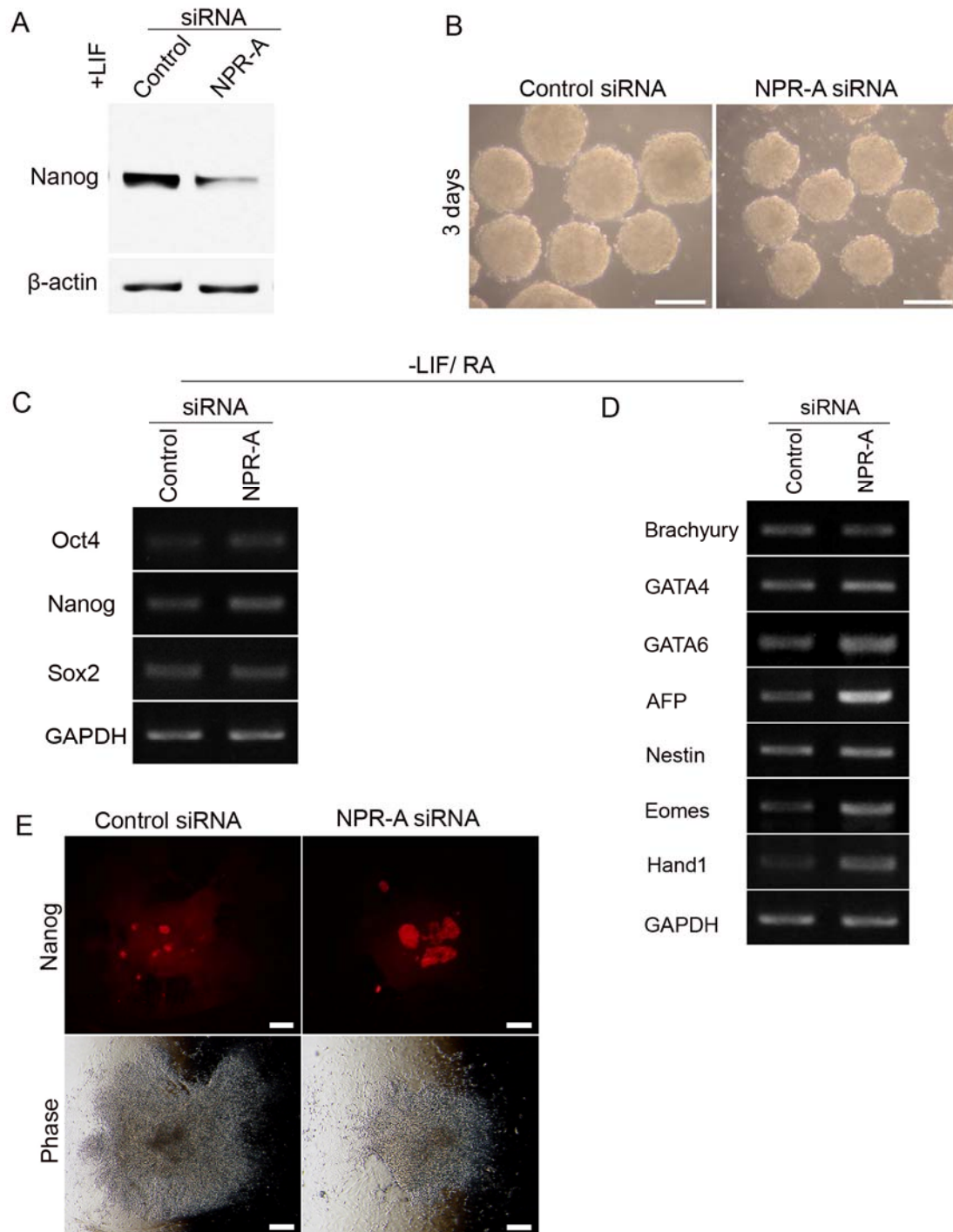


Supplementary Figure 2



Supplementary Figure 2. NPR-A knockdown affects differentiation of ES cells. (A) Western blot analysis of ES cells treated with control siRNA or NPR-A siRNA at 40 hours after transfection (just before induction of differentiation) in the presence of LIF,

showing a marked reduction of protein level of Nanog. β -actin was used as a loading control. (B) Embryoid body (EB) formed from control siRNA and NPR-A siRNA treated ES cells. Note the defective and smaller EB formation in NPR-A-deficient cells compared to control siRNA-treated cells. (C) RT-PCR analysis showing the expression of lineage specific markers for ectoderm (nestin), endoderm (GATA4, GATA6 and AFP), mesoderm (Brachyury) and trophoectoderm (Hand1 and Eomes) layers in EBs generated from control siRNA- and NPR-A siRNA-treated ES cells. (E) Immunofluorescence images of plated EBs after 7 days from induction of differentiation, stained with antibodies against Nanog. Scale bar = 100 μ m