Supporting Information

Park et al. 10.1073/pnas.1013493107

SI Materials and Methods

Transient Transfection and Coimmunoprecipitation. Yeast two-hybrid candidates were amplified by PCR and subcloned into pcDNA3.1 vector containing FLAG-tag. Cos1 cells were transfected with Fugene 6 transfection reagent and harvested 36 h after the transfection, according to the manufacturer's protocol (Roche). Immunoprecipitation was done with anti-MYC antibodies (Sigma), and coprecipitated proteins were analyzed by SDS/PAGE and Western blotting with anti-FLAG antibody (Santa Cruz Biotechnology).

Whole-Mount and Section in Situ Hybridization. For an in situ probe, a 600-bp genomic fragment from exon 2 of *skNAC* was amplified by PCR and cloned into pGEMTEASY vector. Whole-mount in situ hybridization was performed with digoxygenin-labeled RNA probes as previously described (1). Section in situ hybridization was performed on paraffin-embedded sections as previously described (1), using [^{35}S] labeled RNA probes made according to the manufacturer's protocol (Maxiscript In Vitro Transcription kit; Ambion).

Immunohistochemistry. For embryonic heart staining, paraffin-embedded sections were treated with Antigen Retrial Citra solution (BioGenex Laboratories) before immunostaining. Sections were incubated with anti-phosphohistone H3 antibody (Upstate; 1:100) and anti-myosin heavy chain antibody (Hybridoma Bank, MF20; 1:200) together, and then with fluorescein-conjugated anti-rabbit and rhodamin-conjugated anti-mouse secondary antibodies (Vector Laboratories; 1:200).

RNA Isolation and Quantitative Real-Time RT-PCR. Tissues were harvested and homogenized in TRIzol reagent (Invitrogen). RNA was isolated, treated with DNase I, and cleaned with DNA-free kit (Ambion). cDNA was synthesized with SuperScript III First-Strand synthesis kit (Invitrogen). Quantitative real-time RT-PCR was performed on cDNA with TaqMan Gene Expression assays (Applied Biosystems).

Microarray analyses. Mouse mRNA gene expression arrays with E10.5 hearts were performed in triplicate using Affymetrix GeneChip arrays (mouse genome 430 2.0). Hybridization quality was assessed with the affyPLM package (Bioconductor), and processing was performed with the RMA algorithm. Log2 ratio was calculated with the Limma package (Bioconductor). Downstream targets were validated by quantitative real-time RT-PCR (ABI Taqman assays).

Collection of Skeletal Muscle, Staining, and Measurements. Either whole calf muscle or soleus was collected using standardized dissection methods and embedded either in wax or TBS tissue-freezing medium (Fisher Scientific) in isopentane cooled in liquid nitrogen. For histological analysis, 6- to 8-μm sections were stained with either hematoxlin and eosin or Oil Red O. Histochemical assay of succinate dehydrogenase activity was done on frozen section by a standard protocol. Fiber numbers and cross-sectional area of myofibers were determined by capturing an image in the center of each section and analyzing approximately 200 myofibers on a 480,000-μm^2 field by using ImageJ software developed by the National Institutes of Health.

Satellite Cell Isolation and Microwell Culture. Briefly, *tibialis anterior* muscles were isolated from 3-mo-old males and gently digested with collagenase and dispase. Samples were then depleted of nonmuscle cells (CD31$^+$/CD11b$^+$/CD45$^+$) and enriched for muscle cells coexpressing α7 integrin and CD34 by FACS. The sorted cells were spatially segregated as single cells in hydrogel-microwell arrays, which were fabricated using previously described methods (2). Control satellite cells (skNAC$^{+/-}$) were compared with skNAC$^{-\backslash-}$ satellite cells. Clonal proliferation and death of cells were assessed by retrospectively analyzing time-lapse microscope videos that spanned a period of 96 h with images acquired every hour.

1. Gottlieb PD, et al. (2002) Bop encodes a muscle-restricted protein containing MYND and SET domains and is essential for cardiac differentiation and morphogenesis. *Nat Genet* 31:25–32.

2. Gilbert PM, et al. (2010) Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* 329:1078–1081.

Table S1. Partial embryonic lethalty of *skNAC*$^{-/-}$ embryos

	C57BL6/129S6 mixed background			129S6 isogenic background		
Age	WT	HET	MT	WT	HET	MT
E9.5	12	32	13 (23)	16	28	8 (16)
E10.5	13	18	11 (26)	22	35	12 (17)
E11.5	38	67	22 (17)	17	42	11 (16)
E12.5	32	63	18 (16)	14	33	4 (8)
E15.5	17	34	10 (16)	ND	ND	ND
P10	74	122	40 (17)*	34	54	8 (8)*

Genotypes of mice from skNAC$^{+/-}$ (HET) intercrosses were determined. Absolute number (percentage) of mice of each genotype are shown. In both backgrounds, the frequency of homozygous skNAC nulls (MT) was lower than the expected Mendelian ratio at birth. E, embryonic day; P, postnatal day.
*$P < 0.01$.