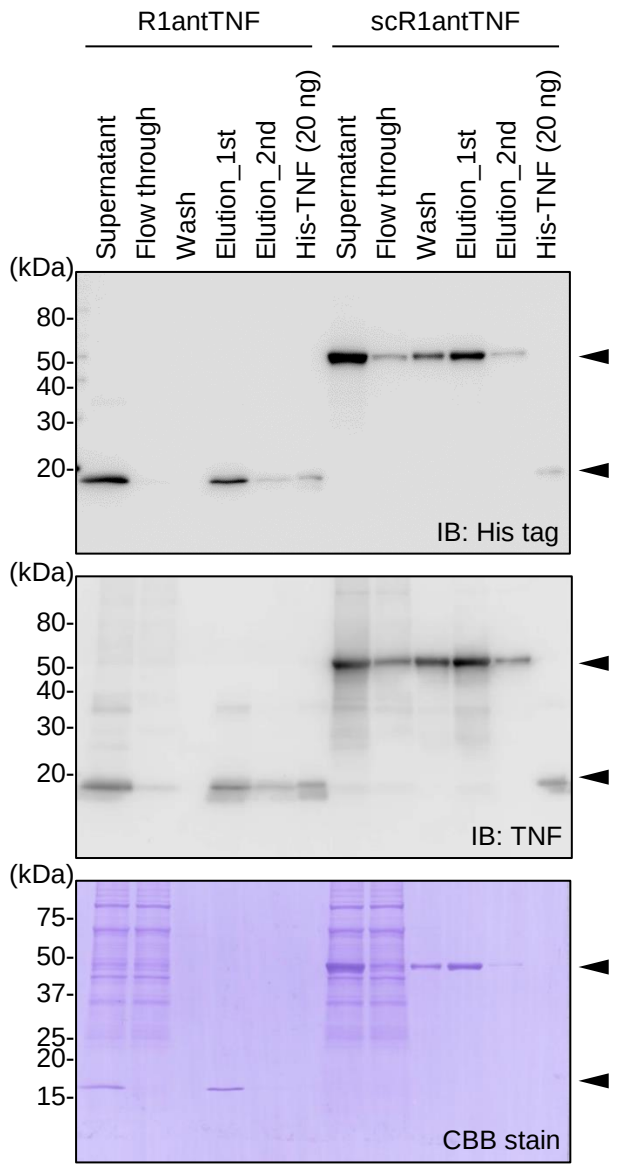
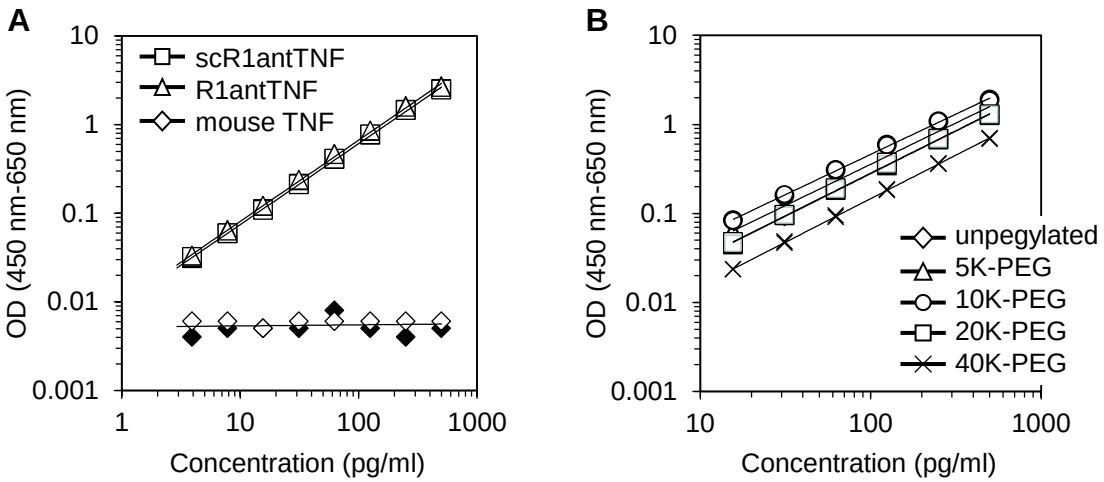


Supplementary Fig. 1 **Purification of TNF mutants using immobilized metal ion chromatography**

After immobilized metal ion chromatography (IMAC), R1antTNF and scR1antTNF in each fraction were detected by western blotting with anti-His-tagged antibodies (upper panel) or anti-TNF antibodies (middle panel). Coomassie brilliant blue (CBB) staining was also performed (lower panel). Protein bands for each TNF mutant are shown by arrowheads.



Supplementary Fig. 2 **Detection of TNF mutants or mouse TNF by ELISA using an anti-human TNF antibody**

(A) Absorbances of R1antTNF (triangles) and scR1antTNF (squares), but not mouse TNF (rhombuses), were increased in a concentration-dependent manner. These data suggested that TNF mutants could be detected by ELISA using an anti-human TNF antibody without being affected by mouse TNF (rhombuses). Each sample was measured in duplicate. (B) Mono-PEGylated R1antTNFs were detected by ELISA using an anti-human TNF antibody. Absolute absorbance values tended to decrease with increasing PEG molecular weight, but that of each PEGylated mutant increased in a concentration-dependent manner. Therefore, it is possible to quantify blood concentrations of the respective PEGylated mutants.