

Supplemental Information Titles and Legends:

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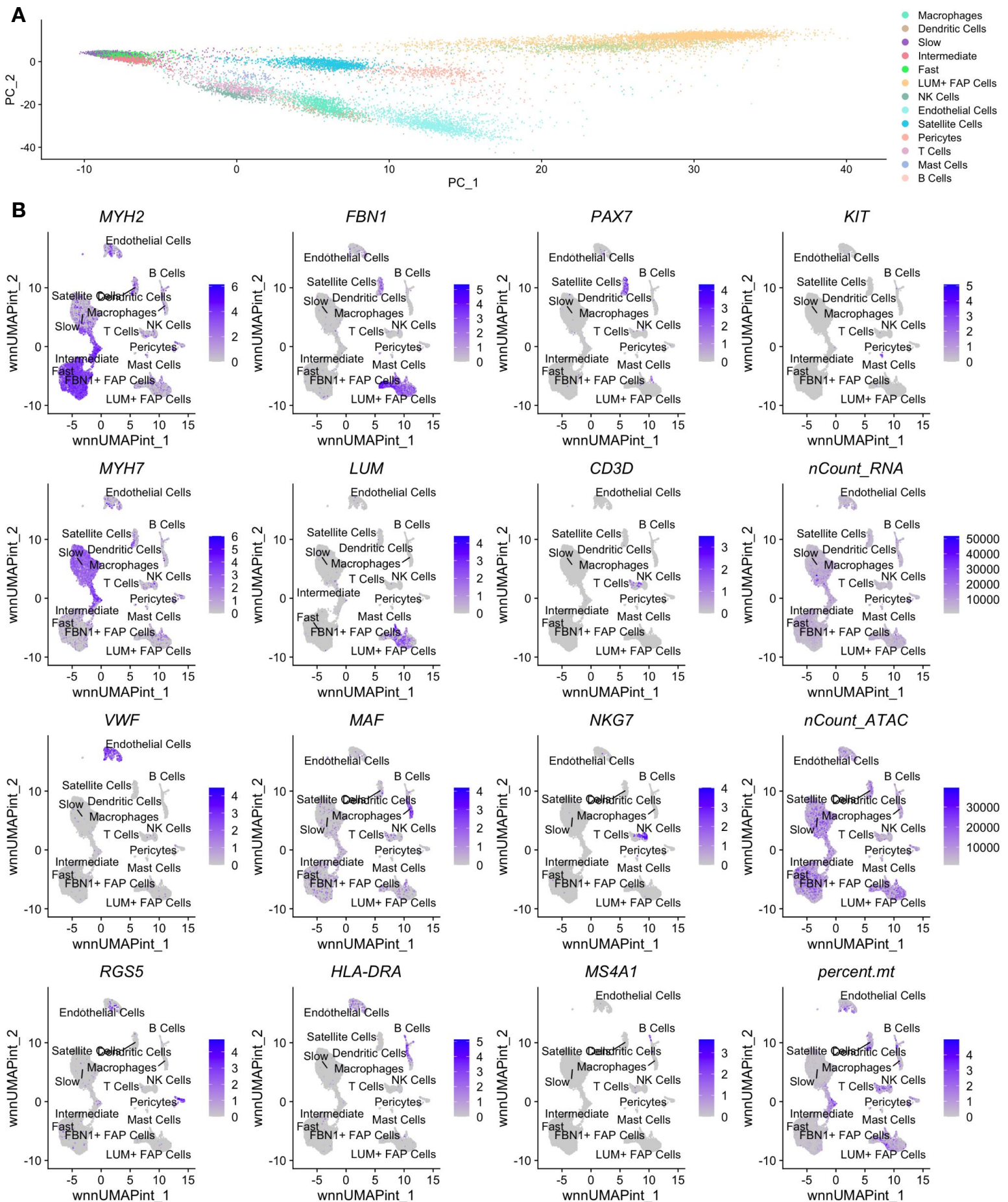
Supplementary Table S1: Subject Characteristics

Supplementary Table S2: Cell-type Markers (separate csv file)

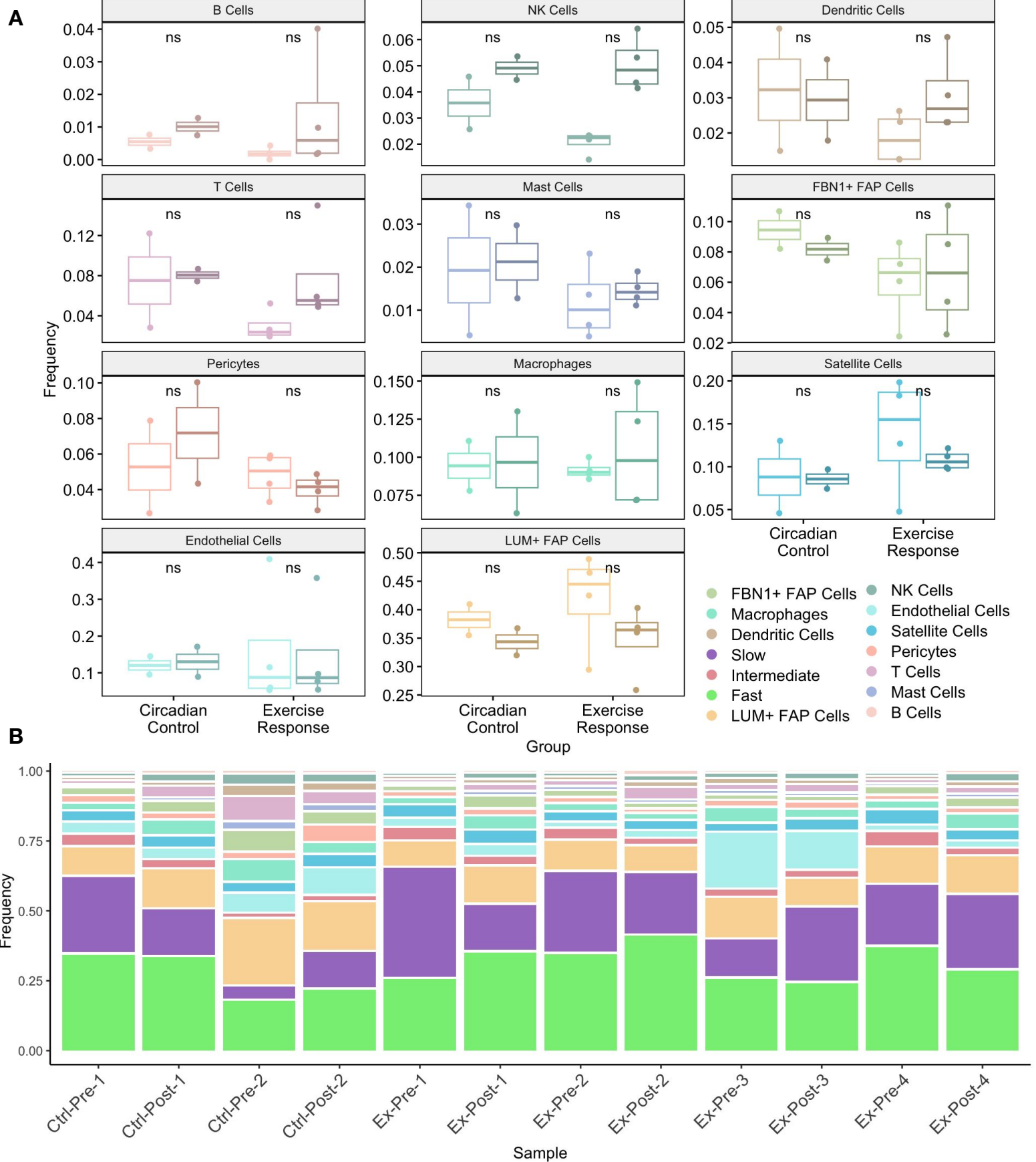
Supplementary Table S3: PPARD Targets

Supplementary Table S4: QC RNA-seq Metrics

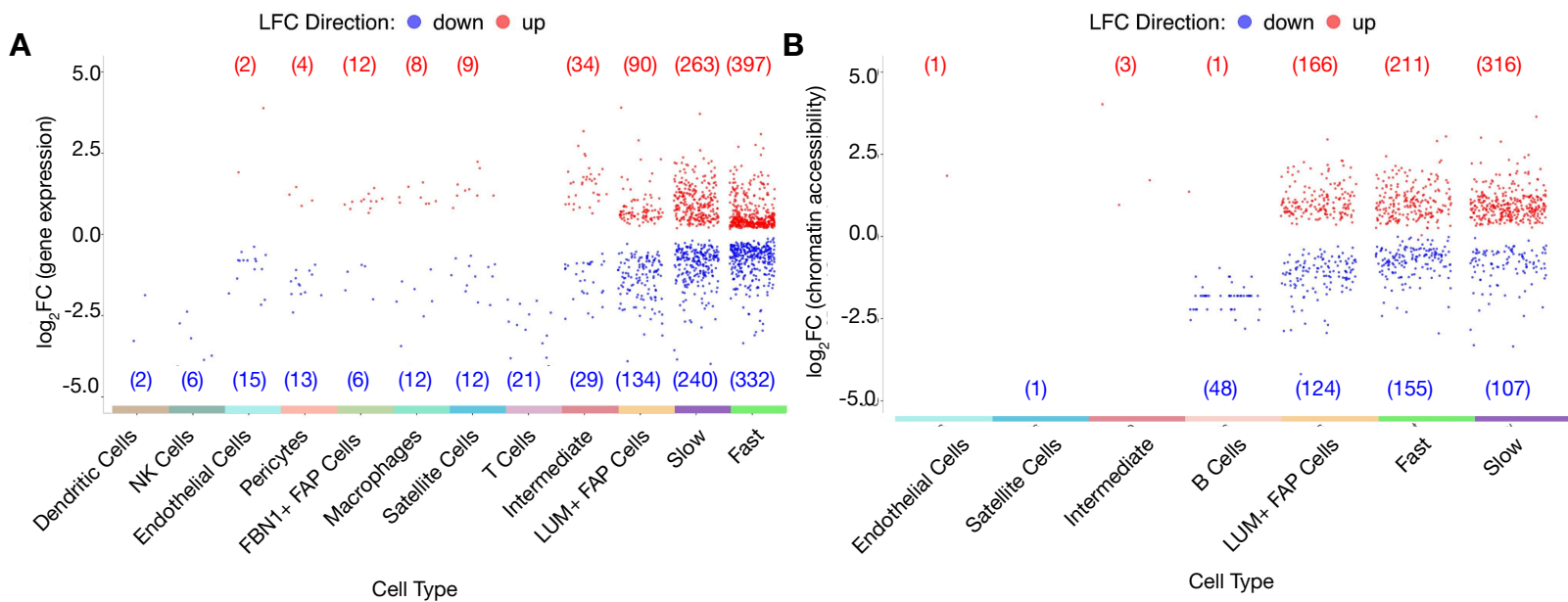
Supplementary Table S5: QC ATAC-seq Metrics



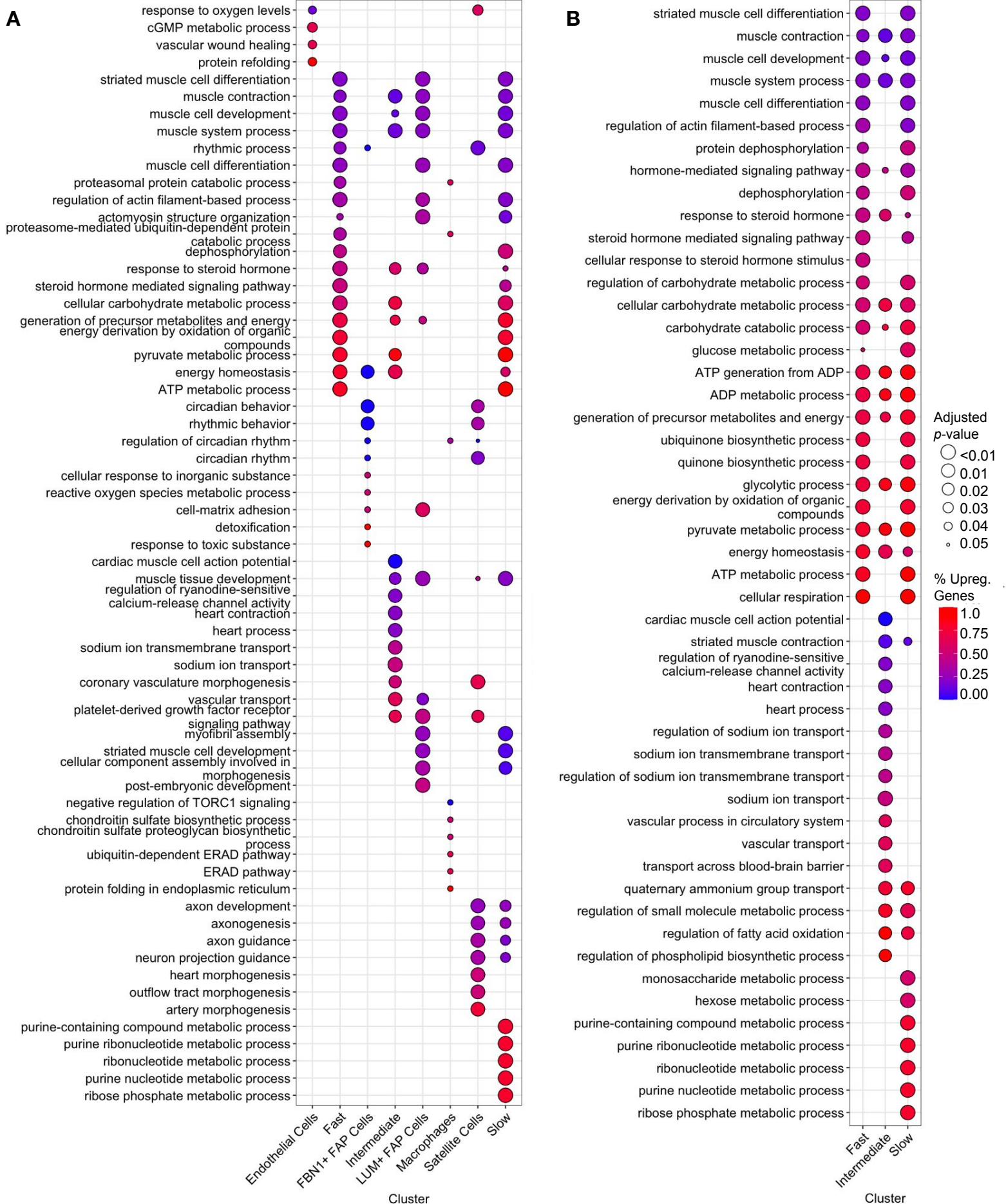
Supplemental Figure S1: Single-cell primary processing. (A) Cell types resolve in PCA as well as UMAP. (B) Feature plots showing marker genes or QC metrics on the UMAP projection. Marker genes include *MYH2* (fast/intermediate), *MYH7* (slow/intermediate), *VWF* (endothelial cells), *RGS5* (pericytes), *FBN1* (FBN1+ FAP cells), *LUM* (LUM+ FAP cells), *MAF* (macrophages), *HLA-DRA* (dendritic cells), *PAX7* (satellite cells), *CD3D* (T cells), *NKG7* (NK cells), *MS4A1* (B cells), *KIT* (mast cells). QC metrics include nCount_RNA (the number of RNA UMIs per cell), nCount_ATAC (the number of ATAC UMIs per cell), and percent.mt (percentage of mitochondrial reads per cell).



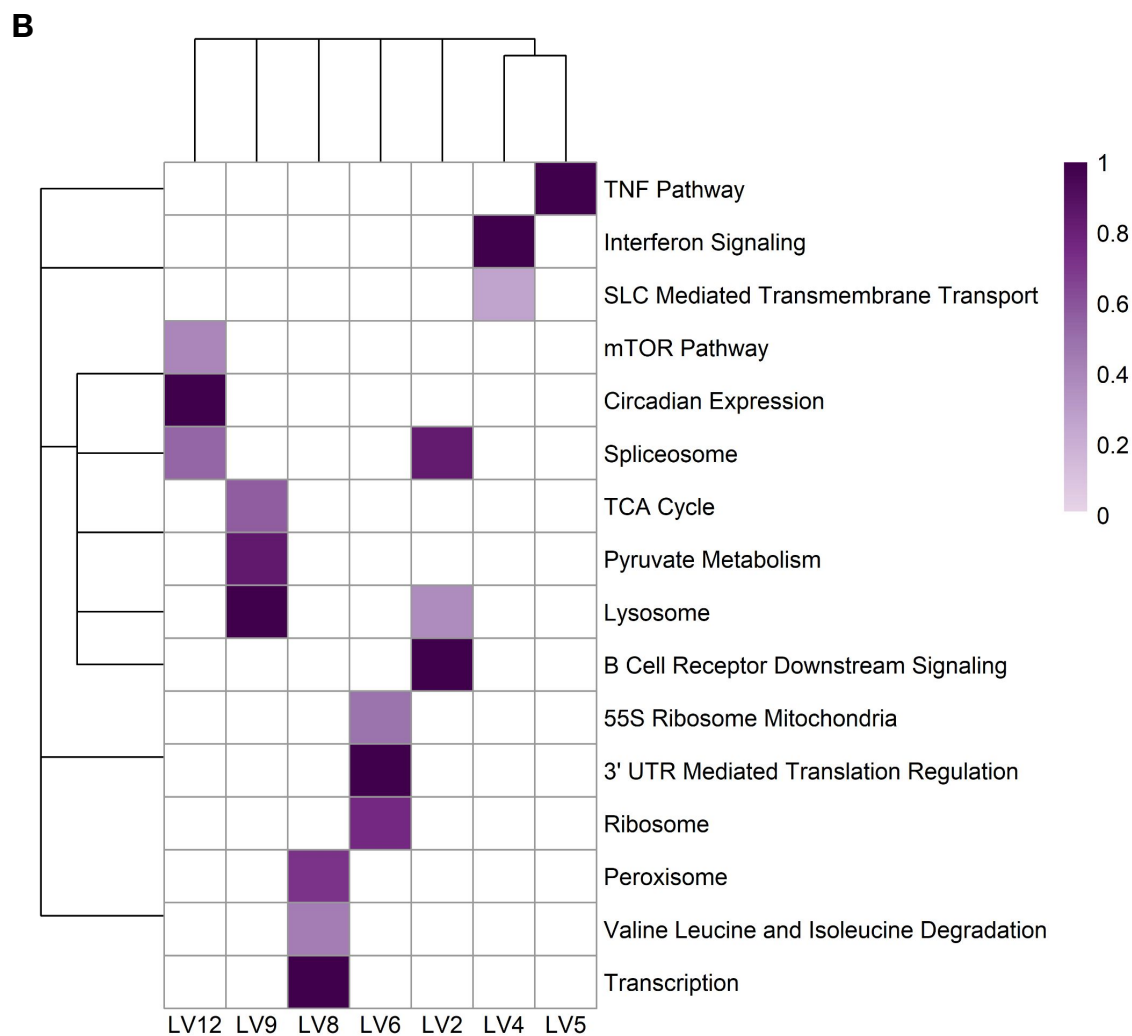
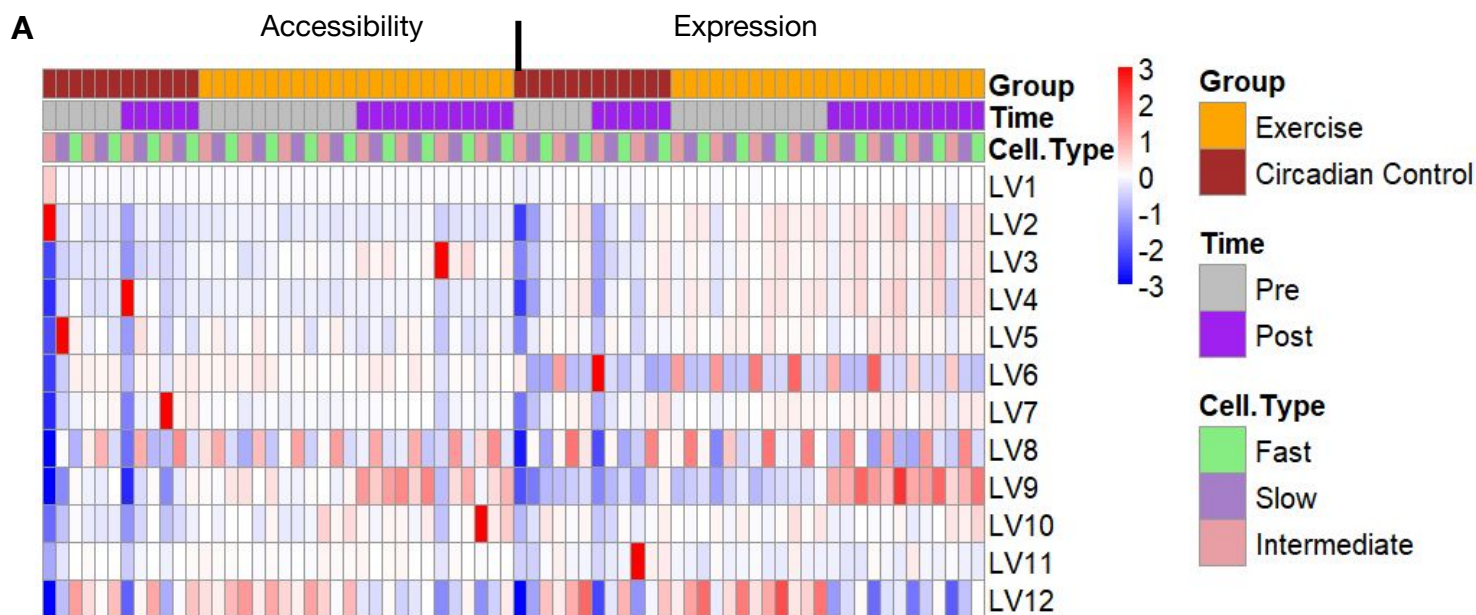
Supplemental Figure S2: Cell type proportions are roughly comparable between pre and post exercise time-points. (A) Boxplots reveal frequency of each cell type within each sample and are split by time-point and group ($n = 2$ control samples per time-point and $n = 4$ exercise samples per time-point). Colors denote cell type with a darker tint indicating the post time-point. To enable more consistent comparison, fibers are excluded during computation of cell type frequency. Change between pre and post cell type proportions is assessed via Holm-Bonferroni adjusted paired t -test with ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$ (B) Barplot indicates frequency of each cell type, including fibers, for each sample. Subjects Ctrl-1, Ex-1, and Ex-2 are female; subjects Ctrl-2, Ex-3, and Ex-4 are male.



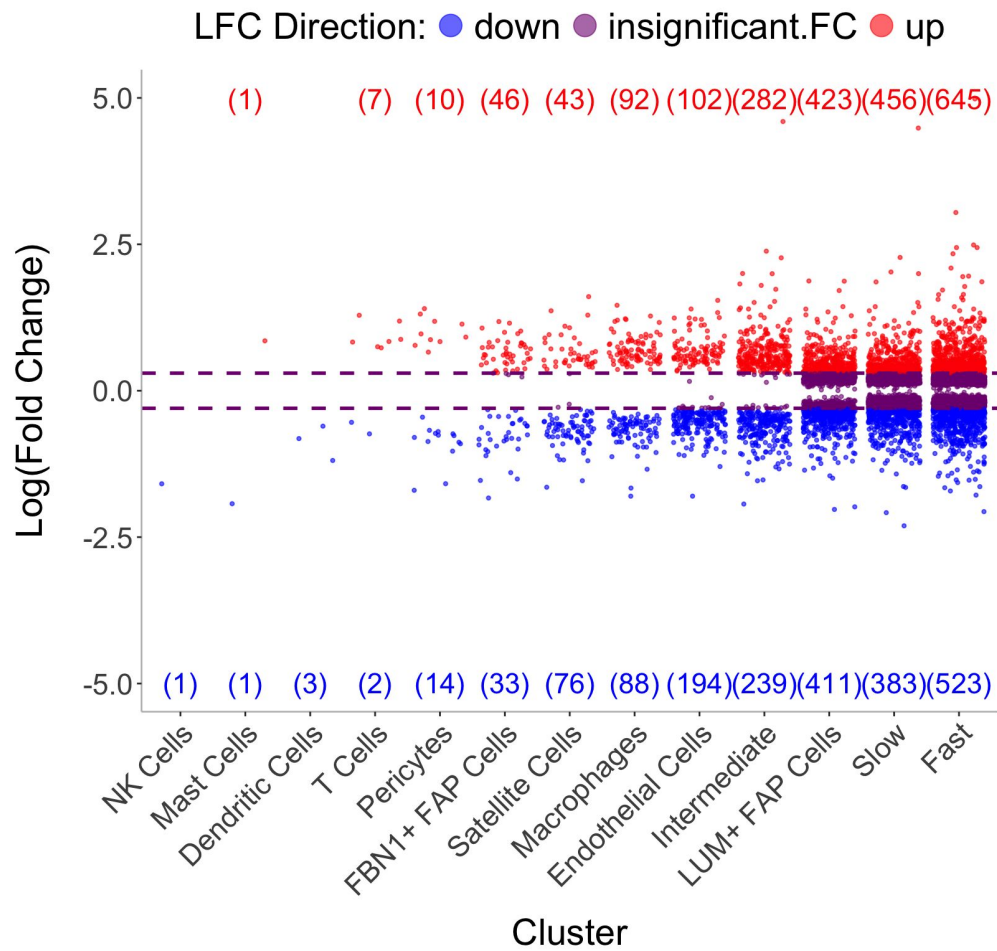
Supplemental Figure S3: Differential analysis of circadian controls. (A-B) Volcano plots show the distributions of upregulated and downregulated genes (A) and chromatin regions (B) for each cell type in circadian controls. These are the features that were excluded from the set of exercise-response features prior to downstream analysis (see Methods).



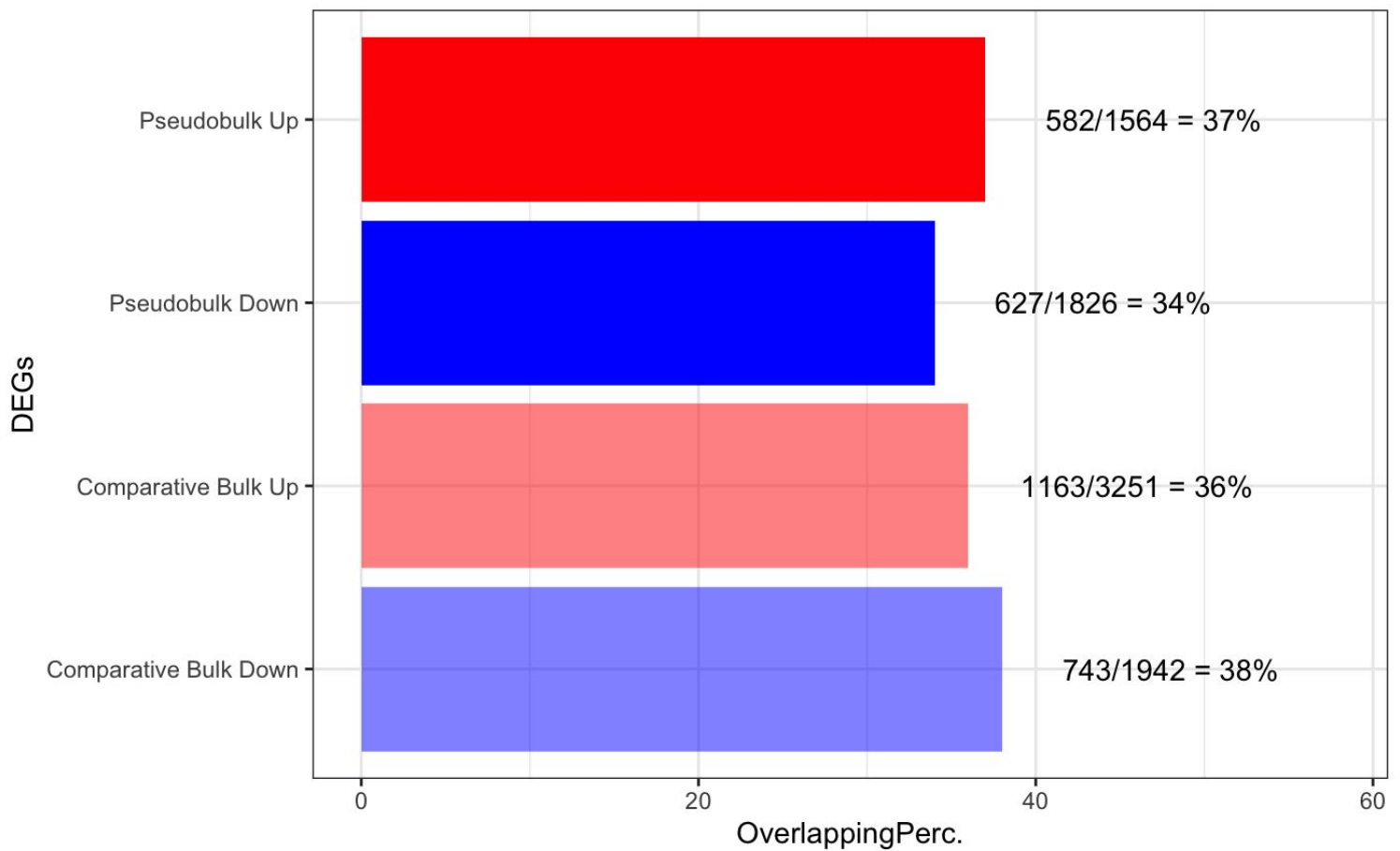
Supplemental Figure S4: Enrichment analysis. (A-B) Enrichment analysis for all cell types (A) and fibers only (B). Enrichment analysis of biological processes in each cell type shows functionally different transcriptome profiles for each cell type. Color represents the percentage of upregulated genes in a given pathway and larger dot radius denotes more significant biological function.



Supplemental Figure S5: PLIER Supplementary Panels. (A) Heatmap represents summary values for all PLIER-identified LVs. Each column represents one fiber-type sample; columns are ordered by -ome type, exercise group, and time-point. Each row represents one LV. (B) Heatmap of enriched pathways associated with select latent variables. Box color reflects AUC for association of pathway to LV.



Supplemental Figure S6: Thresholding parameter sensitivity. Volcano plot shows the distributions of upregulated and downregulated genes due to exercise with a log-fold change of 0.3 and an FDR of 0.05, as determined from single-cell analysis alone.



Supplemental Figure S7: Meta-analysis comparison. Bar plot depicts percentage of overlap of research study DEG in response to exercise to meta-analysis compendium. “Pseudobulk Up” and “Pseudobulk Down” refer to percentage of genes identified as upregulated and downregulated, respectively, using pseudobulk data in the current study that are also identified as regulated in response to exercise in the meta-analysis compendium. “Comparative Bulk Up” and “Comparative Bulk Down” refer to percentage of genes identified as upregulated and downregulated, respectively, using bulk muscle tissue RNA-seq data pre- and post-acute exercise from a published study that are also identified as regulated in response to exercise in the meta-analysis compendium.

Supplemental Table S1: Participant Characteristics

ID	Sex	Group	Age	Height (cm)	Weight (kg)	BMI (kg·m²)	Body Fat %	Absolute VO₂max(L/min)	Relative VO₂max(ml/kg/min)
Ex-1	F	Exercise	23	173	72.90	24.4	34.6	2.86	39.24
Ex-2	F	Exercise	26	164.2	68.9	25.6	30.8	2.78	40.37
Ex-3	M	Exercise	23	175	88.24	28.8	24.4	3.49	39.57
Ex-4	M	Exercise	26	175	91.3	29.8	33.1	4.31	47.24
Ctrl-1	F	Control	24	151	59.63	26.2	34.6	3.03	50.76
Ctrl-2	M	Control	23	184	82.7	24.4	19.4	3.89	47.02

BMI, body mass index; F, female; M, male; VO₂max, maximal oxygen consumption.

Supplemental Table S3: PPARD Targets

Cell type	PPARD targets
Fast fiber	ADORA2A-AS1, P4HA2-AS1, DPP6, C16orf46, TBC1D1, PTTG2, FABP3, PTPRG, CCDC197, RELL1, SERPINB9, ACKR3, SLC25A18, SLC26A9, VEGFA, CASTOR2, SPIRE1, IQCC, RNF144B, TFEB, SERINC2, ANGPTL2, MBP, CDHR3, SLC41A1, SLC38A4, RFTN1, RAB29, ITIH6, RAB7B, RHBDF1, MOCS1, MIA2, PHKG1, STC2, PTPRJ, SLC30A2, FAM83E, PDE11A, CDS2, ADM, OLFM1, RTN4RL1, LANCL1-AS1, NDRG1, GCOM1, CDKN1A, SLC22A23, SLC22A5, TMEM52, HADHB, LARGE-AS1, CES3, SPTBN4, RSPH9, C3orf35, CREB3L2, MID1IP1, SH3BP2, EML1, ZNF366, PSMG4, CPEB4, C4orf19, FAM43A, VGLL2, TMEM164, MIR3936HG, UQCRFS1, LIMD1-AS1, RIPOR1, DAAM2, LARGE1, RRS1, HHATL, POR, ZBTB43, ABCC1, ANKRD9, ERAP1, TSPAN9, PARVA, KLHL40, PSMD8, RANBP10, IGF2R, TMEM140, SCARF1, TRAF5, TECPR2, PPTC7, SLC25A5, DHDDS, KCNC1, LINC01968, SLC16A1-AS1, CALML6, IAH1, SOCS7, FARP1, RAB11FIP3, GANC, SAMD4A, HIVEP2, SLC22A4, ASAP3, DNAJA4, DLGAP4-AS1, IBTK, ZCCHC17, EIF4EBP2, ZMIZ1, NAGLU, SFMBT1, TNS3, CCDC13, CLIP4, KIF13A, MPC1, NCR3LG1, COLQ, RHOBTB2, DLGAP4, RAD9B, C19orf12, EPS15L1, TKT, UBE2O, ABHD3, PTCDD3, GOLGA4, RBMS3-AS3, CRK, AFG3L2, LINC01909, RGS3, OSBPL2, MIR1-1HG, NODAL, RBM38, ZNF385C, PRKCD, LGALS1, PDCD11, SLC22A1, DEK, GBF1, ADHFE1, ABCC8, PIM1, PRAG1, ARMH3, TOGARAM2, IDI2-AS1, SPECC1L, FKBP9, SEPTIN11, CD79A, ZBED6, SH3KBP1, GPR179, LCA5L, PARM1, LINC01754, PALMD, RETSAT, WDR60, CHD2, STAT3, SETD3, ITGB1BP1, SLC37A1, PITPNM2, ACSBG1, BCL2L14, PREB, PAFAH2, PARVB, SLC11A2, SGSM1, C16orf70, PTPN3, MTHFD1, ACACB, SLC4A4, MAN1C1, FNBP1L, ATP5MG, RILPL1, DUS2, NCOA3, GREB1L, JMJD6, UBAC1, ZNF609, FAM181A, ETV6, SLC31A1, LRIG2-DT, PHF20, DPEP2, CES2, PLAGL1, CLIC5, SLC29A1, BCAT2, ATP5MD, MTCH2, CEP68, GLCC1, SNHG1, LACTB, ABHD2, CIAO2A, CFL2, CAMSAP1, RHBDF2, MIOS, CCDC28B, ZCWPW2, ANKRD28, HSPB1, DHRS7C, SELENON, PLEKHA4, SLC66A2, WDR37, PFKFB1, CGREF1, ABHD1, ARHGEF10L, GEM, MIPEP, ANP32A, STK24, GPR157, RDH5, SH2B3, TBC1D22A, PDCD10, GREB1, PPP4C
Slow fiber	CGREF1, KLF13, CNNM1, TKT, GCOM1, JUNB, LINC00598, AMOTL1, IDH3A, ACSBG1, PREB, TRIM63, GDAP1L1, NRP1, STAT3, CCM2, ZMIZ1, ACOT11, MAP4K3-DT, SLC41A1, UBAC1, FAM151A, NOP16, PKIG, ZNF44, ATP5MG, EPDR1, GOT1, HOOK2

Supplemental Table S4: QC RNA-seq Metrics

ID	Time	Replicate	Estimated number of nuclei	Mean reads/cell	Median UMI/cell	Median genes/cell	Reads mapped to transcriptome	Reads mapped to genome	Fraction of transcriptomic reads in cells
<i>Ex-1</i>	<i>Pre</i>	<i>Aggregate</i>	<i>7960</i>	<i>52666</i>	<i>3906</i>	<i>1727</i>	<i>0.30</i>	<i>0.93</i>	<i>0.80</i>
Ex-1	Pre	1	4124	54917	3742	1689	0.31	0.93	0.80
Ex-1	Pre	2	3836	50246	4092	1768	0.29	0.93	0.80
<i>Ex-1</i>	<i>Post</i>	<i>Aggregate</i>	<i>5391</i>	<i>67791</i>	<i>3865</i>	<i>1841</i>	<i>0.32</i>	<i>0.93</i>	<i>0.77</i>
Ex-1	Post	1	2453	75255	3706	1786	0.33	0.93	0.76
Ex-1	Post	2	2938	61559	4050	1880	0.31	0.93	0.78
<i>Ex-2</i>	<i>Pre</i>	<i>*</i>	<i>5854</i>	<i>48101</i>	<i>3660</i>	<i>1652</i>	<i>0.31</i>	<i>0.94</i>	<i>0.84</i>
<i>Ex-2</i>	<i>Post</i>	<i>*</i>	<i>4608</i>	<i>57218</i>	<i>3356</i>	<i>1678</i>	<i>0.31</i>	<i>0.94</i>	<i>0.80</i>
<i>Ex-3</i>	<i>Pre</i>	<i>Aggregate</i>	<i>3003</i>	<i>131480</i>	<i>4386</i>	<i>1950</i>	<i>0.28</i>	<i>0.92</i>	<i>0.80</i>
Ex-3	Pre	1	1333	154393	5252	2140	0.28	0.93	0.78
Ex-3	Pre	2	1670	113191	3662	1763	0.27	0.92	0.81
<i>Ex-3</i>	<i>Post</i>	<i>Aggregate</i>	<i>4335</i>	<i>91301</i>	<i>3328</i>	<i>1660</i>	<i>0.33</i>	<i>0.92</i>	<i>0.78</i>
Ex-3	Post	1	2594	86634	3179	1606	0.32	0.93	0.78
Ex-3	Post	2	1741	98255	3485	1719	0.34	0.93	0.77
<i>Ex-4</i>	<i>Pre</i>	<i>*</i>	<i>8772</i>	<i>32451</i>	<i>3797</i>	<i>1780</i>	<i>0.31</i>	<i>0.94</i>	<i>0.85</i>
<i>Ex-4</i>	<i>Post</i>	<i>*</i>	<i>2524</i>	<i>109593</i>	<i>3944</i>	<i>1874</i>	<i>0.35</i>	<i>0.94</i>	<i>0.81</i>
<i>Ctrl-1</i>	<i>Pre</i>	<i>Aggregate</i>	<i>6499</i>	<i>67568</i>	<i>4064</i>	<i>1805</i>	<i>0.34</i>	<i>0.93</i>	<i>0.72</i>
Ctrl-1	Pre	1	6075	41230	3894	1758	0.28	0.94	0.86
Ctrl-1	Pre	2	424	444939	8663	2838	0.42	0.94	0.54
<i>Ctrl-1</i>	<i>Post</i>	<i>Aggregate</i>	<i>1372</i>	<i>557108</i>	<i>5070</i>	<i>2194</i>	<i>0.29</i>	<i>0.92</i>	<i>0.71</i>
Ctrl-1	Post	1	830	654419	6111	2436	0.30	0.93	0.78
Ctrl-1	Post	2	542	408090	2740	1516	0.28	0.92	0.55
<i>Ctrl-2</i>	<i>Pre</i>	<i>Aggregate</i>	<i>632</i>	<i>667124</i>	<i>4788</i>	<i>2115</i>	<i>0.41</i>	<i>0.91</i>	<i>0.61</i>
Ctrl-2	Pre	1	323	746617	4882	2265	0.41	0.93	0.59
Ctrl-2	Pre	2	309	584029	4703	2037	0.40	0.93	0.63
<i>Ctrl-2</i>	<i>Post</i>	<i>Aggregate</i>	<i>961</i>	<i>502041</i>	<i>4531</i>	<i>2035</i>	<i>0.49</i>	<i>0.91</i>	<i>0.49</i>

Ctrl-2	Post	1	456	<i>558669</i>	4946	2111	0.50	0.93	0.48
Ctrl-2	Post	2	505	<i>450908</i>	4247	1954	0.48	0.92	0.50

* No replicates for sample.

Italicized text represents metrics of final sample used for downstream analysis

Supplemental Table S5: QC ATAC-seq Metrics

ID	Time	Replicate	Median high-quality fragments/cell	Fraction of transposition events in peaks in cells	Number of peaks	Fraction of genome in peaks	TSS enrichment score	Confidently mapped read pairs	Fraction of high quality fragments overlapping peaks
<i>Ex-1</i>	<i>Pre</i>	<i>Aggregate</i>	<i>18055</i>	<i>0.332</i>	<i>84518</i>	<i>0.0230</i>	<i>5.47</i>	<i>0.93</i>	<i>0.33</i>
Ex-1	Pre	1	16472	0.301	77506	0.0214	5.47	0.93	0.33
Ex-1	Pre	2	20201	0.283	76450	0.0211	5.14	0.93	0.32
<i>Ex-1</i>	<i>Post</i>	<i>Aggregate</i>	<i>20117</i>	<i>0.320</i>	<i>78916</i>	<i>0.0220</i>	<i>5.02</i>	<i>0.93</i>	<i>0.32</i>
Ex-1	Post	1	19902	0.271	65845	0.0182	4.84	0.93	0.30
Ex-1	Post	2	20337	0.285	73679	0.0203	5.02	0.93	0.31
<i>Ex-2</i>	<i>Pre</i>	<i>*</i>	<i>10429</i>	<i>0.347</i>	<i>92023</i>	<i>0.0260</i>	<i>6.27</i>	<i>0.94</i>	<i>0.38</i>
<i>Ex-2</i>	<i>Post</i>	<i>*</i>	<i>8209</i>	<i>0.188</i>	<i>54498</i>	<i>0.0150</i>	<i>4.24</i>	<i>0.93</i>	<i>0.21</i>
<i>Ex-3</i>	<i>Pre</i>	<i>Aggregate</i>	<i>25973</i>	<i>0.378</i>	<i>97398</i>	<i>0.0270</i>	<i>6.09</i>	<i>0.92</i>	<i>0.38</i>
Ex-3	Pre	1	29863	0.344	90025	0.0250	6.09	0.92	0.38
Ex-3	Pre	2	23263	0.322	81323	0.0225	5.44	0.92	0.35
<i>Ex-3</i>	<i>Post</i>	<i>Aggregate</i>	<i>18539</i>	<i>0.314</i>	<i>75830</i>	<i>0.0210</i>	<i>4.74</i>	<i>0.92</i>	<i>0.31</i>
Ex-3	Post	1	13667	0.269	64116	0.0177	4.74	0.92	0.30
Ex-3	Post	2	30543	0.277	68359	0.0188	4.60	0.92	0.31
<i>Ex-4</i>	<i>Pre</i>	<i>*</i>	<i>10601</i>	<i>0.348</i>	<i>100678</i>	<i>0.0280</i>	<i>6.05</i>	<i>0.94</i>	<i>0.38</i>
<i>Ex-4</i>	<i>Post</i>	<i>*</i>	<i>25056</i>	<i>0.314</i>	<i>83645</i>	<i>0.0230</i>	<i>5.16</i>	<i>0.93</i>	<i>0.34</i>
<i>Ctrl-1</i>	<i>Pre</i>	<i>Aggregate</i>	<i>10489</i>	<i>0.378</i>	<i>92724</i>	<i>0.0260</i>	<i>6.50</i>	<i>0.93</i>	<i>0.38</i>
Ctrl-1	Pre	1	9959	0.358	91820	0.0255	6.50	0.93	0.38
Ctrl-1	Pre	2	47090	0.293	63042	0.0176	5.74	0.93	0.32
<i>Ctrl-1</i>	<i>Post</i>	<i>Aggregate</i>	<i>23827</i>	<i>0.344</i>	<i>66074</i>	<i>0.0180</i>	<i>5.09</i>	<i>0.92</i>	<i>0.34</i>
Ctrl-1	Post	1	27087	0.316	58204	0.0162	5.09	0.91	0.34
Ctrl-1	Post	2	15607	0.290	52790	0.0147	4.83	0.92	0.31
<i>Ctrl-2</i>	<i>Pre</i>	<i>Aggregate</i>	<i>26889</i>	<i>0.208</i>	<i>35652</i>	<i>0.0100</i>	<i>3.77</i>	<i>0.91</i>	<i>0.21</i>
Ctrl-2	Pre	1	24333	0.160	26657	0.0075	3.45	0.91	0.17
Ctrl-2	Pre	2	28913	0.200	32604	0.0092	3.77	0.91	0.21

<i>Ctrl-2</i>	<i>Post</i>	<i>Aggregate</i>	<i>21707</i>	<i>0.156</i>	<i>29919</i>	<i>0.0080</i>	<i>3.28</i>	<i>0.91</i>	<i>0.16</i>
Ctrl-2	Post	1	21109	0.135	23055	0.0065	3.06	0.91	0.14
Ctrl-2	Post	2	22401	0.135	26529	0.0074	3.28	0.90	0.14

* No replicates for sample.

Italicized text represents metrics of final sample used for downstream analysis