

Supplementary Table 1. Identification of proteins differentially expressed by amastigotes and promastigotes forms from *L. chagasi*.

Spot Code	Protein Name (<i>pI/Mr</i> exp - pred)	GI	Peptide sequence identified (Da)	Coverage %	Protein score*	Abundance Vol.Ratio p < 0.05
1	Alpha tubulin (IsoformA) (5.12/60.97 - 4.89/49.74)	gil606648l	FDGALNVDLTEFQTNLVPYPR (2409.31)	5	40	↑ Amastigote 14.86
2	Hypothetical protein (5,08/29.12 - 5.17/41.05)	gil146105064l	AFTDALAASGAR (1150.54), SECAFFHHEIK + Carbamidomethyl (C) (1551.64), NALSCFVAQLELAR + Carbamidomethyl (C) (1591.77), AVQAAIWTEVFAEGK (1619.78), LQSQVFPDVSTFFR (1670.79)	18	197	↑ Amastigote 2.19
3	Phosphomannomutase (5.58/8.33 – 5.37/28.14)	gil146104294l	DTIAILESLLSESR (1588.78), CLHLIADLDIPVQR (1605.82), VILLFDVDGTLTPPR (1655.87), TSEGGNDYEIYTDCR + Carbamidomethyl (C) (1779.64), TYCLQFVENDFQTIHFFGDK + Carbamidomethyl (C) (2509.07)	32	341	↑ Amastigote 3.14
4	Prostaglandin f2-alpha synthase (6.84/15.22 – 6.78/30.59)	gi 146095209	YGPDPDEAQF (1138.41), VEAWSPLGQGK (1171.55), LLSNPILAAIGAK (1280.73), LGVDYIDL YLIHWPR (1872.92), IEENADVFN FELGAEDVMR (2197.91), LWNTEQGYESTLA AFEESR (2230.93)	31	264	↑ Amastigote 2.97
5	Translation elongation factor 1-beta (Isoform A) (4.25/ 8.43 - 5.14/25.94)	gil146098751l	SSILFDIK (922.49), LVPVAFGVK (929.55), DGLIWGDHK (1040.48), NMATYTEGER (1171.46), LFLGGAKPTAEDVK (1445.75), AWDDTIDLEALAQK (1588.73), VFNDLLGANHVNLYR (1744.86), AA AVAPPSAAAAEEDDDIDLFGETTEEEK (3044.29)	45	593	↑ Amastigote 3.28
6,7,8	Heat shock protein 83-1	gi 146097493	HKPLWTR (937.53), QFYEQFGK + Pyro-glu (N-term Q) (1029.44), EDQLEYLEPR (1291.60),	13	83	↑ Promastigote

	(Isoform A) 6- (4.04/ 119.12 - 5.05/80.68) 7- (4.11/ 119,79) 8- (4.18/ 120,02)		HFSVEGQLEFR (1348.64), KLESSPFIEQAR (1404.73), DVTKEEYAAFYK (1463.68), GVVDSIDLPLNISR (1513.76), YQSLTDPSVLGESPR (1648.80)			13.29 9.55 10.82
9	70 kDa heat shock protein (Isoform A) (4.01/98.99 - 6.45/56.53)	gi 37813097	DAGTIAGLEVLR (1214.54), LVTFTEEFK (1260.52), FEELCGDLFR + Carbamidomethyl (C) (1285.46), LVTFTEEFKR (1416.61), TTPSYVAFTDSER (1473.54), AVVTVPAYFNDSQR (1566.64), DCHLLGTFDLSGIPPAPR + Carbamidomethyl (C) (1965.80), SQIFSTYADNQPGVHIQVFEGER (2622.01)	18	144	↑ Promastigote 3.55
10	Actin (4.72/ 69.45 – 5.40/41.99)	gi 28627572	IWHHTFYNELR (1515.71), YPIEHGIVTNWDDMEK (1946.85), VNPEQHNVLLEAPMNP (2030.98), SANEEAFELPDGNVMMVGNQR (2307.99), TTGIVLDAGDGVTHTVPIYEGYSLPHAVR (3038.51)	25	190	↑ Promastigote 2.41
11	Alpha tubulin (Isoform B) (4.33/67.32 - 4.89/49.74)	gi 606648	LIGQVVSSLTASLR (1443.76), TIQFVDWCPTGFK + Carbamidomethyl (C) (1598.66), SLDIERPSYTNVNR (1663.74), CIFLDLEPTVVDEV + Carbamidomethyl (C) (1804.81), AVCMIANSTAIAEVFAR + Carbamidomethyl (C) (1823.80), AFVHWYVGEGMEEGEFSEAR (2329.89), FDGALNVDLTFQTNLVPYPR (2409.08))	25	102	↑ Promastigote 5.98
12	Beta-tubulin (Isoform A) (4.77/64.48 - 4.69/49.71)	gi 135485	LAVNLVPFPR (1125.56), FPGQLNSDLR (1146.47), VGEQFTGMFR (1171.43), INVYFDESAGGR (1327.49), LHFFMMGFAPLTSR (1654.66), GHYTEGAELIDSVLDVCR + Carbamidomethyl	26	690	↑ Promastigote 3.40

			(C) (2033.76), MSVTFIGNNTCIQEMFR + Carbamidomethyl (C) (2047.74), AGPYGQLFRPDNFIFGQSGAGNNWAK (2812.11)			
13	Paraflagellar rod protein 1 (5.20/111.31- 5.30/69.18)	gi 51704749	EHLEYFR (993.43), SQLDATQLAQVPTR (1527.74), LLELTVYNCDLAMR + Carbamidomethyl (C) (1710.78), RVENAQFLEIAAQHK (1753.86), LAHIQLEFCVETFDPNK + Carbamidomethyl (C) (2131.97), ELYRPEDKQPQFMDIIGVK (2178.04), TACLENEELVQELHISDWSETQR + Carbamidomethyl (C) (2787.20)	18	118	↑ Promastigote 7.83
14	Heat shock 70-related protein 1, mitochondrial precursor (5.30/ 98.79 - 5.80 /71.68)	gil146093966	EQIEQMIR (1046.45), DAGTIAGLNVIR (1199.59), EINDVVLVGGMTR (1402.64), SQTFTAADNQTQVGK (1795.76), GVNPDFAVALGAATLGGVLR (1879.91), VSNAVVTCPAYFNDAQR + Carbamidomethyl (C) (1911.78), EMAADNQMMGQFDLVGIPPAPR (2388.00)	17	241	↑ Promastigote 5.17
15	Chaperonin 60.2 precursor (5.30/83.11 - 5.32 /60.57)	gi 4323557	NVIEQAYGAPK (1302.65), GFLSPYFVTDK (1344.64), EGMLEDIAVFTGAK (1480.68), DRIEQEAVEYNR (1521.67), AAVQEGIVAGGGTALLR (1582.84), YVNMFEAGIIDPTR (1625.74), VLEASEESVGYDAQNDR (1881.78)	17	215	↑ Promastigote 8.58
16	Enolase (5.12/61.88 - 5.33/46.04)	gi 146081643	AQIVGDDLTVTNVER (1629.84), HIDEPLPILMEAIK (1747.92), SAVPSGASTGVHEACELR + Carbamidomethyl (C) (1827.85), GNPTVEVELMTEAGVFR (1848.91), HAGNVLPFQEFMIAPTK (1899.97), YGQDAVNVDGEGGFAPPIK (1933.92), FAICMDCAASEAYDAER + 2 Carbamidomethyl (C) (1979.79), NPEPTYVSAAELQATYER	32	85	↑ Promastigote 9.06

			(2038.97)			
17	Protein disulfide isomerase (5.33/62.10 - 5.42/52.35)	gi 146102742	GFPTLYIFR (1113.52), FPAFVVDFER (1226.53), FYAPWCGHCK + 2 Carbamidomethyl (C) (1325.46), SQVLLTFIDGDQYRPVSR (2093.96), FLATAVL DYFGELGQESFQK (2262.99), FEVSGFPTIYFIPAGKPPIVYEGGR (2741.28)	17	284	↑ Promastigote 8.60
18	Putative eukaryotic initiation factor 4a (6.32/52.44 - 5.83/45.33)	gi 146075141	VLVTDLVAR (1086.64), TGAFSIGLLQR (1162.59), FCETVVGTR + Carbamidomethyl (C) (1173.47), ELALQTAEVISR (1329.66), LQAGVIVAVGTPGR (1337.71), HNLIQGLVLSPTR (1447.76), KLQAGVIVAVGTPGR (1465.81), GIYSYGFEKPSSIQQR (1859.83), GIDVHHVNIVINFDLPTNK (2145.03)	30	106	↑ Promastigote 8.46
19	Hypothetical protein (5.22/29.65 - 5.32/40.84)	gi 146094146	FDGDWVEGR (1080.44), YTG DWTFGR (1102.46)	5	248	↑ Promastigote 3.60
20	Pyruvate dehydrogenase E1 beta subunit (Isoform A) (5.22/29.65 - 5.64/37.85)	gi 146089026	DITLIGFSR (1021.53), DAIHSALDEELAR (1439.66), LAAEGVQAEVINLR (1482.79), VFVIGEEVGQYQGAYK (1786.85), DIEMASQPQVADVMAAAK (1874.78)	20	68	↑ Promastigote 3.60
21	Hypothetical protein (4.73/28.90 - 5.07/36.34)	gi 146081345	TDFFTQPD MAR + Oxidation (M) (1344.49), GQPPIVDGELYSK (1402.61), KTDFFTQPD MAR + Oxidation (M) (1472.57), TIFQGDPEIDLQK (1503.65), YIFSQSLQEA EVR (1569.68), NEAETGATEAFADTGDAPR (1922.70)	22	133	↑ Promastigote 13.81
22	Translation elongation factor 1-beta (Isoform B) (5.27/13.99 - 5.14/25.94)	gi 146098751	SSILFDIK (922.48), LVPVAFGVK (929.54), DGLIWGDHK (1040.47), AWDDTIDLEALAQK (1588.72),	17	112	↑ Promastigote 6.73

23	Hypothetical protein (5.78/9.02 - 5.80/30.40)	gi 146089119	KHDVILGLFLFGTDR (1730.86), YVFPQMYFSPELQAATDK + Oxidation (M) (2150.88), GIDIAFLGQNDLCMSMGLYDGR + Carbamidomethyl (C); Oxidation (M) (2461.98)	20	288	↑ Promastigote 10.77
24	14-3-3 protein-like protein (4.40/9.55 - 4.79/29.73)	gi 146104107	FDEMLLCIR + Carbamidomethyl (C) (1196.54), YYAEIDSGDGQR (1373.55), ENSATLPFVLNMR (1491.72), LPDDLAELIYMAK + Oxidation (M) (1507.71), ATDVANSSLAPTHPIR (1649.81), NVTIQDEVVPKPSDIK (1781.91), LAEEAERFDEMLLCIR + Carbamidomethyl (C) (1994.91), QAYDEAVTELETLDDEAYHESNTIVR (3011.30)	47	675	↑ Promastigote 5.22
25	Nascent polypeptide associated complex homologue, alpha chain (Isoform A) (4.03/7.70 - 4.50/18.14)	gi 8671200	EINVMSQANVSR (1446.68), VGSLSFAMVQPEVYR (1682.80), KVGSLSFAMVQPEVYR (1810.89), FPGTNTFVIFGEAQLEDTSALAQEAAAR (2954.33)	42	105	↑ Promastigote 7.09
26	Hypothetical protein (4.14/7.75 - 4.63/22.41)	gi 146081233	LDPSHICYDEGK + Carbamidomethyl (C) (1433.56), VLAGEELESNGPRPK (1595.76), EAPTIESAHPIKGEK (1606.77), QQFLQHTTVNQSNFR (1847.83), GADV CNDEFTTLSEEMADK + Carbamidomethyl (C) (2131.79)	38	612	↑ Promastigote 6.34
27	Hypothetical protein (4.01/8.55 - 4.15/21.59)	gi 146102004	EQGYWNR (952.36), KEQGYWNR (1080.48), EIVPEESTFK (1178.51), VLGMAIQICAIK + Carbamidomethyl (C) (1316.62), SWLSADWNLWK (1405.60), GSQPHALKDELHLLK (1685.83), LFITVEASTPTDVQVNFQEK (2266.03)	42	212	↑ Promastigote 1.19

28	Rab11 GTPase, putative (4.0/7.01 - 7.60/23.40)	gi 146079278	IQIWDTAGQER (1316.57), SVYEVVVTPQTR (1377.63)	11	41	↑ Promastigote 1,24
29	Iron superoxide dismutase (4.01/7.0 - 8.46/26.21)	gi 146077900	YPAELPTLGFNYK (1512.67), TIEEILATSGSTESK (1678.75)	13	253	↑ Promastigote 1.62
30	IgE-dependent histamine- releasing factor (4.26/8.0 - 4.39/19.45)	gi 146088052	ASYMAHIR (948.41), VVDVVYNNR (1077.49), VFQTNAAAFVK (1195.57), KVFQTNAAAFVK (1323.65), YIDVGGEDYGISANVDEDAEAGATGEVAEGK (3101.19)	42	775	↑ Promastigote 4.11
31	Mitochondrial trypanredoxin peroxidase 4.95/5.19 – 6.90/25.34)	gi 61619796	HSTINDLPVGR (1208.54), GGLGEMHIPVLADK (1436.64), DYGVLIIESGIALR (1534.70), LNTQVVAVSCDSVYSHLAWVNTPR + Carbamidomethyl (C) (2716.15)	28	653	↑ Promastigote 5.95
32	Hypothetical protein Trypanredoxin peroxidase (5.97/7.0 – 7.55/22.19)	gi 134072971 gi 12655871	HGEVCPANWK + Carbamidomethyl (C) (1197.50), QITVNDMPVGR + Pyro-glu (N-term Q) (1212.56), LLEAFQFVEK (1223.62), GLFIIDPNGMVR (1331.67), FNELNCEVLACSMDSYAHLQWTLQDR + 2 Carbamidomethyl (C) (3329.37)	11 25	120 384	↑ Promastigote 1.17
33	Hypothetical protein (4.85/65.02 – 5.17/41.05)	gi 146105064	AFTDALAASGAR (1150.54), SEFCAFFHHEIK + Carbamidomethyl (C) (1551.64), NALSCFVAQLELAR + Carbamidomethyl (C) (1591.77), AVQAAIWTEVFAEGK (1619.78), LQSQVFPDVSTFFR (1670.79)	18	131	No diferencial abundance
34	Eukaryotic translation initiation factor 3 subunit (4.85/65.02 – 5.23/38.55)	gi 146103712	DLATIQVYR (1078.54), VHGHQLGQLR (1144.59), LLGSYTTVGQVEGR (1479.72)	9	249	No diferencial abundance
35	Translation elongation factor	gi 146098751	SSILFDIK (922.49), LVPVAFGVK (929.55),	49	231	No diferencial

	1-beta (Isoform C) (4.05/ 25.77 – 5.14/25.94)		DGLIWGDHK (1040.48), NMATYTEGER (1171.46), LFLGGAKPTAEDVK (1445.75), AWDDTIDLEALAQK (1588.73), VFNDLLGANHVNLYR (1744.86), AAAVAPPSAAAAEEDDDIDLFGETTEEEK (3044.29)			abundance
36	Hypothetical protein (4.40/12.77 – 5.01/31.45)	gi 146078070	WVDPEIFTSR (1249.57), QTCELTCIER + 2 Carbamidomethyl (C) (1309.53), HGLECVQCVVK + 2 Carbamidomethyl (C) (1328.59), RPEVDPILDTVTALR (1694.88), IEAFAAEETAQGRPTR (1746.81), CAVTFLSESTLLLSPK + Carbamidomethyl (C) (1765.88), VVEVDSNEPAGANVLSFSAEK (2161.99)	35	374	No diferencial abundance
37	ATP synthase, epsilon chain (4.32/ 8.12 - 5.02/20.13)	gi 146094218	IGVLESVIQSLK (1285.70), ALAQQQSALNSAHDDK (1696.74), VVDKDIHSAYENMETLR (2019.85)	25	360	No diferencial abundance
38	Eukaryotic initiation factor 5a (4.89/ 8.90 – 4.83/17.88)	gi 146088631	TYPLPAGALK (1030.53), VSIVATDIFTGNR (1392.68), EDLDMPPDVALATQIK (1755.78), LEDQAPSTHNVEVPFVK (1909.87), EVLVVVVSAMGTEQVLQTK (2030.01)	45	68	No diferencial abundance
39	Adenosine kinase (4.95/ 5.34 – 5.30/37.09)	gi 146093656	VAQWIAQAPK (1111.58), AAPYLDVIFGNEVEAK (1735.83), LVVFTQGSQPTVYATR (1766.88), SSFVNYVGCASDDKYGK + Carbamidomethyl (C) (1896.77), GIFEELQHPNVTYVPGGSGLNTR (2685.24), SLVANLSAANLLSADHMHSSDVVETLK (2822.34)	32	86	No diferencial abundance
40	Ribonucleoprotein p18, mitochondrial precursor	gi 146081834	ECVQSGQFR + Carbamidomethyl (C) (1110.39), VKPNEESWTWVLK (1615.69), YYDEAGEVLVNMNVK (1743.66), RVPaelVQQNEANASK (1753.75),	62	189	No diferencial abundance

	(4.95/ 5.34 – 8.53/31.03)		NCPPDLETYNATLQK + Carbamidomethyl (C) (1763.65), FCAMMDLMEEMQHR + Carbamidomethyl (C) (1828.56), EHPSTLAQQQLFDIK (1841.76), KYDLFGYEVDNTALWIDK (2290.91)			
41	Adenosylhomocysteinase (5.28/ 83.20 – 5.75/47.79)	gi 17108371	AGVPVFAWK (974.51), DISLAEWGR (1046.49), SKFDNLYGCR + Carbamidomethyl (C) (1259.54), EHVEIKPQVDR (1349.67), AIELAENEMPGLMELR (1815.84), GETDEEYEWCIQTVK + Carbamidomethyl (C) (1957.78), WSSCNIFSTQDNAAAAIAK + Carbamidomethyl (C) (2054.90), QAEYINCPVNGPFPKPDHYR + Carbamidomethyl (C) (2305.01)	25	316	No diferencial abundance
42	Trypanothione reductase (5.58/ 89.20 – 5.85/53.03)	gil1460767721	LMVTGAQYMDLIR (1510.73), TSVDNIYAIGDVTNR (1637.76), AYDLVVLGAGSGGLEAGWNAAVTHK (2456.19), ISDFHSTIGVHPTSAEELCSMR + Carbamidomethyl (C) (2474.08), NYETVAVYASSFTPLMHNISGSK (2516.13), LGVPGDEFCITSNEAFYLEDAPK + Carbamidomethyl (C) (2572.14), VAVVDVQATHGPPLFAALGGTCVNVGCVPK + 2 Carbamidomethyl (C) (3033.49)	31	60	No diferencial abundance

Mr, molecular weight, kDa; exp, experimental; pred, predicted; score*, the cut off for the protein identification was set by the MASCOT score >33 ($p<0.005$). The Spot Code refers to the spot identification used in the Figure 2.

Supplementary Table 2. Identification of proteins Immunogenic proteins from *L. chagasi* that were recognized by total IgG, IgG1, IgG2 and IgM isotypes as indicated by Western blot analysis.

Spot code	Protein Name (<i>pI/Mr exp- pred</i>)	GI	Antibody Isotypes	Sequence (MS/MS)	Cover age%	Protein score*	Signal Peptide	Glycosyl ation site
19*	Hypothetical protein (5.27/ 59.75 - 5.32/40.8)	gil146094146l	Total IgG, IgG2	DTYEGEWR FDGDWVEGR YTGDWTFGR YEGDWLDDR YEGYWLDK GTLEYADGDRYDGEWK YYYADGGVYEGEWQDGK	21	167	NO	NO
21*	Hypothetical protein (4.92/ 50.21 - 5.07/36.3)	gil146081345l	Total IgG, IgG1, IgG2	TDFFTQPDMAR KTDDFTQPDMAR TIFQGDPEIDLQK YIFSQSLQAEVR NEAETGATEAFADTGDAPR	17	127	YES	NO
32*	Hypothetical protein (4.39/12.80 - 9.59 / 21.9)	gil134072971l	Total IgG, IgG1, IgG2	NPENVVVER KVSTILITLSR TRNPENVVVER ISGLHQVTELGSELVR ISGMGGAIYNAVNI AEIVK SNPGYQEPIPDSEVTEQER	32	186	NO	YES
34*	Eukaryotic translation initiation factor 3 subunit	gil 146103712l	Total IgG IgG1, IgG2	TSHLIDFR DLATIQVYR TYDAAMVALDVNR LLGSYTTVGQVEGR QIHEDENEVINR FSWDPNYLALATASTDK ESTLLATASAGEEVLLWSVESGAL	28	221	NO	YES

	(5.09/ 67.29 - 5.23/ 38.5)			LGSVSR				
35*	Translation elongation factor 1-beta (Isoform C) (5.16/ 38.67 - 4.61/23,2)	gil1461041171	IgG Total	SSILFDIK LVPVAFGVK DGLIWGDHK MASYYQAER AWDDTIDLEALAQK LNAQPFVSGFSPSEDAR IFNEMFGSNVNVIQWVAR IFNEMFGSNVNVIQWVAR + Oxidation (M) MASYYQAERDEILNAGTEK	45	593	NO	NO
36*	Hypothetical protein (5.97/ 41.20 - 5.01/31,4)	gil1460780701	Total IgG IgG2	WVDPEIFTSR RPEVDPILDTVTALR IEAFAAEETAQGRPTR VVEVDSNEPAGANVLSFSAEK	21	207	NO	NO
40*	Ribonucleoprotein p18, mitochondrial precursor (6.45/ 11.87 - 6.74/21.2)	gil1460818341	Total IgG, IgG2	ECVQSGQFR + Carbamidomethyl (C) VKPNEESWTWVLK YYDEAGEVLVNMNVK NCPPDLETYNATLQK + Carbamidomethyl (C) FCAMMDLMEEMQHR.N + Carbamidomethyl (C) EHPSTLAQQQSLFDIK KYDLFGYEVDNTALWIDK	54	457	YES	YES
41*	S-adenosyl-L-homocysteine	gil17108371	Total IgG, IgG1, IgG2	AGVPVFAWK DISLAEWGR SKFDNLYGCR + Carbamidomethyl	19	188	NO	YES

	hydrolase (6.01/ 85.63 - 5.75/47.7)			(C) EHVEIKPQVDR IFGISEETTTGVK AIELAENEMPGLMELR GETDEEYEWCIQTVK + Propionamide (C)				
42*	2- Trypanothione reductase (6.16/ 86.36 - 5.85/52.9)	gil146076772l	Total IgG IgG1, IgG2	TPAYFYESGK LMVTGAQYMDLIR TSVDNIYAIGDVTNR AYDLVVLGAGSGGLEAGWNAAV THK ISDFHSTIGVHPTSAEELCSMR + Carbamidomethyl (C)	22	340	YES	YES
43	Beta-tubulin (Isoform B) (5.26/ 84.19 - 4.74/49.7)	gil13569565l	Total IgG	YLTASALFR LAVNLVPFPR FPGQLNSDLR VGEQFTGMFR INVYFDESTGGR GHYTEGAELIDSVLDVCR + Carbamidomethyl (C)	14	125	NO	YES
44	S- adenosylhomocyst eine hydrolase (5.91/ 71.72 - 5.75/47.7)	gil146103697l	Total IgG, IgG1, IgG2	AGVPVFAWK SKFDNLYGCR.E + Carbamidomethyl (C) EHVEIKPQVDR GNLPISAINVNDSTK AIELAENEMPGLMELR GETDEEYEWCIQTVK + Carbamidomethyl (C)	17	157	NO	YES
45	70 kDa heat shock protein (Isoform	gil10626l	Total IgG IgG2, IgG1	FEELCGDLFR + Carbamidomethyl (C) AVHDVVLVGGSTR + Oxidation (HW)	9	181	NO	YES

	B) (4.96/ 71.44 - 5.64/74.2)			DCHLLGTFDLSGIPPAPR + Carbamidomethyl (C) SQIFSTYADNQPGVHIQVFEGER				
46	Hypothetical protein (5.02/ 72.59 - 5.49/41.9)	gil68127339	Total IgG, IgG1, IgG2	GVNFSDCMDAR + Carbamidomethyl (C) LYDYPSIQSYPIEVR	7	50	YES	YES
47	Hypothetical protein (5.36/ 76.76 - 8.00/65.0)	gil134060528	Total IgG	TQRLVSAVTVSSLGTRGVK	3		YES	YES
48	Heat shock protein 83-1 (Isoform B) (4.99/ 68.10 - 5.12/78.8)	gil146097493	Total IgG, IgG1, IgG2	HKPLWTR ADLVNNLGTIAR EDQLEYLEPR HFSVEGQLEFR DVTKEEYAAFYK YQSLTDPSVLGESPR HSEFIGYDIELMVEK	11	404	NO	YES
49	Heat shock protein 83-1 (Isoform C) (5.04/ 65.70 - 5.12/78.8)	gi146097493	Total IgG IgG2	QFYEQFGK LGIHEDTANR LESSPFIEQAR KLESSPFIEQAR GVVDSIDLPLNISR FYSTESGEEMTTLK LSTSPCILVTSEFGWSAHMEQIMR + Carbamidomethyl (C)	11	331	NO	YES

50	Hypothetical protein (5.22/59.75 - 5.30/32.8)	gil146099894	Total IgG, IgG2	VVMDGSFFLDIR AGDLVVLPAGMYHR ATLDEDDYVALYR WYQEHYTEDEQFR	18	124	NO	NO
51	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor (5.42/ 58.81 - 7.71/38.6)	gil146095259	Total IgG	K.NLESYLER.L R.FLSVPQQR.W R.EYLQMLASDEER.N R.MLQLGETPVADDAR.N 5,26/ 84,19 R.NLGLIDEVAADEESTIEAAYQQ AER.F	18	182	NO	YES
52	Hypothetical protein (5.00/ 56.53 - 5.06/32.8)	gil146096921	Total IgG, IgG1, IgG2	EYDSICPLDDL + Carbamidomethyl (C) SGDYAGAQQIYDHLAR VDEAHDFTFLQAATQYK	15	140	NO	YES
53	Heat shock protein 83; HSP 90 (Isoform A) (4.79/ 47.13 - 5.65/52.7)	gil123669	Total IgG, IgG1	SIYYITGDSK KLESSPFIEQAR EGVHFEESEEEKQQR	8	37	NO	YES
55	Pyruvate dehydrogenase E1 beta subunit	gil146089026	Total IgG, IgG2	DITLIGFSR DAIHSALDEELAR LAAEGVQAEVINLR VFVIGEEVGQYQGAYK	14	228	YES	YES

	(Isoform B) (5.31/ 46.76 - 5.67/37.8)							
59	Proteasome alpha 3 subunit, putative (5.23/ 38.98 - 5.32 /32.2)	gil134068305l	Total IgG, IgG2	MVPHPLFDSENMQDK TTPQFQILTLSELKPHAER IAEHIGCSVAGVTSDAYALLNYAR + Carbamidomethyl (C)	22	49	NO	YES
63	Nascent polypeptide associated complex homologue, alpha chain (Isoform B) (4.93/ 26.08 - 4.50/18.1)	gil8671200l	Total IgG	EINVVMSQANVSR VGSLSFAMVQPEVYR KVGSLSFAMVQPEVYR FPGTNTFVIFGEAQLEDTSALAE AAAR	33	247	NO	YES
64	Hypothetical protein (6.88/ 26.08 - 6.38/538.6)	gil68124247l	Total IgG	IEKMQKNIK			YES	NO
65	Hypothetical protein (6.11/28.78 -	gil146091091l	Total IgG	FLCPLTANK + Carbamidomethyl (C) VAQLSEEEAAAAR YGVIFYQFTIR VLFEVGEAPDGSADGNVNDMLAR	28	199	NO	NO

	4.73/22.3)							
66	Hypothetical protein (5.20/27.92 - 6.09/252.3)	gil70799658l	Total IgG	GSHGDGGDVGALR	1	30	NO	YES
67	Hypothetical protein (6.97/19.40 - 4.76/22.3)	gil146076809l	Total IgG, IgG1	WDNDDFFNQR DYPWFDDAVK DLYIIVHCDDGK + Carbamidomethyl (C) DFSYVVTDKWDNDDFFNQR QCPFIEEAEGEIPFGYPAAVLEHL IR.W + Carbamidomethyl (C)	34	272	NO	NO
70	Hypothetical protein (6.29/88.58 - 6.21 / 55405.07)	gil134070112l	Total IgG, IgG1, IgG2	KMEFVIDR NEAAFQDVGIEYYR LFEVQLQHSGSASDYR ELDVNPHYFQQIESGGEPK	11	49	NO	YES
71	Hypothetical protein (6.95/100.61 - 6.21/55.3)	gil146088184l	Total IgG IgG2	HFMNLEETYK HACLAGVYAMESATR + Carbamidomethyl (C) NEAAFQDVGIEYYR LFEVQLQHSGSASDYR ITNYNENAYTMAANAVLR IGFLGLGTESSSDNSAGAIIVK ELDVNPHYFQQIESGGEPK	22	325	NO	YES
72	Aldose 1-	gil72547339l	Total IgG	K.NWVQPESVR.N R.VIGDCIDDVALLDR.D +	26	208	NO	YES

	epimerase (5.97/69.47 - 5.95/41.4)			Carbamidomethyl (C) R.FNYTSPHMESGFPGEVK.N K.NPEEAYADPDYMGATVGR.C R.MAIP TGEFLSVEGTGLDFR.Q K.VGLTNYAASVASIQVYHPADNK .W				
73	Fatty acid elongase, putative (6.08/73.76 - 8.61 / 34.79)	gil68125282l	Total IgG	MVQFLESFR + Oxidation (M)	3	33	YES	NO
74	Sterol 24-c- methyltransferase (6.45/64.66 - 6.22/39.8)	gil146104460l	IgG Total	HEYFLAAR YAGETFFESLAR CNVIGVNNNDYQISR + Carbamidomethyl (C) GGFMEGDHIVDVGCGVGGPAR + Carbamidomethyl (C) SIPWYQPLVGDYSSLQGLR	21	155	NO	YES
75	Peroxidoxin 1 (4.86/19.70 - 6.31/21.27)	gil11761380l	Total IgG, IgG1	HGEVCPANWK + Carbamidomethyl (C) LLEAFQFVEK GLFIIDPNGMVR GLFIIDPNGMVR + Oxidation (M)	24	131	NO	YES
76	Ubiquitin- conjugating enzyme E2 (4.99/74.30 - 6.08/16.6)	gil72547490l	IgG Total	DQWSPALTISK DPPANTSGGPVSESDLFNWK DLEKDPPANTSGGPVSESDLFNW K	23	196	NO	YES

77	Endoribonuclease L-PSP (pb5) (4.32/75.43 - 5.87/16.9)	gil1460870171	Total IgG, IgG2	PGVVEANLQK SCAVQLIAQIK + Carbamidomethyl (C) LMVGQLGDSLTAEDGK LMVGQLGDSLTAEDGK + Oxidation (M)	22	213	NO	NO
78	Cyclophilin (4.47/94.62 - 5.61/20.3)	gil1460707541	Total IgG	VLDGMDVVLR VLDGMDVVLR + Oxidation (M) DAPLT TENFR QLCTGEHGFYK + Carbamidomethyl (C) VYFDVMIDSEPLGR VYFDVMIDSEPLGR + Oxidation (M) VIPNFMIQGGDFTNFDGTGGK VIPNFMIQGGDFTNFDGTGGK + Oxidation (M)	35	268	YES	YES
54, 56,57, 58, 60, 61, 62	Beta-tubulin (Isoform C) 54- (5.10/ 45.33 - 4.69/49.6) 56- (4.73/ 38.08 - 4.69/49.6) 57- (5.54/ 39.60 - 4.69/49.6) 58- (4.91/ 41.20 - 4.69/49.6)	gil12968321	Total IgG IgG1 (54,57,58) IgG2 (54)	INVYFDESAGGR AVLMDLEPGTMDSVR AVLMDLEPGTMDSVR + Oxidation (M) GHYTEGAELIDSVLDVCR + Carbamidomethyl (C) FWEVIAD EHGVDPTGSYQGDS DL QLER	16	315	NO	YES

	60- (4.38/ 34.62 - 4.69/49.6) 61- (4.44/ 33.45 - 4.69/49.6) 62- (4.98/ 31.29 - 4.69/49.6)							
1M	Heat shock protein 83-1; HSP 90 (Isoform B) (5.55/ 67.88 - 5.65 / 52.69)	gil123669l	IgM	SIYYITGDSK KLESSPFIEQAR EGVHFEESEEEKQQR	9	37	NO	YES
2M	Alpha tubulin (Isoform C) (5.50/55.25 - 4.89/49.7)	gil606648l	IgM	LIGQVVSSLTASLR FDGALNVDLTEFQTNLVPYPR	7	68	NO	NO
3M	Mannose-1-phosphate guanylttransferase (5.45/66.02 - 5.70/41.8)	gil146086987l	IgM	SIVGWNNR AVILVGGFGTR FIPSLVHG NR FTVIGASLIDPSAK ETEQLHTEDMEHQR YGVVVYSPQNYQIER AHGGEGTIMVSQVTQWEK AVGVTEVILAVAYRPEAMK LGVSFVFSVEEEPLGTAGPLALAR	35	248	NO	NO

Spot Code corresponds to the 2D gel shown in figures 4 and 5.

Supplementary Table 3. Peptide sequence and the respective supertypes screened by NetCTL algorithm.

Spot Code	Protein Name	Peptide sequence a.a position	No of Supertypes	Supertypes
18	Putative eukaryotic initiation factor 4a	223-FMRDPVRIL-231	5	A2, A24, B7, B8, B62
47	Hypothetical protein	486-WSSQSPKSF-494	5	A1, A24, B8, B58, B62
64	Hypothetical protein	1326-RMMGVLFDY-1334	5	A2, A3, A24, B27, B62
72	Aldose 1-epimerase	84-FTLDGVKYY-92	5	A1, A3, A24, B58, B62
3M	Mannose-1-phosphate guanyltransferase	75-WSRKLGVSF-83	5	A24, B7, B8, B58, B62
1	Alpha tubulin	164-KSKLGTVY-172	4	A1, A3, B58, B62
2	Hypothetical protein	93-FVQKVMML-101	4	A2, B7, B8, B24
3	Phosphomannomutase	539-GTEPKIKWY-547	4	A1, A3, B58, B62
9	70 kDa heat shock protein	83-ITNPQSTFY-91	4	A1, A3, B58, B62
10	Actin	162-HTVPIYEGY-170	4	A1, A3, B58, B62
19	Hypothetical protein	207-LTYVNGERY-215	4	A1, A3, B58, B62
23	Hypothetical protein	184-GLYDGRYVF-192	4	A2, A24, B27, B62
32	Tryparedoxin peroxidase	108-KTKSIARAY-116 63-ISGMGGAIIY-71	4 3	A1, B8, B58, B62 A1, B58, B62
34	Eukaryotic translation initiation factor 3 subunit	48-KTGKLLGSY-56	4	A1, A3, B58, B62
36	Hypothetical protein	276-LTCCSLLSY-284	4	A1, A3, A24, B62
39	Adenosine kinase	168-FTLTIDVNY-176	4	A1, A24, B58, B62
40	Putative ribonucleoprotein p18, mitochondrial precursor	128-QSGQFRLGY-136	4	A1, A3, B58, B62
41	Adenosylhomocysteina se	59-VLIETLKAL-67	4	A2, A24, B8, B62
44	S-adenosylhomocysteine hydrolase	59-VLIETLKAL-67	4	A2, A24, B8, B62
45	70 kDa heat shock protein	36-RTTPSYVAF-44	4	A1, A24, B58, B62
49	Heat shock protein 83-1	478-FMTEPIDEY-486	4	A1, A2, A24, B62
51	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor	224-WVMPAYAYL-232	4	A2, A24, B7, B62
52	Hypothetical protein	131-WLQQAVRYF-139	4	A2, A24, B8, B62
53	Heat shock protein 83; HSP 90	244-FMTEPIDEY-252	4	A1, A2, A24, B62
55	Pyruvate dehydrogenase E1 beta subunit	1-MRRFASRAL-9	4	A24, B7, B8, B27

59	Proteasome alpha 3 subunit, putative	275-RQRQQEQAL-283	4	B7, B8, B27, B62
66	Hypothetical protein	127-LLPPRTPTL-135	4	A2, A24, B7, B62
71	Hypothetical protein	297-ATSEEHKAY-305	4	A1, A3, B58, B62
73	Fatty acid elongase, putative	257-FILFSKLFY-265	4	A1, A3, A24, B62
74	Sterol 24-c-methyltransferase	274-WYQPLVGDY-282	4	A1, A24, B8, B62
75	Peroxidoxin 1	108-KTKSIARAY-116	4	A1, B8, B58, B62
15	Chaperonin 60.2 precursor	294-GMLEDIAVF-302	3	A2, A24, B62
16	Enolase	168-IAPTKATSF-176	3	A24, B7, B62
21	Hypothetical protein	44-ARRSVQQAM-52	3	B7, B8, B27
24	14-3-3 protein-like protein	196-ALNFSVFYY-204	3	A1, A3, B62
26	Hypothetical protein	33-ITITEDKIY-41	3	A1, B58, B62
30	IgE-dependent histamine-releasing factor	27-IMYVVNGRY-35	3	A3, A24, B62
35	Translation elongation factor 1-beta	43-WVARMASY-51	3	A1, A24, B62
42	Trypanothione reductase	359-HTKVACAVF-367	3	A24, B8, B58
43	Beta-tubulin	428-ATVEEEGEY-436	3	A1, A3, B58
46	Hypothetical protein	53-AMKGATYKL-61	3	A2, A24, B8
50	Hypothetical protein	124-ALRELGVVL-132	3	A2, B7, B8
63	Nascent polypeptide associated complex homologue, alpha chain	55-IRKVGSLSF-63	3	A24, B8, B27
65	Hypothetical protein	80-AARTIRYRF-88	3	A24, B7, B8
67	Hypothetical protein	29-IVHCDDGKY-37	3	A1, A3, B62
76	Ubiquitin-conjugating enzyme E2	55-IHFPSDYPF-63	3	A24, B58, B62
78	Cyclophilin	63-CTGEHGFGY-71	3	A1, B58, B62

The peptides were identified by NetCTL analysis of 81 proteins identified by DIGE as well as 2DE gel and Western blot analysis. The Spot Code refers to the spot identification used in the Figure 2 for DIGE and Figures 4 and 5 for 2DE and Western blot.

Supplementary Table 4. Number of potential T cell epitopes derived from *L. chagasi* proteins identified by DIGE as well as 2DE gel and Western blot analysis.

Spot Code	Protein Name	Protein length (aa)	% predicted peptides	No of predicted peptides with score >1.5
73	Fatty acid elongase, putative	299	33.0	16
53	Heat shock protein 83; HSP 90	452	29.5	16
74	Sterol 24-c-methyltransferase	353	29.3	22
32	Tryparedoxin peroxidase	199	29.3	11
75	Peroxidoxin 1	190	29.1	10
65	Hypothetical protein	197	28.6	9
2	Hypothetical protein	374	27.6	17
24	14-3-3 protein-like protein	258	27.6	12
30	IgE-dependent histamine-releasing factor	170	27.2	9
49	Heat shock protein 83-1	701	26.3	23
4	Prostaglandin f2-alpha synthase	239	26.0	6
76	Ubiquitin-conjugating enzyme E2	148	25.7	4
35	Translation elongation factor 1-beta	211	25.6	4
10	Actin	376	25.3	19
36	Hypothetical protein	286	25.2	12
43	Beta-tubulin	443	25.1	13
26	Hypothetical protein	199	25.1	8
64	Hypothetical protein	4873	24.9	166
23	Hypothetical protein	279	24.7	7
40	Putative ribonucleoprotein p18, mitochondrial precursor	187	24.6	8
19	Hypothetical protein	358	24.3	17
21	Hypothetical protein	322	24.2	8
18	Putative eukaryotic initiation factor 4a	403	24.1	10
70	Hypothetical protein	501	23.7	15
71	Hypothetical protein	501	23.7	15
3M	Mannose-1-phosphate guanylttransferase	379	23.7	10
72	Aldose 1-epimerase	371	23.7	10
51	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor	342	23.4	11
52	Hypothetical protein	291	23.3	12
3	Phosphomannomutase	593	23.2	28
1	Alpha tubulin	451	22.8	17
59	Proteasome alpha 3 subunit, putative	285	22.4	8
39	Adenosine kinase	345	22.3	12
67	Hypothetical protein	196	22.3	6
78	Cyclophilin	187	22.3	5

55	Pyruvate dehydrogenase E1 beta subunit	350	21.9	14
34	Eukaryotic translation initiation factor 3 subunit	356	21.8	12
42	Trypanothione reductase	491	21.7	15
44	S-adenosylhomocysteine hydrolase	437	21.7	16
41	Adenosylhomocysteinase	437	21.2	16
64	Hypothetical protein	609	21.0	21
15	Chaperonin 60.2 precursor	566	20.6	11
77	Endoribonuclease L-PSP (pb5)	163	20.6	7
50	Hypothetical protein	279	20.3	8
63	Nascent polypeptide associated complex homologue, alpha chain	172	20.1	4
46	Hypothetical protein	384	19.9	10
32	Tryparedoxin peroxidase	198	19.5	11
45	70 kDa heat shock protein	678	19.3	15
9	70 kDa heat shock protein	634	19.2	18
27	Hypothetical protein	199	17.8	5
16	Enolase	429	17.3	9
66	Hypothetical protein	2453	17.2	70

The table shows the protein length, number of the nanomers and the predicted peptides with score >1.5 in all *Leishmania* proteins screened for potential T cell epitopes using the NetCTL algorithm. The data sorted in % predicted epitopes. The Spot Code refers to the spot identification used in the Figure 2 for DIGE and Figures 4 and 5 for 2DE and Western blot.