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Supplementary Materials for

Targeting inflammatory sites through collagen affinity enhances the therapeutic efficacy of anti-inflammatory antibodies

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/11/eaay1971/DC1)

Data file S1 (Microsoft Excel format). Original data.

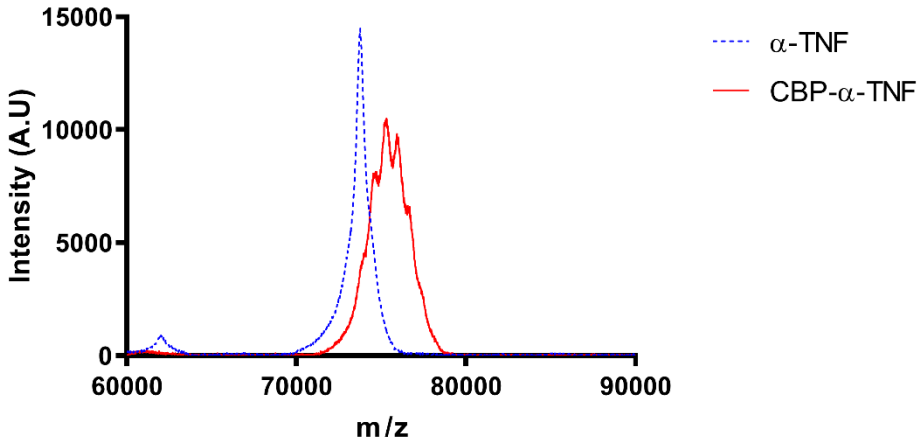

Fig. S1. The molecular weight of α-TNF was increased by CBP conjugation. Unmodified α-TNF and CBP-α-TNF was analyzed by MALDI-TOF MS. Abscissa is mass to charge ratio (m/z) and ordinate is intensity of doubly charged ions.

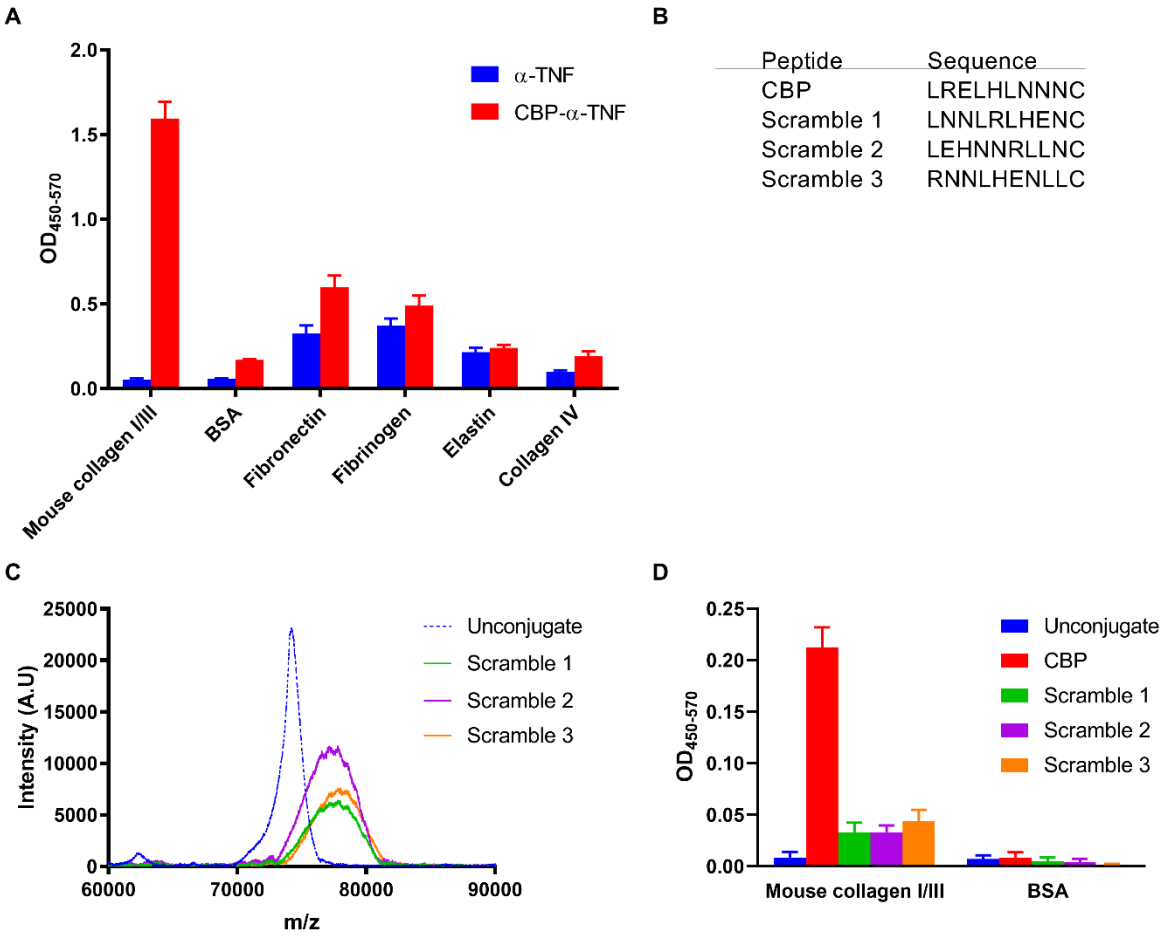

Fig. S2. The target and sequence specificity of CBP. (**A**) Unmodified α-TNF and CBP-α-TNF binding affinities to mouse types I and III collagen, BSA, fibronectin, fibrinogen, and elastin are analyzed by ELISA (n=3, mean + SD). (**B**) Sequences of CBP and its sequence scrambled peptides (Scramble 1, Scramble 2, and Scramble 3), (**C**) Unmodified α-TNF (unconjugate) and scramble peptide-conjugated α-TNF analyzed by MALDI-TOF MS. Abscissa is mass to charge ratio (m/z) and ordinate is intensity of doubly charged ions. (**D**) Unmodified α-TNF, CBP-α-TNF, and scramble peptide-conjugated α-TNF binding affinities to mouse types I and III collagen and BSA are analyzed by ELISA (n=3, mean + SD).

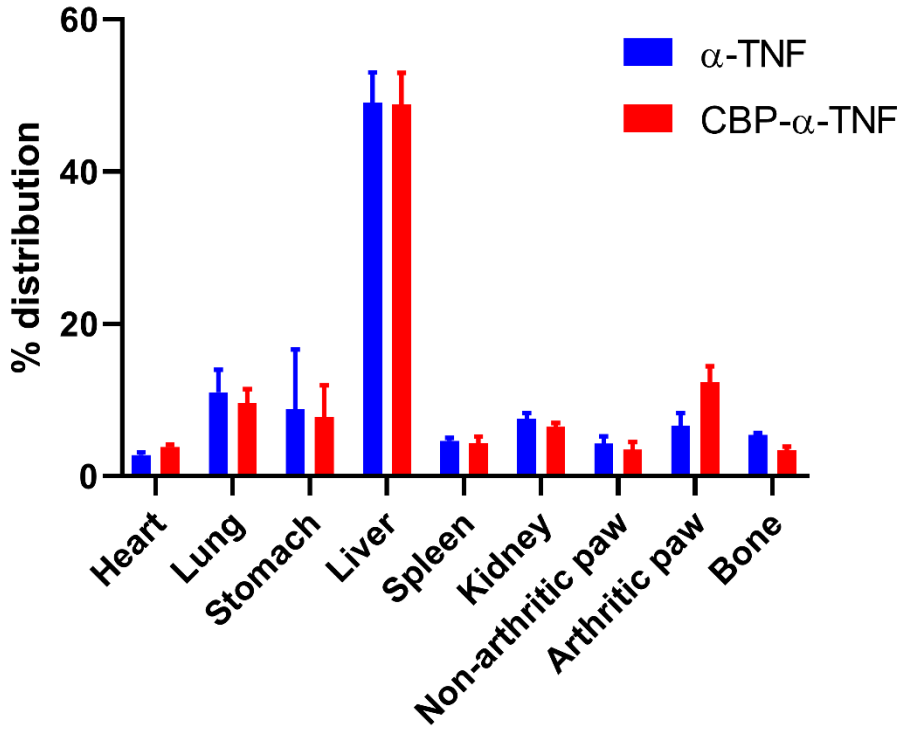

Fig. S3. Distribution of CBP–α-TNF to organs and tissues in the arthritis model. Arthritis (CAIA) was induced selectively in the right hind paw by passive immunization of anti-collagen antibodies, followed by subcutaneous injection of LPS at right hind footpad and of PBS at left hind footpad. On the day following LPS injection, Cy7 labeled CBP-α-TNF and Cy7 labeled α-TNF were intravenously injected into naïve and CAIA mice. One hour after the injection, heart, lung, stomach, liver, spleen, kidney, left hind paw (non-arthritic paw), right hind paw (arthritic paw), and left hind femur/knee/tibia (bone) were collected and their fluorescence levels were measured. Data represent the distribution of Cy7 labeled α-TNF and Cy7 labeled CBP-α-TNF as determined by fluorescence analysis of each organ (n=4, mean + SD).

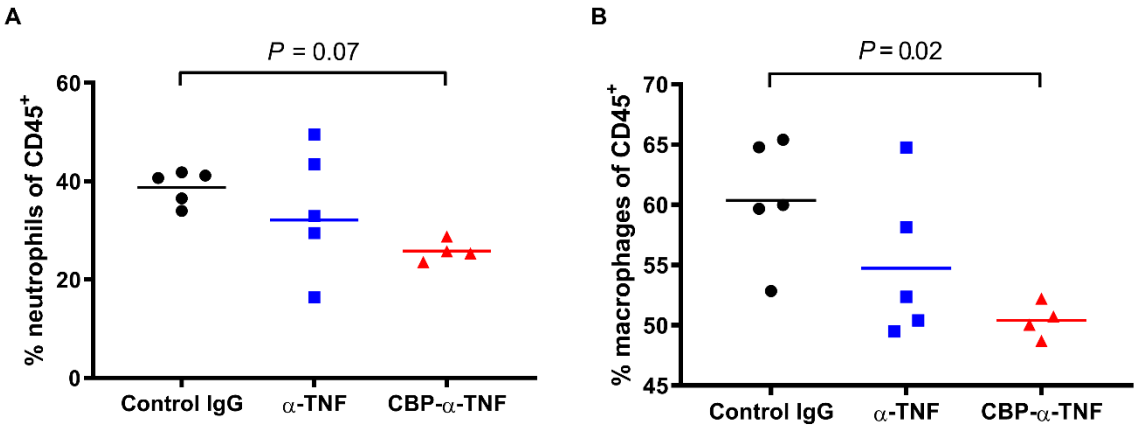

Fig. S4. CBP–α-TNF reduces macrophages and neutrophils within the paws. The number of leukocytes within the paws were analyzed by flow cytometry (n=4-5). Graphs depict (**A**) Ly6ChighLy6G^{+}CD11b^{+} neutrophils within CD45^{+} cells, (**B**) F4/80^{+}CD11b^{+} macrophages within CD45^{+} cells. Bars represent geometric mean. Statistical analyses were performed using Tukey's multiple comparison test.

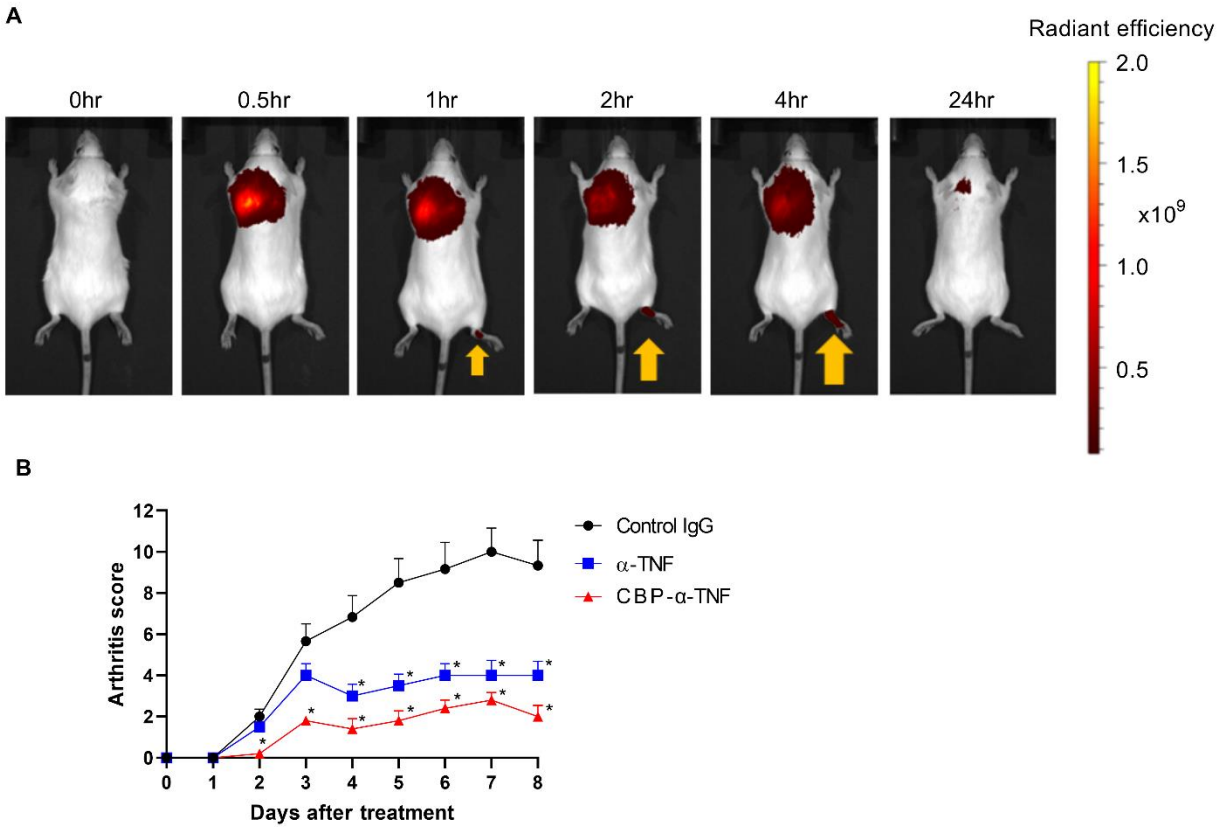

Fig. S5. Effect of subcutaneous injection of CBP–α-TNF in the arthritis model. (**A**) Arthritis was induced selectively in right hind paw by passive immunization of anti-collagen antibodies, followed by subcutaneous injection of LPS at right hind footpad and PBS at left hind footpad. On the day following LPS injection, Cy7 labeled CBP-α-TNF was subcutaneously injected at the back of the mouse. Representative images of accumulation in arthritic or non-arthritic paws of mice injected with CBP-α-TNF (indicated by arrows). (**B**) Arthritis was induced in all paws by passive immunization of anti-collagen antibodies, followed by intraperitoneal injection of LPS. On the day of LPS injection, control IgG, unmodified α-TNF, or CBP-α-TNF was subcutaneously injected. Arthritis scores represent the mean + SE from 5 or 6 mice. * $P <$ 0.05, compared with control (Dunnett's multiple comparison test).

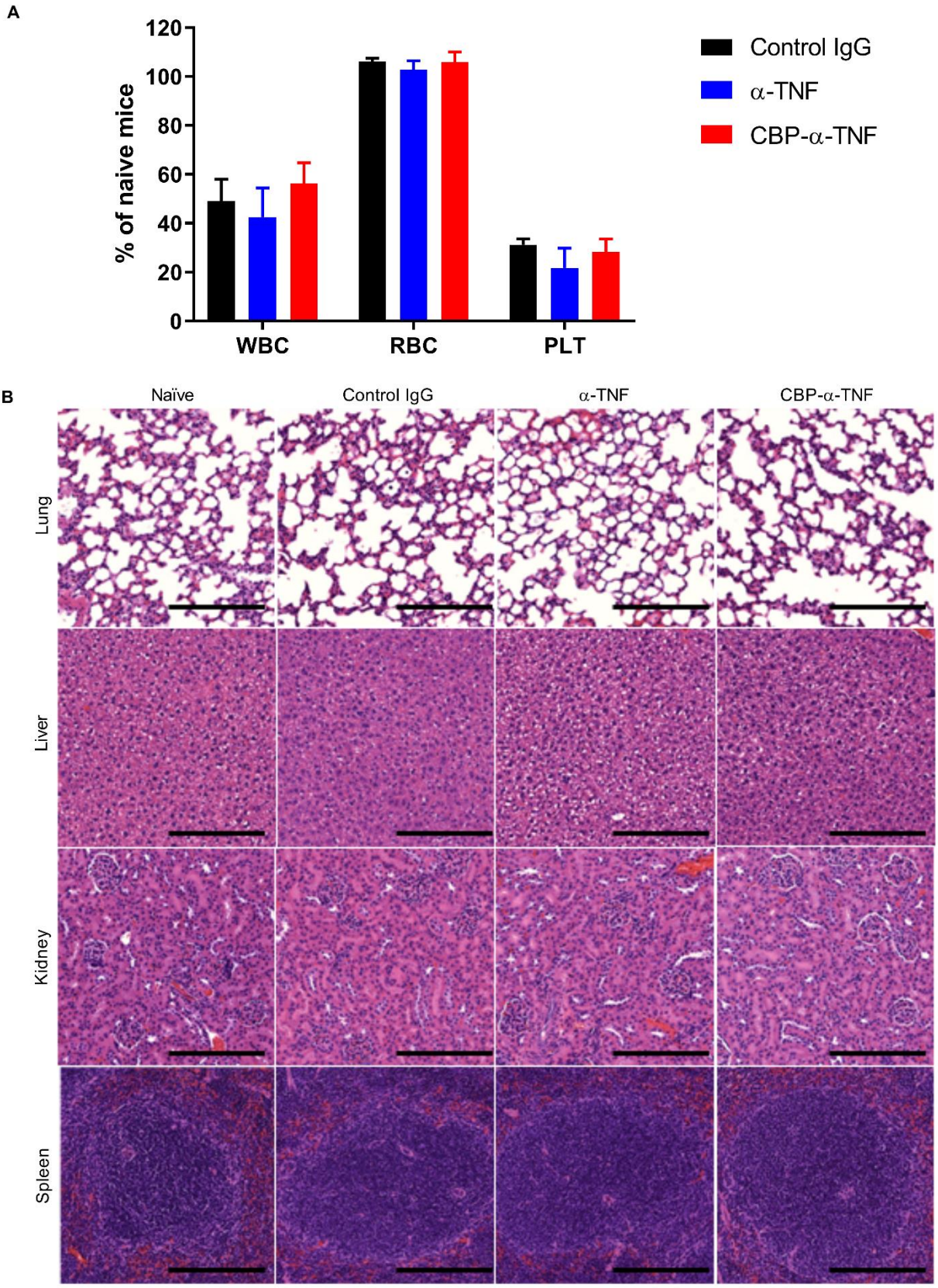
A
120
100
80
60
40
20
0
% of naive mice
WBC
RBC
PLT
Control IgG
α-TNF
CBP-α-TNF
B
Naïve
Control IgG
α-TNF
CBP-α-TNF
Lung
Liver
Kidney
Spleen

Fig. S6. Short-term safety study of CBP–α-TNF in the arthritis model. Mice were given anti-collagen antibody, followed by LPS. On the day of LPS injection, control IgG, unmodified α-TNF, or CBP-α-TNF at 200 μg/mouse was intravenously injected. (**A**) Complete cell counts (white blood cells [WBC], red blood cells [RBC], and platelets [PLT]) on the following day of drug injection (n=3, mean + SD). (**B**) H&E staining of major organs collected three days after drug injection. Scale bar, 200 μm.

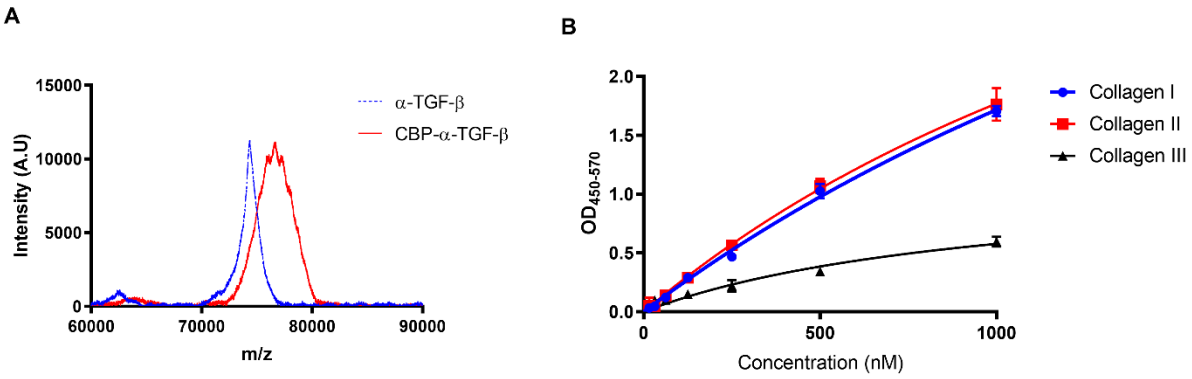

Fig. S7. CBP conjugation provided collagen affinity to α–TGF-β. (**A**) Unmodified α-TGF-β and CBP-α-TGF-β analyzed by MALDI-TOF MS. Abscissa is mass to charge ratio (m/z) and ordinate is intensity of doubly charged ions. (**B**) CBP-α-TGF-β binding affinities to types I, II, and III collagen are analyzed by ELISA (n=3, mean ± SD).