Electronic Supplementary Information

Co-exposure of C_{60} fullerene with benzo[*a*]pyrene results in enhanced biological effects in cells as determined by Fourier-transform infrared spectroscopy

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Supporting Information



Figure S1 Scanning electron microscopy (SEM) images of C_{60} fullerene with Raman spectra of characterization.



Figure S2 Three-D PCA-LDA scores plots (*95% Confidence ellipsoids projected on walls*) derived from post-exposure cells interrogated by FTIR: **a**) Gill cells; and, **b**) MCF-7 cells.



Figure S3 One-D scores plot in LD2 derived from PCA-LDA of spectral dataset (Dataset Total), with corresponding loadings plot: **a**) Gill cells; and, **b**) MCF-7 cells.



Figure S4 One-D scores plot in LD2 derived from PCA-LDA of spectral dataset for single treatment, with corresponding loadings plot.



Figure S5 One-D scores plot derived from PCA-LDA of spectral dataset (Dataset mix), with corresponding loadings plot.



Figure S6 One-D scores plot derived from PCA-LDA of spectral dataset (Dataset B[a]P mix), with corresponding loadings plot.



Figure S7 One-D scores plot derived from PCA-LDA of spectral dataset (Dataset C_{60} mix), with corresponding loadings plot.

One-way ANOVA with Dunnett's Multiple Comparison Test	Gill cells	MCF-7 cells
Control vs C ₆₀ 0.001 mg/L	<i>P</i> >0.05	<i>P</i> >0.05
Control vs C ₆₀ 0.01 mg/L	<i>P</i> < 0.05	<i>P</i> >0.05
Control vs C ₆₀ 0.1 mg/L	<i>P</i> <0.001	<i>P</i> >0.05
Control vs $B[a]P 10^{-8} M$	<i>P</i> <0.001	<i>P</i> >0.05
Control vs $B[a]P 10^{-7} M$	<i>P</i> <0.001	<i>P</i> >0.05
Control vs $B[a]P 10^{-6} M$	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs Mix 1	<i>P</i> >0.05	<i>P</i> >0.05
Control vs Mix 2	<i>P</i> <0.05	<i>P</i> >0.05
Control vs Mix 3	<i>P</i> >0.05	<i>P</i> >0.05
Control vs Mix 4	<i>P</i> >0.05	<i>P</i> <0.001
Control vs Mix 5	<i>P</i> >0.05	<i>P</i> >0.05

Table S1 Ratio of Protein-to-Lipid derived from FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test	Gill cells	MCF-7 cells
Control vs C ₆₀ 0.001 mg/L	<i>P</i> >0.05	<i>P</i> >0.05
Control vs C ₆₀ 0.01 mg/L	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs C ₆₀ 0.1 mg/L	<i>P</i> <0.0001	<i>P</i> <0.0001
Control vs $B[a]P 10^{-8} M$	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs $B[a]P 10^{-7} M$	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs $B[a]P 10^{-6} M$	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs Mix 1	<i>P</i> <0.0001	<i>P</i> <0.05
Control vs Mix 2	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs Mix 3	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs Mix 4	<i>P</i> <0.0001	<i>P</i> <0.05
Control vs Mix 5	<i>P</i> <0.0001	<i>P</i> <0.05

 Table S2 Ratio of Protein-to-Nucleic acid derived from FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test	Gill cells	MCF-7 cells
Control vs C ₆₀ 0.001 mg/L	<i>P</i> >0.05	<i>P</i> >0.05
Control vs C ₆₀ 0.01 mg/L	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs C ₆₀ 0.1 mg/L	<i>P</i> <0.0001	<i>P</i> >0.05
Control vs $B[a]P 10^{-8} M$	<i>P</i> < 0.05	<i>P</i> >0.05
Control vs $B[a]P 10^{-7} M$	<i>P</i> >0.05	<i>P</i> <0.05
Control vs $B[a]P 10^{-6} M$	<i>P</i> >0.05	<i>P</i> <0.0001
Control vs Mix 1	<i>P</i> <0.001	<i>P</i> >0.05
Control vs Mix 2	<i>P</i> >0.05	<i>P</i> >0.05
Control vs Mix 3	<i>P</i> >0.05	<i>P</i> >0.05
Control vs Mix 4	<i>P</i> <0.0001	<i>P</i> <0.05
Control vs Mix 5	<i>P</i> <0.0001	<i>P</i> >0.05

 Table S3 Ratio of RNA-to-DNA derived from FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test		Gill cells		MCF-7 ce	MCF-7 cells	
		LD1	LD2	LD1	LD2	
Dataset	Control vs. C ₆₀ 0.001 mg/L	<i>P</i> < 0.05	P <0.0001	<i>P</i> >0.05	<i>P</i> >0.05	
C_{60}	Control vs. C ₆₀ 0.01 mg/L	P <0.0001	<i>P</i> <0.0001	<i>P</i> >0.05	<i>P</i> <0.0001	
	Control vs. C ₆₀ 0.1 mg/L	P <0.0001	P <0.0001	P <0.0001	<i>P</i> >0.05	
Dataset B[a]P	Control vs. $B[a]P 10^{-8} M$	P <0.0001	<i>P</i> >0.05	P <0.0001	P <0.0001	
נמו	Control vs. $B[a]P 10^{-7} M$	<i>P</i> <0.0001	P <0.001	P <0.0001	<i>P</i> <0.0001	
	Control vs. $B[a]P 10^{-6} M$	P <0.0001	<i>P</i> <0.001	<i>P</i> <0.0001	<i>P</i> <0.0001	
Dataset total	Control vs. C ₆₀ 0.001 mg/L	P <0.0001	P <0.05	<i>P</i> >0.05	<i>P</i> >0.05	
	Control vs. C ₆₀ 0.01 mg/L	<i>P</i> <0.001	<i>P</i> <0.0001	P <0.001	P <0.0001	
	Control vs. C ₆₀ 0.1 mg/L	P <0.001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	
	Control vs. $B[a]P 10^{-8} M$	<i>P</i> <0.0001	P <0.0001	<i>P</i> >0.05	<i>P</i> <0.0001	
	Control vs. $B[a]P 10^{-7} M$	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> < 0.05	<i>P</i> <0.0001	
	Control vs. B[a]P 10 ⁻⁶ M	P <0.0001	P <0.001	P <0.0001	<i>P</i> >0.05	
	Control vs. Mix1	<i>P</i> <0.0001	<i>P</i> <0.001	P <0.0001	<i>P</i> <0.0001	
	Control vs. Mix2	<i>P</i> < 0.001	P <0.0001	<i>P</i> >0.05	<i>P</i> >0.05	
	Control vs. Mix3	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05	P <0.0001	
	Control vs. Mix4	P <0.0001	P <0.0001	P <0.0001	<i>P</i> <0.0001	
	Control vs. Mix5	P <0.0001	P <0.0001	P < 0.05	P <0.0001	

 Table S4 Scores plots derived from PCA-LDA of FTIR spectra of cells

One-way	ANOVA with Dunnett's	Gill cells		MCF-7 cell	S
Multiple	Comparison Test	LD1	LD2	LD1	LD2
Dataset B[a]P	Control vs. $B[a]P 10^{-6} M$	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> >0.05
mix	Control vs. Mix 1	<i>P</i> <0.0001	<i>P</i> <0.001	<i>P</i> <0.0001	<i>P</i> <0.05
	Control vs. Mix 2	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> >0.05	<i>P</i> <0.0001
	Control vs. Mix 3	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> <0.0001	<i>P</i> <0.0001
Dataset	Control vs. C ₆₀ 0.1 mg/L	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001
C ₆₀ mix	Control vs. Mix 3	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> <0.0001	<i>P</i> <0.0001
	Control vs. Mix 4	<i>P</i> <0.001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001
	Control vs. Mix 5	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	P <0.0001
Dataset	Control vs. C ₆₀ 0.1 mg/L	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> >0.05
mix	Control vs. B[a]P 10 ⁻⁶ M	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001
	Control vs. Mix 3	<i>P</i> >0.05	<i>P</i> <0.05	<i>P</i> <0.0001	<i>P</i> <0.0001

 Table S5 Scores plots derived from PCA-LDA of FTIR spectra of cells

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset	1656	Amide I	1658	Amide I
C ₆₀	997	Glycogen	1510	Amide II
	1240	$v_{as}PO_2^-$	1709	Lipid, v(C=O)
	1709	Lipid, v(C=O)	1410	v(COO ⁻)
	951	Protein phosphorylation	1236	$v_{\rm as} PO_2^-$
	1597	Amide I	1097	v _s PO ₂
	1090	$v_{s}PO_{2}^{-}$	1591	Amide I
Dataset B[a]P	1103	Carbohydrates	1699	Lipid, v(C=O)
	1246	$v_{as}PO_2^-$	1003	Glycogen
	1558	Amide II	1516	Amide II
	1645	Amide I	1232	$v_{\rm as} PO_2^-$
	1712	Lipid, v(C=O)	1593	Amide I
	1026	Glycogen	1741	Lipid, v(C=O)
	1421	<i>v</i> (COO ⁻)	955	Protein phosphorylation
Dataset	1232	$v_{as}PO_2^-$	1091	v _s PO ₂
Total	1709	Lipid, v(C=O)	1016	Glycogen
	1664	Amide I	1194	Collagen
	1070	$v_{\rm s} {\rm PO}_2^-$	1408	v(COO ⁻)
	985	Protein phosphorylation	953	Protein phosphorylation
	1417	v(COO ⁻)	1253	$v_{\rm as} PO_2^-$
	1556	Amide II	1570	Amide II

Table S6 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (Gill cells)

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset	1656	Amide I	1649	Amide I
C ₆₀ mix	1705	Lipid, v(C=O)	1512	Amide II
	1548	Amide II	1078	v _s PO ₂
	1624	Amide I	1410	v(COO ⁻)
	991	Protein phosphorylation	1232	vasPO ₂
	1236	v _{as} PO ₂	1587	Amide I
	1088	$v_{\rm s} {\rm PO}_2^-$	1552	Amide II
Dataset	1099	v _s PO ₂	1695	Lipid, v(C=O)
B[a]P mix	1212	$v_{\rm as} PO_2^-$	1548	Amide II
	1710	Lipid, v(C=O)	1506	Amide II
	1668	Amide I	1739	Lipid, v(C=O)
	1554	Amide II	1018	Glycogen
	1421	v(COO ⁻)	1588	Amide I
	1354	Amide III	1090	v _s PO ₂ ⁻
Dataset	1512	Amide II	1236	v _{as} PO ₂
mix	1099	$v_{s}PO_{2}^{-}$	1672	Amide I
	1412	<i>v</i> (COO ⁻)	1512	Amide II
	1234	v _{as} PO ₂ ⁻	1109	Carbohydrates
	1674	Amide I	1714	Lipid, v(C=O)
	1714	Lipid, v(C=O)	1593	Amide I
	1020	Glycogen	1414	v(COO ⁻)

Table S7 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (Gill cells)

	LD1		LD2	
	Wavenumber	Tentative assignment	Wavenumber	Tentative assignment
	(cm ⁻¹)		(cm ⁻¹)	
Dataset	1649	Amide I	1658	Amide I
\mathbf{C}_{60}	1718	Lipid, v(C=O)	1691	Amide I
	1516	Amide II	1022	Glycogen
	1132	Carbohydrates	1090	$v_{\rm s} {\rm PO}_2^-$
	1244	$v_{\rm as} {\rm PO}_2^-$	1489	Protein, CH ₂
	960	Protein phosphorylation	951	Protein phosphorylation
	1095	v _s PO ₂	1593	Amide I
Dataset	1710	Lipid, v(C=O)	1595	Amide I
B[a]P	1097	$v_{\rm s} {\rm PO}_2^-$	1518	Amide II
	1037	Glycogen	1410	v(COO ⁻)
	982	Protein phosphorylation	1132	Carbohydrates
	1602	Amide I	1655	Amide I
	1533	Amide II	962	Protein phosphorylation
	1317	Amide III	1736	Lipid, v(C=O)
Dataset	1101	$v_{\rm s} {\rm PO}_2^-$	1714	Lipid, v(C=O)
Total	1508	Amide II	1672	Amide I
	1026	Glycogen	974	Protein phosphorylation
	1566	Amide II	1525	Amide II
	983	Protein phosphorylation	1597	Amide I
	1406	v(COO ⁻)	1138	Carbohydrates
	1712	Lipid, v(C=O)	1072	$v_{s}PO_{2}$

Table S8 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectraldataset (MCF-7 cells)

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset	1716	Lipid, v(C=O)	1647	Amide I
C ₆₀ mix	1595	Amide I	1606	Amide I
	1516	Amide II	1093	v _s PO ₂
	1132	Carbohydrates	1026	Glycogen
	1674	Amide I	974	Protein phosphorylation
	1068	$v_{s}PO_{2}^{-}$	1196	Collagen
	962	Protein phosphorylation	1437	v(COO ⁻)
Dataset	1101	$v_{\rm s} {\rm PO}_2^-$	1599	Amide I
B[a]P mix	1024	Glycogen	1645	Amide I
	1649	Amide I	1516	Amide II
	1560	Amide II	1088	$v_{s}PO_{2}^{-}$
	1726	Lipid, v(C=O)	1404	v(COO ⁻)
	983	Protein phosphorylation	1002	Glycogen
	1225	v _{as} PO ₂	1464	Protein, CH ₂
Dataset	1103	$v_{s}PO_{2}$	1720	Lipid, v(C=O)
mix	1645	Amide I	1655	Amide I
	1512	Amide II	980	Protein phosphorylation
	1408	<i>v</i> (COO ⁻)	1072	v _s PO ₂
	1024	Glycogen	1153	Carbohydrates
	1585	Amide I	1192	Collagen
	1219	$v_{\rm as} PO_2^-$	1564	Amide II

Table S9 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (MCF-7 cells)

	Gill cells		MCF-7 cells	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
C ₆₀ 0.1	1020	Glycogen	1647	Amide I
mg/L	1097	$v_{\rm s} {\rm PO}_2^-$	1514	Amide II
	956	Protein phosphorylation	1107	Carbohydrates
	1518	Amide II	1410	<i>v</i> (COO ⁻)
	1658	Amide I	1736	Lipid, v(C=O)
	1595	Amide I	1226	$v_{\rm as} PO_2^-$
	1411	v(COO ⁻)	960	Protein phosphorylation
B[a]P 10 ⁻⁶	1512	Amide II	1098	$v_{\rm s} {\rm PO}_2^-$
M	1099	$v_{\rm s} {\rm PO}_2^-$	1024	Glycogen
	1412	v(COO ⁻)	1581	Amide II
	1236	$v_{\rm as} PO_2^-$	1405	v(COO ⁻)
	1714	Lipid, v(C=O)	983	Protein phosphorylation
	1672	Amide I	1214	$v_{\rm as} PO_2^-$
	1022	Glycogen	1666	Amide I
B[<i>a</i>]P 10 ⁻⁶ & C ₆₀ 0.1	958	Protein phosphorylation	1724	Lipid, v(C=O)
	1022	Glycogen	1591	Amide I
	1110	Carbohydrates	1672	Amide I
	1518	Amide II	1629	Amide I
	1599	Amide I	1124	Carbohydrates
	1412	v(COO ⁻)	1022	Glycogen
	1685	Amide I	960	Protein phosphorylation

 Table S10 Primary wavenumbers in cluster vectors plots for PCA-LDA derived from IR

 spectra

	Gill cells		MCF-7 cells	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
B[a]P 10 ⁻⁶	1095	$v_{\rm s} {\rm PO}_2^-$	1098	$v_{\rm s} {\rm PO}_2^-$
Μ	1020	Glycogen	1556	Amide II
	1671	Amide I	1648	Amide I
	1512	Amide II	1031	Glycogen
	1410	v(COO ⁻)	983	Protein phosphorylation
	1593	Amide I	1504	Amide II
	1252	$v_{\rm as} PO_2^-$	1230	$v_{\rm as} PO_2^-$
B[a]P 10 ⁻⁶	1710	Lipid, v(C=O)	1097	$v_{\rm s} {\rm PO}_2^-$
& C ₆₀ 0.001	1101	Carbohydrates	1022	Glycogen
	1223	$v_{\rm as} PO_2^-$	1730	Lipid, v(C=O)
	1664	Amide I	1648	Amide I
	1550	Amide II	1689	Amide I
	1423	v(COO ⁻)	1571	Amide II
	1352	Amide III	948	Protein phosphorylation
B[a]P 10 ⁻⁶	1095	$v_{\rm s} {\rm PO}_2^-$	1022	Glycogen
& C ₆₀ 0.01	1020	Glycogen	1091	$v_{\rm s} {\rm PO}_2^-$
	1512	Amide II	1668	Amide I
	1674	Amide I	1498	Amide II
	1597	Amide I	1552	Amide II
	1408	v(COO ⁻)	1705	Lipid, v(C=O)
	956	Protein phosphorylation	1155	Collagen
B[a]P 10 ⁻⁶	1649	Amide I	1648	Amide I
& C ₆₀ 0.1	1545	Amide II	1726	Lipid, v(C=O)
	1612	Amide I	1606	Amide I
	1712	Lipid, v(C=O)	1095	$v_{\rm s} {\rm PO}_2^-$
	1024	Glycogen	1542	Amide II
	1103	Carbohydrates	1020	Glycogen
	958	Protein phosphorylation	929	Protein phosphorylation

 Table S11 Primary wavenumbers cluster vectors plots for PCA-LDA derived from IR

 spectra

	Gill cells		MCF-7 cells	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
C ₆₀ 0.1	1655	Amide I	1648	Amide I
mg/L	1550	Amide II	1716	Lipid, v(C=O)
	1699	Lipid, v(C=O)	1514	Amide II
	1086	$v_{s}PO_{2}^{-}$	1128	Carbohydrates
	1012	Glycogen	1588	Amide I
	1508	Amide II	1411	v(COO ⁻)
	953	Protein phosphorylation	1023	Glycogen
B[a]P 10 ⁻⁶	1651	Amide I	1597	Amide I
& C ₆₀ 0.1	1545	Amide II	1716	Lipid, v(C=O)
	1616	Amide I	1070	$v_{\rm s} {\rm PO}_2^-$
	960	Protein phosphorylation	964	Protein phosphorylation
	1024	Glycogen	1140	Carbohydrates
	1712	Lipid, v(C=O)	1637	Amide I
	1346	Amide III	1521	Amide II
B[a]P 10 ⁻⁷	1514	Amide II	1604	Amide I
& C ₆₀ 0.1	1591	Amide I	1545	Amide II
	1676	Amide I	1650	Amide I
	1412	v(COO ⁻)	1712	Lipid, v(C=O)
	1232	$v_{\rm as} {\rm PO}_2^-$	1041	Glycogen
	1076	$v_{\rm s} {\rm PO}_2^-$	970	Protein phosphorylation
	985	Protein phosphorylation	1415	v(COO ⁻)
B[a]P 10 ⁻⁸	1514	Amide II	1598	Amide I
& C ₆₀ 0.1	1232	$v_{\rm as} {\rm PO}_2^-$	1716	Lipid, v(C=O)
	1673	Amide I	1070	$v_{\rm s} {\rm PO}_2^-$
	1413	v(COO ⁻)	1643	Amide I
	1074	$v_{\rm s} {\rm PO}_2^-$	1515	Amide II
	1590	Amide I	1136	Carbohydrates
	985	Protein phosphorylation	964	Protein phosphorylation

 Table S12 Primary wavenumbers cluster vectors plots for PCA-LDA of IR spectra