

Figure S1. Dose-response effect of PLX4032, CSPG4-specific 9.2.27 mAb and the combination thereof on the viability of (A) WM164 and (B) M14 melanoma cell lines. Cells were incubated with increasing concentrations of PLX4032, 9.2.27 mAb and 0.1 μ M PLX4032 with increasing concentrations of 9.2.27 mAb for 72 h and cell viability was determined using MTT assay. The results of three independent experiments performed in triplicates are presented as a percentage of viable cells, compared to untreated cells (control). Bars represent the mean \pm SD from triplicate experiments. Statistical analysis was carried out using one-way ANOVA with Tukey's multiple comparisons test. P-values are represented by asterisks (*): *** P <0.0001, ** P <0.01; ns, not significant; CSPG4, chondroitin sulfate proteoglycan 4; mAb, monoclonal antibody.

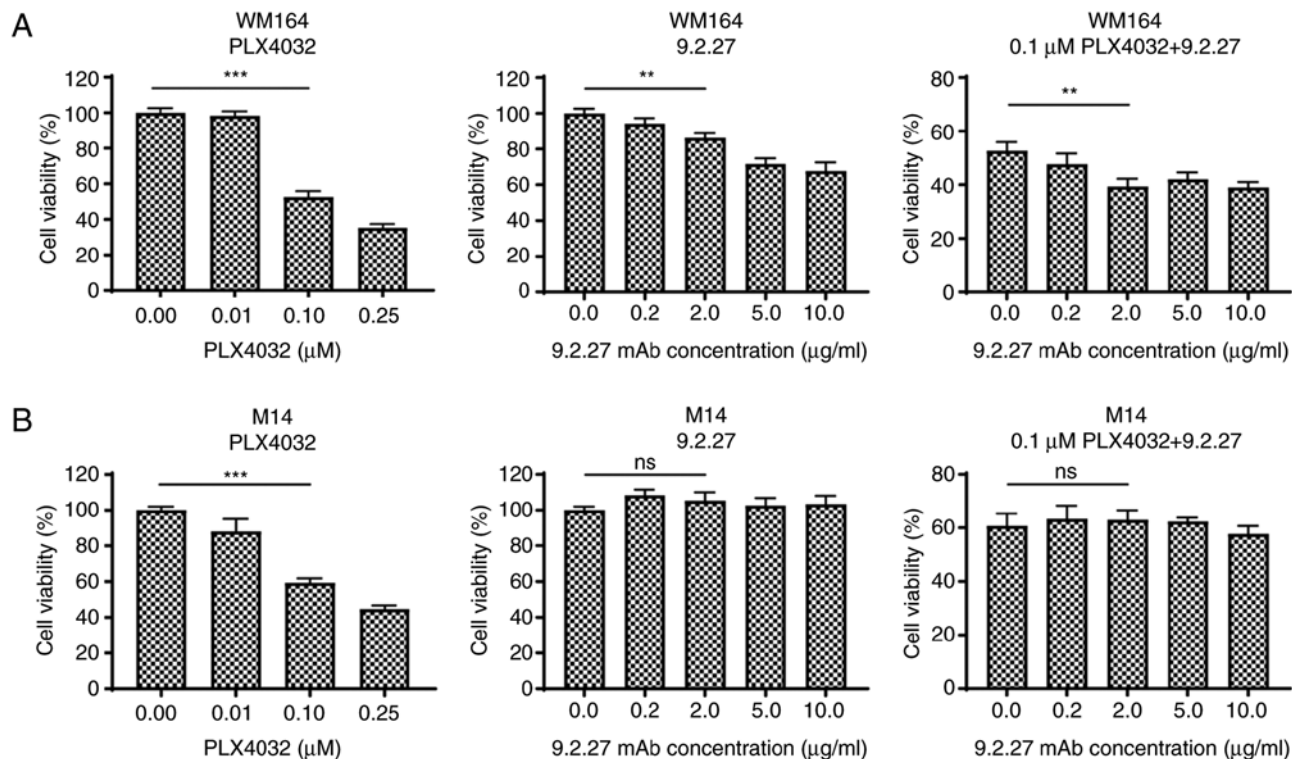


Figure S2. Growth inhibition of melanoma cells following treatment with CSPG4-specific 9.2.27 mAb in relation to CSPG4 expression. WM9, WM35, 451Lu, WM164 and M14 cells were incubated with 9.2.27 mAb for 72 h and inhibition of cell growth was determined using MTT assay. The results of three experiments in triplicate are presented as the percentage inhibition of cell viability, compared with the untreated cells (x-axis). The expression of CSPG4 in WM9, WM35, 451Lu, WM164 and M14 cells was evaluated using flow cytometry. Results are presented as the geometric mean of fluorescence intensity (gMFI) of CSPG4 FITC-A signal (y-axis). CSPG4, chondroitin sulfate proteoglycan 4; mAb, monoclonal antibody.

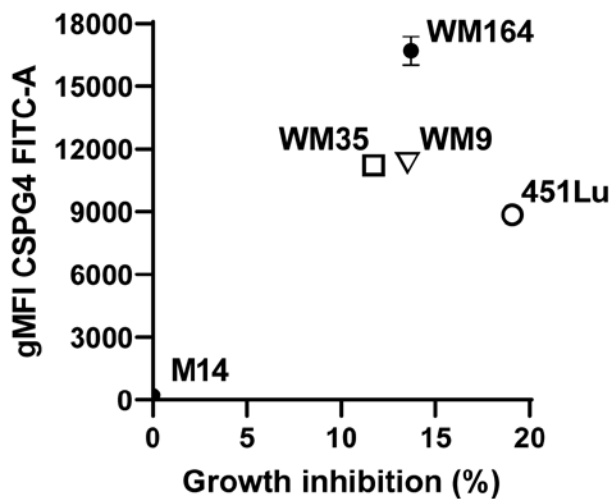


Figure S3. Effect of PLX4032 and the CSPG4-specific 9.2.27 mAb alone or in combination on the growth of melanoma cells. (A) CSPG4-positive WM164 cells and (B) CSPG4-negative M14 cells were exposed to different treatments: PLX4032 (0.1 μ M), 9.2.27 mAb (2 μ g/ml), PLX4032 (0.1 μ M) plus 9.2.27 mAb (2 μ g/ml), IgG control (2 μ g/ml), PLX4032 (0.1 μ M) plus IgG control (2 μ g/ml) and examined using bright-field photography. Representative images were obtained after 24 and 72 h; magnification, x20. CSPG4, chondroitin sulfate proteoglycan 4; mAb, monoclonal antibody.

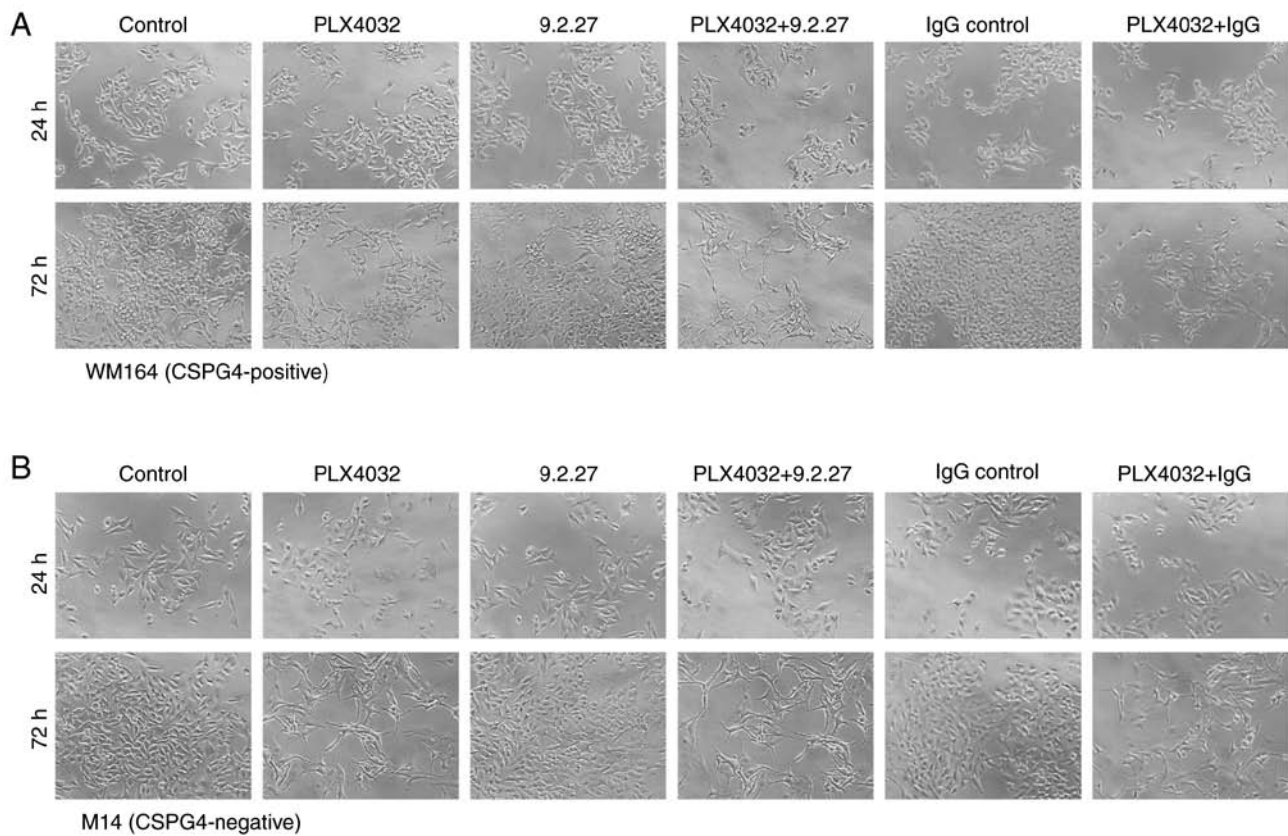


Figure S4. Cell cycle arrest in the S phase of WM164 cells exposed to the CSPG4-specific 9.2.27 mAb is concentration-dependent. CSPG4-positive WM164 cells were incubated with increasing concentrations of the 9.2.27 mAb (2-10 $\mu\text{g/ml}$) for 72 h and analyzed using flow cytometry with propidium iodide staining. Untreated cells served as a control. The left panel illustrates representative histograms, and the right panel presents bar graphs of three independent experiments performed in triplicate indicating the fraction of cells in S phase. Bars represent the mean \pm SD. Statistical analysis was carried out using one-way ANOVA with Tukey's multiple comparisons test. P-values are represented by asterisk's (*): *** $P < 0.001$, ** $P < 0.01$; * $P < 0.05$ ns, not significant; CSPG4, chondroitin sulfate proteoglycan 4; mAb, monoclonal antibody.

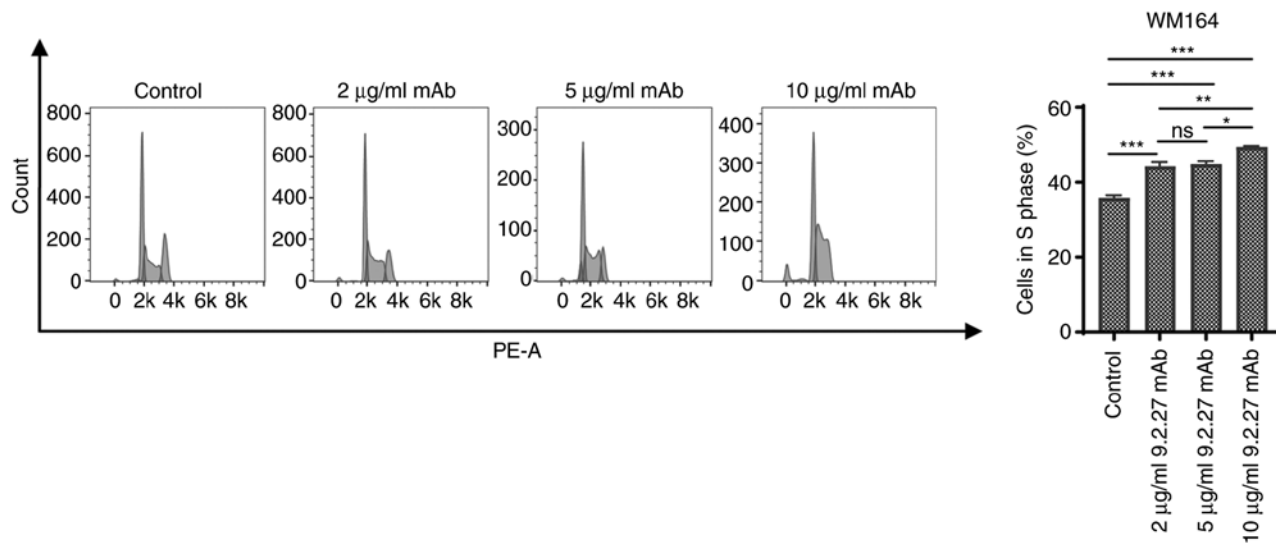


Table SI. P-values^a corresponding to Fig. 5A.

WM164 (CSPG4-positive) cells	Cell cycle phase			
	subG1	G1	S	G2/M
Control vs. 0.1 μ M PLX4032	0.9985	<0.0001	<0.0001	0.0045
Control vs. 2 μ g/ml 9.2.27 mAb	0.9967	0.0384	0.0073	0.6199
Control vs. PLX4032 + 9.2.27 mAb	0.0006	<0.0001	<0.0001	<0.0001
Control vs. 2 μ g/ml IgG control	0.9965	0.9995	0.9690	0.7630
Control vs. PLX4032 + IgG control	0.9984	<0.0001	<0.0001	0.1878
0.1 μ M PLX4032 vs. 2 μ g/ml 9.2.27 mAb	>0.999	<0.0001	<0.0001	0.0648
0.1 μ M PLX4032 vs. PLX4032 + 9.2.27 mAb	0.0011	0.0874	0.9593	<0.0001
0.1 μ M PLX4032 vs. 2 μ g/ml IgG control	0.9490	<0.0001	<0.0001	0.0422
0.1 μ M PLX4032 vs. PLX4032 + IgG control	0.9647	0.9938	>0.9999	0.2827
2 μ g/ml 9.2.27 mAb vs. PLX4032 + 9.2.27 mAb	0.0012	<0.0001	<0.0001	<0.0001
2 μ g/ml 9.2.27 mAb vs. 2 μ g/ml IgG control	0.9307	0.0228	0.0262	0.9998
2 μ g/ml 9.2.27 mAb vs. PLX4032 + IgG control	0.9500	<0.0001	<0.0001	0.9257
PLX4032 + 9.2.27 mAb vs. 2 μ g/ml IgG control	0.0003	<0.0001	<0.0001	<0.0001
PLX4032 + 9.2.27 mAb vs. PLX4032 + IgG control	0.0003	0.2008	0.9556	<0.0001
2 μ g/ml IgG control vs. PLX4032 + IgG control	>0.999	<0.0001	<0.0001	0.8268

^aStatistical analysis was carried out using one-way ANOVA with Tukey's multiple comparisons test. P-values obtained for each comparison are shown. CSPG4, chondroitin sulfate proteoglycan 4; mAb, monoclonal antibody.

Table SII. P-values^a corresponding to Fig. 5B.

M14 (CSPG4-negative) cells	Cell cycle phase			
	subG1	G1	S	G2/M
Control vs. 0.1 μ M PLX4032	0.0793	<0.0001	<0.0001	<0.0001
Control vs. 2 μ g/ml 9.2.27 mAb	0.9815	0.3884	0.7631	0.0065
Control vs. PLX4032 + 9.2.27 mAb	0.0474	<0.0001	<0.0001	<0.0001
Control vs. 2 μ g/ml IgG control	0.9992	0.1822	0.0339	0.2625
Control vs. PLX4032 + IgG control	0.0603	<0.0001	<0.0001	0.0004
0.1 μ M PLX4032 vs. 2 μ g/ml 9.2.27 mAb	0.0256	<0.0001	<0.0001	0.0436
0.1 μ M PLX4032 vs. PLX4032 + 9.2.27 mAb	0.9995	0.0109	0.7631	0.9441
0.1 μ M PLX4032 vs. 2 μ g/ml IgG control	0.0447	<0.0001	<0.0001	0.0011
0.1 μ M PLX4032 vs. PLX4032 + IgG control	>0.9999	0.0102	0.2045	0.5925
2 μ g/ml 9.2.27 mAb vs. PLX4032 + 9.2.27 mAb	0.0152	<0.0001	<0.0001	0.0099
2 μ g/ml 9.2.27 mAb vs. 2 μ g/ml IgG control	0.9993	0.0065	0.2816	0.2784
2 μ g/ml 9.2.27 mAb vs. PLX4032 + IgG control	0.0193	<0.0001	<0.0001	0.4978
PLX4032 + 9.2.27 mAb vs. 2 μ g/ml IgG control	0.0265	<0.0001	<0.0001	0.0003
PLX4032 + 9.2.27 mAb vs. PLX4032 + IgG control	>0.9999	<0.0001	0.0233	0.1933
2 μ g/ml IgG control vs. PLX4032 + IgG control	0.0338	<0.0001	<0.0001	0.0151

^aStatistical analysis was carried out using one-way ANOVA with Tukey's multiple comparisons test. P-values obtained for each comparison are shown. CSPG4, chondroitin sulfate proteoglycan 4; mAb, monoclonal antibody.