ISCI, Volume *15*

Supplemental Information

PBRM1 Regulates Stress Response in Epithelial Cells

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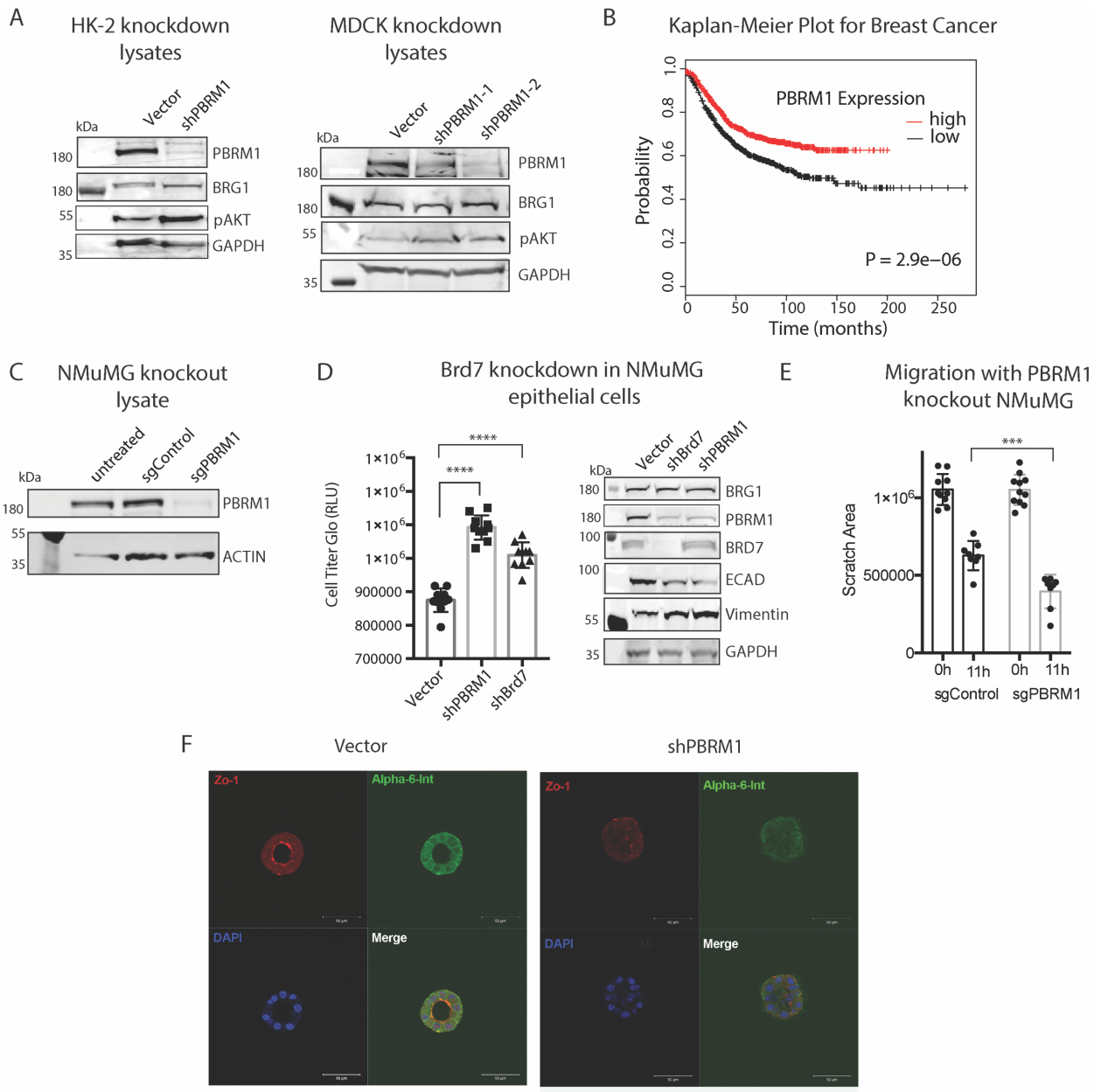
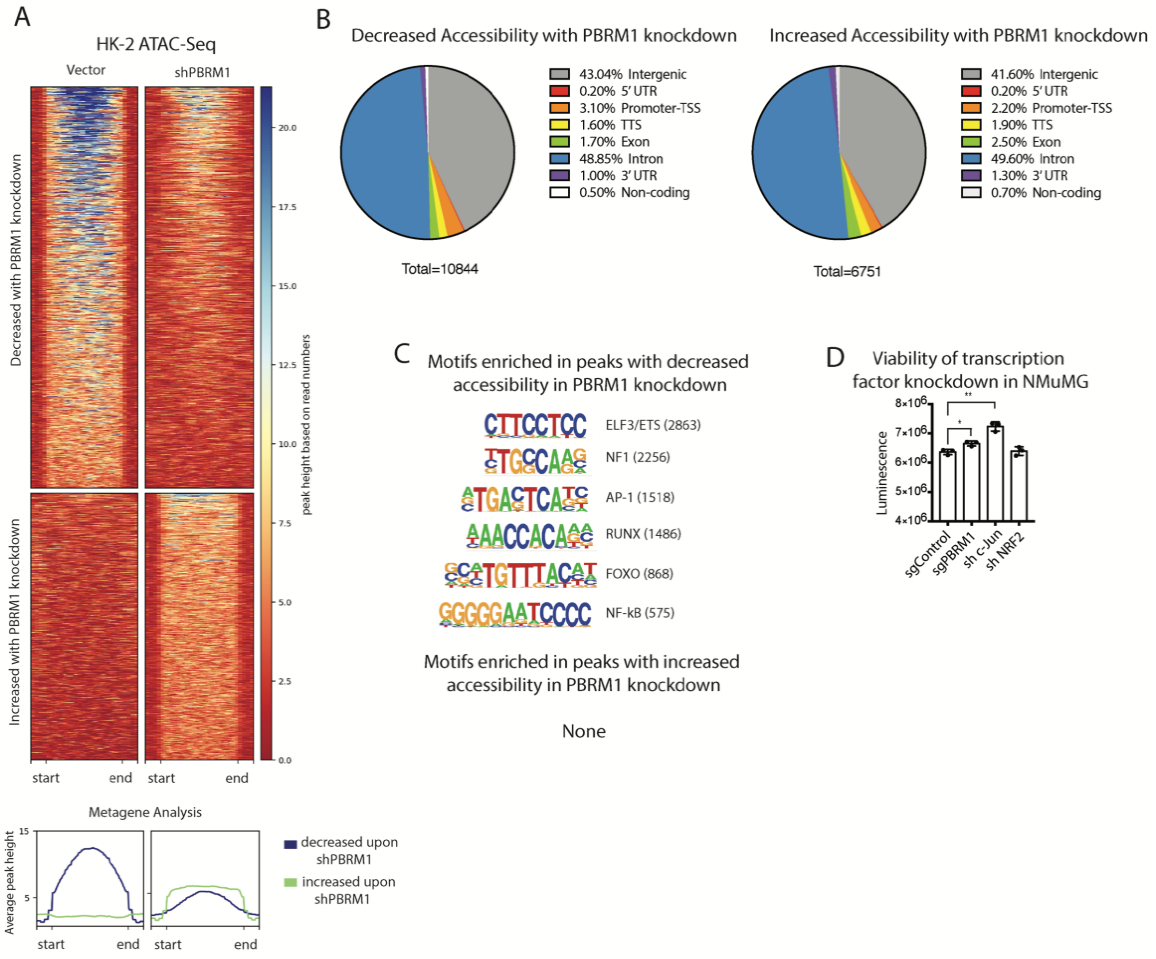
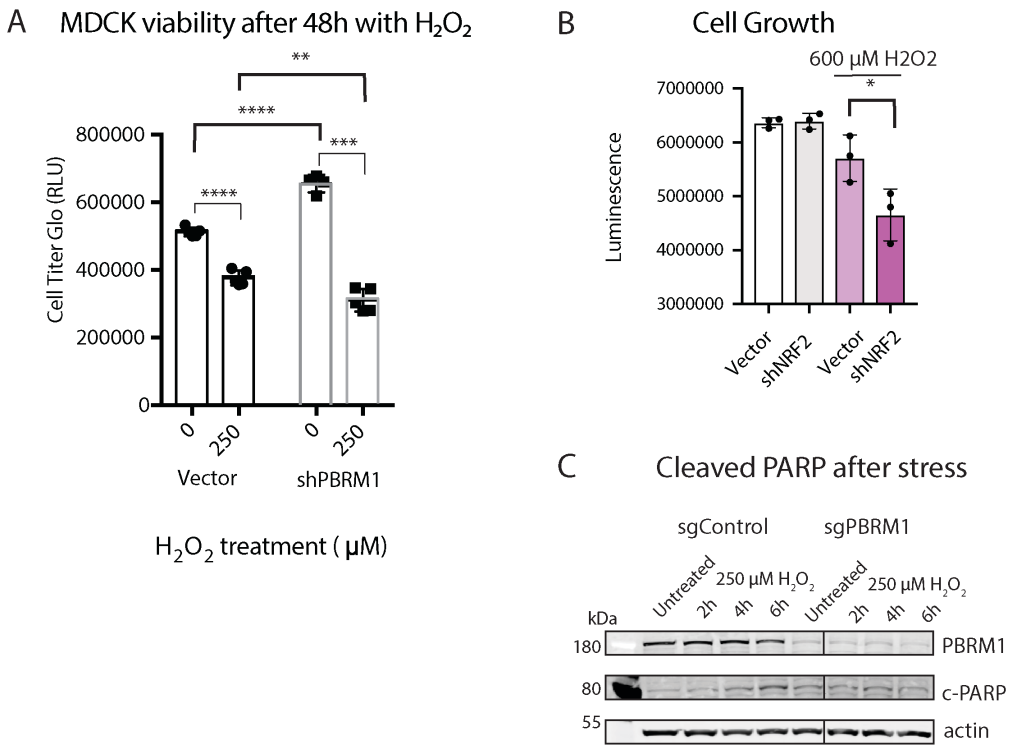


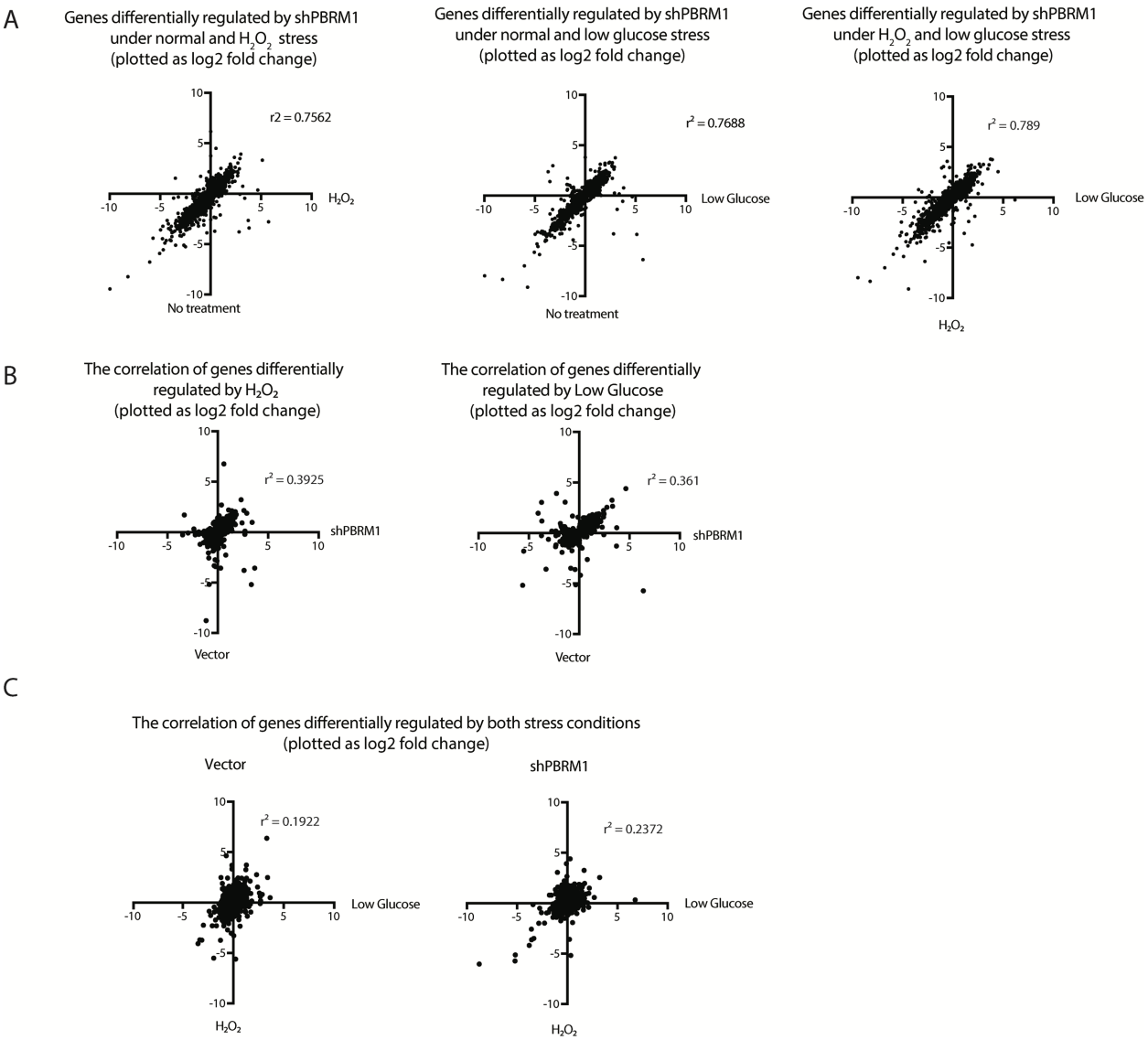
S1: Knockdown of PBRM1 in normal epithelium promotes growth and a loss of epithelial cell maintenance. Related to Figure 1. **A.** Immunoblots of whole cell lysates of epithelial cell lines HK-2 and MDCK with PBRM1 knockdown. **B.** Microarray analysis of breast cancer patients and PBRM1 expression in tumors predicts survival. **C.** Immunoblots of whole cell lysates of epithelial cell line NMuMG with PBRM1 knockout. **D.** NMuMG cells with PBRM1 or BRD7 knockdown were counted after 72h growth and presented as mean ± SD. n = 10. Immunoblot analysis of whole cell lysates from NMuMG cells indicates that PBRM1 or BRD7 knockdown results in decreased E-cadherin expression and increased vimentin expression. **E.** Migration differences between NMuMG control and PBRM1 knockout lines at 0h and 11h. **F.** Representative image of acini from Fig 1E, analyzed using immunofluorescence staining with anti-ZO1 (red) and anti-alpha-6-integrin (green). Nuclei (blue) were visualized by DAPI. *=p <0.05, ** = p <0.01, *** = p < 0.001, **** = p < 0.0001 (paired Student's t test). ns, not significant. Error bars represent S.D



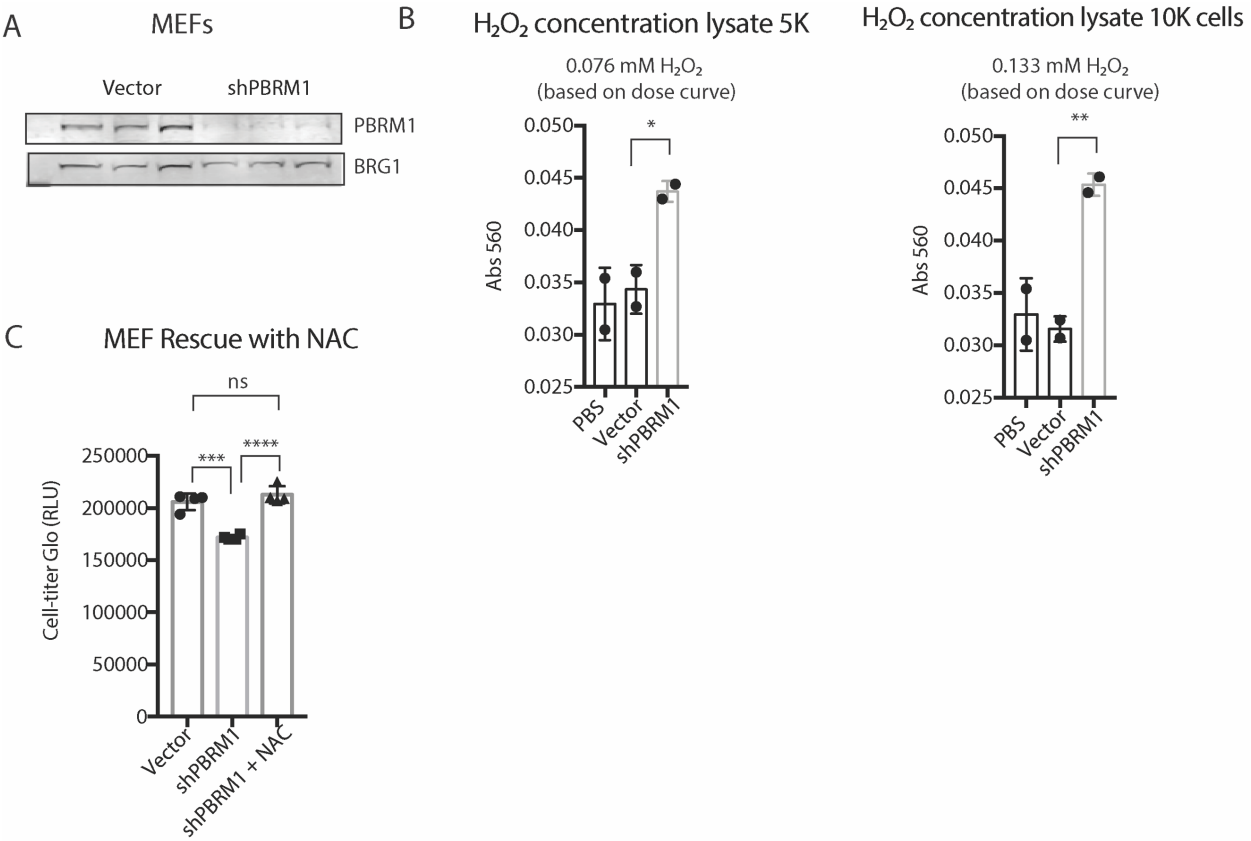
S2: PBRM1 is predicted to cooperate with transcription factors involved in response to stress Related to Figure 2. **A.** Heat maps and metagene plots of regions identified as differentially accessible upon PBRM1 knockdown by ATAC-Seq analysis of HK-2 cells. Regions of at least 1.5-fold differential accessibility were calculated between pooled samples of three biological replicates. **B.** Genomic elements associated with the differentially accessible peaks. The overall distribution was calculated as a percentage of the total differentially accessible regions for each condition. **C.** Motif analysis was performed using HOMER for the differentially accessible peaks. Statistically significant motifs were identified based on relative enrichment over genomic areas with similar AT content. **D.** NMuMG sgcontrol, sgPBRM1, shc-JUN and shNRF2 were cultured and counted after 48h growth. *=p <0.05, ** = p <0.01, *** = p < 0.001, **** = p < 0.0001 (paired Student's t test). Error bars represent S.D. n=3.



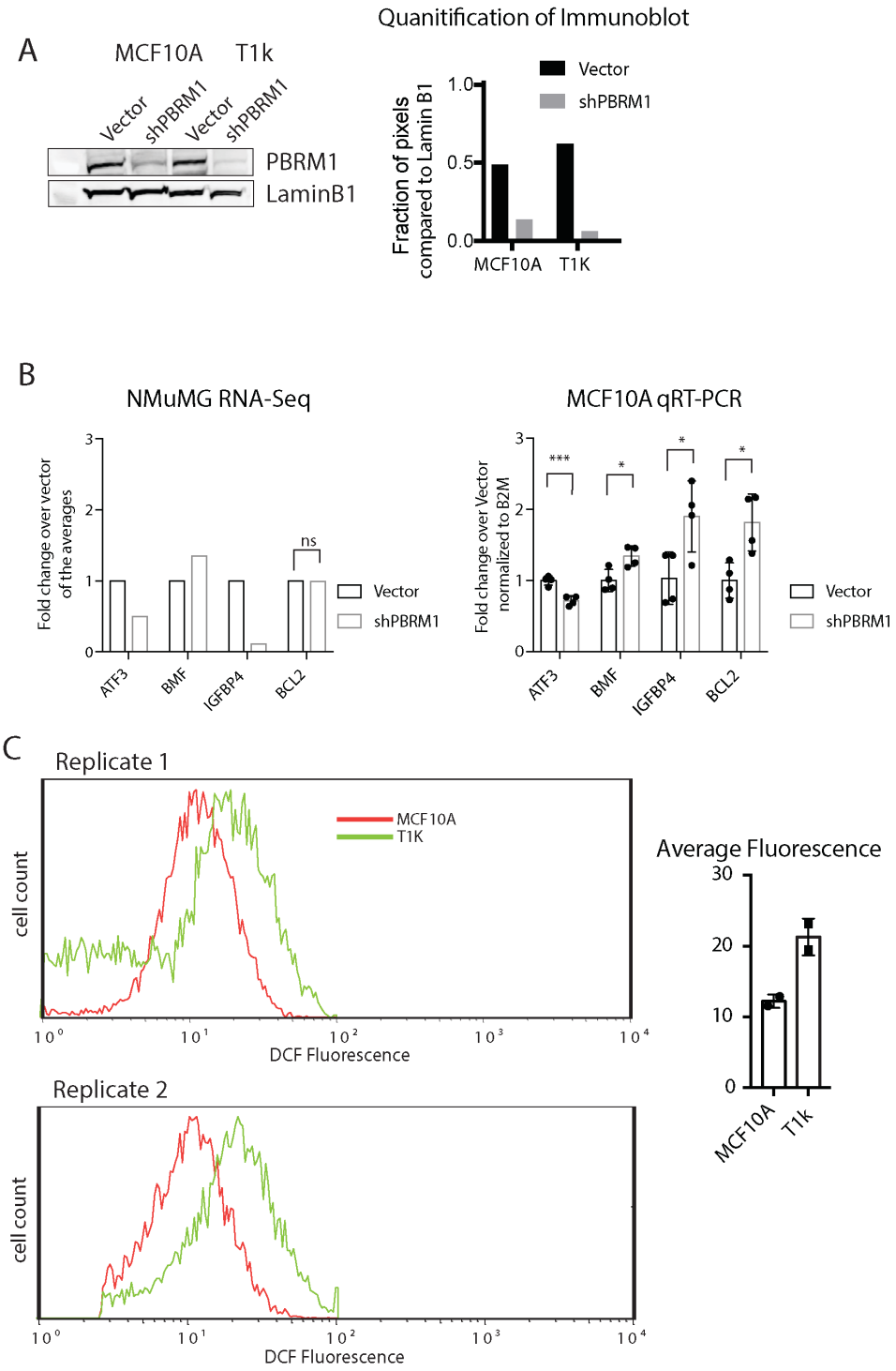
S3: PBRM1 expression is cytoprotective under high stress conditions. Related to Figure 4. **A**. MDCK cells were cultured in normal cell media or 250 µM H_2O_2 for 48 h and luminescence was measured using CellTiter-Glo® assay system and data presented as mean ± SD. n = 4. **B.** NMuMG cells were cultured in normal cell media or 600 µM H_2O_2 for 5 h and luminescence was measured. Data presented as mean ± SD. n = 3. **C.** Immunoblots of whole cell lysates from NMuMG cells treated with 250 µM H_2O_2 for 2h, 4h or 6h. All lanes are from same blot with one irrelevant lane spliced out. *=p <0.05, ** = p <0.01, *** = p < 0.001, **** = p < 0.0001 (paired Student's t test). ns, not significant. Error bars represent S.D.



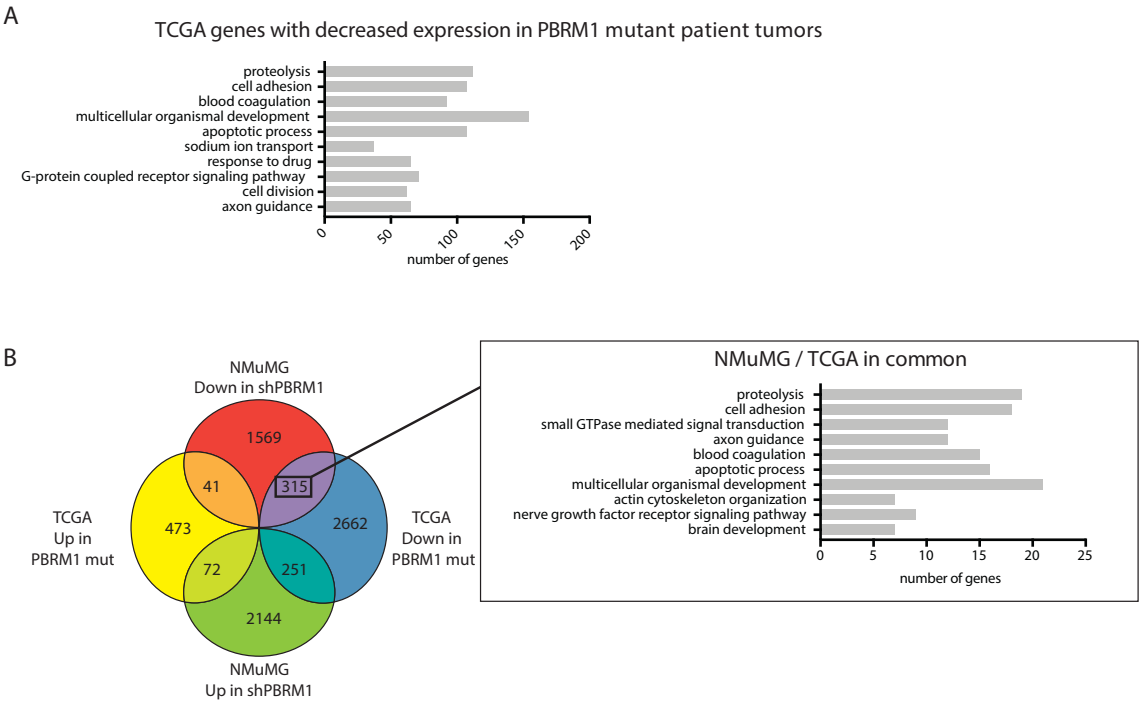
S4: PBRM1-regulated transcriptional effects under cellular stress conditions. Related to Figure 5 and Table 1. **A**. Each data point represents the log2 fold change expression value of a single gene, Left and Center plots- *x axis*: differential expression in H_2O_2 treated cells (left) or low glucose treated cells (center) upon shPBRM1, *y axis*: differential expression in normal cell culture conditions upon shPBRM1 (left and center); Right plot- *x axis:* differential expression under low glucose stress, *y axis:* differential expression under H_2O_2 stress. The degree of correlation was calculated using all differentially expressed genes. **B**. Each data point represents the log2 fold change expression value of a single gene upon 2h H_2O_2 treatment (left) or 6h low glucose treatment (right), *x axis*: differential expression in shPBRM1, *y axis*: differential expression in vector control cells. The degree of correlation was calculated using all differentially expressed genes. **C.** Each data point represents the log2 fold change expression value of a single gene upon 2h H_2O_2 treatment (y axis) or 6h low glucose treatment (x axis) of vector control cells (left) or shPBRM1 cells (right).



S5: PBRM1 has cell-type specific roles on viability. Related to Figure 6. **A**. PBRM1 knockdown in MEFs was analyzed using immunoblot analysis of nuclear lysates. **B**. Hydrogen peroxide levels were quantitated in MEFs using Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit. Whole cell lysates were generated from the indicated number of cells in 50 µL RIPA. **C**. MEFs were cultured for 3 days in normal media or media supplemented with 500 µM NAC. Luminescence was measured using CellTiter-Glo® assay system and data presented as mean ± SD. n = 4. *=p <0.05, ** = p <0.01, *** = p < 0.001, **** = p < 0.0001 (paired Student's t test). ns, not significant. Error bars represent S.D.



S6: PBRM1 has cell-type specific roles on viability. Related to Figure 6. **A.** PBRM1 knockdown in MCF10A and MCF10A-T1k cell lines was analyzed using immunoblot analysis of nuclear lysates. Quantification indicates the intensity of PBRM1 staining over LaminB1. **B.** Comparison of the expression changes of ATF3, BMF, IGFBP4 and BCL2 in PBRM1 knockdown in mouse mammary epithelial cells (NMuMG: RNA-seq) with human mammary epithelial cells (MCF10A: qPCR). **C.** ROS levels for MCF10A and MCF10A-T1k cells were determined using H_2-DCFDA by flow cytometry. *=p <0.05, ** = p <0.01, *** = p < 0.001, **** = p < 0.0001 (paired Student's t test). ns, not significant. Error bars represent S.D.



S7: Pathways deregulated upon PBRM1 knockdown in NMuMG cells correlate with TCGA patient dataset. Related to Figure 7. **A**. Summary of genes with decreased expression in renal clear cell carcinoma patients with PBRM1 mutations from The Cancer Genome Atlas (TCGA). **B.** Overlap of differentially regulated genes in NMuMG cells and TCGA patient data. Summary of genes with decreased expression in both NMuMG shPBRM1 and patients with PBRM1 mutations.

TRANSPARENT METHODS

Cell culture

HK-2 were purchased from ATCC and used within 6 months of purchase and 15 passages. Cells were cultured in RPMI (Corning Mediatech) supplemented with 10% fetal bovine serum (J R Scientific), 1% antibiotics (100 units/ml penicillin and 100 g/ml streptomycin; Corning Mediatech), and 1% L-glutamine (Corning Mediatech) at 37 °C in a humidified atmosphere in a 5% CO_2 incubator.

MEF cells were derived from E13.5 day embryos harvested from pregnant 129 mice. Heads and organs were removed and the rest of the embryo was dissociated in trypsin and DNAseI and cultured for 2 passages before performing knockdowns. Cells were cultured in DMEM (Corning Mediatech) supplemented with 10% fetal bovine serum (J R Scientific), 1% antibiotics (100 units/ml penicillin and 100g/ml streptomycin; Corning Mediatech), 1% nonessential amino acids (Corning Mediatech), 1% L-glutamine (Corning Mediatech) and 0.1% β-mercaptoethanol (Gibco, Thermo Scientific) at 37 °C in a humidified atmosphere in a 5% CO_2 incubator.

MCF10A cells were authenticated by STR DNA profiling at Bio-Synthesis (Lewisville, TX) and cultured in 1:1 DMEM (Corning Mediatech) and F12 (Corning Mediatech) supplemented with 29 mM Hepes (Amresco, LLC), 10 mM Sodium Bicarbonate (Macron), 5% Horse serum (Sigma), 10 µg/mL Insulin (Sigma), 10 ng/mL Epidermal Growth Factor (EGF) (Gold Biotechnology), 0.5 µg/mL hydrocortisone (Sigma), 100 ng/mL cholera toxin (Sigma), and 1% antibiotics (100 units/ml penicillin and 100 g/ml streptomycin; Corning Mediatech) at 37 °C in a humidified atmosphere in a 5% CO_2 incubator.

MCF10A T1K were authenticated by STR DNA profiling at Bio-Synthesis (Lewisville, TX) and cultured in DMEM (Corning Mediatech) supplemented with 10% fetal bovine serum (J R Scientific) 1% L-glutamine (Corning Mediatech), 1% antibiotics (100 units/ml penicillin and 100 g/ml streptomycin; Corning Mediatech), and 1% Sodium Pyruvate (Corning Mediatech) at 37 °C in a humidified atmosphere in a 5% CO_2 incubator.

NMuMG cells were purchased from ATCC and used within 6 months of purchase and 15 passages. Cells were grown in DMEM (Corning Mediatech) supplemented with 10% fetal bovine serum (J R Scientific), 10 µg/mL Insulin (Sigma), 1% L-glutamine (Corning Mediatech), 1% antibiotics (100 units/ml penicillin and 100 g/ml streptomycin; Corning Mediatech), and 1% Sodium Pyruvate (Corning Mediatech) at 37 °C in a humidified atmosphere in a 5% CO_2 incubator.

MDCK cells were purchased from ATCC and used within 6 months of purchase and 10 passages. Cells were cultured in DMEM (Corning Mediatech) supplemented with 10% fetal bovine serum (J R Scientific), 1% L-glutamine (Corning Mediatech), 1% antibiotics (100 units/ml penicillin and 100 g/ml streptomycin; Corning Mediatech), 1% nonessential amino acids (Corning Mediatech), 10 mM Hepes (HyClone) and 1% Sodium Pyruvate (Corning Mediatech), at 37 °C in a humidified atmosphere in a 5% CO_2 incubator.

Caki2 cells were originally purchased from ATCC and subsequently authenticated by STR DNA profiling at Bio-Synthesis (Lewisville, TX). Cells were grown in McCoy's 5A medium (Corning Mediatech) supplemented with 10% fetal bovine serum (J R Scientific), 1% antibiotics (100 units/ml penicillin and 100 g/ml streptomycin; Corning Mediatech), 1% nonessential amino acids (Corning Mediatech), and 1% L-glutamine (Corning Mediatech) at 37 °C in a humidified atmosphere in a 5% CO2 incubator. Caki2 Fuw and Caki2 Fuw+PBRM1 cells were cultured in the presence of doxycycline (1 µg/ml final concentration).

All the media were supplemented with 1:10,000 dilution of Plasmocin™ (InvivoGen).

Cell culture and treatments

Cells were seeded 24-72 h before treatment such that they were 50-80 % confluent at the time of experiment. For hydrogen peroxide treatment, indicated concentrations of freshly prepared hydrogen peroxide were added to the treatment groups for the indicated time periods in their regular media. For glucose starvation studies, the regular media was replaced with glucose free DMEM media (Corning Mediatech) or reduced glucose media for the indicated time periods. Following the completion of

treatment, cells were washed once with PBS, harvested by trypsinization and either processed immediately or flash frozen and stored at -80 ºC for future use.

Generation of cell lines

Knockdown was performed using shRNA-mediated knockdown with lentiviral construct pLKO.1. The shRNA constructs contain the following mature antisense sequences:
Human PBRM1: (TRCN0000015994) TTTGTAGATCAAAGACTCCGG
Mouse PBRM1: (TRCN0000081820) TTCTAGGTTGTATGCCTGTCG
Mouse Brd7 Clone ID: (TRCN0000030015) ATAATCATGGAGTAGCCAGGC
Mouse Brg1: (TRCN0000071386) TTCTCAATAATGTGTCGGGCG
Mouse Arid1a: (TRCN0000071395, Origene TG517733) ATTGTAGGTCATGTCATTTCG
Canine PBRM1-1: ACATCATCATACTCTTCCA
Canine PBRM1-2: ACCAACAGCCATACAACCA
c-Jun (TRCN0000042695): GCTTAAGCAGAAAGTCATGAAC
NRF2 (TRCN0000054658):GCCAAAGCTAGTATAGCAATAA

Caki2 FUW vector and Caki2 FUW PBRM1 as described in Chowdhury et al. 2016.

Short guide RNA for mouse PBRM1 (sgPBRM1) was designed using the MIT CRISPR tool (http://crispr.mit.edu/) and the control sgRNA (sgControl) was taken from Alpsoy and Dykhuizen et al. 2018 (Alpsoy and Dykhuizen, 2018).

Mouse sgControl: GTAGCGAACGTGTCCGGCGT

Mouse sgPBRM1: TTCATCCTTATAGTCTCGGA

The sgRNA were ordered as single strand oligos, annealed and cloned into vector PX459 (pSpCas9(BB)-2A-Puro (PX459) V2.0 was a gift from Feng Zhang, Addgene plasmid # 62988). The constructs were introduced into NMuMG cells by transient transfection using Lipofectamine 3000 (Invitrogen). After 48 h of transfection, selection using puromycin (0.6 µg/ml) was done for 48 h. The efficiency of knockout constructs was confirmed by immunoblotting.

Lentiviral Infection
HEK293T cells were transfected with knockdown and knockout lentivirus constructs along with packaging vectors pMD2.G and psPAX2. After 48 h, the supernatant was collected and concentrated by ultracentrifugation (17,300 rpm for 2 h) and resuspended in 200 µl of PBS. Cells were infected with concentrated virus using spinfection (1500 rpm in swing bucket centrifuge for 1 h). Fresh medium was added 16 h after infection, and cells were allowed to recover for 24 h before selection. Cells were selected for 2 weeks with puromycin (0.6 µg/ml) (Sigma-Aldrich) and hygromycin (200 µg/ml) where applicable (Corning Mediatech). Caki2 cells were cultured with 2 µg/ml doxycycline (EMD Chemicals) for 72 h prior to experiments to induce protein expression which was confirmed by immunoblotting. The efficiency of all constructs was confirmed by immunoblotting.

3D culture
Cells were embedded between 2 layers of Cultrex® Basement Membrane Extract (BME) (R&D Systems) on 8-well Chamber Slide. Wells were pre-coated with BME (200 µl/well) to allow polymerization at 37°C for 15 minutes. Cells were then seeded at 20,000 cells/well density. After attachment (30 minutes at 37°C), cells were covered with a second layer of BME/culture medium (1:19, 5%) to polymerize overnight at 37°C. Cells were incubated for 10 days, and the medium was replenished every 3 days. At the end of incubation, cells were fixed and subjected to immunofluorescence analysis.

Immunofluorescence staining
Cells were washed twice with ice-cold PBS, added 2-3 volumes of ice-cold PBS-EDTA and shaken on ice for 15-30 minutes. BME was detached from the bottom of culture surface by gently scraping the bottom with a pipette tip. The solution was transferred to a conical tube and gently shaken on ice for 15-30 minutes. When BME was dissolved completely, the solution was centrifuged at 120g for 1-2 minutes. The supernatant was carefully aspirated, and cells were gently resuspended in the remaining supernatant.

Pipetted approximated 15 µl of the cell suspension onto a glass bottom dish, allowed cells to settle and adhere to the glass. Cells were fixed using formalin for 20 minutes at room temperature (RT). Next, cells were permeabilized with 0.5% Triton X-100 in PBS for 5 minutes at RT and washed 3 times with 100 mM glycine in PBS at RT. Fixed cells were blocked for 1.5 hours with 10% goat serum. Cells were incubated overnight at 4°C with primary antibodies. The primary antibodies used were as follows: rat anti–α_6-integrin (Millipore; 1:100 in 0.2% Triton X-100, 0.1% BSA, 0.05% Tween 20 in PBS) and rabbit anti–Zo-1 (Invitrogen, 1:100 in 0.2% Triton X-100, 0.1% BSA, 0.05% Tween 20 in PBS). Cells were incubated with secondary antibody for 1 hour, followed by 3 washes at RT. Secondary antibodies were as follows: FITC goat anti-rat and Biotin-SP-conjugated AffiniPure goat anti-rabbit (Jackson ImmunoResearch). Cells were incubated with Texas Red Avidin D (Vector) for 1hour. Cell nuclei were counterstained with DAPI for 10 minutes and washed 3 times with PBS. Cells were incubated in PBS and imaged by confocal microscopy.

Confocal microscopy
Confocal laser scanning microscopy experiments were conducted using the Zeiss LSM 880 Upright Confocal.

TopFlash Reporter Assay
NMuMG cells were transfected with 10:1 ratio of M50 Super 8x TopFlash (Addgene 12456) to pcDNA3.1.CMV-renilla-Neo. The cells were transfected using Lipofectamine with 3:1 ratio of total DNA to lipofectamine reagent. After 24 h, the cells were trypsinized and 20,000 cells/well were plated in 96-well white tissue culture treated plates. After an additional 24 h of growth, the firefly and renilla luciferase levels were measured using the Dual Glo® assay system (Promega).

Annevin V Apoptosis detection in NMuMG cells
NMuMG cells were seeded at a density of ~ 1.5 X 10^6 cells/60mm dish and cultured for 24 h. The cells were then given treatments of 200 µM H_2O_2 in media for 0-4 h, followed by cell harvesting using Accutase (Innovative cell technologies) and apoptosis detection using the FITC Annexin V apoptosis detection kit (BD Pharmingen, Cat. # 556547) as per the manufacturer's instructions. The cells were immediately analyzed by flow cytometry using the Guava EasyCyte Benchtop Flow Cytometer (Millipore Sigma). The results were analyzed using FlowJo software.

Immunoblotting
Cells were given treatments as described before for the indicated time periods, followed by cell harvesting by trypsinization. Whole cell extracts were prepared by dissolving the cell pellets in RIPA buffer (50 mM Tris (pH 8.0), 150 mM NaCl, 0.1% SDS, 0.5% Na Deoxycholate, 1% NP-40) supplemented with freshly added PMSF, aprotinin, leupeptin and pepstatin, and incubation for 30 min at 4 °C. The lysates were centrifuged at 13000 x g for 30 min at 4 °C and the supernatants were preserved. Protein concentration estimations for the supernatants were done using BCA protein assay kit (Pierce Biotechnology) with BSA as standard and whole cell extracts were run on a 4-12 % bis-tris gradient protein gel, transferred to PVDF membrane and probed with primary antibodies in 5% BSA at 4 °C for 16 h.

Antibodies
Cleaved PARP (Asp214) (7C9) (Mouse specific) Cell Signaling #9548
Cleaved PARP (Asp214) (Human Specific) Cell Signaling #9541
PBRM1 (Bethyl Laboratories-PBRM1 Antibody, #A301-591A)
β-actin (Santa Cruz Biotechnology, #sc-47778)
BRG1 (G-7) Santa Cruz sc-17796
BRD7 Bethyl A302-304A
Vimentin BD Biosciences 550513
E-Cadherin BD Biosciences 610182
GAPDH (6C5) (Santa Cruz sc-32233)
LaminB (A-11) sc-377000
Phospho-Akt (Ser473) (D9E) XP® (Cell Signaling #4060)

Migration assay

NMuMG cells were seeded at a density of ~ 1.5 X 10^6 cells/well in a 6-well plate and cultured for 24 h, after which scratches were made in each well. The migration of cells was followed at regular intervals as indicated.

H_2-DCFDA staining for intracellular ROS using flow cytometry
NMuMG cells were seeded at a density of ~ 2 x 10^6 cells/60mm dish and Caki2 cells were seeded at a density of ~ 3 X 10^6 cells/60mm dish. The cells were cultured for 48 h, harvested by trypsinization, washed once with PBS and stained for intracellular ROS by incubation with freshly prepared 10 µM H2-DCFDA (Invitrogen, Cat. # D399) in PBS for 30 min at 37 °C in dark. Following the incubation, the cells were centrifuged at 250 x g for 5 min, resuspended in PBS and immediately analyzed by flow cytometry. The results were analyzed using FlowJo software.

Stress treatments and H_2-DCFDA staining using microplate reader
NMuMG cells were seeded at a density of 6.0 x 10^4 cells/well and Caki2 cells were seeded at a density of 2.5X10^4 cells/well in a 96-well black tissue culture plates. The cells were cultured for 24 h, following which they were subjected to the following stress conditions: H_2O_2 treatment (0-200 µM for NMuMG and 0-800 µM for Caki2) for 1h followed by 10 min recovery in PBS, glucose starvation by culturing in various glucose concentrations for 16 h for NMuMG and culturing in glucose free media for the indicated time periods for Caki2, CoCl2 treatment (0-250µM) for 24h or doxorubicin treatment (0-10 µM) for 24h. At the end of the treatments, cells were washed once with PBS, and stained with freshly prepared 10 µM H_2-DCFDA (Invitrogen, Cat. # D399) in PBS for 30 min at 37 °C in dark. Cells were washed again 2x with PBS and fluorescence measurements were taken using a microplate reader at excitation/emission wavelengths of 485/530 nm. Unstained cells were used as the negative controls.

H_2O_2 detection assay
H_2O_2 levels were measured using Amplex® Red Hydrogen Peroxide/Peroxidase Assay kit (Invitrogen). The H_2O_2 levels were determined from whole cell lysates (in RIPA) according to manufacturer's instruction. The concentration of H_2O_2 was determined for lysates generated from 5,000 and 10,000 cells in 50 µL by plotting fluorescence levels against experimentally determined dose curves.

Viability assays using CellTiter-Glo®
Cells were plated in 96-well white tissue culture plates and cultured for the indicated time under the indicated conditions. Antioxidant rescue experiments were performed with fresh media daily containing 20 µg/mL Vitamin C or 250 µM N-acetylcysteine (NAC). CellTiter-Glo® assay reagent was added directly to cells as per manufacturer's instructions, incubated for 10 min, and the luminescence was measured on a GloMax® microplate reader.

LDH assays using LDH Cytotoxicity Assay Kit II (Abcam, ab65393)
NMuMG cells were seeded at a density of 6.0 x 10^4 cells/well and Caki2 cells were seeded at a density of 1.0 x 10^4 cells/well in 96-well tissue culture plates. The cells were cultured for 24 h, following which they were subjected to H_2O_2 treatment (0-300 µM for NMuMG) for 6h or 24h and 200 µM H_2O_2 for Caki2 for the indicated time periods. Media was harvested from wells (10 µL) and transferred to a separate 96-well assay plate along with negative control (media alone) and positive control (lysed cells). LDH Reaction Mix (100 µl) was added to each well, mixed and incubated for 30 min at room temperature. The absorbance at 490 nm was measured on the GloMax® microplate reader.

H_2-DCFDA staining for MCF10A and MCF10A-T1K followed by flow cytometry
MCF10A and MCF10A-T1K cells were seeded in 60mm dishes in MCF10A media and cultured for 48 h such that they reach 50-80 % confluency at the day of the experiment. The cells were then harvested using trypsin, washed once using serum-free and phenol red-free media and stained for intracellular ROS with freshly prepared 10 µM H_2-DCFDA in PBS-Glucose (1X PBS supplemented with 25 mM glucose) as described before. The cells were immediately examined by flow cytometry and the results were analyzed using FlowJo software.

RNA-seq

RNA isolation, library construction, sequencing and transcriptome analysis was performed as described in our previous publication (Chowdhury et al. 2016). Sequencing was performed in biological triplicates. RNA-seq of NMuMG epithelial cell lines was performed at the Purdue Genomics Core using Illumina HiSeq technology. The resulting reads were trimmed using Trimmomatic utility (Bolger et al., 2014) and mapped to mm9 using STAR (Dobin et al., 2013) using default parameters. Read counts were obtained using HTSeq-count (Anders et al., 2015) in conjunction with a standard gene annotation files from UCSC (University of California Santa Cruz; http://genome.ucsc.edu) and differential expression was determined using DESeq2 pipeline (Love et al., 2014). Differentially expressed genes were filtered using a false discovery rate threshold of < 0.05 and a fold change threshold of > 1.3-fold relative to the reference sample. Gene ontology and transcription factor prediction analyses were performed using GeneCodis (Nogales-Cadenas et al., 2009) iCisTarget (Herrmann et al., 2012), and ToppCluster (Kaimal et al., 2010). Data sets generated in these experiments are available at the Gene Expression Omnibus under accession number GSE113606.

ATAC-seq

The ATAC-seq protocol originally described (Buenrostro et al., 2015) was adapted as follows for HK2 and NMuMG isogenic lines: 50,000 cells were resuspended in Nuclei Lysis buffer containing 0.05% IGEPAL CA-630, incubated for 5 minutes on ice and centrifuged for 10 minutes at 500xg at 4 °C. Nuclei extraction was confirmed by microscopic inspection and the nuclei pellet was resuspended in transposition master mix. Tagmentation, cleanup of tagmented DNA, and PCR enrichment was performed as per original description. High throughput sequencing was performed by HiSeq2500 using 50 bp paired-end at the Purdue Genomics Core. Sequenced reads were mapped by the Bowtie2 aligner (Ben Langmead et al., 2009) using hg19 or mm10 reference genome, respectively. Reads mapping to the mitochondrial genome were discarded. Bigwig files were generated for visual inspection of tracks using the bamCoverage utility of deepTools (Ramírez et al., 2016). Peaks of differential accessibility were identified using the SICER-df-rb utility (Xu et al., 2014) with a false discovery rate threshold of < 0.05 and a fold change threshold of > 1.5-fold difference in accessibility. Scaled heat maps were generated for the peak regions using the computeMatrix and plotHeatmap utilities of deepTools. Peak regions were analyzed for enrichment of sequence motifs and association with genomic elements using the findMotifs and annotatePeaks utilities of HOMER (Heinz et al., 2010).

qRT-PCR

RNA was isolated from cells using Trizol (Ambion, Thermofisher). Total RNA was converted to cDNA with Verso cDNA Synthesis Kit according to manufacturer's instructions (Thermo Scientific). Real-time PCR was performed using a Bio-Rad CFX Connect Real-Time system and Thermo Scientific Maxima SYBR Green qPCR Master Mix (Thermo Scientific). The results were analyzed using the Pfaffl method (Pfaffl, 2001).

Gene	Forward Primer	Reverse Primer
c-Jun	ACTCGGACCTTCTCACGTC	GGTCGGTGTAGTGGTGATGT
BCL2L1	GACAAGGAGATGCAGGTATTGG	TCCCGTAGAGATCCACAAAAGT
HK2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
IL1RL1	ACGCTCGACTTATCCTGTGG	CAGGTCAATTGTTGGACACG
NRF2	GATCCGCCAGCTACTCCCAGGTTG	CAGGGCAAGCGACTCATGGTCATC
HMOX1	GCCGAGAATGCTGAGTTCATG	TGGTACAAGGAAGCCATCACC
NQO1	CGCCTGAGCCCAGATATTGT	GCACTCTCTCAAACCAGCCT
BMF	GTGGCAACATCAAGCAGAGG	CGGTGGAACTGGTCTGCAA
ATF3	CTGCAGAAAGAGTCGGAG	TGAGCCCGGACAATACAC
IGFBP4	CTCTTCCGGTGCTGACCTCT	GGTGCTCCGGTCTCGAAT
BCL2	CTGCACCTGACGCCCTTCACC	CACATGACCCCACCGAACTCAAAGA

TCGA analysis

TCGA data analysis was performed as described in Chowdhury et al. 2016.

Data and software availability

Data sets generated in these experiments are available at the Gene Expression Omnibus under accession number GSE113606.

Supplemental References

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