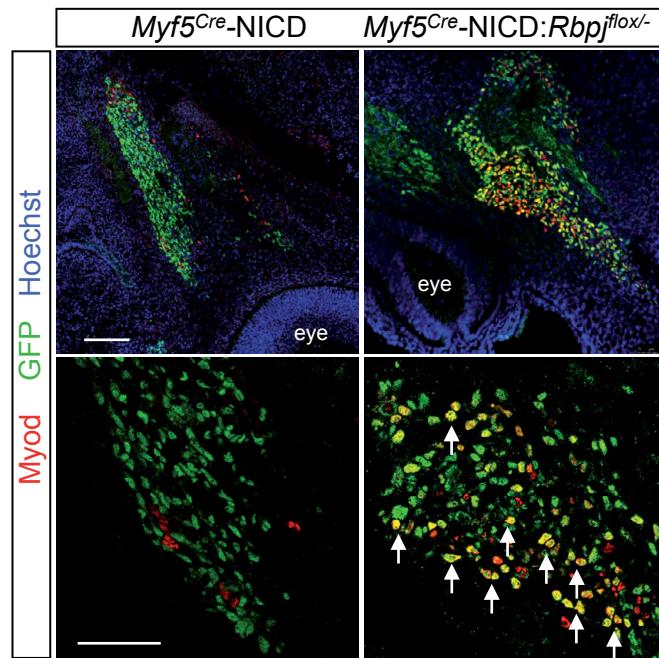
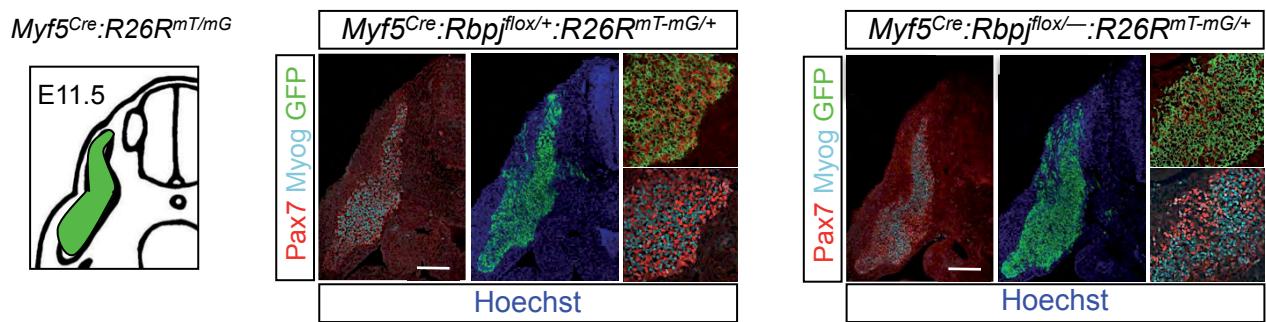


Fig. S1. Induction of muscle-specific and Notch target genes in embryos expressing constitutive NICD. (A) Transcript levels of Notch signalling and muscle factors in FACS-isolated cells from E10.75 control ($En1^{Cre/+};R26R^{mT/mG/+}$) and NICD ($En1^{Cre/+};R26R^{stop-NICD-nGFP/+}$) embryos. Head and limbs were mechanically removed before cell dissociation as represented by the dashed lines on images of live embryos (epifluorescence). Error bars represent s.d.; $n=3$ embryos/genotype. (B) Staining of control E11.5 $En1^{Cre/+};R26R^{mT/mG/+}$ interlimb section for muscle regulatory factors. (C) Whole-mount RNA ISH on E11.5 embryos. The $En1$ targeting domain is visualised by X-Gal staining in $En1^{Cre/+};R26R^{stop-lacZ/+}$ embryo (left panels). Robust expression of $HeyL$ (solid black arrow, lower panel) and upregulation of $Hes1$ transcripts (solid black arrow, lower right image). Red asterisk indicates dorsal root ganglion. Orange arrow in $HeyL$ control image points to $HeyL$ expression in the myotome. Open black arrow indicates region corresponding to black arrow in lower panels. Transverse section showing robust upregulation of $HeyL$, but not $Hes1$, in the region of the dermomyotome/myotome of $En1^{Cre-NICD}$ (solid arrows) compared with control (open arrows) embryos. (D) Immunostaining of dermal progenitors with anti-Alx4 antibody. (Top) $Rbpj$ was deleted using a $Myf5^{Cre}$ line. Some reporter positive cells arising from the DM translocate underneath the ectoderm and express Alx4, both in the control ($Myf5^{Cre/+};Rbpj^{fl/fl};R26R^{mT/mG/+}$) and $Rbpj$ conditional knockout (cKO) ($Myf5^{Cre/+};Rbpj^{fl/fl-/-};R26R^{mT/mG/+}$) samples. (Bottom) E11.5 control ($En1^{Cre/+};Rbpj^{fl/fl};R26R^{mT/mG/+}$) and $Rbpj$ cKO embryos ($En1^{Cre/+};Rbpj^{fl/fl-/-};R26R^{mT/mG/+}$). For quantification, several sections from three different embryos were used per genotype. Error bars indicate s.e.m. Scale bars: 50 μ m in B; 150 μ m in C; 100 μ m in D, left; 35 μ m in D, centre and right.

A



B



C

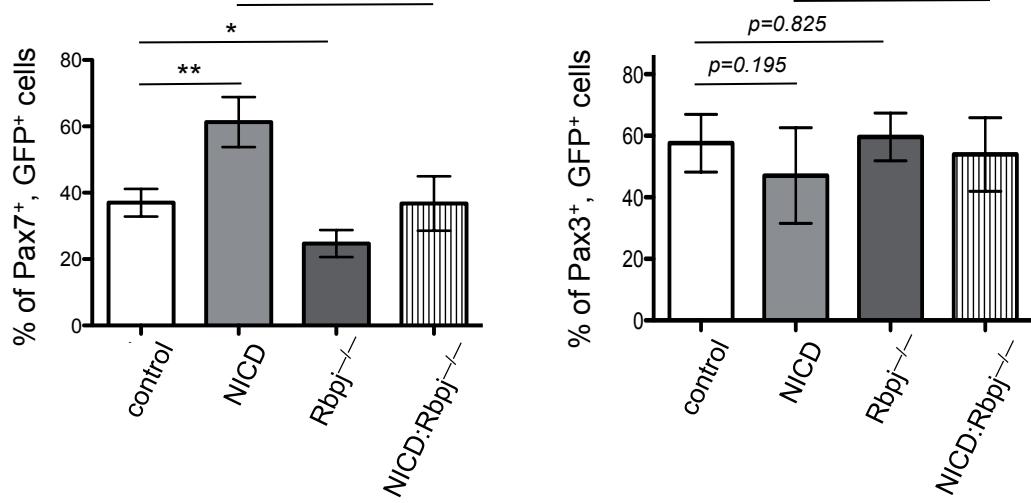


Fig. S2. Activated Notch1 inhibits myogenesis both in trunk and head skeletal muscles exclusively via Rbpj. (A) Inhibition of differentiation exerted by NICD in head muscles of *Myf5^{Cre}-NICD* E12.5 embryos (*Myf5^{Cre}-NICD*, left panels) was rescued when *Rbpj* was genetically removed (*Myf5^{Cre}-NICD:Rbpj^{flox/-}*, right panels), as shown by the presence of NICD (nGFP)/Myod double-positive cells (yellow cells, indicated by arrows in lower panel). (B) *Myf5^{Cre}* targets cells primarily in the myotome (left panel). Conditional deletion of *Rbpj* in *Myf5^{Cre/+}:Rbpj^{flox/+}:R26R^{mT/mG/+}* resulted in a mild decrease in Pax7⁺ cells compared with control (*Myf5^{Cre/+}:Rbpj^{flox/+}:R26R^{mT/mG/+}*). (C) Quantification of Pax7 and Pax3 cells in control (*Myf5^{Cre/+}:Rbpj^{flox/+}:R26R^{mT/mG/+}*), NICD (*Myf5^{Cre}-NICD*), *Rbpj^{-/-}* (*Myf5^{Cre/+}:Rbpj^{flox/-}:R26R^{mT/mG/+}*) and NICD:Rbpj^{-/-} (*Myf5^{Cre}-NICD:Rbpj^{flox/-}*). Values are presented as percentage of Pax7⁺ (or Pax3⁺), GFP⁺ cells over total number of GFP⁺ cells. Error bars indicate s.e.m.; n=3 embryos/genotype. Scale bars: 50 μ m.

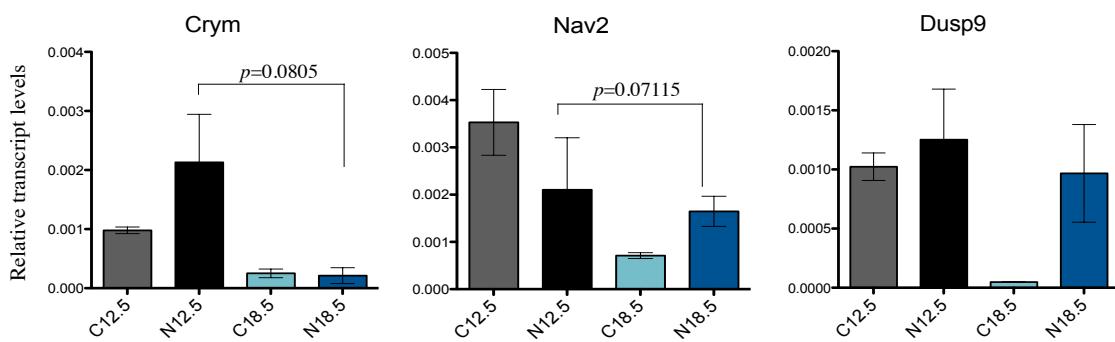
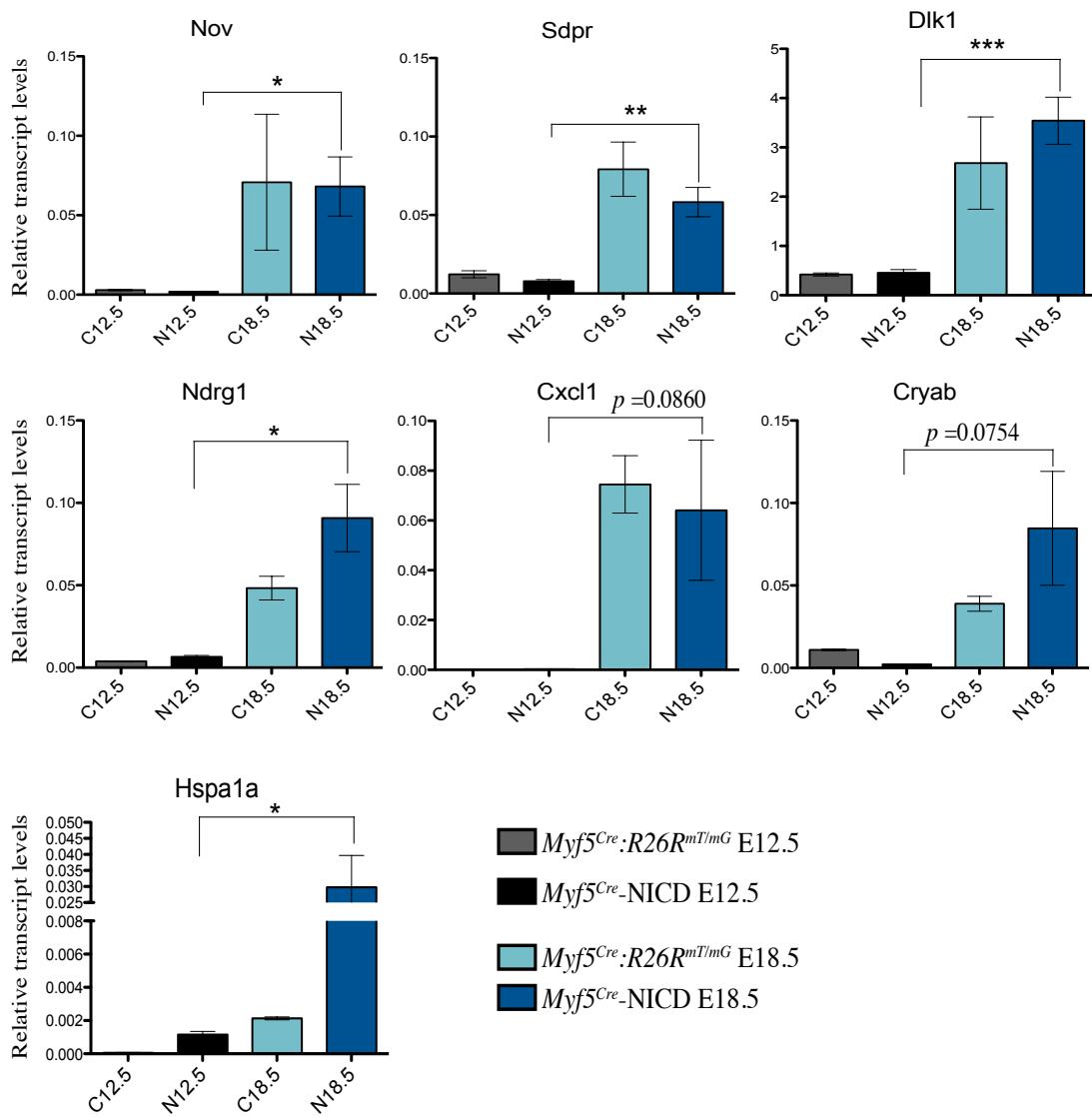
AEmbryonic markers**B**Foetal markers

Fig. S3. Assessment of embryonic and foetal markers in control and NICD-expressing cells. (A) Relative transcript levels of embryonic markers in E12.5 and E18.5 control (*Myf5^{Cre/+}:R26R^{mT/mG/+}*) and *Myf5^{Cre}-NICD* FACS-isolated cells measured by RT-qPCR. (B) Relative expression of foetal markers in E12.5 and E18.5 control and *Myf5^{Cre}-NICD* cells assessed by RT-qPCR. Data are normalised to *Gapdh* ($2^{-\Delta Ct}$). Error bars indicate s.e.m.; $n=3$ embryos/genotype.

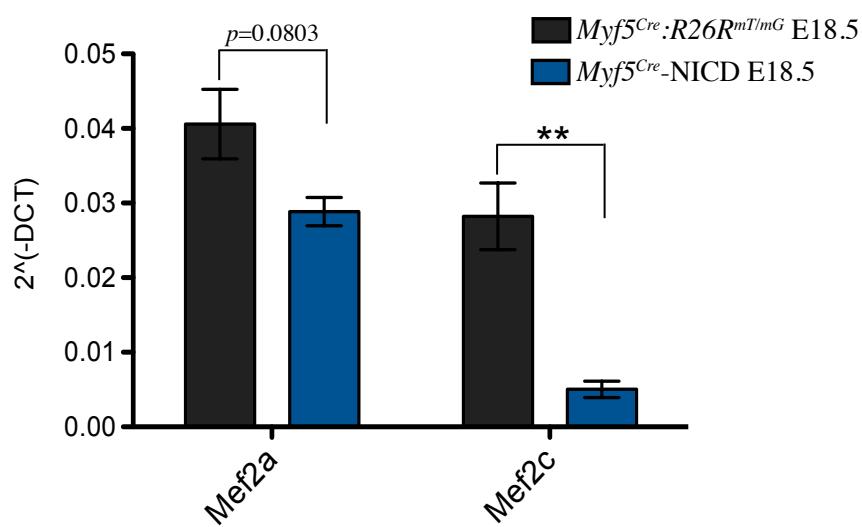
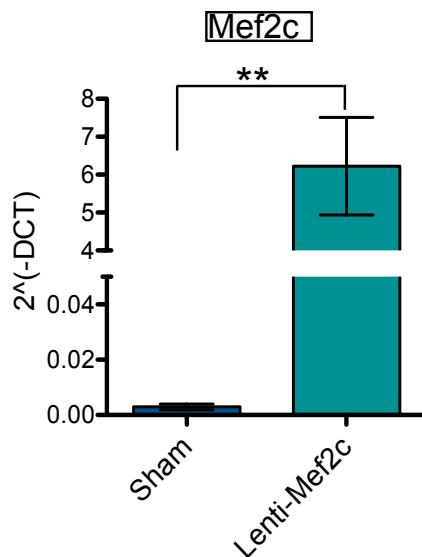
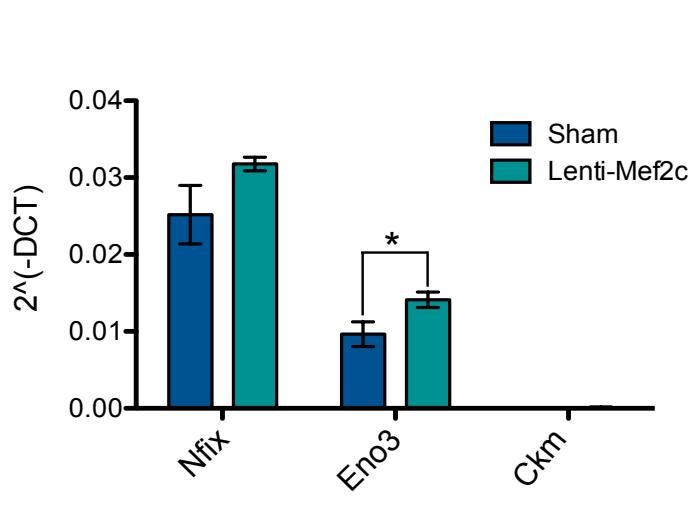
A*Myf5^{Cre}-NICD E18.5***B***Myf5^{Cre}-NICD E18.5***C***Myf5^{Cre}-NICD E18.5*

Fig. S4. Mef2a and Mef2c are not sufficient to rescue the NICD-induced repression of the Nfix target Ckm. (A) Relative transcript levels of *Mef2a* and *Mef2c* in FACS-isolated cells from E18.5 control and *Myf5^{Cre}-NICD* foetuses. Cells were collected for analysis after 2 days in culture. (B) FACS-isolated cells from *Myf5^{Cre}-NICD* from E18.5 foetuses transduced with either control or *Mef2c*-expressing lentiviruses. *Mef2c* transcripts were measured by RT-qPCR. (C) Relative levels of *Nfix*, *Eno3* and *Ckm* in sham and lenti-*Mef2c* transduced E18.5 *Myf5^{Cre}-NICD* cells. Error bars indicate s.e.m.; $n=3$ embryos/genotype.

Table S1. Primers used for RT-qPCR

Gene	Primer sequence	Notes
<i>Alpl</i> (AP)	F: GGCAGCGTCAGATGTTAATTG R: ACTGCGCTCCTTAGGGCT	
<i>Alx4</i>	F: CTCACCCAGGTTGCTCTCTT R: GGACGGTAGCCTCAAGCTC	
<i>Cebpa</i>	F: TTGTTGGCTTATCTCGGC R: CCAAGAACGTGGACAAG	
<i>Cebpd</i>	F: TAAGGAGATGGACCGCTTC R: GTTAGGCCAACTGTTCTCCG	
<i>Cd24a</i>	F: CGTTACTTGGATTGGGAA R: GCTTCTGGCACTGCTCCTAC	
<i>Cd31</i>	F: AGTTGCTGCCATTCATCAC R: CTGGTGCTCTATGCAAGCCT	
<i>Cd44</i>	F: AGCGGCAGGTTACATTCAA R: CAAGTTTGGTGGCACACAG	
<i>Ckm</i>	F: CAGCTGAACCTGTTGTGG R: ACCTCCACAGCACAGACAGA	
<i>Coll5a1</i>	F: CACCAATGAGCACTGTGAG R: GTGGCTGCTCTCCATCATCT	
<i>Colla1</i>	F: TAGGCCATTGTGTATGCAGC R: ACATGTTCAGCTTGTGGACC	
<i>Cryab</i>	F: GTGGAACCTCCCTGGAGATGA R: TCAAAGTCAGGTTCTGGGG	
<i>Crym</i>	F: ATCCAGTTCTGCCAGTCAG R: CAGTCACCATGGCAACAGAG	
<i>Cxcl1</i>	F: TCTCCGTTACTTGGGACAC R: CCACACTAAGAATGGTCGC	
delta-like 1	F: CCGGCTGAAGCTACAGAAC R: GAAAGTCCGCCTTCTTGTG	Holterman et al., 2007
<i>Dermo1</i>	F: TCCATTTCTCCTCTCTGGA R: CATGTCCCGTCCCCACTA	Holterman et al., 2007
<i>Dlk1</i>	F: TGTCATGGAGTCTGCAAGG R: AGGGAGAACCAATTGATCACG	
<i>Dpt</i>	F: GATTGAAAGTAGCGGCTCTG R: TGGTGGGAGGAGATCAACA	
<i>Dusp9</i>	F: TCAGACTCTCCATGGTCGC R: CACTAGCTGTGGCCAGGAC	
<i>Eno3</i>	F: CAGCTTGTCCAGCATGAG R: CTCTACCGACACATCGCAGA	
<i>Epha4</i>	F: CCAGACAGAGTAGCTGTGCG R: CGAGGAGCAGAATGGTGAAT	
<i>Fgf9</i>	F: AGAGGTTGGAAGAGTAGGTGTTG R: ACGAGAACGGGGAGCTGTAT	
<i>Fjx1</i>	F: TCTTCGGATCCAATCTCCAC R: GTTCTGGAAGGGCAAACCTCA	
<i>Gapdh</i>	F: GGCAAAGTGGAGATTGTTGC R: AATTGCCGTGAGTGGAGTC	
<i>Hbb-bh1</i>	F: ACCTCTGGGTGAATTCTT R: TGTGACAAGCTTCATGTGGA	
<i>Hes6</i>	F: AGAAGAACGACCGCAG R: TCTCTAGCTGGCCTGCACC	
<i>Hoxd13</i>	F: TGGTGTAAAGGCACCCTTTC R: CCCATTGGAAATCATCC	
<i>Hspa1a</i>	F: GCAAGGAGAACGAGCAGAGT	

	R: TTTGTGTTGGACTCTCCCC	
<i>Ibsp</i>	F: TGAAGAGTCACTGCCCTCCCT	
	R: GTCTTAAAGTACCGGCCACG	
<i>jagged 1</i>	F: GGTAACACCTTCAATCTCAAGGC	
	R: CCACCAGCAAAGTGTAGGACC	
<i>jagged 2</i>	F: GCGACCAGTACGGCAACAA	
	R: GGGACACACTCGTCACAGAA	
<i>Lhx9</i>	F: GTGCCAGTGCCATTGAAGTA	
	R: TCTTGCAAGGGAAATATCCA	
<i>Matn2</i>	F: CATCTCAAGAGGAGCAGGG	
	R: GGACCACGGAGACTGACACT	
<i>Myo5b</i>	F: CCCCTTCTTGTAGTCCTTGG	
	R: CGTACAGCGAGCTCTACACC	
<i>Nav2</i>	F: ACATTTCGCTCATTCCTGG	
	R: TCCAAACCTGTCACCTCTCC	
<i>Ndrg1</i>	F: GGAGCCATGTAGGGTCTCAA	
	R: AAGCCTCTGGTGGAAAAAGG	
<i>Nfix</i>	F: CACTGGGGCGACTTGTAGAG	
	R: AGGCTGACAAGGTGTGGC	
<i>Ng2</i>	F: ACTCTGGTCAGAGCTGAGGG	
	R: TCTTACCTTGGCCTTGTGG	
<i>Notch1</i>	F: GGTCGCAACTGTGAGAGTGA	Holterman et al., 2007
	R: TTGCTGGCACATTCTATTGAT	Holterman et al., 2007
<i>Notch2</i>	F: GCAGGAGCAGGAGGTATAG	Holterman et al., 2007
	R: GCGTTTCTTGGACTCTCCAG	Holterman et al., 2007
<i>Notch3</i>	F: GTCCAGAGGCCAACAGACTG	Holterman et al., 2007
	R: CAGAAGGAGGCCAGCATCCG	Holterman et al., 2007
<i>Nov</i>	F: CTTGGTGCAGGAGACACTTT	
	R: CGCCAGTGTGAGATGGTAAA	
<i>Osterix</i>	F: CTCTCCATCTGCCTGACTCC	
	R: GGACTGGAGGCCATAGTGAGC	
<i>Pcolce2</i>	F: TCGAAAATTCAAGACGACCAC	
	R: TTGGCAGTGAAGGTTTCCT	
<i>Pdgfb</i>	F: GAAGATCATCAAAGGAGCGG	
	R: CCTTCCTCTGCTGCTTACCC	
<i>Pdgfrb</i>	F: TTGCCAGTTCCACCTTGAATGA	
	R: AGTTGTGCCTCAGGCTCTGCTT	
<i>Pkcθ (Prkcq)</i>	F: AAGATCGACATGCCACACAG	
	R: GTTCATGCCACATGCATCAC	
<i>Pparg</i>	F: TCTTCCATCACGGAGAGGTC	
	R: GATGCACTGCCTATGAGCAC	
<i>Runx2</i>	F: ACACCGTGTCAAGCAAAGC	
	R: GCTCACGTCGCTCATTTG	
<i>Sdpr</i>	F: CTGGCAGGGATCTCACTTT	
	R: GCAGTGTGTCCAGGTGAAGA	
slow MyHC	F: GAGCCTGGATTCTCAAACG	
	R: GTGGCTCCGAGAAAGGAAG	
<i>Sm22a</i>	F: GACTGCACTCTCGGCTCAT	
	R: CCGAAGCTACTCTCCTTCCA	
<i>Socs3</i>	F: AACTGCTGTGGGTGACCAT	
	R: AAGGCCGGAGATTTCGCT	
<i>Thy1</i>	F: GTCAGGCTGGTCACCTTCTG	
	R: AACTCTGGCACCATGAACC	
<i>Lfng</i>	F: CCGTCAAGACCACCAGAAAG	
	R: GTCCTCCCCATCAGTGAAGA	

<i>Mfng</i>	F: AGACTACCTGGGCCTTCCAT R: GCAGTCAAGACTACCTGGGC	
<i>Rfn</i> g	F: GTACCTGGATCTCACGAGCC R: AGCAGAGCAATTGGTGTTGA	
<i>Pofut1</i>	F: CAGCGCCTCCTACAAAGAAC R: CTCCAGGACAGGGAACTGTG	
Transgene		
NICD-F	F: TTTCATTATTGAGGCCACATGG	
ires-R	R: CACCTGAACCTTGCATTCCA	
ires-F	F: AAAGGTACACGTGTCCATGGTGAG	
nEGFP-R	R: TTTACGTCGCCGTCCAGCTC	