



Title	Necessity of p53-binding to the CDH1 locus for its expression defines two epithelial cell types differing in their integrity
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## **Supplementary Information**

**Necessity of p53-binding to the *CDH1* locus for its expression defines two epithelial cell types differing in their integrity**

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### Supplementary figure legends

Figure S1 (related to Figure 2). A549 cells transfected with scramble (Scr) or JMJD3/KDM6B (JMJD3#1 or JMJD3#3) siRNA were subjected to immunoblot analysis with the indicated antibodies.

Figure S2 (related to Figure 3). A, A549 cells were subjected to ChIP-Seq using antibodies against the indicated modified histones, after the transfection of scramble (Scr RNAi) or p53 (p53 RNAi) siRNA. IGV genome browser screenshots of MACS-called peaks are shown. B, Dot plots of TCGA RNA expression profiles of *EZH2* in lung cancer or breast cancer with wild-type (wt) p53 or mutant (mut) p53. Bars represent means  $\pm$  SD. *P*-values calculated by the Student *t*-test are indicated.

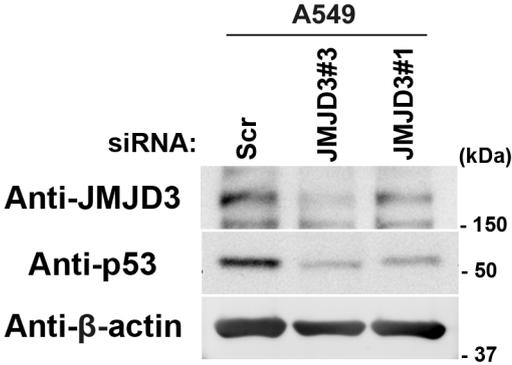
Figure S3 (related to Figure 4). Strategy for deletion of the p53-binding motif at the *CDH1* locus. A, UCSC Genome Browser view of the ENCODE data at the *CDH1* locus. The *CDH1* locus contains the p53 consensus binding motifs (a-e). Among them, the region with a motif to which p53-binding was confirmed (b) is magnified with the sequence: the p53 binding consensus is shown in red; the “TTAA” sequence used for scar-less genome editing in (B) is shown in blue; and the primer sequences used to confirm genome status in (B) are underlined. A549 cells or BJ cells were subjected to ChIP using a normal rabbit IgG or anti-p53 antibody, followed by semi-quantitative RT-PCR analysis with the primers located at the p53 consensus binding motifs (a-e) across the *CDH1* locus. B, Schematic representation of the scar-less genome editing

strategy. A homology repair template was designed to contain a gene cassette with the puromycin N-acetyltransferase gene (Puro<sup>r</sup>) and Ruby3-PEST red-fluorescent gene, which was then removed upon the expression of an excision-only piggybac transposase after the selection. Note that the original TTAA sequence was restored. Primers used to amplify the modified region are shown as horizontal arrows.

Figure S4 (related to Figure 5). A, Lysates of MCF7 cells or BJ cells treated with (+) or without (-) 10 mM butyrate for 24 h were subjected to immunoblot analysis with the indicated antibodies. B, A549 cells were treated with the indicated concentrations of hydroxycitrate for 24 h. 1.5 µg of chromatin fractions were subjected to immunoblot analysis with the indicated antibodies. C, A549 cells were cultured in the medium containing the indicated concentrations of bicarbonate for 18 h. Before cell lysis, pH of the culture medium (pHe) was measured. 1.5 µg of chromatin fractions were subjected to immunoblot analysis with the indicated antibodies.

Figure S5. Full-length blots used in the main figures.

Supplementary Figure S1



# Supplementary Figure S2

A

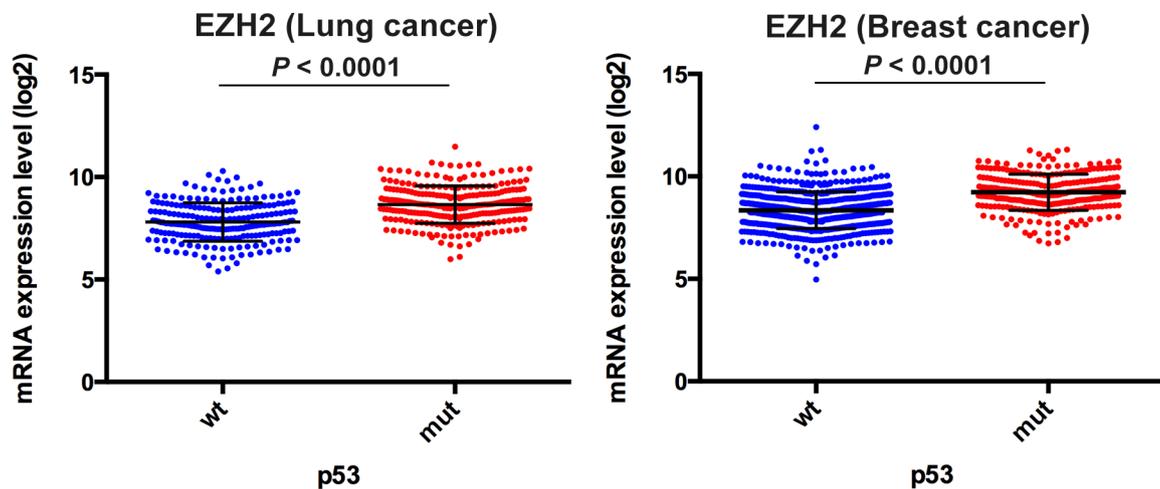


# Supplementary Figure S2

## A (continued)

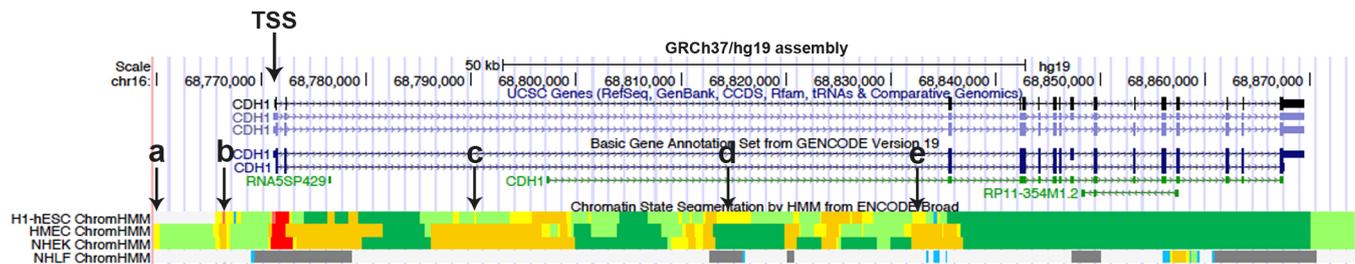


## B



# Supplementary Figure S3

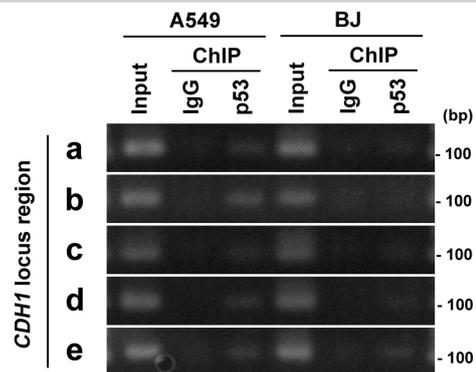
**A**



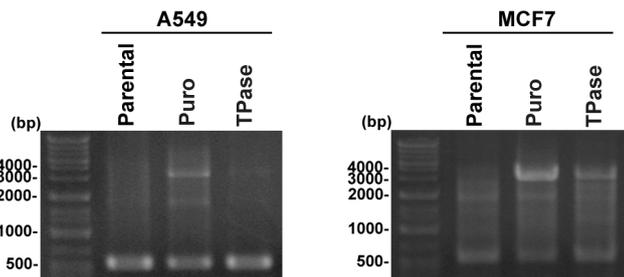
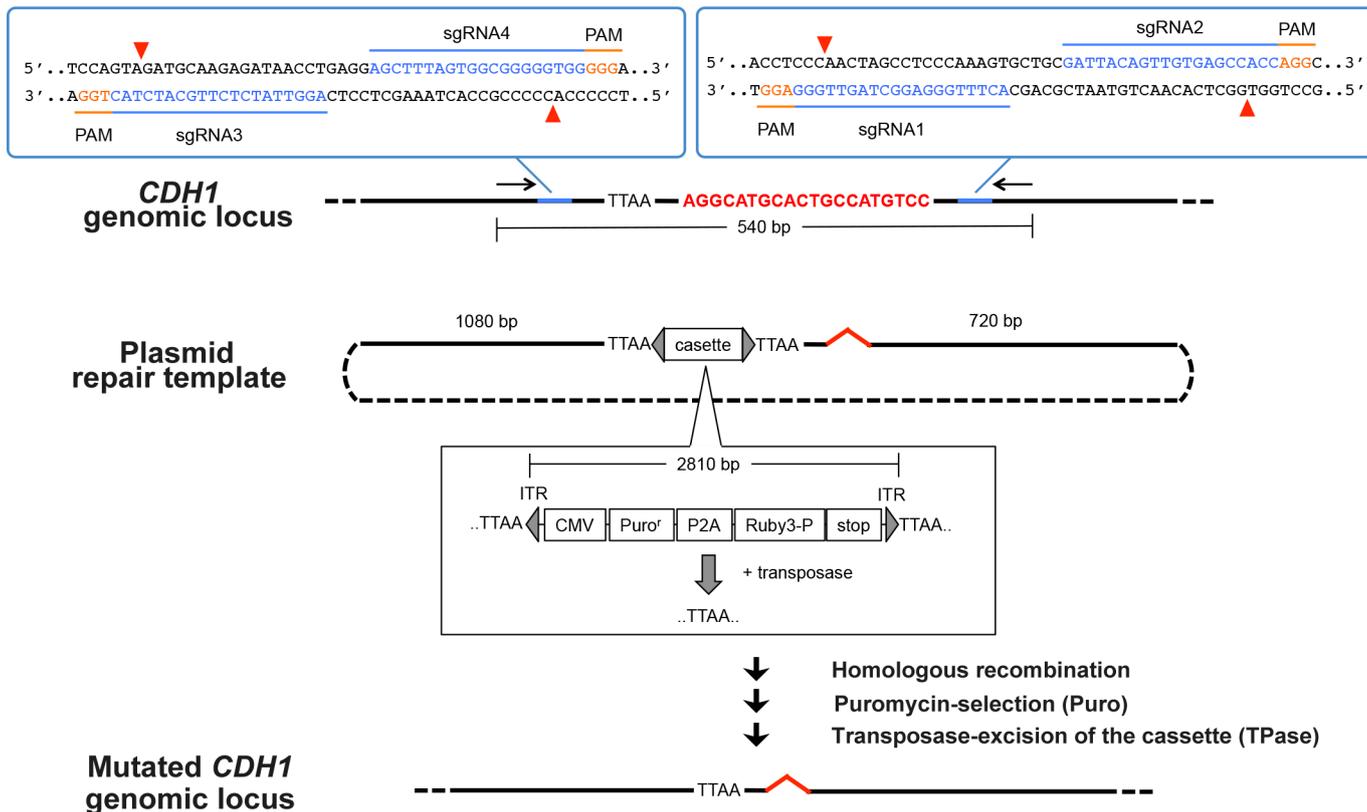
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5'..TTATCACCTGCACGGACAGGTTTGTGTACAGTCTCAGGGATTCAGGGACCCACCAGAGCCATCCACAGACACCAGGTAAAGCCCTGGACAGC
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CAGCCGGGCAACAGAGCAAGACCTTGTCTACTAAATAAATAATAGGAAACAGAGAAGCAATTCAGTGTGTTGCGAAAGTCACACAGCACCTGT..3'
    
```

p53 binding consensus

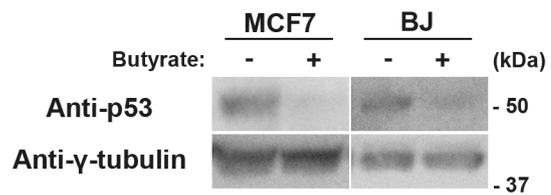


**B**

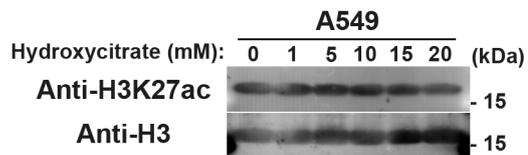


# Supplementary Figure S4

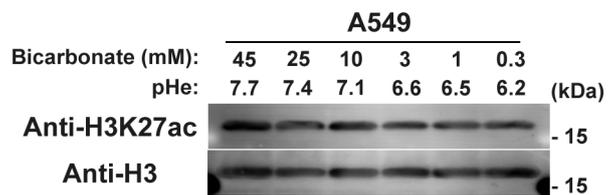
**A**



**B**



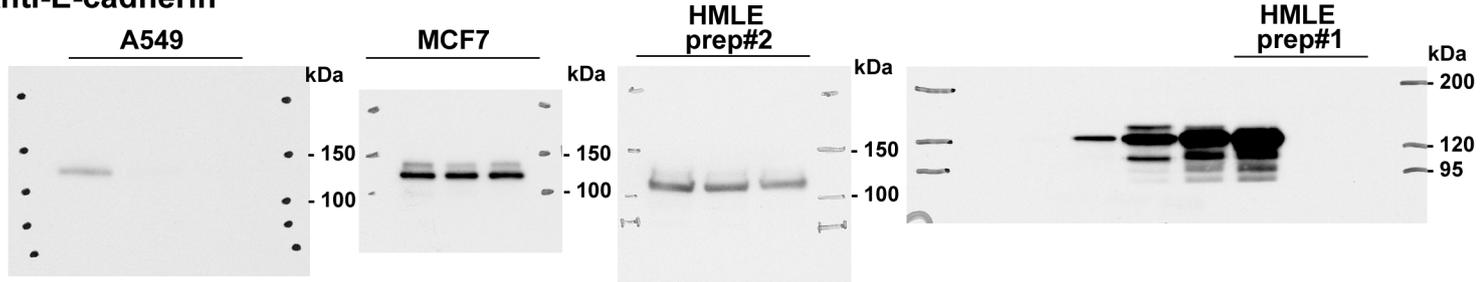
**C**



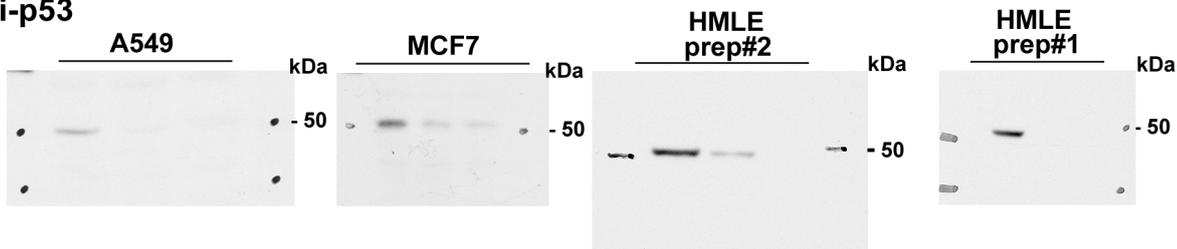
# Supplementary Figure S5

## Full-length blots used in Figure 1A

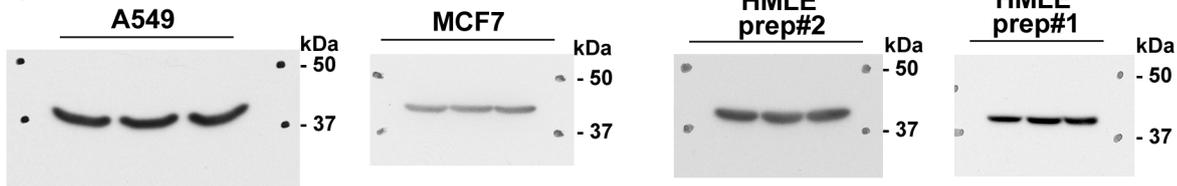
### Anti-E-cadherin



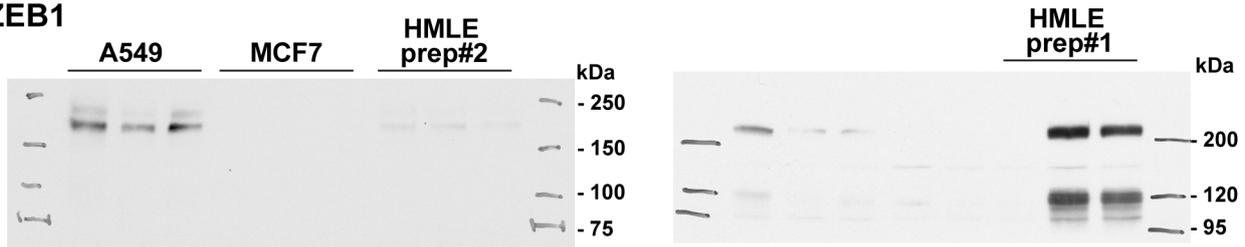
### Anti-p53



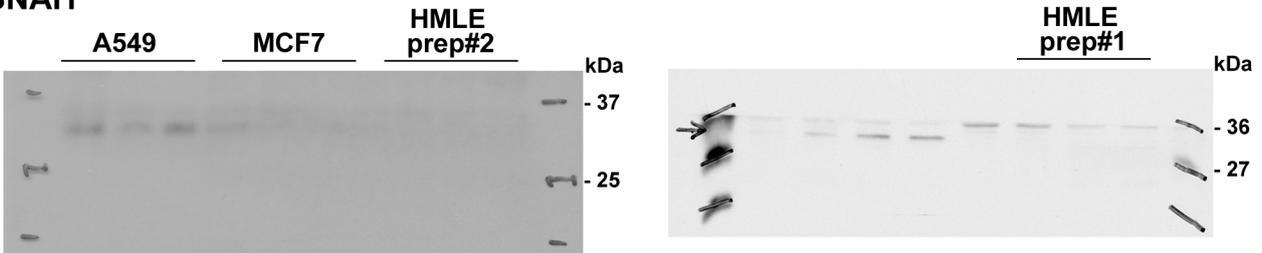
### Anti-β-actin



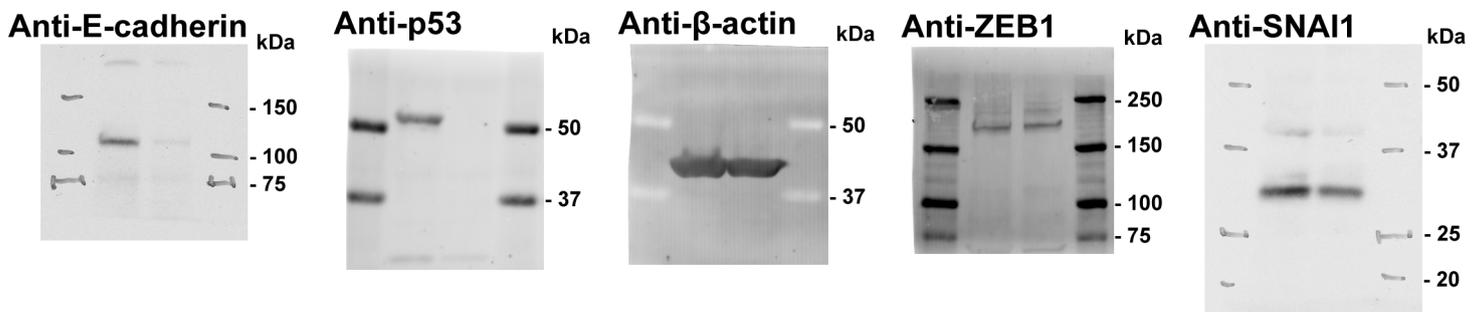
### Anti-ZEB1



### Anti-SNAI1

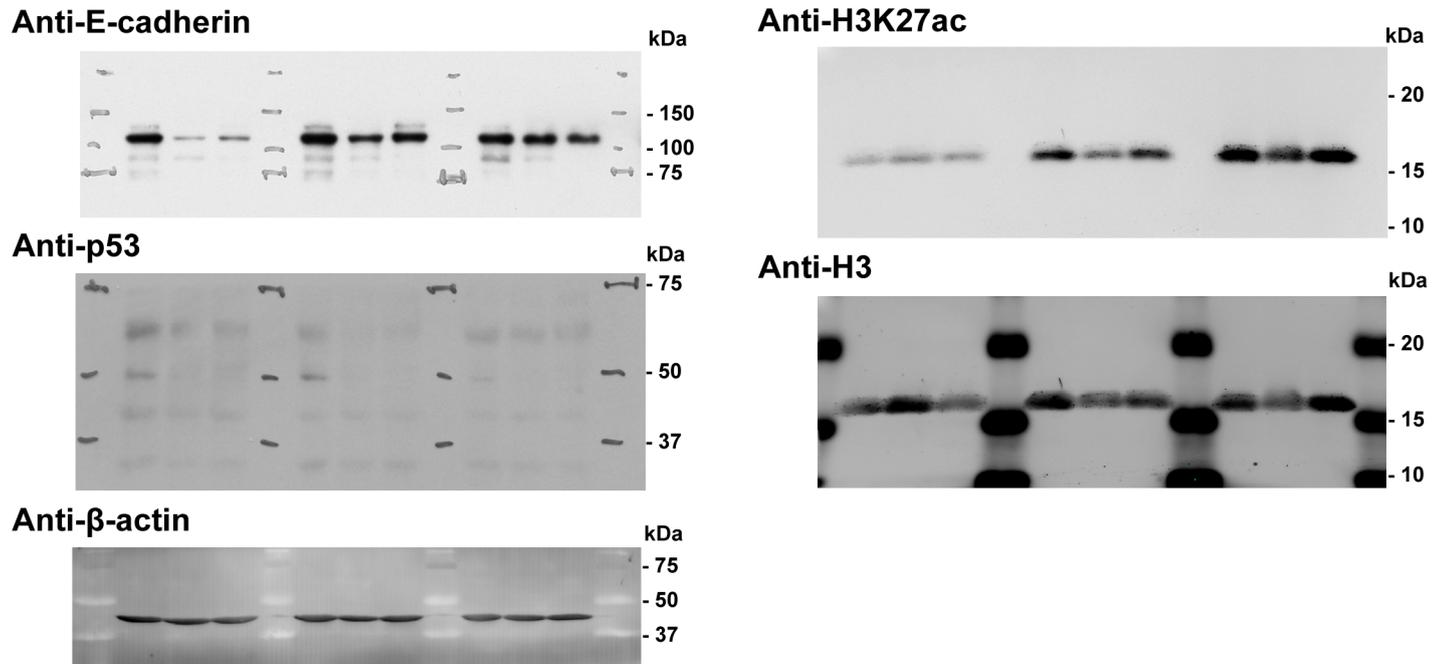


## Full-length blots used in Figure 1C

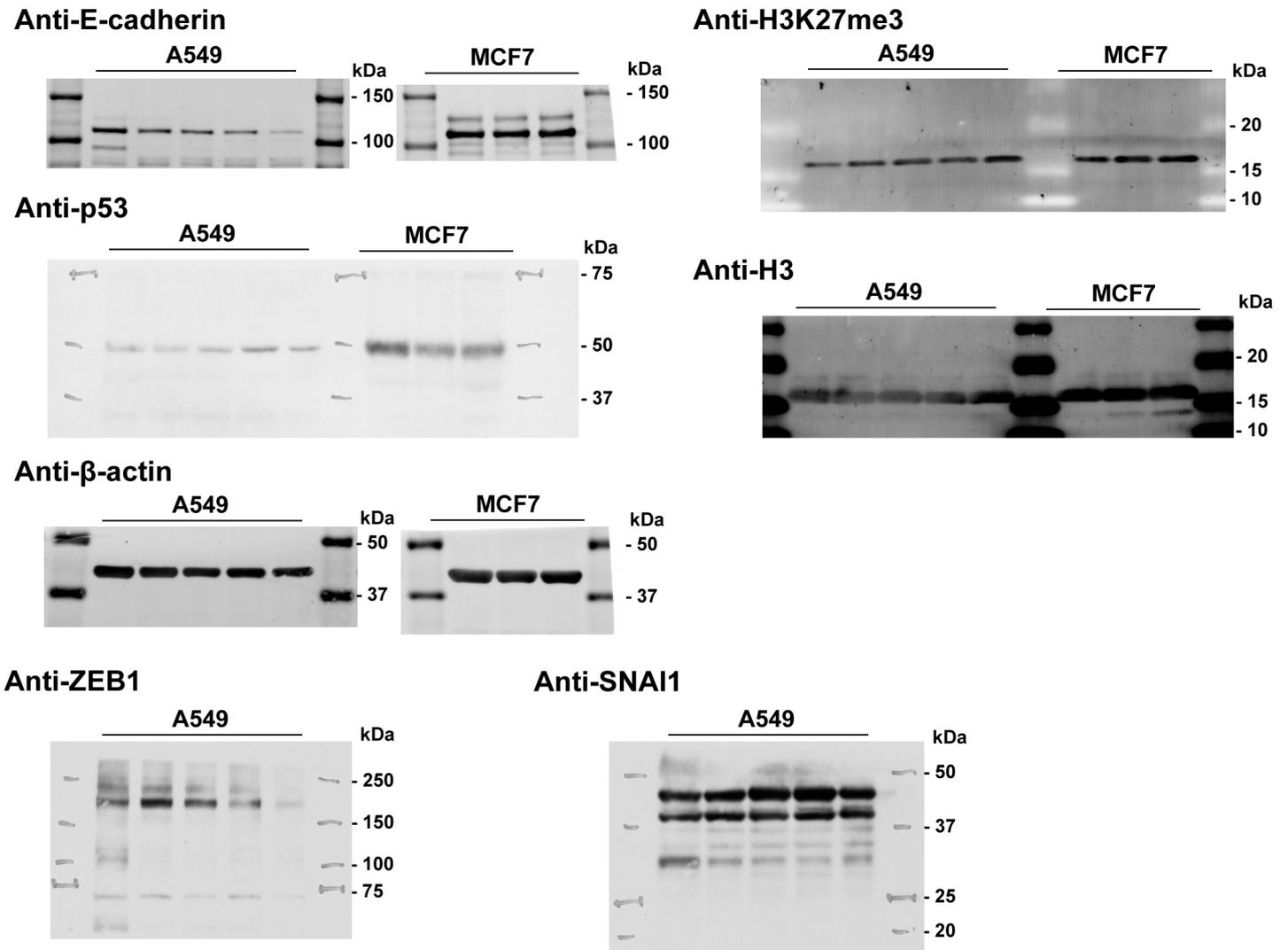


## Supplementary Figure S5

### Full-length blots used in Figure 2B



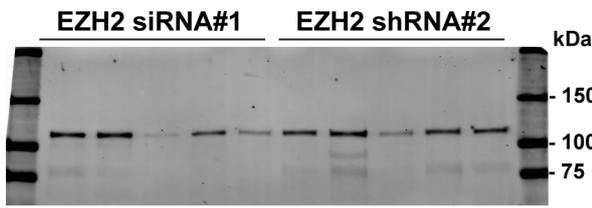
### Full-length blots used in Figure 2C



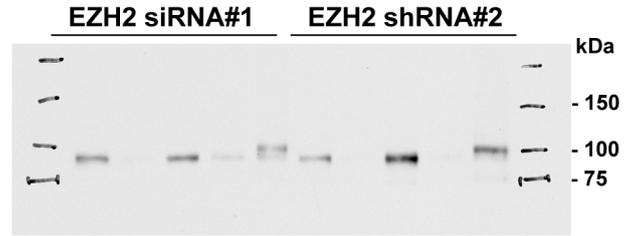
## Supplementary Figure S5

### Full-length blots used in Figure 3A

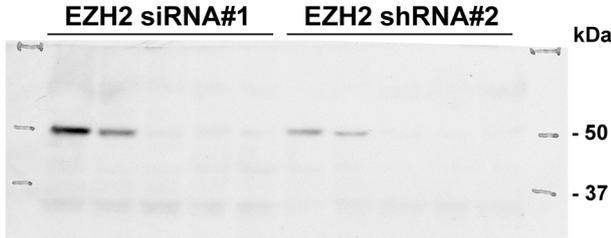
#### Anti-E-cadherin



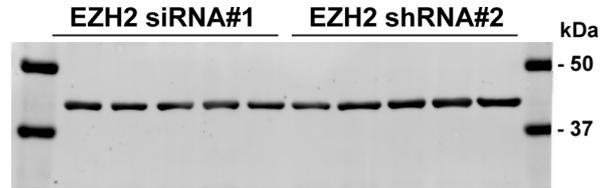
#### Anti-EZH2



#### Anti-p53

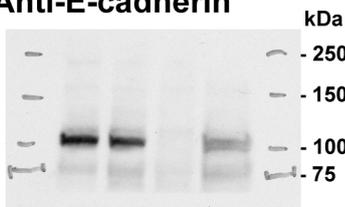


#### Anti-β-actin

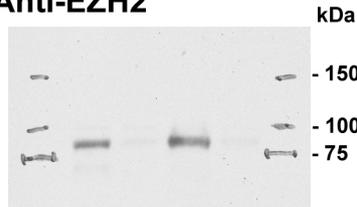


### Full-length blots used in Figure 3C

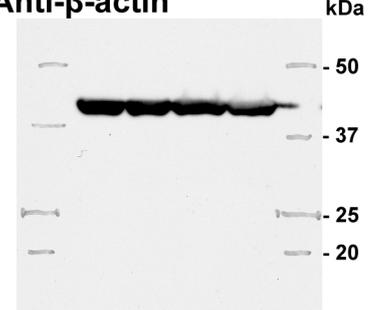
#### Anti-E-cadherin



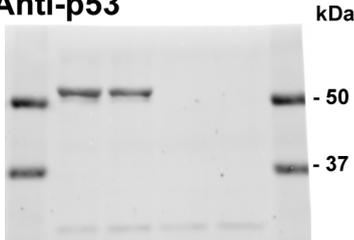
#### Anti-EZH2



#### Anti-β-actin

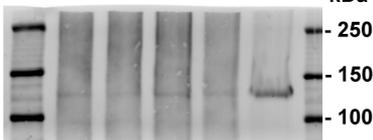


#### Anti-p53

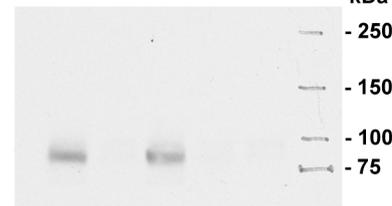


### Full-length blots used in Figure 3D

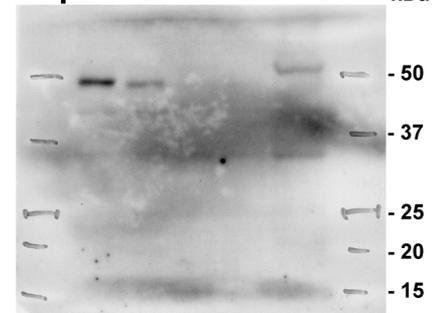
#### Anti-N-cadherin



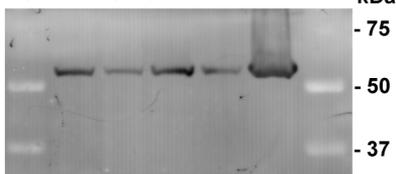
#### Anti-EZH2



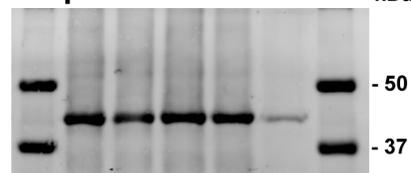
#### Anti-p53



#### Anti-vimentin



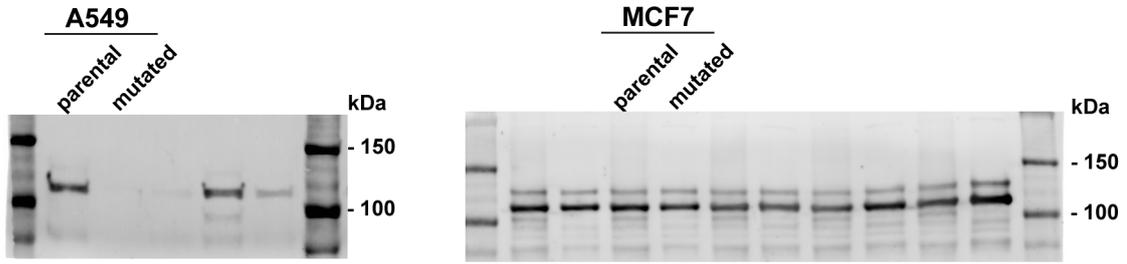
#### Anti-β-actin



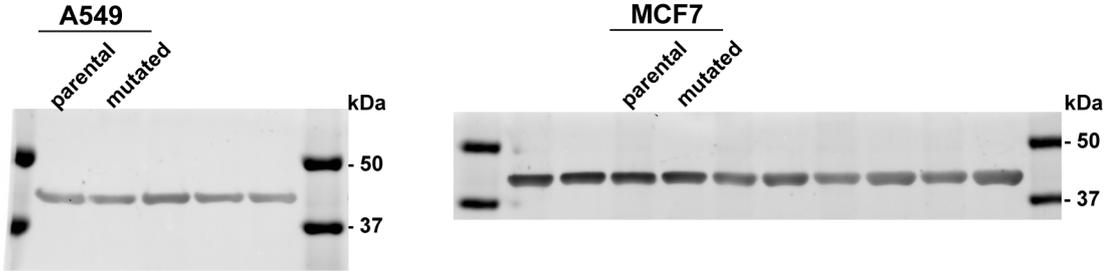
# Supplementary Figure S5

## Full-length blots used in Figure 4E

### Anti-E-cadherin

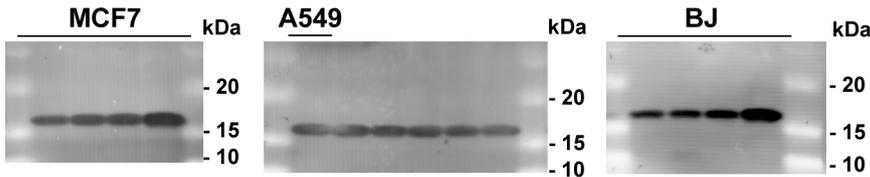


### Anti- $\beta$ -actin

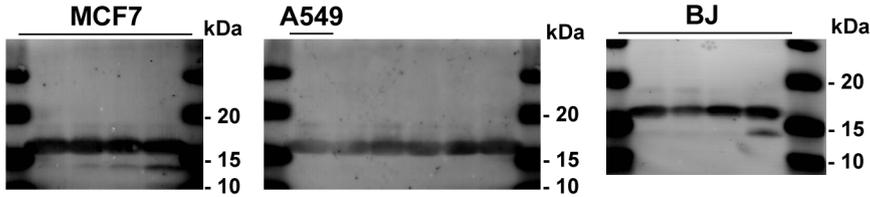


## Full-length blots used in Figure 5B

### Anti-H3K27ac

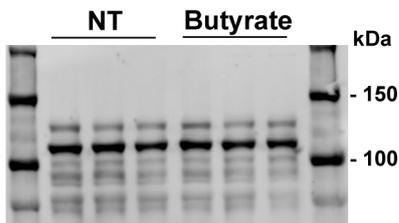


### Anti-H3

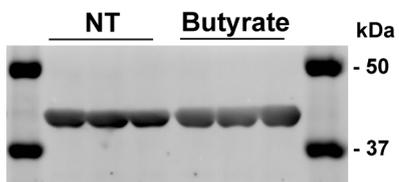


## Full-length blots used in Figure 5E

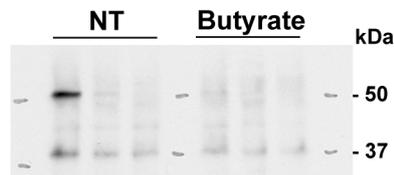
### Anti-E-cadherin



### Anti- $\beta$ -actin



### Anti-p53



long exposure

