

1 **Maternal expression of the novel centrosome assembly factor Wdr8**
2 **is required for vertebrate embryonic mitosis and development**

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10

11 **Abstract**

12 **The assembly of the first centrosome occurs upon fertilisation when the male**
13 **centrioles recruit pericentriolar material (PCM) from the egg cytoplasm. While**
14 **inaccuracy in the assembly of centrosomes leads to infertility and abnormal**
15 **embryogenesis, the mechanism that ensures accurate assembly in vertebrate**
16 **embryos remains obscure. Here we applied a CRISPR-Cas9-mediated gene**
17 **knockout to show that Wdr8, a novel centrosomal protein, is maternally essential**
18 **for PCM assembly during embryonic mitoses of medaka (*Oryzias latipes*).**
19 **Maternal/zygotic Wdr8-null (*Wdr8*^{-/-}) blastomeres exhibit severe defects in PCM**
20 **assembly that cause them to divide asymmetrically and develop multipolar**
21 **mitotic spindles and aneuploidy. We demonstrate that Wdr8 interacts via its**
22 **WD40 domains with the centriolar satellite protein SSX2IP. Strikingly,**
23 **exogenously provided Wdr8 fully rescues *Wdr8*^{-/-} embryos to adulthood, except**
24 **in variants with mutations in the WD40 domains. This combination of targeted**
25 **gene inactivation and *in vivo* reconstitution of the maternally essential Wdr8-**

26 **SSX2IP complex reveals an essential link between maternal PCM and the**
27 **stability of the zygotic genome in the early vertebrate embryo.**

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29

30 **Introduction**

31 The animal microtubule organising centre (MTOC), or centrosome, comprises a pair
32 of centrioles embedded in pericentriolar material (PCM)¹⁻⁴. The centrosome
33 duplicates during every cell cycle to define the two MTOCs of the bipolar mitotic
34 spindle^{2,3}. An abnormal assembly or number of centrosomes can cause cell division
35 failure, genomic instability and tumorigenesis in somatic cells⁵⁻⁷. Their accurate
36 assembly, on the other hand, governs the progression of early cleavages in animal
37 zygotes. Most animal oocytes eliminate centrosomes during oogenesis, supposedly to
38 avoid centriole aging, parthenogenesis or abnormal embryonic mitoses with an excess
39 number of centrosomes^{3,8,9}. The developing oocytes accumulate maternal
40 centrosomal factors to establish the initial zygotic centrosome upon fertilisation by
41 assembling PCM around a paternally introduced centriole^{8,9}. This serves as a
42 template for the numerous centrosomes that are created during subsequent cleavages.
43 Ultimately, therefore, the success of fertilisation and embryonic development depends
44 on the accurate assembly of the initial centrosome from paternal and maternal
45 material during fertilisation⁹.

46 In somatic cells in culture, bipolar mitotic spindle and metaphase progression can
47 occur normally in the absence of the centrosome^{10,11}. In contrast, it has been
48 suggested that centrosomes in the *Drosophila*, *C.elegans* and sea urchin zygotes are
49 essential for embryonic mitoses (i.e. cleavages)¹²⁻¹⁶. For example in *Drosophila*, a
50 PCM protein Spd-2 and a centriolar protein Sas-4 appears to be largely dispensable

51 for *somatic* mitosis in cultured *Drosophila* cells and later development (after
52 midblastula transition) respectively, but is essential for embryonic mitoses via its
53 centrosome assembly functions^{15, 16, 17}. Therefore, embryonic centrosome assembly
54 seems to require a special form of regulation involving specific maternal factors, but
55 an analysis of the mechanisms and factors involved in this process have just begun. In
56 contrast, surprisingly, such crucial role of the centrosome controlled by maternal
57 centrosome factors in vertebrate embryos has been poorly understood. Undoubtedly,
58 there is a compelling need to reveal the centrosomes' physiological roles in vertebrate
59 embryogenesis to provide insights into development and infertility mechanisms in
60 humans. However, it is still elusive partly due to the difficulty of peeling apart the
61 distinct functions of maternal and zygotic factors using the knock out methods at hand.

62 However, a few zebrafish mutants have served as a basis for examining the
63 physiological functions of maternal centrosomal factors in vertebrates¹⁸⁻²⁰. The
64 *cellular atoll* (*cea*) embryo, a maternal-effect Sas-6 mutant, exhibits a failure of
65 centrosome duplication, and has thus revealed functions of centrosomal factors that
66 have been conserved in human somatic cells and teleost embryos¹⁹. The lymphoid-
67 restricted membrane protein (*lrmp*) mutant *fruitless* displays a failure of the attachment
68 of centrosomes to pronuclei immediately after fertilisation, again demonstrating a
69 maternal-specific function²⁰. But many novel factors have scarcely been investigated
70 in vertebrates, making it impossible to model the molecular networks required for
71 centrosome assembly during the initial stages of embryogenesis and later
72 development.

73 To gain molecular insights into the role of maternal-specific centrosomal factors in
74 centrosome assembly *in vivo*, we used the vertebrate model medaka (*Oryzias*
75 *latipes*)²¹. We analysed an uncharacterized WD40 repeat containing protein, Wdr8,

76 (the orthologue of human WRAP73) that we recently identified in a screen for
77 maternal proteins²². We applied CRISPR-Cas9-mediated targeted gene inactivation,
78 and elicited specific centrosome assembly defects in the absence of maternal and
79 zygotic Wdr8. This allowed us to unravel an essential role for Wdr8 in maternal PCM
80 assembly. Subsequently we used mRNA injection, which mimics maternal gene
81 expression, and observed a remarkable reconstitution of centrosome assembly, proper
82 cell divisions and gross development until adulthood. This *in vivo* reconstitution
83 strategy of maternal Wdr8 functions allowed us to perform a straightforward
84 screening of mutant variants that revealed domains/modules of the Wdr8 protein
85 essential for PCM assembly in living vertebrate embryos. Overall, this system clearly
86 delivered molecular insights into Wdr8's essential function in embryonic centrosome
87 assembly.

88

89 **Maternal but not paternal Wdr8 is essential for symmetric cleavages of Medaka** 90 **embryos**

91 In a recent study we identified a number of proteins that were specifically upregulated
92 in *Xenopus laevis* oocytes. Alongside the centrosome assembly factor SSX2IP²², our
93 screen revealed an upregulation of Wdr8, a previously uncharacterized WD40 repeat-
94 containing protein. Particularly interesting was the observation that the two proteins
95 interacted with each other in *Xenopus* egg extracts (OJG, unpublished data).

96 To analyse Wdr8's functions in early vertebrate development, we took advantage
97 of the transparency of medaka embryos to carry out a cell biological analysis
98 combined with a CRISPR-Cas9 genome editing approach to target and inactivate
99 medaka Wdr8 (OIWdr8, hereafter referred to as Wdr8). This inactivation was
100 achieved through the targeted integration of a GFP-stop cassette into exon-3 of the

101 Wdr8 locus (Extended Data Fig. 1a, b). A cross of heterozygous parents yielded
102 Wdr8^{-/-} homozygous offspring that showed no obvious abnormalities during
103 development and were phenotypically undistinguishable from wild-type fish through
104 all stages to adulthood (Extended Data Fig. 2). However, when we compared the four
105 combinations resulting from a cross of Wdr8^{-/-} and wild-type fish (Fig. 1a), we found
106 a severe phenotype in all embryos from homozygous mothers. All maternal/zygotic
107 Wdr8^{-/-} mutants (m/zWdr8^{-/-}) exhibited cleavage cycles that were significantly
108 delayed (by 20-30 min) following the first cleavage (Fig. 1b). The second division
109 and the following cleavage cycles revealed abnormal asymmetric cleavages with a
110 failure of cytokinesis (Fig. 1a), which were sometimes observed during the first
111 division (Supplementary information (SI) Movie 3). In the absence of maternally
112 provided Wdr8, these embryos failed to gastrulate or reach the neurula stage (St.18)²³
113 (Fig. 1a). In contrast, the development of all embryos from homozygous fathers was
114 indistinguishable from that wild-type embryos (Fig. 1a and Extended Data Fig. 3).
115 These results demonstrate that maternally provided Wdr8 is essential for faithful
116 cleavages in the large blastomeres of the early fish embryo.

117

118 **Exogenous expression of Wdr8 can efficiently rescue Wdr8^{-/-} zygotes**

119 Given these indications that the presence of maternal Wdr8 during cleavage divisions
120 suffices for the progression of normal development, we next performed rescue
121 experiments in the maternal/zygotic mutants (Wdr8^{-/-}). We injected mRNA encoding
122 an EYFP-fusion of the human WDR8/WRAP73 orthologue (here called EYFP-
123 huWdr8) into m/zWdr8^{-/-} zygotes within 5-10 min post fertilisation (mpf). At just 60
124 min after injection (about 70 mpf), functional proteins were detected from both the
125 control mRNA, encoding EGFP alone, and EYFP-huWdr8. This was the point at

126 which embryos underwent their first cleavage. While all the $Wdr8^{-/-}$ embryos
127 injected with EGFP showed abortive development, EYFP-huWdr8 expression fully
128 rescued the cleavages and over 90 % of the injected eggs progressed through
129 gastrulation to subsequent developmental stages (Fig. 1c, d). Intriguingly, about 70%
130 of these embryos developed into juveniles indistinguishable from wild-type fish based
131 on gross morphology (data not shown). These results not only confirmed the
132 specificity of the knockout phenotype, but also clearly demonstrated that maternal
133 Wdr8 plays an essential role in early development by maintaining the integrity of
134 embryonic cleavages.

135

136 **Centrosomal localisation of Wdr8 in rescued $Wdr8^{-/-}$ zygotes**

137 The fluorescent tag of the rescue construct allowed us to determine the localisation of
138 EYFP-huWdr8 in living blastomeres. *In vivo* microscopy showed that EYFP-huWdr8
139 localised specifically to one or two distinct dot-like domains in each blastomere of the
140 rescued embryos (Fig. 2a, b). During the cleavage cycles from the one- to the four-
141 cell stage, the two domains remained adjacent to each other, then separated and
142 migrated to opposing sides of the blastomeres during mitosis (Fig. 2a and SI Movie 1).
143 At the end of mitosis (before the emergence of the cleavage furrow), the Wdr8
144 domains were more dispersed (Fig. 2a and SI Movie 1). This localisation was
145 strikingly reminiscent of the pattern that centrosomes follow during the cell cycle as
146 they separate to form mitotic spindle. Strikingly, EYFP-huWdr8 co-localised with γ -
147 tubulin as well as the centriolar satellite (CS)-marker PCM1^{24,25} at both prophase and
148 metaphase, demonstrating that Wdr8 is a novel maternal centrosomal and CS factor in
149 medaka embryos (Fig. 2c). In controls (EGFP-expressing wild-type and m/z $Wdr8^{-/-}$
150 blastomeres), the EGFP signal was found in the cytoplasm, confirming that the

151 centrosomal localisation of the EYFP-huWdr8 signal is due to the Wdr8 sequence (SI
152 Movies 1, 2, and 3). Taken together, our results indicate that Wdr8 acts as a
153 centrosomal organiser in embryonic mitoses.

154

155 **Wdr8 is essential for PCM assembly during rapid embryonic mitoses.**

156 The centrosomal localisation of γ -tubulin and PCM1 in the embryos rescued by
157 EYFP-huWdr8 expression was comparable to that observed in wild-type embryos
158 (Fig. 2d). This indicates that EYFP-huWdr8 successfully rescues the phenotype of
159 Wdr8^{-/-} embryos by restoring the function of embryonic MTOCs. We therefore asked
160 if MTOC assembly is affected in m/zWdr8^{-/-} blastomeres. At prophase and metaphase
161 in these blastomeres, γ -tubulin and PCM1 were severely scattered compared to the
162 wild-type, sometimes forming multiple PCM foci that were unevenly fragmented (Fig.
163 3a). Furthermore, while bipolar mitotic spindles readily formed in wild-type
164 blastomeres, the scattered PCM of m/zWdr8^{-/-} blastomeres induced multipolar spindle
165 assembly from prophase to telophase (Fig. 3b and Extended Data Fig. 4a, b).
166 Importantly, these centrosomal and spindle abnormalities led to severe chromosomal
167 instability (Fig. 3a, b and Extended Data Fig. 4a, b). In contrast, blastomeres
168 expressing exogenous EYFP-huWdr8 recovered from all three defects: abnormal
169 PCM assembly, multipolar spindle formation and aneuploidy (Fig. 2c and Fig. 3b).
170 These results demonstrate that maternal Wdr8 ensures faithful PCM assembly and is
171 essential for the maintenance of functional MTOCs in embryonic mitosis.

172

173 **The WD40 domains of Wdr8 are essential for both localisation and function of**

174 **Wdr8**

175 The fact that exogenously provided Wdr8 mRNA effects a highly efficient rescue
176 during early cleavage stages provides a clear, systematic approach to the
177 identification of interaction partners and the domains of Wdr8 that permit them to
178 bind. Wdr8 is conserved across species from yeast to humans, and vertebrates exhibit
179 four WD40 domains (Fig. 4a). WD40 domains are often involved in protein-protein
180 interactions²⁶, suggesting that they might play an important role in Wdr8's function.
181 We selected amino acids in the WD40 domains of Wdr8 that were conserved across
182 species and mutated either W196D197 (WD40_1) or W359D360 (WD40_3) by
183 Alanine (hereafter each mutant variant called 196/197AA and 359/360AA, Fig. 4a).
184 To assess the functional relevance of the WD40 domain, we performed a rescue assay
185 by injecting mRNA into *m/zWdr8^{-/-}* zygotes. mRNAs of all the variants (EYFP-
186 huWdr8 wild-type, EYFP-196/197AA, or EYFP-359/360AA) were expressed at
187 similar levels (Extended Data Fig. 5 and 359/360AA, data not shown). In contrast to
188 the wild-type construct, Wdr8 lost its centrosomal localisation in both WD mutant
189 variants and was mainly detected in the cytoplasm (Fig. 4b).

190 When blastomeres entered mitosis, a weak but visible centrosomal localisation of
191 the mutant variants was observed in live imaging of either EYFP-196/197AA- or
192 EYFP-359/360AA-injected embryos (Fig. 4c, SI Movie 4, time points at 08:11, 53:12,
193 and 98:12). Individual blastomeres exhibited multiple foci, but they disappeared
194 during the subsequent cleavage cycles (Fig. 4c, SI Movie 4). Interestingly, in
195 *m/zWdr8^{-/-}* blastomeres, both WD variants were ubiquitously dispersed throughout the
196 cytoplasm and slightly co-localised with scattered γ -tubulin and PCM1 at prophase
197 (Fig. 4d). This indicates that WD variants are hypomorphic forms of Wdr8 that
198 inefficiently localise to the centrosome, and demonstrates that the WD40 domain is
199 essential for proper PCM/CS assembly.

200 Importantly, abnormal PCM/CS assembly led to multipolar spindles as well as
201 chromosomal instability (Fig. 4e). This was consistent with the finding that neither of
202 the WD mutant variants was able to rescue the early $m/zWdr8^{-/-}$ phenotypes or their
203 subsequent abortive development (Fig. 4b). In contrast, the mutation of four
204 conserved putative Cdk1-phosphorylation motifs (mutations substituting AP for SP, a
205 site S232 lies in WD40_2)²⁷, affected neither the localisation nor the rescue function
206 of Wdr8 in $m/zWdr8^{-/-}$ embryos (data not shown). These results clearly demonstrate
207 that intact WD40 domains govern the centrosomal localisation of Wdr8 and are
208 required for proper PCM/CS functions in early embryonic mitoses of cleavage stages.
209

210 **The WD40 domains of the Wdr8-SSX2IP complex are essential specifically for**
211 **its localisation and functions**

212 We next took advantage of the mRNA-based *in vivo* reconstitution assay to address
213 the molecular mechanisms underlying Wdr8's function. We hypothesised that WD40
214 domains serve either as centrosome-targeting domains or as modules for interactions
215 with other proteins. We first tested whether the rescue of $m/zWdr8^{-/-}$ zygotes by WD
216 variants was achieved by targeting them to the centrosome. We used the centrosome-
217 targeting motif PACT to facilitate the accumulation of WD mutant variants at the
218 centrosome²⁸. Both PACT fusion constructs weakly localised to the centrosome in
219 some embryos (Fig. 5a, PACT-359/360AA, data not shown). In these embryos,
220 however, the embryonic lethality introduced by Wdr8 knockout was not rescued (1dpf
221 in Fig. 5a, PACT-359/360AA, data not shown). This failure – even when Wdr8 was
222 localised to the centrosome – strongly suggests that the WD40 domains of Wdr8 are
223 not simply centrosome-targeting domains.

224 Next we tested whether WD40 domains are important for interactions with other
225 proteins. Since Wdr8 had initially been identified as an interaction partner of the CS
226 protein SSX2IP, we hypothesised that WD40 domains might be critical for Wdr8's
227 interaction with SSX2IP or other centrosomal or CS proteins. To retrieve amounts of
228 the proteins sufficient to analyse protein-protein interactions, we carried out a
229 biochemical analysis of *Xenopus laevis* egg extracts. Following the expression of
230 either 2xFlag-huWdr8 wild-type (WT), 2xFlag-196/197AA (196/197AA) or 2xFlag-
231 359/360AA (359/360AA) in cytostatic factor (CSF) egg extracts arrested at
232 metaphase²⁹, we performed Flag immunoprecipitations to analyse the proteins that co-
233 precipitated. Strikingly, mass spectrometry analysis revealed that SSX2IP interacted
234 with WT, whereas it was unable to interact with either 196/197AA or 359/360AA
235 (Fig. 5b), clearly demonstrating that WD40 domains are essential for the Wdr8-
236 SSX2IP interactions.

237 Intriguingly, although γ -tubulin was severely scattered in Wdr8^{-/-} blastomeres, γ -
238 tubulin and all the γ -TuRC subunits (including its regulatory protein Nedd1)³⁰⁻³²
239 interacted equivalently with WT and WD variants, indicating that WD40 domains are
240 not required for Wdr8- γ -TuRC/Nedd1 interactions (Fig. 5b). Consistent with this
241 finding, Western blot analysis confirmed that only WT, but not WD mutant variants,
242 interacted with SSX2IP (Fig. 5c). On the other hand, γ -tubulin's interaction with Wdr8
243 was independent of WD40 domains (Fig. 5c). Taken together, these results revealed
244 that WD40 domains are the basis of the complex formed by Wdr8 with SSX2IP, but
245 its interactions with γ -TuRC are independent of the domains. In medaka blastomeres,
246 Wdr8 wild-type almost completely co-localised with SSX2IP, strongly suggesting
247 that Wdr8 is a CS protein which forms a complex with SSX2IP (Fig. 5d). In contrast,
248 SSX2IP was remarkably dispersed in the cytoplasm in both 196/197AA- and

249 359/360AA-expressing blastomeres (Fig. 5d, 359/360AA, data not shown), indicating
250 that the interaction between Wdr8 and SSX2IP is crucial for mutual localisation and
251 the function of Wdr8-SSX2IP as a CS protein complex (Fig. 5d, e).

252 In summary, these results clearly demonstrate that the Wdr8-SSX2IP complex is
253 maternally essential for PCM assembly to ensure rapid embryonic mitoses.

254

255 **Discussion**

256 Although it has long been suggested that centrosomes in vertebrate is absolutely
257 essential for embryonic mitosis, the ultimate impact of centrosomes on vertebrate
258 embryonic development and its molecular mechanism has been ill-defined. In this
259 study, we for the first time reveal the detailed molecular mechanism of centrosome
260 assembly specifically regulated by the novel maternal centrosomal protein Wdr8
261 during medaka embryonic mitoses (Fig. 5e). Centrosomes regulated by maternal
262 Wdr8 are absolutely essential for mitotic spindle bipolarity and accurate inheritance
263 of the zygote genome, which is distinct from a certain dispensability of centrosomes
264 in somatic cells (Fig. 5e). Therefore, we here present a first major step toward solving
265 the long-standing problems of physiological significance of centrosomes in early
266 vertebrate development.

267 Several studies in vertebrate embryos have explored the regulation of the
268 centrosome during embryonic mitoses^{18-20, 33, 34}. A few zebrafish maternal-effect
269 mutants, such as *cea* (Sas-6) or *futile* (Lrmp), have revealed functions in centrosomal
270 regulation in the embryo^{19, 20}. However, these studies failed to reveal further
271 molecular insights due to the lack of precise gene targeting and the immense efforts of
272 forward genetic screens for maternal genes in vertebrate models. Our CRISPR-Cas9
273 knockout medaka line clearly segregates the distinct functional contributions of

274 maternal and zygotic Wdr8 during development. We show that early lethality of
275 Wdr8^{-/-} embryos can be rescued with high efficiency either through maternal effects
276 or the exogenous expression of a functional Wdr8 mRNA, which mimics the presence
277 of a corresponding maternal mRNA. Strikingly, the rescued animals exhibited normal
278 development and grew to become fertile adults. This reliable “reconstitution” rescue
279 system, based on wild-type Wdr8 or several mutant variants, provides an
280 unambiguous readout that directly links the molecular features of Wdr8 structure to
281 its function and interactions with SSX2IP. This indicates that reverse genetic
282 approaches with the CRISPR-Cas9 system are ideally suited to dissect maternal- and
283 zygotic-specific aspects of centrosome regulation, which have been lacking for
284 vertebrate systems.

285 Very recently, Wdr8 was shown to form a ternary complex with SSX2IP and the
286 minus-end-directed kinesin Pkl1 (kinesin-14 homologue), which maintains minus-end
287 pulling forces of microtubules (MTs) in fission yeast³⁵. The SSX2IP-Wdr8-Pkl1
288 ternary complex itself is required for its localisation/function in the spindle pole body
289 (SPB)³⁵. The yeast complex provides a model case in which Wdr8-SSX2IP
290 contributes to the generation of a pulling force by the MTs at the SPB, possibly by
291 capping γ -tubulin^{35, 36}. However, the question of whether Wdr8's functions have been
292 conserved through evolution has yet to be addressed. In cell culture, a Wdr8 partner,
293 SSX2IP is shown to be required in CS for the recruitment of γ -tubulin ring complexes
294 (γ -TuRCs) and other specific components into the PCM for the assembly of expanded
295 mitotic PCM^{22, 37}. While SSX2IP is not absolutely essential for the mitotic spindle
296 bipolarity and metaphase progression in cultured cells^{22, 37}, its function originally
297 identified as maternal protein from *Xenopus* eggs has not been addressed. By
298 unveiling the exclusive function of maternal Wdr8, our data show that Wdr8 interacts

299 with SSX2IP via its WD40 domains and that this complex is essential for their mutual
300 centrosome localisation/function in medaka embryonic mitosis. Although mass
301 spectrometry analysis in our experiments could not identify kinesin-14 in either
302 SSX2IP- or Wdr8-IP samples, the localisation of SSX2IP is dependent on dynein at
303 least in *Xenopus* egg extracts²². Therefore, a complex of Wdr8-SSX2IP bound to
304 dynein as a minus-end-directed motor could be a highly conserved molecular module
305 whose function is crucial to ensure proper PCM assembly and maintain its structure
306 during embryonic mitosis. Since SSX2IP is exclusive to CS^{22, 37}, Wdr8-SSX2IP might
307 be first recognised as a cargo by dynein at the CS granule to transport the complex,
308 together with other PCM components, to the centrosome (Fig. 5e). The formation of
309 the Wdr8-SSX2IP complex via WD40 domains may therefore be a critical step in the
310 assembly of PCM components and the initiation of centrosome maturation (Fig. 5e)³⁸.
311 ³⁹. Intriguingly, our mass spectrometry analysis further demonstrates that Wdr8 also
312 associates with all the γ -TuRC subunits, including its regulatory protein Nedd1, in a
313 manner independent of WD40 domains (Fig. 5b). It is plausible that Wdr8 alone could
314 serve as a platform for γ -TuRC/Nedd1 in either its transport to the centrosome or in
315 stabilising the minus-end of MT.

316 Defects of the assembly of the centrosome after fertilisation have been directly
317 linked to infertility and abnormal embryonic development in humans^{40, 41}. So far the
318 causes of infertility have mainly been attributed to the abnormalities of the sperm
319 centriole^{40, 41}. The effects of maternal centrosomal factors in oocytes have not yet
320 received much attention. Considering that genomic instability in aging eggs causes
321 infertility^{42, 43}, it is possible that the downregulation or absence of maternal
322 centrosomal factors during meiosis may contribute to infertility as well. Since Wdr8 is
323 a critical maternal factor for genomic stability as well as centrosome assembly, the

324 function of Wdr8 in relation to other interaction partners during meiosis needs to be
325 addressed. On the other hand, the rescue of Wdr8^{-/-} embryos through either maternal
326 effects or an introduction of exogenous Wdr8 expression causes them to undergo
327 normal development, which suggests that zygotic Wdr8 is dispensable for later
328 development (Extended Data Fig. 2, 3 and, Fig. 1a, c). It is possible, of course, that
329 zygotic Wdr8 does have functions in later development beyond its role in PCM
330 assembly, or their failure to produce a clear phenotype might be attributable to the
331 presence of other, redundant factors.

332

333 **Methods**

334 **Husbandry of fish and preparation of medaka zygotes.** Husbandry of fish and
335 preparation of zygotes were routinely performed as described previously⁴⁴. The
336 husbandry and all the experiments were performed according to local animal welfare
337 standards (Tierschutzgesetz §11, Abs. 1, Nr. 1, husbandry permit number 35–
338 9185.64/BH Wittbrodt and mutagenesis permit number G-206/09) and in accordance
339 with European Union animal welfare guidelines. The fish facility is under the
340 supervision of the local representative of the animal welfare agency.

341 **Construction of CRISPR/Cas9 materials and generation of Wdr8 knockout**

342 **medaka line.** With CCTop (<http://crispr.cos.uni-heidelberg.de>)⁴⁵, a sgRNA was
343 chosen to target exon-3 of *Oryzias latipes* Wdr8 (OIWdr8, NCBI reference sequence:
344 XM_004070359.2) with least potential off-target sites in the remainder of the genome
345 (Extended Data Table 1). Synthetic sgRNA oligos were annealed and cloned into
346 pDR274 (addgene, #42250). Wdr8-sgRNA forward (5'-
347 TAGGTCTCTCGAGCAGCCGGAC-3') Wdr8-sgRNA reverse (5'-
348 AAACGTCCGGCTGCTCGAGAGA-3'). The donor plasmid was created as

349 described previously^{45, 46} via Golden GATEway cloning comprising the following
350 sequences: 1) a specific sgRNA target site derived from GFP for *in vivo* linearisation
351 of the donor vector (cf sgRNA-1 in ⁴⁵), 2) a 665 bp homology flank corresponding to
352 the upstream genomic sequence of the Wdr8-sgRNA target site, 3) a *GFP* variant
353 (*GFP^{var}*)⁴⁶ that was silently mutated to prevent CRISPR/Cas9-mediated cleavage by
354 the donor-linearising sgRNA (see ⁴⁵), 4) followed by a triple polyadenylation signal.
355 The homology flank was cloned from Cab genomic DNA with primers: Forward (5'-
356 GCCGGATCCATGGTCCTCAGACTCCCTGT-3'), Reverse (5'-
357 GCCGGTACCCGGCTGCTCGAGTGACCACACCT-3') to facilitate cloning into
358 the Golden GATEway entry vector, the forward and reverse primers were extended
359 with a BamHI or KpnI restriction site, respectively. To knock out OIWdr8, *GFP^{var}*
360 with stop codon was inserted by homology directed repair (HDR) to generate
361 nonsense mutation, which resulted in the C-terminal deletion of roughly 500 amino
362 acids, i.e. deletion of >75% of full-length OIWdr8. One-cell stage Medaka zygotes
363 were co-injected with 10 ng/μl of donor plasmid, 15 ng/μl per sgRNA (Wdr8-sgRNA
364 and sgRNA-1⁴⁵), 150 ng/μl Cas9 mRNA diluted in nuclease free water. Founders
365 were screened by genotype PCR (Extended Data Fig. 1b) with FinClip protocol⁴⁷ since
366 *GFP^{var}* expression could hardly be detected, possibly due to impaired protein folding
367 of *GFP* fused with N-terminal short fragment of OIWdr8. To achieve reproducible
368 genotype PCR results, the program and the primer sets for the screening were fixed as
369 below. The primers: for wild-type locus, forward (5'-
370 AGTGTTCAAGCAGTCCAACCA-3') and reverse (5'-
371 TGAGGAGACTAGTCCAATTGAGC-3'), for *GFP^{var}* insertion, forward (5'-
372 AGTGTTCAAGCAGTCCAACCA-3') and reverse (5'-
373 GAACTTGTGGCCGTTTACGT-3'). The genotype PCR reaction with Taq

374 polymerase (NEB): 1. 95°C for 1min, 2. 95 °C for 30s, 3. 65 °C for 30s, 4. 72 °C for
375 40s, repeated the cycle from step 2, 30 cycles. Heterozygous F1 fish were crossed
376 with Cab. Heterozygous F2 fish were crossed with each other to generate F3
377 homozygous fish, which developed normally and grew to viable and fertile adults. By
378 crossing F3 homozygous parents, the maternal effect of Wdr8^{-/-} homozygous zygotes
379 (F4) was obtained to analyse the function of maternal Wdr8.

380 **Whole mount *in situ* hybridisation with medaka embryos.** For whole mount *in situ*
381 hybridisation (WISH), a full-length OIWdr8 cDNA was isolated from cDNA library
382 of wild-type embryos (St. 32)²³, and then cloned into pGEM-T easy vector (Promega)
383 to generate the antisense and the sense RNA probes. WISH was performed as
384 described previously⁴⁴. The pictures were taken under Nikon SMZ18 binocular
385 microscope with the NIS-Elements F4.00.00 imaging software (Nikon).

386 **Preparation of cDNAs, mRNAs, and sgRNAs.** EYFP-huWdr8, 2xFlag-huWdr8, and
387 their mutant variants were cloned into pCS2⁺ for mRNA generation. WD40 mutant
388 variants, 196/197AA and 359/360AA, were generated by mutation PCR. To create
389 PACT fusion construct, PACT domain (kindly provided by Elmar Schiebel, Zentrum
390 für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany)
391 was cloned and N-terminally fused immediately after a WD mutant variant by fusion
392 PCR. mRNAs were transcribed with SP6 mMessage mMachine kit (Life
393 technologies). sgRNAs were transcribed with mMessage mMachine T7 Ultra kit (Life
394 technologies).

395 **Whole mount fluorescent immunostainings with medaka blastomeres.** Whole
396 mount fluorescent immunostainings were performed with 4hpf medaka blastomeres
397 (St. 8) as described previously⁴⁴, except for omission of the heating step, with anti- γ -
398 tubulin (1:200 dilution, Sigma-Aldrich, T6557), anti-PCM1 (rabbit, 1:300 dilution, a

399 gift from A. Merdes, Université de Toulouse, Toulouse, France), anti- α -tubulin (1:100
400 dilution, Sigma-Aldrich, T9026), and anti-GFP (1:300 dilution, Life technologies,
401 A10262) antibodies. Secondary antibodies were Alexa Fluor 488 anti-mouse IgG
402 (Life technologies), Alexa Fluor 488 anti-chicken IgY (Life technologies), Alexa
403 Fluor 546 goat anti-mouse IgG (Life technologies), Dylight 549 goat anti-rabbit IgG
404 (Jackson ImmunoResearch), and Alexa Fluor 647 goat anti-rabbit IgG (Life
405 technologies). All secondary antibodies were incubated at 1:200 dilutions. DNA was
406 counterstained with DAPI (1:200 dilution from 5mg/ml stock, Sigma-Aldrich, D9564).
407 Images were taken with the confocal microscopy (Leica TCS SPE) with either 20x
408 water (Leica ACS APO 20x/0.60 IMM CORR) or 40x oil (Leica ACS APO 40x/1.15
409 Oil CS 0.17/E, 0.27) objectives and processed with Image J. For SSX2IP detection,
410 100 ng/ μ l anti-xlSSX2IP antibody was injected into one-cell stage of medaka zygotes,
411 and then subjected to immunostaining as described previously²².

412 **Live imaging of medaka embryos.** Fluorescence in injected medaka embryos was
413 checked at 70-90 min post injection. The embryo with chorion was incubated in 3%
414 methyl cellulose/1xERM with the glass bottom dishes (MaTeck corporation). Live
415 imaging was performed by Leica SPE confocal microscope with 20x water objective.
416 Images were taken every 37s-49s (Movie 1-4) with a 2-3 μ m- z-step size. Maximum
417 z-stack projection images were processed with Image J and then converted to Quick
418 Time movie files.

419 **Rescue experiments by injection of either EYFP-huWdr8 mRNA or its**
420 **individual mutant variants' mRNA.** Wdr8^{-/-} fertilised eggs were immediately
421 collected after checking mating, and injected with mRNA within 5-10 min post
422 fertilisation in the precooled 0.5x ERM medium. 100 ng/ μ l of mRNA of either EYFP-
423 huWdr8 or its individual mutant variants was injected into Wdr8^{-/-} zygotes at about

424 1/3 volume of one-cell stage. These zygotes were incubated in 0.5x ERM at 28 °C.
425 Embryos at 4 hpf (St. 8) were fixed with 4% PFA in 1x PTw to perform whole mount
426 fluorescent immunostainings. Images of external phenotypes of live embryos were
427 taken under Nikon SMZ18 binocular microscope with the NIS-Elements F4.00.00
428 imaging software (Nikon).

429 **Western blot analysis of medaka embryos at morula stage.** 400 ng/ μ l of mRNA
430 encoding either EYFP-huWdr8 or WD mutant variant was injected into one-cell stage
431 zygotes. The embryos were incubated for 28 °C for 2h, and then treated with hatching
432 enzyme for another 2h to remove chorion. 50 embryos (around St. 8) for each were
433 softly homogenised with 100 μ l cold PBS to remove the yolk. After centrifugation at
434 3,000 rpm for 2 min at 4 °C, the supernatant was removed and the pellet of embryos
435 was frozen at liq N₂ and stored at -80 °C. The pellet was lysed on ice with 25 μ l RIPA
436 buffer (50 mM Tris-HCl at pH 8.0, 150 mM NaCl, 5 mM EDTA, 15 mM MgCl₂, 1%
437 Triton-X100) containing 10 μ M pepstatin, 10 μ g/ml aprotinin, 0.1 mM PMSF, 1 mM
438 Na₃VO₄, and 1 mM NaF. After centrifugation at 14,000 rpm for 5 min at 4 °C, 20 μ l
439 supernatant was mixed with the equal volume of 2x Laemmli sample buffer
440 containing 10% 2-mercaptoethanol, and boiled for 10 min at 100 °C, subjected to
441 SDS-PAGE.

442 **Immunoprecipitation of 2xFlag-huWdr8 from *Xenopus* CSF egg extracts.**

443 *Xenopus* CSF egg extracts were routinely prepared as previously described⁴⁸. 80 μ l of
444 the extracts was incubated with 3 μ g mRNA of either 2xFlag-huWdr8 wild-type,
445 2xFlag-196/197AA, or 2xFlag-359/360AA for 90 min at 23 °C to express Flag-tagged
446 fusion proteins in the extracts. The immunoprecipitations were performed with 2 μ g
447 of either anti-Flag antibody (Sigma-Aldrich, F1804) or normal mouse serum
448 (Invitrogen, #1410) for 45 min at 23 °C, followed by incubation with 20 μ l of Protein

449 G Sepharose 4 Fast Flow (GE Healthcare Life Sciences) slurry (in CSF-XB) for
450 another 25 min at 23 °C by tapping every 5 min. The Protein G Sepharoses were
451 washed twice and one time with 250 µl of TBS-T (10 mM Tris-HCl at pH7.5, 0.5%
452 tween-20, 150 mM NaCl) and 250 µl of TBS (10 mM Tris-HCl at pH7.5, 150 mM
453 NaCl), respectively. After removing TBS, the samples were prepared with 30 µl of 2x
454 Laemmli sample buffer containing 10% 2-mercaptoethanol, boiled for 10 min at
455 100 °C, subjected to Western blot and mass spectrometry analyses as described
456 previously^{22, 49}.

457 **Dimethyl labeling and mass spectrometry.** For quantitative mass spectrometry
458 analysis, comparison of control IgG pull-down and 2xFlag-huWdr8 pull-down
459 samples were performed by after dimethyl-labelling using stable isotopes^{49, 50}.
460 Original mass spectrometry data were analysed using Proteome Discoverer 1.4 and
461 Mascot (Matrix Science; version 2.4) as described previously^{22, 49}. Identified proteins
462 were displayed by Scaffold_4.4.3 (Proteome Software Inc.) to retrieve significantly
463 interacted proteins with either Wdr8 wild-type or WD40 mutant variants.

464

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467 plant and yeast cells. *Int. Rev. Cytol.* **169**, 25-82 (1996).
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594

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597

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609 designed the project with critical input from J.W. D.I. performed all the experiments

610 and analysed the data. M.S. and T.T established CRISPR-Cas9 system. D.I. and O.J.G.

611 wrote the manuscript. J.W. supervised and supported D.I.

612

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617

618 **Figure legends**

619 **Figure 1 | Abnormal cleavage divisions of Wdr8^{-/-} zygotes are rescued by EYFP-**
620 **huWdr8 expression. a,** Phenotypes of paternal and maternal Wdr8^{-/-} embryos before
621 and after midblastula transition (MBT). The maternal Wdr8^{-/-} embryos, but not the
622 paternal, showed disordered cleavage divisions and failure of gastrulation. **b,** Timing
623 of cleavage cycles in Wdr8^{-/-} and wild-type specimens. Data represents median with
624 minimum to maximum. Total embryos, 10 (Cont.), 11 (Wdr8^{-/-}). **c, d,** Full rescue of
625 Wdr8^{-/-} zygotes by exogenous expression of EYFP-huWdr8 (**c**) and its efficiency at
626 St.20 (**d**). n, total embryos from three independent experiments. Data represent mean
627 ± s.e.m. Scale bars, 200 µm.

628 **Figure 2 | Wdr8 localises to the centrosome in the rescued Wdr8^{-/-} blastomeres. a,**
629 **b,** Spatiotemporal localisation of EYFP-huWdr8 in the rescued Wdr8^{-/-} zygotes.
630 Time-lapse images show stereotypic centrosome cycles from one- to 4-cell stage (**a**)
631 and 8-cell stage (**b**). Time, min. **c, d,** Wdr8 is a novel centrosome protein. EYFP-
632 huWdr8 colocalised with γ-tubulin and PCM1 (**c**), resembling the wild-type situation
633 (**d**). Scale bars, 100 µm (**a**), 200 µm (**b**), 10 µm, (**c, d**).

634 **Figure 3 | Centrosome and spindle abnormalities in Wdr8^{-/-} blastomeres.**
635 **a, b,** Abnormal PCM and mitotic spindle assemblies in Wdr8^{-/-} blastomeres. In
636 comparison with wild-type (Fig. 2d), Wdr8^{-/-} eggs exhibited severely scattered γ-
637 tubulin/PCM1 (**a**) and multipolar mitotic spindles (**b**) together with aneuploidy at
638 metaphase (**a, b**). These abnormalities were fully rescued by EYFP-huWdr8
639 expression (Fig. 2c, Fig. 3b). Scale bars, 10 µm.

640 **Figure 4 | WD40 domains are essential for Wdr8 function. a,** The conserved four
641 WD40 domains in huWdr8. Mutations for WD mutant variants are denoted in red. **b,**
642 External phenotypes of WD mutant-injected Wdr8^{-/-} embryos. Note that both variants
643 mostly localised in the cytoplasm, unable to rescue Wdr8^{-/-} embryos. **c,** Time-lapse

644 images of EYFP-196/197AA localisation in $Wdr8^{-/-}$ blastomeres. EYFP-196/197AA
645 weakly localised in the centrosome with multiple foci, which disappeared during
646 cleavages. Time, min. **d, e**, Both mutant variants expression were unable to rescue
647 abnormal PCM assembly (γ -tubulin/PCM1), multipolar mitotic spindles, and
648 aneuploidy. Scale bars, 200 μm (**b**), 100 μm (**c**), 10 μm (**d, e**).

649 **Figure 5 | WD40 domains is essential to form Wdr8-SSX2IP complex and its**
650 **localisation. a**, Centrosome targeting of EYFP-196/197AA-PACT was insufficient
651 for centrosomal localisation and the rescue of $Wdr8^{-/-}$ embryos. **b, c**, Identification of
652 Wdr8 interaction factors by mass spectrometry and immunoblot analyses with
653 *Xenopus* egg extracts. WT, but not WD mutant variants, interacted with SSX2IP (**b, c**).
654 Contrary, γ -TuRC subunits/Nedd1 (**b**) and γ -tubulin (**c**) interacted with Wdr8
655 independently of WD40 domains. *, background. **d**, Interdependent localisation of
656 Wdr8 and SSX2IP via WD40 domains. Due to failure of Wdr8-SSX2IP interaction,
657 EYFP-196/7AA, but not WT, was unable to colocalised with SSX2IP to the
658 centrosome in $Wdr8^{-/-}$ blastomeres. **e**, Wdr8-SSX2IP localises to the centrosome by
659 tethering PCM components (e.g. γ -TuRC, which is essential for maternal PCM
660 assembly). The absence of maternal Wdr8 or dysfunction of its WD40 domains
661 causes PCM assembly defects. Scale bars, 200 μm (**a**), 20 μm (**d**).

662

663 **Extended Data Figure and Supplementary information legends**

664 **Extended Data Figure 1 | CRISPR-Cas9 mediated knockout strategy to generate**
665 **$Wdr8^{-/-}$ medaka line. a**, A sgRNA was designed to knockout (KO) exon-3 of
666 OIWdr8 by inserting 5' homology flank (HF) and GFP with a stop codon (see details
667 in Methods). **b**, The screening of $Wdr8^{-/-}$ fish by genotype PCR. Two sets of primers,
668 Ex1_F-T31_R and Ex1_F-GFP_R, were used to detect wild-type and the KO genomic

669 locus, respectively. Note that WT locus was detected from genomic DNAs of WT and
670 $Wdr8^{-/+}$, but not that of $Wdr8^{-/-}$. On the other hand, the KO locus was detected from
671 genomic DNAs of $Wdr8^{-/+}$ and $Wdr8^{-/-}$, but not that of WT, due to the presence of
672 GFP insertion.

673 **Extended Data Figure 2 | External phenotype of Cab wild-type and $Wdr8^{-/-}$**
674 **hatchlings (F3 generation, see Methods).** $Wdr8^{-/-}$ hatchlings rescued by maternal
675 $Wdr8$ developed normally with no obvious external abnormalities, comparable to
676 wild-type. Scale bars, 500 μ m.

677 **Extended Data Figure 3 | External phenotype of the hatchling from $Wdr8^{-/-}$**
678 **father in comparison with wild-type hatchling.** As in Extended Data Figure 2, there
679 were no obvious differences, demonstrating that paternal $Wdr8$ is not implicated in
680 both early and later stage development of medaka. Scale bars, 500 μ m.

681 **Extended Data Figure 4 | Detailed process of mitotic spindle assembly in Cab**
682 **and $Wdr8^{-/-}$ blastomeres.** Whereas bipolar mitotic spindle was faithfully formed
683 from prometaphase to telophase in wild-type blastomeres, fragmented centrosomes
684 (PCM1) caused multipolar spindles as well as severe chromosome instability during
685 mitosis in $Wdr8^{-/-}$ blastomeres. Scale bars, 10 μ m.

686 **Extended Data Figure 5 | Western blot analysis to compare the protein level**
687 **between EYFP-huWdr8 and EYFP-196/197AA.** Expression levels of both proteins
688 were almost equivalent, demonstrating that mutation in WD40 domain did not affect
689 on protein stability.

690 **Extended Table 1 | The chosen $Wdr8$ -sgRNA target site has only two potential off-**
691 **target sites in the entire medaka genome. Search-parameters (see ⁴⁰ for further**
692 **details): core length = 12, max. core mismatches = 2, max. total mismatches = 4,**
693 **PAM = NGG**

Chromosome	start	end	strand	M	M target_seq	P	A align	M	ment	distance to next exon	position	gene name	gene id
	81	81											
	81	81			GGTCTCT	T					Ex		ENSOR
	46	48			CGAGCAG	G					on	wra	LG0000
chr7	7	9	+	0	CCGGAC	G	PAM			0	ic	p73	0004450
											Int		
	96	96			GGTCACA	A	[-				ro	MA	
chr2	58	58			TGTGCAG	G					ni	PK	XLOC_
3	52	74	+	4	CCGGAC	G	PAM			482	c	11	015134
							-						
	22	22											
	85	85			CGTCCCT	G					Ex		ENSOR
	05	05			CGAGCAT	G]PA				on	csr	LG0000
chr7	30	52	-	4	CCGCAC	G	M			0	ic	np2	0015651

694

695 **SI Movie 1 | Live imaging of EYFP-huWdr8 localisation in the rescued Wdr8^{-/-}**

696 **embryos.** EYFP-huWdr8 localised to the one or two dots in individual blastomeres,

697 showing stereotypic centrosome cycles during embryonic cell cycles from one- to 4-

698 cell stage.

699 **SI Movie 2 | Live imaging of EGFP localisation from one- to 4-cell stage of Cab**

700 **wild-type embryos.** EGFP alone did not localise to the centrosome-like dots as

701 EYFP-huWdr8 did in Wdr8^{-/-} blastomeres.

702 **SI Movie 3 | Live imaging of EGFP localisation from one- to 4-cell stage of Wdr8^{-/-}**

703 **^{-/-} embryos.** EGFP alone did not localise to the centrosome-like dots as EYFP-huWdr8

704 did in Wdr8^{-/-} blastomeres. Note that Wdr8^{-/-} disorderly divided blastomeres with

705 cytokinesis failure.

706 **SI Movie 4 | Live imaging of EYFP-196/197AA localisation from two-cell stage of**

707 **Wdr8^{-/-} embryos.** EYFP-196/197AA mostly localised to the cytoplasm but oscillated

708 weak centrosomal localisation with multiple foci during cleavages. Note that EYFP-

709 196/197AA was not able to rescue the abnormal cleavage divisions of Wdr8^{-/-}

710 embryos.

Figure 1 (Inoue D. et al.)

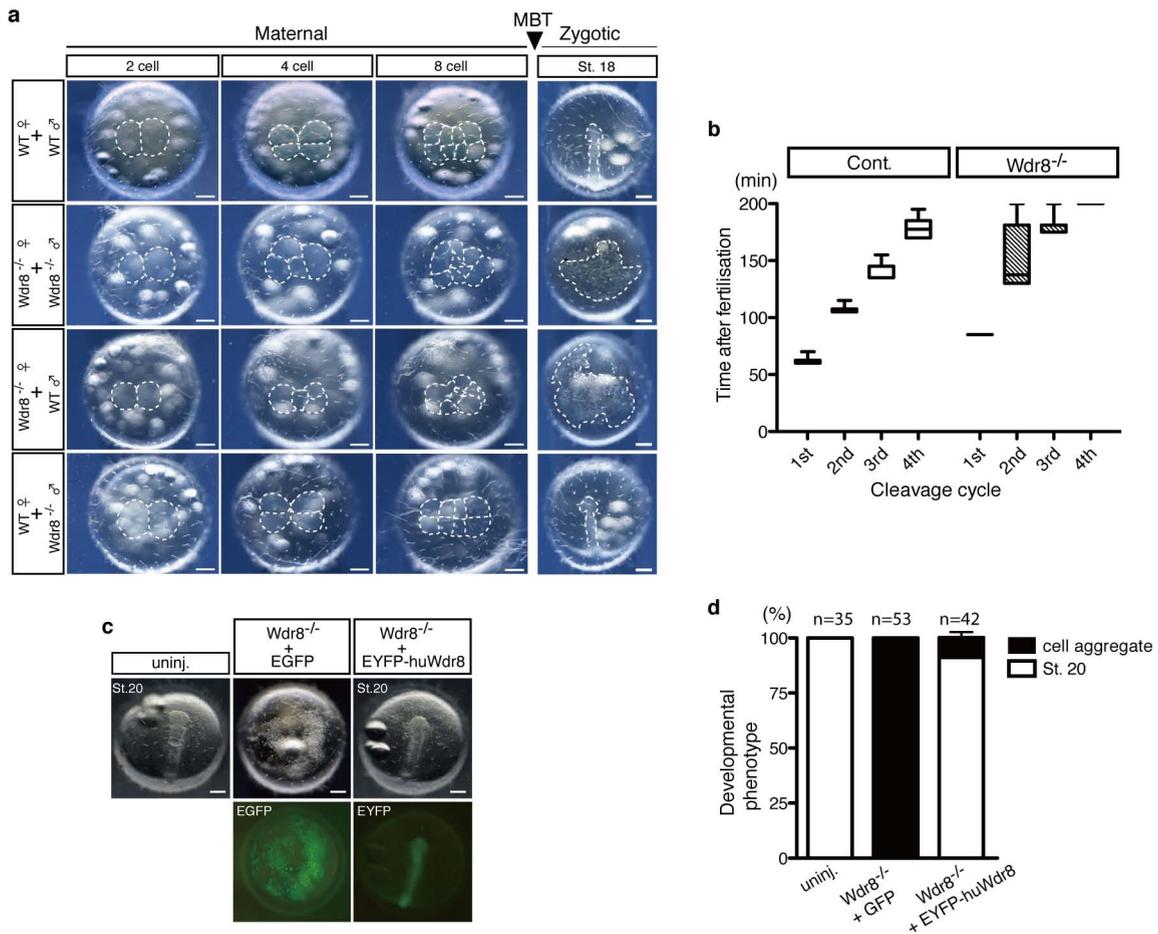


Figure 2 (Inoue D. et al.)

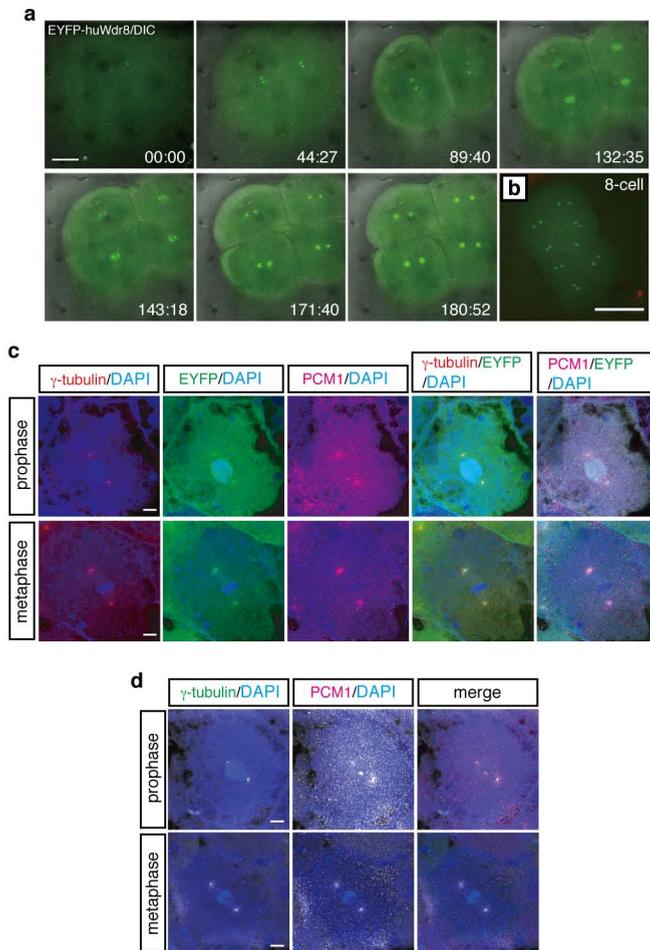


Figure 3 (Inoue D. et al.)

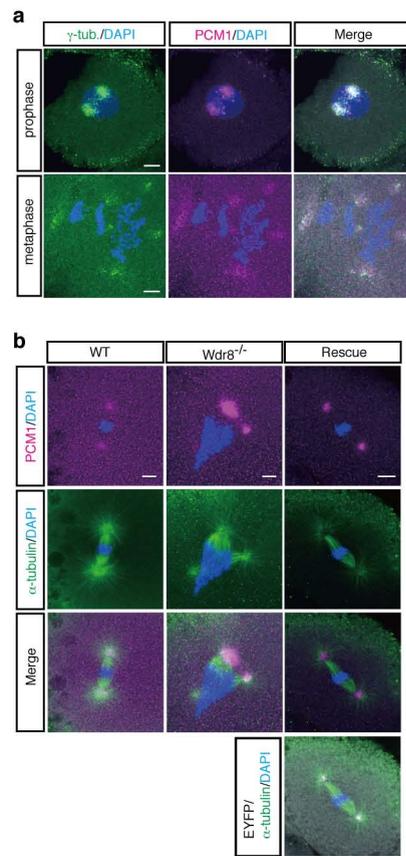
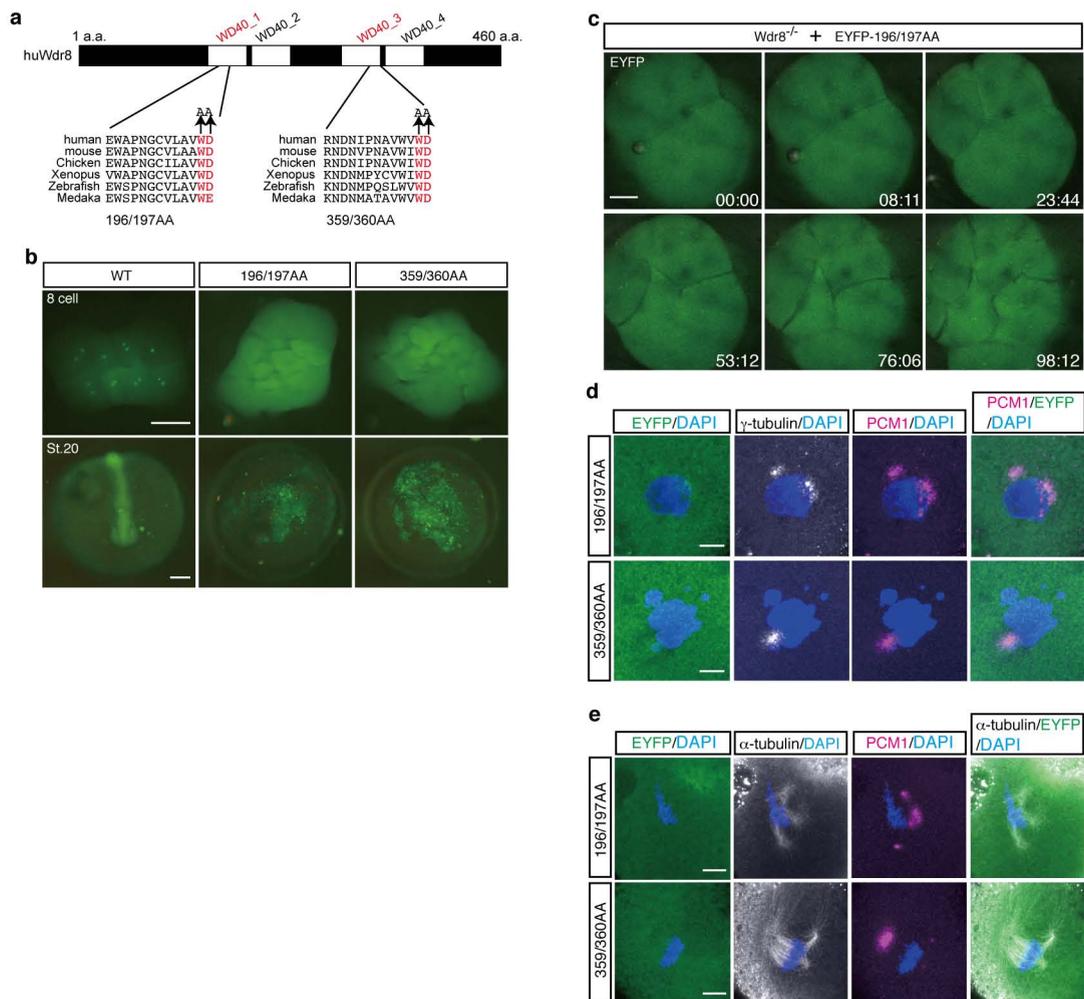
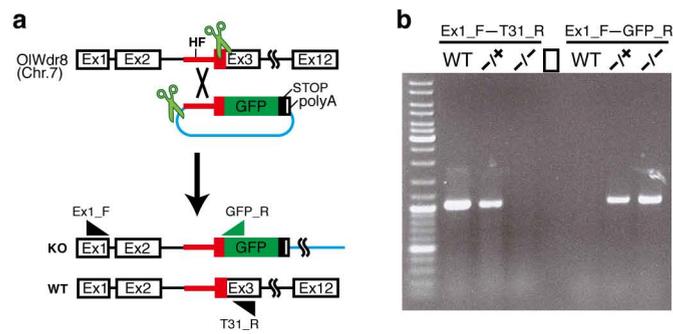


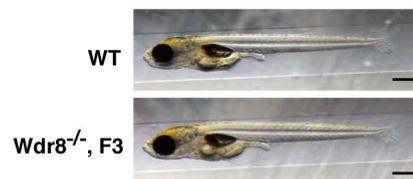
Figure 4 (Inoue D. et al.)



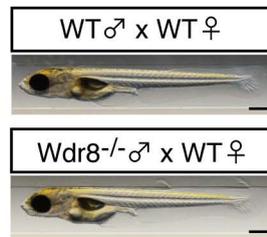
Extended Data Figure 1 (Inoue D. et al.)



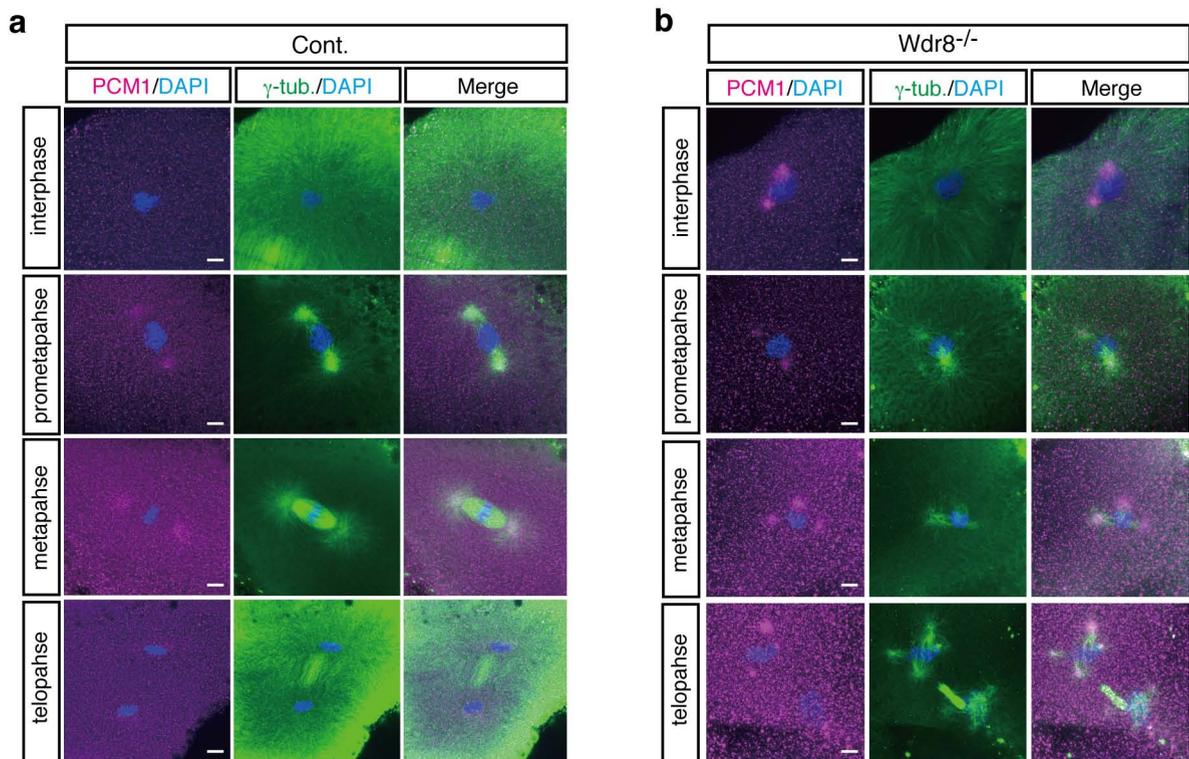
Extended Data Figure 2 (Inoue D. et al.)



Extended Data Figure 3 (Inoue D. et al.)



Extended Data Figure 4 (Inoue D. et al.)



Extended Data Figure 5 (Inoue D. et al.)

