

## **Single cell RNA-seq analysis of the flexor digitorum brevis mouse myofibers**

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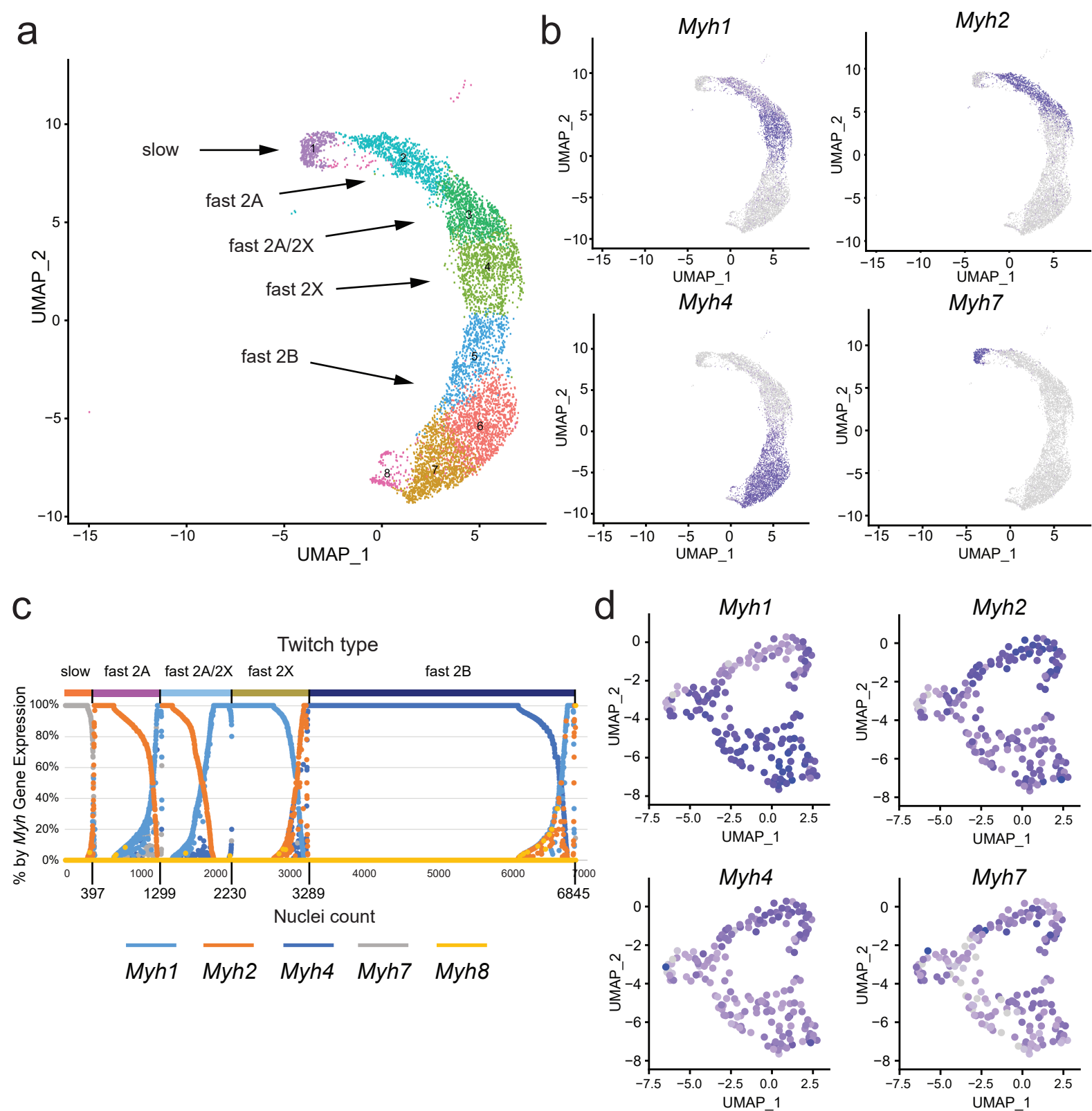
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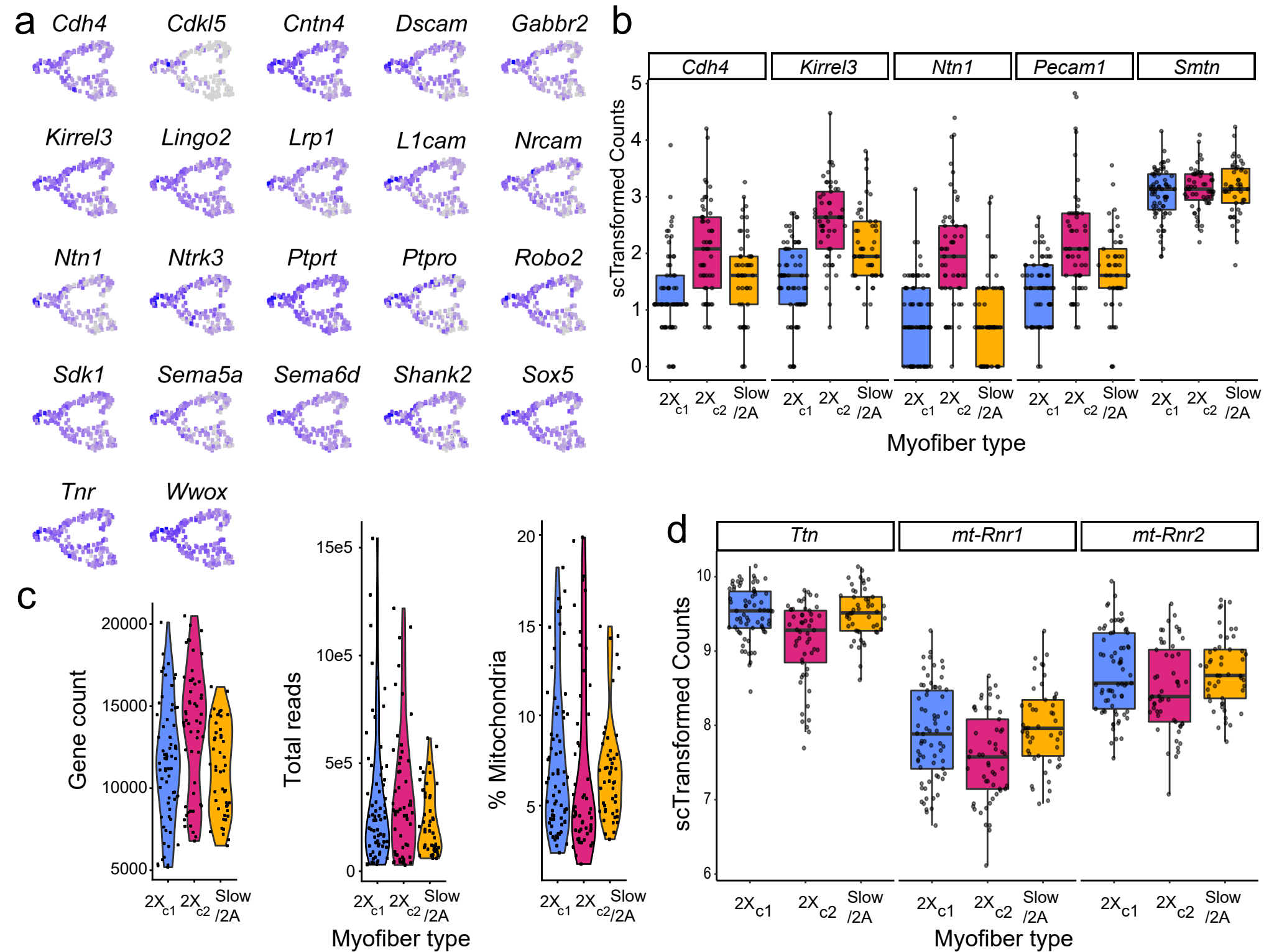
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**Supplementary Figure S1.** Myofiber nuclei analysis. **(a)** A UMAP of all myofiber nuclei with fiber type noted. **(b)** Myosin heavy chain expression used to assign the myofiber types. **(c)** Myosin heavy chain expression and number of nuclei by twitch type demonstrating overlapping expression of Myh genes in some twitch types. **(d)** Myosin heavy chain expression from the whole scRNA-seq data, as a comparison.



**Supplementary Figure S2.** Comparison of Fast 2Xc1 and Fast 2Xc2 subsets. **(a)** Twenty-two neuronal or NMJ-related genes are detected in most cells, but enriched in Fast 2Xc1 (middle left) cells. **(b)** Three neural genes (*Cdh4*, *Kirrel3*, *Ntn1*), an endothelial specific gene (*Pecam1*) and a smooth muscle cell gene (*Smtn*) are all increased in Fast 2Xc2 subsets suggesting overall increase of ambient RNA in these cells. **(c)** Total gene counts are elevated in Fast2Xc2 subsets despite no increase in total reads or % mitochondria. **(d)** Typically abundant genes, *Ttn*, *mt-Rnr1*, and *mt-Rnr2* are all of lower expression in Fast 2Xc2 cells.