Supporting Information for

Original article

Celastrol targets adenylyl cyclase-associated protein 1 to reduce macrophages-mediated inflammation and ameliorates high fat diet-induced metabolic syndrome in mice

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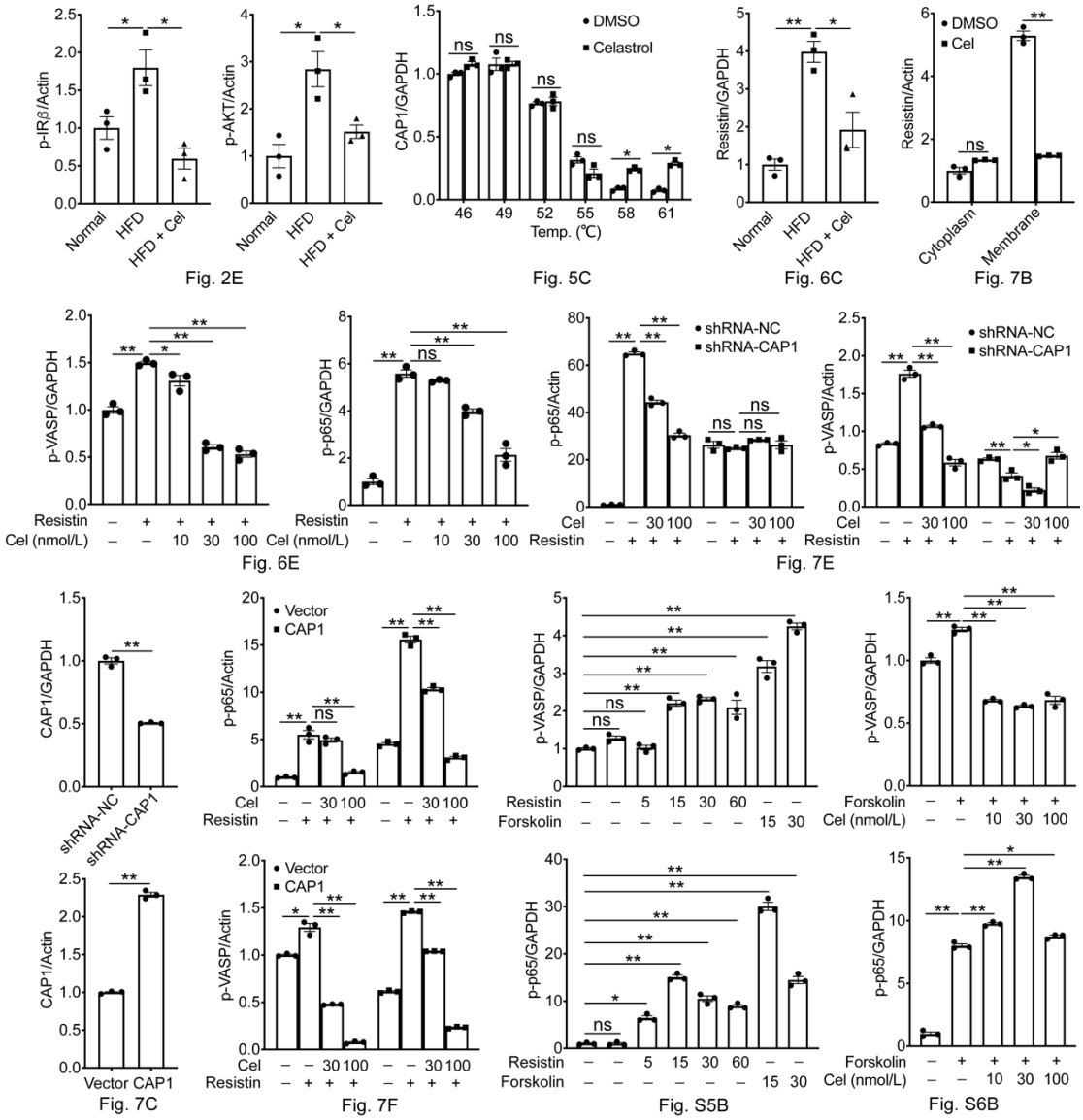

Figure S1 Quantification of data in representative Western blot figures. Data represent as mean ± SEM. *P* values are determined by two-tailed Student's *t* test (*n* = 3). **P* < 0.05, ***P* < 0.01, ns, not significant.

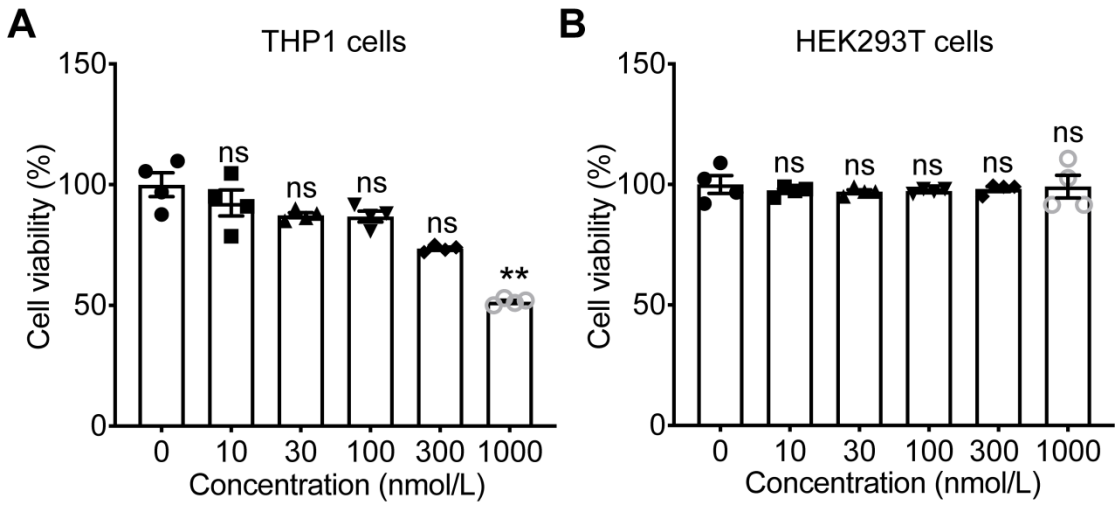

Figure S2 Low dose of celastrol did not affect cell proliferation. (A) THP-1 cells and (B) HEK293T cells were treated with 10–1000 nmol/L of celastrol for 48 h. Cell viability was assessed using the MTT assays. Data represent as mean ± SEM. *P* values are determined by two-tailed Student's *t*-test ($n = 3$). $^{**}P < 0.01$, ns, not significant *vs* medium.

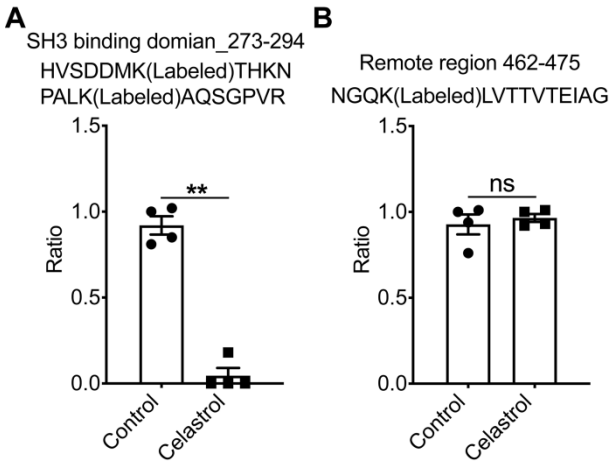

Figure S3 Celastrol-binding domain of CAP1. (A) and (B) Analysis of the combined area of celastrol and CAP1 *via* TRAP experiment. Data represent as mean ± SEM. *P* values are determined by two-tailed Student's *t*-test (n = 3). $^{**}P < 0.01$, ns, not significant.

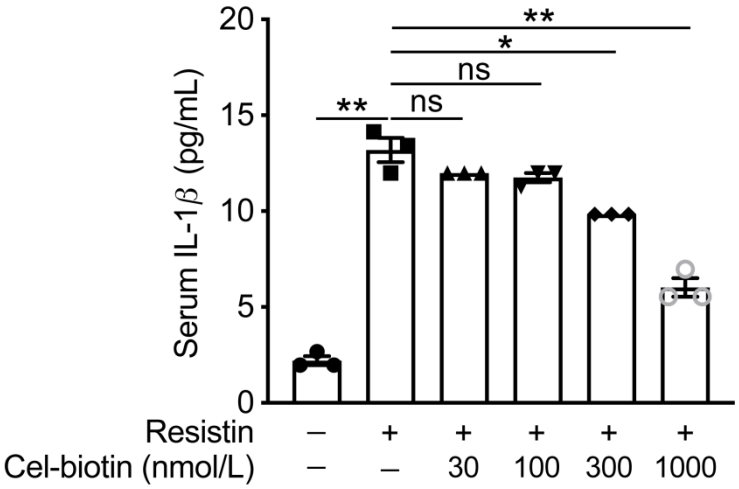

Figure S4 The inhibitory effect of Cel-biotin on IL-1*β*. ELISA quantification of IL-1*β* level in THP-1 cells supernatant. Data represent as mean ± SEM. *P* values are determined by two-tailed Student's *t* test ($n = 3$). *$P < 0.05$, **$P < 0.01$, ns, not significant.

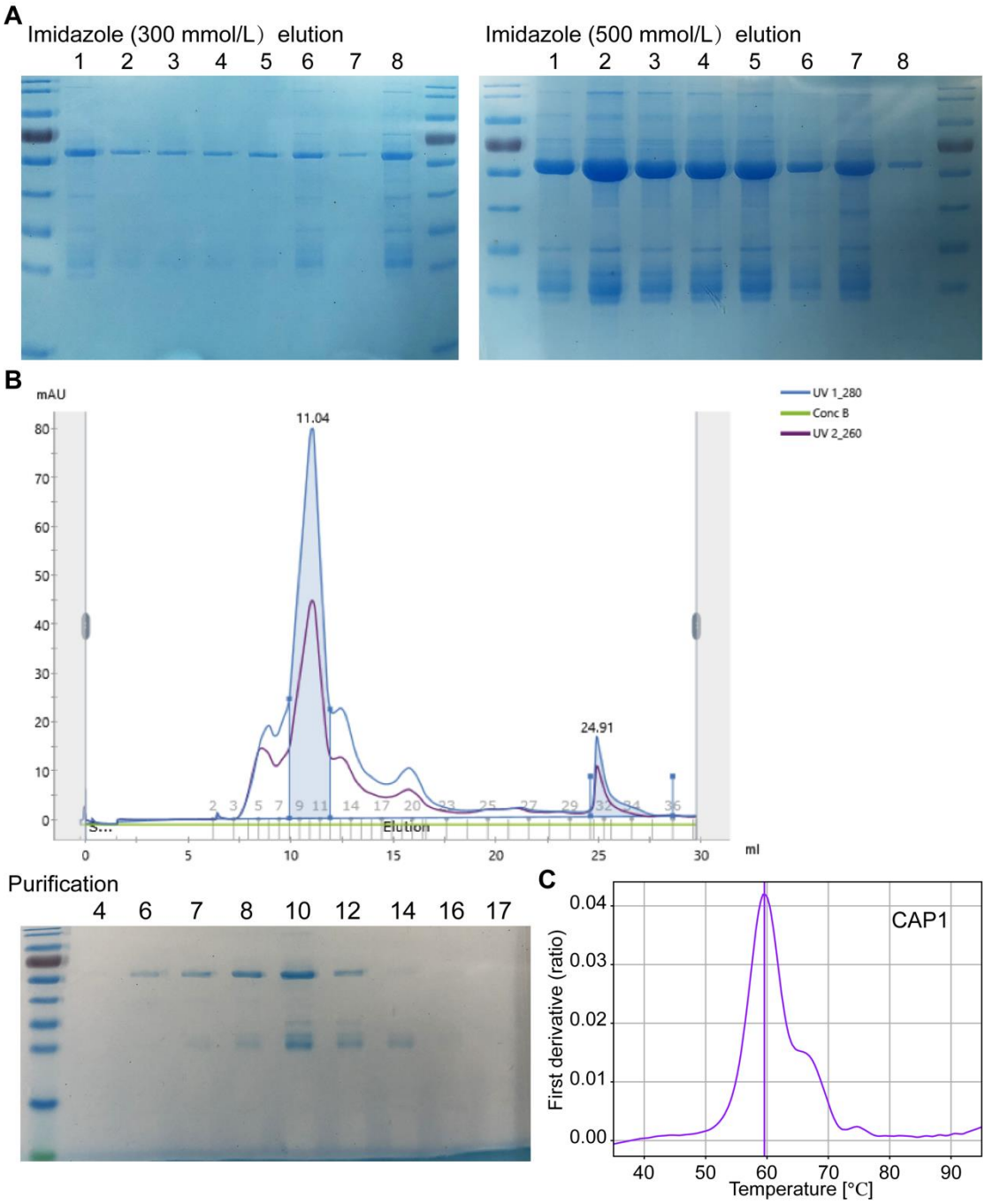

Figure S5 CAP1 expression and purification. (A) SDS-PAGE analysis of CAP1 elution. (B) SDS-PAGE analysis of CAP1 purification after gel filtration chromatography. (C) Analysis of CAP1 quality.

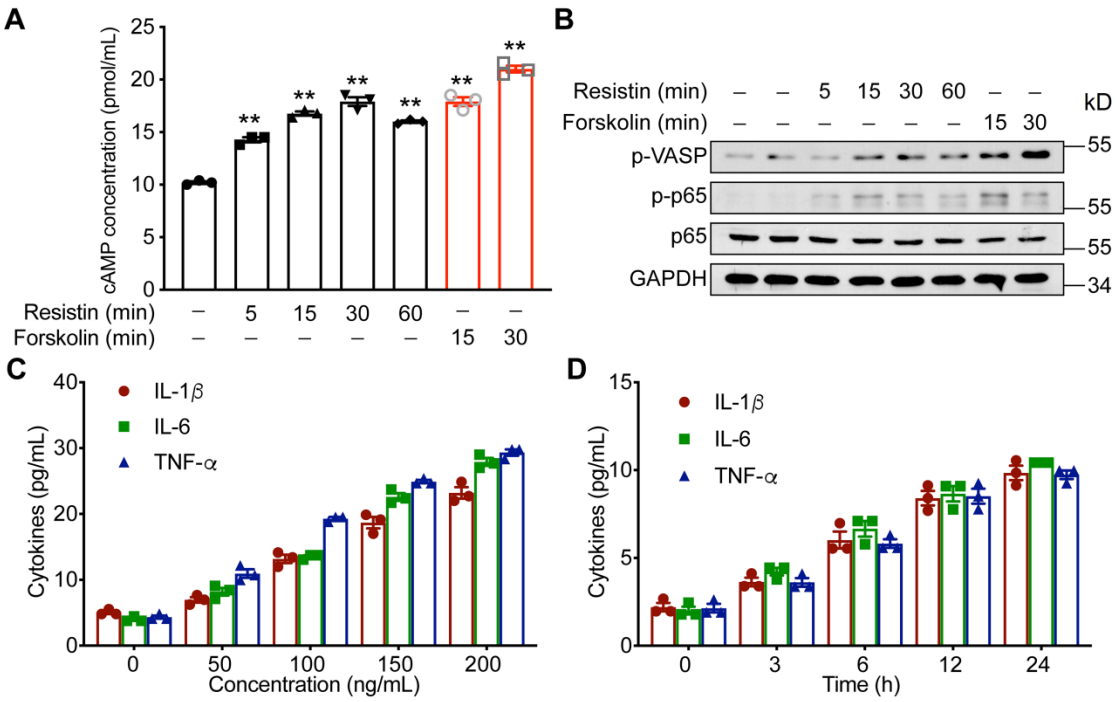

Figure S6 Resistin promotes the activation of PKA–NF-κB signaling and inflammatory cytokine production. (A) ELISA quantification of cAMP level in cell supernatant. (B) PKA–NF-*κ*B signaling in PMA–differentiated THP-1 cells were determined by Western blot analysis after resistin or forskolin treating. (C) and (D) Protein levels of cytokines in cell supernatant were determined by ELISA after resistin dose– or time–dependent treating. Data represent as mean ± SEM. *P* values are determined by two-tailed Student's *t* test ($n = 3$). $^{**}P < 0.01$.

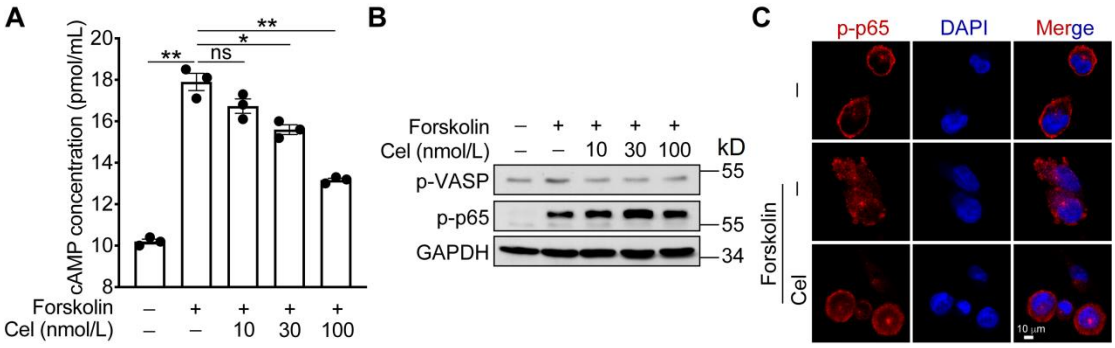

Figure S7 Forskolin activates cAMP/PKA signaling and induces cytokine expression. (A) ELISA quantification of cAMP level in cell supernatant. (B) Proteins in PKA–NF-κB signaling pathways in PMA-differentiated THP-1 cells were determined by Western blot analysis after celastrol treating. (C) Immunofluorescence staining of PMA-differentiated THP-1 cells were treated with celastrol for 2 h, and then stimulated with forskolin for 15 min. Data represent as mean ± SEM. *P* values are determined by two-tailed Student's *t* test (n = 3). *P < 0.05, **P < 0.01, ns, not significant.