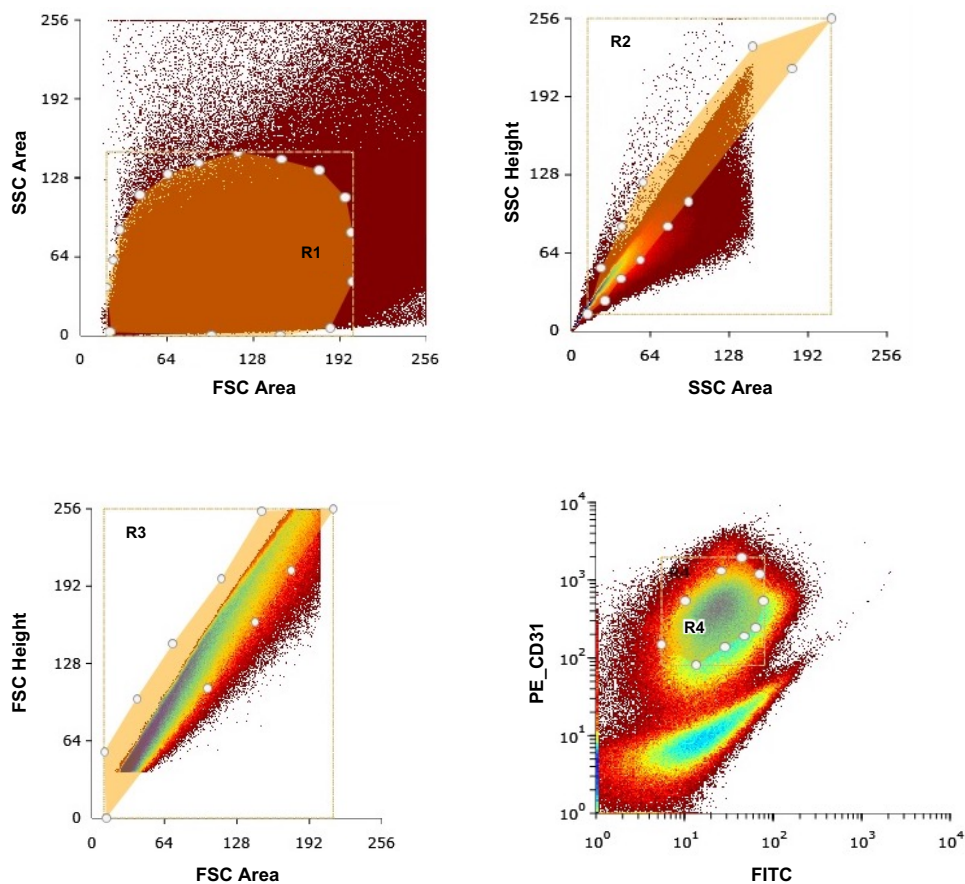

Supplementary information

**The endothelial Dll4–muscular Notch2 axis
regulates skeletal muscle mass**

In the format provided by the
authors and unedited



Supplementary Figure 1: Gating strategy

FSC-A/SSC-A gating strategy was used to remove the debris. FSC-A/FSC-H and SSC-A/SSC-H gating strategies were used to define doublets. Following this, CD31-PE positive cells were gated and collected as endothelial cells.

Supplementary Table 1: Antibody list

Antibody	Host	Clonality	Clone#	Application*	Dilution/Amount	Company	Catalog#
anti-Notch1	Rabbit	Monoclonal	D1E11	IB	1:1,000	Cell Signaling	#3608
anti-Notch2	Rabbit	Monoclonal	D76A6	IB	1:1,000	Cell Signaling	#5732
anti-Pax7	Mouse	Monoclonal	PAX7	IF	1:5	DSHBDevelopmental Studies Hybridoma Bank (DSHB)	PAX7
				IB	1:100		
anti-Myosin heavy chain	Mouse	Monoclonal	MF-20	IF	1:5	DSHB	MF-20
				IB	1:100		
anti-β-Tubulin	Mouse	Monoclonal	10G10	IB	0.5 µg/ml	FUJIFILM-Wako	014-25041
anti-Laminin α2	Rat	Monoclonal	4H8-2	IF	1:600	Santa Cruz	sc-59854
anti-FoxO1	Rabbit	Monoclonal	C29H4	IB	1:1,000	Cell Signaling	#2880
anti-FoxO3a	Rabbit	Monoclonal	75D8	IB	1:1,000	Cell Signaling	#2497
anti-phospho-FoxO1 (Thr24)/ FoxO3a (Thr32)	Rabbit	Polyclonal	-	IB	1:1,000	Cell Signaling	#9464
anti-Akt	Rabbit	Polyclonal	-	IB	1:1,000	Cell Signaling	#9272
anti-phospho-Akt (Ser473)	Rabbit	Polyclonal	-	IB	1:1,000	Cell Signaling	#9271
anti-Histone H3	Rabbit	Monoclonal	D1H2	IB	1:1,000	Cell Signaling	#4499
anti-Dystrophin	Rabbit	Polyclonal	-	IF	1:400	Abcam	ab15277
anti-Dll4	Mouse	Monoclonal	G-12	IF	1:100	Santa Cruz	sc-365429
				IB	1:200		
anti-Dll4	Rabbit	Polyclonal	-	IB (in ED Fig.7)	1:1,000	Abcam	ab7280
anti-CD31	Rat	Monoclonal	MEC13.3	IF, IB	1:400	BioLegend	102501
				IP	6 µg		
PE-conjugated anti-CD31	Rat	Monoclonal	390	CS	1:400	BioLegend	102408
anti-CD102	Rat	Monoclonal	3C4	IP	6 µg	BD Biosciences	553326
anti-CD9	Rabbit	Polyclonal	-	IB	1:1,000	ABclonal	A1703
anti-CD63	Rabbit	Polyclonal	-	IB	1:1,000	ABclonal	A5271
Biotinylated anti-rat IgG	Donkey	Polyclonal	-	IF	1:250	Jackson ImmunoResearch	712-065-153
anti-FLAG	Mouse	Monoclonal	M2	IP	10 µg	Sigma-Aldrich	F1804
anti-GAPDH	Mouse	Monoclonal	686613	IB	0.05 µg/ml	R&D Systems	MAB5718
Alexa Fluor 488-conjugated anti- mouse IgG	Goat	Polyclonal	-	IF	1:1,000	ThermoFisher	A11029
	Donkey	Polyclonal	-	IF	1:250	Jackson ImmunoResearch	715-545-151
Alexa Fluor 488-conjugated anti-rat IgG	Goat	Polyclonal	-	IF	1:500	ThermoFisher	A11006
Alexa Fluor 546-conjugated anti-rat IgG	Goat	Polyclonal	-	IF	1:1,000	ThermoFisher	A11081
Alexa Fluor 594-conjugated anti-mouse IgG antibody	Goat	Polyclonal	-	IF	1:500	ThermoFisher	A11005
Rhodamine Red-conjugated anti-rabbit IgG	Donkey	Polyclonal	-	IF	1:250	Jackson ImmunoResearch	711-295-152
anti-mouse IgG HRP-linked	Horse	Polyclonal	-	IB	1:10,000	Cell Signaling	#7076
anti-rabbit IgG HRP-linked	Goat	Polyclonal	-	IB	1:10,000	Cell Signaling	#7074
Ultra-LEAF purified anti-Dll4	Armenian hamster	Monoclonal	HMD4-1	Block	10 mg/kg B.W. (in vivo) 500 ng/ml (in vitro)	BioLegend	99572 (130802)
Ultra-LEAF purified Armenian hamster IgG control antibody	Armenian hamster	Monoclonal	HTK888	Block	10 mg/kg B.W. (in vivo) 500 ng/ml (in vitro)	BioLegend	40968
anti-human IgG (Fc-specific)	Goat	Polyclonal	-	Coating	10 µg/ml	Jackson ImmunoResearch	109-005-098

*IB: immunoblot, IF: immunofluorescence, IP: immunoprecipitation, CS: cell sorting

Supplementary Table 2: Primer sequences

Gene name	Forward	Reverse
<i>Tbp</i>	ACCGTGAATCTTGGCTGTAA	GTTCTTCACTCTTGGCTCCTG
<i>Actb</i>	CTGGCTCCTAGCACCATGAA	GCAGCTCAGTAACAGTCCGC
<i>Gapdh</i>	CATCTTCCAGGAGCGAGACC	CTCGTGGTTCACACCCATCA
<i>Pecam1</i>	GGAAGTGTCTCCCTTGAGC	GGAGCCTTCCGTTCTTAG
<i>Pax7</i>	GTGCCCTCAGTGAGTTCGATTAGC	CCACATCTGAGCCCTCATCCA
<i>Notch1</i>	GTGTGCGTCAACGTCCGAT	ATTCAGGCAGGTCCCACTTG
<i>Notch2</i>	TACCTACCACAACGGCACAG	ACATCGGCAGGTAGCTTTCC
<i>Rbpj</i>	TGTTCTCAGCAAGCGGATAA	TGCCACCTTCGTTCTGAAG
<i>Hes1</i>	GATAGCTCCCGGCATTCCAA	CGTTCATGCACTCGCTGAAG
<i>Dll1</i>	ACCAAGTGCCAGTCACAGAG	TCCATCTTACACCTCAGTCGC
<i>Dll4</i>	ATGAGTGCATCTGCCGTCC	TTCTTGACGGAGAGTGGTG
<i>Jag1</i>	CAGGGTCTACGCCTGTCATC	AAAGTGTAGACCTCGGCCA
<i>Jag2</i>	ATCAGGTGGACGGCTTCGAG	GCATTAAGGCACGGCTTCCC
<i>Ccng2</i>	CTGCCGAGTTGTCTTCTCCA	TTCCAGCAGTTCCACGGATT
<i>Cdkn1b</i>	AGATACGAGTGGCAGGAGGT	ATGCCGGTCCTCAGAGTTTG
<i>Rbl2</i>	CCATCTTCTGCTGTGCGCATTA	GGGTCTGAGGACGCTTTAGA
<i>Bnip3</i>	GAAGCGCACAGCTACTCTCA	TCCAATGTAGATCCCCAAGCC
<i>Gadd45</i>	CTGCAGAGCAGAAGACCGAA	GGGTCTACGTTGAGCAGCTT
<i>MuRF1</i>	TGATTCTGATGGAAACGCTATGG	ATTCGCAGCCTGGAAGATGTC
<i>Atrogin1</i>	GACAAAGGGCAGCTGGATTGG	TCAGTGCCCTTCCAGGAGAGA
<i>Fbxo31</i>	GTATGGCGTTTGTGAGAACC	AGCCCCAAAATGTGTCTGTA
<i>MUSA1</i>	TCGTGGAATGGTAATCTTGC	CCTCCCGTTTCTCTATCACG
<i>FoxO1</i>	GGCGGGCTGGAAGAATTCAA	GCCCTCGGCTCTTAGCAAAT