

Supplementary files for:

Blood RNA sequencing confirms upregulated *BATF2* and *FCGR1A* expression in children with autism spectrum disorder

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Supplementary Table S1: Primers used for real-time qPCR.

<i>Gene</i>	<i>Forward</i>	<i>Reverse</i>
<i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
<i>RPLP0</i>	AGCCAGAACTGGTCTC	ACTCAGGATTTCATGGTGCC
<i>SERPING1</i>	CCTTGCTCCTCAATGCTATC	CCACAGGGTACTTCTTGCTATT
<i>EFHC2</i>	ATGGCCGGACATTCAAGATT	GGCTCTACGTGTTCTACAACCT
<i>BATF2</i>	CCTCATTGCTCTTTGGACTAGG	TGTGTGAAACCTGGGTGAAG
<i>CDC20</i>	AGACCTGCCGTTACATTCCTTC	GCCAGTACATTCAGAACTCC
<i>FCGR1A</i>	CGCTACACATCAGCAGGAATA	GCCCATGTAGAAGGAGAAGTAAA
<i>MT2A</i>	CCGCTCCAGATGTAAAGAA	CACGGTCAGGGTTGTACATAA
<i>ISG15</i>	CGCAGATACCCAGAAGATCG	TTCGTCGCATTTGTCCACCA
<i>FBXO6</i>	ATCCTACGAAATGTGCCTCAAG	CCAACACGAAGTAGTCAGCCG
<i>LINC00869</i>	CATCAAAGCTACTGCCTACTC	CACCTCTCCCTCCTGTATCT
<i>LY6E</i>	GGGAATCTCGTGACATTTGGC	ACACCAACATTGACGCCTTCT

Supplementary Table S2: Real-time qPCR analyses compared PBMC gene expression levels in children with ASD vs. either all neurotypical controls (left); only their neurotypical siblings (middle) or only unrelated neurotypical children (right). N shows numbers for neurotypical controls/ASD for each comparison. Outlier samples were removed. Note that *SERPING1* was the only significant gene showing differential expression in PBMCs from the ASD group and their neurotypical siblings (bold fonts).

	<i>ASD vs. all neurotypical controls</i>			<i>ASD vs. sibling controls</i>			<i>ASD vs. unrelated controls</i>		
Gene	N	p-value	FD	N	p-value	FD	N	p-value	FD
<i>SERPING1</i>	30/17	0.389	0.71	30/12	0.023	1.90	30/7	0.275	0.82
<i>BATF2</i>	36/17	0.291	0.84	36/11	0.217	1.60	36/6	0.074	1.45
<i>EFHC2</i>	28/18	0.057	1.44	37/12	0.261	1.34	37/6	0.724	1.37
<i>CDC20</i>	30/17	0.275	1.23	30/11	0.988	1.49	30/6	0.113	1.05
<i>FCGR1A</i>	32/17	0.992	0.98	32/11	0.923	1.47	32/6	0.922	1.55
<i>MT2A</i>	32/17	0.222	1.18	32/11	0.276	1.13	32/7	0.928	1.20
<i>ISG15</i>	33/18	0.395	0.89	33/12	0.849	0.79	33/7	0.507	0.98
<i>FBXO6</i>	35/19	0.214	1.20	36/12	0.365	1.63	35/7	0.302	0.65
LINC00869	32/18	0.568	1.03	32/11	0.314	1.24	32/7	0.788	2.01
<i>LY6E</i>	34/18	0.396	1.10	34/11	0.277	1.06	34/7	0.932	3.31

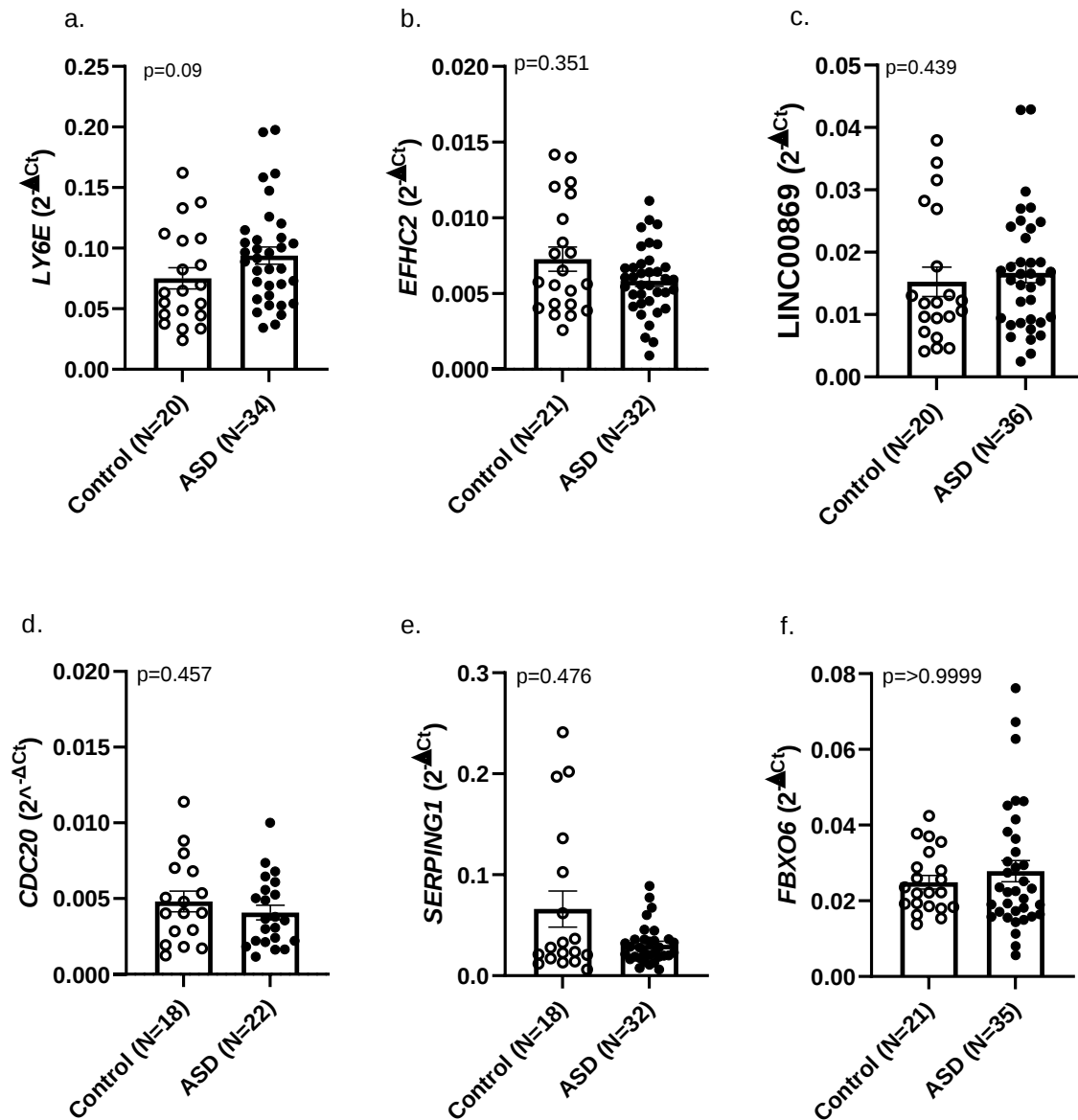
Supplementary Table S3: Summary of Spearman correlation test of top 10 RNA-seq genes and serum endocannabinoids in (a) ASD samples only; (b) neurotypical controls; (c) ASD and neurotypical controls combined. Outlier samples were removed. P-value is two-tailed; N, XY pairs. Serum endocannabinoid levels 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

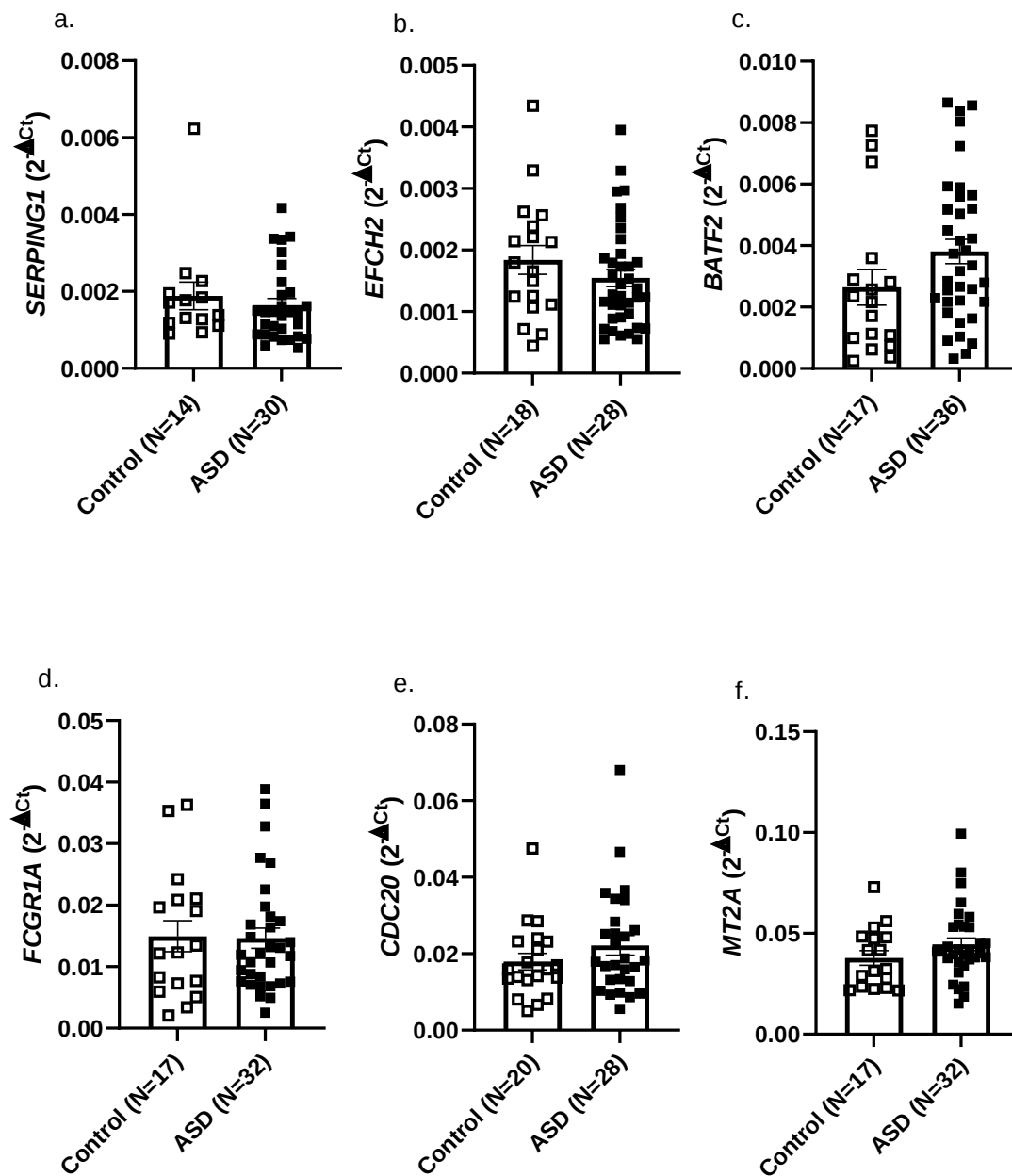
B. Control		<i>SERPING1</i>	<i>EFHC2</i>	<i>BATF2</i>	<i>CDC20</i>	<i>FCGR1A</i>	<i>MT2A</i>	<i>ISG15</i>	<i>FBXO6</i>	<i>LINC00869</i>	<i>LY6E</i>
	N (XY Pairs)	18	19	18	17	17	19	16	19	18	18
OEA	p.val	0.1881	0.4680	0.6042	0.2311	0.9887	0.7372	0.0540	0.5092	0.9384	0.8357
	r	-0.3251	0.1772	-0.1311	-0.3064	-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

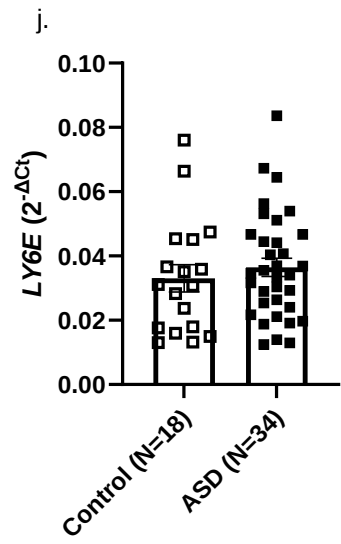
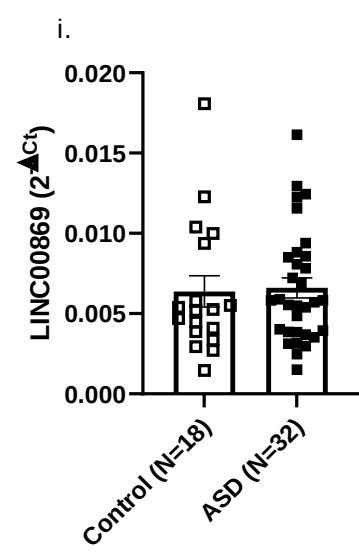
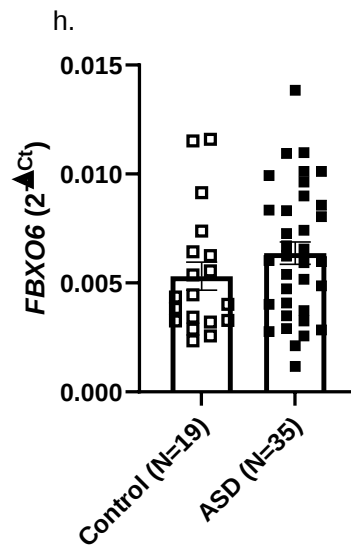
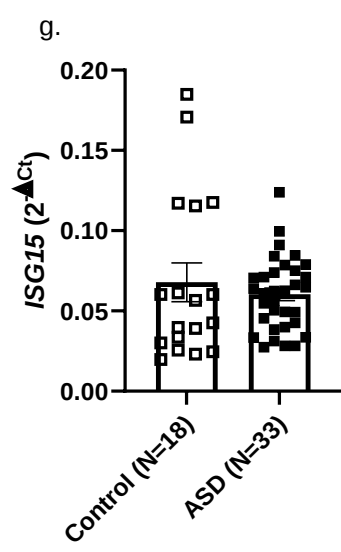
C. ASD and controls		<i>SERPING1</i>	<i>EFHC2</i>	<i>BATF2</i>	<i>CDC20</i>	<i>FCGR1A</i>	<i>MT2A</i>	<i>ISG15</i>	<i>FBXO6</i>	<i>LINC00869</i>	<i>LY6E</i>
	N (XY Pairs)	34	51	49	42	48	51	47	48	52	49
OEA	p.val	0.8055	0.8063	0.6085	0.8773	0.4609	0.0957	0.8464	0.7617	0.2714	0.384
	r	0.04385	-0.0352	0.075	-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 measurements for whole blood RNA expression levels in ASD and control children (Israeli cohort). Box plots show mean $\pm$ SEM RNA levels for neurotypical control vs. ASD whole blood samples. Outliers were removed and analysis was done using a non-parametric Mann Whitney test. As shown, p values for gene expression (qPCR measurements) in ASD vs. control blood samples indicated lack of significant differences for the presented genes.

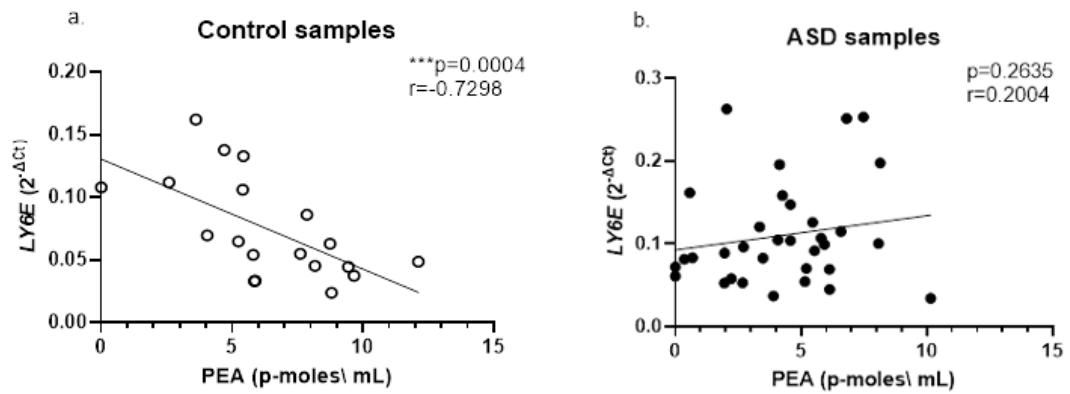


Supplementary Figure. S2: RNA expression by real-time qPCR in PBMCs from children with ASD and all neurotypical control children (U.S. cohort). Graphs show mean $\pm$ SED for control and ASD samples for each of the top 10 genes found by RNA-seq of whole blood samples from the Israeli cohort. Outliers were removed and analysis was done using a non-parametric Mann Whitney test. No significant differences in gene expression were found between ASD and control PBMCs ($p > 0.1$ for the 10 tested genes).





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.

