

***Leishmania donovani* 90 kD Heat Shock Protein – Impact of Phosphosites on Parasite Fitness, Infectivity and Casein Kinase Affinity**

Supplementary Information

Antje Hombach-Barrigah¹, Katharina Bartsch¹, Despina Smirlis^{2,4}, Heidi Rosenqvist^{3,6}, Andrea MacDonald¹, Florent Dingli⁵, Damarys Loew⁵, Gerald F. Späth², Najma Rachidi², Martin Wiese³, and Joachim Clos^{1*}

¹Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

²Institut Pasteur and Institut National de Santé et Recherche Médicale INSERM U1201, Unité de Parasitologie Moléculaire et Signalisation, Paris, France

³Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS) University of Strathclyde, Glasgow, Scotland, UK

⁴Hellenic Pasteur Institute, Athens, Greece

⁵Laboratoire de Spectrométrie de Masse Protéomique, Centre de Recherche, Institut Curie, PSL Research University, Paris, France

⁶current address: Novo Nordisk A/S, Gentofte, Denmark

*) corresponding author:

Joachim Clos, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

phone: +49 40 42818 481

e-mail: clos@bnitm.de

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Table S1. Primer sequences for the mutations of various HSP90 phosphorylation sites are listed in this table. The modified base triplets are highlighted in the sequence.

Mutation	Sequence
T ₂₁ -I ₂₁	AACATCTTCTACTCGAACAAGGAG
T ₂₁ -D ₂₁	AACGACTTCTACTCGAACAAGGAG
T ₂₁ rev	GATGATCAGCGACATCAAC
T ₁₀₀ -I ₁₀₀	GGCATCAAGGCGTTCATGGAGGCG
T ₁₀₀ -D ₁₀₀	GGCGACAAGGCGTTCATGGAGGCG
T ₁₀₀ rev	GGAGCGCGGATCGTGCC
T ₂₁₁ -A ₂₁₁	ACGG CGG GAGAAGGAGGTGACGGACGAGGACGAG
T ₂₁₁ -D ₂₁₁	ACGG ACG GAGAAGGAGGTGACGGACGAGGACGAG
T ₂₁₆ -A ₂₁₆	ACGACGGAGAAGGAGGTGG CGG ACGAGGACGAG
T ₂₁₆ -D ₂₁₆	ACGACGGAGAAGGAGGTGG ACG ACGAGGACGAG
T ₂₁₁ /T ₂₁₆ -A ₂₁₁ /A ₂₁₆	ACGG CGG GAGAAGGAGGTGG CGG ACGAGGACGAG
T ₂₁₁ /T ₂₁₆ rev	CTTCTCCACCATCAGCTCG
T ₂₂₃ -A ₂₂₃	GAGGAT GCG AAGAAGGCCCGCCGAGGAC
T ₂₂₃ -D ₂₂₃	GAGGAT GACA AAGAAGGCCCGCCGAGGAC
T ₂₂₃ rev	CTCGTCCTCGTCCGTCACCTC
S ₂₈₉ -A ₂₈₉	CATCGCCAACGACTGGGAGGAC
S ₂₈₉ rev	GCCTTGTAGAAGGCCGCG
S ₅₂₆ -A ₅₂₆	TTCGAGGAG GCGG AGGAGGAGAAGCAGCAG
S ₅₂₆ rev	GTGCACGCCCTCCTTCGTCAG
S ₅₉₄ -A ₅₉₄	GCGAC GCC AGCATGGCGCAGTACATGATG
S ₅₉₅ -A ₅₉₅	GCGACTCC GCC ATGGCGCAGTACATGATG
S ₅₉₄ /S ₅₉₅ -A ₅₉₄ /A ₅₉₅	GCGAC GCCG CCATGGCGCAGTACATGATG
S ₅₉₄ /S ₅₉₅ -D ₅₉₄ /D ₅₉₅	GCGAC GGACG ACATGGCGCAGTACATGATG
S ₅₉₄ /S ₅₉₅ rev	GCAGCGCCTGGTTGCGCATG
T ₆₉₃ -A ₆₉₃	CGG CGC CTCCAGCATGGAGCAGGTGGAC
T ₆₉₃ -D ₆₉₃	CGG CGACT CCAGCATGGAGCAGGTGGAC
S ₆₉₄ -A ₆₉₄	CGGCAC CGC AGCATGGAGCAGGTGGAC
S ₆₉₄ -D ₆₉₄	CGGCAC CGAC AGCATGGAGCAGGTGGAC
T ₆₉₃ /S ₆₉₄ rev	GCGGTGACCTCCGCGGGGGCCG

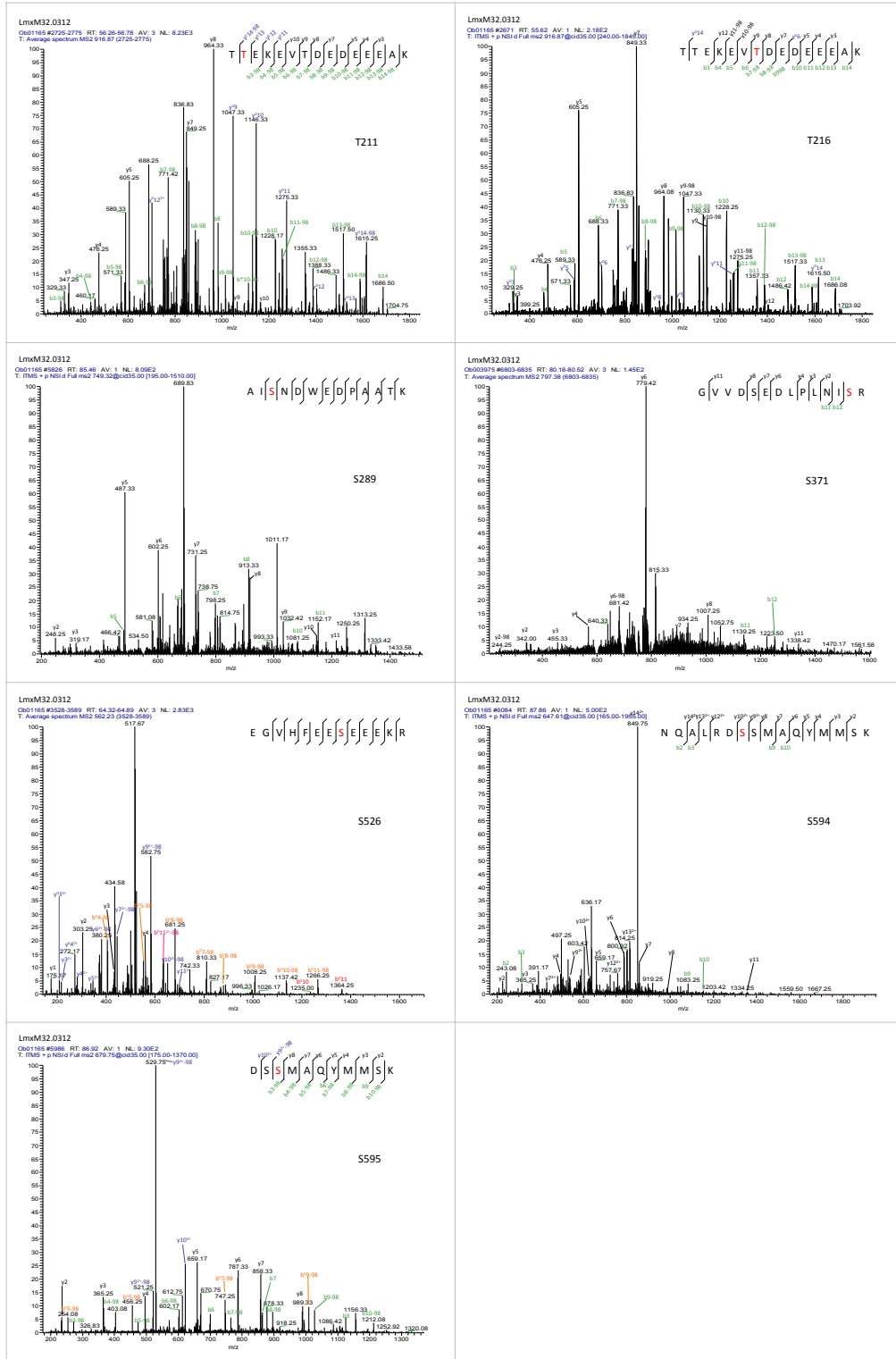
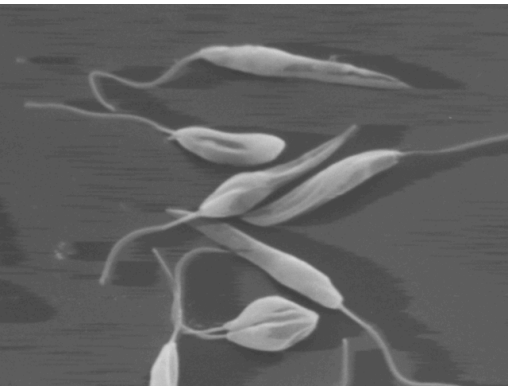
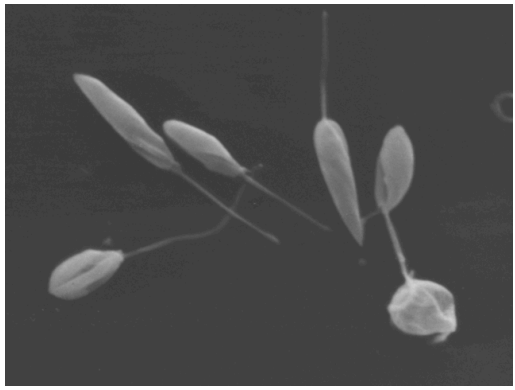

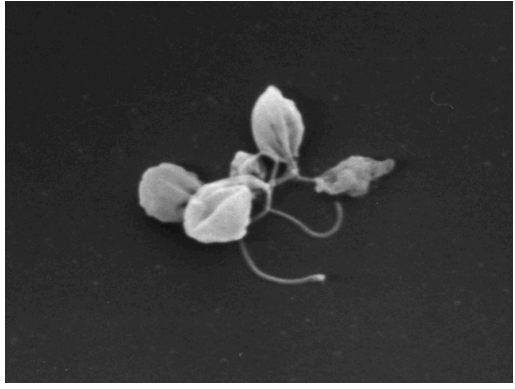
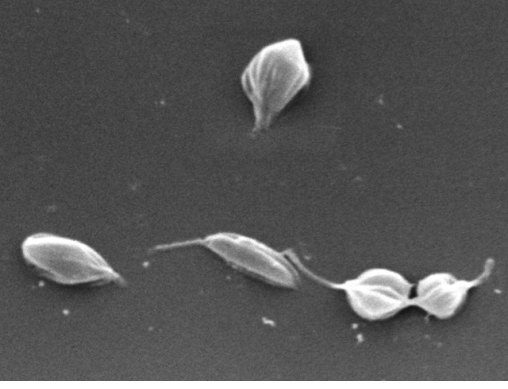

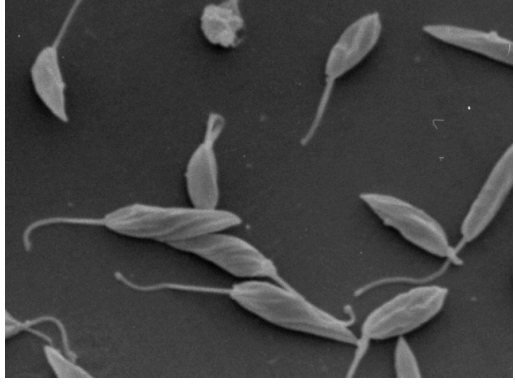


Figure S1: Mass spectra identifying phosphorylated peptides derived from the *Leishmania mexicana* HSP90

	- RAD	+ RAD
HSP90rr		
HSP90wt		
P-site	Ala +RAD	Asp +RAD
T ₂₁₁ /T ₂₁₆		n.a.
T ₂₂₃		

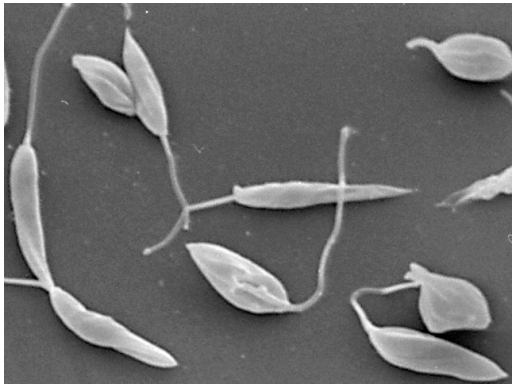
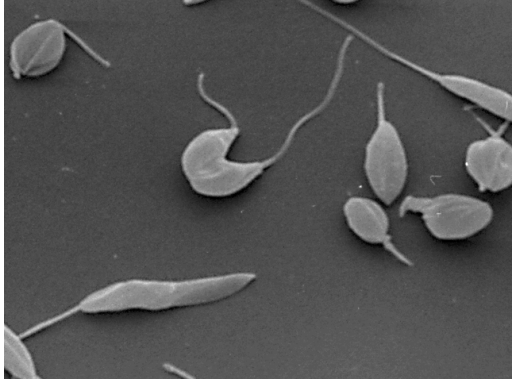
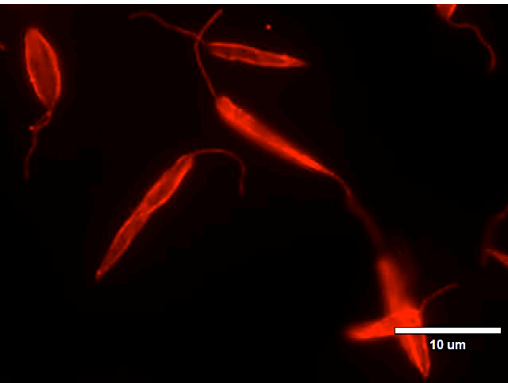
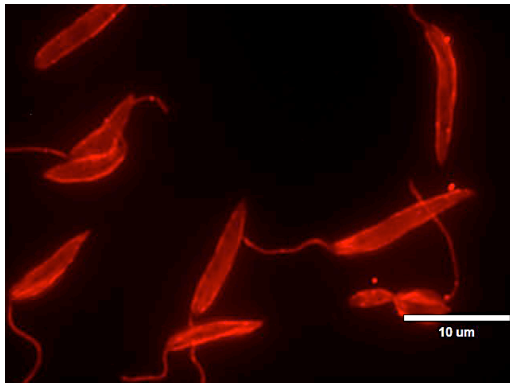
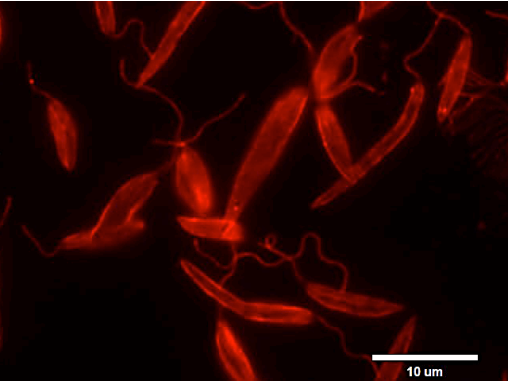
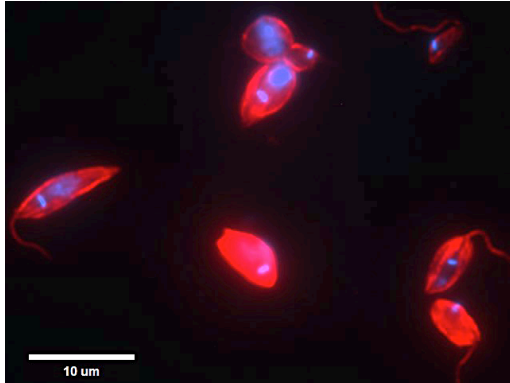
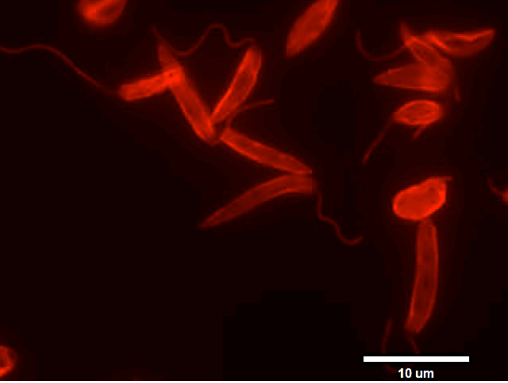
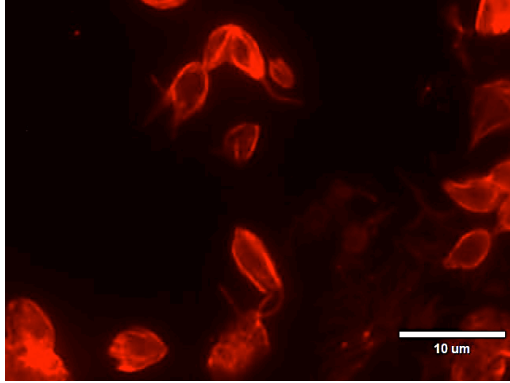
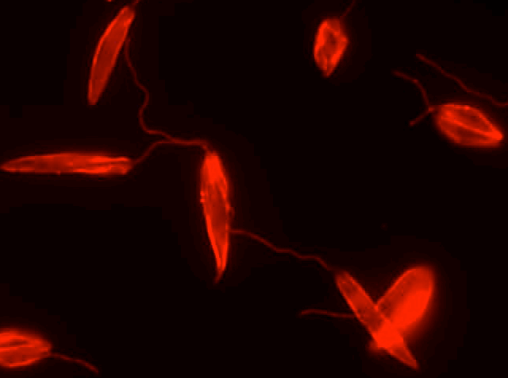
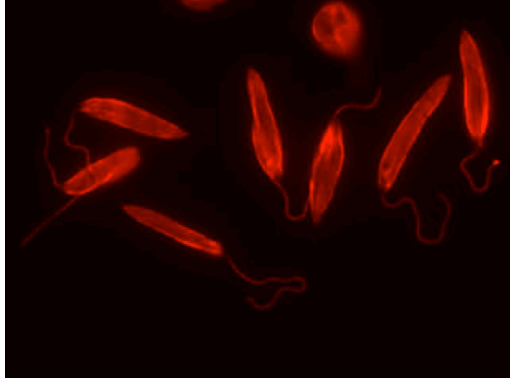
<p>S289</p>		<p>n.a.</p>
<p>S526</p>		<p>n.a.</p>

Figure S2A: Representative images from the morphological analysis summarised in Fig. 3A. Scanning electron micrographs of *L. donovani*, ectopically expressing HSP90rr, HSP90wt, and P-site mutations of HSP90rr (serine or threonine to alanine or aspartic acid) and exposed to RAD at IC₅₀.

	- RAD	+ RAD
HSP90rr		
HSP90wt		
P-site	Ala +RAD	Asp +RAD
S ₅₉₄		
S ₅₉₅		

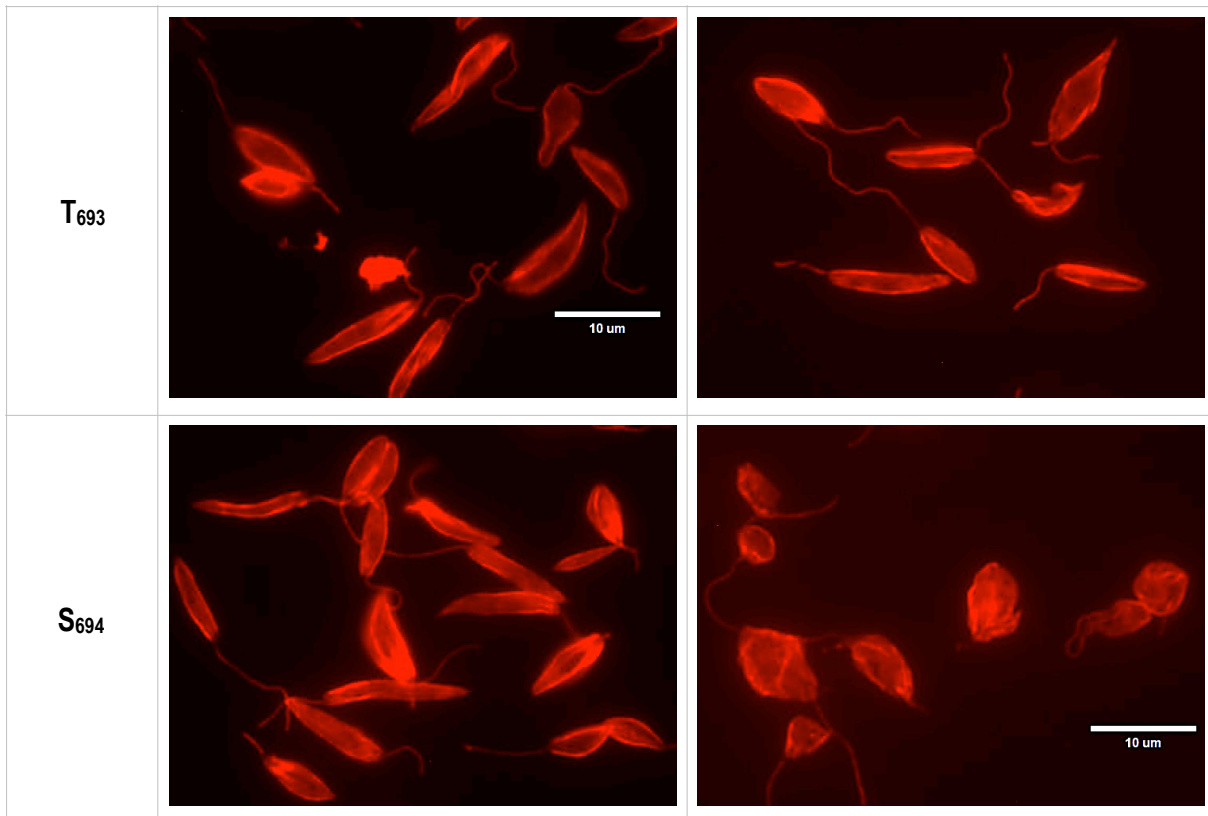


Figure S2B: Representative images from the morphological analysis summarised in Fig. 3B. Anti-tubulin indirect immune micrographs of *L. donovani*, ectopically expressing HSP90rr, HSP90wt, and P-site mutations of HSP90rr (serine or threonine to alanine or aspartic acid) and exposed to RAD at IC₅₀. One image (HSP90wt + RAD) shows DAPI co-staining.