Supplementary Data 1. Alignment of *HSP83* coding sequences

The ten copies of *HSP83* in TriTrypDb (Tb927.10.10890, 10900, 10910, 10920, 10930, 10940, 10950, 10960, 10970, 10980) were aligned using Clustal O (1.2.1). Synonymous variations are highlighted in *green*, nonsynonymous variations are in *yellow*, RNAi sequence is in *red*.

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CLUSTAL O(1.2.1) multiple sequence alignment

Tb...10930      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10970      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10980      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10960      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10950      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10940      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10910      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10920      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10900      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
Tb...10890      ATGACGGAGACATTCGCATTCCAGGCTGAAATCAATCAGCTAATGTCCCTTATTATTAAT    60
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Tb...10930      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10970      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10980      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10960      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10950      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10940      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10910      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10920      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10900      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
Tb...10890      ACCTTCTATTCAAACAAGGAAATTTTTCTTCGCGAGCTAATTAGTAATTCGTCAGATGCG    120
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Tb...10930      TGCGACAAAATCCGGTACCAGAGTCTGACGAGCCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10970      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10980      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10960      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10950      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10940      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10910      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10920      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10900      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
Tb...10890      TGCGACAAAATCCGGTACCAGAGTCTGACGAACCAGTCCGTGCTCGGAGATGAACCCCAT    180
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Tb...10930      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10970      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10980      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10960      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10950      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10940      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10910      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10920      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10900      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
Tb...10890      TTGCGCATTCGCGTAATCCCCGACCGAGTAAATAAGACACTGACGGTGGAGGACAGTGGC    240
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Tb...10930      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC    300
Tb...10970      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC    300
Tb...10980      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC    300
Tb...10960      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC    300
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Tb...10950      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC   300
Tb...10940      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC   300
Tb...10910      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC   300
Tb...10920      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC   300
Tb...10900      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC   300
Tb...10890      ATCGGTATGACGAAAGCCGACCTGGTGAACAACCTTGGTACGATTGCTCGCTCCGGTACC   300
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Tb...10930      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10970      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10980      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10960      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10950      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10940      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10910      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10920      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10900      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
Tb...10890      AAGTCATTCATGGAGGCATTGGAGGCCGGTGGCGATATGAGCATGATCGGGCAGTTCGGC   360
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Tb...10930      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10970      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10980      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10960      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10950      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10940      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10910      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10920      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10900      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
Tb...10890      GTCGGATTTTACTCTGCCTACCTTGTCGCAGACCGCGTAACCGTTGTGTCGAAGAACAAC   420
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Tb...10930      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10970      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10980      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10960      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10950      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10940      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10910      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10920      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10900      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
Tb...10890      GAGGACGATGCCTACACGTGGGAATCCTCTGCAGGTGGTACCTTCACTGTAACCTCCACG   480
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Tb...10930      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10970      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10980      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10960      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10950      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10940      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10910      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10920      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10900      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
Tb...10890      CCCGACTGTGACCTGAAGCGCGGTACGCGTATCGTCTTGCATTTGAAGGAAGACCAGCAG   540
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Tb...10930      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10970      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10980      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10960      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10950      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10940      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10910      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10920      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10900      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
Tb...10890      GAATACCTGGAGGAGCGGCGGCTGAAGGACCTCATCAAAAAACACTCCGAATTTATCGGC   600
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Tb...10930      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10970      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10980      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
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Tb...10960      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10950      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10940      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10910      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10920      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10900      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
Tb...10890      TACGACATCGAGTTAATGGTGGAGAACACCACTGAGAAGGAGGTGACAGATGAAGACGAA   660
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Tb...10930      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10970      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10980      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10960      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10950      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10940      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10910      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10920      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAAGTGGAAGAGGTGAAG   720
Tb...10900      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
Tb...10890      GACGAAGAGGCCGCCAAAAAGGCTGAGGAAGGTGAGGAGCCAAAGGTGGAAGAGGTGAAG   720
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Tb...10930      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10970      GACGGTGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10980      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10960      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10950      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10940      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10910      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10920      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10900      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
Tb...10890      GACGGCGATGATGCCGATGCCAAGAAAAAGAAAACCAAAAAGGTGAAGGAAGTGAAGCAG   780
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Tb...10930      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10970      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10980      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10960      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10950      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10940      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10910      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10920      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10900      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
Tb...10890      GAGTTTGTGGTGCAGAACAAGCACAAGCCACTGTGGACGCGCGACCCGAAAGATGTAACA   840
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Tb...10930      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10970      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10980      AAGGAGGAGTACACCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10960      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10950      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10940      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10910      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10920      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10900      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
Tb...10890      AAGGAGGAGTACGCCTCCTTCTACAAGGCCATCTCGAACGACTGGGAAGAGCAGCTGTCT   900
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Tb...10930      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10970      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10980      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10960      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10950      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10940      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10910      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10920      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10900      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
Tb...10890      ACGAAGCACTTCTCCGTGGAGGGCCAGCTGGAGTTCCGGGCTATCTTATTTCTCCCGAAG   960
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Tb...10930      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10970      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
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Tb...10980      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10960      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10950      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10940      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10910      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10920      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10900      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
Tb...10890      CGCGCACCCTTTGACATGTTCGAACCAAACAAGAAGCGCAACAACATCAAGCTATACGTC   1020
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Tb...10930      CGCCGCGTGTTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10970      CGCCGCGTGTTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10980      CGCCGCGTGTTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10960      CGCCGCGTGTTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10950      CGCCGCGTGTTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10940      CGCCGCGTGTTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10910      CGCCGCGTATTCATCATGGACAACTGTGAGGACCTGTGCCCCGACTGGCTCGGCTTCCTG   1080
Tb...10920      CGCCGCGTATTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10900      CGCCGCGTATTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
Tb...10890      CGCCGCGTATTCATCATGGACAACTGTGAGGACCTGTGCCCCGAGTGGCTCGGCTTCCTG   1080
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Tb...10930      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10970      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10980      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10960      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10950      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10940      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10910      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10920      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10900      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
Tb...10890      CGCGGTGTGGTGGACAGCGAGGACCTGCCACTAAATATCTCCCGTGAGAACCTGCAGCAG   1140
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Tb...10930      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGACCCTGGAGCTCTTCGAG   1200
Tb...10970      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGACCCTGGAGCTCTTCGAG   1200
Tb...10980      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10960      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10950      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10940      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10910      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10920      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10900      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
Tb...10890      AACAAAATCCTCAAGGTGATCCGTAAGAATATTGTGAAGAAGGCCCTGGAGCTCTTCGAG   1200
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Tb...10930      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10970      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10980      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10960      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10950      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10940      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10910      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10920      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10900      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
Tb...10890      GAGCTCGCAGAGAACAAGGAGGACTACAAGAAGTTCTACGAACAGTTCAGCAAGAACGTG   1260
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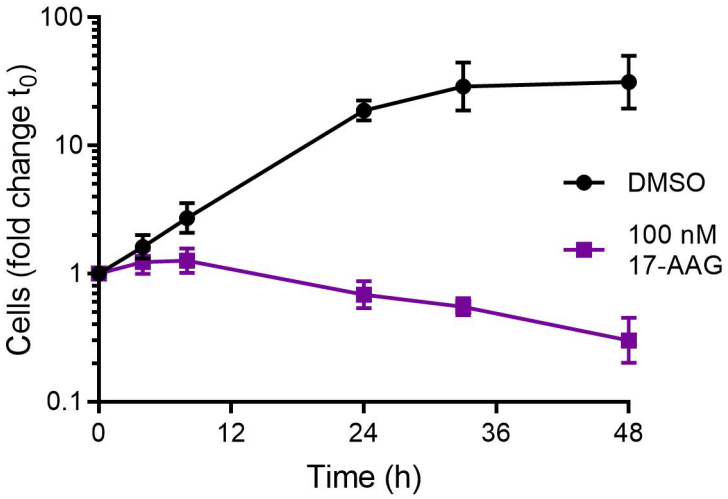
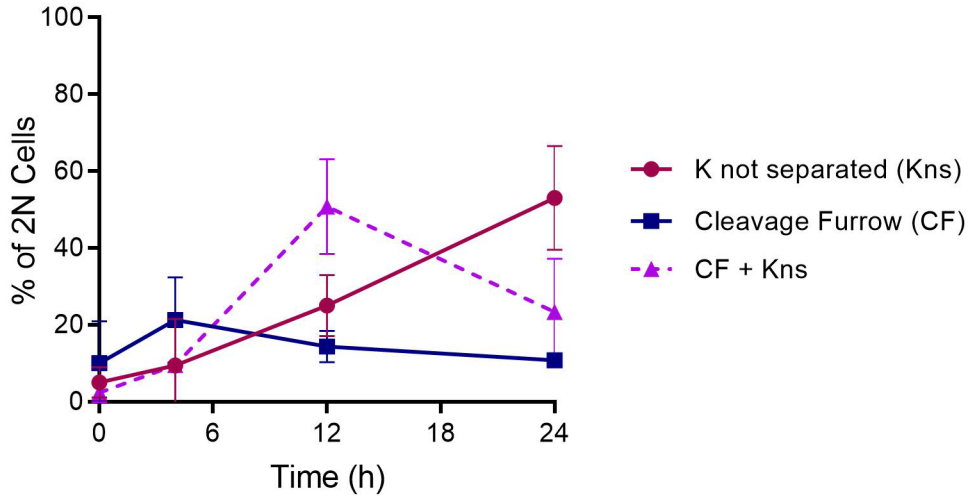
Supplementary Table 1. Primers used in this study

Name	Purpose	Sequence[a]
MX-F	Maxicircle probe for Southern	CTAACATACCCACATAAGACAG
MX-R	blots	ACACGACTCAATCAAAGCC
H83Xb-F	Incorporating restriction sites	CCCCTCTAGATATTGTGAAGAAGGCCCTGG
H83Nd-R	(Xb – XbaI, Nd – NdeI) on RNAi sequence for *HSP83*	CCCCCATATGCTCTTTCATTGCCTTGCACA
H83Xh-F	Incorporating restriction sites	CTCTCTCGAGTATTGTGAAGAAGGCCCTGG
H83As-R	(Xh – XhoI, As – AscI) on RNAi sequence for *HSP83*	CTTTGGCGCGCCCTCTTTCATTGCCTTGCACA
H84Xb-F	Incorporating restriction sites	CCCCTCTAGAGAGCTCTCCTTTTGCACACC
H84Nd-R	(Xb – XbaI, Nd – NdeI) on RNAi sequence for *HSP84*	CCCCCATATGTGTGAACCTGGTCGGTACAA
H84Xh-F	Incorporating restriction sites	CTTCCTCGAGAGCTCTCCTTTTGCACACC
H84As-R	(Xh – XhoI, As – AscI) on RNAi sequence for *HSP84*	GTATGGCGCGCCTGTGAACCTGGTCGGTACAA
H84Kp-F	Incorporating restriction sites	CTATGGTACCCGTTGGCGTCGTCAAATC
H84Xh-R	(Kp – KpnI, Xh – XhoI) on terminal 400 bp of *HSP84*	CTTACTCGAGCTTATCGGCAGTGGGCTCCT
3UH84Bm-F	Incorporating restriction sites	CATTGGATCCGTGCAGAGTAAACATTTTTTC
3UH84Sc-R	(Bm – BamHI, Sc – SacI) on 400 bp of 3'UTR of *HSP84*	CAATGAGCTCCAACGCACAGTAAAGGGTAT
H83m-F	*HSP83* probe for mRNA	CCAGAGTCTGACGAACCAGTC
H83m-R	northern blots	CCAGGTATTCCTGCTGGTCT
H84m-F	*HSP84* probe for mRNA	CTAAAGGGCAATGCAGGGGA
H84m-R	northern blots	CCGGCTGTACACCTTGACAT

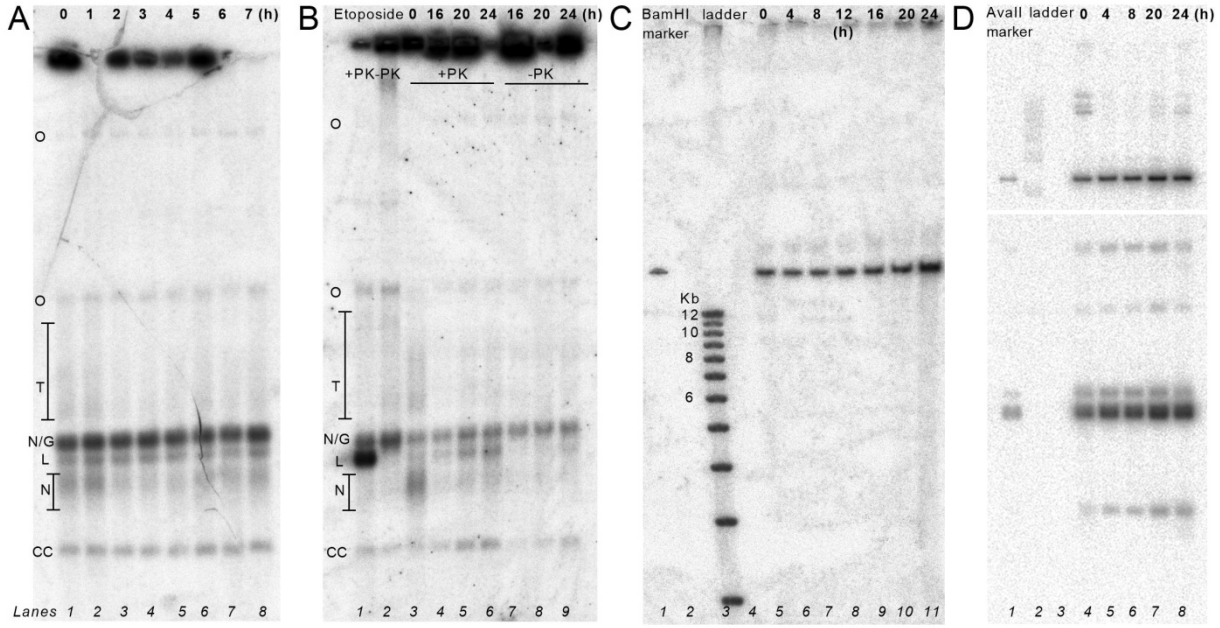
[a]Restriction sites underlined



Supplementary Figure 1. 17-AAG treatment halts trypanosome growth followed by a gradual decline in cell number. 2 x 10^5 Cells were treated with either DMSO or 100 nM 17-AAG and incubated at 37°C. Aliquots were taken across 48 h and live cells counted by hemocytometer and microscopy. Data are from 8 independent growth curves, geometric M ± SD, n = 3-8 for each point.

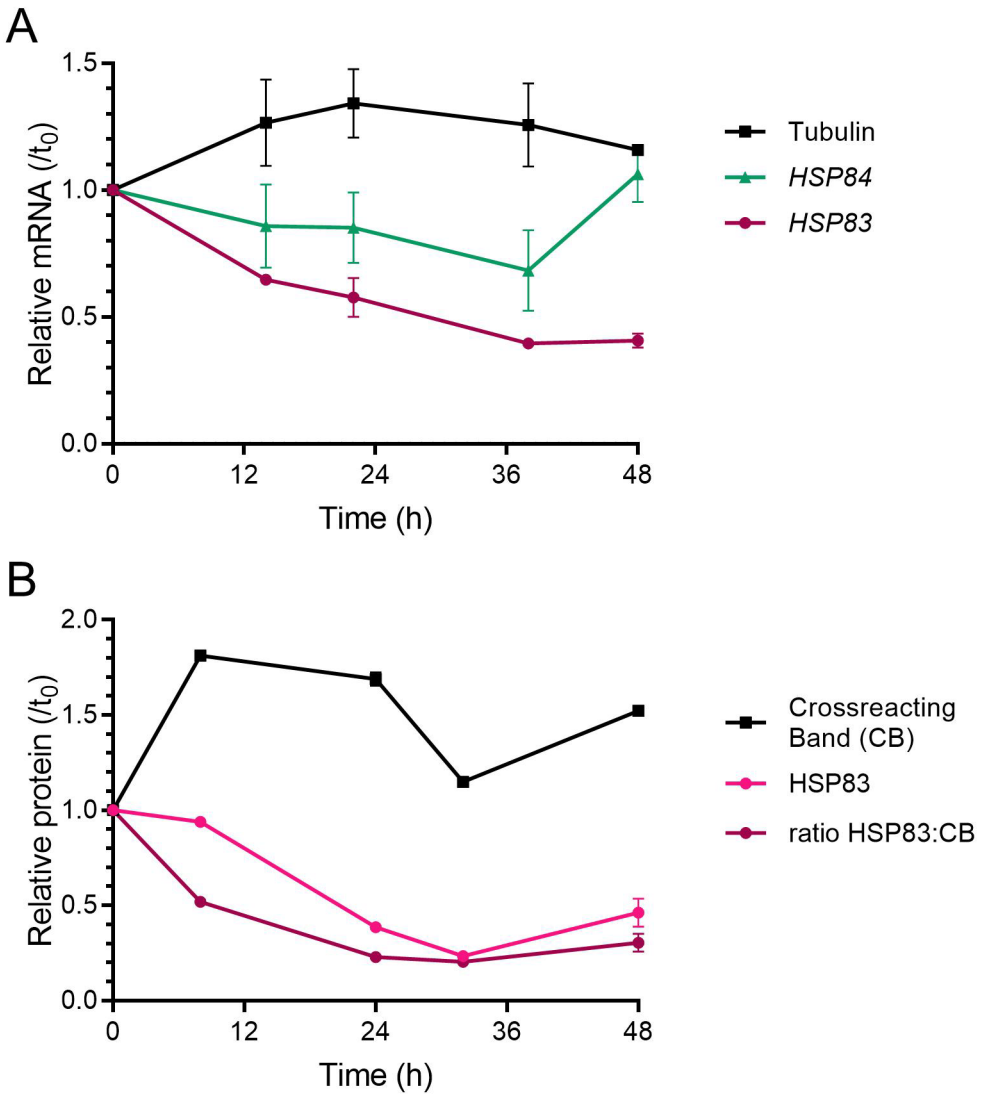


Supplementary Figure 2. Abnormalities in kinetoplast segregation and cleavage furrow ingression after treatment with an Hsp90 inhibitor. Cells treated with 100 nM 17-AAG were harvested at indicated intervals, stained with DAPI, examined by fluorescence microscopy, and 2N cells were scored for presence of a visible cleavage furrow but normal kinetoplasts (*blue*, CF), no cleavage furrow but kinetoplasts not normally separated (*red*, Kns), or presence of both abnormalities (*purple*, CF + Kns). Values are M ± SD; >175 2N cells observed at each time point.

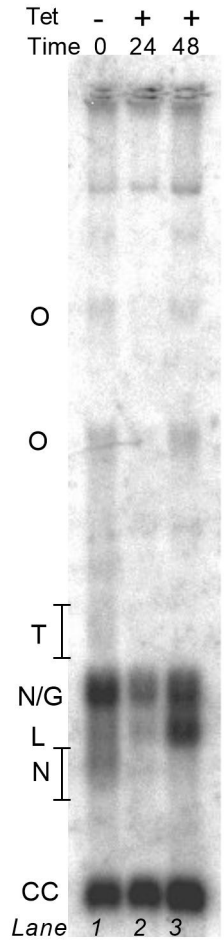


Supplementary Figure 3. 17-AAG treatment leads to changes in free minicircle topology and in the cell content of mini- and maxicircle DNA. (A) Southern blot analysis of free minicircles isolated from trypanosomes treated for short periods with 100 nM 17-AAG. *Lane 1*, DNA from untreated cells; *Lanes 2-8*, increasing duration of 100 nM 17-AAG, as indicated (*bold*, top of lanes). (B) Southern blot analysis of free minicircles isolated from trypanosome lysates treated with or without proteinase K (±*PK*) prior to electrophoresis. *Lanes 1 and 2*, DNA from trypanosomes treated with topoisomerase II poison etoposide; *Lanes 3-9*, DNA from cells treated with 100 nM 17-AAG. Without protease digestion the linear forms are selectively lost from analysis, indicating their covalent linkage to topoisomerase protein [1]. *O*, Oligomers of two or more interlocked circles; *T*, theta structures; *N/G*, mature nicked, or gapped daughters; *L*, Linears, *N*, immature nicked daughters; *CC*, covalently closed template circles. (C) Southern blot analysis of free maxicircles (0.6% gel). *Lane 1*, BamHI-linearized maxicircle marker; *Lane 3,* DNA size ladder; *Lanes 5-11,* increasing duration of 17-AAG (100 nM) treatment. (D) Southern blot analysis in 1.5% gel of total minicircles and total maxicircles after digestion of kDNA (ethanol precipitated from lysates) by AvaII restriction enzyme (overnight). The blot was

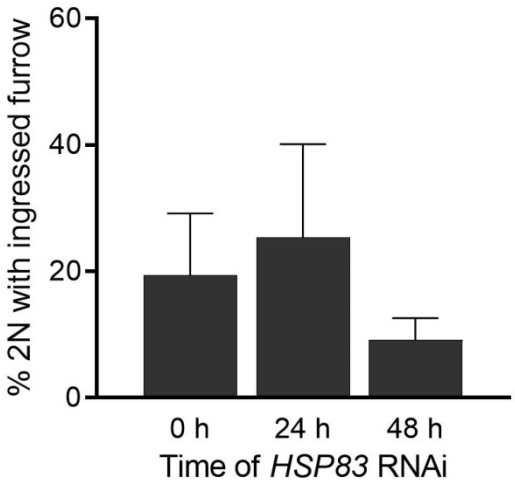
first probed for minicircles (bottom panel), then stripped and reprobed for maxicircles (top panel). *Lane 1,* AvaII-cut network marker; *Lane 2,* DNA size ladder; *Lanes 4-8*, increasing duration of 17-AAG (100 nM) treatment. Samples loaded by equal cell equivalents.



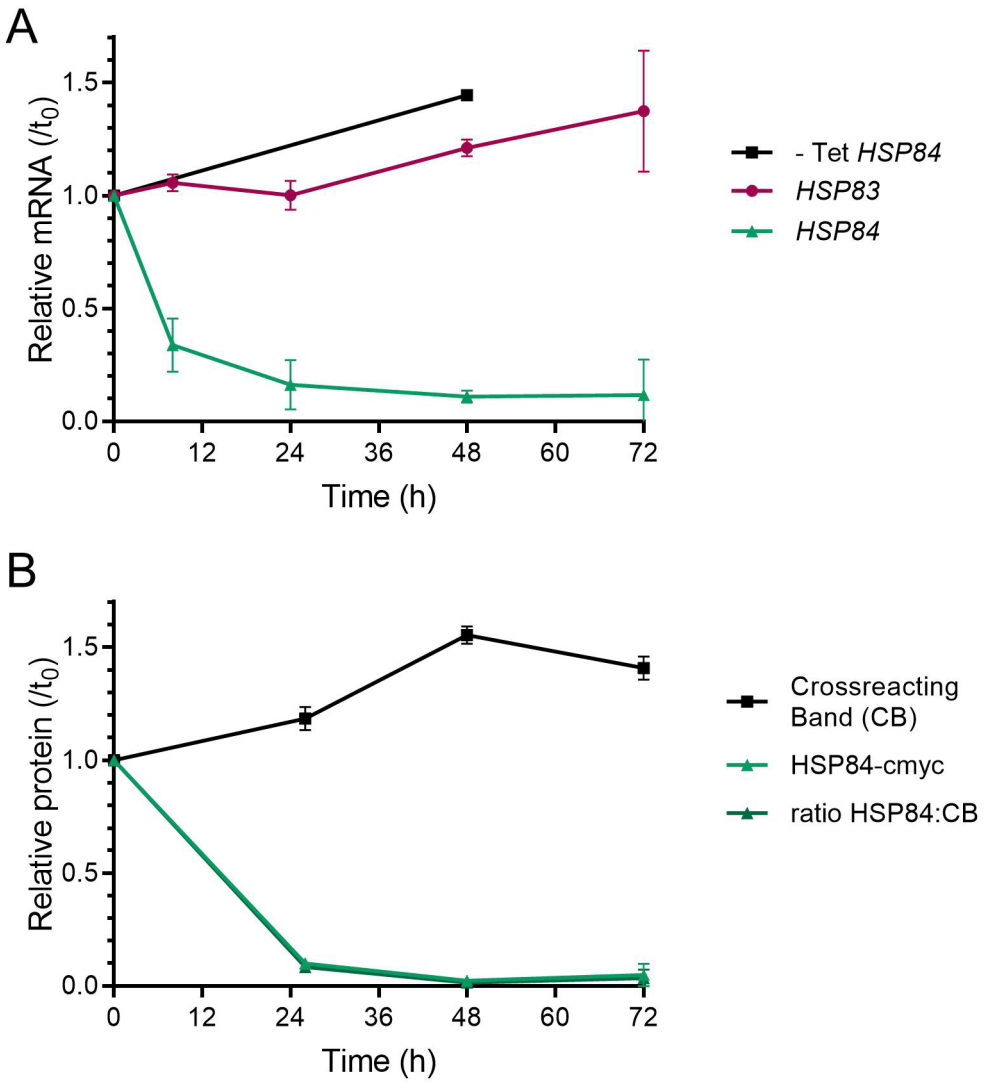
Supplementary Figure 4. Densitometry quantification of *HSP83* mRNA and protein from Figure 4, by mean grey values. (A) Time course of mRNA in northern blots from cells induced for RNAi of *HSP83*. *HSP83 (red)*, tubulin *(black)*, *HSP84 (green)*, shown relative to time 0 h. (B) HSP83 *(pink)* and a cross-reacting protein (50 kDa, *black*) in western blots from cells induced for *HSP83* RNAi over time, shown relative to uninduced cells, and ratioed to each other (*red*). Symbols are M ± SD of three densitometry measurements of the same blot.



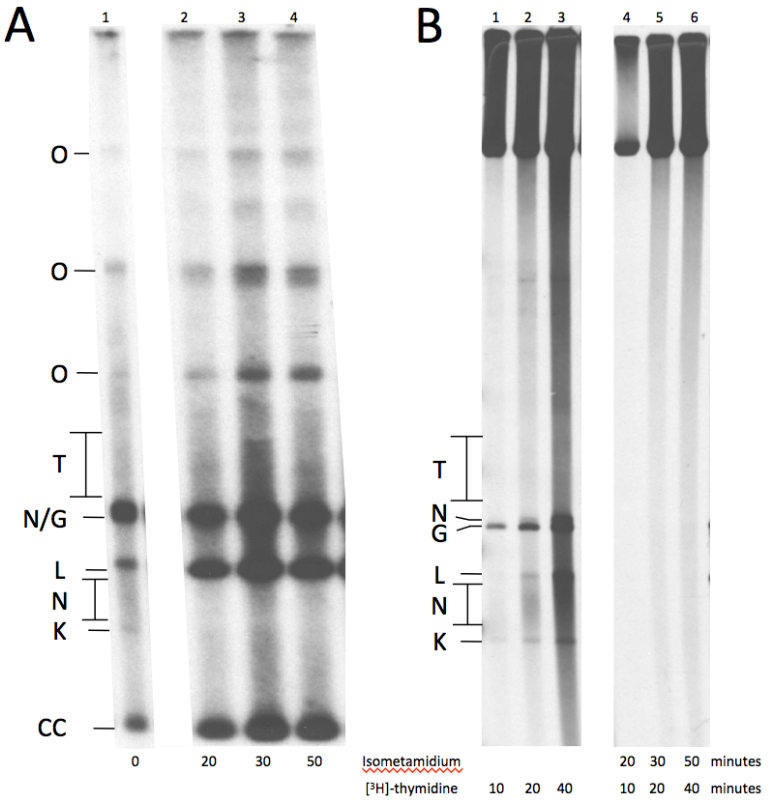
Supplementary Figure 5. Southern blot of free minicircles isolated at indicated times from *HSP83* RNAi-induced trypanosomes and separated in ethidium agarose (biological replicate of Fig. 5A to show theta region). *Lane 1*, uninduced cells at t_{0h}; *Lanes 2 and 3,* increasing hours of RNAi. Lanes loaded by equal cell equivalents. *O*, oligomers of two or more interlocked circles; *T*, theta structures; *N/G*, mature nicked, or gapped daughters; *L*, linears, *N*, immature nicked daughters; *CC*, covalently closed template circles.



Supplementary Figure 6. *Hsp83* RNAi induction does not significantly change the percent of 2N cells with a visibly ingressed cleavage furrow. Merged phase contrast and DAPI fluorescence images of trypanosomes before and after induction of *HSP83* RNAi. M ± SD, n ≥3.



Supplementary Figure 7. Densitometry quantification of mRNA and protein in cells with inducible constructs for RNAi of *HSP84*, by mean grey values (Figure 6 data). (A) mRNA bands of *HSP84*, from uninduced (*black*) or induced (*green*) cells, and of *HSP83 (red)* in induced cells, by northern blot, shown relative to 0 h. (B) HSP84-cmyc *(light green)* and a cross-reacting protein (60 kDa, *black*) bands, and their ratio *(dark green)*, by western blot, across time in induced cells (shown relative to uninduced, time 0 h). Symbols are M ± SD of three densitometry measurements of the same blot.



Supplementary Figure 8. Impact of antitrypanosomal drug isometamidium chloride (Samorin) on free minicircles. African trypanosomes *T. equiperdum* were treated in vitro with 10 μM isometamidium, and minicircle DNA was isolated and imaged as described previously [2,3]. (A) Southern blot of free minicircles obtained before (*Lane 1*) and at indicated intervals after addition of isometamidium to cell culture (*Lanes 3-4*). (B) Fluorographs to detect incorporation of [^{3}H]thymidine into nascent DNA. *Lanes 1-3*, free minicircles isolated from control cells metabolically labeled for increasing times with [^{3}H]thymidine. *Lanes 4-6*, minicircle DNA from cells treated with 10 μM isometamidium for 10 min prior to the addition of [^{3}H]thymidine. All samples obtained in the same experiment; lanes loaded by equal cell equivalents. *O*, Oligomers of two or more interlocked circles; *T*, theta structures; *N/G*, mature nicked, and

gapped daughters; *L*, Linears, *N*, immature nicked daughters; *CC*, covalently closed template circles.

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