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## Supplementary Materials for

### **Synthetic lethal combination targeting BET uncovered intrinsic susceptibility of TNBC to ferroptosis**

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#### **The PDF file includes:**

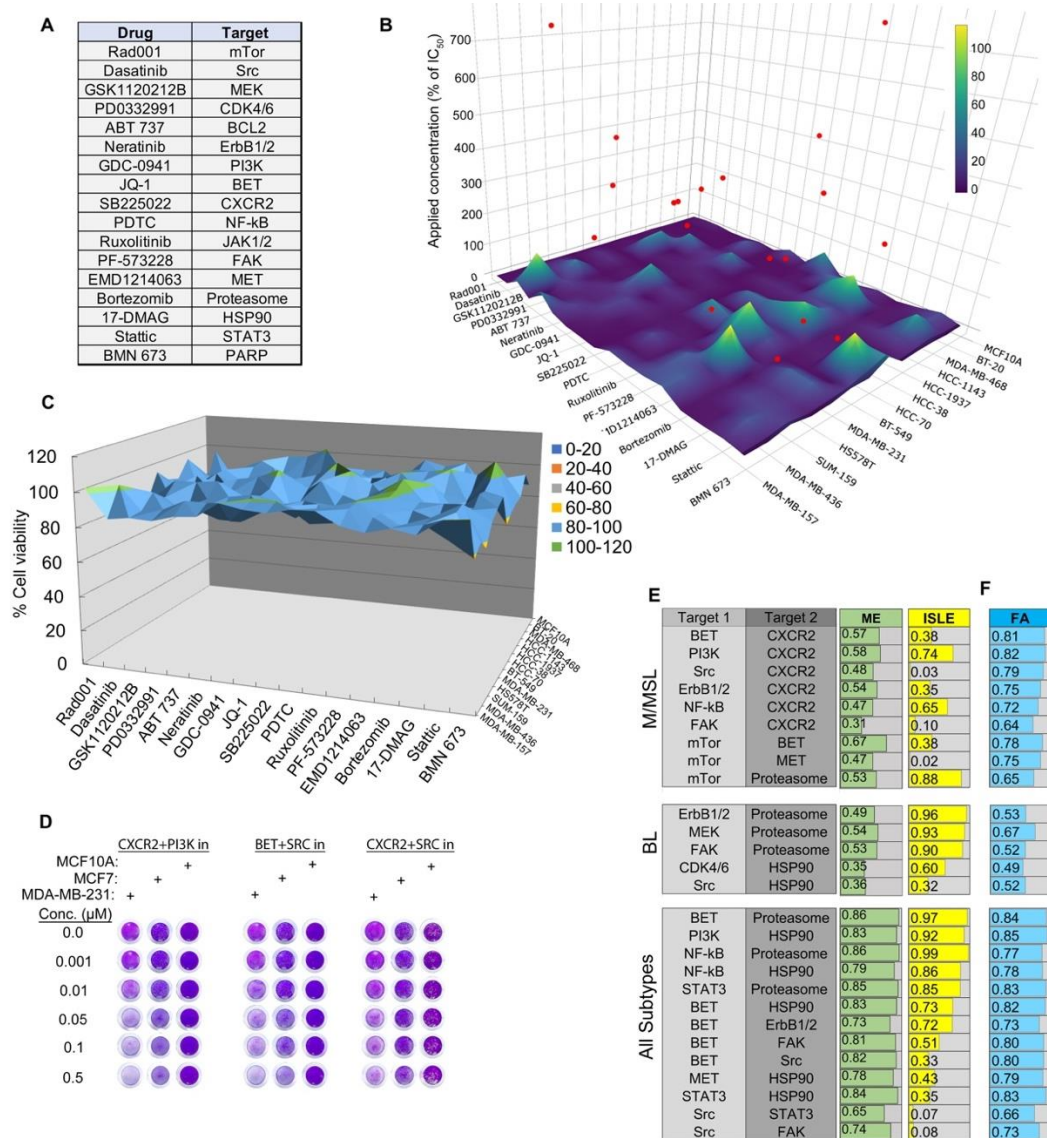
Figs. S1 to S8

#### **Other Supplementary Material for this manuscript includes the following:**

(available at [advances.sciencemag.org/cgi/content/full/6/34/eaba8968/DC1](https://advances.sciencemag.org/cgi/content/full/6/34/eaba8968/DC1))

Tables S1 to S13

## Supplementary Figures



**Figure S1: Screen related information and data**

**(A)** Drugs used in the screen and their corresponding targets.

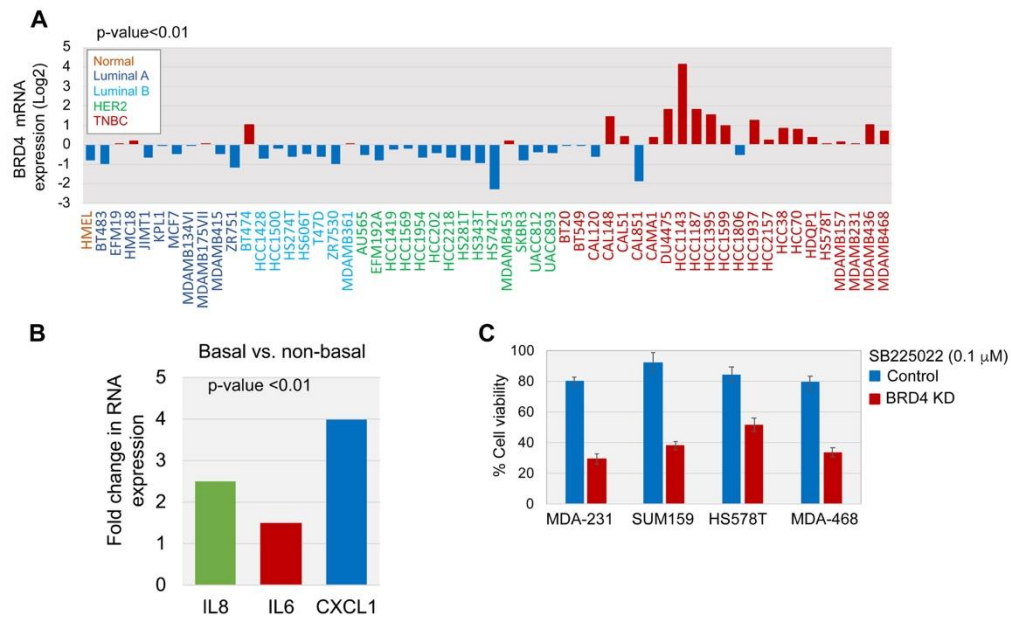
**(B)** Ratio between the apparent  $IC_{50}$  of each drug in the 13 cell lines (Table S1) and the low doses used in the screen (Table S2). Red dots indicate the few cases where the doses in the screen were above the  $IC_{50}$ .

**(C)** 3D plot of cell viability assay using CellTiter-Glo 72 hr after treatment with the 17 different drugs applied at the low doses as indicated in Table S2. Low doses of single agents had minor effects (0-25%) on cell viability. Results are means of 3 experiments.

**(D)** Validation of several hits from primary screen by crystal violet staining. MDA-MB-231 (TNBC), MCF7 (Luminal A) and MCF10A (normal-like) were treated with the indicated concentrations of drugs for 72 hr and then stained with crystal violet. Shown are representative pictures of 2-3 independent experiments.

**(E)** ISLE-significance scores estimate the strength of clinically-relevant synthetic lethal interactions in basal-like breast tumor (330 patients) is shown for each drug pair (yellow). Median value of effectiveness across all 13 cell lines is shown in green (ME- median effectiveness).

**(F)** The fraction affected (FA, represents the fraction of cell death) of each drug pairs was calculated from the screen results. Shown are mean values of FA for each combination within a group of cell lines that belong to M/MSL, BL or all subtypes.

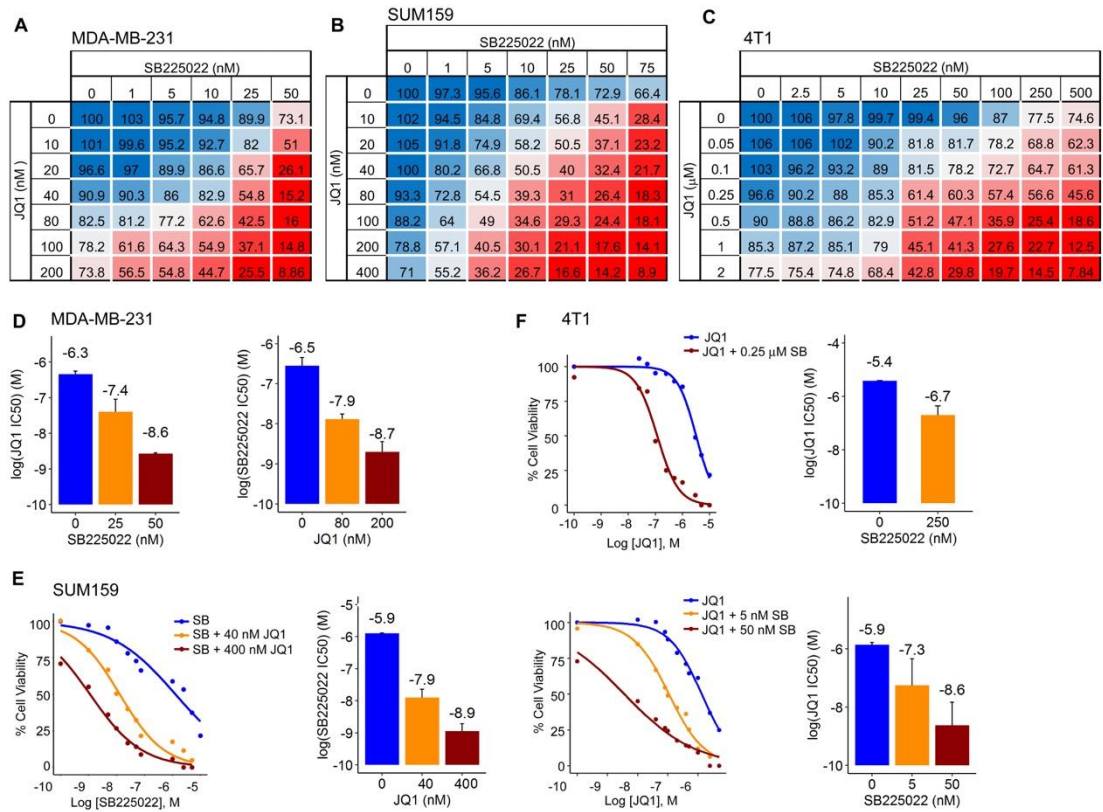


**Figure S2: Expression of BRD4 and CXCR2 ligands in breast cancer and influence of BRD4 on CXCR2 inhibition.**

**(A)** Normalized log2 expression of BRD4 in the 55 breast cancer cell lines of the Cancer Cell Line Encyclopedia (CCLE, Broad). The difference between the TNBC and non-TNBC lines is significant (*t*-test, *p*-value < 0.01).

**(B)** Fold changes in IL8, IL6 and CXCL1 gene expression in basal versus non-basal breast cancer tumors (TCGA). For all three genes the difference is significant (*t*-test, *p*-value < 0.01).

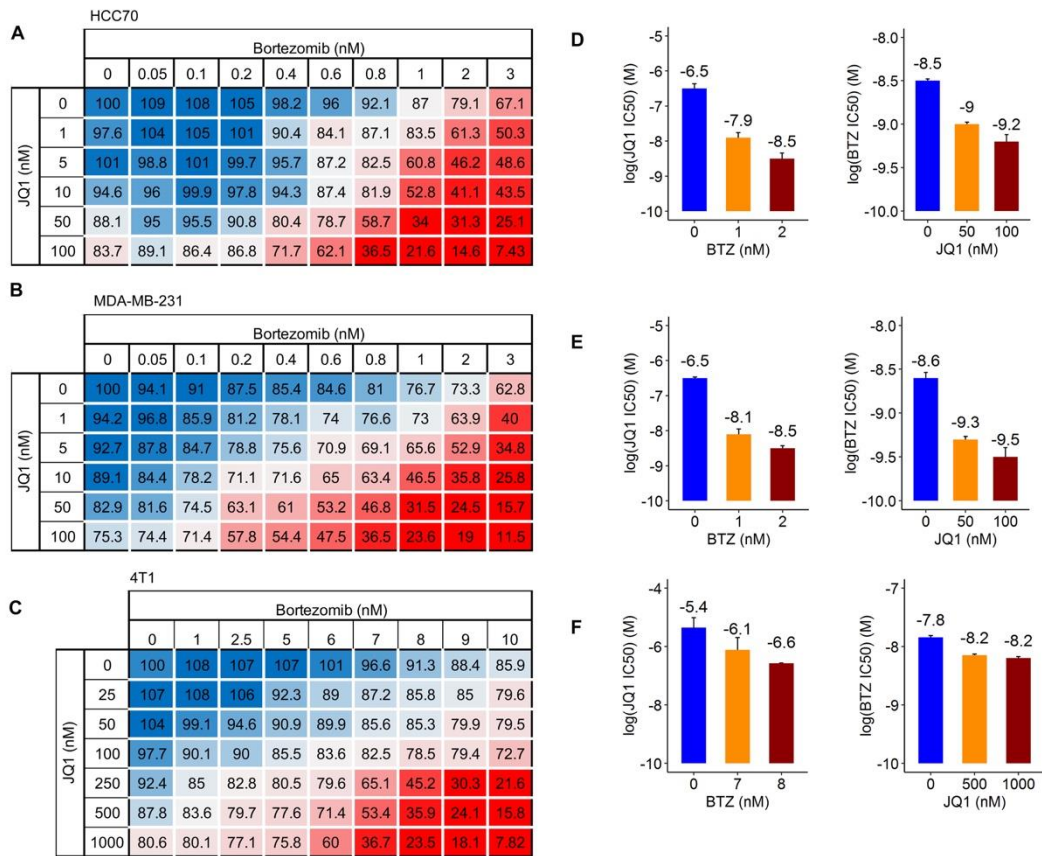
**(C)** BRD4 knockdown sensitizes mesenchymal TNBC to CXCR2 antagonist. Cell viability of the indicated mesenchymal cell lines, control and BRD4 knockdown (KD), was measured 72 h after treatment with SB225022 (0.1 μM) by CellTiter-blue assay and presented as % cell viability of untreated control. Shown are mean values ± SD of cell viability from two independent experiments in duplicates.



**Figure S3: Effects of BET and/or CXCR2 inhibition on cell viability**

**(A-C)** Dose-response matrix for JQ1 and SB225022 combination in MDA-MB-231 **(A)**, SUM159 **(B)**, and 4T1 **(C)**. Cell viability was measured by MTT assay, 72 hr after treatment with the indicated concentrations of drugs. Matrixes shown are mean of four **(A)** or two **(B, C)** independent repeats.

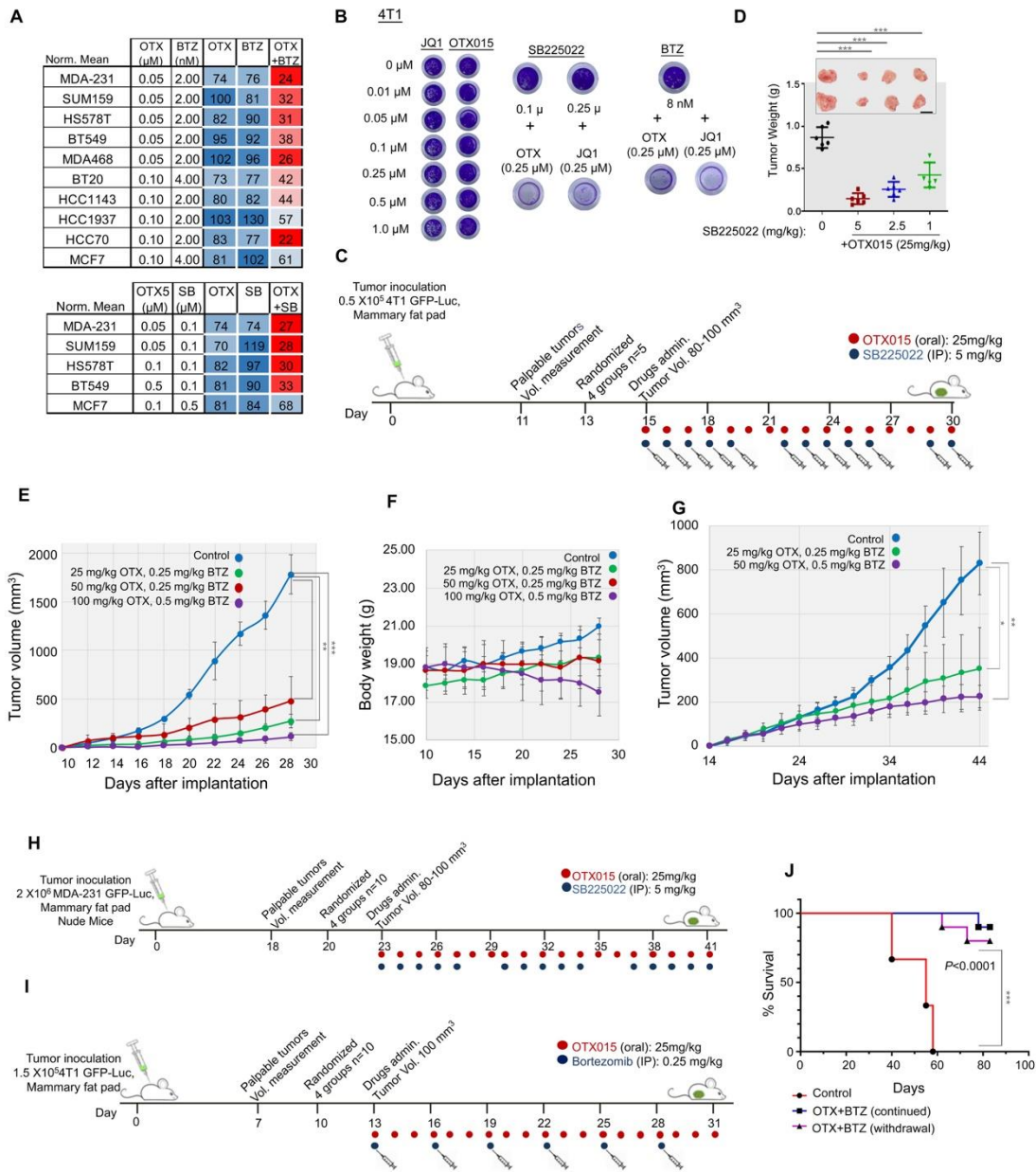
**(D-F)** Dose-response curves of JQ1, SB225022, and their combination at the indicated doses. Viability of MDA-MB-231 **(D)**, SUM159 **(E)**, and 4T1 **(F)** cells was assessed by MTT assay. The effects of SB225022 on the  $IC_{50}$  of JQ1 and vice versa are shown in the bar graphs. Mean values  $\pm$ SD are shown.



**Figure S4: Effects of BET and/or proteasome on TNBC cell viability**

**(A-C)** Dose-response matrix for JQ1 and Bortezomib combination in HCC70 **(A)**, MDA-MB-231 **(B)** and 4T1 **(C)**. Cell viability was measured by MTT assay, 72 hr after drugs treatment. Matrixes shown are mean of three **(A, B)** or two **(C)** repeats each.

**(D-F)** The effects the indicated doses of Bortezomib on  $IC_{50}$  of JQ1 and vice versa in HCC70 **(D)**, MDA-MB-231 **(E)** and 4T1 **(F)** cells are shown in the bar graphs. Results are mean values  $\pm$  SD of three **(D,E)** or two **(F)** repeats.



**Figure S5: Calibration of in vivo studies and drugs administration protocols**

**(A)** Cell viability in response to OTX015 and Bortezomib (BTZ) or OTX015 and SB225022 in the indicated cell lines was measured by MTT assay 72 hr after drug treatments with the indicated drug concentrations. Cell viability is presented as fold of control (%). The mean values  $\pm$  SD of three experiments are shown in Table S4.

**(B)** Effects of JQ1 and OTX015 on viability of 4T1 cells. The effects of JQ1 and OTX015, as single agents or in combination with CXCR2 inhibitor (SB225022) or Bortezomib (BTZ) at the indicated doses on cell viability were assessed by crystal violet staining.

**(C-D)** Dose response of drugs combination in orthotopic 4T1 tumors in BALB/C mice. Orthotopic implantation of 4T1 cells and drugs administration were performed as illustrated in **(C)**. Tumor weights in response to the specified drug doses are shown in the graph together with a picture of excised tumors. (n=3, bilateral tumors) \*\*\*p-value<0.001, calculated by t-test.

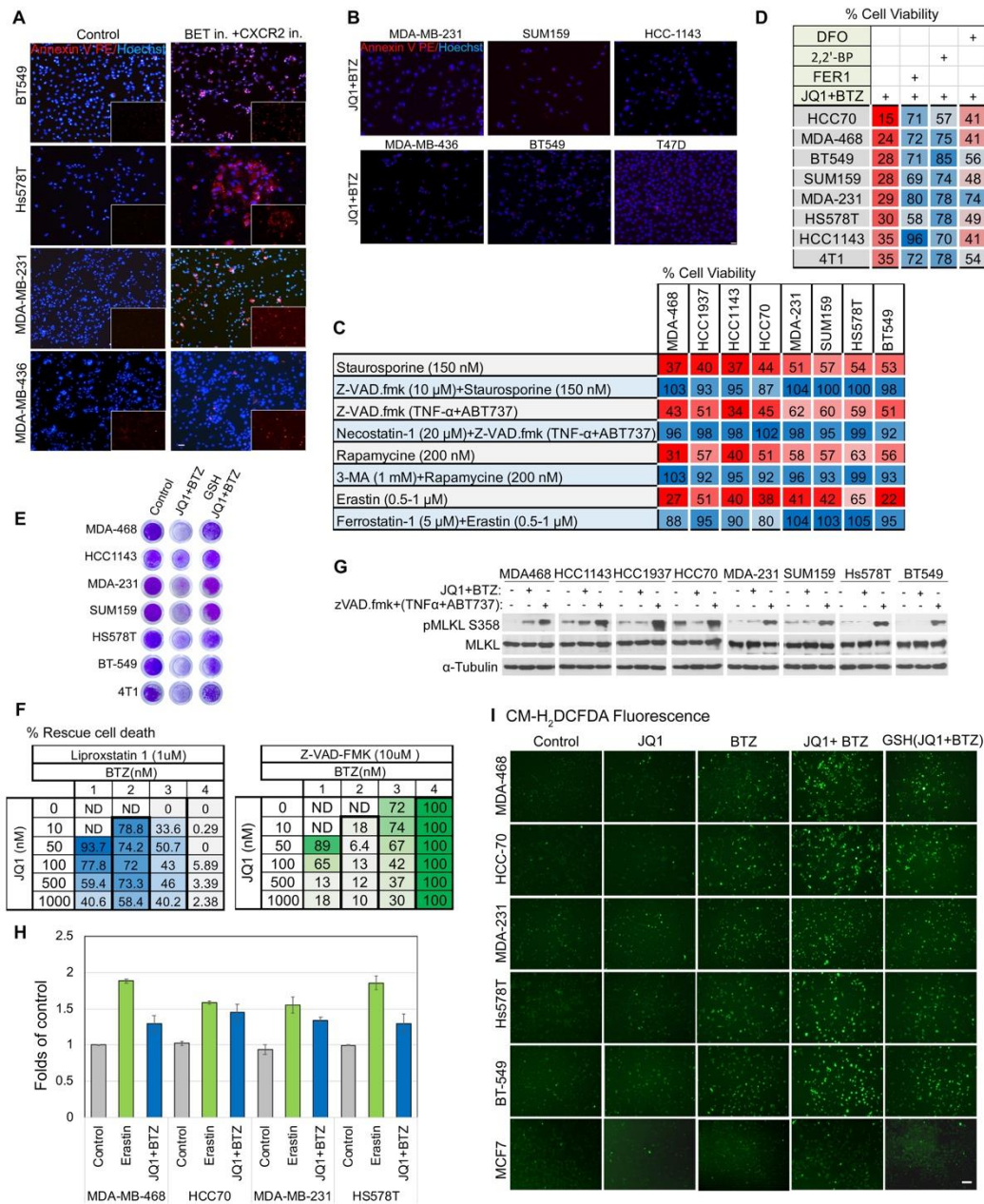
**(E-G)** Effects of OTX015 and Bortezomib on 4T1 allograft and MDA-MB-231 xenograft tumors. OTX015 and Bortezomib (BTZ) were applied at the indicated doses 13 days after orthotopic implantation of 4T1 cells in BALB/c mice **(E, F)** or 23 days after implantation of MDA-MB-231 cells in nude mice (n=3,

bilateral tumors) (**G**). Tumor volume (**E, G**) were measured every 2 days, (Significance was calculated by *t*-test, \*\*\**p*-value<0.001, \*\**p*-value<0.01, \**p*-value<0.05), as well as body weight (**F**).

(**H, I**) Tumor implantation and drugs administration schemes for the tumor xenograft mice model in nude mice (**H**) and the syngeneic mice model (**I**).

(**J**) Kaplan-Meier survival curves (n=11 for control and continued treatment, n=8 for withdrawal treatment) show the effects of continued treatment or withdrawal of OTX015 and Bortezomib (at day 31) on animal survival for 80 days. Differences in survival were determined using the log-rank test of Kaplan–Meier.





**Figure S6: Cell death related assays in response to drugs combinations**

(A, B) Representative images of Annexin V staining in response to either JQ1 plus SB225022 (Methods) in mesenchymal TNBC cell lines (A) or to JQ1 plus BTZ (B) in the indicated breast cancer lines for 24 hr. Scale bar, 10  $\mu$ m

(C) Rescue of cell death induced by the indicated death inducers and corresponding inhibitors. All inhibitors were added before and during treatments with the different death inducers. Apoptosis was induced by Staurosporine (150 nM for 16 hr) and rescued by pre-treatment (1 hr) with caspase inhibitor (Z-VAD.fmk, 10  $\mu$ M). Necrosis was induced by treatment with z-VAD.fmk (20  $\mu$ M) for 30 min followed by TNF- $\alpha$  (20 ng/ml) +ABT-737 (200 nM) for 8 hr and was rescued by 1 hr pretreatment of Necrostatin-1 (20  $\mu$ M). Autophagy was induced by rapamycin (200 nM) and rescued by pretreatment of cells for 1 hr with 3-MA (1mM). Ferroptosis was induced by erastin (0.5- 1  $\mu$ M) and rescued by pretreatment with Ferrosstatin-1 (5  $\mu$ M) for 2 hr. Results are mean values of two experiments.

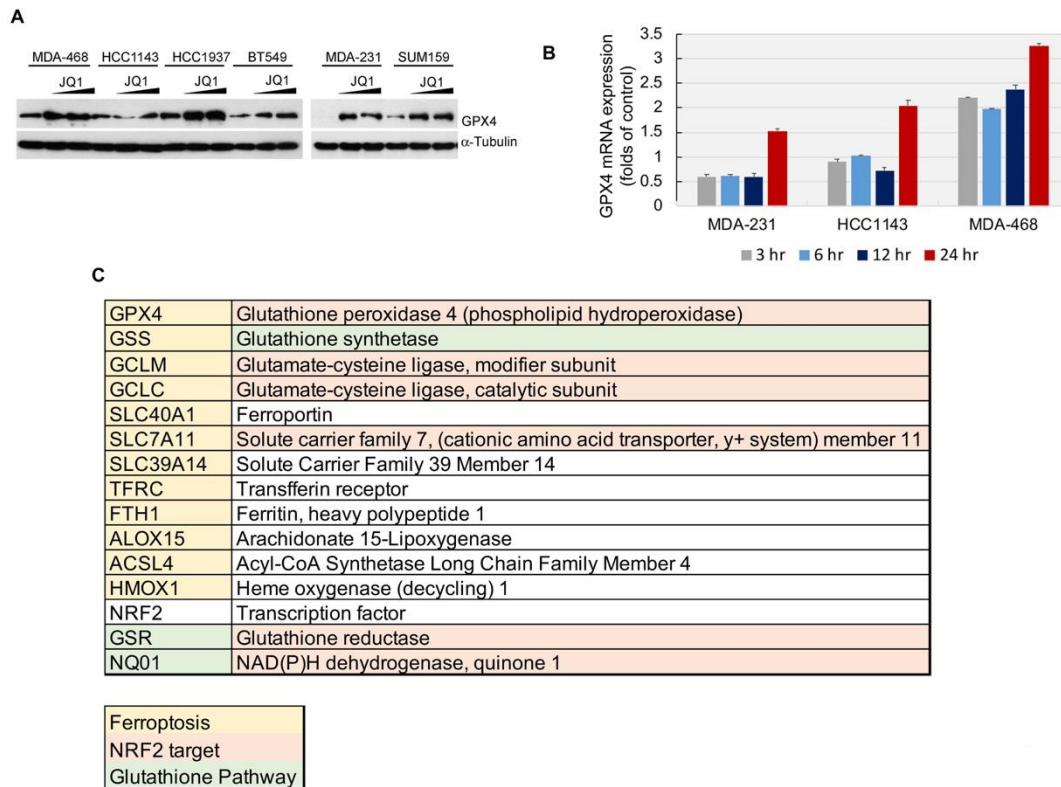
**(D, E)** Viability of indicated cell lines treated with JQ1 and BTZ for 72 h, in the absence or presence of either DFO (1 mM), *2,2'-Bipyridine* (100  $\mu$ M), FER1 (5  $\mu$ M), and/or GSH (1 mM). Cell viability was assessed by MTT assay and presented as percent of control **(D)**, or by crystal violet staining **(E)**. Results shown in **D** are mean values of three independent experiments. Mean values  $\pm$  SD are shown in Table S10.

**(F)** Rescue of cell death induced by BTZ and/or JQ1 across the indicated doses. Liproxstatin 1 (1  $\mu$ M) or z-VAD.fmk (20  $\mu$ M) were added 1 hr before and during JQ1 and/or BTZ treatment. Viability of MDA-MB-468 cells was measured 72 hr later by MTT. The relative rescue of cell death by liproxstatin 1 and z-VAD was calculated as percent of untreated control cells. Shown are representative results of two similar repeats. Ferroptosis or apoptosis associated ranges are marked in blue or green, respectively. ND- No Death.

**(G)** Monitoring necrosis by pMLKL S358. The indicated cells were treated with either JQ1 + BTZ or pretreated with z-VAD.fmk (20  $\mu$ M) for 30 min and then with TNF- $\alpha$  (20 ng/ml) +ABT-737 (200 nM) for 8 hr to induce necrosis and level of pMLKL S358 was examined by WB.

**(H)** Lipid peroxidation was assessed by TBARS assay in response to BET and proteasome inhibition. The indicated TNBC cell lines were incubated either with JQ1 and BTZ or with erastin (0.25  $\mu$ M) as a positive control for 16 hr. Cells were lysed and lipid peroxidation was assessed by TBARS assay as described in Methods. Shown are fold changes compared to untreated controls. The results are mean values  $\pm$  SD of two repeats.

**(I)** Representative images of CM-H<sub>2</sub>DCFDA fluorescence in the indicated cell lines treated with either JQ1, BTZ or both for 12 hr in the absence or presence of GSH (1 mM). Scale bar, 50  $\mu$ m.

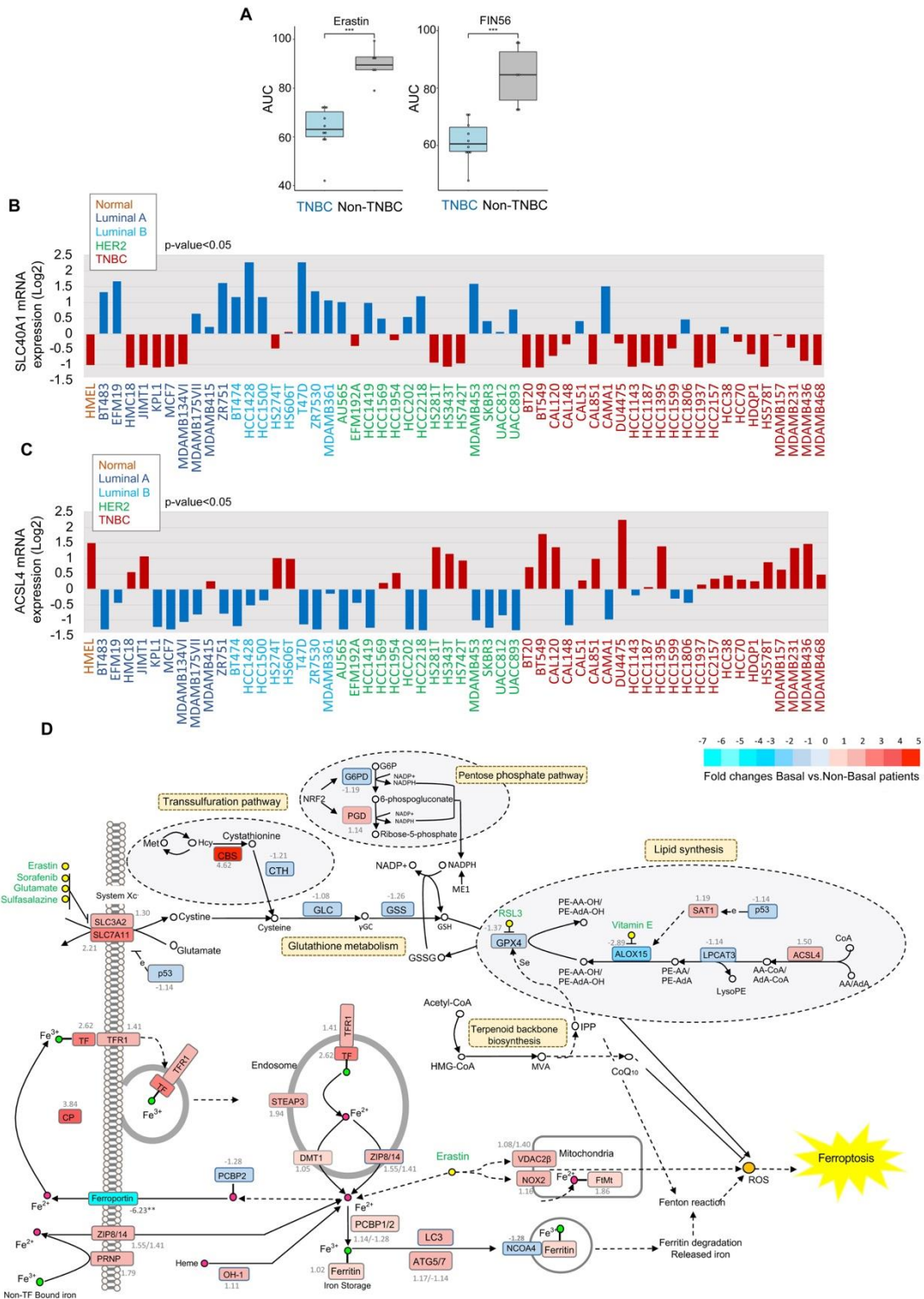


**Figure S7: BET inhibition increases GPX4 transcription**

**(A)** Effects of BET inhibition on level of GPX4 protein. The indicated cell lines were treated with JQ1 (0.05 and 0.25  $\mu$ M) for 24 hr. Cell were lysed and GPX4 level was assessed by WB.

**(B)** GPX4 transcription levels in response to BET inhibition (0.05  $\mu$ M JQ1) for the indicated time periods were assessed by qPCR. Shown are mean values  $\pm$  SD of two experiments.

**(C)** List of ferroptosis related genes used for qPCR analysis.



**Figure S8: Expression of ferroptosis genes in TNBC versus non-TNBC and ferroptosis vulnerability**  
**(A)** Area under the viability curves shown in Fig 7A, and further repeats with additional cell lines (total 10 TNBC and 6 non-TNBC, 3-7 repeats per cell lines). AUCs were measured using the PharmacoGx package in R. The differences between the TNBC and non-TNBC lines are significant (*t*-test, *p*-value<0.01).  
**(B, C)** Normalized log<sub>2</sub> expression of SLC40A1 **(B)** and ACSL4 **(C)** genes in TNBC and non-TNBC cell lines of the CCLE. The differences between the TNBC and non-TNBC lines are significant (*t*-test, *p*-value<0.05).

**(D)** The ferroptosis pathway, adapted from the KEGG ferroptosis pathway (KEGG map hsa04216) was converged with the transsulfuration pathway and pentose phosphate pathway to illustrate the relative expression of each gene in basal relative to non-basal tumors. Colors indicate the fold change between basal and non-basal patients from the TCGA dataset and the actual numbers are labeled in grey.