**Supporting Information:**

# Detection of Ovarian Cancer Using Samples Sourced from the Vaginal Microenvironment

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## Table of Contents

Supporting Information: Materials & Methods……..... S2

Figure S1: Pre-processing Workflow Using the MALDIquant Package in R…….. S3

Figure S2: *In vivo* Fluorescence Imaging of OVCAR-8-RFP Tumors in Athymic Mice…….. S4

Figure S3: Fluorescent Cell Spiking Study……..…….. S5

Figure S4: Mirror Plot Comparing Protein Fingerprints from Week Seven in a HGSOC and NASH Model…….. S7

Figure S5: Mass Spectra of Repeat Murine Lavages from Healthy Mice ……... S8

Figure S6: Consensus Spectra of Vaginal Lavages from Mouse 901…….. S9

Figure S7: Consensus Spectra of Vaginal Lavages from Mouse 902…….. S10

Figure S8: Consensus Spectra of Vaginal Lavages from Mouse 903…….. S11

Figure S9: Consensus Spectra of Vaginal Lavages from Mouse 904…….. S12

Figure S10: Consensus Spectra of Vaginal Lavages from Mouse 905…… S13

Table S1: Up- and Downregulated Spectral Features Differentially Expressed Across Various Time Points Throughout the Study……. S14

References…… S20

## Supporting Information: Materials & Methods

### Cell Culture

OVCAR-8-RFP were grown in DMEM with 10% FBS and 1% penicillin/streptomycin. Cultured cells were maintained in a humidified incubator at 37°C in 5% $CO_2$. Cells were passaged a maximum of 20 times. Cell lines were validated by short tandem repeat analysis and tested mycoplasma-free in 2017.

### *In Vivo* Murine Xenograft Study

Tumor burden was monitored using a Xenogen IVIS® Spectrum *In Vivo* Imaging System (PerkinElmer) as described by Lewellen *et al.* Imaging was done using an exposure time of one second, an Fstop of two, and an excitation and emission wavelength of 535 and 620 nm, respectively.[26] Vaginal lavage samples using 200 μL of sterile PBS as described by McLean *et. al*. at various time points prior to xenografts to collect healthy samples.[25] Mice were housed in facilities managed by the Biological Resources Laboratory at UIC and were provided food and water *ad libitum*. All animals were humanely treated in accordance with the Animal Care and Use Committee guidelines at the University of Illinois at Chicago (UIC) using Protocol #17-174.

Vaginal lavage samples were also collected using 200 μL of sterile PBS of Black 6 age-matched mice (N=9) with NASH for comparative purposes to our ovarian cancer study.

### Cell Counting for MS Analysis

Cells sourced from murine vaginal lavages were counted using a K2 Cellometer (Nexcelom, Lawrence, MA) by pipetting 20 μL of the sample into disposable counting chambers for imaging. Brightfield images were taken of each sample for accurate counts of leukocytes and epithelial cornified cells present which were used to calculate the concentration of the lavage samples using FCS Express software (De Novo). Fluorescent images were also taken using a 660 nm filter to determine if OVCAR-8-RFP cells were present in murine samples. All lavages were spun down at 150 rcf for five minutes, at which point, excess PBS was removed. Cells were resuspended in deionized water to reach a final concentration of 10,000 cells/μL.

### Limit of Detection Spiking Studies

OVCAR-8-RFP cells as well as cells sourced from murine vaginal lavages were separately counted using a K2 Cellometer (Nexcelom, Lawrence, MA) and concentrated to a final concentration of 10,000 cells/μL in deionized water. RFP-tagged cells were spiked into healthy cell mixtures at 1% and 10% for detection. Fluorescent images were taken using a 660 nm filter to quantify the number of OVCAR-8-RFP cells present in the lavage samples.

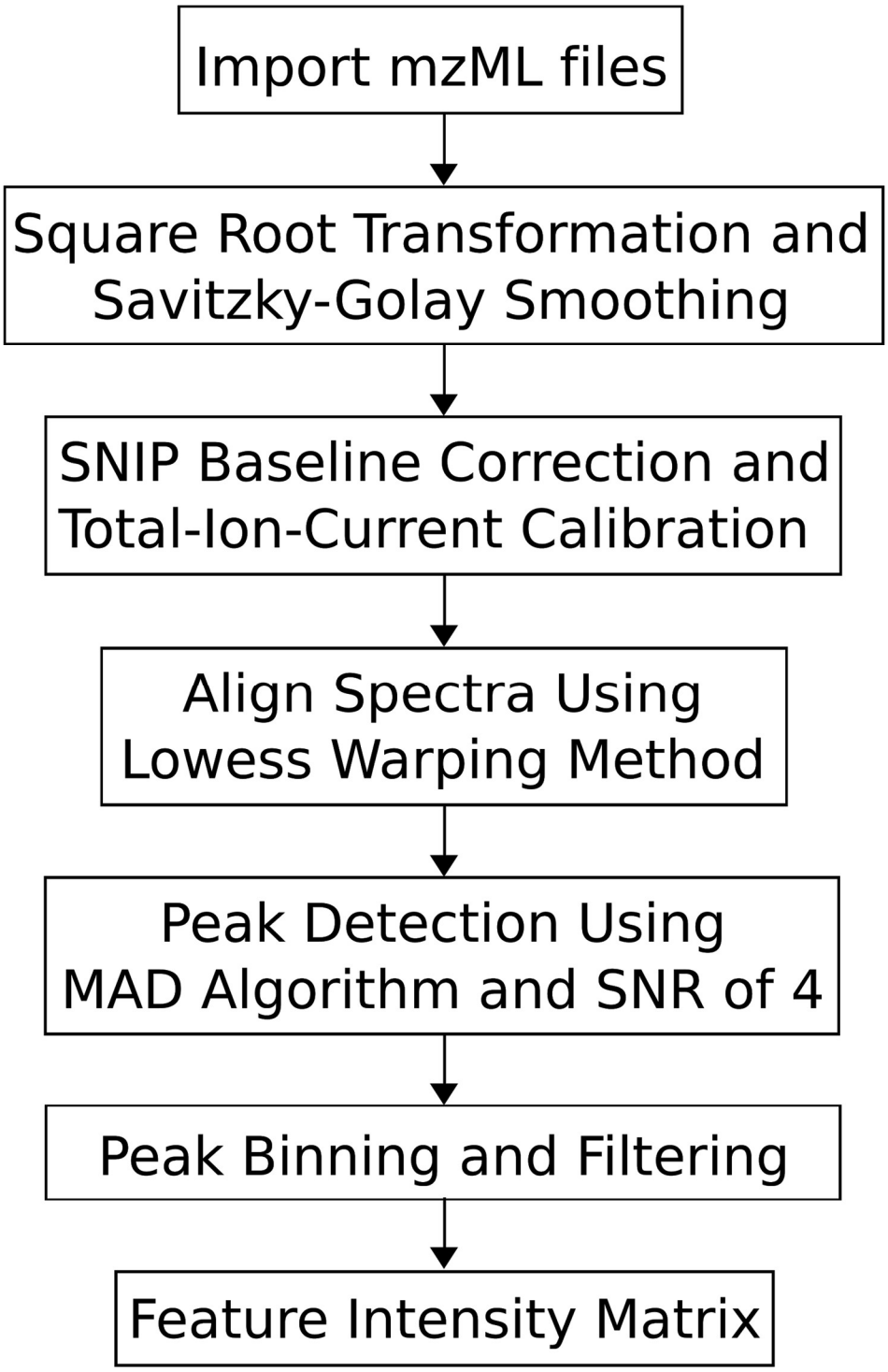



Figure S1: Pre-processing workflow using the MALDIquant package in R. All mzML files were uploaded using the MALDIquant package and batch processed using the outlined parameters, resulting in a feature matrix that consists of feature peaks and their corresponding intensity values.

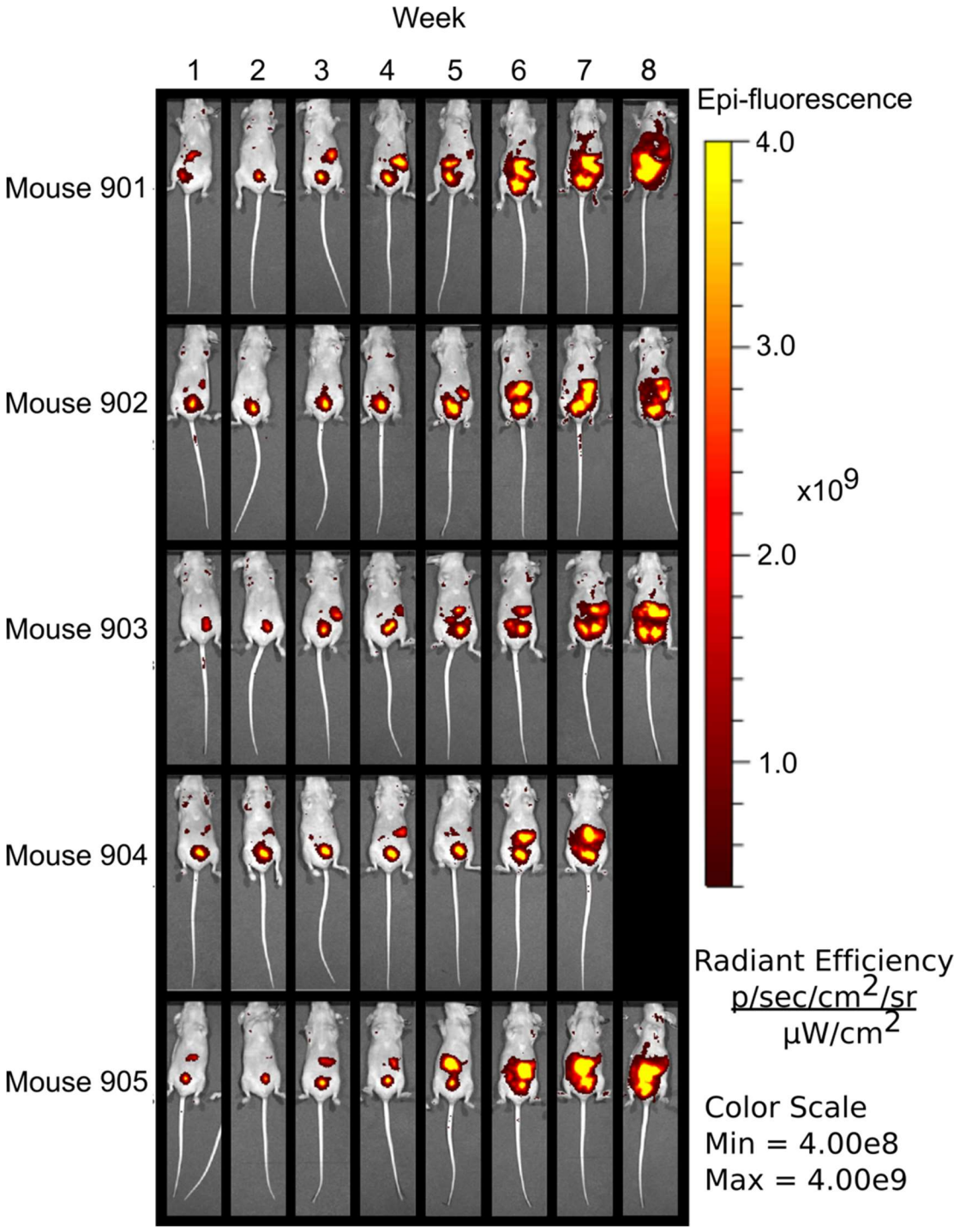
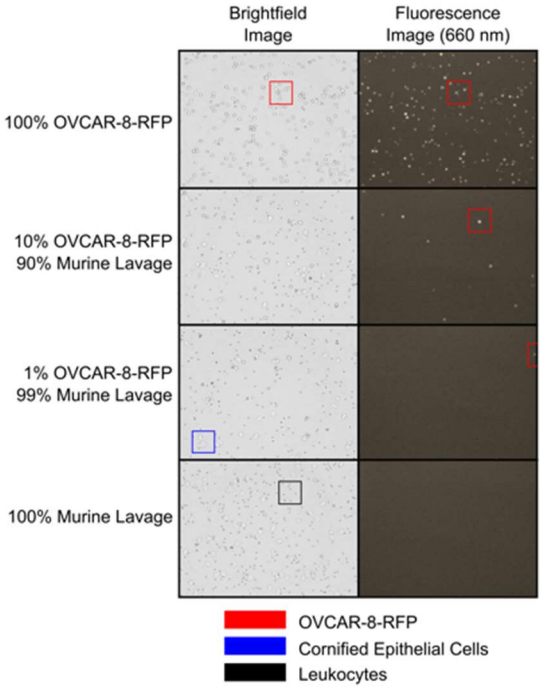



Figure S2: *In vivo* fluorescence imaging of athymic nude mice across the time course of the study showing OVCAR-8-RFP tumor progression. Mice were imaged on a weekly basis for a time span of two months. Mouse 904 expired prior to the conclusion of the study, which is why no image was obtained during the eighth week.

(A)

| Cell Type | Average Cell Count (Fluorescence) | Average Cell Count (Brightfield) | Average Fluorescence (%) | Standard Deviation | Fluorescence Based on FACS (%) |
|---|---|---|---|---|---|
| 100% OVCAR-8-RFP | 913.167 | 1715.500 | 43.552 | 0.154 | 98.52 |
| 10% OVCAR-8-RFP 90% Lavage | 17.500 | 1217.125 | 1.429 | 0.008 | 2.17 |
| 1% OVCAR-8-RFP 99% Lavage | 1.375 | 1326.625 | 0.065 | 0.001 | 0.17 |
| 100% Lavage | 0.000 | 1324.000 | 0.000 | 0.000 | 0.04 |

(B)




(C)

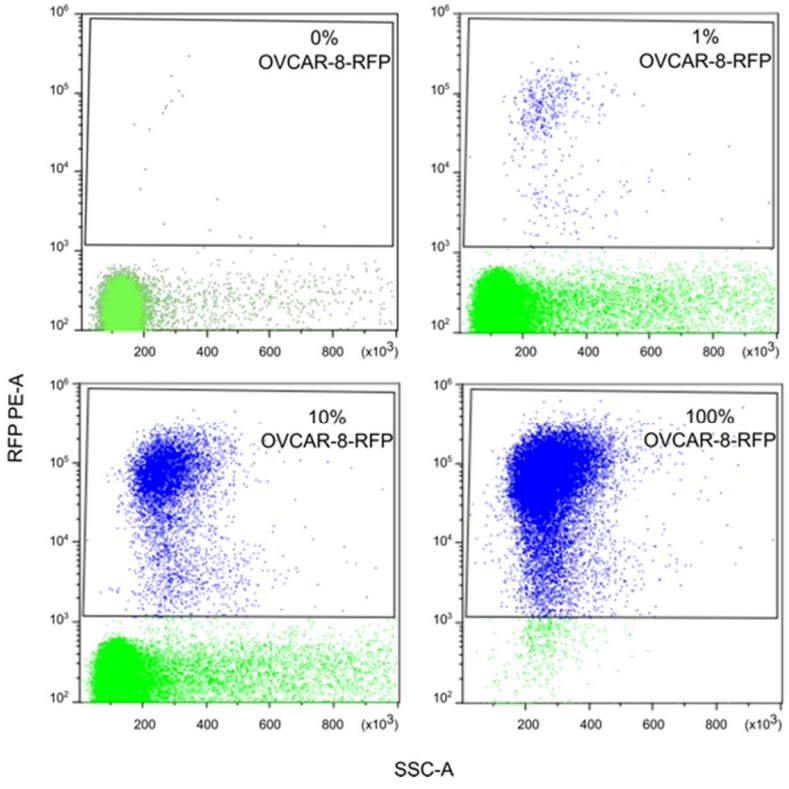


Figure S3: (A) Table detailing the average cell count (fluorescent and brightfield) along with average fluorescence with respective standard deviation values (n=6) and fluorescence based on flow cytometry. (B) Representative brightfield and fluorescence (600 nm) images of OVCAR-8-RFP cells, murine lavages and mixtures of the two components. Colored boxes are used to outline examples of fluorescent OVCAR-8 cells (red), cornified epithelial cells (blue) & leukocytes (black), the latter two being normally found in the murine reproductive system. C) Scatter plots of phycoerythrin (PE) absorbance vs. side scatter (SSC) in our FACS analysis where the blue and green dots are representative of fluorescent and non-fluorescent cell counts, respectively, for four different conditions.

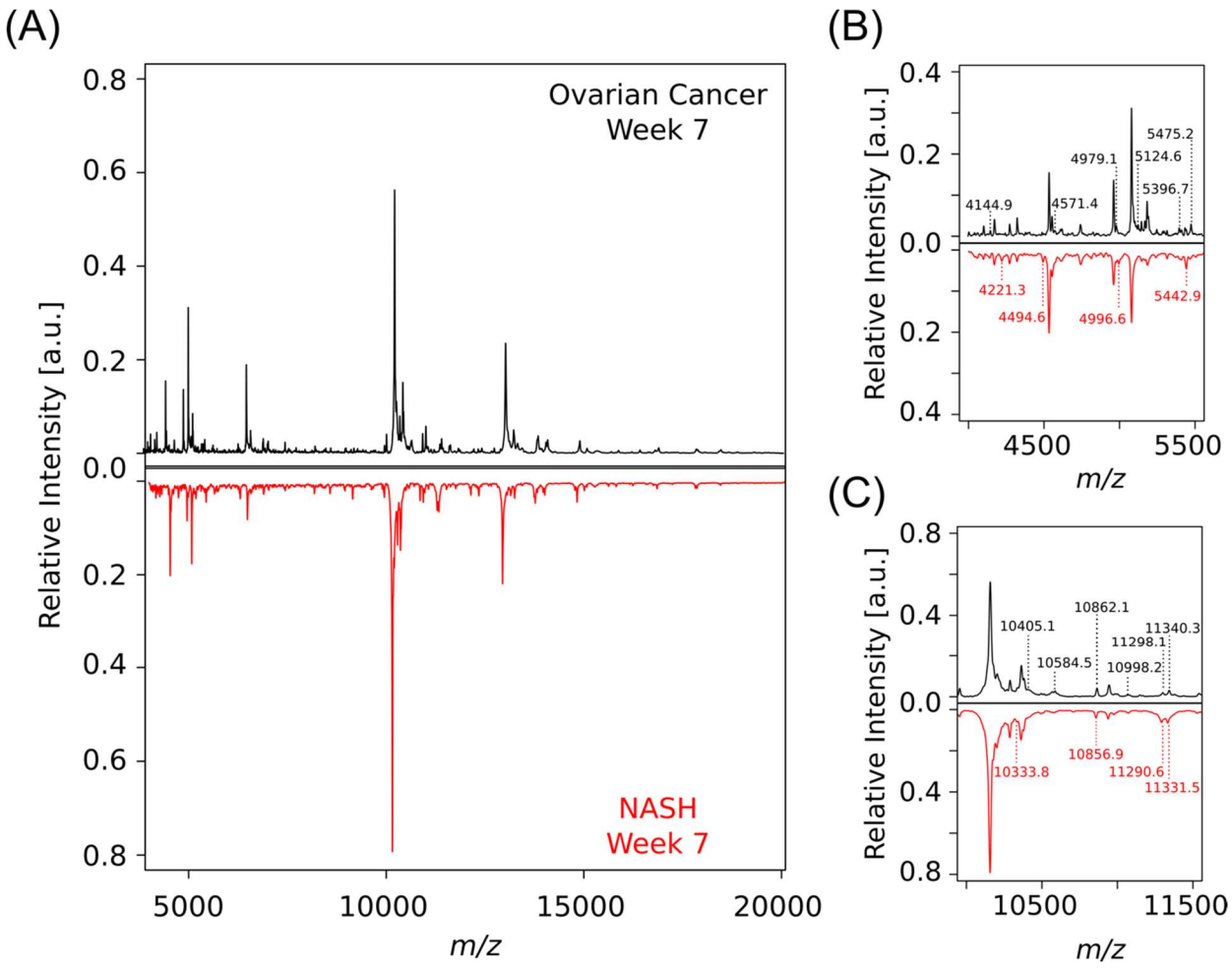



Figure S4: Mirror plot of average protein fingerprints of murine lavages comparing week seven in an ovarian cancer model and week seven in a NASH model (N=5, n=24 and N=9, n=24, respectively). (A) Full spectra (*m/z* 4,000-20,000), regions of interested have been labelled in B and C to display peaks which are different between the spectra. (B) Spectral features in this region (*m/z* 4,000-5,500) differ between both disease states in terms of peaks and intensity. (C) Spectral features in this region (*m/z* 10,000-11,500) present another area in which peaks differ between both disease states in terms of appearance and intensity.

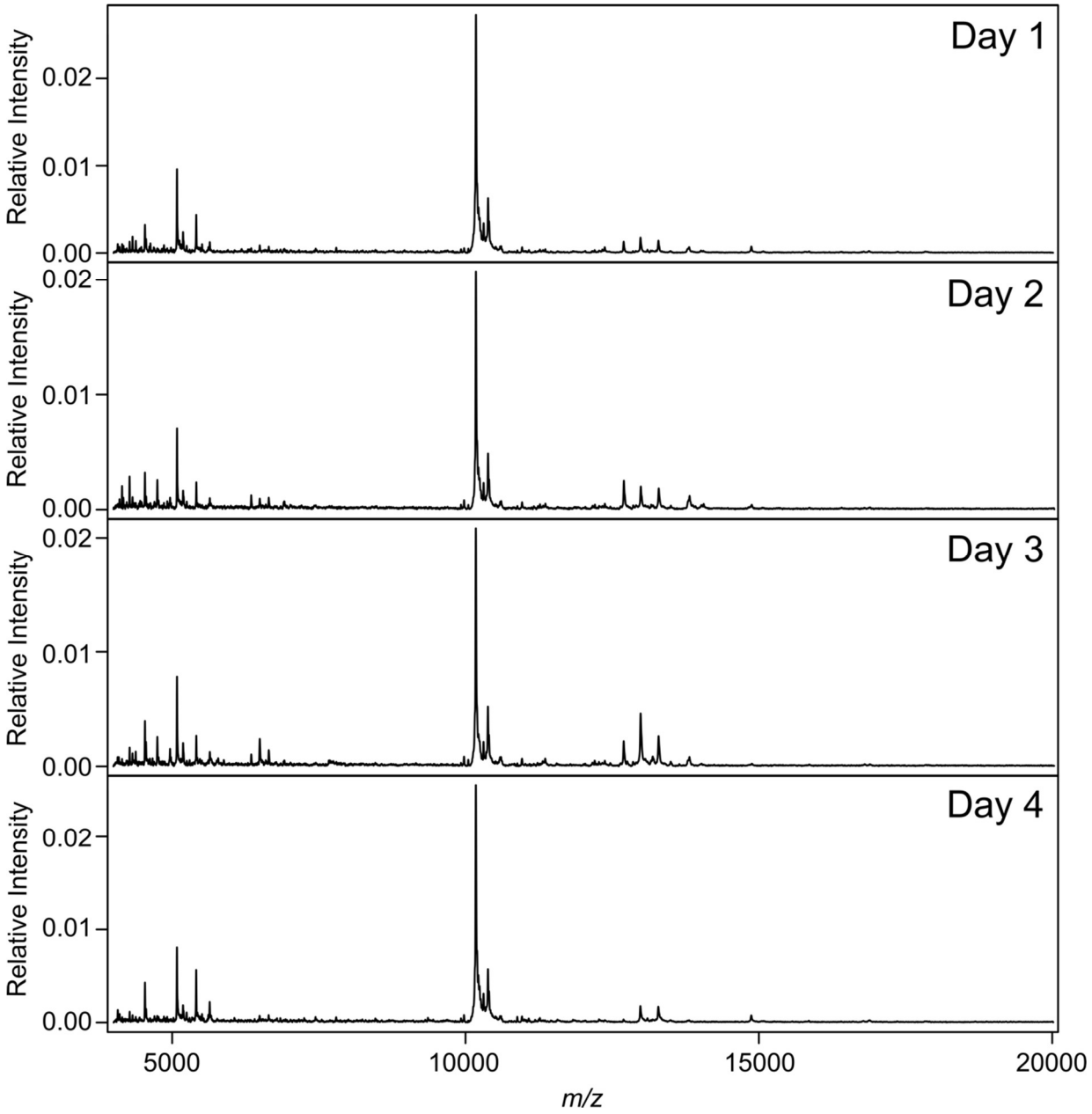



Figure S5: Vaginal lavages from healthy athymic nude mice were collected for a week on a daily basis. This was done to ensure that the signature detected at the conclusion of the study, when mice had heavy HGSOC tumor burden, was not due to inflammation of the vaginal cavity caused by repetitive sampling. It is also of note that the estrous cycle for mice lasts four to five days as that is the time period covered in this experiment.

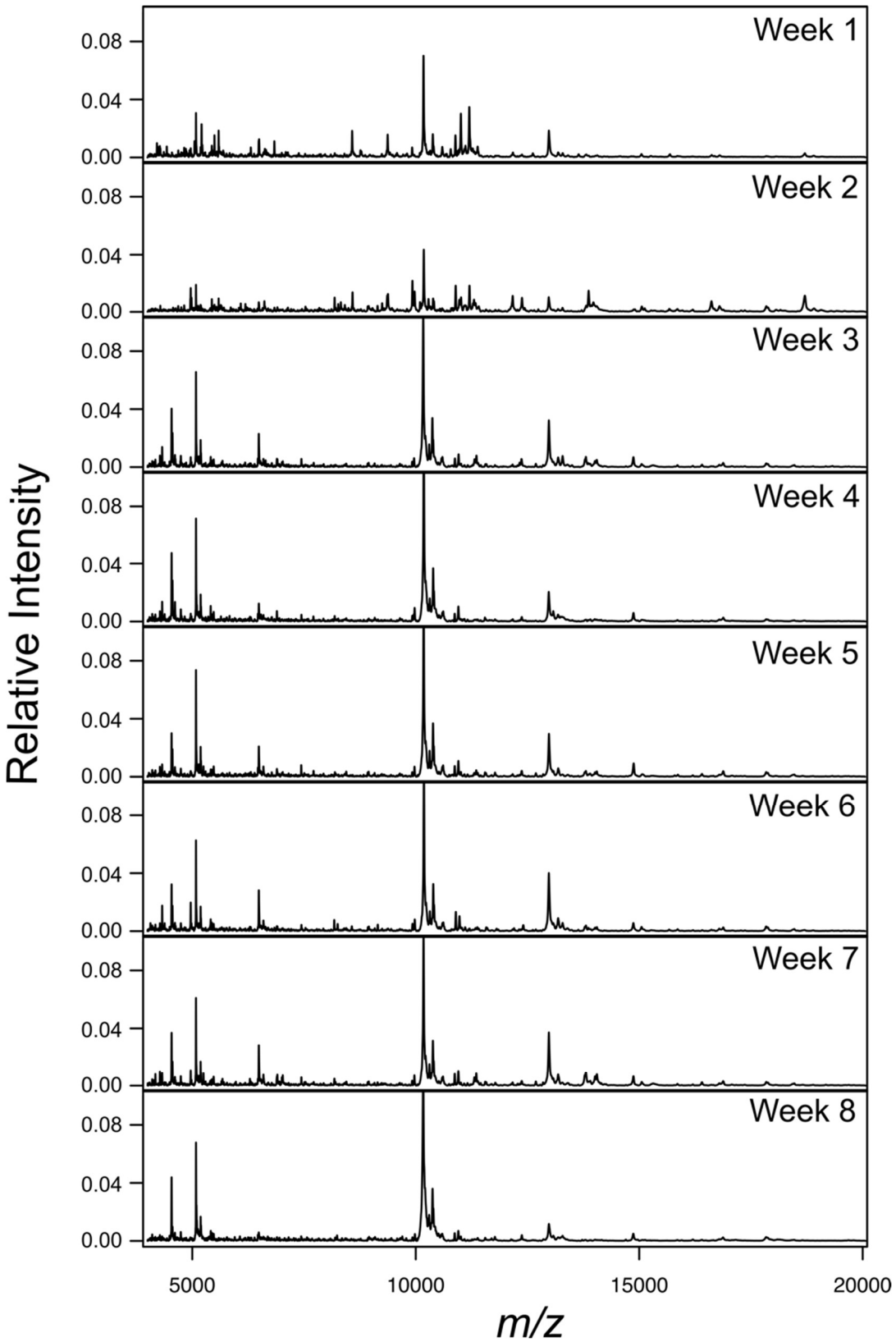



Figure S6: Consensus spectra of vaginal lavages from mouse 901 throughout tumor progression for a total of eight weeks (n=24 for each time point).

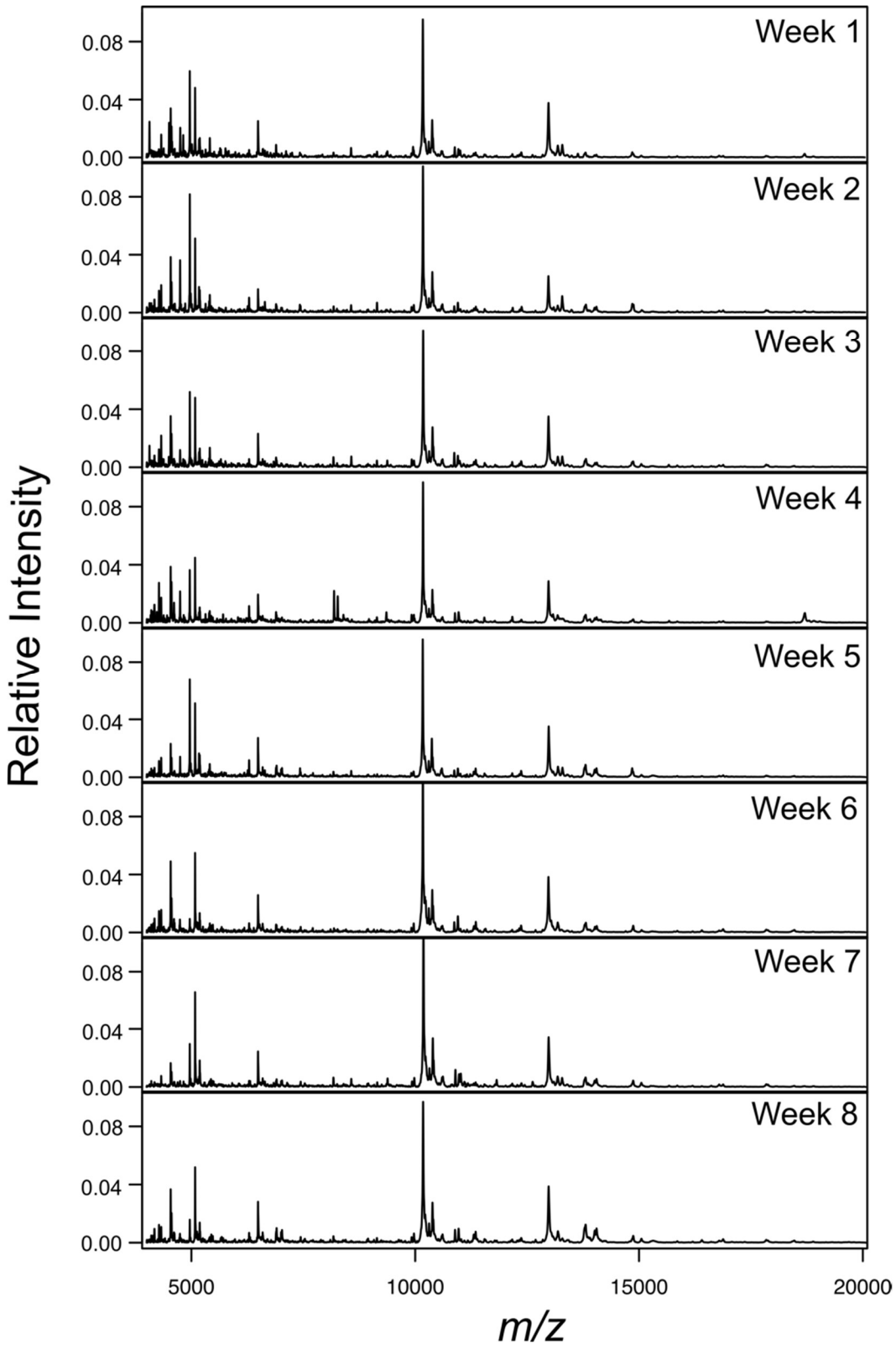



Figure S7: Consensus spectra of vaginal lavages from mouse 903 throughout tumor progression for a total of eight weeks (n=24 for each time point).

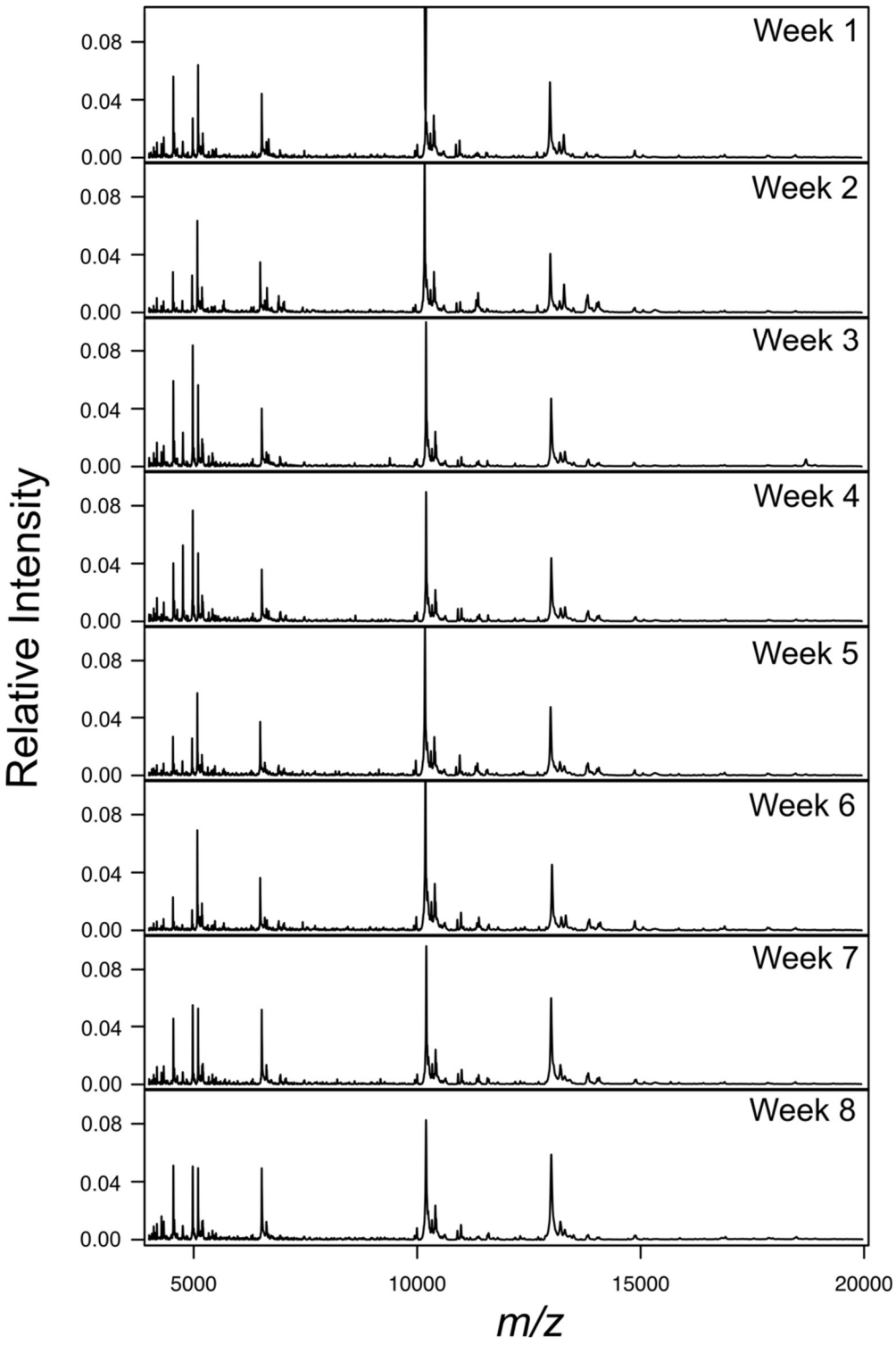



Figure S8: Consensus spectra of vaginal lavages from mouse 903 throughout tumor progression for a total of eight weeks (n=24 for each time point).

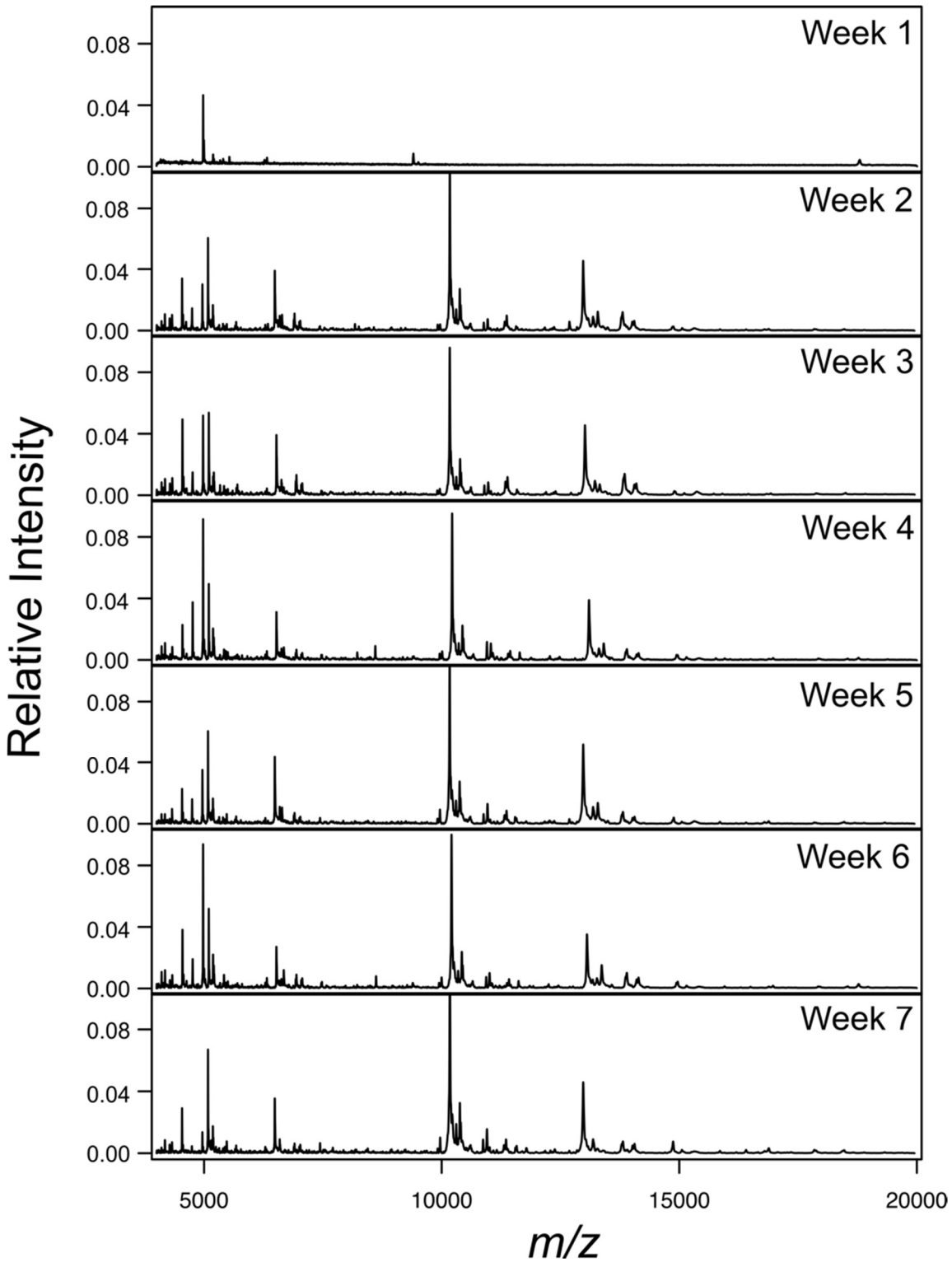



Figure S9: Consensus spectra of vaginal lavages from mouse 904 throughout tumor progression for a total of seven weeks (n=24 for each time point). This mouse expired prior to the conclusion of the study, which is why no lavage was obtained during the eighth week.

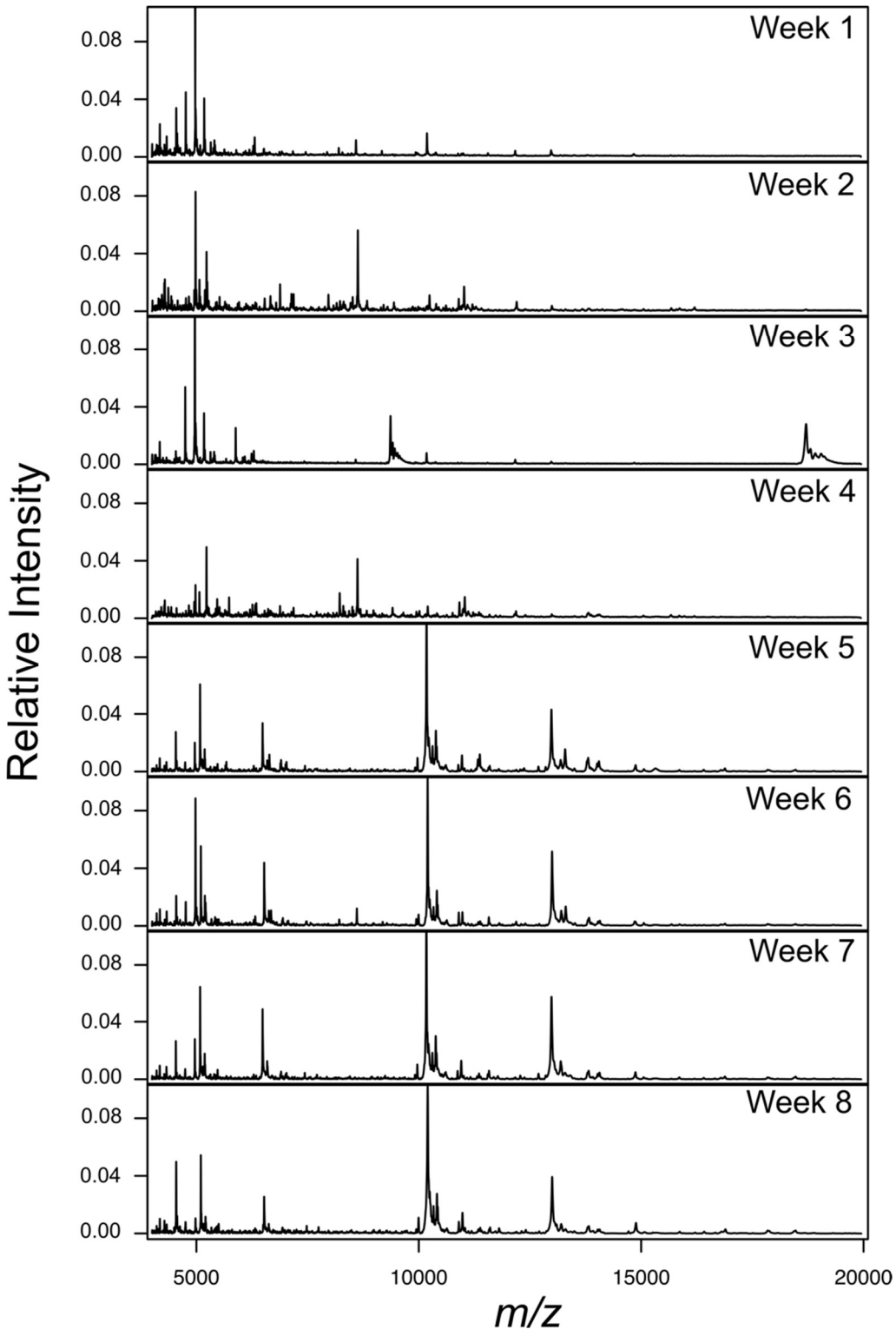



Figure S10: Consensus spectra of vaginal lavages from mouse 905 throughout tumor progression for a total of eight weeks (n=24 for each time point).

Table S1: Pivot tables indicating spectral features that are differentially expressed across various time points throughout the study. (A) Upregulated *m/z* values with a p-value < 0.001 comparing weeks one through seven. (B) Downregulated *m/z* values with a p-value < 0.001 comparing weeks one through seven.

(A) Upregulated Features Shared Across all Five Mice

| | **Week** | **3** | **5** | **6** | **7** |
|---|---|---|---|---|---|
| | | Unique Features | | | |
| **Week 1** | *m/z* | 1 | 9 | 4 | 10 |
| | 4265.9 | - | - | 1 | - |
| | 4269.8 | - | 1 | - | - |
| | 4270.5 | - | 1 | - | - |
| | 4367.7 | - | 1 | - | - |
| | 4369.6 | - | 1 | - | - |
| | 4714.1 | - | 1 | - | 1 |
| | 4792.9 | - | - | - | 1 |
| | 4795.7 | - | - | - | 1 |
| | 4862.5 | - | - | - | 1 |
| | 5508.8 | - | 1 | 1 | - |
| | 5640.7 | - | 1 | - | 1 |
| | 5807.5 | - | - | 1 | - |
| | 5843.8 | - | - | 1 | - |
| | 6744.0 | - | - | - | 1 |
| | 6753.4 | - | - | - | 1 |
| | 6756.0 | - | 1 | - | 1 |
| | 6759.6 | - | - | - | 1 |
| | 7196.1 | - | - | - | 1 |
| | 12620.2 | 1 | 1 | - | - |
| **Week 2** | *m/z* | 0 | 0 | 0 | 6 |
| | 6634.8 | - | - | - | 1 |
| | 6661.3 | - | - | - | 1 |
| | 6744.0 | - | - | - | 1 |
| | 6749.6 | - | - | - | 1 |
| | 6753.4 | - | - | - | 1 |
| | 6759.6 | - | - | - | 1 |
| **Week 3** | *m/z* | 0 | 2 | 0 | 25 |
| | 4048.1 | - | - | - | 1 |
| | 4350.4 | - | - | - | 1 |
| | 4358.6 | - | - | - | 1 |

| | | | | | |
|---|---|---|---|---|---|
| | 4381.7 | - | - | - | 1 |
| | 4567.6 | - | - | - | 1 |
| | 4586.3 | - | - | - | 1 |
| | 4586.8 | - | - | - | 1 |
| | 4587.9 | - | - | - | 1 |
| | 4612.7 | - | - | - | 1 |
| | 4617.0 | - | 1 | - | 1 |
| | 4631.0 | - | - | - | 1 |
| | 4746.9 | - | - | - | 1 |
| | 4753.1 | - | - | - | 1 |
| | 4757.0 | - | - | - | 1 |
| | 4759.0 | - | - | - | 1 |
| | 4762.7 | - | - | - | 1 |
| | 4952.0 | - | - | - | 1 |
| | 5405.3 | - | - | - | 1 |
| | 5412.6 | - | - | - | 1 |
| | 5640.7 | - | - | - | 1 |
| | 5645.6 | - | - | - | 1 |
| | 6744.0 | - | - | - | 1 |
| | 6756.0 | - | - | - | 1 |
| | 6759.6 | - | - | - | 1 |
| | 6767.4 | - | - | - | 1 |
| | 9370.3 | - | 1 | - | - |
| | *m/z* | 0 | 12 | 2 | 25 |
| **Week 4** | 4139.5 | - | 1 | - | - |
| | 4140.7 | - | 1 | 1 | 1 |
| | 4198.4 | - | - | - | 1 |
| | 4226.2 | - | - | - | 1 |
| | 4230.0 | - | - | - | 1 |
| | 4269.8 | - | 1 | - | - |
| | 4358.6 | - | 1 | - | - |
| | 4381.7 | - | 1 | - | - |
| | 4545.4 | - | 1 | - | - |
| | 4566.7 | - | - | - | 1 |
| | 4567.6 | - | 1 | - | 1 |
| | 4617.0 | - | - | - | 1 |
| | 4627.5 | - | - | - | 1 |
| | 4631.0 | - | - | - | 1 |
| | 4636.2 | - | - | - | 1 |

| | | | | | |
|---|---|---|---|---|---|
| | 4757.0 | - | 1 | - | 1 |
| | 4834.7 | - | 1 | - | - |
| | 5412.6 | - | - | - | 1 |
| | 5528.8 | - | 1 | - | - |
| | 5640.7 | - | - | - | 1 |
| | 6041.4 | - | 1 | - | 1 |
| | 6233.9 | - | - | - | 1 |
| | 6236.0 | - | - | - | 1 |
| | 6653.0 | - | - | - | 1 |
| | 6661.3 | - | - | - | 1 |
| | 6759.6 | - | - | - | 1 |
| | 6767.4 | - | - | - | 1 |
| | 6838.4 | - | 1 | - | - |
| | 8261.0 | - | - | 1 | - |
| | 9354.6 | - | - | - | 1 |
| | 18704.5 | - | - | - | 1 |
| | 18716.8 | - | - | - | 1 |
| | 18720.6 | - | - | - | 1 |
| | 18767.5 | - | - | - | 1 |
| **Week 5** | *m/z* | 0 | 0 | 0 | 1 |
| | 9947.8 | - | - | - | 1 |

(B) Downregulated Features Shared Across all Five Mice

| | Week | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| | | Unique Features | | | | |
| | *m/z* | 0 | 3 | 12 | 42 | 19 |
| **Week 1** | 5110.5 | - | - | - | 1 | - |
| | 5127.1 | - | - | - | 1 | - |
| | 5129.8 | - | - | - | 1 | - |
| | 5147.2 | - | - | - | 1 | - |
| | 5149.1 | - | - | - | 1 | - |
| | 6574.9 | - | - | - | - | 1 |
| | 6904.0 | - | - | 1 | 1 | 1 |
| | 7024.0 | - | - | 1 | - | 1 |
| | 8179.0 | - | 1 | - | - | - |
| | 10105.1 | - | - | - | 1 | - |
| | 10108.1 | - | - | - | 1 | - |
| | 10111.1 | - | - | - | 1 | - |
| | 10118.3 | - | - | - | 1 | - |
| | 10126.6 | - | - | - | 1 | - |
| | 10141.9 | - | - | - | 1 | - |
| | 10226.2 | - | - | - | 1 | - |
| | 10296.2 | - | - | - | 1 | - |
| | 10344.0 | - | - | - | 1 | - |
| | 10388.4 | - | - | - | 1 | - |
| | 10430.1 | - | - | - | 1 | - |
| | 10440.8 | - | - | - | 1 | - |
| | 13770.4 | - | - | 1 | 1 | 1 |
| | 13777.6 | - | - | 1 | 1 | 1 |
| | 13786.8 | - | - | 1 | 1 | 1 |
| | 13805.6 | - | - | 1 | 1 | 1 |
| | 13851.0 | - | - | - | 1 | - |
| | 13853.6 | - | - | - | 1 | - |
| | 13861.8 | - | - | - | 1 | 1 |
| | 13870.8 | - | - | - | 1 | - |
| | 13887.0 | - | - | - | 1 | 1 |
| | 13888.7 | - | - | - | - | 1 |
| | 13889.8 | - | - | - | 1 | 1 |
| | 13891.4 | - | - | - | 1 | 1 |
| | 13892.9 | - | - | - | 1 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 13907.1 | - | - | - | 1 | - |
| | 13909.0 | - | - | - | 1 | - |
| | 13910.5 | - | - | - | 1 | - |
| | 13913.5 | - | - | - | 1 | - |
| | 13997.2 | - | 1 | 1 | 1 | 1 |
| | 14008.2 | - | 1 | 1 | 1 | 1 |
| | 14016.7 | - | - | 1 | 1 | 1 |
| | 14025.3 | - | - | 1 | 1 | 1 |
| | 14048.3 | - | - | 1 | 1 | 1 |
| | 14069.3 | - | - | 1 | - | 1 |
| | 14869.0 | - | - | - | 1 | - |
| | 15052.6 | - | - | - | 1 | - |
| | 16879.2 | - | - | - | 1 | - |
| **Week 2** | *m/z* | 1 | 0 | 5 | 1 | 2 |
| | 4065.3 | 1 | - | - | - | - |
| | 6479.9 | - | - | 1 | - | 1 |
| | 6600.4 | - | - | 1 | - | - |
| | 10951.4 | - | - | - | 1 | - |
| | 11774.8 | - | - | - | - | 1 |
| | 12975.8 | - | - | 1 | - | - |
| | 13201.2 | - | - | 1 | - | - |
| | 13204.2 | - | - | 1 | - | - |
| **Week 3** | *m/z* | 0 | 3 | 2 | 2 | 4 |
| | 5147.2 | - | - | 1 | - | - |
| | 5436.7 | - | - | - | - | 1 |
| | 5477.6 | - | - | - | - | 1 |
| | 7713.0 | - | - | 1 | - | - |
| | 8179.0 | - | 1 | - | - | - |
| | 8183.9 | - | 1 | - | - | - |
| | 8261.0 | - | 1 | - | - | - |
| | 9962.3 | - | - | - | 1 | 1 |
| | 10951.4 | - | - | - | 1 | - |
| | 15052.6 | - | - | - | - | 1 |
| **Week 4** | *m/z* | 0 | 0 | 15 | 0 | 5 |
| | 5147.2 | - | - | 1 | - | - |
| | 5676.2 | - | - | 1 | - | - |
| | 6488.6 | - | - | - | - | 1 |
| | 6591.4 | - | - | - | - | 1 |
| | 11296.9 | - | - | 1 | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 11308.0 | - | - | 1 | - | - |
| | 11334.3 | - | - | 1 | - | - |
| | 11349.8 | - | - | 1 | - | - |
| | 11384.4 | - | - | 1 | - | - |
| | 12278.0 | - | - | - | - | 1 |
| | 12284.1 | - | - | - | - | 1 |
| | 13181.8 | - | - | - | - | 1 |
| | 15302.8 | - | - | 1 | - | - |
| | 15309.2 | - | - | 1 | - | - |
| | 15319.2 | - | - | 1 | - | - |
| | 15329.3 | - | - | 1 | - | - |
| | 15330.8 | - | - | 1 | - | - |
| | 15334.7 | - | - | 1 | - | - |
| | 15346.5 | - | - | 1 | - | - |
| | 15359.2 | - | - | 1 | - | - |
| **Week 5** | *m/z* | 0 | 0 | 0 | 0 | 1 |
| | 8179.0 | - | - | - | - | 1 |
| **Week 6** | *m/z* | 0 | 0 | 0 | 0 | 1 |
| | 6581.4 | - | - | - | - | 1 |

References

(1) Petukhova, V. Z.; Young, A. N.; Wang, J.; Wang, M.; Ladanyi, A.; Kothari, R.; Burdette, J. E.; Sanchez, L. M. Whole Cell MALDI Fingerprinting Is a Robust Tool for Differential Profiling of Two-Component Mammalian Cell Mixtures. *J. Am. Soc. Mass Spectrom.* **2019**, *30* (2), 344–354.