Supplementary

Figure S1. Inhibition of binding anti-ferritin autoantibody (IgM) and canine liver ferritin due to the presence of synthesized peptide (DPHLCDF). The synthesized peptide fragment (DPHLCDF) was added to a final concentration of 4.2 μg/mL in 1 mL of PBS containing partially purified FBPs and canine liver ferritin (5 μg) to achieve a 1:500 mole ratio of peptide to ferritin. The mixture was incubated overnight with rotation at 4 °C followed by the addition of rabbit anti-feline liver ferritin antiserum (10 μL). Immunocoprecipitate was coated on plate wells as described in Figure 1. The detection of IgM antibody was performed as described in Figure 1 in the presence (solid bar) or absence (open bar) of peptide. The control indicates the absorbance in the absence of FBPs, and this value was subtracted from the measured ferritin-binding activity for each sample. Inhibition of binding (%) was determined as a percent of control binding activity determined in the absence of peptide and set to 100%. Each bar and error bar represents the mean ± SD of four determinations. * $p < 0.01$, compared to the absence of peptide.

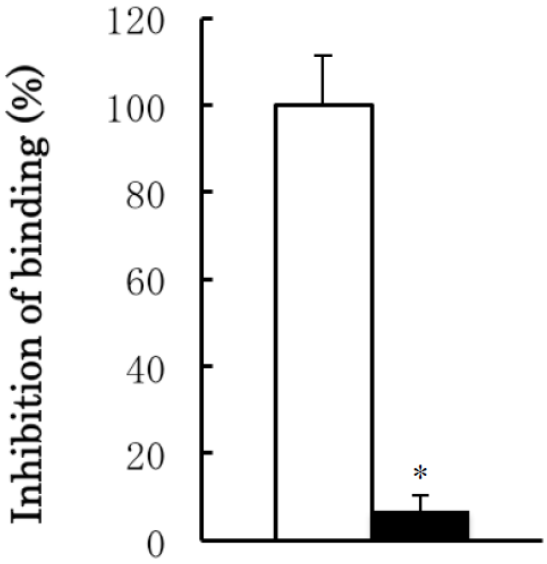

Figure S2. Binding of human serum zinc-binding proteins with synthesized peptide (DPHLCDF). Zn^{2+} immobilized beads (solid bar) or control beads (open bar) were added to 1 mL of PBS containing 100 μL of the human serum samples, which was removed serum components non-specifically binding control beads (Sepharose 4B) from human serum as described in "Experimental Section", in the glass tube (20 μL each), and the mixture was incubated overnight with rotation at 4 °C. After rotation, the mixture was centrifuged at 2,700 × *g* for 15 min. The precipitated gel was washed three times with 1 mL of PBHS at every step with the same centrifugation at 2,700 × *g* for 15 min. The precipitated gel was suspended in 1 mL of PBS containing 0.4 μg/mL of biotinylated peptide (DPHLCDF), and the mixture was incubated overnight at 4 °C. Then, the mixture was centrifuged at 2,700 × *g* for 15 min, and the precipitated was washed three times with 1 mL of PBHS at every step with the same centrifugation. The precipitated gel was suspended with 1 mL of PBS containing ALP-labeled avidin (5 μg/tube) at 37 °C for 1 h. After incubating and washing, the precipitated gel suspended with 1 mL of substrate solution for ALP reaction as described in the "Experimental Section". Control value was absorbance in the corresponding beads in the absence of biotinylated peptide, and each control value was subtracted from the corresponding measured value for peptide-binding activity. Each bar and error bar represents the mean ± SD of four determinations. * $p < 0.01$, compared with the control beads.

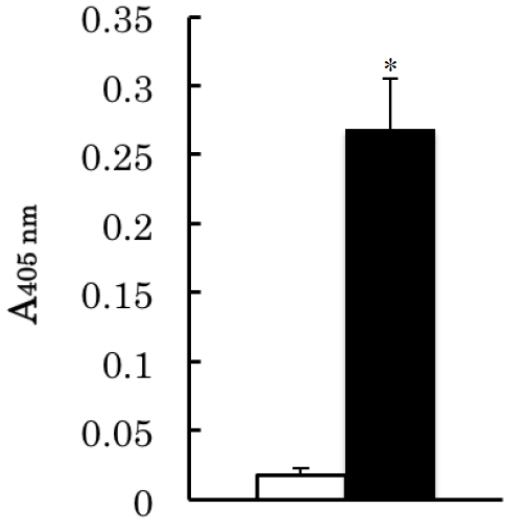

Figure S3. The procedure of detection of human anti-ferritin autoantibodies (IgG, IgM and IgA) by immunocoprecititation method. After immunocomplex was formed between homologous homopolymer ferritin (recombinant H and L subunit homopolymers) or heterohomopolymer ferritin (canine liver ferritin with L/H subunit ratio of 2.3 [35]) and autoantibodies to ferritin in human serum, antibodies to H subunit, L subunit or heterolohous anti-feline liver ferritin antibody were added to immunocoprecipitate the corresponding ferritin. The immunocoprecitate obtained was coated on the plate well, and immunogloblins (IgG, IgM and IgA) on the wells were indirectly detected with antibody specific for each heavy chain of human immunoglobulin (IgG, IgM and IgA).