Supporting Information

Choi *et al.* 10.1073/pnas.0805198105

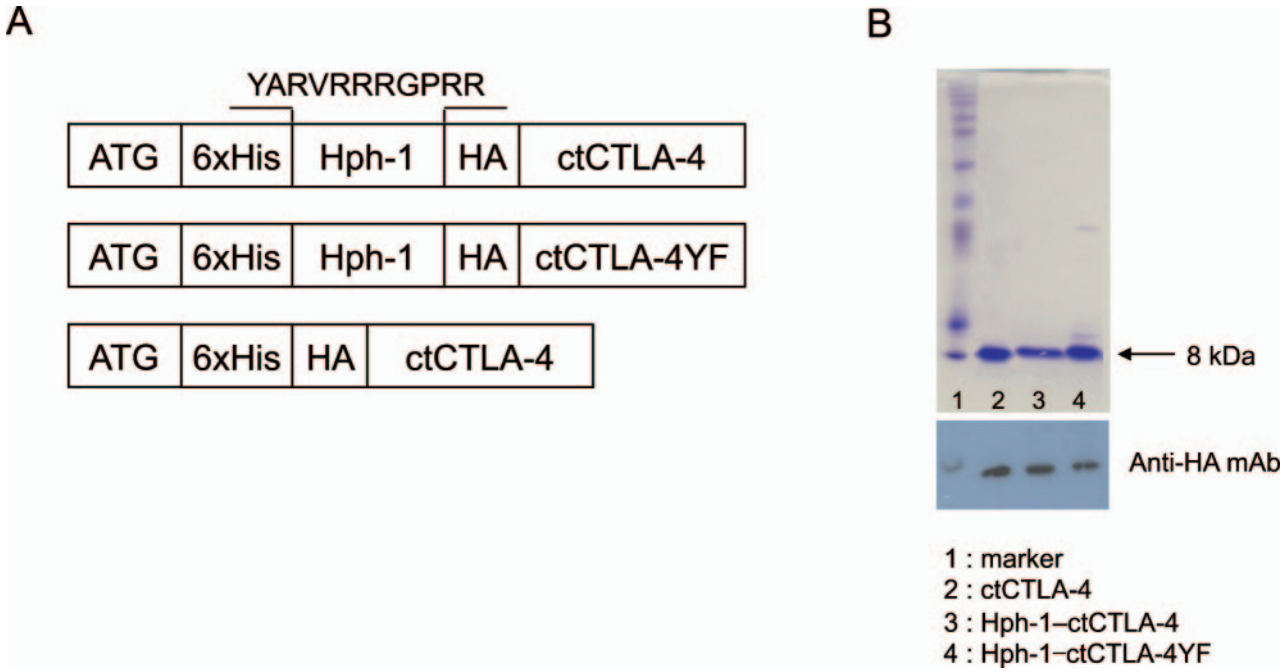

Fig. S1. Purification of cell-permeable recombinant CTLA-4 proteins. Each recombinant DNA plasmid was transformed into BL-21 (DE3 star) *Escherichia coli* and single colonies were picked. After overnight growth protein was expressed by the addition of 1 mM IPTG for 5 h at 37 °C. After induction, bacteria were harvested, sonicated in 6 M urea buffer, and washed using soluble condition buffer containing 20 mM imidazole. High concentrations of imidazole were used as elution buffers (100 mM, 200 mM, 3 M). The eluates were desalted immediately to PBS with 10% glycerol by passing through a PD-10 column and then were aliquoted and stored at −80 °C. Purified proteins were analyzed by Western blot analysis with anti-HA mAb.

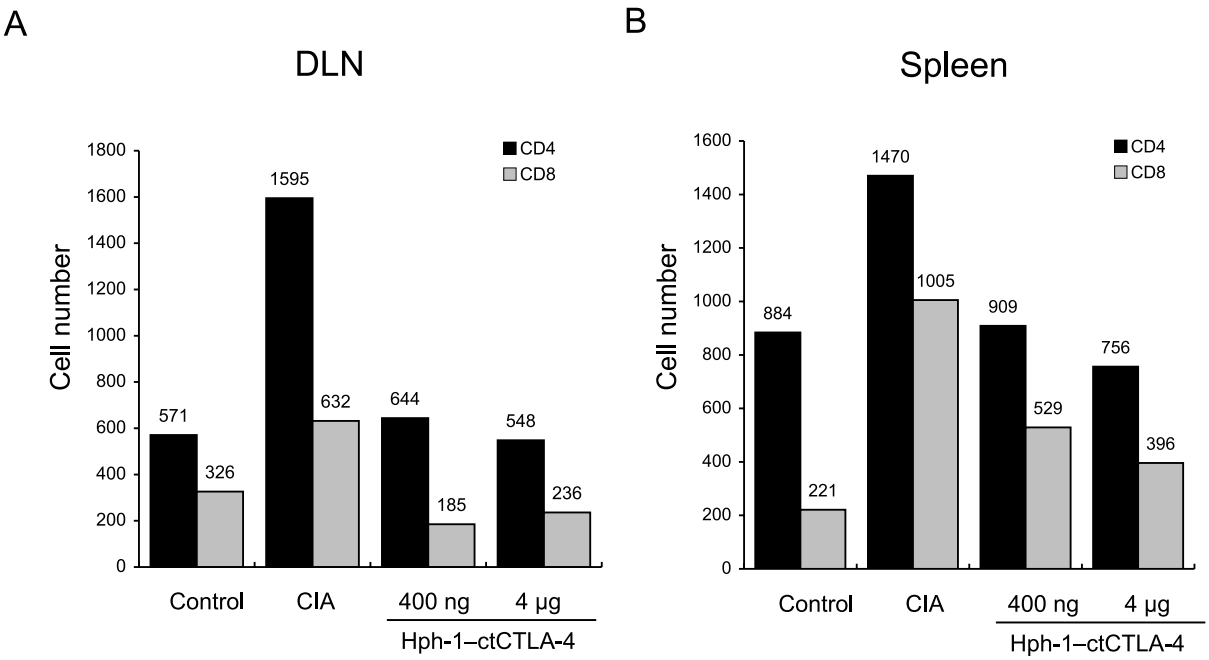

Fig. S2. Hph-1-ctCTLA-4 treatment reduced the number of CD4 and CD8 T cells in CIA mice. CD4 and CD8 T cell populations in drained lymph node and spleen in CIA mice were analyzed by flow cytometry. Data are represented by calculated total absolute cell number depending on total cell count and gated percentage in CD4- or CD8-positive cells.

Table S1. Inhibition of infiltration of inflammatory cells into joint tissue by Hph-1-ctCTLA-4 in the collagen-induced arthritis model

	Joint	
Treatment	Total cells ($\times 10^6$)	$CD11b^+/Gr\text{-}1^+$ ($\times 10^5$)
Control	1.2 ± 0.0	0.5 ± 0.4
CIA	7.7 ± 0.8	19.8 ± 1.5
ctCTLA-4	4.5 ± 0.9	11.8 ± 0.1
Hph-1–ctCTLA-4 (4 μg)	3.2 ± 0.7	4.0 ± 1.6
Hph-1–ctCTLA-4 (40 ng)	3.6 ± 0.5	8.7 ± 3.4
Hph-1–ctCTLA-4YF (4 μg)	5.1 ± 0.5	9.7 ± 5.0

Data are presented as the mean ± SEM. Individual cells from knee joints were stained with FITC-conjugated anti-CD11b mAb and PE-labeled anti-Gr1 mAb to measure $CD11b^+/Gr\text{-}1^+$ granulocytes.