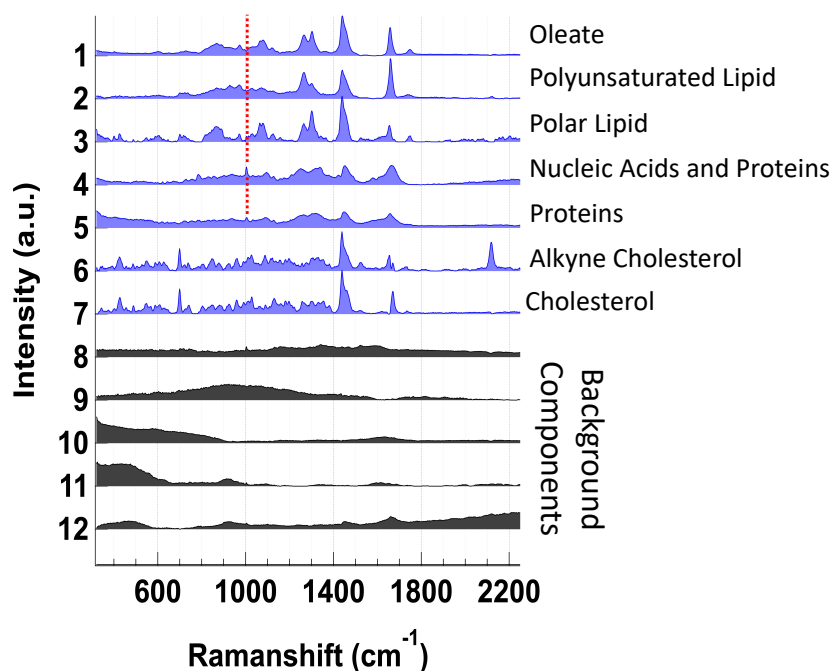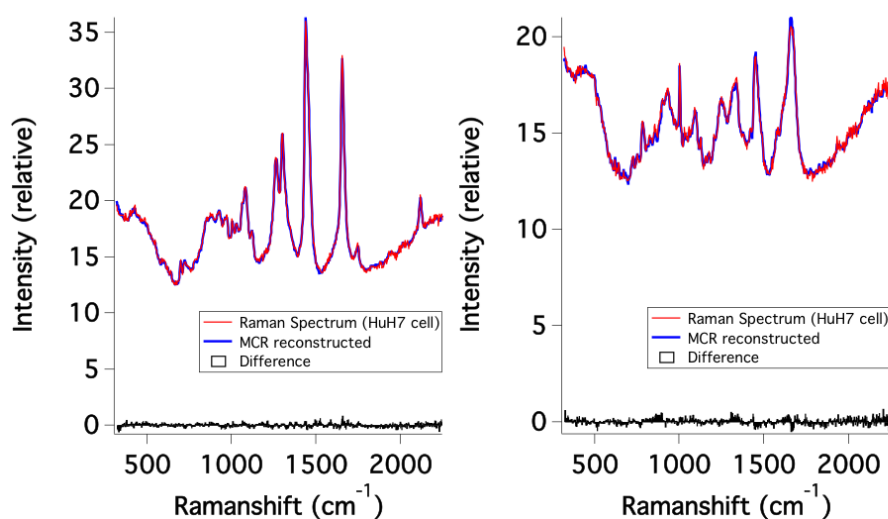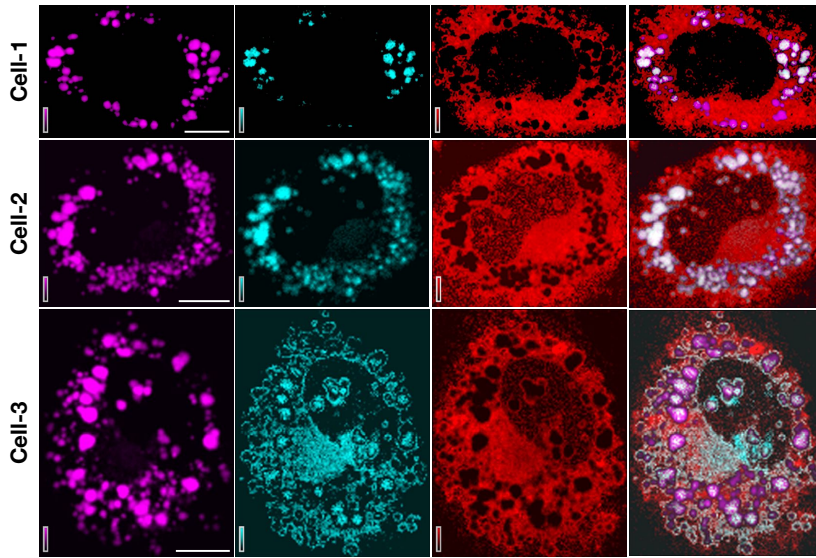## Supplementary Figures

Supplementary Figure 1. Complete list of MCR spectral components and background components (gray). The red line indicates absence of protein signature band at 1003 cm-1 in the lipid spectra suggesting a complete separation of these spectral components (*See* "spectral assignments" in Methods).
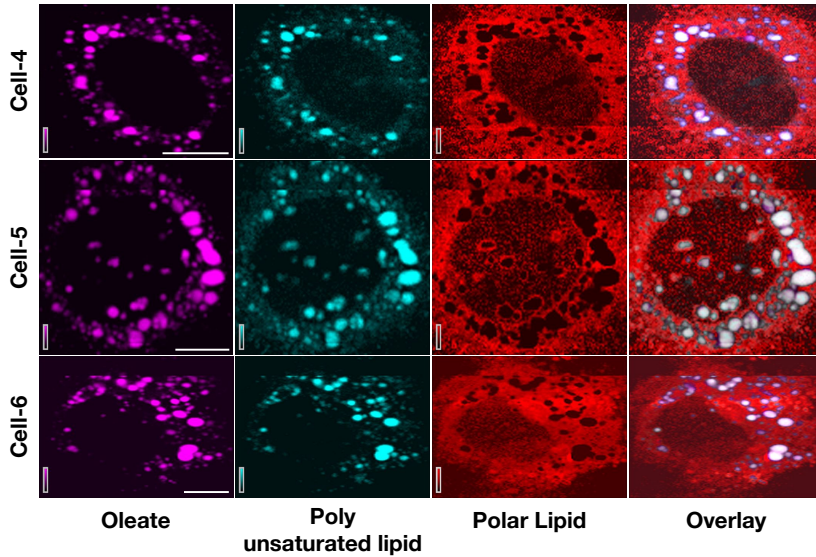

Supplementary Figure 2. Comparison of the signal and the residual in the MCR-ALS analysis.
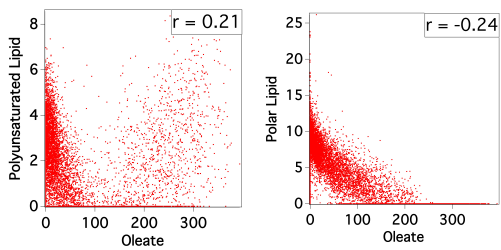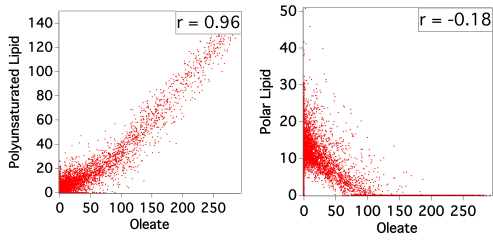
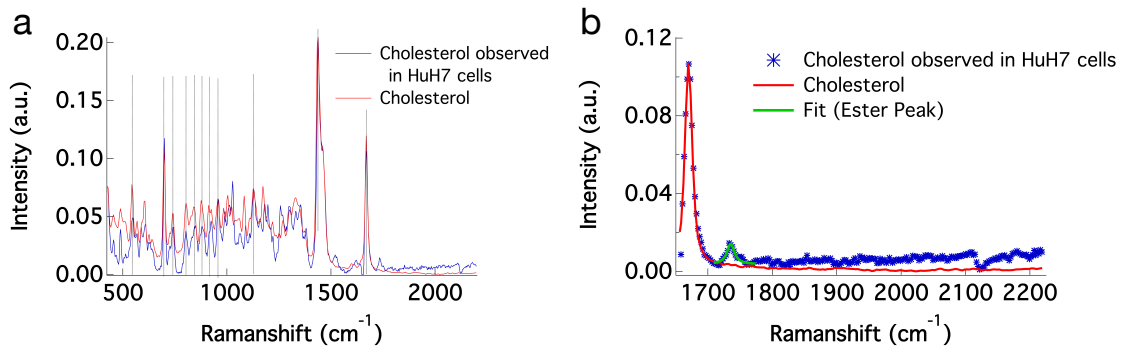**Supplementary Figure 3.** Distribution of lipids in the HuH7 cells. Scale bar 10 μm.

Supplementary Figure 4. Pearsons's correlation between lipid images for cell-3 (Cultured under OL-feed condition).

Supplementary Figure 5. Pearson's correlation between lipid images for cell-6 (Cultured under CH-feed condition).
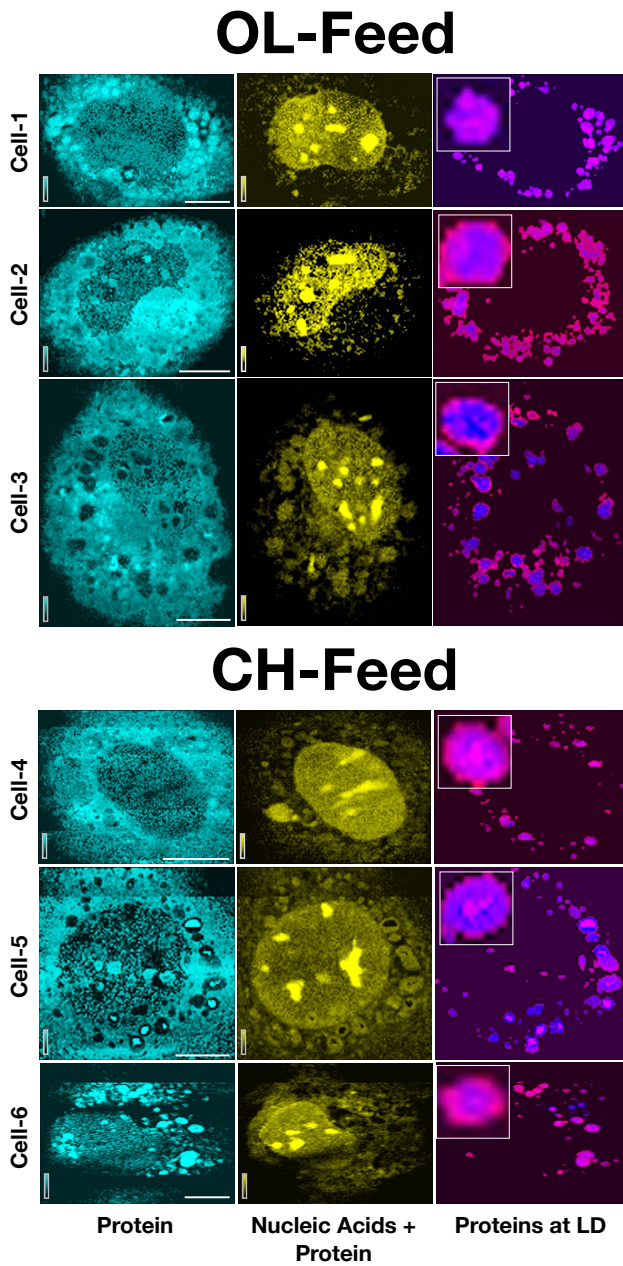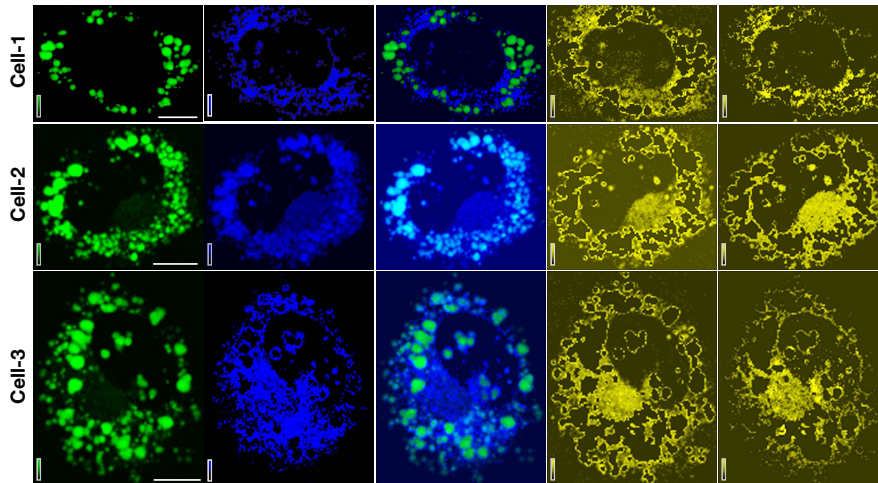

Supplementary Figure 6. Distribution of proteins and nucleic acids in the HuH7 cells. Scale bar 10 μm.

n HuH7 cells

2200

olesterol and

A — Intensity (a.u.): 0.20, 0.15, 0.10, 0.05, 0.00; x-axis 500 1000 1500 2000
Legend: Cholesterol observed in HuH7 cells; Cholesterol

B — Intensity (a.u.): 0.12, 0.08, 0.04, 0.00; x-axis 1700 1800 1900 2000 2100 2200
Legend: Cholesterol observed in HuH7 cells; Cholesterol; Fit (Ester Peak)

OL-Feed

Cell-1

Cell-2

Cell-3

CH-Feed

Cell-4

Cell-5

Cell-6

| Alkyne Cholesterol | Cholesterol | Overlay | Alkyne cholesterol outside LD | Cholesterol outside LD |

095743

0   100

80

ar 10 μm.
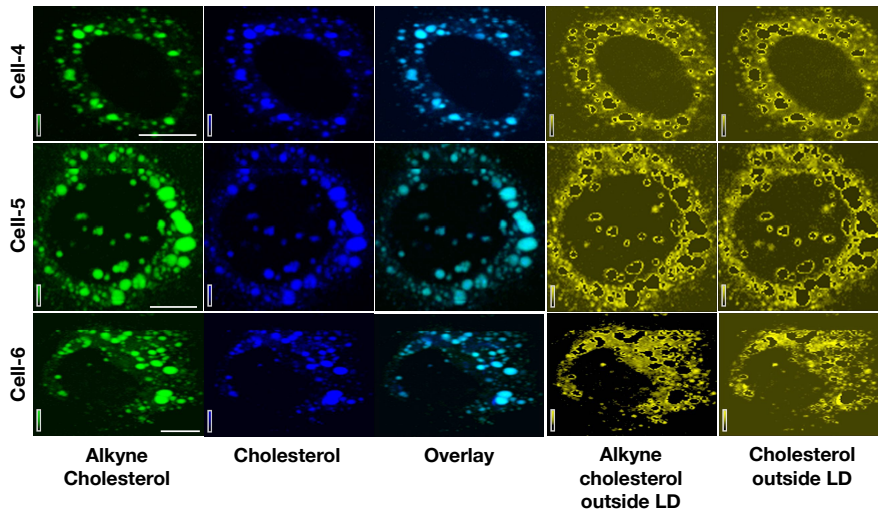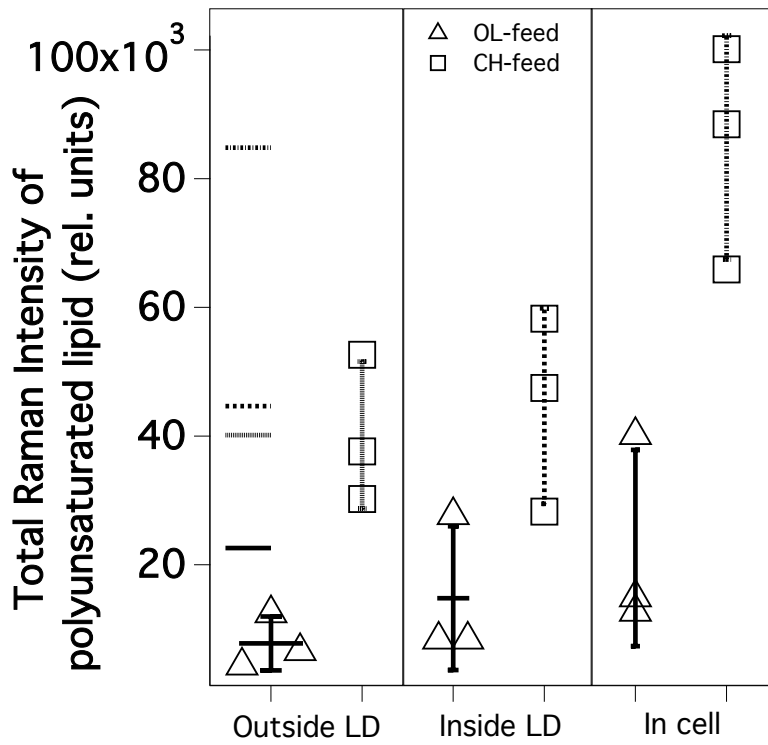
Supplementary Figure 9. Total Raman intensity of polyunsaturated lipid at different locations (e.g. outside LDs) of the cells.

Supplementary Tables

Supplementary Table 1. Pearson's correlates coefficients (PC) for different pairs of Raman images.

Correlation Pair	Pearson's Correlation Coefficient for cells					
	1	**2**	**3**	**4**	**5**	**6**
Lipid 1 & 2	0.78	0.97	0.21	0.93	0.96	0.96
Lipid 2 & 3	-0.12	-0.28	0.42	-0.17	-0.18	-0.13
Lipid 1 & 3	-0.25	-0.35	-0.24	-0.29	-0.37	-0.18
Alkyne Cholesterol & lipid 1	0.99	0.99	0.99	0.99	0.99	0.98
Cholesterol & alkyne cholesterol (Outside LD)	0.63	0.79	0.61	0.96	0.92	0.83
Cholesterol & alkyne cholesterol (Inside LD)	0.11	0.95	0.49	0.99	0.99	0.99