Photoaffinity probe-based antimalarial target identification of artemisinin in the intraerythrocytic developmental cycle of *Plasmodium falciparum*

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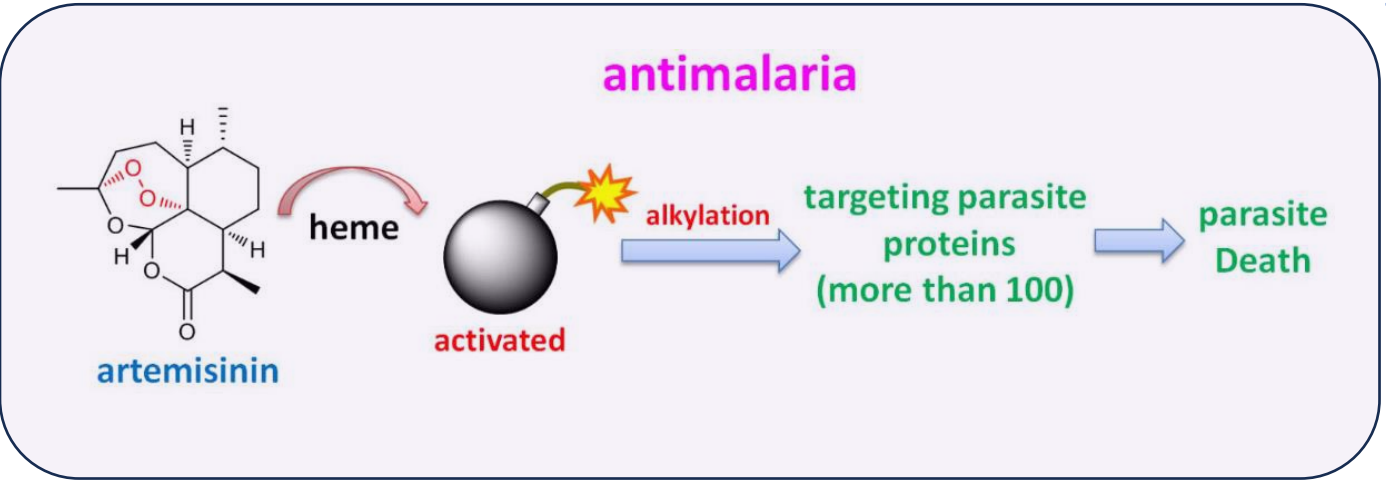
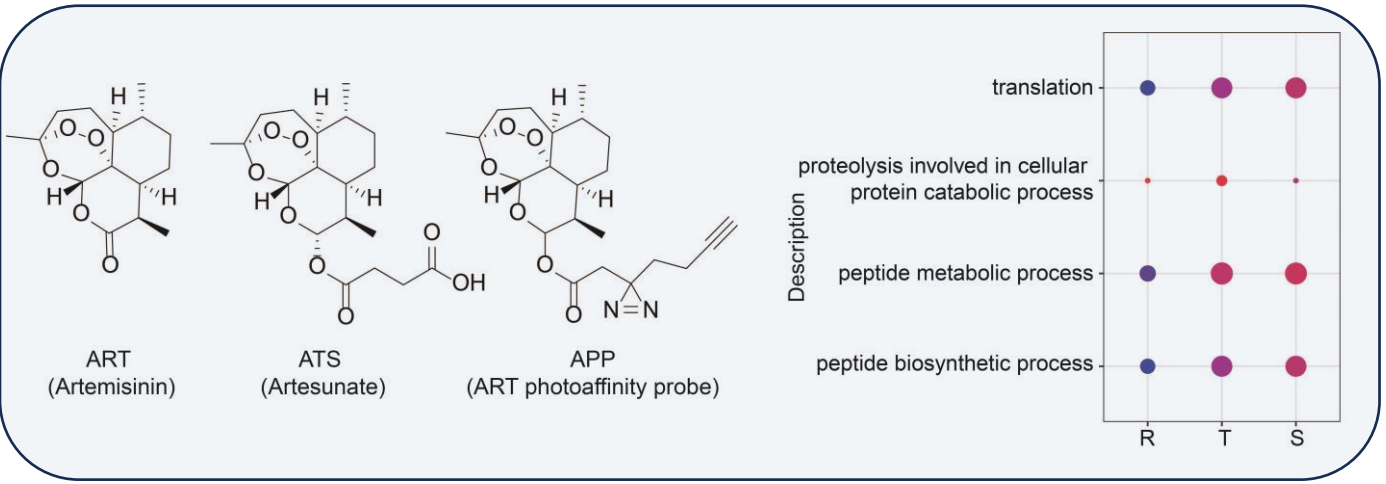
Introduction



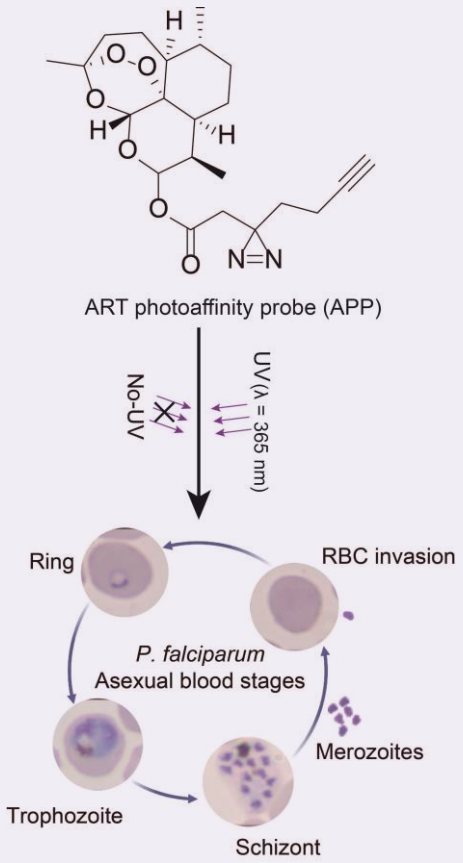
Our group has revealed that the exceptional antimalarial efficacy of artemisinin (ART) results from efficient activation of ART by heme. However, the heme-mediated activation mode suggests that the ART target proteins may differ between different life stages, as the rate of Hb consumption and heme release by parasites varies at different stages.



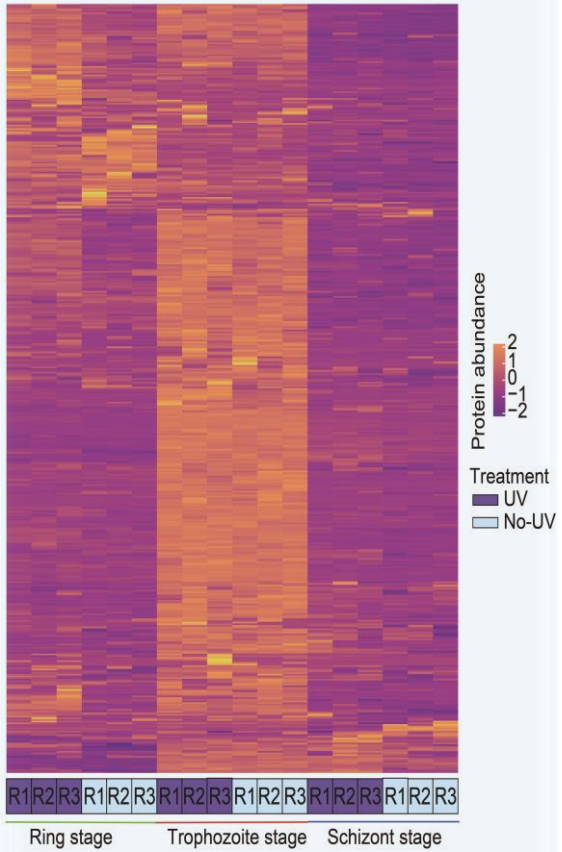
In this work, we identified the protein targets of ART at the ring, trophozoite, and schizont stages using an ART photoaffinity probe (APP), and conducted extensive mechanistic studies by integrating target validation, phenotypic studies, and untargeted metabolomics analysis.

Highlights

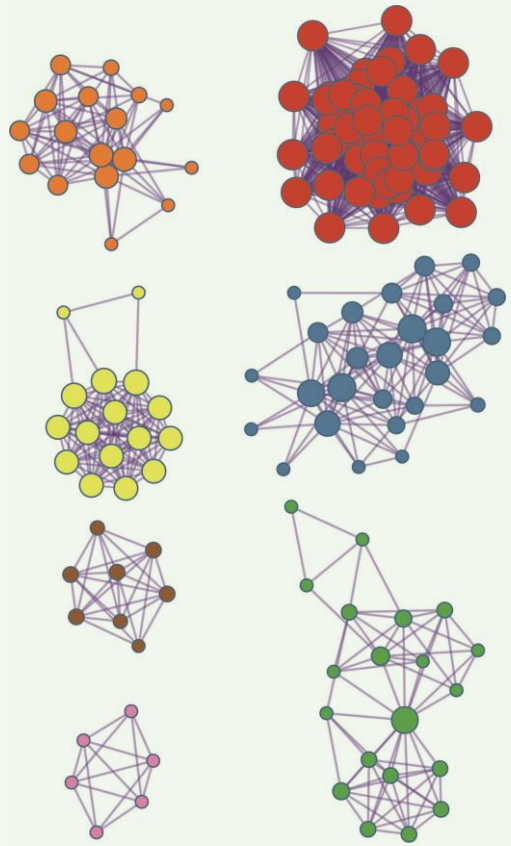
Target identification of different stages of parasite using photoaffinity probe



Artemisinin interact with proteins both in covalent and noncovalent modes

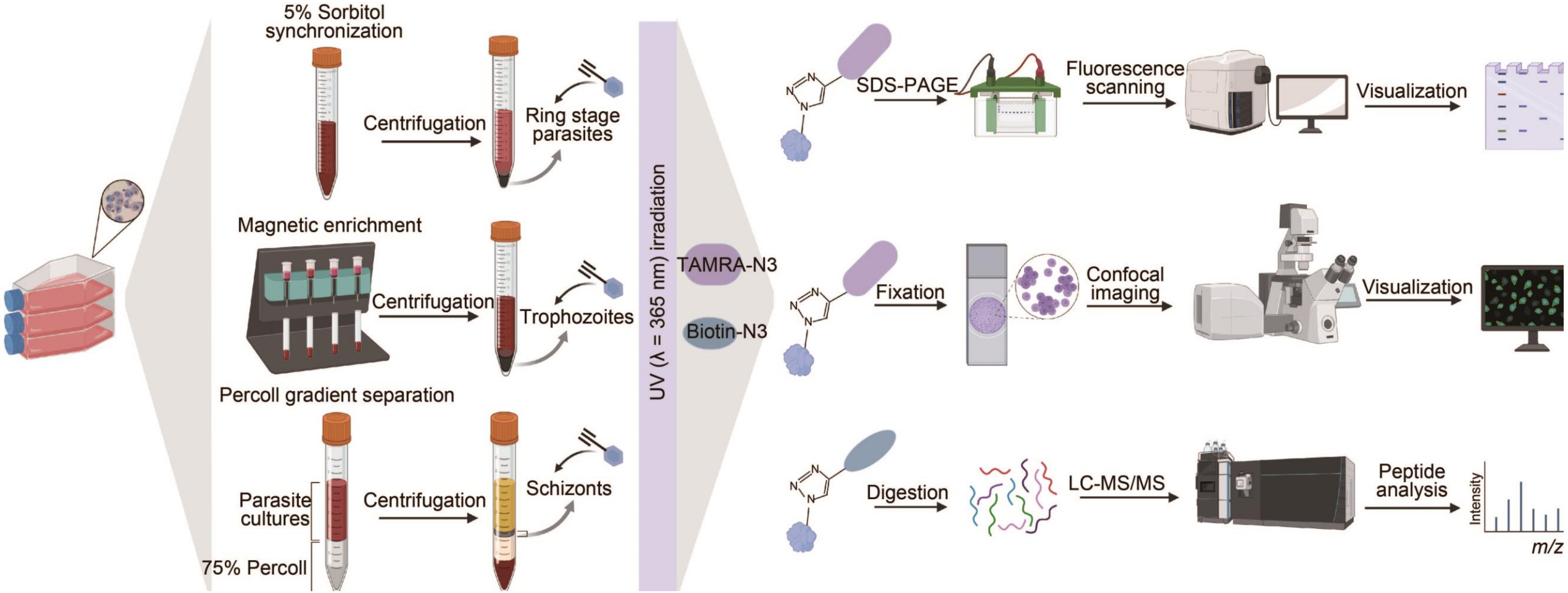


Interfering with the protein synthesis, glycolysis and oxidative homeostasis

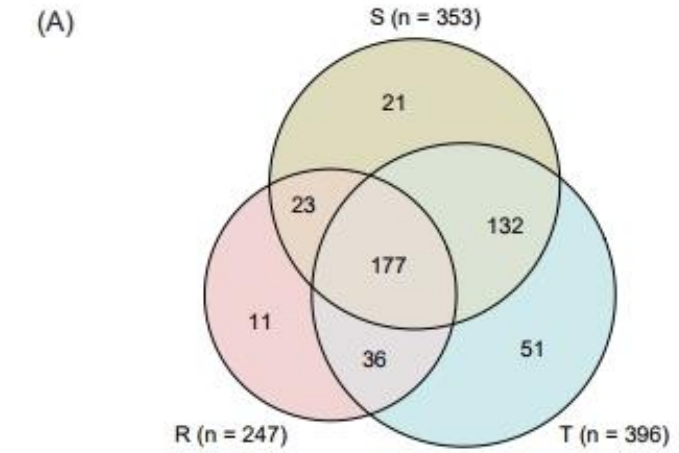


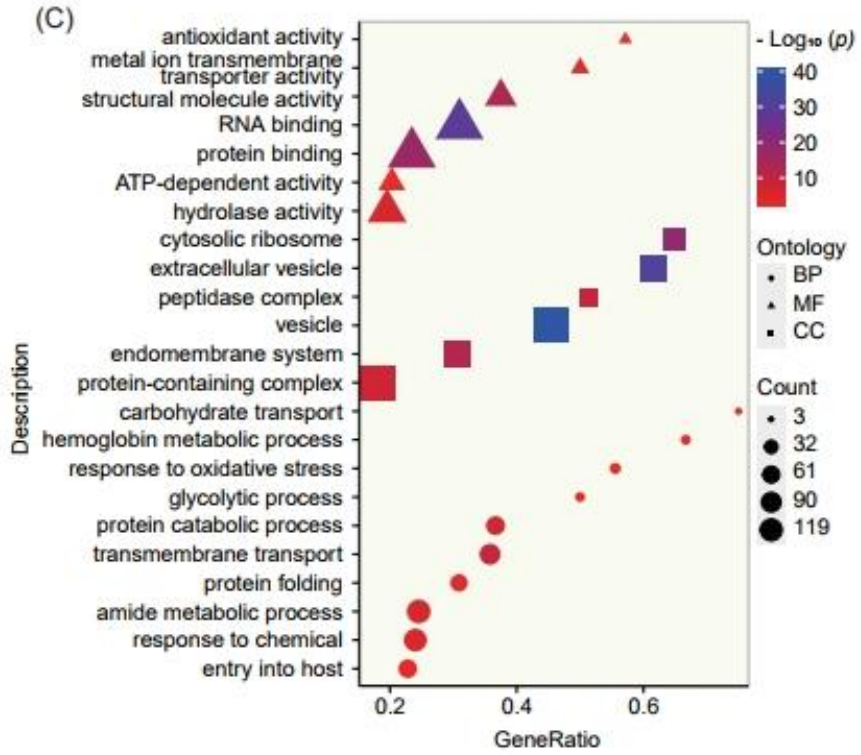
Providing fresh insights into the mechanisms underlying artemisinin's antimalarial effects and its protein targets

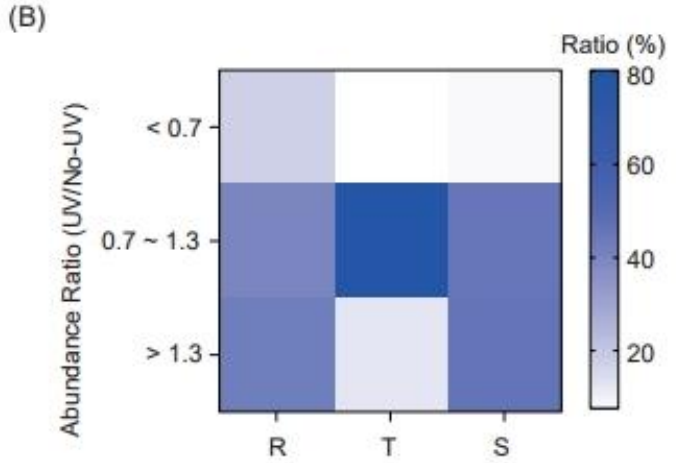
General workflow

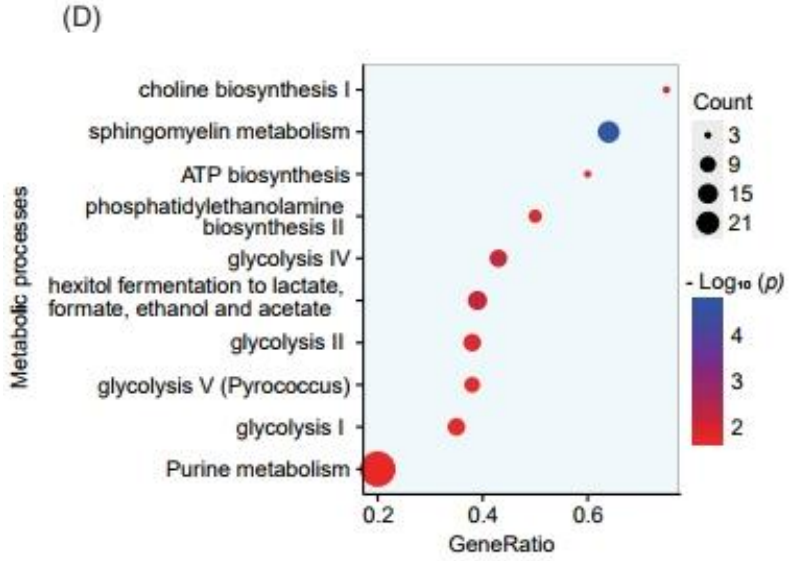


Results

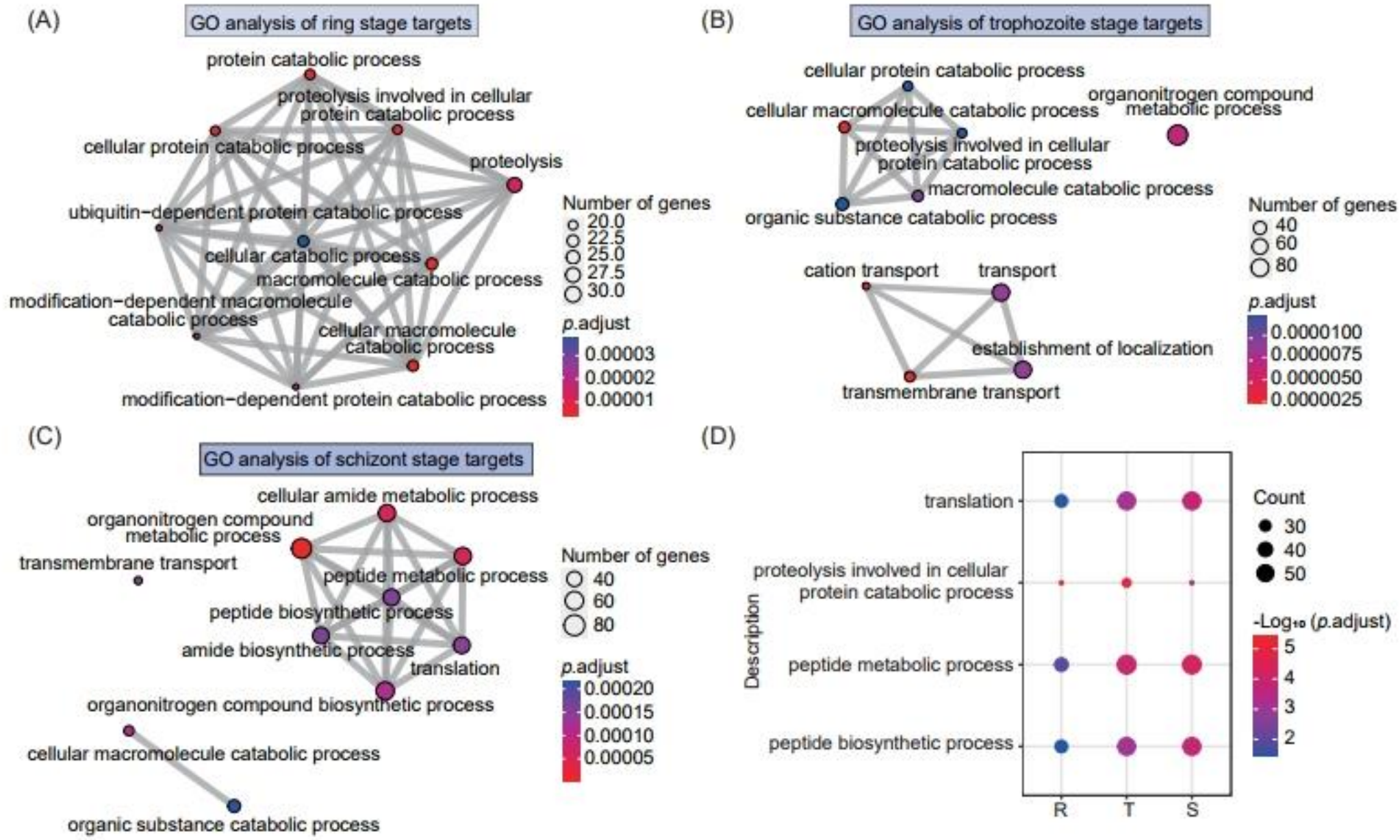

(A)
S (n = 353)
21
23
132
177
11
36
51
R (n = 247)
T (n = 396)


(C)
antioxidant activity
metal ion transmembrane transporter activity
structural molecule activity
RNA binding
protein binding
ATP-dependent activity
hydrolase activity
cytosolic ribosome
extracellular vesicle
peptidase complex
vesicle
endomembrane system
protein-containing complex
carbohydrate transport
hemoglobin metabolic process
response to oxidative stress
glycolytic process
protein catabolic process
transmembrane transport
protein folding
amide metabolic process
response to chemical
entry into host
Description
0.2
0.4
0.6
GeneRatio
- Log10 (p)
40
30
20
10
Ontology
BP
MF
CC
Count
3
32
61
90
119

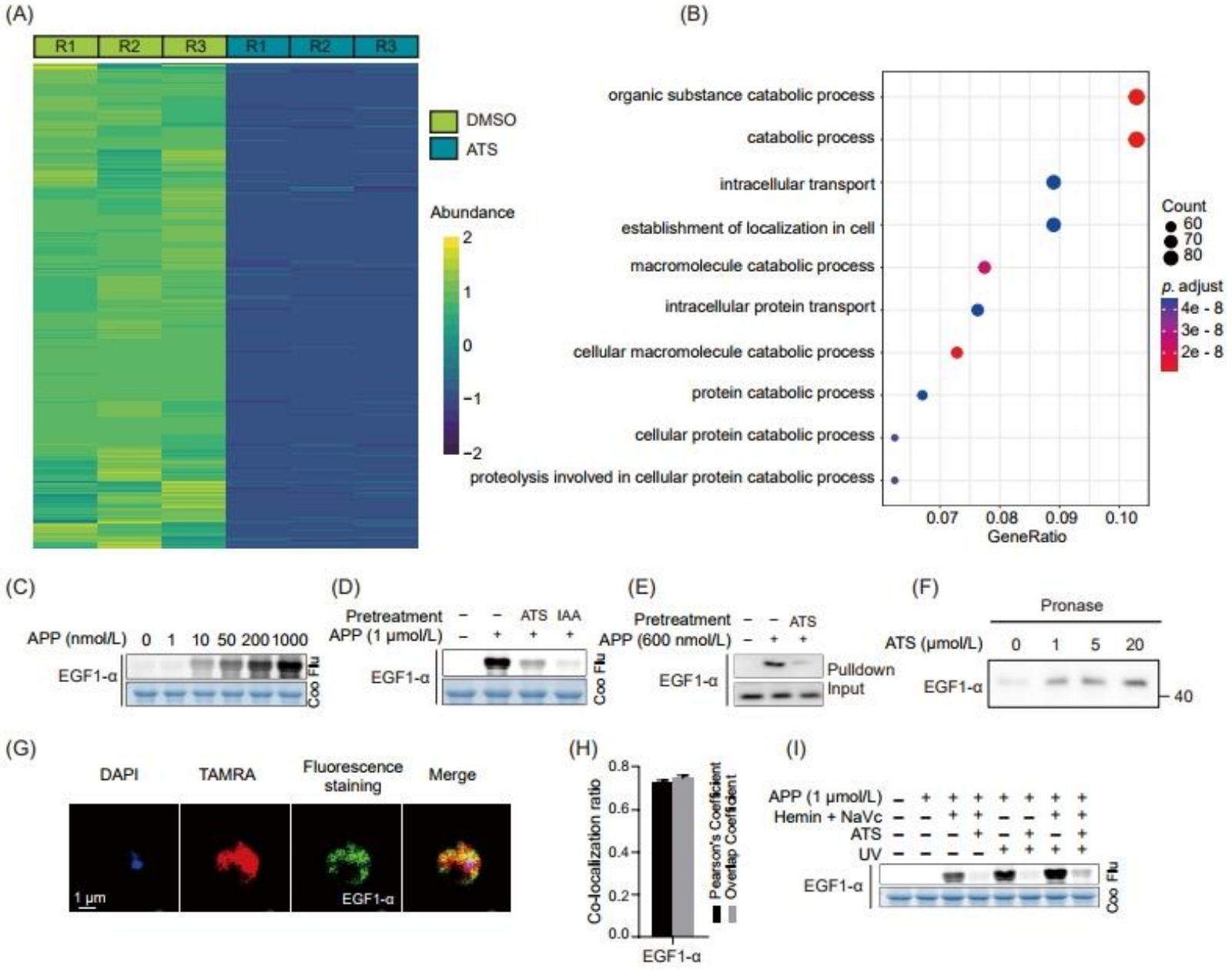

(B)
Ratio (%)
80
60
40
20
Abundance Ratio (UV/No-UV)
< 0.7
0.7 ~ 1.3
> 1.3
R
T
S


(D)
choline biosynthesis I
sphingomyelin metabolism
ATP biosynthesis
phosphatidylethanolamine biosynthesis II
glycolysis IV
hexitol fermentation to lactate, formate, ethanol and acetate
glycolysis II
glycolysis V (Pyrococcus)
glycolysis I
Purine metabolism
Metabolic processes
0.2
0.4
0.6
GeneRatio
Count
3
9
15
21
- Log10 (p)
4
3
2

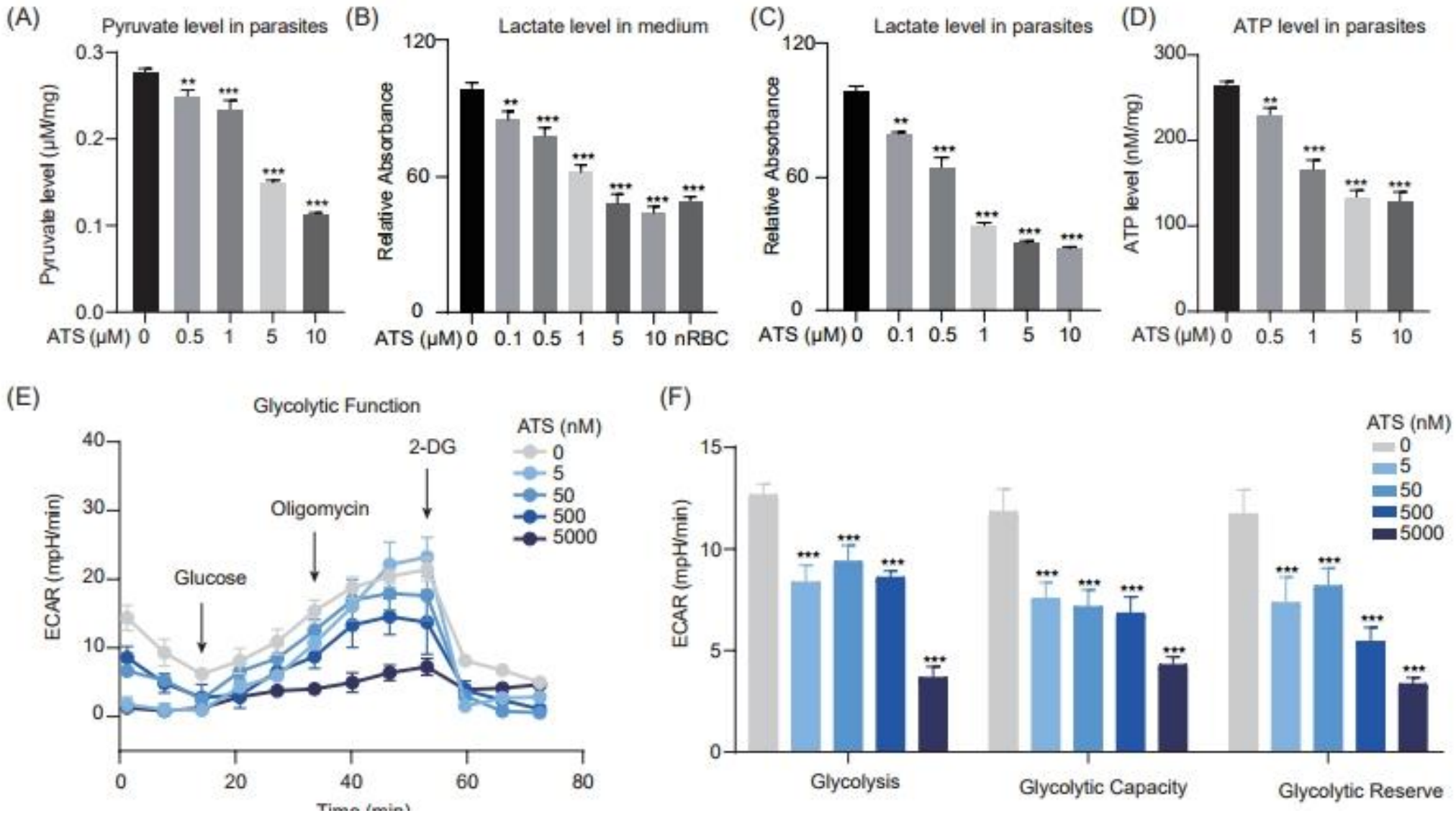
Results


(A)
GO analysis of ring stage targets
protein catabolic process
proteolysis involved in cellular protein catabolic process
cellular protein catabolic process
proteolysis
ubiquitin−dependent protein catabolic process
cellular catabolic process
macromolecule catabolic process
modification−dependent macromolecule catabolic process
cellular macromolecule catabolic process
modification−dependent protein catabolic process
Number of genes
20.0
22.5
25.0
27.5
30.0
p.adjust
0.00003
0.00002
0.00001
(B)
GO analysis of trophozoite stage targets
cellular protein catabolic process
cellular macromolecule catabolic process
organonitrogen compound metabolic process
proteolysis involved in cellular protein catabolic process
macromolecule catabolic process
organic substance catabolic process
cation transport
transport
establishment of localization
transmembrane transport
Number of genes
40
60
80
p.adjust
0.0000100
0.0000075
0.0000050
0.0000025
(C)
GO analysis of schizont stage targets
cellular amide metabolic process
organonitrogen compound metabolic process
transmembrane transport
peptide metabolic process
peptide biosynthetic process
amide biosynthetic process
translation
organonitrogen compound biosynthetic process
cellular macromolecule catabolic process
organic substance catabolic process
Number of genes
40
60
80
p.adjust
0.00020
0.00015
0.00010
0.00005
(D)
translation
proteolysis involved in cellular protein catabolic process
peptide metabolic process
peptide biosynthetic process
Description
R
T
S
Count
30
40
50
-Log10 (p.adjust)
5
4
3
2

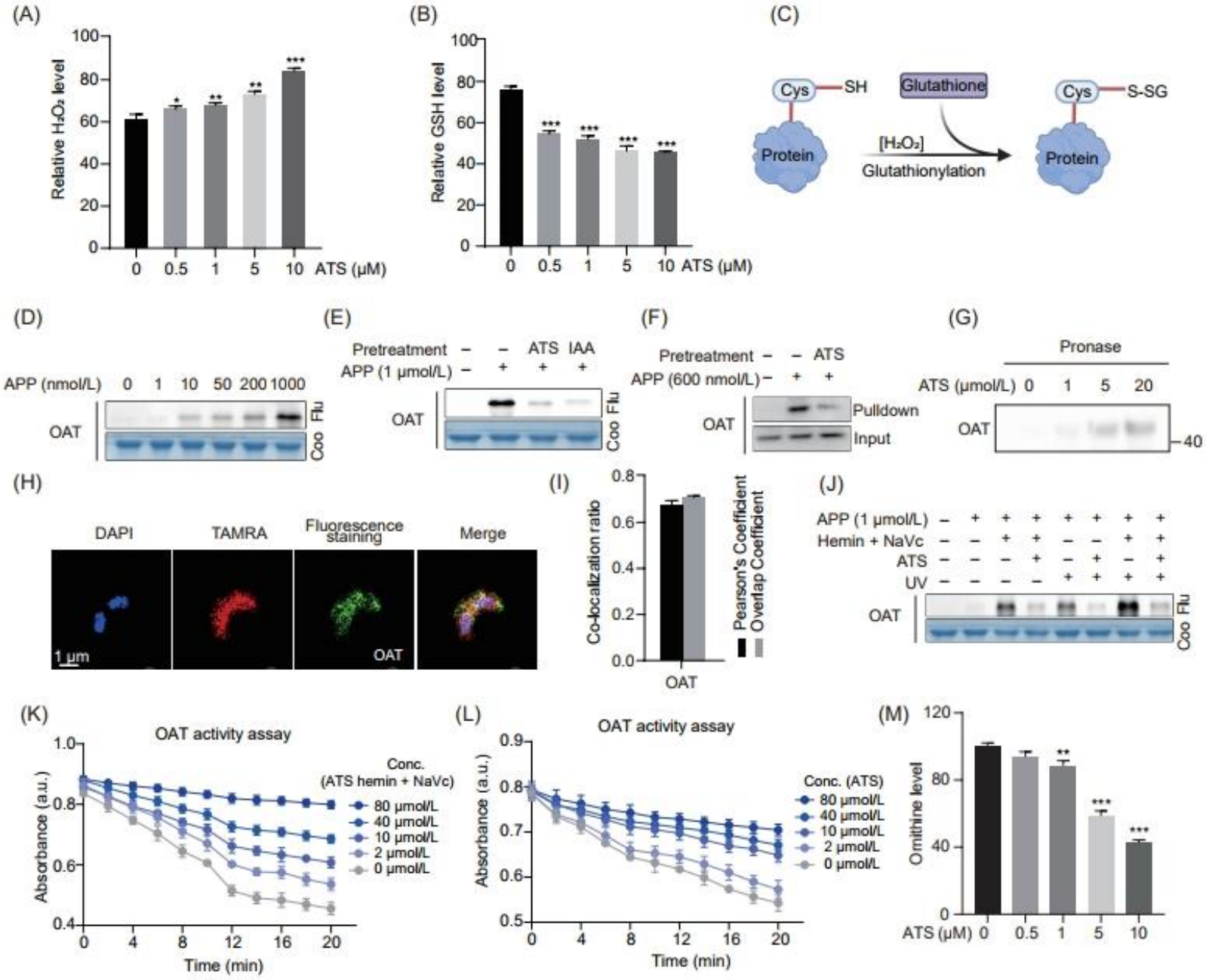
Results


(A)
R1
R2
R3
R1
R2
R3
DMSO
ATS
Abundance
2
1
0
−1
−2
(B)
organic substance catabolic process
catabolic process
intracellular transport
establishment of localization in cell
macromolecule catabolic process
intracellular protein transport
cellular macromolecule catabolic process
protein catabolic process
cellular protein catabolic process
proteolysis involved in cellular protein catabolic process
0.07
0.08
0.09
0.10
GeneRatio
Count
60
70
80
p. adjust
4e - 8
3e - 8
2e - 8
(C)
APP (nmol/L) 0 1 10 50 200 1000
EGF1-α
Coo Flu
(D)
Pretreatment – – ATS IAA
APP (1 μmol/L) – + + +
EGF1-α
Coo Flu
(E)
Pretreatment – – ATS
APP (600 nmol/L) – + +
EGF1-α
Pulldown
Input
(F)
Pronase
ATS (μmol/L) 0 1 5 20
EGF1-α
40
(G)
DAPI
TAMRA
Fluorescence staining
Merge
1 μm
EGF1-α
(H)
Co-localization ratio
0.8
0.6
0.4
0.2
0.0
Pearson's Coefficient
Overlap Coefficient
EGF1-α
(I)
APP (1 μmol/L) – + + + + + + +
Hemin + NaVc – – + + – – + +
ATS – – – + – + – +
UV – – – – + + + +
EGF1-α
Coo Flu

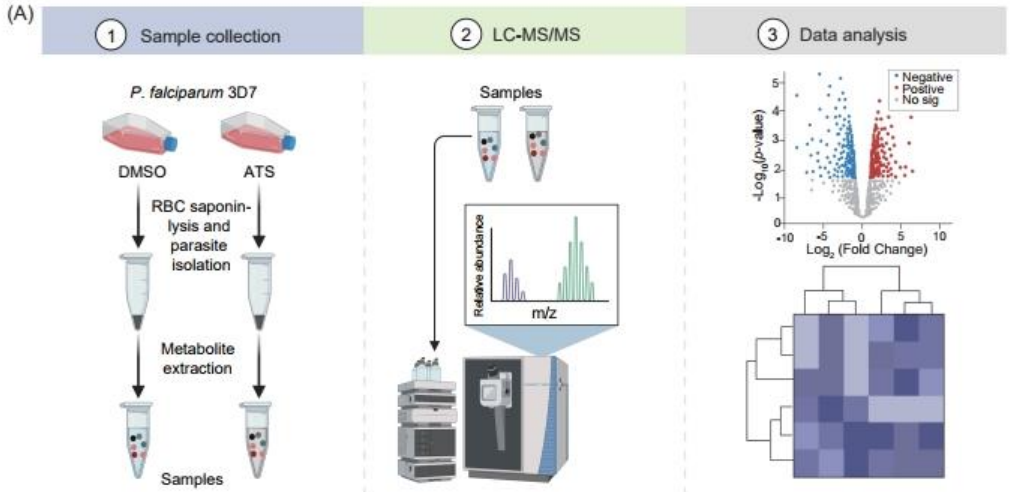
Results

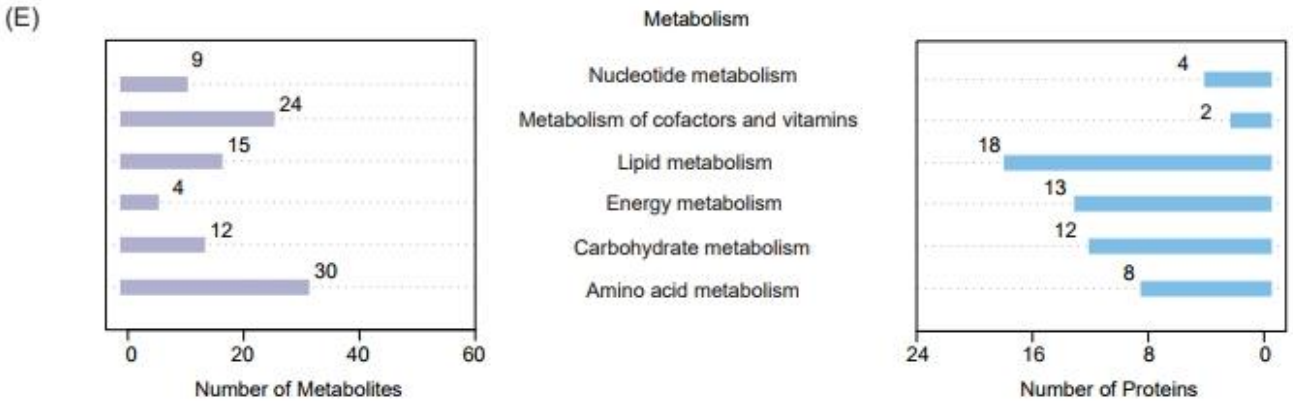

(A)
Pyruvate level in parasites
Pyruvate level (μM/mg)
0.3
0.2
0.1
0.0
ATS (μM) 0 0.5 1 5 10
(B)
Lactate level in medium
Relative Absorbance
120
60
0
ATS (μM) 0 0.1 0.5 1 5 10 nRBC
(C)
Lactate level in parasites
Relative Absorbance
120
60
0
ATS (μM) 0 0.1 0.5 1 5 10
(D)
ATP level in parasites
ATP level (nM/mg)
300
200
100
0
ATS (μM) 0 0.5 1 5 10
(E)
Glycolytic Function
ECAR (mpH/min)
40
30
20
10
0
Glucose
Oligomycin
2-DG
ATS (nM)
0
5
50
500
5000
0 20 40 60 80
Time (min)
(F)
ECAR (mpH/min)
15
10
5
0
ATS (nM)
0
5
50
500
5000
Glycolysis
Glycolytic Capacity
Glycolytic Reserve

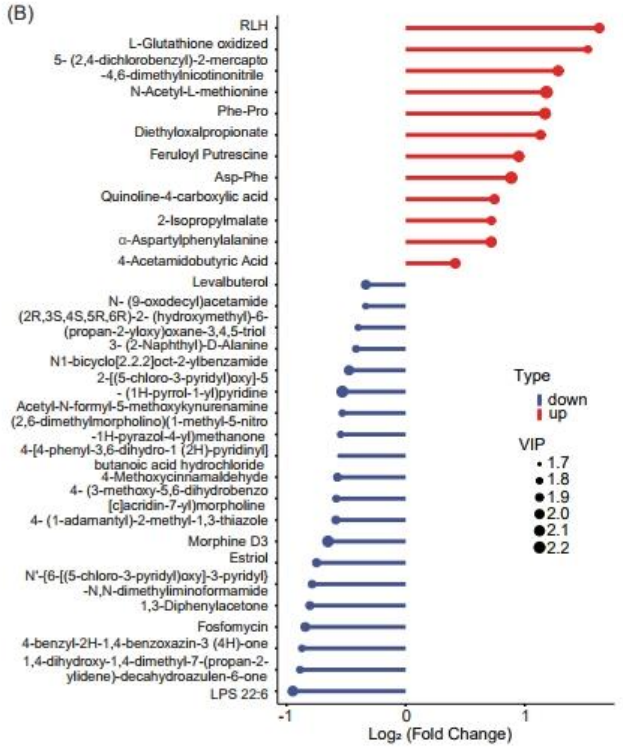
Results

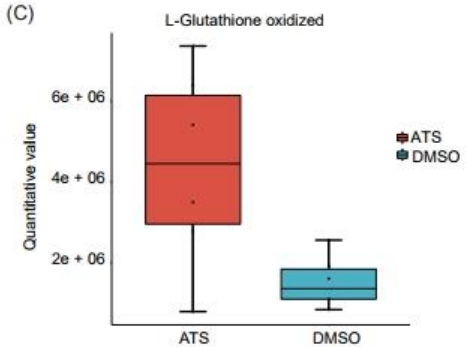

(A)
Relative H2O2 level
ATS (μM)
(B)
Relative GSH level
ATS (μM)
(C)
Cys
SH
Protein
Glutathione
[H2O2]
Glutathionylation
Cys
S-SG
Protein
(D)
APP (nmol/L) 0 1 10 50 200 1000
OAT
Coo Flu
(E)
Pretreatment – – ATS IAA
APP (1 μmol/L) – + + +
OAT
Coo Flu
(F)
Pretreatment – – ATS
APP (600 nmol/L) – + +
OAT
Pulldown
Input
(G)
Pronase
ATS (μmol/L) 0 1 5 20
OAT
40
(H)
DAPI
TAMRA
Fluorescence staining
Merge
1 μm
OAT
(I)
Co-localization ratio
Pearson's Coefficient
Overlap Coefficient
OAT
(J)
APP (1 μmol/L)
Hemin + NaVc
ATS
UV
OAT
Coo Flu
(K)
OAT activity assay
Absorbance (a.u.)
Time (min)
Conc. (ATS hemin + NaVc)
80 μmol/L
40 μmol/L
10 μmol/L
2 μmol/L
0 μmol/L
(L)
OAT activity assay
Absorbance (a.u.)
Time (min)
Conc. (ATS)
80 μmol/L
40 μmol/L
10 μmol/L
2 μmol/L
0 μmol/L
(M)
Ornithine level
ATS (μM) 0 0.5 1 5 10

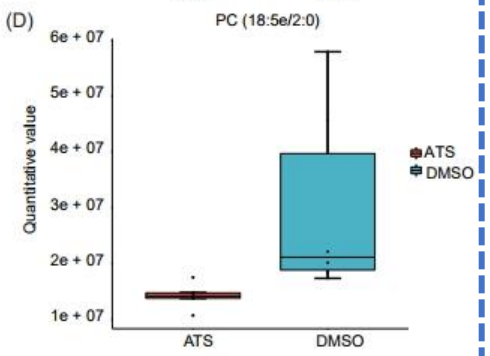
Results











Extensively interfere with multiple metabolic pathways of intraerythrocytic parasites, which may directly or indirectly converge on the collective antimalarial effects of ART.

Summary

- In this work, we comprehensively identified the protein targets of artemisinin at the ring, trophozoite, and schizont stages of *P. falciparum* in situ using an active photoaffinity probe, and investigated the binding mode.

- Our results suggest that ART may exert its antimalarial effect by blocking parasite protein synthesis, interfering with the glycolytic energy supply pathway, and disrupting redox-related processes.

- This work provides new insights for the study of the antimalarial mechanism of artemisinin, which is necessary to optimize current antimalarial drug regimens and offer novel solutions to mitigate artemisinin resistance.

Gao, Peng, Jianyou Wang, Chong Qiu, Huimin Zhang, Chen Wang, Ying Zhang, Peng Sun, et al. 2024. "Photoaffinity Probe-Based Antimalarial Target Identification of Artemisinin in the Intraerythrocytic Developmental Cycle of Plasmodium falciparum." iMeta e176. https://doi.org/10.1002/imt2.176

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