Legends for supplementary information

Table 1: Complete list of primer sequences used for quantitative RT-PCR for all genes assessed in this study.

Fig. S1. (A) Gene expression microarray and GSEA analyses of parental $MCF7^{neo}$ cells expressing an empty plasmid encoding the neomycin resistance cassette compared with MCF^{10X} which expresses elevated levels of SOD2. (B) Quantification of Western blot images in Fig. 1A. (C) Quantification of Western blot images in Fig. 1B. (D) Quantitative RT- QPCR for HIF2α mRNA level in $MCF7^{neo}$, $MCF7^{6X}$, and $MCF7^{10X.}$ Errors represent ±SD. **$p < 0.01$.

Fig. S2. (A) Verification of differential gene expression shown in Fig. S1 was performed by RT-QPCR in $MCF7^{neo}$, $MCF7^{6X}$, and $MCF7^{10X}$ cells. Genes from each of the gene sets were selected randomly base only on expression levels detected using microarray analysis. *$p<0.05$, **$p<0.01$, and were compared to $MCF7^{neo}$. CDKN2D encodes a member of INK4 family of inhibitors of cyclin-dependent kinases that has been shown to be involved in oxidative stress induced senescence. PDLIM1 is involved in assembly, disassembly and direction of stress fiber formation in fibroblasts. PFKP generates fructose 1,6-bisphosphate and has key roles in the regulation of the glycolytic flow. SNAI2 encodes a member of the Snail family of C2H2-type zinc finger transcription factors which represses E-cadherin expression and contributes to EMT. TGFBI is a TGFβ induced gene encoding matrix protein remodeling proteins. GLRX is a gene induced by hypoxia that encodes for glutaredoxin, an enzyme involved in glutathione recycling (B) Western blot analysis of Akt phosphorylation levels used in this experiment as a proxy of PTEN activity. (C) Western blot analysis of PTEN expression and oxidation status. Results indicate that there is not significant differences in the total PTEN protein levels or PTEN oxidation status in $MCF7^{6X}$ and $MCF7^{10X}$ compared to $MCF7^{neo}$.

Fig. S3. Representative flow cytometry plots of the SORE6+ (GFP+, cancer stem cell) subpopulation in parental $MCF7^{neo}$ and $MCF7^{10X}$ cell cultures.

Fig. S4. SOD2 overexpression leads to the accumulation of Ac-SOD2 and an increase in $CD44^{+}/CD24^{-}$ CSC subpopulation. Western blot analysis shows that overexpressing SOD2 in T47D (A) or in ZR75-1 (B) cells leads to upregulation of HIF2α and an increase in the levels of SOD2-K68 acetylation. FACS analysis of $CD44^{+}/CD24^{-}$ expression on the surface of paraformaldehyde fixated cells in T47D (C) and ZR75-1 (D) cells overexpressing SOD2 (OE). N=3, errors represent ±SD .*$P<0.05$.

Fig. S5. (A) Quantification of Western blot images shown in Fig. 3E. (B) Effect of expressing SOD2 acetylation mimetic mutant (K68Q) on SORE6+ subpopulation in MCF7 breast cancer cell cultures. Stem cell population was measured by the genetically encoded SORE6/GFP transfected into MCF7 cells expressing wild type SOD2, SOD2 K68R, or SOD2 K68Q mutation. N=3. Errors represent ±SD. *$p < 0.05$. (C) Western Blot for SOD2K68Ac, SOD2, HIF2α in PyVT mouse tumors compared to non-carrier FVB mouse mammary glands.

Fig. S6. Ingenuity IPA pathway analysis of activated signaling pathways in MCF7 cells expressing SOD2 K68Q vs SOD2 K68R. The red or green arrows indicate the genes that are up or down regulated ($|\log2| > 1$) in SOD2K68Q cells compared with SOD2K68R cells.

Fig. S7. Sirtuin-3 knockdown increases SOD2 K68 acetylation level and HIF2α in MCF7 cells. (A) Western blot of $SOD2^{K68Ac}$ and HIF2α in MCF7 cells with Sirtuin-3 knockdown. (B) Quantification of $SOD2^{K68Ac}$ and HIF2α in (A). (C) Extracellular H_2O_2 accumulation was determined by Amplex Red fluorescence assay. Data were pooled from two independent experiments, N=12. Errors represent ±SD. **$p < 0.01$. (D) H_2O_2 generated in mitochondria was assessed using the probe 3-(Dihydroxyboronyl)Benzyltriphenylphosphonium bromide (MitoB). MitoB and the product of its reaction with H_2O_2 MitoP were quantified using LC-MS, N=4. Errors represent ±SD. *$p < 0.05$. (E) knocking down of the mitochondrial acetyl transferase GCN5L1 leads to reduced SOD2 acetylation levels (F). (G) Quantification $SOD2^{K68Ac}$ normalized on total SOD2 after both are normalized by B-actin in (F). Effect of GCN5L1 knockdown on the expression of Oct4 (H) and Nanog (I) mRNA expression as well as the number of SORE6+ cells (J). N=3. Errors represent ±SD. *$p < 0.05$ and **$p < 0.01$.

Fig. S8. (A) Catalase activity assay measured using a kit by BioVision according to the manufacturer's instructions and (B) Invasion assay (pooled two experiments, with three biological replicates each, N=6) for $MCF7^{10X}$ cells treated with PEG-catalase or transfected with mito-catalase. (C) Colony formation assay stained with Nitroblue for $MCF7^{10X}$ cells treated with 10μM of either Euk8 or Euk134 for 21 days. (D) Quantification of colonies from the experiments shown in (C), performed by counting colonies using a inverted microscope, N=6. (E) Quantification of Oct4 mRNA expression as well as (F) Nanog mRNA level by RT-QPCR in cells treated with Euk8 or Euk8 compared to controls. (G) Effect of Prx3 or (H) Gpx4 overexpression on the levels of Oct4 (I) and Nanog (J) mRNA expression. Representative of two independent experiments with 3 biologic replicates each. (K) GSH/GSSH level was measured in $MCF7^{10X}$ cells compared to control cells. Errors represent ±SD. *$p < 0.05$ and **$p < 0.01$.

Table 1: primer sequences used for quantitative RT-PCR

Gene names	Primer sequences
ADAM12-F	ATGGCGGTTGAGAAAGGAGG
ADAM12-R	TCTGTTCCCACACTTCTGGC
CDKND2-F	TGTCGACATGCTGCTGGAG
CDKND2-R	GGGTGTCCAGGAATCCAGTG
GAPDH-F	ATGACATCAAGAAGGTGGTGAAG
GAPDH-R	CCTGTTGCTGTAGCCAAATTC
GCN5L1-F	GTCCCGCCTCCTAAAAGAAC
GCN5L1-R	GACCTGTAGGGTCTTCACCTCAT
GLRX-F	CCTGGGAAGGTGGTTGTGTT
GLRX-R	GTGGTTACTGCAGAGCTCCA
GPX4-F	GTAACCAGTTCGGGAAGCAG
GPX4-R	GGGTTGGATCTTCATCCACTT
HIF1α F	GACTTGCCTTTCCTTCTCTTCTC
HIF1α R	TTATGTGGAAGTGGCAACTGAT
Hif2α-F	GGAGACGGAGGTGTTCTATGAG
Hif2α-R	CTCCAAGGCTTTCAGGTACAAG
NANOG-F	AGATGCAAGAACTCTCCAACATC
NANOG-R	AAGAGTAAAGGCTGGGGTAGGTAG
Oct4-F	CAGTATCGAGAACCGAGTGAGAG
Oct4-R	AAAATCCTCTCGTTGTGCATAGTC
PDLIM1-F	ACCACCCAGCAGATAGACCT
PDLIM1-R	TCAAGTTGTCTGTGCAGCCT
PFKP-F	GTTCCCGCTACTGTGTCCAA
PFKP-R	GCCCATGGTCTCGATGATGA
PRDX3-F	GACATGTGAGTGCCATTCCTT
PRDX3-R	CTCCATTGACAACGGCTGTA
Sirt3 F1	AAAGCCTAGTGGAGCTTCTGG
Sirt3 R1	ATTGGGATGTGGATGTCTCCTAT
SNAI2-F	AGCGAACTGGACACACATACA
SNAI2-R	ACTCACTCGCCCCAAAGATG
SOD2-F	ACCGAGGAGAAGTACCAGGAG
SOD2-R	ACTTGTCAAAGGAACCAAAGTCAC
TGFBI-F	CTCCCTGGTCAGCAATGTCA
TGFBI-R	CCGGGCACAGTTCACAGTTA

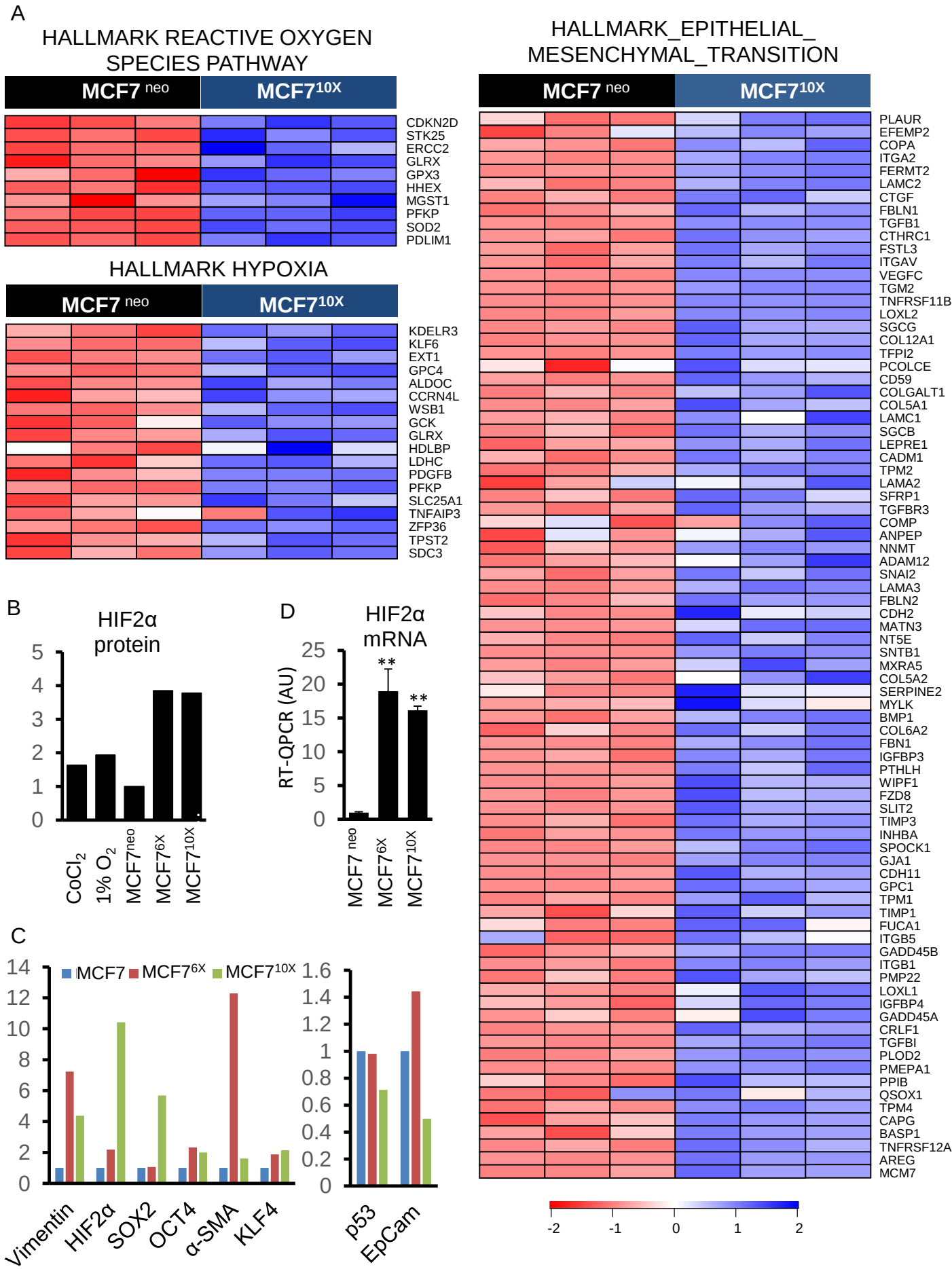

A
HALLMARK REACTIVE OXYGEN SPECIES PATHWAY
MCF7 neo
MCF7 10X
CDKN2D
STK25
ERCC2
GLRX
GPX3
HHEX
MGST1
PFKP
SOD2
PDLIM1
HALLMARK HYPOXIA
MCF7 neo
MCF7 10X
KDELR3
KLF6
EXT1
GPC4
ALDOC
CCRN4L
WSB1
GCK
GLRX
HDLBP
LDHC
PDGFB
PFKP
SLC25A1
TNFAIP3
ZFP36
TPST2
SDC3
HALLMARK_EPITHELIAL_ MESENCHYMAL_TRANSITION
MCF7 neo
MCF7 10X
PLAUR
EFEMP2
COPA
ITGA2
FERMT2
LAMC2
CTGF
FBLN1
TGFB1
CTHRC1
FSTL3
ITGAV
VEGFC
TGM2
TNFRSF11B
LOXL2
SGCG
COL12A1
TFPI2
PCOLCE
CD59
COLGALT1
COL5A1
LAMC1
SGCB
LEPRE1
CADM1
TPM2
LAMA2
SFRP1
TGFBR3
COMP
ANPEP
NNMT
ADAM12
SNAI2
LAMA3
FBLN2
CDH2
MATN3
NT5E
SNTB1
MXRA5
COL5A2
SERPINE2
MYLK
BMP1
COL6A2
FBN1
IGFBP3
PTHLH
WIPF1
FZD8
SLIT2
TIMP3
INHBA
SPOCK1
GJA1
CDH11
GPC1
TPM1
TIMP1
FUCA1
ITGB5
GADD45B
ITGB1
PMP22
LOXL1
IGFBP4
GADD45A
CRLF1
TGFBI
PLOD2
PMEPA1
PPIB
QSOX1
TPM4
CAPG
BASP1
TNFRSF12A
AREG
MCM7
-2
-1
0
1
2
B
HIF2α protein
5
4
3
2
1
0
CoCl2
1% O2
MCF7neo
MCF7 6X
MCF7 10X
D
HIF2α mRNA
RT-QPCR (AU)
25
20
15
10
5
0
**
**
MCF7 neo
MCF7 6X
MCF7 10X
C
MCF7
MCF7 6X
MCF7 10X
14
12
10
8
6
4
2
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Vimentin
HIF2α
SOX2
OCT4
α-SMA
KLF4
p53
EpCam

Figure S1

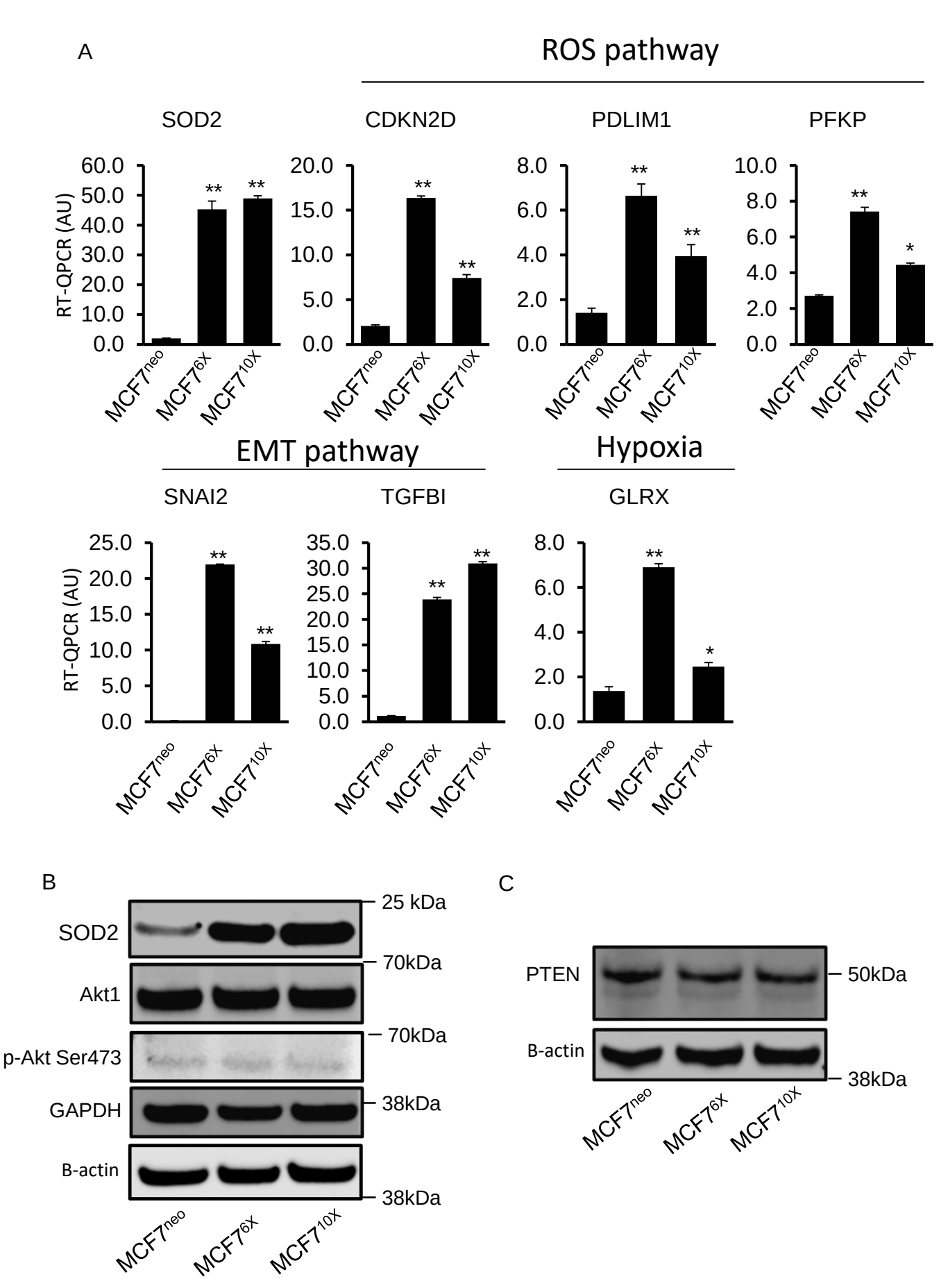

A
ROS pathway
SOD2
CDKN2D
PDLIM1
PFKP
RT-QPCR (AU)
MCF7neo
MCF76X
MCF710X
EMT pathway
Hypoxia
SNAI2
TGFBI
GLRX
B
SOD2
Akt1
p-Akt Ser473
GAPDH
B-actin
25 kDa
70kDa
70kDa
38kDa
38kDa
C
PTEN
B-actin
50kDa
38kDa

Figure S2

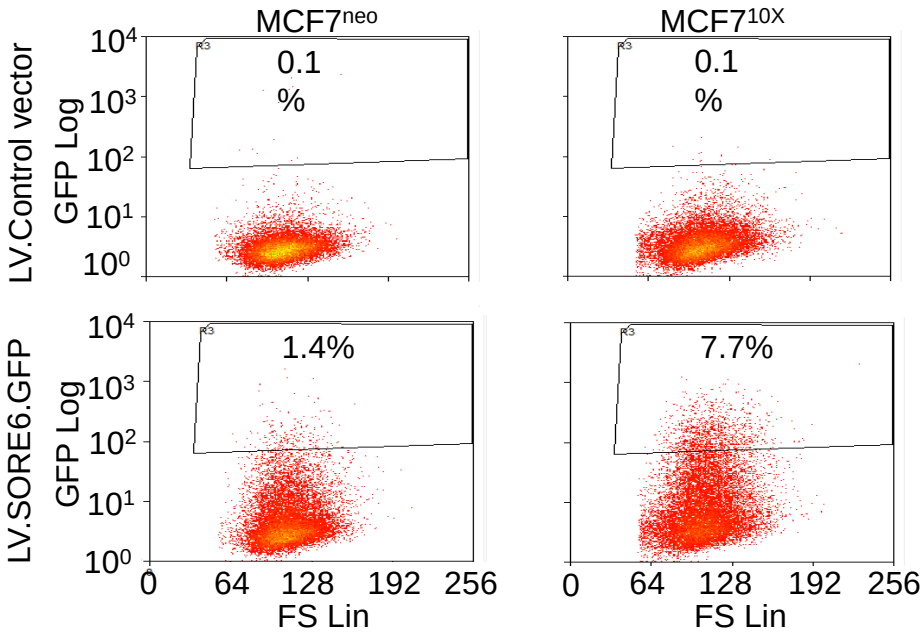

MCF7neo
MCF710X
LV.Control vector
LV.SORE6.GFP
GFP Log
0.1 %
0.1 %
1.4%
7.7%
R3
10^4
10^3
10^2
10^1
10^0
0
64
128
192
256
FS Lin

Figure S3

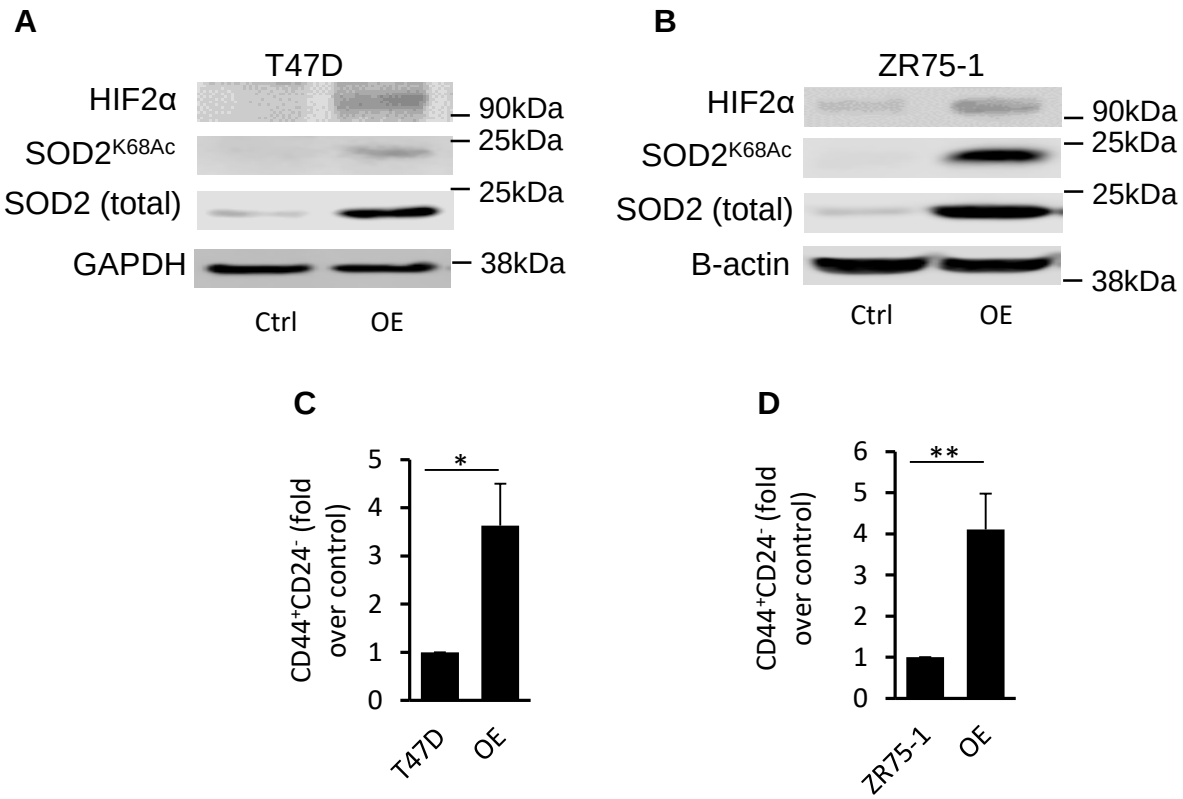

A
T47D
HIF2α
SOD2K68Ac
SOD2 (total)
GAPDH
90kDa
25kDa
25kDa
38kDa
Ctrl
OE
B
ZR75-1
HIF2α
SOD2K68Ac
SOD2 (total)
B-actin
90kDa
25kDa
25kDa
38kDa
Ctrl
OE
C
CD44+CD24- (fold over control)
*
T47D
OE
D
CD44+CD24- (fold over control)
**
ZR75-1
OE

Figure S4

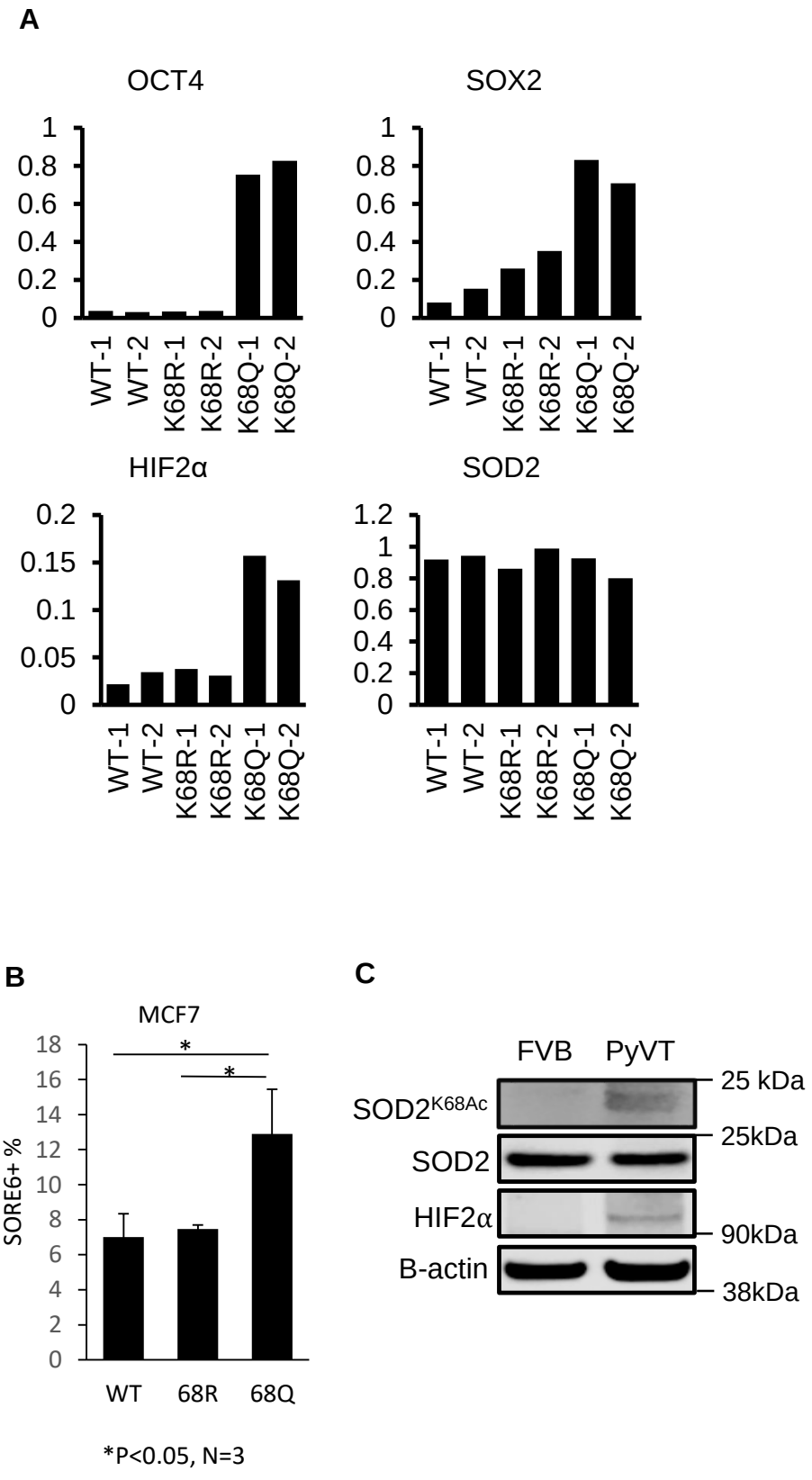
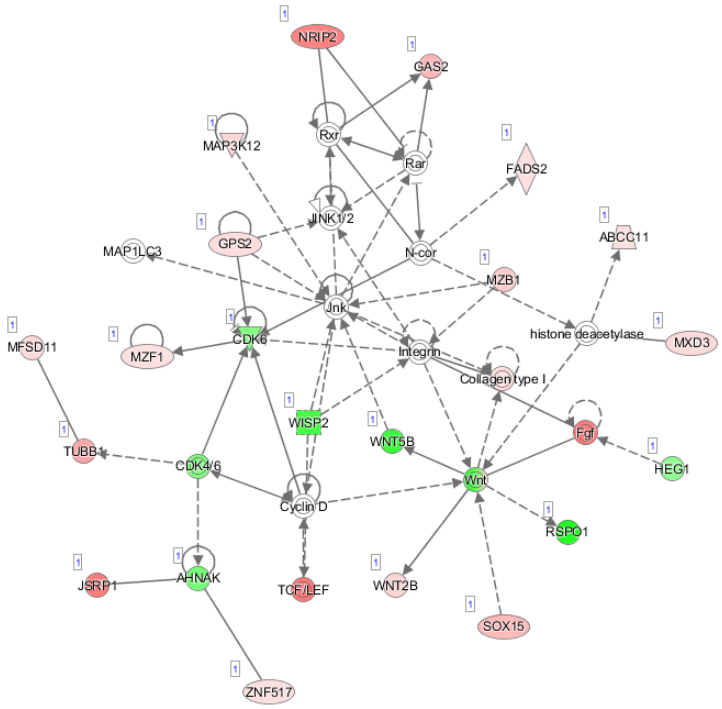

A
OCT4
SOX2
HIF2α
SOD2
WT-1
WT-2
K68R-1
K68R-2
K68Q-1
K68Q-2
B
MCF7
SORE6+ %
WT
68R
68Q
*P<0.05, N=3
C
FVB
PyVT
SOD2K68Ac
SOD2
HIF2α
B-actin
25 kDa
25kDa
90kDa
38kDa

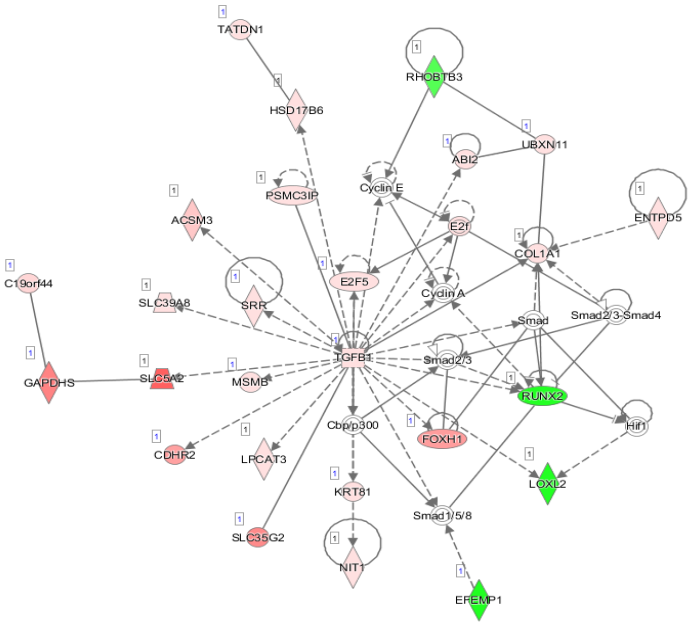
Figure S5

Cancer



ABCC11↑	AHNAK↓	CDK4/6	CDK6↓
Collagen type I	Cyclin D	FADS2↑	Fgf
GAS2↑	GPS2↑	HEG1↓	histone deacetylase
Integrin	JINK1/2	Jnk	JSRP1↑
MAP 1LC3	MAP3K12↑	MFSD11↑	MXD3↑
MZB1↑	MZF1↑	N-cor	NRIP2↑
Rar	RSPO1↓	Rxr	SOX15↑
TCF/LEF	TUBB1↑	WISP2↓	Wnt
WNT2B↑	WNT5B↓	ZNF517↑	

Cellular development



ABI2↑	ACSM3↑	C19orf44↑	Cbp/p300
CDHR2↑	COL1A1↑	Cyclin A	Cyclin E
E2f	E2F5↑	EFEMP1↓	ENTPD5↑
FOXH1↑	GAPDHS↑	Hif1	HSD17B6↑
KRT81↑	LOXL2↓	LPCAT3↑	MSMB↑
NIT1↑	PSMC3IP↑	RHOBTB3↓	RUNX2↓
SLC35G2↑	SLC39A8↑	SLC5A2↑	Smad
Smad1/5/8	Smad2/3	Smad2/3-Smad4	SRR↑
TATDN1↑	TGFB1↑	UBXN11↑	

Figure S6

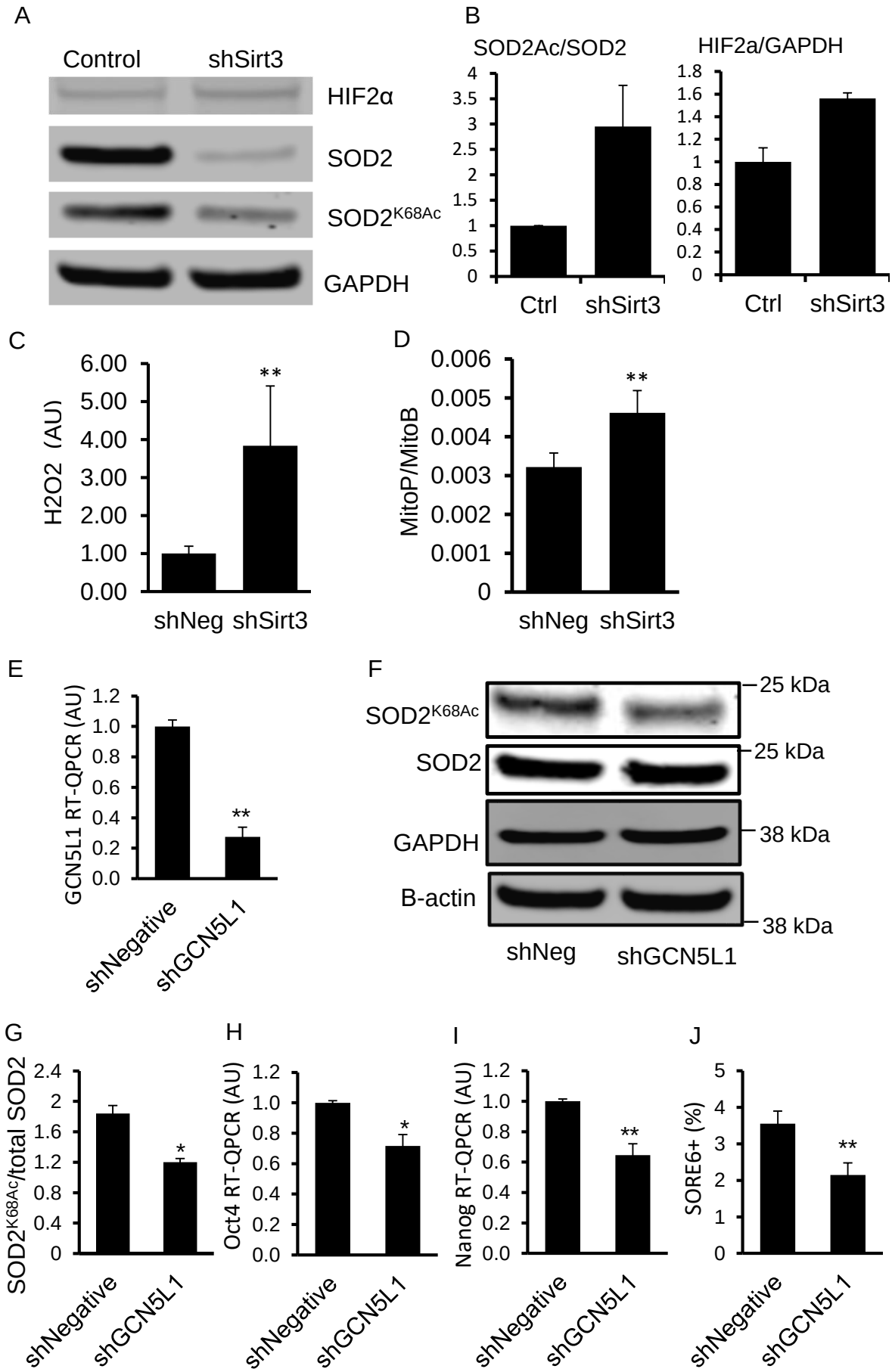

A
Control
shSirt3
HIF2α
SOD2
SOD2K68Ac
GAPDH
B
SOD2Ac/SOD2
HIF2a/GAPDH
Ctrl
shSirt3
C
H2O2 (AU)
shNeg
shSirt3
D
MitoP/MitoB
E
GCN5L1 RT-QPCR (AU)
shNegative
shGCN5L1
F
25 kDa
38 kDa
B-actin
G
SOD2K68Ac/total SOD2
H
Oct4 RT-QPCR (AU)
I
Nanog RT-QPCR (AU)
J
SORE6+ (%)

Figure S7

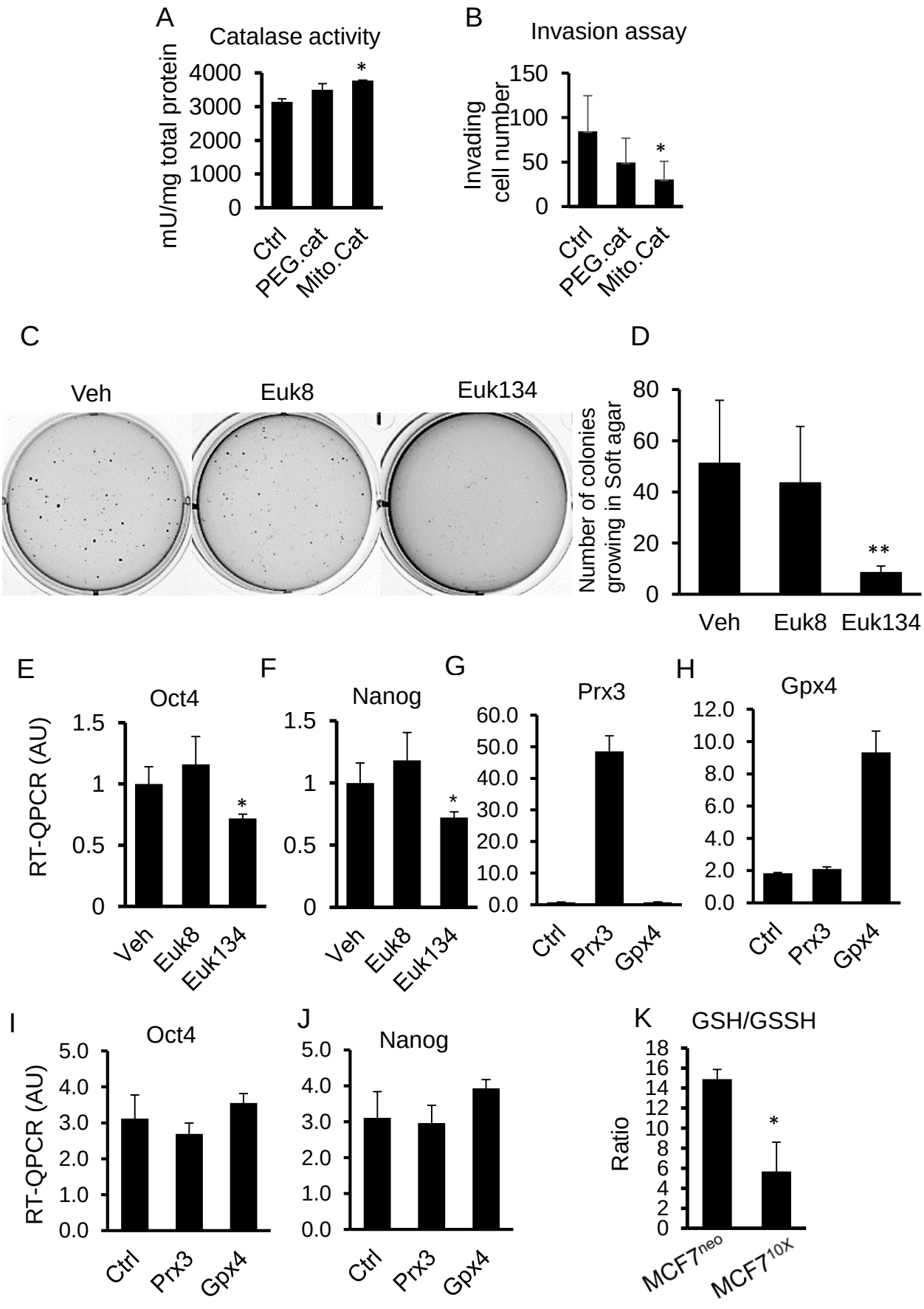

A
Catalase activity
mU/mg total protein
4000
3000
2000
1000
0
*
Ctrl
PEG.cat
Mito.Cat
B
Invasion assay
Invading cell number
150
100
50
0
*
Ctrl
PEG.cat
Mito.Cat
C
Veh
Euk8
Euk134
D
Number of colonies growing in Soft agar
80
60
40
20
0
**
Veh
Euk8
Euk134
E
Oct4
RT-QPCR (AU)
1.5
1
0.5
0
*
Veh
Euk8
Euk134
F
Nanog
1.5
1
0.5
0
*
Veh
Euk8
Euk134
G
Prx3
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Ctrl
Prx3
Gpx4
H
Gpx4
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Ctrl
Prx3
Gpx4
I
Oct4
RT-QPCR (AU)
5.0
4.0
3.0
2.0
1.0
0.0
Ctrl
Prx3
Gpx4
J
Nanog
5.0
4.0
3.0
2.0
1.0
0.0
Ctrl
Prx3
Gpx4
K
GSH/GSSH
Ratio
18
16
14
12
10
8
6
4
2
0
*
MCF7neo
MCF710X

Figure S8