Keratins are asymmetrically inherited fate determinants in the mammalian embryo

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To implant in the uterus, the mammalian embryo first specifies two cell lineages: the pluripotent inner cell mass (ICM) that forms the fetus, and the outer trophectoderm layer that forms the placenta[1]. In many organisms, asymmetrically inherited fate determinants drive lineage specification[2], yet similar mechanisms are thought to be absent during early mammalian development. We found that intermediate filaments assembled by keratins function as asymmetrically inherited fate determinants in the mammalian embryo. Unlike F-actin or microtubules, keratins are the first major cytoskeletal component displaying prominent cell-to-cell variability, triggered by heterogeneities in the BAF chromatin remodeling complex. Live-embryo imaging shows that keratins become asymmetrically inherited by outer daughter cells during cell division, where they stabilize the cortex to promote apical polarization and Yap-dependent Cdx2 expression, thereby specifying the first trophectoderm cells of the embryo. This reveals a mechanism by which cell-cell heterogeneities appearing prior to ICM-trophectoderm segregation bias lineage fate, via differential keratin regulation, and expose an early function for intermediate filaments in development.

The development of multicellular organisms requires the specification of diverse lineages from a small group of cells within the embryo. During mammalian development, the first lineage segregation produces the pluripotent ICM, which forms the fetus and primitive endoderm, and the outer trophectoderm that forms the placenta[1]. How these lineages are specified remains unclear. The "inside-outside" model suggests that lineage fates are specified by local signals after cells segregate into inner-outer positions[3]. Contrary to this model, heterogeneities in histone modifications[4], transcription factor (TF) dynamics[5], non-coding RNA localization[6], and gene expression[7-10] appearing as early as the 4-cell stage bias the acquisition of pluripotent and trophectoderm fates, yet the mechanism is unclear.

On the other hand, the "cell polarity" model proposes that asymmetric inheritance of polarity components during cell division specifies distinct fates[11]. Some suggested that this relies on the asymmetric inheritance of the apical domain, which forms at the 8-cell stage prior to divisions segregating inner and outer cells, via enrichment of F-actin, Par6, and aPKC at the apical

cortex[12-14]. This would be consistent with studies showing that apical polarity at later stages promotes nuclear retention of the TF Yap, which supports high expression of Cdx2, a key TF specifying trophectoderm identity[15]. However, live-embryo imaging revealed that the apical domain disassembles from the cortex before division, instead of being directly inherited[16]. Therefore, it remains open if other polarized components function as asymmetrically inherited fate determinants during mammalian development, similar to those in non-mammalian embryos[2], and how they relate to heterogeneities at earlier stages.

The cytoskeleton is not only composed of F-actin and microtubules, but also various intermediate filaments[17]. During preimplantation development, keratins are the only cytoplasmic intermediate filaments expressed[18-20]. Keratins regulate polarity, signaling, and mechanics in epithelial tissues[17], and have traditionally served as trophectoderm markers[19]. Moreover, keratin knockouts display trophoblast fragility, placental bleeding and post-implantation lethality[21-23]. Yet, keratin functions during preimplantation development remain unknown.

To study their functions, we performed immunofluorescence for keratins 8 and 18 (K8, K18), the subtypes predominantly expressed during preimplantation stages[24]. In contrast to F-actin and microtubules, keratins are the first cytoskeletal component displaying cell-to-cell variability during development (Fig. 1a)[25,26]. Although keratins are well-established trophectoderm markers, the first filaments are already detected in a subset of cells of the 8-cell mouse embryo, before lineage segregation, with a similar pattern in the human embryo (Fig. 1, Extended Data Fig. 1a–c). The proportion of cells assembling filaments increases over time (Fig. 1b–d), and by blastocyst stage the trophectoderm is covered by a dense network, while the ICM is devoid of keratins (Fig. 1b–c, Extended Data Fig. 1d, and Supplementary Video 1)[18,27]. Thus, variability in keratin filament assembly establishes differences in cytoskeletal organization before ICM-trophectoderm segregation.

We next microinjected embryos with mRNA for fluorescently-labelled K18 (K18-Emerald), which display expression patterns resembling endogenous keratins (Extended Data Fig. 1e–g). Live-imaging and immunofluorescence show that keratin filaments start to assemble in the sub-cortical and cortical regions during interphase, prior to apical domain formation (Extended Data Fig. 2a, b, Supplementary Video 2). The size of keratin filaments increases over time, and their motion is unconfined with average speed of 0.45 ± 0.08 μm min^{-1} (Extended Data Fig. 2c–f), similar to measurements in cultured cells[28]. However, when the apical domain forms, keratins become more static and enriched at this structure, suggesting that keratins anchor to the apical domain (Fig. 2a, Extended Data Fig. 2a–b, f–g, and Supplementary Video 3). Consistently, Cytochalasin D treatment blocks apical domain formation and shifts keratin localization to more uniform along the cortex and cytoplasm (Fig. 2a, Extended Data Fig. 2h). Conversely, acute treatment with SiR-Actin, which stabilizes F-actin, increases F-actin density at the apical domain and keratin apical polarization (Fig. 2a, Extended Data Fig. 2h). Therefore, the apical domain serves as scaffold to enrich keratins apically during interphase.

In many tissues, keratins anchor to the cortex via desmosomes[17]. Although mature desmosomes assemble by blastocyst stage, desmosome components are expressed in 8-cell embryos[29,30]. Immunofluorescence for the endogenous desmosome components plakoglobin, plakophilin, and desmoglein2 reveals their localization to the apical domain (Extended Data Fig. 3a–c). Imaging fluorescently-labelled desmoglein2 and K18 in live embryos confirms this pattern (Extended Data Fig. 3d–f). Furthermore, downregulation of desmosome proteins reduces keratin apical polarization (Extended Data Fig. 3g, h). Thus, desmosome components link keratin filaments to the apical domain.

When cells enter mitosis, they largely disassemble their apical domain[16], cortical microtubules, and desmosome components (Extended Data Fig. 3e, 4a). By contrast, keratins are stably retained within mitotic cells across different developmental stages, consistent with fluorescence recovery after photobleaching (FRAP) revealing a larger immobile fraction for keratins than actin (Extended Data Fig. 4b–d). Importantly, live imaging of embryos expressing K18-Emerald shows that keratin filaments become asymmetrically inherited by the outer daughter cell during divisions producing inner-outer daughters, and symmetrically inherited during divisions producing outer-outer cells (Fig. 2b, Extended Data Fig. 4e, f, Supplementary Video 4). We confirmed these inheritance patterns in non-injected embryos (Extended Data Fig. 5a). These findings establish keratins as an asymmetrically inherited component during cell division.

To explore the inheritance mechanism, we used siRNAs targeting Pard6b, which prevent apical domain formation without interfering with completion of mitosis (Extended Data Fig. 5b)[16,31]. Pard6b knockdown reduces keratin apical polarization in interphase (Fig. 2a) and causes a more symmetric inheritance, even during divisions producing inner-outer daughters (Fig. 2c), indicating that apical polarization of keratins before division is required for their asymmetric inheritance. Tracking keratins throughout mitosis shows that they still retain a high apical polarization, even after apical domain disassembly (Extended Data Fig. 2a, f), confirmed via immunofluorescence and in human embryos (Extended Data Fig. 5a, c). This apical retention suggests that some property of the cell hinders keratin movement, as long polymers diffuse more slowly through dense entangled meshworks[32]. Consistently, we found that mitotic cells display a dense cytoplasmic F-actin meshwork similar to earlier stages[33], through which keratin filaments move (at 0.4 μm min^{-1}) (Fig. 2d, Extended Data Fig. 5d, e). Keratin filament speed is inversely proportional to their volume (Extended Data Fig. 5f), in line with polymer studies[34]. Disrupting the F-actin meshwork using Cytochalasin D specifically during mitosis causes keratins to move faster and lose their apical localization (Extended Data Fig. 5g). Moreover, when cells are arrested in metaphase using MG132, keratins have a longer time to move through the F-actin meshwork and eventually lose their apical localization (Extended Data Fig. 5g). As the distance between the apical cortex and cytokinetic furrow is 23.5 ± 1.52 μm, and the time between apical domain disassembly and cytokinesis is 34.9 ± 6.2 min (Extended Data Fig. 5h), our results indicate that the slow movement of keratins through the dense F-actin meshwork during the relatively short duration of mitosis biases their apical retention. Hence, we propose a mechanism for keratin inheritance whereby 1) the apical domain provides a scaffold promoting keratin apical localization during interphase via desmosome proteins, and 2) upon disassembly of this scaffold in mitosis, the cytoplasmic F-actin meshwork hinders keratin movement, maintaining most filaments apically and biasing their inheritance by the outer cell.

Given their asymmetric inheritance by outer cells, we explored whether keratins influence trophectoderm specification. Analysis of 8- to 16-cell embryos shows that after division, cells inheriting keratins rapidly establish a dense network under the cortex (Fig. 3a). Although most 16-cell outer blastomeres reform an apical F-actin ring after division[16], only those inheriting keratins display higher levels of the apical polarity proteins Pard6b and PKCζ (Fig. 3b), and a larger immobile fraction of mRuby2-actin at this ring, compared to keratin-negative cells (Extended Data Fig. 6a–c). Furthermore, manipulation of actin stability *per se* using Cytochalasin D reduces polarization, whereas stabilization with SiR-Actin increases polarization (Extended Data Fig. 6d). Knockdown of desmosome components also reduces actin stability (Extended Data Fig. 6a–c) and disrupts polarity (Extended Data Fig. 6e). Thus, keratins promote apical polarization by regulating F-actin stability.

Apical polarization is thought to oppose cell internalization and trigger Yap-dependent Cdx2 expression to establish trophectoderm identity[12,13]. Consistently, keratin-inheriting cells remain restricted to the outer layer, while most keratin-negative cells can undergo apical constriction[35] to form the ICM (Extended Data Fig. 6f, g, Supplementary Video 5). Keratin-inheriting cells also display the highest levels of nuclear Yap and Cdx2, and lowest Nanog levels (Fig. 3b, c). Consistently, apical Amot, which links apical polarity to Yap localization[36,37], is also enriched in these cells (Extended Data Fig. 6h). To test the role of keratins in trophectoderm specification, we combined siRNAs for K8 and K18, an approach that minimizes compensatory effects from weakly-expressed keratins[38] and extensively eliminates the keratin network (Extended Data Fig. 7a, b). In contrast to cells inheriting keratins, K8/K18 knockdown cells display lower levels of apical Pard6b, PKCζ and Amot, reduced nuclear Yap and Cdx2 expression, and higher Nanog expression (Fig. 3b, c, Extended Data Fig. 6h). Cdx2 and Nanog levels in these knockdown cells are similar to inner cells, suggesting that K8/K18 knockdown cells are not yet specified to the trophectoderm (Fig. 3b, c). Conversely, co-injecting a high concentration of K8 and K18 mRNA triggers the formation of a premature keratin network across all cells (Extended Data Fig. 7c), accompanied by widespread increase in Cdx2 expression (Fig. 3d). Although the inner cells of these embryos inherit some overexpressed keratins, they still display low nuclear Yap and Cdx2 levels, consistent with their lack of apical polarity (Extended Data Fig. 7d, e). Furthermore, keratin-positive cells in Yap knockdown embryos fail to maintain Cdx2 expression, confirming that keratins regulate Cdx2 via Yap (Extended Data Fig. 7f). Finally, microinjecting a rescue keratin construct restores Cdx2 levels in K8/K18 knockdown embryos (Extended Data Fig. 7g). Therefore, keratins control the specification of the first trophectoderm cells of the embryo.

By blastocyst stage, cells that did not inherit keratins eventually assemble a dense keratin network (Fig. 1b–d, Extended Data Fig. 1d) and display apical polarity and trophectoderm markers (Fig. 3b). This coincides with the appearance of junctional desmosomes (Extended Data Fig. 8a) and embryo cavitation, a process requiring mechanical stability to support rising intercellular pressure[39]. While K8/K18 knockdown embryos still cavitate and form a blastocyst, they exhibit decreased volume, higher junctional tortuosity, greater surface curvature indicative of lower apical tension, and reduced cytoplasmic stiffness, assessed by tracking the movement of cytoplasmic nanoparticles[40] (Extended Data Fig. 8b–e). These defects are reversed using rescue keratin constructs (Extended Data Fig. 8f). Thus, in addition to specifying the first trophectoderm cells, keratins subsequently confer mechanical support for blastocyst morphogenesis.

As keratins first appear in a subset of cells and function as fate determinants, they could link heterogeneities within the early embryo to lineage specification[1]. At the 8-cell stage, keratin-forming cells are connected by a microtubule bridge linking sister cells[41], indicating that they originate from the same 4-cell blastomere (Fig. 4a, Extended Data Fig. 9a). Hence, we assessed if they derive from the vegetal blastomere of the 4-cell embryo (Extended Data Fig. 9b), shown to produce more trophectoderm than ICM progeny[4,42]. Selective photoactivation of the vegetal blastomere followed by staining for endogenous keratins at the 8-cell stage, and imaging live embryos expressing K18-Emerald during the 4- to 8-cell window demonstrate that the vegetal blastomere preferentially produces keratin filament-forming cells (Fig. 4b, c, Extended Data Fig. 9c, d).

We finally focused on the BRG1-associated factor (BAF) chromatin remodeling complex, which promotes trophectoderm differentiation and is negatively regulated by the histone methyltransferase Carm1 that biases ICM fate[43,44]. The vegetal blastomere has the highest levels of BAF155, the main regulatory component of the BAF complex[43] (Fig. 4d). Higher BAF155

expression is also maintained in the first 8-cell blastomeres assembling keratin filaments (Fig. 4e). Thus, we tested whether establishing 4-cell embryos with different BAF patterns alters keratin expression at the 8-cell stage, by microinjecting BAF155 siRNAs or high levels of BAF155 mRNA into 1-cell embryos, or one cell of 2-cell embryos (Fig. 4f, Extended Data Fig. 9e). This generates patterns ranging from no detectable BAF155, to higher than normal BAF155 in all blastomeres. BAF155 knockdown or overexpression within all blastomeres triggers a reduction or increase in keratin expression, respectively. Consistently, when BAF155 levels are manipulated in half of the embryo, the resulting embryos display greater variability in keratin expression. To further elucidate how BAF155 regulates keratins, we used the transcriptional inhibitor Actinomycin D, which eliminates keratin expression (Extended Data Fig. 9f). By contrast, facilitating transcription using Trichostatin A (TSA) elicits widespread keratin expression in most cells, and bypassing keratin transcription by microinjection of K8 and K18 mRNAs induces premature and extensive keratin expression (Extended Data Fig. 9f, g), suggesting that keratin expression is transcriptionally regulated. Furthermore, BAF155-overexpressing embryos no longer display high keratin levels when treated with Actinomycin D (Extended Data Fig. 9h).

Carm1 methylates BAF155 at residue R1064[44] and Carm1 knockout embryos display lower BAF155 methylation[43]. Consistently, the vegetal blastomere not only has the lowest Carm1[4] and highest total BAF155 levels (Fig. 4d), but also the lowest methylated BAF155 (Extended Data Fig. 9i). Carm1 overexpression disrupts keratin expression, similarly to BAF155 knockdown, and overexpression of a BAF155^{R1064K} mutant that cannot be methylated by Carm1[44] causes premature keratin expression (Extended Data Fig. 9j–l). Finally, Cdx2 expression is diminished in 16-cell embryos following BAF155 knockdown or Carm1 overexpression (Extended Data Fig. 9m, n). Thus, Carm1 methylation of BAF155 leads to the differential regulation of keratins.

In conclusion, keratins function as asymmetrically inherited factors that specify the first trophectoderm cells of the embryo (Extended Data Fig. 10). Our findings validate a key aspect of the "cell polarity" model[11] by identifying keratins as an asymmetrically inherited fate determinant. Yet, they also highlight important distinctions by showing that 8-cell blastomeres are not equivalent. While all cells initially display apical domains, only a subset expresses keratins. Therefore, even before inner-outer segregation, cells acquire differences in cytoskeletal organization biasing their fate. Moreover, keratin differential expression is traced back to BAF heterogeneities within the 4-cell embryo, providing a mechanism to understand how early cell-to-cell variability is transmitted through divisions to influence lineage fate. This extends the notion that the fate of early blastomeres is predictable[4-6,8,10,43,45-47].

Our study also reveals interactions between the actin cortex and keratins important for trophectoderm specification. The apical domain first promotes apical enrichment of keratins, but following division, keratins stabilize the cortex to prevent cell internalization, support apical polarization and promote acquisition of the first hallmarks of trophectoderm specification. At later stages, Cdx2 was shown to promote keratin expression[27]. Thus, the initial effect of keratins in promoting Cdx2 expression could feedback into the production of more keratins to support the expansion of the keratin network for blastocyst morphogenesis. Finally, the comparable cell-to-cell variability and localization of keratins in the human embryo suggest that keratin asymmetric inheritance may represent a conserved mechanism of lineage specification in early mammalian development.

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Fig. 1. Keratin filaments display cell-to-cell variability before lineage segregation in the mouse and human embryo. a, All cells of the 8-cell embryo show similar F-actin and microtubule organization, yet only a subset assembles keratin filaments. Data are from 5 independent experiments. **b** and **c**, Mouse and human embryos at multiple developmental stages. Keratin filaments initially assemble in a subset of cells at the 8- to 16-cell stage, before inner-outer cell segregation. Blastocysts show dense keratin networks in the trophectoderm, but not ICM. Insets show middle 2D views. **d**, Quantification of keratin filament-forming outer cells of the embryo throughout preimplantation development. ANOVA test; ***$P < 0.0001$. Data are from 3 independent experiments. Scale bars, 10 μm.

Fig. 2. Keratin filaments are asymmetrically inherited during cell division. a, Keratin filaments are apically localized near the apical domain. Cytochalasin D and Pard6b knockdown reduce keratin apical localization; SiR-Actin increases it. Top panels show individual cells. Bottom panels show whole embryo (left) and computationally-rendered filaments within each cell (right). Kruskal-Wallis test; **$P = 0.004$; *$P = 0.02$ for Pard6b KD; *$P = 0.03$ for SiR-Actin. **b**, Imaging fluorescently-tagged keratins within the live embryo reveals their asymmetric inheritance by the outer daughter during divisions producing inner and outer cells. Quantification shows inheritance patterns. Student's *t*-test; NS, not significant; ***$P < 0.0001$. **c**, Pard6b knockdown shifts keratin inheritance from asymmetric to more symmetric in outer-inner divisions. Kruskal-Wallis test; NS, not significant; ***$P = 0.0001$; **$P = 0.0009$. **d**, Immunofluorescence of 8-cell embryo highlights cytoskeletal organization in interphase and mitosis. While the apical domain and cortical microtubules become largely reorganized during mitosis, keratins retain their apical localization. 2D panels show loss of apical domain, but retention of a dense cytoplasmic F-actin meshwork and apically-localized keratins during mitosis. Data are from 5 independent experiments. Scale bars, 5 μm.

Fig. 3. Keratin inheritance specifies the first trophectoderm cells of the embryo. a, Live-embryo imaging shows that outer cells inheriting keratins (K+) establish an extensive network after division, while those that did not remain devoid of filaments (K-). Data are from 5 independent experiments. **b**, Immunofluorescence in non-injected embryos shows that K+ cells are the first to display high levels of apical polarity and trophectoderm fate markers (top), but K8/K18 KD cells fail to establish these features (middle). By 32-cell stage, the remaining cells of the embryo establish a keratin network and trophectoderm identity (bottom). Data are from 3 independent experiments. **c**, Quantification of fluorescence intensities. Kruskal-Wallis test for Pard6b, PKCζ and Cdx2; ANOVA test for Yap and Nanog. For Pard6b, *$P = 0.01$; ***$P = 0.0006$. For PKCζ, **$P = 0.001$; ***$P = 0.0002$. For Yap, ***$P < 0.0001$. For Cdx2, *$P = 0.01$; ***$P < 0.0001$; NS, not significant. For Nanog, ***$P < 0.0001$; **$P = 0.004$; NS, not significant. **d**, Keratin overexpression (K8/K18 OE) causes premature establishment of a keratin network and trophectoderm fate throughout the 16-cell embryo. ANOVA test; *$P = 0.03$ for control 16-cell; *$P = 0.02$ for K8/K18 OE; ***$P < 0.0001$. Data are from 3 independent experiments. Scale bars, 10 μm.

Fig. 4. Keratin expression is regulated by early heterogeneities in the BAF complex. a, A microtubule bridge connecting sister cells reveals that the first 8-cell blastomeres assembling keratins originate from a common 4-cell blastomere. Data are from 3 independent experiments. **b**, Selective H2B-paGFP photoactivation marks the vegetal blastomere that then produces the first keratin-forming cells. Data are from 3 independent experiments. **c**, Live-imaging of K18-Emerald during the 4- to 8-cell stage confirms that the vegetal blastomere produces the first keratin-forming cells. Z-slices show keratin filaments in the cells derived from the vegetal blastomere. Graph shows proportion of embryos where keratin-forming cells derive from vegetal blastomeres. χ^2 test; $^{**}P = 0.002$. **d**, The vegetal blastomere displays the highest endogenous BAF155 levels. Mann-Whitney *U*-test; $^{*}P = 0.01$. **e**, The first cells to form keratin filaments in the 8-cell embryo express higher BAF155 levels than cells without keratins. Mann-Whitney *U*-test; $^{*}P = 0.03$. **f**, Experimental manipulation of BAF155 levels produce different patterns of keratin expression. BAF155 knockdown reduces the proportion of cells expressing keratins at the 8-cell stage, whereas BAF155 overexpression can induce ectopic keratin expression. ANOVA test for 1-cell manipulations; $^{**}P = 0.005$ for KD; $^{**}P = 0.004$ for overexpression. Kruskal-Wallis test for 2-cell manipulations; $^{*}P = 0.03$. Scale bars, 10 μm.

METHODS

Mouse embryo work. Mouse embryo experimentation was approved by the Biological Resource Center Institutional Animal Care and Use Committee (IACUC), Agency for Science, Technology and Research (IACUC Protocol #181370). C57BL/6 wild-type 3-4 weeks old female mice were superovulated using 5 iu of pregnant mare serum (PMS, National Hormone and Peptide Program) gonadotropin given intraperitoneally and 5 iu of recombinant chorionic gonadotrophin (CG, National Hormone and Peptide Program) given 48 h after and immediately before mating, according to animal ethics guidelines of the Agency for Science, Technology and Research, Singapore. Embryos were flushed from oviducts of plugged females using M2 medium (Merck) and cultured in KSOM+AA (Merck) covered by mineral oil (Sigma), at 37ºC and 5% CO_2. Microinjections were performed using a FemtoJet (Eppendorf). mRNA synthesis was performed on linearized plasmids using the mMESSAGE mMACHINE SP6 kit (Ambion), and purified using the RNAeasy kit (QIAGEN). For live imaging experiments, mRNAs diluted in injection buffer (5 mM Tris, 5 mM NaCl, 0.1 mM EDTA) were microinjected as follows: K8-Emerald and K18-Emerald at 150 ng μl^{-1}; mRuby2-Actin at 100 ng μl^{-1}; RFP-Utrophin at 70 ng μl^{-1}; RFP-MAP2c at 80 ng ul^{-1}; memb-mRuby2 at 70 ng μl^{-1}; H2B-RFP and H2B-GFP at 5 ng μl^{-1}; Desmoglein2-Emerald and Desmoglein2-mRuby2 at 150 ng μl^{-1}; H2B-paGFP at 20 ng ul^{-1}. For overexpression experiments, mRNAs were microinjected as follows: K8 and K18 at 300 ng μl^{-1}; BAF155 and BAF155^{R1064K} at 500 ng μl^{-1}; Carm1 at 300 ng μl^{-1}. siRNAs (QIAGEN) were microinjected at the following concentrations: K8 (500 nM), K18 (500 nM), Dsg2 (200 nM), Dsc3 (200 nM), Desmoplakin (200 nM), Plakoglobin (200 nM), Pard6b (200 nM), Yap1 (200 nM), BAF155 (500 nM).
The siRNAs used are: Mm_Krt2-8_1 (AACCATGTACCAGATTAAGTA), Mm_Krt2-8_2 (ATGGATGGCATCATCGCTGAA), Mm_Krt1-18_1 (CAGAGTGGTGTCCGAGACTAA), Mm_Krt1-18_3 (CCGGGAACATCTGGAGAAGAA), Mm_Dsg2_1 (CAGCATTATGCCAATGAAGAA), Mm_Dsg2_2 (CTCCGTCACTTCAGAGATTAA), Mm_Dsc3_2 (CAGAGATAATTCAAGATTATA), Mm_Dsc3_5 (AACTGCGGATGTTCAAATATA), Mm_Dsp_2 (CAGGAAGTTCTTCGATCAATA), Mm_Dsp_4 (ACCGGTTGACATGGCGTATAA), Mm_Jup_4 (CAGACAGTACACACTCAAGAA), Mm_Jup_5 (CACTATGGCTATGGCCACTAA), Mm_Pard6b_3 (CACGGGCCTGCTAGCTGTCAA), Mm_Pard6b_4 (CAGGTGACTGACATGATGATA), Mm_Yap1_6 (ACCCTTGAACATATACATTTA), Mm_Yap1_7 (AACATCCTATTTAAATCTTAA), Mm_Smarcc1_5 (ACGCATCCTGGTTTGATTATA), Mm_Smarcc1_6 (TCGAACTGACATTTACTCCAA).

For drug treatments, all drugs were diluted in KSOM to the following concentrations: Cytochalasin D at 20 $\mu g\ ml^{-1}$, SiR-Actin at 100 nM, MG-132 at 25 µM, Actinomycin D at 100 ng ml^{-1}, Trichostatin A at 75 nM. Drugs were applied for 2 h before embryo fixation, with the exception of Actinomycin D and Trichostatin A, which were both applied for the entire 4- to 8-cell stage window to effectively block or promote transcription respectively.

Human embryo work. Human embryos were donated to the Reproductive Medicine Research Center, Sixth Affiliated Hospital of Sun Yat-sen University for research purposes, following ethical guidelines of the Sixth Affiliated Hospital of Sun Yat-sen University. Experiments were performed according to the guidelines of the Institute of Zoology, Chinese Academy of Sciences and the Sixth Affiliated Hospital of Sun Yat-sen University.

This work was approved by the Ethics Committee of Center for Reproductive Medicine, Sixth Affiliated Hospital of Sun Yat-Sen University (Research license 2019SZZX-008). The Medicine Ethics Committee of Center for Reproductive Medicine, Sixth Affiliated Hospital of Sun Yat-

Sen University is composed of 11 members, including experts of laws, scientists and clinicians with relevant expertise. The Committee evaluated the scientific merit and ethical justification of this study and conducted a full review of the donations and use of these samples.

All embryo donor couples signed informed consent forms for voluntary donations of surplus embryos for research, at the Center for Reproductive Medicine, Sixth Affiliated Hospital of Sun Yat-sen University. Participation in the study was voluntary and no financial inducements were offered for embryo donation. The culture of all embryos was terminated before day 14 post-fertilization. Couples were informed that their embryos would be used to study the developmental mechanisms of human embryos and that their donation would not affect their IVF cycle. The informed consent forms clearly state the goals of the research, clinical procedures used in the study, potential benefits and risks to research participants, and steps taken to ensure that the privacy of each embryo donor was well protected. The participation of embryo donors in the study can only be obtained if eligible participants were provided with all necessary information about the study and the opportunity to receive counselling. These informed consent guidelines are in line with the ethical and regulatory framework set forth by the Center for Reproductive Medicine, Sixth Affiliated Hospital of Sun Yat-sen University, and complied with the International Society for Stem Cell Research (ISSCR) *Guidelines for Stem Cell Research and Clinical Translation* (2016) and *Ethical Guidelines for Human Embryonic Stem Cell Research* (2003) jointly issued by the Ministry of Science and Technology and the Ministry of Health of the People's Republic of China.

All donated samples in this study were obtained from frozen embryos from couples who signed informed consent agreements. The study employed standard clinical protocols for embryo collection, cryopreservation, thawing and culture procedures. Human embryos were frozen-thawed day post-fertilization (d.p.f.) 3 or (d.p.f.) 5. Cryopreserved embryos were thawed using Kitazato Thawing Media Kit VT802 (Kitazato Dibimed) depending on the protocol used for freezing and following the manufacturer's instructions. The embryos were cultured in Single-step embryo culture medium (LifeGlobal) covered with oil (LifeGlobal) (from 4-cell stage to blastocyst stage). Embryos with normal morphology and cleavage patterns were utilized in this study.

Microscopy. Imaging was performed using a laser scanning confocal microscope (LSM 780 and LSM 880, Zeiss) with a water UV-VIS-IR Apochromat 63X 1.2 NA objective. For live imaging, embryos were cultured in LabTek chambers (Nunc) in KSOM+AA (Merck) covered by mineral oil (Sigma), using the incubator system adapted for the microscope (Carl Zeiss, Jena) to maintain the embryos at 37ºC and 5% CO_2. Embryos were scanned every 15 to 20 min for long-term imaging, and selected mitotic cells were imaged at higher temporal resolution of 1 to 3 min intervals in order to track the dynamics of keratin filaments throughout the entire cell division. FRAP was performed at 3.5-times zoom on a 5 μm x 10 μm region of interest, photobleached using the 488 nm laser at 100%, with a pixel dwell time of 6 μs and scanning speed of 6. For photoactivation experiments, H2B-paGFP was selectively illuminated in the nuclei of vegetal blastomeres using an 820 nm two-photon laser (Mai Tai, Spectra-Physics) as described[5,45], followed by live-imaging using a 488 nm laser to track the photoactivated signal in the daughter cells.

For measurements of cell elasticity, 0.1 μm-diameter carboxylate-modified FluoSpheres (Invitrogen) were microinjected into live embryos. The size of these nanoparticles is larger than the average mesh size of the cytoskeletal network[40]. We optimized their concentration to obtain an average of 10 beads per cell, homogenously distributed throughout the cytoplasm at blastocyst

stage. Tracking of the movement of these nanoparticles was performed by imaging individual particles at 30-times zoom, 50 frames s^{-1} for 2 min, as previously described[48].

Immunofluorescence. Embryos were fixed in 4% paraformaldehyde for 30 min at room temperature or overnight at 4ºC, washed twice in PBS with 0.1% Triton X-100, permeabilized for 20 min in PBS with 0.5% Triton X-100, and incubated in PBS with 10% fetal bovine serum (blocking solution) for 30 min. Embryos were then incubated at 4ºC overnight in primary antibodies diluted in blocking solution, at the following concentrations: K8 (DSHB) at 1:20, K18 (Sigma, SAB4501665) at 1:200, K19 (DSHB) at 1:50, pan-Keratin (Cell Signaling, 4545) at 1:50, α-tubulin (Sigma, T6199) at 1:1000, Pard6b (Santa Cruz, 166405) at 1:50, PKCζ (Santa Cruz, 17781) at 1:50, Amot (gift from Hiroshi Sasaki, Osaka University) at 1:200, Yap (Cell Signaling, 8418S) at 1:500, Cdx2 (Abcam, 88129) at 1:200, Nanog (Abcam, 80892) at 1:200, Desmoglein1/2 (Progen, 61002S) undiluted, Plakoglobin (Progen, 61005S) undiluted, Plakophilin (Progen, 651101S) undiluted, Desmoplakin1 (Progen) at 1:100, BAF155 (Santa Cruz, 48350) at 1:50, dimethyl-BAF155 (Merck, ABE1339) at 1:100, and Carm1 (Cell Signaling, 3379S) at 1:150. After primary antibody incubation, embryos were washed 5 times for 20 min in PBS with 0.1% Triton X-100 and incubated 1.5 h at room temperature or overnight at 4ºC in secondary antibodies diluted in blocking solution to 1:500. Phalloidin-Rhodamine (Molecular Probes, R415) diluted to 1:500 and NucBlue Fixed Cell Stain ReadyProbes reagent (Invitrogen) diluted to 1:100 in blocking solution were also used to label the F-actin and chromatin respectively. Embryos were washed three times in PBS with 0.1% Triton X-100 before mounting in PBS covered with mineral oil (Sigma) in an 8-well LabTek chamber (Nunc).

Image analysis. Image analyses were performed using Imaris 8.2 (Bitplane AG), Fiji, and MATLAB. 3D segmentation of whole embryos, individual cells and individual nuclei was performed using the Imaris manual surface rendering module, and 3D segmentation of keratin filaments was done with the automatic surface rendering mode. The Imaris statistics module was used to obtain values for total fluorescence intensities, cell volumes, and nucleus volumes. Measurements of apical fluorescence intensities of Pard6b and PKCζ were performed in Fiji by selecting the apical region of individual cells and averaging the fluorescence intensities across 5 different Z-planes. All quantifications were normalized to background fluorescence to correct for weaker fluorescence with increasing depth through the embryo. M.B. thanks Bitplane AG for an Imaris Developer License.

To quantify the localization of keratin filaments within individual cells, we used a polarization index, adapted from previous work[49,50]. Spatial coordinates for individual keratin filaments and the cell center of mass, volumes of individual keratin filaments, and lengths of the cell apical-basal axis were obtained using Imaris software. The polarization index was calculated by obtaining the difference between the volume-weighted average position of all keratin filaments within the cell and the position of the cell center of mass, normalized to the cell apical-basal axis.

For calculations of keratin filament movement during interphase and mitosis, the spatial coordinates of each filament were obtained from the Imaris statistics module. The mean speed of filament movement was calculated by dividing the distance between the initial and final positions of the filament by the elapsed time.

For analysis of FRAP experiments, mean fluorescence intensity at the photobleached region of interest (ROI) was corrected by background fluorescence and normalized to a non-photobleached reference. The average of the pre-bleach fluorescence intensities was set to 100%, and the fluorescence intensity immediately after photobleaching was set to 0%. The normalized mean fluorescence intensities were then fitted with an exponential function, as previously

described[16]. The immobile fraction was calculated by taking $1 - I_\infty$, where I_∞ is the normalized mean fluorescence intensity when the intensity recovers to a plateau. All fittings were performed in MATLAB, and FRAP kymographs were created using the Montage tool in Fiji.

To characterize the morphology of cell-cell junctions, we used a tortuosity index calculated by measuring the total junction length, normalized to the Euclidean distance.

For measurements of surface curvature, we first segmented individual cells in Imaris and extracted the apical surface of each cell using a custom MATLAB code. The radius of surface curvature was then determined using the radius of the sphere that best fits the cell apical surface.

Statistical analysis. Statistical analyses were performed in GraphPad Prism and Excel. Qualitative data were represented using a contingency table and analyzed using a Fisher's exact test or χ^2 test. All quantitative data were first analyzed for normality using a D'Agostino-Pearson omnibus normality test. Variables showing a normal distribution were then analyzed using an unpaired, two-tailed Student's *t*-test or ANOVA with Tukey's multiple comparisons test for two groups or more than two groups respectively. Variables that did not show a normal distribution were analyzed using an unpaired, two-tailed Mann-Whitney *U*-test or Kruskal-Wallis test with Dunn's multiple comparisons test for two groups or more than two groups respectively. No statistical test was performed to determine sample size, and sample size was determined based on prior experience and in accordance to previous studies. Embryos were randomly allocated into experimental groups and randomly selected for analysis. Reproducibility was confirmed by at least three independent experiments.

Data availability. Source Data behind Figs. 1 to 4 and Extended Data Figs. 3 to 10 are available within the manuscript files.

Code availability. Code for apical surface curvature analysis has been published in a publicly available repository at https://github.com/gracelhy/Analysis-of-embryo-parameters.

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Competing Interests. The authors declare no competing interests.

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Extended Data Fig. 1. Keratin filaments in the preimplantation mouse embryo. a, 3D views of mouse embryos at multiple developmental stages, stained for K18. K18 expression and localization resemble that of K8. Note the initial assembly of filaments in a specific subset of cells in the 8-cell embryo. Data are from 5 independent experiments. **b**, Double immunofluorescence for K8 and K18 shows their colocalization in filament structures within the same embryo. Data are from 3 independent experiments. **c**, Double immunofluorescence using a pan-keratin antibody and K18 shows colocalization in filament structures within the same embryo. Data are from 3 independent experiments. **d**, High-magnification views highlight keratin filament organization at multiple developmental stages (top panels). Surface render of computationally-segmented cells and keratin filaments with top and side views show the changes in cell morphology and keratin filament organization at different developmental stages. The density of the keratin filament network increases over time and the filaments become enriched at cell-cell junctions. Data are from 5 independent experiments. **e**, Live imaging of embryos expressing K18-Emerald. A subset of cells begins to assemble keratin filaments at the 8-cell stage, similar to observations from immunofluorescence for endogenous keratins. No keratin filaments are detected in 4-cell or early uncompacted 8-cell embryos. Data are from 3 independent experiments. **f** and **g**, Colocalization of K18-Emerald and immunofluorescence against K8 or K18. Bottom panels show zoomed views of single cells expressing keratin filaments, with arrows pointing to an example of signal colocalization. Data are from 3 independent experiments. Scale bars, 10 μm.

Extended Data Fig. 2. Tracking and quantitative analysis of keratin filament movement during interphase and mitosis. a, Time series of an embryo expressing K18-Emerald and RFP-Utrophin, with the corresponding major cellular events labelled in the left column. Separate K18-Emerald and RFP-Utrophin channels are shown. Right panels show 2D views through a single cell that assembles keratin filaments, for better visualization of keratin distribution within the cell, relative to the apical domain. Keratin filament assembly is initiated prior to the formation of the apical domain. When the apical domain forms, keratin filaments become enriched apically in close association with F-actin. During mitosis, the apical domain disassembles but keratin filaments remain apically localized, resulting in their asymmetric inheritance by the outer daughter cell. Data are from 3 independent experiments. **b**, Immunofluorescence of endogenous keratins in embryos fixed at different stages of apical domain formation recapitulates the pattern and localization of keratin filaments relative to the apical domain observed in live imaging experiments. Data are from 3 independent experiments. **c**, Computationally-rendered filaments obtained from live imaging data. In this example, five individual filaments were tracked over time with a 10 min interval between frames. Data are from 3 independent experiments. **d**, The log mean square displacement (MSD) versus log lag time graph indicates that the movement of keratin filaments is unconfined and diffusive (slope > 1). Pearson's correlation. **e**, Volume of an individual keratin filament and total filament volume within a single tracked cell increase linearly over time. Pearson's correlation. **f**, Quantification of filament speed, volume of filaments, and polarization index before apical domain formation, after apical domain formation, and during mitosis. After the formation of the apical domain, keratin filaments move more slowly, display a larger total volume, and become more apically polarized than before apical domain formation. During mitosis, keratin filaments move faster, but retain a large volume and high apical polarization. Kruskal-Wallis test for filament speed; ***P = 0.0002; **P = 0.001; ANOVA test for filament volume; **P = 0.003; NS, not significant; Kruskal-Wallis test for polarization index; **P = 0.003; NS, not significant. Scheme shows the parameters used for calculation of the polarization index. d1 is the distance between the volume-weighted center of

mass of the keratin filaments and the center of mass of the cell. d2 is the length of the apical-basal axis of the cell. **g**, High-resolution immunofluorescence images show that keratin filaments align specifically along actin filaments extending from the apical domain. Green arrows indicate examples of keratin-actin colocalization. Data are from 3 independent experiments. **h**, Differences in F-actin accumulation at the apical domain of control embryos and embryos treated with cytochalasin D or a high concentration of SiR-Actin. Insets show zoomed views of individual 8-cell blastomeres, highlighting the loss of the apical domain in cytochalasin D-treated embryos, and a dense accumulation of apical F-actin in SiR-Actin-treated embryos. Data are from 3 independent experiments. Scale bars, 10 μm.

Extended Data Fig. 3. Apical keratin localization requires desmosome protein components. **a** to **c**, Immunofluorescence of endogenous Plakoglobin (**a**), Plakophilin (**b**), and Desmoglein2 (**c**) before and after apical domain formation. Apical accumulation of all three desmosome components is observed after apical domain formation. **d**, Live imaging of embryo expressing Desmoglein2-Emerald, RFP-Utrophin and H2B-RFP recapitulates the endogenous Desmoglein2 expression, both before and after apical domain formation. **e**, Time series of embryo expressing Desmoglein2-Emerald, RFP-Utrophin and H2B-RFP. Desmoglein2-Emerald accumulates with the apical domain (labelled by RFP-Utrophin) during interphase. When the cell enters mitosis, Desmoglein2-Emerald disassembles from the apical surface together with the apical domain. White arrows indicate two different mitotic events within the same embryo. **f**, Live embryo expressing Desmoglein2-Ruby and K18-Emerald shows the enrichment of keratin filaments at the site of apical desmosome accumulation. **g**, Embryos injected with siRNAs against desmosome components do not accumulate desmoglein2 apically with the apical domain. **h**, Desmosome knockdown causes a more homogenous distribution of keratin filaments, as measured by a polarization index. Unpaired, two-tailed Mann-Whitney *U*-test; $^{**}P = 0.01$. Data are from 3 independent experiments. Scale bars, 10 μm.

Extended Data Fig. 4. Keratin filaments are stably retained during mitosis, and become asymmetrically inherited by outer daughter cells. **a**, Immunofluorescence shows the extensive remodeling of cortical F-actin and microtubules during different stages of mitosis. Data are from 6 independent experiments. **b**, Immunofluorescence for K8 shows endogenous keratin filaments retained within mitotic cells in embryos fixed at multiple stages of development. Data are from 6 independent experiments. **c**, FRAP experiments for K18-Emerald and mRuby2-Actin performed in whole live embryos. All cells selected for FRAP were at the 8-cell stage and in interphase, when the actin ring is visible. 3D views of entire pre-FRAP embryos (left panel), zoomed views of the photobleached regions of interest (middle panel), and kymographs of pre- and post-FRAP fluorescence intensities (right panel). Data are from 3 independent experiments. **d**, Analysis of FRAP experiments. Left graphs show fluorescence recovery of K18-Emerald (green) and mRuby2-Actin (red) over time. Thinner lines represent raw data after normalization, and thicker lines indicate fitted exponential curves. Right graph shows that K18-Emerald has a larger immobile fraction than mRuby2-Actin. Unpaired, two-tailed Student's *t*-test; $^{***}P < 0.0001$. **e**, Live imaging of embryos expressing K8-Emerald show a similar pattern of expression and inheritance as K18-Emerald. The outer daughter cell inherits the majority of keratin filaments during an outer-inner division (top panels). Computational segmentation of the same cell at each stage of mitosis (bottom panels). Quantification of proportion of keratin filaments inherited by outer and inner cells in live embryos expressing K8-Emerald shows a comparable asymmetry in keratin inheritance as K18-Emerald. Unpaired, two-tailed Student's *t*-test; $^{**}P = 0.001$. **f**, Time series of a cell expressing K18-Emerald undergoing a symmetric outer-outer division. Keratin filaments are uniformly inherited by both daughter

cells during divisions producing two outer cells (top panels). Computational segmentation of the same cells at each time point (bottom panels), and whole embryo inset highlighting the outer location of both daughter cells (right panel). Data are from 4 independent experiments. Scale bars, 5 μm.

Extended Data Fig. 5. A dense F-actin meshwork within mitotic cells hinders the movement of keratin filaments away from the apical cortex. a, Immunofluorescence of embryos fixed specifically when a cell was undergoing mitosis or cytokinesis. Keratin filaments remain apically-localized throughout different mitotic stages, and become inherited by the prospective outer cell (top panels). Computational segmentation of the same cells highlighting the apical keratin distribution and asymmetric keratin inheritance (bottom panels). Quantification of proportion of endogenous keratin filaments present in the apical and basal regions of mitotic cells, and between prospective outer and inner daughter cells, showing a comparable asymmetry in endogenous keratin localization and inheritance as K18-Emerald dynamics in live embryos. Unpaired, two-tailed Student's *t*-test; ***$P < 0.0001$. **b**, Embryos microinjected with Pard6b siRNAs do not form an apical F-actin ring in the 8-cell embryo. Data are from 3 independent experiments. **c**, Mitotic cell within a fixed human embryo also displays an apical localization of keratins. **d**, A dense cytoplasmic F-actin meshwork is maintained throughout interphase and all stages of mitosis. Data are from 3 independent experiments. **e**, The F-actin meshwork is also present in cells across different stages of development. Representative images of a 3-cell, compacted 8-cell, and 16-cell embryo with all cells displaying a dense cytoplasmic F-actin meshwork. Data are from 3 independent experiments. **f**, Analysis of keratin filament movement during mitosis reveals that filament speed is inversely related to filament volume. n = 8 filaments; Pearson's correlation. **g**, Acute 15 min cytochalasin D treatment specifically during mitosis disrupts the F-actin meshwork, reduces the apical localization of keratins, and increases keratin filament speed. Cells treated with MG132 for 3 hours retain an F-actin meshwork, but keratin apical localization is reduced and filament speed is unchanged. Kruskal-Wallis test for polarization index; **$P = 0.002$ for CytoD; **$P = 0.01$ for MG132; ANOVA test for filament speed; ***$P = 0.0005$; NS, not significant. **h**, Scheme of a cell division producing an inner (green) and an outer (blue) cell. Keratin filaments localize close to the apical cortex of the forming outer daughter cell. The distance between the apical cortex and cytokinetic furrow, time between disassembly of the apical F-actin domain and cytokinesis, and the mean speed of keratin filament movement are indicated. Scale bars, 5 μm.

Extended Data Fig. 6. Keratins promote actin stability and apical polarization. a, FRAP experiments for mRuby2-Actin performed at the apical domain of interphase cells with keratins, cells without keratins, and cells microinjected with desmosome siRNAs. Selected photobleached regions of interest (left panels) and kymographs of pre- and post-FRAP fluorescence intensities (right panels) are shown. Data are from 3 independent experiments. **b**, Analysis of FRAP experiments. Graphs show the fluorescence recovery of mRuby2-Actin over time for each condition. Thinner red lines indicate raw data after normalization, thicker red lines are fitted exponential curves, and thick black lines represent the mean fitted exponential curves. **c**, Cells lacking keratins and cells with reduced desmosome expression show a smaller immobile fraction of mRuby2-Actin compared to cells with keratins. Kruskal-Wallis test; **$P = 0.0002$ for without keratins; **$P = 0.003$ for desmosome KD. **d**, Immunofluorescence of 16-cell stage control embryos and embryos treated with cytochalasin D and SiR-Actin. Disruption of actin stability using cytochalasin D reduces accumulation of apical polarity markers Pard6b and PKCζ. Conversely, increasing actin stability using SiR-Actin increases apical polarity levels. ANOVA

test for Pard6b; *P = 0.03; ***P = 0.0009; Kruskal-Wallis test for PKCζ; **P = 0.003; *P = 0.03. **e**, Desmosome knockdown in 16-cell stage embryos reduces levels of apical polarity markers Pard6b and PKCζ. Unpaired, two-tailed Mann-Whitney *U*-test; ***P = 0.0002 for Pard6b; ***P = 0.001 for PKCζ. **f**, Live imaging of K18-Emerald in an embryo displaying a cell division. Following division, the daughter cell that did not inherit keratins (cyan) undergoes apical constriction to form the pluripotent inner cell mass [35], while the outer daughter cell that inherited keratins (yellow) does not internalize. Data are from 3 independent experiments. **g**, Analysis of internalization events in cells that inherited (K+) or did not inherit (K-) keratin filaments after division. Two-tailed Fisher's exact test; ***P < 0.0001. **h**, Immunofluorescence of endogenous K8 and Amot in a 16-cell stage embryo. Right panels indicate zoomed views of the apical region of cells with and without keratins, with separate K8 and Amot channels for better visualization. Cells with keratins display higher levels of apical Amot than cells lacking keratins and cells with K8/K18 knockdown. Kruskal-Wallis test; *P = 0.04; ***P < 0.0001. Scale bars, 10 μm.

Extended Data Fig. 7. Experimental manipulations of keratin levels show that keratins regulate Cdx2 to specify the first trophectoderm cells of the embryo. a, Immunofluorescence for K8 in embryos microinjected with siRNAs for K8 and K18 at the 1-cell stage, or into only one cell at the 2-cell stage. This double-knockdown approach extensively eliminates keratin filament assembly. Data are from 5 independent experiments. **b**, Knockdown of K8 and K18 in half of the embryo also eliminates filament formation by K19. White arrowheads show knockdown cells. Data are from 3 independent experiments. **c**, Keratin overexpression causes a premature and widespread assembly of a keratin network within the 8- to 16-cell stage embryo. Images show examples of embryos microinjected with high levels of K8 and K18 RNA at the 1-cell stage, or into one cell of the 2-cell embryo. Data are from 3 independent experiments. **d**, Keratin overexpression causes some filaments to be inherited by inner cells of the 16-cell stage embryo (yellow segmented cell indicated by arrow in left panel). 2D view shows keratin filament organization within outer and inner cells of keratin overexpressing embryos (right panel). Data are from 3 independent experiments. **e**, Inner cells in keratin overexpressing embryos express lower levels of nuclear Yap and Cdx2 than outer cells. Unpaired, two-tailed Mann-Whitney *U*-test; ***P < 0.0001; *P = 0.04. **f**, Knockdown of Yap using siRNAs microinjected into one cell of the 2-cell embryo reduces Cdx2 levels, in both keratin-positive and keratin-negative cells. H2B-RFP was co-injected with the siRNAs to identify the knockdown cells (white arrowheads). ANOVA test for Cdx2; ***P < 0.0001; NS, not significant. Right graph shows that our knockdown approach using Yap siRNAs effectively reduced Yap levels. Unpaired, two-tailed Mann-Whitney *U*-test for Yap; *P = 0.03. **g**, Scheme depicting cloning strategy to generate rescue constructs for K8 and K18. The coding regions of K8 and K18 are indicated by thick yellow arrows, and the targeted sequence locations for the keratin siRNAs utilized in this study are indicated by the red arrows. The specific siRNA target sequences are highlighted in yellow, corresponding to the keratin wildtype (WT) sequence (top rows). The rescue construct sequences are indicated (bottom rows). Note the conservation of amino acid sequence despite the scrambling of DNA bases throughout the siRNA target sequence. In each experiment, H2B-RFP was co-injected with the siRNAs and/or mRNAs to label the injected half of the embryo, and 100% of H2B-positive cells displayed keratin filaments when injected with the rescue construct. K8/K18 knockdown cells express lower levels of Cdx2 than control cells with keratins, but this phenotype is rescued when the keratin rescue constructs are co-injected with keratin siRNAs. ANOVA test; ***P < 0.0001; NS, not significant. Scale bars, 10 μm.

Extended Data Fig. 8. Keratins regulate blastocyst morphogenesis. a, Punctate desmosome structures labelled using immunofluorescence for desmoplakin (Dsp) colocalize with K8 along the trophectoderm cell-cell junctions of the blastocyst. Data are from 3 independent experiments. **b**, Analysis of apical surface curvature in control and K8/K18 knockdown blastocysts. Individual cells within the intact embryo were computationally-segmented in 3D. Single cells (blue) are selected for apical surface analysis. Middle panels show rendering of the apical surfaces (orange) of the selected cells. The right panels show fitting of the cell apical surface to a sphere for calculation of radius of apical surface curvature. Data are from 3 independent experiments. **c**, K8/K18 knockdown blastocysts display morphogenetic defects, revealed by smaller blastocyst volume, higher junctional tortuosity, and trophectoderm cells with lower radius of apical surface curvature. Unpaired, two-tailed Mann-Whitney *U*-test; $^{**}P = 0.004$ for blastocyst volume; $^{***}P < 0.0001$ for junctional tortuosity; $^{***}P < 0.0001$ for surface curvature. **d**, 2D confocal planes of live control and K8/K18 knockdown blastocysts, microinjected with fluorescent nanoparticles (yellow). Data are from 3 independent experiments. **e**, Images show nanoparticles within single trophectoderm cells, in control and K8/K18 knockdown embryos. Middle panels show representative trajectories of nanoparticle movement. Graph shows their mean squared displacement (MSD) over lag time. Thicker lines represent the mean of individual curves. The graph has two phases revealing different cytoskeletal properties: a time-independent (short lag times) and a time-dependent (long lag times) phase. These phases are associated with elasticity and viscosity, respectively[40]. Differences in MSD during the time-independent phase reveal higher elasticity, indicative of lower cytoplasmic stiffness, in the K8/K18 knockdown cells. **f**, Co-injection of keratin rescue constructs with K8/K18 siRNAs can restore blastocyst morphology to control conditions. Unpaired, two-tailed Student's *t*-test for blastocyst volume and surface curvature; Unpaired, two-tailed Mann-Whitney *U*-test for junction tortuosity; NS, not significant. Scale bars, 10 μm.

Extended Data Fig. 9. Heterogeneities in BAF155 and Carm1 within the early embryo trigger differential expression of keratins at the 8-cell stage. a, Live-imaging of an embryo expressing K8-Emerald, H2B-RFP and RFP-Utrophin confirms that the first cells to assemble keratin filaments are sister cells. The microtubule bridge connecting sister cells can be identified by RFP-Utrophin accumulation (white arrowheads)[41]. Data are from 3 independent experiments. **b**, Scheme shows the stereotypical 3D organization of a tetrahedral 4-cell embryo. The vegetal blastomere is located distal from the polar body. **c**, Selective photoactivation of the vegetal blastomere. The vegetal blastomere is identified based on its distal position from the polar body. The vegetal cell nucleus is then targeted with a 2-photon laser (820 nm light) to photoactivate H2B-paGFP. 2D confocal planes show efficient photoactivation immediately after 820 nm light illumination. Data are from 3 independent experiments. **d**, The first cells to form keratin filaments are unrelated to the order of cell divisions during the 4- to 8-cell stage transition. χ^2 test. **e**, BAF155 knockdown reduces BAF155 immunofluorescence levels relative to control blastomeres, while BAF155 overexpression increases them. Embryos were microinjected with BAF155 siRNAs or high levels of BAF155 RNA respectively at the 1-cell stage. ANOVA test; $^{***}P < 0.0001$. **f**, Embryos treated with Trichostatin A (TSA) display extensive keratin filament formation, while embryos treated with Actinomycin D (Act D) do not form filaments. ANOVA test; $^{***}P < 0.0001$; $^{*}P = 0.0489$. **g**, Microinjection of K8 and K18 mRNA into the 1-cell embryo causes premature assembly of an extensive keratin filament network throughout early blastomeres prior to the 8-cell stage. Two-sided Fisher's exact test; $^{***}P < 0.0001$. **h**, BAF155 overexpressing embryos treated with Actinomycin D do not form keratin filaments at the 8-cell stage. Data are from 3 independent experiments. **i**, dimethyl-BAF155 is lowest in the vegetal blastomere. Unpaired, two-tailed Student's *t*-test; $^{**}P = 0.004$. **j**, Carm1 overexpression

increases Carm1 immunofluorescence levels relative to control blastomeres. Embryos were microinjected with high levels of Carm1 RNA at the 1-cell stage. Unpaired, two-tailed Mann-Whitney *U*-test; ***$P < 0.0001$. **k**, Carm1 overexpression reduces keratin filament assembly. Unpaired, two-tailed Student's *t*-test; ***$P = 0.0007$. **l**, Overexpression of BAF155 or mutant BAF155^{R1064K} causes premature keratin filament assembly at the 4-cell stage. Two-sided Fisher's exact test; **$P = 0.009$ for BAF155 overexpression; **$P = 0.005$ for BAF155^{R1064K}. **m**, BAF155 knockdown blastomeres (white arrowheads) display lower Cdx2 levels than control cells (orange arrowheads) at the same stage. BAF155 siRNAs were microinjected into only one cell of the 2-cell embryo. ANOVA test; ***$P < 0.0001$; NS, not significant. **n**, Carm1 overexpression blastomeres (white arrowheads) display lower Cdx2 levels than control blastomeres (orange arrowheads) at the same stage. High levels of Carm1 RNA were microinjected into only one cell at the 2-cell stage. ANOVA test; **$P = 0.005$ for control inner cells; *$P = 0.04$ for Carm1 overexpression outer cells; **$P = 0.006$ for Carm1 overexpression inner cells; NS, not significant. Scale bars, 10 μm.

Extended Data Fig. 10. Scheme summarizing the main findings. Keratin expression is regulated by early heterogeneities in the BAF complex. During inner-outer cell segregation, apically-localized keratin filaments are asymmetrically inherited by outer daughter cells, where they stabilize apical F-actin to promote apical polarity and acquisition of a trophectoderm fate. At late stages, keratins also support blastocyst morphogenesis. The numbers indicate the key events.