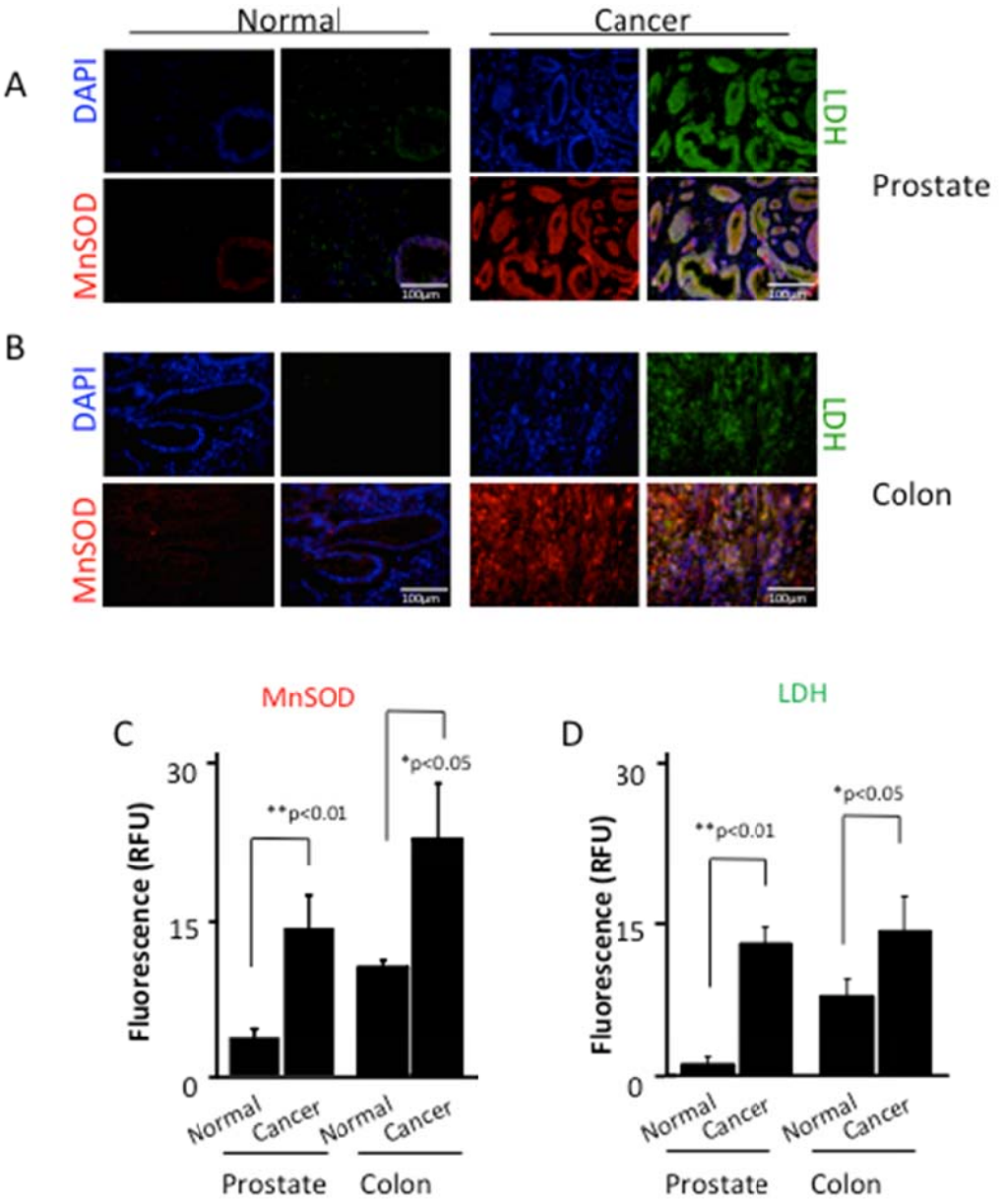
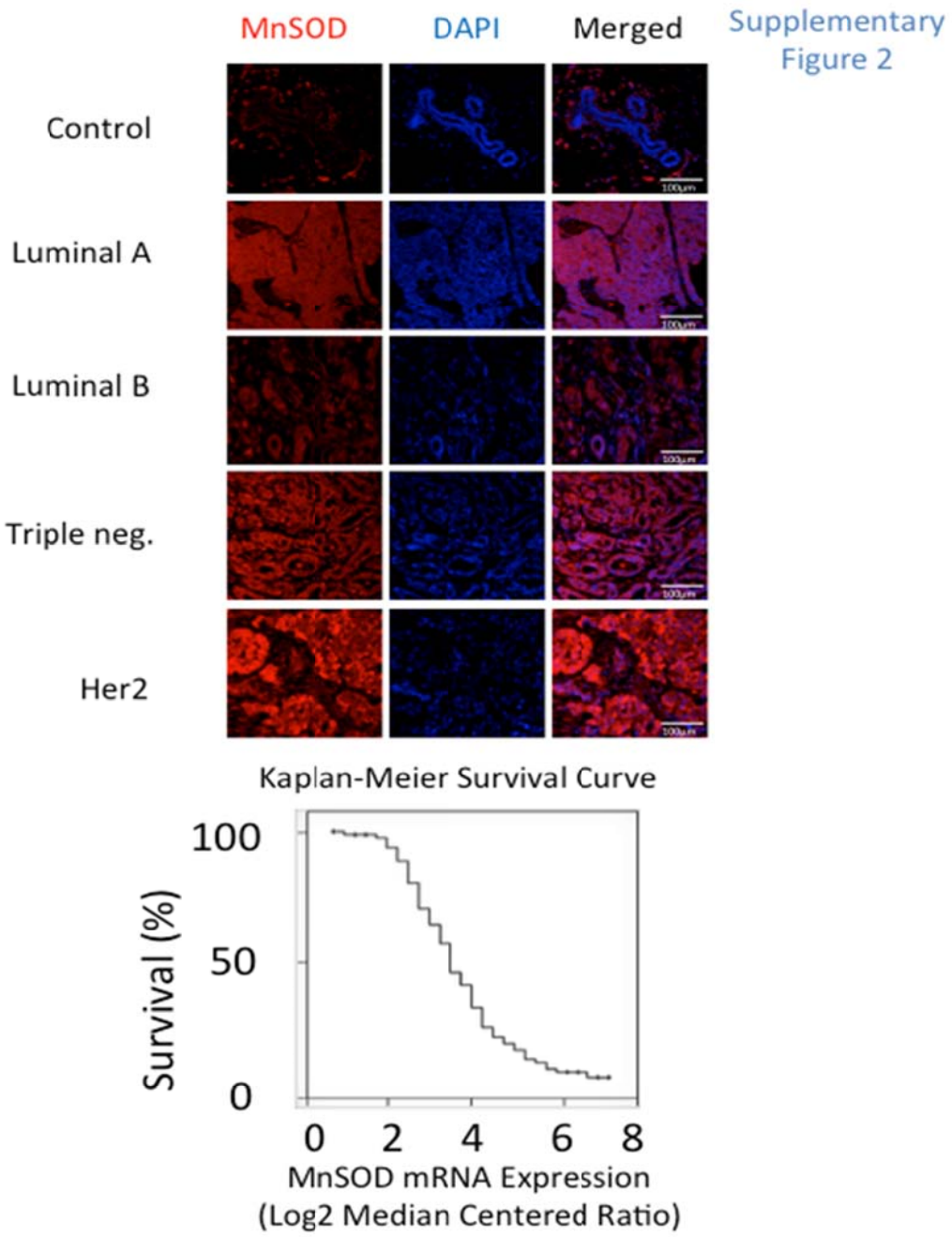
Supplementary Figure 1

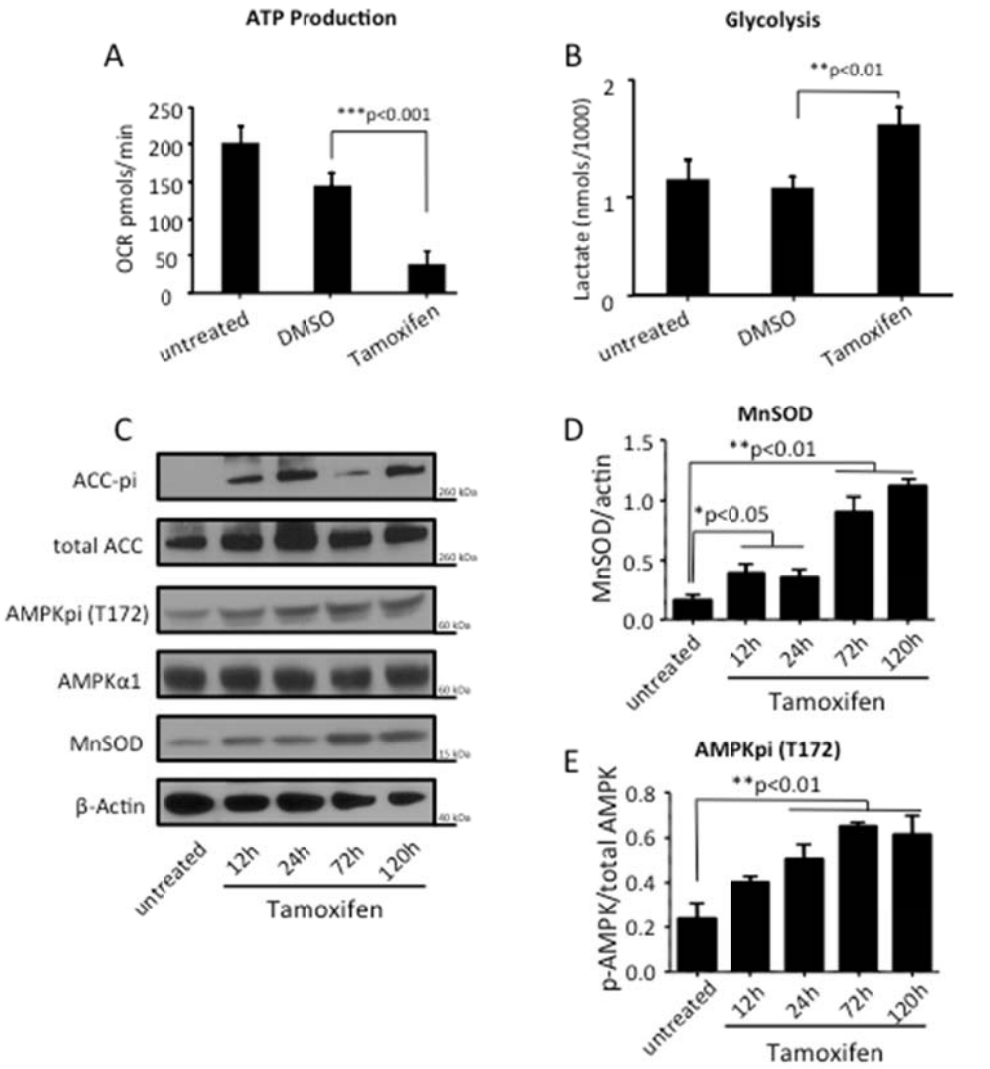


Supplementary Fig. 1 – MnSOD and LDH expression levels in normal and cancerous prostate and colon tissue. Overexpression of MnSOD and LDH was observed in cancerous prostate **(A)** and colon tissue **(B)**, compared to respective normal tissues. Quantification of MnSOD **(C)** and LDH **(D)** expression were performed (Corrected Total Cell Fluorescence, CTCF) and cancerous tissues expressed significantly more MnSOD and LDH compared to their respective control tissues. ($n \geq 6$); *$p<0.05$; **$p<0.01$.



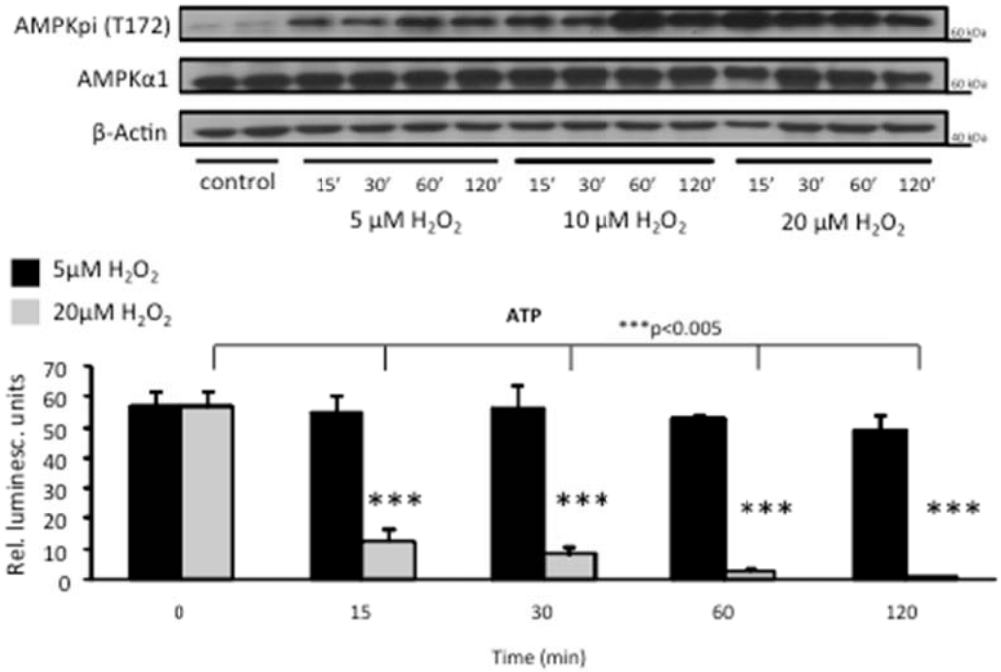
Supplementary Fig. 2 – MnSOD expression in different subtypes of human breast cancer and effects on patient survival. (A) Representative images of MnSOD protein expression in molecular subtypes of human breast cancer using tissue micro-array TMA-1005 (Protein Biotechnologies). **(B)** Kaplan-Meier survival curve stratified by MnSOD mRNA expression as a continuous variable [N = 330]. Relative mRNA is expressed as a log2 median-centered ratio measured from specimen biopsy obtained from the Kao Breast study dataset using the Oncomine database (Compendia Bioscience). Kaplan-Meier cumulative survival was analyzed using IBM SPSS Statistics.

Supplementary Figure 3



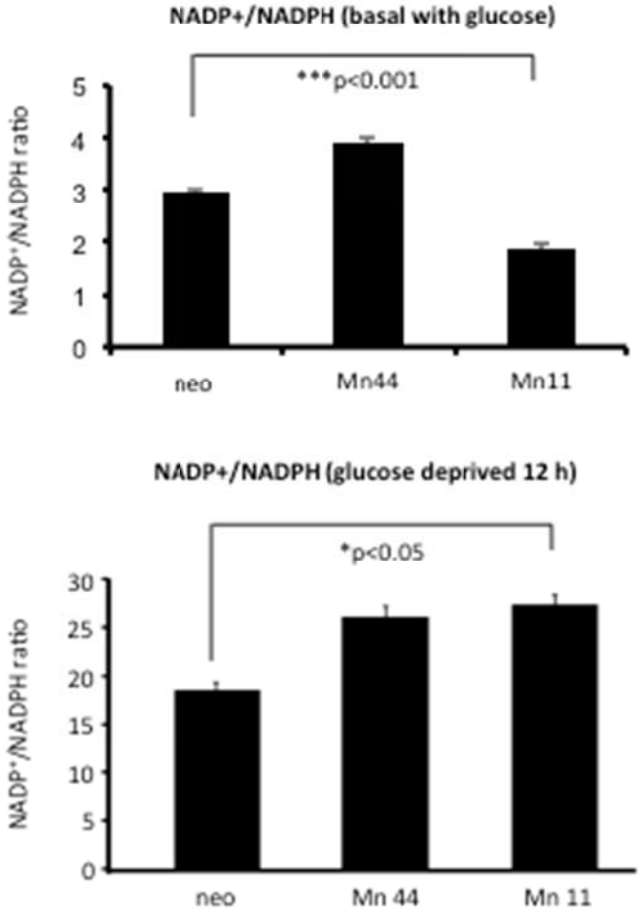
Supplementary Fig. 3 – Effect of induced transformation on MnSOD expression, respiration, glycolysis and AMPK phosphorylation. Non-tumorigenic MCF10A cells expressing transforming v-Src under an inducible estrogen receptor promoter were exposed to tamoxifen (1μM) diluted in full media over the indicated periods of time. **(A)** Basal oxygen consumption rates (OCR) prior and after tamoxifen-induced transformation (72h) were measured using extracellular flow analysis (Seahorse). Because tamoxifen was dissolved in DMSO, pure DMSO was used as the vehicle control. **(B)** Glycolysis was assessed using lactate accumulation as a surrogate in cells treated as in **(A)**. **(C)** Western blot analysis of MnSOD, AMPK (total and phospho-Thr172 AMPK), ACC (downstream AMPK target, total and phospho-Ser79) in transforming MCF10A-Er-Src cells overtime. **(D)** and **(E)** Quantification of MnSOD expression and AMPK phosphorylation at Thr172 at the indicated time points, respectively.

Supplementary Figure 4

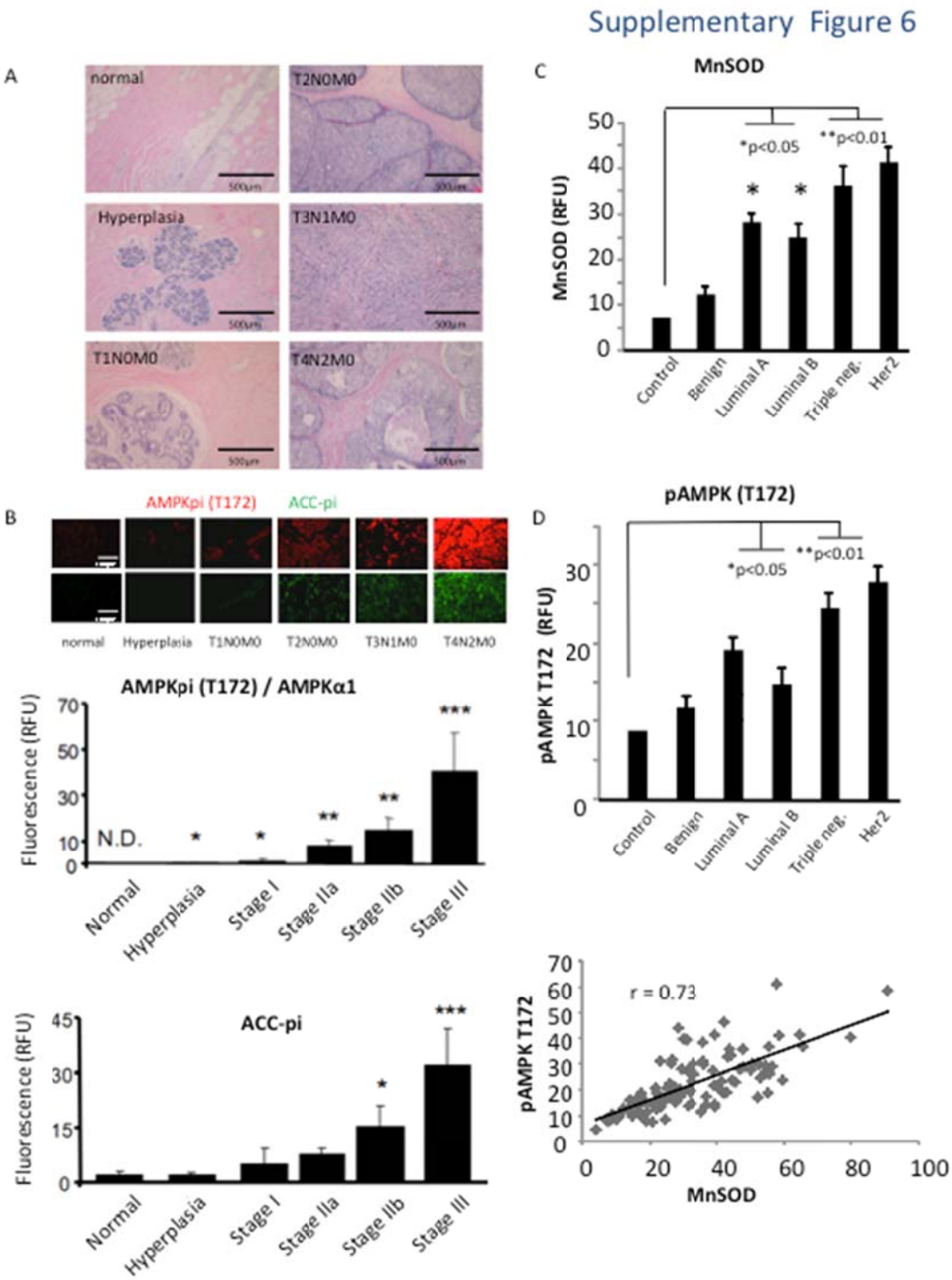


Supplementary Fig. 4 – Effect of low (5μM) or high (20μM) H_2O_2 on AMPK and ACC phosphorylation and steady state levels of ATP overtime. Cells were washed with PBS twice and exposed to the indicated concentrations of H_2O_2 for 15 minutes in RPMI1640 deprived of serum. After 15 minutes, pre-conditioned media was reintroduced and cells were left undisturbed for the incubation times indicated after which the cells were harvested. AMPK and ACC phosphorylation were assessed by Western blot **(A)** and steady state levels of ATP **(B)** were assessed as described in methods.

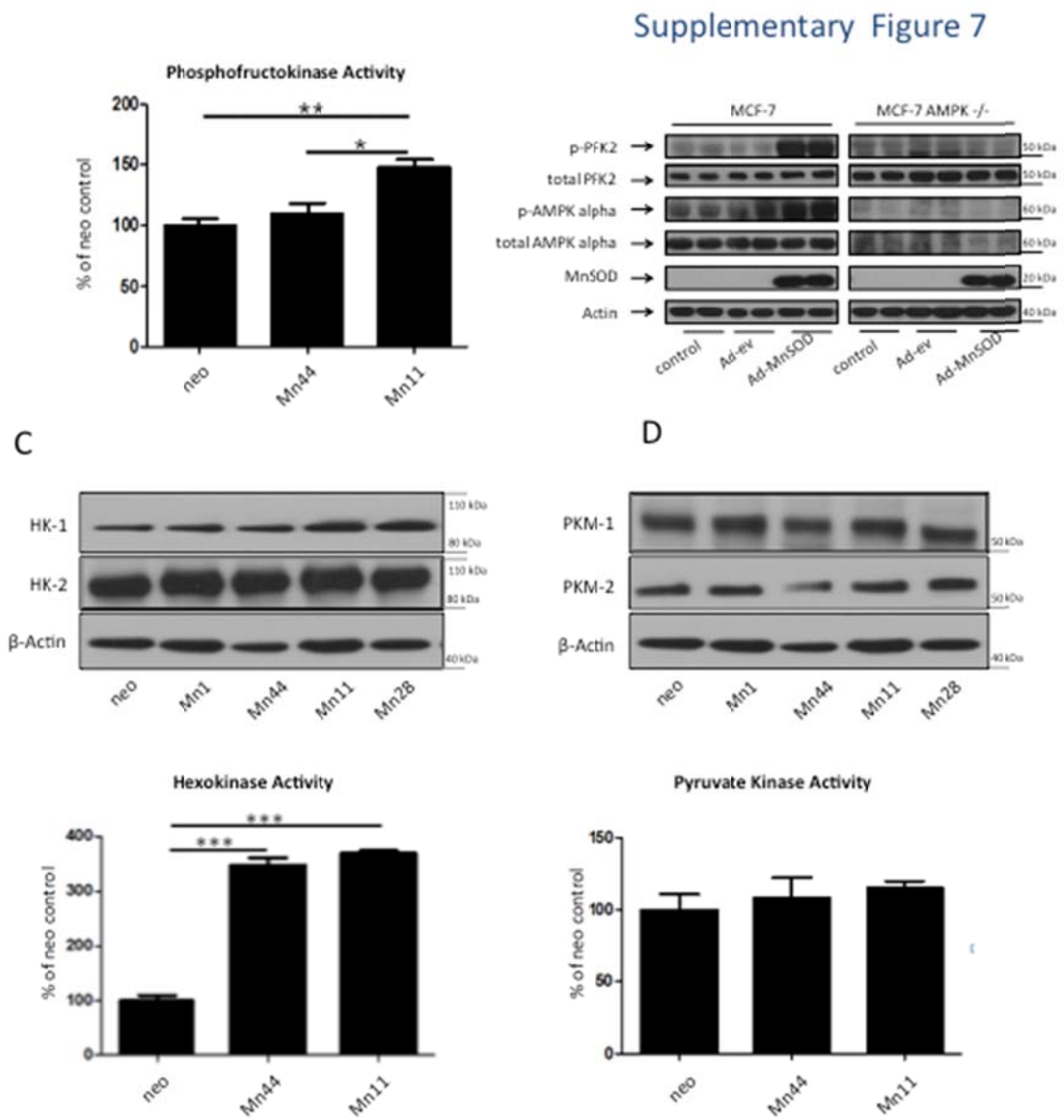
Supplementary Figure 5



Supplementary Fig. 5 – NADP+/NADPH ratio in resting neo, Mn44 and Mn11 cells. (A) Cells were harvested from (5 mM) glucose containing media. NADP+ and NADPH levels were assessed as described before. See methods for a brief description. **(B)** Same experiment as **(A)** but cells were maintained in glucose free media for 12 h prior to harvesting. *p<0.05; ***p<0.001.

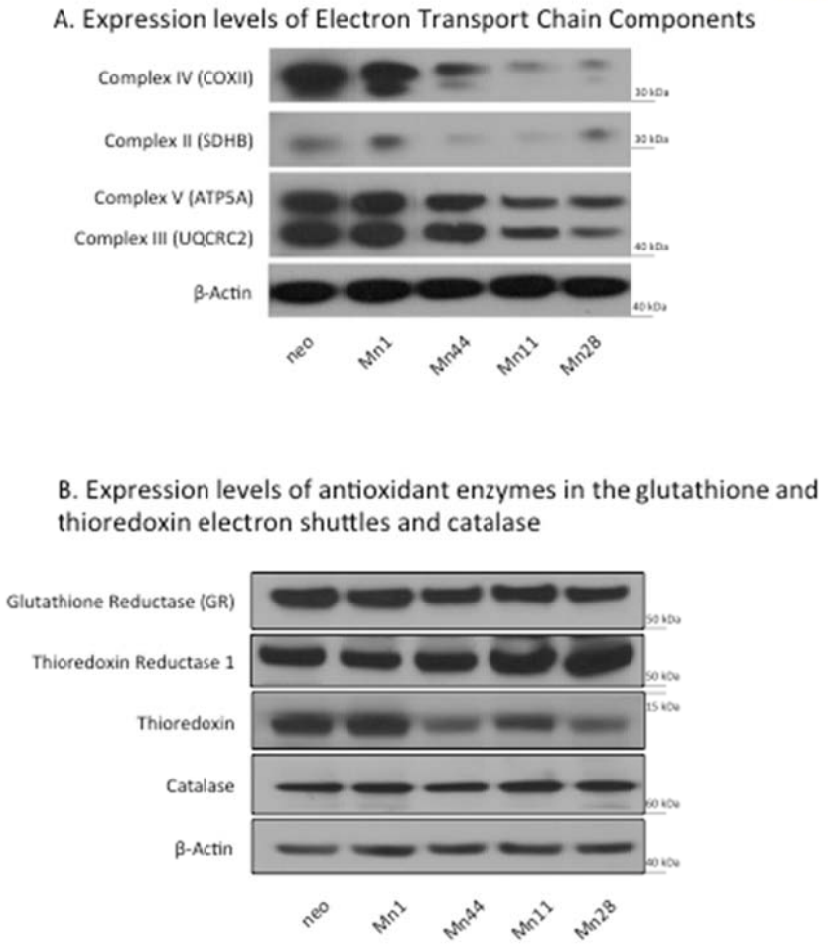


Supplementary Fig. 6 – Detection and quantification of AMPK phosphorylation and activity (pACC used as surrogate) in progressing breast cancer patient samples. (A) Micrographs of representative tissue (normal to stage III breast cancer) composing the analyzed tissue microarray. **(B)** Immunostaining of pAMPK (Thr 172) and pACC (Ser 79) in human mammary normal and cancerous tissue. A minimum of 10 different cases of each type were analyzed. **(C)** Quantification of MnSOD protein expression throughout molecular subtypes of breast cancer using tissue micro-array (TMA BR1503c, Biomax). **(D)** Quantification of AMPK activity (Thr 172) in the same slide as was used in **(C)**. Lower panel shows a strong positive correlation (R = 0.73) of MnSOD and pAMPK (Thr 172). (E) Correlation plot of MnSOD levels and AMPK phosphorylation at the activation site Thr172. Fluorescence was quantified using Image J. Statistical analysis was performed by one-way ANOVA (GraphPad InStat). *p<0.05; **p<0.01; ***p<0.001.



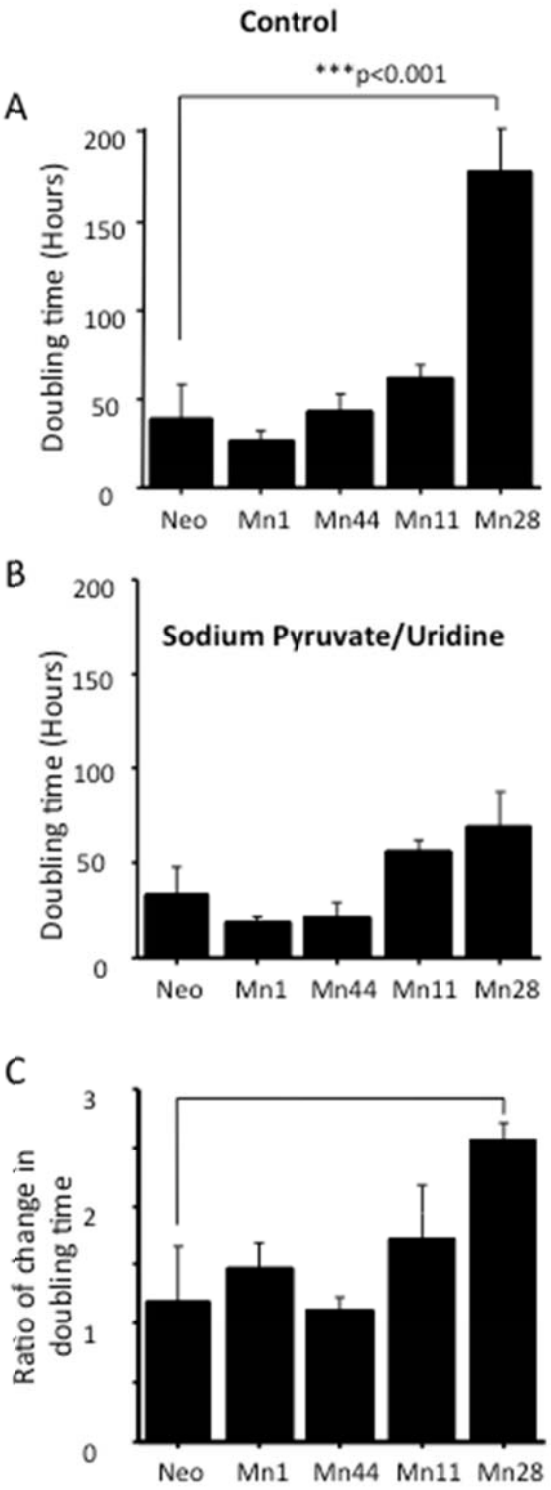
Supplementary Fig. 7 – Activity of key enzymes in the glycolytic pathway in neo and MnSOD overexpressing cells (Mn44 and Mn11). (A) Total phosphofructokinase activity was measured as described in methods. **(B)** Western blot analysis of PFK2 phosphorylation at Ser 466 in AMPK-competent and AMPK-deficient cells transfected with MnSOD, Ad-ev represents cells transfected with adenoviral vectors carrying an empty plasmid only, Ad-MnSOD represents cells transfected with adenovirus carrying an MnSOD gene construct. **(C)** Hexokinase activity assessed as described in methods and Western blot analysis of the hexokinase I (constitutive) and hexokinase II (regulated) isoforms. **(D)** Pyruvate kinase activity and Western blot quantification of the expression levels of PKM1 and PKM2 in neo, Mn44 and Mn11. Please see methods for details on PKM activity assessment.

Supplementary Figure 8



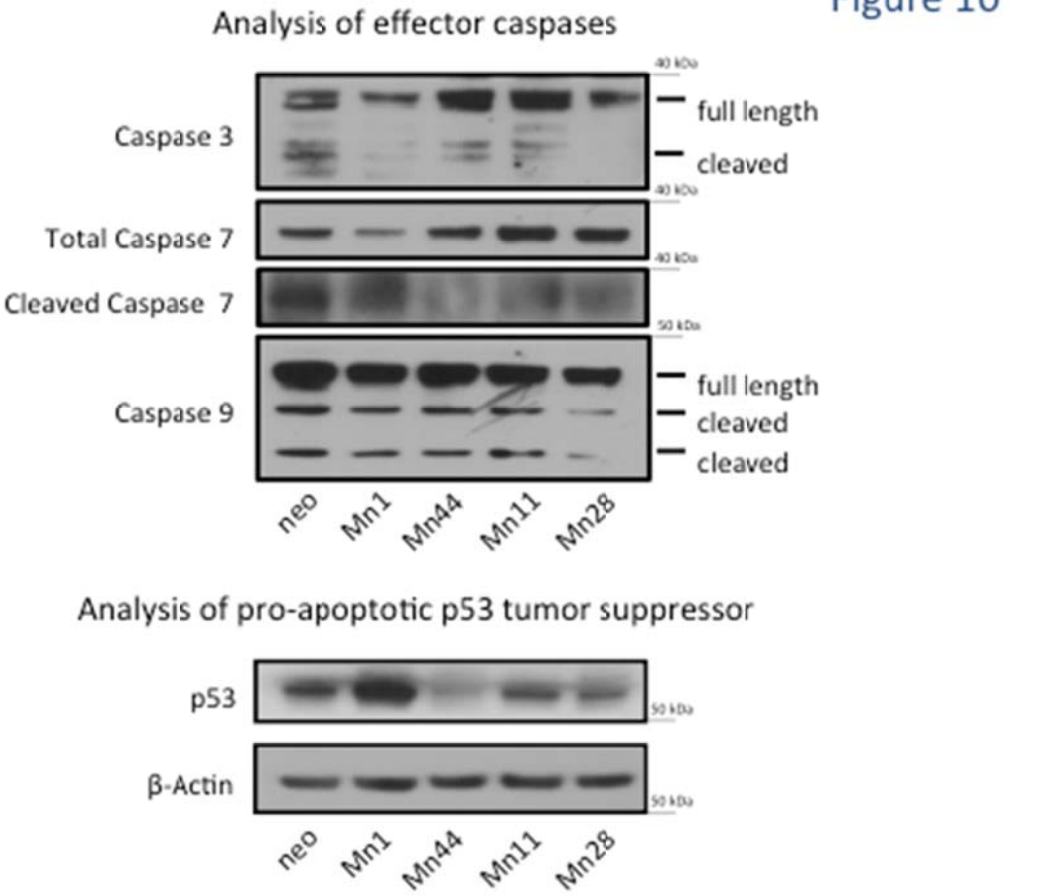
Supplementary Fig. 8 – Effect of MnSOD overexpression on key mitochondrial electron transport chain components and on antioxidant electron shuttle enzymes. (A) Expression levels of electron transport chain components were analyzed by Western blot using mito-profiler antibody cocktail (Mitosciences). **(B)** Expression levels of major components of antioxidant electron shuttle systems (glutathione-dependent, and thioredoxin-dependent). Glutathione Peroxidase-1 was undetectable by Western blot in neo, Mn44 and Mn11 cells.

Supplementary Figure 9



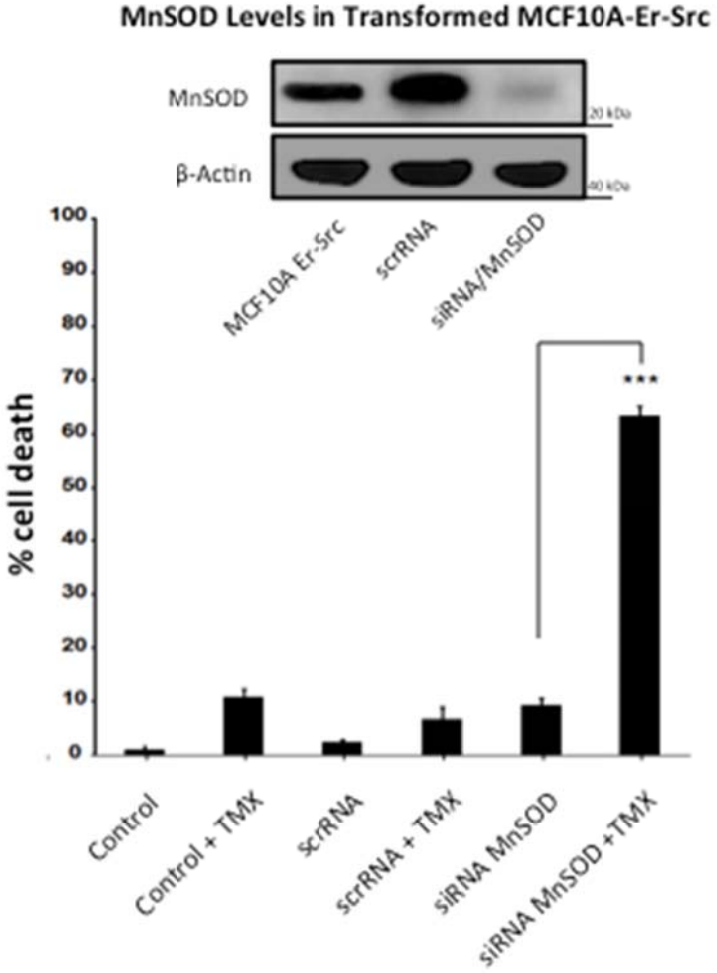
Supplementary Fig. 9 – Proliferation rates of neo and MnSOD overexpressing cells (Mn1, Mn44, Mn11 and Mn28) in the absence and in the presence of glycolytic substrates (pyruvate and uridine). Addition of glycolytic metabolites increased proliferation in MnSOD overexpressing cells. MnSOD overexpressing cells were treated with regular RPMI or RPMI supplemented with 1mM sodium pyruvate and 50 μM uridine for 1 week. Doubling time was measured by cell counting. When exposed to RPMI containing sodium pyruvate and uridine, low expressers (neo and Mn1) had very little change in doubling time, whereas high expressers (Mn11 and Mn28) had significant increases in proliferation rates. Statistical analysis was performed by one-way ANOVA (GraphPad InStat). * $p<0.05$, N = 6 independent assays.

Supplementary Figure 10



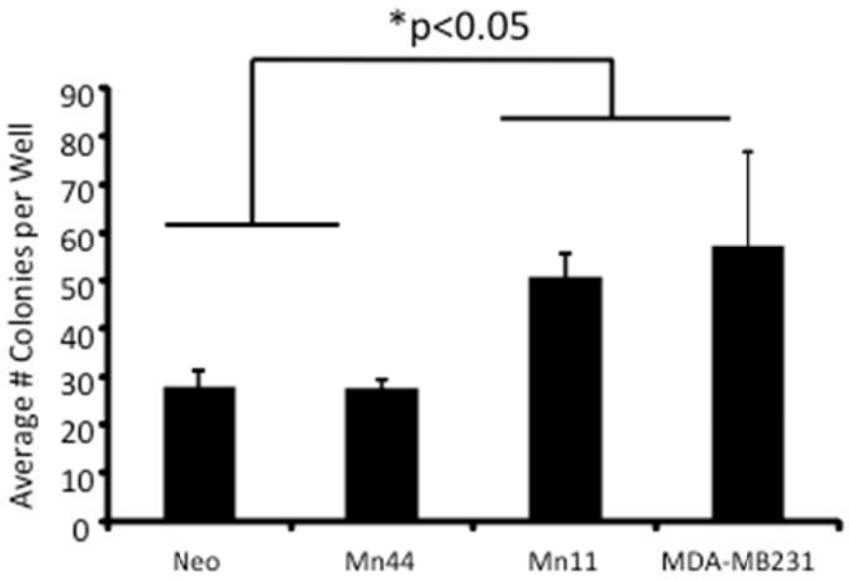
Supplementary Fig. 10 – Effect of MnSOD overexpression on caspase and p53 expression. **(A)** Expression levels of initiator pro-caspase and cleaved caspase 9 and effector pro-caspases and caspases 3 and 7 in neo, Mn1, Mn44, Mn11and Mn28. **(B)** Expression levels of pro-apoptotic p53 protein in neo, Mn1, Mn44, Mn11 and Mn28 analyzed by Western blot.

Supplementary Figure 11



Supplementary Fig. 11 – Effect of MnSOD silencing on the viability of transforming MCF10-Er-Src cells. MnSOD siRNA was introduced into cells overexpressing MnSOD using lipofectamine. Cells were harvested 48h after siRNA delivery. Inset shows a representative Western blot analysis of silencing efficiency in these cells. Panel shows % of cell death 48 h after induction of transformation by tamoxifen (1μM) added directly into full media. Cell death was assessed by membrane permeabilization using the Trypan blue staining. Statistical analysis was performed by one-way ANOVA (GraphPad InStat). N = 3 independent assays.

Supplementary Figure 12



Supplementary Fig. 12 – Colony formation by MCF7 cells expressing different levels of MnSOD and metastatic MDA-MB-231 cells. Soft agar assay analyzing anchorage-independent growth in MCF7 cells expressing increasing MnSOD levels demonstrates that MnSOD promotes clonogenecity of luminal A MCF7 cells. Cells were seeded at 2 x 10^5 in 1mL of .4% soft agar in native media (RPMI 1640, 10% FBS, 1% Anti-Anti) on top of 2mL of .8% soft agar media (2X DMEM, 20% FBS, 1% Pen/Strep) in 12-well dishes. After 4 weeks, colonies were stained with Trypan Blue (Life Technologies), imaged using EVOS inverted microscope (Life Technologies) and quantified using ImageJ (NIH). Significance was determined by Wilcoxon t-test (* = $p < 0.05$, N = 3).

Supplementary Methods

Cell cultures- MCF-7 cells stably expressing an empty vector (neo) or pHβApr-1 MnSOD vector driven by human β-actin promoter (Mn1, Mn44, Mn11 and Mn28) were a generous gift from Dr. Larry Oberley, University of Iowa. MCF-7 cells constitutively expressing AMPKα1 siRNA or scrambled RNA (sc-RNA) were a generous gift from Dr. Kevin P. Claffey, University of Connecticut Health Center. The cells were cultured in either RPMI 1640 medium or DMEM medium (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), 1% Anti-Anti (Invitrogen) and neomycin (50mg/L) (Sigma Aldrich, St. Louis, MO). MB-MDA-231 and U2OS cells were cultured in DMEM:F12 supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% non-essential amino acids and 1% penicillin/streptomycin (Invitrogen). MCF10A-Er-Src cells were a generous gift from Dr. Kevin Struhl, Harvard University, and grown in DMEM:F12 supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin insulin (10mg/mL), human growth factor (20ng/mL), and hydrocortisone (500μg/mL). All cell lines were grown under 5% CO_2 atmosphere at 37°C. Treatments with exogenous H_2O_2 (Sigma) were performed in serum-free medium for 15 min before replenishment with preconditioned medium. Treatments with Compound C, an AMPK inhibitor (Sigma), and 2-Deoxy-D-glucose, a glucose competitor (Sigma), were performed in RPMI 1640 for 24 hours, then in serum free media for 24 hours. Treatments with 1mM Sodium Pyruvate (Sigma) and 40μM Uridine (Sigma) were performed in RPMI 1640 for 7 days.

Mito-Catalase Transfection- Cells engineered to express different MnSOD levels were grown to 50% confluency in a 6-well plate in RPMI with 10% FBS. Mito-catalase adenovirus was added to treatment wells in Opti-MEM without serum and allowed to incorporate for 24 hours. Media was then changed to fresh RPMI+ 10% serum and incubated for 24 hours. Cells were washed and protein lysates were collected for Western blot.

MnSOD/AMPK silencing- MnSOD/AMPK and scrambled siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and incorporated into cells via electroporation using Amaxa Nucleofector Technology (Lonza, Basel, Switzerland). After electroporation, cells were plated in RPMI +10% FBS and incubated for 24 hours. Media was changed and cells were

incubated overnight. Cells were then collected for protein analysis by Western blot, or plated for functional measurements.

Western Blot Analysis – Protein derivatives were analyzed by separating the protein fractions by their molecular weight on 4-12% Bis-Tris gels followed by electroblotting on nitrocellulose membranes. The membranes were blocked overnight in 5% milk/TBS-T (0.05% Tween-20, pH 7.4). After blocking, membranes were washed with TBS-T and incubated with primary antibody [rabbit ant-SOD2-1:1000 (Abcam), mouse anti-β-tubulin- 1:1000 (Cell Signaling Technologies, Beverly, MA), rabbit anti-actin- 1:1000 (Cell Signaling Technologies), rabbit anti-bcl-2-1:100 (Abcam), rabbit anti-Survivin-1:1000 (Abcam), rabbit anti-AMPK alpha-1:1000 (Abcam), rabbit anti-AMPK pThr172-1:1000 (Abcam), rabbit anti-ACCpi- 1:1000 (Cell Signaling Technologies, rabbit anti-ACC-1:1000 (Cell Signaling Technologies), Apoptosis Antibody Sampler Kit- 1:1000 (Cell Signaling Technologies), Mitoprofile- 1:1000 (Mitosciences, Eugene, OR), rabbit anti-CaMKII- 1:1000 (Cell Signaling Technologies), rabbit anti-CaMKII-oxidized- 1:1000 (Millipore, Billerica, MA), rabbit anti-PKM1- 1:1000 (Cell Signaling Technologies), rabbit anti-PKM2- 1:1000 (Cell Signaling Technologies), rabbit anti-HK1- 1:1000 (Abcam), mouse anti-HK2- 1:1000 (Abcam), rabbit anti-PFK- 1:1000 (Santa Cruz), rabbit anti-PFK pSer483- 1:1000 (Santa Cruz), rabbit anti-Glutathione reductase- 1:1000 (Abcam), rabbit anti-Thioredoxin reductase 1- 1:1000 (Abcam), rabbit anti-Thioredoxin- 1:1000 (Abcam), rabbit anti-catalase- 1:1000 (Abcam), mouse anti-p53- 1:1000 (Santa Cruz)] in TBS-T for 60 min. After 3 washes, the secondary antibody, anti-rabbit/mouse IgG-alkaline phosphatase (Life Sciences, Grand Island, NY), 1:5000 in washing buffer, was added and incubated for 60 min. After 3 washes, the antigen-antibody complexes were analyzed by chemiluminescence (Pierce Chemical Co., Rockford, IL).

Human patient sample analysis- Human tissue samples were obtained from the University of Illinois at Chicago tissue bank. Samples were de-identified and were obtained in accordance with the IRB exemption note 20110082-58687-1 from the University of Illinois at Chicago Office of Research Services. Images were taken from at least 6 individual normal and 6 cancerous breast, prostate and colon tissues, as identified by Dr. Andre Kajdacsy-Balla, a clinical

pathologist. Representative images were used for Figures 1, 4 and 7, and fluorescence was quantified as described below.

Fluorescent immunohistochemistry-. Antigen retrieval was done using Antigen Unmasking Solution (Vector Laboratories) and pressure cooked at 20 psi in 10mM sodium citrate buffer for 5 min in a Decloaking Chamber electric pressure cooker (Biocare Medical, Walnut Creek, CA). Slides were blocked with normal serum and incubated with primary antibody overnight (MnSOD 1:1000, LDH 1:500, Abcam, Cambridge, MA) at 4^{o}C. Non-immune IgG was used for negative control. After rinsing in Tris-buffered saline (TBS), sections were incubated with Texas Red fluorescent anti-rabbit secondary antibody (Invitrogen) and Fluorescein fluorescent anti-mouse secondary antibody (Invitrogen) and mounted with Vector Shield Hard Set mounting media (Vector Laboratories). Slides were then examined on a Nikon ECLIPSE E400 microscope and were documented using SPOT Advanced version 4.0.1 software.

Tissue micro-array- MnSOD expression throughout molecular subtypes of breast cancer was assessed using immunofluorescent imaging of tissue micro-array TMA-1005 (Protein Biotechnologies, Ramona, CA). Antigen retrieval was performed as described above. Protein was blocked using 10% FBS in 1X TBS-T for 45 min at room temperature. Goat-anti-MnSOD and rabbit-anti-AMPK-pThr172 primary antibodies (Abcam, Cambridge, MA) were used at 1:100 dilution and incubated overnight at 4˚C. Alexa Fluor 488 and 568 secondary antibodies (Life Technologies, Grand Island, NY) were used at 1:200 dilution and incubated for 2 h at room temperature, in a humid chamber. DAPI (Life Technologies) was used at 50μM for 30 min at room temperature, and slides were mounted using Fluoromount Aqueous Solution (Sigma Aldrich, St. Louis, MO). TMA-1005 was then imaged using Apotome (Zeiss, Jena, Germany). Relative fluorescent intensity was measured using ImageJ, and RFU values were correlated with clinical molecular subtypes [defined by ER, PR and Her2 status]. Representative images were selected by clearest association with the mean RFU within each subtype.

Confocal microscopy- Cells were plated onto MatTek glass-bottomed culture dishes (1.5mm thickness) and allowed to adhere overnight. After treatments were performed, the cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min. After several washes,

cells were permeabilized using 100% methanol for 15 min. Following washing with PBS (3 times for 3 min each), cells were blocked using 10% FBS for 45 min, washed and then incubated with primary antibody at 1:100 overnight at 4˚C. Secondary antibody (Alexafluor 488 and 568, as mentioned above) was then incubated at 1:200 for 2 h at room temperature in a dark humid chamber. DAPI (50 μM) was then incubated for 30 min with agitation. Images were recorded using a Zeiss LSM510UV microscope.

Quantification of Relative Fluorescent Units- Corrected total fluorescence was calculated as described previously (31). Relative fluorescent units as determined by corrected total cell fluorescence were calculated as follows: Integrated density of selection – (area of selection x mean background integrated density). Measurements were recorded using ImageJ. Three background samples were taken per selection to assure proper calibration. Statistical analysis was performed as described below.

Amplex Red Assay- H_2O_2 production from cells was measured using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen/Life Sciences). Samples were prepared in buffer in a 96 well plate and incubated with the reaction mix for 30 min, according to manufacturer's instructions. Fluorescence was read at 560_{EX} /590_{EM} on a spectrophotometer.

ATP Assay- Cells were grown in a white walled, clear bottom 96 well plate in RPMI+ 10% serum to 80% confluency. Cell were transferred to glucose free media with galactose, then analyzed for ATP production using the Mitochondrial Tox-Glo Assay Kit (Promega, Madison, WI). ATP production was measured by luminescence on a spectrophotometer.

Glycolysis Assay- Cells were grown in a 96 well plate in RPMI + 10% serum to 80% confluency. Cells were transferred to serum free media for 24 hours, then analyzed for glycolytic activity using the Glycolysis Cell-Based Assay Kit (Cayman Chemical, Ann Arbor, MI). Lactate concentration in media was measured by absorbance at 480nm on a spectrophotometer.

Visible Spectrometry- All optical measurements were carried out with a Varian Cary 100 Bio spectrophotometer.

JC-1 Assay- Cells were grown to 50% confluency in MatTek confocal dishes. 5mM JC-1 stock was prepared in DMSO then diluted to 5μM into cell media. Cells were incubated in 5μM

JC-1 for 20 minutes at 37°C then washed thoroughly with1X PBS. Cells were imaged on the Zeiss LSM510UV microscope.

Ro-GFP oxidation- Oxidation of mito-roGFP probe was measured by confocal microscopy. Briefly, cells were infected with adenoviral vector expressing mito-roGFP sensors at 100Pfu per cell and incubated for 6 hours after which virus containing media was replaced with regular media and incubated overnight. 10^4 cells were plated on glass-bottom dishes in 1 mL of complete media and imaged by confocal.

Extracellular Flow Analysis- Cells were plated and grown in *Seahorse Bioscience* (North Billerica, MA) custom plates in RPMI + 10% FBS to a uniform monolayer. Cells were then washed and transferred to bicarbonate free RPMI for 2 hours then analyzed on the *Seahorse* Extracellular Flux (XF) Analyzer using the Mitochondria Stress Test Kit. The XF Analyzer measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) at intervals of approximately 2-5 minutes. OCR is an indicator of mitochondrial respiration, and ECAR is predominately the result of glycolysis.

Proliferation Assay- Doubling time was measured by cell counting. Cells were grown in regular media (RPMI + FBS) or RPMI supplemented with 1mM Sodium Pyruvate and 40μM Uridine. Cells were washed, trypsinized and stained with Trypan Blue. Cells were counted using a hemocytometer. Cells were then replated at measured concentrations and recounted over the course of one week. Doubling time is expressed as the number of hours that were required for the cells to double in number from initial concentration.

Soft agar assay- Anchorage-independent growth and clonogenecity were assessed using the soft agar assay. Briefly, 2 x 10^5 cells were seeded using .4% soft agar in native media (RPMI 1640, 10% FBS, 1% Anti-Anti) on .8% soft agar media (2X DMEM, 20% FBS, 1% Pen/Strep) in 12-well dishes. Colonies were allowed to grow for 4 weeks prior to staining with Trypan Blue (Life Technologies, Grand Island, NY) and number of colonies was assessed using EVOS Cell Imaging Systems (Life Technologies). The threshold for scoring as a colony was kept to no fewer than 5 cells within one cluster.

Flow Cytometry- Cells were grown to 80% confluency then treated for 48 hours with 2-deoxy-D-glucose (5 mM). Cells were trypsinized, washed and incubated with YOPRO and PI, using Vybrant® Apoptosis Assay Kit #4 - YO-PRO®-1/Propidium Iodide (Invitrogen). Cells were then analyzed for apoptosis by flow cytometry at the University of Illinois at Chicago Research Resources Flow Cytometry Service.

Epidemiological statistics- MnSOD mRNA expression was obtained from the Oncomine® database (Compendia Bioscience, Ann Arbor, MI) using the Kao-Breast dataset reported by Kao *et.al.*, BMC Cancer, **11:**pp143. Clinical status and Kaplan-Meier estimates were derived using Microsoft Excel (Microsoft, Redmond, WA) and confirmed using IBM SPSS Statistics (International Business Machines, Armonk, NY).

Statistical analysis- Statistical analyses were performed with GraphPad InStat by using one-way ANOVA with Student-Newman-Keuls comparison and also 2-way Contingency Table and Chi-Square Tests. A value of $P<0.05$ was considered significant whereas a value of $P<0.01$ was considered highly significant.