

Supplemental Digital Content 4

Results of manual mitochondrial segmentation and tracking, and comparison to findings from semi-automated analysis of mitochondrial dynamics

Manual and semi-automated ImageJ analyses yielded consistent results. For the manual workflow, each ROI ($n = 12$ per condition) was segmented/tracked twice, independently and in random order. Figures SDC_3 and SDC_4 show the average of the two round-wise means across all 12 ROIs for each treatment condition to provide a compact, condition-level summary. In addition, Tables SDC_5 and SDC_6 report the ROI-wise averages (two manual rounds averaged within each ROI, mean $\pm$ SD) to preserve per-ROI detail.

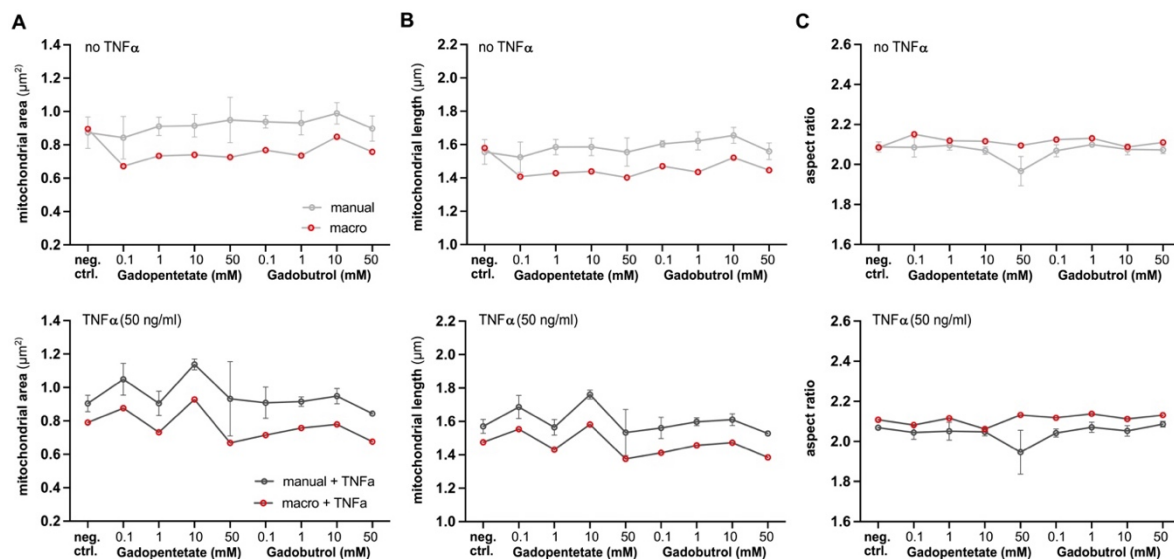


Figure SDC_3. Illustration of semi-automated and manual analysis results on GBCA-induced changes in mitochondrial morphology. Graphs show means per treatment condition *ex vivo* (average of means $\pm$ SD for repeated independent manual analyses ($n=2$) is shown).

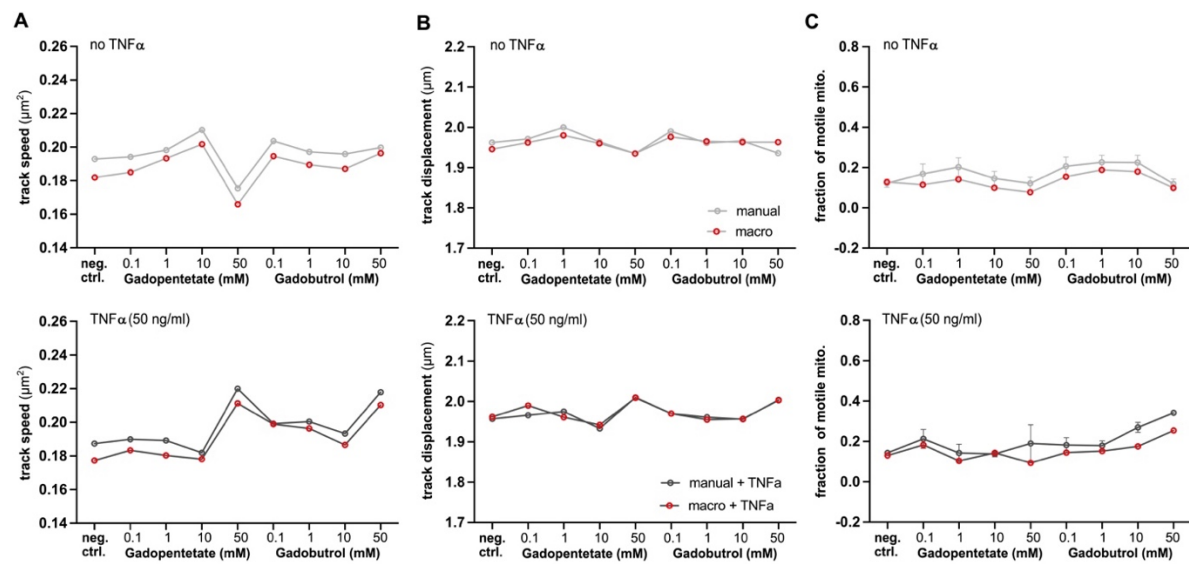


Figure SDC_4. Illustration of semi-automated and manual analysis results on GBCA-induced changes in mitochondrial motility. Graphs show means per treatment condition *ex vivo* (average of means $\pm$ SD for repeated independent manual analyses ($n=2$) is shown). Manual mitochondrial tracking was only performed once per ROI ($n=1$ for track speed and displacement).

Table SDC_5. Manual segmentation 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	0.898 $\pm$ 0.069	0.928	-	0.980 $\pm$ 0.139	0.951	-	>0.1
2.	0.1 mM gadopentetate	0.887 $\pm$ 0.122	0.938	>0.1	1.047 $\pm$ 0.104	1.077	>0.1	*
3.	1 mM gadopentetate	0.894 $\pm$ 0.051	0.900	>0.1	0.938 $\pm$ 0.082	0.960	>0.1	>0.1
4.	10 mM gadopentetate	0.967 $\pm$ 0.096	0.966	>0.1	1.104 $\pm$ 0.073	1.122	>0.1	***
5.	50 mM gadopentetate	0.987 $\pm$ 0.117	1.006	>0.1	1.011 $\pm$ 0.190	1.117	>0.1	>0.1
6.	0.1 mM gadobutrol	0.975 $\pm$ 0.148	0.995	>0.1	0.946 $\pm$ 0.111	0.944	>0.1	>0.1
7.	1 mM gadobutrol	0.990 $\pm$ 0.081	0.987	>0.1	0.960 $\pm$ 0.098	0.994	>0.1	>0.1
8.	10 mM gadobutrol	0.981 $\pm$ 0.056	0.982	>0.1	0.958 $\pm$ 0.070	0.962	>0.1	>0.1
9.	50 mM gadobutrol	0.918 $\pm$ 0.065	0.926	>0.1	0.869 $\pm$ 0.132	0.821	>0.1	>0.1
<u>Mitochondrial length (μm)</u>								
1.	Negative control	1.578 $\pm$ 0.052	1.603	-	1.625 $\pm$ 0.099	1.607	-	>0.1
2.	0.1 mM gadopentetate	1.562 $\pm$ 0.101	1.602	>0.1	1.689 $\pm$ 0.084	1.700	>0.1	**
3.	1 mM gadopentetate	1.575 $\pm$ 0.056	1.593	>0.1	1.596 $\pm$ 0.059	1.607	>0.1	>0.1
4.	10 mM gadopentetate	1.630 $\pm$ 0.078	1.628	>0.1	1.734 $\pm$ 0.057	1.754	*	***
5.	50 mM gadopentetate	1.579 $\pm$ 0.077	1.594	>0.1	1.588 $\pm$ 0.117	1.598	>0.1	>0.1
6.	0.1 mM gadobutrol	1.635 $\pm$ 0.117	1.657	>0.1	1.590 $\pm$ 0.074	1.588	>0.1	>0.1
7.	1 mM gadobutrol	1.662 $\pm$ 0.044	1.668	*	1.628 $\pm$ 0.082	1.638	>0.1	>0.1
8.	10 mM gadobutrol	1.649 $\pm$ 0.027	1.660	*	1.624 $\pm$ 0.055	1.615	>0.1	*
9.	50 mM gadobutrol	1.580 $\pm$ 0.040	1.579	>0.1	1.546 $\pm$ 0.101	1.522	>0.1	>0.1
<u>Aspect ratio (1-∞)</u>								
1.	Negative control	2.086 $\pm$ 0.032	2.087	-	2.052 $\pm$ 0.054	2.053	-	>0.1
2.	0.1 mM gadopentetate	2.081 $\pm$ 0.040	2.081	>0.1	2.053 $\pm$ 0.042	2.067	>0.1	>0.1
3.	1 mM gadopentetate	2.098 $\pm$ 0.039	2.102	>0.1	2.060 $\pm$ 0.049	2.068	>0.1	>0.1
4.	10 mM gadopentetate	2.067 $\pm$ 0.027	2.069	>0.1	2.049 $\pm$ 0.025	2.049	>0.1	>0.1
5.	50 mM gadopentetate	1.961 $\pm$ 0.054	1.945	***	1.932 $\pm$ 0.106	1.924	**	>0.1
6.	0.1 mM gadobutrol	2.064 $\pm$ 0.043	2.064	>0.1	2.036 $\pm$ 0.057	2.033	>0.1	>0.1
7.	1 mM gadobutrol	2.087 $\pm$ 0.059	2.099	>0.1	2.070 $\pm$ 0.033	2.062	>0.1	>0.1
8.	10 mM gadobutrol	2.075 $\pm$ 0.041	2.085	>0.1	2.063 $\pm$ 0.029	2.060	>0.1	>0.1
9.	50 mM gadobutrol	2.058 $\pm$ 0.042	2.060	>0.1	2.078 $\pm$ 0.037	2.073	>0.1	>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).

Table SDC_6. Manual segmentation results on mitochondrial motility

		-TNFα			+ TNFα			-TNFα vs. + TNFα
		<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	0.193 $\pm$ 0.128	0.150	-	0.187 $\pm$ 0.129	0.139	-	>0.1
2.	0.1 mM gadopentetate	0.194 $\pm$ 0.124	0.154	>0.1	0.190 $\pm$ 0.126	0.145	>0.1	>0.1
3.	1 mM gadopentetate	0.198 $\pm$ 0.125	0.153	>0.1	0.189 $\pm$ 0.129	0.139	>0.1	>0.1
4.	10 mM gadopentetate	0.210 $\pm$ 0.130	0.164	>0.1	0.181 $\pm$ 0.126	0.136	>0.1	***
5.	50 mM gadopentetate	0.175 $\pm$ 0.132	0.131	>0.1	0.220 $\pm$ 0.129	0.192	****	****
6.	0.1 mM gadobutrol	0.201 $\pm$ 0.129	0.158	>0.1	0.199 $\pm$ 0.130	0.152	>0.1	>0.1
7.	1 mM gadobutrol	0.197 $\pm$ 0.130	0.151	>0.1	0.200 $\pm$ 0.127	0.155	>0.1	>0.1
8.	10 mM gadobutrol	0.196 $\pm$ 0.126	0.153	>0.1	0.193 $\pm$ 0.123	0.151	>0.1	>0.1
9.	50 mM gadobutrol	0.200 $\pm$ 0.127	0.154	>0.1	0.217 $\pm$ 0.126	0.176	****	*
<u>Track displacement (μm)</u>								
1.	Negative control	1.962 $\pm$ 0.465	1.858	-	1.957 $\pm$ 0.441	1.853	-	>0.1
2.	0.1 mM gadopentetate	1.971 $\pm$ 0.441	1.856	>0.1	1.966 $\pm$ 0.442	1.852	>0.1	>0.1
3.	1 mM gadopentetate	1.999 $\pm$ 0.516	1.852	>0.1	1.975 $\pm$ 0.478	1.835	>0.1	>0.1
4.	10 mM gadopentetate	1.964 $\pm$ 0.441	1.853	>0.1	1.933 $\pm$ 0.408	1.828	>0.1	>0.1
5.	50 mM gadopentetate	1.935 $\pm$ 0.393	1.841	>0.1	2.010 $\pm$ 0.412	1.875	>0.1	*
6.	0.1 mM gadobutrol	1.990 $\pm$ 0.461	1.872	>0.1	1.970 $\pm$ 0.457	1.848	>0.1	>0.1
7.	1 mM gadobutrol	1.961 $\pm$ 0.421	1.859	>0.1	1.961 $\pm$ 0.458	1.841	>0.1	>0.1
8.	10 mM gadobutrol	1.966 $\pm$ 0.452	1.850	>0.1	1.956 $\pm$ 0.437	1.845	>0.1	>0.1
9.	50 mM gadobutrol	1.936 $\pm$ 0.418	1.833	>0.1	2.004 $\pm$ 0.480	1.872	>0.1	****
<u>Fraction of motile mitoch.</u>								
1.	Negative control	0.122 $\pm$ 0.049	0.113	-	0.143 $\pm$ 0.043	0.161	-	>0.1
2.	0.1 mM gadopentetate	0.169 $\pm$ 0.055	0.166	>0.1	0.212 $\pm$ 0.066	0.225	>0.1	>0.1
3.	1 mM gadopentetate	0.202 $\pm$ 0.049	0.196	>0.1	0.143 $\pm$ 0.085	0.119	>0.1	**
4.	10 mM gadopentetate	0.146 $\pm$ 0.056	0.148	>0.1	0.138 $\pm$ 0.068	0.109	>0.1	>0.1
5.	50 mM gadopentetate	0.121 $\pm$ 0.055	0.134	>0.1	0.190 $\pm$ 0.114	0.152	>0.1	>0.1
6.	0.1 mM gadobutrol	0.207 $\pm$ 0.094	0.206	>0.1	0.182 $\pm$ 0.093	0.156	>0.1	>0.1
7.	1 mM gadobutrol	0.227 $\pm$ 0.055	0.222	**	0.179 $\pm$ 0.075	0.149	>0.1	>0.1
8.	10 mM gadobutrol	0.225 $\pm$ 0.097	0.236	*	0.269 $\pm$ 0.051	0.231	>0.1	>0.1
9.	50 mM gadobutrol	0.119 $\pm$ 0.094	0.085	>0.1	0.342 $\pm$ 0.145	0.323	**	***

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