Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary materials

Supplementary Methods

Comparison of commercial transfection reagents with ND

Lipofectamine™ RNAiMAX transfection reagent (Invitrogen) and X-tremeGENE™ HP DNA transfection reagent (Roche) were also used as vehicle controls. Preparation of RNAiMAX-siRNA and X-tremeGENE-siRNA complexes were according to the manufacturer's protocols. The ratio of X-tremeGENE to siRNA was 3:1. Preparation step was done in Opti-MEM reduced serum medium (Gibco). For cell transfection, SNU398 cells were seeded one day before transfection in 6-well plates. For Opti-MEM medium treatment group, normal medium with 10%FBS was aspirated and cells were washed with PBS before replaced with Opti-MEM and transfection complexes were added to the cells in a dropwise manner. For normal medium group (RPMI with 10% FBS) and spheroids group, transfection complexes were directly added into cells in a dropwise manner 24 hours after seeding. Cell pellets were collected after treating for 48 hours for qRT-PCR analysis.

Penetration capacity of RNAiMAX and XtremeGENE in SNU398 spheroids was also evaluated using FACS analysis as described in the article.

Stability test

Stability study of ND-siRNA complexes under different temperature conditions were evaluated both in particle size aspect using DLS method and in biological activity aspect by testing their knockdown efficiency.

For particle size stability test, ND-siRNA complexes were stored under 4°C, 25°C (room temperature) and 37°C (body temperature) conditions. At each time point (0h, 24h, 48h, 72h, 96h, 168h), samples were taken out for DLS measurement.

For biological activity stability test, ND-siSALL4 complexes were placed under 4°C or 37°C for 24 hours before adding into SNU398 spheroids (sample ND-siSALL4 4°C, sample ND-siSALL4 37°C). Meanwhile, bare siSALL4 was placed under 4°C or 37°C for 24 hours before binding to ND and adding into SNU398 spheroids (sample ND-4°C siSALL4, sample ND-37°C siSALL4). After 48-hour-treatment, samples were collected for qRT-PCR analysis.

Supplementary Figures

Table S1. Characterizations of ND-siSALL4 complex

Weight ratio (ND:siRNA)	Size±SD (nm)	Zeta±SD (mV)	Loading efficiency (%)	Surface loading (nmol/mg)	After incubated in medium containing serum and formed a layer of protein corona	Size±SD (nm)	Zeta±SD (mV)
100:1	87.49±2.49	30.03±0.7	100	0.75		/	/
50:1	72.56±1.56	33.33±2.98	100	1.49		107.53±0.01	-18.17±0.69
20:1	152.65±1.25	14.63±0.29	100	3.73		104.77±0.02	-23.13±2.52
10:1	64.36±4.26	-24.8±1.39	68.55	5.12		121.77±0.03	-17.63±0.58

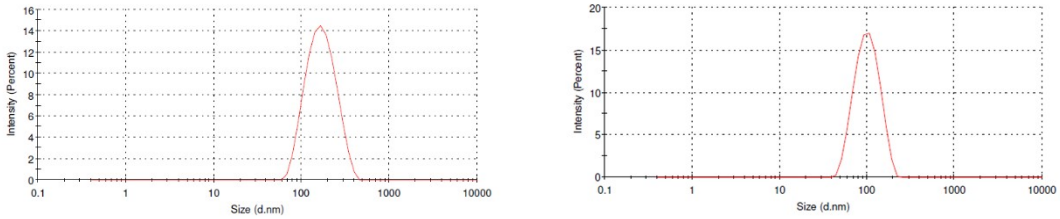


Figure S1. Dynamic light scattering (DLS) results of particle size of ND-siSALL4 at weight ratio of 20:1. (Left) In water, (Right) after incubated in medium containing serum and formed a layer of protein corona.

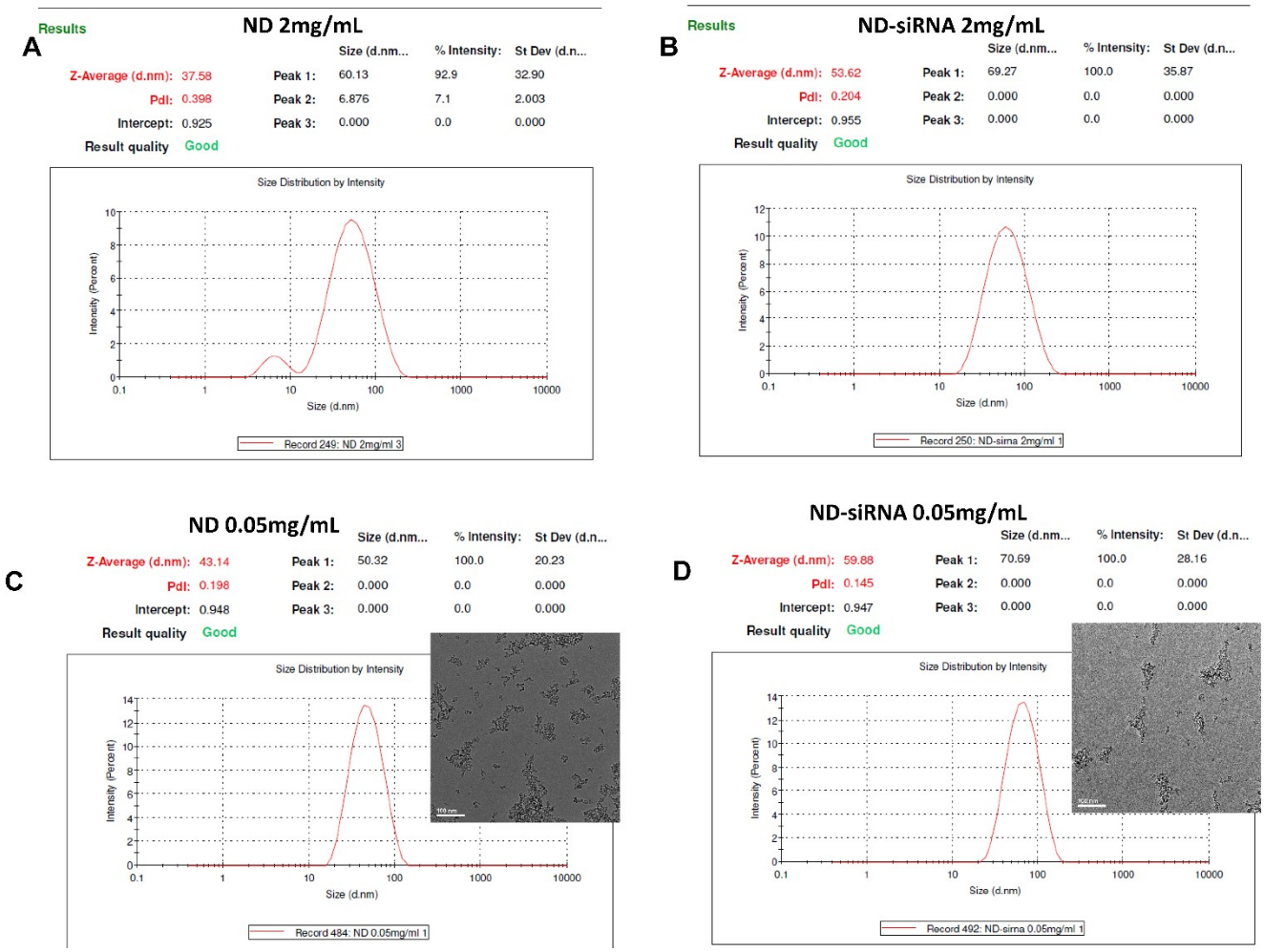


Figure S2. Intensity-weighted particle size distributions of 2mg/mL ND (A), 2mg/mL ND-siRNA (B), 0.05mg/mL ND (C), 0.05mg/mL ND-siRNA (D) and corresponding TEM images (C)(D).

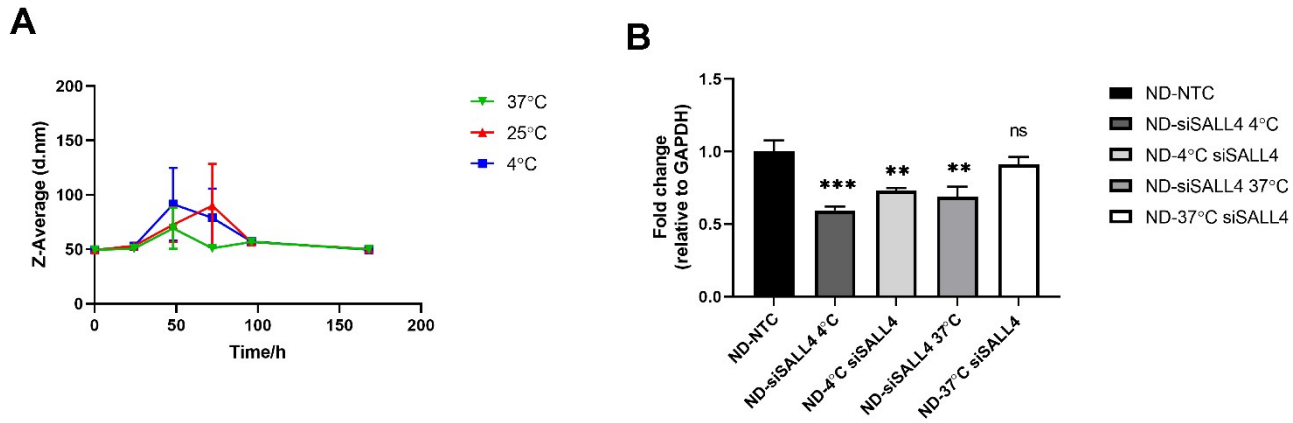
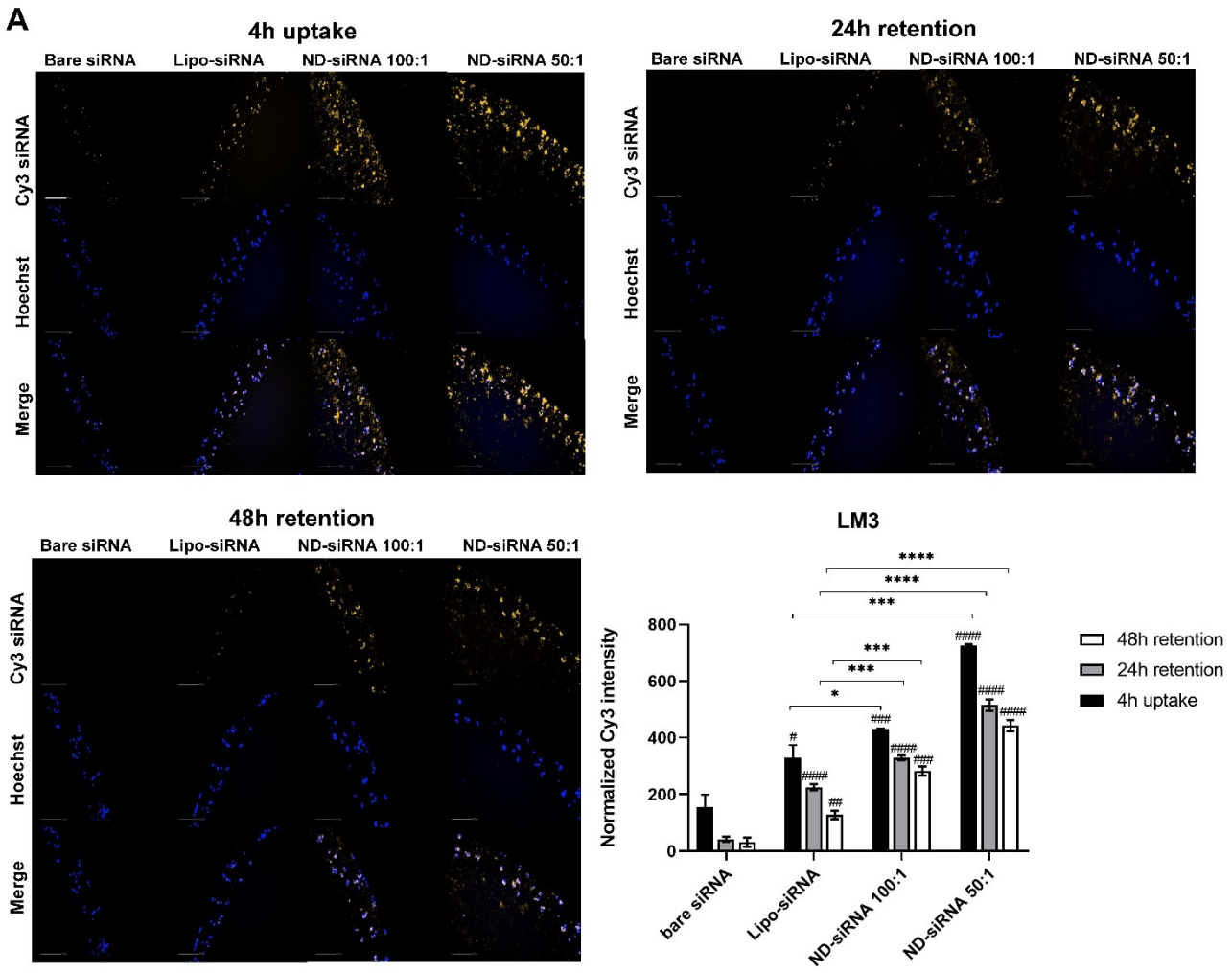


Figure S3. Stability study of ND-siRNA. (A) Particle size stability test. Particle size of ND-siRNA complexes under 4°C, 25°C and 37°C conditions at different time point (0h, 24h, 48h, 72h, 96h, 168h). (B) Biological activity stability test. Knockdown efficiency of different ND-siRNA samples by qRT-PCR. ND-siSALL4 4°C: ND-siSALL4 placed at 4°C for 24 hours. ND-4°C siSALL4: SALL4 siRNA placed at 4°C for 24 hours before binding to ND. ND-siSALL4 37°C: ND-siSALL4 placed at 37°C for 24 hours. ND-37°C siSALL4: SALL4 siRNA placed at 37°C for 24 hours before binding to ND. **p<0.01, ***p<0.001, in comparison with ND-NTC group, n=3.


A
4h uptake
24h retention
48h retention
Bare siRNA
Lipo-siRNA
ND-siRNA 100:1
ND-siRNA 50:1
Cy3 siRNA
Hoechst
Merge
LM3
Normalized Cy3 intensity
800
600
400
200
0
bare siRNA
Lipo-siRNA
ND-siRNA 100:1
ND-siRNA 50:1
48h retention
24h retention
4h uptake

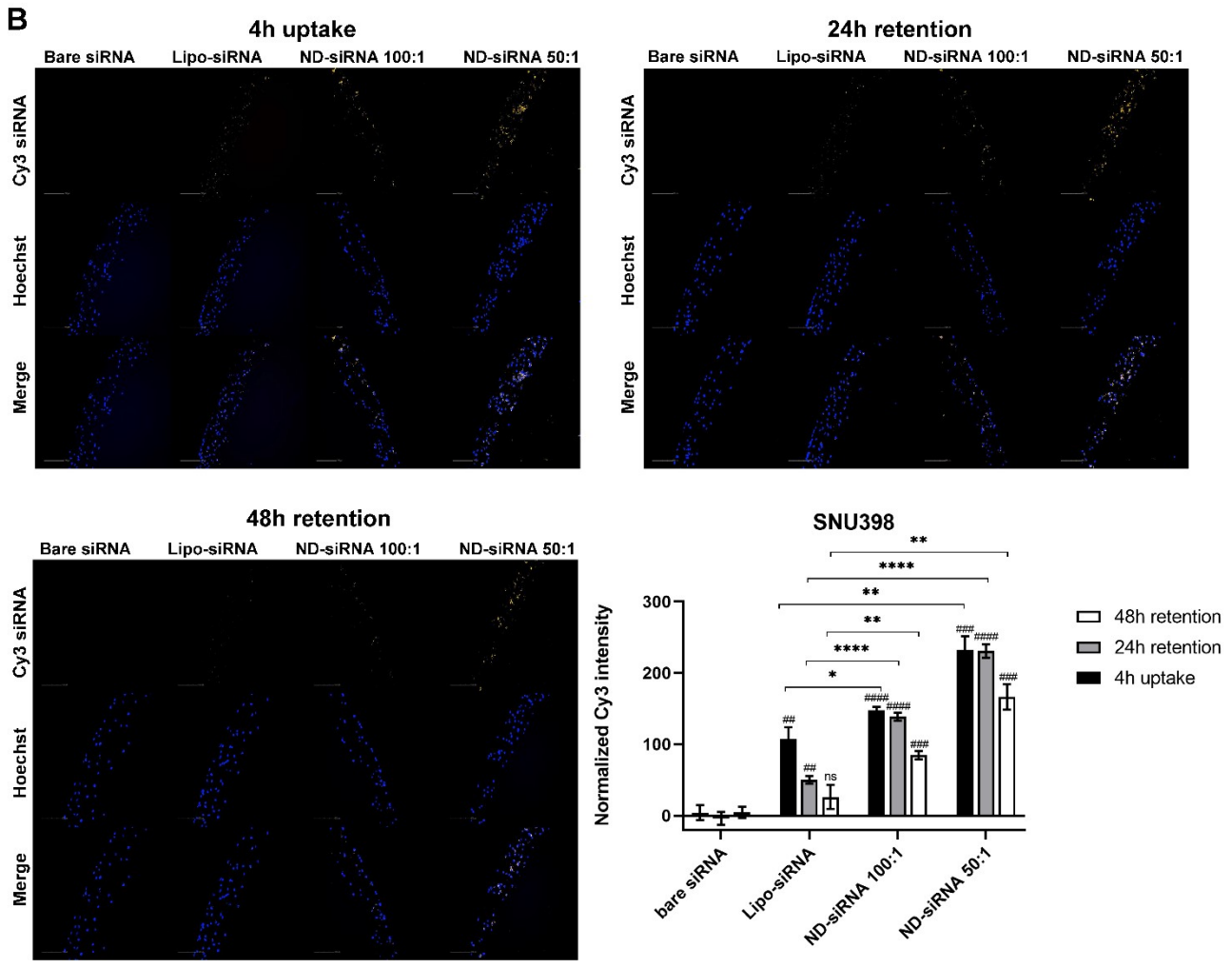


Figure S4. Cellular uptake and retention of siRNA packaged with ND or lipo. Two weight ratios of ND/siRNA (100:1, 50:1) were used, lipofectamine 3000 (lipo) was used as the vehicle control. Scale bars, 200 μm. Representative images of 4-hour cellular uptake, 24-hour and 48-hour retention of siRNA in different groups, in LM3 (A) and SNU398 (B) cell lines and quantification of intracellular fluorescent intensity. Intracellular Cy3 signal was normalized by cell counts using Operetta High-Content Imaging System. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. #: in comparison with bare siRNA group, #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001. n=3.

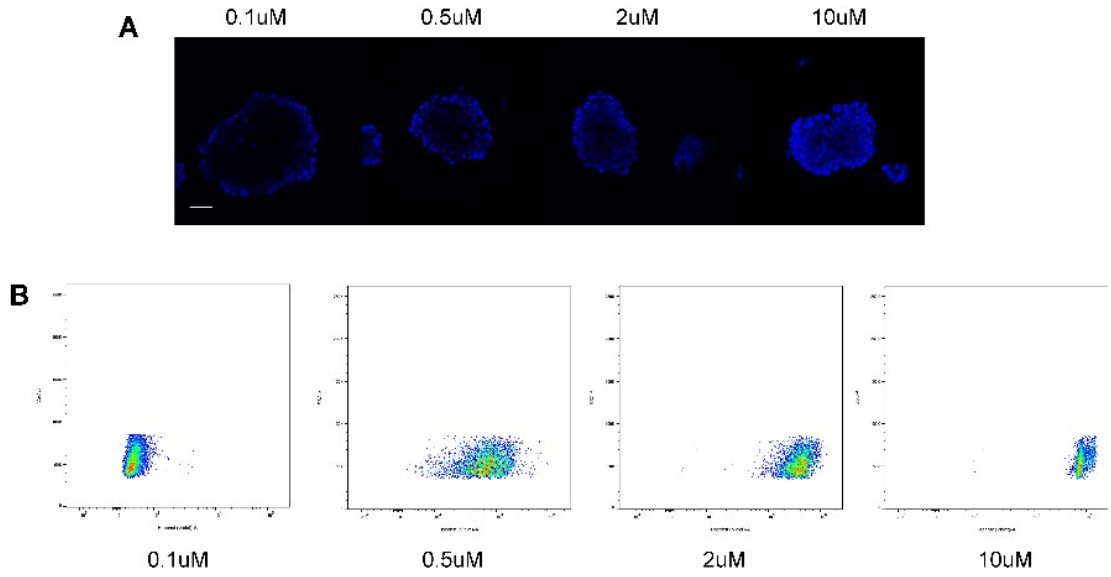


Figure S5. Gradient staining of Hoechst in SNU398 spheroids incubated with different concentrations of Hoechst for 4 hours. (A) Confocal images of spheroids after incubated with 0.1μM, 0.5μM, 2μM or 10μM Hoechst for 4 hours. Different degrees of Hoechst staining were observed. Scale bars, 50 μM. (B) FACS plots representing the distribution of SNU398 cells based on the degree of Hoechst staining at each concentration.

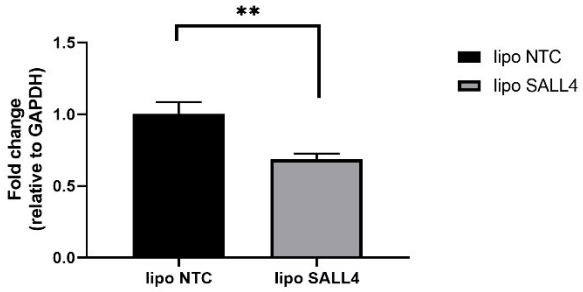


Figure S6. Knockdown efficiency of lipo-siSALL4 in 2D cells under RPMI medium with 10% FBS condition. **p<0.01, n=3.

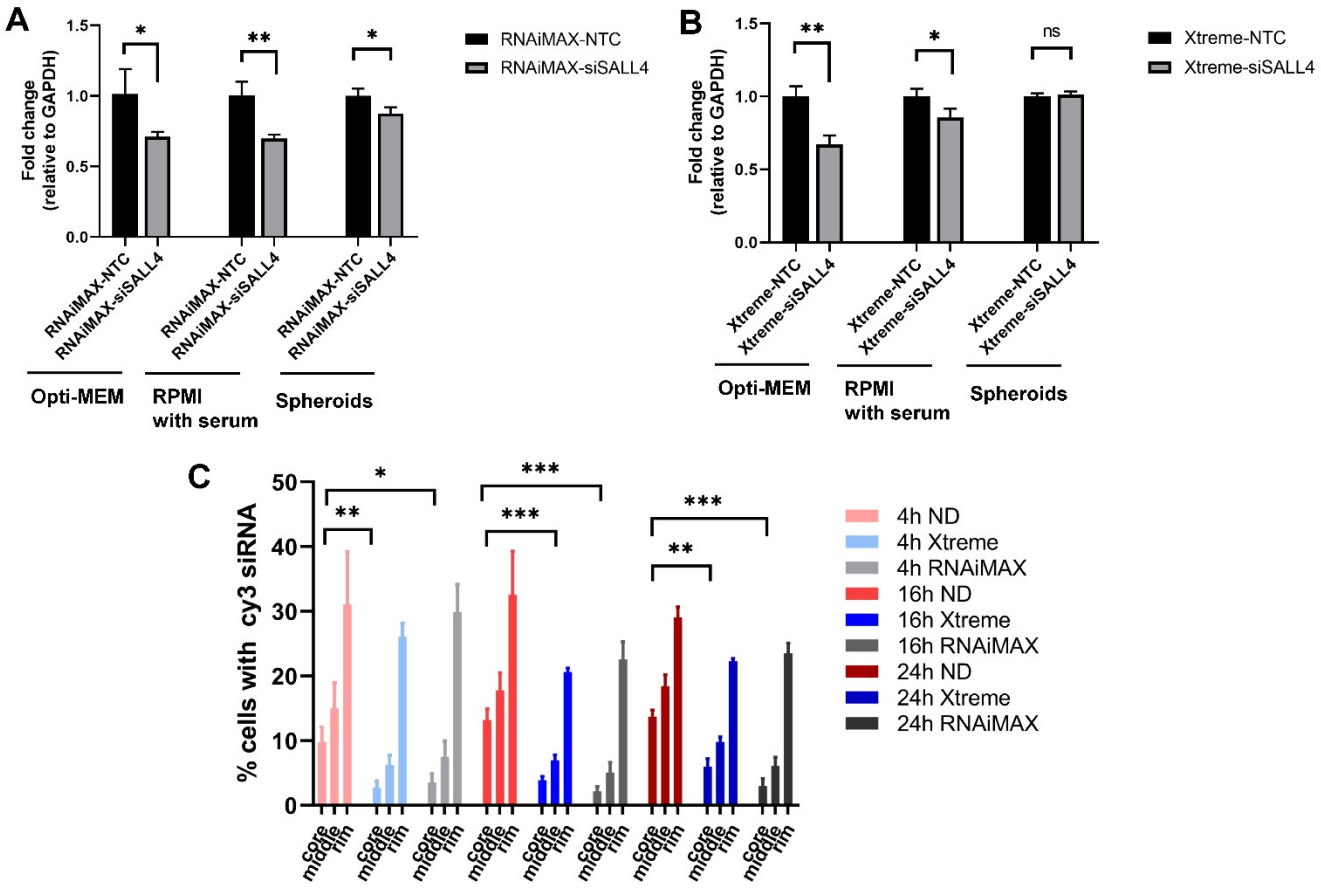


Figure S7. Comparison of commercial transfection reagents with ND. Knockdown capacity test of RNAiMAX-siSALL4 (A) and X-tremeGENE-siSALL4 (B) in 2D cells under Opti-MEM condition or RPMI medium with 10% FBS condition, or in 3D spheroids. (C) siRNA penetration and distribution in SNU398 spheroids using FACS analysis. *p<0.05, **p<0.01, ***p<0.001, n=3. For FACS result, only p-value of core part was evaluated.

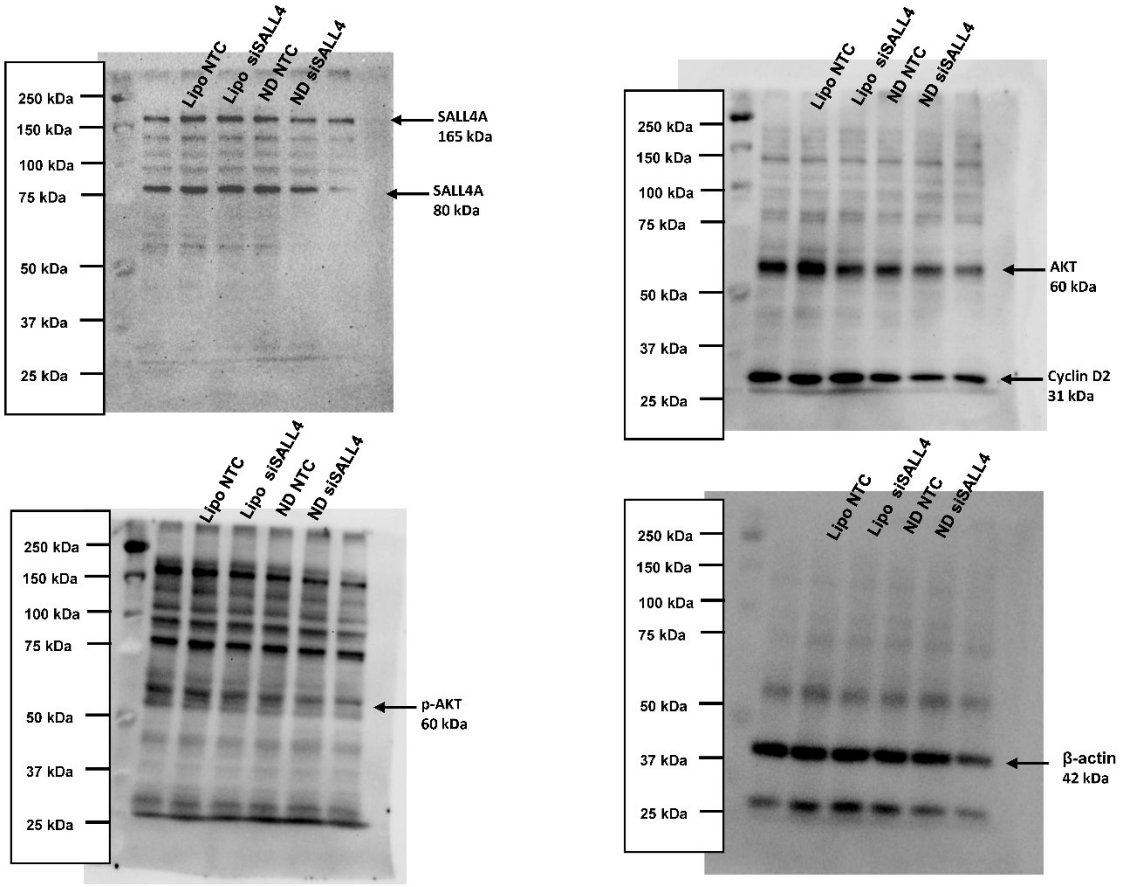


Figure S8. Raw data of Western blot from Figure 6.