Electronic Supplementary Information

to the paper

"Chondrogenic potential of macroporous biodegradable cryogels based on synthetic poly(α-amino acids)"

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1. Yield, swelling and porosity of the gels

Hydrogels were swollen in deionized water for two weeks. The water was changed frequently to wash out all the sol residues and to reach equilibrium. Then, the samples were cut into several

pieces to determine the gel yields and the water content, and to study the rheological properties. The swelling temperature was 25 °C.

The gel yield (*GY%*) of the cryogels was determined gravimetrically by weighing freeze-dried samples (m_d):

$$GY_{\%} = 100 \cdot \frac{m_d}{m_0},$$

where m_0 is the weight of the polymerizable components in the initial solution (of PHEG-MA and comonomers).

The equilibrium swelling regain of the cryogels (*W*) was determined gravimetrically by weighing the samples in their equilibrium-swollen state in water (m_{sw}) and after drying to a constant weight:

$$W = \frac{m_{sw} - m_d}{m_d} = \frac{m_{sw}}{m_d} - 1.$$

The porosity ($P_{\%}$) of the cryogels represents the volume fraction (in %) of water-filled pores in the hydrogels. It has been shown that the volume pore fraction is practically the same for a swollen sample as for a freeze-dried sample[8]. The porosity was therefore determined by a method based on cyclohexane uptake ($P_{\%CH}$) by freeze-dried samples, and was calculated according to the following equation:

$$P_{\%CH} = 100 \cdot \frac{\left(m_{swCH} - m_d\right)/\rho_{CH}}{m_{swCH}/\rho_{swCH}} = 100 \cdot \left(1 - \frac{m_d}{m_{swCH}}\right),$$

where m_{swCH} is the weight of the sample in cyclohexane, m_d is the weight of the freeze-dried sample, ρ_{CH} is the density of the cyclohexane and ρ_{swCH} is the density of the sample with cyclohexane ($\rho_{CH} \approx \rho_{swCH}$ when $P_{\%CH} > 90\%$).

2. The CT measurements

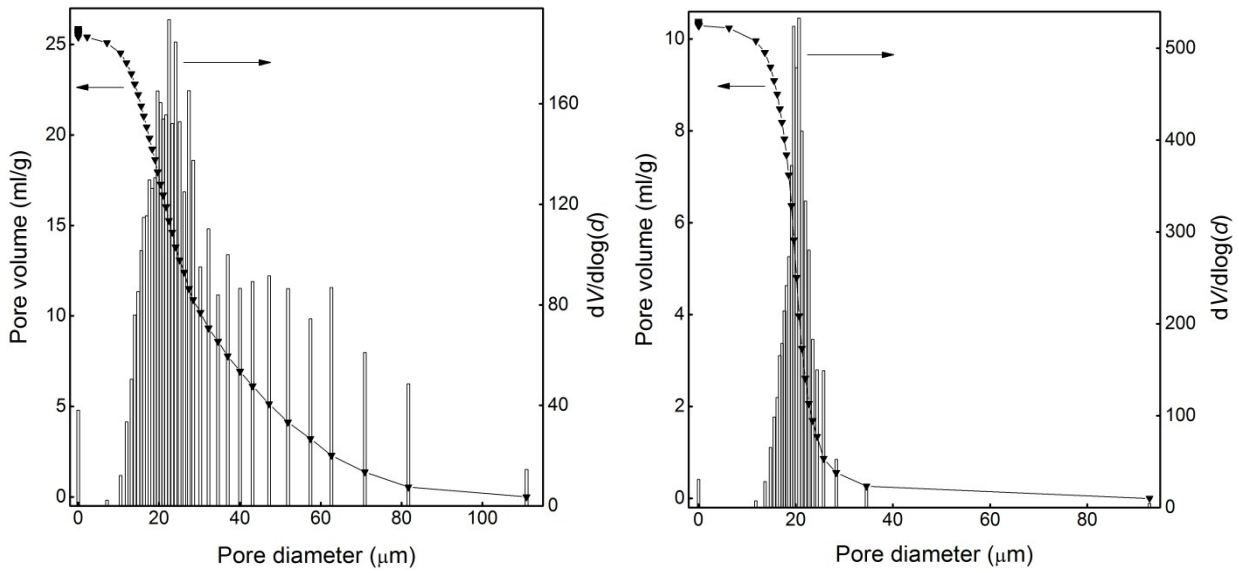
The CT measurements were performed on the RIGAKU Nano3DX system, equipped with a 3300×2500 pixel^2 X-ray CCD camera and a Cr rotatory target working at 35 kV acceleration voltage and 25 mA current. An optical camera with 20× magnification having field of view 0.9 × 0.7 mm^2 was used for the measurements. Both samples were measured under the same conditions. A binning value of 2 was applied to obtain sufficient signal intensity and voxel size of 0.54 µm. The scanning time was 2.5 hours with 10 s acquisition time in each rotatory position. The data were reconstructed using Nano3DX reconstruction software.

The data were analysed and were visualised in VGStudio MAX 3.1 software. A median filter with kernel size 5×5 was applied to the CT data to decrease the noise distortion. The pore analysis was performed using the Foam Structure Analysis module. The software uses the watershed algorithm to create a gradient parametric map, where the local extrema are found. These local extrema correspond to air pores in the original image.

The surface determination tool was used for calculating the volume and the surface area of the material. In addition, the wall thickness was analysed to show differences or similarities in structure thicknesses between the cryogel samples. The wall thickness analysis tool searches the opposite surface by sending a measurement line perpendicular to the current surface. The surface area of the opposite surface is taken into account for the end point of the measurement line, which is defined by a search cone with an angle of 30°. The results are shown on the iso-surface rendered 3D model by a color-coded map.

3. Analysis of porosity by mercury porosimetry

The internal pore structure of freeze-dried C4 and C10 scaffolds was characterized on Pascal 140 and 440 mercury porosimeters (Thermo Finigan, Rodano, Italy). The porosimeters operate in two pressure intervals, 0-400 kPa and 1-400 MPa, so the pore size can be determined in the range of 0.004-116 μm. The pore volume and the most frequent pore diameter were calculated in the Pascal program by means of the Washburn equation, using a cylindrical pore model[S1]. The volume of pores was evaluated as the difference between the end values on the volume/pressure curve. The porosity was obtained via the formula: $p = (V \times 100) / (V + 1/\rho)$ (%), where V is cumulative pore volume and ρ is scaffold density (0.8 $g.ml^{-1}$, pycnometrically).



(a) (b)

Figure S1. Cumulative pore volume (curve) and the derived pore size distribution (bars) of (a) the C4 scaffold and (b) the C10 scaffold, as measured by mercury porosimetry.

Table S1. Mercury porosimetry results.

Sample	Average pore diameter [μm]	Porosity [%]
C4	31.7	93.6
C10	20.2	88.3

References:

S1. Rigby S.P., Barwick D., Fletcher R.S., Riley S.N., Interpreting mercury porosimetry data for catalyst supports using semi-empirical alternatives to the Washburn equation, Appl. Catal. A238, 303-318 (2003).

4. Details of the radioassay (^{125}I)

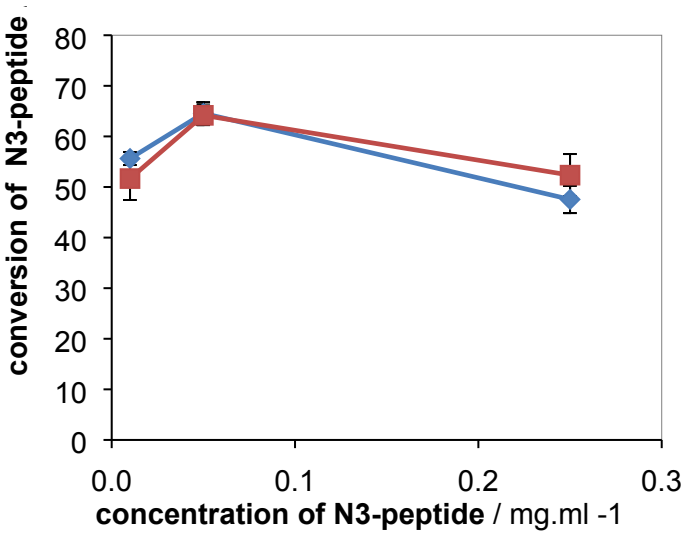


Figure S2. Dependence of azidized peptide conversion on its concentration in the reaction feed: blue – sample C10, red – sample C4

5. Isolation and characterization of hDPSCs cells

The dental pulps were cut into small pieces with the aid of a sterile scalpel in a growth medium containing DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/ mL streptomycin. After mincing, the small pieces of tissue were placed into six-well plates containing growth medium. The media were changed after outgrowth was observed. The well plates were incubated at 37ºC in 5% CO2. The cells that reached confluency were trypsinized for further usage. For cell characterization, the cells were incubated with FITC anti-human CD24, PE anti-human CD45, FITC anti-human CD44, PE anti-human CD90, and PE anti-human CD117 antibodies in darkness for 45 min. Cell surface markers were analyzed by a FACS Calibur Flow Cytometer (BD Biosciences, USA).

The DPSCs were positive (≥94%) for mesenchymal stem cell markers CD90 and CD44, and were negative (≤ 1%) for the hematopoietic markers CD45, CD24 and CD117, for details see Table S2.

Table S2. Expression of surface markers

	CD45+PE	CD24+FITC	CD90+PE	CD44+FITC	CD117+PE
% Positive	0,02	0,07	97,7	94,08	0,17

5. **MTS Assay**

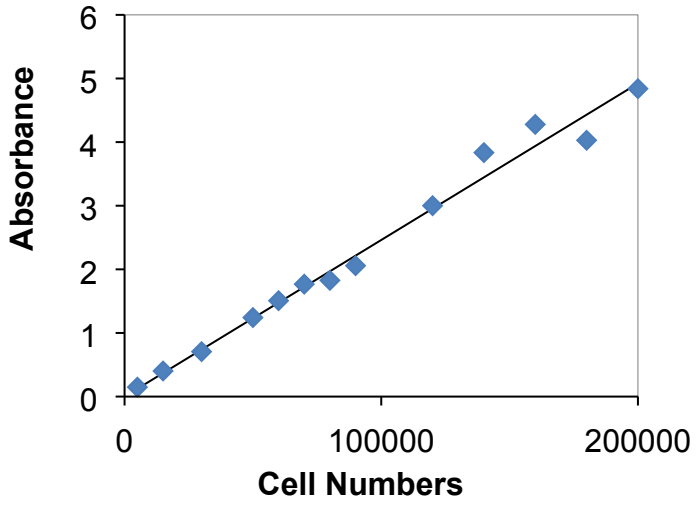


Figure S3. Calibration curve for MTS assay

6. LIVE/DEAD® assay

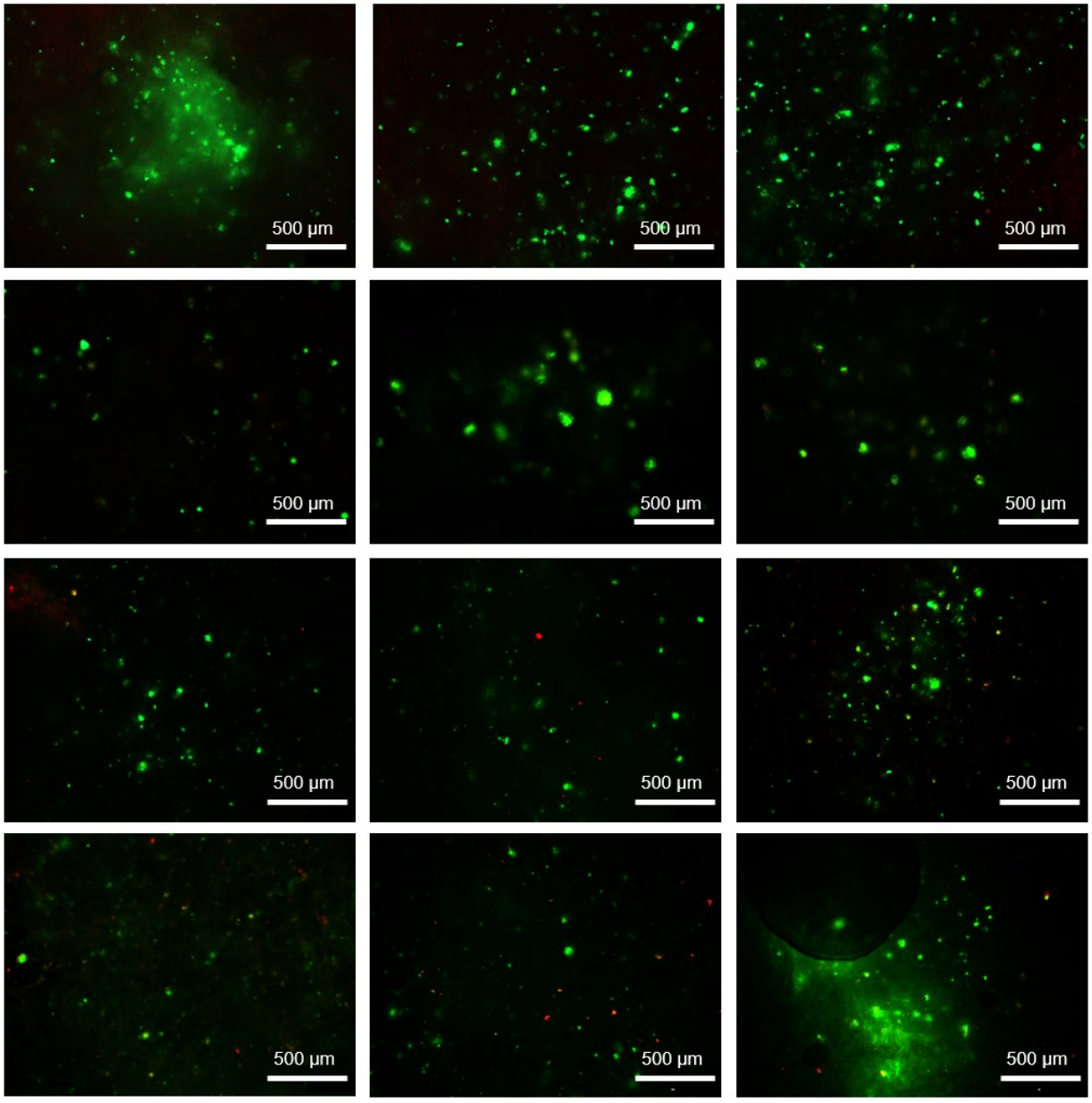


Figure S4. LIVE/DEAD® assay for hDPSCs on C4 cryogel samples; from left to right: C4-blank, C4-low RGDS and C4-high RGDS; top to bottom: day 1, day 10, day 20 and day 30.

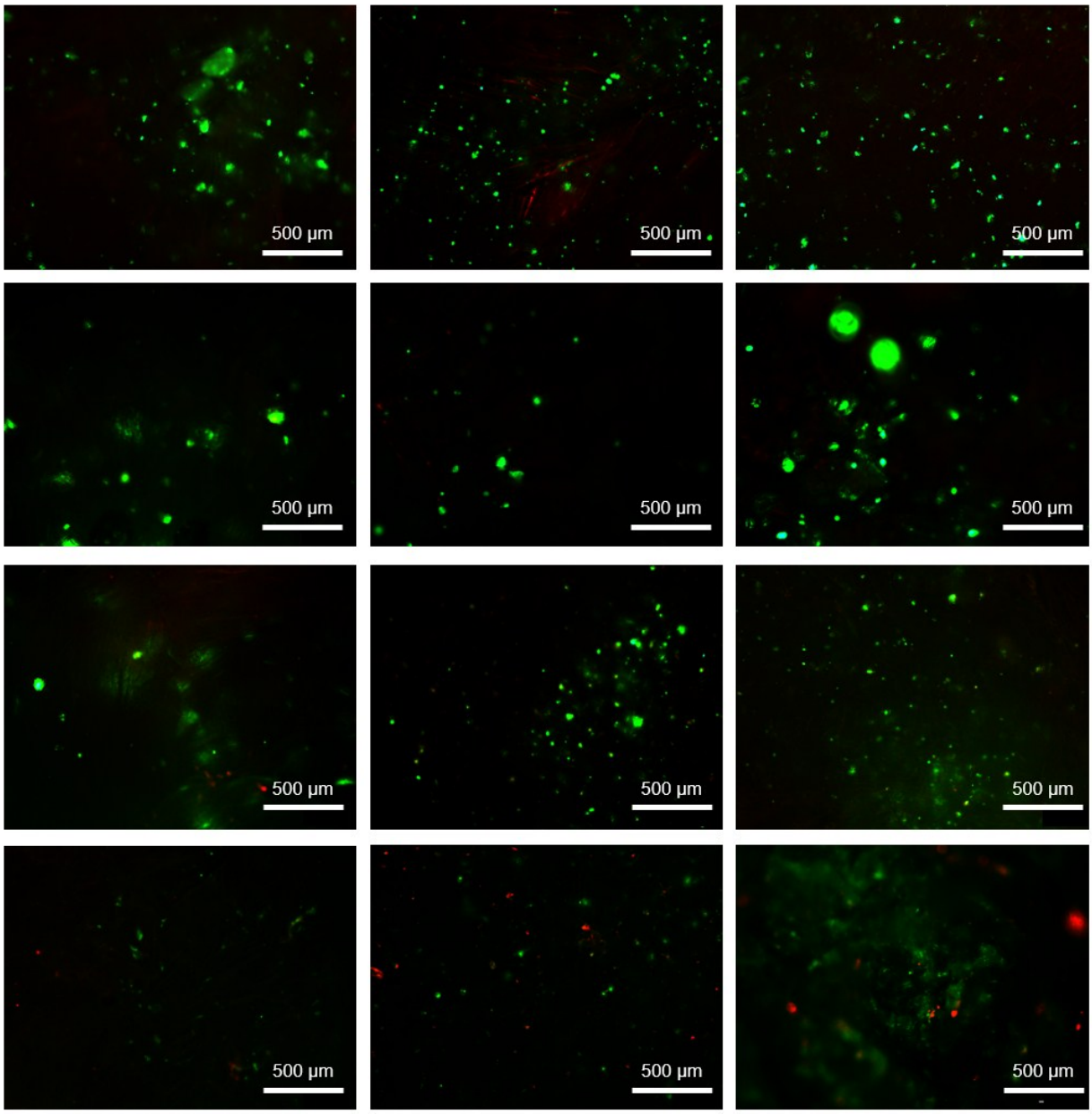
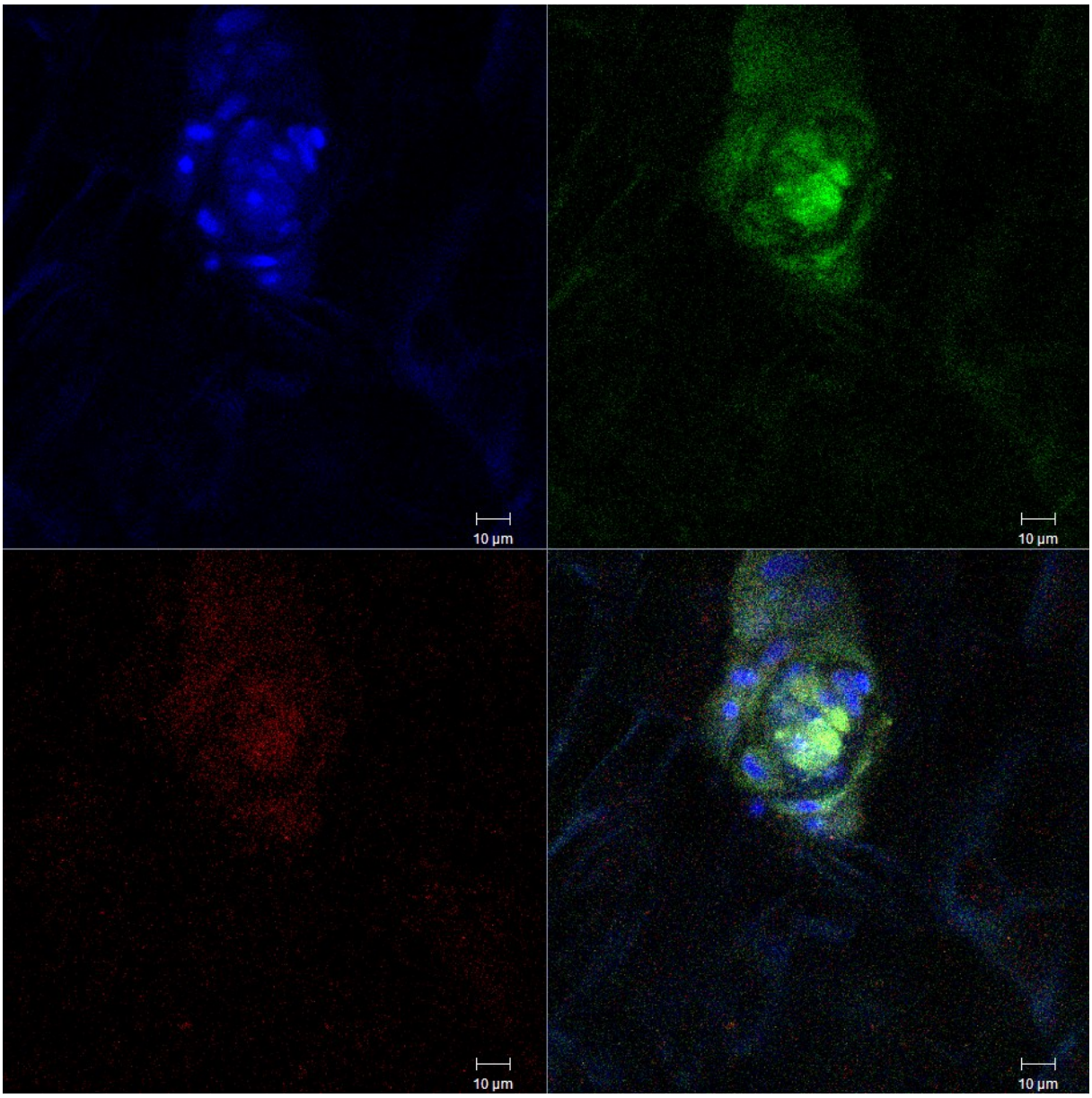


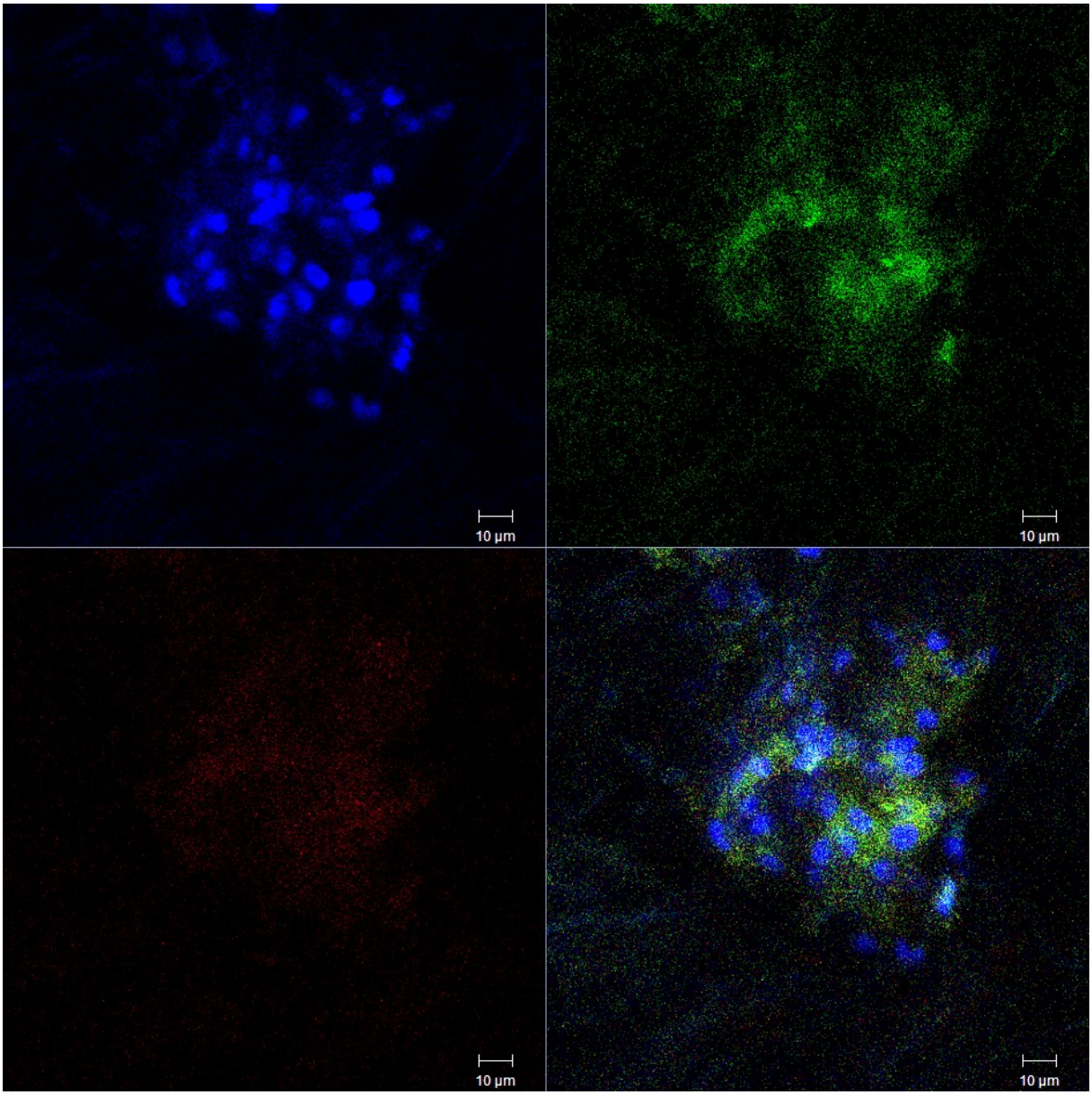
Figure S5. LIVE/DEAD® assay for hDPSCs on cryogel C10 samples; from left to right: C10-blank, C10-low RGDS and C10-high RGDS; top to bottom: day 1, day 10, day 20 and day 30.

7. Immunochemical staining

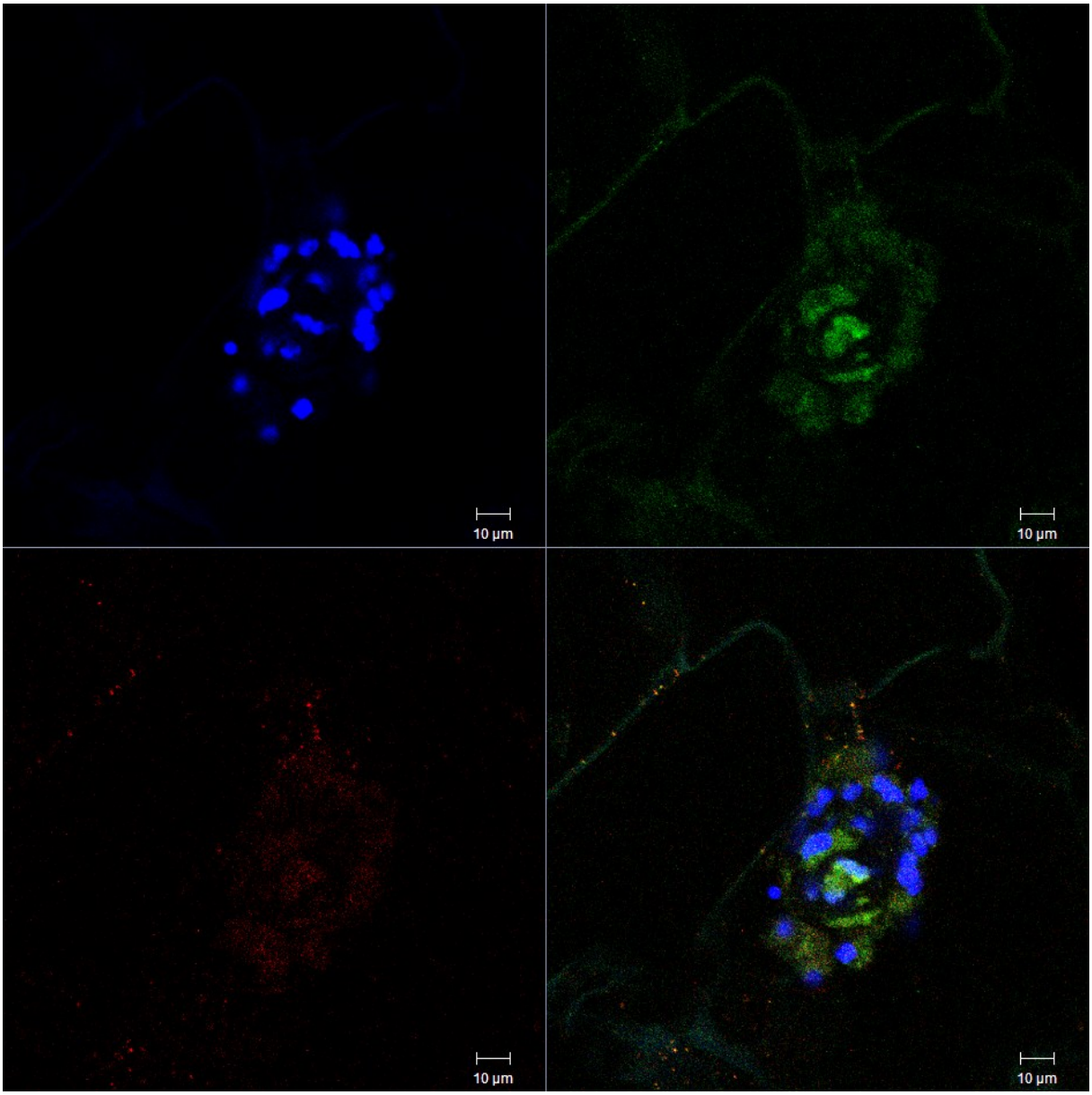
C4-blank, day 20, 40x



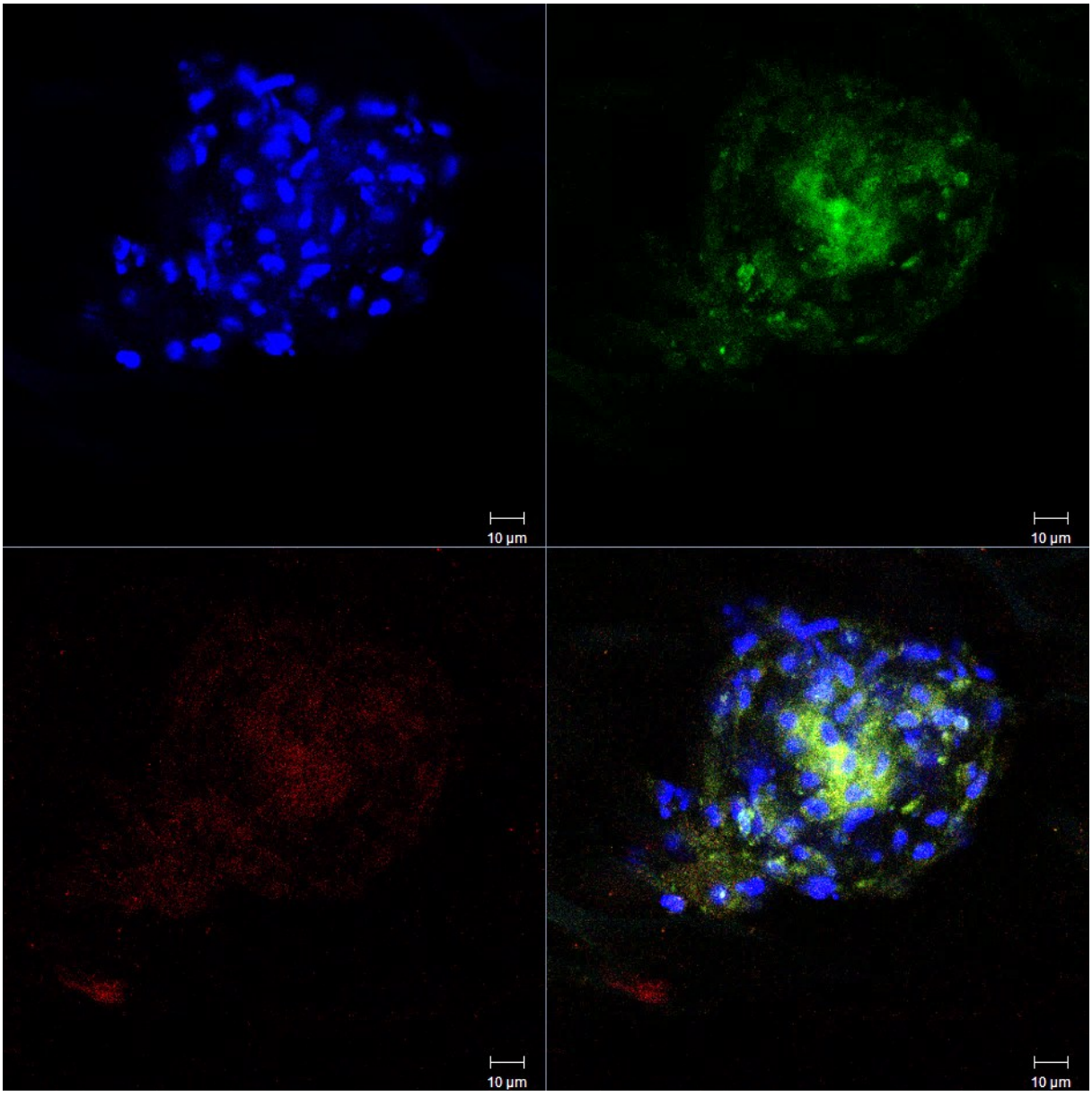
C4-low, day 20, 40x



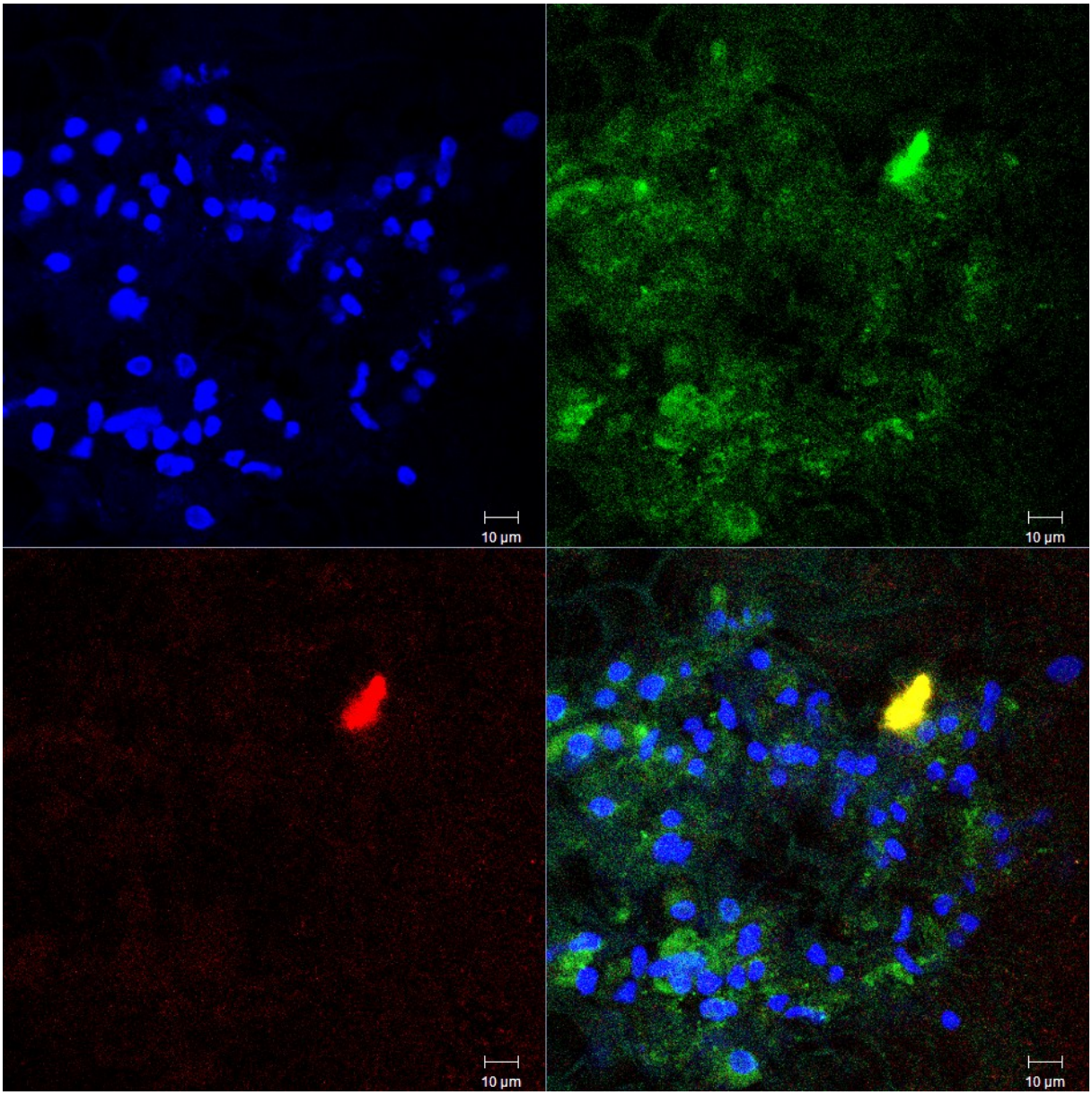
C4-high, day 20, 40x



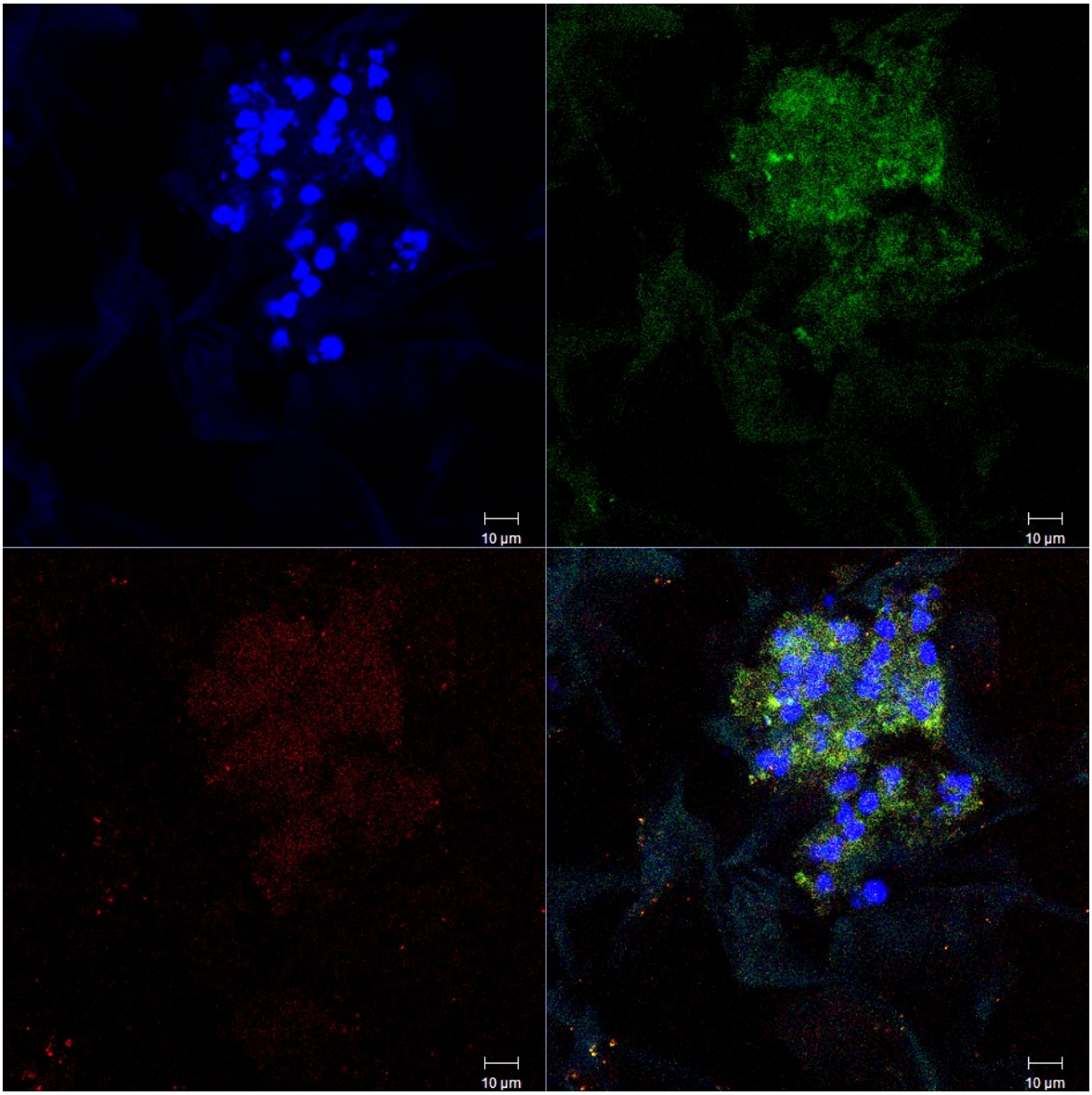
C10-blank, day 20, 40x



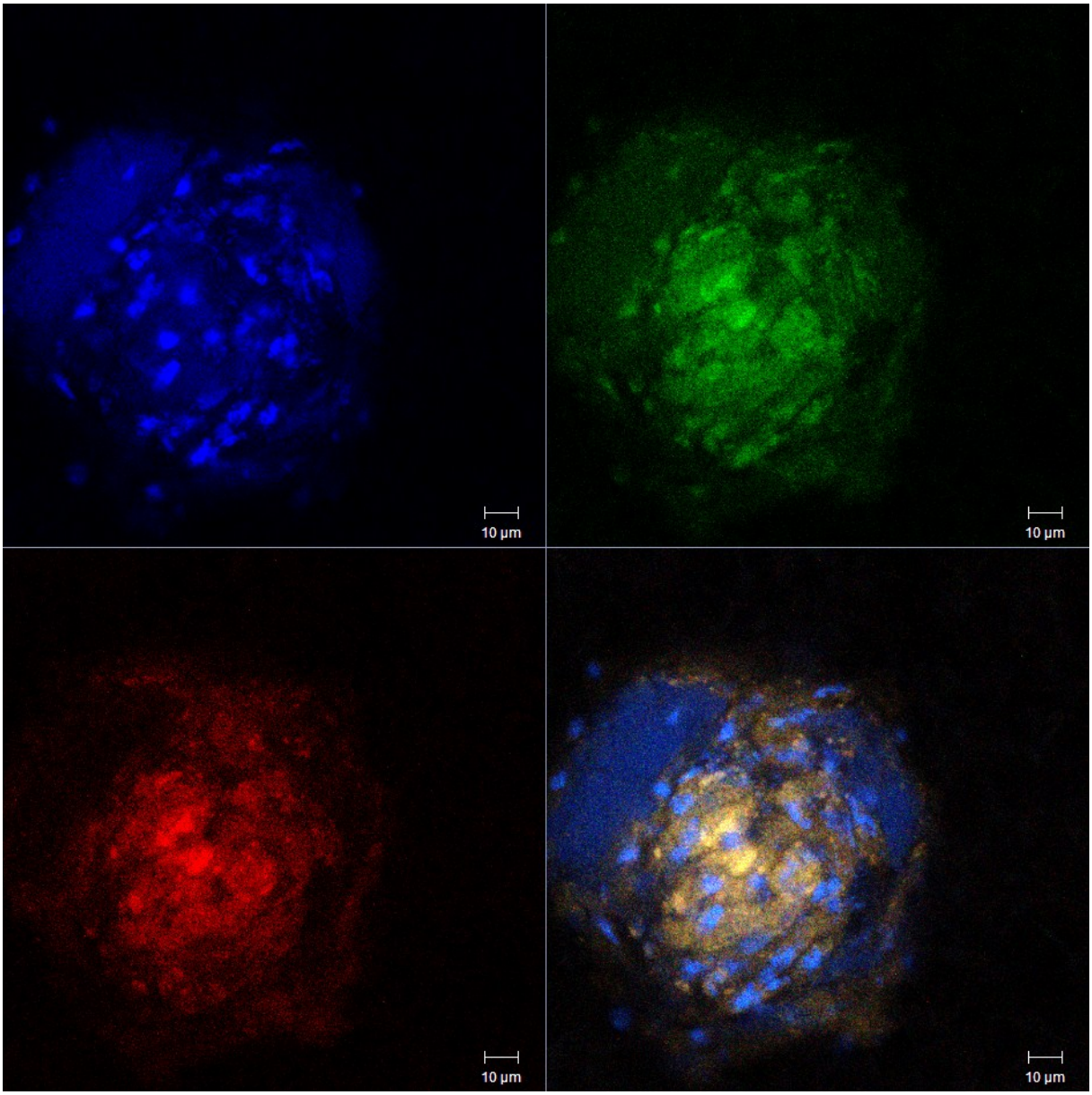
C10-low, day 20, 40x



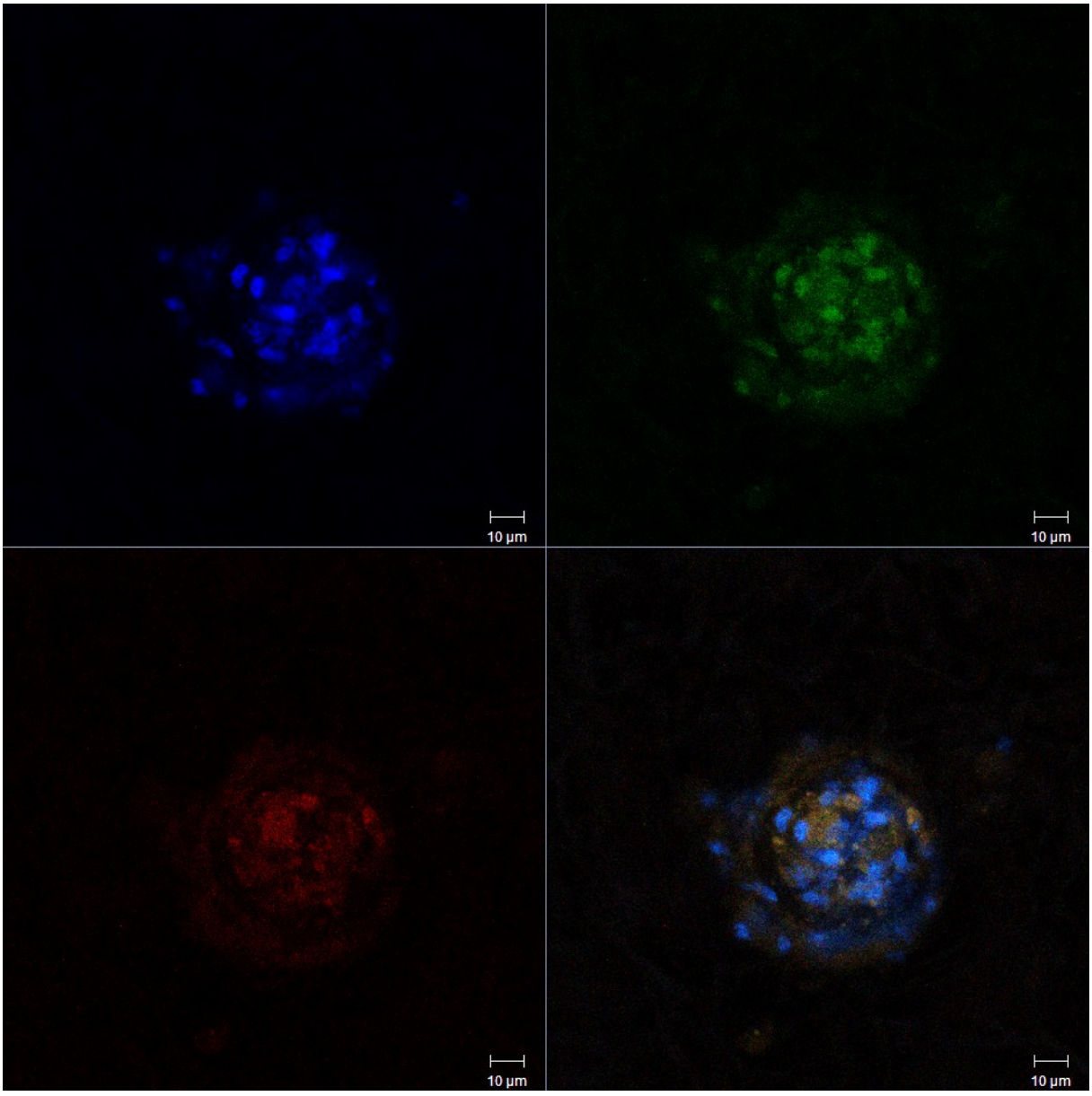
C10-high, day 20, 40x



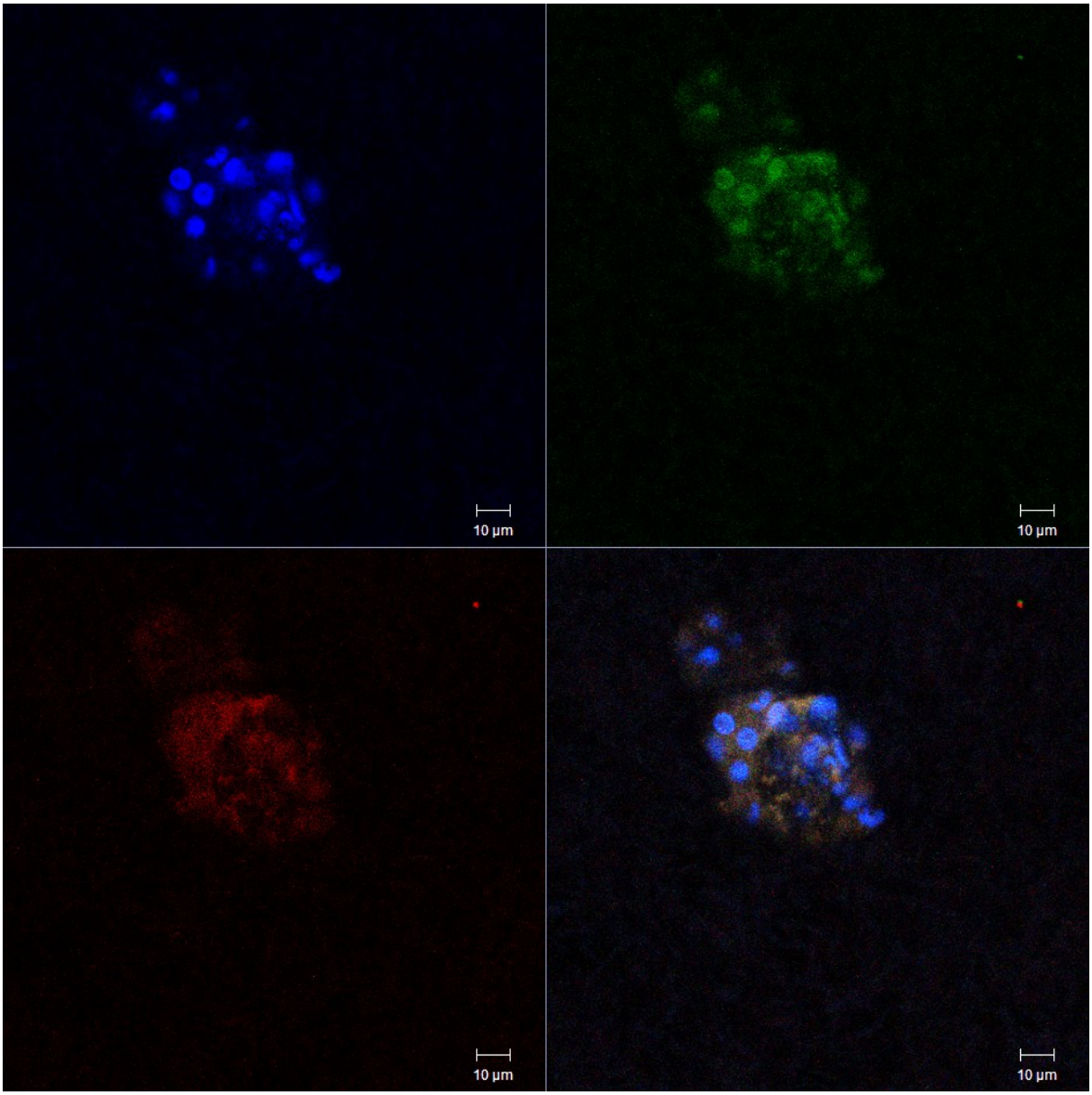
C4-blank, day 30, 40x



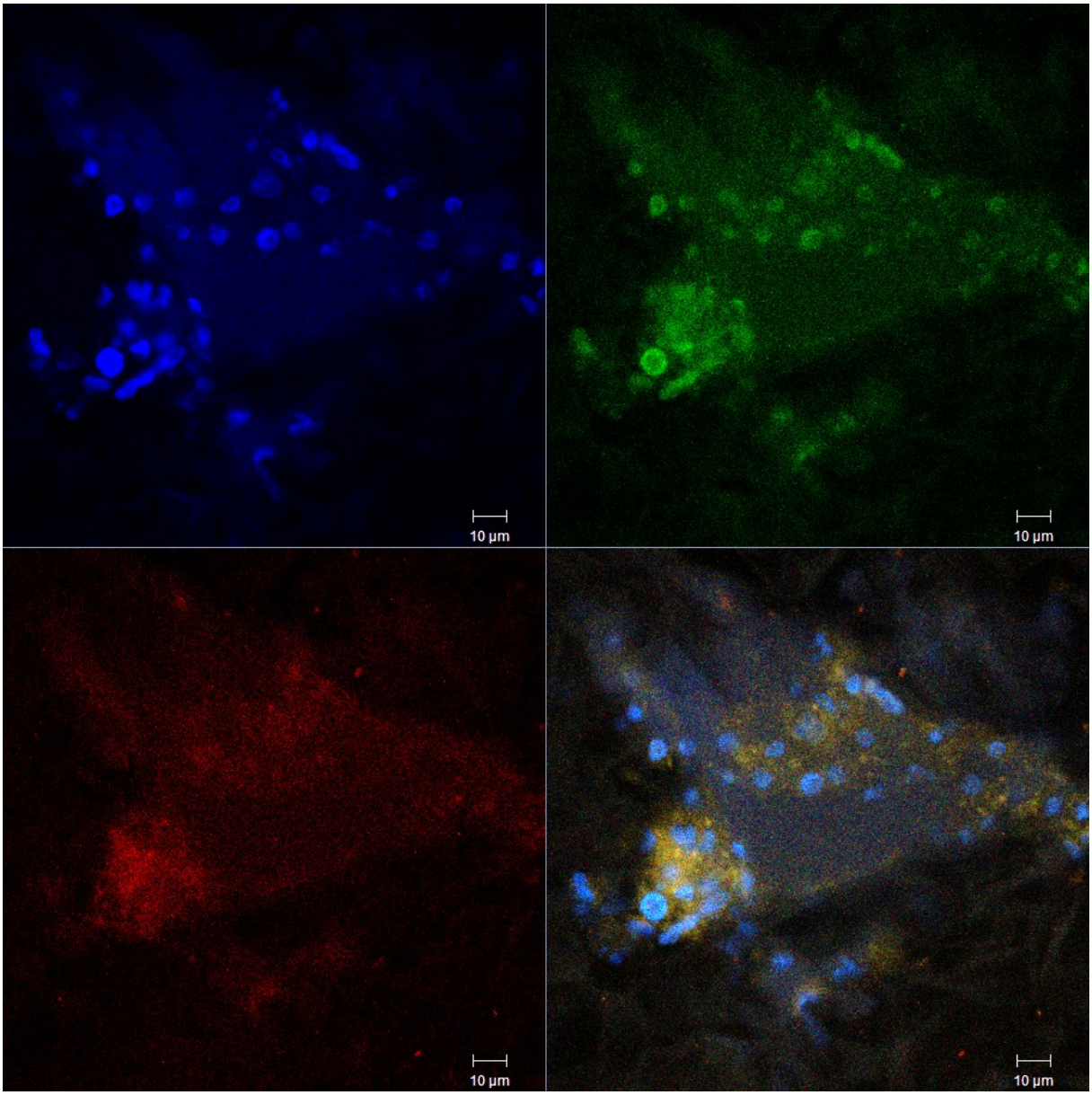
C4-low, day 30, 40x



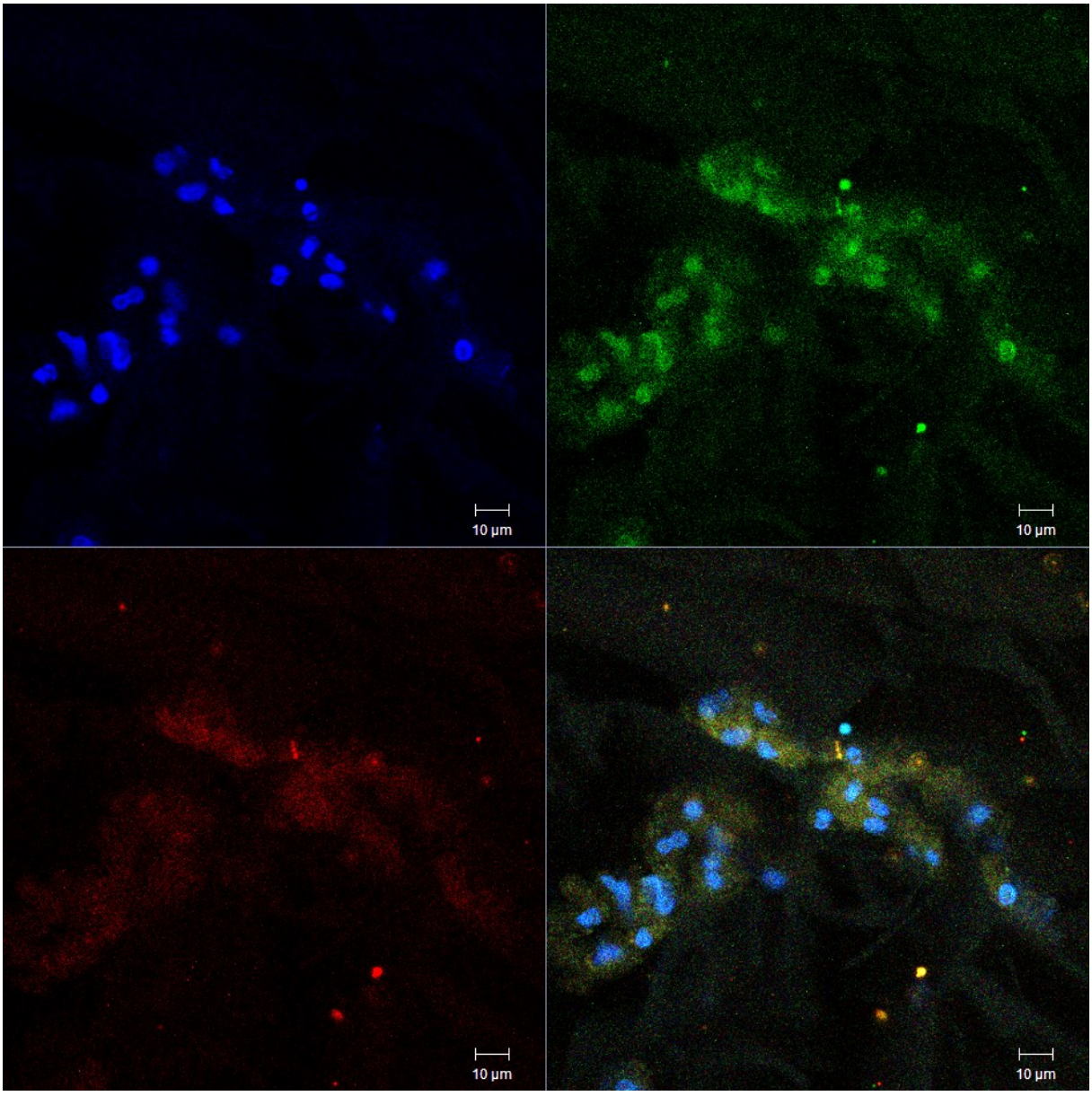
C4-high, day 30, 40x



C10-blank, day 30, 40x



C10-low, day 30, 40x



C10-high, day 30, 40x