

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Blood glucose: Accu-chek (Roche)
Grip strength: 1027SM (Columbus Instruments)
Tetanic muscle force: 1300A (Aurora Scientific)
Cell sorting: S3e (Bio Rad)
RNA-Seq: NovaSeq (Illumina), HiSeq X Ten (Illumina), 10X Chromium (10X Genomics)
qRT-PCR: CFX96 (Bio Rad)
Fluorescent microscope images: IX83 (Olympus), FV-3000 (Olympus)
Bright field microscope images: IX83 (Olympus), BX51 (Olympus)
TEM images: H-7700 (Hitachi)
Western blot: LAS-4000 (GE Healthcare)

Data analysis

Cross-sectional area, myofiber diameter, myotube diameter: Fiji 2.3.0
Fluorescent microscope images: cellSens Dimension 1.17 (Olympus), Photoshop 2020 (Adobe)
TEM images: H-7700, Photoshop 2020
Cell sorting: Pro sort v1.6 (Bio Rad)
RNA-Seq: Strand NGS 3.4 (Strand Life Sciences), Cell Ranger 6.0.1 (10X Genomics), Loupe Browser 5.0 (10X Genomics), Seurat v4 (by Satija lab)
Pathway analysis: DAVID Bioinformatics Resources 6.8 (Laboratory of Human Retrovirology and Immunoinformatics)
Statistics: Graphpad Prism 9

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-seq data have been deposited in the NCBI Gene Expression Omnibus with the accession codes: GSE161693 and GSE185678. The quantification was carried out using the reference genome mm10 (GENCODE: vM23/Ensembl 98). Other raw data are available from the corresponding author upon reasonable requests.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- ☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample-size calculation was performed. Sample sizes were selected based on our prior studies that confirm to the standard in the field and assure statistically meaningful differences and reproducibilities of the results (Fujimaki et al., Stem Cells 2018; Tsuchiya et al., Stem Cell Reports 2020; Yoshioka et al., Sci Adv 2021). Regarding animal experiments, the maximum number of available mice was used for each experiment (at least three mice). In the analysis of myofiber and myotube diameter, one data point represents the average of over 15 individual myofibers and over 50 individual myotubes, respectively.
Data exclusions	Animals that showed signs of illness or were unwell during normal keeping were excluded before starting experiments. CSA analysis excluded the measurement of myofibers whose edges were outside the field of view. Shrunken myofibers were not included when measuring diameter.
Replication	For the purpose of reproducibility, biological replicates were analysed at minimum of three times. This ensured that our results could be reproduced across different days and by different investigators.
Randomization	Samples and mice were randomly allocated to different conditions, and no attempt was made to control for any other variable. In general, litter mates were separated into different groups.
Blinding	For animal experiments, investigators were not blinded to group allocation because investigators needed to take measures for animal models including hindlimb unloading, diabetes, and overloading, and to inject with neutralising antibodies, recombinant proteins, and compounds. For other experiments including biochemical assay, investigators were blinded to group allocation during sample collection and data analysis, when possible.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	anti-Notch1 (D1E11), Rabbit monoclonal, Cell Signaling, #3608, 1:1,000 (for WB) anti-Notch2 (D76A6), Rabbit monoclonal, Cell Signaling, #5732, 1:1,000 (for WB) anti-Pax7 (PAX7), Mouse monoclonal, DSHB, 1:5 (for IF), 1:100 (for WB)
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anti-MyHC (MF-20), Mouse monoclonal, DSHB, 1:5 (for IF), 1:100 (for WB)
 anti-b Tubulin (10G10), Mouse monoclonal, Wako, 014-25041, 0.5 µg/ml (for WB)
 anti-Laminin a2 (4H8-2), Rat Monoclonal, Santa Cruz, sc-59854, 1:600 (for IF)
 anti-FoxO1 (C29H4), Rabbit monoclonal, Cell Signaling, #2880, 1:1000 (for WB)
 anti-FoxO3a (75D8), Rabbit monoclonal, Cell Signaling, #2497, 1:1000 (for WB)
 anti-phospho-FoxO1 (Thr24)/FoxO3a (Thr32), Rabbit polyclonal, Cell Signaling, #9464, 1:1000 (for WB)
 anti-Akt, Rabbit polyclonal, Cell Signaling, #9272, 1:1000 (for WB)
 anti-phospho-Akt (Ser473), Rabbit polyclonal, Cell Signaling, #9271, 1:1000 (for WB)
 anti-Histone H3 (D1H2), Rabbit monoclonal, Cell Signaling, #4499, 1:1000 (for WB)
 anti-Dystrophin, Rabbit polyclonal, abcam, ab15277, 1:400 (for IF)
 anti-Dll4 (G-12), Mouse monoclonal, Santa Cruz, sc-365429, 1:100 (for IF), 1:200 (for WB)
 anti-Dll4, Rabbit polyclonal, abcam, ab7280, 1:1000 (for WB)
 anti-CD31 (MEC 13.3), Rat monoclonal, BD biosciences, 550274, 1:400 (for IF or WB), 6 µg (for IP)
 PE anti-CD31 (390), Rat monoclonal, BioLegend, 102408, 1:400 (for Cell Sorting)
 anti-CD102 (3C4), Rat monoclonal, BD biosciences, 553326, 6 µg (for IP)
 anti-CD9, Rabbit polyclonal, ABclonal, A1703, 1:1000 (for WB)
 anti-CD63, Rabbit polyclonal, ABclonal, A5271, 1:1000 (for WB)
 Biotinylated anti-rat IgG antibody, Donkey polyclonal, Jackson ImmunoResearch, 712-065-153, 1:250 (for IF)
 anti-FLAG (M2), mouse monoclonal, Sigma-Aldrich, F1804, 10 µg (for IP)
 anti-GAPDH antibody (686613), mouse monoclonal, R&D Systems, MAB5718, 0.05 µg/ml (WB)
 Alexa Fluor 488-conjugated anti-mouse IgG antibody, goat polyclonal, ThermoFisher, A11029, 1:1,000 (IF)
 Alexa Fluor 488-conjugated anti-mouse IgG antibody, donkey polyclonal, Jackson ImmunoResearch, 715-545-151, 1:200 (IF)
 Alexa Fluor 488-conjugated anti-rat IgG antibody, goat polyclonal, ThermoFisher, A11006, 1: 500 (IF)
 Alexa Fluor 546-conjugated anti-rat IgG antibody, goat polyclonal, ThermoFisher, A11081, 1:1,000 (IF)
 Alexa Fluor 594-conjugated anti-mouse IgG antibody, goat polyclonal, ThermoFisher, A11005, 1:500 (IF)
 Rhodamine Red-conjugated anti-rabbit IgG antibody, donkey polyclonal, Jackson ImmunoResearch, 711-295-152, 1:250 (IF)
 anti-mouse IgG HRP-linked antibody, horse polyclonal, Cell Signaling, #7076, 1:10,000 (WB)
 anti-rabbit IgG HRP-linked antibody, goat polyclonal, Cell Signaling, #7074, 1:10,000 (WB)
 Ultra-LEAF purified anti-mouse DLL4 antibody, BioLegend, 99572, 10 mg/kg body weight (in vivo), 500 ng/ml (in vitro)
 Ultra-LEAF anti-armenian hamster IgG antibody, BioLegend, 400967, 10 mg/kg body weight (in vivo), 500 ng/ml (in vitro)
 human IgG (Fc-specific) antibody, goat polyclonal, Jackson ImmunoResearch, 109-005-098, 10 µg/ml (coating)

Validation

All antibodies are commercially available and have been validated by the manufacturer as follows:

anti-Notch1 (D1E11) (Cell Signaling, #3608);

<https://www.cellsignal.jp/products/primary-antibodies/notch1-d1e11-xp-rabbit-mab/3608>

anti-Notch2 (D76A6) (Cell Signaling, #5732);

<https://www.cellsignal.jp/products/primary-antibodies/notch2-d76a6-xp-rabbit-mab/5732>

anti-Pax7 (PAX7) (DSHB);

<https://dshb.biology.uiowa.edu/PAX7>

anti-MyHC (MF-20) (DSHB);

<https://dshb.biology.uiowa.edu/MF-20>

anti-b tubulin (10G10) (Wako, 014-25041);

<https://labchem-wako.fujifilm.com/jp/product/detail/W01W0101-2504.html>

anti-Laminin a2 (4H8-2) (Santa Cruz, sc-59854);

<https://www.scbt.com/ja/p/laminin-alpha-2-antibody-4h8-2?requestFrom=search>

anti-FoxO1 (C29H4) (Cell Signaling, #2880);

<https://www.cellsignal.jp/products/primary-antibodies/foxo1-c29h4-rabbit-mab/2880>

anti-FoxO3a (75D8) (Cell Signaling, #2497);

<https://www.cellsignal.jp/products/primary-antibodies/foxo3a-75d8-rabbit-mab/2497>

anti-phospho-FoxO1 (Thr24)/FoxO3a (Thr32) (Cell Signaling, #9464);

<https://www.cellsignal.jp/products/primary-antibodies/phospho-foxo1-thr24-foxo3a-thr32-antibody/9464>

anti-Akt (Cell Signaling, #9272);

<https://www.cellsignal.jp/products/primary-antibodies/akt-antibody/9272>

anti-phospho-Akt (Ser473) (Cell Signaling, #9271);

<https://www.cellsignal.jp/products/primary-antibodies/phospho-akt-ser473-antibody/9271>

anti-Histone H3 (D1H2) (Cell Signaling, #4499);

<https://www.cellsignal.jp/products/primary-antibodies/histone-h3-d1h2-xp-rabbit-mab/4499>

anti-dystrophin, (abcam, ab15277);

<https://www.abcam.co.jp/dystrophin-antibody-ab15277.html>

anti-Dll4 (G-12) (Santa Cruz, sc-365429);

<https://www.scbt.com/ja/p/delta-4-antibody-g-12>

anti-Dll4, (abcam, ab7280);
<https://www.abcam.co.jp/dll4-antibody-ab7280.html> (only the datasheet is available)

anti-CD31 (MEC 13.3) (BD biosciences, 550274);
<https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd31.550274>

PE anti-CD31 (390) (BioLegend, 102408);
<https://www.biolegend.com/ja-jp/products/pe-anti-mouse-cd31-antibody-122?GroupID=BLG1566>

anti-CD102 (3C4) (BD biosciences, 553326);
<https://www.bdbiosciences.com/ja-jp/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd102.553326>

anti-CD9 (ABclonal, A1703);
<https://abclonal.co.jp/catalog-antibodies/CD9RabbitpAb/A1703>

anti-CD63 (ABclonal, A5271);
<https://abclonal.co.jp/catalog-antibodies/CD63RabbitpAb/A5271> (only the datasheet is available)

biotinylated anti-rat IgG antibody (Jackson ImmunoResearch, 712-065-153);
<https://www.jacksonimmuno.com/catalog/products/712-065-153>

anti-FLAG (M2) (Sigma-Aldrich, F1804);
<https://www.sigmaaldrich.com/JP/ja/product/sigma/f1804>

anti-GAPDH antibody (686613) (R&D Systems, MAB5718);
https://www.rndsystems.com/products/human-mouse-rat-gapdh-antibody-686613_mab5718

Alexa Fluor 488-conjugated anti-mouse IgG antibody (ThermoFisher, A11029);
<https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11029>

Alexa Fluor 488-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch, 715-545-151);
<https://www.jacksonimmuno.com/catalog/products/715-545-151>

Alexa Fluor 488-conjugated anti-rat IgG antibody (ThermoFisher, A11006);
<https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11006>

Alexa Fluor 546-conjugated anti-rat IgG antibody (ThermoFisher, A11081);
<https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11081>

Alexa Fluor 594-conjugated anti-mouse IgG antibody (ThermoFisher, A11005);
<https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11005>

Rhodamine Red-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch, 711-295-152);
<https://www.jacksonimmuno.com/catalog/products/711-295-152>

anti-mouse IgG HRP-linked antibody (Cell Signaling, #7076);
<https://en.cellsignal.jp/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076>

anti-rabbit IgG HRP-linked antibody (Cell Signaling, #7074);
<https://en.cellsignal.jp/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074>

Ultra-LEAF purified anti-mouse DLL4 antibody (BioLegend, 99572);
 This is a 0.2 micron sterile-filtered, Ultra-Low Endotoxin, Azide-Free (LEAF) antibody based on the regular antibody (cat #130802, clone: HMD4-1).
<https://www.biolegend.com/ja-jp/search-results/purified-anti-mouse-dll4-antibody-5290>
<https://www.biolegend.com/ja-jp/uleaf>

Ultra-LEAF anti-armenian hamster IgG antibody (BioLegend, 400967);
<https://www.biolegend.com/ja-jp/products/ultra-leaf-purified-armenian-hamster-igg-isotype-ctrl-7728?GroupID=BLG2376>

human IgG (Fc-specific) antibody (Jackson ImmunoResearch, 109-005-098);
<https://www.jacksonimmuno.com/catalog/products/109-005-098>

Specifically for Dll4, the mouse-monoclonal and the rabbit-polyclonal antibodies were also experimentally validated by western blotting analysis using Dll4-knocked down cells and Dll4-deficient muscle tissues, respectively, as provided in Fig. 3k and Extended Data Fig. 7f.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Murine primary satellite cells, murine primary endothelial cells, HEK293 cells (Clontech), Human skeletal muscle myoblasts (HSMs; Lonza), Human umbilical vein endothelial cells (HUVECs; Lonza)
Authentication	Primary satellite cells were isolated by myofiber plating method or preplating method, which are standards in the field and previously reported (Yoshioka et al., 2020; Yoshioka et al., 2021). Primary endothelial cells were isolated by cell sorting (for qRT-PCR) or immunoprecipitation method (for culture), which is standard in the field and previously reported (Muramatsu et al., 2020). HEK293 cells, HSMs, and HUVECs were authenticated by qPCR using human-specific primers.
Mycoplasma contamination	We did not test for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Wild-type C57BL/6J mice were obtained from the Charles River Laboratories Japan or the CLEA Japan. Pax7-CreERT2 mice (Stock No. 012476), Rosa26-LSL-DTA mice (Stock No. 010527), HSA-rtTA;TR-Cre mice (Stock No. 012433), and Notch2-floxed (Stock No. 010525) mice were obtained from the Jackson Laboratories. Rbpj-floxed (RBRC01071) and Hes1-floxed (RBRC06047) mice were obtained from the RIKEN BioResource Research Centre. Rosa26-LSL-N2ICD knock-in mice have been previously described (Fujimura et al., 2010). Mlc1f-Cre mice were kindly provided by Dr. Burden. Ins2Akita mice (AKITA/Slc) were obtained from the Japan SLC. Dll4-floxed mice and Cdh5-CreERT2 mice were obtained from Dr. Shima and Dr. Kubota, respectively. Male mice were used in all experiments with the exception of the experiment shown in Extended Data Fig. 10b–d, in which female mice were used. All experiments were performed using 7- to 16-week-old mice, with the exception of the experiment shown in Extended Data Fig. 2, in which mice used were between 6 and 36 weeks of age. Mice were housed at 22±2 °C and 40–80% humidity on a 12-h light cycle with free access to food and water.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	Institutional Animal Care and Usage Committee (IACUC) of the Kumamoto University (2020-085) or the University of Minnesota

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Endothelial cells belonging to muscle tissues were freshly obtained by cell sorting to assess their gene expression. GAS muscles of mice were minced and digested using 0.2% type II collagenase followed by lysis of red blood cells. Mononuclear cells were incubated with PE-conjugated CD31 antibody at 4 °C for 30 min.
Instrument	S3e Cell Sorter (Bio Rad)
Software	ProSort v1.6 (Bio Rad)
Cell population abundance	Endothelial cells were roughly 15–20% of total cells present in muscle tissue. Endothelial cells were isolated using PE-conjugated antibody for CD31.
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.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.