

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Sequence alignment of GPI8 orthologues. Amino acid sequences of GPI8 from *Leishmania major* (*Lm*), *Leishmania mexicana* (*Lmexi*), *Saccharomyces cerevisiae* (*Sc*), and *Homo sapiens* (*Hs*) were aligned using Clustal Omega. Asterisks (*) indicate fully conserved residues; colons (:) highly similar residues; periods (.) weakly similar residues. The predicted signal peptide (sp) for *L. major* GPI8 was indicated.

Figure S2. Confirmation of YFP-GPI8 expression in *L. major*. (A) The genomic loci of *GPI8* before (top) and after (bottom) N-terminal tagging with *YFP*. Images were adopted from ²⁷. (B) PCR amplification of genomic DNA from WT, WT+Cas9, and WT+YFP-GPI8 (three clones) cells. Top: The binding sites for forward (F) and reverse (R) primers are indicated in A. A 700 bp fragment was amplified from WT+YFP-GPI8 cells as expected. Bottom: PCR amplification of a portion of the beta-tubulin gene was performed as a control. (C) Western blot analyses of whole cell lysate from WT, WT+Cas9, and WT+YFP-GPI8 #1 promastigotes using antibodies against MYC (top) and α -tubulin (bottom). Full-size, uncropped images are included in supplemental Figure S4.

Figure S3. Verification of *GPI8* deletion in *gpi8*[−] mutants. (A) The genomic loci of *GPI8* before (top) and after (bottom) replacement with the drug resistance gene *PAC*. Images were adopted from ²⁷. (B) PCR amplification of genomic DNA from WT, WT+Cas9, *gpi8*[−] #6, *gpi8*[−] #7, *gpi8*[−]/+GPI8 #6, and *gpi8*[−]/+GPI8 #7 cells. The binding sites for forward (F) and reverse (R) primers are indicated in A. A 700 bp DNA fragment was amplified as expected from *gpi8*[−] and add-back cells. Full-size, uncropped images are included in supplemental Figure S4.

Figure S4. Full-size images for gels and blots. The red rectangles represent cropped images.

SUPPLEMENTARY TABLE 1. List of oligonucleotides used in this study.

Name	Purpose	Sequence (5' to 3')
Beta tubulin Forward	Amplify a segment of the Beta tubulin gene	GATTGCCGACGAACATGGTG
Beta tubulin Reverse	Amplify a segment of the Beta tubulin gene	TTCATGTTGGACTCGGCCTC
G00 #825	Amplify sgRNA template	AAAAGCACCGACTCGGTGCCACTTTTTCA AGTTGATAACGGACTAGCCTTATTTTAAC TTGCTATTTCTAGCTCTAAAAC
5' sgRNA #1032	Amplify sgRNA template	ttaatacgactcactataggCTGTAGTGTGCACAAACAGG gttttagagctagaaatagc
3' sgRNA #1033	Amplify sgRNA template	aatacgactcactataggCAGGGGACAACGTCTAGTCA gttttagagctagaaatagc
Upstream forward #1034	Amplify DNA donor	ATATTCCAGCAAACGATAGCGAGTGCAGTTgta taatgcagacctgctgc
Upstream reverse #1035	Amplify DNA donor	CGTCGCGGTAAACCGTGTTGGCGACGTCATacta cccgatcctgatccag
Downstream forward #1036	Amplify DNA donor	GTACCAGTGGACGTTTCGCTACGACCTCGAGgggttc tggtagtgggtccgg
Downstream reverse #1037	Amplify DNA donor	CATTCTTTTCTTTTTTCGTCGACCGGGAAAccaattt gagagacctgtgc
GPI8-ORF-BglII-F #1295	Amplify GPI8 ORF	GCAAGATCTATGAATCTGTACGGCTGCAG
GPI8-ORF-BglII-R #1296	Amplify GPI8 ORF	GCTAGATCTCTACTCGAGGTCGTAGCGAA
GPI 8 5'UTR Forward #990	To verify integration	GCAGCAGAAGGTAGTGG
BSD reverse #833	To verify integration	TCGCGACGATACAAGTCAGG

Supplementary

Fig. S1-S4

sp

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Lm      -----MTSPTRFTATALLVVALLVLTASSCFPDAYAAIRTAVAAAASAPQGATGKGQS
Lmexi   MRTTAYVMTSPTRCIATALIVFAFLVLTA-----AAAASAPLGATGKGQS
Sc      -----MRIAMHLPLLLLYIFLLPLSG-----ANNTDAAHEVIATNT
Hs      -MAVTDSLSTRAATVLATVLLLSFGSVAAS-----HIEDQAEQFFRSGHT
          :      .      *:      :.      .      ::

Lm      NNWAVIVSSSRYLFNRYRHTANALTMHYLLRQHGIDDDHILLFLSDSFACDPRNVYPAEIF
Lmexi   NNWAVIVSSSRYLFNRYRHTANALTMHYLLRQHGIDDDHILLFLSDSFACDPRNVYPAEIF
Sc      NNWAVLVSTSRFWFNRYRHMANVLSMYRTVKRLGIPDSQIILMLSDDVACNSRNLFPGSVF
Hs      NNWAVLVCTSRFWFNRYRHVANTLSVYRSVKRLGIPDSHIVLMLADDMACNPRNPKPATVF
          *****:*.**: ***** *.*:*:*:*:*.**:*.** *. :*

Lm      SQPPGERDANEHASMNLYGCSAQVDYAGSDVDVRRFLSVLQGRYDENTPPTRRLLSDDKS
Lmexi   SQPPGAHDADGRASMNLYGCSAQVDYAGSDVDVRRFLSVLQGRYDENTPPTRRLLSDNTS
Sc      N-----NKDHAIDL YGDSVEVDYRGYEVTVENFIRLLTDRWTEDHPKSKRLLTDENS
Hc      S-----HKNMELNVYGDDVEVDYRSYEVTVENFLRVLTGRIPPSTPRSKRLLSDDRS
          .      .      ::** ..:** . :* *..*: :* .* . * :*:**:*: *

Lm      NIIIIYVAGHGAKSYFKFQDTEFLSSSDISETLMMMHHQQRRYGRVVFMA DTCHAIALCEHV
Lmexi   NIIIIYVAGHGAKSYFKFQDTEFLSSSDISETLTMMHHQQRRYGRVVFMA DTCHAIALCEHV
Sc      NIFIYMTGHGGDDFLKFQDAEEIASEDIADAFQQMYEKKRYNEIFFMIDTCQANTMYSKF
Hs      NIIYMTGHGGNGFLKFQDSEEITNIELADAFEQMWQKRRYNELLFIIDTCQGASMYERF
          **:***:***.....:***:* :. :::: : * :*:**...*: ***:. :. :.

Lm      EAPNVVCLAASDAESESYSYSCQYDQGLGTHLVSFWMNEMYSLLNGTSCSDPLTRRIGNDV
Lmexi   EAPNVVCLAASDAESESYSYSCQYDEQLGTHMVSFWMNEMYSLLNGTSCSNPLTRRIGDDV
Sc      YSPNIIAVGSGSEMDESSYSHHSDVEIGVAVIDRFT---YYCLDFLEQIDKNSTLTQLDLF
Hs      YSPNIMALASSQVGEDSLSHQPDPAIGVHLM DRYT---FYVLEFLEEINPASQTNMNDLF
          :*:.....*: ..* * : * :*. :. : : * : . : : :* .

Lm      SVLHQSWYNFNYPYRVEASRNRSKPAHRDAVNDPTALR-----
Lmexi   SVLHQSWYNFNYPYRVEASRNRSKPAHRDAVNDPTALR-----
Sc      DSFTFEKIHSHVGVRTDLFDRNPSEVLITDFFANVQNVIPDDSKPLSVSHYHHYKDHIDT
Hs      QVCPKSLCVSTPGHRTDLFQRDPKNVLITDFFGSR-----
          .      .      .*: :. * . .

Lm      -----EWIVADFMCGQEQAAPVDVRYDLE-----
Lmexi   -----EWIVADFVCGQVSAAPVDVRYDLE-----
Sc      AQYELNNNVLDLALETYRKNNQSSKIEKKIKDIKSTSVLDVDIDSNECFFTSFKQSATII
Hs      -----KVEITTETIKLQQDSEIMESRYSS-----
          :      :      . :

Lm      -----
Lmexi   -----
Sc      LALIVTILWFMLRGNTAKATYDLYTN
Hs      -----

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Figure S1. Sequence alignment of GPI8 orthologues

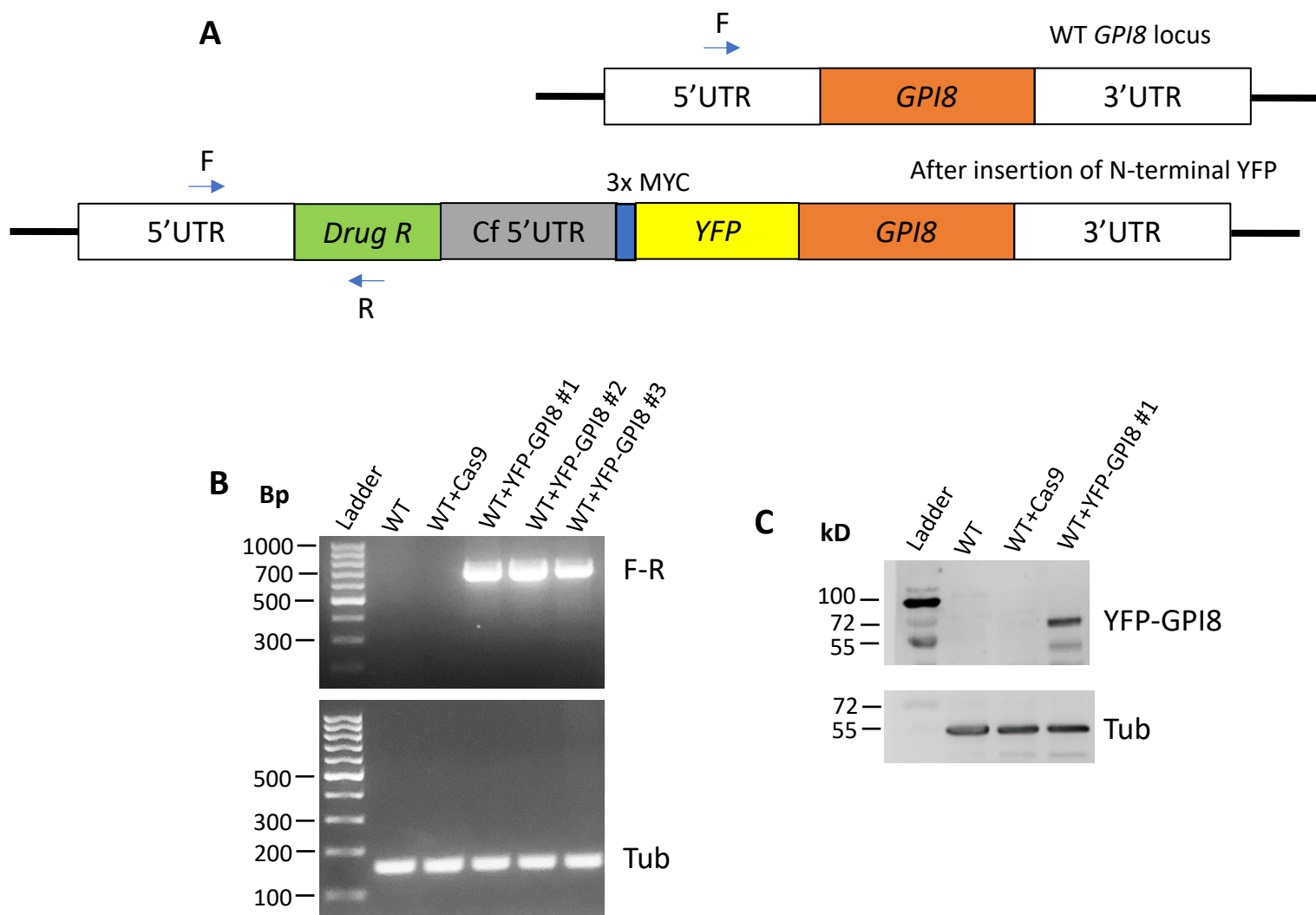


Figure S2. Confirmation of YFP-GPI8 expression in *L. major*.

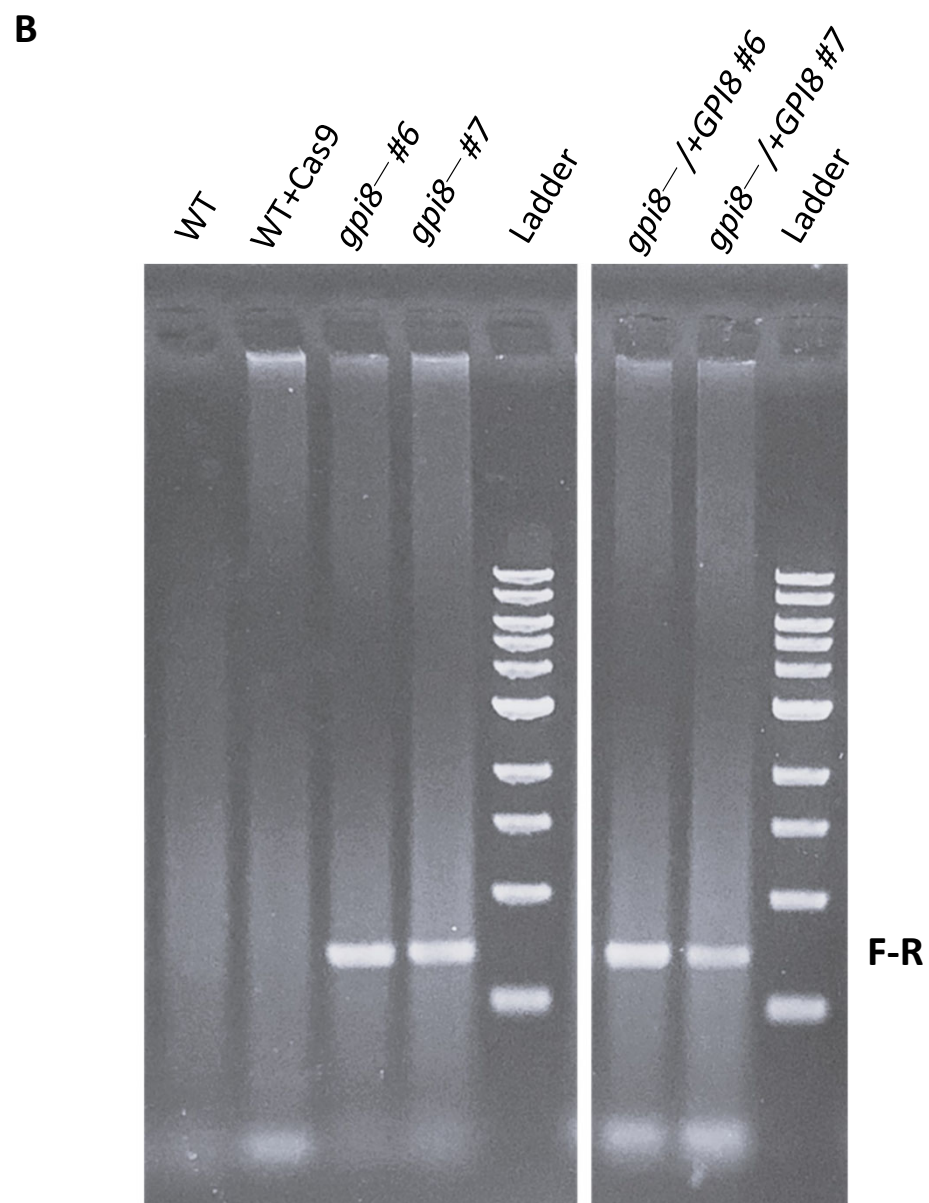
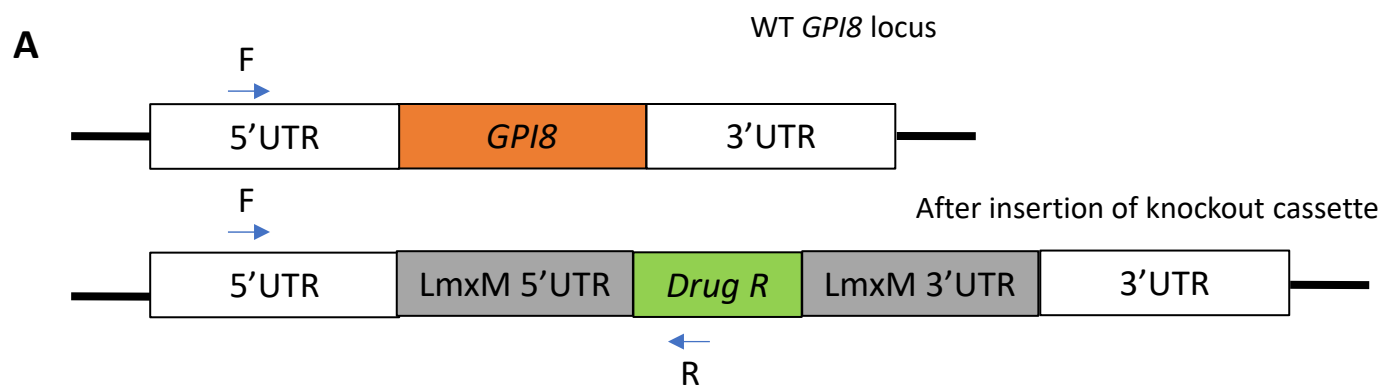


Figure S3. Verification of *GPI8* deletion in *gpi8*⁻ mutants.

Full-size image for Figure 2A

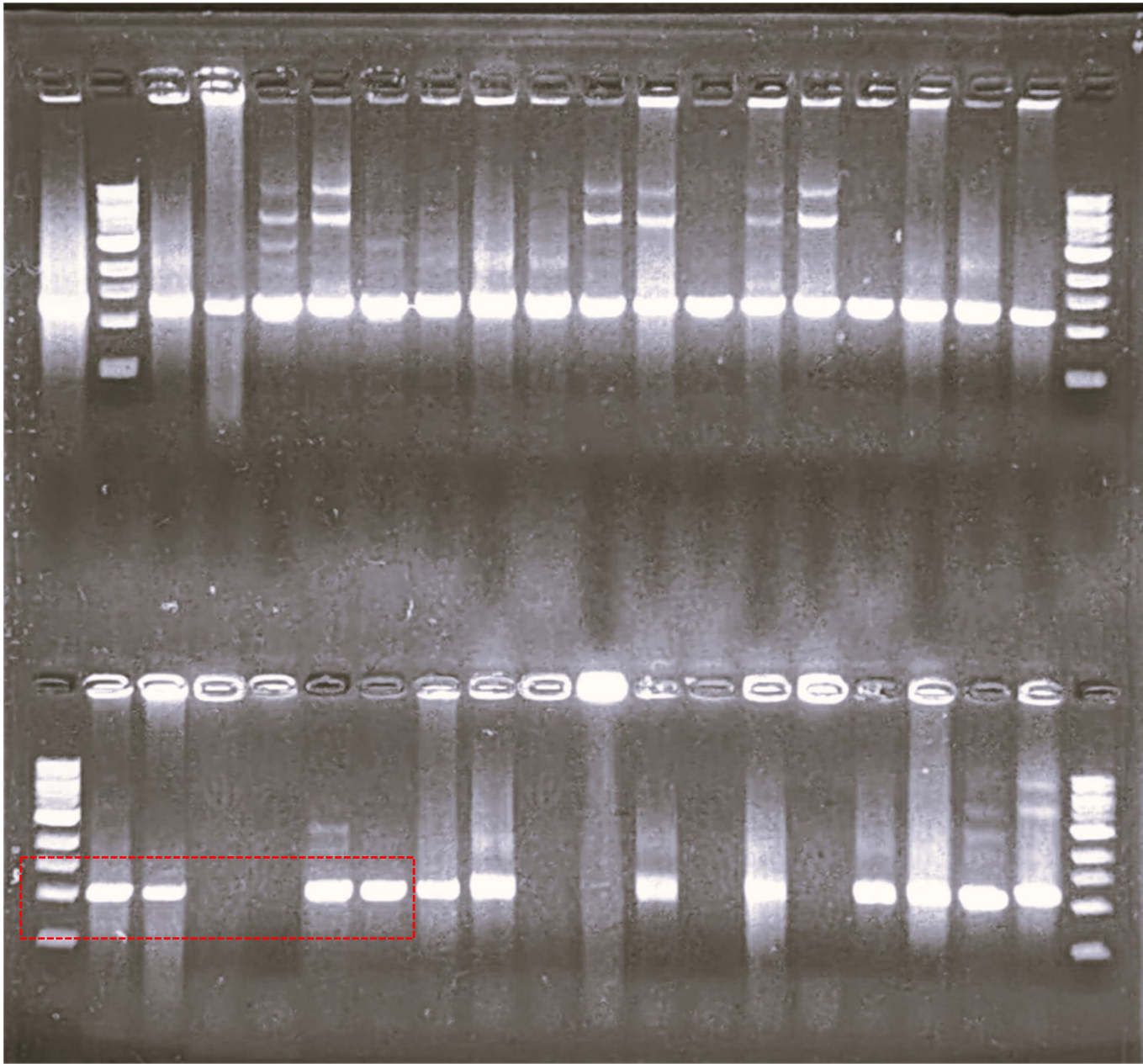


Figure S4. Full-size images for gels and blots.

Full-size image for Figure 2B

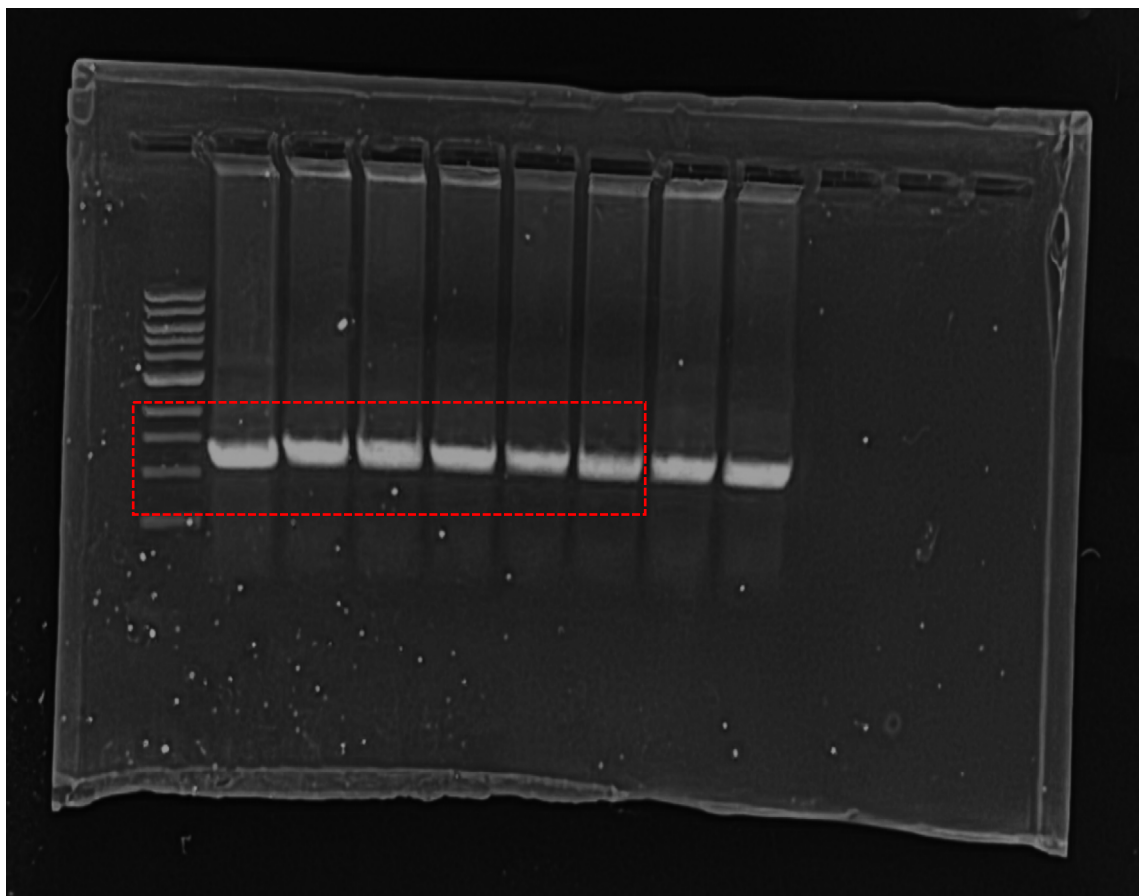


Figure S4. Full-size images for gels and blots.

Full-size image for Figure 2C and 2D

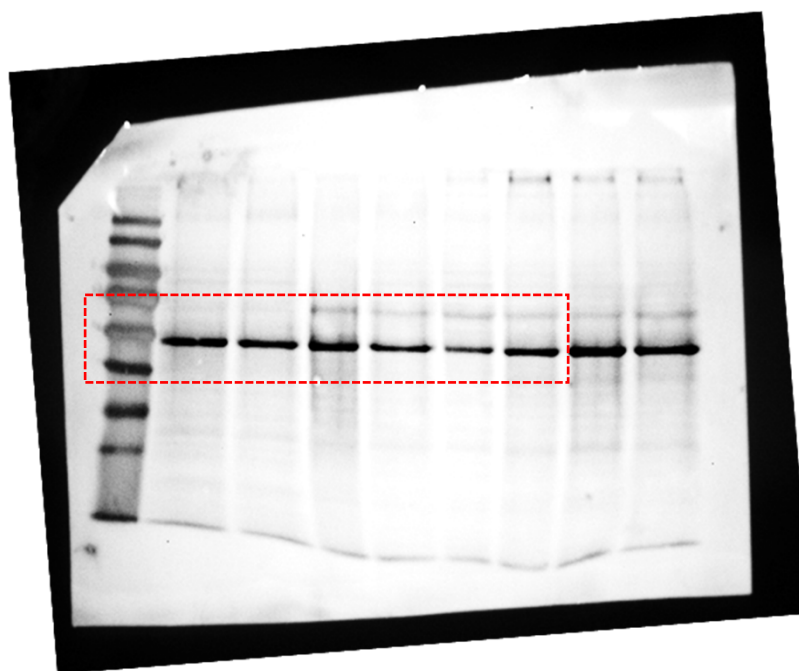
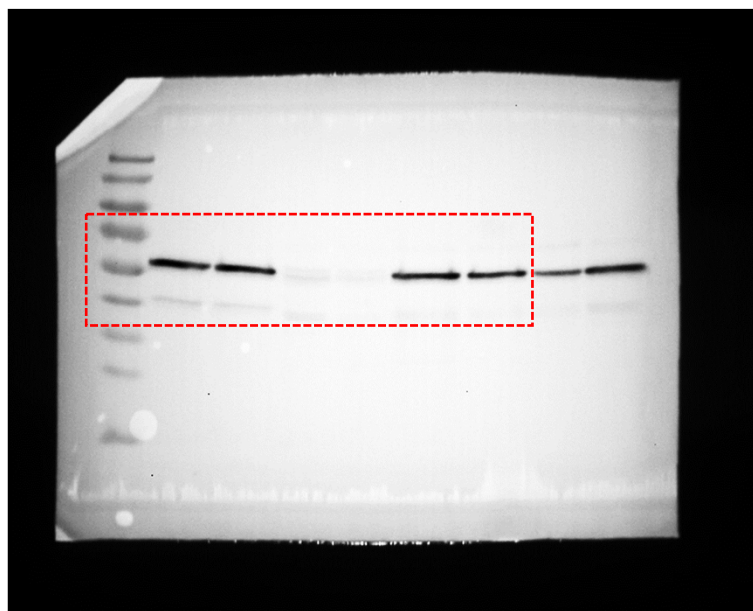


Figure S4. Full-size images for gels and blots.

Full-size image for Figure 2F

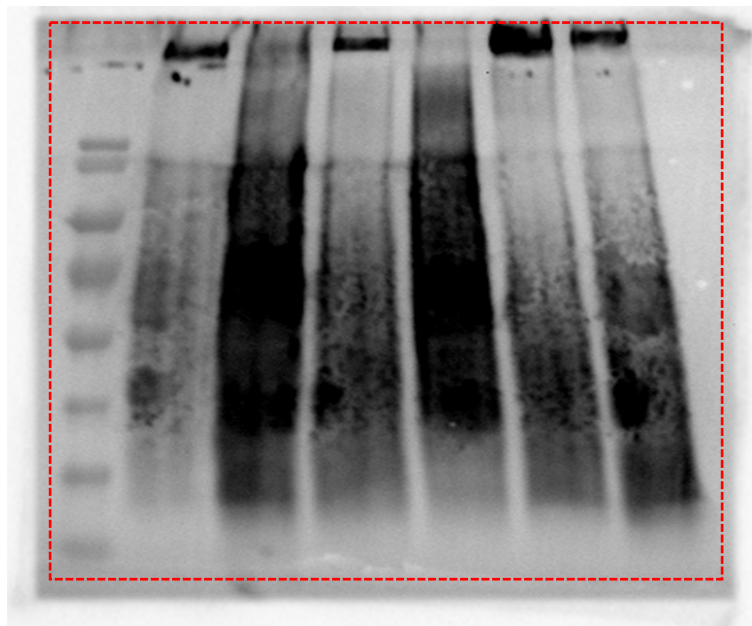


Figure S4. Full-size images for gels and blots.

Full-size image for Figure 2H and 2I

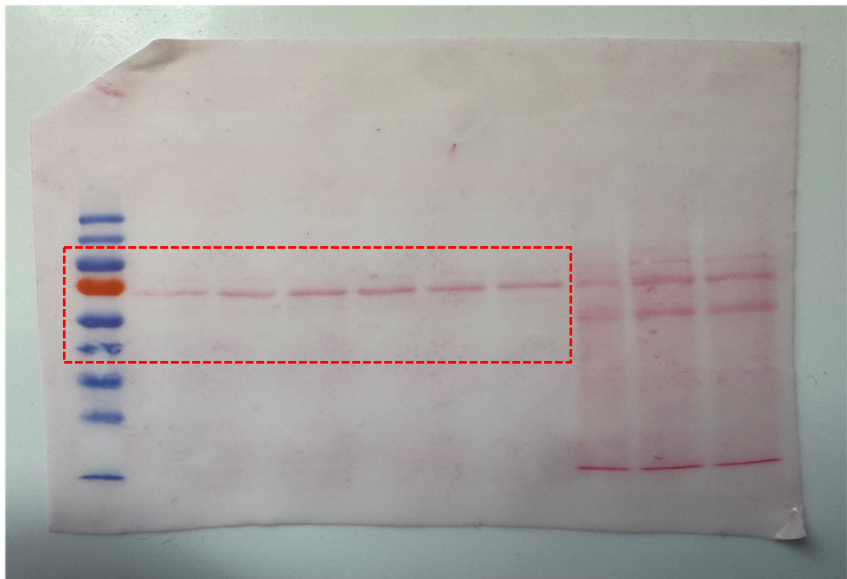
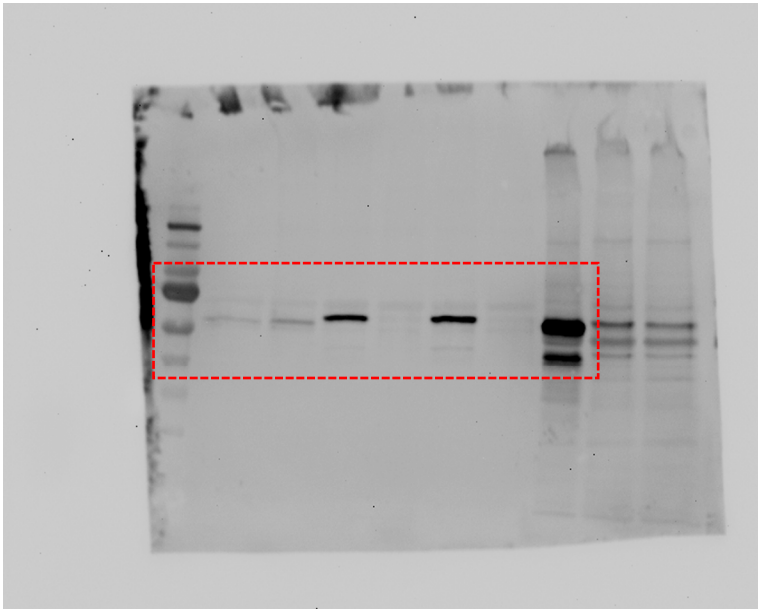


Figure S4. Full-size images for gels and blots.

Full-size image for Figure S2B

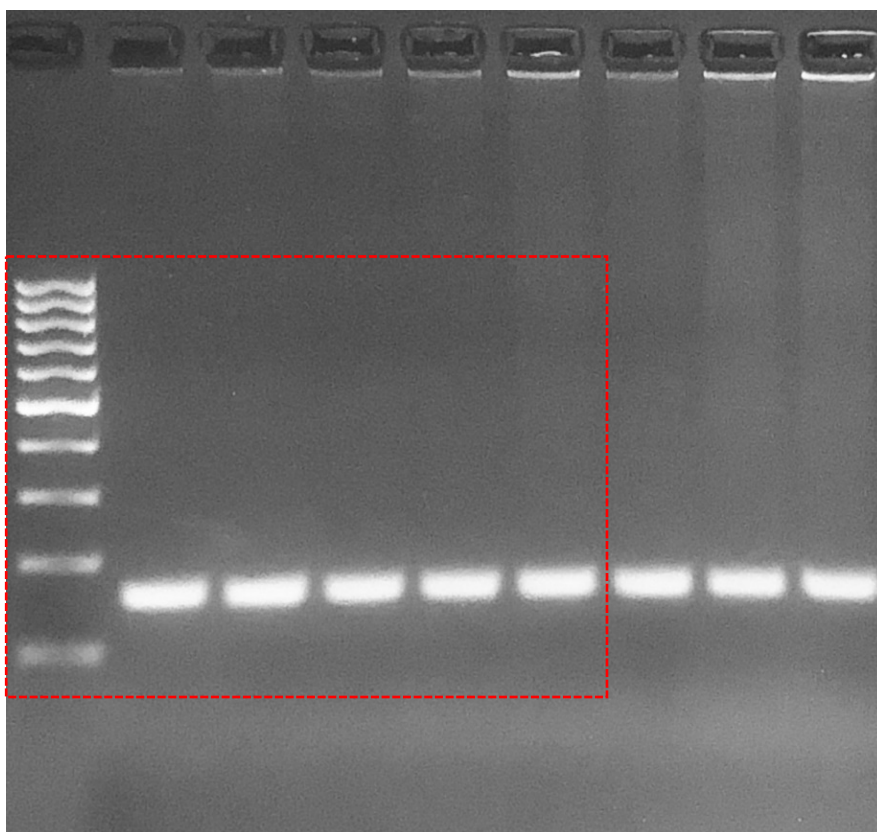
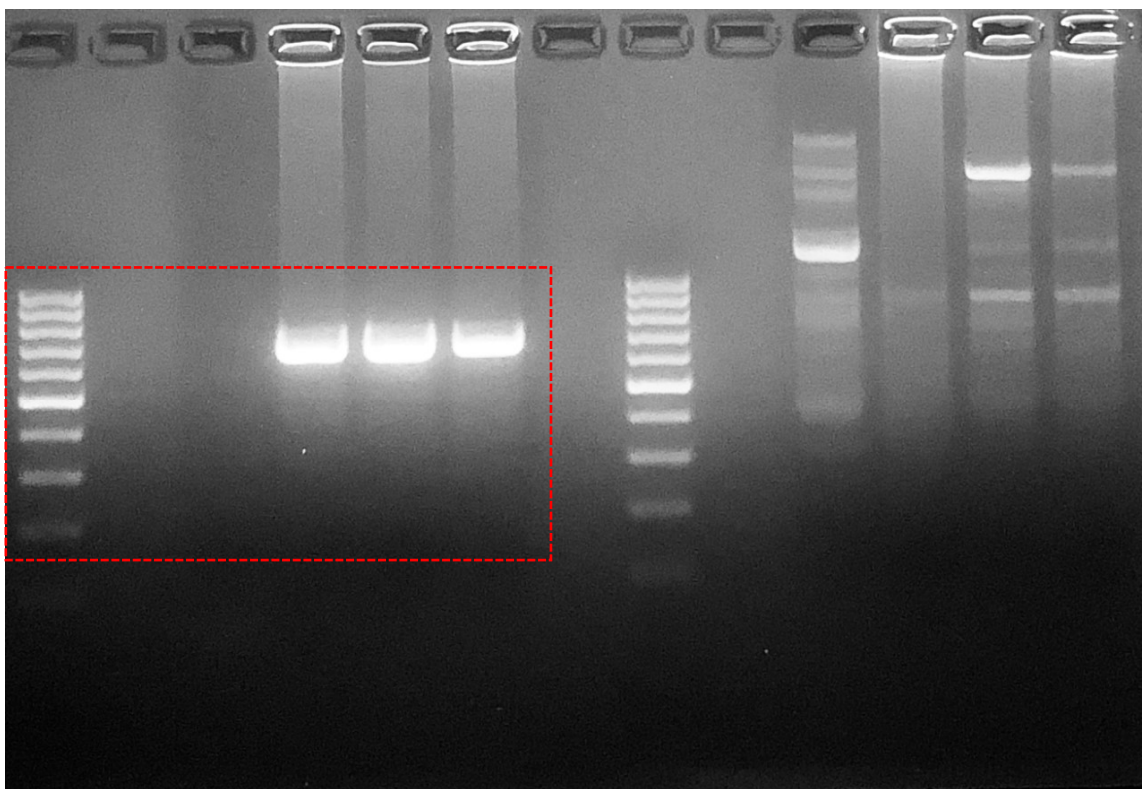


Figure S4. Full-size images for gels and blots.

Full-size image for Figure S2C

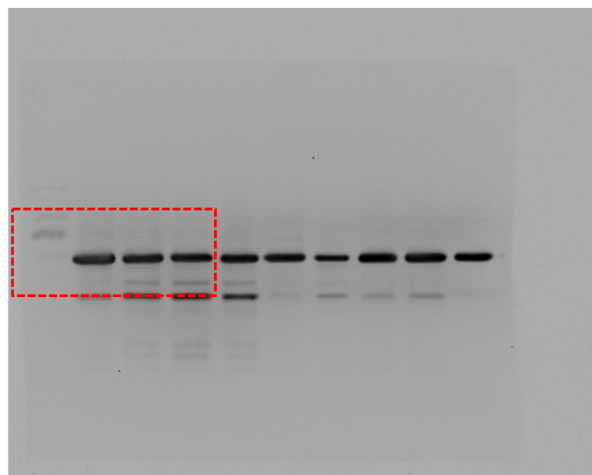
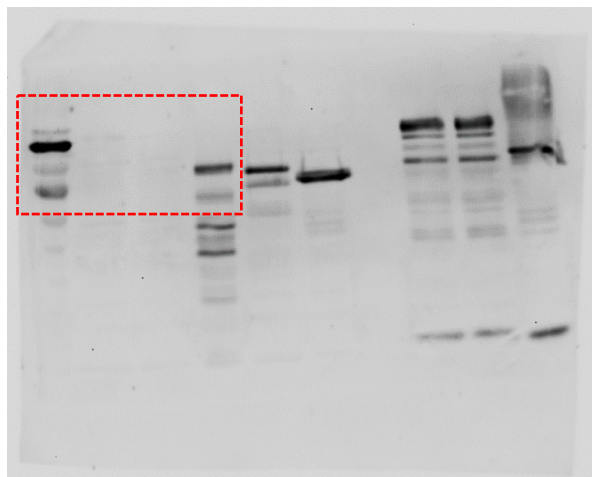


Figure S4. Full-size images for gels and blots.

For Figure S3B

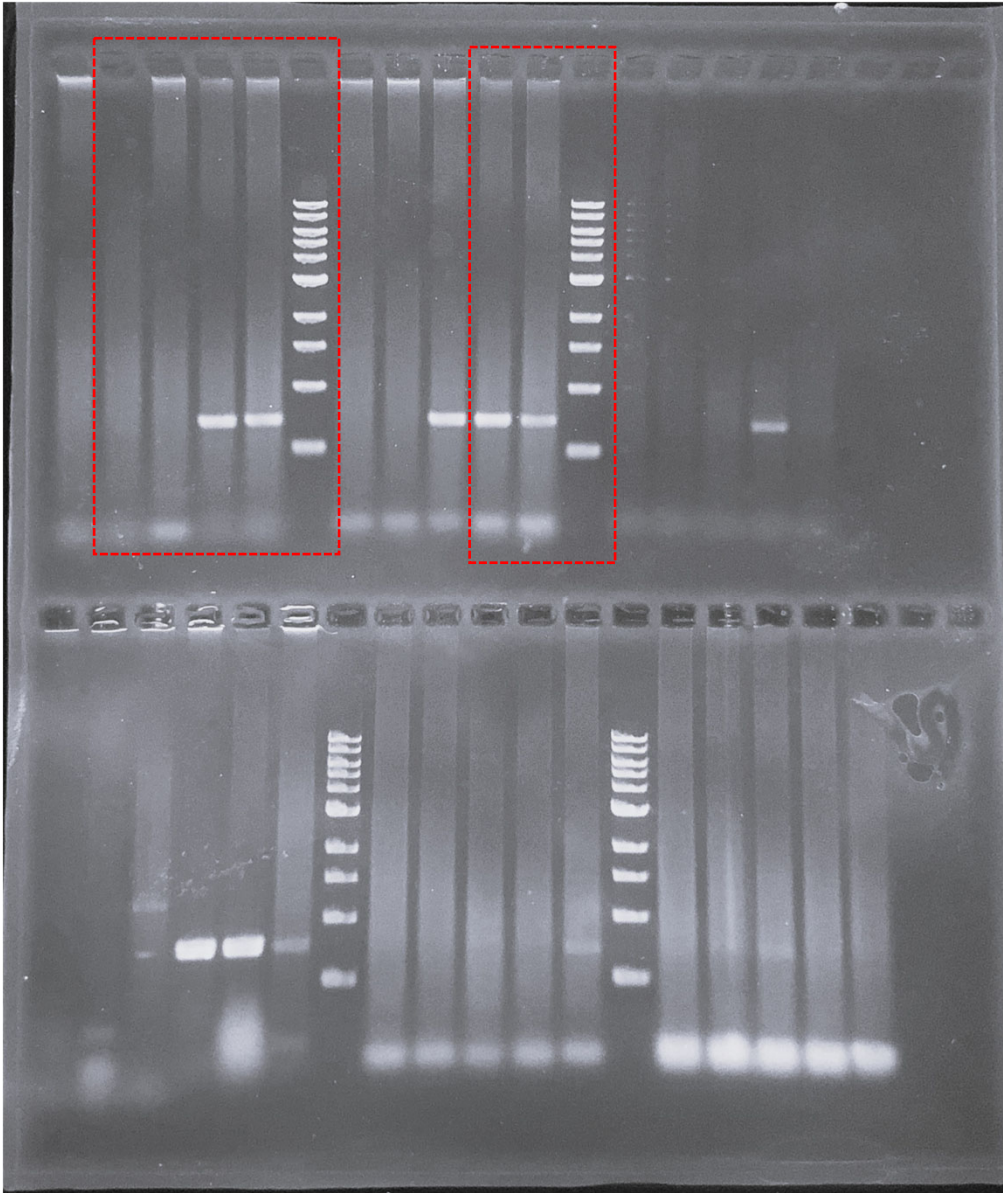


Figure S4. Full-size images for gels and blots.