

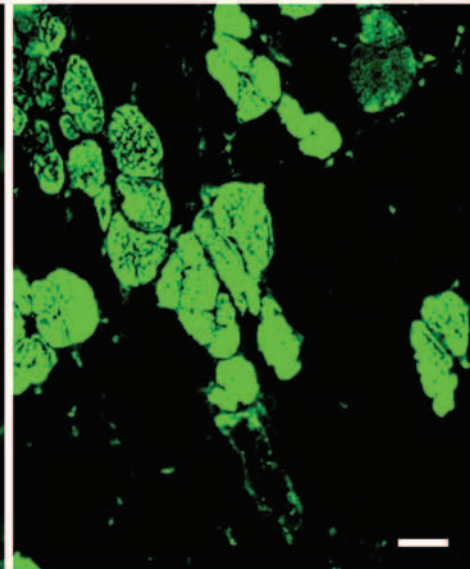
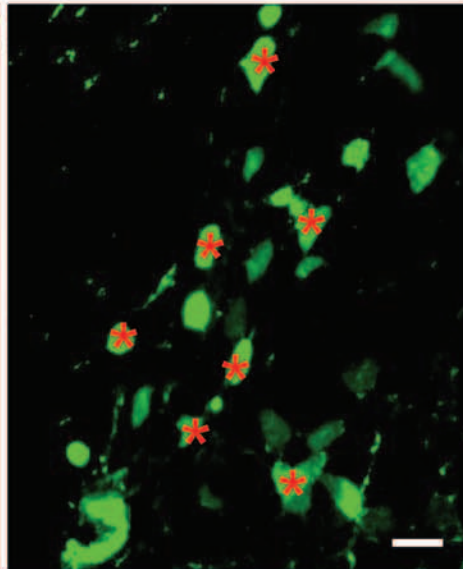
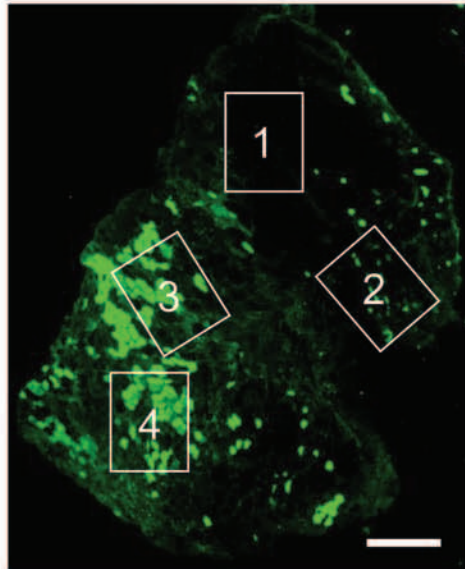
a

Full View

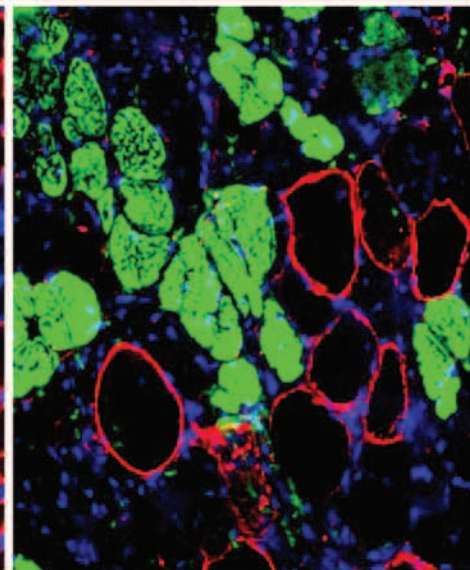
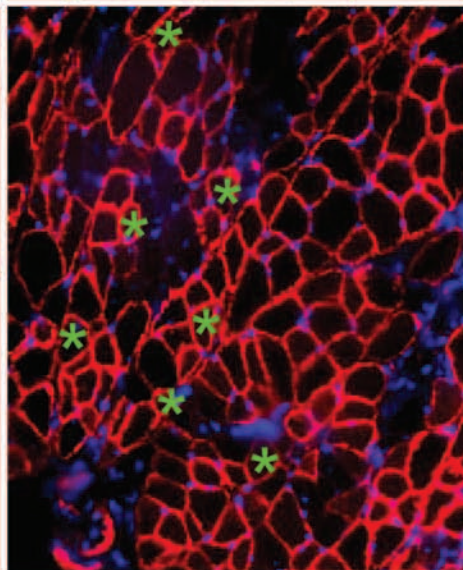
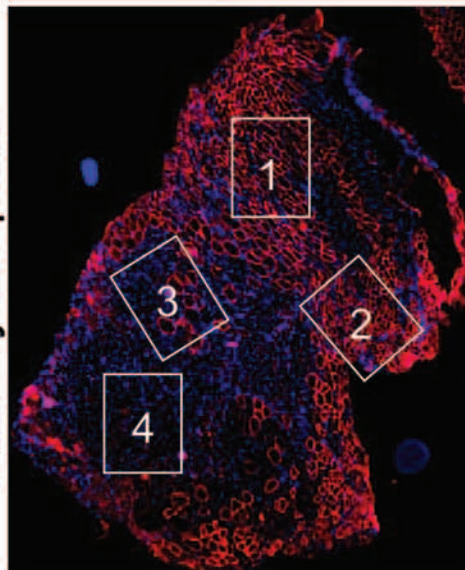
Box 2

Box 3

Anti-Slow

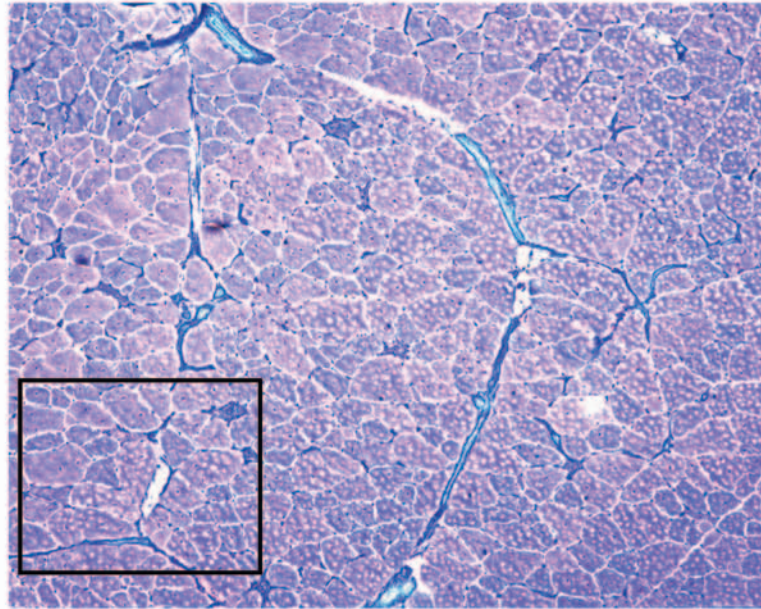


Anti-Dystrophin †

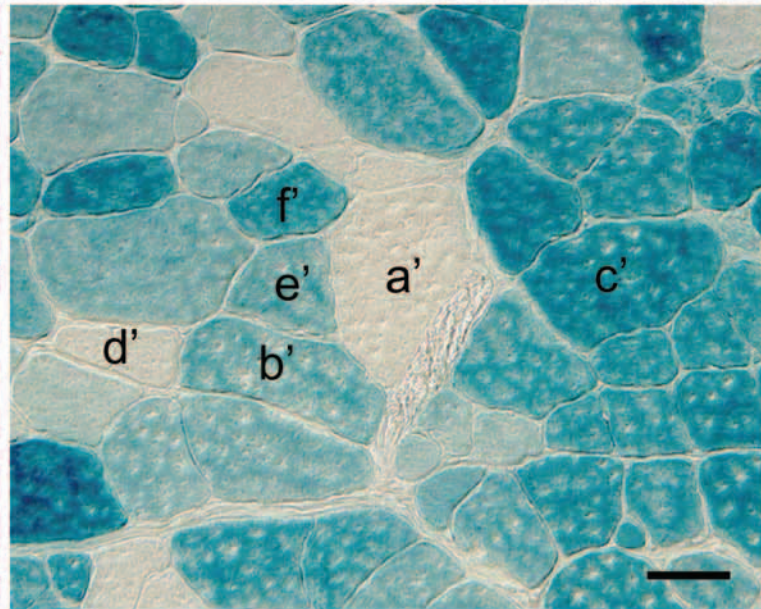
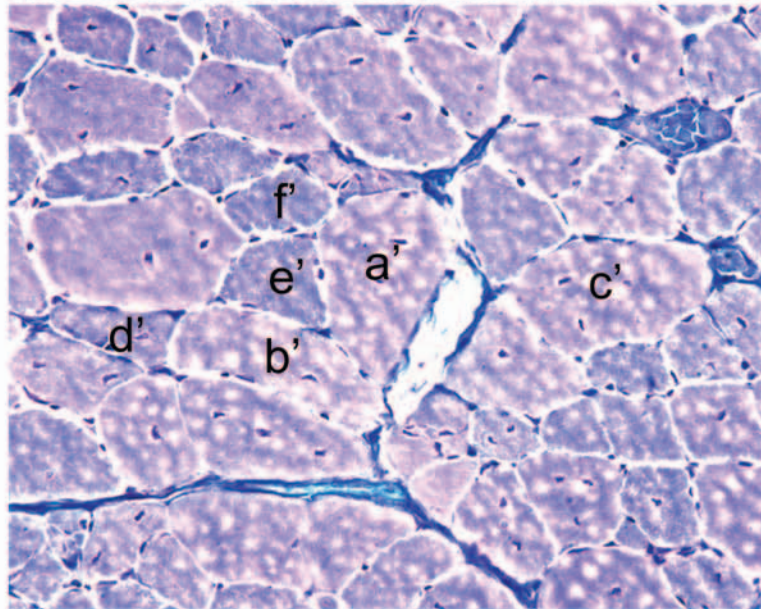
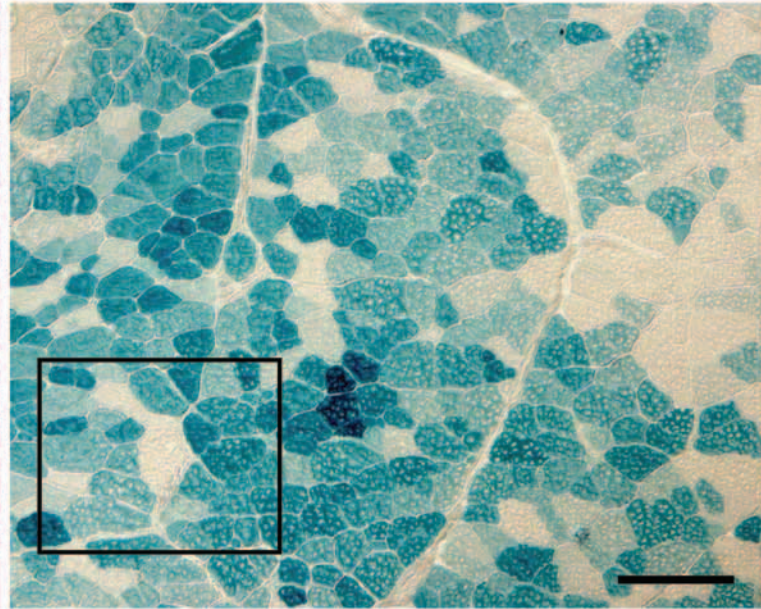


b

Toluidine Blue Stain



LacZ Stain



**Supplementary Figure 6. AAV-6 infects all muscle fiber types, but with a trend towards preferential transduction of fast fibers, in dystrophic *mdx* limb muscle.** AAV-2 has been shown to preferentially infect slow type muscle fiber (Pruchnic R et al. *Hum. Gene Ther.* 11, 521-536, 2000). While AAV-6 was shown to transduce both fast and slow twitch muscles at fairly high efficiency in normal mice (Blankinship MJ et al. *Mol. Ther.* 10, 671-678, 2004). To examine whether there is a muscle fiber type preference for AAV-6 in dystrophic muscle, we compared transgene expression and muscle fiber type staining in *mdx* mice. **a**, The hind limb muscle of 3-day-old *mdx* mice were infected with  $1 \times 10^{10}$  vg particles of AAV-6 carrying the  $\Delta R4-R23$  micro-dystrophin gene ( $n = 4$ ). Micro-dystrophin and slow type fiber (type I) were revealed in serial sections by immunofluorescence staining with antibodies specific for human dystrophin N-terminus and slow fiber myosin heavy chain (clone NOQ7.5.4D), respectively. Representative photomicrographs of gastrocnemius muscle are shown. Four regions in low power photomicrographs are boxed to represent areas with very few (Box 1), some (Boxes 2 and 3) and many (Box 4) slow fibers. Both slow and fast muscle fibers were transduced by AAV-6 (Box 2). However, there is a trend towards preferential transduction of fast fibers (Boxes 1, 3 and 4). Asterisk, representative slow type fibers that were transduced by AAV-6 (Box 2). Cross, the bottom panel of the high power photomicrograph of Box 3 represents a superimposed image from anti-slow fiber immunostaining and anti-dystrophin immunostaining. Scale bar, 400  $\mu\text{m}$  for full view photomicrographs and 50  $\mu\text{m}$  for high power photomicrographs in Boxes 2 and 3. **b**, To further determine whether there is a subtype fiber transduction preference in fast fiber, the TA muscles of 1-month-old *mdx* mice ( $n = 3$ ) were infected with  $5 \times 10^8$  vg particles of AAV-6 LacZ virus. Toluidine blue staining and LacZ staining were performed at one month later to reveal subtype muscle fiber (IIA and IIB) and transgene expression. Representative photomicrographs are shown. High power photomicrographs correspond to boxed areas in the respective low power photomicrographs. **a'**, a type IIA fiber not transduced by AAV-6; **b'**, a type IIA fiber with medium level LacZ expression; **c'**, a type IIA fiber with high level LacZ expression; **d'**, a type IIB fiber not transduced by AAV-6;

e', a type IIB fiber with medium level LacZ expression; f', a type IIB fiber with high level LacZ expression. There is not a significant difference between type IIA and IIB fibers. Scale bar, 200  $\mu\text{m}$  for low power photomicrographs and 50  $\mu\text{m}$  for high power photomicrographs.