

SUPPLEMENTARY MATERIAL

3’Nucleotidase/nuclease is required for *Leishmania infantum* clinical isolate susceptibility to miltefosine

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Supplementary Table 1: Sequence of oligonucleotides.

| OL ID | Target | Oligonucleotide sequence (5' » 3') | Description |
|-------------------|-----------------------------------|--|---|
| Fw, MT | MT(LINF_130020800) | CAAGTGCCTTTCCACCAGAATC | qPCR, product size of 206 bp |
| Rw, MT | | CTCACCTTTTTGAACTCCAACAGG | |
| Fw, β -Ros3 | β -Ros3 (LINF_320010400) | ACGACACGGCTTGATTTTCG | qPCR, product size of 228 bp |
| Rw, β -Ros3 | | GAGTAGTCCACGGAGGCAGTAAAG | |
| Fw, GAPDH | GAPDH | GAAGTACACGGTGGAGGCTG | qPCR, product size of 238 bp |
| Rv, GAPDH | | CGCTGATCACGACCTTCTTC | |
| OL14 | pTB010 on SSU | ATCGCGACACGTTATGTGAG (Annealing upstream of integration region on SSU) | Integration screening on SSU |
| OL2380 | pRIB on SSU | CATTCCGTGCGAAAAGCCGG | Integration screening on SSU |
| OL4613 | LINF_310031200 | ATCTAG ATTATAAAATCCAGTGGATCG | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4614 | LINF_310031200 | TATA AAGCTT CTGTCATCACTCTTGTTAATGCG | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4615 | LINF_310031300 | ATCTAG ACTAGAGGGCGACGTGCTCAT | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4616 | LINF_310031300 | TATA AAGCTT ATGGCTCGAGCTCGTTTCC | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4617 | LINF_310031400 | ATCTAG ACTGCTACGCGCTCCTGTG | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4618 | LINF_310031400 | TATA AAGCTT ATGACCCTGCAGTGGCAT | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4619 | LINF_310031500 | ATCTAG ACAGATTGCAGAATTCACGC | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4620 | LINF_310031500 | TATA AAGCTT GCGTGTTATATACGTGAGCG | Knocked out cell line screening – CRISPR-Cas9 Amplification for re-expression |
| OL4621 | MSL deletion | AGTTGAGTCTGCTCCGGTG | Knocked out cell line screening – CRISPR-Cas9 |
| OL4622 | MSL deletion | TTCACGTCACGGCCAAAG | Knocked out cell line screening – CRISPR-Cas9 |
| OL4747 | pRIB | GCAGAAAGCACTGTCGAATAGG | Integration screening on SSU |
| OL4867 | hSpCas9 | GCCTCCCTGGGCACATAACCAC | hSpCas9 screening |
| OL4868 | hSpCas9 | CCAGCTGTCTTTGATGAAGCCGG | hSpCas9 screening |
| OL8987 | pTB010 on SSU | CGTCCGAGGGCAAAGGAATA (Annealing on construct HYG CDS) | Integration screening on SSU |
| OL9328 | pPLOTv1 blast mNeonGreen-blast | AGAACAGGGGCATCTTGAGC | Repair template (tagged cell lines) integration screening – CRISPR-Cas9 |
| OL9369 | pTBlast_v1 and pTPuro_v1 | GCAGCAGGTCTGCATTATAC | Repair templates (knocked out cell lines) integration screening – CRISPR-Cas9 |
| OL9972 | LINF_310031200 | CTGGTCCTCTCTGCACCGTCGCGCTTCCGgtataatgcagacctgtgc | Upstream forward primer – CRISPR-Cas9 |
| OL9973 | LINF_310031200 | CTGGTCCTCTCTGCACCGTCGCGCTTCCGTAATACGACTCACTATAAAA ACTGGAAGGAGC | Upstream forward primer with barcode – CRISPR-Cas9 |
| OL9974 | LINF_310031200 | TTCGACGG gtataatgcagacctgtgc | Upstream forward primer with barcode – CRISPR-Cas9 |
| OL9974 | LINF_310031200 | GTGGCGCTGCGCAATGAGGGCGACGGCCATactaccgatcctgatccag | Upstream reverse primer – CRISPR-Cas9 |
| OL9975 | LINF_310031200 | gaaattaatcagactcactatagTTGCTCCATCATGCCGCAGagttagagctagaaatagc | 5' sgRNA primer – CRISPR-Cas9 |
| OL9976 | LINF_310031200 | GCCTCAAACAAACAAAAGAAAAGTGGCTGccaatttgagacctgtgc | Downstream reverse primer – CRISPR-Cas9 |

| OL ID | Target | Oligonucleotide sequence (5' » 3') | Description |
|---------|----------------|--|--|
| OL9977 | LINF_310031200 | gaaattaatacactcactataggCGTGGTTGCTTTCAAGGACGgttttagactagaaatagc | 3' sgRNA primer – CRISPR-Cas9 |
| OL9978 | LINF_310031300 | CCTTTCCTCTCCACACCGTGAAGCAAACCTgtataatgcagacctgctgc | Upstream forward primer – CRISPR-Cas9 |
| OL9979 | LINF_310031300 | CCTTTCCTCTCCACACCGTGAAGCAAACCTTAATACGACTCACTATAAACTGGAAG CTAT AATATTA gtataatgcagacctgctgc | Upstream forward primer with barcode – CRISPR-Cas9 |
| OL9980 | LINF_310031300 | CAGAAGCTGAAGGAAACGAGCTCGAGCCATactaccgatcctgatccag | Upstream reverse primer – CRISPR-Cas9 |
| OL9981 | LINF_310031300 | gaaattaatacactcactataggTGTACAGCACGTAATGGCTGgttttagactagaaatagc | 5' sgRNA primer – CRISPR-Cas9 |
| OL9982 | LINF_310031300 | GATAGGATCGAGAGGAGGCACAGGGGAAAccaattgagagacctgctgc | Downstream reverse primer – CRISPR-Cas9 |
| OL9983 | LINF_310031300 | gaaattaatacactcactataggAAGACGCTGCTTCTTCTGCGgttttagactagaaatagc | 3' sgRNA primer – CRISPR-Cas9 |
| OL9984 | LINF_310031400 | CAAGCGCGAGGCTTATCGACTACCAGCCCgtataatgcagacctgctgc | Upstream forward primer – CRISPR-Cas9 |
| OL9985 | LINF_310031400 | CAAGCGCGAGGCTTATCGACTACCAGCCCTAATACGACTCACTATAAACTGGAAG CTC GATCGACGG gtataatgcagacctgctgc | Upstream forward primer with barcode – CRISPR-Cas9 |
| OL9987 | LINF_310031400 | GCGGTAGAGCTCATCGACTGCAGGGTCATactaccgatcctgatccag | Upstream reverse primer – CRISPR-Cas9 |
| OL9988 | LINF_310031400 | gaaattaatacactcactataggCTATGCAGCATACATGGGAAgttttagactagaaatagc | 5' sgRNA primer – CRISPR-Cas9 |
| OL9989 | LINF_310031400 | GCGCGTCTATTCTAAATACAAGAAACACCAccaattgagagacctgctgc | Downstream reverse primer – CRISPR-Cas9 |
| OL9990 | LINF_310031400 | gaaattaatacactcactataggGTTCGGTTGTGAATACTCGGgttttagactagaaatagc | 3' sgRNA primer – CRISPR-Cas9 |
| OL9991 | LINF_310031500 | CGTCTCTTCGCCGCCGCGTCCGCCAGGCCCTgtataatgcagacctgctgc | Upstream forward primer – CRISPR-Cas9 |
| OL9992 | LINF_310031500 | CGTCTCTTCGCCGCCGCGTCCGCCAGGCCCTAATACGACTCACTATAAACTGGAAG TATA TCGATGCC gtataatgcagacctgctgc | Upstream forward primer with barcode – CRISPR-Cas9 |
| OL9993 | LINF_310031500 | GGTGAGAGTGGACGATATGGCGCGGCATactaccgatcctgatccag | Upstream reverse primer – CRISPR-Cas9 |
| OL9994 | LINF_310031500 | gaaattaatacactcactataggATATGGTGGTCTTCGTTGGgttttagactagaaatagc | 5' sgRNA primer – CRISPR-Cas9 |
| OL9995 | LINF_310031500 | CGTGCGCACAAAGAACTCAGAGCCTCTCCTccaattgagagacctgctgc | Downstream reverse primer – CRISPR-Cas9 |
| OL9996 | LINF_310031500 | gaaattaatacactcactataggACGTCTACATGCCCTCATGgttttagactagaaatagc | 3' sgRNA primer – CRISPR-Cas9 |
| OL10358 | LINF_31MSL | ATATATCCATACATATCGATGTGTATATATgtataatgcagacctgctgc | Upstream forward primer – CRISPR-Cas9 |
| OL10359 | LINF_31MSL | ATATATCCATACATATCGATGTGTATATATTAATACGACTCACTATAAACTGGAAG ATAC ATACCATT gtataatgcagacctgctgc | Upstream forward primer with barcode – CRISPR-Cas9 |
| OL10360 | LINF_31MSL | gaaattaatacactcactataggGTAGGTGTGCTGAGGTGTCTgttttagactagaaatagc | 5' sgRNA primer – CRISPR-Cas9 |
| OL10361 | LINF_31MSL | CGATGCAGGTCTAGGGCAGTGGATTCACCAccaattgagagacctgctgc | Downstream reverse primer – CRISPR-Cas9 |
| OL10362 | LINF_31MSL | gaaattaatacactcactataggAACCGACGAGCAGACGGGAGgttttagactagaaatagc | 3' sgRNA primer – CRISPR-Cas9 |
| OL10376 | LINF_310031300 | TCGTACACGTTCGATAGTCAGC | Knocked out cell line screening – CRISPR-Cas9 |
| OL10377 | LINF_310031300 | CGTAGCAGTTCGGTTGTGAA | Knocked out cell line screening – CRISPR-Cas9 |
| OL10640 | LINF_310031200 | CAGCGATGCGAGCCGATCGCACTGGATTTAggttctgtagtggtccgg | Downstream forward primer – CRISPR-Cas9 |
| OL10641 | LINF_310031300 | CGCTTTGGTTCTATGAGCACGTCGCCCTCggttctgtagtggtccgg | Downstream forward primer – CRISPR-Cas9 |
| OL10642 | LINF_310031400 | CTGCAACACGTGATGCCGCACAGGAGCGCGggttctgtagtggtccgg | Downstream forward primer – CRISPR-Cas9 |
| OL10643 | LINF_310031500 | CTGGAACGCCTCAAGAGCCGCTCTCGTAAGggttctgtagtggtccgg | Downstream forward primer – CRISPR-Cas9 |
| OL10855 | LINF_310031200 | GCGGAAGAGCCTCTACAAGA | Knocked out cell line screening – CRISPR-Cas9 |

Fw, forward oligonucleotide; Rv, reverse oligonucleotide. **AAGCTT**, restriction site for HindIII enzyme; **TCTAGA**, restriction site for XbaI enzyme. Barcodes are highlighted in bold/red.

Supplementary Table 2: Read depth coverage differences between MSL mutants/MG11A and *L. infantum* T7/Cas9. Read depth coverage (RDC) of each coding sequence (CDS) were estimated, normalized per chromosome RDC, and compared between mutants/MG11A and T7/Cas9. All CDS with difference [mutants/MG11A - T7/Cas9] higher than 0.5 RDC, which corresponds to approximately one copy per haploid genome, are reported. Table with all CDS normalized was deposited on Figshare, DOI: <https://doi.org/10.6084/m9.figshare.21318240>.

| Chr. | CDS position | | Gene ID | Product Description | RDC | | | | Note |
|---------------------------------|--------------|---------|----------------|--|-------|---------|------------|------------|---|
| | Start | End | | | MA01A | T7/Cas9 | Comparison | Difference | |
| <i>Anuc1</i> vs. T7/Cas9 | | | | | | | | | |
| LinJ.10 | 437644 | 438124 | LINF_100015600 | FKBP-type peptidyl-prolyl cis-trans isomerase - putative | 0.75 | 0.45 | 0.96 | 0.51 | --- |
| LinJ.19 | 697774 | 698152 | LINF_190022700 | ATG8/AUT7/APG8/PAZ2 - putative | 0.93 | 0.77 | 1.27 | 0.50 | --- |
| LinJ.21 | 7847 | 8150 | LINF_210005100 | histone H4 | 0.87 | 0.50 | 1.11 | 0.61 | Multiple paralogues • |
| LinJ.22 | 777241 | 778756 | LINF_220023200 | hypothetical protein | 5.66 | 4.42 | 5.40 | 0.98 | Multi copy gene • |
| LinJ.27 | 1172825 | 1174127 | LINF_270033890 | hypothetical protein - conserved | 3.77 | 3.22 | 4.01 | 0.79 | Multi copy gene • |
| LinJ.28 | 244493 | 245153 | LINF_280011400 | ER lumen retaining receptor-like protein | 0.86 | 1.08 | 0.46 | -0.62 | --- |
| LinJ.30 | 93045 | 93492 | LINF_300008000 | hypothetical protein - conserved | 0.83 | 0.50 | 1.14 | 0.64 | --- |
| LinJ.30 | 1351032 | 1351701 | LINF_300043200 | ribosomal P protein AGP2beta-1 - putative | 0.73 | 0.71 | 1.23 | 0.52 | --- |
| LinJ.31 | 1181281 | 1182328 | LINF_310031200 | NUC1 | 0.80 | 0.71 | 0.00 | -0.71 | MSL gene |
| LinJ.34 | 787427 | 788009 | LINF_340023600 | amastin-like surface protein - putative | 1.27 | 0.69 | 1.38 | 0.69 | Multi copy gene / Multiple paralogues • |
| LinJ.35 | 156279 | 159228 | LINF_350010100 | proteophosphoglycan ppg3 - putative | 3.98 | 3.43 | 3.96 | 0.53 | Multi copy gene • |
| LinJ.35 | 168215 | 175547 | LINF_350010200 | proteophosphoglycan ppg3 - putative | 10.24 | 8.71 | 9.21 | 0.50 | Multi copy gene • |
| <i>Anuc2</i> vs. T7/Cas9 | | | | | | | | | |
| LinJ.12 | 455587 | 456073 | LINF_120014800 | hypothetical protein - conserved | 0.56 | 0.43 | 0.95 | 0.52 | Multiple paralogues |
| LinJ.27 | 1172825 | 1174127 | LINF_270033890 | hypothetical protein - conserved | 3.77 | 3.22 | 3.74 | 0.52 | Multi copy gene • |
| LinJ.31 | 1184204 | 1185341 | LINF_310031300 | NUC2 | 0.76 | 0.78 | 0.00 | -0.78 | MSL gene |
| LinJ.36 | 456312 | 456735 | LINF_360017700 | hypothetical protein - conserved | 0.67 | 1.07 | 0.51 | -0.56 | --- |
| <i>Δhlp</i> vs. T7/Cas9 | | | | | | | | | |
| LinJ.10 | 176091 | 176334 | LINF_100009200 | hypothetical protein | 0.81 | 0.75 | 0.14 | -0.61 | Multiple paralogues |
| LinJ.10 | 231389 | 231845 | LINF_100010500 | hypothetical protein | 2.11 | 1.76 | 2.31 | 0.54 | Multi copy gene • |
| LinJ.22 | 777241 | 778756 | LINF_220023200 | hypothetical protein | 5.66 | 4.42 | 5.16 | 0.74 | Multi copy gene • |
| LinJ.25 | 387568 | 387880 | LINF_250016300 | hypothetical protein | 0.70 | 0.35 | 0.93 | 0.58 | • |
| LinJ.31 | 1185826 | 1188553 | LINF_310031400 | HLP | 0.80 | 0.73 | 0.00 | -0.73 | MSL gene |
| LinJ.32 | 964910 | 965129 | LINF_320031400 | hypothetical protein | 0.89 | 0.46 | 1.02 | 0.56 | --- |
| <i>Δtei</i> vs. T7/Cas9 | | | | | | | | | |
| LinJ.01 | 78067 | 78247 | LINF_010007900 | 4F5 protein family - putative | 0.73 | 0.41 | 0.93 | 0.51 | --- |

| Chr. | CDS position | | Gene ID | Product Description | RDC | | | | Note |
|--------------------------------------|--------------|---------|----------------|--|-------|---------|------------|------------|---|
| | Start | End | | | MA01A | T7/Cas9 | Comparison | Difference | |
| LinJ.08 | 492341 | 493862 | LINF_080017100 | Zinc finger - C3HC4 type (RING finger) - putative | 1.51 | 1.34 | 1.89 | 0.55 | Multi copy gene |
| LinJ.21 | 7847 | 8150 | LINF_210005100 | histone H4 | 0.87 | 0.50 | 1.06 | 0.56 | Multiple paralogues • |
| LinJ.22 | 777241 | 778756 | LINF_220023200 | hypothetical protein | 5.66 | 4.42 | 6.72 | 2.30 | Multi copy gene • |
| LinJ.25 | 114783 | 115389 | LINF_250009100 | hypothetical protein - conserved | 0.79 | 0.37 | 0.96 | 0.60 | --- |
| LinJ.27 | 1172825 | 1174127 | LINF_270033890 | hypothetical protein - conserved | 3.77 | 3.22 | 4.76 | 1.54 | Multi copy gene • |
| LinJ.31 | 1191356 | 1192406 | LINF_310031500 | TEI | 0.81 | 0.70 | 0.08 | -0.62 | MSL gene |
| LinJ.32 | 1462495 | 1462894 | LINF_320044900 | kinetoplast-associated protein p18-2 - putative | 0.95 | 0.56 | 1.06 | 0.51 | --- |
| LinJ.33 | 1520147 | 1520558 | LINF_330042600 | h1 histone-like protein | 0.84 | 0.55 | 1.12 | 0.58 | --- |
| LinJ.34 | 333051 | 333534 | LINF_340012700 | Predicted membrane protein - putative | 0.83 | 0.37 | 0.93 | 0.57 | --- |
| LinJ.35 | 1283 | 3371 | LINF_350005000 | phosphoglycan beta 1,3 galactosyltransferase 7 | 0.74 | 0.56 | 1.16 | 0.60 | Multi copy gene |
| LinJ.35 | 156279 | 159228 | LINF_350010100 | proteophosphoglycan ppg3 - putative | 3.98 | 3.43 | 4.04 | 0.61 | Multi copy gene • |
| LinJ.35 | 168215 | 175547 | LINF_350010200 | proteophosphoglycan ppg3 - putative | 10.24 | 8.71 | 9.42 | 0.71 | Multi copy gene • |
| LinJ.35 | 1921447 | 1921678 | LINF_350056500 | Ribosomal protein L37e - putative | 0.71 | 0.45 | 1.00 | 0.55 | Multiple paralogues |
| LinJ.36 | 99013 | 99064 | LINF_360008450 | hypothetical protein | 0.76 | 0.53 | 1.07 | 0.54 | --- |
| LinJ.36 | 2739457 | 2740183 | LINF_360082400 | PSP1 C-terminal conserved region containing protein - putative | 0.84 | 0.51 | 1.03 | 0.52 | Multiple paralogues |
| <i>Δnuc1/nuc2 vs. T7/Cas9</i> | | | | | | | | | |
| LinJ.21 | 572748 | 572988 | LINF_210022300 | hypothetical protein - conserved | 0.75 | 0.44 | 1.08 | 0.64 | Multiple paralogues |
| LinJ.31 | 1181281 | 1182328 | LINF_310031200 | NUC1 | 0.80 | 0.71 | 0.00 | -0.71 | MSL gene |
| LinJ.31 | 1184204 | 1185341 | LINF_310031300 | NUC2 | 0.76 | 0.78 | 0.00 | -0.78 | MSL gene |
| LinJ.34 | 787427 | 788009 | LINF_340023600 | amastin-like surface protein - putative | 1.27 | 0.69 | 1.26 | 0.57 | Multi copy gene / Multiple paralogues • |
| LinJ.35 | 168215 | 175547 | LINF_350010200 | proteophosphoglycan ppg3 - putative | 10.24 | 8.71 | 9.42 | 0.71 | Multi copy gene • |
| <i>Δmsl vs. T7/Cas9</i> | | | | | | | | | |
| LinJ.22 | 777241 | 778756 | LINF_220023200 | hypothetical protein | 5.66 | 4.42 | 5.42 | 0.99 | Multi copy gene • |
| LinJ.25 | 387568 | 387880 | LINF_250016300 | hypothetical protein | 0.70 | 0.35 | 0.89 | 0.53 | • |
| LinJ.27 | 1172825 | 1174127 | LINF_270033890 | hypothetical protein - conserved | 3.77 | 3.22 | 3.87 | 0.66 | Multi copy gene • |
| LinJ.31 | 1181281 | 1182328 | LINF_310031200 | NUC1 | 0.80 | 0.71 | 0.00 | -0.71 | MSL gene |
| LinJ.31 | 1184204 | 1185341 | LINF_310031300 | NUC2 | 0.76 | 0.78 | 0.00 | -0.78 | MSL gene |
| LinJ.31 | 1185826 | 1188553 | LINF_310031400 | HLP | 0.80 | 0.73 | 0.00 | -0.73 | MSL gene |
| LinJ.31 | 1191356 | 1192406 | LINF_310031500 | TEI | 0.81 | 0.70 | 0.08 | -0.62 | MSL gene |
| LinJ.35 | 156279 | 159228 | LINF_350010100 | proteophosphoglycan ppg3 - putative | 3.98 | 3.43 | 3.97 | 0.54 | Multi copy gene • |
| LinJ.35 | 168215 | 175547 | LINF_350010200 | proteophosphoglycan ppg3 - putative | 10.24 | 8.71 | 9.35 | 0.64 | Multi copy gene • |

| Chr. | CDS position | | Gene ID | Product Description | RDC | | | | Note |
|--------------------------|--------------|---------|----------------|--|-------|---------|------------|------------|--|
| | Start | End | | | MA01A | T7/Cas9 | Comparison | Difference | |
| MG11A vs. T7/Cas9 | | | | | | | | | |
| LinJ.02 | 7750 | 8053 | LINF_020005100 | histone H4 | 1.38 | 1.57 | 0.97 | -0.60 | DNA binding; Protein heterodimerization activity |
| LinJ.02 | 90962 | 92927 | LINF_020006900 | phosphoglycan beta 1 -3 galactosyltransferase | 1.67 | 1.35 | 2.20 | 0.84 | Protein glycosylation |
| LinJ.02 | 99642 | 101961 | LINF_020007000 | phosphoglycan beta 1 -3 galactosyltransferase | 2.01 | 1.76 | 2.64 | 0.88 | Protein glycosylation |
| LinJ.08 | 289982 | 290597 | LINF_080011900 | amastin-like protein | 0.75 | 0.49 | 1.48 | 0.99 | --- |
| LinJ.08 | 295606 | 296212 | LINF_080012000 | amastin-like protein | 0.94 | 0.67 | 2.49 | 1.82 | --- |
| LinJ.08 | 300237 | 300840 | LINF_080012100 | Amastin surface glycoprotein - putative | 0.48 | 0.38 | 1.06 | 0.68 | --- |
| LinJ.08 | 304862 | 305465 | LINF_080012200 | Amastin surface glycoprotein - putative | 0.52 | 0.36 | 1.00 | 0.64 | --- |
| LinJ.08 | 309446 | 310049 | LINF_080012300 | Amastin surface glycoprotein - putative | 0.46 | 0.40 | 1.04 | 0.64 | --- |
| LinJ.08 | 313959 | 314571 | LINF_080012400 | amastin-like protein | 0.48 | 0.38 | 0.98 | 0.59 | --- |
| LinJ.08 | 337858 | 338386 | LINF_080012900 | Amastin-like protein | 1.53 | 1.15 | 1.67 | 0.52 | --- |
| LinJ.10 | 231389 | 231845 | LINF_100010500 | hypothetical protein | 2.11 | 1.76 | 2.27 | 0.51 | Cell adhesion; Proteolysis |
| LinJ.10 | 242240 | 244040 | LINF_100010900 | GP63 - leishmanolysin | 2.25 | 1.86 | 2.38 | 0.52 | Cell adhesion; Proteolysis |
| LinJ.10 | 245287 | 247087 | LINF_100011000 | GP63 - leishmanolysin | 2.12 | 1.79 | 2.33 | 0.53 | Cell adhesion; Proteolysis |
| LinJ.10 | 255010 | 256810 | LINF_100011300 | GP63 - leishmanolysin | 2.08 | 1.75 | 2.25 | 0.50 | Cell adhesion; Proteolysis |
| LinJ.14 | 266070 | 266970 | LINF_140012400 | fatty acid elongase - putative | 0.85 | 0.57 | 1.71 | 1.14 | Fatty acid elongase activity |
| LinJ.15 | 522568 | 524044 | LINF_150020000 | nucleoside transporter 1 - putative | 0.37 | 0.30 | 0.84 | 0.55 | Nucleoside transmembrane transport |
| LinJ.15 | 526293 | 527769 | LINF_150020100 | nucleoside transporter 1 - putative | 0.31 | 0.22 | 0.88 | 0.66 | Nucleoside transmembrane transport |
| LinJ.19 | 361121 | 361487 | LINF_190013900 | ATG8/AUT7/APG8/PAZ2 - putative | 0.32 | 0.24 | 0.74 | 0.50 | --- |
| LinJ.19 | 364703 | 365069 | LINF_190014100 | ATG8/AUT7/APG8/PAZ2 - putative | 0.34 | 0.36 | 0.86 | 0.50 | --- |
| LinJ.22 | 777241 | 778756 | LINF_220023200 | hypothetical protein | 5.66 | 4.42 | 5.76 | 1.34 | --- |
| LinJ.23 | 572031 | 572403 | LINF_230020400 | hypothetical protein - conserved | 0.78 | 0.44 | 0.99 | 0.55 | --- |
| LinJ.24 | 211299 | 211797 | LINF_240010800 | hypothetical protein | 0.79 | 0.46 | 0.96 | 0.51 | --- |
| LinJ.25 | 387568 | 387880 | LINF_250016300 | hypothetical protein | 0.70 | 0.35 | 0.90 | 0.55 | --- |
| LinJ.25 | 886708 | 887011 | LINF_250031800 | histone H4 | 0.28 | 0.24 | 0.90 | 0.66 | DNA binding;protein heterodimerization activity |
| LinJ.27 | 1172825 | 1174127 | LINF_270033890 | hypothetical protein - conserved | 3.77 | 3.22 | 3.79 | 0.57 | --- |
| LinJ.28 | 351787 | 352168 | LINF_280015100 | Tctex-1 family - putative | 0.89 | 0.44 | 0.94 | 0.51 | --- |
| LinJ.28 | 841412 | 842462 | LINF_280027700 | glycerophosphoryl diester phosphodiesterase - putative | 0.80 | 0.41 | 0.91 | 0.50 | Lipid metabolic process |
| LinJ.30 | 220944 | 221145 | LINF_300011800 | 40S ribosomal protein S30 - putative | 0.83 | 0.67 | 1.21 | 0.54 | Translation |
| LinJ.31 | 1017453 | 1017756 | LINF_310027800 | hypothetical protein | 0.72 | 0.44 | 0.96 | 0.52 | --- |
| LinJ.31 | 1181281 | 1182328 | LINF_310031200 | NUC1 | 0.80 | 0.71 | 0.00 | -0.71 | MSL gene: DNA catabolic process |
| LinJ.31 | 1184204 | 1185341 | LINF_310031300 | NUC2 | 0.76 | 0.78 | 0.00 | -0.78 | MSL gene: DNA catabolic process |
| LinJ.31 | 1185826 | 1188553 | LINF_310031400 | HLP | 0.80 | 0.73 | 0.00 | -0.73 | MSL gene: ATP binding |

| Chr. | CDS position | | Gene ID | Product Description | RDC | | | | Note |
|---------|--------------|---------|----------------|--|-------|---------|------------|------------|---|
| | Start | End | | | MA01A | T7/Cas9 | Comparison | Difference | |
| LinJ.31 | 1191356 | 1192406 | LINF_310031500 | TEI | 0.81 | 0.70 | 0.10 | -0.60 | MSL gene: catalytic activity |
| LinJ.32 | 964910 | 965129 | LINF_320031400 | hypothetical protein | 0.89 | 0.46 | 1.01 | 0.55 | --- |
| LinJ.33 | 327305 | 328637 | LINF_330015200 | beta tubulin | 1.61 | 1.17 | 1.69 | 0.52 | Microtubule-based process |
| LinJ.34 | 333051 | 333534 | LINF_340012700 | Predicted membrane protein - putative | 0.83 | 0.37 | 0.87 | 0.50 | --- |
| LinJ.34 | 745976 | 746594 | LINF_340023000 | Amastin surface glycoprotein - putative | 1.07 | 0.69 | 1.22 | 0.53 | --- |
| LinJ.34 | 749230 | 749848 | LINF_340023100 | Amastin surface glycoprotein - putative | 0.94 | 0.73 | 1.34 | 0.61 | --- |
| LinJ.34 | 770988 | 771570 | LINF_340023400 | amastin-like surface protein - putative | 1.22 | 0.92 | 1.44 | 0.53 | --- |
| LinJ.34 | 787427 | 788009 | LINF_340023600 | amastin-like surface protein - putative | 1.27 | 0.69 | 1.38 | 0.69 | --- |
| LinJ.34 | 1258241 | 1258829 | LINF_340034600 | Amastin surface glycoprotein - putative | 1.71 | 1.52 | 2.05 | 0.54 | --- |
| LinJ.35 | 156279 | 159228 | LINF_350010100 | proteophosphoglycan ppg3 - putative | 3.98 | 3.43 | 4.79 | 1.35 | Immune response; Toll-like receptor signaling pathway |
| LinJ.35 | 168215 | 175547 | LINF_350010200 | proteophosphoglycan ppg3 - putative | 10.24 | 8.71 | 11.12 | 2.41 | Immune response; Toll-like receptor signaling pathway |
| LinJ.36 | 2735520 | 2736015 | LINF_360082100 | U1A small nuclear ribonucleoprotein - putative | 0.94 | 0.38 | 0.89 | 0.52 | Nucleic acid binding |

Chr., chromosome. Note: •, CDS that showed RDC difference > 0.5 in more than one MSL knockout line; MSL, miltefosine sensitive locus.

Supplementary Table 3: Summary of variants identified in coding sequence region of the *L. infantum* knockout mutants against T7/Cas9. Variants were filtered for minor allele count >3 to monomorphic variants. Table with all variants identified was deposited on Figshare, DOI: <https://doi.org/10.6084/m9.figshare.21334602>.

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--|---------------|----------------|-----------------|-----------------|----------|---------|---------------|
| | | | | | | | | MA01A | T7/Cas9 | Δ nuc1 |
| LinJ.10 | 175781 | T | G | 319.799 | Moderate | CDS | LINF_100009100 | 0/1 | 0/1 | 0/0 |
| LinJ.11 | 20258 | G | A | 327.929 | Moderate | CDS | LINF_110005600 | 0/1 | 1/1 | 0/0 |
| LinJ.11 | 508343 | G | A | 165.248 | Moderate | CDS | LINF_110018350 | 0/1 | 0/1 | 0/0 |
| LinJ.11 | 508355 | CCGGA | ACGGG | 864.956 | Moderate | CDS | LINF_110018350 | 0/1 | 0/1 | 0/0 |
| LinJ.12 | 407488 | A | G | 635.555 | Moderate | CDS | LINF_120013300 | 0/1 | 0/1 | 0/0 |
| LinJ.12 | 407493 | G | A | 559.85 | Moderate | CDS | LINF_120013300 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 503382 | C | A | 685.08 | Moderate | CDS | LINF_140017400 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 511767 | AAC | AAG | 353.211 | Moderate | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 513700 | A | G | 125.698 | Moderate | CDS | LINF_140017500 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 517675 | C | G | 368.782 | Moderate | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.16 | 594184 | G | T | 206.471 | Moderate | CDS | LINF_160020700 | 1/1 | 0/0 | 0/1 |
| LinJ.22 | 395729 | GGCTGTTGGCCCGCTCTCCGT | GGCTGTTGGCCCGCTCTCCGTTGGCCCG CAGTCTGT | 814.536 | Moderate | CDS | LINF_220014400 | 0/1 | 0/0 | 1/1 |
| LinJ.22 | 603126 | GAG | CAC | 121.468 | Moderate | CDS | LINF_220019300 | 0/1 | 0/1 | 0/0 |
| LinJ.22 | 604346 | T | C | 143.946 | Moderate | CDS | LINF_220019300 | 0/0 | 0/1 | 1/1 |
| LinJ.23 | 10055 | A | C | 40.3924 | Moderate | CDS | LINF_230005300 | 0/0 | 0/0 | 0/1 |
| LinJ.31 | 1151875 | G | C | 38.116 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892355 | AAGGC | AAGGT | 76.1411 | Moderate | CDS | LINF_320029100 | 0/0 | 0/0 | 0/1 |
| LinJ.35 | 158648 | T | C | 508.892 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 1/1 |
| LinJ.35 | 158716 | G | C | 31.1805 | Moderate | CDS | LINF_350010100 | 0/0 | 0/0 | 0/1 |
| LinJ.35 | 171534 | TCGC | GCGT | 50.1271 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.36 | 2068365 | C | G | 149.977 | Moderate | CDS | LINF_360062000 | 1/1 | 0/1 | 0/0 |
| LinJ.02 | 12368 | G | A | 66.2392 | Low | CDS | LINF_020005300 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 510767 | C | T | 64.7176 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 1/1 |
| LinJ.14 | 515174 | G | A | 39.4405 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 515198 | A | G | 468.366 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.15 | 163452 | G | A | 52.7764 | Low | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.27 | 1173671 | T | G | 1685.61 | Low | CDS | LINF_270033890 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892325 | GAGC | AAGG | 50.7191 | Low | CDS | LINF_320029100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 173975 | G | A | 406.402 | Low | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 174494 | G | A | 110.047 | Low | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|---------------|
| | | | | | | | | MA01A | T7/Cas9 | $\Delta nuc2$ |
| LinJ.02 | 346989 | C | T | 392.723 | Moderate | CDS | LINF_020012400 | 1/1 | 1/1 | 0/1 |
| LinJ.11 | 508330 | G | C | 42.6178 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.11 | 508337 | A | G | 46.3174 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.13 | 293651 | T | C | 103.113 | Moderate | CDS | LINF_130013400 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 513700 | A | G | 125.698 | Moderate | CDS | LINF_140017500 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 517590 | A | G | 271.366 | Moderate | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.15 | 164728 | A | G | 82.3111 | Moderate | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.23 | 10055 | A | C | 40.3924 | Moderate | CDS | LINF_230005300 | 0/0 | 0/0 | 0/1 |
| LinJ.31 | 1151875 | G | C | 38.116 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892355 | AAGGC | AAGGT | 76.1411 | Moderate | CDS | LINF_320029100 | 0/0 | 0/0 | 0/1 |
| LinJ.33 | 1445871 | G | T | 615.552 | Moderate | CDS | LINF_330040700 | 0/1 | 0/1 | 0/0 |
| LinJ.34 | 319484 | C | G | 474.818 | Moderate | CDS | LINF_340012400 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 158493 | GTCGG | ATCGC | 904.01 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 158882 | A | G | 1900.74 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 510767 | C | T | 64.7176 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 517676 | GA | GC | 253.8 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.15 | 163452 | G | A | 52.7764 | Low | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.22 | 599299 | T | G | 109.915 | Low | CDS | LINF_220019200 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892325 | GAGC | AAGG | 50.7191 | Low | CDS | LINF_320029100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 158427 | G | A | 429.114 | Low | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171638 | C | G | 47.6726 | Low | CDS | LINF_350010200 | 0/0 | 0/0 | 0/1 |

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|--------------|
| | | | | | | | | MA01A | T7/Cas9 | Δhlp |
| LinJ.11 | 20258 | G | A | 327.929 | Moderate | CDS | LINF_110005600 | 0/1 | 1/1 | 0/0 |
| LinJ.11 | 508337 | A | G | 46.3174 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.13 | 293651 | T | C | 103.113 | Moderate | CDS | LINF_130013400 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 503382 | C | A | 685.08 | Moderate | CDS | LINF_140017400 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 513700 | A | G | 125.698 | Moderate | CDS | LINF_140017500 | 0/0 | 0/0 | 0/1 |
| LinJ.15 | 164644 | GCGG | ACGC | 180.618 | Moderate | CDS | LINF_150009800 | 0/1 | 0/1 | 1/1 |
| LinJ.16 | 594184 | G | T | 206.471 | Moderate | CDS | LINF_160020700 | 1/1 | 0/0 | 0/1 |
| LinJ.22 | 604346 | T | C | 143.946 | Moderate | CDS | LINF_220019300 | 0/0 | 0/1 | 1/1 |
| LinJ.23 | 10055 | A | C | 40.3924 | Moderate | CDS | LINF_230005300 | 0/0 | 0/0 | 0/1 |
| LinJ.31 | 1151875 | G | C | 38.116 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|--------------|
| | | | | | | | | MA01A | T7/Cas9 | Δhlp |
| LinJ.32 | 892355 | AAGGC | AAGGT | 76.1411 | Moderate | CDS | LINF_320029100 | 0/0 | 0/0 | 0/1 |
| LinJ.35 | 158992 | GTCGGCGTCCTCGTCGTCT | GTCGGCGTCGTCTCGTCGTCT | 86.1395 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171534 | TCGC | GCGT | 50.1271 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 1/1 |
| LinJ.02 | 12368 | G | A | 66.2392 | Low | CDS | LINF_020005300 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 510767 | C | T | 64.7176 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 1/1 |
| LinJ.35 | 171638 | C | G | 47.6726 | Low | CDS | LINF_350010200 | 0/0 | 0/0 | 1/1 |
| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
| | | | | | | | | MA01A | T7/Cas9 | Δei |
| LinJ.11 | 20258 | G | A | 327.929 | Moderate | CDS | LINF_110005600 | 0/1 | 1/1 | 0/0 |
| LinJ.11 | 508889 | ATATTC | ATGTTT | 1825.67 | Moderate | CDS | LINF_110018350 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 297402 | C | A | 1501.65 | Moderate | CDS | LINF_140013100 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 513700 | A | G | 125.698 | Moderate | CDS | LINF_140017500 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 516523 | G | C | 109.984 | Moderate | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.15 | 164728 | A | G | 82.3111 | Moderate | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.22 | 396481 | TCT | AGG | 755.038 | Moderate | CDS | LINF_220014400 | 0/1 | 0/1 | 1/1 |
| LinJ.22 | 604346 | T | C | 143.946 | Moderate | CDS | LINF_220019300 | 0/0 | 0/1 | 1/1 |
| LinJ.23 | 10055 | A | C | 40.3924 | Moderate | CDS | LINF_230005300 | 0/0 | 0/0 | 0/1 |
| LinJ.31 | 1151875 | G | C | 38.116 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.31 | 1155400 | C | T | 2764.24 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892355 | AAGGC | AAGGT | 76.1411 | Moderate | CDS | LINF_320029100 | 0/0 | 0/0 | 0/1 |
| LinJ.35 | 158716 | G | C | 31.1805 | Moderate | CDS | LINF_350010100 | 0/0 | 0/0 | 1/1 |
| LinJ.35 | 158992 | GTCGGCGTCCTCGTCGTCT | GTCGGCGTCGTCTCGTCGTCT | 86.1395 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 510767 | C | T | 64.7176 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 1/1 |
| LinJ.15 | 163452 | G | A | 52.7764 | Low | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.15 | 164532 | C | G | 1848.09 | Low | CDS | LINF_150009800 | 0/1 | 0/1 | 1/1 |
| LinJ.16 | 594218 | A | G | 2360.21 | Low | CDS | LINF_160020700 | 0/1 | 0/1 | 1/1 |
| LinJ.22 | 599299 | T | G | 109.915 | Low | CDS | LINF_220019200 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892325 | GAGC | AAGG | 50.7191 | Low | CDS | LINF_320029100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171638 | C | G | 47.6726 | Low | CDS | LINF_350010200 | 0/0 | 0/0 | 1/1 |
| LinJ.35 | 173975 | G | A | 406.402 | Low | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|--------------------|
| | | | | | | | | MA01A | T7/Cas9 | Δ nuc1/nuc2 |
| LinJ.11 | 508330 | G | C | 42.6178 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.11 | 508337 | A | G | 46.3174 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.13 | 293651 | T | C | 103.113 | Moderate | CDS | LINF_130013400 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 515275 | C | G | 2926.33 | Moderate | CDS | LINF_140017500 | 1/1 | 1/1 | 0/1 |
| LinJ.14 | 517339 | C | G | 63.399 | Moderate | CDS | LINF_140017500 | 0/0 | 1/1 | 0/1 |
| LinJ.15 | 164644 | GCGG | ACGC | 180.618 | Moderate | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.22 | 603126 | GAG | CAC | 121.468 | Moderate | CDS | LINF_220019300 | 0/1 | 0/1 | 1/1 |
| LinJ.22 | 778316 | CGC | CGG | 6045.78 | Moderate | CDS | LINF_220023200 | 0/1 | 0/1 | 1/1 |
| LinJ.23 | 10055 | A | C | 40.3924 | Moderate | CDS | LINF_230005300 | 0/0 | 0/0 | 0/1 |
| LinJ.32 | 892355 | AAGGC | AAGGT | 76.1411 | Moderate | CDS | LINF_320029100 | 0/0 | 0/0 | 0/1 |
| LinJ.35 | 158648 | T | C | 508.892 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 1/1 |
| LinJ.35 | 158716 | G | C | 31.1805 | Moderate | CDS | LINF_350010100 | 0/0 | 0/0 | 1/1 |
| LinJ.35 | 158992 | GTCGGCGTCCTCGTCGTCT | GTCGGCGTCGTCTCGTCGTCT | 86.1395 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 170238 | GT | GC | 266.015 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 510767 | C | T | 64.7176 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 1/1 |
| LinJ.14 | 515174 | G | A | 39.4405 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892325 | GAGC | AAGG | 50.7191 | Low | CDS | LINF_320029100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 173189 | TTCAGCGTCGTCGTCGTC | GTCAGCGTCGTCGTCGTC | 501.855 | Low | CDS | LINF_350010200 | 1/1 | 0/1 | 0/0 |

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|--------------|
| | | | | | | | | MA01A | T7/Cas9 | Δ msl |
| LinJ.10 | 175781 | T | G | 319.799 | Moderate | CDS | LINF_100009100 | 0/1 | 0/1 | 0/0 |
| LinJ.11 | 20258 | G | A | 327.929 | Moderate | CDS | LINF_110005600 | 0/1 | 1/1 | 0/0 |
| LinJ.11 | 508330 | G | C | 42.6178 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.11 | 508337 | A | G | 46.3174 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.11 | 508889 | ATATTC | ATGTTT | 1825.67 | Moderate | CDS | LINF_110018350 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 297402 | C | A | 1501.65 | Moderate | CDS | LINF_140013100 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 513700 | A | G | 125.698 | Moderate | CDS | LINF_140017500 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 515275 | C | G | 2926.33 | Moderate | CDS | LINF_140017500 | 1/1 | 1/1 | 0/1 |
| LinJ.14 | 517339 | C | G | 63.399 | Moderate | CDS | LINF_140017500 | 0/0 | 1/1 | 0/1 |
| LinJ.16 | 594184 | G | T | 206.471 | Moderate | CDS | LINF_160020700 | 1/1 | 0/0 | 0/1 |
| LinJ.22 | 396481 | TCT | AGG | 755.038 | Moderate | CDS | LINF_220014400 | 0/1 | 0/1 | 1/1 |
| LinJ.22 | 604346 | T | C | 143.946 | Moderate | CDS | LINF_220019300 | 0/0 | 0/1 | 1/1 |
| LinJ.23 | 10055 | A | C | 40.3924 | Moderate | CDS | LINF_230005300 | 0/0 | 0/0 | 0/1 |

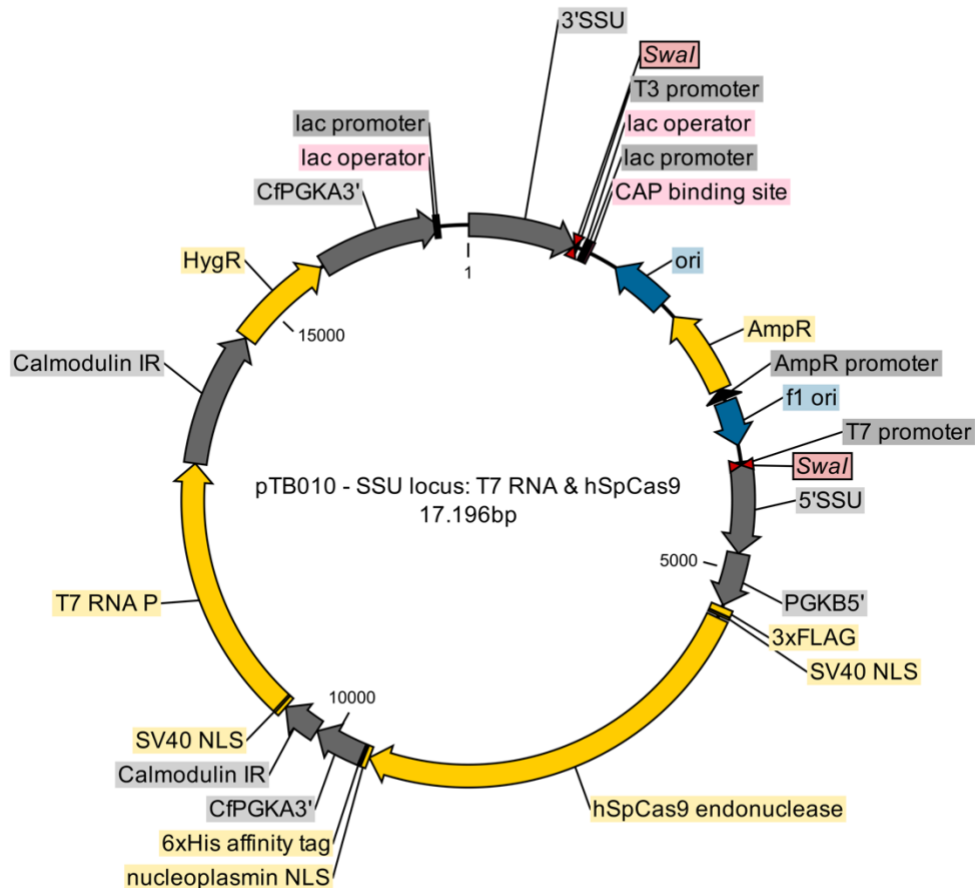
| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|-------------|
| | | | | | | | | MA01A | T7/Cas9 | <i>Δmsl</i> |
| LinJ.31 | 1151875 | G | C | 38.116 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892355 | AAGGC | AAGGT | 76.1411 | Moderate | CDS | LINF_320029100 | 0/0 | 0/0 | 0/1 |
| LinJ.35 | 158648 | T | C | 508.892 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 1/1 |
| LinJ.35 | 158716 | G | C | 31.1805 | Moderate | CDS | LINF_350010100 | 0/0 | 0/0 | 1/1 |
| LinJ.35 | 158992 | GTCGGCGTCCTCGTCGTCT | GTCGGCGTCGTCTCGTCGTCT | 86.1395 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 170320 | T | C | 796.457 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171957 | T | C | 463.911 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 1/1 |
| LinJ.02 | 12368 | G | A | 66.2392 | Low | CDS | LINF_020005300 | 0/0 | 0/0 | 0/1 |
| LinJ.14 | 515174 | G | A | 39.4405 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 516923 | G | A | 1349.11 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 1/1 |
| LinJ.27 | 1173671 | T | G | 1685.61 | Low | CDS | LINF_270033890 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892325 | GAGC | AAGG | 50.7191 | Low | CDS | LINF_320029100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171638 | C | G | 47.6726 | Low | CDS | LINF_350010200 | 0/0 | 0/0 | 1/1 |

Chr., chromosome. CDS, coding sequence.

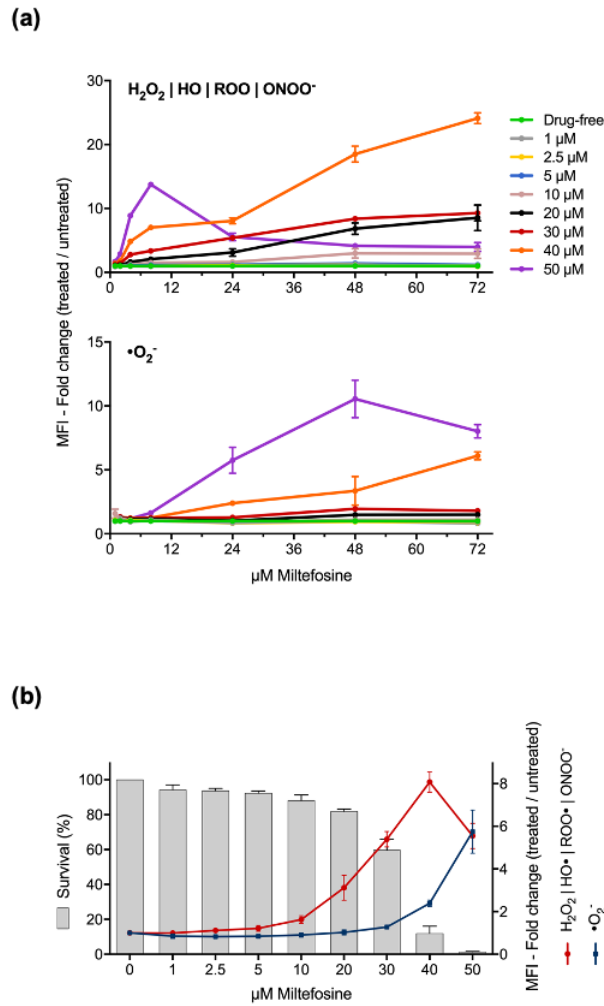
Supplementary Table 4: Summary of variants identified in coding sequence region of the *L. infantum* MG11A against T7/Cas9. Variants were filtered for minor allele count >3 to monomorphic variants. Table with all variants identified was deposited on Figshare, DOI: <https://doi.org/10.6084/m9.figshare.21334602>.

| Chr. | Position | Reference sequence (0) | Altered sequence (1;2;3) | Quality score | Variant impact | Genomic feature | Associated gene | Genotype | | |
|---------|----------|------------------------|--------------------------|---------------|----------------|-----------------|-----------------|----------|---------|-------|
| | | | | | | | | MA01A | T7/Cas9 | MG11A |
| LinJ.02 | 346989 | C | T | 392.723 | Moderate | CDS | LINF_020012400 | 1/1 | 1/1 | 0/0 |
| LinJ.07 | 48196 | G | A | 5073.36 | Moderate | CDS | LINF_070006200 | 0/1 | 0/1 | 0/0 |
| LinJ.11 | 508337 | A | G | 46.3174 | Moderate | CDS | LINF_110018350 | 0/0 | 0/0 | 0/1 |
| LinJ.11 | 508889 | ATATTC | ATGTTT | 1825.67 | Moderate | CDS | LINF_110018350 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 297402 | C | A | 1501.65 | Moderate | CDS | LINF_140013100 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 511767 | AAC | AAG | 353.211 | Moderate | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 515275 | C | G | 2926.33 | Moderate | CDS | LINF_140017500 | 1/1 | 1/1 | 0/1 |
| LinJ.14 | 517590 | A | G | 271.366 | Moderate | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.22 | 396481 | TCT | AGG | 755.038 | Moderate | CDS | LINF_220014400 | 0/1 | 0/1 | 1/1 |
| LinJ.26 | 848805 | A | G | 554.72 | Moderate | CDS | LINF_260027000 | 0/1 | 0/1 | 0/0 |
| LinJ.28 | 400404 | C | G | 2857.97 | Moderate | CDS | LINF_280016400 | 0/1 | 0/1 | 0/0 |
| LinJ.31 | 41621 | C | T | 2443.56 | Moderate | CDS | LINF_310006600 | 0/1 | 0/1 | 0/0 |
| LinJ.31 | 1151875 | G | C | 38.116 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.31 | 1155400 | C | T | 2764.24 | Moderate | CDS | LINF_310030400 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 414996 | A | G | 4397.35 | Moderate | CDS | LINF_320016100 | 0/1 | 0/1 | 0/0 |
| LinJ.33 | 1445871 | G | T | 615.552 | Moderate | CDS | LINF_330040700 | 0/1 | 0/1 | 0/0 |
| LinJ.34 | 319484 | C | G | 474.818 | Moderate | CDS | LINF_340012400 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 158493 | GTCGG | ATCGC | 904.01 | Moderate | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171534 | TCGC | GCGT | 50.1271 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 173776 | C | T | 5759.3 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 173866 | C | T | 1301.79 | Moderate | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.11 | 512015 | T | C | 2050.01 | Low | CDS | LINF_110018350 | 0/1 | 0/1 | 0/0 |
| LinJ.14 | 515174 | G | A | 39.4405 | Low | CDS | LINF_140017500 | 0/1 | 0/1 | 0/0 |
| LinJ.15 | 163452 | G | A | 52.7764 | Low | CDS | LINF_150009800 | 0/1 | 0/1 | 0/0 |
| LinJ.31 | 112149 | C | T | 10083.9 | Low | CDS | LINF_310008900 | 0/1 | 0/1 | 0/0 |
| LinJ.32 | 892325 | GAGC | AAGG | 50.7191 | Low | CDS | LINF_320029100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 158427 | G | A | 429.114 | Low | CDS | LINF_350010100 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 171638 | C | G | 47.6726 | Low | CDS | LINF_350010200 | 0/0 | 0/0 | 1/1 |
| LinJ.35 | 172457 | G | A | 17436.4 | Low | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |
| LinJ.35 | 173975 | G | A | 406.402 | Low | CDS | LINF_350010200 | 0/1 | 0/1 | 0/0 |

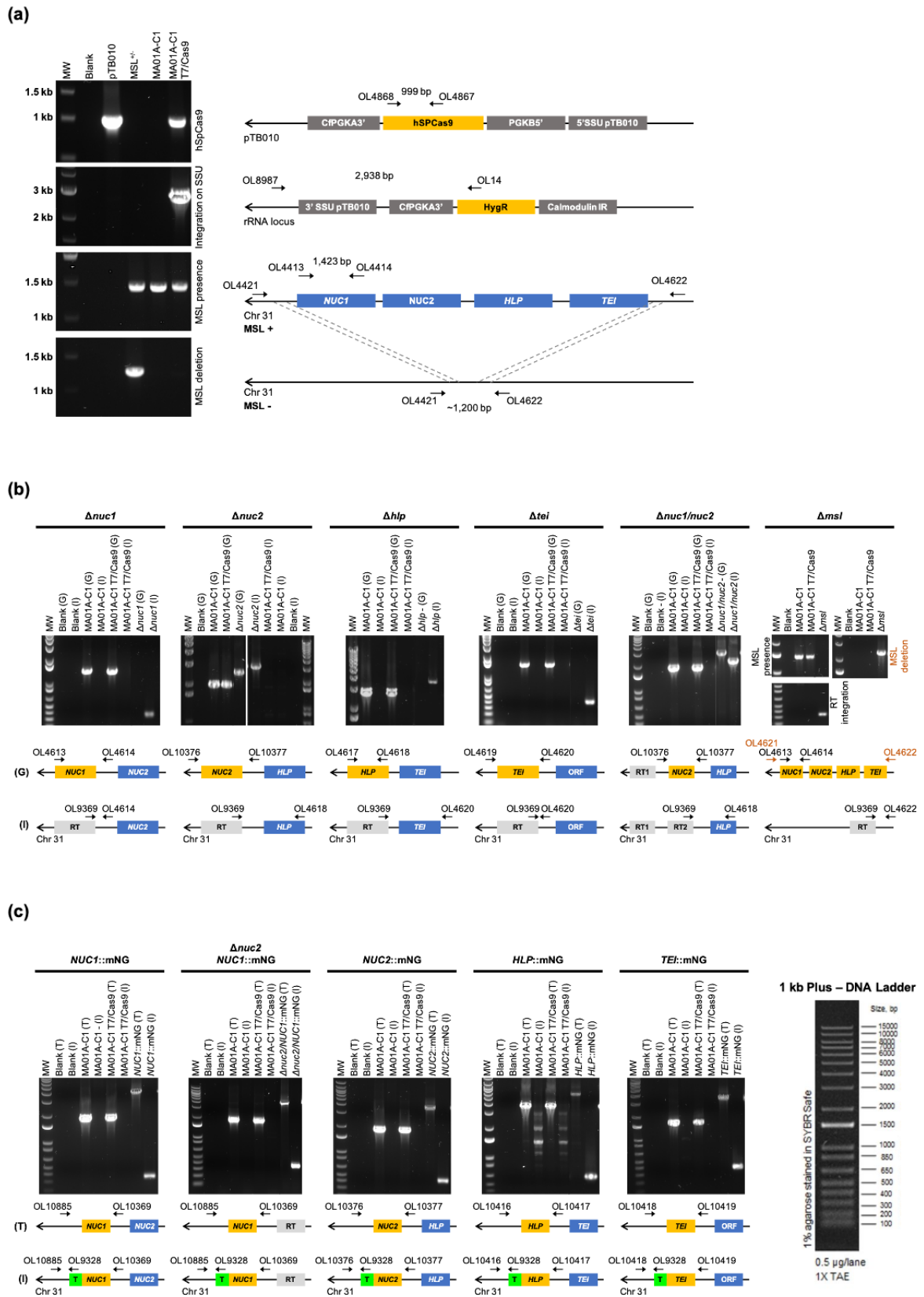
Chr., chromosome. CDS, coding sequence.



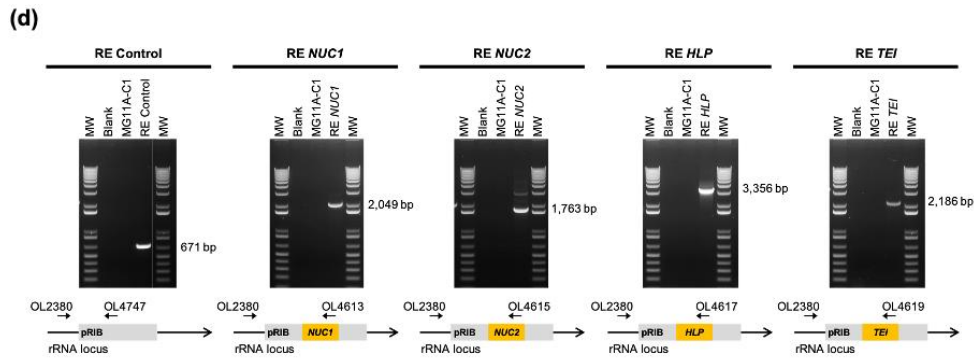
Supplementary Figure 1: Map of the plasmid pTB010. Plasmid used to generate the *L. infantum* cell line expressing T7 RNA polymerase and Cas9 endonuclease. SSU, homologous arm to integration on ribosomal locus of *Leishmania* spp.; T3 promoter, promoter for bacteriophage T3 RNA polymerase; lac operator, the lac repressor binds to the lac operator to inhibit transcription in *E. coli* (this inhibition can be relieved by adding lactose or isopropyl-beta-D-thiogalactopyranoside (IPTG)); lac promoter, promoter for the *E. coli* lac operon; CAP binding site, *E. coli* catabolite activator protein (CAP binding activates transcription in the presence of cAMP); ori, high-copy-number ColE1/pMB1/pBR322/pUC origin of replication; AmpR, product beta-lactamase to confer resistance to ampicillin, carbenicillin, and related antibiotics; f1 ori, f1 bacteriophage origin of replication (arrow indicates direction of (+) strand synthesis); T7 promoter, promoter for bacteriophage T7 RNA polymerase; 3xFLAG, three tandem FLAG(R) epitope tags, followed by an enterokinase cleavage site; NLS40, nuclear localization signal of SV40 large T antigen; hSpCas9 endonuclease, Cas9 (Csn1) endonuclease from the *Streptococcus pyogenes* Type II CRISPR/Cas system; nucleoplasmin NLS, bipartite nuclear localization signal from nucleoplasmin; T7 RNA P, T7 RNA polymerase; HygR, aminoglycoside phosphotransferase from *E. coli* (gene aph(4)-Ia) confers resistance to hygromycin; SwaI, restriction site for SwaI enzyme. The plasmid map was designed on CLC Main workbench v.20.0.3.



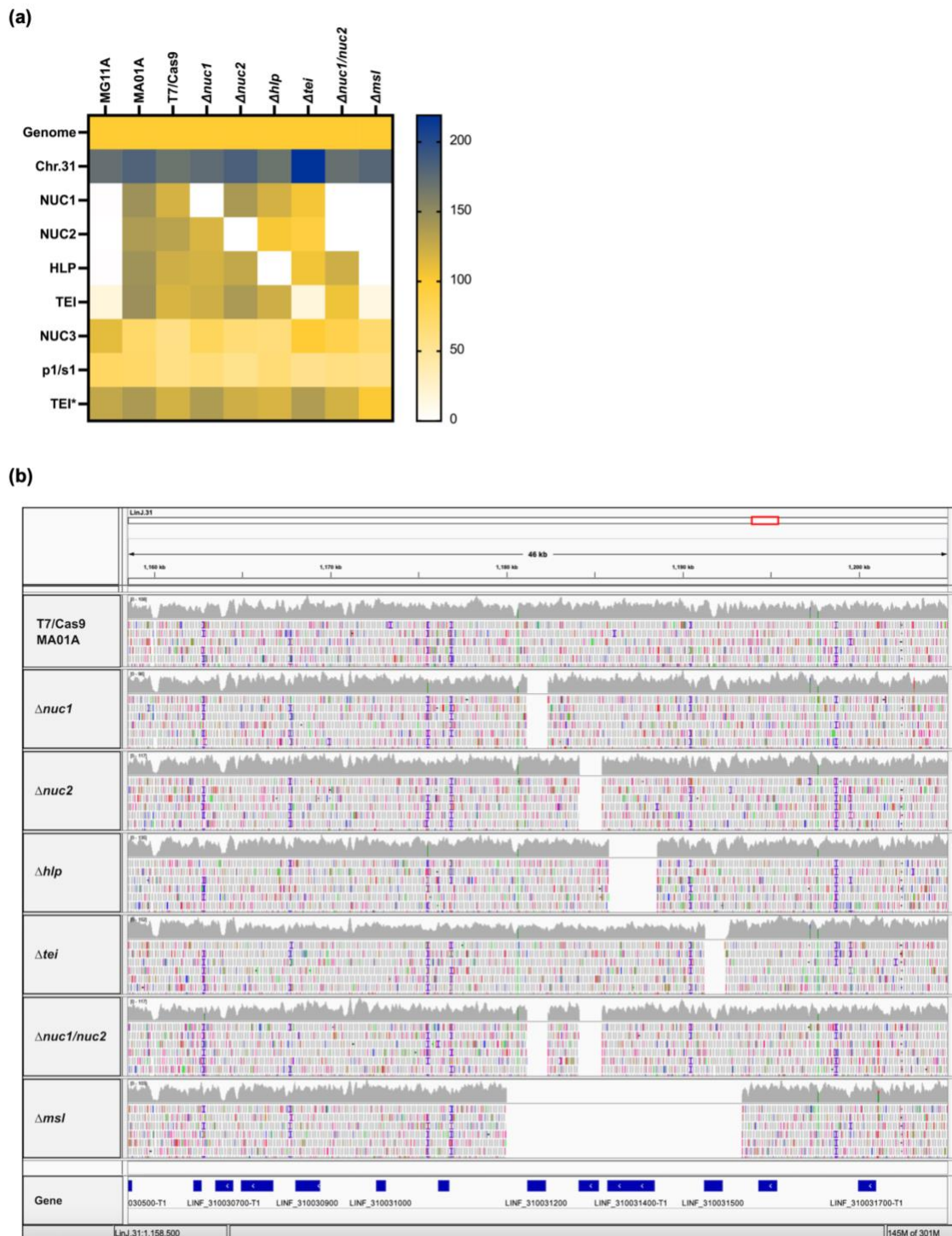
Supplementary Figure 2: Concentration and time effect of miltefosine in ROS accumulation and cell viability on promastigote stage of *L. infantum* parasite. The Brazilian reference strain of *L. infantum* MHOM/BR/74/PP75 was treated with different miltefosine concentrations over 24 h. (a) The reactive oxygen species were measured using the probes H₂DCFDA (H₂O₂, HO•, ROO• and ONOO•) and MitoSOXTM (•O₂⁻). (b) Overlap of miltefosine effect on cell viability and on ROS accumulation in *L. infantum* after 24 h of drug pressure. Data shown represents mean \pm SEM from three independent biological replicates.



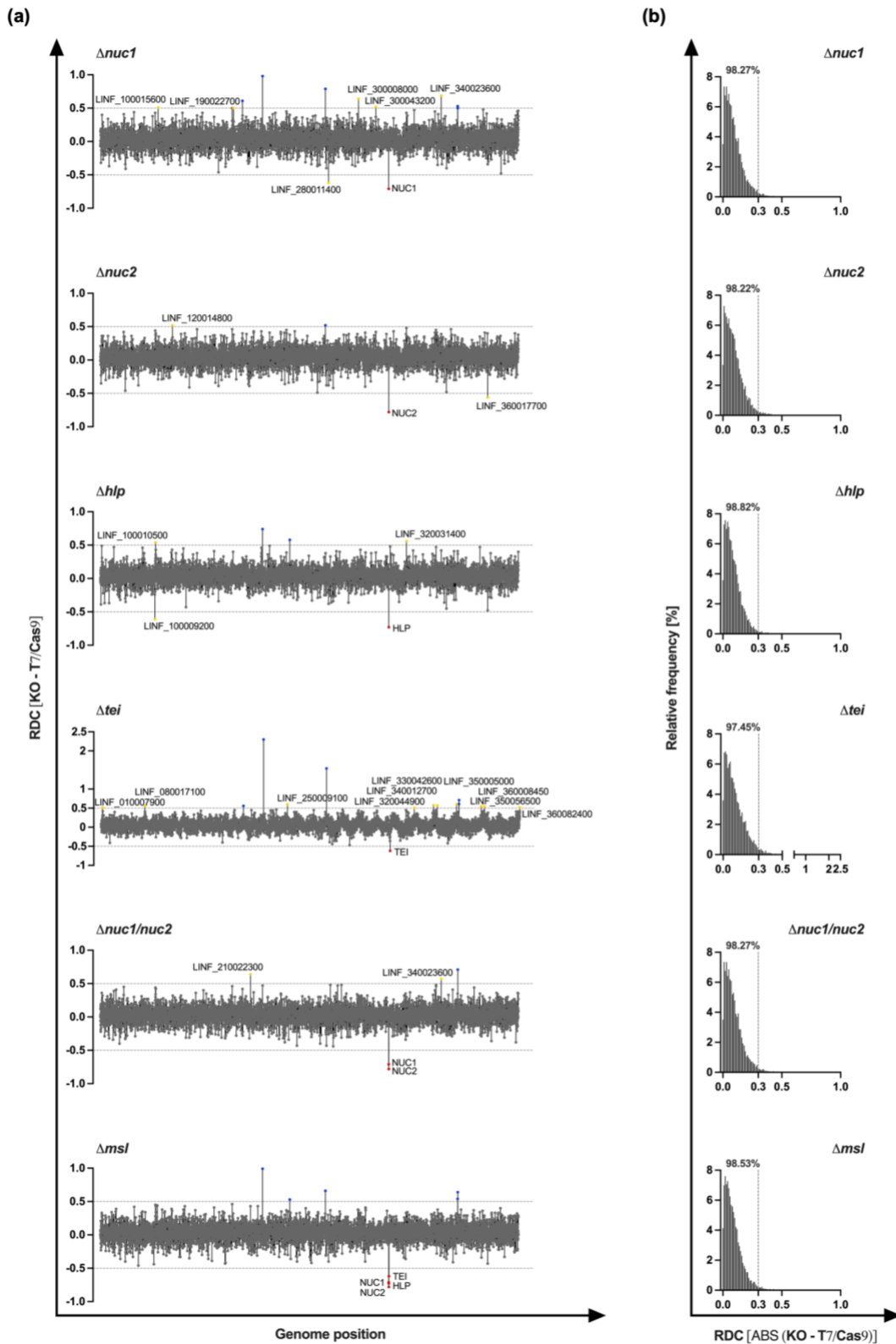
Supplementary Figure 3: PCR screening of the cell lines generated.



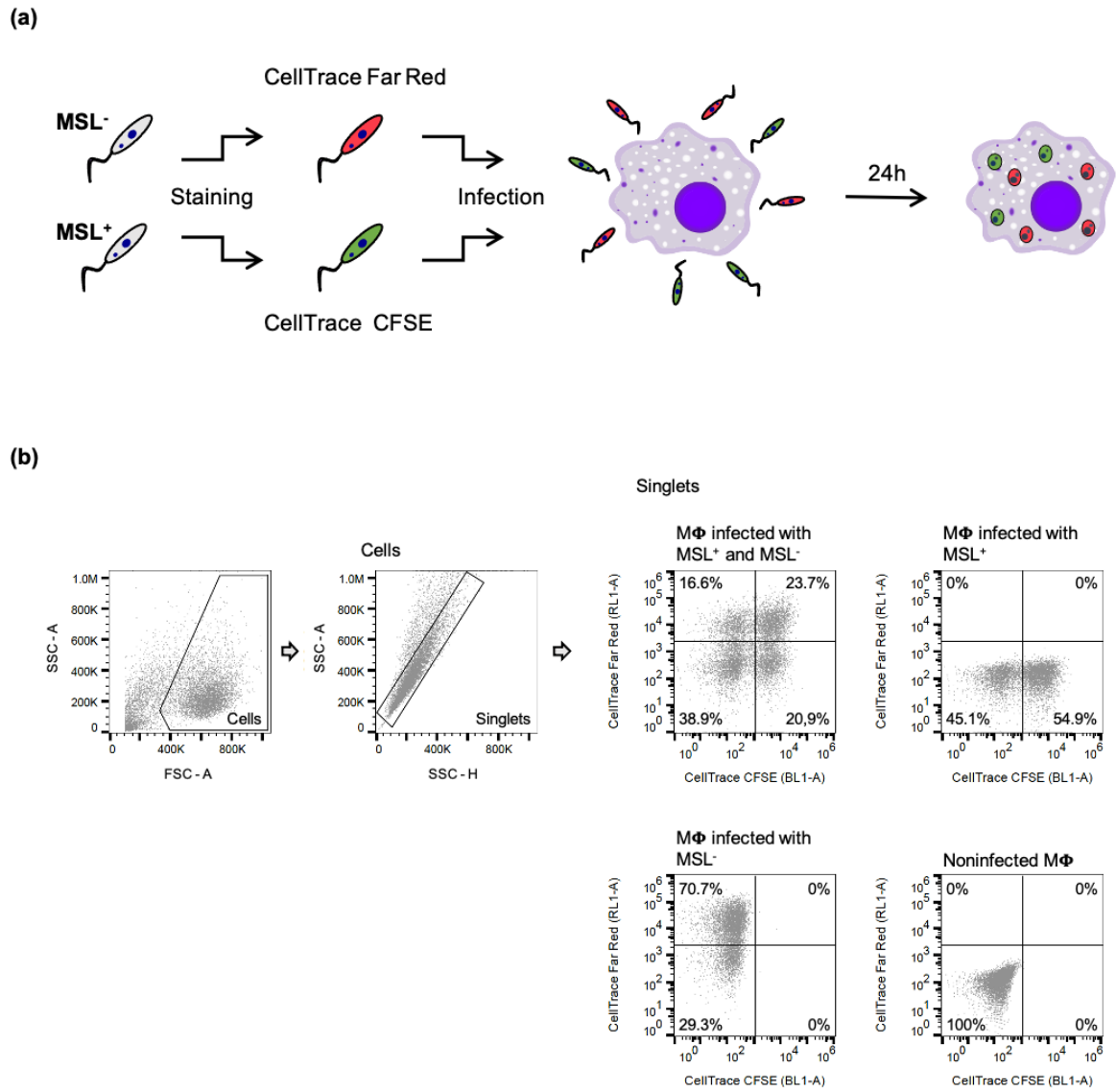
Supplementary Figure 3: PCR screening of the cell lines generated. Cell lines generated in this study were all screened by PCR and each PCR strategy used, including the set of oligonucleotides (OL), is shown. (a) Generation of *L. infantum* expressing T7 RNA polymerase and Cas9 endonuclease. (b) knock out (KO) cell lines generated by CRISPR-Cas9. The target gene is highlighted in yellow, whereas the repair template (RT) and neighbouring ORFs are indicated by grey and blue boxes, respectively. The results of the PCRs to screening for the presence of the target gene (G) and for the integration (I) of RT are interpreted as follow: (i) $\Delta nuc1$, the presence of the target gene is indicated by a PCR product of 1423 bp whereas its absence by a negative PCR. Integration of RT is shown by a PCR product of 408 bp; (ii) $\Delta nuc2$, the presence of the target gene is indicated by a PCR product of 1938 bp whereas its replacement by RT carrying puromycin (PAC) or blasticidin (BSD) resistance genes by a PCR product of 2436 bp and 2305 bp, respectively. Integration of RT is shown by a PCR product of 3177 bp; (iii) Δhlp , the presence of the target gene is indicated by a PCR product of 2727 bp whereas its absence by a negative PCR. Integration of RT is shown by a PCR product of 4331 bp; (iv) Δtei , the presence of the target gene is indicated by a PCR product of 1562 bp whereas its absence by a negative PCR. Integration of RT is shown by a PCR product of 423 bp; (v) $\Delta nuc1/nuc2$, the $\Delta nuc1$ cell line, carrying only PAC resistant mark (RT1), was used to generate $\Delta nuc1/nuc2$. The screening to check deletion of NUC2 was performed as the one mentioned for $\Delta nuc2$; (vi) Δmsl , the presence of MSL locus was checked by a PCR product of 1423 bp, corresponding to the presence of NUC1, whereas its absence by a negative PCR. The deletion of MSL was also checked by a PCR flanking the locus, which generated a fragment of 2412 bp or 2289 bp if the locus was replaced by RT carrying PAC or BSD genes, respectively. Integration of RT is shown by a PCR product of 340 bp. (c) C-terminal tagged cell lines generated by CRISPR-Cas9. The target gene is indicated in yellow and the mNeonGreen tag (T) in green. The PCRs used to show that all alleles were tagged were designed using a set of primers flanking the target gene and are interpreted as follow: (i) NUC1::mNG, tagged and untagged alleles show a PCR product of 4348 bp and 1929 bp, respectively. Integration in the right position is shown by a PCR product of 537 bp. (ii) NUC2::mNG, tagged and untagged alleles show a PCR product of 4,390 bp and 1,938 bp, respectively. Integration in the right position is shown by a PCR product of 576 bp. (iii) HLP::mNG, tagged and untagged alleles show a PCR product of 6194 bp and 3699 bp, respectively. Integration in the right position is shown by a PCR product of 780 bp. (iv) TEI::mNG, tagged and untagged alleles show a PCR product of 4206 bp and 1778 bp, respectively. Integration in the right position is shown by a PCR product of 601 bp. (d) Cell lines re-expressing (RE) MSL genes individually on rRNA locus of the *L. infantum* isolate (MG11A-C1), that lacks this locus on chromosome 31. The re-expression was performed using the pRIB vector. SSU, rRNA locus on *Leishmania* spp.; MSL, miltefosine susceptible locus; NUC1, LINF_310031200; NUC2, LINF_310031300; HLP, LINF_310031400; TEI, LINF_310031500.



Supplementary Figure 4: Whole Genome Sequence analysis of *L. infantum* isolates/mutants. (a) Aneuploidy in natural populations and mutants. The heatmap shows the copy-number status of chromosome 31, the MSL genes (*NUC1*, LINF_310031200; *NUC2*, LINF_310031300; *HLP*, LINF_310031400; and *TEI*, LINF_310031500) and the MSL paralog (*NUC3*, LINF_120009100; *p1/s1*, LINF_300020200) and orthologue (*TEI**, LINF_310030700) genes. (b) Integrative genomics viewer (IGV, v.2.7.2) of the Illumina whole genome sequencing reads from $\Delta nuc1$, $\Delta nuc2$, Δhpl , Δtei , $\Delta nuc1/nuc2$, and Δmsl mutants aligned against the *L. infantum* reference genome JPCM5. Visual inspection of the aligned reads in a genome browser of the sequencing coverage of chromosome 31 region that contain the genes from the MSL locus. Generally, a lack of coverage in sequenced isolates must be interpreted carefully, as it can often represent a region that does not replicate or map well. Visual inspection of the aligned reads in a genome browser, however, supported the calculated predictions with the presence of reads that are split over the predicted deletion indicating that this is a genuine deletion and not a poorly sequenced region. The WGS data generated for all mutants were added to the previous SRA project ID PRJNA494801.

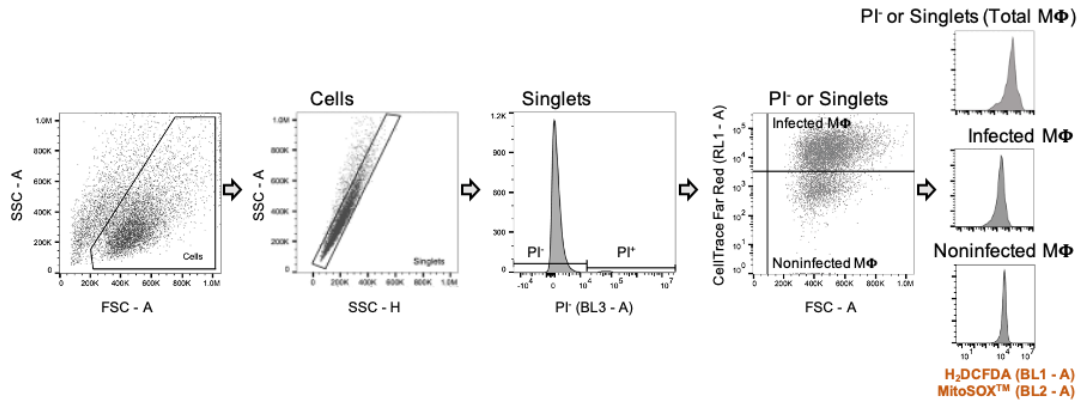


Supplementary Figure 5: Read depth coverage differences between MSL mutants and *L. infantum* T7/Cas9. (a) Read depth coverage (RDC) of each coding sequence (CDS) were estimated, normalized per chromosome RDC, and compared between the MSL knockout mutants and T7/Cas9. All CDS with difference [mutants - T7/Cas9] higher than 0.5 RDC, which corresponds to approximately one copy per haploid genome, are highlighted in yellow or blue (CDS that RDC varied in more than one MSL knockout line). MSL genes are highlighted in red. Each line of the X axis corresponds to a CDS, ordered by its genome position from the smallest to the largest chromosome. (b) Histogram plot of the absolute RDC differences between MSL knockout lines and T7/Cas9. The accumulated frequency for absolute RDC difference until 0.3 is indicated in each plot [%].

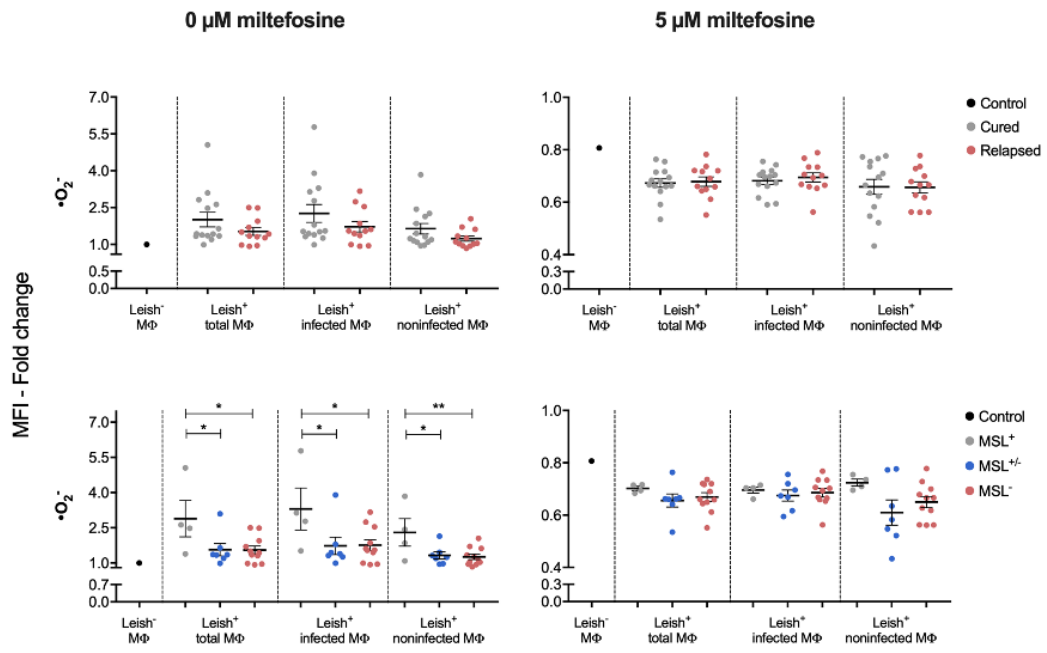


Supplementary Figure 7: Multiple *Leishmania* infection in RAW264.7 macrophage. (a) experimental draw to show if RAW264.7 macrophage can be simultaneously infected with more than one parasite genotype. *L. infantum* MSL⁺ and MSL⁻ were individually stained with CellTrace CFSE and Far red, respectively, mixed and then used to infect RAW264.7. After 24 h of infection the macrophages were analysed by flow cytometry. (b) Flow cytometry plots and gate strategy used to analyse the intracellular infection of RAW264.7 macrophage by *Leishmania* parasites: MSL⁺ and MSL⁻ simultaneously; MSL⁺; MSL⁻; and a noninfected control.

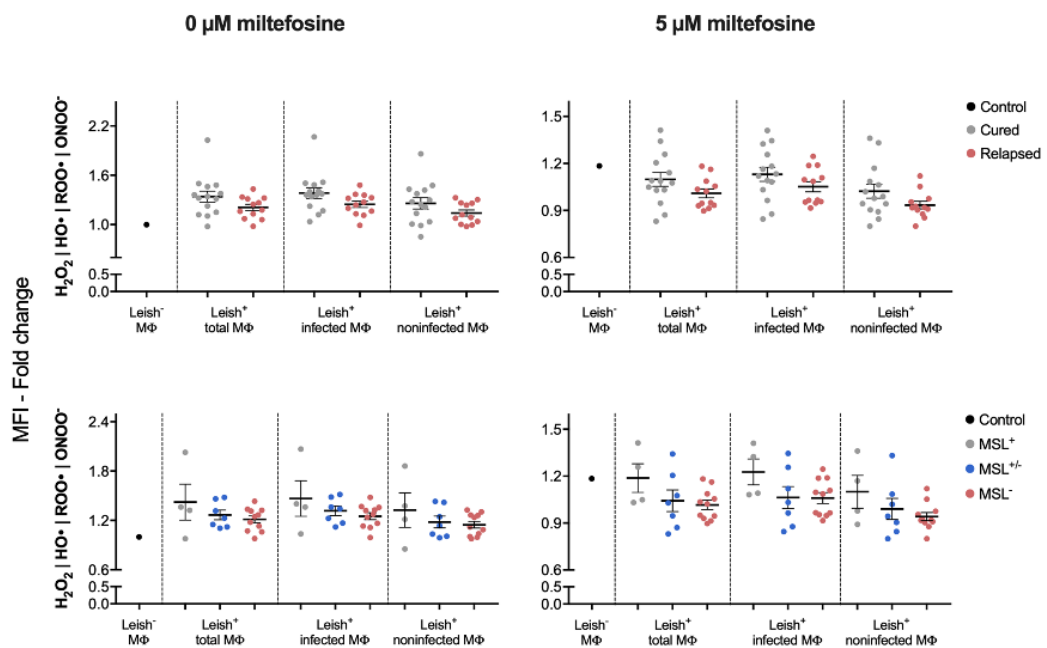
(a)



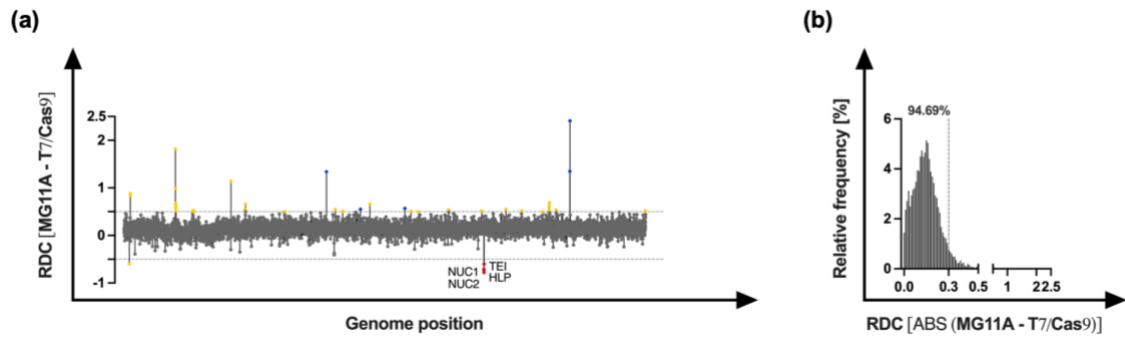
(b)



(c)



Supplementary Figure 8: Intracellular level of ROS into RAW264.7 cells challenged with different *L. infantum* isolates. (a) RAW264.7 cells challenged for 24 h with *L. infantum* (26 isolates: 14 from group cured and 12 from group relapsed). The isolates were also sorted by their MSL genotype/clinical outcome: 4 MSL⁺/cured, 7 MSL^{+/-}/cured, and 11 MSL⁻/relapsed) were subsequently treated with 0 or 5 μ M of miltefosine for an additional 24 h. (a) Gate strategy used to analyse the intracellular level of ROS into different populations. The level of H₂O₂, HO \cdot , ROO \cdot and ONOO $^-$ and mitochondrial superoxide (\cdot O₂ $^-$) for total macrophage (total M Φ) were measured on PI $^-$ and singlets populations, respectively. (b) The reactive oxygen species were measured using the probes H₂DCFDA (H₂O₂, HO \cdot , ROO \cdot and ONOO $^-$) and MitoSOXTM (\cdot O₂ $^-$). In the absence of miltefosine, the cells were normalized by the untreated not challenged RAW264.7 cells (Control Leish $^-$ M Φ), whereas the miltefosine treated RAW264.7 cells (challenged or not with *L. infantum* parasite) were normalized by their untreated pair. Each dot represents the mean of individual *L. infantum* isolate from three independent experiments. Mean and \pm SEM for the groups is shown in black. P values were calculated using unpaired two-tailed Student's t-tests comparing the group cured with relapsed; and using One-way ANOVA test comparing the groups MSL⁺, MSL^{+/-} and MSL⁻ (* p-value <0.05; ** p-value < 0.01). M Φ , macrophage; Leish $^-$, not challenged with *L. infantum* parasite; Leish⁺, challenged with *L. infantum* parasite.



Supplementary Figure 9: Read depth coverage differences between *L. infantum* MG11A and T7/Cas9. (a) Read depth coverage (RDC) of each coding sequence (CDS) were estimated, normalized per chromosome RDC, and compared between MG11A and T7/Cas9. All CDS with difference [MG11A - T7/Cas9] higher than 0.5 RDC, which corresponds to approximately one copy per haploid genome, are highlighted in yellow or blue (CDS that RDC also varied in MSL knockout lines). MSL genes are highlighted in red. Each line of the X axis corresponds to a CDS, ordered by its genome position from the smallest to the largest chromosome. (b) Histogram plot of the absolute RDC differences between MG11A and T7/Cas9. The accumulated frequency for absolute RDC difference until 0.3 is indicated in each plot [%].



Supplementary Figure 10: In-silico protein domain analysis for *L. infantum* 3'nucleotidase/nuclease. Protein domain prediction was performed on online InterProScan tool.

(a)

CLUSTAL O(1.2.4) multiple sequence alignment

| | | |
|----------------|---|-----|
| LINF_300020200 | MPA--LVGLRLPLTVLCLLVSSALCVTEALGWGCVGHMLLAEIARRQLDDKNKEKIDAM | 58 |
| LINF_120009100 | MAAFSAHALLAAVIAML----LLLALPTQAWWDKGHMCIAEIARRNLKPNVQAKVQAC | 56 |
| LINF_310031200 | -----MAVALIAQRHMDPKLVKKANAA | 22 |
| LINF_310031300 | MARARF--LQLLLVTLLTLL----STAALPVSAWWSKGHMSVALIAKRHMGSALVEKAELA | 54 |
| | * : * * * : * : . * : | |
| | | |
| LINF_300020200 | AEVFAQSGPPFPSSPDMVQAACWADDVKLWRQRAMGWSWHYFDAPYNPENIN-I-TDAIATV | 116 |
| LINF_120009100 | ADVLNKIGPFPKSTNIVELGPWADDLKSGLYTMSTWHFIDTIYNP-QDVKVTINPVEIV | 115 |
| LINF_310031200 | AKVFSLAGPFPKSPDMVQLGPWADDLLESGLKTNFNWHFITAPYYPDADFTLELSPAQTV | 82 |
| LINF_310031300 | AKVLSFSGPYPKSPDMVQTAFWADDIKTIGLKTSTWHYITTPYTTDEDFTLDVSPVQTV | 114 |
| | * . * : * * * . * : * : * * * : * : . * * * : * : . * * * : | |
| | | |
| LINF_300020200 | NAVTVSRNMISALKNTKAPLYMLNFAWANLVHIFGDLHQLHTISRYSSEYPHGDKGGNL | 176 |
| LINF_120009100 | NVASVIPMLISAITSPAATSIDIITTSVANLIHFVGDHMLPHSADLFSPEYPLGDGGNK | 175 |
| LINF_310031200 | NAASVIPMLESAIRKTTATTEIITQCLAFMVHLFGDIHQPLHNANLLSDEYPLGDYGGNA | 142 |
| LINF_310031300 | NVASVIPMLQTAIEKPTANSDVIVQSLALLLHFMDIHQPLHNVLNFSNQYPESDLGGNK | 174 |
| | * . . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : | |
| | | |
| LINF_300020200 | IQVMV---GRKSLRLHALWDNICTGTPPR-YQRPLSYTDLFALAATADRLLLETYIF--PE | 230 |
| LINF_120009100 | QIVIVNESAGTSMKLFHAFWDSMCEGPQNN-AVRPLDKDAYAELSAFVDNLVKSYSF--TE | 232 |
| LINF_310031200 | QMVTI-DSNGTKMLLHAYWDSMAEGPASVGYRPLSKDAYEDLKAFVDYLEATYAGNLTT | 201 |
| LINF_310031300 | QLVVI-DSKGTKMLLHAYWDSMAEGKSGEDVPRPLSEADYDDLNNFADYLEATYASTLTD | 233 |
| | * : . . : * * * * . : * * * * . * * * * * : * * * * : | |
| | | |
| LINF_300020200 | ALRTLVDVMAIHEESHMAVNTSYPGVTPGATLSEAYLARCKRVAEARLTLGGYRLGYLL | 290 |
| LINF_120009100 | EQMMTNSTIMAAESYELAVKNVYPGISDRTVLSEYKANGKILAAGRVTLAGYRLATIL | 292 |
| LINF_310031200 | AEKNLQNTTAIRNEGVELAIKYAFPASNGATLSNEYKTNAKKISERQVLLAGYRLAKML | 261 |
| LINF_310031300 | KEKNLQNTTEISKETFDLALKYAYPGADNGATLSDEYKTNAKKISERQVLLAGYRLAKML | 293 |
| | : : : * . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : | |
| | | |
| LINF_300020200 | NTLLSSIHVDEATLEAYR--AARPK--RGA----- | 316 |
| LINF_120009100 | NTALAGVSLDTIMNGTKHMQDEVEVTHGDTYNYAFSGVESGAAAGIFLSSFAIGCLLA | 352 |
| LINF_310031200 | NTTLKSVSMDTILQGLGNIQAEVPRLDNFTIGHTFQEKGLSTGLTVGIAIALFIAGVLIS | 321 |
| LINF_310031300 | NTTLKSVSMDTILQGLKSIQSEVDTENKAEVHNHYDQKGISAAVTAIVAVALFIAGIIIA | 353 |
| | * * * . : * : | |
| | | |
| LINF_300020200 | ----- | 316 |
| LINF_120009100 | TAVVLAALYMRGSSKDERAAAASASHGI | 381 |
| LINF_310031200 | TVVVFLNRPRLVRSYQRCPIALD--L | 348 |
| LINF_310031300 | TLVVLAALKCYLPKRDRFGSYEHVAL---- | 378 |

(b)

| % Identity Matrix | LINF_300020200 | LINF_120009100 | LINF_310031200 (NUC1) | LINF_310031300 (NUC2) |
|-------------------|----------------|----------------|-----------------------|-----------------------|
| LINF_300020200 | 100.00 | 36.01 | 38.57 | 35.16 |
| LINF_120009100 | 36.01 | 100.00 | 45.64 | 43.32 |
| LINF_310031200 | 38.57 | 45.64 | 100.00 | 63.01 |
| LINF_310031300 | 35.16 | 43.32 | 63.01 | 100.00 |

Supplementary Figure 11: *Leishmania infantum* 3’Nucleotidase/Nuclease protein sequences Analysis. (a) Multiple sequence alignment performed with Clustal Omega (1.2.4). (b) Percent Identity Matrix - created by Clustal2.1. NUC1 (LINF_310031200); NUC2 (LINF_310031300); P1/S1 (LINF_300020200); NUC3 (LINF_120009100).