# Supplemental Materials

***Molecular Biology of the Cell***

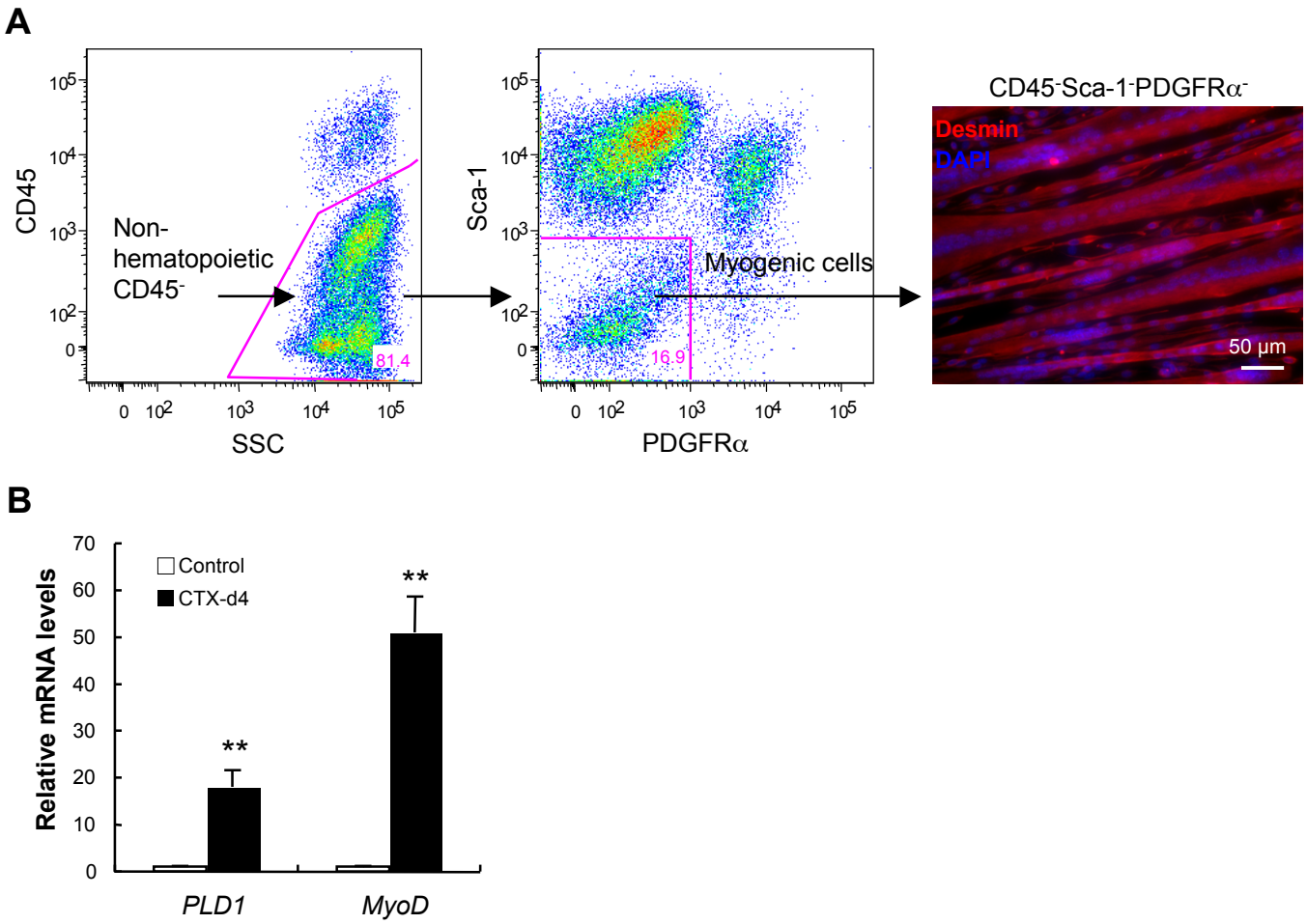
Teng et al.

# Supplementary Materials

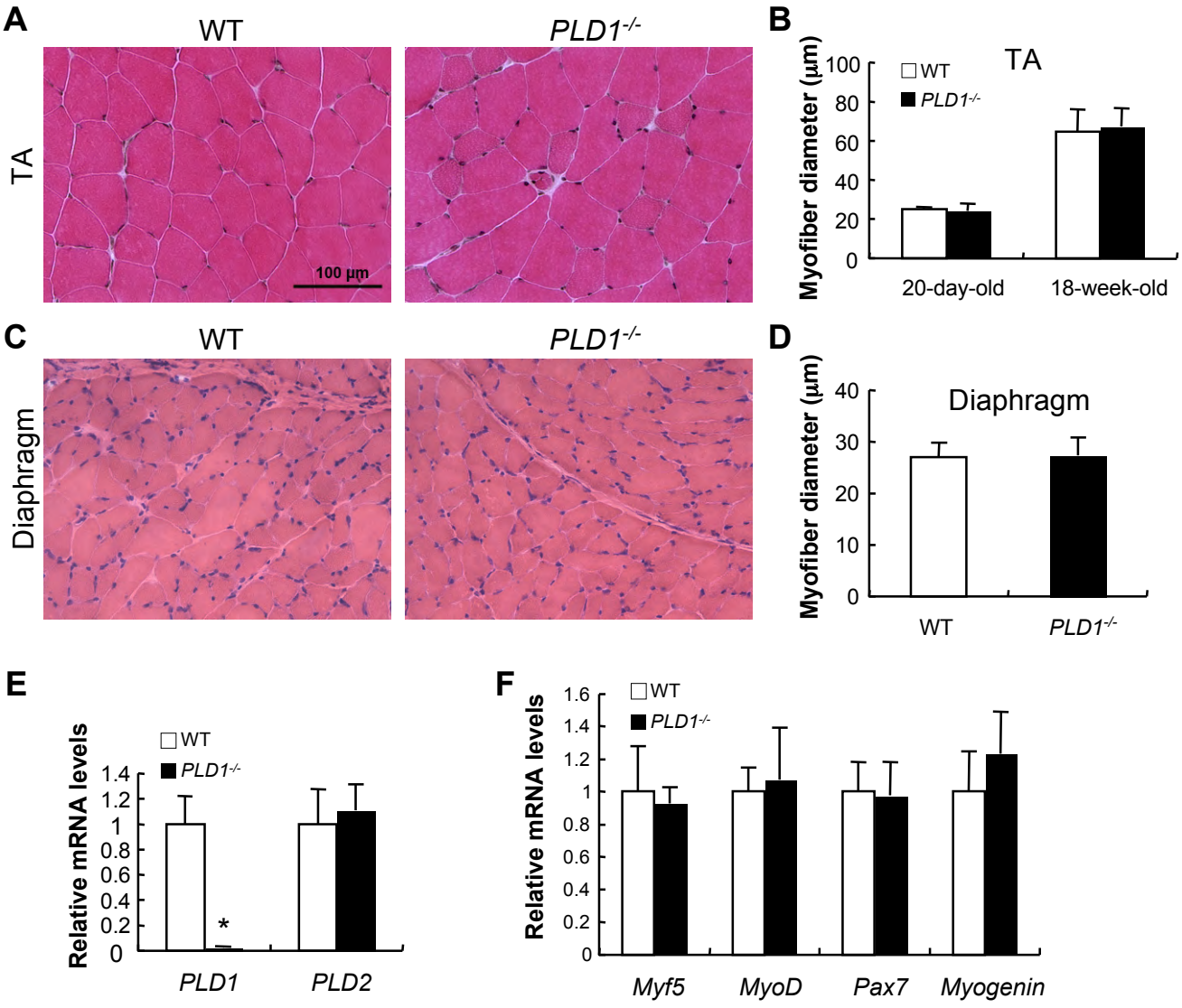
## Supplementary methods

### Fluorescent-Activated Cell Sorting (FACS)

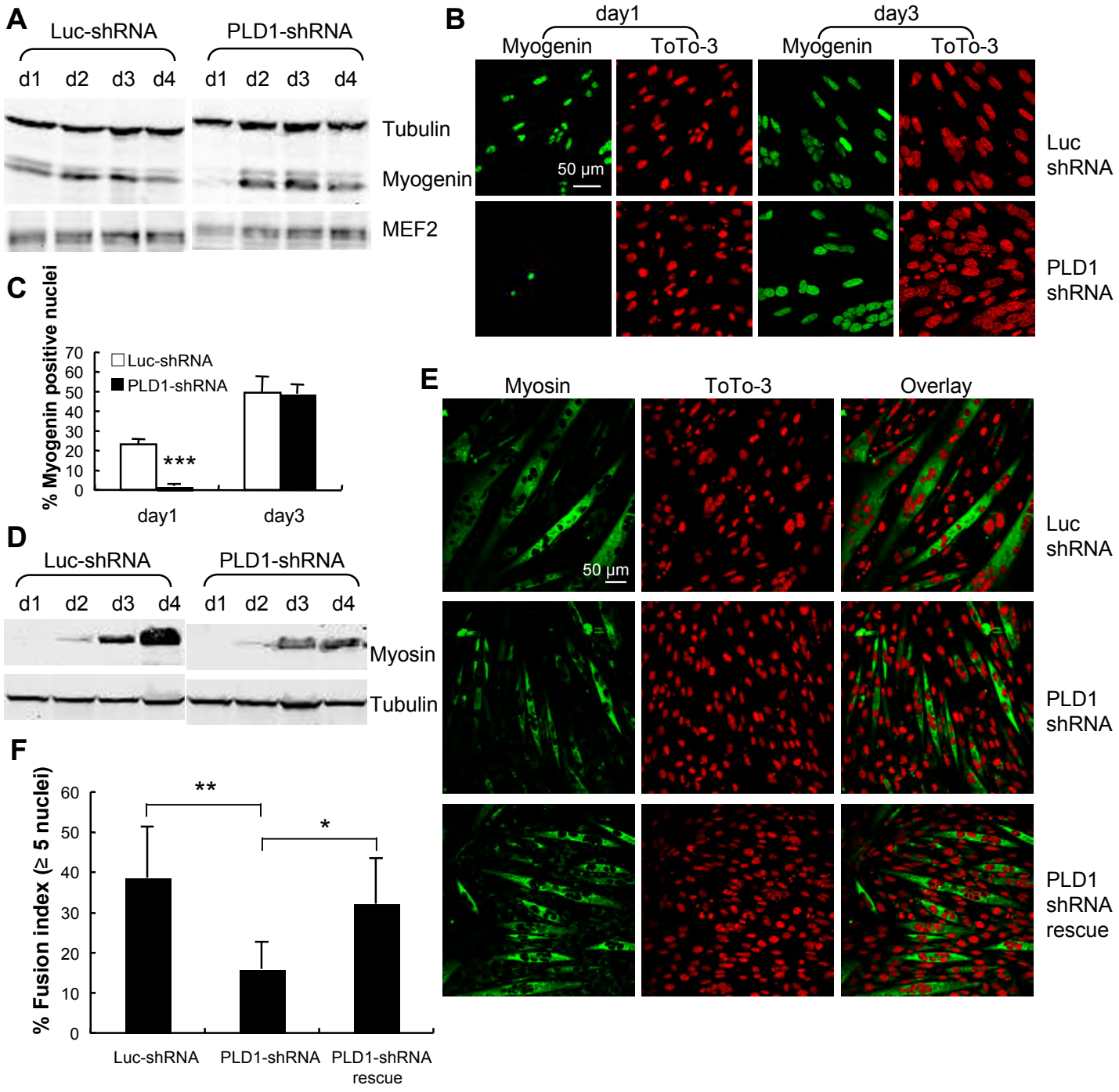
Skeletal muscles were harvested, finely minced using sterile scalpels, and digested at 37ºC for 45 min using 8.4 U dispase II (Roche Applied Science, IN) and 35 mg collagenase D (Roche Applied Science, IN) per gram of tissue with gentle trituration every 15 min. Muscle digests were then filtered through 100-µm and 40-µm cell strainers, spun down and treated with RBC lysis buffer (Invitrogen, CA). Cells were then pelleted and resuspended at a concentration of $2X10^7$ cells/mL in ice-cold 1xHBSS (Hank's buffered salt solution, Invitrogen, CA) containing 0.5% BSA. Live cells were isolated by negative selection for propidium iodide staining (1 µg/mL, Sigma-Aldrich, MO) and positive selection for calcein blue staining (4.65 µg/mL, Invitrogen, CA). Antibodies used were as follows: anti-mouse CD45 (1: 300, PE conjugate, clone 30-F11, BD Biosciences, CA), anti-mouse Sca-1 (Ly-6A/E, 1:100, FITC conjugate, clone E13-161.7, BD Biosciences, CA), anti-mouse PDGFR1$\alpha$ (1:100, APC conjugate, clone APA5, Biolegend, CA). All antibody incubations were carried out on ice for 20 min. Cells were then washed with cold 1xHBSS/ 0.5%BSA and resuspended at a concentration of $10^7$ cells/mL for FACS on the BD FACSAria (Becton Dickinson, NJ). FACS data were collected using DIVA software (Becton Dickinson, NJ) and analyzed using Flowjo software (Tree Star, Inc., Macintosh version 6.4.7).



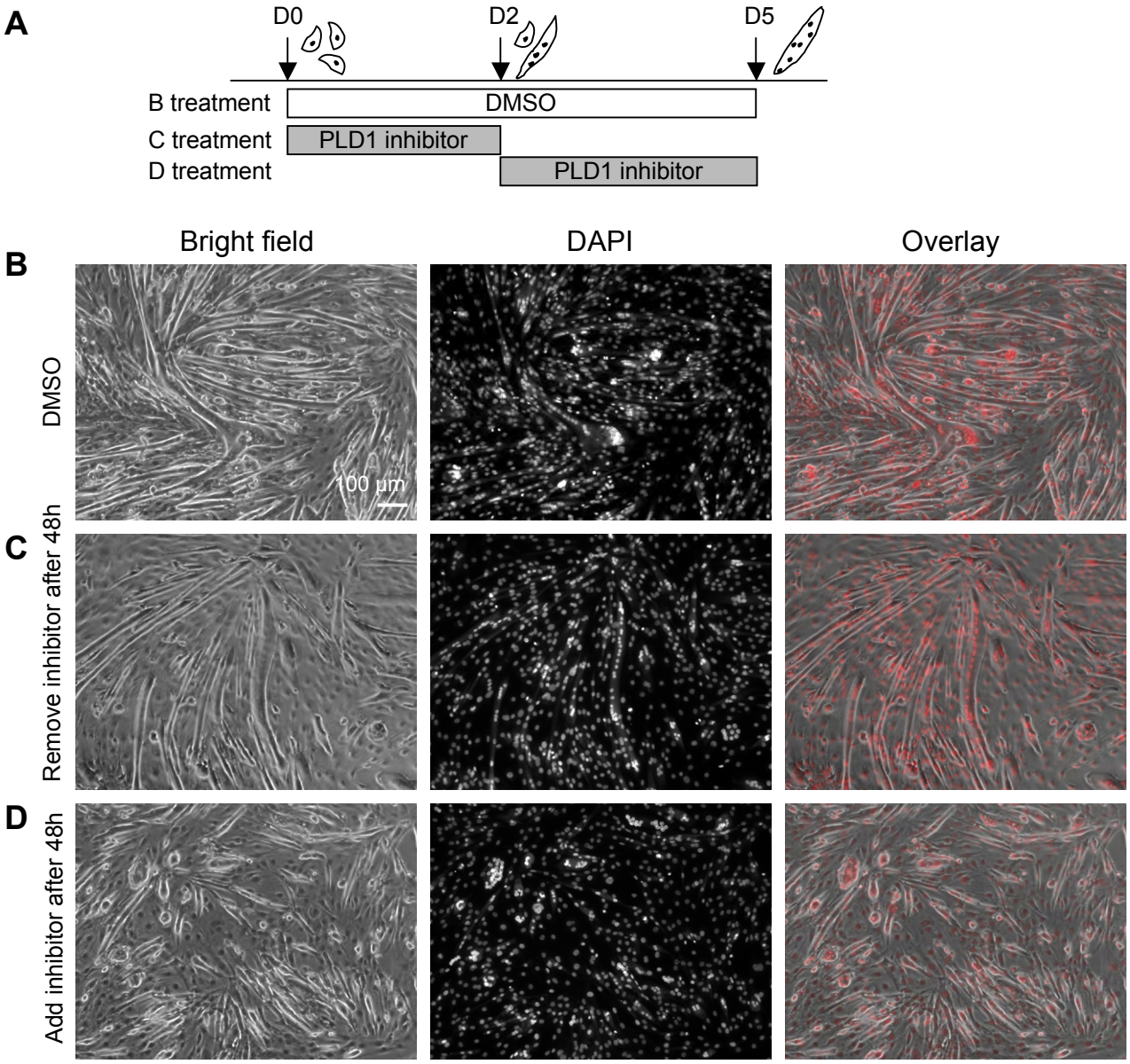

**Supplemental Figure 1. *PLD1* expression is elevated in myogenic cell fractions of CTX-injured muscle at day 4 post injury.** (A) Dot plots showing prospective isolation of myogenic cells (CD45$^-$Sca-1$^-$PDGFR$\alpha^-$) by FACS. The myogenecity of these cells were attested by the expression of myogenic marker desmin and by induction of myotube formation. (B) Expression of *PLD1* and *MyoD* was significantly increased in subfractionated myogenic cells after CTX injury. Data shown are quantitative RT-PCR results on FACS sorted myogenic cells (n = 3, **, p<0.01).

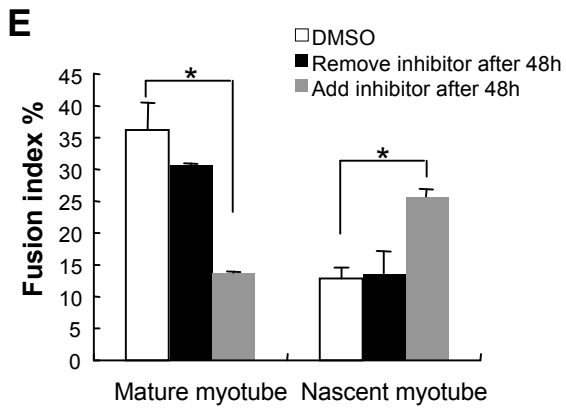



**Supplemental Figure 2.** (A-D) H&E staining of transverse sections of TA muscles (A) and diaphragm (C) of 18-week-old WT and *PLD1*$^{-/-}$ mice. Myofiber size was determined by measuring the myofiber diameter of TA muscle (B) and diaphragm (D) from WT and *PLD1*$^{-/-}$ mice (n = 4/genotype). (E) Analysis of *PLD1* and *PLD2* transcript levels in WT and *PLD1*$^{-/-}$ TA muscles by quantitative RT-PCR (n = 3, *, $p<0.05$). Ablation of PLD1 expression does not induce upregulation of *PLD2*. (F) Examination of *Myf5, MyoD, Pax7* and *myogenin* expression by quantitative RT-PCR in CTX-injured TA muscle at day 3 following injury (n = 3/genotype).

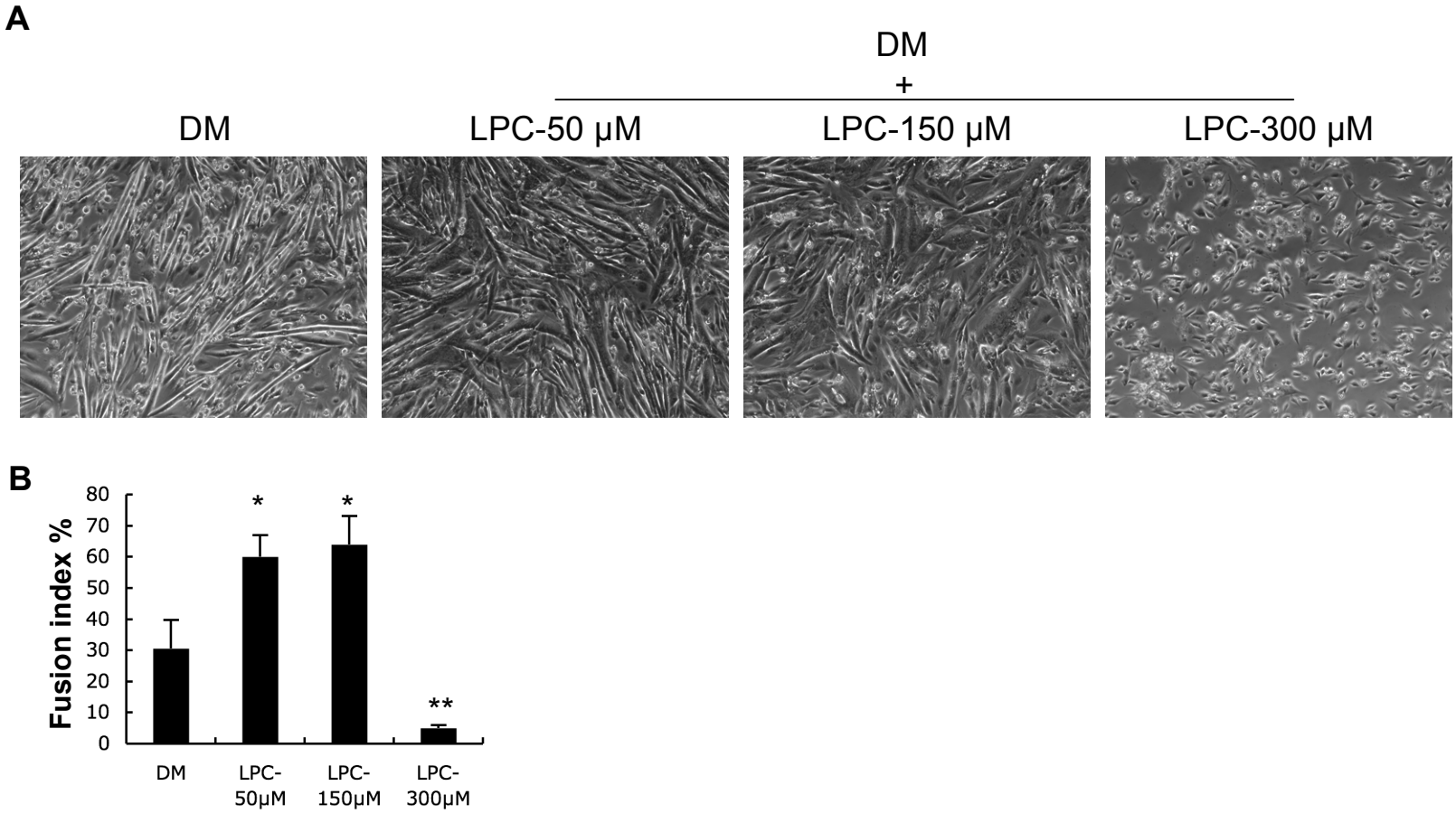



**Supplemental Figure 3. Delayed onset of myogenin and MEF2 expression in PLD1-shRNA cell lines.** (A) Western blot analysis of temporal expression of myogenin and MEF2 in Luc-shRNA and PLD1-shRNA cells during myoblast differentiation. (B, C) Immunofluorescence analysis of myogenin expression in day 1 and day 3 differentiated cultures. Toto-3 was used to image the nuclei. (n = 3, ***, $p<0.001$) (D) Western blot analysis of myosin expression in Luc-shRNA and PLD1-shRNA cells during differentiation. (E, F) Immunofluorescence analysis of myosin expression in day 4 differentiated culture. Mature myotube formation was assessed by fusion index of myotubes with 5 or more nuclei in Luc-shRNA, PLD1-shRNA and PLD1-shRNA rescued cells (n = 3, *, $p<0.05$, **, $p<0.01$).




**Supplemental Figure 4. PLD1 inhibitor specifically blocks mature myotube formation in L6 cells.** (A) Schematic representation of L6 myoblasts treated with or without PLD1 inhibitor during differentiation. L6 myoblasts were differentiated for 5 consecutive days in the following conditions: (B) DM plus DMSO. (C) DM plus 4 μM of PLD1 inhibitor for the first 48 hours followed by 3 days in DM only. (D) DM for the first 48 hours followed by 3 days in DM plus PLD1 inhibitor. DAPI stain of the nucleus was pseudocolored red. (E) Fusion indexes of mature and nascent myotubes were assessed in the above cultures and presented as mean ± SD (n = 3, *, $p<0.05$). Addition of PLD1 inhibitor for the first 48 hours of differentiation had no adverse effect on myoblast fusion, while inhibition of mature myotube formation was observed when PLD1 inhibitor was added after 48 hours of differentiation.




**Supplemental Figure 5.** (A, B) Primary myoblasts from WT mice were differentiated for 48 hrs in DM and then incubated in DM or DM supplemented with LPC for another 15 hrs. Addition of 50 μM or 150 μM LPC significantly enhanced myoblast fusion. On the contrary, 300 μM LPC blocked cell fusion (n=3, *, $p<0.05$ as compared to DM, **, $p<0.01$ as compared to DM).

**Supplementary Table 1.**

Primer sequences for RT-PCR

| Gene | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| mPLD1 | TGCATCCTCAAACGGAAAGC | AGGTACACGCTGGAGGACAC |
| mPLD2 | CGAGAAGCTCCTGGTGGTAG | GGTCACCCAGGTCAGTCAGT |
| rPLD1 | AAGCACAGTTGCTCCGATCT | GATAGCATTGCCCACCTTGT |
| Myf5 | GGCATGCCTGAATGTAACAG | GACACGGAGCTTTTATCTGC |
| Pax7 | GCACGCGTCCAGGTCTGGTT | GGGGCTGGGGCCTGTGTACT |
| MyoD | TACAGTGGCGACTCAGATGC | CGGTGTCGTCGCCATTCTG |
| Myogenin | CTACAGGCCTTGCTCAGCTC | AGATTGTGGGCGTCTGTAGG |
| mGAPDH | AACTTTGGCATTGTGGAAGG | GATGCAGGGATGATGTTCTG |
| rGAPDH | ATGCTTGTGATGGGTGTGAA | ACTGTGGTCATGAGCCCTTC |