

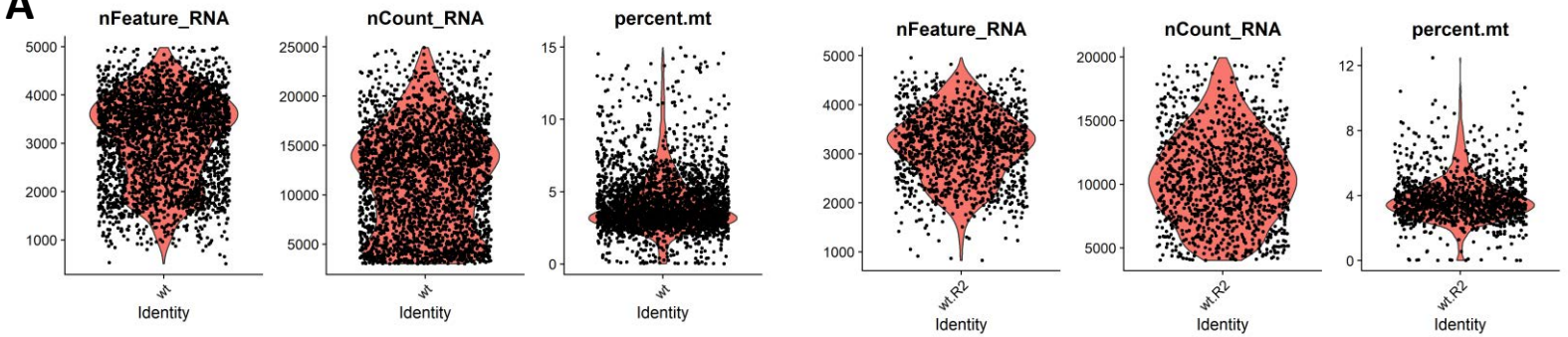
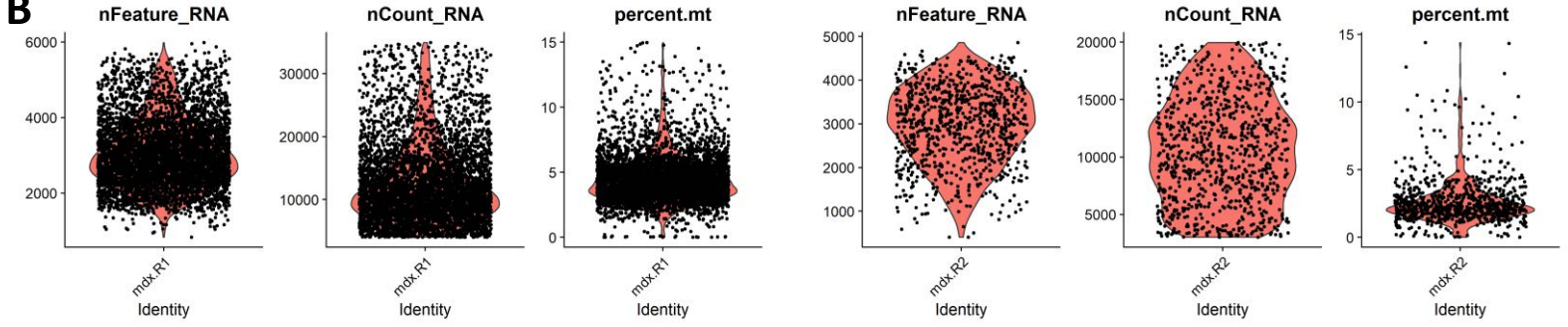
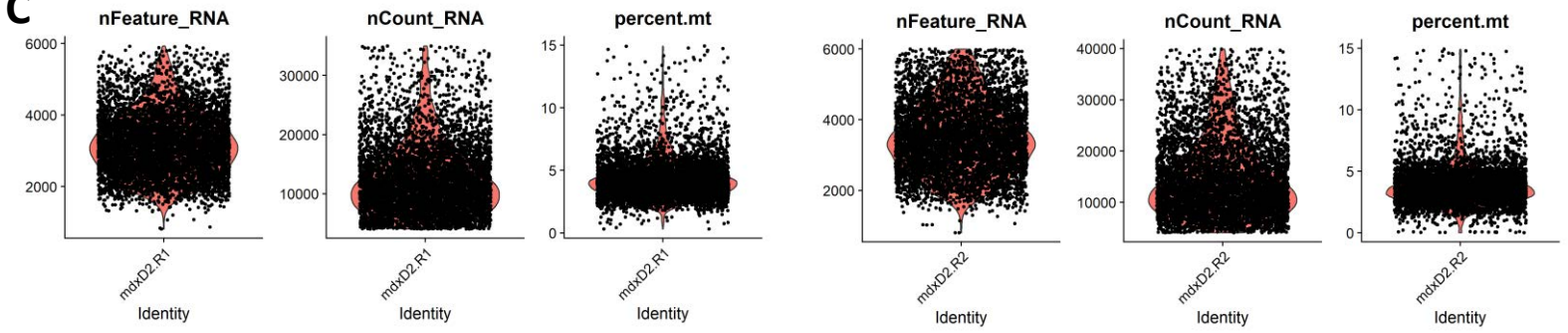
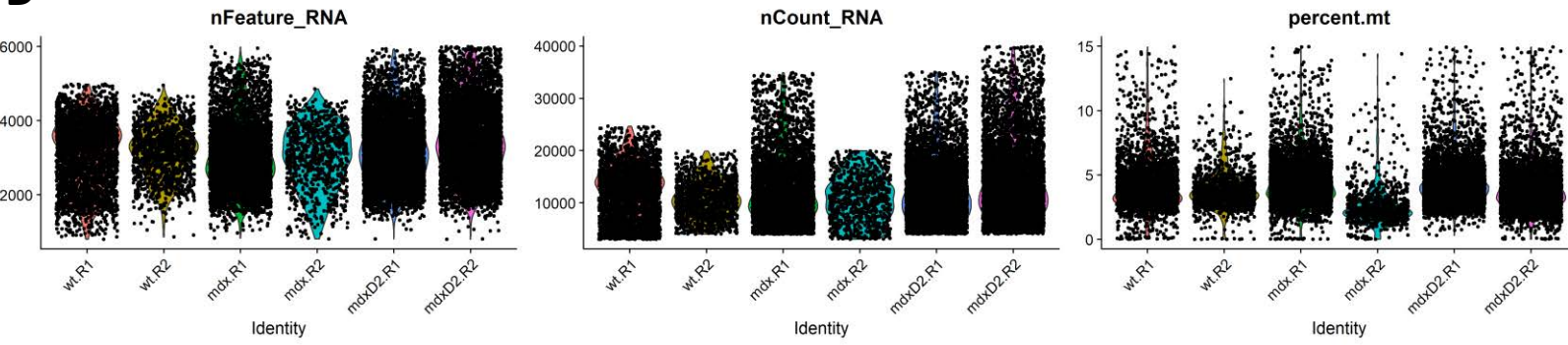
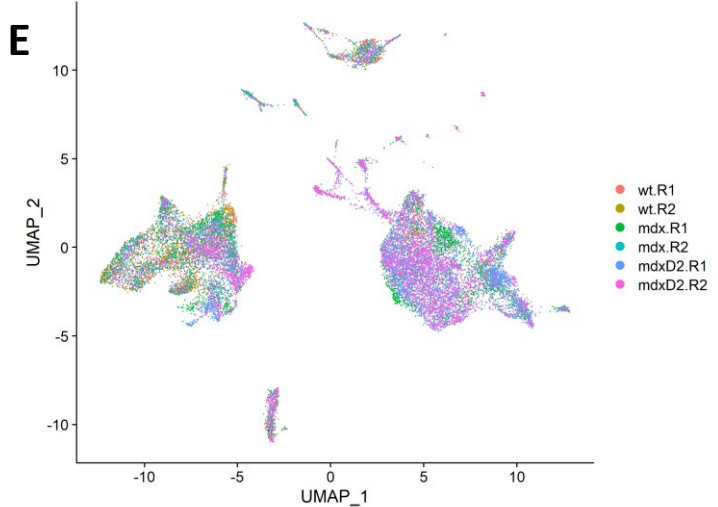
Supplemental information

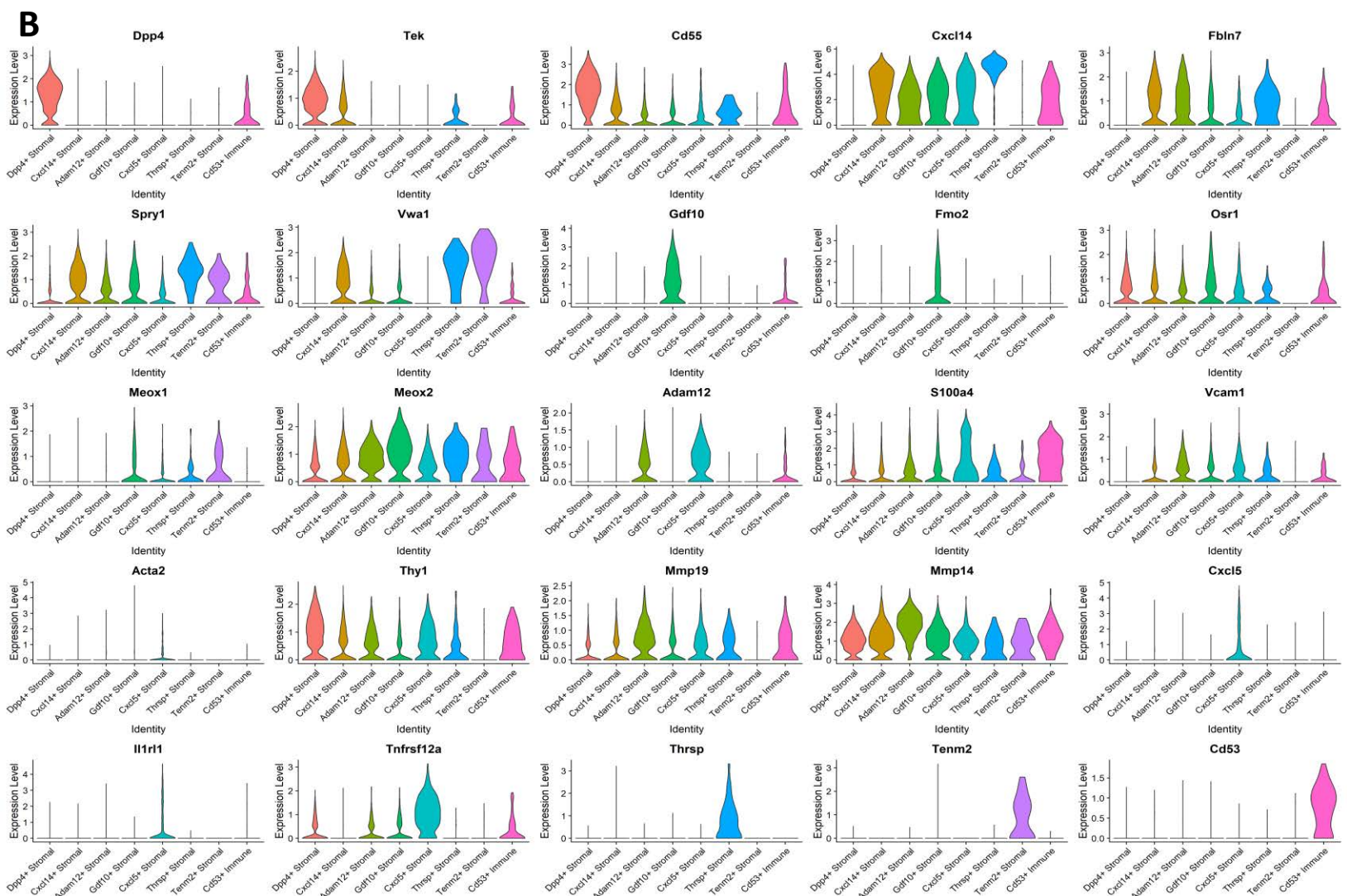
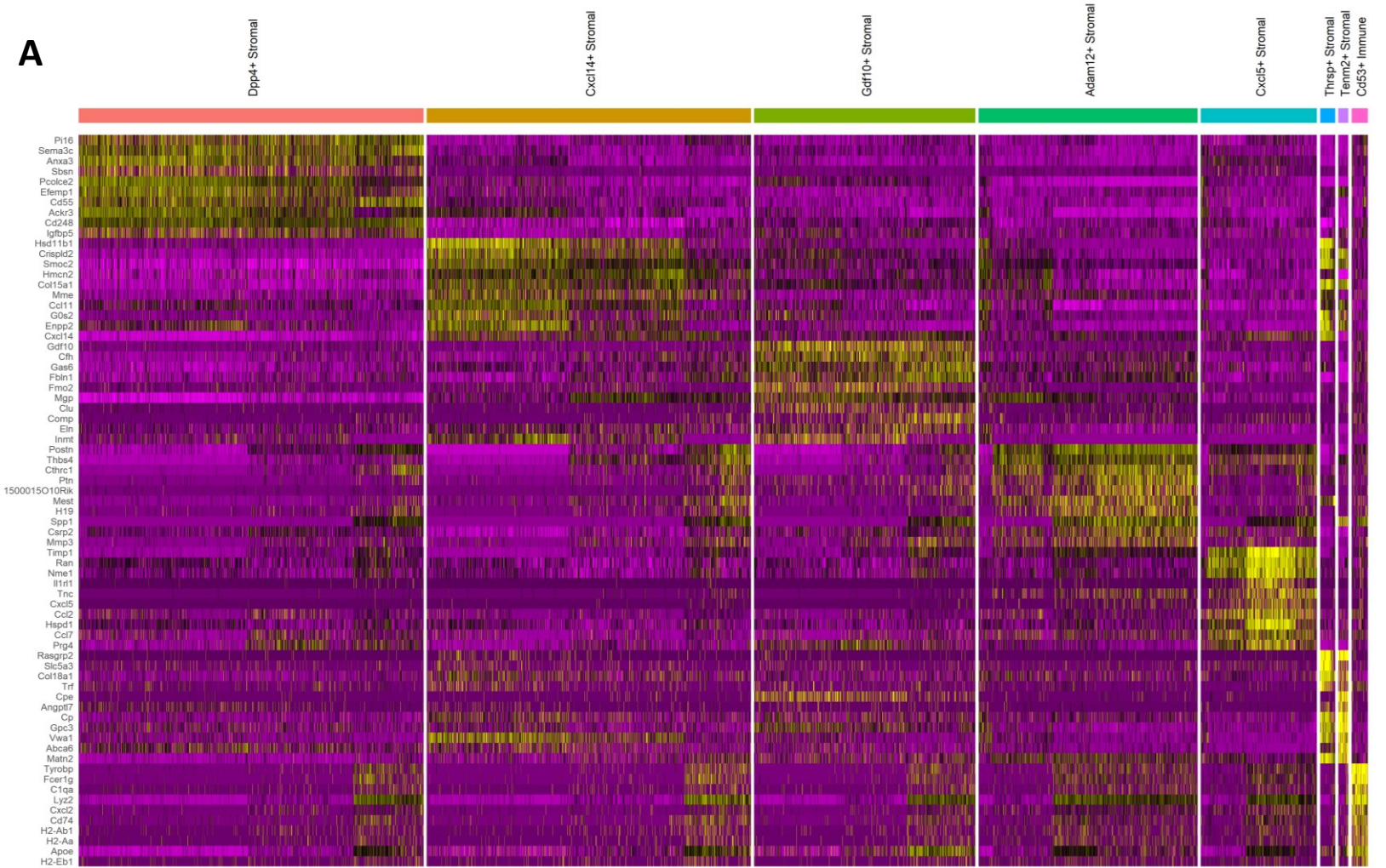
Single cell sequencing maps skeletal muscle

cellular diversity as disease

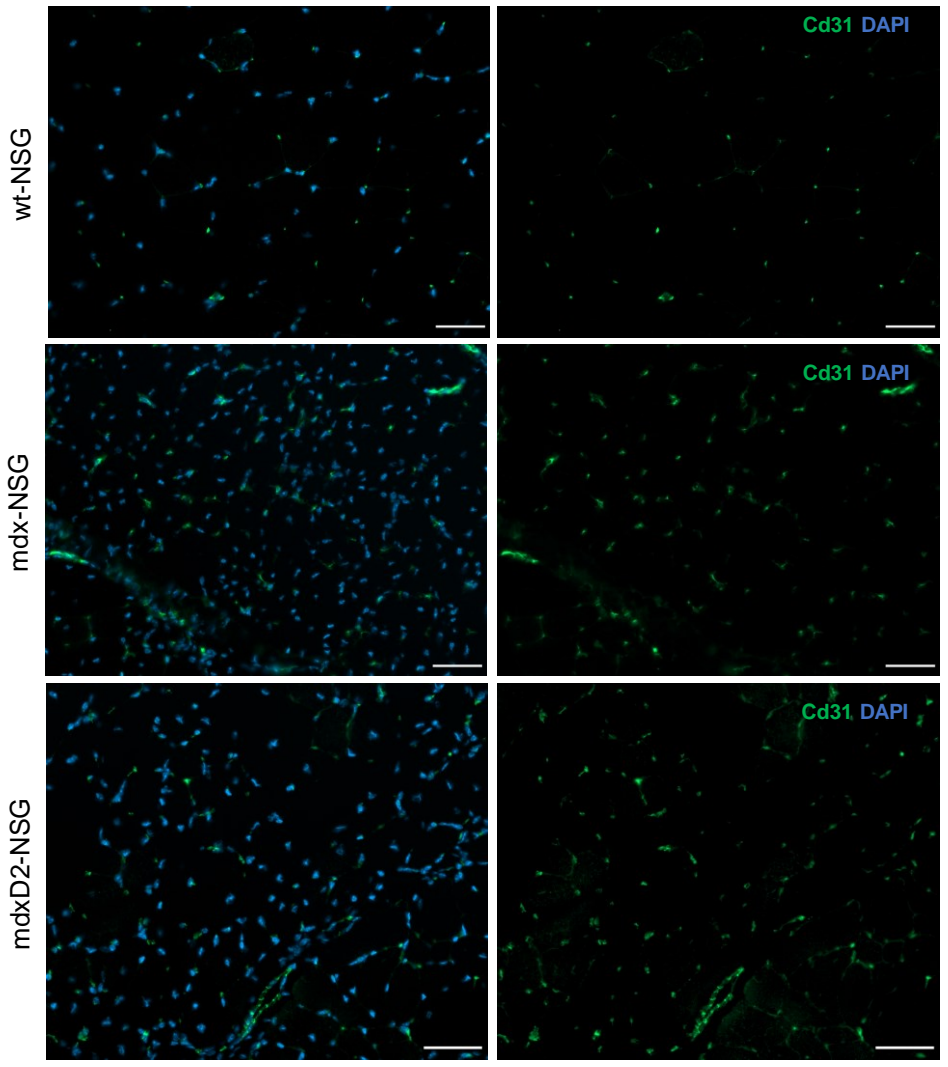
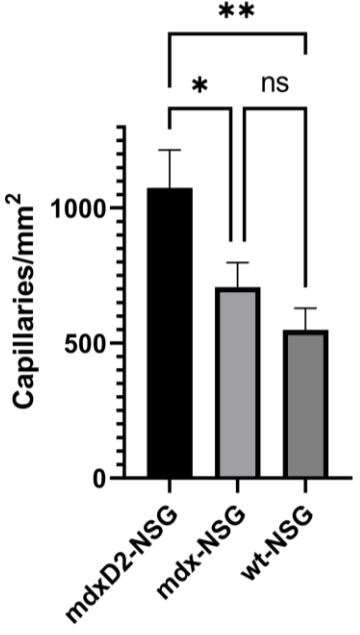
severity increases in dystrophic mouse models

Kholoud K. Saleh, Haibin Xi, Corey Switzler, Emily Skuratovsky, Matthew A. Romero, Peggie Chien, Devin Gibbs, Lily Gane, Michael R. Hicks, Melissa J. Spencer, and April D. Pyle

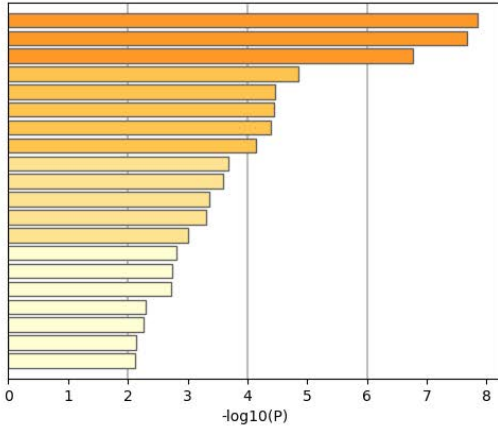
A**B****C****D****E**



A

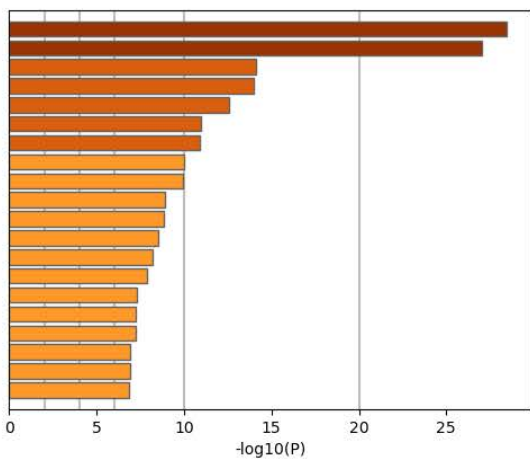


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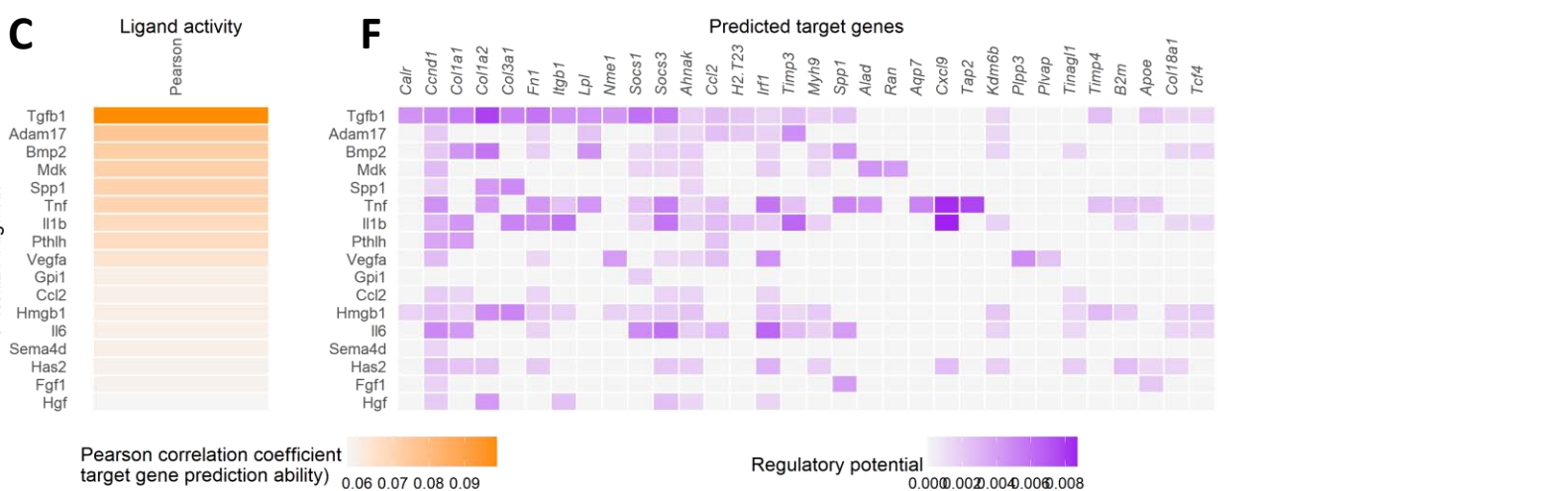
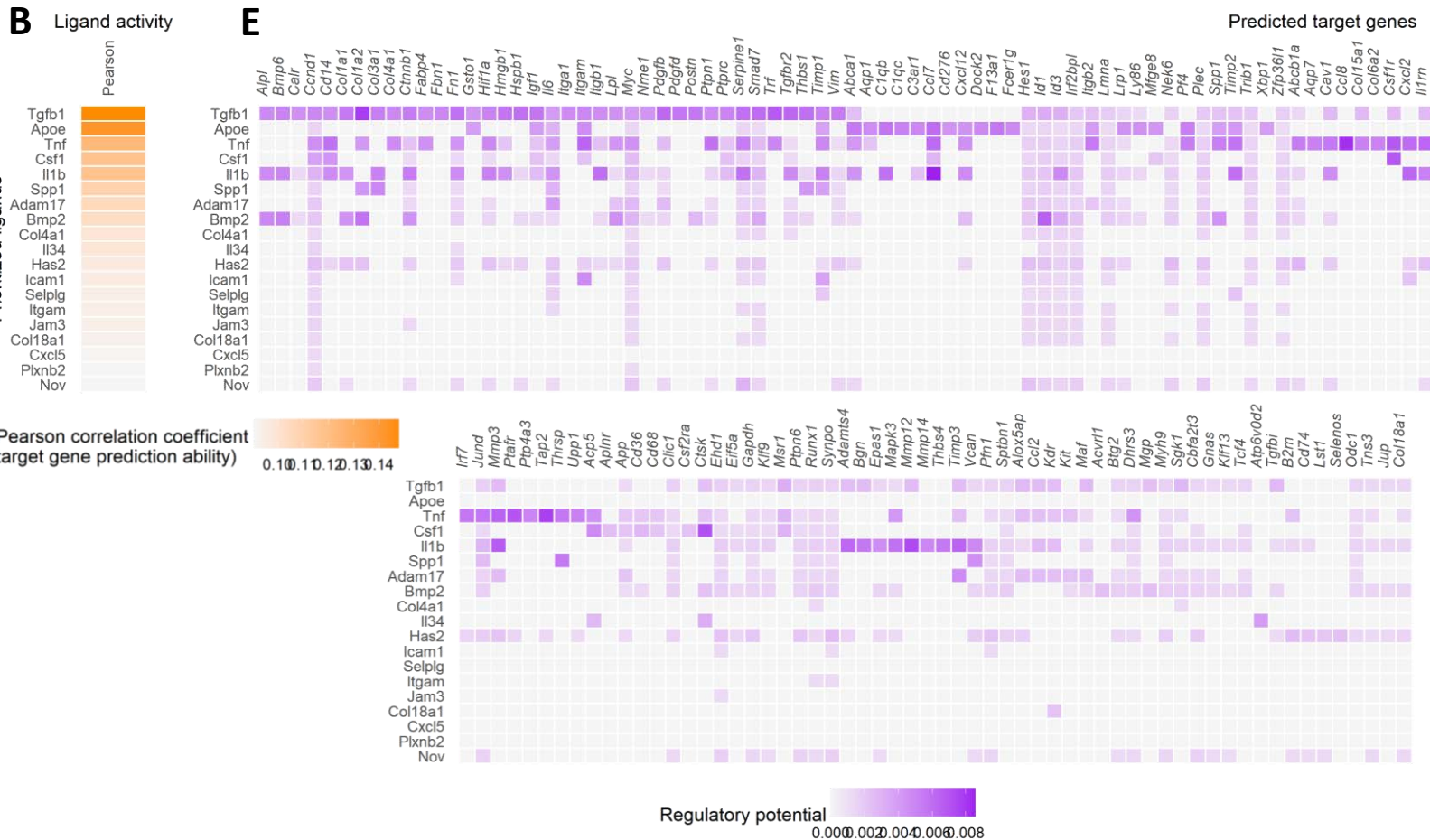
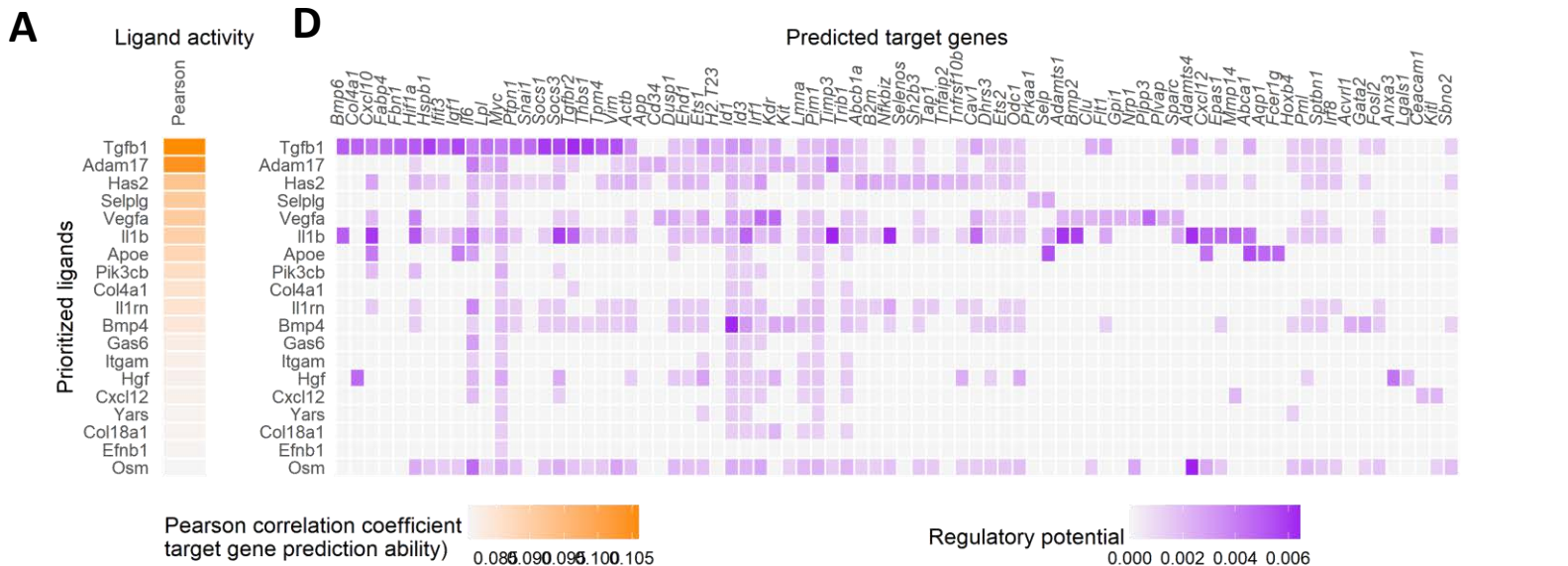


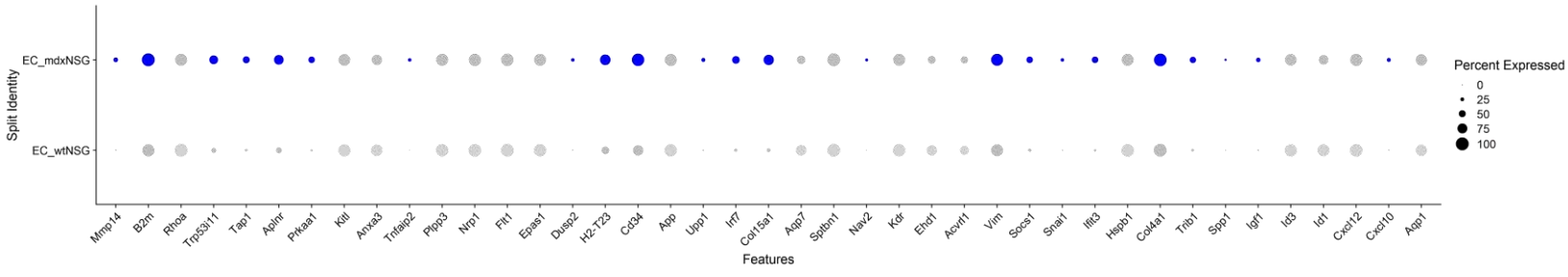
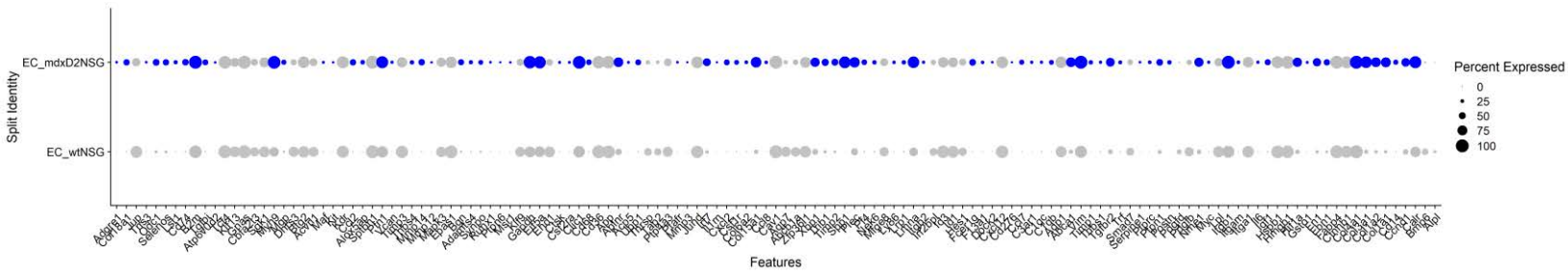
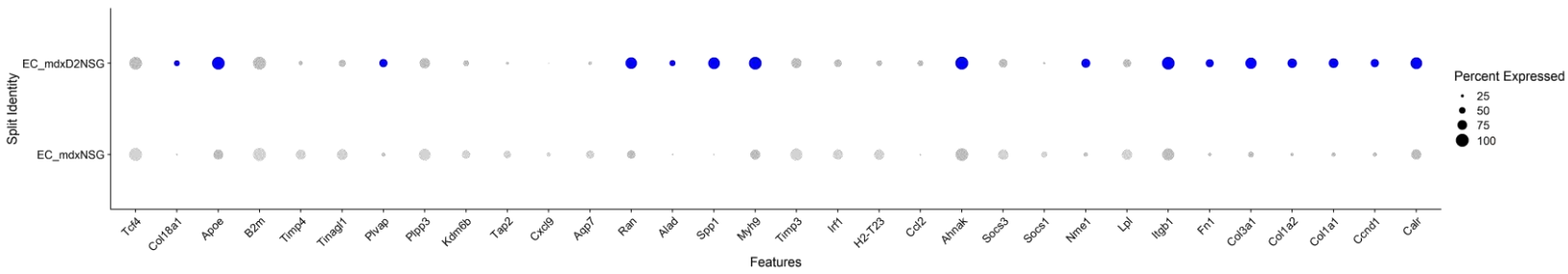
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- GO:0045765: regulation of angiogenesis
- R-MMU-446728: Cell junction organization
- GO:0045446: endothelial cell differentiation
- GO:2000047: regulation of cell-cell adhesion mediated by cadherin
- GO:1903053: regulation of extracellular matrix organization
- GO:0019755: one-carbon compound transport
- GO:0045216: cell-cell junction organization
- GO:0032272: negative regulation of protein polymerization
- GO:0090066: regulation of anatomical structure size
- WP1259: Retinol metabolism
- GO:0050680: negative regulation of epithelial cell proliferation
- GO:0051345: positive regulation of hydrolase activity
- GO:0030335: positive regulation of cell migration
- GO:0022604: regulation of cell morphogenesis
- GO:0007264: small GTPase mediated signal transduction
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- GO:0001666: response to hypoxia
- GO:0007163: establishment or maintenance of cell polarity

C



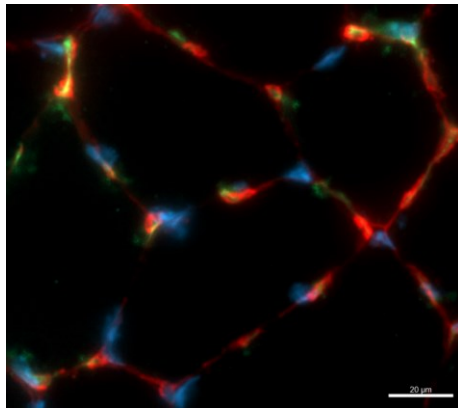
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- R-MMU-1474244: Extracellular matrix organization
- R-MMU-381426: Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)
- GO:0030335: positive regulation of cell migration
- GO:0032103: positive regulation of response to external stimulus
- GO:2000146: negative regulation of cell motility
- GO:0001503: ossification
- GO:0001944: vasculature development
- GO:0008285: negative regulation of cell population proliferation
- GO:0048589: developmental growth
- GO:0032963: collagen metabolic process
- GO:0030155: regulation of cell adhesion
- GO:0001501: skeletal system development
- R-MMU-2243919: Crosslinking of collagen fibrils
- GO:0002683: negative regulation of immune system process
- GO:0022612: gland morphogenesis
- GO:0014910: regulation of smooth muscle cell migration
- GO:0042060: wound healing
- GO:0045667: regulation of osteoblast differentiation
- GO:0050920: regulation of chemotaxis



G**H****I**

A

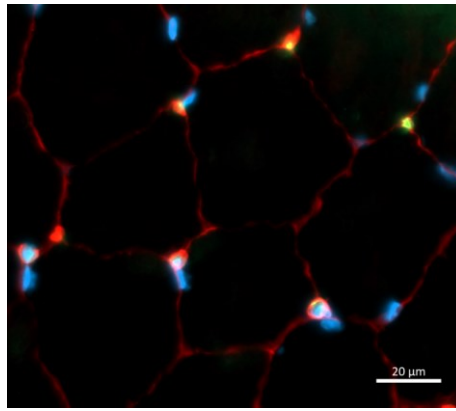
COLIV Cd31 DAPI



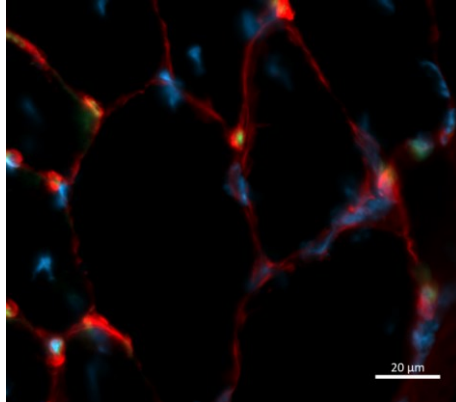
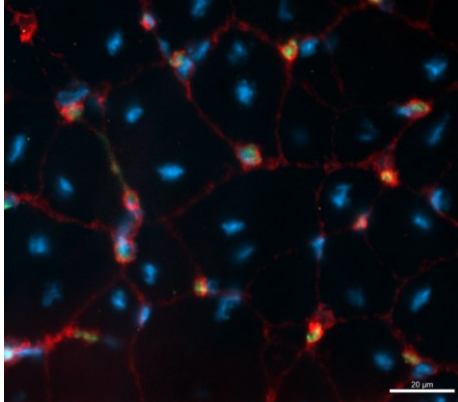
wt-NSG

B

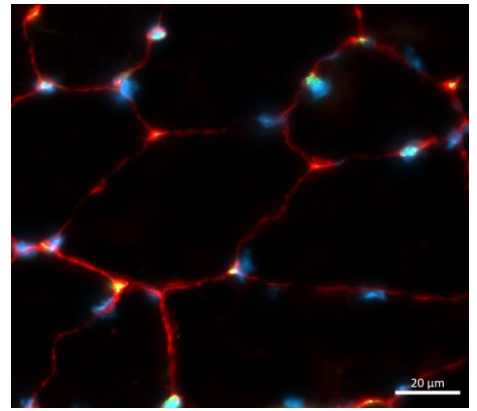
COLI CD31 DAPI



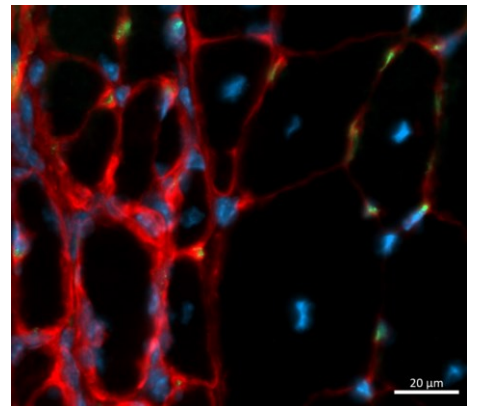
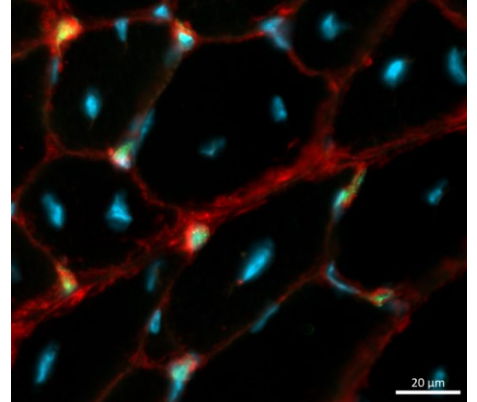
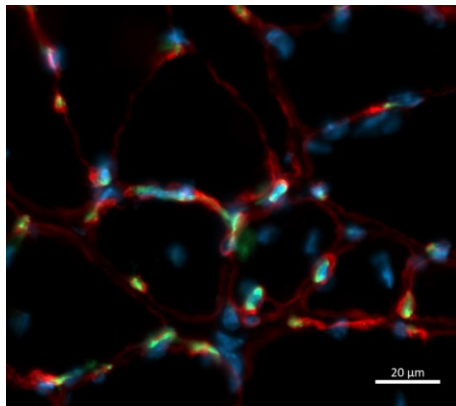
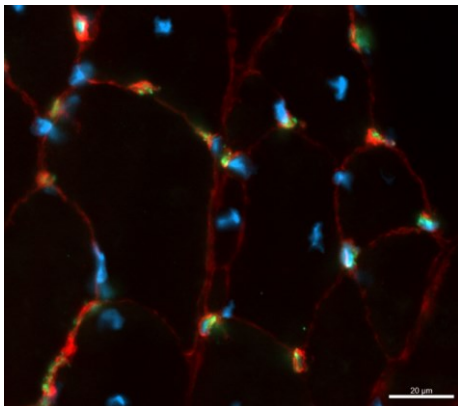
mdx-NSG

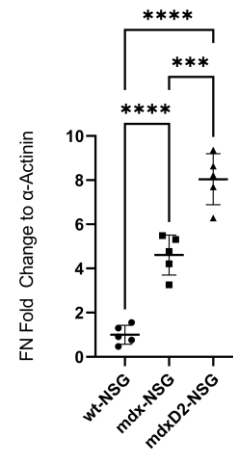
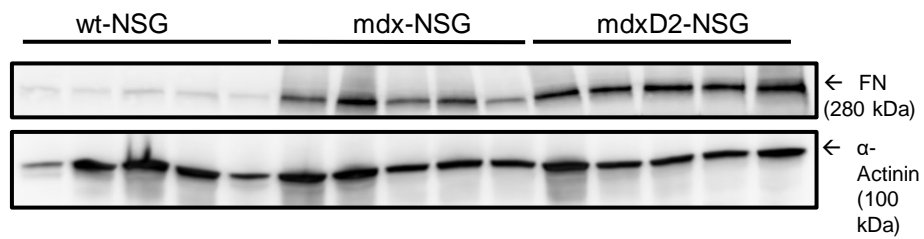
**C**

COLVI CD31 DAPI



mdxD2-NSG

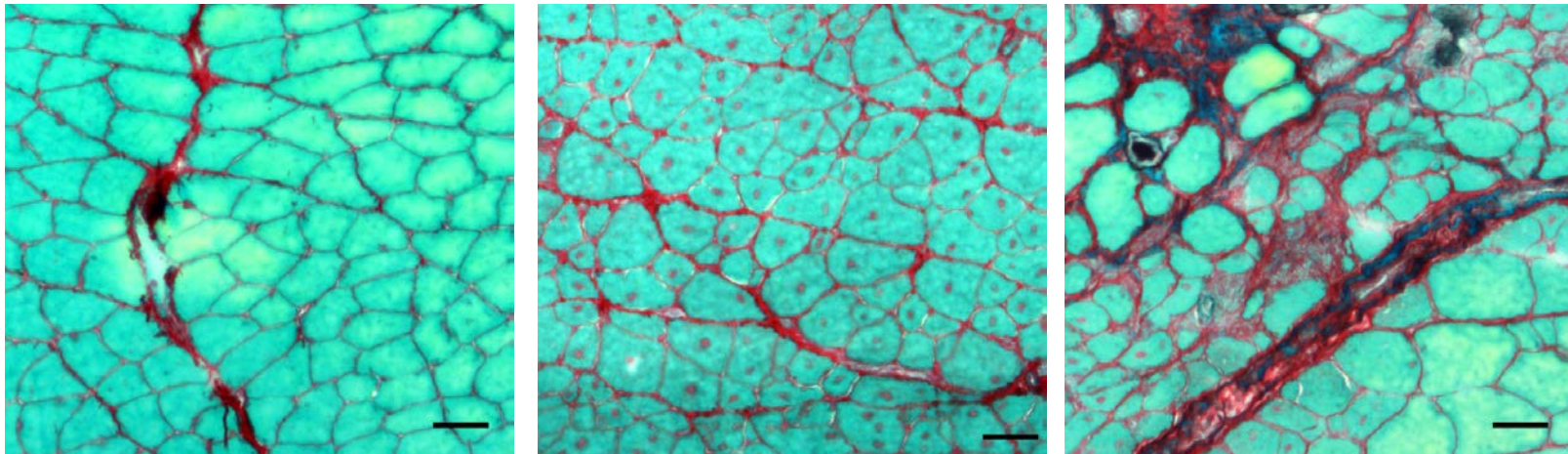
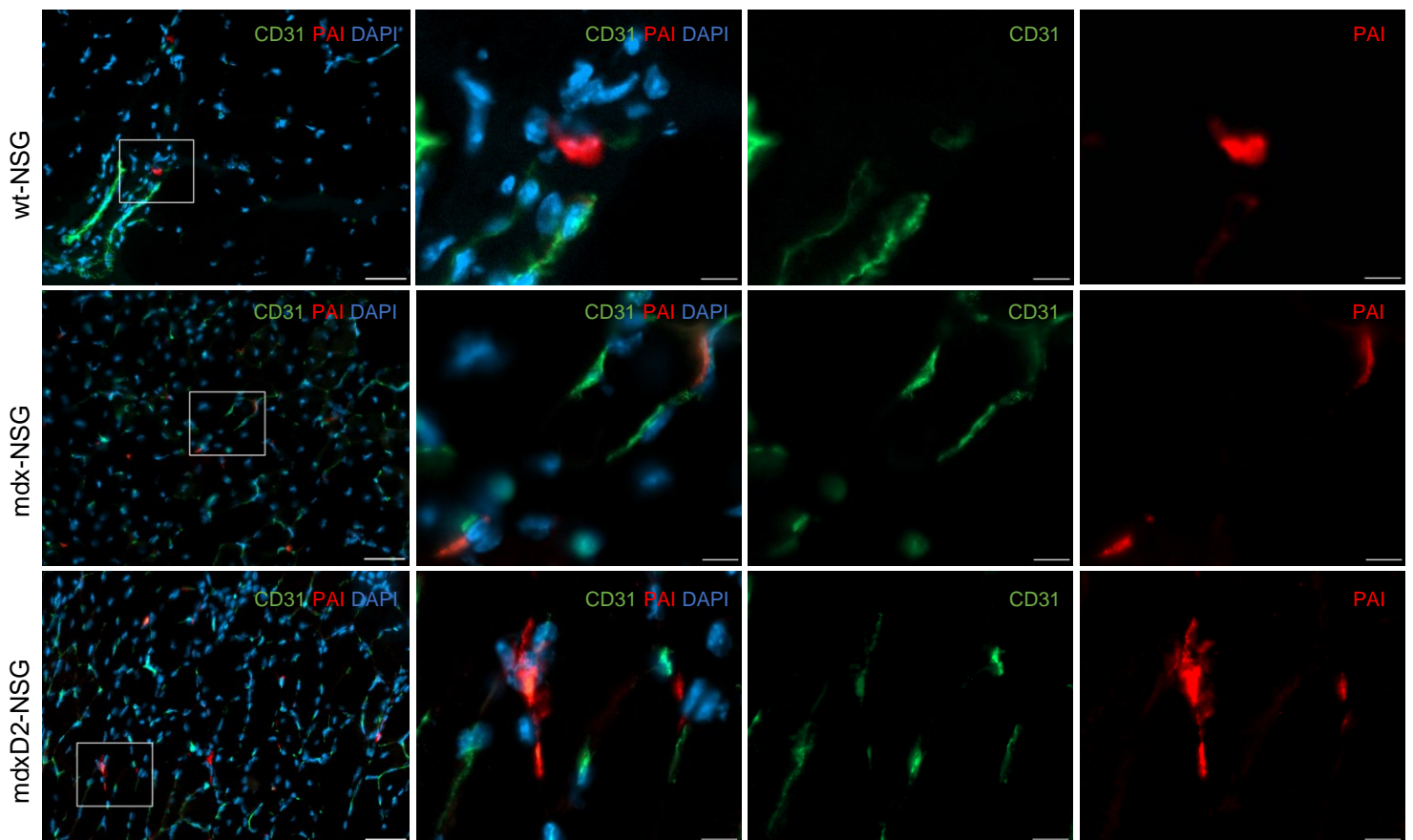


A**B**

wt-NSG

mdx-NSG

mdxD2-NSG

**C**

SUPPLEMENTAL INFORMATION

Supplemental Figures legends

Figure S1 Related to Figure 1: Quality control for scRNA-seq

Violin plots of analyzed samples showing the distribution of the number of genes (nFeature_RNA), number of RNA counts (nCount_RNA) and percent mitochondrial RNA (percent.mt) in (A) replicate 1 and 2 of wt-NSG gastrocnemius muscles. (B) replicate 1 and 2 of mdx-NSG gastrocnemius muscles. (C) Replicate 1 and 2 of gastrocnemius mdxD2-NSG muscles. (D) The integration of all sample replicates. (E) UMAP embedding of all muscles integrated grouped by replicates.

Figure S2 related to Figure 3: Gene expression profile of stromal cell subtypes.

(A) Heatmap of top 10 genes expressed in each stromal cell subtype.

(B) Violin plot of an expanded panel gene expression used to identify stromal cells subtypes.

Figure S3 related to Figure 5: Capillary endothelial cell differences

(A) Left: capillary Density of 8 weeks gastrocnemius muscles in each mouse model. Right: IF images of representative gastrocnemius muscle cross-sections of the mouse models used for quantification, only capillaries were counted per cross-section. Scale bar on IF images are at 20 μm . Quantification was performed using ImageJ on at least 30 images for each mouse, $n=3$ per mouse model. One Way-ANOVA with Tukey's multiple comparison test, mean+SD, * $P<0.05$, ** $P<0.01$

(B) GO enriched terms of DEGs in Capillary EC.1.

(C) GO enriched terms of DEGS in Capillary EC.2.

Figure S4 related to Figure 6: Outcome of NicheNet's ligand activity prediction released from stromal cells and macrophages in dystrophic muscle and their target gene expression regulated on EC.

Outcome of NicheNet's ligand activity prediction of stromal cells and macrophages in (A) mdx-NSG (wt-NSG as reference). (B) mdxD2-NSG (wt-NSG as reference) and (C) mdxD2-NSG (mdx-NSG as reference). Pearson correlation coefficient was used between prior regulatory potential scores and endothelial cell gene set assignments. Pearson correlation indicates the ability of each ligand to predict the endothelial cell (EC) target gene, therefore better predictive ligands are ranked higher.

NicheNet's ligand-target matrix denoting the regulatory potential between predicted released ligands and target gene from EC in (D) mdx-NSG ECs and (E) mdxD2-NSG ECs (F) mdxD2-NSG ECs (mdx-NSG as reference).

(G) Dot plot showing the average expression of NicheNet's predicted target genes on ECs in wt-NSG and mdx-NSG. (H) Dot plot showing the average expression of NicheNet's predicted target genes on ECs in wt-NSG and mdxD2-NSG. (I) Dot plot showing the average expression of NicheNet's predicted target genes on ECs in mdxD2- NSG and mdx-NSG

Figure S5 related to Figure 7: ECM protein staining in wt-NSG, mdx-NSG and mdxD2-NSG of gastrocnemius muscle cross sections

Immunofluorescence staining of gastrocnemius muscle cross-sections for vasculature (CD31, green), and nuclear marker (DAPI, blue) and column (A) collagen type IV (COLIV, red), (B) collagen type I (COLI, red), (C) Collagen type VI (COLVI, red) (Scale bar at 20 μm).

Figure S6 related to figure 7: Validation of fibronectin and plasminogen activator inhibitor-1 upregulation in severely dystrophic gastrocnemius muscle

- (A) Fibronectin (FN) expression analysis by western blotting in whole muscle lysates of healthy, dystrophic and severely dystrophic muscles (graph show individual data points of fold change to α -Actinin, One way ANOVA with Tukey's multiple comparisons, mean \pm SD. *** $P\leq 0.001$, **** $P<0.001$)
- (B) Sirius red stained gastrocnemius skeletal muscle cross-sections of healthy, dystrophic and severely dystrophic mouse models (Scale bar at 50 μm).
- (C) Immunofluorescence staining for plasminogen activator inhibitor-1 (PAI-1) and CD31 colocalization of gastrocnemius skeletal muscles across the mouse models.

Table S1 related to Figure 1: Cell counts per mouse model

Sample	Cell subpopulation	Cell Number	Proportion
wtNSG	MuSC	97	2.320574
wtNSG	MC	62	1.483254
wtNSG	EC	519	12.41627
wtNSG	Stromal	2604	62.29665
wtNSG	Peri	78	1.866029
wtNSG	Teno	130	3.110048
wtNSG	Schwann	10	0.239234
wtNSG	Mphage	639	15.28708
wtNSG	Neutro	30	0.717703
wtNSG	Mast	11	0.263158
mdxNSG	MuSC	123	2.149222
mdxNSG	MC	57	0.995981
mdxNSG	EC	222	3.879084
mdxNSG	Stromal	2446	42.73982
mdxNSG	Peri	33	0.576621
mdxNSG	Teno	216	3.774244
mdxNSG	Schwann	5	0.087367
mdxNSG	Mphage	2560	44.73178
mdxNSG	Neutro	38	0.663987
mdxNSG	Mast	10	0.174734
mdxNSG	DC	13	0.227154
mdxD2NSG	MuSC	49	0.444767
mdxD2NSG	MC	16	0.14523
mdxD2NSG	EC	249	2.260143
mdxD2NSG	Stromal	2738	24.8525
mdxD2NSG	Peri	49	0.444767
mdxD2NSG	Teno	330	2.995371
mdxD2NSG	Schwann	10	0.090769
mdxD2NSG	Mphage	7426	67.40492
mdxD2NSG	Neutro	64	0.58092
mdxD2NSG	Mast	6	0.054461
mdxD2NSG	DC	80	0.72615

Table S3 related to Figure 5: Top 20 genes expressed in each endothelial cell subpopulation cluster

p_val	p_val_adj	avg_logFC	pct.1	pct.2	pct.diff	cluster	gene
1.70E-98	3.37E-94	1.062243	0.99	0.733	0.257	Capillary.EC.1	<i>Gpihbp1</i>
9.88E-82	1.95E-77	1.131859	0.947	0.574	0.373	Capillary.EC.1	<i>Rgcc</i>
2.24E-68	4.43E-64	0.865167	0.962	0.727	0.235	Capillary.EC.1	<i>Kdr</i>
2.98E-68	5.90E-64	0.865231	0.96	0.746	0.214	Capillary.EC.1	<i>Tspan13</i>
1.72E-66	3.39E-62	0.863168	0.916	0.61	0.306	Capillary.EC.1	<i>Cd300lg</i>
1.56E-64	3.08E-60	0.626818	0.999	0.824	0.175	Capillary.EC.1	<i>Cdh5</i>
3.70E-64	7.31E-60	1.065456	0.948	0.636	0.312	Capillary.EC.1	<i>Aqp1</i>
4.18E-58	8.27E-54	0.758685	0.94	0.778	0.162	Capillary.EC.1	<i>Cd200</i>
7.53E-56	1.49E-51	0.891523	0.938	0.763	0.175	Capillary.EC.1	<i>Tcf15</i>
4.85E-55	9.60E-51	1.204337	0.789	0.445	0.344	Capillary.EC.1	<i>Timp4</i>
1.86E-54	3.68E-50	0.80661	0.913	0.737	0.176	Capillary.EC.1	<i>Cav2</i>
6.22E-53	1.23E-48	0.831158	0.808	0.487	0.321	Capillary.EC.1	<i>Clic5</i>
7.30E-53	1.44E-48	0.66859	0.996	0.841	0.155	Capillary.EC.1	<i>Ly6c1</i>
1.03E-52	2.04E-48	0.682497	0.872	0.659	0.213	Capillary.EC.1	<i>Ctnnbip1</i>
2.54E-51	5.02E-47	0.738827	0.914	0.646	0.268	Capillary.EC.1	<i>C1qtnf9</i>
1.17E-49	2.31E-45	0.718219	0.841	0.57	0.271	Capillary.EC.1	<i>Jup</i>
1.31E-49	2.58E-45	0.779908	0.866	0.646	0.22	Capillary.EC.1	<i>Lims2</i>
1.22E-48	2.41E-44	0.685654	0.942	0.767	0.175	Capillary.EC.1	<i>Afdn</i>
6.95E-47	1.38E-42	0.811243	0.897	0.676	0.221	Capillary.EC.1	<i>Tcim</i>
1.07E-44	2.11E-40	0.966124	0.58	0.208	0.372	Capillary.EC.1	<i>Car4</i>
1.62E-44	3.20E-40	0.547063	0.944	0.75	0.194	Capillary.EC.1	<i>Emcn</i>
1.29E-90	2.56E-86	1.137749	0.59	0.013	0.577	Capillary.EC.2	<i>Scara5</i>
4.09E-77	8.10E-73	0.536022	0.41	0.005	0.405	Capillary.EC.2	<i>Mme</i>
6.00E-75	1.19E-70	1.300116	0.692	0.032	0.66	Capillary.EC.2	<i>Abi3bp</i>
1.34E-69	2.65E-65	0.750619	0.538	0.016	0.522	Capillary.EC.2	<i>Svep1</i>
4.34E-66	8.59E-62	0.585115	0.538	0.018	0.52	Capillary.EC.2	<i>Zfhx4</i>
2.94E-64	5.81E-60	0.487362	0.385	0.006	0.379	Capillary.EC.2	<i>Sox9</i>
3.96E-64	7.84E-60	0.964631	0.538	0.02	0.518	Capillary.EC.2	<i>Fbln1</i>
3.81E-62	7.54E-58	0.960262	0.513	0.018	0.495	Capillary.EC.2	<i>Gfpt2</i>
2.43E-58	4.80E-54	1.075102	0.513	0.021	0.492	Capillary.EC.2	<i>Dpep1</i>
1.09E-55	2.15E-51	1.194491	0.667	0.045	0.622	Capillary.EC.2	<i>Fndc1</i>
1.27E-55	2.50E-51	0.655964	0.487	0.019	0.468	Capillary.EC.2	<i>Podn</i>
2.30E-55	4.55E-51	1.001715	0.641	0.041	0.6	Capillary.EC.2	<i>Pdgfra</i>
7.12E-51	1.41E-46	1.281911	0.487	0.023	0.464	Capillary.EC.2	<i>Angptl1</i>
2.36E-49	4.68E-45	0.644649	0.436	0.017	0.419	Capillary.EC.2	<i>Arhgap20</i>
6.31E-49	1.25E-44	1.099917	0.615	0.044	0.571	Capillary.EC.2	<i>Loxl1</i>
6.74E-49	1.33E-44	1.238539	0.59	0.04	0.55	Capillary.EC.2	<i>Htra3</i>
2.29E-48	4.54E-44	0.757599	0.538	0.031	0.507	Capillary.EC.2	<i>Scn7a</i>
7.91E-48	1.57E-43	0.677453	0.487	0.024	0.463	Capillary.EC.2	<i>Lpar1</i>
4.74E-46	9.38E-42	0.564926	0.564	0.036	0.528	Capillary.EC.2	<i>Egfr</i>
6.07E-46	1.20E-41	0.535627	0.41	0.016	0.394	Capillary.EC.2	<i>Scara3</i>
2.82E-152	5.58E-148	0.681372	0.768	0.011	0.757	Activated.EC	<i>Arhgap30</i>

1.20E-148	2.37E-144	1.227475	0.826	0.019	0.807	Activated.EC	<i>Fcgr2b</i>
1.17E-144	2.31E-140	1.308057	0.884	0.03	0.854	Activated.EC	<i>Fyb</i>
6.71E-141	1.33E-136	1.156838	0.725	0.012	0.713	Activated.EC	<i>Ms4a6b</i>
1.32E-139	2.61E-135	1.153249	0.841	0.026	0.815	Activated.EC	<i>Adgre1</i>
5.90E-139	1.17E-134	1.83153	0.899	0.037	0.862	Activated.EC	<i>Cybb</i>
3.69E-138	7.30E-134	0.998384	0.855	0.028	0.827	Activated.EC	<i>Ncf2</i>
1.85E-137	3.67E-133	1.321298	0.826	0.026	0.8	Activated.EC	<i>C3ar1</i>
3.35E-137	6.64E-133	1.099285	0.855	0.03	0.825	Activated.EC	<i>Spi1</i>
2.94E-136	5.81E-132	0.711061	0.609	0.004	0.605	Activated.EC	<i>Tnfaip8l2</i>
4.74E-136	9.38E-132	1.157516	0.812	0.024	0.788	Activated.EC	<i>Aif1</i>
1.54E-135	3.05E-131	0.810659	0.768	0.019	0.749	Activated.EC	<i>Clec4a1</i>
3.62E-135	7.17E-131	1.588221	0.855	0.031	0.824	Activated.EC	<i>Ms4a6c</i>
7.76E-134	1.54E-129	1.39379	0.913	0.041	0.872	Activated.EC	<i>Ptprc</i>
1.04E-133	2.05E-129	0.739551	0.667	0.009	0.658	Activated.EC	<i>Dock2</i>
1.21E-132	2.40E-128	1.230584	0.855	0.031	0.824	Activated.EC	<i>Ly86</i>
1.36E-130	2.70E-126	0.957598	0.768	0.021	0.747	Activated.EC	<i>Csf2ra</i>
3.29E-130	6.51E-126	0.731448	0.667	0.01	0.657	Activated.EC	<i>Epsti1</i>
1.72E-129	3.39E-125	0.966317	0.696	0.014	0.682	Activated.EC	<i>Fcgr1</i>
1.01E-128	1.99E-124	1.990227	0.942	0.055	0.887	Activated.EC	<i>Laptm5</i>
4.66E-87	9.22E-83	1.302079	0.664	0.06	0.604	Arterial.EC	<i>Alpl</i>
1.71E-86	3.38E-82	1.458309	0.744	0.093	0.651	Arterial.EC	<i>Sema3g</i>
1.71E-84	3.38E-80	1.770119	0.8	0.127	0.673	Arterial.EC	<i>Fbln5</i>
2.46E-80	4.87E-76	1.729555	0.88	0.177	0.703	Arterial.EC	<i>Stmn2</i>
5.95E-80	1.18E-75	1.017539	0.456	0.018	0.438	Arterial.EC	<i>Gja5</i>
2.67E-79	5.28E-75	1.148044	0.624	0.06	0.564	Arterial.EC	<i>Col8a1</i>
1.80E-67	3.56E-63	0.789036	0.528	0.045	0.483	Arterial.EC	<i>Eps8l2</i>
4.49E-61	8.89E-57	0.706346	0.472	0.037	0.435	Arterial.EC	<i>Crispld1</i>
1.30E-60	2.58E-56	0.782563	0.488	0.043	0.445	Arterial.EC	<i>St8sia6</i>
1.63E-60	3.22E-56	1.355213	0.784	0.166	0.618	Arterial.EC	<i>Gja4</i>
1.46E-58	2.90E-54	1.206113	0.704	0.13	0.574	Arterial.EC	<i>Hey1</i>
2.07E-58	4.09E-54	0.464141	0.264	0.002	0.262	Arterial.EC	<i>Nebl</i>
1.38E-50	2.74E-46	1.280464	0.6	0.104	0.496	Arterial.EC	<i>Edn1</i>
3.49E-42	6.90E-38	0.933705	0.512	0.09	0.422	Arterial.EC	<i>Vegfc</i>
1.73E-37	3.42E-33	0.476869	0.288	0.022	0.266	Arterial.EC	<i>Alox12</i>
3.96E-35	7.83E-31	0.67643	0.44	0.077	0.363	Arterial.EC	<i>Atp2a3</i>
1.46E-33	2.88E-29	0.760157	0.432	0.077	0.355	Arterial.EC	<i>Tgfb2</i>
1.46E-32	2.89E-28	0.831464	0.464	0.099	0.365	Arterial.EC	<i>Palld</i>
3.06E-32	6.06E-28	0.551942	0.392	0.061	0.331	Arterial.EC	<i>Car7</i>
5.44E-31	1.08E-26	1.000036	0.76	0.351	0.409	Arterial.EC	<i>Pdcd4</i>
7.03E-138	1.39E-133	2.182839	0.782	0.025	0.757	Venous.EC	<i>Selp</i>
1.62E-104	3.21E-100	1.273694	0.828	0.062	0.766	Venous.EC	<i>Chp2</i>
7.25E-81	1.43E-76	1.220817	0.69	0.056	0.634	Venous.EC	<i>Slco2a1</i>
6.41E-77	1.27E-72	2.120923	1	0.217	0.783	Venous.EC	<i>Plvap</i>
4.35E-75	8.60E-71	2.06285	0.897	0.146	0.751	Venous.EC	<i>Lrg1</i>
3.61E-74	7.15E-70	0.845658	0.575	0.039	0.536	Venous.EC	<i>Ctnnal1</i>
3.88E-64	7.67E-60	1.898819	0.954	0.239	0.715	Venous.EC	<i>Vwf</i>

6.09E-63	1.20E-58	1.171017	0.862	0.156	0.706	Venous.EC	<i>Ptgs1</i>
7.75E-61	1.53E-56	1.206662	0.356	0.011	0.345	Venous.EC	<i>Ackr1</i>
1.25E-56	2.48E-52	0.832853	0.644	0.076	0.568	Venous.EC	<i>Cysltr1</i>
4.21E-56	8.33E-52	0.981141	0.529	0.049	0.48	Venous.EC	<i>Vcam1</i>
9.33E-56	1.85E-51	0.773888	0.701	0.1	0.601	Venous.EC	<i>Thsd7a</i>
6.20E-54	1.23E-49	1.820038	0.989	0.421	0.568	Venous.EC	<i>Ehd4</i>
1.45E-52	2.87E-48	0.599418	0.414	0.026	0.388	Venous.EC	<i>Lepr</i>
9.73E-51	1.93E-46	0.473249	0.276	0.007	0.269	Venous.EC	<i>Fam174b</i>
3.17E-49	6.28E-45	1.670995	1	0.503	0.497	Venous.EC	<i>Il6st</i>
1.75E-48	3.46E-44	1.280101	0.816	0.193	0.623	Venous.EC	<i>Rasa4</i>
2.53E-46	5.01E-42	0.499891	0.287	0.01	0.277	Venous.EC	<i>Enpp6</i>
3.45E-46	6.82E-42	0.893335	0.471	0.049	0.422	Venous.EC	<i>Lbp</i>
4.11E-46	8.12E-42	0.530151	0.402	0.031	0.371	Venous.EC	<i>Plekha7</i>
1.36E-42	2.68E-38	1.468284	0.92	0.294	0.626	Venous.EC	<i>Eln</i>
2.60E-178	5.15E-174	2.834275	0.941	0.05	0.891	Pericytes	<i>Vtn</i>
2.07E-174	4.10E-170	2.736126	0.901	0.041	0.86	Pericytes	<i>Kcnj8</i>
3.61E-170	7.15E-166	2.418101	0.954	0.065	0.889	Pericytes	<i>Pdgfrb</i>
6.07E-167	1.20E-162	1.855282	0.888	0.039	0.849	Pericytes	<i>Gucy1a1</i>
1.72E-166	3.40E-162	1.920828	0.921	0.051	0.87	Pericytes	<i>Notch3</i>
1.77E-163	3.51E-159	2.356185	0.934	0.064	0.87	Pericytes	<i>Steap4</i>
3.35E-160	6.63E-156	2.506243	0.901	0.059	0.842	Pericytes	<i>Abcc9</i>
3.95E-156	7.81E-152	1.723156	0.875	0.049	0.826	Pericytes	<i>Gucy1b1</i>
1.68E-155	3.32E-151	1.651181	0.803	0.028	0.775	Pericytes	<i>Rgs4</i>
9.01E-154	1.78E-149	1.352332	0.77	0.024	0.746	Pericytes	<i>Heyl</i>
2.21E-153	4.37E-149	1.867014	0.855	0.048	0.807	Pericytes	<i>Higd1b</i>
2.09E-149	4.14E-145	1.805337	0.882	0.062	0.82	Pericytes	<i>Cox4i2</i>
2.05E-143	4.05E-139	2.107985	0.934	0.092	0.842	Pericytes	<i>Aspn</i>
1.05E-141	2.07E-137	1.196336	0.77	0.035	0.735	Pericytes	<i>Arhgef17</i>
5.20E-134	1.03E-129	2.38312	0.934	0.115	0.819	Pericytes	<i>Ndufa4l2</i>
2.23E-133	4.41E-129	1.686806	0.868	0.079	0.789	Pericytes	<i>Cygb</i>
2.32E-127	4.58E-123	1.32045	0.822	0.069	0.753	Pericytes	<i>Lhfp</i>
2.97E-127	5.88E-123	1.109595	0.664	0.023	0.641	Pericytes	<i>Itga7</i>
1.09E-125	2.15E-121	1.188494	0.724	0.037	0.687	Pericytes	<i>Atp1b2</i>
7.59E-125	1.50E-120	1.122424	0.724	0.04	0.684	Pericytes	<i>Cystm1</i>