

Table S1. Predicted CTL epitopes of selected *L. infantum* proteins to be part of **multi-epitope chimeric protein vaccine** against murine MHC I.

No	Protein Name	Epitope Sequence	IEDB	SYFPEITHI	MHCI
1	CyP2	126-GPNTNGSQF-134	0.6	21	H2-Ld
2	CyP40	4-TYCYLDITI-12	0.5	23	H2-Kd
3		232-KYAKAVRYL-240	0.2	26	H2-Kd
4		119-YYKGEGSFV-127	0.8	24	H2-Kd
5	Gcvl-2	352-NYNVIPGVI-360	0.5	21	H2-Kd
6		439-EYGASSEDL-447	0.7	22	H2-Kd
7	Enol	186-VYHALKVII-194	0.5	23	H2-Kd
8	Cpn60	38-LGPKGRNVI-46	0.3	-	H2-Dd
9	Hyp	92-ESQKSLYEF-100	1	22	H2-Ld
10		169-LFSCMLTSL-177	0.8	22	H2-Ld

Table S2. Predicted HTL epitopes of selected *L. infantum* proteins to be part of **multi-epitope chimeric protein vaccine** against murine MHC II.

No	Protein Name	Epitope Sequence	IEDB	SYFPEITHI	NetMHCII
1		1-MRVVAVLAVVLCALS-15	1.25	-	0.8
2	CyP2	2-RVVAVLAVVLCALS-16	8.87	27	-
3		3-VVAVLAVVLCALS-17	18.002	23	-

4		4-VAVLAVVLCALSFLN-18	17.82	22	-
5		19-VAAEPEVTAKVYFDV-33	19.44	23	-
6		173-DRPVKPKIVASGEL-187	10.73	21	-
7	CyP40	296-RRGTAALKAGDADGA-310	0.31	32	426.8
8		332-SEAKEKVKAKLA-346	0.16	28	299.9
9		19-GGPGGYVAAIKAAQL-33	0.88	28	447.8
10	Gcvl-2	84-ENVTMDVSAMQAQKA-98	0.05	34	88.3
11		87-TMDVSAMQAQKAKGV-101	0.07	22	96.7
12		456-TMSEAVKEACMACFA-470	5.09	23	219.1
13		111-GCSMAISKAAAAG-125	0.07	22	156.4
14	Enol	113-SMAISKAAAAGVP-127	0.48	29	320.4
15		300-DEFSAITMALAGKAQ-314	1.88	20	268.4
16		19-ARRSMQKGVTRAVAA-33	1.39	22	-
17		27-VTRAVAAVATTLGPK-41	1.65	25	-
18		42-GRNVIIQAYGAPKI-56	6.36	21	-
19	Cpn60	220-AELEDALVMSAKKI-234	5.79	27	-
20		272-NKLQGKLVCCVKAP-286	9.55	22	-
21		374-KLKERLAKLSGGVAV-388	4.13	23	-
22		510-PTRVVRVAISDATSV-524	3.91	21	-
23		57-VAITEDVALAAVQAV-71	1.67	26	159.0
24		58-AITEDVALAAVQAVG-72	0.96	21	184.3
25	Hyp	59-ITEDVALAAVQAVGL-73	0.67	29	221.7
26		172-CMLTSLAATVIARDL-186	2.54	22	236.2

27	278-RRAAEELALDGVTKD-292	6.28	21	494.9
28	470-PPRNKAVAVKRLQQV-484	3.27	22	259.2

Table S3. Predicted CTL epitopes of selected *L. infantum* proteins against HLA class I alleles that provide >97% population coverage using IEDB server.

No	Protein Name	Epitope Sequence	HLA class I
1	CyP2	126-GPNTNGSQF-134	HLA-B*07:02, HLA-B*35:01, HLA-B*53:01, HLA-B*44:02
2		4-TYCYLDITI-12	HLA-A*23:01, HLA-A*24:02
3	CyP40	232-KYAKAVRYL-240	HLA-A*24:02, HLA-A*23:01, HLA-A*30:01, HLA-A*31:01, HLA-B*07:02
4		119-YYKGEFSFV-127	HLA-A*24:02, HLA-A*23:01, HLA-A*30:01
5	Gcv1-2	352-NYNVIPGVI-360	HLA-A*24:02, HLA-A*23:01, HLA-B*51:01
6		439-EYGASSEDL-447	HLA-A*24:02, HLA-A*23:01
7	Enol	186-VYHALKVII-194	HLA-A*24:02, HLA-A*23:01, HLA-B*51:01, HLA-A*32:01
8	Cpn60	38-LGPKGRNVI-46	-
9		92-ESQKSLYEF-100	HLA-A*01:01, HLA-A*26:01, HLA-A*23:01, HLA-B*58:01, HLA-A*24:02, HLA-B*15:01, HLA-B*57:01, HLA-B*35:01
10	Hyp	169-LFSCMLTSL-177	HLA-A*02:03, HLA-A*02:06, HLA-B*08:01, HLA-A*23:01, HLA-A*24:02, HLA-B*40:01

Table S4. Predicted HTL epitopes of selected *L. infantum* proteins against HLA class II alleles that provide >97% population coverage using IEDB server.

No	Protein Name	Epitope Sequence	HLA class II
1		1-MRVVAVLAVVLCALS-15	HLA-DRB1*15:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*01:01, HLA-DRB1*08:02, HLA-DQA1*05:01/DQB1*03:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*05:01/DQB1*02:01
2		2-RVVAVLAVVLCALS-16	HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*01:01, HLA-DPA1*03:01/DPB1*04:02
3	CyP2	3-VVAVLAVVLCALSFL-17	HLA-DRB1*01:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*02:01/DPB1*05:01, HLA-DRB1*15:01, HLA-DQA1*05:01/DQB1*02:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DPA1*02:01/DPB1*01:01
4		4-VAVLAVVLCALSFLN-18	HLA-DPA1*02:01/DPB1*05:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DPA1*01:03/DPB1*02:01
5		19-VAAEPEVTAKVYFDV-33	-
6		173-DRPVKPKIVASGEL-187	HLA-DRB1*08:02, HLA-DRB1*07:01, HLA-DRB1*15:01
7	CyP40	296-RRGTAALKAGDADGA-310	HLA-DQA1*05:01/DQB1*03:01
8		332-SEAKEKVKAKKLA-346	HLA-DRB1*09:01, HLA-DRB4*01:01
9		19-GGPGGYVAAIKAAQL-33	HLA-DRB1*08:02, HLA-DRB1*11:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*09:01, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*01:01, HLA-DRB5*01:01, HLA-DQA1*04:01/DQB1*04:02, HLA-DRB1*07:01, HLA-DRB1*15:01
10	Gcvl-2	84-ENVTMDVSAMQAQKA-98	HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*03:01, HLA-DRB1*09:01, HLA-DQA1*04:01/DQB1*04:02, HLA-DRB1*04:01, HLA-DRB1*01:01
11		87-TMDVSAMQAQKAKGV-101	HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*09:01, HLA-DRB1*01:01
12		456-TMSEAVKEACMACFA-470	-
13	Enol	111-GCSMAISKAAAKAG-125	HLA-DRB1*09:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*08:02, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB3*02:02, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DRB5*01:01

14		113-SMAISKAAAAGKAGVP-127	HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*09:01, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*08:02, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB3*02:02, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DRB5*01:01
15		300-DEFSAITMALAGKAQ-314	HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*09:01, HLA-DRB1*08:02, HLA-DRB1*04:01, HLA-DRB1*01:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DQA1*04:01/DQB1*04:02, HLA-DRB1*07:01
16		19-ARRSMQKGVTRAVAA-33	HLA-DPA1*02:01/DPB1*14:01
17		27-VTRAVAAVATTLGPK-41	HLA-DQA1*05:01/DQB1*03:01, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*08:02, HLA-DQA1*01:02/DQB1*06:02
18		42-GRNVIIIEQAYGAPKI-56	HLA-DRB5*01:01
19	Cpn60	220-AELEDALVLSAKKI-234	HLA-DRB5*01:01, HLA-DPA1*02:01/DPB1*05:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*13:02, HLA-DRB1*15:01, HLA-DRB1*11:01, HLA-DRB1*01:01, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*04:01, HLA-DRB1*12:01
20		272-NKLQGKLVCCVKAP-286	HLA-DRB1*12:01, HLA-DRB1*01:01
21		374-KLKERLAKLSGGVAV-388	-
22		510-PTRVVRVAISDATSV-524	HLA-DRB1*08:02, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB4*01:01
23		57-VAITEDVALAAVQAV-71	HLA-DQA1*04:01/DQB1*04:02, HLA-DRB1*03:01, HLA-DQA1*03:01/DQB1*03:02, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*13:02
24		58-AITEDVALAAVQAVG-72	HLA-DQA1*04:01/DQB1*04:02, HLA-DQA1*03:01/DQB1*03:02, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB1*03:01, HLA-DQA1*05:01/DQB1*03:01
25	Hyp	59-ITEDVALAAVQAVGL-73	HLA-DQA1*04:01/DQB1*04:02, HLA-DQA1*03:01/DQB1*03:02, HLA-DRB1*09:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*07:01, HLA-DRB1*01:01
26		172-CMLTSLAATVIARDL-186	HLA-DRB1*01:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*02:01/DPB1*14:01, HLA-DQA1*04:01/DQB1*04:02, HLA-DRB1*09:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB1*12:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*03:01/DQB1*03:02, HLA-DRB1*07:01
27		278-RRAAEELALDGVTKD-292	HLA-DRB1*03:01
28		470-PPRNKAVAVKRLQQV-484	HLA-DRB4*01:01, HLA-DRB1*11:01

Table S5. Conservancy analysis of the predicted sequences among different *Leishmania* species.

No	Protein Name	Epitope Sequence	<i>L. major</i>	<i>L. donovani</i>	<i>L. mexicana</i>	<i>L. braziliensis</i>
1	CyP2	126-GPNTNGSQF-134	100%	100%	100%	n.d.
2		173-DRPVKPVKIVASGEL-187	93.3%	100%	93.3%	n.d.
3	CyP40	232-KYAKAVRYL-240	100%	100%	100%	88.89%
4		332-SEAKEKVKAKAKLA-346	93.3%	100%	93.3%	93.3%
5	Gcv1-2	439-EYGASSEDL-447	100%	100%	n.d.	88.89%
6		19-GGPGGYVAAIKAAQL-33	100%	100%	n.d.	100%
7	Enol	186-VYHALKVII-194	100%	100%	n.d.	88.89%
8		111-GCSMAISKAAAAG-125	100%	100%	n.d.	93.3%
9	Cpn60	38-LGPKGRNVI-46	100%	100%	100%	100%
10		27-VTRAVA AVATTLGPK-41	100%	100%	100%	100%
11	Hyp	169-LFSCMLTSL-177	100%	100%	100%	93.3%
12		57-VAITEDVALAAVQAV-71	88.89%	100%	100%	93.3%

Table S6. Analysis of the physicochemical and immunological characteristics of the designed multi-epitope **protein**-vaccine construct.

Physicochemical properties	
Molecular weight	36458.35
Theoretical pI	6.15
Instability index	31.03
Aliphatic index	87.85
GRAVY	-0.165
Number of amino acids	353
Total no. of negatively charged residues (Asp + Glu)	42
Total no. of positively charged residues (Arg + Lys)	41
Solubility/SOLpro	Soluble/ probability 0.96
Allergenicity	
AlgPred	Non-allergen
Allerdicator	Non-allergen
AllergenFP	Probable non-allergen
Antigenicity	
Vaxigen	0.7132
ANTIGENpro	0.803170

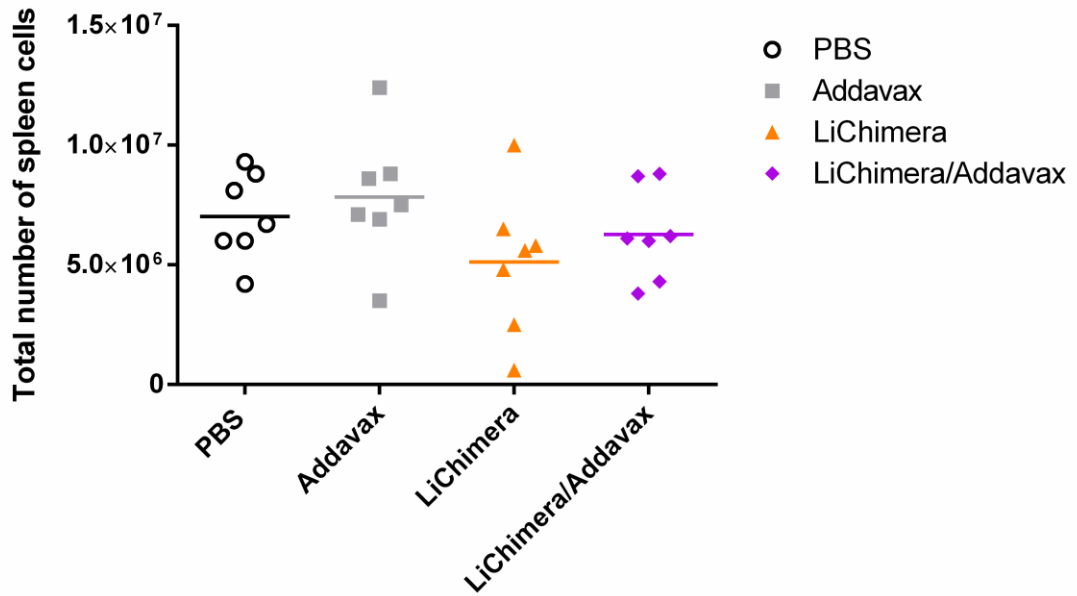


Figure S1. BALB/c mice were vaccinated twice intramuscularly with LiChimera alone or adjuvanted with Addavax. Control groups received PBS or Addavax. Two weeks post boosting, spleen cells were isolated and total cell numbers were determined for each spleen. Dots-Circles, boxes, triangles and reverse triangles represent individual animals from each group and lines represent means. Significant differences between groups are indicated with asterisks measured by one-way ANOVA and Tukey's multiple comparison tests.

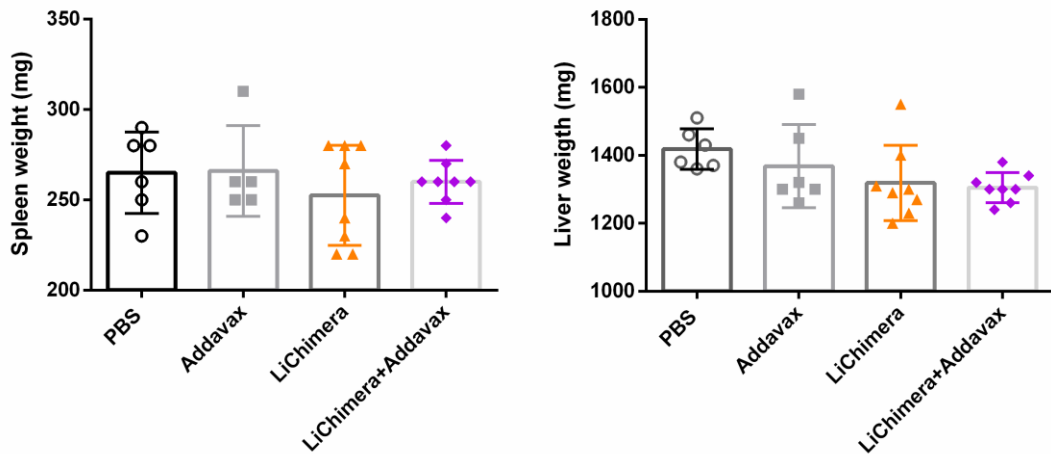


Figure S2. Liver and spleen weights of short-term vaccinated mice 2 months post *L. infantum* challenge. Circles, boxes, triangles and reverse triangles Dots represent individual animals from each group and lines represent means.