

Supplemental Digital Content 3

Quantitative analysis results on mitochondrial morphology and motility using our in-house developed semi-automated image analysis tool

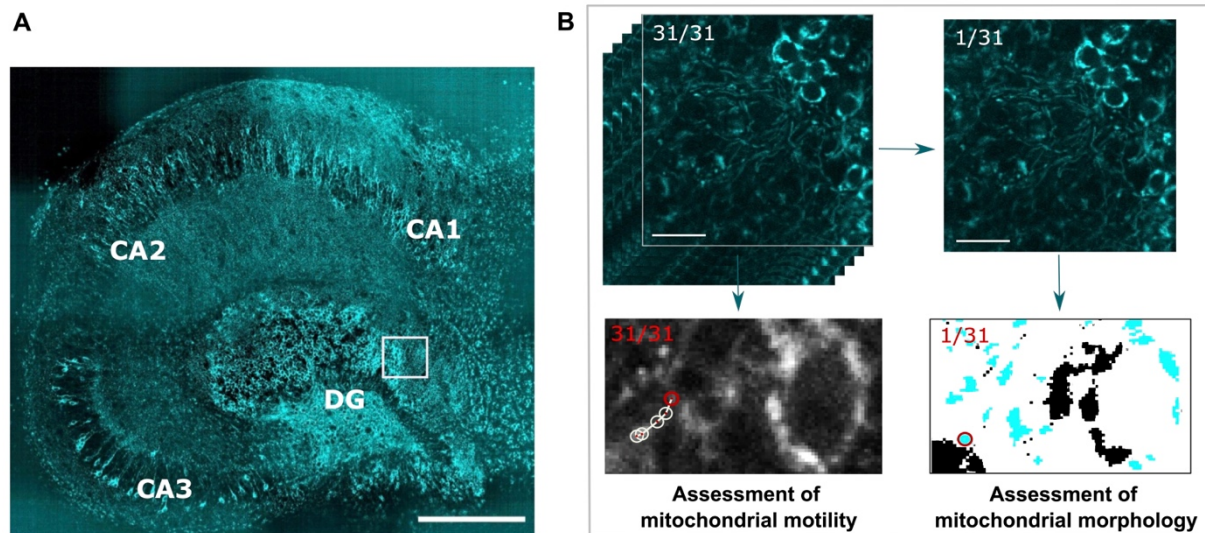


Figure SDC_2. Analysis of mitochondrial dynamics in chronic hippocampal slices generated from *MitoCFP* mice. **A.** Exemplary stitched image of hippocampal section (fluorescence microscopy, CFP channel) indicating the DG as well as the three subfields of cornu ammonis (CA1, CA2, and CA3). *Scale bar: 300 μm .* **B.** Illustration of the major steps of semi-automated image analysis. First, mitochondrial motility was assessed in 3D live imaging stacks consisting of 31 images, respectively. Parameters for particle tracking were set to a blob diameter of 1.5 μm and a displacement threshold of above 1.5 μm . Second, for morphological analysis, only the first of 31 images per stack was considered and the particle size threshold was set to an area of 0.2 μm^2 to 5 μm^2 . Metrics for mitochondrial speed and displacement, mitochondrial area, length and aspect ratio, were extracted and the fraction of motile mitochondria was computed. *Scale bar 20 μm .* More details on the semi-automated analysis are given in (53).

Table SDC_3. Summary of semi-automated analysis results on mitochondrial morphology.

	-TNF α			+ TNF α			-TNF α vs. + TNF α
	<i>mean $\pm$ SD</i>	<i>median</i>	<i>KW</i>	<i>mean $\pm$ SD</i>	<i>median</i>	<i>KW</i>	<i>MW</i>
<u>Mitochondrial area (μm^2)</u>							
1. Negative control	0.896 $\pm$ 0.460	0.848	-	0.790 $\pm$ 0.476	0.724	-	****
2. 0.1 mM gadopentetate	0.682 $\pm$ 0.413	0.578	****	0.877 $\pm$ 0.377	0.838	****	****
3. 1 mM gadopentetate	0.734 $\pm$ 0.394	0.673	****	0.732 $\pm$ 0.400	0.674	>0.1	>0.1
4. 10 mM gadopentetate	0.741 $\pm$ 0.467	0.665	****	0.929 $\pm$ 0.567	0.858	****	****
5. 50 mM gadopentetate	0.726 $\pm$ 0.513	0.625	****	0.669 $\pm$ 0.448	0.578	****	***
6. 0.1 mM gadobutrol	0.769 $\pm$ 0.493	0.694	****	0.716 $\pm$ 0.439	0.636	****	***
7. 1 mM gadobutrol	0.756 $\pm$ 0.432	0.681	****	0.754 $\pm$ 0.515	0.684	**	>0.1
8. 10 mM gadobutrol	0.848 $\pm$ 0.405	0.787	>0.1	0.779 $\pm$ 0.394	0.713	>0.1	****
9. 50 mM gadobutrol	0.758 $\pm$ 0.380	0.703	****	0.676 $\pm$ 0.366	0.617	****	****
<u>Mitochondrial length (μm)</u>							
1. Negative control	1.580 $\pm$ 0.441	1.547	-	1.475 $\pm$ 0.449	1.447	-	****
2. 0.1 mM gadopentetate	1.418 $\pm$ 0.456	1.344	****	1.554 $\pm$ 0.351	1.526	****	****
3. 1 mM gadopentetate	1.429 $\pm$ 0.384	1.391	****	1.431 $\pm$ 0.398	1.402	>0.1	>0.1
4. 10 mM gadopentetate	1.439 $\pm$ 0.452	1.378	****	1.582 $\pm$ 0.506	1.547	****	****
5. 50 mM gadopentetate	1.402 $\pm$ 0.481	1.336	****	1.376 $\pm$ 0.442	1.321	****	>0.1
6. 0.1 mM gadobutrol	1.470 $\pm$ 0.483	1.437	****	1.412 $\pm$ 0.424	1.342	****	****
7. 1 mM gadobutrol	1.455 $\pm$ 0.436	1.402	****	1.453 $\pm$ 0.516	1.419	>0.1	>0.1
8. 10 mM gadobutrol	1.521 $\pm$ 0.375	1.447	*	1.473 $\pm$ 0.373	1.429	>0.1	***
9. 50 mM gadobutrol	1.446 $\pm$ 0.357	1.412	****	1.385 $\pm$ 0.362	1.343	****	****
<u>Aspect ratio (1-∞)</u>							
1. Negative control	2.085 $\pm$ 0.277	2.058	-	2.108 $\pm$ 0.328	2.069	-	>0.1
2. 0.1 mM gadopentetate	2.151 $\pm$ 0.336	2.115	****	2.082 $\pm$ 0.258	2.071	>0.1	****
3. 1 mM gadopentetate	2.119 $\pm$ 0.287	2.096	***	2.116 $\pm$ 0.307	2.091	>0.1	>0.1
4. 10 mM gadopentetate	2.116 $\pm$ 0.309	2.092	*	2.062 $\pm$ 0.350	2.028	*	****
5. 50 mM gadopentetate	2.095 $\pm$ 0.331	2.049	>0.1	2.132 $\pm$ 0.335	2.085	*	****
6. 0.1 mM gadobutrol	2.124 $\pm$ 0.357	2.078	*	2.118 $\pm$ 0.320	2.087	>0.1	>0.1
7. 1 mM gadobutrol	2.131 $\pm$ 0.335	2.083	**	2.138 $\pm$ 0.380	2.075	>0.1	>0.1
8. 10 mM gadobutrol	2.088 $\pm$ 0.259	2.069	>0.1	2.112 $\pm$ 0.247	2.097	>0.1	**
9. 50 mM gadobutrol	2.110 $\pm$ 0.268	2.089	>0.1	2.131 $\pm$ 0.270	2.112	****	**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; SD, standard deviation; KW, Kruskal–Wallis test; MW, Mann-Whitney test (uncorrected).

Table SDC_4. Summary of semi-automated analysis results on mitochondrial motility.

		-TNF α			+ TNF α			-TNF α vs. + TNF α
		<i>mean $\pm$ SD</i>	<i>median</i>	<i>KW</i>	<i>mean $\pm$ SD</i>	<i>median</i>	<i>KW</i>	<i>MW</i>
<u>Track speed (μm/s)</u>								
1.	Negative control	0.182 $\pm$ 0.125	0.137	-	0.177 $\pm$ 0.124	0.134	-	>0.1
2.	0.1 mM gadopentetate	0.185 $\pm$ 0.121	0.146	>0.1	0.183 $\pm$ 0.123	0.141	>0.1	>0.1
3.	1 mM gadopentetate	0.193 $\pm$ 0.124	0.149	**	0.180 $\pm$ 0.122	0.138	>0.1	***
4.	10 mM gadopentetate	0.202 $\pm$ 0.128	0.155	****	0.178 $\pm$ 0.125	0.131	>0.1	****
5.	50 mM gadopentetate	0.166 $\pm$ 0.127	0.126	****	0.211 $\pm$ 0.127	0.179	****	****
6.	0.1 mM gadobutrol	0.195 $\pm$ 0.127	0.150	***	0.199 $\pm$ 0.128	0.155	****	>0.1
7.	1 mM gadobutrol	0.190 $\pm$ 0.126	0.144	>0.1	0.196 $\pm$ 0.127	0.150	****	*
8.	10 mM gadobutrol	0.187 $\pm$ 0.122	0.145	>0.1	0.187 $\pm$ 0.123	0.145	*	>0.1
9.	50 mM gadobutrol	0.196 $\pm$ 0.463	0.126	**	0.210 $\pm$ 0.126	0.167	****	****
<u>Track displacement (μm)</u>								
1.	Negative control	1.946 $\pm$ 0.448	1.838	-	1.962 $\pm$ 0.446	1.866	-	>0.1
2.	0.1 mM gadopentetate	1.962 $\pm$ 0.435	1.850	>0.1	1.990 $\pm$ 0.487	1.866	>0.1	>0.1
3.	1 mM gadopentetate	1.980 $\pm$ 0.491	1.844	>0.1	1.961 $\pm$ 0.453	1.842	>0.1	>0.1
4.	10 mM gadopentetate	1.960 $\pm$ 0.449	1.843	>0.1	1.942 $\pm$ 0.423	1.828	>0.1	>0.1
5.	50 mM gadopentetate	1.935 $\pm$ 0.401	1.839	>0.1	2.009 $\pm$ 0.511	1.874	>0.1	**
6.	0.1 mM gadobutrol	1.976 $\pm$ 0.466	1.858	>0.1	1.970 $\pm$ 0.446	1.848	>0.1	>0.1
7.	1 mM gadobutrol	1.965 $\pm$ 0.434	1.851	>0.1	1.955 $\pm$ 0.443	1.839	>0.1	>0.1
8.	10 mM gadobutrol	1.963 $\pm$ 0.458	1.843	>0.1	1.957 $\pm$ 0.452	1.836	>0.1	>0.1
9.	50 mM gadobutrol	1.963 $\pm$ 0.463	1.836	>0.1	2.003 $\pm$ 0.487	1.869	>0.1	**
<u>Fraction of motile mitoch.</u>								
1.	Negative control	0.128 $\pm$ 0.052	0.123		0.130 $\pm$ 0.053	0.139		>0.1
2.	0.1 mM gadopentetate	0.114 $\pm$ 0.052	0.109	>0.1	0.183 $\pm$ 0.048	0.190	>0.1	**
3.	1 mM gadopentetate	0.141 $\pm$ 0.032	0.148	>0.1	0.104 $\pm$ 0.046	0.093	>0.1	*
4.	10 mM gadopentetate	0.100 $\pm$ 0.057	0.094	>0.1	0.144 $\pm$ 0.100	0.097	>0.1	>0.1
5.	50 mM gadopentetate	0.078 $\pm$ 0.038	0.087	>0.1	0.093 $\pm$ 0.054	0.094	>0.1	>0.1
6.	0.1 mM gadobutrol	0.154 $\pm$ 0.044	0.162	>0.1	0.145 $\pm$ 0.117	0.122	>0.1	>0.1
7.	1 mM gadobutrol	0.188 $\pm$ 0.057	0.176	>0.1	0.152 $\pm$ 0.068	0.133	>0.1	>0.1
8.	10 mM gadobutrol	0.180 $\pm$ 0.073	0.175	>0.1	0.175 $\pm$ 0.055	0.186	>0.1	>0.1
9.	50 mM gadobutrol	0.098 $\pm$ 0.078	0.075	>0.1	0.254 $\pm$ 0.136	0.230	>0.1	**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; SD, standard deviation; KW, Kruskal–Wallis test. MW, Mann-Whitney test (uncorrected).