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.

| Gene   | Forward                      | Reverse                      |
|--------|------------------------------|------------------------------|
| GAPDH  | GGAGCGGAGATCCTCCCAAAAT       | GGCTGTGTCACTTCTCATG          |
| RPLP0  | AGCCCCAGAACACTTGGTCTC        | ACTCAGGATTCAATGGTGC          |
| SERPING1 | CTTGTCTCTCTCAATGTACATC     | CCACAGGTACCTTCTTGCAATTT      |
| EFHC2  | ATGGGCCGACATTTCAAGATT       | GGCTCTACGTGTCTTACAACTCT      |
| BATF2  | CCTCATTGCTCTTTGGGACTAGG     | TGTGTAGAACCTGGGTGAAG         |
| CDC20  | AGACCTGCGGTACATTCTCTCT      | GCCAGTACATCCAGAAGACTCC       |
| FCGR1A | CGCTACACATCACGCGAAGAATA     | GCCCATGTAAGGAGAAGATTAAGA     |
| MT2A   | CCGCTCCAGATGTAAGAAA         | CACGCTACGGGTTGTCATATAA       |
| ISG15  | CGCAAGATCCACCGAGAGAATC     | TTCGTCCACATTGTGTCACCCA       |
| FBXO6  | ATCCCTACAAATGTCCTCAAGA     | CCAACACGGAATGTCACCGG         |
| LINC00869 | CATCACAAGATCCTGCCTACTC   | CACCTCCTCCCCTCTGTATCT        |
| LY6E   | GGGAAATCTCGTGACATTTGGC     | ACACCAAACATTCACGGCCTCT       |
**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).

| Gene       | ASD vs. all neurotypical controls | ASD vs. sibling controls | ASD vs. unrelated controls |
|------------|-----------------------------------|--------------------------|---------------------------|
| **SERPING1** | N: 30/17 p-value: 0.389 FD: 0.71 | N: 30/12 p-value: 0.023 FD: 1.90 | N: 30/7 p-value: 0.275 FD: 0.82 |
| BATF2      | 36/17 0.291 0.84                 | 36/11 0.217 1.60         | 36/6 0.074 1.45           |
| EFHC2      | 28/18 0.057 1.44                 | 37/12 0.261 1.34         | 37/6 0.724 1.37           |
| CDC20      | 30/17 0.275 1.23                 | 30/11 0.988 1.49         | 30/6 0.113 1.05           |
| FCGR1A     | 32/17 0.992 0.98                 | 32/11 0.923 1.47         | 32/6 0.922 1.55           |
| MT2A       | 32/17 0.222 1.18                 | 32/11 0.276 1.47         | 32/7 0.928 1.20           |
| ISG15      | 33/18 0.395 0.89                 | 33/12 0.849 0.79         | 33/7 0.507 0.98           |
| FBXO6      | 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           |
| LY6E       | 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   | SERPING1 | EFHC2 | BATF2 | CDC20 | FCGR1A | MT2A | ISG15 | FBXO6 | LINC00869 | LY6E |
|----------|-----------|-------|-------|-------|--------|------|-------|-------|-----------|------|
|          | N (XY Pairs) |       |       |       |        |      |       |       |           |      |
| OEA      | 22        | 33    | 30    | 25    | 29     | 31   | 31    | 28    | 32        | 30   |
| 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      | 0.0637    | 0.9199 | 0.8702 | 0.5284 | 0.7093 | 0.7767 | 0.9764 | 0.4859 | 0.0779    | 0.2635 |
| p.val    | -0.01822 | 0.05131 | -0.06855 | -0.1323 | 0.005348 | 0.1257 | 0.3162 | 0.2004 | -0.01822 | -0.06855 |
| r        | -0.1056   | -0.2898 | 0.202   | -0.09154 | 0.07144 | 0.1938 | -0.04355 | -0.1692 | -0.2407  | -0.1722 |
| AEA      | 0.64      | 0.1019 | 0.2843 | 0.6634 | 0.7127 | 0.2963 | 0.816  | 0.3895 | 0.1845    | 0.3628 |
| p.val    | -0.1056   | -0.2898 | 0.202   | -0.09154 | 0.07144 | 0.1938 | -0.04355 | -0.1692 | -0.2407  | -0.1722 |
| OS       | 0.1571    | 0.4824 | 0.3038 | 0.8096 | 0.8451 | 0.1035 | 0.4987 | 0.6617 | **0.047** | 0.2331 |
| p.val    | 0.3123    | 0.1267 | 0.1942 | 0.05077 | -0.03793 | 0.298 | -0.1262 | 0.08648 | 0.3537    | 0.2245 |
| r        | 0.4934    | 0.7297 | 0.7516 | 0.4038 | 0.5758 | 0.3308 | 0.4973 | 0.9098 | 0.296     | 0.1326 |
| 2AG      | 0.1542    | -0.0625 | -0.06029 | -0.1746 | 0.1084 | -0.1806 | 0.1266 | -0.02244 | 0.1906    | 0.281  |
| 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 | SERPING1 | EFHC2 | BATF2 | CDC20 | FGFR1A | MT2A | ISG15 | FBXO6 | LINCO00869 | LY6E |
|------------|----------|-------|-------|-------|--------|------|-------|-------|------------|------|
| OEA | 18 | 19 | 18 | 17 | 17 | 19 | 16 | 19 | 18 | 18 |
| 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 | -0.0049 | 0.08246 | -0.4941 | -0.1614 | 0.01961 | -0.05263 |
| PEA | 0.9659 | 0.8700 | 0.5764 | 0.1842 | 0.0617 | 0.9398 | 0.7802 | 0.6045 | 0.7264 | 0.0004 |
| p.val | 0.01053 | 0.03910 | -0.1368 | -0.3382 | -0.4489 | -0.01805 | -0.07353 | 0.1233 | 0.08596 | -0.7298 |
| r | 0.3509 | 0.02851 | -0.2256 | -0.3971 | -0.3612 | 0.06466 | -0.3554 | 0.2211 | 0.2246 | -0.1088 |
| AEA | 0.4415 | 0.9248 | 0.3515 | 0.1156 | 0.8869 | 0.7865 | 0.1619 | 0.349 | 0.3553 | 0.6576 |
| p.val | 0.1877 | -0.20225 | -0.2263 | 0.3971 | 0.06312 | 0.06466 | 0.2211 | 0.2246 | -0.1088 | 0.5075 |
| r | 0.9205 | 0.4237 | 0.7753 | 0.3568 | 0.7851 | 0.3907 | 0.2853 | 0.7005 | 0.6785 | 0.4907 |
| OS | 0.1408 | 0.1408 | 0.5617 | 0.8835 | 0.7048 | 0.5395 | 0.0137 | 0.4778 | 0.101 | 0.9602 |
| p.val | 0.3509 | 0.3414 | 0.1421 | -0.03922 | 0.09598 | -0.1459 | -0.5931 | 0.1684 | 0.3877 | -0.01228 |
| r | -0.3509 | -0.02126 | 0.4273 | 0.7753 | 0.3568 | 0.7851 | 0.3907 | 0.2853 | 0.7005 | 0.6785 |
| ZAG | 0.2206 | 0.7527 | 0.2797 | 0.8686 | 0.2875 | 0.2272 | 0.6322 | 0.1838 | 0.9488 | 0.5375 |
| p.val | 0.2947 | -0.07519 | -0.2614 | -0.04412 | -0.2652 | 0.2827 | -0.125 | -0.3098 | -0.01579 | 0.1509 |
| r | 0.02337 | 0.02851 | -0.2256 | -0.3971 | -0.3612 | 0.06466 | -0.3554 | 0.2211 | 0.2246 | -0.1088 |

| C. ASD and controls | SERPING1 | EFHC2 | BATF2 | CDC20 | FGFR1A | MT2A | ISG15 | FBXO6 | LINCO00869 | LY6E |
|---------------------|----------|-------|-------|-------|--------|------|-------|-------|------------|------|
| OEA | 34 | 51 | 49 | 42 | 48 | 51 | 47 | 48 | 52 | 49 |
| 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 | 0.24 | 0.8426 | 0.5492 | 0.1955 | 0.0098 | 0.152 | 0.1997 | 0.5513 | 0.2708 | 0.0216 |
| p.val | 0.207 | -0.02851 | 0.0876 | -0.2038 | 0.1905 | 0.08816 | 0.1556 | -0.3275 | 0.4019 | 0.1556 |
| r | 0.5675 | -0.1016 | 0.2326 | -0.08832 | -0.1963 | 0.1932 | -0.01532 | -0.04717 | 0.2839 |
| AEA | 0.1573 | 0.8367 | 0.3639 | 0.9647 | 0.9271 | 0.1147 | 0.1111 | 0.7004 | 0.0471 | 0.6837 |
| p.val | 0.248 | 0.02959 | 0.1326 | -0.00705 | 0.01357 | 0.2236 | -0.2354 | 0.05699 | 0.2767 | 0.05969 |
| r | 0.6372 | 0.9362 | 0.3366 | 0.4241 | 0.3184 | 0.0741 | 0.4127 | 0.9947 | 0.0571 | 0.1239 |
| ZAG | 0.08388 | 0.01149 | 0.1402 | -0.1267 | 0.1471 | -0.2523 | -0.1223 | 0.000977 | 0.2655 | 0.2228 |
| 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 | -0.20337 | -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 ± 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 ± 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) FCRG1A 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.