**Supplementary Table 1.** Parametersindicating the enrichment degree of final non-synaptic and synaptic mitochondrial preparations.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | NonTg | | | | 3xTg-AD | | | |
|  |  | **Citrate synthase** | **Urate oxidase** | **AChE** | **Cerebrosides** | **Citrate synthase** | **Urate oxidase** | **AChE** | **Cerebrosides** |
| Brain Homogenate | Activity  (nmol/min./ mg of protein) | 4.52±0.71 | 25.72±3.99 | 0.0119±0.0038 |  | 3.59±0.55 | 33.41±5.52 | 0.0134±0.0048 |  |
| Activity in total extract | 1544.94±231.17 | 8791.04±1364.65 | 4.07±1.30 |  | 1411.39±215.94 | 12442.09±2055.15 | 4.99±1.78 |  |
| Non-synaptic mitochondria | Activity  (nmol/min./ mg of protein) | 83.59±12.36 | 20.17±3.07 | not detected | not detected by LC-MS analysis | 95.26±4.33 | 16.86±1.4 | not detected | not detected by LC-MS analysis |
| Activity in total extract | 634.45±93.81 | 153.11±23.30 | not detected | not detected by LC-MS analysis | 687.78±31.26 | 121.75±10.11 | not detected | not detected by LC-MS analysis |
| Synaptic mitochondria | Activity  (nmol/min./ mg of protein) | 87.25±12.13 | 9.30±1.20 | not detected | not detected by LC-MS analysis | 77.98±5.99 | 9.14±1.03 | not detected | not detected by LC-MS analysis |
| Activity in total extract | 492.96±67.86 | 52.53±6.76 | not detected | not detected by LC-MS analysis | 440.59±33.32 | 76.32±8.60 | not detected | not detected by LC-MS analysis |

Urate oxidade activity was evaluated using Amplex Red Uric acid/uricase Assay Kit by Life Technology (molecular probes). Results are expressed as mean±std of three independent experiments.

**Supplementary Table 2.** Citrate synthase and mitochondrial enzyme complexes I, II, IV, and FoF1-ATPase activities of synaptic and non-synaptic brain mitochondria isolated from nonTg and 3xTg-AD mice with 3 months of age.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Non-synaptic mitochondria | | Synaptic mitochondria | |
|  | **NonTg** | **3xTg-AD** | **NonTg** | **3xTg-AD** |
| Citrate synthase  (nmol/min/mg of protein) | 83.59±12.36 | 95.26±4.33 | 87.25±12.13 | 77.98±5.99 |
| Complex I  (u.a./mg of protein) | 112.43±16.23 | 133.13±17.76 | 105.11±18.86 | 48.40±6.40\* |
| Complex II  (nmol/min/mg of protein) | 59.10±7.43 | 50.95±8.59 | 46.65±7.47 | 41.80±8.06 |
| Complex IV  (nmol O2/min/mg of protein) | 479.36±48.45 | 412.20±55.80 | 514.66±67.18 | 380.80±51.80 |
| FoF1-ATPase  (nmol H+/min/mg of protein) | 175.83±18.94 | 200.67±14.11 | 182.58±17.10 | 201.15±15.45 |

Results are expressed as mean±std of three independent experiments.

**Supplementary Table 3.** Major PC, LPC, and SM molecular species from synaptic and non-synaptic brain mitochondria of nonTg and 3xTg-AD mice

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **[M+H]+ *m/z*** | **Acyl chains** | |  | **[M+H]+ *m/z*** | | **Acyl chains** | |
| **Class** | **(C:N)** | **NonTg** | **3xTg-AD** | **Class** | **(C:N)** | | **NonTg** | **3xTg-AD** |
| **PC** |  |  |  | **LPC** |  | |  |  |
|  |  |  |  |  | **496.5 (16:0)** | | 16:0/0:0 | 16:0/0:0 |
|  | **734.7 (32:0)** | 16:0/16:0 | 16:0/16:0 |  | **516.5 (18:4)** | | 18:4/0:0 | 18:4/0:0 |
|  |  |  | 14:0/18:0 |  | **518.4 (18:3)** | | 18:3/0:0 | 18:3/0:0 |
|  | **756.7 (34:3)** | 16:0/18:3 | 16:0/18:3 |  | **520.5 (18:2)** | | 18:2/0:0 | 18:2/0:0 |
|  | **758.8 (34:2)** | 16:1 / 18:1 | 16:1 / 18:1 |  | **522.5 (18:1)** | | 18:1/0:0 | 18:1/0:0 |
|  |  | 16:0/18:2 | 16:0/18:2 |  | **524.5 (18:0)** | | 18:0/0:0 | 18:0/0:0 |
|  | **760.7 (34:1)** | 16:0/18:1 | 16:0/18:1 |  | **544.5 (20:4)** | | 20:4/0:0 | 20:4/0:0 |
|  |  |  | 16:1/18:0 |  | **546.5 (20:3)** | | 20:3/0:0 | 20:3/0:0 |
|  | **762.7 (34:0)** | 16:0/18:0 | 16:0/18:0 |  | **548.6 (20:2)** | | 20:2/0:0 | 20:2/0:0 |
|  | **782.7 (36:4)** | 16:0/20:4 | 16:0/20:4 |  | **550.6 (20:1)** | | 20:1/0:0 | 20:1/0:0 |
|  |  | 18:1/18:3 | 18:1/18:3 |  | **568.5 (22:6)** | | 22:6/0:0 | 22:6/0:0 |
|  |  | 18:0/18:4 |  |  | **576.4 (22:2)** | | 22:2/0:0 | 22:2/0:0 |
|  | **784.7 (36:3)** | 16:0/20:3 | 16:0/20:3 | **SM** |  | |  |  |
|  |  | 18:1/18:2 | 18:1/18:2 |  | | |  |  |
|  |  | 18:0/18:3 | 18:0/18:3 |  | | **703.7 (34:1)** | d18:1/16:0 | d18:1/16:0 |
|  | **786.7 (36:2)** | 16:0/20:2 | 16:0/20:2 |  | | **729.6 (36:2)** | d18:1/18:1 | d18:1/18:1 |
|  |  | 18:1/18:1 | 18:1/18:1 |  | | **731.6 (36:1)** | d18:1/18:0 | d18:1/18:0 |
|  | **788.7 (36:1)** | 16:0/20:1 | 16:0/20:1 |  | | **733.6 (36:0)** | d18:0/18:0 | d18:0/18:0 |
|  |  | 16:1/20:0 | 14:0/22:1 |  | | **751.7 (38:5)** | d18:1/20:4 | d18:1/20:4 |
|  |  | 18:0/18:1 | 18:0/18:1 |  | | **753.7 (38:4)** | d18:0/20:4 | d18:0/20:4 |
|  | **806.7 (38:6)** | 16:0/22:6 | 16:0/22:6 |  | | **755.6 (38:3)** | d18:0/20:3 | d18:0/20:3 |
|  |  | 18:2/20:4 |  |  | |  |  |  |
|  | **808.7 (38:5)** | 16:0/22:5 | 16:0/22:5 |  | |  | | |
|  |  | 18:1/20:4 | 18:1/20:4 |  | |
|  |  | 18:0/20:5 | 18:0/20:5 |  | |
|  | **810.7 (38:4)** | 18:0/20:4 | 18:0/20:4 |  | |
|  |  | 16:0/22:4 | 16:0/22:4 |  | |
|  |  | 18:1/20:3 |  |  | |
|  | **812.7 (38:3)** | 16:0/22:3 | 18:0/20:3 |  | |
|  |  | 18:0/20:3 | 16:1/22:2 |  | |
|  |  | 18:1/20:2 | 16:0/22:3 |  | |
|  |  | 18:3/20:0 | 18:3/20:0 |  | |
|  | **832.7 (40:7)** | 18:1/22:6 | 18:1/22:6 |  | |
|  |  | 20:3/20:4 |  |  | |
|  | **834.7 (40:6)** | 16:0/24:6 | 20:1/20:5 |  | |
|  |  |  | 16:0/24:6 |  | |
|  | **878.6 (44:12)** | 22:6/22:6 | 22:6/22:6 |  | |

The attribution of the fatty acyl composition to each PL molecular species was done according to the interpretation of the corresponding MS/MS spectrum. Phospholipids are designated as exemplified follows: diacyl-PC (40:6), where 40 indicates the summed number of carbon atoms at both *sn*-1 and *sn*-2 positions, and 6 designates the summed number of double bonds at both positions; SM (d18:1/16:0), where d18:1 indicates the sphingosine chain and 16:0 indicates the fatty acyl residue. These *m/z* values indicate ratios of mass to charge for singly charged [M+H]+ ion.

**Supplementary Table 4.** Major PS molecular species from synaptic and non-synaptic brain mitochondria of nonTg and 3xTg-AD mice

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **[M-H]- *m/z*** | **Acyl chains** | | **[M-H]- *m/z*** | **Acyl chains** | |
| **Class** | **(C:N)** | **NonTg** | **3xTg-AD** | **(C:N)** | **NonTg** | **3xTg-AD** |
| **PS** |  |  |  |  |  |  |
|  | **760.6 (34:1)** | 16:0/18:1 | 16:0/18:1 | **836.6 (40:5)** | 18:0/22:5 | 18:0/22:5 |
|  | **762.6 (34:0)** | 16:0/18:0 | 16:0/18:0 |  | 18:1/22:4 | 18:1/22:4 |
|  | **786.6 (36:2)** | 18:1/18:1 | 18:1/18:1 |  | 20:1/20:4 | 20:1/20:4 |
|  | **788.6 (36:1)** | 18:0/18:1 | 18:0/18:1 | **838.6 (40:4)** | 18:0/22:4 | 18:0/22:4 |
|  |  | 16:0/20:1 | 16:0/20:1 |  | 20:0/20:4 | 20:0/20:4 |
|  | **790.6 (36:0)** | 18:0/18:0 | 18:0/18:0 | **840.6 (40:3)** | 18:0/22:3 | 18:0/22:3 |
|  | **806.6 (38:6)** | 18:2/20:4 | 18:2/20:4 |  | 20:1/20:2 | 20:1/20:2 |
|  |  | 16:0/22:6 | 16:0/22:6 | **842.6 (40:2)** | 18:1/22:1 | 18:1/22:1 |
|  | **808.6 (38:5)** | 16:0/22:5 | 16:0/22:5 |  | 20:1/20:1 |  |
|  |  | 18:1/20:4 | 18:1/20:4 | **844.6 (40:1)** | 16:0/24:1 | 16:0/24:1 |
|  |  | 16:1/22:4 | 16:1/22:4 |  | 18:0/22:1 | 18:0/22:1 |
|  |  | 18:0/20:5 |  |  | 18:1/22:0 | 18:1/22:0 |
|  | **810.6 (38:4)** | 18:0/20:4 | 18:0/20:4 | **846.6 (40:0)** | 18:0/22:0 | 18:0/22:0 |
|  |  | 16:0/22:4 | 16:0/22:4 | **854.6 (42:10)** | 20:4/22:6 | 20:4/22:6 |
|  |  | 18:1/20:3 | 18:1/20:3 | **856.6 (42:9)** | 20:3/22:6 | 20:3/22:6 |
|  | **812.6 (38:3)** | 18:0/20:3 | 18:0/20:3 | **860.5 (42:7)** |  | 20:3/22:4 |
|  |  | 18:1/20:2 | 18:1/20:2 |  | 20:1/22:6 | 20:1/22:6 |
|  | **814.6 (38:2)** | 18:0/20:2 | 18:0/20:2 | **862.5 (42:6)** | 20:0/22:6 | 20:0/22:6 |
|  |  | 18:1/20:1 | 18:1/20:1 | **866.6 (42:4)** | 18:0/24:4 | 18:0/24:4 |
|  | **816.6 (38:1)** | 18:0/20:1 | 18:0/20:1 | **872.5 (42:1)** | 18:0/24:1 | 18:0/24:1 |
|  |  | 18:1/20:0 | 18:1/20:0 |  | 20:1/22:0 | 20:1/22:0 |
|  | **832.6 (40:7)** | 22:6/18:1 | 22:6/18:1 | **878.4 (44:12)** | 22:6/22:6 | 226:6/22:6 |
|  | **834.6 (40:6)** | 20:3/20:3 | 20:3/20:3 | **880.4 (44:11)** | 22:5/22:6 | 22:5/22:6 |
|  |  | 18:0/22:6 | 18:0/22:6 | **882.5 (44:10)** | 22:4/22:6 | 22:4/22:6 |
|  |  | 18:1/22:5 | 18:1/22:5 | **884.5 (44:9)** | 22:3/22:6 | 22:3/22:6 |
|  |  |  |  |  |  |  |

The attribution of the fatty acyl composition to each PL molecular species was done according to the interpretation of the corresponding MS/MS spectrum. Phospholipids are designated as exemplified follows: diacyl-PS (34:1), where 34 indicates the summed number of carbon atoms at both *sn*-1 and *sn*-2 positions, and 1 designates the summed number of double bonds at both positions. These *m/z* values indicate ratios of mass to charge for singly charged [M-H]- ions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **[M-H]- *m/z*** | **Acyl chains** | | **[M-H]- *m/z*** | **Acyl chains** | |
| **Class** | **(C:N)** | **NonTg** | **3xTg-AD** |  | **NonTg** | **3xTg-AD** |
| **PE** |  |  |  | **PE** |  |  |
| **Diacyl species** | |  |  | **Plasmalogens** |  |  |
|  | **716.7 (34:1)** | 16:0/18:1 | 16:0/18:1 | **700.7** | *p*16:0/18:1 | *p*16:0/18:1 |
|  | **738.6 (36:4)** | 16:0/20:4 | 16:0/20:4 |  | *p*18:1/16:0 | *p*18:1/16:0 |
|  | **736.6 (36:5)** | 16:1/20:4 | 16:1/20:4 |  | *p*16:1/18:0 | *p*16:1/18:0 |
|  |  | 18:1/18:4 | 18:1/18:4 | **722.7** | *p*16:0/20:4 | *p*16:0/20:4 |
|  | **742.7 (36:2)** | 18:1/18:1 | 18:1/18:1 | **724.7** |  | *p*16:0/24:3 |
|  |  | 16:0/20:2 | 16:0/20:2 |  | *o*16:0/20:4 | *o*16:0/20:4 |
|  | **744.7 (36:1)** | 18:0/18:1 | 18:0/18:1 | **726.7** | *p*16:0/20:2 | *p*16:0/20:2 |
|  |  | 16:0/20:1 | 16:0/20:1 |  | *p*18:0/18:2 | *p*18:0/18:2 |
|  | **746.6 (36:0)** | 18:0/18:0 | 18:0/18:0 |  | *p*18:1/18:1 | *p*18:1/18:1 |
|  | **762.6 (38:6)** | 16:0/22:6 | 16:0/22:6 | **728.7** | *p*16:0/20:1 | *p*16:0/20:1 |
|  | **764.6 (38:5)** | 16:0/22:5 | 16:0/22:5 |  | *p*18:0/18:1 | *p*18:0/18:1 |
|  |  | 18:1/20:4 | 18:1/20:4 | **746.6** | *p*16:0/22:6 | *p*16:0/22:6 |
|  | **766.6 (38:4)** | 16:0/22:4 | 16:0/22:4 | **748.6** | *o*16:0/22:6 | *o*16:0/22:6 |
|  |  | 18:0/20:4 | 18:0/20:4 |  | *p*18:1/20:4 | *p*18:1/20:4 |
|  | **768.6 (38:3)** | 18:0/20:3 | 18:0/20:3 | **750.7** | *p*18:0/20:4 | *p*18:0/20:4 |
|  |  | 18:1/20:2 | 18:1/20:2 |  | *p*16:0/22:4 | *p*16:0/22:4 |
|  | **772.6 (38:1)** | 18:0/20:1 | 18:0/20:1 | **772.6** | *p*18:1/22:6 | *p*18:1/22:6 |
|  | **792.5 (40:5)** | 18:0/22:5 | 18:0/22:5 | **774.6** | *p*18:0/22:6 | *p*18:0/22:6 |
|  |  | 20:4/20:1 | 20:4/20:1 | **776.6** | *o*18:0/22:6 | *o*18:0/22:6 |
|  | **794.6 (40:4)** | 18:0/22:4 | 18:0/22:4 |  | *p*18:1/22:4 | *p*18:1/22:4 |
|  | **798.5 (40:2)** |  | 20:0/20:2 | **778.6** | *p*18:0/22:4 | *p*18:0/22:4 |
|  |  |  |  | **804.5** | *o*20:0/22:6 | *o*20:0/22:6 |
|  |  |  |  |  |  |  |

**Supplementary Table 5.** Major diacyl-PE and PE plasmalogens species from synaptic and non-synaptic brain mitochondria of nonTg and 3xTg-AD mice

The attribution of the fatty acyl composition to each PL molecular species was done according to the interpretation of the corresponding MS/MS spectrum. Phospholipids are designated as exemplified follows: diacyl-PE (34:1), where 34 indicates the summed number of carbon atoms at both *sn*-1 and *sn*-2 positions and 1 designates the summed number of double bonds at both positions. These *m/z* values indicate ratios of mass to charge for singly charged [M-H]- ions.

**Supplementary Table 6.** Major PA, PG and PI molecular species from synaptic and non-synaptic brain mitochondria of nonTg and 3xTg-AD mice

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **[M-H]- *m/z*** | | **Acyl chains** | | | |
| **Class** | **(C:N)** | | **NonTg** | | **3xTg-AD** | |
| **PA** |  | |  | |  | |
| **Diacyl species** | | |  | |  | |
|  | **665.5 (34:5)** | | 14:0/20:5 | | 14:0/20:5 | |
|  | **673.5 (34:1)** | | 16:0/18:1 | | 16:0/18:1 | |
|  | **695.5 (36:4)** | | 16:0/20:4 | | 16:0/20:4 | |
|  | **699.2 (36:2)** | | 18:1/18:1 | | 18:1/18:1 | |
|  |  | | 18:0/18:2 | | 18:0/18:2 | |
|  | **721.5 (38:5)** | | 18:1/20:4 | | 18:1/20:4 | |
|  | **723.5 (38:4)** | | 16:0/22:4 | | 16:0/22:4 | |
|  |  | | 18:0/20:4 | | 18:0/20:4 | |
|  | **727.6 (38:2)** | | 18:1/20:1 | | 18:1/20:1 | |
|  |  | | 18:0/20:2 | | 18:0/20:2 | |
| **PG** | |  | |  | |  | |
| **Diacyl species** | | | |  | |  | |
|  | | **747.6 (34:1)** | | 16:0/18:1 | | 16:0/18:1 | |
|  | |  | | 16:1/18:0 | | 16:1/18:0 | |
|  | | **749.6 (34:0)** | | 16:0/18:0 | | 16:0/18:0 | |
|  | | **769.5 (36:4)** | | 16:0/20:4 | | 16:0/20:4 | |
|  | | **773.5 (36:2)** | | 18:1/18:1 | | 18:1/18:1 | |
|  | | **775.6 (36:1)** | | 18:0/18:1 | | 18:0/18:1 | |
|  | | **797.6 (38:4)** | | 18:0/20:4 | | 18:0/20:4 | |
| **PI** |  | |  | |  | |
| **Diacyl species** | | |  | |  | |
|  | **857.6 (36:4)** | | 16:0/20:4 | | 16:0/20:4 | |
|  | **865.6 (36:0)** | | 18:0/18:0 | | 18:0/18:0 | |
|  | **881.6 (38:6)** | | 16:0/22:6 | | 16:0/22:6 | |
|  | **883.6 (38:5)** | | 18:1/20:4 | | 18:1/20:4 | |
|  | **885.6 (38:4)** | | 18:0/20:4 | | 18:0/20:4 | |
|  | **887.6 (38:3)** | | 18:0/20:3 | | 18:0/20:3 | |
|  | **909.6 (40:6)** | | 18:0/22:6 | | 18:0/22:6 | |

The attribution of the fatty acyl composition to each PL molecular species was done according to the interpretation of the corresponding MS/MS spectrum. Phospholipids are designated as exemplified follows: diacyl-PA (34:5), where 34 indicates the summed number of carbon atoms at both *sn*-1 and *sn*-2 positions, and 5 designates the summed number of double bonds at both positions. These *m/z* values indicate ratios of mass to charge for singly charged [M-H]- ions.

**Supplementary Table .**- Major CL molecular species from synaptic and non-synaptic brain mitochondria of nonTg and 3xTg-AD mice

|  |  |  |  |
| --- | --- | --- | --- |
| **[M-H]- *m/z*** | **[M-2H]2- *m/z*** | **Acyl chains** | |
| **(C:N)** |  | **NonTg** | **3xTg-AD** |
|  |  |  |  |
| **1397.7 (68:5)** | **698.5** | (16:1)2/18:1/18:2 | (16:1)2/18:1/18:2 |
| **1399.7 (68:4)** | **699.5** | (16:1)2/(18:1)2; 16:0/16:1/18:1/18:2 | (16:1)2/(18:1)2; 16:0/16:1/18:1/18:2 |
| **1403.7 (68:2)** | **701.5** | 16:0/16:1/18:0/18:1; (16:0)2/(18:1)2; | 16:0/16:1/18:0/18:1; (16:0)2/(18:1)2; |
|  |  | (16:0)2/18:0/18:2 | (16:0)2/18:0/18:2 |
| **1421.7 (70:7)** | **710.5** | (16:1)2/18:1/20:4; 16:0/16:1/18:2/20:4 | (16:1)2/18:1/20:4; 16:0/16:1/18:2/20:4 |
| **1423.7 (70:6)** | **711.5** | 16:1/18:1/(18:2)2; 16:0/16:1/18:1/20:4 | 16:1/18:1/(18:2)2; 16:0/16:1/18:1/20:4 |
| **1425.7 (70:5)** | **712.5** | 16:1/(18:1)2/18:2; 16:2/(18:1)3; | 16:1/(18:1)2/18:2; 16:2/(18:1)3; |
|  |  | 16:2/18:0/18:1/18:2; 16:1/18:0/(18:2)2 | 16:2/18:0/18:1/18:2; 16:1/18:0/(18:2)2 |
| **1427.7 (70:4)** | **713.5** | 16:0/(18:1)2/18:2; 16:1/(18:1)3 | 16:0/(18:1)2/18:2; 16:1/(18:1)3 |
| **1429.7 (70:3)** | **714.5** | 16:0/(18:1)3 | 16:0/(18:1)3 |
| **1431.7 (70:2)** | **715.5** | 16:0/18:0/(18:1)2; 16:1/(18:0)2/18:1 | 16:0/18:0/(18:1)2; 16:1/(18:0)2/18:1 |
| **1445.7 (72:9)** | **722.5** | 16:0/16:1/18:3/22:5; 16:0/16:1/(20:4)2 | 16:0/16:1/18:3/22:5; 16:0/16:1/(20:4)2 |
|  |  | 16:1/18:1/18:3/20:4; | 16:1/18:1/18:3/C20:4; |
| **1447.6 (72:8)** | **723.5** | (18:2)4; (16:0)2/(20:4)2; | (18:2)4; (16:0)2/(20:4)2; |
|  |  | 16:1/18:1/18:4/20:2; 16:0/(18:2)2/20:4 | 16:1/18:1/18:4/20:2; 16:0/(18:2)2/20:4 |
| **1449.7 (72:7)** | **724.5** | 16:1/18:1/18:2/20:3; 18:1/(18:2)3; | 16:1/18:1/18:2/20:3; 18:1/(18:2)3; |
|  |  | 16:0/18:1/18:2/20:4; 16:1/(18:1)2/20:4 | 16:0/18:1/18:2/20:4; 16:1/(18:1)2/20:4 |
| **1451.7 (72:6)** | **725.5** | 16:0/(18:1)2/20:4; 16:0/18:0/18:2/20:4; | 16:0/(18:1)2/20:4; 16:0/18:0/18:2/20:4; |
|  |  | 14:1/18:1/20:0/20:4; 16:0/(18:3)2/20:0 | 14:1/18:1/20:0/20:4; 16:0/(18:3)2/20:0 |
| **1453.7 (72:5)** | **726.5** | 16:0/(18:1)2/20:3; (18:1)3/18:2 | 16:0/(18:1)2/20:3; (18:1)3/18:2 |
| **1455.8 (72:4)** | **727.5** | (18:1)4; C18:0/(18:1)2/18:2; 14:0/18:2/(20:1)2 | (18:1)4; C18:0/(18:1)2/18:2; 14:0/18:2/(20:1)2 |
| **1465.6 (74:13)** | **732.5** | 14:1/16:0/(22:6)2 | 14:1/16:0/(22:6)2 |
| **1473.7 (74:9)** | **736.5** | 16:0/18:1/(20:4)2; (18:2)2/18:1/20:4; | 16:0/18:1/(20:4)2; (18:2)2/18:1/20:4; |
|  |  | 16:0/18:2/18:3/22:4 | 16:0/18:2/18:3/22:4 |
| **1475.7 (74:8)** | **737.5** | (18:1)2/18:2/20:4; 16:0/22:4/(18:2)2; | (18:1)2/18:2/20:4; 16:0/22:4/(18:2)2; |
|  |  | 16:0/18:1/18:2/22:5; (18:2)2/18:0/20:4 | 16:0/18:1/18:2/22:5; (18:2)2/18:0/20:4 |
| **1477.7 (74:7)** | **738.5** | (18:1)2/18:3/20:2; (18:1)2/18:2/20:3; | (18:1)2/18:3/20:2; (18:1)2/18:2/20:3; |
|  |  | 16:0/18:0/18:1/22:6; 18:0/(18:2)2/20:3 | 16:0/18:0/18:1/22:6; 18:0/(18:2)2/20:3 |
| **1479.7 (74:6)** | **739.5** | 18:0/(18:1)2/20:4; (18:1)3/20:3 | 18:0/(18:1)2/20:4; (18:1)3/20:3 |
| **1493.7 (76:13)** | **746.5** | 16:1/18:2/20:4/22:6; (18:2)2/C18:3/22:6 | 16:1/18:2/20:4/22:6; (18:2)2/C18:3/22:6 |
| **1495.6 (76:12)** | **747.5** | 16:1/18:2/20:3/22:6; (18:2)2/(20:4)2; 22:6/(18:2)3 | 16:1/18:2/20:3/22:6; (18:2)2/(20:4)2; 22:6/(18:2)3 |
| **1497.6 (76:11)** | **748.5** | 18:1/18:2/(20:4)2 | 18:1/18:2/(20:4)2 |
| **1499.7 (76:10)** | **749.5** | (18:1)2/(20:4)2; 18:0/(18:2)2/22:6 | (18:1)2/(20:4)2; 18:0/(18:2)2/22:6 |
| **1501.7 (76:9)** | **750.5** | (18:1)3/22:6; 18:0/18:1/(20:4)2 | (18:1)3/22:6; 18:0/18:1/(20:4)2 |
| **1503.7 (76:8)** | **751.5** | 18:1/18:2/20:3/20:2; 18:0/(18:1)2/22:6 | 18:1/18:2/20:3/20:2; 18:0/(18:1)2/22:6 |
| **1505.7 (76:7)** | **752.5** | 16:0/18:1/20:3/22:3; 16:0/18:0/20:1/22:6; | 16:0/18:1/20:3/22:3; 16:0/18:0/20:1/22:6; |
|  |  | 18:0/18:1/20:2/20:4; (18:1)2/20:1/20:4 | 18:0/18:1/20:2/20:4; (18:1)2/20:1/20:4 |
| **1517.6 (78:15)** | **758.5** | 16:1/18:2/(22:6)2; 16:1/(20:4)2/22:6 | 16:1/18:2/(22:6)2; 16:1/(20:4)2/22:6 |
| **1519.6 (78:14)** | **759.5** | 18:2/(20:4)3 | 18:2/(20:4)3 |
| **1521.7 (78:13)** | **760.5** | 18:1/(20:4)3; 18:1/18:2/20:4/22:6 | 18:1/(20:4)3; 18:1/18:2/20:4/22:6 |
| **1523.6 (78:12)** | **761.5** | 16:0/18:0/(22:6)2; (18:1)2/20:4/22:6; | 16:0/18:0/(22:6)2; (18:1)2/20:4/22:6; |
|  |  | 18:0/(20:4)3; 16:0/20:2/20:4/22:6 | 18:0/(20:4)3; 16:0/20:2/20:4/22:6 |
| **1525.7 (78:11)** | **762.5** | (18:1)2/20:3/22:6; 18:0/18:1/20:4/22:6 | (18:1)2/20:3/22:6; 18:0/18:1/20:4/22:6 |
| **1531.5 (78:8)** | **765.5** | (18:1)2/20:0/22:6 | (18:1)2/20:0/22:6 |
| **1545.5 (80:15)** | **772.5** | 18:1/(20:4)2/22:6 | 18:1/(20:4)2/22:6 |
| **1547.6 (80:14)** | **773.5** | 18:0/(20:4)2/22:6; (18:1)2/(22:6)2 | 18:0/(20:4)2/22:6; (18:1)2/(22:6)2 |
| **1549.8 (80:13)** | **774.5** | 18:0/18:1/(22:6)2; (18:1)2/22:5/22:6; | 18:0/18:1/(22:6)2; (18:1)2/22:5/22:6; |
|  |  | 16:0/20:3/(22:5)2; 18:0/20:3/20:4/22:6 | 16:0/20:3/(22:5)2; 18:0/20:3/20:4/22:6 |
| **1553.9 (80:11)** | **776.5** | (18:1)2/22:4/22:5; 18:0/18:1/22:4/22:6 | (18:1)2/22:4/22:5; 18:0/18:1/22:4/22:6 |
| **1569.5 (82:17)** | **784.5** | 18:1/20:4/(22:6)2; 20:3/(20:4)2/22:6 | 18:1/20:4/(22:6)2; 20:3/(20:4)2/22:6 |
| **1571.7 (82:16)** | **785.5** | 18:1/20:3/(22:6)2 | 18:1/20:3/(22:6)2 |
| **1575.0 (82:14)** | **787.5** | 18:0/20:2/(22:6)2 | 18:0/20:2/(22:6)2 |

The attribution of the fatty acyl composition to each PL molecular species was done according to the interpretation of the corresponding MS/MS spectrum. Phospholipids are designated as exemplified follows: tetra-acyl-CL (78:8), where 78 indicates the summed number of carbon atoms at the four fatty acyl chains and 8 designates the summed number of double bonds at the four fatty acyl chains. These *m/z* values indicate ratios of mass to charge for singly charged [M-H]- ions and doubly charged ions [M-2H]2-.



**Supplementary Figure 1.** Animal model characterization. Unlike 14 months-old (aging) nonTg brains, 3xTg-AD brains with same age show the deposition of Aβ peptide (A) and presence of hyperphosphorylated tau protein (B).



Supplementary Figure 2. Molecular profile of choline lipids extracted from nonTg (black bars) and 3xTg-AD (gray bars) non-synaptic mitochondria. Typical ESI-MS in positive mode with formation of [M+H]+ ions of PC, LPC, and SM (A). Distribution of PC (B), LPC and SM (C) molecular species. Error bars represent standard deviation for n=3 independent experiments using 3 animals in each one. \* Significantly different from nonTg group, with p ≤0.05

F:\BRAIN_Article Submission\Suplementary data final\SuppFig.3.TIF

Supplementary Figure 3 - Molecular profile of PS, PE, PA, PG, and PI extracted from nonTg (black bars) and 3xTg-AD (gray bars) non-synaptic mitochondria. Typical ESI-MS of (A) PS, (B) PE, (E) PA, (F) PG, and (H) PI obtained in negative ion-mode. Distribution of (C) PS, (D) PE, (G) PA, PG, and PI molecular species. Error bars represent standard deviation for n=3 independent experiments using 3 animals in each one.\* Significantly different from nonTg group, with p≤0.05.

C:\Users\Vera Cardoso\Desktop\Alzheimer's  3 months\imagens tif\Suplementary data final\SuppFig.4.TIF

**Supplementary Figure 4.** Molecular profile of cardiolipin (CL) extracted from nonTg (black bars) and 3xTg-AD (gray bars) synaptic mitochondria. A) Typical ESI-MS of CL obtained in negative mode with formation of [M-H]- ions. B) Distribution of CL molecular species with differences between nonTg and 3xTg-AD groups emphasized into insert (b). Error bars represent standard deviation for n= 3 independent experiments using 3 animals in each one. \* Significantly different from nonTg group, with p ≤0.05.



**Supplementary Figure 5.** Acetylcholinesterase (AChE) activity of nonTg (dark circles) and 3xTg-AD (open circles) brains. AChE data were ﬁtted into a Michaelis–Menten kinetic equation using KaleidaGraph software’s (V3.5, Synergy software, Reading, PA, USA) error-minimization procedure with a χ2 between successive iterations of < 0.001%. Insert Table: Apparent Michaelis–Menten kinetic parameters of brain acetylcholinesterase of nonTg and 3xTg-AD groups. Error bars represent standard deviation for 3 independent experiments.



Supplementary Figure 6. Cardiolipin (CL) profile of synaptic mitochondria isolated from (A) nonTg, (B) 3xTg-AD mice, and (C) mathematical prediction of CL profile considering that the decreased CL content exhibited by synaptic mitochondria of 3xTg-AD mice occurs only as function of the relative abundance of each CL species in nonTg mice.