SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1:

A. Enrichment analysis of methyl arginine proteins for gene sets derived from tachyzoite transcriptomic data percentiles. Gene sets representing expression percentiles at 5 percentile intervals were created from tachyzoite microarray and RNA-seq data downloaded from ToxoDB.org as described previously [27]. These gene sets were used to survey the expression of MMA proteins by applying the hypergeometric statistical test for enrichment against random gene sets. Adjusted p values ($-\log_2$-transformed) are shown with the dotted line indicating significant enrichment (adjusted p-value = 0.05).

B. Arginine monomethylated proteins are enriched for tachyzoite and bradyzoite genes. Enrichment analysis was performed using predefined gene sets representing genes upregulated in different stages or conditions of parasite growth [35]. Adjusted p-values (-log2-transformed) are plotted with the dotted line indicating significant enrichment (adjusted p-value = 0.05).

Supplemental Figure 2: MMA occurs in glycine arginine rich (GAR) motifs in *T. gondii*

A. Comparison of *T. gondii* intracellular, extracellular and Human [33] motif logos of amino acids surrounding detected MMA sites. Residues are colored according to side-

chain chemistry (green = polar uncharged, purple = polar, amide, blue = basic, red = acidic, black = hydrophobic).

B. Heat map of proteins from intracellular wild-type parasites depicting the enrichment of each amino acid at sites surrounding the monomethylated arginine residue. Amino acids that are enriched are green and those that are depleted are red (scale as indicated). Plots were created using iceLogo software (http://iomics.ugent.be/icelogoserver/index.html).

Supplemental Figure 3: Intracellular *T. gondii* MMA motifs identified in protein dataset

Bar graph showing all the methyl arginine motifs found within the proteins of the intracellular wild-type dataset. The number of methyl arginine sites with each motif are plotted and the number following each bar shows the motif score, indicating the probability of a given motif in the target dataset versus the probability of a specific sequence in a background dataset, as described in [61]. Motifs, constructed from six amino acids upstream and downstream of the central arginine, were obtained from Scaffold PTM software.

Supplemental Figure 4: Spectra of AP2 XII-1 and its MMA site.

LC-MS/MS spectrum of a AP2XII-1 derived peptide (DFDAFVR*PPGLTPFR) showing MMA sites on two Arginine, (A) R419 in WT2 with an error of 0.52 ppm and (B)R419 in

PRMT1COMP with and error of 0.73 ppm. (C) Schematic of AP2XII-1 with MMA sites (yellow) and previously mapped serine and threonine phosphorylation sites (green) [42].

Supplemental Figure 5: 2D-PAGE Immunoblots

A. Immunoblot of whole cell lysates from RH*Δprmt1Δhxgprt* (KO) and RHΔ*hxgprt*Δ*prmt1*::*PRMT1RFP* (COMP). Probed with *T. gondii* aldolase (TgALDI): (runs at 40 kDa) as a loading control for the 2D-PAGE in B). The blot shows equal protein loading (240 μg) per lane, which is the same amount used in each of the 2D experiments.

B. 2D-PAGE Immunoblots of whole cell lysate from RH*Δprmt1Δhxgprt* (KO) top left and RHΔ*hxgprt*Δ*prmt1*::*PRMT1RFP* (COMP) top right, probed with R*GG (D5A12) (CST #8711, Cell Signalling Technology, Danvers, MA). . Changes in intensities of spots can be seen in the series of spots labelled 1, ranging from pH 5.2 – 5.5 at 90 kDa, which are visibly more abundant in KO than in COMP. Further the change in methylation pattern between KO and COMP is pronounced in spots 2 (pH3.5, 130kDa), spots 3 (pH4.4, 130kDa), spots 4 (pH3.5, 125kDa) and spots 5 (pH 3.4, 70kDa) of COMP, which are all absent in this region of the KO sample. The COMP sample also shows MMA reactivity in a series of spots labelled 6, with a pH range of 5.6 – 6.1 and 65kDa, which are reduced or absent in the in the same region of the KO sample. The Immunoblots on the bottom left (RH*Δprmt1Δhxgprt* (KO)) and bottom right (RHΔ*hxgprt*Δ*prmt1*::*PRMT1RFP* (COMP)) were probed with an anti- asymmetric dimethyl arginine (ADMA) motif rabbit mAb (CST #13522, Cell Signalling Technology, Danvers, MA). No spots were detected

in the KO sample, most likely due to significantly reduced or absent ADMA deposition upon PRMT1 deletion. ADMA spots reappear in the COMP sample at spots 7 (pH 4.9, 50kDa), spots 8 (pH5.2, 60kDa), spots 9 (pH range 5.6 -5.8, 55kDa), and spots 10 (pH 7.2, 38kDa).

SUPPLEMENTAL TABLE LEGENDS

Supplemental Table 1: PRMTs in *Toxoplasma gondii* and Human.

Summary of PRMTs encoded by genomes of *T. gondii* and human. Type I and II PRMTs are present in *T. gondii.* PRMT classes are based on the terminal arginine modifications produced by the given enzyme [25]. PRMT activity type has not yet been established for all isoforms and was predicted bioinformatically.

Supplemental Table 2: Abundantly modified monomethylarginine proteins.

Arginine monomethylated proteins with the highest numbers (>2 sites) of MMA sites detected in intracellular parasites.

Supplemental Table 3: Apicomplexan AP2 transcription factors

Summary of 10 ApiAP2 transcription factors identified as being MMA modified across all biological samples examined in this study. AP2 domains (found by searching Interpro)

and sites of phosphorylation (toxodb.org) are provided. Not all TgApiAP2 proteins have complete AP2 domains identified by pfam or Interpro analyses.

Supplemental Table 4: Validation of MMA proteins

Summary of 14 proteins found within GCN5b immunoprecipitation mass spectrometry data, identified as being MMA modified across all biological samples examined in this study.

Supplemental table 5: High confidence candidate PRMT1 substrates.

Proteins present in the MMA intracellular union dataset but not in the PRMT1KO dataset were considered to be candidate PRMT1 substrates. High confidence PRMT1 substrates are candidate PRMT1 substrates whose methylation was restored in PRMT1COMP.

Supplemental Table 6: Sample Summary

Summary of all biological and technical replicates analyzed in this study including protein number and overlap between technical replicates.

Supplemental Table 7: Gene IDs in Cell Cycle Enrichment Analysis

Enrichment analysis of G1-regulated gene sets in the arginine methylome. P-value of enrichment is shown (derived from hypergeometric test, $-\log_2$-transformed) and genes overlapping the arginine monomethylome.

Supplemental Table 8: Gene IDs in PTM Enrichment Analysis

Enrichment analysis of gene sets of genes whose protein products are known to be post-translationally modified in the arginine methylome. P-value of enrichment is shown (derived from hypergeometric test, -$\log_2$-transformed) and genes overlapping the arginine monomethylome.

SUPPLEMENTAL EXPERIMENTAL METHODS

2D- SDS-PAGE Immunoblot:

Cell Culture: HFF in 150 cm^2 plates were infected with 2.5 x 10^8 freshly lysed *T. gondii* tachyzoites [strains: RHΔ*hxgprt*Δ*prmt1* (PRMT1KO), RHΔhxgprtΔprmt1::PRMT1RFP (PRMT1COMP)] [29]. The parasites used for the 2D-PAGE experiments were cultured and harvested under the same conditions as the parasites used for mass spectrometry analysis with the exception of the parasite filtration step, which was omitted.

Preparation of Lysates and first dimension: Parasite pellets were solubilized in BioRad Rehydration/Sample Buffer (#1632106), containing 8 M urea, 2% CHAPS, 50 mM DTT, 0.2% Bio-Lyte® 3/10 ampholyte, 0.001% Bromophenol Blue. Samples were stored at -80°C prior to separation in the first dimension. The first dimension was run on 11 cm BioRad ReadyStrip™ IPG Strips with non-linear pH range 3-10, which were passively rehydrated overnight using the BioRad Rehydration/Sample buffer containing 240 µg of the appropriate parasite whole cell lysate. The separation was carried out on

a PROTEAN IEF focusing apparatus (BioRad) using a rapid ramp 35,000 V-hr pre-set program at 50 μA per IPG strip at 20°C.

Equilibration and second dimension: Prior to PAGE separation, the IPG strips were equilibrated for 10 min each with the following buffers: –Equilibration buffer I (6M Urea**,** 2% SDS**,** 0.375M Tris-HCl pH 8.8, 20% glycerol, 130 mM DTT); and Equilibration buffer II (6M Urea, 2% SDS, 0.375M Tris-HCl pH 8.8, 20% glycerol**,** 135 mM Iodoacetmaide). The second dimension was run on 10% Criterion™ Tris-HCl Midi Gels (#3450101) at 150V.

2D Immunoblot: Gels were transferred to either nitrocellulose or PVDF membranes using BioRad Trans-Blot® Turbo™at 2.5A and 25V for 10 min. Blots were probed with R*GG (D5A12) (CST #8711, Cell Signaling Technology, Danvers, MA) or ADMA motif rabbit mAb (CST #13522, Cell Signaling Technology, Danvers, MA). Western analysis of the whole cell lysate probed with rabbit anti-TgALD1 antibody served as a loading control and was a kind gift from Dr. Kentaro Kato at Obihiro University of Agriculture and Veterinary Medicine [62]. For the secondary antibody step, Amersham ECL HRP-linked Donkey anti-Rabbit IgG was used together with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific #34095).