

# Impact of translocator protein 18 kDa (TSPO)-deficiency on mitochondrial function and inflammatory state of human C20 microglia cells

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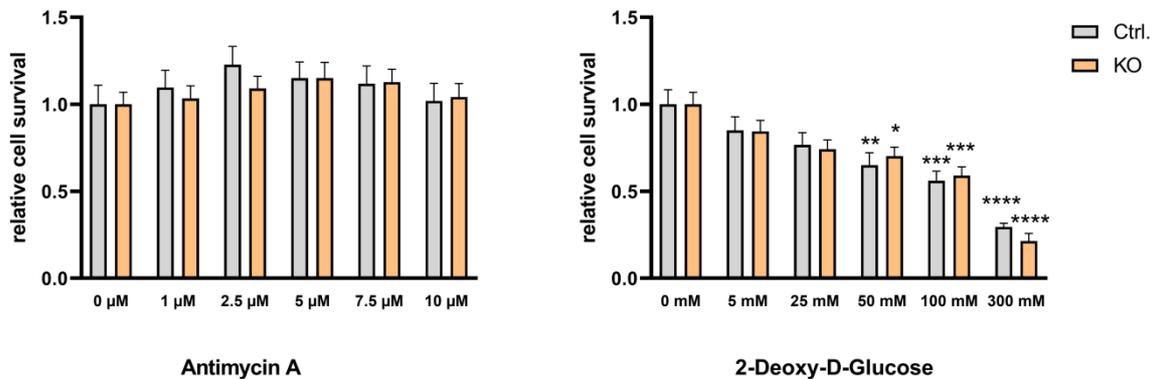
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## SUPPLEMENTARY INFORMATION

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**Supplementary Figure S1:** Effect of Antimycin A and 2-deoxy-D-glucose on viability of cytokine-stimulated TSPO-deficient and control C20 microglia cells. (A) Effect of Antimycin A (1 – 10 μM) or (B) 2-deoxy-D-glucose (5 – 300 mM) on cell viability was tested using the Resazurin assay with TSPO-deficient and control C20 cells. Cells were treated with cytokine cocktail TNF $\alpha$ , IL-1 $\beta$  and INF $\gamma$  (each 1 ng/ml) for 16 h. Values represent survival normalized to conditions without Antimycin A or 2-deoxy-D-glucose. Means and p values see supplementary table S1 and S2.

**Supplementary Table S1:** Effect of Antimycin A on viability of cytokine-stimulated TSPO-deficient (KO) and control C20 microglia cells (Viability assessed with Resazurin assay)

Cell line and condition	Antimycin A concentration	Mean and SEM	P-value compared to baseline at 0 $\mu$ M
Ctrl. basal	0 $\mu$ M	1.000 $\pm$ 0.069	-
Ctrl. basal	1 $\mu$ M	1.078 $\pm$ 0.070	>0.9999
Ctrl. basal	2.5 $\mu$ M	1.139 $\pm$ 0.081	0.9917
Ctrl. basal	5 $\mu$ M	1.146 $\pm$ 0.066	0.9870
Ctrl. basal	7.5 $\mu$ M	1.102 $\pm$ 0.078	0.9995
Ctrl. basal	10 $\mu$ M	1.008 $\pm$ 0.081	0.9999
KO basal	0 $\mu$ M	1.000 $\pm$ 0.098	-
KO basal	1 $\mu$ M	0.956 $\pm$ 0.103	>0.9999
KO basal	2.5 $\mu$ M	1.053 $\pm$ 0.088	>0.9999
KO basal	5 $\mu$ M	1.147 $\pm$ 0.098	0.9869
KO basal	7.5 $\mu$ M	1.154 $\pm$ 0.095	0.9805
KO basal	10 $\mu$ M	1.098 $\pm$ 0.093	0.9996
Ctrl. 16h activated	0 $\mu$ M	1.000 $\pm$ 0.110	-
Ctrl. 16h activated	1 $\mu$ M	1.097 $\pm$ 0.099	>0.9999
Ctrl. 16h activated	2.5 $\mu$ M	1.228 $\pm$ 0.105	0.8178
Ctrl. 16h activated	5 $\mu$ M	1.150 $\pm$ 0.094	0.9893
Ctrl. 16h activated	7.5 $\mu$ M	1.118 $\pm$ 0.102	0.9986
Ctrl. 16h activated	10 $\mu$ M	1.019 $\pm$ 0.102	>0.9999
KO 16h activated	0 $\mu$ M	1.000 $\pm$ 0.070	-
KO 16h activated	1 $\mu$ M	1.034 $\pm$ 0.072	>0.9999
KO 16h activated	2.5 $\mu$ M	1.091 $\pm$ 0.070	0.9999
KO 16h activated	5 $\mu$ M	1.150 $\pm$ 0.090	0.9890

KO 16h activated	7.5 $\mu$ M	1.128 $\pm$ 0.073	0.9971
KO 16h activated	10 $\mu$ M	1.042 $\pm$ 0.077	>0.9999
Ctrl. 40h activated	0 $\mu$ M	1.000 $\pm$ 0.077	-
Ctrl. 40h activated	1 $\mu$ M	1.024 $\pm$ 0.081	>0.9999
Ctrl. 40h activated	2.5 $\mu$ M	1.075 $\pm$ 0.077	>0.9999
Ctrl. 40h activated	5 $\mu$ M	1.026 $\pm$ 0.098	>0.9999
Ctrl. 40h activated	7.5 $\mu$ M	0.966 $\pm$ 0.076	>0.9999
Ctrl. 40h activated	10 $\mu$ M	0.926 $\pm$ 0.079	>0.9999
KO 40h activated	0 $\mu$ M	1.000 $\pm$ 0.082	-
KO 40h activated	1 $\mu$ M	0.947 $\pm$ 0.082	>0.9999
KO 40h activated	2.5 $\mu$ M	1.018 $\pm$ 0.103	>0.9999
KO 40h activated	5 $\mu$ M	0.982 $\pm$ 0.109	>0.9999
KO 40h activated	7.5 $\mu$ M	0.984 $\pm$ 0.104	>0.9999
KO 40h activated	10 $\mu$ M	0.897 $\pm$ 0.091	0.9995

**Supplementary Table S2:** Effect of 2-Deoxy-D-glucose on viability of cytokine-stimulated TSPO-deficient (KO) and control C20 microglia cells (Viability assessed with Resazurin assay)

Cell line and condition	2-Deoxy-D-Glucose concentration	Mean and SEM	P-value compared to baseline at 0 mM
Ctrl. basal	0 mM	1.000 ±0.0708	-
Ctrl. basal	5 mM	0.859 ±0.0731	0.8190
Ctrl. basal	25 mM	0.793 ±0.0786	0.4263
KO basal	0 mM	1.000 ±0.054	-
KO basal	5 mM	0.891 ±0.054	0.9819
KO basal	25 mM	0.819 ±0.047	0.6296
KO basal	50 mM	0.719 ±0.051	0.0752
Ctrl. 16h activated	0 mM	1.000 ±0.083	-
Ctrl. 16h activated	5 mM	0.850 ±0.077	0.8464
Ctrl. 16h activated	25 mM	0.767 ±0.070	0.2522
KO 16h activated	0 mM	1.000 ±0.069	-
KO 16h activated	5 mM	0.844 ±0.063	0.8088
KO 16h activated	25 mM	0.742 ±0.053	0.1385
Ctrl. 40h activated	0 mM	1.000 ±0.087	-
Ctrl. 40h activated	5 mM	0.895 ±0.083	0.9960
Ctrl. 40h activated	25 mM	0.775 ±0.080	0.5332
Ctrl. 40h activated	50 mM	0.668 ±0.076	0.0668
KO 40h activated	0 mM	1.000 ±0.089	-
KO 40h activated	5 mM	0.897 ±0.070	0.9966
KO 40h activated	25 mM	0.806 ±0.062	0.7387
KO 40h activated	50 mM	0.678 ±0.086	0.0668

**Supplementary Table S3:** Cytokine mRNA expression in TSPO-deficient human C20 microglia cells and control cells after treatment with cytokine cocktail (16 h) and related to unstimulated conditions of control or TSPO-KO cells

	<b>Control mean</b>	<b>Control P-value</b>	<b>KO mean</b>	<b>KO P-value</b>
<b>TNF<math>\alpha</math></b>	13.71 $\pm$ 2.845	0.0066	62.70 $\pm$ 14.62	0.0014
<b>IL6</b>	1129 $\pm$ 119.4	0.0002	1007 $\pm$ 155.2	<0.0001
<b>IL12A</b>	7.301 $\pm$ 0.7202	0.0003	4.233 $\pm$ 0.3540	<0.0001
<b>IL1<math>\beta</math></b>	219 $\pm$ 36.70	0.0019	375.3 $\pm$ 29.78	<0.0001
<b>IL8</b>	1368 $\pm$ 164.3	0.0011	2348 $\pm$ 291.6	<0.0001
<b>NOS2</b>	2.776 $\pm$ 0.5568	0.0243	2.851 $\pm$ 0.4841	0.0065