Chemotherapeutic efficacies of a clofazimine and diminazene aceturate combination against piroplasm parasites and their AT-rich DNA-binding activity on *Babesia bovis*

Bumduuren Tuvshintulga[1,2], Mahmoud AbouLaila[1,3], Thillaiampalam Sivakumar[1], Dickson Stuart Tayebwa[1], Sambuu Gantuya[1,4], Khandsuren Naranbaatar[4], Aki Ishiyama[5], Masato Iwatsuki[5], Kazuhiko Otoguro[5], Satoshi Ōmura[5], Mohamad Alaa Terkawi[1,6], Azirwan Guswanto[1], Mohamed Abdo Rizk[1,7], Naoaki Yokoyama[1], Ikuo Igarashi[1]*

[1]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan. [2]Laboratory of Molecular Genetics, Institute of Veterinary Medicine, Ulaanbaatar, Mongolia. [3]Department of Parasitology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32511, Minoufiya, Egypt. [4]Laboratory of Arachno-Entomology and Protozoology, Institute of Veterinary Medicine, Ulaanbaatar, Mongolia. [5]Research Center for Tropical Diseases, Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan. [6]Frontier Research Center for Advanced Material and Life Science, Department of Orthopedic Surgery, School of Medicine, Hokkaido University, Kita 21, Nishi 11, Kita-ku, Sapporo, Hokkaido 001-0021, Japan. [7]Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansura 35516, Dakahlia, Egypt

*Corresponding author: Professor Ikuo Igarashi DVM, PhD,
National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan
E-mail address: igarcpmi@obihiro.ac.jp; Tel.: +81-155-49-5641 (Fax: -5643)

Supplementary information

Table S1. Percentage of nucleic acid contents in genes of *B. bovis*.

Organelles	Genes	A	T	C	G	AT	CG	Total
Nucleus	*18S rRNA*	23%	27%	23%	27%	50%	50%	100%
	Tubulin beta chain	30%	21%	26%	23%	51%	49%	100%
	Arm/cat*	24%	30%	21%	25%	54%	46%	100%
Mitochondria	*cob*	26%	41%	14%	19%	68%	32%	100%
	cox3	31%	40%	13%	15%	71%	29%	100%
Apicoplast	*tufA*	39%	31%	18%	13%	69%	31%	100%
	clpC	43%	37%	10%	10%	79%	21%	100%

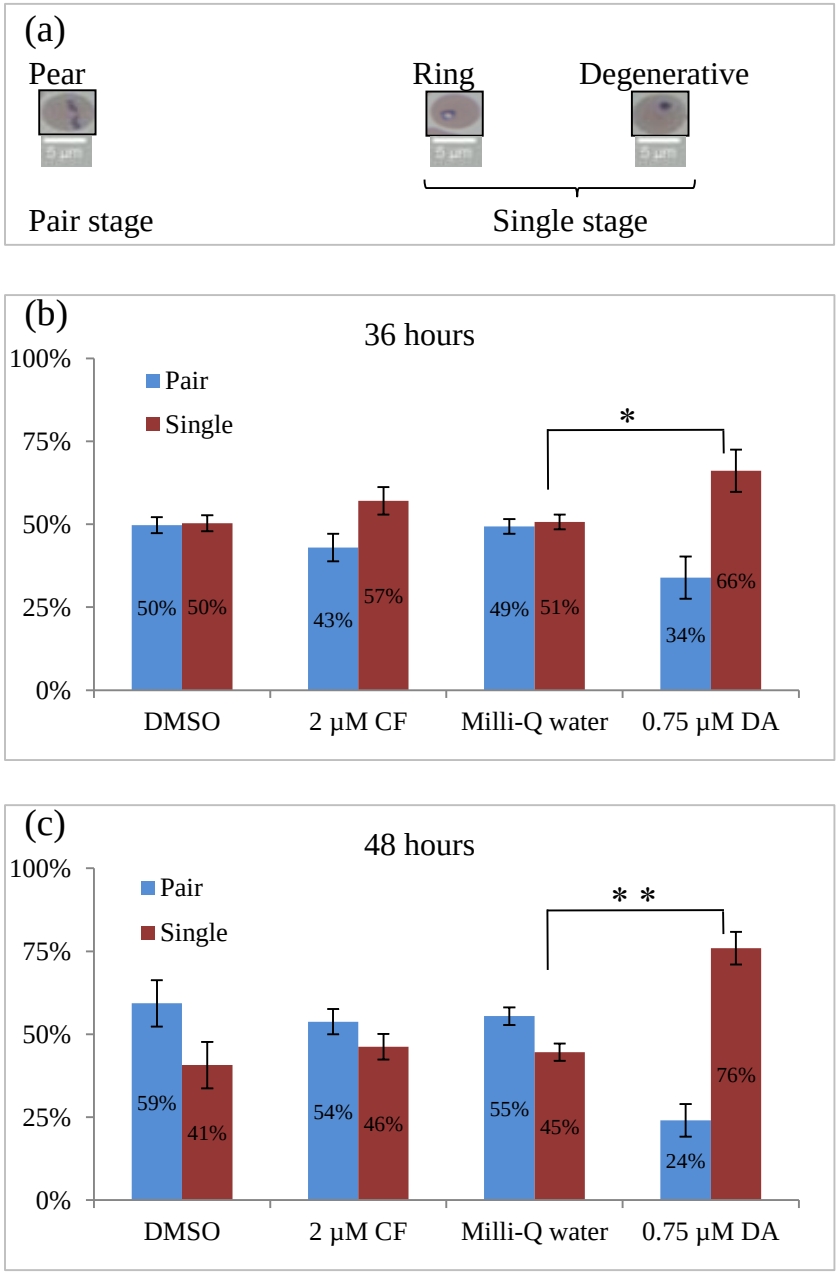
* Armadillo/beta-catenin-like repeat domain containing protein

Table S2. Primers used in this study for qPCR.

Genes	Sequences	Description
18S rRNA	GGACGCCTCGTTACTTTGAGA AGGCGAAACCTGCTTGAAAC	Internal control
Tubulin beta chain	CCTTATCCCCTTCCCCAGGT AGTGGTGCGAATCCGATCAT	Chromosomal gene
Arm/cat*	TTCGTGAACAGGCTGTTTGG CGGGAGAGTCACCTGCGAT	Chromosomal gene
cob	GGTTGGGCAATGCGTTATTT ATGTAGCATCATGAAAAAGAAGCAA	Mitochondrial gene encoding cytochrome b
cox3	TGATGGTTCAAATAGAGCAGAAGATT GTTAAAGCTACCCAGATTAATTCAACAA	Mitochondrial gene of cytochrome c oxidase III
tufA	AAAATATGATAACTGGTGCTGTACAAATG GCATAGGACCGTCTGTTAAAGAAATA	Apicoplast gene for elongation factor
clpC	GTTGTAAAGAGCATAAACAAGTCGTTTT TGCCCCACTAGGACCACAAA	Apicoplast gene encoding chaperon protein

*Armadillo/beta-catenin-like repeat domain containing protein

Figure S1. Development stages of CF– or DA–treated *B. bovis*. Pair and single cell stages were counted within 400 iRBCs. (a) Pair-cell stage is considered to be a pear form of parasites, and single-cell stage is considered to be ring and degenerative forms of parasites. (b) Percentage of pair- or single-cell stages at 36 hours. (c) Percentage of pair- or single-cell stages at 48 hours. Asterisks indicate statistically significant ($P < 0.01$** or 0.05*) differences of percentages based on unpaired *t*-test analysis.



36 hours

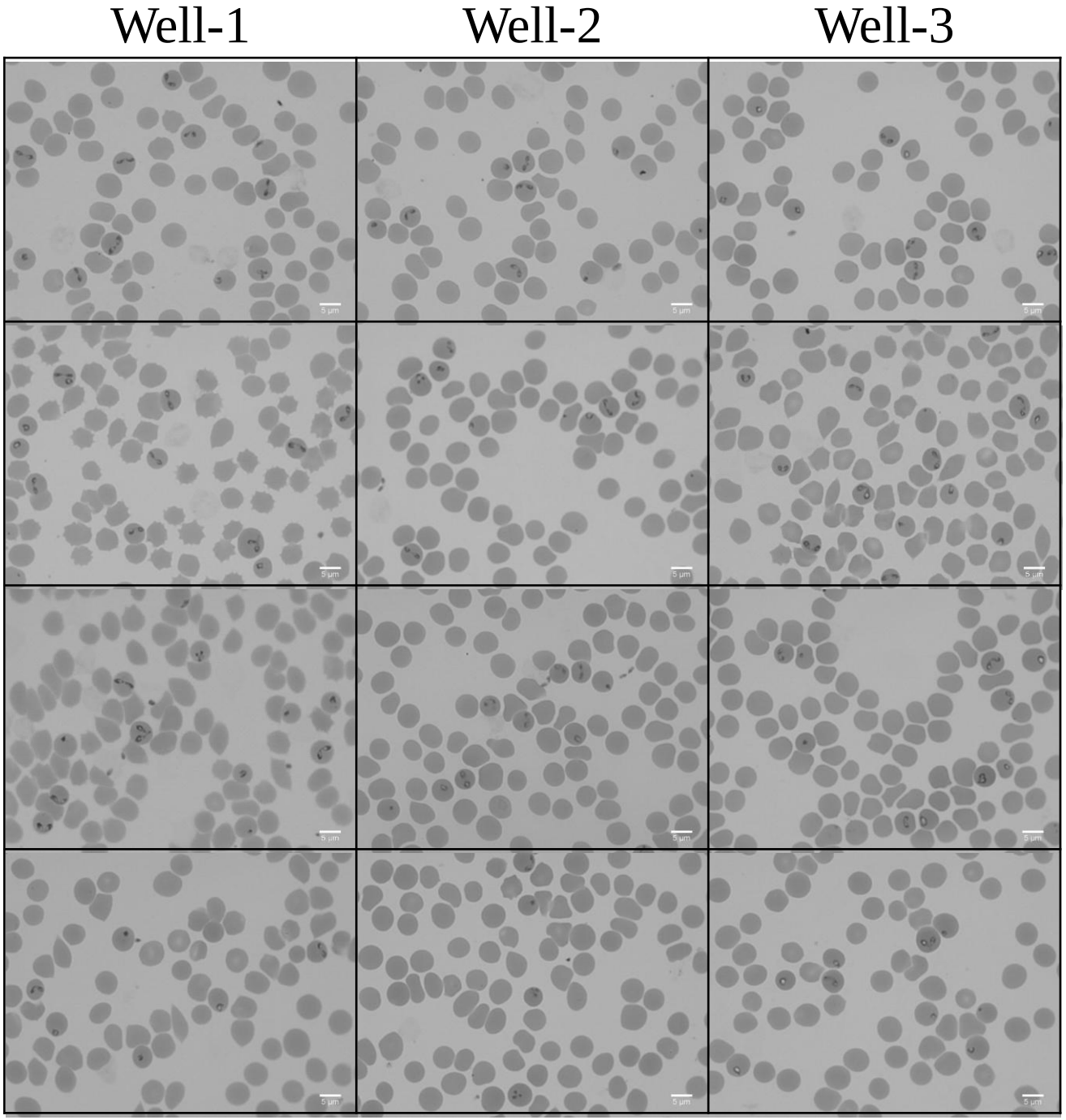


Figure S2

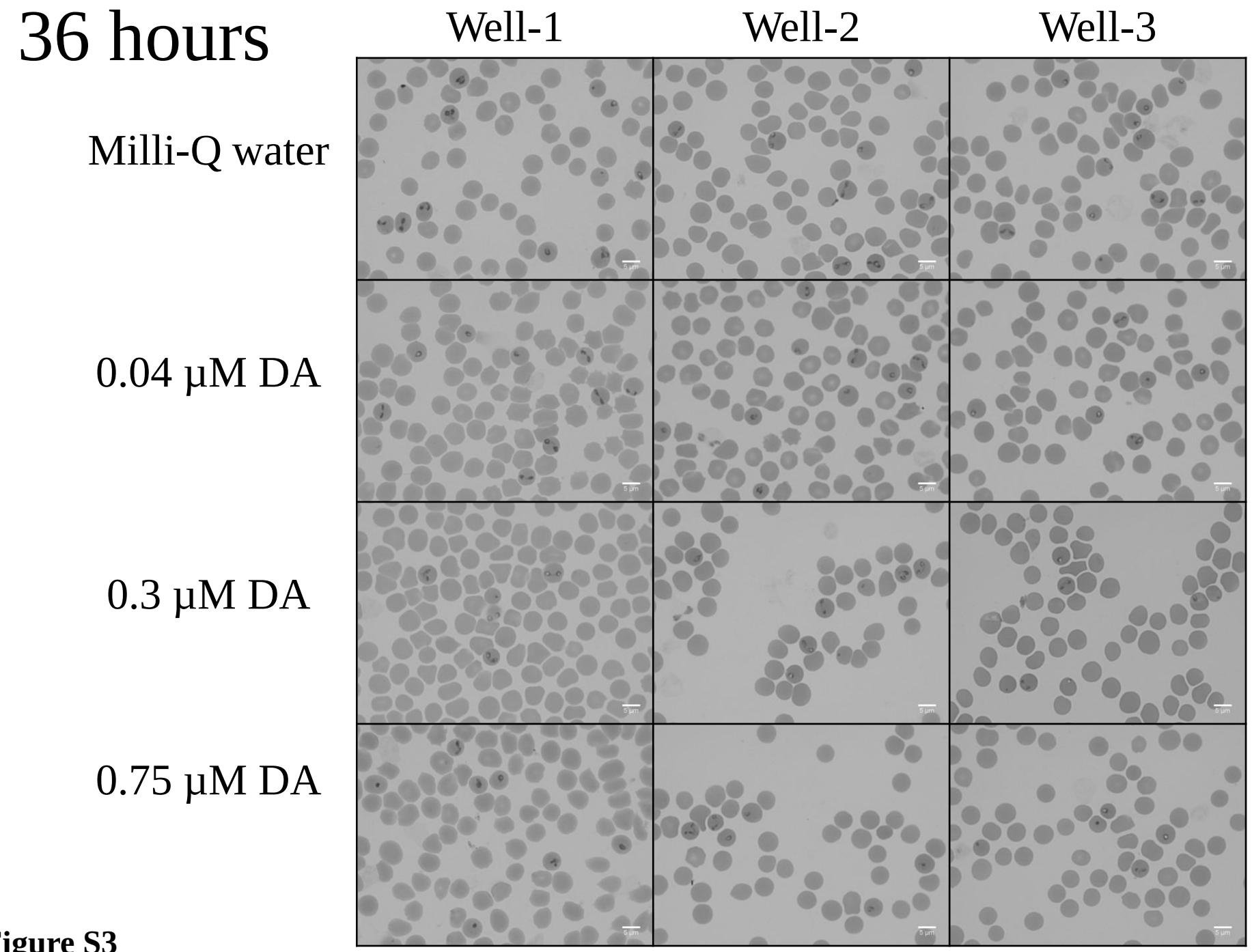

36 hours
Well-1
Well-2
Well-3
Milli-Q water
0.04 µM DA
0.3 µM DA
0.75 µM DA

Figure S3

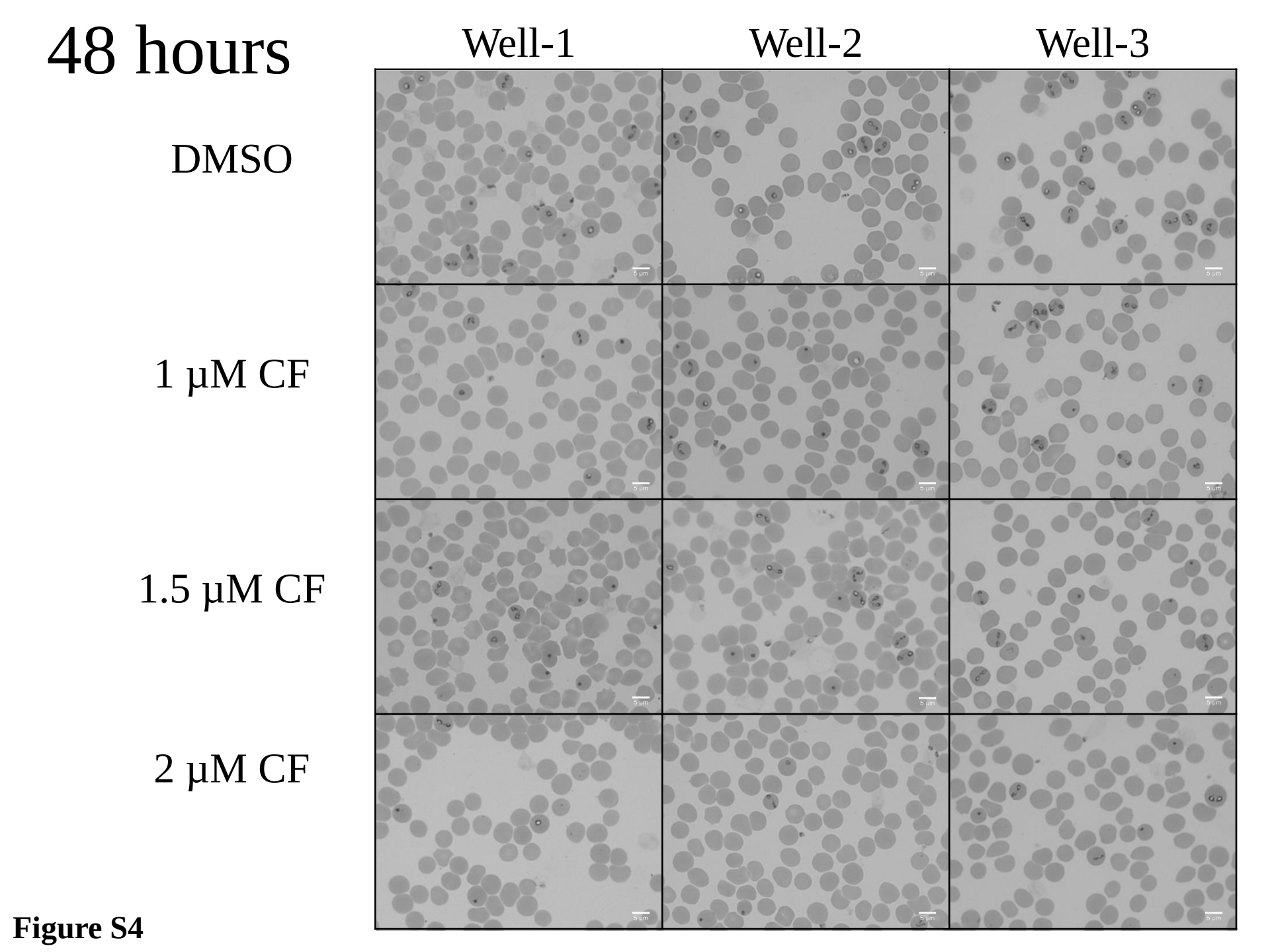


Figure S4

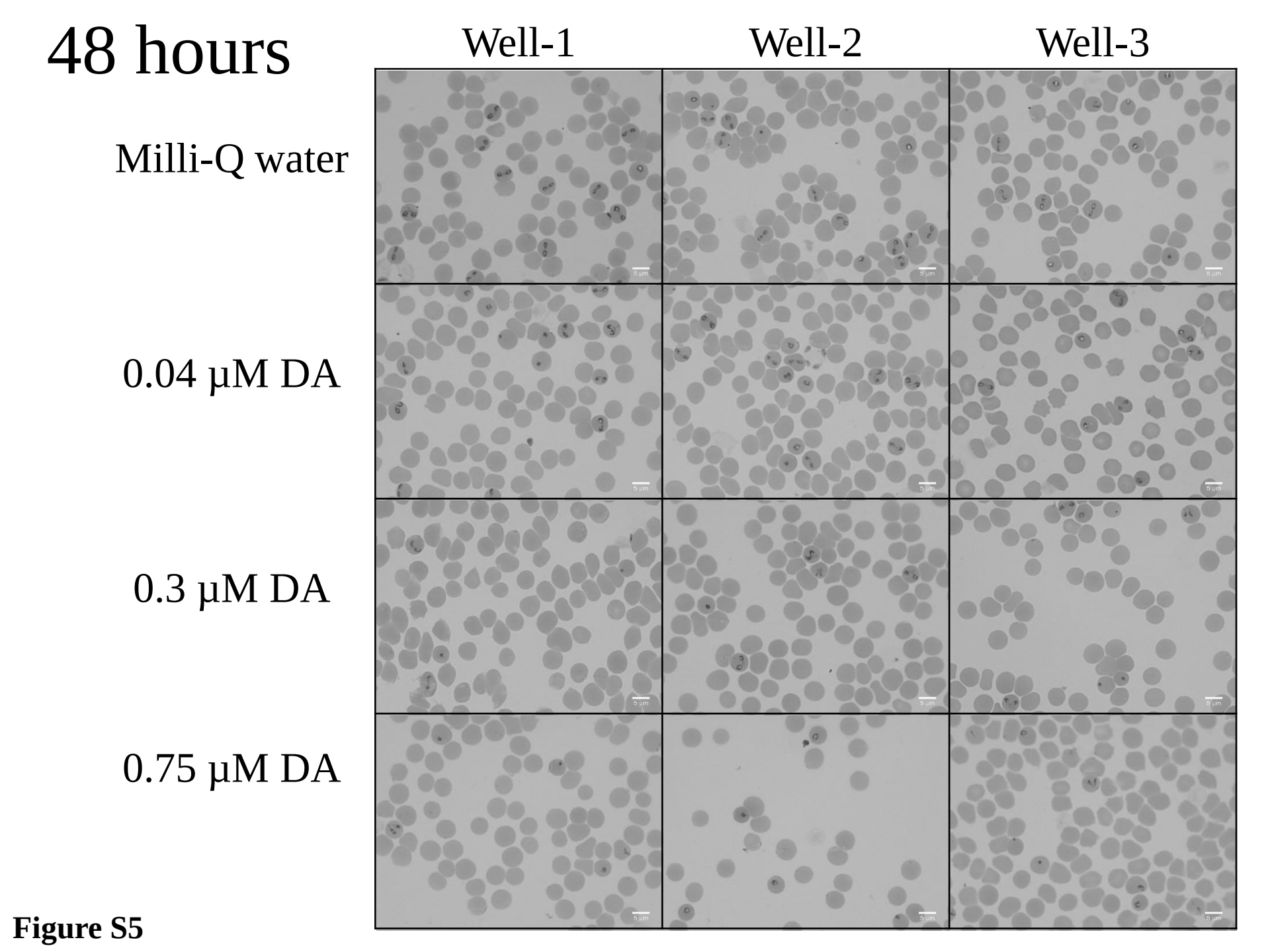

48 hours
Well-1
Well-2
Well-3
Milli-Q water
0.04 µM DA
0.3 µM DA
0.75 µM DA

Figure S5