**Supporting Information**

**Label-free Raman spectroscopy reveals signatures of radiation resistance in the tumor microenvironment**

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**Running title:** Decoding radiation resistance with Raman spectroscopy

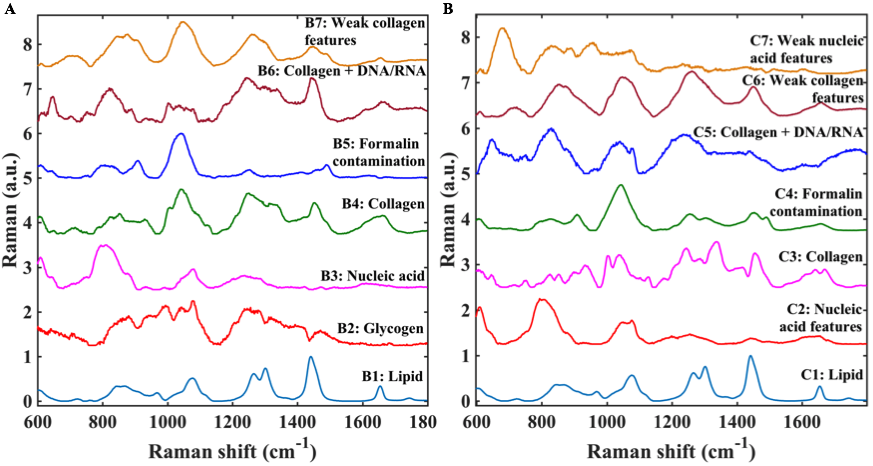
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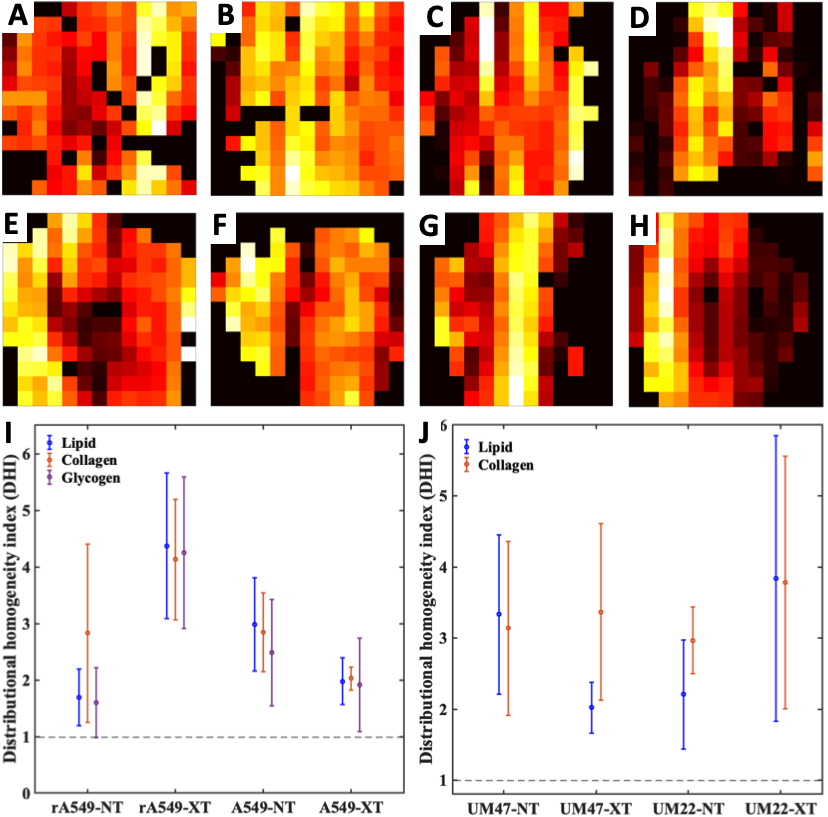
**Conflict of interest:** The authors disclose no potential conflicts of interest.



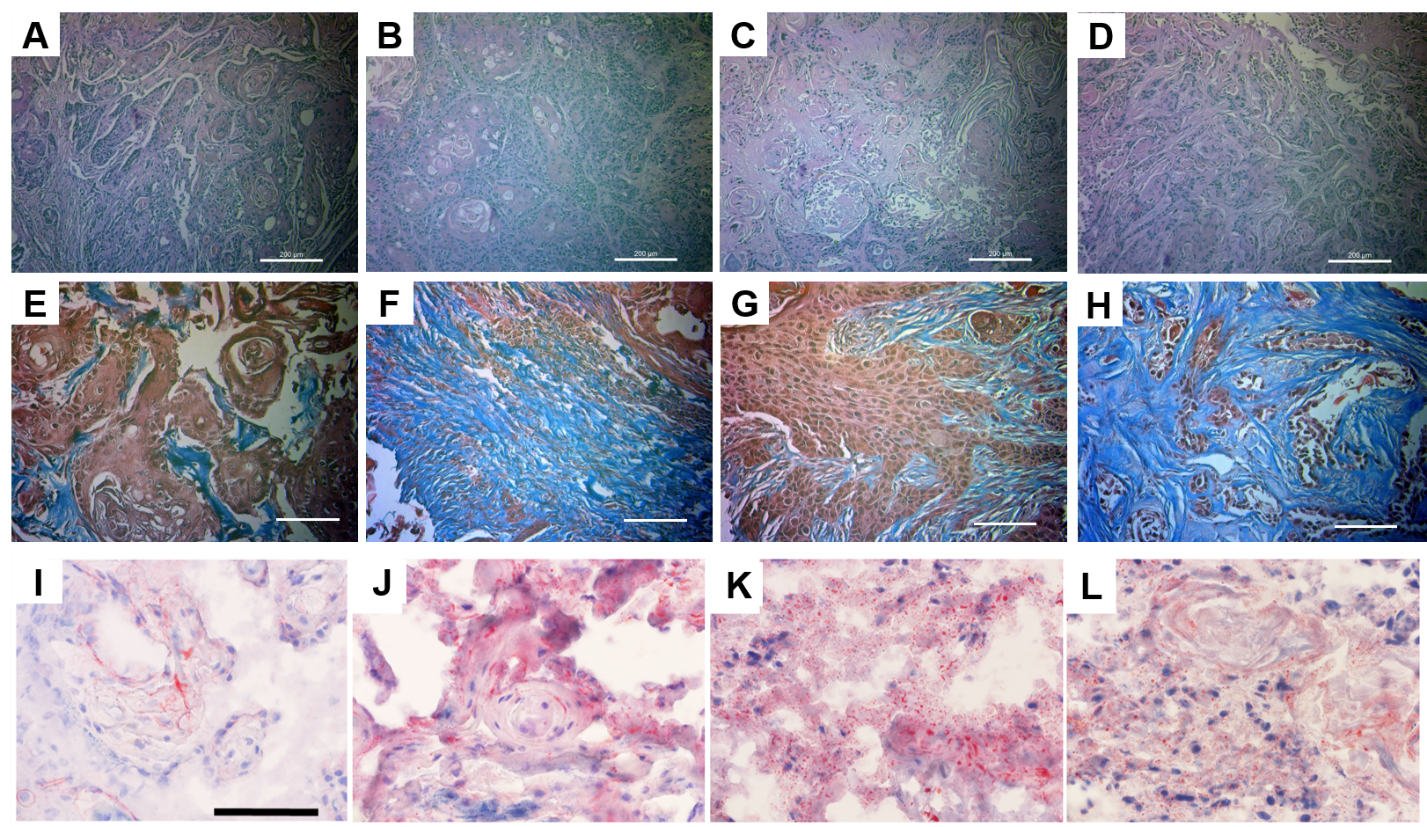
**Fig. SF1:** Tumor growth assays in response to radiation therapy for the **A.** HNSCC tumors and **B.** Lung tumors. Tumors in the XT group were radiated using the radiation schedule described in the methods section (4 x 2 Gy) at tumor volumes between 100 – 200 mm3. NT groups received sham radiation. There were significant differences in tumor volume between NT and XT groups in the UM-SCC-22B and A549 tumors. Differences in tumor volume were evaluated 35-50 days post-treatment. \* indicates significant differences at p < 0.05.



**Figure SF2.** The complete set of MCR-derived pure component spectra are provided here for – (**A**) lung tumor, and (**B**) head and neck tumor datasets. The biological components of interest, whose pure spectra resembles those obtained through MCR-ALS analysis, are identified and marked alongside the spectra.



**Figure SF3. Heterogeneity in biochemical composition of tumors.** Top (**A–D**) and middle (**E–H**) panels display representative MCR-ALS score maps of glycogen-rich and lipid-rich loadings respectively in lung and HN tumors. The columns of panels from left to right - (**A and E**), (**B and F**), (**C and G**) and (**D and H**), respectively, represent fields of view from tumors belonging to the treatment groups - RR-NT, RR-XT, RS-NT and RS-XT, where RR and RS are the radiation resistant and radiation sensitive groups respectively. Each pixel in the images in panels **A-F** spans an area of 1mm x 1mm. The bottom (**I and J**) panels show distributional homogeneity index plots calculated using relevant MCR-ALS loading score maps for tumors in lung and HN tumor datasets, respectively.



**Figure SF4**. Histologic assessment for head and neck tumor dataset**.** Top (**A–D**), middle (**E–H**) and bottom (**I–L**) panels display representative microscopic images of H&E, Masson's trichrome, and Oil Red O stained slides, respectively. The columns of panels from left to right - (**A and I**), (**B and F**), (**C and G**) and (**D and H**), respectively, represent fields of view from tumors belonging to the treatment groups – UM22-NT, UM22-XT, UM47-NT and UM47-XT. The scale bars in panels **A-D** represent 200 µm and panels **E-L** represent 100 µm.

**Table TS1.** Table listing the peak assignments for all the MCR-derived component spectra derived from lung tumor dataset.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Observed Raman peaks in the MCR loadings (cm-1)** | | | | | | | **Raman band assignment from literature** |
| **B1** | **B2** | **B3** | **B4** | **B5** | **B6** | **B7** |  |
|  | 708 |  |  |  |  |  | Glycogen |
|  |  | 790 |  |  |  |  | O-P-O stretching in DNA |
|  |  | 812 |  |  | 816 |  | O-P-O stretching in DNA and RNA |
|  |  |  | 851 |  |  |  | C-C stretch of proline in collagen |
|  |  |  |  |  |  | 878 | C-C stretch of hydroxyproline in collagen |
|  |  |  |  | 908 |  |  | Formalin contamination during tissue fixation |
|  |  |  | 928 |  |  |  | C-C vibration in collagen backbone |
|  | 940 |  |  |  |  |  | Glycogen |
|  |  |  | 1040 |  |  |  | Proline in collagen |
|  |  |  |  | 1042 |  |  | Formalin contamination during tissue fixation |
|  | 1044 |  |  |  |  |  | Glycogen |
|  |  |  |  |  |  | 1047 | Proline in collagen |
|  | 1078 |  |  |  |  |  | Glycogen |
| 1078 |  |  |  |  |  |  | C-C stretch |
|  |  | 1082 |  |  | 1080 |  | PO2- symmetric stretching in DNA |
|  |  | 1237 |  |  |  |  | PO2- asymmetric stretching in DNA |
|  |  |  |  |  | 1242 |  | Amide III in collagen |
|  |  |  | 1251 |  |  |  | Amide III in collagen |
|  |  |  |  | 1251 |  |  | Formalin contamination during tissue fixation |
|  | 1256 |  |  |  |  |  | Glycogen |
|  |  |  |  |  |  | 1259 | Amide III in collagen |
| 1266 |  |  |  |  |  |  | CH2 in-plane deformation (Triglyceride) |
| 1301 |  |  |  |  |  |  | CH vibration (Triglyceride) |
|  | 1320 |  |  |  |  |  | Glycogen |
|  |  |  | 1315 |  |  |  | CH3CH2 twisting modes of collagen |
|  |  |  |  |  | 1337 |  | CH3CH2 wagging modes of collagen and nucleic acids |
| 1442 |  |  |  |  |  |  | CH2 bending mode (Triglyceride) |
|  |  |  | 1453 |  |  | 1448 | CH2 bending mode in collagen |
|  |  |  |  | 1489 |  |  | Formalin contamination during tissue fixation |
| 1654 |  |  |  |  |  |  | C=C lipid stretch |
|  |  |  | 1661 |  | 1657 | 1656 | α-helical structure of amide I in collagen |

**Table TS2.** Table listing the peak assignments for all the MCR-derived component spectra derived from head and neck tumor dataset.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Observed Raman peaks in the MCR loadings (cm-1)** | | | | | | | **Raman band assignment from literature** |
| **C1** | **C2** | **C3** | **C4** | **C5** | **C6** | **C7** |  |
|  | 790 |  |  |  |  |  | O-P-O stretching in DNA |
|  | 806 |  |  |  |  |  | O-P-O stretching in DNA and RNA |
|  |  |  |  | 820 |  |  | O-P-O stretching in DNA and RNA |
|  |  | 852 |  |  |  |  | C-C stretch of proline in collagen |
|  |  |  |  |  | 873 |  | C-C stretch of hydroxyproline in collagen |
|  |  |  | 906 |  |  |  | Formalin contamination during tissue fixation |
|  |  | 932 |  |  |  |  | C-C vibration in collagen backbone |
|  |  | 1038 |  |  |  |  | Proline in collagen |
|  |  |  | 1042 |  |  |  | Formalin contamination during tissue fixation |
|  |  |  |  |  | 1047 |  | Proline in collagen |
| 1078 |  |  |  |  |  |  | C-C stretch |
|  | 1076 |  |  | 1076 |  | 1074 | PO2- symmetric stretching in DNA |
|  |  |  |  | 1235 |  |  | Amide III in collagen |
|  |  | 1245 |  |  |  |  | Amide III in collagen |
|  |  |  | 1256 |  |  |  | Formalin contamination during tissue fixation |
|  |  |  |  | 1259 | 1262 |  | Amide III in collagen |
| 1266 |  |  |  |  |  |  | CH2 in-plane deformation (Triglyceride) |
| 1302 |  |  |  |  |  |  | CH vibration (Triglyceride) |
|  |  |  | 1304 |  |  |  | Formalin contamination during tissue fixation |
|  |  |  |  |  |  |  | CH3CH2 twisting modes of collagen |
|  |  | 1338 |  |  |  | 1335 | CH3CH2 wagging modes of collagen and nucleic acids |
| 1442 |  |  |  |  |  |  | CH2 bending mode (Triglyceride) |
|  |  | 1454 |  |  | 1448 |  | CH2 bending mode in collagen |
|  |  |  | 1451 |  |  |  | Formalin contamination during tissue fixation |
|  |  |  | 1491 |  |  |  | Formalin contamination during tissue fixation |
| 1654 |  |  |  |  |  |  | C=C lipid stretch |
|  |  | 1668 |  |  | 1657 |  | α-helical structure of amide I in collagen |

**Table TS3.** Results of binary leave-one-mouse-out SVM analysis for lung tumor dataset.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **Number of mice classified accurately, unclassified and misclassified** | | | |
| **A549-NT** | **A549-XT** | **rA549-NT** | **rA549-XT** |
| **Binary SVM comparisons** | **A549-NT vs A549-XT** | **(5+0+0)/5** | **(1+2+1)/4** | **-** | **-** |
| **rA549-NT**  **vs**  **rA549-XT** | **-** | **-** | **(5+0+0)/5** | **(4+0+1)/5** |
| **A549-NT vs rA549-NT** | **(5+0+0)/5** | **-** | **(3+2+0)/5** | **-** |
| **A549-XT**  **vs**  **rA549-XT** |  | **(3+0+1)/4** |  | **(4+1+0)/5** |

**Table TS4.** Results of binary leave-one-mouse-out SVM analysis for head and neck tumor dataset.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **Number of mice classified accurately, unclassified and misclassified** | | | |
| **UM22-NT** | **UM22-XT** | **UM47-NT** | **UM47-XT** |
| **Binary SVM comparisons** | **UM22-NT vs UM22-XT** | **(6+0+0)/6** | **(7+0+0)/7** | **-** | **-** |
| **UM47-NT**  **vs**  **UM47-XT** | **-** | **-** | **(4+5+0)/9** | **(8+2+0)/10** |
| **UM22-NT vs UM47-NT** | **(5+1+0)/6** | **-** | **(9+0+0)/9** | **-** |
| **UM22-XT vs UM47-XT** |  | **(7+0+0)/7** |  | **(10+0+0)/10** |