Figure S1

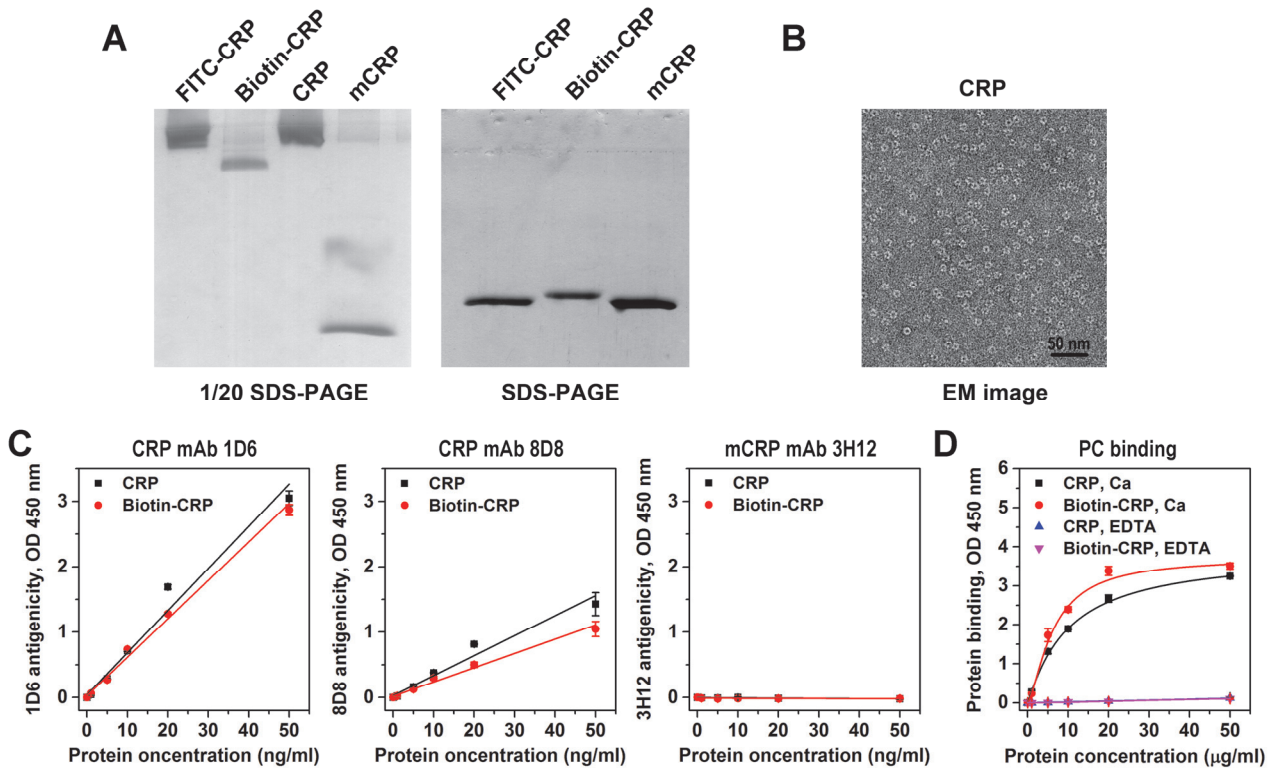

Figure S1. Native structure and normal antigenicity of CRP is preserved after labeling with FITC and biotin. Human CRP, FITC-CRP, and biotin-CRP all migrated in 1/20 SDS-PAGE as large molecular weight pentamers (1) and in regular SDS-PAGE as monomers (**A**; mCRP denotes monomeric CRP). (**B**) By electron-microscopy the pentameric structure of CRP was confirmed. (**C**) In sandwich ELISAs (2) mAbs 1D6 and 8D8 that recognize native CRP (3) also recognized biotin-CRP, whereas mAb 3H12 against a neo-CRP antigen (3) did not. (**D**) Both CRP and biotin-CRP bound to immobilized PC-BSA in a calcium-dependent manner.

Figure S2

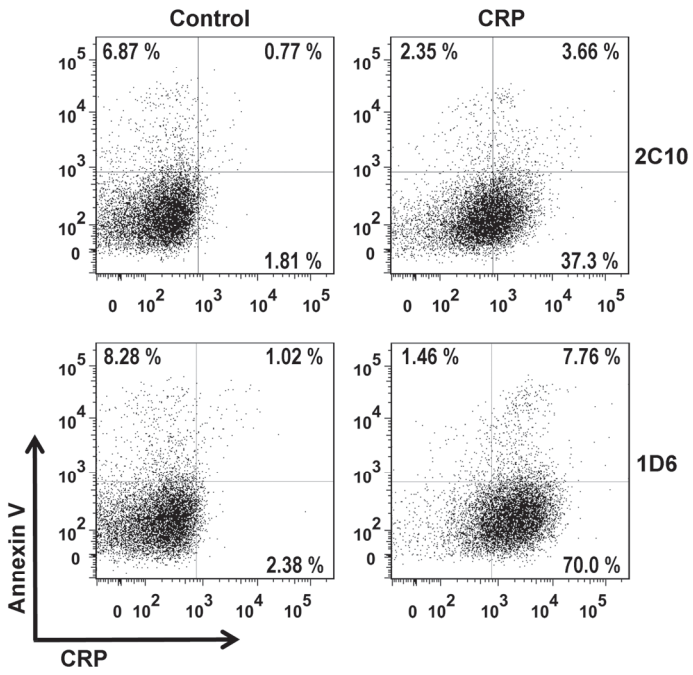

Figure S2. Binding of native CRP to live Jurkat T cells. Based on its detection with mAb 1D6 that recognizes native CRP (3), the majority of human CRP (100 µg/ml) was bound to Annexin V-negative cells (66.4 ± 1.6 % of cells *vs.* 1.3 ± 0.1 % of control cells not exposed to CRP, $p < 0.001$).

Figure S3

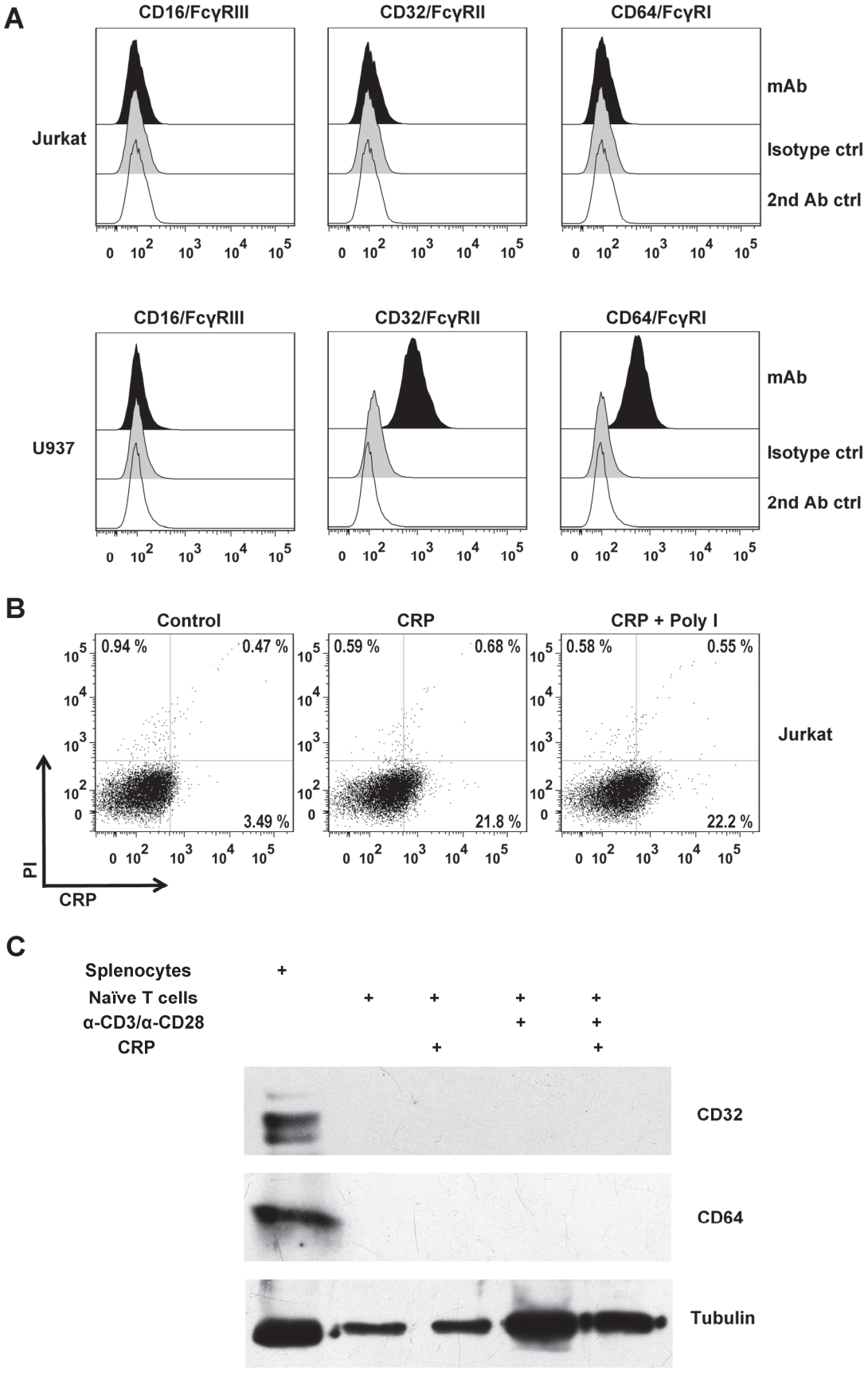

Figure S3. Binding of CRP to human Jurkat and mouse naïve T cells does not require FcγRs nor LOX-1. (**A**) No expression of CD16 (FcγRIII), CD32 (FcγRII) and CD64 (FcγRI) by Jurkat

(human) T cells could be seen by flow cytometry. In contrast, U937 (human) monocytic cells showed robust expression of CD32 and CD64. (**B**) Polyinosinic acid (Poly I, 100 μg/ml), an inhibitor of LOX-1 (4), is unable to inhibit the binding of human CRP (100 μg/ml) to live Jurkat cells. Of note, the lack of LOX-1 expression on mouse $CD3^+$ T cells has also been demonstrated (5). (**C**) Mixed splenocytes or purified mouse naïve T cells were left untreated or activated with anti-CD3/anti-CD28, human CRP, or both for 24 h. Immunoblotting reveals no signal for CD32 and CD64 proteins in naïve T cells.

Figure S4

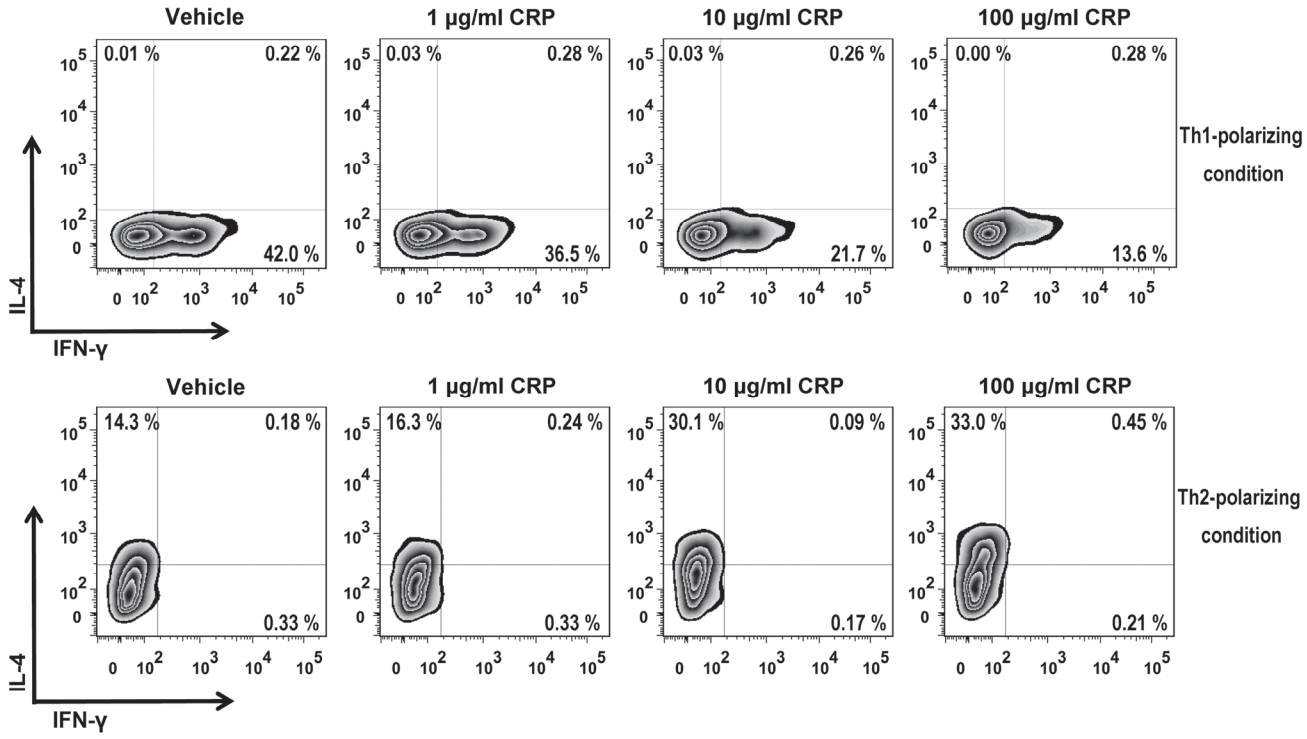

Figure S4. CRP concentration-dependent modulation of Th1/Th2 differentiation. 2 x 10^5 naïve T cells were cultured for 3 days with anti-CD3/anti-CD28 mAbs under Th1 polarizing conditions (10 ng/ml mIL-2, 20ng/ml mIL-12p70, 10 µg/ml anti-IL-4 mAb) or Th2 polarizing conditions (10 ng/ml mIL-2, 20 ng/ml mIL-4, 10 µg/ml anti-IL12 mAb, 10 µg/ml anti-IFN-γ mAb). Human CRP at the indicated concentrations was added to cells 24 h after the beginning of cell differentiation (late treatment as described for Figure 4).

References

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