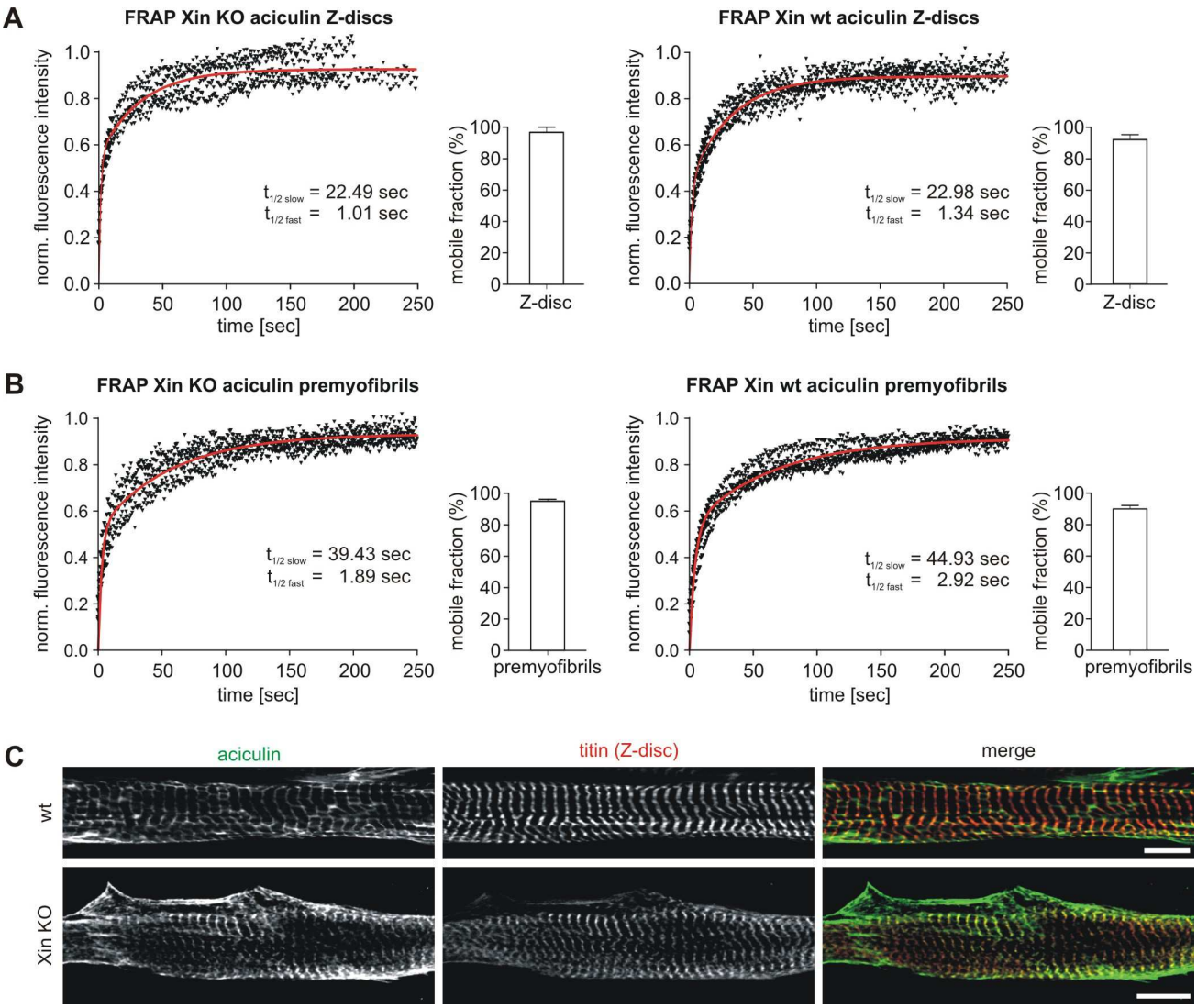
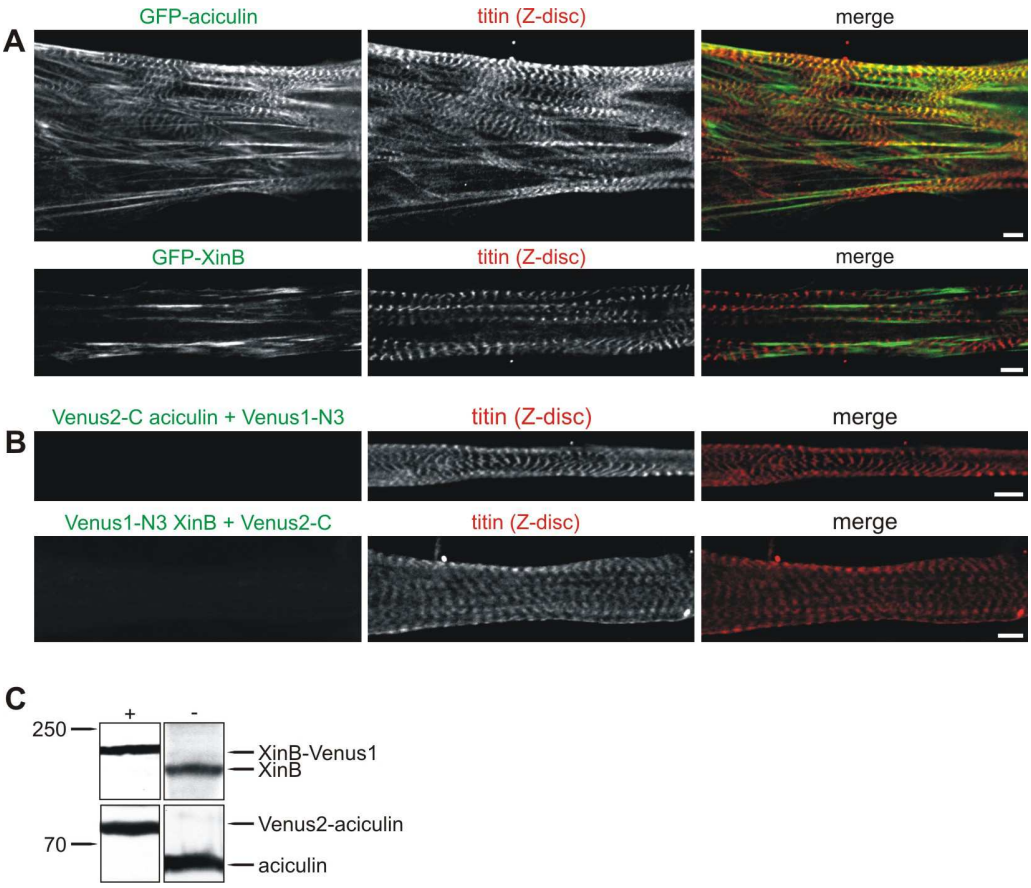
Fig. S1: Aciculin dynamics and localization are independent of Xin. (A, B) FRAP studies reveal equally high mobility and dynamics of aciculin in primary mouse skeletal muscle cells from wildtype (wt) and $Xin^{-/-}$ (Xin KO) mice. Primary cells were transiently transfected with a GFP-aciculin construct and differentiated for seven days. The biphasic curve fit (red line) indicates identical recovery times and mobile fractions of aciculin in Z-discs (A) and premyofibrils (B) in wt and $Xin^{-/-}$ cells. n = 4. (C) Aciculin localization in $Xin^{-/-}$ cells is not altered. Primary wild-type or Xin deficient mouse skeletal muscle cells differentiated for seven days were stained for aciculin and a Z-disc epitope of titin. In both cell types aciculin is localized in lesions bridging the Z-discs and at the sarcolemma pointing to a higher position of aciculin in hierarchy. Scale bars: 10 µm.

Fig. S2: Aciculin localizes to areas of myofibrillar damage and reorganization in C2C12 cells. (A) C2C12 cells transiently expressing GFP-aciculin or GFP-XinB, were differentiated for seven days and EPS treated for 5 hours. Both proteins localize to longitudinal areas bridging two or more Z-dics. Note that aciculin, but not XinB, also localizes to mature Z-discs. (B) For BiFC control experiments XinB and aciculin were co-expressed with the complementary non-fused Venus fragment. The absence of a fluorescent signal substantiates the specificity of the BiFC experiment. (C) To analyze the efficiency of transfectionand size of the fusion proteins, C2C12 cells were co-transfected with XinB-Venus1N and Venus2C-aciculin encoding FLAG-tag-XinB-Venus1-154 and Venus155-238-HA-tag-aciculin fusion protein, respectively, and differentiated for seven days. Transfected cells (+) were lysed and proteins were stained for their specific tags. As a control, lysates from non-transfected C2C12 (-) were stained against the specific proteins. Scale bars: 10 µm.

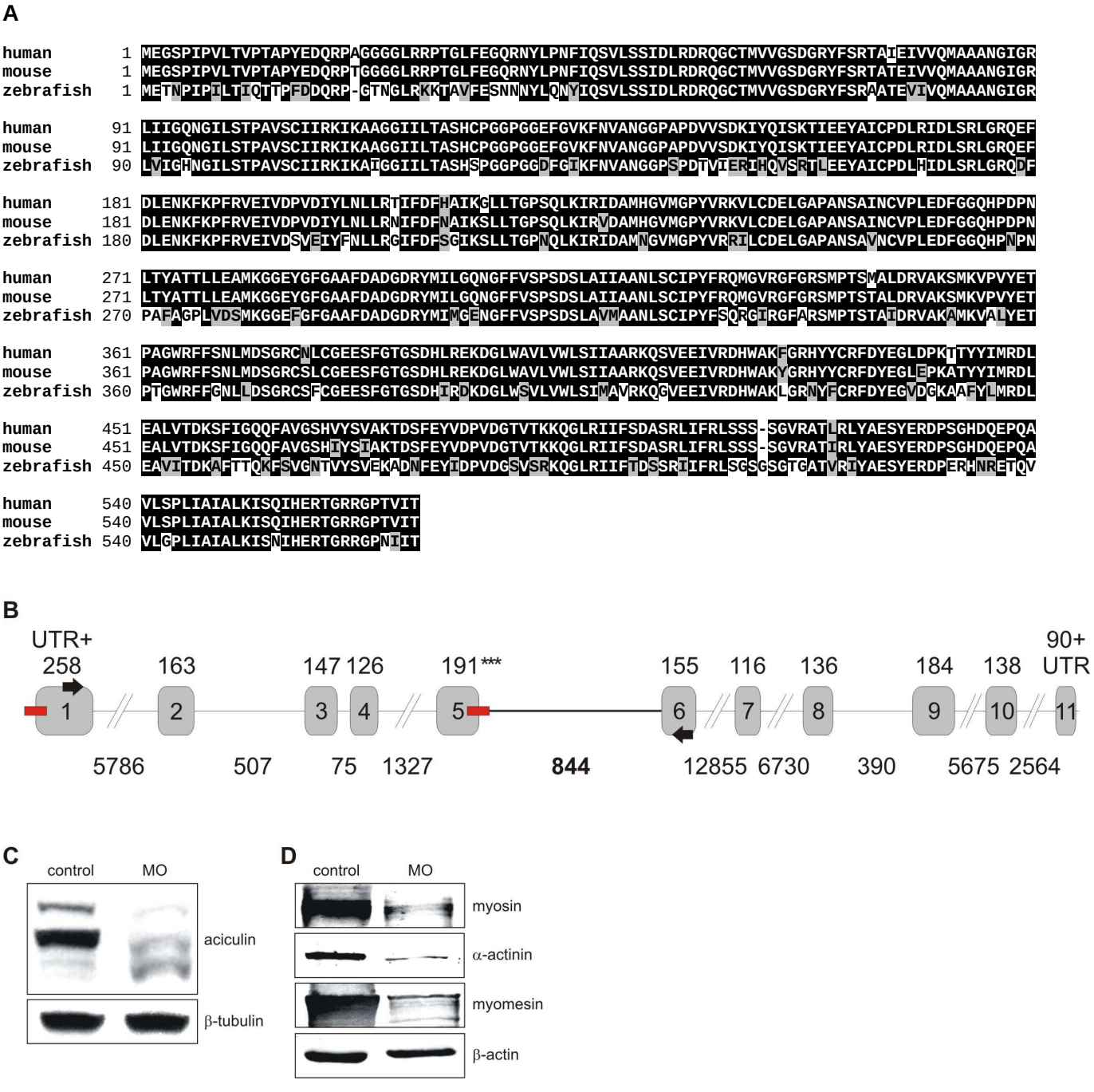
Fig. S3: (A) Amino acid sequence alignment of zebrafish, human and mouse aciculin demonstrating a very high cross-species homology. Black and gray boxes indicate amino acid identity and amino acids with similar chemical properties, respectively. (B) Structure of the zebrafish *pgm5* gene encoding aciculin that is located on chromosome 8. Grey boxes numbered 1 to 11 represent the 11 exons that are separated by introns. Numbers on top of the figure indicate the size of the exons, while the numbers at the bottom show the size of the introns. Red boxes indicate the position of both MOs used in this study: the start site targeting morpholino in exon 1 and the splice site targeting morpholino at the splice donor site of exon 5. The latter MO results in altered splicing and inclusion of intron 5 in the spliced mRNA. Amplification of cDNA prepared from morphant embryos with primers indicated by black arrows and sequencing of the amplicons showed that only mRNA including the 844 base pairs of intron 5 (bold) is synthesized (see Fig. 7). Inclusion of this intron that contains three in frame stop codons (*) in the first 100 basepairs is predicted to result in nonsense mediated decay. (C) Confirmation of aciculin knockdown in zebrafish embryos (72 hpf). (D) Lysates from 72 hpf control and morpholino treated zebrafish embryos were stained against myomesin, α-actinin and β-actin as loading control. The stainings show strong downregulation of myomesin and α-actinin in aciculin knockdown embryos.



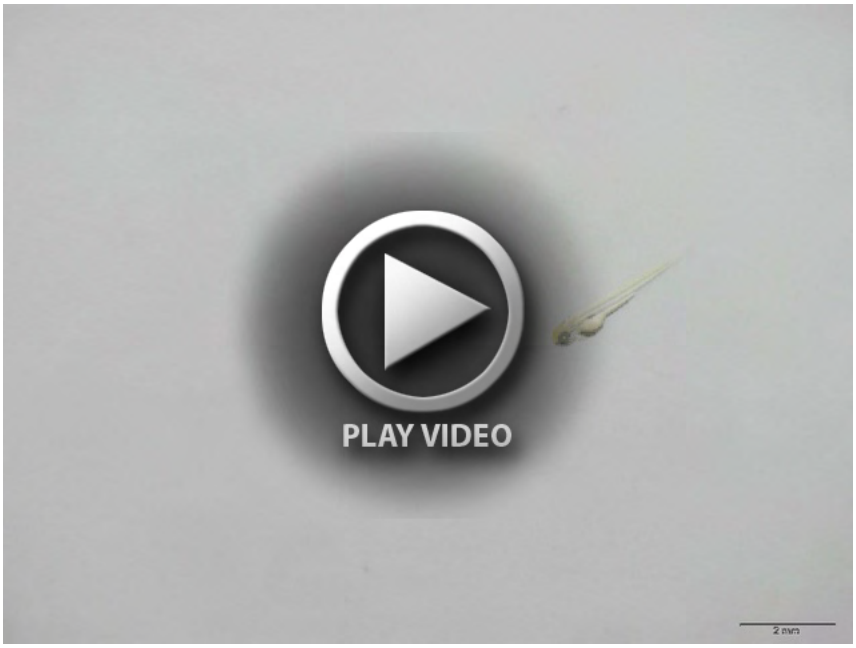
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Movie 1: When touch-stimulated, control MO-injected zebrafish embryos show normal flight response.

Supplementary Movie 2: Aciculin morphant embryos are paralyzed. When touch-stimulated, they fail to show a flight response.

Table S1. Recovery times of aciculin in C2C12 cells and primary mouse skeletal muscle cells are dependent on contraction.

	slow / fast recovery times (s)			
	C2C12 cells		primary SkMC	
	- EPS non-contracting	+ EPS contracting	+ BDM non-contracting	- BDM contracting
premyofibrils	61.73 / 2.0	41.16 / 2.3	56.95 / 4.2	44.93 / 2.9
Z-discs	55.61 / 2.8	23.49 / 1.9	49.0 / 3.2	22.98 / 1.3