

Supplementary Information:-

A novel Monoclonal Antibody against Notch1 Targets Leukemia-associated Mutant Notch1 and Depletes Therapy Resistant Cancer Stem Cells in Solid Tumors

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\*Running Title: Targeting Notch1 Signaling in Cancer Stem Cells

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## **Supplementary Legends:**

**Supplementary Figure 1: Notch1 mutational analysis in T-ALL patients.**

**Supplementary Figure 2: (A) Effect of MAb 604.107 on LIC subpopulation:** The CD34<sup>High</sup>/CD45<sup>High</sup> cells were flow-sorted from the patient derived PBL's followed by culture with control IgG or the MAb 604.107 (10µg/ml) for 72 hours and BrdU incorporation into DNA was determined. (B) Effect of MAb 604.107 on Notch target genes in CCRF-CEM cells.

**Supplementary Figure 3: Effect of MAb604.107 on long-term self-renewing sphere forming cells.** Breast cancer cell lines MCF-7 and MDA-MB-231 cells were allowed to form sphere in semisolid medium in presence of MAb 604.107 for 1 week followed by second generation of sphere formation in the absence of MAb.

**Supplementary Figure 4: Effect of MAb 604.107 on NICD-1, Hes-1, Caspase-3 and BCL-2 expression.** MDA-MB-231 and HCT-116 cells were treated with control of MAb604.107 (20µg/ml) for 48 hours at the end of which whole cell lysate was prepared and level of NICD-1, Hes-1, Caspase-3 and BCL-2 was evaluated in western blot. Beta-actin was used as normalizing control.

**Supplementary Figure 5: Effect of anti-Notch1 antibody treatment on stemness genes in breast and colon cancer cell lines.**

**Supplementary Figure 6: Effect of anti-Notch1 antibody treatment on EMT genes in breast and colon cancer cell lines.**

**Supplementary Figure 7: Effect of MAb604.107 on Dox resistant cell lines.** MDA-MB-231 and HCT-116 cells were cultured in the presence of Dox for three week and Dox-resistant cells lines were established. (A) Expression level of NICD-1 in Dox sensitive and resistant cells. (B) Dox sensitive and resistant MDA-MB-231 and HCT-116 cells were cultured in the presence of control IgG or MAb (20µg/ml) and BrdU incorporation was determined.

**Supplementary Figure 8: Effect of anti-Notch1 antibody treatment on Notch downstream targets and stemness genes in xenografts.**

**Supplementary Figure 9: Expression of NICD-1 in control of MAb treated (A) MDA-MB-231 and (B) HCT-116 xenografts.**

**Supplementary Figure 10: ELISA based epitope mapping of MAb604.107**

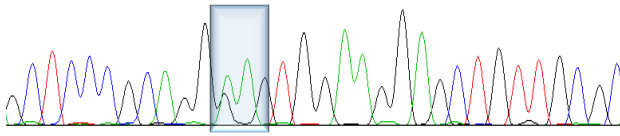
**Supplementary Table 1: Phage-display screening for epitope mapping.**

**Supplementary Table 2: Comparison between MAb604.107 with previously reported inhibitory anti-Notch1 Mab.**

# Supplementary Figure-1

## T-ALL 1

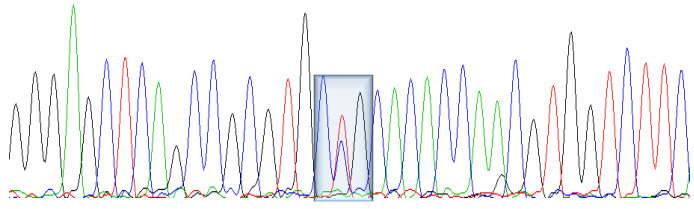
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F1593S

## T-ALL 7

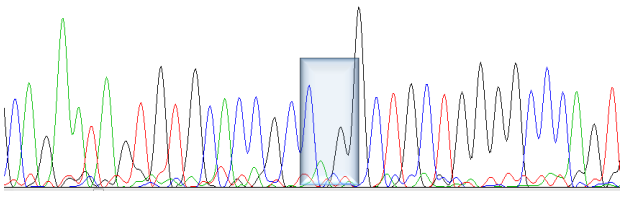
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L1601P CTG - CCG

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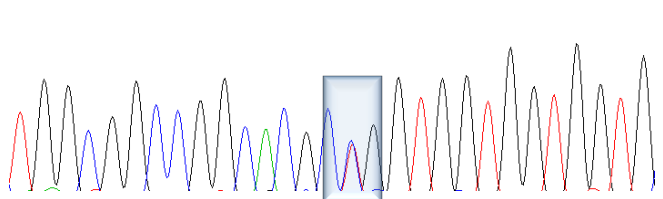
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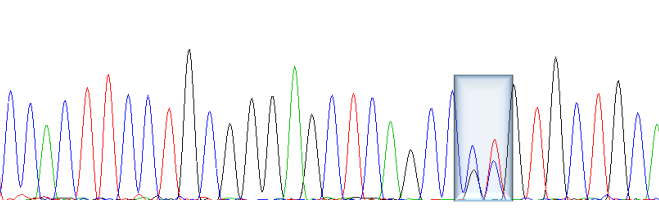
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## T-ALL 12

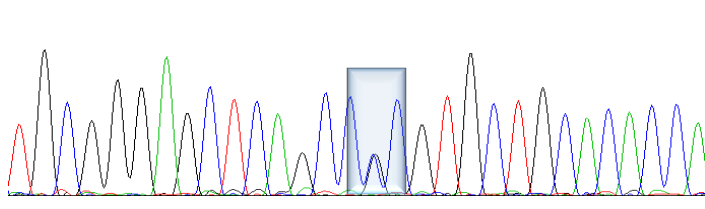
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R1599P CGC-CCC

## T-ALL 17

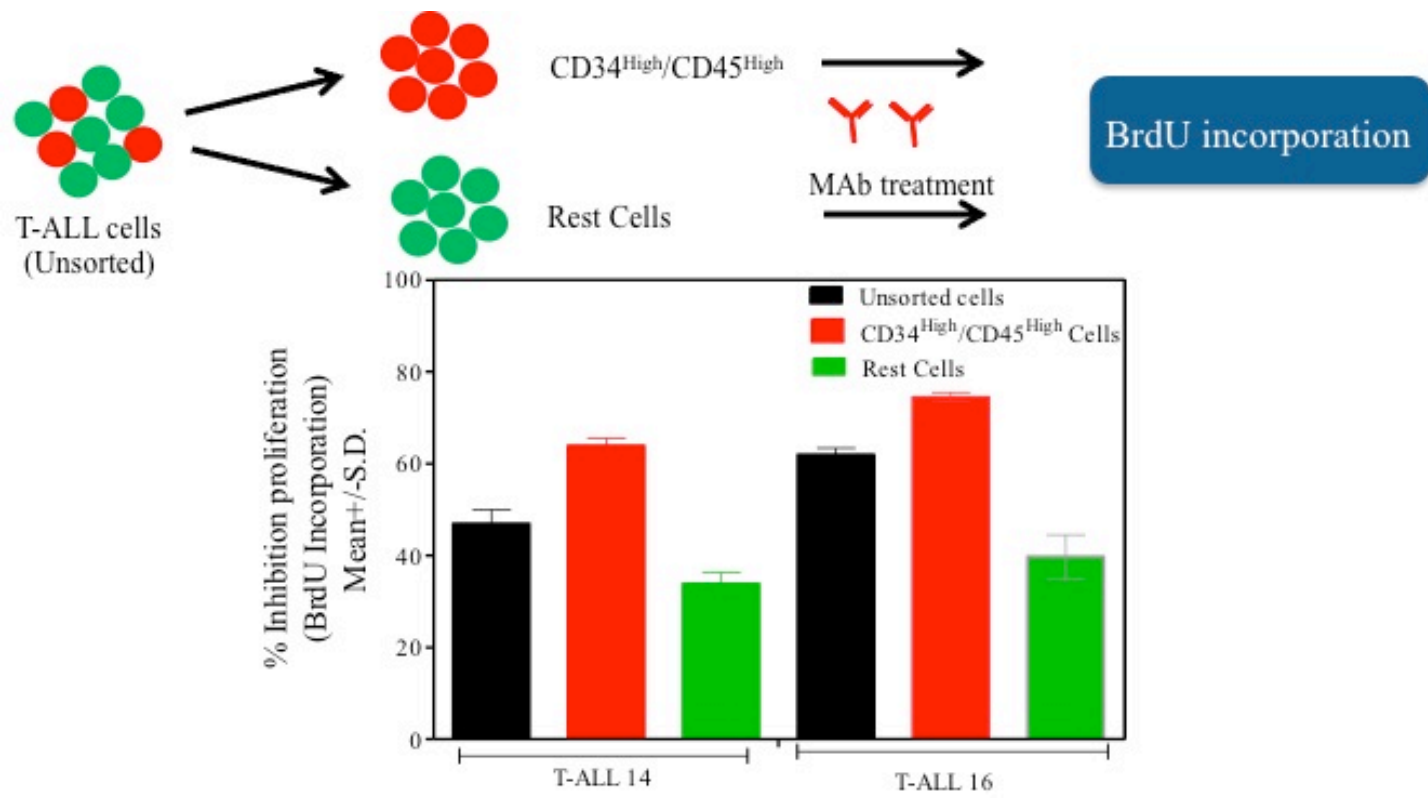
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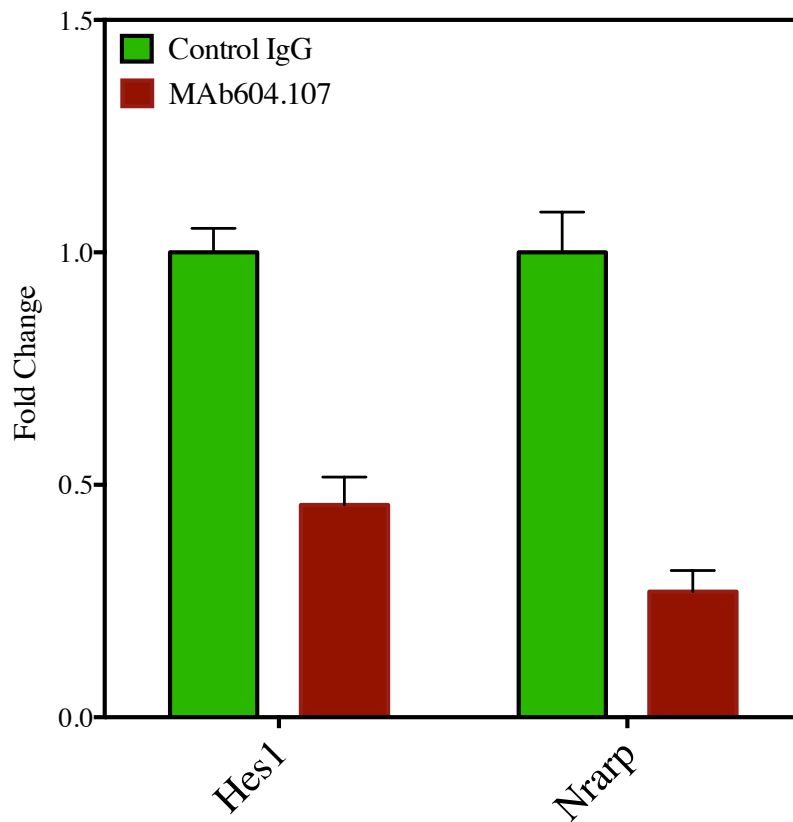
R1599P CGC-CCC

## Supplementary Figure-2

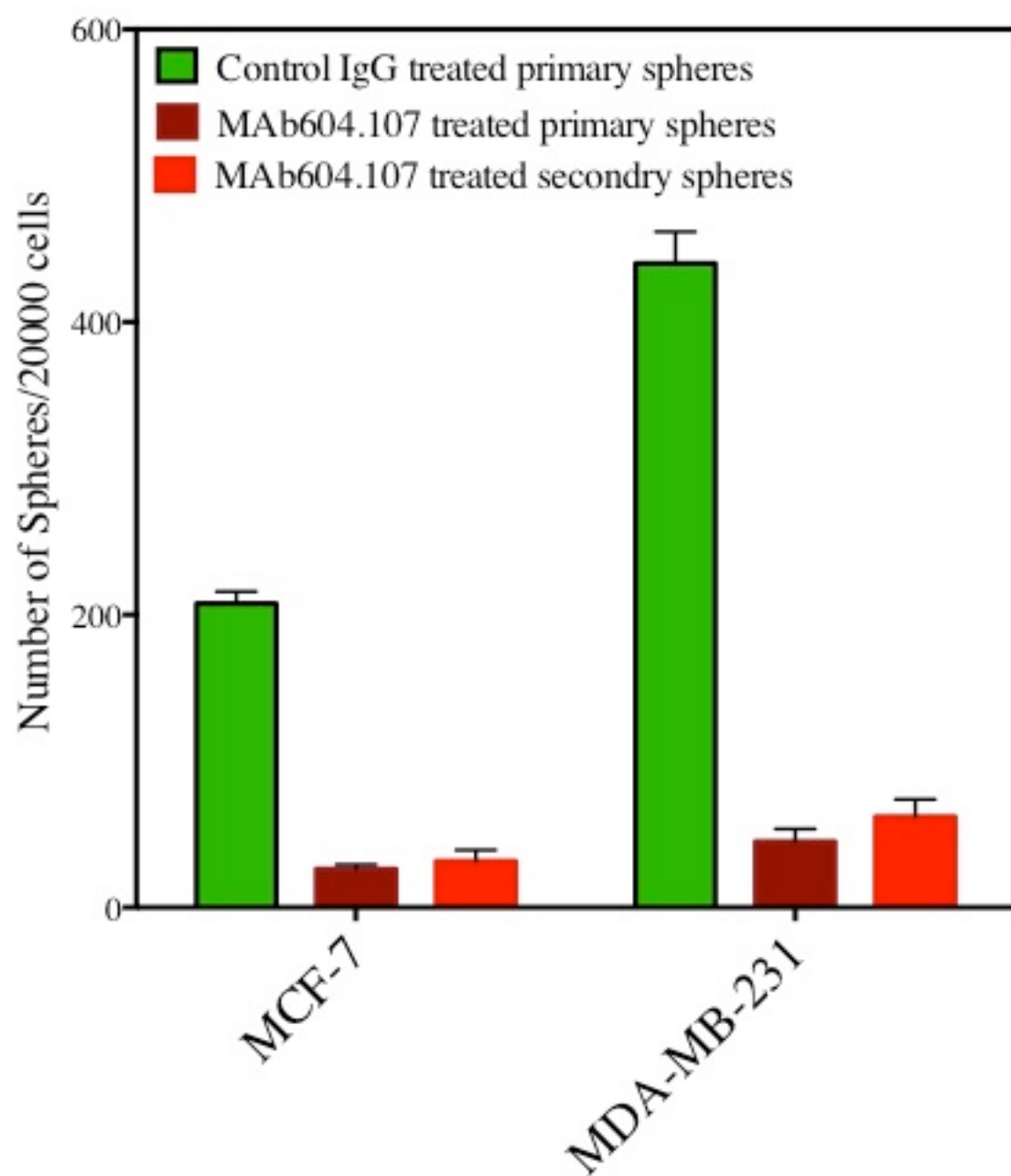
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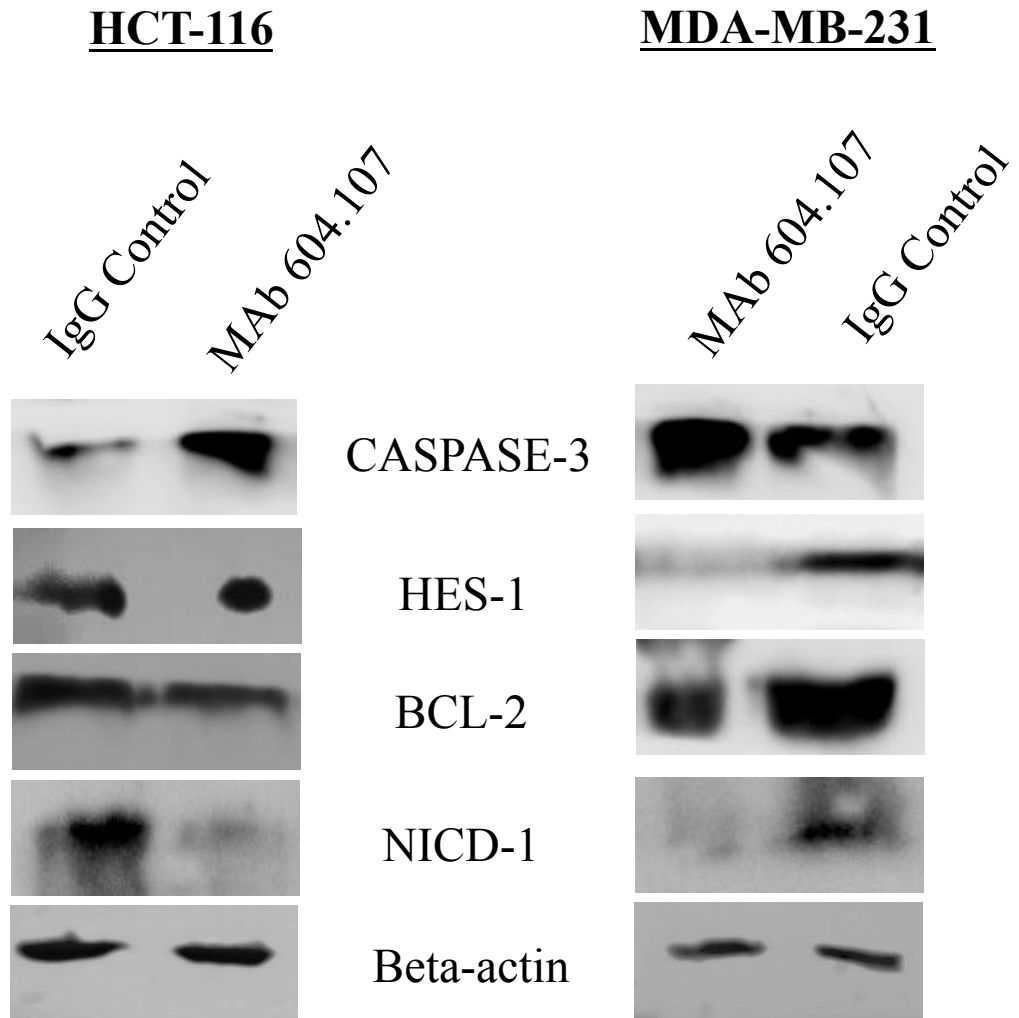
(B)



Supplementary Figure-3

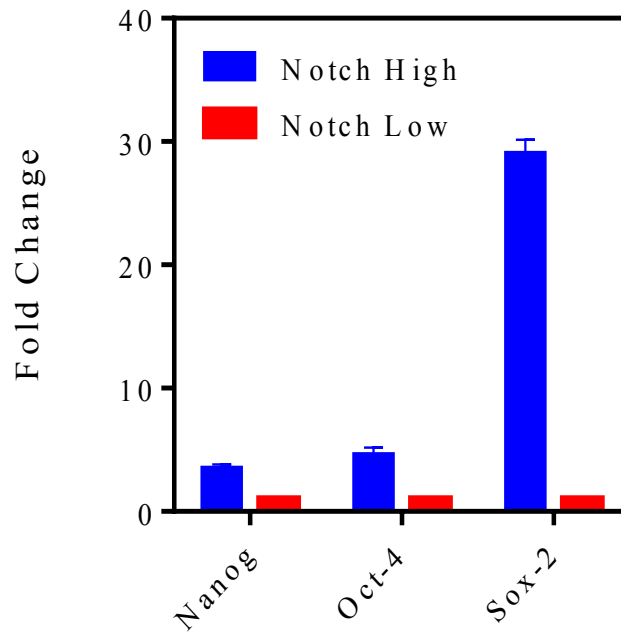


# Supplementary Figure-4

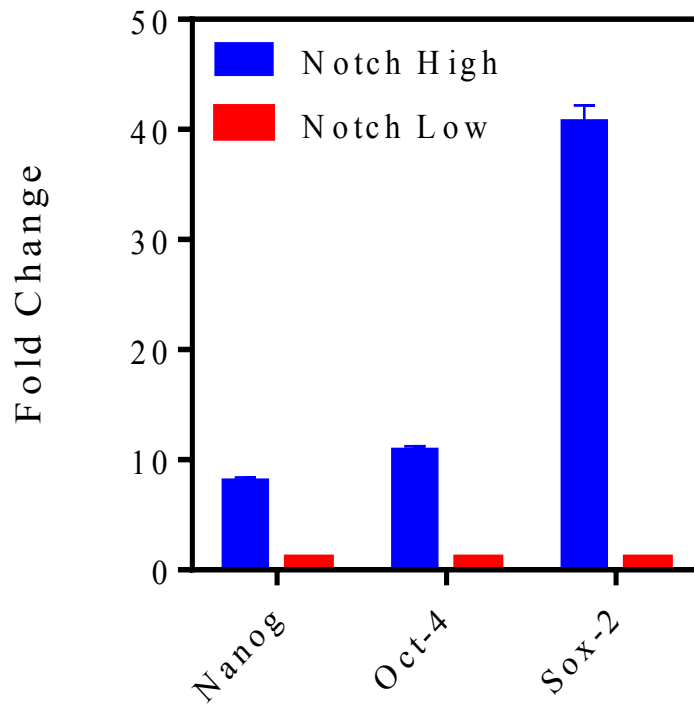


# Supplementary Figure-5

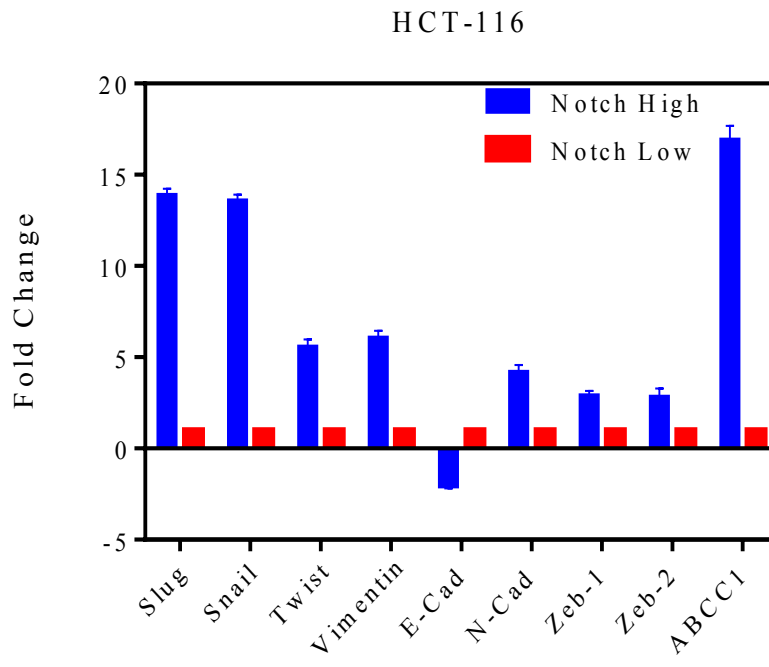
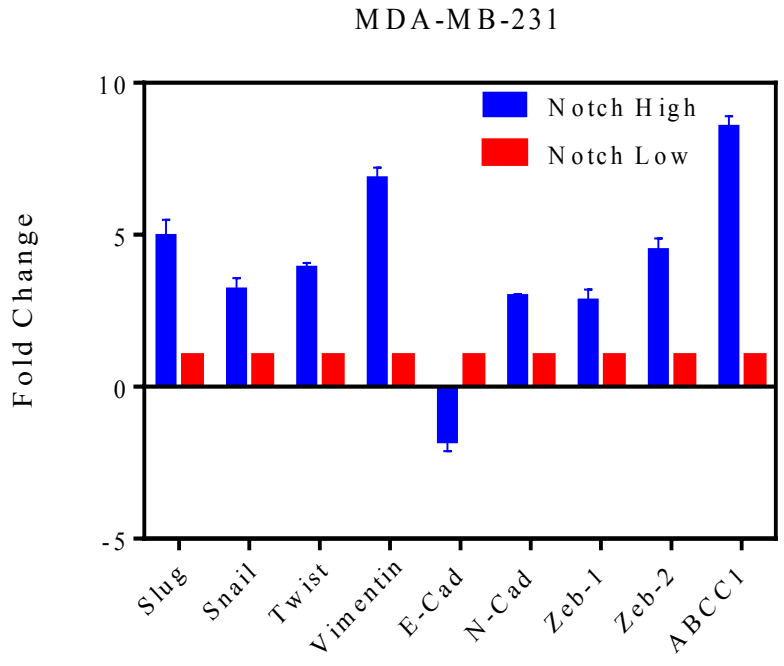
MDA-MB-231



HCT-116

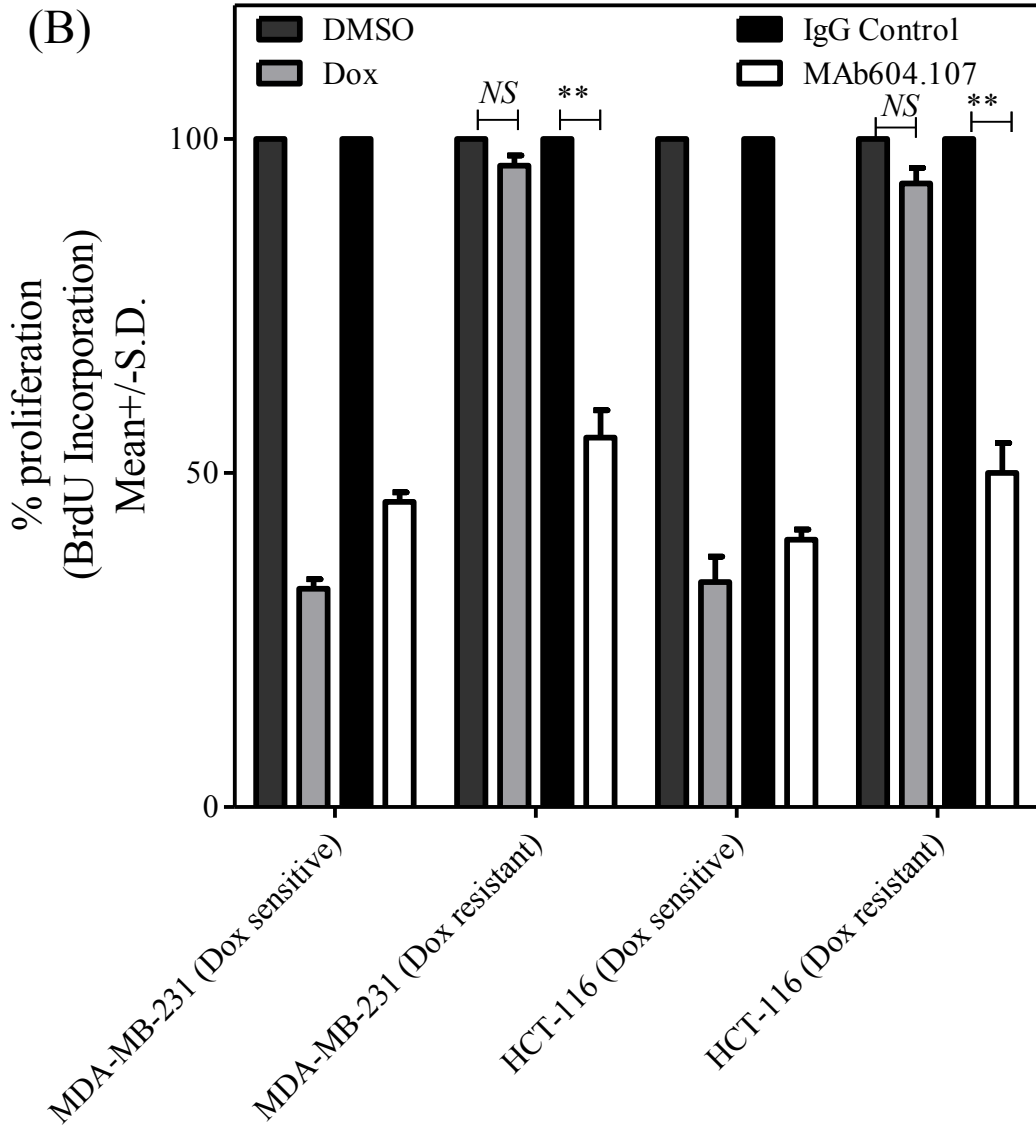
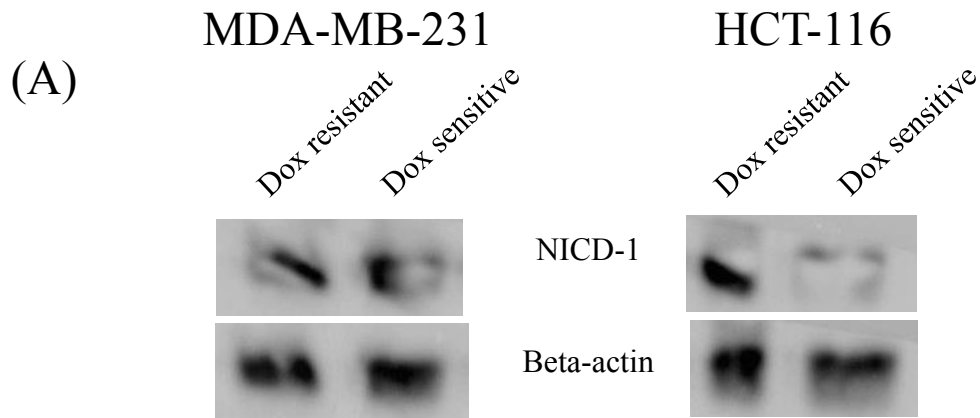


# Supplementary Figure-6



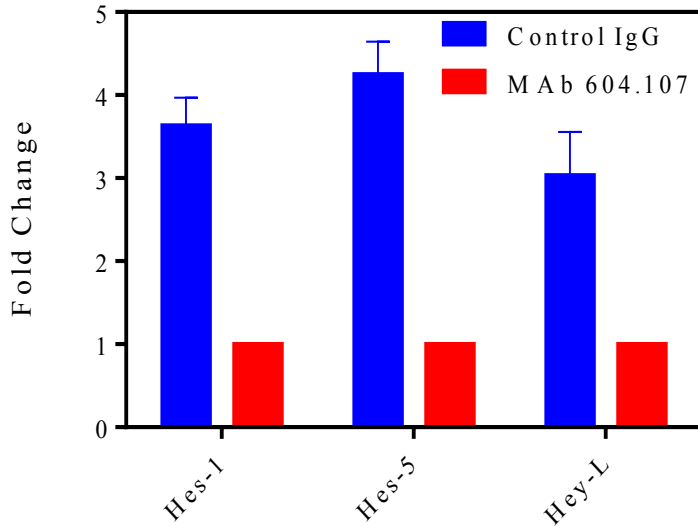


# Supplementary Figure-7

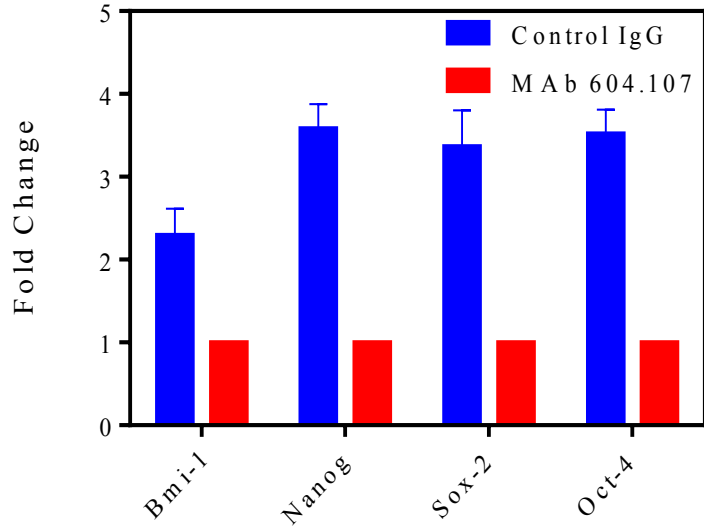


# Supplementary Figure-8

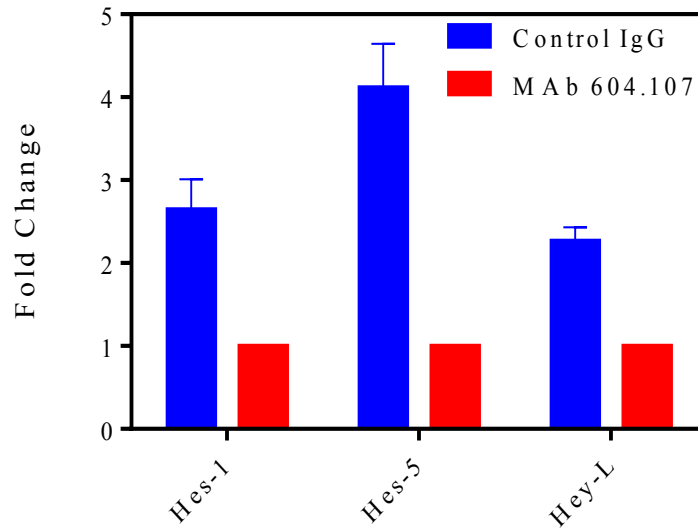
MDA-MB-231



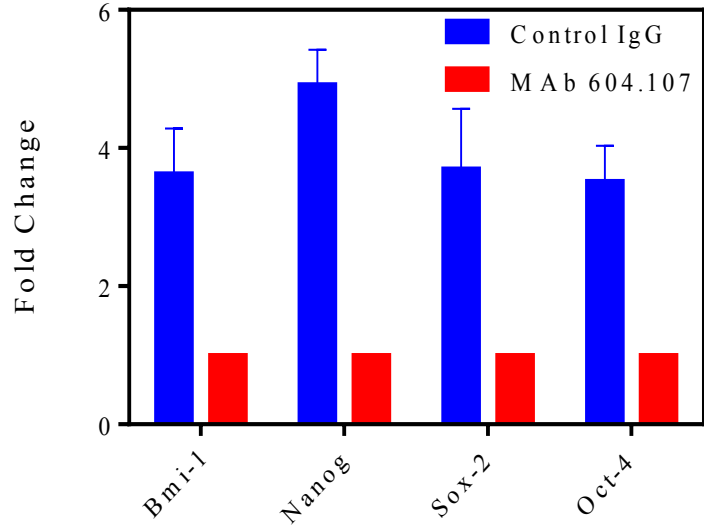
MDA-MB-231



HCT-116



HCT-116

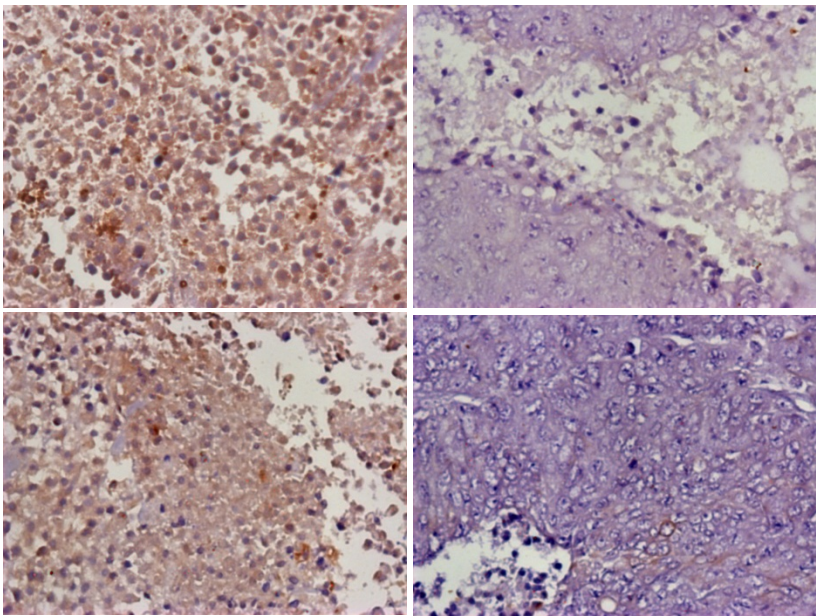


Supplementary Figure-9

Control IgG treated  
tumors

604.107 treated  
tumors

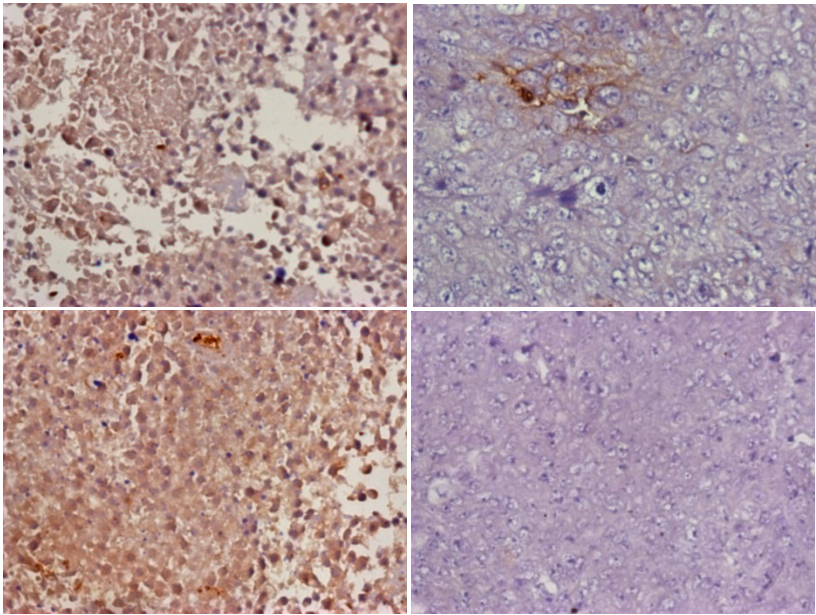
MDA-MB-231



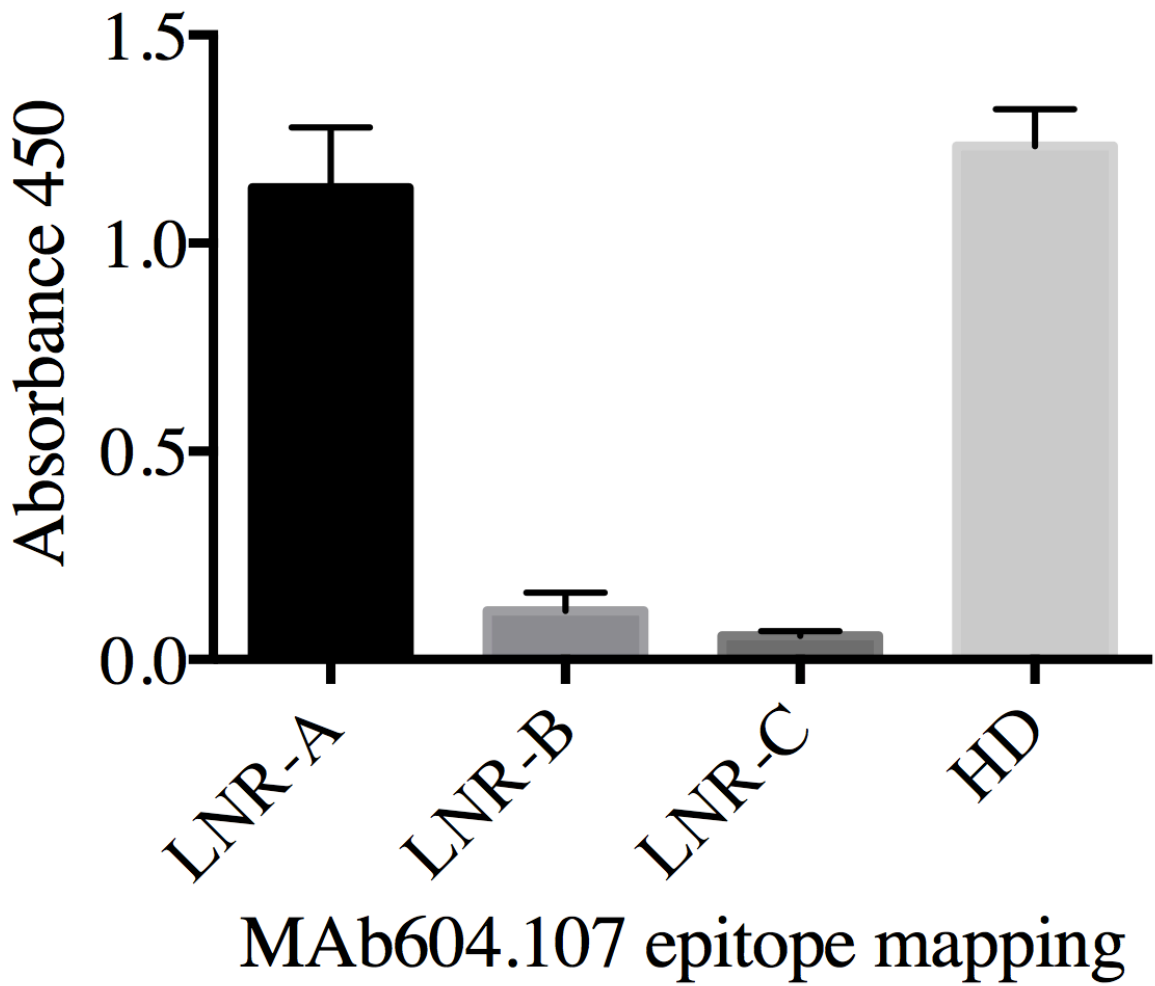
Control IgG treated  
tumors

604.107 treated  
tumors

HCT-116



Supplementary Figure-10



## Supplementary Table-1

<b>MAb</b>	<b>Putative epitopes in Notch1 NRR domain</b>	<b>NRR domain</b>
604.107	LRNSSFHFLRELSRVL HTNVVFKRD	Heterodimerization (HD) domain
604.107	SLNFNDPWKNCTQSL QCWKYFS	LNR-A
604.132	IFPYYGREEELRK	HD
604.132	SLNFNDPWKNCTQSL QCWKYFS	LNR-B
604.164	HGQQMIFPYY	HD

## Supplementary Table-2

Properties of MAb	Present study 604.107 (Sharma et al.,)	Previous study <sup>18</sup> (Wu et al., Nature 2010)
Effect on wild-type Notch signaling	Inhibitory (at higher dose)	Inhibitory
Affinity for wild-type Notch1	$5.08 \times 10^{-8}$	$2.5 \times 10^{-9}$
Effect on mutant Notch1	Inhibitory (both at higher and lower doses)	Inhibitory
Affinity for mutant Notch1	$5.08 \times 10^{-9}$	ND
Specificity for mutant Notch1	At lower concentration specific for mutant Notch1 without affecting wild-type signaling	ND
Effect on LIC subpopulation	Deplete LIC subpopulation	ND
Impact on CSC and chemoresistance cells	Irreversibly inhibit CSCs	ND

ND- not determined