Supplementary Information

Generation of Reactive Oxygen Species is the Primary Mode of Action and Cause of Survivin Suppression by Sepantronium Bromide (YM155)

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Content: Figures and legends

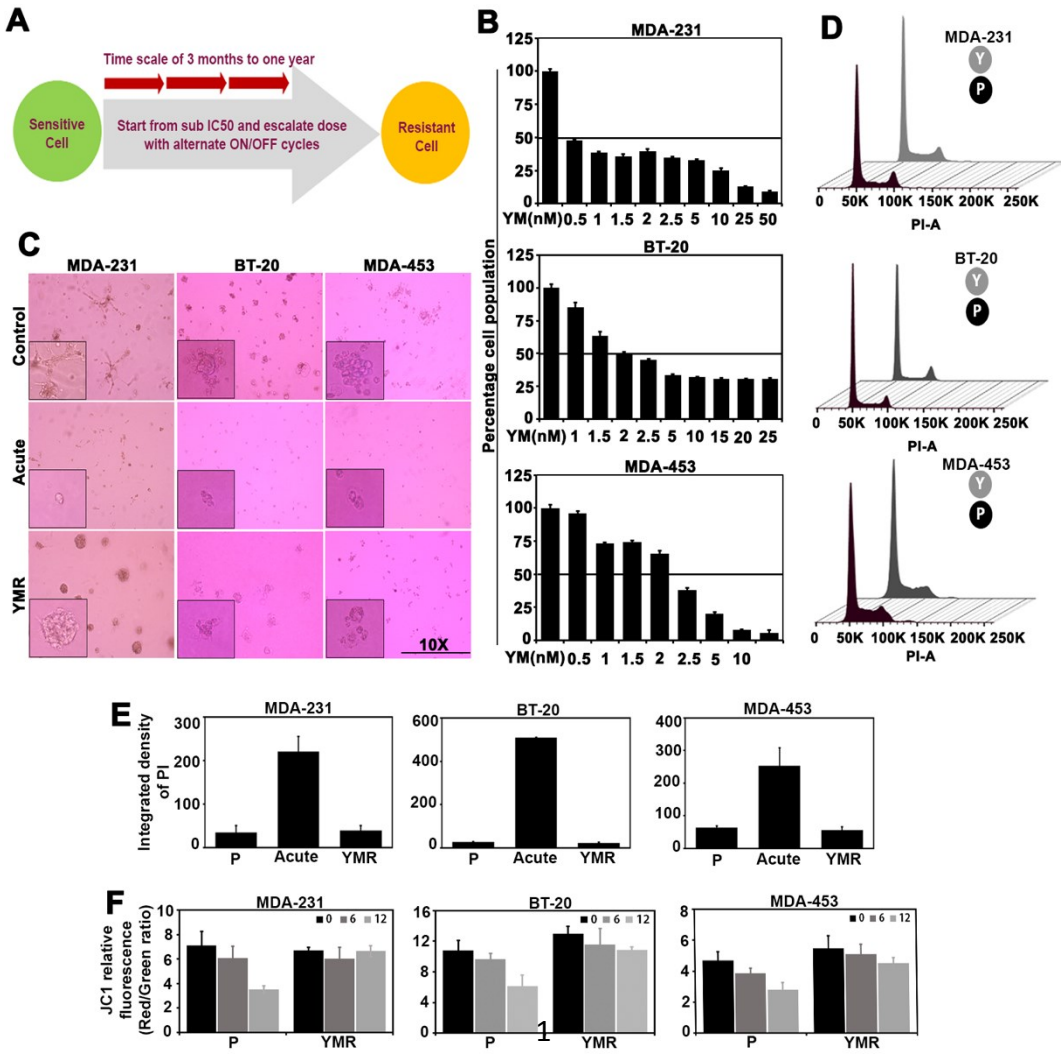

Figure S1: Chronic YM155 exposure led to adaptive drug resistance in TNBC cells. A. Schema for developing isogenic YM155 sensitive (P) and resistant (YMR) cell lines. **B.** Percentage cell population showing IC_{50} for P cell lines following 72h treatment with escalating dosages of YM155. **C.** 3-D Matrigel acini formation assay of P and YMR cell lines. Acute stands for P treated with YM155. **D.** Cell cycle analysis showing peak area for different stages of cell cycle in P versus YMR cell lines. **E.** Quantification of PI staining of P and YMR cells untreated and treated with YM155 for 72h. PI-positive cells indicate cell death. MDA-231 P versus Acute *** P=0.0005, Acute versus YMR *** P=0.0006; BT-20 P versus Acute **** P<0.0001, Acute versus YMR **** P<0.0001; MDA-453 P versus Acute *** P=0.0006, Acute versus YMR *** P=0.0005. P vs YMR is ns in all three cases. **F.** Quantification of JC-1 staining of P and YMR cells at 0, 6 and 12h following YM155 treatment. MDA-231 P 0h versus 12h * P=0.0435; BT-20 P 0h versus 12h * P=0.0344. In all three YMR lines, 0h versus 12h remain ns.

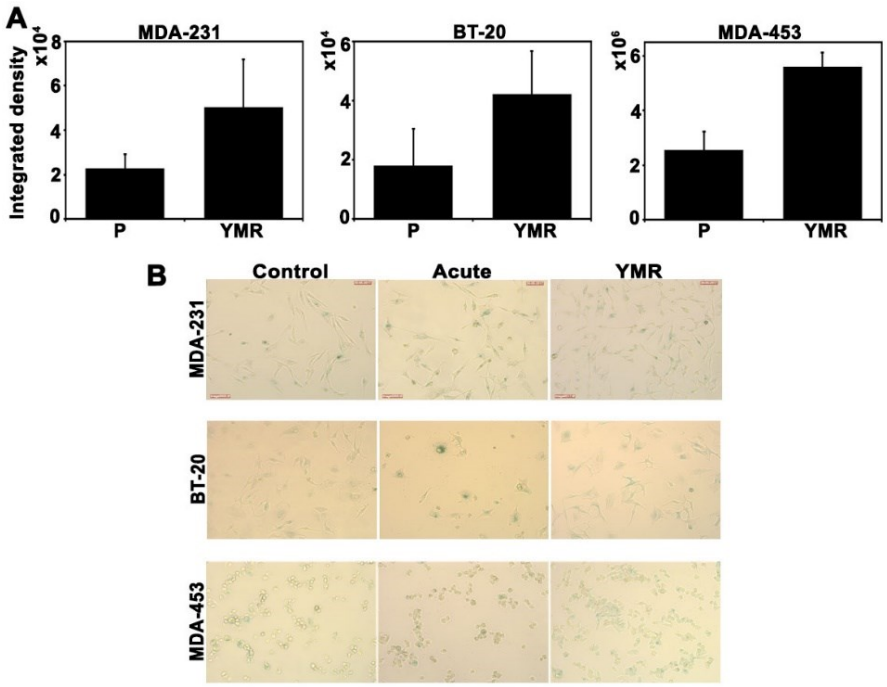

Figure S2: Quantitation of ROS signal obtained from TNBC P versus YMR cell lines at 0h (indicates base-level ROS) and β-gal assay. A. Image-J (NIH) analysis was done to perform quantitation of fluorescent signal obtained from the CellROX staining. **B.** Microscopic images representing SA-βgalactosidase activity in P versus YMR cells.

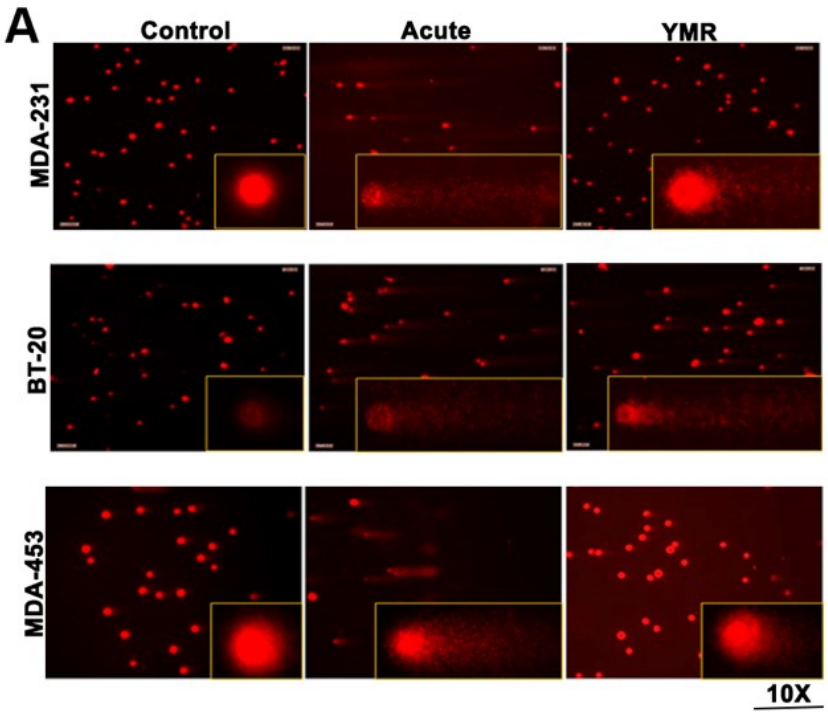

Figure S3: Persistent DNA damage was triggered by chronic YM155 treatment. A. Comet assay examining extent of DNA damage in P (treated: acute, untreated: control) and YMR cells.

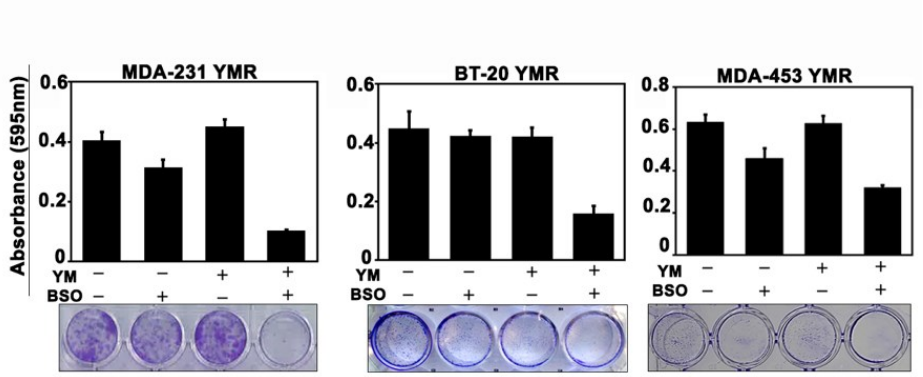

Figure S4: Co-treatment with BSO reversed YM155 resistance. Colonies remaining from YMR cells treated with or without BSO (500μM) alone or in combination with YM155. Upper panels show quantification while the lower panels represent the respective crystal-violet stained colonies.

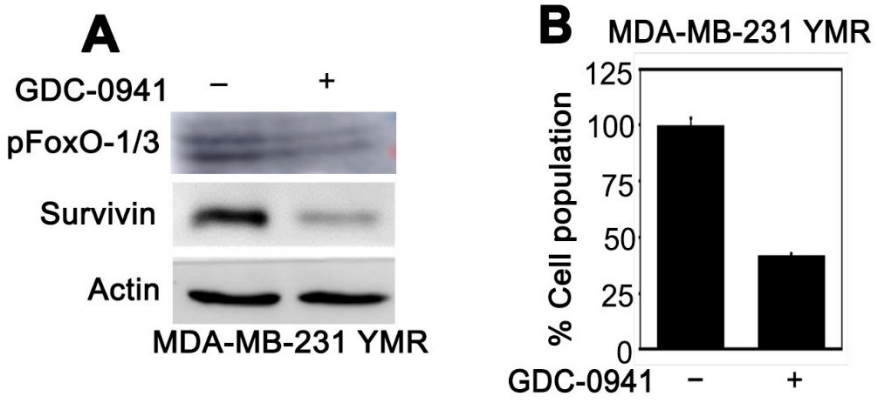

Figure S5: Treatment with GDC-0941 activated FoxO-1/3a and inhibited the MDA-MB-231 YMR cell proliferation. A. Immunoblot for 1μM GDC-0941 treatment comparing survivin and corresponding pFoxO-1/3a levels. **B.** Cell proliferation after 72 h GDC-0941 treatment.