

Three-dimensional Organotypic Culture Models of Human Hepatocellular Carcinoma

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Running title: A three-dimensional organotypic cell culture model exemplifies molecular features of hepatocellular carcinoma

Supplementary information

Supplemental Figure legends

Supplemental Figure 1. Representative images of Huh1 spheres cultured in each matrix examined. Black arrows point to examples of sphere fusion. Scale bars are 50 μ m.

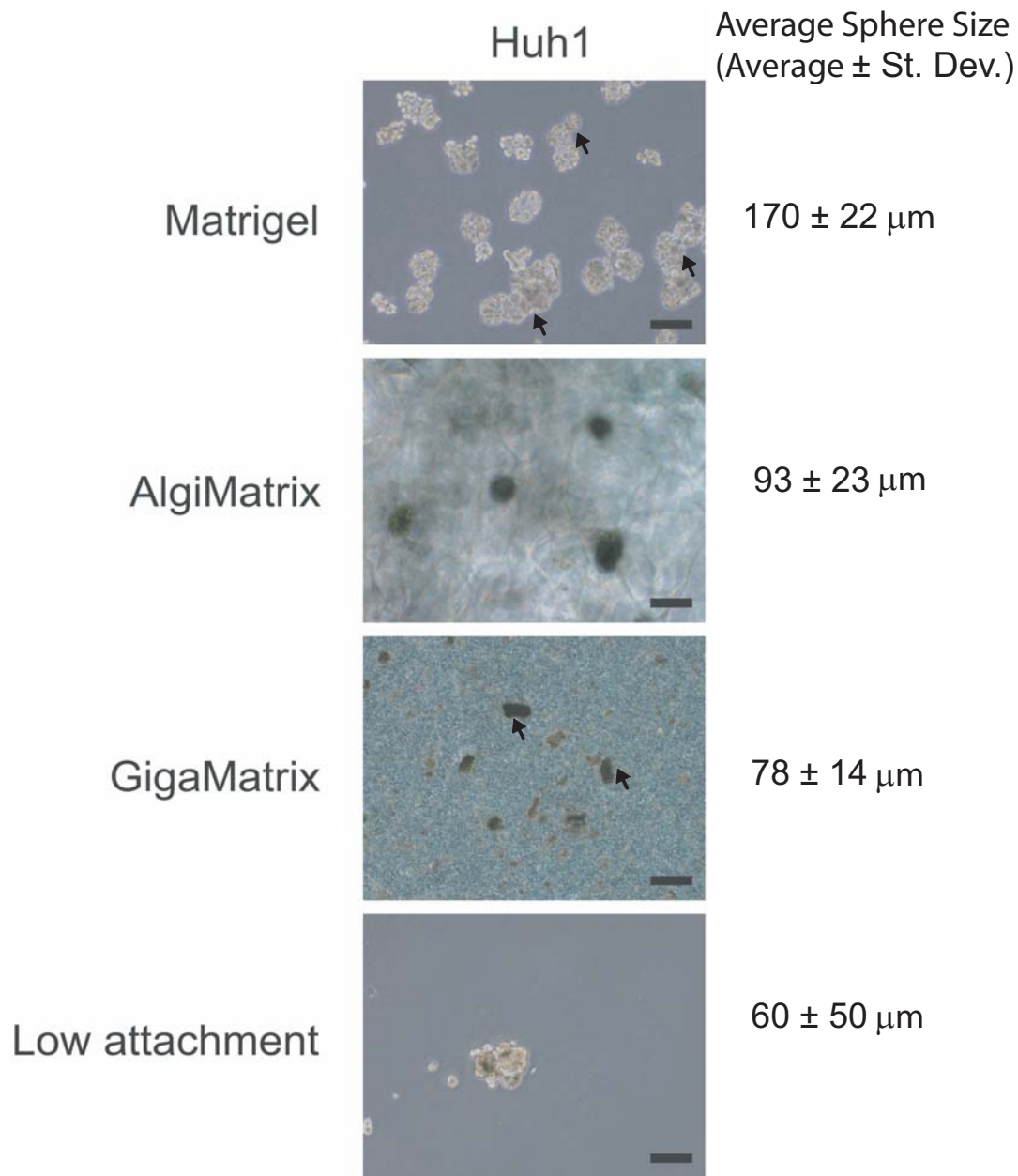
Supplemental Figure 2. Representative images of spheres of various cell lines grown in AlgiMatrix. All the spheres were collected at Day 7 and stained with H&E. Scale bars are 50 μ m.

Supplemental Figure 3. Representative images of Huh-1 spheres with EpCAM shRNA. All the spheres were collected at Day 7 and stained with E-cadherin (green) and EpCAM (red). Scale bars are 50 μ m.

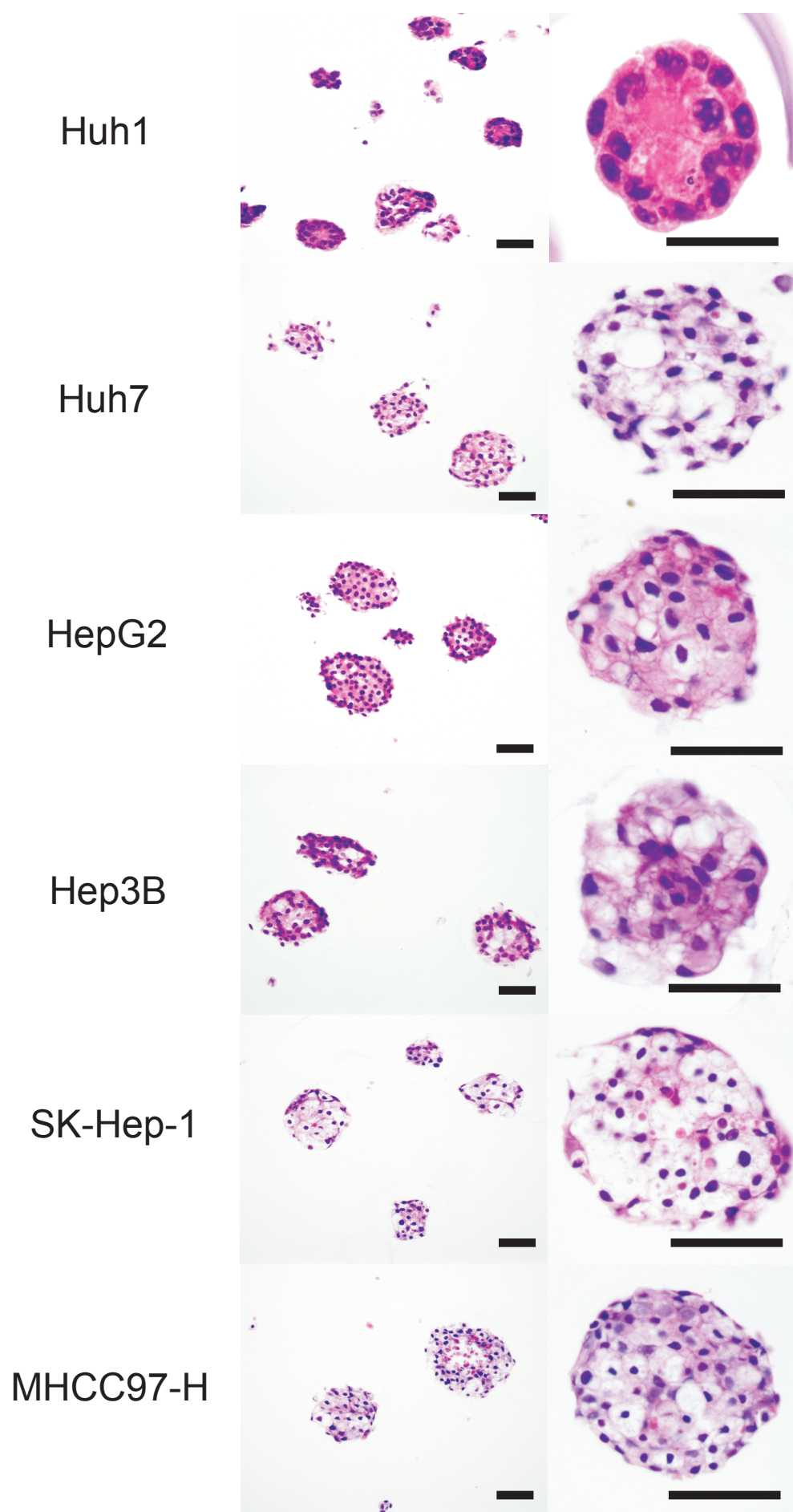
Supplemental Figure 4. Relative number of Huh1 spheres (white bars) and MHCC97-H spheres (black bars) treated with DMSO, AV-606 and fiduxosin are shown.

Supplemental Figure 5. Relative number of Huh1 spheres harvested at Day 7, treated with TGF β beginning at Day 1 of AlgiMatrix culture, is shown.

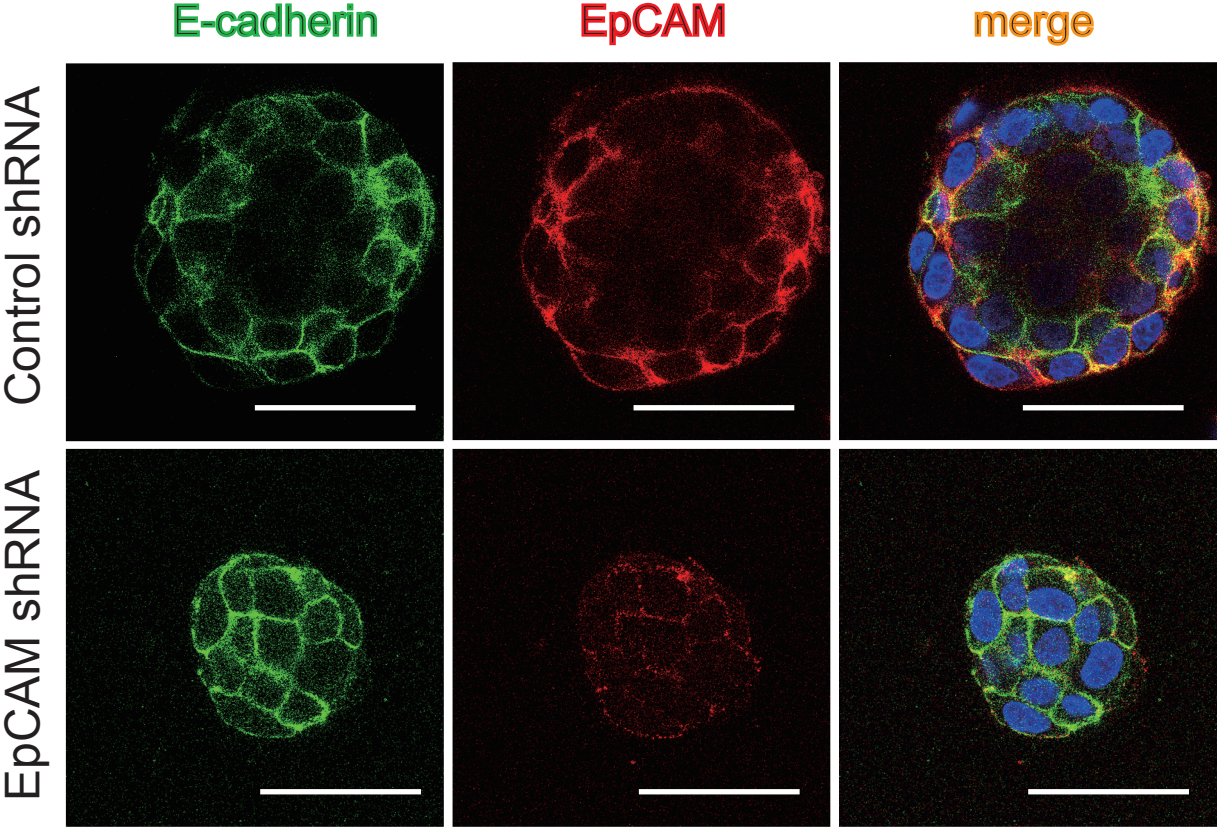
Supplemental Figure 1



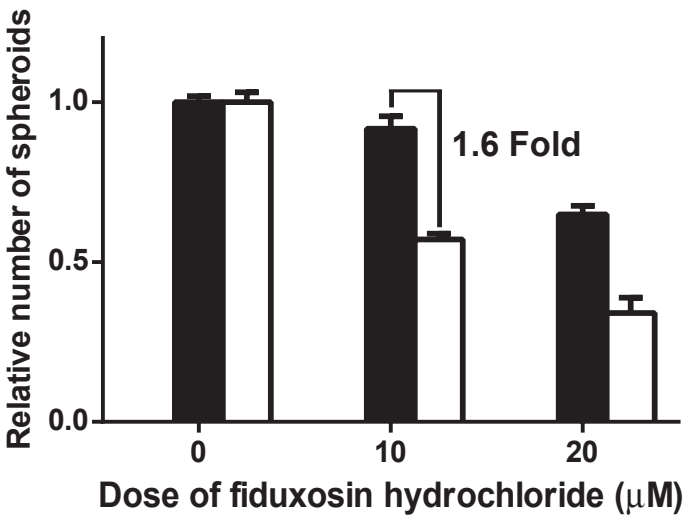
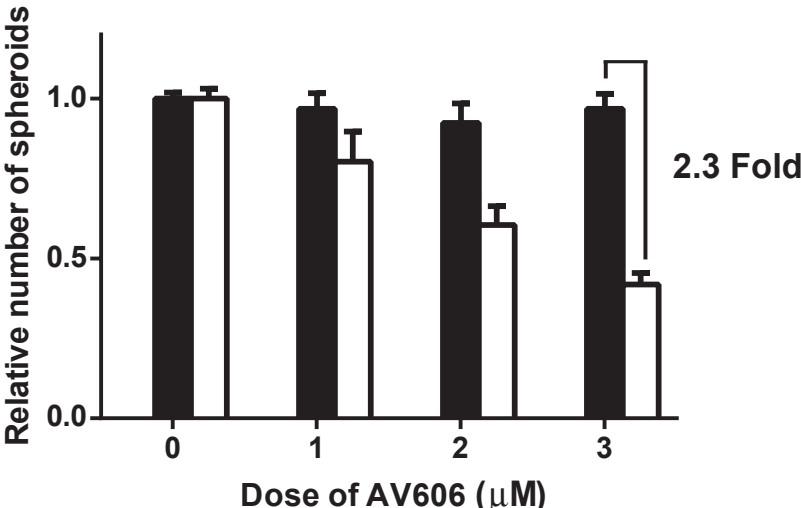
Supplemental Figure 2



Supplemental Figure 3

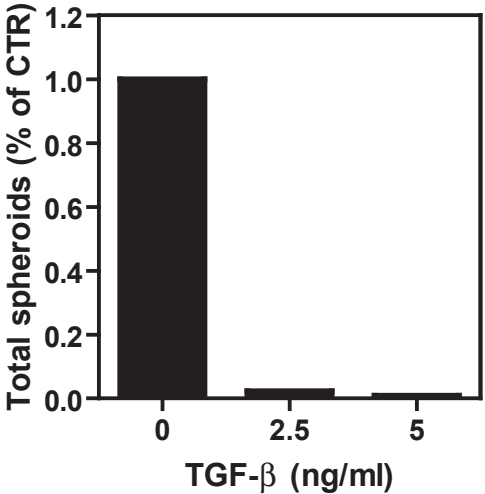


Supplemental Figure 4



□ HUH1 ■ MHCC97

Supplemental Figure 5



Supplemental Table 1. Summary of characteristics of each culture system

	Size Range Observed	Morphology Observed	Sphere Fusion Observed	Ease in Sphere Collection
AlgiMatrix	60-240 μm	Round	No	Easy
Matrigel	50-200 μm	Round/Irregular	Yes	Easy
GigaMatrix	50-125 μm	Irregular	Yes	Difficult
Ultra-Low Attachment	20-300 μm	Round	No	Easy

Supplemental Table 2. Frequencies of HCC and metastasis in orthotopic liver cancer model

	No treatment (3D) (n=9)	TGF treatment (3D) (n=10)	No treatment (2D) (n=9)	TGF treatment (2D) (n=7)
Number of mice with HCC (%)	8 (89)	9 (90)	6 (67)	6 (86)
Peritoneal metastasis (%)	0 (0)	1 (10)	0 (0)	0 (0)
Diaphragm metastasis (%)	0 (0)	1 (10)	0 (0)	0 (0)