

## SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. ClustalW alignment of *Leishmania major* GCVP sequence (*Lm*) with sequences (GenBank sequence reference) from: *At*, *Arabidopsis thaliana* (GCVP2; CAA21210), *Dd*, *Dictyostelium discoideum* (XP\_637330), *Sp*, *Schizosaccharomyces pombe* (CAA91099), *Vc*, *Vibrio cholerae* (ZP\_00746488) and *Xl*, *Xenopus laevis* (AAH42245). N-terminal mitochondrial targeting peptides, predicted by TargetP, are shown by black letters on gray boxes. In the remainder of the sequence, degree of conservation of residues is indicated by increased background shading. Active-site residues are marked (#), with the catalytic lysine marked as (C). Residues predicted to interact with the H-protein are marked (\*).

Fig. S2. Growth of *L. major* WT FV1 and LV39 lines in RPMI-1% fetal bovine serum medium. *A*, *L. major* FV1; *B*, *L. major* LV39. Symbols: ●: RPMI including 285  $\mu$ M L-serine; ○: RPMI without added serine (serum contains  $\sim$ 10  $\mu$ M serine). Error bars indicate standard error of mean counts for triplicate cultures.

Fig. S3. Culture phenotype of *gcvP*- *Leishmania*. Growth in culture of WT, *gcvP*- and *gcvP*-/+GCVP parasites in RPMI media with 1% fetal calf serum and varied glycine and L-serine content. *A*, 133  $\mu$ M glycine and 95  $\mu$ M L-serine; *B*, 13 mM glycine and 95  $\mu$ M L-serine; *C*, 133  $\mu$ M glycine and 9.5  $\mu$ M L-serine; *D*, As *C*, with 10 mM formate added. Symbols: ●: WT; □: *gcvP*-; ▲: *gcvP*-/+GCVP parasites. Error bars indicate standard deviation of mean counts for triplicate cultures.

SUPPLEMENTAL TABLE

Table S1. DNA primers used, listed in order cited in text.

Primer #	Sequence 5'-3' Purpose	Notes
B2057	<u>cgaggatcccacc</u> ATGCTCCGCCGTCTCCTTCGCGTT GCVP ORF PCR	BamHI site underlined. Non-coding nucleotides in lower case
B2062	ataggatcc <b>GAGCT</b> CCGTTTCGCTCTTGTGGCTG GCVP ORF PCR	SacI site in bold. Non-coding nucleotides in lower case
B2063	atacatatgaaa <b>GAGCT</b> CATGCGTTACGCTCAGCATC GCVP ORF PCR	SacI site in bold. Non-coding nucleotides in lower case
B2058	<u>cacggate</u> CTAGTAGAACTCCAATGGGGCACA GCVP ORF PCR	BamHI site underlined. Non-coding nucleotides in lower case
B2137	AGCGTTGCCTTCGGGGTTCACAC PCR assay of gene disruption	
B2013	CCGTGGGCTTGTACTCGGTCAT PCR assay of gene disruption	
B1675	GACGTCGCGGTGAGTTCAGGCT PCR assay of gene disruption	
B2184	GTGGTGATGGAGCGCTGTATGGAG Southern blot probe	
B2185	CTTTGGTCAGACGCAGCACATACG Southern blot probe	
B2086	GAGGCTCACCGCGATGTCTG Amplifying end of GCVP minus stop codon	
B2497	ggaagaa <u>GATCT</u> GTAGAACTCCAATGGGGCACACGAG Amplifying end of GCVP minus stop codon	BglII site underlined. Non-coding nucleotides in lower case

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Lm : -----MLRRLRLRVHGVFAPAG-----40-----LART-STAYLNR-----80-----HIGPTRKETAEMLKTYGKES-----  
At : -----MERARRLAYRGIVKRLVNDT-----KRHRNAETPHLYPHAF-----ARYVSSLSPITSRVSNHTAAAFGRHQQTRISVDAVKPSDTFPRRHSATP  
Dd : -----MLKLLRNNNGINKLKS-----LIRNYSTKS-----IFQALDTPFKR-----HIGPNEINEIMKSTINTSKLSKKNP-----  
Sp : -----MFRACSKLQYHGCVNLSLRH-----FLAKRNLSSACTEAKNSQ-----KFPALDTEPR-----HIGPSTDDQYQIESGYKDFDSFLK-----  
Vc : -----MTELLHS-----LSTONEFVAR-----HNGPDKQEQATMLKTYNAES-----  
X1 : MQSCAKSWGVLRSALRLMPGRMGCRS SAWLRVELMGRSSSSQTAPCAALTSSSPRHFE LLPRHDESER-----HIGPDKKQRMDDTGLQD-----

120 -----HADMTTVLSDILRTPLNFKCLS-----160 -----FTAAISYKSGAOKNVKSMIGGGYECIVSAIMRNVLNEMWYTYPTPQSEIAQGRLESLLN  
At : DEQTHMAKFCGFDHDSILATVFKSIRLDSMKFSKFLA-----GLTESQMLQHVVDLASKNKVFKSTIGMGYYNTHWPTVILRNIMENPAWYTYPTPYCAEISQGRLESLLN  
Dd : -----SEQLIEYTIKDIRLNLRELNIEENK-----VIGENQLKDKKLAERNKVMRSFIGMGYYGTITPHVTCRNLLENPGWYTYPTPYCAEISQGRLESLLN  
Sp : -----DVIIPDSVRTPEQQLMAGFVSNPNKPNPVNYS-----SEFTTIANVANONKLLKSIGMGYYNVKLPAAICRNVLENPEWYTYPTPYCAEISQGRLESMMN  
Vc : -----LCAIAQTVFAQIRLEAPMQLAPAQ-----SEALMATAKSEAKINCLKRTFIGGGYNTFTPNVILRNVNEMENWYTYPTPYCAEISQGRLESLLN  
X1 : -----IEEIDKTVVGSIRLARPLKDDQV-----CENEILESQNLANKNLRVRSIGMGYYNCSVHCALRLNLENAGWYTYPTPYQPEVSQGRLESLLN

240 -----FQTMVTDITKMDISNASLLDGAATAAGECHYALNQRHK-----280 -----FQTMVTDITKMDISNASLLDGAATAAGECHYALNQRHK-----320 -----FQTMVTDITKMDISNASLLDGAATAAGECHYALNQRHK-----  
At : FQTMVTDITKMDISNASLLDGAATAAGECHYALNQRHK-----KKTFTVIAASNCHPCTIDVCTRADGDFLKVVTSDLK-----DIDYSGDVCGLVQVPGTEGVLQMAEFVKMA  
Dd : FQTMVSEHTGEMSNASLLDGAATAAAMCMQVNIKSKGPFATVDKDYCHPCTIDVCTRADGDFLKVVTSDLK-----DFKFT-EDVVCGLVQVPGSSNGVITDMKKAADRA  
Sp : YQTMVADITGHSISNASLLDGAATAAGEAMVLMANDKKK-----RKTFTVDKNYIENYVTLTRASGFGIKELDNITPELITKAKHVFGLFVQVPAADGIFDVGHAATA  
Vc : YQCMVMDLTAMETANASLLDGAATAAAMALQCRAGKSK-----SNLFTVADDVHTQTEVVKTRAFGLFEMKVDSDID-----NIHQEAFCALLQFPTTCEVRLDITIAKA  
X1 : YQTMVCDITGMDVANASLLDGAATAAAMQLGHRHNKRR-----KFTVDSRCHPCTIAVYCTRANIYGVETEILLPH-----EMDFGKDVSGVLFQVPTDNGRVEDRTHVDR

360 -----KANGVVCAGVLDLMSCLVKAGBMCADVVGCAQRFCTPLGYGGPHAAFMATDNLKRLSPGRIVGISKDNAGDPATRALVQTRQEHKRRKATSNICTAQALLANMA  
At : HANGVVKVMAADLLALITVLPKGGEGADIVVGSARQFVPMGYGGPHAAFMATDNLKRLSPGRIVGISKDNAGDPATRALVQTRQEHKRRKATSNICTAQALLANMA  
Dd : HCANALVVAADLLSIALLKKEGEGADIALGNSORFCVPLGFGGPHAAFFSTKDKYARLLPGRILIGVSKDKQCSAFRMAQTRQEHIRRKATSNICTAQALLANMA  
Sp : RSNMHHVVAADLLALITVLPKGGEGADIVVGSARQFVPMGYGGPHAAFFSTKDKYARLLPGRILIGVSKDKQCSAFRMAQTRQEHIRRKATSNICTAQALLANMA  
Vc : QANKTLVTVATDLLASVLLKAGBMCADVVGSAQRFVPMGYGGPHAAFMATRLAHRKTPGRIVGVSDAKNCALRMAQTRQEHIRRKATSNICTAQALLANMA  
X1 : HQNGTLACCATDLLALCIMRFGEGGVDIAGLSSORFCVPLGFGGPHAAFFVKNLVRNMPGRMVGVTRDAACEKVVYRALVQTRQEHIRRKATSNICTAQALLANMA

400 -----KANGVVCAGVLDLMSCLVKAGBMCADVVGCAQRFCTPLGYGGPHAAFMATDNLKRLSPGRIVGISKDNAGDPATRALVQTRQEHKRRKATSNICTAQALLANMA  
At : HANGVVKVMAADLLALITVLPKGGEGADIVVGSARQFVPMGYGGPHAAFMATDNLKRLSPGRIVGISKDNAGDPATRALVQTRQEHKRRKATSNICTAQALLANMA  
Dd : HCANALVVAADLLSIALLKKEGEGADIALGNSORFCVPLGFGGPHAAFFSTKDKYARLLPGRILIGVSKDKQCSAFRMAQTRQEHIRRKATSNICTAQALLANMA  
Sp : RSNMHHVVAADLLALITVLPKGGEGADIVVGSARQFVPMGYGGPHAAFFSTKDKYARLLPGRILIGVSKDKQCSAFRMAQTRQEHIRRKATSNICTAQALLANMA  
Vc : QANKTLVTVATDLLASVLLKAGBMCADVVGSAQRFVPMGYGGPHAAFMATRLAHRKTPGRIVGVSDAKNCALRMAQTRQEHIRRKATSNICTAQALLANMA  
X1 : HQNGTLACCATDLLALCIMRFGEGGVDIAGLSSORFCVPLGFGGPHAAFFVKNLVRNMPGRMVGVTRDAACEKVVYRALVQTRQEHIRRKATSNICTAQALLANMA

480 -----FYAIYHGEGLKQALARELHQAKFAVEMESIG-FSPVNTTYFDLTSFSMEAAPMTAADYARQCVERG-----520 -----FYAIYHGEGLKQALARELHQAKFAVEMESIG-FSPVNTTYFDLTSFSMEAAPMTAADYARQCVERG-----  
At : FYAIYHGEGLKQALARELHQAKFAVEMESIG-FSPVNTTYFDLTSFSMEAAPMTAADYARQCVERG-----EINLVRVVDST-TITASFDETTLLDVKDFKVASGKVPVF--  
Dd : MYAVYHGGQGLHDIANAVHRAKILAEIKKRLG-YTVLDRPFDFVLAITGDKTMMIKELE-----SRQNVVQYCSK-SISLSDETVRSADISALLNGESAHASKPLG-  
Sp : FYAIYHGGNGIQETANRYASTSFLKSALESSGYKIVNKSHEFDLTLVESADKVLAKAL-----H-GYNLRKVDDSD-HVGLSDDETCDKIQALFSTFNINKSVQDYY  
Vc : FYAVYHGGQGLRTIARRAHLTALAAAGITKAC-YEYAHQHFDFTLAINTGAKTLYQAAQ-----QANLNLKLPN-CLGVSFDETTTAVVAEALFAIEGKIEEYVHA--  
X1 : MFCVYHGGNGLKHIARRVHNATVLLAEGIKRAC-HQLQNEMFFDTLKHCGCPTKEVLDRA-----QRQNLNLFVSDG-SLGVSLDETIVKEKLDLILWVGGCESSAEL--

560 -----IEALTRVADTIC-----VPEALLRKSKEFQSTVFNHSHKSEELMRYAQHLQKDYCLTIGMPLGSCCTMKLNSAAAMRALSWEEYALHPYAFEDQARGYHTL  
At : -----TAESEAPEVQNS-----IPSSLTRSEPYLTHEIFNMYTHEHLLRYTHKLSKDLSLCHSMPLGSCCTMKLNATTEMPPVTSSEFDLHPFAVEQAGYQEMF  
Dd : -----LSSPQLEKETSTIS-----VISEFARCTEPLTHEIFNRYSEHLLRYTHKLSKDLGLTTAMPLGSCCTMKLNATTEMPPVTSSEFNSIHPFVFAKSLGKEMF  
Sp : MEIATSPNGNSASTVDNLSCSPENFRFRITLYQHVEVFNRYHSEELMRYTHKLSKDLGLTAMPLGSCCTMKLNATTEMPPITNELFANIHPYVEEOKAGYRRTI  
Vc : -----LSDRIATNELAA-----IPESCRCROSAFTHEVFVNTTHSETQMLRYMKLEKDFSLTIGMPLGSCCTMKLNATAMIPVTWEEFALHPFVFAKQAGYAHA  
X1 : -----VASMGEIEKGT-----LGTAFKRTSKFTEHLEFNYSYHSETNIVRYMKLEKDFSLTIGMPLGSCCTMKLNSSSILTEITTSAFANIHPFVBLDQAGYQCF

680 -----ADLKQKLDITGMAACSOPNSGAGEYAGLRIITAYHESRGEAHRVDFEIPISAHGTFNFAAVAGLVVVVVKCLDQSVLMVDTETKCVKHARDLACIMITYPSTYGL  
At : ENLGDLLCTITGFEFSFOPNAGAGEYAGLVMVIRAYHMSRGDHRNVCILIPVSAHGTNFAAAMCGMKIITVGTDAKGNINIEBVRKAEEKANLALAMITYPSTHGV  
Dd : ESISNMLCEVTFDGSOPNAGAGEYAGLVMVIRAYHMSRGDHRNVCILIPVSAHGTNFAAAMCGMKIITVGTDAKGNINIEBVRKAEEKANLALAMITYPSTHGV  
Sp : EDLQMLTITGFEAACSOPNSGAGEYAGLRSVIRAYQRSICGGHNRNICLIPVSAHGTNFAAAMAGETVIVKCLNGLYLMQDLKEKASKHAKLAAEMVITYPSTFGI  
Vc : EDLKQKLEITGYALNISOPNSGAGEYAGLVAIQRYHQSREGHRVCLIPSSAHGTFNFAAAMVSMKVVVVKCDENGLMDIADKIEKHKHLSSIMITYPSTHGV  
X1 : QELEKDLCEITGYENISOPNSGAGEYAGLVAIQRYHQSREGHRVCLIPSSAHGTFNFAAAMVSMKVVVVKCDENGLMDIADKIEKHKHLSSIMITYPSTHGV

800 -----YDQNRKRTISAGVHEHGGQCYIDCANLNAVGYTGPFGICGDVCHLNMHKTESIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----840 -----YDQNRKRTISAGVHEHGGQCYIDCANLNAVGYTGPFGICGDVCHLNMHKTESIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----  
At : YBEGHDEICNLIHENGQYVMDGANMNAQVGTSTPGFAGADVCHLNLHKTFCIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----AFQVQVAGNCSASIT  
Dd : FEEGANDCDIIFHANGQYVMDGANMNAQVGTSTPGFAGADVCHLNLHKTFCIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----AMSAVAGVGSASILP  
Sp : FEPDVKALEVHEHGGQYVMDGANMNAQVGTSTPGFAGADVCHLNLHKTFCIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----ITVSVSGEAGSAGILP  
Vc : YEQQVREVCBMVHAAGQYVMDGANMNAQVGTSTPGFAGADVCHLNLHKTFCIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----FAVSAADLGSASILP  
X1 : FEEEDSNVCDLHFKNGGQYVMDGANMNAQVGTSTPGFAGADVCHLNLHKTFCIPHGGGGPGLGPIVREHHLAFLFNSTYGFVAVGSGQ-----SISGTISAAVGSASILP

920 -----ISYAFMMMLGSHGLKTCREYAVLNANYIKRREEHYTHCFDLHDSQFOAHEFILDTRPFRKTAHIDAEVAKRILIDYGFHAPTIAFPVECTIMIEPTESSEKRELDRLADA  
At : ISYAFMMMLGSHGLKTCREYAVLNANYIKRREEHYTHCFDLHDSQFOAHEFILDTRPFRKTAHIDAEVAKRILIDYGFHAPTIAFPVECTIMIEPTESSEKRELDRLADA  
Dd : ITYVYKFLMGCGGLKTAQVALLNANYMARSRLKHYKILYTGSHGLVAHEFILDTRPFRKTAHIDAEVAKRILIDYGFHAPTIAFPVECTIMIEPTESSEKRELDRLADA  
Sp : ISWAYMMMLGSHGLKTCREYAVLNANYIKRREEHYTHCFDLHDSQFOAHEFILDTRPFRKTAHIDAEVAKRILIDYGFHAPTIAFPVECTIMIEPTESSEKRELDRLADA  
Vc : ISWAYMMMLGSHGLKTCREYAVLNANYIKRREEHYTHCFDLHDSQFOAHEFILDTRPFRKTAHIDAEVAKRILIDYGFHAPTIAFPVECTIMIEPTESSEKRELDRLADA  
X1 : ISWAYMMMLGSHGLKTCREYAVLNANYIKRREEHYTHCFDLHDSQFOAHEFILDTRPFRKTAHIDAEVAKRILIDYGFHAPTIAFPVECTIMIEPTESSEKRELDRLADA

1000 -----LISIRREIAAVERGDQPKDNNVLTNAPHITAKVTADEWNRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----1040 -----LISIRREIAAVERGDQPKDNNVLTNAPHITAKVTADEWNRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----  
At : LISIRREIAAVERGDQPKDNNVLTNAPHITAKVTADEWNRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----SSKFWPTTRVDNVYGRKLVCTLLREEVQAAVSA-----  
Dd : LILIRREIAAVERGDQPKDNNVLTNAPHITAKVTADEWNRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----ASKFWPTTRVDNVYGRKLVCTLLREEVQAAVSA-----  
Sp : LISIRREIAAVERGDQPKDNNVLTNAPHITAKVTADEWNRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----ASKFWPTTRVDNVYGRKLVCTLLREEVQAAVSA-----  
Vc : LIAIRGEIDKVKNGEWSPLVHAPHTQADLREEKDRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----ASKFWPTTRVDNVYGRKLVCTLLREEVQAAVSA-----  
X1 : MISIRREIAAVERGDQPKDNNVLTNAPHITAKVTADEWNRPYSRILAYPTTRHQY-REKFWPSPGRVDYIGDRNLMCSAPLEFY-----ASKFWPTTRVDNVYGRKLVCTLLREEVQAAVSA-----

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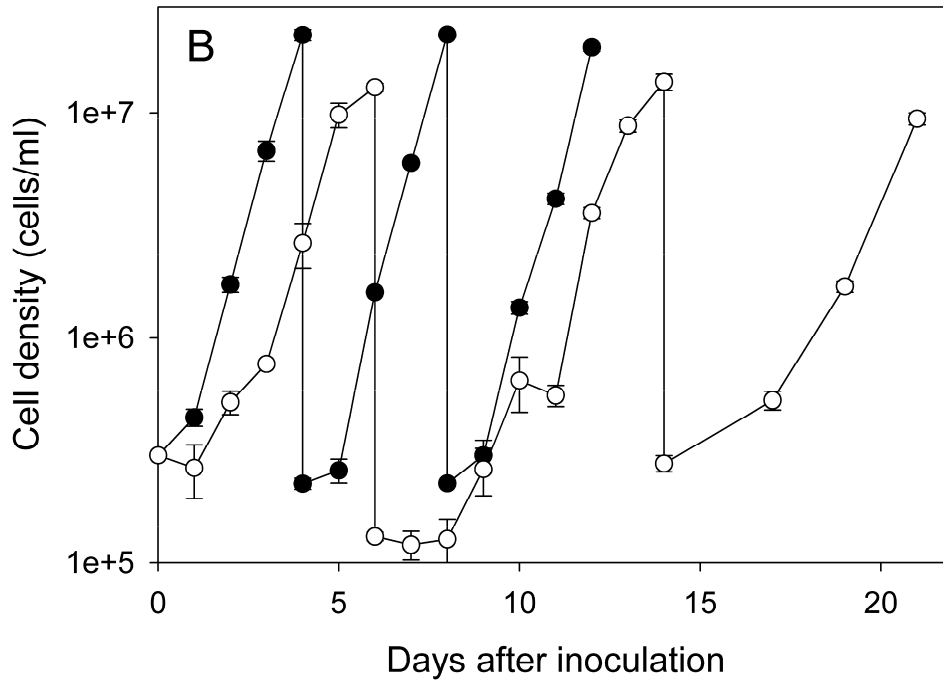
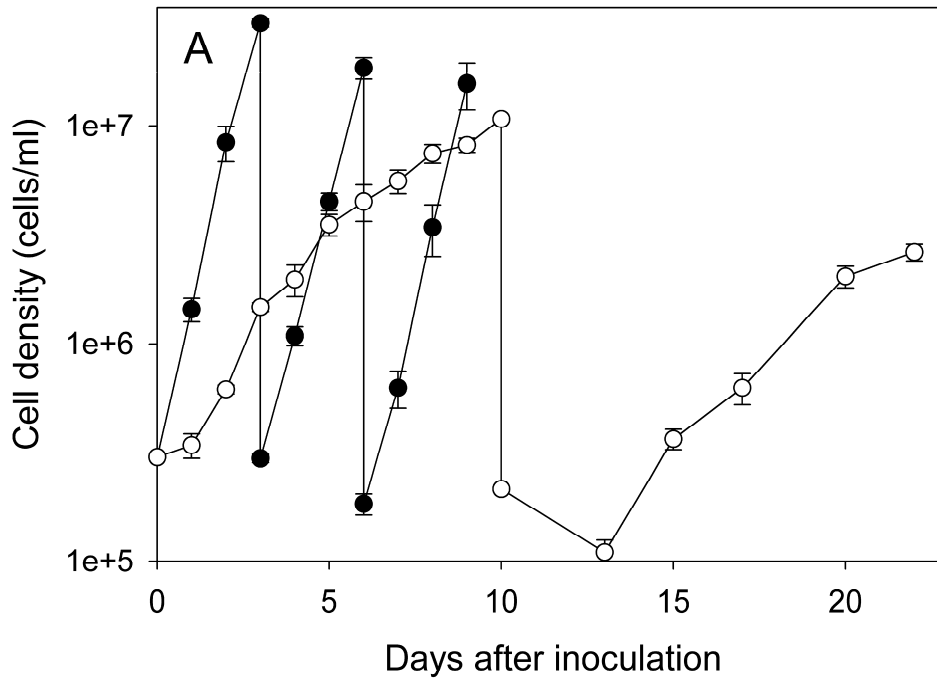


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