Methods:

Synthesis of poly(Pro-Hyp-Gly)-graft-(PCCL-Rhodamine 110): First, the poly(Pro-Hyp-Gly) film was succinylated and HOSu activated as described in the main text. Next, 6.42 μmol of H-Lys-Lys-OH hydrochloride and 19.3 μmol of DIPEA was dissolved in 170 μL Milli-Q H_2O:DMF = 1:1 solution. The solution was poured onto the film and reacted for 12 hrs. The following HOSu-activated PCCL conjugation, succinylation, HOSu activation, and Rhodamine 110 conjugation was conducted as described in the main text and Scheme 1.

Synthesis of poly(Pro-Hyp-Gly)-graft-Rhodamine 110 and poly(Pro-Hyp-Gly)-graft-BFP: First, the poly(Pro-Hyp-Gly) film was succinylated and HOSu activated as described in the main text. The following Rhodamine 110 or BFP conjugation was conducted as described in the main text and Scheme 1.

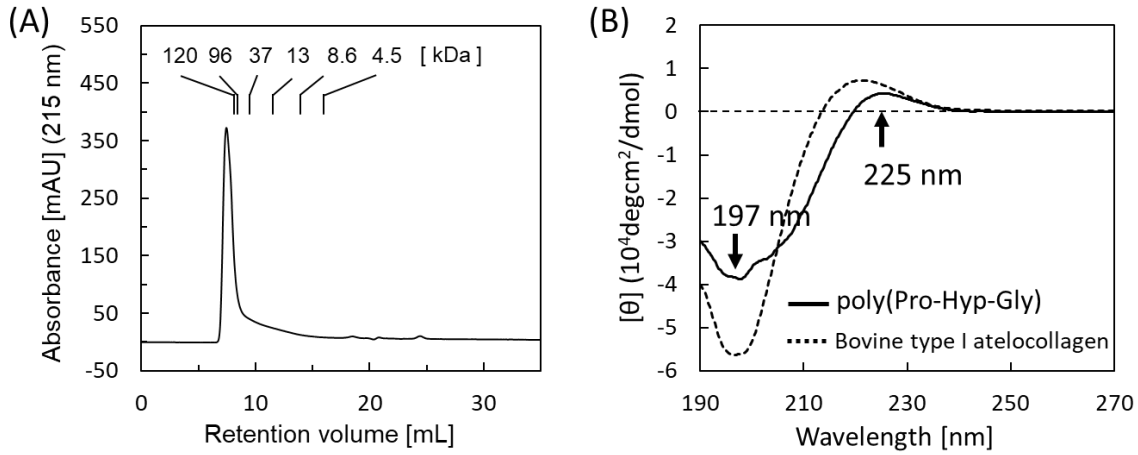

Figure S1. (A) Gel permeation chromatography profile of poly(Pro-Hyp-Gly). A Superdex 200 HR 10/300 GL column was used with 10 mM phosphate buffer containing 150 mM NaCl (PBS, pH 7.4) as an elution buffer. The flow rate was 0.5 mL/min at room temperature. The concentration of injected poly(Pro-Hyp-Gly) was 0.50 mg/mL in PBS. The detection wavelength was set at 215 nm. The molecular weight standards were poly(ethylene glycol). (B): Circular dichroism spectra of poly(Pro-Hyp-Gly) (solid line) and bovine type I atelocollagen (dotted line). Sample concentrations in water were 0.25 mg/mL. Using a quartz cell with the optical length of 0.1 cm, spectra were acquired with five scans at a scan speed of 50 nm/min and a resolution of 0.2 nm at room temperature on a spectropolarimeter (J-820, JASCO, Tokyo, Japan).

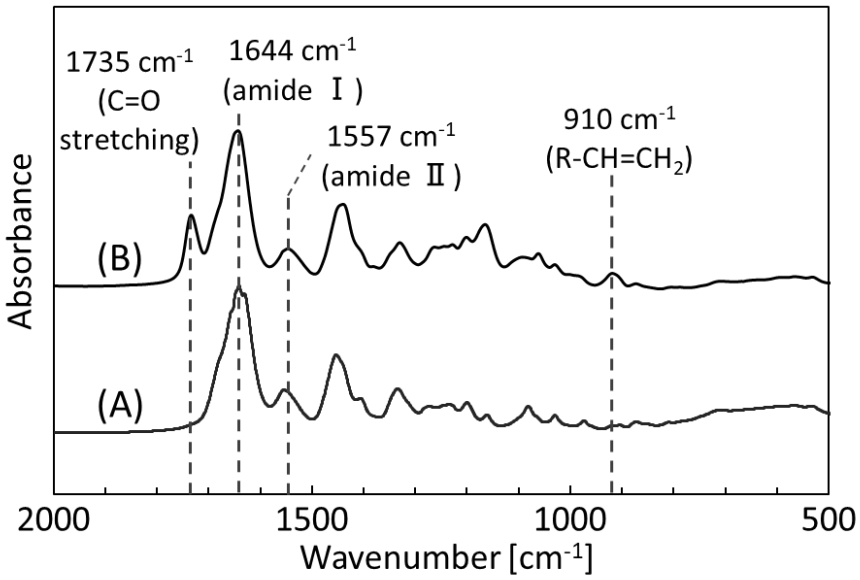

Figure S2. Fourier transform infrared (FTIR) spectra of poly(Pro-Hyp-Gly) (A), and poly(Pro-Hyp-Gly)-*graft*-4-pentenoate (B). FTIR spectra of the poly(Pro-Hyp-Gly)-derived scaffolds showed peaks at 1644 and 1557 cm^{-1} that were assigned as amide I and amide II of the poly(Pro-Hyp-Gly) backbone, respectively. The FTIR spectrum of poly(Pro-Hyp-Gly)-*graft*-4-pentenoate showed additional peaks at 1735 and 910 cm^{-1} that were assigned to the carbonyl stretching of the ester group and the C-H wagging of the vinyl group.

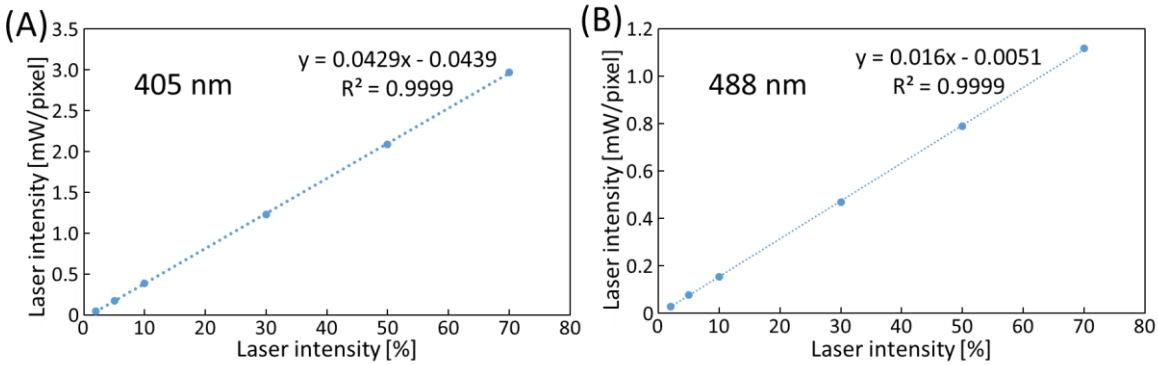

Figure S3. Power calibration curves of 405 nm (A) and 488 nm (B) lasers on a LSM710 confocal laser scanning microscope (CLSM, Carl Zeiss). Actual laser power values (mW/pixel) at 2, 5, 10, 30, 50 and 70% laser intensity setting were measured by the supplier. Using other imaging parameters (resolution, scanning speed, size of irradiation area and duration), actual laser power values on the samples (mW/cm^2) were calculated.

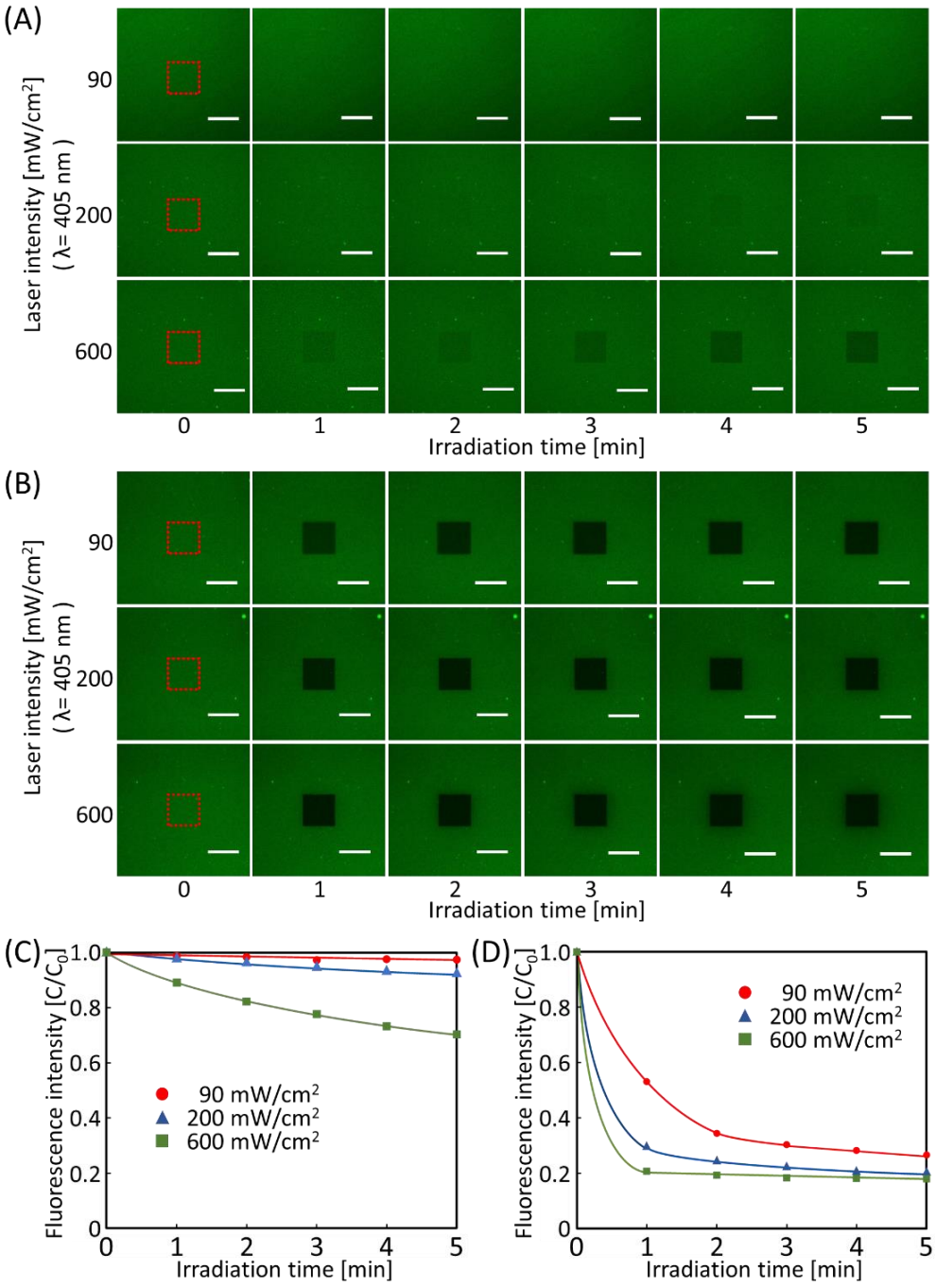

Figure S4. A functional chemical moiety is detached with visible light. (A,C) Results using a scaffold directly modified with Rhodamine 110. (B,D) Results using a scaffold modified via PCCL. In (A) and (B), red squares indicate areas irradiated with 405 nm laser. Scale bars indicate 200 μm. Graphs in (C) and (D) are based on fluorescent intensity in images of irradiated areas presented in (A) and (B). C_0 and C indicate fluorescent intensity before and after the laser irradiation.

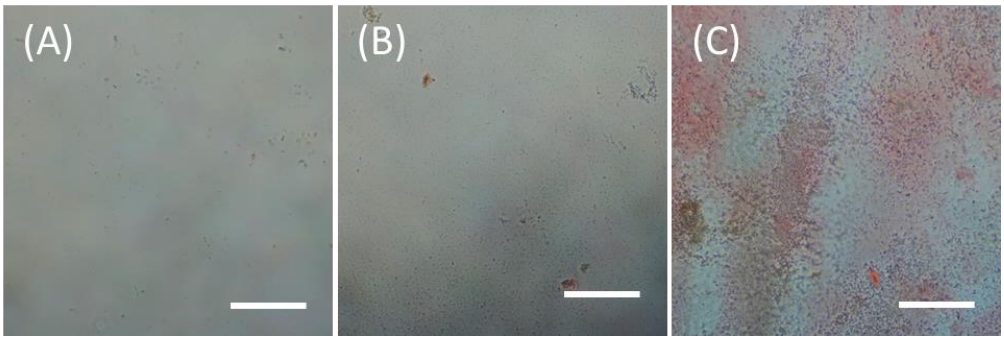

Figure S5. Calcium deposition is induced by scaffold-tethered BFP. Rat bone marrow stromal cells (rBMSCs) were cultured for 3 weeks on poly(Pro-Hyp-Gly) (A), poly(Pro-Hyp-Gly)-*graft*-4-pentenoate (B), or poly(Pro-Hyp-Gly)-*graft*-BFP (C). For preparation of scaffolds used in (A) and (B), mock BFP conjugation reaction was conducted without HOSu activation of the scaffolds. Physically attached BFP, if any, on the scaffolds did not induce bone differentiation of the cells. Scale bars indicate 200 μm.