Supplementary Information

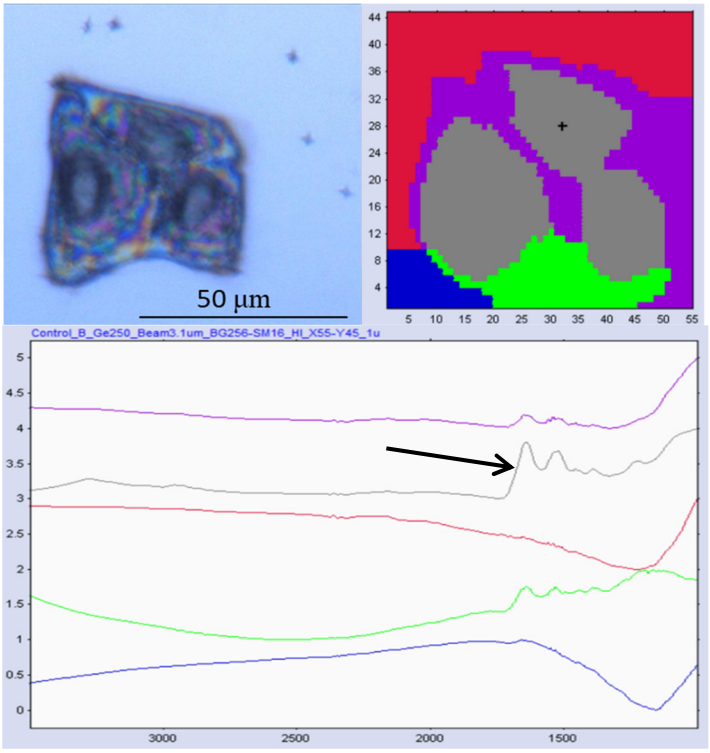

Figure S1. *Top left*: microscope image of fixed, untreated HaCat cells prior to the synchrotron macro-ATR-FTIR imaging. *Top right*: HCA image obtained from the corresponding synchrotron macro-ATR-FTIR dataset of the same HaCat cells. *Bottom*: five average spectra extracted from each of the five colored clusters. Note: black arrow indicates the spectral cluster selected for subsequent PCA analysis.

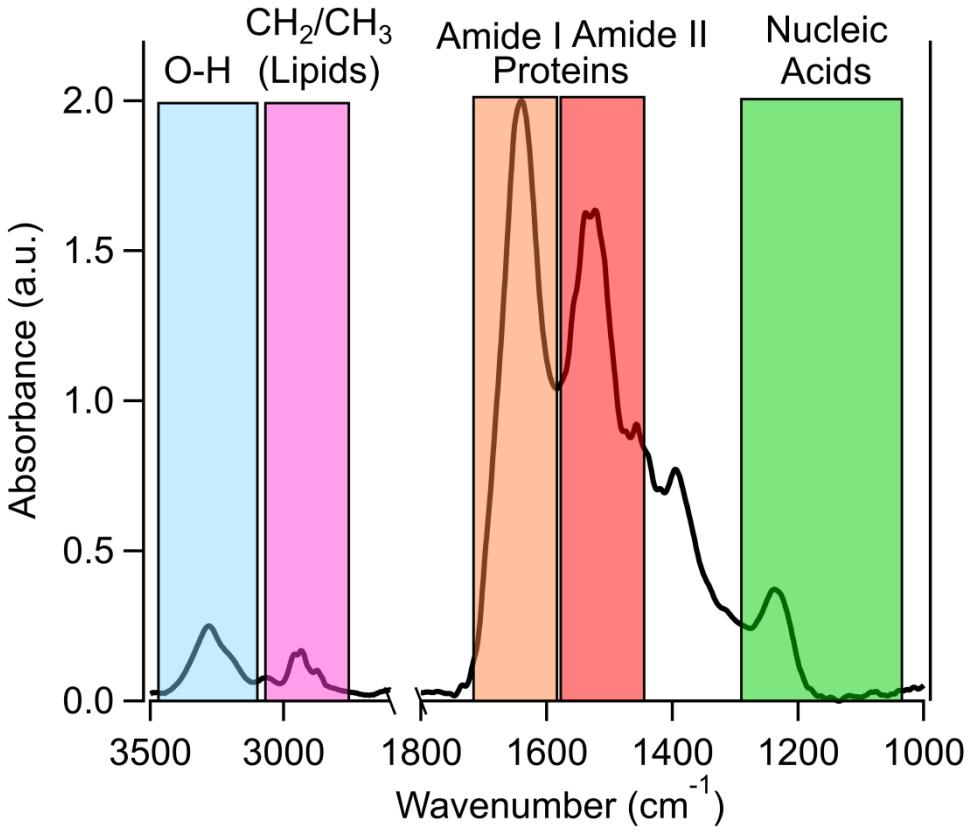

Figure S2. Regions of interest containing the key biochemical information in the acquired synchrotron macro-ATR-FTIR spectra.

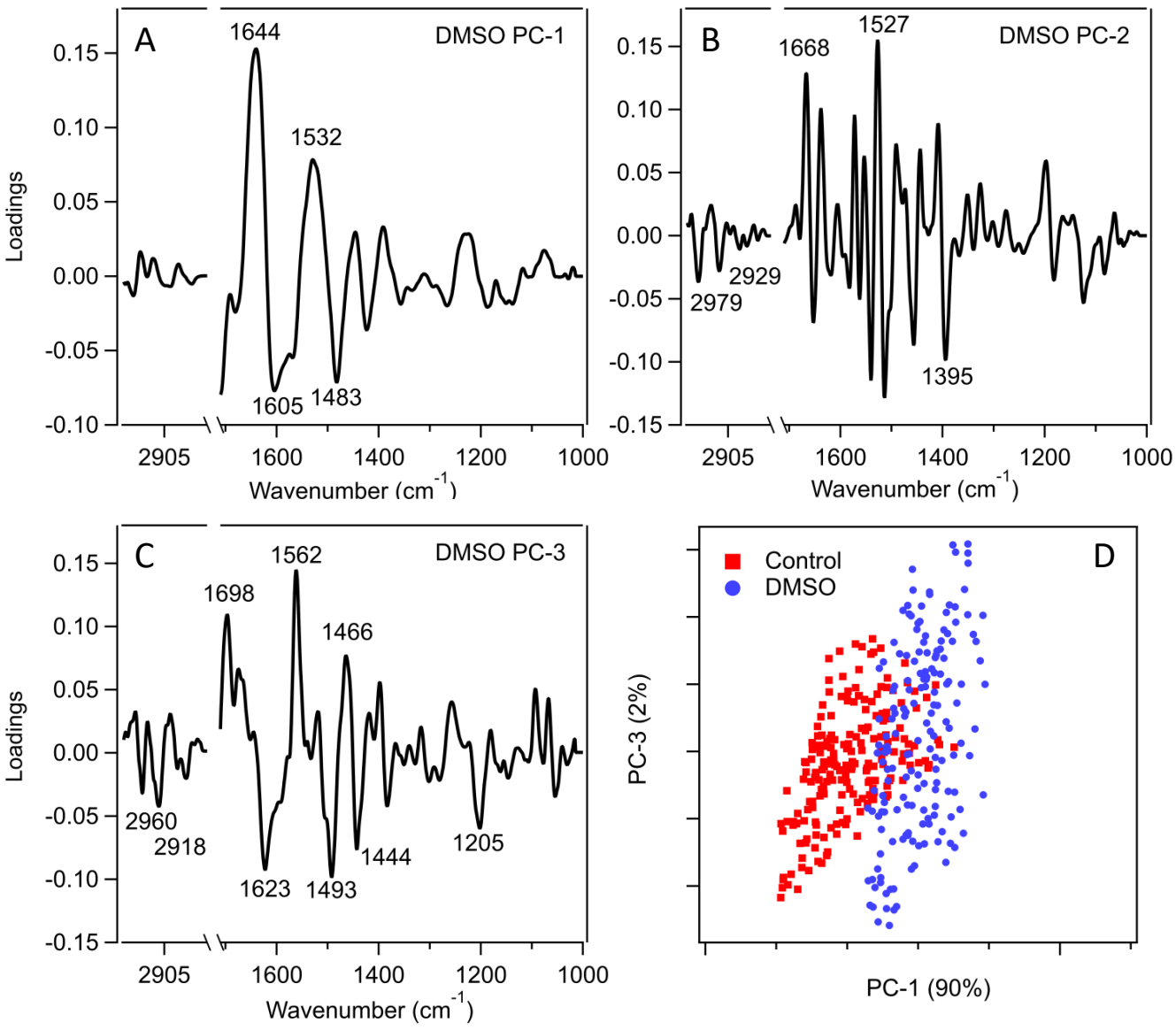

Figure S3. A-C) PCA loadings plots of HaCat cells treated with DMSO compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. A significance cut-off of 0.07 was applied to PC-1 and 3, and of 0.1 was applied to PC-2. D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

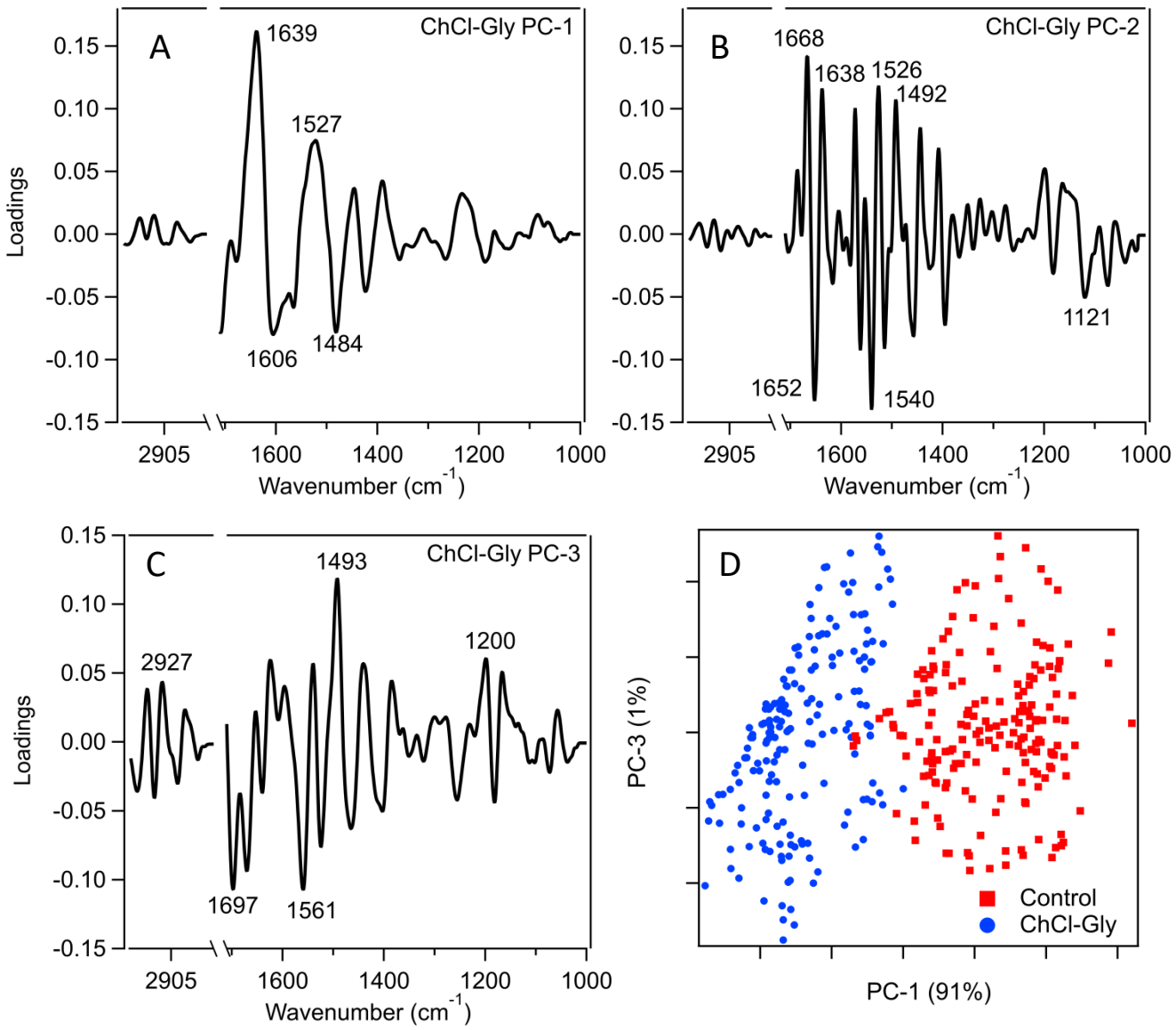

Figure S4. A-C) PCA loadings plot of HaCat cells treated with ChCl-Gly compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. Significance cut off of 0.07 applied to PC-1 and of 0.1 to PC-2 and PC-3. D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

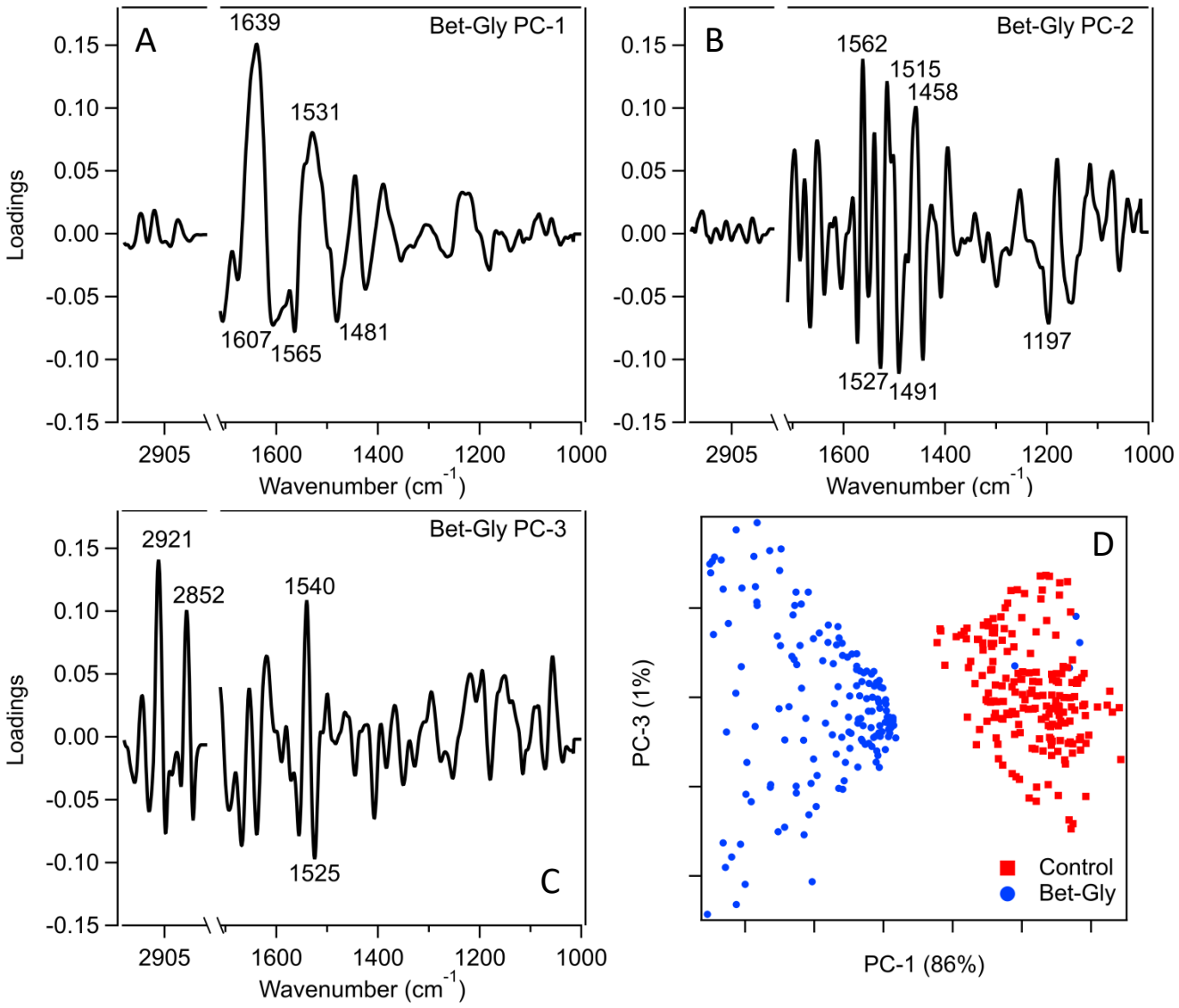

Figure S5. A-C) PCA loadings plots of HaCat cells treated with Bet-Gly compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. Significance cut off of 0.07 applied to PC-1 and of 0.1 to PC-2 and PC-3. D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

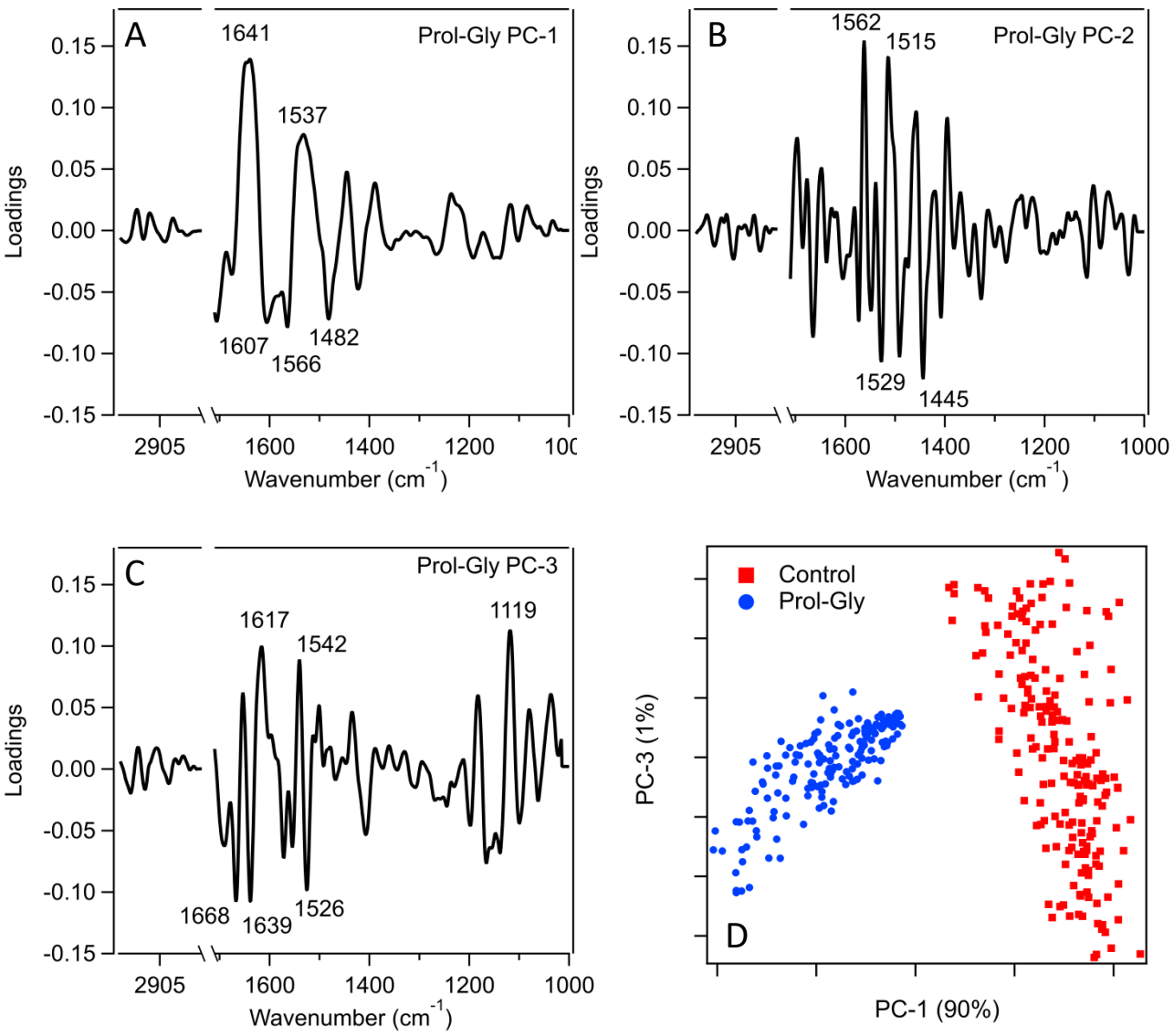

Figure S6. A-C) PCA loadings plots of HaCat cells treated with Prol-Gly compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. A significance cut-off of 0.07 was applied to PC-1 and of 0.1 was applied to PC-2 and PC-3. D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

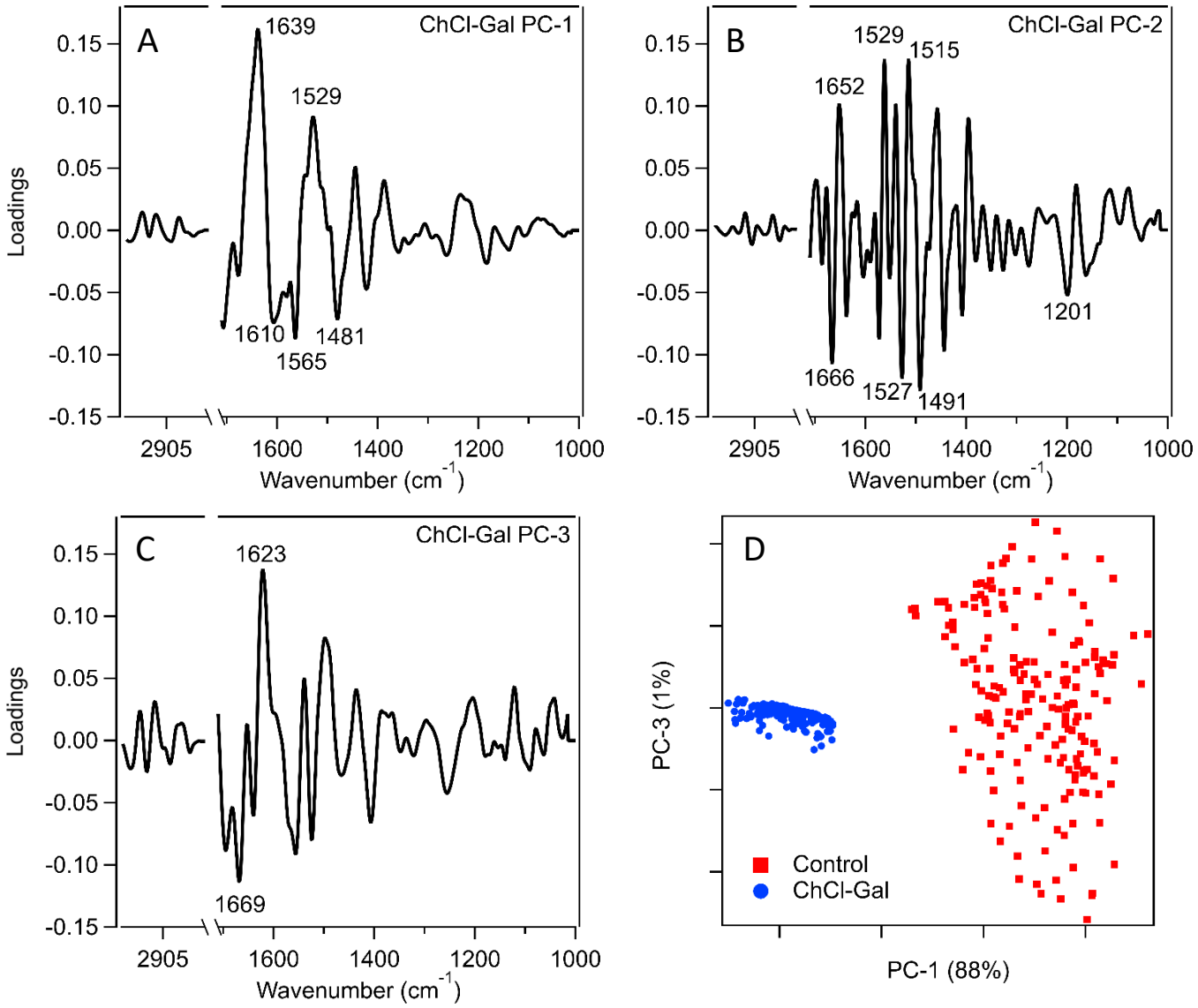

Figure S7. PCA loadings plots of HaCat cells treated with ChCl-Gal compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. Significance cut off of 0.05 was applied to PC-1 and of 0.1 to PC-2 and PC-3. D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

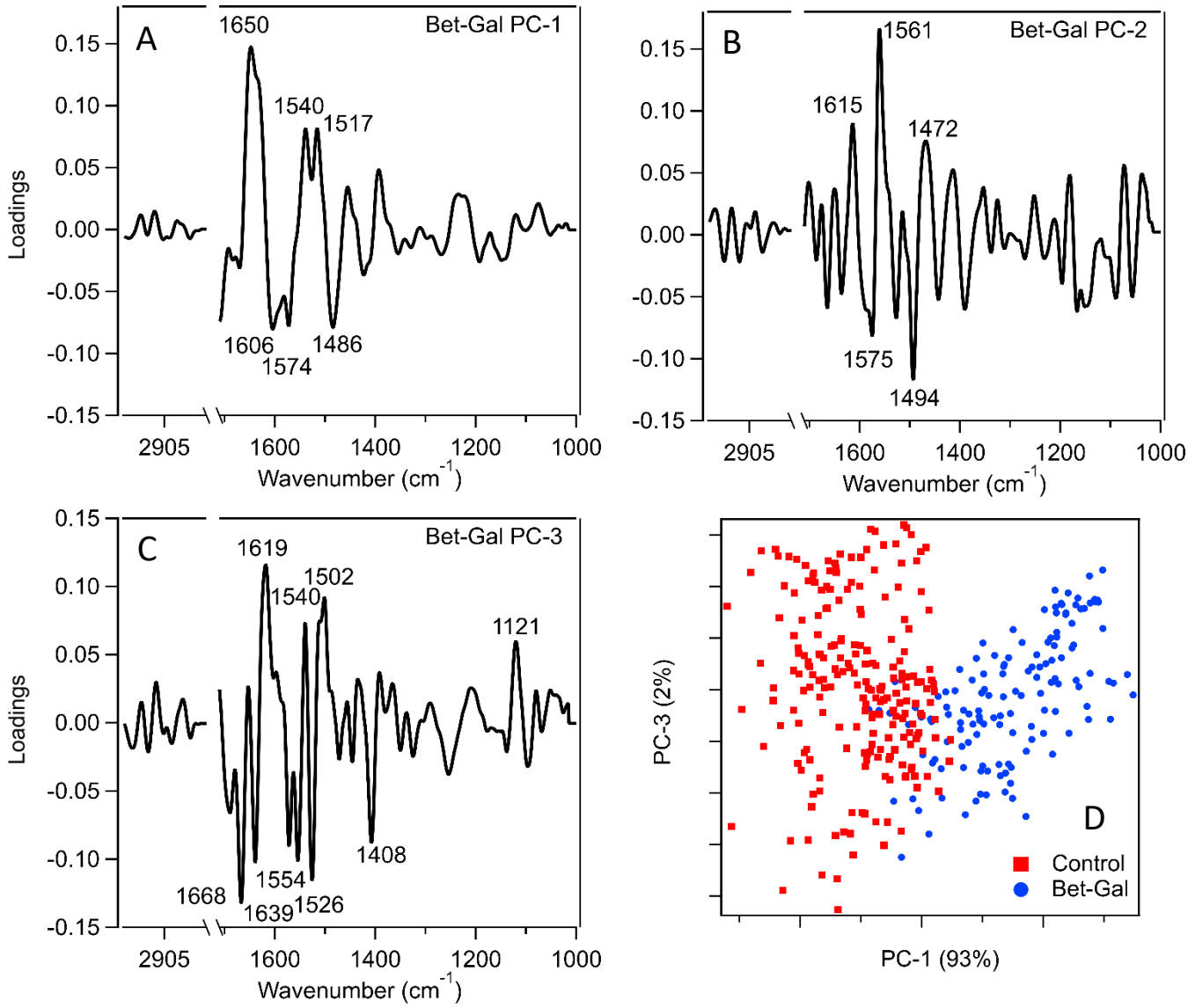

Figure S8. A-C) PCA loadings plots of HaCat cells treated with Bet-Gal compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. A significance cut off of 0.07 was applied to PC-1 and PC-2 and of 0.1 to PC-3 D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

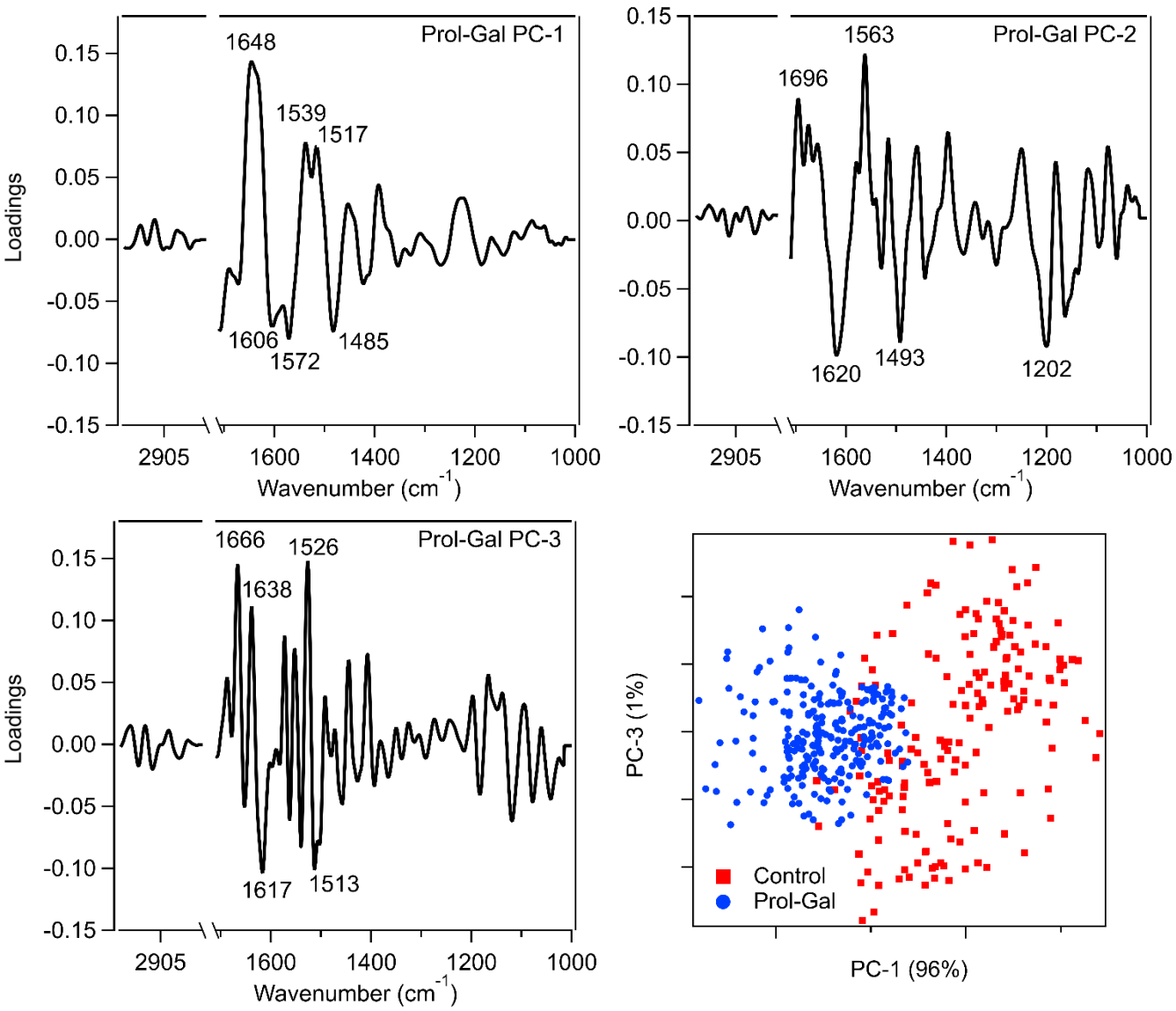

Figure S9. PCA loadings plots of HaCat cells treated with Prol-Gal compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. Significance cut offs of 0.05, 0.07 and 0.1 were applied for PC-1, 2 and 3 respectively. D) PCA scores plot showing the separation of the cell clusters along PC-1 and PC-3 axes.

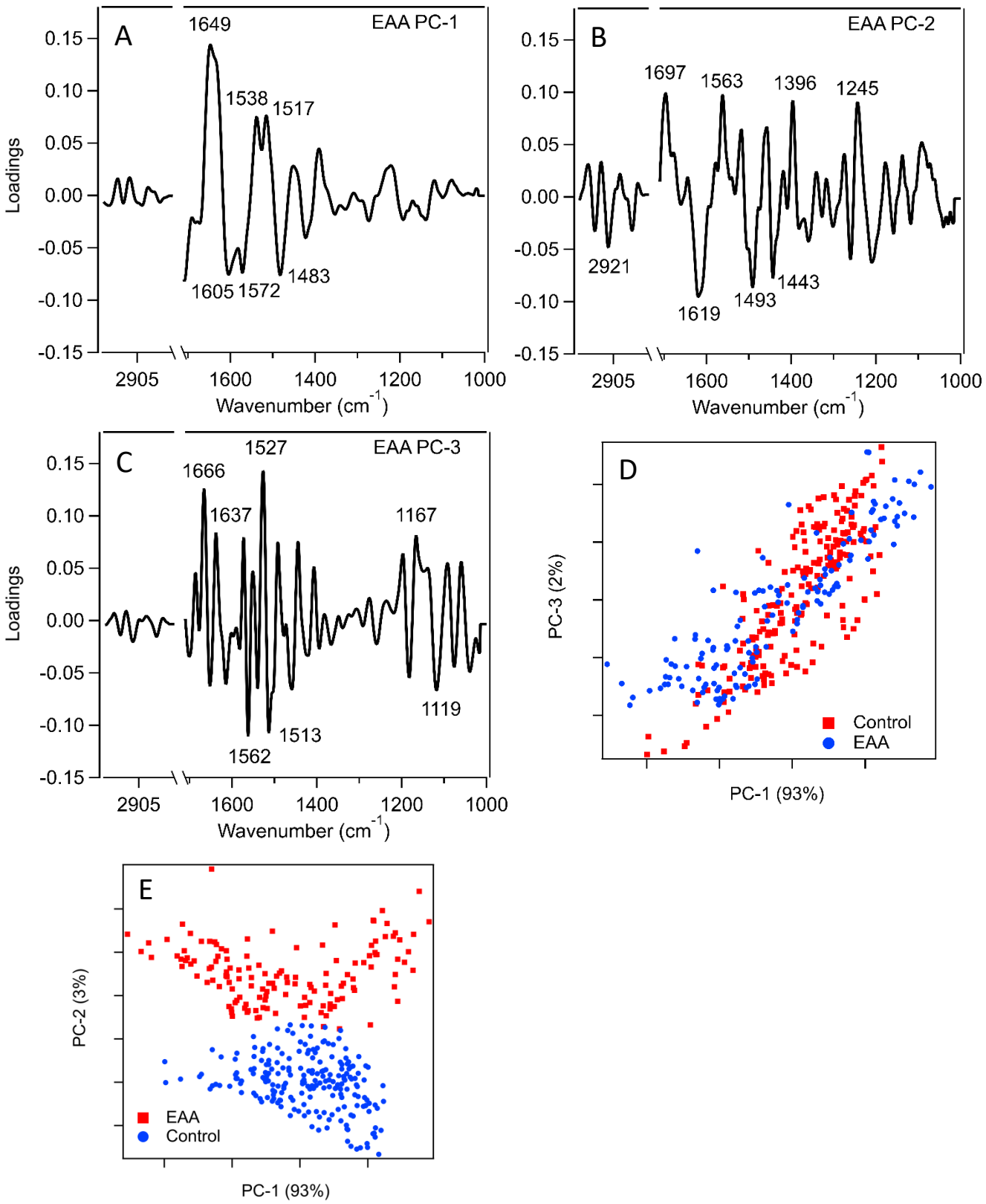

Figure S10. A-C) PCA loadings plots of HaCat cells treated with EAA compared to the untreated control. The assignment of key absorption peaks is shown in Table S1. A significance cut-off of 0.07 was applied to PC-1 and PC-2 and of 0.1 was applied to PC-3. D and E) PCA scores plot showing the separation of the cell clusters along PC-1, PC-2 and PC-3 axes.

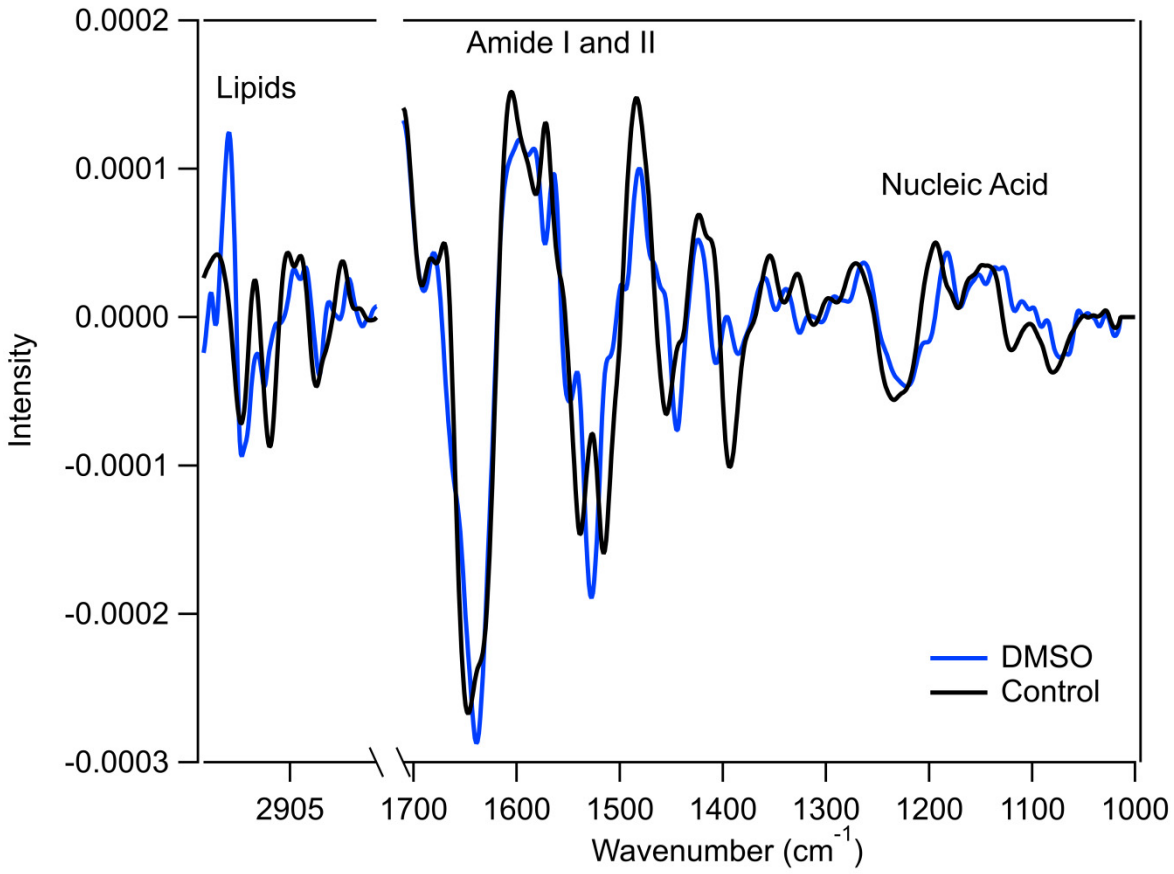

Figure S11. Comparison of average 2nd derivative spectra of the DMSO-treated and untreated control cells, showing the differences in the key biochemical compositions (i.e., lipids, proteins and nucleic acids).

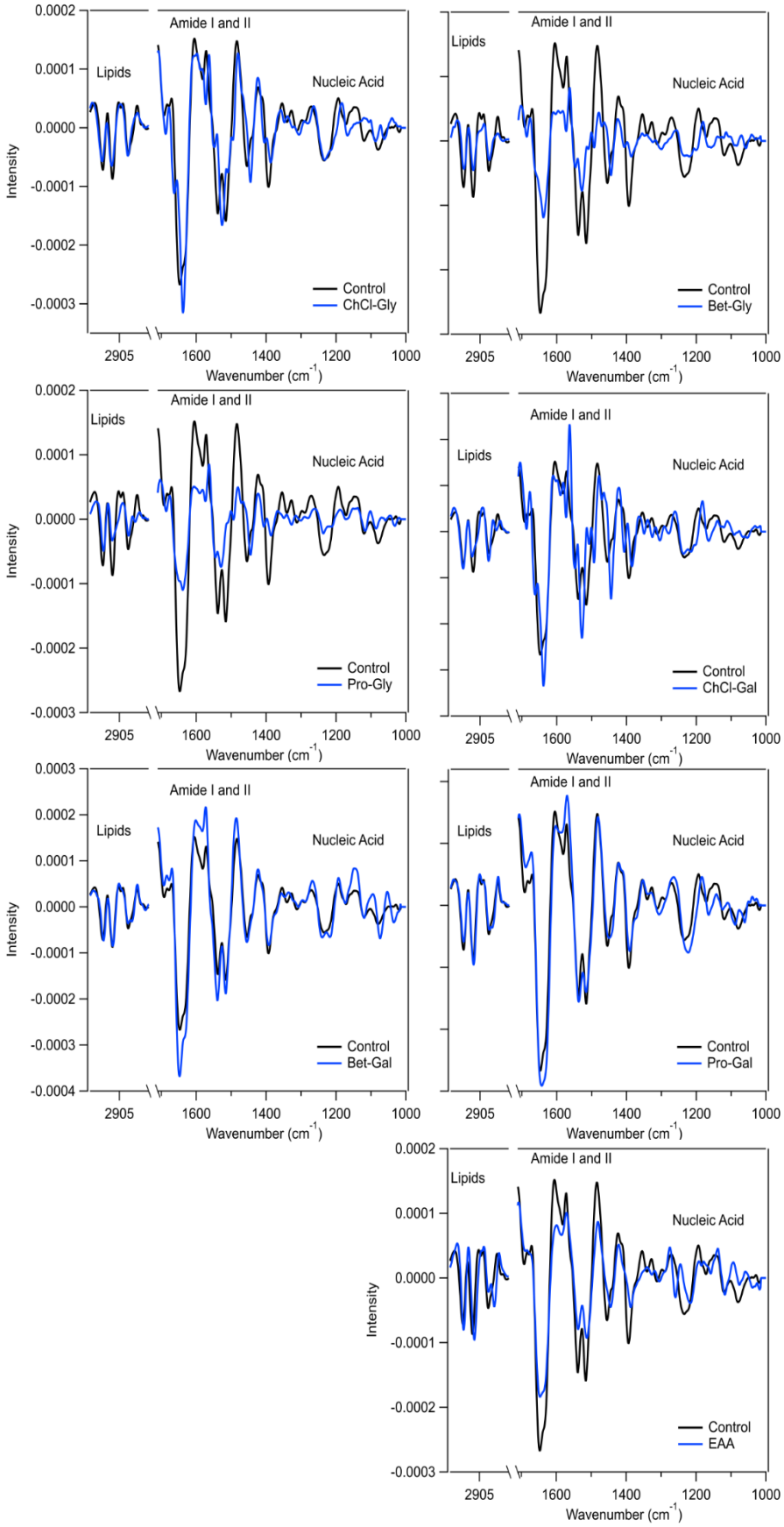

Figure S12. Comparison of average 2nd derivative spectra of treated cells and untreated control cells, showing the differences in the key biochemical compositions (i.e., lipids, proteins and nucleic acids).

Table S1. Band assignment of the loaded peaks observed in the PCA loadings plots for each treatment.

Treatment	PC	Wavenumber (cm^{-1})	Assignment	Broad Assignment	References
DMSO	PC-1	1644	Amide I (C=O stretch)	Amide I	[1, 2]
		1605	ν_{as} (COO^-) (polysaccharides, pectin)	Amide I	[1]
		1532	C=N	Nucleic Acid	[1, 3]
		1483	C_8-H couple with a ring vibration[a] assigned to 1482/3/5	Nucleic Acid	[4]
	PC-2	2979	Undefined	N/A	[1]
		2929	C-H stretching	Lipids	[3]
		1668	Amide I (anti-parallel β-sheet)	Amide I	[1]
		1527	Stretching C=N, C=C	Amide II	[1, 3]
		1514	ν (C=C) diagnostic of the presence of carotenoid structure	N/A	[1]
		1395	Undefined	N/A	[1]
	PC-3	2960	ν_{as} (CH_3)	Lipids	[5]
		2918	C-H stretching	Lipids	[3]
		1698	C_2=O guanine N-H thymine	Nucleic Acid	[1, 3]
		1623	Base carbonyl stretching and ring breathing[k Assigned to 1620]	Nucleic Acid	[1, 6]
		1562	Ring Base	Ring Base	[1, 3]
		1493	In-plane CH bending vibration	Amide II	[1]
		1466	Overlapping peak region	N/A	[1]
		1444	Overlapping peak region	N/A	[1]
		1205	C-O-C, C-O dominated by the ring vibrations of polysaccharides C-O-P, P-O-P	Nucleic Acid	[1, 7, 8]
ChCl-Gly	PC-1	1639	Amide I	Amide I	[17]
		1606	Adenine vibration	Nucleic Acid	[1, 9]
		1527	Stretching C=N, C=C	Amide II	[1, 3]
		1484	C_8-H couple with a ring vibration[a] assigned to 1482/3/5	Nucleic Acid	[4]
	PC-2	1668	Amide I (anti-parallel β-sheet)	Amide I	[1]
		1652	Amide I	Amide I	[1, 3]
		1638	Overlapping peak region	N/A	[1]
		1540	Amide II (β-sheet)	Amide II	[1, 10]
		1526	C=N	Nucleic Acid	[1, 3]
		1492	Overlapping peak region	N/A	[1]
		1121*	Symmetric phosphodiester stretching band	Nucleic Acid	[1, 11]
	PC-3	2927	C-H stretching	Lipids	[3]
		1697	Amide I (anti-parallel β-sheet)	Amide I	[1, 8]
		1561	Ring Base	Ring Base	[1, 3]
		1493	In-plane CH bending vibration	Amide II	[1]
		1200*	Phosphate (P=O) band	Nucleic Acid	[1, 12]
Bet-Gly	PC-1	1639	Amide I	Amide I	[17]
		1607	Adenine vibration	Nucleic Acid	[1, 9]
		1565	Ring Base	Ring Base	[1, 3]
		1531	Undefined	N/A	[1]

		1481	Amide II	Amide II	[1, 8]
	PC-2	1562	Ring Base	Ring Base	[1, 3]
		1527	Stretching C=N, C=C	Amide II	[1, 3]
		1515	ν (C=C) diagnostic of the presence of carotenoid structure	N/A	[1]
		1491	Overlapping peak region	N/A	[1]
		1458	$\delta_{as}CH_3$ of collagen	N/A	[1, 13]
		1197*	Phosphate (P=O) band	Nucleic Acid	[1, 12]
	PC-3	2921	Asymmetric vibrations of CH_2 of acyl chains	Lipid	[1, 14]
		2852	ν_s CH_2	Lipid	[1, 5]
		1540	Amide II (β-sheet)	Amide II	[1, 10]
		1525	C=N	Nucleic Acid	[1, 3]
Prol-Gly	PC-1	1641	C=O stretch	Nucleic Acid	[1, 3]
		1607	ν_{as} (COO^-) (polysaccharides, pectin)	Amide I	[1]
		1566	Ring Base	Ring Base	[1, 3]
		1537	C=N and C=C stretch	Nucleic Acid	[1, 3]
		1482	C_8-H couple with a ring vibration[a] assigned to 1482/3/5	Nucleic Acid	[1, 3]
	PC-2	1562	Ring Base	Ring Base	[1, 3]
		1529	C=N	Nucleic Acid	[1, 3]
		1515	ν (C=C) diagnostic of the presence of carotenoid structure	N/A	[1]
		1445	Overlapping peak region	N/A	[1]
	PC-3	1668	Amide I (anti-parallel β-sheet)	Amide I	[1]
		1639	Amide I	Amide I	[17]
		1617	Ring C-C stretch of aromatic	Amide I	[1]
		1542	Amide II	Amide II	[1, 15]
		1526	C=N	Nucleic Acid	[1, 3]
		1119	C-O stretching mode	N/A	[1]
ChCl-Gal	PC-1	1639	Amide I	Amide I	[17]
		1610	Adenine vibration	Nucleic Acid	[1, 9]
		1565	Ring Base	Ring Base	[1, 3]
		1529	Stretching C=N, C=C	Amide II	[1, 3]
		1481	Amide II	Amide II	[1, 8]
	PC-2	1666	Amide I	Amide I	[1]
		1652	Amide I	Amide I	[1, 3]
		1529	Stretching C=N, C=C	Amide II	[1, 3]
		1527	Stretching C=N, C=C	Amide II	[1, 3]
		1491	Overlapping peak region	N/A	[1]
		1201*	PO_2^- asymmetric (phosphate I)	Nucleic Acid	[1, 3]
	PC-3	1669	Amide I (anti-parallel β-sheet)	Amide I	[1]
		1623	Base carbonyl stretching and ring breathing[k Assigned to 1620]	Nucleic Acid	[1, 6]
Bet-Gal	PC-1	1650	Amide I	Amide I	[1, 3]
		1606	Overlapping peak region	N/A	[1]
		1574	C=N Adenine	Nucleic Acid	[1, 3]
		1540	Amide II (β-sheet)	Amide II	[1, 10]
		1517	Amide II	Amide II	[1]
		1486	Overlapping peak region	N/A	[1]
	PC-2	1615	Ring C-C stretch of aromatic	Amide I	[1]

		1574	C=N Adenine	Nucleic Acid	[1, 3]
		1561	Ring Base	Ring Base	[1, 3]
		1494	Overlapping peak region	N/A	[1]
		1472	Overlapping peak region	N/A	[1]
	PC-3	1668	Amide I (anti-parallel β-sheet)	Amide I	[1]
		1639	Amide I	Amide I	[17]
		1619	Ring C-C stretch of aromatic	Amide I	[1]
		1554	Amide II (α-sheet)	Amide II	[18]
		1540	Amide II (β-sheet)	Amide II	[1, 10]
		1526	C=N	Nucleic Acid	[1, 3]
		1502	Amide II	Amide II	[1]
		1408	Overlapping peak region	N/A	[1]
		1121*	Symmetric phosphodiester stretching band	Nucleic Acid	[1, 11]
Prol-Gal	PC-1	1648	Unordered random coils	Amide I	[1]
		1606	ν_{as} (COO^-) (polysaccharides, pectin)	Amide I	[1]
		1572	Amide II	Amide II	[1]
		1539	Amide II	Amide II	[1]
		1517	Amide II	Amide II	[1]
		1485	C_8-H couple with a ring vibration[a] assigned to 1482/3/5	Nucleic Acid	[4]
	PC-2	1696	Amide I (anti-parallel β-sheet)	Amide I	[1, 8]
		1620	Base carbonyl stretching and ring breathing[k Assigned to 1620]	Nucleic Acid	[1, 6]
		1563	Ring Base	Ring Base	[1, 3]
		1493	In-plane CH bending vibration	Amide II	[1]
		1202	PO_2^- asymmetric (phosphate I)	Nucleic Acid	[1, 3]
	PC-3	1666	Amide I	Amide I	[1]
		1638	Overlapping peak region	N/A	[1]
		1617	Ring C-C stretch of aromatic	Amide I	[1]
		1526	C=N	Nucleic Acid	[1, 3]
		1513	ν (C=C) diagnostic of the presence of carotenoid structure	N/A	[1]
EAA	PC-1	1649	Unordered random coils	Amide I	[1]
		1605	ν_{as} (COO^-) (polysaccharides, pectin)	Amide I	[1]
		1572	Amide II	Amide II	[1]
		1538	C=N and C=C stretch	Nucleic Acid	[1, 3]
		1517	Amide II	Amide II	[1]
		1483	C_8-H couple with a ring vibration[a] assigned to 1482/3/5	Nucleic Acid	[1, 3]
	PC-2	2921	Asymmetric vibrations of CH_2 of acyl chains	Lipid	[1, 14]
		1697	Amide I (anti-parallel β-sheet)	Amide I	[1, 8]
		1619	Ring C-C stretch of aromatic	Amide I	[1]
		1563	Ring Base	Ring Base	[1, 3]
		1493	In-plane CH bending vibration	Amide II	[1]
		1443	Overlapping peak region	N/A	[1]
		1396	Overlapping peak region	N/A	[1]
		1245	PO_2^- asymmetric	Nucleic Acid	[1]
	PC-3	1666	Amide I	Amide I	[1]
		1637	Amide I (β-sheet)	Amide I	[1, 8, 14]

		1562	Ring Base	Ring Base	[1, 3]
		1527	Stretching C=N, C=C	Amide II	[1, 3]
		1513	ν(C=C) diagnostic of the presence of carotenoid structure	N/A	[1]
		1167	Overlapping peak region	N/A	[1]
		1119	C-O stretching mode	N/A	[1]

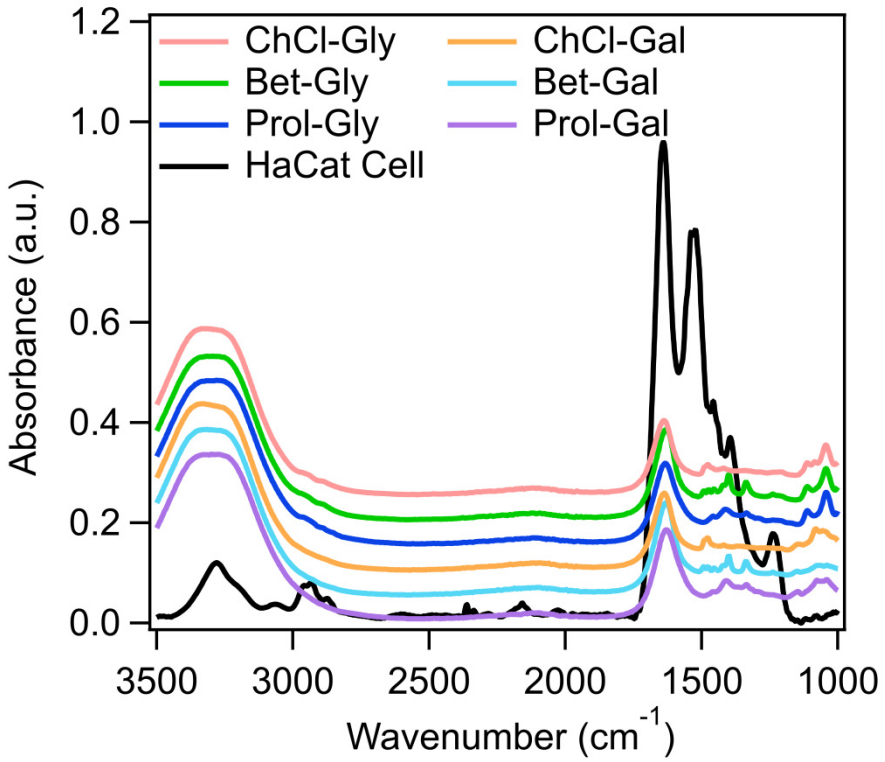

Figure S13. FTIR spectra of the deep eutectic solvents used in this study.

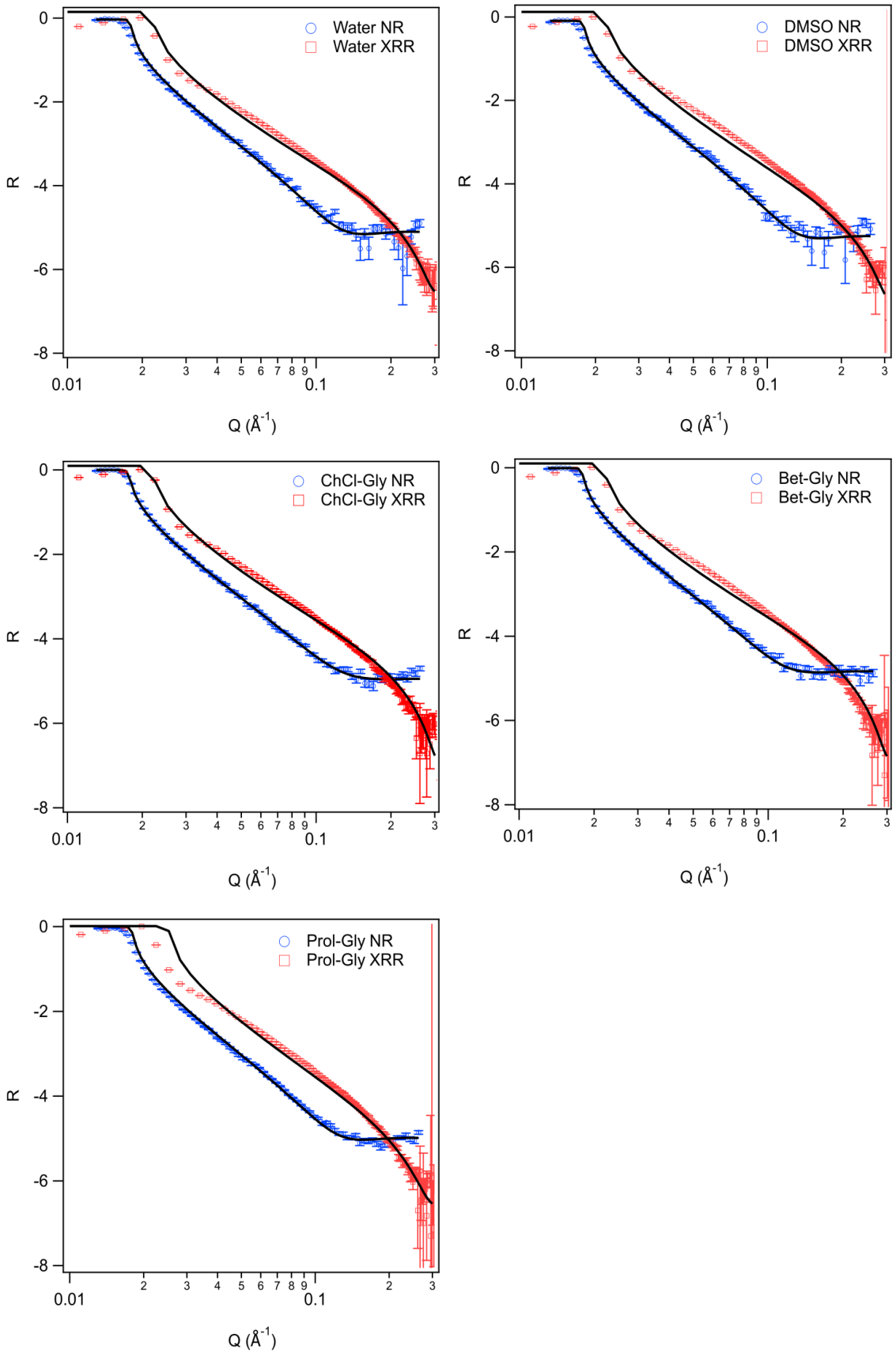

Figure S14. Neutron and X-ray reflectivity data with best fits for a POPC monolayer.

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