Supplemental information

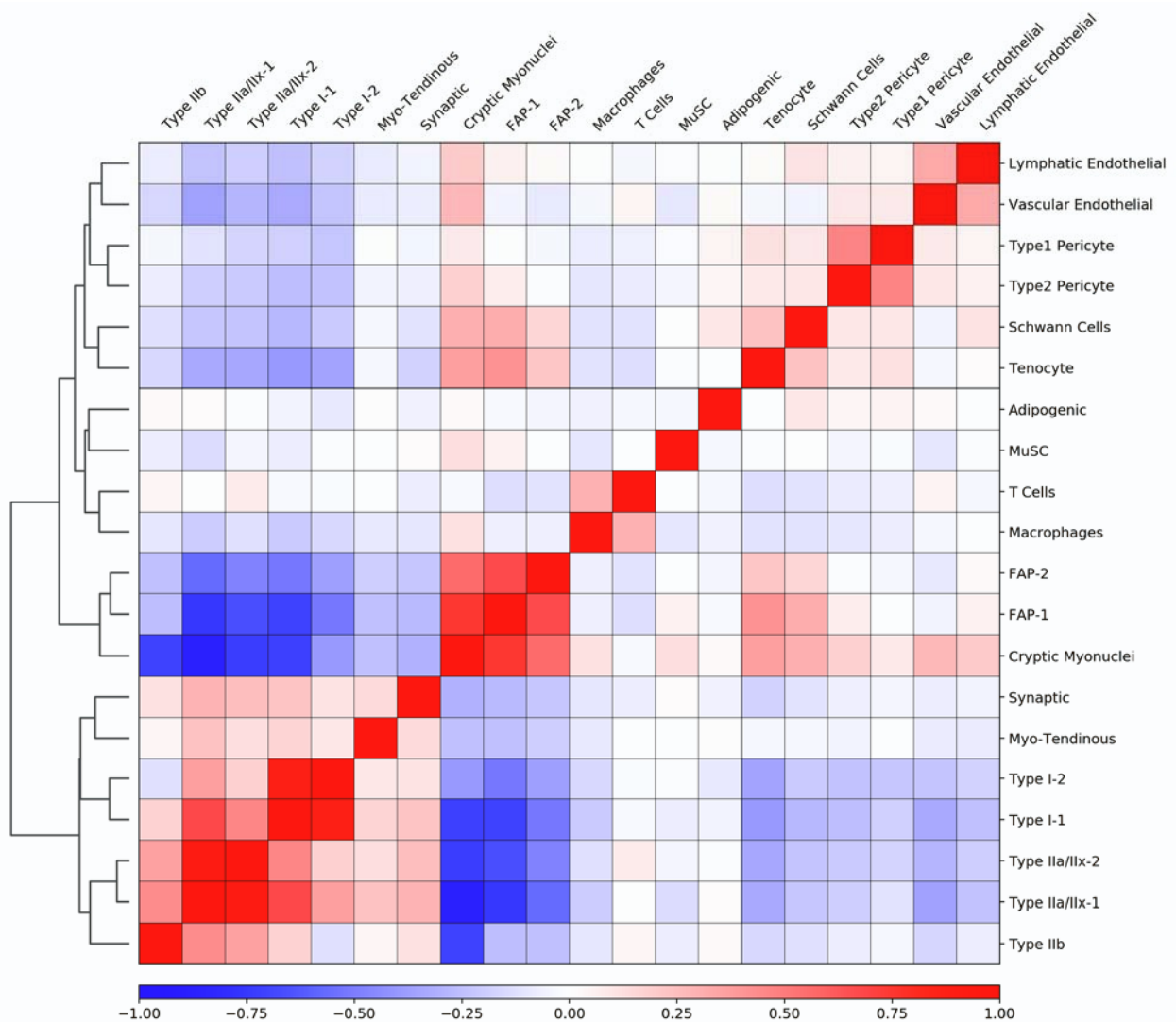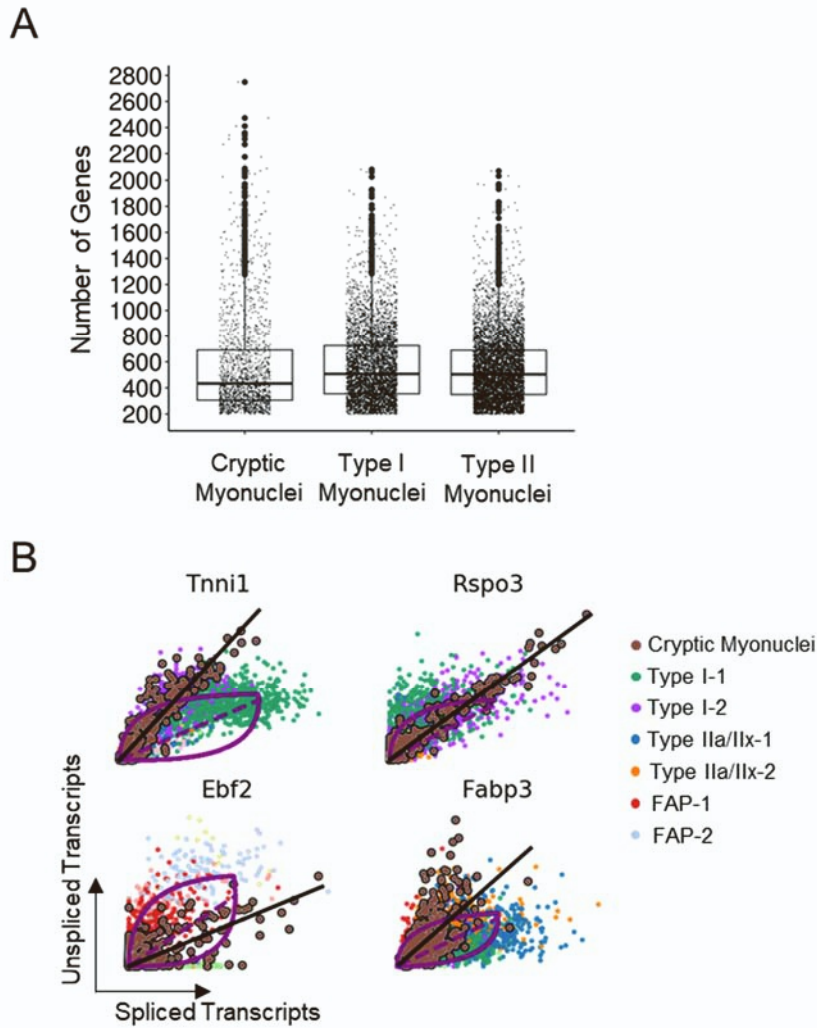# Myonuclear transcriptional dynamics

in response to exercise following

satellite cell depletion

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Figure S1. **Pairwise gene expression correlation analysis comparing all cluster, related to Figure 2**. Gene expression correlation demonstrates that the ambiguous nuclear cluster with MyHC gene expression is highly correlated with fibroadipogenic precursors (FAP-1 and 2). The ambiguous myonuclear cluster has moderate positive correlation with tenocytes and endothelial cells but is negatively correlated with the main Type I and II myonuclear clusters. Red and blue represent Pearson correlation coefficients of 1 and -1, respectively.

Figure S2. **Evaluation of cryptic myonuclei cluster, related to Figure 2**. **A)** The number of genes in the cryptic myonuclei are not higher than those in the canonical Type I and Type II myonuclei. **B)** Transcriptional dynamical modeling of RNA velocity for four genes showing differential kinetics for nuclei of different cell types. The cryptic myonuclei are distinct from other types of nuclei. Colors are coded to match the main clusters in Fig. 2A.

Cluster	ID	Description	GeneRatio	p.adjust	-log10(adj.p)	Count
Type I	GO:0003779	actin binding	27/219	5.51E-12	11.26	27
	GO:0051015	actin filament binding	16/219	2.70E-08	7.57	16
	GO:0003995	acyl-CoA dehydrogenase activity	3/219	1.25E-02	1.90	3
	GO:0000062	fatty-acyl-CoA binding	4/219	1.25E-02	1.90	4
	GO:0050660	flavin adenine dinucleotide binding	6/219	1.35E-02	1.87	6
Type IIa-1	GO:0003779	actin binding	25/293	2.07E-07	6.68	25
	GO:0051087	chaperone binding	9/293	1.46E-03	2.84	9
	GO:0005496	steroid binding	9/293	1.98E-03	2.70	9
	GO:0050662	coenzyme binding	14/293	3.29E-03	2.48	14
	GO:0019829	ATPase-coupled cation transmembrane transporter activity	6/293	3.78E-03	2.42	6
Type IIb-1	GO:0004674	protein serine/threonine kinase activity	17/187	1.14E-04	3.94	17
	GO:0004683	calmodulin-dependent protein kinase activity	5/187	5.60E-04	3.25	5
	GO:0005516	calmodulin binding	10/187	6.22E-04	3.21	10
	GO:0008081	phosphoric diester hydrolase activity	7/187	7.47E-04	3.13	7
	GO:0042578	phosphoric ester hydrolase activity	13/187	7.47E-04	3.13	13

Table S1, Gene ontology enrichment analysis of main myonuclear clusters, related to Figure 1. Significant marker genes for each Type I, IIa, and IIb main clusters show few significantly enriched GO terms with the most significant being components of the sarcomere classified under actin binding. Other GO terms are less enriched with -log10(adj.p) < 4.