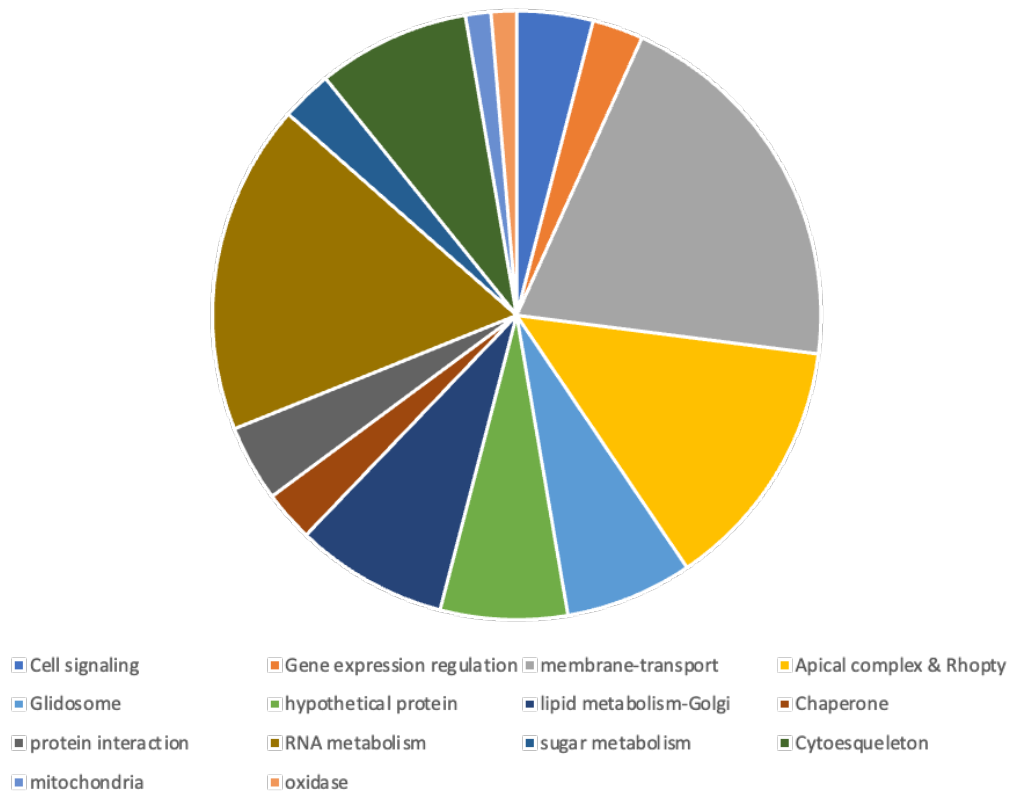


| Purpose  | Name                | Sequence  |
|--|---------------------|---|
| Generate pSag1-Cas9-U6-sgGSK-TG-HXG CRSPR/Cas9 plasmid for endogeneous tagging   | GSK-tg-sgRNA.For    | GTCTTTTTTTGTTTTAGAGCTAGAAATAGC  |
|  | GSK-tg-sgRNA.Rev    | CGACAGCTGCAACTTGACATCCCCATTTAC  |
| Amplify 3xHA-DHFR cassette from the plasmid pLIC-3xHA-DHFR                       | GSK-TG-insert.For   | ATGTATTCCGAAGCATATCGCCAGTGCAAACAACC<br>GTGGCTTAATTAATAATTGGAAGTGGAGG  |
|  | GSK-TG-insert.Rev   | AGCATAAGAGAAGCTCCCCATCCCTAGTAGGTGTA<br>GGGAGGTTTTCCAGTCACGACG         |
| Generate pSag1-Cas9-U6-sgGSK-TATI-HXG CRSPR/Cas9 plasmid for endogeneous tagging | GSK-TATI-sgRNA.For  | AAGAAGGGGTGTTTTAGAGCTAGAAATAGC  |
|  | GSK-TATI-sgRNA.Rev  | TCCTTCGTCCAAGTTGACATCCCCATTTAC  |
| Amplify TATi cassette from the plasmid 5'COR-pT8TATi1-HX-tetO7S1mycNtCOR.dna     | GSK-TATI-insert.For | CACTCATCTTTTCTGGCCTTTGTGCGAGAAGGCAG<br>AAGTCTCTTCTCATGTTTGCGGATCCG    |
|  | GSK-TATI-insert.Rev | CTACTCTTCTGAGCAGCTGCGGGATCGTACTGCG<br>GGTCCGGCATTGATATCCCTAGGAATTCCTC |

**Supplemental table 1.** Primers used in this work. Sequences are 5' to 3'.



**Supplemental figure S1. Classification of hypothetical proteins with TgGSK-dependent phosphopeptides.** Hypothetical proteins with peptides differentially phosphorylated in the knockdown vs the parental were analyzed based on annotations in the *Toxoplasma* genome database or homology to proteins in other Apicomplexan species. Additionally, the protein domains were analyzed based on known conserved functions. Some of these proteins remained classified as hypothetical.