

## Supplementary Materials

### **Metformin improves cognitive functions and reduces neuroinflammation after traumatic brain injury**

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## Supplementary Figure Legends

**Supplemental Figure 1. Overall Experimental Design.** Timeline of surgery, treatments and behavioral testing.

**Supplemental Figure 2. Memory retained in both vehicle and metformin treated injured animals.** Following six days of Morris Water Maze training, adult CD-1 mice were tested using a probe trial on day 7. The platform was removed from the target quadrant. Animals were placed in the center of the pool and allowed to swim for 60 seconds. The amount of time spent and number of crossings into the target quadrant and overall swim speed were measured. Data represent the mean  $\pm$ SEM. A 2-way ANOVA was performed. N (animals = Vehicle: Naive=8, Sham=12, TBI=10; Metformin: Naive=10, Sham=12, TBI=11).

**Supplemental Figure 3. Animals show no deficiencies in motor function following CCI-TBI.** Adult CD-1 mice were subjected to the pole test at 1, 4 and 7 days post-surgery to examine motor skills and coordination. No significant changes in latency to reach the floor were observed, suggesting that the mice have no deficiency in motor function skills following a CCI-TBI. Data represents the mean  $\pm$  SEM. A 2-way ANOVA was performed. N (animals = Vehicle: Naive=2, Sham=3, TBI=3; Metformin: Naive=2, Sham=3, TBI=5).

**Supplemental Figure 4. Nesting behavior as a measure for stress.** Adult CD-1 mice had their home cage nests ranked each morning, prior to all other testing, starting 1-day post surgery to assess for stress, anxiety, and other emotional disturbances. Nests were ranked on a scale from 1 to 5, with a score of 1 representing no nest and a score of 5 representing a full nest. Old nestlets were removed and new nestlets replaced each morning after scoring.

**Supplemental Figure 5. No significant change in tissue disruption following Controlled Cortical Impact (CCI) injury between vehicle or metformin treatments. Light microscope images were acquired from brain sections at the center of the impact and the percent area of cortical disruption was quantified using ImageJ. No significant difference in % area was found between vehicle or metformin treated CCI-TBI mice. Data represent the percent average  $\pm$  SEM. N= vehicle:15; metformin:17.**

**Supplemental Figure 6. Microglia response following treatment with metformin post-surgery.** Naïve, sham-operated control or CCI-TBI mice were treated with either vehicle or metformin beginning at 1-hour post-surgery and daily thereafter. At 7 days post-injury, mice were sacrificed, their brains sectioned and immunostained for microglia marker Iba1 (red) and nuclear marker DAPI (cyan). Confocal images were acquired of the ipsi-lateral cortex for all mouse tissue at 20x. Scale bar = 30 $\mu$ m.

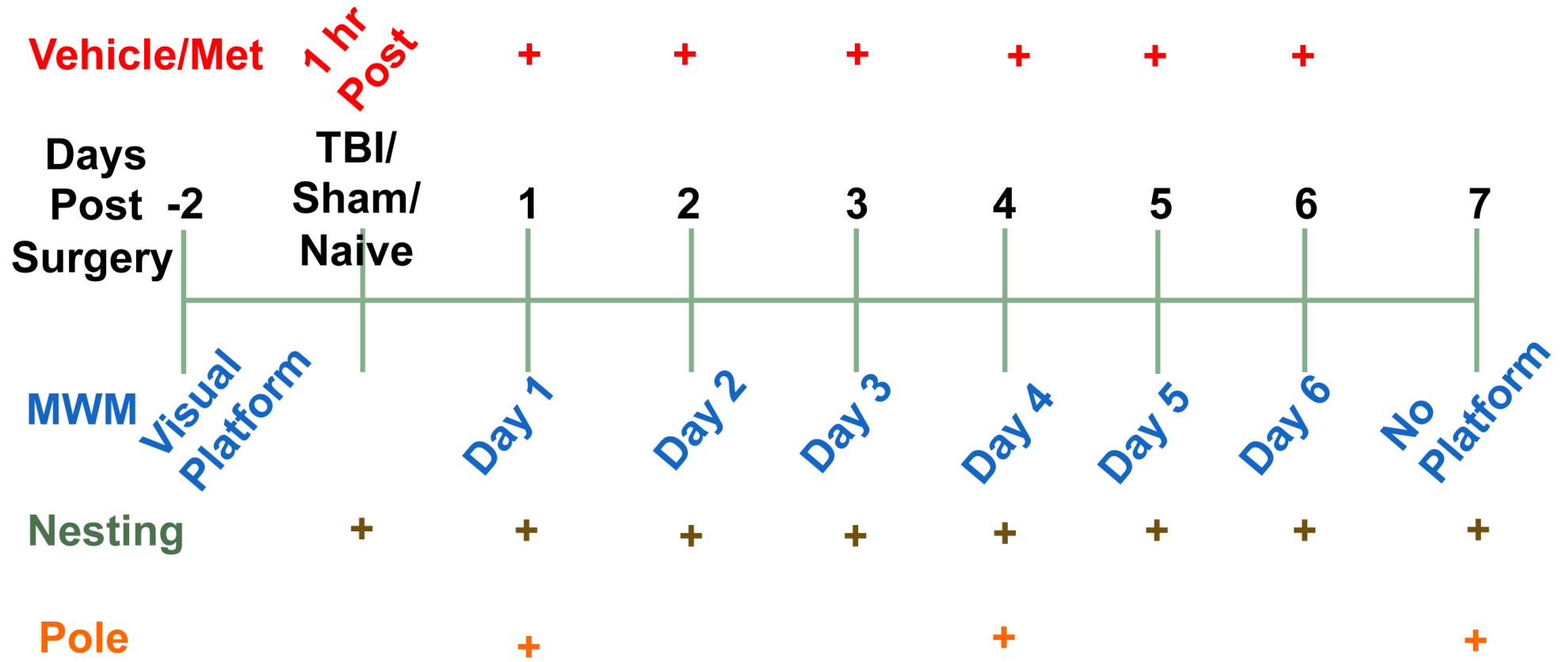
## Supplementary Figure Legends

[Supplemental Figure 7. 3D renderings of microglia following treatment with metformin. 3D renderings of individual microglia were created using Neurolucida software. Shown are representative reconstructions of microglia from naïve, sham-operated control or CCI-TBI mice following treatment with either vehicle or metformin.](#)

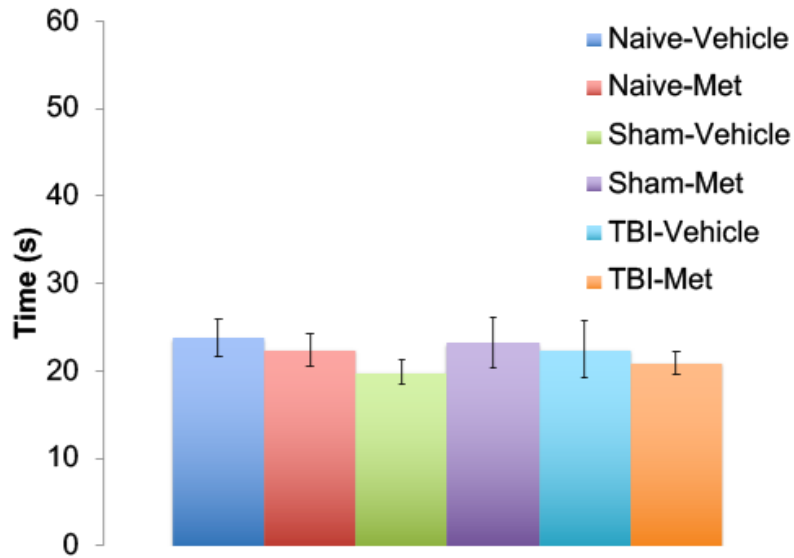
**Supplemental Figure 8. No significant change in microglia density following metformin treatment.** Confocal images were acquired of the ipsi-lateral cortex of CCI-TBI, sham-operated and naïve control mouse tissue and total microglia density manually quantified using ImageJ. No significant difference in microglia density was found between vehicle or metformin treated Naïve, sham-operated control or CCI-TBI mice.

Data represent the mean  $\pm$  SEM. A 2-way ANOVA was performed. N (animals/images) = Vehicle: Naive=8/30, Sham=11/33, TBI=10/31; Metformin: Naive=10/27, Sham=12/36, TBI=10/35.

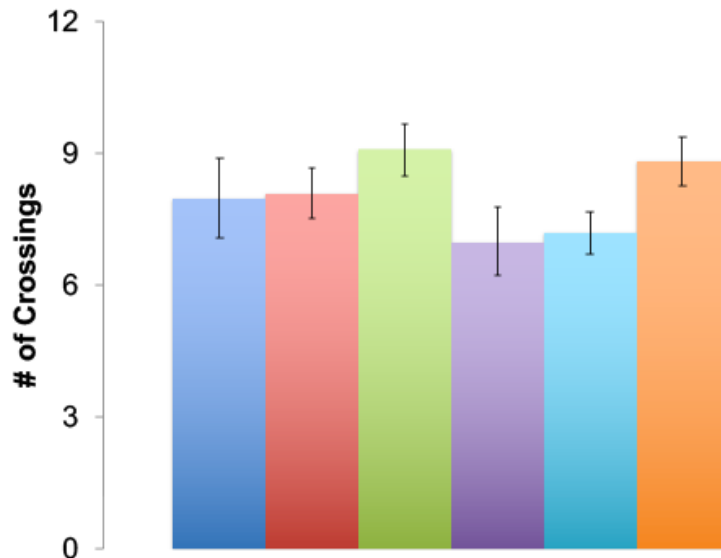
Supplemental Figure 1



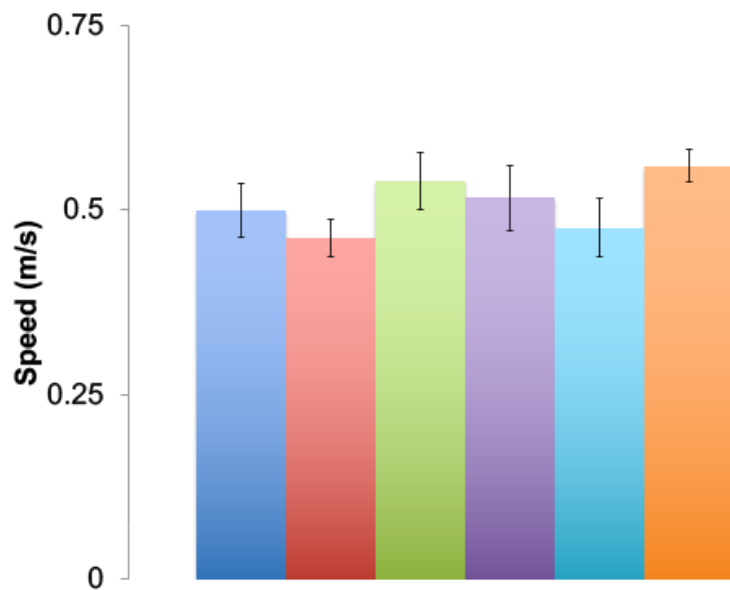
**Supplemental Figure 2 Morris Water Maze - 7 day hidden probe trial - Average time spent in target quadrant**



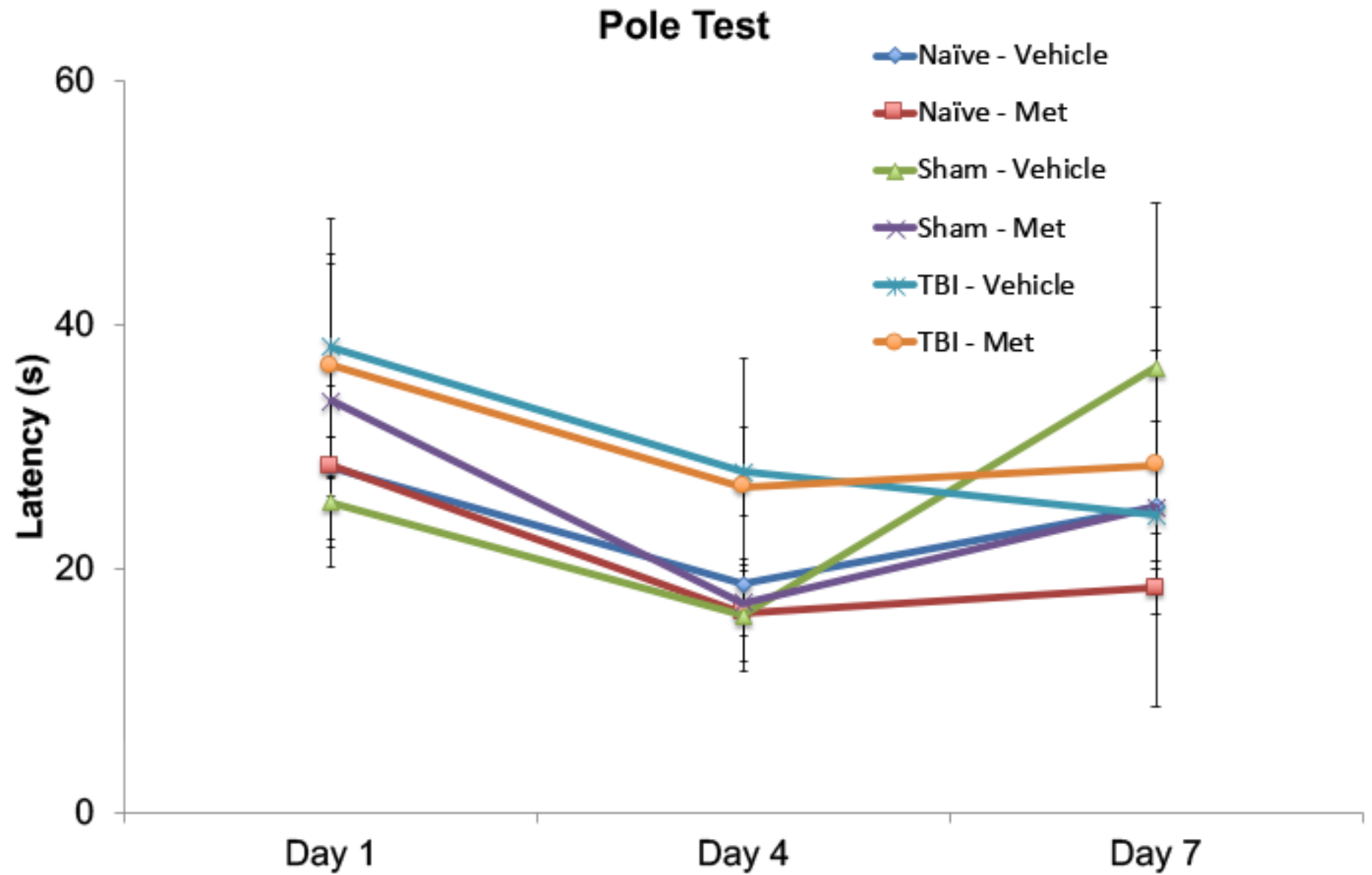
**Morris Water Maze - 7 day hidden probe trial - Average # of crossings into target quadrant**



**Morris Water Maze - 7 day hidden probe trial - Mean swim speed of overall trial**



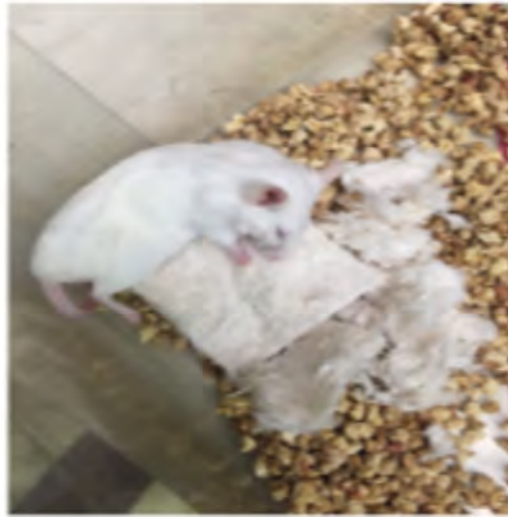
Supplemental Figure 3



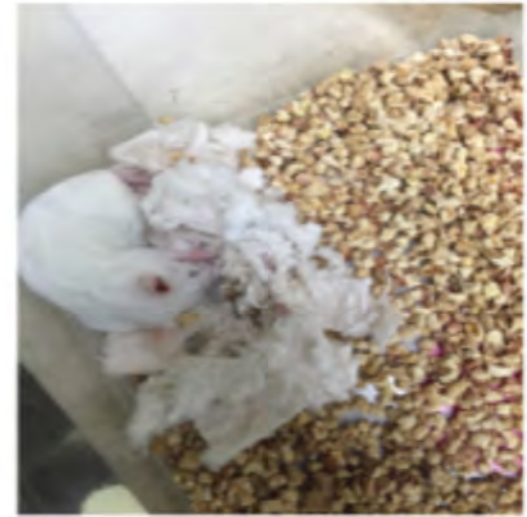
Supplemental Figure 4



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**Score=2**



**Score=3**

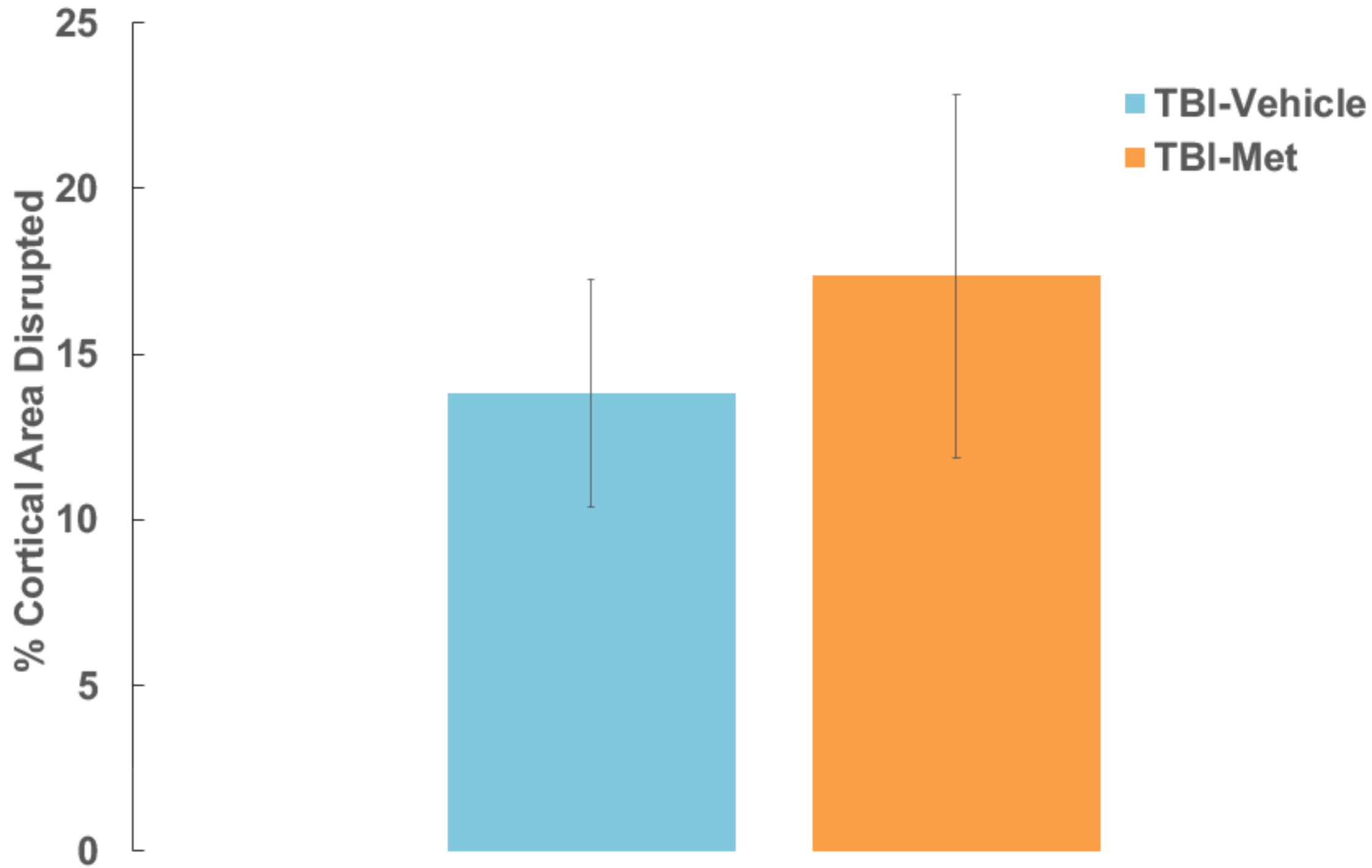


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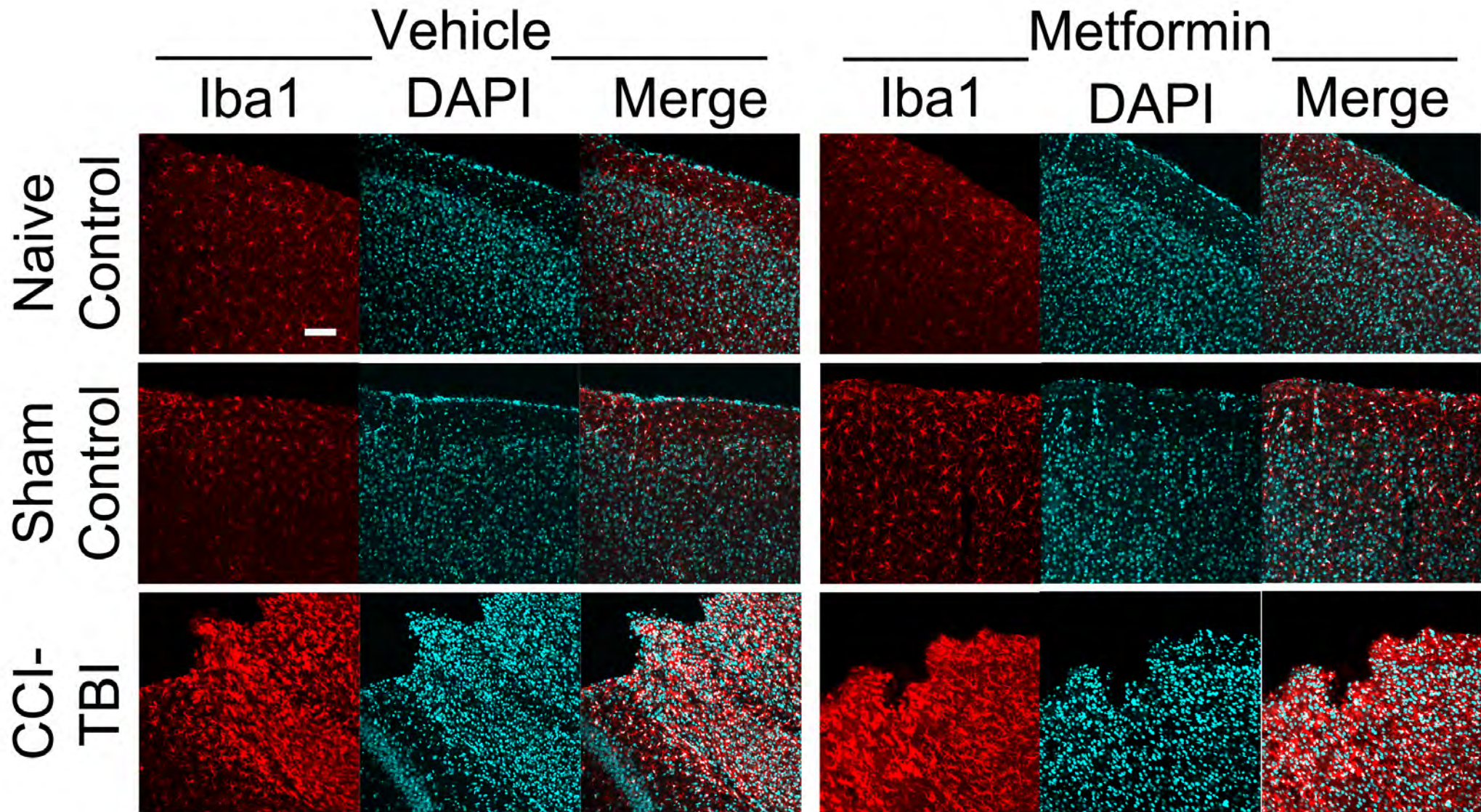
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# Average Cortical Tissue Disruption





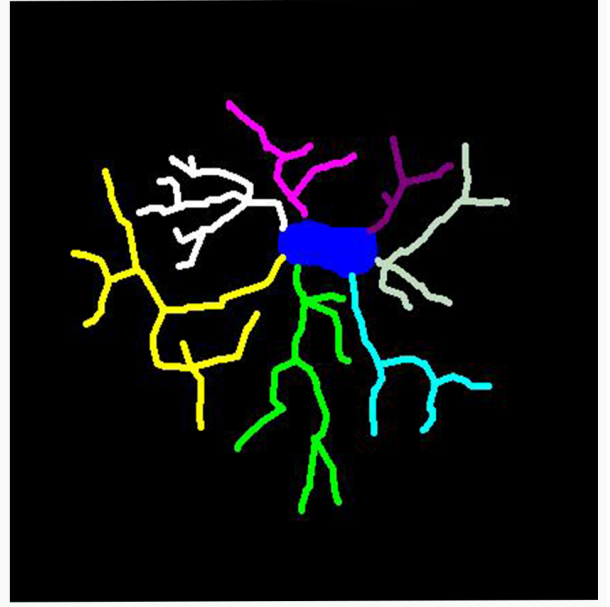
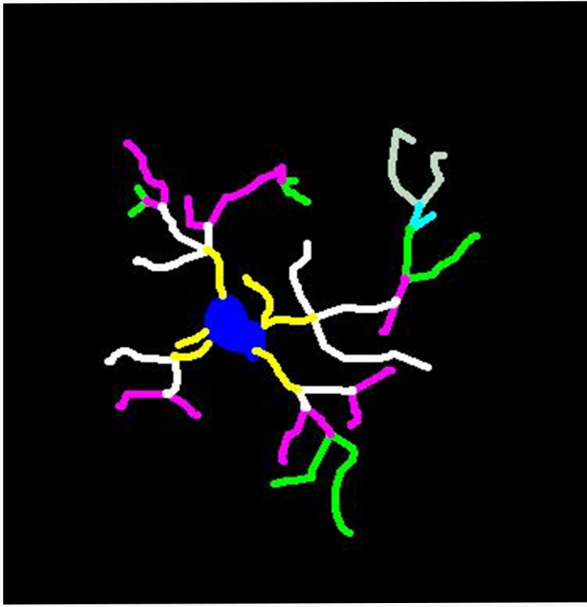
Supplemental Figure 6



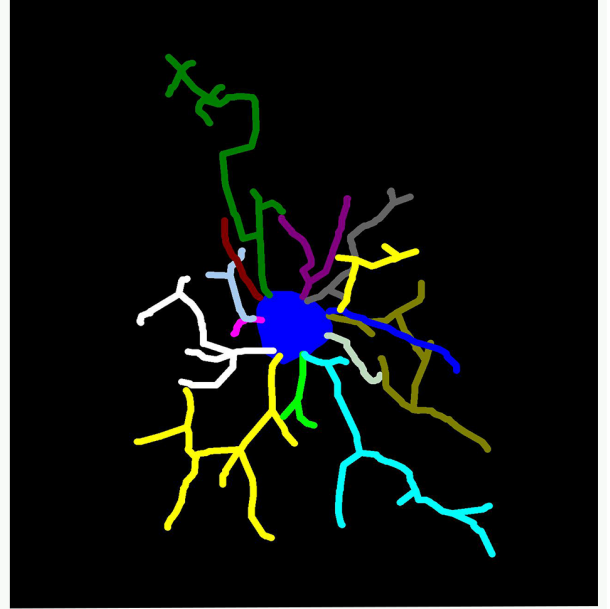
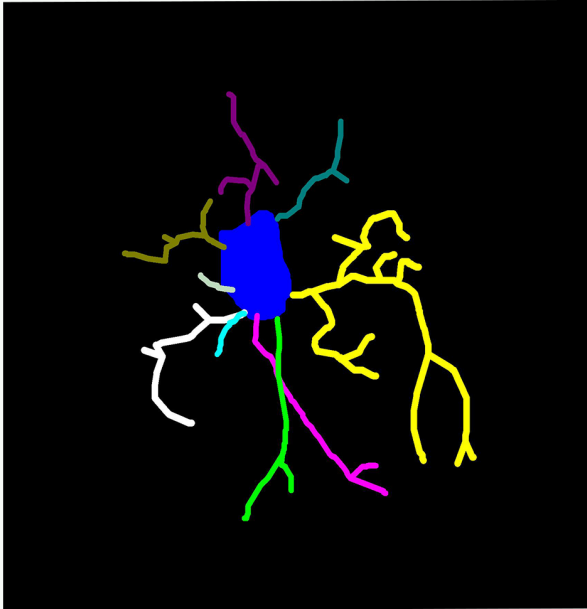
# Vehicle

# Metformin

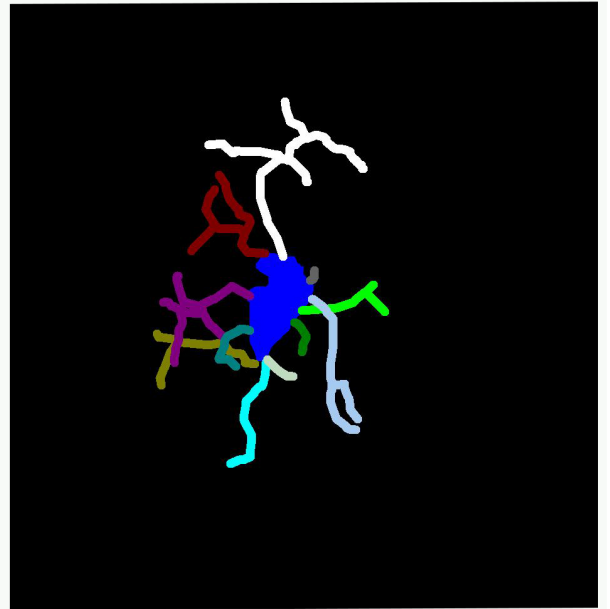
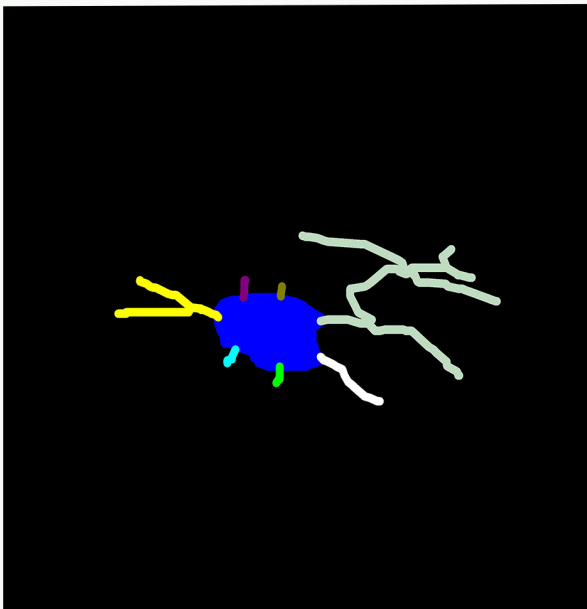
**Naïve**



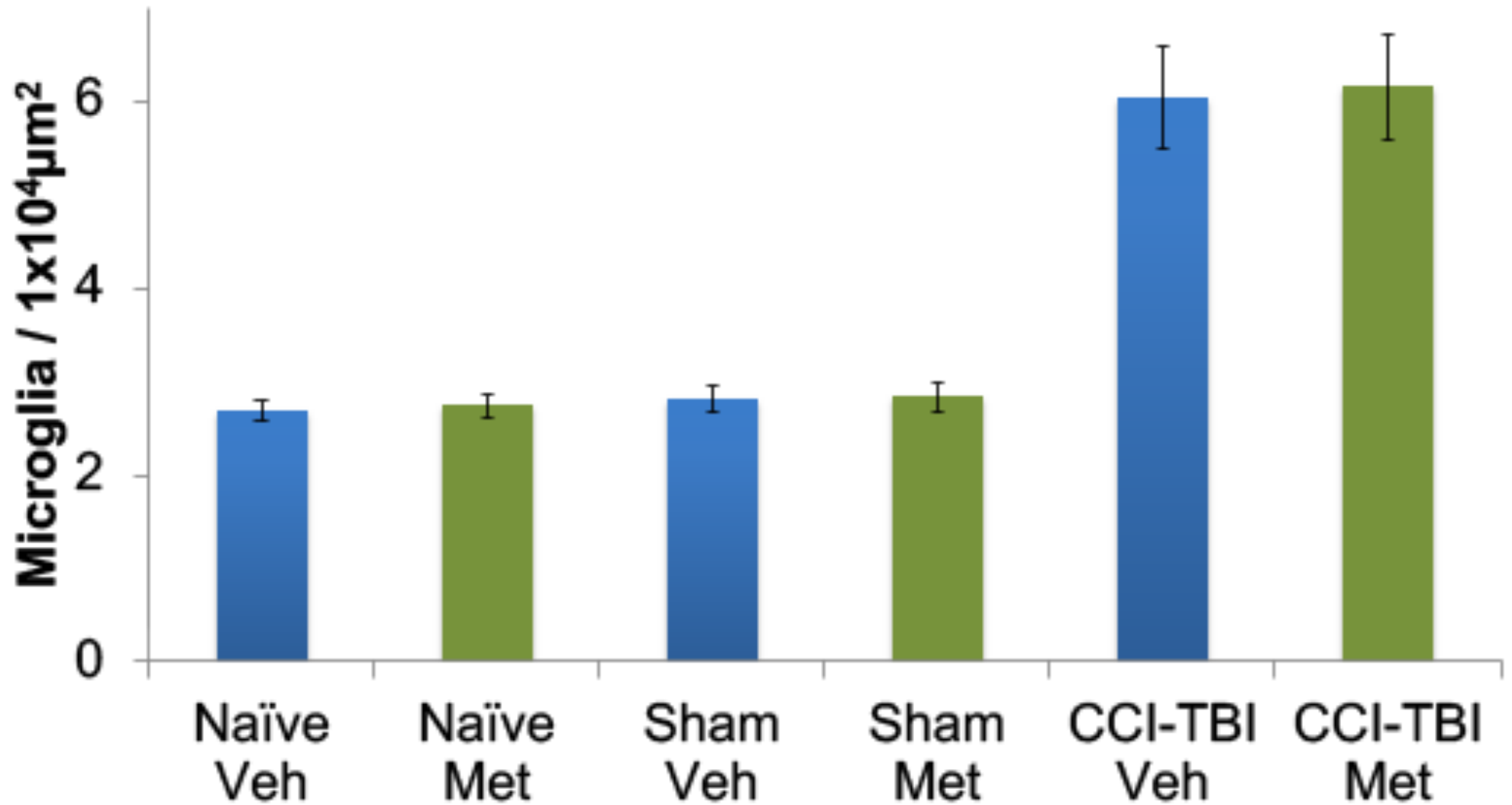
**Sham**



**TBI**



## Microglia Density (Ipsilaterally)



Supplemental Table 1: Two-Way ANOVA MORRIS WATER MAZE TEST

dpi	Surgery	Treatment	n-mice	Mean	Stand Dev	SEM	df factor	df	F	p	Sig.
Day 1	Naive	Vehicle	8	27.580	10.437	3.690	Surgery	2	5.865	0.005	**
	Naive	Metformin	10	32.234	8.864	2.803	Treatment	1	0.921	0.341	
	Sham	Vehicle	12	27.571	11.624	3.355	Surgery x Treatment	2	1.560	0.219	
	Sham	Metformin	12	36.471	17.062	4.925	Within Groups	57			
	TBI	Vehicle	10	45.353	11.720	3.706	Total	62			
Day 2	Naive	Vehicle	8	25.581	12.038	4.256	Treatment	2	2.405	0.099	
	Naive	Metformin	10	23.034	15.708	4.967	Surgery	1	0.363	0.549	
	Sham	Vehicle	12	20.353	11.043	3.188	Treatment x Surgery	2	1.902	0.159	
	Sham	Metformin	12	25.581	8.942	2.581	Within Groups	57			
	TBI	Vehicle	10	36.223	16.828	5.322	Total	62			
Day 3	Naive	Vehicle	8	24.003	9.312	3.292	Treatment	2	4.705	0.013	*
	Naive	Metformin	10	18.415	7.826	2.475	Surgery	1	0.349	0.557	
	Sham	Vehicle	12	14.491	9.279	2.679	Treatment x Surgery	2	5.160	0.009	**
	Sham	Metformin	12	24.151	9.668	2.791	Within Groups	57			
	TBI	Vehicle	10	35.411	16.710	5.284	Total	62			
Day 4	Naive	Vehicle	8	18.533	8.752	3.094	Treatment	2	2.236	0.116	
	Naive	Metformin	10	18.534	8.840	2.795	Surgery	1	0.108	0.743	
	Sham	Vehicle	12	15.111	10.130	2.924	Treatment x Surgery	2	1.397	0.256	
	Sham	Metformin	12	21.282	12.848	3.709	Within Groups	57			
	TBI	Vehicle	10	26.703	9.243	2.923	Total	62			
Day 5	Naive	Vehicle	8	15.866	10.814	3.823	Treatment	2	3.335	0.043	*
	Naive	Metformin	10	16.917	4.330	1.369	Surgery	1	0.312	0.578	
	Sham	Vehicle	12	12.086	11.003	3.176	Treatment x Surgery	2	1.420	0.250	
	Sham	Metformin	12	13.967	12.484	3.604	Within Groups	57			
	TBI	Vehicle	10	25.307	13.218	4.180	Total	62			
Day 6	Naive	Vehicle	8	10.411	3.709	1.311	Treatment	2	2.486	0.092	
	Naive	Metformin	10	11.090	3.296	1.042	Surgery	1	1.532	0.221	
	Sham	Vehicle	12	13.134	8.798	2.540	Treatment x Surgery	2	2.400	0.100	
	Sham	Metformin	12	14.001	9.540	2.754	Within Groups	57			
	TBI	Vehicle	10	21.339	11.818	3.737	Total	62			
All Days Combined	Naive	Vehicle	8	20.329	5.010	1.771	Surgery	2	7.502	0.001	**
	Naive	Metformin	10	20.037	4.963	1.569	Treatment	1	0.112	0.739	
	Sham	Vehicle	12	17.124	5.962	1.721	Surgery x Treatment	2	4.617	0.014	*
	Sham	Metformin	12	22.575	8.666	2.502	Within Groups	57			
	TBI	Vehicle	10	31.723	8.290	2.622	Total	62			
7dpi probe time target quad	Naive	Vehicle	8	23.835	6.198	2.191	Surgery	5	0.017	1.000	
	Naive	Metformin	10	22.410	5.801	1.834	Treatment	3	39.333	<0.0001	***
	Sham	Vehicle	12	19.956	4.773	1.378	Surgery x Treatment	15	1.339	0.180	
	Sham	Metformin	12	23.251	9.892	2.855	Within Groups	228			
	TBI	Vehicle	10	22.485	10.129	3.203	Total	251			
7dpi probe crossings target quad	Naive	Vehicle	8	8.000	2.563	0.906	Surgery	5	3.711	0.003	**
	Naive	Metformin	10	8.100	1.792	0.567	Treatment	3	11.955	<0.0001	***
	Sham	Vehicle	12	9.083	2.065	0.596	Surgery x Treatment	15	0.427	0.970	
	Sham	Metformin	12	7.000	2.663	0.769	Within Groups	228			
	TBI	Vehicle	10	7.200	1.549	0.490	Total	251			
7dpi probe Mean swim speed overall trial	Naive	Vehicle	6	0.500	0.104	0.042	Surgery	2	1.104	0.342	
	Naive	Metformin	8	0.462	0.078	0.028	Treatment	1	<0.000	0.991	
	Sham	Vehicle	9	0.540	0.094	0.031	Surgery x Treatment	2	1.607	0.213	
	Sham	Metformin	9	0.517	0.107	0.036	Within Groups	39			
	TBI	Vehicle	7	0.476	0.119	0.045	Total	44			
TBI	Metformin	6	0.560	0.059	0.024	Total					

dpi	Post-Hoc	Post hoc Tukey	Post hoc Sig.	Figure
Day 1	Naive Vehicle vs. Sham Vehicle	1.000		
	Naive Vehicle vs. TBI Vehicle	0.015	*	1C
	Sham Vehicle vs TBI Vehicle	0.006	**	1C
	Naive Metformin vs Sham Metformin	0.727		
	Naive Metformin vs TBI Metformin	0.291		
	Sham Metformin vs TBI Metformin	0.701		
	Naive: Vehicle vs Metformin	0.452		
	Sham: Vehicle vs Metformin	0.098		
	TBI: Vehicle vs Metformin	0.427		
	Day 2	Naive Vehicle vs. Sham Vehicle	0.655	
Naive Vehicle vs. TBI Vehicle		0.205		
Sham Vehicle vs TBI Vehicle		0.016	*	1C
Naive Metformin vs Sham Metformin		0.891		
Naive Metformin vs TBI Metformin		0.832		
Sham Metformin vs TBI Metformin		0.990		
Naive: Vehicle vs Metformin		0.681		
Sham: Vehicle vs Metformin		0.329		
TBI: Vehicle vs Metformin		0.087		
Day 3		Naive Vehicle vs. Sham Vehicle	0.174	
	Naive Vehicle vs. TBI Vehicle	0.100		
	Sham Vehicle vs TBI Vehicle	<0.0001	***	1C
	Naive Metformin vs Sham Metformin	0.478		
	Naive Metformin vs TBI Metformin	0.538		
	Sham Metformin vs TBI Metformin	0.987		
	Naive: Vehicle vs Metformin	0.309		
	Sham: Vehicle vs Metformin	0.044	*	
	TBI: Vehicle vs Metformin	0.024	*	1D
	Day 4	Naive Vehicle vs. Sham Vehicle	0.764	
Naive Vehicle vs. TBI Vehicle		0.249		
Sham Vehicle vs TBI Vehicle		0.037	*	1C
Naive Metformin vs Sham Metformin		0.821		
Naive Metformin vs TBI Metformin		0.706		
Sham Metformin vs TBI Metformin		0.974		
Naive: Vehicle vs Metformin		1.000		
Sham: Vehicle vs Metformin		0.163		
TBI: Vehicle vs Metformin		0.346		
Day 5		Naive Vehicle vs. Sham Vehicle	0.715	
	Naive Vehicle vs. TBI Vehicle	0.153		
	Sham Vehicle vs TBI Vehicle	0.014	*	1C
	Naive Metformin vs Sham Metformin	0.792		
	Naive Metformin vs TBI Metformin	0.994		
	Sham Metformin vs TBI Metformin	0.716		
	Naive: Vehicle vs Metformin	0.835		
	Sham: Vehicle vs Metformin	0.664		
	TBI: Vehicle vs Metformin	0.093		
	Day 6	Naive Vehicle vs. Sham Vehicle	0.749	
Naive Vehicle vs. TBI Vehicle		0.018	*	1C
Sham Vehicle vs TBI Vehicle		0.059		
Naive Metformin vs Sham Metformin		0.687		
Naive Metformin vs TBI Metformin		0.931		
Sham Metformin vs TBI Metformin		0.885		
Naive: Vehicle vs Metformin		0.862		
Sham: Vehicle vs Metformin		0.797		
TBI: Vehicle vs Metformin		0.015	*	1D
All Days Combined		Naive Vehicle vs. Sham Vehicle	0.608	
	Naive Vehicle vs. TBI Vehicle	0.005	**	1E
	Sham Vehicle vs TBI Vehicle	<0.0001	***	1E
	Naive Metformin vs Sham Metformin	0.701		
	Naive Metformin vs TBI Metformin	0.469		
	Sham Metformin vs TBI Metformin	0.912		
	Naive: Vehicle vs Metformin	0.934		
	Sham: Vehicle vs Metformin	0.075		
	TBI: Vehicle vs Metformin	0.017	*	1F
	7dpi probe time target quad	Naive Vehicle vs. Sham Vehicle	0.936	
Naive Vehicle vs. TBI Vehicle		0.999		
Sham Vehicle vs TBI Vehicle		0.983		
Naive Metformin vs Sham Metformin		1.000		
Naive Metformin vs TBI Metformin		0.999		
Sham Metformin vs TBI Metformin		0.988		
Naive: Vehicle vs Metformin		0.999		
Sham: Vehicle vs Metformin		0.946		
TBI: Vehicle vs Metformin		0.998		SF2
7dpi probe crossings target quad		Naive Vehicle vs. Sham Vehicle	0.983	
	Naive Vehicle vs. TBI Vehicle	0.996		
	Sham Vehicle vs TBI Vehicle	0.870		
	Naive Metformin vs Sham Metformin	0.977		
	Naive Metformin vs TBI Metformin	0.997		
	Sham Metformin vs TBI Metformin	0.673		
	Naive: Vehicle vs Metformin	1.000		
	Sham: Vehicle vs Metformin	0.828		
	TBI: Vehicle vs Metformin	0.917		SF2
	7dpi probe Mean swim speed overall trial	Naive Vehicle vs. Sham Vehicle	0.708	
Naive Vehicle vs. TBI Vehicle		0.900		
Sham Vehicle vs TBI Vehicle		0.396		
Naive Metformin vs Sham Metformin		0.479		
Naive Metformin vs TBI Metformin		0.159		
Sham Metformin vs TBI Metformin		0.676		
Naive: Vehicle vs Metformin		0.475		
Sham: Vehicle vs Metformin		0.612		
TBI: Vehicle vs Metformin		0.127		SF2

**Supplemental Table 2: Two-Way ANOVA POLE TEST OF MOTOR FUNCTION**

dpi	Surgery	Treatment	n-mice	Mean	Stand Dev	SEM	df factor	df	F	p	Sig.
Day 1	Naïve	Vehicle	2	28.328	3.359	2.375	Surgery	2	0.519	0.608	
	Naïve	Metformin	2	28.368	9.251	6.542	Treatment	1	0.155	0.700	
	Sham	Vehicle	3	25.456	9.157	5.287	Surgery x Treatment	2	0.111	0.896	
	Sham	Metformin	3	33.693	19.588	11.309	Within Groups	12			
	TBI	Vehicle	3	38.131	18.300	10.565	Total	17			
Day 3	TBI	Metformin	5	36.572	20.532	9.182					
	Naïve	Vehicle	2	18.707	0.226	0.160	Surgery	2	1.107	0.362	
	Naïve	Metformin	2	16.355	5.560	3.932	Treatment	1	0.007	0.936	
	Sham	Vehicle	3	16.182	8.030	4.636	Surgery x Treatment	2	0.020	0.980	
	Sham	Metformin	3	17.181	4.563	2.634	Within Groups	12			
Day 7	TBI	Vehicle	3	27.939	6.310	3.643	Total	17			
	TBI	Metformin	5	26.72	23.514	10.516					
	Naïve	Vehicle	2	25.063	23.203	16.407	Surgery	2	0.303	0.744	
	Naïve	Metformin	2	18.442	3.071	2.172	Treatment	1	0.181	0.678	
	Sham	Vehicle	3	36.434	23.393	13.506	Surgery x Treatment	2	0.336	0.721	
Day 7	Sham	Metformin	3	25.008	12.310	7.107	Within Groups	12			
	TBI	Vehicle	3	24.329	7.529	4.347	Total	17			
	TBI	Metformin	5	28.515	21.056	9.416					

dpi	Post-Hoc	Post hoc Tukey	Post hoc Sig.
Day 1	Naïve Vehicle vs. Sham Vehicle	0.981	
	Naïve Vehicle vs. TBI Vehicle	0.802	
	Sham Vehicle vs TBI Vehicle	0.636	
	Naïve Metformin vs Sham Metformin	0.936	
	Naïve Metformin vs TBI Metformin	0.831	
	Sham Metformin vs TBI Metformin	0.970	
	Naïve: Vehicle vs Metformin	0.998	
	Sham: Vehicle vs Metformin	0.559	
Day 3	TBI: Vehicle vs Metformin	0.901	
	Naïve Vehicle vs. Sham Vehicle	0.980	
	Naïve Vehicle vs. TBI Vehicle	0.767	
	Sham Vehicle vs TBI Vehicle	0.591	
	Naïve Metformin vs Sham Metformin	0.998	
	Naïve Metformin vs TBI Metformin	0.675	
	Sham Metformin vs TBI Metformin	0.647	
	Naïve: Vehicle vs Metformin	0.873	
Day 7	Sham: Vehicle vs Metformin	0.934	
	TBI: Vehicle vs Metformin	0.910	
	Naïve Vehicle vs. Sham Vehicle	0.770	
	Naïve Vehicle vs. TBI Vehicle	0.999	
	Sham Vehicle vs TBI Vehicle	0.693	
	Naïve Metformin vs Sham Metformin	0.915	
	Naïve Metformin vs TBI Metformin	0.783	
	Sham Metformin vs TBI Metformin	0.961	
Day 7	Naïve: Vehicle vs Metformin	0.717	
	Sham: Vehicle vs Metformin	0.449	
	TBI: Vehicle vs Metformin	0.754	

Supplemental Table 3: Two-Way ANOVA NESTING TEST

dpi	Surgery	Treatment	n-mice	Mean	Stand Dev	SEM	df factor	df	F	p	Sig.
Day 1	Naïve	Vehicle	7	4.571	0.535	0.202	Surgery	2	6.557	0.003	**
	Naïve	Metformin	9	4.056	1.130	0.377	Treatment	1	1.735	0.194	
	Sham	Vehicle	10	4.150	1.203	0.380	Surgery x Treatment	2	0.206	0.814	
	Sham	Metformin	10	3.600	0.876	0.277	Within Groups	48			
	TBI	Vehicle	9	3.000	1.677	0.559	Total	53			
	TBI	Metformin	9	2.556	1.685	0.562					
Day 2	Naïve	Vehicle	8	4.688	0.458	0.162	Surgery	2	2.994	0.058	
	Naïve	Metformin	10	4.500	0.667	0.211	Treatment	1	0.373	0.544	
	Sham	Vehicle	12	4.083	0.925	0.267	Surgery x Treatment	2	0.632	0.535	
	Sham	Metformin	12	4.125	0.882	0.255	Within Groups	57			
	TBI	Vehicle	10	3.600	1.125	0.356	Total	62			
	TBI	Metformin	11	4.091	1.221	0.368					
Day 3	Naïve	Vehicle	8	4.563	0.496	0.175	Surgery	2	0.169	0.845	
	Naïve	Metformin	10	4.300	1.252	0.396	Treatment	1	0.005	0.945	
	Sham	Vehicle	12	4.333	1.155	0.333	Surgery x Treatment	2	0.361	0.699	
	Sham	Metformin	12	4.333	0.778	0.225	Within Groups	57			
	TBI	Vehicle	10	4.100	0.994	0.314	Total	62			
	TBI	Metformin	11	4.364	0.809	0.244					
Day 4	Naïve	Vehicle	8	4.688	0.458	0.162	Surgery	2	0.142	0.868	
	Naïve	Metformin	10	4.750	0.540	0.171	Treatment	1	0.573	0.452	
	Sham	Vehicle	12	4.542	0.542	0.156	Surgery x Treatment	2	0.016	0.984	
	Sham	Metformin	12	4.708	0.865	0.250	Within Groups	57			
	TBI	Vehicle	10	4.550	0.956	0.302	Total	62			
	TBI	Metformin	11	4.682	0.462	0.139					
Day 5	Naïve	Vehicle	8	4.750	0.463	0.164	Surgery	2	0.308	0.736	
	Naïve	Metformin	10	4.650	0.474	0.150	Treatment	1	0.134	0.716	
	Sham	Vehicle	12	4.458	0.722	0.208	Surgery x Treatment	2	0.285	0.753	
	Sham	Metformin	12	4.708	0.396	0.114	Within Groups	57			
	TBI	Vehicle	10	4.500	0.972	0.307	Total	62			
	TBI	Metformin	11	4.500	1.204	0.363					
Day 6	Naïve	Vehicle	8	5.000	0.000	0.000	Surgery	2	2.624	0.081	
	Naïve	Metformin	10	4.950	0.158	0.050	Treatment	1	0.992	0.323	
	Sham	Vehicle	12	4.833	0.326	0.094	Surgery x Treatment	2	3.439	0.039	*
	Sham	Metformin	12	4.708	0.450	0.130	Within Groups	57			
	TBI	Vehicle	10	4.450	0.685	0.217	Total	62			
	TBI	Metformin	11	4.909	0.302	0.091					
Day 7	Naïve	Vehicle	8	4.750	0.463	0.164	Surgery	2	0.074	0.928	
	Naïve	Metformin	10	4.550	0.832	0.263	Treatment	1	0.175	0.677	
	Sham	Vehicle	12	4.708	0.450	0.130	Surgery x Treatment	2	0.635	0.534	
	Sham	Metformin	12	4.542	0.722	0.208	Within Groups	57			
	TBI	Vehicle	10	4.600	0.516	0.163	Total	62			
	TBI	Metformin	11	4.773	0.410	0.124					

dpi	Post-Hoc	Post hoc Tukey	Post hoc Sig.	Figure
Day 1	Naïve Vehicle vs. Sham Vehicle	0.779		
	Naïve Vehicle vs. TBI Vehicle	0.045	*	1C
	Sham Vehicle vs TBI Vehicle	0.129		
	Naïve Metformin vs Sham Metformin	0.716		
	Naïve Metformin vs TBI Metformin	0.040	*	1D
	Sham Metformin vs TBI Metformin	0.182		
	Naïve: Vehicle vs Metformin	0.423		
	Sham: Vehicle vs Metformin	0.337		
	TBI: Vehicle vs Metformin	0.460		
Day 2	Naïve Vehicle vs. Sham Vehicle	0.339		
	Naïve Vehicle vs. TBI Vehicle	0.045	*	1C
	Sham Vehicle vs TBI Vehicle	0.453		
	Naïve Metformin vs Sham Metformin	0.619		
	Naïve Metformin vs TBI Metformin	0.579		
	Sham Metformin vs TBI Metformin	0.996		
	Naïve: Vehicle vs Metformin	0.674		
	Sham: Vehicle vs Metformin	0.913		
	TBI: Vehicle vs Metformin	0.234		
Day 3	Naïve Vehicle vs. Sham Vehicle	0.861		
	Naïve Vehicle vs. TBI Vehicle	0.571		
	Sham Vehicle vs TBI Vehicle	0.838		
	Naïve Metformin vs Sham Metformin	0.996		
	Naïve Metformin vs TBI Metformin	0.988		
	Sham Metformin vs TBI Metformin	0.997		
	Naïve: Vehicle vs Metformin	0.567		
	Sham: Vehicle vs Metformin	1.000		
	TBI: Vehicle vs Metformin	0.532		
Day 4	Naïve Vehicle vs. Sham Vehicle	0.884		
	Naïve Vehicle vs. TBI Vehicle	0.903		
	Sham Vehicle vs TBI Vehicle	1.000		
	Naïve Metformin vs Sham Metformin	0.989		
	Naïve Metformin vs TBI Metformin	0.971		
	Sham Metformin vs TBI Metformin	0.995		
	Naïve: Vehicle vs Metformin	0.846		
	Sham: Vehicle vs Metformin	0.547		
	TBI: Vehicle vs Metformin	0.656		
Day 5	Naïve Vehicle vs. Sham Vehicle	0.688		
	Naïve Vehicle vs. TBI Vehicle	0.775		
	Sham Vehicle vs TBI Vehicle	0.991		
	Naïve Metformin vs Sham Metformin	0.983		
	Naïve Metformin vs TBI Metformin	0.897		
	Sham Metformin vs TBI Metformin	0.795		
	Naïve: Vehicle vs Metformin	0.786		
	Sham: Vehicle vs Metformin	0.431		
	TBI: Vehicle vs Metformin	1.000		
Day 6	Naïve Vehicle vs. Sham Vehicle	0.623		
	Naïve Vehicle vs. TBI Vehicle	0.012	*	1C
	Sham Vehicle vs TBI Vehicle	0.066		
	Naïve Metformin vs Sham Metformin	0.327		
	Naïve Metformin vs TBI Metformin	0.969		
	Sham Metformin vs TBI Metformin	0.442		
	Naïve: Vehicle vs Metformin	0.789		
	Sham: Vehicle vs Metformin	0.438		
	TBI: Vehicle vs Metformin	0.010	**	1B
Day 7	Naïve Vehicle vs. Sham Vehicle	0.987		
	Naïve Vehicle vs. TBI Vehicle	0.853		
	Sham Vehicle vs TBI Vehicle	0.903		
	Naïve Metformin vs Sham Metformin	0.999		
	Naïve Metformin vs TBI Metformin	0.664		
	Sham Metformin vs TBI Metformin	0.617		
	Naïve: Vehicle vs Metformin	0.477		
	Sham: Vehicle vs Metformin	0.491		
	TBI: Vehicle vs Metformin	0.505		

**Supplemental Table 4: Two-Way ANOVA IMMUNOHISTOCHEMISTRY**

Surgery	Treatment	n-mice	n-image	Mean	Stand Dev	SEM	df factor	df	F	p	Sig.
Naïve	Vehicle	8	30	0.027	0.011	0.001	Surgery x Treatment	2	0.013	0.987	
Naïve	Metformin	10	27	0.027	0.011	0.001	Surgery	2	61.270	<0.0001	*
Sham	Vehicle	11	33	0.028	0.015	0.001	Treatment	1	0.047	0.828	
Sham	Metformin	12	36	0.028	0.016	0.002	Residual	570			
TBI	Vehicle	10	31	0.060	0.053	0.005					
TBI	Metformin	10	35	0.062	0.057	0.006					

Post-Hoc	Post hoc Tukey	Post hoc Sig.
Naïve Vehicle vs. Sham Vehicle	0.9998	
Naïve Vehicle vs. TBI Vehicle	<0.0001	***
Sham Vehicle vs TBI Vehicle	<0.0001	***
Naïve Metformin vs Sham Metformin	>0.9999	
Naïve Metformin vs TBI Metformin	<0.0001	***
Sham Metformin vs TBI Metformin	<0.0001	***
Naïve: Vehicle vs Metformin	>0.9999	
Sham: Vehicle vs Metformin	>0.9999	
TBI: Vehicle vs Metformin	0.9999	

Supplemental Table 5 Type III Test of Fixed Effects/Mixed Model MICROGLIA DEVELOPMENTAL MORPHOLOGY - STRUCTURAL CHARACTERISTICS

Experimental Group: TBI	n-mice	n-microglia	
Vehicle	10	120	
Metformin	12	144	

Morphology Structural Characteristics of Branching	Experimental Group: TBI	Mean	Stand Dev	SEM	Coeff Var	Tests of Fixed Effects					Figure	
						Source	Numerator df	Denominator df	F	p		Sig.
Intersections - Radii 30	Vehicle	1.490	1.983	0.181	1.230	Treatment	1	262.000	6.607	0.019	*	4B
	Metformin	2.190	2.700	0.225	1.329							
Avg. Branch Length - SUM TOTAL	Vehicle	210.458	94.596	8.635	0.449	Treatment	1	262.000	4.258	0.040	*	4F
	Metformin	237.033	111.556	9.296	0.471							
Nodes - Radii 15	Vehicle	3.030	1.756	0.160	0.580	Treatment	1	262.000	5.241	0.023	*	4H
	Metformin	3.620	2.341	0.195	0.647							
Nodes - Radii 20	Vehicle	2.280	1.919	0.175	0.841	Treatment	1	262.000	6.928	0.009	**	4H
	Metformin	2.940	2.121	0.177	0.720							
Nodes - Radii 25	Vehicle	1.310	1.565	0.143	1.196	Treatment	1	262.000	4.799	0.029	*	4H
	Metformin	1.760	1.774	0.148	1.006							
Nodes - Radii 30	Vehicle	0.540	0.916	0.084	1.691	Treatment	1	262.000	6.319	0.013	*	4H
	Metformin	0.910	1.369	0.114	1.504							
Nodes - SUM TOTAL	Vehicle	9.408	4.949	0.452	0.526	Treatment	1	74.322	6.412	0.013	*	4I
	Metformin	11.826	6.933	0.578	0.586							
Endings - Radii 10	Vehicle	2.330	1.600	0.146	0.686	Treatment	1	262.000	4.680	0.031	*	4K
	Metformin	2.820	1.981	0.165	0.703							
Endings - Radii 25	Vehicle	3.490	2.580	0.236	0.739	Treatment	1	262.000	4.681	0.031	*	4K
	Metformin	4.220	2.806	0.234	0.666							
Endings - SUM TOTAL	Vehicle	19.692	6.504	0.594	0.330	Treatment	1	491.337	5.642	0.018	*	4L
	Metformin	22.431	8.458	0.705	0.377							

Morphology Structural Characteristics of Complexity	Experimental Group: TBI	Mean	Stand Dev	SEM	Coeff Var	Tests of Fixed Effects					Figure	
						Source	Numerator df	Denominator df	F	p		Sig.
Primary	Vehicle											5A-C
	Metformin											NS
Secondary	Vehicle											5D-F
	Metformin											NS
Tertiary - Radii 20	Vehicle	12.965	11.498	1.050	0.887	Treatment	1	262.000	4.249	0.040	*	5H
	Metformin	16.117	13.050	1.088	0.810							
Tertiary - SUM TOTAL	Vehicle	39.639	31.447	2.621	0.793	Treatment	1	262.000	6.205	0.013	*	5I
	Metformin	49.846	34.503	2.875	0.692							
Quarternary - Radii 20	Vehicle	4.007	6.025	0.550	1.503	Treatment	1	262.000	7.897	0.005	**	5K
	Metformin	6.783	9.317	0.776	1.374							
Quarternary - Radii 25	Vehicle	5.361	7.805	0.650	1.456	Treatment	1	262.000	4.832	0.029	*	5K
	Metformin	7.831	10.034	0.836	1.281							
Quarternary - SUM TOTAL	Vehicle	19.203	22.767	1.897	1.186	Treatment	1	262.000	6.701	0.010	**	5L
	Metformin	27.420	27.877	2.323	1.017							



**Supplemental Table 6 Type III Test of Fixed Effects/Mixed Model ASTROCYTE (GFAP+) FLUORESCENT INTENSITY**

Surgery	Treatment	n-mice	n-images	Mean	Stand Dev	SEM	Coeff Var	df factor	df	F	p	Sig.
Naive	Vehicle	8	24	5.115	3.661	0.747	0.716	Intercept	1	298.755	<0.0001	***
Naive	Metformin	10	27	6.815	4.093	0.788	0.601	Surgery	3	37.548	<0.0001	***
Sham	Vehicle	11	32	8.308	6.220	1.100	0.749	Treatment	2	2.001	0.159	N.S
Sham	Metformin	12	37	9.038	7.959	1.308	0.881	Surgery + Treatment	5			
TBI	Vehicle	10	31	17.379	8.575	1.540	0.493					
TBI	Metformin	11	33	20.449	14.902	2.594	0.729					

Post-Hoc	Test of Fixed Effects	Post hoc Sig.
Naive: Vehicle vs Metformin	0.126	N.S
Sham: Vehicle vs Metformin	0.676	N.S
TBI: Vehicle vs Metformin	>0.9999	N.S

**Supplemental Table 7****One-Way ANOVA****PRIMARY NEURONAL TREATMENT**

Treatment	n	Mean	SEM	df factor	df	F	p	Sig.	Post hoc Tukey	Post hoc Sig.
Control	6	0.250	0.099	between groups	1	11.097	0.008	**	0.008	**
Metformin	6	1.236	0.279	within groups	10					