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Supplemental Information

The Delivery of a Wnt Pathway Inhibitor Toward CSCs Requires Stable Liposome Encapsulation and Delayed Drug Release in Tumor Tissues

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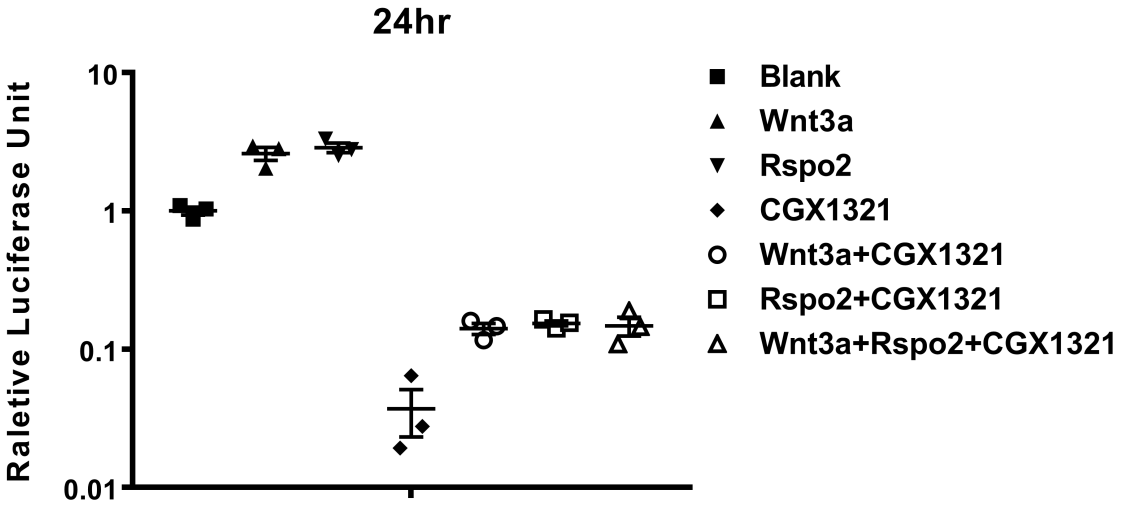
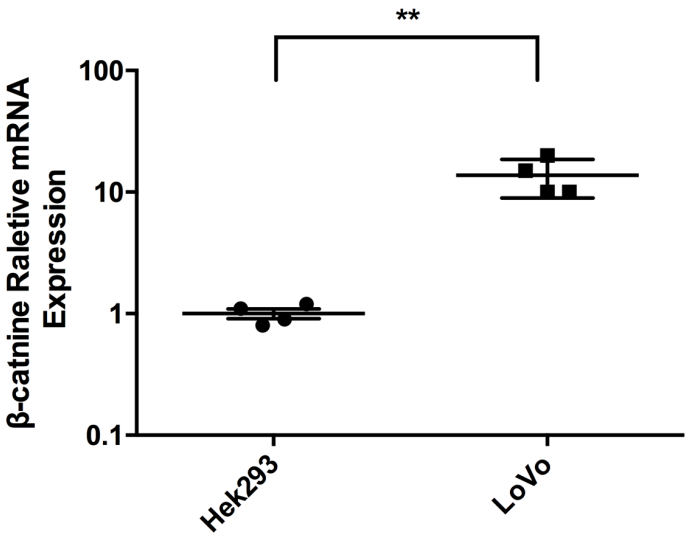
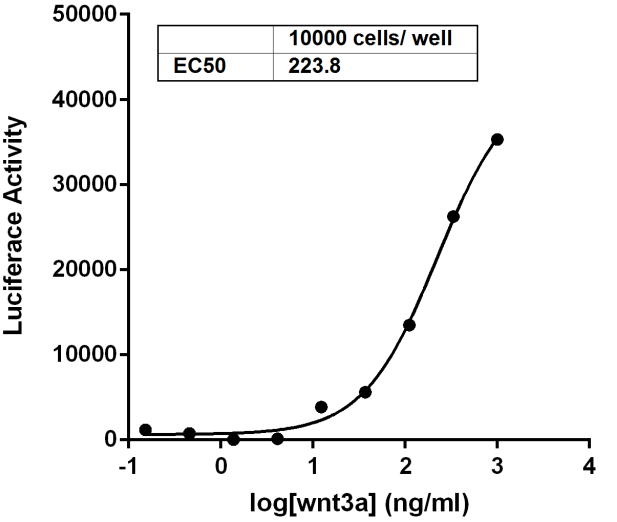
S1


24hr
Raletive Luciferase Unit
10
1
0.1
0.01
Blank
Wnt3a
Rspo2
CGX1321
Wnt3a+CGX1321
Rspo2+CGX1321
Wnt3a+Rspo2+CGX1321

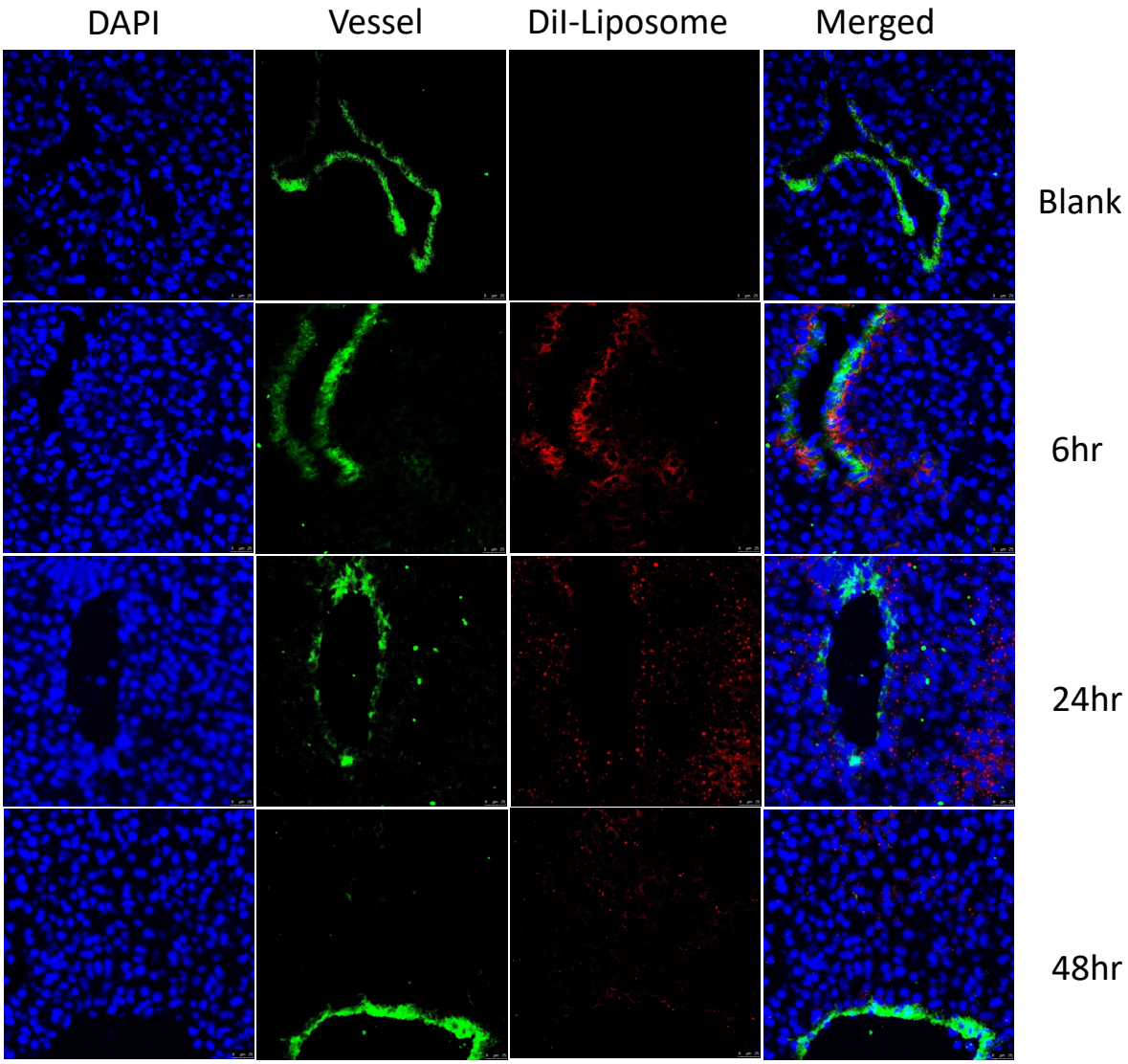
S2


**
β-catnine Raletive mRNA Expression
100
10
1
0.1
Hek293
LoVo

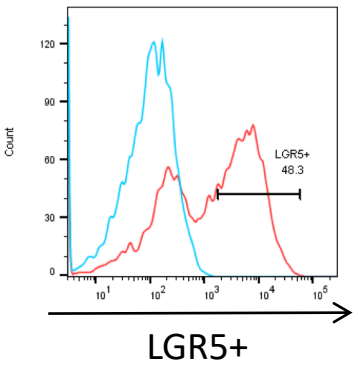
S3


10000 cells/ well
EC50
223.8
Luciferace Activity
50000
40000
30000
20000
10000
0
-1
0
1
2
3
4
log[wnt3a] (ng/ml)

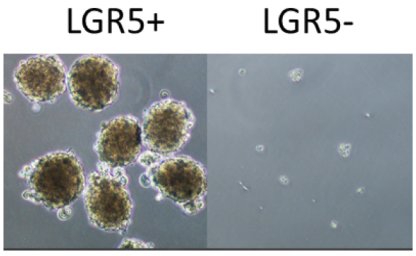
S4


DAPI
Vessel
DiI-Liposome
Merged
Blank
6hr
24hr
48hr

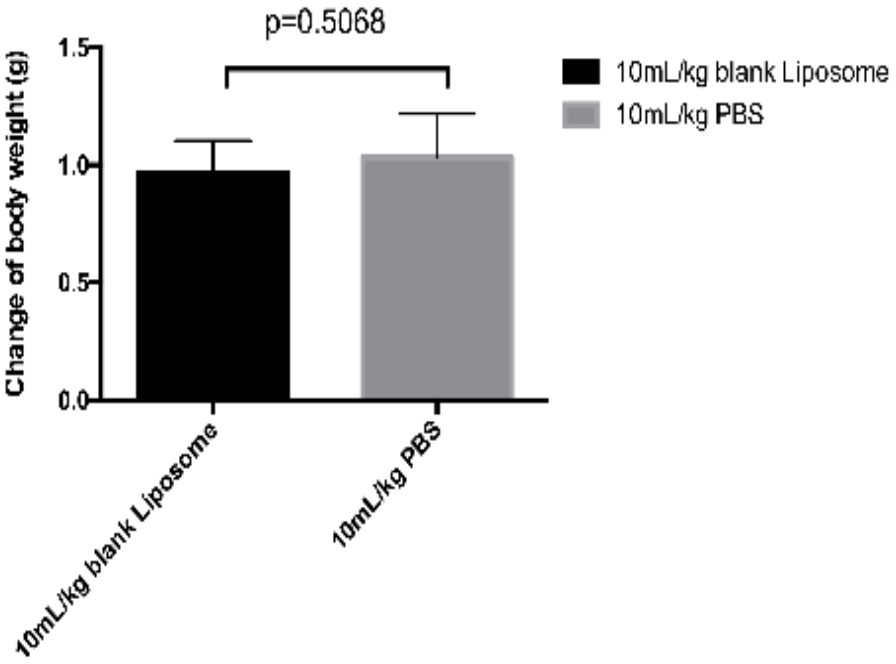
S5


Count
LGR5+
48.3
LGR5+

S6


LGR5+
LGR5-

S7



- S1, Luciferase activity assays in HEK293-TCF cells. Luciferase units were detected at 24 hrs after co-culture with RSPO2, Wnt3a and CGX1321. The concentrations of CGX1321 in medium is 20μM, Wnt3a concertation is 200ng/ml, Rspo2 concentration is 10ng/ml.
- S2, The confirmation of β-catenin expression in LoVo cells line by q-PCR.
- S3, Wnt3a or RSPO2 Cellular assay in HEK293-TCF cells.
- S4, Immunofluorescence staining in liver from GA007 PDX mice model after a single dosing of 10mg/kg liposome-CGX1321 (liposome concentration is 50mg/mL) via i.v. injection, detected by confocal microscopy. Scale Bar is 25 μm.
- S5-6, Flow cytometry of LGR5+ cells from the GA007 PDX tumor single cells suspension. The gate shows LGR5 positive cells (s6). We sorted out these LGR5+ cells and 3D cultured them *in vitro*. They quickly grew into tumor-like spheroids, while the LGR5- cells had a low survival rate (S6). These LGR5+ cells were also injected subcutaneously into nude mice in order to analyze their tumor-initiating properties. As summarized, as few as 100 LGR5+ cells resulted in tumor formation in five out of the six mice, while as many as 100,000 LGR5- cells failed to initiate formation of any tumors (Supplemental table 2). Mice (n=6) were assessed over a time period of 21–55 days
- S7, Bodyweight change in liposome injection group and PBS injection group. There is no significant difference between blank liposome group and PBS group. P=0.5068, n=5

Supplemental Table 1

Primers used in this study

LGR5-F	CTGAACTAAGAACACTGA
LGR5-R	TTGAGGAAGAGATGAGAT
GAPDH-F	GAAGGTGAAGGTCGGAGT
GAPDH-R	GAAGATGGTGATGGGATTTC
β-catenin-F	GGTTGCCTTGCTCAACAAAA
β-catenin-R	TCCCAAGGAGACCTTCCATC
Axin2-F	ATGTCTGGCAGTGGATGTTAG
Axin2-R	GACTCCAATGGGTAGCTCTTTC

Supplemental Table 2

Ex vivo 100 Lgr5+ cells induced Xenograft

	Cells injected				
	10	10^2	10^3	10^4	10^5
Lgr5+cell	0/6	5/6	6/6	6/6	6/6
Lgr5-cell	0/6	0/6	0/6	0/6	0/6