

## Supplementary Materials

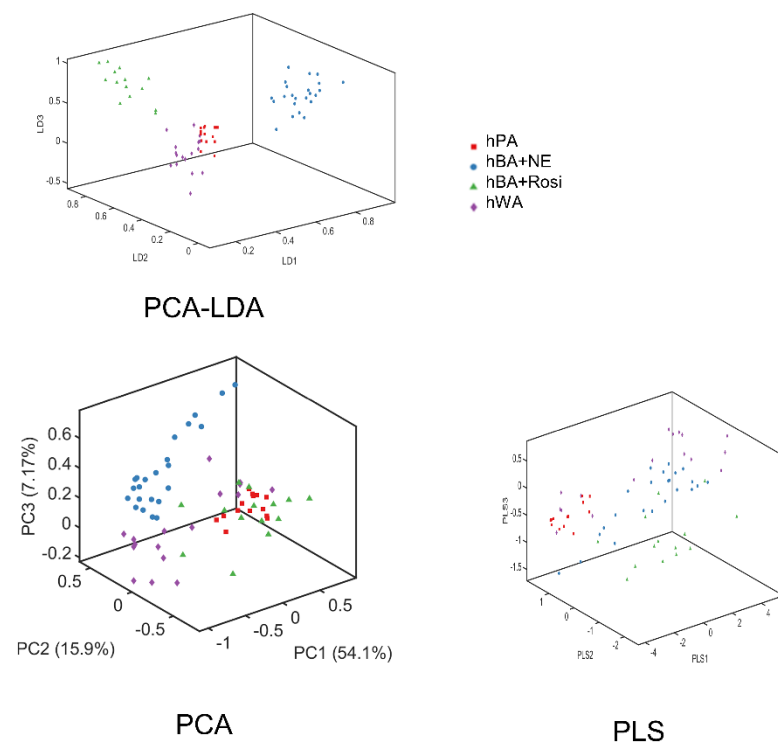


Figure S1. The 3D scatter plots of PCA-LDA, PCA, and PLS using FTIR spectra of human adipocytes.

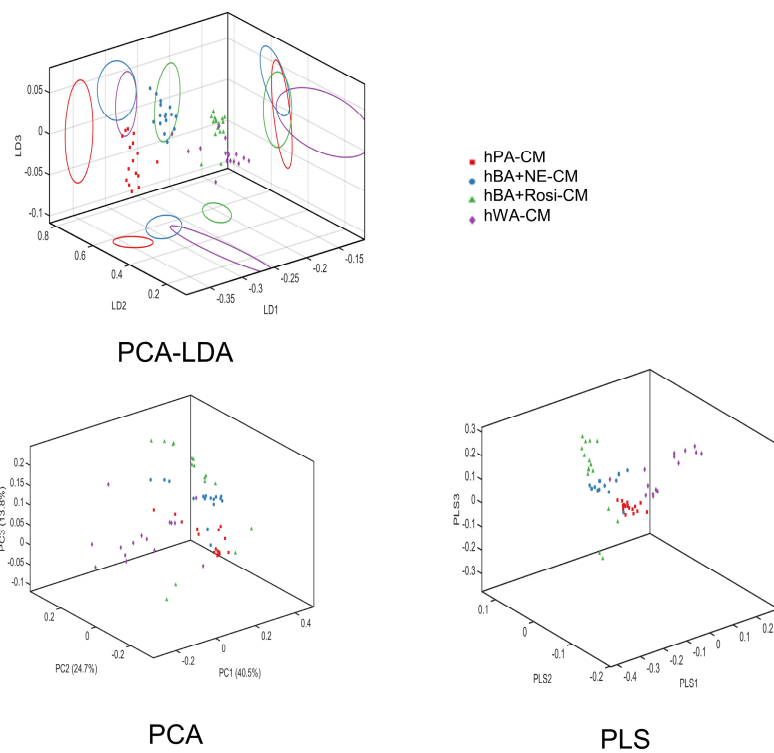


Figure S2. The 3D scatter plots of PCA-LDA, PCA, and PLS using FTIR spectra of human adipocytes conditioned media.

(A) **Component Values**

Used Rank: 4

File Name	True	Prediction	Difference	Possible Outl
EXTRACT_IR 100		83.41	16.6	
EXTRACT_IR 100		114.2	-14.2	
EXTRACT_IR 100		92.22	7.78	
EXTRACT_IR 100		82.38	17.6	
EXTRACT_IR 0		8.035	-8.04	
EXTRACT_IR 0		-12.47	12.5	

(B) **Component Values**

Used Rank: 4

File Name	True	Prediction	Difference
EXTRACT_gel 100		89.8	10.2
EXTRACT_gel 100		84.82	15.2
EXTRACT_gel 100		91.14	8.86
EXTRACT_gel 100		85.91	14.1
EXTRACT_gel 0		22.55	-22.6
EXTRACT_gel 0		-8.818	8.82

(C) **Component Values**

Used Rank: 5

File Name	True	Prediction	Difference
EXTRACT_nor 100		91.91	8.09
EXTRACT_nor 100		104.9	-4.91
EXTRACT_nor 100		109.7	-9.73
EXTRACT_nor 100		97.02	2.98
EXTRACT_nor 0		22.11	-22.1
EXTRACT_nor 0		26.86	-26.9

Figure S3. Test set validation (A) Test set validation of PLSR for hBA. (B) Test set validation of PLSR for hBA on a slide glass. (C) Test set validation of PLSR for hBA-CM on a slide glass. Top 2 samples (hBA+NE), middle 2 samples (hBA+Rosi), bottom 2 samples (hWA)

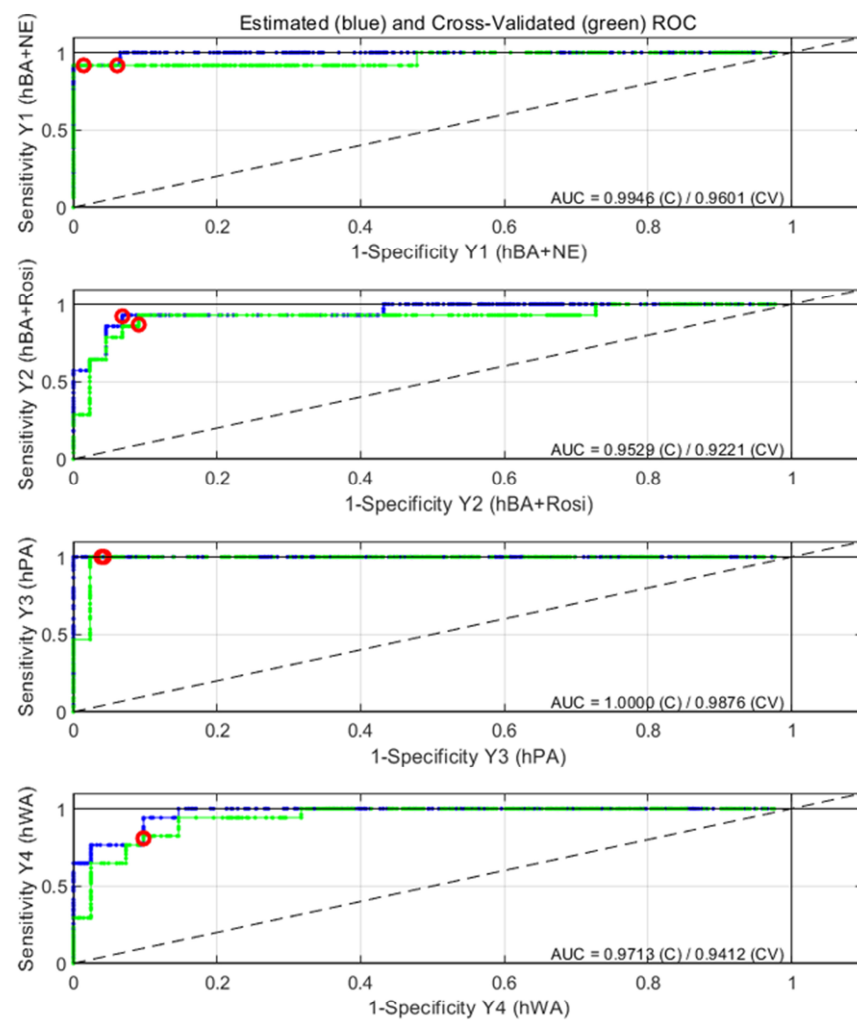


Figure S4. ROC of PLS-DA used in classification of adipocytes.

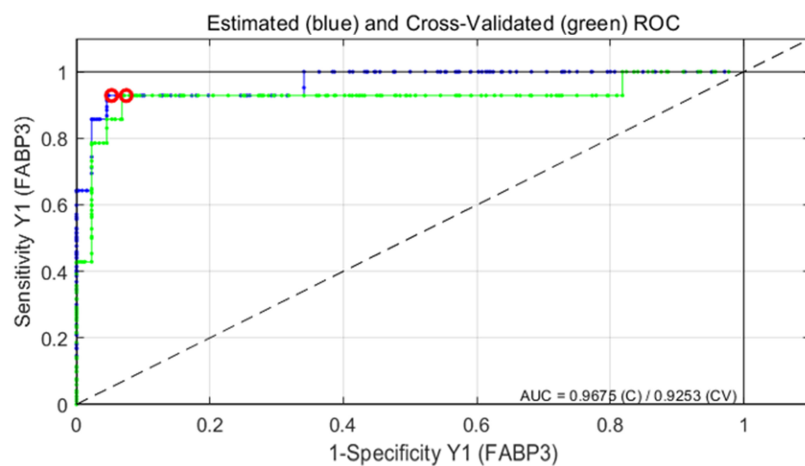
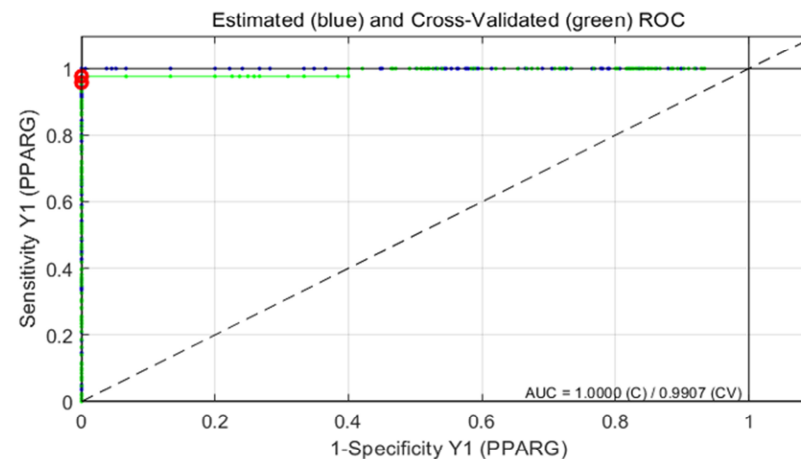
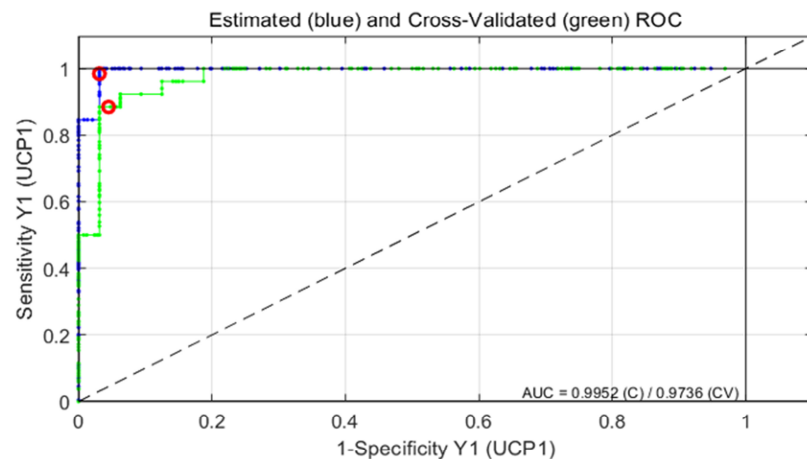


Figure S5. ROC of PLS-DA used in expression distribution of adipogenic genes in adipocytes.

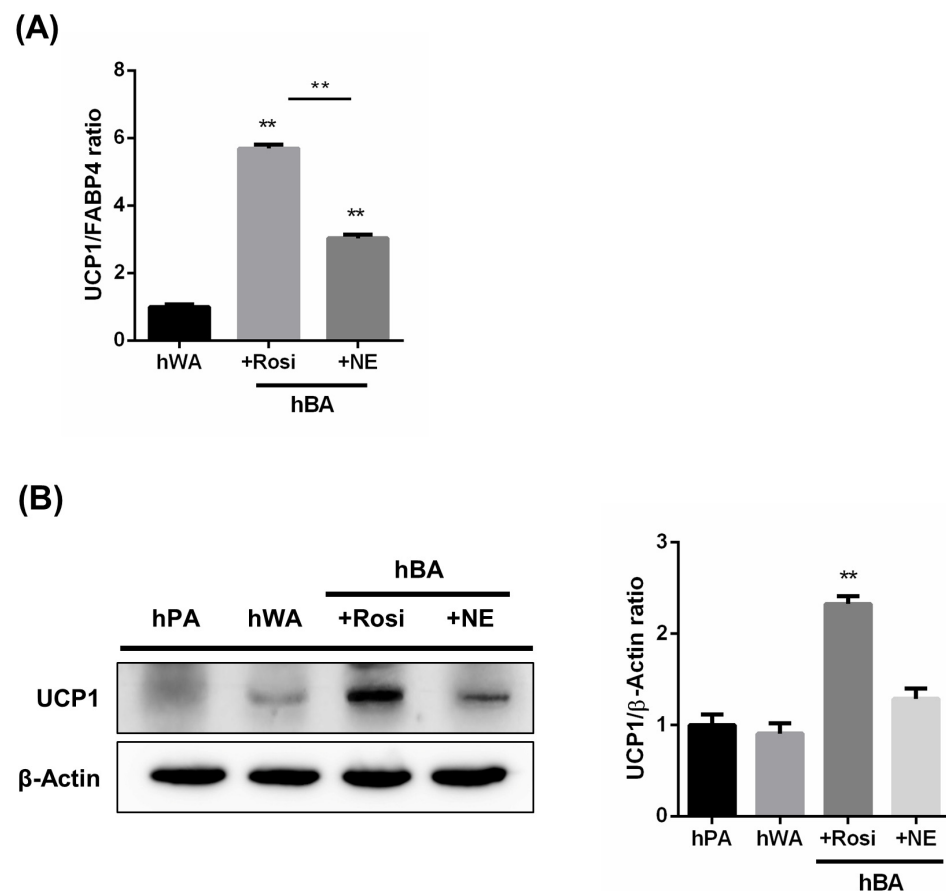


Figure S6. Analysis of brown-like adipocyte characteristics of adipocytes in differentiated hBA. (A) The expression ratio of *UCP1/FABP4* in differentiated hWA, hBA + Rosi, and hBA + NE were investigated by qRT-PCR. Results are shown as mean  $\pm$  SEM (n = 3). \*, p < 0.05; \*\*, p < 0.01 compared to the each groups. (B) The protein level of UCP1 was analyzed by Western blotting. The protein levels of UCP1 was quantified using the ImageJ software. Results are shown as mean  $\pm$  SEM (n = 3). \*, p < 0.05; \*\*, p < 0.01 compared to the hPA, hWA, and hBA + NE groups.

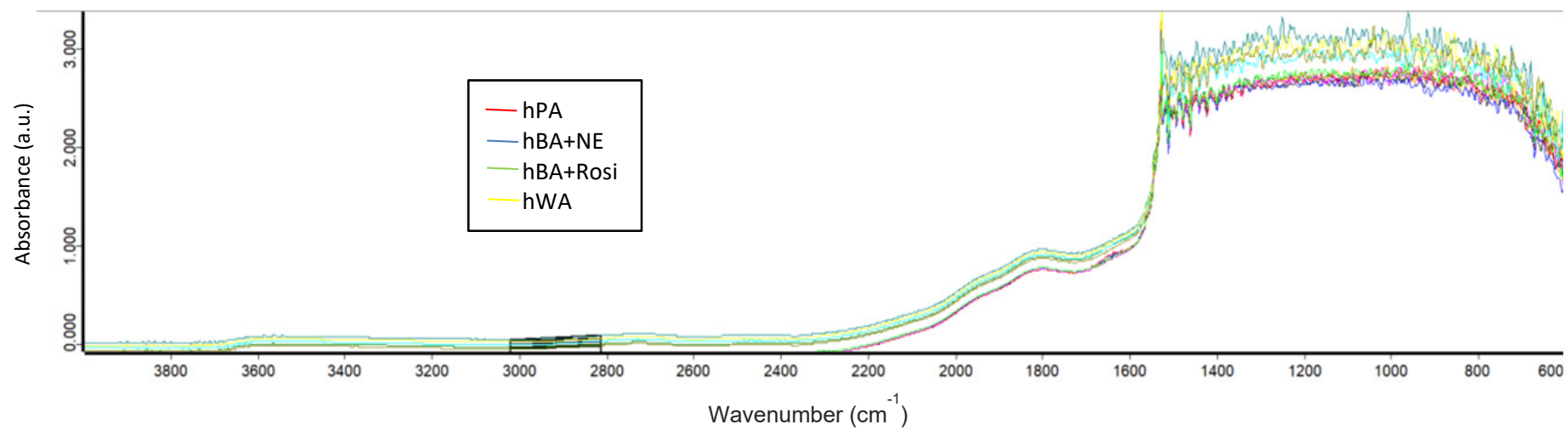


Figure S7. Raw spectra of hBA, hPA and hWA on a slide glass.

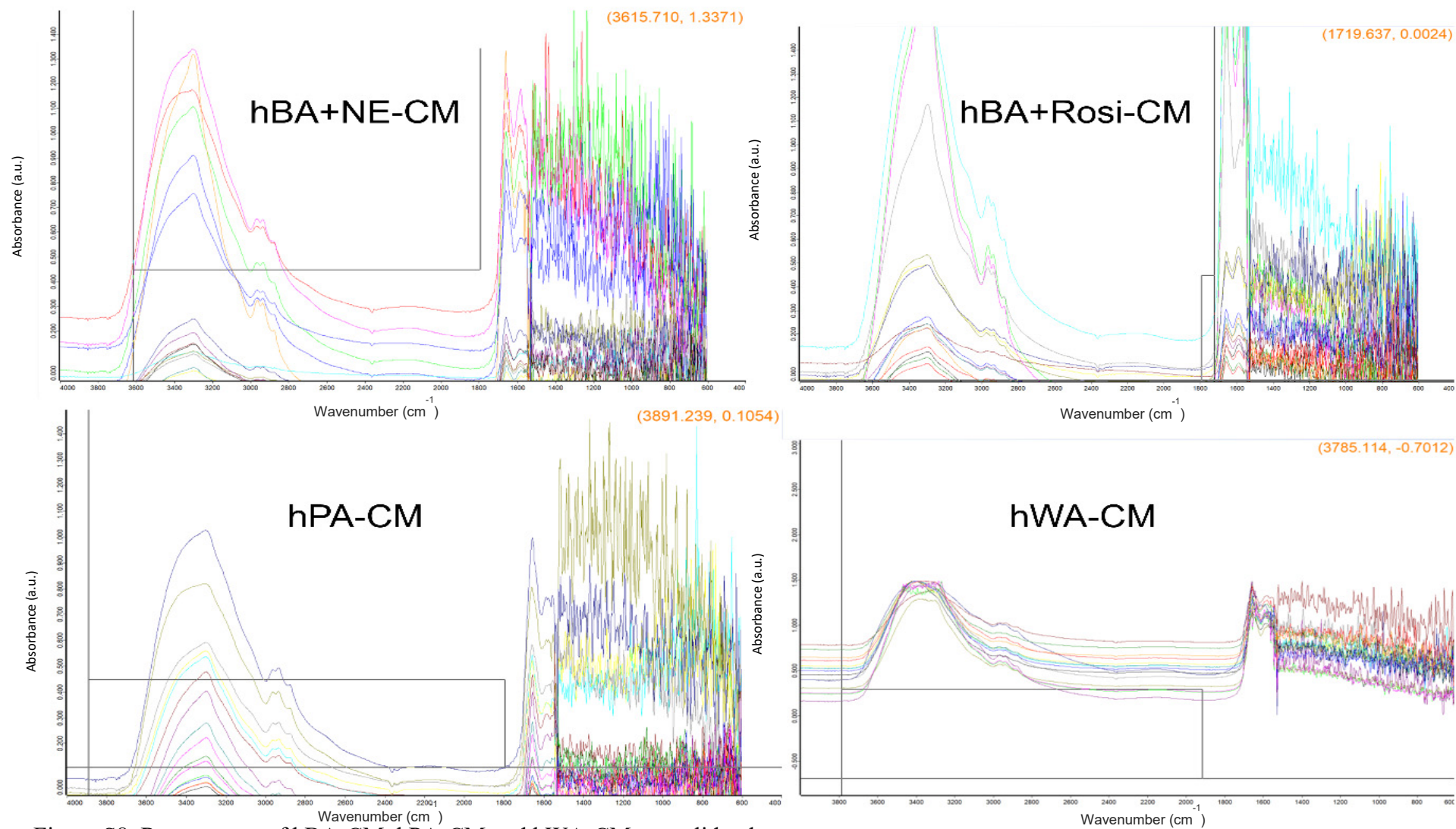


Figure S8. Raw spectra of hBA-CM, hPA-CM and hWA-CM on a slide glass.



Table S1. Summarization of objective, methods, results, and discussion of this study.

Objective	Chemometrics			Materials and Methods					Result		Validation	Limitation	Significance
Discrimination of beige adipocytes	PLSR [32,33,34,40]	Types	Used software	No. of spectra from 3 tests (n=3)	Preaction before preprocessing	Preprocessing	Used wavenumbers	Input values	Predicted values of hBA (Table 2)	Cros-Validation-R <sup>2</sup> , RMSECV, RPD (Table 2)	Predicted values of hBA+NE and +Rosi showed over 70 in test set validation (Figure S3)	Limit to screening actual drug candidates that are not a strong activator such as Rosi or NE.	Predictive model of PLSR based on less than 35 as negative effects, 35-70 as uncertainty, and more than 70 as positive effects.  Liquid samples (conditioned media) can be analyzed by FTIR [44,45]  Comment: Figures (spectra of PLSRs) included hPA to compare.
										Calibration-R <sup>2</sup> , RMSE, RPD			
		hBA (Figure 1F)	OPUS 8.5	12(+NE), 11(+Rosi), 15(hWA)	n.a.	1 <sup>st</sup> derivative, Vector Normalization, 17 smoothing points	3997-3656, 1618-938 cm <sup>-1</sup>	hWA 0 +NE, +Rosi 100	68.83-134.5	88.95, 2.13, 3.01			
										95.65, 1.16, 4.79			
		hBA on a slide glass (Figure 2A)		15(+NE on a slide glass), 15(+Rosi on a slide glass), 14(hWA on a slide glass)	Removed 1600-400cm-1 (Figure S7)		3997-3756, 3278-3037, 2798-1838 cm <sup>-1</sup>	hWA 0 +NE, +Rosi 100	66.73-122.7	92.11, 1.72, 3.56			
										97.22, 0.85, 6			
		hBA-CM on a slide glass (Figure 2C)		16(+NE-CM), 16(+Rosi-CM), 13(hWA-CM)	Removed 1600-400cm-1 (Figure S8)		3997-3338, 3118-2898, 2678-2459, 2239-1800 cm <sup>-1</sup>	hWA-CM 0 +NE/+Rosi-CM 100	80.55-111.9	93.39, 1.53, 3.89			
										97.11, 0.87, 5.88			
	Infrared spectral biomarkers on hBA	Difference between mean spectra [50]	IRootLab [35] toolbox in MATLAB 2021a	12(+NE), 14(+Rosi), 17(hWA)	n.a.	Wavelet de-noising, rubberband baseline coorection, normalization(mean-centering)	Full range (4000-400 cm <sup>-1</sup> )	n.a.	1635, 879-882, 860-3, 629-38 cm <sup>-1</sup>	Selecting peaks (Table 4 and Figure 3A)	n.a.		Finding biomarkers as infrared spectrum in beige adipocytes
		PCA-LDA cluster [50]		15(hPA), 14(+NE, +Rois), 17(hWA)						Selecting peaks (Table 4 and Figure 3B)			
		u-test & Fisher's score [50]								-log(0.05)=1.3, significance level (Table 4 and Figure 3C-D)			

	Infrared spectral biomarkers on hBA-CM	Difference between mean spectra [50]		16(+NE/+Rosi-CM), 15(hWA-CM)			4000-1600 cm <sup>-1</sup> (Figure S7)		1728-33, 1656-9, and 1640-8 cm <sup>-1</sup>	Selecting peaks (Table 4 and Figure 4A)			
		PCA-LDA cluster [50]		16(hPA-CM, +NE/ +Rosi-CM), 15(hWA-CM)						Selecting peaks (Table 4 and Figure 4B)			
		u-test & Fisher's score [50]								-log(0.05)=1.3, significance level (Table 4 and Figure 4C)			
	PLS-DA [54]		PLS Toolbox (Eigenvector Research, Inc., USA) in MATLAB 2021a	12(+NE), 14(+Rosi, hPA), 17(hWA)	n.a.	Extended scatter correction, normalization (1-norm)	Full range (4000-400 cm <sup>-1</sup> )	Assigning class (hBA+NE/+Rosi, hPA, and hWA)	Figure 5A	n.a.	AUC=0.9946 (+NE), 0.9529(+Rosi) (Figure S4)		As predictive model
Lipidomics of beige adipocytes	Infrared spectral biomarkers	difference between mean spectra [35]	IRootLab [35] toolbox in MATLAB 2021a	12(+NE), 14(+Rosi), 17(hWA)	n.a.	Wavelet de-noising, rubberband baseline coorection, normalization(mean-centering)	Full range (4000-400 cm <sup>-1</sup> )	n.a.	1635, 879-882, 860-3, 629-38 cm <sup>-1</sup>	Selecting peaks (Table 4 and Figure 3A)	n.a.	n.a.	The spectral biomarkers can be linked to genes, especially thermogenic markers (e.g., UCP1), of beige adipocytes.
		PCA-LDA cluster, u-test and Fisher's score [50]		15(hPA),14(+NE, +Rois), 17(hWA						-log(0.05)=1.3, significance level	n.a.		
	PLS-DA [54]		PLS Toolbox (Eigenvector Research, Inc., USA) in MATLAB 2021a	12(+NE), 14(+Rosi, hPA), 17(hWA)	n.a.	Extended scatter correction, normalization (1-norm)	Full range (4000-400 cm <sup>-1</sup> )	Assigning class (UCP1, PPARG, FABP3)	Figure 5B-D	n.a.	AUC=0.9952(UCP1), 1(PPARG), 0.9675(FABP3) (Figure S5)	Need cross-validation with single cell analysis	Expecting expression distribution of adipogenesis genes in adipocytes

n.a., Not Applicable; [], indicating reference number