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Supporting Information

Single-cell infrared phenomics: phenotypic screening with infrared microspectroscopy

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Author Contributions

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Materials and Methods

1 Cell culture, drug treatment and sample preparation

Human liver cancer cell HepG2 were cultured in DMEM (Hyclone) containing 10% fetal bovine serum (obtained from Zhejiang Tianhang Biotechnology Co., China), 1% penicillin and streptomycin. Cells were incubated at 37 ℃ under 5% CO_2 and saturated humidity. The cells were incubated in petri dishes (or 6-well culture plates deposited with a piece of CaF_2 window on the bottom) until 80% density.

Protopanaxadiol (PPD, obtained from Shandong International Biotechnology Park) was dissolved in DMSO and diluted using culture medium to a final concentration of 0, 10, 30 and 50 μM to stimulate the cells, respectively. Then the cells were let to grow in hypoxia incubator with 1% O_2 for 48 hours.

After that, the medium was abandoned and the cells were washed by PBS for 3 times. (a) The cells cultured on petri dishes were detached using a cell scraper and then transferred into centrifuge tubes. After centrifugation, the cells were fixed with 4% paraformaldehyde for 30 minutes. The fixed cells were washed three times using ultrapure water. 5 μl suspension was deposited on a piece of BaF_2 window and let to dry completely under room temperature, then single cells would spread on the BaF_2 window. (b) The cells grew on CaF_2 windows in 6-well culture plates were also fixed using 4% paraformaldehyde for 30 minutes and washed 3 times using ultrapure water. Then the CaF_2 windows with single cells attached on were took out and let to dry completely. All of the samples were preserved at 4 ℃ before experiments.

2 Synchrotron Fourier transform infrared (SR-FTIR) microspectroscopy

Single-cell SR-FTIR measurements were carried out at beamline BL01B of Shanghai Synchrotron Radiation Facility (SSRF) which equipped with Nicolet 6700 Fourier transform infrared spectrometer, Continuμm infrared microscope and 32× infrared objective. Spectra within mid-infrared region (4000-600 cm^{-1}) were collected with spectral resolution set to 4 cm^{-1}, 64 co-added scans each spectrum. (a) Single spectra of individual cells on BaF_2 window were collected one by one, a total of about fifty single-cells were collected for each group, the aperture size was set to 20× 20 μm; (b) when collecting mapping images of single cells on CaF_2 window, the aperture size was set to 12×12 μm with a step size of 6×6 μm.

3 Data analysis

3.1 Spectra pre-processing

For single-cell spectral data, the original spectra were smoothed (9-point) and automatic baseline corrected on OMNIC (Thermo Fisher Scientific Inc.). The Mie scatter of all the spectra were corrected using RMieS-EMSC correction [1]. After that, the corresponding second-derivative spectra were calculated on OMNIC.

Mapping data were analyzed on CytoSpec. The original spectral was cut and the region 4000-1000 cm^{-1} was retained, then the mapping spectra was smoothed (15-point) and baseline corrected by AsLs method. The chemical images were acquired based on the area of absorption band regions listed in Table S1.

For whole spectral region, the range 3000-2800 cm^{-1} together with 1800-900 cm^{-1}; fatty acid component, 3000-2800, 1480-1300 cm^{-1}; proteins, 1800-1480 cm^{-1}; carbohydrates, 1200-900 cm^{-1} were selected, respectively.

3.2 Intercellular Euclidean distance calculation

The similarity of infrared spectra between cells were evaluated by Euclidean distance: $D_{Eu}(p,q) = \sqrt{\sum_{i=1}^{n}(p_i - q_i)^2}$, where p and q are two single cells represented by n dimensional vectors ("n" is corresponding to the number of points of infrared spectra). The value of $D_{Eu}(p,q)$ is negative correlation with intercellular similarity.

The intercellular Euclidean distance matrix was constructed by calculating all the cell-to-cell distances in a group (in-group) or between two groups (inter-group). For a group with "x" number of cells, a total of $C_x^2 = x(x-1)/2$ Euclidean distances can be acquired. For two groups (group A and B with "x" and "y" numbers, respectively), a total of x×y Euclidean distances can be acquired.

Intercellular Euclidean distance matrixes were calculated on MATLAB R2014a. The intercellular Euclidean distances heatmaps and frequency distributions were drawn using Origin Pro 2016.

3.3 Principle component analysis (PCA) and hierarchical cluster analysis (HCA)

PCA was carried out on the second-derivative spectra of single-cells. The first two principle components (PCs) were used to construct the score plots using OriginPro 2016, the corresponding

loading plots of PC1 and PC2 were also acquired.

HCA was also carried out on the second-derivative spectra, “Euclidean distance” calculation and “Ward” linkage were chosen.

Both PCA and HCA were carried out on MATLAB R2014a.

Table S1 Main infrared spectral absorption bands of HepG2.

Number	Assignment	Absorption bands (cm^{-1})
1	ν (N-H) of amide A	3201-3355
2	ν_{as} (C-H_3)	2966-2948
3	ν_{as} (C-H_2)	2943-2908
4	ν_s (C-H_2)	2864-2845
5	ν (C=O) of esters	1755-1725
6	Amide I	1692-1615
7	Amide II	1566-1509
8	δ (C-H_2)	1478-1428
9	ν_s (C=O) of COO^-	1426-1353
10	ν_{as} (P=O) of PO_2^-	1332-1193

ν_s/ ν_{as} : symmetric/asymmetric stretching vibration

δ: scissoring vibration

Figures

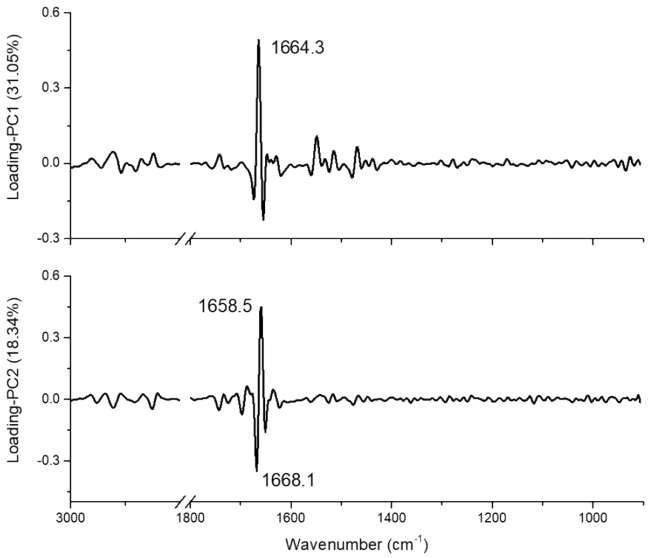

Figure S1 The corresponding loading plots of PC1 and PC2 of Fig. 1B.

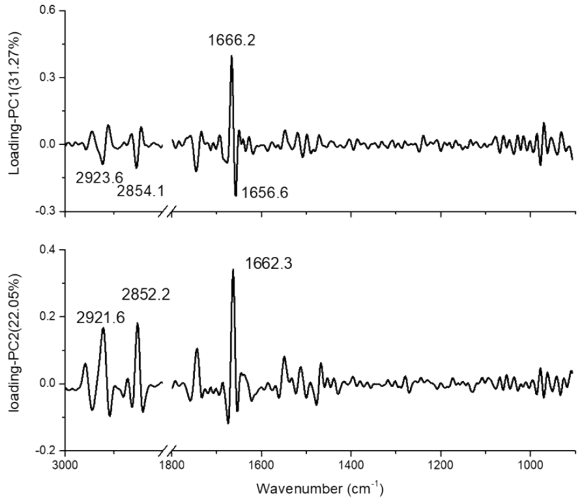

Figure S2 The corresponding loading plots of PC1 and PC2 of Fig. 2B.

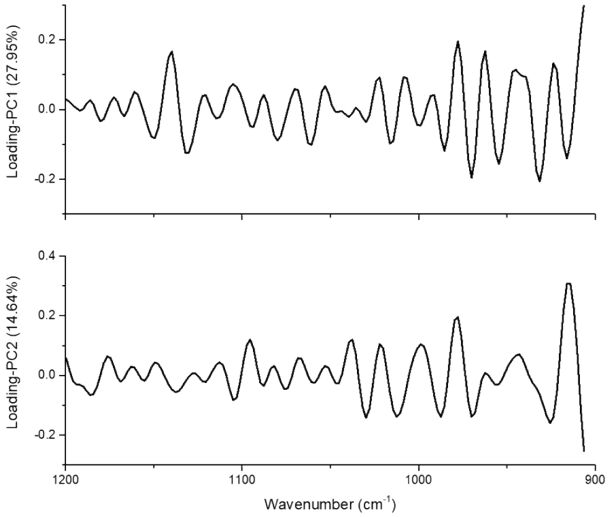

Figure S3 The corresponding loading plots of PC1 and PC2 of Fig. 3A.

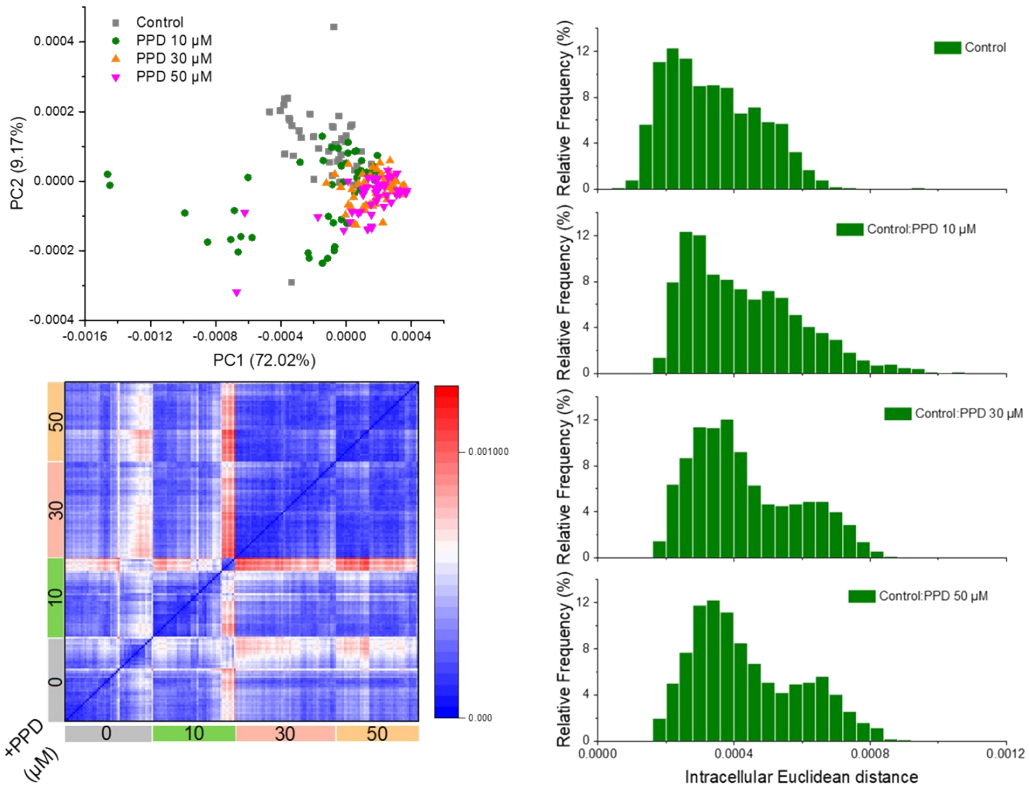

Figure S4 HepG2 cells were treated with 0, 10, 30 and 50 μM of PPD, respectively, the spectral region 3000-2800 and 1480-1300 cm^{-1} was used to evaluate the fatty acid difference between cells: (A) PCA on 0, 10, 30 and 50 μM PPD treated cells based on the second-derivative spectra within 3000-2800 and 1480-1300 cm^{-1}. (B) The heatmap derived from intercellular Euclidean distance between each two cells among 0 (grey), 10 (green), 30 (red) and 50 (orange) μM PPD treatment groups, about fifty cells each group. (C) For control group, its in-group intercellular Euclidean distance distribution histogram were shown on the top; while for PPD treatment group, their distribution histogram of intergroup intercellular Euclidean distance compared with control group were showed as indicated on the graph

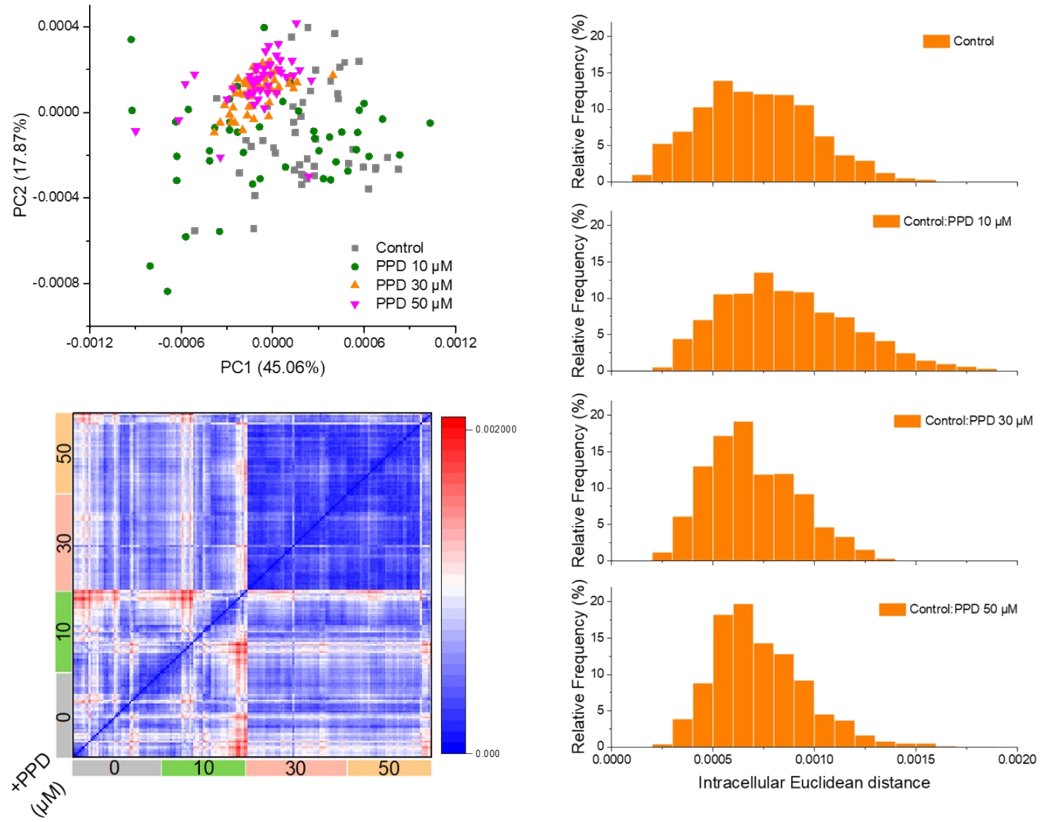

Figure S5 HepG2 cells were treated with 0, 10, 30 and 50 μM of PPD, respectively, the spectral region 1800-1480 cm^{-1} was used to evaluate the protein difference between cells: (A) PCA on 0, 10, 30 and 50 μM PPD treated cells based on the second-derivative spectra within 1800-1480 cm^{-1}. (B) The heatmap derived from intercellular Euclidean distance between each two cells among 0 (grey), 10 (green), 30 (red) and 50 (orange) μM PPD treatment groups, about fifty cells each group. (C) For control group, its in-group intercellular Euclidean distance distribution histogram were shown on the top; while for PPD treatment group, their distribution histogram of intergroup intercellular Euclidean distance compared with control group were showed as indicated on the graph.

References:

[1] Bassan, P., Kohler, A., Martens, H. *et al.* Resonant Mie Scattering (RMieS) correction of infrared spectra from highly scattering biological samples. *Analyst*. 2010;135(2):268-77.