**Label-free Raman spectroscopy detects stromal adaptations in pre-metastatic lungs**

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**Supplementary Information**

**Table ST1.** Band assignment for spectral features observed in PC loadings derived from the Raman spectra of the mouse lungs.

|  |  |
| --- | --- |
| **Observed Raman Peaks in the PC loadings (cm-1)** | **Raman band assignment from literature** |
| 859 | C-C stretch of proline (Collagen) |
| 917 | C-C stretch of proline ring (Collagen) |
| 1003 | C–C stretching vibration of the aromatic ring in the phenylalanine side chain (Collagen) |
| 1061 | OSO3 symmetric stretching (Proteoglycans) |
| 1304 | In-plane CH2 twisting modes of lipids |
| 1442 | CH2 deformation of lipids |
| 1592 | Carbon particles (source of particles unknown) |
| 1653 | Amide I (symmetric C=O stretching mode of proteins) and C=C lipid stretch (Lipids and proteins) |

**Table ST2.** Confusion matrix for PLS-DA derived classification model showing correct classification rates (%) averaged over 1000 iterations.

|  |  |
| --- | --- |
|  | **Predicted Class Labels** |
|  |  | **Control** | **MCL** | **MDL** |
| **Reference Labels** | **Control** | 90.1 | 0.1 | 9.8 |
| **MCL** | 1.0 | 97.7 | 1.3 |
| **MDL** | 20.9 | 0.7 | 78.4 |

**Table ST3.** Correct classification rates (%) of the PLS-DA-derived model using leave-one-mouse-out protocol (MD and MC refer to mouse models with MDA-MB-231 and MCF-7 tumor xenografts, respectively) with background subtracted spectra.

|  |  |  |
| --- | --- | --- |
| **Mouse excluded** | **Correct classification rate (%)** | **Chauvenet’s criterion for MDL**(n = 10; τmax = 1.96) |
| **Control** | **MCL** | **MDL** | **τ = |xi - xmean|/σ** | **Result** |
| None  | 95.4 | 95.6 | 75.1 | 0.71 | Retain |
| MD #1 | 85.7 | 94.2 | 81.5 | 0.04 | Retain |
| MD #2 | 90.1 | 94.8 | 76.8 | 0.52 | Retain |
| MD #3 | 100.0 | 96.0 | 100.0 | 2.18 | Eliminate |
| MC #1 | 88.5 | 96.3 | 83.0 | 0.21 | Retain |
| MC #2 | 96.3 | 95.5 | 74.5 | 0.77 | Retain |
| MC #3 | 96.2 | 96.5 | 73.1 | 0.95 | Retain |
| Control #1 | 96.7 | 93.4 | 73.7 | 0.87 | Retain |
| Control #2 | 98.0 | 94.0 | 84.8 | 0.42 | Retain |
| Control #3 | 95.6 | 94.1 | 89.6 | 0.97 | Retain |



**Figure S1.** Radial visualization plot showing clusters formed by spectra recorded from lung samples of sacrificed mice bearing MDA-MB-231 and MCF-7 breast cancer xenografts as well as controls without xenografts after autofluorescence background subtraction.