

**Fig. S1.** Changes in fatty acid composition of minor fatty acids in seed lipid during seed development of WT and  $PLD_{\zeta}$ . (A) Arachidic acid (20:0). (B) Eicosadienoic acid (20:2). (C) Eicosatrienoic acid (20:3). (D) Docosanoic acid (22:0). (E) Erucic acid (22:1) (SD, n=3). Significant differences (*T* test, *P* <0.05) between  $PLD_{\zeta}$  and WT are denoted with an asterisk.





**Fig. S2.** Total radioactivity incorporation into fatty acids of seed lipids during [<sup>14</sup>C]acetate labeling of WT (A) and  $PLD_{\zeta}$  (B) developing seeds (SD, n=3, time points: 3, 6, 10, 30, 60, 180 min).



**Fig. S3.** Incorporation of  $[{}^{14}C]$  acetate in to fatty acids of PE/PG, PA, PI and MGDG in WT (A) and PLD<sub> $\zeta$ </sub> (B) developing embryos (SD, n=3, time points: 3, 6, 10, 30, 60, 180 min).



**Fig. S4.** Regiochemistry of  $[{}^{14}C]$ acetate labeling in fatty acids incorporated into TAG. Radioactive TAG was digested with *Rhizomucor miehei* lipase as described in Materials and Methods. Products of lipase digestion of  $[{}^{14}C]$ TAG were measured and percentage of each product over total was calculated. (A) WT. (B) PLD<sub> $\zeta$ </sub> (SD, n=3, time points: 3, 6, 10, 30, 60, 180 min).



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**Fig. S5.** Total radioactivity incorporation into fatty acids of seed lipids during [<sup>14</sup>C]glycerol labeling of WT (A) and PLD<sub> $\zeta$ </sub> (B) developing seeds (SD, n=3, time points: 5, 10, 20, 30, 60, 180).





Fig. S6. Accumulation of total labeled PC, DAG and TAG in WT (A) and  $PLD_{\zeta}$  (B) developing embryos from [<sup>14</sup>C]glycerol labeling (SD, n=3, time points: 5, 10, 20, 30, 60, 180). The early labeling time frame is redrawn in (C, D) for WT and  $PLD_{\zeta}$  respectively.





**Fig. S7.** Accumulation of labeled PE/PG, PA/PI and MGDG in WT (A) and  $PLD_{\zeta}$  (B) developing embryos from [<sup>14</sup>C]glycerol labeling (SD, n=3, time points: 5, 10, 20, 30, 60, 180).





48 49 **Fig. S8.** Incorporation of  $[^{14}C]$ -glycerol into acyl chains of glycerolipids during labeling of WT and PLD<sub> $\zeta$ </sub> 50 developing embryos. (A)  $[^{14}C]$ -glycerol into acyl chains of TAG, DAG and PC in WT embryos. (B)  $[^{14}C]$ -glycerol 51 into acyl chains of TAG, DAG and PC in PLD<sub> $\zeta$ </sub> embryos (SD, n=3, time points: 5, 10, 20, 30, 60, 180). The early 52 labeling time frame is redrawn in (C, D) for WT and PLD<sub> $\zeta$ </sub> respectively.

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**Table S1.** Initial labeling of glycerol backbone. 5 and 10 minute time points from  $[^{14}C]$ -glycerol incorporation into the backbone of lipids were linearly regressed to obtain the initial rate of labeling (i.e. slope) and the ratio of slopes, PC/TAG was calculated to evaluate the relative use of *de novo* DAG for PC and TAG (n=3). 56 57 58

	DAG	PC	TAG	PC/TAG
WT	124.5 ± 64.20	141.3 ± 29.45	36.50 ± 4.482	3.9
OE	235.6 ± 32.33	119.8 ± 35.55	28.13 ± 11.89	4.3