Supplementary information

ICM conversion to epiblast by FGF/ERK inhibition is limited in time and requires transcription and protein degradation

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Running title: Temporal dynamics of ICM specification

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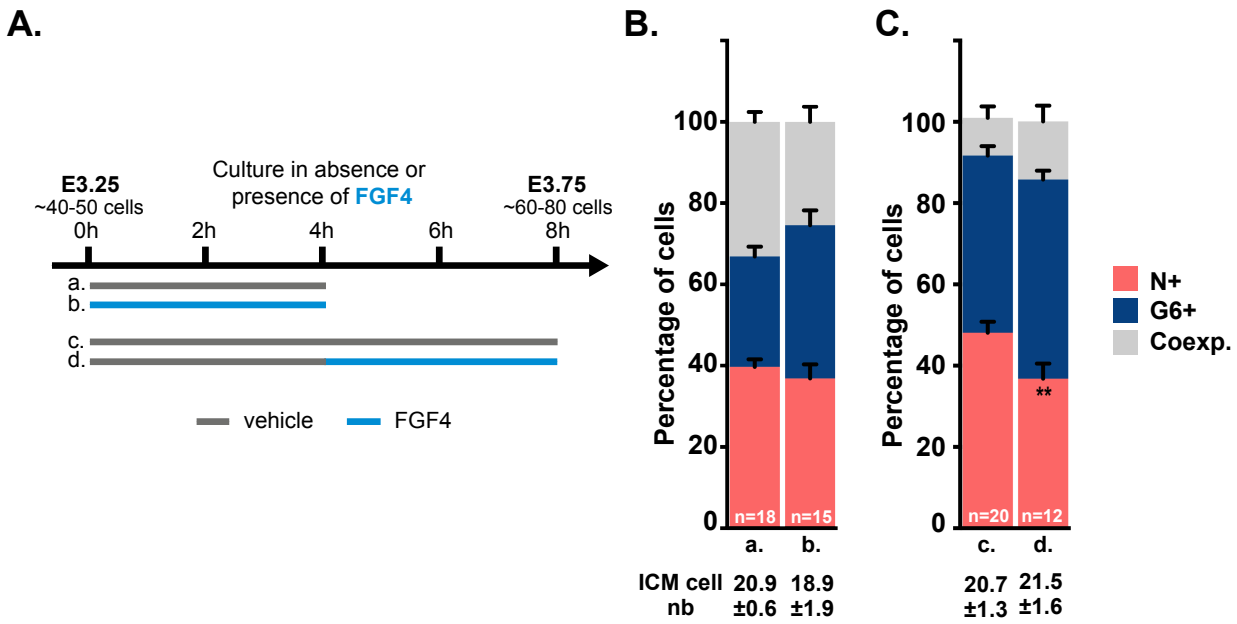
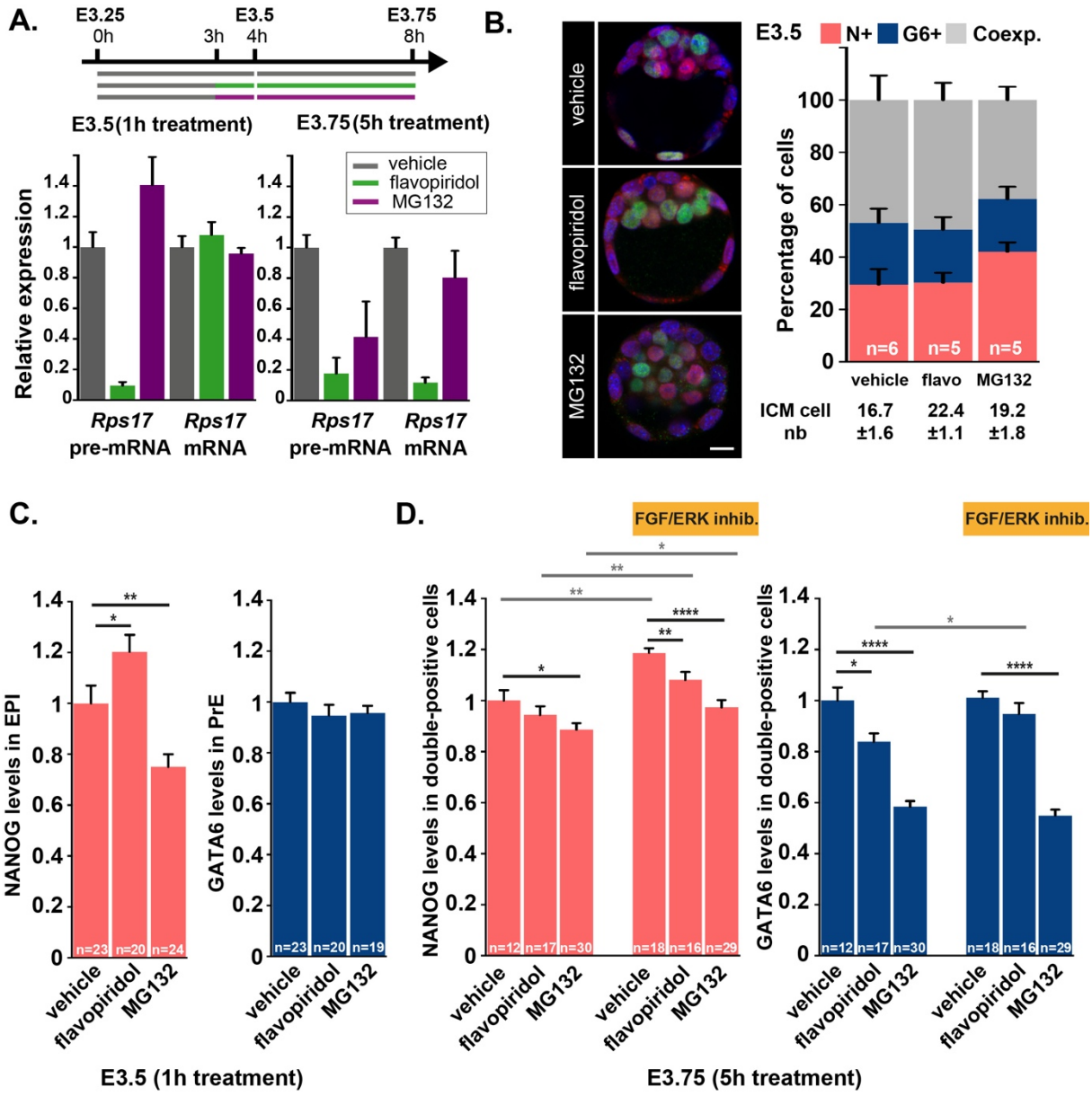


Supplementary Figure S1 (related to Figures 3 and 4). Effect on ICM specification of exogenous FGF 4 stimulation between E3.25 and E3.75. (A) Schematic of the time schedule of vehicle (grey) and FGF4 (blue) treatment. **(B-C)** Distribution of ICM cells expressing NANOG (N+, red), GATA6 (G6+, blue) or both markers (Coexp., grey) in embryos cultured in presence or absence of FGF4. Control embryos plotted in Figure S2B are similar to Figure 1B. Error bars indicate SEM. *n*, number of embryos analyzed. Statistical Mann–Whitney tests are indicated when significant (**, $p < 0.01$).



Supplemental Figure S2 (related to Figure 5). Effect of modulating transcription/proteasome activity during ICM specificationw. (A) Schematic of the time schedule of inhibitor treatment. Green, purple and grey lines indicate the culture periods in the presence of flavopyridol, MG132 and DMSO (vehicle), respectively. RT-qPCR expression analysis of *Rps17* pre-mRNA and *Rps17* mRNA in embryos cultured 1h and 5h with/without the drugs. **(B)** Immunodetection of NANOG (green) and GATA6 (red) in embryos cultured 1h (from 3h to 4h) in the presence/absence of drugs. Pictures correspond to a projection of 5 confocal optical slices. Scale bar: 20µm. Distribution of ICM cells expressing NANOG (N+, red), GATA6 (G6+, blue) or both markers (Coexp., grey) in embryos cultured for the indicated period of times. Error bars indicate SEM. *n*, number of embryos analyzed. **(C)** Quantification of NANOG levels

in Epi (NANOG-positive) progenitors and of GATA6 levels inPrE (GATA6-positive) in E3.5 embryos after 1 hours of treatment. **(D)** Quantification of NANOG and GATA6 levels in co-expressing ICM cells in E3.75 embryos after 5 hours of treatment. Error bars indicate SEM. *n*, number of cells analyzed. Statistical Mann–Whitney tests are indicated when significant (*, $p < 0.05$; **, $p < 0.01$;, ****, $p<0.0001$).