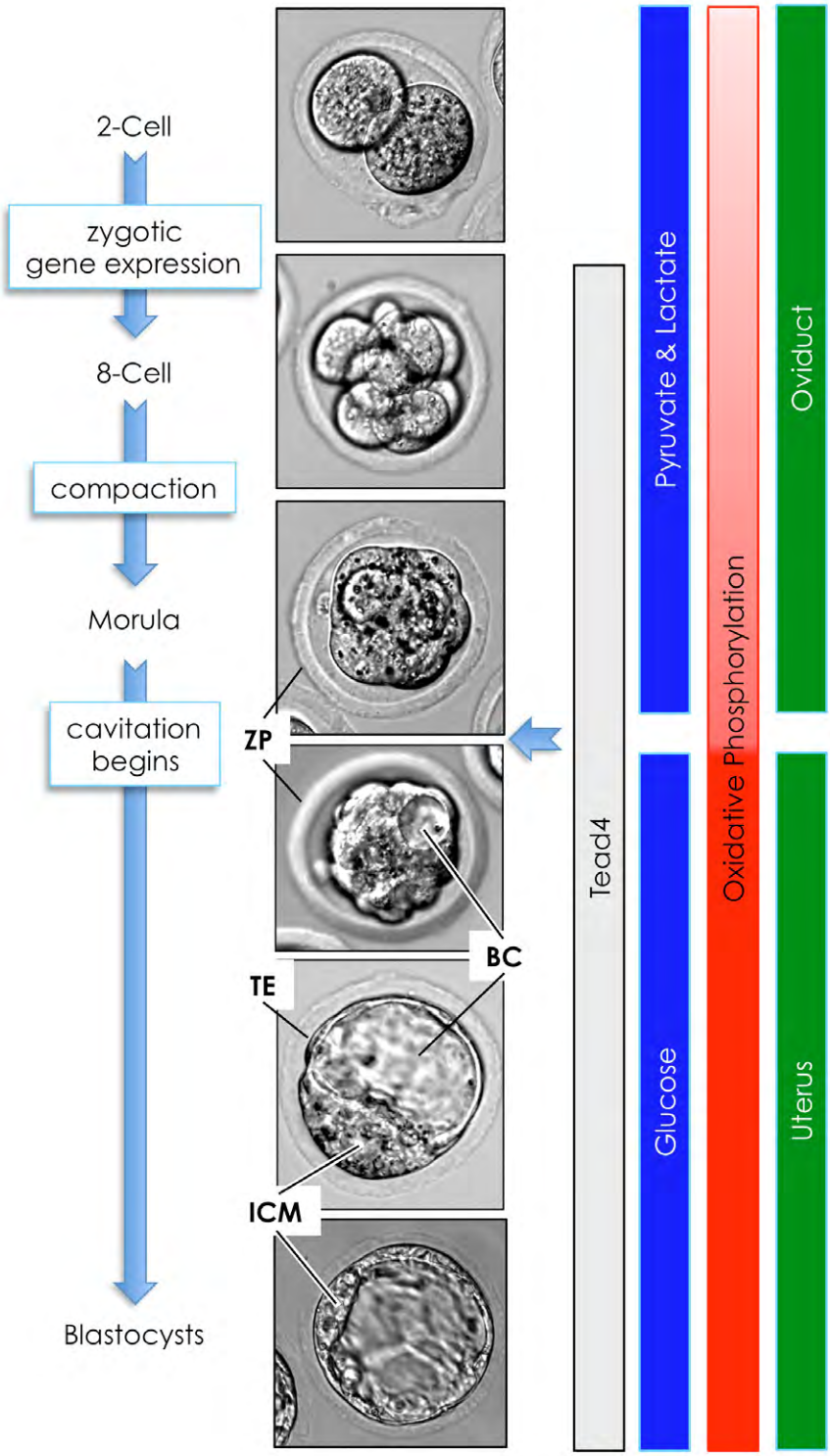


**Fig. S1. Changes in energy metabolism accompany blastocoel formation and expansion.** Zygotic gene activation in mice begins at the two-cell stage. At the eight-cell stage to the 16-cell stage, the blastomeres 'compact' to form a morula. During the morula to blastocyst transition, the outer cells become the trophectoderm (TE), cells essential to produce the blastocoel (BC). This process uses the energy-expensive $Na^+/K^+$ ATPase. As the blastocoel expands, the remaining pluripotent cells become the inner cell mass (ICM), which is clearly visible even in partially expanded blastocysts. The fully expanded blastocysts hatches by rupturing the outer glycoprotein layer termed zona pellucida (ZP). As the embryo transverses from the oviduct to the uterus, there is a major shift in energy source from pyruvate and lactate to glucose. This change in energy substrates is accompanied by an increase in oxygen consumption and oxidative phosphorylation that occurs specifically in the TE in order to accommodate the demands of the $Na^+/K^+$ ATPase. Thus, the appearance of a blastocoel is a bioassay for functional TE. Phase-contrast photographs of developing embryos are at 40×. *Tead4* is a transcription factor that is first expressed at the eight-cell stage and continues to be express throughout preimplantation development. One-cell embryos that lack a functional *Tead4* gene arrest development *in utero* as abnormally shaped morula with no detectable blastocoel (Yagi et al., 2007).

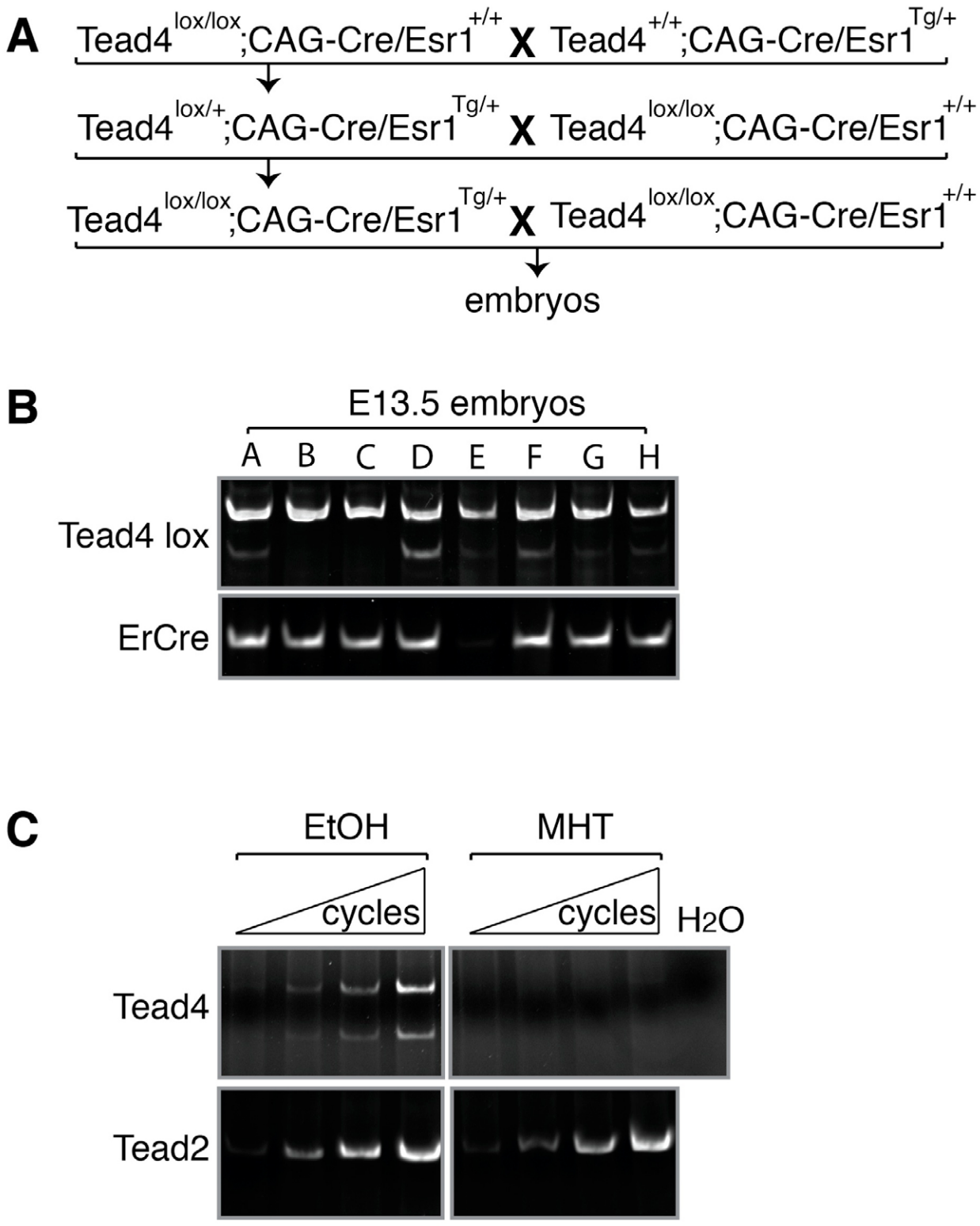


**Fig. S2. The *Tead4* gene can be ablated in embryonic cells using a monohydroxytamoxifen (MHT)-dependent Cre recombinase.** (**A**) Breeding protocol for isolating *Tead4*$^{lox/lox}$; *CAG-Cre/Esr1*$^{Tg/+}$ embryos. (**B**) DNA from 13.5-day-old embryos (lanes A-H) was genotyped to identify the *Tead4 lox* and *CAG-Cre/Esr1* (ErCre) alleles. For *Tead4* alleles, three primers were used (Genomic lox/null-F, Genomic lox-R, Genomic null-R) in order to amplify both the *lox* allele and the *null* allele in the same PCR reaction (supplementary material Table S1). (**C**) Primary mouse embryo fibroblasts (PMEFs) were isolated from each of the *Tead4*$^{lox/lox}$; CAG-Cre/*Esr1*$^{Tg/+}$embryos shown in panel B and then treated for 48 hours with either ethanol (EtOH) vehicle as a control or MHT to activate the Cre recombinase. Total RNA was isolated from PMEFs and assayed by RT-PCR to determine whether or not MHT converted *Tead4*$^{lox/lox}$ into *Tead4*$^{-/-}$ using RT-PCR assay. Results with PMEFs from embryo A in panel B are shown as an example. The primer set for *Tead4* RNA amplifies two transcript variants (Kaneko et al., 1997). Negative control used $H_2O$ was instead of cDNA. *Tead2* expression was used as a control to show that MHT treatment did not randomly suppress overall gene expression.

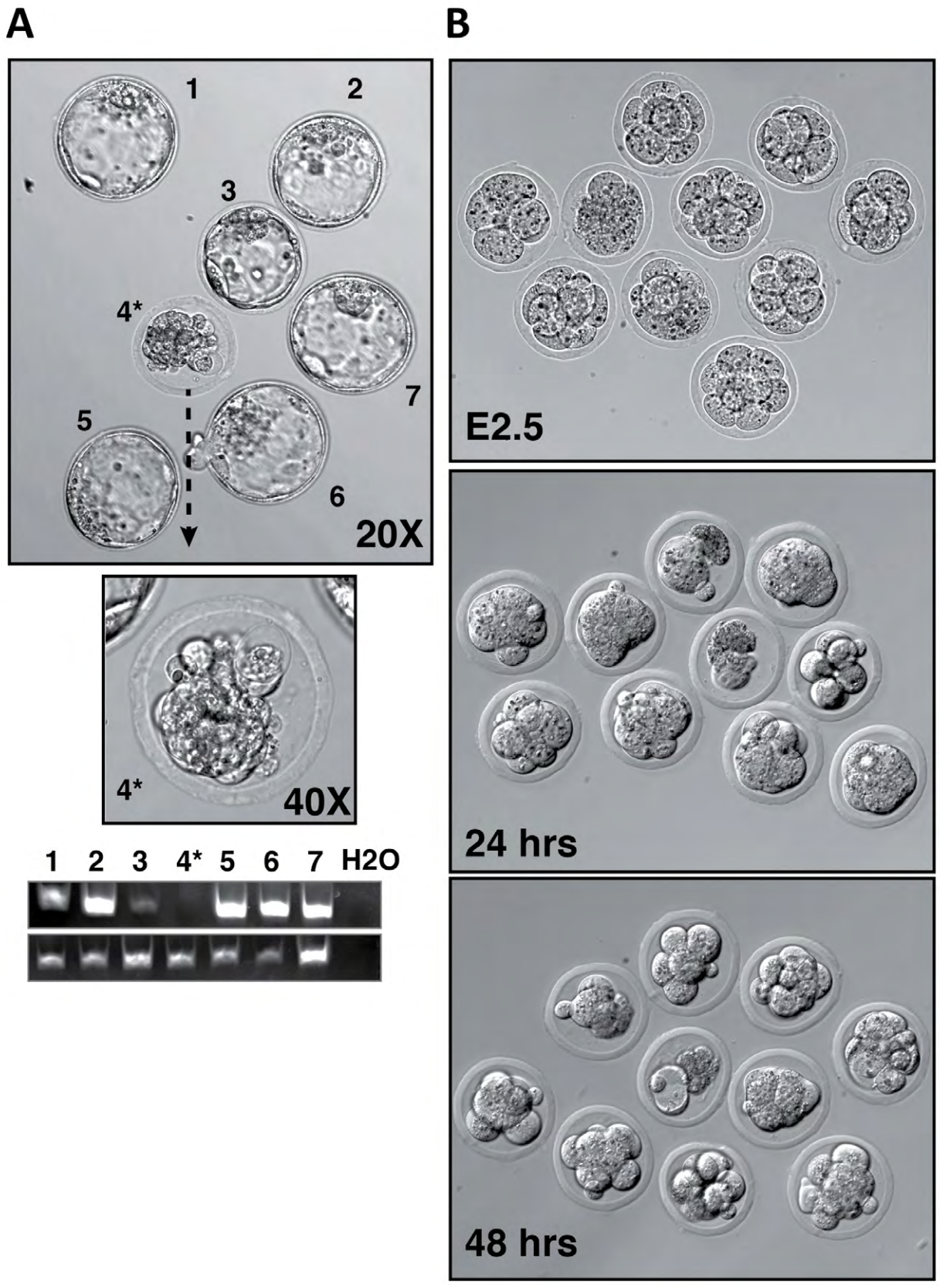


**Fig. S3. $O_2$ is crucial for embryo development but *Tead4*$^{-/-}$ embryos are hypersensitive to 21% $O_2$.** (**A**) Two-cell embryos (numbered 1-7) from a single litter of *Tead4*$^{+/-}$ matings were isolated and cultured in KSOM under atmospheric $O_2$. After 72 hours, the embryos were photographed using 20× or 40× objectives as indicated and genotyped as described for Fig. 1. The *Tead4*$^{-/-}$ embryo (4*) failed to form a blastocoel (BC), confirming previous report that at least one wild-type *Tead4* allele is required for BC to form under these conditions (Nishioka et al., 2008). (**B**) Eight-cell *Tead4*$^{+/+}$ embryos from *Tead4*$^{+/+}$ matings were isolated (top, E2.5) and cultured in KSOM under 0.5% $O_2$/5% CO2 (maintained in Sanyo MCO-5M tri-gas incubator) and photographed after 24 and 48 hours using 20× objectives.

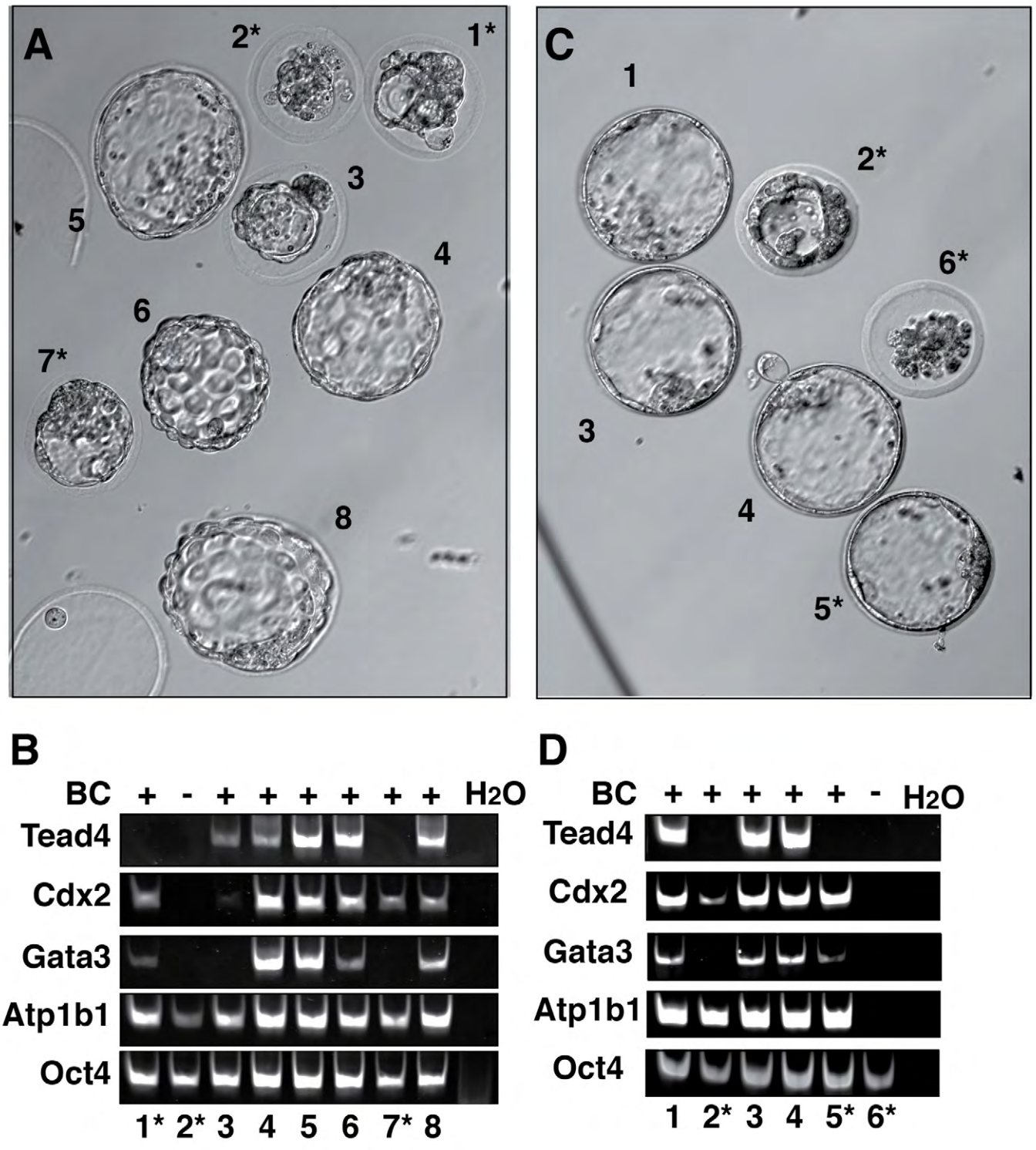


**Fig. S4. Neither the expression of TE-associated markers nor blastocoel formation depends on expression of *Tead4*.** (**A-D**) Two-cell embryos were isolated from two separate *Tead4*$^{+/-}$ matings and cultured in KSOM/5% $O_2$ for 72 hours. RNA was prepared from individual embryos and analyzed as described for Fig. 3. $H_2O$ was used in place of RNA to provide a negative control. In panel A, *Tead4*$^{-/-}$ embryos were embryos 1*, 2* and 7*, and in panel C, *Tead4*$^{-/-}$ embryos were embryos 2*, 5* and 6*, because those embryos did not express *Tead4* RNA (B,D). The presence or absence of blastocoel (BC) is indicated. As in Fig. 3, *Tead4* expression was not essential for expression of TE-associated genes, *Cdx2*, *Gata3* and *Atp1b1*. All embryos, including those *Tead4*$^{-/-}$ embryos that did not form a blastocoel, expressed ICM-specific *Oct4*. Intriguingly, the two *Tead4*$^{-/-}$ embryos that did not form a blastocoel also did not express *Cdx2*, suggesting that *Tead4* and *Cdx2* may play complimentary roles in mediating oxidative stress.

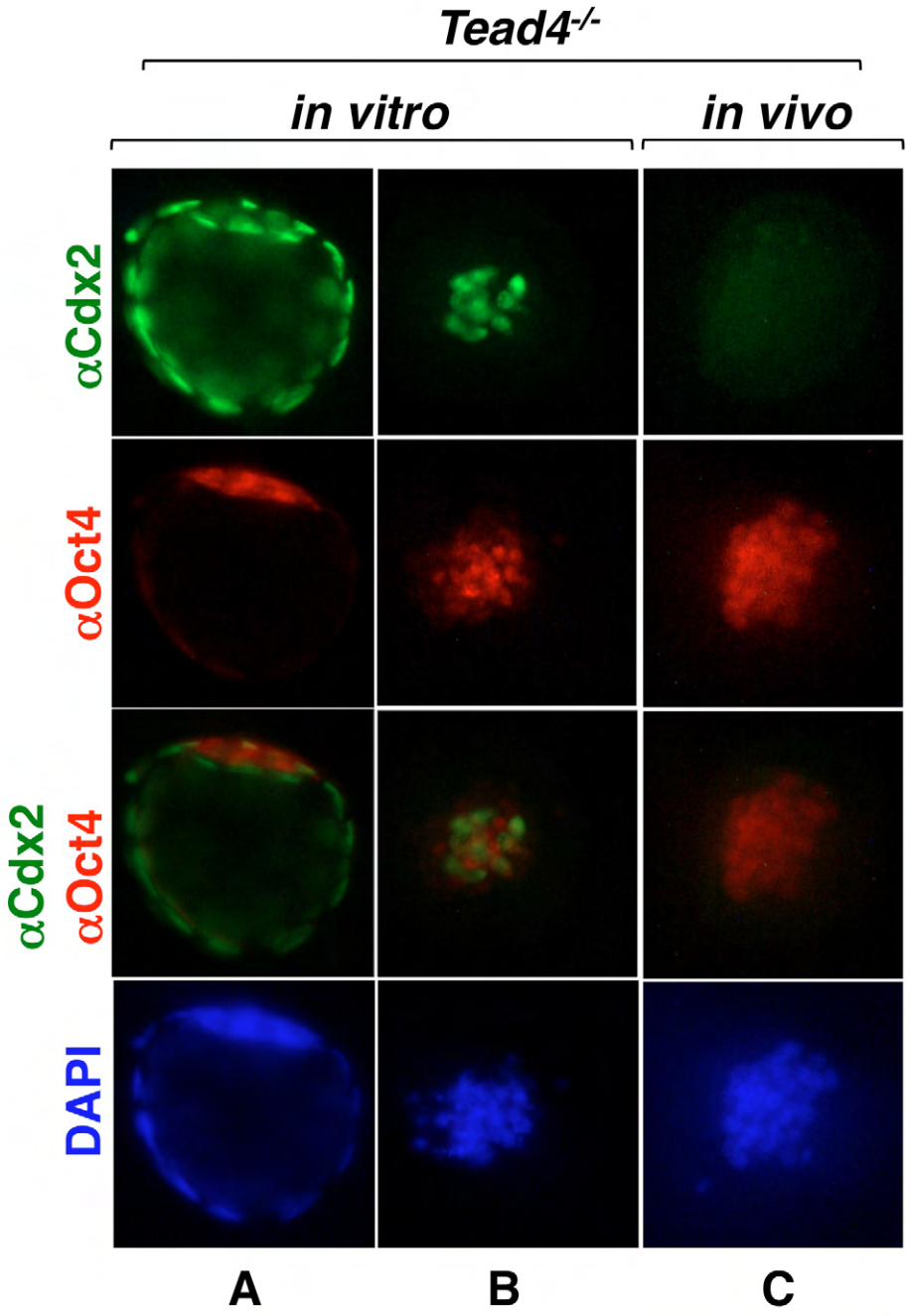


**Fig. S5. *Tead4*$^{-/-}$ embryos that developed in KSOM/5% $O_2$ expressed both OCT4 and CDX2 proteins.** *Tead4*$^{-/-}$ males were mated to *Tead4*$^{lox/lox}$; *ZP3-Cre*$^{Tg/+}$ females. Six *Tead4*$^{-/-}$ embryos were isolated from a single female at E2.5, cultured in KSOM/5% $O_2$ for 48 hours, then immunostained for OCT4 (red) and CDX2 (green) proteins, and stained for nuclear DNA with DAPI (blue). (**A**,**B**) Five of the embryos developed blastocoels (example in A) and one did not (B). Thus, *Tead4*$^{-/-}$ embryos developed under these *in vitro* conditions, even those that did not form a blastocoel, expressed both OCT4 and CDX2 proteins. (**C**) Embryos were isolated from the same mating scheme by flushing the uterus of the female at E3.5, fixed immediately and stained as above. All of seven embryos recovered expressed OCT4 protein but none of them expressed CDX2 or formed a blastocoel, as expected (Yagi et al., 2007). A representative example is shown. Embryos were stained (Ralston and Rossant, 2008) using mouse anti-CDX2 (CDX-88, Biogenex; 1:200), rabbit anti-OCT4 (Cell Signaling #2840S; 1:400), Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen; 1:400) and Alex Fluor 488 goat anti-mouse IgG (Invitrogen; 1:400). Stained embryos were photographed using an epifluorescent Nikon E600 photomicroscope (Vassilev et al., 2001).

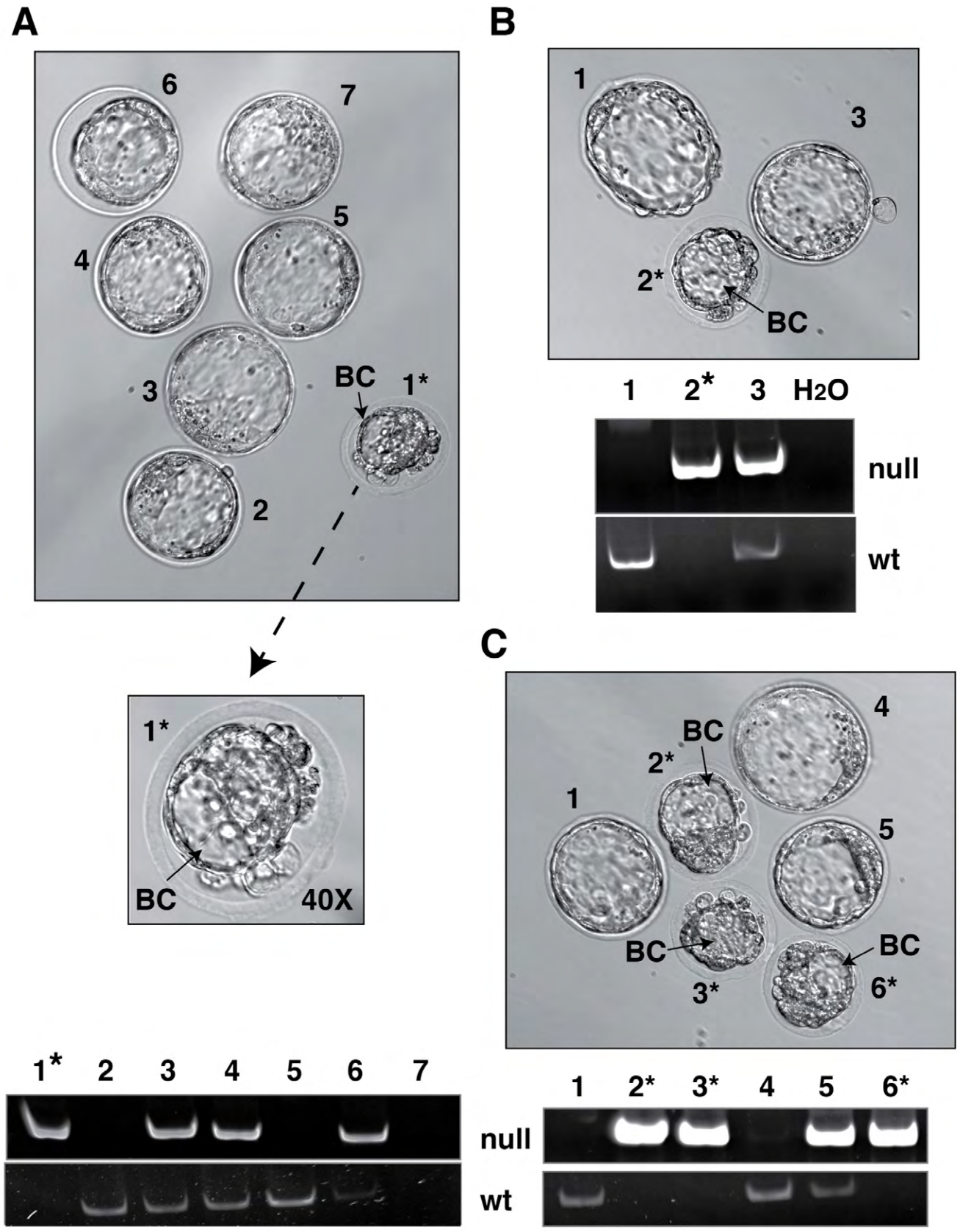


**Fig. S6. *Tead4*$^{-/-}$ eight-cell embryos could form blastocoels in KSOM with high glucose and either glutamine or essential amino acids.** (**A-C**) Eight-cell embryos at day E2.5 were isolated from *Tead4*$^{+/-}$ matings and cultured for 48 hours in KSOM medium in which the glucose concentration was raised from 0.2 to 3.4 mM. In this medium, embryo 1*, genotyped as *Tead4*$^{-/-}$, formed a blastocoel (BC). Furthermore, *Tead4*$^{-/-}$ eight-cell embryos formed a blastocoel in the same medium supplemented with either glutamine (B) or essential amino acids (C). Individual embryos were genotyped using PCR primers specific for either the wild-type (wt) or *Tead4* null allele as in Fig. 1. *Tead4*$^{-/-}$ embryos are indicated by an asterisk.

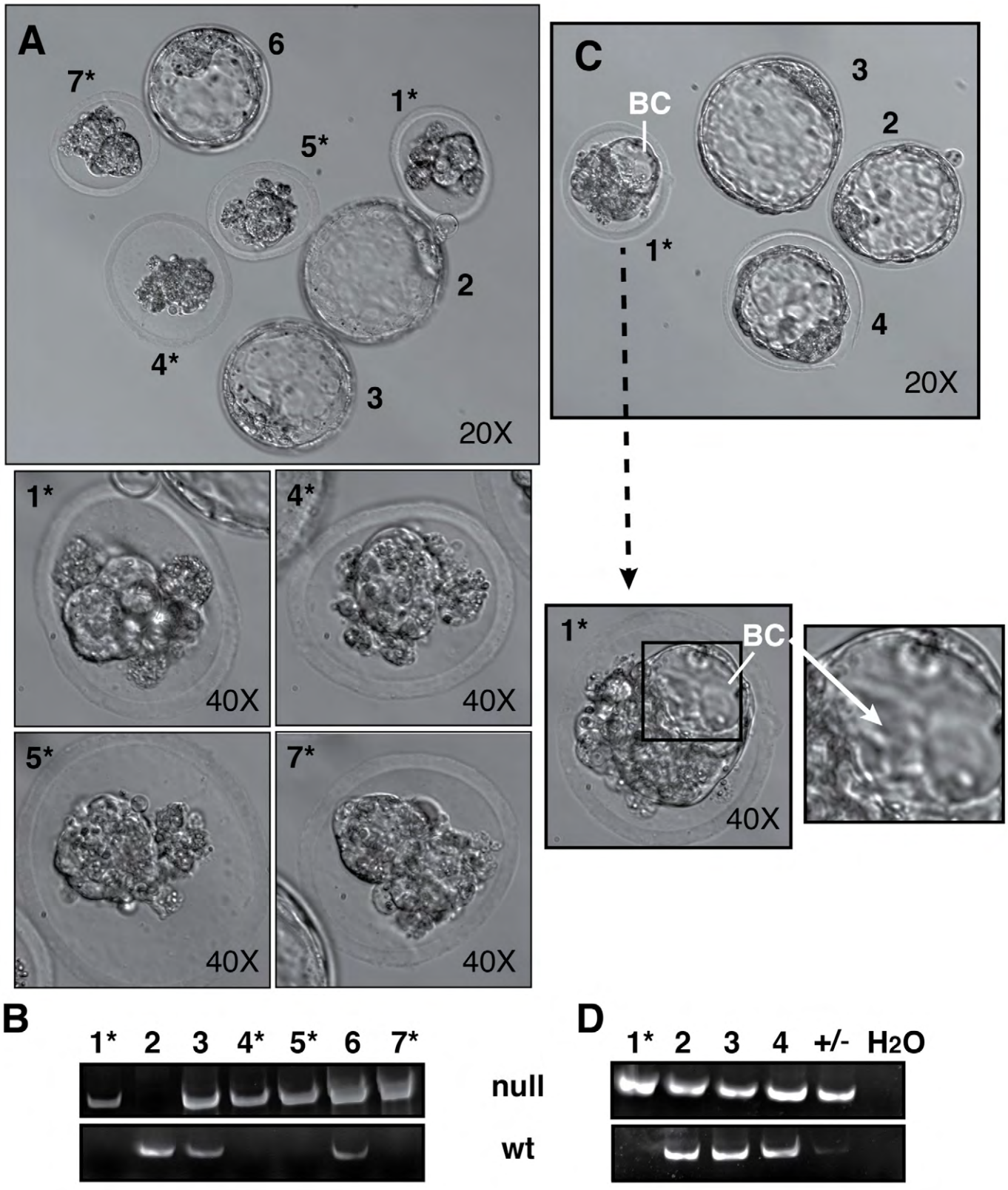


**Fig. S7. *Tead4*$^{-/-}$ eight-cell embryos could not form blastocoels in the presence of 3.4 mM glucose, glutamine (Gln), and essential amino acids (EAAs), but they could form blastocoels if N-acetylcysteine was included in the culture medium.** (**A**) Seven eight-cell embryos from a single litter were isolated from *Tead4*$^{+/-}$ matings at E2.5 and then cultured for 48 hours in KSOM with 3.4 mM glucose, Gln and EAAs. (**B**) Embryos 1*, 4*, 5* and 7* were genotyped as *Tead4*$^{-/-}$, and none of them possessed a blastocoel (shown at higher magnification in panel A, bottom). (**C**) Four eight-cell embryos from a single litter were cultured as in panel A, except that the culture medium also contained 500 μM N-acetylcysteine. All of them, including embryo 1*, contained a blastocoel (BC). (**D**) Embryo 1* was genotyped as *Tead4*$^{-/-}$.

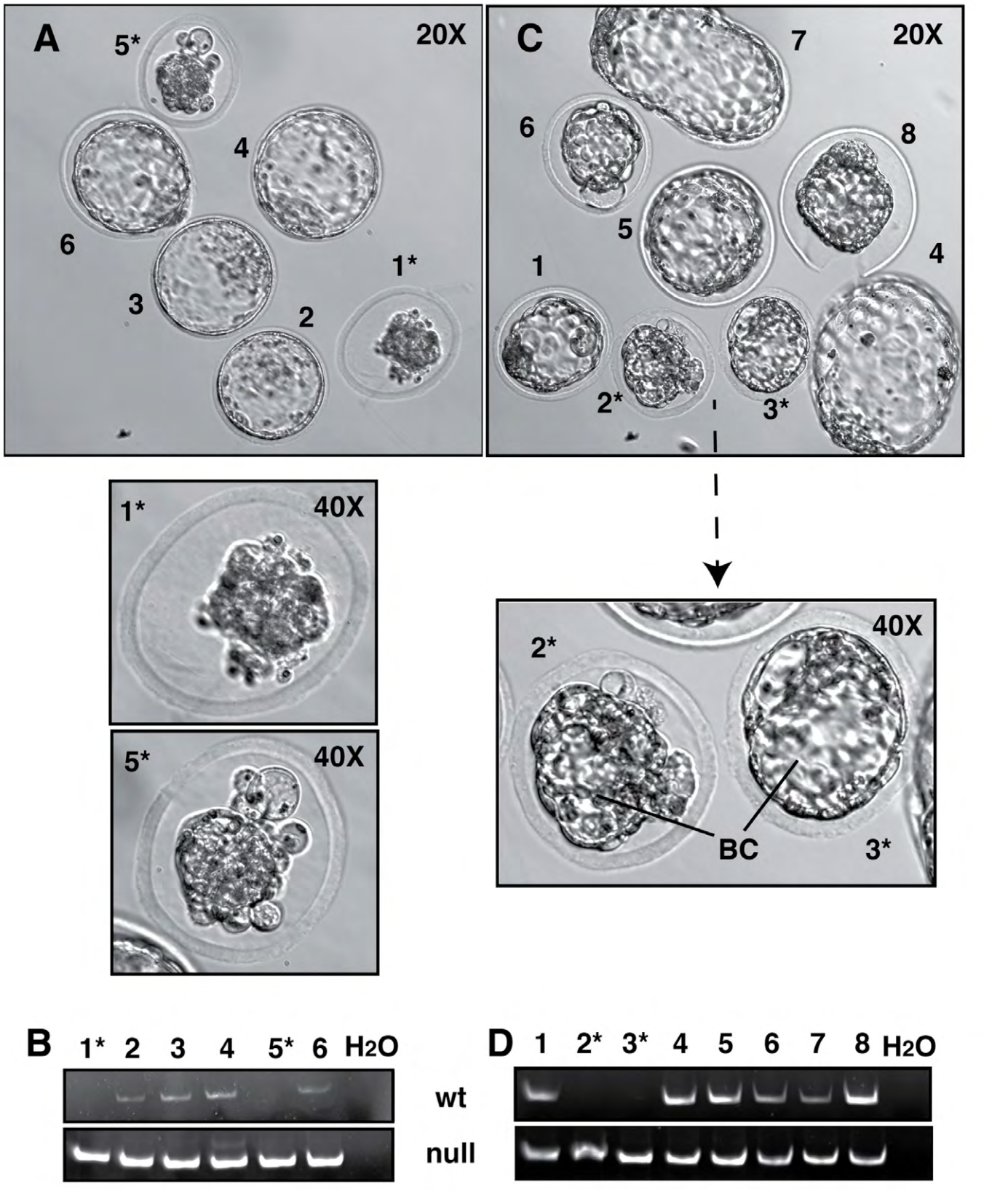


**Fig. S8. *Tead4*$^{-/-}$ two-cell embryos could not adapt to glucose starvation in the absence of an antioxidant.** (**A**,**B**) Two-cell embryos were isolated from *Tead4*$^{+/-}$ matings and cultured in 5% $O_2$ for 72 hours in KSOM lacking glucose and amino acids. Whereas all embryos reached at least the morula stage after 48 hours (data not shown), embryos 1* and 5* did not form a blastocoel and began to disintegrate by 72 hours of culture (A; microphotographs at higher magnification are shown below). Embryos 1* and 5* were genotyped as *Tead4*$^{-/-}$ (B). (**C**,**D**) Two-cell embryos were cultured for 72 hours in 5% $O_2$ in KSOM lacking glucose and amino acids as in panel A but supplemented with 500 μM N-acetylcysteine. Microphotographs of embryos 2* and 3* at higher magnification are shown below (C). Embryos 2* and 3* were genotyped as *Tead4*$^{-/-}$ (D).

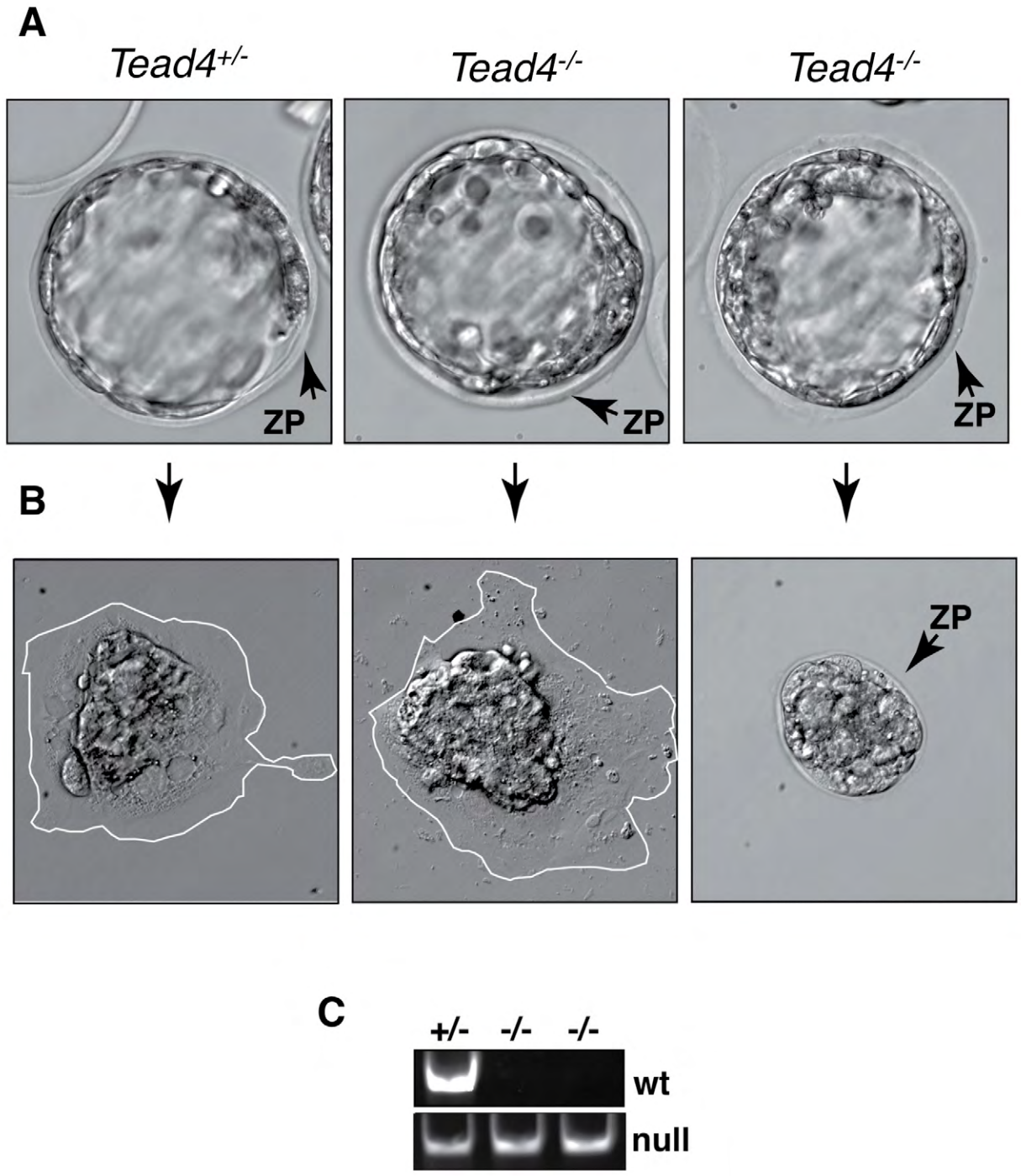


**Fig. S9. TEAD4 was not required for hatching from zona pellucida or formation of a blastocyst outgrowth.** (**A**) Two-cell embryos from *Tead4*$^{+/-}$ matings were cultured for 72 hours in KSOM at 5% $O_2$ and photographed using 40× objective. (**B**) Blastocysts formed were then transferred to trophoblast giant cell (TGC) media for blastocyst outgrowth assay as described (Yagi et al., 2007). Whereas most of *Tead4*$^{+/+}$ and *Tead4*$^{+/-}$ blastocysts (11/12 blastocysts) hatched from zona pellucida (ZP) and formed an outgrowth within 48-72 hours (example on left panel), most *Tead4*$^{-/-}$ (6/8 blastocysts) failed to hatch from ZP (example on right panel; Fig. 1C, Fig. 2B,–AT). However, an example of *Tead4*$^{-/-}$ blastocyst that was able to successfully hatch from ZP is shown in the middle panel; these blastocysts were capable of forming an outgrowth that was indistinguishable from those formed by *Tead4*$^{+/+}$ and *Tead4*$^{+/-}$ blastocysts. When ZP was removed by Acidic Tyrode's treatment, all *Tead4*$^{-/-}$ blastocysts (9/9) attached and formed an outgrowth (Fig. 2B; data not shown). All microphotographs were taken using 40× (A) or 20× (B) objectives. (**C**) Embryos/outgrowths were genotyped as described in Materials and methods.

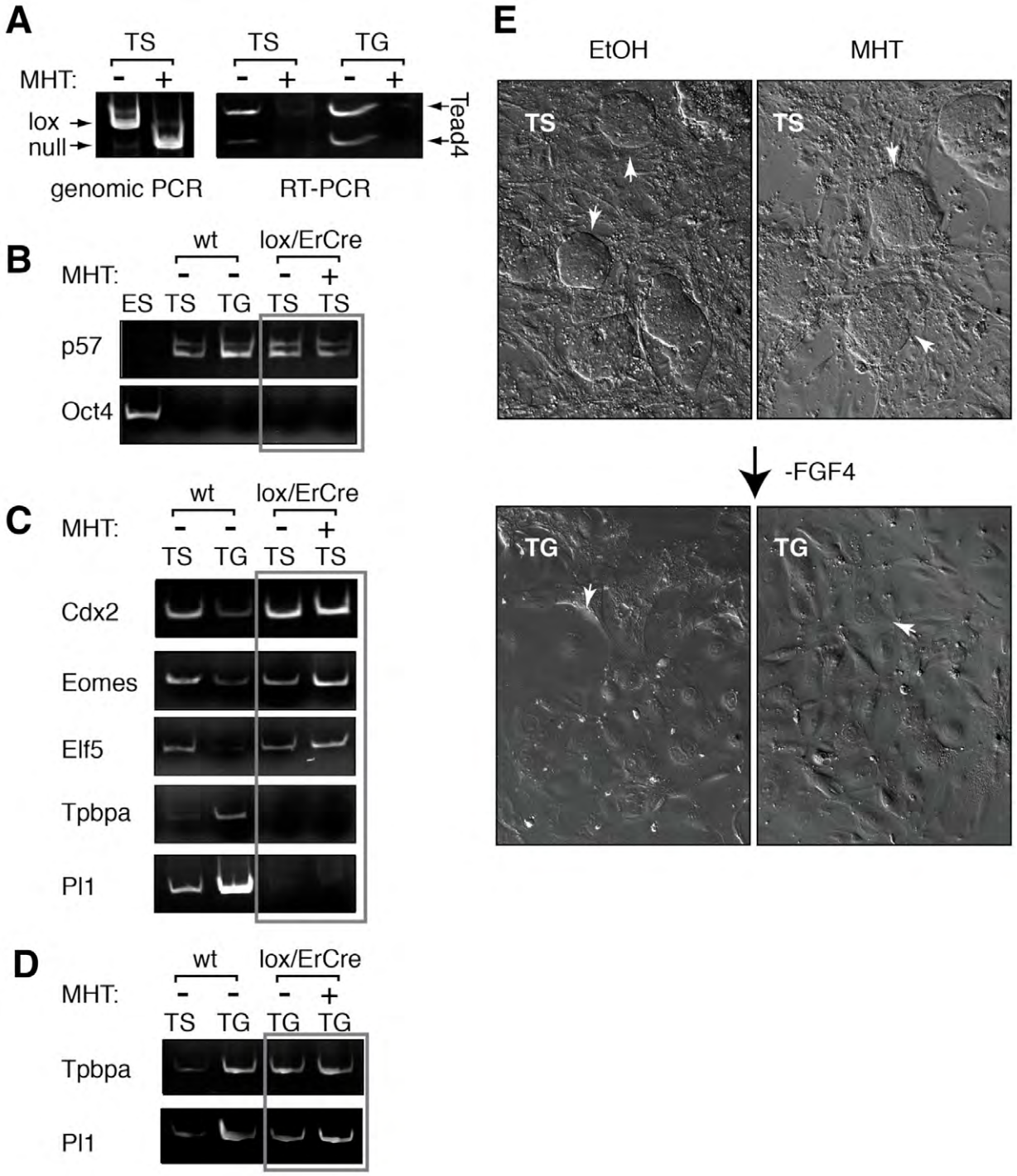


**Fig. S10. TEAD4 was not required for trophoblast stem (TS) cells either to proliferate or to differentiate into trophoblast giant (TG) cells.** *Tead4*$^{lox/lox}$; *CAG-cre/Esr1*$^{Tg/+}$ TS cells were cultured with either monohydroxytamoxifen (MHT) or ethanol control for 48 hours to produce *Tead4*$^{-/-}$ or *Tead4*$^{lox/lox}$ TS cells. TS cells were then induced to differentiate into TG cells by FGF4 deprivation for 5 days. (**A**) *Tead4 lox* and null alleles were detected by genomic PCR as described in supplementary material Fig. S2B. *Tead4* mRNA was detected by RT-PCR. MHT eliminated exon 2 from the *Tead4* gene as well as its corresponding mRNA. Amplicon sizes (supplementary material Table S1) were confirmed from DNA size markers (not shown) on the same gel. (**B**) RNA was prepared from *Tead4*$^{lox/lox}$ or *Tead4*$^{-/-}$ TS cells and assayed for presence or absence of the ICM marker *Oct4*, or the TE marker *p57*. RT-PCR using RNA prepared from control ES cells showed expression of *Oct4* but not *p57*, whereas RNA prepared from control TS cells or TG cells showed expression of *p57* but not *Oct4*. *Tead4* was essential neither for continued expression of *p57* nor for suppression of *Oct4* in TS cells or TG cells. (**C**) RNA from *Tead4*$^{lox/lox}$ or *Tead4*$^{-/-}$ TS cells was examined by RT-PCR for expression of genes normally upregulated in undifferentiated TS cells (*Cdx2*, *Eomes*, *Elf5*) as well as genes normally upregulated in differentiated TS cells (*Tpbpa* and *Pl1*). RT-PCR using RNA from control TS and TG cells are shown on the left. (**D**) RNA from *Tead4*$^{lox/lox}$ or *Tead4*$^{-/-}$ TG cells was examined by RT-PCR for expression of genes normally up regulated in TG cells (*Tpbpa* and *Pl1*). RT-PCR using RNA from control TG cells is shown on the left. (**E**) Phase-contrast microphotographs of *Tead4*$^{lox/lox}$ (EtOH) or *Tead4*$^{-/-}$ TS cells (MHT; top panel) and *Tead4*$^{lox/lox}$ or *Tead4*$^{-/-}$ TG cells (bottom panel) are shown.

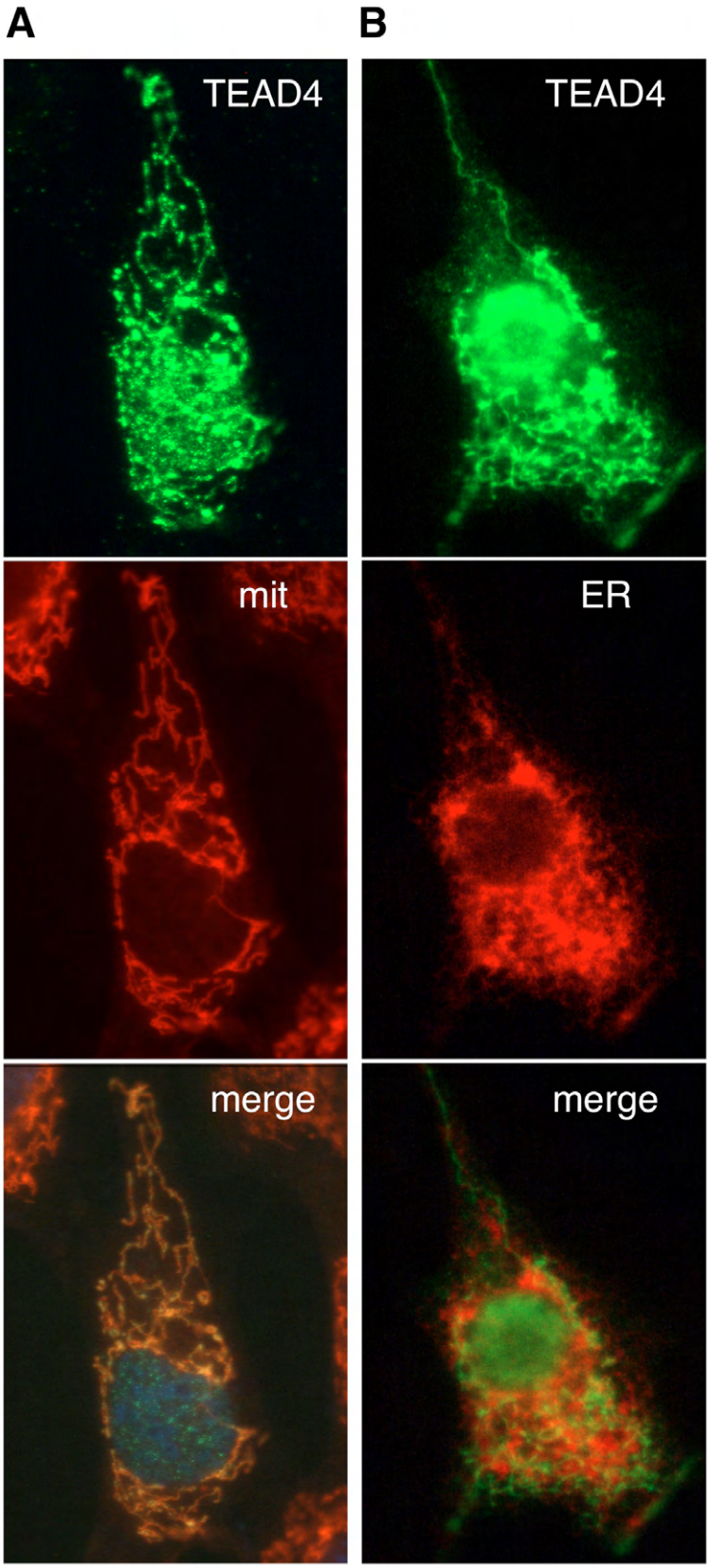


**Fig. S11. HA-epitope tagged TEAD4 protein colocalizes with mitochondria, but not endoplasmic reticulum.** (**A**) NIH3T3 fibroblasts were transfected with the pCI-Tead4-HA expression vector, and 48 hours later, the cells were stained with Mitotracker Red, fixed, and then stained with anti-HA antibody that was visualized using Alexa Fluor 488 (green). HA-TEAD4 (green) accumulated in the mitochondria (mit) as well as in the nucleus. Merged images included DAPI stained cells (blue) to reveal nuclear DNA. (**B**) pDsRed2-ER vector (Clontech) was co-transfected with pCI-Tead4-HA into NIH3T3 fibroblasts and 48 hours later, the cells were fixed and then stained with anti-HA antibody that was visualized using Alexa Fluor 488 (green). DsRed that is targeted to endoplasmic reticulum (ER) stains red. HA-stain (green) does not colocalize with DsRed-ER (merge).

**Table S1. Deoxyoligonucleotide primers**

| Primer | Sequence (5′-3′) | Amplicon (bp) |
|---|---|---|
| Genomic lox/null-F | GAGGTAAAACAACCTGTTCAAGCCTCCCTG | 321 (lox) |
| Genomic lox-R | ACCCTCTGCATCATTGTCGATGGGCTTGTC | |
| Genomic lox/null-F | GAGGTAAAACAACCTGTTCAAGCCTCCCTG | 191 (null) |
| Genomic null-R | ATAGCCTGCTGGTCCTTCCCATATGGTTG | |
| *Gata3*-forward | AGTACAGCTCTGGACTCTTC | 431 |
| *Gata3*-reverse | GCTAGACATCTTCCGGTTTC | |
| *Atp1b1*-forward | AACTGAAGCCCACATACCAG | 244 |
| *Atp1b1*-reverse | CCTCGTTCGTGATTGATGTC | |
| *p57*-forward | AGCAGGACGAGAATCAAGAG | 272 |
| *p57*-reverse | AAGTTCTCTCTGGCCGTTAG | |
| *Elf5*-forward | ACTACTACCCTGCCTTTGAG | 307 |
| *Elf5*-reverse | ATGCCAGTCTTGGTCTCTTC | |
| *Pl1*-forward | CCCTGTGTCATACTGCTTCCATC | 419 |
| *Pl1*-reverse | AACTCGGCACCTCAAGACTTTG | |
| *Tpbpa*-forward | CTGAACTGCAAGAGCAGAAG | 331 |
| *Tpbpa*-reverse | TTCGCTCGTTGCCTAACTTC | |