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Supplemental Information

In Vivo Delivery and Therapeutic Effects of a MicroRNA on Colorectal Liver Metastases

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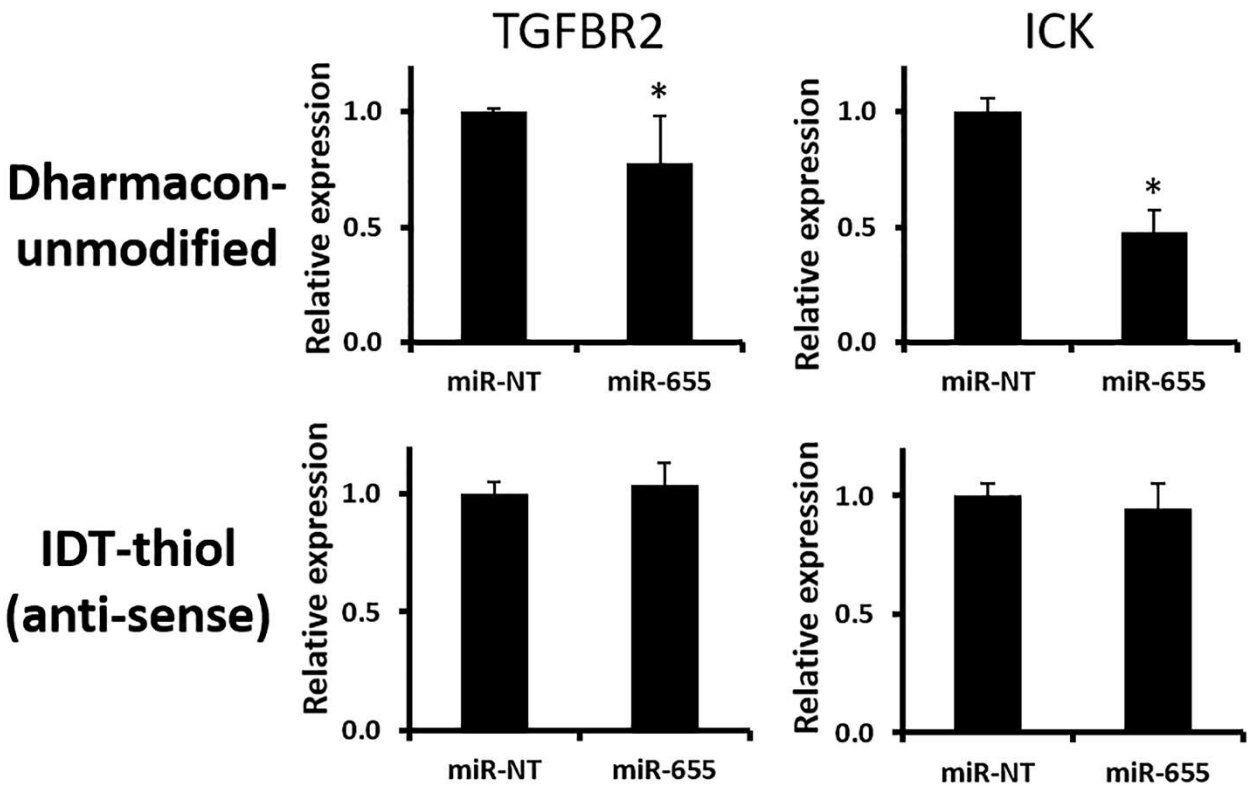

Figure S1 Functional validation of micro-RNAs modified with thiol on an antisense strand

Expression of miR-655-3p-targeted genes TGFBR2 and ICK quantified by qPCR in HCT116L2T cells transfected with each of the tested microRNA mimics (n=6, unpaired *t* test, $p < 0.05$, *).

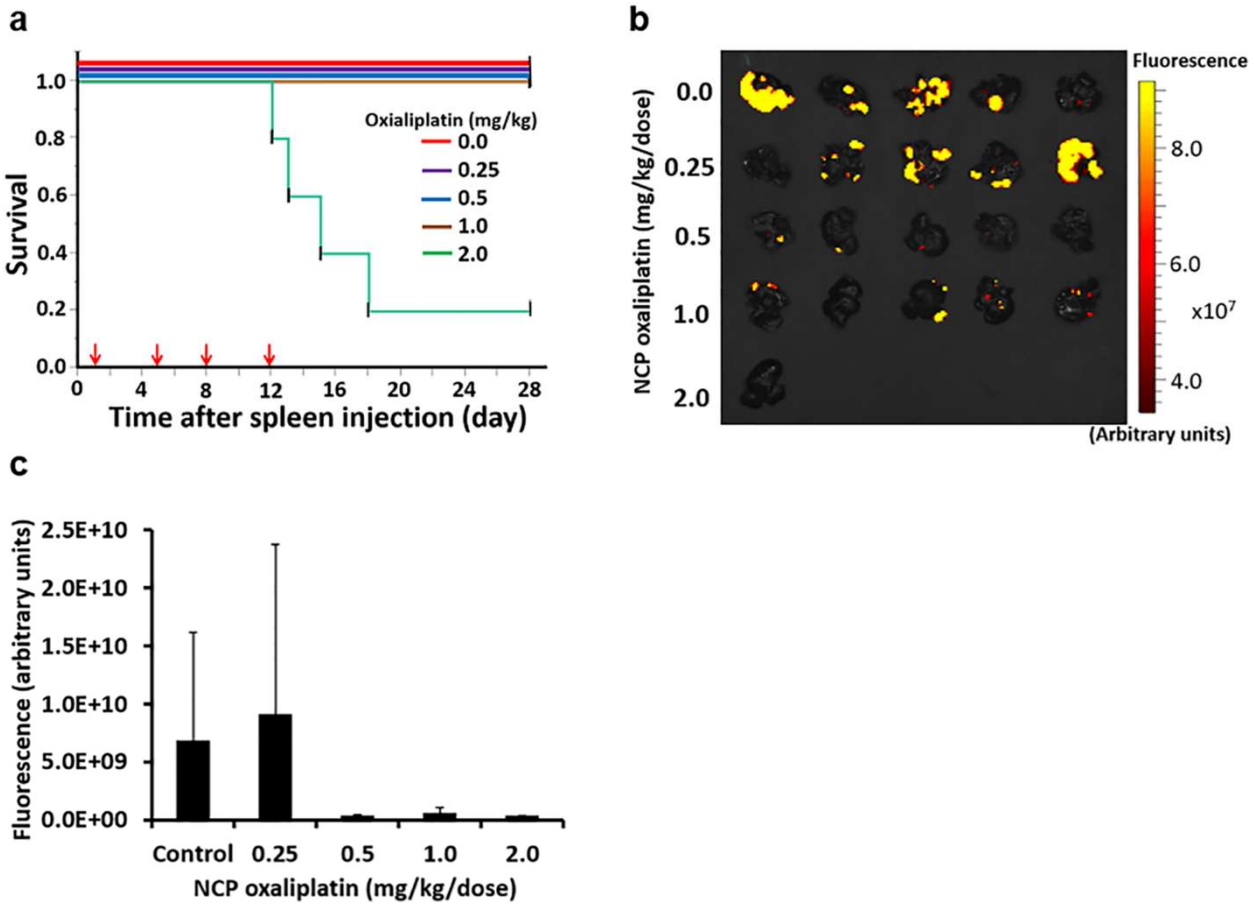

Figure S2 Toxicity of oxaliplatin-based NCPs in a liver metastases model

NCPs containing oxaliplatin in concentrations of 0.0, 0.25, 0.5, 1.0, and 2.0 mg/kg were injected intraperitoneally twice a week for 2 weeks in a liver metastases model. (a) Survival curve after NCPs injection. (b) Ex vivo fluorescence imaging of harvested livers with metastases at 4 weeks after cell injection. (c) Quantification of liver tumor burden by ex vivo fluorescence imaging. Data are expressed as means ± SD (n = 5).

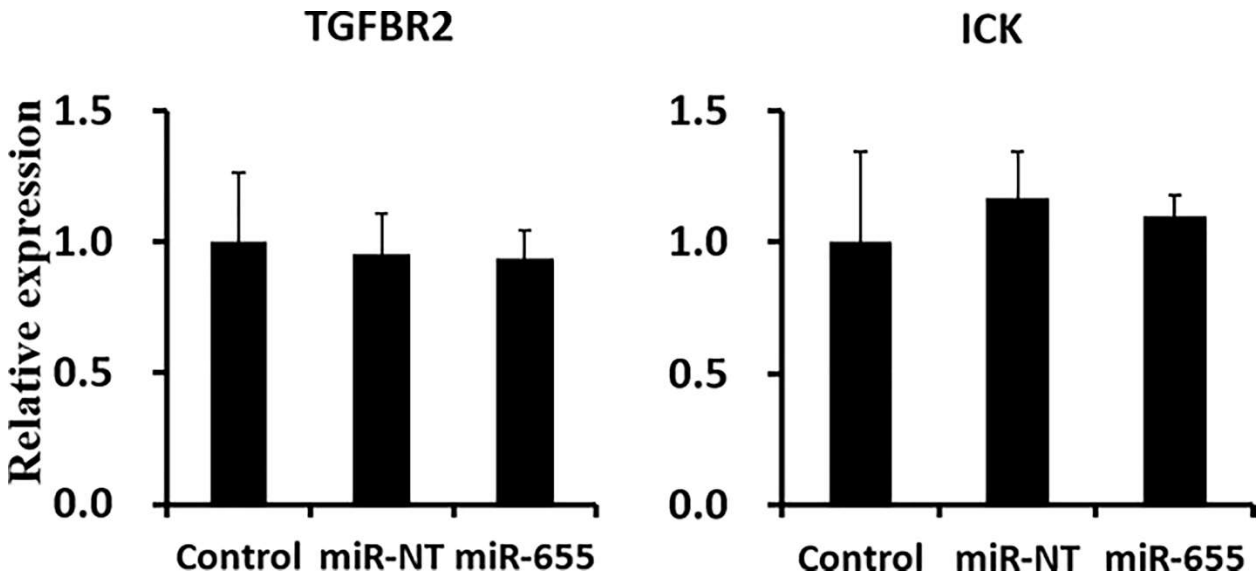

Figure S3 In vivo gene expression treated with NCP carrying miRNA, RNA extracted from tumor 16 days after the last NCP injection

	KEGG pathway	p-value
1	Glycosaminoglycan biosynthesis - chondroitin sulfate (hsa00532)	2.31E-19
2	Sphingolipid metabolism (hsa00600)	2.39E-05
3	Axon guidance (hsa04360)	4.41E-05
4	p53 signaling pathway (hsa04115)	9.35E-05
5	Focal adhesion (hsa04510)	9.35E-05
6	Renal cell carcinoma (hsa05211)	9.35E-05
7	Transcriptional misregulation in cancer (hsa05202)	1.85E-04
8	Ubiquitin mediated proteolysis (hsa04120)	1.97E-03
9	TGF-beta signaling pathway (hsa04350)	1.97E-03
10	Glutamatergic synapse (hsa04724)	1.97E-03
11	Inositol phosphate metabolism (hsa00562)	5.03E-03
12	Acute myeloid leukemia (hsa05221)	6.42E-03
13	Pathways in cancer (hsa05200)	7.71E-03
14	Prostate cancer (hsa05215)	9.52E-03
15	Gap junction (hsa04540)	1.02E-02
16	Fc gamma R-mediated phagocytosis (hsa04666)	1.34E-02
17	MAPK signaling pathway (hsa04010)	1.52E-02
18	Protein processing in endoplasmic reticulum (hsa04141)	2.56E-02
19	Long-term potentiation (hsa04720)	3.39E-02
20	Bacterial invasion of epithelial cells (hsa05100)	3.39E-02
21	Phosphatidylinositol signaling system (hsa04070)	3.52E-02
22	Arrhythmogenic right ventricular cardiomyopathy (ARVC) (hsa05412)	3.52E-02
23	Peroxisome (hsa04146)	3.53E-02
24	Chronic myeloid leukemia (hsa05220)	3.53E-02
25	Pathogenic Escherichia coli infection (hsa05130)	3.73E-02
26	T cell receptor signaling pathway (hsa04660)	3.76E-02
27	RNA degradation (hsa03018)	4.75E-02
28	Adherens junction (hsa04520)	4.75E-02
29	Jak-STAT signaling pathway (hsa04630)	4.75E-02

Table S1 Predicted target KEGG pathway by miR-655-3p

KEGG pathways targeted by miR-655-3p were identified using hypergeometric testing via Diana miRPath v2 ($p \leq 0.05$) (See methods).

Gene	Forward	Reverse
humanTGFBR2	GTAGCTCTGATGAGTGCAATGAC	CAGATATGGCAACTCCCAGTG
human ICK	AGCTCAACCATGCCAATGTAG	AGTCTCGATGAAAGAAGCCGT
human GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
mouse TGFBR2	CCGCTGCATATCGTCCTGTG	AGTGGATGGATGGTCCTATTACA
mouse ICK	TTCTTCCACCGGGACTTAAAAC	CGGAGGTCTTGATCGGATTTCT
mouse GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Table S2 Sequences of primers used in qPCR assays

Supplementary Methods

microRNA target pathway prediction. Predicted targets of micro-RNAs were determined using Diana microT-CDS (MicroT threshold 0.6). KEGG pathways targeted by micro-RNAs were identified using hypergeometric testing via Diana miRPath v2 ($p \leq 0.05$). Statistical significance of each KEGG pathway was calculated using Fisher's combined probability method via Diana miRPath v2.