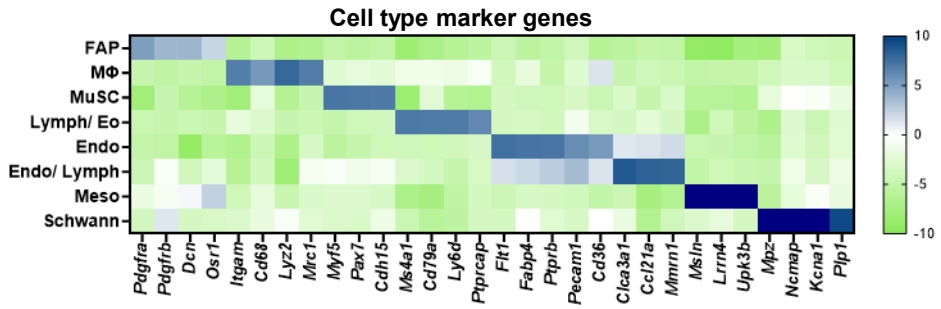
Figure S1: Diaphragm mononuclear cell annotation, FAP1 marker genes

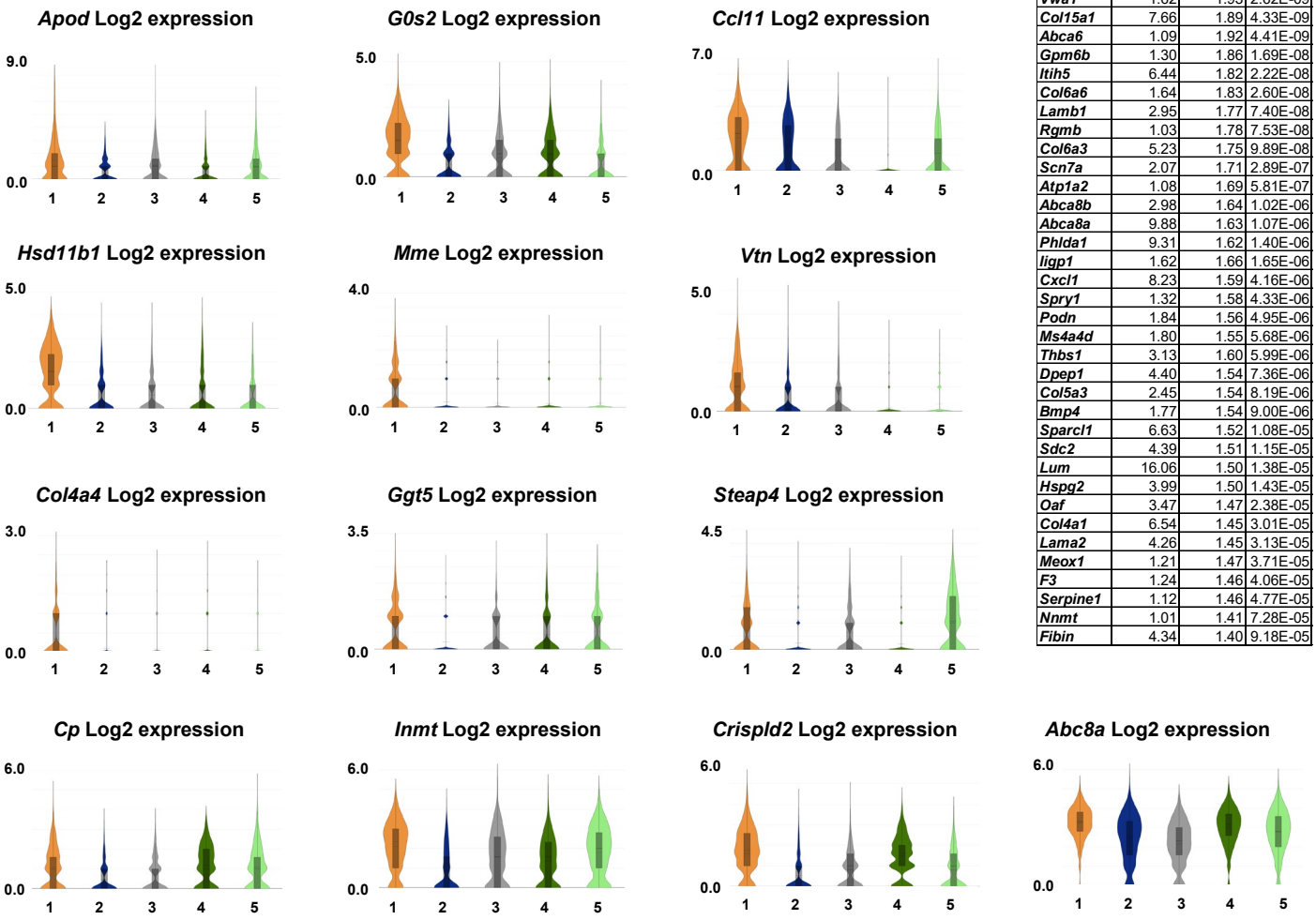
A



B

Gene	Average	Log2 FC	p-val
Cxcl14	12.85	4.01	2.17E-43
Hsd11b1	2.97	2.61	3.17E-18
Vtn	1.73	2.69	3.25E-18
Kcnk2	1.06	2.45	3.73E-15
Apod	6.31	2.72	1.03E-14
Crispld2	3.38	2.37	1.23E-14
Plau	1.92	2.37	1.45E-14
Ccl11	6.20	2.39	1.62E-14
Ces1d	1.80	2.31	9.48E-14
Gask1b	1.33	2.24	8.58E-13
G0s2	2.66	2.19	6.05E-12
Lifr	1.61	2.14	1.09E-11
Adm	2.89	2.08	8.72E-11
Smoc2	14.41	1.97	7.16E-10
Inmt	4.84	1.95	1.28E-09
Vwa1	1.62	1.93	2.62E-09
Col15a1	7.66	1.89	4.33E-09
Abca6	1.09	1.92	4.41E-09
Gpm6b	1.30	1.86	1.69E-08
Itih5	6.44	1.82	2.22E-08
Col6a6	1.64	1.83	2.60E-08
Lamb1	2.95	1.77	7.40E-08
Rgmb	1.03	1.78	7.53E-08
Col6a3	5.23	1.75	9.89E-08
Scn7a	2.07	1.71	2.89E-07
Atp1a2	1.08	1.69	5.81E-07
Abca8b	2.98	1.64	1.02E-06
Abca8a	9.88	1.63	1.07E-06
Phlda1	9.31	1.62	1.40E-06
Iigp1	1.62	1.66	1.65E-06
Cxcl1	8.23	1.59	4.16E-06
Spry1	1.32	1.58	4.33E-06
Podn	1.84	1.56	4.95E-06
Ms4a4d	1.80	1.55	5.68E-06
Thbs1	3.13	1.60	5.99E-06
Dpep1	4.40	1.54	7.36E-06
Col5a3	2.45	1.54	8.19E-06
Bmp4	1.77	1.54	9.00E-06
Sparcl1	6.63	1.52	1.08E-05
Sdc2	4.39	1.51	1.15E-05
Lum	16.06	1.50	1.38E-05
Hspg2	3.99	1.50	1.43E-05
Oaf	3.47	1.47	2.38E-05
Col4a1	6.54	1.45	3.01E-05
Lama2	4.26	1.45	3.13E-05
Meox1	1.21	1.47	3.71E-05
F3	1.24	1.46	4.06E-05
Serpine1	1.12	1.46	4.77E-05
Nnmt	1.01	1.41	7.28E-05
Fibin	4.34	1.40	9.18E-05

C



D

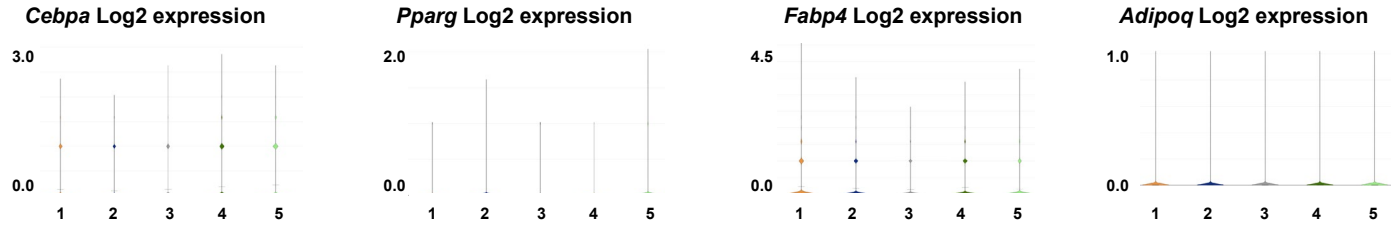


Figure S1. (A) Heat map demonstrating cell-specific gene expression used for diaphragm mononuclear cell annotation. **(B)** FAP1 sub-population: Top 50 enriched genes. **(C)** Violin plots demonstrating expression of adipocyte precursor genes in FAP sub-populations. **(D)** Violin plots demonstrating limited expression of committed preadipocyte and adipocyte markers in FAP sub-populations. FAP1, FAP2, FAP3, FAP4 and FAP5 respectively labeled 1, 2, 3, 4, 5.

Figure S2: FAP2 marker genes

A

Gene	Average	Log2 FC	p-val
Stmn4	1.25	3.62	1.36E-40
Efhd1	1.09	3.60	1.36E-40
Sema3c	2.70	3.17	1.23E-31
Ccn3	1.45	3.05	1.78E-28
Sbsn	1.11	2.85	7.31E-22
Cd55	6.85	2.75	2.87E-24
Fbn1	16.62	2.70	1.85E-23
Pla1a	2.51	2.67	1.99E-22
Thy1	1.06	2.39	3.26E-17
Uap1	4.24	2.38	8.13E-18
Cadm3	1.04	2.35	6.58E-17
Dbn1	1.74	2.31	1.50E-16
Cd248	6.26	2.28	2.46E-16
Gfpt2	4.05	2.22	2.59E-15
Adgrd1	1.54	2.22	3.68E-15
Ugdh	2.95	2.20	7.35E-15
Dpp4	1.39	2.18	1.84E-14
Pcolce2	5.08	2.11	8.04E-14
Vcan	3.01	2.09	1.61E-13
Anxa3	3.88	2.09	1.60E-13
Mustn1	1.34	2.08	6.58E-13
Mfap5	26.85	2.08	1.68E-13
Zfp385a	1.27	2.01	2.50E-12
Pi16	29.97	1.97	4.39E-12
Fstl1	15.37	1.96	5.11E-12
Tmem100	3.08	1.96	9.55E-12
Pcsk6	6.32	1.96	7.44E-12
Creb5	2.08	1.94	1.51E-11
Adamts5	4.98	1.90	4.16E-11
Has1	1.89	1.87	5.86E-10
Ugp2	3.38	1.84	2.02E-10
Axl	4.52	1.81	4.48E-10
Ackr3	5.79	1.80	6.52E-10
Aspn	11.72	1.79	9.45E-10
Emilin2	2.27	1.74	3.71E-09
Fndc1	4.34	1.72	4.92E-09
Efemp1	3.33	1.71	8.77E-09
Postn	1.78	1.66	7.10E-07
Prss23	1.81	1.66	3.60E-08
Hspb8	1.11	1.62	8.55E-08
Thbd	1.39	1.60	1.25E-07
Loxl2	1.61	1.58	1.65E-07
Lrrn4cl	1.51	1.58	1.77E-07
Cd34	9.65	1.57	1.40E-07
Ddr2	2.22	1.55	3.27E-07
Tnxb	9.14	1.52	4.57E-07
Bmp1	1.68	1.52	6.01E-07
Ahnak2	1.81	1.52	6.32E-07
Rhoq	1.61	1.51	7.25E-07
Tppp3	7.58	1.49	9.38E-07

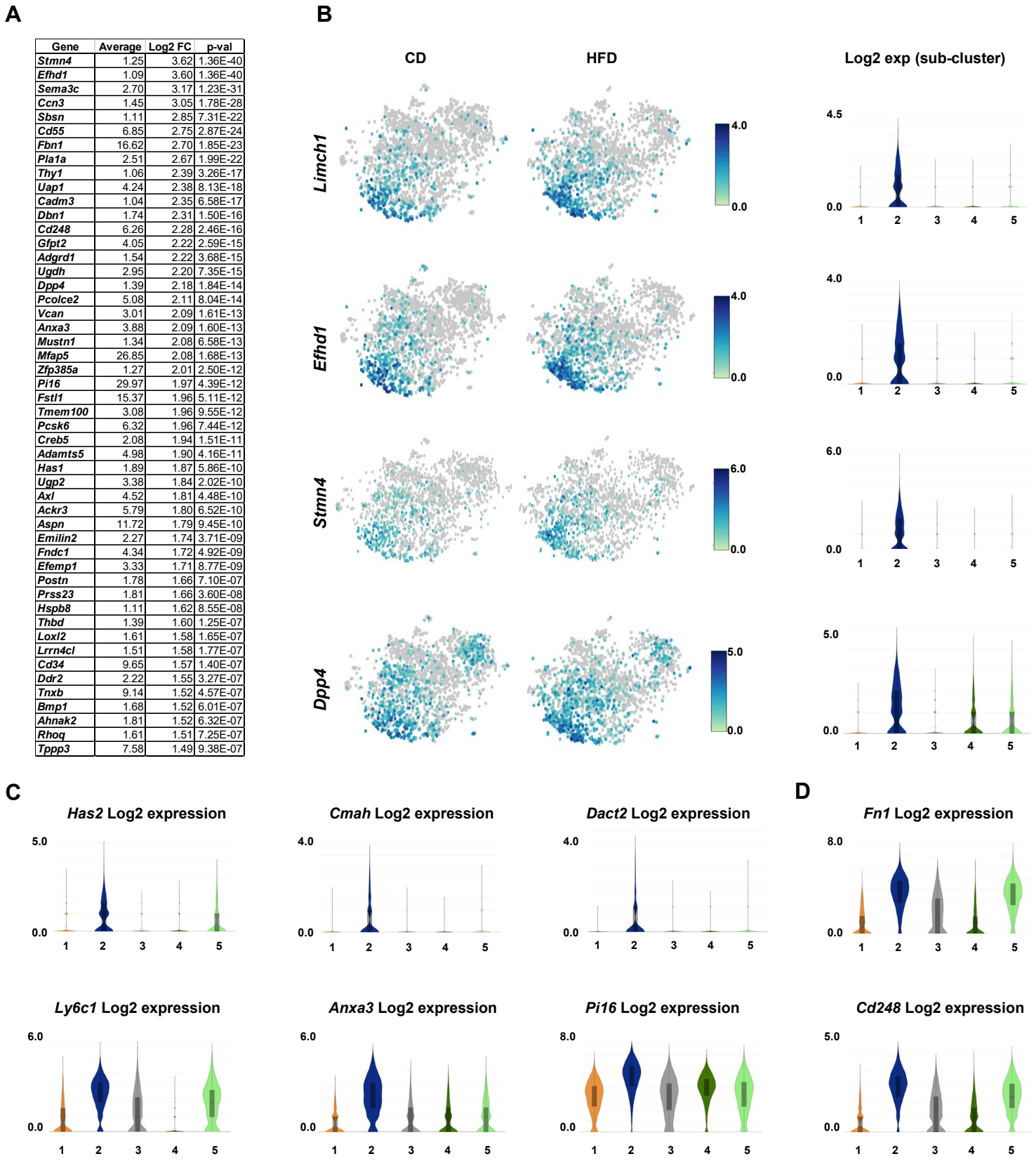


Figure S2. (A) FAP2 sub-population: Top 50 enriched genes. **(B)** FAP2-enriched genes with tSNE plots demonstrating expression in 6-month control diet (CD) versus high fat diet (HFD)-fed FAPs. Color gradient indicates Log2 expression. Violin plots show FAP sub-population-specific expression of the same genes. **(C)** Violin plots indicating sub-population-specific expression of genes previously associated with adipose fibroinflammatory progenitors (FIPs). **(D)** Violin plots indicating sub-population-specific expression of fibronectin (*Fn1*) and endosialin (CD248) (*Cd248*). FAP1, FAP2, FAP3, FAP4 and FAP5 respectively labeled 1, 2, 3, 4, 5.

Figure S3: Marker genes: FAP3, FAP4, FAP5 subclusters

A

Gene	Average	Log2 FC	p-val
Timp1	1.10	1.77	1.79E-05
Sult1e1	0.94	1.58	2.68E-04
Col6a5	2.15	1.52	1.10E-04
Sfrp4	1.78	1.52	3.60E-04
Penk	2.50	1.49	8.34E-05
Apod	3.70	1.48	1.34E-03
Spon2	0.91	1.37	7.81E-04
Rbp1	5.95	1.31	9.33E-04
Igfbp7	17.77	1.30	1.01E-03
Serpinf1	21.00	1.28	1.22E-03
Sfrp1	2.24	1.28	1.63E-03
C7	2.55	1.27	1.87E-03
Phlda3	1.74	1.25	1.93E-03
Pcolce	8.22	1.23	2.23E-03
Cygb	4.85	1.22	2.68E-03
Steap4	0.89	1.22	3.76E-03
Mgp	24.54	1.21	3.54E-03
Cst3	44.70	1.21	3.50E-03
Olfml3	2.03	1.21	3.38E-03
Mmp23	2.31	1.18	4.52E-03
Il11ra1	5.13	1.18	4.23E-03
Mfap2	0.82	1.17	5.88E-03
Gpx3	10.32	1.17	4.98E-03
Inmt	3.30	1.17	5.62E-03
Igfbp4	13.73	1.15	5.95E-03
Gstm2	1.65	1.14	7.05E-03
Nbl1	7.90	1.13	7.62E-03
Mustn1	0.91	1.11	1.25E-02
Gpx8	1.65	1.11	1.00E-02
Selenom	7.40	1.11	9.43E-03
Sod3	1.79	1.11	1.07E-02
Entpd2	4.39	1.10	1.05E-02
Fbln1	4.68	1.09	1.27E-02
F3	1.03	1.07	1.74E-02
Lbp	2.14	1.07	1.60E-02
Lysmd2	1.22	1.06	1.67E-02
Gsn	147.09	1.04	1.89E-02
Mgst1	3.38	1.04	1.94E-02
Nenf	4.91	1.04	2.00E-02
Pdlim2	1.97	1.04	2.16E-02
Csrp2	3.33	1.03	2.32E-02
Ctsk	1.38	1.02	2.53E-02
Dpep1	3.37	1.02	2.56E-02
Tmed3	3.81	1.01	2.66E-02
Tmem100	2.07	1.01	3.00E-02
Vkorc1	2.84	1.01	2.74E-02
Serping1	16.48	1.00	2.84E-02
Maged2	1.81	1.00	3.21E-02
Bgn	14.18	0.99	3.04E-02
Cd302	4.76	0.98	3.47E-02

B

Gene	Average	Log2 FC	p-val
Col6a5	4.05	2.96	2.81E-20
Sult1e1	1.48	2.55	2.08E-12
Cyp1b1	1.04	2.45	5.67E-14
Has1	2.37	2.16	2.27E-10
Cxcl13	1.66	2.10	3.15E-04
Steap4	1.31	1.99	5.99E-09
C3	28.98	1.98	3.40E-09
Fst	2.18	1.87	8.72E-08
Ptgs2	2.79	1.80	4.87E-07
Ifi204	2.10	1.76	4.51E-07
C4b	2.60	1.76	2.09E-06
Tnfaip6	3.80	1.68	2.16E-06
Scara3	1.10	1.67	2.87E-06
Myc	1.85	1.53	3.17E-05
Mmp14	1.29	1.53	3.19E-05
C1s1	5.99	1.53	2.35E-05
Lgi2	1.38	1.51	4.22E-05
Ifi205	2.86	1.47	8.16E-05
Aebp1	9.97	1.46	7.35E-05
Steap3	1.84	1.45	1.06E-04
Ebf2	1.83	1.44	1.27E-04
Ddr2	2.24	1.43	1.33E-04
Penk	2.35	1.38	3.56E-04
Slit3	1.46	1.37	3.60E-04
Cdon	1.37	1.35	4.48E-04
Pdgfra	4.09	1.32	5.74E-04
Ugdh	2.13	1.32	7.44E-04
Hk2	1.87	1.32	8.52E-04
Scara5	3.13	1.32	6.51E-04
Man2a1	2.34	1.30	7.94E-04
Gfpt2	2.88	1.30	8.68E-04
Ifi211	1.64	1.30	8.45E-04
Cd248	4.30	1.28	1.13E-03
C1ra	3.56	1.27	1.23E-03
Igf1	4.43	1.27	1.34E-03
Lox	1.60	1.26	1.80E-03
Tshz2	2.21	1.21	2.55E-03
Arhgap20	1.04	1.21	2.94E-03
Lrp1	10.37	1.20	2.85E-03
Gpc3	2.30	1.19	3.84E-03
Pdgfrb	1.06	1.18	4.08E-03
Ace	2.25	1.18	3.99E-03
Ar	1.90	1.17	4.10E-03
Antxr1	1.23	1.17	4.38E-03
Adgrd1	1.02	1.17	4.86E-03
Col6a6	1.18	1.17	4.82E-03
Gpx3	10.21	1.15	4.97E-03
Plpp3	6.74	1.15	5.34E-03
Adamts2	1.54	1.14	6.26E-03
Efemp1	2.67	1.14	6.76E-03

C

Gene	Average	Log2 FC	p-val
Sfrp2	3.66	4.86	2.90E-59
Fmod	1.63	4.63	3.20E-35
Tnmd	1.37	4.23	2.60E-45
Thbs2	3.62	3.39	1.92E-30
Thbs4	18.67	3.11	9.69E-26
Igfbp3	2.57	3.01	1.69E-21
Col12a1	2.17	2.88	4.50E-21
Pdgfrl	4.03	2.75	8.05E-20
Cpxm2	1.78	2.66	7.81E-18
Mfap4	14.16	2.64	4.68E-18
Angptl1	16.18	2.62	6.04E-18
Lox	2.91	2.53	3.40E-16
Reck	1.60	2.48	1.45E-15
Itgbl1	2.50	2.43	6.11E-15
Eln	3.92	2.42	2.15E-14
C1qtnf2	1.66	2.41	1.29E-14
Igsf10	2.05	2.39	2.51E-14
Ntrk2	3.02	2.35	5.46E-14
Fibin	6.73	2.30	3.33E-13
Tmem119	1.13	2.17	1.44E-11
Cilp	16.72	2.16	1.34E-11
Tcf7l2	2.01	2.08	1.02E-10
Col14a1	10.32	2.06	1.34E-10
Sfrp1	3.28	2.05	3.47E-10
Mfap2	1.22	1.96	2.57E-09
Gas1	19.28	1.96	1.57E-09
Spon2	1.18	1.90	3.01E-08
Olfml3	2.81	1.85	2.37E-08
Col8a1	4.00	1.84	4.15E-08
Ccn2	5.66	1.84	6.08E-08
Ogn	15.44	1.81	4.42E-08
Pcsk5	1.37	1.81	6.81E-08
Pam	5.10	1.80	5.86E-08
Sfrp4	2.01	1.79	2.50E-06
Angptl2	1.03	1.79	1.15E-07
Fxyd6	3.54	1.78	1.04E-07
Svil	2.82	1.77	1.36E-07
Col1a1	12.58	1.76	1.59E-07
Smoc2	13.19	1.76	1.63E-07
Col1a2	30.30	1.73	2.28E-07
Svep1	2.62	1.73	3.22E-07
Mgp	31.77	1.72	4.23E-07
Cpxm1	2.45	1.70	6.74E-07
Rbp1	7.13	1.67	8.81E-07
Mmp23	2.96	1.67	1.04E-06
Itm2a	5.62	1.65	1.33E-06
Nbl1	10.18	1.63	2.01E-06
Aspn	11.67	1.62	2.53E-06
Fbln1	6.17	1.62	2.33E-06
C7	3.04	1.62	3.55E-06

D

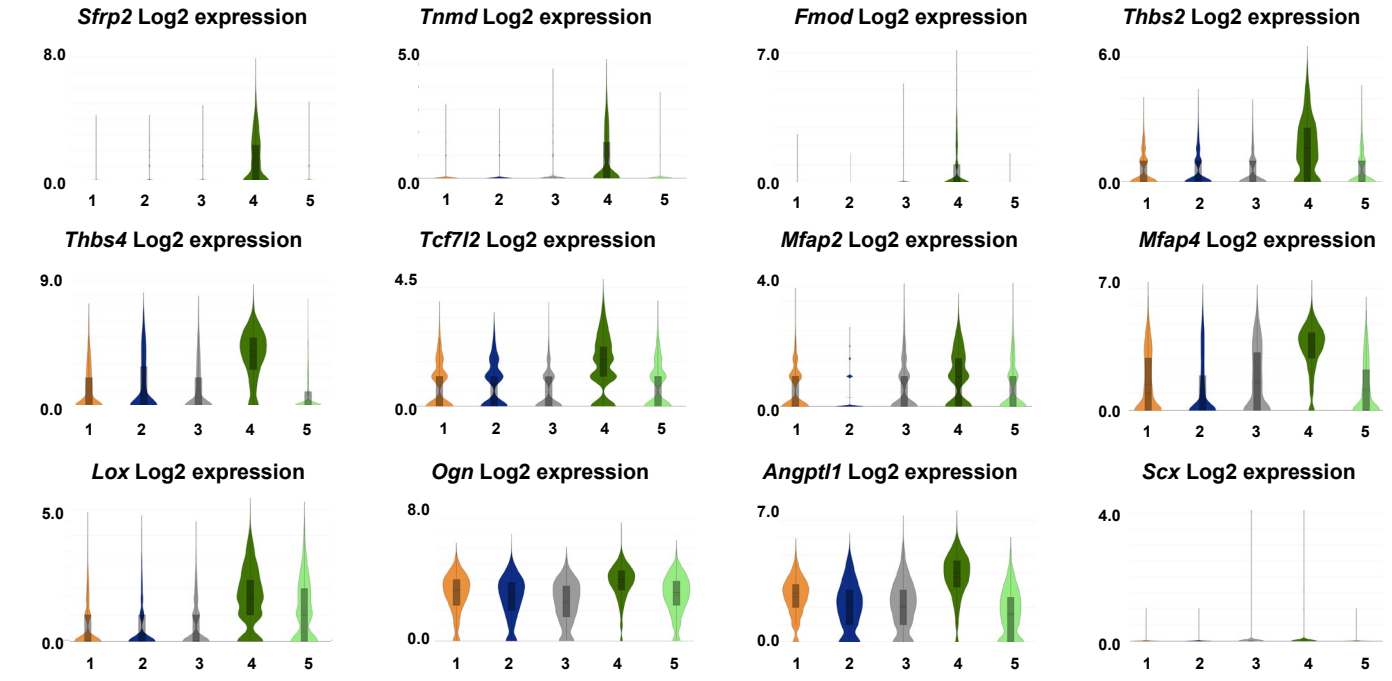


Figure S3. (A) FAP3 sub-population: Top 50 enriched genes. **(B)** FAP5 sub-population: Top 50 enriched genes. **(C)** FAP4 sub-population: Top 50 enriched genes. **(D)** Violin plots demonstrating sub-population-specific expression of selected matricellular and regulatory molecules enriched in FAP4, as well as tenocyte marker scleraixs (*Scx*). FAP1, FAP2, FAP3, FAP4 and FAP5 respectively labeled 1, 2, 3, 4, 5.

Figure S4: Genes enriched in *Thy1*-expressing cells

A

Gene	Average	Log2 FC	p-val
Thy1	1.58	4.16	1.96E-58
Sema3c	1.98	2.29	1.52E-16
Pla1a	2.12	2.26	2.10E-16
Ccn3	1.08	2.24	2.55E-15
Cd55	5.43	2.16	3.56E-15
Fbn1	12.59	2.01	5.95E-13
Pcsk6	6.22	1.98	1.28E-12
Adamts5	4.59	1.76	7.37E-10
Adgrd1	1.24	1.73	2.16E-09
Uap1	3.11	1.66	1.35E-08
Pcolce2	4.09	1.64	1.84E-08
Cd248	4.69	1.63	2.57E-08
Dbn1	1.29	1.62	3.34E-08
Gfpt2	3.05	1.58	8.61E-08
Dpp4	1.06	1.59	1.08E-07
Has1	1.66	1.62	1.27E-07
Zfp385a	1.03	1.56	1.39E-07
Pi16	24.34	1.54	1.97E-07
Creb5	1.72	1.54	2.10E-07
Prss23	1.69	1.54	2.51E-07
Mfap5	20.78	1.52	2.64E-07
Ugdh	2.19	1.54	2.91E-07
Vcan	2.32	1.52	3.27E-07
Fstl1	12.30	1.49	4.64E-07
Ackr3	4.97	1.49	4.82E-07
Tnxb	8.74	1.46	9.36E-07
Anxa3	2.91	1.46	1.14E-06
Axl	3.75	1.43	1.94E-06
Loxl2	1.48	1.43	1.98E-06
Ugp2	2.76	1.42	2.15E-06
Fndc1	3.71	1.41	2.83E-06
Col5a3	2.16	1.41	3.16E-06
Bmp1	1.57	1.40	3.57E-06
Lrrn4cl	1.36	1.39	5.36E-06
Ccl11	3.65	1.39	7.36E-06
Oaf	3.08	1.36	8.24E-06
Myoc	12.15	1.36	9.14E-06
Efemp1	2.78	1.35	1.22E-05
Aspn	9.41	1.34	1.38E-05
Fap	1.04	1.32	1.92E-05
Nid1	8.41	1.31	1.97E-05
Sdc2	3.72	1.31	2.21E-05
Clec3b	14.83	1.30	2.21E-05
Cd34	8.35	1.30	2.51E-05
Itih5	4.64	1.28	3.45E-05
Lamb1	2.18	1.28	3.94E-05
Emilin2	1.81	1.28	3.95E-05
Dpt	12.97	1.27	4.34E-05
Tmem100	2.23	1.28	4.64E-05
Ddr2	1.90	1.24	7.45E-05

B

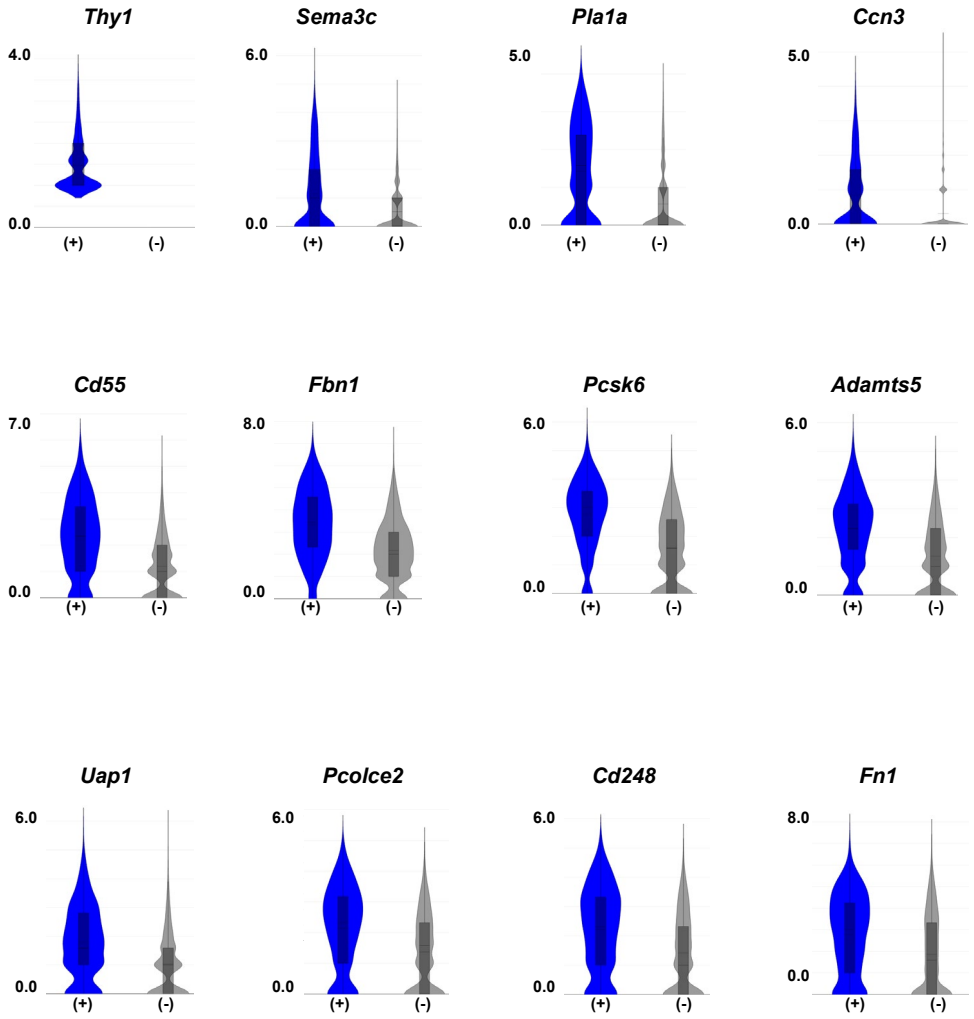


Figure S4. (A) *Thy-1* expressing FAPs: Top 50 enriched genes. FAP2 marker genes highlighted in blue. **(B)** Violin plots indicating expression of selected FAP2-enriched genes in *Thy1*-expressing (+) and *Thy1*-negative (-) cells.

Figure S5: High fat diet-fed *Thbs1*$^{-/-}$ mice show similar weight gain, total body adiposity and glucose intolerance to wild type mice fed the same diet

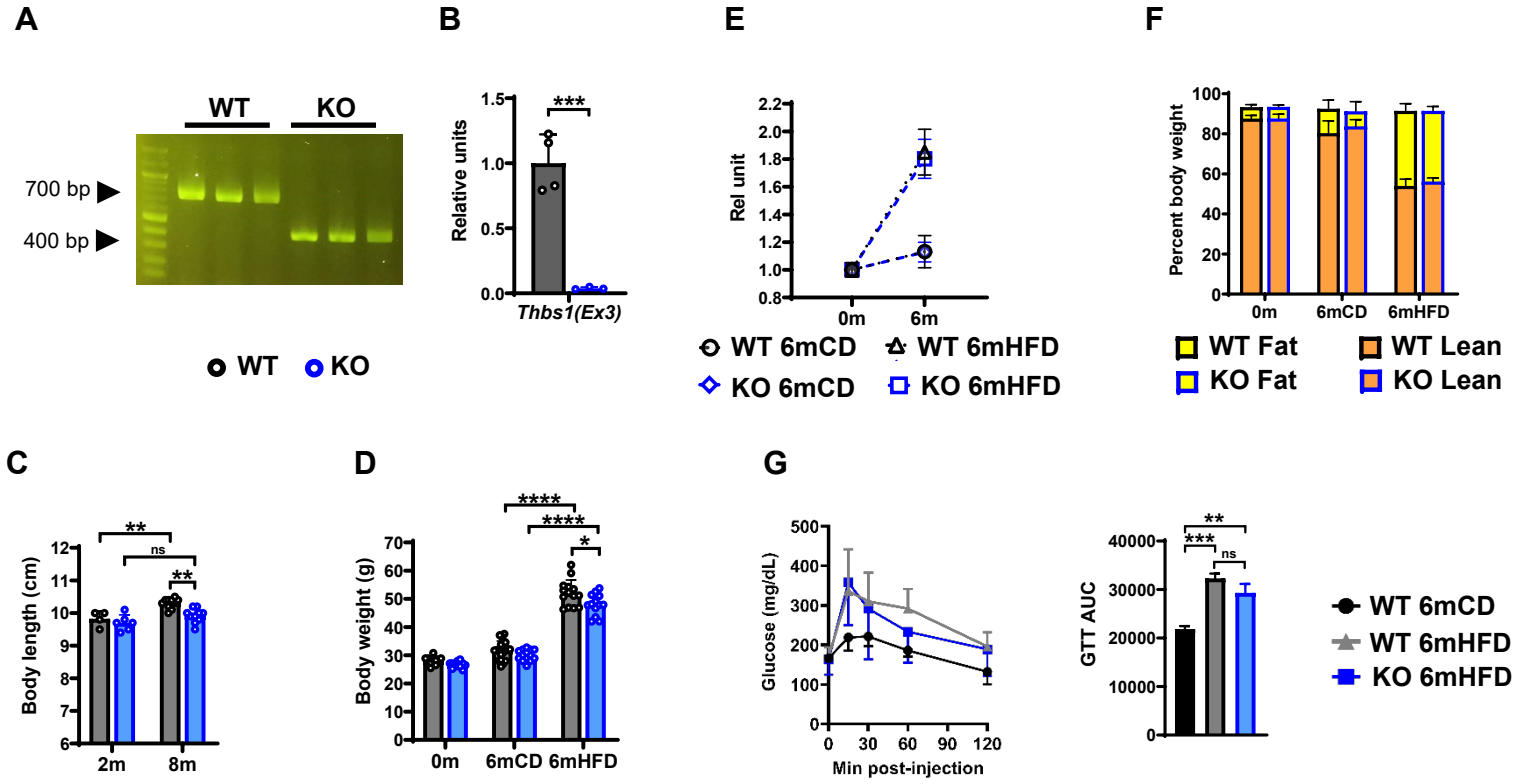


Figure S5. (A) Tail genotyping of *Thbs1*$^{-/-}$ (KO) mouse model. Representative gel showing PCR bands specific to wild type C57Bl/6J (WT) and KO animals. **(B)** QPCR analysis of costal diaphragm tissue from WT and KO mice using primers specifically directed against exon 3, ablated in this strain. n= 3-4 samples per group. **(C)** Body length (nose to anus) of 2-month-old (2m) and 8-month-old (8m) WT and KO mice. n= 4-10 mice per group. **(D)** Total body weight of WT and KO mice subjected to 6-month diet time course beginning at 2-months-old (0m time point) and ending at 8-months-old (6m time point). Control diet (CD), high fat diet (HFD). n= 10-20 mice per group. **(E)** Weight gain (normalized to baseline) in CD and HFD-fed WT and KO mice. n= 10-20 mice per group. **(F)** NMR-based body composition of WT and KO mice at baseline (2-months-old, 0m time point) and following 6-month CD or HFD feeding. n= 5-10 mice per group. **(G)** Intraperitoneal glucose tolerance test performed on 6m HFD-fed WT and KO mice; as well as age matched WT mice fed CD. Line graph indicates blood glucose values at baseline (0) and 15, 30, 60, 90 and 120 minutes (min) following intraperitoneal injection of 1g /kg dextrose. Bar graph compares area under the curve (AUC) for each line. n= 4-7 mice per group. Statistical analysis with two tailed t-test for individual comparisons, one-way ANOVA for multiple comparisons, and two-way ANOVA with Tukey's multiple comparison test for multiple variables. Error bars indicate SDM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.001.

Figure S6: *Thbs1* ablation has minimal effect on organ and muscle weights in HFD-fed mice

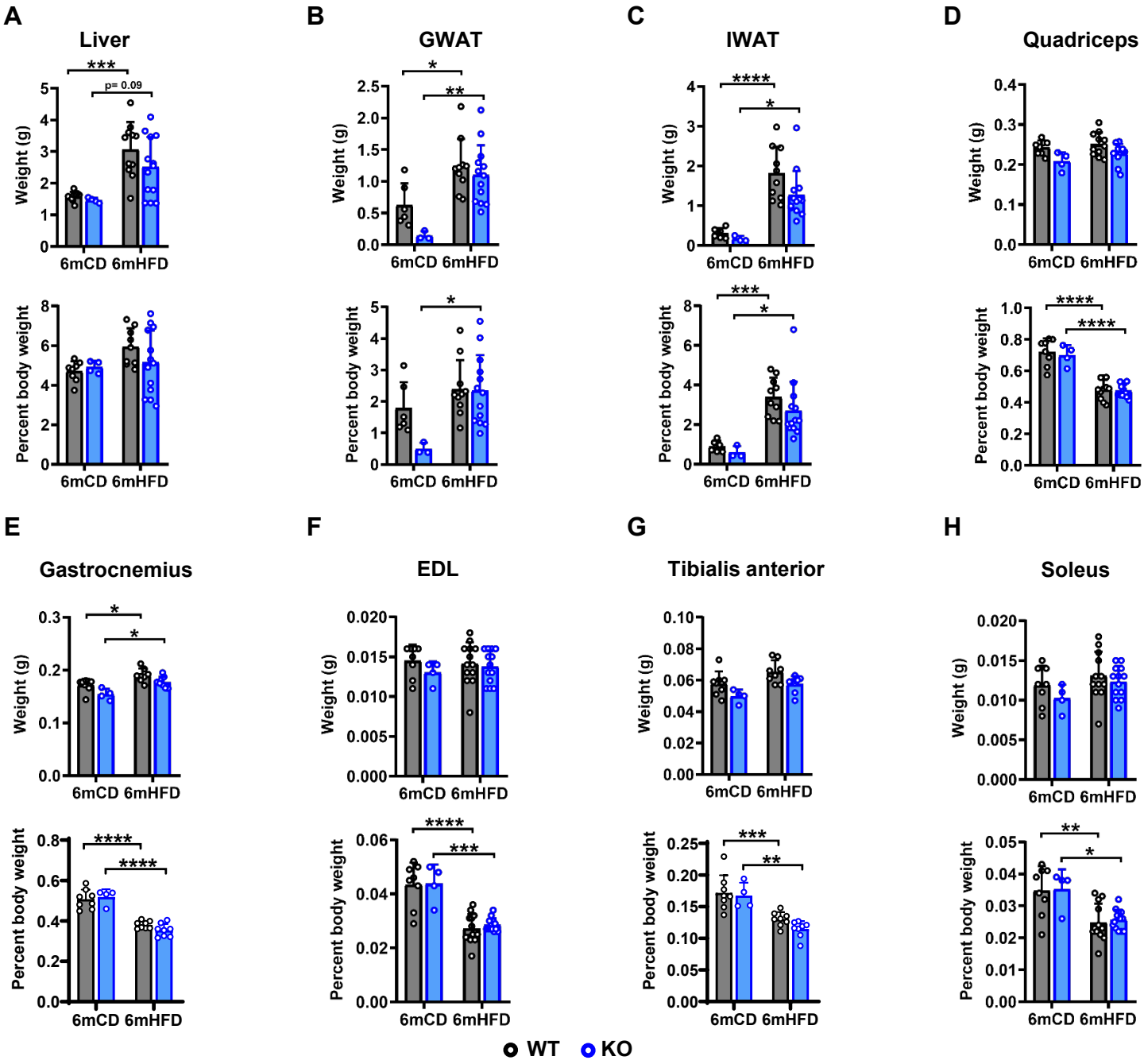
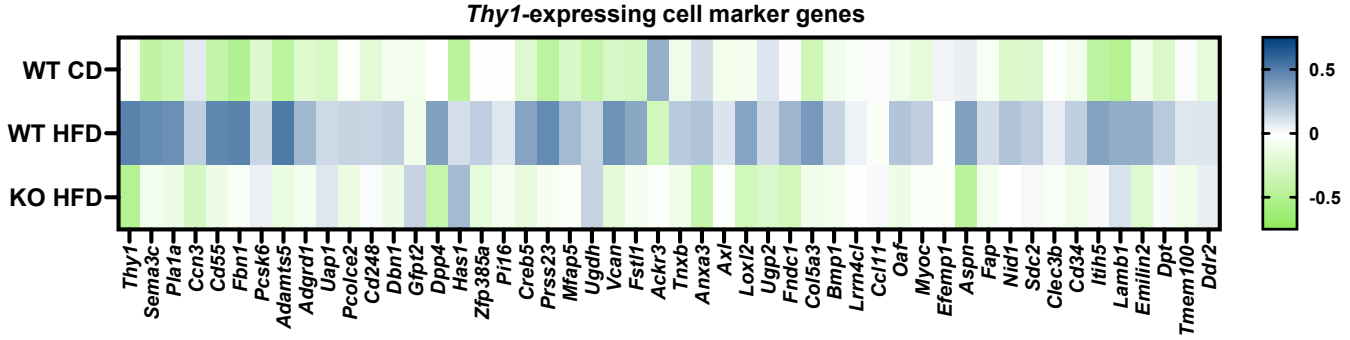


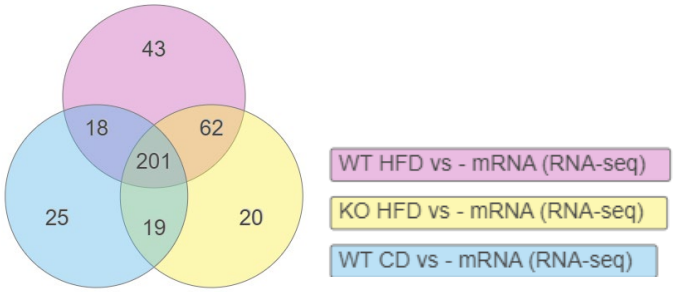
Figure S6. Weights (absolute, top panels; % total body weight, bottom panels) of **(A)** Liver, **(B)** Gonadal white adipose tissue (GWAT), **(C)** Inguinal white adipose tissue (IWAT), **(D)** Quadriceps, **(E)** Gastrocnemius, **(F)** Extensor digitorum longus (EDL), **(G)** Tibialis anterior, and **(H)** Soleus. All isolated from WT and KO mice after 6-month control diet (6mCD) or 6-month high fat diet (6mHFD) feeding. Fat depot weights represent combined weight of bilateral depots. Muscle weights indicate weight of a single muscle. n= 4-13 animals per group. Statistical analysis with two-way ANOVA with Tukey's multiple comparison test. Error bars indicate SDM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S7: Inter-group comparisons: Differentially expressed genes

A



B



C

Common to WT HFD and KO HFD

Gene	WT HFD	pval	KO HFD	pval	WT CD	pval
Fbn1	1.392	1.20E-06	0.956	0.002	0.452	0.371
Adamts5	1.357	2.48E-06	0.773	0.019	0.452	0.37
Sema3c	1.341	6.48E-06	0.9	0.005	0.514	0.276
Itih5	1.286	1.06E-05	0.977	0.001	0.502	0.275
Dclk1	1.279	1.43E-05	0.983	0.001	0.539	0.222
Cd55	1.261	2.03E-05	0.75	0.025	0.47	0.338
Col6a3	1.249	2.25E-05	1.021	6.31E-04	0.538	0.217
Col5a3	1.25	2.34E-05	0.818	0.011	0.541	0.215
Col3a1	1.246	2.37E-05	0.87	0.006	0.583	0.159
Lamb1	1.173	9.81E-05	1.006	8.28E-04	0.392	0.49
Ccdc80	1.164	1.08E-04	0.957	0.002	0.563	0.183
Sparc	1.119	2.19E-04	0.769	0.019	0.512	0.254
Ahnak2	1.088	4.21E-04	0.641	0.074	0.512	0.261
Golim4	1.073	5.13E-04	0.623	0.086	0.514	0.255
Igsf10	1.072	6.02E-04	1.225	1.95E-05	0.532	0.235
Ly6a	1.034	9.03E-04	0.642	0.071	0.283	0.696
Lama4	1.026	0.001	0.78	0.018	0.372	0.534
Creb5	1.016	0.001	0.589	0.116	0.466	0.345
Has1	1.038	0.002	1.25	2.53E-05	0.477	0.357
Steap3	0.996	0.002	1.278	5.29E-06	0.385	0.503
Egfr	0.989	0.002	0.792	0.015	0.45	0.373
Amotl1	0.98	0.002	0.838	0.009	0.512	0.259
Ppic	0.98	0.002	0.815	0.012	0.445	0.384
Csf1	0.968	0.003	0.983	0.001	0.556	0.195
Ugdh	0.971	0.003	1.036	6.18E-04	0.463	0.36
Prss23	0.971	0.003	0.597	0.114	0.184	0.887
Uap1	0.963	0.003	0.935	0.003	0.565	0.186
Fbln5	0.948	0.003	0.823	0.01	0.376	0.521
Hspg2	0.93	0.004	0.758	0.022	0.563	0.183
Col4a1	0.926	0.004	0.69	0.044	0.183	0.88
Antxr2	0.924	0.004	0.585	0.117	0.434	0.398
Ifi205	0.917	0.005	1.132	1.02E-04	0.531	0.231
Sntb2	0.882	0.007	0.716	0.034	0.399	0.472
Frmd6	0.843	0.012	0.702	0.04	0.481	0.313
Col4a2	0.832	0.014	0.598	0.108	0.154	0.939
Nfix	0.818	0.015	0.757	0.021	0.568	0.174
Akap12	0.821	0.016	0.698	0.044	0.472	0.333
Ablim1	0.806	0.018	0.629	0.08	0.572	0.173
Agap1	0.805	0.019	0.676	0.053	0.514	0.258
Cavin3	0.792	0.021	0.657	0.061	0.457	0.355
Serpinb6a	0.791	0.021	0.735	0.027	0.499	0.274
Timp2	0.78	0.023	0.683	0.047	0.543	0.205
Vat1	0.784	0.023	0.688	0.046	0.495	0.287
Ifi211	0.783	0.024	1.045	4.59E-04	0.438	0.395
Ebf1	0.776	0.025	0.638	0.073	0.477	0.316
Colec12	0.76	0.03	0.801	0.014	0.542	0.213
Nav1	0.744	0.035	0.826	0.01	0.49	0.292
Cyth3	0.742	0.037	0.732	0.03	0.484	0.309
Sash1	0.736	0.038	0.683	0.048	0.158	0.929
Nedd4	0.72	0.043	0.716	0.033	0.392	0.482
Itm2a	0.699	0.055	0.699	0.042	0.448	0.375
Zbtb20	0.692	0.056	0.674	0.051	0.35	0.557
Lima1	0.681	0.064	0.594	0.108	0.437	0.393
Nfia	0.678	0.065	0.68	0.049	0.468	0.332
C3	0.676	0.069	1.061	3.40E-04	0.526	0.237
Ifi204	0.668	0.076	0.885	0.005	0.236	0.79
Nfib	0.644	0.086	0.739	0.026	0.41	0.444
Thbs1	0.658	0.097	0.601	0.123	0.361	0.559
Man1a	0.593	0.131	0.618	0.087	0.486	0.298
Bmp4	0.598	0.133	0.78	0.019	0.536	0.228
Tspan3	0.576	0.15	0.608	0.096	0.508	0.262
Fcgrt	0.573	0.151	0.673	0.052	0.539	0.213
Ecm1	0.397	0.427	0.631	0.078	0.387	0.497
Errfi1	0.389	0.444	0.778	0.017	0.142	0.953

D

WT HFD-specific

Gene	WT HFD	pval	KO HFD	pval	WT CD	pval
Zeb1	0.881	0.008	0.566	0.138	0.491	0.297
Tgfbr2	0.863	0.009	0.517	0.194	0.437	0.393
Prg4	0.892	0.011	0.227	0.767	0.072	1
Igf2r	0.853	0.011	0.555	0.149	0.402	0.468
Emilin2	0.845	0.012	0.392	0.419	0.461	0.356
Cald1	0.805	0.018	0.569	0.132	0.322	0.618
Marcks	0.755	0.03	0.508	0.204	-0.058	1
P4ha1	0.757	0.031	0.53	0.179	0.489	0.297
Arl4a	0.752	0.032	0.571	0.131	0.548	0.202
Enpp2	0.759	0.035	0.462	0.294	0.578	0.178
S100a16	0.727	0.042	0.463	0.277	0.425	0.415
Sec31a	0.726	0.042	0.508	0.207	0.486	0.302
Mast4	0.705	0.053	0.567	0.137	0.396	0.48
Rhoj	0.685	0.061	0.566	0.135	0.437	0.392
Thra	0.686	0.063	0.423	0.355	0.507	0.267
Glg1	0.672	0.07	0.465	0.276	0.223	0.809
Nfic	0.649	0.083	0.563	0.138	0.509	0.26
Fermt2	0.646	0.085	0.46	0.281	0.417	0.432
Dap	0.645	0.087	0.501	0.216	0.449	0.371
Hdlbp	0.631	0.097	0.392	0.41	0.321	0.617
Sar1a	0.626	0.101	0.421	0.355	0.429	0.408
Sptbn1	0.626	0.101	0.341	0.525	0.298	0.664
Rhoc	0.624	0.103	0.483	0.244	0.469	0.332
Wls	0.625	0.104	0.519	0.193	0.498	0.283
Dnajc3	0.619	0.107	0.266	0.672	0.567	0.175
Arl1	0.615	0.11	0.405	0.387	0.406	0.454
Tcf4	0.612	0.112	0.472	0.26	0.33	0.595
Ski	0.611	0.114	0.475	0.256	0.4	0.468
Lman1	0.61	0.115	0.334	0.54	0.361	0.547
Anxa1	0.607	0.117	0.521	0.187	0.337	0.58
Ramp2	0.603	0.123	0.538	0.169	0.282	0.701
Dpysl2	0.599	0.125	0.444	0.31	0.52	0.242
Copb2	0.599	0.125	0.43	0.34	0.217	0.823
P4hb	0.597	0.126	0.529	0.176	0.469	0.329
Ly6c1	0.601	0.131	0.523	0.194	-0.064	1
Add3	0.588	0.135	0.447	0.304	0.467	0.336
Dst	0.587	0.141	0.336	0.541	0.154	0.939

E

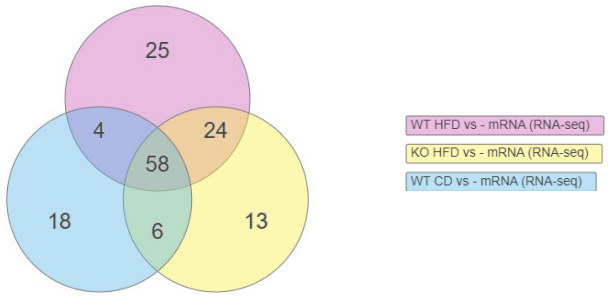
KO HFD-specific

Gene	WT HFD	pval	KO HFD	pval	WT CD	pval
Col6a5	0.256	0.726	1.882	1.00E-06	0.359	0.573
Phlda1	0.324	0.567	1.005	8.11E-04	0.577	0.167
Cxcl1	0.203	0.811	1.002	0.001	0.313	0.642
Errfi1	0.389	0.444	0.778	0.017	0.142	0.953
Adamts1	0.209	0.794	0.717	0.035	0.374	0.526
Myc	0.044	1	0.711	0.04	0.169	0.913
Tent5a	0.316	0.588	0.683	0.05	0.395	0.484
Pnp	0.229	0.749	0.676	0.05	0.528	0.229
Gem	0.267	0.686	0.678	0.051	0.43	0.408
Fcgrt	0.573	0.151	0.673	0.052	0.539	0.213
Nr4a1	-0.126	0.988	0.661	0.06	0.424	0.419
Ifi203	-0.006	1	0.655	0.066	-0.045	1
Ecm1	0.397	0.427	0.631	0.078	0.387	0.497
Klf4	0.269	0.678	0.61	0.093	0.389	0.49
Bag3	0.148	0.911	0.614	0.093	0.432	0.407
Tspan3	0.576	0.15	0.608	0.096	0.508	0.262
Egr1	0.115	0.964	0.597	0.105	0.444	0.378
Fos	0.293	0.623	0.591	0.109	0.332	0.59
Cebpd	0.191	0.825	0.591	0.111	0.077	1

Figure S7. (A) Heat map indicating the expression of *Thy1*-expressing cell marker genes in WT CD, WT HFD and KO HFD groups. **(B)** Venn diagram showing differentially expressed genes in diaphragm FAPs from WT CD, WT HFD, KO HFD mice. **(C)** Transcripts enriched in WT HFD and KO HFD (n= 62; orange section of Venn diagram) . **(D)** Genes uniquely enriched in WT HFD (n= 43; pink section of Venn diagram). **(E)** Genes uniquely enriched in KO HFD (n= 20; yellow section of Venn diagram). Indicated p-values are corrected for false discovery rate (FDR).

Figure S8: Inter-group comparisons: Differentially represented gene ontology terms

A



B

Common to all groups

Biological Process	WT CD pval	WT HFD pval	KO HFD pval
External encapsulating structure organization	1.51E-09	1.06E-15	1.69E-17
Extracellular structure organization	1.51E-09	1.06E-15	1.69E-17
Extracellular matrix organization	1.51E-09	1.06E-15	1.69E-17
System development	3.04E-06	4.90E-10	3.04E-08
Anatomical structure development	2.94E-06	2.62E-09	2.87E-07
Anatomical structure morphogenesis	3.35E-04	1.76E-08	4.37E-06
Multicellular organism development	1.01E-05	2.37E-08	3.37E-07
Skeletal system development	2.26E-04	7.54E-08	4.05E-08
Multicellular organismal process	3.73E-06	1.01E-07	2.17E-07
Developmental process	1.28E-05	1.51E-07	4.40E-06
Animal organ development	2.83E-05	2.56E-07	8.63E-07
Cell adhesion	1.58E-06	7.96E-07	3.61E-07
Biological adhesion	1.90E-06	1.16E-06	5.06E-07
Connective tissue development	2.10E-02	1.29E-06	8.71E-07
Cartilage development	3.00E-02	1.98E-06	2.14E-05
Animal organ morphogenesis	5.92E-04	2.33E-06	2.44E-05
Collagen fibril organization	3.83E-05	2.34E-06	1.01E-05
Cell migration	1.28E-05	2.74E-06	6.33E-08
Tissue development	3.35E-04	9.26E-06	1.44E-06
Circulatory system development	2.00E-03	1.38E-05	3.50E-07
Localization of cell	4.53E-05	1.57E-05	3.27E-07
Cell motility	4.53E-05	1.57E-05	3.27E-07
Enzyme linked receptor protein signaling pathway	1.25E-04	1.57E-05	8.29E-04
Locomotion	4.81E-05	1.57E-05	2.87E-07
Tube development	3.00E-03	1.66E-05	7.38E-06
Vasculature development	4.00E-03	1.77E-05	8.54E-07
Blood vessel development	7.00E-03	2.87E-05	4.10E-06
Tube morphogenesis	2.60E-02	3.64E-05	2.62E-05
Cellular response to growth factor stimulus	1.90E-02	4.69E-05	2.00E-03
Movement of cell or subcellular component	6.95E-04	1.25E-04	1.67E-06
Response to growth factor	4.80E-02	1.40E-04	8.00E-03
Cell differentiation	2.26E-04	1.92E-04	2.62E-05
Cell-substrate adhesion	5.00E-03	4.24E-04	2.53E-04
Cellular developmental process	4.71E-04	5.78E-04	8.77E-05
Cell surface receptor signaling pathway	1.56E-04	6.83E-04	1.96E-04
Regulation of multicellular organismal process	1.40E-02	6.83E-04	1.00E-03
Supramolecular fiber organization	2.00E-03	1.00E-03	2.00E-02
Ossification	3.60E-02	2.00E-03	7.00E-03
Regulation of developmental process	2.30E-02	2.00E-03	1.30E-02
Regulation of BMP signaling pathway	2.00E-03	2.00E-03	6.00E-03
Biomineral tissue development	2.00E-03	2.00E-03	1.00E-03
Biomineralization	2.00E-03	2.00E-03	1.00E-03
Wound healing	1.60E-02	5.00E-03	1.00E-02
Biological regulation	2.90E-02	5.00E-03	1.00E-02
Collagen metabolic process	4.15E-04	5.00E-03	3.87E-04
Response to wounding	7.00E-03	5.00E-03	3.00E-03
Response to BMP	4.00E-03	6.00E-03	2.00E-03
Cellular response to BMP stimulus	4.00E-03	6.00E-03	2.00E-03
BMP signaling pathway	4.00E-03	6.00E-03	2.00E-03
Transmembrane receptor protein tyrosine kinase signaling pathway	3.00E-03	7.00E-03	4.00E-03
Blood vessel remodeling	4.80E-02	1.30E-02	7.00E-03
Bone mineralization	2.00E-03	1.30E-02	1.00E-03
Regulation of cell motility	4.40E-02	1.60E-02	2.00E-03
Regulation of locomotion	4.90E-02	2.00E-02	2.00E-03
Regulation of response to stimulus	4.00E-03	2.40E-02	5.00E-03
Negative regulation of multicellular organismal process	4.00E-02	2.40E-02	7.00E-03
System process	6.95E-04	3.60E-02	6.00E-03
Negative regulation of cell population proliferation	2.80E-04	3.60E-02	9.00E-03

C

Specific to WT CD

Biological Process	P-val (corr for FDR)
Negative regulation of signal transduction	0.0080
Hormone metabolic process	0.0100
Negative regulation of cell communication	0.0120
Negative regulation of signaling	0.0120
Regulation of blood pressure	0.0140
Regulation of systemic arterial blood pressure	0.0180
Sensory perception	0.0210
Circulatory system process	0.0210
Blood vessel diameter maintenance	0.0290
Regulation of tube size	0.0290
Regulation of tube diameter	0.0290
Cell development	0.0290
Regulation of hormone levels	0.0360
Regulation of signal transduction	0.0400
Regulation of tissue remodeling	0.0480
Regulation of response to external stimulus	0.0480
Regulation of insulin-like growth factor receptor signaling pathway	0.0480

D

Specific to KO HFD

Biological Process	P-val (corr for FDR)
Renal system development	0.0020
Regulation of ossification	0.0050
Regulation of epithelial cell proliferation	0.0060
Kidney development	0.0060
Nephron development	0.0150
Regulation of cell population proliferation	0.0170
Negative regulation of peptidase activity	0.0170
Negative regulation of endopeptidase activity	0.0250
Angiogenesis	0.0250
Cell population proliferation	0.0270
Regulation of bone mineralization	0.0320
Regulation of phosphatidylinositol 3-kinase signaling	0.0350
Glomerulus development	0.0350

E

Common to WT HFD and KO HFD

Biological Process	P-val (corr for FDR)
Bone development	6.80E-04
Skeletal system morphogenesis	7.00E-03
Extracellular matrix assembly	2.00E-03
Negative regulation of cellular response to growth factor stimulus	3.50E-02
Blood vessel morphogenesis	2.00E-03
Chondrocyte differentiation	1.40E-02
Regulation of multicellular organismal development	1.40E-02
Heart development	2.00E-03
Complement activation	5.00E-03
Anatomical structure formation involved in morphogenesis	1.50E-02
Complement activation, classical pathway	7.00E-03
Response to stimulus	1.00E-02
Urogenital system development	3.87E-04
Regulation of biological process	3.80E-02
Epithelium development	1.10E-02
Regulation of cell migration	3.00E-03
Reproductive structure development	1.90E-02
Reproductive system development	1.90E-02
Response to chemical	1.70E-02
Regulation of cellular component movement	4.00E-03
Prostate gland morphogenesis	2.10E-02
Prostate gland epithelium morphogenesis	2.10E-02
Elastic fiber assembly	2.10E-02
Humoral immune response mediated by circulating immunoglobulin	3.20E-02

Figure S8. (A) Venn diagram showing differentially enriched gene ontology terms (biological processes) in WT CD, WT HFD and KO HFD diaphragm FAPs. **(B)** Biological processes common to all groups (n= 58). **(C)** Biological processes enriched in WT CD diaphragm FAPs (n= 18; blue section of Venn diagram). **(D)** Biological processes enriched in KO HFD diaphragm FAPs (n= 13; yellow section of Venn diagram). **(E)** Biological processes common to WT HFD and KO HFD FAPs (n= 24; orange section of Venn diagram). Indicated p-values are corrected for false discovery rate (FDR).

Figure S9: Impact of TGFβ signaling blockade on THBS1-induced FAP proliferation and fibronectin deposition

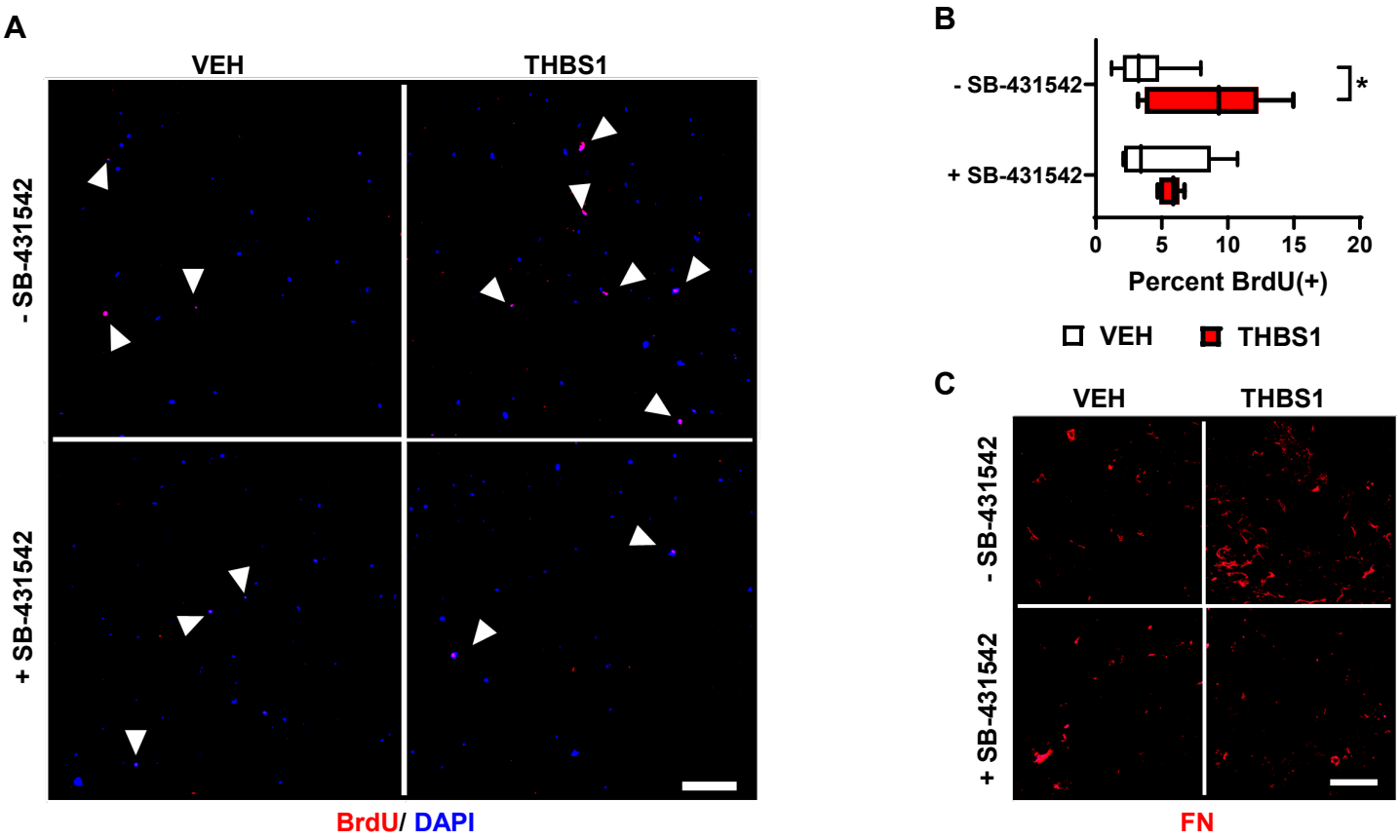


Figure S9. (A) Nuclear BrdU staining in isolated FAPs treated with THBS1 (5μg/mL) or vehicle (VEH)+/- application of SB431542 (SB) (10μg/mL), an inhibitor of TGFβ signaling via ALK5. Scale 200μm. **(B)** Percent BrdU(+) nuclei in images from groups described in (A). Data include average of 5-7 samples per group. **(C)** Fibronectin (FN) immunocytochemistry in samples treated with THBS1 or VEH +/- application of SB431542. Scale 400 μm. Statistical analysis with two-way ANOVA with Tukey's multiple comparison test. Error bars indicate SDM. *p<0.05

Figure S10: TGFβ, PDGF and THBS1 receptor expression in FAP sub-populations

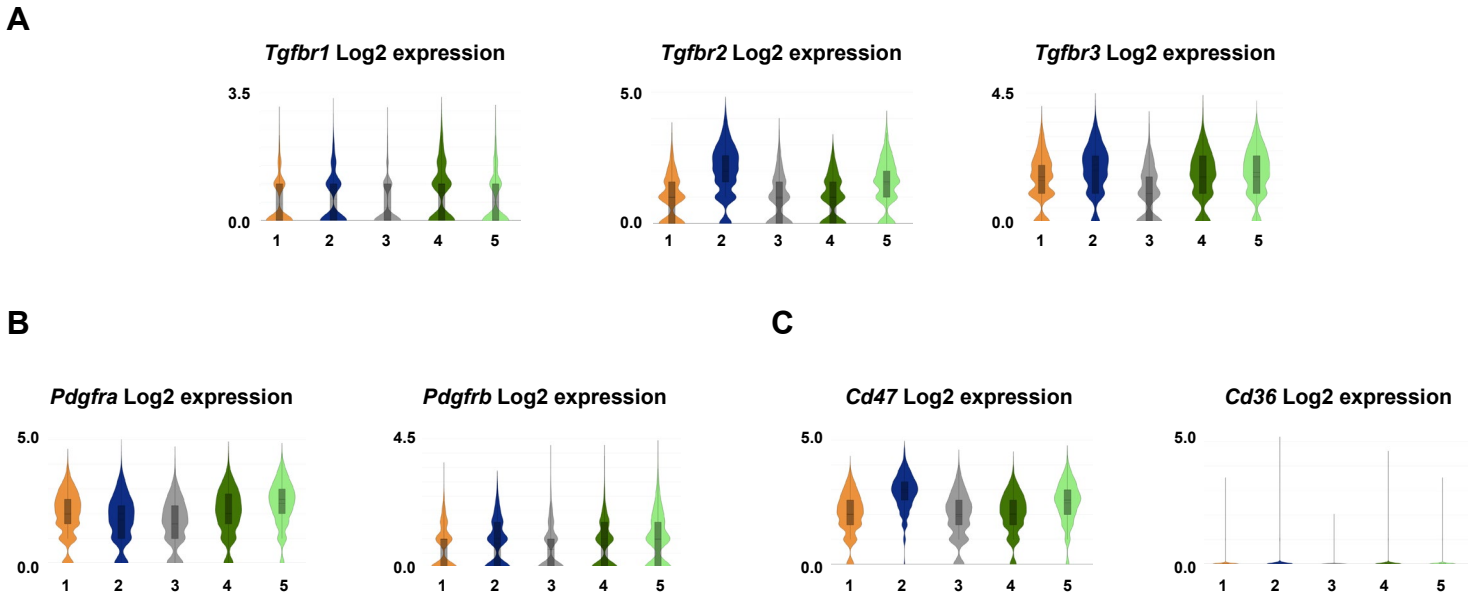


Figure S10. (A-C) Expression of TGFβ receptors (*Tgfbr1*, *Tgfbr2*, *Tgfbr3*); PDGF receptors (*Pdgfra*, *Pdgfrb*) and THBS1 receptors (*Cd36*, *Cd47*) in individual FAP sub-populations. FAP1, FAP2, FAP3, FAP4 and FAP5 respectively labeled 1, 2, 3, 4, 5.

Figure S11: Effect of DIO and *Thbs1* ablation on diaphragm macrophages

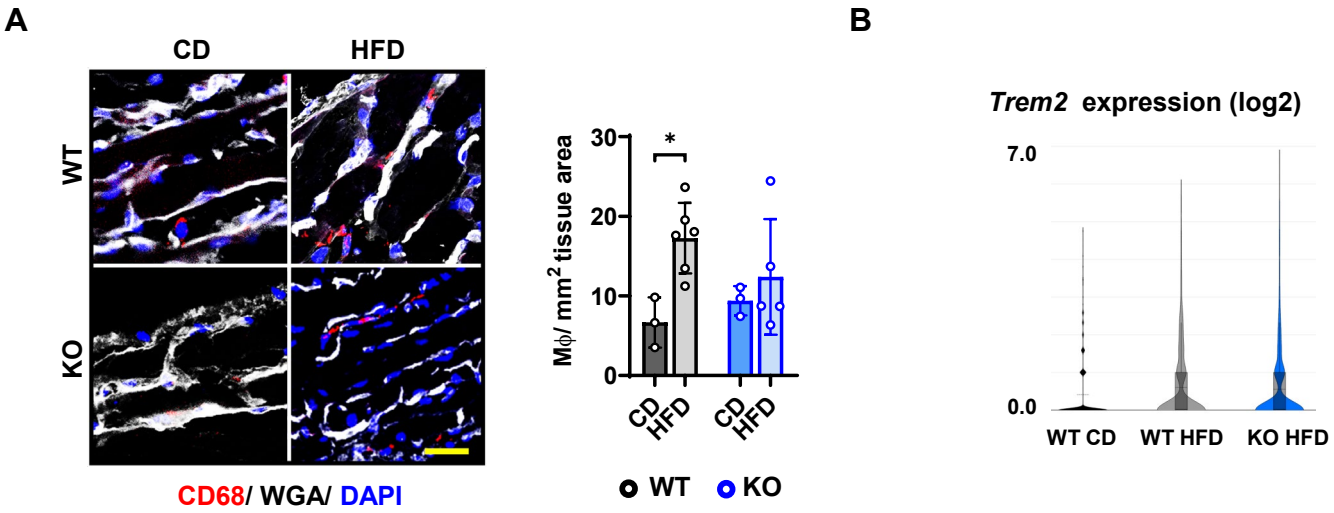


Figure S11. (A) CD68 immunohistochemistry in costal diaphragm longitudinal sections from wild type (WT) and *Thbs1*$^{-/-}$ (KO) mice fed either control diet (CD) or high fat diet (HFD) for 6 months. Wheat germ agglutinin, WGA. Scale 20 μm. Bar graph shows number of CD68 immunopositive cells per mm^2 cross sectional area. n= 4-5 per group. **(B)** Expression of obesity-associated macrophage-specific *Trem2* in macrophage sub-clusters and (WT CD, WT HFD and KO HFD) groups. Statistical analysis with two-way ANOVA with Tukey's multiple comparison test. Error bars indicate SDM. *p<0.05.

Figure S12: Fibro-adipogenic remodeling of the mouse and human diaphragm

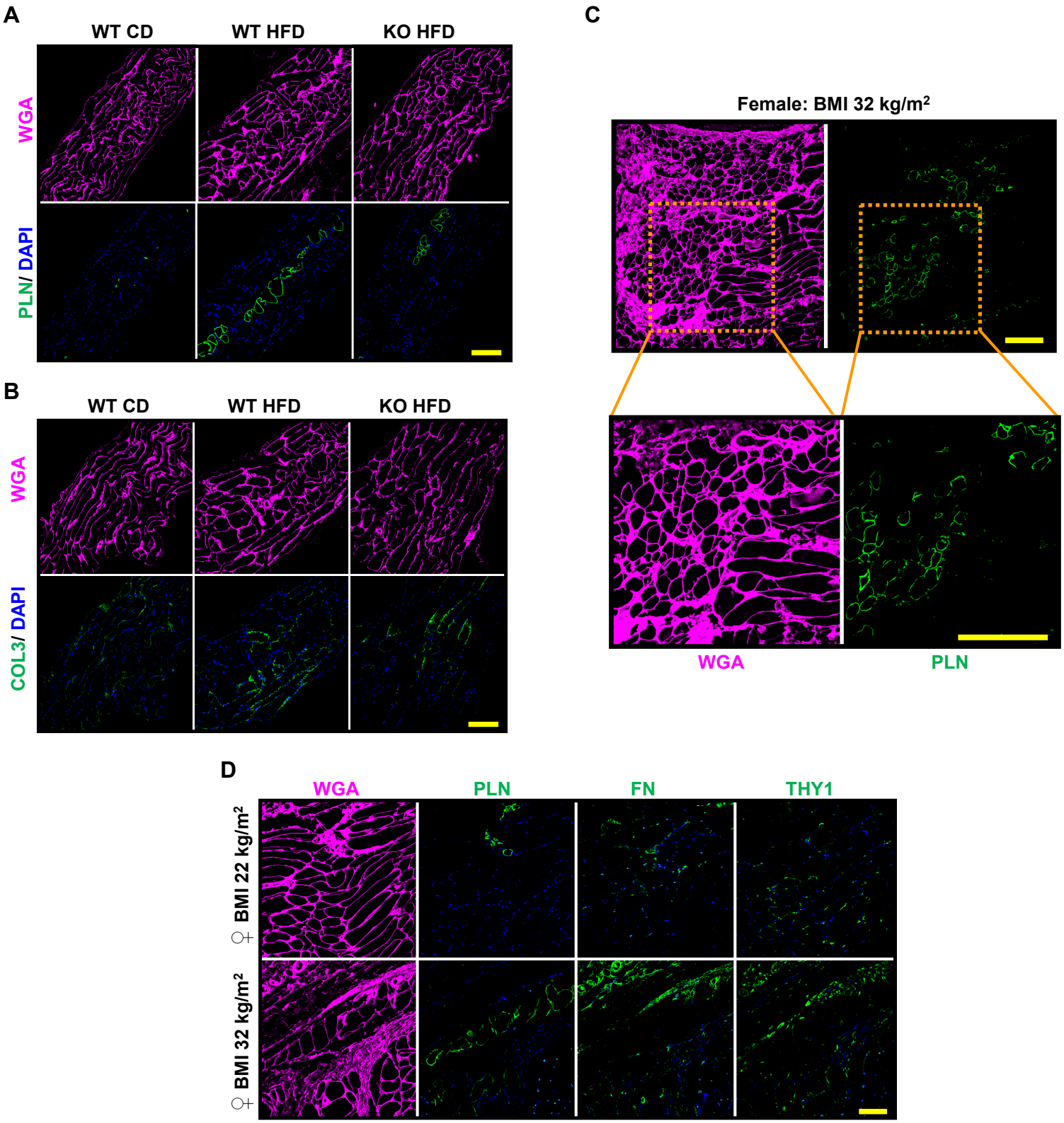


Figure S12. (A) Immunofluorescent staining of wheat germ agglutinin (WGA) and perilipin (PLN) on adjacent 7 μm-thick longitudinal sections from wild-type mice fed control diet (WT CD) or HFD (WT HFD) for 6 months; and *Thbs1*$^{-/-}$ (KO) mice fed HFD for 6 months (KO HFD). Representative images from analysis of 5-6 mice per group. Scale 200 μm. **(A)** Immunofluorescent staining of WGA and type 3 collagen (COL3) in sections from samples indicated in (A). **(C)** Immunofluorescent staining of perilipin (with WGA counter-stain) on 7 μm-thick sectiosn of human diaphragm sample obtained from a female cadaveric donor 62-years-old, body mass index (BMI) 32 kg/m^2. Images obtained at 10X and 20X magnification. Scale 200μm. **(D)** Immunofluorescent staining of PLN, fibronectin (FN) and THY1 (with WGA counter-stain) 7 μm-thick sections of human diaphragm samples obtained from female cadaveric donors: 62-years-old, body mass index (BMI) 32 kg/m^2; 72-years-old BMI 22kg/m^2. Scale 100 μm.

Figure S13: Mouse diaphragm histology and physiology parameters

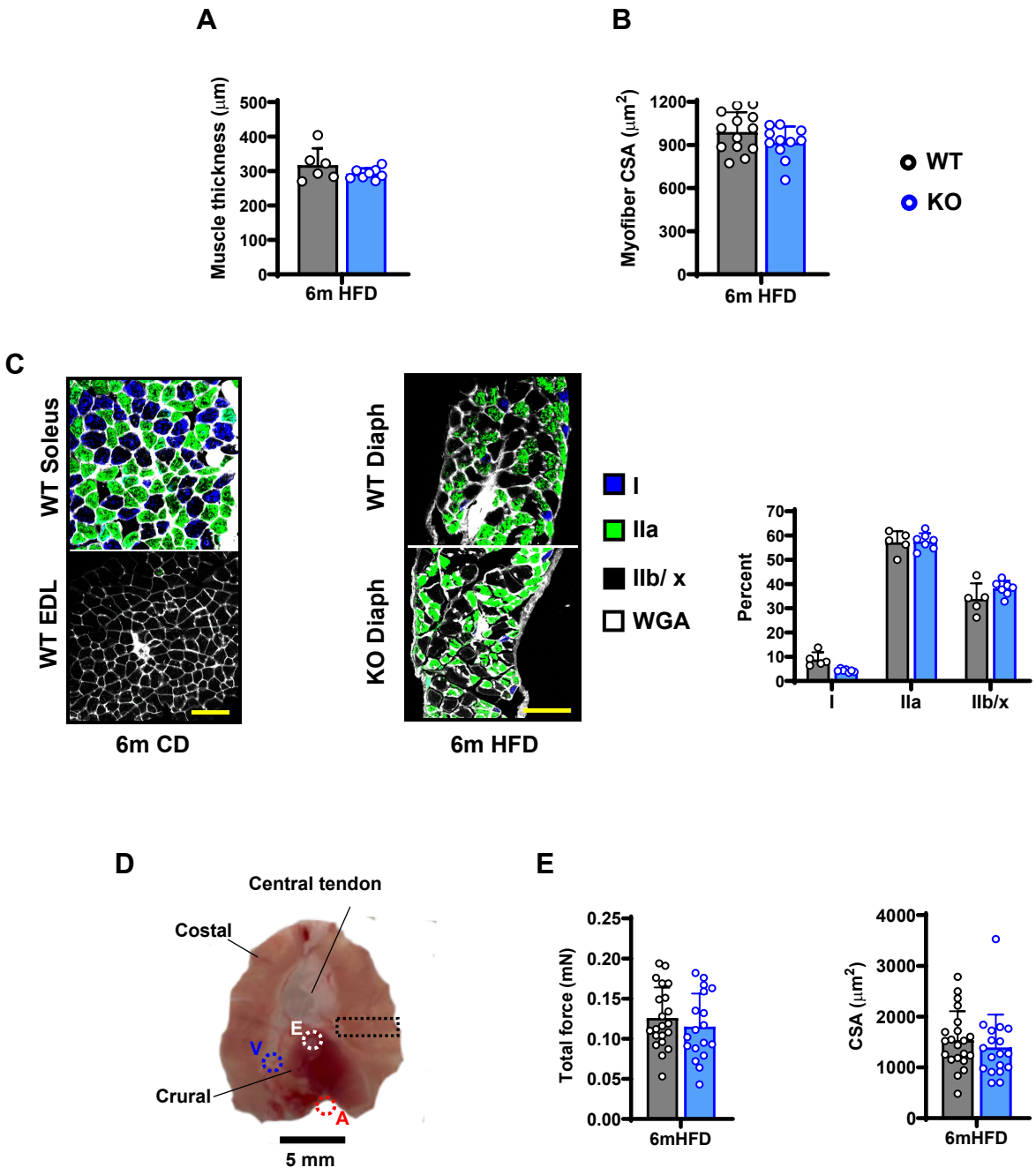


Figure S13. (A) Diaphragm thickness measured on FFPE longitudinal sections from wild type (WT) and *Thbs1*$^{-/-}$ (KO) mice fed high fat diet for 6 months (6mHFD). Average value from 10-15 thickness measurements made on 3 non-consecutive sections from 6-8 mice per group. **(B)** Myofiber size quantification (fiber cross-sectional area) from animals indicated in (A). Each point represents average myofiber cross sectional area from an individual diaphragm cross section. **(C)** Transverse sections from unfixed frozen samples of wild type (WT) extensor digitorum longus (EDL)—these act as positive controls for fiber typing antibodies. Type I (slow oxidative), type IIa (fast oxidative), type IIb/ x (fast glycolytic). Transverse sections from unfixed frozen samples of diaphragm stained with fiber typing antibodies. Bar graph shows percentage of each fiber type in diaphragm samples from each group. n= 4-8 animals per group. **(D)** Diagram of mouse diaphragm indicating costal muscle, crural muscle and central tendon region, as well as positions of aorta (A), descending vena cava (V) and esophagus (E). B lack dashed line indicates position of strips isolated for isometric force testing. **(E)** Isometric force measurement performed on single myofibers isolated from 6mHFD WT and KO mice (n= 4-5 animals per group; 5 fibers per animal). Graphs indicate total isometric force and fiber cross sectional area. Statistical analysis with t-test. Error bars indicate SDM.

Table S1: Antibodies

	Primary antibodies		Secondary antibodies	
Experiment	**Target, animal, conjugate, company, catalog #**	**Dilution**	**Target, animal, conjugate, company, catalog #**	**Dilution**
Cell culture ICC	Ki67, rabbit, unconjugated Abcam, ab15580	1:250	Goat anti-rabbit, Alexa Fluor 594, Invitrogen, A11072	1:250
	BrdU, rat, unconjugated Abcam, ab6326	1:200	Donkey anti-rat, Alexa Fluor 594, Invitrogen, A-21209	1:250
	Fibronectin, rabbit, unconjugated, Sigma Aldrich F3648	1:250	Goat anti-rabbit, Alexa Fluor 594, Invitrogen, A11072	1:250
Tissue (frozen secions) IHC	PDGFRα, goat, unconjugated R&D Systems, AF1062	1:400	Donkey anti-goat, Alexa Fluor 594, Invitrogen, A-32758	1:2000
	CD68, rat, unconjugated, BioRad, MCA1957	1:200	Donkey anti-rat, Alexa Fluor 594, Invitrogen, A-21209	1:200
	Myosin heavy chain Type I, mouse, DHSB, BA-D5-c	1:300	Goat anti-mouse, Alexa Fluor 647, Invitrogen, A-21242	1:300
	Myosin heavy chain Type IIA, mouse, DHSB, SC-71-c	1:300	Goat anti-mouse, Alexa Fluor 488, Invitrogen, A-21121	1:300
Tissue (FFPE sections) IHC	Perilipin-1, rabbit, unconjugated, Cell Signaling, 3470S	1:250	Goat anti-rabbit, Alexa Fluor 594, Invitrogen, A-11072	1:1000
	THY1/ CD90, rabbit, unconjugated, Bioss, BS-0778R	1:400	Goat anti-rabbit, Alexa Fluor 594, Invitrogen, A-11072	1:1000
	Fibronectin, rabbit, unconjugated, Sigma Aldrich F3648	1:400	Goat anti-rabbit, Alexa Fluor 594, Invitrogen, A-11072	1:1000
Flow cytometry	CD31, rat, PE, eBioscience, 12-0311-81	1:1000	N/A	N/A
	CD45, rat, PE, eBioscience, 12-0451-82	1:1000	N/A	N/A
	Integrin α7, rat, PE, Invitrogen, MA5-23608	1:1000	N/A	N/A
	Sca-1, rat, APC, eBioschence, 17-5981-82	1:1000	N/A	N/A

Table S1. ICC, immunocytochemistry. IHC, immunohistochemistry. FFPE, formalin-fixed, paraffin-embedded. DHSB, Developmental Studies Hybridoma Bank.