

Supplementary information for

Porcine myogenesis in cloned wildtype and *MYF5/MYOD/MYF6*-null porcine embryo

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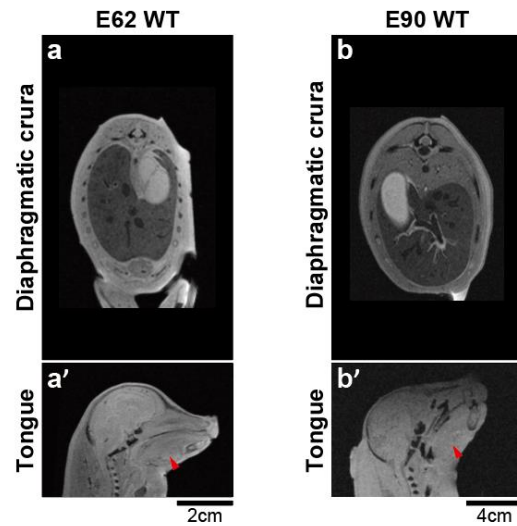
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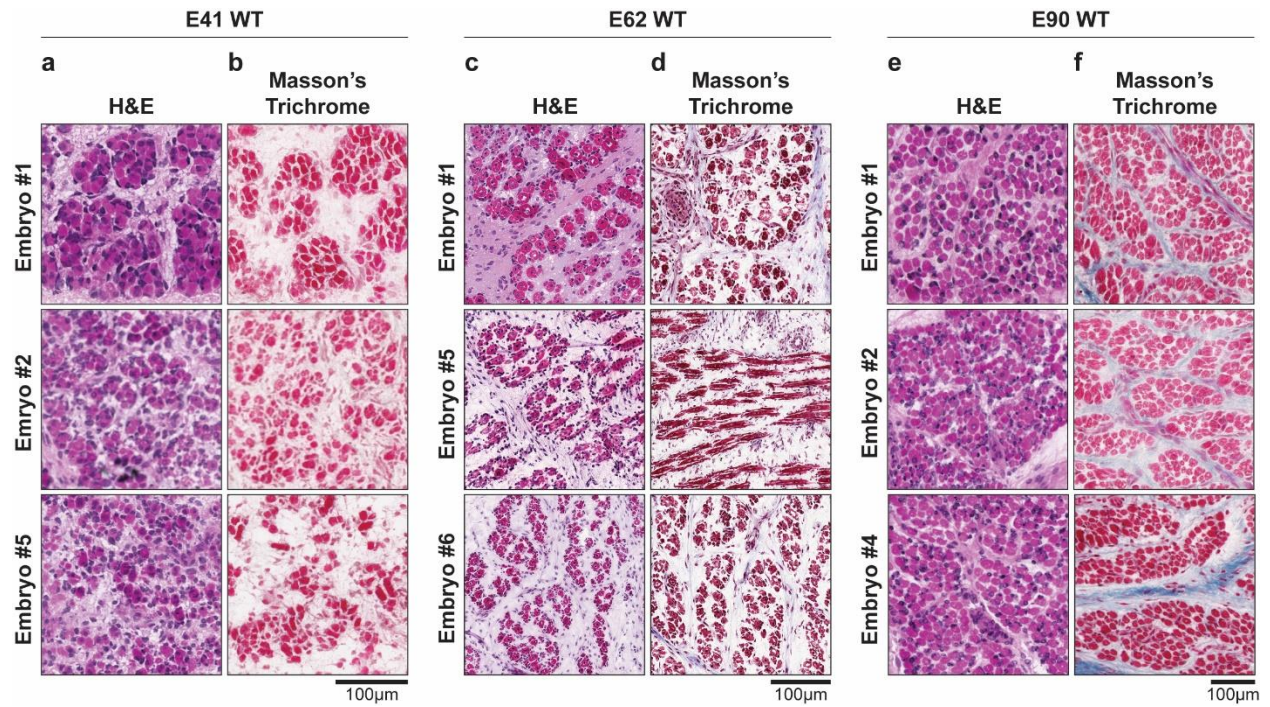
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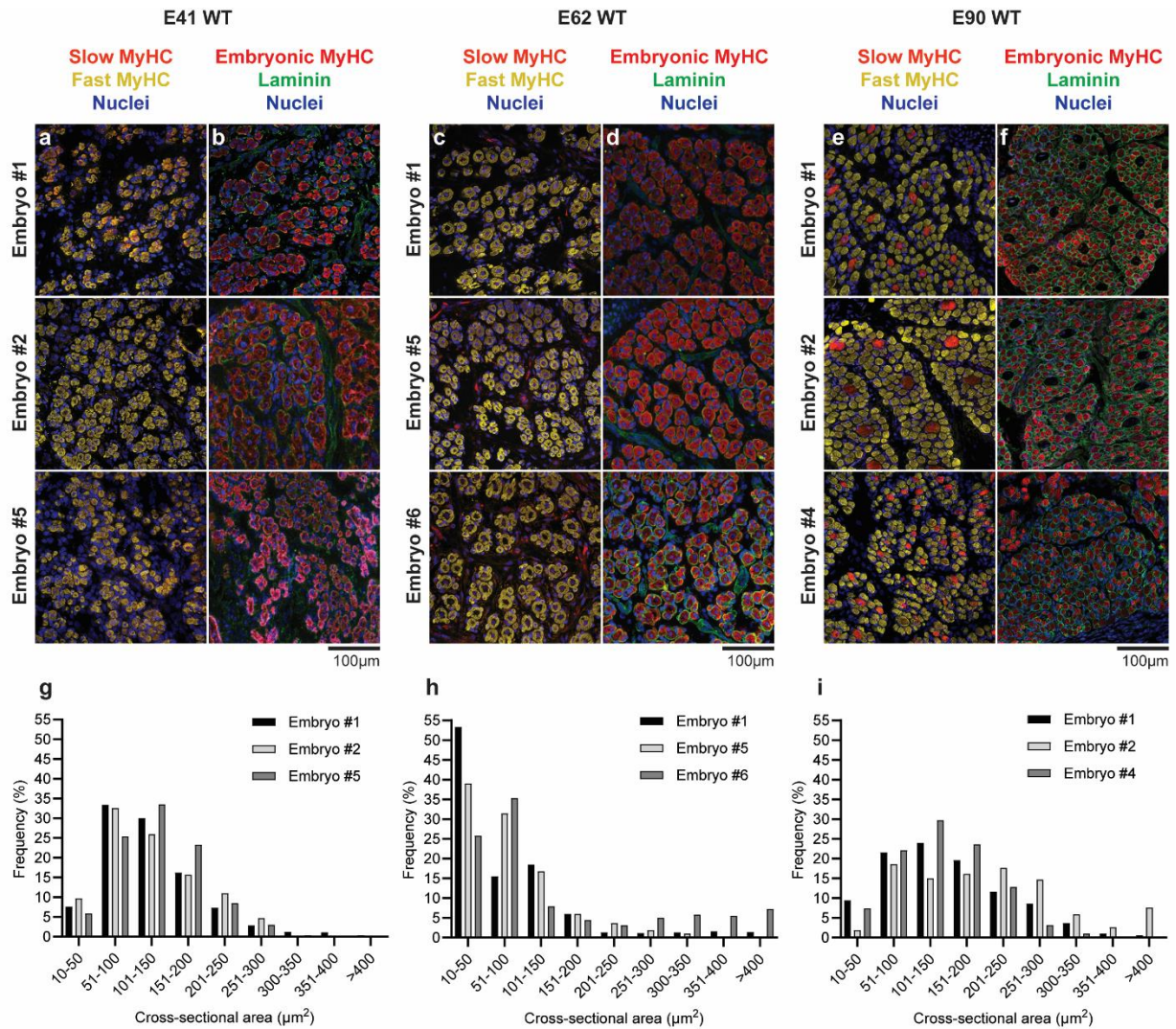
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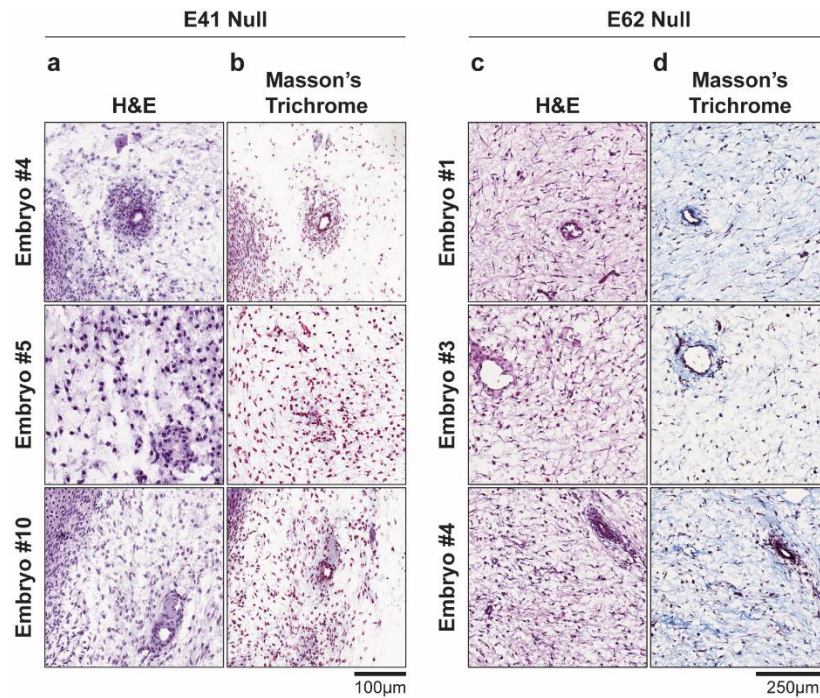
Supplementary Fig. 1 | Additional MRI scans of cloned WT porcine embryos at E62 and E90. **a, b** Transverse MRI image of E62 WT (**a**) and E90 WT (**b**) showing diaphragmatic crura. **a' b'** Parasagittal MRI image of a head of E62 WT (**a'**) and E90 WT (**b'**) demonstrate the development of tongue (red arrowhead). Scale bar, 2 cm (**a, a'**), 4 cm (**b, b'**).



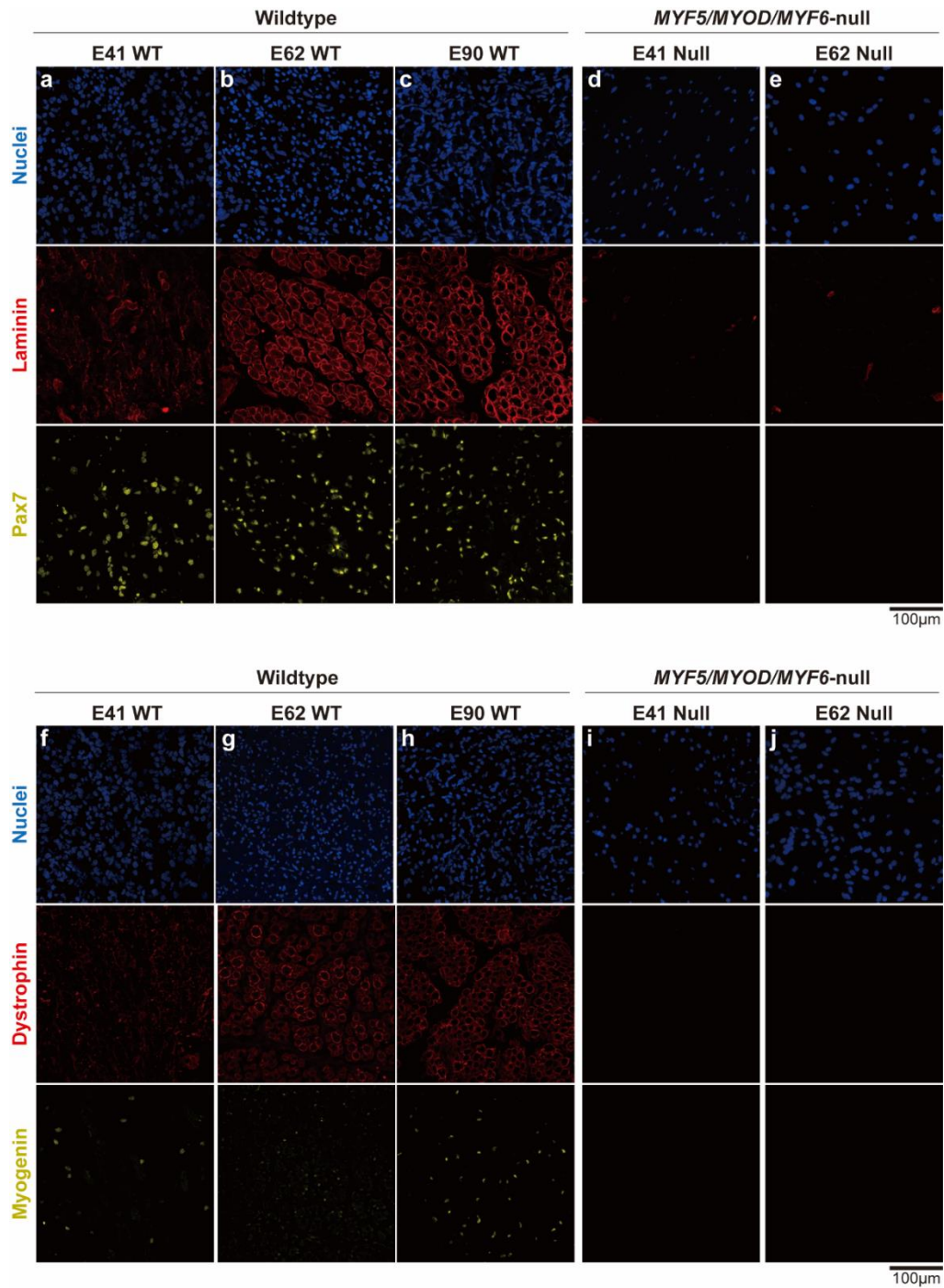
Supplementary Fig. 2 | Histological imaging of additional cloned WT porcine embryos across developmental stages. a, b H&E staining (a) and Masson's Trichrome staining (b) of hindlimb sections from three additional embryos at E41. Sections of each embryo are arranged vertically in each panel. **c-f** For E62 (c, d) and E90 (e, f), H&E (c, e) and Masson's Trichrome (d, f) stained. Sections from three different embryos are arranged vertically in each panel. Scale bar, 100 µm.



Supplementary Fig. 3 | Fiber typing of additional cloned wildtype WT porcine embryos across developmental stages. **a, c, e** Immunohistochemical staining of hindlimb section with myosin heavy chain slow (red), myosin heavy chain fast (yellow), and nuclei (blue). Merged channels of three additional embryos at E41 (**a**), E62 (**c**), and E90 (**e**) are presented. Scale bar, 100 μm . **b, d, f** Immunohistochemical staining of hindlimb section with embryonic myosin heavy chain (red), laminin (green), and nuclei (blue). Merged channels of three additional embryos at E41 (**b**), E62 (**d**), and E90 (**f**) are presented. Scale bar, 100 μm . **g-i** Across gestational stages, E41 (**g**), E62 (**h**), and E90 (**i**), the distribution of muscle fiber cross-sectional areas of each corresponding additional embryo, ranging from 10 to 400 μm^2 , segmented into intervals of 50 μm^2 is presented.

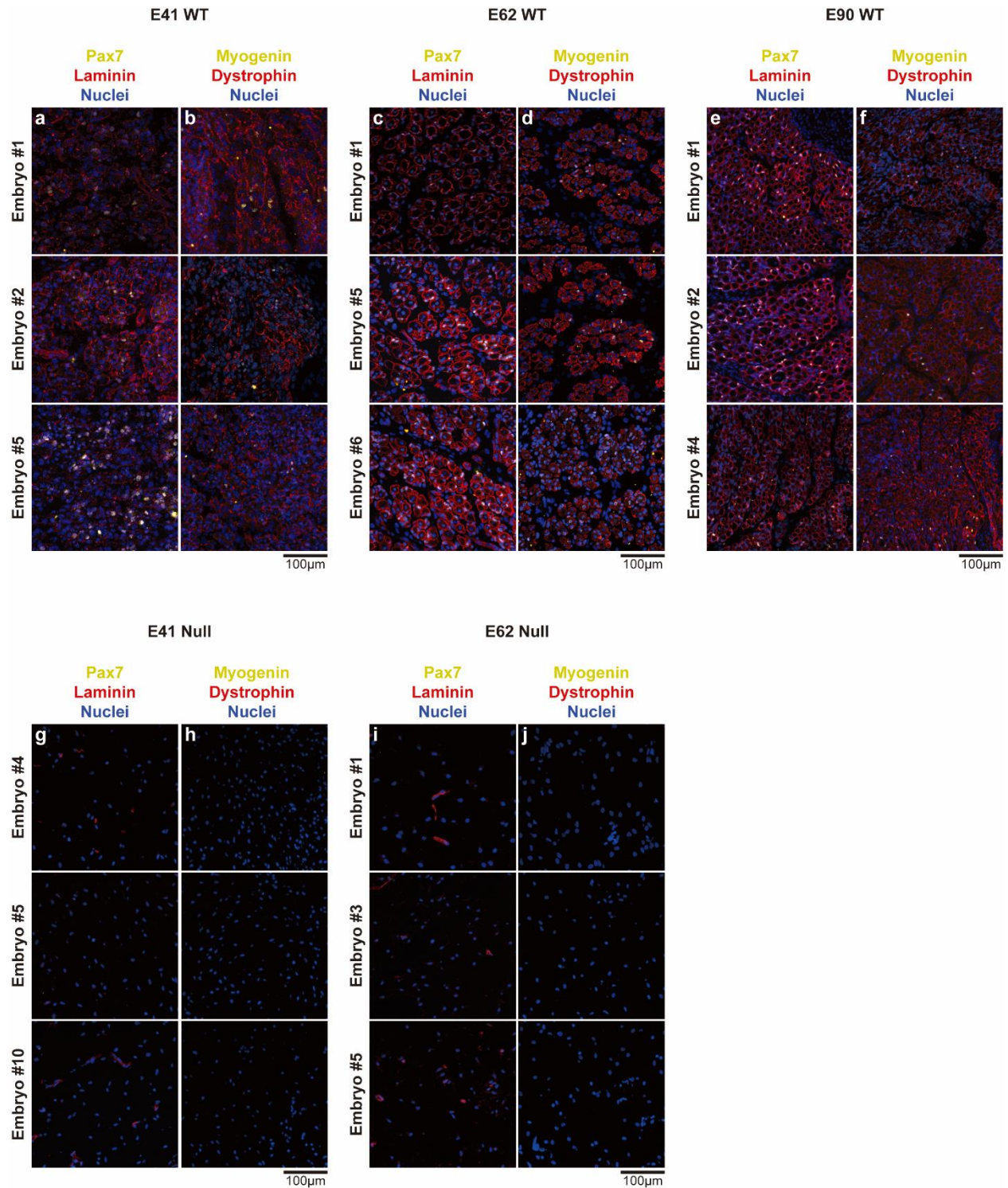


Supplementary Fig. 4 | Histological imaging of additional *MYF5/MYOD/MYF6*-null porcine embryos. a,b H & E staining (a) and Masson's Trichrome staining (b) of hindlimb sections from three additional embryos at E41. Sections of each embryo are arranged vertically in each panel. Scale bar, 100 µm. **c, d** For E62, H&E (c) and Masson's Trichrome (d) stained sections. Sections from three different embryos are arranged vertically in each panel. Scale bar, 250 µm.



Supplementary Fig. 5 | Immunohistochemical imaging using satellite cell and muscle differentiation markers. Single-channel immunofluorescence images of merged image in **Fig. 4 a-e** and **Fig. 4 g-k** are provided for a detailed view of the specific markers. **a-e** Separate images for each marker, including nuclei (blue), Laminin (red), and Pax7 (yellow), across E41 (**a**), E62 (**b**), and E90 (**c**) stages in WT hindlimbs, followed by *MYF5/MYOD/MYF6*-null hindlimbs at E41 (**d**) and E62 (**e**). **g-k** Separate images for each marker, including nuclei (blue), Dystrophin (red), and Myogenin (yellow), across E41 (**g**),

E62 (**h**), and E90 (**i**) stages in WT embryos, followed by *MYF5/MYOD/MYF6*-null hindlimbs at E41 (**j**) and E62 (**k**). Scale bar, 100 μm .



Supplementary Fig. 6 | Immunohistochemical imaging of satellite cell and muscle differentiation in additional WT and *MYF5/MYOD/MYF6*-null hindlimbs of porcine embryos. For analysis of satellite cell distribution, cross sections of a hindlimb are labeled with DAPI (nuclei, blue), Laminin (basement membrane, red), and Pax7 (satellite cell, yellow). **a, c, e** Satellite cell distribution of muscle sections from three additional WT

embryos at E41(**a**), E62 (**c**), and E90 (**e**). **g, i**. Satellite cell distribution of hindlimb sections from three additional *MYF5/MYOD/MYF6*-null embryos at E41(**g**) and E62 (**i**). For analysis of muscle differentiation marker, cross sections of a hindlimb are labeled with DAPI (nuclei, blue), Dystrophin (muscle membrane, red), and Myogenin (muscle differentiation marker, yellow). **b, d, f** The expression of Dystrophin and Myogenin in three additional WT embryos at E41(**b**), E62 (**d**), and E90 (**f**). **h, j** The expression of Dystrophin and Myogenin in three additional *MYF5/MYOD/MYF6*-null embryonic hindlimbs at E41(**h**) and E62 (**j**). Sections of each embryo are arranged vertically in each panel. Scale bar, 100 μm .

Supplementary Table 1. Embryo length of wildtype embryos

E41		E62		E90	
Embryo #	C-R (cm)	Embryo #	C-R (cm)	Embryo #	C-R (cm)
1	5.5	1	11.7	1	21.9
2	5.2	2	11.4	2	19.5
3	5.2	3	10.3	3	20.6
4	5.2	4	11.1	4	19.5
5	6.8	5	10.8		
		6	13.4		
Mean±S.D.	5.6 ± 0.7	11.5 ± 1.1		20.4 ± 1.1	

Supplementary Table 2. Summary of the production for *MYF5/MYOD/MYF6*-null porcine embryo

Donor cell line for SCNT	Transferred embryos	Transferred gilts	Pregnant gilts	E-age at harvest	Harvested embryos
<i>MYF5/MYOD/MYF6</i> -null	694	5	1	E42	10*
<i>MYF5/MYOD/MYF6</i> -null	520	4	1	E62	6
<i>MYF5/MYOD/MYF6</i> -null	348	3	3	E90	4**

*One was identified as a resorbing embryo

**All four embryos harvested were identified as resorbing embryos

Supplementary Table 3. Embryo length of *MYF5/MYOD/MYF6*-null embryos

E41		E62		E90	
Embryo #	C-R (cm)	Embryo #	C-R (cm)	Embryo #	C-R (cm)
1	4.6	1	11.0	1	13.5
2*	N/A	2	11.0	2	11.9
3	4.5	3	12.5	3	13.7
4	3.9	4	12.5	4	9.1
5	5.1	5	12.5		
6	2.1				
7	3.7				
8	3.7				
9	5.1				
10	5.1				
Mean±S.D.	4.2 ± 1.0	11.9 ± 0.8		12.1 ± 2.1	

*Resorbing embryo