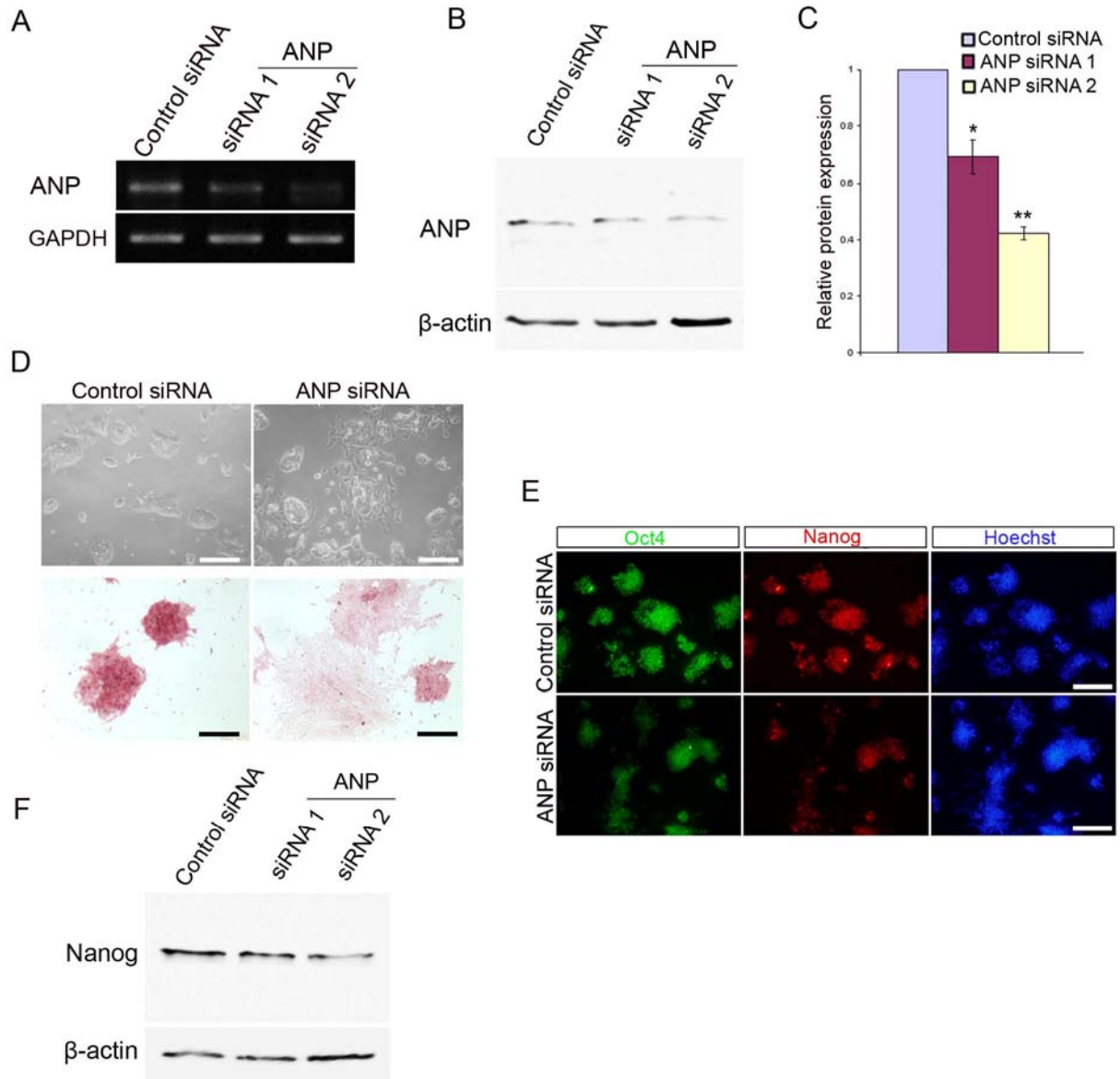


Supplementary Figure 4



Supplementary Figure 4. Knockdown of ANP in ES cells and its effect on ES cell self-renewal. (A) RT-PCR analysis of ES cells transfected with the control siRNA or ANP siRNAs, showing knockdown of the ANP gene 48 hours after siRNA transfection. GAPDH was used as the internal control. (B) Western blot analysis of ES cells treated as in A, showing a reduced level of ANP protein 48 hours after siRNA transfection. β -actin was

used as a loading control. (C) Quantification of **B**. (D) *Upper panels*, Morphology of ES cells treated with control siRNA or NPR-A siRNA2 at 2 days after transfection in the presence of LIF. *Lower panels*, AP-staining of the ES cells treated as in D, 4 days after transfection. (E) Immunofluorescence images of ES cells treated as in D for 48 h, stained with antibodies against Oct-4 and Nanog, and counterstained with Hoechst reagent. (F) Western blot analysis of ES cells treated as in D, showing reduced protein levels of Nanog 48 h after siRNA transfection. β -actin was used as a loading control. Data represent mean \pm s.d (n=3); * $P < 0.05$, ** $P < 0.01$ (two-tailed t -test). Scale bar = 100 μ m.