222 (FR), apicidin (API), or solvent (cntl) and analyzed by immunofluorescence. Number
22 of parasites expressing CWP1 was expressed as percentage of the total number of parasites (TOT), assessed by
23 nuclear staining, \pm standard errors ($n = 6$).

1 **Figure S5: FR235222 treatment of trophozoites does not induce nuclear translocation of 14-3-3.** Trophozoites
2 were treated for 24 h with 2 μ M FR235222 (FR) or solvent (cntl), stained with anti-giardial 14-3-3 antibody and
3 analyzed by immunofluorescence. DAPI, nuclear staining. Scale bar: 3 μ m.

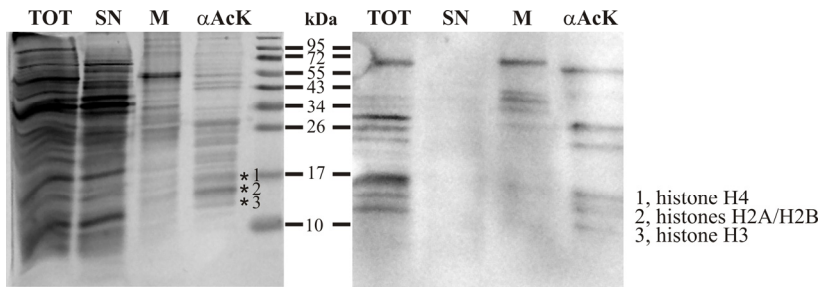
4 **Figure S6: List of FR235222 regulated genes.** Microarray analysis was performed on encysting cells (E) and
5 trophozoites (T) treated with 2 μ M FR235222, as described in the Experimental procedures section. Genes whose
6 regulation reached the significance threshold are shown. Orange, up-regulation; blue, down-regulation.

7 **Figure S7: Analysis of regulated gene location in genomic scaffolds.** Microarray analysis was performed on
8 encysting cells (E) and trophozoites (T) treated with 2 μ M FR235222, as described in the Experimental procedures
9 section. A. Correlation between the number of regulated genes and the number of genes present in the indicated
10 genomic scaffolds. B. Distribution of regulated genes over 9 genomic scaffolds. Scaffolds containing less than three
11 regulated genes were not included in the analysis. C. Potential plots for regulated genes with threshold $\mu_{\text{pot}} + \lambda \cdot s_{\text{pot}}^+$
12 with $\lambda=1$. Asterisks indicate the scaffolds with non-randomly distributed co-regulated genes (tested on a significance
13 level $\alpha=0.05$).

14 **Figure S8: Cluster analysis of regulated genes.** Microarray analysis was performed on encysting cells (E) and
15 trophozoites (T) treated with 2 μ M FR235222, as described in the Experimental procedures section. A. Expression
16 profiles of scaffolds CH991762 and CH991763 containing non-randomly distributed co-regulated genes. Yellow,
17 trophozoite profiles; Green, encysting cell profiles. B. Dendrograms of regulated genes in scaffolds CH991762 and
18 CH991763 based on the potential-induced metric M_f . Cluster candidates are boxed in red (tested on a significance
19 level $\alpha=0.05$).

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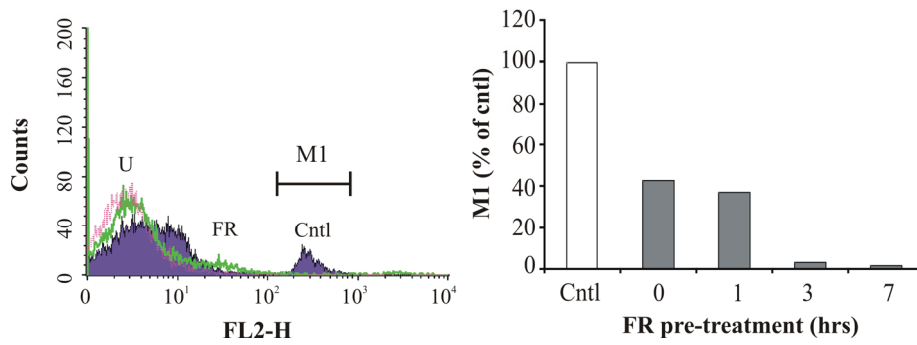
1 Figure S1



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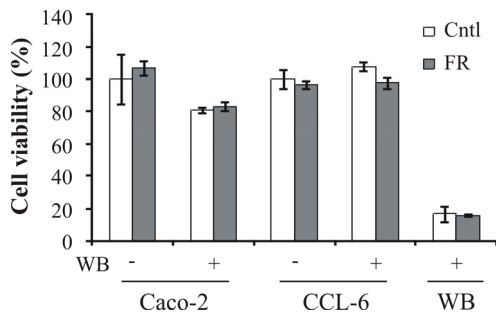
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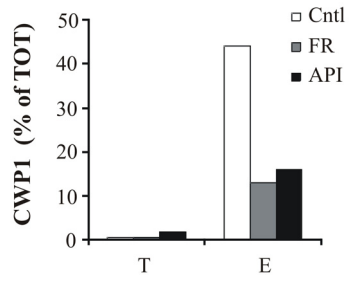
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1 Figure S4



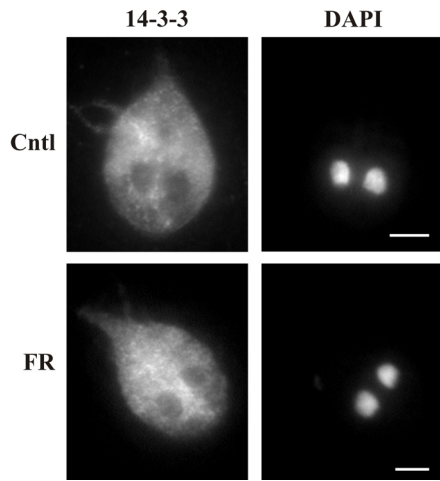
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6 Figure S5



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1 Figure S6

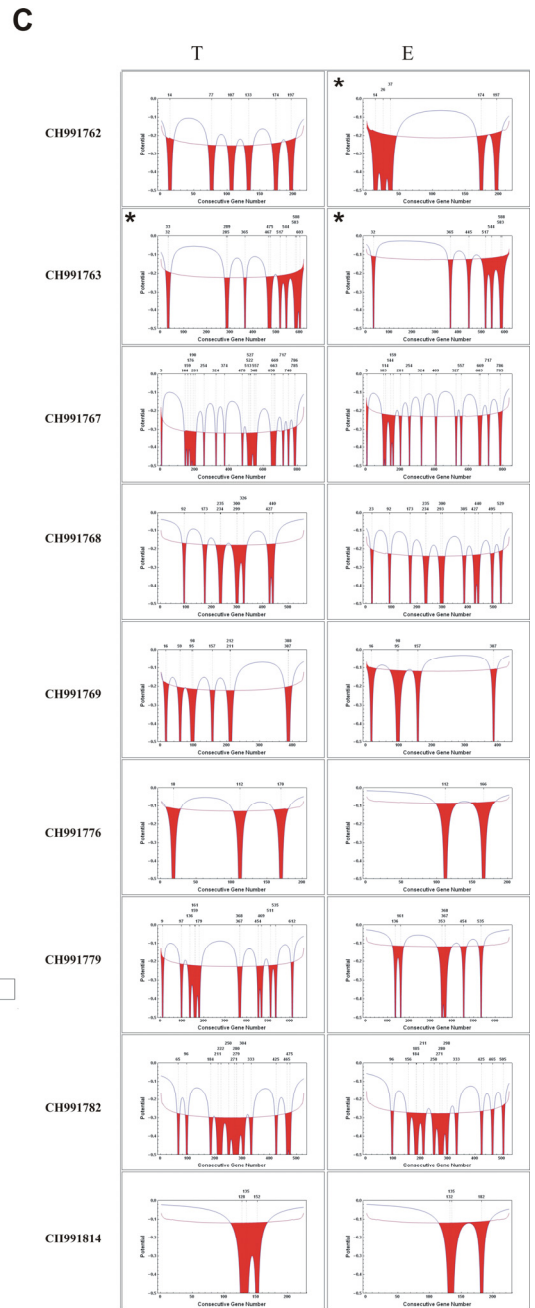
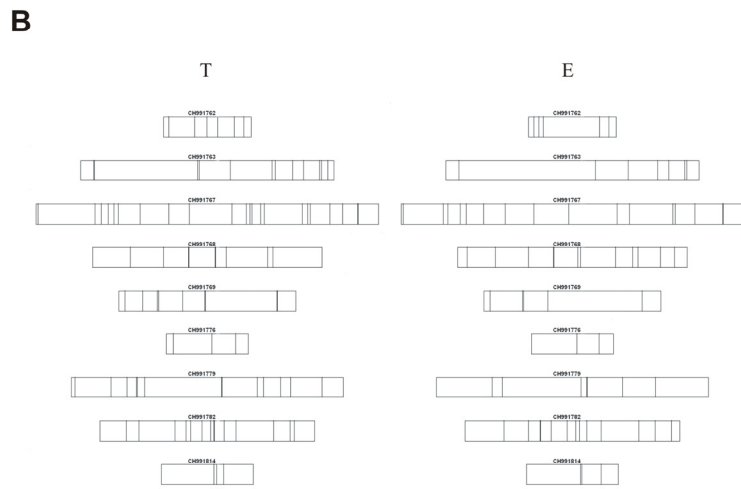
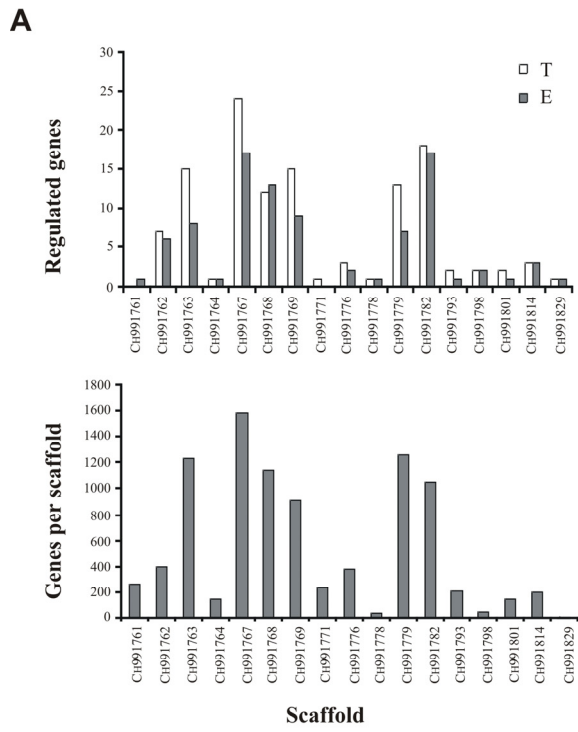
| Gene ID | Description | T | | E | | Gene ID | Description | T | | E | |
|---------|---|-----------------|-----------------|-----------------|-----------------|---------|---|-----------------|-----------------|-------|--|
| | | Fold regulation | Fold regulation | Fold regulation | Fold regulation | | | Fold regulation | Fold regulation | | |
| 114930 | high cysteine membrane protein Group 4 | 26.21 | 18.05 | | | 9605 | predicted protein | 2.44 | | | |
| 112673 | high cysteine membrane protein VSP-like | 23.83 | 19.40 | | | 114779 | Hypothetical Protein | 2.44 | | 2.28 | |
| 102575 | Hypothetical Protein | 16.40 | 10.48 | | | 11930 | retinoic acid induced 17; PIAS-like protein hZimp10 | 2.43 | | | |
| 3269 | Hypothetical Protein | 16.25 | 9.93 | | | 113512 | high cysteine membrane protein Group 6 | 2.41 | | 3.48 | |
| 112135 | high cysteine membrane protein VSP-like | 14.88 | 8.99 | | | 113741 | blasthit 3e-05 putative integral membrane protein | 2.41 | | 4.31 | |
| 14259 | Glucose 6-phosphate N-acetyltransferase | 12.35 | 6.63 | | | 6492 | blasthit 2e-12 hypothetical protein AN0137.2 | 2.38 | | | |
| 8505 | Hypothetical Protein | 10.70 | 5.89 | | | 6291 | Hypothetical Protein | 2.36 | | 2.45 | |
| 118710 | Hypothetical Protein | 10.27 | 6.33 | | | 14261 | Kinase | 2.34 | | | |
| 112135 | high cysteine membrane protein VSP-like | 10.26 | 6.37 | | | 17604 | blasthit 2e-08 HUMANHypothetical protein KIAA1223 | 2.34 | | | |
| 112305 | high cysteine membrane protein VSP-like | 10.10 | 9.36 | | | 6639 | Protein 21.1 | 2.32 | | | |
| 6175 | Nitroreductase family protein | 10.06 | 7.24 | | | 101451 | Hypothetical Protein | 2.27 | | | |
| 14247 | TM efflux prot | 9.11 | 7.83 | | | 4846 | Protein 21.1 | 2.23 | | | |
| 9548 | Cathepsin L precursor | 8.60 | 10.23 | | | 15030 | Protein 21.1 | 2.22 | | | |
| 31886 | Hypothetical Protein | 8.40 | 7.19 | | | 112937 | blasthit 8e-05 HUMANHypothetical protein KIAA1223 | 2.21 | | 2.18 | |
| 92983 | Protein 21.1 | 7.88 | 5.21 | | | 35352 | Hypothetical Protein | 2.21 | | | |
| 36351 | Hypothetical Protein | 7.83 | 3.21 | | | 9051 | Hypothetical Protein | 2.20 | | | |
| 113512 | high cysteine membrane protein Group 6 | 7.78 | 7.90 | | | 21924 | kinase, NEK | 2.19 | | | |
| 114215 | Hypothetical Protein | 7.17 | 3.41 | | | 39824 | Hypothetical Protein | 2.17 | | 2.11 | |
| 32453 | Hypothetical Protein | 6.58 | 4.04 | | | 8960 | blasthit 2e-08 unnamed protein product | 2.17 | | 2.19 | |
| 91707 | high cysteine membrane protein Group 1 | 6.44 | 4.25 | | | 127181 | Hypothetical Protein | 2.16 | | | |
| 9338 | Hypothetical Protein | 6.44 | 5.63 | | | 92919 | Hypothetical Protein | 2.15 | | | |
| 17120 | CEGP1 protein | 5.54 | 2.04 | | | 114210 | blasthit 4e-12 LRP1 | 2.13 | | | |
| 115066 | high cysteine membrane protein VSP-like | 5.43 | 4.96 | | | 13272 | blasthit 3e-11 LOC402865 protein | 2.13 | | | |
| 10659 | high cysteine membrane protein Group 1 | 4.97 | 4.16 | | | 17012 | Hypothetical Protein | 2.12 | | | |
| 37605 | Hypothetical Protein | 4.52 | 3.27 | | | 137726 | ABC transporter ABCA.1 | 2.12 | | | |
| 20020 | Hypothetical Protein | 4.49 | 3.61 | | | 8048 | Hypothetical Protein | 2.12 | | 2.06 | |
| 117655 | Hypothetical Protein | 4.44 | 2.83 | | | 36776 | Hypothetical Protein | 2.12 | | 2.15 | |
| 32578 | Hypothetical Protein | 4.10 | | | | 16519 | AstB/chuR-related protein | 2.11 | | | |
| 17476 | CXC-rich protein | 3.93 | 2.49 | | | 3546 | kinase, NEK | 2.11 | | | |
| 100877 | blasthit 2e-12 variant-specific surface protein AS7 | 3.90 | 2.81 | | | 32701 | high cysteine protein (HCp) | 2.11 | | 2.31 | |
| 34163 | Hypothetical Protein | 3.48 | 2.24 | | | 15125 | Hypothetical Protein | 2.10 | | | |
| 121724 | Hypothetical Protein | 3.42 | 2.11 | | | 16283 | amino acid transporter family | 2.08 | | | |
| 112008 | blasthit 1e-08 HUMANHypothetical protein KIAA1223 | 3.07 | 2.29 | | | 102180 | high cysteine protein | 2.07 | | 2.25 | |
| 3855 | Hypothetical Protein | 3.03 | | | | 38584 | Hypothetical Protein | 2.07 | | | |
| 22217 | Hypothetical Protein | 3.02 | | | | 117437 | blasthit 6e-11 HUMANSerine/threonine-protein kinase RIPK4 | 2.06 | | | |
| 10829 | Glucosamine-6-phosphate deaminase | 3.00 | | | | 14331 | variant-specific surface protein AS1 | 2.06 | | 5.37 | |
| 8377 | Hypothetical Protein | 2.96 | 2.43 | | | 12229 | Hypothetical Protein | 2.05 | | | |
| 120906 | Hypothetical Protein | 2.93 | 2.45 | | | 13922 | blasthit 2e-08 Large exoproteins | 2.03 | | 2.72 | |
| 32139 | Hypothetical Protein | 2.90 | 2.44 | | | 13412 | 60S acidic ribosomal protein P0 | 2.03 | | | |
| 11040 | kinase, NEK | 2.89 | 2.62 | | | 9210 | Hypothetical Protein | 2.02 | | | |
| 114470 | high cysteine membrane protein Group 6 | 2.88 | 3.68 | | | 7760 | Aldose reductase | 2.02 | | | |
| 37595 | Hypothetical Protein | 2.81 | | | | 5435 | cyst wall protein 2 | 2.01 | | -4.04 | |
| 8585 | Hypothetical Protein | 2.76 | 2.03 | | | 5638 | cyst wall protein 1 | 2.01 | | -2.96 | |
| 3279 | ribonuclease | 2.75 | 2.14 | | | 14863 | Hypothetical Protein | 2.01 | | | |
| 4507 | CTP synthase/UTP-ammonia lyasen | 2.69 | | | | 14759 | 6-phosphogluconate dehydrogenase, decarboxylating | 2.00 | | | |
| 36327 | Hypothetical Protein | 2.64 | | | | 3593 | Alcohol dehydrogenase | -2.04 | | | |
| 113030 | kinase, NEK | 2.62 | 2.61 | | | 17198 | Leucine-rich repeat protein | -2.05 | | -2.78 | |
| 114210 | blasthit 4e-12 LRP1 | 2.59 | 2.16 | | | 11448 | Phosphomannomutase-2 | -2.10 | | | |
| 11976 | Hypothetical Protein | 2.56 | | | | 8958 | Hypothetical Protein | -2.11 | | | |
| 98867 | Hypothetical Protein | 2.51 | 2.15 | | | 16111 | Elongation factor 1-alpha | -2.13 | | | |
| 37897 | Hypothetical Protein | 2.48 | | | | 37109 | Hypothetical Protein | -2.13 | | | |
| 25816 | high cysteine membrane protein Group 1 | 2.47 | 3.15 | | | 9620 | high cysteine membrane protein Group 2 | -2.15 | | | |
| 102542 | kinase, NEK | 2.46 | 2.25 | | | 9008 | Aldose reductase | -2.17 | | | |
| | | | | | | 31956 | Hypothetical Protein | -2.21 | | | |

| Gene ID | Description | T | | E | |
|---------|---|-----------------|-----------------|-----------------|-----------------|
| | | Fold regulation | Fold regulation | Fold regulation | Fold regulation |
| 103829 | Hypothetical Protein | -2.25 | | | |
| 16670 | Hypothetical Protein | -2.36 | | | |
| 9099 | blasthit 0.0002 Probable COP9 signalosome complex subunit 7 | -2.41 | | | |
| 36663 | Hypothetical Protein | -2.50 | | -2.12 | |
| 112359 | Hypothetical Protein | -2.51 | | -2.09 | |
| 11209 | Cathepsin L precursor | -2.81 | | -2.10 | |
| 20781 | Hypothetical Protein | -2.88 | | -2.42 | |
| 14200 | Molybdenum cofactor sulfurase | -3.30 | | -4.25 | |
| 11449 | Predicted ATPase of the PP-loop superfamily | -4.02 | | -2.23 | |
| 7421 | Casein kinase II beta chain | -4.08 | | -3.36 | |
| 114474 | Hypothetical Protein | -4.08 | | -3.60 | |
| 3470 | ABC transporter family protein | | 2.18 | | |
| 8227 | ATP-binding cassette protein 5 | | 2.22 | | |
| 103709 | blasthit 0.0004 unnamed protein product | | 2.09 | | |
| 12082 | blasthit 1e-10 Biotin-(acetyl-CoA carboxylase) ligase | | -2.11 | | |
| 10552 | blasthit 4e-10 HUMANThyroglobulin precursor | | -2.34 | | |
| 2421 | Cyst wall protein 3 | | -3.13 | | |
| 8245 | Glucosamine-6-phosphate deaminase | | -2.64 | | |
| 15250 | high cysteine membrane protein Group 6 | | 2.31 | | |
| 135231 | Histone H3 | | -2.05 | | |
| 33672 | Hypothetical Protein | | -2.38 | | |
| 32657 | Hypothetical Protein | | -2.75 | | |
| 17188 | kinase, NEK | | 2.19 | | |
| 11436 | Molybdopterin biosynthesis MoeB protein | | -2.55 | | |
| 8722 | Myb 1-like protein (gMyb2) | | -2.70 | | |
| 14626 | Oxidoreductase, short chain dehydrogenase/reductase family | | -2.21 | | |
| 102444 | Protein 21.1 | | -2.03 | | |
| 115831 | S4:AY14125; vsp with INR | | 4.63 | | |
| 23015 | Serine palmitoyltransferase 1 | | -2.12 | | |
| 9046 | Sugar transport family protein | | -3.16 | | |
| 137740 | vsp with INR | | 2.00 | | |

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1 Figure S7



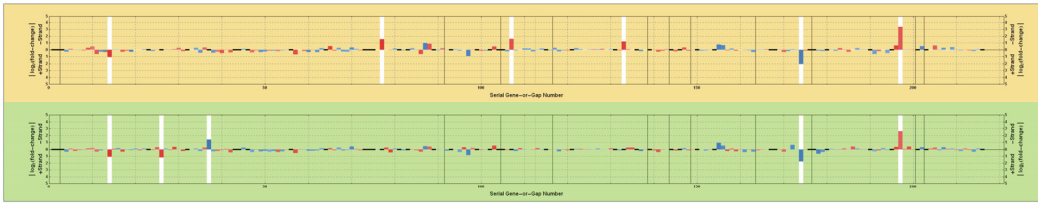
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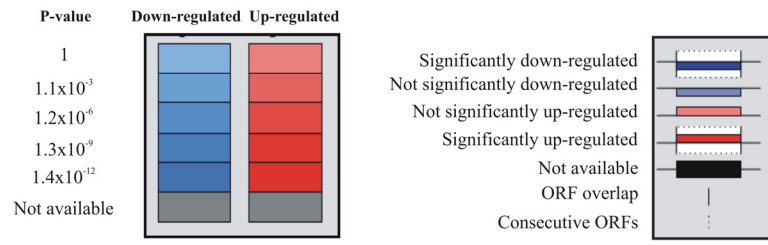
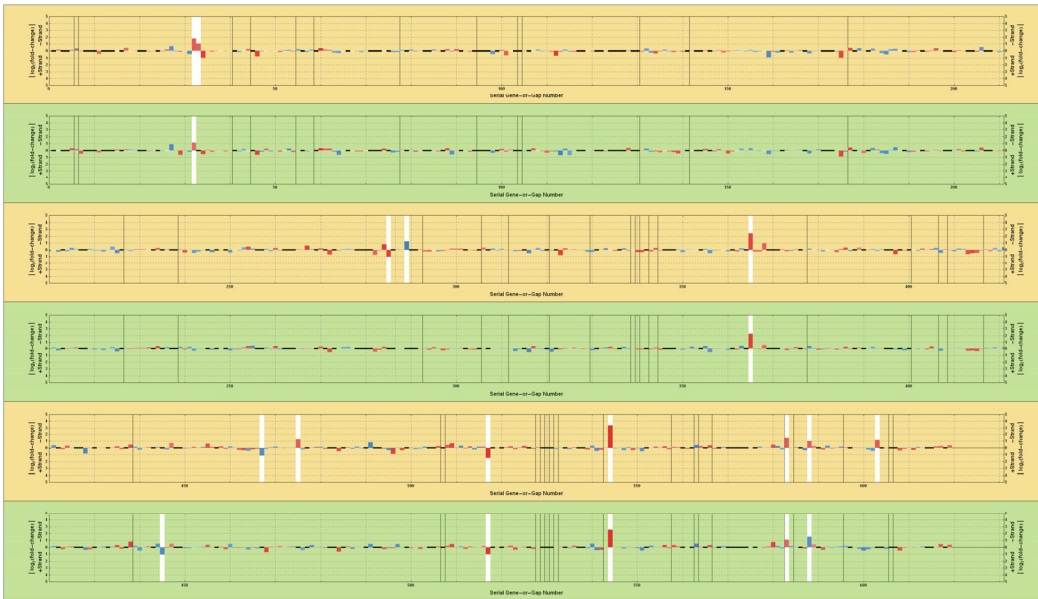
1 Figure S8

A

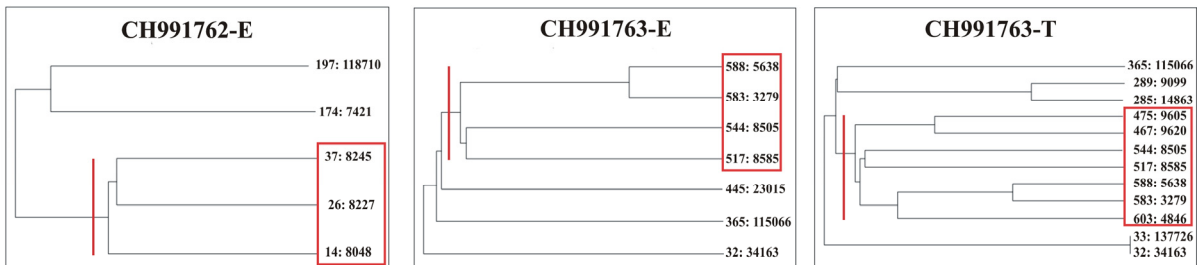
CH991762



CH991763



B



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