Supplementary Figures and Legends

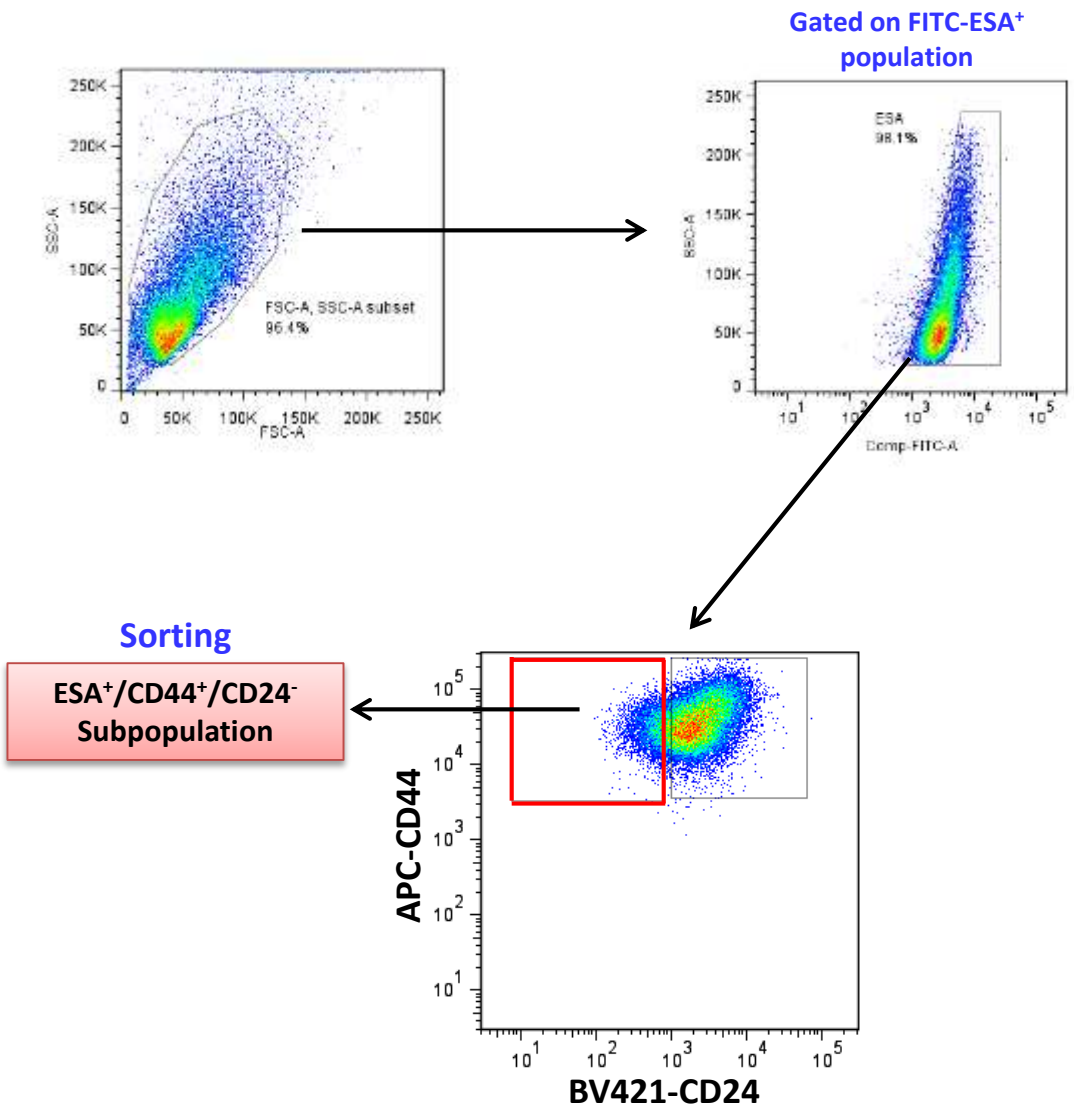

Figure S1. Shown is the flow cytometry gating strategy for sorting of ESA$^+$/CD44$^+$/CD24$^-$ breast CSCs. Flow cytometry was employed to sort breast CSCs from SUM159 and SUM149 human TNBC cell lines using a previously published protocol (1).

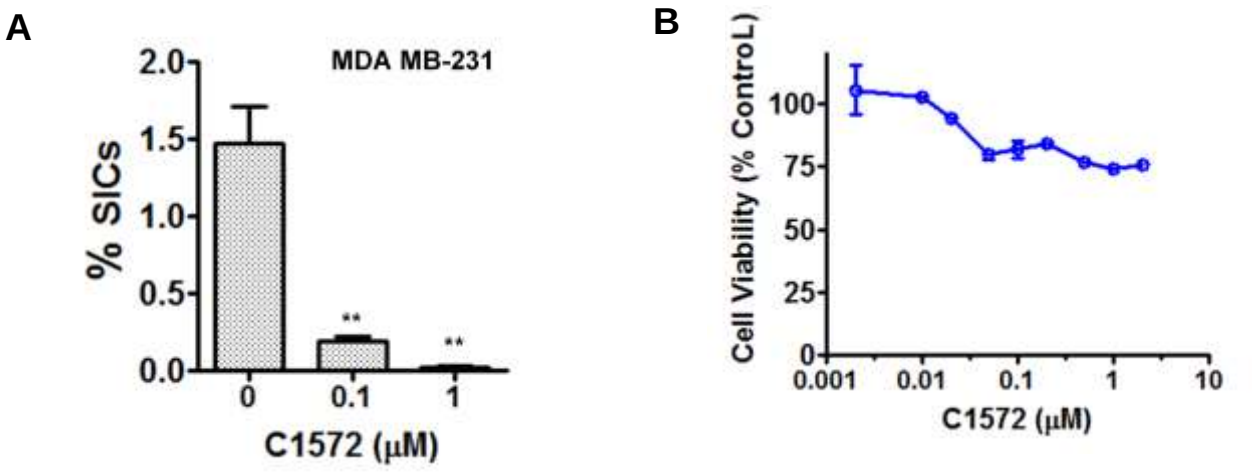

Figure S2. C1572 selectively depletes SICs in human TNBC cells. A, The frequency of SICs in residual MDA-MB-231 human TNBC cells following different doses of C1572 treatment is presented as mean ± SEM of three independent experiments. **B**, Human cord blood derived hematopoietic stem/progenitor cells (HSPCs) were prepared as previously reported (3) and treated with different doses of C1572. Cell viability was measured by MTS assays (4) at 24 h after drug treatment. ** $p < 0.01$ vs. vehicle (DMSO) control.

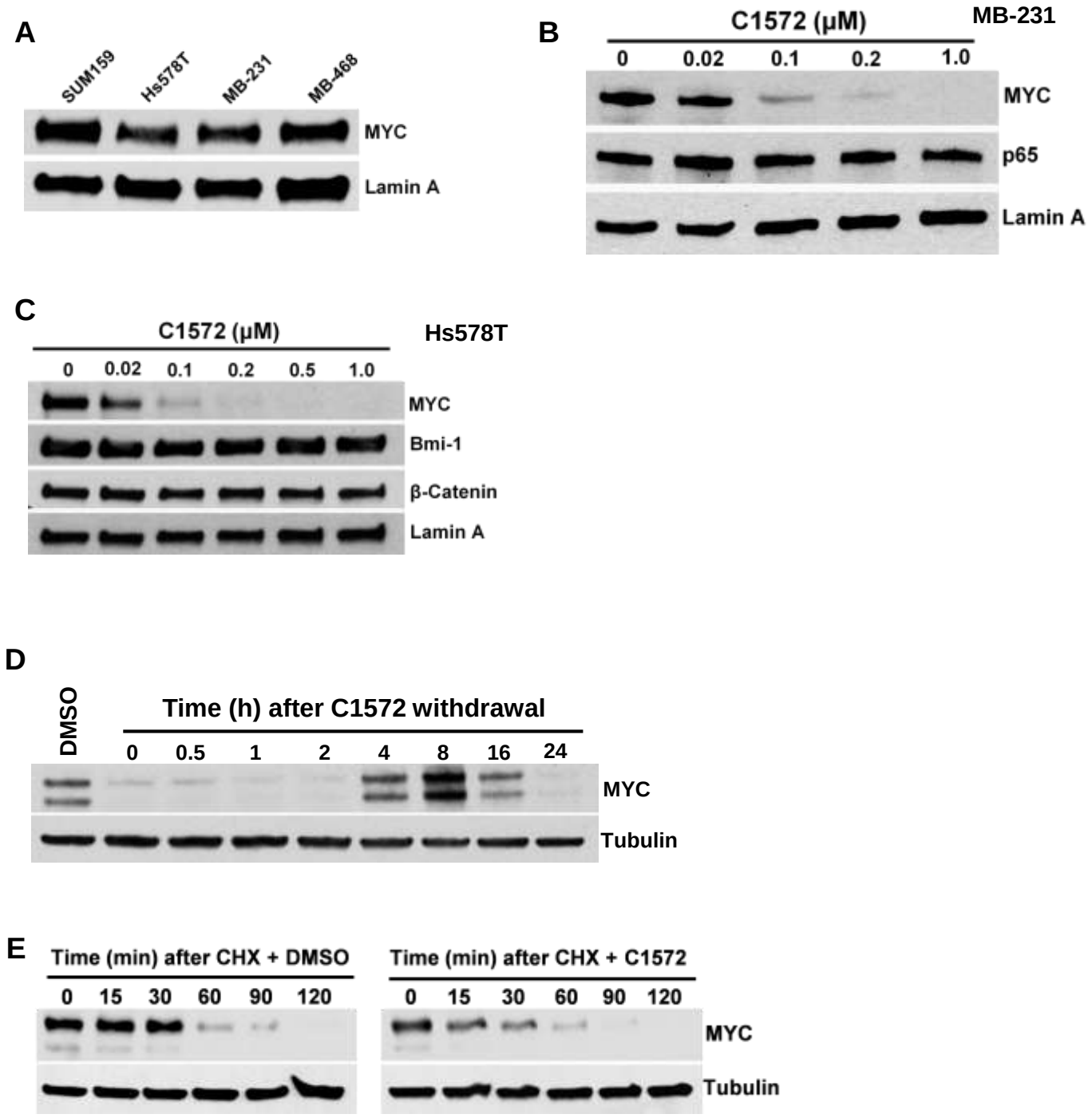

Figure S3. C1572 reduces MYC protein levels in a dose-dependent fashion in human TNBC cells. A, MYC expression levels were measured in various human TNBC cell lines using Western blot analysis. **B**, C1572 reduces MYC protein levels in MDA-MB-231 cells. **C**, C1572 decreases MYC protein levels in Hs578T cells. **D**, SUM159 cells were dosed with C1572 (0.2 μM) for 6 h and then drug was removed. MYC protein levels were measured at different time points after drug withdrawal using Western blot analysis. **E**, C1572 treatment shortens the half-life of MYC protein.

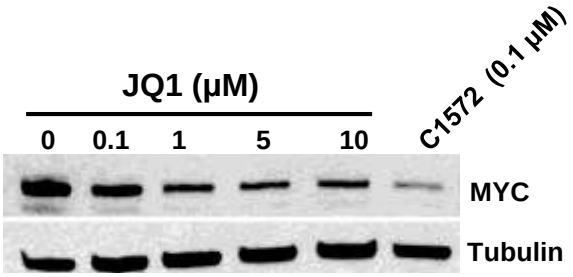

Figure S4. C1572 is 100-fold more potent than JQ1 in inhibiting MYC. SUM159 cells were treated with different doses of JQ1 or C1572 for 16 h. MYC expression levels after treatments were determined by Western blotting.

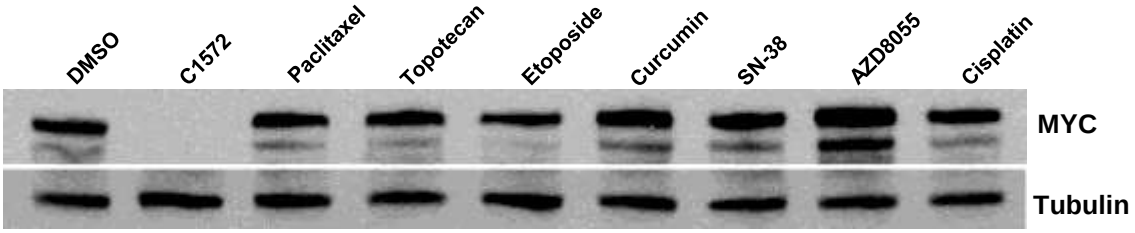

Figure S5. C1572 is the only compound of a set of tested drugs that depletes MYC. SUM159 cells were treated with C1572 (0.2 μM), Paclitaxel (0.1 μM), Topotecan (10 μM), Etoposide (20 μM), Curcumin (20 μM), SN-38 (0.1 μM), AZD8055 (10 μM), and Cisplatin (50 μM). MYC expression levels were determined by Western blotting at 24 h after treatments.

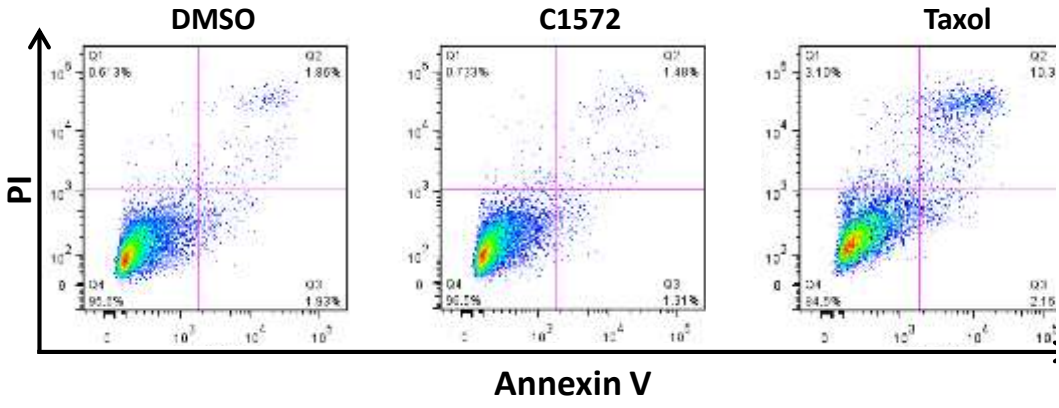

Figure S6. Effects of C1572 and Taxol on apoptosis in TNBC cells. SUM159 cells were treated with C1572 (0.2 μM), JQ1 (10 μM) and Taxol (0.1 μM) for 24 h. Flow cytometry was employed to measure apoptotic cells after Annexin V and PI staining as described in methods.

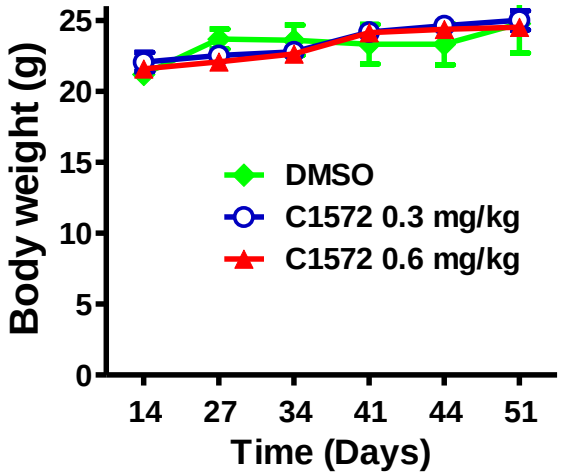

Figure S7. Treatment with C1572 has no significant impact on body weight in NSG mice. To gain insight into the possible toxicity of C1572, mouse body weight was monitored during drug treatment. Changes in body weight versus time (days) after tumor cell transplantation are shown.

Supplementary references

1. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-8.

2. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25:402-8.

3. Yang A, Xiao X, Zhao M, LaRue AC, Schulte BA, Wang GY. Differential Reponses of Hematopoietic Stem and Progenitor Cells to mTOR Inhibition. Stem Cells Int 2015; 2015: 561404.

4. Zheng G, Peng C, Jia X, Gu Y, Zhang Z, Deng Y, Wang C, Li N, Yin J, Liu X, Lu M, Tang H, He Z. ZEB1 transcriptionally regulated carbonic anhydrase 9 mediates the chemoresistance of tongue cancer via maintaining intracellular pH. Mol Cancer 2015; 14: 84.