Supplementary Materials

Polymeric Nanomedicine for overcoming Resistance Mechanisms in Hedgehog and MYC-amplified Medulloblastoma

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Figures. S1 to S8
Table S1

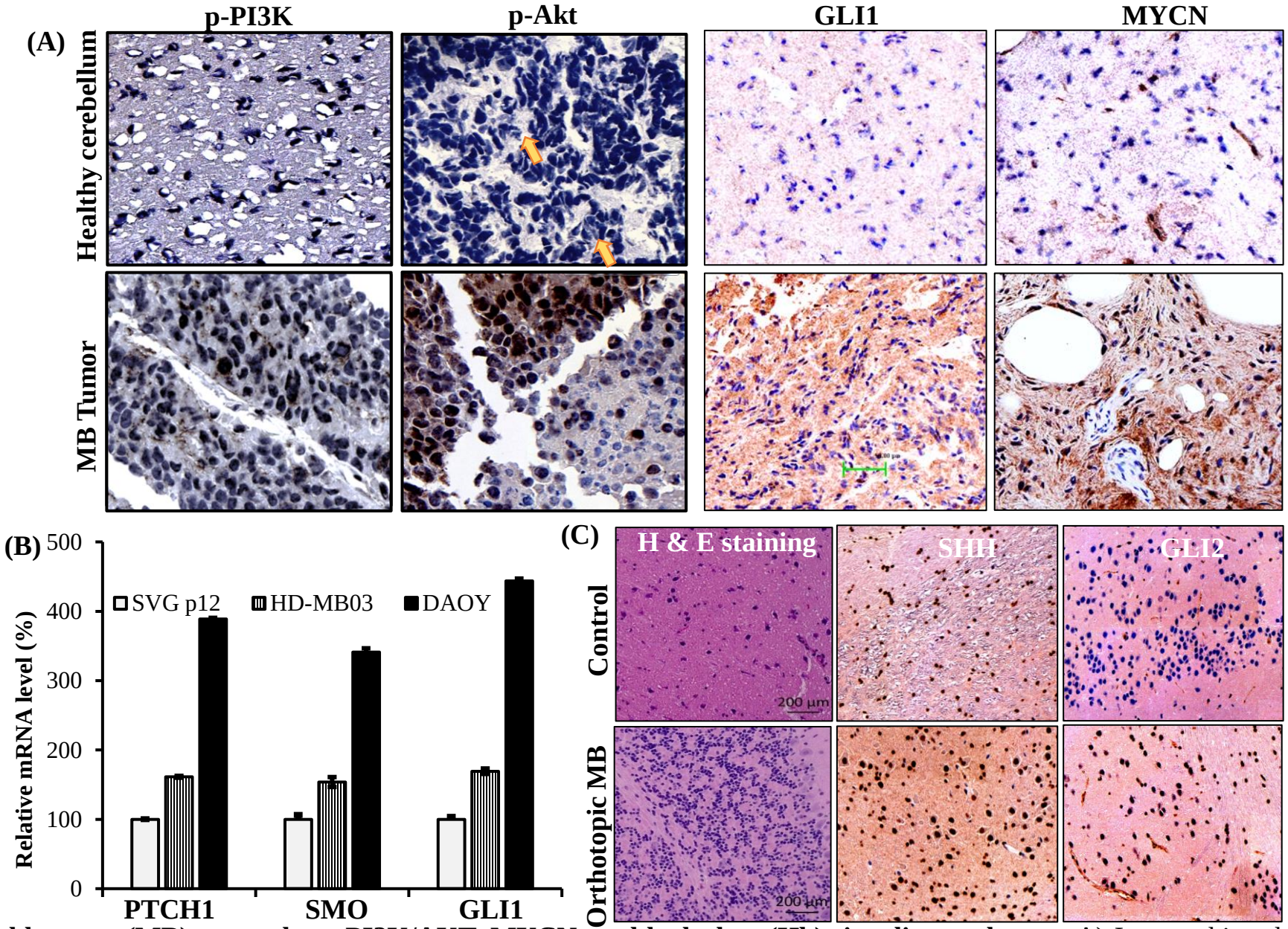


Figure S1. Human medulloblastoma (MB) upregulates PI3K/AKT, MYCN, and hedgehog (Hh) signaling pathways. A) Immunohistochemical (IHC) images of tumors and adjacent healthy cerebellum stained for p-PI3K, p-AKT , GLI1 and MYCN expression (magnification 20 X). B) Expression levels of PTCH1, SMO and GLI1 in SVG p12 (healthy cells), HD-MB03 (Group 3) and DAOY (SHH) cells as determined by real-time RT-PCR. C) IHC staining of normal brain tissue and MB tissue isolated from orthotopic MB tumor generated after stereotaxically injection of luciferase-expressing DAOY cells 2 mm deep into the cerebellum of NSG mice..

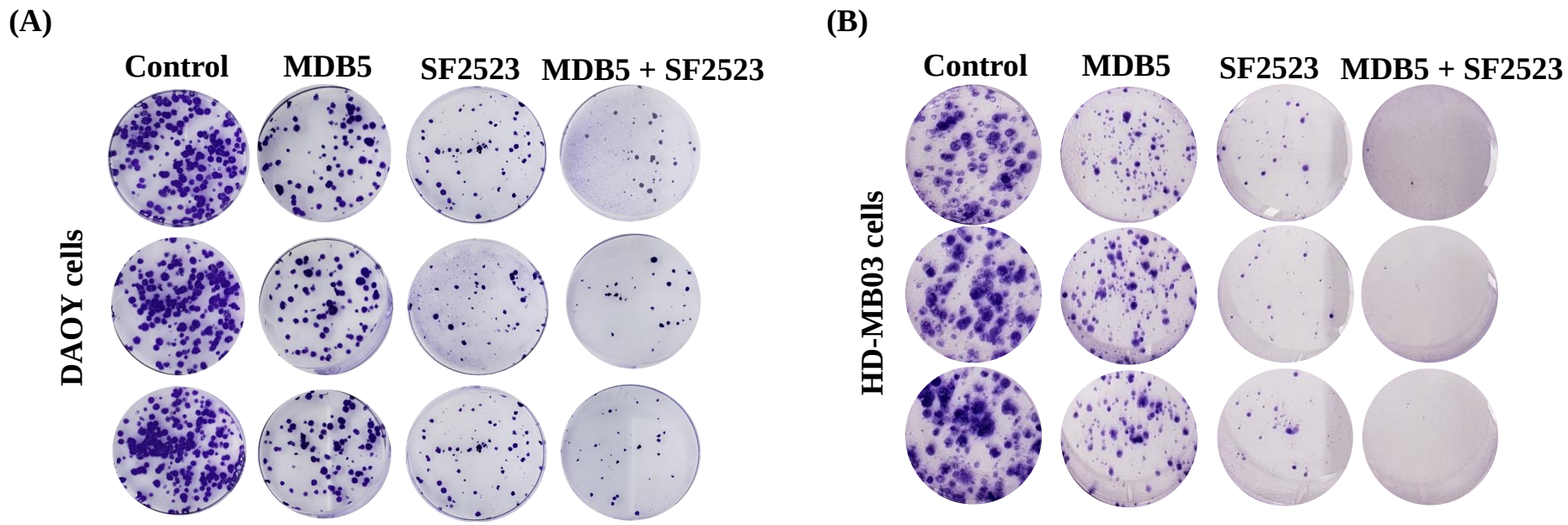


Figure S2. Colony formation ability of DAOY and HD-MB03 cells after different treatments. A) Representative colony formation assay of DAOY cells. B) Representative colony formation assay of HD-MB03 cells. The figure is a representative of three experiments with similar results.

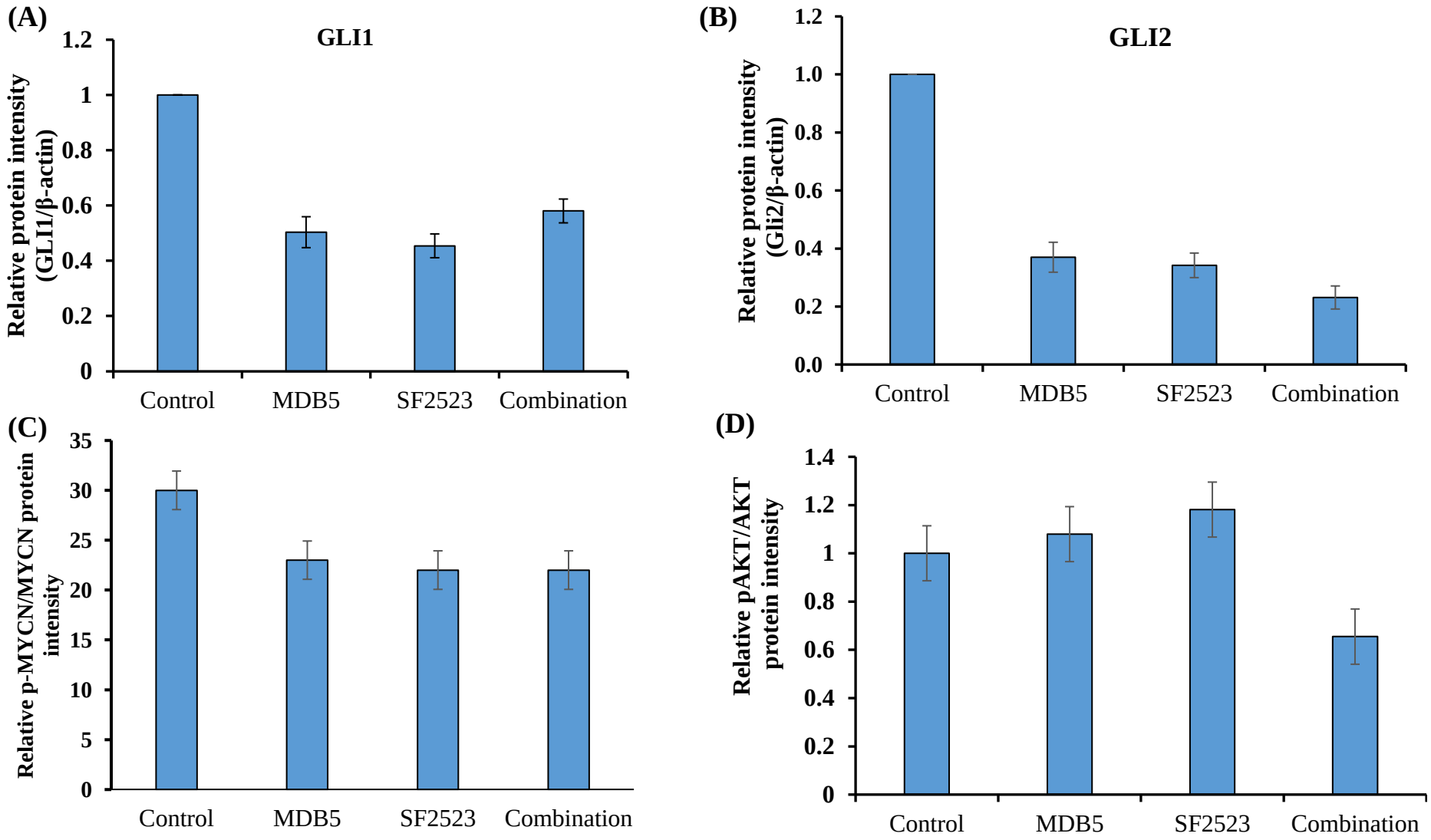


Figure S3. Western blot analysis. Intensity quantification of A) GLI1, B) GLI2 normalized to β-actin. C) p-MYCN and D) p-AKT levels normalized to total MYC and total AKT, respectively. Data are presented as mean ± SD; Statistics were done by one-way ANOVA. (* $p <$ 0.05, vs. Vehicle).

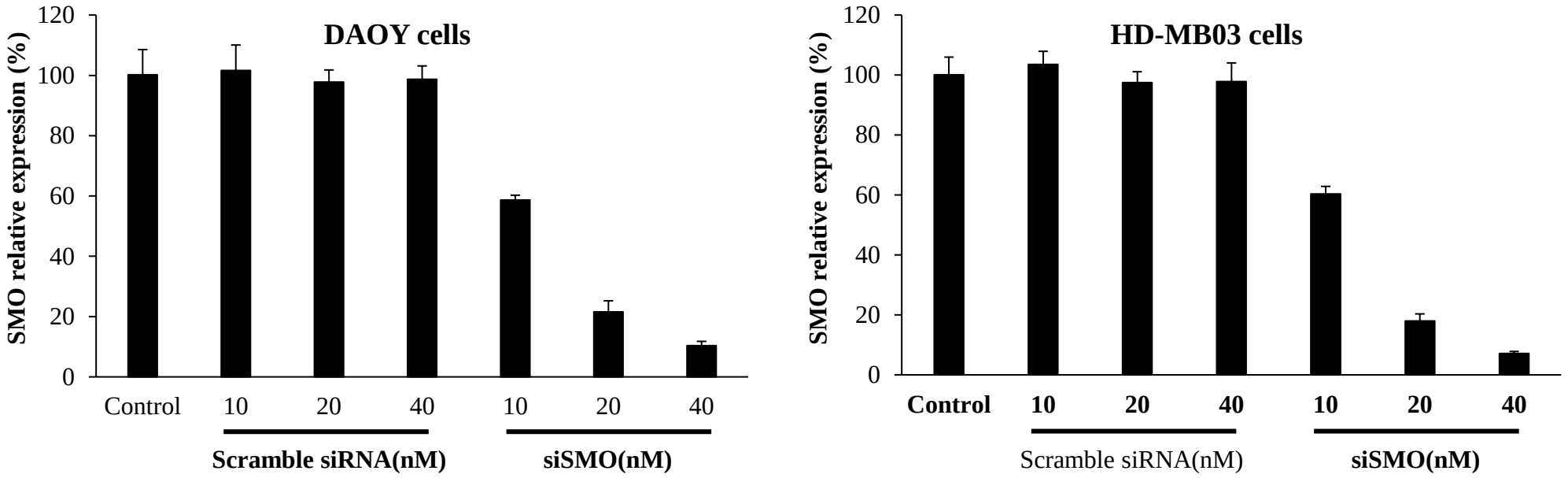


Figure S4. siRNA against SMO (siSMO) but not scrambled siRNA inhibits SMO expression after transfection of DAOY and HD-MB03 cells with Lipofectamine/siSMO or Lipofectamine/scrambled siRNA complexes for 24 h.

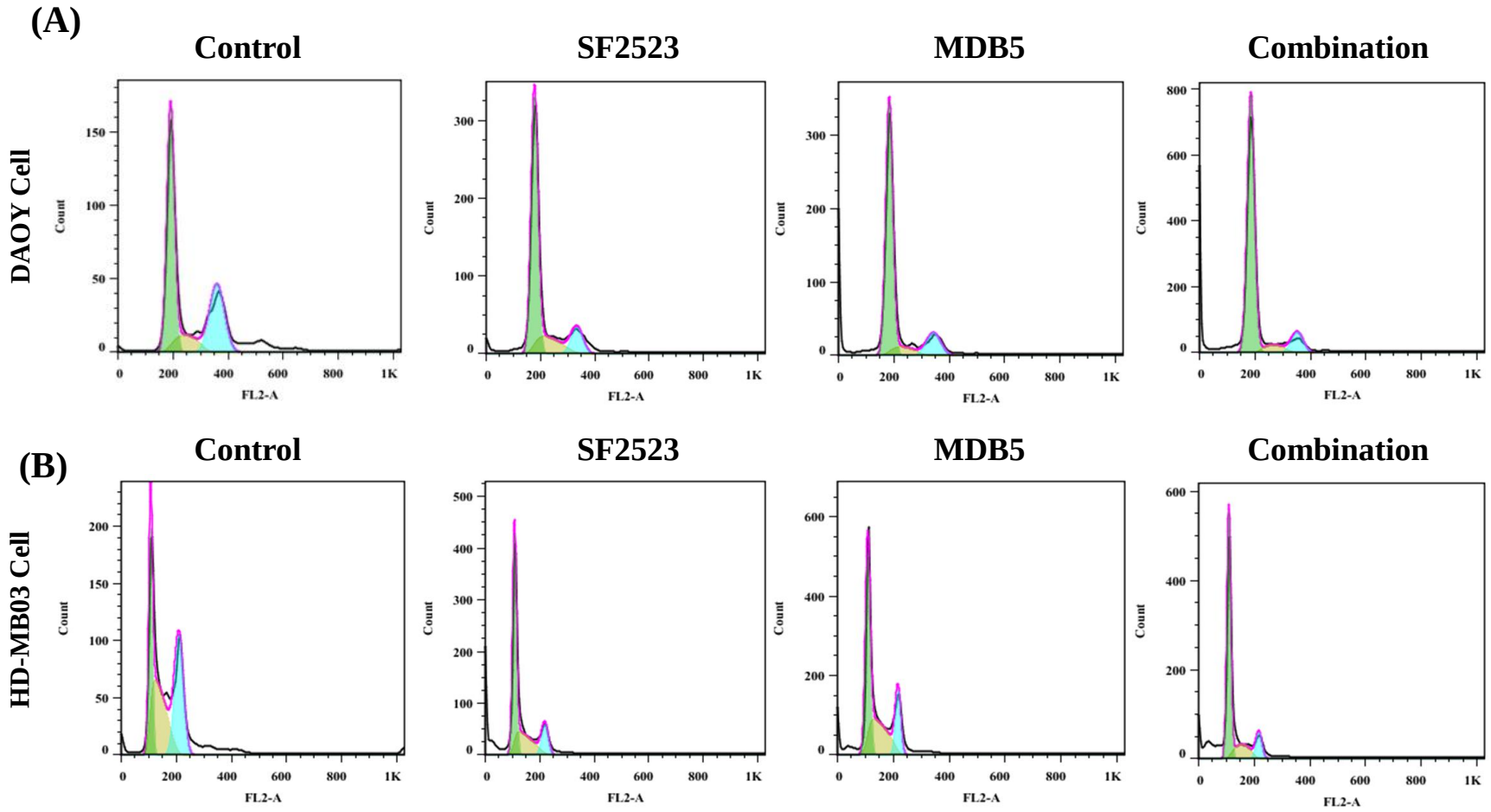


Figure S5. Representative flow cytometry histograms of SF2523 and MDB5 on cell cycle of MB cells (A) cell cycle after incubation of DAOY and (B) HD-MB03 cells for 48h. The DNA histograms show the distribution of cell populations in each phase of the cell cycle. Flow cytometry data from three independent experiments.

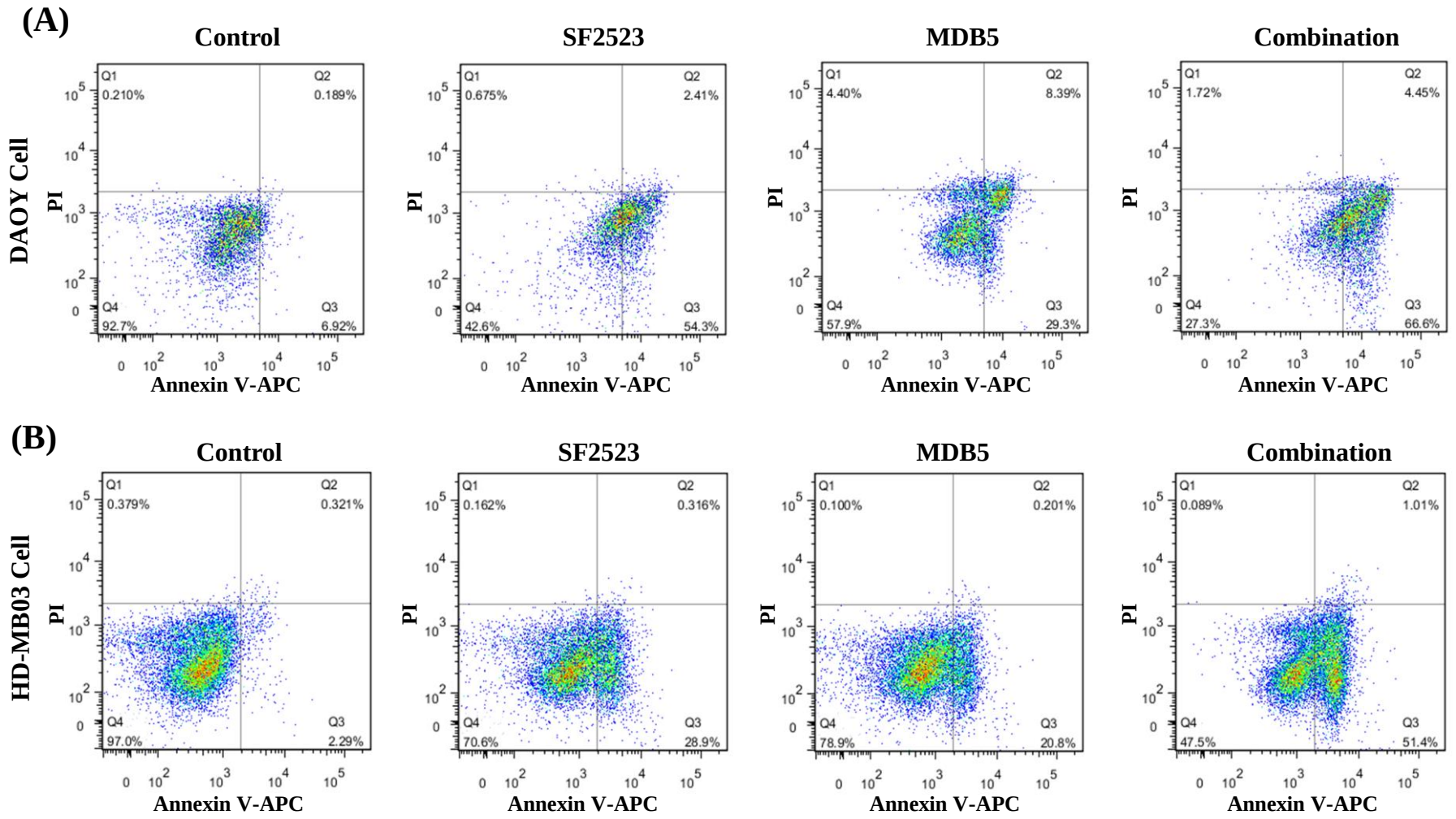


Figure S6. Effect of MDB5, SF2523 and their combination on apoptosis. A) Analysis of Annexin V-APC and propidium iodide-stained cells by flow cytometry in DAOY cells. B) HD-MB03 cells. Data represents three different independent experiments.

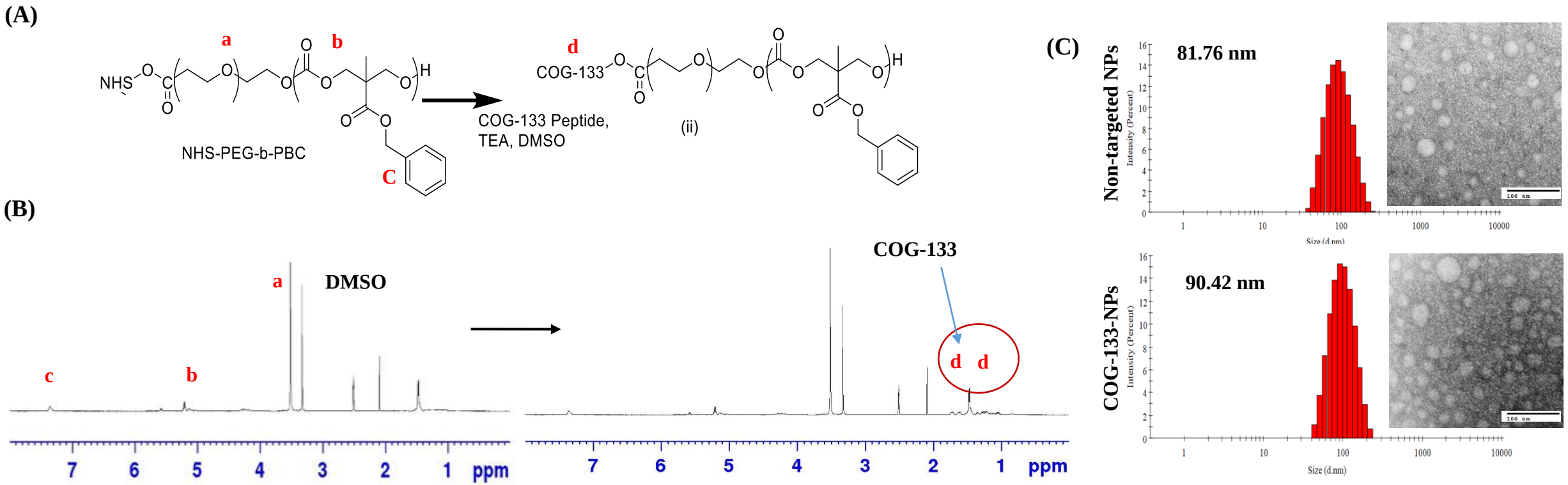


Figure S7. Synthesis and characterization of brain targeted COG-133-NPs. A) Synthesis scheme of apolipoprotein E mimetic peptide COG-133 conjugated polymer COG-133-PEG-b-PBC. **B)** ^{1}H NMR spectrum of polymers without and with COG-133 peptide. **C**) Effect of COG-133 decoration on NP size distribution, morphology determined by DLS and TEM.

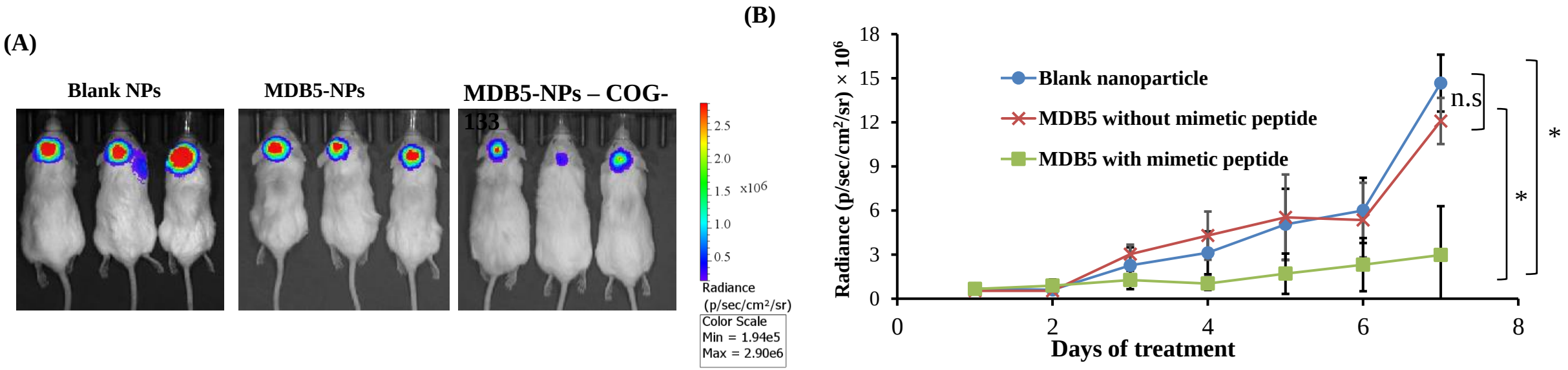
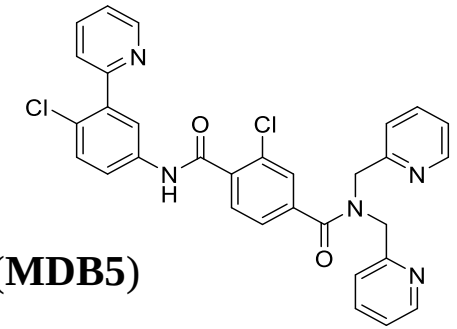
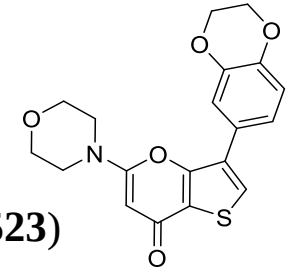


Figure S8. Antitumor efficacy of COG-133 decorated NP loaded with MDB5. A) Representative bioluminescence images (BLI) of mice after treatment. B) BLI photon intensity (*p < 0.01 vs. blank NPs; n=4; n.s.= non-significant). Drug formulations were injected at the dose of 20mg/kg every alternate days.

Chemical Structure	Molecular weight	Solubility (PBS, pH 5.0) (µg/mL)	Solubility (PBS, pH 7.4) (µg/mL)
 (**MDB5**)	568.46	15.15±0.37	14.72±2.47
 (**SF2523**)	371.41	4.10±0.06	3.71±0.611
Ac-LRVRLASHLRKLRKRLL-amide (**COG-133**)	2169.73	-	47.5 mg/ml (water)

Supplementary Table S1. Structures and physicochemical properties of MDB5 and SF2523.

Table S2A. Combination index data for non constant combination of (SF2523 and MDB5) in DAOY cells.

S.N.	SF2523 (µM)	MDB5 (µM)	Inhibitory Effect	Combination index (CI)
1.	3.0	15	0.269	1.94
2.	30	30	0.364	1.68
3.	3.0	45	0.408	1.76
4.	3.0	60	0.566	0.87
5.	6.0	15	0.384	1.34
6.	6.0	30	0.522	0.89
7.	6.0	45	0.534	1.05
8.	6.0	60	0.591	0.91
9.	9.0	15	0.455	1.22
10.	9.0	30	0.536	1.03
11.	9.0	45	0.551	1.14
12.	9.0	60	0.605	0.99
13.	12.0	15	0.546	0.97
14.	12.0	30	0.568	1.04
15.	12.0	45	0.640	0.83
16.	12.0	60	0.669	0.79

Table S2B. Combination index data for non constant combination of (SF2523 and MDB5) in HD-MB03 cells.

S.N.	SF2523 (µM)	MDB5 (µM)	Inhibitory Effect	Combination index (CI)
1.	1.5	25	0.364	1.60
2.	1.5	50	0.454	0.95
3.	1.5	75	0.516	0.68
4.	1.5	100	0.561	0.54
5.	3.0	25	0.498	0.66
6.	3.0	50	0.564	0.45
7.	3.0	75	0.577	0.49
8.	3.0	100	0.589	0.51
9.	4.5	25	0.558	0.51
10.	4.5	50	0.606	0.39
11.	4.5	75	0.615	0.41
12.	4.5	100	0.627	0.42
13.	6.0	25	0.580	0.53
14.	6.0	50	0.635	0.35
15.	6.0	75	0.642	0.37
16.	6.0	100	0.646	0.40