## **Supporting Information**

## Shen et al. 10.1073/pnas.1501555112



**Fig. 51.** c-Myc regulates glucose metabolism in TNBC. The IC<sub>50</sub> for the GLS1 inhibitor CB-839 in the indicated TNBC cell lines were reported recently (1). We used this information to bin the cell lines into insensitive, low-sensitivity, medium-sensitivity, and high-sensitivity groups. (A) The Myc pathway score for each cell line is plotted against the sensitivity category. The Pearson's correlation coefficient is presented. (*B*) mRNA levels for TXNIP and its paralog ARRDC4 were determined in MDA-MB-231 cells in which we had knocked down c-Myc using a specific siRNA pool. (C and D) Levels of the indicated proteins were measured in MDA-MD-231 (C) or MDA-MB-157 (D) cells following 24-h treatment with the indicated dose of JQ1. (*E*) The indicated cell lines were treated with increasing concentrations of JQ1 for 24 h, and levels of TXNIP mRNA were determined by qPCR. JQ1 was used at 100, 200, and 500 nM. MCF7 and MDA-MB-361 cells are ER-positive cell lines, and MDA-MB-157 and MDA-MB-231 are TNBC cell lines. (*F*-*I*) Levels of the indicated metabolites in TN HCI-014 cells were determined following 24-h treatment with 100 nM JQ1: (*F*) Glucose 6-phosphate (G6P). (*G*) 3-Phosphoglycerate (3-PG). (*H*) 2-Phosphoglycerate (2-PG). (*I*) Glutamate. \**P* < 0.05 as determined using *t* tests. *n*, number of independent biological replicates. In *B* and *F*-*I*, values are reported as means  $\pm$  SEM.

1. Gross MI, et al. (2014) Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. Mol Cancer Ther 13(4):890-901.



**Fig. 52.** TXNIP up-regulation following Myc suppression requires MondoA. (*A* and *B*) MDA-MB-157 (*A*) or MDA-MB-231 (*B*) cells were transfected with either a wild-type TXNIP luciferase reporter construct or a TXNIP promoter construct carrying mutations in the ChoRE MondoA:Mlx binding site. Transfected cells were treated for 24 h with 500 nM JQ1 before analysis. (C) Wild-type MEFs or MondoA-null (Mon<sup>-/-</sup>) MEFs were transfected with a wild-type TXNIP luciferase reporter construct. c-Myc levels were decreased in these experiments by 24-h treatment with 500 nM JQ1. (*D*) Wild-type or MondoA-null MEFs were treated for 24 h with 500 nM JQ1, and the levels of the TXNIP mRNA were determined by qPCR. (*E* and *F*) Quiescent MDA-MB-157 cells were released into serum for the indicated times. In *F*, quiescent cells were pretreated with either DMSO or 500 nM JQ1 for 1 h before serum treatment. (*G* and *H*) The ECAR (*G*) and OCR (*H*) were determined in control or TXNIP-knockdown MDA-MB-157 cells. (*I* and *J*) The OCR (*I*) and glutamine uptake (*J*) were determined in vector control MDA-MB-157 cells (Vec) or after doxycycline induction of TXNIP-V5. *n*, number of independent biological replicates; NS, not significant. In *A–D*, values are reported as  $\pm$  SD.



**Fig. S3.** A Myc<sub>high</sub>/TXNIP<sub>low</sub> gene-expression signature correlates with poor patient outcome. Shown are Kaplan–Meier plots correlating overall survival or death from breast cancer for the gene-expression patterns indicated at the top of each panel. Statistics in *A–E* were determined using Mantel–Cox log-rank tests. *n*, numbers of tumors analyzed. (A) Data available from the Netherlands Cancer Institute were analyzed (1). (*B*) Data from a compendium of data assembled from five independent studies were analyzed (2). (*C–G*) Data from the METABRIC dataset were analyzed (3). (*C*) Death from breast cancer was correlated with the gene expression pattern indicated at the top of each panel. (*D*) Data were separated into the indicated four intrinsic breast cancer subtypes. Only outcomes from the indicated Myc<sub>high</sub>/TXNIP<sub>low</sub> gene-expression signature are shown. (*E*) The Myc<sub>high</sub>/TXNIP<sub>low</sub> gene signature was correlated with outcome based on p53 status as indicated at the bottom of each panel. (*F*) The correlation between overall survival and Myc<sub>high</sub>/TXNIP<sub>low</sub> or Myc<sub>high</sub>/TXNIP<sub>low</sub> or Myc<sub>high</sub>/TXNIP<sub>low</sub> or Myc<sub>high</sub>/TXNIP<sub>low</sub> are determined using Kaplan–Meier methods to determined confidence intervals. The Cox proportional hazard model with associated Wald test was used to determine hazard ratios.

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1. van de Vijver MJ, et al. (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347(25):1999-2009.

2. Cunha S, et al. (2014) The RON receptor tyrosine kinase promotes metastasis by triggering MBD4-dependent DNA methylation reprogramming. *Cell Reports* 6(1):141–154. 3. Curtis C, et al.; METABRIC Group (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 486(7403):346–352.

Features	Myc activity score	TXNIP expression	Basal subtype
0230_184A1N4.CEL	0.053893	3.273637284	Yes
0231_184B5.CEL	0.20427	3.764303989	Yes
0232_600MPE.CEL	0.057588	4.151907479	
0233_AU565.CEL	0.55753	2.849166329	
0234_BT20.CEL	0.11256	2.37280326	Yes
0235_BT474.CEL	0.2072	4.01348457	
0236_BT483.CEL	0.11121	3.987326175	
0237_CAMA1.CEL	0.61071	2.401074761	
0240_HCC38.CEL	0.6544	1.391522472	Yes
0241 HCC70.CEL	0.032056	2.635696436	Yes
0242 HCC202.CEL	0.21535	4.110151672	
0243 HCC1143.CEL	0.27101	2.770793918	Yes
0244 HCC1187.CEL	0.48066	1.027664704	Yes
0245 HCC1395.CEL	0.40289	2.221118206	Yes
0246 HCC1419.CEL	0.12898	3.17229511	
0247 HCC1428.CEL	0.37848	4.008091807	
0248 HCC1500 CEL	0.27735	1,924921055	Yes
0249 HCC1569 CEL	1	2,554209951	Yes
0250 HCC1599 CEL	0 41077	3 014923564	Yes
0251 HCC1806 CEL	0.67017	2 606612601	Yes
0252 HCC1937 CEL	0 16583	2 512470139	Yes
0254 HCC1954 CEL	0 12074	2 637075297	Yes
0255 HCC2185 CEL	0.28045	4 557883171	105
0256 HCC2218 CEL	0.53406	4 192826589	
0250_HCC3153_CEL	0.28253	2 850060709	Voc
0258 HS578T CEL	0.20255	0.862854758	Vos
0250_1155701.CEL	0.42720	3 478641548	163
	0.26836	4 707125722	Vor
	0.20830	4.707133732	Vor
	0 2572	4.270333037	163
	0.3572	2 66070429	
	0.30313	2 452057944	Vor
	0.20079	2.433037644	Tes
	0.000002	4.408485715	Vor
	0.14122	4 49609462	163
	0.14122	4.48008403	
	0.030387	2 150015092	Vor
	0.41037	2 656627042	Tes
	0.4078	0.015370005	Vec
	0.35987	2 205554606	Tes
	0.40557	3.303334090	
	0.120	3.401133314	
	0.10916	3.001007970	Vor
	0.030143	4.803000333	Yes
	0.23310	3.0/0/32337	Yes
	0.30324	2.040375000	Tes
	0.2409	4.01152656	
	0.053279	4.710603629	Vee
0285_30101131510102.CEL	0.03499	1.20094254	res
0286_147D.CEL	0.32144	2.796718678	
	0.30471	3./3912928/	
	0.10114	2.558834297	
U289_2K/51.CEL	0.38406	4.258/30651	
UZ9U_ZK/53U.CEL	0.049989	4.121688422	
U291_ZK/5B.CEL	0.82359	3.60161/	.,
0293_B1549.CEL	0.61104	1.989558963	Yes
0294_MCF10A.CEL	0.55842	3.074354649	Yes

Table S1	Myc gene s	ignature score	is inversely	correlated with	
Table 31.	iviye gene s	ignature score	is inversely	correlated with	

Myc activity as measured by gene signature score was determined in the listed breast cancer cell lines using the procedures described in *Materials and Methods*. TXNIP expression levels in each cell line are listed, and whether the cell lines are of the basal intrinsic subtype is stated.

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