Supplemental Figure 1. MKK4 and MKK7 are not required for ESC maintenance and EB formation. (A) Wild type, *Mkk4 (-/-)*, *Mkk7(-/-)* and *Mkk4/7(-/-)* ES cells were maintained in stem cell culture condition and expression of stem cell specific genes, including *Sox-2, Nanog* and *Oct4* was examined by RT-PCR. The ESCs were subjected to differentiation using the embryoid body (EB) protocol. (B) The cells at various days of differentiation were examined by Western blotting using antibodies as indicated. (C) At day 3 of the "handing drop", the formation of EB was observed under microscopy and the efficiency of EB formation was quantified. The percentage of EB verse total handing drops was calculated. The scale bars represent 100 μm. (D) The relative cell number in the 13 days EB was estimated by examining the Hoechst staining intensity using Axio fluorescence microscopy. Results are shown as mean ± SD from at least 3 slides and at least 3 areas from each slide. Statistical analyses were done by comparison to the levels in wild type cells, and $^{**}p < 0.01$ were considered significant.

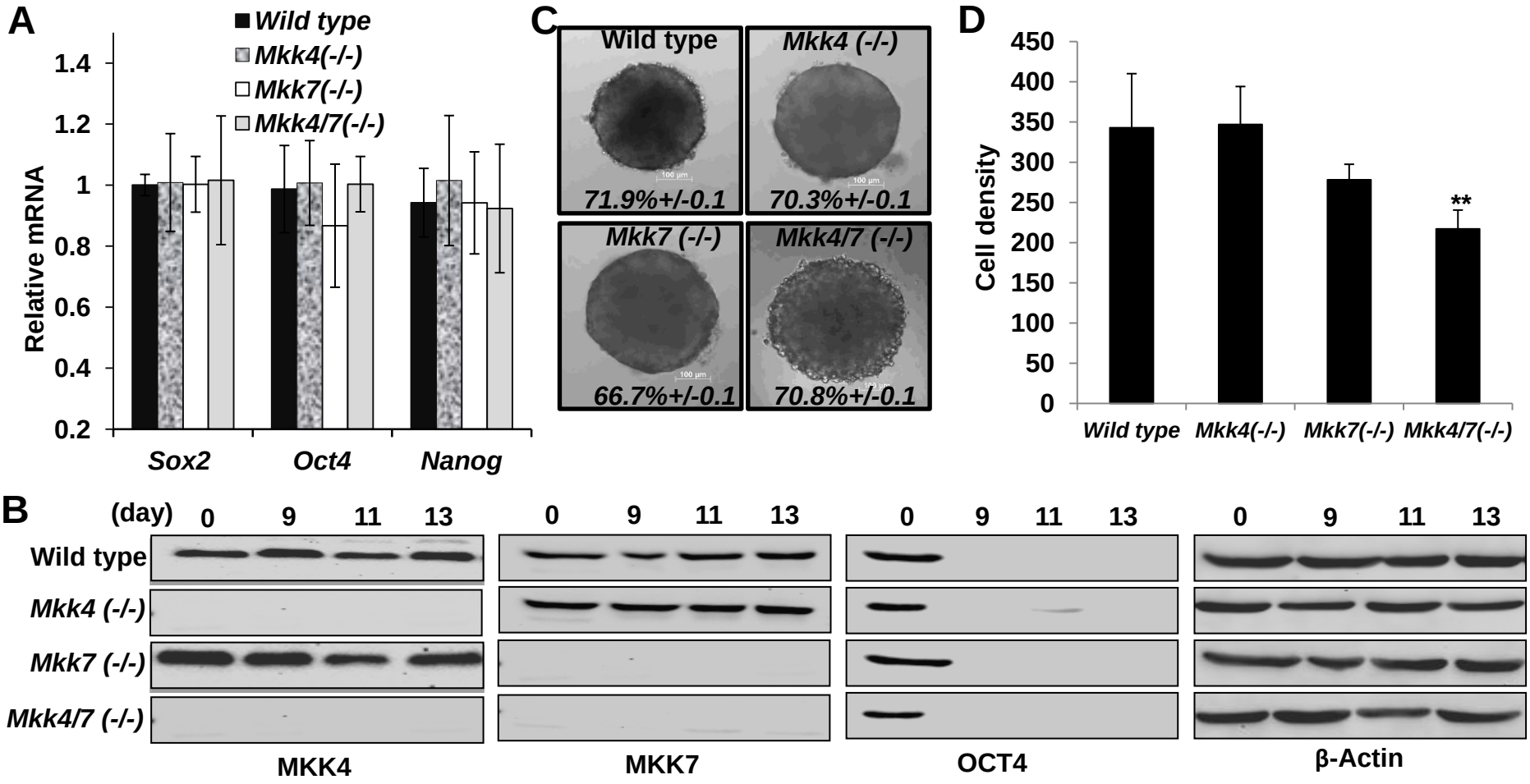
Supplemental Figure 2. Roles of MKK4 and MKK7 in mesodermal lineage differentiation. (A) A schematic illustration of mesodermal lineage differentiation. The ESCs first commit to primitive mesoderm, expressing *Fgf5, T (Brachyury),* and *Nodal,* followed by early myogenesis, expressing *Nkx2.5*. These cells can further different into smooth muscle, expression *a-sm-actin*, or cardiac muscle, expressing myosin light chain (*Mlc*) and myosin heavy chain (*Mhcs*). The wild type, *Mkk4 (-/-)* and *Mkk7(-/-)* ESCs were either undifferentiated or subjected to EB differentiation for different days and the expression of lineage-specific genes were examined by real time RT-PCR. The relative expression value was normalized to that of *Gapdh* in each sample and results are shown as mean ± SD from triplicates of at least three experiments. Statistical analyses were done by Student *t-test*, comparing to the expression in knockout to that in wild type cells at the same differentiation stage.

Supplemental Figure 3. Activation of the JNK-c-Jun pathways during ESC differentiation. (A) Wild type and *Mkk7(-/-)* ESCs were either maintained under stem cell condition or subjected to EB differentiation for 13 days in the presence or absence of the p38 inhibitor SB202190 (5 μM) at days 6-13. The cell lysates were analyzed by Western blotting using antibodies as indicated. (B) Wild type and *Mkk7(-/-)* ESCs were either undifferentiated or differentiated under the EB protocol for 13 days. In some cases, the EBs were exposed to the JNK inhibitor, SP600125 (5 μM) and ERK inhibitor PD98059 (5 μM) at days 6-13. The number of EBs that developed rhythmic beating was counted under light microscopy and the percentage versus total number of EBs was calculated. Results are shown as mean ± SD from at least three experiments.

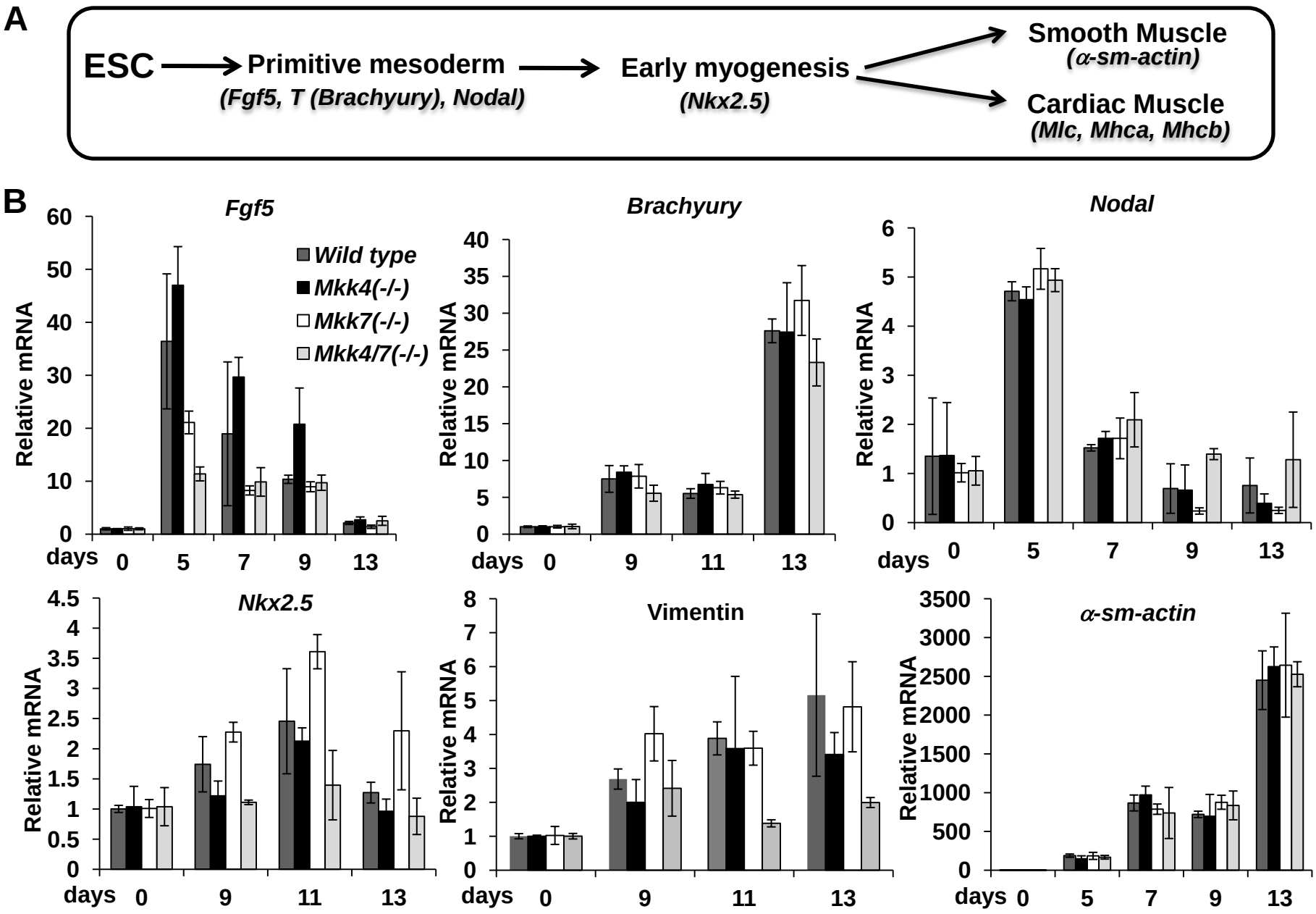
Supplemental Figure 4. Characterization the pNkx2.5PuroIRES2eGFP stable cells. The pNkx2.5PuroIRES2eGFP ESCs were treated with or without puromycine (3μg/ml) from day 4-6 of differentiation in the presence or absence of p38 inhibitor from days 6-13. The GFP positive cells were observed undera fluorescent microscopy, while the scale bars represent 100 μm.

Supplemental Figure 5. Activation of c-Jun and ATF2 in ESC differentiation. Wild type and *Mkk4 (-/-)*, *Mkk7(-/-)* and *Mkk4/7(-/-)* ES cells were analyzed at different days of EB differentiation by Western blotting using antibodies as indicated.

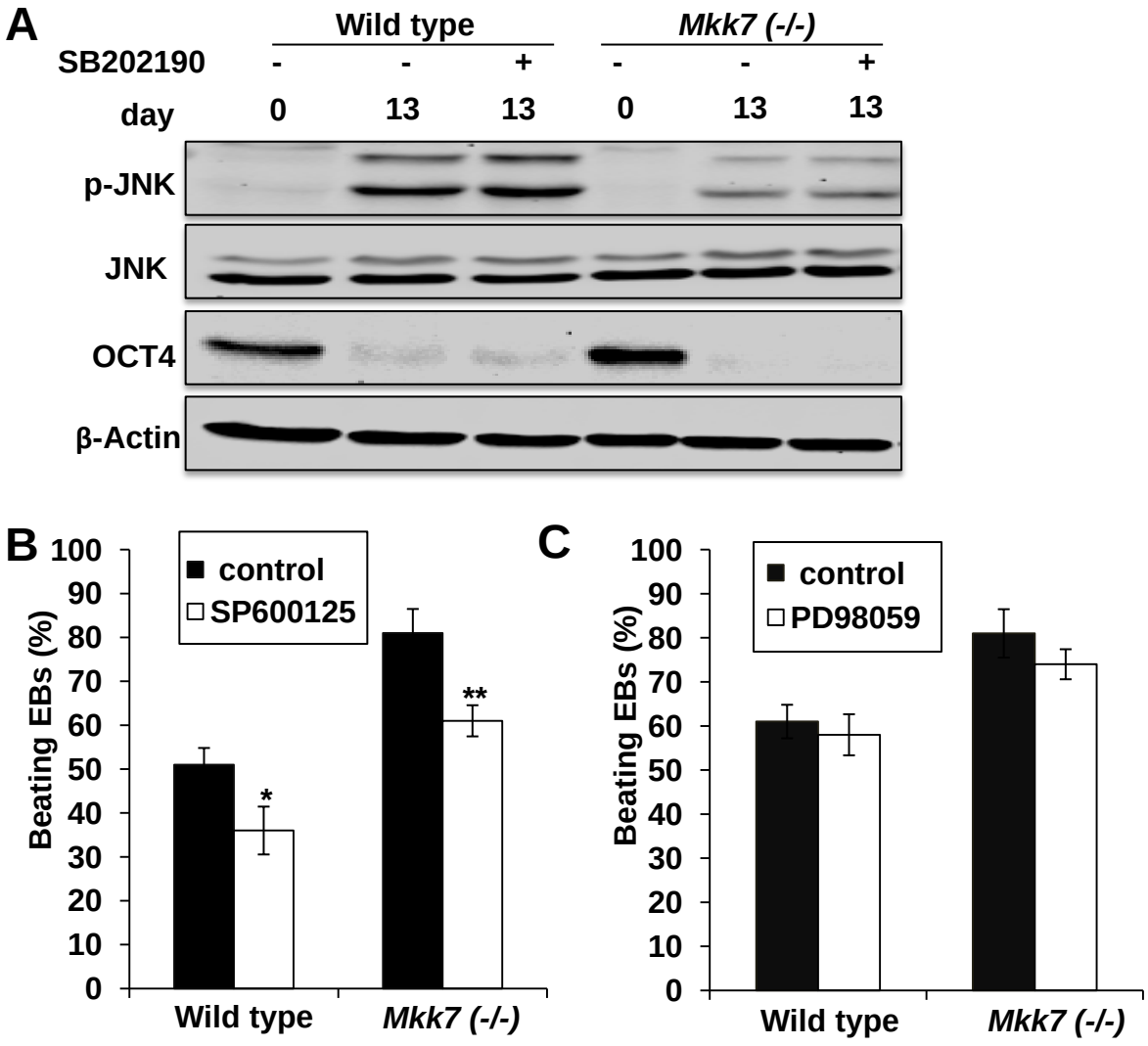
Supplemental Figure 1, Wang, et. al.



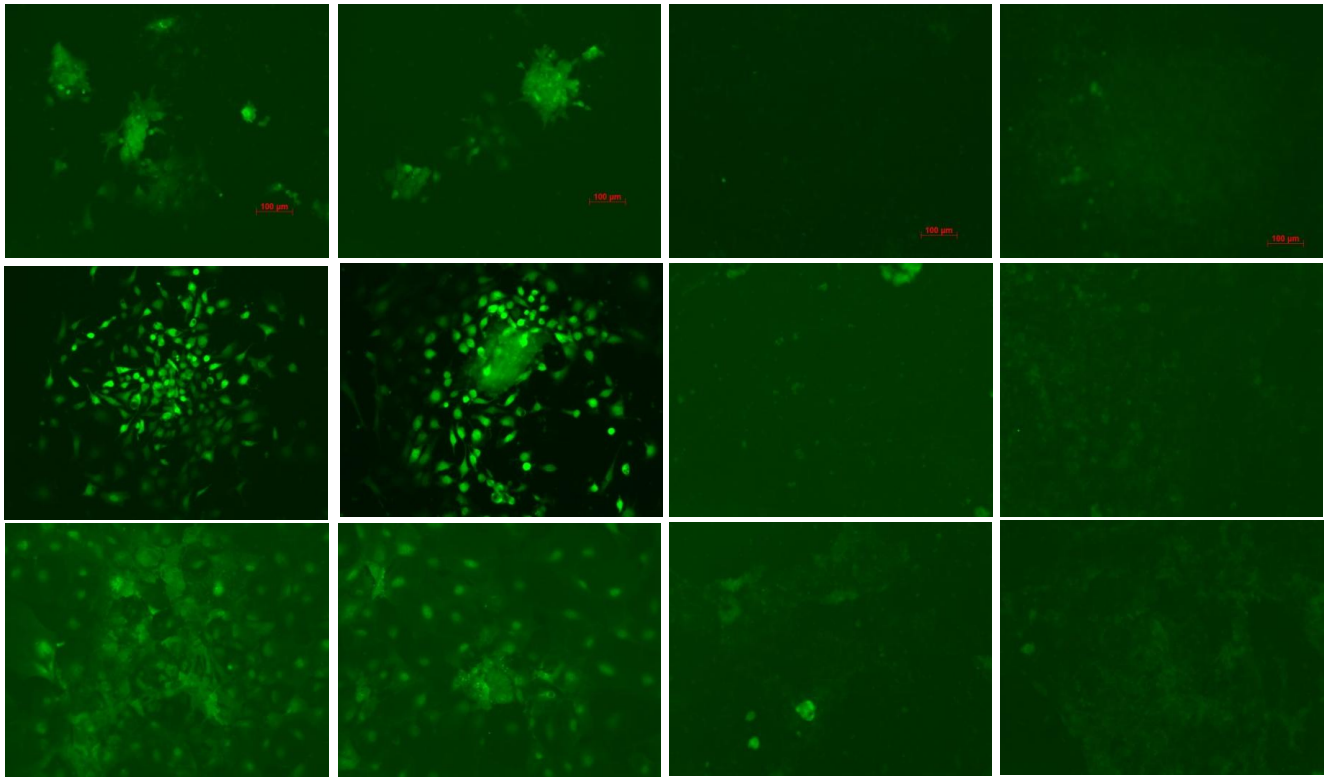
Supplemental Figure 2, Wang, et. al.



Supplemental Figure 3, Wang, et. al.



Supplemental Figure 4, Wang, et. al.



Supplemental Figure 5, Wang, et. al.