Supplemental Figures

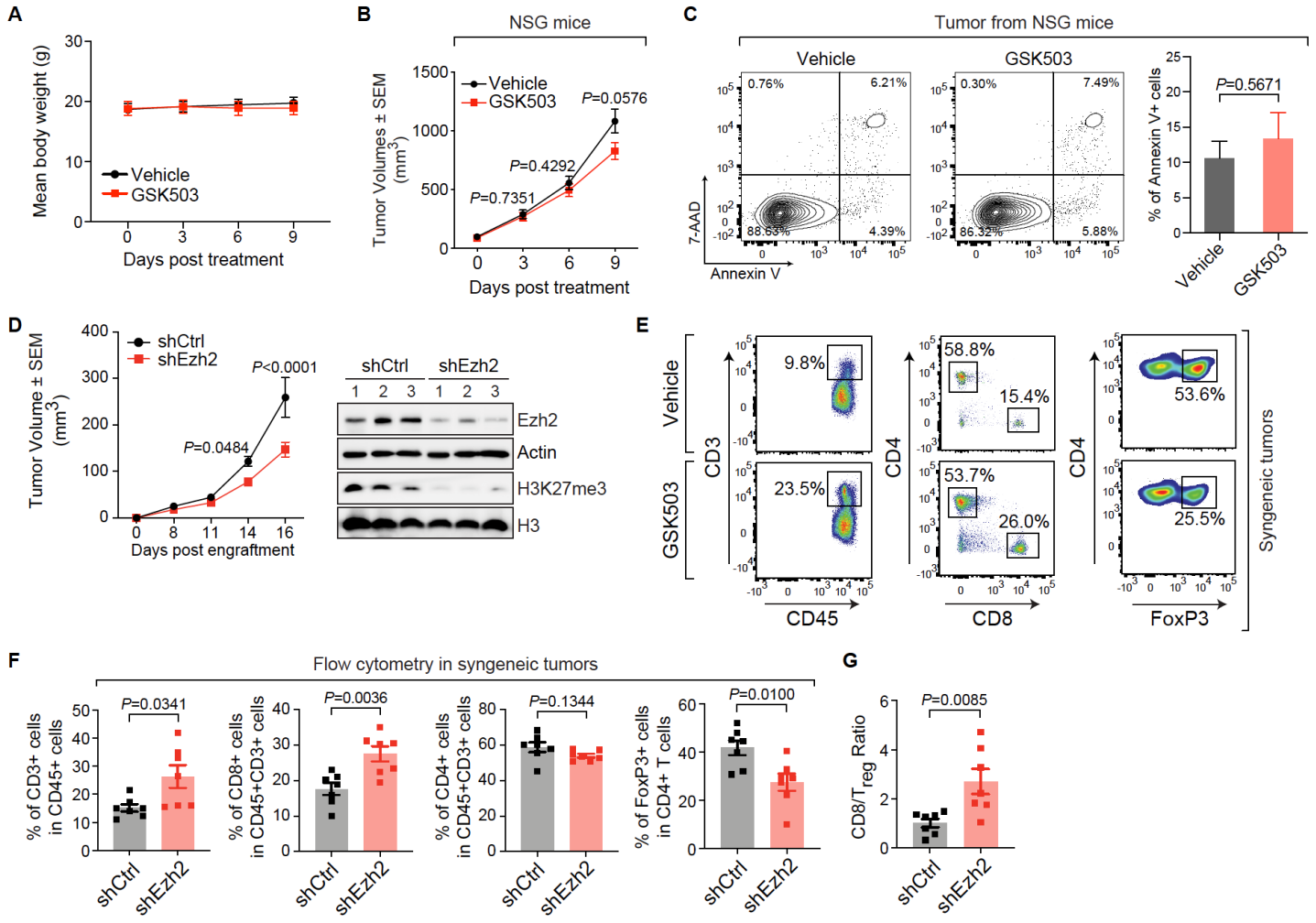

Fig. S1. Depletion of Ezh2 in ERα-positive (ERα+) breast cancer cells retards tumor growth and induces an antitumor microenvironment in immunocompetent mice. (**A**) Body weights of 67NR-derived syngeneic BALB/c mice receiving vehicle or 150 mg/kg GSK503 over the treatment period (n=12 per group). (**B-C**) Growth (n=10 per group, **B**) and Annexin V/7-amino-actinomycin (AAD) staining (n=3 per group, **C**) of allograft tumors developed by injecting 67NR cells into NSG mice followed by the treatment with vehicle or 150 mg/kg GSK503. Left and right panels in (**C**), representative density plots and quantification of Annexin V-positive (Annexin V+) cells in total cells analyzed in flow cytometry, respectively. (**D**) Growth (left panel, n=7 per group) and immunoblotting analysis (right panel) of breast tumors developed by injecting 67NR clones stably expressing control shRNA (shCtrl) or Ezh2-specific shRNA (shEzh2) in immunocompetent BALB/c mice. Numbers in right panel, three independent allograft tumors. (**E**) Density plots from the flow cytometry analysis of T cells with specified markers in the allograft tumors treated with vehicle or 150 mg/kg GSK503. (**F-G**) Quantification of flow cytometry results showing proportions of T cells with specified markers **(F)** and ratios of $CD8^+$ T cells to Treg cells **(G)** in allograft tumors expressing control shRNA (shCtrl) or Ezh2-targeting shRNA (shEzh2).

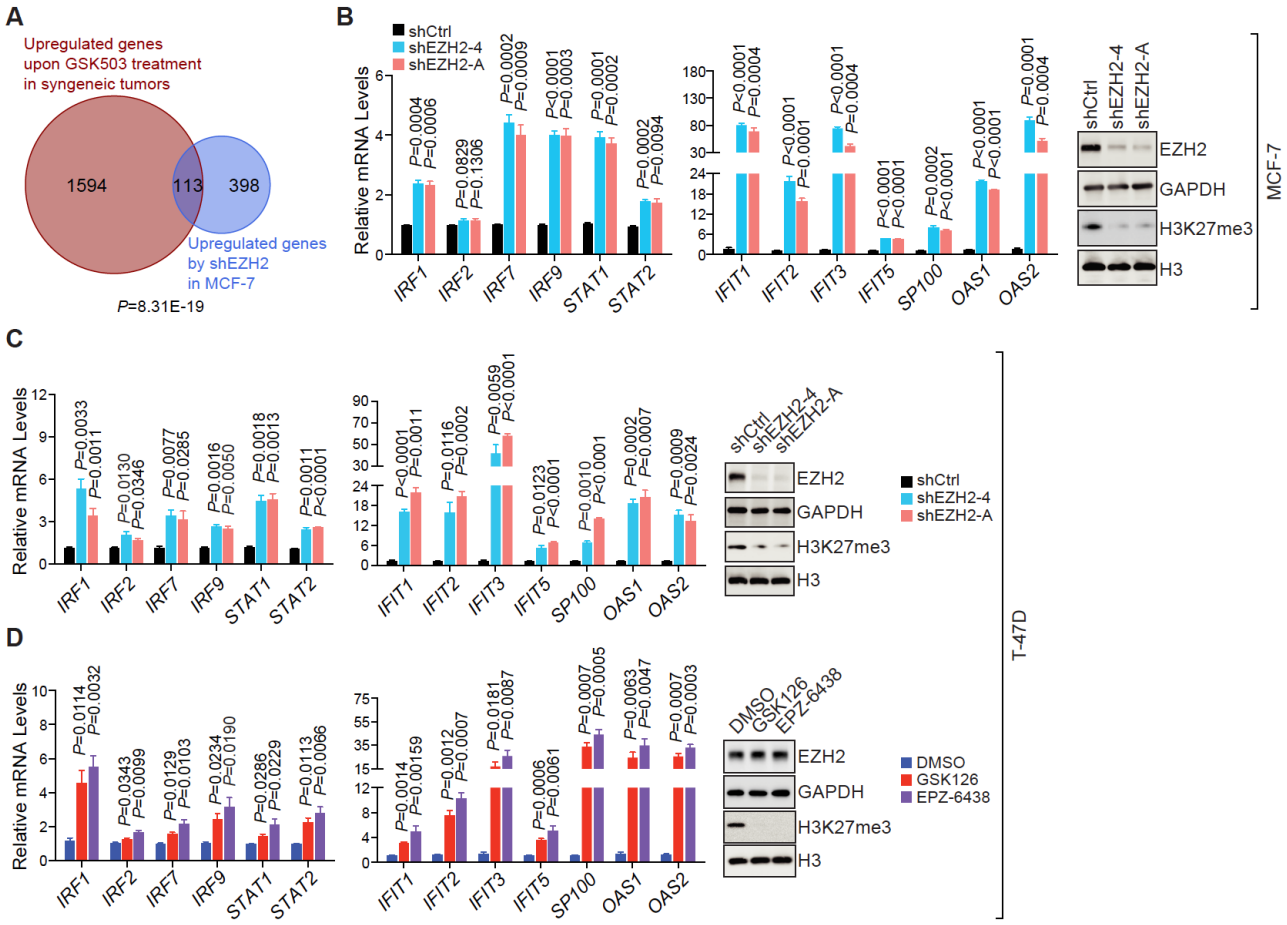

Fig. S2. EZH2 inhibition increases the expression of interferon-stimulated genes (ISGs) in ERα+ breast cancer cells.

(**A**) Venn diagram showing the overlaps between genes upregulated by EZH2 inhibitor (GSK503) in 67NR-derived syngeneic mouse model and EZH2 knockdown (shEZH2) in MCF-7 cells. (**B-D**) Expression of paradigm ISGs (left panel) and immunoblotting analysis (right panel) in MCF-7 (**B**) and T-47D (**C**) cells that were infected with control shRNA (shCtrl) or two independent shRNAs targeting EZH2 (shEZH2-4 and -A) or in T-47D cells that were treated with DMSO or 5 μM EZH2 inhibitor (GSK126 and EPZ-6438) for 7 days (**D**).

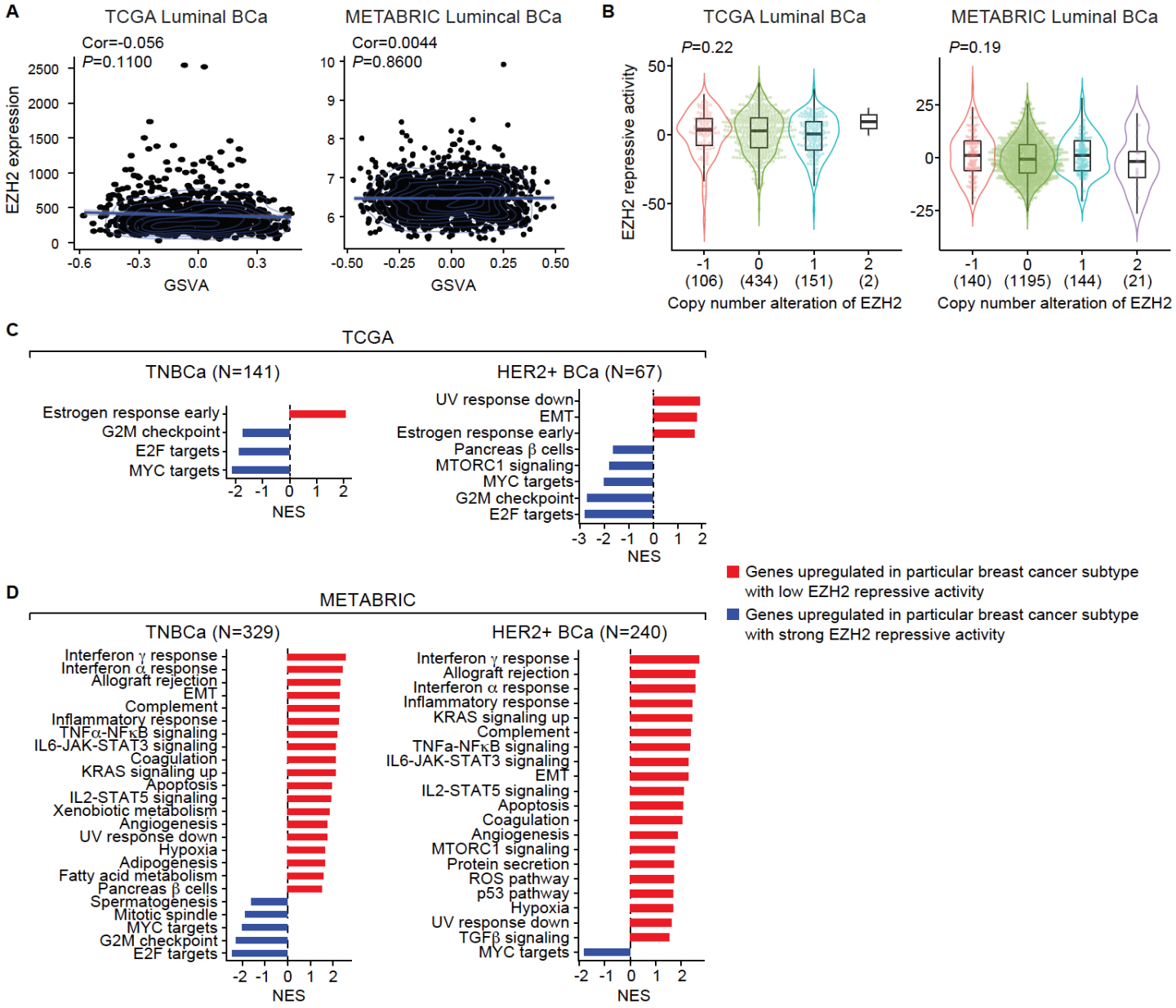

Fig. S3. Association between the repressive activity of EZH2 and interferon signaling is verified in different subtypes of breast cancer. (**A**) Correlation between the Gene Set Variation Analysis (GSVA) scores of the 53 signature genes representing EZH2 repressive activity and the expression of EZH2 gene in patients with luminal breast cancer (BCa) in the TCGA (1) and METABRIC (2) dataset. Correlation coefficients (Cor) were determined by Pearson correlation. (**B**) Violin plots showing GSVA scores of the 53 signature genes reflecting the repressive activity of EZH2 in four groups of ERα+ breast cancer (Luminal BCa) with specified copy-number levels of EZH2 gene (-1, 0, 1 and 2) in the TCGA (1) and METABRIC (2) datasets. Numbers in brackets, patient numbers in each group. (**C-D**) Cancer hallmarks enriched in genes that are upregulated in breast cancer with weak EZH2 repressive activity (red bars) or in those with strong EZH2 repressive activity (blue bars). Data were retrieved from the TCGA (1) (**C**) and METABRIC (2) (**D**) datasets and only HER2-positive (HER2+ BCa) or triple-negative (TNBCa) breast cancer was considered. NES, normalized enrichment score; N in brackets, numbers of patients with particular subtype of breast cancer in specified dataset.

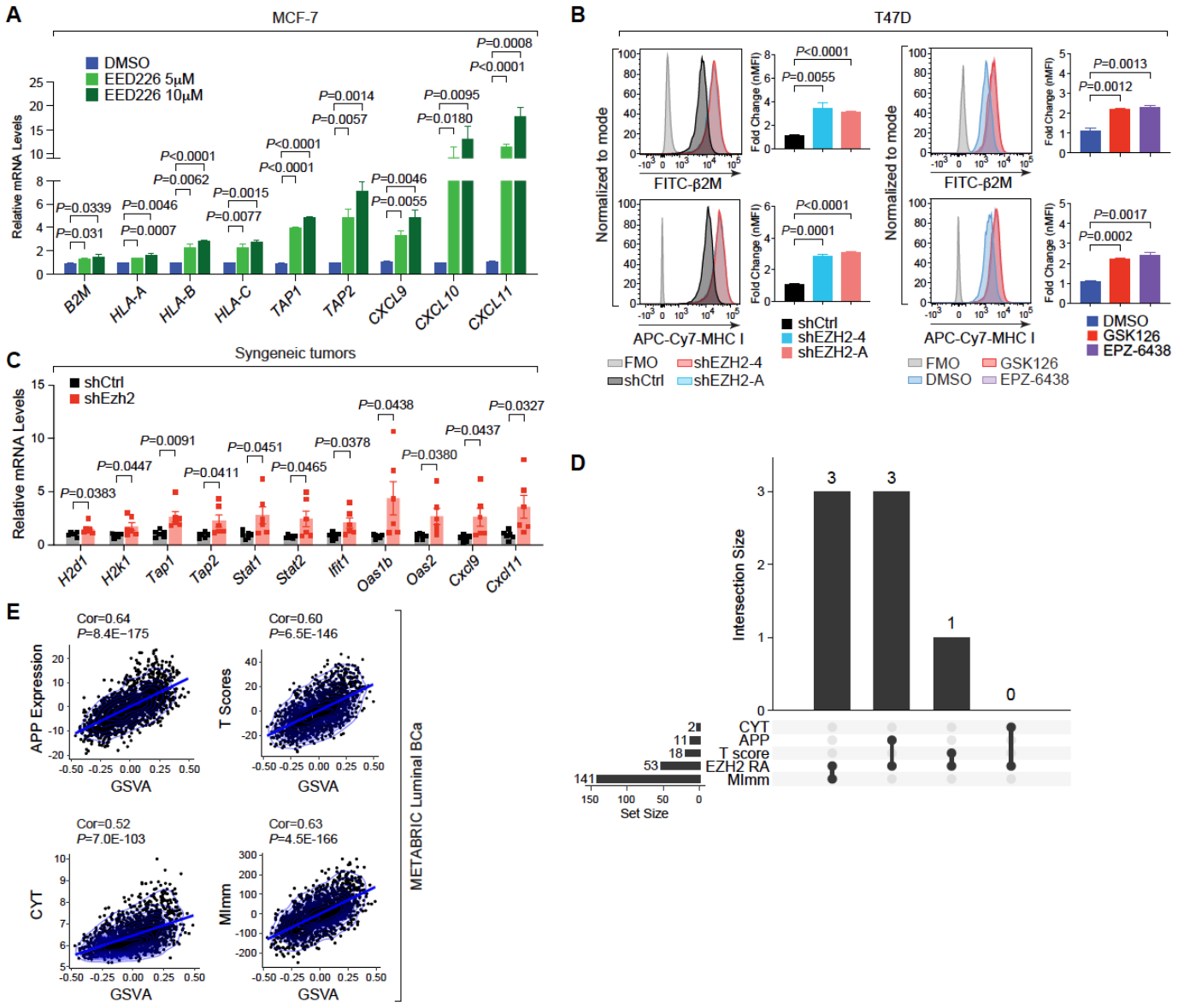

Fig. S4. PRC2 inhibition de-represses ~~the~~ downstream target genes of the IFN signaling that play important roles in immune surveillance. (**A**) Expression of IFN-responsive genes that encode antigen processing and presentation (APP) molecules or chemokines in MCF-7 cells upon 5 or 10 μM EED226 treatment for 7 days. (**B**) Representative histograms of flow cytometry analysis and quantification of normalized mean fluorescence intensity (nMFI) showing the surface levels of MHC class-I (MHC I) and β2M proteins in T-47D cells upon EZH2 knockdown (shEZH2-4 and -A, left panel) or 5 μM EZH2 inhibitor (GSK126 and EPZ-6438) treatment for 7 days (right panels). (**C**) Expression of IFN-activated, immune-related genes in 67NR-derived allograft tumors expressing control shRNA (shCtrl) or Ezh2-specific shRNA (shEzh2). (**D**) Overlaps between the 53 signature genes we identified to reflect the repressive activity of EZH2 (EZH2 RA) and the signature genes used to calculate the scores that are associated with active immune responses. Lists of signature genes that were overlapped are indicated by the black dots and vertical lines. Numbers on top of the vertical bars, overlapped genes between the sets of signature genes compared; numbers on top of the horizontal bars, sizes of individual sets of signature genes. **(E)** Correlation between EZH2 repressive activity represented by the GSVA scores of the 53 signature genes and the expression of APP genes, T scores, CYT scores or MImm scores in patients with luminal breast cancer (Luminal BCa). Clinical information was retrieved from the METABRIC (2) dataset. Correlation coefficients (Cor) were determined by Pearson correlation.

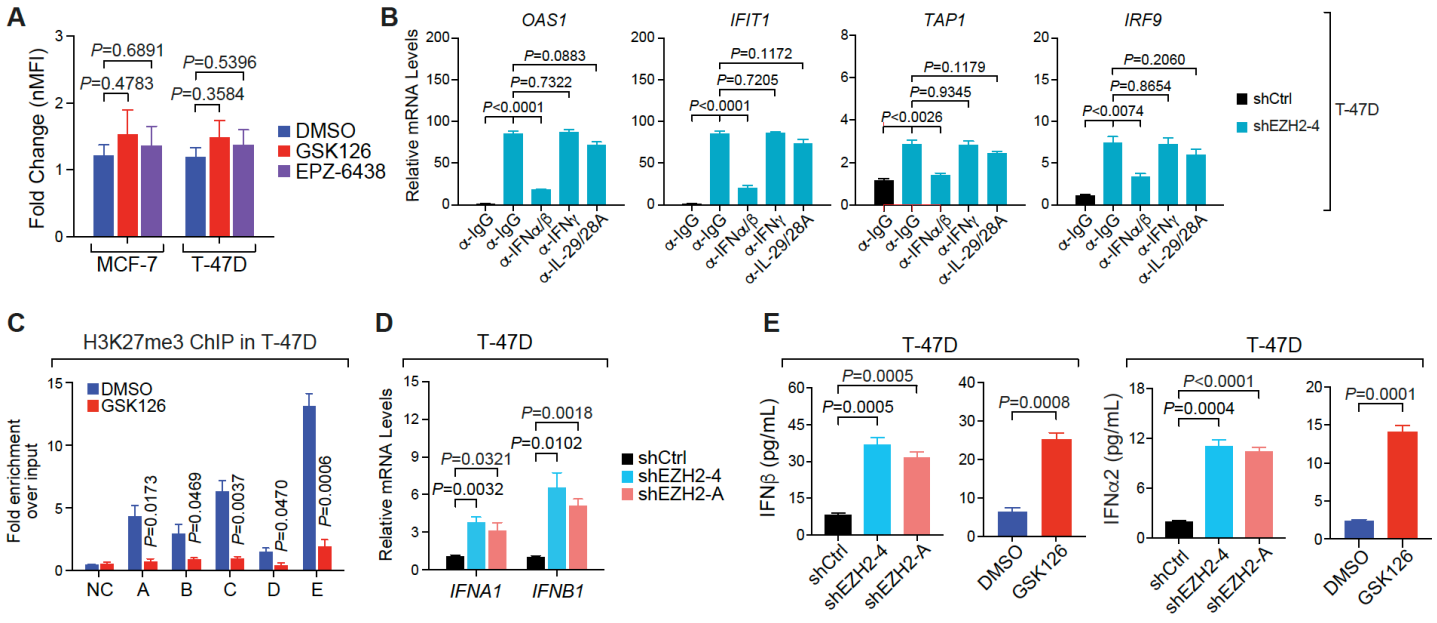

Fig. S5. Type I ligands are transcriptionally suppressed by EZH2-mediated H3K27me3. (**A**) Quantification of the expression of double-stranded RNAs by flow cytometry analysis, presented as the relative normalized mean fluorescence intensity (nMFI), in MCF-7 and T-47D cells treated with DMSO or 5 μM EZH2 inhibitor (GSK126 and EPZ-6438) for 7 days. (**B**) Expression of specified genes in T-47D cells treated with 2.5 μg/mL IgG isotype control antibody (α-IgG) or 2.5 μg/mL neutralizing antibodies against type I (α-IFNα/β), 2 μg/mL against type II (α-IFNγ) and 4 μg/mL against type III (α-IL29/28A) IFN ligands for 24 hours. (**C**) ChIP of H3K27me3 at five representative chromatin sites we designated across type I IFN gene cluster in T-47D cells treated with DMSO or 5 μM GSK126 for 7 days. (**D**) Expression of *IFNA1* and *IFNB1* genes in T-47D cells stably expressing control shRNA (shCtrl) or two separate EZH2-specific shRNAs (shEZH2-4 and -A). (**E**) Measurement of secreted IFNβ and IFNα2 protein levels by ELISA in the culture medium of T-47D cells upon EZH2 knockdown (shEZH2-4 and -A) or 5 μM GSK126 treatment for 7 days.

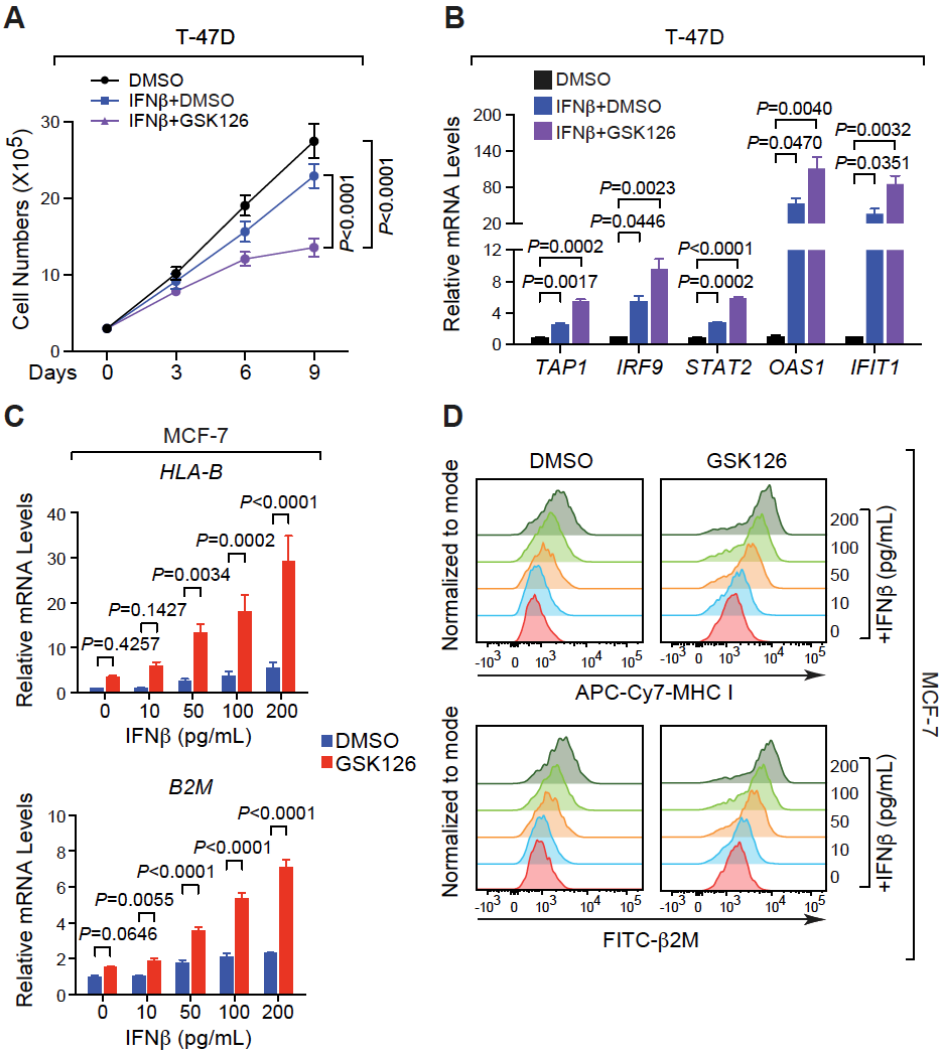

Fig. S6. Inhibition of EZH2 potentiates the antineoplastic and immunomodulatory effects of type I IFN signaling. (**A**-**B**) Growth (**A**) and expression of selected ISGs (**B**) in T-47D cells treated with 200 pg/mL IFNβ, either alone or with 3 μM GSK126, for indicated period of time (**A**) or for 9 days (**B**). (**C**-**D**) Expression of HLA-B and β2M genes (**C**) and flow cytometry analysis of the MHC class-I (MHC I) and β2M proteins on the cell surface (**D**) in MCF-7 pretreated with 5 μM GSK126 for 8 days and then incubated with IFNβ at indicated concentrations for 24 hours.

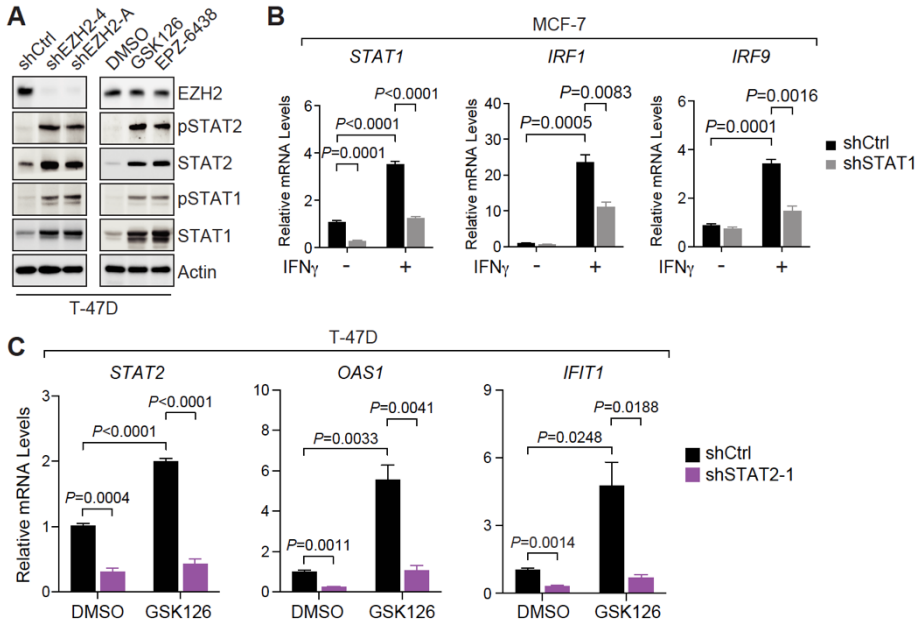

Fig. S7. STAT1 is dispensable for the type I IFN signaling that is activated upon EZH2 inhibition.
(**A**) Immunoblotting analysis in T-47D cells upon EZH2 knockdown (shEZH2-4 and -A) or upon treatment with 5 μM EZH2 inhibitors (GSK126 and EPZ-6438) for 8 days. (**B**) Expression of indicated genes in control (shCtrl) and STAT1-knockdown (shSTAT1) MCF-7 cells upon treatment with (+) or without (-) 10 μg/mL IFNγ for 24 hours. (**C**) Expression of indicated genes in control (shCtrl) and STAT2-knockdown (shSTAT2-1) T-47D cells upon treatment with DMSO or 5 μM GSK126 for 7 days.

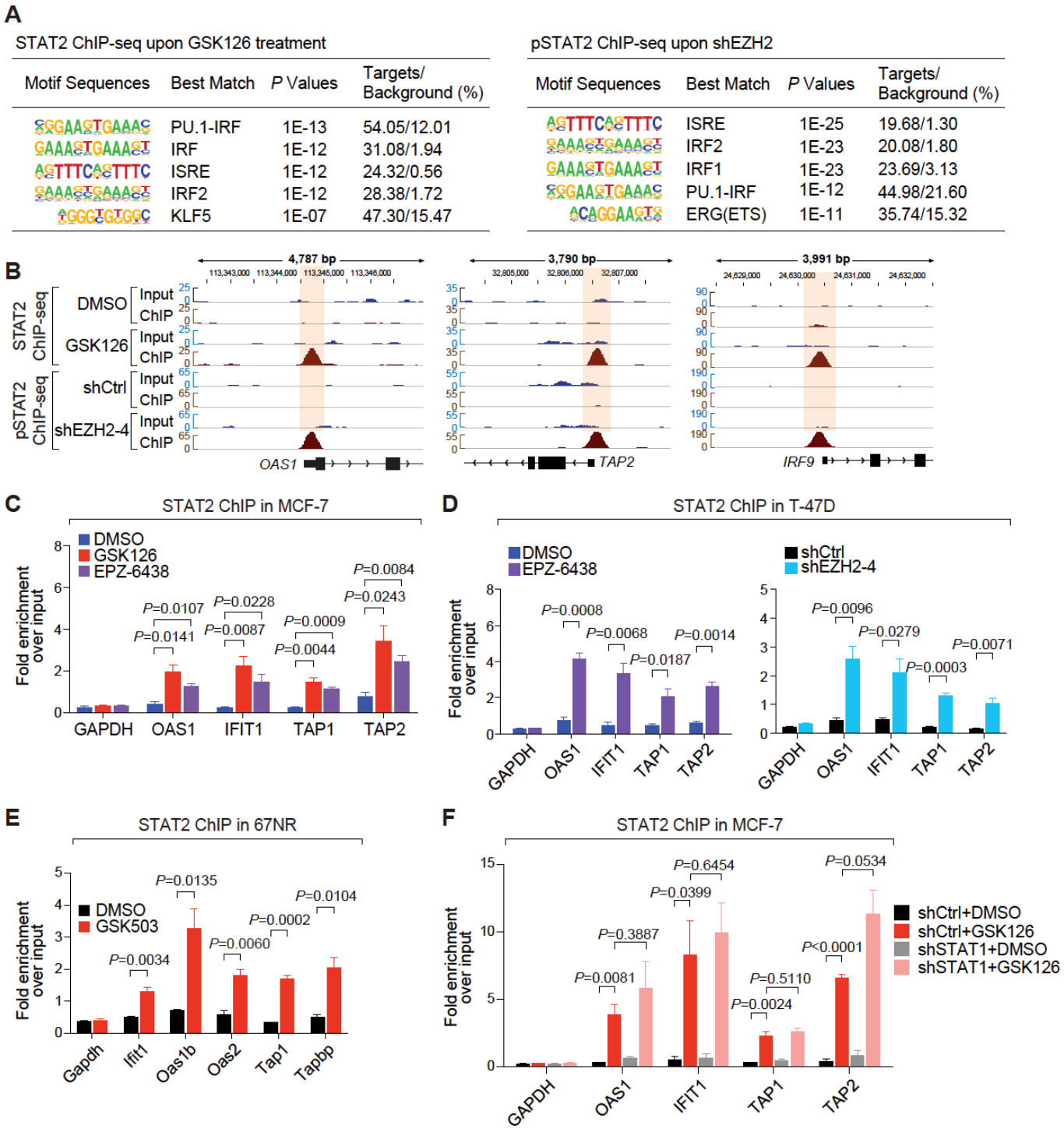

Fig. S8. STAT2 is recruited to the classical interferon-sensitive response element (ISRE) upon EZH2 inhibition in ERα+ breast cancer cells. (**A**) Motifs enriched in the chromatin regions bound by total STAT2 upon GSK126 treatment (left panel) or the phosphorylated STAT2 (pSTAT2) upon EZH2 knockdown (shEZH2, right panel). (**B**) Snapshots of Integrative Genomics Viewer (IGV) showing the input and ChIP-seq signals of total STAT2 and phosphorylated STAT2 (pSTAT2) at the promoters of representative ISGs under control (DMSO or shCtrl) and experimental (GSK126 or shEZH2-4) conditions. (**C-E**) STAT2-targeted ChIP in MCF-7 cells treated with 5 μM EZH2 inhibitors (GSK126 and EPZ-6438) for 7 days (**C**), in T-47D cells treated with 5 μM EPZ-6438 for 8 days (left panel) or infected with control (shCtrl) and EZH2-specific (shEZH2-4) shRNA (right panel) (**D**), or in 67 NR cells treated with 10 μM GSK503 for 8 days (**E**). (**F**) ChIP of STAT2 in control (shCtrl) and STAT1-knockdown (shSTAT1) MCF-7 cells in the presence of DMSO or 5 μM GSK126 for 7 days.

Table S1. Primers for construction of sgRNA-expressing plasmids.

Names	Primer Sequence
sgCtrl	5’-CACCGGAATAGCTCAGAGGCCGAGG-3’ 5’- AAACCCTCGGCCTCTGAGCTATTCC-3’
sgEzh2	5’-CACCG GTGGTGGATGCAACCCGAAA-3’ 5’-AAACTTTCGGGTTGCATCCACCACC-3’
sgStat2	5’- CACCGGCTGGGCAGTGATGACGCCA-3’ 5’- AAACTGGCGTCATCACTGCCCAGCC-3’

Table S2. Antibodies used in CyTOF.

Names of Antibodies	Vendor	Catalog Numbers
174 Yb Monoclonal anti-mouse Ly-6G/C (RB6-8C5)	Fluidigm	3174008B
141Pr Monoclonal anti-mouse Ly-6G (1A8)	Fluidigm	3141008B
142Nd Monoclonal anti-mouse CD11c (N418)	Fluidigm	3142003B
145Nd Monoclonal anti-mouse CD4 (RM4-5)	Fluidigm	3145002B
146Nd Monoclonal anti-mouse CD8a (53-6.7)	Fluidigm	3146003B
147Sm Monoclonal anti-mouse CD45 (30-F11)	Fluidigm	3147003B
148Nd Monoclonal anti-mouse CD11b (M1/70)	Fluidigm	3148003B
152Sm Monoclonal anti-mouse CD3e (145-2C11)	Fluidigm	3152004B
159Tb Monoclonal anti-mouse F4/80 (BM8)	Fluidigm	3159009B
162Dy Monoclonal anti-mouse Ly-6C (HK1.4)	Fluidigm	3162014B
169Tm Monoclonal anti-mouse CD206 (C068C2)	Fluidigm	3169021B
166Er Monoclonal anti-mouse CD326 (G8.8)	Fluidigm	3166014B
176Yb Monoclonal anti-mouse B220 (RA3-6B2)	Fluidigm	3176002B
150Nd Monoclonal anti-mouse CD25 (3C7)	Fluidigm	3150002B
158Gd Monoclonal anti-mouse Foxp3 (FJK-16s)	Fluidigm	3158003A
153Eu Monoclonal anti-mouse CD335 (29A1.4)	Fluidigm	3153006B

Table S3. Primers for RT-qPCR.

Gene Names	Primer Sequence
Human genes	
B2M	5'-GAGGCTATCCAGCGTACTCCA-3' 5'-CGGCAGGCATACTCATCTTTT-3'
HLA-A	5'-AGTTGAGAGCCTACCTGGAT-3' 5'-TGGTGGGTCATATGTGTCTTG-3'
HLA-B	5'-GACACTGAGCTTGTGGAGAC-3' 5'-GGCATGTGTATCTCTGCTCTT-3'
HLA-C	5'-CTGGACAAGAGCAGAGATACAC-3' 5'-AGCAACGATGCCCATGAT-3'
TAP1	5'-GGACAAGAGCCACAGGTATTT-3' 5'-CAGCTGTGATTTCCTCCATAGT-3'
TAP2	5'-CAGGACCAGGTGAACAACAA-3' 5'-CAGCAAGGACAAGGAAGAAGA-3'
CXCL9	5'-CCAGTAGTGAGAAAGGGTCGC-3' 5'-AGGGCTTGGGGCAAATTGTT-3'
CXCL10	5'-CCATTCTGATTTGCTGCCTTATC-3' 5'- TACTAATGCTGATGCAGGTACAG-3'
CXCL11	5'-AGCCTTGGCTGTGATATTGT-3' 5'-GGGTACATTATGGAGGCTTTCT-3'
IRF1	5'-CAAGGCCAAGAGGAAGTCAT-3' 5'-CATGTAGCCTGGAACTGTGTAG-3'
IRF2	5'-CTTCCTATGCAGAAAGCGAAAC-3' 5'-CATGTTGCTGAGGTACTGTTTG-3'
IRF7	5'-GTGTGTCTTCCCTGGATAGC-3' 5'-GCTGCTCCAGCTCCATAAG-3'
IRF9	5'-ATGTTGCTGAGCCCTACAAG-3' 5'-ACTGTGCTGTCGCTTTGAT-3'
STAT1	5'-CACCTACGAACATGACCCTAT-3'C 5'-GCTGTCTTTCCACCACAAAC-3'
STAT2	5'-CCCTCCCTGTGGTGATTATTT-3' 5'-AGAACTGCTGGTTCTGAAGG-3'
IFIT1	5'-CGCCTGGATGGCTTTAAATTAG-3' 5'-CAGGGCAAGGAGAACCTTAATA-3'
IFIT2	5'-ACTATGCCTGGGTCTACTATCA-3' 5'-TCAAGCTCTGGACTCTCAATTC-3'
IFIT3	5'-CCATTGAGCTGAGTCCTGATAA-3' 5'-GCTTCTTCAACAAACTGCTCTC-3'
IFIT5	5'-CCAACATGTACGCTGAAGGA-3' 5'-GGCCATAGTGGTAATGGATCTG-3'
SP100	5'- GTGAGGTGTGCAACAAATGG -3' 5'- AACTCCACGGGTTCTTGTTAG -3'
OAS1	5'-CAGTTGACTGGCGGCTATAA-3' 5'-TGTGAAGCAGGTGGAGAAC-3'
OAS2	5'-GGAGCTTCCTGATTGGCAGA-3' 5'-ATGTAGGGTGGCAAGCACTG-3'
IFNA1	5'-AATTCTGCACCGAACTCTACC-3' 5'-ATGGAGTCCGCATTCATCAG-3'
IFNB1	5'-GCTTCTCCACTACAGCTCTTTC-3' 5'-CAGTATTCAAGCCTCCCATTCA-3'

GAPDH	5’-TGCACCACCAACTGCTTAGC-3’ (3) 5’-GGCATGGACTGTGGTCATGAG-3’ (3)
Mouse genes	
H2d1	5’-CCTCACTTCCACACTGAGAATAA-3’ 5’-CATGTGGTTGCTGGGATTTG-3’
H2k1	5’-CCTCATTCTCTAGCGTGAAGAC-3’ 5’-CACAGGGAACATCAGACACTT-3’
Tap1	5’-GGACTTGCCTTGTTCCGAGAG-3’ 5’-GCTGCCACATAACTGATAGCGA-3’
Tap2	5’- CTGGCGGACATGGCTTTACTT-3’ 5’- CTCCCACTTTTAGCAGTCCCC-3’
Stat1	5’-GCTGCCTATGATGTCTCGTTT-3’ 5’-TGCTTTTCCGTATGTTGTGCT-3’
Stat2	5’-TCCTGCCAATGGACGTTCG-3’ 5’-GTCCCACTGGTTCAGTTGGT-3’
Ifit1	5’-CACCAGTATGAAGAAGCAGAGAG-3’ 5’-GCCATAGCGGAGGTGAATATC-3’
Oas1b	5’-CTTCCTGAACTGTCGCCCAA-3’ 5’-GACTCCCATACTCCCAGGCAT-3’
Oas2	5’-CTGAGTCACCTGCACCATATT-3’ 5’-GCTGGAGAAAGTCCTTGATGA-3’
Cxcl9	5’-GAGCAGTGTGGAGTTCGAGG-3’ 5’-TCCGGATCTAGGCAGGTTTG-3’
Cxcl11	5’-GGCTTCCTTATGTTCAAACAGGG-3’ 5’-GCCGTTACTCGGGTAAATTACA-3’
Gapdh	5’-TGCACCACCAACTGCTTAGC-3’ (3) 5’-GGCATGGACTGTGGTCATGAG-3’ (3)

Table S4. Primers for ChIP-qPCR.

Gene Names	Primer Sequence
Human genes	
GAPDH	5'-TACTAGCGGTTTTACGGGCG-3' (4) 5'-TCGAACAGGAGGAGCAGAGAGCGA-3' (4)
KIAA0066	5'-GCCCCAAACAGGAGTAATGA-3' (5) 5'-CTAGGAGGGTGGAGGTAGGG-3' (5)
IFIT1	5'-TAGGTTTCCAACTTGCAAGG-3' 5'-GCTCCTCTGAGATCTGGCTA-3'
OAS1	5'-GTCAGCAGAAGAGATAAAAGCAAAC-3' 5'-GGTATTTCTGAGATCCATCATTGAC-3'
TAP1	5'-GCGAGAAGCTCAGCCATTTA-3' 5'-TGATTTCCACGCTTGCTACC-3'
TAP2	5'-CAGCTTTCGCTTTCGCTTC-3' 5'-GTACAGTGCGAACCAGAGTT-3'
A	5'-CCTGTTCACCCTGATGATTGA-3' 5'-CATTGGCATGGGCAAAGATT -3'
B	5'-GGCTAATGACAGCCCATATGTA-3' 5'-GGTGCTGTGATTGGAGGAAT-3'
C	5'-GAGAGATCAATCTGGGCTCAAC-3' 5'-CCATCCACAGATCCTGTCATTC-3'
D	5'-CTGCTTCTGAGACCATCACTTT-3' 5'-CTAGCAGAATTCCCTCCTTCTTC-3'
E	5'-TGTTGAATAAGAGCGGTGAGAG-3' 5'-TGATAAACCCACAGCCACTATC-3'
Mouse genes	
Gapdh	5'-GGGTTCCTATAAATACGGACTGC-3' 5'-CTGGCACTGCACAAGAAGA-3'
Ifit1	5'-GTTTCAGAGCCTTCTCCTCATC-3' 5'-CTGCAGTTTATCCAGGGAGAC-3'
Oas1b	5'-ACCTGTTCAGAAGCCCTAAC-3' 5'-GGGACTTTCAGTTTCCATTTCC-3'
Oas2	5'-TGTCAATTGTCGAGGCAGAG-3' 5'-CTAGGCTTGGCTGTGTTAGG-3'
Tap1	5'-GAGCAAGCCAGTCTCAGAAG-3' 5'-TGGTGGAGCTGACTAGAAGT-3'
Tapbp	5'-AGACTTGGTAGGAGAGATCGTAG-3' 5'-CCCAGTGTGTCTATTCCTAAACAA-3'

Table S5. Details of the 53 signature genes reflecting the repressive activity of EZH2.

Gene Names	Log_2FC	P values	TSS to the nearest H3K27me3 peak (bp)
ABCA6	2.945315214	0.003445449	0
ADCY5	2.63778093	6.5353E-142	60
ASCL1	3.225927209	9.7432E-121	1073
ATP10D	3.072490052	1.75508E-05	677
BDKRB1	4.038391276	0.000150199	123
BDKRB2	2.920438774	1.06851E-24	2028
CD36	4.653379002	3.47581E-91	4598
CDC20B	3.942614188	0.002856067	637
CDH12	4.960269697	4.69059E-06	770
CMPK2	4.675406182	5.95433E-18	502
CSMD3	2.984779359	4.30563E-08	0
CYP1A1	4.641186558	1.05408E-10	824
DACH1	4.592392111	6.45473E-15	53
EMX1	3.917370947	9.5903E-05	610
ETV7	3.622805694	3.444E-07	560
FAM71D	3.090837783	0.023754337	0
FRMPD1	2.433428599	0.000844062	417
GALNT13	2.541506311	1.38171E-05	676
GJA1	2.504887959	2.20531E-45	666
GPRIN3	3.499509498	1.30533E-05	0
HDAC9	3.940671228	6.14563E-22	0
HLA-DPA1	3.826057675	1.6912E-09	2570
HLA-DPB1	2.912268572	0.000101477	1919
KCNE4	4.857152867	6.7068E-14	0
KCNH1	2.794829645	1.88863E-15	380
KCNK2	3.452193297	6.35799E-26	1863
LIN7A	2.604184113	2.82957E-35	0
LINC01686	3.040355764	0.010208757	131
LMO3	3.108768145	0.008695737	669
LUM	3.888066469	0.001537068	276
LUZP2	3.79158805	0.000507397	0
MAPK4	3.494598738	0.012737004	712
MMP13	2.797354256	0.026108682	2606
PAK6	2.728607543	0.036395432	314
PGLYRP4	2.387577111	0.0002202	1313
PSMB8	4.853351488	2.14445E-37	596
PSMB8-AS1	3.384103439	0.000103082	0
PSMB9	4.118465719	9.06787E-23	608
RGS16	3.189265613	2.17066E-37	481
RHBDL3	3.634653625	0.000913816	0
RSAD2	4.154082231	4.04855E-05	237
S100A7	3.962299778	2.98989E-57	2162

S100A8	3.406566147	4.2615E-118	3522
SLC5A8	2.780913529	8.6222E-39	427
STAT1	2.376463544	0	1258
TAP1	2.803419191	3.4357E-178	798
TAP2	3.2443403	1.7221E-300	4783
TSPAN2	3.244236242	1.57098E-07	19
TSPAN5	2.67139504	9.67101E-62	0
UBE2QL1	2.656479195	0.001048391	420
UNC5C	3.46687006	5.15203E-14	894
VNN3	4.568806072	0.000126515	2696
WNT10B	2.537059331	1.39555E-06	164

Notes:
1. Log2FC, log2-transformed fold change between the expression of corresponding gene in EZH2-knockdown MCF-7 cells and the control cells.
2. TSS, transcription start site
3. bp, base pair.

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