

Supporting Information

Microbiome-Gut-Brain-Axis communications regulating metabolic switch in the mosquito *Anopheles culicifacies*

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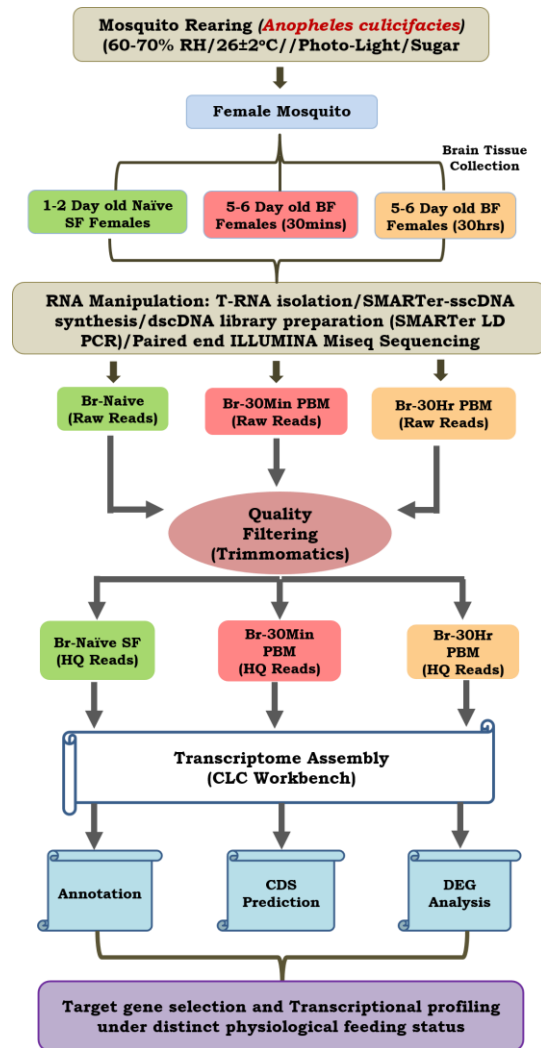


Fig. S1: A technical overview to decode the hard-wired genetic structure of brain system of *Anopheles culicifacies* mosquito.

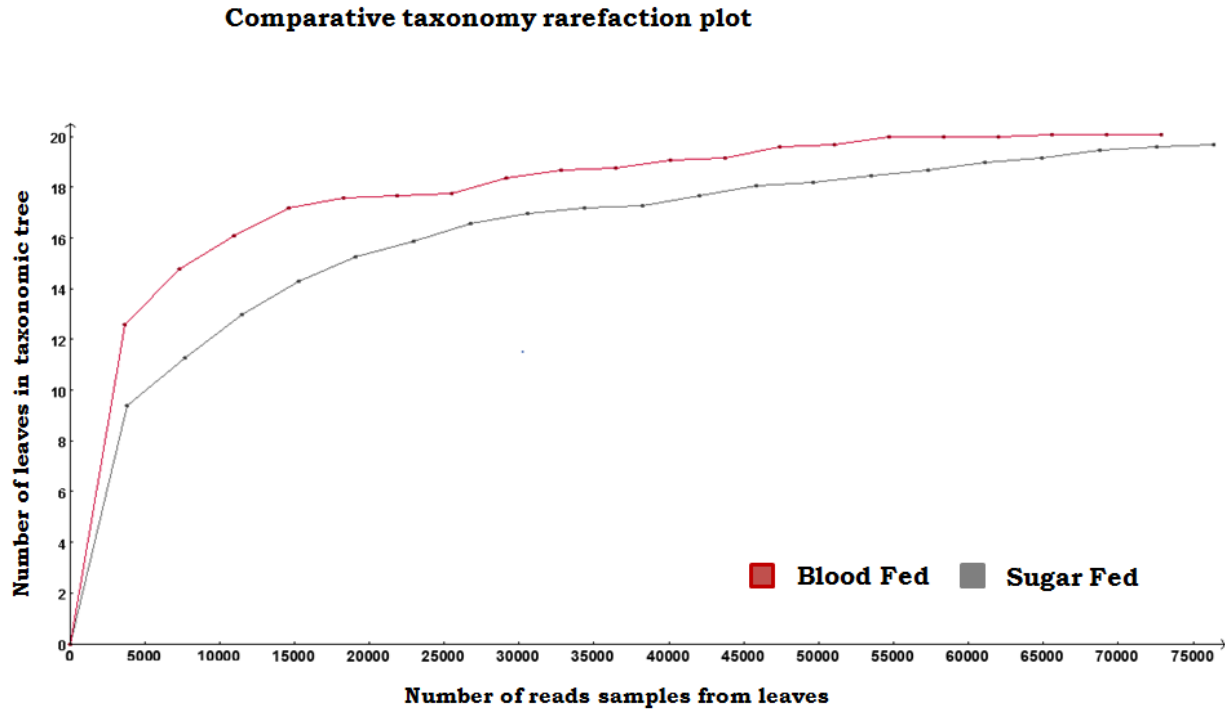


Figure S2: Rarefaction curve plot showing alpha diversity for the sugar fed (Gray line) and blood fed (Red line) at family level

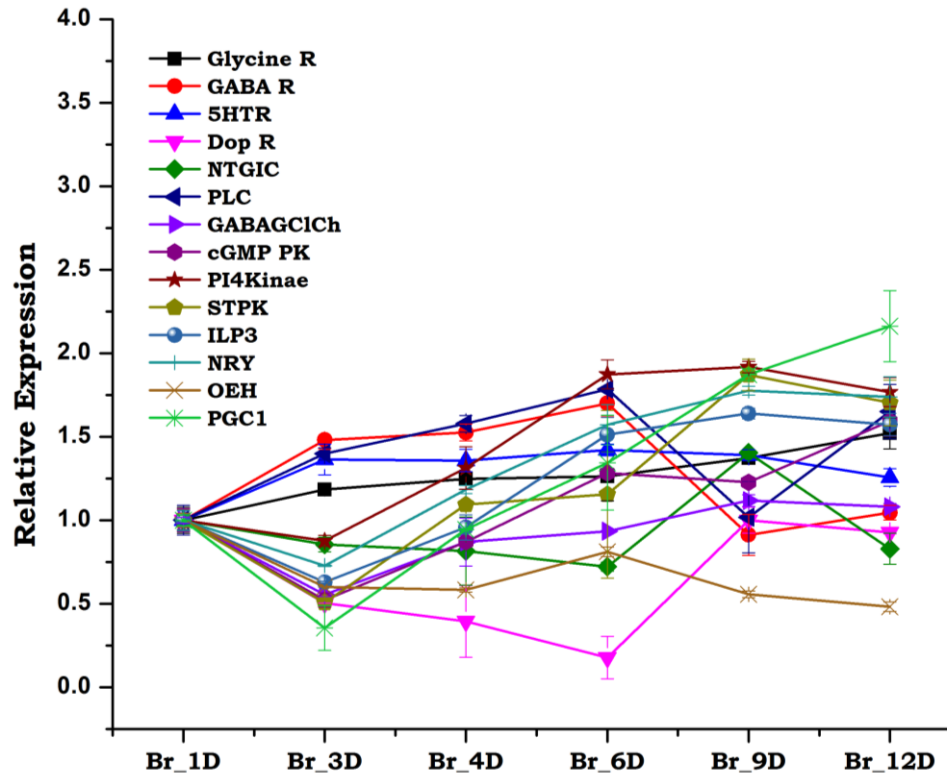


Fig. S3: Differential gene expression analysis in aging non-blood fed female mosquito brain. No significant modulation of neuronal genes was observed when compared to 0-1-day old teneral mosquitoes with 12 day-old non-blood fed mosquitoes. Statistical analysis using two-way ANOVA followed by Tukey Test has implied at 0.05 level, the expression pattern of the respective genes was not statistically significant in aging mosquitoes ($n = 25$, $N = 3$).

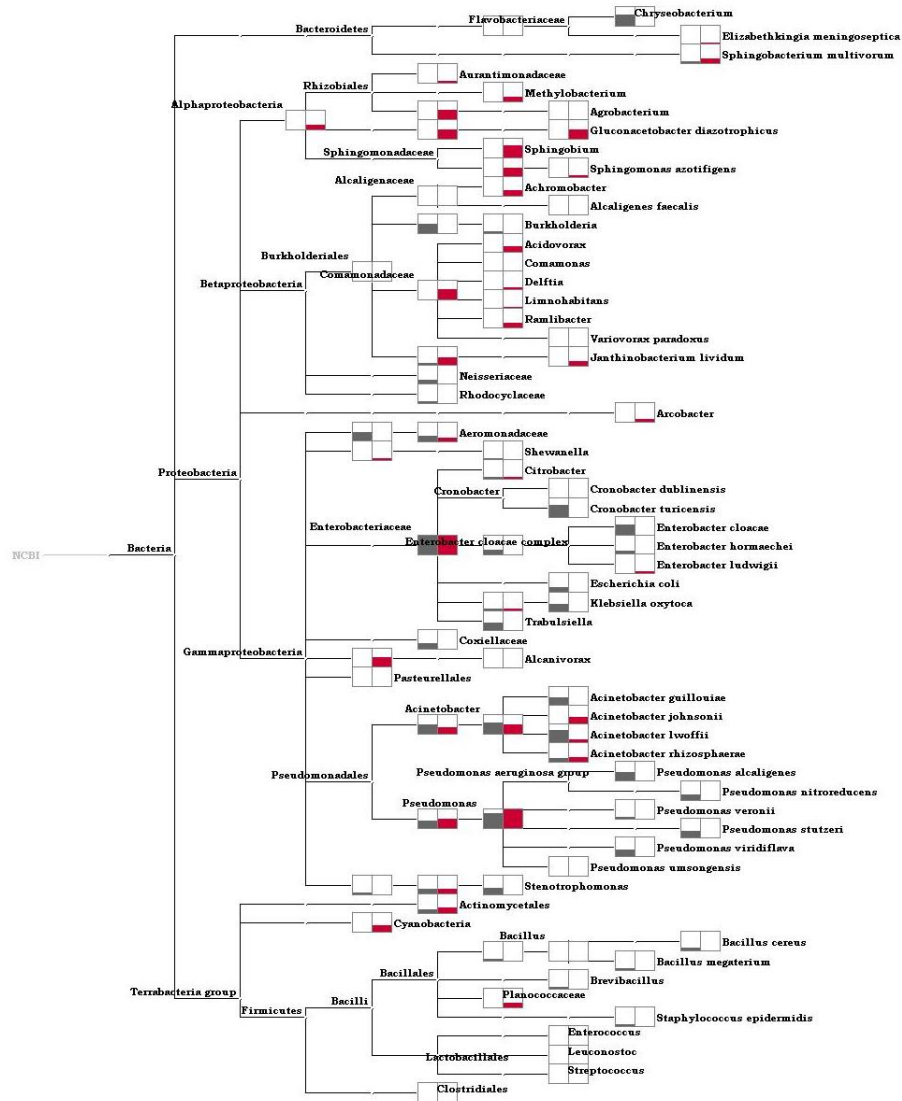


Figure S4a: Heat map of the comparative sugar fed (Gray bar) and blood fed (Red bar) midgut metagenomic data showing values at log scale for species level

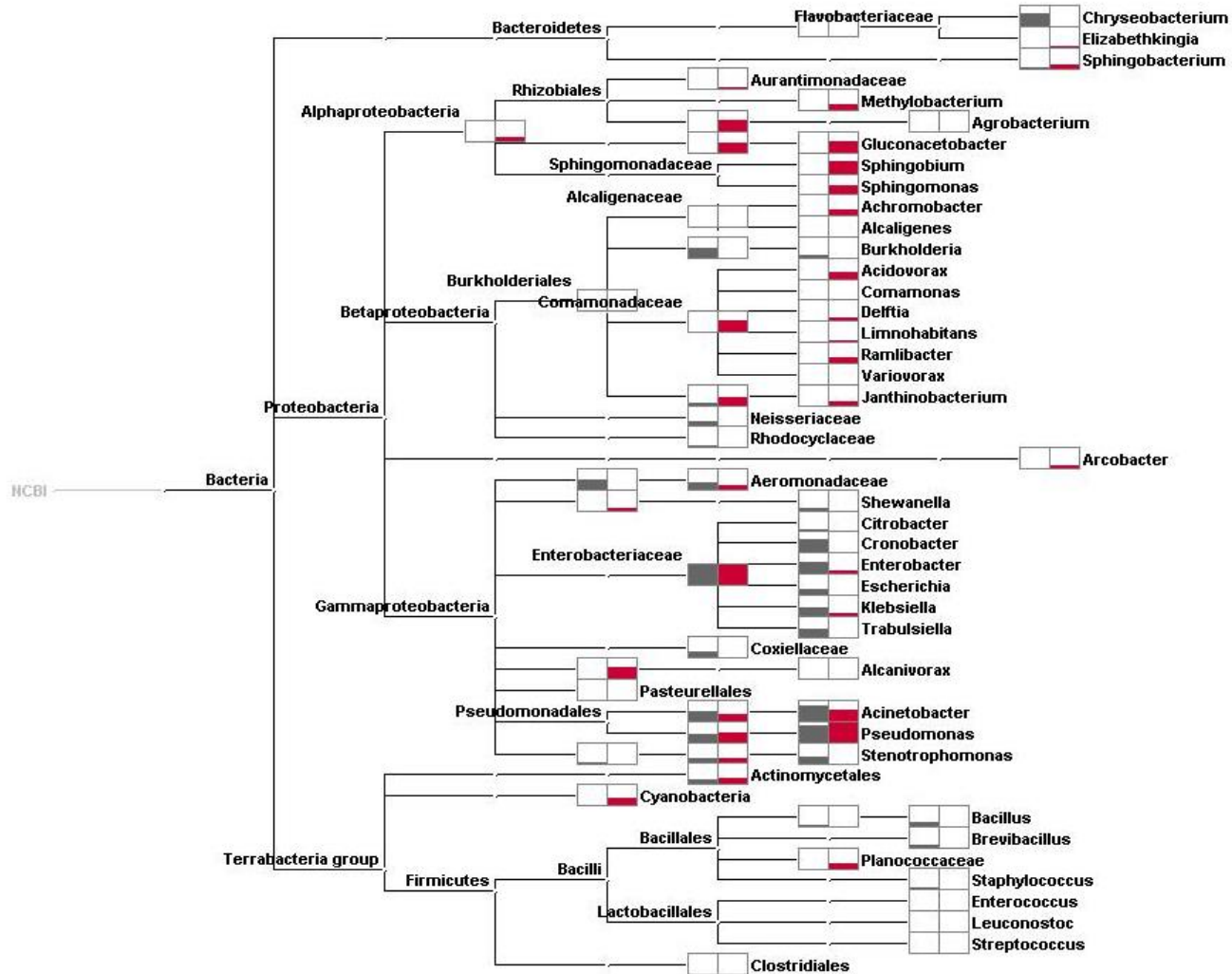


Figure S4b: Heat map of the comparative sugar fed (Gray bar) and blood fed (Red bar) midgut metagenomic data showing values at log scale for genus level

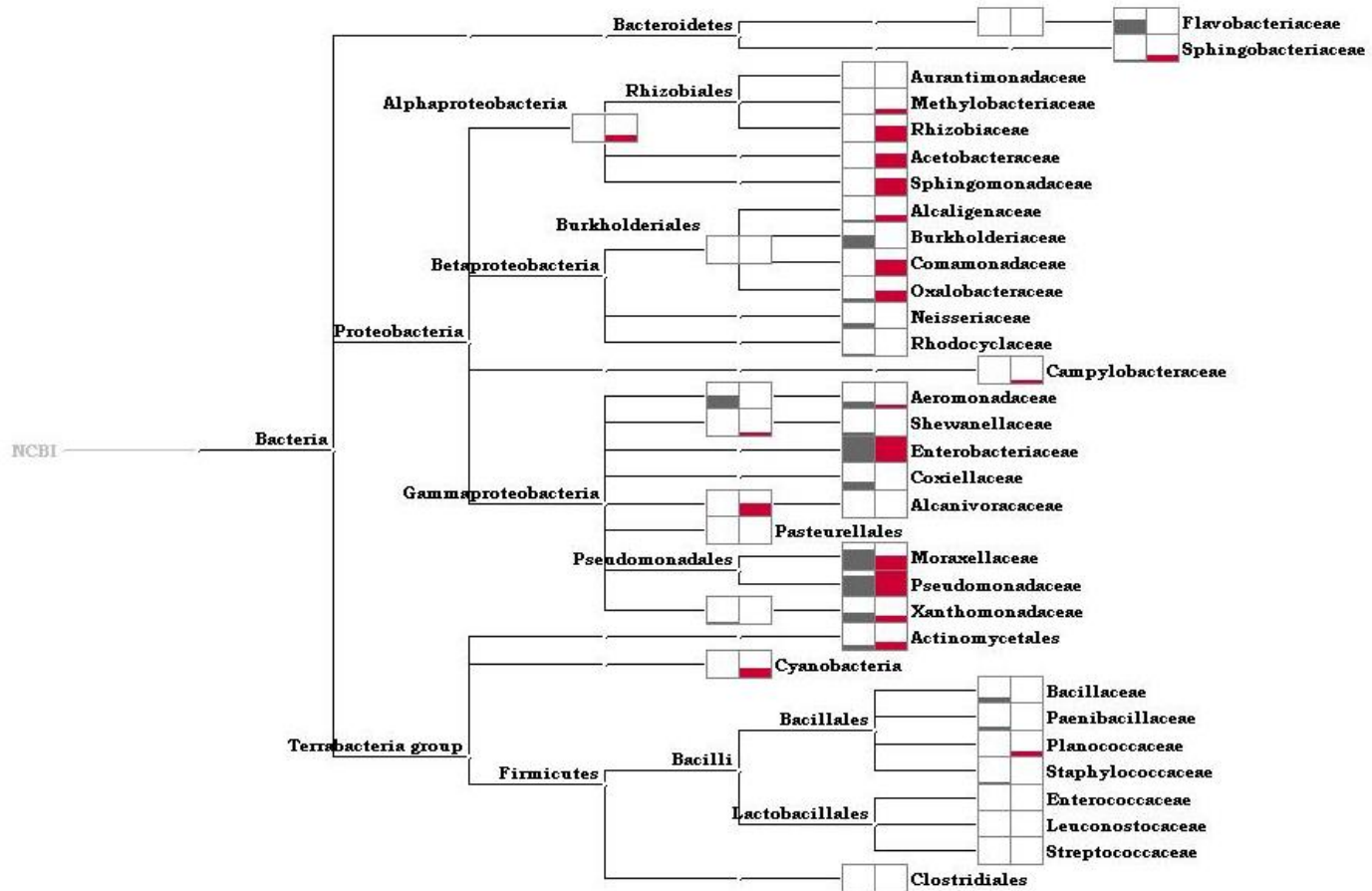
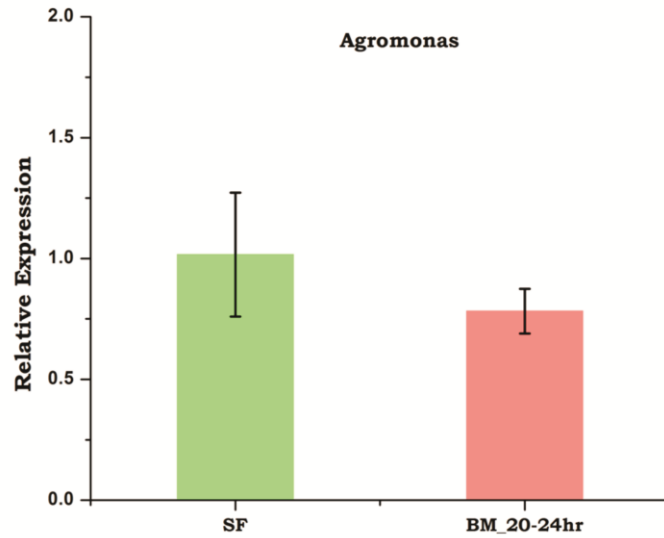


Figure S4c: Heat map of the comparative sugar fed (Gray bar) and blood fed (Red bar) midgut metagenomic data showing values at log scale for family level

(a)



(b)

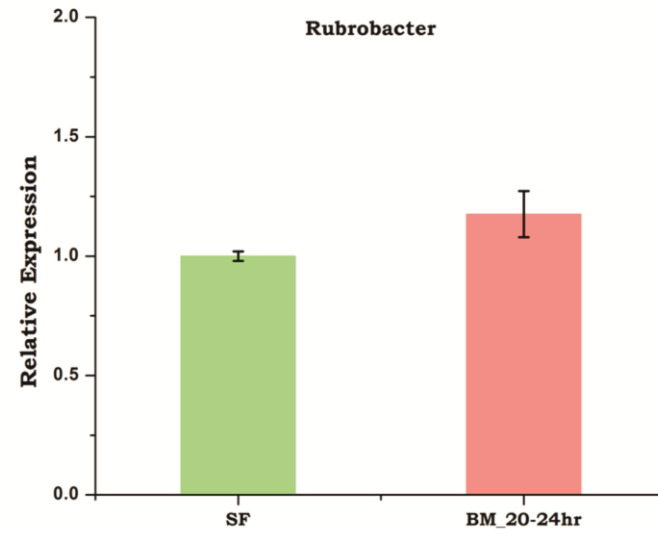


Figure S5: Relative expression of *Agromonas* and *Rubrobacter* (gram positive bacteria) at Sugar fed and 20-24hr Post blood fed condition shows no significant change during the two feeding status

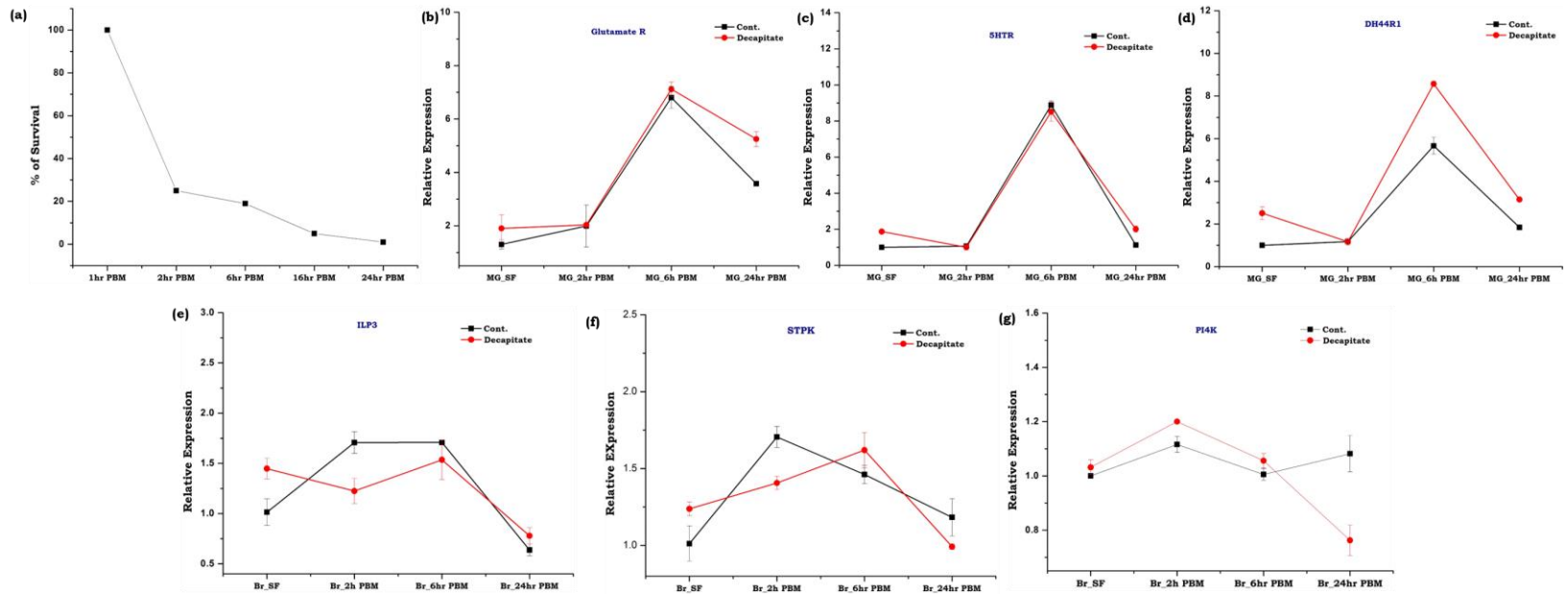


Fig. S6: Transcriptional Response of neuromodulator receptor genes in naïve and decapitated blood-fed female *An. culicifacies* mosquitoes. (a) Percentage of mosquitoes that survived till 24 h after decapitation which was performed after 1h of blood-feeding. 5-6 days old sugar-fed mosquitoes were provided blood meal and mosquitoes heads were decapitated after 1 h. from the full-fed gravid females. After that, the decapitated mosquitoes were kept in a cage for recovery and count the live (mosquitoes that vibrate/move their legs or other body parts are considered as live) and dead mosquitoes (non-movable mosquitoes with visible shrinkage of the body parts at the respective time points are considered as dead) at different time points until we observed 100% mortality. The percentage of survival was calculated until 24h after blood feeding. (b-d) Relative gene expression analysis of neuromodulator receptor genes in the gut of blood-fed and decapitated female mosquitoes. (e-g) Relative gene expression analysis of neuromodulator receptor genes in the gut of blood-fed and decapitated female mosquitoes. Statistical analysis using two-way ANOVA implied that at 0.05 level the expression level the respective genes in control and decapitated females are not statistically different.

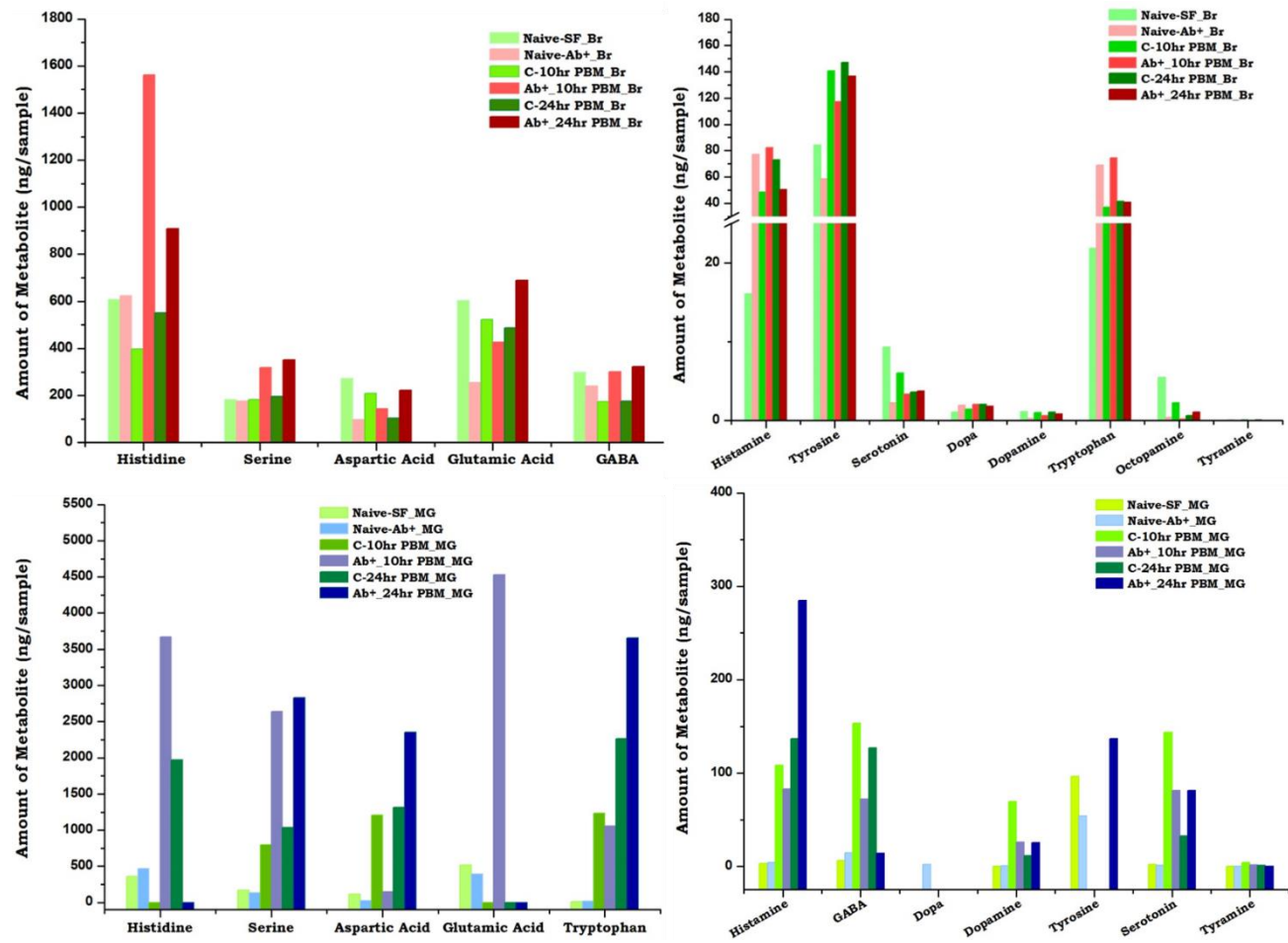


Fig. S7: Neurotransmitters dynamics of naive and aseptically reared mosquitoes collected from sugar fed and blood fed conditions

Table S1: List of Primer Sequence used in the study

Sl. No.	Primer Name and Sequence
1.	Actin_Fw: 5' TGCCTGACATCAAGGAGAAG 3' Actin_Rev: 5' GATTCCATACCCAGGAACGA 3'
2.	Ac_PyruvateKinase_Fw: 5' CGCACTTGATCTCCAAGTAT 3' Ac_PyruvateKinase_Rev: 5' TTCCAGCCAGTAACAACAA 3'
3.	Ac_Solute Carrier 7_Fw: 5' TCAATAGCTCCGAAATCAGT 3' Ac_Solute Carrier 7_Rev: 5' TGATAACGAACAGCAAGACA 3'
4.	Ac_AATransporter_Fw: 5' CAATGCCTATGGTTACAGGT 3' Ac_AATransporter_Rev: 5' GCTGGTAAGTGTCCTTCTTG 3'
5.	Ac_TrehalaseTransporter_Fw: 5' CGATGGGACTGTA CT TCTTC 3' Ac_TrehalaseTransporter_Rev: 5' GTCTAGATCGGCGAAAAAC 3'
6.	Ac_PGC1_Fw: 5' ACCTTACGGTAAAATCGTCA 3' Ac_PGC1_Rev: 5' GTACGGTAGCTGATGTTCGT 3'
7.	Ac_OxoglutarateDHS_Fw: 5' GCAACTACTTCCATCTGCTC 3' Ac_OxoglutarateDHS_Rev: 5' GAGCCTTCAACAAGTCGTAA 3'
8.	cGMP PK_Fw: 5' GCGTTTGATTATCTGCACTC 3' cGMP PK_Rev: 5' AAGGACTCCAAGTGACCAGT 3'

9.	GlutamateR_Fw: 5'AGTGGTATCAACGCAGAGTG 3' GlutamateR_Rev: 5' GAGTTTAAGCACTGCTCCAC 3'
10.	Glycine R_Fw: 5' GATACTGCCACTACCTCGTC 3' Glycine R_Rev: 5' CTTGGAGACCGAATTGAATC 3'
11.	GABA R_Fw: 5' CAGAACGAAGAAGGCTACTC 3' GABA R_Rev: 5' AGTATCCACGCATACTCAGC 3'
12.	ARMAA_Decarboxylase_Fw: 5'GGTAACCAAGTCCTTCAGTG 3' ARMAA_Decarboxylase_Rev: 5' TAGAACAGACGACCTCGAAC 3'
13.	ILP1_Fw: TCCACTACATGGAAAACCTCC ILP1_Rev: GTCATCAGTGCCTGGTAGAT
14.	ILP3_Fw: TAGCAATGATGAGTGGATGA ILP3_Rev: ACAACACTCCTCTACGATGC
15.	Leukokinin_Fw: AAACATCGCATAGCAGAGAT Leukokinin_rev: TCAGATAATCCTGCACCATA
16.	NRY_Fw: TACTGTACGGCTGGTTGAAT NRY_Rev: TTAGTTCCGGCAGTGTTT
17.	OEH_Fw: GACAAGAATGCGGTGATAAT OEH_Rev: CGTTGCTGTAGTAATCGAAG
18.	DH44R1_Fw: CTCGAAATAGAATGCTCCTG DH44R1_Rev: AGATGACGATGAGGTAGGTG

19.	LKR_Fw: AAGAGGGAACACGACAAAC LKR_Rev: GCTCGATATAATTGGTGGTC
20.	DH44_Fw: AACGAACAGGAAGATCTCAA DH44_Rev: ATACCGTAGACGTACCGTGA
21.	CCHAR2_Fw: CCACTCCGAAAACACTACAGAC CCHAR2_Rev: GTGGCAGGAAGTAGTAAACG
22.	V-Type ATPase_Fw: TTACATGTACACCGATTTGG V-Type ATPase_Rev: GACTTCATCAGACGTGACAG
23.	DopR_Fw:5' GTTATGGGCGTGTTTATTGT 3' DopR_Rev:5' GCTGGTACTTGCGTCTTATC 3'
24.	AKT Kinase_Fw: GATGGAGGAGGTAAAGTTCC AKT Kinase_Rev: GAACTCACGGTCGAAGTAAC
25.	CYP314A1_Fw: GAGATTGCGCAAGAATTTAG CYP314A1_Rev: GGAAGTTGTCCTCACTCTGA
26.	PTTH_Fw: CTTCACCTCTGAATTGCTTC PTTH_Rev: ACAAGAAGACGGGTTACTGTG
27.	KDNaCaExchanger_Fw: GTGAGATGGGTATCAGCAAC KDNaCaExchanger_Rev: CTTCCAATCAAGTTTGAAGC

28.	GABA ClCh_Fw: 5' GGAAGGTGTTTGGTAAGTCA 3' GABA ClCh_Rev: 5' GGTGATCGTGTTTCGAGTAAT 3'
29.	PLC_Fw: 5' TGGATTCGTCCAACACTATCAT 3' PLC_Rev: 5' TTCACGATCACCTCGTTC 3'
30.	PI-4Kinase_Fw: 5' ACATCATCTCCTCACTGTCC 3' PI-4Kinase_Rev: 5'GTGTGCCACTGTTGTAATCA 3'
31.	ST ProteinKinase_Fw: 5'TTTATAGTGCCGTGTGTTGA 3' ST ProteinKinase_Rev: 5'CTTAATGTGGAACCGATCAT 3'
32.	Trehalase_Fw: 5' GAAGAGGACAAACAGGACTA 3' Trehalase_Rev: 5' GTTCCGGTAACCATAGAAC 3'
33.	5-HT Receptor_Fw: 5' ATGATCTCGCGTAACTCCTC 3' 5-HT Receptor_Rev: 5' ATCGGATTGACCAGACTGC 3'
34.	TOR_Fw: GTAGAATGTTGGTGGTCGAT TOR_Rev: ACCATCTGCTAGGTTATTGC
35.	Octopamine Receptor_Fw: 5'CTACTGGCGGATCTATCGGG 3' Octopamine Receptor_Rev: 5' TGGTGGAAAGGCTGTGTTTTG 3'
36.	Calcitonin R_Fw: AATAGAATGCTGGATGAACG Calcitonin R_Rev: GGACGAAACGGTGTAAGTAT

37.	NTGated IonCh_Fw: 5' ACGTTTCGAAAGTCAAACAC 3' NTGated IonCh_Rev: 5' GCTGTAGAATGCACAAATGA 3'
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Table S2: Comparative alpha diversity indices estimation of gut-bacterial population of naïve sugar fed and blood fed mosquito *An. culicifacies*

Sample	Taxonomy Rank: Class	
	Shannon index	Simpson index
Ac_SF_MG	0.088	1.019
Ac_BF_MG	0.225	1.063

Table S3a: Annotation kinetics of RNA-Seq data

Molecular Features	Ac-Br-Naive	Ac-Br-30M PBM	Ac-Br-30Hr PBM
Total No. of Raw Reads	5268211	3947521	3760078
Total No. of Contigs	32118	32984	38512
Total Transcripts	9460	9146	7387
Total BLASTx hits (NR)	8,668 (~91%)	8,336 (~91%)	6,548 (~88%)
Transcripts with GO Match			
Molecular Function	4773	4556	3575
Biological process	4446	4299	3381
Cellular component	2523	2424	1888

Table S3b: Percentage of differentially expressed transcripts

Sample	No. of Transcripts	Transcripts showing Differential gene Expression (DGE)	Upregulated Transcripts	Downregulated Transcripts	Percentage of Transcripts showing DGE
Ac_Br_naive vs Ac_Br_30min	(9460 + 9146) = 18606	Total - 4747 Significant – 3183 Not significant - 1564	622 (3%)	2110 (11%)	14% CDS show differential expression
Ac_Br_Naive vs Ac_Br_30hr	(9460 + 7387) = 16847	Total -3966 Significant – 3174 Not significant - 792	482 (2%)	2469 (14%)	16 % show differential expression

Table S4: Quantitative estimation of 13 different neurotransmitters in the brain and the gut of mosquitoes under different physiological conditions.

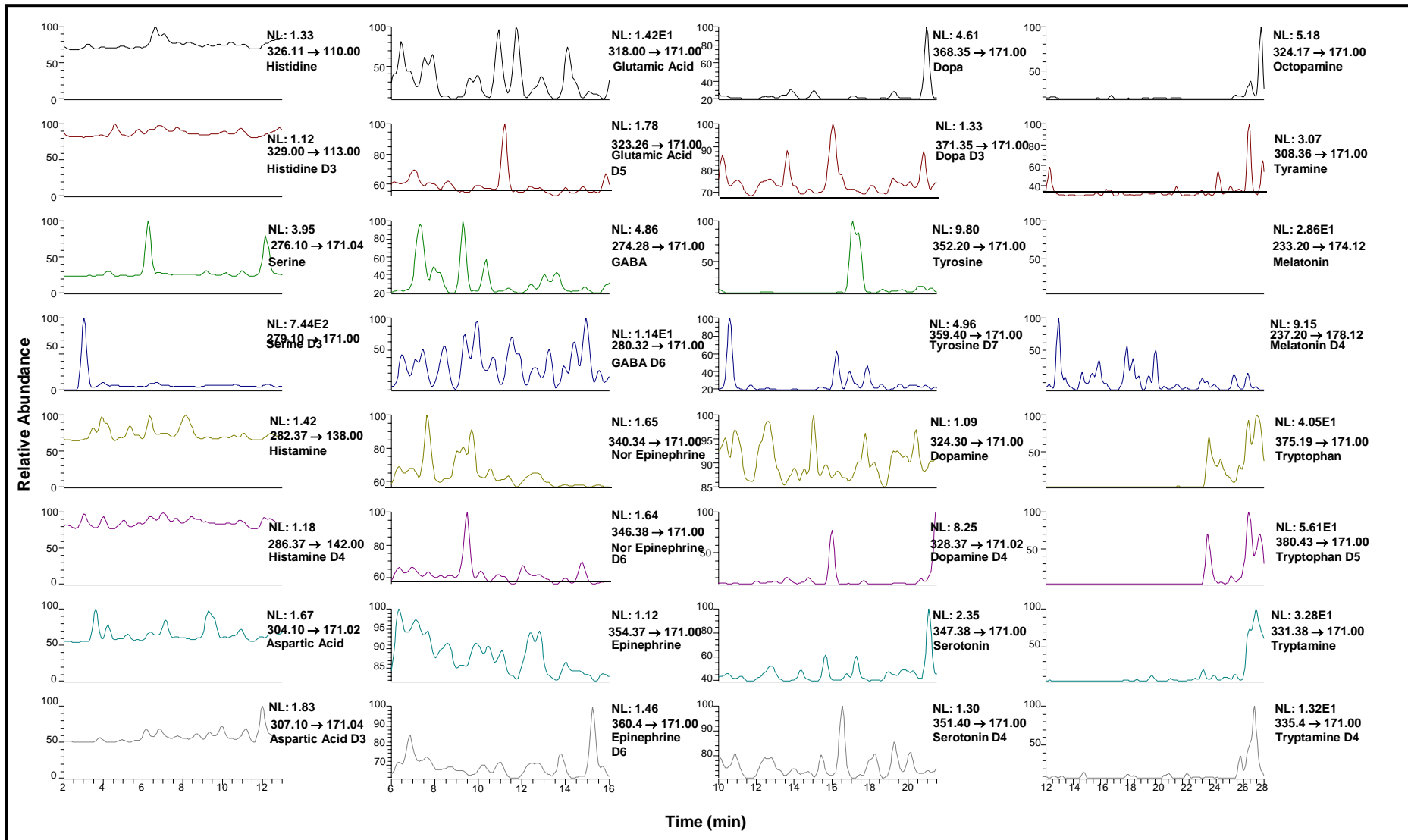
Name of NT	Control SF_Br	Ab+ SF BR	Control SF_MG	Ab+ SF _MG	10hr PBM_Br	Ab+ 10hr PBM_Br	10hr PBM_MG	Ab+ 10hr PBM_MG	24hr PBM_Br	Ab+ 24hr PBM_Br	24hr PBM_MG	Ab+ 24hr PBM_MG
Histidine	607.77	622.52	356.52	469.70	397.58	1562.75	DC	3672.30	550.28	908.02	1968.73	DC
Serine	182.75	176.07	167.62	134.24	182.04	318.04	798.51	2636.08	197.18	350.75	1034.20	2830.31
Histamine	16.08	76.89	3.35	3.83	48.28	82.21	108.12	83.16	73.12	50.42	136.69	284.97
Aspartic Acid	272.35	96.74	115.69	24.02	209.89	143.05	1205.17	146.59	102.76	222.80	1312.08	2348.14
Glutamic Acid	604.78	255.68	513.97	397.82	523.25	425.24	DC	4530.44	486.92	690.13	DC	DC
GABA	300.01	240.89	6.38	14.50	174.40	300.76	153.31	72.01	175.86	321.81	126.79	13.94
Dopa	1.06	1.94	DC	2.00	1.45	2.04	DC	DC	2.08	1.84	DC	DC
Octopamine	5.49	0.39	BLQ	BLQ	2.29	0.20	NF	DC	0.65	1.11	NF	NF
Tyrosine	84.26	58.49	96.41	53.93	140.86	117.51	DC	DC	147.28	136.63	DC	136.63
Dopamine	1.12	0.28	0.13	0.46	1.00	0.62	69.17	26.02	1.06	0.83	11.59	25.18
Serotonin	9.33	2.25	1.92	1.35	6.06	3.39	143.62	81.14	3.59	3.72	32.70	81.37
Tyramine	0.08	BLQ	0.05	0.06	0.10	0.05	4.06	1.73	0.07	0.05	0.90	0.22
Tryptophan	21.84	68.84	11.59	16.44	36.93	74.66	1230.41	1054.85	41.40	40.70	2263.10	3650.53

* BLQ= Below Limit of Quantitation

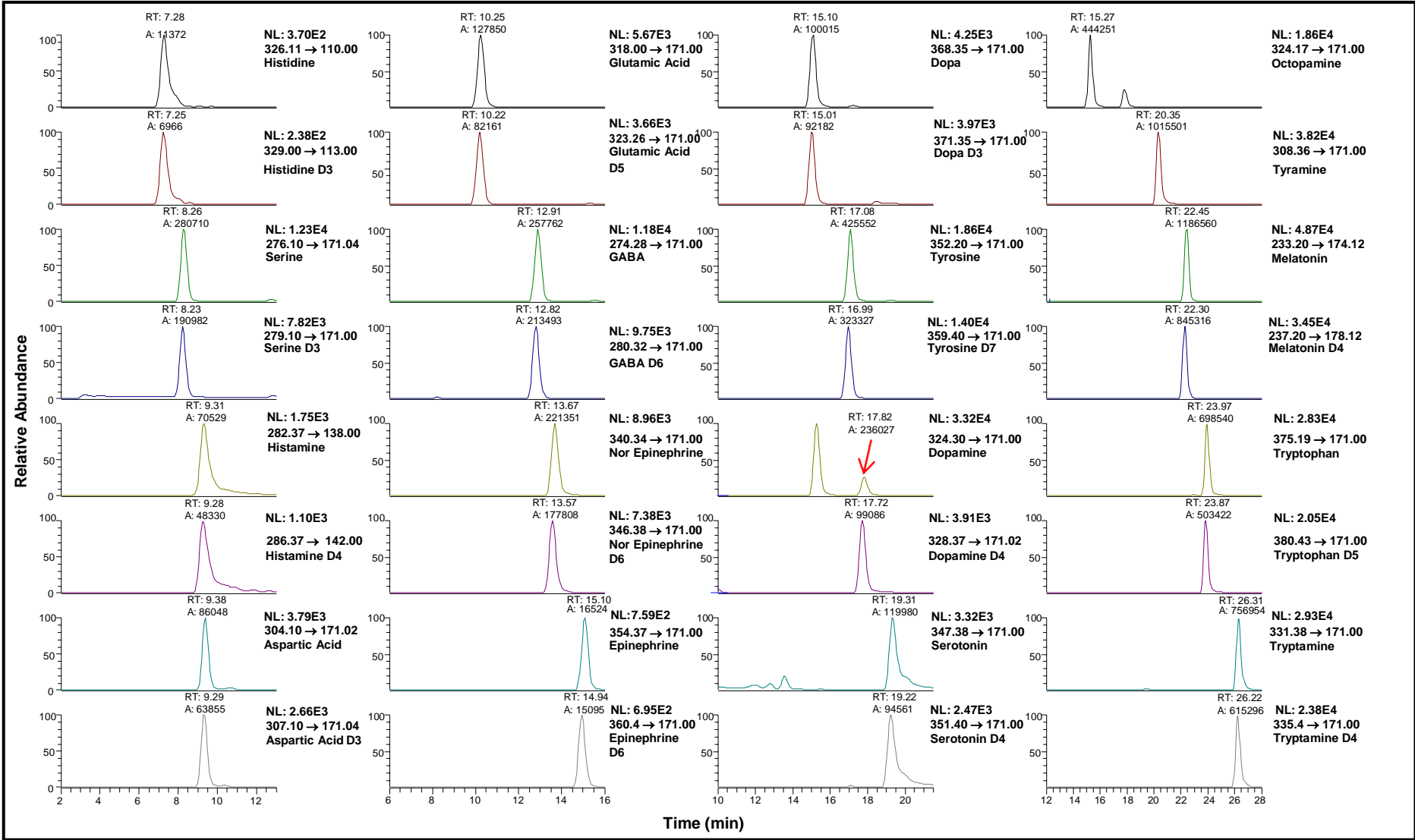
NF= Not Found

DC= Detected but not calculated due to the highly suppressed Internal Standard signal

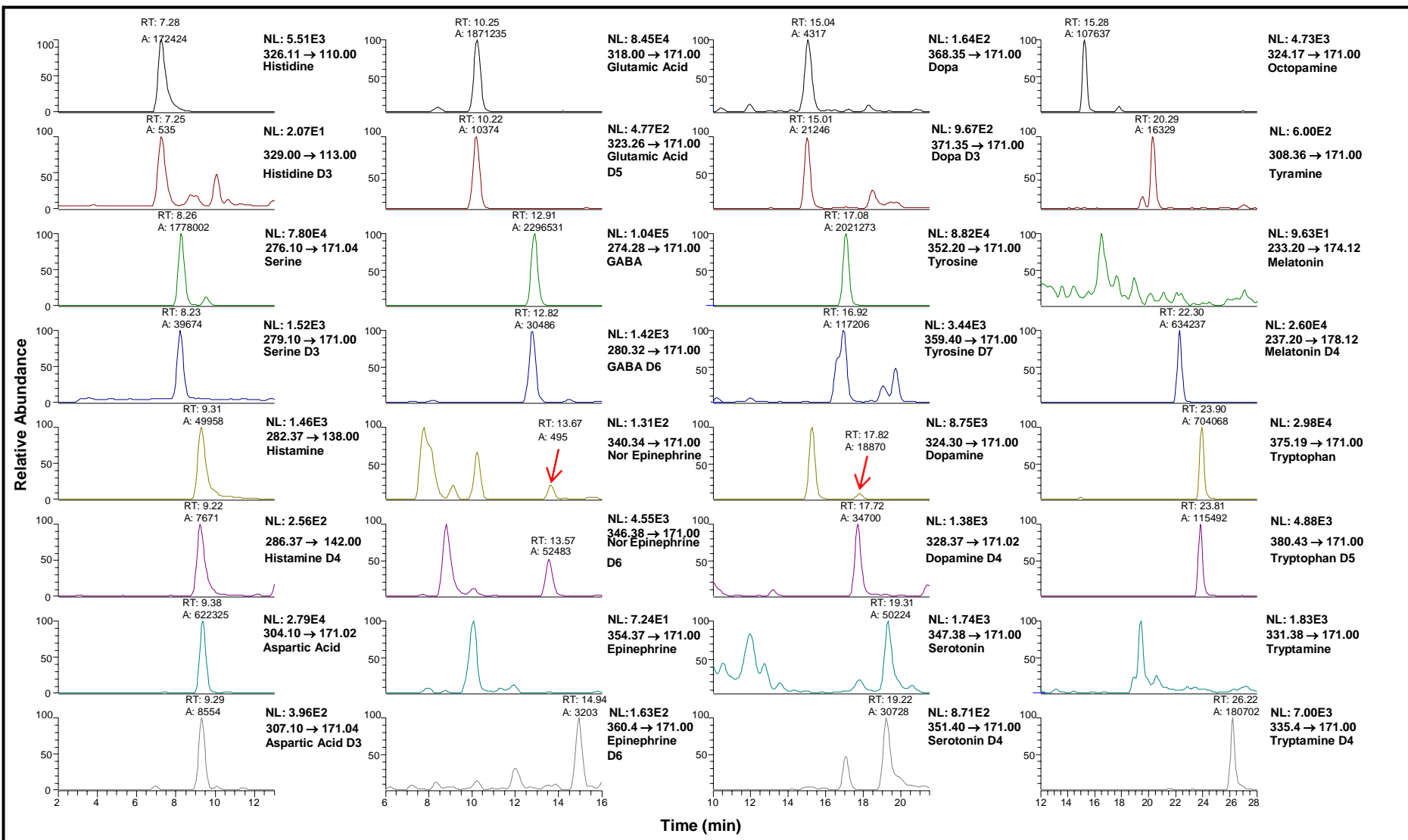
Representative UHPLC-MS/SRM chromatogram of Blank:



Representative UHPLC-MS/SRM chromatogram of Standard:



Representative UHPLC-MS/SRM chromatogram of sample Ac_SF_Br:



Representative UHPLC-MS/SRM chromatogram of sample Ac_SF_MG:

