Bentzinger et al., http://www.jcb.org/cgi/content/full/jcb.201310035/DC1

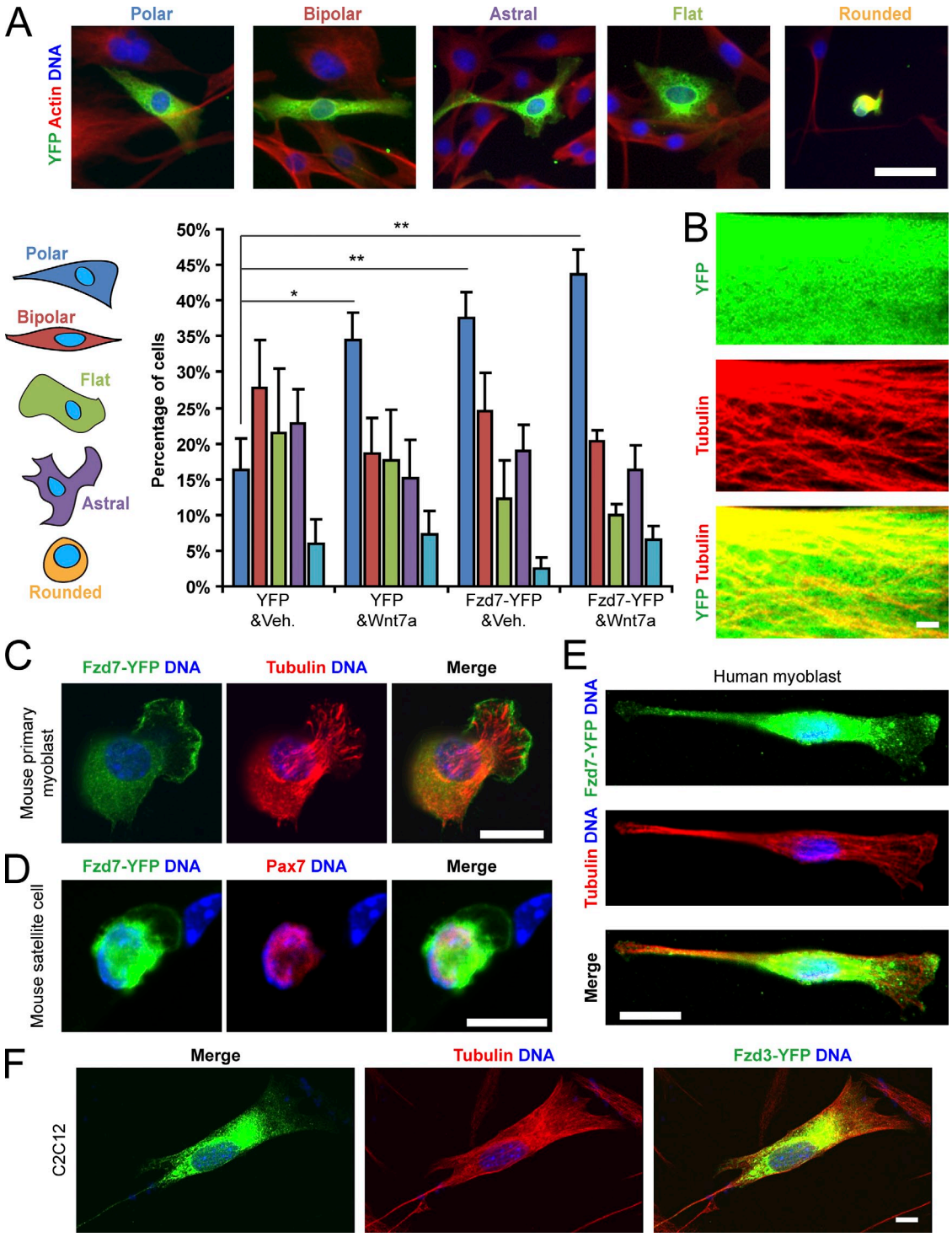


Figure S1. **Wnt7a and Fzd7 polarize myogenic cells.** (A) Morphological quantification of C2C12 cells transfected with the indicated constructs and treated with either Wnt7a or vehicle. Representative pictures of morphologies are depicted with YFP-transfected cells above. Bars represent means ± SEM; $n \geq 4$. **, $P < 0.01$; *, $P < 0.05$. (B) Subcellular localization of YFP with respect to the tubulin cytoskeleton. Bar, 2 µm. (C) Localization of Fzd7-YFP in primary myoblasts. Note the accumulation of Fzd7 in the cellular periphery. Bar, 10 µm. (D) Localization of Fzd7-YFP in satellite cells that were transfected on single muscle fibers. Accumulation of Fzd7 in the cellular periphery can be observed. Bar, 10 µm. (E) A human primary myoblast that was transfected with Fzd7-YFP. Similar to other myogenic cell types, Fzd7 shows accumulation in the periphery of the cell. Bar, 10 µm. (F) Little to no peripheral localization could be observed for Fzd3-YFP in C2C12 cells. Bar, 10 µm.

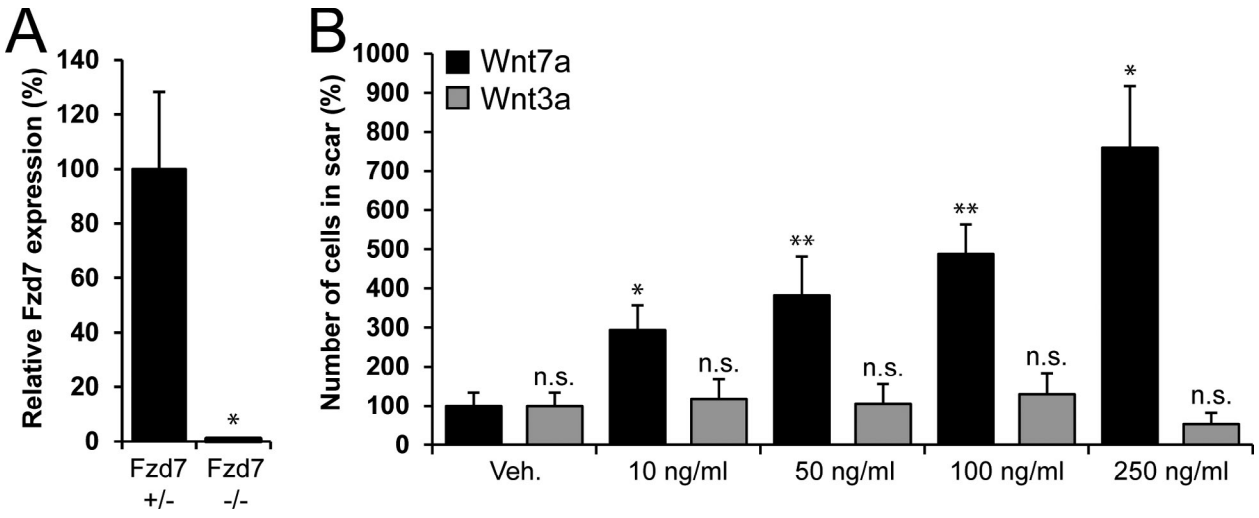


Figure S2. **Fzd7 mRNA levels in Fzd7 knockout muscle tissue and dose dependency of Wnt7a-mediated cell migration.** (A) qPCR comparing Fzd7 expression in muscles of $Fzd7^{+/-}$ and $Fzd7^{-/-}$ mice. Bars represent means ± SEM; *n* = 3. *, P < 0.05. (B) Scratch assay using mouse primary myoblasts that were exposed to different concentrations of Wnt3a or Wnt7a. Bars represent means ± SEM; *n* = 3. *, P < 0.05; **, P < 0.01. n.s., no significant difference.

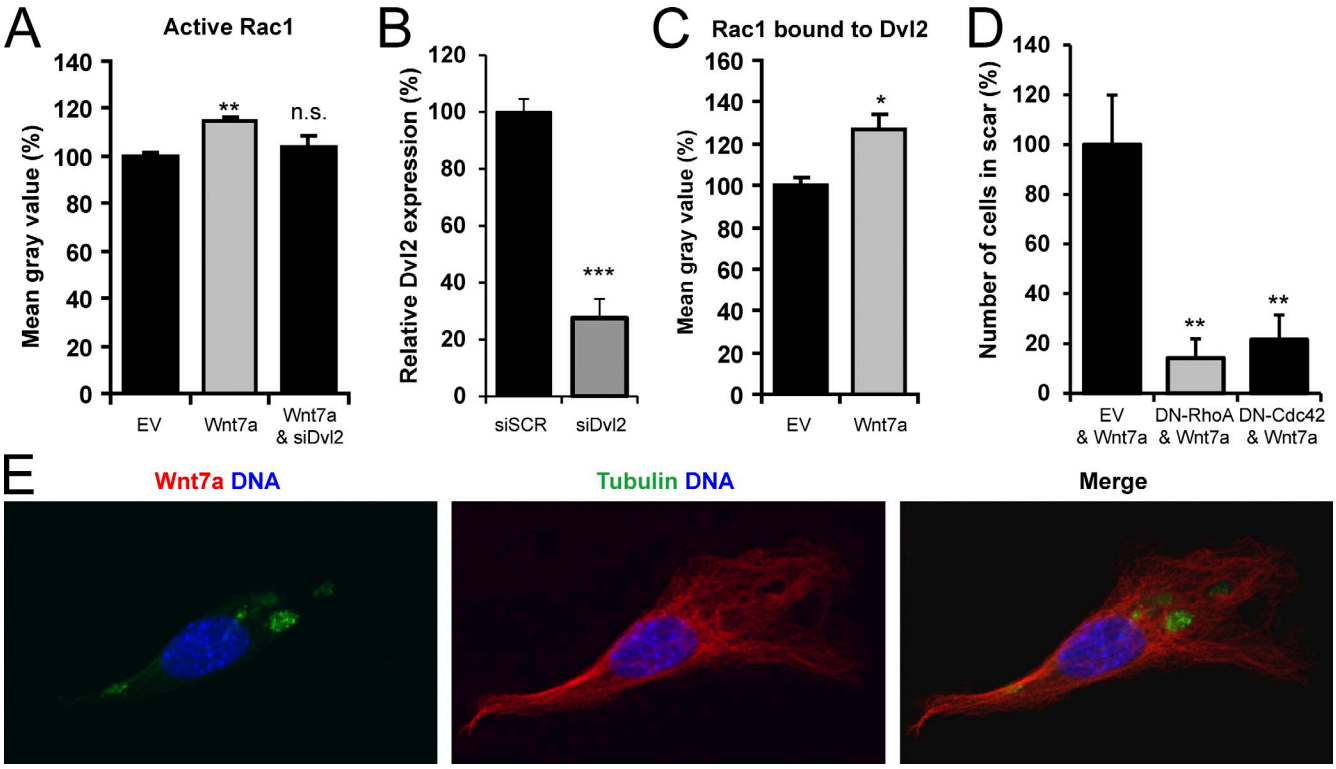


Figure S3. **Gray value quantification of Western blots, Dvl2 knockdown, and Wnt7a endocytosis in C2C12 cells.** (A) Gray value quantification of Western blots for active Rac1 as shown in Fig. 3 B. Bars represent means ± SEM; *n* = 3. **, P < 0.01. n.s., no significant difference. (B) qPCR comparing Dvl2 expression upon siDvl2 or siSCR treatment in primary myoblasts. Bars represent means ± SEM; *n* = 3. ***, P < 0.001. (C) Gray value quantification from Western blots of Rac1 bound to Dvl2 as shown in Fig. 3 C. Bars represent means ± SEM; *n* = 5. *, P < 0.05. (D) Scratch assay using Wnt7a-stimulated mouse primary myoblasts expressing either EV, RhoA-DN, or Cdc42-DN. Bars represent means ± SEM; *n* = 3. **, P < 0.01. (E) A C2C12 cell that was loaded for 3 h with Wnt7a-HA from conditioned medium produced in COS-1 cells, washed extensively, and then cultured for >72 h. Wnt7a was detected by staining for the HA epitope. Bar, 10 µm.

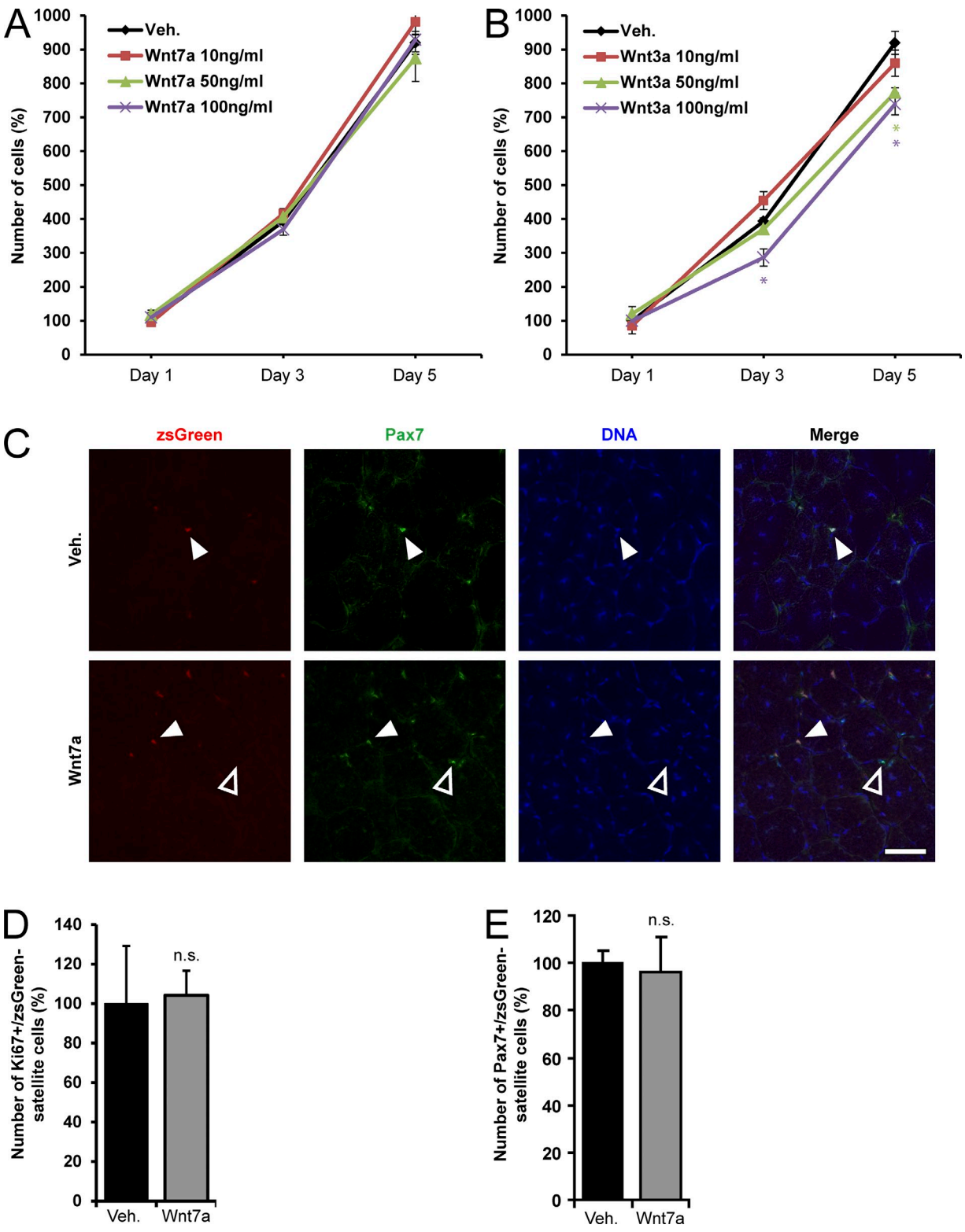


Figure S4. **Wnt7a loading does not influence the cell cycle, increases engraftment, and does not alter the number of endogenous satellite cells.** (A and B) The proliferation of equal numbers of primary myoblasts in the presence of different concentrations of Wnt7a and Wnt3a was assayed over five days. Data points represent means ± SEM; $n = 3$. *, $P < 0.05$. (C) Immunostaining for engrafted zsGreen-positive satellite cells (full arrowheads) upon Wnt7a and vehicle treatment. Host-derived satellite cells are negative for zsGreen (empty arrowhead). Bar, 50 µm. (D) $Pax7^+/zsGreen^+$ engrafted satellite cells that were treated with Wnt7a do not show more Ki67 staining than vehicle-treated cells. Bars represent means ± SEM; $n = 3$. (E) The number of endogenous $Pax7^+/zsGreen^-$ satellite cells is not significantly changed by transplantation of Wnt7a or vehicle-treated satellite cells. Bars represent means ± SEM; $n = 3$. n.s., no significant difference.

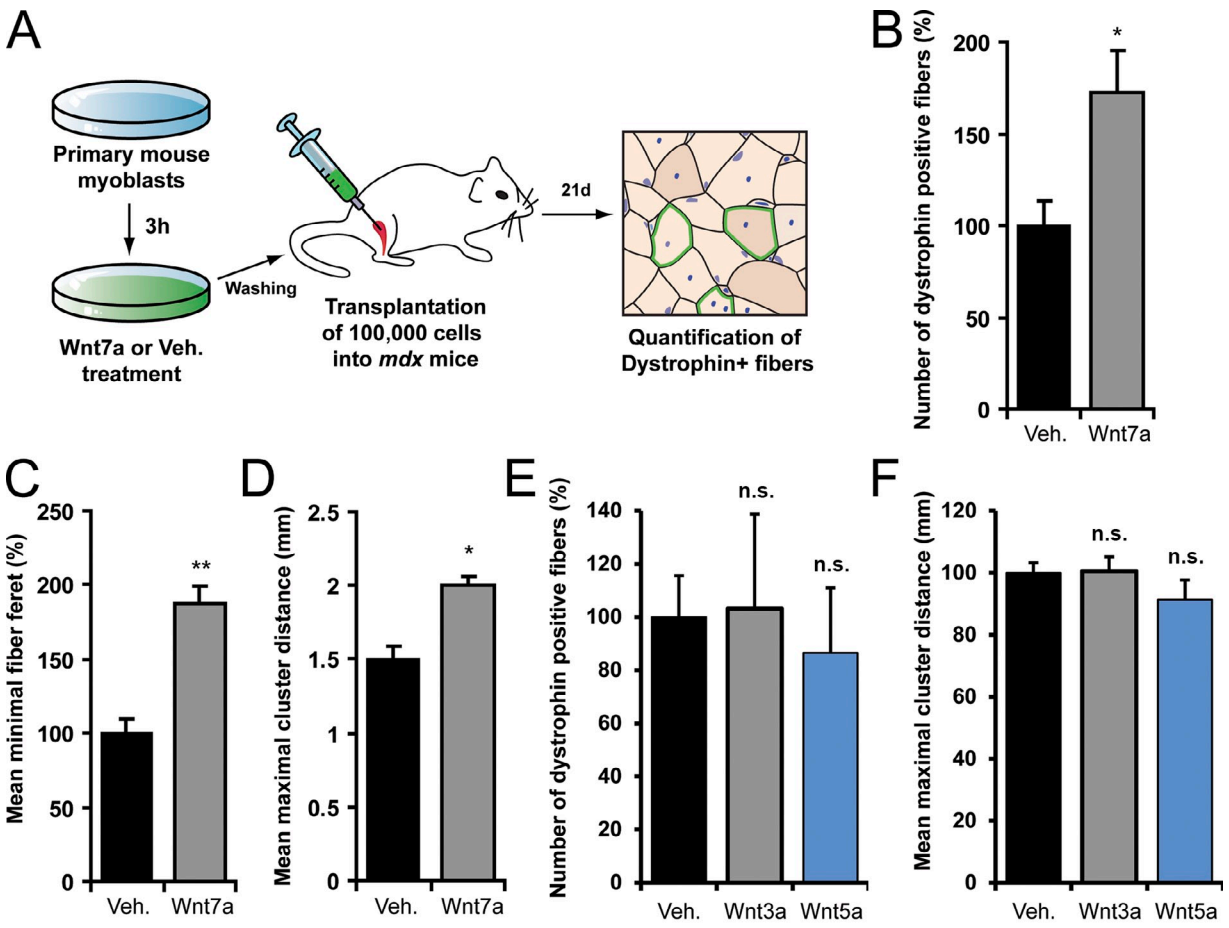
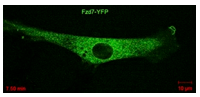
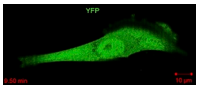


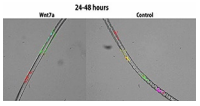
Figure S5. **Wnt7a loading improves the engraftment of mouse primary myoblasts.** (A) Strategy used for Wnt7a or vehicle treatment and subsequent transplantation of mouse primary myoblasts into *mdx* mice. (B) Number of dystrophin-positive fibers upon transplantation of Wnt7a or vehicle-treated mouse primary myoblasts. Bars represent means ± SEM; $n = 3$. *, $P < 0.05$. (C) Minimal fiber feret of dystrophin-positive fibers generated from fusion with Wnt7a or vehicle-treated mouse primary myoblasts. Bars represent means ± SEM; $n = 3$. **, $P < 0.01$. (D) Mean maximum cluster distance in muscles transplanted with Wnt7a or vehicle-treated mouse primary myoblasts. Bars represent means ± SEM; $n = 3$. *, $P < 0.05$. (E and F) Three weeks after transplantation of Wnt3a-and Wnt5a-treated zsGreen$^+$ myoblasts, no difference in the number of dystrophin-positive fibers or in the mean maximal cluster distance is observed when compared with vehicle. Bars represent means ± SEM; $n = 3$. n.s., no significant difference.



Video 1. **Localization of Fzd7-YFP in migrating C2C12 cells.** C2C12 cells were transfected with Fzd7-YFP. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM710; Carl Zeiss). Frames were taken every 15 s for 29.5 min.



Video 2. **Localization of YFP in migrating C2C12 cells.** C2C12 cells were transfected with YFP. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM710; Carl Zeiss). Frames were taken every 15 s for14.75 min.



Video 3. **Effect of Wnt7a on satellite cell migration.** Satellite cell time-lapse microscopy was performed using an inverted microscope (DMI 5100; Leica) and MetaMorph 7.6.1 software (Molecular Devices) with time points being acquired every 7 min in a stage-top incubator (LiveCell Imaging) for 24 h.