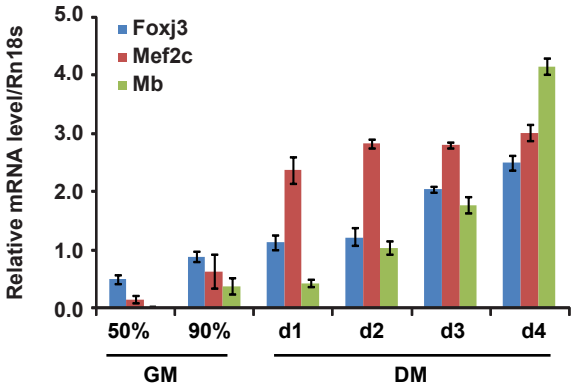
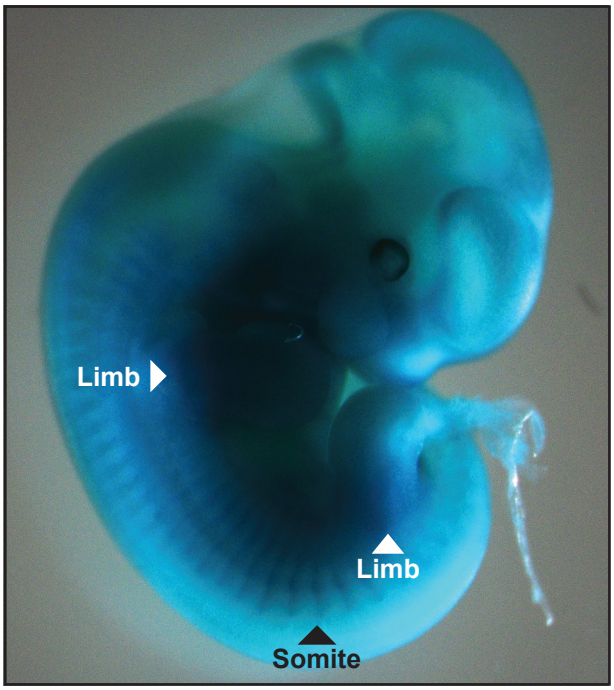


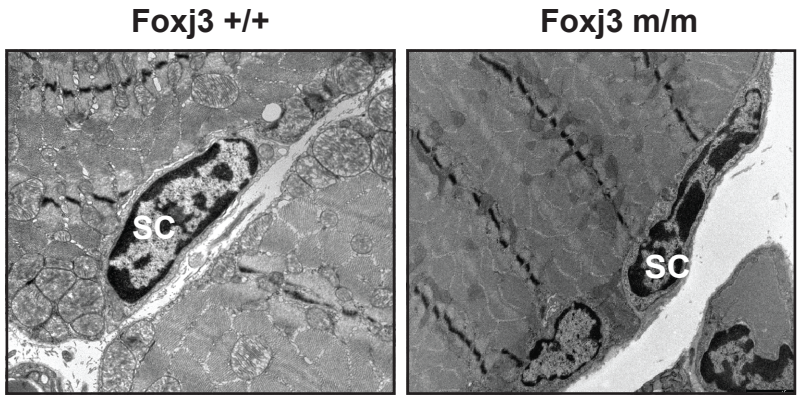
Alexander, Supplemental Figure S1

	Foxj3 +/+	Foxj3 +/m	Foxj3 m/m	Total
Number	21	46	17	84
Percentage (%)	25	55	20	100

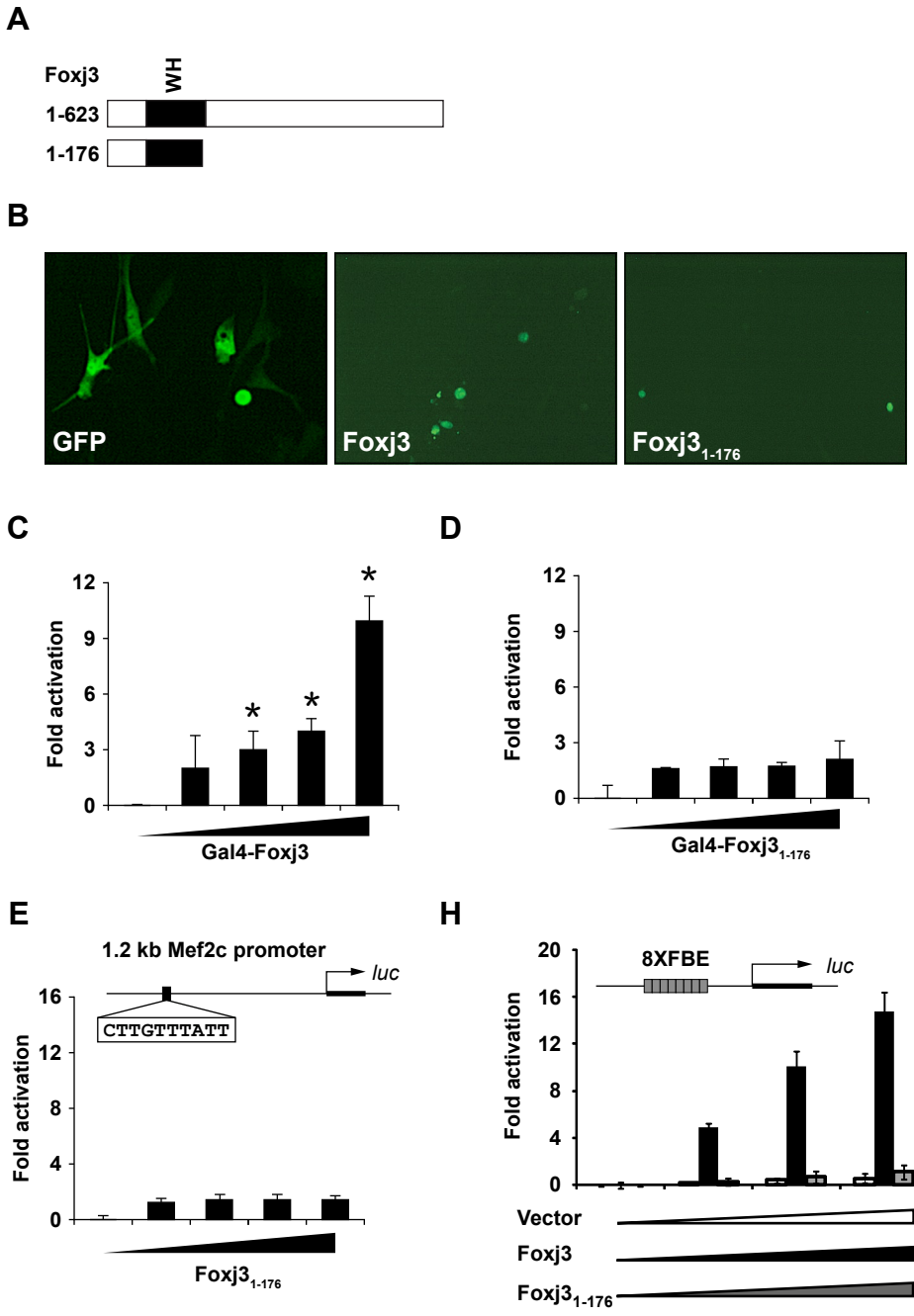
Alexander, Supplemental Figure S2



Alexander, Supplemental Figure S3



Alexander, Supplemental Figure S4



Alexander, Supplemental Figure S5

RT-PCR Primers

Primer	Sequence
Foxj3_F	5’ AGCCTAACATCTATGGACTGGT 3’
Foxj3_R	5’ GGTCAAGGAGTGCATTCTTCTTA 3’
Mef2c_F	5’ GTCTCACCTGGTAACCTGAACAAG 3’
Mef2c_R	5’ GCAGATGGCGGCATGTTATGTAGG 3’
Mef2d_F	5’ CCACACGAGAGCCGCACCAATGC 3’
Mef2d_F	5’ GGCAAAGTTGGGGGCCGGAACAG 3’
Tnni1_F	5’ GAAATCCAAGATCACTGCCTCCC 3’
Tnni1 _R	5’ CCACCACCTCTACCTTGGCATG 3’
Tnnt2 _F	5’ GCCACCCAAGATCCCCGATGGAG 3’
Tnnt2 _R	5’ CCTCCTCCTCACGCCGGGCCCTC 3’
Cdkn1a_F	5’ TTGCACTCTGGTGTCTGAGC 3’
Cdkn1a_R	5’ CTGCGCTTGGAGTGATAGAA 3’
Gapdh_F	5’ GTGGCAAAGTGGAGATTGTTGCC 3’
Gapdh_R	5’ GATGATGACCCGTTTGGCTCC 3’
18sRb_F	5' CTCAACACGGGAAACCTCAC 3'
18sRb_R	5' TGCCAGAGTCTCGTTCGTTAT 3'
Mb_F	5’ CCTGGGTACCATCCTGAAGA 3’
Mb_R	5’ GAGCATCTGCTCCAAAGTCC 3’

Alexander, Supplemental Table 1

Supplemental Materials and Methods

Genotyping of Foxj3 mutant mice. The β-*geo* cassette from the XL913 BayGenomics cell line was mapped to approximately 1.8 kb from the end of exon 5 of the Foxj3 locus on the *cis*-DNA genomic strand. Tail clippings were digested in Proteinase K (Sigma) at 55°C for 3 hours. 2 µL of tail DNA was used to amplify an 800 base pair WT amplicon, and a 300 base pair mutant band using the following primers: Foxj3GT-F: 5' GATGTCTGGGAAAGGAAGCTT 3'; Foxj3GT-R: 5' CTTTTTGGTTGCTTGGTTGGT 3'; β-*gal*vector_R: 5' CGTGTCCTACAACACACACTCCAACC 3'.

LacZ staining. Transgenic embryos were harvested at E11.5, fixed in 4% PFA (pH7.4) for 45 minutes at 4ºC, rinsed with the rinse buffer (100mM NaCl, 2mM MgCl2, 0.01% sodium deoxycholate, and 0.02% NP-40) for 10 min at room temperature three times and stained with fresh staining solution (rinse buffer plus 5mM potassium ferricyanide, 5mM potassium ferrocyanide, 1mg/ml X-gal) overnight at 37 ºC, followed by post-fix in 4% PFA overnight at 4ºC. The stained embryos were washed with PBS, visualized by Zeiss Stereo Discovery v.20 macro/stereo microscope with AxioCam MRc5 camera and photographed with Axio Vision 6 software.

Supplemental Figure Legends

Supplemental Figure S1. Foxj3 is expressed in C2C12 myoblasts. Quantitative PCR analysis of transcript expression during C2C12 differentiation

from myoblasts (50% or 90% confluency, cultured in growth medium or GM) to myotubes (day 1 or d1 to day 4 or d4, cultured in differentiation medium or DM) demonstrates that Foxj3 and Mef2c are expressed in myoblasts prior to myoglobin (Mb) expression.

Supplemental Figure S2. Foxj3m/m pups are born at Mendelian ratios. Table indicating that Foxj3m/m pups are viable and follow Mendelian genetic ratios (n = 8 Foxj3+/m matings).

Supplemental Figure S3. Foxj3 is expressed in the developing somites during murine embryogenesis. LacZ staining in the E11.5 Foxj3+/m embryo reveals Foxj3 expression within the developing somites (black arrowhead) and limbs (white arrowheads).

Supplemental Figure S4. Foxj3 mutant mice have satellite cells. Transmission electron microscopy of TA muscles reveals the presence of satellite cells (SC) in skeletal muscle of both wild type and Foxj3 mutant mice.

Supplemental Figure S5. Foxj3 functions as a transcriptional activator of gene expression. A) Schematic of Foxj3 constructs used in the transfection assays (WH, winged helix). **B)** Transfection of the respective constructs and the GFP control. **C)** Using the Gal4 assay, Foxj3 in a dose dependent fashion functions as a transcriptional activator (n = 3; *p < 0.05). **D)** Transcriptional assay using the Foxj3 mutant construct that lacks the C-terminal region lacks

transcriptional activity. **E)** Transcriptional assay using the Foxj3 mutant construct that lacks the C-terminal region lacks transcriptional activity using the 1.2 kb Mef2c promoter-luc plasmid. **F)** Using transcriptional assays and the multimerized FBE (8XFBE, *forkhead* binding element) fused to the luciferase reporter reveals that full length Foxj3 functions in a dose dependent fashion as a transcriptional activator. Note that vector alone or the Foxj3 mutant construct ($Foxj3_{1\text{-}176}$) lack transcriptional activity.

Supplemental Table 1. RT-PCR primers used in this study.