Supplementary Materials

ZMYND8 expression in breast cancer cells blocks T-lymphocyte surveillance to promote tumor growth

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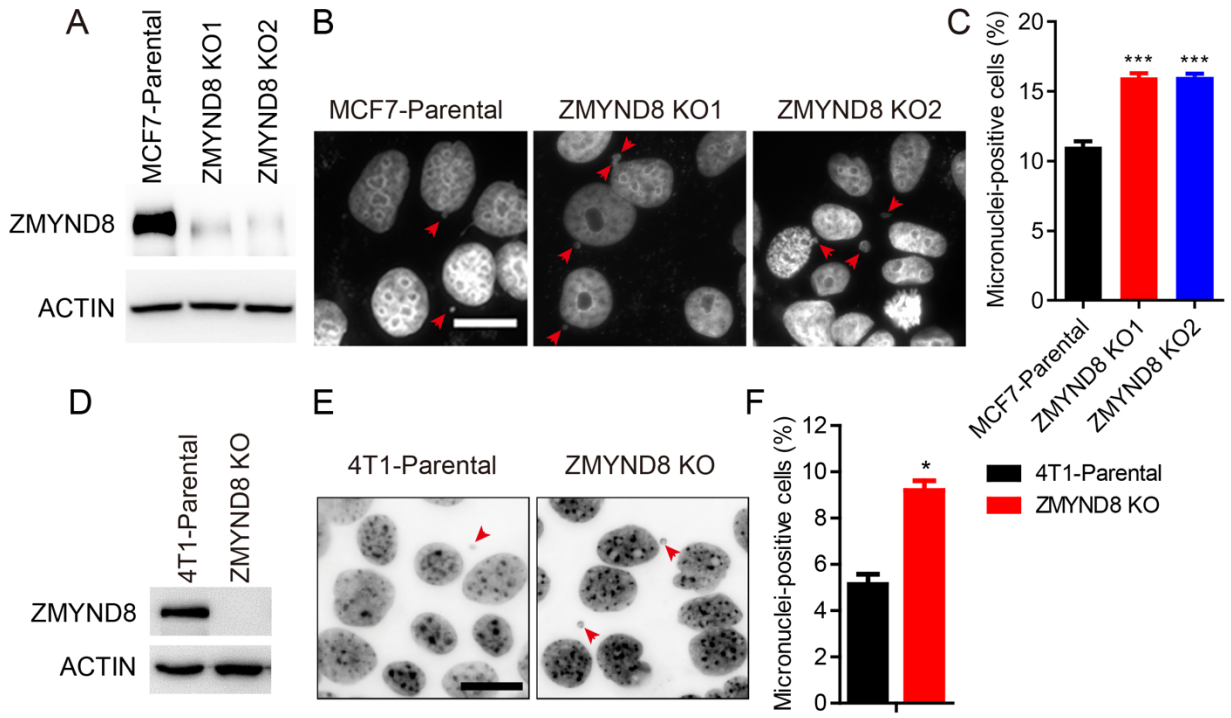
Supplementary Table 1. The sgRNA Sequences

Target genes	Sequences	Target location
mouse ZMYND8 #1	5’-GCAGTTGTGCAGGATCCACT-3’	exon 8
mouse ZMYND8 #2	5’-CGGGTTTATCACGCTAAGTG-3’	exon 5
mouse cGAS #1	5’-CCGAGGCGCGGAAAGTCGTA-3’	exon 1
mouse cGAS #2	5’-AAAGGGGGGCTCGATCGCGG-3’	exon 1
mouse STING #1	5’-GATGATCCTTTGGGTGGCAA-3’	exon 1
mouse STING #2	5’-GTTGAAAAACCTCTGCTGTC-3’	exon 3
mouse P65 #1	5’-ATCGAACAGCCGAAGCAACG-3’	exon 3
SC	5’-ACGGAGGCTAAGCGTCGCAA-3’	

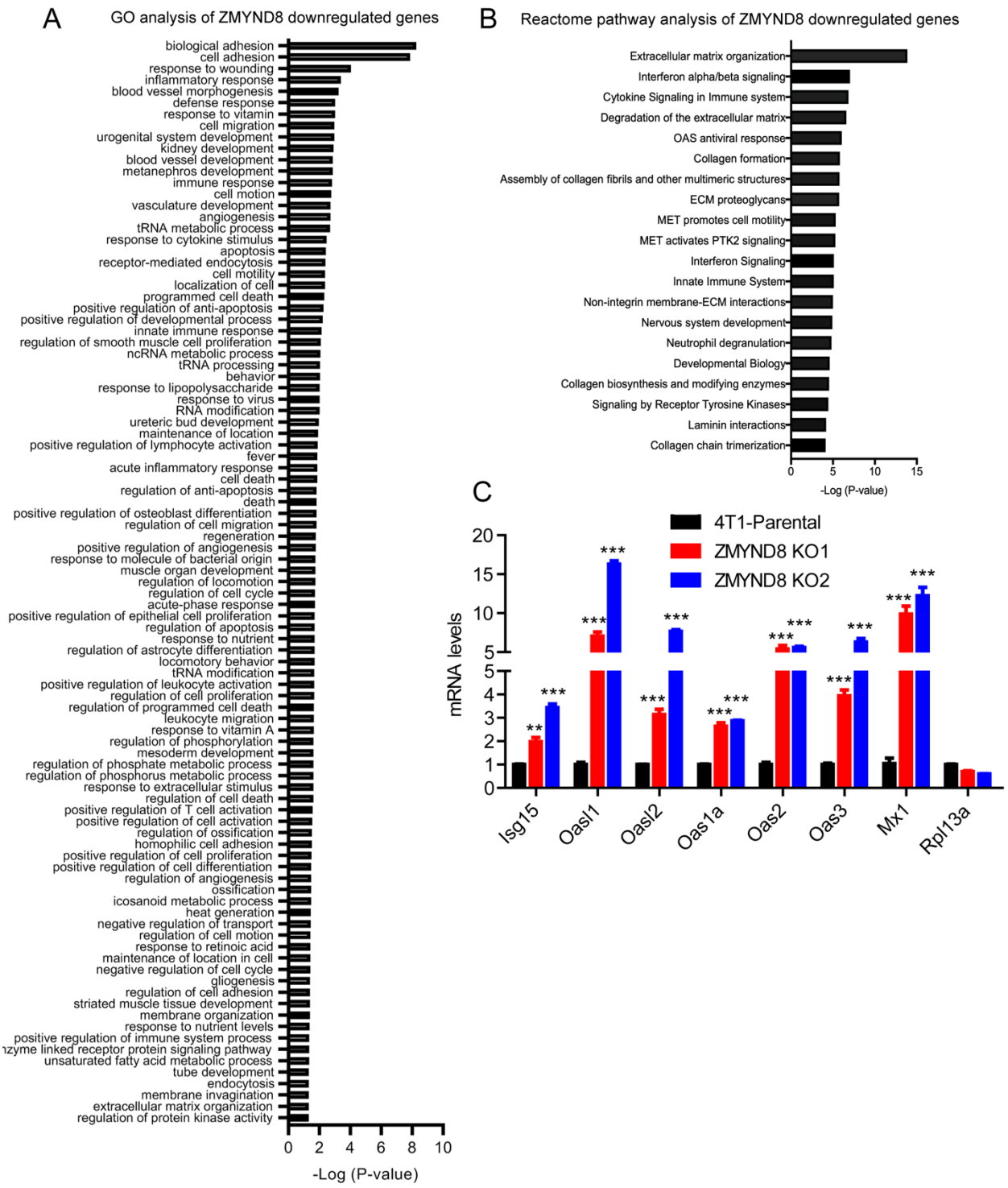
Supplementary Table 2. qPCR Primers

human IFN-β (RT-qPCR)	Forward: 5’-ATGACCAACAAGTGTCTCCTCC-3’
	Reverse: 5’-GGAATCCAAGCAAGTTGTAGCTC-3’
human ISG15 (RT-qPCR)	Forward: 5’-ACTCATCTTTGCCAGTACAGG-3’
	Reverse: 5’-CAGCTCTGACACCGACATG-3’
human OSAL (RT-qPCR)	Forward: 5’-GTGGCAGAAGGGTACAGATG-3’
	Reverse: 5’-CTGTCAAGTGGATGTCTCGTG-3’
human OAS1 (RT-qPCR)	Forward: 5’-CATCTGTGGGTTCCTGAAGG-3’
	Reverse: 5’-GAGAGGACTGAGGAAGACAAC-3’
human OAS2 (RT-qPCR)	Forward: 5’-GTTATCCTCTCCCTGCTTCAAG-3’
	Reverse: 5’-GGATTCTATGGCTTCTGGATGAG-3’
human RPL13A (RT-qPCR)	Forward: 5’-CTCAAGGTCGTGCGTCTG-3’
	Reverse: 5’-TGGCTTTCTCTTTCCTCTTCTC-3’
human 18S rRNA (RT-qPCR)	Forward: 5’-CGGCGACGACCCATTCGAAC-3’
	Reverse: 5’-GAATCGAACCCTGATTCCCCGTC-3’
mouse Ifn-β (RT-qPCR)	Forward: 5’-AGCTCCAAGAAAGGACGAACA-3’
	Reverse: 5’-GCCCTGTAGGTGAGGTTGAT-3’
mouse Isg15 (RT-qPCR)	Forward: 5’-CCCAGTGCACAGTGATCAAG-3’
	Reverse: 5’-TCCAATGCTATCCCAAAAGTCC-3’
mouse Oasl1 (RT-qPCR)	Forward: 5’-CTGTATCTACTGGACCAAGCAC-3’
	Reverse: 5’-GCCACTATGTCCCATCTGTAG-3’
mouse Oasl2 (RT-qPCR)	Forward: 5’-ATCATTGTCCTTACCCACAGAG-3’
	Reverse: 5’-TGCTGGTTTTGAGTCTCTGG-3’
mouse Oas1a (RT-qPCR)	Forward: 5’-AGAGATGCTTCCAAGGTGC-3’
	Reverse: 5’-ACTGATCCTCAAAGCTGGTG-3’
mouse Oas2 (RT-qPCR)	Forward: 5’-GCCTGAATACTGAGTCACCTG-3’
	Reverse: 5’-TTCAGTAAGTGGCTTGGAGTG-3’
mouse Oas3 (RT-qPCR)	Forward: 5’-TGCTACTGGTCAAACACTGG-3’
	Reverse: 5’-CTGAACTTTTGCTCTCCACAG-3’

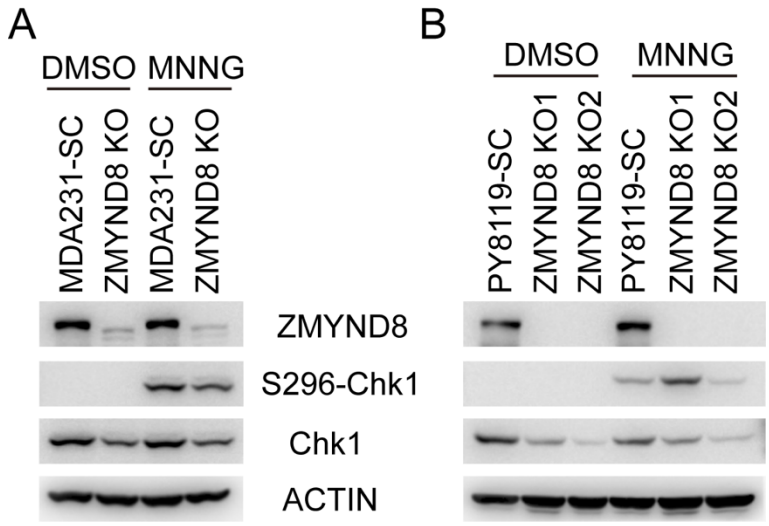
mouse Mx1 (RT-qPCR)	Forward: 5’-GACCATAGGGGTCTTGACCAA-3’
	Reverse: 5’-AGACTTGCTCTTTCTGAAAAGCC-3’
mouse Cxcl10 (RT-qPCR)	Forward: 5’-TCAGCACCATGAACCCAAG-3’
	Reverse: 5’-CTATGGCCCTCATTCTCACTG-3’
mouse Rpl13a (RT-qPCR)	Forward: 5’-GGGCAGGTTCTGGTATTGGAT-3’
	Reverse: 5’-GGCTCGGAAATGGTAGGGG-3’
mouse Actin (RT-qPCR)	Forward: 5’-GGCTGTATTCCCCTCCATCG-3’
	Reverse: 5’-CCAGTTGGTAACAATGCCATGT-3’
mouse Ifnb1 (ChIP-qPCR)	Forward: 5’-ATTCCTCTGAGGCAGAAAGGACCA-3’
	Reverse: 5’-GCAAGATGAGGCAAAGGCTGTCAA-3’
mouse Isg15 (ChIP-qPCR)	Forward: 5’-CCTTTCCTTCCCAACTACAG-3’
	Reverse: 5’-TTCCAGAAACCAGAGCTATTT-3’
mouse Oas2 (ChIP-qPCR)	Forward: 5’-GCTAGCTGGAAGCAAACA-3’
	Reverse: 5’-GAAAGAGGAAATCAAGTCCAAAG-3’
mouse Oas3 (ChIP-qPCR)	Forward: 5’-GGAGACAGCAGAACCATAAA-3’
	Reverse: 5’-GTCACCTCCCTTCACTTTC-3’
mouse Mx1 (ChIP-qPCR)	Forward: 5’-CGGTTTCAATTCTCCTCTGG-3’
	Reverse: 5’-CTGTGCAATACTCACCACTC-3’



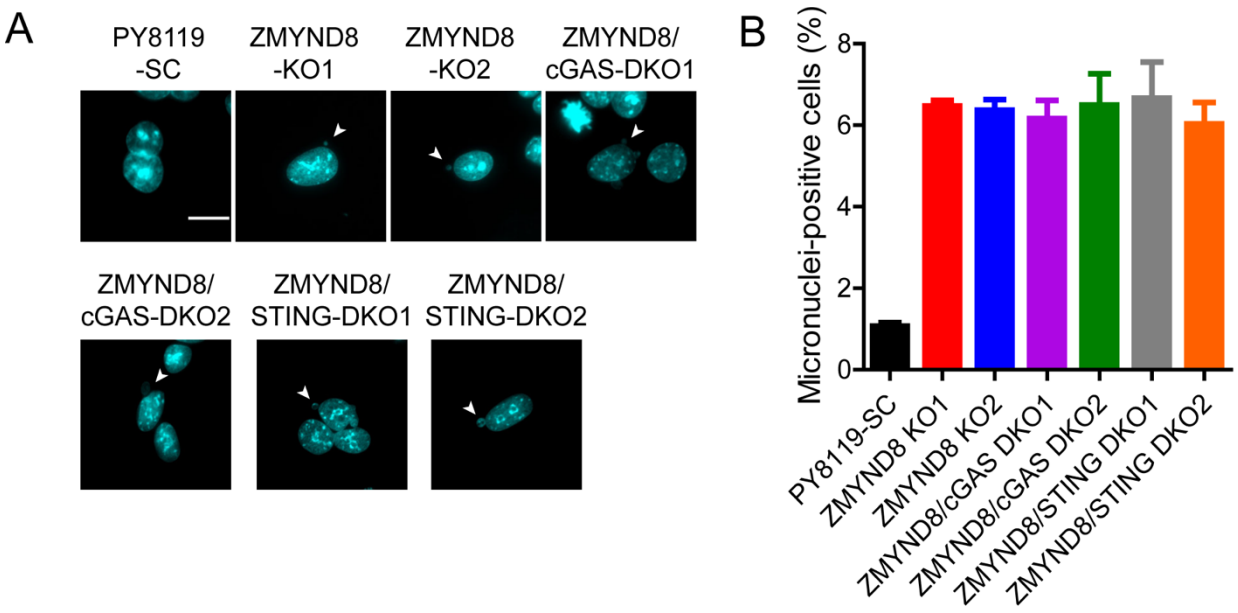
Supplementary Figure 1. **ZMYND8 KO increases micronucleus formation in breast cancer cells.** (**A**) Immunoblot analysis of ZMYND8 and actin in parental, ZMYND8 KO1, and ZMYND8 KO2 MCF-7 cells. (**B**) Representative images of micronuclei (indicated by red arrows) in parental, ZMYND8 KO1, and ZMYND8 KO2 MCF-7 cells. Scale bar, 10 μm. (**C**) Quantification of the number of micronuclei in parental, ZMYND8 KO1, and ZMYND8 KO2 MCF-7 cells (n = 200-300, mean ± SEM). ***$p < 0.001$ vs. MCF-7 by one-way ANOVA with Dunnett's test. (**D**) Immunoblot analysis of ZMYND8 and actin in parental and ZMYND8 KO 4T1 cells. (**E**) Representative images of micronuclei (indicated by red arrows) in parental and ZMYND8 KO 4T1 cells. Scale bar, 10 μm. (**F**) Quantification of the number of micronuclei in parental and ZMYND8 KO 4T1 cells (n = 458-521, mean ± SEM). *$p < 0.05$ vs. 4T1 by Student's t test.



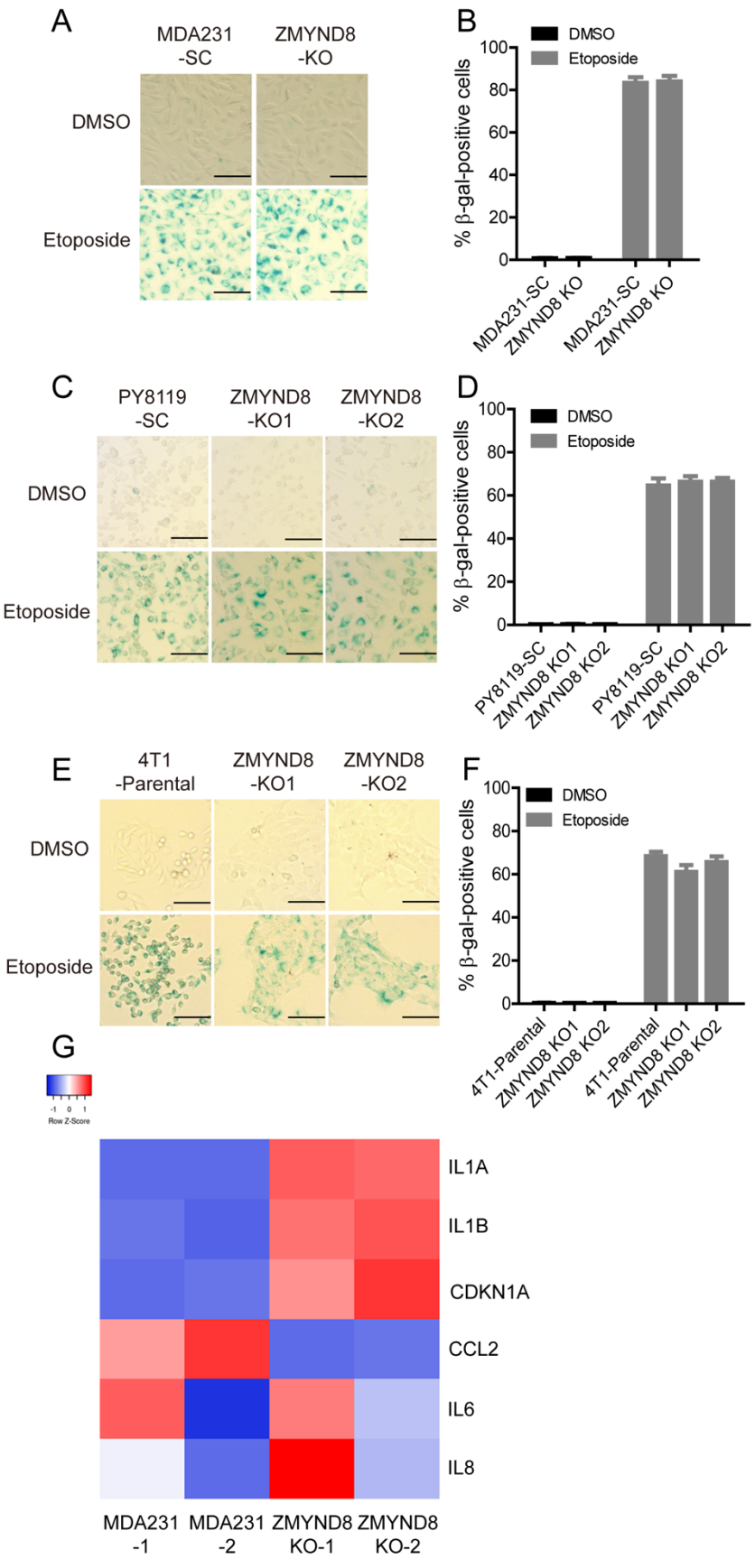
Supplementary Figure 2. ZMYND8 suppresses ISGs in breast cancer cells. (**A**) GO analysis of ZMYND8-downregulated genes. (**B**) Reactome pathway analysis of ZMYND8-downregulated genes. Top 20 pathways are shown. (**C**) RT-qPCR analysis of ISGs and Rpl13a mRNAs in SC, ZMYND8 KO1, and ZMYND8 KO2 4T1 cells ($n = 3$, mean ± SEM). $^{**}p < 0.01$; $^{***}p < 0.001$ vs. SC by one-way ANOVA with Dunnett's test.



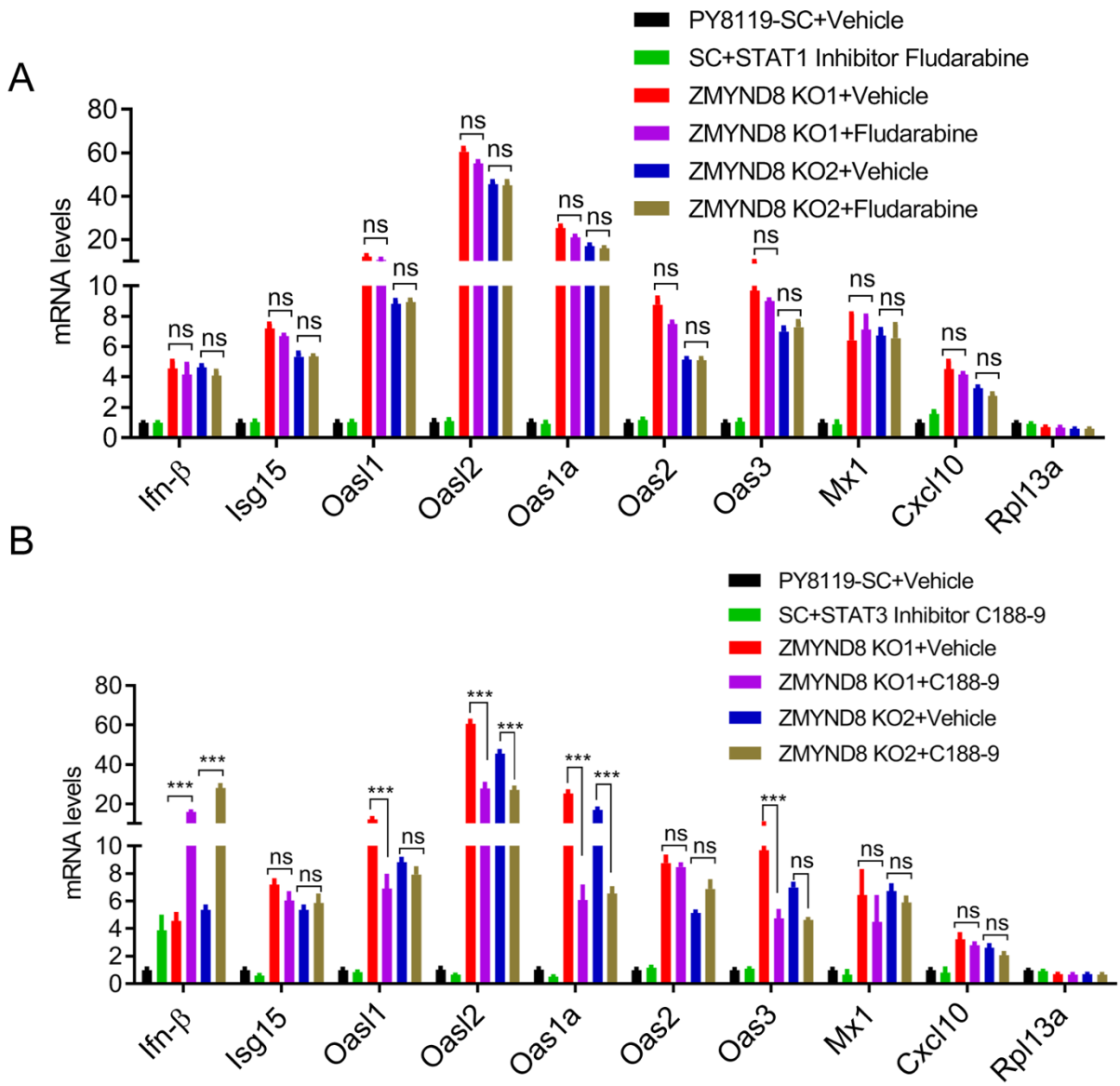
Supplementary Figure 3. ZMYND8 loss fails to induce the cell-cycle checkpoint response in breast cancer cells. SC and ZMYND8 KO MDA-MB-231 (MDA231, **A**) and PY8119 (**B**) cells were treated with DMSO or MNNG (75 μM) for 15 min and then incubated for additional 4 hours. Immunoblot assay was performed with indicated antibodies. Representative immunoblots from two independent experiments are shown.



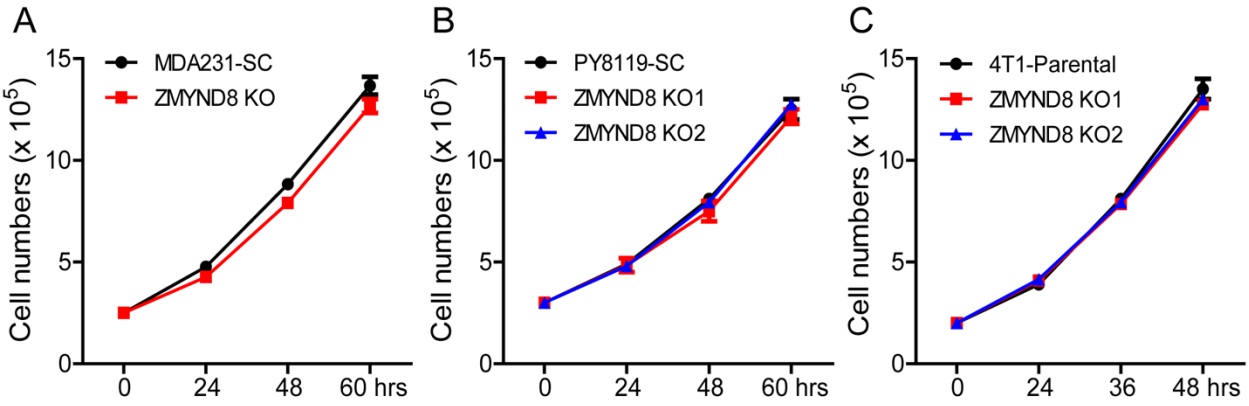
Supplementary Figure 4. Deletion of cGAS or STING does not lead to a further increase of micronuclei in ZMYND8 KO cells. (**A**) Representative immunostaining of micronuclei in SC, ZMYND8 KO1, ZMYND8 KO2, ZMYND8/cGAS DKO1, ZMYND8/cGAS DKO2, ZMYND8/STING DKO1, and ZMYND8/STING DKO2 PY8119 cells. Scale bar, 10 μm. (**B**) Quantification of micronuclei in SC, ZMYND8 KO1, ZMYND8 KO2, ZMYND8/cGAS DKO1, ZMYND8/cGAS DKO2, ZMYND8/STING DKO1, and ZMYND8/STING DKO2 PY8119 cells (n = 300, mean ± SEM).


A
MDA231 -SC
ZMYND8 -KO
DMSO
Etoposide
B
DMSO
Etoposide
% β-gal-positive cells
100
80
60
40
20
0
MDA231-SC
ZMYND8 KO
MDA231-SC
ZMYND8 KO
C
PY8119 -SC
ZMYND8 -KO1
ZMYND8 -KO2
DMSO
Etoposide
D
DMSO
Etoposide
% β-gal-positive cells
100
80
60
40
20
0
PY8119-SC
ZMYND8 KO1
ZMYND8 KO2
PY8119-SC
ZMYND8 KO1
ZMYND8 KO2
E
4T1 -Parental
ZMYND8 -KO1
ZMYND8 -KO2
DMSO
Etoposide
F
DMSO
Etoposide
% β-gal-positive cells
100
80
60
40
20
0
4T1-Parental
ZMYND8 KO1
ZMYND8 KO2
4T1-Parental
ZMYND8 KO1
ZMYND8 KO2
G
-1 0 1
Row Z-Score
IL1A
IL1B
CDKN1A
CCL2
IL6
IL8
MDA231 -1
MDA231 -2
ZMYND8 KO-1
ZMYND8 KO-2

Supplementary Figure 5. ZMYND8 loss fails to induce senescence in breast cancer cells. (**A-F**) SC/parental and ZMYND8 KO MDA-MB-231 (MDA231, **A** and **B**), PY8119 (**C** and **D**), and 4T1 (**E** and **F**) cells were treated with DMSO or etoposide (12.5 μM) for 24 hours and subjected to senescence-associated β-galactosidase assay. Representative images are shown in **A**, **C**, and **E**. The percentage of β-galactosidase (gal)-positive cells is quantified in **B**, **D**, and **F** (n = 3, mean ± SEM). Scale bar, 10 μm. (**G**) The heatmap of senescence-associated gene expression in parental and ZMYND8 KO MDA-MB-231 cells. Gene expression data were retrieved from RNA-seq (GSE108833).



Supplementary Figure 6. STAT1 and STAT3 are not involved in ZMYND8 KO-induced IFN-β and ISGs expression in PY8119 cells. (**A**) SC, ZMYND8 KO1, and ZMYND8 KO2 PY8119 cells were treated with a STAT1 inhibitor Fludarabine or vehicle for 24 hours, and subjected to RT-qPCR analysis of IFN-β, ISGs, and Rpl13a mRNAs (n = 3, mean ± SEM). ns, not significant. (**B**) SC, ZMYND8 KO1, and ZMYND8 KO2 PY8119 cells were treated with a STAT3 inhibitor C188-9 or vehicle for 24 hours, and subjected to RT-qPCR analysis of IFN-β, ISGs, and Rpl13a mRNAs (n = 3, mean ± SEM). ***$p < 0.001$ by two-way ANOVA with Tukey's test.



Supplementary Figure 7. ZMYND8 loss has no effect on cell proliferation *in vitro*. SC or parental and ZMYND8 KO MDA-MB-231 (MDA231, **A**), PY8119 (**B**), and 4T1 (**C**) cells were subjected to *in vitro* cell growth assay using trypan blue 24, 48, and 60 hours after culture. Data are shown in mean ± SEM (n = 3).