

## Supplementary information (Verma, et al)

### Supplementary Figure 1. TA muscle of *mdx:Flt-1<sup>+/-</sup>* mice exhibits more oxidative fiber type (type I) and less muscle hypertrophy.

(A) Immunostaining against slow myosin isoform in TA muscle of *mdx:Flt-1<sup>+/+</sup>* and *mdx:Flt-1<sup>+/-</sup>* mice (green color). (B) Quantification of slow MHC to evaluate the relative frequency of type I fibers per area of TA muscle of *mdx:Flt-1<sup>+/+</sup>* and *mdx:Flt-1<sup>+/-</sup>* (n=6 each) mice. (C, D) Distributions of mean fiber diameter in TA muscle and diaphragm of *mdx:Flt-1<sup>+/-</sup>* mice were skewed toward the smaller fiber size and less hypertrophic compared to the control *mdx:Flt-1<sup>+/+</sup>* mice (n=4 each). Values are mean  $\pm$  SEM. Asterisks indicate experimental pairs where differences between the compared values were statistically significant ( $p < 0.05$ ).

### Supplementary Figure 2. No change in the immune response in *mdx:Flt-1<sup>+/-</sup>* mice.

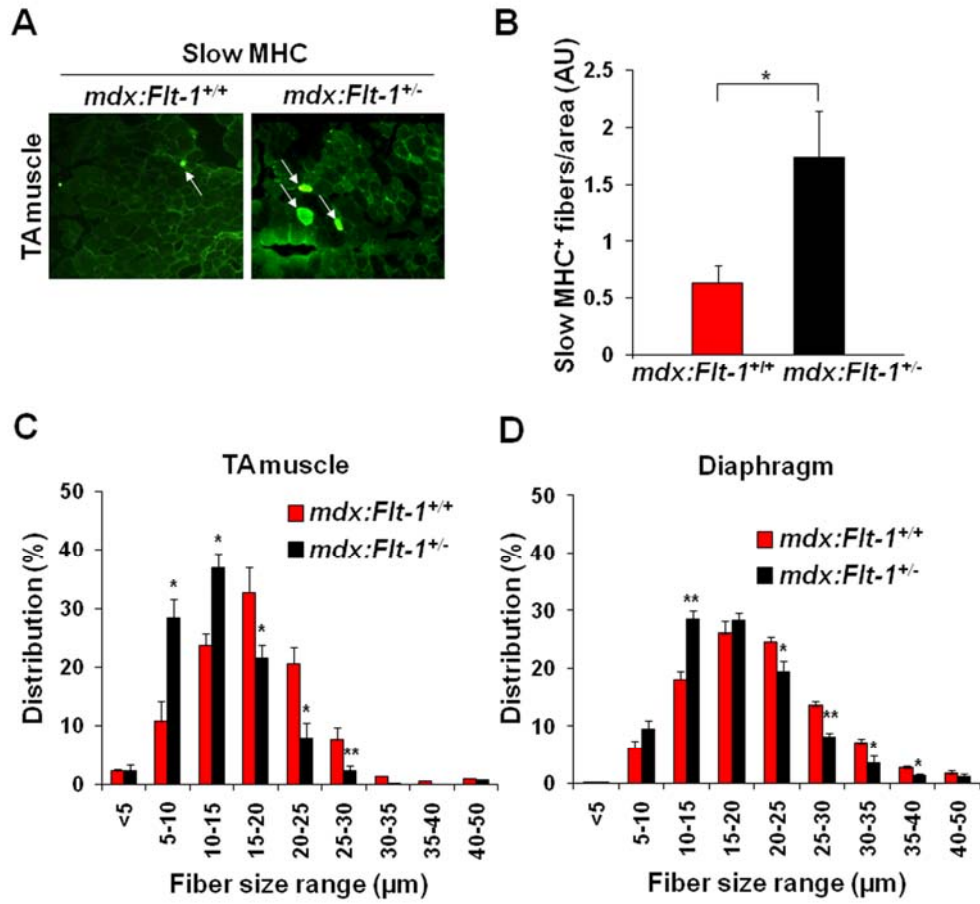
(A) Cryosections from the TA muscle were stained for Mac-1 to detect monocytes and macrophages. (B) Quantification of Mac-1<sup>+</sup> monocytes/macrophages in the cryosections of TA muscle of *mdx:Flt-1<sup>+/+</sup>* (n=4) and *mdx:Flt-1<sup>+/-</sup>* (n=9) mice. (C) Quantification of Gr-1<sup>+</sup> cells monocytes/granulocytes in the cryosection of TA muscle of *mdx:Flt-1<sup>+/+</sup>* and *mdx:Flt-1<sup>+/-</sup>* mice (n=3 each). Values are mean  $\pm$  SEM.

### Supplementary Figure 3. Myogenic cells do not express Flt-1 or Flk-1.

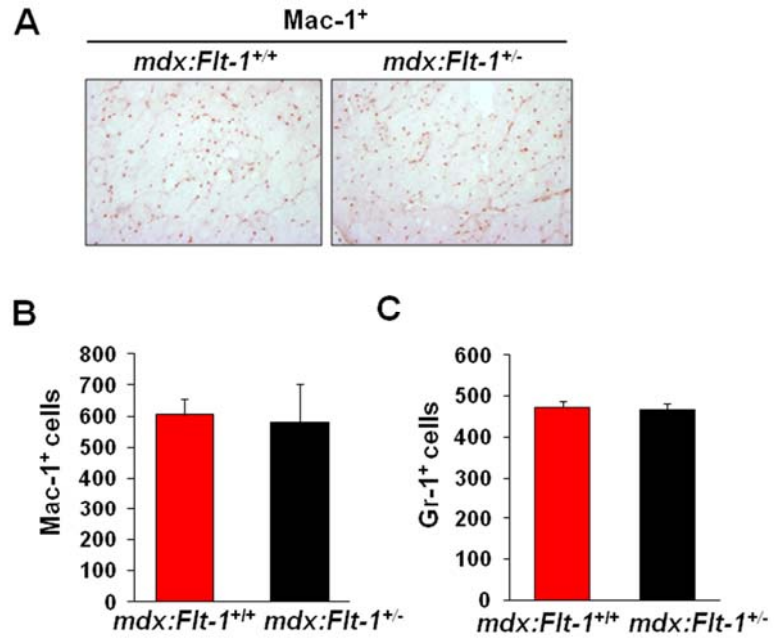
(A) Whole mount TA muscle from knock-in mice where lacZ expression recapitulates the expression of Flt-1, Flk-1 and Myf5 was stained with X-gal, and then transverse sections were stained with anti-laminin antibody (upper panels: AEC colorimetric staining, lower panels: Alexa

488 green fluorescent staining). Myf5-nlacZ<sup>+</sup> cells were located in the satellite cell nuclei (blue color: arrows) within the laminin-positive basal lamina (brown color), whereas the Flt-1-lacZ<sup>+</sup> and Flk-1-lacZ<sup>+</sup> cells (blue color: arrows) were located around vasculatures outside the laminin-positive basal lamina (brown color). (B) Quiescent satellite cells, proliferating myoblasts and differentiating myocytes expressed nuclear lacZ (arrows) in single muscle fiber cultures from *Myf5<sup>+/nLacZ</sup>* knock-in mice. Single muscle fiber cultures from *Flt-1-lacZ* or *Flk-1-lacZ* knock-in mice show that none of isolated muscle fibers or myogenic cells expressed lacZ. (C) Immunostaining conformed that satellite cell-derived myoblasts did not express Flt-1 or Flk-1, while they expressed the muscle marker desmin. By contrast, human umbilical vein endothelial cells (HUVECs) expressed Flt-1 and Flk-1 at their cell membranes but did not express the muscle marker desmin. Nuclei were counterstained with DAPI (blue).

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## Supplementary Figure 2 (Verma et al.)



## Supplementary Figure 3 (Verma et al.)

