

Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: Comparative transcriptome analysis of day-0, day-3, day-4.5 Rax::GFP⁺ and day-4.5 Rax::GFP⁻ samples

File Name: Supplementary Data 2

Description: Analysis of gene ontology biological processes (GOBPs) in upregulated genes of the Rax::GFP⁺ rostral cells.

File Name: Supplementary Data 3

Description: List of gene ontology biological processes (GOBPs), which were analyzed by using upregulated genes in Rax::GFP⁻

File Name: Supplementary Movie 1

Description: Rax::GFP⁺ rostral formation in ESC-derived aggregate during days 1 to 7 (related to Figure 1a) Left, merged image of DIC and Rax::GFP⁺ (green); right, Rax::GFP⁺ were observed by time-lapse imaging. The movie was taken at 1 frame per hour and played at 15 frame per second (fps).

File Name: Supplementary Movie 2

Description: Six3::Venus⁺ rostral and Irx3::Tomato⁺ caudal formation in ESC-derived aggregate during days 3 to 7 (related to Figure 1f, g) Left, merged image of DIC, Six3::Venus⁺ (green) and Irx3::Tomato⁺ (red); center, merged image of Six3::Venus⁺, Irx3::Tomato⁺; right, Irx3::Tomato⁺ (enhanced images) were observed by time-lapse imaging. The movie was taken at 1 frame per hour and played at 15 fps.

File Name: Supplementary Movie 3

Description: Six3::Venus⁺ and Fgf5::Turq⁺ expression dynamics in ESC-derived aggregate during days 1.3 to 4 (related to Figure 2b) Left, merged image of Six3::Venus⁺ (green) and Fgf5::Turq⁺ (cyan); center, Six3::Venus⁺ (green); right, Fgf5::Turq⁺ (pseudo color) were observed by time-lapse imaging. The movie was taken at 1 frame per 4 hours and played at 15 fps.

File Name: Supplementary Movie 4

Description: 7Tcf::Cherry⁺ expression of ESC and day-4 aggregate for 24 hours after addition of Wnt agonist, CHIR99021 (related to Supplementary Figure 5B) Part 1, ESCs; part 2, day-4 aggregate. Left, merged image of DIC and 7Tcf::mCherry⁺ (red); right, 7Tcf::mCherry⁺ were observed by time-lapse imaging. The movie was taken at 1 frame per 15 minutes (ESCs) or 1 frame per 40 minutes (day-4 aggregate) and played at 15 fps.

File Name: Supplementary Movie 5

Description: Rax::GFP⁺ and 7Tcf::Cherry⁺ expression dynamics in ESC-derived aggregate during days 1.25 to 6.25 (related to Figure 5a) Left, merged image of DIC, Rax::GFP⁺ (green) and 7Tcf::mCherry⁺ (red); right, Rax::GFP⁺ and 7Tcf::mCherry⁺ were observed by time-lapse imaging. The movie was taken at 1 frame per 40 minutes and played at 15 fps.