

1 **Running Title:** Zebrafish lipid droplet reporter lines

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10 **Imaging cytoplasmic lipid droplets *in vivo* with fluorescent perilipin 2 and**  
11 **perilipin 3 knockin zebrafish**

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41 **Keywords:** Lipid droplet, perilipin, zebrafish

42 **Abstract**

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44 Cytoplasmic lipid droplets are highly dynamic storage organelles; their rapid synthesis,  
45 expansion, and degradation, as well as their varied interactions with other organelles allow cells  
46 to maintain lipid homeostasis. While the molecular details of lipid droplet dynamics are  
47 currently a very active area of investigation, this work has been primarily performed in cultured  
48 cells and *in vitro* systems. By taking advantage of the powerful transgenic and *in vivo* imaging  
49 opportunities afforded by the zebrafish model system, we have built a suite of tools to allow  
50 lipid droplets to be studied in real-time from the subcellular to the whole organism  
51 level. Fluorescently-tagging the lipid droplet associated proteins, perilipin 2 and perilipin 3, in  
52 the endogenous loci, permits visualization of lipid droplets in the intestine, liver, lateral line and  
53 adipose tissue. Using these transgenic lines we have found that perilipin 3 is rapidly loaded on  
54 intestinal lipid droplets following a high fat meal and then largely replaced by perilipin 2 a few  
55 hours later. These powerful new tools will facilitate studies on the role of lipid droplets in  
56 different tissues and under different genetic and physiological manipulations.

## 57 **Introduction**

58

59 Cytoplasmic lipid droplets are cellular organelles composed of a core of neutral lipids  
60 surrounded by a monolayer of phospholipids and coated with a variety of proteins. While  
61 initially believed to be passive storage depots for lipids, it is now appreciated that lipid droplets  
62 are dynamic organelles with roles in cellular lipid homeostasis, protection from lipotoxicity and  
63 ER stress, viral and parasitic infection, and in host defense (Bosch et al., 2020; Cloherty et al.,  
64 2020; Coleman, 2020; Farese and Walther, 2009; Olzmann and Carvalho, 2019; Roberts and  
65 Olzmann, 2020).

66 Lipid droplets are typically coated by one or more perilipins, an evolutionarily related  
67 protein family defined by two conserved protein motifs, the N-terminal ~100 amino acid  
68 hydrophobic PAT domain followed by a repeating 11-mer helical motif of varying length  
69 (Kimmel and Sztalryd, 2016). Perilipins (PLINs) are recruited to the lipid droplet surface directly  
70 from the cytosol, mediated at least in part by the 11-mer repeat regions which fold into  
71 amphipathic helices (Kimmel and Sztalryd, 2016; Rowe et al., 2016). Perilipins act to regulate  
72 lipid storage by through their role in preventing or modulating access of lipid droplets to lipases  
73 and lipophagy (Sztalryd and Brasaemle, 2017).

74 Perilipins have been found in species ranging from *Dictyostelium* to mammals, with  
75 more divergent variants in fungi and *Caenorhabditis* (Bickel et al., 2009; Gao et al., 2017;  
76 Kimmel and Sztalryd, 2016; Sztalryd and Brasaemle, 2017). The human genome contains five  
77 perilipin genes, now designated PLIN1 – 5. Perilipins 2 and 3 are expressed ubiquitously  
78 (Brasaemle et al., 1997; Diaz and Pfeffer, 1998; Heid et al., 1998; Wolins et al., 2001), whereas  
79 perilipin 1 is predominantly expressed in white and brown adipocytes (Greenberg et al., 1991;  
80 Lu et al., 2001). PLIN4 is expressed in adipocytes, brain, heart and skeletal muscle, and PLIN5 is  
81 found in fatty acid oxidizing tissues such as heart, brown adipose tissue, and skeletal muscle, as  
82 well as in the liver (Dalen et al., 2007; Wolins et al., 2006; Yamaguchi et al., 2006). The  
83 genomes of rayfin fish, including zebrafish, have orthologs of human PLIN1, PLIN2 and PLIN3 in  
84 addition to a unique PLIN variant, perilipin 6 which targets the surface of pigment-containing  
85 carotenoid droplets in skin xanthophores (Granneman et al., 2017).

86           While fluorescently-tagged perilipin reporter proteins are used extensively in cell culture  
87 to visualize lipid droplets (for example (Chung et al., 2019; Granneman et al., 2017; Kaushik and  
88 Cuervo, 2015; Miura et al., 2002; Schulze et al., 2020; Targett-Adams et al., 2003)), lipid  
89 droplets *in vivo* have been historically studied in fixed tissues using immunohistochemistry  
90 (Frank et al., 2015; Lee et al., 2009), staining with lipid dyes (Oil red O, Sudan Black, LipidTox),  
91 or by electron microscopy (Chughtai et al., 2015; Marza et al., 2005; Zhang et al., 2010). Lipid  
92 droplets can also be labeled in live organisms with fluorescent lipophilic dyes such as BODIPY  
93 (Mather et al., 2019) & Nile red (Minchin and Rawls, 2017b), fed with fluorescently-tagged fatty  
94 acids (BODIPY & TopFluor) which are synthesized into stored fluorescent triglycerides or  
95 cholesterol esters (Ashrafi et al., 2003; Carten et al., 2011; Furlong et al., 1995; Quinlivan et al.,  
96 2017), or imaged in the absence of any label using CARS or SRS microscopy (Chien et al., 2012;  
97 Chughtai et al., 2015; Wang et al., 2011). However, expression of fluorescently-tagged lipid  
98 droplet associated proteins *in vivo* has been primarily limited to yeast (Gao et al., 2017),  
99 *Drosophila* (Bi et al., 2012; Gronke et al., 2005) and *C. elegans* (Chughtai et al., 2015; Xie et al.,  
100 2019; Zhang et al., 2010), although a transgenic zebrafish *plin2-tdtomato* line was very recently  
101 described (Lumaquin et al., 2020).

102           Here, we report the generation and validation of zebrafish perilipin reporter lines,  
103 including *Fus(EGFP-plin2)* and *Fus(plin3-RFP)*, in which we inserted fluorescent reporters in-  
104 frame with the coding sequence at the genomic loci. These reporter lines faithfully recapitulate  
105 the endogenous expression of *plin2* and *plin3* in larval zebrafish, allowing for *in vivo* imaging of  
106 lipid droplet dynamics in live animals at the subcellular, tissue, organ and whole larvae level.  
107 Using these lines, we describe the ordered recruitment of *plin3* and then *plin2* to lipid droplets  
108 in intestinal enterocytes following the consumption of a high fat meal, reveal a delay in hepatic  
109 expression of *plin2* and *plin3* during development and identify a population of *plin2*-positive  
110 lipid droplets adjacent to neuromasts in the posterior lateral line.

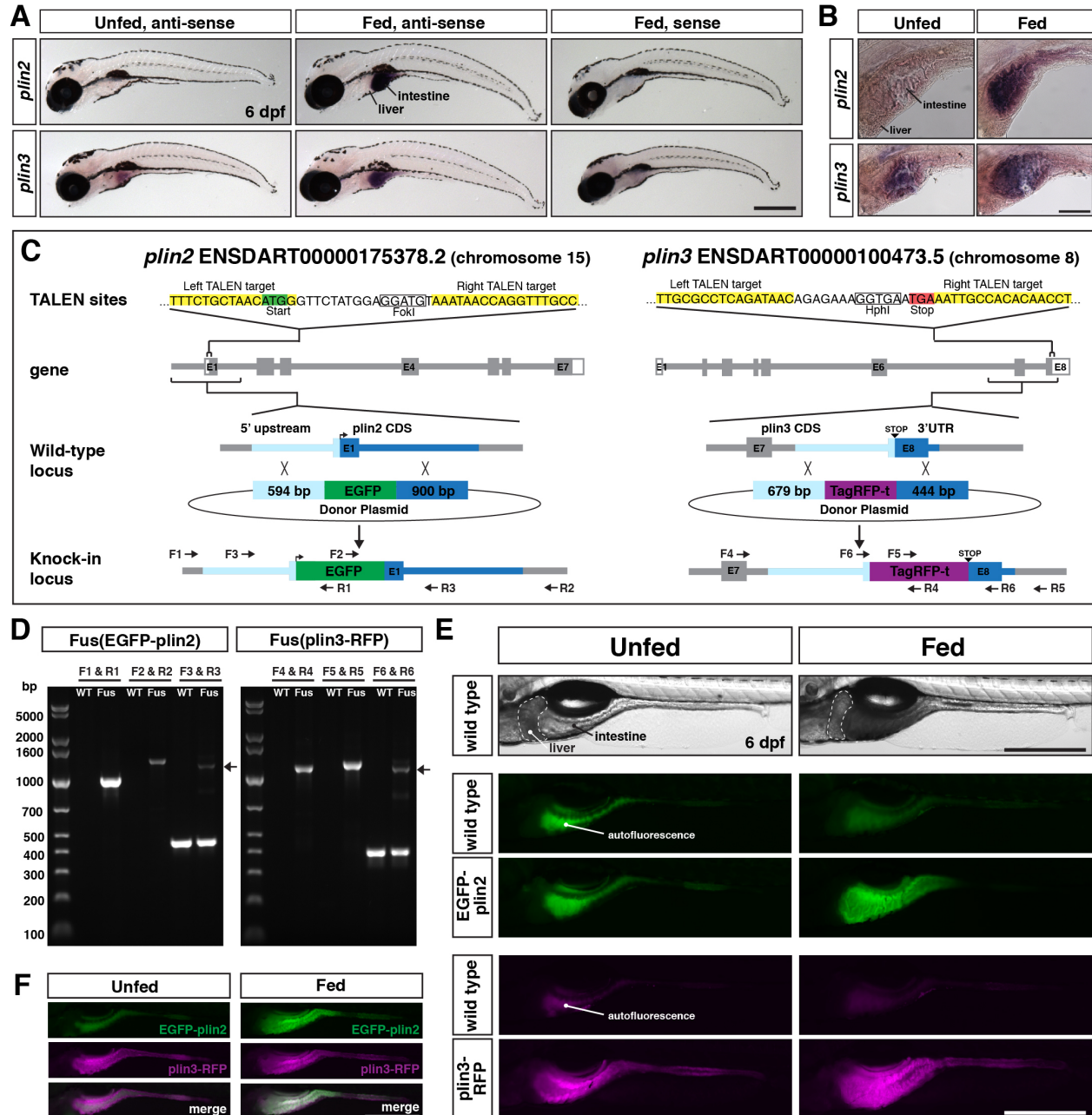
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## 116 **Results and Discussion**

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118 ***Perilipin 2 and perilipin 3 are expressed in the intestine of larval zebrafish.*** To determine the  
119 tissue localization of *plin2* and *plin3* mRNA expression in larval zebrafish, we performed whole  
120 mount *in situ* hybridization. Our data indicate that *plin2* is not expressed in any tissues of unfed  
121 larvae at 6 days post fertilization (dpf) (Figure 1A,1B). However, following a high-fat meal, *plin2*  
122 mRNA expression is strongly induced in the intestine, consistent with findings in mice (Lee et  
123 al., 2009) and with our previous RNAseq and qRT-PCR data (Zeituni et al., 2016). *plin3* mRNA is  
124 present in the intestines of both unfed and fed larvae, and the signal in the intestine is stronger  
125 in high-fat fed larvae (Figure 1A & 1B), again consistent with previous data in fish and mice (Lee  
126 et al., 2009; Zeituni et al., 2016). Surprisingly, neither *plin2* nor *plin3* mRNA expression was  
127 noted in the liver or in other tissues of unfed or high-fat fed zebrafish larvae at 6 dpf (Figure 1A  
128 & 1B) as was expected from studies in mouse and human tissues (Brasaemle et al., 1997; Heid  
129 et al., 1998; Than et al., 1998; Wolins et al., 2006).

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134 **Figure 1: Generation of in-frame fluorescent reporters in the endogenous *plin2* and *plin3* loci.**

135 (A,B) Representative images of whole mount *in situ* hybridization with probes against zebrafish

136 *plin2* (ENSDARG00000042332) and *zgc:77486* (*plin3/4/5*) (ENSDARG00000013711) at 6 dpf

137 either unfed or following feeding with a high fat meal for 90 min. ISH was performed 3 times

138 for each gene with n = 10 larvae per probe per experiment; scale = 500 μm (A), scale = 100 μm

139 (B). *Plin2* is expressed in the intestine only following a high-fat meal whereas *plin3* is expressed

140 in the intestine in unfed fish and has stronger expression following a high fat meal. (C)  
141 Overview of the location and strategy used for TALEN-mediated genome editing. EGFP was  
142 fused in-frame at the N-terminus of *plin2*. TALEN targets in *plin2* are located in exon 1 of the  
143 *plin2-203 ENSDART00000175378.2* transcript and flank a FokI restriction site, loss of which was  
144 used to confirm cutting activity. A donor plasmid with the coding sequence for EGFP and *plin2*  
145 homology arms was co-injected with TALEN mRNA into 1-cell stage embryos to be used as a  
146 template for homology directed repair. mTag-RFP-t was fused in frame at the C-terminus of  
147 *plin3*. TALEN targets were located in exon 8 of the *plin3 ENSDART00000100473.5* transcript  
148 and flank the termination codon and a HphI restriction site, loss of which was used to confirm  
149 cutting activity. A donor plasmid with the coding sequence for mTagRFP-t and *plin3* homology  
150 arms was co-injected with TALEN mRNA into 1-cell stage embryos to be used as a template for  
151 homology directed repair. (D) Following identification of fluorescent embryos in the F1  
152 generation, RT-PCR and sequencing of genomic DNA using the primers noted on the knock-in  
153 loci depicted in (C) were used to confirm successful in-frame integration of the fluorescent tags.  
154 The size of the amplicons expected for correct integration were as follows: F1-R1 1033bp, F2-  
155 R2 1340bp, F3-R3 440bp for WT & 1224bp for *Fus(EGFP-plin2)* fusion, F4-R4 1218bp, F5-R5  
156 1274bp, F6-R6 401bp for WT & 1187 for *Fus(plin3-RFP)*. Arrows indicate the larger amplicon in  
157 heterozygous fish carrying the fusion alleles. (E) Imaging in live larvae (6 dpf) reveals expression  
158 of EGFP-*plin2* only in the intestine of larvae fed a high-fat meal (7 h post-start of 2 h meal) and  
159 *plin3*-RFP is expressed in the intestine of both unfed and fed larvae (4.5 h post-start of 2 h meal,  
160 larvae are heterozygous for the fusion proteins; the lumen of the intestine has strong  
161 autofluorescence in wild-type and transgenic fish; see Figure 1 – figure supplement 2 for images  
162 of whole fish). Scale = 500  $\mu$ m. (F) Examples of larvae expressing both EGFP-*plin2* and *plin3*-  
163 RFP (7 h post start of meal). Scale = 500  $\mu$ m.

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166 **Generation of knock-in/fusion lines to study lipid droplets *in vivo*.** To study how *plin2* and  
167 *plin3* regulate lipid droplet dynamics *in vivo*, we generated fluorescent *plin2* and *plin3* zebrafish  
168 reporter lines (Figure 1C). We specifically wanted to explore the precise timing of *plin2* and  
169 *plin3* association with lipid droplets immediately following a high fat meal. We also recognized

170 that overexpression of plin proteins can alter lipid droplet dynamics by altering the rate of  
171 lipolysis and lipophagy, which can result in altered levels of cytoplasmic lipid and lipoprotein  
172 secretion (Bell et al., 2008; Bosma et al., 2012; Fukushima et al., 2005; Listenberger et al., 2007;  
173 Magnusson et al., 2006; McIntosh et al., 2012; Tsai et al., 2017). Therefore, we chose to tag the  
174 endogenous proteins by engineering knock-in alleles. We used TALENs to introduce a double  
175 strand break near the start codon in exon 1 in *plin2* (ENSDART00000175378.2) or adjacent to  
176 the termination codon in the last exon of *plin3* (ENSDART00000100473.5). TALEN mRNA was  
177 injected into 1-cell stage embryos, together with donor constructs including either *EGFP* for  
178 *plin2* or *tagRFP-t* for *plin3*, flanked by the noted homology arms to direct homology directed  
179 repair (Figure 1C). The left homology arm for *plin2* included the 54-bp variable sequence we  
180 discovered upstream of exon 1, that may contain a regulatory element for control of *plin2*  
181 expression (Figure 1 – figure supplement 1). From the injected F0 adult fish, we identified a  
182 single founder for each knock-in allele by out-crossing and screening progeny for fluorescence  
183 in the intestine at 6 dpf either following a meal (*Fus(EGFP-plin2)*) or prior to feeding (*Fus(plin3-*  
184 *RFP)*). Correct integration of the fluorescent constructs was confirmed by PCR on genomic DNA  
185 of individual larvae, followed by sequencing (Figure 1D).

186 Consistent with our whole mount *in situ* hybridization data, we observe EGFP-plin2 only  
187 in the intestine of larvae fed a high-fat meal, whereas plin3-RFP is detected in the intestine of  
188 both unfed and fed larvae (Figure 1E). No fluorescence is noted in the liver or in other tissues  
189 at 6 dpf, regardless of feeding status (for images of whole fish see Figure 1 – figure supplement  
190 2). Fish carrying the knock-in alleles can be in-crossed and resulting progeny express RFP-plin3  
191 in the intestine prior to feeding and both transgenes are expressed subsequent to consuming a  
192 high-fat meal (Figure 1F). Thus, these knock-in alleles faithfully recapitulate the endogenous  
193 tissue mRNA expression patterns of *plin2* and *plin3* as revealed by *in situ* hybridization of fixed  
194 larval zebrafish (Fig 1A).

195  
196 **EGFP-plin2 and plin3-RFP decorate lipid droplets in intestinal enterocytes.** To confirm that  
197 EGFP-plin2 and plin3-RFP decorate the surface of lipid droplets in the intestine, we performed  
198 confocal imaging in live larvae following a high-fat meal. *Fus(EGFP-plin2)/+* larvae were fed  
199 liposomes containing the fluorescent fatty acid analogue BODIPY 558/568-C12. This fatty acid



200 analogue can be incorporated into both phospholipid and triglycerides for storage in lipid  
201 droplets in larval zebrafish (Quinlivan et al., 2017). As expected, EGFP-plin2 decorates the  
202 surface of BODIPY 558/568-C12-positive lipid droplets in the intestine, which is visualized as  
203 rings of EGFP fluorescence in single confocal z-slices (Figure 2A). Similarly, plin3-RFP localizes to  
204 the surface of intestinal lipid droplets labeled with the green fluorescent BODIPY FL-C12 fatty  
205 acid analogue (Figure 2A). In larvae heterozygous for both *Fus(EGFP-plin2)* and *Fus(plin3-RFP)*,  
206 we found that lipid droplets can be labeled by both plin3-RFP and EGFP-plin2 proteins (Figure  
207 2B). While resolving cell membranes in the anterior intestine of larvae is difficult with bright-  
208 field or differential interference contrast microscopy due to the three-dimensional nature of  
209 the intestinal folds and small cell size, these perilipin reporter lines could be crossed to fish  
210 carrying transgenic markers of the cell membranes (Alvers et al., 2014) to better elucidate to  
211 localization of lipid droplets within individual enterocytes if desired.

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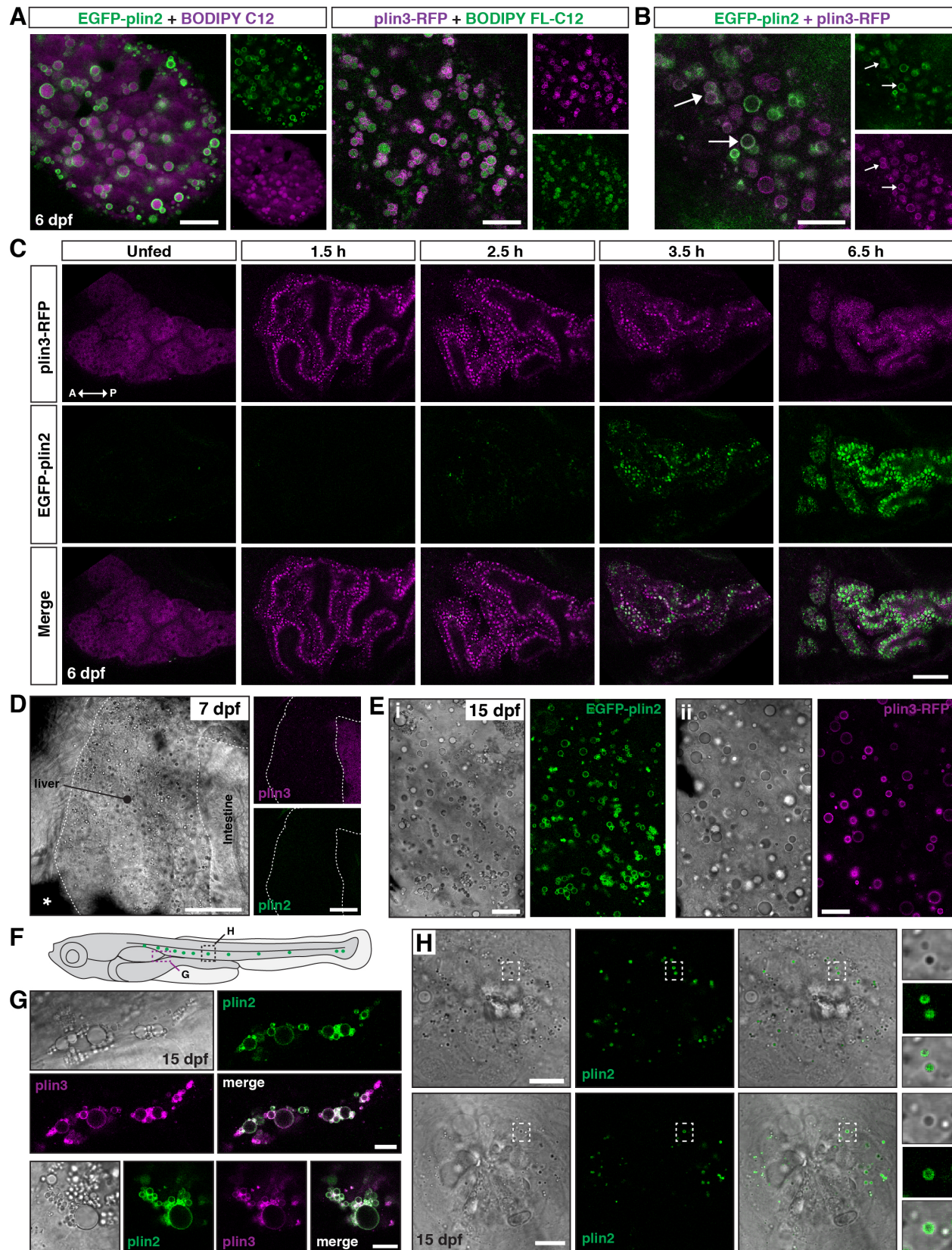
### 213 **Investigating the temporal dynamics of EGFP-plin2 and plin3-RFP in live intestinal**

214 **enterocytes.** The localization of perilipin 2 and perilipin 3 on lipid droplets in enterocytes in  
215 response to high fat remains poorly understood. Prior studies in mice have suggested that  
216 PLIN3 is located on lipid droplets following an acute high-fat meal, but not following chronic  
217 high-fat feeding (D'Aquila et al., 2015; Lee et al., 2009). In contrast, despite upregulation of  
218 PLIN2 protein in the intestine following an acute feed, PLIN2 was only present on lipid droplets  
219 in enterocytes following chronic high-fat feeding (D'Aquila et al., 2015; Lee et al., 2009).  
220 However, these findings were based on single time-points and no studies have imaged  
221 perilipins in the intestine at the level now possible with these transgenic zebrafish lines.

222 Plin3 is expressed throughout the cytoplasm in cells, including enterocytes, in the  
223 absence of lipid droplets and binds to nascent lipid droplets as they emerge from the  
224 endoplasmic reticulum (Bulankina et al., 2009; Chung et al., 2019; Lee et al., 2009; Skinner et  
225 al., 2009; Wolins et al., 2005). In contrast, plin2 is only stable when bound to lipid droplets, and  
226 is quickly ubiquitinated and degraded in the absence of droplets (Xu et al., 2005). In keeping  
227 with these data, in unfed larval zebrafish, in the absence of lipid droplets, plin3-RFP is  
228 distributed throughout the cytoplasm of the intestinal enterocytes (Figure 2C, Unfed). After

229 consuming a high-fat meal, the plin3-RFP pattern changes considerably, there is less  
230 cytoplasmic signal and bright puncta are present, which correspond to lipid droplets of various  
231 sizes (1.5 h). However, weak EGFP-plin2 signal, exclusively on lipid droplets, only emerges ~2-3  
232 hr after the start of a meal, which is consistent with the need to both transcribe (Figure 1A &  
233 (Zeituni et al., 2016)) and translate the protein after the start of the meal. As time continues,  
234 the EGFP-plin2 signal on droplets increases strongly and the plin3-RFP fluorescence becomes  
235 predominantly cytoplasmic again by 6-7 h.

236 Because plin3-RFP is already present in the cytoplasm before the emergence of lipid  
237 droplets, the timing of the appearance of this transgene on lipid droplets is likely similar to the  
238 unlabeled plin3 allele. However, while we suspect that the additional time necessary to  
239 translate and fold the EGFP tag (~30-60 min, (Balleza et al., 2018; Heim et al., 1995)) likely  
240 delays the appearance of EGFP-plin2 on lipid droplets compared to unlabeled plin2, the visible  
241 detection of EGFP-plin2 starting at ~2-3 h is still consistent with the peak in *plin2* mRNA  
242 expression between 1-3 h following the start of a high-fat meal (Zeituni et al., 2016). It is also  
243 unclear whether the fluorescent tags alter the stability or removal of the perilipins from the  
244 lipid droplets. Despite these caveats, these reporters indicate a clear progression from plin3 to  
245 plin2 on intestinal lipid droplets in zebrafish. The shift from plin3 to plin2 over time is consistent  
246 with displacement of PLIN3 by PLIN2 as lipid droplets grow in size in cultured 3T3-L1 adipocytes  
247 (Wolins et al., 2005). However, it is unclear whether this ordered recruitment in the intestine is  
248 specific to fish, or whether it would also be observed in mammals if more frequent sampling  
249 was performed following an acute high-fat meal. The physiological significance of this shift in  
250 perilipin lipid droplet association in enterocytes remains to be determined, though we  
251 hypothesize that it may aid in the regulation of chylomicron production and post-prandial  
252 plasma lipid levels.



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255 **Figure 2: EGFP-plin2 and plin3-RFP decorate lipid droplets in the intestine, liver, adipocytes**  
256 **and in cells surrounding neuromasts.** (A) EGFP-plin2 (green) and plin3-RFP (magenta) label the  
257 lipid droplet surface in the intestine of 6 dpf larvae fed with a high-fat meal containing either  
258 BODIPY 558/568 C12 (magenta) or BODIPY FL-C12 (green) to label the stored lipids. Note the  
259 558/568 C12 is not fully incorporated into stored lipid and is also found diffuse in the  
260 cytoplasm. Scale = 10  $\mu\text{m}$ . (B) EGFP-plin2 and plin3-RFP can decorate the same lipid droplets in  
261 the intestine. Arrows denote examples of dual-labeled droplets, scale = 10  $\mu\text{m}$ . (C) Lateral  
262 views of the anterior intestine in unfed larvae and in larvae at different time-points following  
263 the start of feeding with a high-fat meal for 1 h. Fish were heterozygous for both *Fus(plin3-RFP)*  
264 and *Fus(EGFP-plin2)*. Images are representative of 3 independent experiments (15-25 fish per  
265 experiment); data presented are from one experiment. Scale = 50  $\mu\text{m}$ . (D) Lateral view of the  
266 liver in a 7 dpf larvae heterozygous for both *Fus(plin3-RFP)* and *Fus(EGFP-plin2)*. Scale = 50  $\mu\text{m}$ .  
267 (E) Liver micrographs from 15 dpf larval zebrafish fed a diet of Gemma + 4% cholesterol for 10  
268 days. Lipid droplets in hepatocytes can be labeled with EGFP-plin2 (i) and with plin3-RFP (ii).  
269 Scale = 20  $\mu\text{m}$ . (F) Cartoon of 15 dpf larval zebrafish showing the general location of images in  
270 panels G and H. (G) EGFP-plin2 and plin3-RFP can both decorate lipid droplets in adipocytes.  
271 Fish are heterozygous for both transgenes and were fed Gemma + 4% cholesterol for 10 days.  
272 Scale bars = 10  $\mu\text{m}$ . (H) Examples of lipid droplets around neuromasts in fish heterozygous for  
273 *Fus(EGFP-plin2)* at 15 dpf. Panels on right show droplets in the boxed regions to the left. Scale  
274 bars = 10  $\mu\text{m}$ .

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276

277 **Plin2 and plin3 decorate hepatic lipid droplets in older larvae.** Brightfield imaging of livers in  
278 larvae at 6-7 dpf suggest they contain lipid droplets (Figure 2D) and we have shown previously  
279 that these droplets store lipids synthesized with BODIPY fatty acid analogues (Carten et al.,  
280 2011; Quinlivan et al., 2017). However, confocal imaging in unfed and fed fish indicates that  
281 these droplets are only very rarely labeled with EGFP-plin2 or plin3-RFP (Figure 2D). In  
282 contrast, hepatic lipid droplets in older larvae can be labeled with EGFP-plin2 and plin3-RFP  
283 (Figure 2E). While labeled lipid droplets are present in fish fed our standard Gemma diet, when

284 this diet was supplemented with 4% cholesterol, the hepatic lipid droplets tend to be more  
285 abundant and are more often decorated by perilipins 2 and 3 (Figure 2E). Studying the  
286 differential expression of perilipins in hepatocyte of young vs. older larvae using these  
287 transgenic lines provide an opportunity to yield novel insights into the transcriptional regulation  
288 of perilipins and how the perilipins influence hepatic lipid storage and mobilization *in vivo*.

289

290 **EGFP-*plin2* and *plin3*-RFP decorate adipocyte lipid droplets.** Using fluorescent lipophilic dyes,  
291 Minchin & Rawls have described 34 distinct regions of adipose tissue in zebrafish, including 5  
292 visceral and 22 subcutaneous adipose tissue depots (Minchin and Rawls, 2017a; Minchin and  
293 Rawls, 2017b). One of the earliest depots to develop is the abdominal visceral adipose tissue  
294 (AVAT), which appears at the posterior aspect of the swim bladder at ~5 mm standard length  
295 (Minchin and Rawls, 2017b). Confocal imaging in fish carrying the *plin* knock-in alleles indicates  
296 that the lipid droplets in the adipocytes of the AVAT tissue are labeled with both EGFP-*plin2*  
297 and *plin3*-RFP (Figure 2F & 2G). Thus, we expect that the perilipin knock-in alleles will be a  
298 valuable tool to assist in studies of adipocyte lipid droplet dynamics *in vivo* during development  
299 and in pathological conditions.

300

301 **EGFP-*plin2* indicates the presence of lipid droplets around neuromasts.** Unexpectedly, when  
302 imaging larvae at 15 dpf, we consistently noted small EGFP-*plin2*-positive lipid droplets in the  
303 neuromasts of the posterior lateral line (Figure 2H). The neuromasts are sensory epithelial  
304 receptor organs that contain hair cells that respond to changes in movement and pressure of  
305 the surrounding water (Chitnis et al., 2012; Thomas et al., 2015). The lipid droplets appear at  
306 the edge of the organs, suggesting that they are likely not within the hair cells, but may be  
307 located in either the support cells, mantle cells (Chitnis et al., 2012; Thomas et al., 2015) or in  
308 the neuromast border cells (Seleit et al., 2017). These findings are consistent with the report of  
309 lipid droplets adjacent to neuromasts in zebrafish imaged with lattice light sheet PAINT  
310 microscopy (Legant et al., 2016). Crossing the *Fus(EGFP-*plin2*)* line to transgenic reporter lines  
311 for the different cell-types (Parinov et al., 2004; Steiner et al., 2014; Thomas and Raible, 2019)

312 will allow the cellular location of these organelles to be identified and may provide insight into  
 313 the possible role the lipid droplets play in neuromast physiology.

314

315 **Additional transgenic lines are also available for overexpression of human PLIN2 and PLIN3.**

316 While the knock-in lines are superior for imaging plin2 and plin3 in the zebrafish because they  
 317 are expressed under the control of the endogenous promoter and regulatory elements, we also  
 318 have a number of additional Tol2-based transgenic lines available which could be useful in  
 319 specific contexts or for specific purposes. These lines express human PLIN2 or PLIN3 under the  
 320 control of the zebrafish FABP2, FABP10a or Hsp70I promoters for over-expression in the yolk  
 321 syncytial layer & intestine, liver or throughout the larvae following heat shock, respectively  
 322 (Table 1 & Table 1 - figure supplement 1).

323

324 **Table 1: Comparison of available transgenic perilipin lines**

Transgenic Line	Promoter	Coding sequence	Tissue Expression
<i>Fus(EGFP-plin2)</i>	Integration into the endogenous plin2 locus	zebrafish plin2 ENSDART00000175378.2	Intestine, liver, adipose, neuromasts, rare LDs in YSL
<i>Tg(FABP2: EGFP-PLIN2)</i>	Zebrafish intestinal fatty acid binding protein (FABP2)	Human perilipin 2 ENST00000276914.7	Yolk syncytial layer, intestine
<i>Tg(FABP10a: EGFP-PLIN2)</i>	Zebrafish liver fatty acid binding protein (FABP10a)	Human perilipin 2 ENST00000276914.7	Liver
<i>Tg(Hsp70I: EGFP-PLIN2)</i>	Zebrafish heat shock cognate 70-kd protein, like	Human perilipin 2 ENST00000276914.7	Widespread tissue expression following heat shock Labeled LDs observed in intestine and liver
<i>Fus(plin3-RFP)</i>	Integration into the endogenous plin3 locus	zebrafish plin3 ENSDART00000100473.5	Intestine, liver, adipose Cytoplasmic in addition to LDs
<i>Tg(FABP2: PLIN3-EGFP)</i>	zebrafish intestinal fatty acid binding protein (FABP2)	Human perilipin 3 ENST00000221957.9	Yolk syncytial layer, intestine Cytoplasmic in addition to labeled LDs in intestine
<i>Tg(Hsp70I: PLIN3-EGFP)</i>	Zebrafish heat shock cognate 70-kd protein, like	Human perilipin 3 ENST00000221957.9	Widespread tissue expression following heat shock; often mosaic Cytoplasmic in addition to labeled LDs in intestine and liver

325

326

327 In summary, the *Fus(EGFP-plin2)* and *Fus(plin3-RFP)* knock-in zebrafish lines provide the  
328 opportunity to study perilipins and lipid droplet biology *in vivo* at the organelle, cell, tissue,  
329 organ and whole animal level. These lines exploit the advantages of the zebrafish model and  
330 will be important tools to understand how lipid droplet dynamics are affected by different  
331 genetic and physiological manipulations. Future studies with these fish may also help us  
332 explain the poorly understood genetic association of the PLIN2 locus with a host of highly  
333 prevalent metabolic diseases such as fatty liver, insulin resistance and type 2 diabetes,  
334 cardiovascular disease and atherosclerosis (Conte et al., 2016).

335

336

## 337 **Methods and materials**

338

### 339 **Zebrafish husbandry and maintenance**

340 All procedures using zebrafish (*Danio rerio*) were approved by the Carnegie Institution  
341 Department of Embryology Animal Care and Use Committee (Protocol #139). Zebrafish stocks  
342 (AB line) were maintained at 27°C in a circulating aquarium facility with a 14:10 h light:dark  
343 cycle. For propagation and stock maintenance, starting at 5.5 dpf, larvae were fed with  
344 GEMMA Micro 75 (Skretting) 3x a day until 14 dpf, GEMMA Micro 150 3x a day + Artemia 1x  
345 daily from 15-42 dpf and then GEMMA Micro 500 1x daily supplemented once a week with  
346 Artemia. The nutritional content of GEMMA Micro is: 59% Protein 59%; 14% Lipids, 0.2% Fiber;  
347 14% Ash; 1.3% Phosphorus; 1.5% Calcium; 0.7% Sodium; 23000 IU/kg Vitamin A; 2800 IU/kg  
348 Vitamin D3; 1000 mg/kg Vitamin C; 400 mg/kg Vitamin E. Embryos were obtained by natural  
349 spawning and raised in embryo medium at 28.5°C in culture dishes in an incubator with a 14:10  
350 h light:dark cycle. Zebrafish sex is not determined until the juvenile stage, so sex is not a  
351 variable in experiments with embryos and larvae.

352

### 353 **High-fat & high-cholesterol diets**

354 To feed 6 dpf larvae a high-fat, high-cholesterol meal, larvae were immersed in a solution of 5%  
355 chicken egg yolk liposomes in embryo media for 1-2 h on an orbital shaker at 29°C as described  
356 in (Zeituni and Farber, 2016). Where noted, BODIPY (558/568)-C12 (D3835, Thermo Fisher  
357 Scientific) or BODIPY FL-C12 (D3822, Thermo Fisher Scientific) were included in the egg yolk  
358 solution at 4 µg/ml. Following feeding, larvae were washed in embryo media, and screened for  
359 full guts by examining intestinal opacity under a stereomicroscope. Fed larvae were either  
360 maintained in embryo media until imaging, fixed immediately for *in situ* hybridization or guts  
361 were extracted and frozen for qRT-PCR. A high-cholesterol diet was made by soaking Gemma  
362 Micro 75 in a diethyl ether and cholesterol (Sigma-Aldrich C8667) for a final content of 4% w/w  
363 cholesterol after ether evaporation (based on (Stoletov et al., 2009)). Larvae were fed with this  
364 high-cholesterol diet 3x daily from 5.5 dpf to 15 dpf where noted.

365

366



### 367 ***In situ* hybridization**

368 Zebrafish embryos were staged according to (Kimmel et al., 1995) and fixed 4%  
369 paraformaldehyde in phosphate buffered saline overnight at 4°C, washed twice with MeOH and  
370 stored in MeOH at -20°C. To generate riboprobes, 754 base pairs of the *perilipin 2* (*plin2*;  
371 *ENSDARG00000042332*; *ENSDART00000175378.2* transcript) and 900 base pairs of the  
372 *zgc:77486* (*perilipin 3*; *plin3*; *ENSDARG00000013711*, *ENSDART00000100473.5*  
373 transcript)(GRCz11) mRNA sequences were amplified from cDNA using the primers noted in  
374 Supplementary Table 1 and TA cloned into the dual promoter pCRII-TOPO® (Thermo Fisher  
375 Scientific, K207020). Sense and anti-sense probes were synthesized using the DIG RNA labeling  
376 kit (Roche 11277073910) using T7 and SP6 polymerases (Roche 10881767001 & 10810274001).  
377 Whole mount *in situ* hybridization was performed as previously described (Thisse and Thisse,  
378 2008) on 6 dpf unfed and high-fat fed larvae. Larvae were mounted in glycerol and imaged  
379 using a Nikon SMZ1500 microscope with HR Plan Apo 1x WD 54 objective, Infinity 3 Lumenera  
380 camera and Infinity Analyze 6.5 software or a Nikon E800 microscope with a 20X/0.75 Plan Apo  
381 Nikon objective and Canon EOS T3 camera using EOS Utility image acquisition software.

382

### 383 **DNA extraction and genotyping**

384 Genomic DNA was extracted from embryos, larvae or adult fin clips using a modified version of  
385 the HotSHOT DNA extraction protocol (Meeker et al., 2007). Embryos or tissue were heated to  
386 95°C for 18 minutes in 100 µL of 50 mM NaOH, cooled to 25°C and neutralized with 10 µL of 1  
387 M Tris-HCL pH 8.0. The gDNA extractions and PCR verifying integration of the fluorescent tags  
388 into the genomic loci was performed using the REExtract-N-Amp Tissue PCR kit (Sigma-  
389 Aldrich). PCR amplicons were run on 1 or 2% agarose gels in TBE and gels were imaged with Bio-  
390 Rad Gel ChemiDoc XRS system and Quantity One software.

391

### 392 **RNA isolation, cDNA synthesis and quantitative RT-PCR**

393 Following a 90 min feed with 5% chicken egg yolk, guts were dissected from larvae (6 dpf, 10  
394 guts pooled per sample) and stored in *RNAlater* (Thermo Fisher Scientific AM7020) at 4°C for 1  
395 week. RNA was isolated using a Trizol-based RNA prep adapted from (N.J. and T.L., 2014).

396 Samples were subsequently treated with DNase I and purified using the RNA Clean and  
397 Concentrator kit (Zymo Research R1013). cDNA was synthesized using the iScript™ cDNA  
398 Synthesis Kit (1708891, Bio-Rad Laboratories, Inc.). qRT-PCR samples were prepared using  
399 SsoAdvanced™ Universal SYBR® Green Supermix (1725271, Bio-Rad Laboratories, Inc.). Primers  
400 targeting zebrafish *plin2* transcripts were previously validated (See Supplementary Table  
401 1)(Zeituni et al., 2016) and zebrafish 18S (*rps18*) was used as the reference gene (Otis et al.,  
402 2015). qRT-PCR was performed in triplicate for each sample with the Bio-Rad CFX96 Real-Time  
403 System with 45 cycles: 95°C for 15 seconds, 59°C for 20 seconds, and 72°C for 20 seconds.  
404 Results were analyzed with the Bio-Rad CFX Manager 3.0 software and relative gene expression  
405 was calculated using the  $\Delta\Delta CT$  method (Livak and Schmittgen, 2001).

406

#### 407 **Genome editing to create perilipin fusion lines**

408 *Fus(EGFP-plin2)* and *Fus(plin3-RFP)* lines were created with TALEN-mediated genome editing.  
409 The genomic region around the location targeted for editing in the *plin2* and *plin3* genes was  
410 amplified by PCR and sequenced from multiple wild-type AB fish to identify any discrepancies  
411 between the published sequences and Farber lab stocks. During this process we identified a  
412 variable 54-bp region prior to exon 1 in the *plin2-203* transcript (*ENSDART00000175378.2*)(See  
413 Figure 1 – figure supplement 1) and discovered a polymorphism (T>C) in the ATG designated as  
414 the start codon in the *plin2-202* *ENSDART00000129407.4* transcript. We designed our editing  
415 strategy to fuse the EGFP coding sequence in-frame with the *ENSDART00000175378.2*  
416 transcript in fish carrying the 54-bp intronic sequence and performed editing only in fish  
417 carrying this full-length sequence. Two pairs of TALENs were designed per gene using the Mojo  
418 Hand design tool (Neff et al., 2013) and cloned with the FusX assembly system and the pKT3Ts-  
419 goldyTALEN vector (Ma et al., 2013; Ma et al., 2016; Welker et al., 2016). The designed TALEN  
420 pairs for *plin2* (pair 1 TTTCTGCTAACATGG & AAATAACCAGGTTTGCC; pair 2  
421 TTTCTGCTAACATGGGT & AATAACCAGGTTTGCC) flank a Fok1 restriction site just downstream of  
422 the endogenous start codon. The designed TALEN pairs for *plin3* (pair 1 TTGCGCCTCAGATAAC  
423 & AATTGCCACACAACCT; pair 2 CAGATAACAGAGAAA & CACACAACCTAAATA) flank a Hph1 site  
424 immediately upstream of the endogenous termination codon. TALEN mRNA was in vitro

425 transcribed using the T3 Message Machine Kit (Thermo Fisher Scientific, AM1348), injected into  
426 1-cell stage zebrafish embryos, and cutting efficiency of each pair was assessed by monitoring  
427 the loss of either FokI (NEB R0109) or HphI (NEB R0158) digestion due to TALEN nuclease  
428 activity. Nuclease activity was higher for *plin2* TALEN pair 1 and *plin3* TALEN pair 1 and these  
429 were used subsequently for genome integration. Donor plasmids used as templates for  
430 homology directed repair were assembled using the three-fragment MultiSite gateway  
431 assembly system (Invitrogen, 12537-023). For *plin2*, the 5' element consisted of 594bp of  
432 genomic sequence upstream of the *plin2* start codon, the middle-entry element contained the  
433 *plin2* kozak sequence followed by the EGFP coding sequence lacking a termination codon that  
434 was in-frame with the 3' element which consisted of 900bp of genomic sequence including and  
435 downstream of *plin2* start codon. For *plin3*, the 5' element consisted of the 679 bp of genomic  
436 sequence immediately upstream of the termination codon, a middle entry element of in-frame  
437 tagRFP-t (amplified from Addgene # 61390, which has been codon modified for zebrafish  
438 (Horstick et al., 2015)) with a C-terminal termination codon, and a 3' element consisting of the  
439 444 bp genomic sequence downstream of the *plin3* termination codon. Genome integration  
440 was accomplished by co-injection of 150 pg of TALEN mRNA and 100 pg of donor plasmid into  
441 1-cell stage embryos. Injected embryos were raised to adulthood, out-crossed to wild-type fish  
442 and resulting F1 progeny were screened for either EGFP or RFP fluorescence; it was necessary  
443 to feed the *Fus(EGFP-plin2)* fish with a high-fat meal in order to detect EGFP-*plin2* fluorescence  
444 when integrated correctly. Correct in-frame integration of the fluorescent reporters was  
445 confirmed by PCR and sequencing. *Fus(EGFP-plin2)* fish can also be genotyped using primers  
446 for EGFP. For primer information, see Supplementary Table 1.

447

#### 448 **Additional transgenic zebrafish**

449 Additional transgenic zebrafish expressing human *perilipin 2* (*PLIN2*, *ENSG00000147872*  
450 *GRCh38.p13*) or *perilipin 3* (*PLIN3*, *ENSG00000105355*) under the control of various promoters  
451 were generated with the Tol2-Gateway molecular cloning system (Kwan et al., 2007). The  
452 coding sequence of human *PLIN2* with an N-terminal EGFP tag was provided by John  
453 McLauchlan (Targett-Adams et al., 2003) and re-cloned into pCR8 (ThermoFisher Scientific).

454 The human *PLIN3* (TIP47) coding sequence was obtained from Flexgene clones collection  
455 (Harvard Medical School, clone ID: HsCD00004695) and re-cloned into pCR8. The intestine-  
456 specific intestinal fatty acid binding protein (*fabp2*) promoter was provided by Michel Bagnat;  
457 the liver-specific liver fatty acid binding protein 10a (p5E *fabp10a*, (-2.8 kb)), originally  
458 described in (Her et al., 2003), was provided by Brian Link. The heat shock cognate 70-kDa  
459 protein, like (*hsp70l*) promoter (p5E-*hsp70l*), p3E-EGFPpA, pME-EGFP no stop, and p3E-polyA  
460 plasmids were originally provided by Chi-bin Chien (Kwan et al., 2007). Gateway recombination  
461 was used to combine entry plasmids into the pDestTol2Pa2 plasmid to create *tg(fabp2: EGFP-*  
462 *PLIN2)*, *tg(fabp10a: EGFP-PLIN2)*, *tg(hsp70l: EGFP-PLIN2)*, *tg(fabp2: PLIN3-EGFP)* and *tg(hsp70l:*  
463 *PLIN3-EGFP)* transgene constructs. Plasmids were injected (25-50 pg) along with 40 pg tol2  
464 transposase mRNA into 1-cell stage AB embryos. Zebrafish were raised to adulthood, out-  
465 crossed to wild-type fish and resulting embryos were screened for progeny stably expressing  
466 the fluorescent constructs. Transgenic larvae expressing a heat shock-inducible construct were  
467 incubated at 37°C for 45 min in 15 mL of embryo media and screened a few hours later.  
468 Embryos expressing a *fabp2*-driven construct were screened at 2-4 dpf for EGFP expression in  
469 the yolk syncytial layer and embryos expressing the *fabp10a*-driven construct were screened at  
470 5 or 6-dpf following liver development. At least two stable lines per construct were initially  
471 generated, the pattern of expression was verified to be the same in each line and subsequently,  
472 a single line for each construct was used for experiments and propagated by out-crossing to  
473 wild-type AB fish.

474

#### 475 **Fluorescence microscopy**

476 Zebrafish larvae at 6 dpf were mounted in 3% methylcellulose in embryo media on glass slides  
477 and imaged live with a Zeiss Axio Zoom V16 microscope equipped with a Zeiss PlanNeoFluar Z  
478 1x/0.25 FWD 56 mm objective, AxioCam MRm camera, EGFP and Cy3 filters and Zen 2.5 Blue  
479 edition software. For confocal imaging of lipid droplets in the tissues of live larvae at 6, 7 and  
480 15 dpf, larvae were anesthetized with tricaine (Sigma-Aldrich A5040) and mounted in 3%  
481 methylcellulose between on glass slides with bridged coverslips. Images were obtained with a  
482 Leica DMI6000 inverted microscope and Leica 63x/1.4 HCX PL Apo oil-immersion objective with

483 a Leica TCS-SP5 II confocal scanner with photomultiplier detectors using Leica Application Suite  
484 Advanced Fluorescence 2.7.3.9723 image acquisition software. Images were obtained using 4-  
485 line average, and recorded with 12-bit dynamic range. EGFP and BODIPY-FL were excited with  
486 an argon laser (488 nm) and had a collection window of 498 – 530 nm. BODIPY (558/568)-C12  
487 was imaged with 561 laser and collection window of 571 – 610 nm and mTagRFP-t was imaged  
488 with 561 laser and collection window of 575 – 650 nm.

489

#### 490 **Additional software**

491 Graphing was performed with GraphPad Prism (GraphPad Software). DNA, mRNA and protein  
492 sequence alignments were performed with MacVector V15.5 (MacVector, Inc.). Micrographs  
493 were adjusted and cropped as needed in Fiji (NIH) and figures were assembled in Adobe  
494 Illustrator CS5 (Adobe Systems). Microsoft Word and Excel were used for manuscript  
495 preparation and data analysis, and references were compiled with EndNote 8x.

496

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511

512

513 **Author Contributions**

514

515 M.H.W. contributed to study conception and design, resources, data acquisition, analysis and

516 interpretation of data, data presentation, manuscript writing and revision and funding

517 acquisition. S.C.E. contributed methodology, resources and funding acquisition. S.A.F.

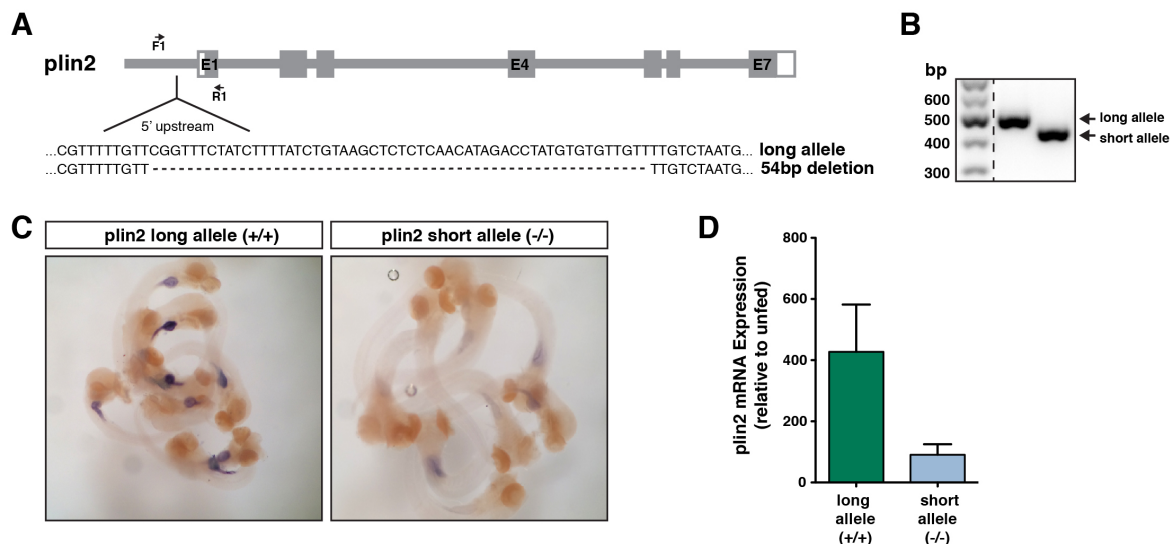
518 contributed to study conception and design, resources, manuscript review and editing,

519 supervision, project administration and funding acquisition.

520

521 **Supplementary Figures**

522



523

524

525

526 **Figure 1 – figure supplement 1: A deletion upstream of exon 1 in *plin2* impacts gene**

527 **expression following a high fat meal. (A) A 54-bp deletion was noted in the 5' untranslated**

528 **region upstream of exon 1 in the *plin2* ENSDART00000175378.2 transcript in AB wild-type**

529 **stocks. (B) RT-PCR using the primers noted in A reveal the long vs. short (deletion) alleles. (C)**

530 ***In situ* hybridization indicates that larvae homozygous for the long allele have stronger**

531 **expression of *plin2* in the intestine following a high-fat meal than fish homozygous for the short**

532 **allele (~10 larvae shown at 6 dpf, fed 90 min prior to fixation). (D) Quantitative RT-PCR**

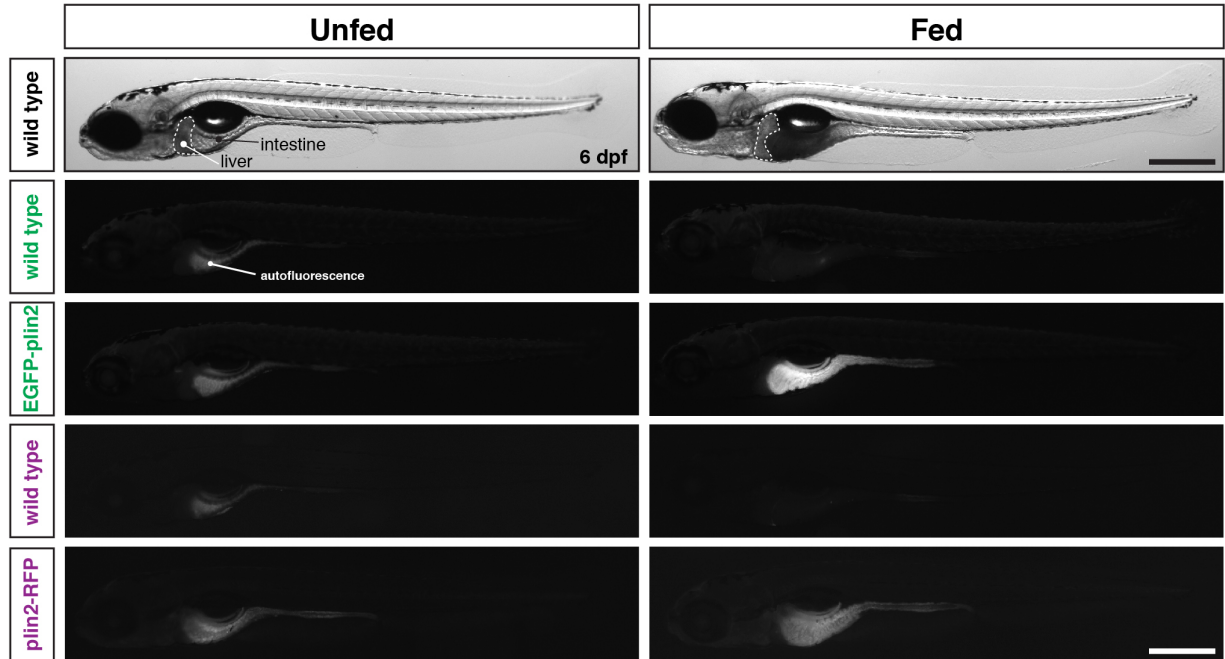
533 **confirmed the difference in *plin2* expression induction between larvae with the long vs. short**

534 **allele following a high fat meal relative to unfed controls (N = 5 samples of isolated guts from 10**

535 **larvae per sample following a 90 min feed, samples are from two independent experiments;**

536 **mean +/- SD).**

537



538

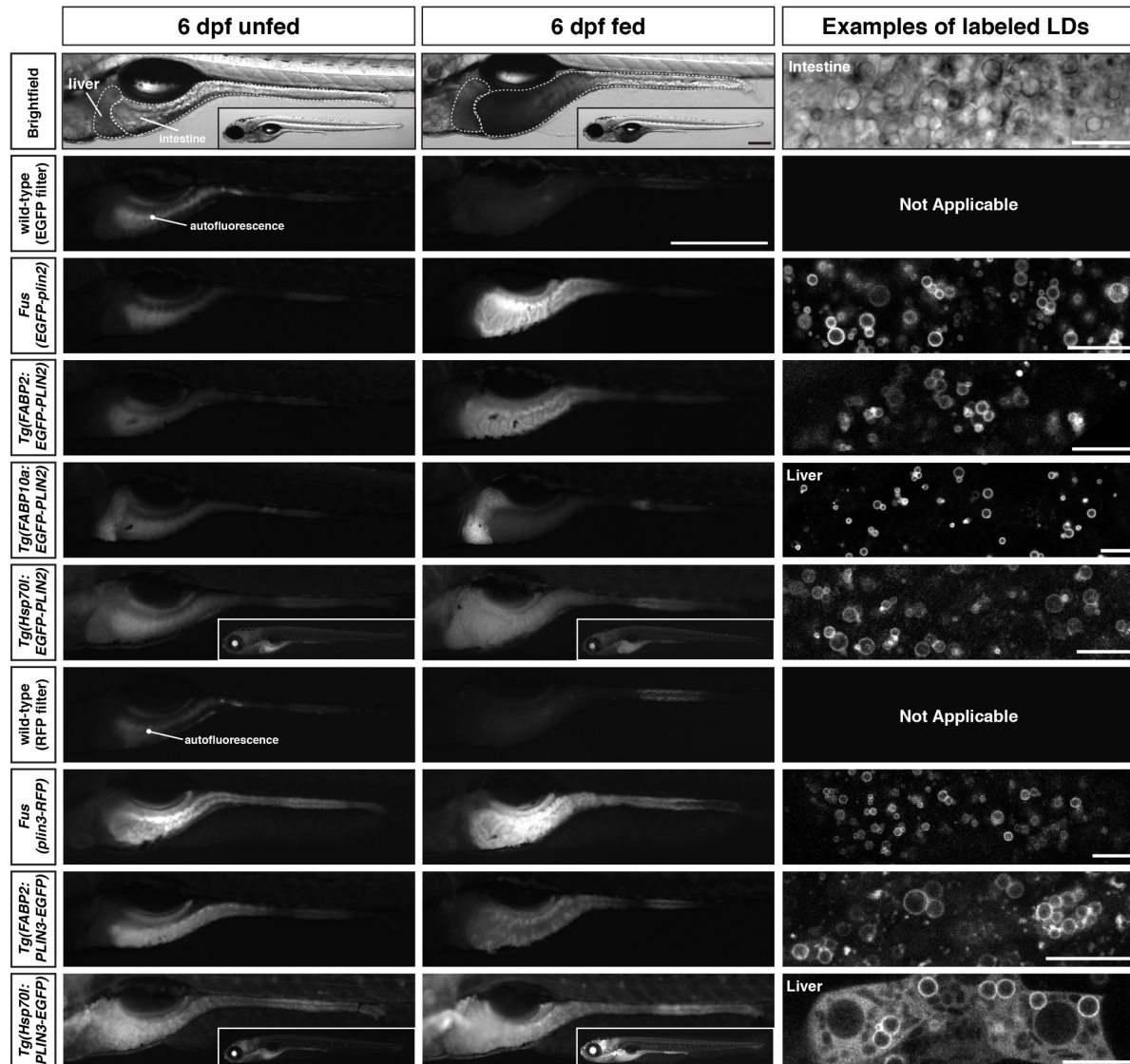
539

540 **Figure 1 – figure supplement 2: Whole fish images corresponding to Figure 1E**

541 Whole mount images of the same fish shown in figure 1E. Scale = 500  $\mu$ m.

542





543  
544

545 **Table 1 – figure supplement 1: Whole mount images and examples of perilipin-labeled lipid**  
546 **droplets corresponding to the transgenic zebrafish lines noted in Table 1.** All fish are  
547 heterozygous for the noted transgene. Heat shock transgenic lines were incubated at 37°C for  
548 45 min prior to feeding. For whole mount images, larvae were fed for 2 h with a high-fat meal  
549 and imaged 3-4.5 h (*plin3* lines) or 5-8 h (*plin2* lines) following the start of the feed. Where  
550 appropriate, images of whole fish are included as insets. Scale = 500 μm for main images and  
551 insets. In the right column, examples of confocal micrographs are included to show the  
552 fluorescent perilipin proteins labeling lipid droplets in the various transgenic lines following a  
553 high-fat meal. Unless noted, images are from the intestine. Scale = 10 μm for each image.

554  
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557

558 **Supplementary Table 1: Primers**

Gene/Figure #	Purpose	Sequence
<i>plin2</i> Fig 1A,B	<i>In situ</i> probe	F: CGT GCA AAG GAC TGG ATA AG
<i>plin2</i> Fig 1A,B	<i>In situ</i> probe	R: AGA CCC CTG AGA CTG GAC AC
<i>plin3</i> Fig 1A,B	<i>In situ</i> probe	F: AGA CCG ACT GGA ACC TCA GA
<i>plin3</i> Fig 1A,B	<i>In situ</i> probe	R: CTG GCG TGT CTG CAG TAA GA
<i>plin2</i> Fig 1 – S1 F1	TALEN target site verification	F: TGC ACC TTA AAC TCA AAC CGT G
<i>plin2</i> Fig 1 – S1 R1	TALEN target site verification	R: AGG ATT AAA GTG GCA AAC CTG G
<i>plin2</i> Fig 1 – S1	qRT-PCR	F: TTC ACT AAT GGG CTG GAA GA
<i>plin2</i> Fig 1 – S1	qRT-PCR	R: CAC CAC ACA TGT GCT CTG AA
<i>rps18</i> Fig 1- S1	qRT-PCR	F: TGC AGA ACC CTC GCC AGT ACA AAA TCC CAG
<i>rps18</i> Fig 1 – S1	qRT-PCR	R: CCA GAA GTG ACG GAG ACC ACG GTG AGC CCT
<i>plin2</i>	Amplification of left homology arm for donor plasmid	F: GGG GAC AAC TTT GTA TAG AAA AGT TGA AGC CCT GAT ACA ACA TAT TCG C
<i>plin2</i>	Amplification of left homology arm for donor plasmid	R: GGG GAC TGC TTT TTT GTA CAA ACT TGA GTT AGC AGA AAA TCT GCA AAA G
<i>plin2</i>	Amplification of right homology arm for donor plasmid	F: GGG GAC AGC TTT CTT GTA CAA AGT GGA AAT GGG TTC TAT GGA GGA TGT
<i>plin2</i>	Amplification of right homology arm for donor plasmid	R: GGG GAC AAC TTT GTA TAA TAA AGT TGA GTG ATT GGA TGT GTT TTG GAT TG
<i>EGFP</i>	pME EGFP for donor plasmid	F: GGG GAC AAG TTT GTA CAA AAA AGC AGG CTG CTA ACA TGG TGA GCA AGG GCG AGG AGC TGT
<i>EGFP</i>	pME EGFP for donor plasmid	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TCC ACC GCC CTT GTA CAG CTC GTC CAT GCC GAG A
<i>plin3</i>	Amplification of left homology arm for donor plasmid	F: GGG GAC AAC TTT GTA TAG AAA AGT TGA ACC AGC AGA TTG GCC AGG TAG
<i>plin3</i>	Amplification of left homology arm for donor plasmid	R: GGG GAC TGC TTT TTT GTA CAA ACT TGA TTC ACC TTT CTC TGT TAT CTG AGG
<i>plin3</i>	Amplification of right homology arm for donor plasmid	F: GGG GAC AGC TTT CTT GTA CAA AGT GGA AAA TTG CCA CAC AAC CTA AAT AAA TCT G
<i>plin3</i>	Amplification of right homology arm for donor plasmid	R: GGG GAC AAC TTT GTA TAA TAA AGT TGA ACT TCT TCA TAG AAT CCT GTG TCC A
<i>tagRFP-t</i>	pME tagRFP-t for donor plasmid	F: GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT GAT GGT GAG CAA AGG AGA GGA AC
<i>tagRFP-t</i>	pME tagRFP-t for donor plasmid	R: GGG GAC CAC TTT GTA CAA GAA AGC TGG GTT TAC TTG TAC AGC TCA TCC ATT CC
<i>plin2</i> Fig 1C F1	Integration confirmation PCR	F: TGC TGA AGA AGA GTG ATC TCA TCC
<i>plin2</i> Fig 1C R1	Integration confirmation PCR	R: GTG CGC TCC TGG ACG TAG CCT TCG
<i>plin2</i> Fig 1C F2	Integration confirmation PCR	F: CAA GGA GGA CGG CAA CAT CCT GGG
<i>plin2</i> Fig 1C R2	Integration confirmation PCR	R: AAA TGT TTG CAC ATC AGA CTA CAG
<i>plin2</i> Fig 1C F3	Integration confirmation PCR	F: TGG TCT CAG CGT GAA ATC CC
<i>plin2</i> Fig 1C R3	Integration confirmation PCR	R: TCC TTG CTT TGT CAA CCT ACC A
<i>plin3</i> Fig 1C F4	Integration confirmation PCR	F: AGG AAC AGC TTC TCA ATG CTC G
<i>plin3</i> Fig 1C R4	Integration confirmation PCR	R: GCA TCA CAG GTC CAT TGC TA
<i>plin3</i> Fig 1C F5	Integration confirmation PCR	F: CTT CCC TAG CAA TGG ACC TG
<i>plin3</i> Fig 1C R5	Integration confirmation PCR	R: AAC TAT TCG GTG GCG CAG AA
<i>plin3</i> Fig 1C F6	TALEN target site verification &	F: CGG CAG TCT CTT GAT GGA GT

	Integration confirmation PCR	
<i>plin3</i> Fig 1C R6	TALEN target site verification & Integration confirmation PCR	R: ACA ACA ACT ATA AAG TAT GGC TTG C
<i>EGFP</i>	Genotyping Fus( <i>EGFP-plin2</i> )	F: GGT GAA CTT CAA GAT CCG CCA
<i>EGFP</i>	Genotyping Fus( <i>EGFPplin2</i> )	R: GAA CTC CAG CAG GAC CAT GT

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