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Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering

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Supplementary info

Part 1. Nucleotide sequences produced during cloning:

atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATCACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCGTTCGGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGCTCAACGGCACCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtacccccAGCTT

CBDΔ(FTN) in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATCACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCGTTCGGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGCTCAACGGCACCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtaccGGTACAGGTGGTT
CTGGTG
GTGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACG
GCCACAAG
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GGCAAGCT
GCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCA
CATGAAGC
AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGG
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GAACACC
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CCCAACGA
GAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
AAGTCCG
GACTCAGATCTCGAGCTCAgGCTTCGAATTCTGCAGTCGACGGcACCGCGGGCccAggaTCtACCGGATCTAGAggt
acc
cccAGCTT

CBD(G)-ECFP in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWTYTAGQRIQQLWNGTASTNGGQVS
VTSLPW
NGSIPTGGTASFGFNGSWAGSNPTPASFSLNGTTCTGTVPTTSPTGT**GTGGSGG**VSKGEELFTGVVPILVELDGDVN
GHK
FSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD
GNY
KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYISHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNT
PIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKSGLRSRAQASNSAVDGTAGPGSTGS
RGT
PS

Primary structure of **CBD(G)-ECFP protein**. 402 aa, 43,1 kDa, pI 6.6. Gly-rich linker is highlighted in yellow.

atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATCACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
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CCGGCGTC
GTTCTCGCTCAACGGCACCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtaccGGTACAGGTGGTT
CTGGTG
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CATGGTC
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CGTCTA
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CACCCAG
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TCACTCT
CGGCATGGACGAGCTGTACAAGGGCGGcACCGGAGGGAGCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGG
GGTGGTGC
CCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGTGAGGGCGATG
CCACCTAC
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TGACCTA
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GGCTACA
TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGA
CACCCTG
GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA
ACACGCG
TGACCAACTGACTGAAGAGCAGATCGCAGAATTTAAAGAGGCTTTCTCCCTATTTGACAAGGACGGGGATGGGA
CAATAA
CAACCAAGGAGCTGGGGACGGTGATGCGGTCTCTGGGGCAGAACCCCACAGAAGCAGAGCTGCAGGACATGA
TCAATGAA
GTAGATGCCGACGGTAATGGCACAATCGACTTCCCTGAGTTCCTGACAATGATGGCAAGAAAAATGAAAGACAC
AGACAG
TGAAGAAGAAATTAGAGAAGCGTTCCGTGTGTTTGATAAGGATGGCAATGGCTACATCAGTGCAGCAGAGCTTC
GCCACG
TGATGACAAACCTTGGAGAGAAGTTAACAGATGAAGAGGTTGATGAAATGATCAGGGAAGCAGACATCGATGGG
GATGGT
CAGGTAAACTACGAAGAGTTTGTACAAATGATGACAGCGAAGggtacccccAGCTT

CBD(G)-GCaMP2 in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWTYTAGQRIQQLWNGTASTNGGQVS
VTSLPW
NGSIPTGGTASFGFNGSWAGSNPTPASFSLNGTTCTGTVPTTSPTGTGTGGSGGMASMTGGQQMGRDLYDDDDK
DLATMV
DSSRRKWNKTGHAVRAIGRLSSLENVYIMADKQKNGIKANFKIRHNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLS
TQ
SKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGTGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGD
ATY
GKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGD
TL
VNRIELKGIDFKEDGNILGHKLEYNTRDQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMIN
E
VDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDG
DG

QVNYEEFVQMMTAKGTPS

Primary structure of **CBD(G)-GCaMP2** protein. 578 aa, 63,2 kDa, pI 4.9. Gly-rich linker is highlighted in yellow.

Table S1. Primer pairs used for cloning of ECFP and GCaMP2.

ECFP (no linker peptide)	Forward: 5'-GATCGGTACCGTGAGCAAGGGCGAGGA Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT
GCaMP2 (no linker peptide)	Forward: 5'-GATCGGTACCATGGCTAGCATGACTGGTGGAC Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTTGTA
ECFP (with linker peptide GTGGSGG)	Forward: 5'-GATCGGTACCGGTACAGGTGGTTCTGGTGGTGTGAGCAAGGGCGAGGA Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT
GCaMP2 (with linker peptide GTGGSGG)	Forward: 5'-AGTCGGTACCGGTACAGGTGGTTCTGGTGGTATGGCTAGCATGACTGGT Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTTGTA

Part 2. Supplementary figures

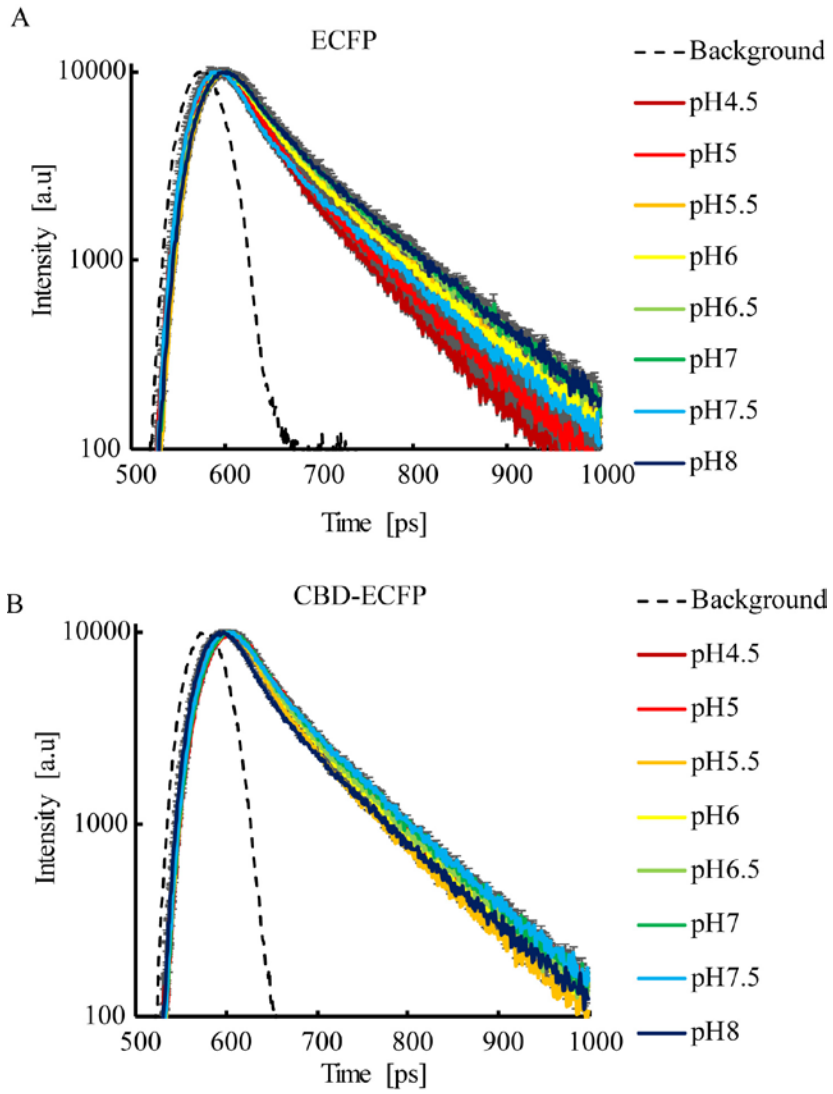


Figure S1. Fluorescence lifetime decays for ECFP and CBD-ECFP proteins measured in solution at different pH. A: Observed decay times for ECFP were 1.35 ns (pH 4.5) and 2 ns (pH 8). B: Observed decay times for CBD-ECFP were 1.89 ns (pH 4.5) and 2.18 ns (pH 8). Standard deviations are shown in gray. Prompt (background) fluorescence signals are also shown. N=3.

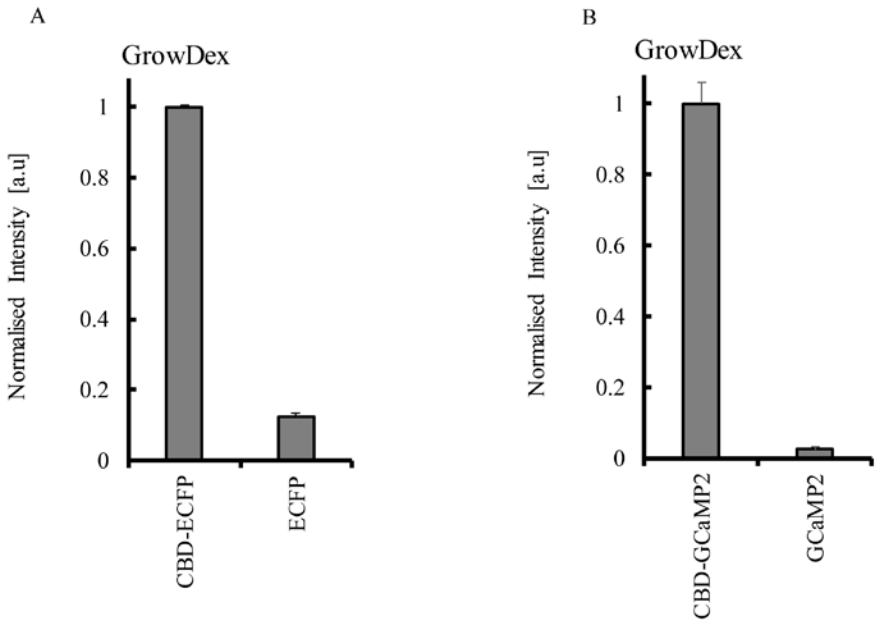


Figure S2. Comparison of specific / non-specific staining of GrowDex with CBD-ECFP (A) and CBD-GCaMP2 proteins (B) compared to untagged ECFP and GCaMP2 proteins. Matrices were incubated with 10 µM of respective proteins for 15 min in PBS, washed and measured on fluorescence microscope (470 nm exc., 510-560 nm em.). N=3.

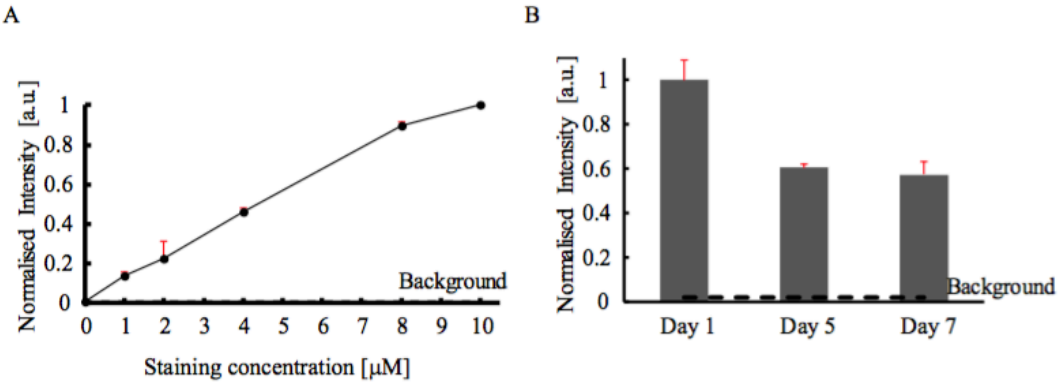


Figure S3. A: Concentration-dependent staining of GrowDex with CBD-GCaMP2 (incubation time 15 min, in PBS). B: Stability of stained (5 µM, 15 min) GrowDex scaffold. Matrices were measured on a fluorescence microscope (470 nm exc., 510-560 nm em.), using the same acquisition settings for different experimental points. N=3.

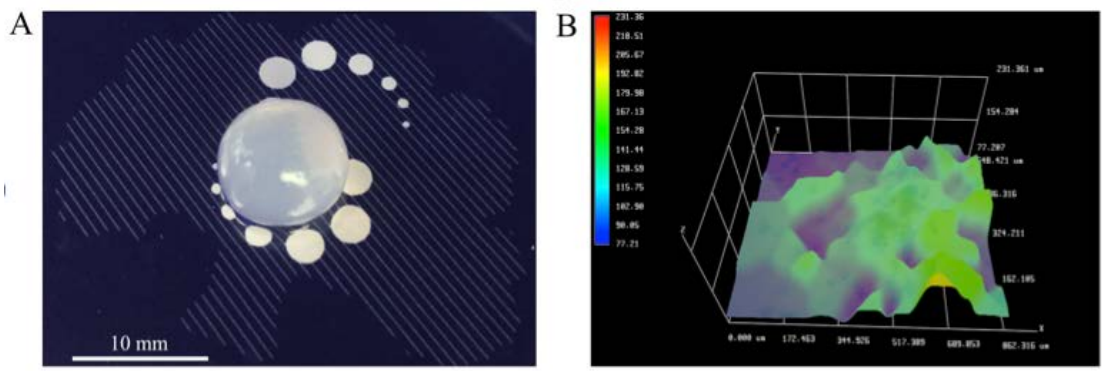


Figure S4. Characterization of bacterial cellulose. A: Photograph of cellulose sphere produced by *Gluconacetobacter hansenii* GH-1/2008 (VKPM B-10547). B: 3D microscopy and nanoindentation reveals homogenous surface of the bacterial cellulose. Young's modulus is 1.93±0.76 kPa.

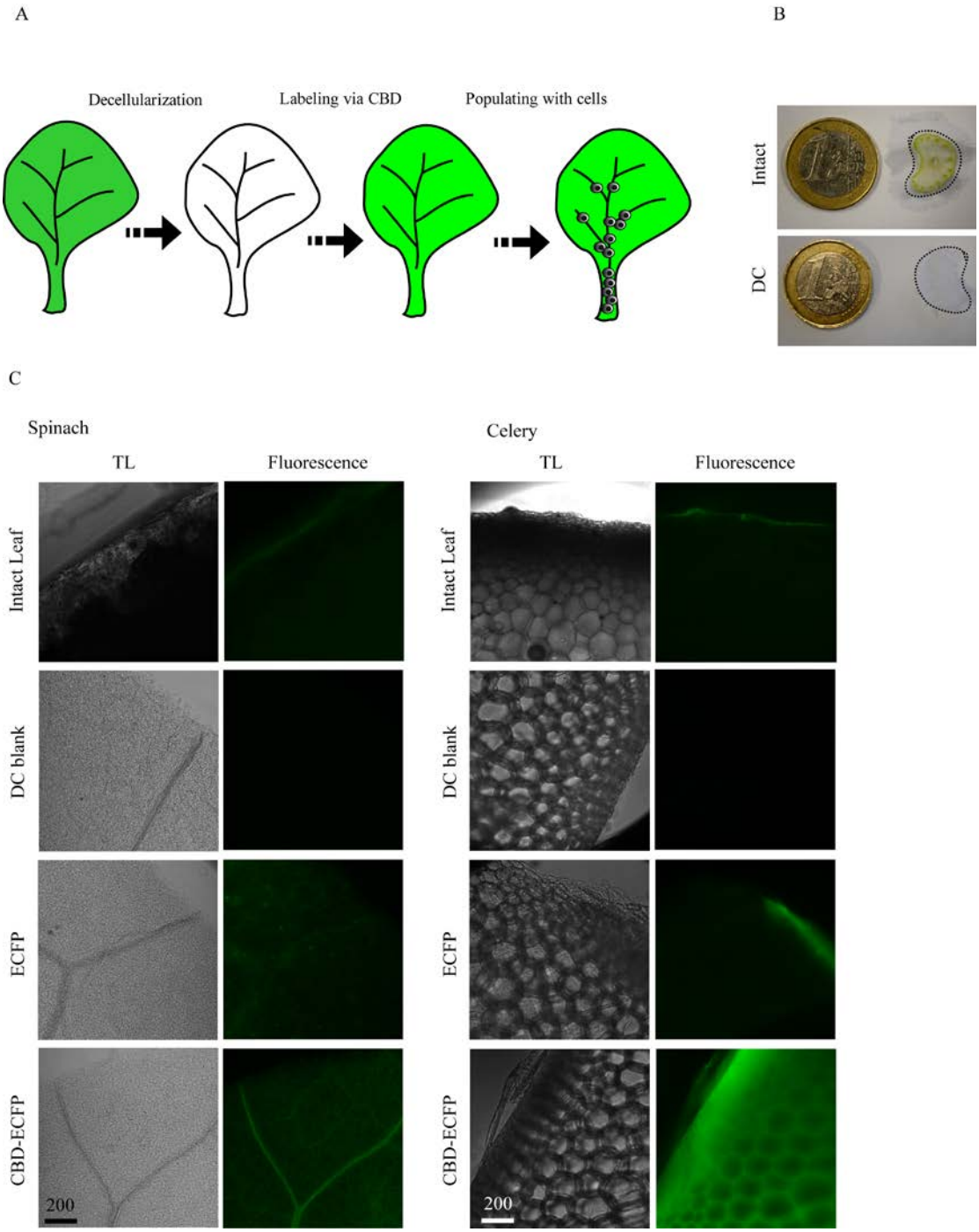


Figure S5. Overview of plant decellularization (DC) procedure and studied examples. A: cartoon representation of the use of DC plant for cell-based application with CBD-tagged biosensors. B: Photographs of celery stem section before and after decellularization. 1 euro coin is shown for scale. C: Transmission light and widefield fluorescence (470 nm exc., 510-560 nm em.) microscopy images for spinach leaf and celery stem before (intact), after DC and following labeling either with ECFP (non-specific binding) or CBD-ECFP. The same acquisition settings were used for fluorescence images. Scale bar is in μm.

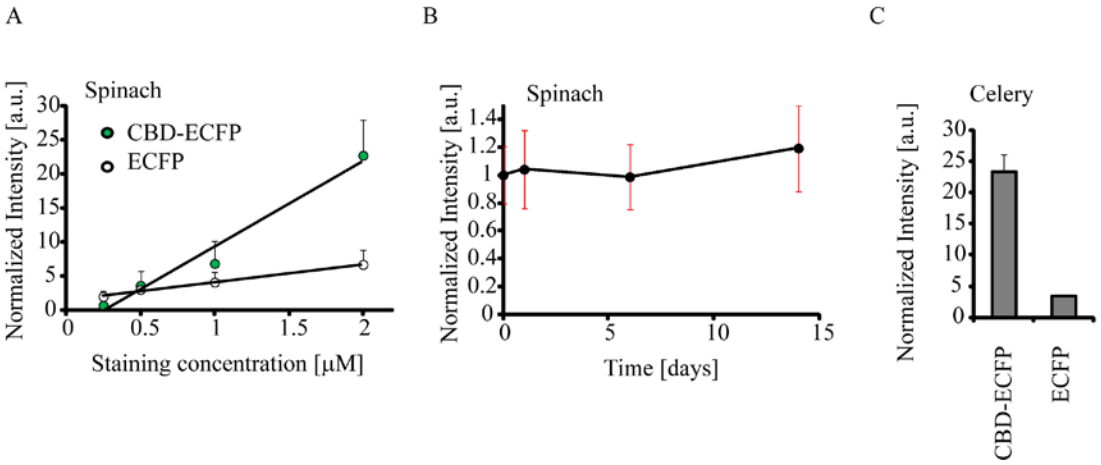


Figure S6. Staining and stability of decellularized (DC) celery and spinach. DC samples were stained with various concentrations of CBD-ECFP or ECFP and measured on a fluorescence microscope (470 nm exc., 510-560 nm em.). A: Concentration-dependence of staining of DC spinach (16 h incubation in PBS). B: Storage stability of stained DC spinach (5 μM, 16 h) over 2 weeks time. C: Staining of DC celery with CBD-ECFP (5 μM, 16 h). N=3.

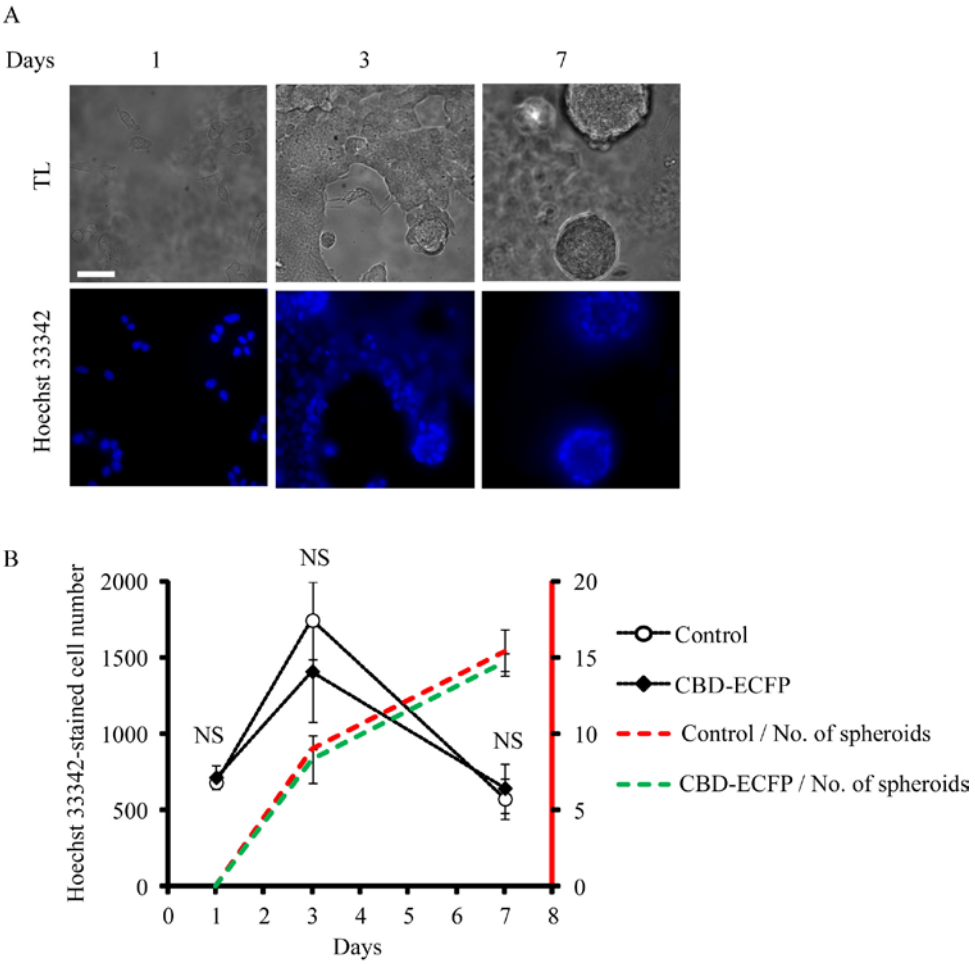


Figure S7. Cell growth in unstained and CBD-ECFP-stained Growdex. HCT116 cells were seeded in stained (5 μM CBD-ECFP, 15 min) or unstained (control) Growdex matrices, grown for 0-7 days and quantified by using fluorescence microscopy with the help of Hoechst 33342 (1 μM, 30 min) staining. A: Fluorescence microscopy images showing cell growth (CBD-ECFP-stained sample). TL-transmission light. Scale bar is 50 μm. B: Calculated cell

numbers (per region of interest) for different time points of culture in Growdex. No significant differences (indicated as 'NS') between CBD-ECFP-stained and unstained samples were found for mean values (*t*-test, p=0.05). Cells grown in Growdex cells also formed spheroids over time (shown as green and red dashed lines, red Y axis). N=3.

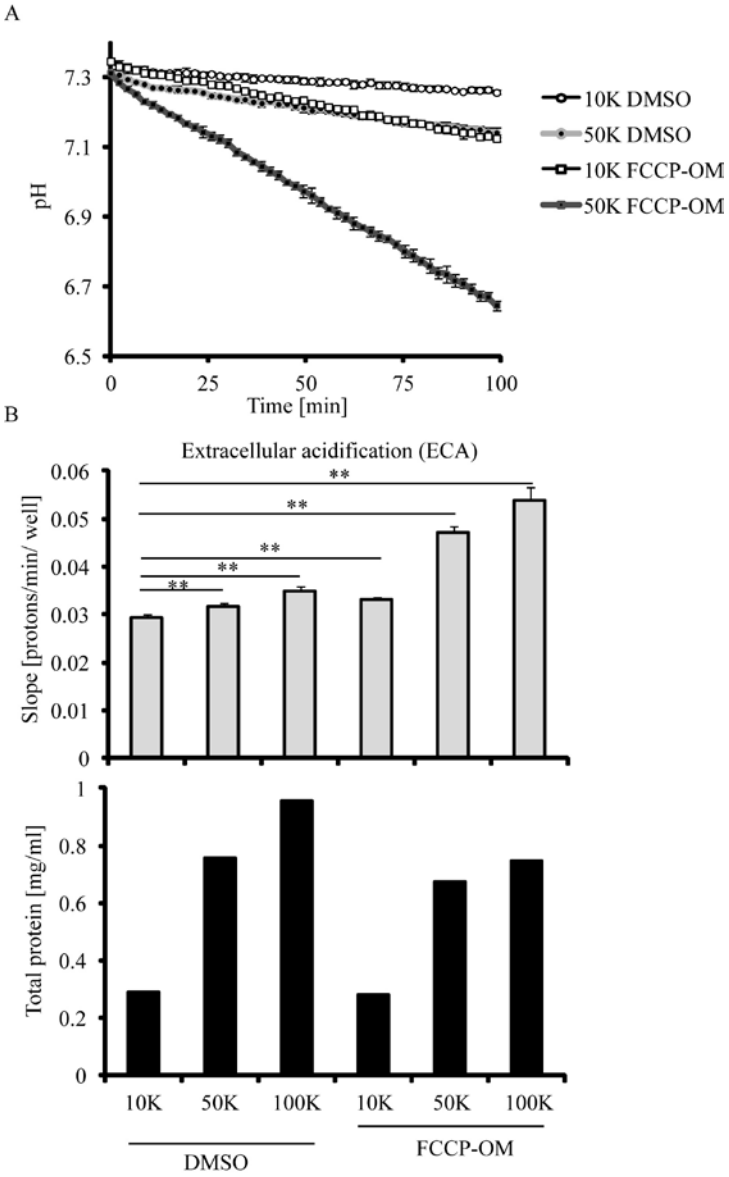


Figure S8. Dependence of extracellular acidification rates from cell number, measured on a microplate reader by ECA method. HCT116 cells seeded and grown at different densities (10,000, 50,000 and 100,000 designated as 10K, 50K and 100K, respectively) were measured by ECA method (unsealed system, glycolytic flux) in the presence of 1 μM FCCP and 10 μM Oligomycin (FCCP-OM, maximal acidification) and DMSO (mock). A: time-dependence of extracellular acidification. B: extracellular acidification rates (top) and average total protein concentrations (bottom). N=8. Asterisks indicate significant difference (*t*-test, p=0.001)