***Supplementary Material***

Biophysical Control of Bile Duct Epithelial Morphogenesis in natural and synthetic scaffolds.

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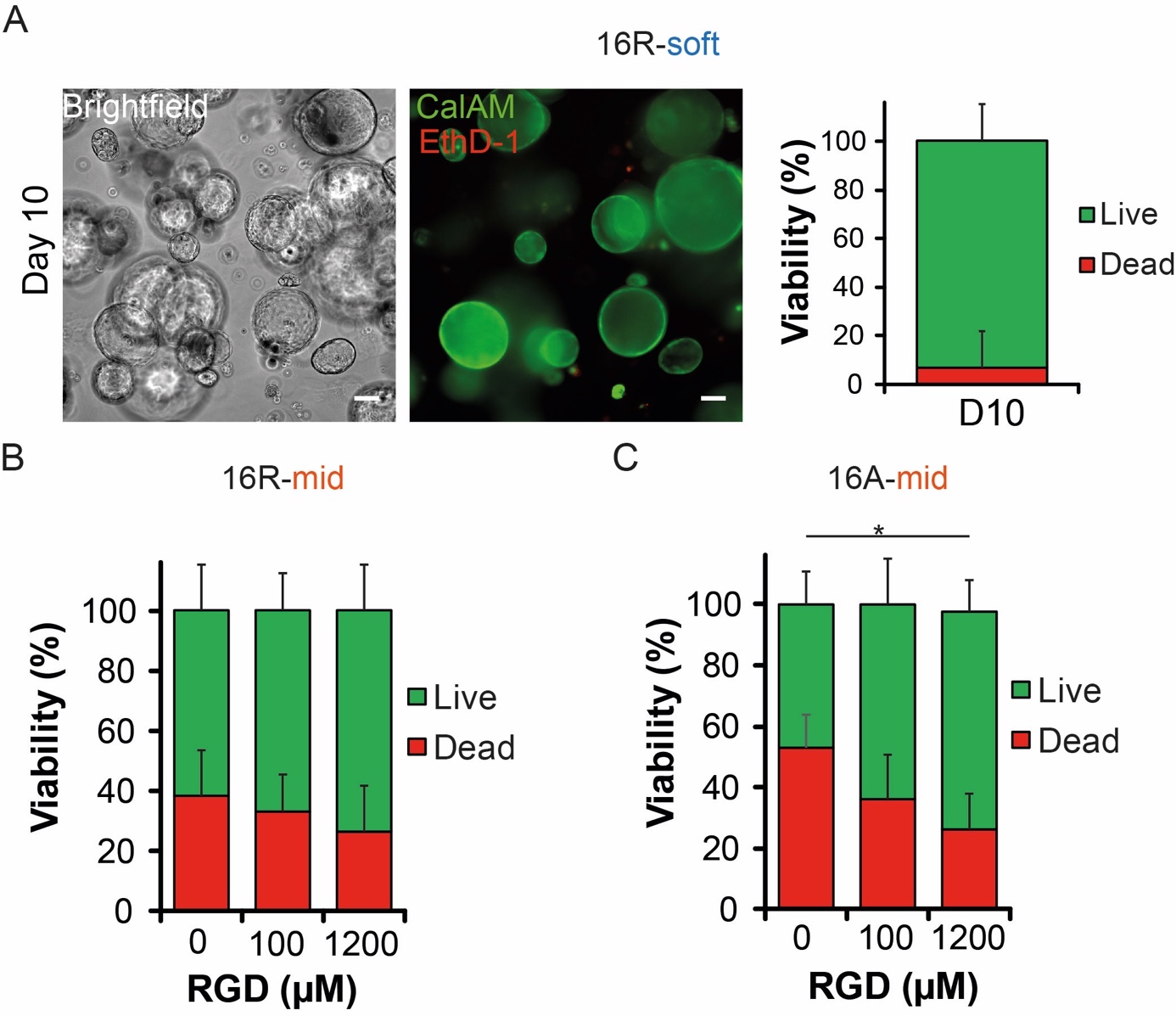
[pascale.dupuis-williams@u-psud.fr](mailto:pascale.dupuis-williams@u-psud.fr)

Dr. Samy Gobaa

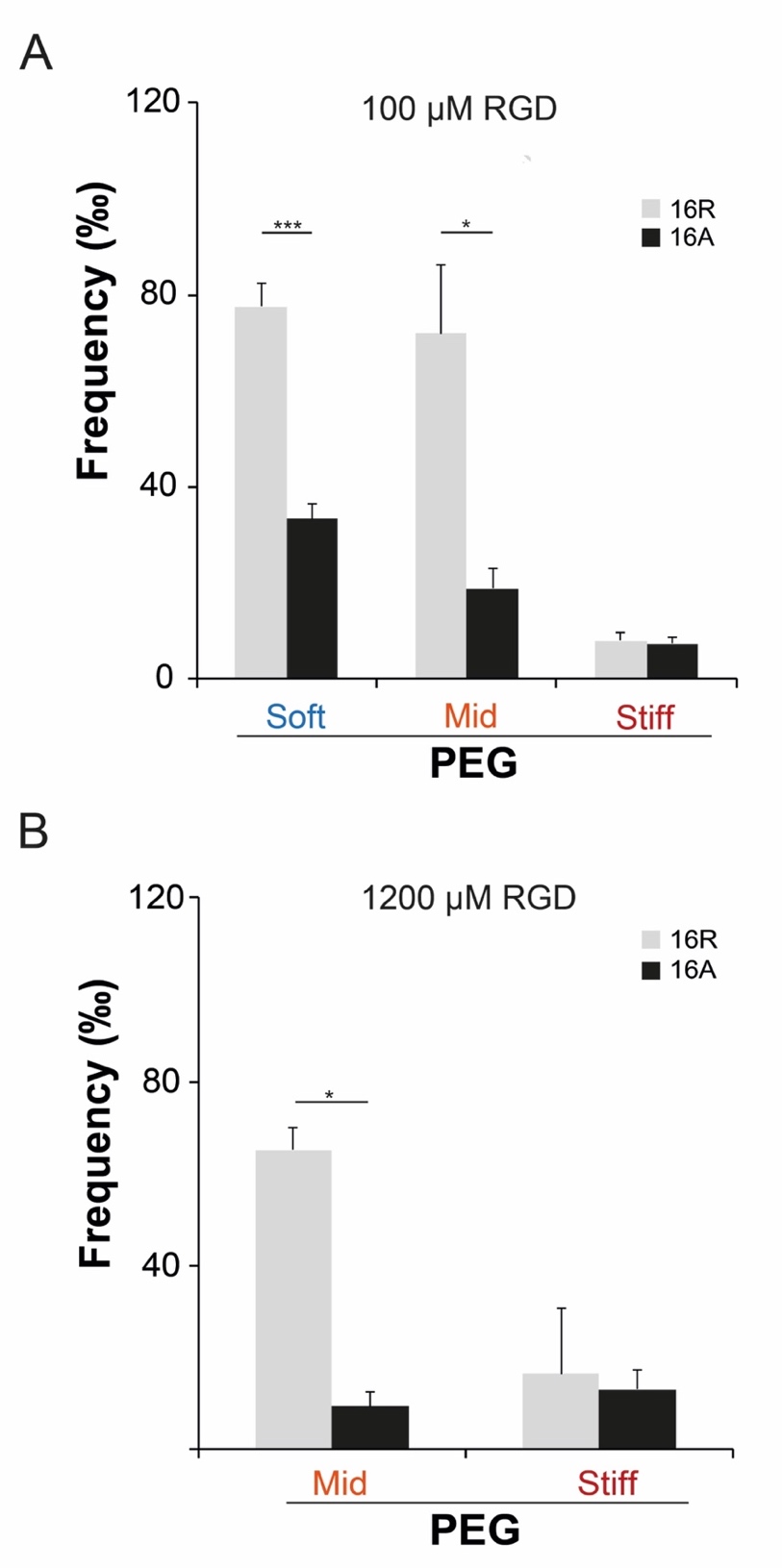
[samy.gobaa@pasteur.fr](mailto:samy.gobaa@pasteur.fr)

**Movie S1.** Three-dimensional nucleus location of NRC cells within different 10-day old cysts cultured in 4.5% PEG-VS hydrogels supplemented with 1200µM RGD. Scale bar:100µm

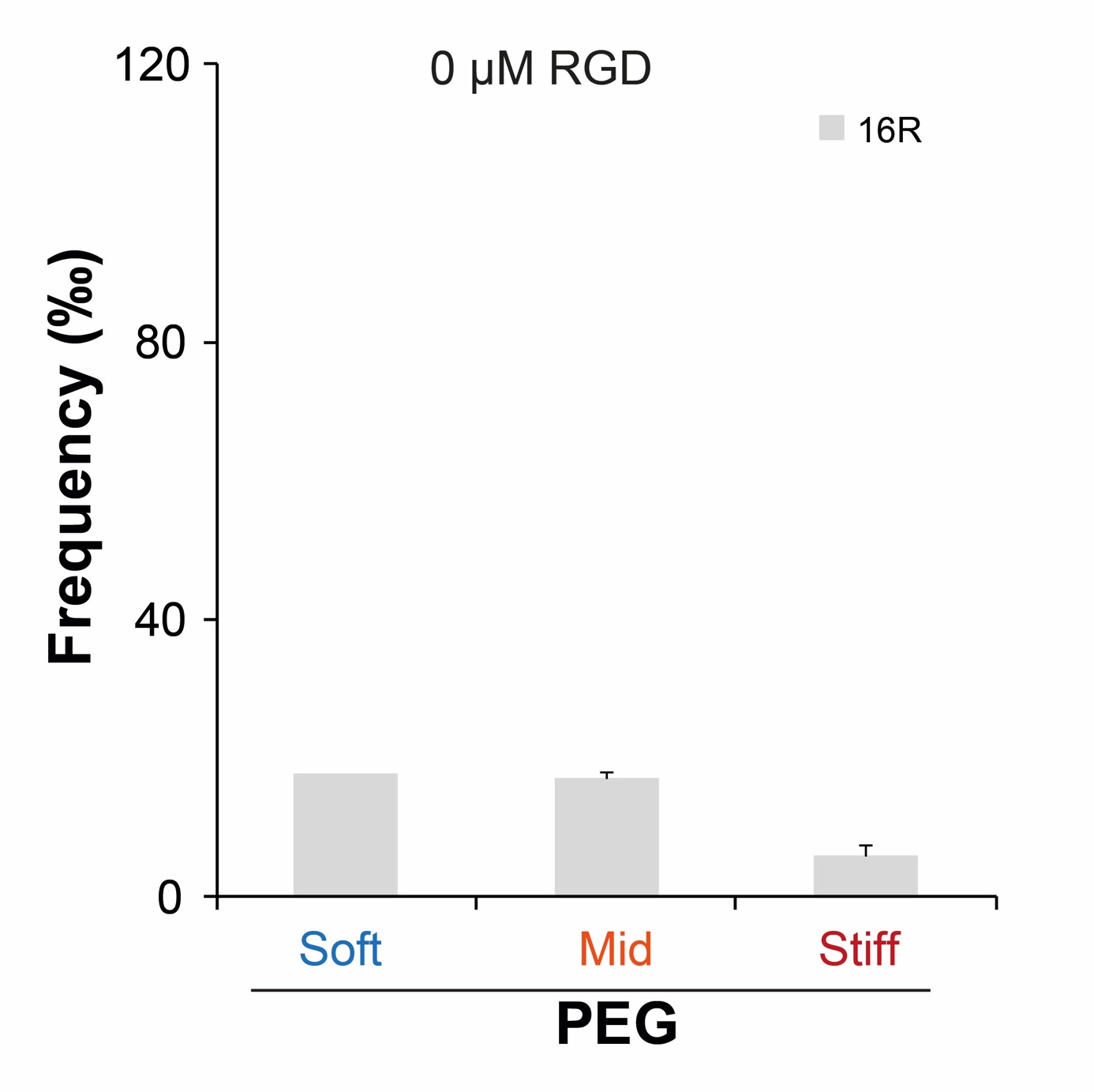
**Movie S2.** NRC cysts growth filmed over 48h in a PEG-VS hydrogel with intermediate stiffness containing 1200µM RGD.



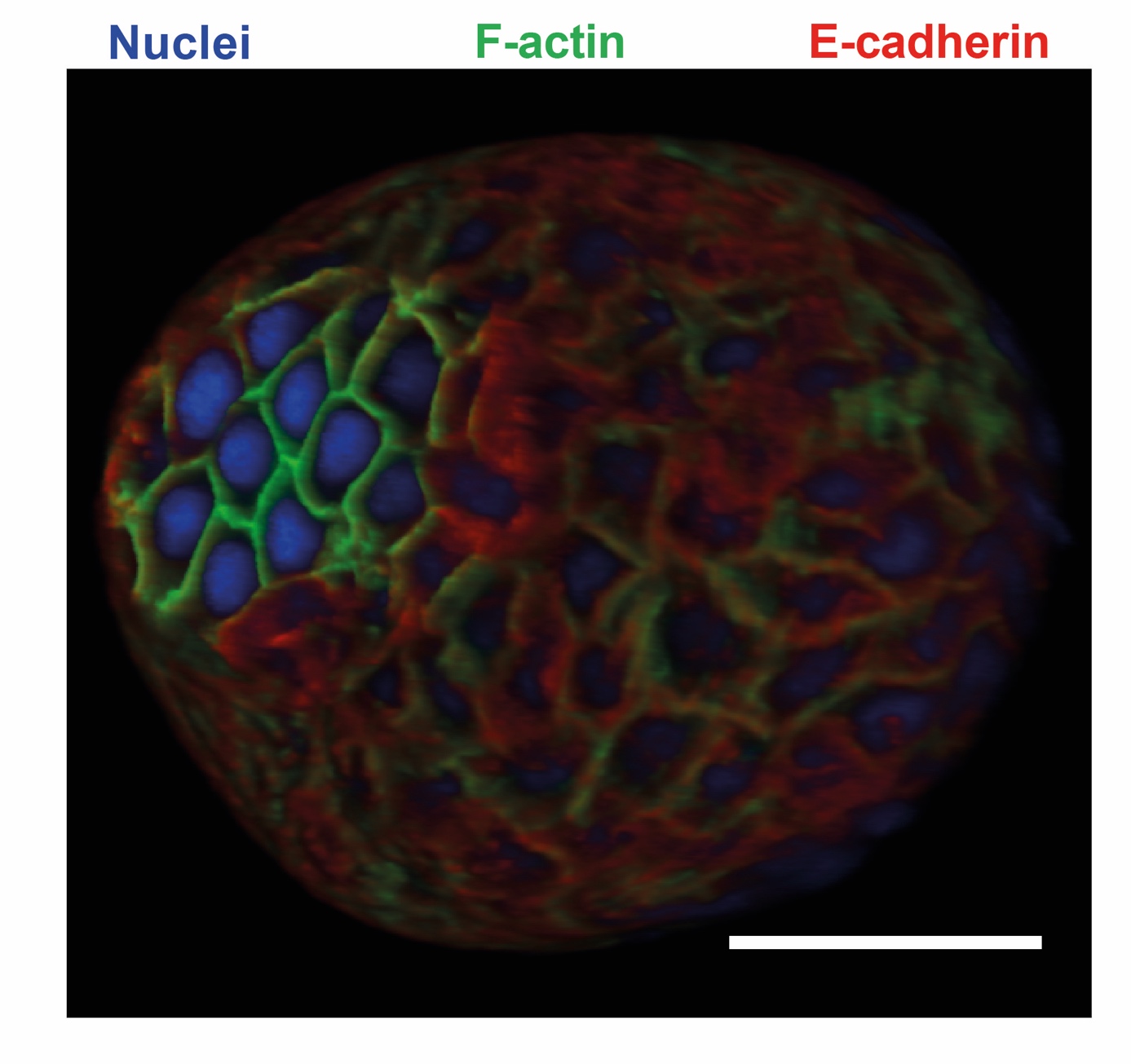
**Figure S3.** NRC cyst viability at Day 10 for PEG-VS hydrogels crosslinked with a fast (16R) and slow (16A) MMP-degradable peptide sequence.A) Representative brightfield and z-stack confocal images of 10-d-old NRC cysts cultured in a soft PEG-VS hydrogel crosslinked with a fast degradable crosslinker sequence and functionalized with 1200µM RGD (left) and percentage of cell viability at day 10 assessed by CalAM (Live) and EthD-1 (Dead) staining (right). B) Percentage of cell viability for different RGD ligand concentration assessed by CalAM (Live) and EthD-1 (Dead) staining of 10-d-old NRC cysts cultured in 4.5% PEG-VS hydrogel crosslinked with a fast MMP-degradable peptide. C) Percentage of cell viability for different RGD ligand concentration assessed by CalAM (Live) and EthD-1 (Dead) staining of 10-day old NRC cysts cultured in 4.5% PEG-VS hydrogel crosslinked with a slow MMP-degradable peptide. \* significant at p<0.05. Error bars represent SD. Scale bar: 100µm.



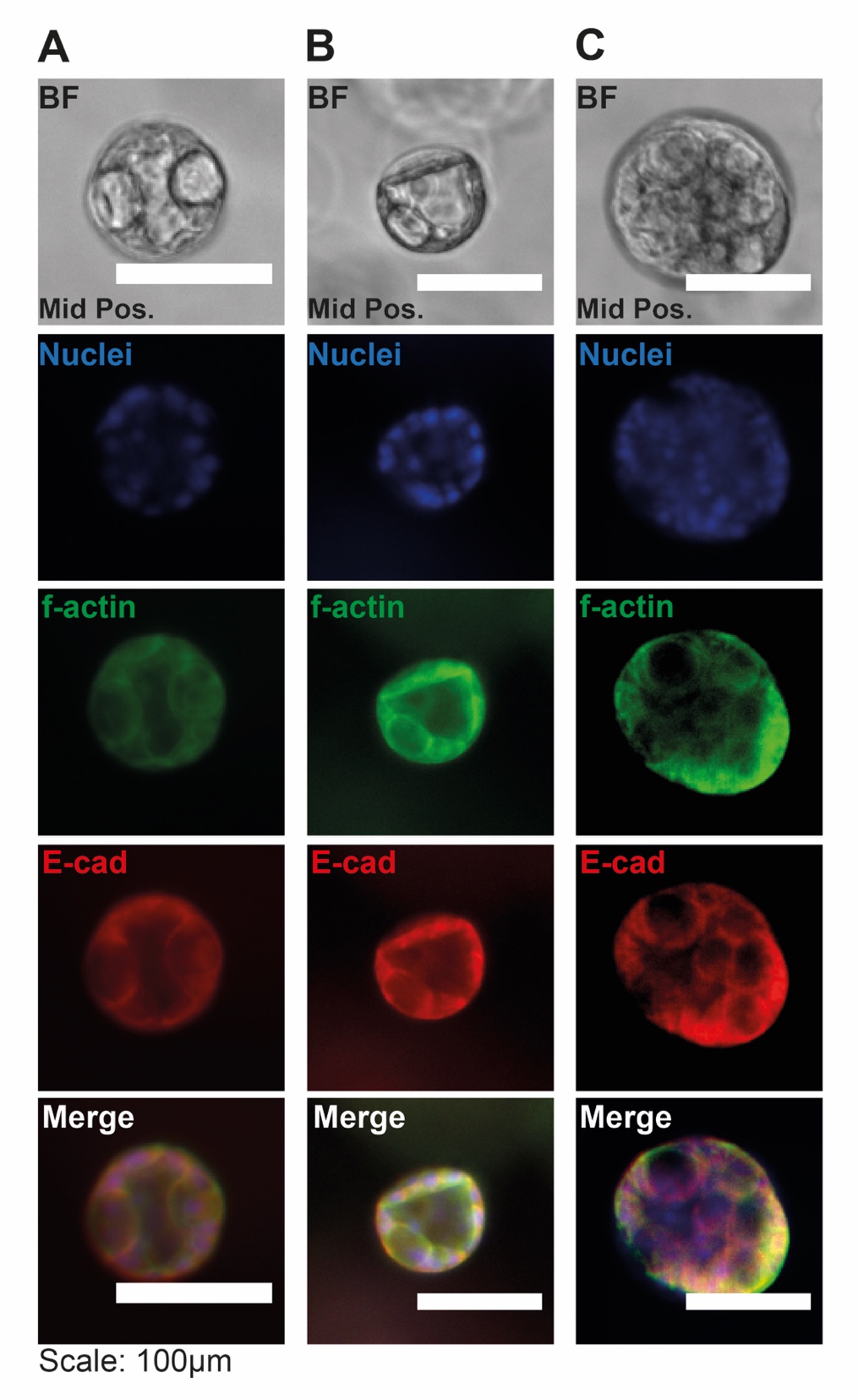
**Figure S4.** NRC cyst formation. (A) Comparison of cyst formation efficiency between fast and slow MMP-degradable hydrogels with soft, intermediate and stiff matrix when containing 100µM RGD. (B) Comparison of cyst formation efficiency between fast (16R) and slow MMP-degradable (16A) hydrogels with intermediate and stiff matrix when containing 1200µM RGD. \*, \*\*, \*\*\* significant at p<0.05, p<0.01 and p<0.001 respectively. Error bars represent SEM.



**Figure S5.** NRC cyst formation.Number of cysts formed in soft, intermediate and stiff hydrogels in absence of the RGD ligand. Error bars represent SEM.



**Figure S6.** NRC cyst cultured in PEG-VS hydrogels express apical-basolateral polarization. 3D immunostaining image of a cysts marked for basolateral marker E-cadherin and apical marker f-actin. Scale bar: 100µm.

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**Figure S7.** Normal Rat Cholangiocyte (NRC) cysts can retain a multi-lumen phenotype over 18 days of culture in 4.5% PEG-VS hydrogels containing 1200 µM RGD. (A) Three-lumen cyst, (B) two-lumen cyst and (C) 8+ lumen cyst. BF: Brightfield. E-cad: E-cadherin. Scale bar: 100µm.