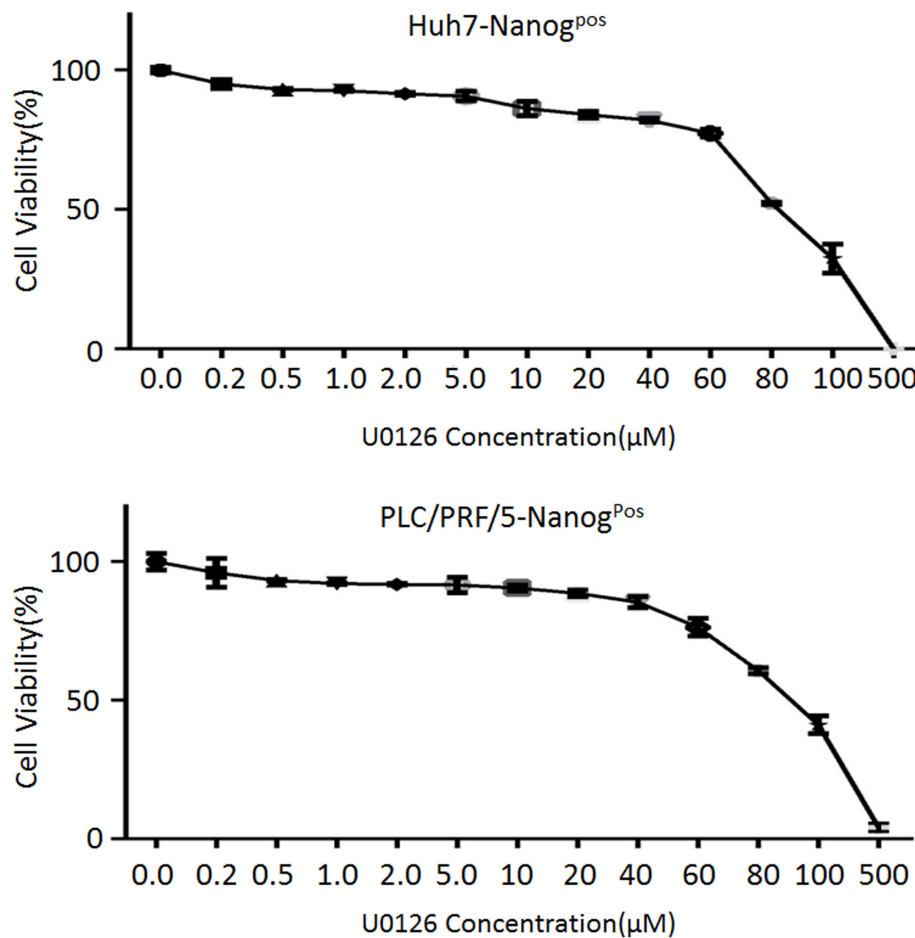
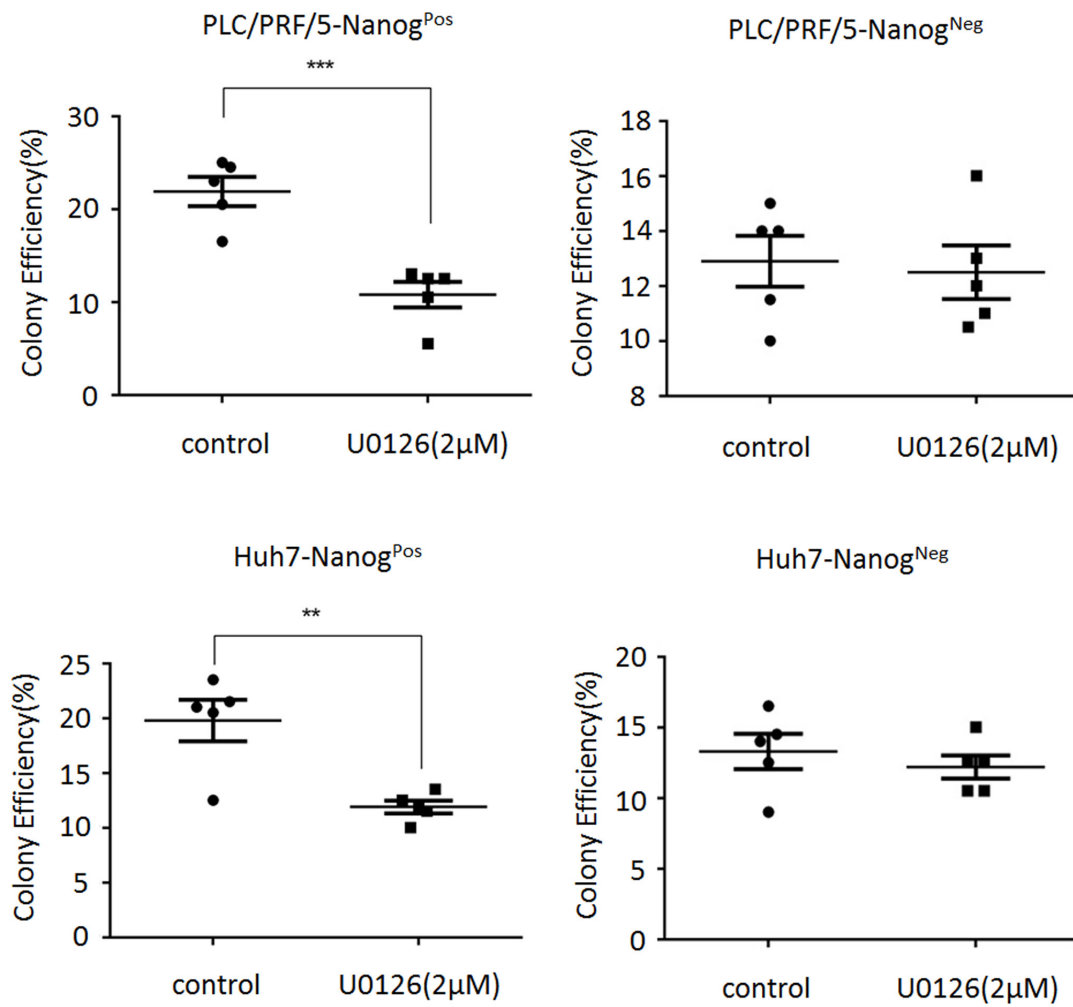


## MEK1 signaling promotes self-renewal and tumorigenicity of liver cancer stem cells via maintaining SIRT1 protein stabilization

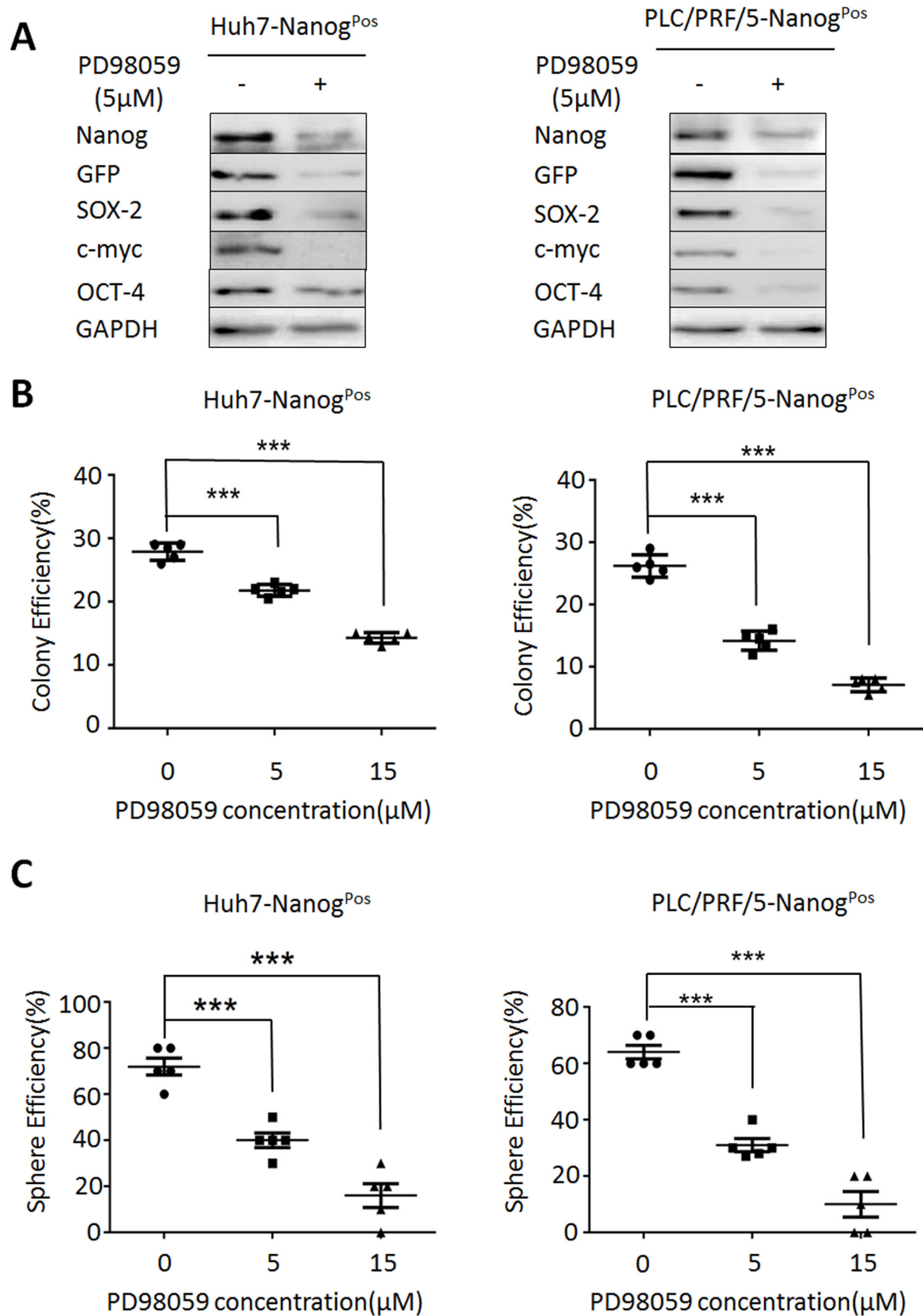
### Supplementary Materials



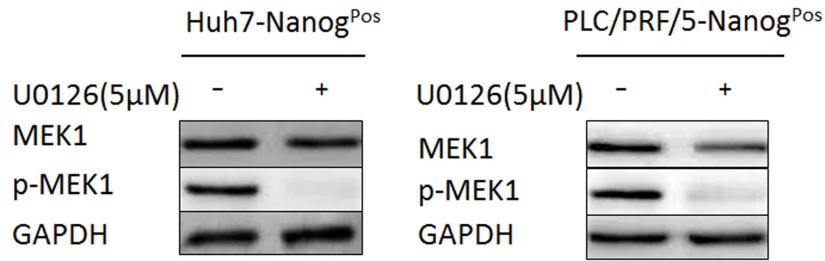
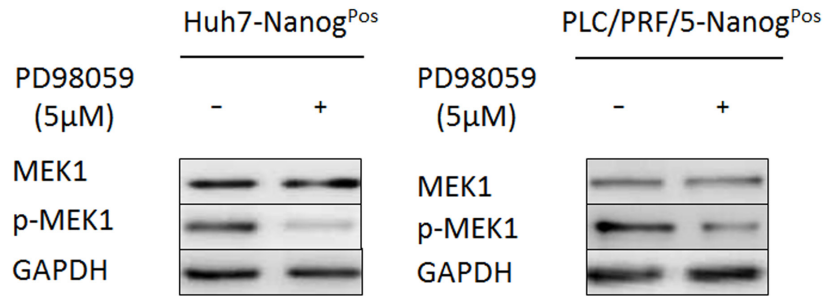
**Supplementary Figure S1: Toxicity test of U0126 to liver CSCs.** CSCs (Huh7- and PLC/PRF/5- Nanog<sup>pos</sup> cells) were treated with indicated concentration of U0126 for 24 hours, before being analyzed with the CCK8 assay.



**Supplementary Figure S2: U0126 inhibited CSCs and non-CSCs discrepantly in Colony capacity.** CSCs and non-CSCs were treated with 2 µM U0126 for 14 days and stained with crystal violet.



**Supplementary Figure S3: PD98059 significantly inhibited CSCs self-renewal and tumorigenesis.** (A) Proteins were obtained after 5 μM PD98059 treatment of CSCs for 48 hours. Western blots analysis of Nanog, GFP, SOX2, OCT4 and c-myc expression level compared with negative control. (B) CSCs were co-cultured with indicate dose of PD98059 for 14 days before stained with crystal violet and counted. (C) CSCs were treated with 5 μM or 15 μM PD98059 in non-adhesion system for 7 days before counted.

**A****B**

**Supplementary Figure S4: MEK1 inhibitor inhibited phosphorylated-MEK1 expression.** (A–B) CSCs (Huh7- and PLC/PRF/5-Nanog<sup>Pos</sup> cells) were treated with 5 µM U0126 or 5 µM PD98059 for 48 hours. Cells were harvested and analysis by western blots about the expression of MEK1 and phosphorylated-MEK1.

## Detail of Antibody

Name	Company	Catalog Number	Dilutions
SOX2	Cell Signaling Technology	2748	1:1000 for WB
MEK1	Cell Signaling Technology	12671	1:500 for WB
phospho-MEK1	Cell Signaling Technology	2338	1:500 for WB, 1:50 for IHC
GAPDH	Cell Signaling Technology	2118L	1:1000 for WB
Ki-67	Cell Signaling Technology	9449	1:800 for IF
SIRT1 (H-300)	Santa Cruz	sc-15404	1:500 for WB, 1:200 for IP, 1:50 for IHC
c-Myc (9E10)	Santa Cruz	sc-40	1:500 for WB
Ub	Santa Cruz	sc-8017	1:500 for WB, 1:300 for IP
GFP	abcam	ab290	1:1000 for WB
OCT4	abcam	ab19857	1:1000 for WB
Nanog	abcam	ab109250	1:1000 for WB, 1:100 for IHC
ERK1/2	Millipore	05-1152	1:1000 for WB
Phospho-ERK1/2	Millipore	05-797R	1:1000 for WB
Alexa Flour 647	life technologies	A31571	1:1000 for IF