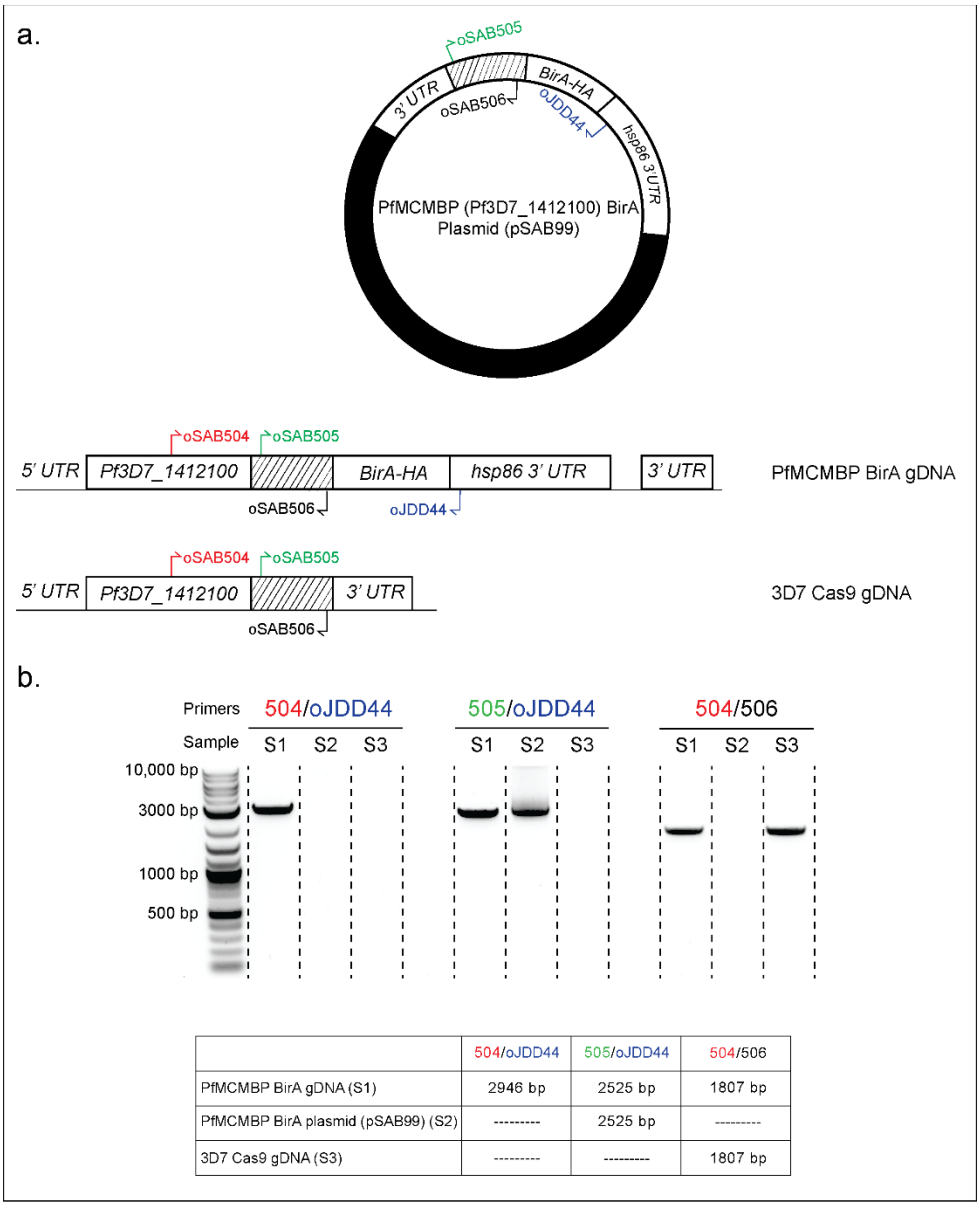
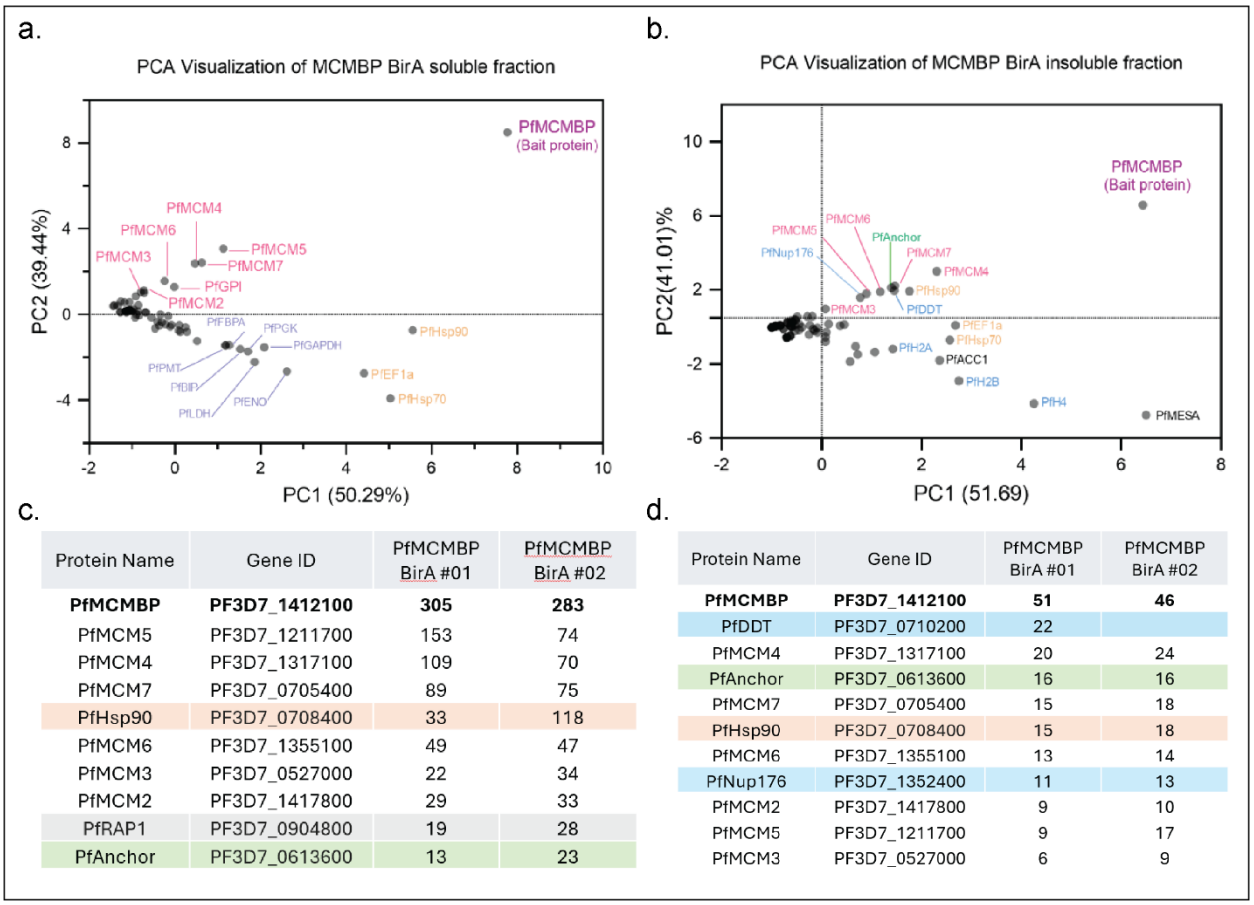


	504/oJDD44	505/oJDD44	504/506
PfMCMBP BirA gDNA (S1)	2946 bp	2525 bp	1807 bp
PfMCMBP BirA plasmid (pSAB99) (S2)	---------	2525 bp	---------
3D7 Cas9 gDNA (S3)	---------	---------	1807 bp

Figure S1: Generation and confirmation of the PfMCMBP BirA-HA cell line.

a. Schematic representation of the PfMCMBP (Pf3D7_1412100) plasmid and locus before and after integration of the BirA-HA tag. 3D7 Cas9 parasites were transfected with pSAB99 containing a 3' homology region (marked with hashed lines) of PfAnchor, followed by a C-terminal BirA-HA. (**b**) PCR confirmation of successful integration of the BirA-HA tag into the *PfMCMBP* locus in the PfMCMBP BirA-HA cell line. PCR was performed using the primers indicated in the schematic to confirm correct integration.



c.

Protein Name	Gene ID	PfMCMBP BirA #01	PfMCMBP BirA #02
PfMCMBP	**PF3D7_1412100**	**305**	**283**
PfMCM5	PF3D7_1211700	153	74
PfMCM4	PF3D7_1317100	109	70
PfMCM7	PF3D7_0705400	89	75
PfHsp90	PF3D7_0708400	33	118
PfMCM6	PF3D7_1355100	49	47
PfMCM3	PF3D7_0527000	22	34
PfMCM2	PF3D7_1417800	29	33
PfRAP1	PF3D7_0904800	19	28
PfAnchor	PF3D7_0613600	13	23

d.

Protein Name	Gene ID	PfMCMBP BirA #01	PfMCMBP BirA #02
PfMCMBP	**PF3D7_1412100**	**51**	**46**
PfDDT	PF3D7_0710200	22	
PfMCM4	PF3D7_1317100	20	24
PfAnchor	PF3D7_0613600	16	16
PfMCM7	PF3D7_0705400	15	18
PfHsp90	PF3D7_0708400	15	18
PfMCM6	PF3D7_1355100	13	14
PfNup176	PF3D7_1352400	11	13
PfMCM2	PF3D7_1417800	9	10
PfMCM5	PF3D7_1211700	9	17
PfMCM3	PF3D7_0527000	6	9

Figure S2: Proximal labeling using PfMCMBP as bait identifies PfAnchor in both nuclear and cytosolic fractions.

(**a-b**). Principal component analysis (PCA) of mass spectrometry data from proximal labeling using PfMCMBP as bait. (**a**) shows the nuclear (soluble) fraction, and (b) shows the cytosolic (insoluble) fraction. Labeled proteins represent enriched interactors identified in each fraction. (**c-d**). Tables listing the number of peptides detected for proteins uniquely present in the +biotin condition from the nuclear (**c**) and cytosolic (**d**) fractions in the PfMCMBP proximity labeling experiments.

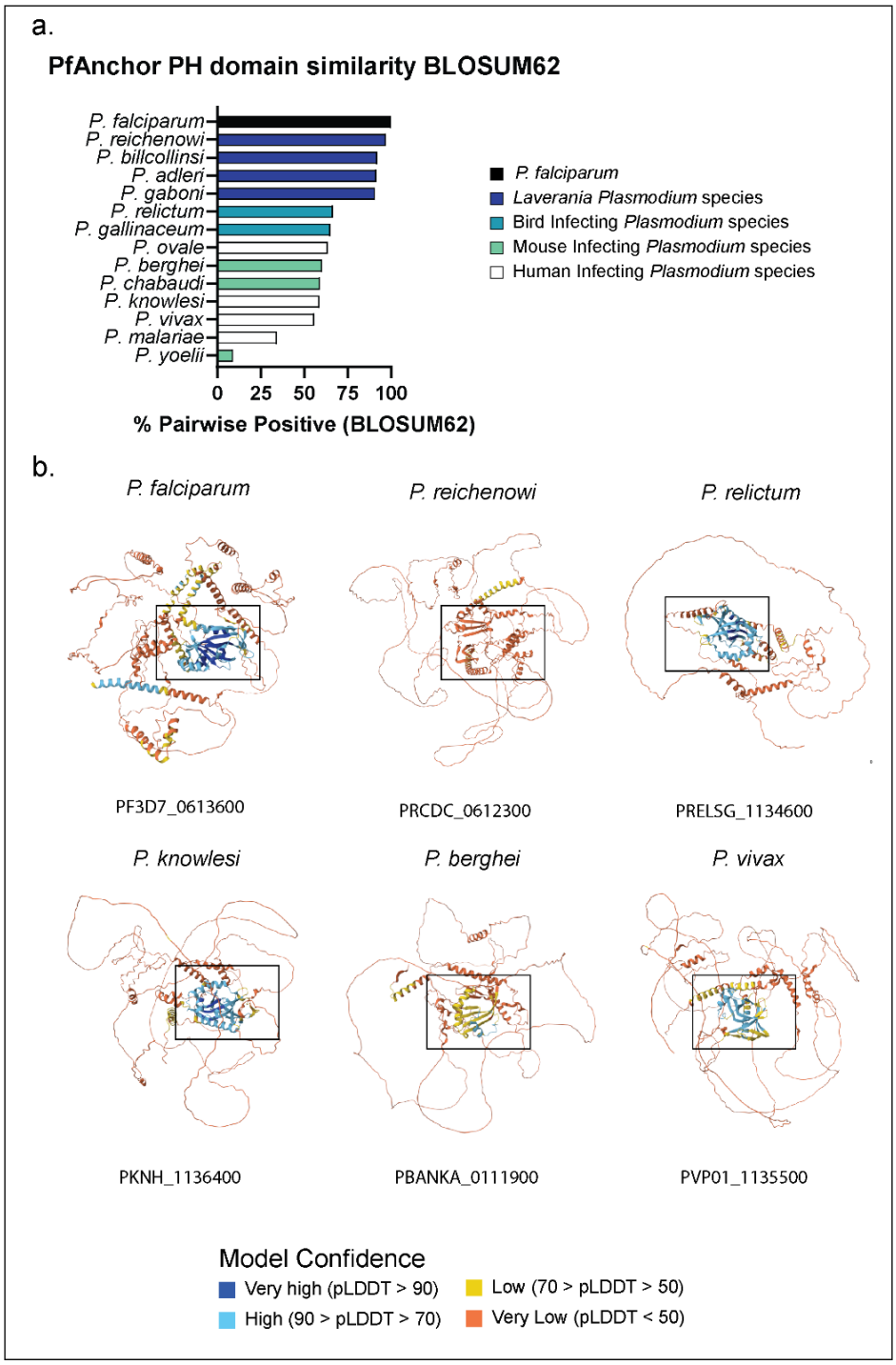
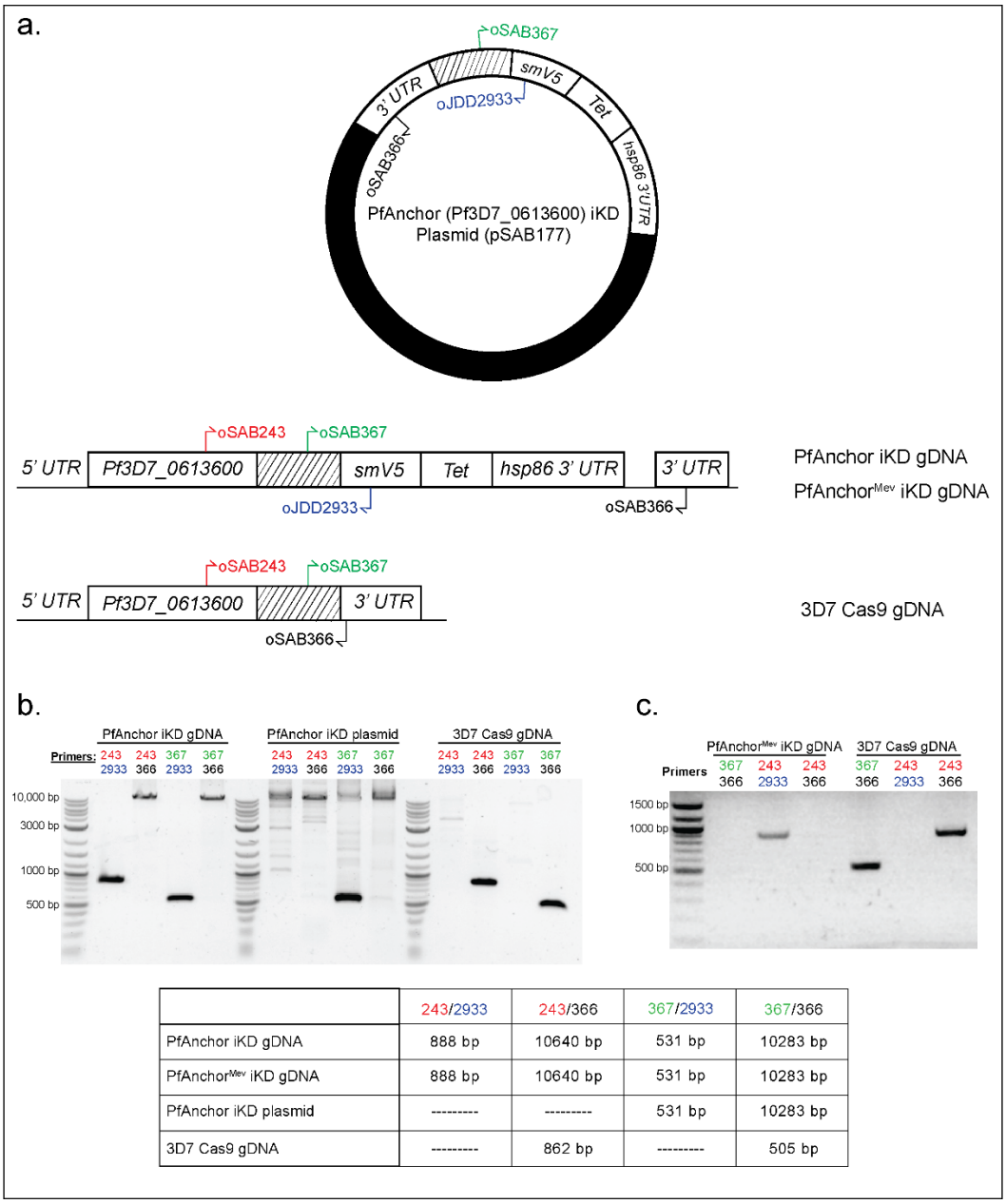


Figure S3: Conservation of the PfAnchor PH domain across *Plasmodium* species. a. Pairwise sequence similarity of the predicted PH domain across *Plasmodium* species based on BLOSUM62 scoring. *Plasmodium* species from the *Laverania* subgenus exhibit the highest similarity to PfAnchor. **b.** Alphafold structure predictions of PfAnchor across various *Plasmodium* species. The predicted PH domain (black box) is structurally conserved and modeled with high confidence in most *Plasmodium* species.



	243/2933	243/366	367/2933	367/366
PfAnchor iKD gDNA	888 bp	10640 bp	531 bp	10283 bp
PfAnchor^{Mev} iKD gDNA	888 bp	10640 bp	531 bp	10283 bp
PfAnchor iKD plasmid	---------	---------	531 bp	10283 bp
3D7 Cas9 gDNA	---------	862 bp	---------	505 bp

Figure S4: Confirmation of PfAnchor iKD and PfAnchor^{Mev} iKD cell lines. **a.** Schematic representation of the PfAnchor (Pf3D7_0613600) locus before and after integration of the smV5-Tet tag. 3D7 Cas9 parasites were transfected with a plasmid containing a 3' homology region of PfAnchor, followed by a C-terminal smV5-Tet tag. (**b-c**) PCR confirmation of successful integration of the smV5-Tet tag into the *PfAnchor* locus in both PfAnchor iKD and PfAnchor^{Mev} iKD cell lines using primers indicated in the schematic.

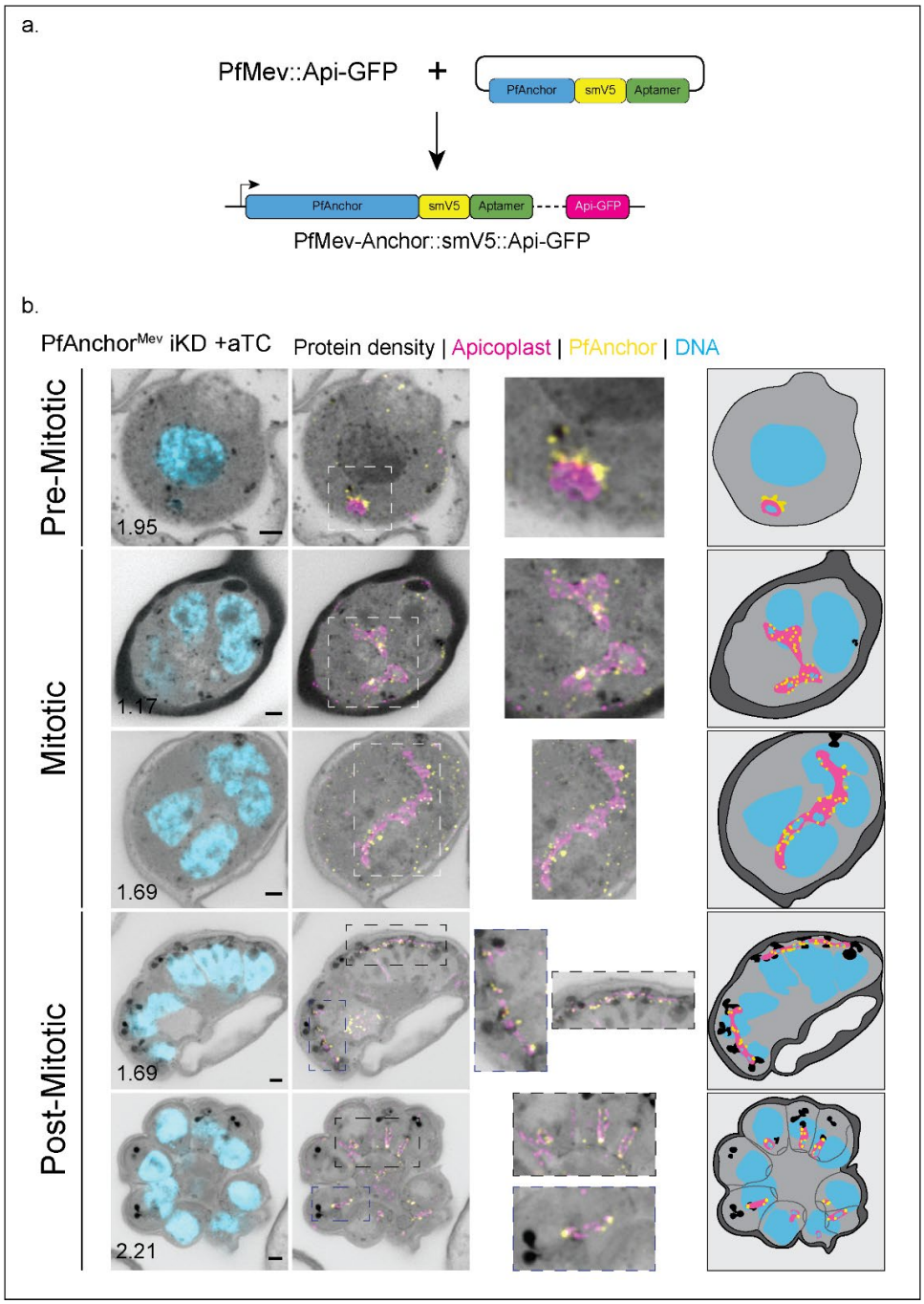


Figure S5: PfAnchor is expressed throughout the asexual life cycle and localizes around the apicoplast.

a. Schematic representation of the PfAnchor^{Mev} iKD cell line. This cell line expresses PfAnchor tagged with an smV5-Tet tag in the PfMev background, which also contains a GFP-tagged apicoplast marker **c.** U-ExM images showing the localization of PfAnchor with respect to the apicoplast throughout the asexual blood-stage cycle. Scale bars: 2 µm, with image depth (µm) indicated.

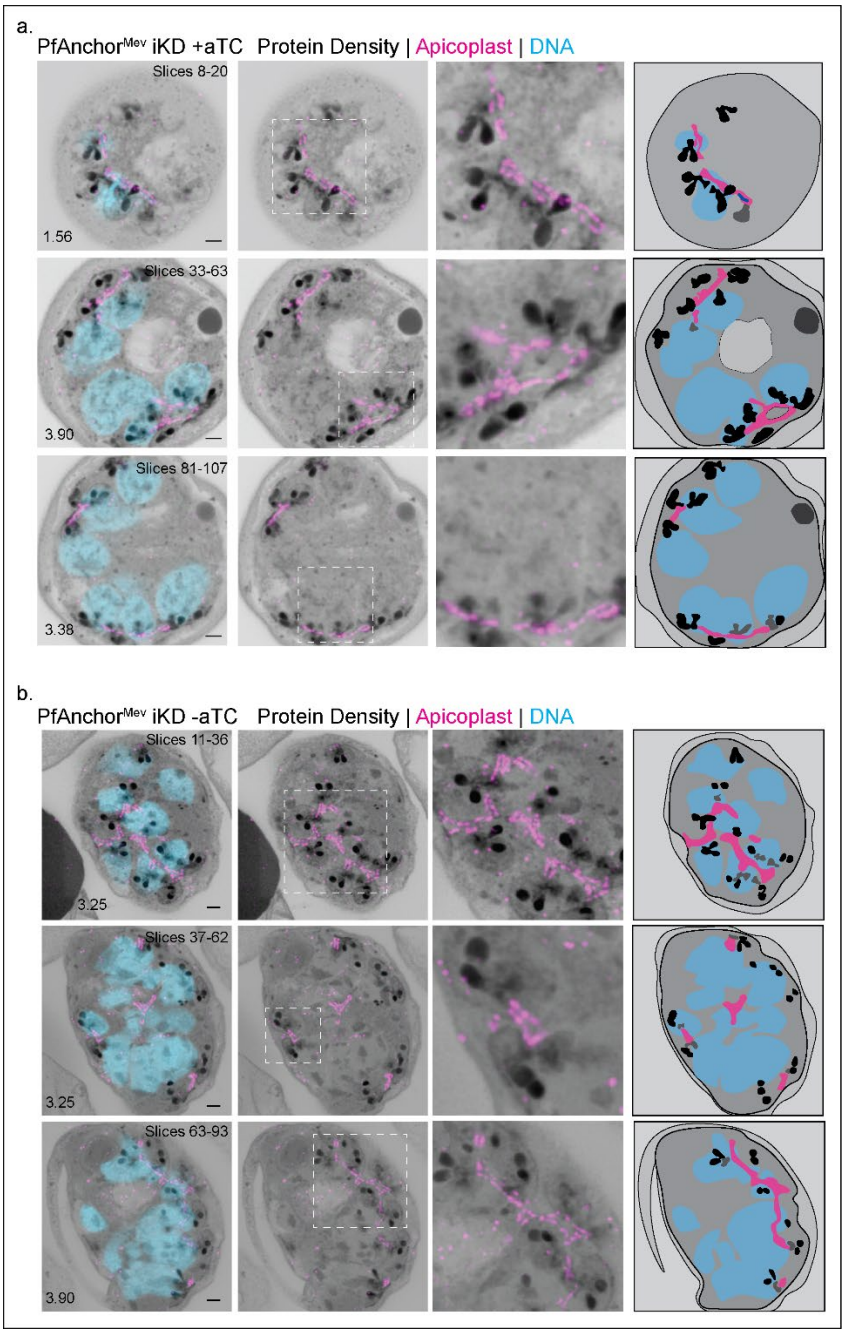


Figure S6: PfAnchor knockdown does not affect apicoplast positioning or "crown" morphology upon fission.

Representative U-ExM images showing the apicoplast's characteristic "crown" morphology upon fission in PfAnchor-expressing (**+aTC, panel a**) and PfAnchor-deficient (**-aTC, panel b**) parasites. In both conditions, the apicoplast remains a single branching organelle that associates with the centriolar plaques (CPs) during early segmentation, suggesting that PfAnchor depletion does not impair apicoplast positioning before cytokinesis. Apicoplasts (magenta), DNA (cyan), and protein density (grayscale) are shown. Scale bars: 2 μm.

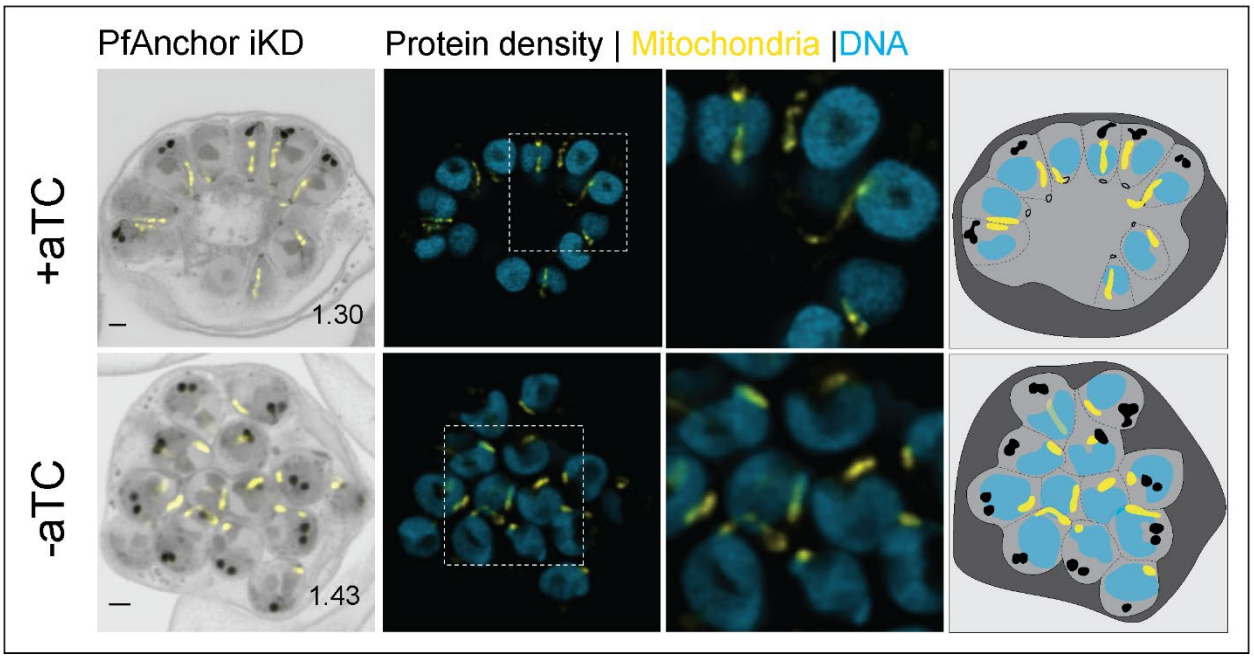


Figure S7: PfAnchor knockdown does not affect mitochondrial division and inheritance. U-ExM images of parasites during cytokinesis, showing mitochondrial organization in PfAnchor-expressing (+aTC) and PfAnchor-deficient (–aTC) conditions. Mitochondrial signal (yellow) is observed to undergo proper segmentation in both conditions, indicating that PfAnchor depletion does not impair mitochondrial division and inheritance. Scale bars: 2 µm, with image depth (µm) indicated.

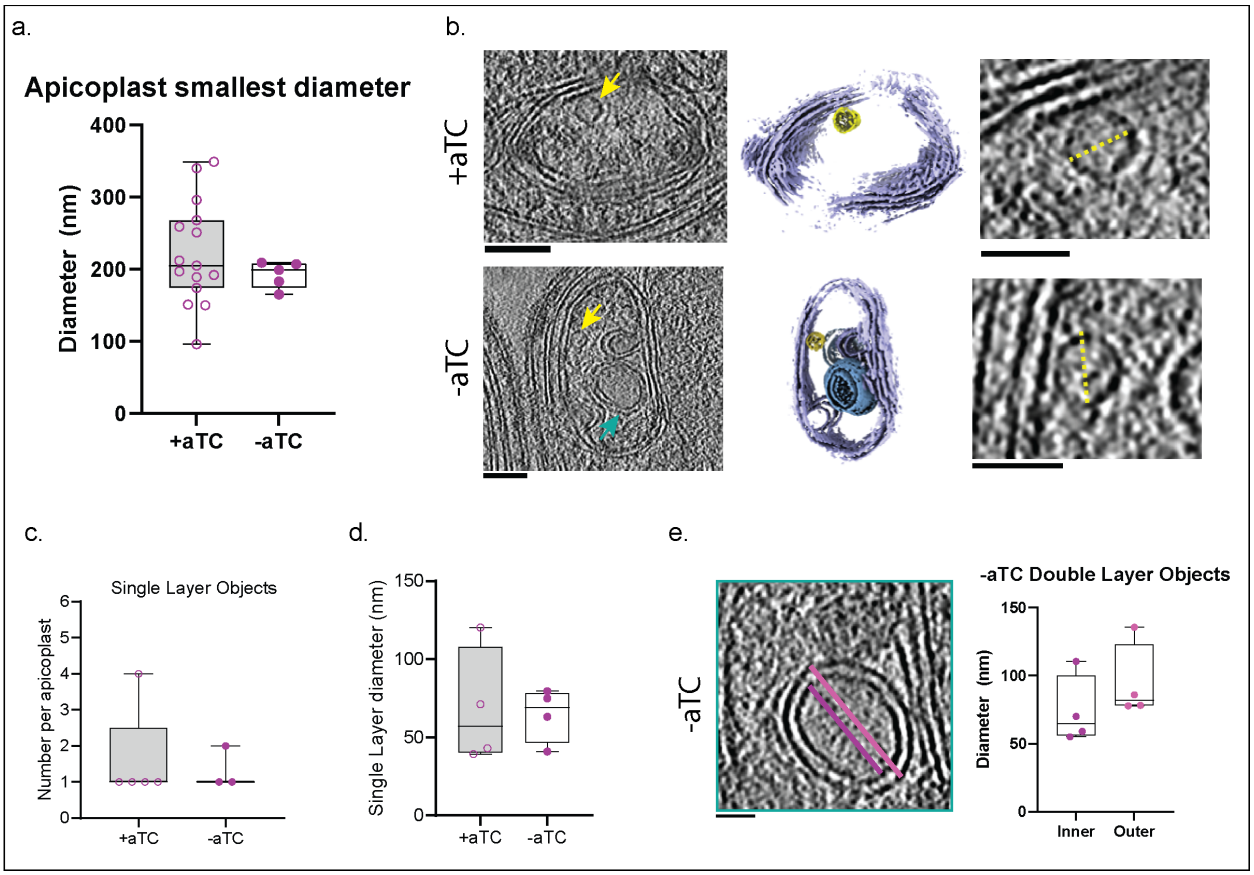


Figure S8: Cryo-electron tomography (Cryo-ET) reveals internal apicoplast structures in PfAnchor-expressing and deficient parasites.

a. Measurement of the smallest internal apicoplast diameter shows no significant difference between PfAnchor-expressing (+aTC) and PfAnchor-deficient (−aTC) parasites. **b.** Representative Cryo-ET images show single- and double-layered membranous structures inside the apicoplast lumen in PfAnchor-expressing and deficient parasites. Yellow arrows highlight these structures. **c.** Quantification of the number of single-layered membrane structures per apicoplast in PfAnchor-expressing and PfAnchor-deficient parasites. **d.** Quantification of the diameter of the single-layered membrane structures in the apicoplast lumen in PfAnchor-expressing and deficient parasites. **e.** Measurement of the inner (purple) and outer (magenta) diameters of double-layered membrane structures found inside the apicoplast lumen of PfAnchor-deficient parasites. All scale bars: 100 nm.

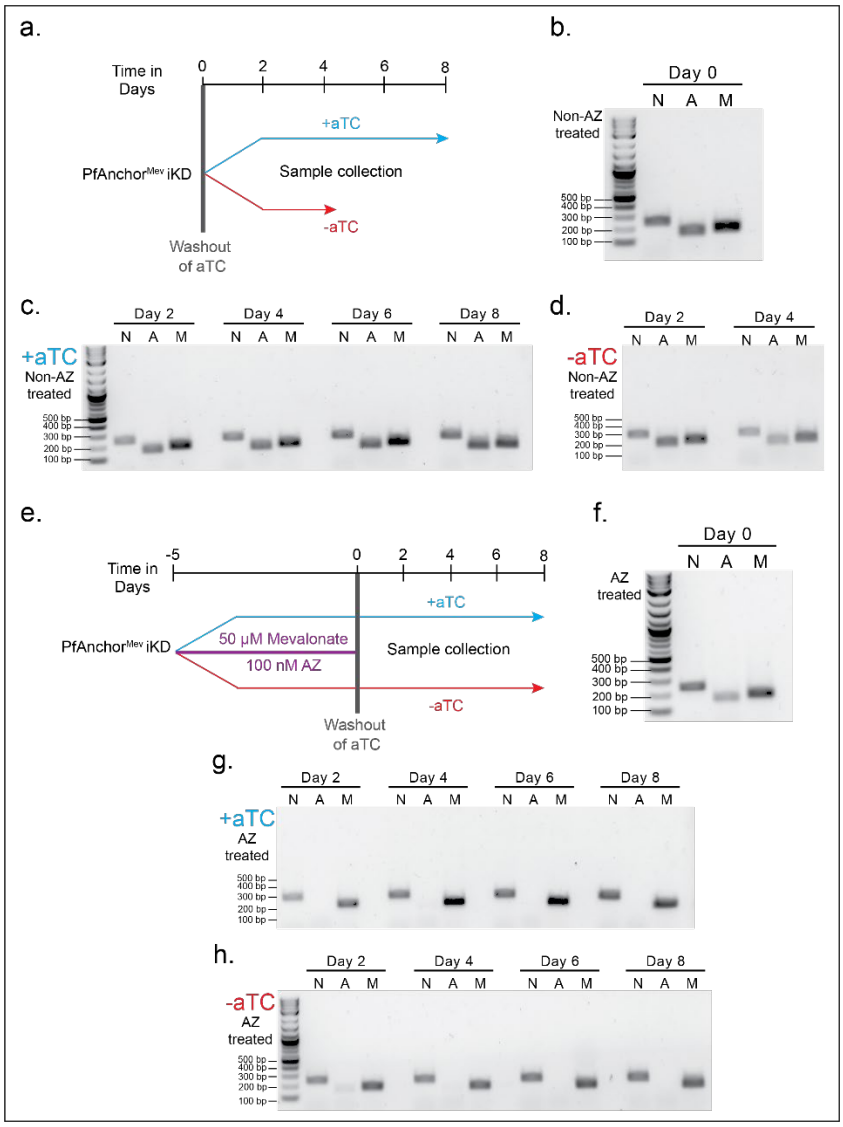


Figure S9: Loss of the apicoplast genome upon azithromycin (AZ) treatment in PfAnchor^{Mev} iKD parasites.

(**a, e**) Schematic representation of the experimental design. PfAnchorMev iKD parasites were grown in the presence or absence of aTC, with or without prior treatment with azithromycin (AZ) and mevalonate (Mev). For panels (**a–d**), parasites were cultured without mevalonate supplementation, thus mimicking PfAnchor depletion. After washout of aTC, samples were collected at the indicated time points. (**b, f**) DNA gel showing the presence of nuclear (N), apicoplast (A), and mitochondrial (M) genomes at Day 0. (**c, d**) Time-course DNA analysis of parasites without AZ treatment, in the absence (**c**) or presence (**d**) of aTC. In -aTC conditions, no parasites were detected after Day 4 due to lack of PfAnchor and Mev, so no samples were collected beyond this point. (**g, h**) In AZ-treated parasites (with Mev), apicoplast genome (A) was progressively lost over time in both +aTC and -aTC conditions, while nuclear (N) and mitochondrial (M) DNA remained detectable. Primers used: see Table S1

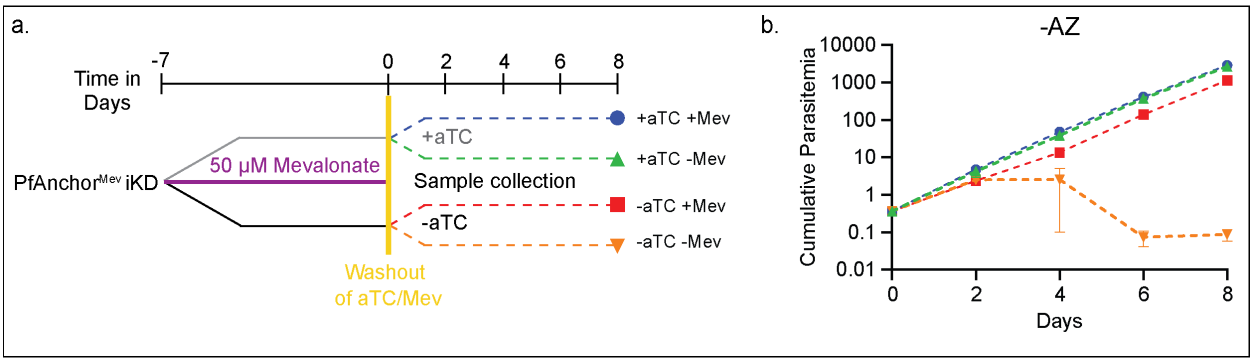


Figure S10: Supplementation of PfAnchor-deficient PfAnchor^{Mev} iKD -AZ parasites with Mev rescues parasite growth after one cycle.

a. Schematic of the experimental setup. PfAnchor^{Mev} iKD parasites were pre-treated with mevalonate (+Mev) for 7 days before prior to cell washing and maintaining under the indicated conditions (±aTC, ±Mev) for downstream analyses. **b.** Cumulative parasitemia of PfAnchor-expressing (+aTC) and PfAnchor-deficient (-aTC) parasites over 8 days in +AZ conditions. In AZ-treated parasites, disruption of the apicoplast resulted in the formation of apicoplast-like vesicles, which rescued the growth defect of PfAnchor-deficient parasites, but only in the presence of mevalonate (+Mev). Parasites lacking both PfAnchor and mevalonate (-Mev) failed to propagate. Parasites were sampled every 2 days for growth and PCR analysis, with cultures diluted 1:8 every 2 days to ensure continued replication. Data represent mean ± SD from 2 biological replicates in quadruplicate.

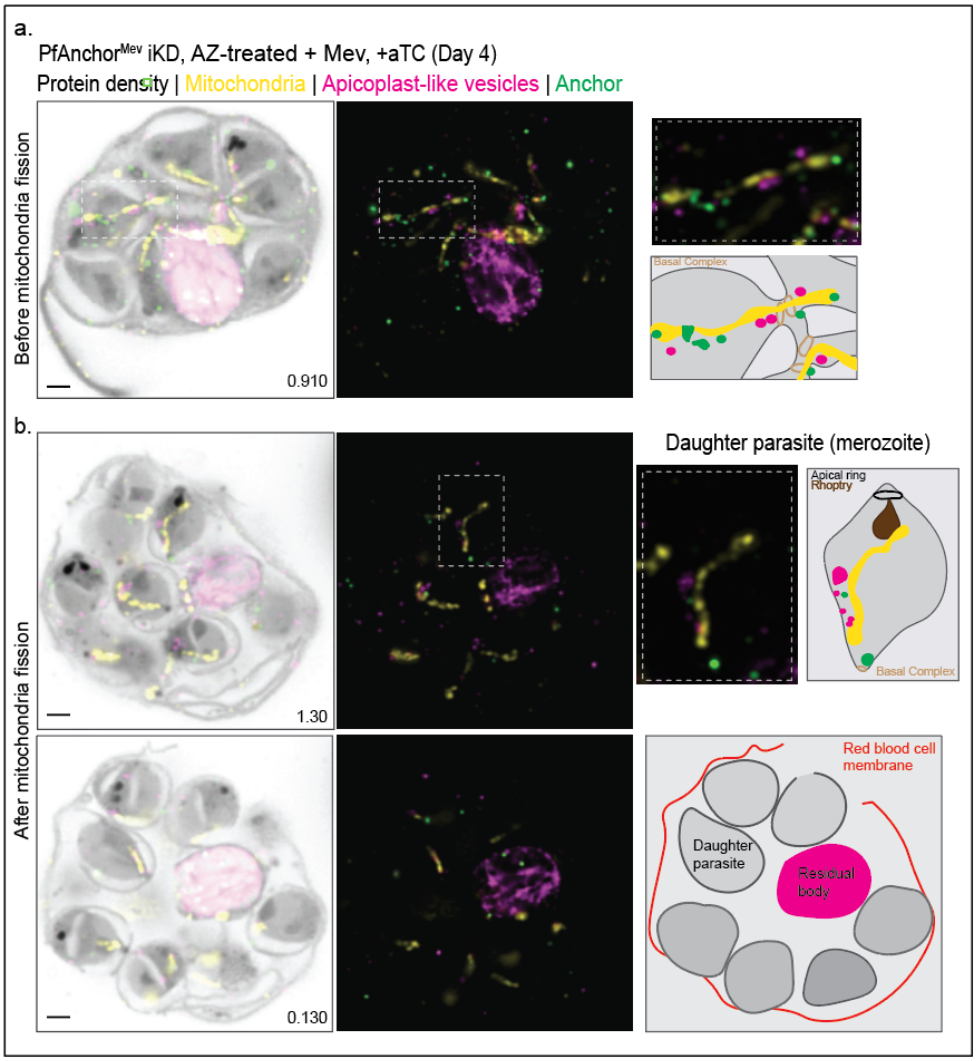


Figure S11: Apicoplast-like vesicles retained within daughter parasites are predominantly associated with mitochondria.

a. U-ExM image of a PfAnchor-expressing (+aTC) parasite treated with AZ/Mev, collected on Day 4 of the time-course experiment prior to mitochondrial fission. Most apicoplast-like vesicles (ACP-GFP, magenta) are localized to the residual body, with only a subset retained within developing daughter parasites. These retained vesicles are predominantly associated with mitochondria (yellow) and often colocalize with PfAnchor foci (green). Insets show zoomed-in views and a schematic representation of vesicle-mitochondria associations at the basal complex. **b**. U-ExM images of parasites collected after mitochondrial fission. Apicoplast-like vesicles are now more frequently retained within daughter parasites and remain associated with mitochondria. Right panels show higher magnification of the vesicle-mitochondrion association within an individual merozoite (top), and a schematic overview of parasite organization within the red blood cell (bottom). Protein density is shown in grayscale. Scale bars: 2 µm, with image depth (µm) indicated.

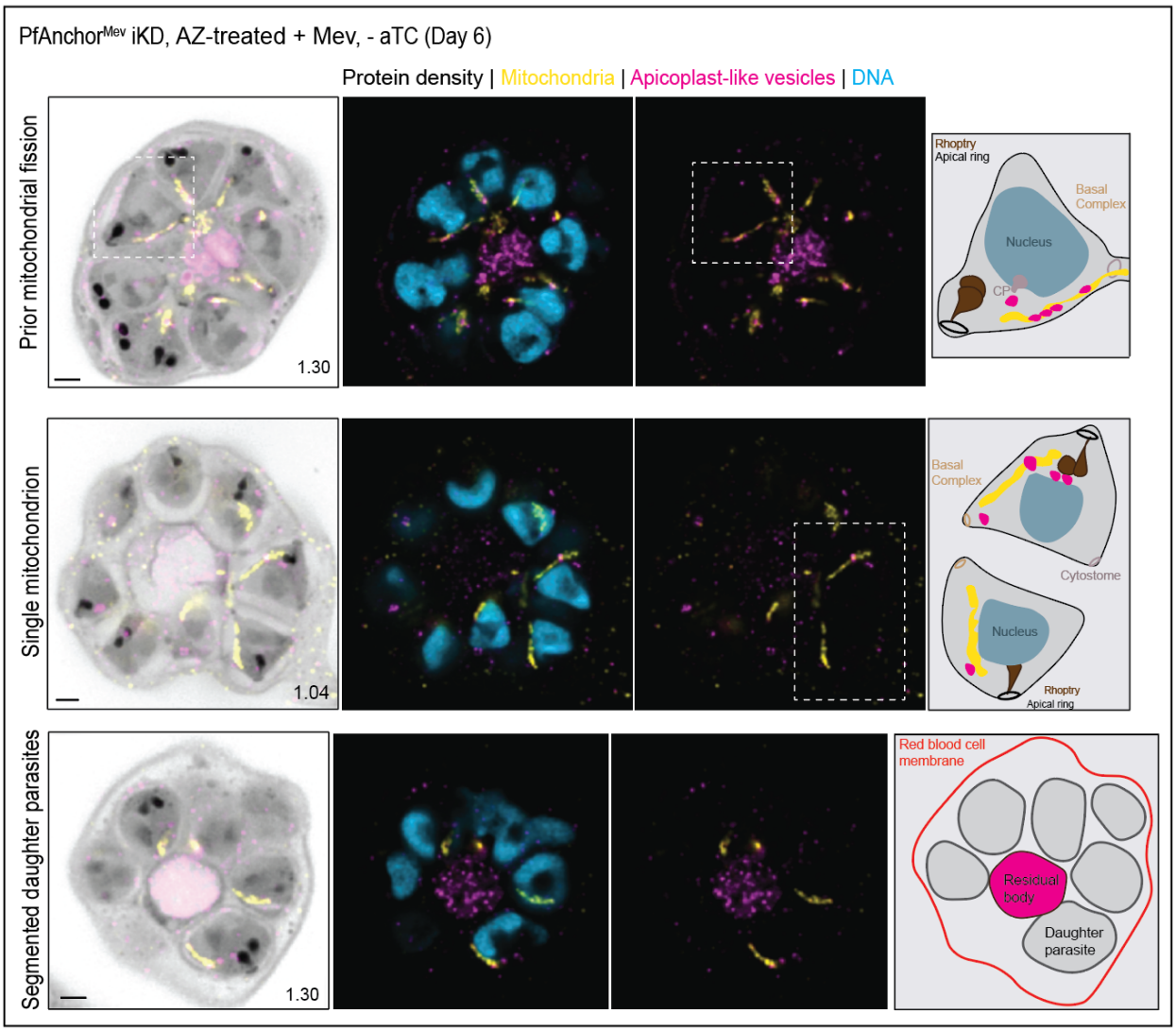
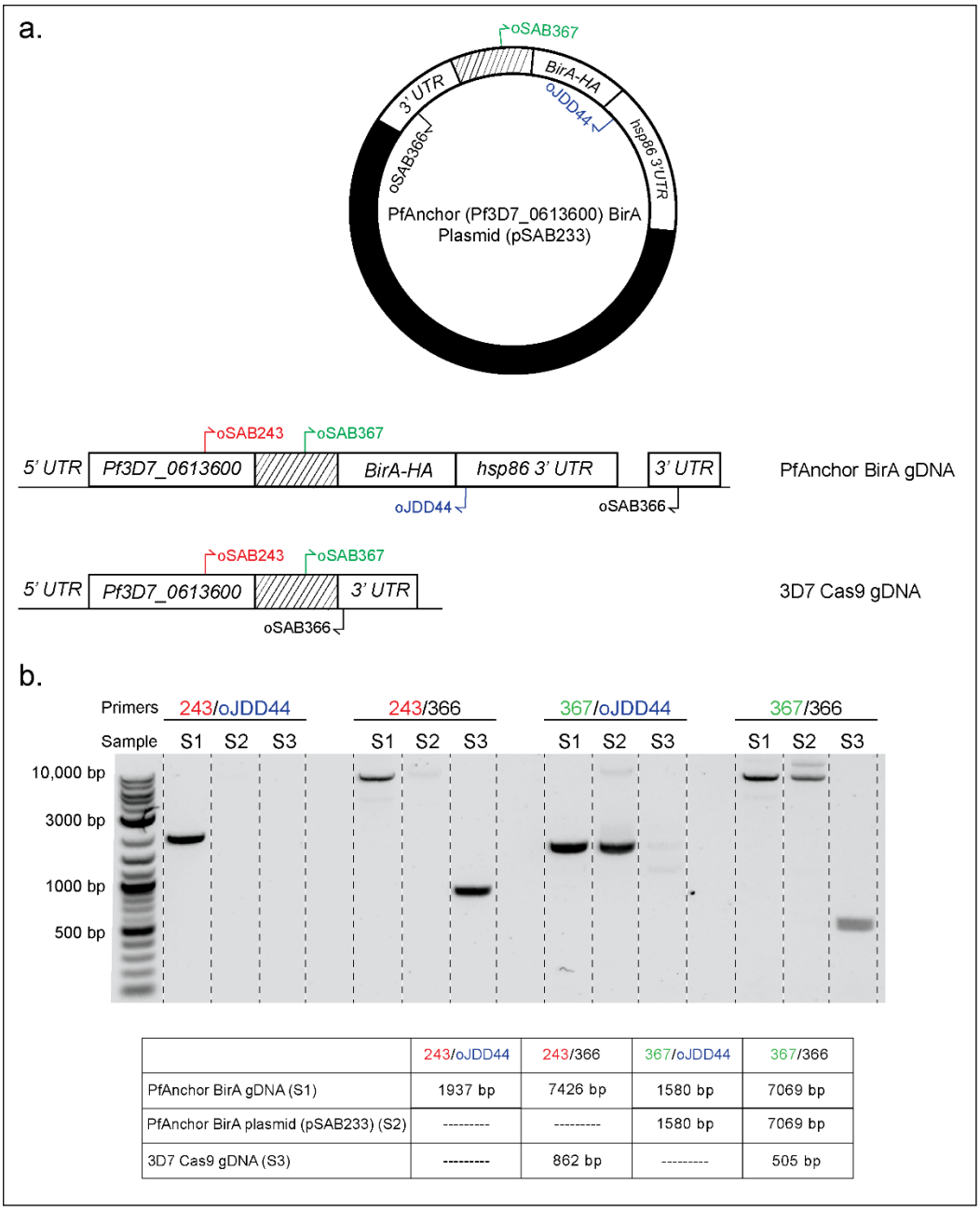


Figure S12: PfAnchor is not required for apicoplast-like vesicle inheritance.
U-ExM images of PfAnchor-deficient (-aTC) parasites treated with AZ/Mev, collected six days after aTC removal during cytokinesis. Similar to PfAnchor-expressing (+aTC) parasites, the majority of apicoplast-like vesicles (ACP-GFP, pink) localize to the residual body, while a subset remains associated with mitochondria (yellow) within daughter parasites. DNA is labeled in blue. Insets highlight vesicle-mitochondria interactions and their spatial organization within the developing parasite. These findings indicate that PfAnchor is not required for apicoplast-like vesicle inheritance. Scale bars: 2 µm, with image depth (µm) indicated.



	243/oJDD44	243/366	367/oJDD44	367/366
PfAnchor BirA gDNA (S1)	1937 bp	7426 bp	1580 bp	7069 bp
PfAnchor BirA plasmid (pSAB233) (S2)	---------	---------	1580 bp	7069 bp
3D7 Cas9 gDNA (S3)	---------	862 bp	---------	505 bp

Figure S13: Generation and confirmation of the PfAnchor BirA-HA cell line.

a. Schematic representation of the PfAnchor (Pf3D7_0613600) locus before and after integration of the BirA-HA tag. 3D7 Cas9 parasites were transfected with pSAB233 containing a 3' homology region (marked with hashed lines) of PfAnchor, followed by a C-terminal BirA-HA. (**b**) PCR confirmation of successful integration of the BirA-HA tag into the *PfAnchor* locus in the PfAnchor BirA-HA cell line. PCR was performed using the primers indicated in the schematic to confirm correct integration.

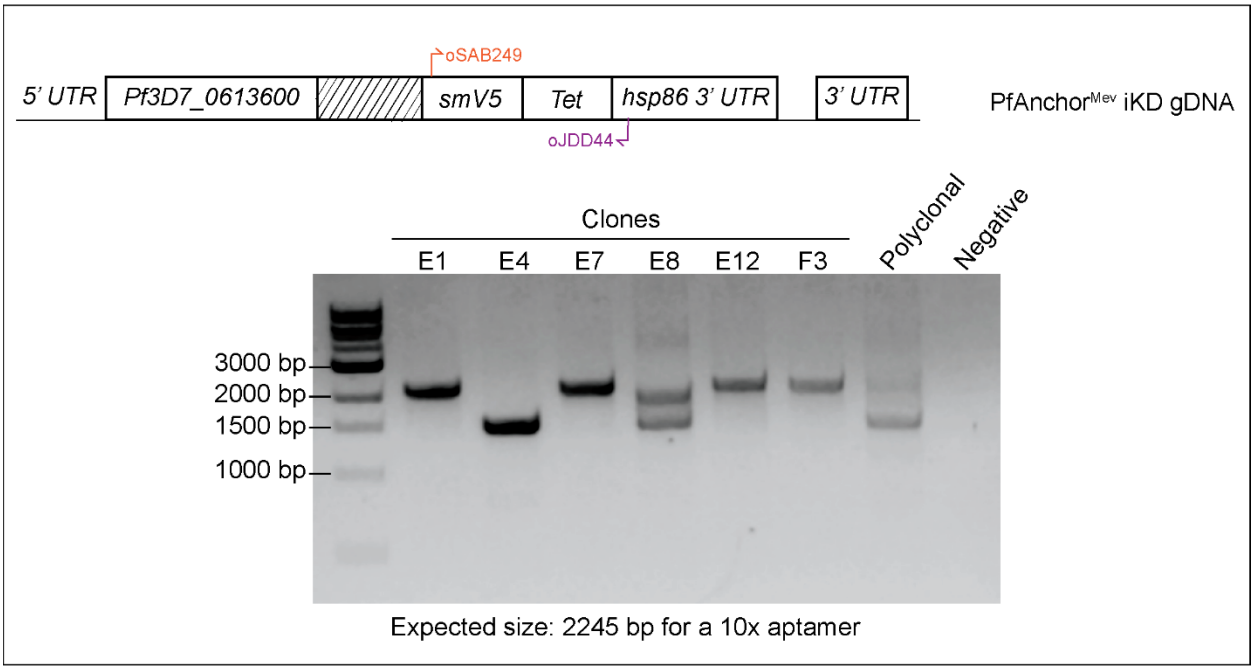


Figure S14: PCR Confirmation of 10x aptamer in the PfAnchor^{Mev} iKD cell line.
a. PfAnchor^{Mev} iKD parasites were subcloned by limiting dilution before parasite clones were selected and screened for a 10x aptamer for the TetR-DOZI aptamer knockdown system. PCR primers oSAB249 and oJDD44 were used to amplify genomic DNA. Clones E1, E7, E12, and F3 showed a 10x aptamer (2245 bp PCR product) and clone F3 was selected for future experiments.

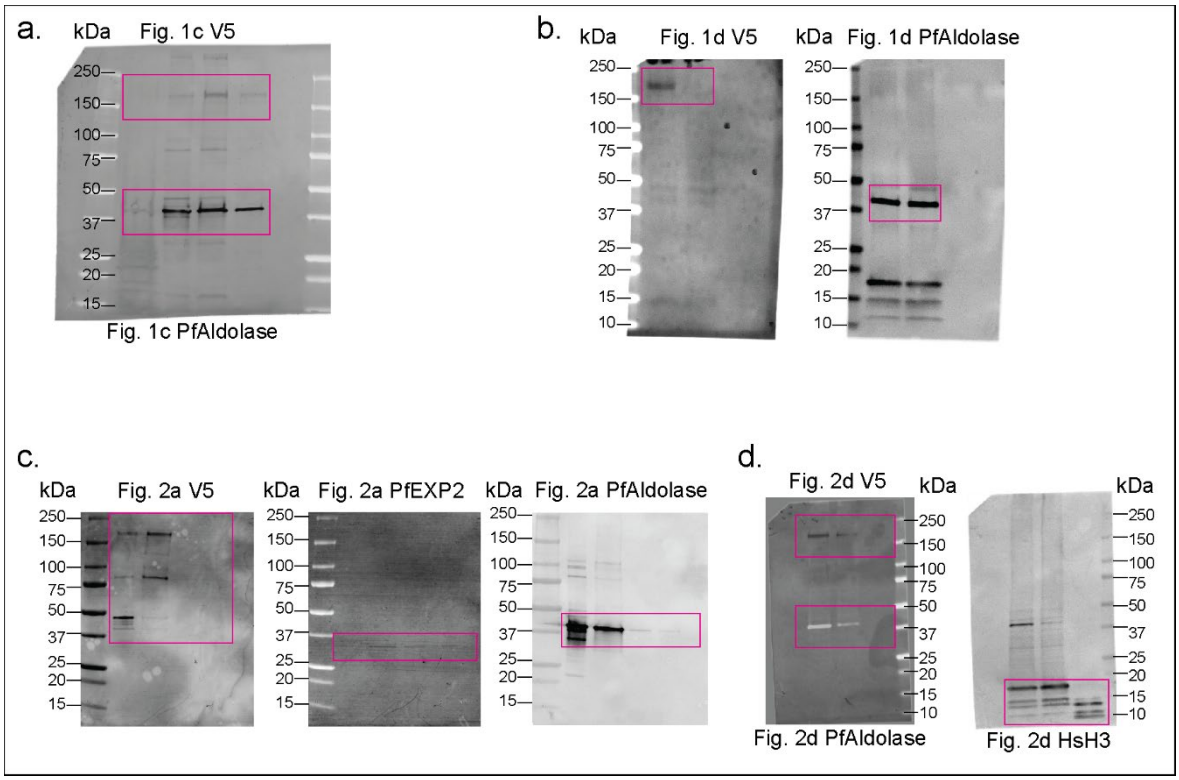


Figure S16: Full-sized images of all immunoblots. Full-sized immunoblots from Figure 1 (a-c) and Figure S4 (d) are shown. The magenta box on each immunoblot indicates the cropped area displayed for the figures.

Table S1: PCR primers used in this study.

Primer	Use	Sequence
oJDD44	PfAnchor aptamer hsp86 3' UTR reverse	TGGGGTGATGATAAAATGAAAG
oJDD56	PfMCMBP reverse plasmid integration	ACACTTTATGCTTCCGGCTCGTATGTTGTG
oJDD2933	PfAnchor integration smV5 reverse	CTGCTGCTGAGTACTATCAAGTC
oJDD4307	PfAnchor plasmid building 3HR forward	ggccgcAAGGGCACAAAATAAAATCAAGCATATATGGA
oJDD4308	PfAnchor plasmid building 5HR forward	cacgtgaggcctccgcggACATCAGCTTAATAATTTAGAGCTAGCTATAC
oJDD4309	PfAnchor plasmid building 3HR reverse	TAAGCTGATGTccgcggaggcctcacgtgCTACATTTATGGGGTCAAGGATATA
oJDD4310	PfAnchor plasmid building 5HR reverse	ACGTTAACAAAAAGTTAAACCAGtggatCcaTgtCttTaaAcAAGAGAGAAACATAAAGCACCTGTGAccatggATGGGAAAAC
oJDD4347	PfAnchor guide plasmid building forward	TATAATATTgatggatacacgtattcaagcGTTTTAGAGCTAGAAATAGCAAGTTAA
oJDD4348	PfAnchor guide plasmid building reverse	GCTCTAAAACgcttgaatacgtgtatccatcAATATTATATACTTAATATGAAATATG
oJDD4803	PfAnchor SOE-PCR forward	TGACCCCATAAATGTAGccgcgggatatcaggcctCATCAGCTTAATAATTTAGAGCTAGCTATAC
oJDD4804	PfAnchor SOE-PCR reverse	GATccatggCAGATGCTTGATGTTCCTCTCTTgTttAaaCacAtgGatccaCTGATTTAATTTTTTATTAACATAATG
oSAB243	PfAnchor integration Anchor sequence forward	CAGATCAAAGAAGTGTCACAGGAATATC
oSAB249	PfAnchor aptamer number smV5 forward	CCATGGATGGGAAAACCTATACCGAAC
oSAB356	PfAnchor plasmid building 3HR forward	CATCGCGGCCGCAAGGGCACAAAATAAAATCAAGCATATATGGA
oSAB358	PfAnchor guide plasmid building 5HR reverse with XhoI cutsite	ATATTGATGGATACACGTATTCAAGC
oSAB366	PfAnchor integration 3' UTR reverse	CCATATATGCTTGATTTTATTTTGTGCCC
oSAB367	PfAnchor integration plasmid forward	CATCAGCTTAATAATTTAGAGCTAGCTATAC
oSAB484	PfGAPDH forward for Apicoplast genome analysis	ATCAAAGGGTGGTAAGGACTGG
oSAB485	PfGAPDH reverse for Apicoplast genome analysis	AGTGGACCTTCAGCAGCTTTTT
oSAB486	PfTufA forward for Apicoplast genome analysis	GATATTGATTCAGCTCCAGAAGAAA
oSAB487	PfTufA reverse for Apicoplast genome analysis	ATATCCATTTGTGTGGCTCCTATAA

oSAB488	PfCytb3 forward for Apicoplast genome analysis	AGATACATGCACGCAACAGG
oSAB489	PfCytb3 reverse for Apicoplast genome analysis	TCATTTGACCCCATGGTAAGA
oSAB504	PfMCMBP forward plasmid integration	TTCGGTAGCCTCATCAACGGACATATCT
oSAB505	PfMCMBP forward plasmid integration	GACGTAATCGAAATTATTGGAATATATCGT
oSAB506	PfMCMBP reverse plasmid integration	CATAATATAATCCCAGTGATCCCTA

Table S2: Cell lines used in this study.

Cell line	Description
pSAB99	3D7 PfMCMBP^{BioID}
pSAB177	3D7 PfAnchor$^{smV5\text{-}Tet}$
PfMev-AnchorsmV5-Tet	NF54 PfMev-Anchor$^{smV5\text{-}Tet}$
pSAB233	3D7 PfAnchor^{BioID}

Table S3: Primary antibodies, secondary antibodies, and stains used in this study.

Antibody/Stain	Catalog number	Concentration	Use
Rabbit Anti-GFP	OriGene (TP401)	1:2000	U-ExM
Chicken Anti-GFP	Abcam (ab13970)	1:2000	U-ExM
Anti-Aldolase	Abcam (ab207494)	1:1000	Western Blot
Anti-Histone H3	Abcam (ab1791)	1:2000	Western Blot
Anti-V5 (Sy30-01)	biorad serotech MCA1360	1:250, 1:1000	U-ExM, Western Blot
Anti-HSP60	Invitrogen: PA5-34760	1:100	U-ExM
Anti-EXP2	Gift from Dr. Vasant Muralidarhan	1:1000	Western Blot
Anti-mouse IgG Alexa Fluor 555	Thermo Fisher (A21428)	1:500	U-ExM
Anti-mouse IgG Alexa Fluor 568	Thermo Fisher (A11004)	1:500	U-ExM
Anti-rabbit IgG Alexa Fluor 488	Thermo Fisher (A11034)	1:500	U-ExM
Anti-rabbit IgG Alexa Fluor 555	Thermo Fisher (A21428)	1:500	U-ExM
Anti-rabbit IgG Alexa Fluor 633	Thermo Fisher (A21070)	1:500	U-ExM
Anti-rat IgG Alexa Fluor 488	Thermo Fisher (A11006)	1:500	U-ExM
Anti-Chicken Alexa Fluor 488	Thermo Fisher (A78948)	1:500	U-ExM
Anti-mouse Starbright Blue 520	BioRad 12005867	1:2000	Western Blot
Anti-Rabbit Starbright Blue 700	BioRad 12004159	1:2000	Western Blot
NHS ester Alexa Fluor 405	Thermo Fisher (A30000)	1:250 (8 µM) in DMSO	U-ExM
SYTOX Deep Red	Thermo Fisher (S11381)	1:1000 (1 µM) in DMSO	U-ExM