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Trappc4 is required for early mammalian development

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Research Article

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ABSTRACT

Trafficking Protein Particle Complex Subunit 4 (TRAPPC4) is essential for protein transport from the endoplasmic reticulum to the Golgi apparatus. It plays a crucial role in tethering COPII vesicles and assembling SNARE complexes, ensuring precise protein delivery, which is vital for cellular communication and growth. Mutations in TRAPPC4 and other TRAPP subunits are associated with a group of human diseases collectively known as TRAPPopathies, often manifesting as neurological disorders. This study investigates the role of TRAPPC4 during early embryonic development by analysing the phenotypic consequences when the gene is knocked out in the mouse. Our results show that TRAPPC4 knockout leads to early embryonic lethality, where TRAPPC4 homozygous mutant embryos form morphologically normal blastocysts but fail to implant in the uterus and are not present at embryonic day 7.5. We show that mutant embryos can hatch from the zona pellucida, but do not form proper inner cell mass (ICM) outgrowths. Unlike the tightly compressed ICM colonies in control littermates, the mutant ICM appears necrotic with cells that are not adherent. This dispersion suggests that ICM cells may begin deteriorating at peri-implantation stages, underscoring TRAPPC4's pivotal role in maintaining cellular viability during early embryonic development.

Introduction

Approximately one-third of all mammalian proteins are synthesized in the endoplasmic reticulum (ER), transported to the Golgi apparatus and processed in a destination-dependent manner (Kanapin *et al.,* 2003). These proteins, including growth factors and hormones, are essential for differentiation and cellular communication. ER-to-Golgi trafficking is a highly conserved and essential process for the proper sorting and delivery of membrane-bound and secretory proteins (Barlowe and Miller, 2013). TRAnsport Protein Particle (TRAPP) complexes are tethering factors for COPII vesicles, and the TRAPPIII complex specifically mediates the assembly of the COPII vesicle outer layer during their formation (Zhao *et al.,* 2017). TRAPP complexes are integral for intracellular vesicle transportation (Hall *et al.,* 2024).

The TRAPP complexes also function in final vesicle fusion to target membranes, a molecular event physically orchestrated by soluble NSF-attachment protein (SNAP) receptors (SNAREs) and mediated by molecular switches (Galindo and Munro, 2023). These molecular switches, Rabs and Arfs, are active when GTP is bound and inactive when GDP is bound (Cherfils and Zeghouf, 2013). Rabs and Arfs are controlled by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) (Galindo and Munro, 2023). The TRAPPII complex is a GEF for Rab11, which regulates Golgi-to-plasma membrane transport, and the TRAPPIII complex is a GEF for Rab1, which regulates ER to Golgi transport (Hall *et al.,* 2024). Mammalian TRAPPCII and III share a core set of subunits that includes TRAPPC1–6B (Galindo and Munro, 2023). TRAPPC1 and TRAPPC4 form the catalytic site at which TRAPPCII and III will activate Rab11 or Rab1, respectively. *In vivo* activation of Rabs requires additional subunits of TRAPPCII and TRAPPCIII (Hall *et al.,* 2024).

The majority of known cellular and molecular functions of TRAPP proteins has been performed primarily *in vitro* and in yeast models. The shared core TRAPP subunits are highly conserved with mammalian orthologs of all yeast TRAPP subunits (Riedel *et al.*, 2018). When gene function is removed in mouse models, four complex members are homozygous lethal prior to organogenesis: *Trapp1, Trappc4, Trappc11, Trappc12* (Dickinson *et al.,* 2016). *Trappc8* is sub-viable at birth, and five subunits are homozygous viable: *Trappc2, Trappc2l, Trappc6b, Trappc9, Trappc10* (IMPC). The remaining five subunits have not been studied *in vivo* (*Trappc3, Trappc3l, Trappc5, Trappc6a, Trappc13*).

There are multiple human disease associations with TRAPP complex members, collectively known as TRAPPopathies, which include Spondyloepiphyseal Dysplasia Tarda, neuro-developmental disorder, limb girdle muscle dystrophy and cancer (Hall *et al.,* 2024). TRAPPC4 is one of two proteins that have limb girdle muscular dystrophy and cancer phenotypes (Hall *et al.,* 2024).

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Early development is a tightly regulated process, with carefully orchestrated and essential morphogenetic events making the first third of gestation particularly susceptible to genetic perturbations. Following initial post-fertilization cleavage divisions, the first differentiation events are completed by embryonic day 3.5 (E3.5) in the mouse, with the formation of a morphologically evident blastocyst, which remains unattached to maternal uterus. Successful blastocyst development requires specification of the first lineages: initially the inner cell mass (ICM) and trophectoderm (TE). The ICM is pluripotent and gives rise to both embryonic and extraembryonic lineages, while the TE contributes only to extra-embryonic tissues. During these preimplantation stages, the blastocyst remains within a spherical protein matrix, the zona pellucida (ZP). At approximately ~E4.0, the blastocyst expands, hatches from the ZP and implants into the maternal epithelium. Each step is essential for continued development. Here we present characterization of Trappc4 mutant mouse embryos, which fail to implant resulting in early embryonic lethality.

Results/discussion

Expression of murine TRAPP complex subunits

We first examined expression of all TRAPP complex subunits to identify embryonic stage (Figure 1A) and/or adult tissue (Figure 1B) specific expression. Pooled RNA from wild-type embryos at zygote, 2-cell, morula, blastocyst, E6.5, and E7.5 stages were used to synthesize cDNA. Intron-spanning primers were used to determine where each gene is expressed. *Trappc1, −3, −4, −6b,* and *−8* are expressed at all embryonic stages tested (Figure 1A). *Trappc2* shows dynamic expression – being robustly expressed in zygotes, weakly in 2 cell embryos, not expressed in morula but then transcribed again in blastocysts and gastrula. *Trappc2l, −5, −11 and −13* are each expressed in all stages except for 2-cell embryos, which is consistent with transcriptomics data that also show low expression at the zygote and 2-cell stages compared to later stages (Lab 2025). *Trappc3l* is robustly expressed in zygotes but is not expressed in later stages, suggesting it is an oocyte specific transcript (also consistent with other data sets, Lab 2025). *Trappc6a* was not expressed in any of the embryonic stages tested. *Trappc9* is robustly expressed in zygotes but then decreases as development proceeds, being absent at E7.5. Interestingly, a unique transcript variant of *Trappc9* appears to be expressed only at the 2-cell stage (Figure 1A). *Trappc12* levels appear to vary and it is not expressed in morula (Figure 1). *Trappc2, Trappc3l, Trappc6b, Trappc8 and Trappc11* have robust zygotic expression compared to other embryonic stages, suggesting maternal loading of RNA into oocytes, and possible roles during early post-fertilization events. Although there is relatively low expression of several TRAPP subunits in 2-cell and morula, each one of these genes exhibits robust expression in zygotes, suggesting that the proteins (and TRAPP complexes) are still functional despite low or no RNA at these stages.

We also examined expression of each subunit in 8 adult tissues – brain, heart, kidney, liver, lung, ovary, testis and tongue (Figure 1B), representing diverse lineages. *Trappc1, Trappc2, Trappc2l, Trappc3, Trappc4, Trappc5, Trappc6b, Trappc8,* and *Trappc13* are expressed in all tissues. Tongue showed very low or no expression of *Trappc3l, Trappc6a, Trappc9, Trappc10, Trappc11* and *Trappc12.* Consistent with its embryonic expression, *Trappc3l* was only expressed in ovary, and very weakly in testis, further suggesting a germ cell-specific transcript is present in oocytes (and possibly spermatogonia). *Trappc6a, Trappc11* and *Trappc12* were highly expressed in ovary and testis compared to the other tissues tested. Overall, these results indicate widespread expression of TRAPP subunits in adult tissues, with some developmental differences suggesting TRAPP sub-complexes may form during specific embryonic stages. Widespread expression also suggests that both TRAPPCII and TRAPPCIII complexes are active in all cell types, perhaps not surprising given the fundamental role of ER-to-Golgi trafficking.

Trappc4 mutant preimplantation embryos are indistinguishable from littermates

Initial findings from the IMPC indicated the Trappc4 homozygous mutant embryos could not be recovered at E9.5. Consistent with our early lethality phenotyping pipeline, we performed *Trappc4*$^{+/-}$ x *Trappc4*$^{+/-}$ timed matings to identify and analyse *Trappc4* knockout embryos during early development. The mutant allele used for these studies has a genomic deletion removing exons 2–5 (Figure 2A), allowing for robust genotyping of every embryo to distinguish wild-type (WT), heterozygous (HET) and homozygous mutant (MUT) embryos (see Materials and Methods). Dissections at E7.5 indicated that *Trappc4* mutant embryos are not present at gastrulation stages. Four E7.5 litters yielded 32 embryos with zero homozygous mutants recovered ($n = 11$ WT, $n = 21$ HET, $n = 0$ MUT, Figure 2B).

Since mutant embryos were not recovered at E7.5, post-implantation, we next flushed heterozygous intercross litters at E3.5 and found that *Trappc4* mutant embryos form morphologically normal blastocysts which are indistinguishable from littermates (Figure 2C). At E3.5 mutants are recovered at expected Mendelian ratios (Figure 2B). To confirm that the mutants lack WT *Trappc4* transcript, we designed RT-PCR primers within the CRISPR-deleted exons. As expected, no WT *Trappc4* RNA can be amplified from mutant embryos (Figure 2D).

To assess if the initial lineage specification is occurring in the absence of TRAPPC4, we performed whole-mount immunofluorescence with antibodies identifying cells of TE (caudal type homeobox 2 (CDX2)), and ICM (sex determining region Y-box two (SOX2)) (Figure 2E). Careful analysis and counting of cells indicate no significant differences between genotypes with regard to total cell number, or the quantity or percentage of ICM and TE lineages (Figure 2F–H), indicating that Trappc4 mutants properly segregate ICM and TE.

Trappc4 mutants do not form normal ICM outgrowths

Because we could not recover mutants at E7.5, and E3.5 mutants appear normal, we performed outgrowth assays on cultured blastocysts to assess if Trappc4 mutants hatch and form typical outgrowths where we can assess both ICM colony formation and TE differentiation (Figure 3A). Control blastocysts hatch from the ZP, forming a single layer of adherent TE cells containing large nuclei, and a tightly compacted ICM colony with stem cell characteristics (Figure 3A, dotted lines). Although mutant embryos are able to hatch from the ZP and a TE monolayer is evident, there is no mutant ICM colony, but instead each mutant culture contained a loose collection of necrotic cells that were not attached to the rest of the outgrowth, which easily floated away when the dish was moved (arrowheads, Figure 3A). Based on these findings, we suspect that in mutants the ICM is dying – either at E3.5 or during the 72 h *in vitro* in culture, which might have clinical relevance for assisted reproductive technology (ART). Trappc4 mutant blastocysts are morphologically normal prior to implantation (E3.5), yet

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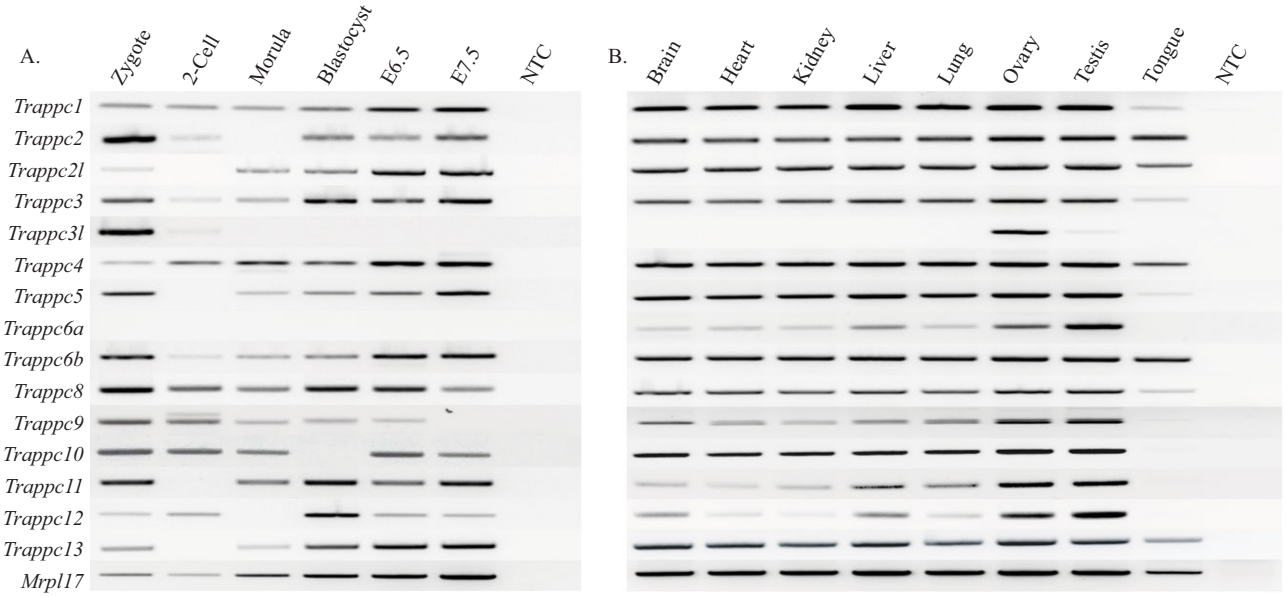


Figure 1. Expression of the 15 murine TRAPP complex subunit genes. Intron spanning primers designed against the 15 TRAPP complex subunit transcripts were used to amplify cDNA from pooled litters of different stages of embryo development (zygote, 2-cell, morula, blastocyst, E6.5, E7.5) and adult tissues (brain, heart, kidney, liver, lung, ovary, testis, tongue). *Mrpl17* serves as cDNA control. NTC, no template control.

no mutants can be recovered *in vivo* four days later (E7.5). Studies report that blastocyst TE grade is a strong predictor for live birth (Belal *et al.*, 2026). Since *Trappc4* mutant blastocysts have morphologically normal TE, successfully hatch from the ZP, but fail to produce post-implantation embryos, there may be clinical relevance to anembryonic pregnancies (Carlson and Tafti, 2026).

To test the possibility that the Trappc4 mutant ICM is dying at E3.5, we performed immunofluorescence using phospho-p53 (P-p53) on blastocysts from *Trappc4*$^{+/-}$ x *Trappc4*$^{+/-}$ ($n = 4$ WT, $n = 19$ HET, $n = 4$ MUT, Figure 3B). Surprisingly, we found no difference in the total number of P-p53-positive cells between genotypes (one-way ANOVA, $p = 0.3875$, Figure 3C–E). Additionally, there is no difference in the number of P-p53-positive cells in specific lineages when P-p53 was examined in concert with either CDX2 (TE) or SOX2 (ICM) immunofluorescence (Figure 3C–E).

Materials and methods

Animals

The authors assert that all use of animals and experiments contributing to this work were approved by the UMass Institutional Animal Care and Use Committee. Knockout (KO) alleles used were produced by Baylor College of Medicine: C57BL/6NJ-Trappc4$^{em1(IMPC)Bay}$/Mmmh. The *Trappc4* KO allele was generated by a deletion of exons 2, 3 and 4 via injection of CAS9 protein and guide sequences 5'-CCGCACCAGCCCACTTCCGCAGC-3' and 5'-CCTACGGAAACCCTGTGTCCTCA-3'. Further allele details are available at https://www.mmrrc.org/catalog/sds.php?mmrrc_id=66947

Embryo retrieval, culture and genotyping

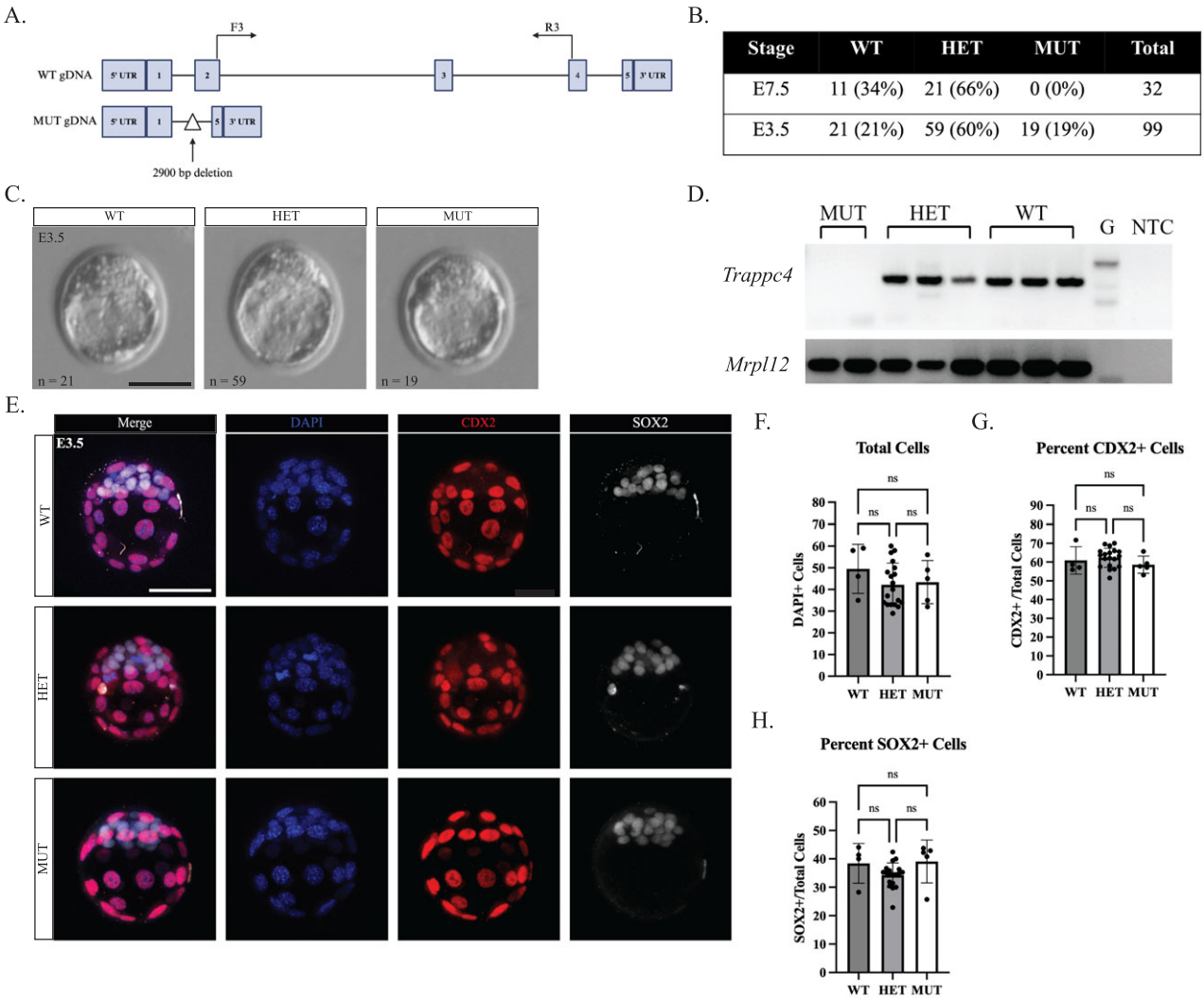
Embryos were generated by heterozygote intercrosses. Males and females were housed together, and the presence of a vaginal plug in the morning indicated E0.5 of development. E3.5 embryos were collected by flushing the uterus with outgrowth media (described below) for outgrowths, or with phosphate-buffered saline (PBS) with 0.03% (w/v) polyvinylpyrrolidone for other purposes. Embryos were cultured at 37°C and 5% O_2, 5% CO_2 atmosphere in a humidified incubator. Genotypes of individual embryos were determined by genomic PCR using the following primers: *Trappc4* WT (515 bp) F1: 5′-ACCAGTGGGACAGCTATTCG-3′ and R1: 5′-TAGCCGGCCTAGAGACAGAT-3′ and MUT (339 bp) F2: 5′-ACCAGTGGGACAGCTATTCG-3′ and R2: 5′-CCAAAAGT GCCAGCCTTCTC-3′. Loss of WT *Trappc4* transcript in mutants was demonstrated using RT-PCR primers designed within the deletion (F3: 5'- CCATGCAGTGTTGGCCATCA -3' and R3: 5'-AGTTTGTGGTCCTGGCAGAC -3'). For all results shown, a minimum of three embryos of each genotype were assessed (more in most cases, as indicated in results).

RNA extraction, complementary DNA synthesis, RT-PCR analysis and quantitative RT-PCR

RNA was isolated from pooled wild-type embryo litters collected at zygote, 2-cell, morula and blastocyst stages. RNA was also isolated from single wild-type embryos collected at E6.5 and E7.5 stages. Brain, heart, kidney, liver, lung, ovary, testis and tongue samples were collected from adult wild-type C57BL/6NJ mice and RNA was isolated from those samples as well. RNA was isolated using the Zymo Quick-DNA/RNA Microprep Plus Kit (Zymo Research, D7005) and was converted into complementary DNA (cDNA) using the BioRad iScript cDNA Synthesis Kit (BioRad 1708890), according to the manufacturer's protocol. RT-PCR was performed for 38 cycles of 30 s at 60°C, 72°C and 95°C with the following primers:

Mrpl17 (414 bp), 5'-CCATCTGCTGCGGAACTTG-3′, 5'-TT GCGTCCTGGTTATGGTG-3′; *Trappc1* (142 bp), 5'- CCATTCG TTCGTTTGTCAGCA -3′, 5'- CCCACGCCCAAGTCAGTATT -3′; *Trappc2* (194 bp), 5'- CTCATCTCGGCTCCTCCCTA -3′, 5'-TCGTCCACAAGGTCAAGAGC -3′; *Trappc2l* (455 bp), 5'- TCC GACAGAGAGTGAGCTCA -3′, 5'- ACTCAGGGAGCAGAAA GGGA -3′; *Trappc3* (296 bp), 5'- ACCTATGGAGCGCTTGT

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Stage	WT	HET	MUT	Total
E7.5	11 (34%)	21 (66%)	0 (0%)	32
E3.5	21 (21%)	59 (60%)	19 (19%)	99

Figure 2. *Trappc4* knockout validation and lineage assessment. (A) Schematic comparison of WT and MUT *Trappc4* alleles (not to scale). Elbowed arrows indicate primers used for RT-PCR shown in D. Triangle represents CRISPR Cas9-mediated deletion of 2900 bp. (B) Table showing genotypes of embryos recovered through timed *Trappc4*$^{+/-}$ x *Trappc4*$^{+/-}$ matings dissected at E7.5 and E3.5. (C) Representative brightfield images of *Trappc4* Wild type (WT) ($n = 21$), Heterozygote (HET) ($n = 59$), and Mutant (MUT) ($n = 19$) E3.5 embryos. (D) RT-PCR results of single E3.5 embryos indicating complete absence of normal *Trappc4* mRNA. *Mrpl12* is loading control, "G" is genomic DNA control, "NTC" is no template control. (E) Representative maximum confocal intensity projection of immunofluorescence of E3.5 embryos. DAPI stains nuclei (blue), CDX2 marks TE cells (red), SOX2 marks ICM cells (white). $n = 4$ WT, $n = 19$ HET, $n = 4$ MUT. (F) Bar chart comparing total cell number (DAPI + cells) between each genotype. (G) Bar chart comparing TE lineage (CDX2+) cell numbers. (H) Bar chart comparing ICM (SOX2+) cell numbers. Each circle represents one embryo analysed in each group. Statistical analyses were performed using one-way ANOVAs. "ns" indicates not statistically significant. Scale bars represent 50 μm.

CAC -3′, 5'- TCGGGAAGCTCCACAAAGTC -3′; *Trappc3l* (305 bp), 5'- AGAGCAAAGGCAGTAGAGCG -3′, 5'- CCAGCCGTG TTCCAATGTTG -3′; *Trappc4* (385 bp), 5'- TCCCAGCTATC TCCGGAACA -3′, 5'- AAGGCTGCTACAACAGGACC -3′; *Trappc5* (378 bp), 5'- CAGCACAGTTCCGGTTGAGA -3′, 5'- CCTTGACGAAGAGCAATGCG -3′; *Trappc6a* (319 bp), 5'- TA CCTCCCGAGCTGACCAAT -3′, 5'- AGTCCGTCCATGTGCTT CTG -3′; *Trappc6b* (255 bp), 5'- TCATTCAGCTGTCTGCAGGA -3′, 5'- AACGATTTGCTCCAACGCAG -3′; *Trappc8* (274 bp), 5'- CTCGCCTCACTTCCGAAGTT -3′, 5'- TCTGATGCTGGCATG GACTG -3′; *Trappc9* (241 bp), 5'- TGTGCTCGGCTTCAGTC ATT -3′, 5'- CATGCCACGCTTCTGAATCG -3′; *Trappc10* (370 bp), 5'- AGTGCACCTTGCTCCTCTTC -3′, 5'- GCTGTGTTGG CTGTTTCAGG -3′; *Trappc11* (339 bp), 5'- ACAATACCCCAC TGGATGCG -3′, 5'- TCCAGGGCCTCTGTCCATAA -3′; *Trappc12* (435 bp), 5'- GGCTGTGTTTGCTGGAAGTG -3′, 5'- AACCAGAGCTGCAAGGAGTC -3′; *Trappc13* (397 bp), 5'- AG GCCATCCCAGACACTGTA -3′, 5'- TCACTGCACAAAGCTG ACGA -3′.

Outgrowth assays

Blastocysts were harvested at E3.5 and cultured individually in iBiTreat dishes (μ-slide 8 well, 80826-90) with Dulbecco Modified Eagle Medium (Lonza, Allendale, NJ, USA) containing 10% (v/v) fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA, USA), 1% (v/v) GlutaMAX (Thermo Fisher Scientific) and 1% (v/v) Penn-strep. Embryos were kept in culture for 72 h at 37°C and 5% O_2, 5% CO_2 atmosphere in a humidified incubator, followed by imaging and genotyping.

Immunofluorescence

Freshly dissected E3.5 embryos and outgrowths were fixed in 4% (w/v) paraformaldehyde for 10 minutes at room temperature,

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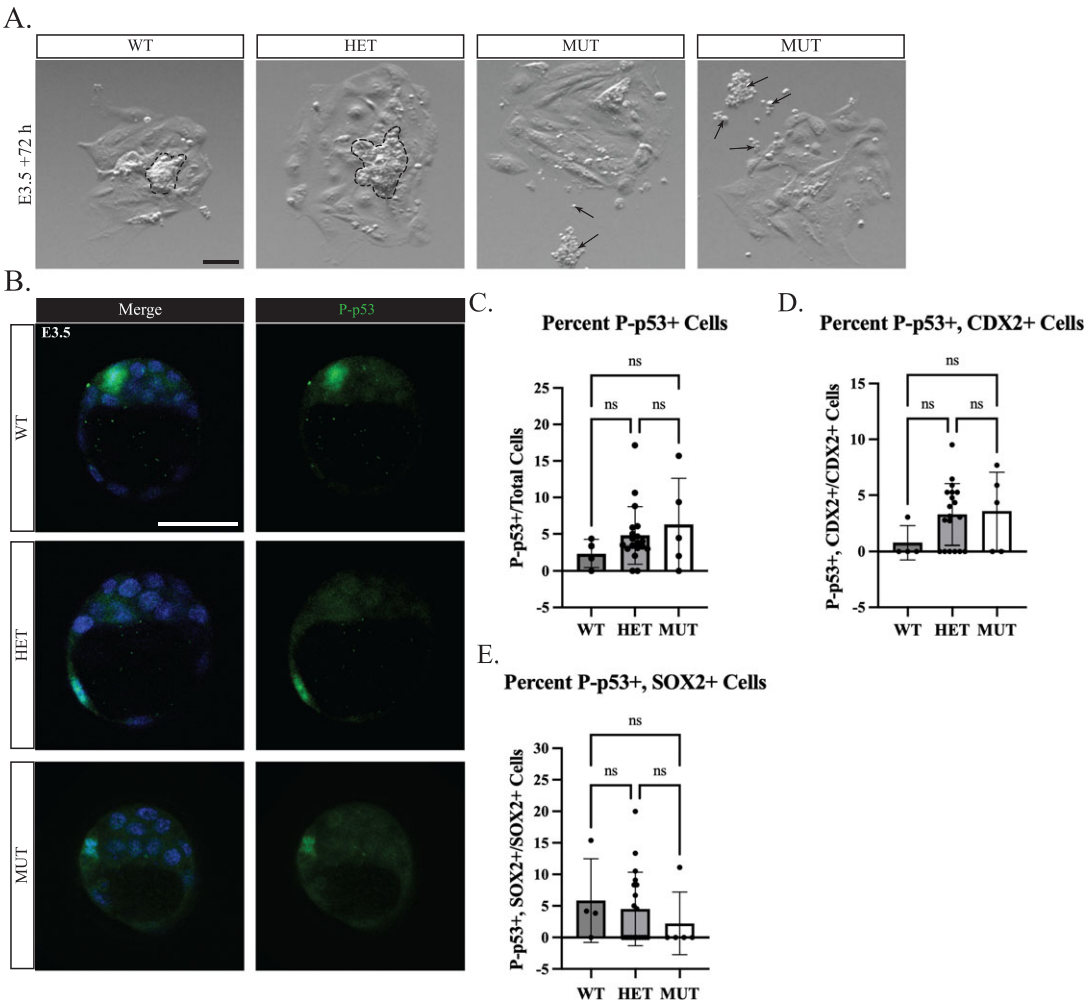


Figure 3. Cell death assessment in mutants. (A) Outgrowth assay of *Trappc4*$^{+/-}$ x *Trappc4*$^{+/-}$ litters ($n = 4$ WT, $n = 9$ HET, $n = 9$ MUT). E3.5 embryos cultured for 72 hours at 37°C in 5% O_2, 5% CO_2 atmosphere in a humidified incubator. Dotted circles enclose inner cell mass (ICM). Arrowheads indicate necrotic floating cells. Scale bar represents 100 µm. (B) Phospo-p53 (P-p53) -mediated cell death assessment in E3.5 embryos. Representative z-section images of immunofluorescence of E3.5 embryos. DAPI stains nuclei (blue) and P-p53, an apoptotic marker (green). Scale bars represent 50 µm. $n = 4$ WT, $n = 19$ HET, $n = 4$ MUT. Scale bar represents 50 µm. (C–E) Bar charts comparing P-p53+ cells between genotypes and lineage-specific cell populations. The circles represent the embryos analyzed in each group. Statistical analyses were performed using one-way ANOVAs. "ns" indicates not statistically significant.

permeabilized in 1% (v/v) Triton-X and blocked with 0.1% (w/v) bovine serum albumin (BSA) supplemented with 0.01% (v/v) Tween20 and 20 µg/mL Fab fragment. Primary antibodies were diluted in 0.1% BSA supplemented with 0.01% Tween 20 (block) and incubated overnight at 4°C. The embryos were then rinsed with block for 30 min, followed by incubation with secondary antibodies at room temperature for an hour. Next, embryos were stained with DAPI in PBS (1 µg/mL) for 3 min and rinsed thoroughly. Images were taken with a Nikon Eclipse Ti Series inverted microscope with a C2 confocal attachment.

The primary antibodies used and their dilution include SOX2 (R and D Systems Cat# AF2018, RRID:AB_355110, 1:200), CDX2 (BioGenex Cat# MU392A, RRID:AB_2923402, 1:200); phospho-p53 (Ser15) (P53) (Cell Signaling Technology Cat# 9284S, RRID: AB_331464, 1:50).

The secondary antibodies used include Alexa Fluor 488 donkey anti-mouse (Thermo Fisher Scientific, A21202, 1:500); Alexa Fluor 546 donkey anti-rabbit (Thermo Fisher Scientific, A10040, 1:500); Alexa Fluor 647 donkey anti-goat (Thermo Fisher Scientific, A21447, 1:500); and DAPI (Molecular Probes, 1 µg/mL).

Quantification of cell number

The analysis was conducted using the Nikon Elements Analysis 3.1 software. The quantity of DAPI+, CDX2+, SOX2+ and P-p53+ cells were manually counted for statistical comparison. Statistical analyses were performed using Prism (Version 10). To assess statistical differences among three or more groups, one-way analysis of variance (ANOVA) was conducted. Multiple one-way ANOVA tests were used to compare several metrics between wild types, heterozygotes and mutants. Measurements compared were the quantities of total cells, SOX2+ cells relative to total cells, CDX2+ cells relative to total cells, P-p53+ cells relative to total cells, P-p53+ and SOX2+ cells relative to SOX2+ cells and P-p53+ and CDX2+ cells relative to CDX2+ cells. Statistical significance was set as $p = 0.05$.

Conclusions

Here we show that nearly all mammalian TRAPP complex subunits are widely expressed throughout early development and

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in adult tissues of the mouse (Figure 1). *Trappc4* mutant embryos are present and appear morphologically indistinguishable from littermates at E3.5, but none are recovered at E7.5, suggesting that *Trappc4* is required for survival at post-implantation stages (Figure 2B, C). Mutant and control E3.5 embryos exhibit no difference in the total number of cells per embryo (Figure 2F), nor in number or percentage of ICM and CDX2-positive cells (Figure 2G, H). However, when subjected to outgrowth assays, mutant embryos form highly irregular cultures with apparent dying ICM after 72 hours *in vitro*. Instead of a compact and tightly adhered ICM colony, loose clumps of small, rounded cells are detached and readily float away from where the ICM should be (Figure 3A). Analysis of cell death with P-p53 revealed no difference in cell death between mutants and controls (Figure 3C–E). These results indicate that loss of *Trappc4* is early embryonic lethal at peri-implantation stages between embryo hatching (~E4.0) and gastrulation stages (~E7.5) and may provide a model for clinical scenarios where anembryonic pregnancies detected by ultrasound after transfer of morphologically normal blastocysts (Carlson and Tafti, 2026). Additionally, although there are several documented cases of Trappc4 mutations resulting in neurodevelopmental disorder with epilepsy, spasticity and brain atrophy (OMIM, 2023). These are predominantly splice site and/or hypomorphic mutations and likely not null alleles. Further investigation of the role of *Trappc4* in early development is needed to understand the mechanism by which Trappc4 mutations induce neurodevelopmental disorders in humans.

Author contributions. **Sarah S. Mirza:** Conceptualization-Equal, Formal analysis-Equal, Writing – original draft-Equal, Writing – review & editing-Equal; **Louise Bassom:** Formal analysis-Equal, Investigation-Equal, Writing – review & editing-Supporting; **Minjun Ahn:** Formal analysis-Equal, Investigation-Supporting; **Jesse Mager:** Conceptualization-Equal, Resources-Lead, Supervision-Lead, Writing – original draft-Equal, Writing – review & editing-Equal.

Competing interests. The authors declare none.

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