

## **Supplementary Material**

Maas et al., Antioxidant treatment ameliorates prefrontal hypomyelination and cognitive deficits in a rat model of schizophrenia

Supplementary Table 1 – Primer sequences.

Gene and abbreviation		Forward primer 5'-3'	Reverse primer 5'-3'
<b>Housekeeping genes</b>			
<b><i>β-actin</i></b>	Beta-actin	CCTTCCTGGGTATGGAATCCTGT	TAGAGCCACCAATCCACACA
<b><i>Ppia</i></b>	Peptidyl-prolyl cis-trans isomerase A	AGCACTGGGGAGAAAGGATT	AGCCACTCAGTCTTGGCAGT
<b><i>Gapdh</i></b>	Glyceraldehyde-3-phosphate	GGGTGTGAACCACGAGAAAT	ACTGTGGTCATGAGCCCTTC
<b><i>Ywhaz</i></b>	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	TTGAGCAGAAGACGGAAGGT	GAAGCATTGGGGATCAAGAA
<b>Myelin-related genes</b>			
<b><i>Cldn11</i></b>	Claudin 11	CGCAAATGGACGAACTGGG	TGCACGTAACCAGGGAGGAT
<b><i>Mag</i></b>	Myelin-associated glycoprotein	AAGCCAGACCATCCAACCTTC	CTCCTGATTCCGCTCCAAGT
<b><i>Mbp</i></b>	Myelin basic protein	CCCTACTCCATCCTCAGACTTTC TT	TGGCGGTGTGCCTGTCTAT
<b><i>Mobp</i></b>	Myelin-associated oligodendrocytebasic protein	AATACCTGCAGGGCAACAAAG	TCTGGTTCTTGGAGAGCCTGG
<b><i>Mog</i></b>	Myelin oligodendrocyte glycoprotein	CGCCGTGGAGTTGAAAGTAG	GCACGGAGTTTTCTCTAGT
<b><i>Opalin</i></b>	Oligodendrocytic Myelin Paranodal And Inner Loop Protein	ACCCTGATCCAGCGAAGAAG	TGACTGCCTAGGATTCTCGGAT A
<b><i>Plp1</i></b>	Proteolipid protein 1	GGGCCTGAGCGCAACGGTAA	CAGGCACAGCAGAGCAGGCAA
<b>Glutathione-related genes</b>			
<b><i>Gsta4</i></b>	Glutathione-S-transferase alpha4	GCCGCCAAGTACAACCTTGTA	CACTGCTAAAGCTAGGCTCTCTT CTT
<b><i>Gstm4</i></b>	Glutathione-S-transferase mu 4	GCCTAGGCCCTGGTTTTTC	TCTTCACAGCAGCACAGCAACT
<b><i>Prdx6</i></b>	Peroxiredoxin 6	TGACTGGAAGAAGGGAGAGAG TGT	ATGGGAGCTCTTTGGTGAAGAC
<b><i>Gclc</i></b>	Glutamate—cysteine ligase catalytic subunit	AGAGGACAAACCCCAACCAC	TCGTGCAAAGAGCCTGATGT
<b><i>Gss</i></b>	Glutathione synthetase	AGCGTGCCATAGAGAACGAG	GCTTCCCAGTTCTGTGCGTT
<b><i>Gstm1</i></b>	Glutathione-S-transferase mu 1	GTCATGCCACATAGTCTTCATT C	AGTTCAGGGCAGACCTCAAATC

<b>Gstm6</b>	Glutathione-S-transferase mu 6	CTGAGCGTTGCTATCTCGGAG	TTCTGTGTATTCCAGGAGCAGC
<b>Anpep</b>	Alanyl Aminopeptidase	CCCATCAGTGGTTTGGCAAC	CATAGTCAGCACCCAGAAATTC C

**Supplementary Table 2** – Statistical values of Independent samples T-test with Benjamini Hochberg multiple comparisons correction on qPCR expression data presented in Figure 1.

Age	Gene	t-value	df	p-value	n-number before outlier exclusion	n-number after outlier exclusion
<b>P0</b>	gstm1	-3.184	19	0.005*	APO-SUS n=12 APO-UNSUS n=9	No significant outliers
	gstm4	4.841	19	0.000*	APO-SUS n=12 APO-UNSUS n=9	No significant outliers
	gsta4	3.652	18	0.002*	APO-SUS n=12 APO-UNSUS n=8	No significant outliers
	prdx6	-1.402	19	0.177	APO-SUS n=12 APO-UNSUS n=9	No significant outliers
	gstm6	-3.468	19	0.003*	APO-SUS n=12 APO-UNSUS n=9	No significant outliers
	anpep	-3.070	8.378	0.015*	APO-SUS n=12 APO-UNSUS n=9	No significant outliers
<b>P7</b>	gstm1	-3.184	19	0.005*	APO-SUS n=11 APO-UNSUS n=11	No significant outliers
	gstm4	5.494	15.065	0.000*	APO-SUS n=11 APO-UNSUS n=12	No significant outliers
	gsta4	7.191	20	0.000*	APO-SUS n=11 APO-UNSUS n=11	No significant outliers
	prdx6	-3.100	20	0.006*	APO-SUS n=10 APO-UNSUS n=12	No significant outliers
	gstm6	-8.665	21	0.000*	APO-SUS n=11 APO-UNSUS n=12	No significant outliers
	anpep	-3.979	20	0.001*	APO-SUS n=11 APO-UNSUS n=11	No significant outliers
<b>P14</b>	gstm1	-2.296	20	0.033*	APO-SUS n=12 APO-UNSUS n=12	No significant outliers
	gstm4	10.977	22	0.000*	APO-SUS n=12 APO-UNSUS n=12	No significant outliers
	gsta4	8.099	20	0.000*	APO-SUS n=11 APO-UNSUS n=11	No significant outliers
	prdx6	-8.349	22	0.000*	APO-SUS n=12 APO-UNSUS n=12	No significant outliers
	gstm6	-8.912	22	0.000*	APO-SUS n=12 APO-UNSUS n=12	No significant outliers
	anpep	-2.374	22	0.027*	APO-SUS n=12 APO-UNSUS n=12	No significant outliers

<b>P21</b>	gstm1	-4.385	22	0.000*	APO-SUS n=12 APO-UNSUS n=12	No significant outliers
	gstm4	6.433	20	0.000*	APO-SUS n=10 APO-UNSUS n=12	No significant outliers
	gsta4	8.913	18	0.000*	APO-SUS n=9 APO-UNSUS n=11	No significant outliers
	prdx6	-6.683	16.914	0.000*	APO-SUS n=10 APO-UNSUS n=12	No significant outliers
	gstm6	-4.280	20	0.000*	APO-SUS n=10 APO-UNSUS n=11	No significant outliers
	anpep	-2.784	19	0.012*	APO-SUS n=10 APO-UNSUS n=11	No significant outliers
<b>P90</b>	gstm1	-3.829	16.795	0.001*	APO-SUS n=10 APO-UNSUS n=11	No significant outliers
	gstm4	4.446	5.128	0.006*	APO-SUS n=6 APO-UNSUS n=6	APO-SUS n=4 APO-UNSUS n=6
	gsta4	2.526	10	0.030*	APO-SUS n=6 APO-UNSUS n=6	No significant outliers
	prdx6	-3.513	10	0.006*	APO-SUS n=6 APO-UNSUS n=6	No significant outliers
	gstm6	-3.111	10	0.011*	APO-SUS n=6 APO-UNSUS n=6	APO-SUS n=5 APO-UNSUS n=6
	anpep	-1.783	10	0.105	APO-SUS n=6 APO-UNSUS n=6	APO-SUS n=6 APO-UNSUS n=5
<b>P365</b>	gstm1	-3.760	10	0.004*	APO-SUS n=6 APO-UNSUS n=6	APO-SUS n=6 APO-UNSUS n=5
	gstm4	3.921	14	0.002*	APO-SUS n=8 APO-UNSUS n=8	No significant outliers
	gsta4	2.399	14	0.031*	APO-SUS n=8 APO-UNSUS n=8	No significant outliers
	prdx6	-3.719	14	0.002*	APO-SUS n=8 APO-UNSUS n=8	No significant outliers
	gstm6	-1.673	14	0.117	APO-SUS n=8 APO-UNSUS n=8	No significant outliers
	anpep	-1.170	14	0.261	APO-SUS n=8 APO-UNSUS n=8	No significant outliers
	gstm1	0.684	14	0.505	APO-SUS n=8 APO-UNSUS n=8	No significant outliers

\* Significant

**Supplementary Table 3** – Statistical values of Independent samples T-test with Benjamini Hochberg multiple comparisons correction of qPCR expression data presented in Figure 2.

APO-SUS versus APO-UNSUS	APO-SUS versus APO-SUS+NAC	n-number before	n-number after
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Gene	t-value	df	p-value	t-value	df	p-value	outlier exclusion	outlier exclusion
<b>Prdx6</b>	-3.157	15	0.007*	0.021	16	0.983	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	No significant outliers
<b>Gstm4</b>	1.115	10.736	0.289	-0.197	11.573	0.847	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	No significant outliers
<b>Anpep</b>	-2.362	14	0.033*	-3.070	16	0.007*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=7 APO-UNSUS + NAC n=8
<b>Gstm6</b>	-2.837	6.303	0.028*	-3.139	8.533	0.013*	APO-SUS n=8 APO-SUS + NAC n=9 APO-UNSUS n=7 APO-UNSUS + NAC n=8	APO-SUS n=8 APO-SUS + NAC n=9 APO-UNSUS n=6 APO-UNSUS + NAC n=8
<b>Gsta4</b>	-1.190	14	0.254	-6.897	15	0.000*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=7 APO-UNSUS + NAC n=8
<b>Gstm1</b>	-2.568	14	0.022*	-5.476	15	0.000*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=8 APO-UNSUS n=7 APO-UNSUS + NAC n=8
<b>Gclc</b>	-1.782	14	0.096	-4.570	15	0.000*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=7 APO-UNSUS + NAC n=8
<b>Gss</b>	-2.415	7.096	0.046*	-3.013	9.440	0.014*	APO-SUS n=9 APO-SUS + NAC n=9	No significant outliers

							APO-UNSUS n=7	
							APO-UNSUS + NAC n=8	
<b>Gstm7</b>	-3.175	13	0.007*	-6.484	13	0.000*	APO-SUS n=8 APO-SUS + NAC n=8 APO-UNSUS n=7 APO-UNSUS + NAC n=8	APO-SUS n=8 APO-SUS + NAC n=7 APO-UNSUS n=7 APO-UNSUS + NAC n=8

\* Significant

**Supplementary Table 4** – Statistical values of Independent samples T-test with Benjamini Hochberg multiple comparisons correction of qPCR expression data presented in Figure 3.

Gene	APO-SUS versus APO-UNSUS			APO-SUS versus APO-SUS+NAC			n-number before outlier exclusion	n-number after outlier exclusion
	t-value	df	p-value	t-value	df	p-value		
<b>Mog</b>	-3.719	15	0.002*	-6.022	15	0.002*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=8 APO-UNSUS n=8 APO-UNSUS + NAC n=8
<b>Plp</b>	-2.495	14	0.026*	-4.769	15	0.026*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=7 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=8 APO-UNSUS n=7 APO-UNSUS + NAC n=8
<b>Mag</b>	-2.901	13	0.012*	-4.253	15	0.012*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8	APO-SUS n=9 APO-SUS + NAC n=8 APO-UNSUS n=7 APO-UNSUS + NAC n=8
<b>Mobp</b>	-2.929	14	0.011*	-2.942	14	0.011*	APO-SUS n=8 APO-SUS + NAC n=8 APO-UNSUS n=8 APO-UNSUS + NAC n=8	No significant outliers
<b>Cldn11</b>	-3.740	13	0.002*	-4.424	14	0.002*	APO-SUS n=8 APO-SUS + NAC n=8 APO-UNSUS n=7 APO-UNSUS + NAC n=8	No significant outliers
<b>Mbp</b>	-2.162	14	0.048*	-3.010	16	0.048*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=8	APO-SUS n=9 APO-SUS + NAC n=0 APO-UNSUS n=7

							APO-UNSUS + NAC n=8	APO-UNSUS + NAC n=8
<b>Opalin</b>	-4.025	13	0.001*	-4.667	15	0.001*	APO-SUS n=9 APO-SUS + NAC n=9 APO-UNSUS n=7 APO-UNSUS + NAC n=8	APO-SUS n=8 APO-SUS + NAC n=9 APO-UNSUS n=8 APO-UNSUS + NAC n=8

\* Significant

**Supplementary Table 5** – Ingenuity pathway analysis (IPA) of RNA-sequencing data from medial prefrontal cortex (mPFC) of APO-SUS versus APO-UNSUS rats.

Ingenuity Canonical Pathways	-log(B-H p-value)	Ratio
<b>Glutathione-mediated Detoxification</b>	2.69	0.258
<b>Glutathione Redox Reactions I</b>	2.02	0.25
<b>Complement System</b>	1.87	0.189
<b>Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells</b>	1.6	0.188
<b>Calcium-induced T Lymphocyte Apoptosis</b>	2.56	0.167

**Supplementary Table 6** - Genes in Ingenuity pathway analysis (IPA) canonical pathway ‘Glutathione-mediated detoxification’. Transcriptome-wide analysis of APO-SUS and APO-UNSUS medial prefrontal cortex (mPFC) combined with IPA revealed that this pathway represents the most significantly enriched pathway.

Symbol	Entrez Gene Name	Ratio	Corrected p-value
<b>Gsta1</b>	glutathione S-transferase alpha 1	1.95	0.0264
<b>GSTA1</b>	glutathione S-transferase alpha 1	-2.01	0.00111
<b>Gsta4</b>	glutathione S-transferase, alpha 4	2.02	0.00025
<b>GSTM4</b>	glutathione S-transferase mu 4	1.2	0.0354
<b>GSTM5</b>	glutathione S-transferase mu 5	-1.21	0.000000969
<b>Gstt1</b>	glutathione S-transferase, theta 1	-1.65	0.00713
<b>HPGDS</b>	hematopoietic prostaglandin D synthase	10.42	0.000794
<b>MGST1</b>	microsomal glutathione S-transferase 1	1.46	0.00282

**Supplementary Table 7** - Genes in Ingenuity pathway analysis (IPA) canonical pathway ‘Glutathione redox reactions I’. Transcriptome-wide analysis of APO-SUS and APO-UNSUS medial prefrontal cortex (mPFC) combined with IPA revealed that this pathway represents the second-most significantly enriched pathway.

Symbol	Entrez Gene Name	Ratio	Corrected p-value
<b>GPX3</b>	glutathione peroxidase 3	-1.2	0.0183
<b>Gsta1</b>	glutathione S-transferase alpha 1	1.95	0.0264
<b>GSTA1</b>	glutathione S-transferase alpha 1	-2.01	0.00111

<b>Gstt1</b>	glutathione S-transferase, theta 1	-1.65	0.00713
<b>MGST1</b>	microsomal glutathione S-transferase 1	1.46	0.00282
<b>PRDX6</b>	peroxiredoxin 6	-1.64	0.00E+00

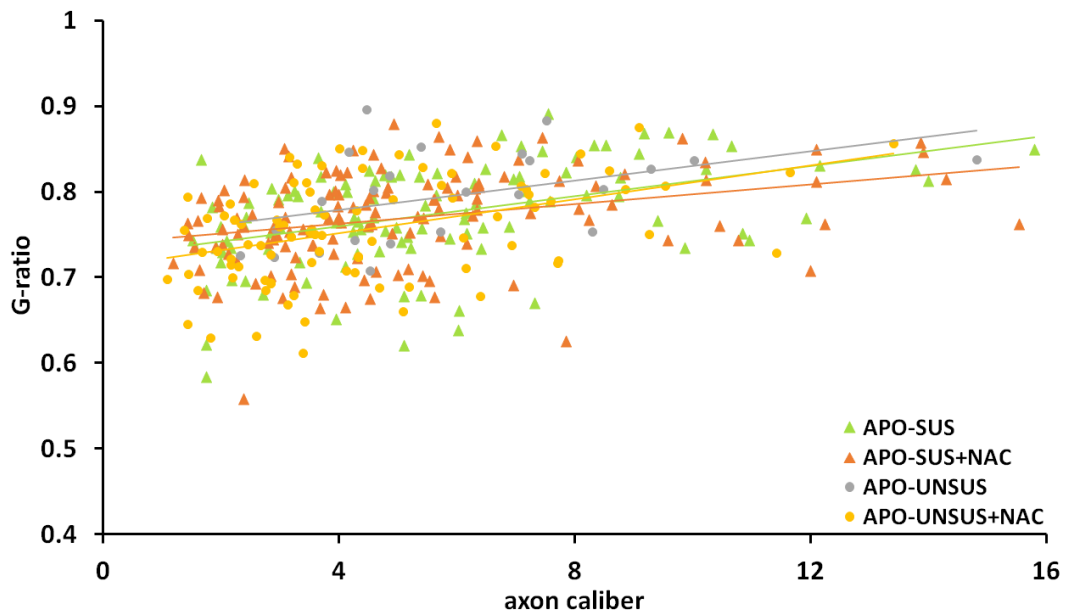
**Supplementary Table 8** - Top 35 most significantly differentially expressed genes in APO-SUS *versus* APO-UNSUS medial prefrontal cortex (mPFC) as revealed by RNA-sequencing analysis.

#	Ratio	Corrected p-value	Symbol	Gene name
1	-109.31	0.00E+00	<b>Igh-1a</b>	Immunoglobulin heavy chain 1a (serum IgG2a)
2	-68.51	0.00E+00	<b>690499</b>	rCG64263-like
3	-60.45	0.00E+00	<b>100361105</b>	Igh protein-like
4	12.53	0.00E+00	<b>Spag6</b>	Sperm associated antigen 6
5	-7.92	0.00E+00	<b>309627</b>	RT1 class I, locus A3
6	5.81	0.00E+00	<b>365613</b>	hypothetical gene supported by X60212
7	4.71	0.00E+00	<b>Vwa3a</b>	Von Willebrand factor A domain containing 3A
8	-4.6	0.00E+00	<b>Ghdc</b>	GH3 domain containing
9	-3.97	0.00E+00	<b>Prss12</b>	Protease, serine, 12 neurotrypsin (motopsin)
10	-3.42	0.00E+00	<b>Acsn3</b>	Acyl-CoA synthetase medium-chain family member 3
11	-3.05	0.00E+00	<b>LOC362863</b>	First gene upstream of Nt5dc3
12	3	0.00E+00	<b>RGD1303232</b>	Phytn_dehydro and Pyr_redox domain containing protein RGD1303232
13	-2.85	0.00E+00	<b>Aph1b</b>	Anterior pharynx defective 1 homolog B (C. elegans)
14	-2.81	0.00E+00	<b>306478</b>	ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit d-like 1
15	-2.69	0.00E+00	<b>368153</b>	RT1 class I, locus CE7
16	-2.63	0.00E+00	<b>292539</b>	similar to 60S ribosomal protein L17 (L23)
17	2.49	0.00E+00	<b>Ifi271</b>	Interferon, alpha-inducible protein 27-like
18	2.31	0.00E+00	<b>Zfp61</b>	Zinc finger protein 61

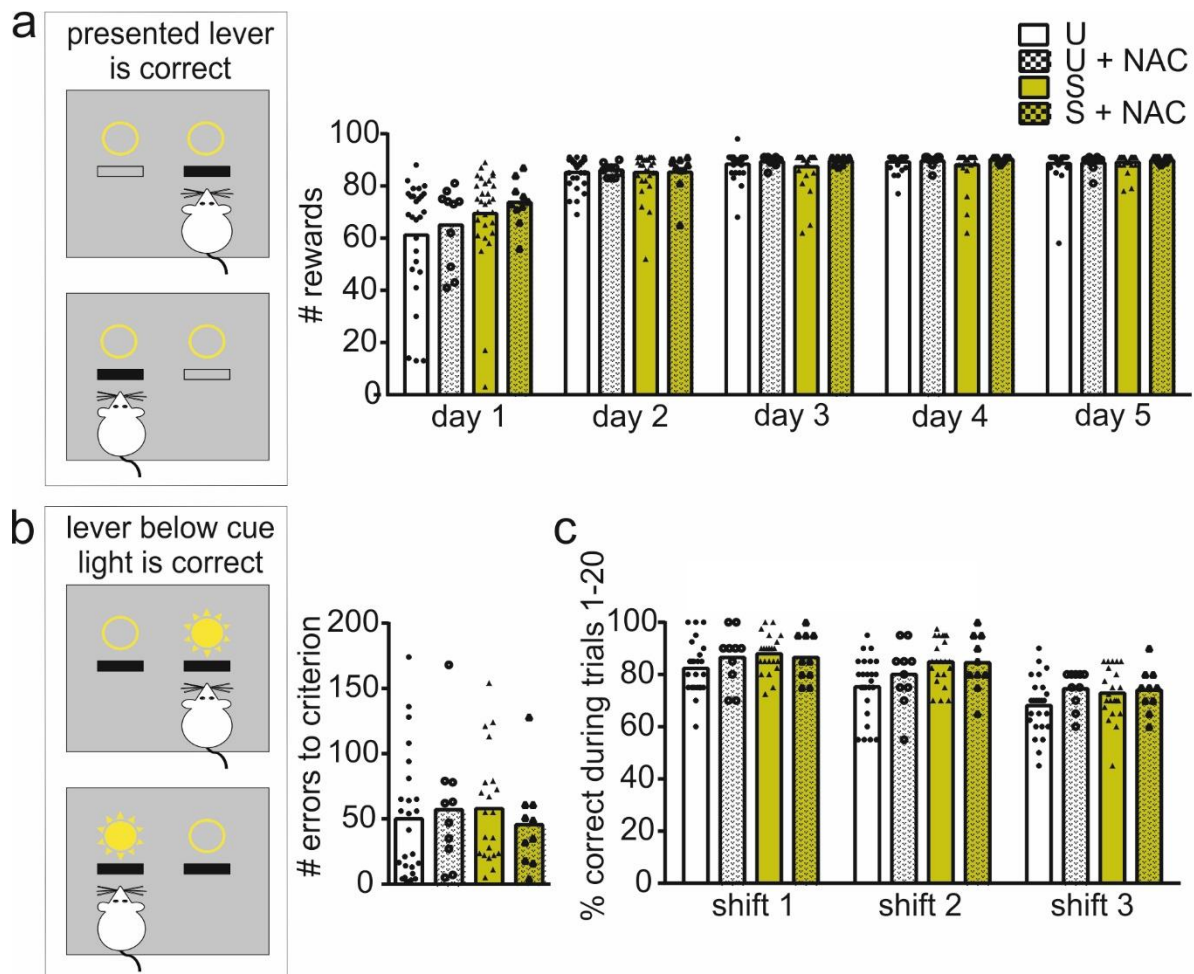


<b>19</b>	-2.28	0.00E+00	<b>Thumpd2</b>	THUMP domain containing 2
<b>20</b>	2.19	0.00E+00	<b>Rpl17</b>	Ribosomal protein L17
<b>21</b>	2.02	0.00E+00	<b>Pla2g4e</b>	Phospholipase A2, group IVE
<b>22</b>	-2	0.00E+00	<b>Pex11a</b>	Peroxisomal biogenesis factor 11 alpha
<b>23</b>	1.87	0.00E+00	<b>LOC361963</b>	Similar to phosphoglycerate mutase (EC 5.4.2.1) B chain – rat
<b>24</b>	-1.79	0.00E+00	<b>Mobp*</b>	Myelin-associated oligodendrocyte basic protein
<b>25</b>	-1.79	0.00E+00	<b>Cables2</b>	Cdk5 and Abl enzyme substrate 2
<b>26</b>	-1.77	0.00E+00	<b>St7l</b>	Suppression of tumorigenicity 7-like
<b>27</b>	1.75	0.00E+00	<b>100362124</b>	intraflagellar transport 140
<b>28</b>	-1.72	0.00E+00	<b>Tmem119</b>	Transmembrane protein 119
<b>29</b>	-1.71	0.00E+00	<b>RGD735029</b>	SEL1 domain containing protein RGD735029
<b>30</b>	-1.7	0.00E+00	<b>Opalin*</b>	Oligodendrocytic myelin paranodal and inner loop protein
<b>31</b>	-1.69	0.00E+00	<b>Mbp*</b>	Myelin basic protein
<b>32</b>	-1.67	0.00E+00	<b>Mag*</b>	Myelin-associated glycoprotein
<b>33</b>	-1.64	0.00E+00	<b>Prdx6</b>	Peroxiredoxin 6
<b>34</b>	-1.63	0.00E+00	<b>Plp1*</b>	Proteolipid protein 1
<b>35</b>	-1.59	0.00E+00	<b>Ptgds</b>	Prostaglandin D2 synthase (brain)

\* Myelin-related genes



**Supplementary Figure S1 – Myelin thickness is not affected in APO-SUS medial prefrontal cortex (mPFC).** G-ratio versus axon caliber for all myelinated axons in the infralimbic (IL) subregion of the mPFC of APO-SUS and APO-UNSUS rats with and without NAC treatment.



**Supplementary Figure S2 – No differences in the performance of APO-SUS and APO-UNSUS rats during retractable lever-press training and visual cue discrimination with and without NAC treatment.**

**(a)** Schematic representation and performance during retractable lever press training. Rats were required to press the lever they were presented with during 5 consecutive sessions, 1 session per day. The number of rewards in each session involving APO-SUS versus APO-UNSUS rats with and without NAC treatment is depicted. **(b)** Schematic representation and quantification of performance during the acquisition of the initial rule. Rats were required to press the lever above which a cue light was illuminated in order to receive a reward pellet. The number of errors until criterion of a streak of 10 correct trials was reached for APO-SUS and APO-UNSUS rats with and without NAC treatment is depicted (Two-way ANOVA interaction  $F[1,77]=0.603$ ,  $p=0.440$  main effects rat line  $F[1,77]=0.033$ ,  $p=0.857$  treatment  $F[1,77]=0.027$ ,  $p=0.870$ ). **(c)** Percentage correct trials during the first 20 trials of the extra-dimensional set-shifts in APO-SUS and APO-UNSUS rats with and without NAC treatment. Shift 1 Two-way ANOVA interaction  $F[1,77]=0.990$ ,  $p=0.323$  main effects rat line  $F[1,77]=0.990$ ,  $p=0.323$  treatment  $F[1,77]=0.280$ ,  $p=0.598$ ; Shift 2 Two-way ANOVA interaction  $F[1,77]=1.070$ ,  $p=0.304$  main effects rat line  $F[1,77]=6.457$ ,  $p=0.013$  treatment  $F[1,77]=0.489$ ,  $p=0.486$ ; Shift 3 Two-way ANOVA interaction  $F[1,77]=0.718$ ,  $p=0.400$  main effects rat line  $F[1,77]=0.439$ ,  $p=0.510$  treatment  $F[1,77]=1.792$ ,  $p=0.185$ .