

## Supplementary Data

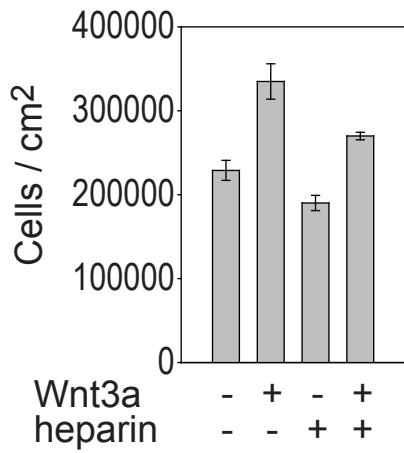
**Figure S1. Heparin did not enhance mitogenic effects of Wnt3a.** *A*, MC3T3-E1 cells were treated by either 50 ng/ml Wnt3a, 50 µg/ml heparin or their combination for 72 h. The cell numbers were then determined using GUAVA PCA-96 Viacount system. *B* and *C*, The effects of Wnt3a and heparin on the mRNA expression levels of several key cell cycle regulators were determined by Taqman quantitative PCR. The data represents the mean ± S.E. of three independent experiments.

**Figure S2. BMPs or FGF/FGFR signaling were not required for Wnt3a/heparin stimulated ALP activity.** *A*, MC3T3-E1 cells transfected with Id1-LUC reporter were challenged with either 50 ng/ml Wnt3a, 50 µg/ml heparin, their combination or 100 ng/ml BMP2 (as positive control ). *B*, The gene silencing efficiency of RNA interference was determined by Western blotting 24 h after cells were transfected with siRNA specific for Smad4 or scramble siRNA. *C*, Cells transfected with siRNAs as in *B* were treated with Wnt3a (50 ng/ml) and heparin (50 µg/ml). After 72 h, the ALP activity was assayed. *D* and *E*, The mRNA and protein expression of FGFR1, R2 or R3 in MC3T3-E1 cells was assessed after treatments as indicated using Taqman quantitative PCR and Western blot analysis, respectively. *F*, MC3T3-E1 cells pre-treated by various doses of SU5402 were pulsed in combination with Wnt3a and heparin. At 72 h post-stimulation, ALP activity was measured. *G*, The level of phosphorylated ERKs were determined by Western blot analysis in MC3T3-E1 cells exposed to Wnt3a and heparin.

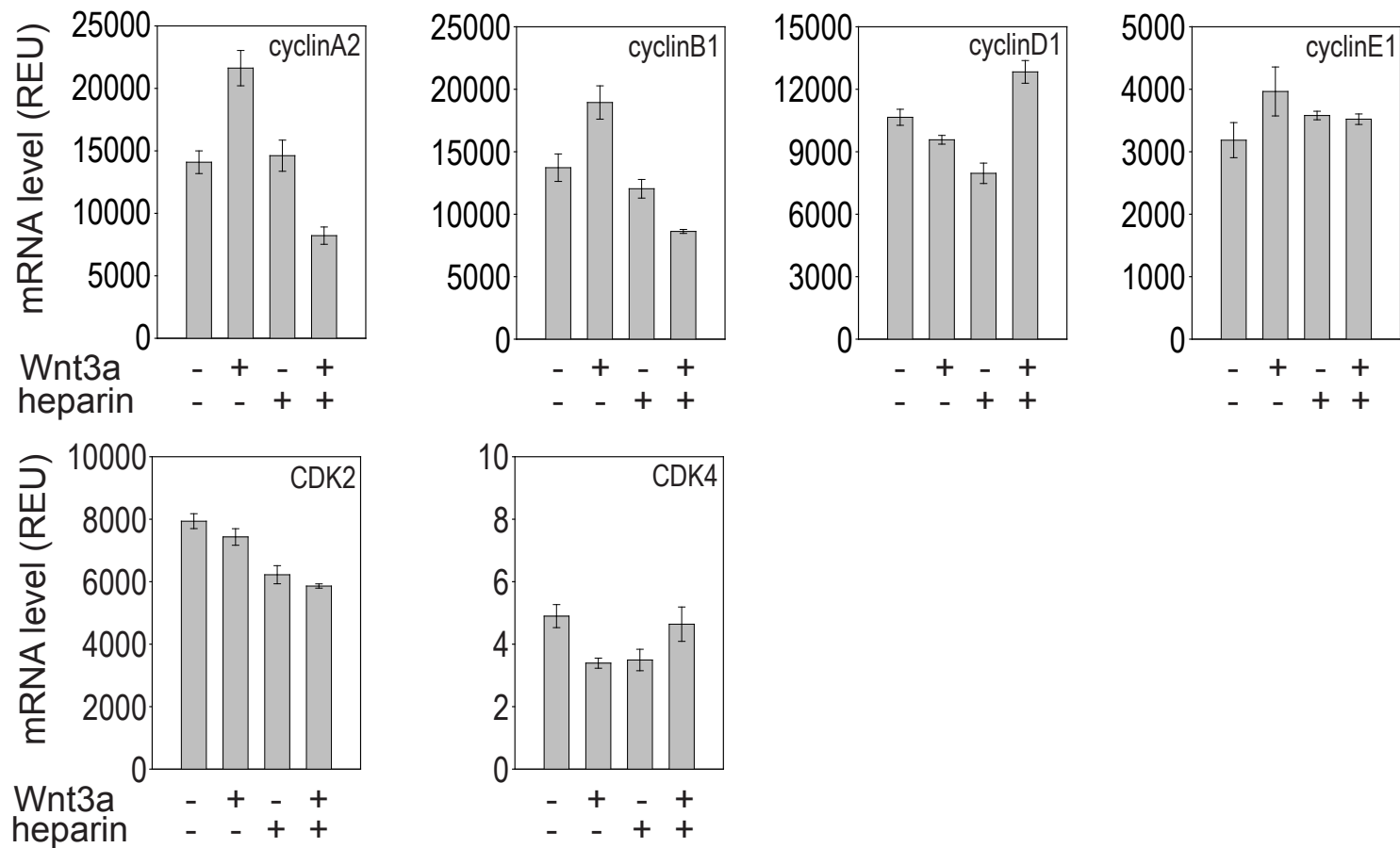
**Figure S3.** *A*, MC3T3-E1 cells were treated with 100 ng/ml IGF-1, 50 µg/ml heparin, or their combination for 72 h before ALP activity was determined. *B*, Cells were pretreated with 5 µM cycloheximide (CHX) or equal amount of DMSO as vehicle control for 1 h before stimulated with the combination of Wnt3a and heparin. 24 h later, the level of phosphorylated Akt was detected by Western Blot analysis.

Fig. S1

A



B



C

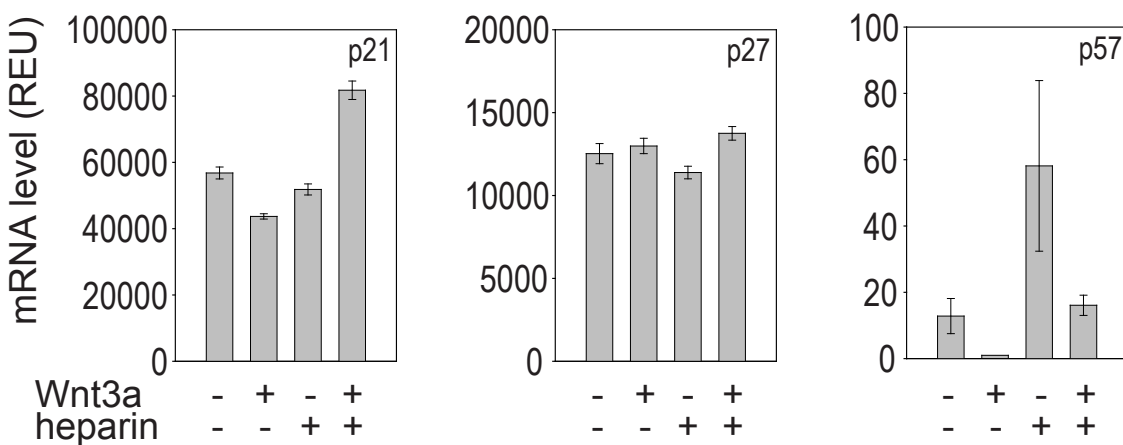


Fig. S2

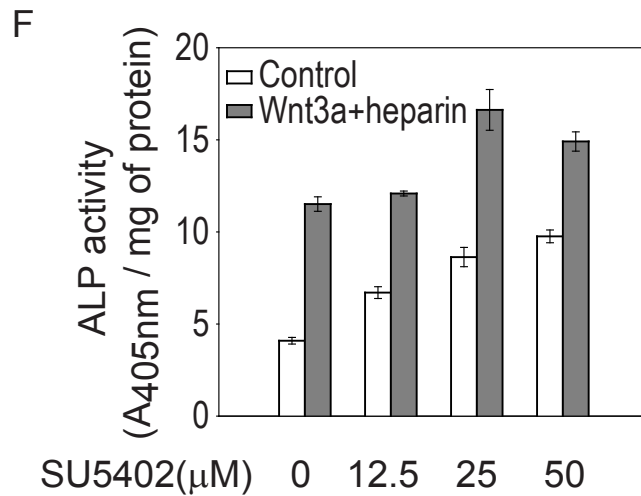
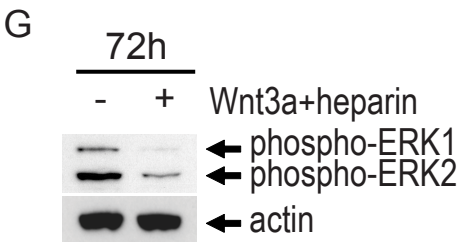
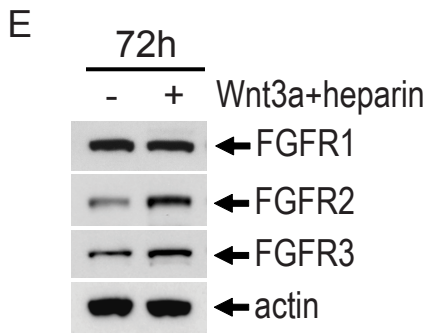
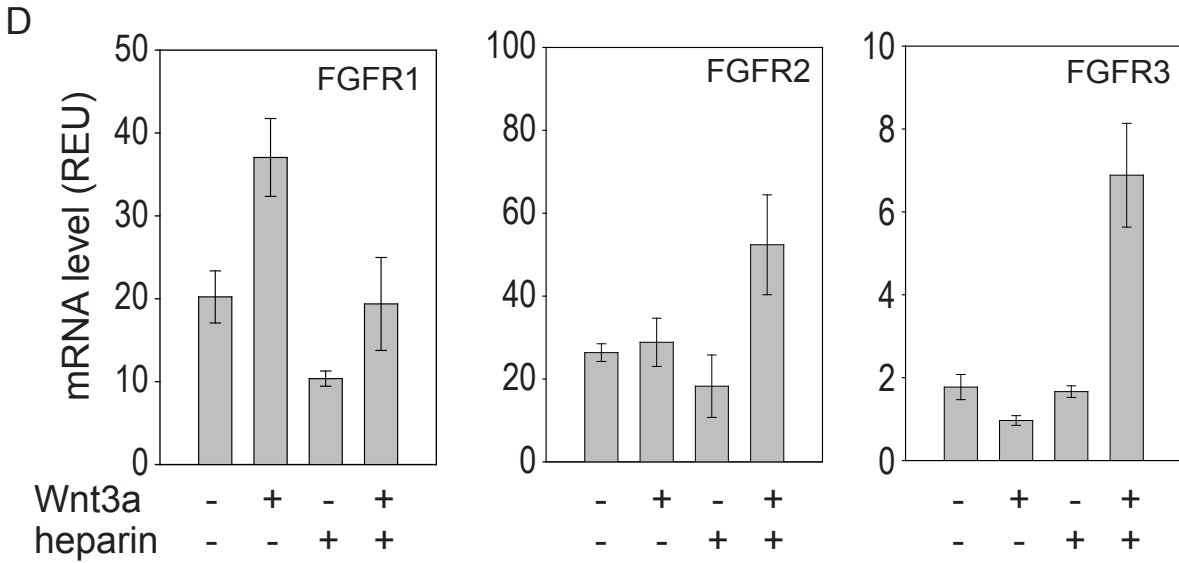
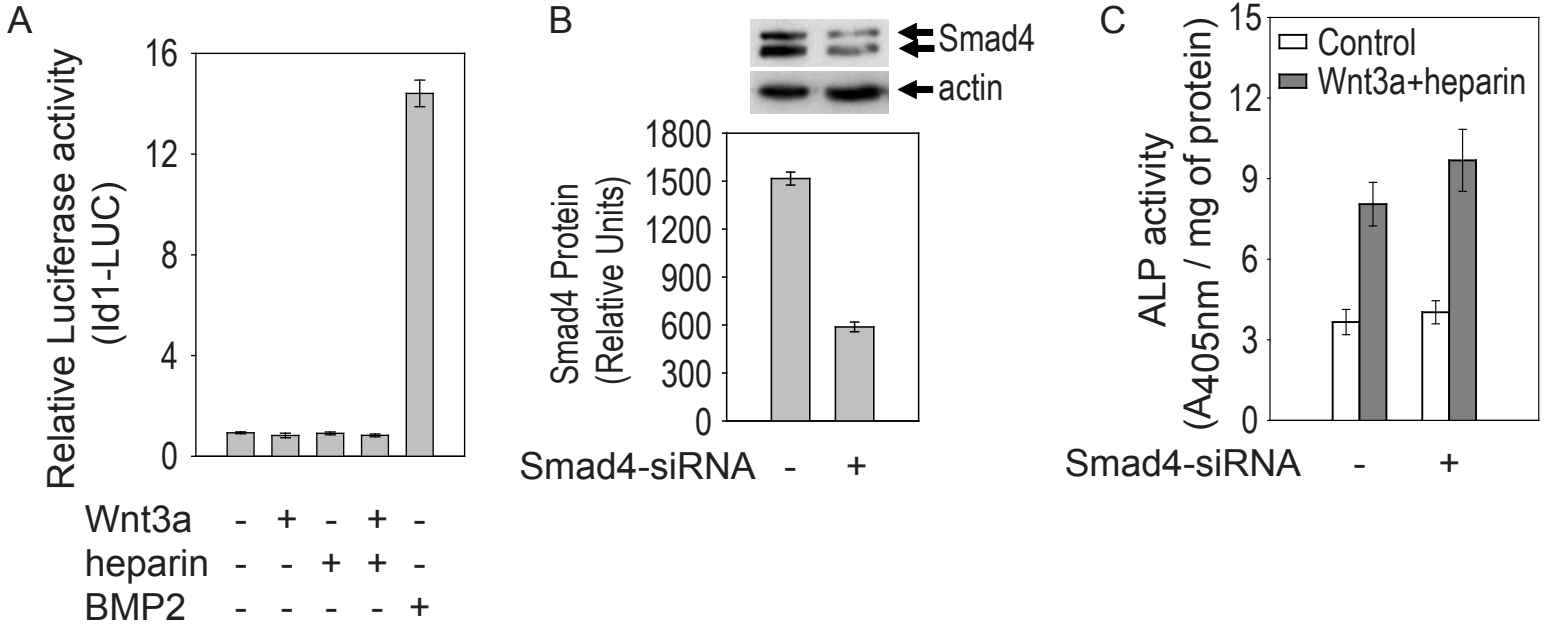
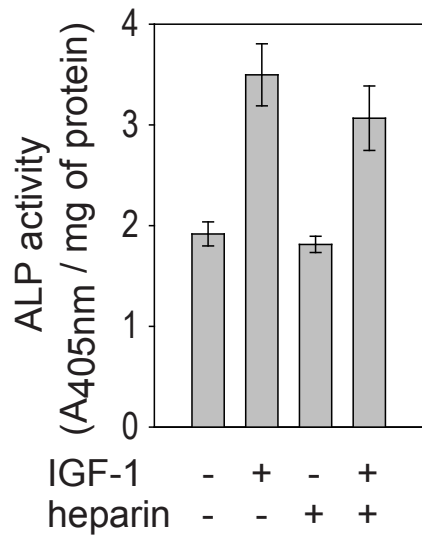


Fig. S3

A



B

