Supplemental Figure S1A

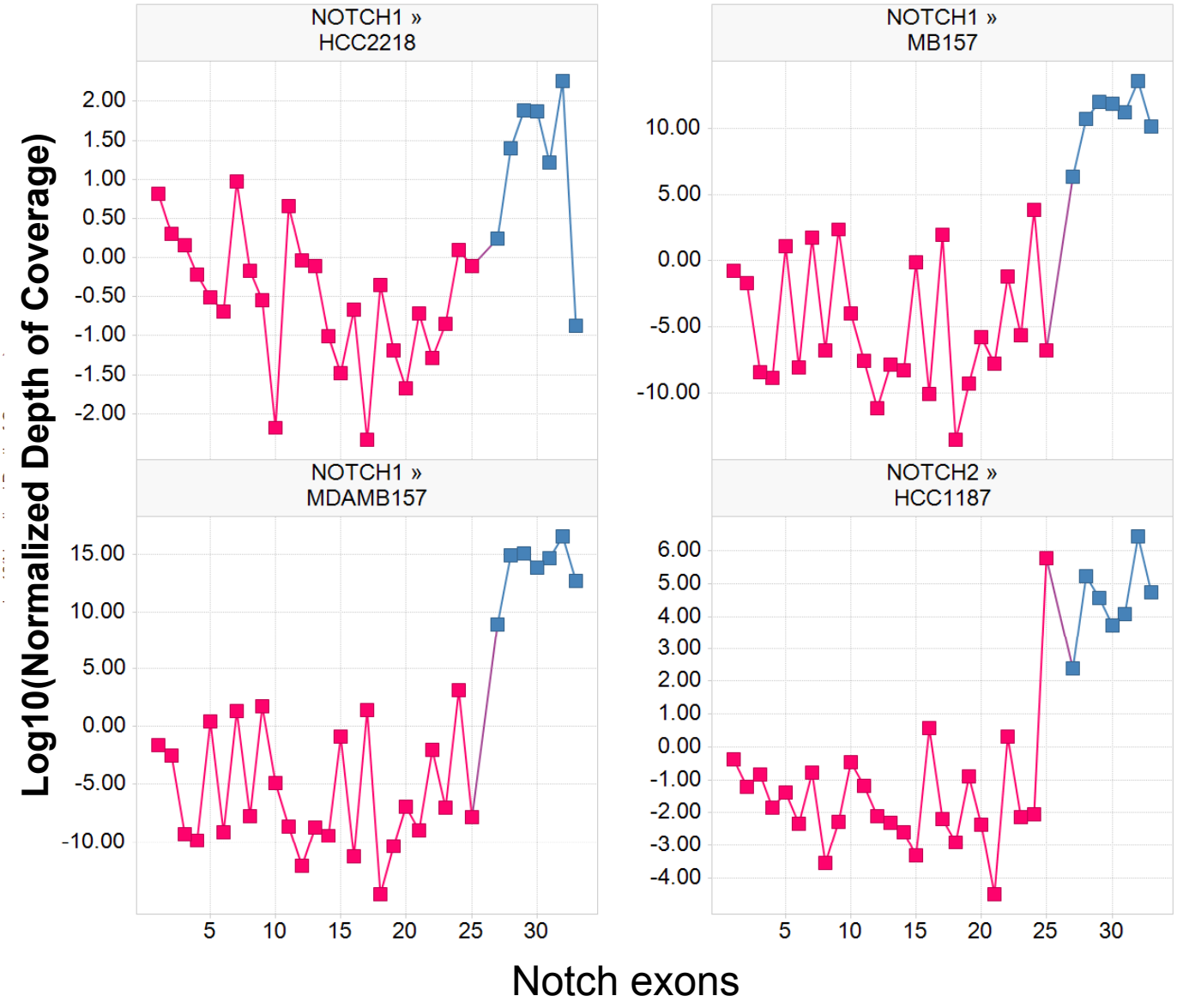


Figure S1: A) Relative coverage of Notch1 and Notch 2 exons in HCC2218, HCC1187, MB157, MDA-MB157 cell lines. Blue color corresponds to NICD domain and encompasses exons 27-34. Red color corresponds to exons 1 to 26. The cell lines show significant (pvalue < 10-5) and at least 10 fold difference in coverage between exons 27-36 and 1-26.

Supplemental Figure S1B

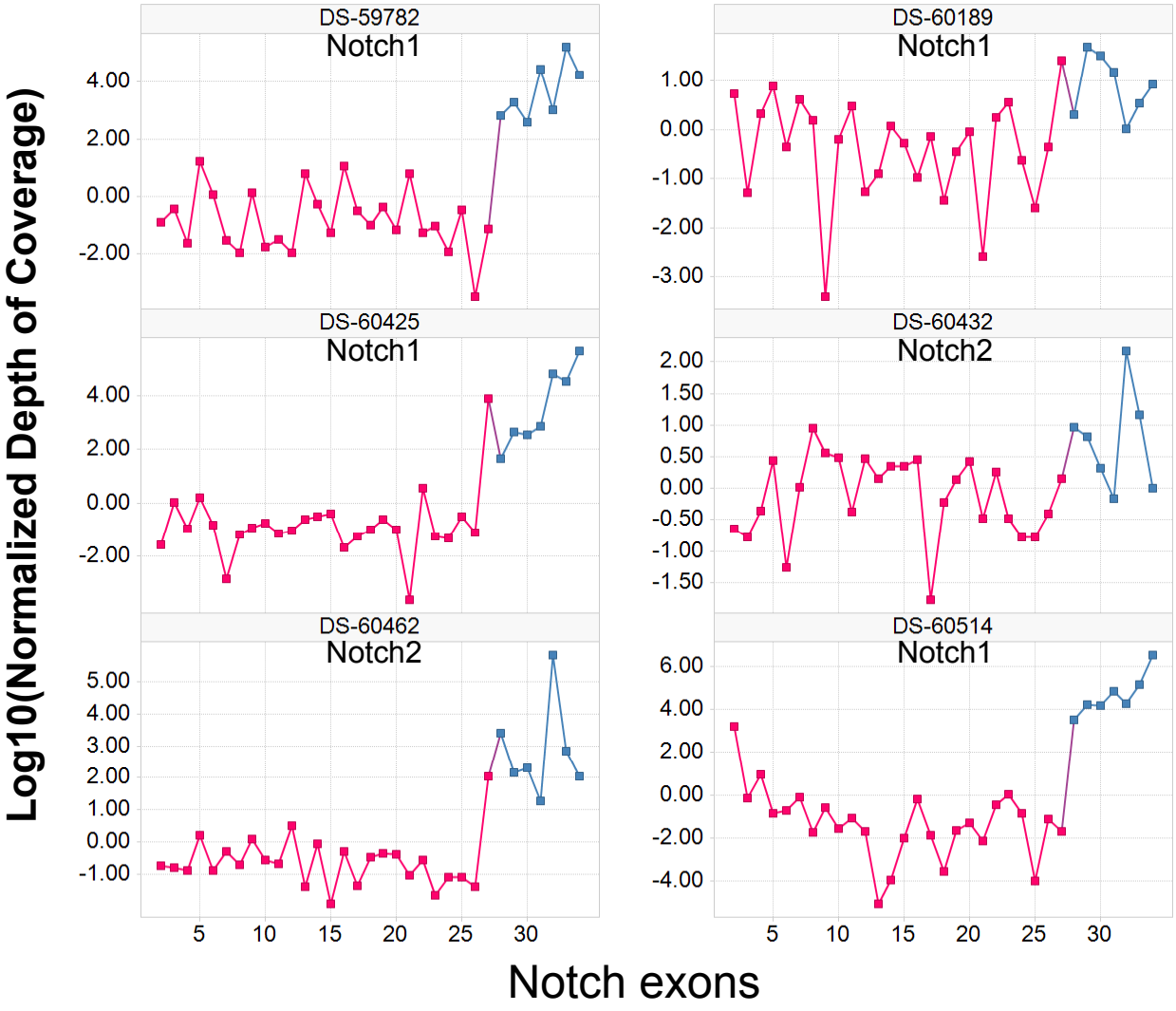
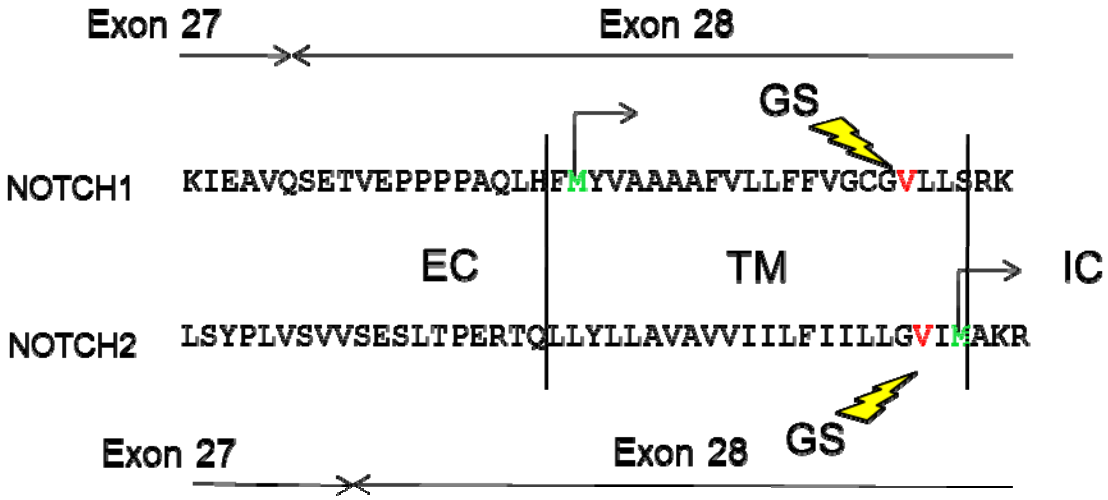


Figure S1: B) Relative coverage of Notch1 and Notch2 exons in triple negative breast tumors. Blue color corresponds to NICD domain and encompasses exons 27-34. Red color corresponds to exons 1 to 26. The depicted patient derived samples show significant (pvalue < 10-4) and at least 5 fold difference in coverage between exons 27-36 and 1-26.

Supplemental Figure S1C



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Supplemental Figure S2

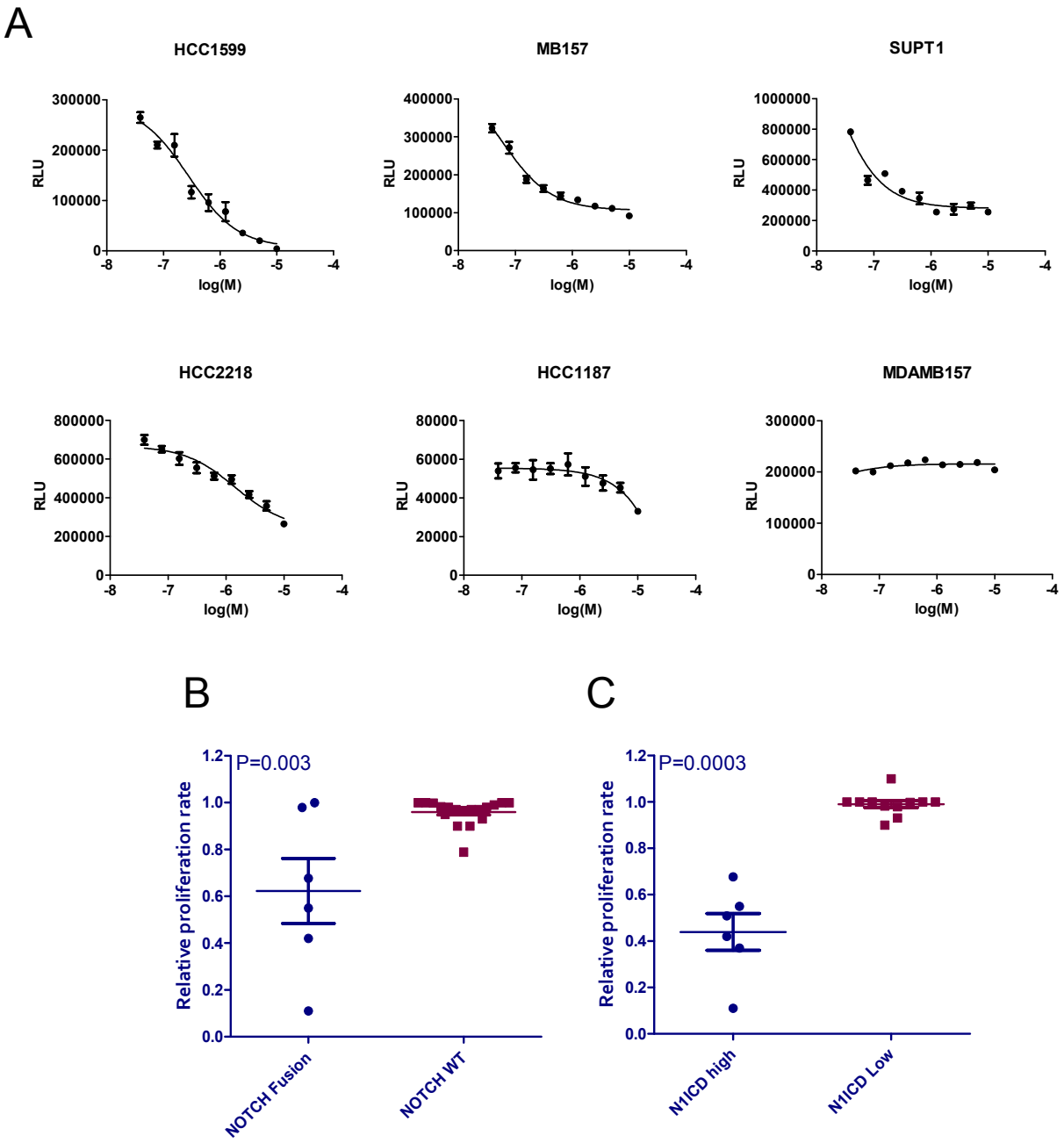

Figure S2: A) Dose dependent growth inhibition of cell lines with NOTCH gene re-arrangement. Sensitivity to MRK-003 in cell lines with recurrent NOTCH1 or NOTCH2 gene re-arrangement in which the N-terminal region is deleted is shown. Cell proliferation was analyzed at the end of 7 days and IC50 values are shown. The data is representative of 3 independent experiments. All cell lines with the exception of SUPT1 is of breast cancer origin. **B)** Relative proliferation rate at day 7 in the presence of MRK-003 (1.25 uM). Growth rate of cell lines harboring NOTCH1 or NOTCH2 gene-rearrangement or NOTCH WT were measured. MRK-003 treatment resulted in significant growth inhibition in a subset of NOTCH rearranged cell lines (p=0.003; t-test) **C)** Notch1 ICD levels are correlated with MRK-003 response. Cell lines were classified into N1ICD high or low based on the western blot analysis and quantification (Figure 2). Response to MRK-003 was enriched in N1ICD high group (P=0.0003; t-test)

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Supplemental Figure S2

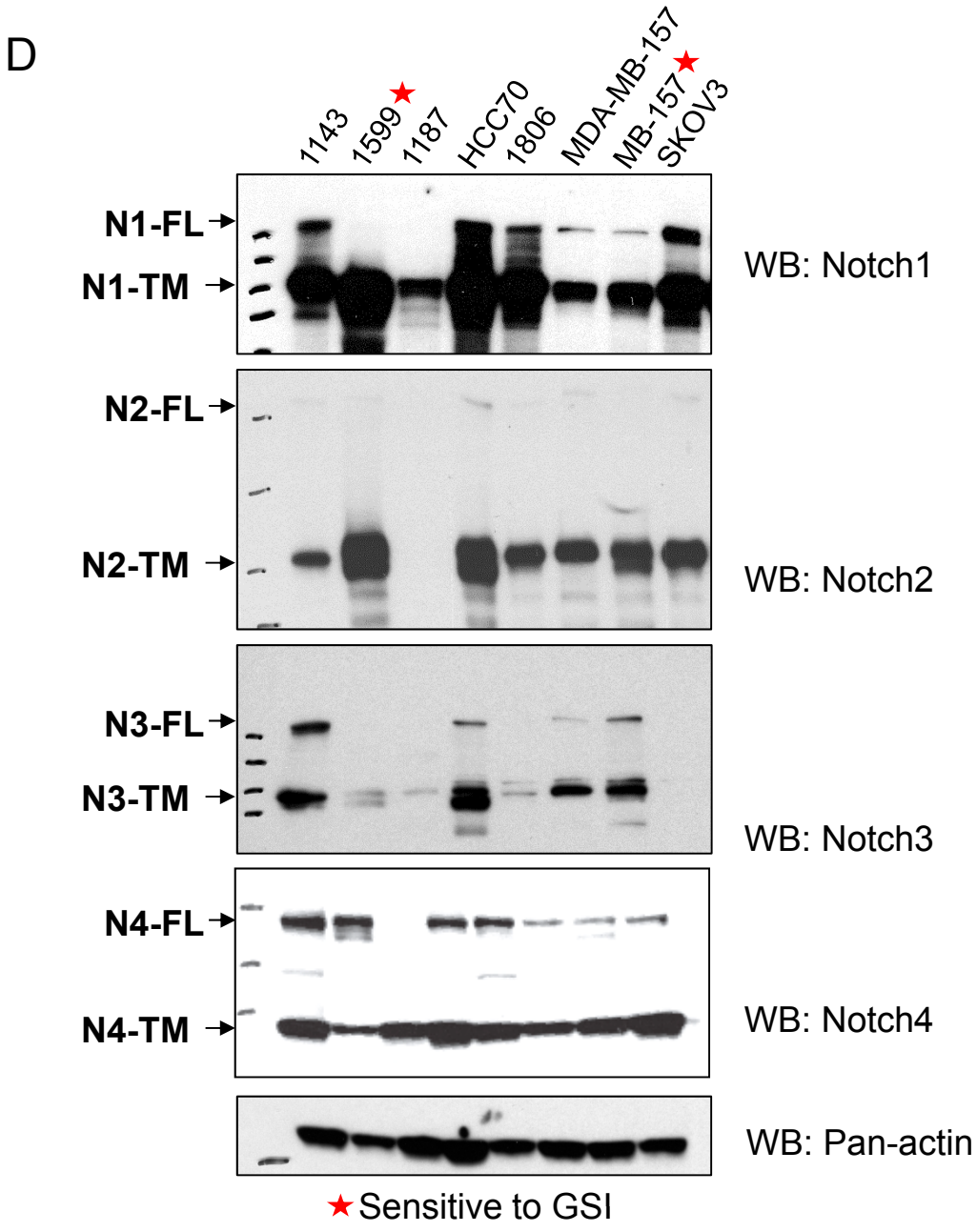


Figure S2D: NOTCH protein levels are not associated with response to MRK-003 treatment in breast cancer. Protein levels of NOTCH 1-4 were evaluated by western blot analysis using protein lysates from the breast cancer cell panel. Cell lines harboring NOTCH gene re-arrangement and sensitive to GSI therapy are indicated (red star). Both full length (FL) and the transmembrane region (TM) regions of the NOTCH proteins are indicated. Actin was used as a loading control.

Supplemental Figure S3

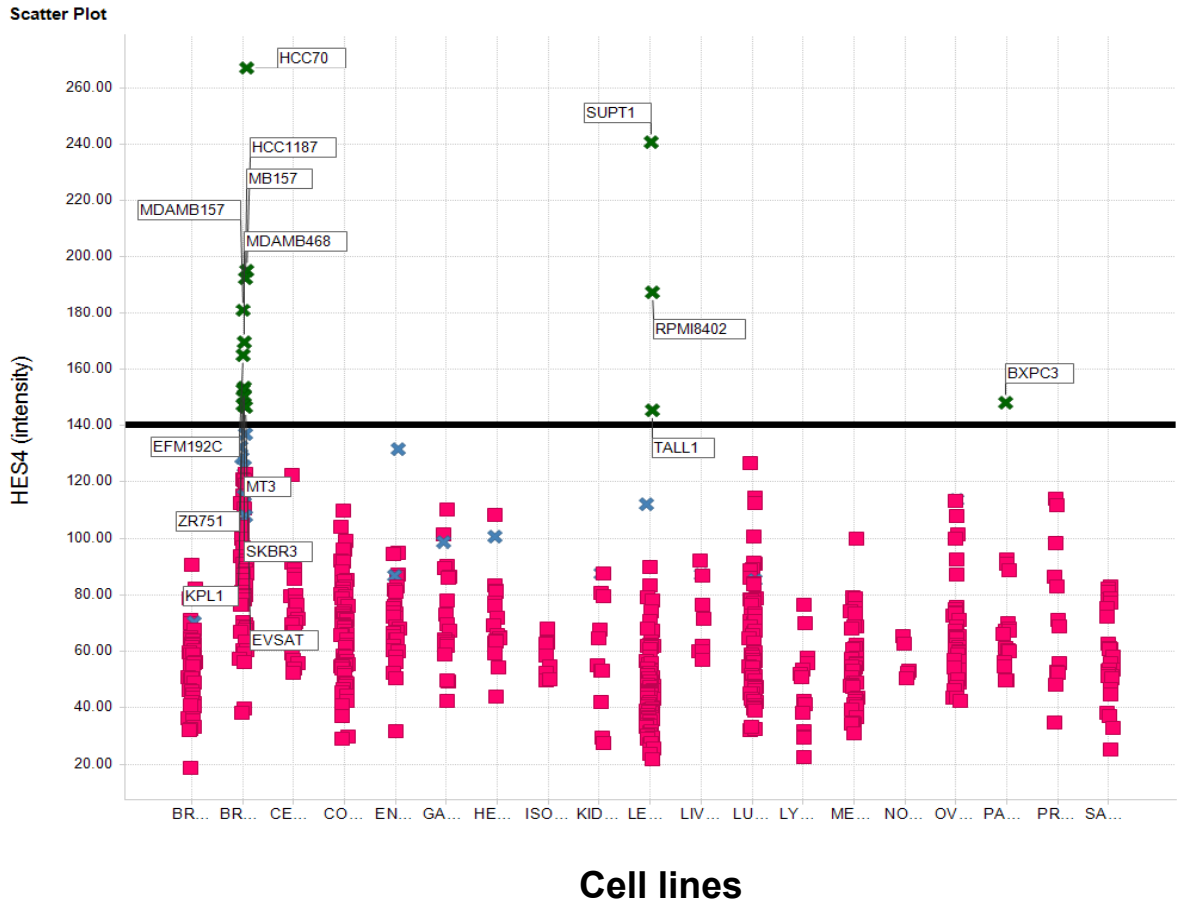


Figure S3: NOTCH gene-rearrangement or activating mutation is associated with elevated HES4 expression across tumor cell lines of multiple indications. Hes4 expression in 619 cell lines was analyzed by gene-expression profiling. Relative normalized probe intensities are provided. Cell lines harboring activating mutations or gene-rearrangements in NOTCH are indicated.

Supplemental Figure S4

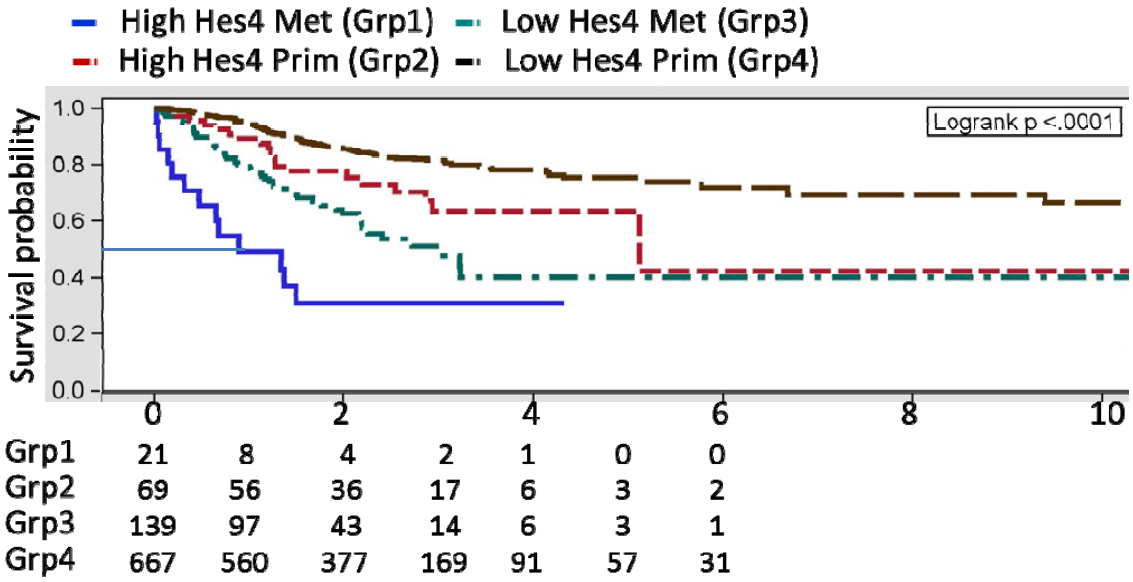


Figure S4: Elevated HES4 expression is a poor prognostic indicator in TNBC.

Supplemental Figure S5

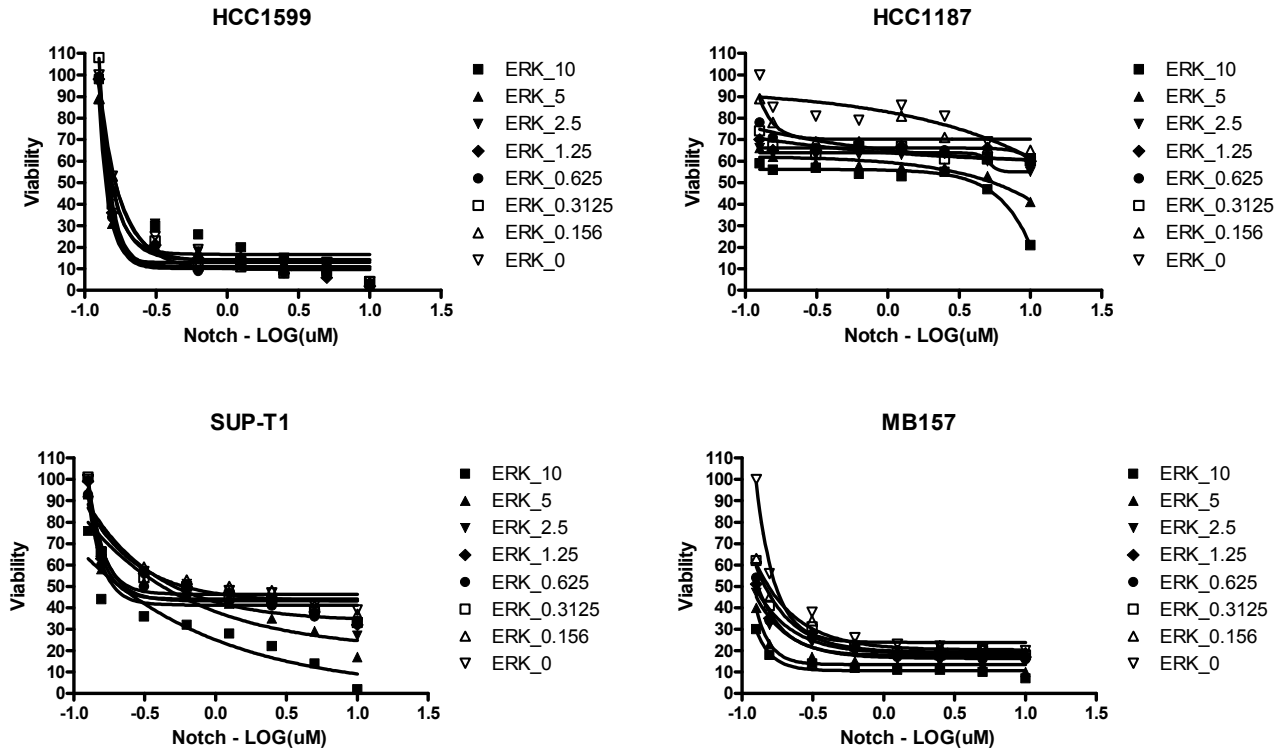
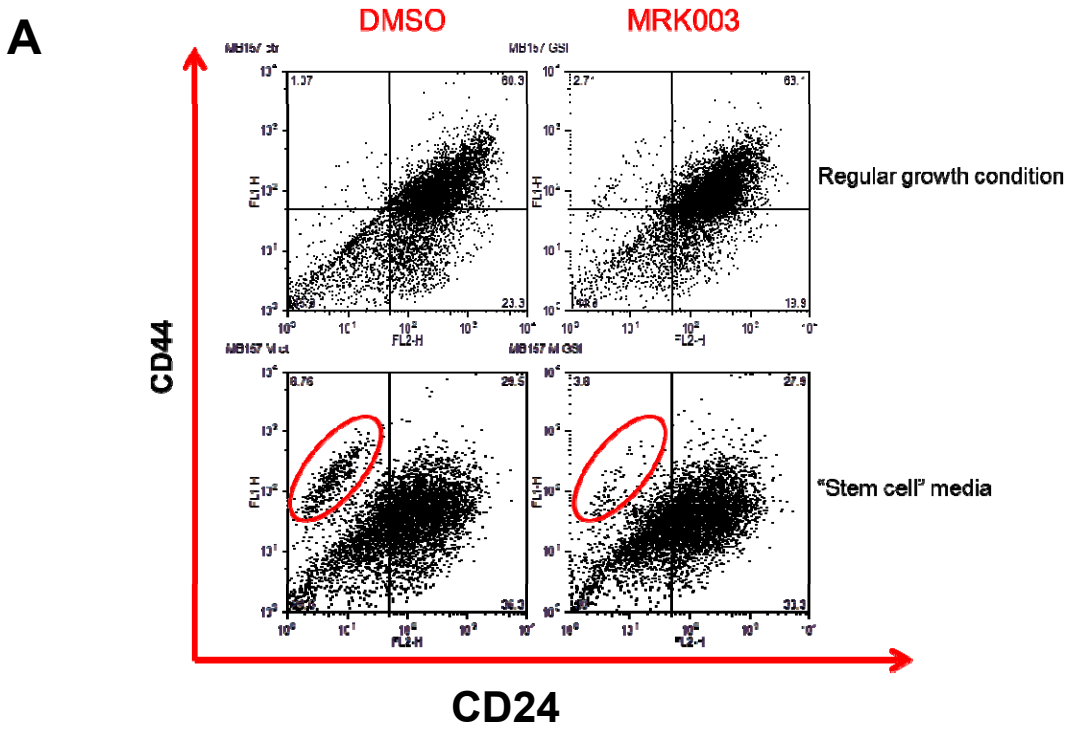


Figure S5: Proliferation inhibition by combination therapy with MRK-003 and ERK inhibitor (SCH772984). Cells were treated with increasing concentrations of both inhibitors in combination and viability was determined after 72h and is shown as percentage of untreated control. No significant combination benefit was observed.

Supplemental Figure S6



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Supplemental Figure S6

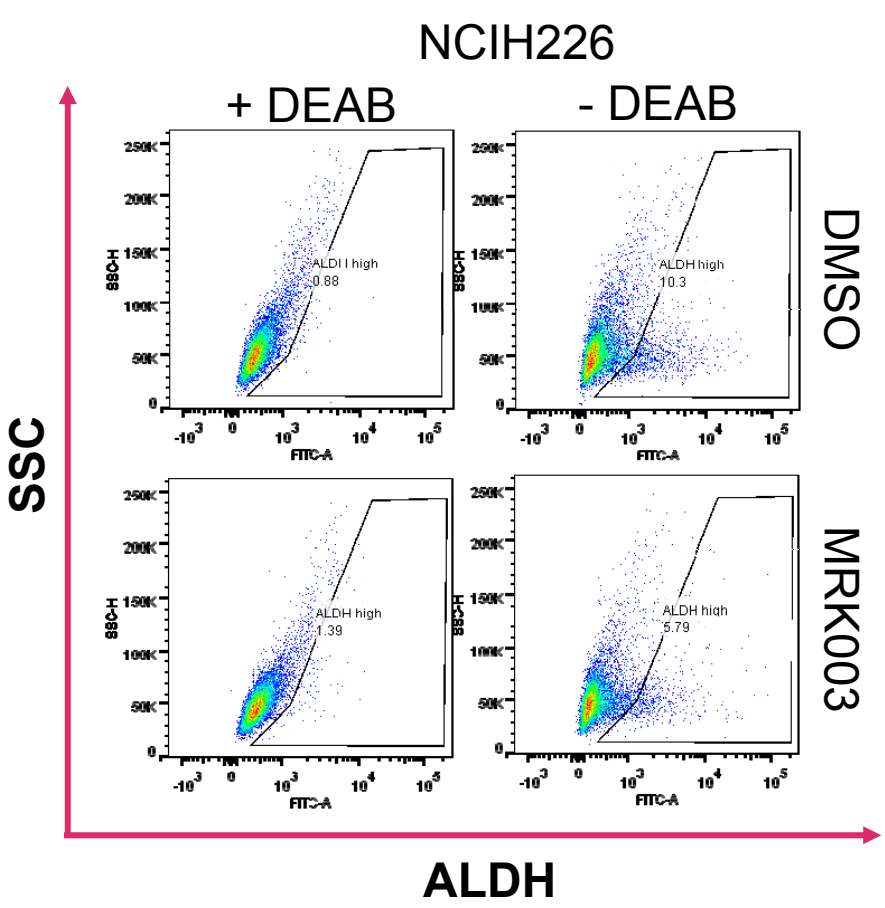
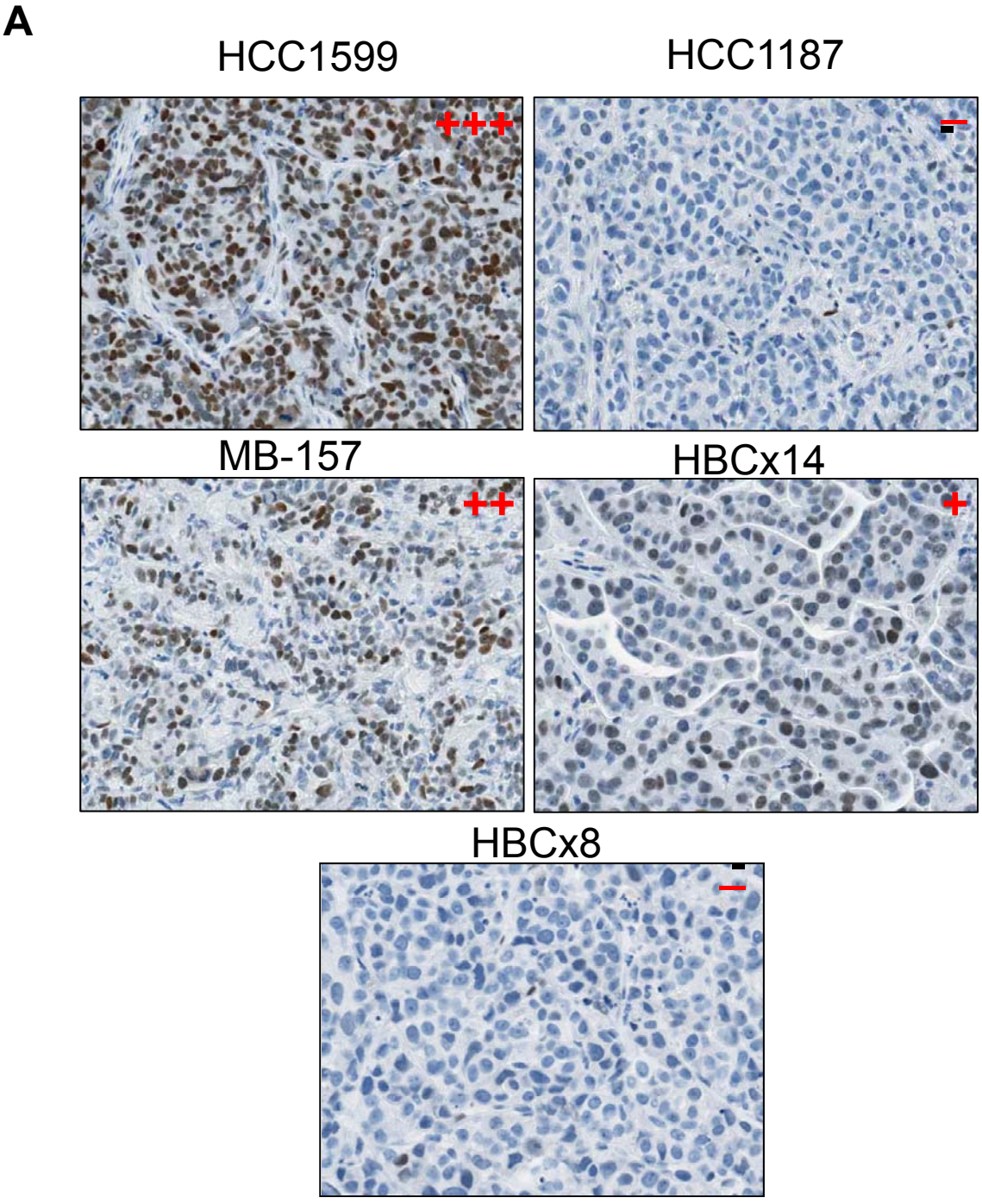


Figure S6: Effect of MRK-003 treatment on CD44^{+}/CD24^{high} stem-like cell population. A) Relative abundance (%) of double positive cells in MB-157 TNBC cell line is indicated .**B)** and **C)** MB157 and NCIH226 cells were treated with 500nM MRK003 or DMSO as control in duplicates. Side populations of ALDH high expressing cells were determined by utilizing the Aldefluor^{TM} kit according to manufacturers protocol. In both cell lines percentage of the ALDH high population was suppressed by treatment with control reagent DEAB or the gamma-secretase inhibitor MRK003.

Supplemental Figure S7



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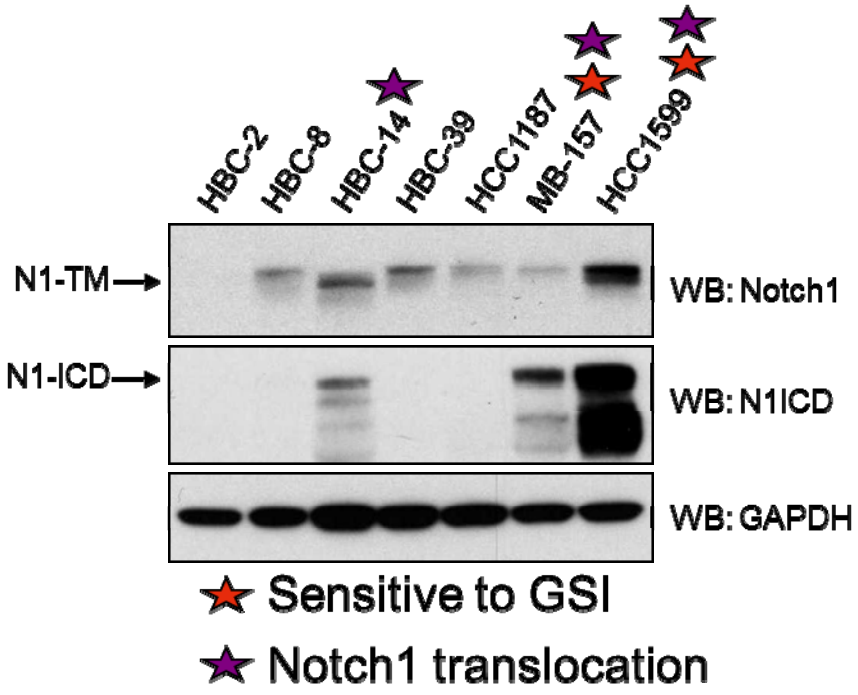
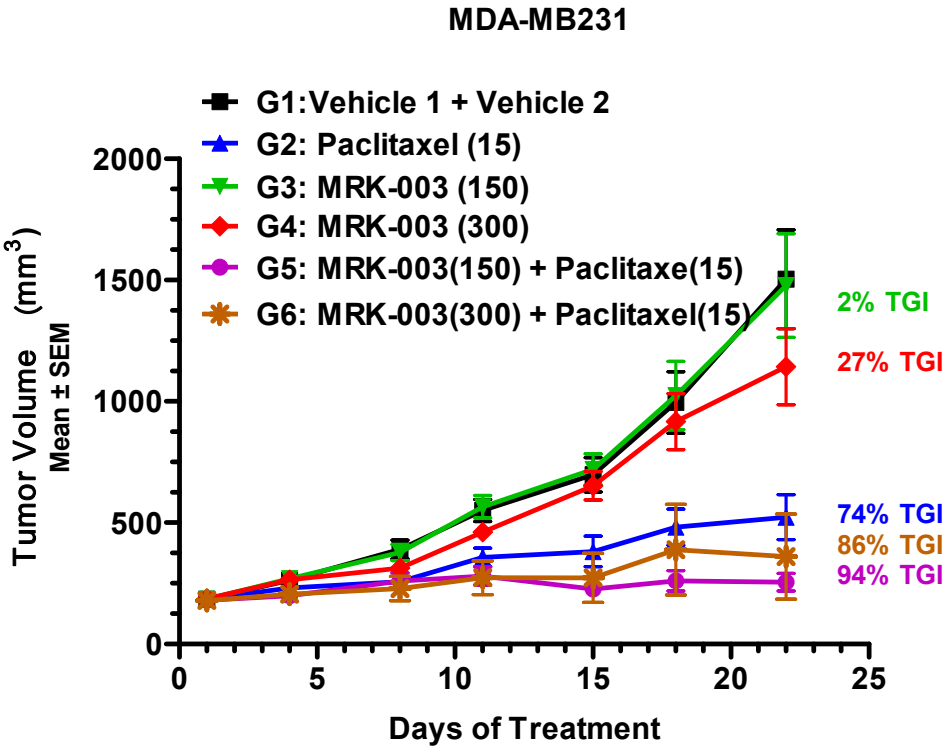
B


HBC-2
HBC-8
HBC-14
HBC-39
HCC1187
MB-157
HCC1599
N1-TM
N1-ICD
WB: Notch1
WB: N1ICD
WB: GAPDH
Sensitive to GSI
Notch1 translocation

C


MDA-MB231
G1:Vehicle 1 + Vehicle 2
G2: Paclitaxel (15)
G3: MRK-003 (150)
G4: MRK-003 (300)
G5: MRK-003(150) + Paclitaxe(15)
G6: MRK-003(300) + Paclitaxel(15)
Tumor Volume (mm3)
Mean ± SEM
2000
1500
1000
500
0
0
5
10
15
20
25
Days of Treatment
2% TGI
27% TGI
74% TGI
86% TGI
94% TGI

D

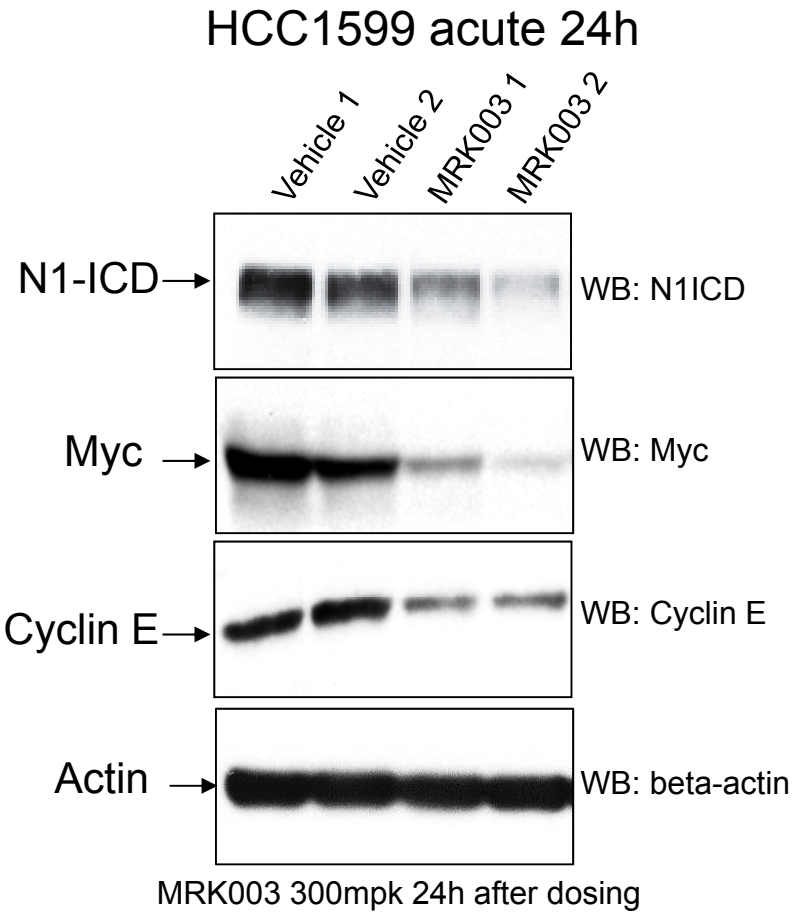


Figure S7: N1ICD correlates with mutation status and is predictive of MRK-003 response in xenograft models. A) Paraffin embedded tissue sections from xenografts were stained with anti-N1ICD rabbit monoclonal antibody as described in material and methods. The HCC1599 xenograft model harboring a Notch1 gene-rearrangement showed high levels of nuclear N1ICD (+++), followed by MB-157 (++) and HBCx14 (+). HBCx8 with rearrangement and HCC1187 cells WT for Notch1 were negative for N1ICD (-). **B)** Western blot analysis of tumor tissues from patient derived and cell line derived xenografts with antibodies against total Notch1 and N1ICD and GAPDH as loading control. N1ICD was only detected in samples harboring the Notch translocation including the patient derived TNBC model HBC14. Sensitivity to MRK003 treatment correlates with constitutive NICD levels observed. **C**) Tumor growth inhibition following MRK-003 and Paclitaxel treatment in MDA-MB-231. Combination treatment with paclitaxel did not show combination benefit in MDA-MB231 xenografts as TGI was not significantly higher compared to Paclitaxel alone. **D**) Acute changes in MYC and cyclin E following MRK-003 treatment. HCC1599 xenografts were treated with a single dose of 300mg/kg MRK003. Tumor tissues were harvested 24h after dosing. Western blot analysis was performed with antibodies against N1ICD, cMyc , Cycline E and Pan-Actin as loading control. Reduced NICD, Myc and Cycline E was observed in both animals treated with MRK003 compared to animals treated with vehicle control.