Analysis of the metabolome of *Anopheles gambiae* mosquito after exposure to *Mycobacterium ulcerans*

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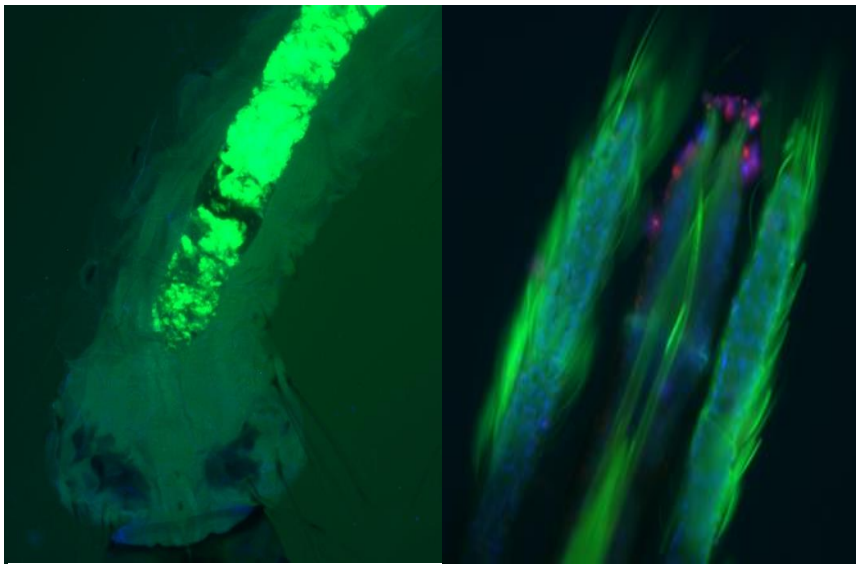
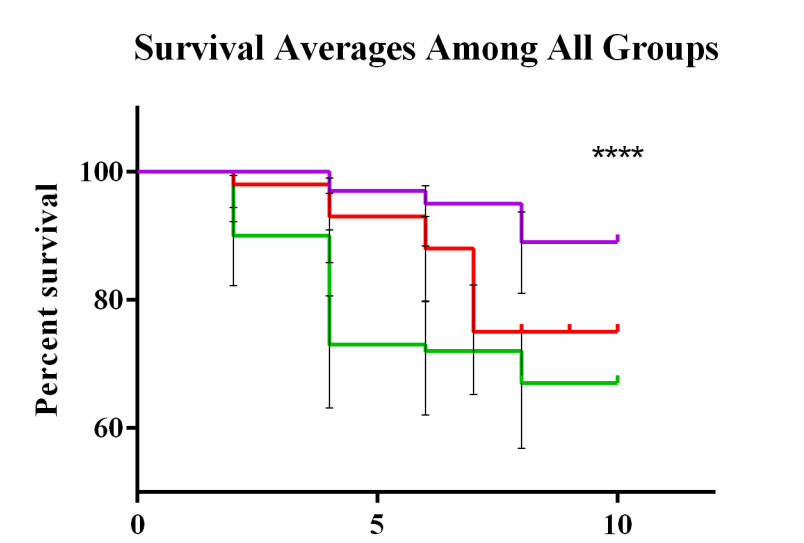
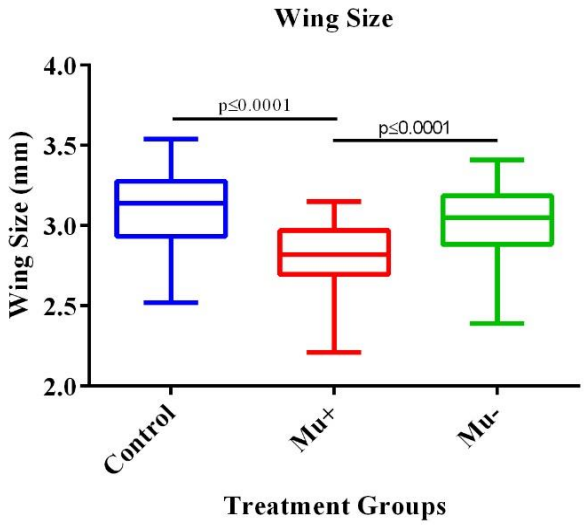
Supplementary Figures and Table for SREP-14-07711



Supplemental Figure S1: Alimentary canal of larval *A. gambiae* mosquito packed with *M. ulcerans* 1615-GFP, viewed under a fluorescent microscope (10x) (Left). Immunofluorescence image of the mosquito proboscis and maxillary palps at 40x magnification. *M. ulcerans* bacilli (labelled in red) contaminated the tip of the labellum (Right).



Supplemental Figure S2: Kaplan-Meier plot of Survival to adulthood for *Anopheles gambiae* mosquitoes from all treatment groups. Significant (****=*P≤0.0001)* trend for survival among all groups and significant difference (****=*P≤0.0001)* in final survival between mosquitoes exposed to live compared to dead *M. ulcerans*. Mosquitoes exposed to dead, ɣ-irradiated *M. ulcerans* (top), control mosquitoes (middle), mosquitoes exposed to live *M. ulcerans* (bottom).



Supplemental Figure S3: Comparison of the wing size of emerged female mosquitoes as a proxy for body size. Adult mosquitoes are significantly smaller than their control counterparts as a result of exposure to live *M.*

Supplementary Table S1

ID[a]	Compound Annotation[b]	KEGG ID[c]	Retention time[d]	m/z[e]	*P*-value[f] Mu+ vs Ctrl	*P*-value[f] Mu- vs Ctrl	ID confidence[g]	Fold Change Control vs Mu+[h]	Fold Change Control vs Mu-[i]
C186	1-hexadecanoyl-sn-glycero-3-PC	C04317	522.7	518.325	0.036	0.012	II	1.660	0.740
C449	ACTH-like	C02017	508.4	468.309	0.009	0.051	II	1.356	1.410
C794	locustachykinin II	C16098	589.5	1065.693	0.417	0.008	II	1.121	1.712
C104	GPCHo (17:1(9Z)/22:5(4Z,7Z,10Z,13Z,16Z))	C05212	520.3	494.324	0.086	0.007	II	1.100	1.400
C100	GPCho (17:1(9Z)/22:5(4Z,7Z,10Z,16Z))	C05212	586.2	508.340	0.066	0.003	II	0.600	0.170
C641	Dodecylbenzenesulfonic acid	n/a	849.2	703.217	<0.001	0.001	II	0.524	0.482
C814	GPEtn 42:3	C04475	858.6	804.557	0.044	0.042	III	2.753	0.483
C355	GPEtn (20:0/20:0)	C04475	737.7	782.579	0.008	0.755	III	2.438	1.067
C426	Lyso-PC 24:0	C04317	575.7	570.355	0.004	0.562	III	2.215	1.114
C686	GPEtn 42:3	C04475	720.4	804.579	<0.001	0.691	III	2.002	1.070
C456	peptide fragment	n/a	556.4	546.284	0.005	0.259	III	1.874	0.807
C465	oxytocin-like	C00746	550.0	955.584	0.012	0.921	III	1.851	0.970
C24	Dodecylbenzenesulfonic acid	n/a	752.6	554.177	0.007	0.710	III	1.830	1.050
C493	Sphingomyelin 40:5	C00550	825.5	786.509	0.027	0.304	III	1.759	0.806
C555	dynorphin-like	C01574	646.2	508.341	0.006	0.049	III	1.592	1.306
C326	GPEtn(7:0/20:4(5E,8E,11E,14E))	C04475	513.6	472.364	0.016	0.042	III	1.550	1.415
C52	Lyso-PC 16:0	C05209	520.0	476.279	0.034	0.068	III	1.523	0.850
C217	Sphingomyelin (d19:1(4E)/26:1(17Z))	C00550	948.0	85.086	0.019	0.201	III	1.420	1.220
C240	Lyso-PC 18:0	C04317	618.5	524.372	0.034	0.844	III	1.400	0.978

C71	GPSer (15:0/25:0)	C18125	768.1	761.609	0.004	0.320	III	1.380	0.920
C183	Lyso-PE 18:1	C05209	586.4	959.613	0.021	0.016	III	1.350	1.570
C575	ACTH-like	C02017	587.9	587.940	0.255	0.037	III	1.309	1.780
C243	2-oleoyl glycerol	n/a	595.1	542.299	0.020	0.308	III	1.300	1.080
C344	ACTH-like	C02017	500.0	468.308	0.012	0.017	III	1.290	1.285
C83	GPEtn (5:0/24:4(5Z,8Z,11Z,14Z)	C04475	558.3	500.395	0.027	0.881	III	1.234	0.986
C335	GPEtn(9:0/18:3(6Z,9Z,12Z))	C04475	545.1	474.379	0.034	0.158	III	1.230	0.856
C245	peptide fragment	n/a	528.1	452.277	0.056	<0.001	III	1.220	1.930
C444	Lyso-PE 29:5	C05209	530.0	498.379	0.130	0.018	III	1.212	0.692
C562	Lyso-PE 20:4	C05209	574.1	361.274	0.230	0.010	III	1.202	0.395
C560	angiotensin-like	C15850	578.8	1031.713	0.018	0.011	III	1.197	1.376
C107	neurotensin-like	n/a	552.3	502.294	0.042	0.148	III	1.150	0.890
C194	Lyso-PE	C05209	543.5	502.294	0.05	0.05	III	1.140	0.840
C123	Lyso-PE 16:1	C05209	536.0	311.259	0.375	0.001	III	1.090	1.800
C315	isoprene	n/a	575.6	975.633	0.133	0.014	III	1.070	1.165
C590	Lyso-PC 20:1	C04317	520.3	266.639	0.397	0.008	III	1.060	1.360
C790	GPEtn 28:6	C04475	574.1	621.303	0.485	0.008	III	1.057	0.512
C712	adrenosterone-like	C05285	546.2	582.300	0.784	0.011	III	1.016	0.749
C22	Lyso-PE 16:1	C05209	517.6	474.260	0.590	0.005	III	0.980	1.610
C120	1-octadecanoyl-sn-glycero-3-PE	C04475	623.8	482.324	0.498	0.017	III	0.964	0.856
C223	Lyso-PE 21:0	C05209	264.2	520.338	0.001	0.097	III	0.951	1.122
C205	Oleic acid-like	C00712	749.0	283.264	0.479	0.044	III	0.943	0.851
C203	GPEtn(17:1(9Z)/17:1(9Z))	C04475	749.1	689.561	0.603	0.022	III	0.922	0.589

C95	1-octadecanoyl-sn-glycero-3-PE	C04475	716.0	357.300	0.558	0.031	III	0.921	0.642
C234	unknown	C11045	708.9	281.248	0.001	0.007	III	0.880	0.830
C180	prostaglandin-like	C00639	697.3	329.248	0.009	<0.001	III	0.870	0.670
C408	GPEtn (20:0/18:2)	C04475	670.1	379.283	0.352	0.001	III	0.867	0.586
C688	pimelic acid-like	C02656	708.8	245.227	0.001	0.023	III	0.850	0.781
C125	Lyso-PC 20:5	C04317	516.8	564.306	0.103	0.002	III	0.840	0.620
C414	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid	n/a	708.6	239.202	0.036	0.047	III	0.829	0.809
C416	GPEtn(15:0/18:2(2E,4E))	C04475	708.7	264.241	0.094	0.006	III	0.820	0.799
C415	3-cyclohexyl-1-propanol	n/a	708.8	83.086	0.019	0.011	III	0.818	0.809
C93	Androstane-like	C03772	669.9	267.212	0.004	0.056	III	0.806	0.836
C322	peptide fragment	n/a	534.3	641.271	0.035	0.240	III	0.796	0.907
C38	histamine-like	C00388	990.5	130.159	0.035	0.042	III	0.730	0.694
C102	Lyso-PE 16:0	C05209	528.0	245.616	0.732	0.027	III	0.730	1.600
C151	Lyso-PC 20:0	C04317	646.5	468.345	0.004	0.240	III	0.710	0.790
C336	Lyso-PE 17:1	C05209	549.6	506.323	0.040	0.004	III	0.680	0.391
C421	GPCho (14:0/17:1(9Z))	C05212	598.9	508.339	0.069	<0.001	III	0.654	0.213
C589	unknown	n/a	521.3	440.277	0.003	<0.001	IV	4.150	2.810
C341	unknown	n/a	484.9	199.133	0.002	<0.001	IV	3.450	2.390
C828	unknown	n/a	781.3	297.279	0.051	0.012	IV	3.392	0.536
C753	unknown	n/a	792.7	738.627	0.003	0.412	IV	2.346	1.181
C321	unknown	n/a	583.6	510.356	0.009	0.032	IV	2.320	1.590
C209	unknown	n/a	807.7	826.541	0.016	0.231	IV	2.260	0.755
C506	unknown	n/a	792.0	800.528	0.045	0.033	IV	2.199	0.765

C591	unknown	n/a	560.4	307.227	0.001	0.003	IV	2.110	1.800
C78	unknown	n/a	650.0	309.243	0.006	0.346	IV	1.917	0.851
C643	unknown	n/a	772.2	760.593	0.031	0.946	IV	1.860	0.985
C795	unknown	n/a	589.5	1001.670	0.119	0.008	IV	1.688	2.027
C482	unknown	n/a	734.1	331.263	0.005	0.982	IV	1.661	1.006
C443	unknown	n/a	530.8	522.379	0.002	0.042	IV	1.600	0.750
C187	unknown	n/a	517.5	452.277	0.298	<0.001	IV	1.570	1.050
C338	unknown	n/a	549.4	509.345	0.007	0.021	IV	1.560	1.490
C703	unknown	n/a	522.9	544.268	0.009	0.944	IV	1.560	1.011
C750	unknown	n/a	763.6	684.581	<0.001	0.053	IV	1.449	1.311
C379	unknown	n/a	774.7	712.611	0.020	0.405	IV	1.394	0.850
C442	unknown	n/a	536.3	544.341	0.001	0.614	IV	1.353	0.921
C390	unknown	n/a	916.8	804.556	0.015	0.480	IV	1.326	0.922
C461	unknown	n/a	540.6	498.379	0.038	0.142	IV	1.259	0.837
C799	unknown	C07443	528.1	253.100	0.186	0.002	IV	1.217	1.958
C195	unknown	n/a	548.1	466.293	0.076	0.018	IV	1.200	2.160
C328	unknown	n/a	520.2	516.306	0.149	0.003	IV	1.180	1.600
C705	unknown	n/a	520.3	517.311	0.545	0.018	IV	1.087	1.410
C807	unknown	n/a	520.2	1014.612	0.336	0.009	IV	1.069	1.773
C711	unknown	n/a	546.1	299.132	0.400	0.045	IV	1.065	0.799
C422	unknown	n/a	633.1	283.263	0.989	<0.001	IV	0.999	2.127
C550	unknown	n/a	708.6	179.143	0.471	0.028	IV	0.971	0.819
C818	unknown	n/a	728.8	308.265	0.651	0.011	IV	0.952	0.692

C709	unknown	n/a	539.7	743.395	0.283	0.026	IV	0.923	0.770
C577	unknown	n/a	536.3	571.290	0.225	0.033	IV	0.910	1.529
C118	unknown	n/a	704.2	235.169	0.367	0.017	IV	0.900	0.600
C447	unknown	n/a	525.6	299.628	0.086	0.011	IV	0.885	0.744
C583	unknown	n/a	516.7	298.626	0.189	0.010	IV	0.850	0.539
C178	unknown	n/a	669.9	171.122	0.024	0.047	IV	0.828	0.753
C568	unknown	n/a	568.3	318.131	0.118	0.014	IV	0.825	0.730
C430	unknown	n/a	568.6	279.232	0.017	0.035	IV	0.798	0.846
C663	unknown	n/a	946.6	304.299	0.017	0.356	IV	0.797	1.120
C366	unknown	n/a	825.0	529.881	<0.001	0.476	IV	0.783	0.958
C704	unknown	n/a	523.3	270.120	0.006	0.006	IV	0.760	0.851
C227	unknown	n/a	360.3	98.512	0.046	0.003	IV	0.743	0.770
C548	unknown	n/a	708.5	139.112	0.001	0.010	IV	0.727	0.663
C177	unknown	n/a	356.4	98.512	0.035	0.111	IV	0.710	0.790
C593	unknown	n/a	554.9	530.320	0.103	0.007	IV	0.670	0.279
C578	unknown	n/a	534.3	424.324	0.044	0.015	IV	0.488	0.275
C528	unknown	n/a	24.6	173.021	<0.001	0.228	IV	0.463	0.784
C699	unknown	n/a	517.6	398.327	0.033	0.047	IV	0.454	0.485

Supplementary Table S1 key a) Compound identifier. b) identification of compound based on match of mass spectra, m/z ratio, and/or retention time to available databases. c) compound identifier from Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg/) d) retention time (seconds) generated via liquid chromatography. e) mass to charge ratio of the base peak from the mass spectra. f) T-test p-value of pairwise comparison between specified treatment and control group. g) confidence level of identification based on matching of chromatographic and mass spectral characteristics for each compound to validated compound library or publicly available databases, from Sumner et al. 2007. Metabolomics. h/i) fold change in abundance of compound compared between control and treatment groups. Abbreviations: PC: phosphocholine; GPCho: glycerophosphocholine; GPEtn: glycerophosphoethanolamine; GPSer: glycerophosphoserine; ACTH: adrenocorticotropic hormone; X:N represents number of carbons to double bonds in a given compound.