Supplementary Information

Supplementary Figure 1

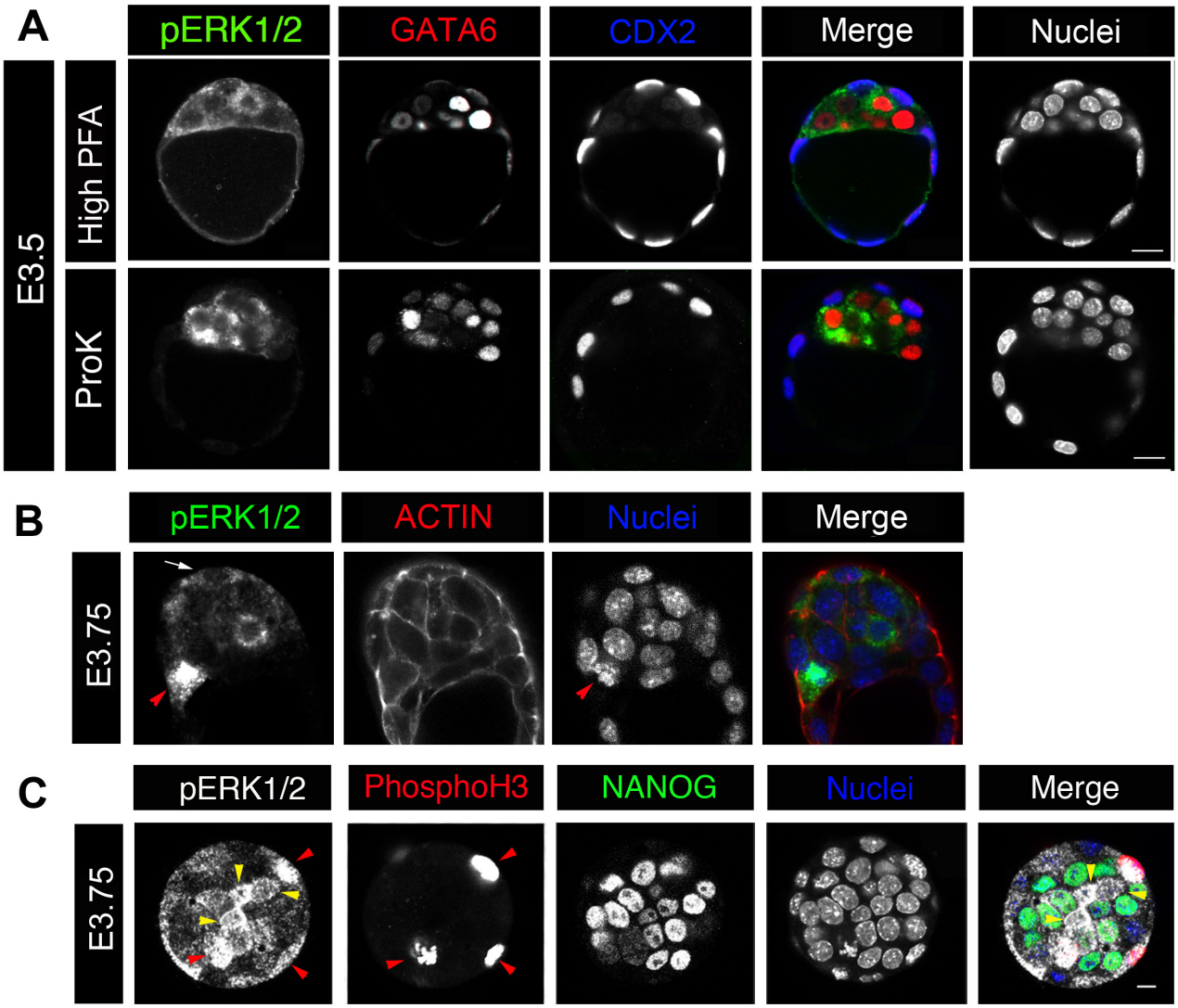

Figure S1. pERK labelling in blastocysts. A. Parallel staining of pERK, GATA6 and CDX2 with the High PFA and ProK protocols. B. ERK phosphorylation at E3.75 with signal in the TE (arrow) and ICM. The red arrowhead points toward a mitotic cell. C, Section through the ICM of an E3.75 embryo, stained with antibodies against pERK, NANOG and phospho-Histone3 to detect mitotic cells (n=8). Yellow arrowheads indicate cytoplasmic pERK while red arrowheads indicate nuclear pERK. Nuclear pERK cells are labelled by phospho-Histone3. Scale bars: 10 microns.

Supplementary Figure 2

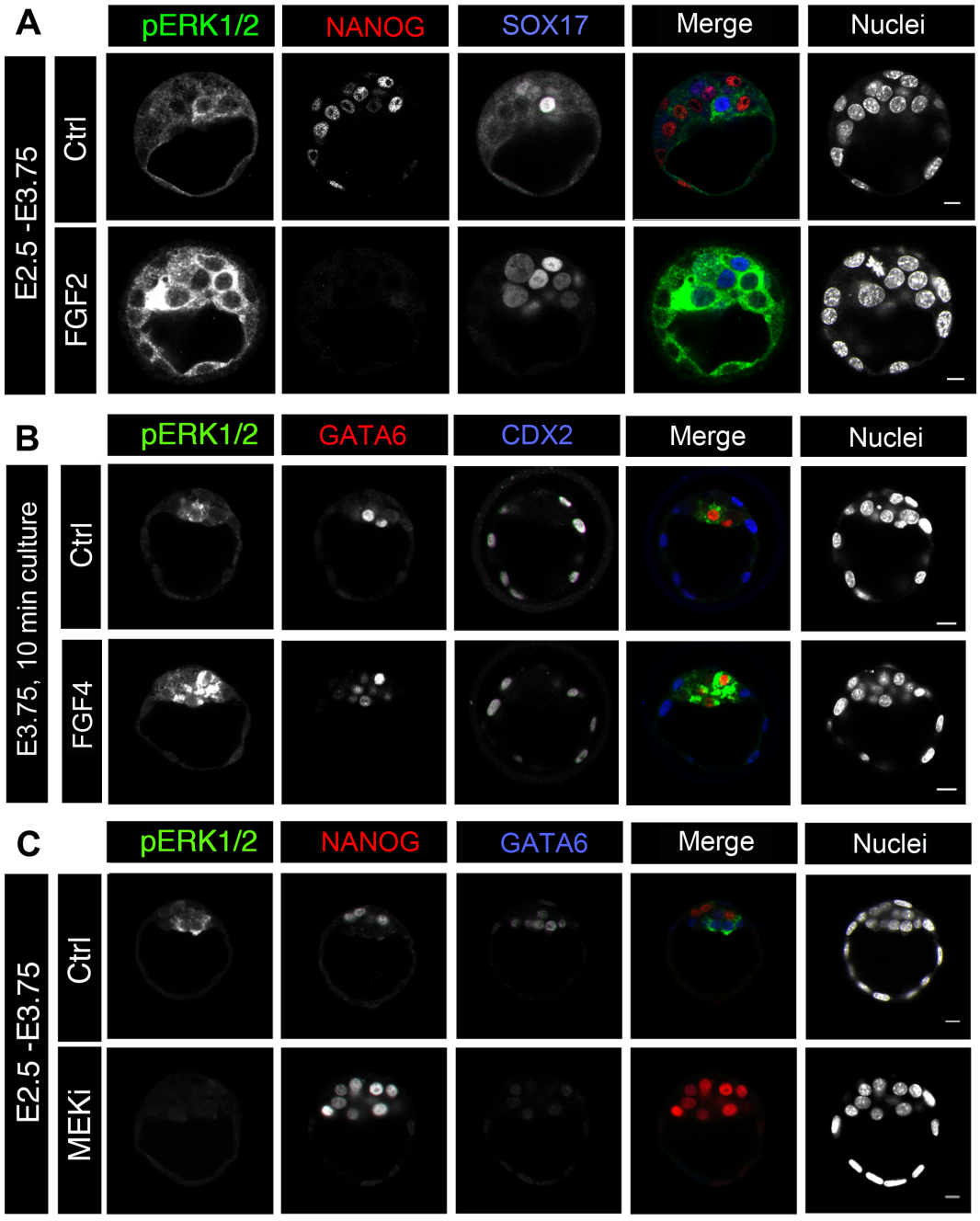

Figure S2. Validation of pERK staining in blastocysts. A, B. ERK phosphorylation after embryo cultures with FGF for 30h (A) (Ctrl, n=21; FGF4 n=22) or 10 min (B) (Ctrl, n=12; FGF4 n=14). C. pERK immunostaining after cultures with MEK inhibitor (Ctrl, n=18; MEK or MEK+FGFR inhibitors, n=18). Scale bars: 10 microns.

Supplementary Figure 3

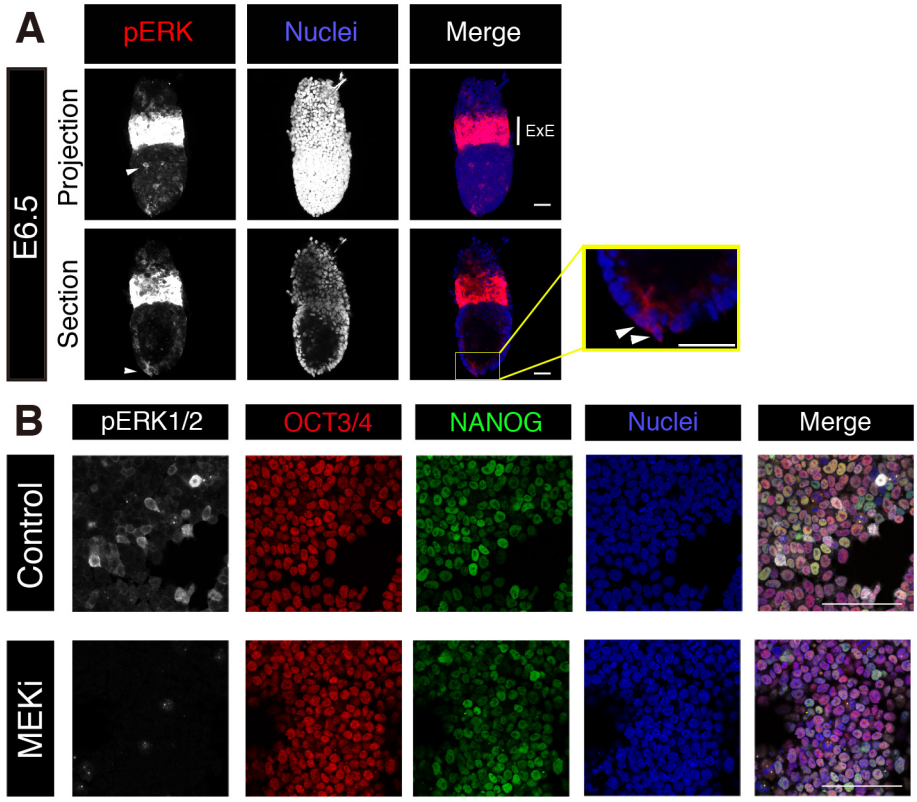

Figure S3: pERK immunostaining after implantation and in ES cells. **A**. ERK phosphorylation at E6.5 with a strong labelling in the extraembryonic ectoderm (ExE) and a weaker labelling in some PrE cells (arrowheads). The right panel is a magnification of the boxed area to show outside (VE) pERK-labelled cells (arrowheads). B. ERK phosphorylation in ES cells, co-stained with OCT3/4 and NANOG, cultured in absence (top panel) or presence (bottom panel) of the MEK inhibitor. Scale bars: 10 (A) and 100 (C) microns.

Supplementary Figure 4

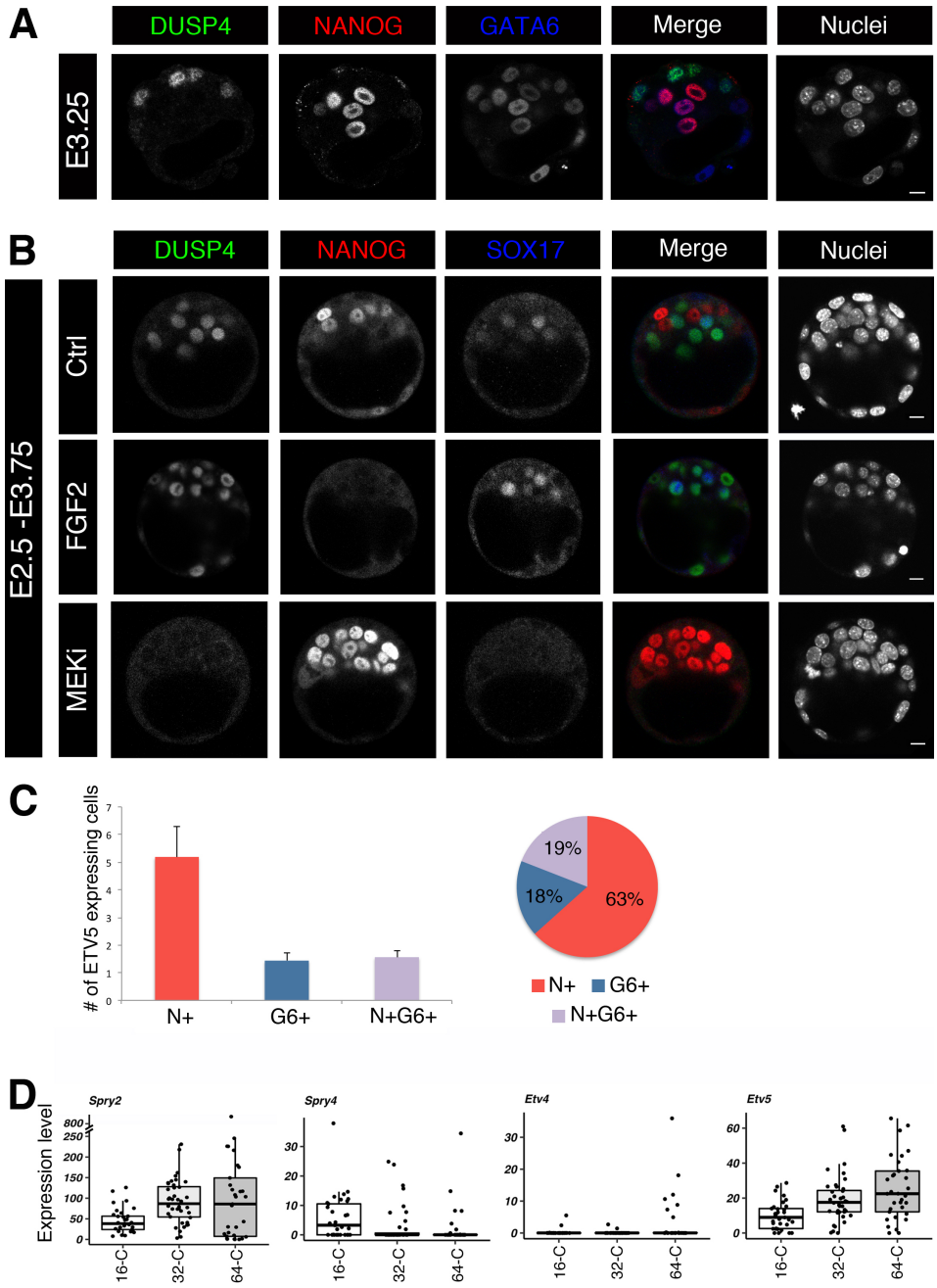

Figure S4. Expression of factors related to the FGF pathway. **A.** DUSP4, NANOG and GATA6 detection at E3.25 (n=15). **B**. DUSP4, NANOG and SOX17 immunolabelling in embryos cultured with either MEK inhibitor or FGF2 from E2.5 to E3.75 (Ctrl, n=20; FGF2, n= 15; MEK or MEK+FGFR inhibitors, n= 22). Scale bars: 10 microns. **C**. Number of cells labelled by ETV5 per embryo at E3.5-E3.75 (n=16 embryos) and their distribution between precursor (N+G6+), Epi (N+) or PrE cells (G6+). Data are represented as mean ± SEM. **D**. Expression of indicated genes by single-cell RNA-seq extracted from (Posfai et al., 2017) at the 16-cell stage (33 inner cells), E3.25 (32-C, 40 ICM cells) and E3.5 (64-C, 33 ICM cells). Expression levels are in RPKM.

Table S1: List of primary antibodies used

Epitope	Host	Supplier	Reference	Dilution
pERK	rabbit	Cell Signaling	4370	1/50
GATA6	goat	R&D	AF1700	1/300
pHistone 3	rabbit	Millipore	06-570	1/100
NANOG	rabbit	Abcam	ab80892	1/100
NANOG	rat	e-bioscience	14-5761	1/100
DUSP4	rabbit	Abcam	ab216576	1/100
ETV5	rabbit	Proteintech	13011-1-AP	1/100
SOX17	goat	R&D	AF1924	1/100
CDX2	mouse	Abcam	ab89949	1/1
OCT3/4	mouse	SantaCruz	Sc-5279	1/100

Table S2: list of primers used for the single-cell RTqPCR

RefSeq #	Gene	Forward	Reverse
NM_176933.4	*Dusp4*	AGCTCCTGGTTCATGGAAGC	ACTCAAAAGCCTCCTCCAGC
NM_001316365	*Etv4*	GCAGGGAAAGCTCATGGAC	GAGCCACGTCTCTTGGAAGT
NM_023794	*Etv5*	CAGAACCTGGATCACAGCAA	GACTGAGGAGGGAAGGGATG
NM_010202	*Fgf4*	ACTACCTGCTGGGCCTCAA	ACTCCGAAGATGCTCACCAC
NM_001079908	*Fgfr1*	GCTATAACCCCAGCCACAAC	AGCCAAAGTCTGCGATCTTC
NM_010207	*Fgfr2*	CACCAACTGCACCAATGAAC	GAATCGTCCCCTGAAGAACA
NM_001163485	*Rpl30*	AGTCTCTGGAGTCGATCAACT	AGCCAGTGTGCATACTCTGTAG
NM_009092	*Rps17*	ATGACTTCCACACCAACAAGC	GCCAACTGTAGGCTGAGTGAC