Supplemental Table 1

			HFD (for 25 wks)	
Variable	**Wild type**	***db/db***	**Wild type**	**MCM; FoxO1$^{L/L}$**
MV E (mm/s)	573 ± 66	658 ± 48	621 ± 71	567 ± 58
MV A (mm/s)	400 ± 62	439 ± 42	348 ± 59	400 ± 52
MV E/A	1.43 ± 0.02	1.49 ± 0.01	1.78 ± 0.20	1.41 ± 0.02
IVCT (ms)	**16.3 ± 1.2**	9.6 ± 1.8	7.5 ± 1.4	**15.8 ± 1.2**
IVRT (ms)	**14.2 ± 1.6**	22.9 ± 1.8	19.2 ± 1.6	**13.3 ± 1.5**
MVDT (ms)	**21.4 ± 0.4**	9.6 ± 0.6	11.7 ± 0.3	**18.8 ± 0.1**
MV ET (ms)	67.4 ± 5.7	60.0 ± 3.5	53.3 ± 6.2	64.2 ± 5.8
Heart rate (beats/min)	410 ± 20	400 ± 15	390 ± 20	400 ± 10

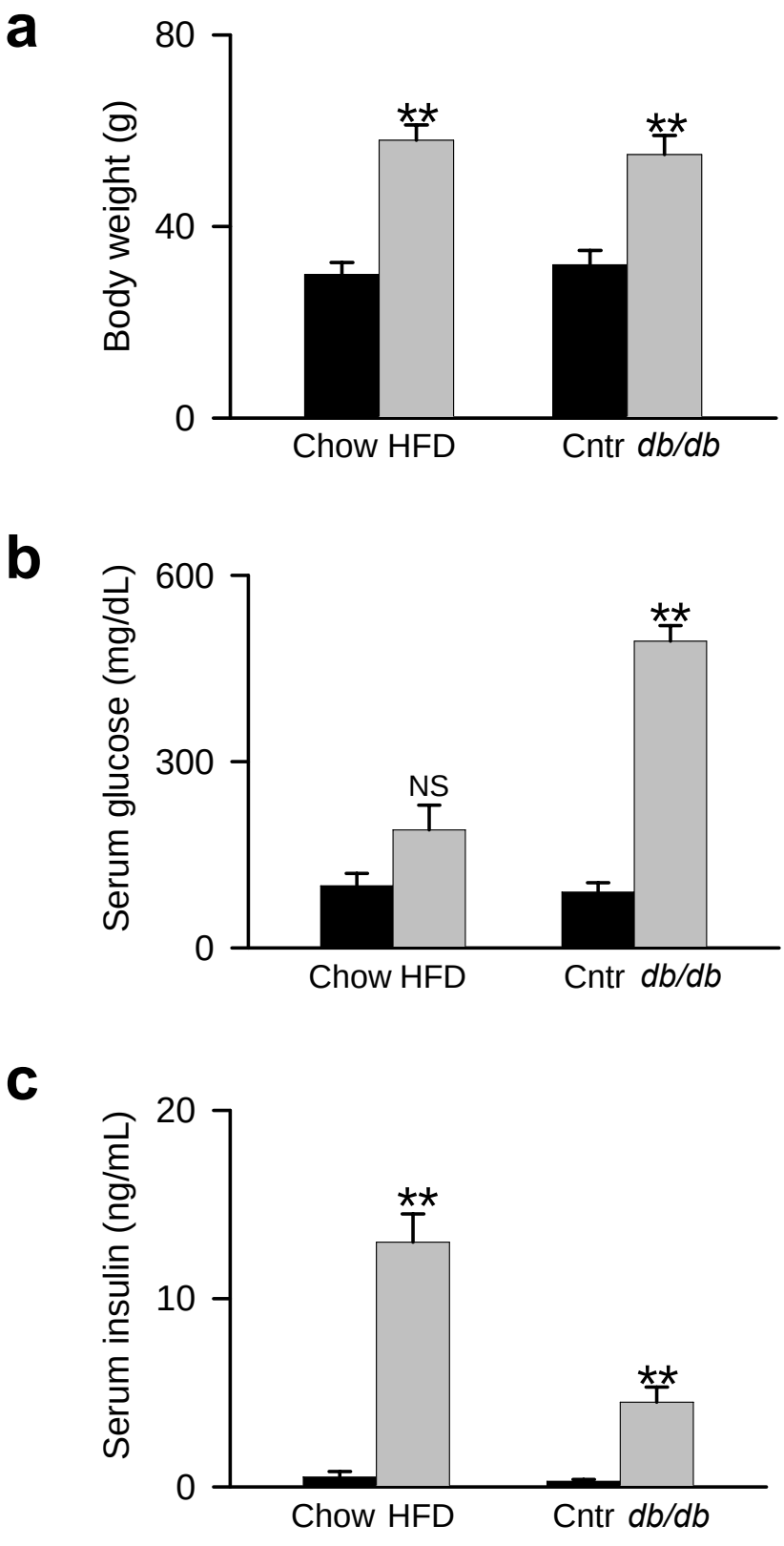
Animals were **anaesthetized** to obtain these variables, n= 3 animals per group.

Supplemental Table 2

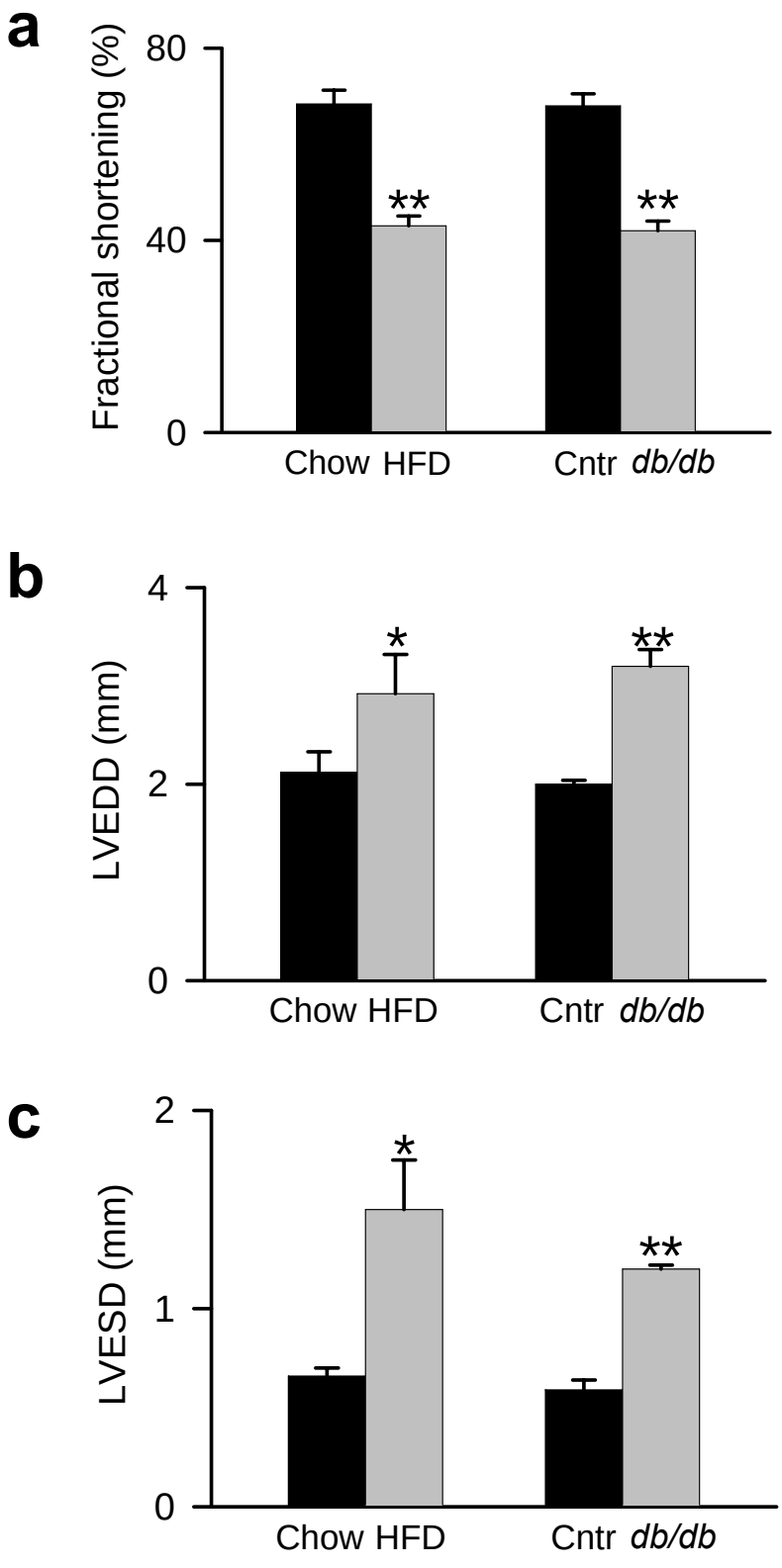
Primer pairs used for quantitative RT-PCR

Gene	Primer Pair	
	Forward (5'-3')	**Reverse (5'-3')**
18s RNA	ACCGCAGCTAGGAATAATGGA	TCTCCTCCAGGTGGTCTAGCA
ANF	CTTCTTCCTCGTCTTGGCCT	CTGCTTCCTCAGTCTGCTCA
ATROGIN-1	CCATCAGGAGAAGTGGATCTATGTT	GTTCATGAAGTT CTTTTGGGCGATGC
BNF	CATGGATCTCCTGAAGGTGC	CCTTCAAGAGCTGTCTCTGG
BNIP3	TTCCACTAGCACCTTCTGATGA	GAACACCGCATTTACAGAACAA
CD36	GCCAAGCTATTGCGACATGA	AAGGCATTGGCTGGAAGAAC
CPT1M	TGTCACTTCTGTCGCCACCT	CACCTCATAACGCTGGCTTC
FOXO1	GCAGCCAGGCATCTCATAA	CCTACCATAGCCATTGCAGC
FOXO3	AGATCTACGAGTGGATGGTG	CGGCTCTTGGTGTACTTGTT
GLUT1	AACATGGAACCACCGCTACG	CCATAAGCACAGCAGCCACA
GLUT2	AGGAAGAGGCATCGACTGAG	CACAGAGACAGCCGTGAAGA
GLUT4	CACTGCTTCTGGCTCTCACA	GCTCTCTCTCCAACTTCCGT
HK1	TCACCGAGCTGAAGGATGAC	GCCGAGATCCAGTGCAATGA
LPL	GTG TGA TTG CAGA GAGA GGA	TTC TAC AA CTC AGG CAG AGC
MURF-1	ACAACCTCTGCCGGAAGTGT	CGGAAACGACCTCCAGACAT
PDK4	GCTTGCCAATTTCTCGTCTC	CTTCTCCTTCGCCAGGTTCT
PFKFB2	GTCCATCCTCGCACCATCTA	TCCTCTTCAACTGGCTCGTC
PFKM	TTGACCTCTGGTGGAGATGC	TTCTCGCTCTCGGCAGTCCT
PGC1α	CTCTCCTTGCAGCACCAGAA	CCATCCATGGCTAGTCCTGA
PPARα	TCACAAGTGCCTGTCTGTCG	CAGGTAGGCTTCGTGGATTC
SMA	AGACCACCGCTCTTGTGTGT	GTCAGGATACCTCGCTTGCT
αMHC	CGGAACAAGACAACCTCAAT	TGGCAATGATTTCATCCAGC
βMHC	AAGCAGCAGTTGGATGAGCG	CCTCGATGCGTGCCTGAAGC

Supplemental Figure 1

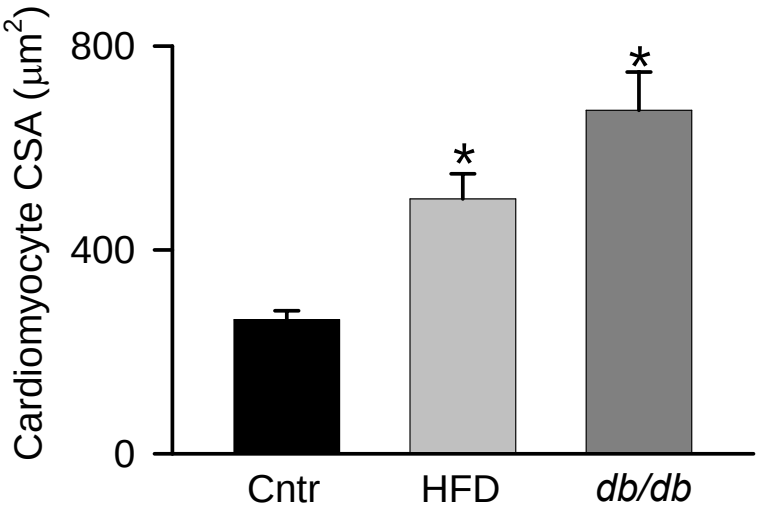


Supplemental Figure 2



Supplemental Figure 3

a



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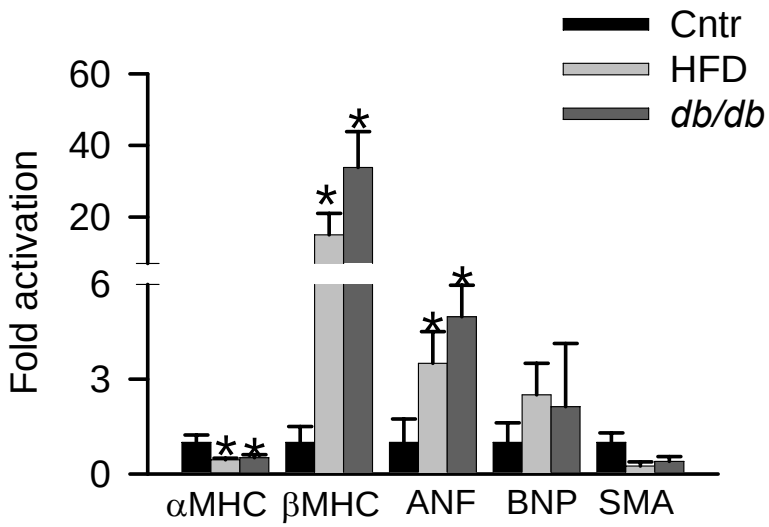
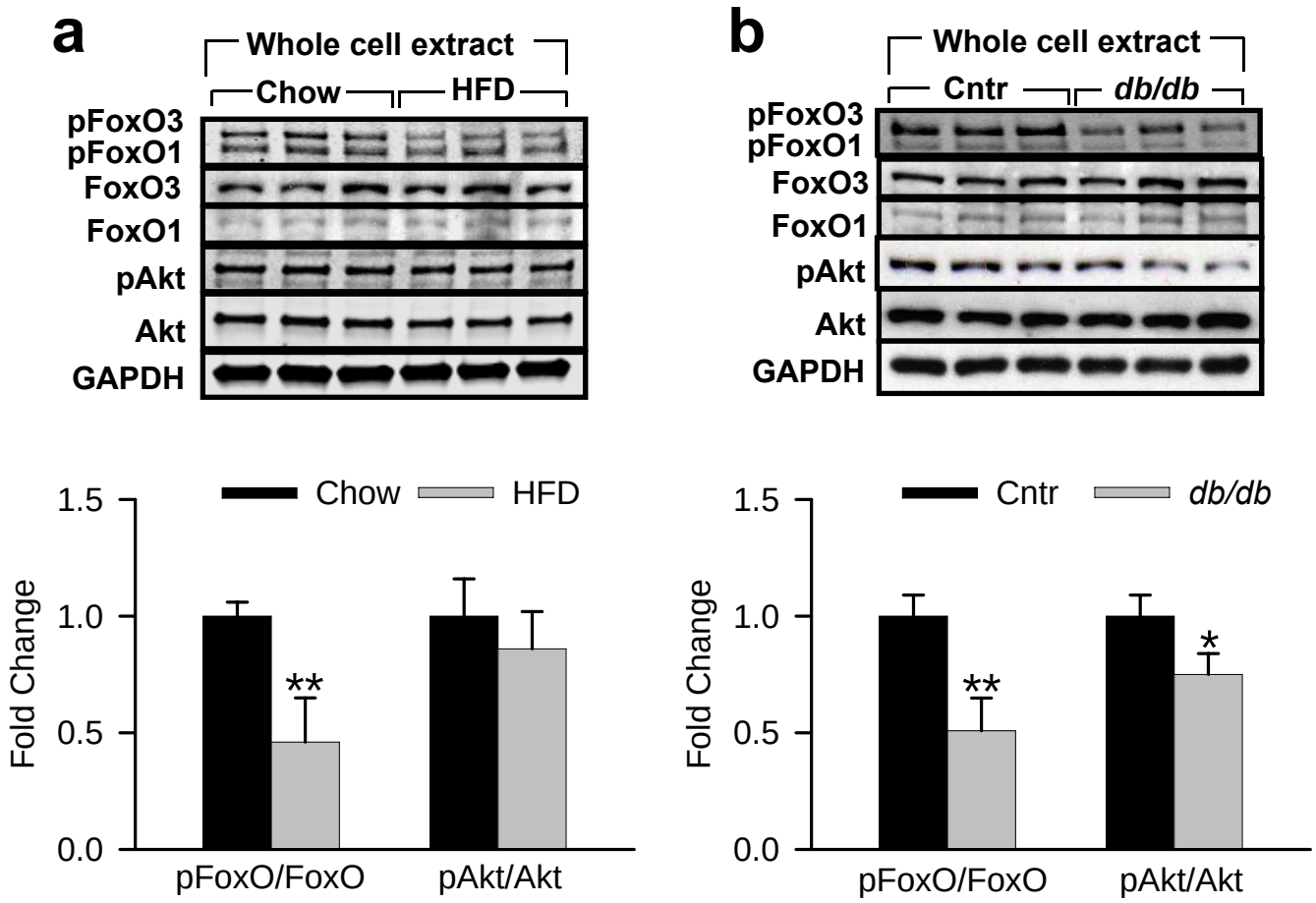


Supplemental Figure 4



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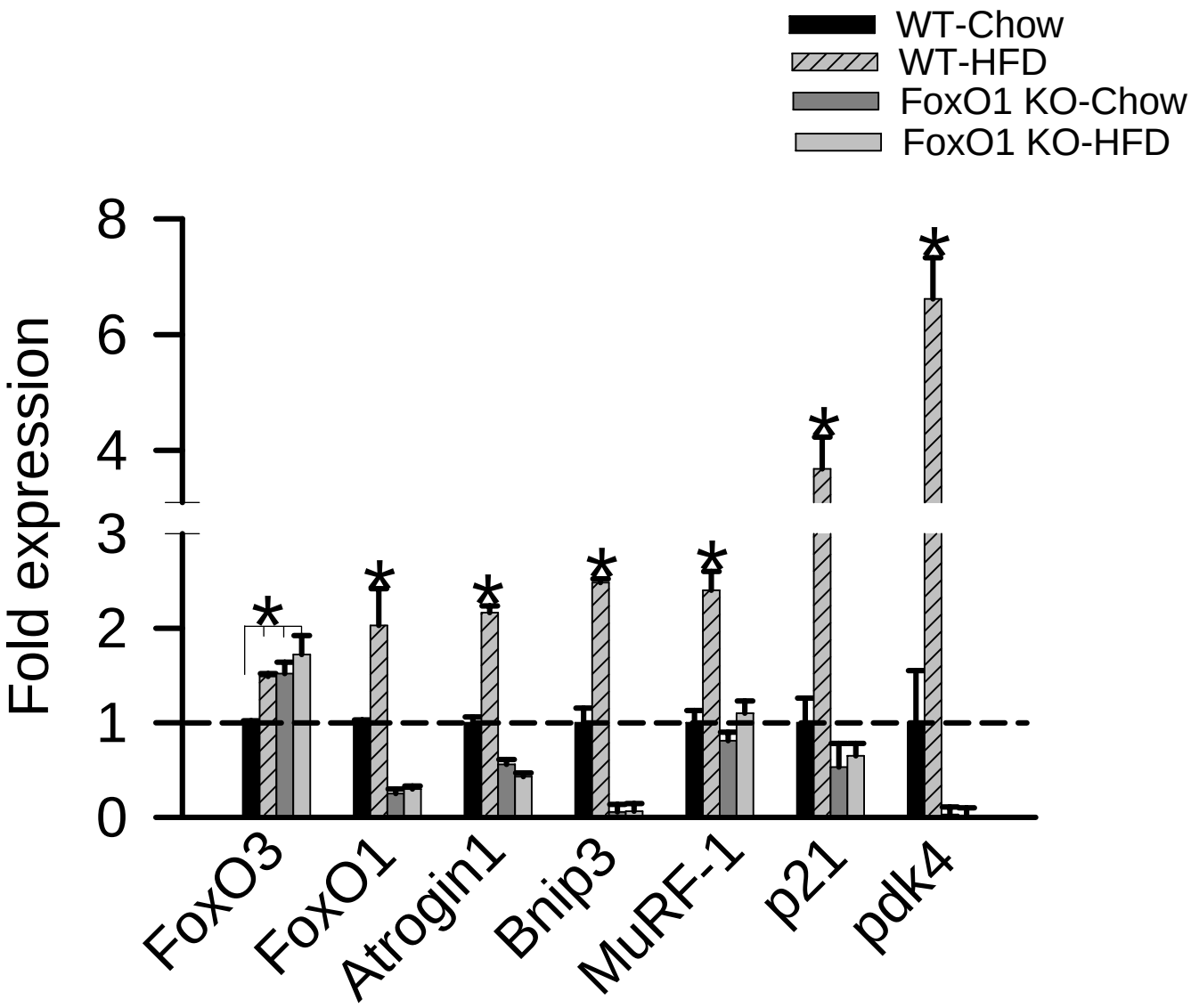

Supplemental Figure 6

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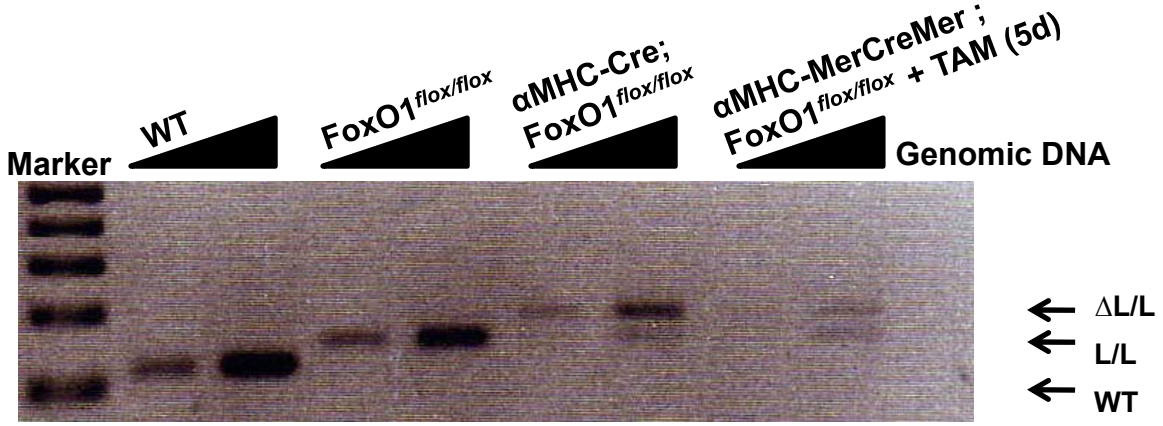

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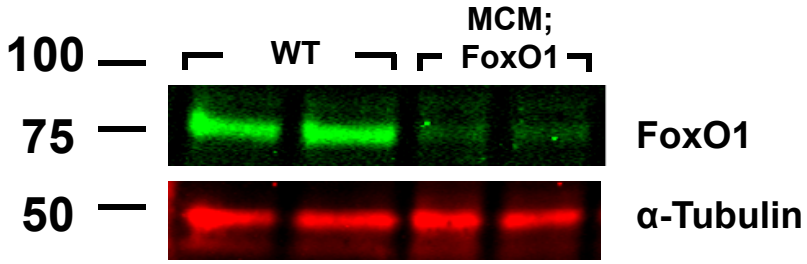
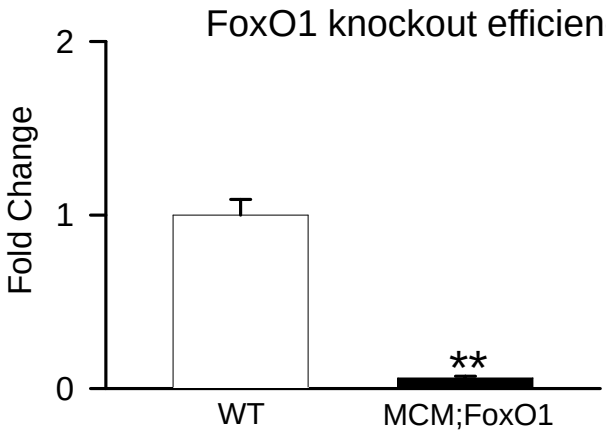
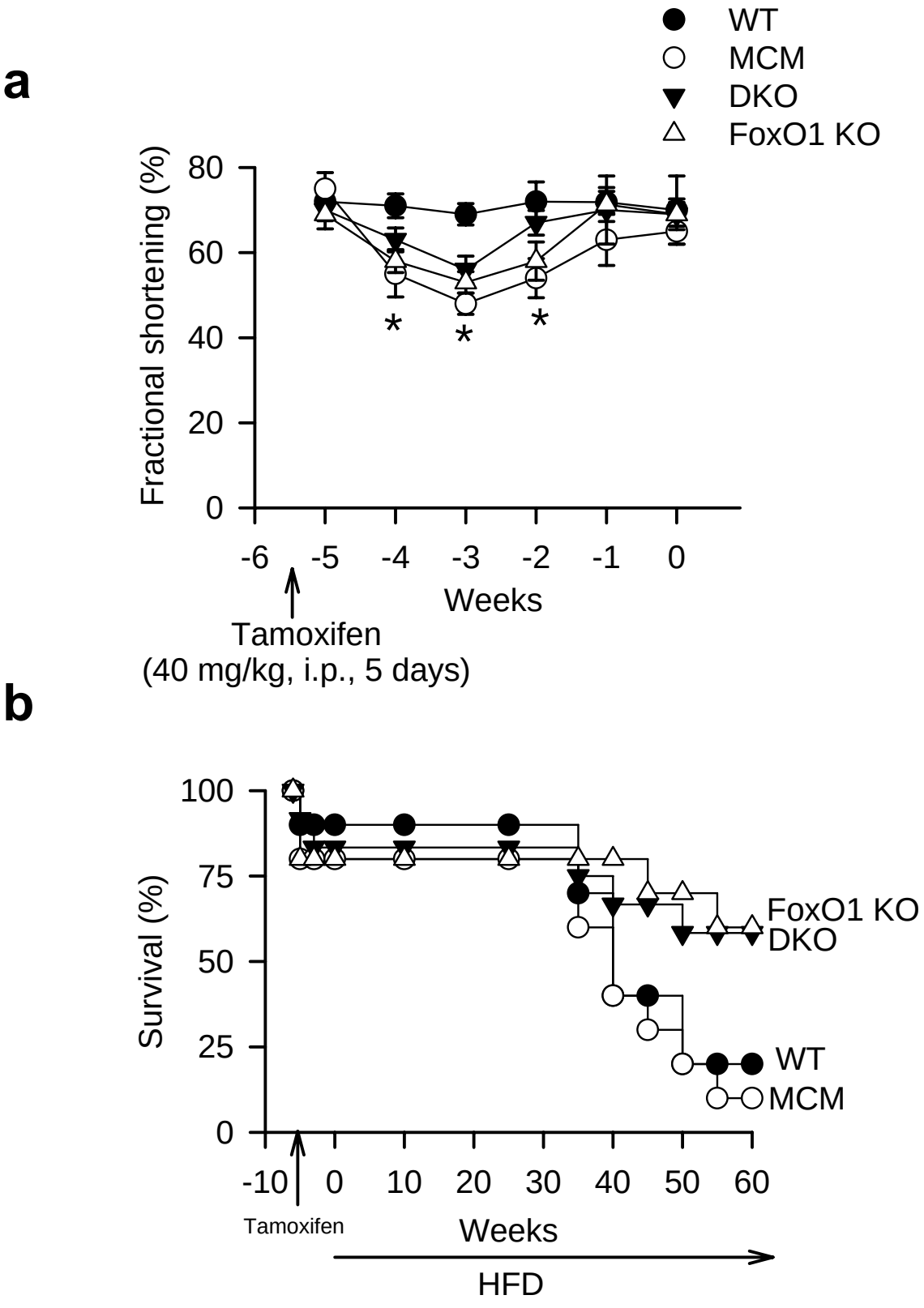
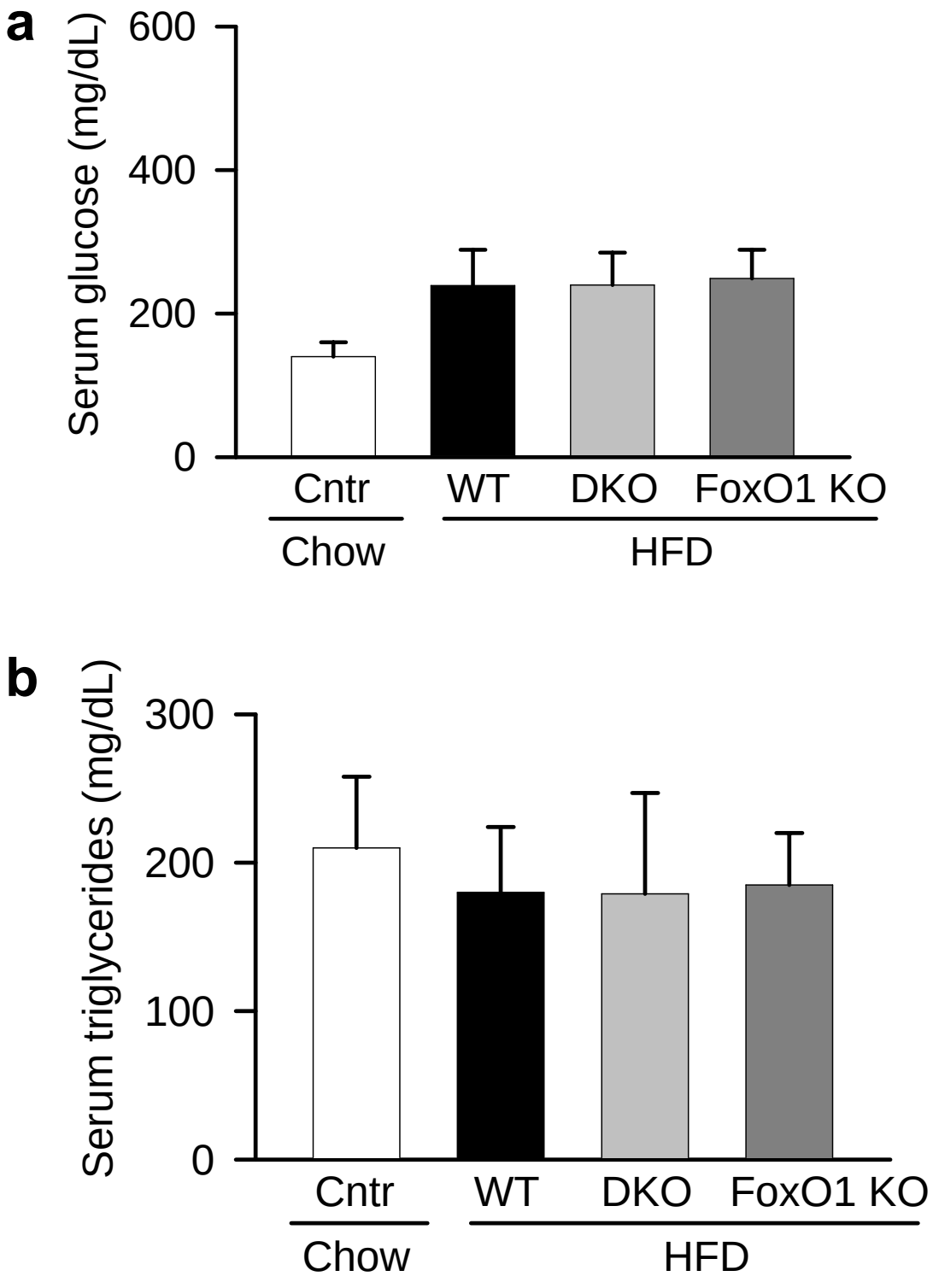




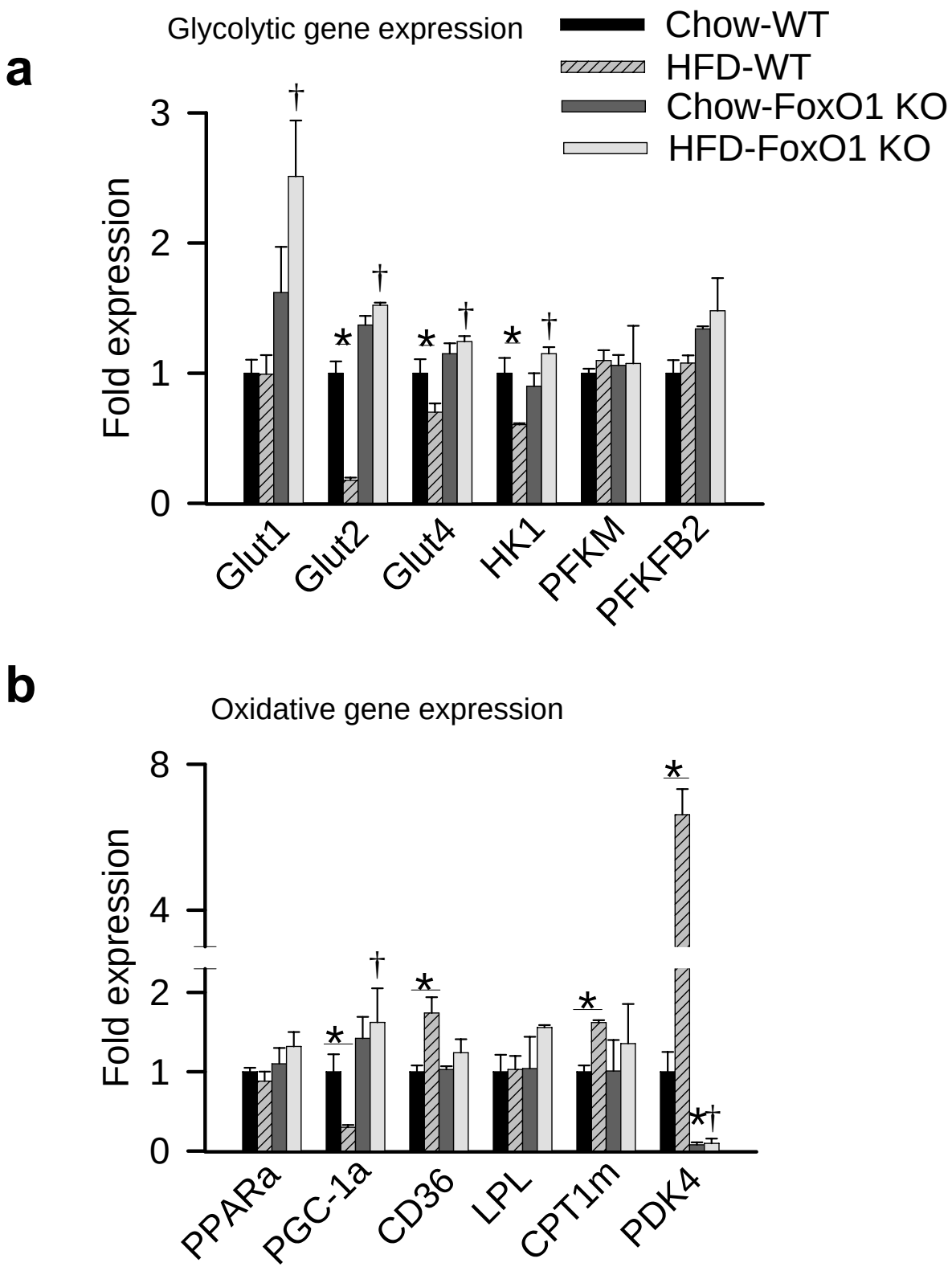
Supplemental Figure 7



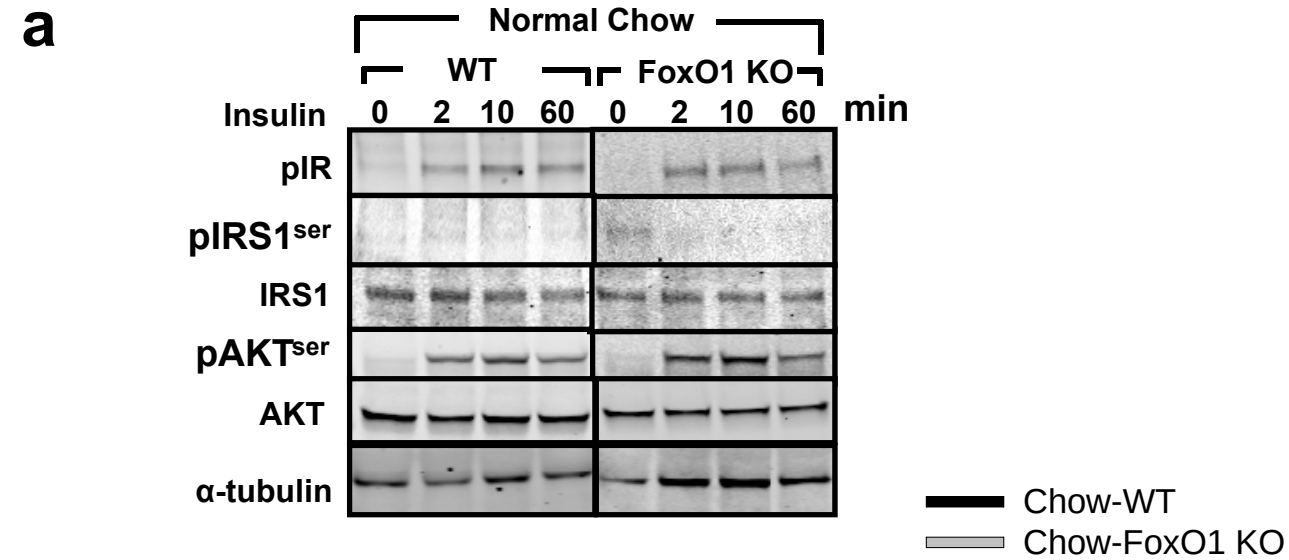
Supplemental Figure 8

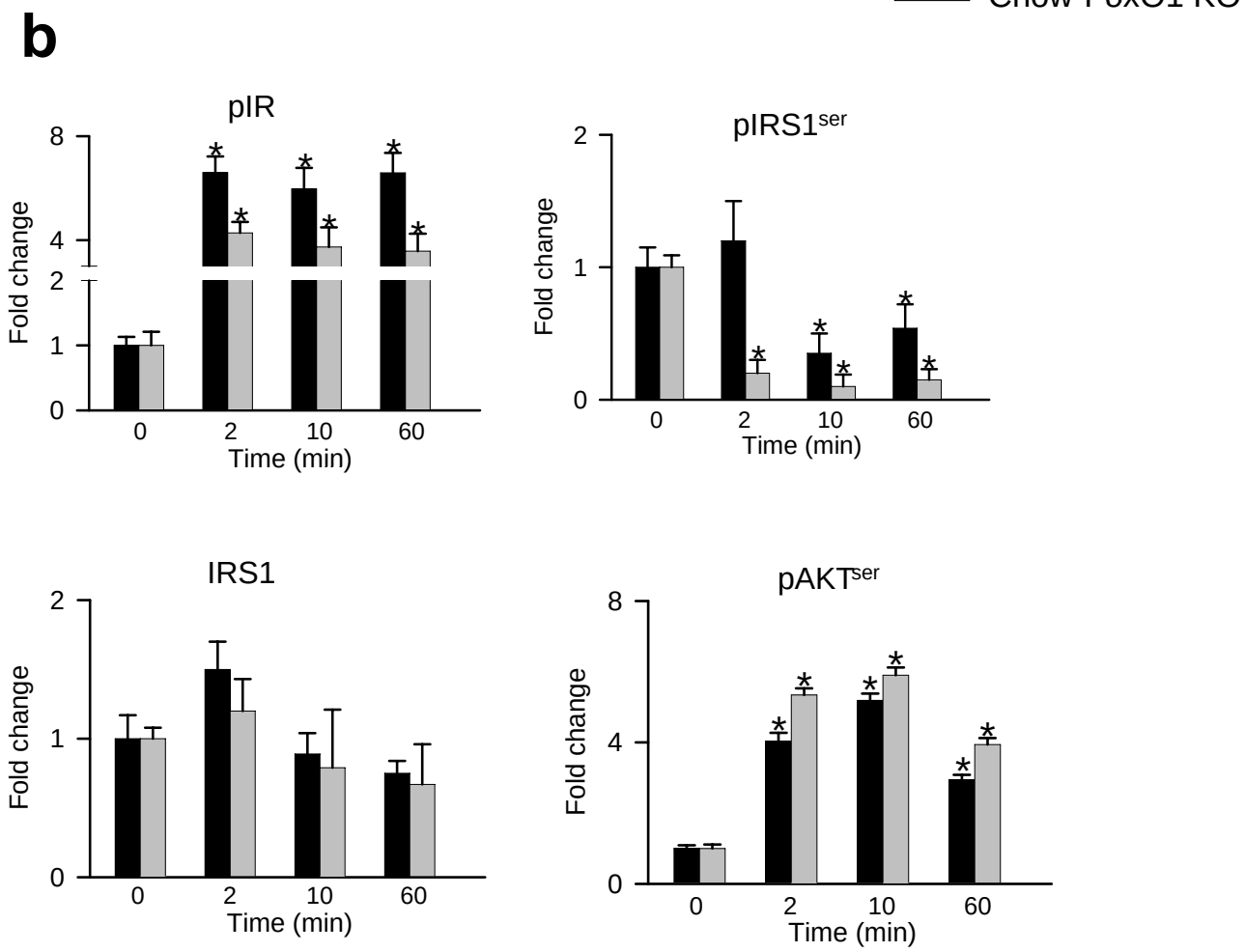


Supplemental Figure 9



Supplemental Figure 10





Online Data Supplement Figure Legends

Supp. Figure 1. Measurement of body weight, serum glucose and serum insulin levels in chow vs. HFD and Cntr vs. *db/db* mice. Increased body weight **(a)**, hyperglycemia **(b)** and hyperinsulinemia **(c)** were observed in HFD and *db/db* mice compared with their respective age-matched controls**.** HFD refers to high fat diet group, Cntr refers to *db*/+ and WT combined. The results are presented as mean±s.e.m., n=8 per group. ** $P < 0.01$ vs. respective controls.

Supp. Figure 2. Cardiac dysfunction in models of type II diabetes. (a-c) Echocardiography of gently restrained, unanaesthetized mice revealed decreased percent fractional shortening **(a)** and increased left ventricular dimensions in end-diastole (LVEDD) **(b)** and end-systole (LVESD) **(c)** in both HFD and *db/db* mice compared with their respective controls. Cntr refers to *db*/+ and WT combined. The results are presented as mean±s.e.m., n=8 per group. *$P < 0.05$; ** $P < 0.01$ vs. respective controls.

Supp. Figure 3. Cardiac hypertrophy in models of type II diabetes. (a,b) Cardiomyocyte cross-sectional areas (CSA) were greater in HFD and *db/db* groups compared with controls (Cntr) **(a)**. CSA was determined from at least 15 cells per section and 3 sections per group stained for wheat-germ agglutinin (sections not shown). Further, mRNA measurements by real-time RT-PCR (normalized to 18S RNA) revealed reduced αMHC and elevated βMHC and ANF levels **(b)**. Cntr refers to *db*/+ and WT combined. Results are presented as mean±s.d., n=3 per group. *$P < 0.05$ vs. Cntr.

Supp. Figure 4. FoxO factors are active in HFD and *db/db* hearts. (a,b) Top, immunoblot detection of phosphorylated FoxO3 and FoxO1 (Threonine 24 and 32 residues, respectively), Akt (Serine 473 residue) and total FoxO3, FoxO1, Akt proteins from whole cell cardiac extracts in chow vs. high fat diet (HFD) **(a)** and Cntr vs. *db/db* hearts **(b)**. Bottom, quantification of band density normalized to respective total protein. Decreased pFoxO/FoxO levels are indirectly suggestive of activation of FoxO proteins. Cntr refers to *db*/+ and WT combined. Histograms are presented as mean±s.d. (n=5). *$P < 0.05$; ** $P < 0.01$ vs. respective controls.

Supp. Figure 5. Expression of FoxO1-specific targets. mRNA levels of FoxO1 downstream target genes in hearts from chow- and HFD-fed WT and FoxO1 KO mice measured using real-time RT-PCR. Data are normalized to 18S RNA and are presented as mean±s.d. (n=3-5). *P<0.05 vs WT controls.

Supp. Figure 6. Recombination and knockout efficiency of FoxO1. Recombination efficiency was determined from genomic DNA prepared from isolated adult cardiomyocytes from WT, *FoxO1$^{flox/flox}$, αMHC-Cre; FoxO1$^{flox/flox}$, αMHC-MerCreMer; FoxO1$^{flox/flox}$* post TAM (tamoxifen) injection (40 mg/kg IP x 5d) **(a)**. Immunoblotting of whole cell extracts prepared from WT and MCM;FoxO1 revealed significant reduction in FoxO1 levels normalized to α-tubulin following 5-day TAM injection **(b)**. Histograms are presented as mean±s.d. (n=5). ** P < 0.01 vs. WT controls.

Supp. Figure 7. Tamoxifen–induced transient cardiac dysfunction and mortality. A significant, transient decline in percent fractional shortening **(a)** and 10-15% mortality **(b)** were observed in MerCreMer (MCM), *αMHC-Cre; FoxO3&1$^{flox/flox}$*(DKO), *αMHC-MerCreMer; FoxO1$^{flox/flox}$* (FoxO1 KO) mice following 5-day tamoxifen i.p., injection (40 mg/kg). Data are presented as mean±s.e.m., n=12, P < 0.01 vs. controls.

Supp. Figure 8. Serum glucose and triglycerides. Serum glucose **(a)**, and serum triglycerides **(b)** in chow-fed controls (Cntr) compared to WT, DKO and FoxO1 KO mice fed HFD. Data are presented as mean±s.d. n=5. No statistical significance observed at P < 0.05.

Supp. Figure 9. Expression of glycolytic and oxidative genes in cardiac tissue from chow and HFD-fed, WT, and FoxO1 KO mice. Real-time RT-PCR analysis revealed significant decreases in mRNA levels of glycolytic (Glut4, HK1) and significant increases in oxidative (CD36, CPT1m) genes in WT-HFD group. Data are presented as mean±s.d., n=3. *P < 0.05 vs. WT-Chow group, $^{\dagger}P$ < 0.05 vs. WT-HFD group.

Supp. Figure 10. Insulin responses in adult cardiomyocytes isolated from chow-fed WT and FoxO1 KO mice. Top, immunoblot detection of proteins involved in insulin signaling cascade. Bottom, quantification of band density normalized to α-tubulin. Proteins were blotted from whole cell extracts of isolated adult cardiomyocytes treated with insulin (100 nM) for 0, 2, 10, 60 min. pIR, phosphorylated-insulin receptor β (Tyr1150/1151); $pIRS1^{ser}$, phosphorylated-insulin receptor substrate-1 (serine 636/639 residue); IRS1, insulin receptor substrate-1; $pAKT^{ser}$, phosphorylated-AKT (serine 473 residue). Histograms are presented as mean±s.d., n=3. *$P < 0.05$ vs. 0 time-point controls within each group.