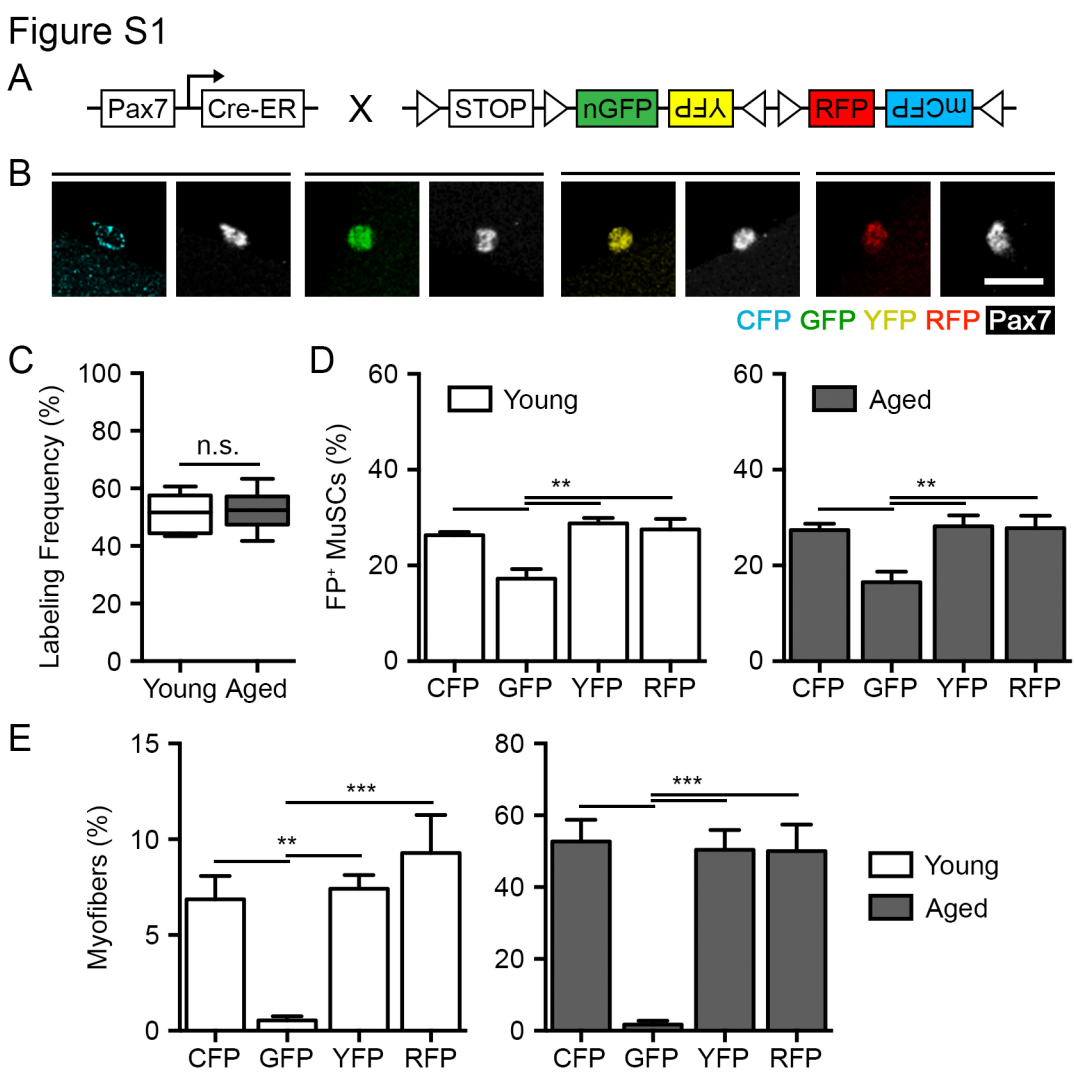

Figure S1, related to Figure 1.

(**A**) Scheme of the genetic strategy for multicolor lineage tracing of MuSCs.

(**B**) Images of $Pax7^{+}FP^{+}$ myofiber-associated MuSCs isolated from young muscles and fixed immediately upon isolation. Scale bar, 20 μm.

(**C**) Quantification of FP^{+} labeling frequency in myofiber-associated MuSCs isolated from young and aged muscles and fixed immediately upon isolation (n = 5-6).

(**D-E**) Quantification of the percentage of young and aged freshly isolated myofiber-associated MuSCs and uninjured myofibers expressing each FP in tissue sections (n = 5-6).

Data are represented as average ± SEM (***P < 0.001, **P < 0.01).

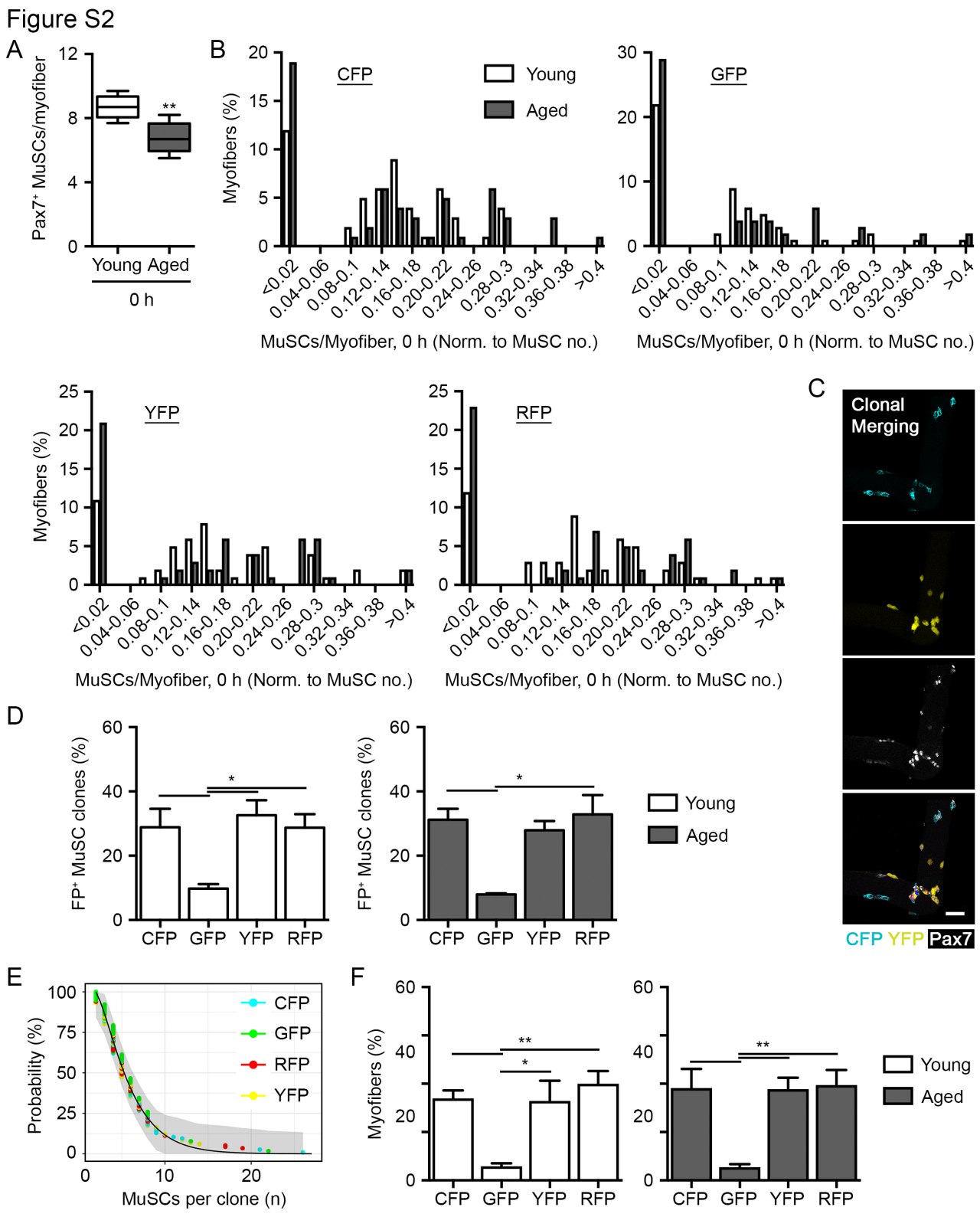

Figure S2, related to Figure 2.

(**A**) Quantification of Pax7$^+$ MuSCs per myofiber and fixed immediately upon isolation (n = 5-6).

(**B**) Histogram depicting the local distribution of FP^+ MuSCs within individual young and aged freshly isolated single myofibers (n = 54-56). Gaussian distribution not assumed; statistical comparison using Kolmogorov-Smirnov test ($p > 0.05$ for all FPs).

(**C**) Example of inter-FP clonal merging in composite images of myofiber-associated MuSCs after 3 d in suspension culture. Scale bar, 50 μm.

(**D**) Quantification of the percentage of young and aged MuSCs expressing each FP 3 d post-$BaCl_2$ injury (n = 2-3).

(**E**) Young MuSC cumulative clone size distributions for each FP 3 d post-$BaCl_2$ injury. Shaded area denotes 95% Kolmogorov-Smirnov confidence intervals of empirical distribution.

(**F**) Quantification of the percentage of young and aged regenerating myofibers expressing each FP 25 d post-$BaCl_2$ injury (n = 5).

Data are represented as average ± SEM (**P < 0.01, *P < 0.05).

Figure S3

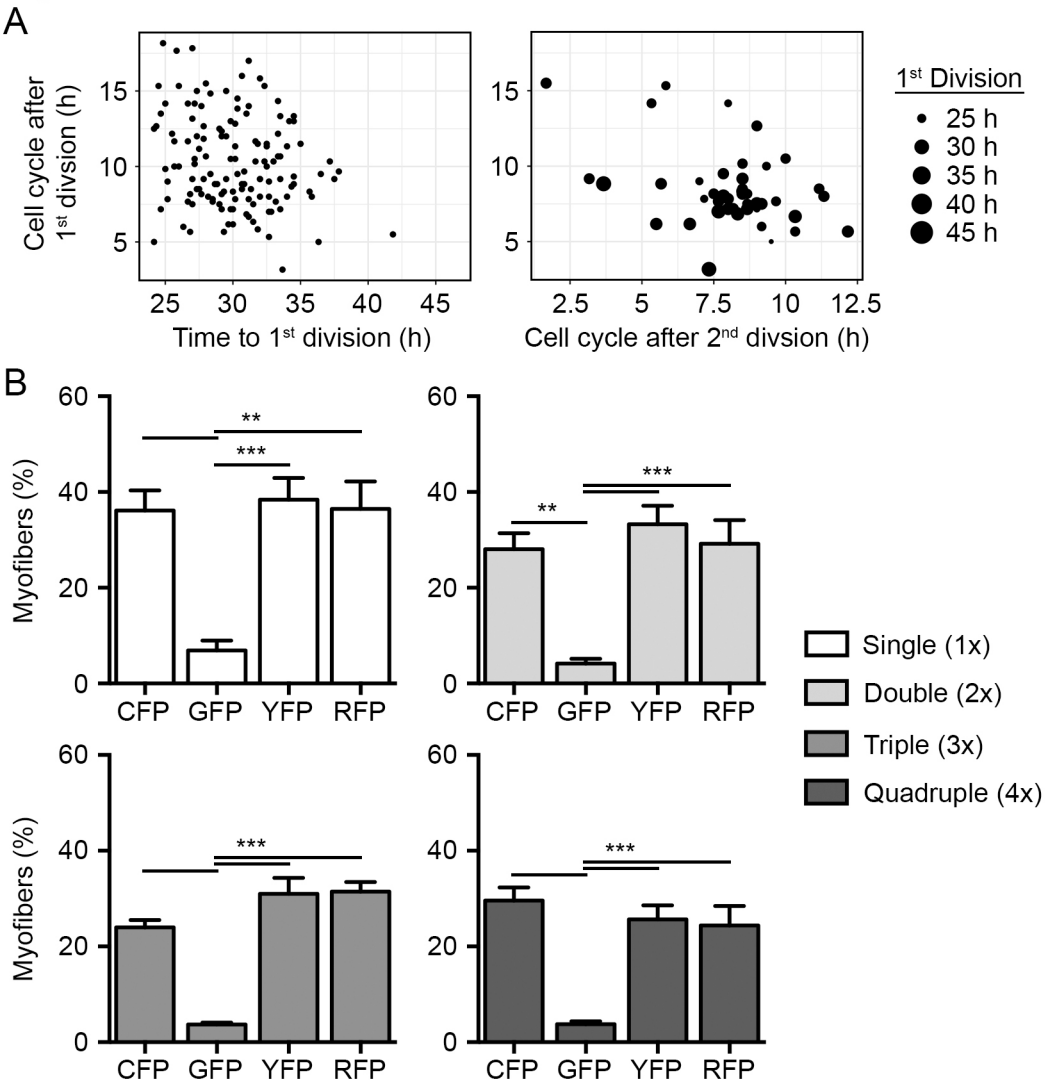

Figure S3, related to Figure 3.

(**A**) Relationship between the time to first division and cell cycle length after the first (left) or second (right) divisions in myofiber-associated MuSCs, obtained from (Siegel et al., 2011).

(**B**) Quantification of the percentage of young regenerating myofibers expressing each FP following serial $BaCl_2$ injury (n = 5).

Data are represented as average ± SEM (***P < 0.001, **P < 0.01).

Figure S4

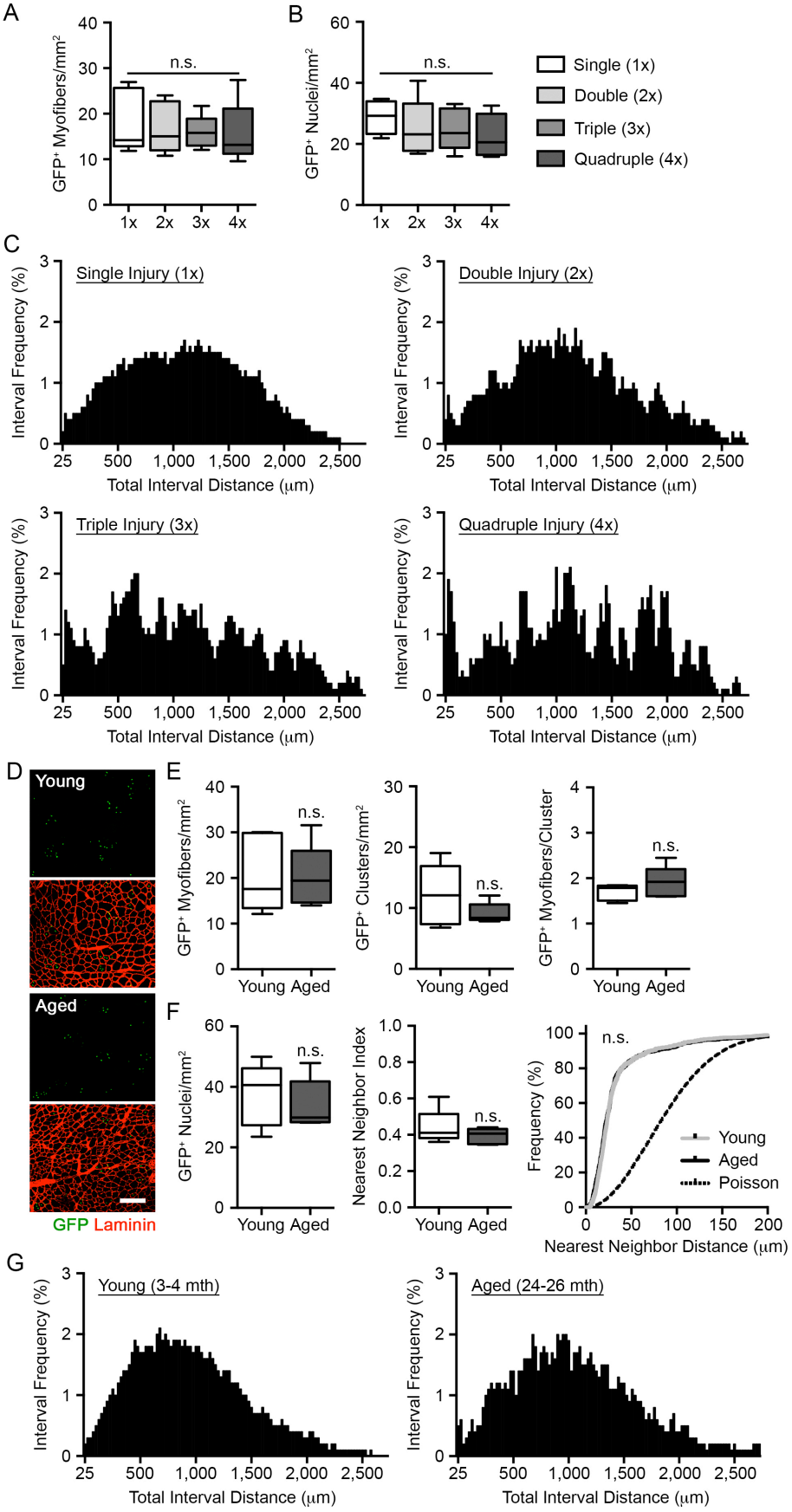
A
B
GFP⁺ Myofibers/mm²
GFP⁺ Nuclei/mm²
n.s.
1x 2x 3x 4x
Single (1x)
Double (2x)
Triple (3x)
Quadruple (4x)
C
Single Injury (1x)
Double Injury (2x)
Triple Injury (3x)
Quadruple Injury (4x)
Interval Frequency (%)
Total Interval Distance (μm)
D
Young
Aged
GFP Laminin
E
GFP⁺ Clusters/mm²
GFP⁺ Myofibers/Cluster
Young Aged
F
GFP⁺ Nuclei/mm²
Nearest Neighbor Index
Frequency (%)
Young
Aged
Poisson
Nearest Neighbor Distance (μm)
G
Young (3-4 mth)
Aged (24-26 mth)

Figure S4, related to Figure 4.

(**A**) Quantification of GFP^+ myofiber density in regenerated muscles following serial $BaCl_2$ injury (n = 5).

(**B**) Quantification of GFP^+ nuclei density in regenerated muscles following serial $BaCl_2$ injury (n = 5).

(**C**) Representative plots of total distance interval distributions of GFP^+ nuclei following serial $BaCl_2$ injury (n = 5).

(**D**) Composite images of GFP^+ myonuclei in young and aged regenerated muscles 25 d post-$BaCl_2$ injury. Scale bar, 100 μm.

(**E**) Quantification of GFP^+ myofiber density (left), myofiber cluster density (center) and the number of regenerated myofibers per cluster (right) in young and aged muscles 25 d post-$BaCl_2$ injury (n = 5).

(**F**) Quantification of GFP^+ nuclei density (left), nearest neighbor index (center) and cumulative distribution frequency (right) derived from spatial analyses in young and aged muscles 25 d post-$BaCl_2$ injury (n = 5).

(**G**) Representative plots of total distance interval distributions of GFP^+ nuclei in young and aged muscles 25 d post-$BaCl_2$ injury (n = 5).

Data are represented as average ± SEM ($P > 0.05$).