

## SUPPLEMENTAL MATERIAL

### **Systematic interrogation of angiogenesis in the ischemic mouse hindlimb: vulnerabilities and quality assurance**

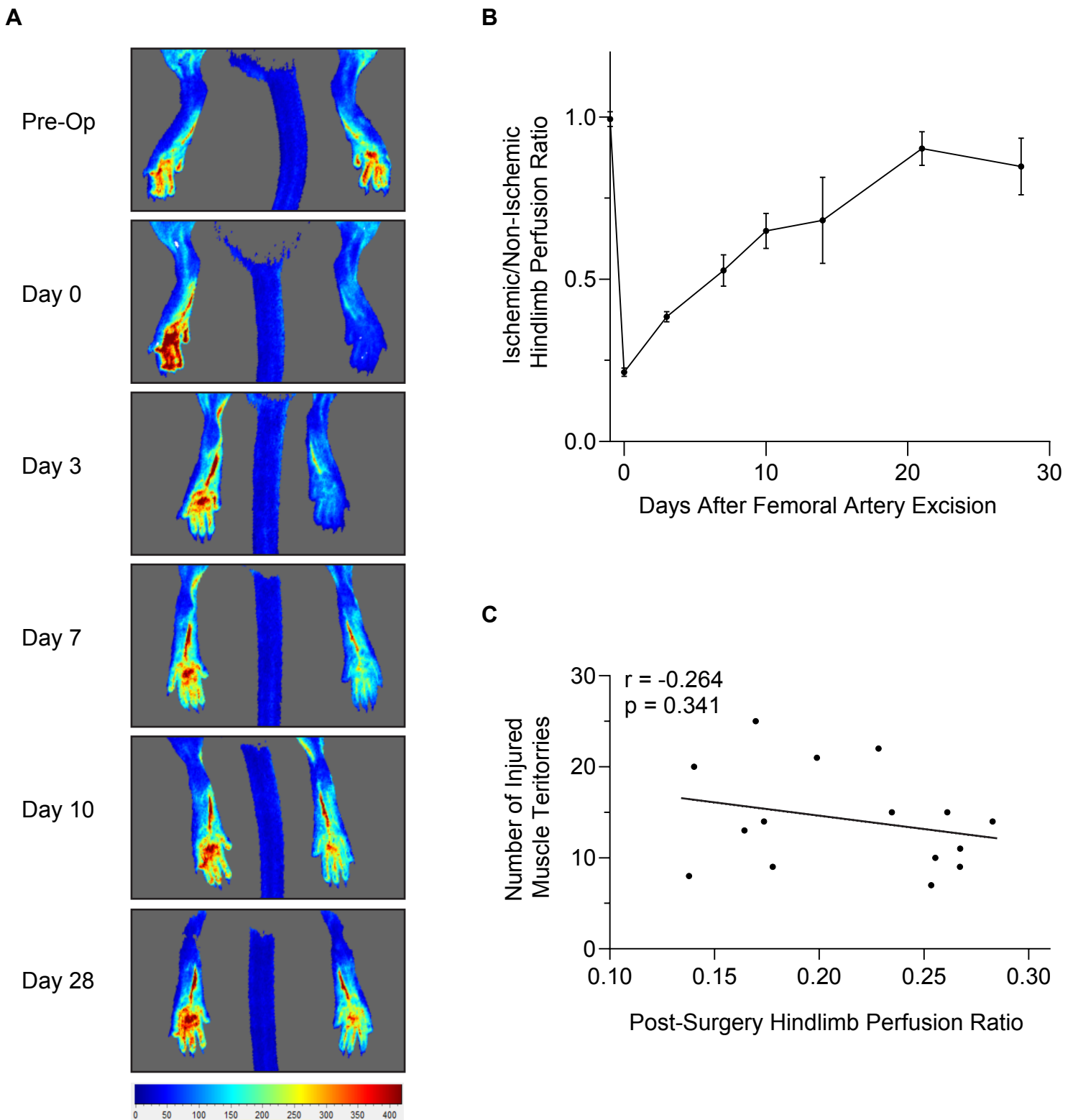
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**Supplemental Figure I. Hindlimb perfusion in C57BL/6J mice subjected to right femoral artery excision**

**A.** Laser speckle contrast images depicting paw perfusion before and on the designated days after femoral artery excision. **B.** Graph of hindlimb blood flow before and after femoral artery excision. Data are presented as perfusion ratios of the injured ischemic limb to the non-injured non-ischemic limb, up to 28 days after surgery. Day 0 corresponds to immediately after the surgical procedure (mean  $\pm$  SEM,  $n = 15$  mice). **C.** Plot of post-operative perfusion ratios vs. the number of muscles showing injury 10 days after surgery, among mice with a post-operative perfusion ratio that had declined to at least 0.3 ( $n = 15$  mice). No correlation between these indices was detected.

**Supplemental Table I.** Distribution and size of injury zones, 10 days after hindlimb ischemia in C57BL6/J mice

Proximal Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Biceps Femoris Anterior (Prox)			Vastus Lateralis (Prox)			Rectus Femoris (Prox)			Vastus Medialis (Prox)			Pectineus and Adductor Longus (Prox)					
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC			
Mouse 1	100	0	0	100	0	0	89	11	0	95	5	0	100	0	0			
Mouse 2	100	0	0	100	0	0	100	0	0	49	51	0	82	18	0			
Mouse 3	100	0	0	100	0	0	100	0	0	85	15	0	82	18	0			
Mouse 4	100	0	0	100	0	0	100	0	0	100	0	0	67	33	0			
Mouse 5	100	0	0	100	0	0	97	3	0	37	63	0	28	62	10			
Mouse 6	100	0	0	100	0	0	71	28	1	0	100	0	0	100	0			
Mouse 7	100	0	0	100	0	0	99	1	0	82	18	0	93	7	0			
Mouse 8	100	0	0	100	0	0	100	0	0	88	12	0	100	0	0			
Mouse 9	100	0	0	100	0	0	90	10	0	44	47	9	0	100	0			
Average	100%	0%	0%	100%	0%	0%	94%	6%	0%	64%	35%	1%	61%	38%	1%			
	Biceps Femoris Posterior (Prox)			Semi-membranosus (Prox)			Quadratis Femoris			Gracilis Anterior (Prox)			Semi-tendinosus (Prox)			Caudofemoralis (Prox)		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 3	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 4	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 5	100	0	0	22	78	0	36	62	2	100	0	0	100	0	0	0	100	0
Mouse 6	100	0	0	100	0	0	92	8	0	100	0	0	100	0	0	100	0	0
Mouse 7	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 8	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 9	22	78	0	100	0	0	85	14	1	100	0	0	100	0	0	0	98	2
Average	91%	9%	0%	91%	9%	0%	90%	9%	0%	100%	0%	0%	100%	0%	0%	78%	22%	0%

Mid Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Vastus Lateralis (Mid)			Rectus Femoris (Mid)			Vastus Medialis (Mid)			Vastus Intermedius			Caudofemoralis (Mid)			Adductor Magnus		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	80	20	0	62	38	0	30	70	0	100	0	0	97	3	0
Mouse 2	100	0	0	95	5	0	78	22	0	40	60	0	100	0	0	100	0	0
Mouse 3	100	0	0	100	0	0	100	0	0	83	17	0	100	0	0	100	0	0
Mouse 4	100	0	0	100	0	0	90	10	0	100	0	0	100	0	0	100	0	0
Mouse 5	100	0	0	99	1	0	17	61	22	100	0	0	0	100	0	25	67	8
Mouse 6	100	0	0	69	23	8	50	46	4	54	46	0	100	0	0	100	0	0
Mouse 7	100	0	0	100	0	0	100	0	0	42	58	0	100	0	0	100	0	0
Mouse 8	100	0	0	100	0	0	100	0	0	59	0	41	100	0	0	66	34	0
Mouse 9	100	0	0	96	4	0	34	60	6	83	17	0	0	96	4	0	92	8
Average	100%	0%	0%	93%	6%	1%	70%	26%	4%	66%	30%	5%	78%	22%	0%	76%	22%	2%
	Biceps Femoris Anterior (Mid)			Semi-membranosus (Mid)			Biceps Femoris Posterior (Mid)			Semi-tendinosus (Mid)			Gracilis (Mid)			Adductor Longus (Mid)		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 3	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 4	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 5	100	0	0	30	62	8	100	0	0	100	0	0	100	0	0	12	58	30
Mouse 6	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 7	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 8	88	12	0	100	0	0	93	7	0	100	0	0	100	0	0	100	0	0
Mouse 9	80	20	0	100	0	0	0	100	0	100	0	0	100	0	0	77	23	0
Average	96%	4%	0%	92%	7%	1%	88%	12%	0%	100%	0%	0%	100%	0%	0%	88%	9%	3%

Distal Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Popliteus			Tibialis Posterior			Tibialis Anterior			Flexor Digitorum Longus			Peroneus Longus			Extensor Digitorum Longus		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	0	100	0	0	43	57	0	62	38	0	71	29	0	88	12	0	48	52
Mouse 2	21	79	0	0	54	46	2	72	26	0	74	26	2	75	23	0	90	10
Mouse 3	5	95	0	0	52	48	0	69	31	0	82	18	0	100	0	0	64	36
Mouse 4	0	100	0	0	40	60	0	59	41	0	72	28	0	74	26	0	53	47
Mouse 5	14	62	24	0	18	82	0	61	39	0	59	41	0	75	25	0	61	39
Mouse 6	0	94	6	0	20	80	0	68	32	0	75	25	0	64	36	0	70	30
Mouse 7	35	65	0	0	70	30	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 8	0	100	0	0	61	39	0	86	14	0	78	22	0	100	0	0	95	5
Mouse 9	0	95	5	0	16	84	0	52	48	0	32	68	0	51	49	0	63	37
Average	8%	88%	4%	0%	42%	58%	11%	59%	30%	11%	60%	29%	11%	70%	19%	11%	60%	28%
	Gastrocnemius			Soleus/Plantaris			Semi-tendinosus (Distal)			Biceps Femoris (Distal)								
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC						
Mouse 1	47	53	0	0	100	0	100	0	0	100	0	0						
Mouse 2	100	0	0	67	33	0	100	0	0	100	0	0						
Mouse 3	74	26	0	0	100	0	100	0	0	100	0	0						
Mouse 4	79	21	0	0	100	0	100	0	0	100	0	0						
Mouse 5	23	61	16	0	56	44	67	33	0	100	0	0						
Mouse 6	37	60	3	0	83	17	100	0	0	100	0	0						
Mouse 7	97	3	0	0	100	0	100	0	0	100	0	0						
Mouse 8	52	48	0	0	100	0	100	0	0	100	0	0						
Mouse 9	0	61	39	0	40	60	100	0	0	100	0	0						
Average	57%	37%	6%	7%	79%	13%	96%	4%	0%	100%	0%	0%						

Individual C57BL6/J mouse data depicting the relative areas of uninjured (NML), injured-regenerating (RGN), and injured-necrotic (NEC) muscle in 33 hindlimb skeletal muscle territories, 10 days after femoral artery excision. (Prox=Proximal)

**Supplemental Table II.** Distribution and size of injury zones, 28 days after hindlimb ischemia in C57BL6/J mice

Proximal Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Biceps Femoris Anterior (Prox)			Vastus Lateralis (Prox)			Rectus Femoris (Prox)			Vastus Medialis (Prox)			Pectineus and Adductor Longus (Prox)					
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC			
Mouse 1	100	0	0	100	0	0	100	0	0	47	53	0	94	6	0			
Mouse 2	100	0	0	100	0	0	99	1	0	0	100	0	60	40	0			
Mouse 3	100	0	0	100	0	0	81	19	0	54	46	0	100	0	0			
Mouse 4	100	0	0	100	0	0	100	0	0	31	69	0	100	0	0			
Mouse 5	100	0	0	100	0	0	100	0	0	67	33	0	100	0	0			
Average	100%	0%	0%	100%	0%	0%	96%	4%	0%	40%	60%	0%	91%	9%	0%			
	Biceps Femoris Posterior (Prox)			Semi-membranosus (Prox)			Quadratis Femoris			Gracilis Anterior (Prox)			Semi-tendinosus (Prox)			Caudofemoralis (Prox)		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 2	23	77	0	23	77	0	0	100	0	100	0	0	0	100	0	69	31	0
Mouse 3	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 4	88	12	0	0	100	0	100	0	0	100	0	0	100	0	0	46	54	0
Mouse 5	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Average	82%	18%	0%	65%	35%	0%	80%	20%	0%	100%	0%	0%	80%	20%	0%	83%	17%	0%

Mid Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Vastus Lateralis (Mid)			Rectus Femoris (Mid)			Vastus Medialis (Mid)			Vastus Intermedius			Caudofemoralis (Mid)			Adductor Magnus		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	92	8	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	100	0	0	0	100	0	0	100	0	0	100	0	0	100	0
Mouse 3	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 4	100	0	0	100	0	0	0	100	0	89	11	0	0	100	0	0	100	0
Mouse 5	100	0	0	100	0	0	68	32	0	100	0	0	100	0	0	100	0	0
Average	100%	0%	0%	100%	0%	0%	52%	48%	0%	78%	22%	0%	60%	40%	0%	60%	40%	0%
	Biceps Femoris Anterior (Mid)			Semi-membranosus (Mid)			Biceps Femoris Posterior (Mid)			Semi-tendinosus (Mid)			Gracilis (Mid)			Adductor Longus (Mid)		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	0	100	0	35	65	0	0	100	0	100	0	0	0	100	0
Mouse 3	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 4	100	0	0	0	100	0	41	59	0	100	0	0	55	45	0	46	54	0
Mouse 5	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Average	100%	0%	0%	60%	40%	0%	75%	25%	0%	80%	20%	0%	91%	9%	0%	69%	31%	0%

Distal Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Popliteus			Tibialis Posterior			Tibialis Anterior			Flexor Digitorum Longus			Peroneus Longus			Extensor Digitorum Longus		
	Nor	Reg	Nec	Nor	Reg	Nec	Nor	Reg	Nec	Nor	Reg	Nec	Nor	Reg	Nec	Nor	Reg	Nec
Mouse 1	85	15	0	0	100	0	0	100	0	46	54	0	87	13	0	15	85	0
Mouse 2	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0
Mouse 3	0	100	0	0	100	0	0	83	17	0	100	0	0	100	0	0	100	0
Mouse 4	0	100	0	0	77	23	0	89	11	0	100	0	0	100	0	0	0	100
Mouse 5	22	78	0	0	100	0	88	12	0	100	0	0	100	0	0	100	0	0
Average	21%	79%	0%	0%	95%	5%	18%	77%	6%	29%	71%	0%	37%	63%	0%	23%	57%	20%
	Gastrocnemius			Soleus/Plantaris			Semi-tendinosus (Distal)			Biceps Femoris (Distal)								
	Nor	Reg	Nec	Nor	Reg	Nec	Nor	Reg	Nec	Nor	Reg	Nec						
Mouse 1	96	4	0	0	100	0	100	0	0	100	0	0						
Mouse 2	0	100	0	0	100	0	0	100	0	100	0	0						
Mouse 3	100	0	0	28	72	0	100	0	0	100	0	0						
Mouse 4	0	100	0	0	100	0	100	0	0	100	0	0						
Mouse 5	99	1	0	0	100	0	100	0	0	100	0	0						
Average	59%	41%	0%	6%	94%	0%	80%	20%	0%	100%	0%	0%						

Individual C57BL6/J mouse data depicting the relative areas of uninjured (NML), injured-regenerating (RGN), and injured-necrotic (NEC) muscle in 33 hindlimb skeletal muscle territories, 28 days after femoral artery excision. (Prox=Proximal)



**Supplemental Table III.** Distribution and size of injury zones, 10 days after hindlimb ischemia in C57BL6/N mice

Proximal Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Biceps Femoris Anterior (Prox)			Vastus Lateralis (Prox)			Rectus Femoris (Prox)			Vastus Medialis (Prox)			Pectineus and Adductor Longus (Prox)					
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC			
Mouse 1	100	0	0	100	0	0	100	0	0	20	80	0	70	30	0			
Mouse 2	100	0	0	100	0	0	87	13	0	30	70	0	100	0	0			
Mouse 3	100	0	0	100	0	0	100	0	0	43	57	0	64	36	0			
Mouse 4	100	0	0	100	0	0	100	0	0	94	6	0	93	7	0			
Mouse 5	100	0	0	100	0	0	100	0	0	0	100	0	64	36	0			
Average	100%	0%	0%	100%	0%	0%	97%	3%	0%	37%	63%	0%	78%	22%	0%			
	Biceps Femoris Posterior (Prox)			Semi-membranosus (Prox)			Quadratis Femoris			Gracilis Anterior (Prox)			Semi-tendinosus (Prox)			Caudofemoralis (Prox)		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 3	100	0	0	100	0	0	60	40	0	100	0	0	100	0	0	36	64	0
Mouse 4	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 5	100	0	0	0	100	0	100	0	0	100	0	0	100	0	0	100	0	0
Average	100%	0%	0%	80%	20%	0%	92%	8%	0%	100%	0%	0%	100%	0%	0%	87%	13%	0%

Mid Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Vastus Lateralis (Mid)			Rectus Femoris (Mid)			Vastus Medialis (Mid)			Vastus Intermedius			Caudofemoralis (Mid)			Adductor Magnus		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	81	19	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	100	0	0	76	24	0	100	0	0	100	0	0	100	0	0
Mouse 3	100	0	0	100	0	0	23	77	0	100	0	0	0	100	0	100	0	0
Mouse 4	100	0	0	100	0	0	35	65	0	95	5	0	100	0	0	100	0	0
Mouse 5	100	0	0	100	0	0	0	100	0	100	0	0	0	100	0	100	0	0
Average	100%	0%	0%	100%	0%	0%	43%	57%	0%	99%	1%	0%	60%	40%	0%	100%	0%	0%
	Biceps Femoris Anterior (Mid)			Semi-membranosus (Mid)			Biceps Femoris Posterior (Mid)			Semi-tendinosus (Mid)			Gracilis (Mid)			Adductor Longus (Mid)		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 2	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 3	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Mouse 4	100	0	0	79	19	2	96	4	0	100	0	0	100	0	0	100	0	0
Mouse 5	100	0	0	0	100	0	100	0	0	100	0	0	100	0	0	28	72	0
Average	100%	0%	0%	76%	24%	0%	99%	1%	0%	100%	0%	0%	100%	0%	0%	86%	14%	0%

Distal Hindlimb	Percent Area of Uninjured, Injured-Regenerating, and Injured-Necrotic Muscle																	
	Popliteus			Tibialis Posterior			Tibialis Anterior			Flexor Digitorum Longus			Peroneus Longus			Extensor Digitorum Longus		
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC
Mouse 1	14	86	0	0	38	62	0	94	6	0	100	0	0	89	11	0	85	15
Mouse 2	100	0	0	61	39	0	0	89	11	0	100	0	0	100	0	0	100	0
Mouse 3	0	92	8	0	100	0	0	97	3	0	100	0	0	94	6	0	100	0
Mouse 4	0	88	12	0	50	50	0	100	0	0	90	10	0	70	30	0	84	16
Mouse 5	0	67	33	0	20	80	0	76	24	0	84	16	0	79	21	0	73	27
Average	23%	67%	11%	12%	49%	38%	0%	91%	9%	0%	95%	5%	0%	86%	14%	0%	88%	12%
	Gastrocnemius			Soleus/Plantaris			Semi-tendinosus (Distal)			Biceps Femoris (Distal)								
	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC	NML	RGN	NEC						
Mouse 1	100	0	0	79	21	0	100	0	0	100	0	0						
Mouse 2	100	0	0	55	45	0	100	0	0	100	0	0						
Mouse 3	12	77	5	0	98	2	100	0	0	100	0	0						
Mouse 4	0	78	22	0	100	0	100	0	0	100	0	0						
Mouse 5	10	76	14	0	100	0	100	0	0	100	0	0						
Average	44%	46%	8%	27%	73%	0%	100%	0%	0%	100%	0%	0%						

Individual C57BL6/N mouse data depicting the relative areas of uninjured (NML), injured-regenerating (RGN), and injured-necrotic (NEC) muscle in 33 hindlimb skeletal muscle territories, 10 days after femoral artery excision. (Prox=Proximal)

#### Supplemental Table IV. Studies included in the systematic manuscript review

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## Major Resources Tables

### Animals (in vivo studies)

<b>Species</b>	<b>Vendor or Source</b>	<b>Background Strain</b>	<b>Sex</b>
Mouse	The Jackson Laboratory	C57BL/6J	Male
Mouse	Charles River Laboratories	C57BL/6N	Male
Mouse	The Jackson Laboratory / Inbreeding	C57BL/6J	Male

### Antibodies

<b>Target antigen</b>	<b>Vendor or Source</b>	<b>Catalog #</b>	<b>Working concentration</b>
CD31	Dianova (SZ31), Rat	DIA 310	1:20
Ki-67	Abcam, Rabbit	Ab16667	1:400
Rat IgG	Thermo Fisher	A11006	1:100
Rabbit IgG	Thermo Fisher	A11012	1:100