Le Grand et al., http://www.jcb.org/cgi/content/full/jcb.201201050/DC1

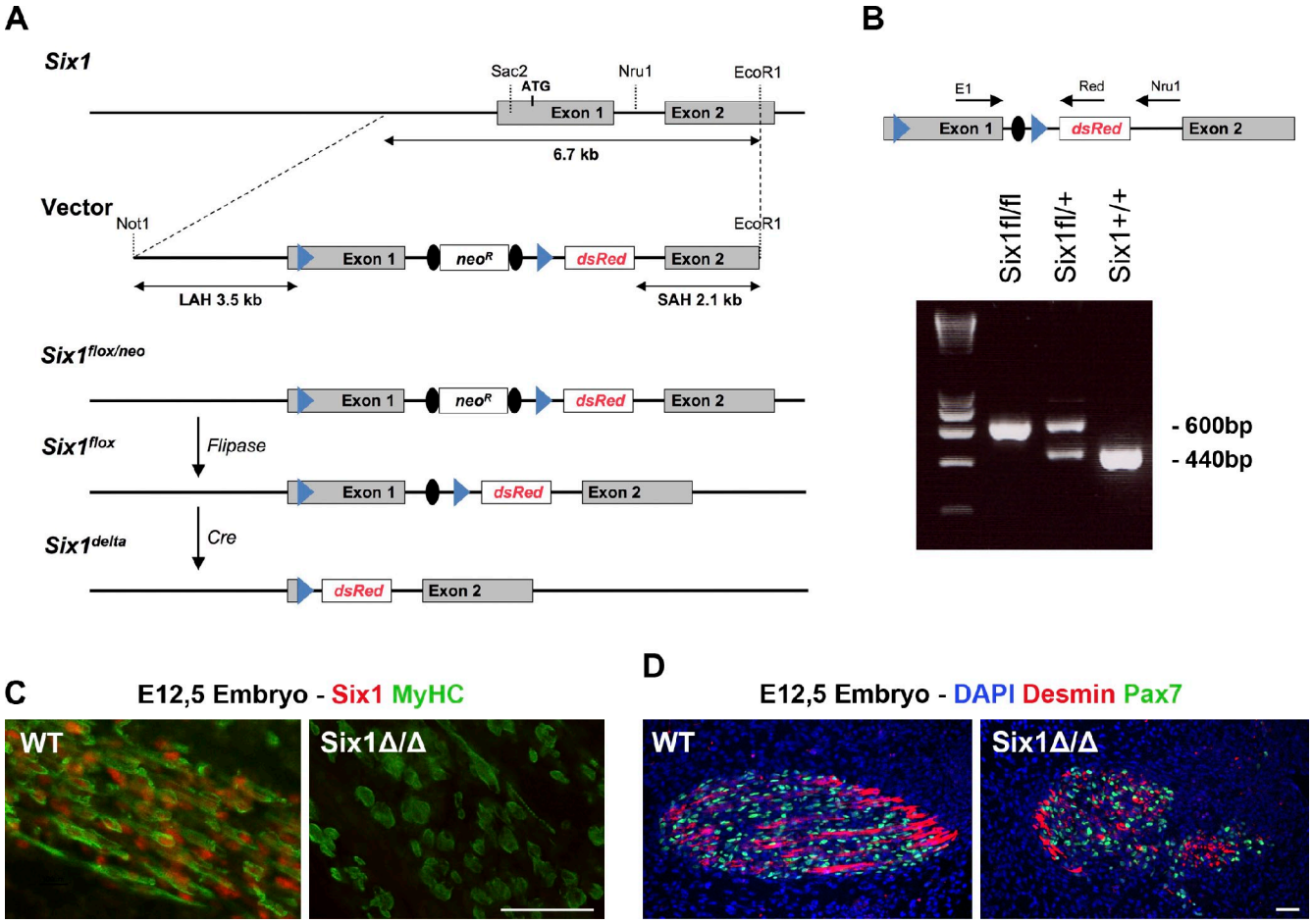

Figure S1. **The *Six1-flox* allele used for Cre-mediated *Six1* gene inactivation.** (A) Schematic representation of the wild-type *Six1* locus, with the restriction sites used in the present study. The 3.5-kb 5′ and 2.1-kb 3′ homologous fragments are indicated by two-headed arrows. The Six1-loxP targeting fragment is shown with loxP sites as blue arrowheads. The 5′ lox site is integrated in the 5′ untranslated region of Six1 first exon at the Sac2 restriction site. The targeted *Six1* mutant alleles after Flip (Schaft et al., 2001) and Cre excisions are depicted below. Schematic representations are not drawn to scale. (B) Schematic representation of the *Six1-flox* locus, with PCR primers used for genotyping shown with black arrows. PCR analysis of genomic DNA extracted from wild-type, heterozygous, and homozygous mutant mice and amplified with the three primers. (C) We generated *Six1Δ* mice by crossing *Six1-flox* mice with *Ella-Cre* mice (provided by F. Relaix, Institut de Myologie, Paris, France; Rodríguez et al., 2000) to mediate total recombination of the *Six1-flox* allele. Cryosections of 12.5-d post-coitum wild-type and $Six1^{\Delta/\Delta}$ mouse embryos at the dorsal level are shown. MyHC protein immunolocalization marks embryonic myofibers and Six1 protein immunolocalization marks nuclei of myogenic cells. No Six1 proteins could be detected on $Six1^{\Delta/\Delta}$ cryosections. (D) Cryosections of 12.5-d post-coitum wild-type and $Six1^{\Delta/\Delta}$ mouse embryos at the hind limb level. Desmin protein immunolocalization marks differentiated muscle cells, and Pax7 protein immunolocalization marks proliferating myogenic progenitors. $Six1^{\Delta/\Delta}$ embryos exhibit altered primary myogenesis at the distal anterior part of developing limb, recapitulating the phenotypes caused by the germline *Six1-LacZ* mutation (Laclef et al., 2003). Bars, 10 µm.

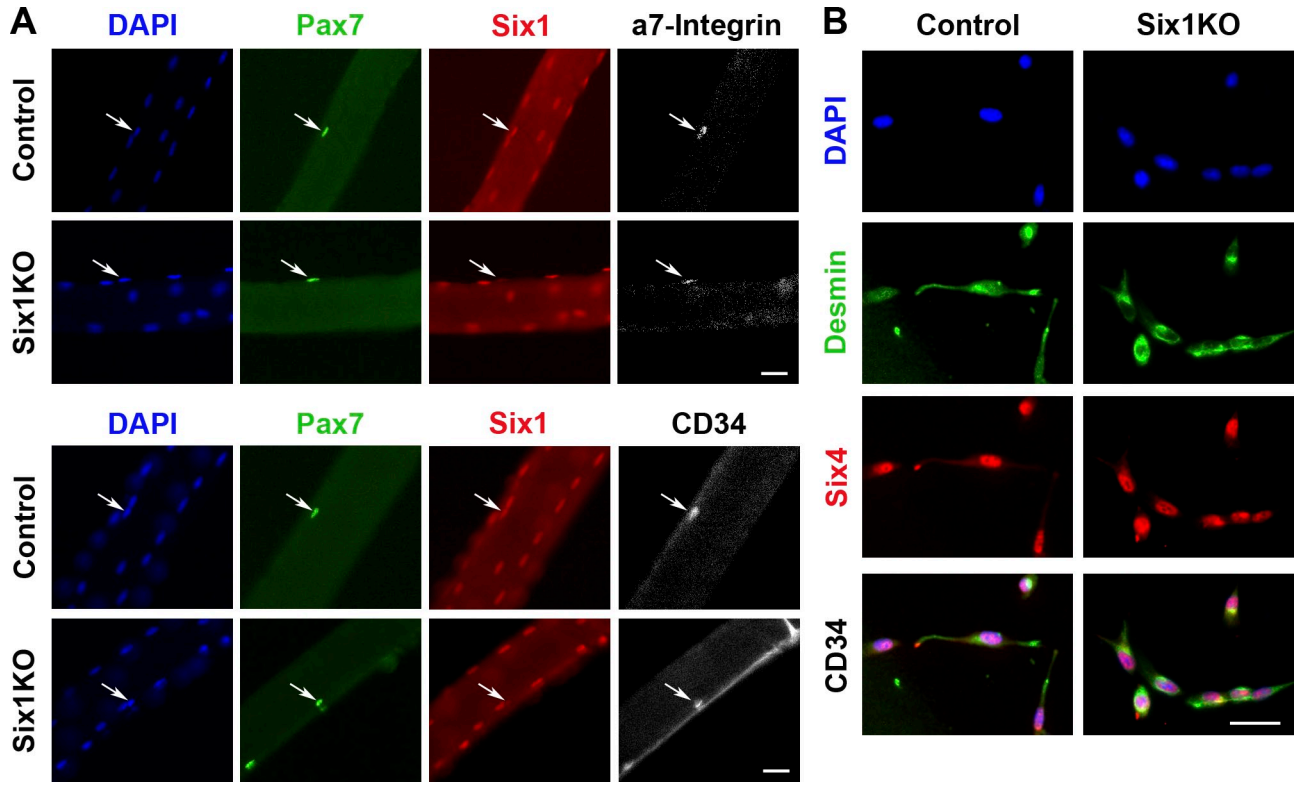

Figure S2. **Conditional *Six1* mutant cells do not lose SC characteristics.** (A) EDL single myofibers from control and Six1KO muscles were immunolocalized for Pax7 and Six1 proteins as well as the SC markers α7-Integrin and CD34 proteins at 6 wk after TM injections. Pax7^{+}/Six1^{-} cells retain expression of specific SC markers. (B) Control and Six1KO myogenic cells grown for 6 d were immunolocalized for Desmin and Six4 proteins. *Six1* gene disruption does not induce up-regulation of Six4 expression. Bars, 10 µm.

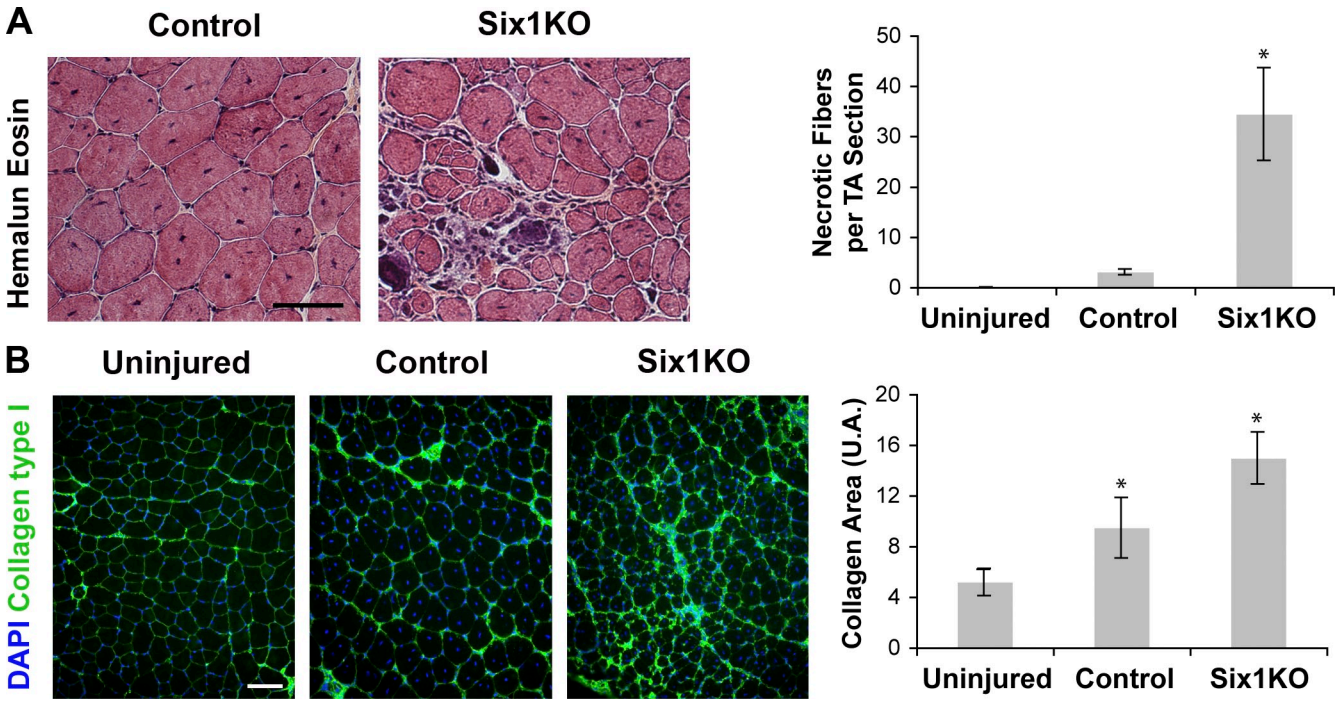

Figure S3. **Impaired tissue repair in regenerated SixKO muscles.** Cryosections of control and Six1KO regenerated TA muscles 14 d after CTX injection. (A) Hemalun-Eosin staining. Regeneration of the tissue induces necrosis of numerous myofibers in Six1KO animals. (B) Collagen type I staining shows fibrous/connective tissue. Note the increased fibrosis in Six1KO muscles compared with controls. Error bars indicate standard deviations. *, $P < 0.01$.

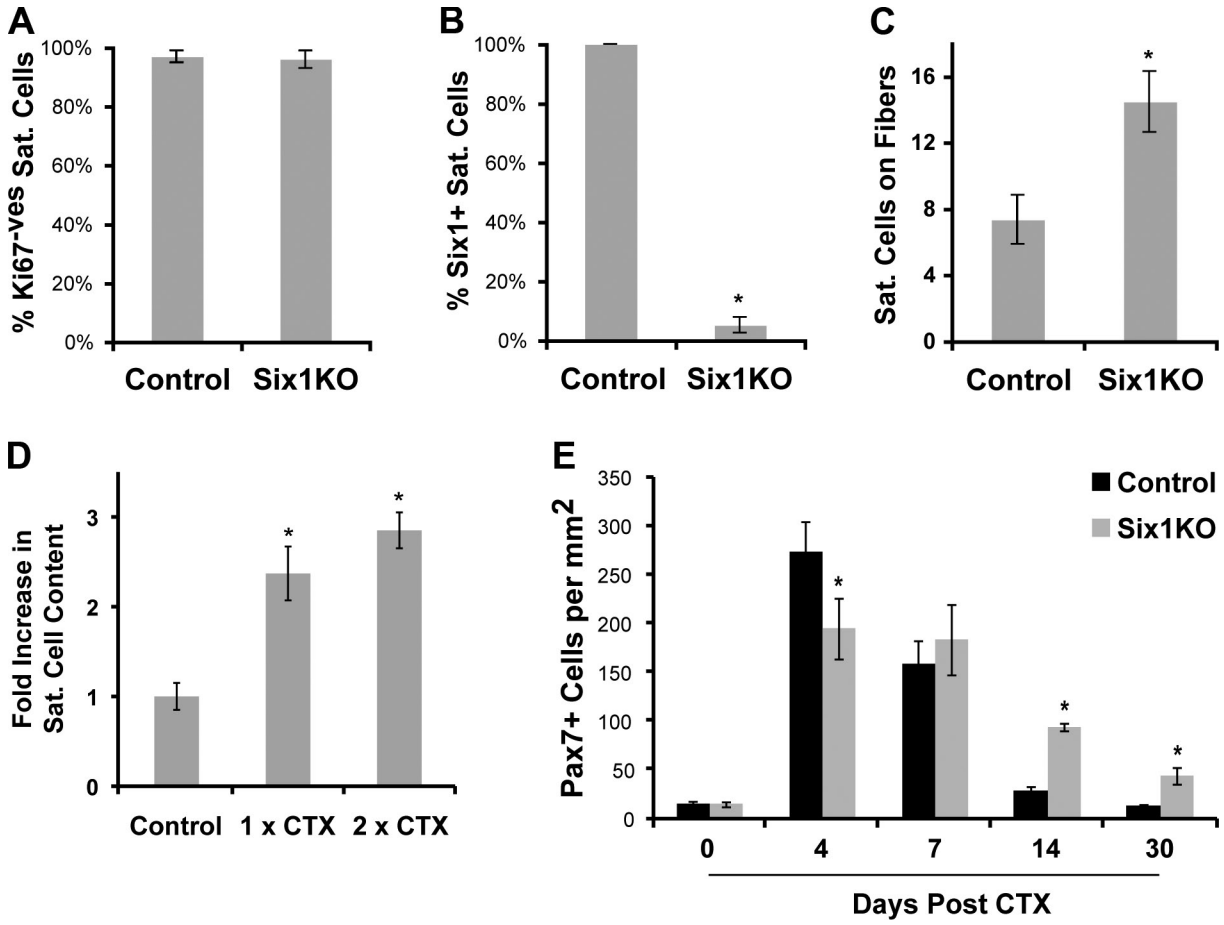

Figure S4. **Satellite cell states within regenerated muscles.** Muscles were analyzed 30 d after CTX injury. (A) More than 97% of renewed sublaminar Pax7$^+$ SCs from both control and Six1KO mice were quiescent (n = 2). (B) More than 95% of renewed SCs from Six1KO mice were negative for Six1 expression (n = 3). (C) The SC pool was increased 2.2-fold in regenerated Six1KO EDL muscles (n = 4). (D) The SC pool was increased 2.8-fold in regenerated Six1KO TA after two rounds of regeneration muscles (n = 4). (E) The Pax7$^+$ cell population was quantified on TA muscle cryosections at various times during the regeneration process. Pax7$^+$ cells accumulate in Six1KO muscles between 7 and 14 d after CTX injection. Error bars indicate standard deviations. *, $P < 0.01$.

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