Appendix I

Materials and methods

Cell culture. The mouse myeloma cell line SP2/0 were purchased from the American Type Culture Collection (ATCC) and cultured in RPMI-1640 medium with 10% FBS in a 5% CO_2-humidified atmosphere at 37˚C.

MTT assay for SP2/0 cell viability. The SP2/0 cells ($2x10^4$ cells/well) were seeded in a 96-well culture plate. Then, the cells were treated with 0-10 ng/ml activin A for 12 and 24 h, and incubated with 0.5 mg/ml MTT for 4 h. After the supernatant was discarded carefully, 100 μl DMSO per well was added to dissolve the formazan crystals. Then the absorbance values of the samples were read using a plate reader (BioTek Instruments, Inc.) at 540 nm to evaluate the cell viability.

Proliferation assay by Cell Counting Kit-8. Proliferation of SP 2/0 cells was determined by Cell Counting Kit-8 (CCK-8) assay. Briefly, the cells were seeded in a 96-well plate at a density of $2x10^4$ cells/well in 100 μl of culture medium with or without activin A and cultured in a CO_2 incubator at 37˚C for 12 and 24 h. Then 10 μl of the CCK-8 solution was added to each well and the plate was cultured for 2 h. SDS 1% w/v (10 μl) was added to each well, and the absorbance was measured at 450 nm using a microplate reader.

Weights of mice and tumors at the endpoint. The weights of the mice and tumors were measured at the endpoint, and the ratio of tumor weight/body weights was analyzed.

Results

Activin A inhibits the viabilities and proliferation of SP2/0 cells. To verify the effect of activin A on the viability of mouse myeloma cells, the viability of a second mouse myeloma cell line SP2/0 was further examined using an MTT assay. The results revealed that after treatment with activin A for 12 and 24 h, the viability of SP2/0 cells decreased in a dose-dependent manner (Fig. S1A). This result was similar to the effect of activin A on the viability of mouse myeloma cell line NS-1. Then, the proliferation of NS-1 cells was further evaluated by Cell Counting Kit-8 after treatment with activin A for 12 and 24 h. The results revealed that the proliferation of SP2/0 cells significantly decreased with the treatment of activin A, compared with that in the control group (Fig. S1B). These results indicated that activin A could also inhibit SP2/0 cell proliferation.

Weight of mice and tumors at endpoint. After the solid tumors of NS-1cells were formed in mice, exogenous activin A was injected into the solid tumors and the same volume of saline was injected in the control group. On the sixth day, the weights of mice and tumors were measured and the ratio of tumor weight/body weights was analyzed. Although there was no significant difference in the body weight of mice between the control group and activin A group, the ratio of tumor weight/body weights of mice treated with exogenous activin A was significantly decreased compared to the control group (Fig. S2).

Figure S1. Effect of activin A on the viability and proliferation of mouse SP2/0 cells. (A) Cell viability was determined by MTT after treatment with activin A for 12 and 24 h. (B) Cell proliferation was examined using Cell Counting Kit-8 after treatment with activin A for 12 and 24 h. $^{*}P<0.05$, $^{**}P<0.01$, compared with the 0-ng/ml group.

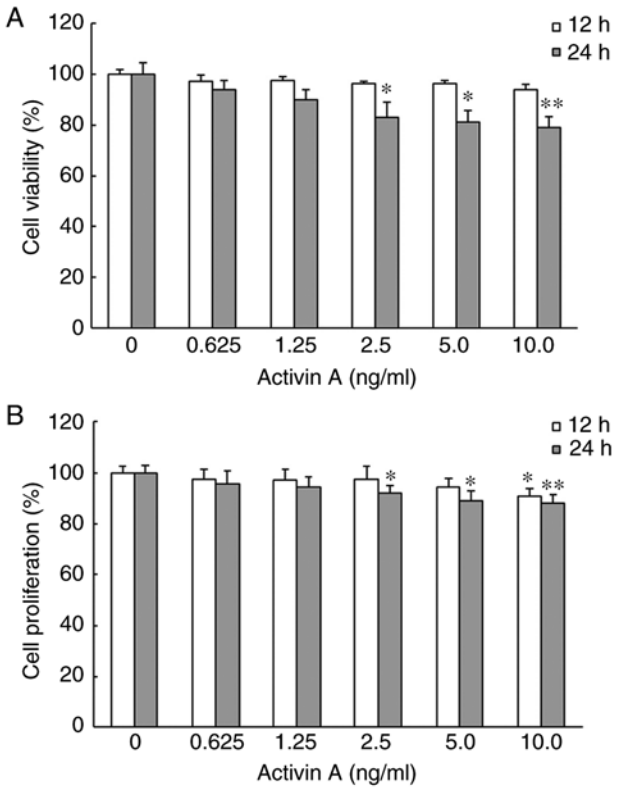

Figure S2. Mice body weight and ratio of tumor weight to body weight at the endpoint. (A) Body weight of mice. (B) Ratio of tumor weight to body weight. *P<0.05, activin A (Act A) group compared with the control (Cont) group.