a TCGA (Firehose Legacy, n = 1108)

PTEN RNA (z-score)

ATAD1 RNA (z-score)

$R^2 = 0.4103$
****$P < 0.0001$

b PTEN expression

Normalized PTEN expression (Nanostring)

- Normal
- Tumor
- Matched Normal-Tumor pair

PTEN Nanostring probe

c PTEN-loss associated gene signature (Saal *et al.* PNAS, 2007)

Normalized expression (Nanostring)

ATAD1 CNN2 DKK3 ECE1 MAP3K12   MCM6 STMN1 ATAD2 MELK MLF1

PTEN signature down genes   PTEN signature up genes

d Western blot data from Fig. 1e

e TCGA (Firehose Legacy, n = 841)

PTEN RNA (z-score)

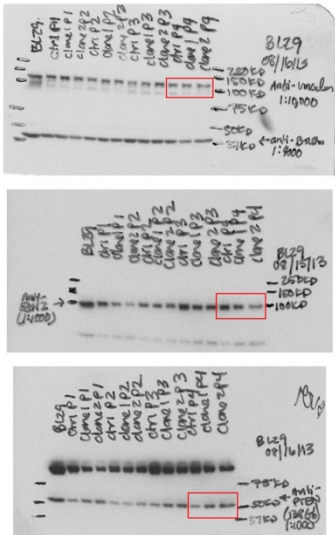PTEN RPPA (z-score)

$R^2 = 0.1786$
****$P < 0.0001$

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6   **Supplementary Figure 1: Transcriptional repression of *PTEN* and associated poor-**

7   **prognosis gene expression signature is present in tumor but not adjacent normal**

8   **breast samples. a** Scatter plot showing PTEN and ATAD1 levels by RNA-seq (z-score)

9   in breast cancer cases (n= 841). Linear regression was performed; trendline, R-squared

10   (R^2), and P-value displayed on chart [1,2]. Nanostring was used to measure transcript levels

11   of **b** PTEN and **c** various members of the PTEN-loss associated gene signature [3] in 4

12   normal-tumor pairs and 4 additional normal breast tissue biopsy samples. Normal shown

13   in black, tumor in red, N-T pairs are connected by gray line. See Supplementary

14   Spreadsheet S1 for Nanostring probes. Error Bars are mean ± s.e.m. Significance: 2-tailed

15   t-test, Holm-Sidak correction for multiple comparisons. (***P<0.001, **P<0.01, *P<0.05)

16   **d** Uncropped immunoblot images from Figure 1e. Vinculin is loading control. **e** Scatter

17   plot showing PTEN transcript levels by RNA-seq and PTEN protein levels by RPPA in

18   breast cancer cases (n= 841). Linear regression was performed; trendline, R-squared (R^2),

19   and P-value displayed on chart [1,2].

a

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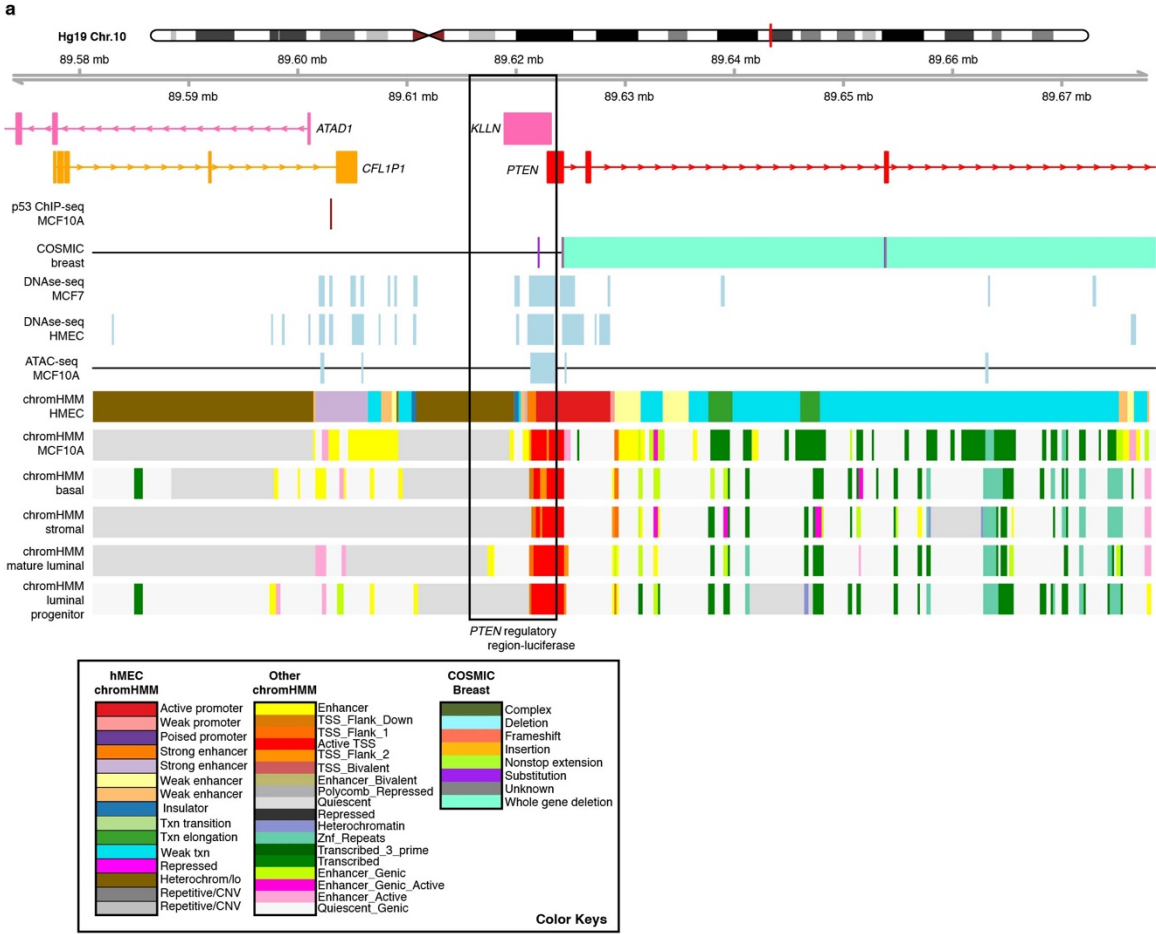
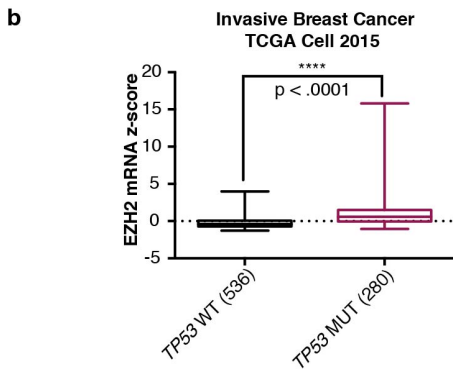Western blot data from Fig. 2d   Western blot data from Fig. 2e-f
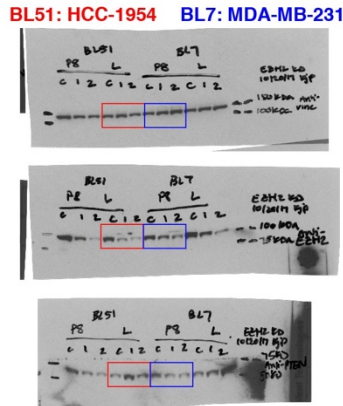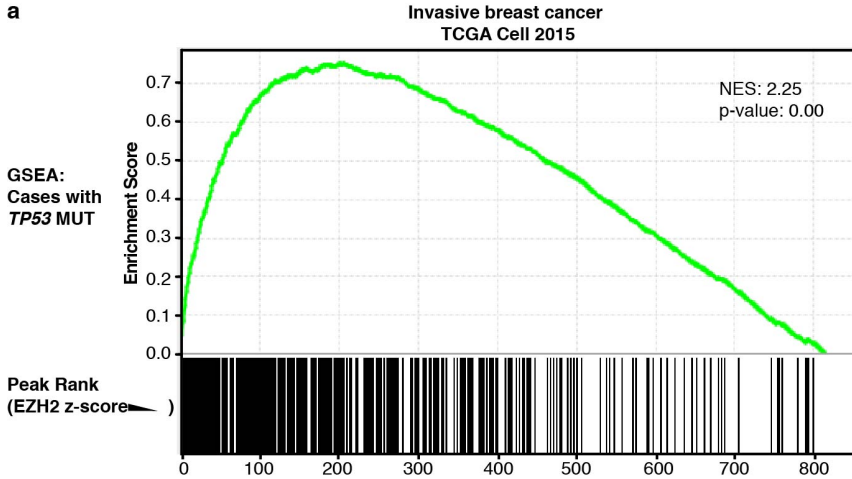
23   **Supplementary Figure 2: Transcriptional landscape of the *PTEN* promoter and**

24   **regulatory region.** Characterization of the chromatin accessibility and transcriptional

25   landscape of the *PTEN* promoter and upstream regulatory region including p53 ChIP-seq

26   in MCF10A non-tumorigenic mammary epithelial cells, COSMIC genetic alterations in

27   breast cancer, DNAse-seq in MCF7 breast cancer cells and non-tumorigenic human

28   mammary epithelial cells (HMEC), and chromHMM in HMEC and MCF10A cells (non-

29   tumorigenic cell lines), and in basal, stromal, and luminal (mature and progenitor) breast

30   cells. *PTEN* regulatory region analyzed in the luciferase assay in Fig. 2 is highlighted

31   (black box). See Materials and Methods for additional detail and dataset references. **b**

32   Uncropped blot images from Figure 2d-f. Vinculin is loading control.

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a

Invasive breast cancer
TCGA Cell 2015

GSEA:
Cases with
TP53 MUT

NES: 2.25
p-value: 0.00

Enrichment Score

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

Peak Rank
(EZH2 z-score)

0   100   200   300   400   500   600   700   800

b

Invasive Breast Cancer
TCGA Cell 2015

EZH2 mRNA z-score

20
15
10
5
0
-5

****
p < .0001

TP53 WT (536)   *TP53* MUT (280)

c

Cell Line	*TP53* Status
HCC-1187	MUT
HCC-1954	MUT
HCC-2218	MUT
MDA-MB-231	MUT
HCC-1143	MUT
MDA-MB-175-VII	WT

d   Western blot data from Fig. 5b   Western blot data from Fig. 5e

BL51: HCC-1954   **BL7: MDA-MB-231**

 β-actin (42 kDa)
38 kDa

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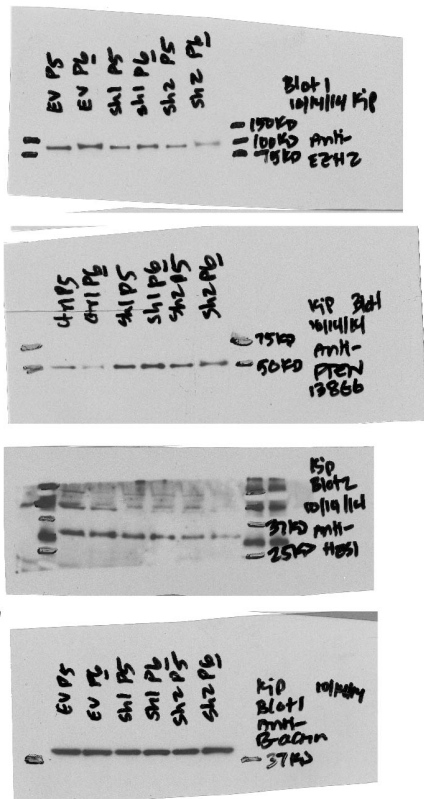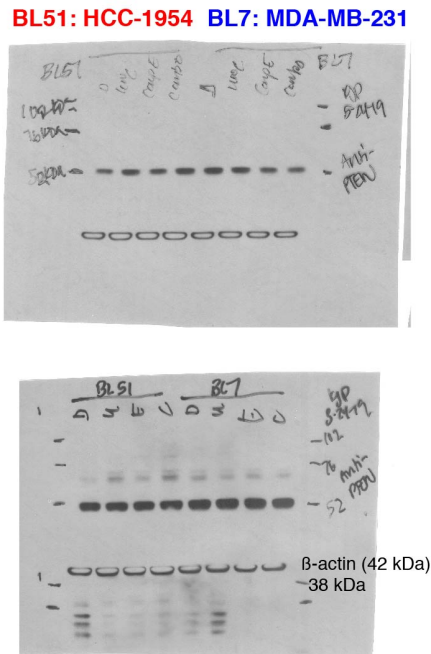
35 **Supplementary Figure 3:** *TP53* **mutation associates with increased expression of**
36 **EZH2 in breast cancer, but does not associate with restoration of PTEN expression**
37 **in response to EZH2 inhibition. a** Gene set enrichment analysis (GSEA) was performed
38 on a list of 816 invasive breast cancer cases [4] pre-ordered for EZH2 RNA-seq z-score for
39 mutation of *TP53*. Normalized enrichment score (NES) and p-value are displayed. **b**
40 Boxplots of EZH2 expression in *TP53*-mutant versus wild-type case. Significance: Mann-
41 Whitney test. Number of cases indicated for each group (total n = 816). (****P<0.0001) **c**
42 *TP53* mutation status of cell lines used in knockdown and EZH2 inhibitor studies
43 (cancer.sanger.ac.uk) [5]. **d** Uncropped blot images from Figure 5b and e. β-actin is loading
44 control.

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Cell Line	Subtype	ER	PR	HER2
HS758T	TNBC	Neg	Neg	Neg
HCC-202	HER2	Neg	Neg	Pos
HCC-1187	TNBC	Neg	Neg	Neg
HCC-1143	TNBC	Neg	Neg	Neg
HCC-1599	TNBC	Neg	Neg	Neg
SUM-159	TNBC	Neg	Neg	Neg
HCC-2218	HER2	Neg	Neg	Pos
MDA-MB-330	LUMB	Pos	Neg	Pos
HCC-1954	TNBC	Neg	Neg	Neg
BT-474	LUMB	Pos	Pos	Pos
HCC-1806	TNBC	Neg	Neg	Neg
ZRC75-30	LUMB	Pos	Neg	Pos
L56BR	TNBC	Neg	Neg	Neg
MDA-MB-361	LUMB	Pos	Pos/Neg*	Pos
HCC-1419	LUMB	Pos/Neg*	Neg	Pos
MDA-MB-157	TNBC	Neg	Neg	Neg
DU-4475	TNBC	Neg	Neg	Neg
UACC-812	LUMB	Pos	Pos/Neg*	Pos
HCC-38	TNBC	Neg	Neg	Neg
UACC-893	HER2	Neg	Neg	Pos
BT-483	LUMA	Pos	Pos/Neg*	Neg
MDA-MB-231	TNBC	Neg	Neg	Neg
SUM-1315	TNBC	Neg	Neg	Neg
HCC-1428	LUMA	Pos	Pos	Neg
MDA-MB-175-VII	LUMA	Pos	Neg	Neg
BT-20	TNBC	Neg	Neg	Neg
HCC-1569	HER2	Neg	Neg	Pos
MDA-MB-435-S	TNBC	Neg	Neg	Neg

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60 **Supplementary Table 1: Molecular characteristics of breast cancer cell lines** [6,7]. Table

61 contains (left to right) cell line name, breast cancer subtype, immunohistochemical status

62 of estrogen receptor (ER), progesterone receptor (PR), and Her2 listed as positive (Pos) or

63 negative (Neg). *Cell lines listed as Pos/Neg have published values that are contradictory.

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BlockID	PatientID	N/T	ER	PR	Her2
2656	1589	N	n/a	n/a	n/a
2568	1590	N	n/a	n/a	n/a
8207	3937	N	n/a	n/a	n/a
8216	3931	N	n/a	n/a	n/a
2611	1601	N	n/a	n/a	n/a
2614	1602	N	n/a	n/a	n/a
3206	1978	N	n/a	n/a	n/a
3194	1972	N	n/a	n/a	n/a
2613	1601	T	Pos/100	Pos/50	Neg/0
2615	1602	T	Neg/00	Neg/00	Pos/3+
3207	1978	T	Pos/80	Pos/30	Neg/1+
3196	1972	T	Neg/00	Neg/00	Pos/3+

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73 **Supplementary Table 2: Molecular characteristics of breast cancer tumor samples**

74 **from CUMC tumor bank.** Table contains (left to right) BlockID and PatientID, normal

75 (adjacent) or tumor sample (N/T), and clinical immunohistochemistry scoring and status

76 of ER, PR, and Her2 (only available for tumor samples). Score is numerical, status is Pos

77 or Neg.

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Supplementary References:

1   Cerami, E. *et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer discovery* **2**, 401-404, doi:10.1158/2159-8290.CD-12-0095 (2012).

2   Gao, J. *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* **6**, pl1, doi:10.1126/scisignal.2004088 (2013).

3   Saal, L. H. *et al.* Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proceedings of the National Academy of Sciences* **104**, 7564-7569, doi:10.1073/pnas.0702507104 (2007).

4   Ciriello, G. *et al.* Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. *Cell* **163**, 506-519, doi:10.1016/j.cell.2015.09.033 (2015).

5   Forbes, S. A. *et al.* COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res* **45**, D777-D783, doi:10.1093/nar/gkw1121 (2017).

6   Dai, X., Cheng, H., Bai, Z. & Li, J. Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *J Cancer* **8**, 3131-3141, doi:10.7150/jca.18457 (2017).

7   Johannsson, O. T. *et al.* Characterization of a novel breast carcinoma xenograft and cell line derived from a BRCA1 germ-line mutation carrier. *Lab Invest* **83**, 387-396 (2003).