

1 **Supplemental Materials and Methods**

2 **RNA-seq**

3 RNA-seq libraries (GSE89977) were prepared using SMART-Seq Ultra Low Input RNA Kit
4 (Clontech) with the manufacturer's instructions. Libraries were sequenced (51 bp, paired end) on
5 an Illumina HiSeq2500 machine. Raw reads were first trimmed of the first 6 bp using
6 Trimmomatic v0.32 (1) and filtered through Bowtie2 v2.2 using default options to remove
7 ribosomal RNAs. The remaining reads were aligned to known *Mus musculus* mm9 RefSeq genes
8 with the TopHat2 v2.0 and Bowtie2 v2.2 tools (2) using options '--no-coverage-search --
9 prefilter-multihits --no-discordant --no-mixed -N 0 --b2-very-sensitive'. Reads were counted
10 across each gene with HTSeq v0.6 (3) using the default options for non-stranded, paired-end
11 reads. The subsequent counts were normalized for each replicate using the R package DESeq2
12 (4), and the normalized replicates were then averaged together.

13 To calculate fold-change in expression, (1) genes with non-zero, non-normalized expression
14 values (from HTSeq) in at least two replicates each from satellite cells and iPax7 (+Dox and -
15 Dox) cells were selected and (2) the corresponding average normalized expression values (from
16 DESeq) were used. Pearson correlation calculations were performed with the R function cor
17 using the normalized expression of genes that have a combined non-normalized read count of at
18 least 3 (across all conditions and cell types).

19 All ChIP-seq, ATAC-seq, and RNA-seq data have been submitted to the Gene Expression
20 Omnibus (GEO, accession number GSE89977).

21

22 **Gene ontology analysis**

23 For ChIP-seq, Pax7 sites were annotated to the nearest TSS, and this gene list was used for Gene
24 Ontology (GO) term enrichment analysis using DAVID (5, 6). Redundant groups were removed
25 from the list and sorted by $-\log_{10}$ of the p-value assigned by DAVID. For RNA-seq, genes shown
26 to be expressed in satellite cells and upregulated or downregulated by at least two-fold were used
27 for GO term enrichment analysis using DAVID.

28

29 **Principal component analysis**

30 Principal component analysis was performed using the multiBigwigSummary tool of deepTools
31 version 2.2.3 (7). All ATAC regions called by MACS2 in all samples were collectively
32 considered for PCA. In analyses examining Pax7-bound sites, ATAC regions also bound by
33 Pax7 were considered for PCA. Output from multiBigwigSummary tool was subsequently used
34 to generate PCA plots using the plotPCA tool of deepTools.

35

36 ***k*-means clustering and heat map generation**

37 For each analysis, an equal number of reads was randomly selected from each experiment. Reads
38 within a 3 or 6 kb window around each RefSeq Transcriptional Start Sites (TSS) or center of a
39 Pax7-bound region were collected. These regions were then clustered and visualized with a +/-
40 1.5 kb window around each given Pax7 peak-center as a reference using SeqMINER version 1.3.

41

42 **Western blotting**

43 Cells were collected, washed with ice-cold PBS, and lysed with lysis buffer (50 mM HEPES pH
44 7.0, 150 mM NaCl, 5 mM EDTA, 0.1% NP-40, 1 mM dithiothreitol (DTT) and protease and
45 phosphatase inhibitors (aprotinin, leupeptin, pepstatin A, 4-(2-aminoethyl) benzenesulfonyl

46 fluoride hydrochloride (AEBSF), sodium fluoride and β -glycerophosphate)) for 30 min at 4
47 °C. The whole cell lysate was then spun at 14,000 rpm for 10 min at 4 °C, and the resulting
48 supernatant was collected and analyzed by immuno-blotting.

49

50 **DNA sequence motif Analysis**

51 To search for motifs near Pax7 binding sites, we converted bed files to fasta files with sequences
52 250 bp upstream and 250 bp downstream of the peak center of Pax7 binding sites. Bed files were
53 converted to fasta-format using the UCSC table browser, where repetitive sequences were
54 masked with N, sequences were used for discovery of DNA sequence motifs using MEME, and
55 transcription factor binding motifs were extracted from CentriMo (8, 9).

56

57 **Antibodies used in this study**

58 Antibodies directed against the following proteins and modified histones were used: MHC
59 (Developmental Studies Hybridoma Bank (DSHB) MF20, 1:500 for Western blot, 1:20 for
60 immunofluorescence), Pax7 (DSHB, 1:500 for Western blot, 1:200 for immunofluorescence and
61 3 μ g for ChIP), Myf5 (Santa Cruz C-20 sc-302, 1:1000 for Western blot, 1:500 for
62 immunofluorescence), Myogenin (DSHB F5D, 1:500 for Western blot, Rb SC576 1:100 for
63 Immunofluorescence), α -tubulin (Sigma T5168, 1:5000 for Western blot), H3K4me1 (Abcam
64 ab8895, 2.5 μ g for ChIP), H3K4me3 (Active Motif 39159, 2.5 μ g for ChIP), H3K27Ac (Abcam
65 ab4729, 2.5 μ g for ChIP), and H3K27me3 (Millipore 07-449, 2.5 μ g for ChIP).

66

67

68 1. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence
69 data. *Bioinformatics* (Oxford, England). 2014;30(15):2114-20.

- 70 2. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate
71 alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome*
72 *biology*. 2013;14(4):R36.
- 73 3. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput
74 sequencing data. *Bioinformatics (Oxford, England)*. 2015;31(2):166-9.
- 75 4. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for
76 RNA-seq data with DESeq2. *Genome biology*. 2014;15(12):550.
- 77 5. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene
78 lists using DAVID bioinformatics resources. *Nature protocols*. 2009;4(1):44-57.
- 79 6. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward
80 the comprehensive functional analysis of large gene lists. *Nucleic acids research*. 2009;37(1):1-
81 13.
- 82 7. Ramirez F, Ryan DP, Gruning B, Bhardwaj V, Kilpert F, Richter AS, et al. deepTools2: a
83 next generation web server for deep-sequencing data analysis. *Nucleic acids research*.
84 2016;44(W1):W160-5.
- 85 8. Bailey TL, Machanick P. Inferring direct DNA binding from ChIP-seq. *Nucleic acids*
86 *research*. 2012;40(17):e128.
- 87 9. Machanick P, Bailey TL. MEME-ChIP: motif analysis of large DNA datasets. *Bioinformatics*
88 *(Oxford, England)*. 2011;27(12):1696-7.
- 89