Supplementary Material

Characterizing the Natural History of Acute Radiation Syndrome of the Gastrointestinal Tract: Combining high mass and spatial resolution using MALDI-FTICR-MSI

Claire L. Carter*, Kim Hankey[†], Cath Booth[‡], Greg Tudor[‡], George Parker[§], Jace W. Jones*, Ann M. Farese[†], Thomas J. MacVittie[†], and Maureen A. Kane*

*University of Maryland, School of Pharmacy, Department of Pharmaceutical Sciences

[†]University of Maryland, School of Medicine, Department of Radiation Oncology,

[‡]Epistem Ltd, Manchester, UK

[§]Charles River Laboratories, Pathology Associates, Raleigh-Durham, North Carolina

Figure 1. Probabilistic latent semantic analysis (pLSA) score images showing 7 components from the control A) and irradiated samples taken from each day post-IR, B-F for days 4-21 respectively.

Figure 2. MALDI-MS images of PE's and PI's localized to the crypt-villi axis in control and at days 4, 8, 11, 15 and 21 post-exposure.

Figure 3. MALDI-MS images of glycophosphingolipids mapped to the villi epithelium in control and days 4, 8, 11, 15 and 21 post-irradiation.

Figure 4. MALDI-MS images of PL's localized to the lamina Propria and muscularis externa in the control and at days 4, 8, 11, 15, and 21 post-exposure.

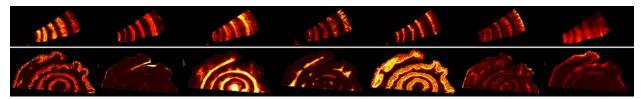
Figure 5. MALDI-MS images of cardiolipins detected in the jejunum of control and irradiated animals taken at days 4, 8, 11, 15 and 21 post-exposure.

Figure 6. MALDI-MS images of GSLs detected in the jejunum of control and irradiated animals taken at days 4, 8, 11, 15 and 21 post-exposure.

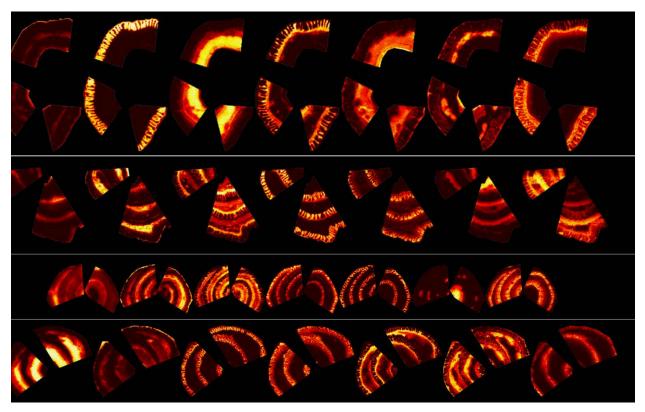
 Table 1. Ki67 IHC staining protocol.

 Table 2. CD 13 IHC staining protocol.

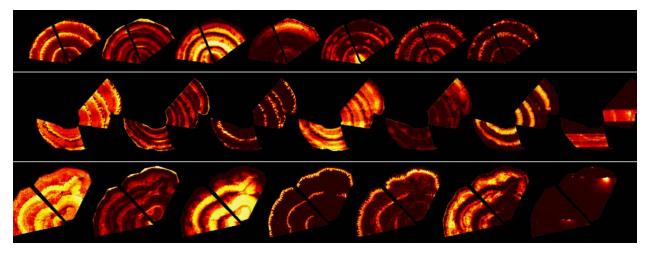
A) Control



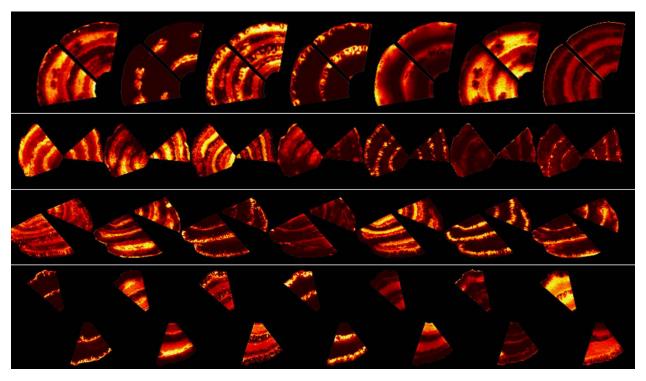
B) Day 4



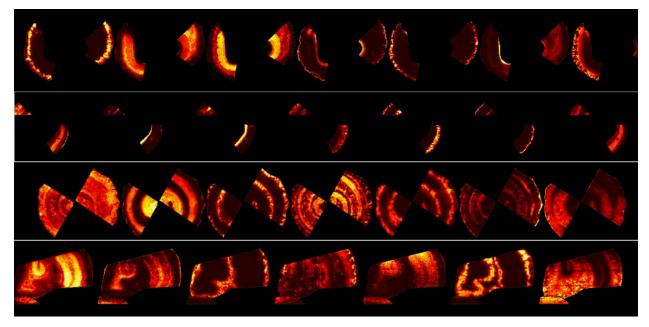
C) Day 8



D) Day 11



E) Day 15



F) Day 21

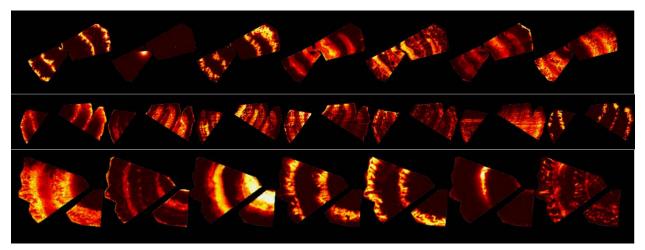


Figure S1.

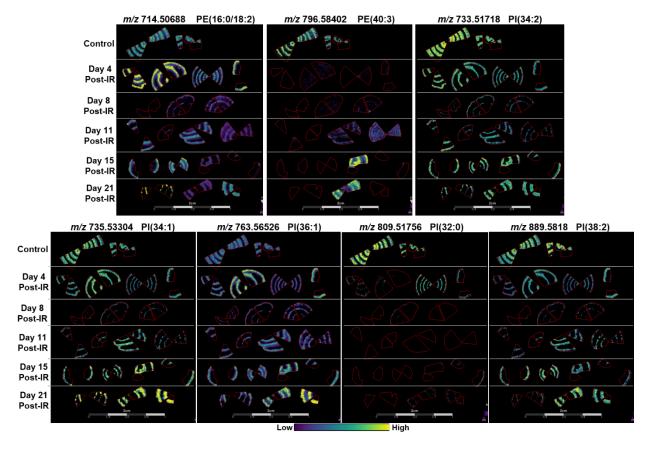


Figure S2.

	<i>m/z</i> 778.51296 ST(d34:1)	m/z 796.52338 ST(t34:0)	m/z 878.60252 ST(t40:1)	<i>m/z</i> 878.60252 ST(t40:0)
Control	100 MIL SPI	the mu EF.	Mean Bos	Incarry East
Day 4 Post-IR	((m)) (m) (m)	C ((col) (C) 2	(((∞)) ((∞)) £	
Day 8 Post-IR	\$ \$ \$ \$			
Day 11 Post-IR				THE SAL
Day 15 Post-IR	(10 B B C)	() (M 8 °)	60MB 2	COM BOOD
Day 21 Post-IR		Zen st		
	<i>m</i> /z 896.61293	m/z 904.6193 ST(t42:2)	<i>m</i> /z 922.62941	<i>m/z</i> 924.65155
Control	42 Bu	m/z 904.6193 ST(t42:2)	m/z 922.62941	m/z 924.65155
Control Day 4 Post-IR	ILE AND CALL			
Day 4		198 Mar 11	Illean East	ILLE MILL F. C.
Day 4 Post-IR Day 8			Illean East	ILLE MILL F. C.
Day 4 Post-IR Day 8 Post-IR Day 11		198 Mar 11	Illean East	ILLE MILL F. C.
Day 4 Post-IR Day 8 Post-IR Day 11 Post-IR Day 15			A C E CA	The and Ed CA

Figure S3.

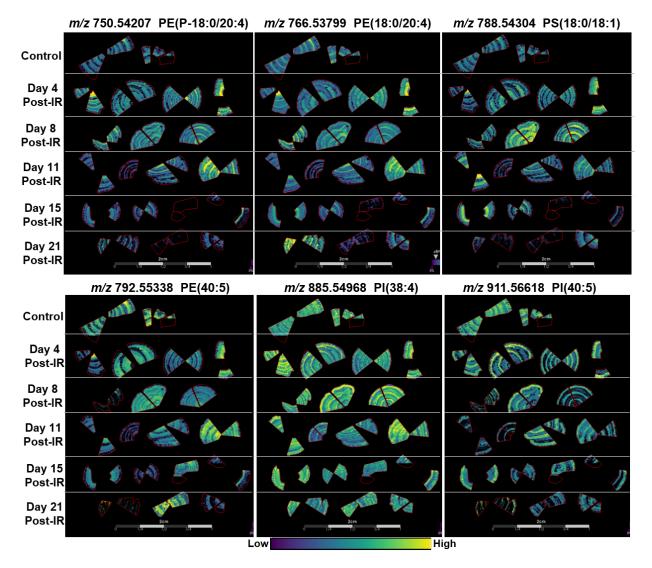


Figure S4.

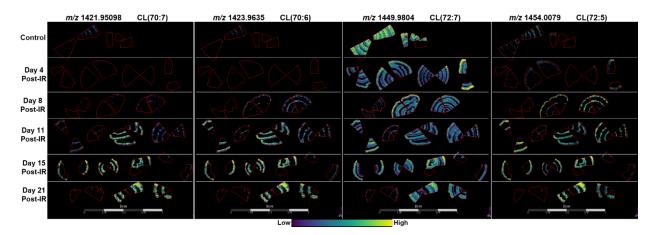


Figure S5

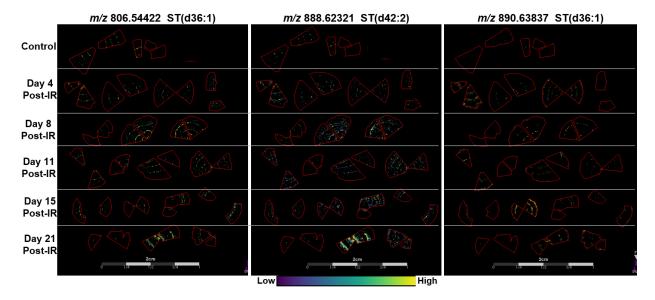


Figure S6.

	Ki-67 (ab15580)(Rb) Immunohistochemistry Staining Procedure
Step	Procedure on Ventana Discovery Ultra #11
1	Warm Slides to 69°C for 3 cycles of 8mins on Ultra.
2	Deparaffinize with EZ prep (Ventana, Reference No. 950-100).
3	Apply Liquid Coverslip (Ventana, reference No. 650-010).
4	Antigen retrieval with RiboCC incubation at 95°C for 32 minutes (Ventana, reference No. 760-107).
5	Rinse slide with Reaction Buffer (Ventana, reference No. 950-300).
6	Apply Liquid Coverslip.
7	Apply Background Sniper block option for 8 Minutes (Biocare medical, reference No. BS966M)
8	Rinse slide with Reaction Buffer.
9	Apply Inhibitor CM and incubate for 4 minutes (Ventana, reference No. 760-159)
10	Rinse slide with Reaction Buffer.
11	Apply primary antibody anti-Ki-67 for 32 minutes (abcam, reference No. Ab15580) Dilution:
	1:500.
12	Rinse slide with Reaction Buffer.
13	Apply OmniMap anti-rabbit and incubate for 12 minutes (Ventana, reference No. 760-4311).
14	Rinse slide with Reaction Buffer.
15	Apply DAB CM and DAB H2O2 CM and incubate for 8 minutes (Ventana, reference No. 760-159).
16	Rinse slide with Reaction Buffer.
17	Apply Copper CM and incubate for 4 minutes (Ventana, reference No. 760-159).
18	Rinse slide with Reaction Buffer.
19	Apply Hematoxylin and incubate for 8 minutes (Ventana, reference No. 760-2021).
20	Rinse slide with Reaction buffer.
21	Apply Bluing Reagent and incubate for 4 minutes (Ventana, reference No. 760-2037).
22	Rinse slide with Reaction Buffer.
23	Remove slides, dehydrate, and coverslip.

Table 1.

	CD13 (NHP) Immunohistochemistry Staining Procedure				
Step	Procedure on Ventana Discovery XT, Protocol #116				
1	Warm slides to 75°C.				
2	Deparaffinize with EZ prep (Ventana, reference no. 950-100).				
3	Apply Liquid Coverslip (Ventana, reference no. 650-010).				
4	Antigen retrieval with Protease 2 (Ventana, reference no. 760-2019) for 4 minutes at 37 degree				
	Celsius.				
5	Rinse slide with Reaction Buffer (Ventana, reference no. 950-300).				
6	Apply Liquid Coverslip.				
7	Apply Inhibitor CM and incubate for 4 minutes (Ventana, reference no. 760-159).				
8	Rinse slide with Reaction Buffer.				

9	Apply primary antibody anti-CD13 ¹ for 32 minutes at 37 degree Celsius (abcam, reference no.
	ab108310).
10	Rinse slide with Reaction Buffer.
11	Apply OmniMap anti-rabbit HRP and incubate for 12 minutes (Ventana, reference no. 760-4311).
12	Rinse slide with Reaction Buffer.
13	Apply DAB CM and DAB H_2O_2 CM and incubate for 8 minutes (Ventana, reference no. 760-159).
14	Rinse slide with Reaction Buffer.
15	Apply Copper CM and incubate for 4 minutes (Ventana, reference no. 760-159).
16	Rinse slide with Reaction Buffer.
17	Apply Hematoxylin and incubate for 8 minutes. (Ventana, reference no. 760-2021).
18	Rinse slide with Reaction Buffer.
19	Apply Bluing Reagent and incubate for 4 minutes (Ventana, reference no. 760-2037).
20	Rinse slide with Reaction Buffer.
21	Remove slides, rinse, dehydrate, and coverslip.

Table 2.