**Supporting information**

**Supplementary Experimental Section**

**S.4.1. Lysosomal pH assay**

Lysosomal pH was measured using the Lysotracker Red (LTR) dye DND-99 (Molecular Probes, L7528, Carlsbad, CA). The lysotracker probes are weak base and fluorescent acidotropic probes for labeling and tracking acidic organelles in live cells. Therefore, the more acidic organelles become, the more LTR is taken up. Briefly, cells $6x10^5$ were seeded in 60 mm dish overnight and then media were replaced by fresh media containing either vehicle or 10μM LCL521. At indicated time points, 200nM LTR was added and incubated for another 20min before media were completely removed. Kept in the dark, cells were then washed twice with cold PBS before lifted by trypsin. After two more washes with cold PBS, cells were re-suspended in 500μl PBS. LTR fluorescence was measured using FACS analysis. Minimum of 10,000 events were scored for each sample. Results were presented as % Control with means ± SD of duplicate. * $p$ value <0.05 (*vs* Control).

**S.4.2. Dihydroceramide desaturase protein expression by Western Blot**

Membranes (**Fig.3A.)** were incubated with strip buffer (15g glycine, 1g SDS, 10ml Tween 20, adjust pH to 2.2, 1L) at room temperature for 10 min twice, then washed with PBS 10 min and TBST 10 min prior to the blocking stage. The primary antibody (ab80654, Abcam) was used at a dilution of 1:1000; the secondary anti-rabbit antibody was at 1:15,000.

### S.4.3 Light chain 3B (LC-3B) protein expression by Western Blot

Cells were treated with vehicle or 1 and 10μM LCL521 for 1, 5, 8 and 24h before collection. LC3B protein expression was visualized by western blot. The primary antibody (#2775, Cell Signaling) was used at a dilution of 1:1000; the secondary anti-rabbit antibody was at 1:15,000. Actin was utilized to monitor protein loading and transfer.

### S.4.4 Effects of LCL521 on ACDase mRNA expression

Cells were treated with vehicle or 10μM LCL521 for 1, 8 and 24h before collection. RNA molecules longer than 200 nucleotides were purified following the protocol provided in the RNeasy mini Kit (#74104, Qiagen). cDNA was synthesized from 1 μg of the total RNA using iScript™ cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). RT-PCR was performed on a Bio-Rad CFX96 detection system. Bio-Rad SsoAdvanced™ Universal SYBR GREEN Supermix™ with the primers of ACDase (Bio-Rad, #10025636) and the housekeeping gene product RPL13A1 were used. Initial steps of RT-PCR were 2 min at 95˚C, followed by 40 cycles consisting of a 5 sec at 95˚C, and 30 sec at 60˚C right after. Determination of the relative normalized expression of corresponding

ACDase mRNA against the expression of housekeeping gene-encoded protein RPL13A1 was performed by $\Delta\Delta C_T$ provided by CFX96 manager software 3.0 (Bio-Rad). Results were presented as relative normalized expression with means ± SD of triplicate. * *p* value <0.05 (*vs* Control).

**Additional figure Legends:**

**Figure 1A. Chemical structure of LCL521.**

**Supplementary Figure legends**

**S. Figure 1. Effects of LCL521 on ACDase protein expression at 1h.** MCF7 cells were treated with vehicle or with 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5 and 10μM LCL521 for 1h. **α**-ACDase and p-ACDase protein expression was visualized by western blot as described; actin was utilized to monitor protein loading and transfer.

**S. Figure 2. Effects of 10μM LCL521 on DES-1 protein expression over time.** Cells were treated with vehicle or 10μM LCL521 for 1, 2, 5, 8, 15 and 24h. ACDase (**α**- and P-ACDase) and DES-1 protein expressions were then visualized by western blot as described; actin was utilized to monitor protein loading and transfer.

**S. Figure 3. Effects of 10μM LCL521 on lysosomal pH alteration over time.** Cells were treated with vehicle or 10μM LCL521 for 1, 5, 15 and 24h. 200nM LTR was then added and evaluated as described; a minimum of 10,000 events were scored for each

sample. Results were presented as % control with means ± SD of duplicate. * *p* value <0.05 (*vs* Control)

**S. Figure 4. Effects of LCL521 on LC-3B protein expression.** Cells were treated with vehicle or 1 and 10μM LCL521 for 1, 5, 8 and 24h before collection. LC3B protein expression was visualized by western blot as described; actin was utilized to monitor protein loading and transfer.

**S. Figure 5. Effects of LCL521 on ACDase protein expressions in SCC14 A and PPC-1 cells at 5h.** Cells were treated with vehicle or 5, 10μM LCL521 for 5h. ACDase protein expression was visualized by western blot as described; actin was utilized to monitor protein loading and transfer.

**S. Figure 6. Effects of 10μM LCL521 on ACDase mRNA expression.** Cells were treated with vehicle or 10μM LCL521 for 1, 8 and 24h before collection. 1μg purified RNA from each sample was then used for cDNA synthesis. RT-PCR was performed with the primers for ACDase and the housekeeping gene product RPL13A1. The results of the relative normalized expression of corresponding ACDase mRNA against the expression of RPL13A1 was measured. Results were presented as relative normalized expression with means ± SD of triplicate. * *p* value <0.05 (*vs* Control).

**S. Figure 7. CA-074me prevents the effect of LCL521 on a-ACDase protein expression.** Cells were treated with vehicle or 4μM CA-074me (#205531, Sigma) for 30

min, then they were treated with vehicle or 10μM LCL521 for additional 5h. a-ACDase protein expression was then visualized by western blot as described; actin was utilized to monitor protein loading and transfer.

LCL521 exhibits significant effects on *ACDase* in dose and time dependent manner, and higher dose of LCL521 additionally inhibits *DES-1*, another therapeutic target for cancer. Thus, LCL521 inhibits MCF7 cell growth through these dual actions.