



**Figure S1. Experimental design for late overexpression in porcine embryos**

- (A) Control and NANOG mRNA-injected porcine D6 embryos at the one-cell stage.
- (B) Transcription levels of control and NANOG mRNA-injected D6 blastocysts. The sample size was  $n = 30$ . Each group contained three replicates. Error bars represent the mean SEM. \* corresponds to significant differences (\*\*\*\*:  $P < 0.001$ )
- (C) Experimental scheme of the overexpression assay using lipofectamine and the EGFP mRNA complex in eight-cell stage porcine embryos.
- (D) Transcription levels of EGFP mRNA-transfected D7 blastocysts with and without zona pellucida. The sample size was  $n = 30$ . Each group contained three replicates. Error bars represent the mean SEM. \* corresponds to significant differences (\*:  $P < 0.05$ ).
- (E) EGFP mRNA-transfected D7 blastocysts with and without zona pellucida were hatched. Sample size

was  $n = 30$ .