Supplementary Information

FoxK1 and FoxK2 are Components in Insulin Regulation of Cellular and Mitochondrial Metabolism

Sakaguchi et al

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Proteomics screening of IR/IGF1R binding proteins in the insulin dependent manner

Total: 1469proteins

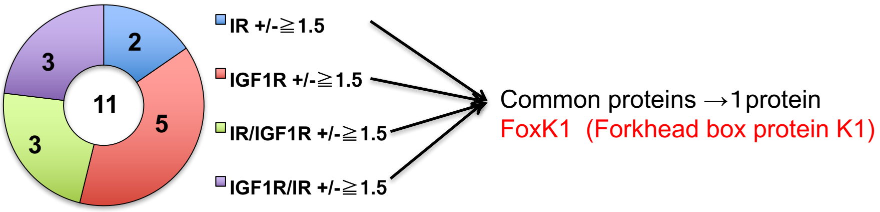
↓

≧5 quantified peptide: 707 proteins (Including IRS1 and IRS2)

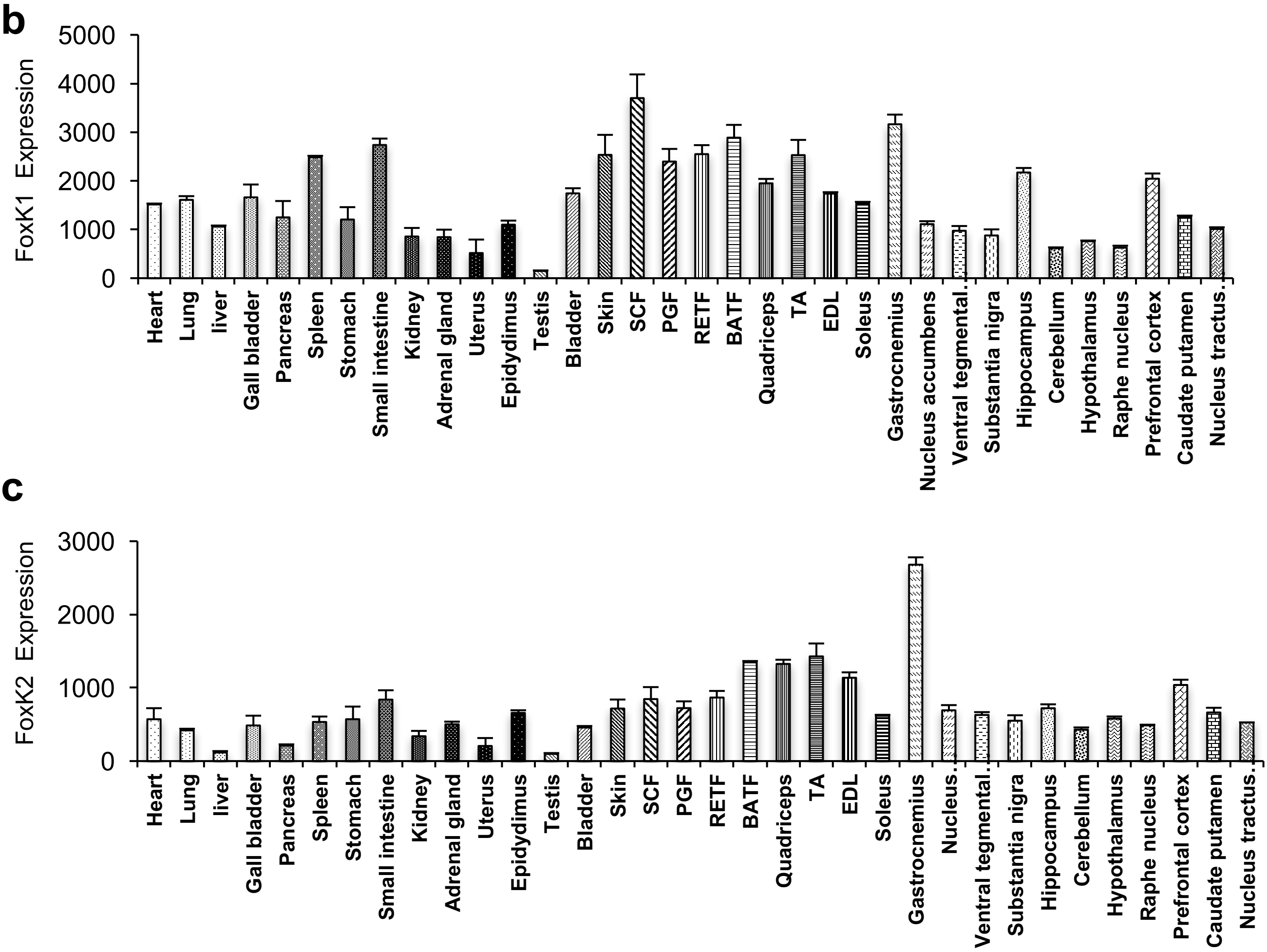
↓

(IR+/IR-≧1.5, IGF1R+/IGF1R-≧1.5, IR/IGF1R+/ IR/IGF1R-≧1.5)
IGF1R/IR+/ IGf1R/IR-≧1.5) Total 11 proteins

↓



Common proteins →1protein
FoxK1 (Forkhead box protein K1)



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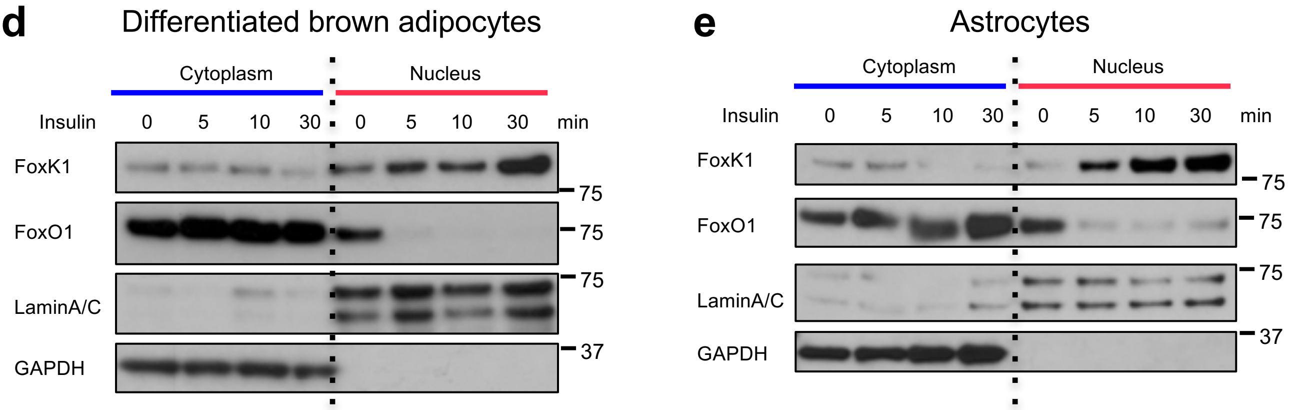
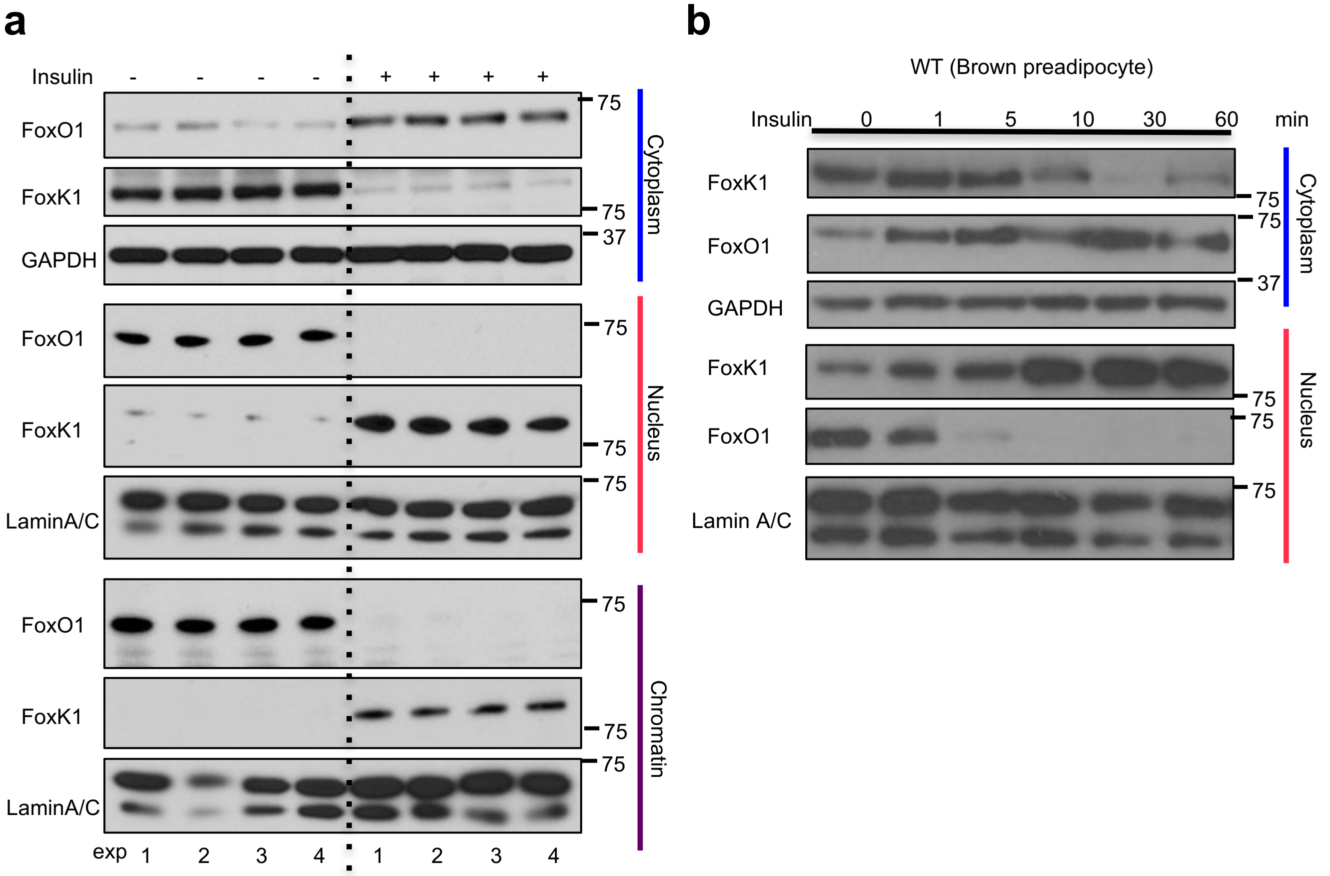
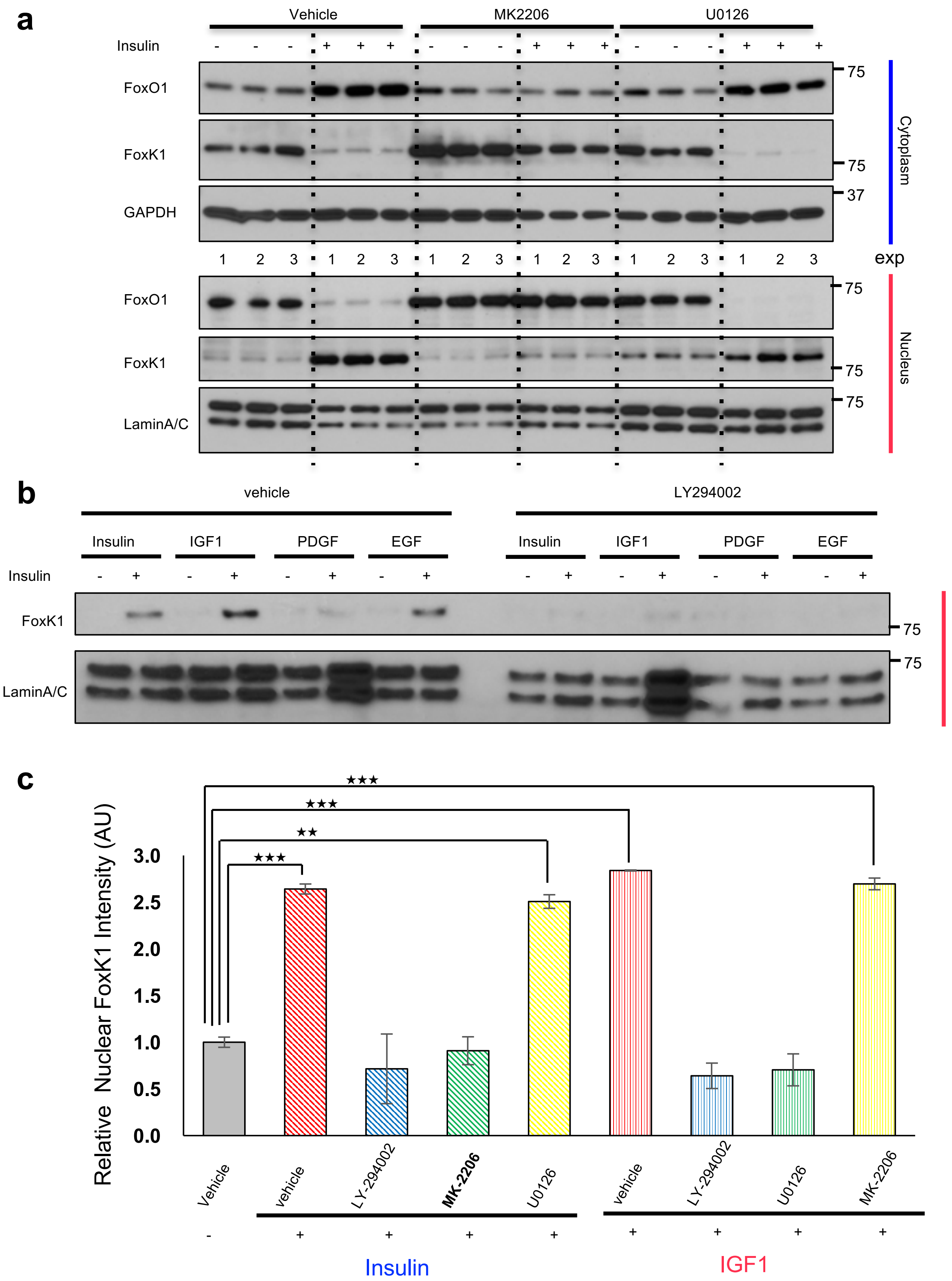


Supplementary Figure 1. FoxK1 is a novel component of IR- and IGFR-mediated signaling complex.
(**a**) FoxK1 is identified by the proteomics screening of IR/IGF1R binding proteins. (**b**) FoxK1 and (**c**) FoxK2 expression (qPCR) from 34 tissues from 3-month-old C57BL/6J mice (All data are represented as mean ± SEM, n=3). Data were normalized to *Tbp*. Immunoblotting of FoxO1 and FoxK1 in nuclear and cytoplasmic fractions extracted from differentiated brown preadipocytes **(d)** or astrocytes **(e)** before and after 100 nM insulin stimulation for indicated times.



Supplementary Figure 2. Insulin-induced FoxK1 translocation is reciprocal to FoxO1. (a) Subcellular fractions of cytoplasm, nucleus and chromatin were prepared from DKO brown preadipocytes reexpressing the IR without (0 min) and with 100 nM insulin stimulation for 30 min. FoxO1 and FoxK1 in each fraction were assessed by western blotting, as were markers for different fractions, cytosol (GAPDH), nuclear and chromatin (Lamin A/C) (n = 4). **(b)** Immunoblotting of FoxO1 and FoxK1 in nuclear and cytoplasmic fractions extracted from wild-type brown preadipocytes before and after 100 nM insulin stimulation for 30 min.



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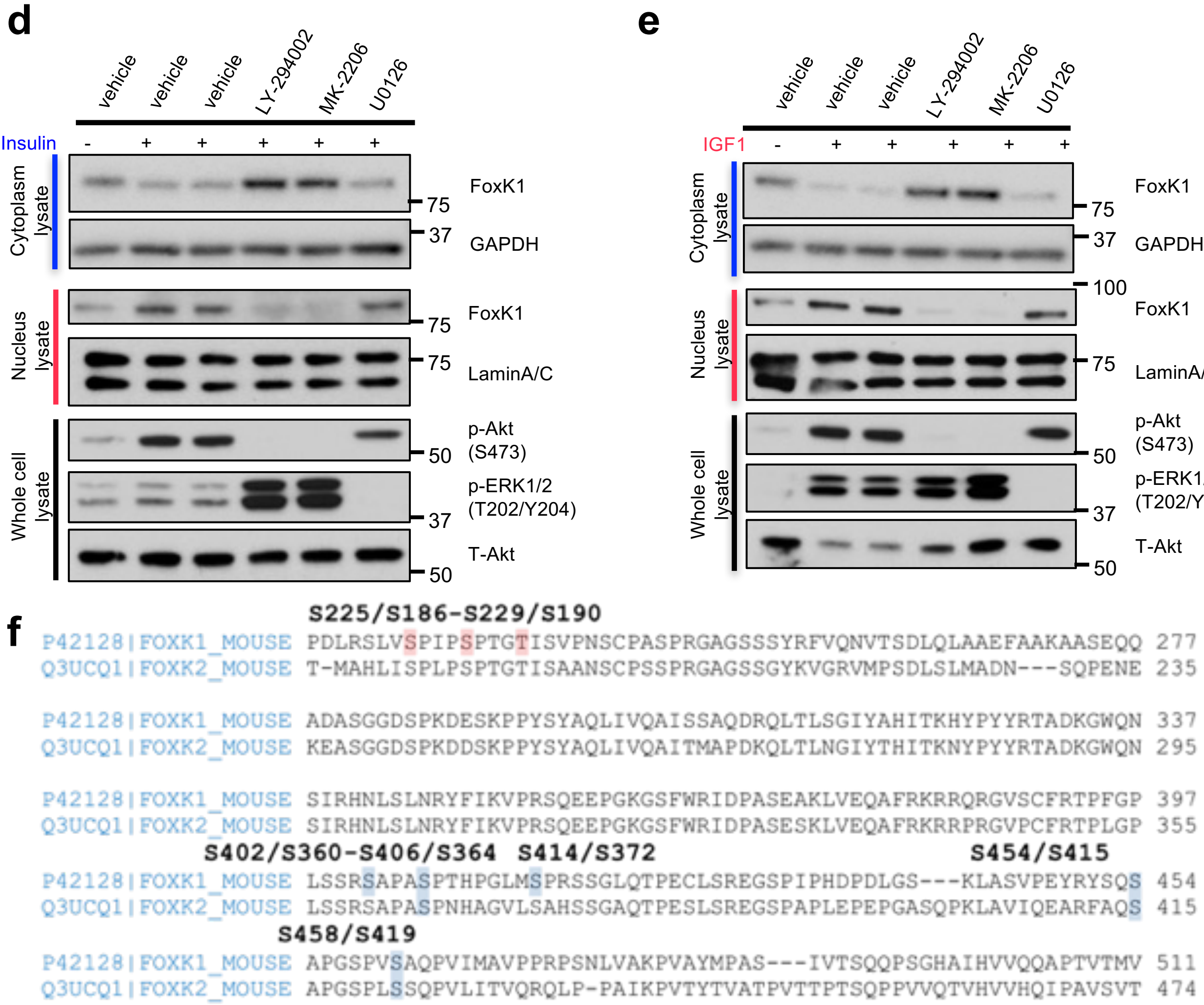
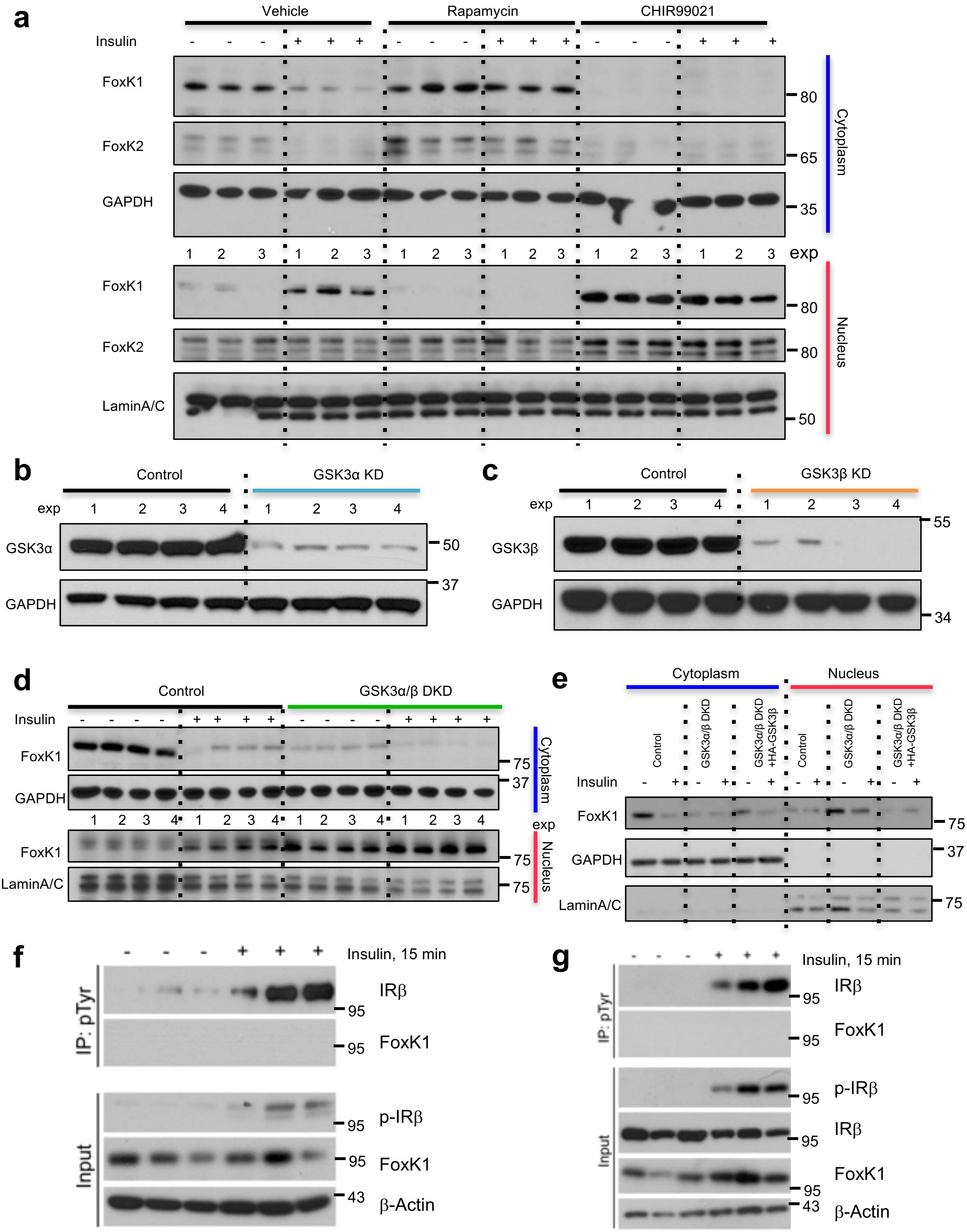


Supplementary Figure 3. Insulin-induced FoxK1 translocation is regulated by PI3K-Akt rather than ERK MAPK pathway. (a) Immunoblotting of FoxO1 and FoxK1 in nuclear and cytoplasmic fractions extracted from IR-expressing brown preadipocytes before and after 10 nM insulin stimulation for 30 min in the presence or absence of the Akt inhibitor MK2206 (5 μM) or the MEK1/2 inhibitor U0126 (20 μM) (n = 4). **(b)** Immunoblotting of FoxK1 in nuclear fraction extracted from WT-brown preadipocytes before and after stimulation with insulin, IGF1, PDGF and EGF at 15 min in the presence or absence of the PI3K inhibitor (LY-294002). (**c**) Relative FoxK1 fluorescence intensity in nuclear (DAPI$^+$) area quantified by Image J in Figure 2d. All data are represented as mean ± SEM. ($P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) (n = 4). **(d,e)** Immunoblotting of FoxO1 and FoxK1 in nuclear and cytoplasmic fractions extracted from AML12 cells before or after stimulation with 100 nM insulin and IGF1 for 30 min in the presence or absence of the Akt inhibitor MK2206 (5 μM) or the MEK1/2 inhibitor U0126 (20 μM). **(e)** Comparison of the phosphorylation clusters between FoxK1 and FoxK2 after Insulin/IGF1 stimulation. The significant increase after Insulin/IGF1 stimulation shown in red and the decrease shown in blue.



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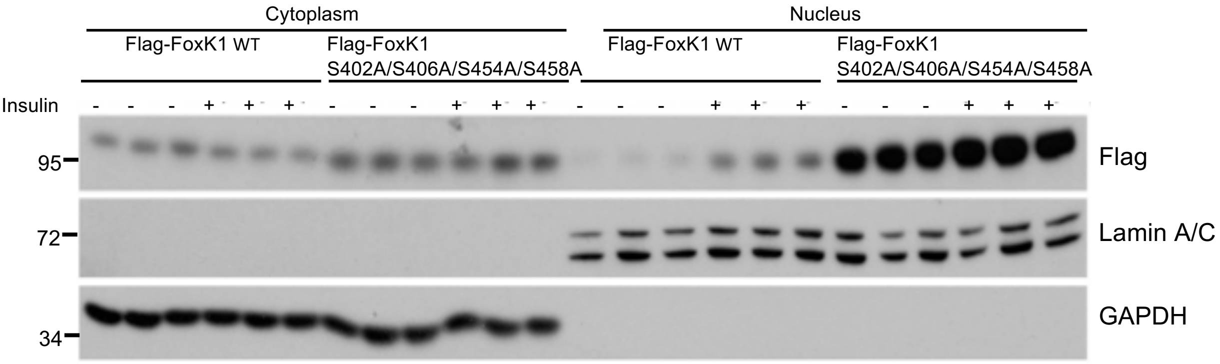
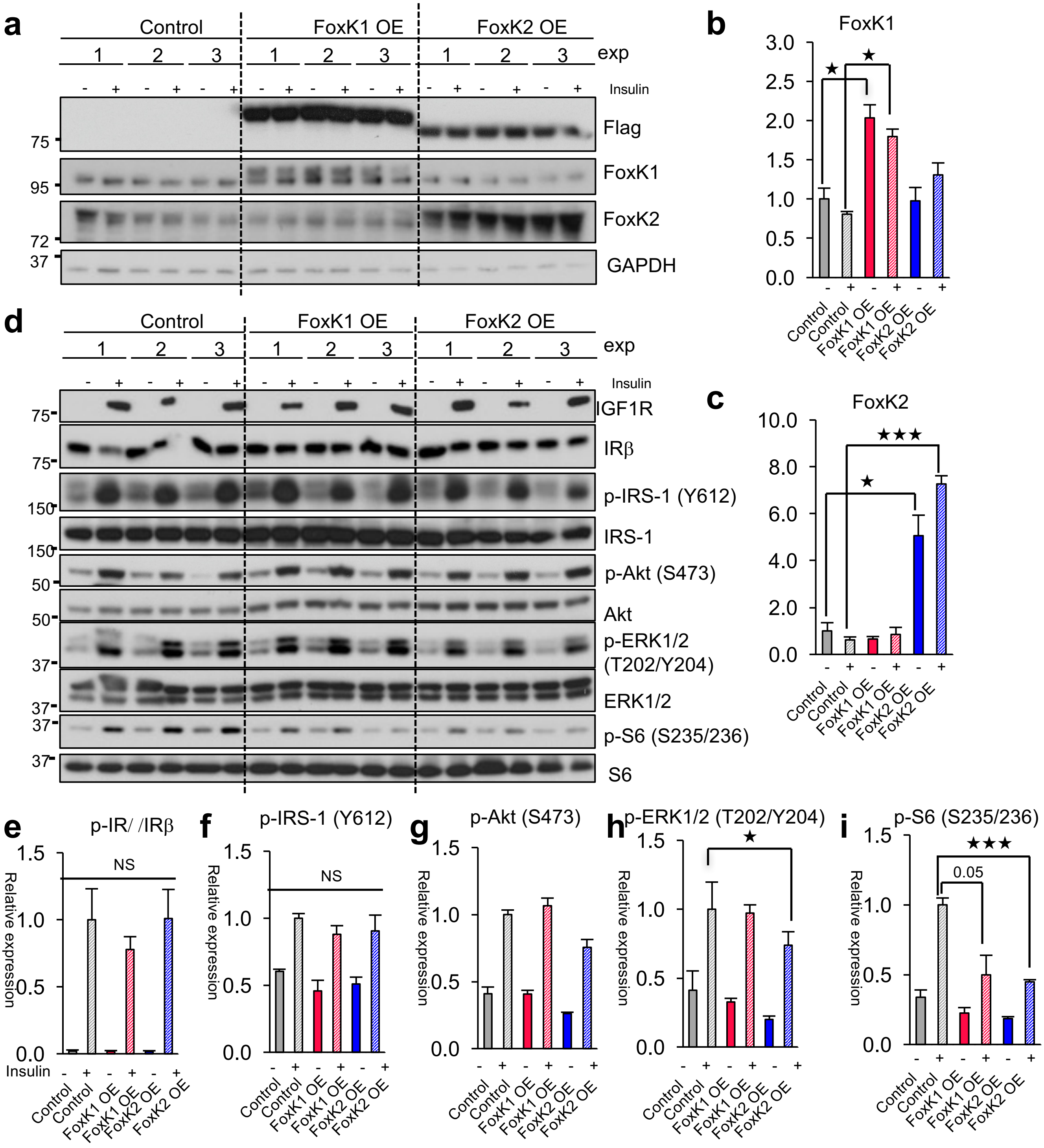


Supplementary Figure 4. Insulin-induced FoxK1 translocation is regulated by not only mTOR but also GSK signaling. (a) Nuclear cytoplasmic fractionation and immunoblotting for FoxK1 and FoxK2 extracted from the AML12 cells before and after 100 nM insulin at 30 min the presence or absence of 100 nM rapamycin (mTOR inhibitor) or/and 10 μM CHIR99201 (GSK3 inhibitor). GAPDH is cytosolic marker and Lamin A/C is nuclear marker (n = 3). Immunoblotting Control (NS siRNA), GSK3α-knockdown (GSK3α KD) **(b)** and GSK3β-knockdown (GSK3β KD) **(c)** cells by siRNAs with anti-GSK3α and GSK3β antibodies (n = 4). **(d)** Nuclear and cytoplasmic fractionation and immunoblotting of FoxK1 extracted from the Control (NS siRNA) or GSK3α/β double knockdown cells before (0 min) and after 100 nM insulin stimulation for 30 min. (n = 4). (**e**) Nuclear and cytoplasmic fractionation and immunoblotting of FoxK1 extracted from the Control (NS siRNA) , GSK3α/β DKD cells and re-expressed with HA-GSK3β in GSK3α/β DKD cells. before (0 min) and after 100 nM insulin stimulation for 30 min.(**f**) Immunoblotting with anti-IRβ, p-IRβ and FoxK1 antibody in lysates extracted from AML12 cells which were immunoprecipitated with p-Tyr antibody following insulin stimulation. (**g**) Immunoblotting with anti-IRβ, p-IRβ and FoxK1 antibody in lysates extracted from liver samples which were immunoprecipitated with p-Tyr antibody following insulin stimulation via vena cava injections. (**h**) Nuclear and cytoplasmic fractionation and immunoblotting of Flag extracted from the overexpressed 3XFlag-FoxK1 wild type (WT) cells or overexpressed 3XFlag-FoxK1 S402A/S406A/S454A/S458A mutant cells before (0 min) and after 100 nM insulin stimulation for 30 min.



Supplementary Figure 5. Regulation of insulin-mediated signal transduction in overexpression of FoxK1 or FoxK2 cells. **(a)** Immunoblotting for Flag, FoxK1 and FoxK2 in lysates from Control (Mock) AML12 cells or cells overexpressed either 3XFlag-FoxK1 (FoxK1 OE) or 3XFlag-FoxK2 (FoxK2 OE) with 100 nM insulin for 5 min. **(b-c)** Densitometric analysis of phosphorylated FoxK1 and FoxK2 following 5 min stimulation. Data are mean ± SEM of n = 3 (One-way ANOVA followed by *t*-test with Bonferroni correction , *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.). **(d)** Immunoblotting of the phosphorylation of IR, IRS-1, Akt, ERK, and S6 in lysates from Control (Mock) AML12 cells or cells overexpressed either 3XFlag-FoxK1 (FoxK1 OE) or 3XFlag-FoxK2 (FoxK2 OE) and stimulated with 100 nM insulin for 5 min. **(e-i)** Densitometric analysis of phosphorylated IR, IRS1, Akt, ERK, and S6 following 5 min insulin stimulation. Data are mean ± SEM of n = 3 (One-way ANOVA followed by *t*-test with Bonferroni correction , *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$).

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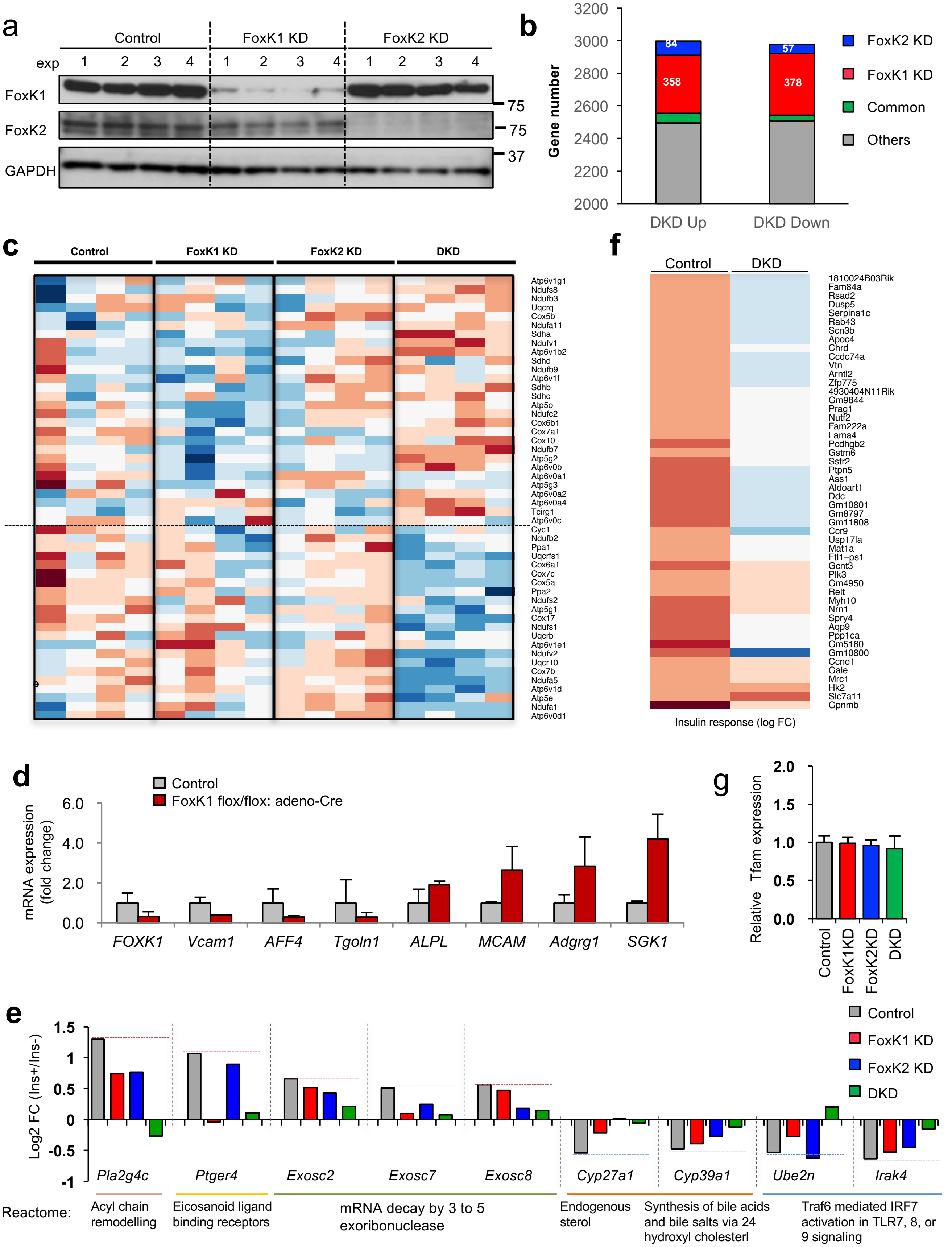
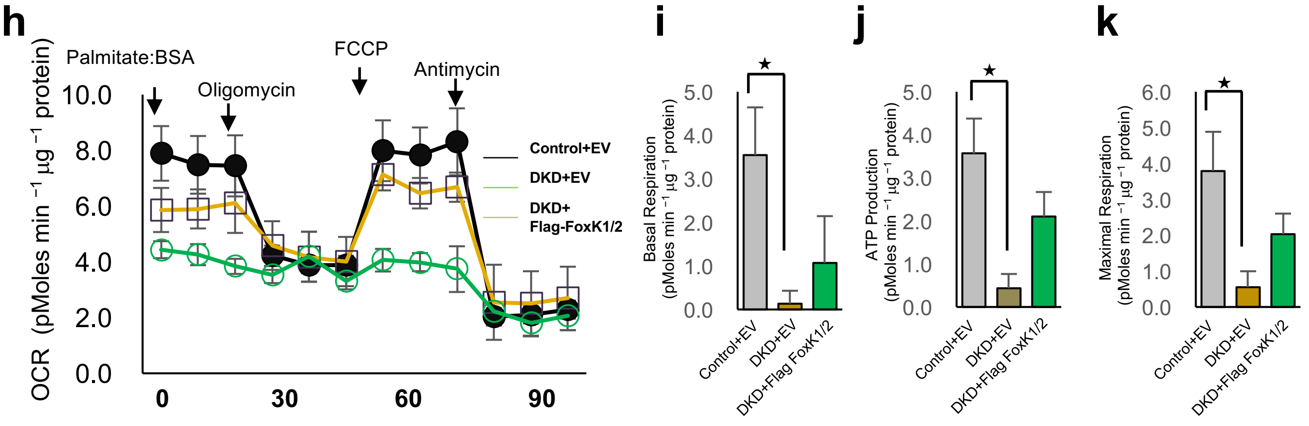


Sakaguchi et al., Supplementary Figure 6



Supplementary Figure 6. Differential gene regulation by loss of FoxK1 and FoxK2. **(a)** Immunoblotting Control (NS siRNA), FoxK1 knockdown (FoxK1 KD) and FoxK2 knockdown (FoxK2 KD) cells by siRNAs with anti-FoxK1 and FoxK2 antibodies. (n = 4). (**b**) Among the 2996 genes, 358 (12%) genes were selectively upregulated and 378 (12.6%) genes were selectively downregulated in FoxK1 KD cells, whereas only 84 (3%) genes were selectively upregulated and 57 (2%) genes were selectively downregulated in FoxK2 KD cells. **(c)** Heat map of mitochondrial oxidative phosphorylation genes in all groups. (**d**) mRNA abundance measured by real-time qPCR in liver from Contol (FoxK1$^{flox/flox}$: adeno-GFP) *n* = 4 and FoxK1KO (FoxK1$^{flox/flox}$: adeno-Cre) mice. All data are represented as mean ± SEM. **(e)** Heatmap of top 50 significant changed insulin response genes differentially regulated by Control and DKD (SHOWN only up in Control). (**f**) Alteration of reactome transcripts in KD cells compared with Controls (gray). Fold change is shown as insulin +/insulin -. (**g**) mRNA abundance measured by real-time qPCR from Control ,FoxK1KD,FoxK2KD and DKD cells *n* = 4/group. All data are represented as mean ± SEM. (**h**) Measurement of fatty acid oxidation (FAO) using Seahorse Bioanalyzer. 0.175 mM palmitate:BSA was used as substrate. One day before cells were incubated with substrate-limited medium (DMEM, 0.5 mM glucose, 1 mM GlutaMAX, 0.5 mM carnitine, 1% FBS) to prime cells for fatty acid utilization. One hour before the assay, cells were given 2.5 mM glucose and 0.5 mM carnitine in the running medium (111 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 2 mM $MgSO_4$, 1.2 mM NaH_2PO_4 and 5 mM HEPES), followed by addition of 0.175 mM palmitate-BSA (pal), 1µM oligomycin (oligo) from, 0.5 mM FCCP, and 2 µM antimycin (Ant). All data are represented as mean ± SEM. **(i-k)** Quantitation of basal (**i**), ATP-coupled (**j**) and maximal (**k**) oxygen consumption rates (OCRs) of fatty acid oxidation (FAO). For the quantifications in the bar graphs, data points for all wells were averaged, and error bars represent SEM. (One-way ANOVA followed by *t*-test with Bonferroni correction , *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, n = 5/group).

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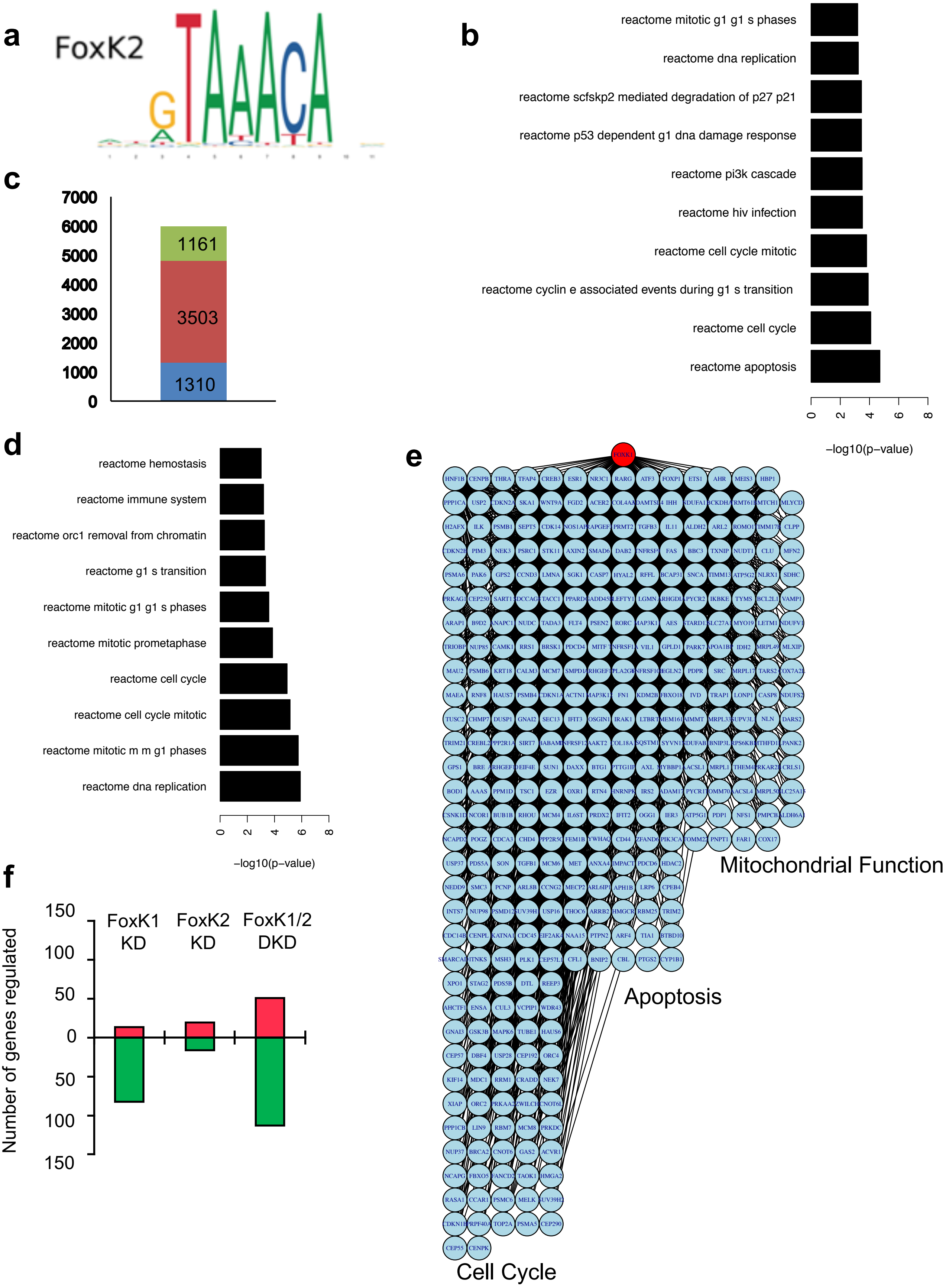

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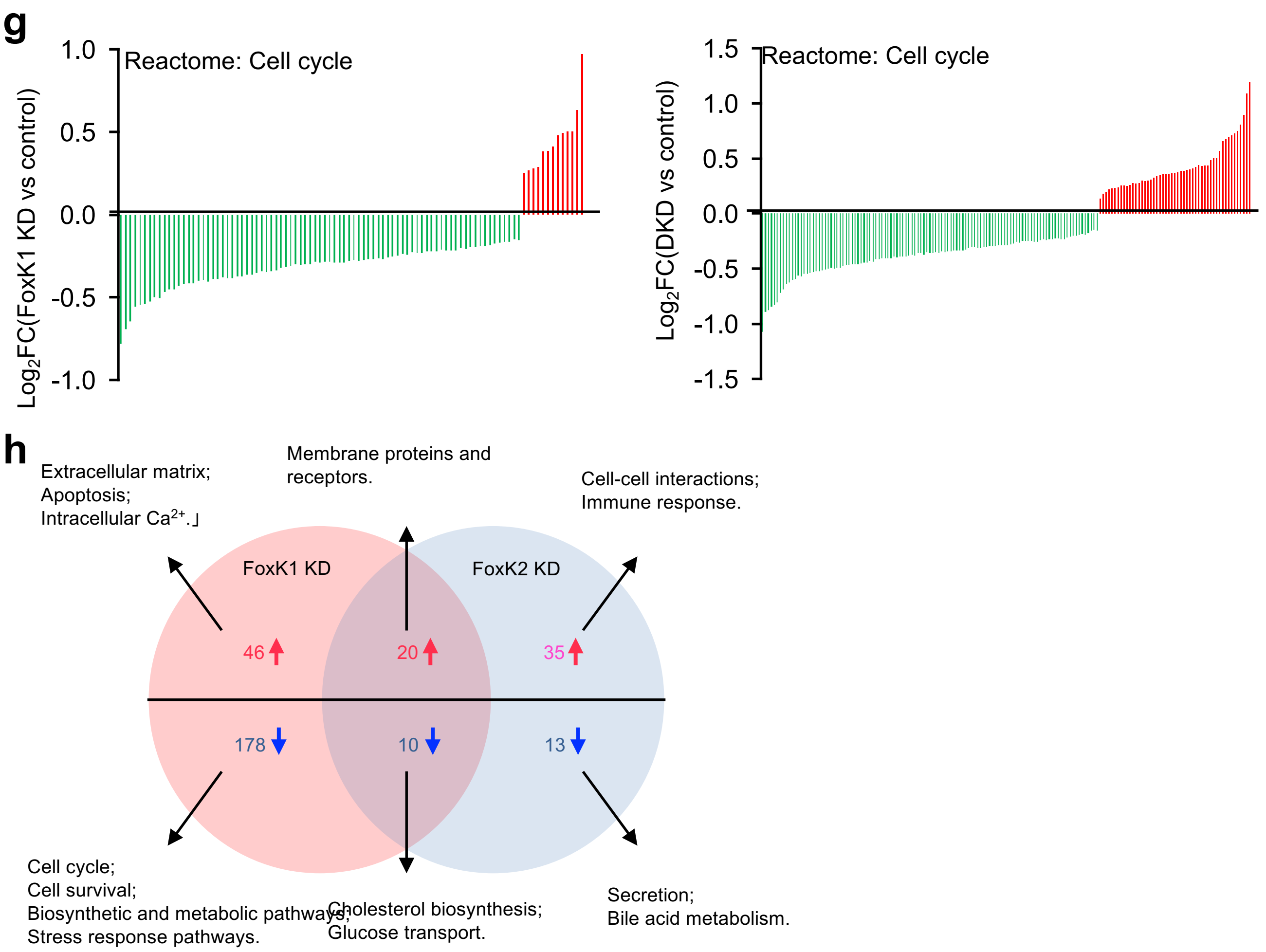
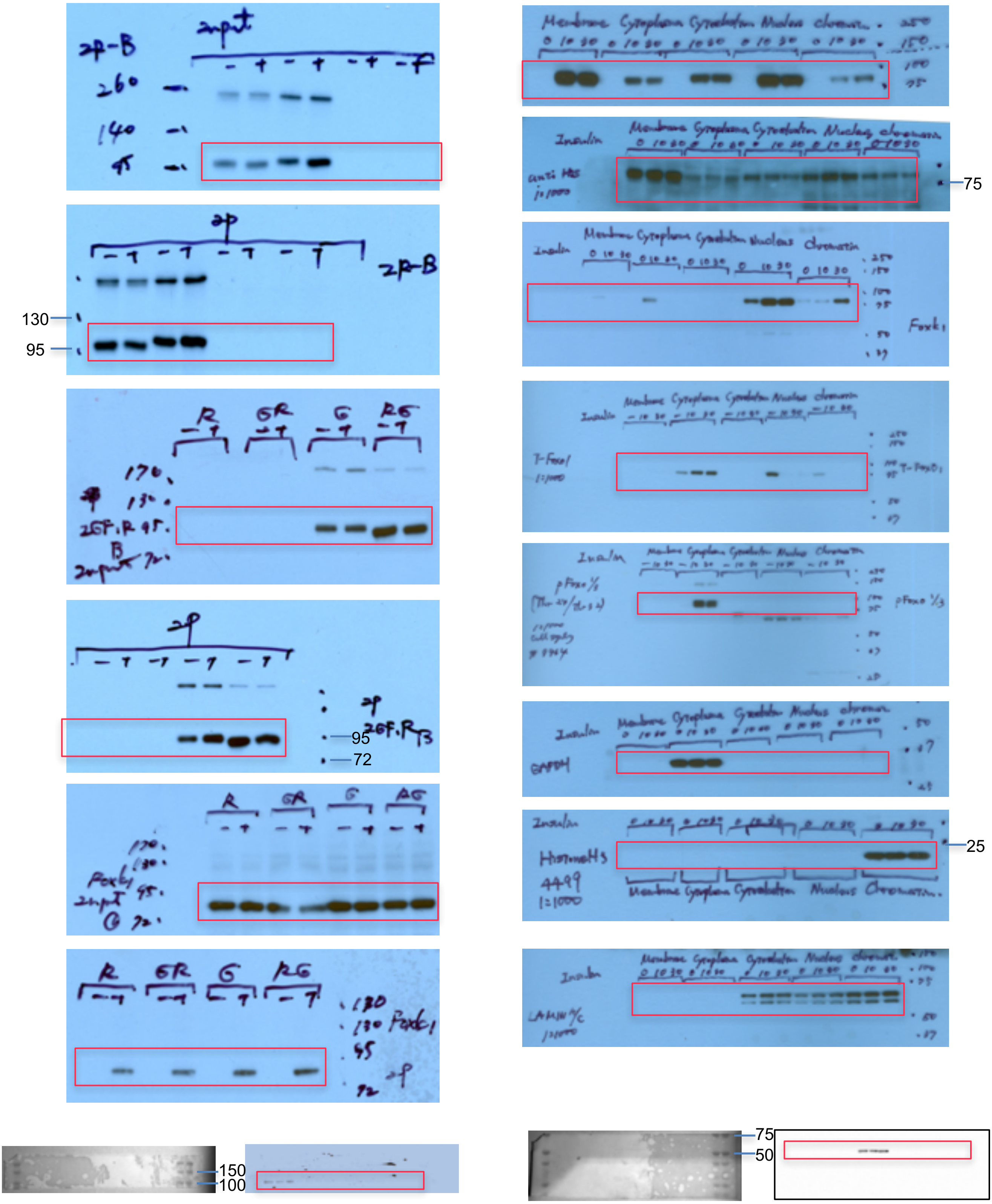
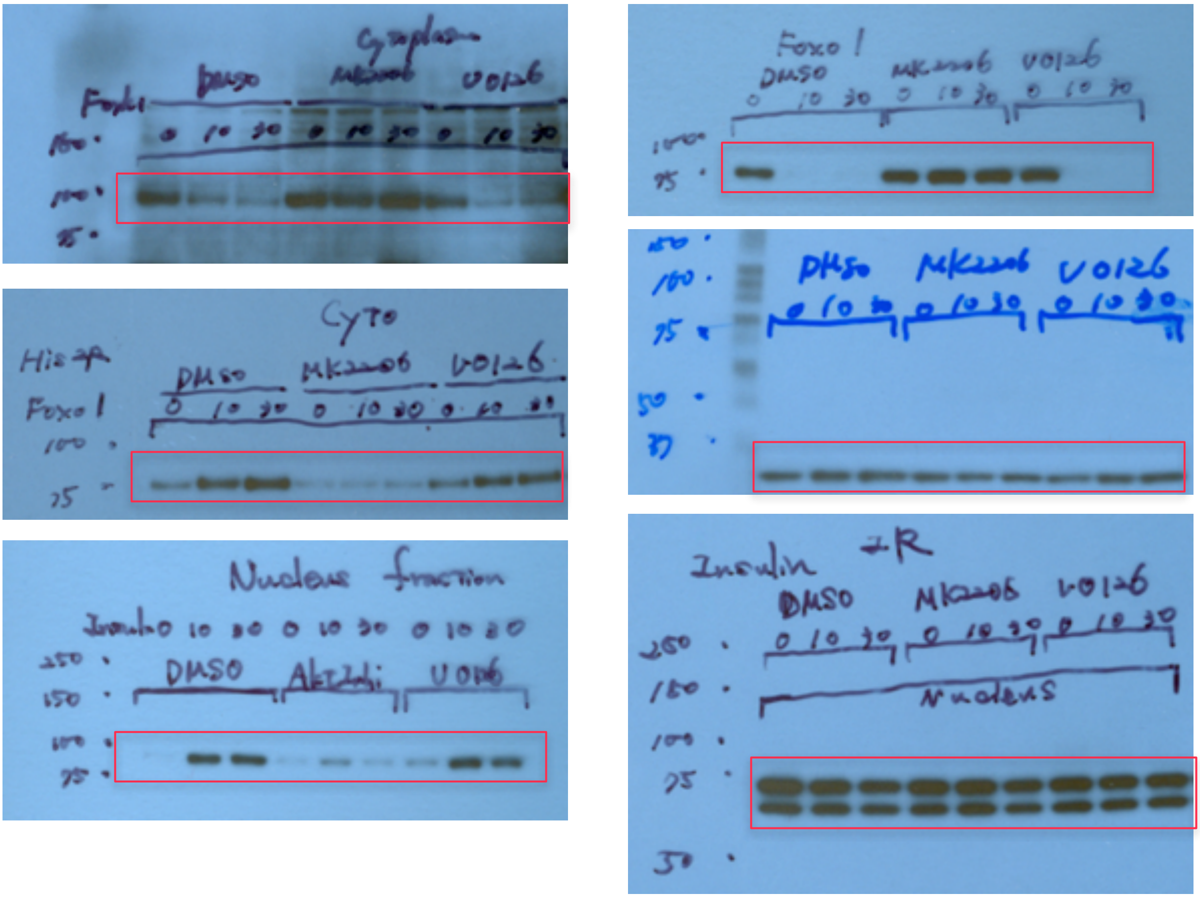


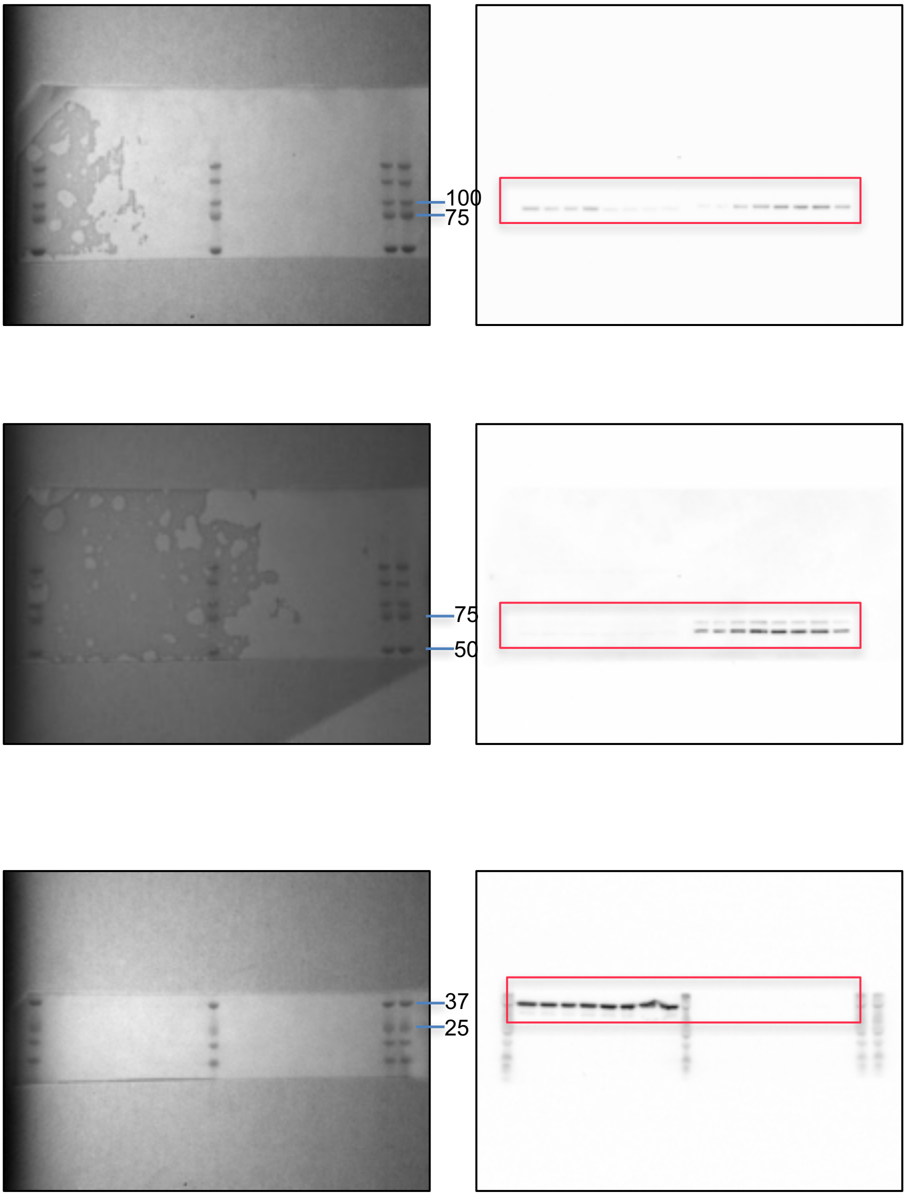
Supplementary Figure 7. Altered target genes regulated by loss of FoxK1 and FoxK2. (**a**) The logo shows the promoter consensus motif for FoxK2 (human) used to search the targets affected by loss of FoxK1 and FoxK2. (**b**) Top regulated pathways by insulin stimulation in Control and DKD. (**c**) The bar graph shows the number of genes (1310) including 49 transcription factors carrying the consensus FoxK1/2 motifs in the promoter regions among 5,974 genes that were differentially regulated in the DKD cells at the basal condition compared with those of the Control. Another 3,503 genes have promoter regions with the motifs that could be targeted by the 49 FoxK1-regulated transcription factors. Thus 4,813 (1,310+3,503) genes contain the FoxK1/2 motif or the motifs in the promoter regions regulated by downstream transcription factors. The remaining 1,161 genes do not contain any of these motifs, implicating that they are regulated by further downstream events. **(d)** Top differentially regulated pathways at basal condition in Control and DKD. (**e**) The network profile showed three groups of genes with the FoxK1/2 motifs in the promoter regions as involved in the cell cycle, apoptosis and mitochondria-related genes that were also highly affected at the basal condition in the Control and DKD cells. (**f**) Total number of genes within Reactome: Cell cycle that were significantly up- or down-regulated in FoxK1KD, FoxK2KD and DKD cells than control cells. (P < 0.05, basal conditions) (**g**) Within cell cycle pathway, the majority of the genes are significantly down-regulated in FoxK1 KD cells (left) and DKD cells (right). (**h**) Venn diagram showing the numbers of significantly regulated pathways (Reactome) in FoxK1KD and FoxK2KD cells (FDR<0.25).



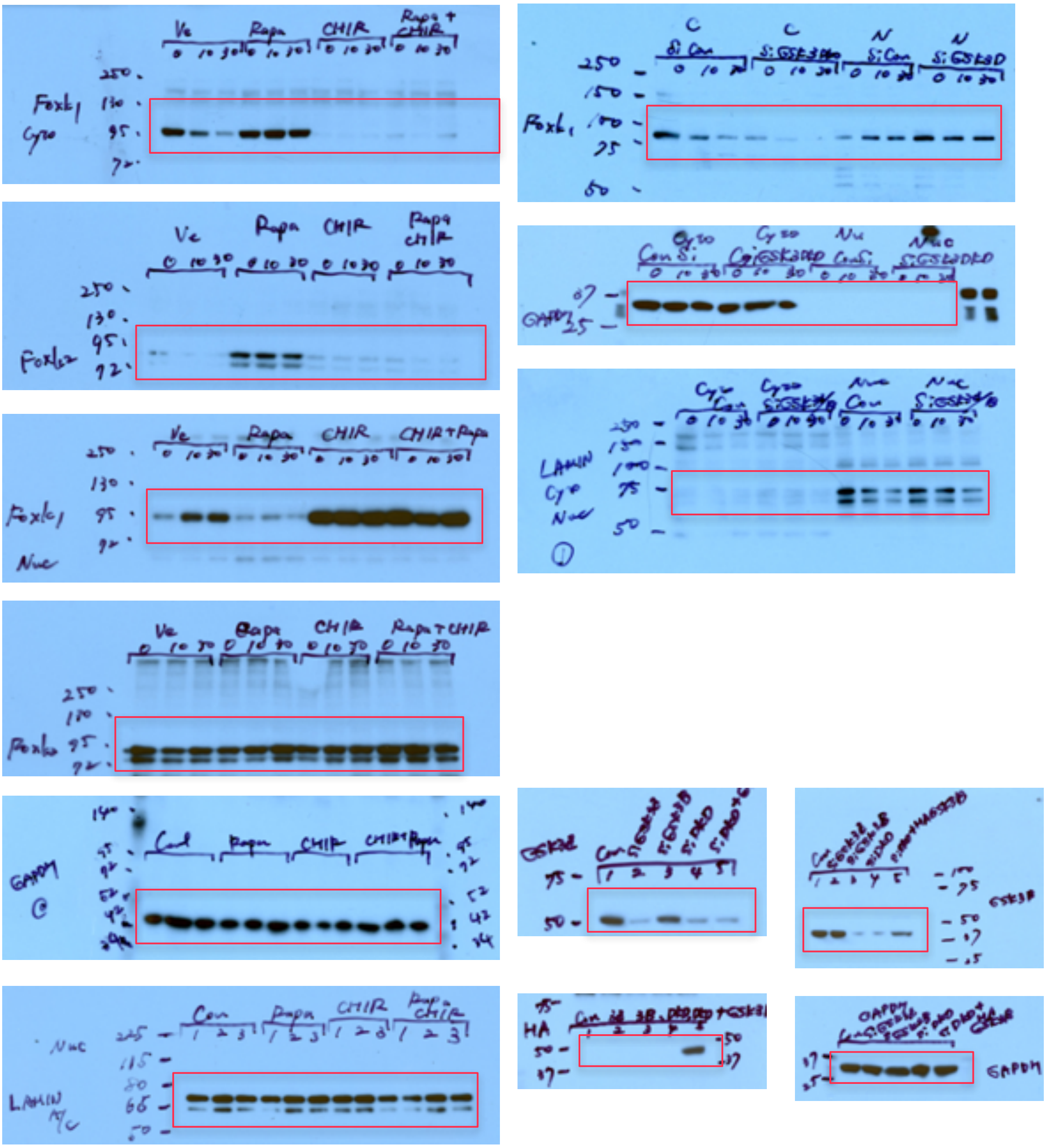
Supplementary Figure 8. Uncropped blots. The red sections indicate blot results shown in Figure 1.



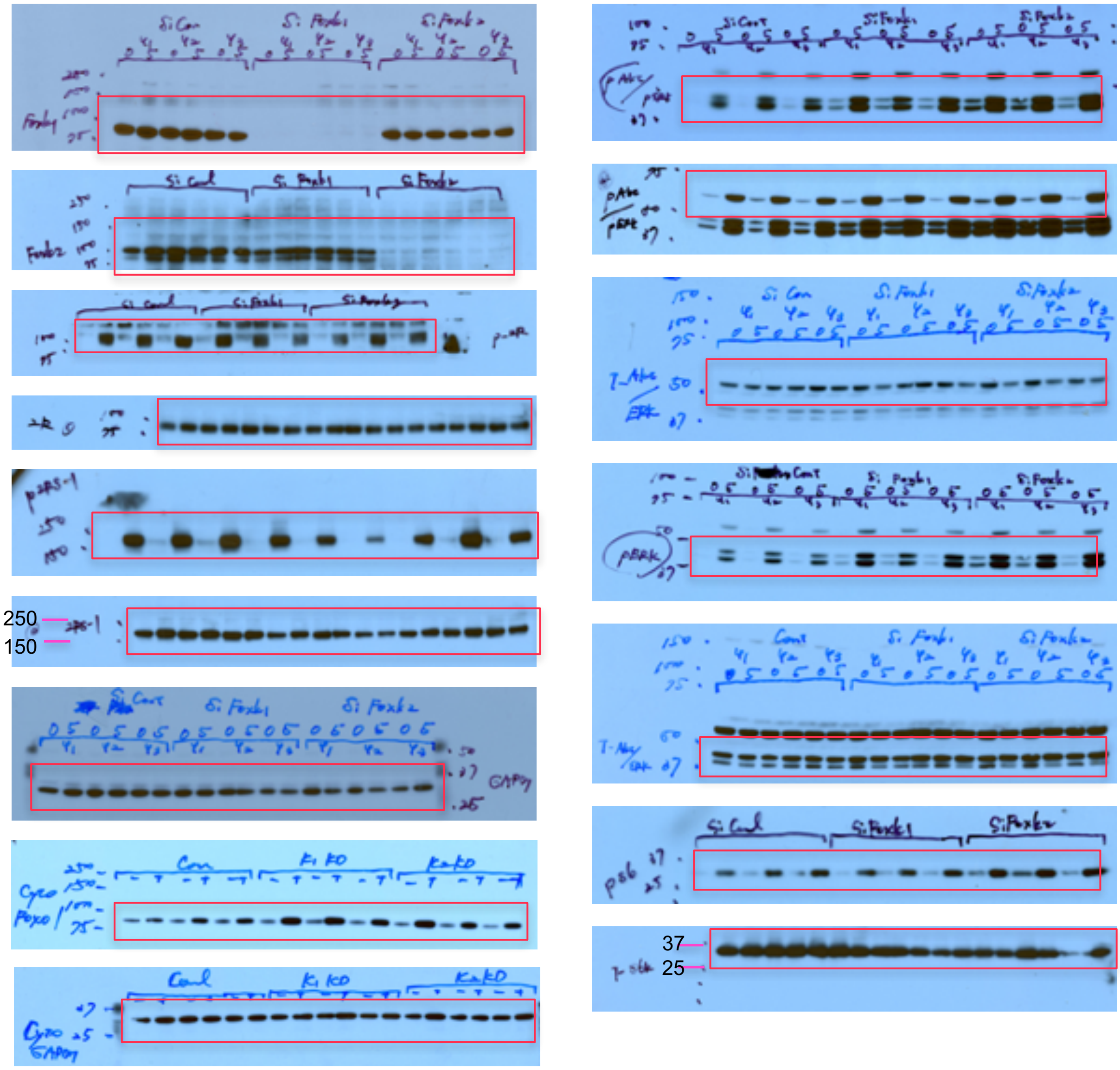
Supplementary Figure 9. Uncropped blots. The red sections indicate blot results shown in Figure 2.



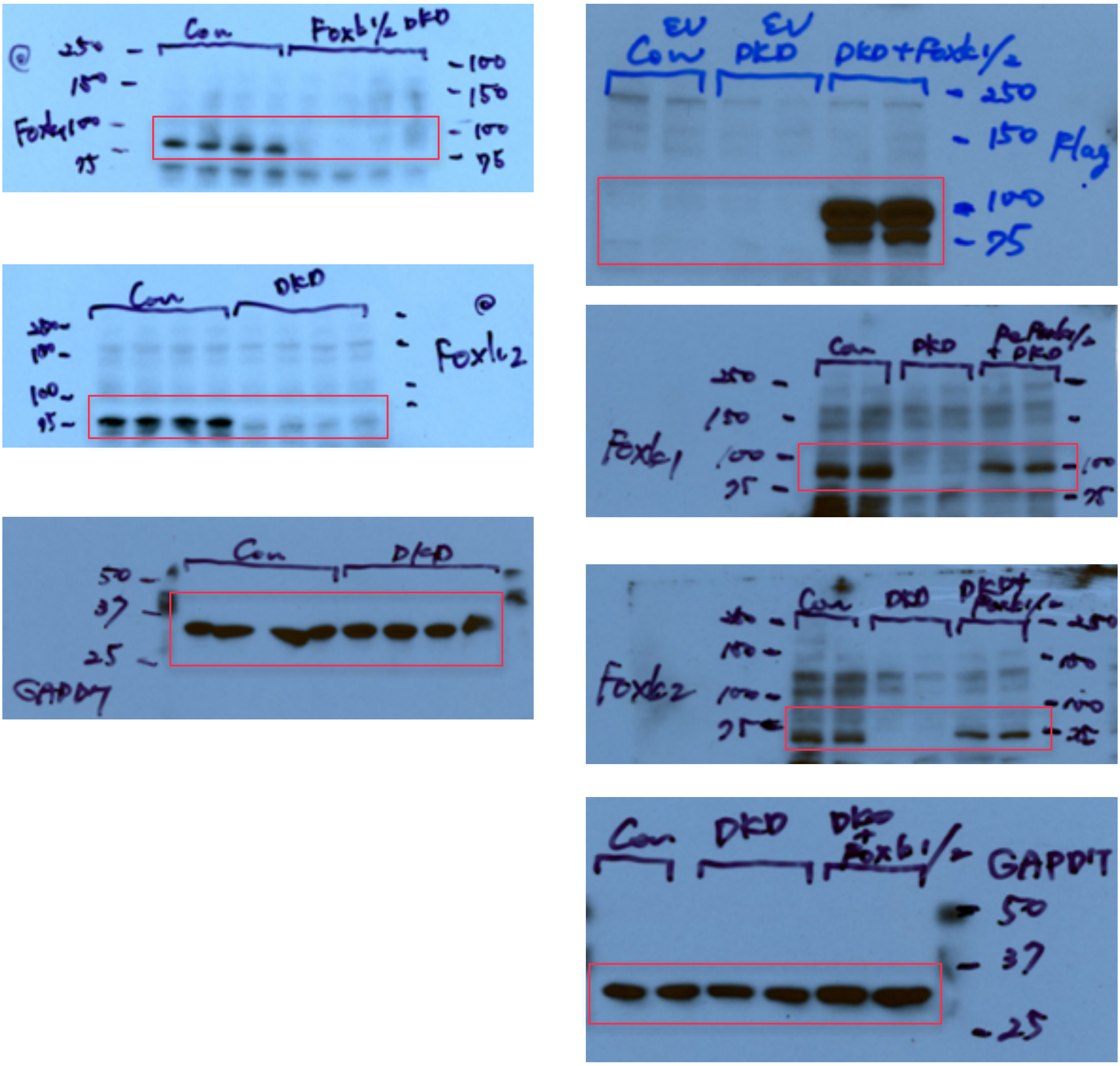
Supplementary Figure 10. Uncropped blots. The red sections indicate blot results shown in Figure 2.



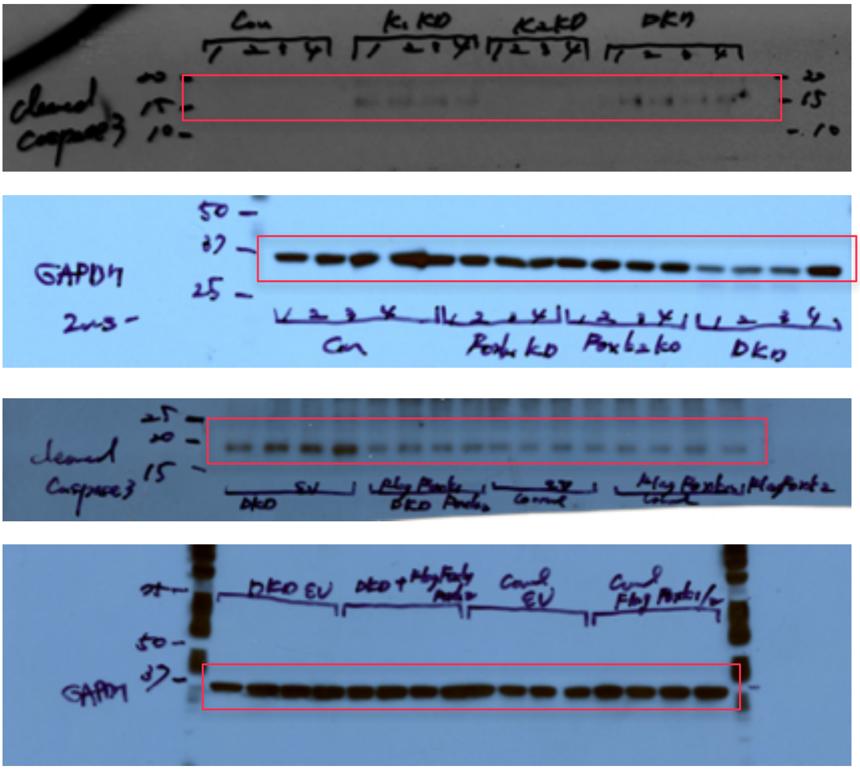
Supplementary Figure 11. Uncropped blots. The red sections indicate blot results shown in Figure 3.



Supplementary Figure 12. Uncropped blots. The red sections indicate blot results shown in Figure 4.



Supplementary Figure 13. Uncropped blots. The red sections indicate blot results shown in Figure 5 and 6.



Supplementary Figure 14. Uncropped blots. The red sections indicate blot results shown in Figure 7.

Selected top differentially regulated pathways in Control and DKD cells (Reactome).

Pathway	Compared with Control	p-value	FDR
Extrinsic pathway for apoptosis	Up	6.68.E-09	4.33.E-06
Regulation of pyruvate dehydrogenase PDH complex	Down	1.52.E-06	7.00.E-05
Synthesis of very long chain fatty acyl CoAs	Down	1.52.E-05	3.19.E-04
G2 M DNA damage checkpoint	Down	5.12.E-05	8.19.E-04
Apoptotic cleavage of cell adhesion proteins	Down	1.38.E-04	1.82.E-03
Caspase mediated cleavage of cytoskeleton proteins	Up	2.32.E-04	2.60.E-03
Mitotic G2 G2 M phases	Down	3.06.E-04	3.16.E-03
Fatty acid triacyglycerol and ketone body metabolism	Down	4.10.E-04	3.83.E-03
Mitotic M M G1 phases	Down	8.21.E-04	6.34.E-03
Cell cycle	Down	1.57.E-03	9.78.E-03
Cell cycle mitotic	Down	2.08.E-03	1.13.E-02
G2 M checkpoints	Down	5.45.E-03	2.54.E-02
G0 and early G1	Down	8.92.E-03	3.57.E-02
Triglyceride biosynthesis	Down	1.10.E-02	4.17.E-02
Pyruvate metabolism and citric acid TCA cycle	Down	1.98.E-02	6.26.E-02
Metabolism of lipids and lipoproteins	Down	2.04.E-02	6.39.E-02
Peroxisomal lipid metabolism	Down	3.20.E-02	9.01.E-02
Mitochondrial fatty acid beta oxidation	Down	3.82.E-02	1.04.E-01
Intrinsic pathway for apoptosis	Up	3.20.E-02	8.85.E-02
Apoptosis induced DNA fragmentation	Down	2.34.E-02	6.98.E-02

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Top genes significantly altered in FoxK1KD, FoxK2KD and DKD cells.

			FoxK1KD compared to Control			FoxK2KD compared to Control			DKD compared to Control		
Symbol	Entrez	Name	FC	P-value	FDR	FC	P-value	FDR	FC	P-value	FDR
Mcam	84004	melanoma cell adhesion molecule	5.01	1.19E-07	5.88E-05	2.02	0.0129	0.331	3.78	7.84E-06	0.000244
Lcn2	16819	lipocalin 2	1.51	0.0128	0.163	2.72	1.77E-08	6.10E-05	3.50	7.36E-11	3.18E-08
Fxyd2	11936	FXYD domain-containing ion transport regulator 2	2.36	1.68E-06	0.000527	1.93	9.34E-05	0.0364	3.38	2.69E-10	9.72E-08
Thbs1	21825	thrombospondin 1	2.68	3.19E-14	1.47E-10	1.29	0.00713	0.264	2.39	1.23E-12	1.55E-09
Ptgds	19215	prostaglandin D2 synthase (brain)	1.26	0.0275	0.239	1.40	0.00091	0.11	2.28	3.13E-11	1.73E-08
Serpina1a	20700 /// 20703	serine (or cysteine) peptidase inhibitor, clade A, member 1A	1.90	2.49E-07	0.000114	1.76	1.57E-06	0.00309	2.20	1.57E-09	4.25E-07
C3	12266	complement component 3	1.27	0.0264	0.234	2.13	8.79E-10	6.06E-06	2.20	3.79E-10	1.22E-07
4-Sep	18952	septin 4	2.13	8.56E-13	2.95E-09	1.41	3.51E-05	0.0193	2.15	3.83E-13	8.80E-10
Bcam	57278	basal cell adhesion molecule	1.62	3.48E-06	0.000792	1.33	0.00201	0.155	2.13	4.96E-11	2.44E-08
Saa3	20210	serum amyloid A 3	1.63	0.00205	0.0587	2.26	5.19E-07	0.00119	2.07	8.23E-06	0.000255
Rab11fip5	52055	RAB11 family interacting protein 5 (class I)	1.62	3.86E-07	0.000161	1.40	9.14E-05	0.0364	2.00	3.54E-11	1.81E-08
Slc6a18	22598	solute carrier family 6 (neurotransmitter transporter), member 18	1.55	4.84E-06	0.000997	1.26	0.00664	0.259	1.99	9.21E-11	3.85E-08
Serpina1b	20701	serine (or cysteine) preptidase inhibitor, clade A, member 1B	1.63	5.67E-06	0.00111	1.77	1.14E-07	0.000313	1.97	3.74E-09	7.87E-07
Notch3	18131	notch 3	2.07	2.35E-09	2.95E-06	1.21	0.0601	0.508	1.92	3.51E-08	4.57E-06
Rap1gap	110351	Rap1 GTPase-activating protein	1.72	1.92E-06	0.000563	1.26	0.0221	0.393	1.92	3.48E-08	4.57E-06
Wdr45	54636	WD repeat domain 45	1.40	0.000598	0.0271	1.50	2.64E-05	0.0182	1.90	3.77E-09	7.87E-07
Bag1	12017	BCL2-associated athanogene 1	1.18	0.0298	0.248	1.41	1.02E-05	0.0128	1.87	2.30E-11	1.47E-08
Gstm1	14862	glutathione S-transferase, mu 1	1.31	0.00188	0.0563	1.29	0.00243	0.168	1.85	9.55E-10	2.69E-07
Stc2	20856	stanniocalcin 2	1.39	8.07E-05	0.00752	1.33	0.000268	0.0637	1.81	2.75E-10	9.72E-08
Myo7a	17921	myosin VIIA	1.68	8.54E-07	0.00031	1.44	0.000149	0.0438	1.75	1.66E-07	1.46E-05
Alpl	11647	alkaline phosphatase, liver/bone/kidney	1.70	9.15E-07	0.000324	1.31	0.00436	0.219	1.67	1.35E-06	6.47E-05
Lgals3bp	19039	lectin, galactoside-binding, soluble, 3 binding protein	1.13	0.0269	0.236	1.28	9.16E-06	0.0126	1.65	1.08E-12	1.55E-09
Ccnd1	12443	cyclin D1	1.55	1.93E-11	4.43E-08	1.24	3.42E-05	0.0193	1.59	1.22E-12	1.55E-09
Adgrg1	14766	adhesion G protein-coupled receptor G1	1.52	7.29E-09	6.70E-06	1.12	0.0484	0.486	1.56	8.33E-10	2.42E-07
Fhod3	225288	formin homology 2 domain containing 3	1.79	1.12E-11	3.09E-08	1.23	0.00179	0.146	1.55	1.60E-08	2.40E-06
Dab2	13132	disabled 2, mitogen-responsive phosphoprotein	2.00	6.53E-11	1.13E-07	1.15	0.0969	0.571	1.54	3.69E-06	0.000142
Sgk1	20393	serum/glucocorticoid regulated kinase 1	1.47	2.20E-06	0.000633	1.26	0.00136	0.13	1.46	2.47E-06	0.000104
Lbp	16803	lipopolysaccharide binding protein	1.36	3.37E-07	0.00015	1.10	0.0558	0.503	1.41	9.82E-09	1.76E-06
Lss	16987	lanosterol synthase	0.74	6.29E-08	4.27E-05	0.88	0.00618	0.256	0.81	2.61E-05	0.000585
Tgoln1	22134 /// 22135	trans-golgi network protein	0.69	3.12E-09	3.58E-06	-0.92	0.0656	0.518	0.76	1.52E-06	7.15E-05
Acat2	110460	acetyl-Coenzyme A acetyltransferase 2	0.65	4.78E-09	5.07E-06	0.88	0.0262	0.411	0.75	6.92E-06	0.000223
Abcc2	12780	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	0.81	5.65E-05	0.00604	0.87	0.00197	0.153	0.69	3.42E-10	1.12E-07
Gldc	104174	glycine decarboxylase	0.79	0.000109	0.00906	0.82	0.000486	0.0918	0.69	4.02E-08	5.03E-06
Ssr3	67437	signal sequence receptor, gamma	0.79	0.000421	0.0216	0.83	0.00141	0.134	0.66	1.34E-08	2.12E-06
Tspan12	269831	tetraspanin 12	0.82	0.000623	0.0278	0.83	0.00119	0.127	0.65	2.97E-10	1.03E-07
Scd2	20250	stearoyl-Coenzyme A desaturase 2	0.85	0.000528	0.025	0.87	0.000911	0.11	0.64	5.65E-14	1.56E-10
Chmp2b	68942	charged multivesicular body protein 2B	0.82	0.000891	0.0347	0.86	0.00836	0.284	0.64	4.29E-10	1.32E-07
Top2a	21973	topoisomerase (DNA) II alpha	0.79	6.65E-05	0.00674	0.79	4.69E-05	0.0239	0.63	5.28E-11	2.51E-08
Tfrc	22042	transferrin receptor	0.80	9.12E-05	0.00811	0.85	0.00193	0.152	0.62	1.20E-11	9.22E-09
Foxk1	17425	forkhead box K1	0.48	3.96E-11	7.80E-08	0.87	0.0531	0.498	0.59	3.27E-08	4.37E-06
Cnih1	12793	cornichon family AMPA receptor auxiliary protein 1	0.82	0.00267	0.0709	0.85	0.00776	0.273	0.59	5.83E-11	2.68E-08
Aff4	93736	AF4/FMR2 family, member 4	0.70	5.39E-09	5.31E-06	0.91	0.0514	0.493	0.58	3.75E-14	1.56E-10
Scd1	20249	stearoyl-Coenzyme A desaturase 1	0.56	2.08E-14	1.43E-10	0.85	0.00176	0.146	0.57	5.24E-14	1.56E-10
Vcam1	22329	vascular cell adhesion molecule 1	0.51	5.57E-16	7.68E-12	-0.93	0.186	0.681	0.56	1.75E-14	1.21E-10
Ube2d3	66105	ubiquitin-conjugating enzyme E2D 3	0.89	0.078	0.387	0.75	1.85E-05	0.0182	0.54	2.36E-12	2.51E-09
Cmtm8	70031	CKLF-like MARVEL transmembrane domain containing 8	0.67	5.61E-05	0.00604	0.78	0.00448	0.219	0.51	8.35E-09	1.56E-06
Hmgn5	50887	high-mobility group nucleosome binding domain 5	0.63	0.000394	0.0209	0.56	8.42E-06	0.0126	0.48	6.62E-07	3.95E-05
mt-Nd6	17722	mitochondrially encoded NADH dehydrogenase 6	0.90	0.158	0.52	0.74	9.45E-05	0.0364	0.47	1.23E-12	1.55E-09
Sprr1a	20753	small proline-rich protein 1A	0.35	6.86E-08	4.27E-05	0.59	0.000203	0.0509	0.46	4.18E-06	0.000153
Foxk2	68837	forkhead box K2	0.84	0.00574	0.108	0.34	2.26E-20	3.12E-16	0.36	4.80E-19	6.61E-15

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Selected common regulated pathways by insulin stimulation in Control and DKD cells (Reactome).

	Control insulin + vs -			DKD insulin + vs -			DKD vs Control	
		p-value	FDR		p-value	FDR	p-value	FDR
Glucose transport	Up	6.37E-13	4.14E-10	Up	6.18E-13	2.01E-10	0.301	0.785
Cholesterol biosynthesis	Up	1.12E-10	1.82E-08	Up	2.19E-12	2.8E-10	0.508	0.86
Metabolism of carbohydrates	Up	2.93E-10	0.000000025	Up	2.19E-10	1.78E-08	0.402	0.807
Diabetes pathways	Up	3.08E-10	0.000000025	Up	1.33E-10	1.23E-08	0.601	0.9
Triglyceride biosynthesis	Up	0.00000232	0.0000334	Up	3.83E-09	0.000000118	0.169	0.636
Glycolysis	Up	0.0000132	0.00014	Up	0.000000802	0.0000102	0.724	0.956
Glucose metabolism	Up	0.0000742	0.000618	Up	0.000000278	0.00000475	0.262	0.761
Metabolism of vitamins and cofactors	Up	0.00015	0.00114	Up	1.03E-08	0.000000277	0.0174	0.303
Integration of energy metabolism	Up	0.000179	0.0013	Up	0.0000085	0.0000788	0.625	0.911
Metabolism of amino acids and derivatives	Up	0.000461	0.00285	Up	0.00103	0.00455	0.667	0.933

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Top significant genes that were differentially regulated in Control, FoxK1KD, FoxK2KD and DKD cells in response to insulin stimulation.

			Fold change to basal				P-value (Compared to Control)		
Symbol	Entrez	Name	Control	FoxK1KD	FoxK2KD	DKD	FoxK1KD	FoxK2KD	DKD
Gpnmb	93695	glycoprotein (transmembrane) nmb	22.20	7.66	15.40	2.11	0.207	0.638	0.000959
Gm5160		predicted gene 5160	8.96	5.26	5.01	0.91	0.434	0.361	0.000536
Aqp9	64008	aquaporin 9	6.72	1.65	1.38	1.24	0.0595	0.024	0.0258
Ppp1ca	19045	protein phosphatase 1, catalytic subunit, alpha isoform	6.22	5.67	6.74	1.05	0.884	0.896	0.00398
Aldoart1	353204	aldolase 1 A, retrogene 1	6.03	2.97	2.47	0.69	0.322	0.138	0.00044
Spry4	24066	sprouty homolog 4 (Drosophila)	5.72	2.43	2.36	1.29	0.112	0.092	0.00765
Sstr2	20606	somatostatin receptor 2	4.86	0.90	0.86	0.82	0.035	0.0228	0.0267
Ddc	13195	dopa decarboxylase	4.85	1.63	0.79	0.56	0.187	0.0198	0.00877
Ptpn5	19259	protein tyrosine phosphatase, non-receptor type 5	4.75	1.43	2.67	0.69	0.15	0.459	0.0272
Gm8797		predicted pseudogene 8797	4.35	5.08	1.47	0.57	0.841	0.152	0.00799
Ass1	11898	argininosuccinate synthetase 1	4.31	0.99	0.48	0.69	0.0712	0.00826	0.0321
Gcnt3	72077	glucosaminyl (N-acetyl) transferase 3, mucin type	4.29	1.38	1.55	1.35	0.0274	0.0728	0.0323
Gm11808	22186	predicted gene 11808	4.19	5.28	2.08	0.52	0.726	0.271	0.00154
Gstm6	14867	glutathione S-transferase, mu 6	4.08	0.86	0.99	0.81	0.0255	0.0249	0.0195
Vtn	22370	vitronectin	3.75	1.35	0.72	0.68	0.215	0.0273	0.0334
Ftl1-ps1		ferritin light polypeptide 1, pseudogene 1	3.41	3.65	2.80	1.18	0.789	0.403	0.0000357
Chrd	12667	chordin	3.38	1.26	1.60	0.78	0.0967	0.2	0.0217
Arntl2	272322	aryl hydrocarbon receptor nuclear translocator-like 2	3.30	1.92	1.77	0.54	0.473	0.469	0.0328
Zfp775	243372	zinc finger protein 775	3.24	0.42	1.32	0.53	0.00476	0.163	0.00746
Fam222a	433940	family with sequence similarity 222, member A	3.18	0.93	1.37	0.88	0.0194	0.0895	0.0151
Ccne1	12447	cyclin E1	3.17	3.44	2.41	1.85	0.713	0.187	0.017
Gale	74246	galactose-4-epimerase, UDP	2.96	3.28	3.02	1.80	0.612	0.91	0.0128
Ccr9	12769	chemokine (C-C motif) receptor 9	2.95	1.56	2.09	0.36	0.319	0.571	0.00479
Nutf2	68051 /// 621832	nuclear transport factor 2	2.85	1.27	1.77	0.93	0.0968	0.308	0.0209
Dusp5	240672	dual specificity phosphatase 5	2.80	1.50	1.12	0.74	0.0961	0.0114	0.00113
Pnldc1	240023	poly(A)-specific ribonuclease (PARN)-like domain containing 1	0.33	1.88	1.64	1.77	0.0234	0.0457	0.0365
Cyp2d26	76279	cytochrome P450, family 2, subfamily d, polypeptide 26	0.32	2.48	2.80	1.23	0.0223	0.0122	0.106
Nupr1l	69034	nuclear protein transcriptional regulator 1 like	0.31	0.56	0.51	1.19	0.431	0.502	0.0833
Ikzf4	22781	IKAROS family zinc finger 4	0.30	1.41	0.32	1.24	0.0805	0.929	0.0932
Nat14	269854	N-acetyltransferase 14	0.30	0.76	0.65	0.74	0.0496	0.0882	0.0525
Fhl1	14199	four and a half LIM domains 1	0.30	0.78	0.81	1.15	0.114	0.0791	0.0228
Gprin3	243385	GPRIN family member 3	0.29	1.18	1.51	1.38	0.076	0.0353	0.0319
Fn3k	63828	fructosamine 3 kinase	0.29	0.47	0.41	0.53	0.0853	0.22	0.0382
Hist1h4i	319158	histone cluster 1, H4i	0.28	1.17	1.40	1.31	0.00979	0.00501	0.00615
Fbxo32	67731	F-box protein 32	0.28	0.31	0.26	0.38	0.36	0.606	0.00631
Zfp960	449000	zinc finger protein 960	0.27	1.78	1.17	2.12	0.0273	0.0582	0.0112
Ttc6	70846	tetratricopeptide repeat domain 6	0.26	1.00	0.28	1.23	0.0324	0.911	0.026
Tcf7l1	21415	transcription factor 7 like 1 (T cell specific, HMG box)	0.26	1.32	0.71	0.85	0.021	0.139	0.0939
Slc52a3	69698	solute carrier protein family 52, member 3	0.25	0.96	1.06	1.74	0.0875	0.0531	0.0118
Mturn	68235	maturin, neural progenitor differentiation regulator homolog (Xenopus)	0.24	0.34	0.30	0.43	0.0593	0.185	0.00215
Dclk3	245038	doublecortin-like kinase 3	0.24	0.40	0.40	0.42	0.148	0.156	0.105
Gpr160	71862	G protein-coupled receptor 160	0.23	0.75	0.53	0.99	0.0394	0.136	0.0128
Xkrx	331524	X-linked Kx blood group related, X-linked	0.22	0.72	0.75	2.25	0.14	0.129	0.00712
Me3	109264	malic enzyme 3, NADP(+)-dependent, mitochondrial	0.22	0.52	0.47	0.56	0.0333	0.0646	0.0237
Chst4	26887	carbohydrate (chondroitin 6/keratan) sulfotransferase 4	0.20	1.16	0.81	0.84	0.0362	0.0927	0.0859
Hoxb6	15414	homeobox B6	0.19	1.01	1.39	1.68	0.0169	0.00455	0.00596
H2-T23	15040	histocompatibility 2, T region locus 23	0.17	0.50	0.68	0.53	0.115	0.0379	0.0993
Slamf9	98365	SLAM family member 9	0.15	0.91	0.57	0.93	0.0233	0.0951	0.0241
Corin	53419	corin	0.14	0.35	0.46	0.44	0.128	0.0468	0.0543
C330021F23Rik	546049	RIKEN cDNA C330021F23 gene	0.12	0.52	1.12	0.59	0.0318	0.00197	0.0226

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Comparison of mitochondrial related genes in Control, FoxK1KD, FoxK2KD and DKD cells.

			FoxK1KD compared to Control		FoxK2KD compared to Control		DKD compared to Control	
Symbol	Entrez	Name	FC	P-value	FC	P-value	FC	P-value
Bckdha	12039	branched chain ketoacid dehydrogenase E1, alpha polypeptide	1.17	0.363	1.19	0.297	2.10	0.0000244
Rab11fip5	52055	RAB11 family interacting protein 5 (class I)	1.62	0.000000386	1.40	0.0000914	2.00	3.54E-11
Mtch1	56462	mitochondrial carrier 1	0.88	0.0735	1.09	0.178	1.51	0.000000102
Ndufs8	225887	NADH dehydrogenase (ubiquinone) Fe-S protein 8	1.11	0.24	1.22	0.0188	1.50	0.0000147
Pink1	68943	PTEN induced putative kinase 1	1.04	0.641	1.17	0.0744	1.48	0.0000354
Bbc3	170770	BCL2 binding component 3	1.08	0.322	1.06	0.476	1.41	0.0000582
Casp7	12369	caspase 7	0.75	0.00212	1.04	0.627	1.41	0.000115
Phb	18673 /// 237880	prohibitin	0.93	0.371	1.11	0.221	1.39	0.000349
Pgs1	74451	phosphatidylglycerophosphate synthase 1	1.19	0.0217	1.15	0.0489	1.38	0.0000295
Mfn2	170731	mitofusin 2	1.20	0.00605	1.08	0.201	1.37	0.0000062
Me3	109264	malic enzyme 3, NADP(+)-dependent, mitochondrial	1.44	0.0301	0.95	0.786	1.35	0.0731
Fis1	66437	fission, mitochondrial 1	0.94	0.397	1.11	0.123	1.31	0.000359
Slc25a22	68267	solute carrier family 25 (mitochondrial carrier, glutamate), member 22	1.06	0.48	1.01	0.894	1.31	0.00117
Letm1	56384	leucine zipper-EF-hand containing transmembrane protein 1	0.96	0.51	0.92	0.167	1.25	0.000402
Phb2	12034	prohibitin 2	0.84	0.033	1.19	0.0202	1.24	0.00523
Shmt2	108037	serine hydroxymethyltransferase 2 (mitochondrial)	0.74	0.000569	0.92	0.24	1.22	0.0114
Idh2	269951	isocitrate dehydrogenase 2 (NADP+), mitochondrial	1.04	0.529	1.13	0.03	1.21	0.000768
Tbrg4	21379	transforming growth factor beta regulated gene 4	0.89	0.102	1.03	0.655	1.21	0.00426
Bphl	68021	biphenyl hydrolase-like (serine hydrolase, breast epithelial mucin-associated antigen)	1.20	0.0213	1.11	0.173	1.19	0.0223
Glyat	107146	glycine-N-acyltransferase	1.48	0.0999	1.27	0.296	1.12	0.658
Cox18	231430	cytochrome c oxidase assembly protein 18	0.74	0.0744	1.09	0.546	1.11	0.481
Timm50	66525	translocase of inner mitochondrial membrane 50	0.77	0.000841	1.04	0.596	1.11	0.116
Abcf2	27407	ATP-binding cassette, sub-family F (GCN20), member 2	0.90	0.0635	1.11	0.0509	1.10	0.0762
Msrb3	320183	methionine sulfoxide reductase B3	1.16	0.0783	1.00	0.974	1.10	0.25
Slc25a11	67863	solute carrier family 25 (mitochondrial carrier oxoglutarate carrier), member 11	0.94	0.375	1.05	0.448	1.07	0.295
Mthfd1	108156	methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthase	0.96	0.455	0.99	0.867	1.04	0.444
Ogdh	18293	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	0.98	0.673	0.92	0.0861	0.94	0.277
Cs	12974	citrate synthase	0.94	0.22	0.94	0.233	0.91	0.0565
Amacr	17117	alpha-methylacyl-CoA racemase	0.78	0.0018	0.85	0.0216	0.91	0.206
Hscb	100900	HscB iron-sulfur cluster co-chaperone	1.03	0.782	1.09	0.358	0.90	0.311
Acadsb	66885	acyl-Coenzyme A dehydrogenase, short/branched chain	1.15	0.0304	1.05	0.41	0.88	0.0352
Ptrf	19285	polymerase I and transcript release factor	0.87	0.0562	0.83	0.00887	0.88	0.058
Supv3l1	338359	suppressor of var1, 3-like 1 (S. cerevisiae)	0.94	0.42	0.97	0.727	0.86	0.0474
Gpx4	625249	glutathione peroxidase 4	0.94	0.462	1.25	0.00525	0.85	0.0519
Acadm	11364	acyl-Coenzyme A dehydrogenase, medium chain	1.01	0.869	1.01	0.846	0.84	0.00406
Vdac1	22333	voltage-dependent anion channel 1	0.99	0.831	0.97	0.531	0.80	0.0000363
Slc25a13	50799	solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 13	0.85	0.00154	1.03	0.589	0.79	0.0000118
Pdha1	18597	pyruvate dehydrogenase E1 alpha 1	0.93	0.263	1.00	0.937	0.78	0.000271
Oxsm	71147	3-oxoacyl-ACP synthase, mitochondrial	0.92	0.267	0.93	0.27	0.78	0.00156
Mut	17850	methylmalonyl-Coenzyme A mutase	0.96	0.527	0.93	0.184	0.77	0.0000381
Abce1	24015	ATP-binding cassette, sub-family E (OABP), member 1	0.89	0.0623	0.86	0.0132	0.76	0.0000329
Clpx	270166	caseinolytic mitochondrial matrix peptidase chaperone subunit	0.88	0.0554	1.02	0.769	0.75	0.0000336
Asah2	54447	N-acylsphingosine amidohydrolase 2	1.12	0.211	1.04	0.603	0.75	0.00288
Pdk1	228026	pyruvate dehydrogenase kinase, isoenzyme 1	0.76	0.0000736	0.90	0.0754	0.67	0.000000117
Dld	13382	dihydrolipoamide dehydrogenase	0.89	0.0857	0.90	0.0781	0.67	4.65E-08
Tomm22	223696	translocase of outer mitochondrial membrane 22 homolog (yeast)	0.79	0.00579	0.87	0.0676	0.67	0.00000429
Mthfd2	17768	methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	0.70	0.000517	0.86	0.0942	0.65	0.0000653
Fpgs	14287	folylpolyglutamyl synthetase	0.78	0.00681	0.92	0.289	0.65	0.0000176
Coq4	227683	coenzyme Q4	0.74	0.00611	0.72	0.00231	0.63	0.000104
Bnip3	12176	BCL2/adenovirus E1B interacting protein 3	0.73	0.00385	0.89	0.24	0.53	0.000000583

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Changes of genes associated with mitochondrial oxidative phosphorylation in Control, FoxK1KD, FoxK2KD and DKD cells.

Symbol	Entrez	Name	FoxK1KD compared to Control		FoxK2KD compared to Control		DKD compared to Control	
			FC	P-value	FC	P-value	FC	P-value
Ndufa11	69875 /// 239760	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 11	1.89	0.0147	2.04	0.0043	2.24	0.0019
Ndufb7	66916	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7	0.81	0.21	0.99	0.932	2.01	1.73E-05
Atp6v0a4	140494	ATPase, H+ transporting, lysosomal V0 subunit A4	1.55	0.00454	1.10	0.517	1.64	0.00146
Ndufs8	225887	NADH dehydrogenase (ubiquinone) Fe-S protein 8	1.11	0.24	1.22	0.0188	1.50	1.47E-05
Atp5g2	67942	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C2 (subunit 9)	0.79	0.023	0.85	0.0873	1.35	0.00199
Tcirg1	27060	T cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein A3	1.17	0.0168	1.01	0.892	1.34	1.79E-05
Sdhc	66052	succinate dehydrogenase complex, subunit C, integral membrane protein	0.97	0.743	1.12	0.231	1.33	0.00435
Cox6b1	110323	cytochrome c oxidase, subunit VIb polypeptide 1	0.72	0.0368	1.08	0.591	1.32	0.0491
Atp6v0b	114143	ATPase, H+ transporting, lysosomal V0 subunit B	0.92	0.225	0.99	0.915	1.30	0.000401
Ndufv1	17995	NADH dehydrogenase (ubiquinone) flavoprotein 1	0.89	0.101	0.95	0.473	1.24	0.0015
Sdhb	67680	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	0.92	0.429	1.17	0.124	1.21	0.0726
Atp6v1f	66144	ATPase, H+ transporting, lysosomal V1 subunit F	0.97	0.736	1.35	0.00189	1.20	0.0595
Cox5b	12859	cytochrome c oxidase subunit Vb	1.07	0.506	1.35	0.00284	1.18	0.116
Atp6v1b2	11966	ATPase, H+ transporting, lysosomal V1 subunit B2	0.97	0.464	1.07	0.117	1.15	0.00267
Sdha	66945	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	0.93	0.114	0.97	0.573	1.14	0.00875
Ndufc2	68197	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2	0.80	0.117	1.13	0.327	1.14	0.323
Atp6v1g1	66290	ATPase, H+ transporting, lysosomal V1 subunit G1	0.97	0.657	1.02	0.77	1.12	0.108
Ndufb9	66218	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	0.87	0.119	1.09	0.283	1.11	0.228
Uqcrq	22272	ubiquinol-cytochrome c reductase, complex III subunit VII	0.93	0.503	1.05	0.627	1.07	0.547
Atp6v1e1	11973	ATPase, H+ transporting, lysosomal V1 subunit E1	1.19	0.0214	1.09	0.228	1.06	0.434
Cox10	70383	cytochrome c oxidase assembly protein 10	0.88	0.241	0.92	0.405	1.05	0.632
Atp6v0a2	21871	ATPase, H+ transporting, lysosomal V0 subunit A2	1.00	0.955	0.93	0.175	1.03	0.617
Ndufb3	66495	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 3	0.88	0.334	0.83	0.122	1.03	0.815
Sdhd	66925	succinate dehydrogenase complex, subunit D, integral membrane protein	1.00	0.969	1.08	0.179	1.01	0.838
Atp5o	28080	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	0.75	0.00647	1.12	0.211	0.98	0.861
Atp6v0a1	11975	ATPase, H+ transporting, lysosomal V0 subunit A1	0.91	0.0536	1.02	0.693	0.95	0.271
Ndufs2	226646	NADH dehydrogenase (ubiquinone) Fe-S protein 2	0.95	0.346	0.94	0.281	0.91	0.0854
Ndufb2	68198	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2	0.88	0.207	1.11	0.289	0.91	0.357
Atp6v0d1	11972	ATPase, H+ transporting, lysosomal V0 subunit D1	0.97	0.639	1.01	0.846	0.88	0.0294
Atp5g3	228033	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C3 (subunit 9)	0.78	0.00857	0.99	0.879	0.85	0.0682
Ndufs1	227197	NADH dehydrogenase (ubiquinone) Fe-S protein 1	1.04	0.459	0.96	0.468	0.85	0.00356
Uqcrb	67530	ubiquinol-cytochrome c reductase binding protein	0.93	0.558	0.96	0.775	0.83	0.162
Ppa1	67895	pyrophosphatase (inorganic) 1	0.88	0.18	1.06	0.493	0.81	0.0225
Ppa2	74776	pyrophosphatase (inorganic) 2	0.98	0.803	0.99	0.905	0.81	0.0195
Cyc1	66445	cytochrome c-1	0.80	0.0234	0.92	0.364	0.81	0.026
Ndufv2	72900	NADH dehydrogenase (ubiquinone) flavoprotein 2	1.01	0.914	1.04	0.584	0.80	0.00153
Atp5g1	11951	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C1 (subunit 9)	0.86	0.253	0.99	0.923	0.76	0.0444
Atp5e	67126	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	0.98	0.917	1.28	0.116	0.75	0.089
Atp6v1d	73834	ATPase, H+ transporting, lysosomal V1 subunit D	0.99	0.866	0.99	0.839	0.75	6.23E-06
Ndufa1	54405	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1	1.11	0.319	1.19	0.0875	0.75	0.012
Cox7a1	12865	cytochrome c oxidase subunit VIIa 1	0.35	0.00384	0.30	0.000649	0.74	0.306
Atp6v0c	11984	ATPase, H+ transporting, lysosomal V0 subunit C	0.85	0.436	0.64	0.0413	0.73	0.166
Uqcrfs1	66694	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	0.90	0.133	0.92	0.205	0.71	1.31E-05
Cox6a1	12861	cytochrome c oxidase subunit VIa polypeptide 1	0.90	0.32	1.03	0.79	0.69	0.00148
Uqcr10	66152	ubiquinol-cytochrome c reductase, complex III subunit X	0.98	0.883	1.19	0.0881	0.69	0.00146
Cox5a	12858	cytochrome c oxidase subunit Va	0.90	0.208	0.91	0.203	0.63	4.40E-07
Cox7b	66142	cytochrome c oxidase subunit VIIb	0.89	0.189	1.00	0.994	0.59	4.59E-07
Cox17	12856	cytochrome c oxidase assembly protein 17	0.89	0.242	0.94	0.486	0.57	3.67E-06
Ndufa5	68202	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5	0.86	0.157	0.97	0.733	0.56	6.26E-06
Cox7c	12867	cytochrome c oxidase subunit VIIc	0.72	0.0364	0.96	0.771	0.47	1.15E-05

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Primer Sequences for Quantitative Real-Time PCR

Gene	Direction	Sequence
TBP	Forward	ACCCTTCACCAATGACTCCTATG
TBP	Reverse	TGACTGCAGCAAATCGCTTGG
FoxK1	Forward	ACCCACGAATAGCTTGACGG
FoxK1	Reverse	GCATTAGCGGCTACTGAGACG
Vcam1	Forward	CGGGAA AGCCTCGACTTCCA
Vcam1	Reverse	TTATGTCTATGAGCTTTGTA
AFF4	Forward	GGGATCGCCCGACGCGTCCT
AFF4	Reverse	ACTTGAATGTCTCTAACCGG
Tgoln1	Forward	TCGAAGGCTTTTTGCTCCGG
Tgoln1	Reverse	GTCTATAGTTTCGTAACTTT
ALPL	Forward	CCCGCCCCTCCGGCCGCCCA
ALPL	Reverse	GGTTTATAAAGACTCCGGTC
Mcam	Forward	TGCGCCCCGCCCCGGCCCGC
Mcam	Reverse	CACAATAACAAGGTTTTCGT
Adgrg1	Forward	AGTTCTCCTCGAGTCGGTAC
Adgrg1	Reverse	CGTCGTGTCAAACCTCTCAA
SGK1	Forward	CCTTGGATGTGTTAGTTTTT
SGK1	Reverse	AACTTTTGATAAACGTATTT
GAPDH	Forward	GTATTGGGCGCCTGGTCACC
GAPDH	Reverse	CGCTCCTGGAAGATGGTGATG
mt-Nd1	Forward	TGCCAGCCTGACCCATAGCC
mt-Nd1	Reverse	ATGGGCCGGCTGGGTATTCT
mt-Nd6	Forward	CCCGCAAACAAAGATCACCCAGC
mt-Nd6	Reverse	GTTGGAAGGAGGGATTGGGGTAGC

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SiRNA Sequences

Dharmacon On Target plus SMART pool SiRNA			
Gene	Species	Name	Target Sequence
FoxK1	mouse	siFoxk1#1	5'-GCAUGGGCCUGUCGAGCUU
FoxK1	mouse	siFoxk1#2	5'-UCGAGUUCCUGAUGCGACA
FoxK1	mouse	siFoxk1#3	5'-GAACAAGCAUUCCGAAAGC
FoxK1	mouse	siFoxk1#4	5'-UCACAUCGCUAUACCAUAA
FoxK2	mouse	siFoxk2#1	5'-UGUCAUCAUCAUAACGUUA
FoxK2	mouse	siFoxk2#2	5'-CGGAAUGUCUGUCUCGUAA
FoxK2	mouse	siFoxk2#3	5'-UCACAGAGUAGCCGUAGUA
FoxK2	mouse	siFoxk2#4	5'-CCAGAGCUCAAGCGAGUUA
GSK3β	mouse	siGSK3β#1	5'-GGACCCAAAUGUCAAACUA
GSK3β	mouse	siGSK3β#2	5'-CCACAGGAAGUCAGUUAUA
GSK3β	mouse	siGSK3β#3	5'-UCAGAAGUCUAGCCUAUAU
GSK3β	mouse	siGSK3β#4	5'-GAUUACACGUCCAGUAUAG
Dharmacon On Target plus Non-targeting pool SiRNA			
Non-targeting		siControl#1	5'-UGGUUUACAUGUCGACUAA
Non-targeting		siControl#2	5'-UGGUUUACAUGUUGUGUGA
Non-targeting		siControl#3	5'-UGGUUUACAUGUUUUCUGA
Non-targeting		siControl#4	5'-UGGUUUACAUGUUUUCCUA

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