Fig S1

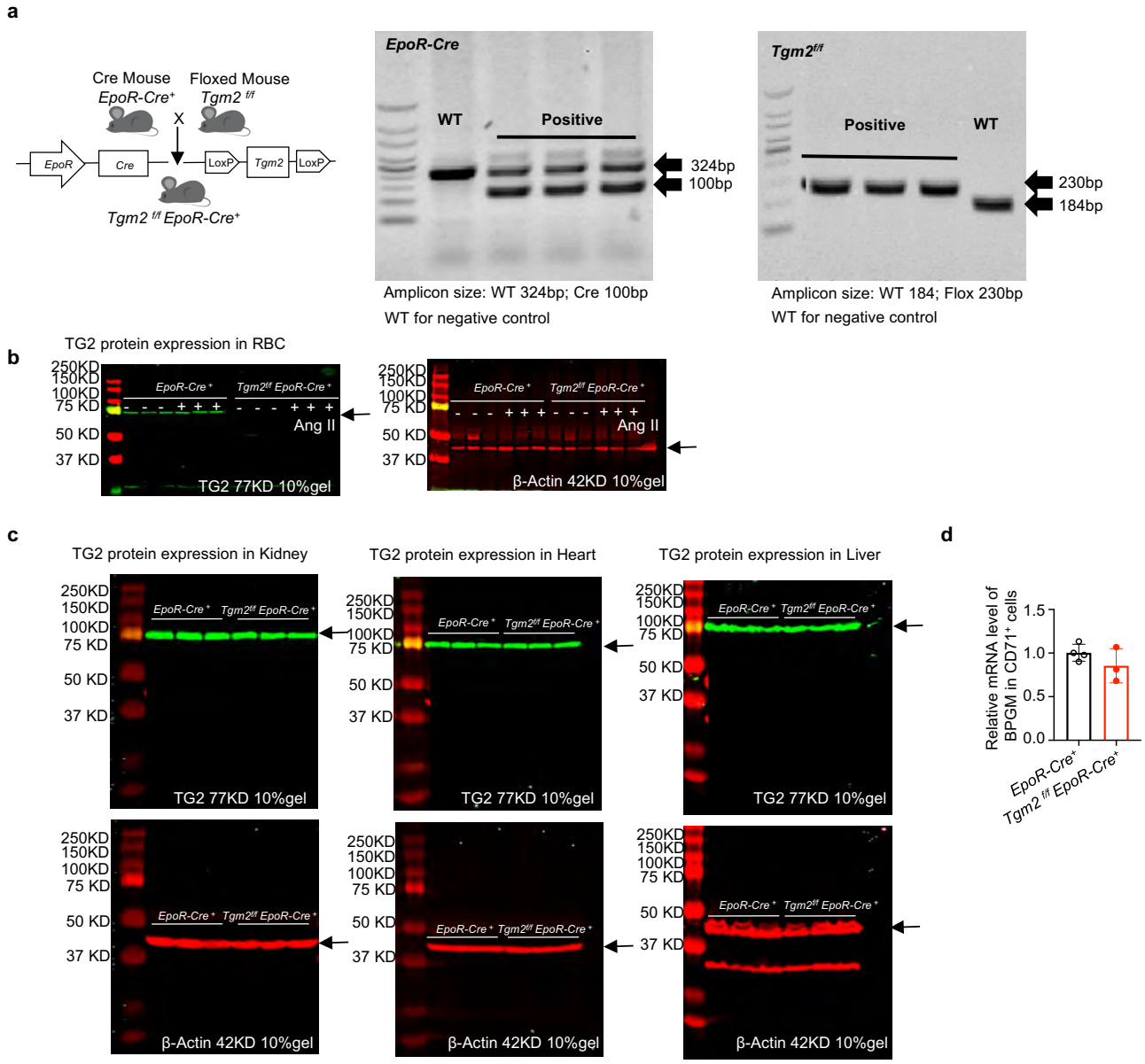


Figure S1. Generation of mice with deletion of *Tgm2* in erythroid cells, related to Figure 1 and STAR Methods.

a. Schematic representation of erythrocyte specific *Tgm2* deletion mice (*Tgm2$^{f/f}$EpoR-Cre^{+}*) generation (left panel); Representative genotyping results of experimental mice (right panel).

b. TG2 protein levels in *EpoR-Cre^{+}* and *Tgm2$^{f/f}$EpoR-Cre^{+}* mouse RBC.

c. TG2 protein levels in kidney, heart and liver of *EpoR-Cre^{+}* and *Tgm2$^{f/f}$EpoR-Cre^{+}* mouse.

d. Relative mRNA levels in CD71^{+} cells. Data are expressed as mean ± SD. Data were analyzed by unpaired t-test, (n=3 or 4).

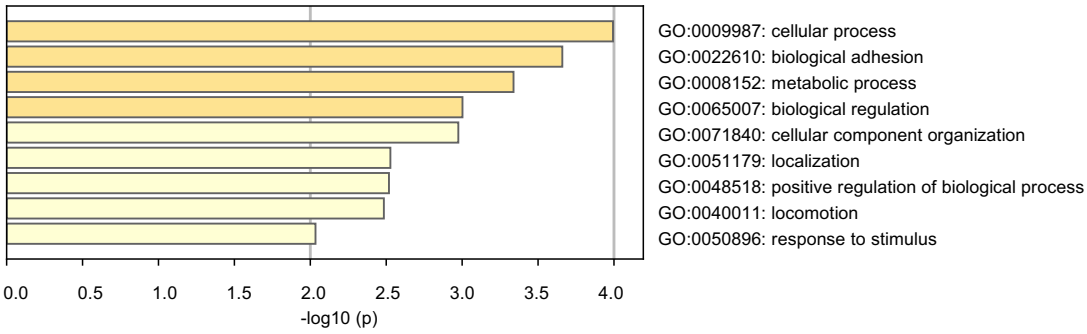
Fig S2

a

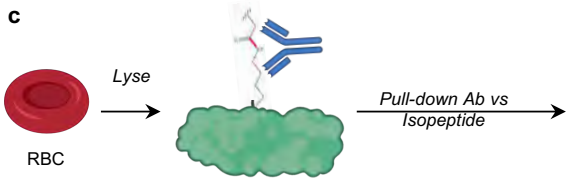
Table 1. Potential TG2 substrates

	Identified Proteins (23)	Alternate ID
1	Phosphatidylinositide phosphatase SAC1	Sacm1l
2	Actin-related protein 2/3 complex subunit 2	Arpc2
3	Ras GTPase-activating protein 3	Rasa3
4	Tubulin beta-5 chain	Tubb5
5	Flavin reductase (NADPH)	Blvrb
6	Aldehyde dehydrogenase family 16 member A1	Aldh16a1
7	Heat shock protein HSP 90-beta	Hsp90ab1
8	Ketosamine-3-kinase	Fn3krp
9	COP9 signalosome complex subunit 4	Cops4
10	Gamma-adducin	Add3
11	Beta-parvin	Parvb
12	Flotillin-2	Flot2
13	Casein kinase II subunit alpha	Csnk2a1
14	Phospholipid transfer protein C2CD2L	C2cd2l
15	Integrin-linked protein kinase	Ilk
16	DnaJ homolog subfamily B member 4	Dnajb4
17	ATP-binding cassette sub-family F member 1	Abcf1
18	Prohibitin-2	Phb2
19	Bisphosphoglycerate mutase	Bpgm
20	Guanine nucleotide-binding protein G(i) subunit alpha-2	Gnai2
21	Integrin alpha-IIb	Itga2b
22	Transferrin receptor protein 1	Tfrc
23	ATP-binding cassette sub-family B member 6	Abcb6

b



c



PMGE_MOUSE
Bisphosphoglycerate mutase (Mus musculus)
Sequence coverage: 75% ***(green)***

MSKHKLIILR HGEGQWNKEN RFCSWVDQKL NNDGLEEARN CGRQLKALNF
EFDLVFTSIL NRSIHTAWLI LEELGQEWVP VESSWRLNER HYGALIGLNR
EKMALNHGEE QVRLWRRSYN VTPPPIEESH PYFHEIYSDR RYKVCDVPLD
QLPRSESLKD VLERLLPYWK ERIAPEILKG KSILISAHGN SSRALLKHLE GISDEDIINI
TLPTGVPILL ELDENLRAVG PHQFLGNQEA IQAAIKKVDD QGKVKQGKQ

Figure S2. BPGM and 22 additional potential proteins were identified as TG2 substrates, related to Figure 1.

a. Potential targets modified by eTG2 based on isopeptide pulldown of membrane proteins.

b. The Gene Ontology biological processes of genes from isopeptide pulldown proteins between *EpoR-Cre* $^{+}$ and *Tgm2* $^{f/f}$*EpoR-Cre* $^{+}$ mice analyzed by Metascape.

c. Proteomics analysis of BPGM.

Fig S3

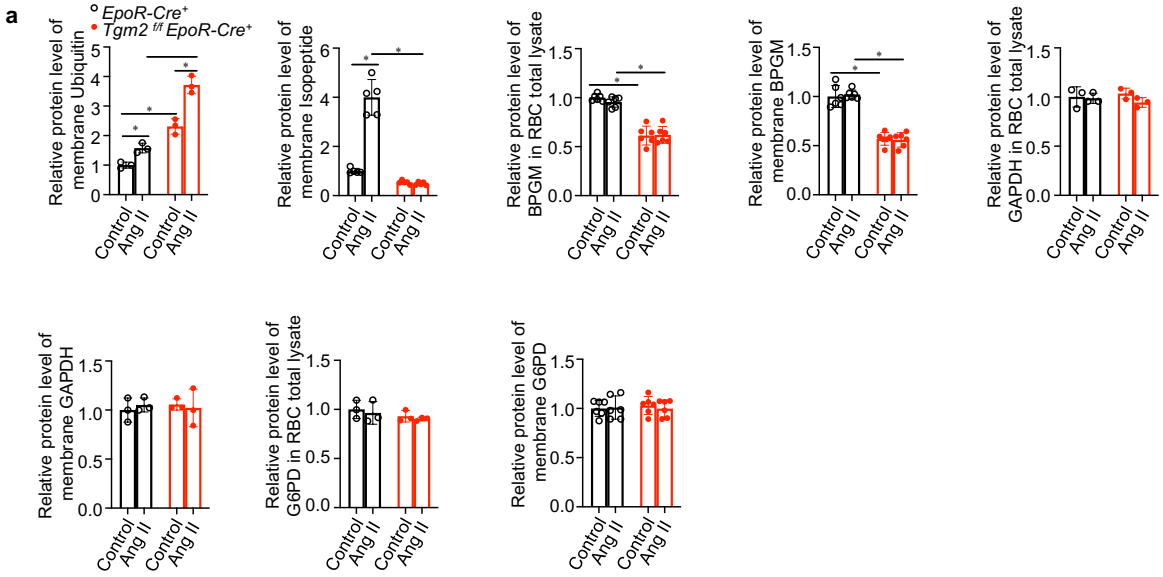


Figure S3. Effect of Ang II-mediated pathological hypoxia on the protein levels of BPGM, GAPDH, G6PD, ubiquitin and isopeptide, related to Figure 3.

a. Protein levels of ubiquitin, isopeptide, BPGM, GAPDH and G6PD as quantified by densitometry. Data are expressed as mean ± SD, **P*<0.05, (n=3-6). Data in this figure were analyzed by two-way ANOVA followed with Sidak's multiple comparisons test.

Fig S4

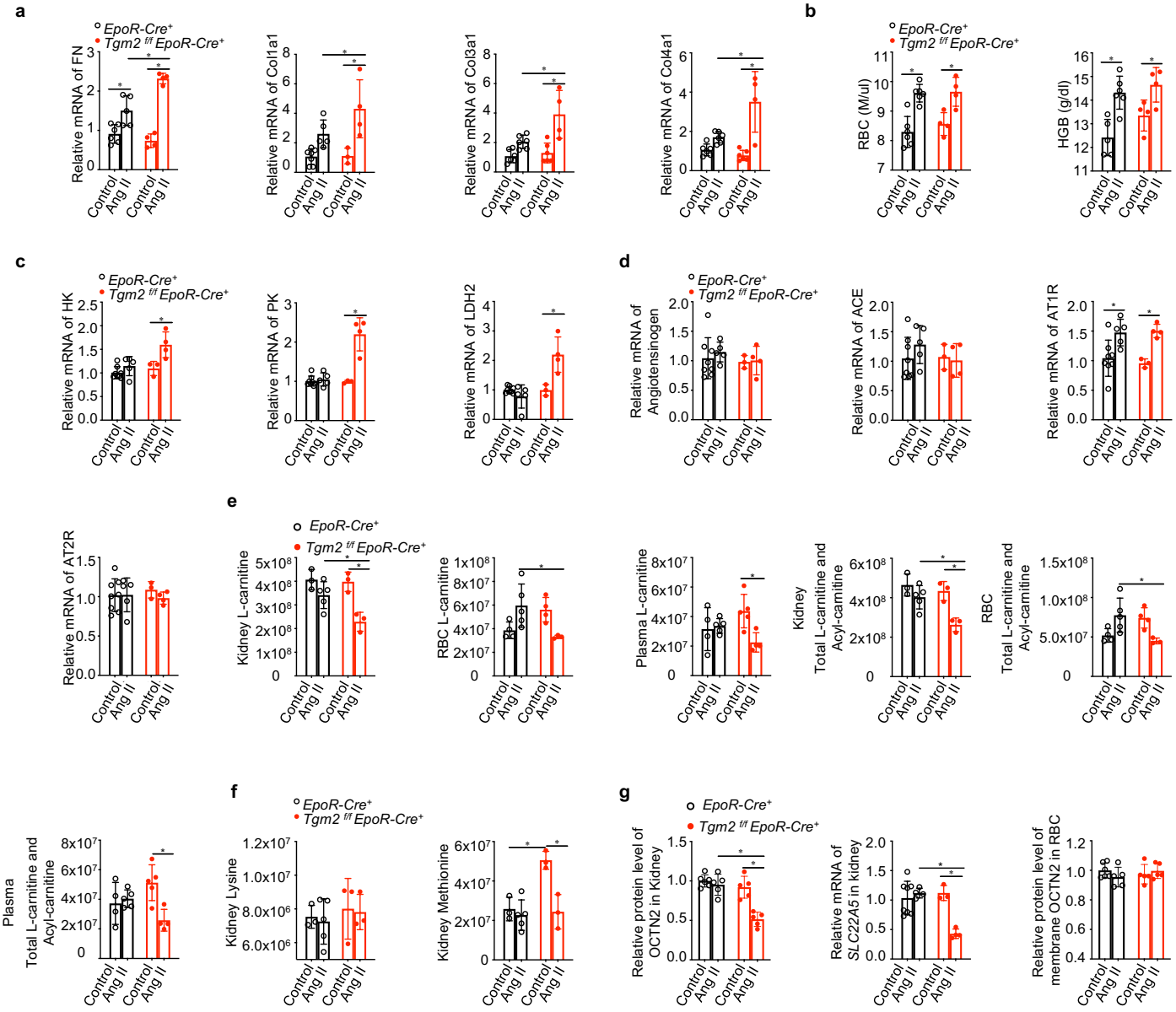


Figure S4. Erythrocyte TG2 deletion promoted the expression of fibrotic genes and glycolytic genes, related to Figure 4.

a. Relative levels of mRNAs in kidneys for fibronectin (FN) and collagens (Col1a1, Col3a1and Col4a1). **P* < 0.05, (n=3-7).

b. Erythrocyte number and Hemoglobin (Hb) concentration were determined by complete blood count (CBC). **P* < 0.05, (n=4-6).

c. Relative mRNA levels of hexokinase (HK), pyruvate kinase (PK) and lactate dehydrogenase 2 (LDH2) in kidney. **P* < 0.05, (n=3-8).

d. qRT-PCR analyses of mRNAs encoding renin-angiotensin system (RAS) components in kidney tissues from *EpoR-Cre*$^{+}$ and *Tgm2*$^{f/f}$*EpoR-Cre*$^{+}$ mice with or without Ang II treatment; angiotensinogen, angiotensin-converting enzyme (ACE), Angiotensin II type I receptor (AT1R) and Angiotensin II type 2 receptor (AT2R). **P* < 0.05, (n=3-8).

e. L-carnitine and acyl-carnitine levels of kidney, RBC and plasma were quantified by metabolomic screening. **P* < 0.05, (n=3-5).

f. Kidney lysine and methionine levels were quantified by metabolomic screening. **P* < 0.05, (n=3-5).

g. Protein levels of OCTN2 in kidneys and erythrocyte membranes and the mRNA levels of *Slc22a5* (the gene encoding OCTN2) in kidney, were quantified, **P* < 0.05, (n=3-7). All data are expressed as mean ± SD and were analyzed by two-way ANOVA followed with Sidak's multiple comparisons test.

Fig S5

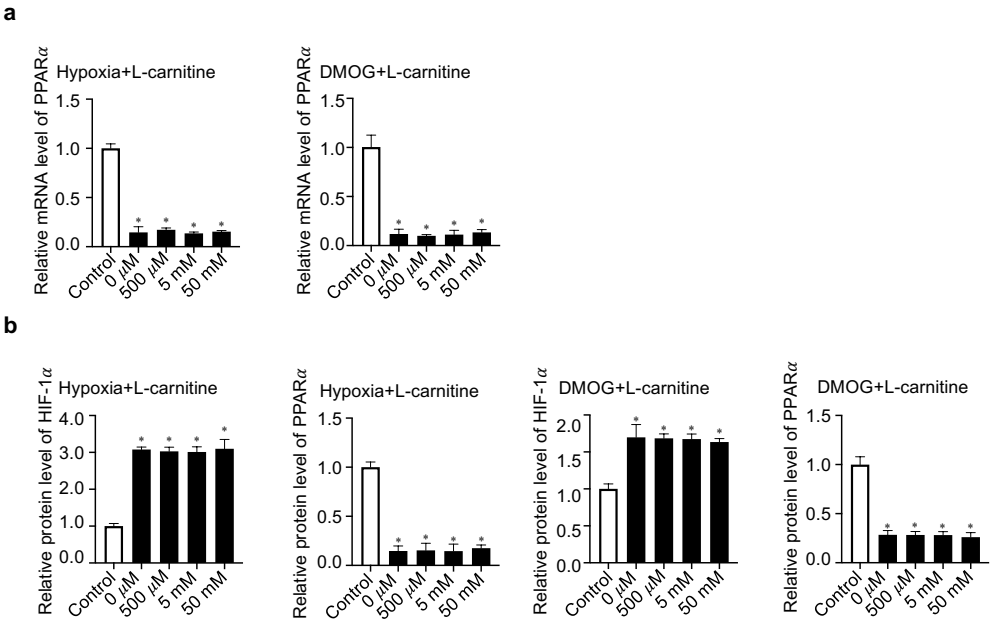


Figure S5. L-carnitine has no effect on the protein and mRNA expression of HIF-1α and PPARα In primary kidney organ cultures, related to Figure 7.

a. PPARα mRNA levels were detected after hypoxia or DMOG treatment of primary kidney organ cultures in the presence or absence of various concentrations of L-carnitine. Data are expressed as mean ± SD, **P*<0.05 versus Control, (n=3).

b. Quantification of western blot data in Figure 7d. Data are expressed as mean ± SD, **P*<0.05 versus Control, (n=3). Data were analyzed by two-way ANOVA followed with Tukey's multiple comparisons test.

Table S1. Human volunteer characteristics (Related to Figure 1)

	Male	Female
Age	21.09±1.30	20.57±1.62
HT (cm)	181.3±4.26	169.2±5.77
WT (kg)	75.71±6.39	63.47±6.55
BMI (kg/m2)	22.99±1.68	22.1±1.87
Number	11	7

Data are expressed as mean ± SEM; height (HT), weight (WT), body mass index (BMI), n=18. Related to Figure 1.

Table S2. Sequences of RT-PCR primers. (Related to STAR Methods.)

Gene Name	Forward primers (5'-3')	Reverse primers (5'-3')
Hexokinase (HK)	GGAGCAGTGGACCAGGGTA	GAAGTTCAGCTGTTTTTGAATTG
Pyruvate kinase (PK)	AAGAAGGGAGCCACTCTGAA	CTTGTAGTCCAGCCACAGGAT
Lactate dehydrogenase 2 (LDH2)	GGCACGTTCAAGTTGGTCTT	GAAATGGACTCGTCGGTGTT
mCol4a	GACAGCCAGGTTTGACAGGT	GGCAGCTCTCTCCTTTCTGA
mCol3a	ACAGCTGGTGAACCTGGAAG	ACCAGGAGATCCATCTCGAC
mCol1a	GCTCTTTTTAGATACTGTGGTGAGGAA	GTTTCCACGTCTCACCATTG
Mouse fibronectin (mFn)	ACAAGGTTCGGGAAGAGGTT	CCGTGTAAGGGTCAAAGCAT
Slc22a5	GGA CCA GAA ACT TAA CAA CGA CG	CAG GCT GTG TGA ATG GAC CT
angiotensinogen	TCTCCTTTACCACAACAAGAGCA	CTTCTCATTCACAGGGGAGGT
angiotensin-converting enzyme (ACE)	AGGTTGGGCTACTCCAGGAC	GGTGAGTTGTTGTCTGGCTTC
Angiotensin II type I receptor (AT1R)	ATGCTTGGGGCAACTTCACTA	GCAGCAAGAGAAGGGCTTCA
Angiotensin II type 2 receptor (AT2R)	GAAGCTCCGCAGTGTGTTTA	TGGCTAGGCTGATTACATGC
PPAR-α	TGCAAACTTGGACTTGAACG	GATCAGCATCCCGTCTTTGT

Data S1. Original Images of Representative Western blot and PCR Agarose Gel. Related to Figures 1-7 and S1.

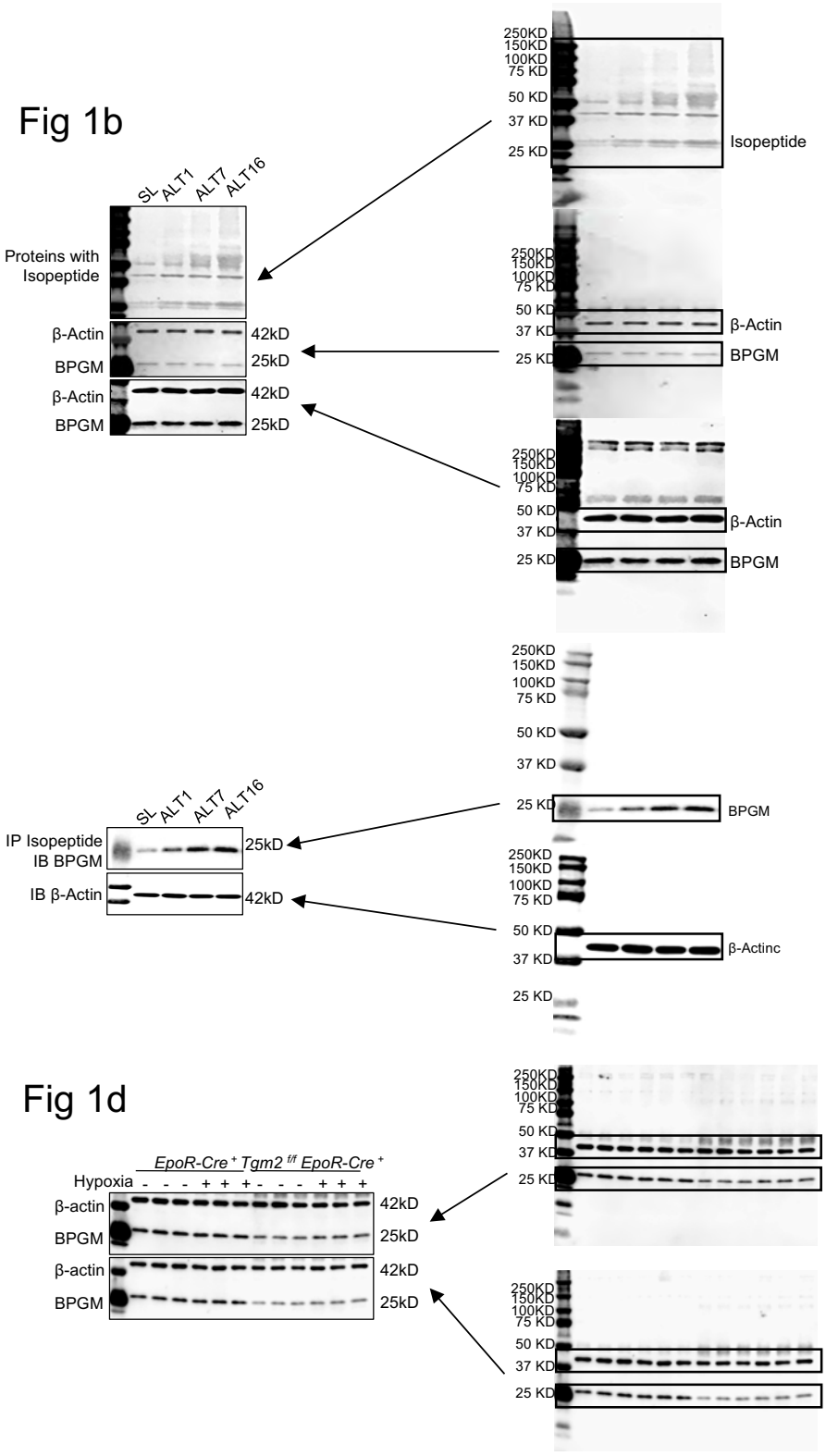
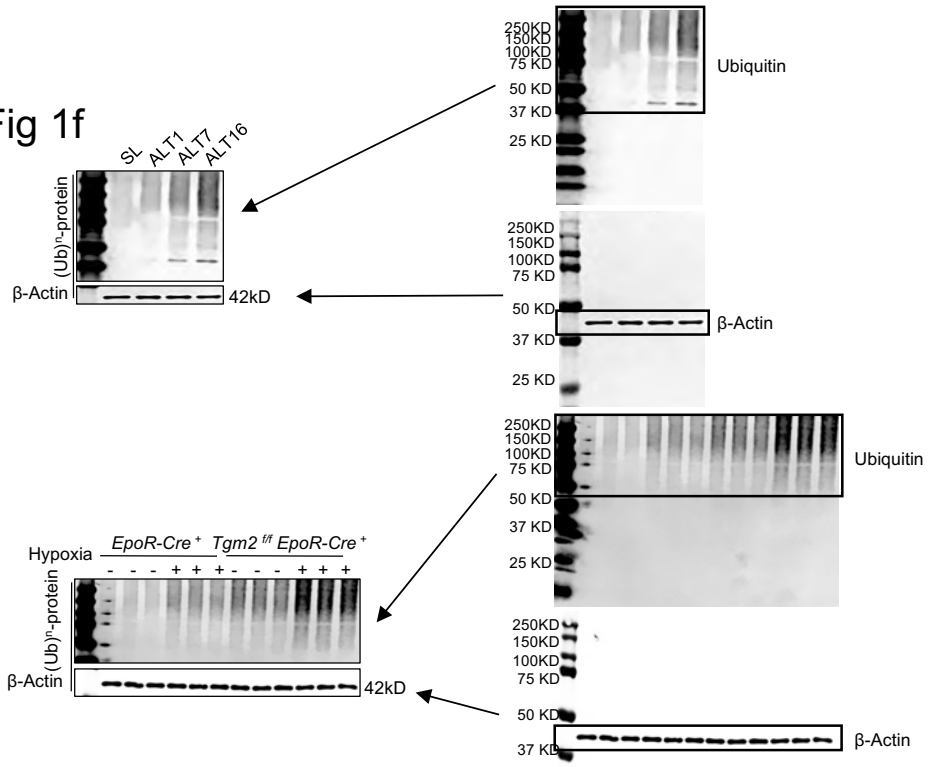


Fig 1f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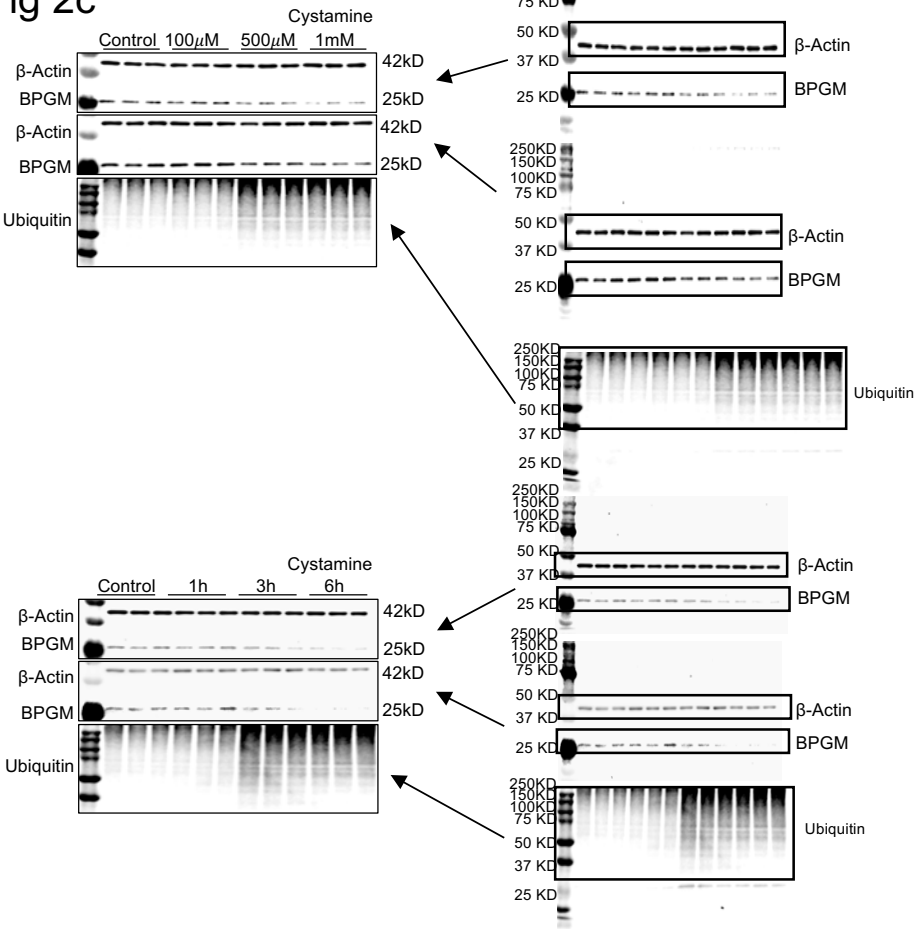
ALT7
ALT16
(Ub)n-protein
β-Actin
42kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
Ubiquitin
β-Actin
Hypoxia
EpoR-Cre +
Tgm2 f/f EpoR-Cre +

Fig 2c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Control
100μM
500μM
1mM
β-Actin
BPGM
Ubiquitin
42kD
25kD
250KD
150KD
100KD
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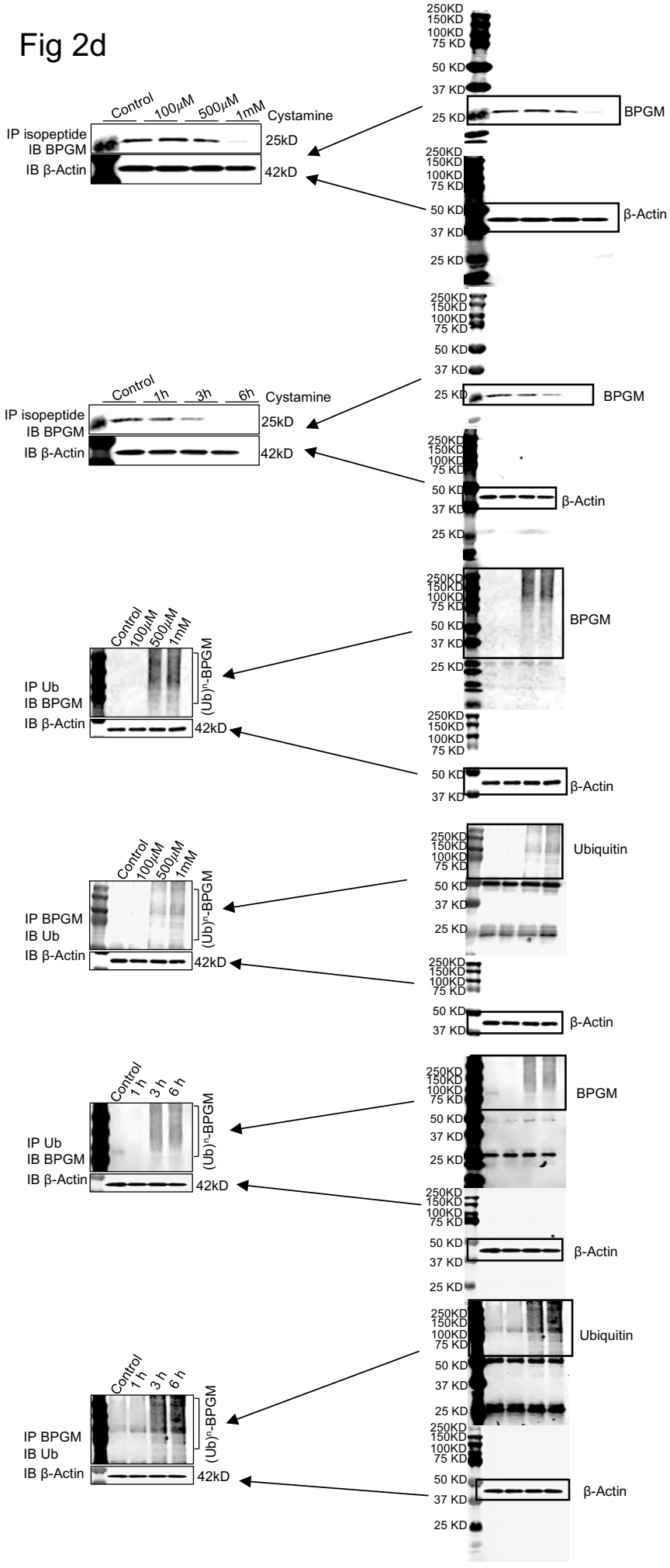
50 KD
37 KD
25 KD
Control
1h
3h
6h

Fig 2d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500μM
1mM
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IB BPGM
IB β-Actin
25kD
42kD
250KD
150KD
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75 KD
50 KD
37 KD
25 KD
BPGM
β-Actin
Control
1h
3h
6h
Cystamine
IP isopeptide
IB BPGM
IB β-Actin
25kD
42kD
BPGM
β-Actin
Control
100μM
500μM
1mM
IP Ub
IB BPGM
IB β-Actin
(Ub)n-BPGM
42kD
BPGM
β-Actin
Control
100μM
500μM
1mM
IP BPGM
IB Ub
IB β-Actin
(Ub)n-BPGM
42kD
Ubiquitin
β-Actin
Control
1 h
3 h
6 h
IP Ub
IB BPGM
IB β-Actin
(Ub)n-BPGM
42kD
BPGM
β-Actin
Control
1 h
3 h
6 h
IP BPGM
IB Ub
IB β-Actin
(Ub)n-BPGM
42kD
Ubiquitin
β-Actin

Fig 2f

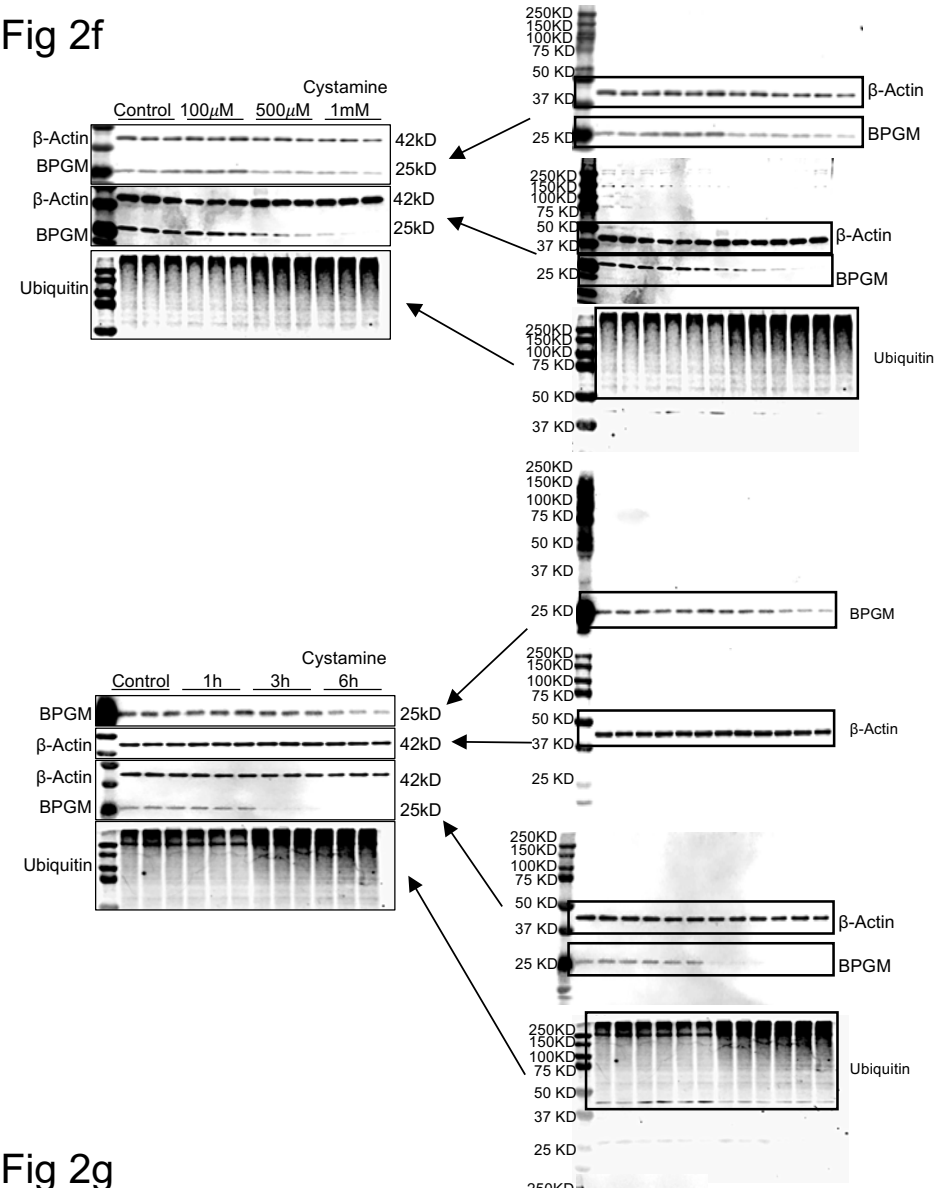
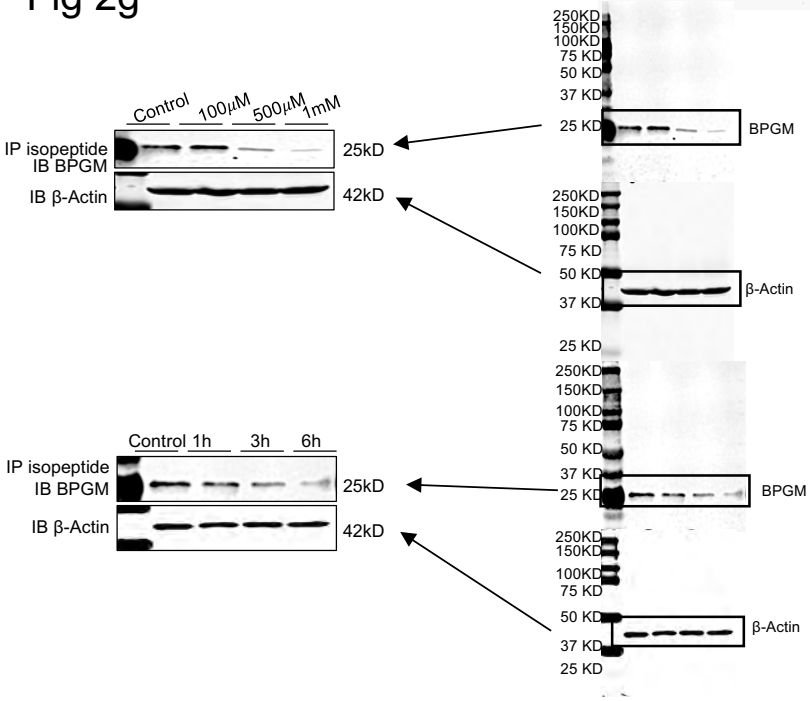

Cystamine
Control 100μM 500μM 1mM
β-Actin
BPGM
β-Actin
BPGM
Ubiquitin
42kD
25kD
42kD
25kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
β-Actin
BPGM
Ubiquitin
Cystamine
Control 1h 3h 6h
BPGM
β-Actin
β-Actin
BPGM
Ubiquitin
25kD
42kD
42kD
25kD

Fig 2g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IP isopeptide
IB BPGM
IB β-Actin
25kD
42kD
Control 1h 3h 6h
IP isopeptide
IB BPGM
IB β-Actin
25kD
42kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
BPGM
β-Actin
BPGM
β-Actin

Fig 2g

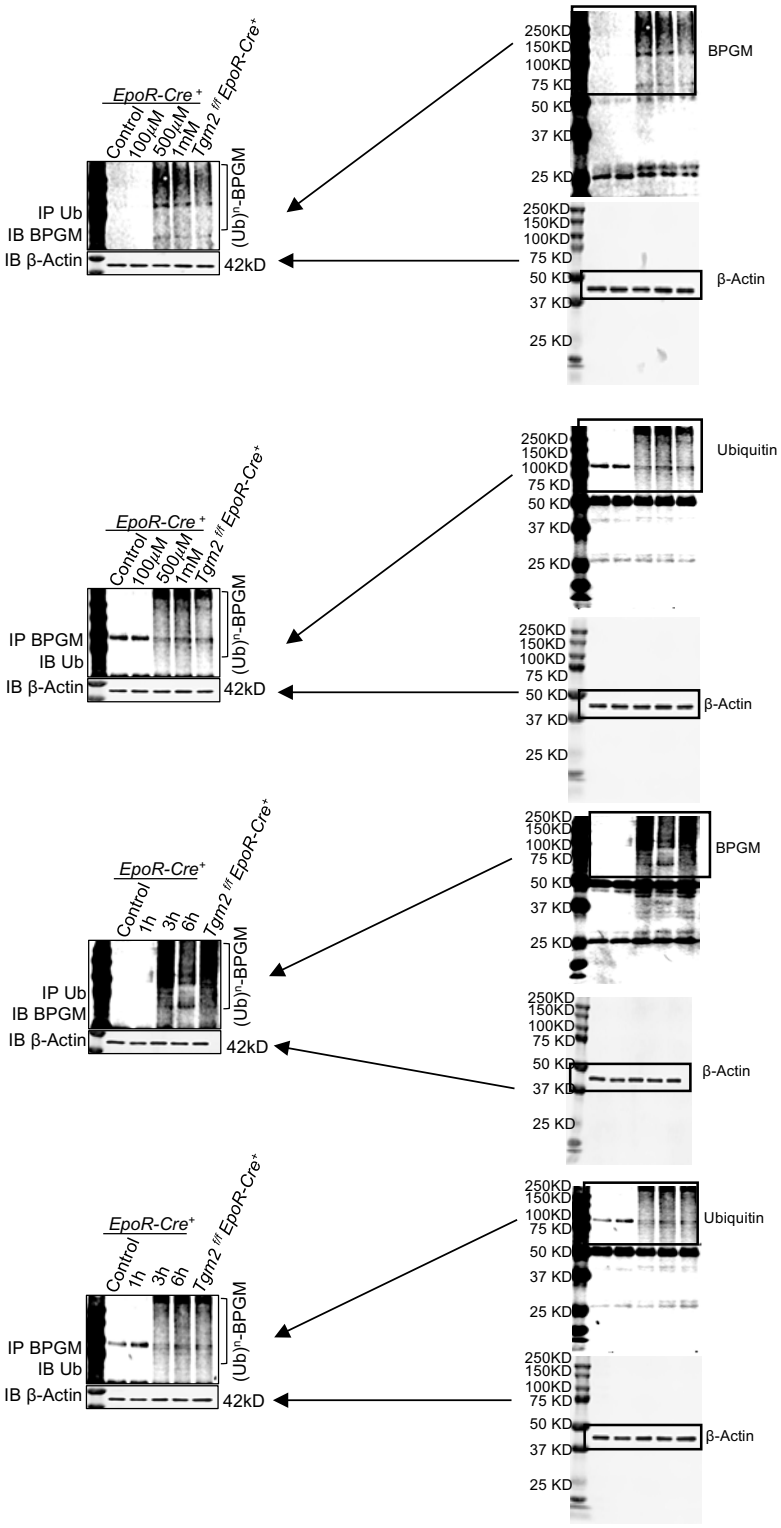

EpoR-Cre+
Control
100μM
500μM
1mM
Tgm2 f/f EpoR-Cre+
IP Ub
IB BPGM
IB β-Actin
(Ub)n-BPGM
42kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
BPGM
β-Actin
EpoR-Cre+
Control
100μM
500μM
1mM
Tgm2 f/f EpoR-Cre+
IP BPGM
IB Ub
IB β-Actin
(Ub)n-BPGM
42kD
Ubiquitin
β-Actin
EpoR-Cre+
Control
1h
3h
6h
Tgm2 f/f EpoR-Cre+
IP Ub
IB BPGM
IB β-Actin
(Ub)n-BPGM
42kD
BPGM
β-Actin
EpoR-Cre+
Control
1h
3h
6h
Tgm2 f/f EpoR-Cre+
IP BPGM
IB Ub
IB β-Actin
(Ub)n-BPGM
42kD
Ubiquitin
β-Actin

Fig 3b

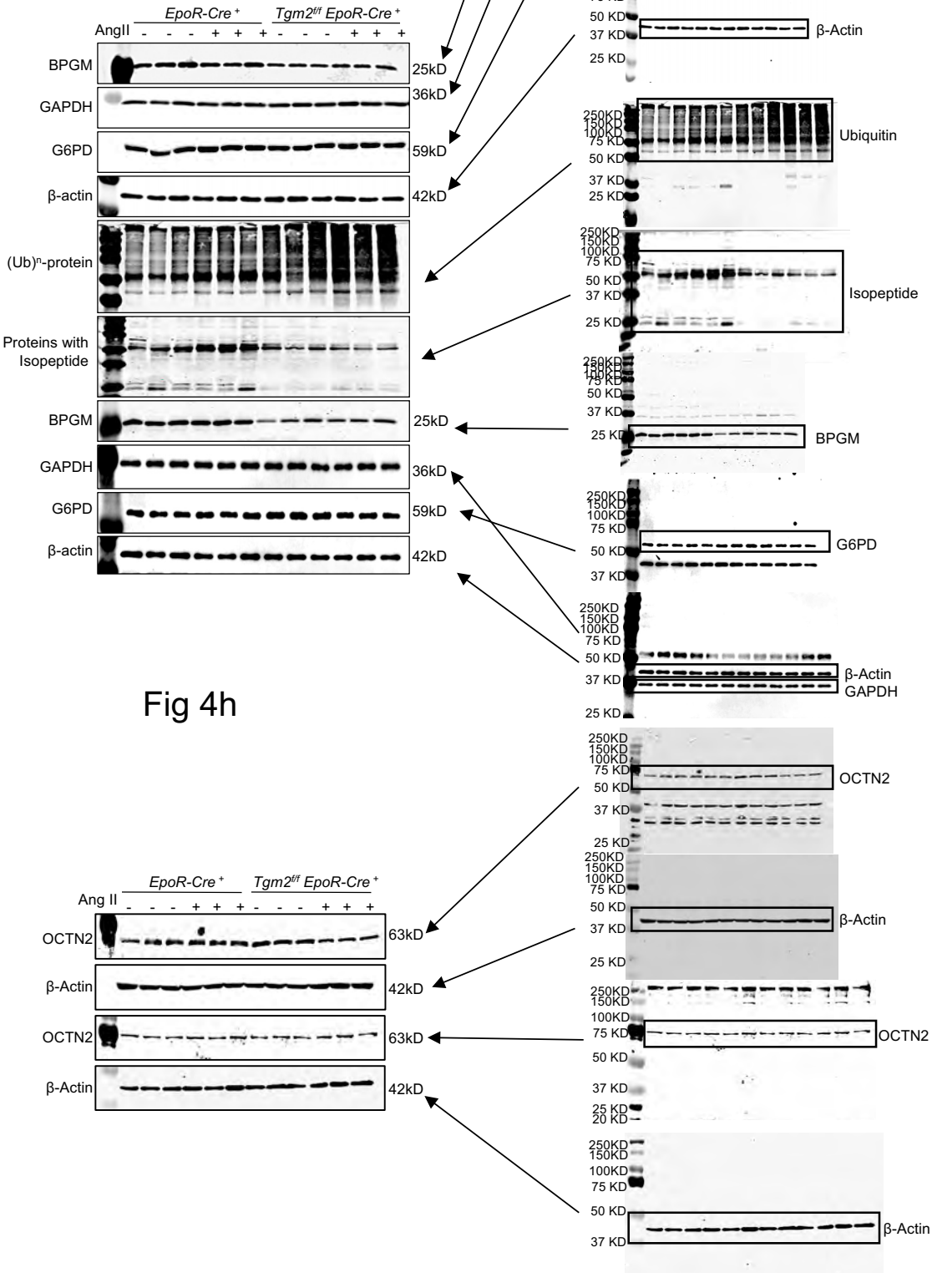
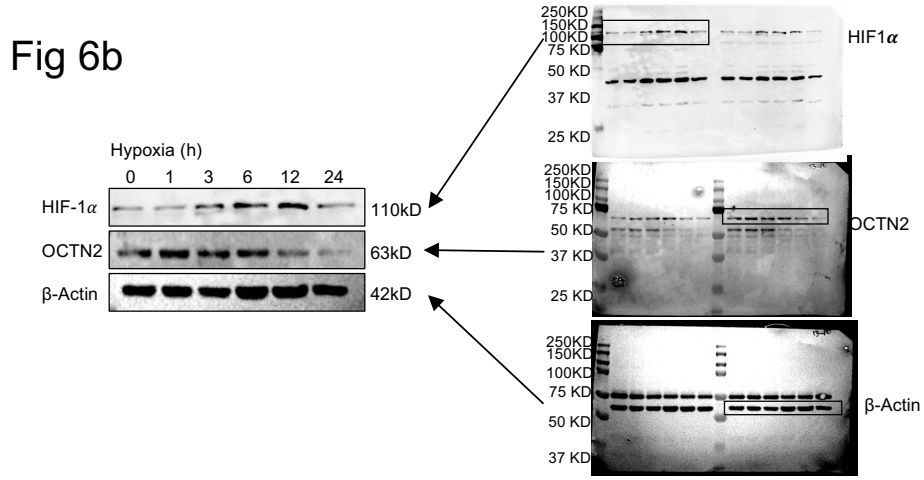

EpoR-Cre+
Tgm2f/f EpoR-Cre+
AngII
BPGM
GAPDH
G6PD
β-actin
(Ub)n-protein
Proteins with Isopeptide
25kD
36kD
59kD
42kD
Ubiquitin
Isopeptide
β-Actin

Fig 4h

EpoR-Cre+
Tgm2f/f EpoR-Cre+
Ang II
OCTN2
β-Actin
63kD
42kD

Fig 6b


Hypoxia (h)
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HIF-1α
OCTN2
β-Actin
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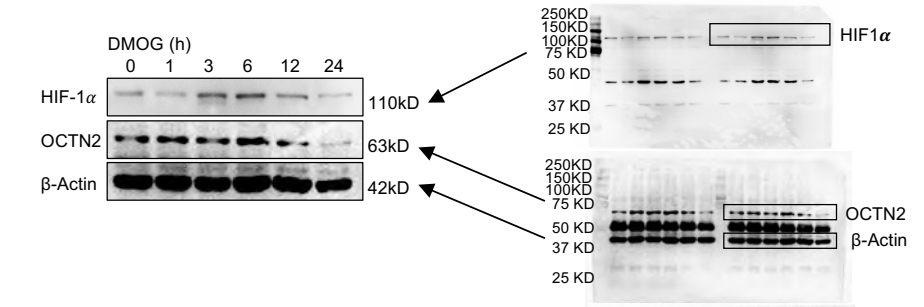
63kD
42kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
HIF1α
OCTN2
β-Actin


DMOG (h)
0 1 3 6 12 24
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OCTN2
β-Actin
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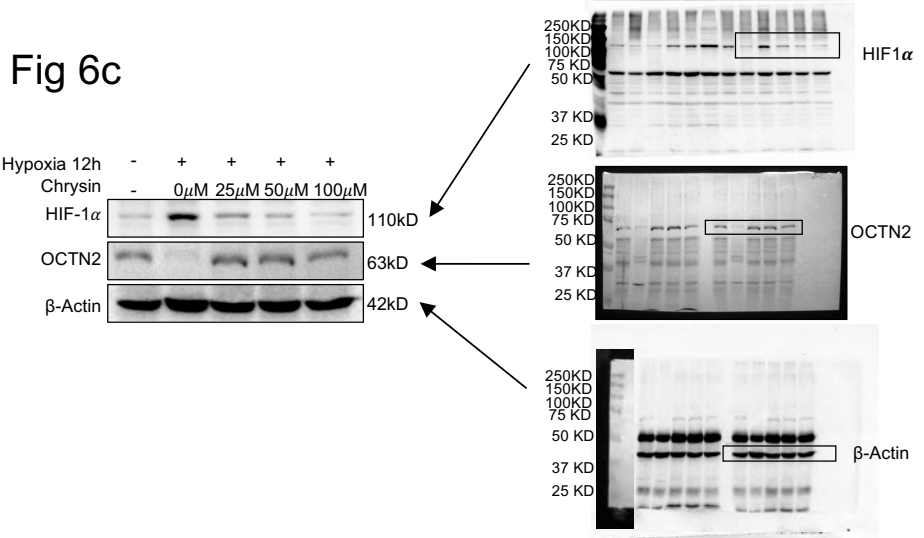
75 KD
50 KD
37 KD
25 KD
HIF1α
OCTN2
β-Actin

Fig 6c


Hypoxia 12h - + + + +
Chrysin - 0μM 25μM 50μM 100μM
HIF-1α
OCTN2
β-Actin
110kD
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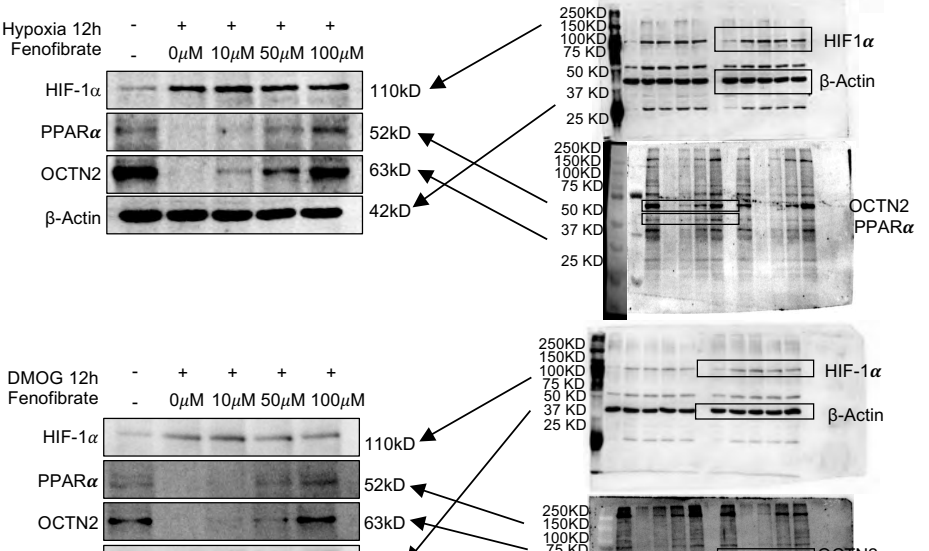
42kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
HIF1α
OCTN2
β-Actin


Hypoxia 12h - + + + +
Fenofibrate - 0μM 10μM 50μM 100μM
HIF-1α
PPARα
OCTN2
β-Actin
110kD
52kD
63kD
42kD
250KD
150KD
100KD
75 KD
50 KD
37 KD
25 KD
HIF1α
β-Actin
OCTN2
PPARα

DMOG 12h - + + + +
Fenofibrate - 0μM 10μM 50μM 100μM
HIF-1α
PPARα
OCTN2
β-Actin
110kD
52kD
63kD
42kD
250KD
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100KD
75 KD
50 KD
37 KD
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β-Actin
OCTN2
PPARα

Fig 7b

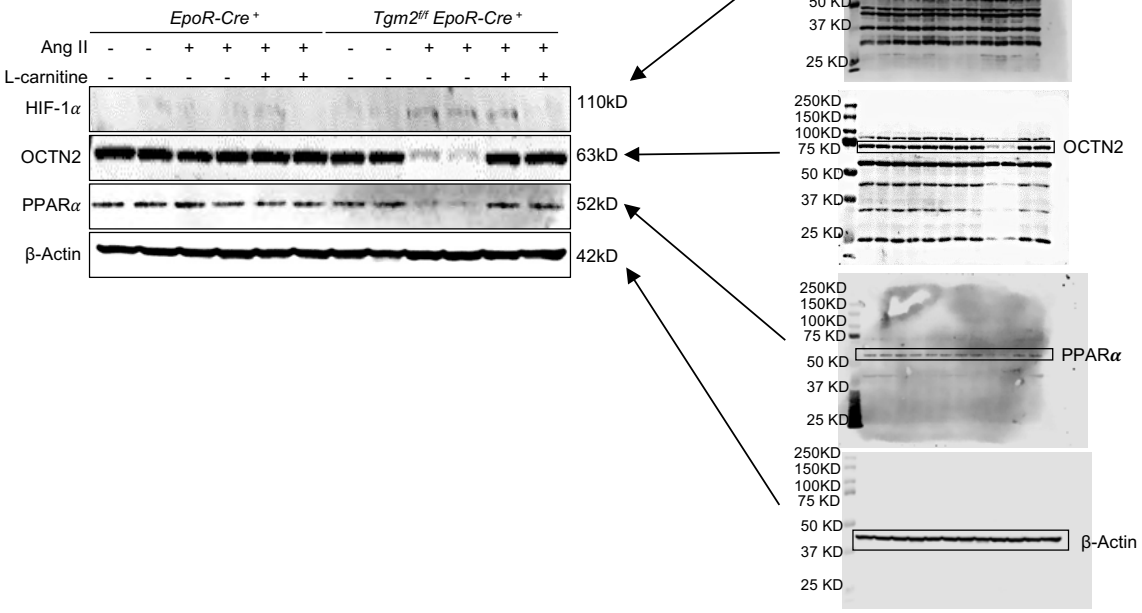


Fig 7d

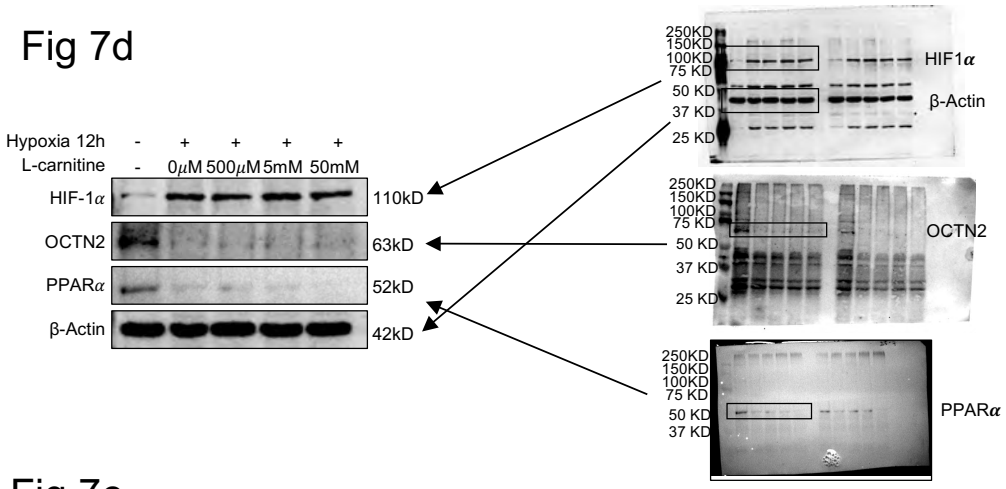


Fig 7e

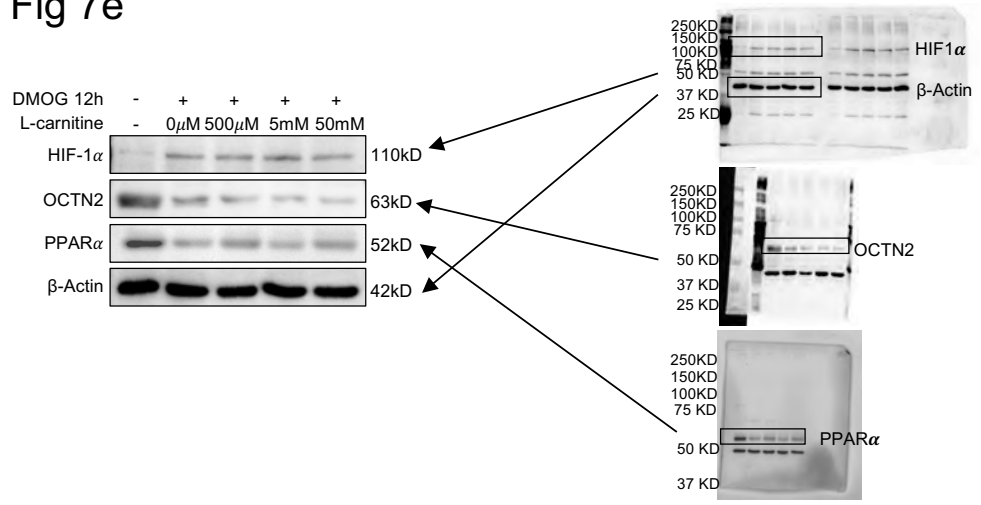
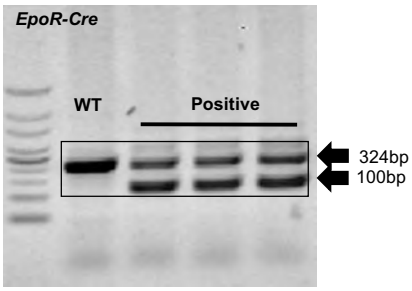
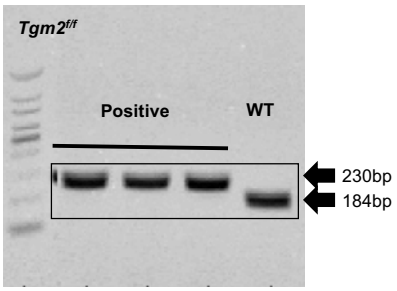


Fig S1a



Amplicon size: WT 324bp; Cre 100bp

WT for negative control



Amplicon size: WT 184; Flox 230bp

WT for negative control

Fig S1b

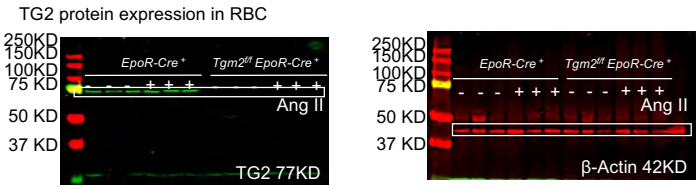


Fig S1c