A novel triazole NMK-T-057 induces autophagic cell death in breast cancer cells by inhibiting γ-secretase-mediated activation of Notch-signaling.

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Running title: NMK-T-057 inhibits γ-secretase mediated Notch activation

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Table I: *In vivo* toxicity assessment of NMK doses in Swiss Albino mice

Parameters	GRP- I Control	GRP- II: NMK-T-057 (25 mg/kg / BW)	GRP III: NMK-T-057 (50 mg/kg / BW)
Body weight (g)	26.02 ± 0.9	24.14 ± 1.7	24.88 ± 2.27
Hb (g/dl)	15.66 ± 0.7	14.98 ± 0.6	14 ± 0.7
Total WBC count (X 10^3 cells/ mm^3)	6413 ± 0.7	6140 ± 0.6	5167 ± 2
Total RBC blood (X 10^6 cells/ mm^3)	5.5 ± 0.8	5.22 ± 0.7	5.42 ± 0.6
Neutrophil %	40.5 ± 6	41.5 ± 4.3	35.5 ± 4*
Lymphocyte %	51.5 ± 4	50.5 ± 6.7	40.5 ± 6*
Monocyte %	1.3 ± 0.7	1.6 ± 0.6	1.7 ± 1.1
Eosinophil %	0.9 ± 0.8	1.2 ± 0.7	1.4 ± 0.5*
Basophil %	0.2 ± 0.5	0.4 ± 0.6*	0.4 ± 0.6*
SGPT (U/l)	32±3.2	35±4	40±3.7*
SGOT (U/l)	84±4.3	90±3.5	105±4.1*
ALP (U/l)	110±10.1	115±5.7	118±5.0

Table I: Haematology and hepatic parameters of control and NMK-T-50 treated Swiss Albino mice (25 mg/kg body weight and 50 mg/kg body weight, 7 days treatment). Impact on the hematological parameters such as Hb, RBC, WBC, Neutrophil %, Monocyte % and Eosinophil % and hepatic parameters such as SGOT, SGPT and ALP, were monitored in each group † Results expressed as mean ± S.D. (n=5).

Table II : Details of interactions of ligands docked at γ-Secretase (PDB ID: 5FN2)

Docked molecule	Interaction Type						
	Hydrogen bond		Hydrophobic				Van der Waals
	onventional	Carbon Hydrogen	Pi-Sigma	Pi-Pi T-Shaped	Alkyl	Pi-Alkyl	
DAPT -7Kcal/mole	--	--	PHE (C21)	PHE (B411)	ALA (C39) VAL (B412) LEU (B415) VAL (C36) LEU (C35) ILE (C127) ILE (C128)	ILE (B408)	VAL (C32) VAL (C131)
NMKT -8 kcal/mole	TYR (C119)	SER (A683) PRO (C16)	LEU (C169)	PHE (C173)	--	LEU (C20) ILE (A690) VAL (C176) ALA (C228) ALA (C232)	THR (C172) PHE (A698) THR (A687)

Table II: Different interactions stabilizing the binding of DAPT and NMK-T-057 to γ-Secretase (PDB ID: 5FN2)

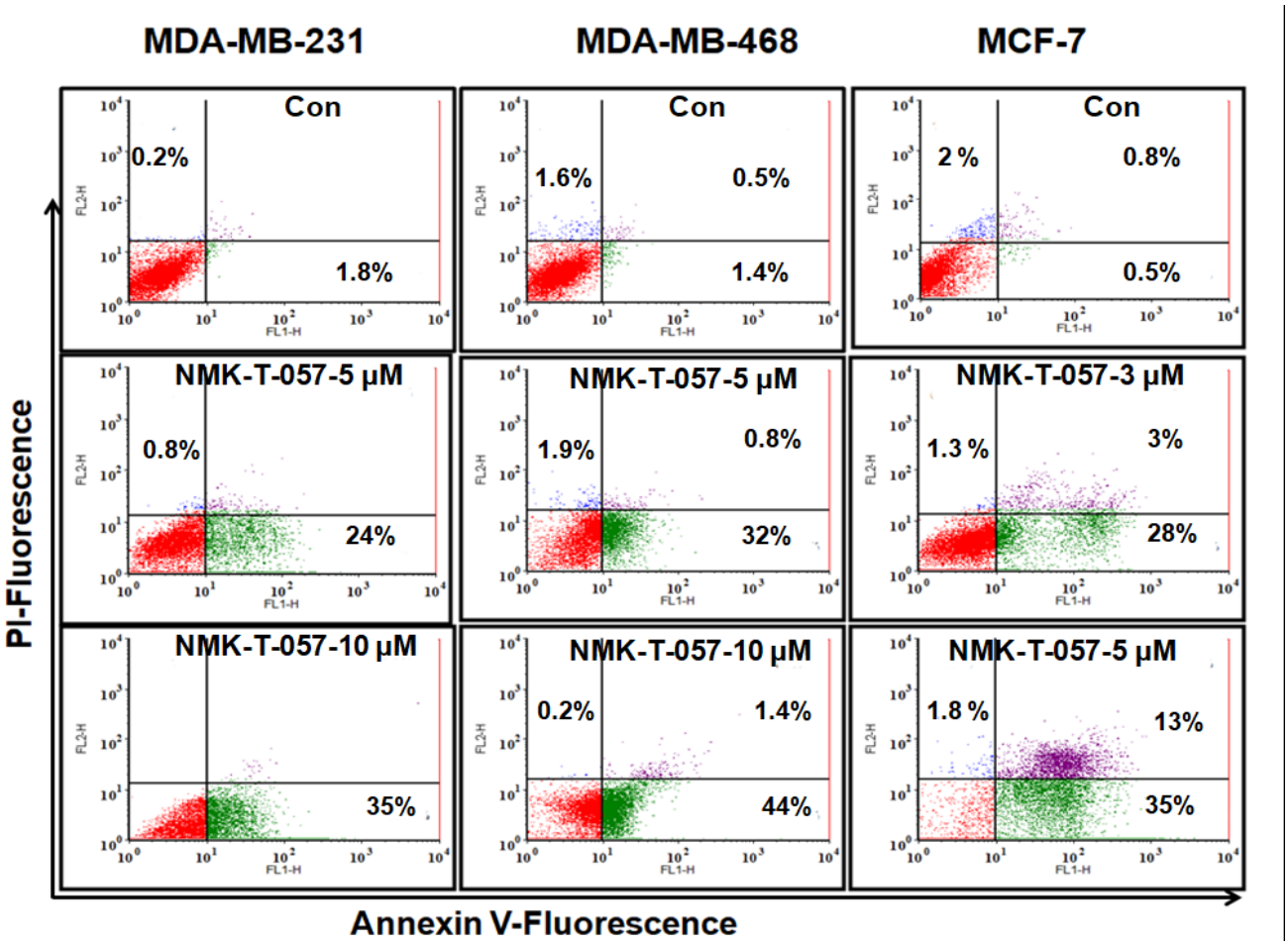

Fig.S1. Induction of apoptosis in BC cells by NMK-T-057

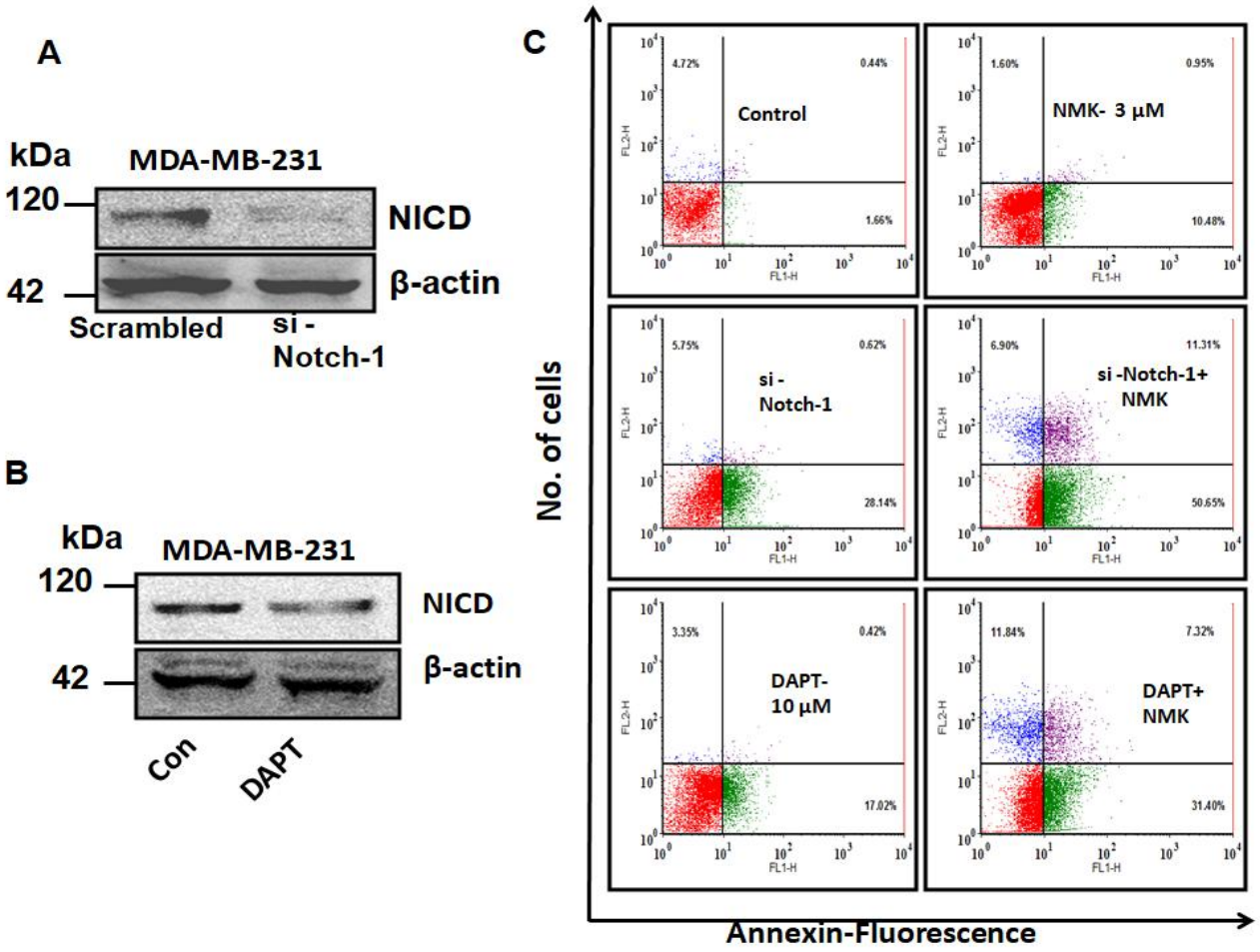

Fig. S2. Depletion of Notch induces apoptosis in MDA-MB-231 cells. A. NICD-expression in MDA-MB-231 cells transfected with scrambled/ siNotch-1 (This blot is also used as Fig. 5C in the main text). B. NICD-expression in MDA-MB-231 cells treated with DAPT. C. Induction of apoptosis in Notch-depleted MDA-MB-231 cells alone or in combination with NMK-T-057.

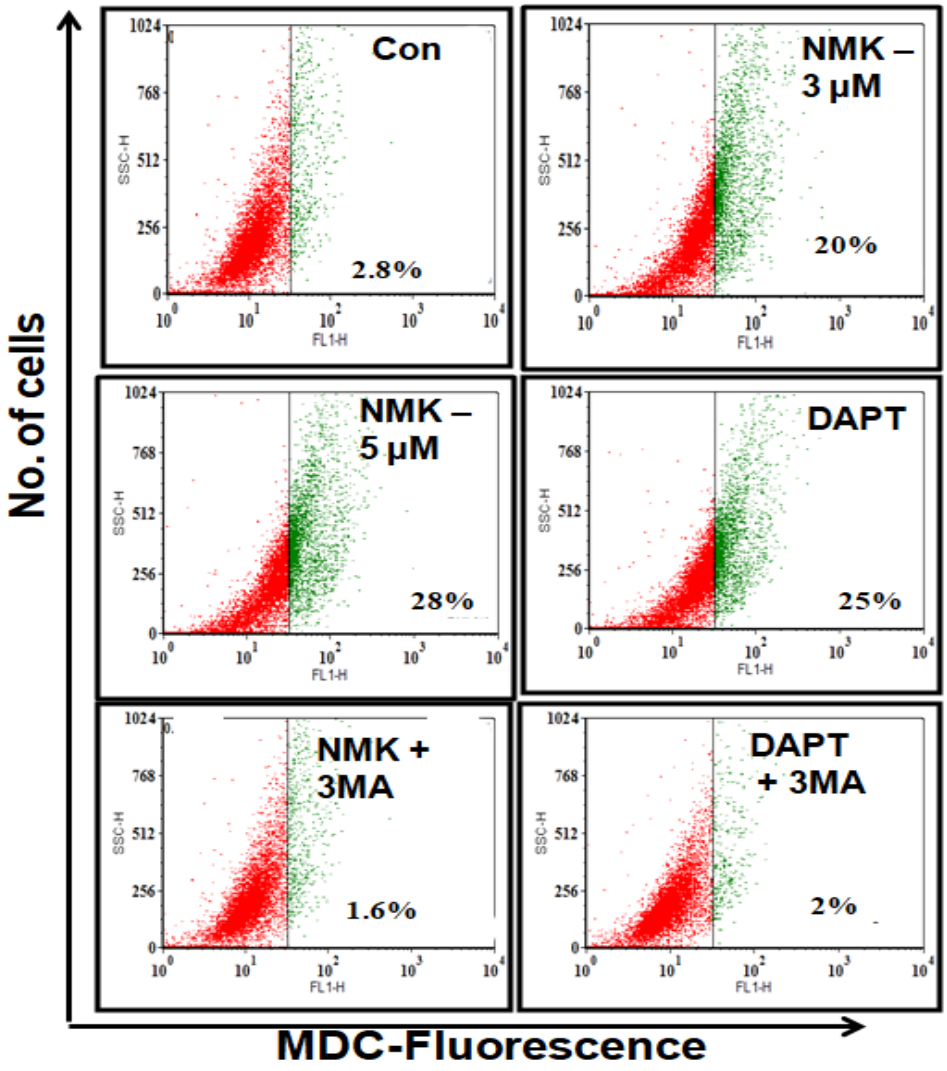

Fig. S3: FACS analysis of autophagic population stained with MDC in control and DAPT/ NMK-treated MDA-MB-231 cells, in the absence and presence of the autophagy inhibitor 3-MA, presented as dot plot.

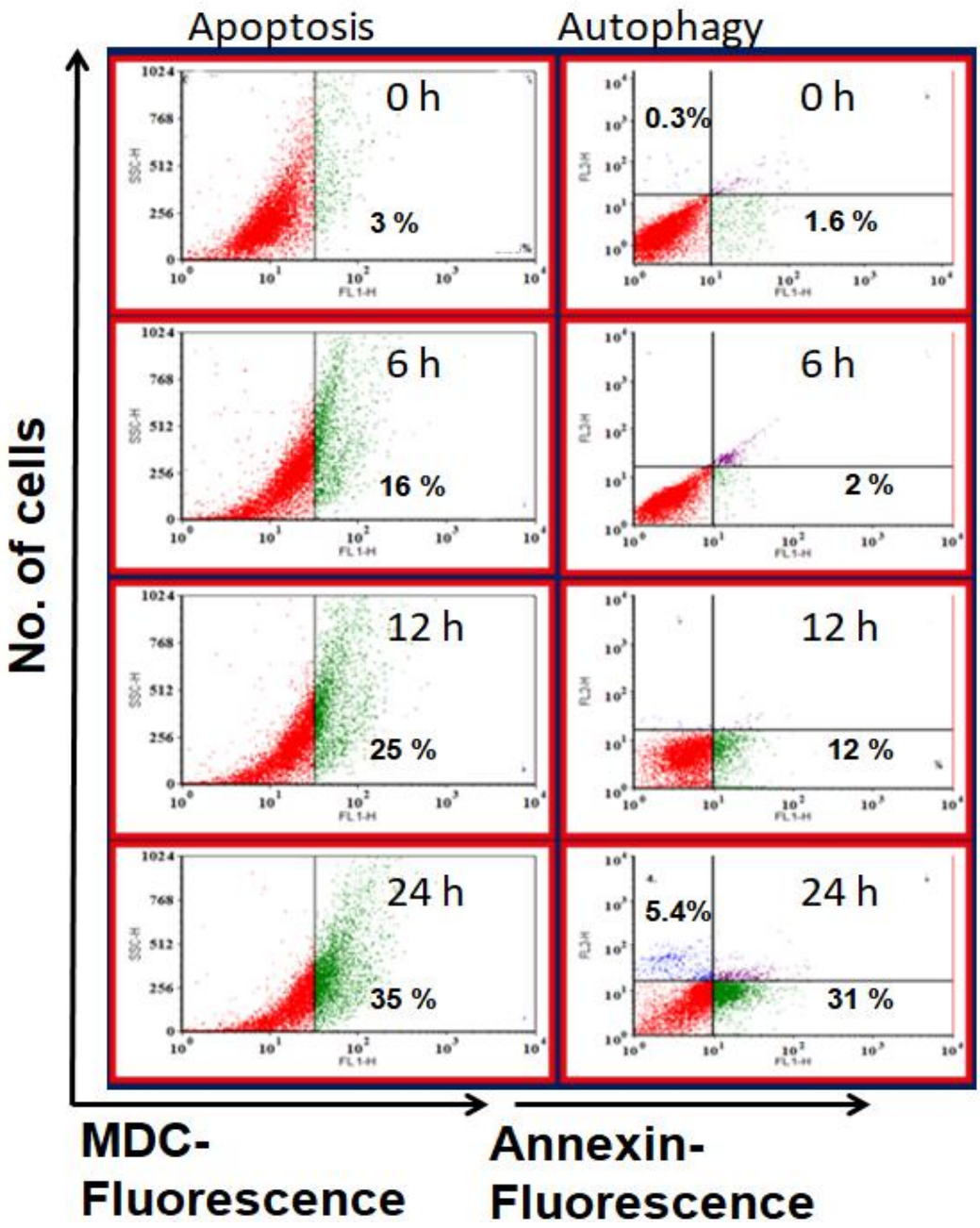

Fig. S4: FACS analysis of time-dependent induction of autophagy and apoptosis in control and NMK-treated MDA-MB-231 cells, stained with MDC and Annexin V-FITC in conrol and presented as dot plot.

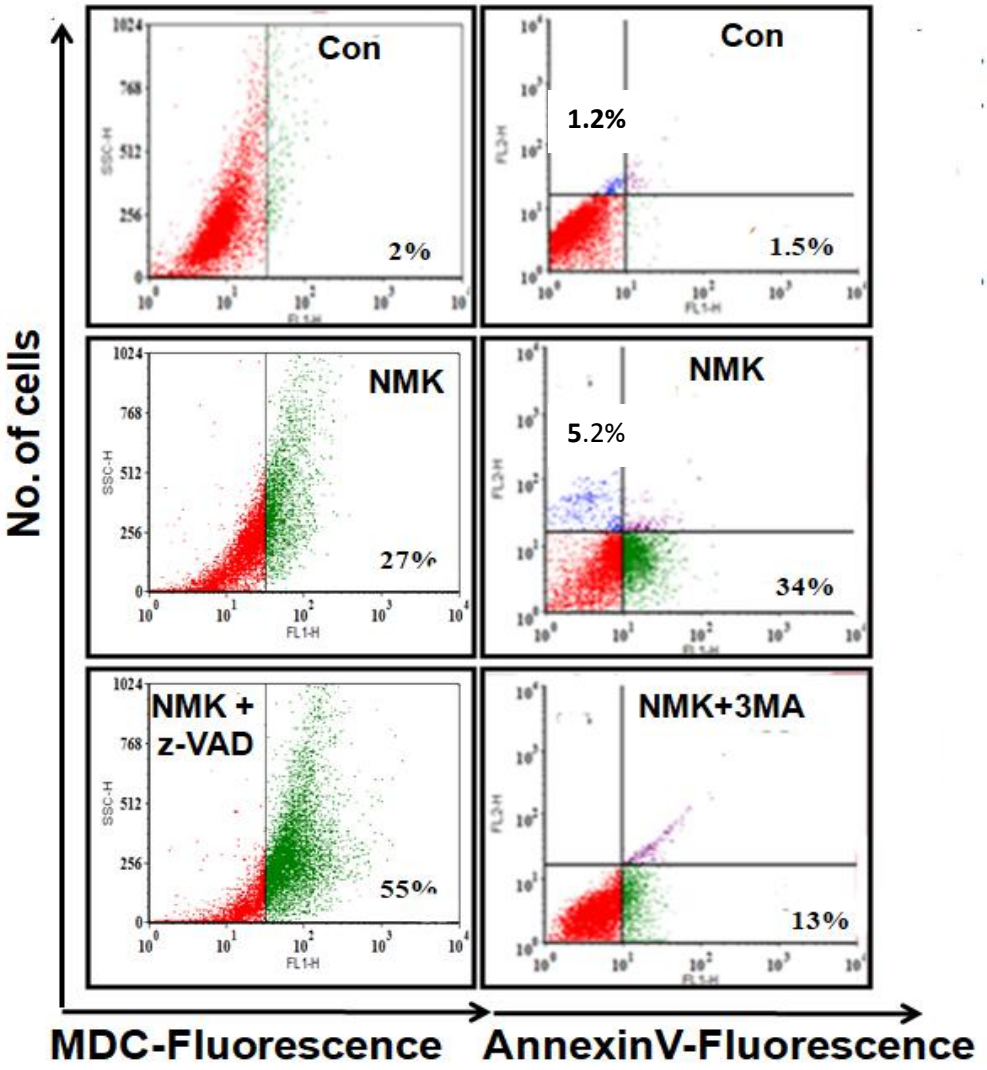

Fig. S5: FACS analysis induction of autophagy and apoptosis in control and NMK-treated MDA-MB-231 cells, stained with MDC and Annexin V-FITC in conrol and presented as dot plot.