Supplementary Material for:

Dystrophin is required for muscle stem cell asymmetric division

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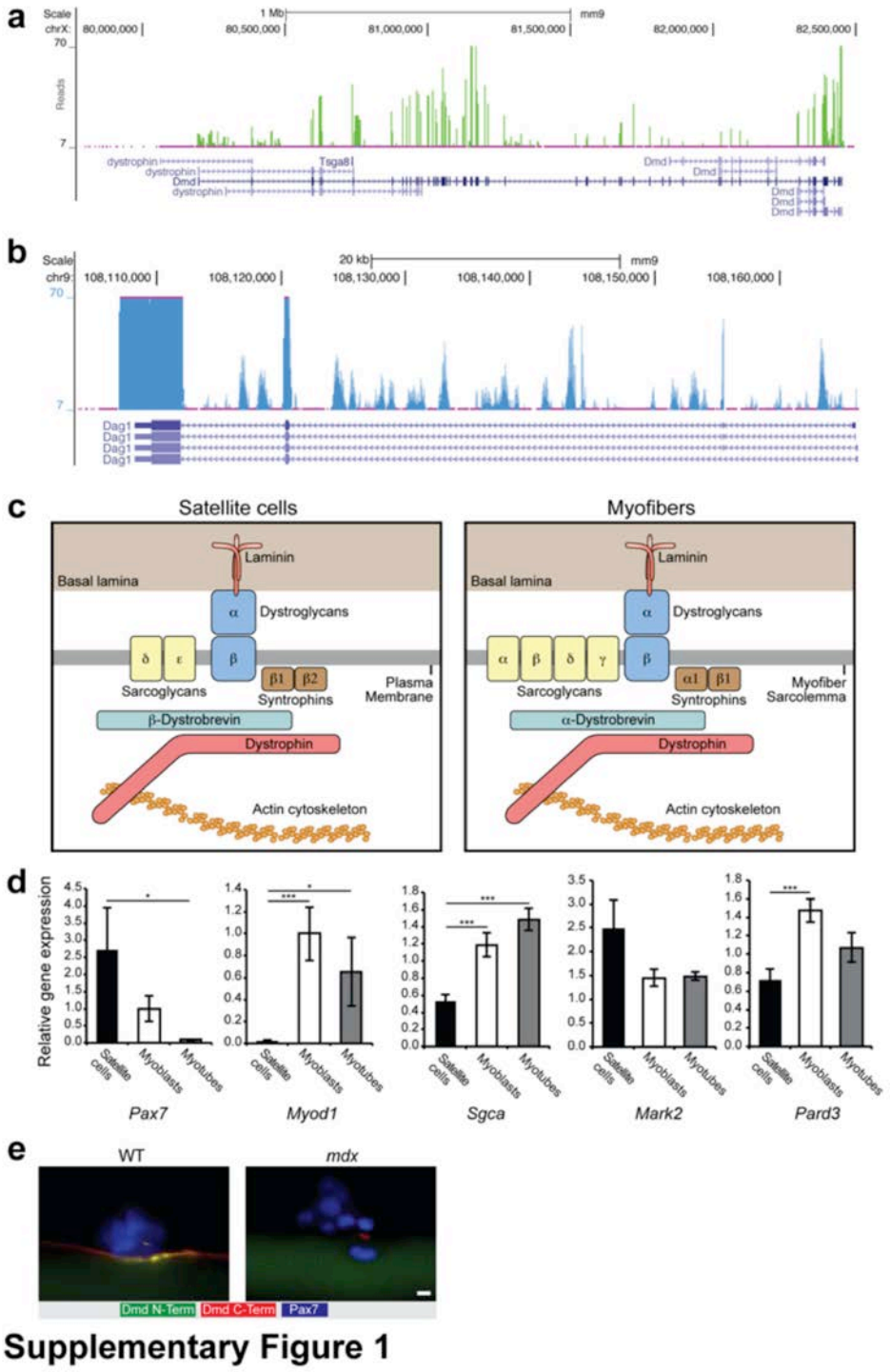
Supplementary Legends and Figures 1 to 7

Supplementary Table 1

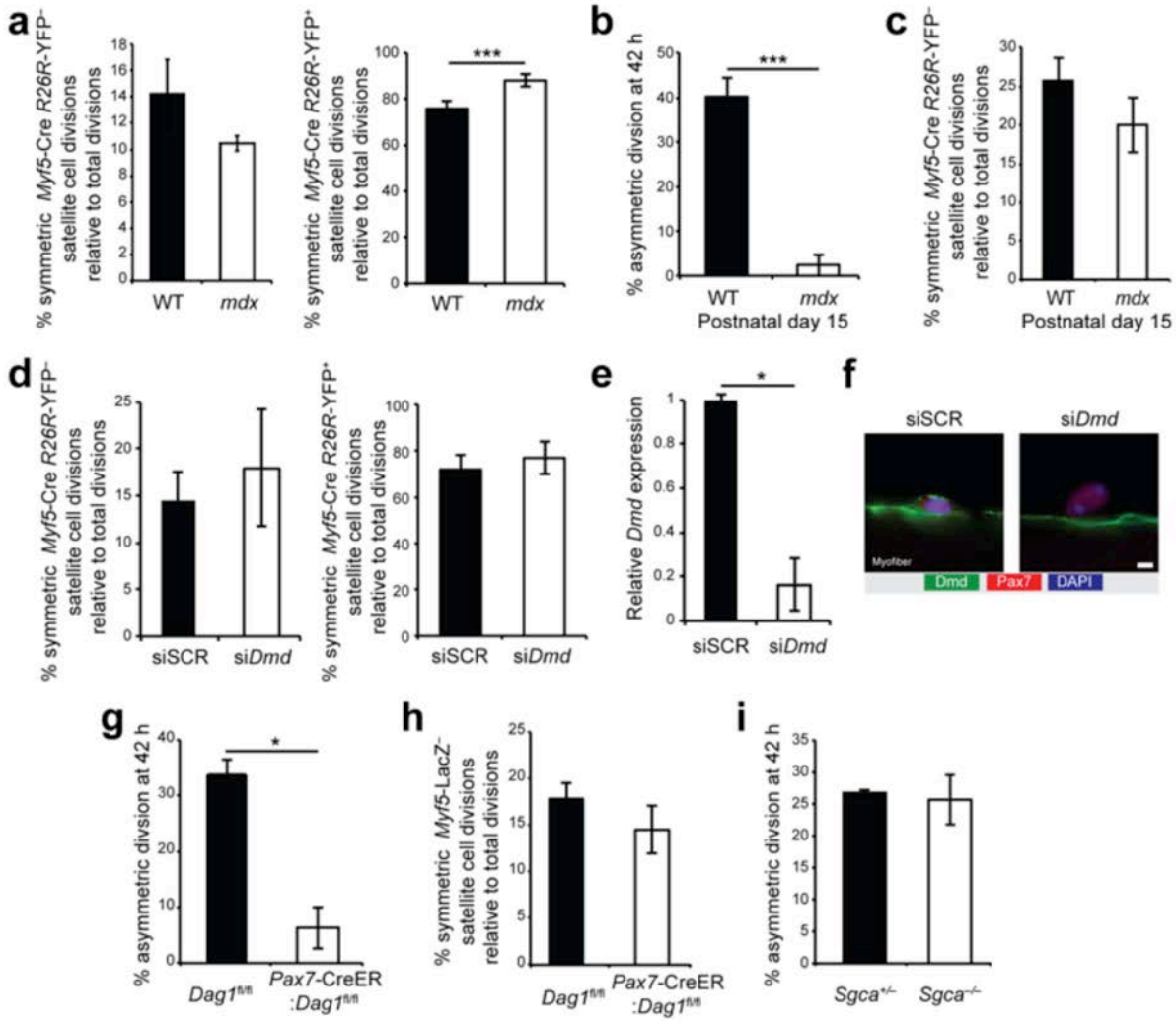
Legend for supplementary movie 1



Supplementary Figure 1

Supplementary Figure 1 DGC components expression in satellite cells. (**a**,**b**) RNA-seq reads at (**a**) the *Dmd* locus and (**b**) the *Dag1* locus in prospectively isolated satellite cells. $n = 4$ mice (**c**) Schematic representation of the distinct DGC components isoforms expressed in satellite cells

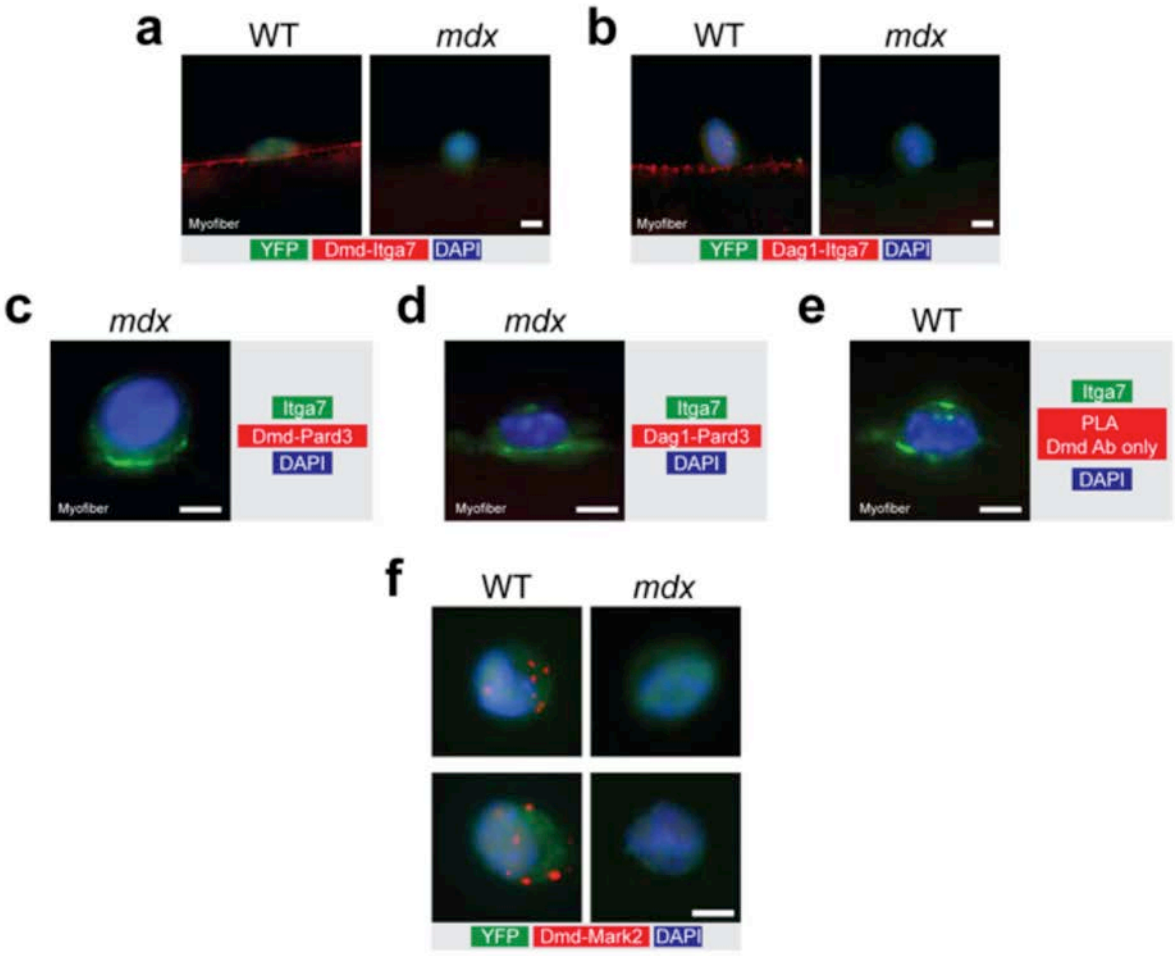
versus myofibers based on clustering analysis shown in **Fig. 1a**. (**d**) Quantitative Real-time PCR for *Pax7*, *Myod1*, *α-sarcoglycan (Sgca)*, *Mark2*, and *Pard3* in satellite cells, myoblasts, and myotubes. $n = 3$ for myoblasts and myotubes, and $n = 1$ for satellite cells obtained from pooled freshly isolated satellite cells of nine mice. Error bars represent means ± SEM; p-values: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$. Statistical significance was calculated by Student's *t* test. (**e**) Representative pictures ($n = 20$ pictures per condition) of myofibers from WT and *mdx* mice cultured for 72 h and immunostained for Dmd C-terminal (red), Dmd N-terminal (green), and Pax7 (blue). Scale bar, 5 μm.



Supplementary Figure 2

Supplementary Figure 2 Satellite stem cell division in DGC-deficient satellite cells. (**a**) Proportion of YFP-negative and YFP-positive symmetric divisions relative to total cell divisions in EDL myofibers cultured for 42 h from WT and *mdx Myf5-Cre*:*R26R-YFP* mice. *n* = 3 mice, 20-40 myofibers per mice (**b**,**c**) Myofibers cultured for 42 h from WT and *mdx Myf5-Cre*:*R26R-YFP* mice at postnatal day 15. (**b**) Quantification of asymmetric divisions relative to total YFP-negative satellite stem cell divisions and (**c**) symmetric YFP-negative satellite cell divisions relative to total cell divisions. *n* = 3 mice, 20-40 myofibers per mice (**d**) Proportion of YFP-

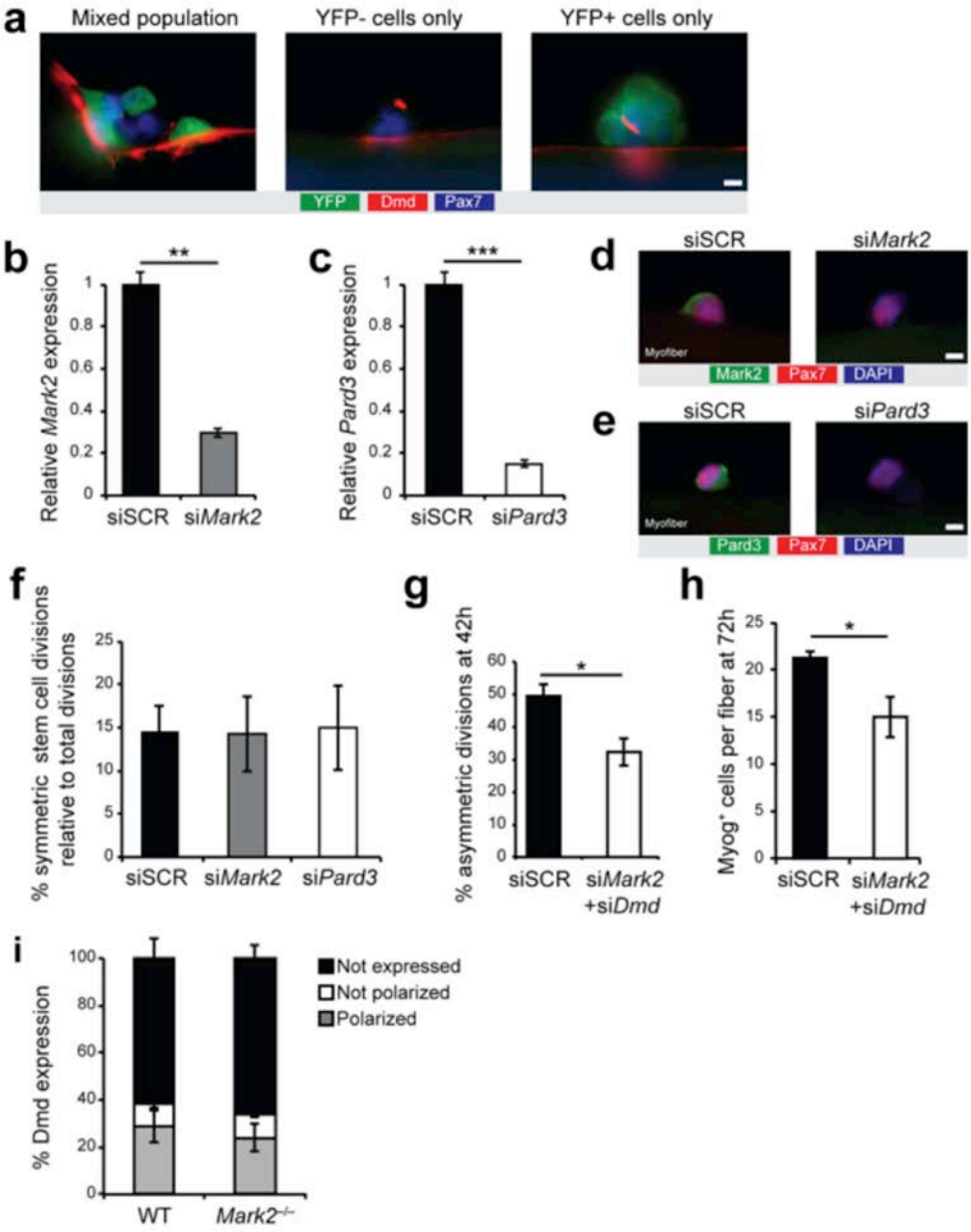
negative and YFP-positive symmetric divisions relative to total cell divisions in cultured myofibers (at 42h) of *Myf5-Cre*:*R26R-YFP* mice following knockdown of *Dmd* (si*Dmd*) compared to scramble siRNA (siSCR). *n* = 5 mice, 30-40 myofibers per mice (**e**,**f**) Knockdown efficiency of si*Dmd* measured by (**e**) qPCR on differentiating primary myocytes (*n* = 2 in technical quadruplicates) and (**f**) by immunostaining on myofibers for Dmd C-terminal (green), Pax7 (red), DAPI (blue). Scale bar, 5 μm. Representative pictures of *n* = 20-30 pictures (**g**) Quantification of asymmetric divisions relative to total YFP-negative satellite stem cell divisions and (**h**) symmetric YFP-negative satellite cell divisions relative to total cell divisions in myofibers cultured for 42 h from tamoxifen-treated $Dag1^{fl/fl}$*:Myf5-LacZ* and *Pax7-CreER:*$Dag1^{fl/fl}$*:Myf5-LacZ* mice. *n* = 3 mice, 20-40 myofibers per mice (**i**) Quantification of asymmetric divisions relative to total YFP-negative satellite stem cell divisions in cultured myofibers from $Sgca^{+/-}$*:Myf5-LacZ* and $Sgca^{-/-}$*:Myf5-LacZ* mice at 42 h. *n* = 3 mice, 20-40 myofibers per mice. Error bars represent means ± SEM. p-values: **P* < 0.05 ****P* < 0.005. Statistical significance was calculated by Student's *t* test.



Supplementary Figure 3

Supplementary Figure 3 Dystrophin and PAR proteins in satellite cells from WT and *mdx* mice. (**a**,**b**) Representative pictures (*n* = 10 pictures per condition) of proximity ligation assay (PLA) for (**a**) Dmd and itga7 and (**b**) Dag1 and itga7 was performed on myofibers from WT and *mdx* mice cultured for 36 h. (**c**,**d**) Representative pictures (*n* = 10-20 pictures per condition) of PLA for (**c**) Dmd and Pard3 and (**d**) Dag1 and Pard3 was performed on myofibers from *mdx* mice cultured for 36h. (**e**) Representative pictures (*n* = 10-20 pictures per condition) of negative control for PLA using a single antibody (Dmd only, rod domain antibody) on myofibers from WT mice cultured for 36 h. (**f**) Representative pictures (*n* = 10 pictures per condition) of PLA for

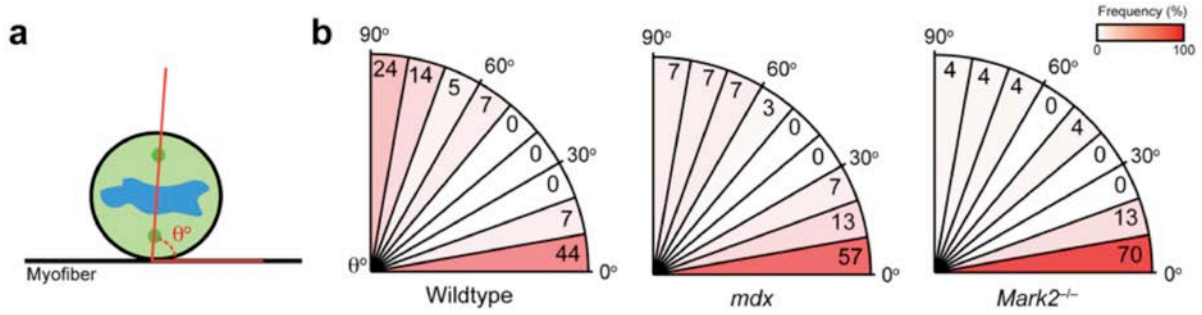
Dmd and Mark2 on activated satellite cells from WT and *mdx Myf5-Cre:R26R-YFP* mice isolated by FACS 2 days after CTX injury. (**a**–**f**) PLA (red) was performed along with immunostaining for (**a**,**b**,**f**) YFP (green) and DAPI (blue) or (**c**–**e**) itga7 (green) and DAPI (blue). Scale bar, 5 μm.



Supplementary Figure 4

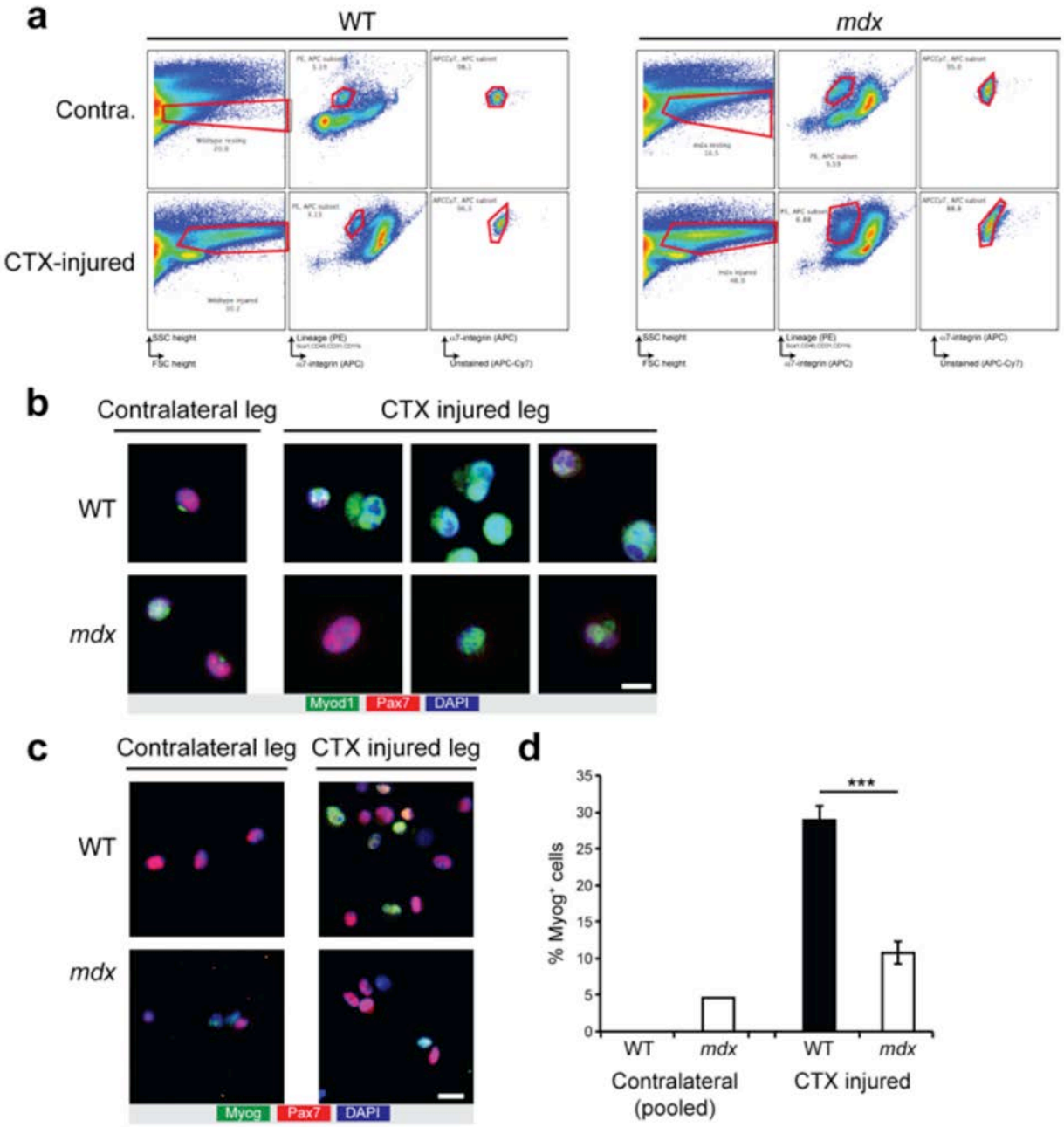
Supplementary Figure 4 Dmd and PAR expression in symmetric and asymmetric divisions. (**a**) Representative pictures (*n* = 20-30 pictures) of myofibers from WT mice cultured for 72 h and immunostained for Dmd C-terminal (red), YFP (green), and Pax7 (blue). Scale bar, 5 μm. (**b**–**e**)

Knockdown efficiency of si*Mark2* and si*Pard3* compared to siSCR measured by (**b**,**c**) qPCR on primary myoblasts (n = 2 in technical quadruplicates) and (**d**,**e**) immunostaining for Mark2 or Pard3 (green), Pax7 (red), and DAPI (blue) on myofibers from WT mice cultured for 36 h. Representative pictures from n = 20 pictures per condition. (**f**) Proportion of symmetric YFP-negative satellite cell divisions relative to total cell divisions in myofibers from WT mice cultured for 42 h and treated with si*Mark2*, si*Pard3*, or siSCR. n = 3 mice, 30-40 myofibers per mice. Quantification of (**g**) asymmetric divisions relative to total YFP-negative satellite cell divisions (at 42 h) and (**h**) Myog-expressing cells per fiber (at 72 h) in cultured myofibers of *Myf5-Cre*:*R26R-YFP* mice following double knockdown for si*Mark2* and si*Dmd* compared to siSCR. n = 5 mice, 30-40 myofibers per mice. (**i**) Quantification of Dmd expression (using rod domain antibody) and localization in satellite cells of myofibers from WT and *Mark2*$^{-/-}$ mice cultured for 36 h. Only undivided cells were quantified. n = 3 mice, 40-60 cells per mouse. (**a**–**j**) Error bars represent means ± SEM. Scale bar, 5 μm. p-values: *P < 0.05, **P < 0.01, ***P < 0.005. Statistical significance was calculated by Student's t test.



Supplementary Figure 5

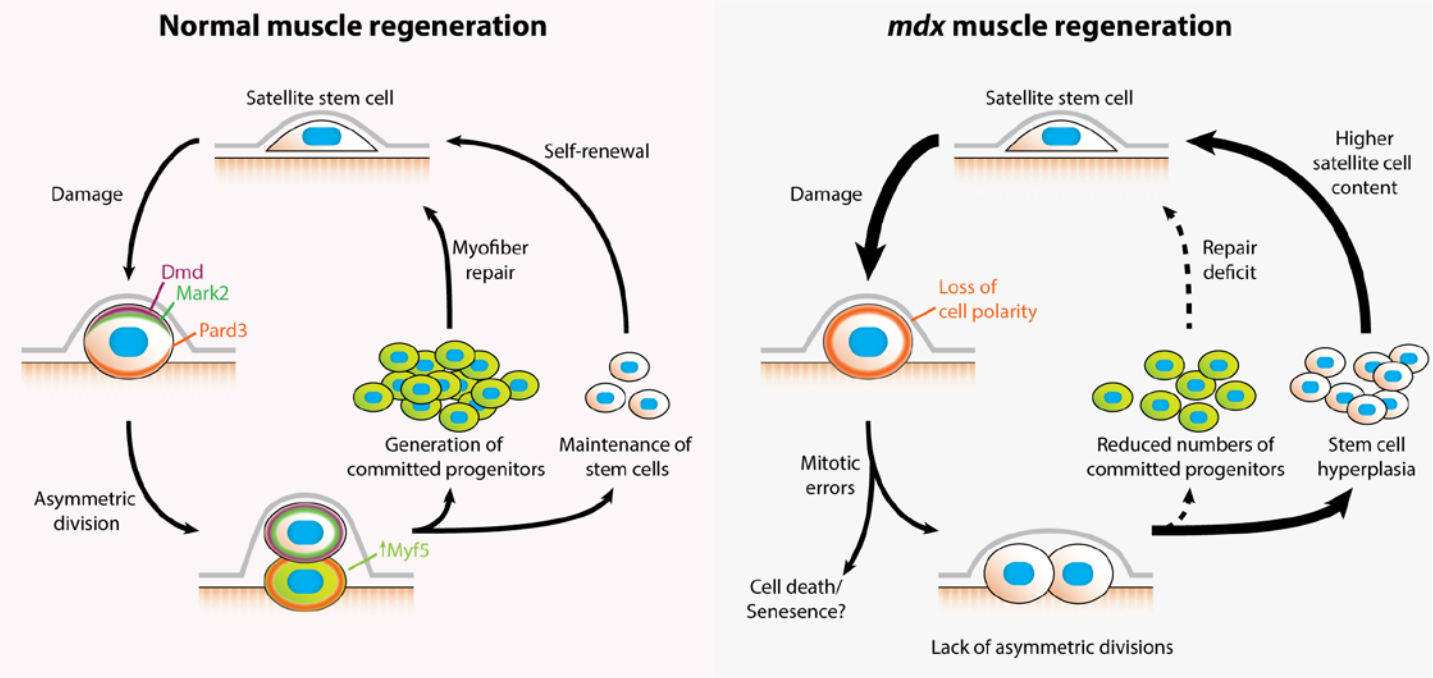
Supplementary Figure 5 Satellite cell division orientation in WT, *mdx*, and *Mark2*$^{-/-}$ mice. (**a)** Cartoon schematic for the determination of satellite cell division orientation. (**b**) Binned frequency of mitotic orientations (θ) of satellite cells on myofiber cultured for 36 h from WT, *mdx*, and *Mark2*$^{-/-}$ mice. Relative frequencies are indicated as a percentage and visualized as intensity of red. n = 3 mice, >40 cells per condition.



Supplementary Figure 6

Supplementary Figure 6 FACS gating strategy and validation of cell purity. **(a**) FACS gating strategy for the isolation of quiescent satellite cells and activated myogenic cells from resting contralateral (Contra.) and CTX-injured muscles of WT and *mdx Myf5-Cre*:*R26R-YFP* mice.

Cells were selected based on side and forward scatter profiles, purified by selecting itga7-high (APC) lineage-low (Sca1, CD45, CD31, CD11b; PE), and autofluorescent cells were further removed by gating APC against an unstained APC-Cy7 channel. (**b**,**c**) Representative pictures (*n* > 20 pictures per condition) of immunostaining for (**b**) Myod1 (green) and (**c**) Myog (green) along with Pax7 (red) and DAPI (blue) in prospectively isolated myogenic cells from resting (contralateral) and CTX-injured muscles of WT and *mdx Myf5-Cre*:*R26R-YFP* mice. Scale bars, 10 μm (in **b**) and 20 μm (in **c**). **(d**) Quantification of Myog-expressing cells relative to total myogenic cells (Pax7-expressing and Myog-expressing cells) in prospectively isolated myogenic cells from resting (contralateral) and CTX-injured muscles of WT and *mdx Myf5-Cre*:*R26R-YFP* mice. Error bars represent means ± SEM; $n = 5$ mice, samples from resting muscles were pooled together. p-values: ***$P < 0.005$. Statistical significance was calculated by Student's *t* test.



Supplementary Figure 7

Supplementary Figure 7 Schematic of the cell-autonomous defects in satellite cells from WT and *mdx* mice. Left panel shows activated satellite cell from WT mice that expresses Dmd leading to the polarization of Mark2 and Pard3 at opposite poles of the dividing cell. Polarization of PAR proteins promotes apicobasal asymmetric cell division leading to the maintenance of the satellite stem cell (YFP-negative cell) and the generation of a committed progenitor (YFP-positive cell). YFP-positive myogenic progenitors promote myofiber repair, while YFP-negative satellite stem cells maintain the satellite cell pool through self-renewal. Right panel shows loss of cell polarity in dystrophin-deficient satellite cells leading to mitotic errors and lack of apicobasal asymmetric divisions. Impaired asymmetric divisions reduce the generation of YFP-positive myogenic progenitors and lead to higher number of YFP-negative satellite stem cells that do not contribute efficiently to muscle regeneration.

Asymmetric divisions YFP-/YFP+

	YFP + only	YFP - only	Both cells	No expression
Mark2	13%	74%	13%	0%
Pard3	80%	10%	10%	0%
Dmd	0%	78%	5%	17%

Symmetric divisions YFP-/YFP-

	YFP + only	YFP - only	Both cells	No expression
Mark2	0%	0%	100%	0%
Pard3	0%	0%	100%	0%
Dmd	0%	0%	76%	24%

Supplementary table 1 Distribution of Dmd and PAR proteins in symmetric and asymmetric divisions. Table quantifying the distribution of Mark2, Pard3, and Dmd in the daughter cells after asymmetric (YFP^-/YFP^+ pairs) and symmetric divisions (YFP^-/YFP^- pairs). *n* = 3 mice, 10-20 cell divisions per condition.

Supplementary Movie 1 3D reconstruction of a satellite cell expressing DGC components on a myofiber. Myofibers cultured for 36h were immunostained for Dmd C-terminal (green), Dag1 (red), itga7 (cyan), and DAPI (dark blue). Series of pictures were taken by confocal microscopy and reconstructed into a 3D image using Zen software. Movie shows a satellite cell (delineated by itga7 staining) juxtaposed to a myofiber. Expression of Dmd and Dag1 in satellite cell is clearly distinguishable from their expression in the myofiber.