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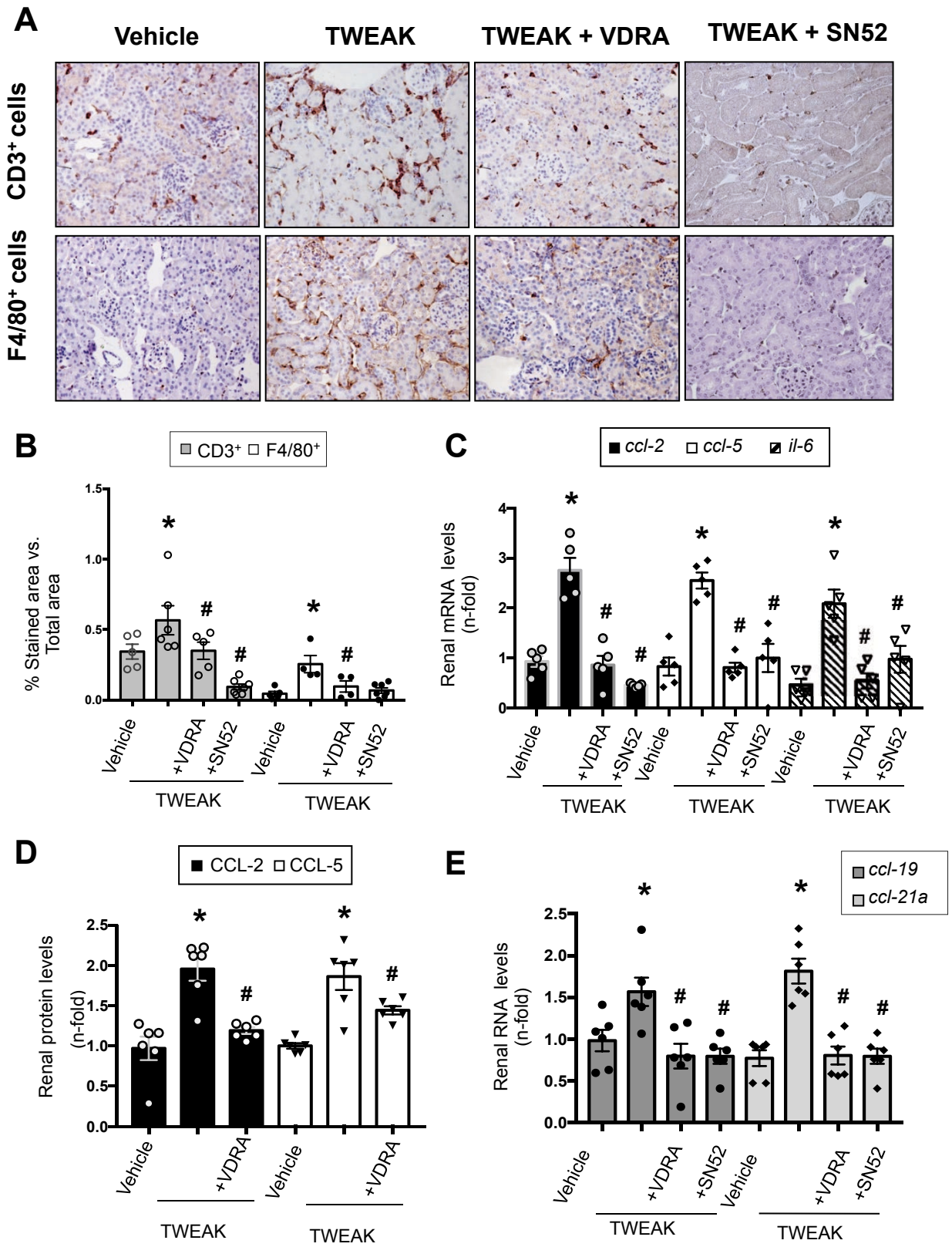
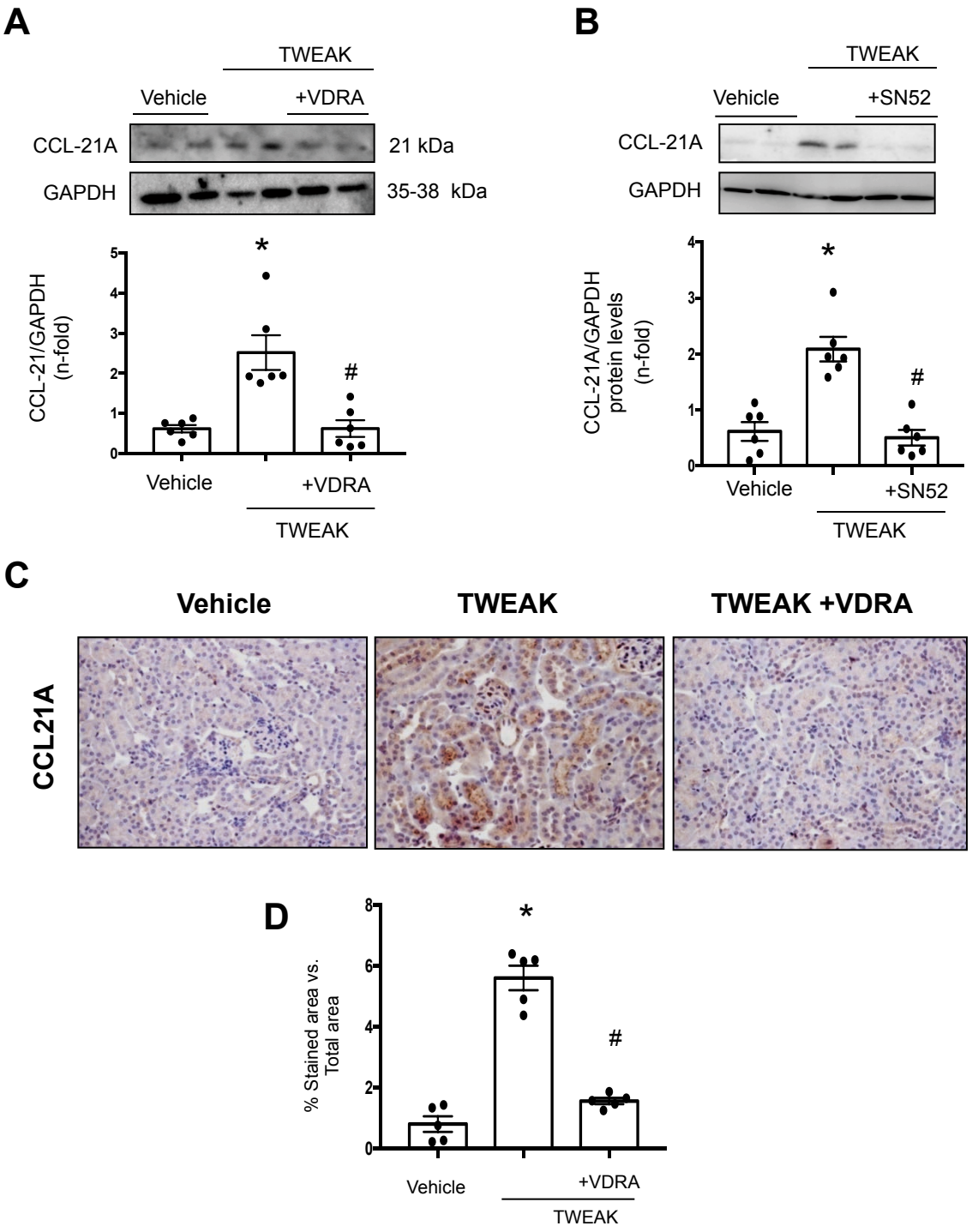
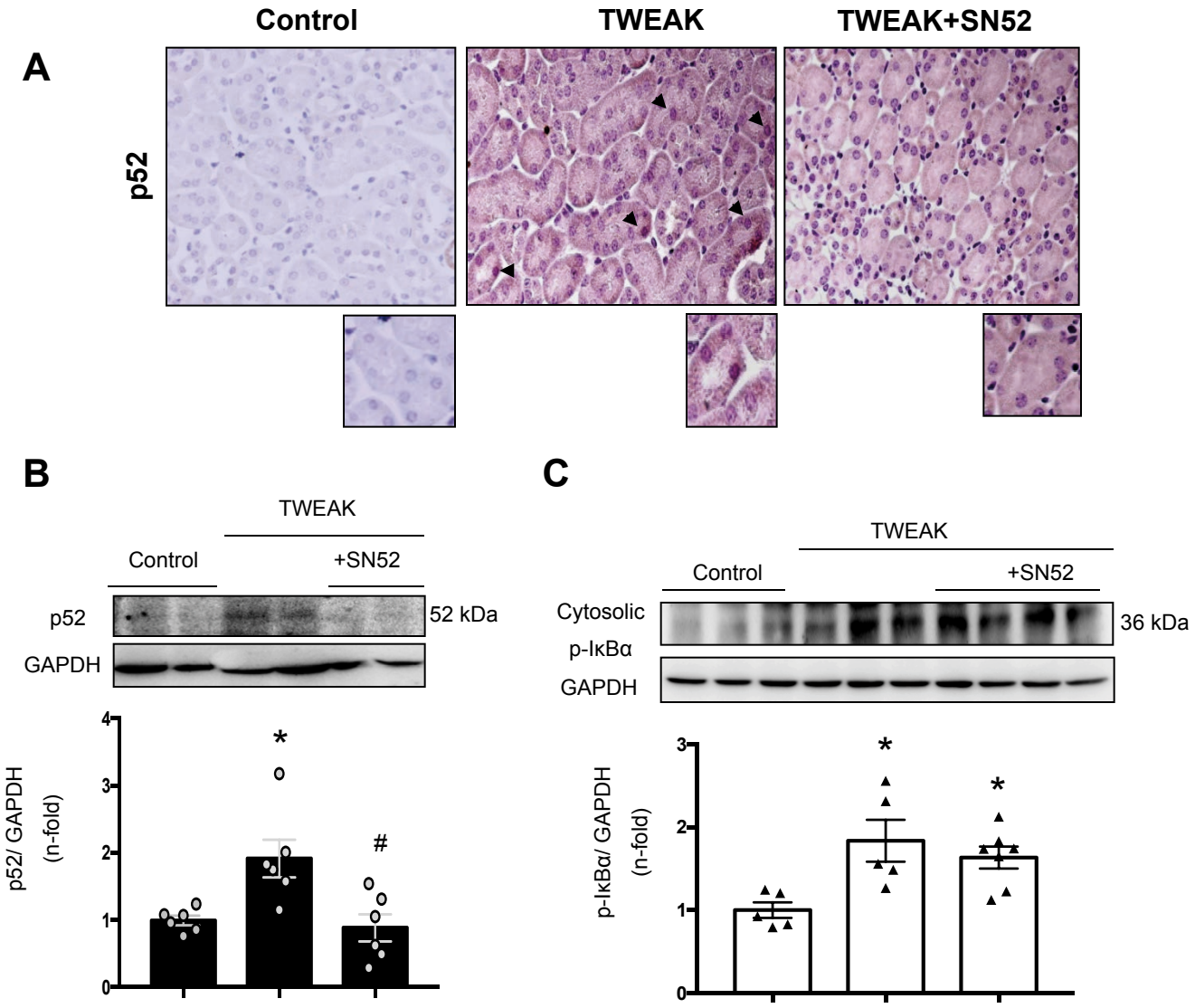


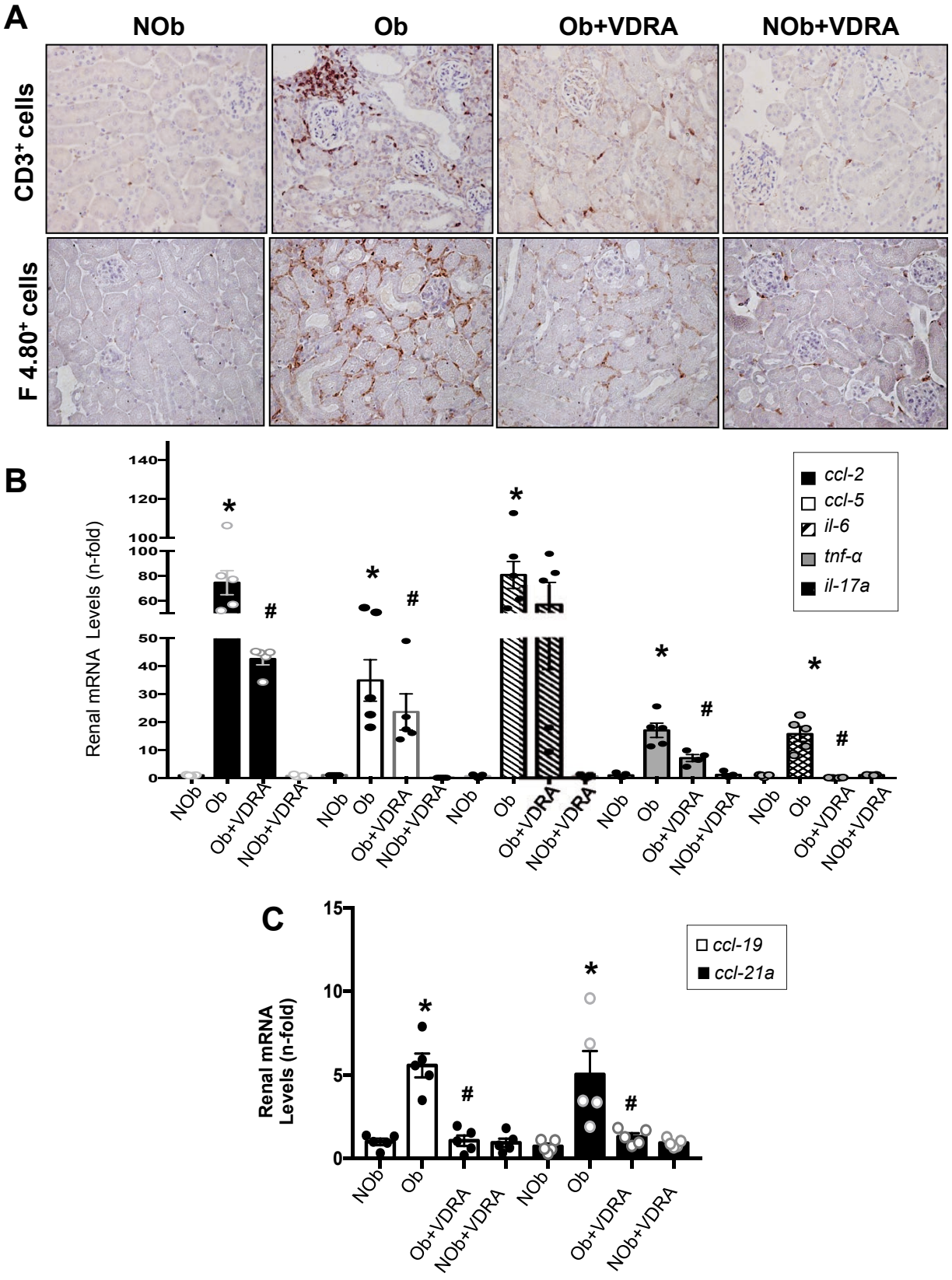
Supplementary Figure 1. Paricalcitol or NF-κB2 blockade decreased TWEAK-induced renal inflammation. C57BL/6 mice were treated with paricalcitol 750 ng/Kg/day or the NF-κB2 inhibitor SN52 0.7 mg/day (two doses; day -1; day 0) starting 48 hours before TWEAK 0.5 μg, and sacrificed 24 hours after TWEAK administration. (**A)** Immunohistochemistry using anti-F4/80 and anti-CD3 identified monocyte/macrophages and T lymphocytes, respectively. Representative animal from each group. Magnification 200X. (**B)** Staining quantification **(C)** RNA was obtained from total renal extracts and proinflammatory gene expression levels (*ccl-2, ccl-5* and *il-6*) were determined by Real Time PCR. (**D)** Kidney CCL-2 and CCL-5 protein levels were evaluated by ELISA. (**E)** NF-κB2-regulated cytokines, *ccl-21a* and *ccl-19* gene expression levels were determined by Real Time PCR. Data expressed as mean±SEM of 5-8 animals per group. Differences between intervention and control groups were assessed by Mann–Whitney test. *p<0.05 *vs* control. # p<0.05 *vs* TWEAK.



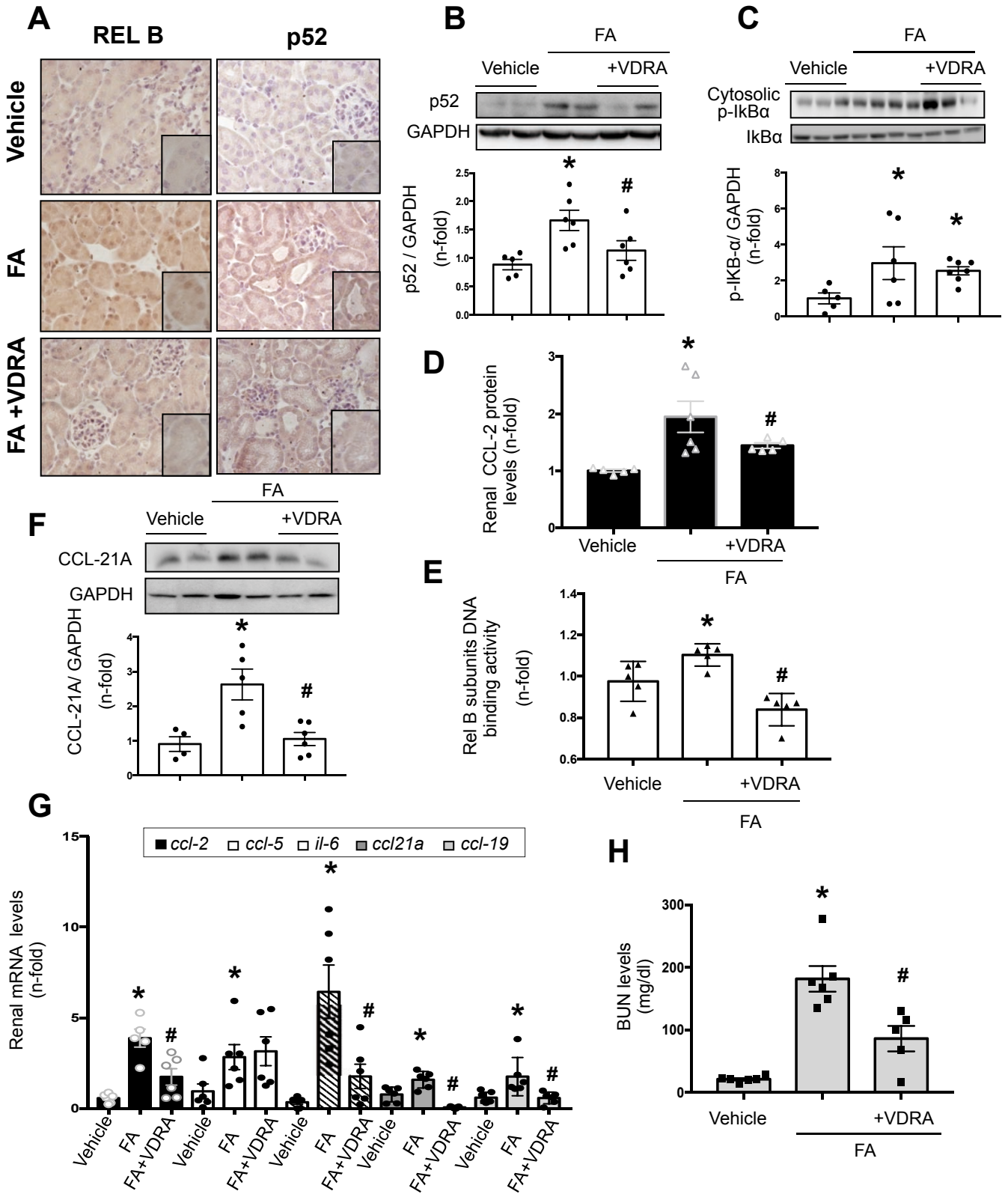
Supplementary Figure 2. Paricalcitol inhibits TWEAK-induced upregulation of specific NF-κB2 targets in the kidney. Mice were treated with paricalcitol 750 ng/Kg/day or the NF-κB2 inhibitor SN52 0.7 mg/ day (two doses; day -1; day 0) starting 48 hours before TWEAK 0.5 µg, and sacrificed 24 hours after TWEAK administration. (**A and B)** CCL21A protein levels were evaluated by western blot in total kidney proteins**.** GAPDH was used as loading control. (**C)** CCL21A immunohistochemistry located CCL21A expression to tubular cells. Representative mouse from each group. (**D)** Quantification of CCL21A stained area vs total area. Data expressed as mean ± SEM of 5-8 mice per group. Differences between intervention and control groups were assessed by Mann–Whitney test. *p<0.05 vs control. # p<0.05 vs TWEAK



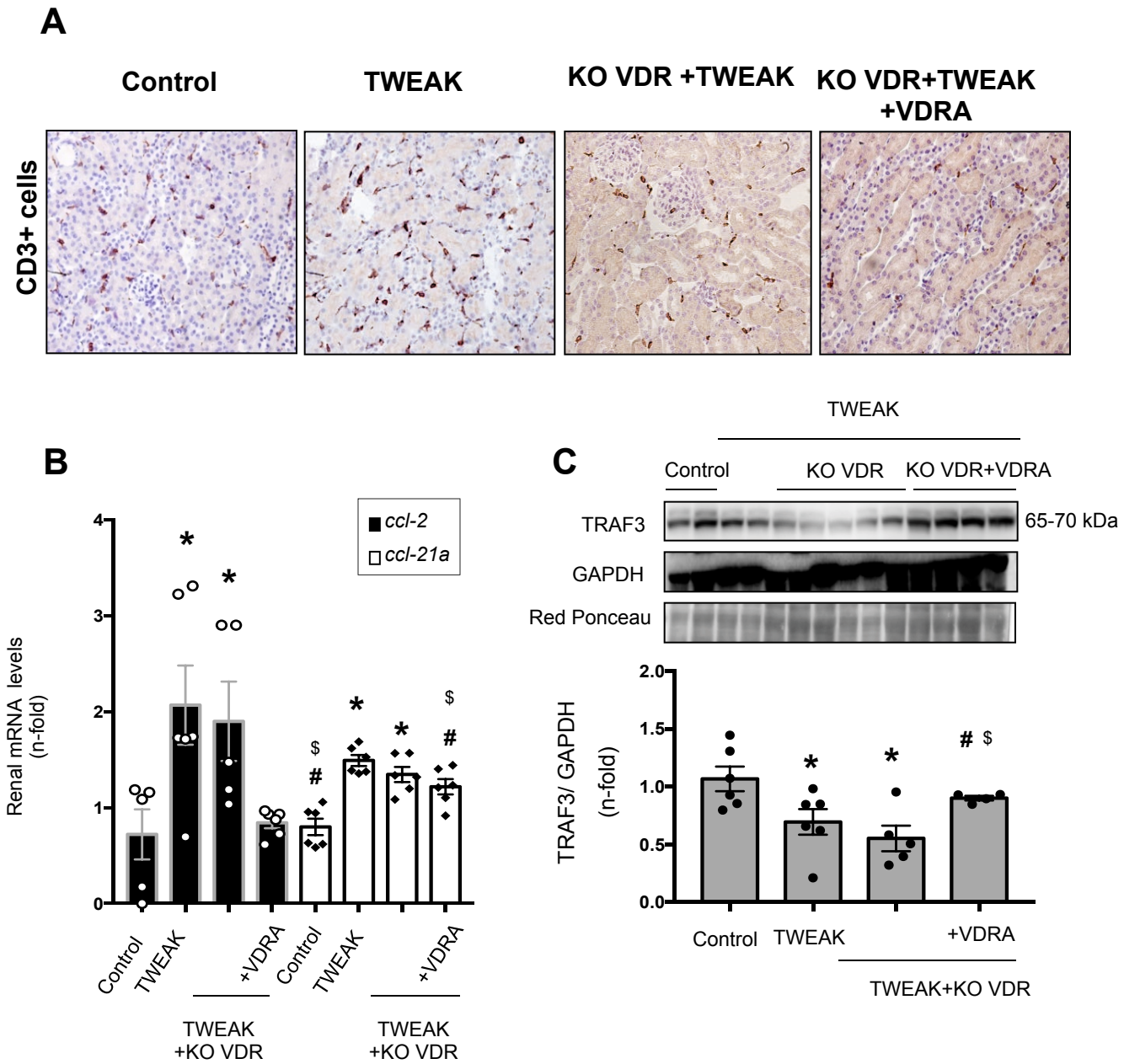
Supplementary Figure 3. SN52 peptide blocks NF-κB2 activation and NF-κB2 nuclear translocation in a experimental TWEAK-induced renal damage. Mice were pretreated with the NF-κB2 inhibitor SN52 (0.7 mg/day; two doses; day -1; day 0) starting 48 hours before administration of TWEAK 0.5 μg, and were sacrificed 24 hours after TWEAK administration. (**A)** P52 immunohistochemistry in paraffin-embedded kidney sections. Representative animal from each group (magnification 200x). (**B and C)** Western blot for p52 **(B)** and IκBα phosphorylation **(C)**. Data expressed as mean ± SEM of 5-8 animals per group. Differences between intervention and control groups were assessed by Mann–Whitney test. *p<0.05 *vs* control. # p<0.05 *vs* TWEAK.



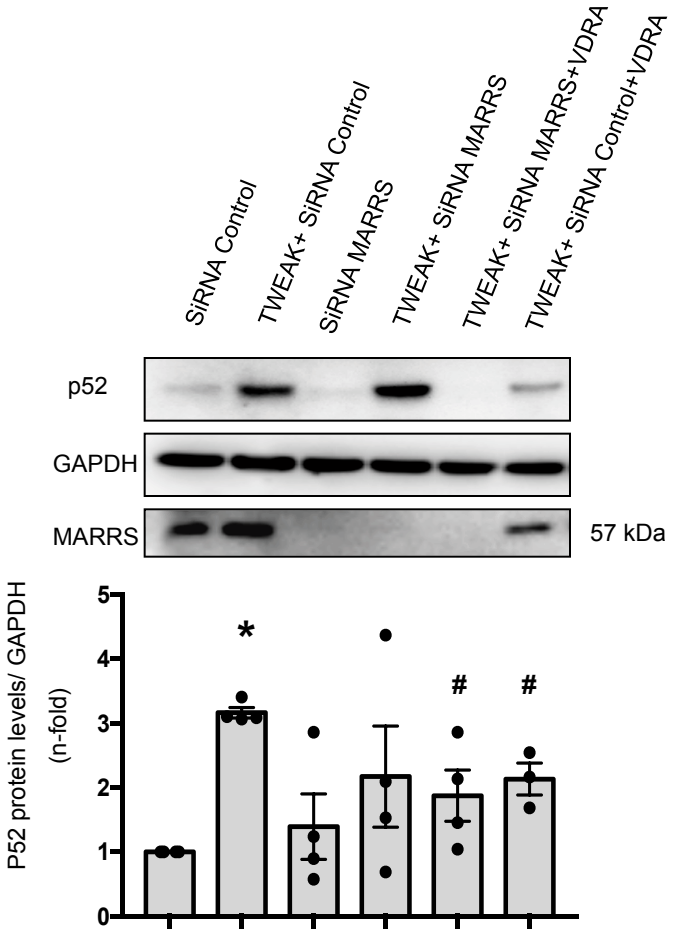
Supplementary Figure 4. Paricalcitol decreases renal inflammation in experimental Unilateral Ureteral Obstruction (UUO) in mice. Mice were treated with paricalcitol 750 ng/Kg/day, starting 24 hours before UUO, and studied 5 days after UUO. In paraffin-embedded kidney sections, immunohistochemistry using anti-F4/80 and anti-CD3 identified monocyte/macrophages and T lymphocytes, respectively. A. Representative animal from each group. Magnification 200X. B, C. In RNA obtained from total renal extracts, proinflammatory gene expression (*ccl-2, ccl-5, il-6, tnf-α* and *il-17a*) and specific NFκB2-regulated gene expression (*ccl-19* and *ccl-21*) were determined by Real Time PCR. Data expressed as mean±SEM of 4-8 animals per group. Differences between intervention and control groups were assessed by Mann–Whitney test. *p<0.05 *vs* contralateral non-obstructed (NOb) kidney; #p<0.05 *vs* obstructed (Ob) kidneys.



Supplementary Figure 5. Paricalcitol inhibits NF-κB2, but not NF-κB1 activation in folic acid-induced renal injury. Mice were treated with paricalcitol 25 μg/Kg/day starting 24 hours before folic acid (FA) 300 mg/kg or vehicle (sodium bicarbonate 0.3 mol/L) administration, and studied 24 hours after FA injection. (**A)** Immunohistochemistry disclosed nuclear localization of p52 and RelB that was decreased by paricalcitol. Representative animal from each group (magnification 200x). (**B and C)**. Western blotting of p52, as evidence of NF-κB2 activation **(B)** and IκBα phosphorylation as evidence of NF-κB1 activation **(C)**. (**D)** CCL2 protein levels evaluated by ELISA. (**E).** In isolated renal nuclear proteins, RelB DNA binding activity was assessed by ELISA. (**F)** CCL21 protein levels evaluated in total renal protein extracts by Western Blot. (**G)** RNA was obtained from total renal extracts, and proinflammatory gene expression levels were determined by Real Time PCR. H. Data of serum BUN levels are shown. Data expressed as mean±SEM of 5-8 animals per group. Differences between intervention and control groups were assessed by Mann–Whitney test.. *p<0.05 *vs* control; #p<0.05 *vs* folic acid kidneys.



Supplementary Figure 6. Paricalcitol reduces TWEAK-induced renal inflammation in VDR KO mice. Some animals were pretreated with paricalcitol 750 ng/kg/day, starting 48 hours before a single dose of TWEAK 0.5 µg, and were sacrificed 24 hours after TWEAK administration. (**A)** Immunohistochemistry using anti-CD3 identified T lymphocytes. Representative animal from each group. Magnification 200X. (**B)** RNA was obtained from total renal extracts, and proinflammatory gene expression levels were determined by Real Time PCR. *p<0.05 *vs* WT; #p<0.05 *vs* TWEAK; $p<0.05 *vs* TWEAK+KO VDR. (**C)** Paricalcitol restored TRAF3 levels in experimental renal damage induced by TWEAK in VDR KO mice. TRAF3 protein levels evaluated by Western blot. Data expressed as mean±SEM of 5-8 animals per group. *p<0.05 *vs* control; #p<0.05 *vs* injured kidney. Differences between intervention and control groups were assessed by Mann–Whitney test. *p<0.05 *vs* control; #p<0.05 *vs* injured-kidney.



Supplementary Figure 7. Paricalcitol inhibits TWEAK-induced upregulation of specific NF-κB2 targets in MARRS gene silenced cells. MARRS gene silencing was achieved in cultured cells using a predesigned and validated SiRNA against MARRS. Cells were stimulated with recombinant human soluble TWEAK 100 ng/ml. In some experiments, cells were preincubated for 48 hours with paricalcitol 12 μmol/L prior to TWEAK stimulation. NF-κB2 pathway activation was assessed by western blot of NFκB2 p52. Data expressed as mean±SEM of 3-5 independent experiments. *p<0.05 vs control; #p<0.05 *vs* TWEAK-treated cells.