Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Information for:

Physiological formation of fluorescent and conductive protein microfibers in live fibroblasts upon spontaneous uptake of biocompatible fluorophores

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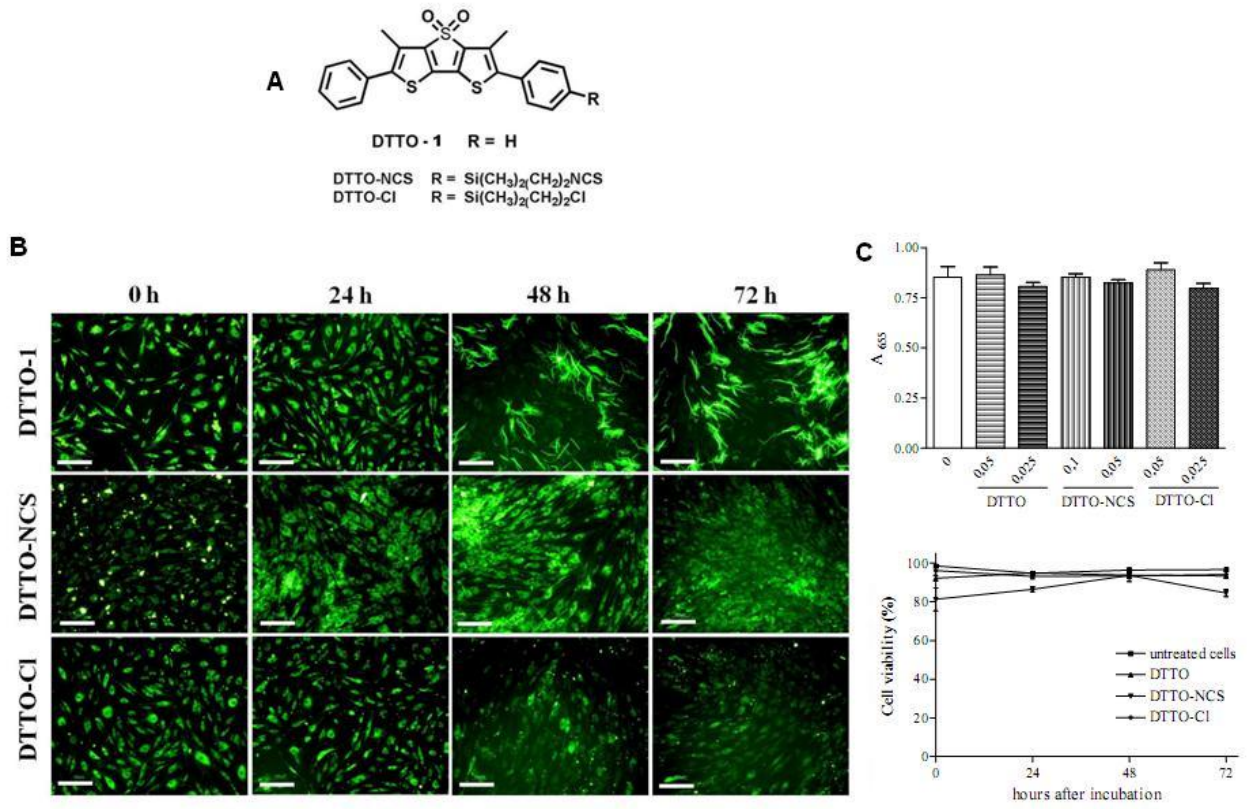

Figure S1. (**A,B**) Live human skin fibroblasts form fluorescent fibers upon incubation with DTTO-1 while incubation with selectively functionalized DTTO-1 derivatives (DTTO-NCS and DTTO-Cl) - analyzed for comparison - do not lead to fibers formation but only to staining of the cytoplasm. The figure shows the fluorescence images of treated cells up to 72h after uptake of the fluorophores (0.12 mM solution). *Scale bars*: 200 µm. (**C**) Proliferation and viability of the cells treated with DTTO-1, DTTO-NCS and DTTO-Cl. No statistically significant differences between treated and untreated cells is found and a substantial number of cells remain viable (range: 81% to 96%).

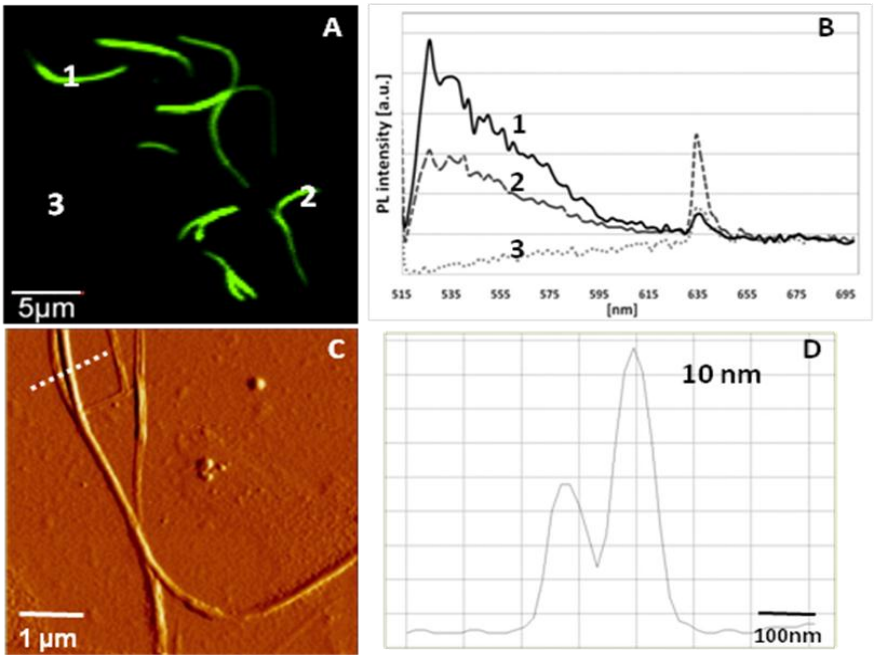

Figure S2. (A) LSCM image of DTTO-1 deposited on a mica substrate from a 0.12 mM solution of DTTO. The DTTO self-assembly shows the formation of rod-like structures. *Scale bar*: 5µm. (B) Spatially resolved photoluminescence (PL) spectra of the self-assembled dye structures. The extra peak at 640 nm is probably due to D-PBS. (C-D) Atomic force microscopy (AFM) planar view (C) and corresponding cross-section (D) of the DTTO-1 formations in film.

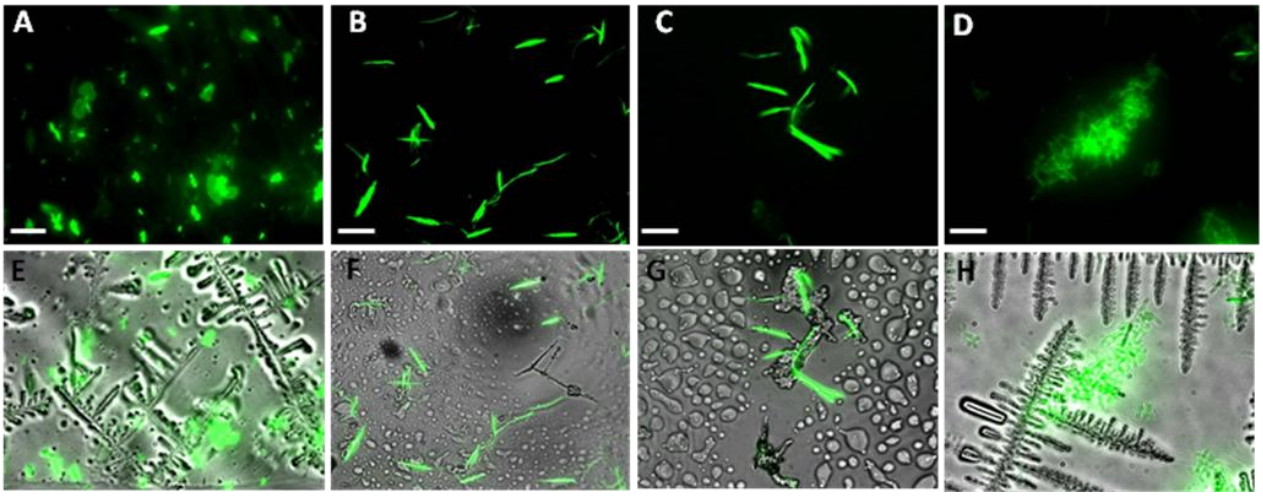

Figure S3. Fluorescence microscopy images, in reflection (A-D) and merged with transmission (E-H), of DTTO (0.12 mM) in solution with the main ECM synthetic proteins: (A-E) fibronectin; (B-F) collagen I; (C-G) collagen IV; (D-H) laminin. *Scale bars*: 10 µm.

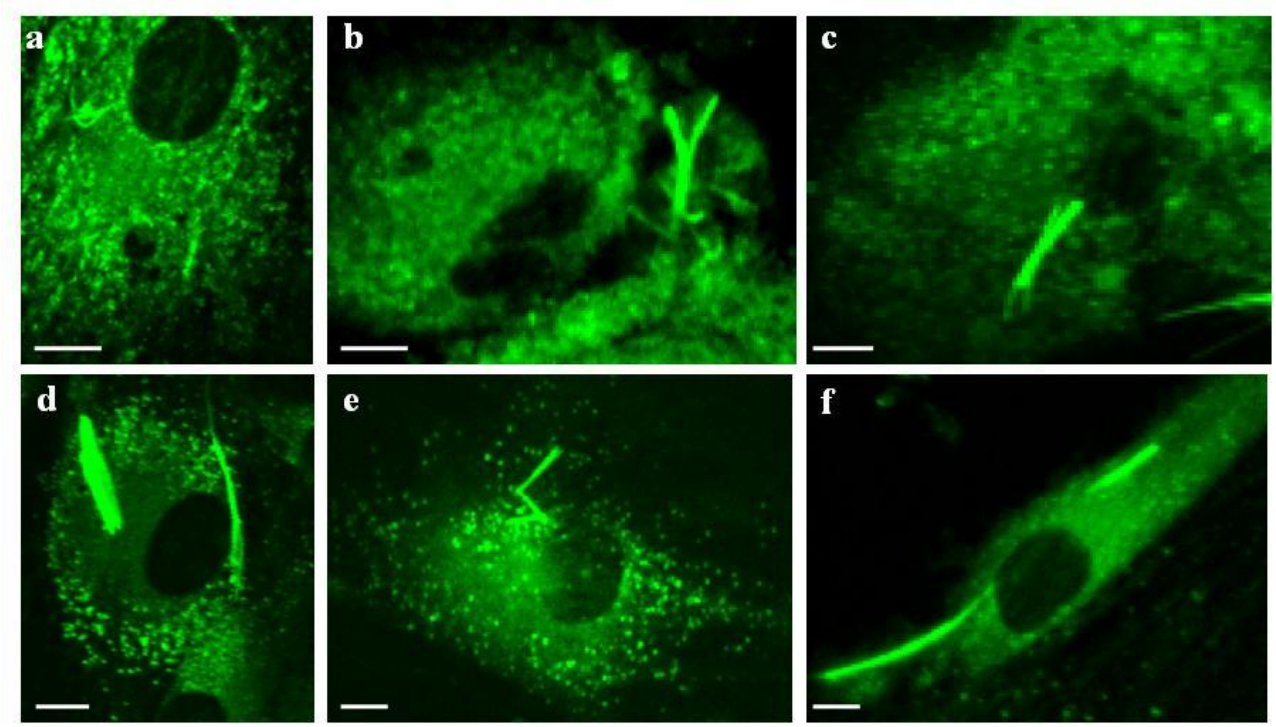

Figure S4. Fibers formation in different cells of the same population at 6h upon DTTO- uptake. *Scale bars:* 10µm.

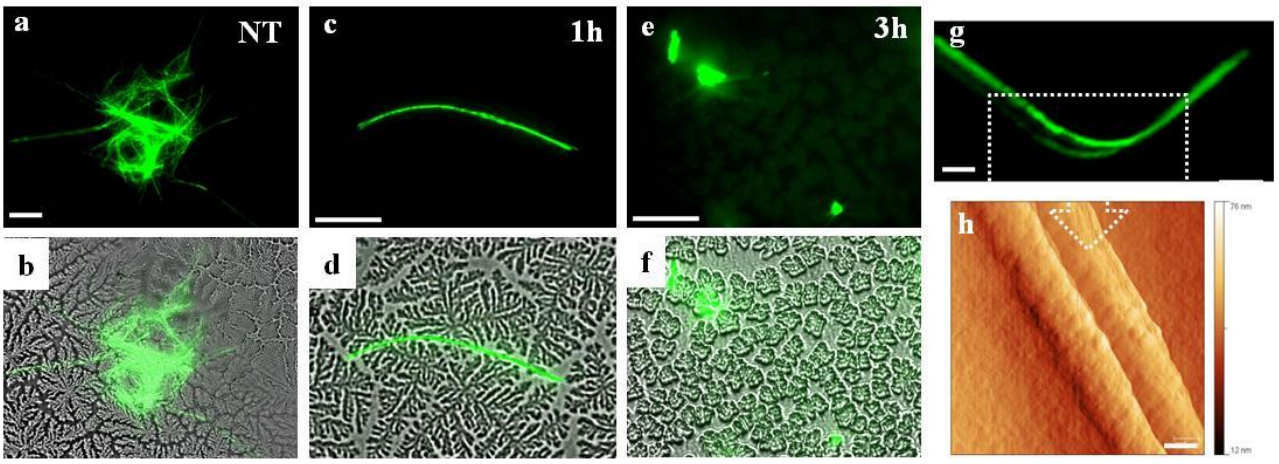

Figure S5. The fluorescent microfibers after a collagenase treatment. Fluorescence optical images, in reflection (a,c,e) and merged with transmission (b,d,f) of untreated (NT) fibers isolated from the cell-conditioned medium after 120h upon DTTO-1 uptake (a,b); after a collagenase digestion at 37°C for 1h (c,d) and 3h (e,f). (g) LCSM image and (h) NC-AFM topography of a fiber after 1h collagenase digestion. *Scale bars*: 10 µm (a,c,e); 2 µm (g); 500 nm (h).

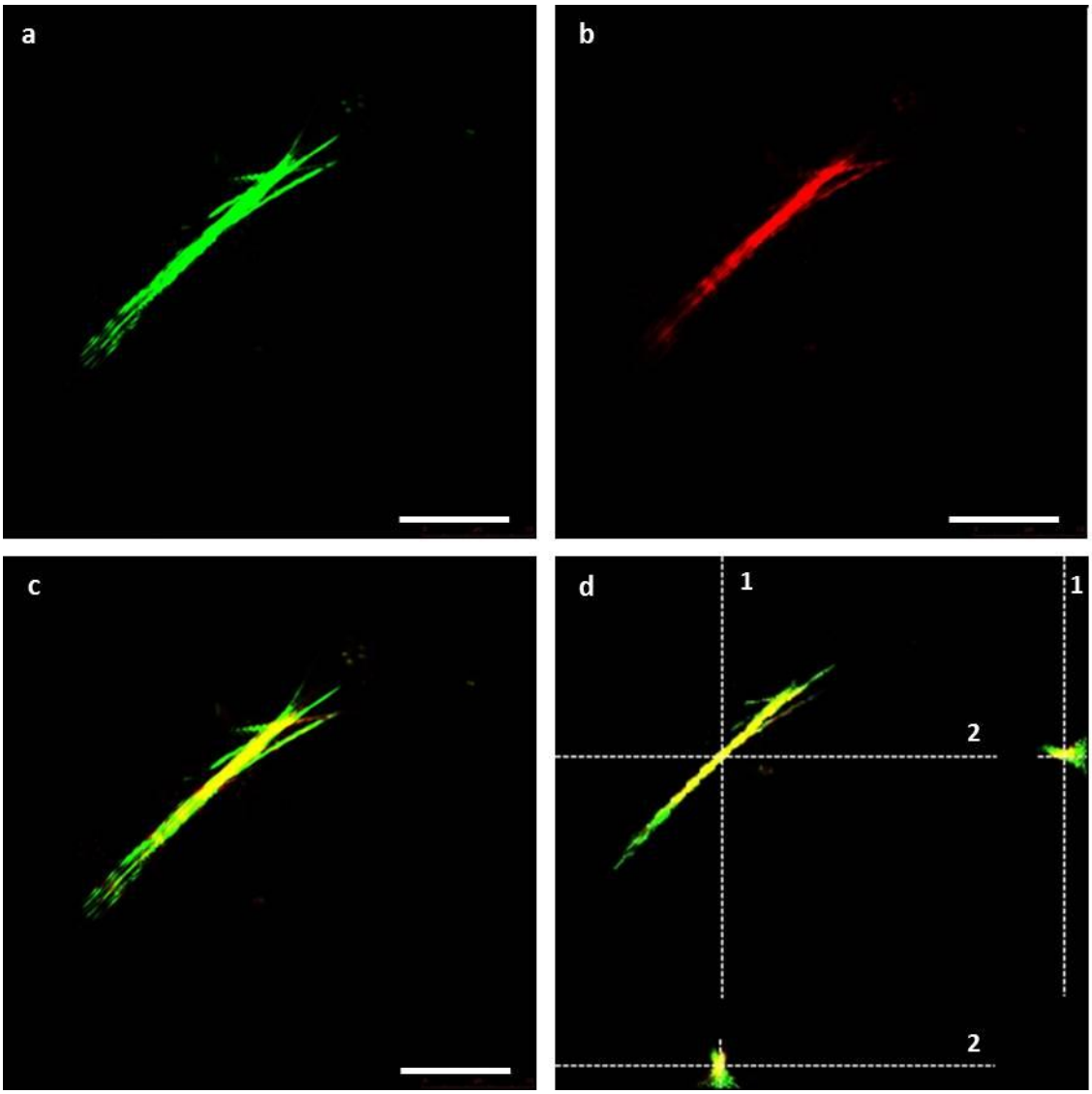

Figure S6. LCSM images of co-localization of microfibers (a, green) isolated by NIH-3T3 fibroblasts with anti-collagen type-I antibody (**b**, red), merge image (c) and z- stack sections (d), show the spatial co-localization of DTTO-1(green) with collagen I (red) inside microfibers. *Scale bar*: 10 µm

Sample	σ, mS/cm
1	$0{,}13 \times 10^{-2}$
1- mfb[a]	$0{,}17 \times 10^{-3}$
1- mfb[b]	$0{,}13 \times 10^{-4}$
2	$0{,}27 \times 10^{-2}$
2- mfb	$1{,}03 \times 10^{-1}$
3	$0{,}22 \times 10^{-2}$
3- mfb	$0{,}45 \times 10^{-2}$

Table S1. Conductivity (σ, mS/cm) measured by Tr-TUNA AFM on self-assembled structures of DTTO-1,2,3 and the corresponding microfibers (mfb) obtained after the uptake of fibroblasts. 1-mfb indicates the microfibers obtained from the uptake of DTTO-1 from BMFbs (a) and NIH-3T3 (b).