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The functionality of stem cells declines during ageing, and this decline contributes to age-associated impairments in tissue regeneration and function1. Alterations in developmental pathways have been associated with declines in stem-cell function during ageing2–6, but the nature of this process remains poorly understood. Hox genes are key regulators of stem cells and tissue patterning during embryogenesis with an unknown role in ageing7,8. Here we show that the epigenetic stress response in muscle stem cells (also known as satellite cells) differs between aged and young mice. The alteration includes aberrant global and site-specific induction of active chromatin marks in activated satellite cells from aged mice, resulting in the specific induction of Hox9 but not other Hox genes. Hox9 in turn activates several developmental pathways and represents a decisive factor that separates satellite cell gene expression in aged mice from that in young mice. The activated pathways include most of the currently known inhibitors of satellite cell function in ageing muscle, including Wnt, TGFβ, JAK/STAT and senescence signalling2–4,6. Inhibition of aberrant chromatin activation or deletion of Hox9 improves satellite cell function and muscle regeneration in aged mice, whereas overexpression of Hox9 mimics ageing-associated defects in satellite cells from young mice, which can be rescued by the inhibition of Hox9-targeted developmental pathways. Together, these data delineate an altered epigenetic stress response in activated satellite cells from aged cells, which limits satellite cell function and muscle regeneration by Hox9-dependent activation of developmental pathways.

Age-dependent declines in the number and function of Pax7+ satellite cells (SCs) impair the regenerative capacity of skeletal muscle2,4,9. Genes and pathways that contribute to this process2–6 often also have a role in regulating embryonic development10–13. Despite these parallels, the function of the master regulators of development, Hox genes, has not been determined in SC ageing. An analysis of freshly isolated, in vivo activated SCs from young adult and aged mice (Extended Data Fig. 1a–e) revealed a specific upregulation of Hox9 in SCs from aged mice, both at the mRNA (Fig. 1a, Extended Data Fig. 2a, b) and protein level (Fig. 1b, c). Similar results were obtained by immunofluorescence staining of SCs (Extended Data Fig. 2c) and myofibre-associated SCs (Fig. 1d, e, Extended Data Fig. 2d) that were activated in culture (Extended Data Fig. 1f, g).

Ageing reduces the proliferative and self-renewal capacity of SCs in wild-type mice2,8,11,12 (Hox9+; Extended Data Fig. 3). Homozygous deletion of Hox9 (Hox9−/−) did not affect the colony-forming capacity of SCs from young adult mice but ameliorated ageing-associated impairment in colony formation of single-cell-sorted SCs in culture (Fig. 2a). Hox9 deletion also increased the self-renewal of myofibre-associated SCs from aged mice in culture but had no effect on SCs from young adult mice under these conditions (Extended Data Fig. 4a–c). Similar results were obtained by short interfering RNA (siRNA)-mediated knockdown of Hox9 in myofibre-associated SC cultures derived from aged mice (Extended Data Fig. 4d–h). The number of SCs decreases in resting tibialis anterior muscle of ageing wild-type mice2,4,9, this phenotype was not affected by Hox9 gene status (Extended Data Fig. 5a). However, homozygous deletion or siRNA-mediated knockdown of Hox9 increased the total number of Pax7+ SCs (Fig. 2b, Extended Data Fig. 5b–e) and improved myofibre regeneration.

Figure 1 | Upregulation of Hox9 in aged activated SCs. a–c. Analysis of freshly isolated, in vivo activated SCs (3 days after muscle injury with BaCl2) from young adult and aged mice. a. Heatmap showing the mRNA expression of all Hox genes as determined by RNA-seq analysis. b. Representative immunofluorescence staining for Hox9 and Pax7. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). c. Corrected total cell fluorescence (CTCF) for Hox9 per SC as shown in b. AU, arbitrary units. d. e. Immunofluorescence (IF) staining for Hox9 and Pax7 in myofibre-associated SCs that were quiescent (freshly isolated (FI) myofibres) or activated (act; 24 h culture of myofibres). d. Representative images with arrowheads denoting Pax7+ cells. e. CTCF for indicated Hox genes. Note the specific induction of Hox9 in activated SCs isolated from aged mice. Scale bars, 5 μm (b) and 20 μm (d). P values were calculated by two-sided Mann–Whitney U-test (c) or two-way analysis of variance (ANOVA) (e). NS, not significant. n = 3 mice in a; n = 134 nuclei (young), n = 181 nuclei (aged) from 3 mice in c; n = 12/13/17/56 nuclei (Hox7), n = 9/42/102/62 nuclei (Hox9), n = 7/35/34/25 nuclei (Hox9) from 2 young and 4 aged mice in e.

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injured muscle of aged mice almost to the levels in young adult mice (Fig. 2c, Extended Data Fig. 5f), albeit without affecting overall SC proliferation rates seven days after muscle injury (Extended Data Fig. 5g, h). Hoxa9 gene deletion also improved the cell-autonomous, in vivo regenerative capacity of transplanted SCs derived from aged donor mice but did not affect the capacity of SCs derived from young adult donors (Fig. 2d, e, Extended Data Fig. 6a). Similarly, Hoxa9 downregulation by short hairpin RNA (shRNA) infection rescued the regenerative capacity and the engraftment of transplanted SCs derived from aged mice almost to the level of SCs from young adult mice (Extended Data Fig. 6b–h). When transduced at similar infection efficiency (Extended Data Fig. 6i), Hoxa9 shRNA compared to scrambled shRNA improved the self-renewal of serially transplanted SCs from aged mice in primary recipients (Fig. 2f, Extended Data Fig. 6j) as well as the regenerative capacity of 500 re-isolated SCs from primary donors that were transplanted for a second round into the injured tibialis anterior muscle of secondary recipients (Fig. 2g, Extended Data Fig. 6k). Together, these results demonstrate that the induction of Hoxa9 limits SC self-renewal and muscle regeneration in aged mice, and that the deletion of Hoxa9 is sufficient to reverse these ageing-associated deficiencies.

The expression of Hoxa9 in development and leukaemia is actively maintained by Mll1-dependent tri-methylation at lysine 4 of histone 3 (H3K4me3)16–18. Chromatin immunoprecipitation (ChIP) revealed that H3K4me3 is strongly enriched at the promoter and first exon of Hoxa9 in activated SCs from aged compared to young adult mice, which was not detected to the same extent for other Hoxa genes (Fig. 3a, Extended Data Fig. 7a). ChIP analyses for Mll1 and Wdr5 (a scaffold protein of the Mll1 complex) revealed increased recruitment of these factors to the Hoxa cluster with Wdr5 enrichment being confined to the Hoxa9 locus (Fig. 3b, c). Although no changes were observed for Mll1, both H3K4me3 and Wdr5 showed significantly increased levels in nuclei of myofibre-associated SCs from aged versus young adult mice upon activation (Extended Data Fig. 7b–e). Of note, knockdown of either Mll1 (also known as Kmt2a) or Wdr5 reduced H3K4me3 levels as well as Mll1 recruitment to the Hoxa9 locus and ameliorated Hoxa9 induction in activated myofibre-associated SCs from aged mice (Fig. 3d, e, Extended Data Fig. 7f–i). Similar results were obtained by treatment of aged myofibre-associated SCs with OICR-9429, an inhibitor of the Mll1–Wdr5 interaction19 (Extended Data Fig. 7j, k). Moreover, both Mll1 knockdown and OICR-9429 treatment increased the self-renewal and lowered the myogenic commitment of myofibre-associated SCs from aged mice (Extended Data Fig. 7l–q), resulting in increased SC numbers in cultures of purified SCs or myofibre-associated SCs derived from aged mice (Extended Data Fig. 7r, s). Notably, Mll1 inhibition by either stable shRNA knockdown (Extended Data Fig. 7l) or OICR-9429 treatment improved the regenerative capacity of SCs from aged mice when transplanted into injured muscle of recipient mice (Fig. 3f–h). Taken together, these experiments demonstrate that the Mll1 complex contributes to Hoxa9 induction in activated SCs from aged mice, resulting in impairment in SC function and muscle regeneration. Pax7 expression was downregulated in activated SCs of aged mice (Extended Data Fig. 7a–w) and did not correlate with Hoxa9 expression (Extended Data Fig. 7x, y), indicating that Mll1-dependent regulation of Pax7 target genes20 was not involved in the Mll1-dependent induction of Hoxa9 in activated SCs from aged mice.

Next, a global analysis of histone post-translational modifications was carried out on freshly isolated SCs obtained before muscle injury (quiescent state) or two, three and five days after in vivo SC activation mediated by muscle injury (Fig. 4a, b, Extended Data Fig. 8a). Using a recently developed mass-spectrometry-based proteomic strategy21, 46 histone H3 and H4 lysine acetylation and methylation motifs were quantified. Quiescent SCs from aged mice compared to young adult mice showed increased levels of repressive marks (H3K9me2 and H3K9me3).
a | Heatmap of mass spectrometry (LC–MS) analysis displaying significant (P < 0.05) relative changes in abundance of the indicated histone modifications (measured at the indicated peptides) at the indicated days post injury (dpi). c, d, Trajectory time-course plots showing relative abundance of H4K5acK8acK12acK16ac (c) or H3K27me3 (d) in freshly isolated quiescent (q) or in vivo activated SCs purified at indicated time points post muscle injury. e–g, Fluorescence in situ hybridization of freshly isolated quiescent or in vivo activated SCs with the indicated probes spanning the *Hoxa* cluster (e); an exemplary image (f); and the average probe distance (g). Scale bar, 1 μm. h, i, Relative changes in SC number 4 days after transfection of freshly isolated SCs from aged mice with the indicated siRNAs. j, k, Pearson correlation of relative cell number and *Hoxa9* immunofluorescence signal of SCs from young adult and aged mice 4 days after transfection with a selection of siRNAs targeting different classes of chromatin modifiers. RFU, relative fluorescence units. P values were calculated by two-way ANOVA (c, d, g), two-sided Student’s t-test (a, b, h, i), or Pearson correlation (j, k). n = 4 mice in a–d, n = 3 mice with 50 nuclei per replicate in g, n = 7 mice (Esh2 siRNA), 8 mice (all others) in h, i; n = 6 mice (aged), n = 3 mice (young) in j, k.

H3K27me3; Extended Data Fig. 8a; consistent with ref. 22), and lower amounts of histone modifications typically enriched on active genes (for example, various H4 acetylation motifs, H3K18ac and H3K36me2; Extended Data Fig. 8a). A time-dependent shift towards a heterochromatome state occurred during SC activation in young adult mice, whereas activation in aged SCs generated the opposite response (Fig. 4a, b). Although selective active marks such as H3 and H4 acetylation motifs declined in SCs from young adult mice during activation, there was a substantial increase in these marks in aged SCs (Fig. 4a–c). Conversely, repressive marks (for example, H3K27me3) decreased in SCs from aged mice but remained stable in SCs from young adult mice during activation (Fig. 4a, b, d). The observed shift of the chromatin towards a more permissive state after SC activation appeared to also affect the *Hoxa* cluster as this locus displayed an increased chromatin decompaction after SC activation in aged mice but not in young adult mice (Fig. 4e–g).

To analyse the functional contribution of different types of chromatin modifications in activated SCs from aged mice, a set of genetic and pharmacological experiments was conducted. The expression of key enzymes involved in chromatin modifications detected by RNA-sequencing analysis was similar in activated SCs from young adult and aged mice (Extended Data Fig. 8b). However, knockdown of the acetyltransferases MOF (also known as Kat8), CRP (Crebbp) or Pcaf (Kat2b) improved the proliferative capacity of SCs from aged mice in bulk culture, whereas knockdown of histone deacetylases led to a reduction (Fig. 4h). Furthermore, knockdown of the H3K27 demethylases Utx (also known as Kdm6a), Uty or Kdm7a promoted the proliferation of aged SCs, which was instead inhibited by knockdown of Suz12 and Ezh2 (Fig. 4i), members of the PRC2 protein complex responsible for H3K27me3. Multi-acetylation motifs, as observed in activated SC from aged mice (Fig. 4b, c), are preferred binding sites for bromodomain-containing proteins23. Eight out of eleven non-toxic bromodomain inhibitors available from the Structural Genomics Consortium exhibited positive effects on the proliferative capacity of SCs from aged mice (Extended Data Fig. 8c, d, P = 4.2 × 10⁻⁴). Targeting major classes of chromatin modifiers by a selected set of siRNAs (Supplementary Table 1) revealed a significant inverse correlation (r = −0.612) between siRNA-mediated changes in *Hoxa9* protein expression and the proliferative capacity of SCs from aged mice, with no such effects observed in SCs from young adult mice (Fig. 4j, k). Similarly, siRNAs against MOF and Utx as well as bromodomain inhibitors led to significant decreases in the *Hoxa9* protein level in activated myofibre-associated SCs from aged mice (Extended Data Fig. 8e–g). In summary, activated SCs from aged mice exhibit site-specific and global aberrations in the epigenetic stress response, resulting in *Hoxa9* activation and profound negative effects on SC function, which are ameliorated by targeting the respective enzymes underlying these alterations.

By analysing the downstream effects of *Hoxa9* induction through lentiviral-mediated *Hoxa9* overexpression, we found a strong reduction in the colony forming and proliferative capacity of SCs from young adult mice (Extended Data Fig. 9a–c). The overexpression of other Hox genes exerted similar effects (Extended Data Fig. 9d) but the *Hoxa9* results are probably most relevant for physiological ageing because only *Hoxa9* was upregulated in activated SCs from aged mice (Fig. 1). The impaired myogenic capacity of SCs in response to *Hoxa9* overexpression was associated with increased rates of apoptosis and decreased cell proliferation (Extended Data Fig. 9e–h). Furthermore, *Hoxa9* induction associated with the suppression of several cell cycle regulators and induction of cell cycle inhibitors and senescence-inducing genes (Extended Data Fig. 9i) as well as with increased staining for senescence-associated β-galactosidase (Extended Data Fig. 9j, k). Microarray expression analysis of *Hoxa9*-overexpressing SCs compared to controls revealed that among the top 12 pathways regulated by *Hoxa9* were several major developmental pathways that have previously been shown to impair SC function and muscle regeneration in the context of ageing23,25,9,24,25 (Fig. 5a, Extended Data Fig. 9i–o). ChIP analysis of putative *Hoxa9*-binding sites (Supplementary Table 1) in *Hoxa9*-overexpressing primary myoblasts indicated that a high number of these genes are probably direct targets of *Hoxa9* (Extended Data Fig. 9p; cumulative P value over tested genes: P = 1 × 10⁻⁴). *Hoxa9* strongly induced downstream targets of the Wnt, TGFβ and JAK/STAT pathways, but targeted activation of each one of these pathways alone only led to slight changes in the expression of target genes of the other two pathways (Extended Data Fig. 9q–s), suggesting that *Hoxa9* acts as a central hub required for the parallel induction of these pathways in aged SCs. Of note, the inhibition of Stat3, Bmp4 or Ctnnb1 (encoding β-catenin) by shRNAs as well as pharmacological inhibition of the Wnt, TGFβ or JAK/STAT pathway was sufficient to improve the myogenic colony forming capacity of SCs overexpressing *Hoxa9* (Fig. 5b, Supplementary Note 3).
Extended Data Fig. 10a, b). In line with previous results, knockdown of Stat3 also increased the total number and lowered early differentiation of myofibre-associated SCs from aged mice, and in addition, increased the regenerative capacity of transplanted SCs from aged mice to a similar extent as Hoxa9 knockdown (Extended Data Fig. 10c–g).

Differentially expressed genes were determined using RNA-sequencing data of freshly isolated, in vivo activated SCs from young adult and aged wild-type mice as well as from aged Hoxa9−/− mice. There was a highly significant overlap between genes induced by Hoxa9 overexpression in SCs from young adult mice with those genes that were dysregulated in in vivo activated SCs from aged compared to young adult mice (P = 2.2 × 10−12; Extended Data Fig. 10h). Pathways that are currently known to be associated with SC ageing were again among the highest ranked pathways differentially expressed in activated SCs from aged compared to young adult mice including MAPK, TGFβ, Wnt and JAK/STAT signalling (Fig. 5c). Of note, Hoxa9 deletion abrogated the separate clustering of gene expression profiles of activated SCs from aged compared to young adult mice (Fig. 5d, e). Comparing transcriptomes of activated SC from aged Hoxa9−/− to aged Hoxa9−/− mice re-established the separate clustering (Fig. 5f) characterized by enrichment of the same set of developmental pathways that associate with SC ageing in wild-type mice (Fig. 5g, compare to Fig. 5c).

Taken together, the current study provides experimental evidence that an aberrant epigenetic stress response impairs the functionality of SCs from aged mice by Hoxa9-dependent activation of developmental signals (Extended Data Fig. 10i). Notably, a proof of concept is provided that key enzymes that promote global and site-specific alterations in the epigenetic stress response of aged SCs are druggable, and that the inhibition of these targets leads to improvement in SC function and muscle regeneration during ageing. These findings provide experimental support for the recent hypothesis that a ‘shadowed’ dysregulation of developmental pathways represents a driving force of stem-cell and tissue ageing.26,27

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 16 October 2015; accepted 3 November 2016.

Published online 30 November 2016.
transplantation. Work on this project in K.L.R.’s laboratory was supported by the DGF (RU-745/10, RU-745/12), the ERC (2012-AdG 323136), the state of Thuringia, and intramural funds from the Leibniz association. J.V.M. was supported by a grant from the DFG (MA-3975/2-1). C.F. acknowledges support by the DFG (FE-1544/1-1) and EMBO (long-term postdoctoral fellowship ALTF 55-2015). R.A. was supported by the ERC (AdvGr 670821 (Proteomics 4D)). The funding for the Hoxa9−/− mice to K.L.M. was provided by a grant of the NIH (HL096108). R.R. was supported by a grant from the NIH (RO1GM106056). This work was further supported by grants to H.A.K. from the DFG (SFB 1074 project Z1), the BMBF (GerontoSys II, Forschungskern SyStaR, project ID 0315894A), and the European Community’s Seventh Framework Programme 390 (FP7/2007-2013, grant agreement 602783).

**Author Contributions** S.S. designed and performed most experiments, analysed data, interpreted results and wrote the manuscript. F.B. designed and performed RNAi, ChIP and FISH experiments on isolated SCs, analysed data, interpreted results and wrote the manuscript. C.F. and R.A. designed and performed LC–MS experiments, analysed data, interpreted results and wrote the manuscript. A.H.B., U.K., H.H., C.S.V. and M.S. performed individual experiments and analysed data. A.L. performed microarray experiments. D.B.L. provided support and suggestions for ChIP experiments. K.L.M. provided Hoxa9−/− mice. J.M.K. and H.A.K. performed microarray and pathway analysis, analysed putative Hoxa9-binding sites and provided support for statistical analysis. B.X. and R.R. conducted analysis of putative Hoxa9-binding sites. F.N. analysed RNA-sequencing data and performed correlation analysis. J.V.M. and S.T. conceived the project, designed and performed individual experiments, interpreted results and wrote the manuscript. K.L.R. conceived the project, designed and performed individual experiments, interpreted results and wrote the manuscript.

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**Reviewer Information** Nature thanks J. Gil and the other anonymous reviewer(s) for their contribution to the peer review of this work.
METHODS

Data reporting. No statistical methods were used to estimate sample size. No randomization was used. No animals were excluded. The evaluator was blinded to the identity of the specific sample as much as the nature of the experiment allowed it.

Mice. We purchased female young adult C57BL/6J mice (3–4 months) and aged C57BL/6J mice (22–28 months) from Janvier (wild-type mice). Female and male Hoxa9−/− mice have been described36 and were obtained together with age- and gender-matched littermate controls from K. L. Medina. Mice were housed in a pathogen-free environment and fed with a standard diet ad libitum. Animal experiments were approved by the Thüringer Landesamt für Verbraucherschutz (Germany) under Reg.-Nr. 35/9185.81-3/919.

Muscle injury. Mice were anaesthetized using isoflurane in air and oxygen through a nose cone. For SC activation, muscles were injured by injecting a total volume of 50 μl of 1.2% BaCl2 (Sigma) into approximately 20 sites in the hindlimb muscles. For regeneration and transplantation experiments, tibialis anterior muscle of the right leg was injected with 50 μl cardiotxin (CTX, 10 μl, Sigma).

SC isolation and FACS. Muscles from hindlimbs from young adult or aged mice were dissected and collected in PBS on ice. Muscles were rinsed with PBS, minced with scissors and incubated in DMEM with Collagenase (0.2%, Biochrom) for 90 min at 37 °C and 70 °C. R跂ed muscles were washed with 10% FBS in PBS, triturated and incubated in Collagenase (0.0125%) and Dispase (0.4%, Life Technologies) for 30 min at 37 °C and 100 μm. The muscle slurry was diluted with 10% FBS in PBS, filtered through 100-μm cell strainers and spun down at 500 g for 5 min. Cell pellets were resuspended in FACS buffer (2% FBS in HBSS) and filtered through 40-μm cell strainers and pelleted at 500 g for 5 min. Pellet cell were resuspended in FACS buffer and stained with anti-mouse CD45 PE conjugate (D7, BioLegend), anti-mouse CD31 PE/Cy7 conjugate (30B, BioLegend) and anti-mouse O7/4-34 integrin Alexa Fluor 647 conjugate (R2F2, AbLab) for 20 min at 4 °C on a rotating wheel. Cells were washed with FACS buffer. Live cells were identified as calcein blue positive (1:1,000, Invitrogen) and propidium iodide negative (PI, 1 μg/ml−1, BD Biosciences). SCs were identified as CD45−/Sca-1−/CD11b−/CD31−/α7-integrin−. Cell sorting was performed on a FACSAriaIII with Diva Software (BD).

Culture of SCs. SCs and SC-derived primary myoblasts were cultured at 37 °C, 5% CO2, 3% O2 and 95% humidity in growth medium on collagen/laminin-coated tissue culture plates for the indicated time periods. Growth medium was comprised of F10 (Life Technologies) with 20% horse serum (GE), 1% penicillin/streptomycin (Life Technologies) and 5 μg/ml−1 bFGF (Sigma). For coating, tissue culture plates were incubated with 1 mg/ml−1 collagen (Sigma) and 10 μg/ml−1 laminin (Life Technologies) in ddH2O for at least 1 h at 37 °C and allowed to air-dry. For passaging or FACS analysis, cultured cells were incubated with 0.5% trypsin in PBS for 3 min at 37 °C and collected in FACS buffer. Treatment of SCs with noggin (Peprotech) or DKK1 (Peprotech) was done at 100 μg/ml−1 concentration. SCs and SC-derived primary myoblasts were treated with 1 μM of chemical probes provided by the Structural Genomics Consortium (SGC, http://www.thesgc.org/) and SC-derived primary myoblasts were treated with 1 μM of chemical probes provided by the Structural Genomics Consortium (SGC, http://www.thesgc.org/) and SC-derived primary myoblasts were treated with 1 μM of chemical probes provided by the Structural Genomics Consortium (SGC, http://www.thesgc.org/). SCs were seeded in growth medium into individual wells of a 384-well plate pre-filled with transfection mix. For floating cultures of single myofibres, transfections were performed 4 h after isolation in myofibre culture medium. Transfections were done using Lipofectamine RNAiMAX (Life Technologies) according to manufacturer’s instructions. For gene knockdown either Silencer Select siRNAs (Life Technologies) or ON-TARGETplus siRNA SMART-pools (Dharmacon) were used. Respective Silencer Select or ON-TARGETplus SMART-pool non-targeting siRNAs were used as negative control. siRNA sequences are listed in Supplementary Table 1. Transfection efficiency was monitored using a Cy3-labelled control siRNA (Life Technologies). After transfection, FACS-sorted SCs or myofibre-associated SCs were cultured for the indicated time periods and fixed in 2% PFA in PBS. In vivo knockdown experiments were performed as described earlier41. siRNA sequences were modified to the Accell self-delivering format (Dharmacon) and 100 μg Accell siRNA was injected into tibialis anterior muscle 2 days after CTX injury. In vivo knockdown was evaluated from SCs isolated from injected tibialis anterior muscle 3 days after transfection. Transfected muscles were collected 5 days after siRNA injection, frozen in 10% sucrose/OCT and maintained throughout the entire time of engraftment. Engrafted muscles were collected 3 weeks after transplantation and fixed in 4% PFA for 30 min at room temperature followed by incubation in 30% sucrose/PBS overnight at 4 °C. Fixed muscles were frozen in 10% sucrose/OCT in liquid nitrogen and stored at −80 °C.

Lentivirus production and transduction. Lentivirus was produced in Lenti-X cells (Clonetech) after co-transfection of 15 μg shRNA or cDNA plasmid, 10 μg psPAX2 helper plasmid and 5 μg pMD2.G according to standard procedures42. Virus was concentrated by centrifugation for 2.5 h at 106,000 g and 4 °C, and virus pellet was resuspended in sterile PBS. Lentiviral transduction was carried out in growth medium supplemented with 8 μg/ml−1 polybrene (Sigma).

Plasmids. cDNA was inserted into the SF-LV-cDNA-eGFP plasmid43. Primers used for cloning of individual Hox cDNAs are listed in Supplementary Table 1. shRNA was inserted into the SF-LV-shRNA-eGFP plasmid using mir30 primers (Supplementary Table 1). shRNA sequences are listed in Supplementary Table 1.

SC transplantation. SCs were FACS purified and transplanted with a lentivirus on Retronectin (Takara) coated 48-well plates4. After 8–10 h, SCs were obtained by resuspension and washed several times with FACS buffer. For each engraftment, 10,000 SCs were resuspended in 0.9% NaCl and immediately transplanted into tibialis anterior muscles of adult immunosuppressed mice that had been injured with CTX 2 days before. Immunosuppression with FK506 (5 mg kg−1 body weight, Sigma) was started at the day of injury using osmotic pumps (model 2004, Alzet) and maintained throughout the entire time of engraftment. Engrafted muscles were collected 3 weeks after transplantation and fixed in 4% PFA for 30 min at room temperature followed by incubation in 30% sucrose/PBS overnight at 4 °C. Fixed muscles were frozen in 10% sucrose/OCT in liquid nitrogen and stored at −80 °C.

Immunohistochemistry. Cryosections of 10 μm were cut from frozen muscle using the Microm HM 550. Cryosections were rinsed once with PBS and fixed in 95% ethanol for 5 min at room temperature. Sections were rinsed three times for 5 min with PBS, permeabilized with 0.5% Triton X-100/0.1 M glycine in PBS for 5 min at room temperature followed again by rinsing them three times with PBS. Sections were blocked in PBS supplemented with 5% horse serum and 1:40 mouse on mouse blocking reagent (Vector labs) for 1 h at room temperature. Incubation with primary antibodies was carried out overnight at 4 °C. The next day, sections were rinsed three times with PBS followed by incubation with secondary antibodies for 1 h at room temperature. Sections were rinsed again with PBS and nuclei were counterstained with 1:1,000 DAPI in PBS before mounting with Permafluor (Thermo Scientific). Slides were stored at 4 °C until analysis. The following primary antibodies were used: 1:1,000 anti-chicken IgG Alexa-Fluor 488, anti-rabbit IgG Alexa-Fluor 488, anti-mouse IgG Alexa-Fluor 594 (Life Technologies).
Immunofluorescence. Freshly isolated SCs were allowed to settle on poly-L-lysine-coated diagnostic microscope slides for 30 min at room temperature. All cells and myofibers were fixed with 2% PFA, permeabilized with 0.5% Triton X-100 and blocked with 10% horse serum in PBS for 1 h at room temperature. Cells and fibres were stained with primary antibodies in blocking solution overnight at 4°C. Samples were washed three times with PBS and incubated with secondary antibodies for 1 h at room temperature. Nuclei were counterstained with DAPI. Cultured cells were kept in PBS, freshly isolated SCs and myofibers were mounted with ProLong Gold Antifade Reagent with DAPI (ThermoFisher). The following primary antibodies were used: undiluted mouse anti-Pax7 (DSHB), 1:300 rabbit anti-Hox9a (07-178, Millipore), 1:500 mouse anti-Mll1 (65-755, Millipore), 1:500 rabbit anti-Wdr5 (A302-429A, Bethyl Laboratories), 1:300 rabbit anti-H3K4me3 (C15410003-50, Diagenode), 1:200 rabbit anti-MyoD (sc-304, Santa Cruz). The following secondary antibodies were used at 1:1,000: anti-rabbit IgG Alexa-Fluor 488, anti-mouse IgG Alexa-Fluor 594, anti-mouse IgG1 Alexa-Fluor 594 (Life Technologies).

Fluorescence in situ hybridization (FISH). Chromatin compaction FISH was done as described previously. DNA of the 3′- and 5′ probe (Fosmid clones WIBR1-1312N03 and WIBR1-2209G09, CHORI) was labelled with digoxigenin or biotin by nick-translation (Roche). 100 ng of probe DNA was used per slide, together with 5 μg mouse Cot DNA (Life Technologies) and 5 μg single-stranded DNA (Ambion). Approximately 5,000 freshly sorted SCs were allowed to settle on poly-L-lysine-coated diagnostic microscope slides for 30 min at room temperature and were fixed with 2% PFA for 5 min. After washing three times with PBS, slides were incubated with 0.1 M HCl for 5 min and permeabilized with 0.5% Triton X-100 in 0.5% saponin for 10 min before freeze–thaw in 20% glycerol in PBS. Denaturation was performed in 50% formamide, 1% Tween-20 and 10% dextran sulfate/2× SSC for 5 min at 75°C before applying the hybridization cocktail. Probes were hybridized overnight at 37°C in a humidified chamber. Slides were rinsed three times with 2× SSC, blocked with 2% BSA in 0.1% Tween-20 in PBS for 1 h at room temperature, and hybridized probes were visualized with anti-digoxigenin-rhodamine (ST715, Millipore) and Streptavidin-Cy2 (016-220-084, IR USA) for 30 min at room temperature. Nuclei were counterstained with DAPI.

Digital image acquisition and processing. Immunofluorescence images of muscle sections, myofibers and freshly isolated SCs were acquired using the upright microscope Axio Imager (Zeiss) with 10×, 20× and 100× objectives and a monochrome camera. Brightfield and immunofluorescence images of cultured SCs were captured using the microscope Axio Observer (Zeiss) with 5×, 10× and 20× objectives and a monochrome camera. Image acquisition and processing was performed using the ZEN software (2012) (Zeiss). Brightness and contrast adjustments were applied to the entire image before the region of interest was selected. For the analysis of muscle sections, several images covering the whole area of the section were acquired in a rasterized manner and assembled in Photoshop CS6 (Adobe) to obtain an image of the entire section. Images were analysed using ImageJ software. The number of Pax7+ cells in regeneration experiments was normalized to the area of the entire muscle section. CTGF was determined for each SC using the calculation: integrated density (area of selected cell + background readings) (ref. 45).

Immunoprecipitation and qPCR analysis. Chromatin was immunoprecipitated overnight with 20 μg of antibody (MII: A300-086A, Wdr5: A302-429A, Bethyl Laboratories; HA-tag: ab9110, Abcam). Chromatin-antibody complexes were captured with 20 μl Protein A/G bead mix (1:1, Dynabeads, Invitrogen) for 2 h. Beads were washed twice with sonication buffer, twice with NaCl buffer (0.1% SDS, 1% Triton X-100, 0.1% Na-deoxycholate, 1 mM EDTA, 500 mM NaCl, 50 mM HEPEs, pH 7.9) with LiCl buffer and once with TE buffer. Decussolysis and elution was performed in 30 μl decussolysis buffer (1% SDS, 100 mM NaHCO3, 250 mM NaCl) for 4 h at 65°C with continuous shaking and subsequent Proteinase K treatment for 1 h at 45°C. DNA was purified using Agencourt AMPure XP beads (Beckman Coulter) with a beads:sample ratio of 1.81 or MinElute PCR Purification Kit according to manufacturer's protocols.

Quantitative PCR. Quantitative PCR (qPCR) was performed with an ABI 7500 Real-Time PCR System (Applied Biosystems) in technical duplicates from the indicated number of biological replicates. The qPCR was carried out in a volume of 12 μl using the Absolute qPCR Rox Mix (Thermo Scientific) and the Universal Probe Library (Roche). Primer and probe sets for the detection of single genes are listed in Supplementary Table 1. Gapdh was detected with rodent Gapdh control reagents (Applied Biosystems). Relative expression values were calculated using the ∆Ct method.

\[
\Delta C_t = C_t \left[\text{gene of interest}\right] - C_t \left[\text{control}\right]
\]

Relative expression = \(2^{-\Delta C_t}\)

qPCR analysis of ChIP samples was performed using SYBR Green Supermix (Biorad) in a final reaction volume of 10 μl and 0.75 μl final primer concentration. Primers are listed in Supplementary Table 1. HA-tag ChIP signals were calculated as percentage of the input fraction. The ∆∆Ct method was used to calculate fold enrichment of a genomic locus over the ChIP specific background control (Arb intergenic region for H3K4me3 or gene desert for MII and Wdr5), both normalized to the signal in the input fraction:

\[
\Delta \Delta C_t = \Delta C_t \left[\text{gene of interest}\right] - \Delta C_t \left[\text{control region}\right]
\]

Fold enrichment = \(2^{-\Delta \Delta C_t}\)

Nanostring analysis. Pellets of freshly isolated SCs were lysed with 3 μl RL RT buffer (QIAGEN) and subjected to Nanostring analysis according to manufacturer's instructions using a custom-made Hox gene nCounter Elements TagSet (Nanostring Technologies). Relative expression to the housekeeping genes Gapdh, Hmbs and Plo2sa was calculated using nSolver Software (v2.0) after background correction and normalization to hybridized probe signals.

Proteomic analysis of histone modifications. Preparation of histones for mass spectrometry, data acquisition and analysis were essentially performed as described previously without modifications described below. In brief, histones were isolated by acid extraction, derivatized by d6-acetylated anhydride (CD6, Aldrich) and digested with sequencing-grade trypsin (Promega) overnight at a trypsin:protein ratio of 1:20. To acetylate free peptide N termini, trypsinised histones were derivatised again for 45 min at 37°C using 1:20 (v/v) d6-acetyl anhydride (CD6, Aldrich) in 50 mM ammonium bicarbonate buffered to pH 8 by ammonium hydroxide solution. After derivatization, peptides were evaporated in a speed-vac at 37°C to near dryness, resuspended in 50 μl of 0.1% formic acid and purified by a StageTip protocol using two discs of C18 followed by one disc of activated carbon (3 M Empore). After StageTip purification, the samples were evaporated in a speed-vac to near dryness, resuspended in 20 μl of 0.1% formic acid and stored at −80°C until mass spectrometry acquisition. The histone samples were separated on a reversed-phase liquid chromatography column (75-μm, New Objective) that was packed in-house with a 15-μm stationary phase (ReproSil-Pur C18-AQ, 1.9 μm). The column was connected to a nano-flow HPLC (EASY-nLC 1000; Thermo Scientific) and peptides were electrosprayed in a Q Exactive mass spectrometer (Thermo Fisher Scientific). Buffer A was composed of 0.1% formic acid in HPLC-grade water and buffer B was 0.1% formic acid in ACN. Peptides were eluted in a linear gradient with a flow rate of 300 nl per minute, starting at 3% B and ramping to 35% in 52 min, followed by an increase to 50% B in 4 min, followed by an increase to 98% B in 4 min and then holding at 98% B for another 6 min. Mass spectrometry was operated in a combined shotgun-PRM mode targeting positional isomers. Ion chromatograms were extracted with Thermo Xcalibur and Skyline and data summarization and statistical analysis was performed in Excel and R. Relative abundances were calculated from the raw signal reads, according to the formulas described previously without further normalizations.

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**Microarray and bioinformatics analysis.** Gene expression analysis was performed using the Mouse GE 8x60K Microarray Kit (Agilent Technologies, Design ID 028005). 100 ng total RNA isolated from SCs were used for the labelling. Samples were labelled with the Low Input Quick Amp Labelling Kit (Agilent Technologies) according to the manufacturer’s instructions. Slides were scanned using a microarray scanner (Agilent Technologies). Expression data were extracted using the Feature Extraction software (Agilent Technologies). Preprocessing of expression data was performed according to Agilent’s standard workflow. Using five quality flags (gIsPosAndSignif, gIsFeatNonUnifOL, gIsWellAboveBG, gIsSaturated, and gIsFeatPopnOL) from the Feature Extraction software output, probes were labelled as detected, not detected, or compromised. Gene expression levels were background corrected, and signals for duplicated probes were summarized by geometric mean of non-compromised probes. After log transformation, a percentile shift normalization at the 75% level and a baseline shift to the median baseline of all probes was performed. All computations were performed using the R statistical software framework (http://www.R-project.org). Differentially expressed genes were calculated by the shrinkage T-statistic and controlled for multiple testing by maintaining a false discovery rate (FDR) < 0.05 (ref. 47).

**RNA-sequencing analysis.** Sequencing reads were filtered out for low quality sequences and trimmed of low quality bases by using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Mapping to mm9 genome was performed by using TopHat software48. Gene quantification was performed by using HT-Seq and differentially expressed genes (DEGs) were estimated by using DESEQ2 (refs 49, 50) within the R statistical software framework (http://www.R-project.org) with \( P < 0.01 \). Pearson correlation heatmaps were generated by using custom R scripts by selecting genes having more than 10 read counts in all the samples of at least one condition and an interquartile range (IQR) > 0.5. Significance of overlapping DEGs was calculated by normal approximation of hypergeometric probability.

**Identification of Hoxa9-binding sites.** Transcription start and end sites of putative Hoxa9 target genes were collected from the UCSC Genome Browser with mm8 track. Sequences in gene body regions (from transcription start to end sites), promoter regions (\(-2/-+1\) kb relative to transcription start sites), and distal intergenic regions (\(-50/-+50\) kb relative to transcription start sites) of 26 genes were prepared for identification of Hoxa9 binding sites. These sequences were aligned based on the previously reported consensus motifs for Hoxa9-Meis1-Pbx1 (ATGATTATGGGC)52 and Meis1 (TGTC)53. Putative Hoxa9-binding sites were aligned when they contained either no mismatch or one mismatch, and Meis1 motifs were aligned with no mismatch allowed. Hoxa9-binding sites with at least one Meis1-binding site within 300 bp on the same DNA strand were selected for further analysis. Identified Hoxa9-binding sites are listed in Supplementary Table 1.

**Statistics.** If not stated otherwise, results are presented as mean and s.e.m. from the number of samples indicated in the figure legends. Two groups were compared by two-sided Student’s t-test or two-sided Mann–Whitney U-test. For multiple comparisons a two-way ANOVA was performed using a FDR < 0.5 to correct for multiple comparisons. \( *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 \). Statistical analysis was done using GraphPad Prism 6 software and R (v3.3.1).

**Data availability statement.** Microarray and RNA-sequencing data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) with the accession code GSE87812. Further data that support the findings of this study are available from the corresponding authors upon reasonable request. Source data for the Figures and Extended Data Figures are provided with the paper.

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Extended Data Figure 1 | SC activation. a, Immunofluorescence staining for Pax7 and MyoD of freshly isolated SCs from injured (activated SCs) and uninjured muscles (quiescent SCs) from young adult mice. Nuclei were counterstained with DAPI (blue). b, c, Quantification of Pax7⁷⁺ cells (b) and MyoD⁺ cells (c) in a. d, e, qPCR analysis of Spry1 (d) and Myod1 (e) expression in freshly isolated quiescent and in vivo activated SCs of young adult and aged mice. f, Immunofluorescence staining for Pax7 and MyoD on freshly isolated and 24-h cultured myofibre-associated SCs from aged mice. Nuclei were counterstained with DAPI (blue). g, Corrected total cell fluorescence (CTCF) for MyoD per SC as in f. Scale bars, 10 μm (a) and 20 μm (f). P values were calculated by two-sided Student’s t-test (b, c) or two-way ANOVA (d, e, g). n = 2 mice in b; n = 4 mice in c; n = 3 mice (young activated), n = 4 mice (all others) in d; n = 4 mice in e; n = 33/24 nuclei (young), n = 35/20 nuclei (aged) from 3 mice in g.
Extended Data Figure 2 | Expression of Hox genes in SCs. **a,** Nanostring analysis of mRNA expression of Hoxa genes and Hoxa9 paralogues (b9-c9-d9) in *in vivo* activated (a) and quiescent (b) freshly isolated SCs from young adult and aged mice. **c,** Relative fluorescence units (RFU) for Hoxa9 per SC in 4-day cultured SCs from young adult and aged mice. **d,** Corrected total cell fluorescence (CTFC) for Hoxa9 per activated SC on 24-h cultured myofibres as in Fig. 1d. *P* values were calculated by two-way ANOVA (**a, b**) or two-sided Mann–Whitney U-test (**c, d**). *n* = 3 mice in **a, b;** *n* = 3 mice (young), *n* = 5 mice (aged) in **c;** *n* = 34 nuclei (young), *n* = 32 nuclei (aged) from 4 mice in **d.**
Extended Data Figure 3 | Functional decline in aged SCs. a, SCs from young adult and aged mice were sorted as single cells. After 5 days, the frequency of myogenic colonies was assessed. b, Equal numbers of FACS-isolated SCs from young adult and aged mice were cultured for 4 days and Alamar Blue assay was performed. c, TUNEL staining of SCs isolated from young adult or aged mice after 4 days of culture. Nuclei were counterstained with DAPI (blue). d, Quantification of apoptosis based on TUNEL staining in c. e, BrdU staining of SCs isolated from young adult or aged mice after 4 days of culture. Nuclei were counterstained with DAPI (blue). f, Quantification of proliferation based on BrdU staining in e. g, Immunofluorescence staining for Pax7 and MyoD on myofibres isolated from young adult and aged mice after 72 h in culture. Nuclei were counterstained with DAPI (blue). h–j, Quantification of the number of SC-derived clusters with at least 3 adjacent cells (h), average number of all Pax7+ cells (i), or proportion of Pax7+/MyoD− cells (j) within clusters as in g. Scale bars, 20 μm (c, g) and 50 μm (e). P values were calculated by two-sided Student’s t-test. n = 8 mice (young), n = 10 mice (aged) in a; n = 7 mice (young), n = 5 mice (aged) in b; n = 3 mice in d; n = 4 mice in f; n = 4 mice (aged) in j, n = 5 mice (all others) in h–j.
Extended Data Figure 4 | Deletion or knockdown of Hoxa9 improves SC function in myofibre cultures. a, Immunofluorescence staining for Pax7 and MyoD on 72 h cultured myofibre-associated SCs from aged Hoxa9+/+ and Hoxa9−/− mice. b, c, Average number of all Pax7+ cells (b) or Pax7+/MyoD+ cells (c) within clusters from aged or young adult Hoxa9+/+ and Hoxa9−/− mice as shown in a. d, Immunofluorescence staining for Pax7 and MyoD on 72-h cultured myofibres isolated from aged mice transfected with Hoxa9 or scrambled (Scr) siRNAs. Nuclei were counterstained with DAPI (blue). e, qPCR analysis of Hoxa9 expression in SCs transfected with Hoxa9 siRNA or scrambled control. Two Hoxa9 siRNAs with different target sequences (Supplementary Table 1) were used. f–h, Analysis of 72-h cultured myofibre-associated SCs from d. Quantification of the number of SC-derived clusters with at least 3 adjacent cells (f), average number of all Pax7+ cells (g), or proportion of Pax7+/MyoD− cells (h) within clusters. Scale bars, 20 μm (a, d). Dashed lines outline myofibres. P values were calculated by two-sided Student’s t-test. n = 3 mice (aged), n = 4 mice (young) in b, c; n = 3 mice in e; n = 5 mice in f–h.
Extended Data Figure 5 | Inhibition of Hoxa9 improves muscle regeneration in aged mice. a, Quantification of Pax7+ cells per area in uninjured tibialis anterior muscles from young adult and aged Hoxa9+/+ and Hoxa9−/− mice. b, Representative immunofluorescence staining for Pax7 and laminin on tibialis anterior muscles from aged Hoxa9+/+ and Hoxa9−/− mice that were collected 7 days after cardiotoxin (CTX) injury. c, qPCR analysis of Hoxa9 expression in SCs isolated from tibialis anterior muscles injected with a self-delivering Hoxa9 or scrambled siRNA and collected 5 days after muscle injury. d, Representative immunofluorescence staining for Pax7 and laminin of injured tibialis anterior muscles from young adult and aged mice that were injected with a self-delivery siRNA and collected 7 days after muscle injury. Nuclei were counterstained with DAPI (blue). Arrowheads denote Pax7+ cells. e, Quantification of Pax7+ cells from d per area. f, Frequency distribution minimal Feret’s diameter of muscle fibres from d. g, Exemplary immunofluorescence staining for Pax7 and Ki67 on tibialis anterior muscles from aged Hoxa9+/+ and Hoxa9−/− mice collected 7 days after muscle injury. Nuclei were counterstained with DAPI (blue). h, Quantification of proliferating SCs (Ki67+/Pax7+) as depicted in g. Scale bars, 50 μm. P values were calculated by two-sided Student’s t-test (c, h) or two-way ANOVA (a, e, f). n = 3 mice in a; n = 3 mice in c; n = 3 mice in e, f; n = 4 mice in h.
Extended Data Figure 6 | Inhibition of Hoxa9 improves regenerative capacity of aged SCs. **a,** Quantification of donor-derived (eGFP⁺) myofibres from transplantation of SCs from young adult Hoxa9⁺/⁺ and Hoxa9⁻/⁻ mice. **b,** qPCR analysis of Hoxa9 expression in SCs transduced with scrambled control or Hoxa9 shRNA encoding lentivirus. **c–g,** Transplantation of eGFP-labelled SCs from young adult and aged mice that were targeted with shRNAs against Hoxa9 or a scrambled control. **c,** Representative immunofluorescence staining for Pax7 and eGFP of transplanted muscle sections. Nuclei were counterstained with DAPI (blue). Arrowheads denote Pax7⁺/eGFP⁺ cells, asterisks label Pax7⁻/eGFP⁻ cells. **d,** Quantification of donor-derived (eGFP⁺) Pax7⁺ cells in e. Representative immunofluorescence staining for eGFP and laminin of transplanted muscle sections, nuclei were counterstained with DAPI (blue). **f,** Quantification of donor-derived (eGFP⁺) myofibres in e for two different Hoxa9 shRNAs in two independent experiments.

**h,** Exemplary immunofluorescence staining for eGFP and laminin in tibialis anterior muscles engrafted with untransduced aged SCs. Nuclei were counterstained with DAPI (blue). **i,** Flow cytometric analysis of transduction efficiency of donor SCs used for transplantation in primary recipients analysed in Fig. 2f. **j,** Representative flow cytometry plots for re-isolation of transplanted aged SCs that were untransduced as control or transduced with scrambled control or Hoxa9 shRNA encoding lentivirus as quantified in Fig. 2f. **k,** Representative immunofluorescence staining for eGFP and laminin in engrafted tibialis anterior muscles from secondary recipients quantified in Fig. 2g. Nuclei were counterstained with DAPI (blue). Scale bars, 20 μm (c), 50 μm (h) and 100 μm (e, k). P values were calculated by two-sided Student's t-test (a, b) or two-way ANOVA (d, f, g). n = 4 recipient mice in a; n = 3 mice in b; n = 6 recipient mice (young donors), n = 4 recipient mice (aged donors) in d, f; n = 5 recipient mice in g.
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | Inhibition of Mll1 rescues H3K4me3 induction. *Hoxa9* overexpression, and functional impairment of activated SCs from aged mice. **a,** ChIP for H3K4me3 at promoters or exons of indicated Hox genes in activated SCs (4 day culture) from young adult and aged mice. **b,** Representative immunofluorescence staining for Pax7 and H3K4me3 on myofibre-associated SCs from aged mice that were freshly isolated or activated by 24-h culture of myofibres. **c,** Corrected total cell fluorescence (CTCF) for H3K4me3 on activated SCs shown in **b.** **d,** Representative immunofluorescence staining for Pax7 and Wdr5 on myofibre-associated SCs from young adult and aged mice that were freshly isolated or activated by 24-h culture of myofibres. **e,** CTCF for Mll1 and Wdr5 per activated SC as shown in **d.** **f,** qPCR analysis of Mll1 in SCs transduced with Mll1 siRNA or scrambled control. **g,** ChIPs for H3K4me3 (g) and Mll1 (h) in primary myoblasts 3 days after transfection with the indicated siRNAs. **i,** Immunofluorescence staining for Pax7 and Hoxa9 in myofibres from aged mice after transfection with Mll1 siRNA or scrambled control (i, quantification in Fig. 3d) or after treatment with OICR-9429 or vehicle (j, k). **l,** CTCF for Hoxa9 per SC as shown in **j.** **m,** Average number of Pax7+/MyoD+ cells (m) or Pax7+/MyoD− cells (n) within clusters as shown in **l.** **o,** Representative immunofluorescence staining for Pax7 and MyoD on siRNA-treated myofibre-associated SCs from aged mice after 72-h culture. Nuclei were counterstained with DAPI (blue). **p–r,** Average number of Pax7+/MyoD+ cells (p), Pax7+/MyoD− cells (q) or Pax7+ cells (r) within clusters in **m–n**. Relative changes in cell number of aged SCs after treatment with OICR-9429 and 4 days of culture, compared to vehicle control. **t,** qPCR analysis of Mll1 in SCs transduced with Mll1 shRNA or scrambled control. **u–w,** Analysis of Pax7 expression in *in vivo* activated SCs from young adult and aged mice by RNA-sequencing (u), qPCR (v), or immunofluorescence as depicted in Fig. 1b (w). **x, y,** Pearson correlation comparing the Hoxa9 immunofluorescence signal (quantification in Fig. 1c) and the Pax7 immunofluorescence signal (quantification in **w**) of activated SCs from aged (x) and young adult (y) mice. Note, there is no correlation between Hoxa9 expression level and Pax7 expression level in activated SCs from aged mice. Scale bars, 20 μm. **P** values were calculated by two-way ANOVA (a, g, h), two-sided Student’s *t*-test (**f, m, n, p–v**), two-sided Mann–Whitney *U*-test (**c, e, k, w**) or Pearson correlation (**x, y**). *n* = 4 mice (young), *n* = 7 mice (aged) in **a;** *n* = 27 nuclei from 2 mice (young), *n* = 27 nuclei from 4 mice (aged) in **c;** *n* = 40/52 nuclei (Mll1), *n* = 44/99 nuclei (Wdr5) from 3 young/aged mice in **e;** *n* = 3 mice in **f;** *n* = 3 biological replicates (Wdr5 siRNA), *n* = 2 biological replicates (Mll1 siRNA) in **g;** *n* = 3 biological replicates in **h;** *n* = 173 nuclei (DMSO), *n* = 324 nuclei (OICR-9429) from 4 mice in **k;** *n* = 3 mice in **m, n;** *n* = 7 mice in **p–r;** *n* = 6 mice in **s;** *n* = 3 mice in **t;** *n* = 3 mice in **u;** *n* = 2 mice in **v;** *n* = 134 nuclei (young), *n* = 181 nuclei (aged) from 3 mice in **w–y.**
Extended Data Figure 8 | Alterations in the epigenetic stress response of activated SCs from aged mice. a, Heatmap displaying relative changes in abundance of different histone modifications (measured at the indicated peptides) in freshly isolated SCs from aged compared to young adult mice. SCs were analysed in quiescence (Q, derived from uninjured muscle) or at the indicated time points after activation mediated by muscle injury. Relative abundances at indicated days after injury are first normalized to quiescent SCs, and then compared between SCs isolated from aged and young adult mice and log2 scaled. Only significant changes are shown ($P < 0.05$). b, Expression analysis of the indicated genes in freshly isolated in vivo activated SCs from young adult and aged mice based on RNA-sequencing. c, Viability of primary myoblasts after 48-h treatment with bromodomain inhibitors (1 μM) from the Structural Genomics Consortium probe set, measured by Alamar Blue assay. d, Relative changes in cell number of aged SCs after treatment with non-toxic bromodomain inhibitors (1 μM) from c and 4 days of culture, compared to vehicle control. A Wilcoxon rank-sum test on the ratio of all cell counts being equal to 1 was performed to test the hypothesis of a general effect of the inhibitors on cell number. e, Representative immunofluorescence staining for Pax7 and Hoxa9 in siRNA-treated myofibre-associated SCs from aged mice. Scale bar, 20 μm. f, CTCF for Hoxa9 per SC as shown in e. g, Quantification of immunofluorescence staining for Hoxa9 in Pax7+ cells on myofibre-associated SCs from aged mice treated with bromodomain inhibitors. P values were calculated by two-sided Student’s t-test (a–c), Wilcoxon rank-sum test (d) or two-sided Mann–Whitney U-test (f, g). n = 4 mice in a; n = 6 mice in b; n = 4 biological replicates in c; n = 6 mice in d; n = 71 nuclei (scrambled siRNA), n = 48 nuclei (MOF siRNA), n = 98 nuclei (Utx siRNA) from 3 mice in f; n = 60 nuclei (vehicle), n = 59 nuclei (1-BRD9), n = 38 nuclei (LP99), n = 62 nuclei (PFI-3) from 3 mice in g.
Extended Data Figure 9 | See next page for caption.
Overexpression of Hox genes inhibits SC function. a, Expression of Hoxa9 in SCs transduced with Hoxa9 cDNA or eGFP as control. b, c, FACS-isolated SCs from young adult mice were transduced with a lentivirus either containing both eGFP and Hoxa9 cDNA or only eGFP. Infected (eGFP+) cells were isolated after 3 days. d, Frequency of myogenic colonies from single-cell-sorted SCs. e, f, g, TUNEL (e) or BrdU (g) staining of SCs overexpressing Hoxa9 or eGFP. Infected (eGFP+) cells were isolated 3 days after transduction and analysed 3 days later. Nuclei were counterstained with DAPI (blue). Arrowheads mark TUNEL- or BrdU-positive cells. h, i, Quantification of apoptosis (f) or proliferation (h) based on TUNEL or BrdU staining, respectively. j, Quantification of senescence per field of view (FOV) based on SA-β-Gal staining in j. k, Heatmap displaying log2 fold changes of expression of selected genes from microarray analysis in Fig. 5a. l–o, qPCR validation of differentially expressed genes annotated to Wnt (m), TGFβ (n) and JAK/STAT pathways (o) as in l. p, Identification of Hoxa9-binding sites by anti-HA ChIP of primary myoblasts overexpressing HA-tagged Hoxa9 cDNA or eGFP as control. Shown is the qPCR for 1 or 2 putative Hoxa9-binding sites at the indicated loci. Hoxa9-binding sites at target genes were identified as described in the Methods and are listed in Supplementary Table 1. A two-sided block bootstrap test on the difference of the percentage of bound DNA for all binding sites being equal to 0 was performed to test the hypothesis of a generally increased binding of Hoxa9. q–s, SCs were infected with lentiviruses expressing Hoxa9, Wnt3a, Bmp4 or Stat3 cDNAs or eGFP. qPCR analysis of expression of the indicated target genes at 5 days after infection: Axin2 (q), Bmp4 (r) and Stat3 (s). Scale bars, 20 μm (e, g) and 50 μm (j). P values were calculated by two-sided Student’s t-test (a–d, f, h, k, q–s) or two-way ANOVA (i, m–o). n = 4 mice in a; n = 3 mice in b; n = 7 mice in c; n = 3 mice in d; n = 4 mice in f, h, k; n = 3 mice (p15, p21), n = 6 mice (p16), n = 4 mice (all others) in i; n = 4 pools of 3 mice in l; n = 4 mice in m–o; n = 3 biological replicates for p; n = 3 mice (Wnt3a, Bmp4, Stat3), n = 4 mice (eGFP, Hoxa9) in q–s.
Extended Data Figure 10 | Validation of Hoxa9 downstream targets.

a, Knockdown efficiency of two shRNAs (red bars) for Stat3, Bmp4 and Ctnnb1. b, SCs from young adult mice were transduced with an Hoxa9 and eGFP-encoding lentivirus. eGFP+ cells were sorted as single cells and cultured in the presence of noggin, DKK1 or 0.1% BSA in PBS as vehicle. Colony frequency was assessed after 5 days and is compared to Hoxa9 cDNA expressing cells treated with vehicle control. c, Representative immunofluorescence staining for Pax7 and MyoD on siRNA-transfected myofibres from aged mice after 72 h of culture. Nuclei were counterstained with DAPI (blue). d, e, Average number of Pax7+ cells (d) or Pax7+/MyoD+ cells (e) within clusters in c. f, Representative immunofluorescence staining for eGFP and laminin in tibialis anterior muscles engrafted with siRNA-transfected SCs isolated from eGFP transgenic aged mice. Nuclei were counterstained with DAPI (blue). g, Quantification of donor-derived (eGFP+) myofibres in f. h, Area-proportional Venn diagram of differentially expressed genes from indicated transcriptomes. i, Model for the Hoxa9-mediated impairment of SC function during ageing: quiescent SCs become activated upon muscle injury and proliferate as myoblasts to repair damaged muscle tissue. After activation, aged SCs display global and locus-specific alterations in the epigenetic stress response resulting in overexpression of Hoxa9, which in turn induces developmental pathways inhibiting SC function and muscle regeneration in aged mice. Scale bars, 20 μm (c), and 100 μm (f). P values were calculated by two-way ANOVA (a, b) or two-sided Student’s t-test (d, e, g). n = 3 mice in a; n = 4 mice in b; n = 5 mice in d; n = 5 recipient mice in g; n = 3 mice per group (activated SCs), n = 4 pools of 3 mice (Hoxa9 overexpression) in h.
Author Correction: Epigenetic stress responses induce muscle stem-cell ageing by Hoxa9 developmental signals

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Correction to: Nature https://doi.org/10.1038/nature20603, published online 30 November 2016.

In this Letter, errors occurred in the following figures. In Extended Data Fig. 6e, the 'shScr, Aged donor' image is a duplicate of the 'Vehicle, Aged donor' image in Fig. 3f. The images in Extended Data Fig. 6e represent differences in engraftment levels under four experimental conditions; however, these reflect the lower end of the observed overall engraftment rate in the experiment. Figure 1 of this Amendment shows the corrected panels for Extended Data Fig. 6e, with images from the original experiment that best reflect the differences in, and the overall level of, the engraftment rates between the conditions under study (the original images from Extended Data Fig. 6e are shown for comparison).

In addition, there are errors in the Source Data for Figs. 3d, 4k, Extended Data Figs. 4f–h, 7f, s, t and 9m–o, q–s due to copy-and-paste errors or due to the presentation of controls that were used for the calculation of P values or error bars shown in the figures. One value that was identified as an outlier in Extended Data Fig. 10g was not labelled as such in the original Source Data and was erroneously included for graphical depiction. See Supplementary Information to this Amendment for the corrected Source Data files, and Figs. 2, 3 and 4 of this Amendment for the corrected and original panels for Figs. 3d, 4k and Extended Data Fig. 4f, g, respectively. For the calculation of the P value in Extended Data Fig. 6b, we applied a one-sided paired ratio Student’s t-test (not, as stated, a two-sided Student’s t-test).

In addition, in Extended Data Fig. 7m, n, p, r, 9i, q–s and 10d, e of the original Letter, errors occurred in data scaling that affect the calculation of P values and the graphical presentation of the data. See Figs. 5, 6 and 7 of this Amendment for the corrected and incorrect panels for Extended Data Fig. 7f, m, n, p–t, 9i, m, q–s and 10d, e, g, respectively, and Supplementary Information to this Amendment for the corrected Source Data. The errors in data scaling occurred because two methods of data scaling were used throughout the study. In some experiments, data of the experimental groups were scaled to the average of the control values; in other experiments, data of the experimental groups were scaled to each of the corresponding controls of a biological repeat, set to 1 or 100. Although both methods of scaling are valid, they should not be combined within one experiment, which happened in the aforementioned figures. This has now been corrected and we include a detailed description of our scaling approach in the Supplementary Information to this Amendment.

The outlined corrections do not change the conclusions of the original Letter, and we apologize for any confusion that these errors may have caused. The original Letter has not been corrected.

Supplementary Information is available in the online version of this Amendment.

![Original Extended Data Fig. 6e](image1)

![Corrected Extended Data Fig. 6e](image2)

Fig. 1 | This is the corrected Extended Data Fig. 6e (right) and the original Extended Data Fig. 6e (left) published in the original Letter. All images have been replaced in the corrected figure.
**CORRECTIONS & AMENDMENTS**

**Fig. 2** | This is the corrected Fig. 3d (right) and the original Fig. 3d (left) published in the original Letter. Changes are highlighted in red.

**Fig. 3** | This is the corrected Fig. 4k (right) and the original Fig. 4k (left) published in the original Letter. Changes are highlighted in red. The data point that changes is also marked in red in the original figure as well as in the corrected figure.

**Fig. 4** | This is the corrected Extended Data Fig. 4f, g (right) and the original Extended Data Fig. 4f, g (left) published in the original Letter. Changes are highlighted in red.
Fig. 5 | This is the corrected Extended Data Fig. 7f, m, n, p, r–t (right) and the original Extended Data Fig. 7f, m, n, p, r–t (left) published in the original Letter. Changes are highlighted in red.
Fig. 6 | This is the corrected Extended Data Fig. 9i, m, q, r, s (right) and the original Extended Data Fig. 9i, m, q, r, s (left) published in the original Letter. Changes are highlighted in red.
Fig. 7 | This is the corrected Extended Data Fig. 10d, e, g (right) and the original Extended Data Fig. 10d, e, g (left) published in the original Letter. Changes are highlighted in red.