Supporting Information for

Original article

Targeted inhibition of GRK2 kinase domain by CP-25 to reverse fibroblast-like synoviocytes dysfunction and improve collagen-induced arthritis in rats

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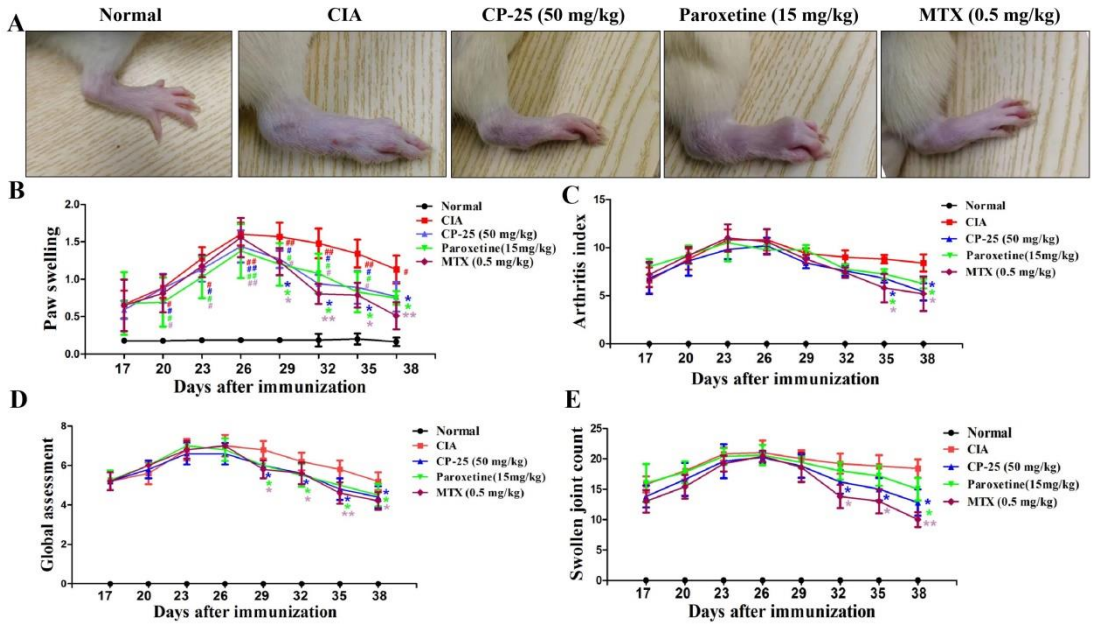

Figure S1 CP-25 treatment obviously attenuates the manifestation of CIA rats. Successfully establishing CIA rats model, CP-25 (50 mg/kg/day), paroxetine (15 mg/kg/day), and MTX (0.5 mg/kg/3 days) with continuous administration for 21 days, and meanwhile there were age-matched normal control groups. The onset of assess was on around Day 17 after the first injection. The representative pictures of swelling joints were presented (A). Paw swelling (B), arthritis index (C), global assessment (D), swollen joints count (E) were evaluated following the published standard. The grading scheme consisted of ordinal categories ranging from 0 (no effect) to 3 (severe effect). Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* CIA group ($\bar{x} \pm s$, $n = 6$~8).

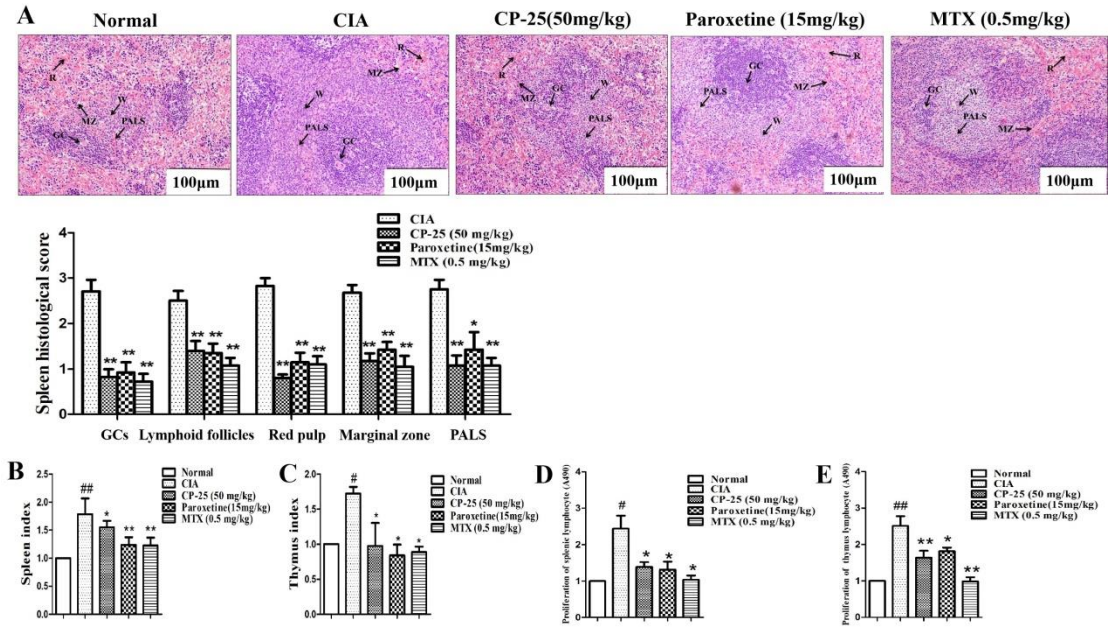

Figure S2 CP-25 treatment improves spleen and thymus function of CIA rats. (A) Representative micrographs of HE-stained histological sections of the spleens are shown. The histology section shows the red pulp (R), the marginal zone (MZ), the germinal centre (GC), and the periarteriolar lymphoid sheaths (PALS). The grading scheme consisted of ordinal categories ranging from 0 (no effect) to 3 (severe effect). Scale bars = 100 μm. (B) and (C) Effects of CP-25 on spleen index (B) and thymus index (C) of CIA rats. (D) and (E) Effects of CP-25 on proliferation of splenic lymphocyte (D) and thymus lymphocyte (E) in CIA rats. Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* CIA group ($\bar{x} \pm s$, $n = 6$).

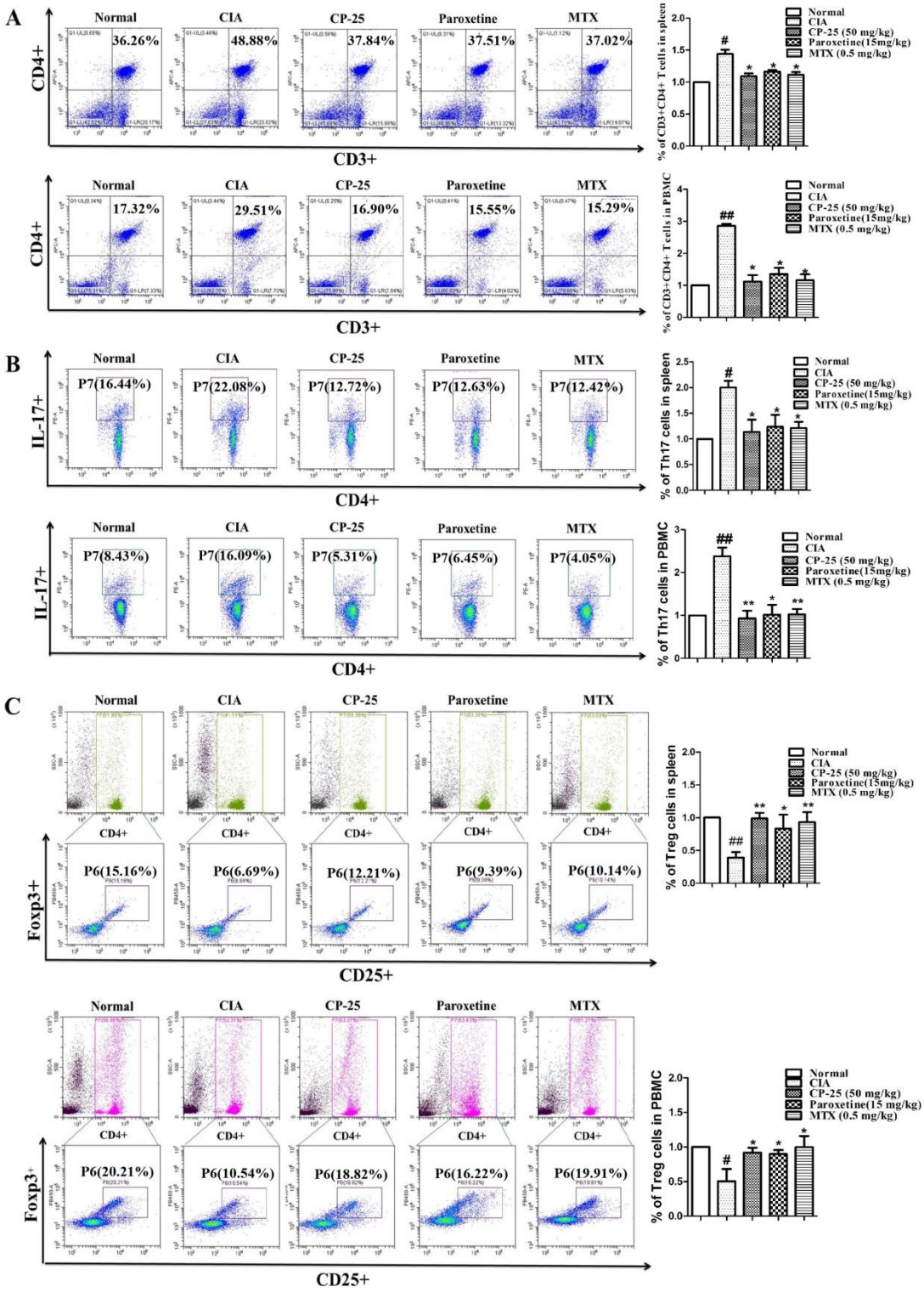

Figure S3 CP-25 treatment regulates the percentage of T cells in PBMC and spleen of CIA rats. Flow cytometry was used to detect the percentage of $CD3^+CD4^+$ T cells (A), Th17 (B) and Treg cells (C) in spleen and PBMC of CIA rats. Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* Normal group; $*P < 0.05$, $**P < 0.01$ *vs.* CIA group ($\bar{x} \pm s$, $n = 6$).

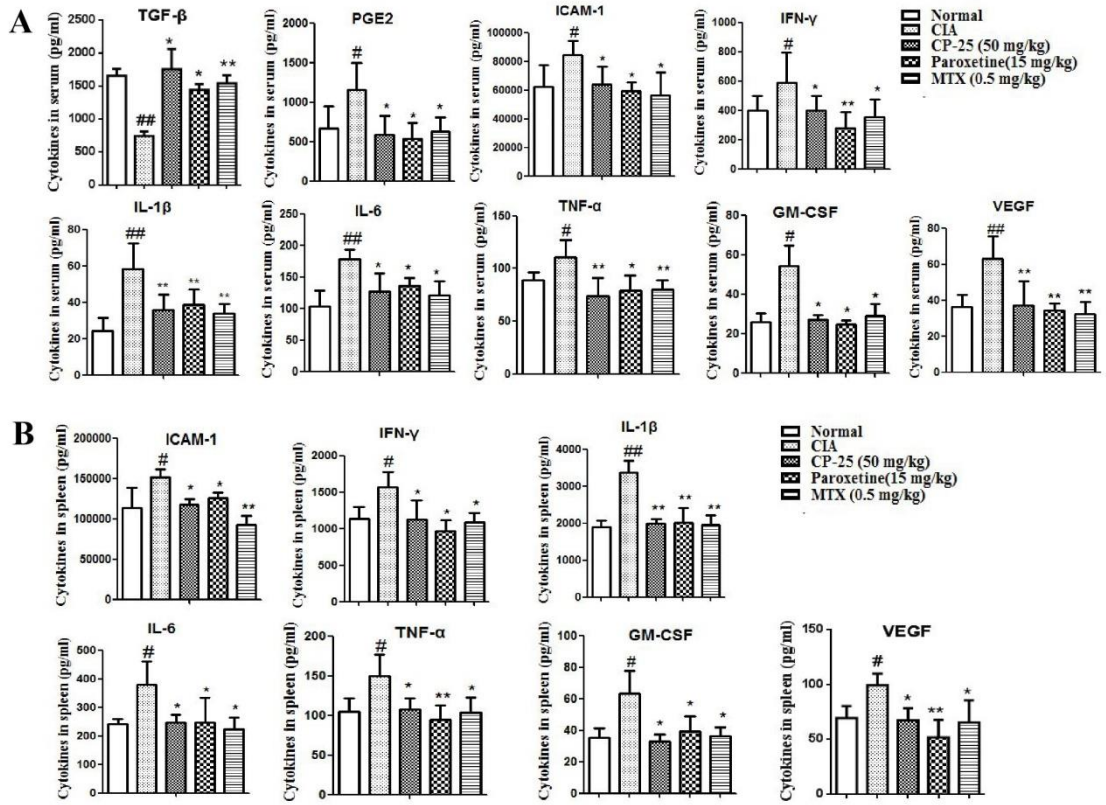

Figure S4 CP-25 treatment regulated cytokines serum and spleen of CIA rats. PGE2, TNF-*α*, IFN-*γ*, TGF-*β*, VEGF, ICAM-1, IL-1*β*, GM-CSF and IL-6 cytokines level in serum (A) and spleen (B) of CIA rats. Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* CIA group ($\bar{x} \pm s$, $n = 6$).

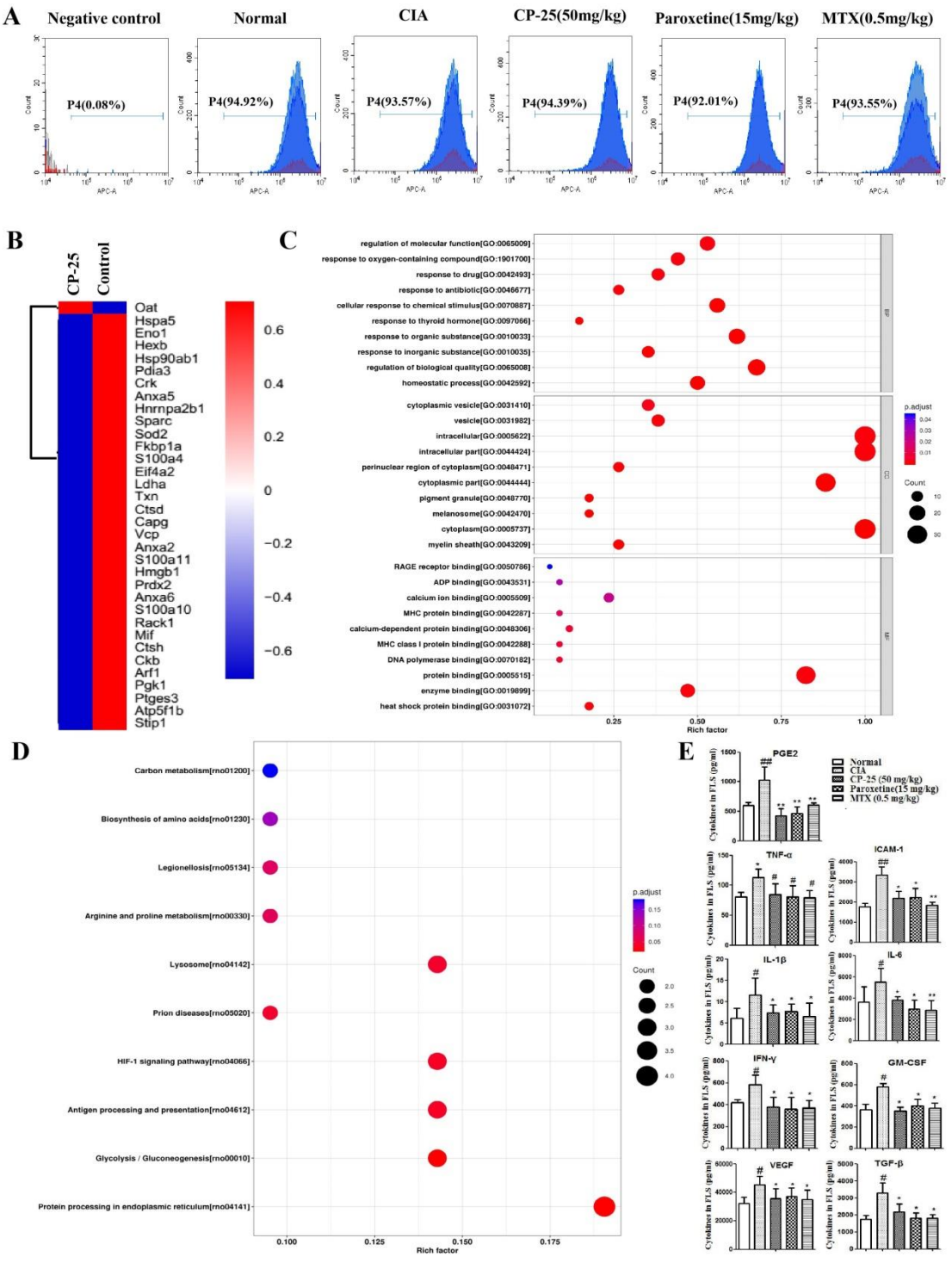

Figure S5 Identification of target proteins in FLS for CP-25. (A) The FLS of CIA rats were identified by flow cytometry (n = 4). (B) Thermodynamic diagram of differentially expressed proteins by hierarchical cluster analysis. (C) GO enrichment analysis. BP: biological process; MF: molecular function; CC: cellular component. The X-axis represents the Rich factor value of the degree of enrichment. The size of the circle indicates the number of differentially expressed proteins mapped, and the

larger the circle, the more the number. The color represents the significance of enrichment, the redder the color, the smaller the *P* value. (D) KEGG pathway enrichment analysis. The *X*-axis represents the Rich factor value of the degree of enrichment. The size of the circle indicates the number of differentially expressed proteins mapped, and the larger the circle, the more the number. The color represents the significance of enrichment, the redder the color, the smaller the *P* value. (E) CP-25 modulates cytokines production in the supernatant fluid of FLS (n = 6). Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* CIA group.