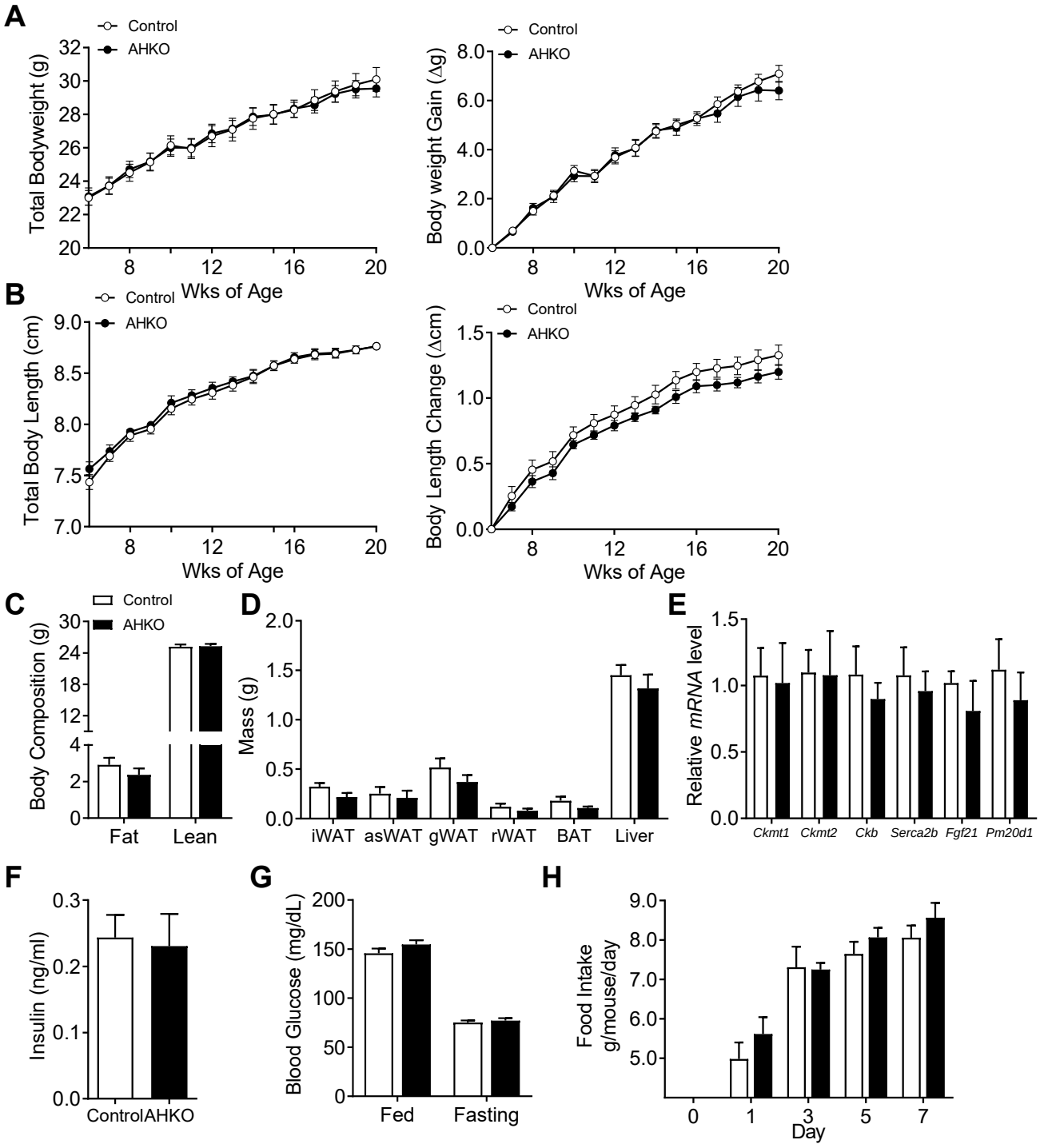
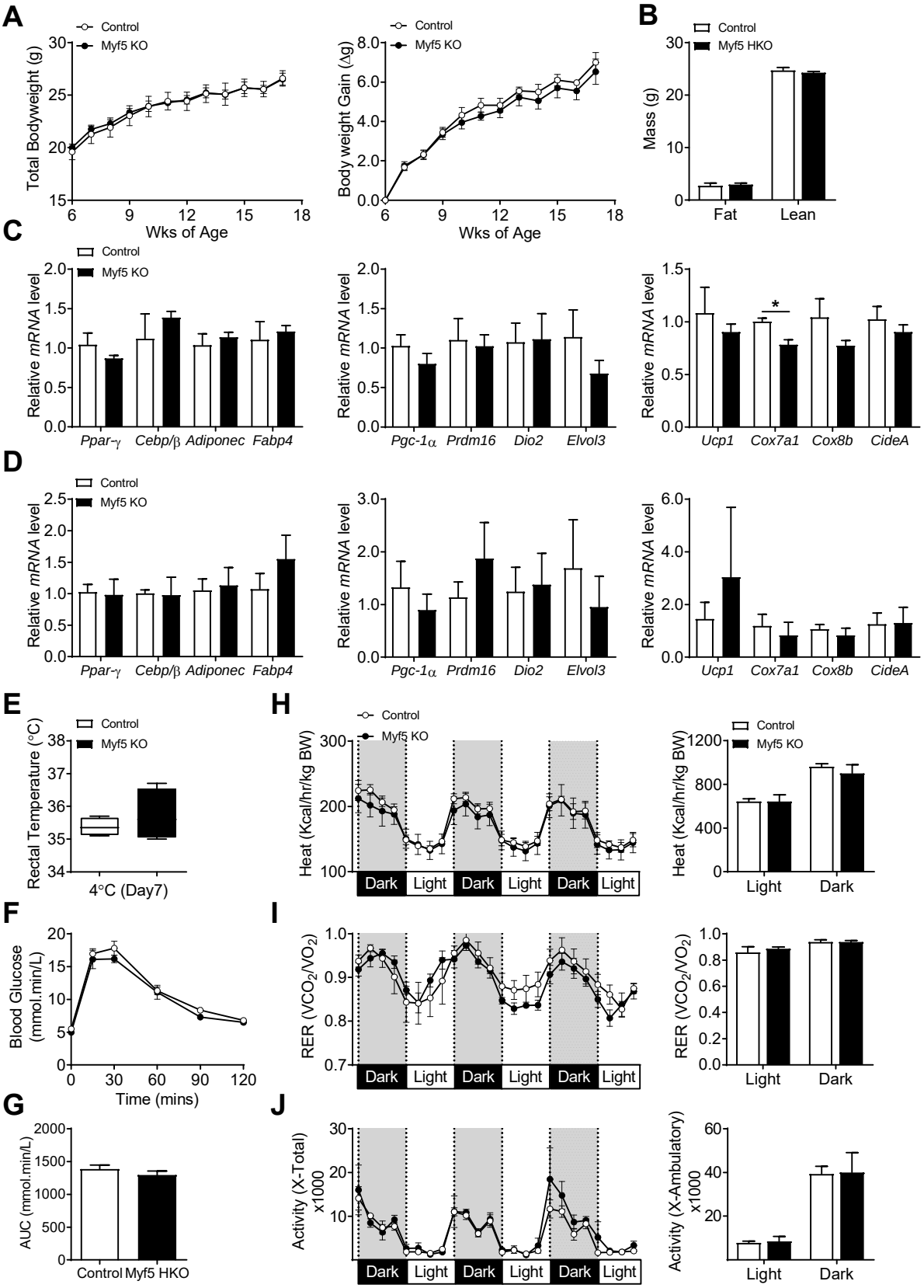
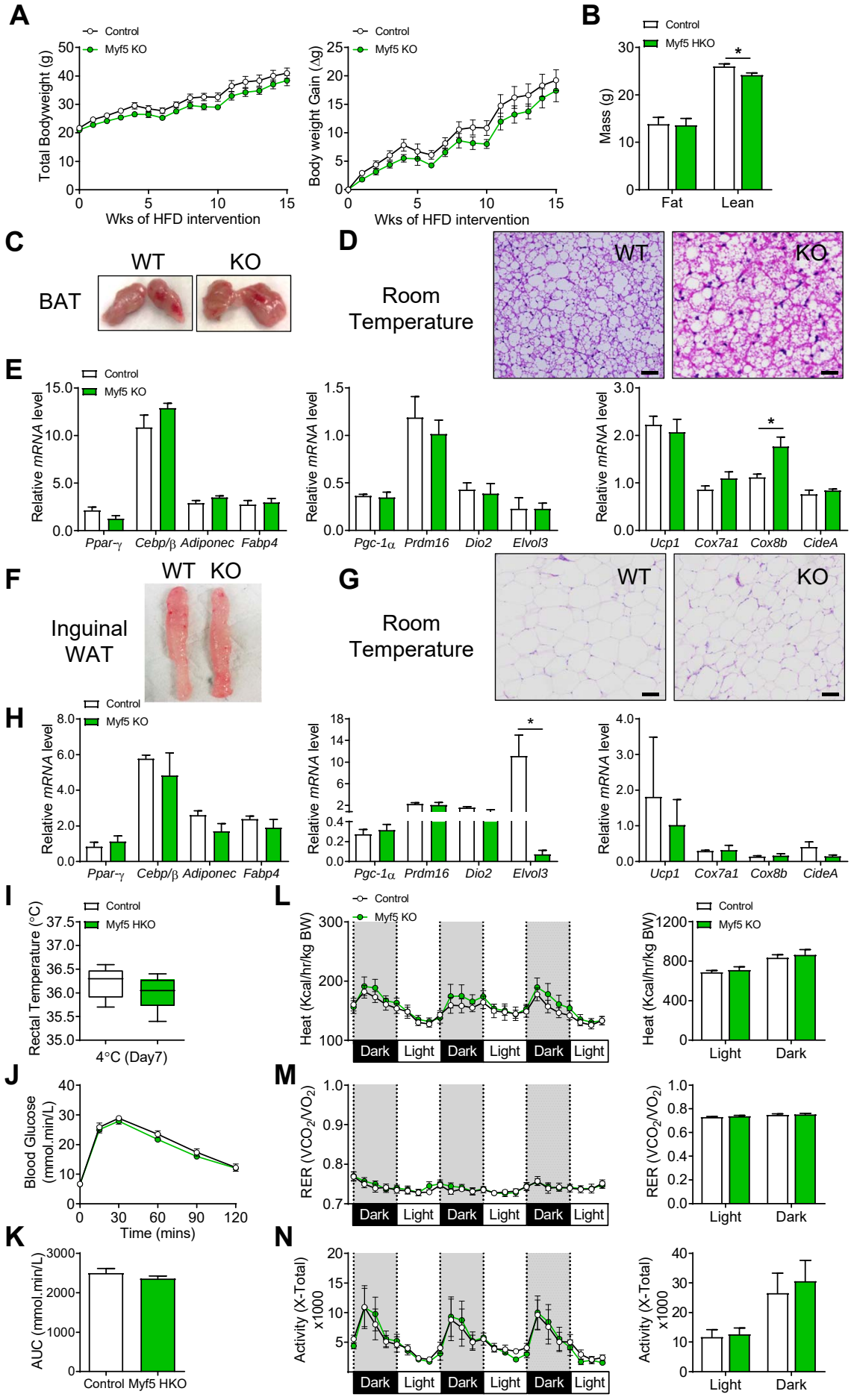


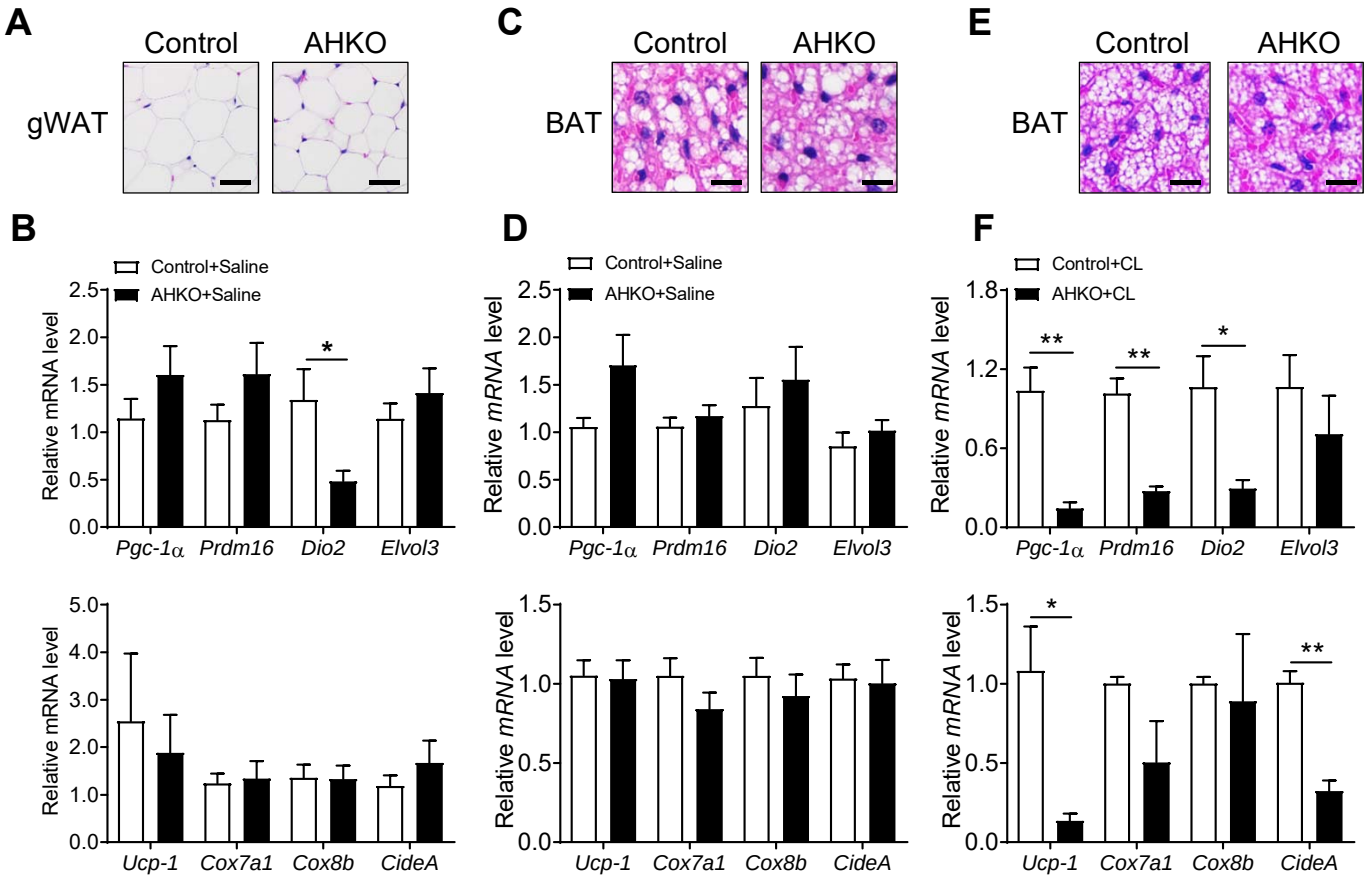
Supplemental Figure 1: Phenotypic analysis of AHKO mice fed regular chow diet. (A) Growth curve illustrating weekly total body weight and cumulative body weight change and (B) total body length and cumulative body length change in AHKO mice compared to wild type controls starting from 6 weeks of age, kept at room temperature and fed chow diet, n=14. (C) In vivo body composition by EchoMRI of total fat and total lean mass and (D) isolated adipose tissue depots and liver of control and AHKO mice at 20 weeks of age. (E) Relative mRNA levels of Ucp-1-independent thermogenic markers, n=10. (F) Plasma insulin levels and (G) Fed and fasting blood glucose levels in control and AHKO mice. (H) Average daily food consumption in control and AHKO mice.


A
Control
Myf5 KO
Total Bodyweight (g)
Wks of Age
Body weight Gain (Δg)
B
Myf5 HKO
Mass (g)
Fat
Lean
C
Relative mRNA level
Ppar-γ
Cebp/β
Adiponec
Fabp4
Pgc-1α
Prdm16
Dio2
Elvol3
Ucp1
Cox7a1
Cox8b
CideA
*
D
E
Rectal Temperature (°C)
4°C (Day7)
F
Blood Glucose (mmol.min/L)
Time (mins)
G
AUC (mmol.min/L)
Control
Myf5 HKO
H
Heat (Kcal/hr/kg BW)
Dark
Light
I
RER (VCO2/VO2)
J
Activity (X-Total) x1000
Activity (X-Ambulatory) x1000

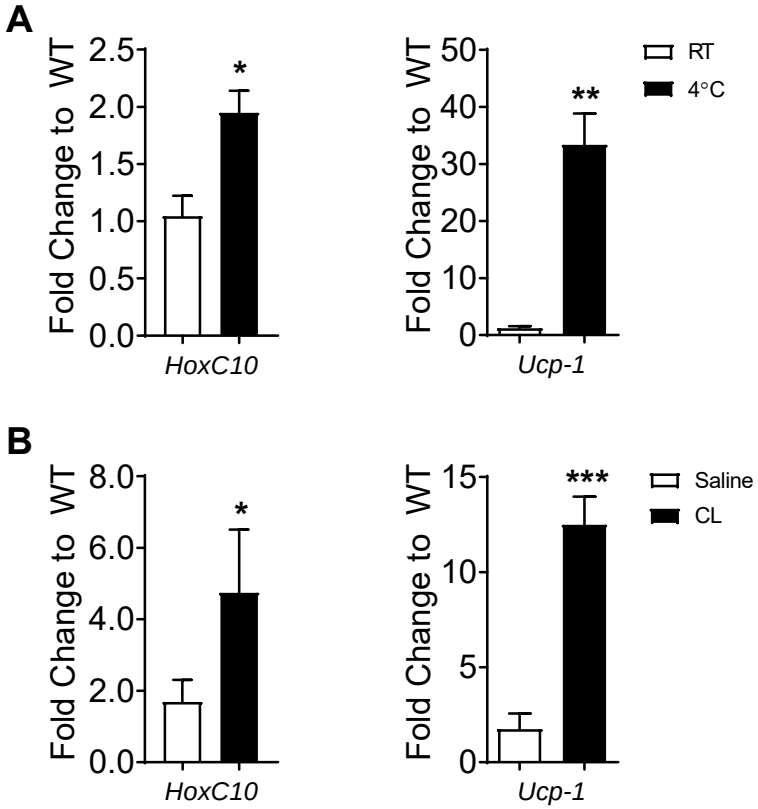
Supplemental Figure 2: Phenotypic and metabolic analysis of Myf5 HKO mice fed regular chow diet. (A) Total body weight and cumulative body weight change starting from 6 weeks of age, kept at room temperature. (B) In vivo body composition by EchoMRI of total fat and total lean mass in control and Myf5 HKO mice at 18 weeks of age. Common adipogenic and thermogenic browning genes in (C) brown adipose tissue and (D) subcutaneous WAT. (E) Body temperature was measured following 7-day cold challenge at 10weeks of age. (F) Glucose tolerance test and (G) area under curve of control and Myf5 HKO mice between 15-16 weeks of age. (H) Mean average heat production, (I) respiratory exchange ratio (RER) and (J) activity level determined at 14 weeks of age, by indirect calorimetry in metabolic chambers during the 3-day measurement (after an initial 48 hours of acclimation period). Dark and light phase cumulative means within dark or light phase and each time point were compared by student's t-test between Myf5 HKO mice and their littermate male control mice. Data are presented as mean ± SEM. *$p<0.05$ (n=4).


A
Control
Myf5 KO
Total Bodyweight (g)
Wks of HFD intervention
Body weight Gain (Δg)
B
Control
Myf5 HKO
Mass (g)
Fat
Lean
C
WT
KO
BAT
D
Room Temperature
WT
KO
E
Control
Myf5 KO
Relative mRNA level
Ppar-γ
Cebp/β
Adiponec
Fabp4
Pgc-1α
Prdm16
Dio2
Elvol3
Ucp1
Cox7a1
Cox8b
CideA
F
WT KO
Inguinal WAT
G
Room Temperature
WT
KO
H
Control
Myf5 KO
Relative mRNA level
I
Control
Myf5 HKO
Rectal Temperature (°C)
4°C (Day7)
L
Control
Myf5 KO
Heat (Kcal/hr/kg BW)
Dark
Light
J
Blood Glucose (mmol.min/L)
Time (mins)
M
RER (VCO2/VO2)
K
AUC (mmol.min/L)
Control
Myf5 HKO
N
Activity (X-Total) x1000

Supplemental Figure 3: Phenotypic and metabolic analysis of Myf5 HKO mice on high fat diet intervention for 15 weeks. (A) Total body weight and cumulative body weight change starting from 6 weeks of age, kept at room temperature. (B) In vivo body composition by EchoMRI of total fat and total lean mass in control and Myf5 HKO mice at 20 weeks of age. (C) Representation of photograph and (D) H&E staining of BAT of control and Myf5 HKO mice. Scale bar = 20μm. (E) Relative mRNA levels of common adipocyte genes and brown/beige-selective thermogenic markers in BAT. (F) Representation of photograph and (G) H&E staining of subcutaneous WAT of control and Myf5 KO mice. Scale bar = 20μm. (H) Relative mRNA levels of common adipocyte genes and brown-selective thermogenic markers in subcutaneous WAT. (I) Body temperature was measured following 7-day cold challenge at 10weeks of age. (J) Glucose tolerance test and (K) area under curve of control and Myf5 HKO mice between 15-16 weeks of age. (L) Mean average heat production, (M) respiratory exchange ratio (RER) and (N) activity level determined at 14 weeks of age, by indirect calorimetry in metabolic chambers during the 3-day measurement (after an initial 48 hours of acclimation period). Dark and light phase cumulative means within dark or light phase and each time point were compared by student's t-test between Myf5 HKO mice and their littermate male control mice. Data are presented as mean ± SEM. *$p<0.05$ ($n=8$).



Supplemental Figure 4: Effects of β-Adrenergic Receptor Activation in BAT of AHKO mice. Representative H&E staining and corresponding relative mRNA levels of brown fat selective markers in (A-B) gonadal WAT (gWAT) and (C-D) BAT from control and AHKO mice at 20weeks of age fed on chow diet. Scale bar = 100μm. n=10. (E) Representative H&E staining and (F) Relative mRNA levels of brown fat selective markers of BAT of control and AHKO mice treated with CL316,243 to induce browning. Scale bar = 100μm. n=4. Data are presented as mean ± SEM. Student's t test, *p<0.05, **p<0.01, ***p<0.001. n=4.



Supplemental Figure 5: *HOXC10* mRNA expression is increased in subcutaneous WAT following cold exposure and β3-Adrenergic Activation. (A) *HOXC10* and corresponding *Ucp-1* mRNA levels in subcutaneous WAT isolated from wild type C57BL6/J mice held at room temperature (RT) or exposed to 4°C. (B) *HOXC10* and corresponding *Ucp-1* mRNA levels in subcutaneous WAT isolated from wild type C57BL6/J mice treated daily with CL-316,243 (1 mg/kg) or vehicle (saline) for 7days (n=5mice per group). Data are presented as mean ± SEM. Student's t test, *p<0.05, **p<0.01, ***p<0.001.

Protein IDs	Peptide counts (all)	Protein names	Gene names	Sequence coverage [%]	Mol. weight [kDa]	Sequence length	Ratio H/L L3	Ratio H/L normalized L3	Ratio H/L count L3	Ratio H/L L5	Ratio H/L normalized L5	Ratio H/L count L5
P0CG50;P0C	3;3;3;3;3;3	Polyubiquitin-C;Ubiquitin;Ubiquitin-related 1;Ubiquitin-related 2;Polyubiquitin-B;Ubiquitin;Ubiquitin-40S ribosoı	Ubc;Ubb;2810422J05Rik	41.7	82.549	734	NaN	NaN	0	8.2338	7.9405	4
Q8BTM8;B7I	2;2;2;1;1	Filamin-A;Filamin-B	Flna;Flnb	0.7	281.22	2647	NaN	NaN	0	2.8836	2.3412	2
F7B6A4;F7C	4;1	BTB/POZ domain-containing protein KCTD17	Kctd17	21.4	23.641	206	19.254	20.272	2	0.71763	0.9469	3
Q99NF7;P36	11;9;9;9;9;9;	Protein phosphatase 1B	Ppm1b	30.8	52.11	477	20.025	20.023	13	0.80499	0.96815	10
P31257;P31:	12;2;2;2;2;1;	Homeobox protein Hox-C10	Hoxc10	41.8	38.195	342	20.215	18.278	14	0.75607	0.85878	11
Q8CFE4;G5E	4;3;2;1	SCY1-like protein 2	Scyl2	4.4	103.32	930	13.503	16.879	2	NaN	NaN	0
A2A9V9;F6S	3;3;3;1	BTB/POZ domain-containing protein KCTD2	Kctd2	9.5	36.162	338	11.84	12.467	2	0.77432	1.0217	2
Q8VC57	5	BTB/POZ domain-containing protein KCTD5	Kctd5	32.1	26.163	234	11.11	10.09	1	0.72193	0.7397	5
Q60597-3;Q	5;5;5;5;4	2-oxoglutarate dehydrogenase, mitochondrial	Ogdh;Ogdhl	5.3	118.18	1038	5.9499	5.9522	4	0.8201	0.83764	2
P63017;Q50	17;17;8;4;3;:	Heat shock cognate 71 kDa protein	Hspa8	26	70.87	646	1.8072	2.0589	8	2.1156	2.1112	15
O08749	2	Dihydrolipoyl dehydrogenase, mitochondrial	Dld	4.5	54.272	509	1.6481	1.6387	2	NaN	NaN	0
Q9D0E1;Q9I	4;4;4;4;1;1;1	Heterogeneous nuclear ribonucleoprotein M	Hnrnpm	5.2	77.648	729	1.0061	1.4943	1	3.7195	3.5358	3
CON__P607	8;8;7;7;6;6;6	Actin, cytoplasmic 1;Actin, cytoplasmic 1, N-terminally processed;Actin, cytoplasmic 2;Actin, cytoplasmic 2, N-tern	Actb;Actg1;Acta2;Actc1	25.3	41.736	375	1.2643	1.277	10	0.90703	1.1766	4
Q8VDD5;E9(	44;7;6;6;3;3;	Myosin-9	Myh9	27.3	226.37	1960	1.3136	1.1067	44	1.2619	1.0188	7
E9QPD7;G5E	3;3;3	Pyruvate carboxylase;Pyruvate carboxylase, mitochondrial	Pcx;Pc	3.2	129.83	1179	1.0485	1.1031	2	NaN	NaN	0
CON__ENSE	2;2;2;2	Tubulin alpha-1B chain;Tubulin alpha-1A chain;Tubulin alpha-1C chain	Tuba1b;Tuba1a;Tuba1c	5.3	50.179	451	1.1239	1.0762	2	NaN	NaN	0
P11499;P07	2;1;1	Heat shock protein HSP 90-beta;Heat shock protein HSP 90-alpha	Hsp90ab1;Hsp90aa1	3.6	83.28	724	0.85947	1.0744	2	NaN	NaN	0
P20029	24	78 kDa glucose-regulated protein	Hspa5	42.4	72.421	655	1.0452	1.0687	25	1.0697	1.036	11
P38647	28	Stress-70 protein, mitochondrial	Hspa9	45.9	73.46	679	1.0285	1.064	41	1.1179	1.0405	21
D3Z0Z9;D3Y	2;2;2;2;1	Glyceraldehyde-3-phosphate dehydrogenase	Gm5069;Gm7293;Gapdh	7.7	36.487	337	1.0546	1.0587	2	NaN	NaN	0
Q03265;D3Z	2;2	ATP synthase subunit alpha, mitochondrial;ATP synthase subunit alpha	Atp5a1	5.1	59.752	553	1.1347	1.0377	2	1.1707	1.3245	1
P56480	2	ATP synthase subunit beta, mitochondrial	Atp5b	6.6	56.3	529	1.0833	1.0373	2	NaN	NaN	0
Q3UH59;Q5	12;12;12	Myosin-10	Myh10	7.4	233.45	2013	1.1313	1.0065	6	NaN	NaN	0
Q8CAQ8-5;(	3;3;3;3;3;2;1	Mitochondrial inner membrane protein	Immt	4.4	87.693	790	0.92508	0.97325	1	1.6832	1.6093	2
E9Q447;A3K	2;2;2;2;2	Spectrin alpha chain, brain	Spna2;Sptan1	1	285.34	2478	1.0033	0.93034	2	NaN	NaN	0
P35980;G3U	2;2;1;1	60S ribosomal protein L18	Rpl18	12.2	21.644	188	0.93321	0.8162	2	NaN	NaN	0
Q8VEK3;G3	2;2	Heterogeneous nuclear ribonucleoprotein U	Hnrnpu	3	87.917	800	0.84459	0.75266	2	NaN	NaN	0
E9Q616	4		Ahnak	4	604.25	5656	0.91395	0.71565	4	2.2746	1.757	2
Q99PL5;A2A	7;7;1;1;1;1;1	Ribosome-binding protein 1	Rrbp1	4.5	172.88	1605	0.84377	0.6907	6	1.4952	1.276	1
P62918	1	60S ribosomal protein L8	Rpl8	4.3	28.024	257	0.69273	0.68421	2	NaN	NaN	0
E9Q3X0;Q9E	13;13;2	Major vault protein	Mvp	19.4	96.856	870	0.46087	0.65921	14	NaN	NaN	0
P62631;P10	3;3;2;2	Elongation factor 1-alpha 2;Elongation factor 1-alpha 1	Eef1a2;Eef1a1	7.8	50.454	463	0.52534	0.50302	1	6.2594	7.0817	3
P20152;P03	6;2;2;1	Vimentin	Vim	12	53.687	466	0.46239	0.45832	3	1.1787	1.3335	1
Q8BMF4	4	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	Dlat	8.3	67.941	642	0.49323	0.43075	4	NaN	NaN	0
CON__Q7RT	4;4			6.9	56.964	521	0.32041	0.30192	2	NaN	NaN	0
Q62191;Q3U	2;2	E3 ubiquitin-protein ligase TRIM21	Trim21	4.7	54.174	470	0.14686	0.14062	2	NaN	NaN	0
P01783;P18	2;1;1;1;1;1	Ig heavy chain V region MOPC 21;Ig heavy chain V region 5-84;Ig heavy chain V region 345;Ig heavy chain V region 5-76;Ig heavy chain V reg		16.2	15.071	136	0.1285	0.12304	2	NaN	NaN	0
P01878	3	Ig alpha chain C region		21.8	36.875	344	0.11959	0.11328	4	NaN	NaN	0
CON__P136	22			34.9	62.378	590	0.077668	0.081776	13	0.039396	0.044571	2
Q9QZM3	1	Cardiotrophin-like cytokine factor 1	Clcf1	3.6	25.261	225	0.06775	0.062822	3	0.042485	0.049185	1
CON__P025	30;11;7;7;5;5;3;2			50.7	60.044	564	0.067082	0.05876	26	0.32875	0.32303	2
CON__P025	20;10;7;7;3	Keratin, type I cytoskeletal 14	Krt14	52.8	51.621	472	0.048073	0.05475	8	NaN	NaN	0
CON__P027	10			21.4	69.293	607	0.051934	0.050938	9	0.056651	0.069217	4
CON__Strep	3			31.2	16.622	160	0.06649	0.050189	3	0.33738	0.37235	1
F6TQW2;P0	2;1	Ig gamma-2A chain C region secreted form	Ighg2c	11.2	44.095	403	0.054968	0.048005	3	NaN	NaN	0
P14106	3	Complement C1q subcomponent subunit B	C1qb	13.8	26.717	253	0.040821	0.04298	2	NaN	NaN	0
Q3UV17	7	Keratin, type II cytoskeletal 2 oral	Krt76	8.8	62.844	594	0.051011	0.038505	3	0.24555	0.22127	1
P01865;P01	5;5;1	Ig gamma-2A chain C region, membrane-bound form;Ig gamma-2A chain C region, A allele	Igh-1a;Ighg	18.8	43.949	398	0.042837	0.037411	4	NaN	NaN	0
P01872-2;P(	5;5	Ig mu chain C region secreted form	Igh-6	14.1	52.526	475	0.029031	0.03629	4	NaN	NaN	0
CON__P087	20;6;6;6;5;5;5;5;3;3;2;2;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1;1			50.1	51.267	473	0.041175	0.033123	17	NaN	NaN	0
CON__ENSE	6;5			23.4	43.9	401	0.037092	0.032475	7	NaN	NaN	0
P01867;P01	5;5	Ig gamma-2B chain C region	Igh-3	19.8	44.259	404	0.030654	0.029344	11	NaN	NaN	0
F6XWB2;P0	1;1	Ig kappa chain V-II region 26-10	Igkv1-115	11.4	12.428	114	0.0225	0.025626	4	NaN	NaN	0
CON__Q046	15			34.5	48.105	432	0.026426	0.024504	5	NaN	NaN	0
CON__P127	4			14.8	38.418	359	0.025918	0.023701	2	NaN	NaN	0
CON__P026	3			18.6	22.975	199	0.023593	0.023689	4	NaN	NaN	0
Q792Z1	2		Try10	8.1	26.221	246	0.024062	0.022986	5	NaN	NaN	0
CON__Q86Y	9			11.3	282.39	2850	0.023674	0.021952	2	NaN	NaN	0
CON__P359	36;4			68.7	65.865	645	0.019849	0.019799	61	0.015421	0.0163	15
CON__P355	27			60.7	62.129	623	0.018167	0.016647	73	0.028635	0.025035	16
P01869;P01	15;15	Ig gamma-1 chain C region, membrane-bound form;Ig gamma-1 chain C region secreted form	Ighg1	50.9	43.386	393	0.014338	0.015773	30	0.020752	0.025298	18
CON__P136	31;10;10;10;10;10;4;4;3;3;2			60.9	59.51	593	0.015365	0.015761	85	0.032831	0.032633	28
CON__P042	35			57.5	66.017	644	0.015477	0.015691	91	0.014103	0.017925	28
P01837	10	Ig kappa chain C region		95.3	11.778	106	0.0096714	0.0091747	14	0.0083416	0.0092062	11
CON__P007	5			31.6	24.409	231	0.0038625	0.005496	19	0.0041091	0.0051895	14
D3YZ71;S4R	1;1		Ap1m1	7.7	20.734	181	0.0045414	0.0042694	3	0.011638	0.011287	2

Supplemental Table 1: SILAC experiment to identify potential HOXC10 interacting proteins. L3: Total cell lysates from differentiated adipocytes expressing flag-tagged HOXC10 (light and heavy labelled) were used for IP with anti-Flag antibody (heavy sample, H) and mouse IgG (light sample, L). Flag peptide elution were used for both samples. L5: Differentiated adipocytes expressing flag-tagged HOXC10 (light and heavy labelled) were treated with 10μM forskolin (heavy sample, H) or DMSO (light sample, L) for 2.5h. Total cell lysates were used for IP with anti-Flag antibody and Flag peptide elution was performed on both samples. The E3 ligases KCTD2, KCTD5 and KCTD17 (highlighted in yellow) were found to potentially interact with HOXC10 (high ratio H/L, L3).