SUPPORTING INFORMATION

Noninvasive imaging of protein metabolic labeling in single human cells using stable isotopes and Raman microscopy

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This Supporting Information contains the following material:

1. Description of Materials
2. Description of principal component analysis (PCA) results on Raman spectra of Phe-d_5-labeled HeLa cells
3. Raman spectral images of a Tyr-d_4-labeled HeLa cell
4. Description of principal component analysis (PCA) results on Raman spectra of Met-d_3-labeled HeLa cells
5. Signal-to-noise analyses of Raman band intensities

1. Materials. L-Leucine (cat. no. P5482), L-phenylalanine (cat. no. P5482), L-tyrosine (cat. no. T8566) and L-methionine (cat. no. M5308) were obtained from Sigma. L-Methionine-(methyl-d_3) (cat. no. 300616) was obtained from Sigma-Isotec. Toluene-(ring-d_5) (cat. no. DLM-1176), L-phenylalanine-(ring-d_5) (cat. no. DLM-1258) and L-tyrosine-(ring-d_4) (cat. no. DLM-451) were a gift from Cambridge Isotope Laboratories Inc. (Andover, MA). DMEM medium deficient in Leu, Phe, and Tyr (composition A0387) was obtained from Biowest (Nuaillé, France). DMEM medium deficient in Met, Cys, and Gln was obtained from Sigma. SILAC culture media (containing either Phe-h_5, Phe-d_5, Tyr-d_4, or Met-d_3) were prepared from these media by adding the appropriate amounts of nondeuterated amino acids and deuterated or nondeuterated Phe, Tyr, or Met stock solutions, together with 10% (v/v) fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin.

2. Principal component analysis (PCA) on Raman spectra of Phe-d_5-labeled cells

PCA was used to identify the major differences between Raman spectra of HeLa cells incubated with Phe-h_5 for 8 h, Phe-d_5 for 8 h, or Phe-d_5 for 28 h. These 3 groups are clearly separated in the PC1-PC2 score plot as shown in Figure S-1A. They differ mainly in their scores on PC2. The loading vector of PC2 (Figure S-1C) is composed of positive contributions from Phe-d_5 and negative contributions from Phe-h_5, which is evident from a comparison of Figure S-1C with Figure 2A (in the primary article). Together, the loadings and score values of PC2 thus show that the 1001 cm^{-1} band decreases and the 959 cm^{-1} band increases in going from spectra of cells incubated with Phe-h_5 for 8 h to those of cells incubated with Phe-d_5 for 8 h and 28 h.

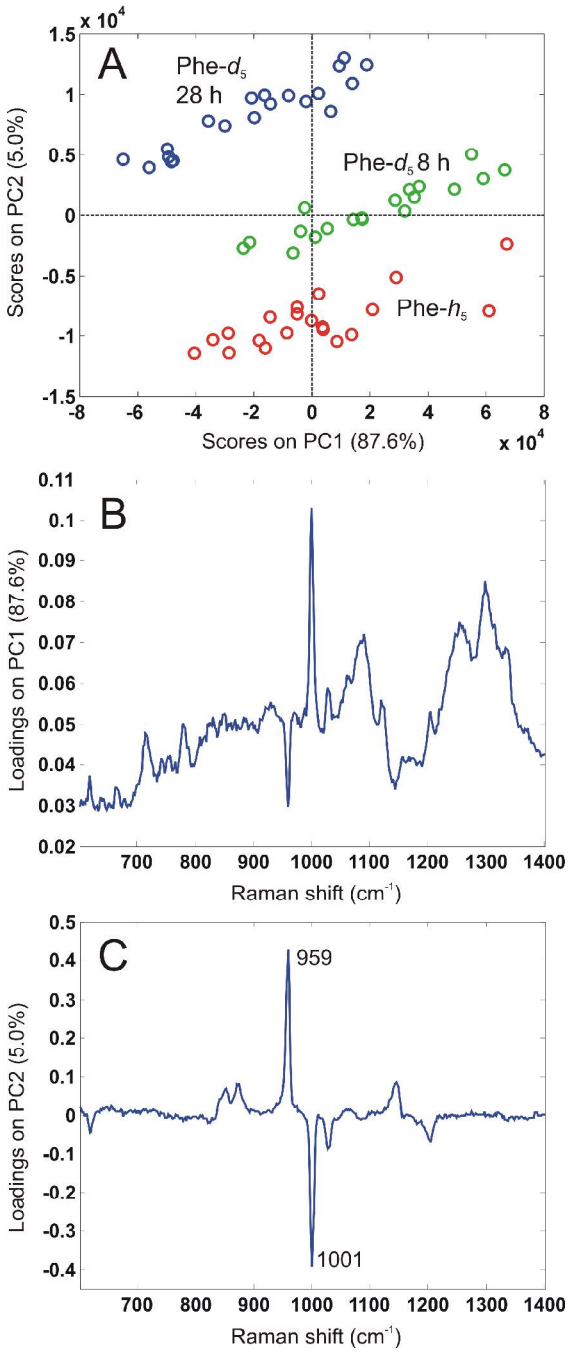

Figure S-1. (A) PC1-PC2 score plot resulting from principal component analysis of Raman spectra of cells incubated with Phe-h_5 for 8 h (20 spectra; red circles), Phe-d_5 for 8 h (19 spectra; green circles), and Phe-d_5 for 28 h (20 spectra; blue circles). Raman spectra were mean-centered and truncated to the 600–1400 cm^{-1} spectral region before PCA. (B) Loading vector of PC1. (C) Loading vector of PC2.

3. Raman spectral images of a Tyr-d_4-labeled HeLa cell

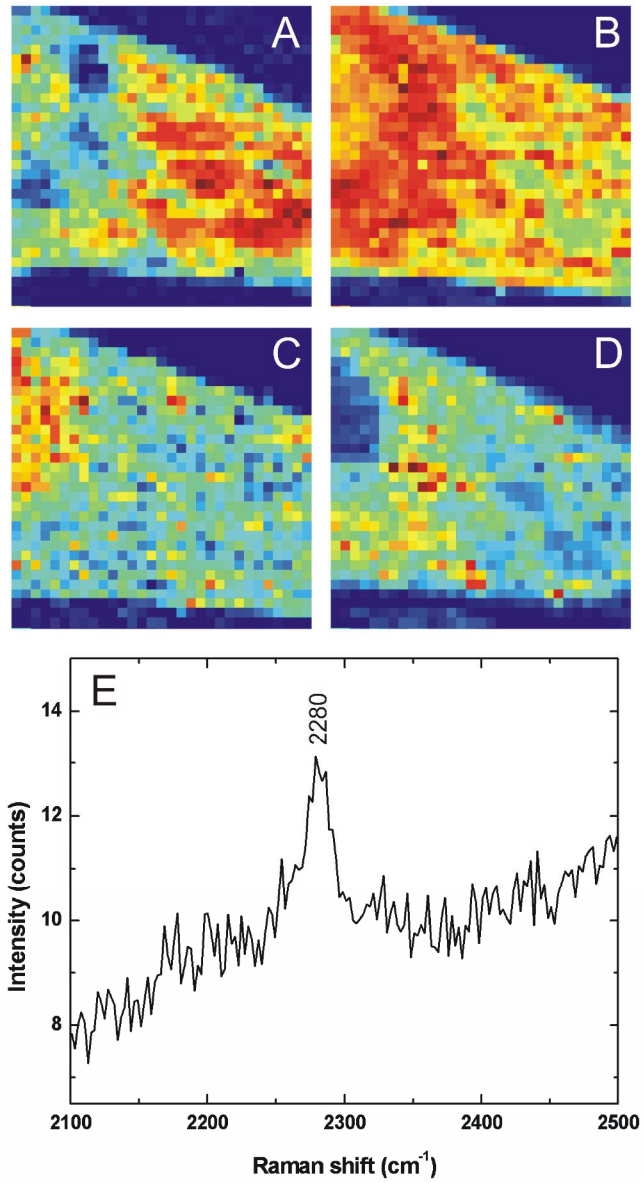

Figure S-2. Raman imaging of a HeLa cell incubated for 8 h with Tyr-d_4. (A) Nucleotide image (770–790 cm^{-1}). (B) Phe-h_5 (995–1005 cm^{-1}). (C) Tyr-d_4 image (2280 cm^{-1}). (D) Phospholipid image (700–730 cm^{-1}). (E) Average spectrum of the cell in the 2100–2500 cm^{-1} range. Image acquisition parameters are identical as described in the caption to Figure 4 in the main text.

4. Principal component analysis (PCA) on Raman spectra of Met-d_3-labeled cells

PCA was also used to identify the major differences between Raman spectra of HeLa cells incubated with Met-d_3 or Met-h_3 for 6 days. Two clusters are clearly separated in the PC1-PC2 score plot as shown in Figure S-3A. They differ mainly in their scores on PC1, which accounts for 99.9% of the variance. The loading vector of PC1 (Figure S-3B) is composed of positive contributions from Met-d_3, which is evident from a comparison of Figure S-3B with Figure 6 (bottom trace). Together, the score plot and loading vector PC1 show that cells incubated with Met-d_3 and Met-h_3 can be clearly distinguished by Raman spectroscopy.

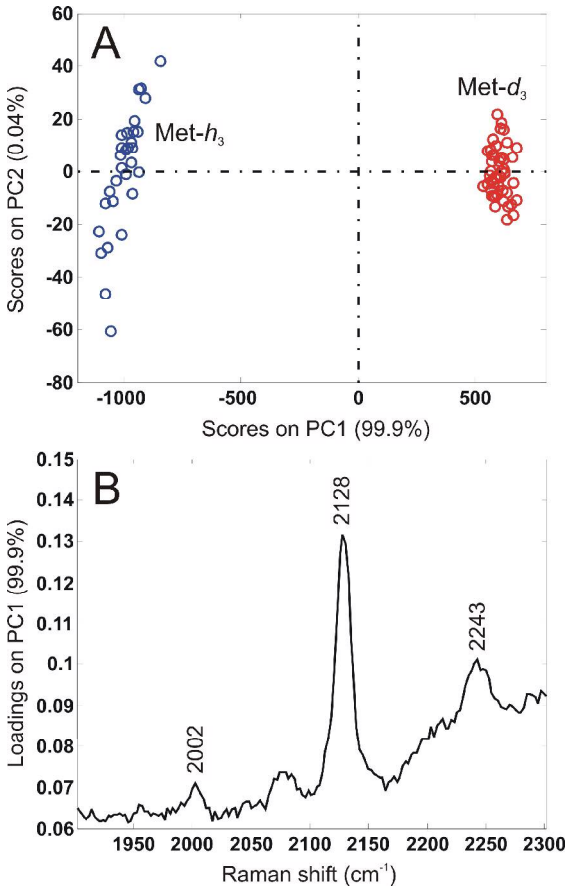

Figure S-3. (A) PC1-PC2 score plot resulting from principal component analysis of Raman spectra of cells incubated with Met-d_3 for 6 days (50 spectra; red circles) and Met-h_3 for 6 days (30 spectra; green circles). Raman spectra were mean-centered and truncated to the 1900–2300 cm^{-1} spectral region before PCA. (B) Loading vector of PC1.

5. Signal-to-noise analysis of the average Phe-d_5 959 cm^{-1} band intensity in the nucleoli of the cell shown in Figure 4

Selected spectra (50 in total; each spectrum 1 s exposure time) from the nucleoli of the HeLa cell shown in Figure 4B were averaged. The background-corrected intensity of the Phe-d_5 Raman band centered at 959 cm^{-1} is 335 counts for the average spectrum. To estimate the noise for the 959 cm^{-1} band, the difference spectrum of two very similar spectra from the nucleolus was

calculated and the standard deviation of the residual intensities in the 950–965 cm^{-1} region was calculated at 1.4 counts. Thus, the S/N ratio of the 959 cm^{-1} band intensity is 335/1.4 = 239. Since the average Phe-d_5 incorporation level of cells incubated for 28 h with Phe-d_5 (such as the cell shown in Fig. 4) is 55%, the detection limit for the incorporation level is estimated at 55%/√239 = ~3.6%.

5. Signal-to-noise analysis of the average Tyr-d_4 2282 cm^{-1} band intensity in the nucleus of HeLa cells

Selected spectra (32 in total; each spectrum 1 s exposure time) from the nucleolus of a HeLa cell incubated for 8 h with Tyr-d_4 were averaged. The background-corrected intensity of the Tyr-d_4 Raman band centered at 2282 cm^{-1} is 36 counts for the average spectrum. To estimate the noise for the 2282 cm^{-1} band, the difference spectrum of two very similar spectra from the nucleolus was calculated and the standard deviation of the residual intensities in the 2260–2295 cm^{-1} region was calculated at 0.5 counts. Thus, the S/N ratio of the 2282 cm^{-1} band intensity is 36/0.5 = 72. Since the average Tyr-d_4 incorporation level of cells incubated for 8 h with Tyr-d_4 is 28%, the detection limit for the incorporation level is estimated at 28%/√72 = ~3.3%.

5. Signal-to-noise analysis of the average Met-d_3 2128 cm^{-1} band intensity in the nucleus of HeLa cells

Selected spectra (50 in total; each spectrum 3 s exposure time) from the nucleus of a HeLa cell incubated for 6 days with Met-d_3 were averaged. The background-corrected intensity of the Met-d_3 Raman band centered at 2128 cm^{-1} is 615 counts for the average spectrum. To estimate the noise for the 2128 cm^{-1} band, the difference spectrum of two very similar spectra from the

nucleus was calculated and the standard deviation of the residual intensities in the region 2110–2150 cm^{-1} was calculated at 0.8 counts. Thus, the S/N ratio of the 2128 cm^{-1} band intensity is 615/0.8 = 769. Since the Met-d_3 incorporation level of cells incubated for 6 days with Met-d_3 is 100% (see main text), the detection limit for the incorporation level is estimated at 100%/√769 = ~3.6%.