Supporting information

In Situ Imaging of Tissue Remodeling with Collagen Hybridizing Peptides

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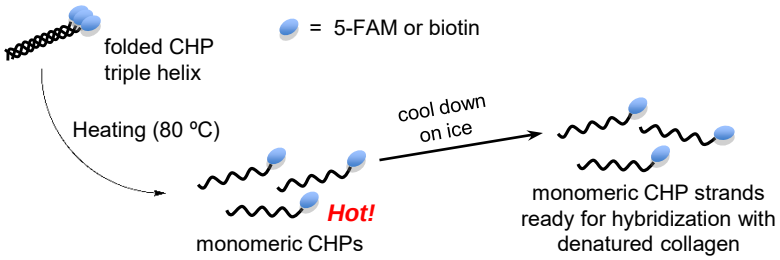

Figure S1. The thermal activation step used to generate monomeric CHP strands for collagen hybridization. A dilute CHP solution (15-30 μM) was heated in a water bath at 80 °C for 5 min to dissociate the folded CHP homotrimers. The hot solution was then quenched in an ice-water bath for 15-90 s (depending on the volume) to room temperature. The solution of monomeric CHP was then ready for immediate use to hybridize with denatured collagen in tissue samples. The self-trimerizing of CHP takes several hours to complete in this concentration range, and the effect of the dead time due to quenching (< 2 min) is negligible.

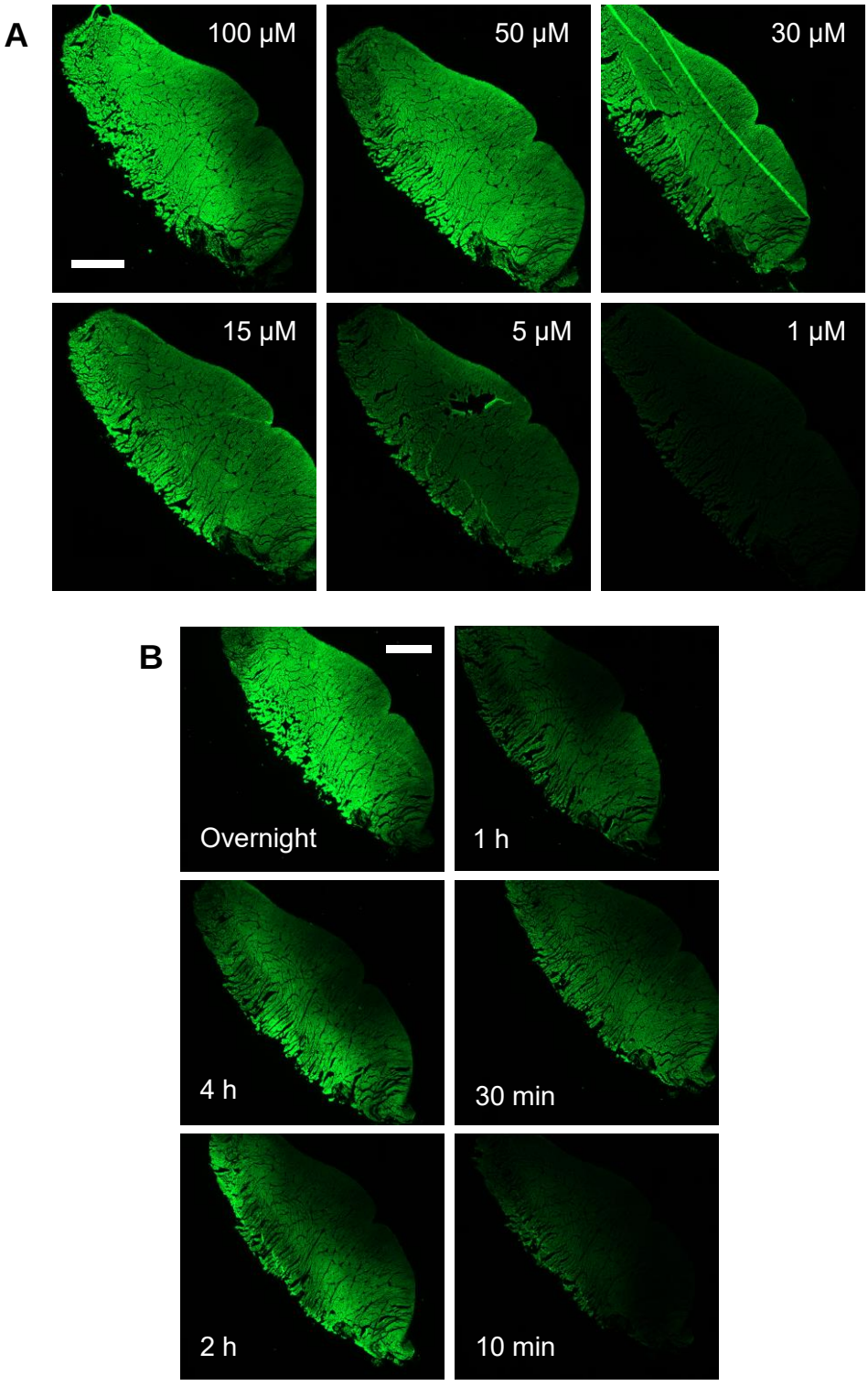

Figure S2. (A) Fluorescence images of neighboring cryosections of a porcine ligament thermally denatured in 80 °C water and subsequently stained with 100 μL of F-CHP solutions at varying concentrations are shown. The staining time was set at 2 h for all samples. (B) Fluorescence images of a set of heated ligament tissue stained with F-CHP (100 μL, 15 μM) for different incubation time periods are shown. Scale bars: 2 mm.

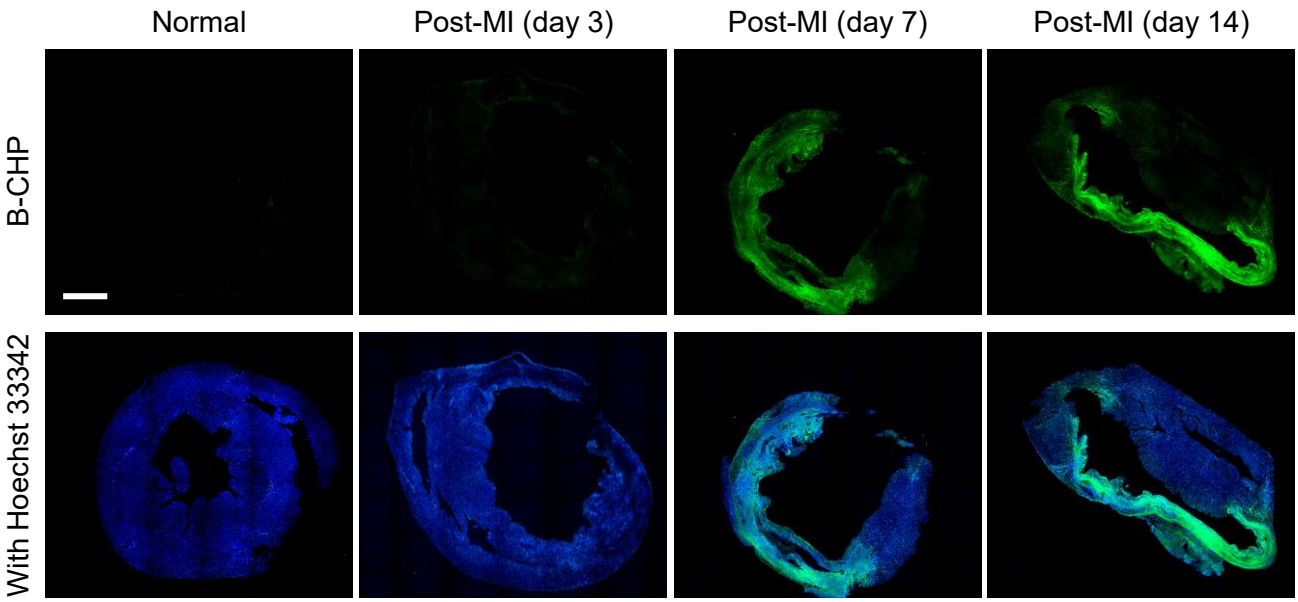

Figure S3. Representative fluorescence scans of axial cross-sections of fixed and paraffin-embedded mouse hearts before (normal), and at three time points after myocardial infarction that were stained with Hoechst 33342 and B-CHP (detected by AlexaFluor647-streptavidin). Scale bar: 1 mm.

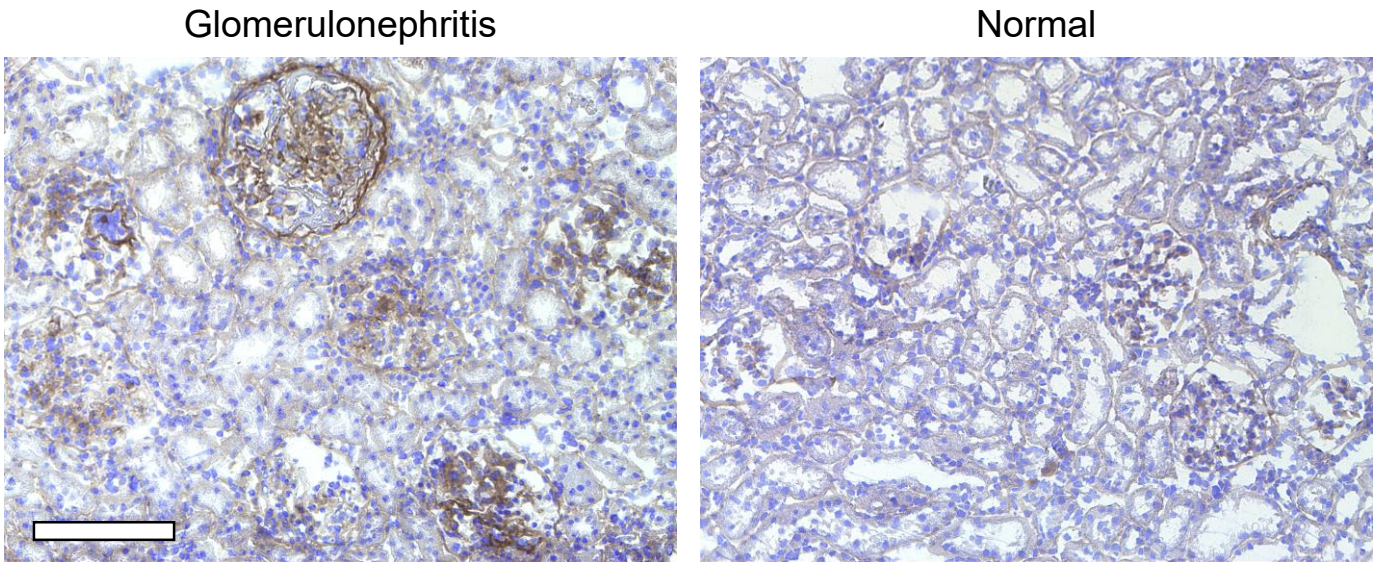

Figure S4. Representative micrographs of kidney cryosections from anti-Thy-1 nephritic and normal control rats stained with B-CHP, and further visualized with neutravidin-conjugated horseradish peroxidase (HRP). Tissue-bound CHP was visualized *via* the HRP-mediated chromogenic reaction using a DAB substrate. The cell nuclei were counter-stained with hematoxylin. Scale bar: 100 μm.

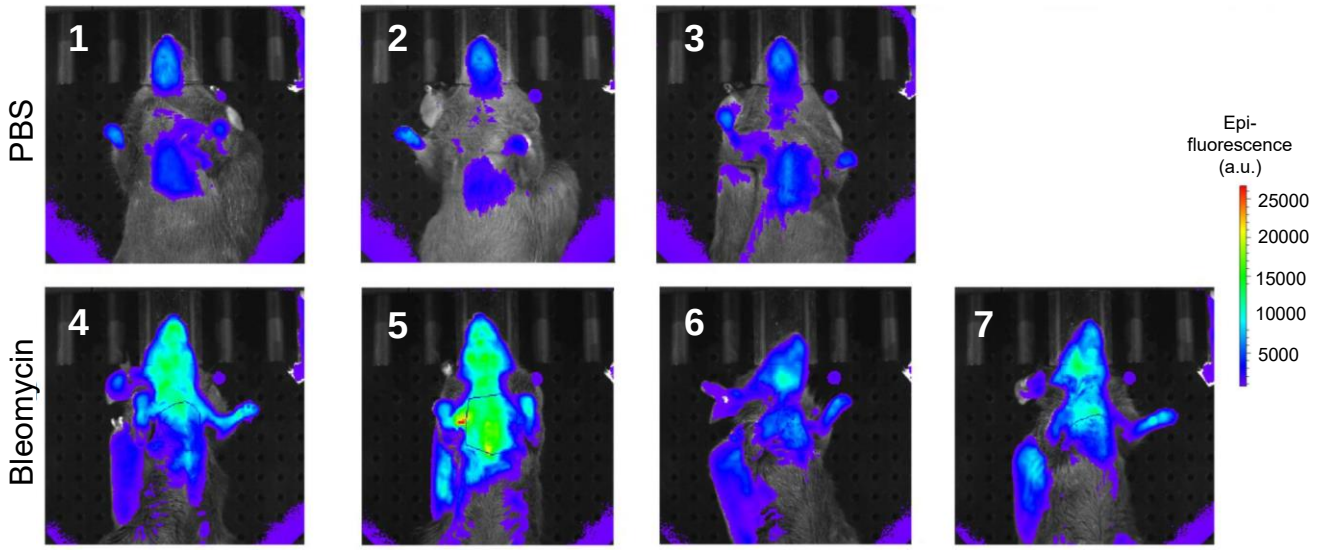

Figure S5. *In vivo* near-infrared epi-luminescence imaging of MMP activity in C57BL/6 mice which had been treated with bleomycin (bleomycin group, n = 4) or PBS (PBS group, n = 3) through subcutaneous minipumps for 3 weeks. The mice were administered 2 nmol of MMPsense 680 (PerkinElmer) through tail vein injection, and anesthetized with 2% isofluorane 24 h post injection. The surface fluorescence of the lungs was detected with an IVIS Spectrum imager [PerkinElmer, Ex/Em = 675/720 nm, Epi=illumination, Bin:(M)8, FOV: 6.6, f-stop 2, exposure 0.5 s]. Hair on the ventral surface of the thoracic region was removed with a depillitator (Nair) at least 3 days prior to imaging. Remarkably higher signals were detected in the thoracic region of the bleomycin-treated mice, indicating elevated MMP activity. All images were acquired with identical instrument settings and set to the same scale (a.u. = arbitrary unit).

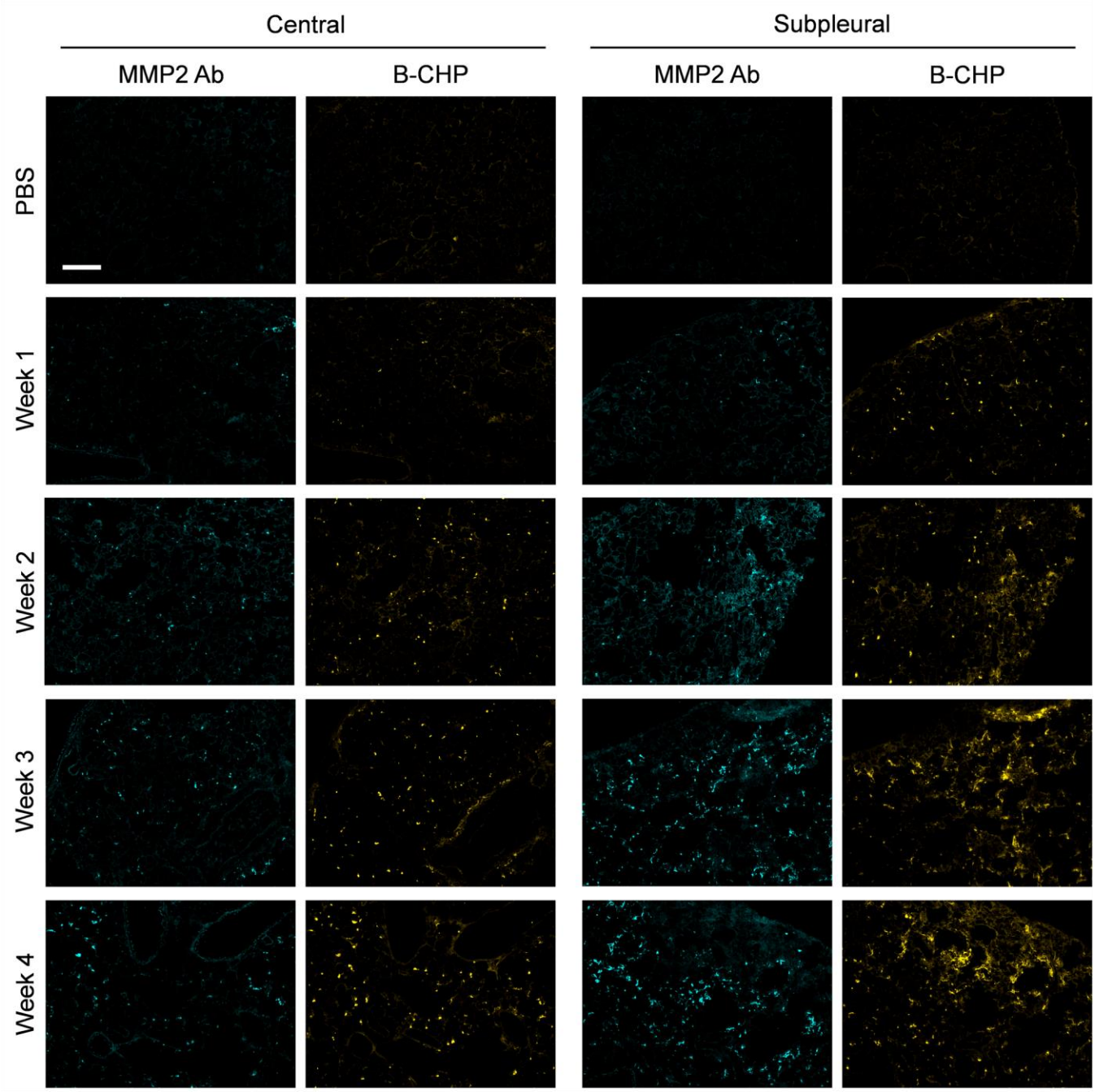

Figure S6. Representative fluorescence micrographs from the central and subpleural areas of lung cryosections harvested from bleomycin-treated or control mice dosed with PBS for 1 week. The tissues were double stained with B-CHP and an anti-MMP2 antibody followed by AlexaFluor647-labeled streptavidin and AlexaFluor555-labeled donkey anti-rabbit IgG H&L, respectively. The spatial distribution of the signals from the two probes are similar. Selected micrographs shown here are representative of images collected from 3 animals in each group. Scale bar: 200 μm.

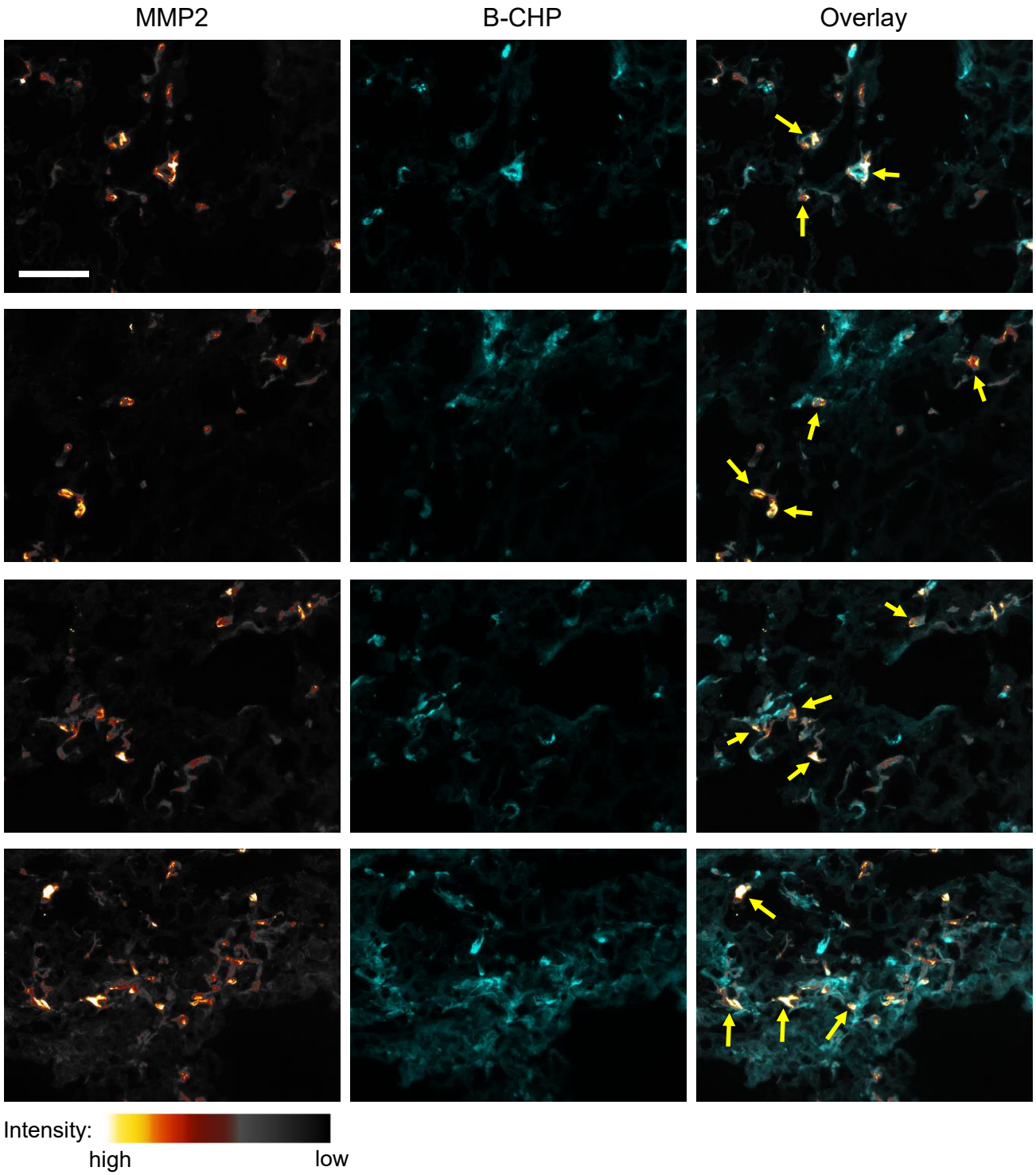

Figure S7. Micrographs showing multiple views of lung tissue (cryosections) harvested from the mice dosed with bleomycin for 3 weeks. The tissues were double-stained with B-CHP and an anti-MMP2 antibody followed by AlexaFluor647-labeled streptavidin and AlexaFluor555-labeled donkey anti-rabbit IgG H&L, respectively. Yellow arrows mark the locations where high MMP2 signals overlap with decreased CHP signals. Scale bar: 50 μm.

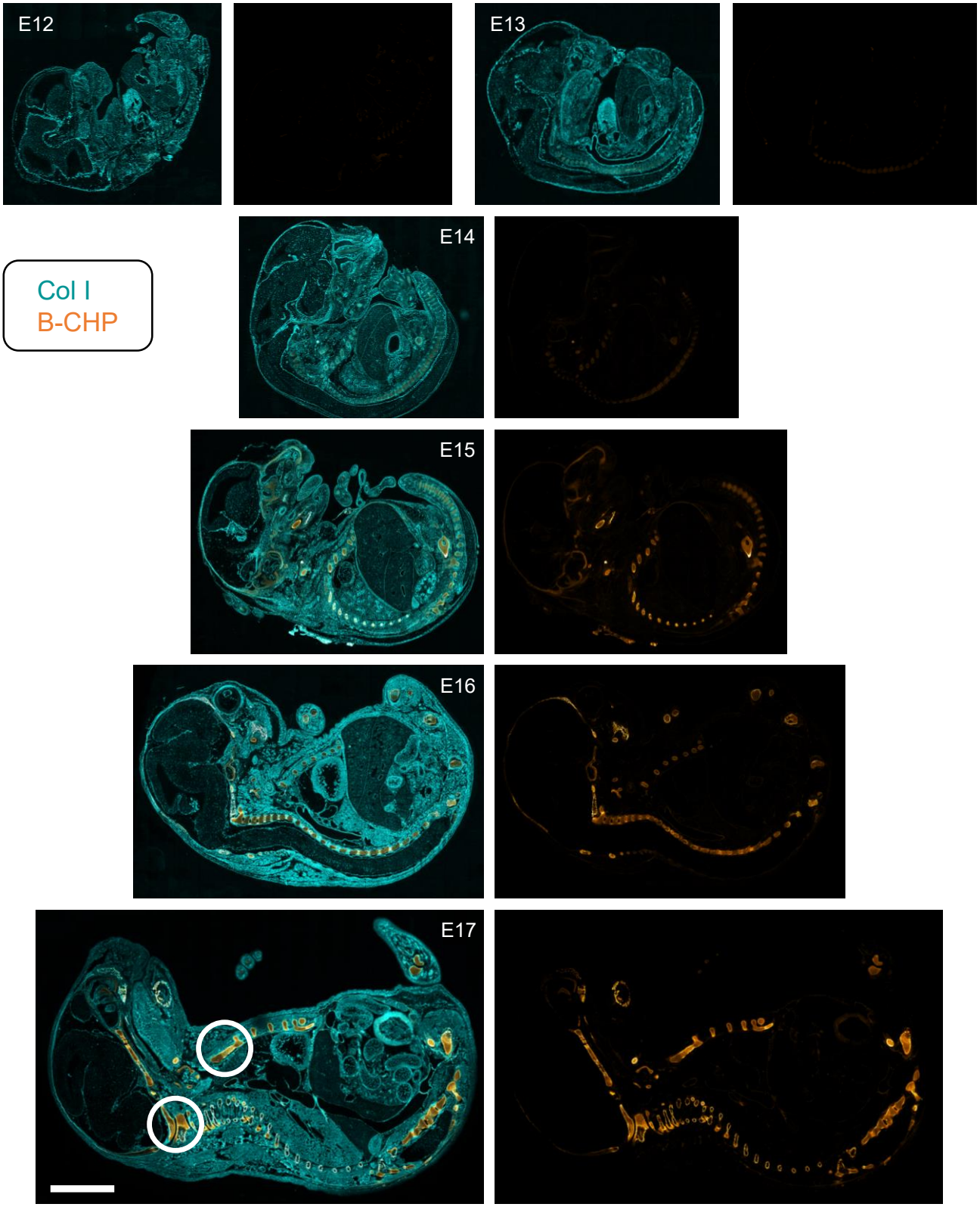

Figure S8. Localization of CHP binding in a series of sagittal cryosections of mouse embryos from 12 to 17 d.p.c. (labeled as E12-17) that were double-stained with B-CHP (detected by AlexaFluor647-streptavidin) and an anti-collagen I antibody (detected by AlexaFluor555-labeled donkey anti-rabbit IgG H&L). Regions in the circles in E17 are presented in magnified views in Fig. 6B. Scale bar: 3 mm.

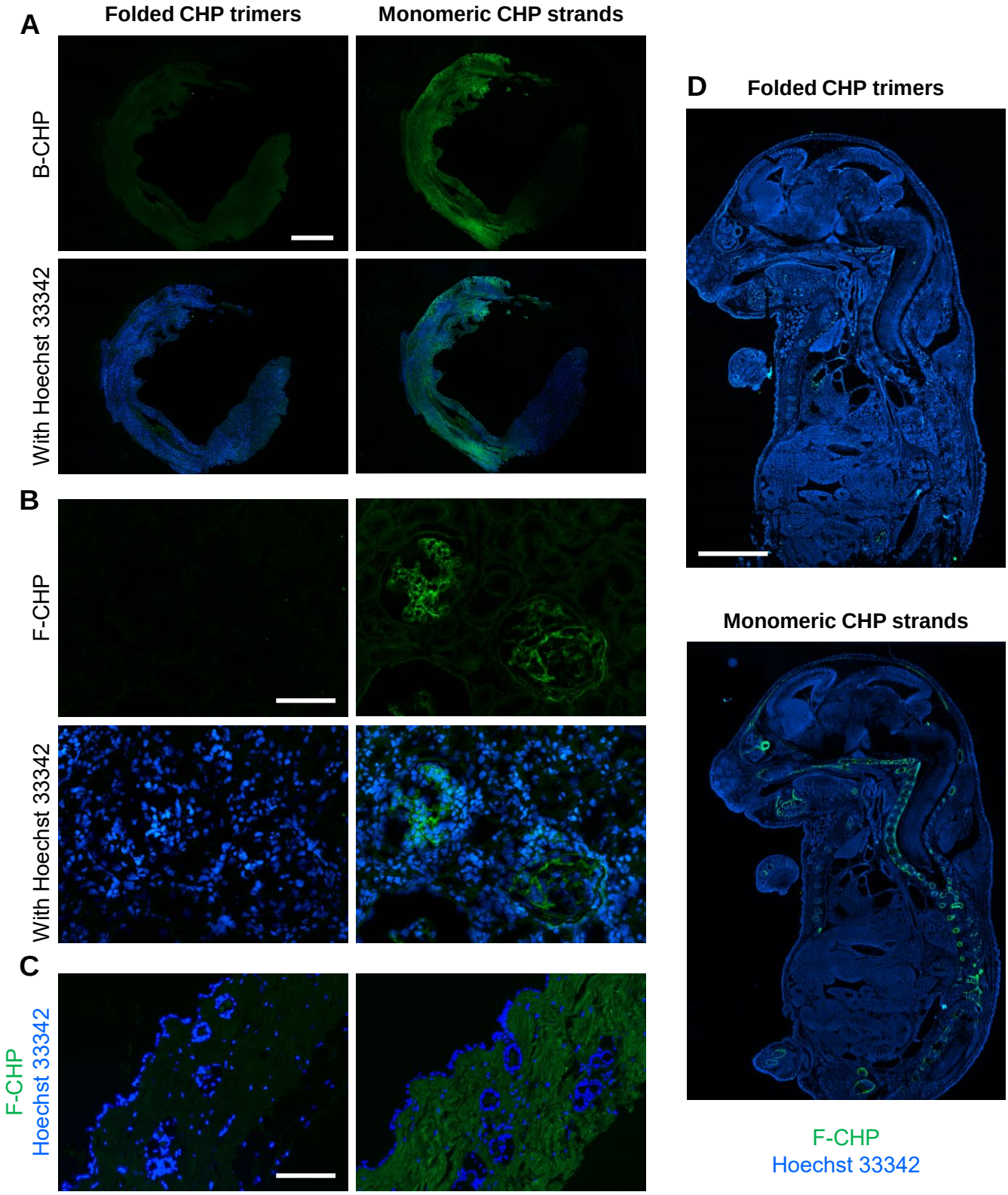

Figure S9. Neighboring sections from a formalin-fixed paraffin-embedded (FFPE) mouse heart harvested 7 days after myocardial infarction (A), a frozen nephritic rat kidney (B), frozen skin of a 9-month old mouse (C), and a frozen 18 d.p.c. mouse embryo (D) were stained with monomeric CHP strands or unheated, folded CHP trimers. CHP stock solutions (100 μM) were incubated at 4 °C for days to allow adequate CHP self-trimerization, and the diluted solutions were directly added to tissue sections without heating. Cell nuclei were counter-stained with Hoechst 33342. B-CHP was detected using AlexaFluor647-streptavidin. Paired images in each panel were acquired under identical microscopic settings. Results are representative of three experiments for each group. Scale bars: 1 mm (A), 100 μm (B, C), 3 mm (D).

Sample		W*	Endogenous biotin-blocking	W	CHP Primary antibody	W	Streptavidin Secondary antibody	W	Color development / Nuclei staining	W	Data
Human cartilage (frozen)	Blocking with 5% goat serum				F-CHP (15 μM)	✓			Hoechst	✓	Fig. 2B
Mouse heart (frozen)		✓	✓	✓	B-CHP (15 μM)	✓	AlexaFluor647-streptavidin (0.005 mg/mL)		Hoechst	✓	Fig. 3A
Mouse heart (frozen)		✓	✓	✓	B-CHP (15 μM) anti-CD68 antibody (0.01 mg/mL)	✓	AlexaFluor647-streptavidin (0.005 mg/mL), Goat F(ab')2 anti rat IgG: FITC (0.005 mg/mL)		Hoechst	✓	Fig. 3C, D
Rat kidney (frozen)					F-CHP (50 μM) anti-collagen IV antibody (0.01 mg/mL)	✓	AlexaFluor555 labeled donkey anti-rabbit IgG H&L (0.002 mg/mL)		Hoechst	✓	Fig. 4B
Mouse lung (frozen)					F-CHP (15 μM) anti-MMP2 antibody (0.01 mg/mL)	✓	AlexaFluor555 labeled donkey anti-rabbit IgG H&L (0.005 mg/mL)		Hoechst	✓	Fig. 5A
Mouse lung (frozen)		✓	✓	✓	B-CHP (15 μM) anti-MMP2 antibody (0.01 mg/mL)	✓	AlexaFluor647-streptavidin (0.005 mg/mL), AlexaFluor555 labeled donkey anti-rabbit IgG H&L (0.005 mg/mL)		Hoechst	✓	Fig. 5C, Fig. S6 & S7
Mouse embryo (frozen)		✓	✓	✓	B-CHP (15 μM) anti-collagen I antibody (0.005 mg/mL)	✓	AlexaFluor647-streptavidin (0.005 mg/mL) AlexaFluor555 labeled donkey anti-rabbit IgG H&L (0.005 mg/mL)		Hoechst	✓	Fig. 6, Fig. S8
Mouse skin (FFPE)		✓	✓	✓	B-CHP (15 μM)	✓	AlexaFluor647-streptavidin (0.005 mg/mL)		Hoechst	✓	Fig. 7A
Mouse skin (frozen)					F-CHP (15 μM)	✓					Fig. 7B
Porcine ligament (frozen)					F-CHP	✓					Fig. S2
Mouse heart (FFPE)		✓	✓	✓	B-CHP (15 μM)	✓	AlexaFluor647-streptavidin (0.005 mg/mL)		Hoechst	✓	Fig. S3
Rat kidney (frozen)		✓	✓	✓	B-CHP (15 μM)	✓	neutravidin-HRP (0.002 mg/mL)	✓	DAB peroxidase substrate kit, hematoxylin**	✓	Fig. S4

Table S1. The specific protocol steps for antibody and CHP staining in each histological experiment.
*W, washing: the slides were incubated in 100 mL of PBS at room temperature for three 5-min cycles to remove unbound materials.
**The DAB color development and the hematoxylin counterstain procedures were performed following the manufacturer's recommendation.