

# 1 Haploinsufficient maternal effect of Epidermal Growth Factor Receptor A mutation in zebrafish

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3 Margherita Ciano<sup>1,2</sup>[ORCID](#), Paul R. Kemp<sup>3</sup>, S. Amanda Sathyapala<sup>3</sup>[ORCID](#) and Simon M. Hughes<sup>1</sup>[ORCID](#)\*

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5 <sup>1</sup> Randall Centre for Cell and Molecular Biophysics, King's College London, London UK.

6 <sup>2</sup> Department of Medicine, Imperial College London, London UK

7 <sup>3</sup> National Heart and Lung Institute, Imperial College London, London UK

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9 \* Author for correspondence [s.hughes@kcl.ac.uk](mailto:s.hughes@kcl.ac.uk)

10 Randall Centre for Cell and Molecular Biophysics, New Hunt's House, Guy's Campus, King's College

11 London, SE1 1UL, UK

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## 14 Abstract

15 Generation of viable offspring depends both on genetic and environmental factors of both mother and child.  
16 Analysis of a likely amorphic allele of the zebrafish *epidermal growth factor receptor a* (*egfra*) gene revealed  
17 that heterozygous females were infertile due to death of all fertilized eggs during embryonic and early larval  
18 life with cardiac, tail and other defects. Comparison of the severe dominant maternal effect with previous  
19 studies using pharmacological inhibitors of Egfrs or antisense morpholino injection indicate that a normal  
20 level of maternal *Egfra* is required for viability of offspring both during egg development and in the embryo  
21 after fertilisation. As heterozygous mothers were not fertile, the homozygous zygotic *egfra*<sup>kg134</sup> phenotype  
22 could not be analysed. Heterozygous *egfra*<sup>+/*kg134*</sup> males crossed to wild type females produced fully viable  
23 offspring, among which *egfra*<sup>+/*kg134*</sup> individuals had increased slow muscle but no functional motility defect.  
24 Our findings suggest that *Egfra* activity is crucial for early development both before and after fertilisation and  
25 are likely to constitute a rare example of a haploinsufficient maternal effect in a species lacking imprinting.

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27

## 28 Introduction

29 Regulation of fertility is important for humans and food production. Development of oocytes and early  
30 embryos depends on the contribution of protein and RNA encoded by the maternal genome prior to the final  
31 meiotic division to yield the mature gamete. Maternal effect genetic screens have been a powerful tool in  
32 unravelling early embryo development across vertebrates (Marlow, 2010). A particularly important aspect of  
33 oocyte development is its interaction with other cells within the ovary, such as follicular or nurse cells, which  
34 are mediated by a wide range of reciprocal extracellular signals (Richani and Gilchrist, 2018). Mutation of  
35 genes required for such signals have been shown to have effects on the developing oocyte or embryo when  
36 the mother is homozygous for the mutation, irrespective of ultimate embryonic genotype (Marlow, 2010). In  
37 general, however, maternal effect mutations are recessive, such that heterozygous females yield offspring  
38 that develop normally unless the offspring inherit an allelic combination causing a zygotic phenotype. Here  
39 we describe dominant maternal effect caused by mutation in the zebrafish *egfra* gene.

40 Epidermal growth factor (EGF) signalling through EGFR receptors of the ErbB family is of central  
41 importance in human reproduction (Richani and Gilchrist, 2018). In mammals, several EGFR ligands and

42 the EGFR itself are required for oocyte maturation and ovulation (Hsieh et al., 2007). The EGFR is not  
43 thought to be present on the oocyte itself, but acts via the surrounding cumulus cells, in which EGFR  
44 signalling triggers expansion of the cumulus and cumulus-derived extracellular matrix and promotes oocyte  
45 maturation through downregulation of cyclic nucleotides that are shared with the oocyte through gap junctions  
46 and suppress meiotic resumption. EGFR signaling within the follicle also enhances oocyte ATP production  
47 and subsequent oocyte developmental competence (that is, the ability of an oocyte to yield, upon fertilization,  
48 an embryo that successfully develops to term; (Richani and Gilchrist, 2018) and references therein). Indeed,  
49 levels of the EGFR ligand Amphiregulin in follicles correlate with infertility and successful IVF treatment  
50 (Ambekar et al., 2015; Huang et al., 2015). Strikingly, in mouse, maturation of EGFR signalling competence  
51 in cumulus cells is itself triggered by oocyte-derived signals, supporting the view that stepwise maturation of  
52 the follicle involves continual crosstalk between oocyte and its support cells that modulate EGFR signaling  
53 (Richani and Gilchrist, 2018). It is, therefore, of great importance to understand the roles of EGFR in oocyte  
54 maturation and acquisition of developmental competence.

55 EGF signalling has been extensively characterized in cultured cells and in mammals, in large part due to  
56 its importance in a variety of cancers (<https://omim.org/entry/131550> ). During murine development, *Egfr* is  
57 an essential gene; null mutation leads to genetic background-dependent prenatal death due to placental  
58 insufficiency, and additional lung, epidermis, whisker and eye defects (Miettinen et al., 1995; Sibilias and  
59 Wagner, 1995; Threadgill et al., 1995). Numerous other mutant alleles affect hair and skin and cause defects  
60 in a variety of organs including heart and lung. However, genetic loss of function analysis in vertebrates  
61 beyond the mouse has not been performed.

62 In the zebrafish, *egfra* function has been implicated in cardiac outflow tract formation, aorta thickness,  
63 adult intestinal adaptation and ovarian follicle development (Aizen and Thomas, 2015; Goishi et al., 2003;  
64 Peyton and Thomas, 2011; Schall et al., 2015; Wang and Ge, 2004; Zhao and Lin, 2013). In embryos/larvae,  
65 inhibition of zebrafish *Egfra* with either AG1478 or PK166, two drugs that inhibit mammalian EGFRs, leads  
66 to death from pericardial oedema, an effect that can be phenocopied by *egfra* antisense morpholino injection  
67 (Goishi et al., 2003). We previously described generation of the putative null *egfra*<sup>kg134</sup> allele in the zebrafish  
68 in which a frameshift 12 amino acids from the N-terminus of the protein leads to a severe truncation.  
69 Heterozygous *egfra*<sup>+/<sup>kg134</sup> larvae have a mild increase in slow muscle but are viable, whereas homozygous  
70 mutants die early in larval life (Ciano et al., 2019). However, in our initial report, more extensive analysis of  
71 the genetics of the *egfra*<sup>kg134</sup> allele was not reported.</sup>

72 Here we analyse the genetics of the *egfra* loss of function mutation in more detail and report the striking  
73 finding that the *egfra*<sup>kg134</sup> allele shows a maternal effect in heterozygous mothers, such that no offspring  
74 survive beyond early larval life, irrespective of zygotic genotype. In contrast, heterozygous males yield viable  
75 heterozygous offspring of both sexes.

76

## 77 Results

78 The *egfra* mutant allele *egfra*<sup>kg134</sup> generated by CRISPR/Cas9 genome editing has a small indel that leads  
79 to a frameshift after 12 amino acids in the first coding exon. This results in termination of the resultant  
80 polypeptide with a 70 amino acid nonsense tail at a normally out-of-frame stop codon in the second coding  
81 exon (Ciano et al., 2019). The predicted protein lacks essential EGF binding regions, the transmembrane  
82 domain and the intracellular tyrosine kinase of EGFR and is therefore highly unlikely to have any residual

83 positive or dominant negative function. Further evidence for lack of a dominant negative effect is a) that the  
84 mutant allele causes nonsense-mediated mRNA decay, so little truncated protein will be produced (Ciano et  
85 al., 2019) and b) that heterozygous *egfra*<sup>+/*kg134*</sup> larvae do not show any of the severe defects reported in *Egfr*  
86 null mutant mice (Miettinen et al., 1995; Sibilias and Wagner, 1995; Threadgill et al., 1995).

87

### 88 **Fertilized eggs from *egfra*<sup>+/*kg134*</sup> heterozygous females die early**

89 Heterozygous F1 *egfra*<sup>+/*kg134*</sup> fish were in-crossed to generate embryos homozygous for the mutation.  
90 However, all embryos died in the lays. Four F1 *egfra*<sup>+/*kg134*</sup> in-crosses between the single available adult F1  
91 carrier *egfra*<sup>+/*kg134*</sup> female (designated  $\alpha$ ) and different *egfra*<sup>+/*kg134*</sup> F1 males were performed. No embryos  
92 survived beyond 4 days post fertilisation (dpf). Most deaths occurred between 1 and 2 dpf (Fig. 1A).  
93 Genotyping of resultant F2 embryos failed to reveal differences based on zygotic genotype.

94 F2 generations were bred by out-crossing carrier *egfra*<sup>+/*kg134*</sup> males to wild type females. After genotyping  
95 to identify *egfra*<sup>+/*kg134*</sup> F2 males and females, three females (designated  $\beta$ ,  $\gamma$  and  $\delta$ ) and various males were  
96 obtained. Repeatedly, embryos laid by F2 *egfra*<sup>+/*kg134*</sup> females all died (Fig. 1B). In contrast, embryos laid  
97 from wild type *egfra*<sup>+/+</sup> sibling F2 females developed normally and survived (Fig. 1B). Paternal genotype had  
98 no discernible effect; fertilisation of wild type-derived eggs by sperm from either wild type or *egfra*<sup>+/*kg134*</sup> F2  
99 sibling males yielded viable embryos (Fig. 1B). An F3 generation was bred by out-crossing an *egfra*<sup>+/*kg134*</sup>  
100 F2 male to a wild type female. Female F3 *egfra*<sup>+/*kg134*</sup> carriers again failed to yield viable offspring (data not  
101 shown). The number of eggs produced by *egfra*<sup>+/*kg134*</sup> females was not significantly different from that  
102 produced by their wild type siblings. These data suggest that female zebrafish possessing only a single  
103 functional *egfra* allele are not fertile because they produce eggs that cannot develop normally.

104

### 105 **Heterozygous maternal effect causes abnormal embryo development**

106 Maternal effect is often caused by the requirement of gene function in the egg or fertilized embryo from  
107 mRNA placed in the egg during oogenesis (Marlow, 2010). We performed in situ mRNA hybridization on  
108 newly-laid eggs and embryos prior to activation of the zygotic genome and observed *egfra* mRNA  
109 accumulation in the early embryos (Fig. 2A). This finding indicates that *egfra* mRNA derived from maternal  
110 chromosomes is accumulated in the egg.

111 Fertilisation and egg activation rates appeared normal in clutches derived from *egfra*<sup>+/*kg134*</sup> females (Fig.  
112 1). Chorion elevation was indistinguishable from wild type (data not shown). Although small numbers of  
113 unfertilized embryos were observed in some lays, as reflected by the slight drop in survival during the first  
114 dpf in some control lays (Fig. 1B), such unfertilized embryos were readily distinguished from fertilized  
115 embryos undergoing cleavage in lays from *egfra*<sup>+/*kg134*</sup> females. No defects in cleavage stage development  
116 were noted. Moreover, epiboly and gastrulation were not grossly defective (data not shown). By 18 hpf,  
117 however, almost all embryos in clutches from *egfra*<sup>+/*kg134*</sup> females were defective (Fig. 2B). Subsequently, a  
118 range of defects of variable severity appeared. Moderately affected embryos had a curved tail tip sometimes  
119 accompanied by cardiac oedema. 'Severe' embryos had unusual yolk shapes and showed altered tailbud  
120 morphology and poor yolk extension. Very severely affected embryos lacked a tail and showed signs  
121 compatible with widespread apoptosis (Fig. 2B). The severity of defects did not correlate with zygotic *egfra*  
122 genotype. For example, the moderately affected embryo shown in Fig. 2B was a zygotic mutant derived from  
123 an *egfra*<sup>+/*kg134*</sup> female crossed to an *egfra*<sup>+/*kg134*</sup> male. In contrast, more severely defective embryos could be

124 *egfra*<sup>+/*kg134*</sup> or wild type, whether derived from a heterozygote in-cross or an *egfra*<sup>+/*kg134*</sup> female crossed to a  
125 wild type male (Fig. 2B and data not shown). We conclude that reduction of *Egfra* function derived from the  
126 mother causes a severe developmental defect.

127

### 128 **Behaviour of mutant and wild type larvae**

129 To examine the role of zygotic *egfra* further, bearing in mind the increase in slow muscle fibres previously  
130 observed (Ciano et al., 2019), differences in burst swimming in response to touch were investigated in F3  
131 *egfra*<sup>+/*kg134*</sup> and wt siblings. No significant difference in motility were observed (Fig. 3). Heterozygous  
132 *egfra*<sup>+/*kg134*</sup> and wt sibling larvae grew to adulthood equally well and were indistinguishable as adults (data  
133 not shown).

134

### 135 **Discussion**

136 The data presented strongly suggest that *egfra* has an essential role on early embryonic development  
137 and that the level of the expressed *Egfra* receptor is crucial. As we have only isolated and characterised a  
138 single mutant allele, we cannot rule out the possibility that the sgRNA used generated an off-target mutation  
139 closely-linked to the *egfra*<sup>*kg134*</sup> allele on chromosome 2. We think this unlikely because i) no close matches  
140 with adjacent PAM sites are present nearby on chromosome 2 (CRISPRdirect shows zero 20 bp or 12 bp  
141 matches and the nearest 7/8 bp seed match with an adjacent PAM site are 3 Mb distal and 1.5 Mb proximal  
142 on chromosome 2 and neither lies in or near a known coding region), ii) two other lines with F1s carrying  
143 distinct *egfra* mutant alleles generated with the same sgRNA did not yield fertile F1 females and were  
144 discarded before the nature of the maternal effect became apparent, iii) *egfra* is expressed in the developing  
145 oocyte, egg and early embryo, consistent with a maternal effect (Goishi et al., 2003), and iv) *Egfr* has  
146 previously been implicated in oocyte development in zebrafish (Peyton and Thomas, 2011; Van Der Kraak  
147 and Lister, 2011).

148 The correct level of the *Egfra* receptor appears to be important for zebrafish fertility. We previously  
149 reported that *egfra* mRNA is reduced by 50% in *egfra*<sup>+/*kg134*</sup> fish (Ciano et al., 2019), consistent with nonsense  
150 mediated decay of the mutant mRNA, bearing in mind that the minor sequence change in exon 1 is not  
151 predicted to affect transcription, splicing, or translational initiation. It is therefore likely that during some or  
152 all of egg development and early embryonic life, *Egfra* protein level in the developing gamete/embryo may  
153 be reduced. It has been suggested that oocyte maturation depends on the balance of *Egfr* (also known as  
154 *ErbB1*) and other *ErbB* receptors, such as *ErbB2*, that can heterodimerise (Aizen et al., 2018). Other work  
155 has suggested a role for *Egfr* activity in the follicle cells during oocyte maturation in zebrafish (Wang and Ge,  
156 2004) and ovulation in mice (Ashkenazi et al., 2005; Hsieh et al., 2007; Jamnongjit et al., 2005). Treatment  
157 of female fish with the environmental dioxin pollutant TCDD reduces both fertility and *egfra* level in the ovary  
158 (Heiden et al., 2008). However, we observe normal egg abundance, mating behaviour, fertilisation rates and  
159 oocyte activation from *egfra*<sup>+/*kg134*</sup> females. Moreover, early stages of cleavage and epiboly also appear  
160 normal. Our *egfra*<sup>*kg134*</sup> maternal effect mutation has late and pleiotropic effects. We therefore hypothesise  
161 that the relevant period for the requirement for the correct level of *Egfra* may extend into early embryonic life.  
162 But an alternative possibility is that lack of appropriate EGFR signalling during oocyte development reduces  
163 oocyte quality such that subsequent embryo development is compromised. The fact that injection of *egfra*-  
164 targeting morpholino antisense oligonucleotides into 1-cell stage embryos does not phenocopy the

165 *egfra*<sup>+/*kg134*</sup> maternal effect (Goishi et al., 2003), suggests the maternal Egfra protein functions before  
166 fertilisation and/or may be stockpiled in the egg.

167 Our data add an evolutionary perspective to the increasing understanding of the importance of EGFR  
168 signalling in mammalian oocyte development (Richani and Gilchrist, 2018). Our data show that reduction of  
169 maternal Egfra function generates poor quality eggs, irrespective of zygotic genotype. Despite fertilisation  
170 and apparently normal cleavage, embryos from *egfra*<sup>+/*kg134*</sup> mothers develop poorly and die. In humans and  
171 other mammals, oocyte maturation and high oocyte developmental competence depend on EGFR signalling  
172 within the follicle that acts on cumulus cells and may affect in vitro maturation procedures that could offer  
173 significant benefit to women with polycystic ovary syndrome or cancer (Richani and Gilchrist, 2018). Such  
174 signalling is thought to enhance metabolic capacity and regulate post-transcriptional events that prepare  
175 oocytes for their future developmental role in the embryo (Chen et al., 2013; Richani et al., 2014; Sugimura  
176 et al., 2015). Our finding that EGFR signalling controls developmental competence in zebrafish suggests that  
177 EGFR signalling is an ancient and important pathway in oogenesis that is conserved across vertebrate  
178 evolution.

179 Treatment of zebrafish embryos with Egfr-blocking drugs causes a variety of defects (Budi et al., 2008;  
180 Goishi et al., 2003), but those described are not as severe as the early death we observe. This supports the  
181 view that Egfra activity is required both prior to egg laying for correct egg development and after egg laying  
182 for later developmental steps. Morpholino knockdown of Egfra leads to cardiac and vascular abnormalities  
183 that have some similarities to the variable penetrance cardiac oedema we observe (Goishi et al., 2003).  
184 Altered Egfra activity has also been described to cause biliary atresia and to alter gut repair (Ningappa et al.,  
185 2015; Schall et al., 2015), processes that may contribute the phenotypes we observe, but are unlikely to  
186 cause the gross embryonic defects and early death.

187 We also report further characterisation of the phenotype of *egfra*<sup>+/*kg134*</sup> fish, which have extra slow muscle  
188 fibres in their somitic myotome (Ciano et al., 2019). These *egfra*<sup>+/*kg134*</sup> fish did not change speed of swimming  
189 triggered by a touch, a validated physiological measure that is fast fibre-dependent (Naganawa and Hirata,  
190 2011). As the level of Hedgehog (Hh) signaling also controls the number of slow muscle fibres in zebrafish  
191 somite, it is possible that the EGF-repeat containing protein Scube2, which is required for normal slow fibre  
192 formation could provide a link to EGF/Egfr function (Blagden et al., 1997; Hollway et al., 2006). However,  
193 because Scube2 lacking the EGF domains can rescue Hh palmitoylation and signaling in vitro and in vivo  
194 (Creanga et al., 2012), it is unclear if reduction of Egfra function affects slow myogenesis through modulating  
195 Hh signalling. Alternatively, Egfra function may suppress the later Hh-independent additional slow  
196 myogenesis, as patterned expression is observed in the somite after 1 dpf (Fig. 2B)(Barresi et al., 2001;  
197 Ciano et al., 2019). To date we have been unable to discern a zygotic functional phenotype in *egfra*<sup>+/*kg134*</sup>  
198 fish. Unfortunately, *egfra*<sup>*kg134*</sup> homozygote mutants do not survive well or long enough for meaningful  
199 functional studies.

200 Finally, the most notable aspect of our findings is the existence of such a highly penetrant heterozygous  
201 maternal effect that we attribute to haploinsufficiency. Female mice lacking one allele of *Egfr* do not show a  
202 similar infertility (Miettinen et al., 1995; Sibia and Wagner, 1995; Threadgill et al., 1995). To our knowledge,  
203 few dominant maternal effect genes have been described, although many may have been discarded in the  
204 extensive screens for recessive zygotic and maternal effect mutations (Marlow, 2010). Rare known  
205 examples of maternal haploinsufficiency are the unmapped *mel-23*<sup>ct45</sup> allele in *C. elegans* and the Minute

206 allele *RpS17<sup>4</sup>* in *D. melanogaster* (Boring et al., 1989; Mains et al., 1990). In mammals, genetics mimicking  
207 maternal haploinsufficiency can be observed after uneven X-chromosome inactivation or in imprinted genes  
208 where the paternal allele is not expressed. However, zebrafish have neither sex chromosomes, nor parental  
209 imprinting. Moreover, because we used genome editing to create and select *egfra<sup>kg134</sup>* as a likely loss of  
210 function *egfra* allele, we suggest that the apparent dominant maternal effect is caused by maternal  
211 haploinsufficiency of *Egfra*. Although to date searches of OMIM and extant QTL data have failed to reveal  
212 linkage of human *EGFR* variation to infertility, a possible genetic influence on EGFR signalling in fertility in  
213 humans and other vertebrates should be borne in mind.

214

## 215 **Methods**

216

### 217 **Zebrafish lines and maintenance**

218 Zebrafish were reared at King's College London on a 14/10 hours light/dark cycle at 28.5 °C with adults kept  
219 at 26.5°C, with staging and husbandry as described (Westerfield, 2000). Founder and subsequent  
220 generations were out-crossed to AB fish. Fish were genotyped by High Resolution Melt Analysis and/or DNA  
221 sequencing in the mutant alleles, following PCR amplification using primers indicated (Supplementary Table  
222 1). Individual genotyped carrier *egfra<sup>kg134/+</sup>* females were kept in separate tanks to ensure reproducibility. All  
223 experiments were performed in accordance with licences held under the UK Animals (Scientific Procedures)  
224 Act 1986 and later modifications and conforming to all relevant guidelines and regulations.

225

### 226 **Imaging and in situ mRNA hybridization**

227 ISH was performed as described (Ganassi et al., 2014). Briefly, fish were fixed in 4% paraformaldehyde (PFA)  
228 in phosphate-buffered saline (PBS) for 30 min or 3 hours at room temperature, stored in 100% methanol at  
229 -20°C and rehydrated in PBS prior to ISH. Digoxigenin-labelled probes were against *egfra* as described  
230 (Ciano et al., 2019) and embryos were imaged under a Leica MZ16F with Olympus camera.

231

### 232 **Motility Assay**

233 Embryos touch response was measured on F3 larvae at 2 and 5 dpf and recorded with a Leica DFC490  
234 colour camera at 500 frame per seconds, as previously described (Naganawa and Hirata, 2011). Each  
235 embryo was imaged in a petri dish of fish water under a Leica MZ16F microscope, the tail touched with  
236 forceps to trigger movement and behaviour recorded for 10s. Videos were analysed blind with Tracker  
237 software 4.97 (<https://physlets.org/tracker/>) and mean and maximum velocity calculated. DNA was  
238 subsequently extracted from each embryo for genotyping.

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## 241 **References**

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243 **Aizen, J., Pang, Y., Harris, C., Converse, A., Zhu, Y., Aguirre, M. A. and Thomas, P. (2018).**  
244 Roles of progesterone receptor membrane component 1 and membrane progesterin  
245 receptor alpha in regulation of zebrafish oocyte maturation. *Gen Comp Endocrinol* **263**,  
246 51-61.

- 247 **Aizen, J. and Thomas, P.** (2015). Role of Pgrmc1 in estrogen maintenance of meiotic arrest  
248 in zebrafish oocytes through Gper/Egfr. *J Endocrinol* **225**, 59-68.
- 249 **Ambekar, A. S., Kelkar, D. S., Pinto, S. M., Sharma, R., Hinduja, I., Zaveri, K., Pandey, A.,**  
250 **Prasad, T. S., Gowda, H. and Mukherjee, S.** (2015). Proteomics of follicular fluid from  
251 women with polycystic ovary syndrome suggests molecular defects in follicular  
252 development. *J Clin Endocrinol Metab* **100**, 744-753.
- 253 **Ashkenazi, H., Cao, X., Motola, S., Popliker, M., Conti, M. and Tsafriri, A.** (2005). Epidermal  
254 growth factor family members: endogenous mediators of the ovulatory response.  
255 *Endocrinology* **146**, 77-84.
- 256 **Barresi, M. J., D'Angelo, J. A., Hernandez, L. P. and Devoto, S. H.** (2001). Distinct  
257 mechanisms regulate slow-muscle development. *Current biology : CB* **11**, 1432-1438.
- 258 **Blagden, C. S., Currie, P. D., Ingham, P. W. and Hughes, S. M.** (1997). Notochord induction  
259 of zebrafish slow muscle mediated by Sonic Hedgehog. *Genes Dev* **11**, 2163-2175.
- 260 **Boring, L. F., Sinervo, B. and Schubiger, G.** (1989). Experimental phenocopy of a minute  
261 maternal-effect mutation alters blastoderm determination in embryos of *Drosophila*  
262 *melanogaster*. *Dev Biol* **132**, 343-354.
- 263 **Budi, E. H., Patterson, L. B. and Parichy, D. M.** (2008). Embryonic requirements for ErbB  
264 signaling in neural crest development and adult pigment pattern formation.  
265 *Development* **135**, 2603-2614.
- 266 **Chen, J., Torcia, S., Xie, F., Lin, C. J., Cakmak, H., Franciosi, F., Horner, K., Onodera, C., Song,**  
267 **J. S., Cedars, M. I., et al.** (2013). Somatic cells regulate maternal mRNA translation and  
268 developmental competence of mouse oocytes. *Nat Cell Biol* **15**, 1415-1423.
- 269 **Ciano, M., Mantellato, G., Connolly, M., Paul-Clark, M., Willis-Owen, S., Moffatt, M. F.,**  
270 **Cookson, W., Mitchell, J. A., Polkey, M. I., Hughes, S. M., et al.** (2019). EGF receptor  
271 (EGFR) inhibition promotes a slow-twitch oxidative, over a fast-twitch, muscle  
272 phenotype. *Scientific reports* **9**, 9218.
- 273 **Creanga, A., Glenn, T. D., Mann, R. K., Saunders, A. M., Talbot, W. S. and Beachy, P. A.**  
274 (2012). Scube/You activity mediates release of dually lipid-modified Hedgehog signal  
275 in soluble form. *Genes Dev* **26**, 1312-1325.
- 276 **Ganassi, M., Badodi, S., Polacchini, A., Baruffaldi, F., Battini, R., Hughes, S. M., Hinitz, Y.**  
277 **and Molinari, S.** (2014). Distinct functions of alternatively spliced isoforms encoded  
278 by zebrafish *mef2ca* and *mef2cb*. *Biochimica et biophysica acta* **1839**, 559-570.
- 279 **Goishi, K., Lee, P., Davidson, A. J., Nishi, E., Zon, L. I. and Klagsbrun, M.** (2003). Inhibition  
280 of zebrafish epidermal growth factor receptor activity results in cardiovascular  
281 defects. *Mech Dev* **120**, 811-822.
- 282 **Heiden, T. C., Struble, C. A., Rise, M. L., Hessner, M. J., Hutz, R. J. and Carvan, M. J., 3rd**  
283 (2008). Molecular targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) within the  
284 zebrafish ovary: insights into TCDD-induced endocrine disruption and reproductive  
285 toxicity. *Reprod Toxicol* **25**, 47-57.
- 286 **Hollway, G. E., Maule, J., Gautier, P., Evans, T. M., Keenan, D. G., Lohs, C., Fischer, D.,**  
287 **Wicking, C. and Currie, P. D.** (2006). Scube2 mediates Hedgehog signalling in the  
288 zebrafish embryo. *Dev Biol* **294**, 104-118.
- 289 **Hsieh, M., Lee, D., Panigone, S., Horner, K., Chen, R., Theologis, A., Lee, D. C., Threadgill,**  
290 **D. W. and Conti, M.** (2007). Luteinizing hormone-dependent activation of the  
291 epidermal growth factor network is essential for ovulation. *Mol Cell Biol* **27**, 1914-  
292 1924.
- 293 **Huang, Y., Zhao, Y., Yu, Y., Li, R., Lin, S., Zhang, C., Liu, P. and Qiao, J.** (2015). Altered  
294 amphiregulin expression induced by diverse luteinizing hormone receptor reactivity  
295 in granulosa cells affects IVF outcomes. *Reprod Biomed Online* **30**, 593-601.

- 296 **Jamnongjit, M., Gill, A. and Hammes, S. R.** (2005). Epidermal growth factor receptor  
297 signaling is required for normal ovarian steroidogenesis and oocyte maturation. *Proc*  
298 *Natl Acad Sci U S A* **102**, 16257-16262.
- 299 **Mains, P. E., Sulston, I. A. and Wood, W. B.** (1990). Dominant maternal-effect mutations  
300 causing embryonic lethality in *Caenorhabditis elegans*. *Genetics* **125**, 351-369.
- 301 **Marlow, F. L.** (2010). *Maternal Control of Development in Vertebrates: My Mother Made Me*  
302 *Do It!* San Rafael (CA): Morgan & Claypool Life Sciences.
- 303 **Miettinen, P. J., Berger, J. E., Meneses, J., Phung, Y., Pedersen, R. A., Werb, Z. and Derynck,**  
304 **R.** (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal  
305 growth factor receptor. *Nature* **376**, 337-341.
- 306 **Naganawa, Y. and Hirata, H.** (2011). Developmental transition of touch response from slow  
307 muscle-mediated coilings to fast muscle-mediated burst swimming in zebrafish. *Dev*  
308 *Biol* **355**, 194-204.
- 309 **Ningappa, M., So, J., Glessner, J., Ashokkumar, C., Ranganathan, S., Min, J., Higgs, B. W.,**  
310 **Sun, Q., Haberman, K., Schmitt, L., et al.** (2015). The Role of ARF6 in Biliary Atresia.  
311 *PLoS One* **10**, e0138381.
- 312 **Peyton, C. and Thomas, P.** (2011). Involvement of epidermal growth factor receptor  
313 signaling in estrogen inhibition of oocyte maturation mediated through the G protein-  
314 coupled estrogen receptor (Gper) in zebrafish (*Danio rerio*). *Biol Reprod* **85**, 42-50.
- 315 **Richani, D. and Gilchrist, R. B.** (2018). The epidermal growth factor network: role in oocyte  
316 growth, maturation and developmental competence. *Hum Reprod Update* **24**, 1-14.
- 317 **Richani, D., Sutton-McDowall, M. L., Frank, L. A., Gilchrist, R. B. and Thompson, J. G.**  
318 (2014). Effect of epidermal growth factor-like peptides on the metabolism of in vitro-  
319 mated mouse oocytes and cumulus cells. *Biol Reprod* **90**, 49.
- 320 **Schall, K. A., Holoyda, K. A., Grant, C. N., Levin, D. E., Torres, E. R., Maxwell, A., Pollack, H.**  
321 **A., Moats, R. A., Frey, M. R., Darehzereshki, A., et al.** (2015). Adult zebrafish intestine  
322 resection: a novel model of short bowel syndrome, adaptation, and intestinal stem cell  
323 regeneration. *Am J Physiol Gastrointest Liver Physiol* **309**, G135-145.
- 324 **Sibilia, M. and Wagner, E. F.** (1995). Strain-dependent epithelial defects in mice lacking the  
325 EGF receptor. *Science* **269**, 234-238.
- 326 **Sugimura, S., Ritter, L. J., Rose, R. D., Thompson, J. G., Smitz, J., Mottershead, D. G. and**  
327 **Gilchrist, R. B.** (2015). Promotion of EGF receptor signaling improves the quality of  
328 low developmental competence oocytes. *Dev Biol* **403**, 139-149.
- 329 **Threadgill, D. W., Dlugosz, A. A., Hansen, L. A., Tennenbaum, T., Lichti, U., Yee, D.,**  
330 **LaMantia, C., Mourton, T., Herrup, K., Harris, R. C., et al.** (1995). Targeted disruption  
331 of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science*  
332 **269**, 230-234.
- 333 **Van Der Kraak, G. and Lister, A. L.** (2011). The inhibitory control of oocyte maturation in the  
334 zebrafish (*Danio rerio*): the role of the G protein-coupled estrogen receptor and  
335 epidermal growth factor. *Biol Reprod* **85**, 6-8.
- 336 **Wang, Y. and Ge, W.** (2004). Cloning of epidermal growth factor (EGF) and EGF receptor from  
337 the zebrafish ovary: evidence for EGF as a potential paracrine factor from the oocyte  
338 to regulate activin/follistatin system in the follicle cells. *Biol Reprod* **71**, 749-760.
- 339 **Westerfield, M.** (2000). *The Zebrafish Book - A guide for the laboratory use of zebrafish (Danio*  
340 *rerio)*: University of Oregon Press.
- 341 **Zhao, Y. and Lin, S.** (2013). Essential role of SH3-domain GRB2-like 3 for vascular lumen  
342 maintenance in zebrafish. *Arterioscler Thromb Vasc Biol* **33**, 1280-1286.
- 343



344

345 **Author contributions**

346 SMH and SAS conceived the project and obtained finance. MC performed all experiments and analysis.  
347 PRK provided advice. SMH and MC wrote the paper with input from other authors.

348

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352

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357

358 **Competing interests**

359 The authors have declared that no competing interests exist.

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363 **Figure Legends**

364

365 **Figure 1 Survival of embryos from *egfra*<sup>+/*kg134*</sup> and sibling females.**

366 **A, B.** Survival curves for embryos from the indicated crosses. The single F1 female obtained (designated  $\alpha$ )  
367 was crossed repeatedly and survival of progeny monitored every twelve hours (A). Three separate F2  
368 *egfra*<sup>+/*kg134*</sup> females (designated  $\beta$ ,  $\gamma$  and  $\delta$ ) were also tested (B). Note that lays from wild type (wt) sibling  
369 females crossed with wt or heterozygous *egfra*<sup>+/*kg134*</sup> males survive, whereas lays from heterozygous  
370 *egfra*<sup>+/*kg134*</sup> females die, irrespective of the partner male genotype.

371

372 **Figure 2 Heterozygous maternal effect may arise from maternal *egfra* expression in early embryos.**

373 **A.** In situ mRNA hybridisation for antisense (left) and sense (right) *egfra* probes at 32 cell stage, after  
374 gastrulation and at 1 dpf. Bars = 500  $\mu$ m. Note abundant expression at 32 cells, prior to zygotic genome  
375 activation and widespread but declining mRNA until 24 hpf. **B.** Representative timelapse imaging of single  
376 progeny of a wild type sibling female (left) and three maternal effect phenotypes (right) from *egfra*<sup>+/*kg134*</sup>  
377 females crossed to *egfra*<sup>+/*kg134*</sup> males. As all embryos from the wild type female appeared wild type the  
378 progeny were not individually genotyped and are therefore designated *egfra*<sup>+/?</sup>. All embryos from the carrier  
379 female showed growth retardation, with a moderate group characterised by cardiac oedema (arrow 1) and  
380 curvature at the tail tip (arrow 2), of which a zygotic mutant is shown. A severe group presented with a bean-  
381 shaped yolk at 18 hpf that, by the end of day 1, showed curvature of the trunk and tail (arrow 3), which  
382 persisted even after a day outside of the chorion; a zygotic wild type is shown. A very severe group had poor  
383 tailbud formation at 18 hpf, cardiac oedema (arrow 4) and failure of tail development (arrow 5) during day 1.  
384 Such embryos die by 2 dpf and the one shown was therefore not genotyped. All three phenotypes were also  
385 obtained from *egfra*<sup>+/*kg134*</sup> females crossed to *egfra*<sup>+/+</sup> males.

386

387 **Figure 3 F3 *egfra*<sup>+/*kg134*</sup> and wild type siblings swim similarly.**

388 **A.** Images extracted from burst response videos of F3 2 dpf sibling wt (A-A2) and *egfra*<sup>+/*kg134*</sup> (B-B2) larvae  
389 and 5 dpf sibling wt (C-C2) and *egfra*<sup>+/*kg134*</sup> (D-D2) larvae. Timepoints are shown in milliseconds (ms). Arrows  
390 indicate the plot of the tracking analysis with the X and Y coordinates, the red asterisk in the plot indicates  
391 the start point of the fish, black symbol: WT fish, blue symbols: *egfra*<sup>+/*kg134*</sup> fish. Bar 5 mm. **B.** Diagram to  
392 illustrate how measurement of Velocity was performed. **C.** Motility of 2 dpf and 5 dpf F3 *egfra*<sup>+/*kg134*</sup> and wt  
393 sibling larvae were compared. No difference was found at either age (2 dpf N=22/group from 2 crosses,  
394 mean velocity p=0.68 and maximum velocity p=0.12; 5 dpf N=11/group from 2 crosses, mean velocity p=0.21  
395 and maximum velocity p=0.32, Mann-Whitney U test).

396

397 **Supplementary Table 1 Primers for genotyping**

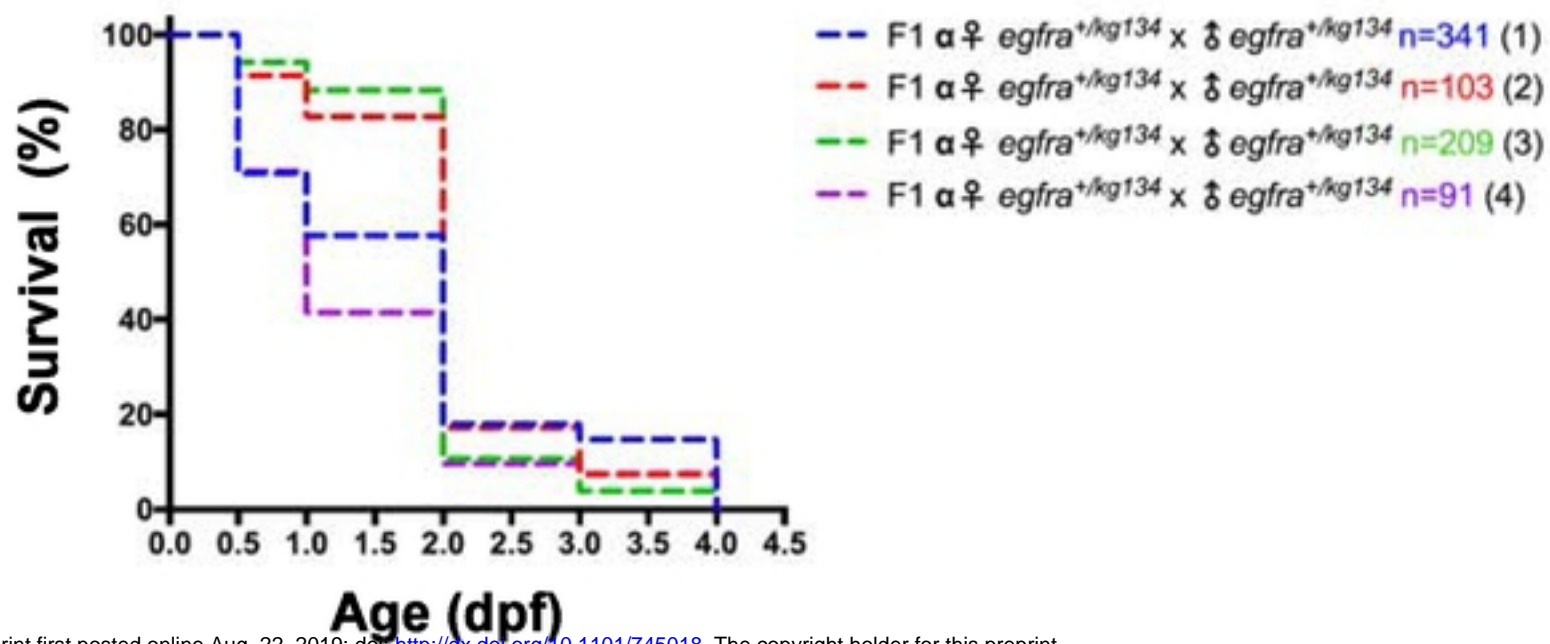
398 HRMA primers 5'-CCCGATAGCTTACAAACGCA-3' 5'-GCCGTTTCACAATAGTCCTACC-3'

399 Sequencing primers 5'-GGAGGAGGAGCTGTCAAAGT-3' 5'-GCGATGTTCCCAAATCATTTTCC-3'

400

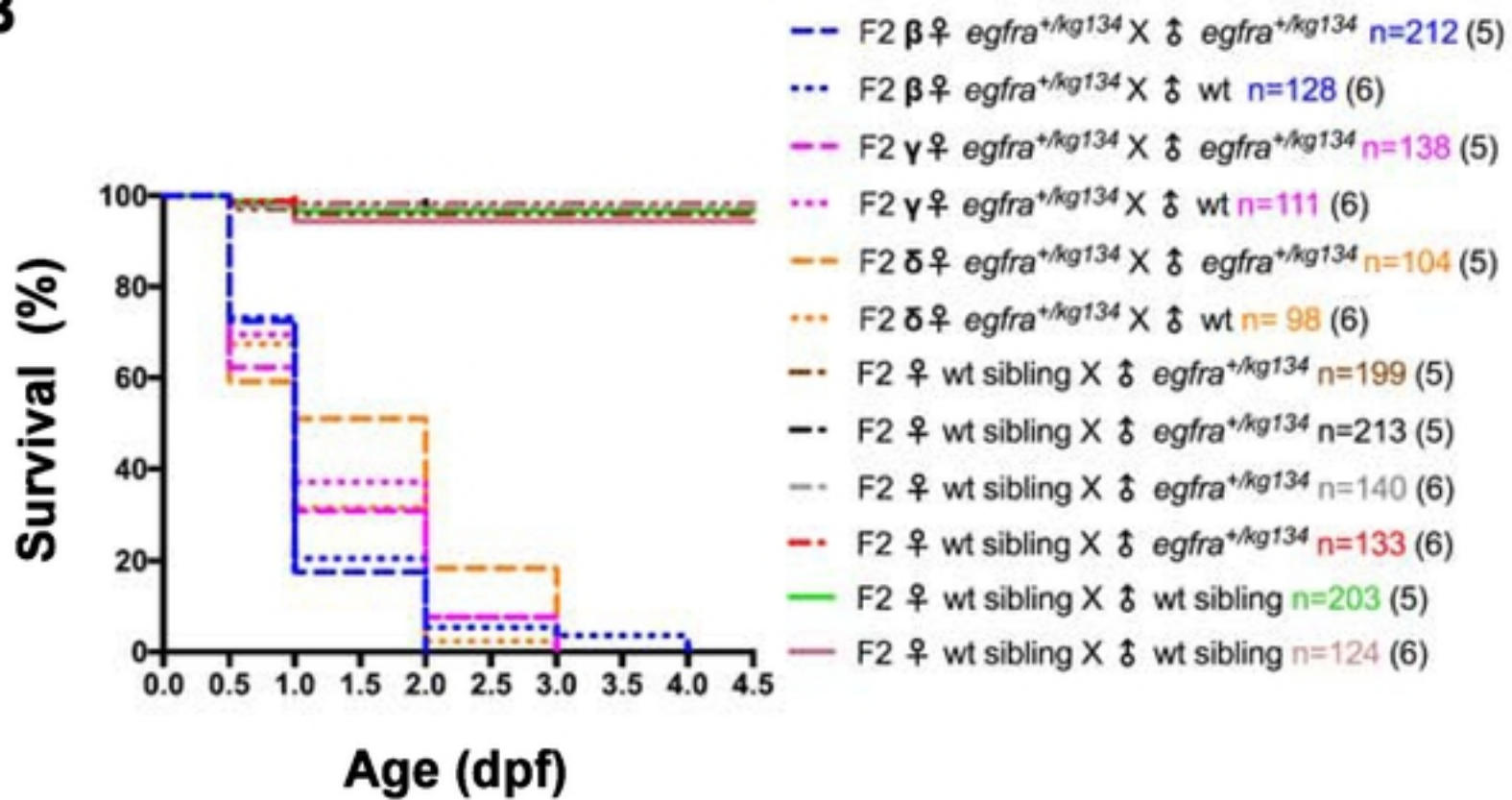
**Fig. 1**

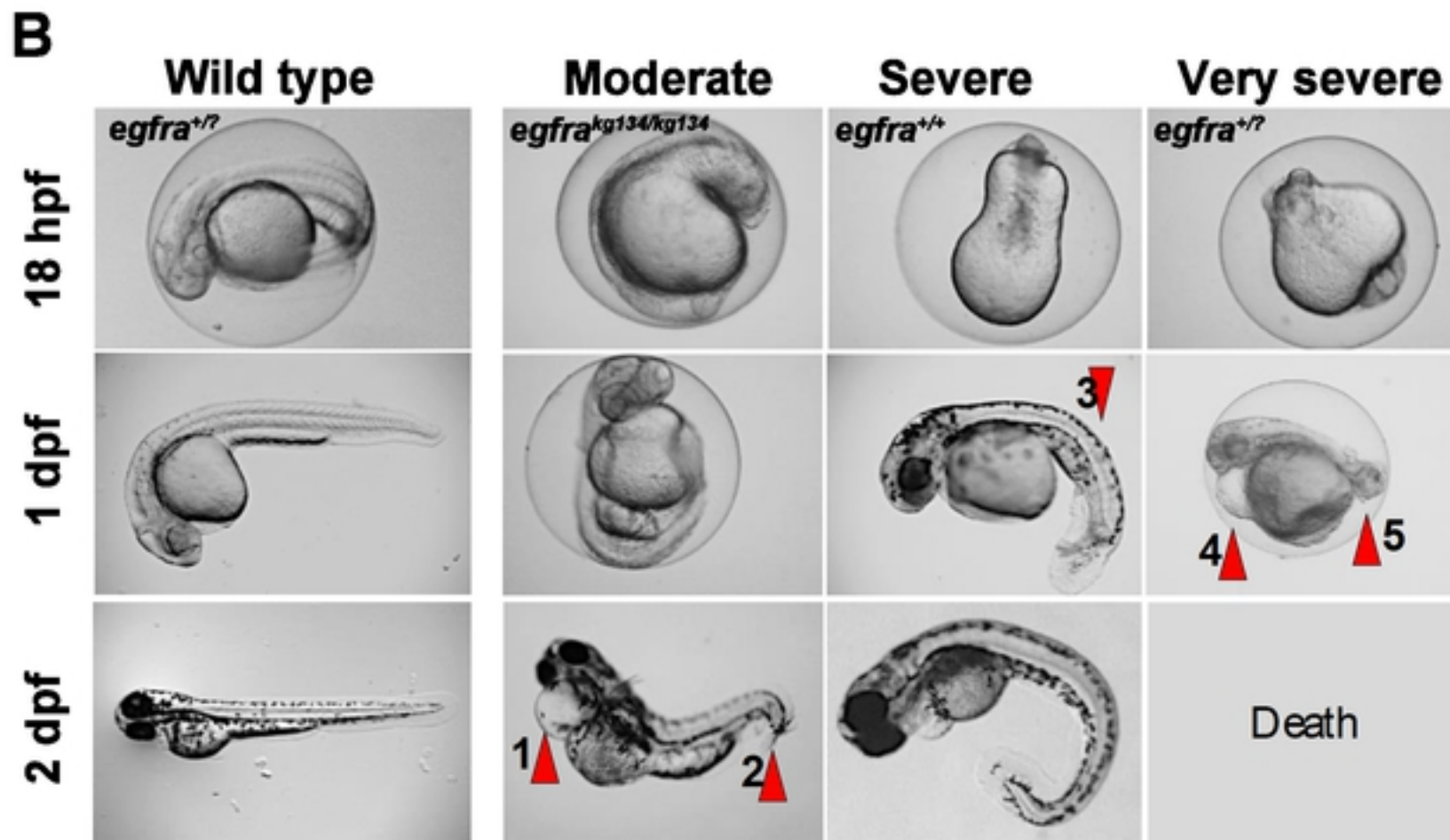
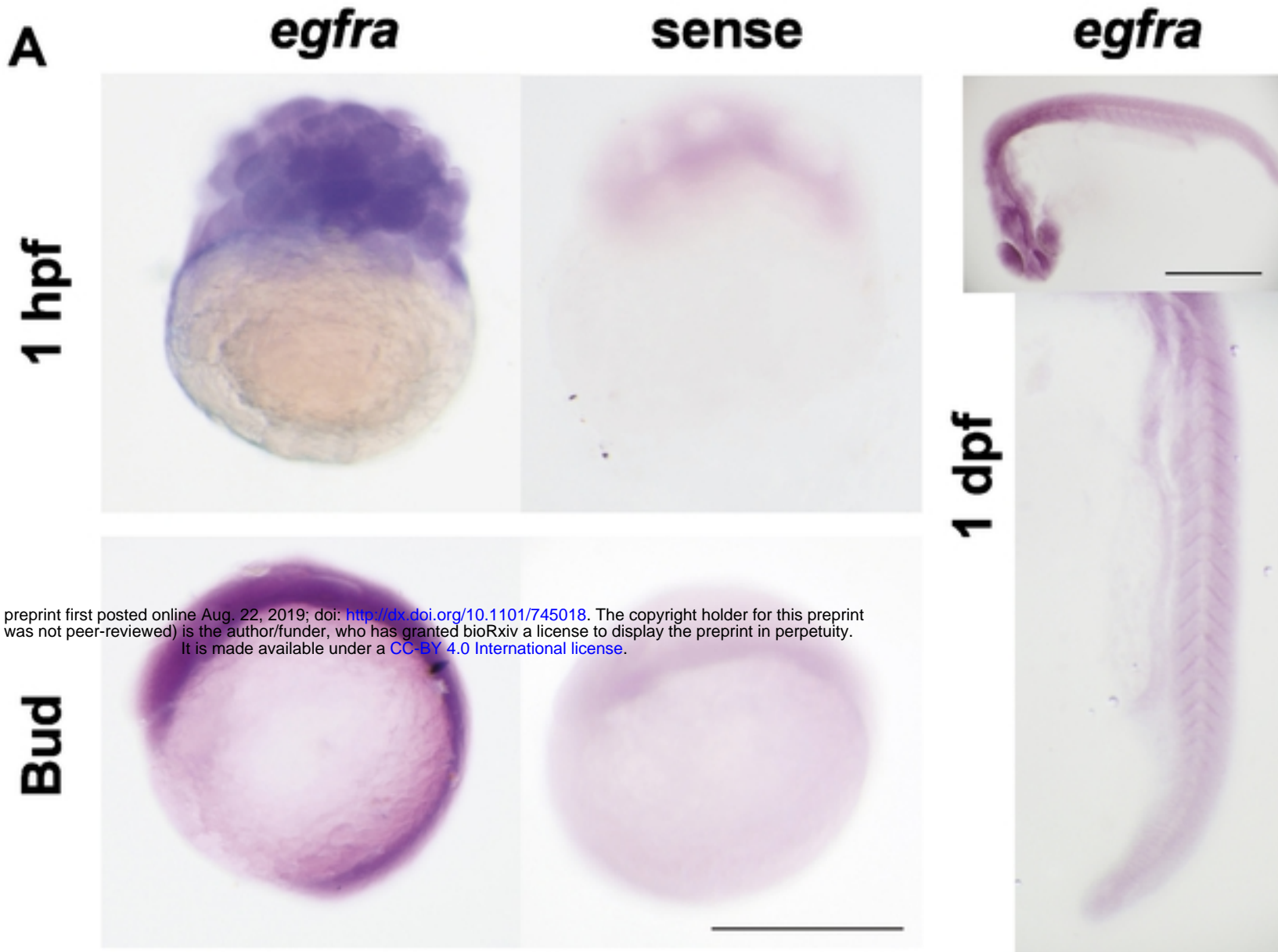
**A**



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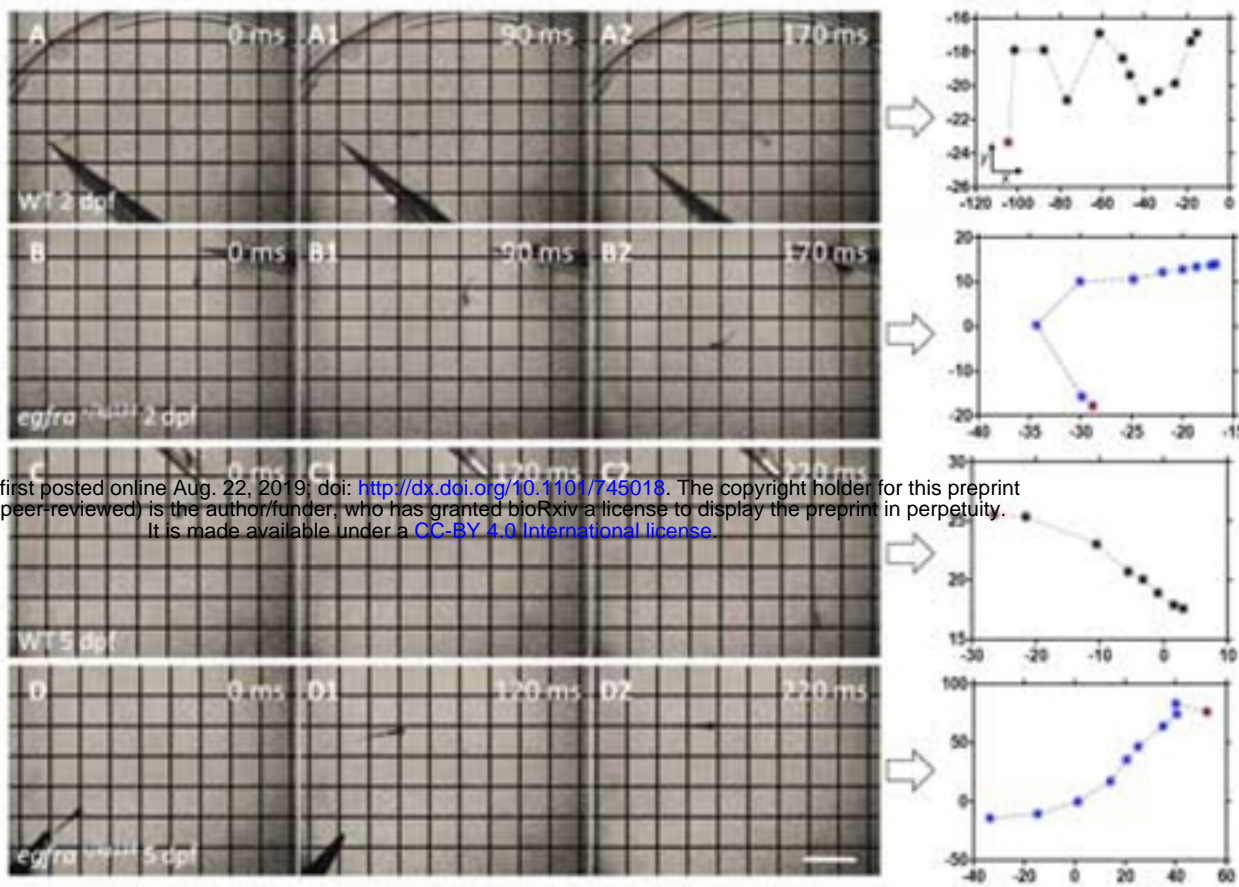
**B**



**Fig. 2**

# Fig 3

## A



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## B

