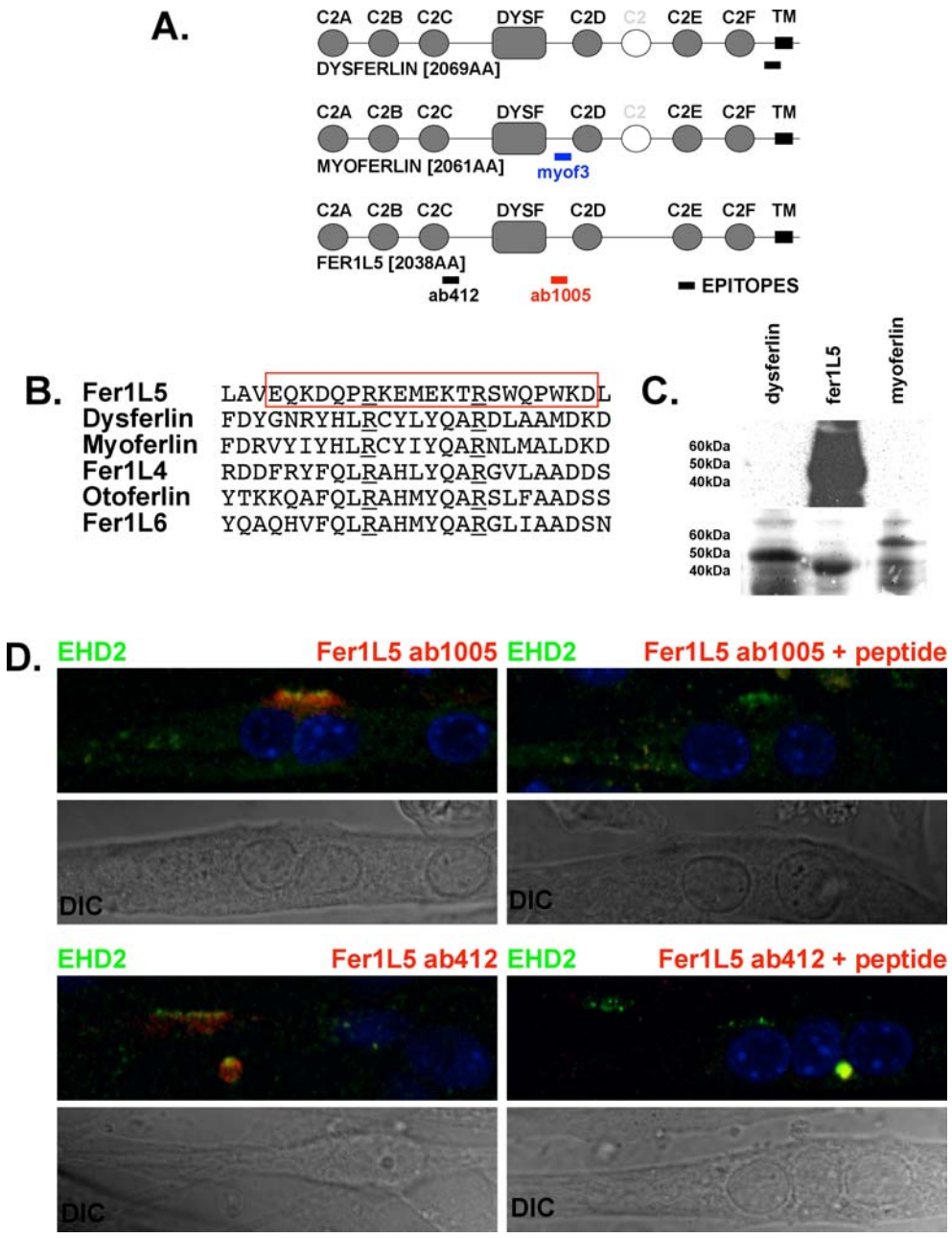
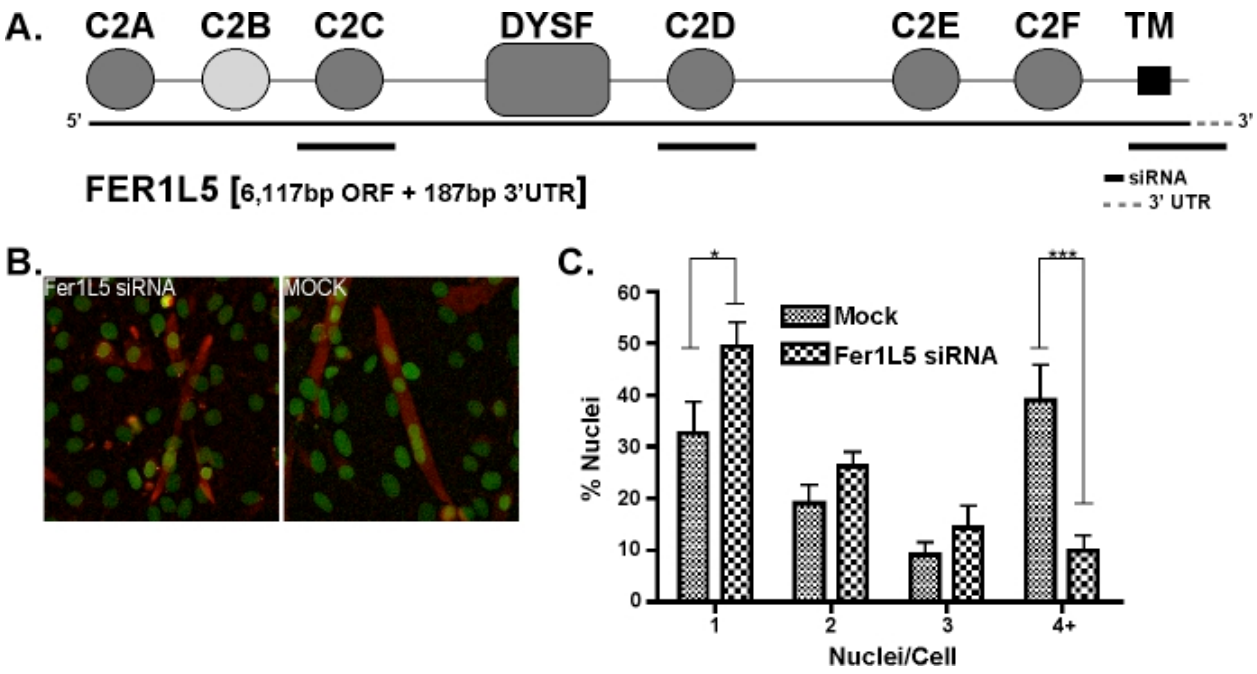


Supplemental Fig. 1. The anti-Fer1L5 antibody is specific for Fer1L5. *A.* Protein schematics of dysferlin, myoferlin, and Fer1L5 showing the antibody epitopes for anti-dysferlin, anti-myoferlin (myof3), and anti-Fer1L5 (ab1005 and ab412) antibodies used in this study. *B.* Alignment of the epitope used to generate the anti-Fer1L5 (ab1005) antibody. This region was selected because it is unique to Fer1L5. *C.* GST-fusion proteins of the DysF domain and the adjacent region that contains the epitope used to generate anti-Fer1L5 (ab1005) antibody. The corresponding regions from dysferlin and myoferlin were also expressed in BL21 cells and cell lysates were separated on a 10% polyacrylamide gel and transferred to a PVDF membrane (upper panel). The membrane was immunoblotted with the anti-Fer1L5 (ab1005) antibody and immunoreactivity was only seen in the Fer1L5 lane. The lower panel is the GelCode staining to demonstrate loading. *D.* Immunostaining of myotubes with 2μg of Fer1L5 (ab1005) or Fer1L5 (ab412) antibodies alone or with 40μg of respective peptides demonstrates the antibodies specifically target Fer1L5 structures that colocalize with EHD2 at the plasma membrane.



Supplemental Fig. 2. Partial reduction of Fer1L5 by siRNA and inhibition of myoblast fusion. *A.* Schematic of the Fer1L5 protein and corresponding mRNA. Along the mRNA three ~500bp regions were selected for siRNA generation. These regions were selected for their lower homology to the other ferlins. The siRNA was introduced into C2C12 cells. By densitometry, Fer1L5 was reduced by 33% after siRNA treatment compared to mock transfected, and desmin levels were similar between siRNA and mock treated (not shown). *B.* C2C12 myoblasts are shown after Fer1L5 siRNA or mock transfection. *C.* Quantification of the number of nuclei found within desmin-stained C2C12 cells after four days of differentiation. Partial reduction of Fer1L5 was sufficient to reduce the number of multinucleated myotubes (four or greater nuclei) formed in culture. (* = $p<0.05$, *** = $p<0.001$).