

Supplemental information for

**Experimental Genetics of *Plasmodium berghei* NFU
in the Apicoplast Iron-Sulfur Cluster Biogenesis Pathway**

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Contents:

- **Supplemental Figure S1**
- **Supplemental Table S1 and S2**

Haussig et al., Figure S1

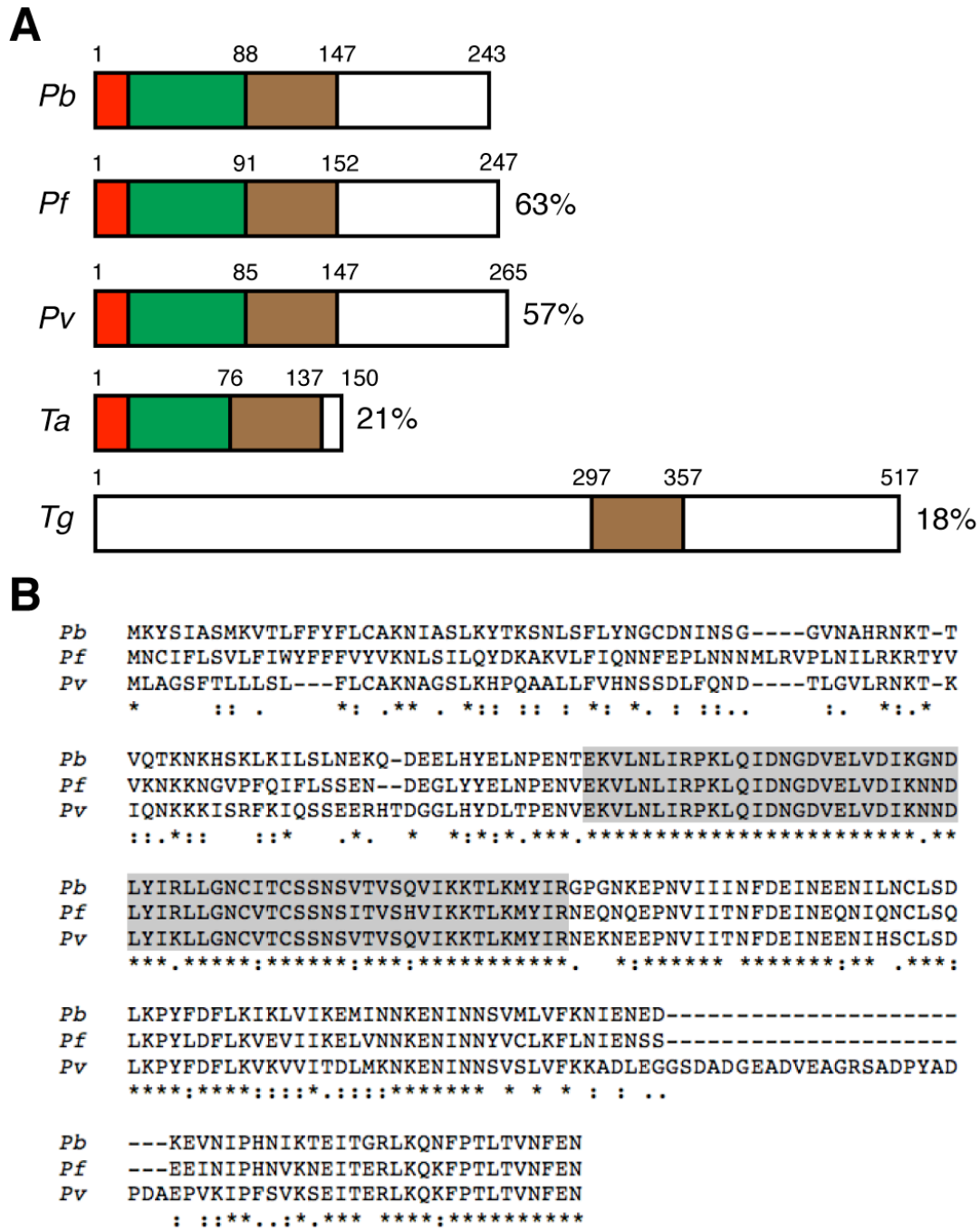


Figure S1. Apicomplexan NFUapi proteins. (A) Primary structure of representative apicomplexan NFUapi proteins. Shown are the overall sequence structures and amino acid sequence identities of NFUapi orthologs in *P. falciparum* (PF3D7_0921400), *P. vivax* (PVX_099490), *Theileria annulata* (TA19885), and *Toxoplasma gondii* (TGME49_021920) compared with *P. berghei* NFUapi (PBANKA_082230). Signal peptide (red), apicoplast-targeting sequence (green), and the NifU-like domain (brown) are shown. (B) Sequence alignment of three *Plasmodium* NFUapi proteins. The NifU-like domain (gray shading) is well conserved.

Table S1. NifU-like domain containing proteins.

Species	Gene Name	Gene ID	Source	Species	Gene Name	Gene ID	Source
<i>APICOMPLEXA</i>				<i>KINETOPLASTIDA</i>			
<i>Plasmodium berghei</i>	<i>PbNFUapi</i>	PBANKA_082230	PlasmoDB	<i>Leishmania major</i>	<i>LmjNFU1</i>	LmjF.26.0190	TriTrypDB
	<i>PbNFU1</i>	PBANKA_083170	PlasmoDB		<i>LmjNFU2</i>	LmjF.36.0880	TriTrypDB
	<i>PbISU</i>	PBANKA_131820	PlasmoDB		<i>LmjISU</i>	LmjF.35.3620	TriTrypDB
<i>Plasmodium chabaudi</i>	<i>PcNFUapi</i>	PCHAS_082260	PlasmoDB	<i>Trypanosoma brucei</i>	<i>TbNFU1</i>	Tbg972.7.1730	TriTrypDB
	<i>PcNFU1</i>	PCHAS_083200	PlasmoDB		<i>TbNFU2</i>	Tbg972.10.6450	TriTrypDB
	<i>PcISU</i>	PCHAS_132150	PlasmoDB		<i>TbISU</i>	Tbg972.9.6980	TriTrypDB
<i>Plasmodium yoelii</i>	<i>PyNFUapi</i>	PY02489	PlasmoDB	<i>Trypanosoma cruzi</i>	<i>TcNFU1</i>	Tc00.1047053508207.130	TriTrypDB
	<i>PyNFU1</i>	PY06884	PlasmoDB		<i>TcNFU2</i>	Tc00.1047053506679.60	TriTrypDB
	<i>PyISU</i>	PY00856	PlasmoDB		<i>TcISU</i>	Tc00.1047053507009.90	TriTrypDB
<i>Plasmodium knowlesi</i>	<i>PkNFUapi</i>	PKH_071860	PlasmoDB	<i>PLANTAE</i>			
	<i>PkNFU1</i>	PKH_072920	PlasmoDB	<i>Chlamydomonas reinhardtii</i>	<i>CrNIFU1</i>	XP_001695148	GenBank
	<i>PkISU</i>	PKH_125200	PlasmoDB		<i>CrNIFU2</i>	XP_001690805	GenBank
<i>Plasmodium vivax</i>	<i>PvNFUapi</i>	PVX_099490	PlasmoDB		<i>CrNIFU3</i>	XP_001691526	GenBank
	<i>PvNFU1</i>	PVX_087160	PlasmoDB	<i>CrISU1</i>	XP_001693712	GenBank	
	<i>PvISU</i>	PVX_117755	PlasmoDB	<i>Arabidopsis thaliana</i>	<i>AtNFU1</i>	NP_567219	GenBank
<i>Plasmodium falciparum</i>	<i>PfNFUapi</i>	PF3D7_0921400	PlasmoDB		<i>AtNFU2</i>	NP_568715.1	GenBank
	<i>PfNFU1</i>	PF3D7_0930900	PlasmoDB		<i>AtNFU3</i>	NP_567735	GenBank
	<i>PfISU</i>	PF3D7_1454500	PlasmoDB		<i>AtNFU4</i>	NP_566673	GenBank
<i>Babesia bovis</i>	<i>BbNFU1</i>	BBOV_III006970	PiroplasmaDB		<i>AtNFU5</i>	NP_175550	GenBank
	<i>BbISU</i>	BBOV_IV004050	PiroplasmaDB	<i>AtISU1</i>	NP_193953	GenBank	
<i>Theileria annulata</i>	<i>TaNFUapi</i>	TA19885	PiroplasmaDB	<i>AtISU2</i>	NP_186751	GenBank	
	<i>TaNFU1</i>	TA10900	PiroplasmaDB	<i>AtISU3</i>	NP_192317	GenBank	
	<i>TaISU</i>	TA19345	PiroplasmaDB	<i>FUNGI</i>			
<i>Theileria parva</i>	<i>TpNFUapi</i>	TP01_0062	PiroplasmaDB	<i>Cryptococcus neoformans</i>	<i>CnNfu_N</i>	XP_571879	GenBank
	<i>TpNFU1</i>	TP04_0610	PiroplasmaDB		<i>CnlscU</i>	XP_569269	GenBank
	<i>TpISU</i>	TP01_0192	PiroplasmaDB	<i>Saccharomyces cerevisiae</i>	<i>ScNfu1</i>	NP_012884	GenBank
<i>Cryptosporidium muris</i>	<i>CmISU</i>	CMU_027160	CryptoDB		<i>ScIsu1</i>	NP_015190	GenBank
	<i>CpISU</i>	cgd6_1050	CryptoDB		<i>ScIsu2</i>	NP_014869	GenBank
<i>Eimeria tenella</i>	<i>EtNFUapi</i>	ETH_00036850 + ETH_00021275	ToxoDB	<i>ANIMALIA</i>			
<i>Neospora caninum</i>	<i>EtNFU1</i>	ETH_00027735	ToxoDB	<i>Mus musculus</i>	<i>MmNfu1</i>	NP_001164062	GenBank
	<i>EtISU</i>	ETH_00024750	ToxoDB		<i>Mmlscu</i>	NP_079802	GenBank
	<i>NcNFUapi</i>	NCLIV_005330	ToxoDB	<i>Homo sapiens</i>	<i>HsNFU1</i>	NP_056515	GenBank
<i>NcNFU1</i>	NCLIV_013030	ToxoDB	<i>HsISCU</i>		NP_055116	GenBank	
<i>Toxoplasma gondii</i>	<i>NcISU</i>	NCLIV_051300	ToxoDB	<i>BACTERIA</i>			
	<i>TgNFUapi</i>	TGME49_021920	ToxoDB	<i>Azotobacter vinelandii</i>	<i>AvNifU</i>	YP_002797400	GenBank
	<i>TgNFU1</i>	TGME49_012930	ToxoDB				
<i>TgISU</i>	TGME49_037560	ToxoDB					

Table S2. Primer sequences.

Primer Name ^a	Primer Sequence (REase sites underlined)	REase	Size WT (bp) ^b	Size INT (bp) ^c
5'PbHSP70rev	CAATTTGTTGTACATAAAATAGGCAG			
5'PbDHFRrev	ATGAAATACCGCTCCATTTTTCC			
mCherryRev	CCCTCCATGTGAACCTTGAAG			
TV-5'NFU-F	TATCCGCGGTTTTTCTATAAAATGTGTGTGTAATGC	SacII	599	
TV-5'NFU-R	AAAGATATCACTCACAAAAATAAGCACATGATTG	EcoRV		
TV-C-NFU-F	AAACCGCGGCGTGTAGCTCAAATAGTGTAAGT	SacII	576	
TV-C-NFU-R	TAAGATATCATTTTTCAAATTAACAGTTAATGTTGGAAA	EcoRV		
TV-3'NFU-F	TATAAGCTTCAGCGGCTAGTCAATCTAATC	HindIII	439	
TV-3'NFU-R	TAAGGTACCGTTCATTTAAAACACCAGCGAG	KpnI		
GT-5'NFU-F	ATTTTTCTCTGGTTTTTCATTTATATTTTC		960	958
GT-5'NFU-R	TTGTTTTATTTCTGTGTGCATTAACC			
GT-C-NFU-F	GCCCCAAATTACAAATAGATAATGG		1106 ^d	758 ^d
GT-3'NFU-F	GAAAATGAAGATAAGGAAGTAAATATACC		702	
GT-3'NFU-R	TAATATACATAAATGTTATTGGGATCTTTTG			1210

^a TV and GT indicate primers designed for the generation of Transfection Vectors and GenoTyping.

^b Sizes of the PCR products of forward (F) and reverse (R) primers on WT gDNA.

^c Sizes of the respective integration-specific PCR products; forward 5' gene-specific primers combined with 5'PbHSP70rev and reverse 3' gene-specific primers combined with 5'PbDHFRrev.

^d WT-specific reaction with TV-3'NFU-R; integration-specific reaction with mCherryRev.