Live imaging YAP signalling in mouse embryo development

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YAP protein is a critical regulator of mammalian embryonic development. By generating a near-infrared fusion YAP reporter mouse line, we have achieved high-resolution live imaging of YAP localization during mouse embryonic development. We have validated the reporter by demonstrating its predicted responses to blocking LATS kinase activity or blocking cell polarity. By time lapse imaging preimplantation embryos, we revealed a mitotic reset behaviour of YAP nuclear localization. We also demonstrated deep tissue live imaging in post-implantation embryos and revealed an intriguing nuclear YAP pattern in migrating cells. The YAP fusion reporter mice and imaging methods will open new opportunities for understanding dynamic YAP signalling in vivo in many different situations.

1. Background

Yes-associated protein 1 (Yap1)—commonly referred to as YAP—is a signalling protein that serves as a hub of biochemical and mechanical sensing [1–3]. YAP plays crucial regulatory roles in enormously diverse processes in development and disease, from the very beginning of life, such as during mammalian preimplantation development [4], to the very late stage of disease, such as in cancer metastasis [5]. Real-time tracking of the dynamic YAP signalling status in vivo would offer crucial insights into the fundamental regulatory mechanisms of mammalian development and disease.

YAP is a transcriptional co-activator for the TEA domain (TEAD) family transcription factors [6]. In response to numerous signals, YAP shifts its sub-cellular distribution to the nucleus where it associates with TEAD proteins to activate gene expression. Thus, monitoring the nuclear/cytoplasmic localization of YAP is the most broadly applied method to evaluate YAP activity [6,7]. Currently, this is primarily achieved by immunostaining of fixed embryos or tissue sections, precluding the acquisition of dynamic information. Although live imaging has been achieved in Drosophila and human cell lines and revealed intriguing YAP behaviours [8,9], no appropriate tools exist to date for live imaging endogenous YAP protein in mammals in vivo. Here, we report a YAP fusion reporter mouse line that allows in vivo live imaging of YAP behaviour.

2. Methods

2.1. Designs of guide RNAs and knock-in repair donors

A guide RNA target spanning the Stop TAG codon: TCACGTGGTTATAGAGCTGCAGG was selected using the CRISPOR algorithm (http://crispor.royalsocietypublishing.org/journal/rsob).

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5777459.
tefor.net) based on specificity scores [10]. The chemically modified single guide RNA (sgRNA) with the sequence of UUGCGCGGGCUCCAUGGCUG was synthesized by Synthego Inc. The repair donor for YAP-emiRFP670 reporter was designed as illustrated in Extended figure 1 in the electronic supplementary material. The emiRFP670 and the linker coding sequences in proper orientation were flanked by long homology arms (813 bp 5’ arm and 717 bp 3’ arm) on each side and replaced the stop codon of the Yap1 gene. The donor DNA sequence was synthesized by Epoch Life.

Figure 1. Characterization and validation of YAP-emiRFP670 reporter in preimplantation embryos: (a) Live imaging of YAP-emiRFP670 localization in preimplantation embryos. (b) Immunofluorescence images of the YAP-emiRFP670, endogenous YAP protein (Immunofluorescence) and CDX2 protein (Immunofluorescence) in a mouse blastocyst, showing perfect colocalization of YAP and CDX2 in the embryo. (c) Manipulation of YAP-emiRFP670 localization by expressing a dominant-negative LATS2. Left: A schematic for mRNA injection experiment. mRNAs of H2B-RFP (control group) or H2B-RFP + dnLATS2 (experimental group) were injected into one of the two cells of a 2-cell stage mouse embryo and then cultured to early blastocyst stage for analysis. Right upper panel, a control embryo shows that the expression of H2B-RFP did not cause nuclear YAP localization and CDX2 expression. Right bottom panel, a dnLATS2 injected embryo showed nuclear YAP and CDX2 expression in inside cells. Cells of interest were circled by dotted lines. (d) Manipulation of YAP-emiRFP670 localization by treating with the ROCK inhibitor Y27632. Left: A schematic for the treatment experiment. Right, Snapshots from live imaging series electronic supplementary material, movie S1 showing ROCK inhibitor treatment leads to cytoplasmic Yap localization in embryos.
2.2. Generating knock-in (KI) reporter mouse lines by 2C-HR-CRISPR

The KI reporter mouse line was generated following our published protocol using 2C-HR-CRISPR on the CD1 background [11,12]. Briefly, Cas9 monomeric streptavidin (mSA) mRNAs were produced by in vitro transcription using the mMessage mMachine SP6 Kit (Thermo Scientific). PCR templates were generated by PCR reaction with biotinylated primers using high fidelity ClonAmp HiFi PCR mix (Takara Inc.). A mixture of Cas9-mSA mRNA (75 ng µl⁻¹), sgRNA (50 ng µl⁻¹) and biotinylated repair donor (20 ng µl⁻¹) was microinjected into the cytoplasm of 2-cell stage mouse embryos and transferred the same day to pseudo-pregnant females. Founder pups were obtained from the pregnancies.

We established founder mice with the correct insertion at high efficiency (2/2 live born pups). A founder was outcrossed five generations to wild-type CD1 mice to breed out any potential off-target mutations introduced by CRISPR–Cas9 and then bred to homozygosity at N6 generation. The mouse line was then maintained by homozygous breeding. In the early generations of homozygous breeding, a cataract phenotype was observed in some mice. This phenotype was then removed by selectively breeding YAP-emiRFP670 homozygous reporter mice without such a phenotype. The homozygous mice are otherwise healthy and fertile without apparent phenotype.

For generating Yap-emiRFP670/Cdx2-eGFP embryos, the Yap-emiRFP670 mouse line was bred to the Cdx2-eGFP mouse line to generate double homozygous mice [13]. Embryos were then collected from the double homozygous mouse line for imaging.

All animal work was carried out following the Canadian Council on Animal Care Guidelines for Use of Animals in Research and Laboratory Animal Care under protocols approved by the Centre for Phenogenomics Animal Care Committee (20–0026H).

2.3. Genotyping and genetic quality controls

Founder mice were genotyped by PCR amplification with primers spanning homology arms using the following primers: 5’ arm gtF: GTTCTAAGGTAGACACTGTGTGCTTCAGTT and 5’ arm gtR: TCATTGTCAGGTCAGGTT and 5’ arm gtF: CTGGTTGTCTGTCACCATTATCTGC and 3’ arm gtR: AACACCTGCCAATGTCCACCACCC. Founder mice were outcrossed to CD1 mice to generate N1 mice. The N1 mice were genotyped by PCR. Additionally, genomic regions spanning the targeting cassette and 3’ and 5’ homology arms were Sanger-sequenced to validate correct targeting and insertion copy number was evaluated by droplet digital PCR (performed by the Centre for Applied Genomics at the Research Institute of The Hospital for Sick Children, Toronto). Heterozygous N1 mice have only one insertion copy, demonstrating single-copy insertion.

2.4. Embryo isolation, culture and treatments

Preimplantation embryos were isolated from superovulated females that were mated with males, both homozygous for the C-YAP reporter. Embryos were isolated from oviducts or uterus at appropriate stages for each experiment—E0.5 for zygote, E1.5 for 2-cell embryos, E2.5 for 8-cell embryos and E3.5 for blastocysts. Embryos were flushed with M2 medium. They were then cultured in small drops of KSOM-AA medium under mineral oil at 37°C, with 6% CO2 for specified times. For the ROCK inhibitor treatment experiment, 8-cell stage embryos were cultured in KSOM-AA medium supplemented with 20 µM Y-27632.

2.5. Time lapse live imaging preimplantation embryos

For imaging the YAP dynamics during the formation of 16-cell embryos, embryos were flushed from the oviduct at 4-cell or 8-cell stage and cultured in a 3 µl drop of KSOM-AA medium under mineral oil on a MatTek glass-bottom dish. Live imaging was performed at 20 min frame⁻¹ for 24–36 h. For the ROCKi inhibitor treatment, embryos were flushed from the oviduct at 8-cell stage and cultured in a 3 µl drop of KSOM-AA medium with 20 µM Y-27632 under mineral oil on a MatTek glass-bottom dish. Live imaging was performed every 90 min for 24 h.

2.6. dnLATS2 mRNA injection

Homozygous Yap-emiRFP670 embryos were collected at 2-cell stage. mRNAs were microinjected into one of the two blastomeres as previously described [7,14]. For control experiments, embryos were injected with H2B-RFP mRNAs at 300 ng µl⁻¹. Treated embryos were injected with dnLATS2 mRNA at 1000 ng µl⁻¹ plus H2B-RFP mRNA at 300 ng µl⁻¹. The embryos were then cultured to the early blastocyst stage for immunofluorescent analysis.

2.7. Whole-mount immunofluorescent staining of embryos

Whole-mount immunofluorescent staining of embryos was performed as previously described [15]. Briefly, embryos were fixed in 4% paraformaldehyde at room temperature for 15 min, washed once in PBS containing 0.1% Triton X-100 (PBS-T), permeabilized for 15 min in PBS 0.5% Triton X-100 and then blocked in PBS-T with 2% BSA (Sigma) and 5% normal donkey serum (Jackson ImmunoResearch Laboratories) at room temperature for 2 h, or overnight at 4°C. Primary and secondary antibodies were diluted in blocking solution, staining was performed at room temperature for approximately 2 h or overnight at 4°C. Washes after primary and secondary antibodies were done three times in PBS-T. Nuclear staining was performed using Hoechst 33258 (Thermo scientific) at a concentration of 10 µg ml⁻¹ for 20 min at room temperature. Embryos were mounted in PBS in wells made with Secure Seal spacers (Molecular Probes, Thermo Fisher Scientific) and placed between two cover glasses for imaging. Primary antibodies: Goat anti-ltTomato (Biorybt orb182397 Lot AR2150) at 1: 200 dilution, Rabbit anti YAP (Cell Signalling Technology (D8H1X) XP Ref 11/ 2018 Lot4) at 1: 100 dilution and Mouse anti Cdx2(Biogenex MU392A-UC Lot MU392A0714) at 1: 100 dilution. Secondary antibodies all from Thermo Scientific and at 1: 400 dilution: Donkey-anti-goat IgG AF 546 (A11056 Lot 1714714), Donkey-
2.8. Confocal imaging of preimplantation embryos

Both live and immunostained images of preimplantation embryos were acquired using a Zeiss Axiovert 200 inverted microscope equipped with a Hamamatsu C9100-13 EM-CCD camera, a Quorum spinning disk confocal scan head, and Volocity acquisition software v. 6.3.1. Single plane images or Z-stacks (at 1 µm intervals) were acquired with a 40x air (NA = 0.6) or a 20x air (NA = 0.7) objective. Images were analysed using Volocity software. Live imaging was performed in an environment controller (Chamlide, Live Cell Instrument, Seoul, South Korea) on the same microscope.

Time lapse imaging was performed on the same microscope equipped with an environment controller (Chamlide, Live Cell Instrument, Seoul, South Korea). Embryos were placed in a glass-bottom dish (MatTek) in KSOM-AA covered with mineral oil. A 20x air (NA = 0.7) objective lens was used. Images were acquired at 1–3 µm Z intervals with time lapse settings as indicated in the legend to figure 2a.

2.9. Image quantification analysis for preimplantation embryos

Preimplantation images were visualized using the Volocity 6.3 software (see https://www.perkinelmer.com/en-ca/lab-products-and-services/resources/whats-new-volocity-6-3.html). The live images of preimplantation embryos were traced manually by carefully inspecting the movie and tracking the cell nucleus marked by the live DNA dye and recording the presence or absence of the YAP-emiRFP670 reporter. For quantifying fluorescent intensity, images were exported as TIF files and measured using the region of interest (ROI) function in ImageJ 1.53a software (see https://imagej.nih.gov/ij/notestools.html). The average fluorescent intensity of each nuclear ROI was measured and subtracted against a general background fluorescent intensity in the corresponding image.

2.10. Lightsheet imaging of post-implantation embryos

Three-dimensional static live imaging was performed on a Zeiss Lightsheet Z.1 lightsheet microscope. Embryos were suspended in a solution of DMEM without phenol red containing 12.5% filtered rat serum and 1% low-melt agarose (Invitrogen) in a glass capillary tube. Once the agarose solidified, the capillary was partially extruded from the glass capillary tube until the portion containing the embryo was completely outside of the capillary. The temperature of the imaging chamber was maintained at 37°C with 5% CO2. Images were acquired using a 20x objective with dual-side illumination in a multi-view mode (four evenly spaced views spanning 360° for E8.0 embryos imaging) or tile scanning mode (for E8.5 and E9.5 embryos imaging). The light sheet images were processed using Zen (Zeiss), Aravis Vision4D (Aravis), Imaris (Bitplane) and ImageJ.

Time lapse imaging was performed similarly to the static live imaging with the following modifications: (i) 2% fluorescent beads (1:1000, diameter: 2 µm; Sigma-Aldrich) were added to the low melting point agarose for drift compensation. (ii) Images were acquired for 3 h with 5 min intervals.

2.11. In vivo drift-compensated cell tracking and mean squared displacement calculation

The light sheet time lapse image was first rendered in Imaris (Bitplane). Nuclear YAP positive cells were determined by mean thresholding of fluorescence intensity. For each embryo, the cut-off intensity was set to be 50% of its maximum intensity. Small bright spots of YAP (diameter less than 7 µm) due to local chromatin condensation were excluded from nuclear YAP positive cell identification. The proportions of nuclear YAP positive cells were tracked over time using an autoregressive motion algorithm. The tracking data were then imported into MATLAB (MathWorks) for drift compensation using a program reported before [16].

Mean squared displacement (MSD) is an unbiased metric to evaluate cell migration [17]. For an arbitrary trajectory, the MSD and time delay follows a power-law relation, with power of 0 representing the random walk motion, and power of 2 representing the straight-line motion. On a log-log plot, these two cases translate into a line with slope 0 (i.e. a horizontal line) for the random walk motion, and a line of slope 2 for the straight-line motion. The mean MSDs of nuclear YAP positive cells’ migration trajectories are characterized by a line of slope 1.433 as shown in figure 4c, which deviates significantly from the random walk motion suggesting a persistent cell migration [18].

2.12. Statistical analysis

Statistics on numerical data were performed using the Prism 9 software (GraphPad Software, LLC). For intergroup comparison, the data were first subjected to the D’Agostino and Pearson normality test. Datasets that conform to a normal distribution were then subjected to the unpaired Student’s t-test, and the ones that did not conform to a normal distribution were then subjected to the Mann–Whitney test. Two-tailed analysis were used in all cases. Exact p-values were presented in the figures. For the one-group t-test for the proportion of relative position of cell division pairs at the 16-cell stage, we tested against a null hypothesis that the proportion = 50%, which resulted in a p-value of 0.0381.

3. Results and discussion

We set out to engineer a knock-in (KI) fusion reporter of Yap in mice (Extended figure 1 in the electronic supplementary material). The function of mammalian YAP seems to be easily disrupted by fusion tags. We tested different combinations of tagging position and linker sequences and finally successfully generated a healthy reporter mouse line by C-terminal tagging with a long (30 amino acid) flexible linker (Extended figure 1a). Other designs, such as N-terminal tagging with the same linker led to embryo death at embryonic days 8.5 (E8.5), similar to Yap knockout embryos, suggesting functional interference [19]. To allow good light penetration for imaging deep tissue layers in post-implantation mammalian embryos and other tissues, we chose to use a bright near-infrared (NIR) fluorescent protein (FP)-enhanced miRFP670 (emiRFP670), as the fluorescent indicator [20]. We performed extensive quality control to confirm single-copy insertion of the fusion reporter with the correct sequence, as detailed in the methods section and extended figure 1b and
our published protocol [11,21]. The homozygous mice are healthy and fertile. The mouse line was maintained in the homozygous state after outcrossing for five generations to ensure no carry-over of any possible off-target alterations.

We validated the reporter in mouse preimplantation embryos where the YAP distribution and its responses to various interventions are well characterized. The nuclear/cytoplasmic distribution of YAP from 8-cell stage onward is well established from previous research: At 8-cell stage, all blastomeres have nuclear localized YAP. From 16-cell stage to early mid blastocyst stage (E3.5), nuclear YAP is restricted to the outer cells that will give rise to the trophectoderm [7].

By the expanded blastocyst stage (E4.5), some epiblast cells will start to present nuclear YAP status [22]. Our live imaging reproduced this pattern (figure 1a). Before the 8-cell stage, the localization of YAP distribution is more debatable. Our live imaging showed that YAP was evenly distributed in the cytoplasm and nucleus of blastomeres until the late 4-cell stage, at which point YAP begins to be more restricted to the nucleus (figure 1a). We further validated the colocalization of the YAP-emiRFP670 reporter with the endogenous YAP protein and with the expression of CDX2 protein in the trophectoderm (TE) of blastocysts by immunofluorescence (figure 1b).

We then investigated whether the YAP-emiRFP670 reporter protein can respond appropriately to exogenous signals. In mouse early embryos, YAP localization is controlled by HIPPO, polarity signalling pathways and cortical tension [23–28]. From the HIPPO signalling pathway, LATS1/2 kinase is responsible for the phosphorylation of YAP, which leads to its sequestration in the cytoplasm [7]. Overexpressing a kinase-dead LAT kinase (dnLATS2) can promote the nuclear localization of YAP and the expression of CDX2 even in inside cells [7]. This result was replicated when we injected a dnLATS2 mRNA into homozygous Yap-emiRFP670 embryos (figure 1c). As for polarity and cortical tension, it has been shown that the inhibition of the Rho-associated protein kinase (ROCK) kinase using a small molecular inhibitor Y27632 resulted in cytoplasmic YAP localization across all cells of the embryo, and as a result, failure to establish the TE [29]. We cultured Yap-emiRFP670 reporter embryos from 8- to 16-cell stages in Y27632 and live imaged them. As shown in figure 1d and electronic supplementary material, movie S1, ROCK inhibition indeed resulted in a cytoplasmic YAP localization in all cells (figure 1d).

We then used the validated reporter mouse line to analyze the behaviour of YAP during embryonic development in real-time. A key event that YAP regulates during preimplantation development is the initiation of inner cell mass (ICM)-TE segregation at the 16-cell stage. When 8-cell embryos transition to 16-cell embryos through cell division, YAP becomes localized in the nucleus of blastomeres located on the surface of the embryos (outside cells) and excluded from the nucleus in inside cells [7]. The presence of nuclear YAP drives the expression of the TE-specific transcription factor CDX2 in outside cells, initiating TE lineage differentiation. By contrast, the enclosed blastomeres with cytoplasmic localized YAP initiate Sox2 expression and ICM differentiation [30]. However, the exact process that leads up to this asymmetric YAP signalling status is still debatable. Some models suggest a fixed determination of YAP localization at the time of generation of inside and outside 16-cell blastomeres, while other recent studies suggest a more dynamic process [31,32]. Direct observation of the YAP dynamics through the 8-16-cell stage is the best way to resolve these models.

We time lapse imaged homozygous YAP-emiRFP670 reporter embryos every 20 min at the transition between the 8-cell to 16-cell stage for a period of 20 h. Cell nuclei were marked by a live DNA dye (electronic supplementary material, movies S2 and S3, with additional movie from 4-cell stage-electronic supplementary material, movie S4). To rule out the possibility of the imaging process affecting normal development, we further cultured the embryos to E4.5 and validated a high blastocyst formation rate (8/9) (Extended figure 2a in the electronic supplementary material). The movies revealed profound dynamic movements of blastomeres in 8–16-cell stage embryos. Many cells changed their relative outside-inside position from where they were localized right after the cell division that generates them (electronic supplementary material, movies S2 and S3). We discovered an intriguing mitotic reset behaviour pattern of YAP during this transition through closer inspection and tracking cells from the movies.

When an 8-cell blastomere divides to form two daughter cells—16-cell blastomeres—both always show nuclear localization of YAP, regardless of the direction of the cell division axis (figure 2a). It took roughly 100–300 min for the two daughter cells to adopt their final position in the embryo (figure 2b). The final position that a cell adopted determined its YAP distribution—when a cell adopted an outside position, it presented nuclear YAP whereas when a cell adopted an inside position, it presented cytoplasmic YAP (examples in figure 2a and quantifications in Extended figure 2b–c). There was an almost equal chance for two 16-cell blastomeres from a single 8-cell blastomere to adopt one of the two relative positions—outside-inside or outside-inside (Extended figure 2d).

Previous reports have suggested that, rather than oriented cell division, a significant proportion of inside cells at the 16-cell stage are internalized by cell movement, or asymmetrical constriction of the apical domains [25,32]. Both of these scenarios are consistent with our observations. However, because we did not include a reporter of the apical domain, our imaging studies cannot distinguish these two scenarios and further studies will be needed. The YAP-emiRFP670 reporter allowed us to relate this dynamic cell movement to a dynamic regulation of YAP signalling. Interestingly, the movie of embryos treated with ROCK inhibitor (electronic supplementary material, movies S1 and S5 and figure 2h) showed that although ROCK inhibition eventually inhibited YAP nuclear localization in all 16-cell blastomeres, the initial YAP nuclear localization right after cell division was not affected, suggesting a differential involvement of ROCK-related processes—such as polarity and mechanical tension—in regulating these two distinct YAP nuclear localization processes. How this dynamic behaviour is controlled remains an open question, which can now be addressed by live imaging the YAP reporter model in combination with additional KI reporters tracking polarity and mechanical tension.

Preimplantation embryos are small and transparent. To demonstrate the broader application of the YAP reporter in more challenging samples, we live imaged the YAP-emiRFP670 embryos at E8.0 (before turning; electronic supplementary material, movie S6), E8.5 (after turning, electronic supplementary material, movie S7) and E9.5 (electronic supplementary material, movie S8) using light sheet imaging technology. We achieved high-resolution imaging of multiple tissues including those located in deep tissue layers (up to 200 μm), such as the heart tube (figure 3 and Z-stack in electronic supplementary material, movie S9). In most regions, tissues consisted primarily of cells...
with cytoplasmic YAP, with only small populations of cells with strong nuclear signals (figure 3b–d, examples marked by arrows). In the heart tube, on the other hand, most cells showed nuclear YAP, which may be consistent with the mechanical load these cells are subjected to and suggests a crucial role for YAP in heart development [33,34]. All the raw imaging data of the sub-cellular distribution of YAP in post-implantation embryos will be deposited on open access databases and will serve as a rich resource for studying YAP signalling in mouse embryos.

To reveal the dynamic behaviour of cells with active YAP signalling, we time lapse imaged E8.5 embryos every 5 min.
for 3 h (electronic supplementary material, movie S10). This movie revealed a population of cells with strong nuclear YAP signal migrating within the head region (figure 4a and electronic supplementary material, movie S10). We conducted tracking of the time-dependent motion of these nuclear YAP cells [16]. The persistence of these cellular...
The YAP-emiRFP670 mouse line will open up broad opportunities in biomedical research. For example, existing studies in early embryos suggest that at the very early totipotent stages of development (zygote to pre-compaction 8-cell stage), YAP primarily serves to open up the zygotic genome [35,36], and then it transforms to a lineage determinant around the 16-cell stage [7]. We conducted a time lapse imaging series with YAP-emiRFP670 and a trophectoderm reporter Cdx2-eGFP (electronic supplementary material, movies S11–S13). The movie revealed very little correlation between the YAP nuclear localization and Cdx2 expression up to the late 16-cell stage. In addition, although ROCK inhibitor treatment caused the cytoplasmic localization of YAP at late 16-cell stage, the Cdx2-eGFP persisted (electronic supplementary material, movies S11–S13). The movie S13 is an example of YAP relocating from the cytoplasm to the nucleus during 3 h time-lapse imaging (Methods). Grey colour marks the standard error of mean.

3.1. Open up

The YAP-emiRFP670 mouse line will open up broad opportunities in biomedical research. For example, existing studies in early embryos suggest that at the very early totipotent stages of development (zygote to pre-compaction 8-cell stage), YAP primarily serves to open up the zygotic genome [35,36], and then it transforms to a lineage determinant around the 16-cell stage [7]. We conducted a time lapse imaging series with YAP-emiRFP670 and a trophectoderm reporter Cdx2-eGFP (electronic supplementary material, movies S11–S13). The movie revealed very little correlation between the YAP nuclear localization and Cdx2 expression up to the late 16-cell stage. In addition, although ROCK inhibitor treatment caused the cytoplasmic localization of YAP at late 16-cell stage, the Cdx2-eGFP persisted (electronic supplementary material, movie S14). These data suggested a more complex relationship between the YAP nuclear activity and the expression of TE markers such as CDX2. Live imaging could help define the precise timeline over which YAP acts as a lineage determinant and lead to further understanding of the transition of YAP functions in early embryos. In addition, the deep imaging capability provided by this reporter can illuminate previously unknown YAP activity status in embryos or other three-dimensional model systems such as organoids. Our validation of a knock-in fusion design that maintains the normal functions of endogenous YAP can also serve as the ground-plan for developing other powerful genetic tools such as degrons and optogenetic tools for further functional interrogation of YAP signalling in vivo.

In summary, we present the first KI fusion reporter mouse model to enable the readout of YAP nuclear–cytoplasmic localization by live imaging. Using this line, we reveal new aspects of dynamic YAP behaviour in preimplantation mouse embryos and the capacity to live-image post-implantation embryos with penetration at depths of up to 200 µm. This live imaging YAP reporter can be combined with other appropriate reporters to study multiple developmental and disease processes.

Data accessibility. The raw data that support the findings of this study have been uploaded to: https://figshare.com/articles/dataset/Yap_live_imaging/19319885.

Authors’ contributions. B.G.: conceptualization, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; B.B.: investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; M.Z.: investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; Y.S.: funding acquisition, investigation, supervision; S.H.: funding acquisition, investigation, supervision; J.R.: conceptualization, funding acquisition, investigation, project administration, supervision, writing—original draft, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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