Supplementary information

Increased extracellular matrix density disrupts E-cadherin/ß-catenin complex in gastric cancer cells

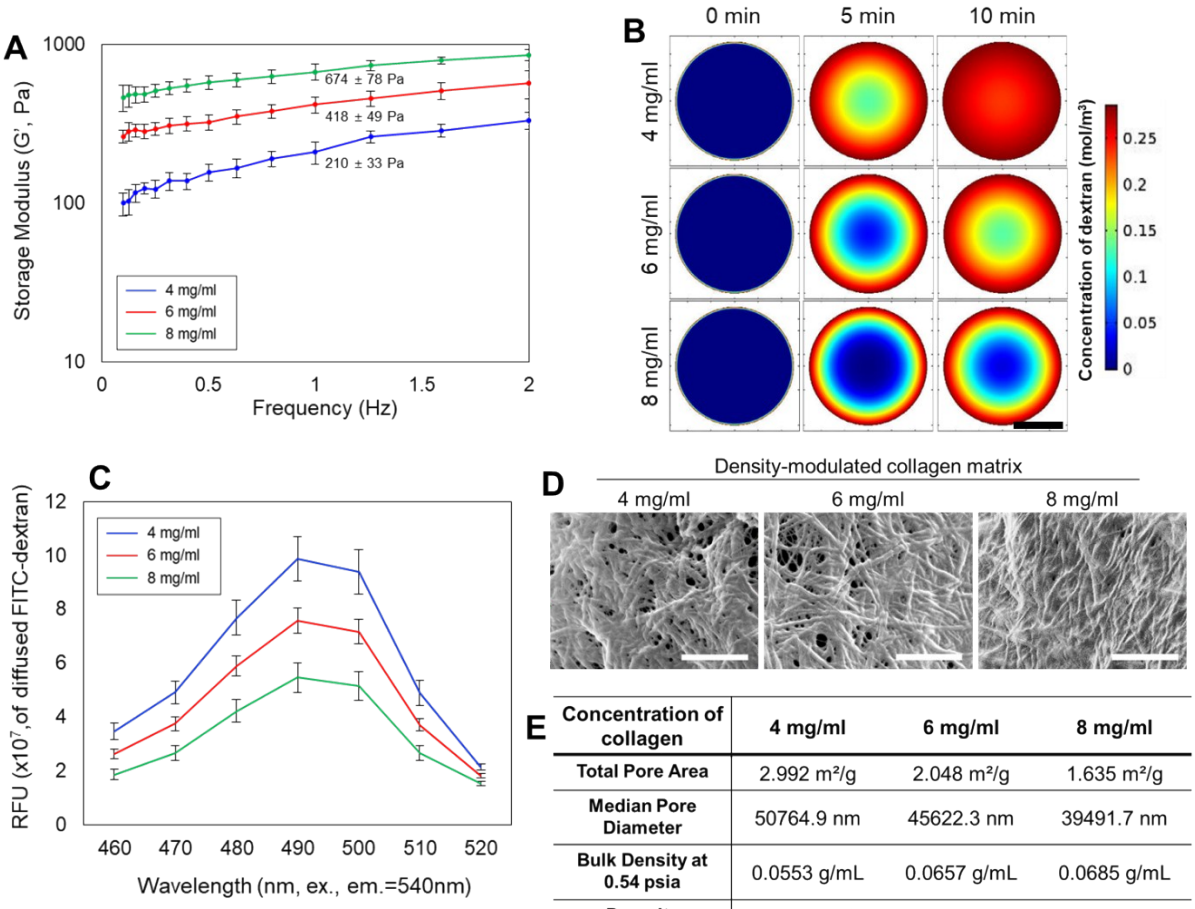
Minjeong Jang,[a] Ilkyoo Koh,[a] Jae Eun Lee,[b] Ju Yeon Lim,[b] Jae-Ho Cheong,[*b] and Pilnam Kim[*a]

[a]Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea.

[b]Department of Surgery, Severance Hospital, Yonsei University College of Medicine, Seoul 03722, Korea.

***** Corresponding author. Email: [a*]pkim@kaist.ac.kr, [b*]JHCHEONG@yuhs.ac

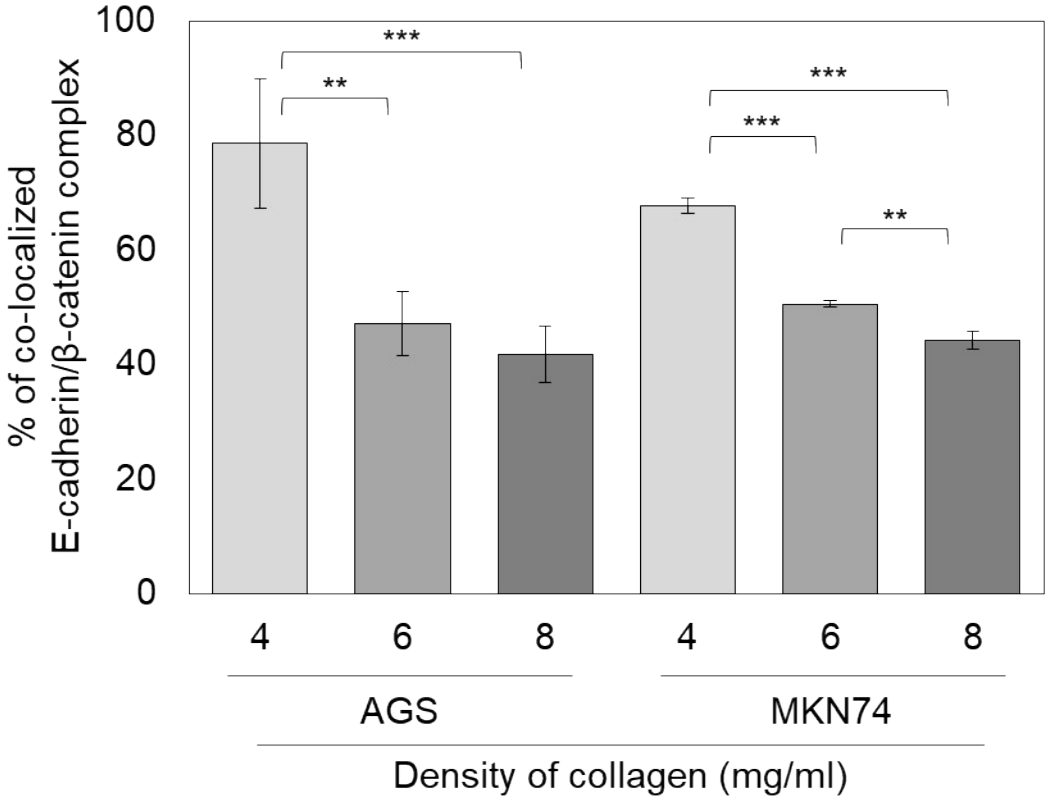
[*] Corresponding Author: Pilnam Kim, Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea. Phone: +82-42-350-4332; E-mail: pkim@kaist.ac.kr, and Jae-Ho Cheong, Department of Surgery, Severance Hospital, Yonsei University College of Medicine, Seoul 03722, Korea. Phone: +82-2-2228-2094; Fax: +82-2-303-8289; E-mail: JHCHEONG@yuhs.ac



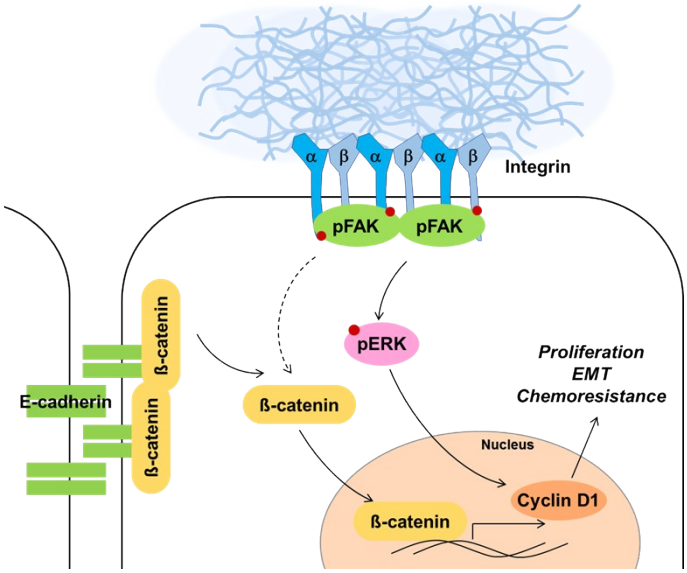
E

Concentration of collagen	4 mg/ml	6 mg/ml	8 mg/ml
Total Pore Area	2.992 m²/g	2.048 m²/g	1.635 m²/g
Median Pore Diameter	50764.9 nm	45622.3 nm	39491.7 nm
Bulk Density at 0.54 psia	0.0553 g/mL	0.0657 g/mL	0.0685 g/mL
Porosity	99.7391 %	91.0965 %	88.7759 %

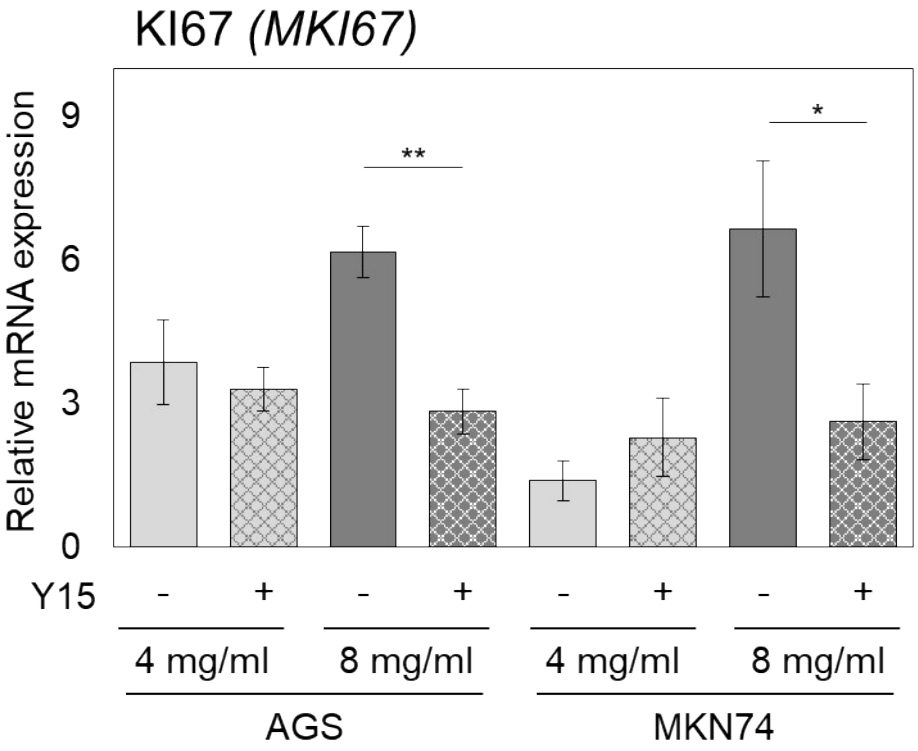
Supplementary Figure S1. Characterization of density-varied collagen matrix (4, 6, and 8 mg/ml). A. Storage modulus in 4, 6, and 8 mg/ml of collagen gels. B. COMSOL simulation of diffusion of FITC-dextran (70kDa) through density-varied collagen matrix. Scale bar = 200 μm C. Permeability of fluorescein isothiocyanate (FITC)-dextran (70 kDa) in density-varied collagen matrix. With increased collagen density, the fluorescence intensity of FITC-dextran (70 kDa) diffused from the hydrogel increased (n = 3). D. Scanning electron microscopy (SEM) images of collagen fiber matrices with varying density. Scale bar = 1 μm. E. Evaluation of porosity depending on the concentration of collagen by mercury intrusion porosimetry (MIP).



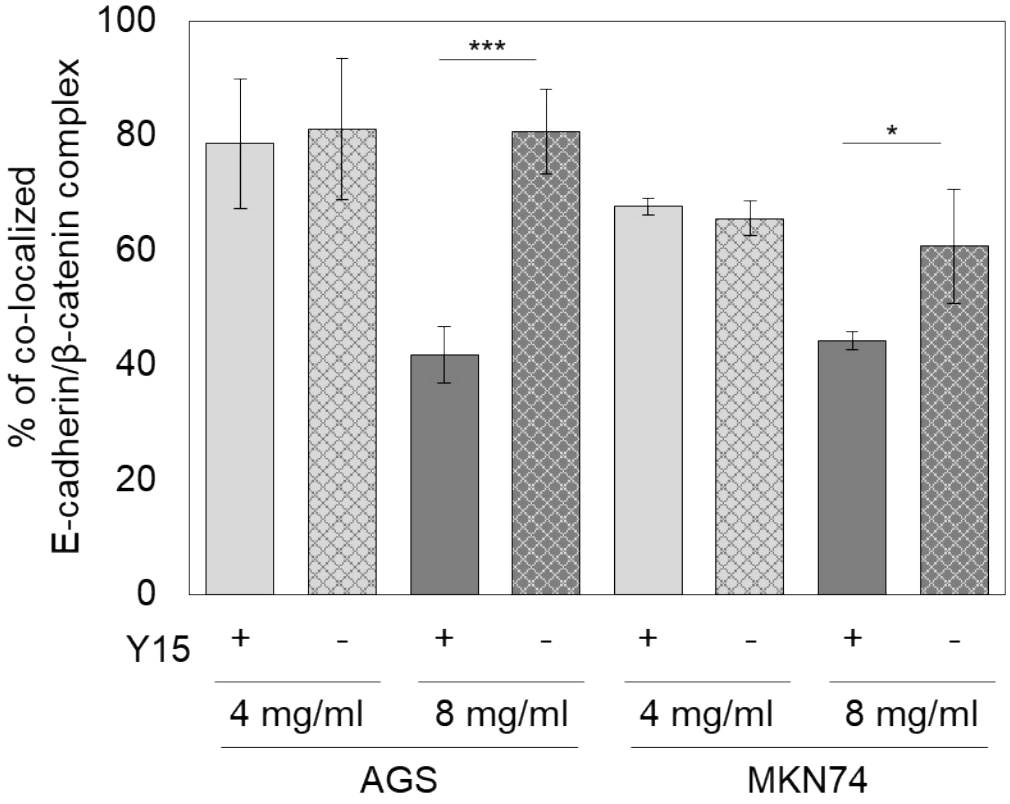
Supplementary Figure S2. Percentage of co-localized E-cadherin/β-catenin complex of AGS and MKN74 in density-varied collagen matrix (4~8 mg/ml) (n=3-5, **:$p<0.001$, ***:$p<0.001$)



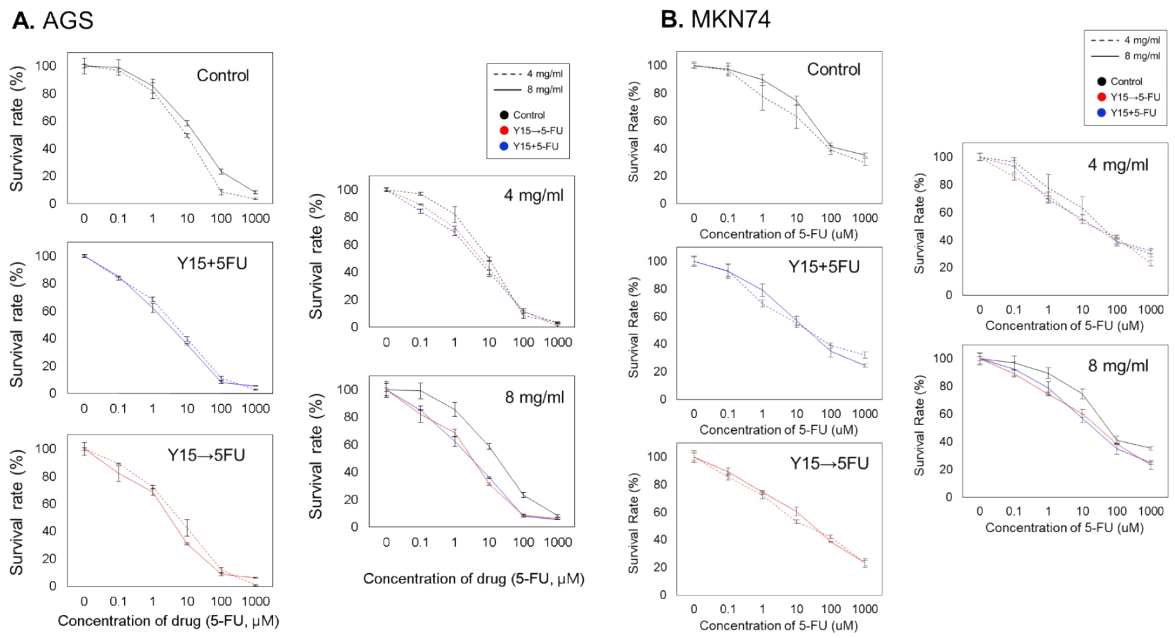
Supplementary Figure S3. Schematics of proposed integrin-mediated cell-ECM interaction and E-cadherin/β-catenin-mediated cell-cell adhesion depending on matrix density.



Supplementary Figure S4. Effect of Y15 treatment on GC proliferation by collagen density. Expression of the proliferation-related gene, *MKI67*, of GC cell lines, AGS and MKN74, in density-varied ECM by treating Y15. *:$p<0.05$, **:$p<0.01$



Supplementary Figure S5. Percentage of co-localized E-cadherin/β-catenin complex of GC cell lines, AGS and MKN74, in density-varied ECM by treating Y15. (n=3-5, *:$p<0.05$, ***:$p<0.001$)



Supplementary Figure S6. Dose-dependent survival rates were assessed in the presence of Y15. A. The survival rates of AGS represented 4 mg/ml (dotted line) and 8 mg/ml (straight line) in a dose-dependent manner of 5-FU, without (black), with Y15 (blue), and after Y15 treatment (red). B. The survival rates of MKN74 represented 4 mg/ml (dotted line) and 8 mg/ml (straight line) in a dose-dependent manner of 5-FU, without (black), with Y15 (blue), and after Y15 treatment (red).Y15 affected drug resistance in a dense matrix.

Gene		Sequence	Refs.
ITGB1	F	CAA GAG AGC TGA AGA CTA TCC CA	PrimerBank ID: 182507160c3
	R	TGA AGT CCG AAG TAA TCC TCC T	
PTK2	F	CCA GGA GAG AAT GAA GCA AA	
	R	CAA CAA ACT AAA GGG AGG GTA T	
MAPK1 (ERK2)	F	TCT GGA GCA GTA TTA CGA CCC	PrimerBank ID: 75709179c3
	R	CTG GCT GGA ATC TAG CAG TCT	
MAPK3 (ERK1)	F	CTA CAC GCA GTT GCA GTA CAT	PrimerBank ID: 91718898c1
	R	CAG CAG GAT CTG GAT CTC CC	
CCND1	F	CAA TGA CCC CGC ACG ATT TC	PrimerBank ID: 77628152c3
	R	CAT GGA GGG CGG ATT GGA A	
MKI67	F	GGT GCT TGA GGT CTG CTA	
	R	CTG CTT CTC CTT TCC CTT TC	
GAPDH	F	GTA TGA CAA CAG CCT CAA GAT	
	R	AGT CCT TCC ACG ATA CCA AA	

Supplementary Table 1. Primer sequences. Forward (F) and Reverse (R) primer sequence summarized. Target genes are *ITGB1, PTK2, MAPK1/3, CCND1, MKI67* and *GAPDH*.