

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	No public or other external data were used in this study. For the bulk RNA sequencing in this study, we used embryo samples and data that we generated ourselves.
Data analysis	As described in manuscript, the sequencing reads were aligned to the Sus scrofa (pig) genome assembly Sscrofa11.1 (susScr11), submitted by the Swine Genome Sequencing Consortium (SGSC), using kallisto (v0.48.0) with the following parameters: -t 8 -b 100. Subsequently, we employed tximport (v1.26.0) to determine the read count for each transcript and quantified transcript abundance as transcripts per kilobase per million reads mapped (TPM). We summed the read counts and TPM of all alternative splicing transcripts of a gene to obtain gene expression levels. To identify statistically significant differences in gene abundance between the WT and MYF5/MYOD/MYF6-null embryos, as well as differences among WT embryos at E41 and E62, a Wald test (corrected for multiple testing using the Benjamini and Hochberg method) was applied using DESeq2 (v1.38.3). The test was conducted for a fold change difference greater than 2x (log2 fold change threshold of 1), rejecting the null hypothesis when the adjusted p-value was less than 0.05, followed by differential expression analysis. Gene Ontology (GO) enrichment analysis for biological process (BP) terms was then performed with clusterProfiler (v4.7.0), using a p-value-adjusted false discovery rate < 0.05.

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data will be available upon request. The Bulk RNA-seq data from WT and null embryos and fetal pigs have been deposited in NCBI Sequence Read Archive (SRA) database with the project accession number (GSE288246). All unique materials used in these studies are readily available from the authors or from commercial sources. Gene edited primary cell lines are limited in number but will be available pending their supply.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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](https://www.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	Sample sizes were determined to achieve statistical significance wherever possible.
Data exclusions	No data were excluded
Replication	All attempts at replication for standard assays were successful. We observed that the production of null embryos was reliable and reproducible.
Randomization	Random allocation (as was possible)
Blinding	Investigators were blinded whenever possible (for cell counting, CSA measurement, and bulk-RNA sequencing, in particular). Embryos were processed, however, in the order that they were delivered to maximize preservation and to inform next steps, which limited investigator blinding in some instances.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Pax7 (Pax7; Developmental Studies Hybridoma Bank; 5 µg/ml with Alexa Fluor 647 Goat anti-mouse IgG1), embryonic myosin heavy chain (F1.652; Developmental Studies Hybridoma Bank; 5 µg/ml with Alexa Fluor 546 Goat anti-mouse IgG1), myosin heavy chain all fast (F-59; Developmental Studies Hybridoma Bank; 5 µg/ml with Alexa Fluor 647 Goat anti-mouse IgG1), myosin heavy chain slow (BA-F8; Developmental Studies Hybridoma Bank; 5 µg/ml with Alexa Fluor 546 Goat anti-mouse IgG2B), myogenin (PCRP-MYOG-1C5; Developmental Studies Hybridoma Bank; 5 µg/ml with Alexa Fluor 647 Goat anti-mouse IgG2B), laminin (L9393; Sigma Aldrich; 1 µg/ml with Alexa Fluor 546 goat anti-rabbit IgG), dystrophin (15277; abcam; 1:200 with Alexa Fluor 546 goat anti-mouse IgG). Nuclei Hoechst 33342 Dye 20mM Solution (62249; Pierce; 1:1000) or DAPI (D21490; Invitrogen; 1:1000). For NMJ, S100 (GA50461-2; Agilent Dako Omnis; 1:200 with Alexa Fluor 647 goat anti-rabbit IgG), neurofilament (2H3; DSHB; 0.25 µg/ml with Alexa Fluor 488 goat anti-mouse IgG1), synaptic vesicles (SV2; DSHB; 0.9 µg/ml with Alexa Fluor 488 goat anti-mouse IgG1) and DAPI (1 µg/ml).
Validation	Validation statements are provided on the manufacturer's website for each antibody used. All catalog number, clone number, concentration (or dilution) and supplier information are listed above. Appropriate positive and negative control were used in each experiment to validate the manufacturer's statement for our system.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Porcine fetal fibroblasts were derived from E35 wild-type embryos. Wild-type fibroblasts were gene-edited using CRISPR/Cas9 to generate MYF5/MYOD/MYF6-null fibroblasts.
Authentication	All cell lines were authenticated using karyotypic analysis
Mycoplasma contamination	The cell line tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Mixed strains of Landrace x Yorkshire X Duroc porcine gilts were used
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex-based analyses were not performed in this study. The research design did not account for sex as a variable, and data disaggregated by sex were not collected. The focus of the study was not influenced by sex-based differences, and thus, sex was not considered during the study design.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	IACUC (International Animal Care and Use Committee), SCRO (Stem Cell Research Oversight)

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

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A