

SUPPORTING INFORMATION:

Localization of Fe-S Biosynthesis Machinery in *Cryptosporidium parvum* Mitosome by
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Figure S1: Conservation of functionally important residues in *Cryptosporidium* cysteine desulphurase (IscS) sequences.

Figure S2: Conservation of functionally important residues in *Cryptosporidium* scaffold protein (IscU) sequences.

Figure S3: Identification of candidate functionally important residues in *Cryptosporidium* Frataxin sequences.

Figure S4: Detection of CpIscS via indirect immunofluorescence.

Figure S5: Detection of CpIscU via indirect immunofluorescence.

Figure S6: Detection of CpFrataxin via indirect immunofluorescence.

Figure S7: Work flow for the yeast-based functional complementation assay.

Figure S8: Electron Microscope Images of *C. parvum* life cycle stages in an infected COLO-680N culture.

Table S1: Identified proteins potentially involved in mitochondrial Fe-S cluster assembly in various *Cryptosporidium* species as extracted from NCBI and CryptoDB using BLASTP.

Table S2: In silico mitochondrial prediction of proteins involved in Fe-S cluster assembly in *Cryptosporidium* species.

Video S1: Animation of cellular staining of *Cryptosporidium parvum* cell using confocal microscopy

Supplementary Figure S1

C. parvum ----- MIVHRYCROWAPS VVRGISKLAFFSSMSSIAKK-----RPAYFDYQATTPVDPRLDKMM
C. hominis_UdeA01 ----- MIVHRYCROWAPS VVRGISKVAFFSSMSSIAKK-----RPAYFDYQATTPVDPRLDKMM
C. hominis_TU502 ----- MIVHRYCROWAPS VVRGISKVAFFSSMSSIAKK-----RPAYFDYQATTPVDPRLDKMM
C. ubiquitum ----- MIVQGYYRQLIPGVMRSVAKVAFFSSMTSITKK-----RPVYFDYQATTPVDPRLDKMM
C. andersoni ----- MNMRRTLPLGVFSIRHSKYLRSFSTKLKNQ-----SIQRPLYFDYQATTPVDPRLDKML
C. muris ----- MNMKTLPLGVFSIRRSKYLRSFSSKLKRO-----SIQRPLYFDYQATTPVDPRLDKML
S. cerevisiae ----- MLKSTATRSITRLSQVYNPAATYRACLVSRRFYSPPAAGVKLDDNFSLETHDIQAAAKAQASARASASGTTDAVVASGSTAMSHAYQENTGFGRPIYLDMQATTPTDPRVLDTML
H. sapiens ----- MLLRAAWRRAAVAVTAAPGPKPAAPTRGLRL-----RVGDRAPQSAPVADTAAPEV-----GPVLRPLYMDVQATTPLDPRVLDAHL
E. coli ----- MLLRAAWRRAAVAVTAAPGPKPAAPTRGLRL-----MKL-----PIYLDYSATTPVDPRVAEKMM

C. parvum PFFT--EKFG₁SHSRTHGYGWEAEEAVENARTNIANLIKCLPKEIFIFTSGATESNNNTIIRGVCDIYGDIENKKNHIITTOIEHKCVLSTLRELELKGRFRVTYLKVNNKGLISLEELEKSI
C. hominis_UdeA01 PFFT--EKFG₁SHSRTHGYGWEAEEAVENARTNIANLIKCLPKEIFIFTSGATESNNNTIIRGVCDIYGDIENKKNHIITTOIEHKCVLSTLRELELKGRFRVTYLKVNNKGLISLEELEKSI
C. hominis_TU502 PFFT--EKFG₁SHSRTHGYGWEAEEAVENARTNIANLIKCLPKEIFIFTSGATESNNNTIIRGVCDIYGDIENKKNHIITTOIEHKCVLSTLRELELKGRFRVTYLKVNNKGLISLEELEKSI
C. ubiquitum PFFT--EKFG₁SHSRTHGYGWEAEDAVESARTNIANLIKCPKEIFIFTSGATESNNNTIIRGVCDVYGDIKSKKNHIITTOIEHKCVLSTLRELELKGRFRVTYLKVNNKGLISLEELEKSI
C. andersoni PLYT--ELYG₁SHSRTHGYGWETEDIENARDEVAKLINCLSKEVIFTSGATESNNNTIIRGVCENYGNIKEGRNHIIITQIEHKCVLSTLRELELKGFKVTVYLKVDRDGSLKSLESAI
C. muris PLYT--ELYG₁SHSRTHGYGWETEDIENARDEVAKLINCLSKEVIFTSGATESNNNTIIRGVCENYGNIKEGRNHIIITQIEHKCVLSTLRELELKGFKVTVYLEVDRDGSLKDLESAI
S. cerevisiae KFYT--GLYC₁PHSNTHSYGWETNTAVENARAHVAKMINADPKEEIFIFTSGATESNNMVLKGVPRFY---KTKHHIITTRTEHKCVLEAARAMMKEGFEVTFLNVDDQGLIDLKELEDAI
H. sapiens PYLI--NYYG₁PHSRTHAYGWESEAAMERARQOVASLIGADPREIIFTSGATESNNIAIKGVARFY---RSRKHHLITTQTEHKCVLDSCRSLEAEGFQVTLPVQKSGIIDLKELEAAI
E. coli QFMTMDGTG₁PASRSHRGWQAAEAVDIARNQIADLVGADPREIVFTSGATESDNLAIGAANFY---QKKGKHIITSKTEHKAVLDTCRQLEREGFEVTYLAQQRNGIIDLKELEAAI

C. parvum IPGETILASIMHV₁NEIGVIQPMNLIGEICKKYNVLFHSDVAQGLGKINIDVDKWNADFLSLSAHKVYGPKGIGAFYIRSKP₂RRRIKPLIFGGGQERGRMSGTMPVPLAVGFGEACKIAS
C. hominis_UdeA01 IPGETILASIMHV₁NEIGVIQPMNLIGEICKKYNVLFHSDVAQGLGKINIDVDKWNADFLSLSAHKVYGPKGIGAFYIRSKP₂RRRIKPLIFGGGQERGRMSGTMPVPLAVGFGEACKIAS
C. hominis_TU502 IPGETILASIMHV₁NEIGVIQPMNLIGEICKKYNVLFHSDVAQGLGKINIDVDKWNADFLSLSAHKVYGPKGIGAFYIRSKP₂RRRIKPLIFGGGQERGRMSGTMPVPLAVGFGEACKIAS
C. ubiquitum IPGETILVSIMHV₁NEIGVIQPMNLIGELCKKHNVLFHSDVAQGLGKINIDVDKWNADFLSLSAHKVYGPKGIGAFYIRSKP₂RRRIKPLIFGGGQERGRMSGTIPVPLAVGFGEACKIAS
C. andersoni ESGK₁TILVSVMFV₁NEIGVIQPMESIGEVCRKHGVLFHSDVAQGLGKLQIDVNWKVDFLSMSGH₂KIYGPKGIGAFYIRSKP₂RRRIKPLIFGGGQERGRSGTLAVPLVVGFGEACRIAN
C. muris ESGK₁TILVSVMFV₁NEIGVIQSMESIGEVCRKHGVLFHSDVAQGLGKLQIDVNWKVDFLSMSGH₂KIYGPKGIGAFYIRSKP₂RRRIKPLIFGGGQERGRSGTLAVPLVVGFGEACRIAN
S. cerevisiae RP-DTCLVSVMAV₁NEIGVIQPIKEIGAICRKNKIYFHTDAAQAYGKIHIDVNEMNIDLLSIS₂SSH₃KIYGPKGIGAIYVRRRPRVLEPLLSGGGOERGLSGTLAPPLVAGFGEARLMK
H. sapiens QP-DTSLSVSVMTV₁NEIGVKQPIAEIGRICSSRKVYFHTDAAQAVGKIPLDVNDMKIDLMSISGH₂KIYGPKGVGAIYIRRPRVRVEALQSGGGQERGRMSGTVPPLVVGAAUCEAQ
E. coli RD-DTILVSIMHV₁NEIGVVQDIAAIGEMCRARGIYHV₂DATOSVGKLPIDLSQLKVDLMSFSGH₃KIYGPKGIGALYVRRKPRVRIEAQMHHGGHERGRMSGTLPVHQIVGMGEAYRIAK

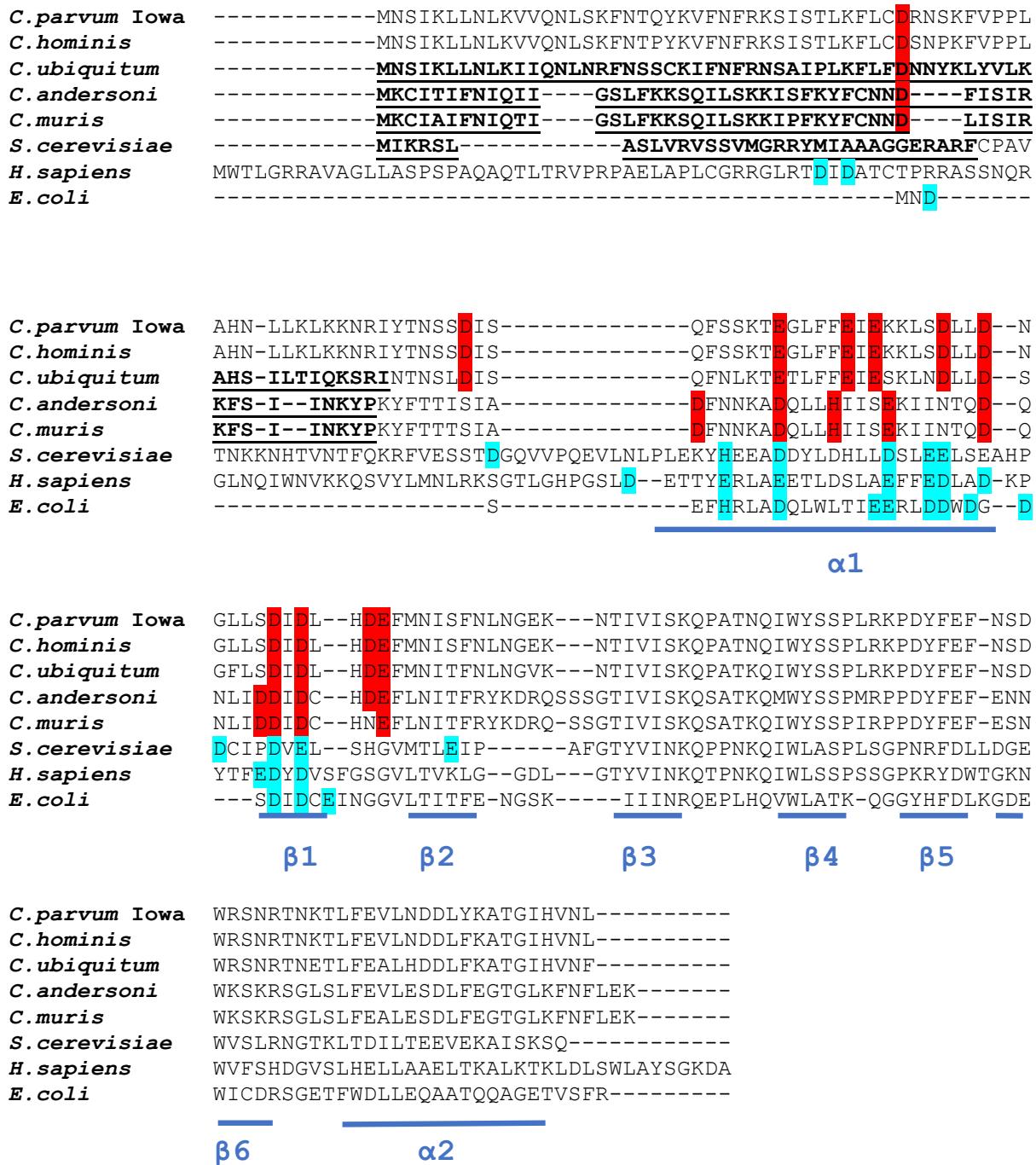
C. parvum SEMNSDSIHVKS₁LYDKLYKGITTQLPVELNGCGVNRMFGNLNL₂SFTGV₃VEGESLMMKLYSLALSSGSACTSASLEPSYVLRAIGVGEDVAHTSIRFGLG₄FTKHEDVDKAVKEIVESVTL
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C. hominis_TU502 SEMNSDSIHVKS₁LYDKLYKGITTQLPVELNGCGVNRMFGNLNL₂SFTGV₃VEGESLMMKLYSLALSSGSACTSASLEPSYVLRAIGVGEDVAHTSIRFGLG₄RFTKHEDVDKAVKEIVESVTL
C. ubiquitum SEMNLD₁IHVKS₂LYDKLYKGITTQLPVELNGCSINRMFGN₃NLNL₄SFAGVEGESLMMKLYSLALSSGSACTSSSLEPSYVLRAIGVGEDVAHTSIRFGLG₅RFTRHEDVDKAVKEIVESVTL
C. andersoni DEMVRDS₁IHKKKLYHR₂LHG₃IQSQV₄PNIKLNGSP₅HRCFNN₆NLNL₇SFAGVEGESLMMKLYSLALSSGSACTSASLEPSYVLRAIGIDEETAHTSIRFGLG₈RFTTPSEIDLAVDEVVKAVSS
C. muris DEMVRDS₁IHKKKLYHR₂LHG₃IQSQV₄PNIKLNGSS₅HRCFNN₆NLNL₇SFAGVEGESLMMKLYSLALSSGSACTSASLEPSYVLRAIGVDEETAHTSIRFGLG₈RFTTPSEIDLAVDEVVKAVSS
S. cerevisiae KEFDNDQAH₁IHKRLSD₂DKLVKG₃LL-SAEHTT₄LNGSP₅DHYPGCVN₆FAYVEGES₇LLMALRDIALSSGSACTSASLEPSYVLHALGKDD₈DALAHSSIRFGIGRFTSEEV₉DYVVKA₁₀SDRVKF
H. sapiens QEMEYDHKRISK₁L₂SERL₃IQNIMKSLPD₄VVMNGDP₅HHYPGC₆INLSFAYVEGES₇LLMALKD₈VALSSGSACTSASLEPSYVLRAIGTDED₉LAHSSIRFGIGRFT₁₀TEE₁₁EVDYTVEKC₁₂IQHVKR
E. coli EEMATEMERLRLGRNRLWNGIK-DIEEVYLN₂GDLEH₃GAPN₄ILN₅VSFNYVEGES₆LIM₇ALKD₈LAVSSGSACTSASLEPSYVL₉RALGLN₁₀DELAHSSIRF₁₁SLGRFT₁₂EEE₁₃IDY₁₄TIELVRKSIGR

C. parvum LRKMSPLWDSITDTKV₁KDEDSLKWT--
C. hominis_UdeA01 LRKMSPLWDSITDTKV₁KDEDSLKWT--
C. hominis_TU502 LRKMSPLWDSITDTKV₁KDEDSLKWT--
C. ubiquitum LRKMSPLWD₁DTGTRV₂KNE₃ESLKWT--
C. andersoni LRNMSPIWDQ₁INSTNPSDISS₂SP₃WT--
C. muris LRNMSPIWDQ₁INSTN₂SSDIS₃GP₄WT--
S. cerevisiae LRELSP₁LWEMVQEG--IDLNSIKWSGH
H. sapiens LREMSP₁LWEMVQDG--IDLKS₂IKW₃TOH
E. coli LRDLSP₁LWEMYKQG--VDLNSIEWAHH

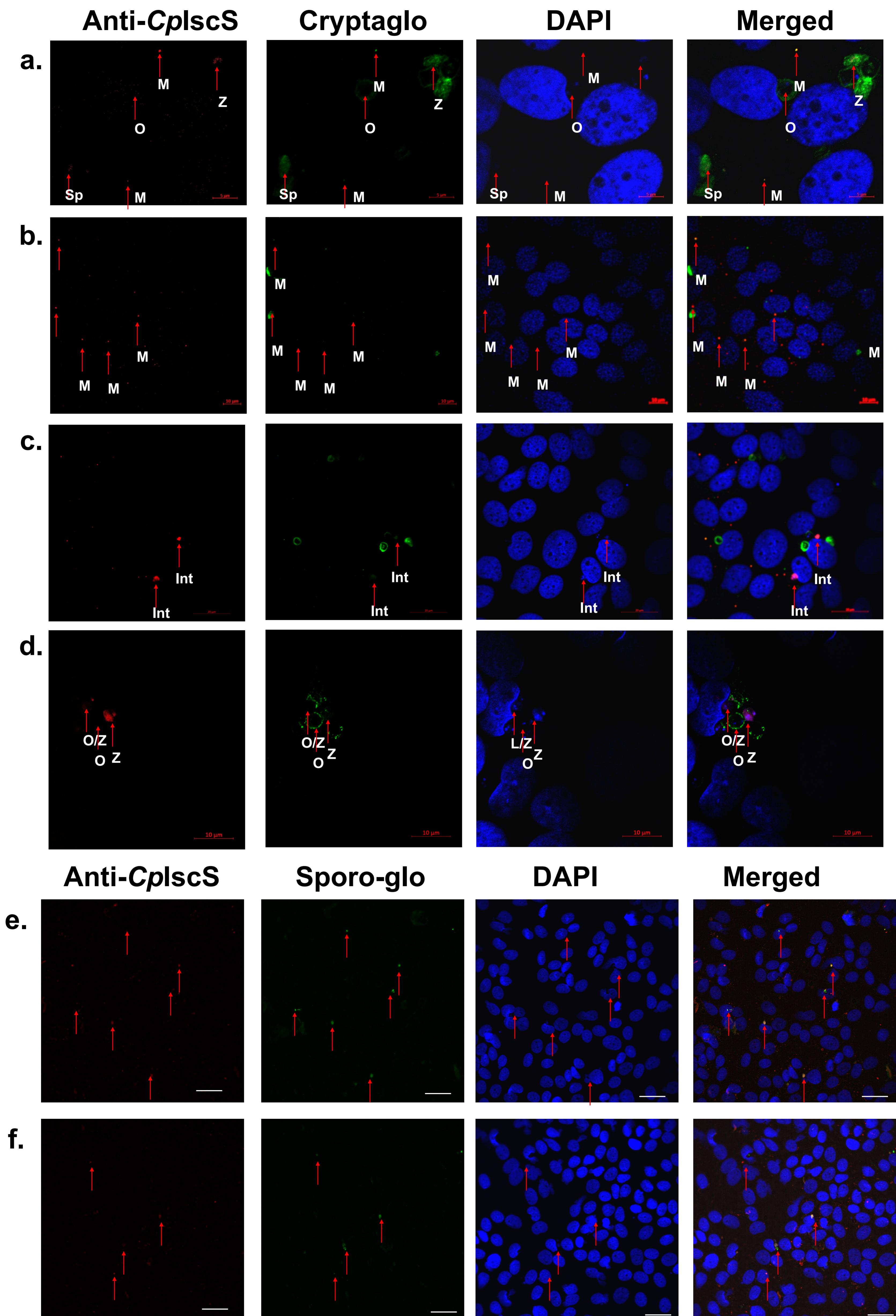
Supplementary Figure S2

<i>C. parvum</i>	-MLQLRQLIDKRILIKKCVPICQRLF	SDTVHDHFRNPRNVGSLPSDDKNVGTAVVGKASC D VVKLQVDIRD--GIIKDAFKTFGCGSAIASTSYATELIIGKTEEALKINNKTIAD
<i>C. hominis</i> UdeA01	-MLQLRQLIDKRILIKKCVPICQRLF	YSDTVHDHFRNPRNVGSLPSDDKNVGTAVVGKASC G DVVKLQVDIRD--GIIKDAFKTFGCGSAIASTSYATELIIGKTEEALKINNKTIAD
<i>C. hominis</i> TU502	-MLQLRQLIDKRILIKKCVPICQRLF	YSDTVHDHFRNPRNVGSLPSDDKNVGTAVVGKASC G DVVKLQVDIRD--GIIKDAFKTFGCGSAIASTSYATELIIGKTEEALKINNKTIAD
<i>C. ubiquitum</i>	-MLQLRNFVDKRILLKKCVPICQRLF	YSNVVNDHFRNPRNVGSLPSDDKNVGTAVVGKAACGDVVKLQVSIKD--GVIKDAFKTFGCGSAIASTSYATELIIGKTEEALKVNNTIAN
<i>C. andersoni</i>	MMIVVRSCITR--CKQHLSLIAQKAMY	SEAVHDHFRNPRNVGTLPTDKKNVGTAVVGKAAC G DVVKLQVSIED--GIIRDAFKTFGCGSAIASSSYVTELIGKTPEDALKIKNTDIAN
<i>C. muris</i>	MMIAVRNCITR--CKQHLSLIAQKAMY	SEAVHDHFRNPRNVGTLPSDKKNVGTAVVGKAAC G DVVKLQVSIED--GVIRDAFKTFGCGSAIASSSYVTELIGKTPEDALKIKNTDIAN
<i>S. cerevisiae</i>	MFARLANPAHFKP L TGS H TRAAKRL	YHPKV I DHYTNPRNVGSM D KSLANVGTGIVGA P ACGD V IKLQI Q VNDKSGIIENVKF K TFGCGSAIASSSYMTELVRGMSLDEAVKIKNTEIAK
<i>H. sapiens</i>	-----	MVLIDMSV D LSTQVVDHYENPRNVG S LDKTS T KNVGT G LVGA P ACGD V M K LQI Q VDEK-GKIVDARFKTFGCGSAIASSSLATEWVKGKTVEEALTIKNTDIAK
<i>E. coli</i>	-----	-----MAY A SEKVIDHYENPRNVG S FDNNDENVG G M V GAPACGD V M K LQI Q VNDE-GIIEDARFKTYGC G SAIASSSLVTEWVKG K SLDEAQAIKNTDIAE
<i>C. parvum</i>	HLNLPP I KLHC S LLAEDA I KA I KNY Q D Q QLKS-----	
<i>C. hominis</i> UdeA01	HLNLPP I KLHC S LLAEDA I KA I KNY Q D Q QLKS-----	
<i>C. hominis</i> TU502	HLNLPP I KLHC S LLAEDA I KA I KNY Q D Q QLKS-----	
<i>C. ubiquitum</i>	HLNLPP I KLHC S LLAEDA I KA I KNY Q E K QQES-----	
<i>C. andersoni</i>	YLNLP P V K LHC S LLAEDA V HLAI K DY Q SK Q STS D DKSSSVNISY S DDNKVNPN T K-----	
<i>C. muris</i>	YLNLP P V K LHC S LLAEDA V HLAI K DY Q SK Q STS D DKSSSVNISY S DDNKVNPN T K-----	
<i>S. cerevisiae</i>	ELSLPP V KLHC S MLAEDA I KA A AI K DY K TKRNP S VLH-----	
<i>H. sapiens</i>	ELCLPP V KLHC S -----KSVLFPAE E KT Q LP-----	
<i>E. coli</i>	ELELPP V KI H C S ILAEDA I KA A ADY K SK R EAK-----	

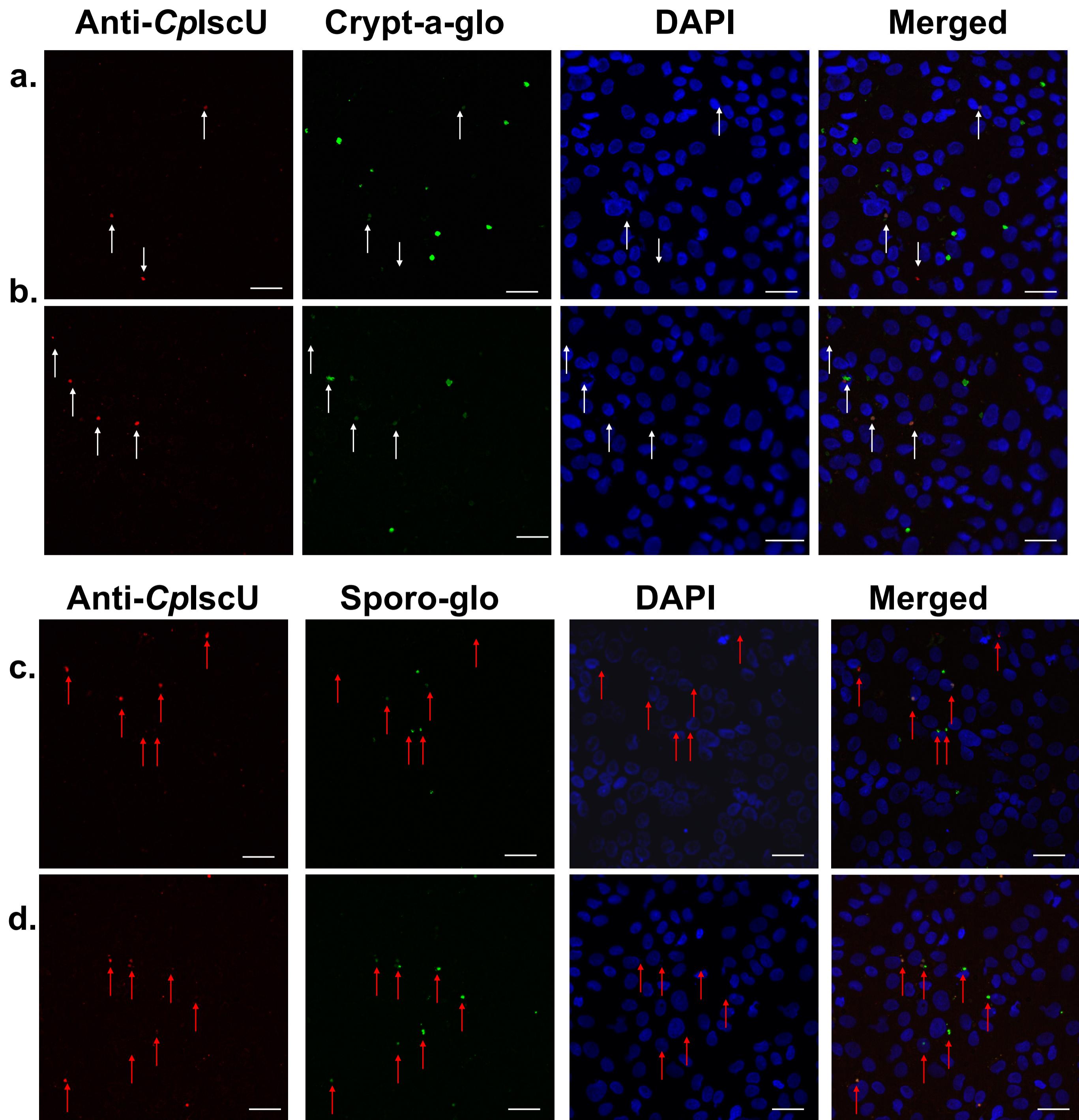
Supplementary Figure S3

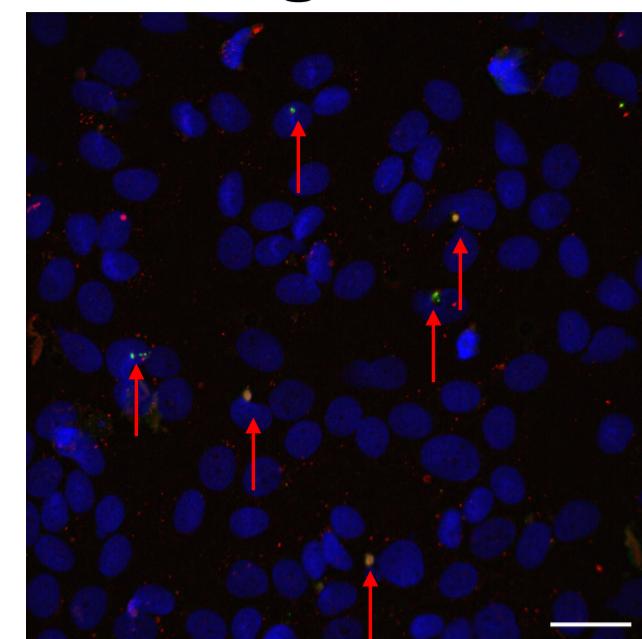
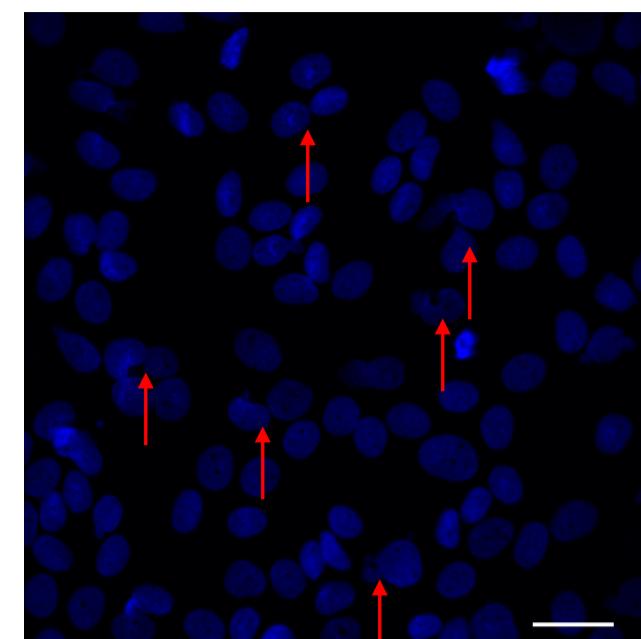
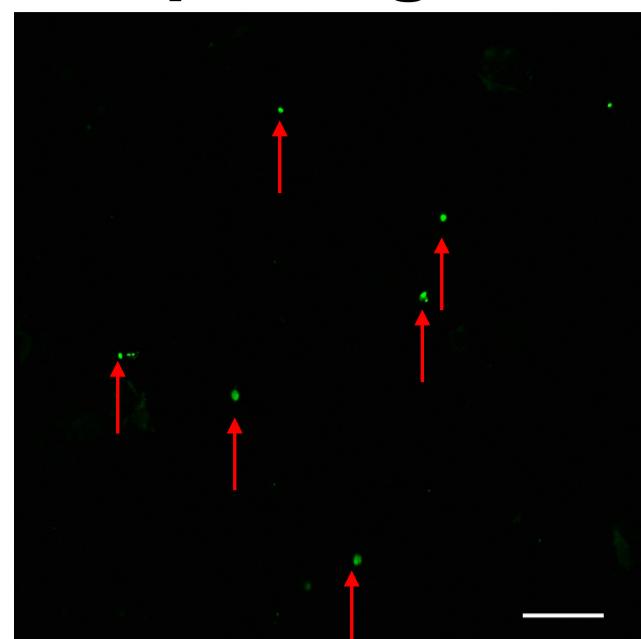
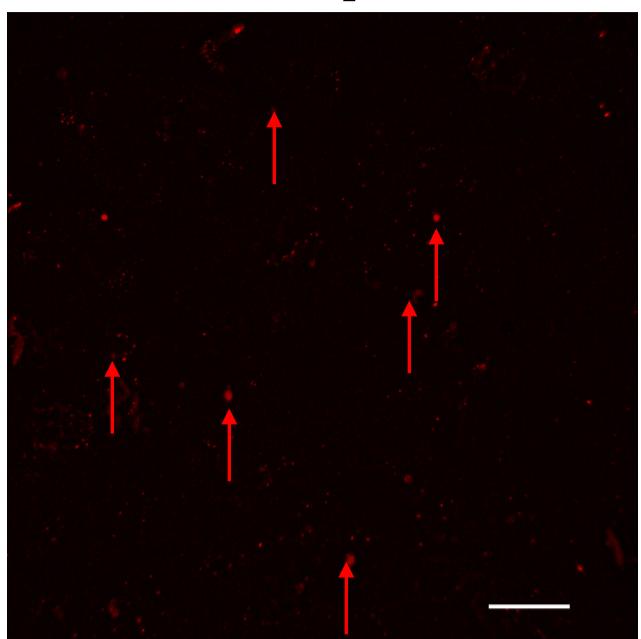
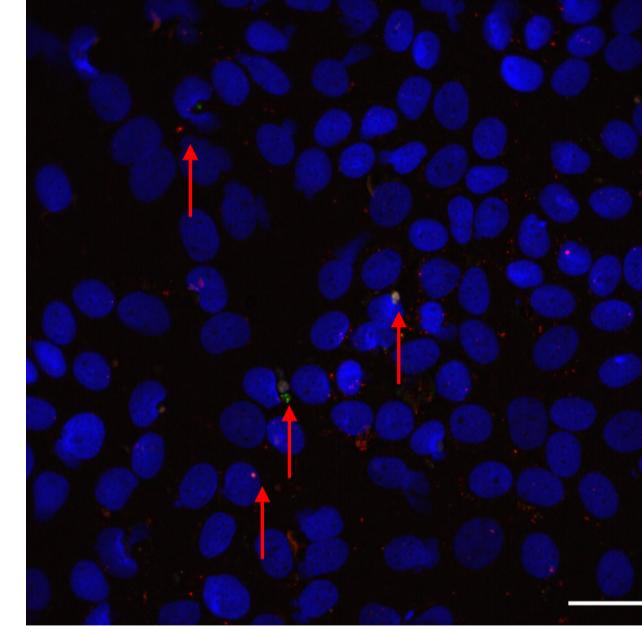
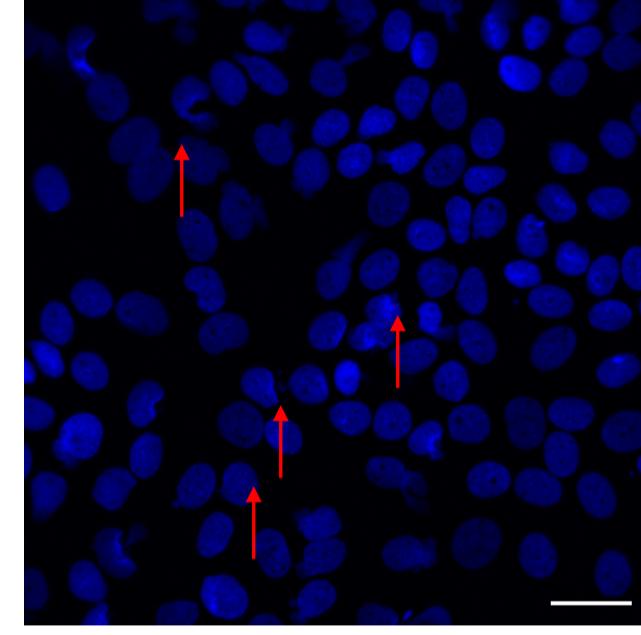
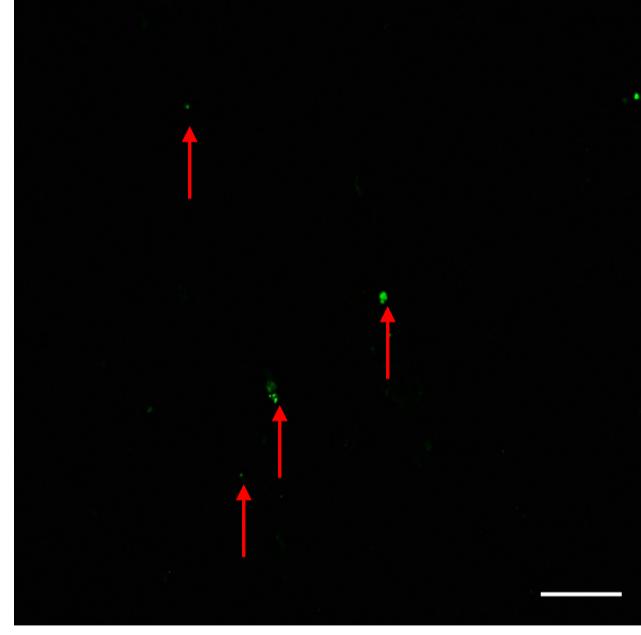
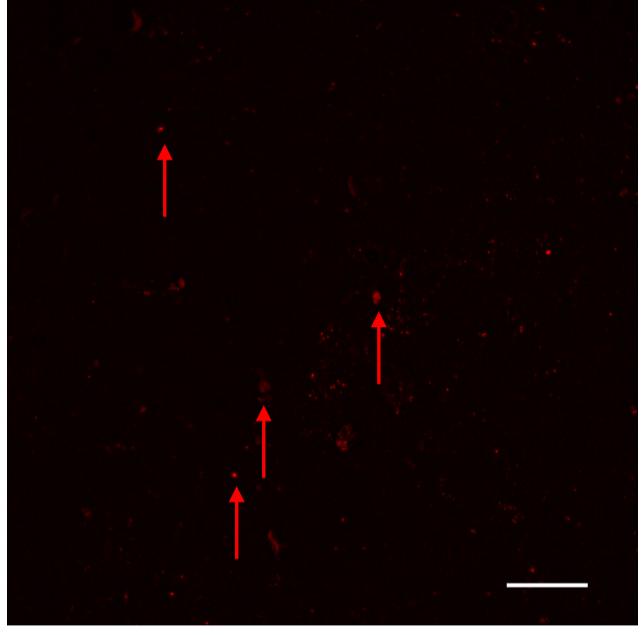


Supplementary Figure 4



Supplementary Figure S5

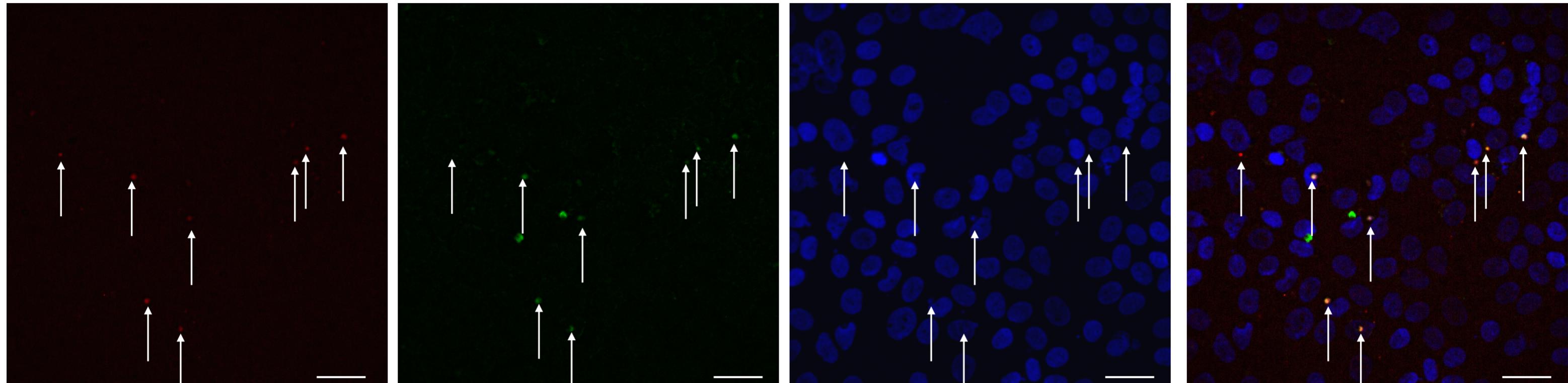


Anti-CpIScU**Sporo-glo****DAPI****Merged****e.****f.**

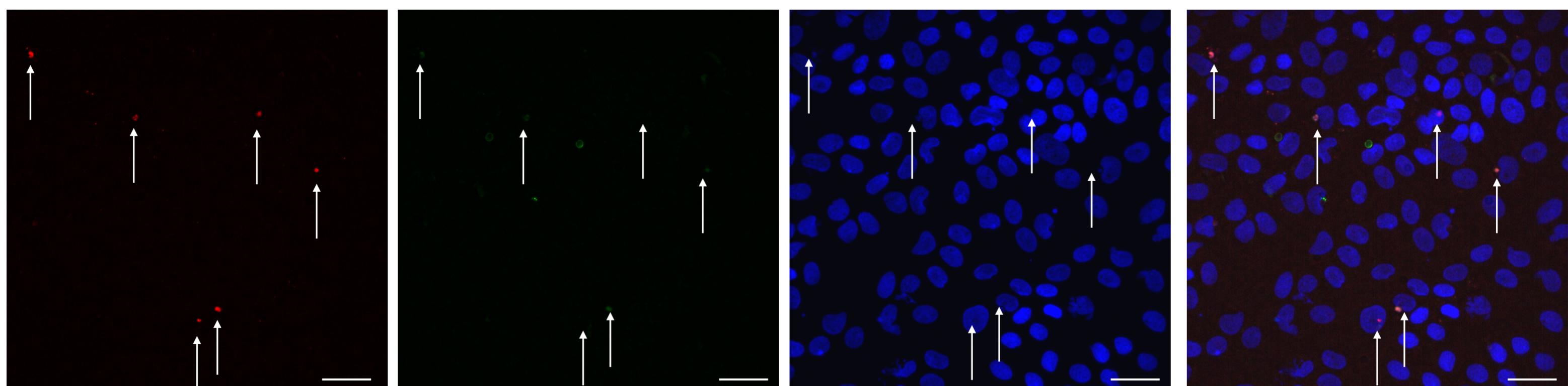
Supplementary Figure 6

Anti-CpFrataxin Crypt-a-glo DAPI Merged

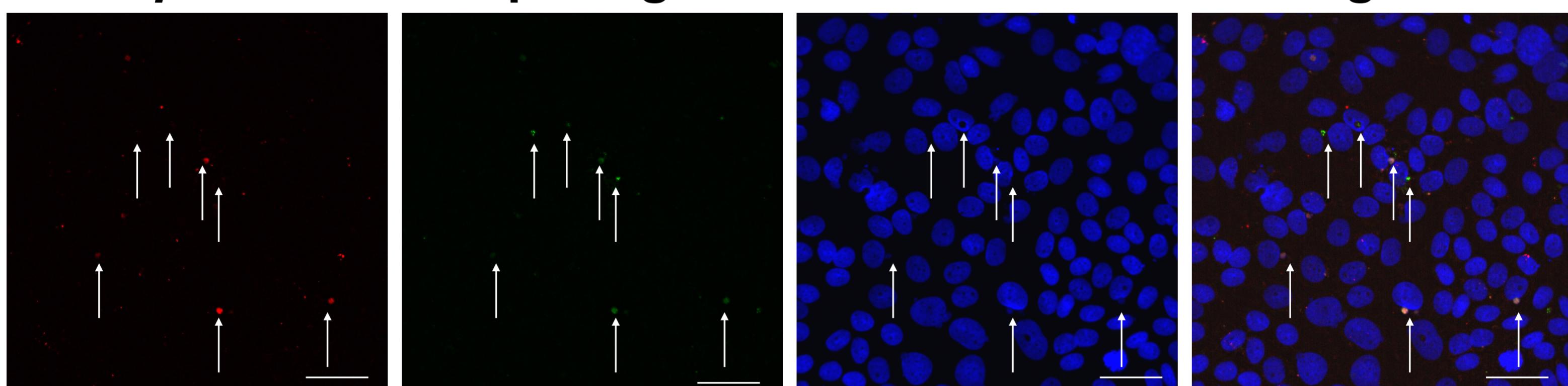
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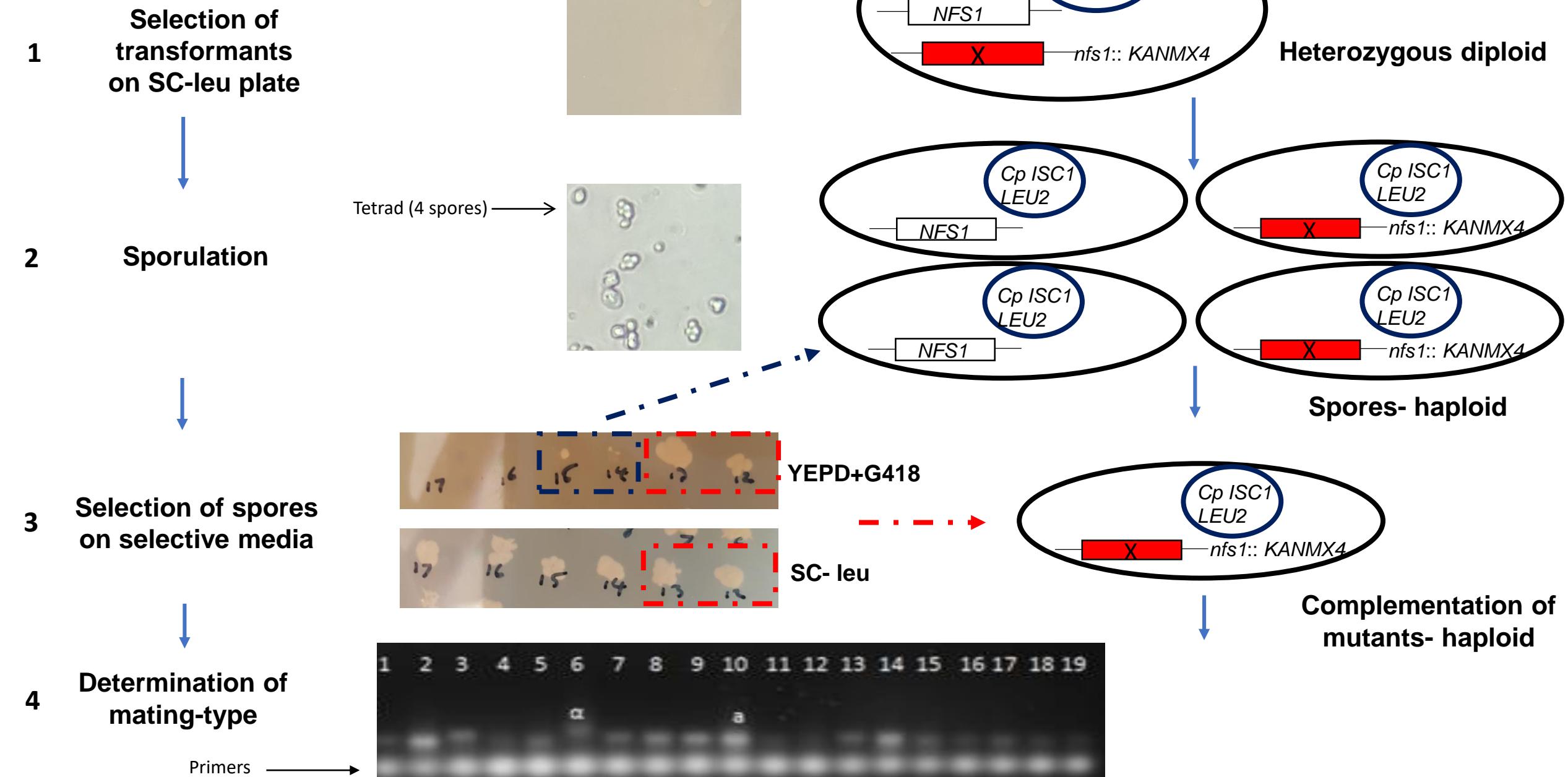
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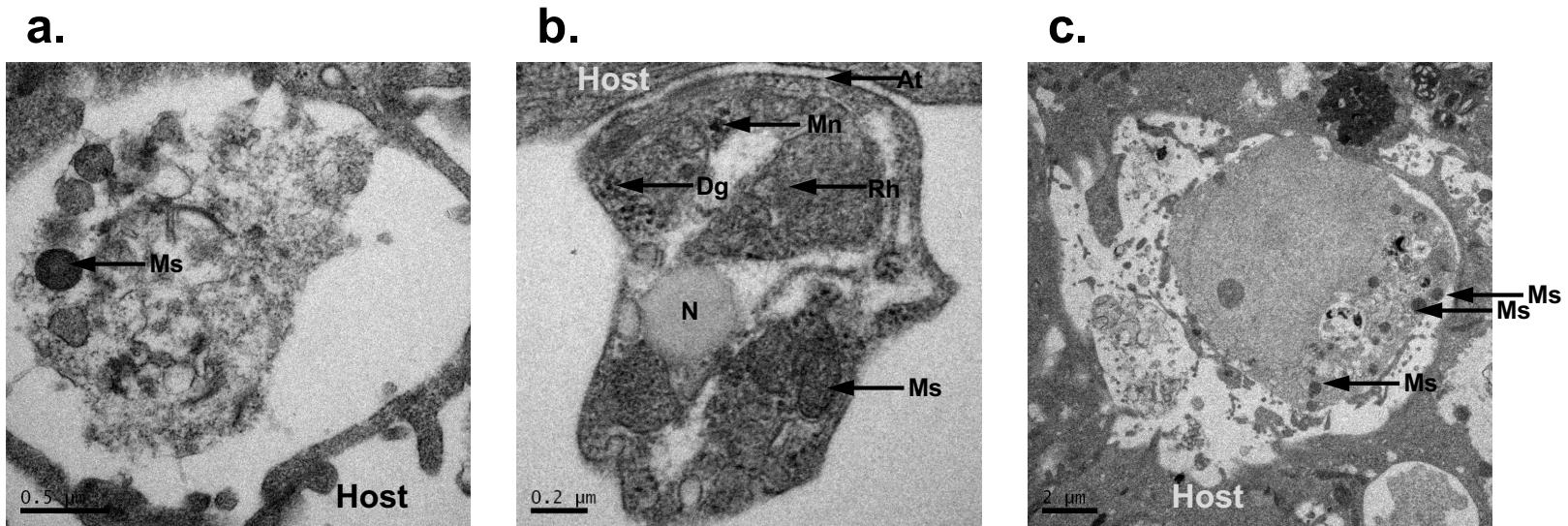
c.



Supplementary Figure S7



Supplementary Figure S8



Supplementary Table 2: *In silico* mitochondrial prediction of proteins involved in Fe-S cluster assembly in *Cryptosporidium* species

Species	Protein	MitoFates	Predotar	MitoProt	TargetP
<i>C. parvum</i>	IscS	0.877	0.76	0.9930	0.849
	IscU	0.889	0.50	0.9617	0.418
	Frataxin	0.273	0.13	0.9485	0.586
	Arh1	0	0	0.1862	0.012
	Yah1	0.932	0.91	0.9941	0.946
	MFRN1/Mrs3	0.017	0.00	0.0230	0.042
	MFRN2/Mrs4	0.04	0.00	0.2602	0.016
	Grx5	0	0.00	0.0965	0.021
	mtHsp70	0.258	0.08	0.9877	0.741
	Jac1	0.247	0.00	0.2205	0.187
	Mge1	0.854	0.69	0.9674	0.887
	Ind1	0.008	0.00	0.0737	0.093
	Isa1	-	-	-	-
	Isa2	-	-	-	-
	Atm1	0.373	0.69	0.6661	0.581
	Erv1	-	-	-	-
<i>C. hominis</i>	IscS	0.819	0.72	0.9888	0.852
	IscU	0.889	0.50	0.9617	0.418
	Frataxin	0.243	0.16	0.8810	0.583
	Arh1	0.045	0.06	0.4090	0.148
	Yah1	-	-	-	-
	MFRN1/Mrs3	0.020	0.00	0.0230	0.041
	MFRN2/Mrs4	0.051	0.01	0.1975	0.020
	Grx5	0.004	0.00	0.0406	0.067
	mtHsp70	0.44	0.05	0.9329	0.548
	Jac1	0.009	0.00	0.0091	0.244
	Mge1	0.864	0.69	0.9695	0.866
	Ind1	0.117	0.02	0.6708	0.337
	Isa1	-	-	-	-
	Isa2	-	-	-	-
	Atm1	0.228	0.69	0.7737	0.573
	Erv1	-	-	-	-
<i>C. ubiquitum</i>	IscS	0.887	0.52	0.9750	0.691
	IscU	0.958	0.48	0.9303	0.582
	Frataxin	0.492	0.10	0.9706	0.671
	Arh1	0.055	0.24	0.1361	0.049
	Yah1	0.716	0.24	0.9762	0.753
	MFRN1/Mrs3	0.016	0.00	0.0472	0.039
	MFRN2/Mrs4	-	-	-	-
	Grx5	0	0.00	0.2028	0.067
	mtHsp70	0.131	0.14	0.9588	0.717

	Jac1	0.009	0.00	0.0069	0.244
	Mge1	0.622	0.23	0.9778	0.748
	Ind1	0.277	0.03	0.8964	0.569
	Isa1	-	-	-	-
	Isa2	-	-	-	-
	Atm1	0.372	0.49	0.8801	0.619
	Erv1	-	-	-	-
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<i>C. andersoni</i>	IscS	0.953	0.58	0.9230	0.942
	IscU	0.865	0.34	0.8425	0.640
	Frataxin	0.079	0.12	0.8360	0.156
	Arh1	0.835	0.87	0.7677	0.882
	Yah1	0	0.02	0.1832	0.176
	MFRN1/Mrs3	0.010	0.00	0.0579	0.077
	MFRN2/Mrs4	-	-	-	-
	Grx5	0.022	0.13	0.2324	0.068
	mtHsp70	0.543	0.00	0.9197	0.863
	Jac1	0.006	0.61	0.0354	0.255
	Mge1	0.117	0.01	0.9886	0.462
	Ind1	0.012	0.02	0.0537	0.040
	Isa1	0.003	0.02	0.3211	0.163
	Isa2	0.580	0.47	0.8120	0.749
	Atm1	-	-	-	-
	Erv1	0.009	0.02	0.0777	0.064
<hr/>					
<i>C. muris</i>	IscS	0.931	0.77	0.9843	0.952
	IscU	0.897	0.19	0.8456	0.656
	Frataxin	0.083	0.17	0.7823	0.196
	Arh1	0.792	0.90	0.8725	0.915
	Yah1	0	0.01	0.1844	0.092
	MFRN1/Mrs3	0.008	0.00	0.0565	0.064
	MFRN2/Mrs4	0.018	0.00	0.0348	0.045
	Grx5	0	0.00	0.1164	0.032
	mtHsp70	0.382	0.16	0.9464	0.798
	Jac1	0.010	0.00	0.0252	0.282
	Mge1	0.112	0.64	0.9896	0.621
	Ind1	0.019	0.01	0.0706	0.062
	Isa1	0	0.01	0.4522	0.166
	Isa2	0.976	0.63	0.8293	0.759
	Atm1	0.320	0.46	0.9362	0.683
	Erv1	0.013	0.02	0.0982	0.071

In silico prediction of potential mitochondrion-targeted proteins using MitoFates (Fukasawa et al. 2015) and displaying the “possibility of presequence”, MitoProt (Claros 1995) while displaying the “probability of export to mitochondria”, Predotar (Small et al. 2004) and TargetP (Emanuelsson et al. 2007), by displaying the probability of prediction of mitochondrial targeting signal. Possibilities above 85 % are shaded in light gray for MitoFates, MitoProt and Target P and above 65 % for Predotar.