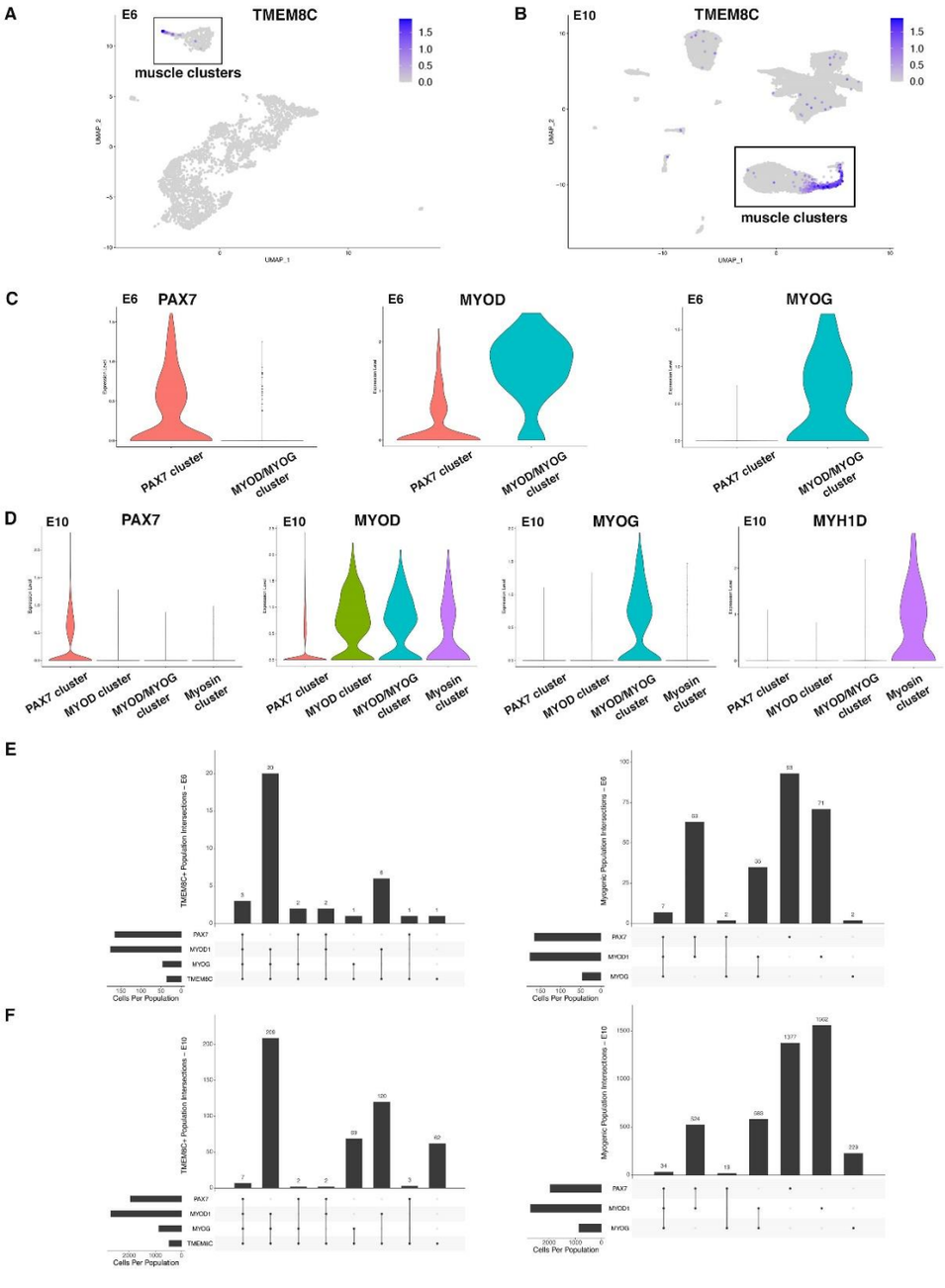

G

	Percentage in TMEM8C+ population	
Major gene combinations	E6	E10
TMEM8C/MYOD/MYOG	55,5	44,1
TMEM8C/MYOD	16,7	25,3
TMEM8C/MYOG	2,8	14,5
3 combinations altogether	75	84

	Percentage in myogenic population	
Corresponding gene combinations	E6	E10
MYOD/MYOG	12,8	13,5
MYOD	26	36,1
MYOG	0,7	5,3
3 combinations altogether	39,6	54,8

Figure S1

Fig. S1. *TMEM8C* is co-expressed with *MYOD* and *MYOG* in limb foetal muscles

(**A,B**) Feature plots showing the distribution of *TMEM8C*+ cells across all limb clusters at E6 (A) and E10 (B). The muscle clusters are boxed. (**C**) Violin plots showing Log-normalized expression levels of the *PAX7, MYOD* and *MYOG* genes in cells grouped by muscle clusters at E6. (**D**) Violin plots showing Log-normalized expression levels of the *PAX7, MYOD, MYOG* and *MYH1D* genes in cells grouped by muscle clusters at E10. (**E,F**) UpSet plots showing cell numbers for the gene combinations present within the *TMEM8C*+ population (left panel) or within the myogenic (*PAX7*+, *MYOD*+ or *MYOG+)* population (right panel) at E6 (E) and E10 (F). The genes considered for the combinations are *PAX7*, *MYOD* and *MYOG*. The size of each population expressing a gene of interest is shown at the bottom left of the plot. (**G**) Left panel: percentage of gene combinations among the *TMEM8C*+ cell population at E6 and E10. Right panel: percentage of gene combinations among the myogenic (*PAX7*+, *MYOD*+ or *MYOG+)* population at E6 and E10. Numbers used for percentage calculations are those of panels E and F.

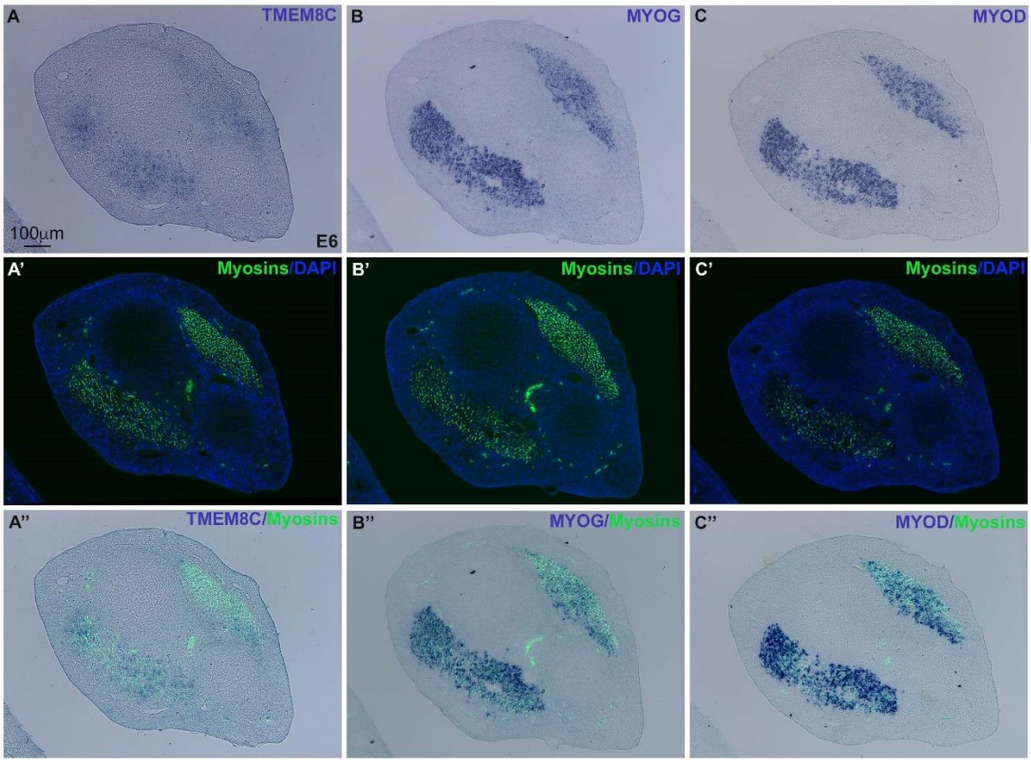

Figure S2

Fig. S2. *MYOD, MYOG* and *TMEM8C* transcripts show a uniform distribution in limb muscle masses of E6 chicken embryos

In situ hybridization to adjacent limb sections of E6 chicken embryos with the TMEM8C (A,A',A''), MYOG (B,B',B'') and MYOD (C,C',C'') probes (blue), followed by immunohistochemistry with the MF20 antibody to visualize myosins (green) and the nuclear marker DAPI (n=4 embryos). (A-C) show in situ hybridization, (A'-C') show myosin expression, (A''-C") show combined in situ hybridization and myosin expression.

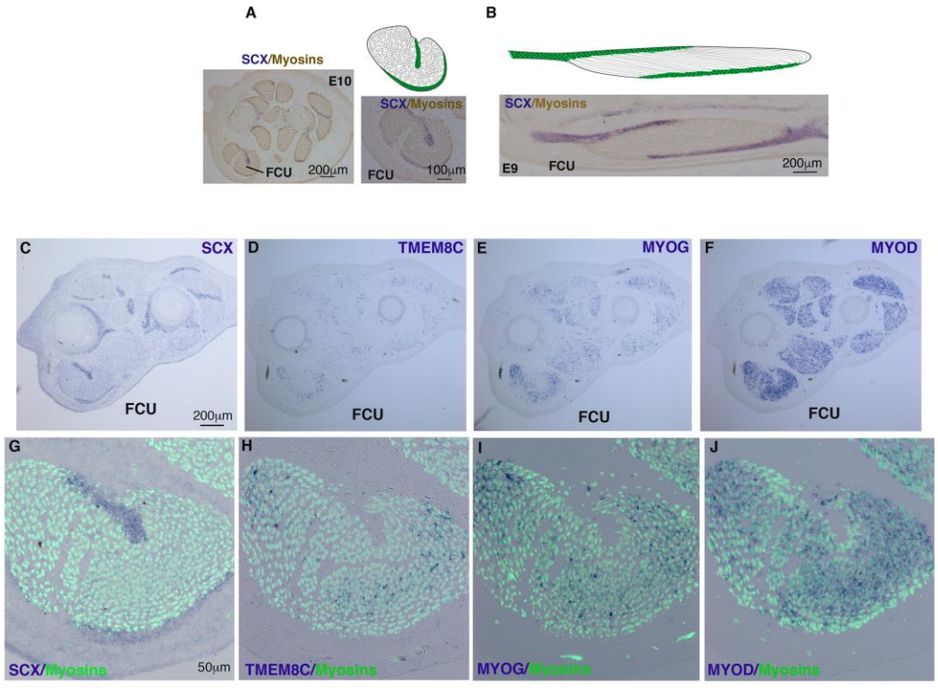

Figure S3

Fig. S3. *TMEM8C* and *MYOG* transcripts are regionalized in foetal muscles of E10 chicken limbs

(**A,B**) Schematic representation of FCU muscle and adjacent tendons. Transverse (A) and longitudinal (B) views to visualise the muscle and tendon interface. (A,B) Tendon are in green in the schematics; In situ hybridization to transverse (A) and longitudinal (B) sections of the FCU muscle with SCX probe (blue) followed by immunohistochemistry with the MF20 antibody to visualize myosins (brown). (**C-J**) In situ hybridization to adjacent and transverse limb sections of E10 chicken embryos with the SCX (C,G), TMEM8C (D,H), MYOG (E,I) and MYOD (F,J) probes (blue), followed by immunohistochemistry with the MF20 antibody to visualize myosins (green) (n=5 embryos). (G-J) are high magnification of the FCU muscle shown in (C,F). *TMEM8C* and *MYOG* transcripts display a regionalized expression in muscles with less intense expression close to SCX+ tendons.

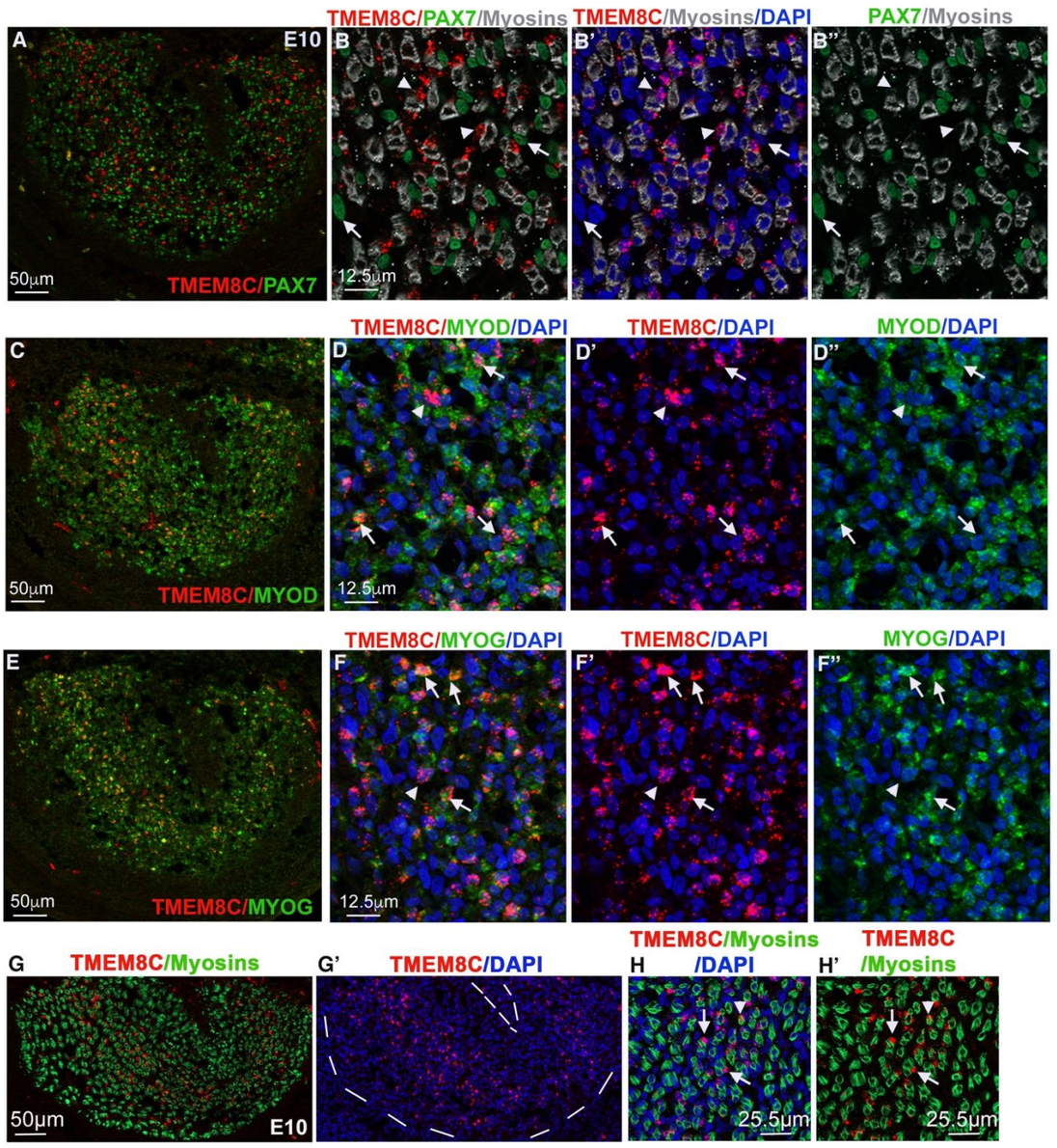

Figure S4

Fig. S4. *TMEM8C* is preferentially expressed in *MYOG*+ cells outside myosin+ myotubes and excluded from PAX7+ progenitors.

(A,B,B',B'') Fluorescent RNA in situ hybridization to transverse limb muscle sections of E10 chicken embryos with the *TMEM8C* probe (red), followed by immunohistochemistry with the PAX7 antibody (green) and MF20 antibody to visualize myosins (grey). (B,B',B'') are high magnification of the FCU muscle shown in (A). Arrowheads point to *TMEM8C* expressing cells (red), while arrows point to PAX7+ cells (green). **(C,D,D',D'')** Double in situ hybridisation to transverse limb muscle section at E10 with *TMEM8C* (red) and *MYOD* (green) probes. (D,D',D'') are high magnification of the FCU muscle shown in (C). Arrows point to *TMEM8C+MYOD+* cells (red and green), while arrowheads point to *TMEM8C+MYOD-* cells (red). **(E,F,F',F'')** Double in situ hybridisation to transverse limb muscle section at E10 with *TMEM8C* (red) and *MYOG* (green) probes. (F,F',F'') are high magnification of the FCU muscle shown in (E). Arrows point to *TMEM8C+MYOG+* cells (red and green), while arrowheads point to *TMEM8C+* cells (red) that are not *MYOG+* cells. (**G,G',H,H'**) Fluorescent in situ hybridization to transverse limb muscle sections of E10 chicken embryos with the *TMEM8C* probe (red), followed by immunohistochemistry with the MF20 antibody to visualize myosins (green). (H,H') is a higher magnification of (G). *TMEM8C* transcripts (red, arrows) are preferentially observed outside myotubes (green), although we could detect rare myotube expressing *TMEM8C*, n=5 embryos.

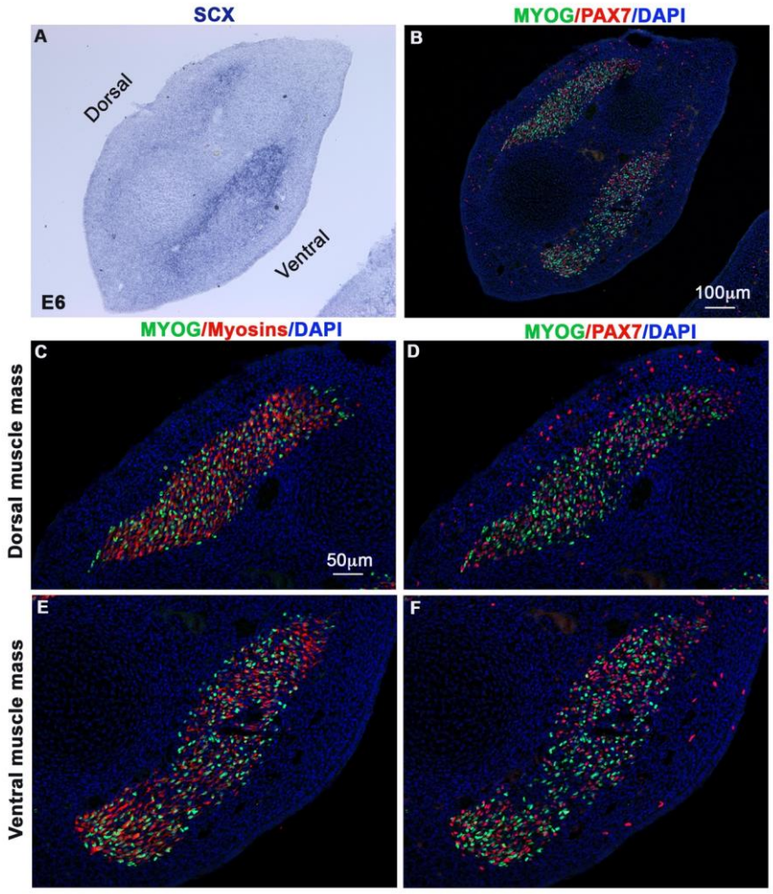

Figure S5

Fig. S5. MYOG + cells are not regionalized in limb muscle masses of E6 chicken embryos

(**A,B**) Adjacent limb transverse sections of E6 chicken embryos were hybridized with the SCX probe (A) and co-immunostained with MYOG (green) and PAX7 (red) antibodies, and DAPI to visualise nuclei (n=3 embryos). **(C-F)** Adjacent limb transverse sections of E6 chicken embryos were co-immunostained with MYOG (green) and MF20 (red), to visualise myosins, (C,E) and MYOG (green) and PAX7 (red) (D,F), with DAPI to visualise nuclei (C-F). (D, F) are high-magnifications of B. (C,D) and (D,F) show dorsal and ventral muscle masses, respectively.

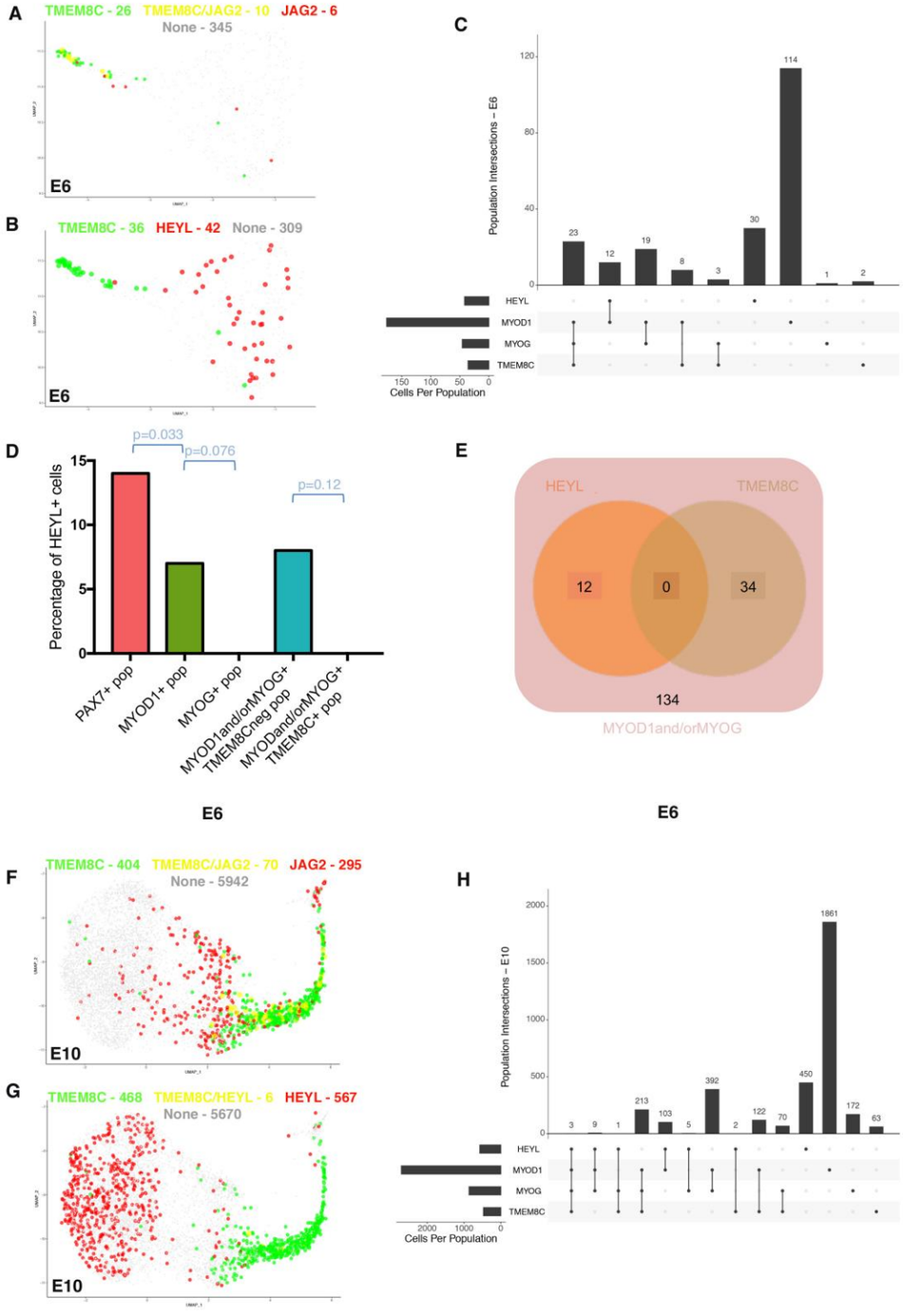

Figure S6

Fig. S6. Unlike *JAG2*, *HEYL* expression does not overlap with *TMEM8C* expression at E6 and E10.

(**A,F**) Feature plot showing the distribution of *TMEM8C*+ cells (green dots) and *JAG2*+ cells (red dots) and double *TMEM8C*+/*JAG2*+ cells (yellow dots) within muscle clusters at E6 (A) and E10 (F). (**B,G**) Feature plot showing the distribution of *TMEM8C*+ cells (green dots) and *HEYL*+ cells (red dots) and double *TMEM8C*+/*HEYL*+ cells (yellow dots) within muscle clusters at E6 (B) and E10 (G). (**C,H**) UpSet plots showing cell numbers for the gene combinations present within the *MYOD1*+ and/or *MYOG*+ population at E6 (C) and E10 (H). The genes considered for the combinations are *HEYL*, *MYOD1*, *MYOG* and *TMEM8C*. The size of each population expressing a gene of interest is shown at the bottom left of the plot. **(D)** Bar plot showing the percentage of *HEYL*+ cells within the *PAX7*+ (red), *MYOD1*+ (green), *MYOG*+ (blue) populations as well as within the *MYOD1*+ and/or *MYOG*+/*TMEM8Cneg* and *TMEM8C*+ (blue) populations at E6. Statistical test used is two-sided Fisher's exact test. Sample size: 3268 cells from three E6 embryos. The exact p values are indicated on the graph. Numbers used for percentage calculations in the *PAX7*+, *MYOD1*+ and *MYOG*+ populations are those of the corresponding co-expression feature plots (data not shown). Numbers used for percentage calculations in the two *MYOD1*+ and/or *MYOG*+ populations are those of panel C. **(E)** Visual representation in the form of a Venn diagram showing the distribution of *HEYL*+ and *TMEM8C*+ cells within the *MYOD1*+ and/or *MYOG*+ population at E6.

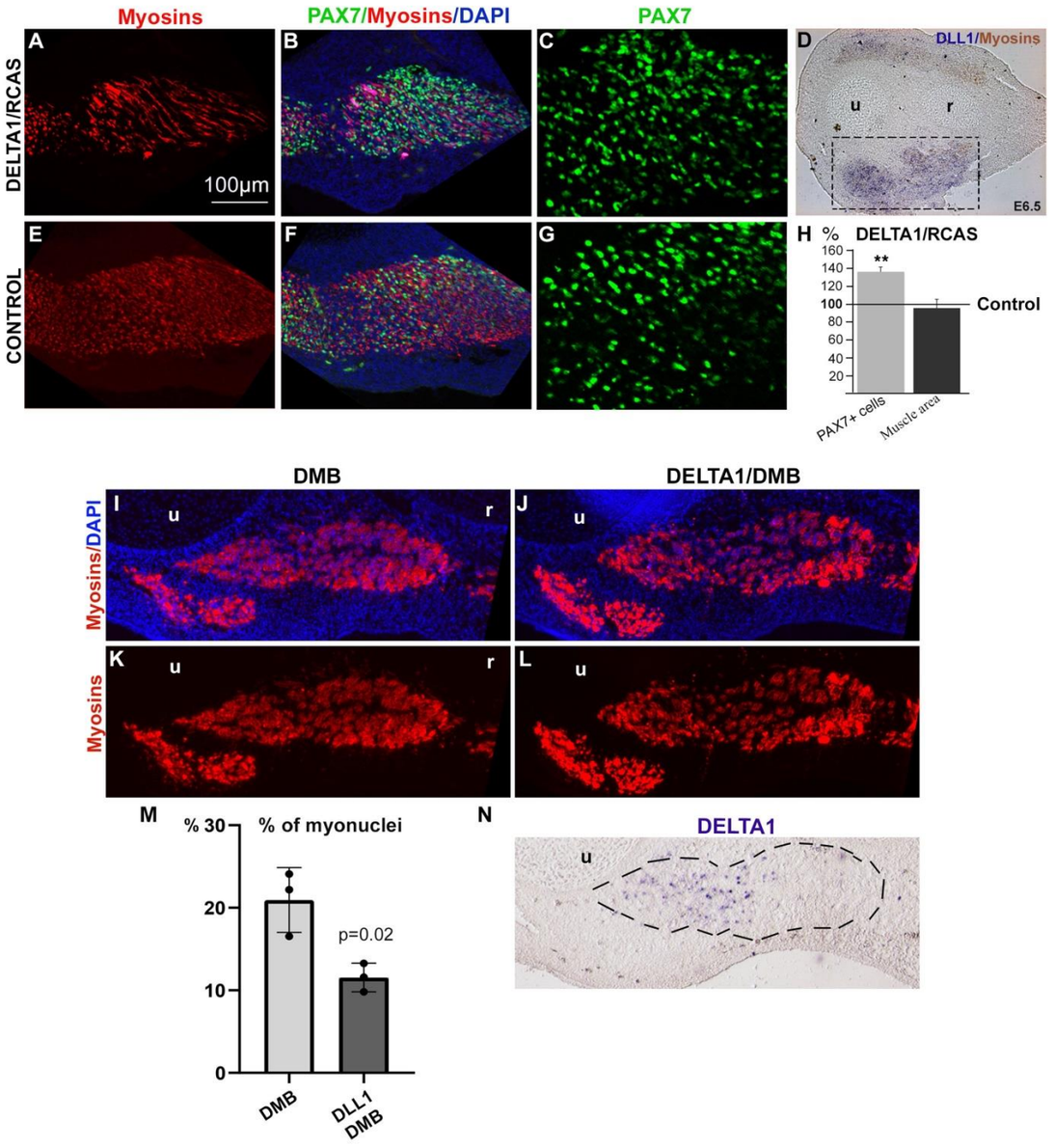

Figure S7

Fig. S7. DELTA1 activated NOTCH prevents the increase of myoblast fusion observed in DMB limbs

(**A-G**) DELTA1/RCAS-expressing cells were grafted into the presumptive right forelimb buds of E4.5 chicken embryos, the contralateral limb of the same embryo was used as control (n=3 embryos). DELTA1-grafted right (A-D) and control left (E-G) forelimbs from E6.5

chicken embryos were cut transversely and analysed for *DLL1* transcripts (D) and immunostained with the PAX7 (green) and MF20 (myosins, red) antibodies (A-C,E-G). (**H**) Percentage of PAX7+ cells with respect to the muscle area and percentage of the muscle area (MF20 area), in muscle regions of DELTA1-grafted and control forelimbs from E6.5 chicken embryos (n=3 embryos). (**I-L**) Limb transverse sections of E9.5 DMB-treated embryos (I,J) and DELTA1-grafted embryos treated with DMB (K,L) immunostained with MF20 to visualise myosins (red) and DAPI (blue) to visualise the nuclei. (**M**) Quantification of the number of myonuclei in MF20+ myotubes versus total DAPI. Graph shows the percentage of cells per area ± s.d. (n=3 embryos). (**N**) In situ hybridization for *DLL1* (blue) shows ectopic expression of *DLL1* in DELTA1-grafted embryos treated with DMB. (N) is an adjacent section to (J,L) of the same embryo.

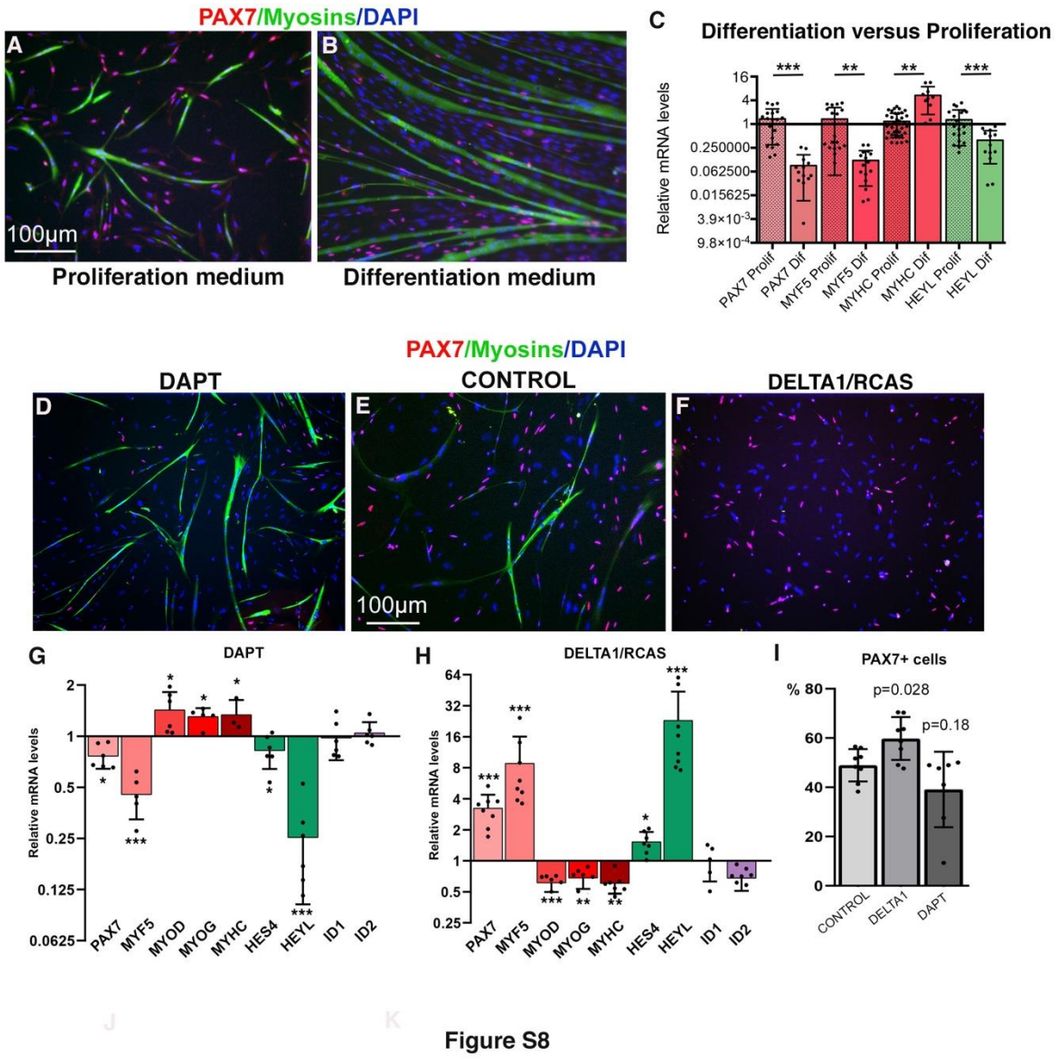

Figure S8

Fig. S8. A myoblast culture system that mimics in vivo myogenesis

(**A,B**) Representative fields of proliferating (A) and differentiating (B) chicken myoblasts labelled with PAX7 (red) and MF20 (green), to visualise myosins, and with the nuclear marker DAPI (n=12 independent cultures). (**C**) RT-qPCR analyses of the mRNA expression levels for the muscle markers, *PAX7*, *MYF5*, *MYHC* and the NOTCH target gene *HEYL*, in differentiation (*PAX7* n=12, *MYF5* n=15, *MYHC* n=9, *HEYL* n=12) versus proliferation (*PAX7* n=18, *MYF5* n=17, *MYHC* n=35, *HEYL* n=20) culture conditions. The mRNA levels

of the genes of myoblasts in proliferation conditions were normalised to 1 (control) and the relative mRNA levels of the genes in differentiation conditions was calculated versus the expression in proliferating conditions. Graph shows mean±s.d. (**D-F**) Representative fields showing PAX7+ (red) and myosin+ (green) cells in chicken foetal myoblasts treated with DAPT (D), transfected with Empty/RCAS (E) or transfected with DELTA1/RCAS (F), and cultured in proliferation conditions (n=5 independent cultures). (**G,H**) RT-qPCR analyses of the expression levels for muscle markers, NOTCH target genes and BMP target genes in DAPT-treated (G) (*PAX7* n=6, *MYF5* n=5, *MYOD* n=6, *MYOG* n=5, *MYHC* n=3, *HES4* n=6, *HEYL* n=6, *ID1* n=6, *ID2* n=6) and DELTA1/RCAS-infected (H) (*PAX7* n=8, *MYF5* n=8, *MYOD* n=6, *MYOG* n=6, *MYHC* n=7, *HES4* n=7, *HEYL* n=8, *ID1* n=5, *ID2* n=7) myoblasts cultured in proliferation conditions. The relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method. For each gene, the mRNA levels of Empty/RCAS-infected myoblasts (control) were normalised to 1. Graph shows mean±s.d. *p<0.05; ** p<0.01; *** p<0.001. (**I**) Percentage of PAX7+ cells with respect to all DAPI+ cells in control, DAPT-treated and DELTA1 cultures (control n=8, DELTA1 n=8, DAPT n=7).

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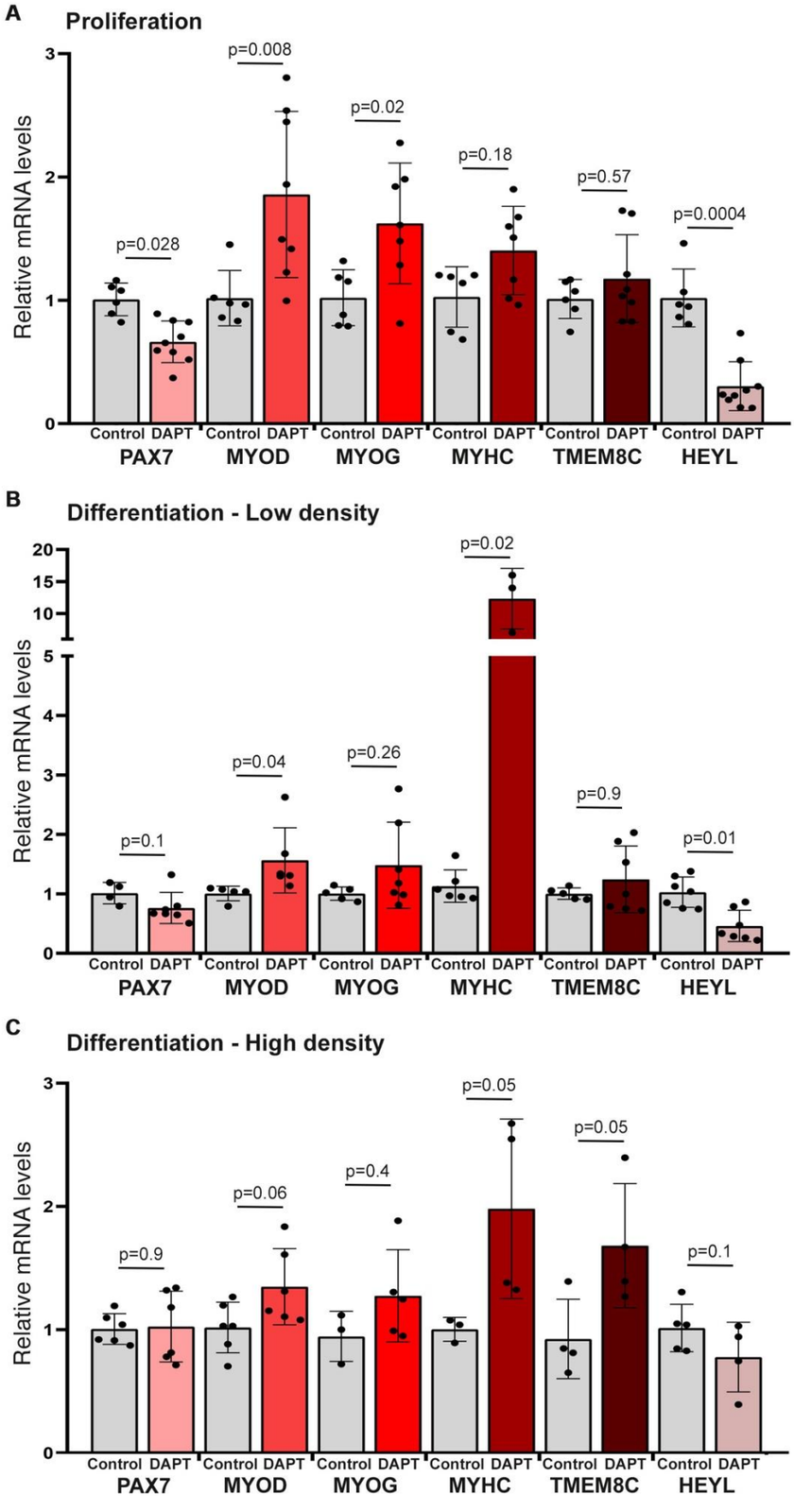

Figure S9

Fig. S9. Inhibition of NOTCH increases *TMEM8C* expression in the differentiation and high confluence condition in the 2-step culture system.

(**A**) Myoblasts cultured at low density with proliferation medium. RT-qPCR analyses of the expression levels for muscle markers and *HEYL* (NOTCH target gene) in control myoblasts (*PAX7* n=6, *MYOD* n=6, *MYOG* n=6, *MYHC* n=6, *TMEM8C* n=6, *HEYL* n=6) and DAPT-treated myoblasts (*PAX7* n=9, *MYOD* n=8, *MYOG* n=7, *MYHC* n=7, *TMEM8C* n=8, *HEYL* n=9) cultured at low density and in proliferation conditions. (**B**) Myoblasts cultured at low density with differentiation medium. RT-qPCR analyses of the expression levels for muscle markers and *HEYL* (NOTCH target gene) in control myoblasts (*PAX7* n=4, *MYOD* n=5, *MYOG* n=5, *MYHC* n=6, *TMEM8C* n=5, *HEYL* n=7) and DAPT-treated myoblasts (*PAX7* n=7, *MYOD* n=6, *MYOG* n=6, *MYHC* n=3, *TMEM8C* n=7, *HEYL* n=7) cultured at low density and in differentiation conditions. (**C**) Myoblasts cultured at high density with differentiation medium. RT-qPCR analyses of the expression levels for muscle markers and *HEYL* (NOTCH target gene) in control myoblasts (*PAX7* n=6, *MYOD* n=6, *MYOG* n=3, *MYHC* n=3, *TMEM8C* n=4, *HEYL* n=5) and DAPT-treated myoblasts (*PAX7* n=6, *MYOD* n=6, *MYOG* n=5, *MYHC* n=4, *TMEM8C* n=4, *HEYL* n=4) cultured at high density and in differentiation conditions. The relative mRNA levels were calculated using the $2^{\wedge-\Delta\Delta Ct}$ method. For each gene, the mRNA levels of control myoblasts were normalised to 1. Graph shows mean±s.d. p values are indicated on the graphs.

Table S1. List of antibodies.

Antibody	Validation	Source	Reference	Dilution
Mouse monoclonal IgG2b anti-MYHC	https://dshb.biology.uiowa.edu/MF-20	DSHB	MF20	IF supernatant
Mouse monoclonal IgG1 anti-PAX7	https://dshb.biology.uiowa.edu/PAX7	DSHB	PAX7, lot 20ea1/24/19	IF (1:200)
Mouse polyclonal anti-MYOD	Manceau et al., 2008	produced in the lab	554130, lot 9011506	IF (1:100)
Rabbit polyclonal anti-MYOG	Manceau et al., 2008	produced in the lab	lot HL1510	IF supernatant
Rabbit polyclonal anti-Collagen type XII	Koch et al, 1992	produced in the lab	Clone 522	IF (1:100)
Rabbit polyclonal anti-pSMAD1/5/9	https://www.cellsignal.com/products/primary-antibodies/phospho-smad1-5-ser463-465-41d10-rabbit-mab/9516	Cell Signalling	9516, lot 9	IF (1:100)
Rabbit polyclonal anti-HEYL	Fukada et al., 2007	produced in the lab	NM_013905	ChIP (5µg/IP)

Table S2. List of primers.

RT-qPCR	Forward	Reverse
PAX7	5'-AGAAGAAGGCCAAGCACAGCATAG-3'	5'- ATTCGACATCGGAGCCTTCATCCA-3'
MYF5	5'-ACCAGAGACTCCCCAAAGTG-3'	5'-TCGATGTACCTGATGGCGTT-3'
MYOD	5'-CGACAGCAGCTACTACACGGAAT-3'	5'-CTCTTCCCATGCTTTGGGTC-3'
MYOG	5'-AGGCTGAAGAAGGTGAACGAAG-3'	5'-CAGAGTGCTGCGTTTCAGAGC-3'
MYHC	5'-TGACAACTCCTCACGCTTTG-3'	5'- CTCTGGCTTCTTGTTGGA-3'
TMEM8C	5'-TGGGTGTCCCTGATGGC-3'	5'-CCCGATGGGTCCTGAGTAG-3'
HEYL	5'-CCAAGCTGGAGAAGGCAGA-3'	5'-CCAGAGCACGAGCATCCA-3'
HES4	5'-GCCGGACAAACCTCGGA-3'	5'-CATCCGCTGCCATTTACCTT-3'
ID1	5'-CCGGAGGGTCTCTAAAGTGG-3'	5'-GCAGGTCCCAGATGTAGTCG-3'
ID2	5'-GAAGAACGGCCTTTCGGAG-3'	5'-TCATGTTGTACAGCAGGCTCA-3'
ChIP RT-qPCR	**Forward**	**Reverse**
R1	5'-GTGCATCACAGCCAGCATG-3'	5'-GGTGGGCAGGAGGAGTTT-3'
R2	5'-CACAGTTGCACGTCCATGC-3'	5'-CAACCTGAATGATTCTGTGGTTCTG-3'
R3	5'-GCTGCAAATCAAGCACCGG-3'	5'-CAAACCGTTGTCACACAACCC-3'