

Electronic supplementary information

Murine cutaneous leishmaniasis investigated by MALDI mass spectrometry imaging

Fernanda Negrão,^{1,2*} Daniele F. O. Rocha,¹ Caroline F. Jaeger¹, Francisca J. S. Rocha,³ Marcos N. Eberlin¹, Selma Giorgio,²

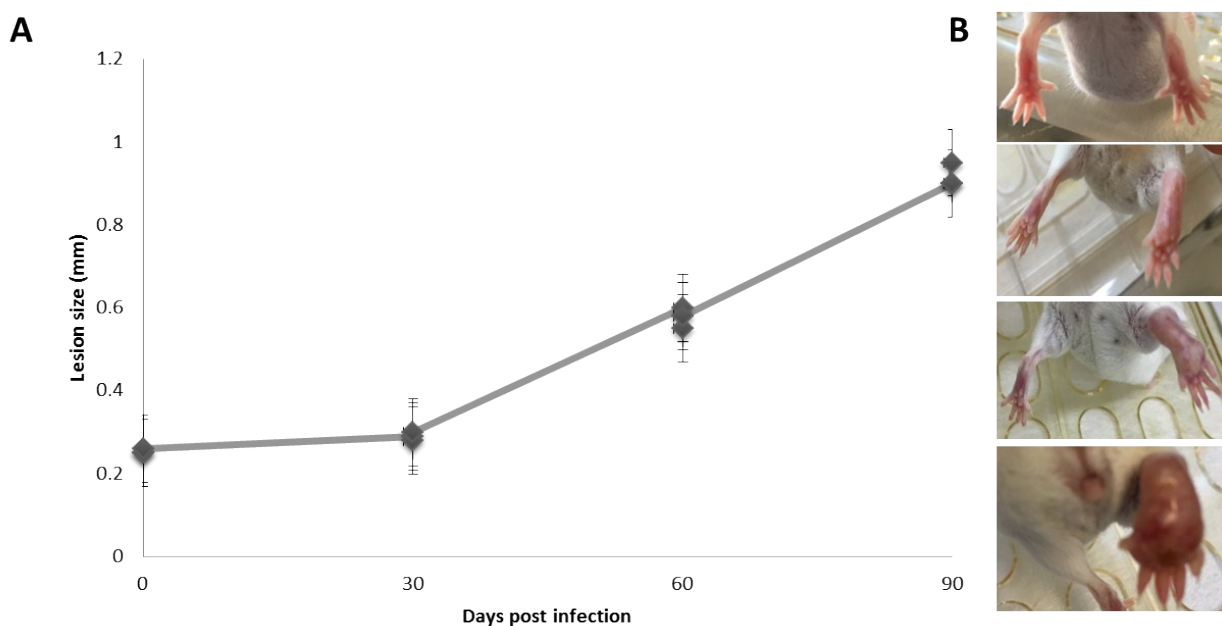


Figure S1: Evaluation of lesions on the course of *L. major* infection in mice. (A) Standard deviation of lesion size from Balb/c footpads. (B) Balb/c footpad lesions at 0, 30, 60 and 90 days p.i. (up to bottom).

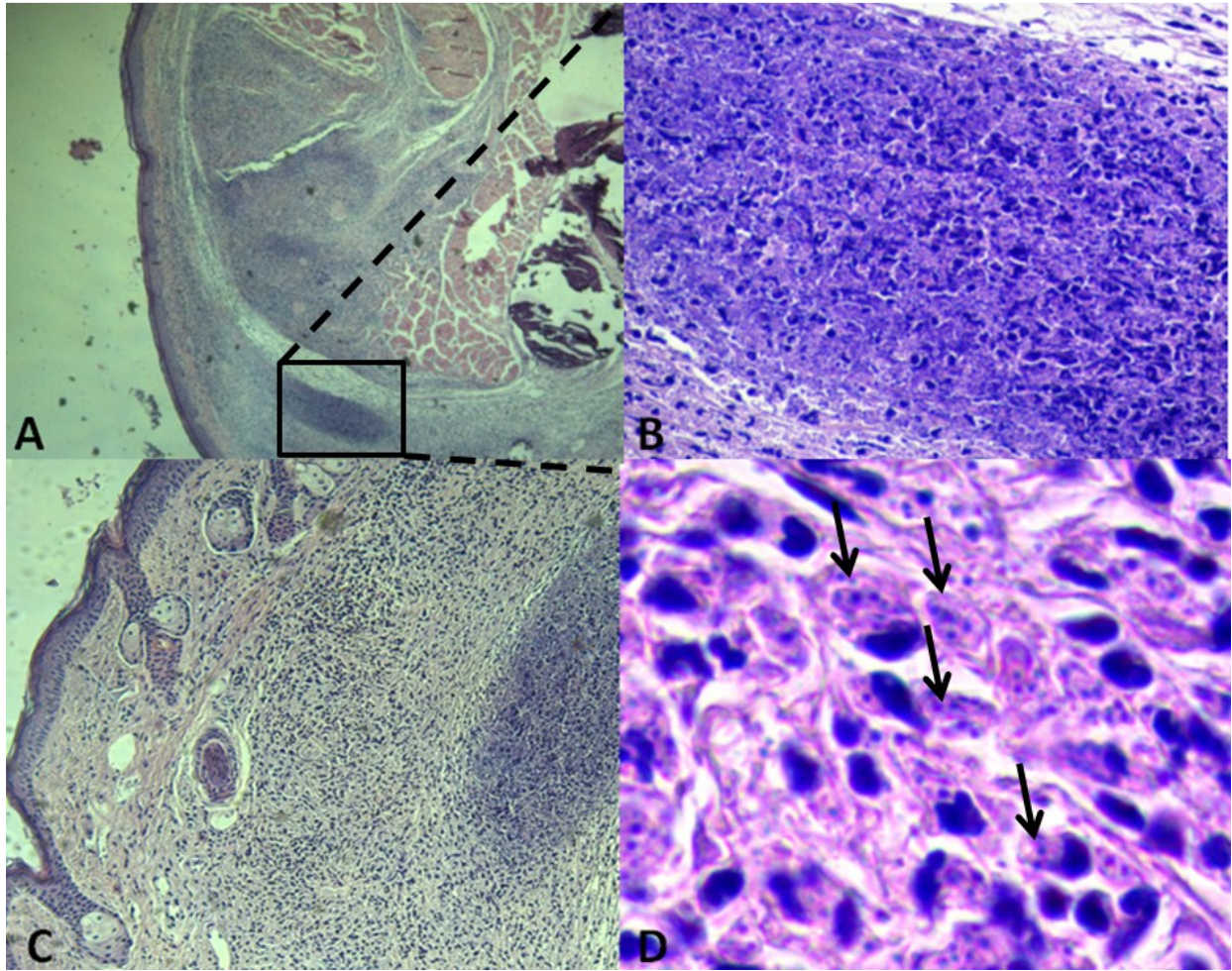


Figure S2: Histological analysis of footpads infected with *L. major* at 30 days p.i. (A) Panoramic view of skin layers. Skeletal muscle and blood vessels are also observed (40x). (B) Signs of edema revealed by a permeability increase in blood vessels. Amastigotes are less abundant than *L. amazonensis* infection (1000x). (C) Panoramic view of skin layers showing abundant inflammatory infiltrates (100x). (D) Lymphocytes are abundant (arrows). Infected macrophages and amastigotes are barely observed (1000x)

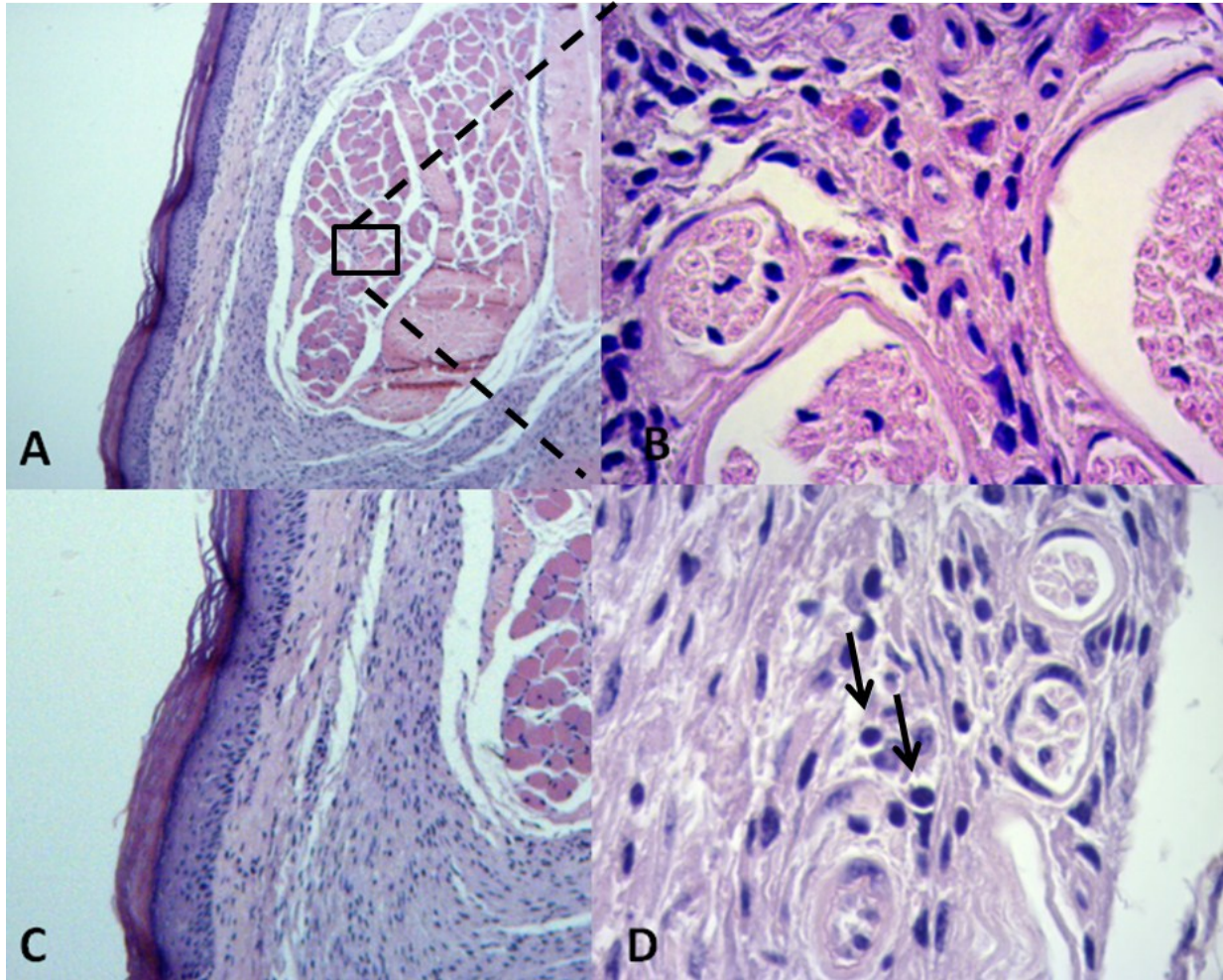


Figure S3: Histological analysis of footpads infected with *L. major* at 30 days p.i. (A) Panoramic view of skin layers. Skeletal muscle and blood vessels are also observed (40x). (B) Signs of edema revealed by a permeability increase in blood vessels. Amastigotes are less abundant than *L. amazonensis* infection (1000x). (C) Panoramic view of skin layers showing abundant inflammatory infiltrates (100x). (D) Lymphocytes are abundant (arrows). Infected macrophages and amastigotes are barely observed (1000x)

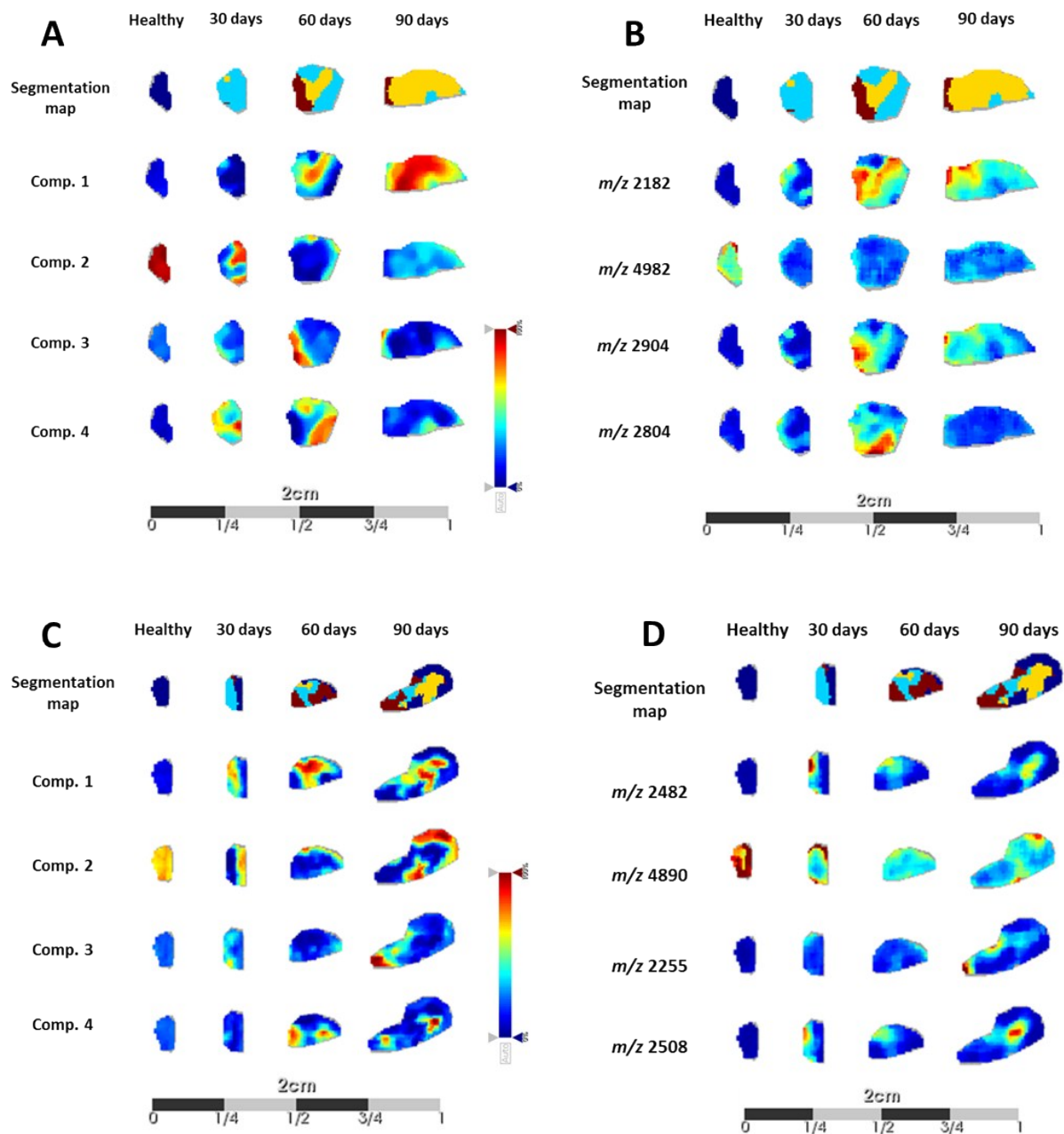


Figure S4: Segmentation map analysis and pLSA components visualized in SCiLS Lab. (A) Segments and pLSA components for *L. amazonensis* disease progression. (B) Relevant m/z intervals (pLSA loadings) responsible for discrimination of segments for *L. amazonensis*. (C) Segments and pLSA components for *L. major* disease progression. (D) Relevant m/z intervals (pLSA loadings) responsible for discrimination of segments for *L. major*.

Table S1*m/z* intervals and AUC values most relevant for *L. amazonensis* infection

m/z	± [Da]	AUC^a	m/z	± [Da]	AUC	m/z	± [Da]	AUC
2181	10	0.9862	2339	10	0.8177	3350	10	0.7944
2232	10	0.9742	2070	10	0.8155	2518	10	0.7942
2350	10	0.9095	2423	10	0.8152	3083	10	0.7942
3835	10	0.8926	4625	10	0.8152	2580	10	0.7914
2248	10	0.8843	2246	10	0.8151	2802	10	0.7897
2446	10	0.8799	2305	10	0.8151	2140	10	0.7891
2902	10	0.8754	3048	10	0.8141	4204	10	0.7888
3837	10	0.8754	2293	10	0.8136	3005	10	0.7869
2114	10	0.8749	2317	10	0.8122	3920	10	0.7821
2818	10	0.8744	2737	10	0.8110	2933	10	0.7808
2474	10	0.8643	2866	10	0.8096	2362	10	0.7786
2084	10	0.8531	3704	10	0.8089	3766	10	0.7751
2165	10	0.8438	3103	10	0.8066	3120	10	0.7742
2502	10	0.8405	2258	10	0.8064	3821	10	0.7735
2378	10	0.8371	2065	10	0.8052	2097	10	0.7734
2485	10	0.8318	2721	10	0.8052	2593	10	0.7724
3879	10	0.8304	2358	10	0.8038	3588	10	0.7724
2129	10	0.8269	2792	10	0.8024	4231	10	0.7633
2277	10	0.8269	2153	10	0.7979	2912	10	0.7629
2887	10	0.8228	2636	10	0.7970	3278	10	0.7525
3680	10	0.8214	3190	10	0.7960	2990	10	0.7522

^aArea Under the ROC Curve

Table S2*m/z* intervals and AUC values most relevant for *L. major* infection (AUC>0.75)

m/z	± [Da]	AUC	m/z	± [Da]	AUC	m/z	± [Da]	AUC
2561	10	0.8969	2906	10	0.801154	2811	10	0.781189
2804	10	0.8959	2257	10	0.798709	2236	10	0.780786
2477	10	0.8592	2088	10	0.798709	2727	10	0.780786
2434	10	0.8513	3158	10	0.79836	2426	10	0.780688
2250	10	0.8474	4630	10	0.798082	2514	10	0.779397
2482	10	0.8470	2416	10	0.797541	4643	10	0.779397
2508	10	0.8445	2312	10	0.797498	3835	10	0.773445
2558	10	0.8222	3167	10	0.795918	2441	10	0.772291
2639	10	0.8222	3840	10	0.794367	2254	10	0.771714
2487	10	0.8195	2232	10	0.793428	2317	10	0.768434
2326	10	0.8168	2666	10	0.793097	4991	10	0.762937
2642	10	0.8168	2287	10	0.791148	2437	10	0.762092
3799	10	0.8097	2341	10	0.79	2301	10	0.75823
2494	10	0.8085	4759	10	0.789925	2818	10	0.757925
4773	10	0.8085	4977	10	0.784842	2910	10	0.756013
2185	10	0.8018	2190	10	0.783953	-	-	-

^aArea Under the ROC Curve

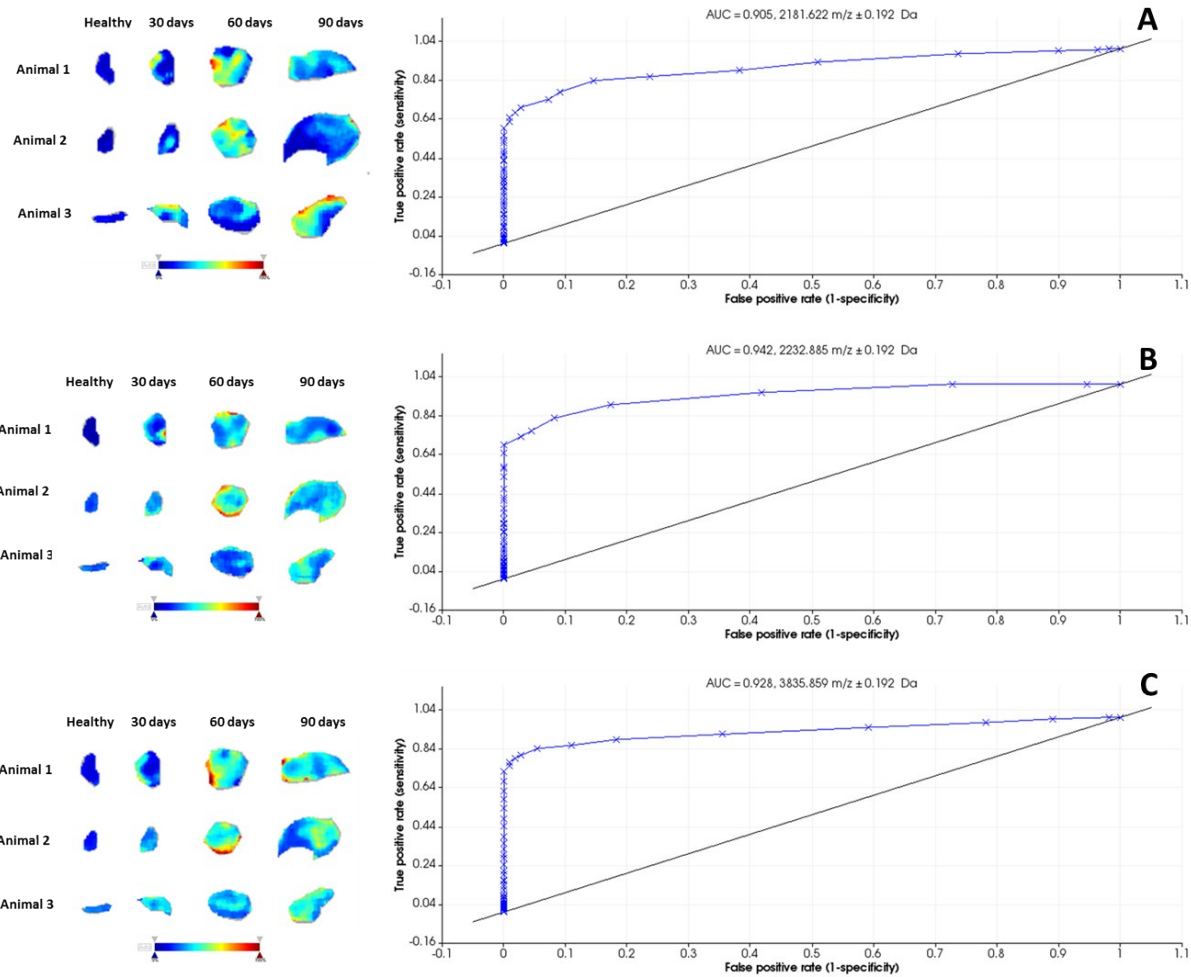


Figure S5: MALDI-IMS for *L. amazonensis* infection progress and the respective Area Under the ROC Curve (AUC) graphs. Graphs are representing the m/z intervals (A) 2181, (B) 2232 and (C) 3835.

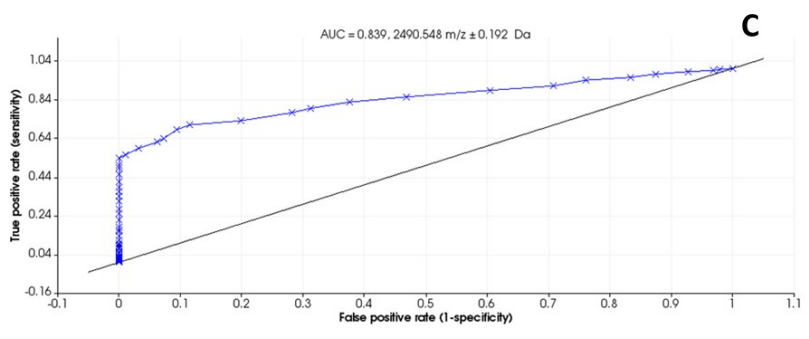
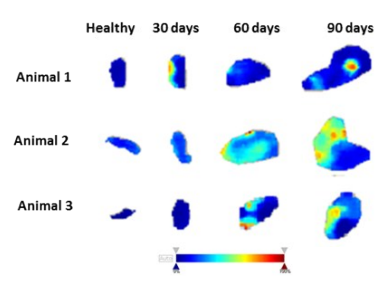
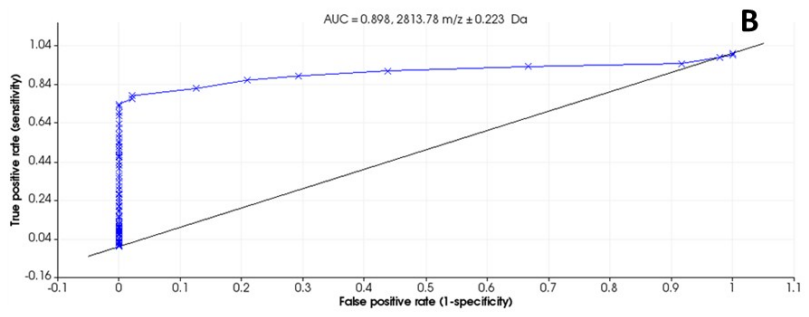
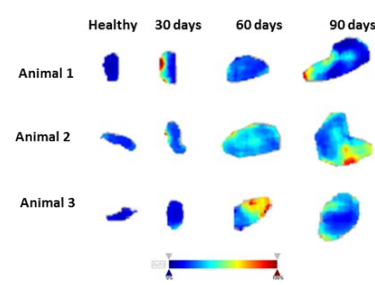
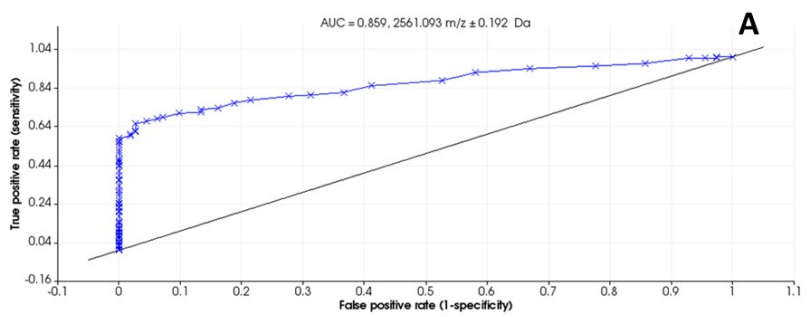
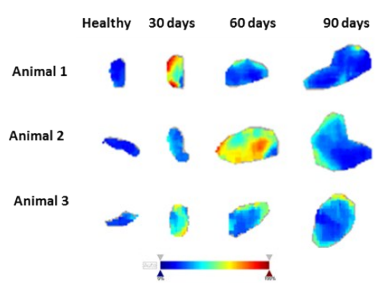


Figure S6: MALDI-IMS for *L. major* infection progress and the respective Area Under the ROC Curve (AUC) graphs. Graphs are representing the *m/z* intervals (A) 2561, (B) 2813 and (C) 2940.

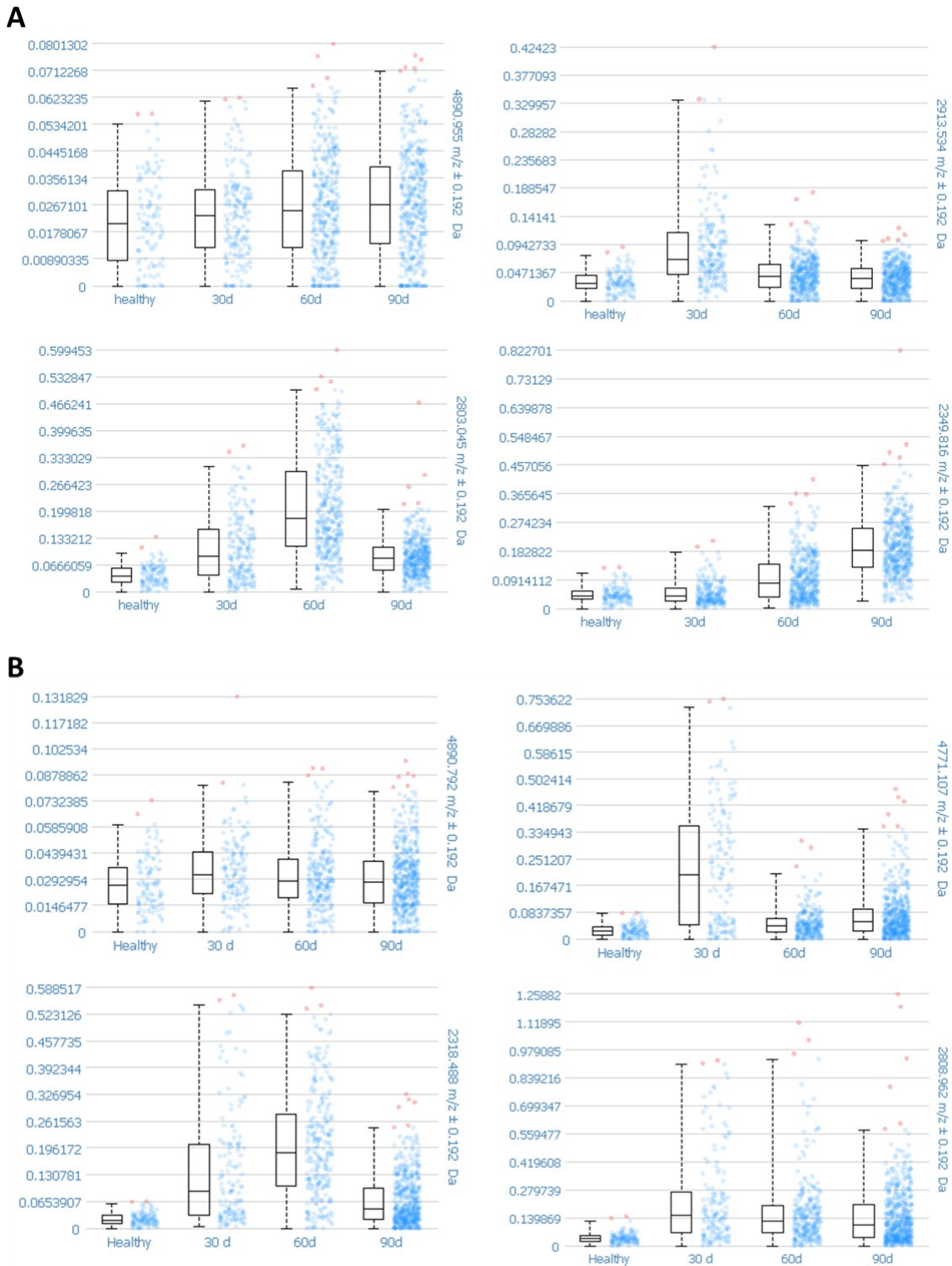


Figure S7: Every box describes a single m/z interval and a single stage of disease progression. Every plot consists of two parts, a box part and a cloud part. The box part represents a median intensity whilst the cloud part shows how spectra of a given tissue section are spread by intensity of a given m/z interval. Blue dots represent the spectra in which intensities of a given m/z interval are between the lower and upper quantiles. Red dots represent outliers. (A) Box plots for *L. amazonensis* disease

progression showing m/z 4890, which is correlated to preserved tissue; m/z 2913 being most intense at 30 days p.i.; m/z 2803 being most intense at 60 days p.i and m/z 2349, which is progressing with the infection. (B) Box plots for *L. major* disease progression showing m/z 4890, which is correlated to preserved tissue; m/z 4771 being most intense at 30 days p.i.; m/z 2318 being most intense at 60 days p.i and m/z 2808, which is progressing with the infection.