SUPPLEMENTAL DATA

GIARDIA DUODENALIS 14-3-3 PROTEIN IS POLYGLYCYLATED BY TTLL3 AND DEGLYCYLATED BY TWO METALLOCARBOXYPEPTIDASES

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Running head: Identification of g14-3-3 polyglycylase and deglycylases

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Supplemental Experimental Procedures.

Construction of the Giardia *expression vectors.* To obtain the pTUB-FLAGpac vector, a short linker encoding the FLAG epitope and containing a BamHI and PspOMI site, for PCR fragment cloning in frame with the FLAG (5'-ACATGTTGGATTATAAGGATGATGATGATAAG**GGATCC***GGGCCC*AAA**TGATCA**-3', the PciI site is underlined, the BamhI site is in bold, the PspOMI site is in italic, and the BclI site is in bold and underlined) was cloned under the alpha-tubulin gene promoter in the NcoI/BamHI-digested PtubApaH7-HApac vector (1), instead of the *VSPH7* gene and of 3xHA tags coding sequence. The primer FLAGforw (5'-CATGTTGGATTATAAGGATGATGATGATAAGGGATCCGGGCCCAAAT-3') and the partially complemental primer FLAGrev (5'-AACCTAATATTCCTACTACTACTATTCCCTAGGCCCGGGTTTACTAG-3') were annealed to produce PciI protrusion at the 5' end and BamHI protrusion end at the 5' end and then cloned in the NcoI/BamHI-digested PtubApaH7-HApac vector.

To obtain the pTUB-FLAG_HApac vector, a second linker encoding the HA epitope (hemmaglutinin; GSYPYDVPDYAGS) and containing a BamHI site for PCR product cloning (5'-TGATCATACCCATACGATGTTCCAGATTACGCT**GGATCC**-3', the BclI site is underlined and the BamhI site is in bold) was introduced in frame with FLAG in the BamHI-digested pTub-FLAGpac vector.

The primer HAforw (5'-GATCATACCCATACGATGTTCCAGATTACGCTG-3') and the partially complemental primer HArev (5'-GATCCAGCGTAATCTGGAACATCGTATGGGTAT-3') were annealed to produce BclI protrusion at the 5' end and BamHI protrusion end at the 5' end and then cloned in the BamHI-digested pTUB-FLAGpac vector.

Sequence analysis. General homology searches with protein sequences were conducted on non-redundant GenBank databases using the BLAST algorithm, available at http://www.ncbi.nlm.nih.gov/BLAST/. *Giardia* genome databases were analyzed on-line at http://giardiadb.org/giardiadb/. Multiple alignments were performed using the Clustal W program at http://www.ebi.ac.uk/clustalw/. Phylogenetic and molecular evolutionary analyses were conducted with MEGA version 4.0.2 (2) using the neighbor joining method and 10000 bootstrap replications.

SUPPLEMENTAL REFERENCES.

1. Touz, M.C., Lujan, H.D., Hayes, S.F., and Nash, T.E. (2003) *J. Biol. Chem.* **278**, 6420–6426.
2. Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) *Mol. Biol. Evol.* **24**, 1596-1599.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. S1. A). Representation of the encoded gTTLL proteins. The position of the TTL domain (green oval) in each protein is shown. Numbering of aminoacids is according the first Met of each protein. The protein size in aminoacid residues (aa) is reported on the left. B). Phylogenetic tree of TTLLs calculated using a neighbor-joining method. All known human proteins that have a TTL catalytic domain were included. TTLL sequences from National Center for Biotechnology Information (NCBI) were: *Homo*

sapiens (Hs): hTTL, NP_714923.1; hTTLL1, CAG30485.1; hTTLL2, AAK20169.1; hTTLL3, NP_056459.3; hTTLL4, Q14679.2; hTTLL5, Q6EMB2.1; hTTLL6, BAC05032.1; hTTLL7, NP_078962.4; hTTLL8, XP_943304.2; hTTLL9, EAW76406.1; hTTLL10, Q6ZVT0.2; hTTLL11, NP_919228.2; hTTL12, NP_055955.1; hTTLL13, NP_001025135.2); *Macaca mulatta* (Mm) MmTTLL10, NP_001156552.1; *Danio rerio* (Dr) TTLL3, AAI17657.1; *Saccharomyces cerevisiae* (Sc) ScPby1p, NP_009652.1. From FlyBase (http://flybase.org/) for *Drosophila melanogaster* (Dm): DmTTLL3A, CG11323; DmTTLL3B, CG11201; Dm_CG32238; Dm_CG8918; Dm_CG16716; Dm_CG5987; Dm_CG16833; Dm_CG3964; Dm_CG1550; Dm_CG31108; Dm_CG4089. From Tetrahymena genome database (http://www.ciliate.org/) for *Tetrahymena thermophila* (Tt): TtTTLL1, TTHERM_00136210; TtTTLL2, TTHERM_00193570; TtTTL3A, TTHERM_00666600; TtTTL3B, TTHERM_00125600; TtTTL3C, TTHERM_00770730; TtTTL3D, THERM_00316230; TtTTL3E, TTHERM_00378750; TtTTL3F, TTHERM_00196050; TtTTLL4A, TTHERM_00758970; TtTTLL6A, TTHERM_00284020; TtTTLL6E, TTHERM_00077290; TtTTLL9, TTHERM_00773080; TtTTLL10A, TTHERM_00586750; TtTTLL12A, TTHERM_00913400; TtTTLL14A, TTHERM_00502360; TtTTLL14H, TTHERM_01084300; TtTTLL15C, TTHERM_00530520. From WormBase (http://www.wormbase.org/) for *Caenorabditis elegans* (Ce): CeTTLL4, ZK1128.6; CeTTLL5, C55A6.2; CeTTLL9, F25C8.5; CeTTLL11, H23L24.3; CeTTLL12, D2013.9; CeTTLL15, K07C5.7. Glutamylases with demonstrated activity are in light blue, glycylases are in green and, *Giardia* TTLLs homologs are in red. Bootstrap values >50 are reported.

Supplemental Figure S2. Confocal Laser Scanning Immunofluorescence Microscopy of WBC6 and FLAG-tagged gTTLL3 and gDIPs transfected *G. duodenalis* encysting parasites (at 12h post encystations induction) stained with anti polyGly antibody. Wild type parasite WBC6 (A), parasites transfected with FLAG-gTTLL3 (B), FLAG-gDIP1 (C) and FLAG-gDIP2 (D). FITC-conjugated anti-FLAG mAb (shown in green), anti-polyGly Ab revealed by Alexa Fluor-594 goat anti-rabbit Ab (red), Cy3-conjugated anti-CWP mAb (pseudocolor grey), and DAPI (blue). Displayed micrographs correspond to the single stack encompassing the center of the two nuclei of trophozoite or encysting parasite or at least two of the four cyst nuclei. Merged+DAPI, merged images with DAPI-stained nuclei. 3D, three dimensional reconstructions of the complete stack series for each acquisition. T, transmission light acquisition. Scale bars, 2 μm. Arrows indicate the position of median body (mb) and flagella (fl).

Supplemental Table 1. PCR primers' list

Gene (ID)	Primer forward[a]	Primer reverse
gDIP1 (GL50803_15832)	5'-CCC**TGATCA**TCCAATAAATACAATATTTACACTACAAATGCCG-3'	5'-GGG*gcggccgc*TTACTGGAGGGTGGCAATCTC-3'
gDIP2 (GL50803_8407)	5'-CCCGGATCCCTTGACGCTAACAGGGCTGTG-3'	5'-GGG*gcggccgc*TTACTTGTACTTCGCCGCCAAC-3'
gTTLL1 (GL50803_95661)	5'-CCC**TGATCA**ACAGATAACTTCGAGGATTATG-3'	5'-CCC*gcggccgc*TTATTTCTTGGCAAAGAGGCC-3'
gTTLL2 (GL50803_10382)	5'-CCCGGATCCCCGTCCTTCCAGAAAGGAATTATC-3'	5'-CCC*gcggccgc*CTACAGGGAAGATACCATCATC-3'
gTTLL3 (GL50803_8456)	5'-CCCGGATCCAATAATGATATTACAGCATTAGAC-3'	5'-CCC*gcggccgc*CTAGTACTCAGTAGCTATGTCAGAG-3'
gTTLL4 (GL50803_8592)	5'-CCCGGATCCCTTCCAATTAAAAAAGATGCTCTCA-3'	5'-CCC*gcggccgc*CTAAATAAGCTCGTGCCACGG-3'
gTTLL5 (GL50803_9272)	5'-CCCGGATCCAAAAAACTTAAAATGTACTCC-3'	5'-CCC*gcggccgc*TTAAATTATGAGACCAGAAGCTGAC-3'
gTTLL6 (GL50803_10801)	5'-CCC**TGATCA**GGAGCTGATGGCGTTTGTGAT-3'	5'-CCC*gcggccgc*TCATTGCGCCTCTCCCTTATC-3'
gTTLL7 (GL50803_14498)	5'-CCCTGATCACCGCCCATAAAATACAGG-3'	5'-CCC*gcggccgc*TTACTTACTTGAGAGGGCACC-3'

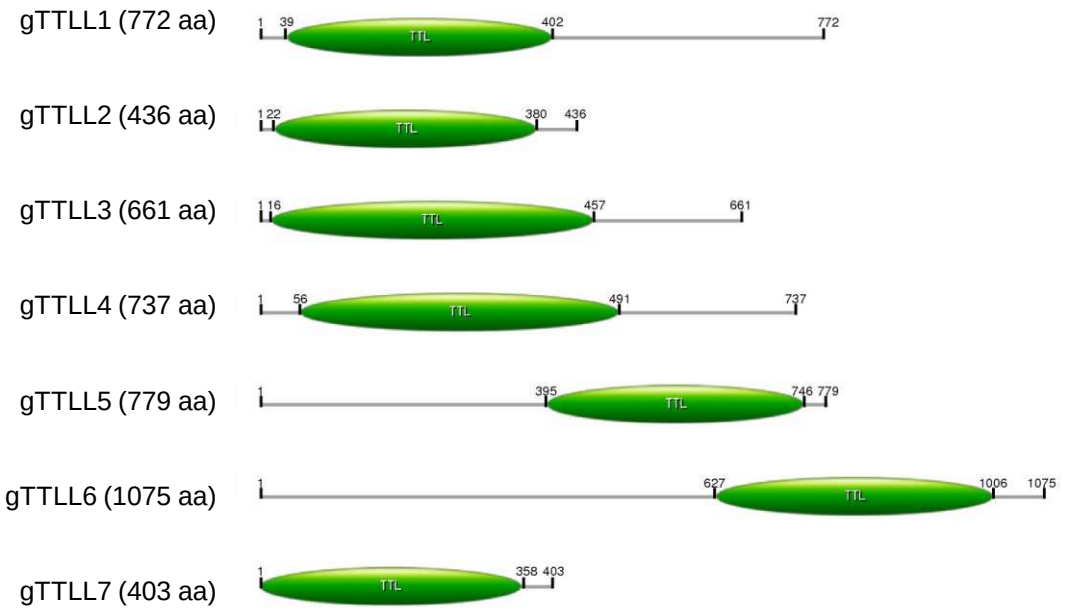
[a]BclI site is in bold; BamHI site is underlined, NotI site is in italics

Supplemental Table 2. Real Time PCR primers' list

Gene (ID)	Primer forward[a]	Primer reverse
gCWP1	5'-CTGCATCAATGAGCTTCAATTT-3'	5'-TGCCTGACAGCTGATTGC-3'
gGAP1	5'-ACAGGTCGCTTTACAACGAAG-3'	5'-AGATGATGACACGCTTGCAG-3'
gTTLL3	5'-CTCATGGACCTGACGATTGA-3'	5'-CCACAGCTTATCACGGTACG-3'
gDIP1	5'-AAGTATAGCGGCAAGCTGGA-3'	5'-CAGCTTGGAGTGAGGATTCG-3'
gDIP2	5'-ACTGAATTCTCTCTTCTGGGTAGTG-3'	5'-GACGCCATCCAATTCAGACT-3'

Figure S1

A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1 16 TTL 457 661
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1 56 TTL 491 737
gTTLL5 (779 aa)
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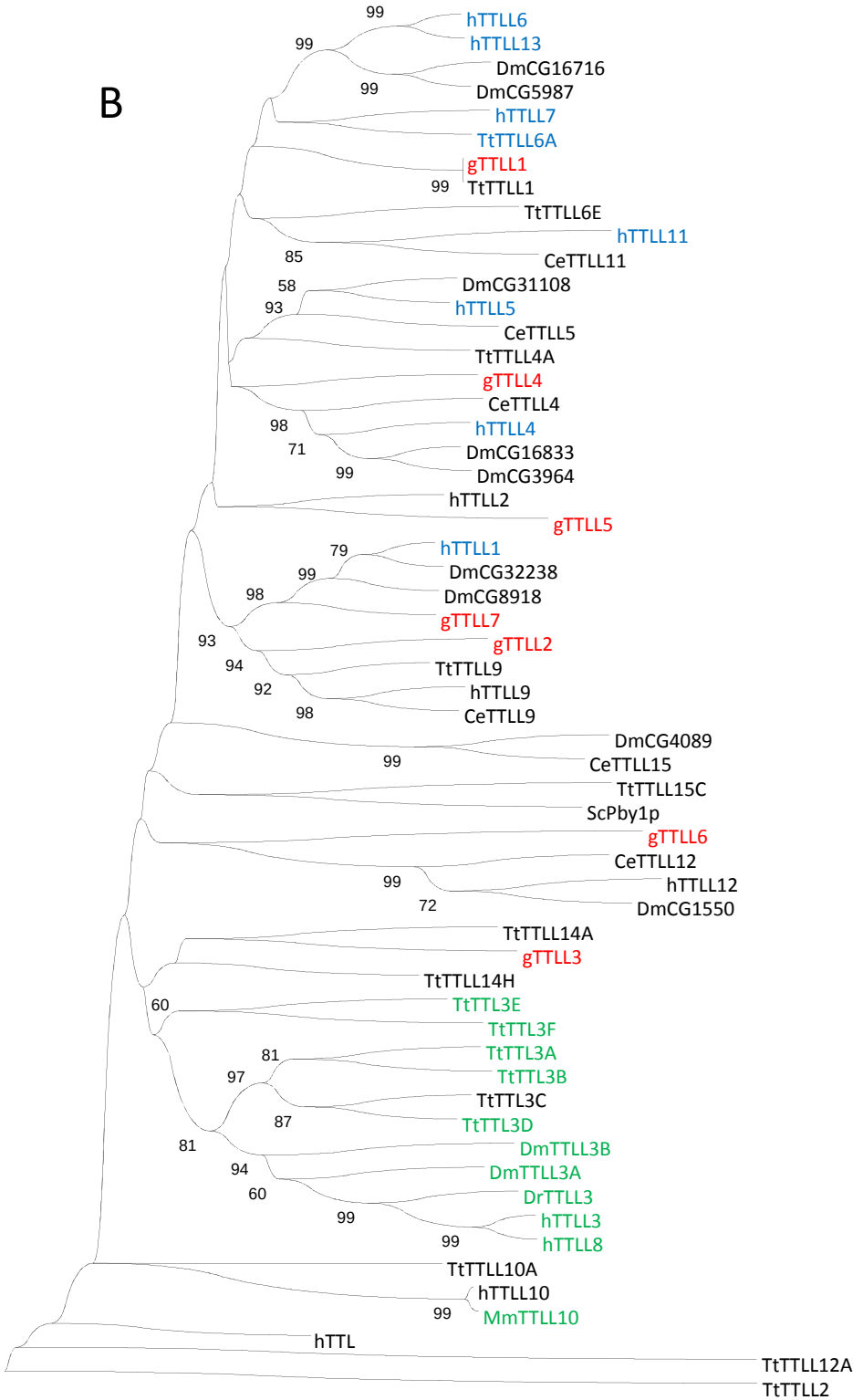
1 627 TTL 1006 1075
gTTLL7 (403 aa)
1 TTL 358 403

B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CeTTLL11
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CeTTLL5
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CeTTLL4
hTTLL4
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gTTLL2
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CeTTLL9
DmCG4089
CeTTLL15
TtTTLL15C
ScPby1p
gTTLL6
CeTTLL12
hTTLL12
DmCG1550
TtTTLL14A
gTTLL3
TtTTLL14H
TtTTL3E
TtTTL3F
TtTTL3A
TtTTL3B
TtTTL3C
TtTTL3D
DmTTLL3B
DmTTLL3A
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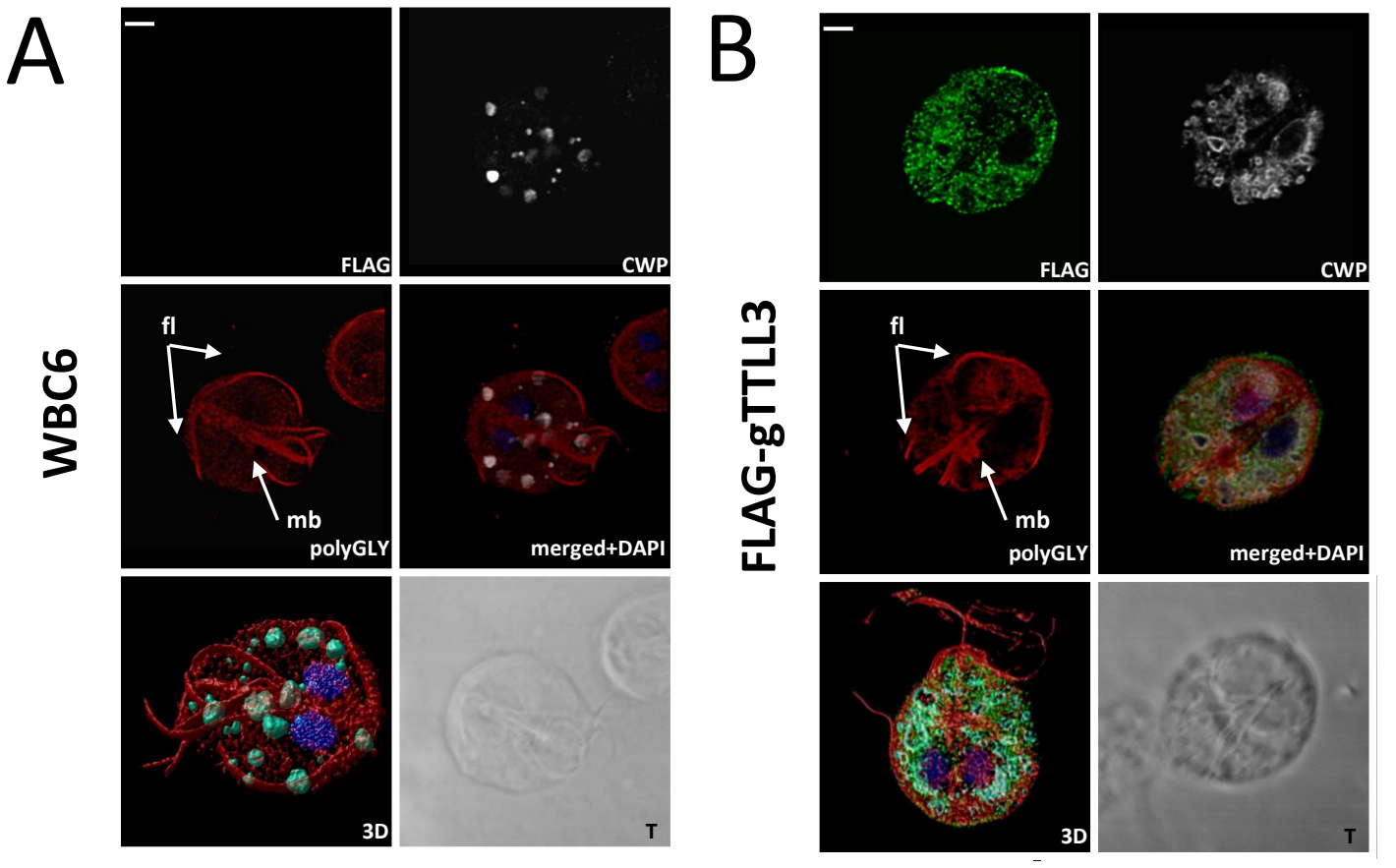
MmTTLL10
hTTL
TtTTLL12A
TtTTLL2

Figure S2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