Electronic supplementary information

Tumor-suppressing miR-141 Gene-complex Loaded Tissue-adhesive Glue for the Locoregional Treatment of Hepatocellular Carcinoma

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Supplementary Methods

- Making NPX-glue

Supplementary Figures

- **Figure S1.** Generating of NPX-glue
- **Figure S2.** Estimation of cytotoxicity of the nanoplex with various NP (PAA/NCmiR) ratios

Supplementary Methods. For making NPX-glue, Partially oxidized-alginate (OA) was prepared as described previously with slight modifications (Artzi, Shazly, Baker, Bon, & Edelman, 2009) and characterized. Sodium alginate (3.0 g, 15.1 mmol uronate) was dissolved in 70 ml of distilled water and mixed with 30 ml of sodium periodate solution (total 4.7, 9.4, or 14.0 mmol). The mixed solutions were incubated in a 50˚C water bath for 1 to 4 h and the reaction was stopped by adding 10 ml of 10% (v/v) ethylene glycol. Sodium chloride (8 g) was added and then precipitated in 600 ml of ethanol. The supernatant was removed, and the precipitates were dissolved in distilled water. The solution was washed twice and dialyzed against distilled water

for 3 days (molecular-weight cutoffs 12-14 kDa, Spectrum Laboratories Inc. Rancho Dominguez, CA). The solution was lyophilized for 3 days and kept in a desiccator. The aldehyde contents in oxidized alginate were measured by the hydroxylamine hydrochloride titration method (Pardridge, 2004; Rutz & Scheffold, 2004). Oxidized alginate was added to the nanoplex (2:1, v:v) and designated it as the tissue-adhesive nanoplex (NPX-glue).

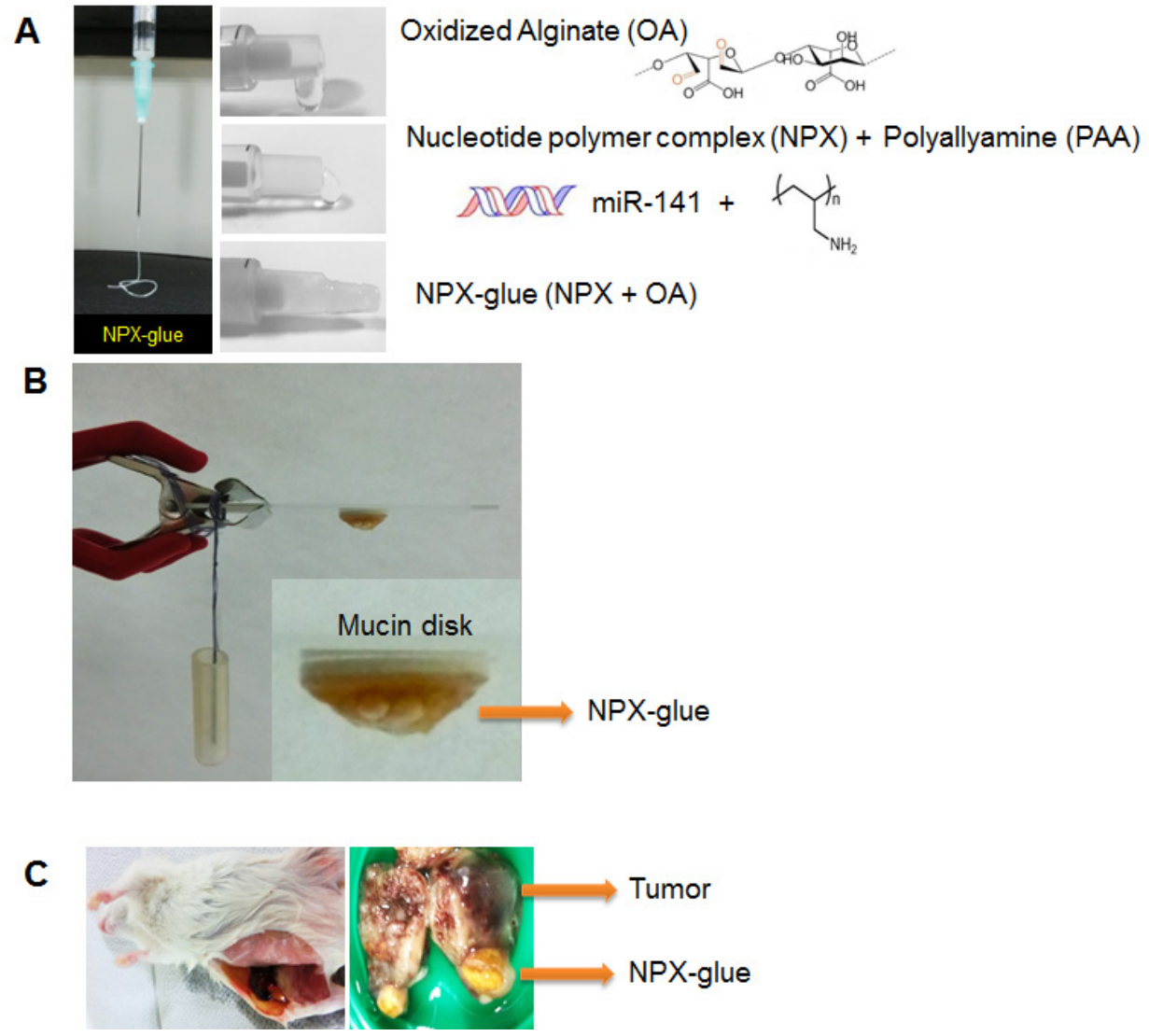

Fig. S1 Generating of NPX-glue (**A**) Formation of injectable NPX-glue. (**B**) The adhesiveness of NPX-glue to a mucin disc. (**C**) Adhesiveness of the NPX-glue to tumor tissues, tumor was

recovered 1 week after the subcutaneous injection of NPX-glue.

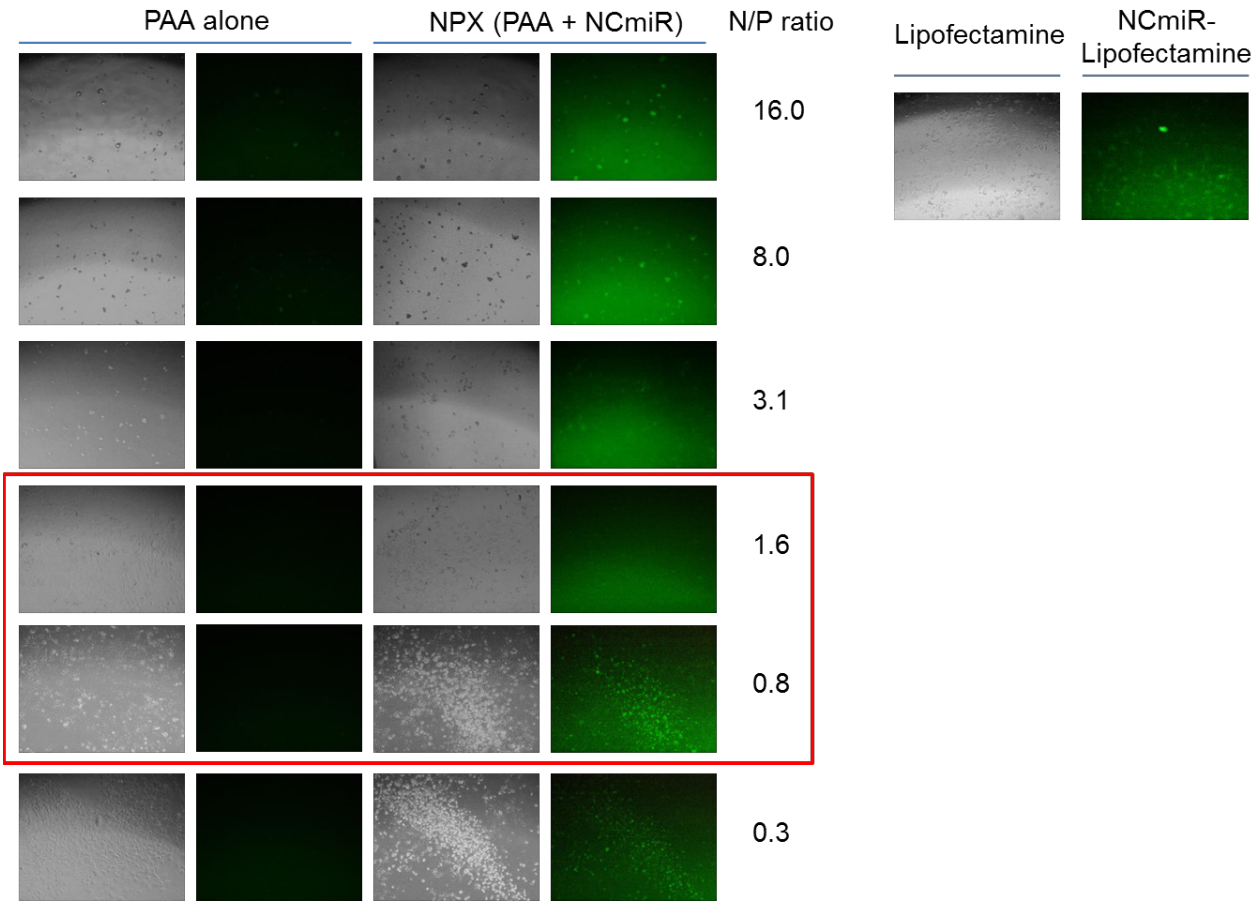

Fig. S2 Estimation of cytotoxicity of the NPX with various NP (PAA/NCmiR) ratios. The cell (GFP-HEP3B) morphology was determined on day 3 after transfection. Lipofectamine was selected for positive control that is the cells transfected with NCmiR using Lipofectamine 2000.

Supplementary Table

Supplementary Table 1. The sequences of the primers that were used in this study.

	Forward (5' to 3')	Reverse (5' to 3')
miR oligo dT	CAGGTCCAGTTTTTTTTTTTTTT	
miR-141	CAGCATCTTCCAGTACAGTGT	CAGGTCCAGTTTTTTTTTTTTTTTCCAACAC
U6	CTCGCTTCGGCAGCACA	GTCCAGTTTTTTTTTTTTTTTAACGCTTCACGA-ATTTGCGT
Tiam1	AAGACGTACTCAGGCCATGTCC	GACCCAAATGTCGCAGTCAG
Turbo RFP	CAACACCGAGATGCTGTACC	GGTTCTTAGCGGGTTTCTTG
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
MAP4K4	CATCTCCAGGGAAATCCTCAGG	TTCTGTAGTCGTAAGTGGCGTCTG
TM4SF1	ACCACTATGTCTTGATTCCCTC	ATTGTGGCTCTGTCCTGGGT
KEAP1	ATTGGCTGTGTGGAGTTGC	CAGGTTGAAGAACTCCTCTTGC
HDGF	GAGGGTGACGGTGATAAGA	GAAACATTGGTGGCTACAGG