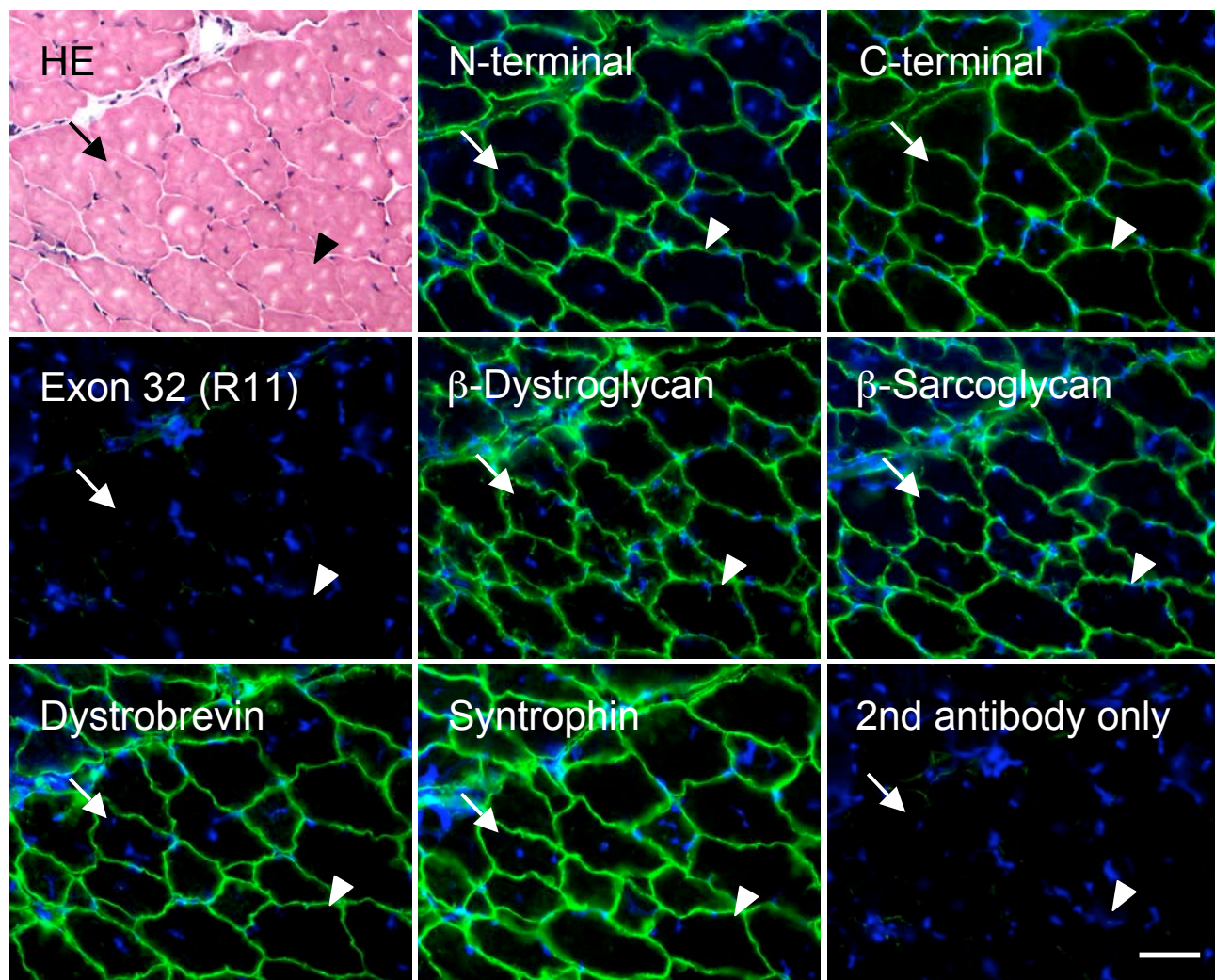
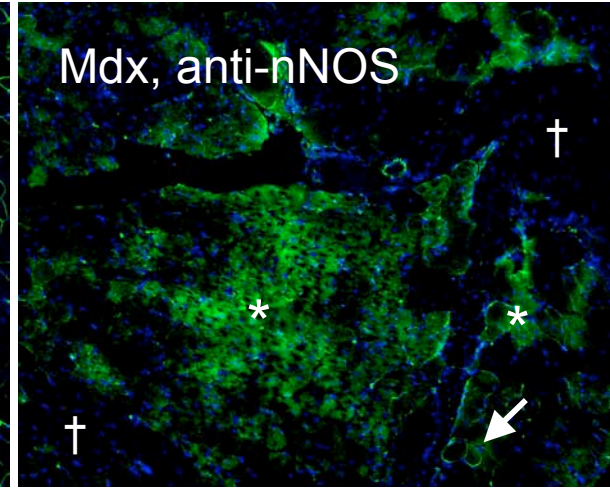
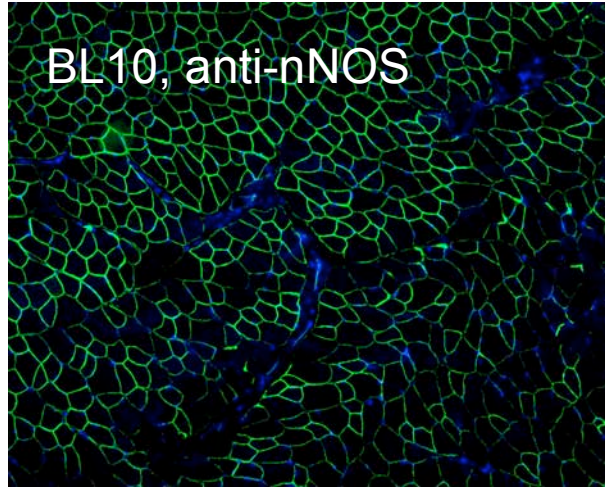


a

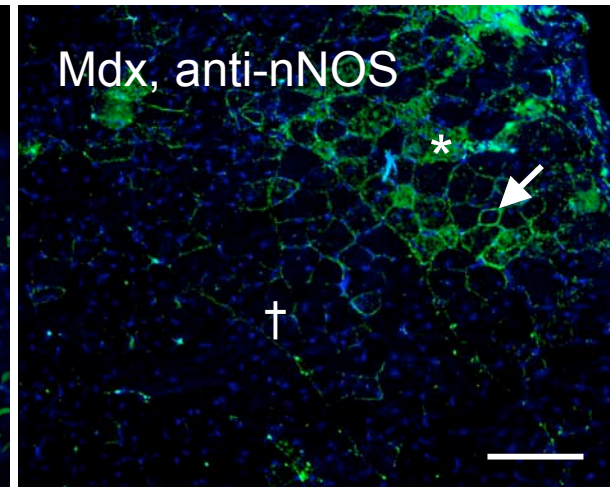
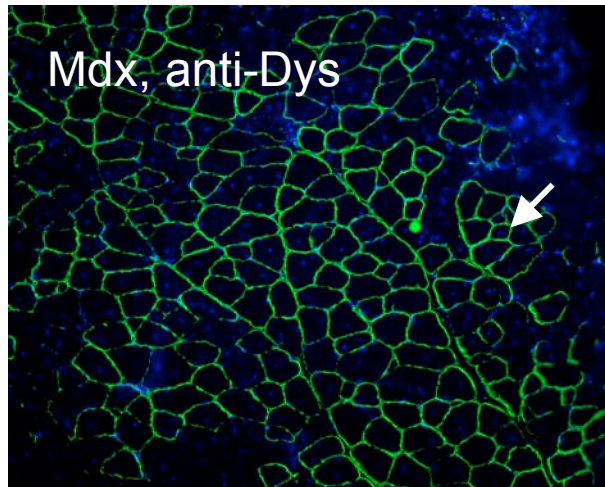


b

No AAV Infection



AV.Donor.60 +
AV.Acceptor.60



Supplementary Figure 4. Mini-dystrophin expression restores the entire DGC complex but not nNOS to the sarcolemma. **a**, Immunostaining of serial sections from the co-infected TA muscle with antibodies for the N-terminal domain, the C-terminal domain and the spectrin-like repeat 11 (exon 32, this exon is not presented in the Δ H2 mini-dystrophin gene) of dystrophin, β -dystroglycan, β -sarcoglycan, dystrobrevin and syntrophin, respectively. Secondary antibody alone is included as a negative control for non-specific staining. Nuclei are revealed by DAPI staining. HE staining shows muscle morphology. Arrow, a mini-dystrophin positive fiber with a centrally located nucleus. Arrowhead, a mini-dystrophin positive fiber with a peripherally located nucleus. Scale bar, 50 μ m. **b**, A polyclonal anti-nNOS antibody (1:2000, Santa Cruz, Santa Cruz, California) was used to evaluate nNOS expression by immunostaining. In the absence of AAV infection, C57Bl/10 (BL10) TA muscle shows normal sarcolemma expression of nNOS. *Mdx* TA muscle displays sarcoplasmic nNOS expression (asterisk) in some regions while no nNOS expression in other regions (cross). The only exception is sarcolemmal expression in a few revertant fibers (arrow). Efficient mini-dystrophin expression was achieved in the majority of myofibers in *trans*-splicing AAV infected *mdx* TA muscle (immunostaining with a polyclonal anti-dystrophin N-terminal domain antibody). However, nNOS immunostaining in adjacent section does not show sarcolemmal expression except in a few revertant myofibers (arrow). Sarcoplasmic nNOS expression is seen in some myofibers (asterisk), but nNOS is missing in the majority of myofibers (cross). Scale bar, 200 μ m.