Supplementary information

#### **Direct Visualization of De-novo Lipogenesis in Single Living Cells**

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Supplementary figure captions.

Movie showing the dynamic motions of newly synthesized lipid droplets in living cells.

**Supplementary figure 1: Lipogenesis derived from glucose-d7 or glutamine-d5 metabolic pathway.** PANC1 cells were treated with 25 mM glucose-d7 or 4 mM glutamine-d5 or both in glucose, glutamine-free DMEM media for 3 days. SRS imaging at C-D and C-H vibration were taken.

**Supplementary figure 2: Lipogenesis in pancreatic cancer MIA PaCa2 cells. a.** MIA PaCa2 cells were treated with 25 mM glucose-d7 in glucose-free DMEM media supplemented without or with 10% FBS for 3 days. SRS imaging at C-D and C-H vibration were taken. The ratio of C-D/C-H was used to analyze the level of de-novo lipogenesis. b. Quantitative analysis of de-novo lipogenesis level in MIA PaCa2 cells. The ratio of C-D signal to C-H signal intensity on single LD was measured. Totally 10 lipid droplets were analyzed for each sample. The data was represented by Mean + SD.

**Supplementary figure 3: Lipogenesis and lipid uptake in different cancer cell lines. a.** Glucose-d<sub>7</sub> derived lipogenesis were measured in different cancer cell lines, including breast cancer MCF7, lung cancer A549, prostate cancer PC3 and pancreatic cancer PANC1 by SRS imaging at C-D and C-H vibration. **b.** Quantification of SRS signal at C-D vibration per cell. Data were shown as mean + SD. N= 5 for each group.

# Glu-d<sub>7</sub> + Gln-d<sub>5</sub>

#### Gln-d<sub>5</sub>

### Glu-d<sub>7</sub>





C-D









## Glu-d<sub>7</sub> + FBS



a





