

**Investigating the cause of cardiovascular dysfunction in chronic kidney disease: capillary rarefaction and inflammation may contribute to detrimental cardiovascular outcomes.**

Basic Research in Cardiology

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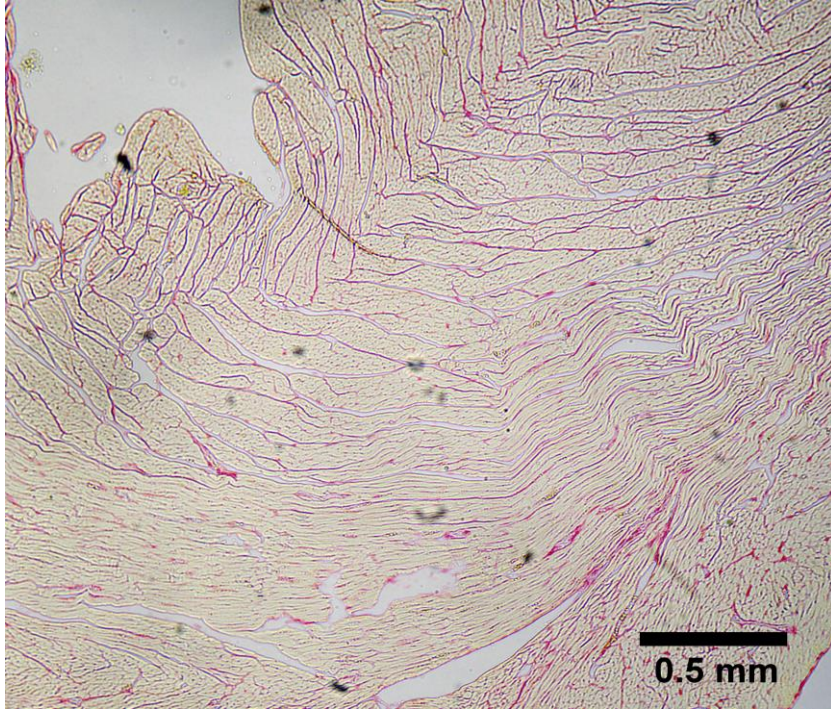
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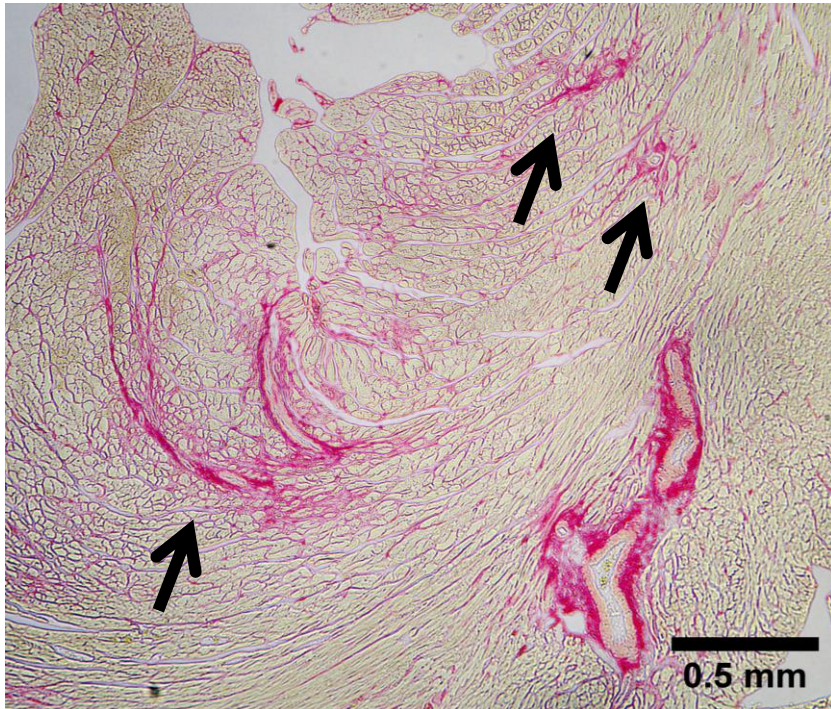
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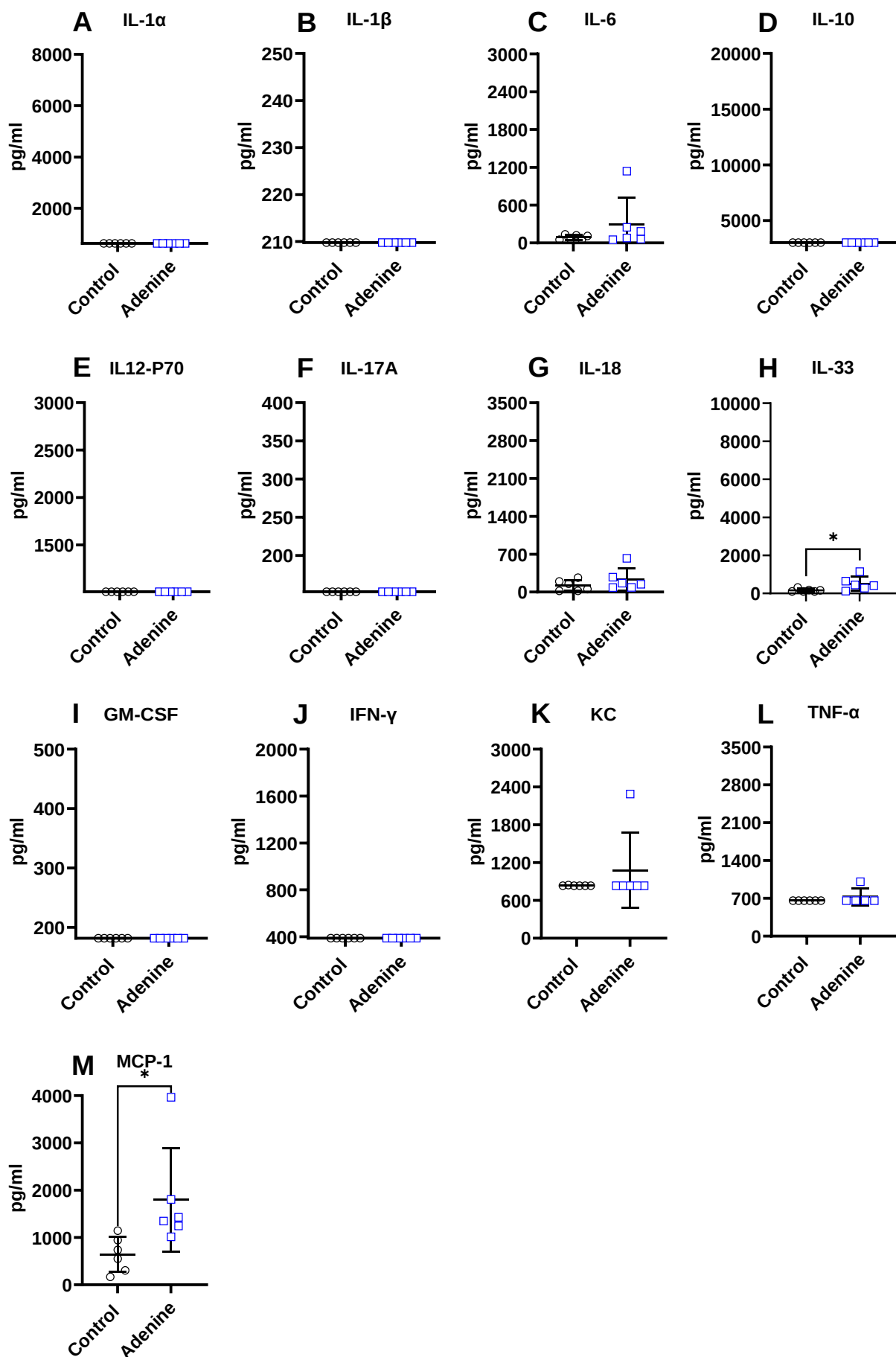
**Control**



**Adenine**

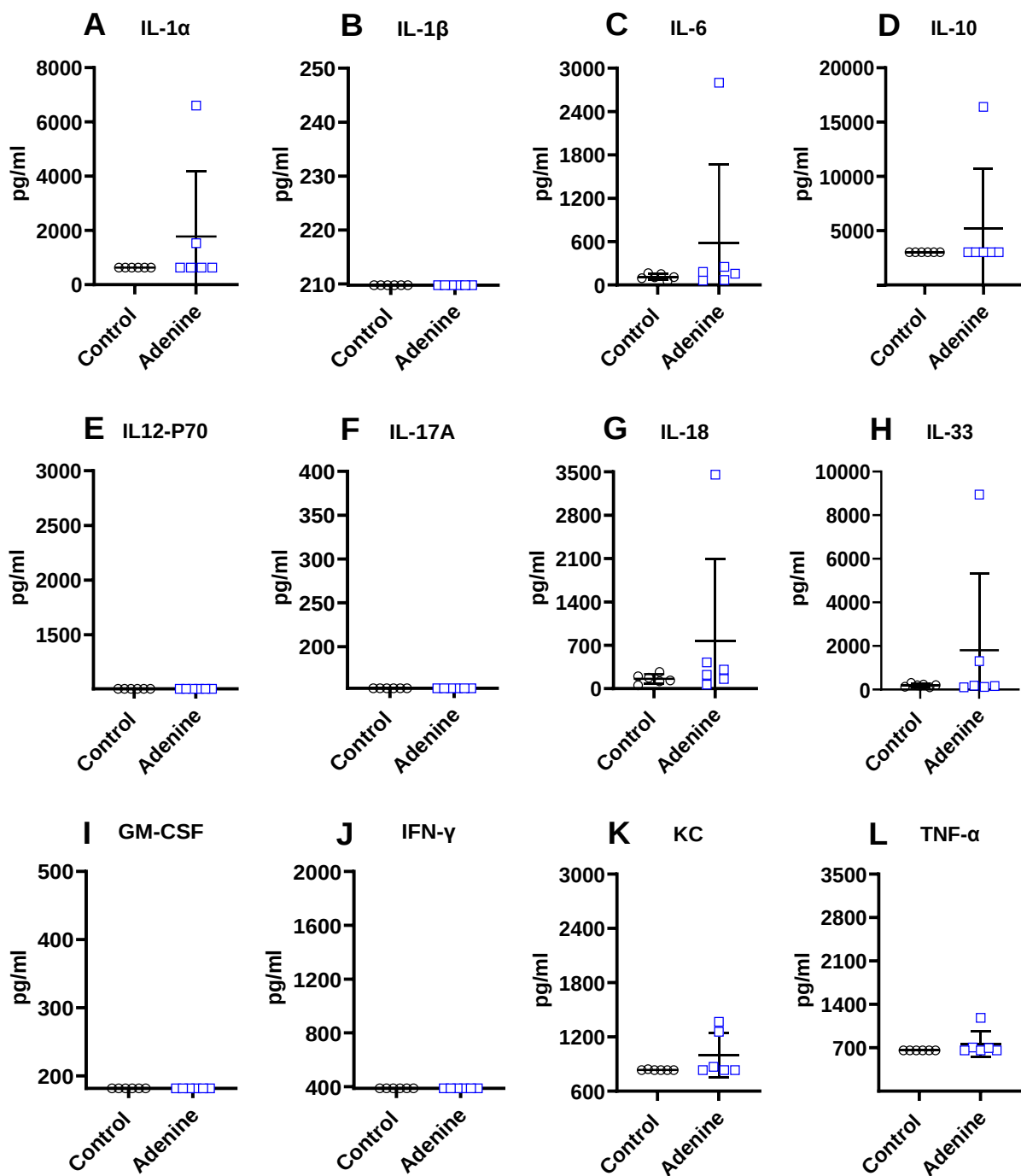


**Supplementary Fig. 1.** Picrosirius red staining of the LV. Areas of fibrosis can be seen in dark red staining indicated by black arrows.



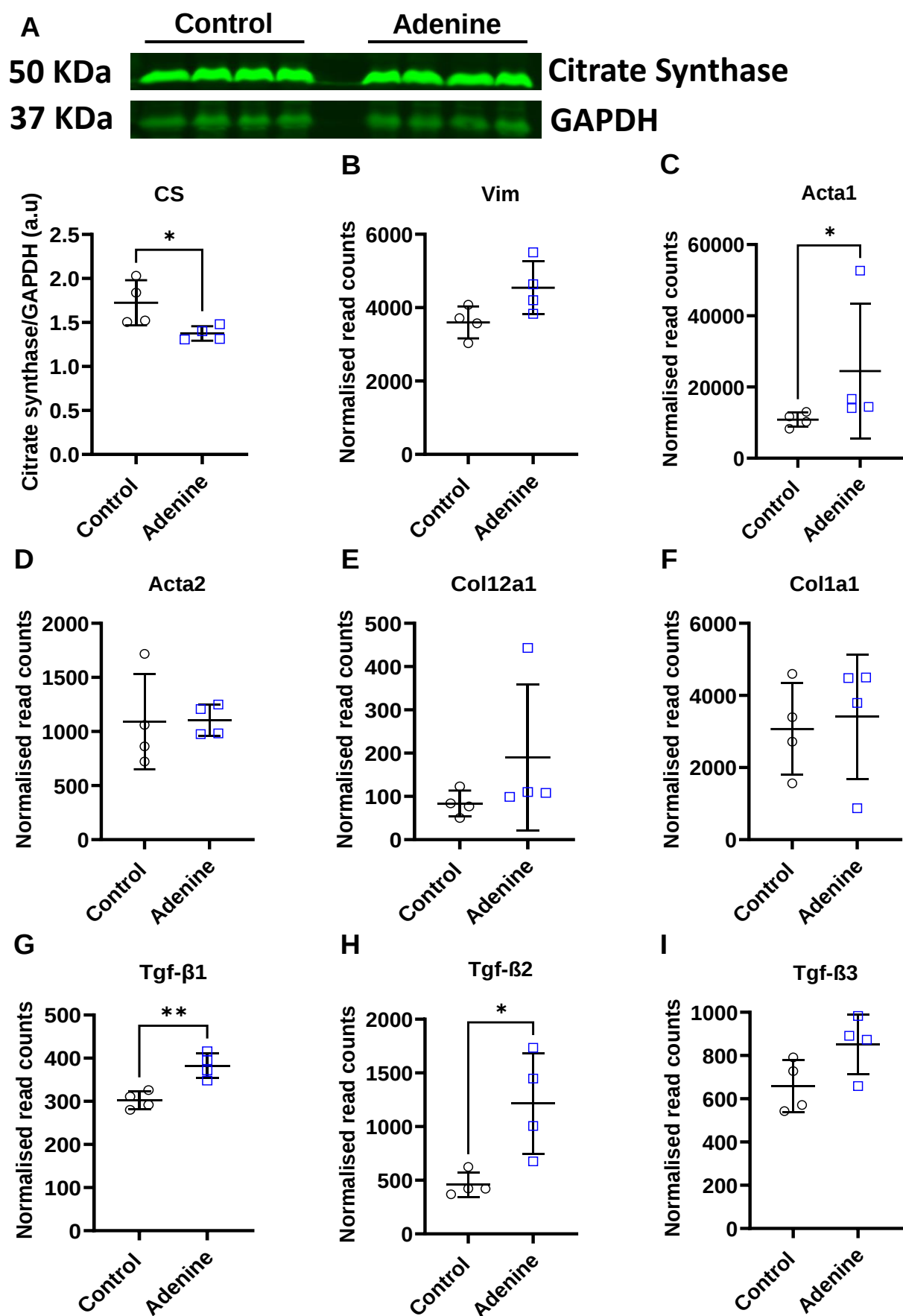
**Supplementary Fig. 2.** The levels of circulating inflammatory cytokines at week 11.

There data indicates the changes in protein levels of different inflammatory markers in the plasma of control and adenine-fed rats. All analysis was conducted using Student's t-test. (Data is expressed as mean  $\pm$  SD).



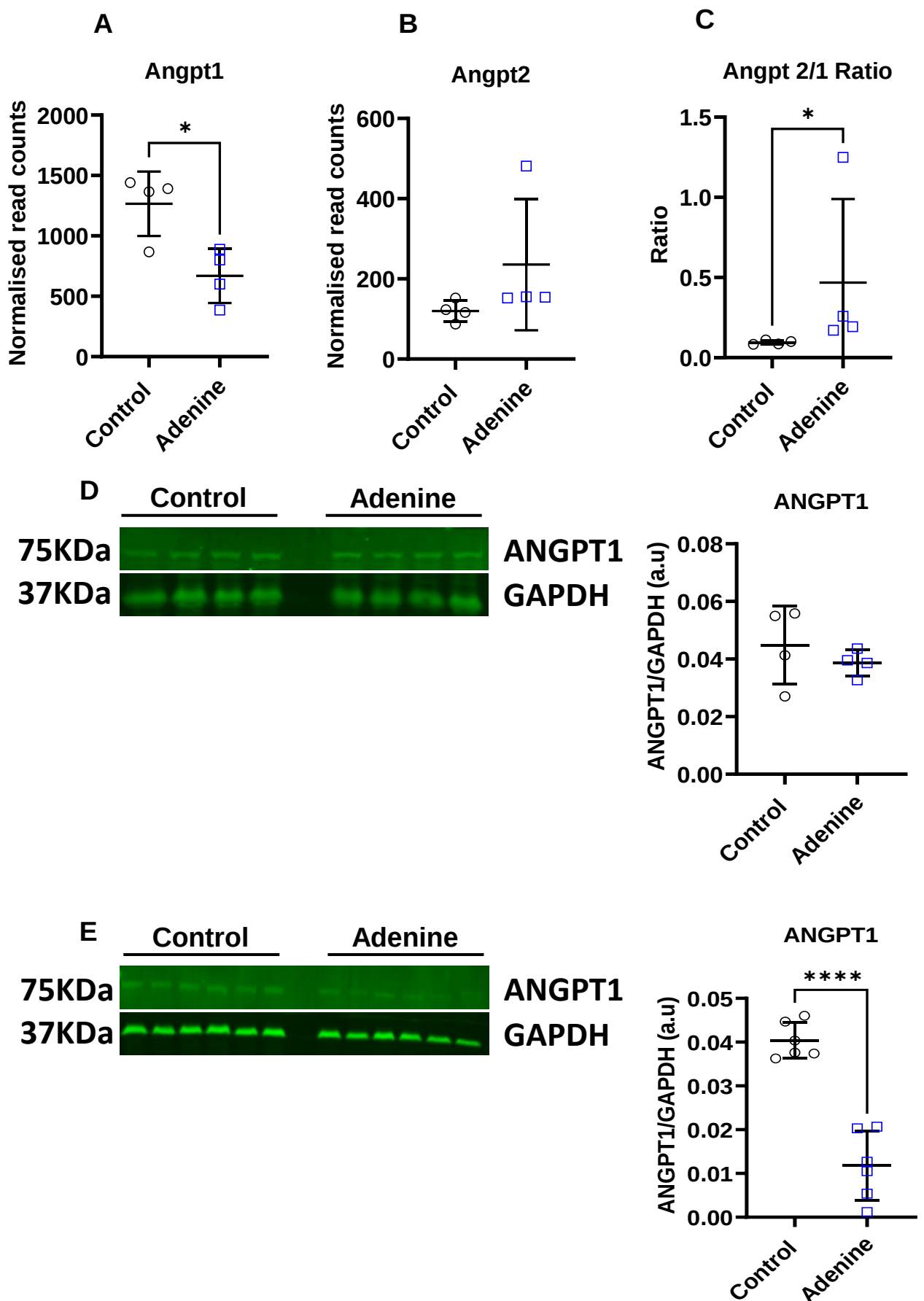
**Supplementary Fig. 3.** The levels of circulating inflammatory cytokines at week 18.

There data indicates the changes in protein levels of different inflammatory markers in the plasma of control and adenine-fed rats. All analysis was conducted using Student's t-test. (Data is expressed as mean  $\pm$  SD).



**Supplementary Fig 4.** Alteration of a mitochondrial protein and fibrosis-related genes in the adenine group.

(A) shows the protein level of citrate synthase in the heart of control versus adenine –fed rats. Normalised read counts of the mRNA expression from different genes involved in fibrosis are shown in (B-I). Acta1 levels were analysed using Mann-Whitney U-test. All other analysis was conducted using Student's t-test. (Data is expressed as mean  $\pm$  SD).



**Supplementary Fig 5. Alteration of angiopoietin protein and genes in the heart and kidneys of rats with CKD.**

(A) and (B) show the normalised read counts of mRNA level of Angpt1, Angpt2 and Angpt2/Angpt1 ratio in the heart of control versus adenine-fed rats, respectively. Protein levels of ANGPT1 in the heart and kidney from control and adenine group are shown in (D) and (E), respectively. All analysis were conducted using Student's t-test. (Data is expressed as mean  $\pm$  SD).

**Supplementary Table 1.** Outputs of two-way ANOVA analysis.

| Figure number | Source of Variation | % of total variation | P value |
|---------------|---------------------|----------------------|---------|
| Fig. 2A       | Interaction         | 19.37                | <0.0001 |
|               | Weeks               | 19.99                | <0.0001 |
|               | Treatments          | 30.50                | <0.0001 |
| Fig. 2B       | Interaction         | 6.99                 | <0.0001 |
|               | Weeks               | 52.72                | <0.0001 |
|               | Treatments          | 28.00                | <0.0001 |
| Fig. 2C       | Interaction         | 3.54                 | <0.0001 |
|               | Days                | 4.82                 | <0.0001 |
|               | Treatments          | 86.19                | <0.0001 |
| Fig. 2D       | Interaction         | 3.57                 | 0.1644  |
|               | Weeks               | 49.86                | <0.0001 |
|               | Treatments          | 0.45                 | 0.3161  |
| Fig. 2E       | Interaction         | 4.14                 | 0.2599  |
|               | Weeks               | 17.02                | 0.0002  |
|               | Treatments          | 11.17                | <0.0001 |
| Fig. 2F       | Interaction         | 3.22                 | 0.1665  |
|               | Weeks               | 50.93                | <0.0001 |
|               | Treatments          | 2.43                 | 0.0155  |
| Fig. 2G       | Interaction         | 4.84                 | 0.2468  |
|               | Weeks               | 16.47                | 0.0007  |
|               | Treatments          | 1.62                 | 0.1347  |
| Fig. 4A       | Interaction         | 11.14                | 0.0058  |
|               | Weeks               | 19.87                | <0.0001 |
|               | Treatments          | 0.00                 | 0.9874  |
| Fig. 4B       | Interaction         | 2.35                 | 0.5802  |
|               | Weeks               | 30.90                | <0.0001 |
|               | Treatments          | 0.07                 | 0.7391  |
| Fig. 4C       | Interaction         | 9.89                 | 0.0056  |
|               | Weeks               | 13.90                | 0.0004  |
|               | Treatments          | 15.30                | <0.0001 |
| Fig. 4D       | Interaction         | 1.06                 | 0.8760  |
|               | Weeks               | 34.68                | <0.0001 |
|               | Treatments          | 0.22                 | 0.5437  |
| Fig. 4E       | Interaction         | 7.26                 | 0.0589  |
|               | Weeks               | 21.64                | <0.0001 |
|               | Treatments          | 0.00                 | 0.9519  |

**Supplementary Table 2.** Statistical parameters of differentially expressed genes.

| <b>Gene symbol and Loci</b> | <b>log2 Fold Change</b> | <b>Adjusted P value</b> |
|-----------------------------|-------------------------|-------------------------|
| <b>Thbs4</b>                | 3.28                    | 9.58E-05                |
| <b>Tmem119</b>              | 3.22                    | 3.02E-02                |
| <b>Myl4</b>                 | 3.19                    | 4.85E-02                |
| <b>LOC102554231</b>         | 2.55                    | 2.14E-02                |
| <b>Pnma2</b>                | 2.55                    | 3.02E-02                |
| <b>Tnfrsf11b</b>            | 2.26                    | 5.73E-03                |
| <b>Dact1</b>                | 2.20                    | 1.89E-02                |
| <b>Fos</b>                  | 2.00                    | 5.61E-02                |
| <b>Bok</b>                  | 1.77                    | 8.01E-02                |
| <b>Camk2b</b>               | 1.65                    | 2.78E-02                |
| <b>Ildr2</b>                | 1.50                    | 2.52E-02                |
| <b>Rarres1</b>              | 1.47                    | 2.14E-02                |
| <b>Slfn4</b>                | 1.42                    | 7.98E-02                |
| <b>Tgfb2</b>                | 1.40                    | 3.45E-03                |
| <b>Junb</b>                 | 1.40                    | 2.14E-02                |
| <b>Ldlr</b>                 | 1.40                    | 1.79E-03                |
| <b>Apold1</b>               | 1.32                    | 7.91E-02                |
| <b>Sypl2</b>                | 1.32                    | 9.37E-02                |
| <b>Vcam1</b>                | 1.29                    | 2.14E-02                |
| <b>Ifit3</b>                | 1.20                    | 1.31E-03                |
| <b>Ncam1</b>                | 1.17                    | 4.27E-03                |
| <b>Cdkl3</b>                | 1.15                    | 9.26E-02                |
| <b>Myl9</b>                 | 1.13                    | 6.64E-09                |
| <b>Myo3b</b>                | -2.32                   | 1.31E-03                |
| <b>LOC120093103</b>         | -2.19                   | 2.78E-02                |
| <b>PCDH11X</b>              | -2.11                   | 5.56E-02                |
| <b>PRP2L1</b>               | -1.49                   | 4.70E-03                |
| <b>Csdc2</b>                | -1.43                   | 3.69E-07                |
| <b>Atp6ap1l</b>             | -1.17                   | 2.78E-02                |
| <b>Mme</b>                  | -1.15                   | 2.06E-02                |

**Supplementary Table 3.** Statistical parameters of Hallmark pathways analysis.

| Pathway                           | Adjusted<br>P Value | Normalised<br>Enrichment<br>score | Size of<br>gene set |
|-----------------------------------|---------------------|-----------------------------------|---------------------|
| TNFA SIGNALING VIA NFKB           | 2.29E-18            | 2.540235                          | 187                 |
| EPITHELIAL MESENCHYMAL TRANSITION | 3.61E-12            | 2.268322                          | 182                 |
| INTERFERON GAMMA RESPONSE         | 2.46E-11            | 2.234137                          | 187                 |
| INTERFERON ALPHA RESPONSE         | 1.39E-07            | 2.194609                          | 95                  |
| APOPTOSIS                         | 1.05E-09            | 2.164746                          | 156                 |
| P53 PATHWAY                       | 1.06E-09            | 2.135754                          | 189                 |
| INFLAMMATORY RESPONSE             | 1.25E-09            | 2.12858                           | 189                 |
| IL6 JAK STAT3 SIGNALING           | 1.98E-06            | 2.114865                          | 82                  |
| CHOLESTEROL HOMEOSTASIS           | 1.58E-05            | 2.085405                          | 71                  |
| APICAL JUNCTION                   | 5.91E-08            | 2.015113                          | 190                 |
| HYPOXIA                           | 1.55E-07            | 1.978497                          | 186                 |
| COAGULATION                       | 1.58E-05            | 1.91081                           | 113                 |
| IL2 STAT5 SIGNALING               | 4.87E-06            | 1.869762                          | 190                 |
| TGF BETA SIGNALING                | 0.000979            | 1.865311                          | 51                  |
| REACTIVE OXYGEN SPECIES PATHWAY   | 0.002003            | 1.865106                          | 48                  |
| COMPLEMENT                        | 2.27E-05            | 1.840493                          | 174                 |
| ALLOGRAFT REJECTION               | 8.78E-06            | 1.813164                          | 184                 |
| KRAS SIGNALING UP                 | 2.25E-05            | 1.775194                          | 186                 |
| ANGIOGENESIS                      | 0.005872            | 1.760716                          | 31                  |
| UV RESPONSE UP                    | 0.000272            | 1.703807                          | 154                 |
| MTORC1 SIGNALING                  | 0.003725            | 1.55341                           | 195                 |
| KRAS SIGNALING DN                 | 0.030263            | -1.36589                          | 153                 |
| HEME METABOLISM                   | 0.018298            | -1.39028                          | 186                 |
| ADIPOGENESIS                      | 0.003725            | -1.49645                          | 194                 |
| BILE ACID METABOLISM              | 0.00981             | -1.53655                          | 107                 |
| OXIDATIVE PHOSPHORYLATION         | 1.58E-05            | -1.78335                          | 197                 |
| FATTY ACID METABOLISM             | 2.39E-05            | -1.84328                          | 150                 |