Figure S1, Related to Figure 1.

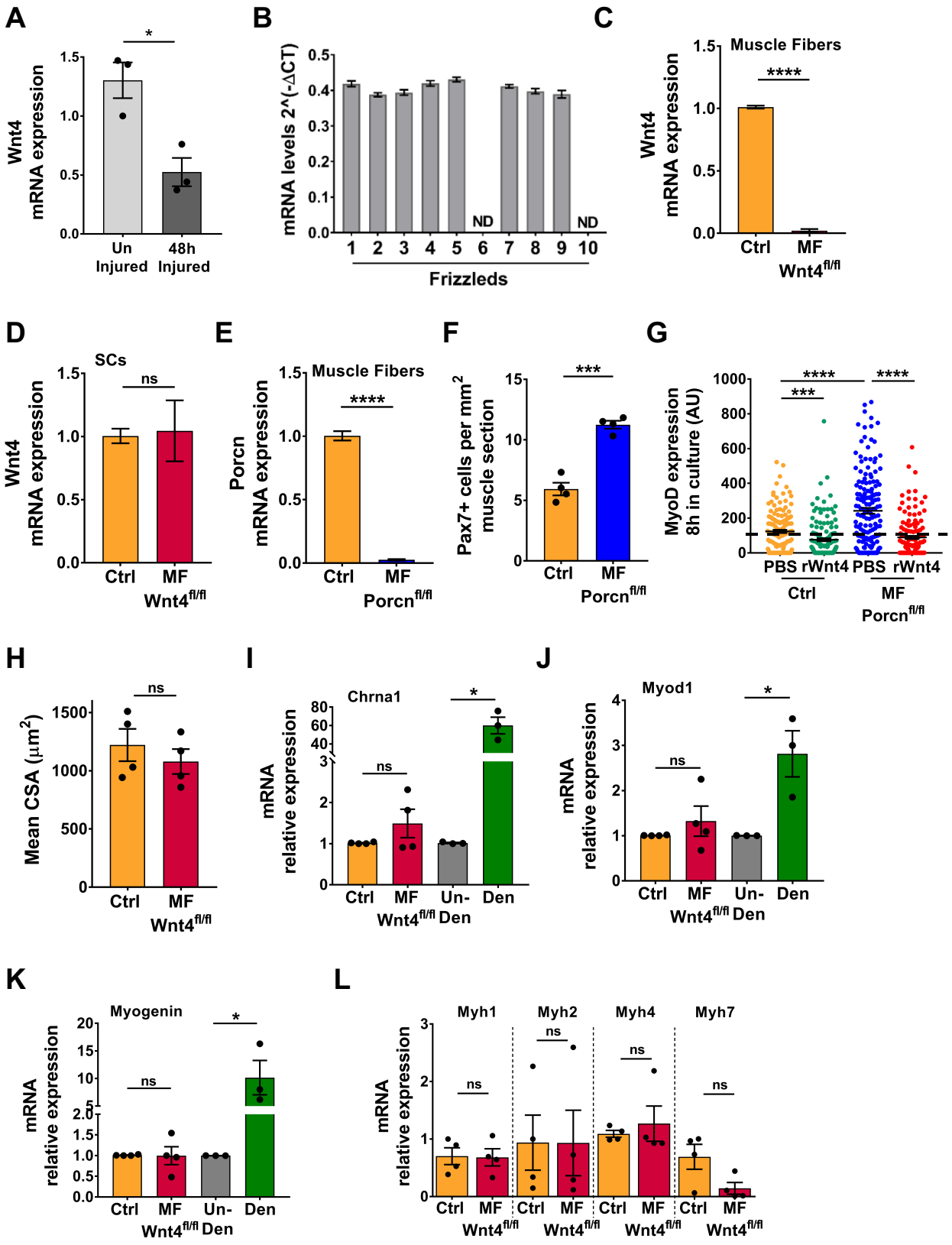

Figure S1, related to Figure 1. Wnt4 deletion has no effect on muscle fibers at tissue homeostasis.

(A) Wnt4 mRNA expression by qRT-PCR from wildtype uninjured and 48h injured whole TA muscle.

(B) qRT-PCR of Frizzled expression in FACS isolated wildtype adult SCs.

(C and D) qRT PCR for Wnt4 expression in control and Myofiber-Wnt4$^{fl/fl}$ single muscle fibers (n=7) (C) and in isolated SCs (n=3) (D), analyzed 14d post tmx.

(E) Porcn mRNA expression in Control and Myofiber-Porcn$^{fl/fl}$ muscle fibers, 14d post tmx (n=3).

(F) Quantification of Pax7^{+} cells per 1mm^2 TA muscle section from Control and Myofiber-Porcn$^{fl/fl}$ mice, 14d post tmx (n=4).

(G) MyoD expression in SCs on single muscle fibers from Control and Myofiber-Porcn$^{fl/fl}$ treated with recombinant Wnt4 (rWnt4) for 8h *in vitro* (n=3). The dashed line represents the threshold of intensity visible by eye.

(H) Quantification of the cross-sectional area of fibers in uninjured Control and Myofiber-Wnt4$^{fl/fl}$ TA muscle sections at tissue homeostasis (n=4).

(I, J and K) qRT PCR for denervation genes, Chrna1 (I), Myod1 (J) and Myogenin (K) in control and Myofiber-Wnt4$^{fl/fl}$ single muscle fibers, 14d post tmx (n=>3). Fibers from EDL muscle of denervated and un-denervated mice were used as controls (n=3).

(L) qRT PCR for myosin expression in control and Myofiber-Wnt4$^{fl/fl}$ muscle fibers isolated from the EDL muscle, analyzed 14d post tmx (n=4)

Error bars, s.e.m.; *P<0.05, ***P<0.001, ****P<0.0001.

Figure S2, Related to Figure 2.

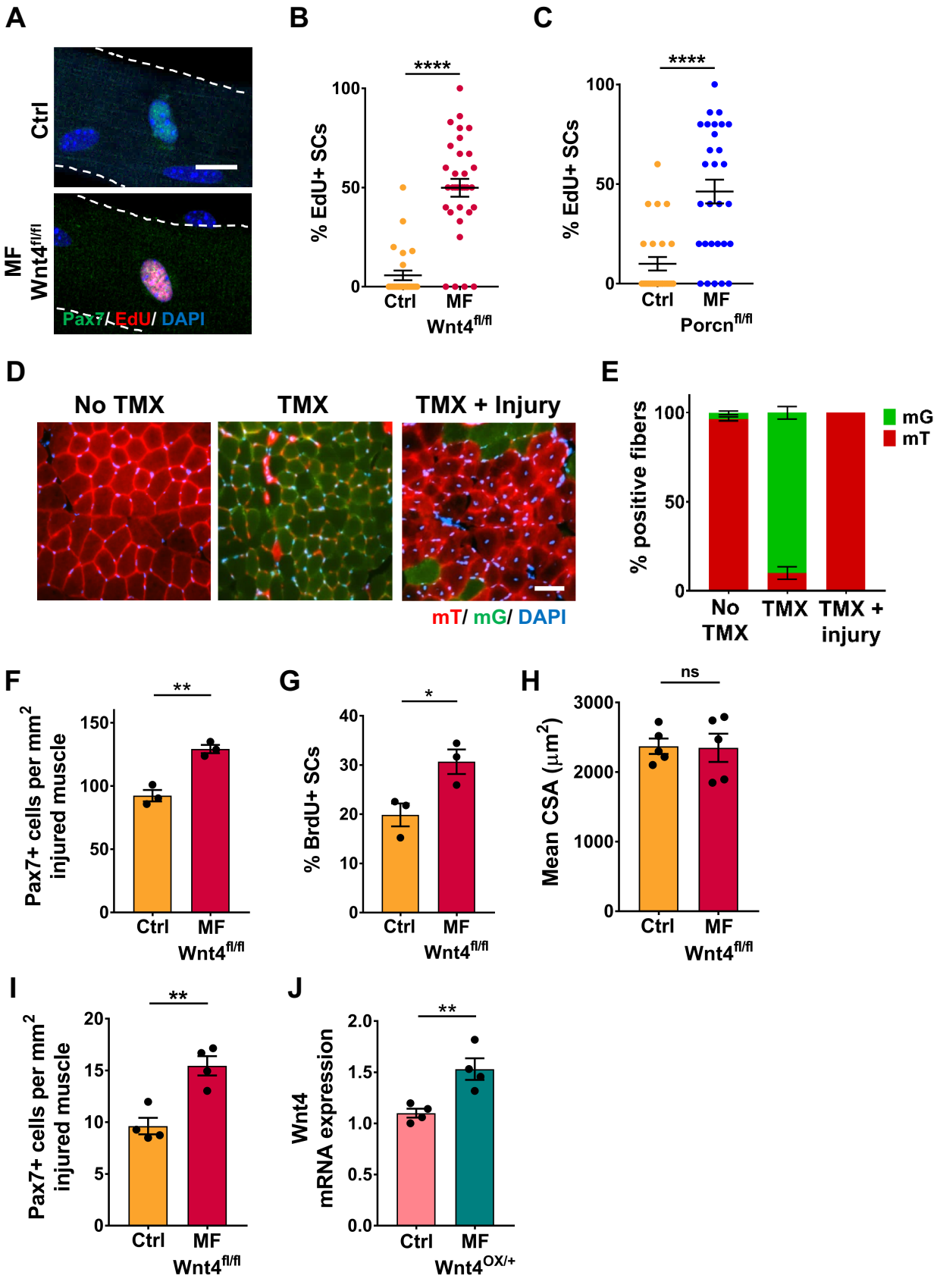

Figure S2, related to Figure 2. Loss of myofiber Wnt4 causes SCs to proliferate faster *in vitro* and *in vivo* after injury.

(A and B) Representative images (A) and quantification (B) of the percentage of EdU^+ SCs after culturing Control and Myofiber-$Wnt4^{fl/fl}$ single muscle fibers for 30hrs after isolation (n=3).

(C) Percentage of EdU^+ SCs after culturing Control and Myofiber-$Porcn^{fl/fl}$ single muscle fibers for 30hrs after isolation (n=3).

(D and E) Representative images (D) and quantification (E) of HSA^{CreMER} - mTmG reporter expression.

(F) Quantification of the number of Pax7 SCs per mm^2 of Control and Myofiber-$Wnt4^{fl/fl}$ TAs injured and regenerated for 6d (n=3).

(G) Percentage of $BrdU^+$ SCs in control and Myofiber-$Wnt4^{fl/fl}$ TAs injured and regenerated for 6d. The mice were injected with BrdU 12h before the TA muscle was isolated (n=3).

(H) Quantification of the cross-sectional area of fibers in injured Control and Myofiber-$Wnt4^{fl/fl}$ TA muscle sections, 35d post injury (n=5).

(I) Quantification of the number of $Pax7^+$ SCs per mm^2 of Control and Myofiber-$Wnt4^{fl/fl}$ TAs injured and regenerated for 35d (n=4).

(J) Wnt4 mRNA expression by qRT-PCR on Control and Myofiber-$Wnt4^{OX/+}$ single muscle fibers, 14d post tmx (n=4).

Error bars, s.e.m.; *P<0.05, **P<0.01, ****P<0.0001. Scale bar 10μm in (A), 50μm in (D).

Figure S3, Related to Figure 3.

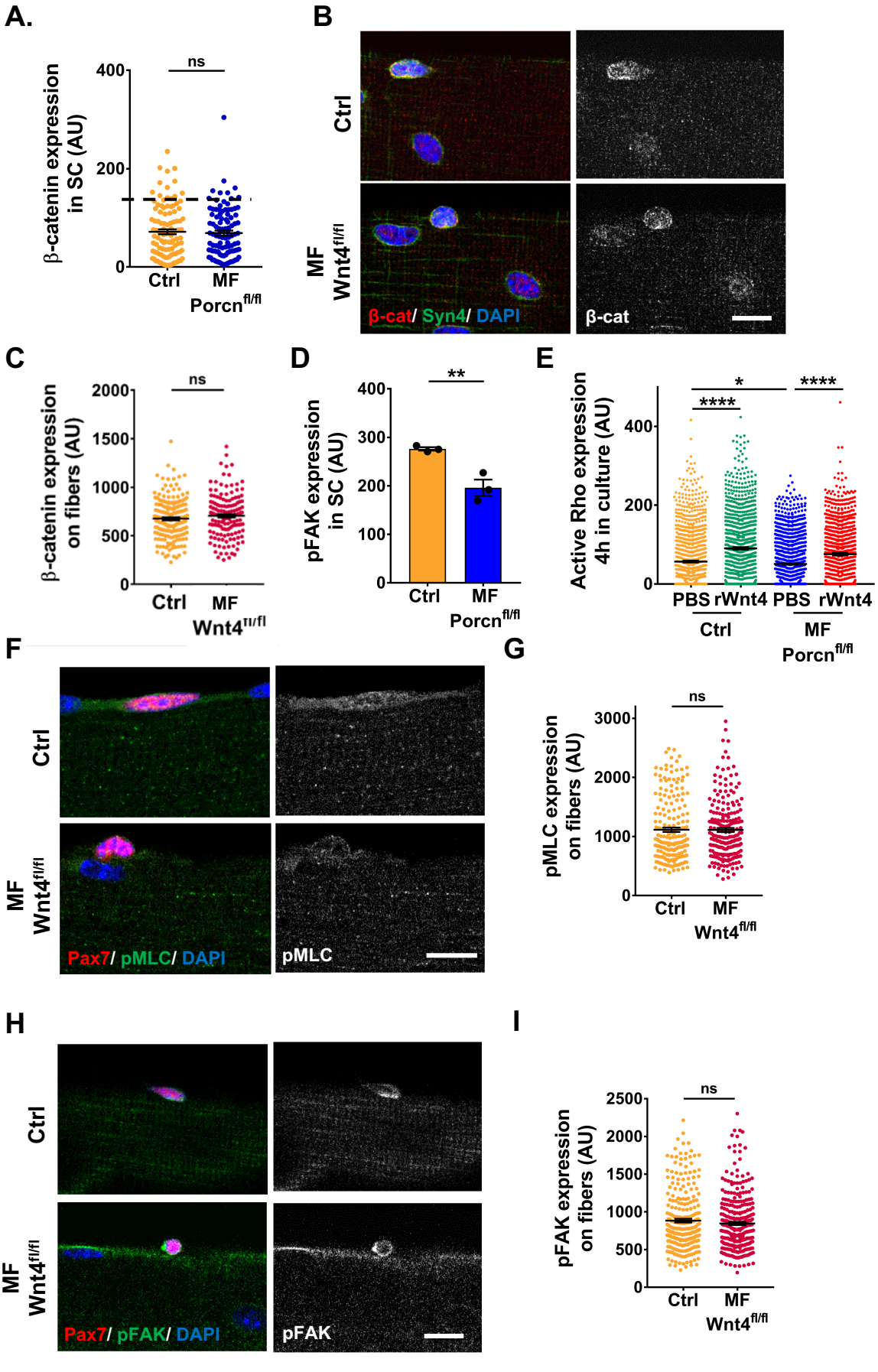

Figure S3, related to Figure 3. Myofiber Wnt4 does not affect canonical β-catenin and cytoskeletal signaling in muscle fibers at tissue homeostasis.

(A) Quantification of active β-catenin levels in fiber associated SCs from Myofiber-Porcn$^{fl/fl}$ compared to control SCs, 14d post tmx (n=3). The dashed line represents the threshold of intensity visible by eye.

(B and C) Representative images (B) and quantification (C) of active β-catenin expression in muscle fibers of control and Myofiber-Wnt4$^{fl/fl}$, 14d post tmx (n=3)

(D) Quantification of pFAK expression in Control and Myofiber-Porcn$^{fl/fl}$ SCs, 14d post tmx (n=3).

(E) Active Rho levels in FACS isolated SCs from Control and Myofiber-Porcn$^{fl/fl}$ treated with recombinant Wnt4 (rWnt4) for 4h *in vitro* (n=3).

(F and G) Representative images (F) and quantification (G) of pMLC expression in control and Myofiber-Wnt4$^{fl/fl}$ muscle fibers, 14d post tmx (n=3).

(H and I) Representative images (H) and quantification (I) of pFAK expression levels in control and Myofiber-Wnt4$^{fl/fl}$ muscle fibers, 14d post tmx (n=3).

Error bars, s.e.m.; *P<0.05, **P<0.01, ****P<0.0001. Scale bars 10μm in (B, F and H).

Figure S4, Related to Figure 4.

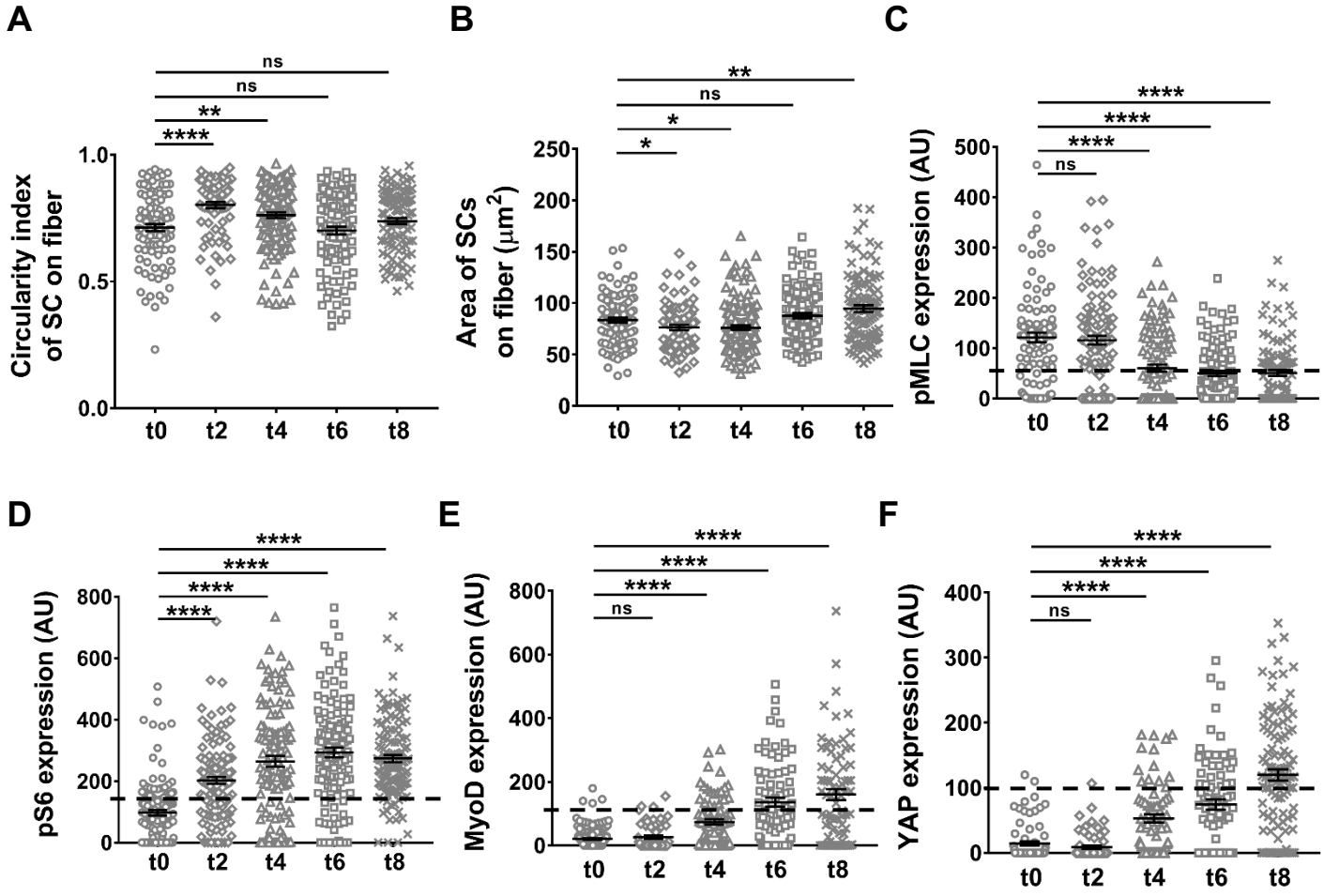

Figure S4, related to Figure 4. Quiescence to activation transition of SCs is characterized by morphological and cytoskeletal changes preceding onset of activation markers.

(A) Circularity index of WT SCs on single muscle fibers cultured *in vitro* for different time points (n=3).

(B) Area of WT SCs on muscle fibers cultured *in vitro* for different time points (n=3).

(C) pMLC expression of WT SCs on single muscle fibers cultured *in vitro* for different time points (n=3).

(D) pS6 expression of WT SCs on single muscle fibers cultured *in vitro* for different time points (n=3).

(E) MyoD expression of WT SCs on single muscle fibers cultured *in vitro* for different time points (n=3).

(F) YAP expression of WT SCs on single muscle fibers cultured *in vitro* for different time points (n=3).

The dashed line in Figures S4C, S4D, S4E and S4F represents the threshold of intensity visible by eye.

Error bars, s.e.m; *P<0.05, **P<0.01, ****P<0.0001.

Figure S5, Related to Figure 5.

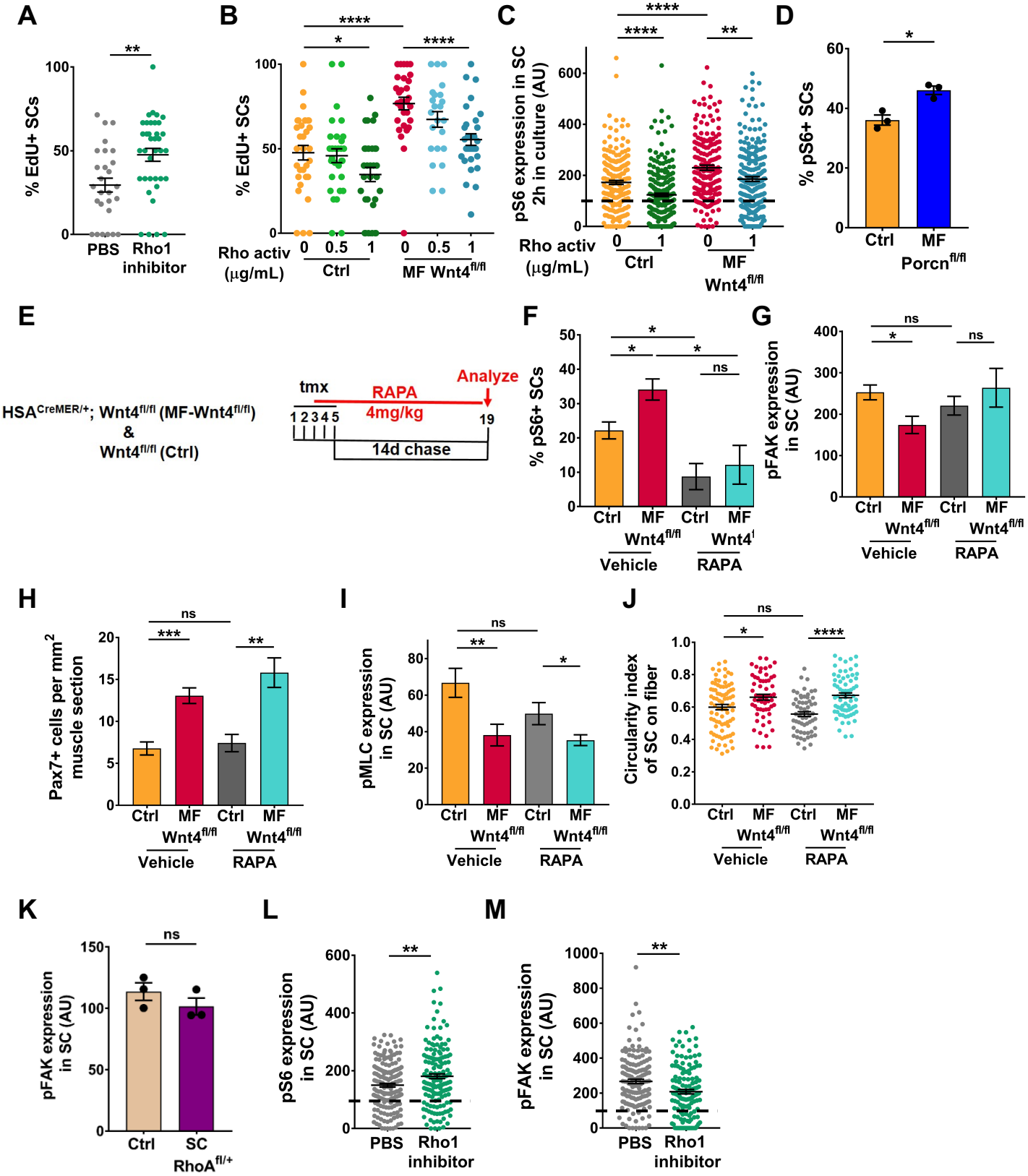

Figure S5, related to Figure 5. Wnt4-RhoA signaling axis in QSC does not require activation of mTORC1 to enter cell cycle.

(A) Quantification of the percentage of EdU^+ SCs on single muscle fibers isolated from wildtype mice treated with PBS or Rho1 inhibitor for 30h *in vitro* (n=2).

(B) Quantification of the percentage of EdU^+ SCs on single muscle fibers from Control and Myofiber-$Wnt4^{fl/fl}$ treated with Rho activator for 36h *in vitro* (n=3).

(C) pS6 expression in SCs on single muscle fibers from Control and Myofiber-$Wnt4^{fl/fl}$ treated with Rho activator for 2h *in vitro* (n=3).

(D) Quantification of the percentage of $pS6^+$ SCs on Control and Myofiber-$Porcn^{fl/fl}$ muscle fibers, 14d post tmx (n=3).

(E) Schematic representation of the experimental design.

(F) Quantification of the percentage of $pS6^+$ SCs in Control and Myofiber-$Wnt4^{fl/fl}$ mice, *in vivo* treated with either Vehicle or Rapamycin (n=5).

(G) Quantification of pFAK expression in SCs of Control and Myofiber-$Wnt4^{fl/fl}$ mice, *in vivo* treated with either Vehicle or Rapamycin (n=5).

(H) Quantification of the number of $Pax7^+$ SCs per mm^2 TA muscle in Control and Myofiber-$Wnt4^{fl/fl}$ mice, *in vivo* treated with either Vehicle or Rapamycin (n=5).

(I) Quantification of pMLC expression in fiber-aaociated SCs of Control and Myofiber-$Wnt4^{fl/fl}$ mice, *in vivo treated* with either Vehicle or Rapamycin (n=4).

(J) Quantification of the circularity index of fiber-associated SCs in Control and Myofiber-$Wnt4^{fl/fl}$ mice, *in vivo* treated with either Vehicle or Rapamycin (n=5).

(K) Quantification of pFAK expression in fiber associated SCs in SC-$RhoA^{fl/+}$ SCs compared to Control SCs, 14d post tmx (n=3).

(L) Isolated wildtype single muscle fibers treated with PBS or Rho1 inhibitor for 2h *in vitro* and analyzed for pS6 expression in SCs (n=2).

(M) Isolated wildtype single muscle fibers treated with PBS or Rho1 inhibitor for 2h *in vitro* and analyzed for pFAK expression in SCs (n=2).

The dashed line in Figures S5C, S5L and S5M represents the threshold of intensity visible by eye.

Error bars, s.e.m.; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Table S1, related to Figure 1. Microarray expression profile of Wnt ligands in adult muscle fibers (Log2 values).

	Adult muscle fibers (n1)	Adult muscle fibers (n2)
Wnt1	4.563	4.982
Wnt2	5.640	5.734
Wnt2B	5.432	6.331
Wnt3	5.077	5.623
Wnt3A	5.526	6.062
Wnt4	8.193	8.047
Wnt5A	5.686	6.660
Wnt5B	6.257	6.851
Wnt6	6.143	6.156
Wnt7A	5.074	5.478
Wnt7B	5.430	6.238
Wnt8A	5.755	5.554
Wnt8B	4.075	4.869
Wnt9A	7.254	7.419
Wnt9B	5.877	6.607
Wnt10A	6.395	7.160
Wnt10B	5.640	6.048
Wnt11	6.763	7.288
Wnt16	5.840	5.999

Table S2, related to STAR Methods. Primers used for qRT-PCR analysis.

Primer	Forward Sequence 5' to 3'	Reverse Primer 5' to 3'
Fzd1	CAAGGTTTACGGGCTCATGT	GTAACAGCCGGACAGGAAAA
Fzd2	GAGGTGCATCAGTTCTACCC	ATGGCCTGCTCCAGCACT
Fzd3	GCAGATAGGTGGGCACAGTT	AAAGAAATGGCCGAAAATCC
Fzd4	CACGCCGCTCATCCAGTA	GCCGATGGGGATGTTGAT
Fzd5	CAACCATGACACGCAGGAC	GGGCGTGTACATAGAGCACA
Fzd6	TTCCCTAACCTGATGGGTCA	ACATTTCAATGTTTGGTGAACA
Fzd7	ATATCGCCTACAACCAGACCATCC	AAGGAACGGCACGGAGGAATG
Fzd8	ATGGAGTGGGGTTACCTGTTG	CACCGTGATCTCTTGGCAC
Fzd9	GTCCGCGTTGTGTTTCTTCT	CAGACCCTCCTGGATCACAT
Fzd10	GTACCCCGAACGTCCTATCA	GTGCTCTCCAGTCCTTCCTG
Wnt1	TTCGGCAAGATCGTCAACCG	GCCAAAGAGGCGACCAAAATC
Wnt2	CTCGGTGGAATCTGGCTCTG	CACATTGTCACACATCACCCT

Wnt2B	GGAGGCAGCGTTCGTCTATG	CGCGGGTATATGGGTCACAG
Wnt3	AGCGTAGCAGAAGGTGTGAAG	CCAGGTGGCCCCTTATGATG
Wnt3A	CAGGAACTACGTGGAGATCATGC	CGTGTCACTGCGAAAGCTACT
Wnt4	AGACGTGCGAGAAACTCAAAG	GGAACTGGTATTGGCACTCCT
Wnt5A	CAACTGGCAGGACTTTCTCAA	CATCTCCGATGCCGGAACT
Wnt5B	TCCTGGTGGTCACTAGCTCTG	TGCTCCTGATACAACTGACACA
Wnt6	TCCTCTACGCAGCCGATTCA	CGGCACAGACAGTTCTCCTC
Wnt7A	TGAACTTACACAATAACGAGGCG	GTGGTCCAGCACGTCTTAGT
Wnt7B	CTTCACCTATGCCATCACGG	TGGTTGTAGTAGCCTTGCTTCT
Wnt8A	AGTGAACAACTTCCTGATAACCG	GAATGAAGGATGTCTCTCTCGTG
Wnt8B	CCCGTGTGCGTTCTTCTAGTC	AGTAGACCAGGTAAGCCTTTGG
Wnt9A	GGCCCAAGCACACTACAAG	AGAAGAGATGGCGTAGAGGAAA
Wnt9B	TCCTGTGCTGTTCGTACCTG	CCGTGTCATAGCGTAGCTTCA
Wnt10A	GCTCAACGCCAACACAGTG	CGAAAACCTCGGCTGAAGATG
Wnt10B	GAAGGGTAGTGGTGAGCAAGA	GGTTACAGCCACCCCATTCC
Wnt11	GCTGGCACTGTCCAAGACTC	CTCCCGTGTACCTCTCTCCA
Wnt16	CAGGGCAACTGGATGTGGTT	CTAGGCAGCAGGTACGGTT
Porcn	CTGCCTACTGTCCAACAGGG	GCATGCTTCAGGTAAGACGG
Axin2	TGACTCTCCTTCCAGATCCCA	TGCCCACACTAGGCTGACA
MyoD	CCACTCCGGGACATAGACTTG	AAAAGCGCAGGTCTGGTGAG
Myogenin	GAGACATCCCCCTATTTCTACCA	GCTCAGTCCGCTCATAGCC
Chrna	ACCTGGACCTATGACGGCTCT	AGTTACTCAGGTCGGGCTGGT
Myh1	GCGAATCGAGGCTCAGAACAA	GTAGTTCCGCCTTCGGTCTTG
Myh2	GCACCCATCCTCATTTCGTGA	GGAATGGCACTTGCGTTTAACA
Myh4	AGGACCAACTGAGTGAAGTGA	GGGAAAACTCGCCTGACTCTG
Myh7	ACTGTCAACACTAAGAGGGTCA	TTGGATGATTTGATCTTCCAGGG
GAPDH	GGCAAAGTGGAGATTGTTGC	AATTTGCCGTGAGTGGAGTC