

## ORIGINAL RESEARCH ARTICLE

# *In silico* evaluation of heat shock proteins reveals an interplay with polyamines as a survival strategy for the *Plasmodium falciparum*

## Supplementary Files

### Supplementary S1. Bioinformatics databases and tools used in this study

Bioinformatics tool	Website address	Tool use
PlasmoDB	<a href="https://plasmodb.org/plasmo/app">https://plasmodb.org/plasmo/app</a>	Sequence retrieval of <i>Plasmodium falciparum</i> 3D7 HSPs
NCBI	<a href="https://www.ncbi.nlm.nih.gov/">https://www.ncbi.nlm.nih.gov/</a>	Sequence retrieval of <i>Escherichia coli</i> and <i>Saccharomyces cerevisiae</i>
Bioedit	<a href="http://bioedit.software.informer.com">bioedit.software.informer.com</a>	Global alignment
Phyre2	<a href="http://www.sbg.bio.ic.ac.uk/phyre2">http://www.sbg.bio.ic.ac.uk/phyre2</a>	Prediction of 3D structures
PyMol	<a href="http://pymol.org/academic">http://pymol.org/academic</a>	Viewing structures
PDBSum	<a href="https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html">https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html</a>	Structure validation
SiteMap	<a href="https://www.schrodinger.com/products/sitemap">https://www.schrodinger.com/products/sitemap</a>	Identification of binding sites of the HSPs
PubChem	<a href="https://pubchem.ncbi.nlm.nih.gov/">https://pubchem.ncbi.nlm.nih.gov/</a>	Retrieval of ligands (Putrescine, Spermidine, and Spermine)
QM Conformer and Tautomer Predictor	<a href="https://forum.knime.com/t/qm-conformer-tautomer-predictor/26153">https://forum.knime.com/t/qm-conformer-tautomer-predictor/26153</a>	To find the lowest energy tautomers or conformers for the ligands
OPLS4	<a href="https://www.schrodinger.com/products/opls4">https://www.schrodinger.com/products/opls4</a>	Molecular Docking
Desmond Molecular Dynamics System	<a href="https://www.schrodinger.com/products/desmond">https://www.schrodinger.com/products/desmond</a>	Molecular Dynamics Simulations

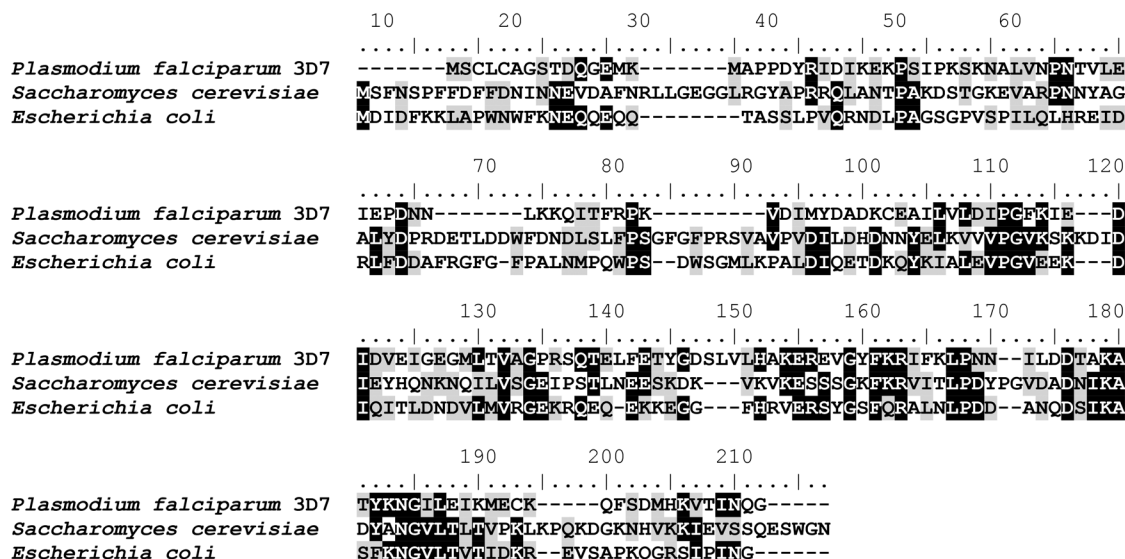
**Supplementary S2. Number of Na<sup>+</sup> or Cl<sup>-</sup> counterions used assigned to each system for neutralization during molecular docking**

Protein	Counterions
HSP20	3 Na <sup>+</sup>
HSP40	5 Na <sup>+</sup>
HSP60	0
HSP70	7 Na <sup>+</sup>
HSP90	10 Na <sup>+</sup>
HSP20-Put	1 Na <sup>+</sup>
HSP20-Spd	0
HSP20-Spn	1 Cl <sup>-</sup>
HSP40-Put	3 Na <sup>+</sup>
HSP40-Spd	2 Na <sup>+</sup>
HSP40-Spn	1 Na <sup>+</sup>
HSP60-Put	2 Cl <sup>-</sup>
HSP60-Spd	3 Cl <sup>-</sup>
HSP60-Spn	4 Cl <sup>-</sup>
HSP70-Put	5 Na <sup>+</sup>
HSP70-Spd	4 Na <sup>+</sup>
HSP70-Spn	3 Na <sup>+</sup>
HSP90-Put	8 Na <sup>+</sup>
HSP90-Spd	7 Na <sup>+</sup>
HSP90-Spn	6 Na <sup>+</sup>

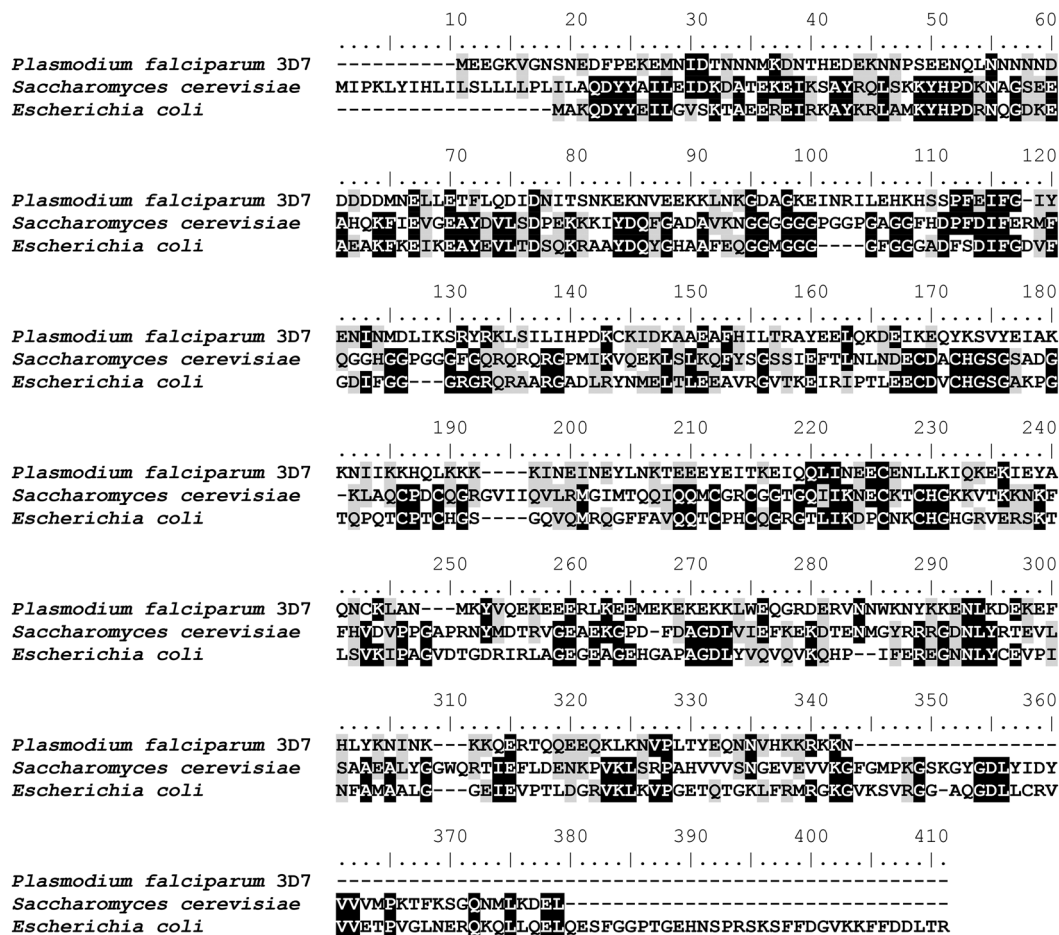
**Supplementary S3. Genomic characterization of *Plasmodium falciparum* 3D7 strain major heat shock proteins**

Protein	Accession #	Genomic locus	Gene ID	Exons	Introns
HSP20	XP_001349359.1	Chromosome: 8; NC_004329.3	2655428	3	2
HSP40	XP_001349799.1	Chromosome: 13; NC_004331.3	814015	1	-
HSP60	CZT98408.1	Chromosome: 10; NC_037281.1	810311	2	1
HSP70	XP_001349336.1	Chromosome: 8; NC_004329.3	2655254	1	-
HSP90	XP_001348998.1	Chromosome: 7; NC_004328.3	2655065	2	1

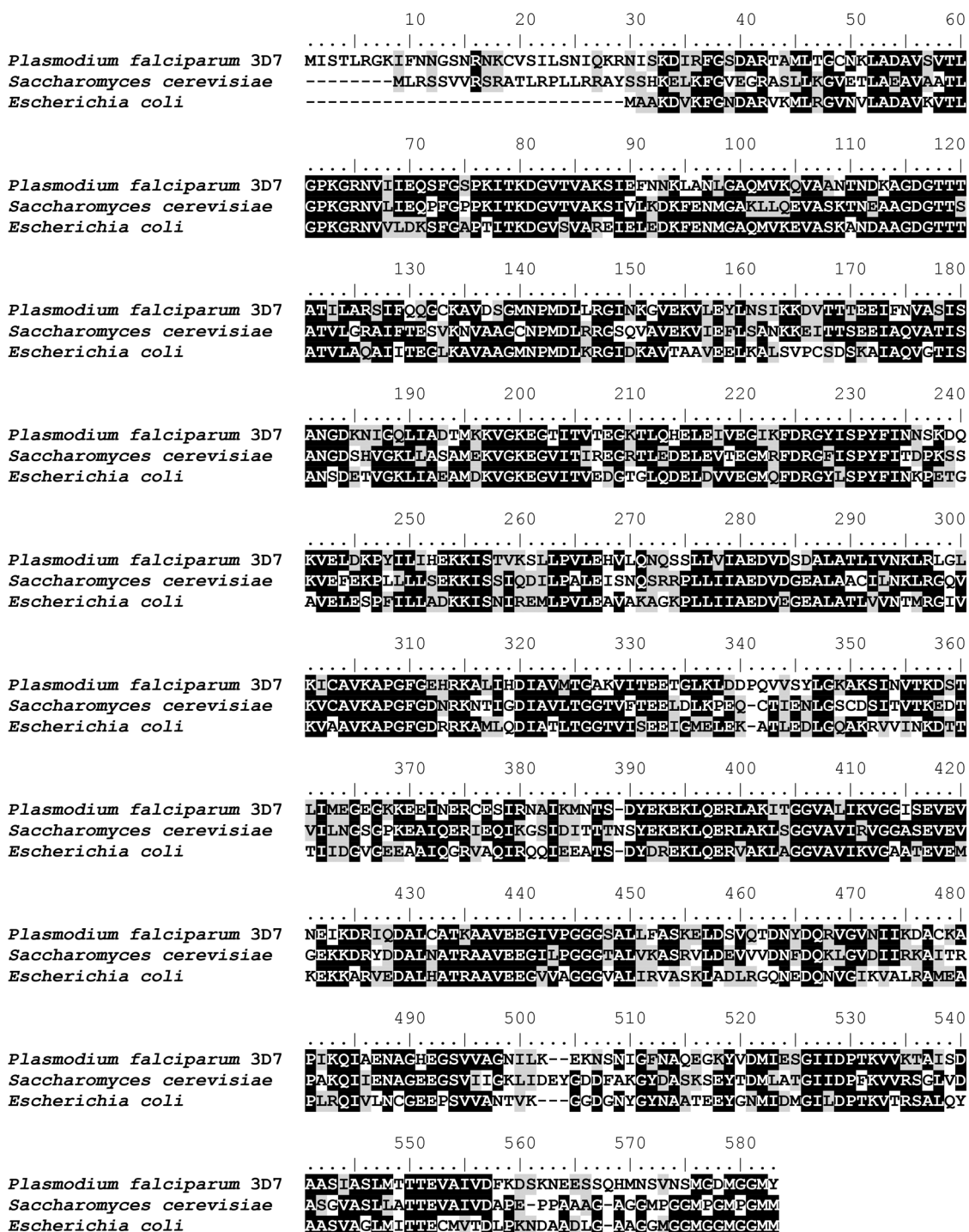
**Supplementary 4. Multiple sequence alignment of *Plasmodium falciparum* HSP 20, 40, 60, 70, and 90 with *Saccharomyces cerevisiae* and *Escherichia coli*.**



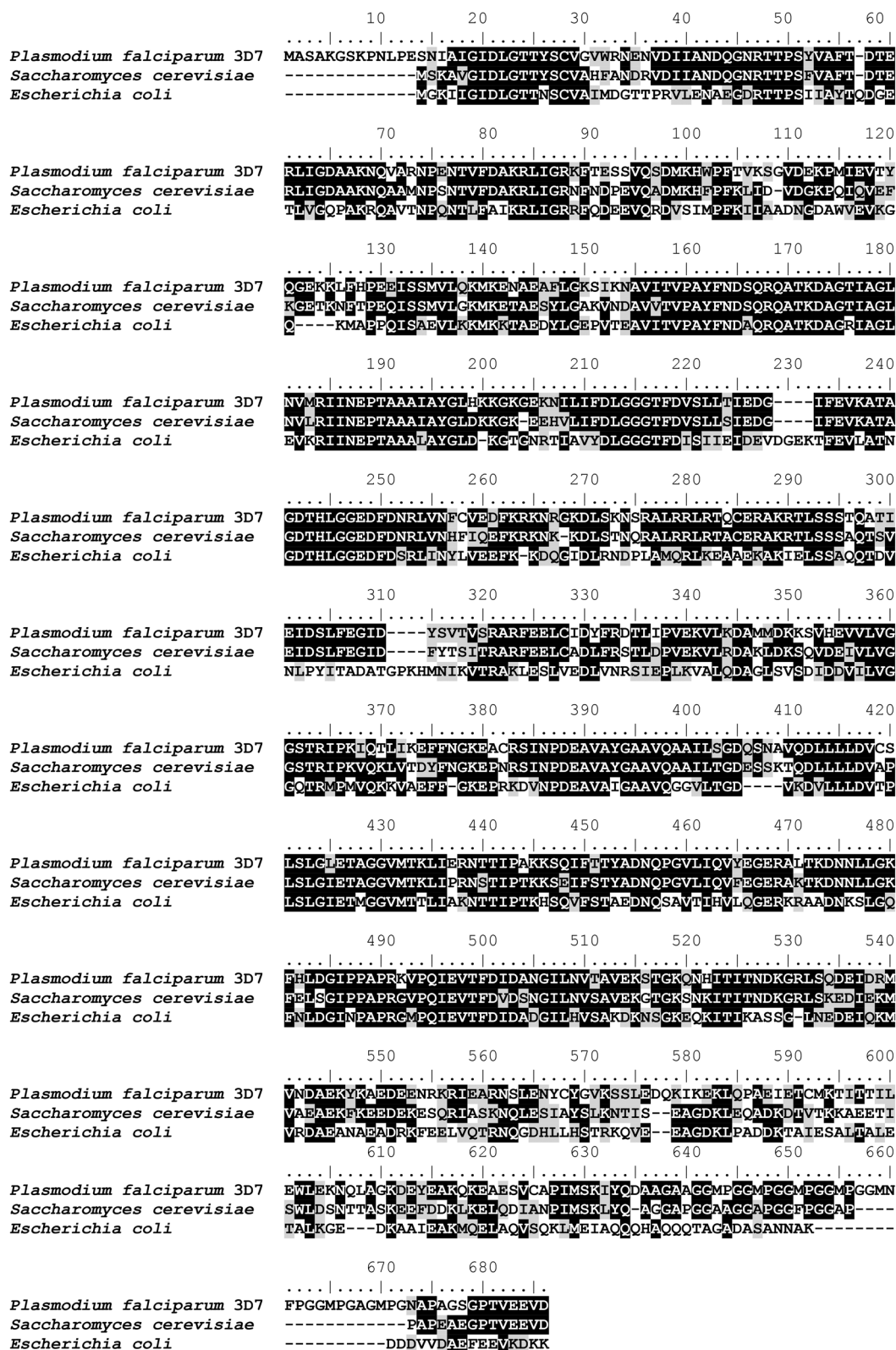
Multiple sequence alignment of HSP20 in *P. falciparum* 3D7, *S. cerevisiae*, and *E. coli* indicating identical (black), similar (grey), and non-identical (white) residues.



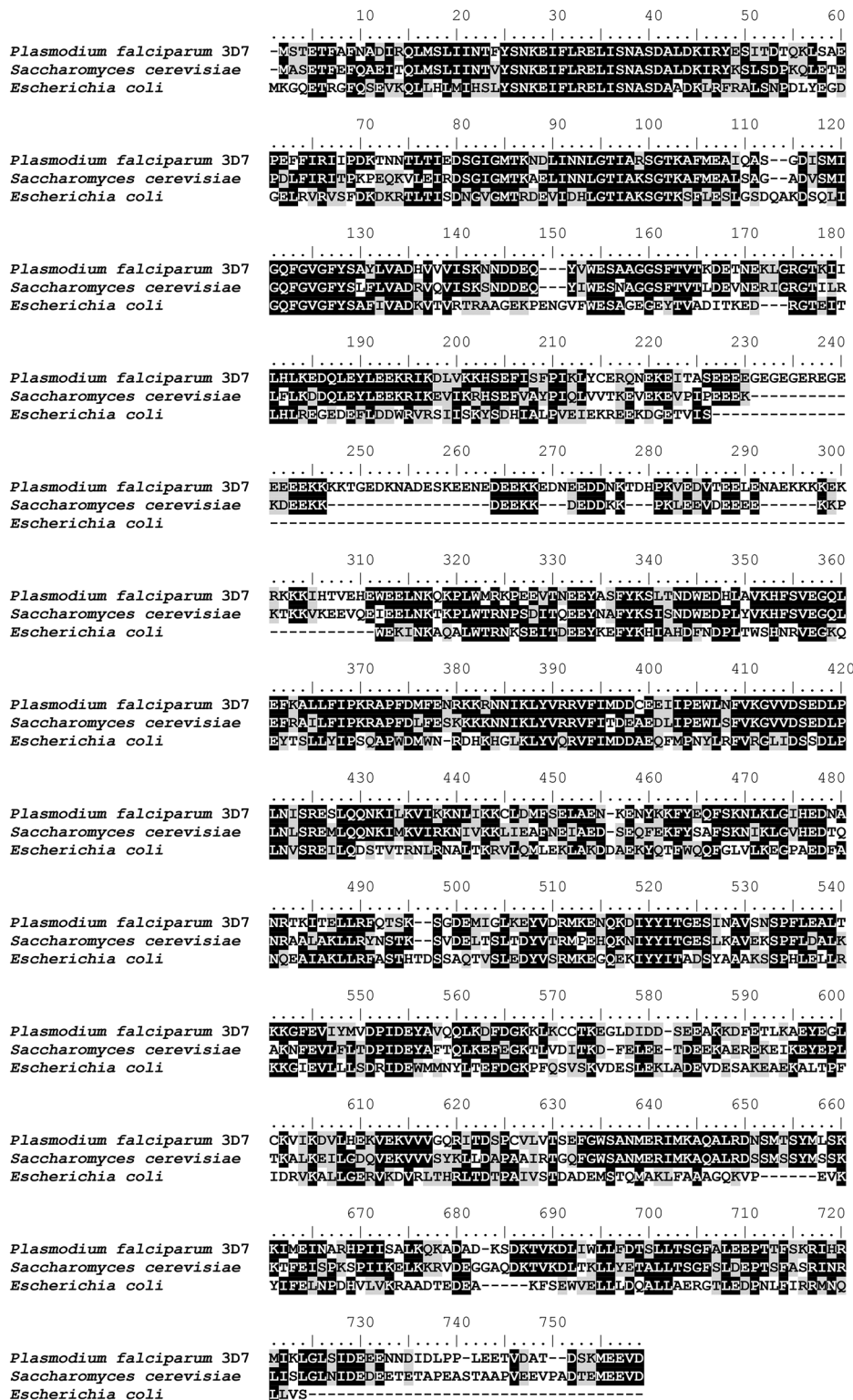
Multiple sequence alignment of HSP40 in *P. falciparum* 3D7, *S. cerevisiae*, and *E. coli* indicating identical (black), similar (grey), and nonidentical (white) residues.



Multiple sequence alignment of HSP60 in *P. falciparum* 3D7, *S. cerevisiae*, and *E. coli* indicating identical (black), similar (grey), and nonidentical (white) residues.



Multiple sequence alignment of HSP70 in *P. falciparum* 3D7, *S. cerevisiae*, and *E. coli* indicating identical (black), similar (grey), and nonidentical (white) residues.



Multiple sequence alignment of HSP90 in *P. falciparum* 3D7, *S. cerevisiae*, and *E. coli* indicating identical (black), similar (grey), and nonidentical (white) residues.

**Supplementary S5. SiteScore, docking score, and volume of *Plasmodium falciparum* 3D7 selected heat shock proteins obtained for the identified active sites obtained from SiteMap**

<b>Protein</b>	<b>Site</b>	<b>SiteScore</b>	<b>DScore</b>	<b>Volume</b>
HSP20	1	1.028	0.990	543.098
	2	0.888	0.758	149.419
	3	0.772	0.774	151.049
	4	0.672	0.595	130.297
HSP40	1	1.051	1.033	2112.623
	2	0.970	0.896	251.934
	3	0.863	0.868	115.977
	4	0.796	0.658	118.549
	5	0.712	0.518	86.951
HSP60	1	1.156	1.014	268.998
	2	1.069	0.958	243.873
	3	1.018	0.978	1547.659
	4	0.988	0.954	321.605
	5	0.978	0.928	148.519
HSP70	1	1.123	0.866	502.066
	2	1.067	0.940	1224.982
	3	1.059	0.877	273.028
	4	0.976	1.027	267.454
	5	0.966	0.953	377.300
HSP90	1	1.092	0.898	488.775
	2	1.025	0.932	242.887
	3	1.024	1.035	196.496
	4	1.023	0.925	453.446
	5	0.874	0.879	203.999