Supplementary Materials: Therapeutic targeting of Stat3 using lipopolyplex nanoparticle-formulated siRNA in a syngeneic orthotopic mouse glioma model

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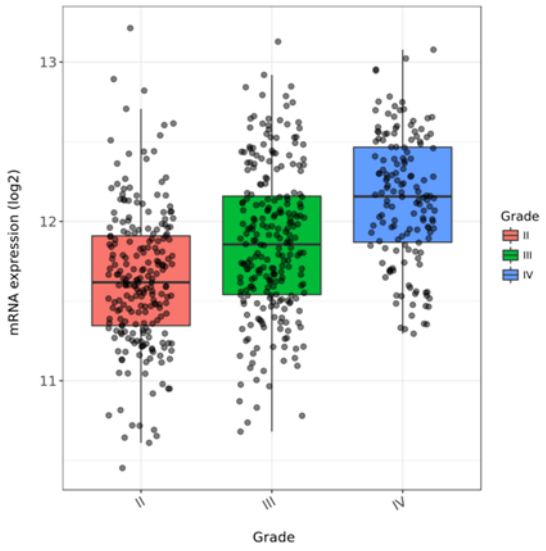
Supplementary Methods:

Cell cycle analysis with Nocodazole-induced G2/M-block

Cell cycle distribution in U87 and Mz18 cells was analyzed following the same protocol; however, 100 ng/ml nocodazole was added 24 h after transfection for inducing a G2/M arrest. Thus, smaller fractions of cells reaching the G2/M block at 8 h after nocodazole addition indicate slower cell cycle progression.

Supplementary Figures:

a



b

Comparison	diff	lwr	upr	p.adj	Significance
IV-II	0.50	0.39	0.61	0.00	***
IV-III	0.27	0.16	0.38	0.00	***
III-II	0.23	0.13	0.32	0.00	***

Figure S1. STAT3-mRNA expression from the TCGA dataset GBMLGG [62] plotted using the GlioVis portal (gliovis.bioinfo.cnio.es [63]). (**a**) Box-Plots depicting the expression levels of STAT3-mRNA in different glioma subtypes. (**b**) Statistical comparison of STAT3-mRNA expression using Tukey HSD-test as provided via the GlioVis-Portal. The table shows the difference between pairs, the 95% confidence interval and the p-value of the pairwise comparisons. *** $p < 0.001$; ns, not significant.

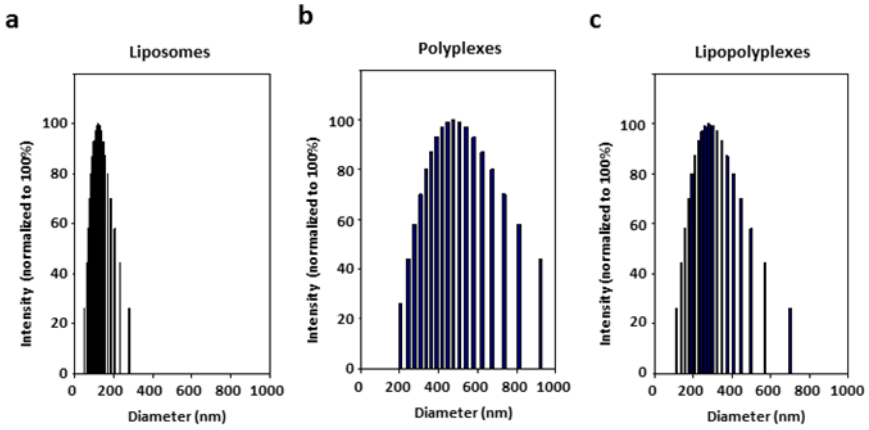


Figure S2. Nanoparticle sizes as determined by Zetasizer (dynamic light scattering) (**a–c**) Results of size measurements by Zetasizer. Two independent samples were measured 10 times.

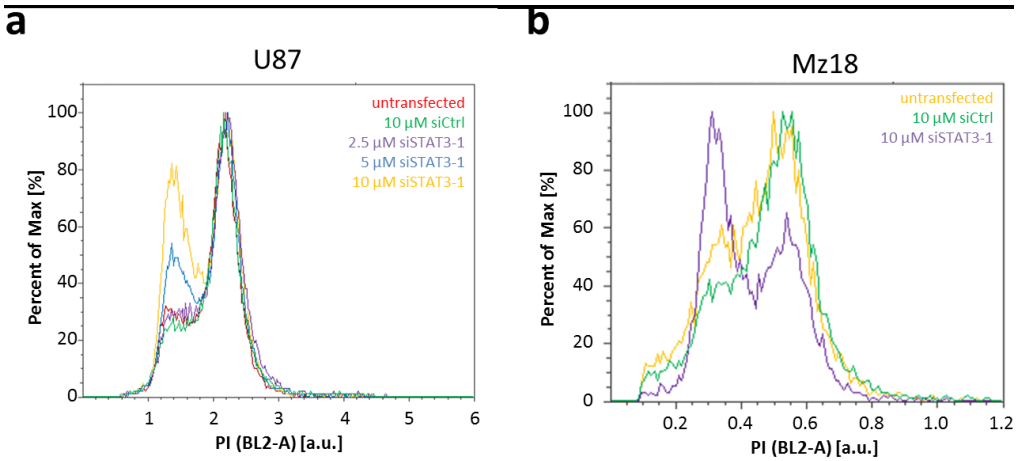


Figure S3. siStat3 hinders cell cycle progression in human GBM cell lines. (**a** and **b**) Cell cycle analysis in (**a**) U87 and (**b**) Mz18 cells after transfection with siRNA as indicated in the figures. Note that nocodazole (100 ng/ml) was added 48 h after transfection for inducing a G2/M arrest. Thus, larger percentages of cells in G0/G1 indicate smaller fractions of cells reaching the G2/M block at 16 h after nocodazole addition and thus slower cell cycle progression. In Figure S3a, an siSTAT3 dose-dependent decrease in cell cycle deceleration can be observed.