SUPPLEMENTAL MATERIAL

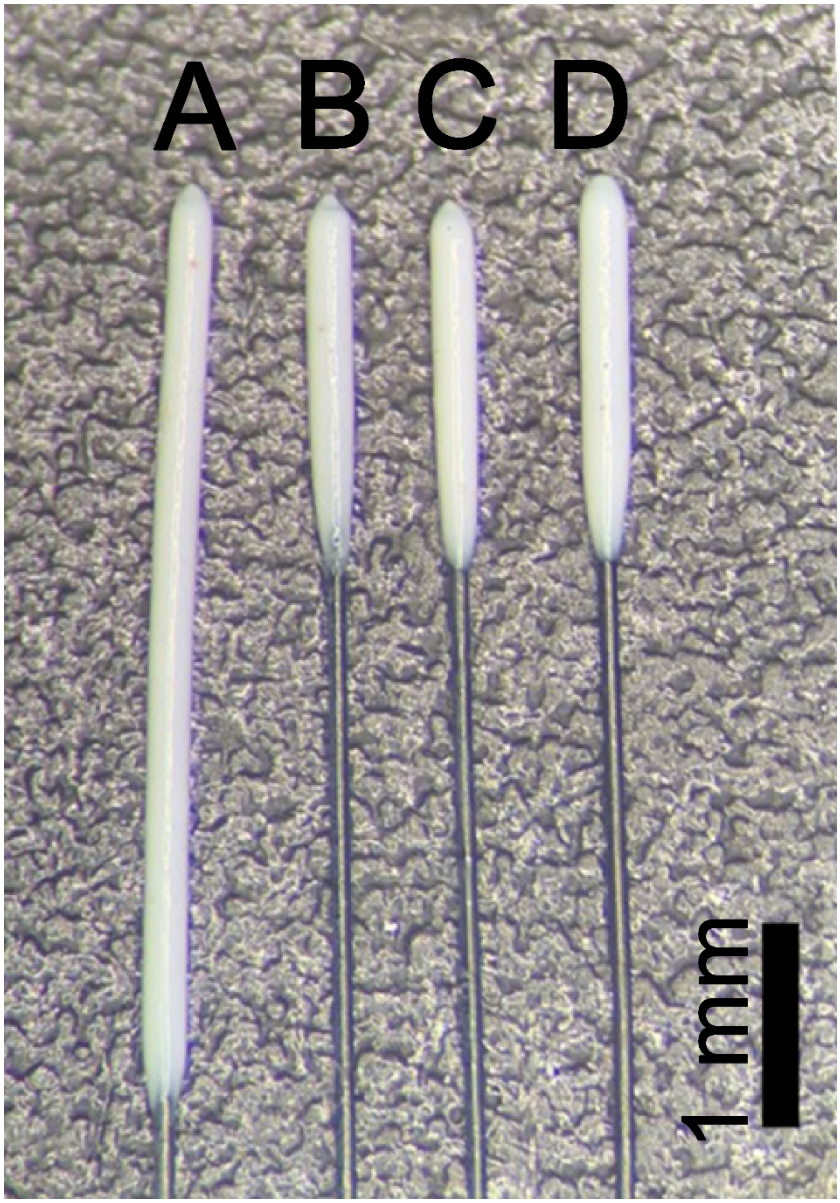

Figure S1. Representative filaments with varying lengths and thicknesses of silicone coating.

A, long filament (4-6 mm) with 0.22 mm thickness; B-D, short filaments (1-2 mm) with 0.21, 0.22, and 0.23 mm thickness (from left to right). Vertical bar = 1 mm.

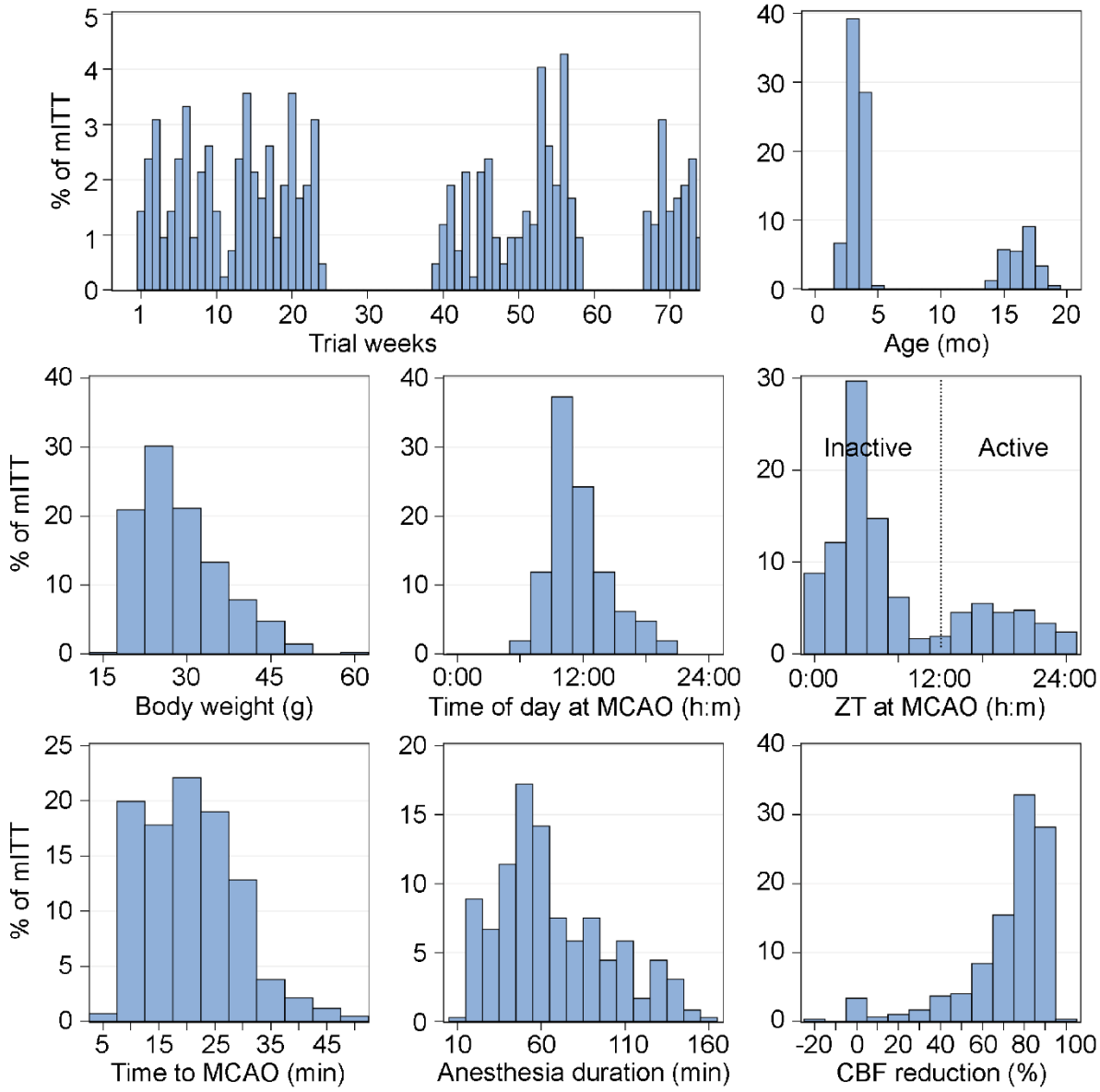

Figure S2. Basic characteristics of the biological and procedural numerical variables.

Histograms are displayed where the horizontal axis shows the center of each bin (numerical data are also presented in Supplemental Table 3).

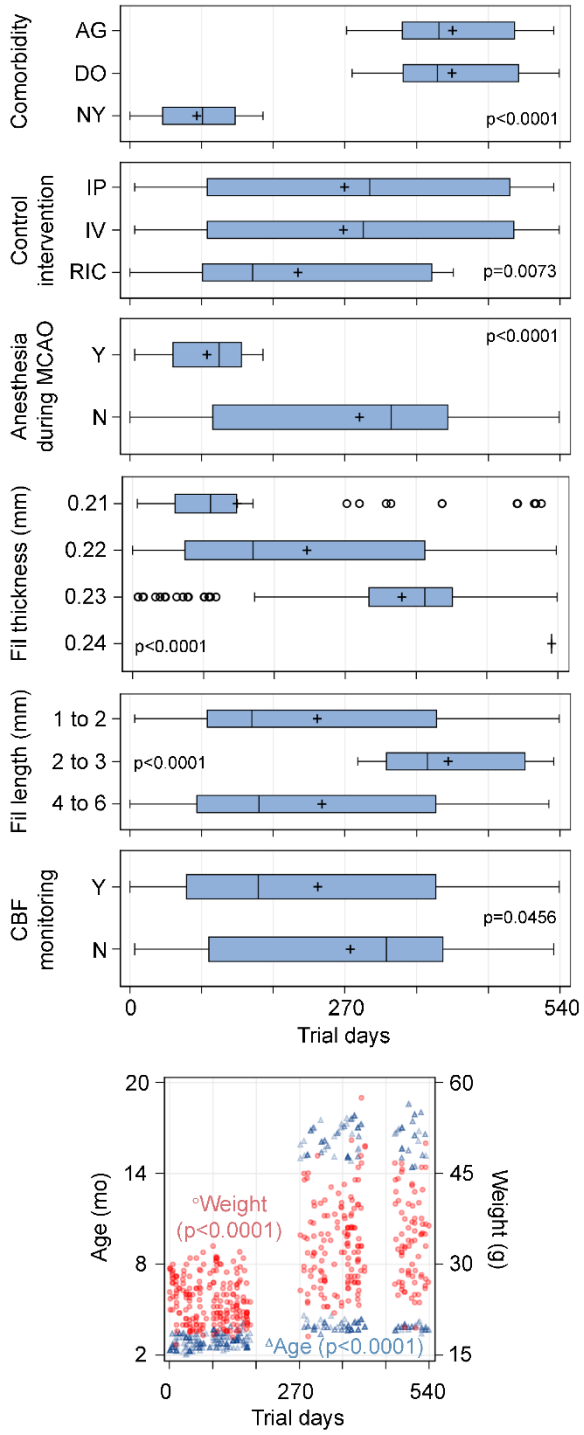

Figure S3. Temporal heterogeneity throughout the trial.

Whisker-box and scatter plots show the statistically significant temporal changes in independent variables during the trial (t-test, one-way ANOVA, or general linear model). Circles on whisker-box plots indicate data points outside the 1.5x interquartile range (IQR). The

scatter plot shows the association of age (blue triangles) and weight (red circles) with trial days. All p-values are adjusted by FDR.

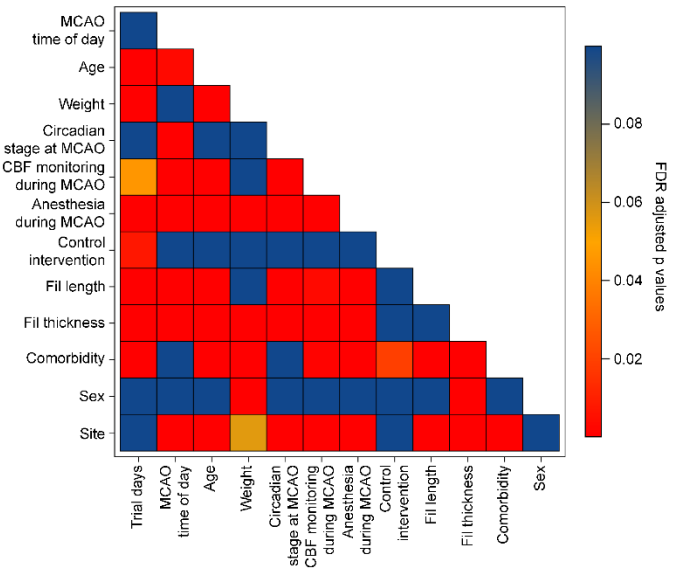

Figure S4. Covariance matrix among the independent variables.

More than half of pairwise associations between independent variables were statistically significant (t-test, one-way ANOVA, or general linear model). All p-values are adjusted by FDR. The direction of the relationship and effect size for the statistically significant associations are shown in Figures 2-5, Supplemental Table 3.

Table S1. List of SPAN investiators.

Site/Names	Highest Degree
NINDS	
Francesca Bosetti	PhD
James I. Koenig	PhD
Coordinating Center	
Patrick D. Lyden	MD
Jessica Lamb	BS
Karisma Nagarkatti	MS
Augusta University	
David C. Hess	MD
Pradip K. Kamat	PhD
Mohammad Badruzzaman Khan	PhD
Krishnan Dhandapani	PhD
Ali S. Arbab	MD, PhD
Shahneela Siddiqui	MS
Cameron Smith	MS
Mohammad Nisar	BS
University of Iowa	
Enrique C. Leira	MD, MS
Anil K. Chauhan	PhD
Nirav Dhanesha	PhD
Rakesh B. Patel	PhD
Mariia Kumskova	PhD
Daniel Thedens	PhD
Kai Wang	PhD
Mass General	
Cenk Ayata	MD, PhD
Andreia Morais	PhD
Takahiko Imai	PharmD, PhD
Tao Qin	BS
Xuyan Jin	PhD
Taylan Denis Erdogan	BS
Lili Yu	MD
Joseph B. Mandeville	PhD
William Taylor Kimberly	MD, PhD
Jonah Patrick Weigand Whittier	BS
Eng Lo	PhD
Ken Arai	PhD
Klaus Van Leyen	PhD
Yale	

Lauren H. Sansing	MD
Fahmeed Hyder	PhD
Jelena M. Mihailovic	PhD
Basavaraju G. Sanganahalli	PhD
Sebastian Diaz-Perez	BS
Sofia E. Velazquez	BS
Hannah E. Beatty	BS
Conor Johnson	BS
Alison L. Herman	BA
Ligia S. B. Boisserand	PhD
Emma Immakavar	BS
Johns Hopkins	
Raymond C. Koehler	PhD
Ted Dawson	PhD
Valina Dawson	PhD
Yanrong Shi	MD, MS
Brooklyn Avery	BS
Steven Lannon	BS
Adnan Bibic	PhD
Kazi Akhter	PhD
Senthilkumar S. Karuppagounder	PhD
UT Houston	
Jaroslaw Aronowski	MD, PhD
Louise D. McCullough	MD, PhD
Lidiya Obertas	PhD
Andrew Goh	MS
Shuning Huang	PhD
Anjali Chauhan	PhD

Table S2. Basic characteristics of study population, biological and procedural variables: categorical data.

		N (% of mITT)
Enrollment site	1	48 (12)
	2	81 (19)
	3	58 (14)
	4	64 (15)
	5	81 (19)
	6	89 (21)
Comorbidity model	AG	106 (25)
	DO	109 (26)
	NY	206 (49)
Sex	Female	210 (50)
	Male	211 (50)
Control intervention	IP	137 (33)
	IV	131 (31)
	RIC	153 (36)
Circadian stage (at time of MCAo)	Active	111 (26)
	Inactive	310 (74)
CBF (i.e., LDF) monitoring	No	123 (29)
	Yes	298 (71)
Anesthesia throughout MCAo	No	332 (79)
	Yes	89 (21)
Filament silicone thickness (mm)	0.21	69 (16)
	0.22	212 (50)
	0.23	137 (33)
	0.24	2 (0)
Filament silicone length (mm)	1 to 2	233 (55)
	2 to 3	26 (6)
	4 to 6	161 (38)

A, Aged; DO, diet-induced obesity; NY, normal young; IP, intraperitoneal; IV, intravenous; RIC, remote ischemic conditioning; LDF, laser Doppler flowmetry. Numerical variables are shown in Figure 1 and Supplemental Table 3.

Table S3. Basic characteristics of study population, biological and procedural variables: numerical data.

	N	Mean ± SD	Median	Min	IQR	Max
Age (months)	421	6.6±5.7	3.7	2.0	11.5	18.6
Weight (g)	421	28.9±7.4	27.2	16.7	9.8	57.5
Days into the trial	421	247.7	279	0	299	539
Time of MCAo (real time of day)	421	11:30±2:51	10:55	5:40	3:12	19:47
Time of MCAo (ZT)	421	7:46±6:38	4:56	0:00	9:33	23:54
Time to MCAo (min)	421	20.7±8.5	20	7	12	50
Anesthesia duration (min)	360	66.3±33.3	58	11	45	160
CBF drop (%)	299	71.5±21.6	79	-19	18	95

IQR, interquartile range, ZT zeitgeber time.

Table S4. Distribution of categorical independent variables across sites.

		1	2	3	4	5	6	χ^2 p (adjusted)
Comorbidity model	AG	18	10	26	0	26	26	*<0.0001*
	DO	10	28	0	27	17	27	
	NY	20	43	32	37	38	36	
Sex	Female	24	38	31	31	40	46	0.9905
	Male	24	43	27	33	41	43	
Control intervention	IP	20	26	16	20	23	32	0.4585
	IV	20	23	19	19	24	26	
	RIC	8	32	23	25	34	31	
Filament thickness	0.21mm	23	0	1	6	23	16	*<0.0001*
	0.22mm	10	80	44	40	9	29	
	0.23mm	15	0	13	18	4	42	
Filament length	1-2mm	48	0	32	64	0	89	*<0.0001*
	2-3mm	0	0	26	0	0	0	
	4-6mm	0	80	0	0	81	0	
CBF monitoring	No	48	0	0	0	55	20	*<0.0001*
	Yes	0	81	58	64	26	69	
Anesthesia throughout MCAo	No	28	81	26	27	81	89	*<0.0001*
	Yes	20	0	32	37	0	0	
Circadian stage at MCAo	Active	0	0	55	54	2	0	*<0.0001*
	Inactive	48	81	3	10	79	89	

Values represent the number of mice. p values were adjusted for multiplicity using false discovery rate. A, Aged; DO, diet-induced obesity; NY, normal young; IP, intraperitoneal; IV, intravenous; RIC, remote ischemic conditioning.

Table S5. Covariance among categorical independent variables.

		Comorbidity model			**χ^2 p (adjusted)**
		AG	DO	NY	
Filament thickness	0.21mm	4	7	58	*<0.0001*
	0.22mm	44	43	125	
	0.23mm	58	56	23	
Filament length	1-2mm	44	64	125	*<0.0001*
	2-3mm	26	0	0	
	4-6mm	36	44	81	
Control intervention	IP	37	42	58	*0.0195*
	IV	40	35	56	
	RIC	29	32	92	
Anesthesia throughout MCAo	No	106	109	117	*<0.0001*
	Yes	0	0	89	
CBF monitoring	No	46	27	50	*0.0018*
	Yes	60	82	156	
		Filament thickness			
		0.21 mm	0.22 mm	0.23 mm	
Anesthesia throughout MCAo	No	48	145	136	*<0.0001*
	Yes	21	67	1	
CBF monitoring	No	41	19	61	*<0.0001*
	Yes	28	193	76	
Circadian stage at MCAo	Active	7	73	31	0.0008
	Inactive	62	139	106	
		Filament length			
		1-2 mm	2-3 mm	4-6 mm	
Anesthesia throughout MCAo	No	144	26	161	*<0.0001*
	Yes	89	0	0	
CBF monitoring	No	68	0	55	0.0032
	Yes	165	26	161	
Circadian stage at MCAo	Active	86	23	2	*<0.0001*
	Inactive	147	3	159	

Only statistically significant associations are shown. Please see Supplemental Figure 4 for a full covariance matrix. A, Aged; DO, diet-induced obesity; NY, normal young; IP, intraperitoneal; IV, intravenous; RIC, remote ischemic conditioning.

Table S6. Multivariable analyses for CBF drop, time to MCAo, and anesthesia duration.

	CBF drop			Time to MCAo			Anesthesia duration		
	Initial	**Final**	**SDS**	**Initial**	**Final**	**SDS**	**Initial**	**Final**	**SDS**
Comorbidity	0.4005			< 0.0001	< 0.0001	< 0.0001	0.0037	0.0427	0.0799
Sex	0.6635			0.4463	0.0007	0.0037	0.1590	0.0126	0.0372
Age	0.1901			0.7001			0.7041		
Age x Age	0.6715			0.5664			0.8400		
Weight	0.6780			0.3184	0.0237	0.0694	0.2624		
Weight x Weight	0.9098			0.5762			0.5164		
Sex x Age	0.3705			0.7154			0.1996		
Sex x Weight	0.2900			0.8986			0.6064		
Age x Weight	0.4488			0.7059			0.2411		
CBF monitoring	NA			< 0.0001	< 0.0001	0.0001	0.0004	0.0001	0.0007
Filament thickness	0.0045	0.0006	0.0006	0.5716			0.7294		
Filament length	0.0058	0.0001	0.0001	0.0913			< 0.0001	< 0.0001	< 0.0001
Control intervention	0.8218			0.9332			< 0.0001	< 0.0001	< 0.0001
Trial days	0.0259			0.0368	0.0009	0.0037	0.2335	0.0048	0.0239
Trial days x Trial days	0.0246			0.4382			0.9037		
Anesthesia throughout MCAo	0.4074			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Surgery time	0.8482			0.1273	0.1215	0.1215	0.3310	0.0408	0.0799
Surgery time x Surgery time	0.7618			0.0534	0.0435	0.0852	0.2000		
Circadian stage	0.5526			0.0919			0.0045	0.0052	0.0239
Site	0.1423	0.1164		0.0924	0.0607		.	.	
R^2 without site	0.2871	0.1818		0.3674	0.1639		0.9190	0.9163	
R^2 with site	0.4627	0.4315		0.5407	0.5299		0.9190	0.9163	

Table shows initial and final p-values (Type 3 Tests of Fixed Effects) as well as the final p-values adjusted for multiplicity using stepdown Sidak (SDS). The final model represents significant associations remaining after stepwise backward elimination. Effect sizes are shown on Table 2 and Figure 4. The R^2 values without and with the site are also shown to highlight the influence of site.