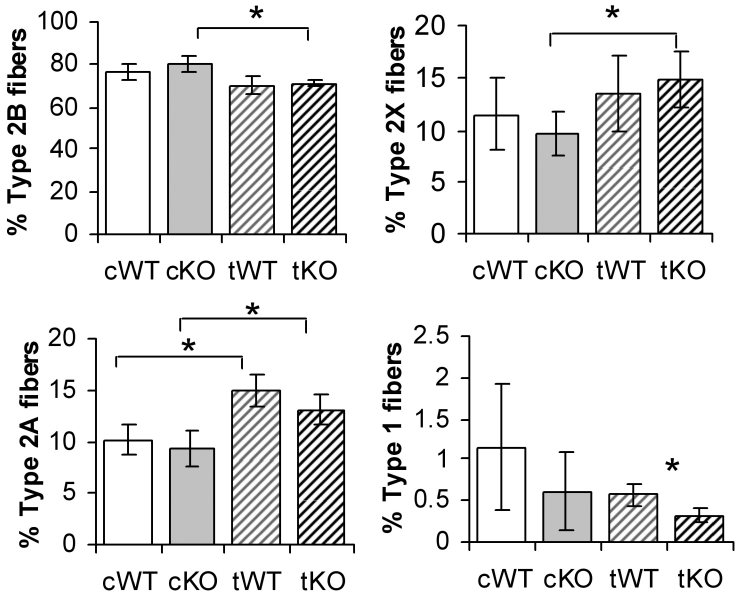
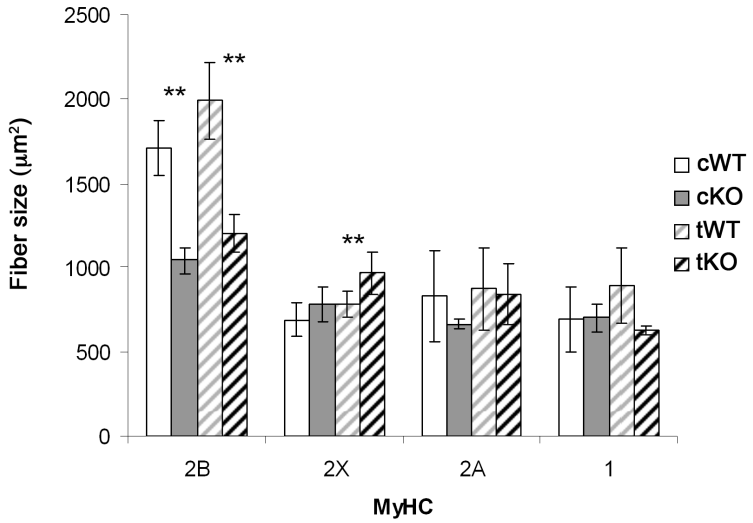
Supplementary Figures



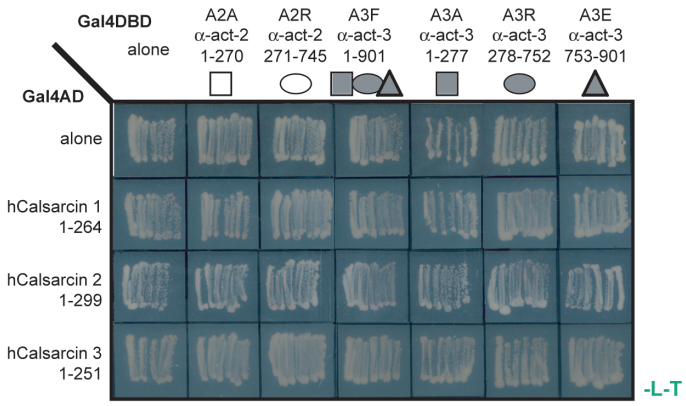
Supplementary Figure 1. Training induces greater shifts in fiber type proportions in KO muscles.

Endurance training decreased the proportion of 2B and type 1 fibers and increased the proportion of 2X and 2A fibers in both WT and KO muscles, but the shift from 2B to 2X fibers was significantly greater in the KO (2B: -9.1%; P=0.004, 2X: +5.2%; P=0.015). Except for type 1 fibers, fiber type proportions between WT and KO are not significantly different in either untrained or trained states. (Mean±SEM; *P<0.05, Mann-Whitney U test; n=6-7 muscles, mean calculated from >5000 fibers/muscle for all groups)



Supplementary Figure 2. Training induces muscle fiber hypertrophy in both WT and KO muscles.

Endurance trained KO muscles demonstrated greater hypertrophy of 2X (2X: 24.4%; 2A: 26.5%) compared to WT (2X: 13.5%, 2A: 5.5%). (Mean±SEM; $^{**}P<0.01$, Mann-Whitney U test; n=6-7 muscles, mean calculated from >5000 fibers/muscle for all groups)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A2R α-act-2 271-745
A3F α-act-3 1-901
A3A α-act-3 1-277
A3R α-act-3 278-752
A3E α-act-3 753-901
Gal4AD
alone
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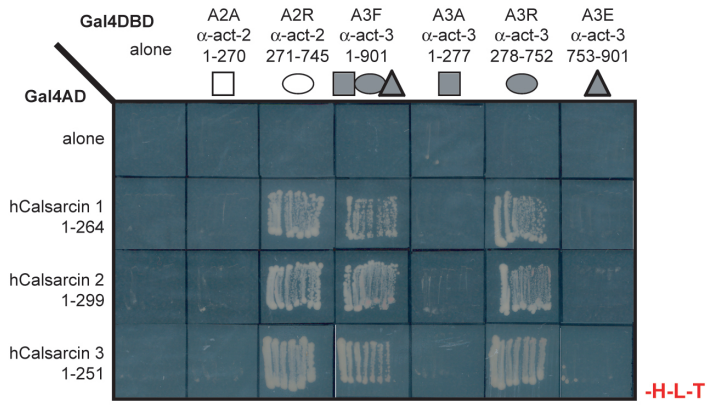
hCalsarcin 2 1-299
hCalsarcin 3 1-251
-L-T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A3R α-act-3 278-752
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Gal4AD
alone
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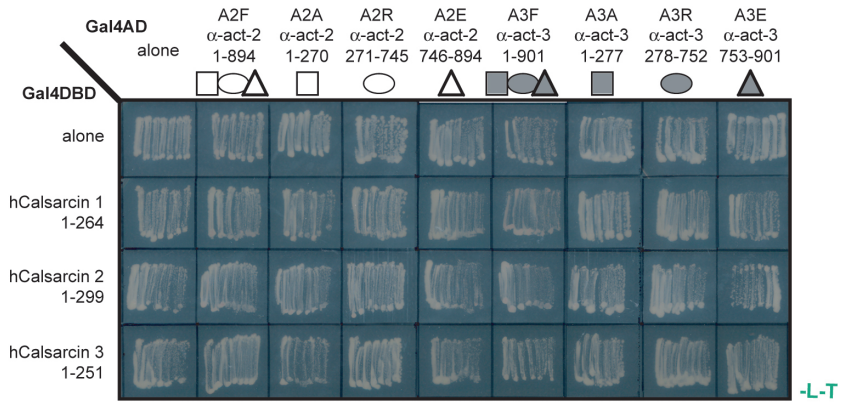
hCalsarcin 2 1-299
hCalsarcin 3 1-251
-H-L-T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A3R α-act-3 278-752
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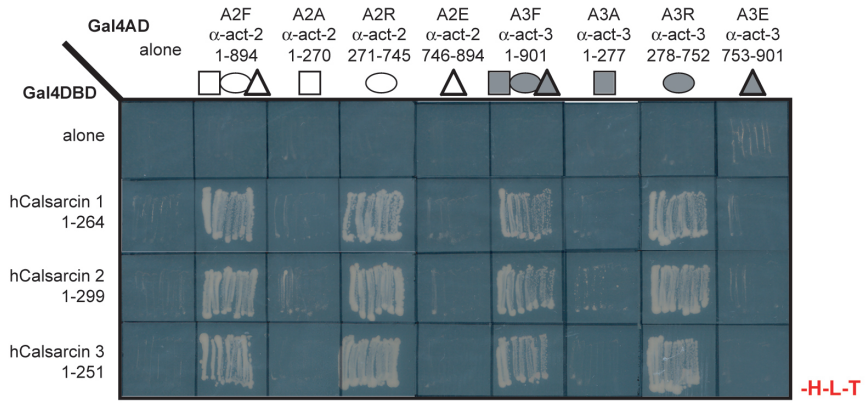
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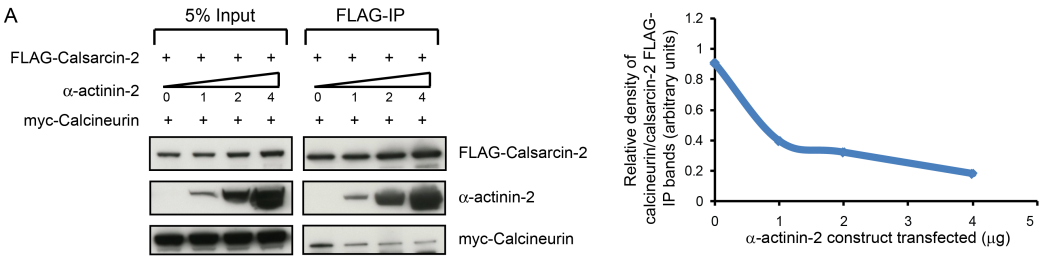
A3E α-act-3 753-901
Gal4DBD
alone
hCalsarcin 1 1-264
hCalsarcin 2 1-299
hCalsarcin 3 1-251
-H-L-T

Supplementary Figure 3. Calsarcin 1, 2 and 3 interact with α-actinin-2 and -3.

Yeast two-hybrid assays were performed to compare the interactions of human α-actinin-2 and human α-actinin-3 with human Calsarcin 1 (full length, amino acids 1-264), Calsarcin 2 (full length, amino acids 1-299) and Calsarcin 3 (full length, amino acids 1-251) . These assays were performed with each of the test proteins fused to the C-terminus of either Gal4AD or Gal4DBD. Growth on plates lacking leucine and tryptophan (-L-T) indicates that the yeast have taken up both the Gal4AD and the Gal4DBD fusion protein plasmids. Growth on plates lacking leucine, tryptophan and histidine (-H-L-T) indicates that the protein fused to Gal4AD interacts with the protein fused to Gal4DBD. The amount of growth on the –H-L-T plates gives an indication of the relative strength of the interaction. A2F = full length α-actinin-2 (amino acids 1-894), A3F = full length α-actinin-3 (amino acids 1-901), A2A = α-actinin-2 actin binding domain (amino acids 1-270), A3A = α-actinin-3 actin binding domain (amino acids 1-277), A2R = α-actinin-2 rod domain (amino acids 271-745), A3R = α-actinin-3 rod domain (amino acids 278-752), A2E = α-actinin-2 EF-hand (amino acids 746-894), A3E = α-actinin-3 EF-hand (amino acids 753-901). Both full length human α-actinin-2 and human α-actinin-3 interact with all three of the calsarcins and the rod domain of the α-actinins is responsible for this interaction.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α-actinin-2
myc-Calcineurin
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α-actinin-2
myc-Calcineurin
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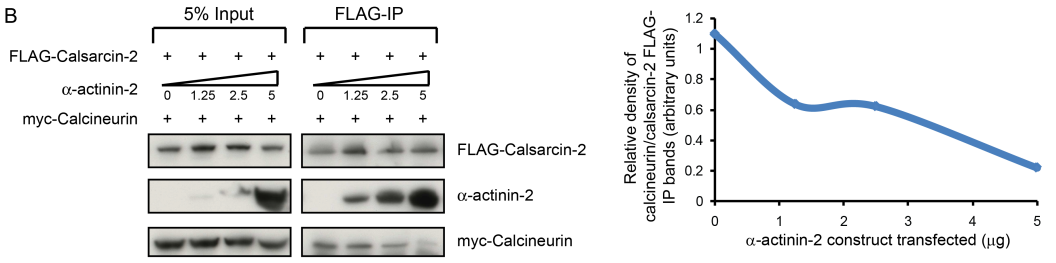
α-actinin-2 construct transfected (μg)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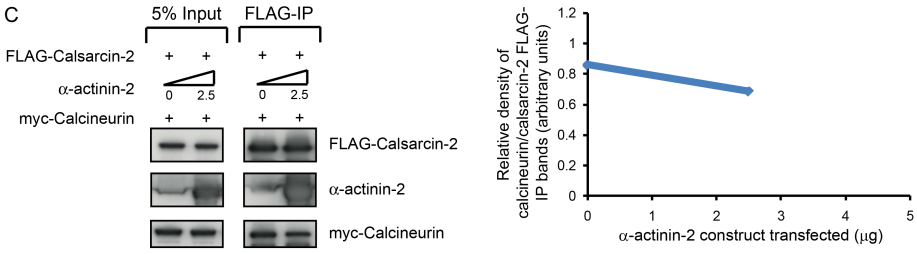
α-actinin-2 construct transfected (μg)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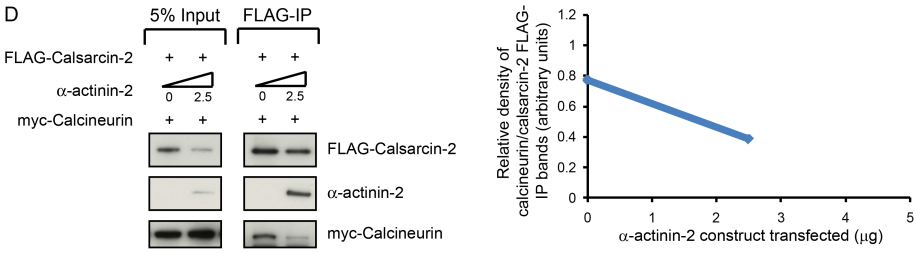
Relative density of calcineurin/calsarcin-2 FLAG-IP bands (arbitrary units)
α-actinin-2 construct transfected (μg)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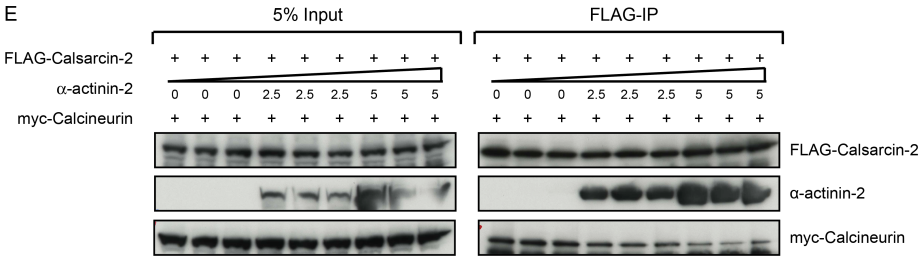
α-actinin-2 construct transfected (μg)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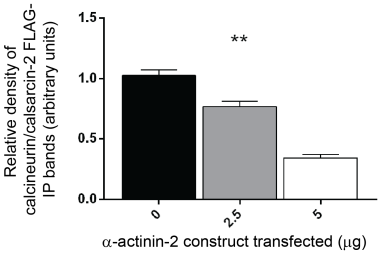
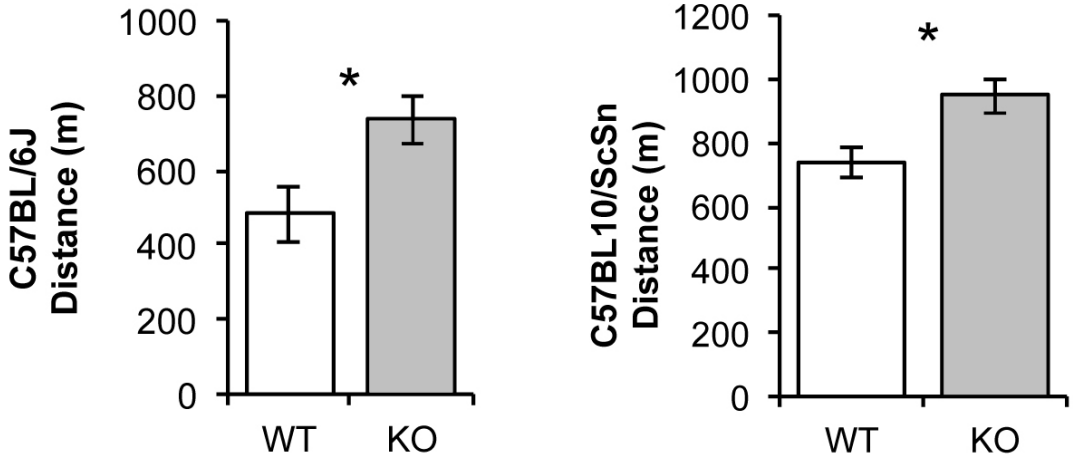

**
Relative density of calcineurin/calsarcin-2 FLAG-IP bands (arbitrary units)
α-actinin-2 construct transfected (μg)

Supplementary Figure 4. (A-D)Four independent co-immunoprecipitations of calcineurin and α-actinin-2 with calsarcin-2. Equal amounts of calsarcin-2 and calcineurin expression construct was transfected in each experiment with the amount of transfected plasmid for actinin-2 presented in μg. Densitometry was performed for each calsarcin-2 and calcineurin bands in the FLAG-IP blots. The ratio of calcineurin/calsarcin-2 band density for each amount of α-actinin-2 transfected was graphed and presented to the right of their corresponding blots. (E) Co-immunoprecipitation of calcineurin and α-actinin-2 with calsarcin-2 with each dosage of α-actinin-2 (0, 2.5 and 5 μg DNA) transfected in triplicates. Densitometry was performed for each calsarcin-2 and calcineurin bands in the FLAG-IP blots and graphed as the ratio of calcineurin/calsarcin-2 band density for each amount of α-actinin-2 transfected. Error bars represents mean ± SEM. Statistical analyses of the data were carried out using the 1-way ANOVA Kruskal-Wallis test. ***P*=0.0036.



Supplementary Figure 5. *Actn3* KO mice on C57BL/6J and C57BL/10ScSn backgrounds also demonstrate increased endurance performance (greater fatigue resistance) compared to WT.

(Mean±SEM; **P*<0.05, Mann-Whitney U test; n= 12 for all groups (C57BL/6J); n=17 for all groups (C57BL10/ScSn)).