

Supplementary Tables and Figures for:

Blood RNA sequencing indicates upregulated *BATF2* and *LY6E* and downregulated *ISG15* and *MT2A* expression in children with autism spectrum disorder

Irena Voinsky, Yazeed Zoabi, Noam Shomron, Moria Harel, Hanoach Cassuto, Joseph Tam, Shannon Rose, Adrienne C. Scheck, Mohammad A. Karim, Richard E. Frye, Adi Aran, and David Gurwitz

Supplementary Table S1: Real-time qPCR primers used for real-time qPCR.

<i>Gene</i>	<i>Forward</i>	<i>Reverse</i>
<i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
<i>SERPING1</i>	CCTTGTCCTCCTCAATGCTATC	CCACAGGGTACTTCTTGCTATT
<i>EFHC2</i>	ATGGCCGGACATTCAAGATTT	GGCTCTACGTGTTCTACAACCT
<i>BATF2</i>	CCTCATTGCTCTTTGGACTAGG	TGTGTGAAACCTGGGTGAAG
<i>CDC20</i>	AGACCTGCCGTTACATTCCTTC	GCCAGTACATTCCCAGAACTCC
<i>FCGR1A</i>	CGCTACACATCAGCAGGAATA	GCCCATGTAGAAGGAGAAGTAAA
<i>MT2A</i>	CCGCTCCCAGATGTAAAGAA	CACGGTCAGGGTTGTACATAA
<i>ISG15</i>	CGCAGATCACCCAGAAGATCG	TTCGTCGCATTTGTCCACCA
<i>FBXO6</i>	ATCCTACGAAATGTGCCTCAAG	CCAACACGAAGTAGTCAGCCG
<i>LINC00869</i>	CATCCAAAGCTACTGCCTACTC	CACCTCTCCCTTCCTGTATCT
<i>LY6E</i>	GGGAATCTCGTGACATTTGGC	ACACCAACATTGACGCCTTCT

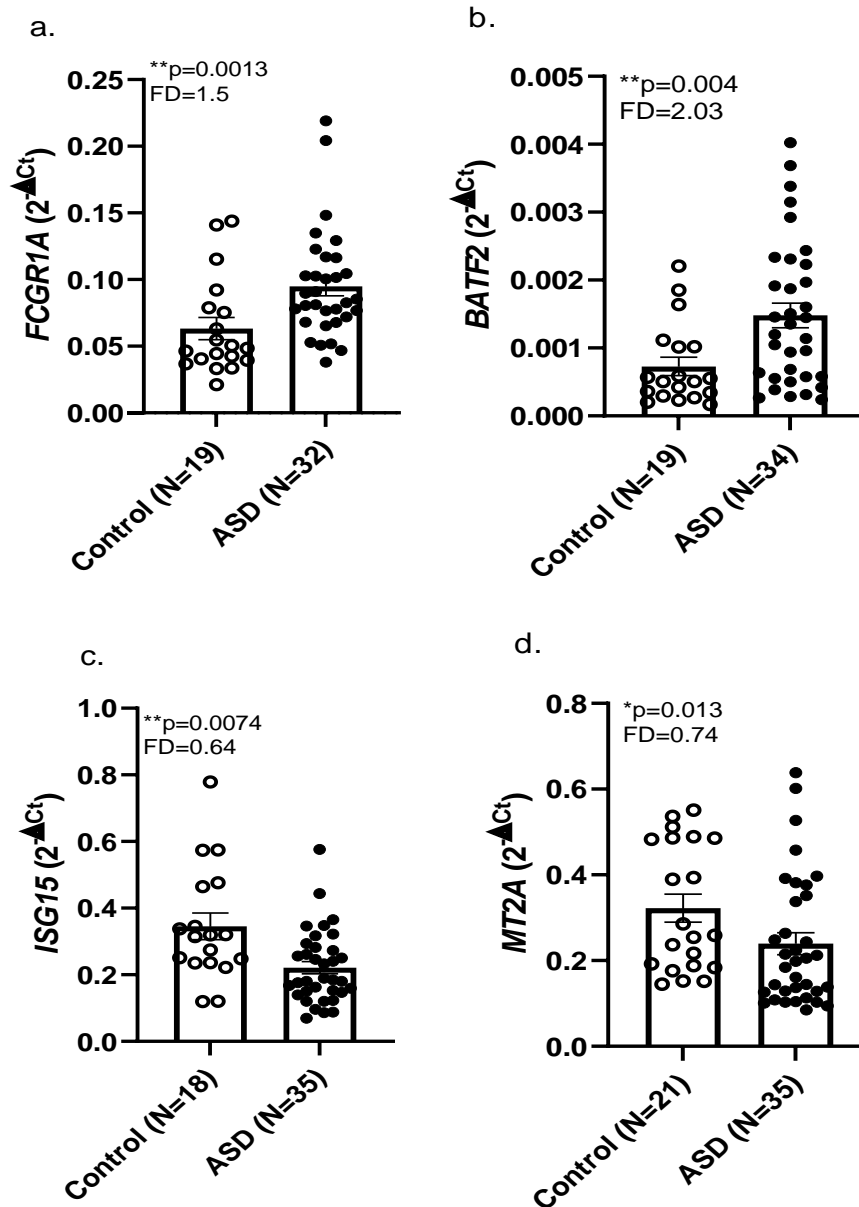
Supplementary Table S2: Summary of Spearman correlation test of top 10 RNA-seq genes and serum endocannabinoids. (a) ASD samples only; (b) neurotypical controls; (c) ASD and neurotypical controls combined. Outlier samples were removed. P-value is two-tailed; N, XY pairs. Serum endocannabinoid levels are taken from Aran et al. 2019. Correlations with $p < 0.05$ are shown in bold fonts.

A. ASD		<i>SERPING1</i>	<i>EFHC2</i>	<i>BATF2</i>	<i>CDC20</i>	<i>FCGR1A</i>	<i>MT2A</i>	<i>ISG15</i>	<i>FBXO6</i>	<i>LINC00869</i>	<i>LY6E</i>
	N (XY Pairs)	22	33	30	25	29	31	31	28	32	30
OEA	p.val	0.4166	0.9617	0.0302	0.3610	0.7452	0.2111	0.7679	0.7148	0.2676	0.8491
	r	0.1824	-0.00869	0.3962	0.1908	0.06305	0.231	0.05524	0.07225	0.202	0.03626
PEA	p.val	0.0637	0.9199	0.8702	0.5284	0.7093	0.7767	0.9764	0.4859	0.0779	0.2635
	r	-0.01822	0.05131	-0.06855	-0.1323	0.005348	0.1257	0.3162	0.2004	-0.01822	-0.06855
AEA	p.val	0.64	0.1019	0.2843	0.6634	0.7127	0.2963	0.816	0.3895	0.1845	0.3628
	r	-0.1056	-0.2898	0.202	-0.09154	0.07144	0.1938	-0.04355	-0.1692	-0.2407	-0.1722
OS	p.val	0.1571	0.4824	0.3038	0.8096	0.8451	0.1035	0.4987	0.6617	0.047	0.2331
	r	0.3123	0.1267	0.1942	0.05077	-0.03793	0.298	-0.1262	0.08648	0.3537	0.2245
2AG	p.val	0.4934	0.7297	0.7516	0.4038	0.5758	0.3308	0.4973	0.9098	0.296	0.1326
	r	0.1542	-0.0625	-0.06029	-0.1746	0.1084	-0.1806	0.1266	-0.02244	0.1906	0.281
AA	p.val	0.9106	0.1811	0.973	0.5828	0.9697	0.0718	0.0399	0.083	0.645	0.2182
	r	-0.02541	-0.2386	-0.00645	-0.1154	0.007389	0.3278	-0.371	-0.3333	-0.08468	0.2316

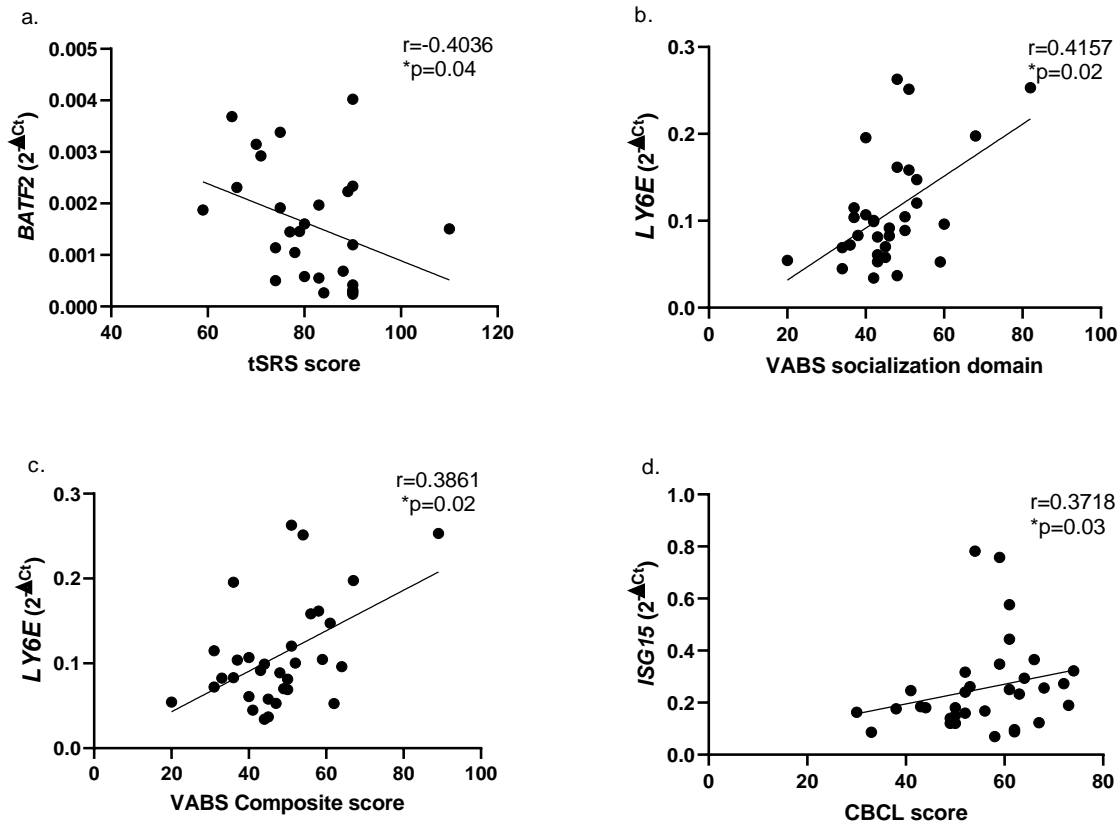
B. NT controls		<i>SERPING1</i>	<i>EFHC2</i>	<i>BATF2</i>	<i>CDC20</i>	<i>FCGR1A</i>	<i>MT2A</i>	<i>ISG15</i>	<i>FBXO6</i>	<i>LINC00869</i>	<i>LY6E</i>
	N (XY Pairs)	18	19	18	17	17	19	16	19	18	18
OEA	p.val	0.1881	0.4680	0.6042	0.2311	0.9887	0.7372	0.0540	0.5092	0.9384	0.8357
	r	-0.3251	0.1772	-0.1311	-0.3064	-0.00490	0.08246	-0.4941	-0.1614	0.01961	-0.05263
PEA	p.val	0.9659	0.8700	0.5764	0.1842	0.0617	0.9398	0.7802	0.6045	0.7264	0.0004
	r	0.01053	0.03910	-0.1368	-0.3382	-0.4489	-0.01805	-0.07353	0.1233	0.08596	-0.7298
AEA	p.val	0.4415	0.9248	0.3515	0.1156	0.8869	0.7865	0.1619	0.349	0.3553	0.6576
	r	-0.1877	-0.02256	-0.2263	-0.3971	0.03612	0.06466	-0.3554	0.2211	0.2246	-0.1088
OS	p.val	0.9205	0.4237	0.7753	0.3568	0.7851	0.3907	0.2853	0.7005	0.6785	0.4907
	r	-0.02456	0.1895	-0.07018	-0.2377	0.06914	0.203	-0.2745	-0.09173	0.1018	-0.1684
2AG	p.val	0.1408	0.1408	0.5617	0.8835	0.7048	0.5395	0.0137	0.4778	0.101	0.9602
	r	-0.3509	0.3414	0.1421	-0.03922	0.09598	-0.1459	-0.5931	0.1684	0.3877	-0.01228
AA	p.val	0.2206	0.7527	0.2797	0.8686	0.2875	0.2272	0.6322	0.1838	0.9488	0.5375
	r	0.2947	-0.07519	-0.2614	-0.04412	-0.2652	0.2827	-0.125	-0.3098	-0.01579	0.1509

C. ASD and NT controls		<i>SERPING1</i>	<i>EFHC2</i>	<i>BATF2</i>	<i>CDC20</i>	<i>FCGR1A</i>	<i>MT2A</i>	<i>ISG15</i>	<i>FBXO6</i>	<i>LINC00869</i>	<i>LY6E</i>
	N (XY Pairs)	34	51	49	42	48	51	47	48	52	49
OEA	p.val	0.8055	0.8063	0.6085	0.8773	0.4609	0.0957	0.8464	0.7617	0.2714	0.384
	r	0.04385	-0.0352	0.075	-0.02455	-0.109	0.2358	-0.02902	0.04494	0.1554	-0.1271
PEA	p.val	0.24	0.8426	0.5492	0.1955	0.0098	0.152	0.1997	0.5513	0.2708	0.0216
	r	0.207	-0.02851	-0.08766	-0.2038	-0.3693	0.2035	0.1905	0.08816	0.1556	-0.3275
AEA	p.val	0.5675	0.1005	0.5462	0.2127	0.2017	0.1744	0.9186	0.9259	0.7398	0.0481
	r	-0.1016	-0.2326	-0.08832	-0.1963	-0.1876	0.1932	-0.01532	-0.01379	-0.04717	-0.2839
OS	p.val	0.1573	0.8367	0.3639	0.9647	0.9271	0.1147	0.1111	0.7004	0.0471	0.6837
	r	0.248	0.02959	0.1326	-0.00705	0.01357	0.2236	-0.2354	0.05699	0.2767	0.05969
2AG	p.val	0.6372	0.9362	0.3366	0.4241	0.3184	0.0741	0.4127	0.9947	0.0571	0.1239
	r	0.08388	-0.01149	0.1402	-0.1267	0.1471	-0.2523	-0.1223	0.000977	0.2655	0.2228
AA	p.val	0.5781	0.1857	0.8734	0.6996	0.7157	0.0708	0.0314	0.0242	0.6809	0.0995
	r	0.09885	-0.1883	-0.02337	-0.06134	-0.05395	0.2551	-0.3143	-0.325	-0.0584	0.2381

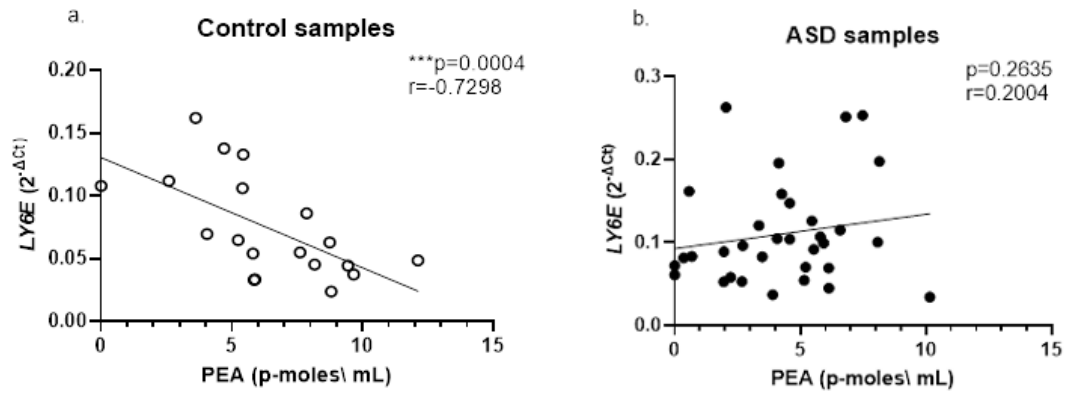
Supplementary Figure S1: Real-time qPCR validation for whole blood RNA expression levels in ASD and control children from the Israeli cohort alone. Box plots show mean \pm SEM mRNA levels for ASD vs. neurotypical control whole blood samples. Outliers were removed and analysis was done using a non-parametric Mann Whitney test. FD and p values are shown for the genes with differential expression in ASD vs. neurotypical controls.



Supplementary Figure S2: Whole blood mRNA expression levels of *BATF2*, *LY6E* and *ISG15* correlate with ASD behavioral scores (Israeli cohort). Correlations for mRNA levels (real-time Pare shown for (a) *BATF2* vs. tSRS score (N=27); (b) *LY6E* vs. VABS socialization domain score (N=32); (c) *LY6E* vs. VABS Composite score (N=32); (d) *ISG15* vs. CBCL score (N=33). The *r* and *p* values for each correlation plot (Spearman test) are shown in each panel. See Methods for further information on behavioral scores.



Supplementary Figure S3: Correlations for whole blood *LY6E* expression levels with serum palmitoylethanolamide (PEA) levels in children with ASD and neurotypical controls (Israeli cohort). Correlations are shown for (a) neurotypical control children (N=19); (b) ASD children (N=30). The *r* and *p* values for each correlation plot (Spearman test) are shown in each panel. PEA levels were taken from Aran *et al.* 2019. See Methods for further details.



Supplementary Figure S4: Correlations for whole blood mRNA expression levels with serum endocannabinoid levels in children with ASD and neurotypical controls combined (Israeli cohort). Correlations are shown for (a) *FCGR1A* and palmitoylethanolamide (PEA); (b) *LY6E* and PEA; (c) *FBXO6* and arachidonic acid (AA); (d) *ISG15* and AA; (e) *LINC00869* and oleoyl serine (OS); (f) *LY6E* and anandamide (AEA). The r and p values for each correlation plot (Spearman test) are shown in each panel. Open circles indicate control children, while closed circles indicate ASD children; individual findings for whole blood mRNA expression and serum EC levels were combined for controls (open circles) and children with ASD (closed circles) for calculating r and p values for each correlation. Endocannabinoid levels were taken from Aran *et al.* 2019.

