Legends to Supplementary Figures

Supplementary Figure S1

a, qPCR analysis of mRNAs in inguinal (subcutaneous), epididymal (visceral) or interscapular brown fat (BAT) depots for indicated genes. Samples were harvested from mice after three weeks of free wheel running or sedentary controls. The exercised mice were rested for 12 hours prior to analysis. Each group had n=10 mice. **b-c**, qPCR analysis of epididymal (visceral) (**b**) and inguinal (subcutaneous) (**c**) fat against indicated genes in mice after three weeks of swimming exercise (methods). Each group had n=10 mice. * $p<0.05$ compared to control using students T-TEST.

Supplementary Figure S2

A, QPCR analysis of mRNAs for indicated genes from muscle of PGC1α muscle-specific knockout mice or flox/flox control animals. Each group had 5 mice. **b**, qPCR analysis of mRNA for indicated genes in muscle from mice subjected to 1 hour of acute exercise compared to sedentary mice. The mice were rested for 5 hours prior to analysis. **c**, qPCR analysis of indicated genes in muscle from mice subjected to 6 hours of cold exposure (4 degrees) compared to mice housed in room temperature. **d**, qPCR analysis of mRNA for indicated genes in inguinal fat from mice subjected to 1 hour of acute exercise compared to sedentary mice. The mice were rested for 5 hours prior to analysis. * $p<0.05$ compared to control using students T-TEST

Supplementary Figure S3

Affymetrix analysis shows all mRNAs significantly altered in expression arrays after 6 days of Fndc5 treatment (50nM) of SVF cells during differentiation compared to saline control.

Genes up regulated >4-fold or down regulated to < 0,4 fold are shown. FC= fold change and the “p=” column indicates p-value using T-TEST.

Supplementary Figure S4

a, This shows 8 genes whose expression was significantly induced with $p<0.05$ on gene expression arrays, with highest fold change in SVF treated with Fndc5 for 8 days. Brown fat-related genes are marked in bold. **b**, representative images of immunohistochemistry against UCP1 in SVF differentiated into adipocytes for 6 days in the presence of saline or recombinant Fndc5 (20 nM). Right graph shows BioPix quantification of UCP1 positive cells in 40 random images per group. **c**, representative electron microscopy of SVF differentiated into adipocytes for 6 days in the presence of saline or recombinant Fndc5 (20 nM). d, QPCR against indicated mRNAs in SVF from BAT, differentiated for 6 days in the presence or absence of 20nM Fndc5.

Supplementary Figure S5

a, qPCR from SVF from the inguinal fat depot, differentiated into adipocytes for 6 days and treated with 20nM Fndc5 at different days of differentiation, as indicated. This experiment was repeated once with similar results. **b**, mRNA expression of indicated mitochondrial genes from inguinal SVF treated with saline or Fndc5 for 6 days during differentiation. Data is presented as mean ±SEM. * $p<0.05$ compared to control using students one-way ANOVA.

Supplementary Figure S6

a, Homology between the mouse and human Fndc5. Red underline marks irisin. Amino acid sequence for homo sapiens, mus musculus, danio rerio and gallus gallus is shown, whereas homology is clear for regions other than the signal peptide at the n-terminal side. Presentation is formatted as MView with colors marking consensus/80% property. **b**, RT-PCR analysis of mRNAs encoding indicated genes in adipocytes differentiated for 6 days from the stromal-vascular fraction (SVF) cells. This was done in the presence of conditioned media from primary myocytes with forced expression of GFP or PGC1α and with antibodies against control/Fndc5 as indicated. Data presented is representative of 2 independent experiments. Data is presented as mean ±SEM. * $p<0.05$ compared to IgG control and § $p<0.05$ compared to IgG + PGC1α conditioned media using one-way ANOVA.

Supplementary Figure S7

a, anti-FLAG western blot against Albumin/IgG cleared and deglycosylated plasma from mice injected with GFP or n-terminally flag-tagged Fndc5 expressing adenovirus. **b**, western blot analysis of four human serum samples where the primary antibody has been pre-incubated with either recombinant Fndc5 (right) or BSA (left). **c**, standard curve of recombinant, n-terminally tagged Fndc5 for semi quantitative plasma irisin-measurements.

Supplementary Figure S8

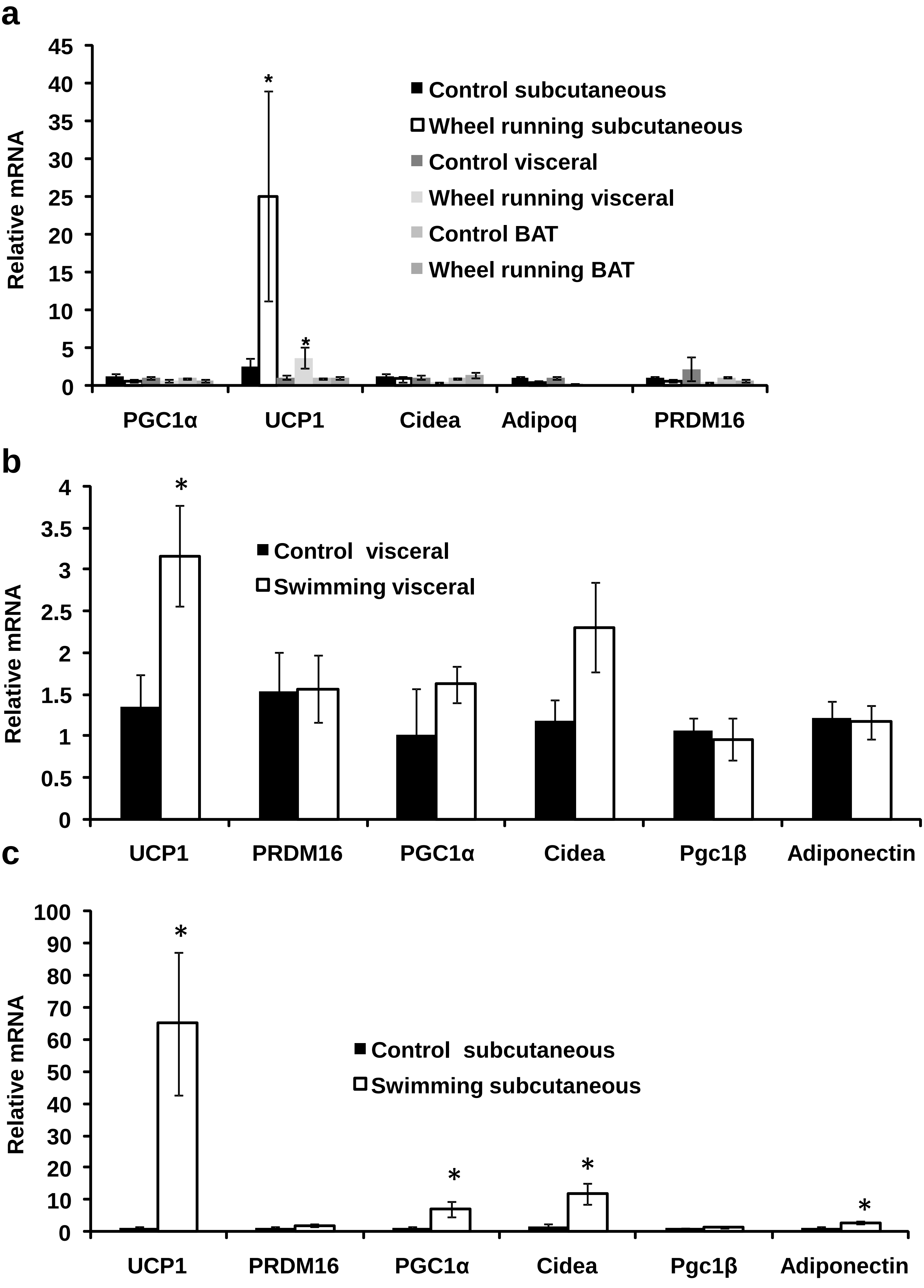
a, QPCR analysis of interscapular brown fat (BAT) against indicated genes in high-fat diet treated mice, 10 days after injection with Fndc5 or GFP expressing adenovirus. **b**, qPCR analysis for indicated mRNAs in inguinal WAT in high-fat diet treated mice, 10 days after injection with Fndc5 or GFP expressing adenovirus. **c**, qPCR analysis against indicated mRNAs in liver of high-fat diet treated mice, 10 days after injection with Fndc5 or GFP expressing adenovirus. d, Aspartate transaminase (AST) activity in plasma from mice 10 days after injection with Fndc5 or GFP expressing adenovirus (n=7 for each group). Data is presented as relative to control. * $p<0.05$ compared to control using students T-TEST.

Supplementary Figure S9

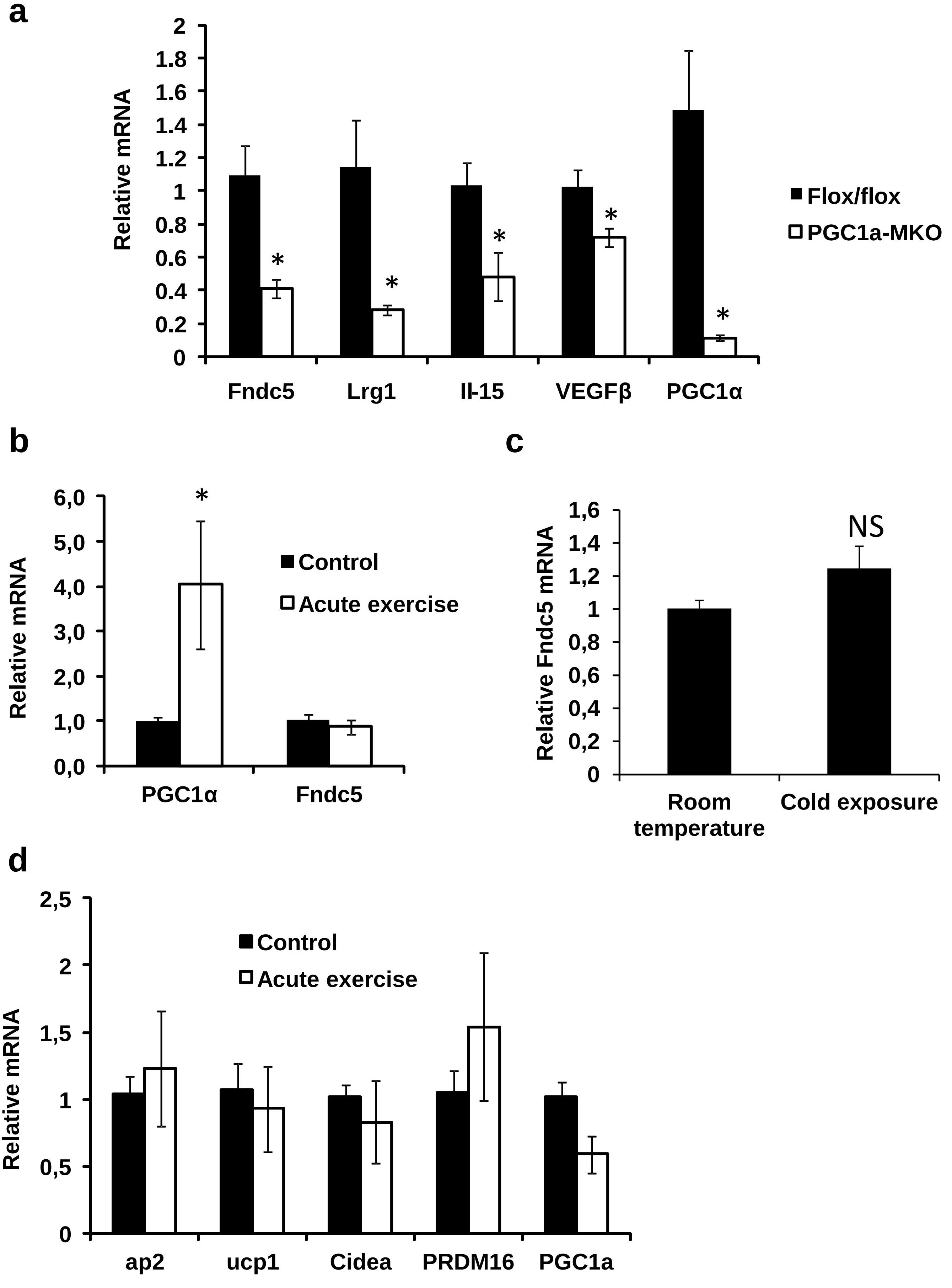
a, qPCR analysis of indicated genes from inguinal WAT in mice treated with HFD and injected with GFP or Fndc5 adenovirus. **b,** qPCR against indicated genes from inguinal WAT in 12 weeks B/6 mice on chow diet treated with GFP or Irisin expressing virus (n=7)**.** These results were confirmed in an independent mouse cohort. * p<0.05 compared to GFP adenovirus injection.

Supplementary Figure S10

a, qPCR against indicated mRNAs from primary myotubes 48h after treatment with recombinant Fndc5 (100 nM) or saline control. **b**, qPCR analysis muscle (quadriceps) of indicated mRNAs in high-fat diet treated mice, 10 days after injection with Fndc5 or GFP expressing adenovirus.



Bostrom et al supplementary figure S1



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gene	FC	p=
Ucp1: uncoupling protein 1 (mitochondrial. proton carrier)	80.41	8.62E-05
Otop1: otopetrin 1	25.98	6.87E-05
Aadac: arylacetamide deacetylase (esterase)	10.52	0.000211
Cox7a1: cytochrome c oxidase. subunit VIIa 1	7.73	1.01E-08
Acaa1b: acetyl-Coenzyme A acyltransferase 1B	7.50	5.36E-05
Ubd: ubiquitin D	7.03	3.45E-05
Ncan: neurocan	7.02	0.000319
Elovl3: elongation of very long chain fatty acids-like 3	6.91	0.000219
Plin5: perilipin 5	6.57	1.23E-05
Cox8b: cytochrome c oxidase. subunit VIIIb	6.48	5.62E-07
Scd3: stearoyl-coenzyme A desaturase 3	6.17	0.011329
carnitine palmitoyltransferase 1b. Muscle	6.14	0.000143
Itih4: inter alpha-trypsin inhibitor. heavy chain 4	5.65	0.000318
Car4: carbonic anhydrase 4	5.59	0.000885
Cyp2e1: cytochrome P450. family 2. subfamily e. polypeptide 1	5.34	0.000716
Slc25a34: solute carrier family 25. member 34	5.21	3.17E-05
Arg1: arginase. liver	5.02	0.013321
Car4: carbonic anhydrase 4	4.80	0.000302
Sptlc3: serine palmitoyltransferase. long chain base subunit 3	4.63	0.00014
Acaa2: acetyl-Coenzyme A acyltransferase 2	4.48	0.00187
Mpzl2: myelin protein zero-like 2	4.36	0.000588
Reep1: receptor accessory protein 1	4.31	0.000319
Acer2: alkaline ceramidase 2	4.30	0.000991
Gys2: glycogen synthase 2	4.14	3.59E-07
Gpr120: G protein-coupled receptor 120	4.01	0.000986
Gpld1: glycosylphosphatidylinositol specific phospholipase D1	0.39	0.031602
Asb4: ankyrin repeat and SOCS box-containing 4	0.39	0.019371
Mm.207312.1	0.38	0.001832
Cyp2f2: cytochrome P450. family 2. subfamily f. polypeptide 2	0.37	0.000688
Odz3: odd Oz/ten-m homolog 3 (Drosophila)	0.37	0.000236
Zfp207: zinc finger protein 207	0.36	0.039105
Ntn4: netrin 4	0.36	0.001783
Higd1b: HIG1 domain family. member 1B	0.35	0.000626
Myh11: myosin. heavy polypeptide 11. smooth muscle	0.34	0.000105
Serpina1b: serine (or cysteine) preptidase inhibitor. clade A. member 1B	0.32	7.31E-06
Aplnr: apelin receptor	0.31	5.19E-06
Mtx3: metaxin 3	0.28	0.001888
Serpina1a	0.27	1.95E-05
Oxtr: oxytocin receptor	0.24	6.85E-05
4921521F21Rik: RIKEN cDNA 4921521F21 gene	0.24	0.000241
Rgs5: regulator of G-protein signaling 5	0.19	0.00216
Lep: leptin	0.15	0.000173
Mup5: major urinary protein 5	0.12	0.00016
Mup3: major urinary protein 1	0.07	1.64E-06

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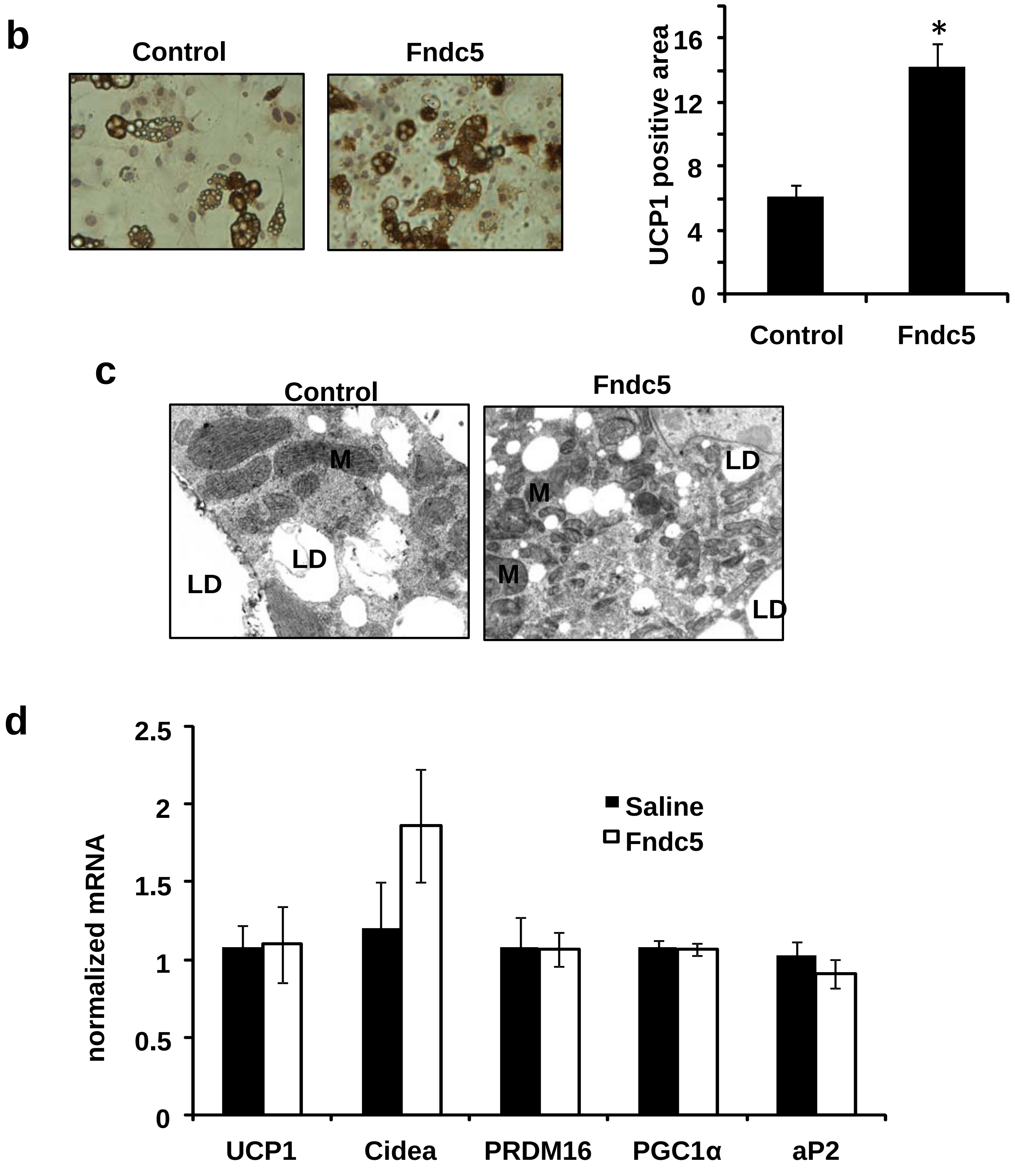
a

gene	fold change	p
Ucp1	80.41	0.0001
Otop1	25.98	0.0001
Aadac	10.52	0.0002
Cox7a1	7.73	0.0000
Acaa1b	7.50	0.0001
Ubd	7.03	0.0000
Ncan	7.02	0.0003
Elovl3	6.91	0.0002



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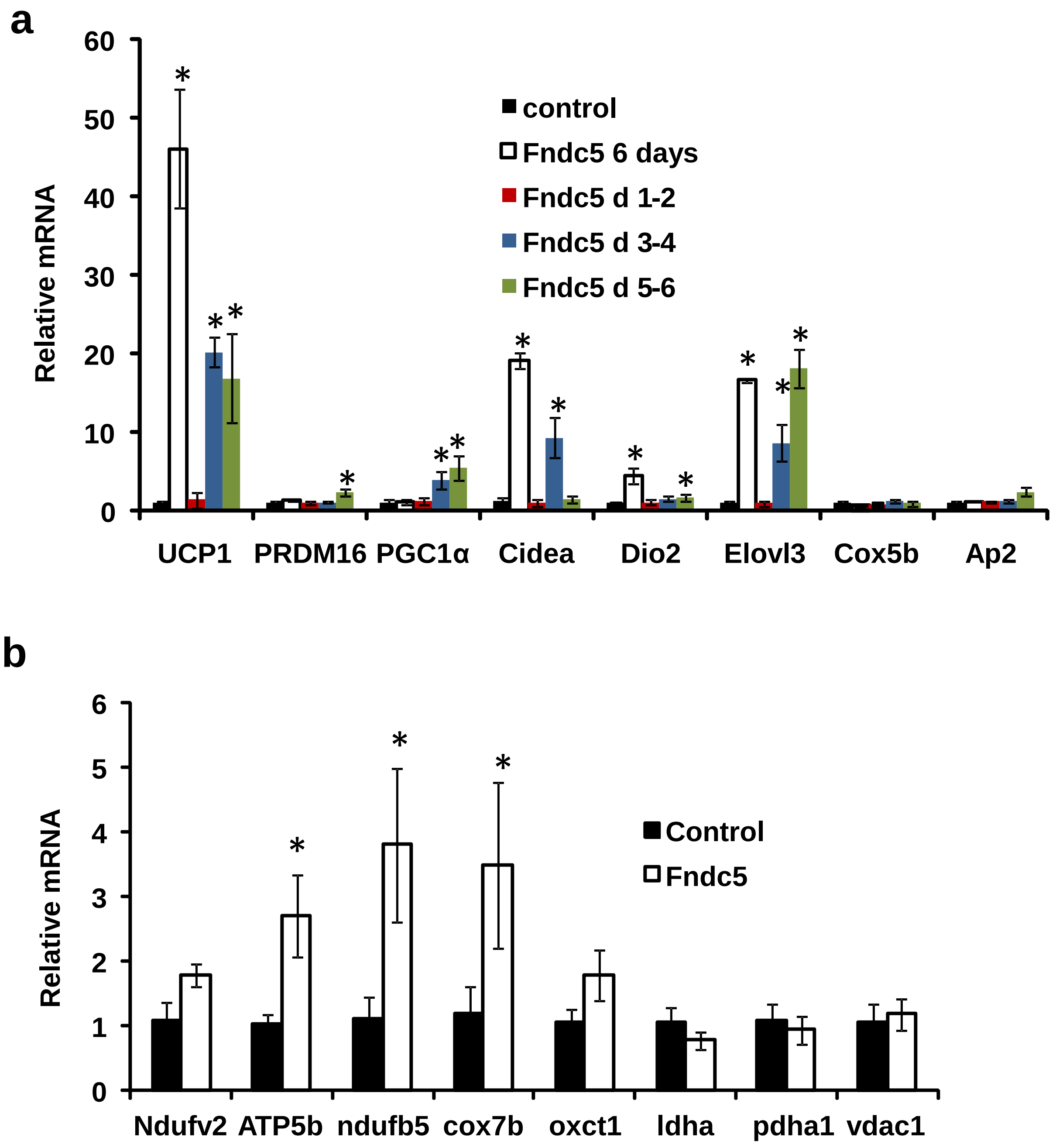

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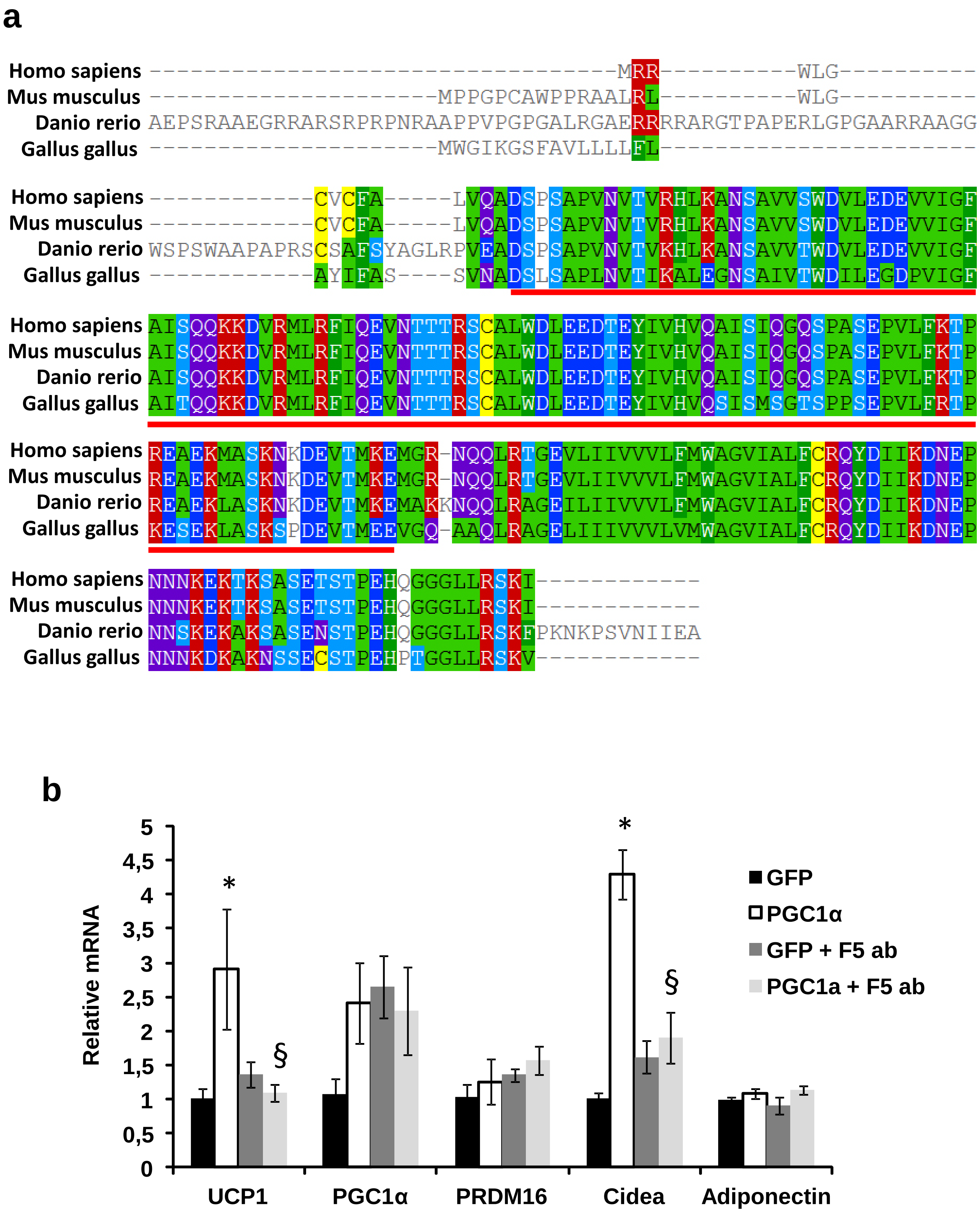

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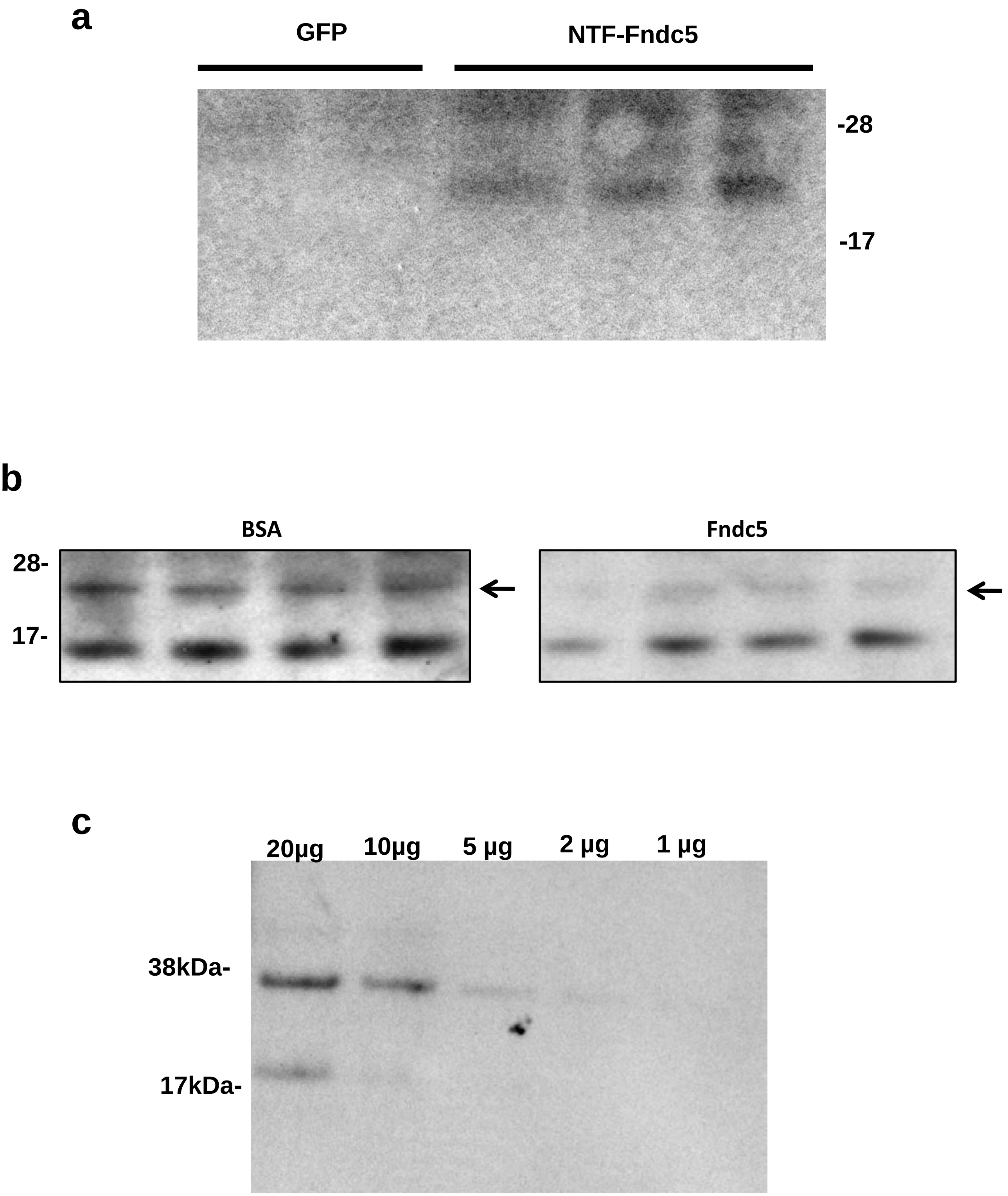

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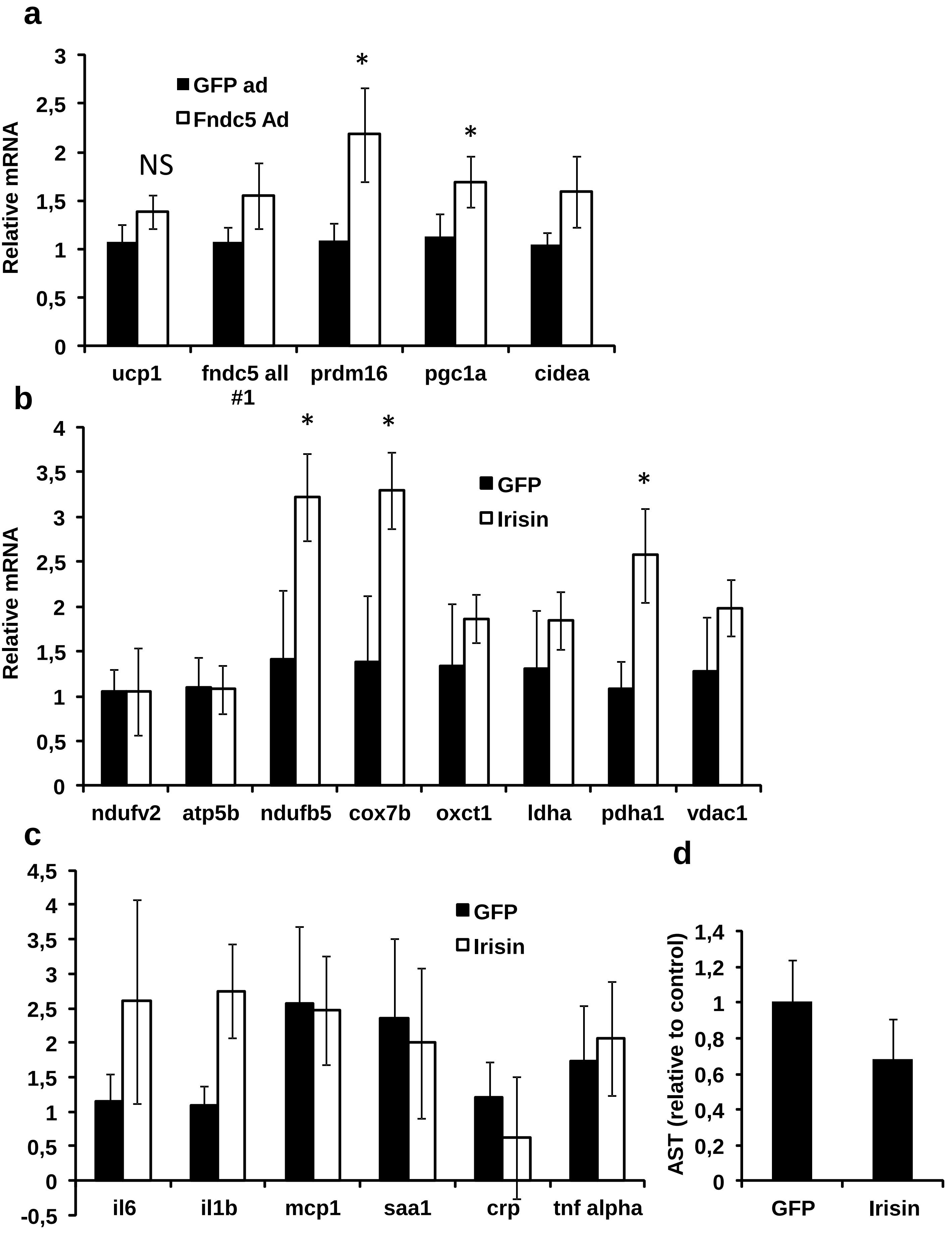

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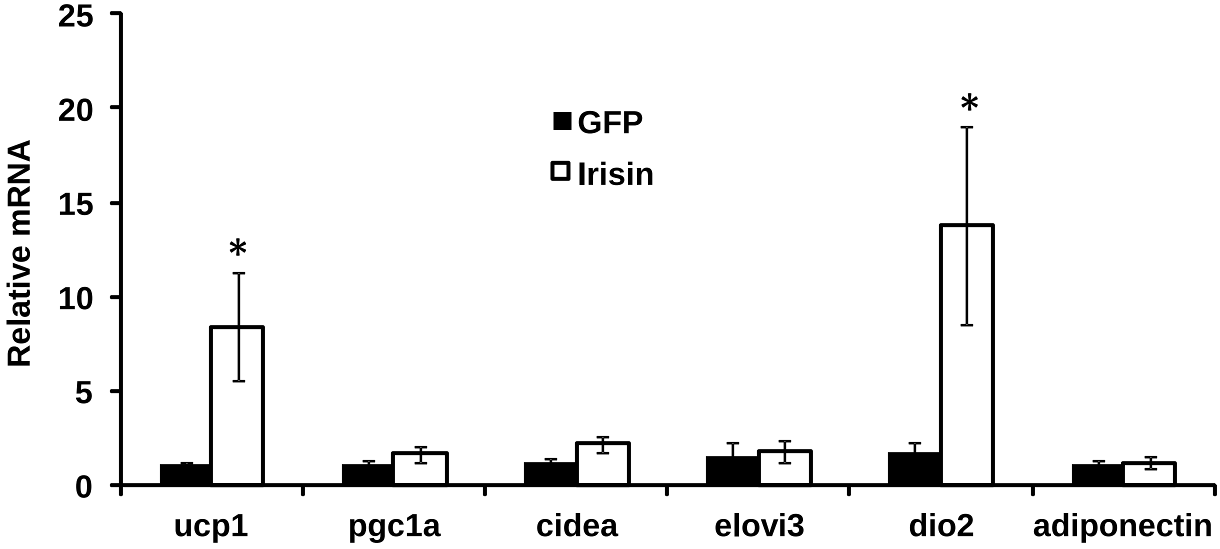

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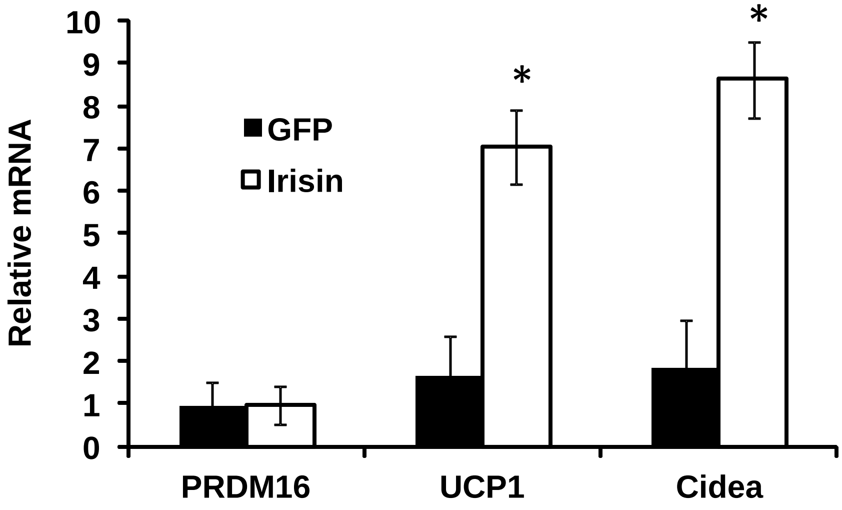

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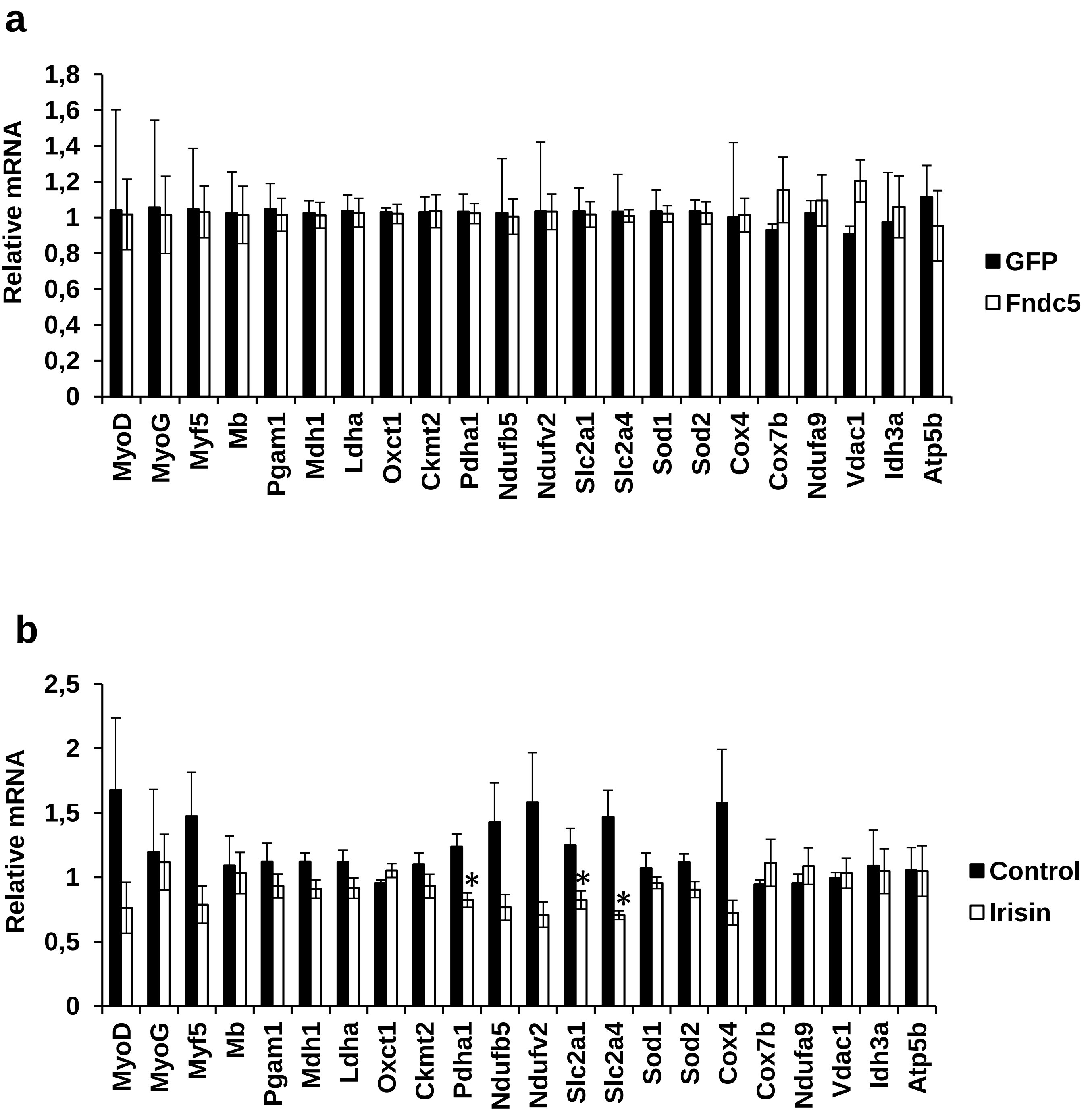

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Gene	Forward primer	Reverse primer
m18s	TCAAGAACGAAAGTCGGAGG	GGACATCTAAGGGCATCAC
mFndc5 1 UTR	ggactcttggaaaacaccactg	tccacacagatgatctcaccac
mFndc5 2	atgaaggagatggggaggaa	gcggcagaagagagctataaca
mAp2	ACA CCG AGA TTT CCT TCA AAC TG	CCA TCT AGG GTT ATG ATG CTC TTC A
mCidea	TGC TCT TCT GTA TCG CCC AGT	GCC GTG TTA AGG AAT CTG CTG
mPGC1a	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
mPRDM16	CAG CAC GGT GAA GCC ATT C	GCG TGC ATC CGC TTG TG
mTBP	GAA GCT GCG GTA CAA TTC CAG	CCC CTT GTA CCC TTC ACC AAT
mUCP1	ACT GCC ACA CCT CCA GTC ATT	CTT TGC CTC ACT CAG GAT TGG
mAdiponectin	GCA CTG GCA AGT TCT ACT GCA A	GTA GGT GAA GAG AAC GGC CTT GT
mVEGFb	tatctcccagagctgccatcta	agccagaagatgctcacttgac
mIl15	gaggccaagaagagttctggat	tgcccaggtaagagcttcaa
mTIMP4	gaccctgctgacactcaaaaac	ggaagagtcaaatggcgtgtag
mLrg1	cctcaaggaatgcctgatactg	ttggagaattccaccgacag
hTIMP4	caggtcctcagtgatggaaaag	gtgatttggcagccacagtt
hFndc5	aagcacaaggactgactcaagc	catgtccttgatggctggat
hLrg1	ctagaacacacgatgggctttc	tcagctggaaggaaggacaa
hIL-15	tcagtgcagggcttcctaaa	tggggtgaacatcactttcc

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