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SUPPLEMENTARY INFORMATION

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Targeting protein and peptide therapeutics to the heart via tannic acid modification

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Supplememtary figures

Supplementary Figure 1.

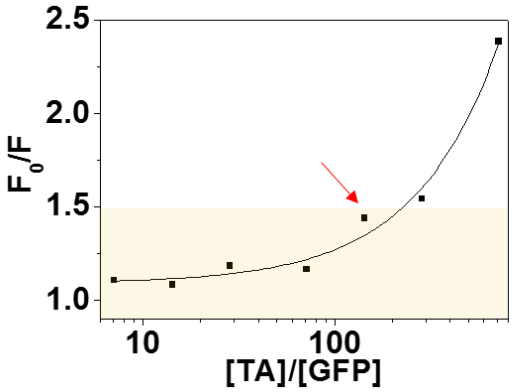

Figure S1. GFP quenching associated with TA. The red arrow shows the GFP quenching at the stoichiometric ratio of [TA] / [GFP], 143.

Supplementary Figure 2.

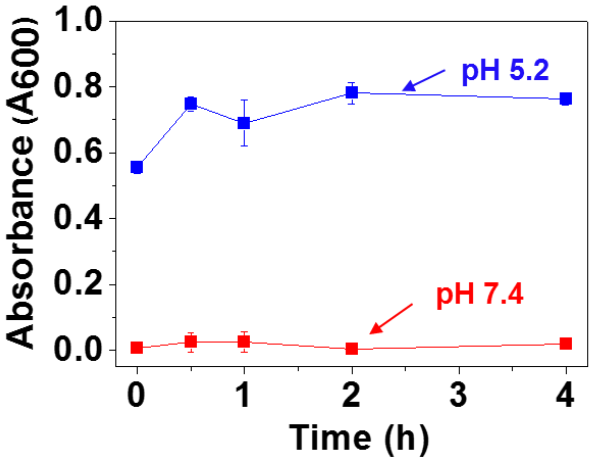

Figure S2. The stability of TANNylated GFP ([TA] / [GFP] = 72) at pH 7.4 (red) or 5.2 (blue). Data are mean ± s. d. (n = 3).

Supplementary Figure 3.

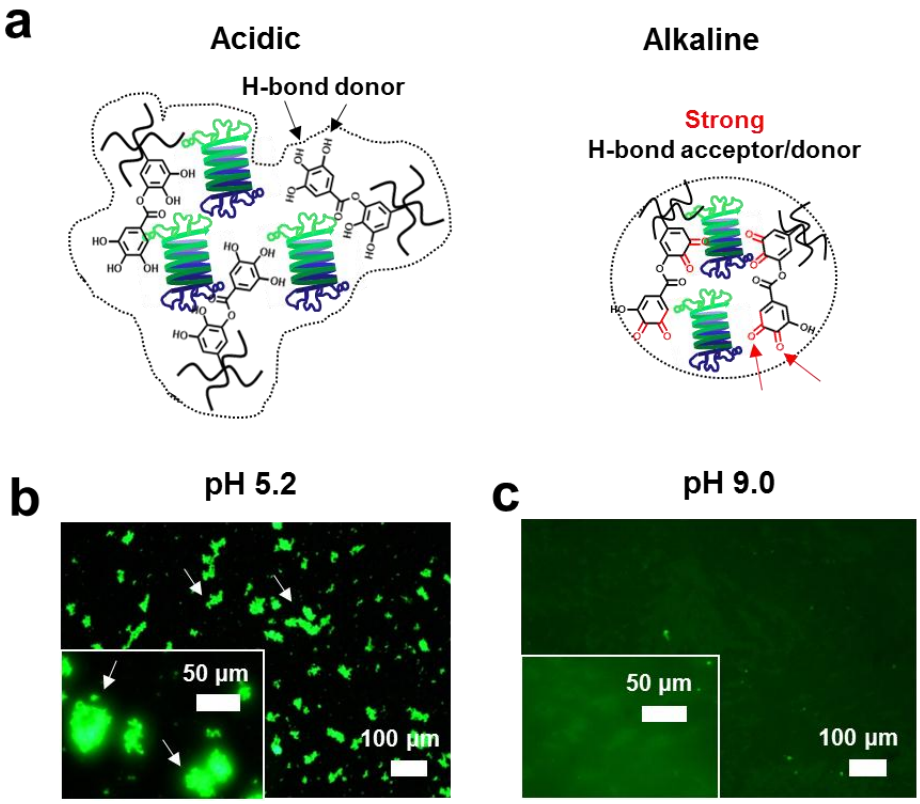

Figure S3. The pH-dependent size changes in TANNylated GFP (the ratio of 714): a, Schematic illustration for the hydrogen bond formations between proteins and TA either acidic or alkaline pH value. The reds are galloylquinone, an effective H-bond acceptor, formed by pH increases. **b, c**, Fluorescent microscopic images of TANNylated GFP at pH 5.2 (**b**) or pH 9.0 (**c**). The white arrows in '**b**' panel means the formation of large aggregates.

Supplementary Figure 4.

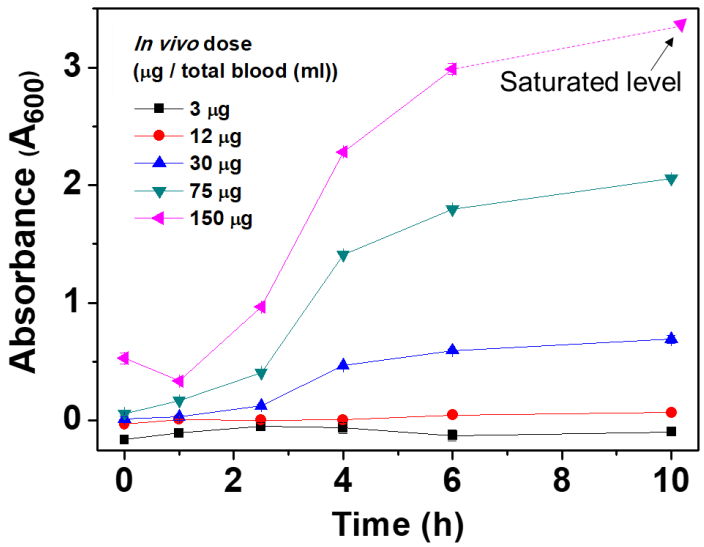

Figure S4. The colloidal stability and dose responsive turbidity assay of the TANNylated GFP in rat blood plasma. Plasma/TANNylated GFP complex mixture with a concentration of 3 μg (black), 12 μg (red), 30 μg (blue), 75 μg (cyan), and 150 μg (pink) per ml of blood plasma. Data are mean ± s. d. (n = 3).

Supplementary Figure 5.

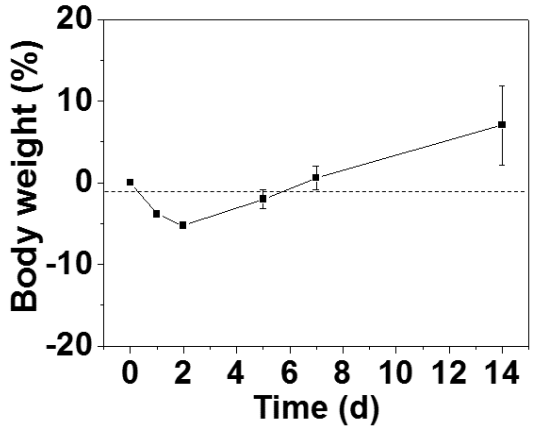

Figure S5. The changes in body weight (%) for evaluating the toxicity of TANNylated GFP. Data are mean ± s. d. (n = 3).

Supplementary Figure 6.

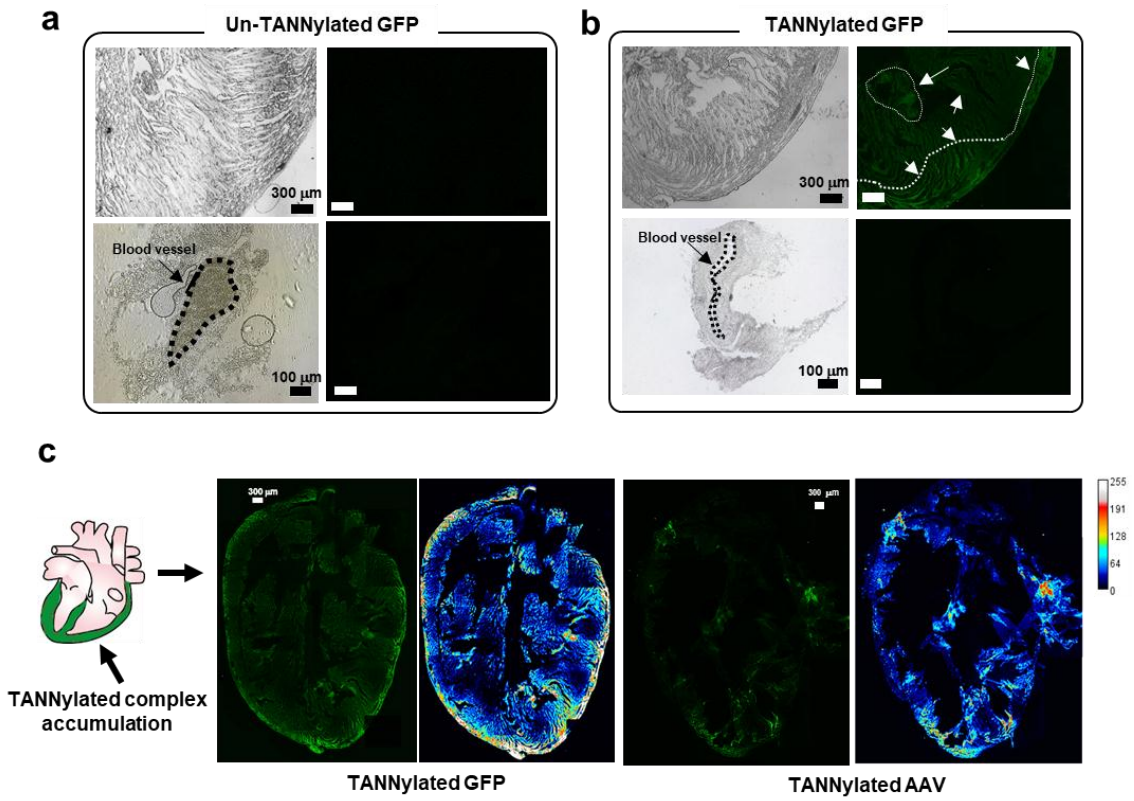

Figure S6. Immunohistochemical heart section analysis showing the distribution of the heart targeted TANNylated GFPs. a, The heart (top) and abdominal vena cava (bottom) after an injection of un-TANNylated GFP. The cross-sectional images using optical (left) and fluorescent microscopy (right). **b,** The heart (top) and abdominal vena cava (bottom) after injection of the TANNylated one. The cross-sectional images using optical (left) and fluorescent microscopy (right). The arrows shown in 'b' indicate the delivered GFP via TANNylation. **c**, A schematic diagram for the accumulated location to the heart after injection of TANNylated complex (left). The cross-sectional whole images of the heart after injection of TANNylated GFP (1st and 2nd images) or TANNylated GFP-encoding AAV (3rd and 4th images). The 2nd and 4th images are for quantitative comparisons of the produced Image J software.

Supplementary Figure 7.

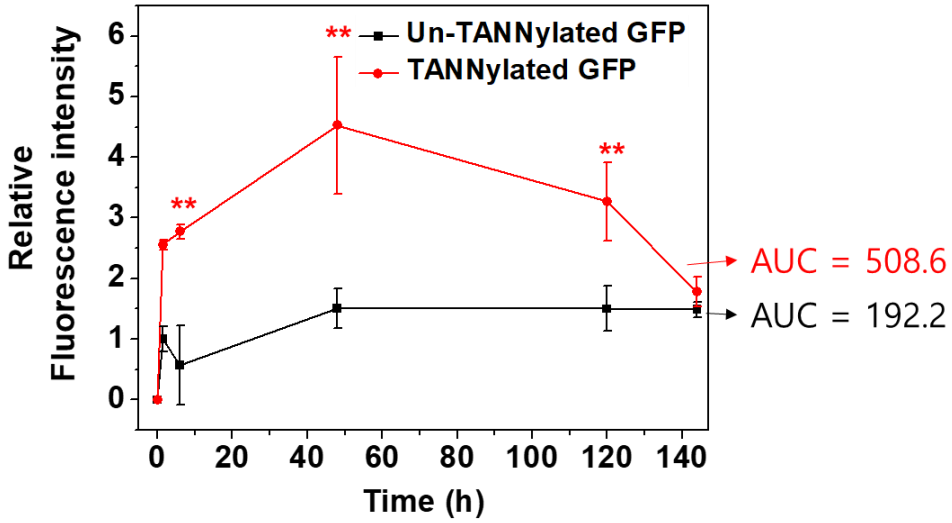

Figure S7. Relative quantitative analysis for the fluorescence intensity of the TANNylated GFP (red) and un-TANNylated GFP (black) accumulated heart as a function of time. The fluorescence intensity was normalized to the value for 1.5 h of un-TANNylated GFP ($6.78 \times 10^7 \pm 1.41 \times 10^7$). The AUC stands for area under the curves. Data are mean ± s. d. (n = 3). **P < 0.01, Tukey test, one-way ANOVA.

Supplementary Figure 8.

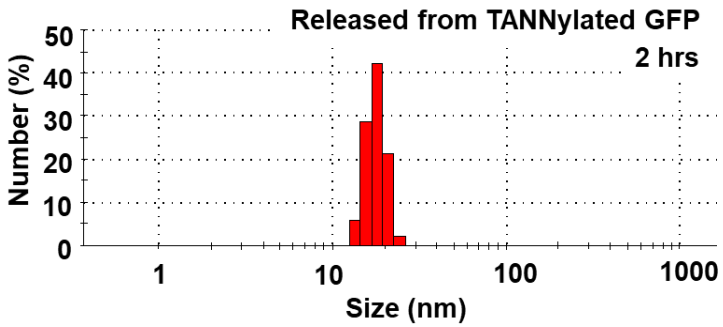

Figure S8. The size distribution of the small complexes released from TANNylated GFP at a ratio of 143 ([TA] /[GFP]) after 2 h incubation.

Supplementary Figure 9.

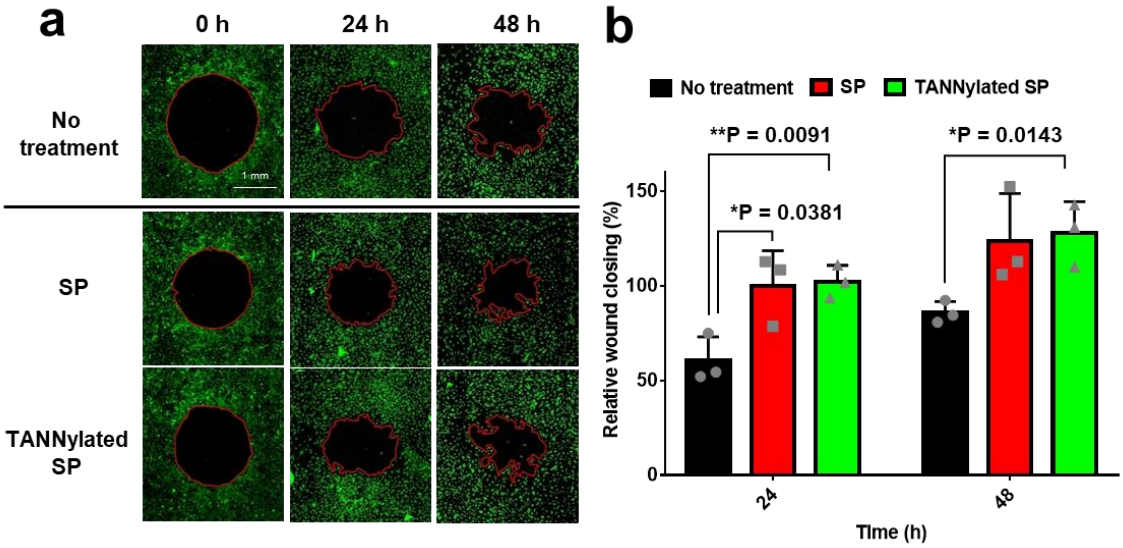

Figure S9. TANNylated SP activity test using *in vitro* wound closing assay using human mesenchymal stem cells. a, Fluorescent images for the migration of the stem cells as a function of time without treatment (1st row), with SP alone (2nd row) or TANNylated SP (3rd row). **b,** Quantitative analysis for the wound closing (relative %) (black bars for no treatment, red bars for SP, and green bars for TANNylated SP). (Data are mean ± s. d. (n = 3). unpaired t-test, *P < 0.05, **P < 0.01).

Supplementary Figure 10.

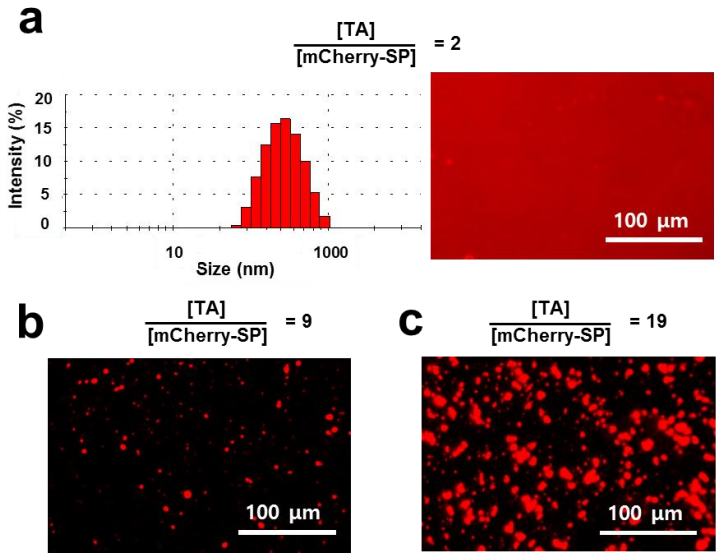

Figure S10. Determination for the stoichiometric ratio of TANNylated mCherry-SP conjugates: a, DLS (left) and the fluorescence image (right) of TANNylated mCherry-SP. **b, c,** Fluorescence images of the TANNylated mCherry-SP at the ratios of 9 (**b**) and the one of 19 (**c**).

Supplementary Figure 11.

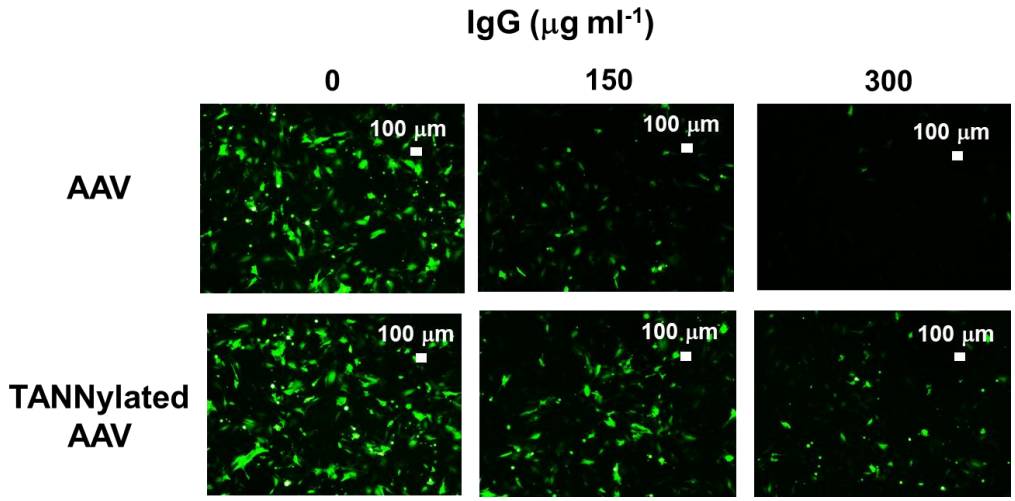

Figure S11. ***In vitro*** **evaluation of antibody evading effect of TANNylated AAV2 as a function of human immunoglobulin G (IgG) concentration, 0, 150, and 300 μg ml^{-1}.**

Supplementary Figure 12.

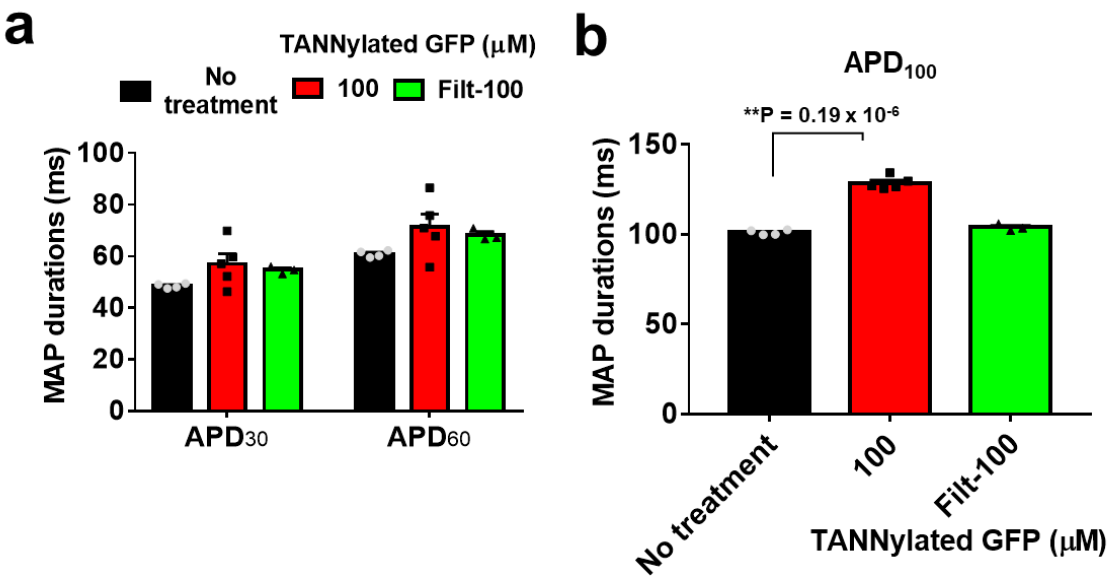

Figure S12. Effects of TANNylated GFP on Monophasic action potentials (MAPs) recorded from Langendorff- perfused rat hearts. a, Statistical analysis of MAP durations at 30 % (APD_{30}) (left bars) and 60 % (APD_{60}) (right bars). **b,** MAP durations at 100 % of full repolarization (APD_{100}) in a vehicle control (K-H solution only) and TANNylated GFP at concentrations of 100 μM or centrifugally filtered one ('Filt-100'). Mean ± s.e.m. each n = 4 for no treatment, each n = 5 for the 100 μM [TA] + GFP group and each n = 3 for the Filt-100 group. **P value compared with the no treatment group. Tukey test, one-way ANOVA.

Supplementary Figure 13.

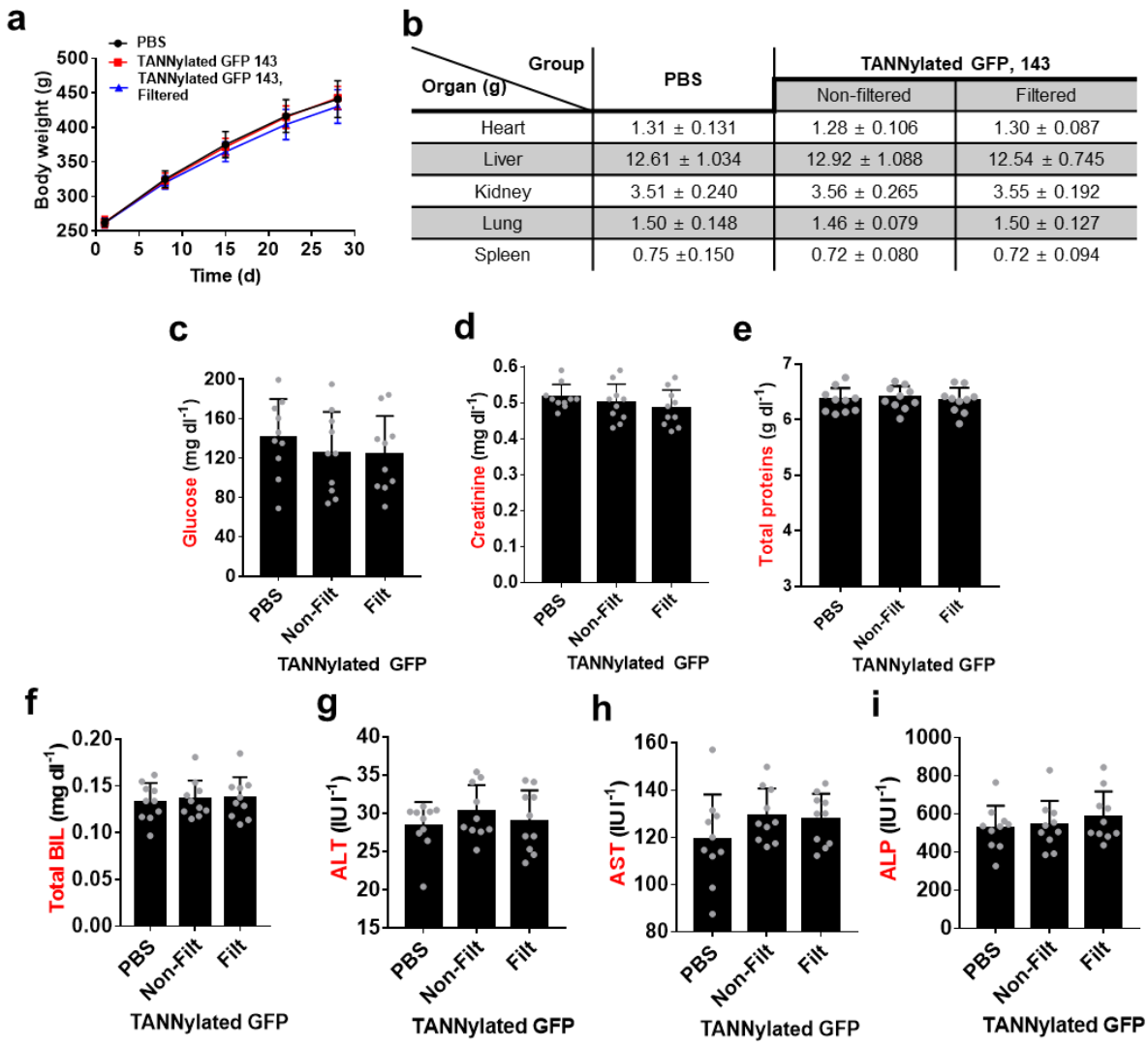

Figure S13. ***In vivo*** **long-term toxicity of TANNylated GFP injected into rats' tail veins (n=30). a,** The changes in total body weights for 29 days post-injection of TANNylated GFP with non-filtration (red, n = 10) and filtration (blue, n = 10), or PBS as a control (black, n =10). **b,** Comparison of the weights of main organs such as heart, liver, kidney, lung, and spleen, at 29 days post-injections. **c to i,** Clinical chemistry analysis for constituents in blood and urine, including glucose (**c**), creatinine (**d**), total proteins (**e**), total bilirubin (BIL) (**f**), aminotransferase (ALT and AST) (**g** and h), and alkaline phosphatase (ALP) (**i**).

Supplementary Figure 14.

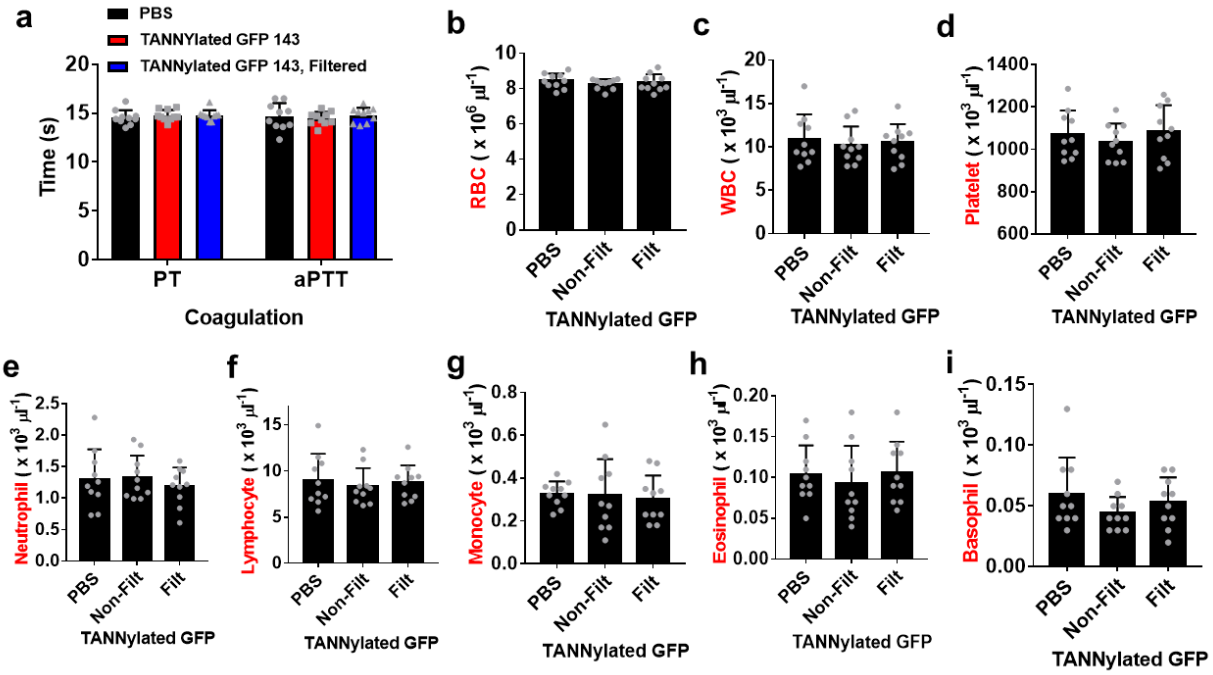

Figure S14. ***In vivo*** **hematological analysis for TANNylated GFP injected into rats' tail veins (n = 30). a,** Prothrombin time (PT) (left bars) and activated partial thromboplastin time (aPTT) (right bars) tests to evaluate coagulation times of the whole blood samples after injecting TANNylated GFP with non-filtration (red, n = 10) and filtration (blue, n = 10), or PBS as a control (black, n = 10). **b to i**, The blood cell count levels at 29 days post-injection, including red blood cells (RBC) (**b**), white blood cells (**c**), platelet (**d**), neutrophil (**e**), lymphocyte (**f**), monocyte (**g**), eosinophil (**h**), and basophil (**i**).

Supplementary Table S1.

Table S1. Evaluation for *in vivo* hemodynamic parameters, blood pressure and volume, at 12 or 24 h post-injection of TANNylated GFP.

	Control	TANNylated GFP 12 h	TANNylated GFP 24 h
n	6	6	6
ESP, mmHg	93.5 ± 3.4	92.7 ± 6.1	96.5 ± 6.3
EDP, mmHg	5.6 ± 0.7	7.6 ± 1.3	7.2 ± 1.3
ESV, µl	66.5 ± 8.4	71.0 ± 9.4	75.2 ± 6.4
EDV, µl	256.9 ± 17.4	246.3 ± 15.0	236.3 ± 27.4
SW, mmHg·ml·kg	3.06 ± 0.31	3.02 ± 0.24	3.05 ± 0.29
Ea, mmHg μl^{-1}	0.52 ± 0.07	0.54 ± 0.03	0.60 ± 0.07

Values are means ± s.e.m; n, no. of rats; ESP, end systolic pressure; EDP, end diastolic pressure; ESV, end systolic volume; EDV, end diastolic volume; SW, Stroke work; Ea, arterial elastance (measure of ventricular afterload).

Supplementary Table S2.

Table S2. Therapeutic evaluation for *in vivo* hemodynamic parameters such as blood pressure and volume after 28 days of injection of TANNylated bFGF or bFGF alone injections.

	Sham	MIR model		
		No treatment	bFGF	TANNylated bFGF
n	6	7	7	7
HR, bpm	310.0 ± 17.7	329.8 ± 15.2	316.5 ± 12.9	322.7 ± 18.0
ESP, mmHg	99.7 ± 1.4	74.3 ± 4.7*	85.3 ± 1.7*	103.8 ± 4.6
EDP, mmHg	8.7 ± 1.5	5.7 ± 1.2	8.5 ± 0.9	9.8 ± 1.6
ESV, μl	48.6 ± 11.0	280.2 ± 13.5*	254.5 ± 13.8*	133.9 ± 19.5*
EDV, μl	255.2± 39.8	396.2 ± 13.4*	365.3 ± 27.5*	295.9 ± 23.3
SW, mmHg·ml·kg	3.23 ± 0.54	1.39 ± 0.18*	1.51 ± 0.14*	3.00 ± 0.53
Ea, mmHg μl^{-1}	0.57 ± 0.18	0.77 ± 0.21	0.86± 0.12	0.77 ± 0.14
Tau, ms	11.2 ± 1.1	10.2 ± 0.3	11.7 ± 0.5	11.7 ± 1.1

Values are means ± s.e.m.; n, no. of rats; HR, heart rate; ESP, end systolic pressure; EDP, end diastolic pressure; ESV, end systolic volume; EDV, end diastolic volume; SW, Stroke work; Ea, arterial elastance (measure of ventricular afterload), Tau, relaxation time constant calculated by Weiss' method. *$P < 0.05$, compared with the sham group. Dunnett's test.