Supporting Information

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SI Materials and Methods

Cell Lines and Culture Conditions. MCF-10A ER-Src cells were grown as previously described in DMEM/F12 media supplemented with charcoal stripped FBS, penicillin/streptomycin, puromycin, EGF, hydrocortisone, insulin, and choleratoxin (1). Transformation via Src activation was induced by addition to 1 μM tamoxifen (Sigma) for 24 h. Metformin (300 μM) or phenformin (10 μM) was added, together with tamoxifen. CAMA-1 cells were grown in DMEM media containing 10% FBS and antibiotics.

Mammosphere Culture Conditions. CAMA-1 cells were trypsinized and counted, and 10,000 cells/mL were seeded in ultra-low attachment plates in serum-free mammosphere media as previously described (2). Cells were passaged every 7 d and collected in 50-mL tubes, and the plate was washed once with PBS and combined with the collected cells. Spheres were collected by gentle centrifugation and resuspended in 0.5% Trypsin for 8 min. Trypsin was quenched with media containing FBS, pelleted by centrifugation, and resuspended in mammosphere media. Cells were further dissociated mechanically by passing through a 23-G syringe six times, and the single cell suspension was verified microscopically. Mammospheres were passaged multiple times to ensure enrichment for cancer stem cells (CSCs).

Metabolic Profiling by Target LC/MS/MS. Cells were washed once with PBS and lysed in 80% (vol/vol) methanol at −78 °C to extract intracellular polar metabolites. Cell debris was removed by centrifugation at 4 °C. The supernatant containing metabolites was evaporated using a refrigerated Speed Vac. LC/MS/MS-based metabolomics analysis was done as previously described (3).

Lipogenesis. De novo lipogenesis was measured in MCF-10A ERSrc cells 24 h after treatment ± tamoxifen and ± biguanide. Cells were pulsed for 4 h with 0.8 μCi ^{14}C-glucose (Perkin-Elmer) per 800 μL media ± biguanide. Cells were rinsed twice with PBS and then lysed in 0.5% Triton X-100. The lipid fraction was obtained by chloroform and methanol (2:1 vol/vol) extraction, followed by the addition of water. Samples were centrifuged, and the bottom phase was collected to measure ^{14}C incorporation into lipids. All scintillation counts were normalized to protein concentrations.

Statistical Analysis. To identify significantly altered metabolites with either metformin or phenformin treatment in comparison with control treatment, metabolites from each sample were normalized to total metabolite counts. A Student t test was performed, and changed metabolites with a $P < 0.05$ were used for further analysis. To identify unique effects of one biguanide over the other, unbiased by a consistent potency effect, fold changes of metformin or phenformin over control cells for all metabolites were plotted against each other. Ratios of these twofold changes for each metabolite were calculated, and a 99.7% CI over all measured metabolites was determined to address whether some metabolites are uniquely altered by either biguanide. Metabolites outside of this interval were considered to be differentially regulated.

Glucose, Glutamine, Lactate, and Ammonia Measurement (NOVA Analysis). Cell supernatant was collected 24 h after tamoxifen and biguasnide treatment, and cells were counted for normalization. Analysis was performed using the BioProfile FLEX analyzer (Nova Biomedicals) as previously described (4).

1. Iliopoulos D, Hirsch HA, Struhl K (2009) An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 139(4): 693–706.
2. Iliopoulos D, Hirsch HA, Struhl K (2011) Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. *Cancer Res* 71(9):3196–3201.
3. Shyh-Chang N, et al. (2013) Influence of threonine metabolism on S-adenosylmethionine and histone methylation. *Science* 339(6116):222–226.
4. Finley LW, et al. (2011) SIRT3 opposes reprogramming of cancer cell metabolism through HIF1α destabilization. *Cancer Cell* 19(3):416–428.

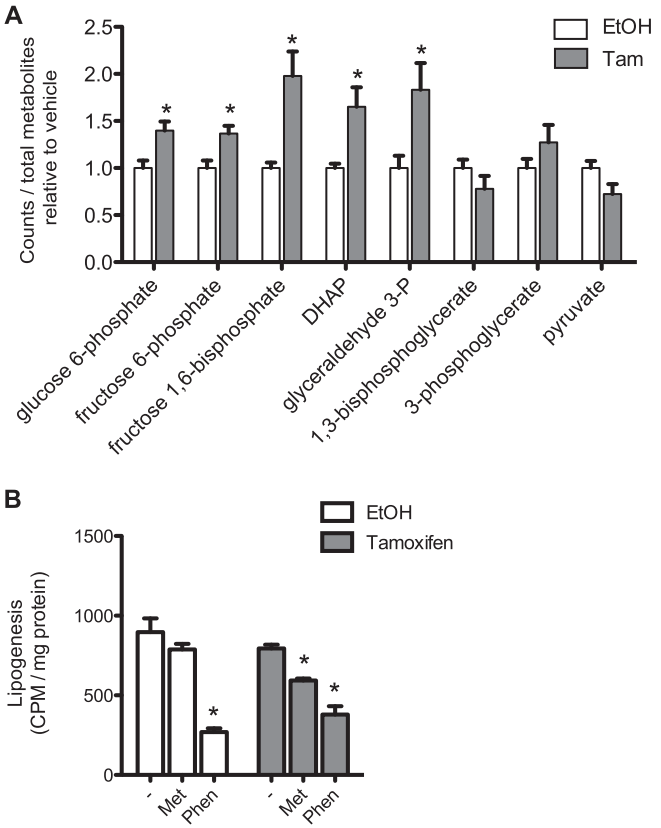

Fig. S1. Glycolytic induction during transformation and increase in fatty acid oxidation by phenformin treatment. Relative levels of glycolytic intermediates measured by LC-MS/MS in MCF10A ER-Src cells were treated with tamoxifen or ethanol for 24 h (*A*), $n = 4$. De novo lipogenesis from 14C-glucose in ER-Src cells pretreated with tamoxifen or ethanol ± biguanide for 24 h before 4-h lipogenesis analysis (*B*), $n = 3$. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle control. Error bars indicate SEM.

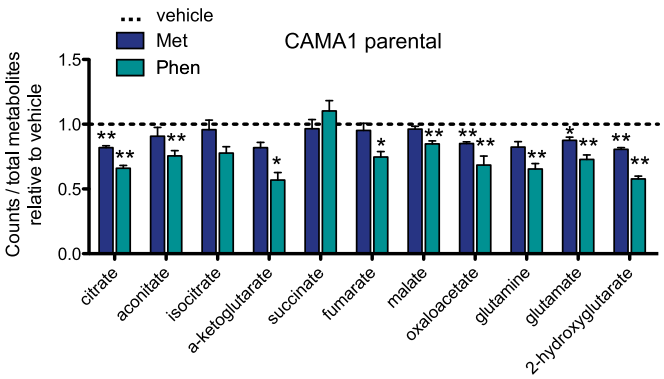

Fig. S2. Tricarboxylic acid (TCA) cycle regulation by biguanides in transformed CAMA-1 breast cancer cells. Twenty-four hours after metformin or phenformin treatment, TCA cycle intermediates were measured in CAMA-1 breast cancer cells. $n = 4$, $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle control. Error bars indicate SEM.

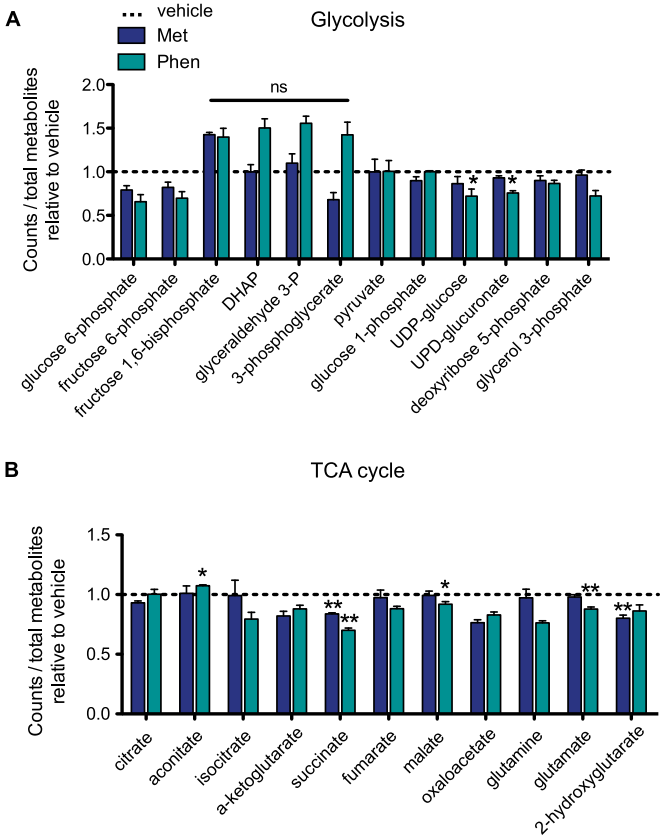

Fig. S3. Glycolysis and TCA cycle regulation by metformin and phenformin in CSCs. After 24-h treatment with metformin and phenformin, intermediates of glycolysis (*A*) and TCA cycle (*B*) in cancer stem cells were measured. $n = 3$, $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle control. Error bars indicate SEM.

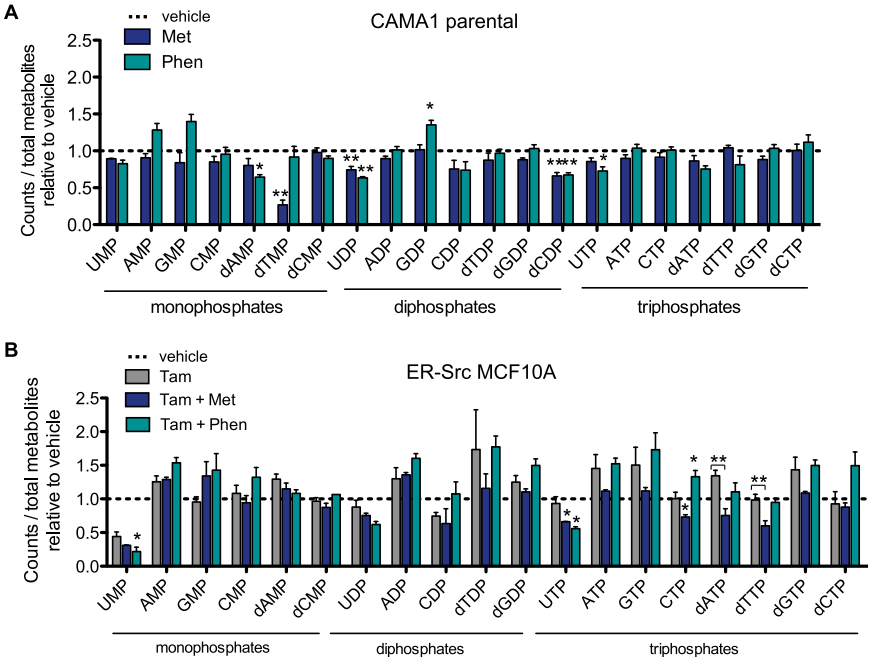

Fig. S4. Nucleoside regulation by metformin and phenformin in parental CAMA-1 cells and during the transformation process of MCF-10A ER-Src. Parental CAMA-1 (*A*) and transformation-induced MCF-10A ER-Src (*B*) cells were treated with metformin or phenformin for 24 h, and changes in nucleotide metabolism were measured, $n = 4$. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with tamoxifen treatment alone. Error bars indicate SEM.

Table S1. Metabolites analyzed by LC-MS/MS

1-Methyl-histidine	Carbamoyl phosphate	Fructose-1,6-bisphosphate	Methylnicotinamide	Spermine
1-Methyladenosine	Carnitine	Fructose-6-phosphate	Myo-inositol	Succinate
1,3-Diphosphateglycerate	CDP	Fumarate	*N*-acetyl spermidine	Succinyl-CoA
2-Aminooctanoic acid	CDP-choline	GDP	*N*-acetyl spermine	Taurine
2-Dehydro-D-gluconate	CDP-ethanolamine	Geranyl-PP	*N*-acetyl-glucosamine	Thiamine pyrophosphate
2-DFeoxyglucose-6-phosphate	cholesteryl sulfate	Glucono-lactone	*N*-acetyl-glucosamine-1-phosphate	Thiamine-phosphate
2-Hydroxy-2-methylbutanedioic acid	choline	Glucosamine	*N*-acetyl-glutamate	Threonine
2-Hydroxygluterate	citraconic acid	Glucose-1-phosphate	*N*-acetyl-glutamine	Thymine
2-Isopropylmalic acid	citrate	Glucose-6-phosphate	*N*-acetyl-L-alanine	Trans, transfarnesyl diphosphate
2-Keto-isovalerate	citrulline	Glutamate	*N*-acetyl-L-ornithine	Trehalose-6-phosphate
2-Ketohaxanoic acid	CMP	Glutamine	*N*-carbamoyl-L-aspartate	Trehalose-sucrose
2-Oxo-4-methylthiobutanoate	CoA	Glutathione	N6-acetyl-L-lysine	Tryptophan
2-Oxobutanoate	creatine	Glutathione	NAD^+	Tyrosine
2,3-Dihydroxybenzoic acid	creatinine	Glutathione disulfide	NADH	UDP
2,3-Diphosphoglyceric acid	CTP	Glutathione disulfide	$NADP^+$	UDP-D-glucose
3-Methylphenylacetic acid	cyclic-AMP	Glycerate	NADPH	UDP-D-glucuronate
3-Phospho-serine	cystathionine	Glycerophosphocholine	Nicotinamide	UDP-*N*-acetyl-glucosamine
3-Phosphoglycerate	cysteine	Glycolate	Nicotinamide ribotide	UMP
3-*S*-methylthiopropionate	cystine	Glyoxylate	Nicotinate	Uracil
4-Aminobutyrate	cytidine	GMP	*O*-acetyl-L-serine	Urea
4-Pyridoxic acid	cytosine	GTP	Ornithine	Uric acid
5-Methoxytryptophan	D-erythrose-4-phosphate	Guanidoacetic acid	Orotate	Uridine
5-Methyl-THF	D-glucarate	Guanine	Orotidine-5-phosphate	UTP
5-Phosphoribosyl-1-pyrophosphate	D-gluconate	Guanosine	oxaloacetate	Valine
6-Phospho-D-gluconate	D-glucono-lactone-6-phosphate	Guanosine 5-diphosphate, 3-diphosphate	p-Aminobenzoate	Xanthine
7-Methylguanosine	D-glucosamine-1-phosphate	Hexose-phosphate	p-Hydroxybenzoate	Xanthosine
7,8-Dihydrofolate	D-glucosamine-6-phosphate	Histidine	Pantothenate	Xanthurenic acid
a-Keoglutarate	D-glyceraldehdye-3-phosphate	Homocysteic acid	Phenylalanine	
Acadesine	D-sedoheptulose-1–7-phosphate	Homocysteine	Phenyllactic acid	
Acetoacetate	dAMP	Homoserine	Phenylpropiolic acid	
Acetyl-CoA	dATP	Hydroxyisocaproic acid	Phosphoenolpyruvate	
Acetylcarnitine	dCDP	Hydroxyphenylacetic acid	Phosphorylcholine	
Acetyllysine	dCMP	Hydroxyphenylpyruvate	Pipecolic acid	
Acetylphosphate	dCTP	Hydroxyproline	Proline	
Aconitate	Deoxyadenosine	Hypoxanthine	Purine	
Adenine	Deoxyguanosine	IDP	Putrescine	
Adenosine	Deoxyinosine	Imidazole	Pyridoxamine	
Adenosine 5-phosphosulfate	Deoxyribose-phosphate	Imidazoleacetic acid	Pyridoxine	
ADP	Deoxyuridine	IMP	Pyroglutamic acid	
ADP-D-glucose	Dephospho-CoA	Indole	Pyrophosphate	
Alanine	Dephospho-CoA	Indole-3-carboxylic acid	Pyruvate	
Allantoate	dGDP	Indoleacrylic acid	Quinolinate	
Allantoin	dGMP	Inosine	Riboflavin	
Aminoadipic acid	dGTP	Isocitrate	Ribose-phosphate	
Aminoimidazole carboxamide ribonucleotide	Dihydroorotate	Kynurenic acid	*S*-adenosyl-L-homocysteine	
AMP	Dihydroxy-acetone-phosphate	Kynurenine	*S*-adenosyl-L-homocysteine	
Anthranilate	Dimethylglycine	L-arginino-succinate	*S*-adenosyl-L-methionine	
Arginine	dTDP	Lactate	*S*-methyl-5-thioadenosine	
Ascorbic acid	dTMP	Lipoate	*S*-ribosyl-L-homocysteine	
Asparagine	dTMP	Lysine	*S*-ribosyl-L-homocysteine	
Aspartate	dTTP	Malate	Sarcosine	
ATP	dUMP	Maleic acid	Sedoheptulose-bisphosphate	
Atrolactic acid	Ethanolamine	Methionine	Serine	
Betaine	FAD	methionine sulfoxide	shikimate	
Betaine aldehyde	Flavone	Methylcysteine	sn-Glycerol-3-phosphate	
Biotin	Folate	Methylmalonic acid	Spermidine	