Supporting Information

Citrulline as a biomarker in the murine total-body irradiation model: correlation of circulating and tissue citrulline to small intestine epithelial histopathology.

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SUPPORTING INFORMATION: FIGURE AND TABLE CAPTIONS

Fig. S1. Linear regression analysis for plasma citrulline versus IR dose per day. Linear regression analysis for a given day from plasma citrulline versus IR dose where inset displays linear regression equation, R^2, and *p-value*. A.) day 1, B.) day 2, C.) day 3, D.) day 4, E.) day 5, F.) day 6.

Fig. S2. Linear regression analysis plots for mean values from plasma and jejunal citrulline at specific IR doses. Linear regression analysis plot for jejunal and plasma citrulline at a given dose with 95% confidence intervals. A.) 8 Gy, B.) 10 Gy, C.) 12 Gy, D.) 14 Gy.

Fig. S3. Linear regression analysis for CCN versus IR dose per day. Linear regression analysis for a given day from CCN versus IR dose. Inset displays linear regression equation, R^2, and *p-value*. A.) day 1, B.) day 2, C.) day 3, D.) day 4, E.) day 5, F.) day 6.

Fig. S4. Linear regression analysis for temporal variation of plasma citrulline versus CCN per IR dose. Linear regression analysis at a given dose for plasma citrulline versus CCN over days 1-6, except for 14 Gy and 15 Gy which are over days 1-5. Inset displays linear regression equation, R^2, and *p-value*. A.) 6 Gy, B.) 7 Gy, C.) 8 Gy, D.) 9 Gy, E.) 10 Gy, F.) 11 Gy, G.) 12 Gy, H.) 13 Gy, I.) 14 Gy, J.) 15 Gy.

Fig. S5. Linear regression analysis for radiological variation of plasma citrulline versus CCN per day post IR. Linear regression analysis for a given day for plasma citrulline versus CCN over 6-15 Gy, except day 6 which is over 6-13 Gy. Inset displays linear regression equation, R^2, and *p-value*. A.) day 1, B.) day 2, C.) day 3, D.) day 4, E.) day 5, F.) day 6.

Table S1. Tabular representation of the number of mice per radiological dose and time point in the TBI mouse model for plasma citrulline. The number in each box represented the number of mice per the specific radiological dose and time point. Plasma samples for day 6 at 14 and 15 Gy were not accessible due to euthanasia criteria and were sampled on day 5. In a few cases plasma was not obtained from the mouse due to euthanasia criteria. In those cases, the number in the parenthesis corresponded to the actual number of mice whose blood plasma was collected. There were a total of 315 mice in the experiment and a total of 302 mice whose plasma was analyzed for citrulline.

Table S2. Plasma citrulline concentrations expressed per radiological dose and time point. Plasma samples for day 6 at 14 and 15 Gy were not accessible due to euthanasia criteria and were sampled on day 5. Concentrations were expressed in μM; sem, standard error of the mean; N, number of independent plasma samples.

Table S3. Tabular representation of the number of mice per radiological dose and time point in the TBI mouse model for jejunal citrulline. The number in each box represented the number of mice per the specific radiological dose and time point. There were a total of 74 mice in the

experiment whose jejunal tissue was analyzed for citrulline. Jejunum samples for day 6 at 14 Gy were not accessible due to euthanasia criteria and were sampled on day 5.

Table S4. Jejunal citrulline concentrations expressed per radiological dose and time point. Jejunal samples for day 6 at 14 Gy were not accessible due to euthanasia criteria and were sampled on day 5. Concentrations were expressed in pmol/mg of tissue; sem, standard error of the mean; N, number of independent tissue samples.

Table S5. CCN values expressed per radiological dose and time point. CCN values for day 6 at 14 and 15 Gy were not accessible due to euthanasia criteria and were determined on day 5. Values were expressed as mean; sem, standard error of the mean; N, number of independent plasma samples.

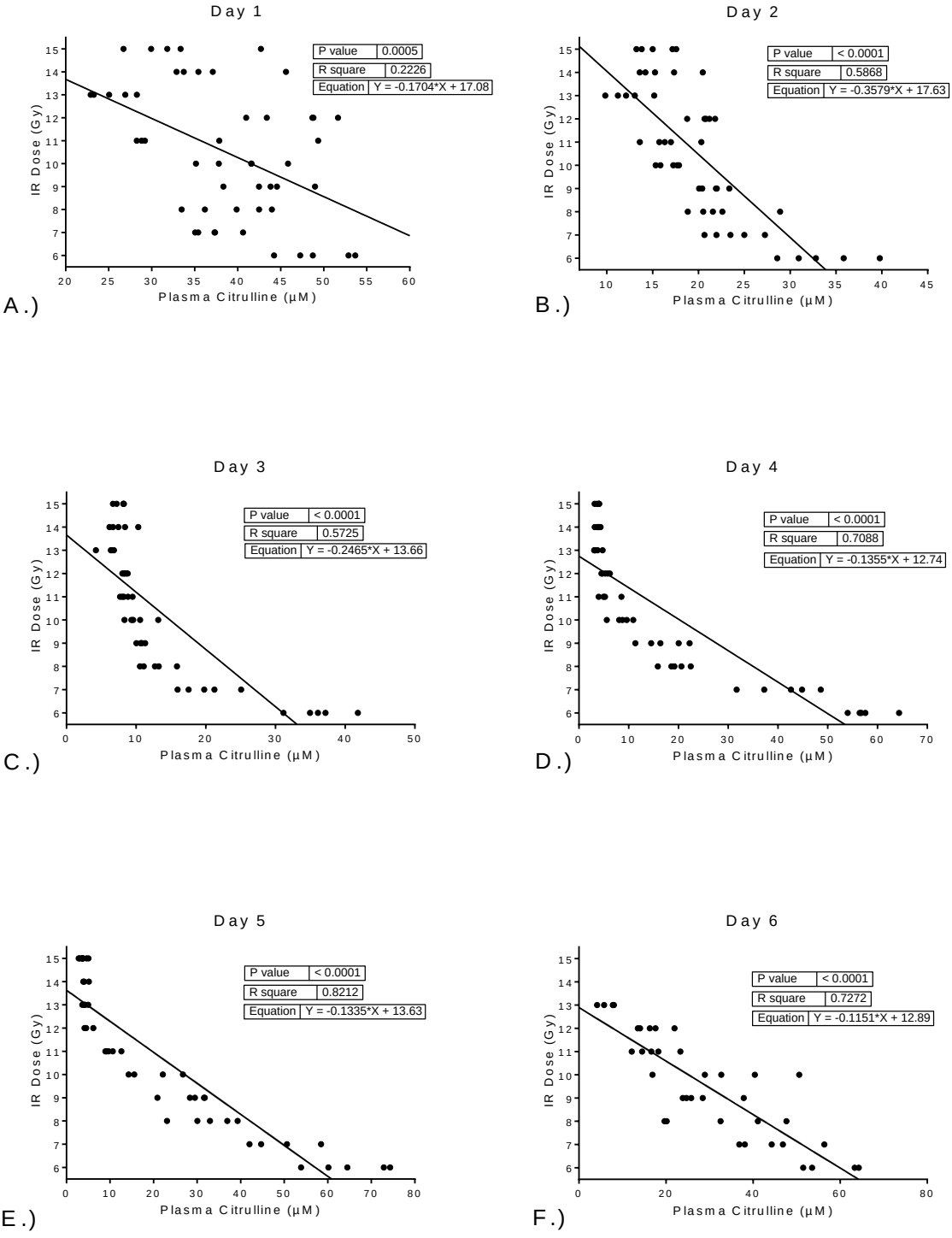
Day 1
P value 0.0005
R square 0.2226
Equation Y = -0.1704*X + 17.08
IR Dose (Gy)
Plasma Citrulline (µM)
A.)
Day 2
P value < 0.0001
R square 0.5868
Equation Y = -0.3579*X + 17.63
IR Dose (Gy)
Plasma Citrulline (µM)
B.)
Day 3
P value < 0.0001
R square 0.5725
Equation Y = -0.2465*X + 13.66
IR Dose (Gy)
Plasma Citrulline (µM)
C.)
Day 4
P value < 0.0001
R square 0.7088
Equation Y = -0.1355*X + 12.74
IR Dose (Gy)
Plasma Citrulline (µM)
D.)
Day 5
P value < 0.0001
R square 0.8212
Equation Y = -0.1335*X + 13.63
IR Dose (Gy)
Plasma Citrulline (µM)
E.)
Day 6
P value < 0.0001
R square 0.7272
Equation Y = -0.1151*X + 12.89
IR Dose (Gy)
Plasma Citrulline (µM)
F.)

Figure S1.

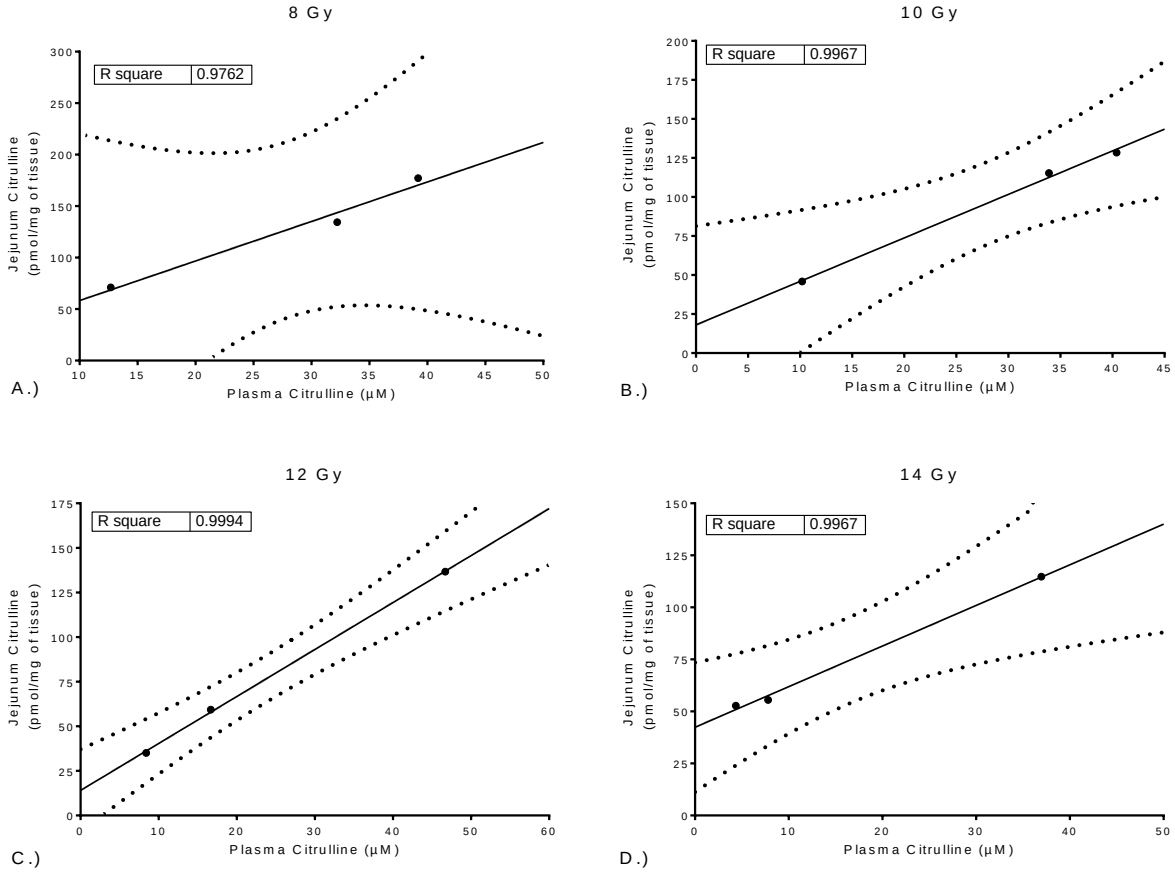
8 Gy
R square 0.9762
Jejunum Citrulline (pmol/mg of tissue)
Plasma Citrulline (µM)
A.)
10 Gy
R square 0.9967
Jejunum Citrulline (pmol/mg of tissue)
Plasma Citrulline (µM)
B.)
12 Gy
R square 0.9994
Jejunum Citrulline (pmol/mg of tissue)
Plasma Citrulline (µM)
C.)
14 Gy
R square 0.9967
Jejunum Citrulline (pmol/mg of tissue)
Plasma Citrulline (µM)
D.)

Figure S2.

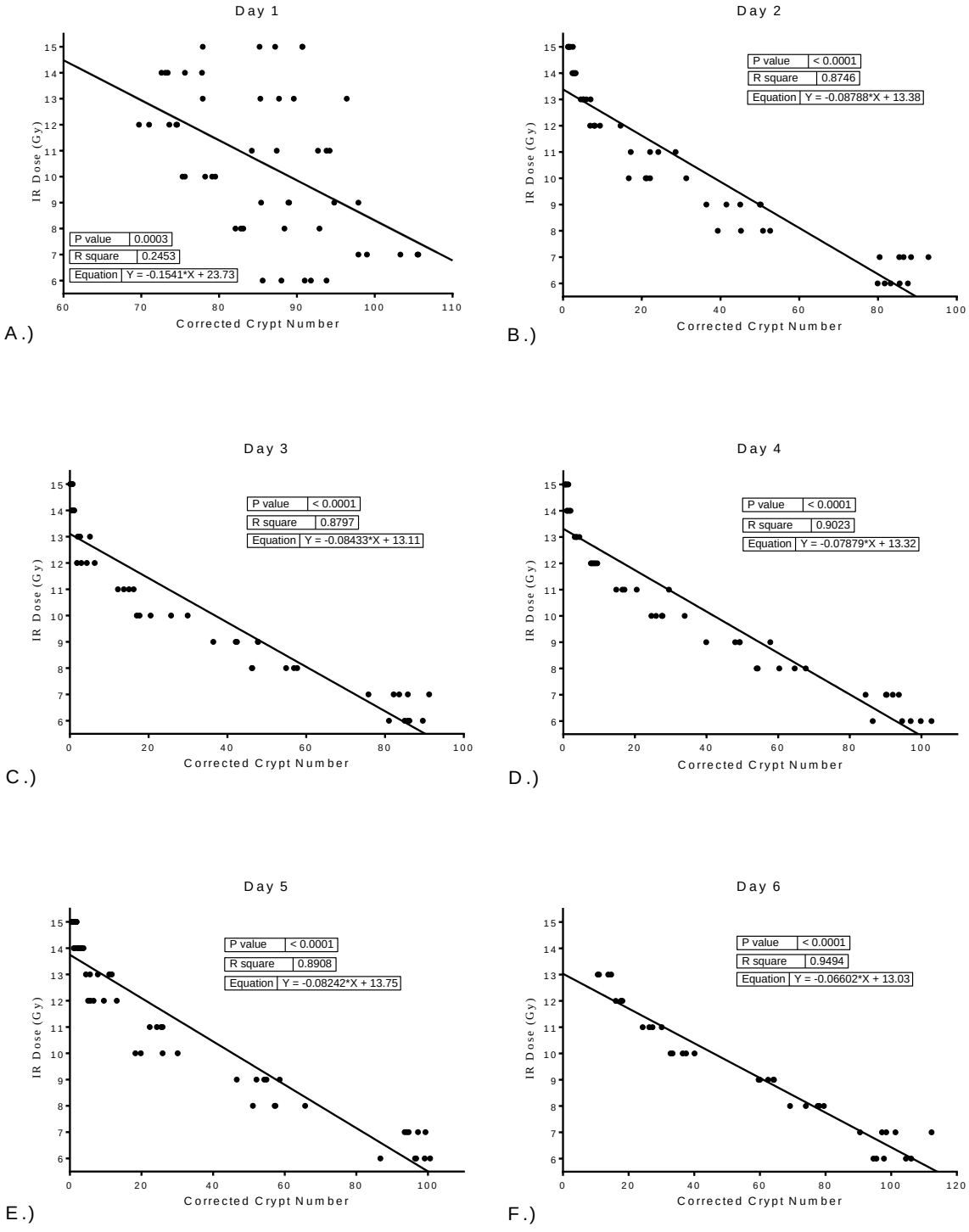
Day 1
IR Dose (Gy)
Corrected Crypt Number
P value 0.0003
R square 0.2453
Equation Y = -0.1541*X + 23.73
A.)
Day 2
IR Dose (Gy)
Corrected Crypt Number
P value < 0.0001
R square 0.8746
Equation Y = -0.08788*X + 13.38
B.)
Day 3
IR Dose (Gy)
Corrected Crypt Number
P value < 0.0001
R square 0.8797
Equation Y = -0.08433*X + 13.11
C.)
Day 4
IR Dose (Gy)
Corrected Crypt Number
P value < 0.0001
R square 0.9023
Equation Y = -0.07879*X + 13.32
D.)
Day 5
IR Dose (Gy)
Corrected Crypt Number
P value < 0.0001
R square 0.8908
Equation Y = -0.08242*X + 13.75
E.)
Day 6
IR Dose (Gy)
Corrected Crypt Number
P value < 0.0001
R square 0.9494
Equation Y = -0.06602*X + 13.03
F.)

Figure S3.

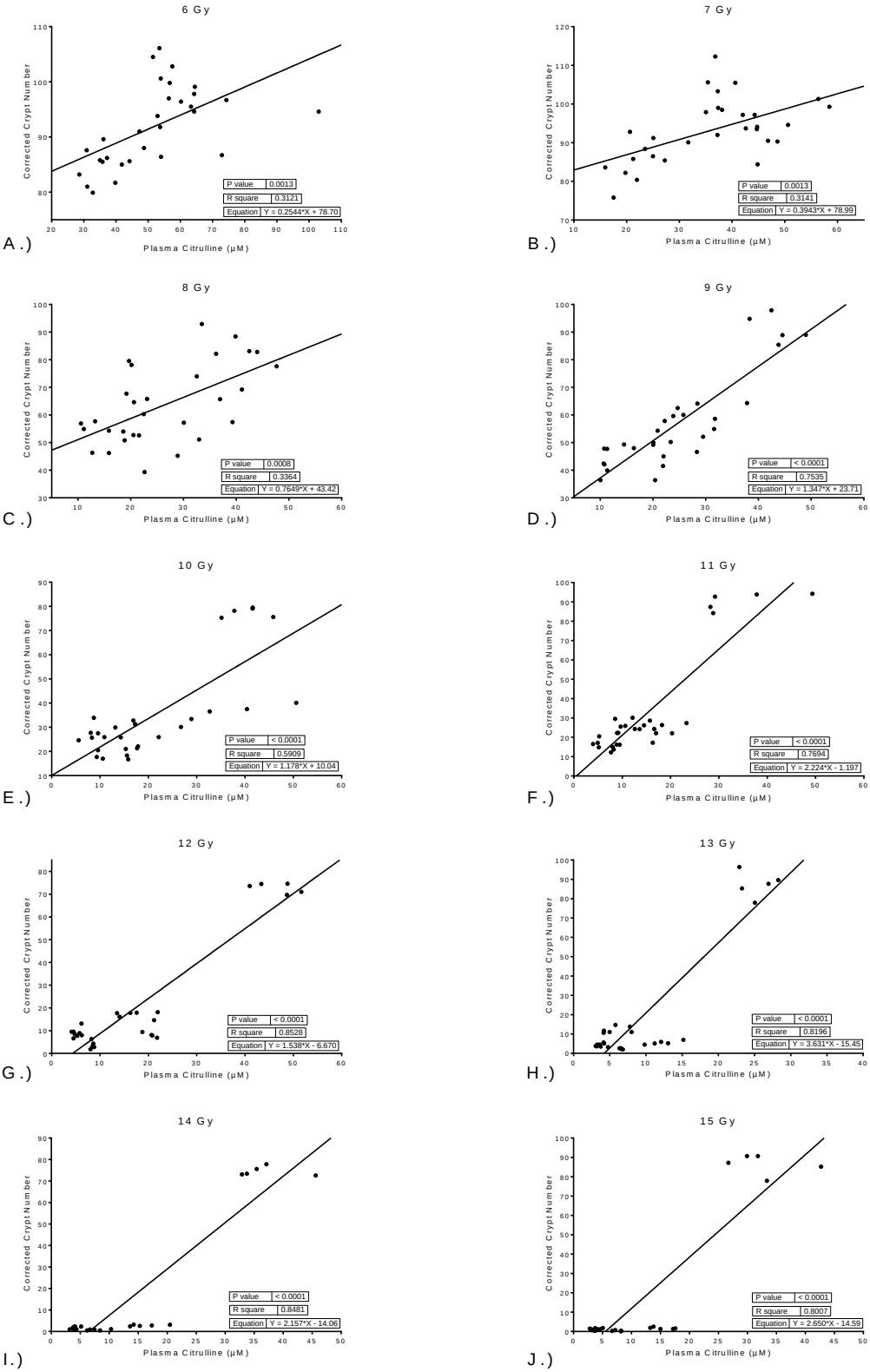
6 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value 0.0013
R square 0.3121
Equation Y = 0.2544*X + 78.70
A .)
7 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value 0.0013
R square 0.3141
Equation Y = 0.3943*X + 78.99
B .)
8 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value 0.0008
R square 0.3364
Equation Y = 0.7649*X + 43.42
C .)
9 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.7535
Equation Y = 1.347*X + 23.71
D .)
10 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.5909
Equation Y = 1.178*X + 10.04
E .)
11 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.7694
Equation Y = 2.224*X - 1.197
F .)
12 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.8528
Equation Y = 1.538*X - 6.670
G .)
13 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.8196
Equation Y = 3.631*X - 15.45
H .)
14 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.8481
Equation Y = 2.157*X - 14.06
I .)
15 Gy
Corrected Crypt Number
Plasma Citrulline (µM)
P value < 0.0001
R square 0.8007
Equation Y = 2.650*X - 14.59
J .)

Figure S4.

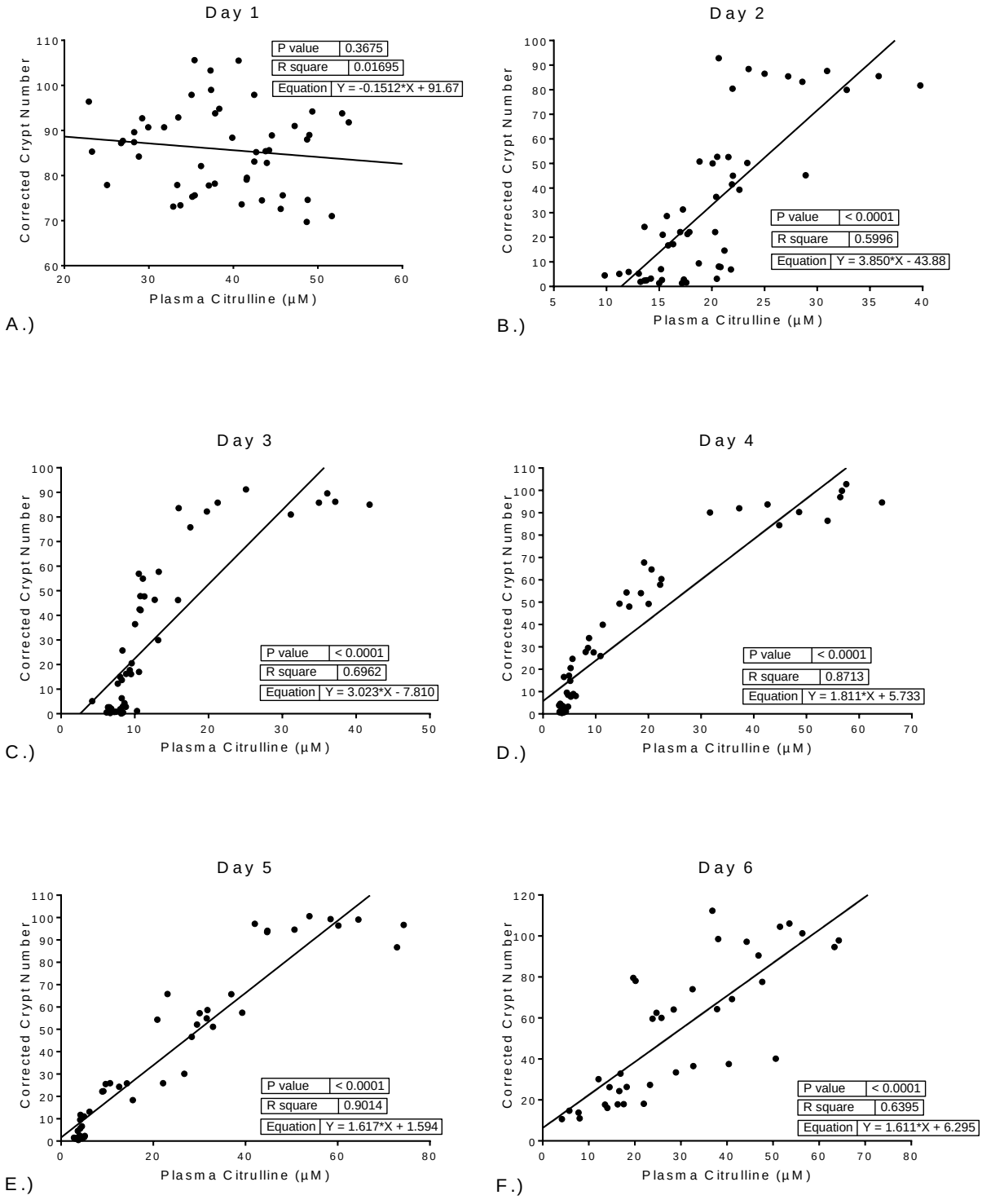
Day 1
P value 0.3675
R square 0.01695
Equation Y = -0.1512*X + 91.67
Corrected Crypt Number
Plasma Citrulline (µM)
A.)
Day 2
P value < 0.0001
R square 0.5996
Equation Y = 3.850*X - 43.88
Corrected Crypt Number
Plasma Citrulline (µM)
B.)
Day 3
P value < 0.0001
R square 0.6962
Equation Y = 3.023*X - 7.810
Corrected Crypt Number
Plasma Citrulline (µM)
C.)
Day 4
P value < 0.0001
R square 0.8713
Equation Y = 1.811*X + 5.733
Corrected Crypt Number
Plasma Citrulline (µM)
D.)
Day 5
P value < 0.0001
R square 0.9014
Equation Y = 1.617*X + 1.594
Corrected Crypt Number
Plasma Citrulline (µM)
E.)
Day 6
P value < 0.0001
R square 0.6395
Equation Y = 1.611*X + 6.295
Corrected Crypt Number
Plasma Citrulline (µM)
F.)

Figure S5.

Radiological Dose (Gy)	Day Post IR								
	-7	0	1	2	3	4	5	6	Total # of Mice per dose
Control	5	5						5	15
6			5	5	5	5	5	5	30
7			5	5	5	5	5	5	30
8			5	5	5	5	5	5	30
9			5	5	5	5	5	5	30
10			5	5	5	5	5 (4)	5	30 (29)
11			5	5	5	5	5	5	30
12			5	5	5	5	5 (3)	5	30 (28)
13			5	5	5	5	5 (4)	5 (4)	30 (28)
14			5	5	5	5	5 (3)	5 (0)	30 (23)
15			5	5	5	5	5 (9)	5 (0)	30 (29)
							Total # of Mice		**315 (302)**

Table S1.

Day Post IR

Radiological Dose	Day -7			Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	Conc (μM)	sem	n	Conc (μM)	sem	n	Conc (μM)	sem	n	Conc (μM)	sem	n	Conc (μM)	sem	n	Conc (μM)	sem	n	Conc (μM)	sem	n	Conc (μM)	sem	n
Control	38.5	1.9	5	41.4	4.4	5																38.0	2.4	5
6 Gy							49.4	1.8	5	33.6	1.9	5	36.2	1.7	5	57.8	1.7	5	65.1	3.8	5	67.1	9.3	5
7 Gy							37.1	1.0	5	23.7	1.2	5	19.9	1.6	5	41.0	3.0	5	48.1	2.9	5	44.5	3.5	5
8 Gy							39.2	1.9	5	22.5	1.7	5	12.7	0.9	5	19.3	1.1	5	32.5	2.8	5	32.2	5.6	5
9 Gy							43.6	1.7	5	21.6	0.6	5	10.7	0.2	5	16.9	1.9	5	28.4	2.0	5	28.1	2.6	5
10 Gy							40.4	1.8	5	16.8	0.5	5	10.2	0.8	5	8.6	0.9	5	19.7	2.9	4	33.9	5.6	5
11 Gy							34.7	4.1	5	16.6	1.1	5	8.5	0.3	5	5.6	0.8	5	10.2	0.7	5	17.0	1.9	5
12 Gy							46.7	2.0	5	20.6	0.5	5	8.4	0.1	5	5.3	0.3	5	4.9	0.6	3	16.7	1.5	5
13 Gy							25.3	1.0	5	12.3	0.9	5	6.1	0.5	5	3.6	0.3	5	4.2	0.3	4	6.4	0.9	4
14 Gy							37.0	2.3	5	16.2	1.2	5	7.8	0.7	5	3.8	0.2	5	4.4	0.4	3			
15 Gy							32.9	2.7	5	15.4	0.9	5	7.7	0.3	5	3.7	0.2	5	3.8	0.2	9			

Table S2.

Radiological Dose (Gy)	Day Post IR					
	-7	0	1	3	6	Total # of Mice per dose
Control	5	5			4	14
8			5	5	5	15
10			5	5	5	15
12			5	5	5	15
14			5	5	5*	15
				Total # of Mice		**74**

Table S3.

		Day Post IR														
		Day -7			Day 0			Day 1			Day 3			Day 6		
		Conc (pmol/mg)	sem	n	Conc (pmol/mg)	sem	n	Conc (pmol/mg)	sem	n	Conc (pmol/mg)	sem	n	Conc (pmol/mg)	sem	n
Radiological Dose	Control	173.8	13.5	5	144.5	17.1	5							181.7	12.0	4
	8 Gy							177.1	12.3	5	71.0	6.6	5	134.4	18.2	5
	10 Gy							128.4	16.5	5	45.9	5.1	5	115.3	8.1	5
	12 Gy							136.7	23.8	5	35.1	6.1	5	59.3	6.2	5
	14 Gy							114.7	12.2	5	55.5	13.6	5	52.8*	11.9	5

Table S4.

Day Post IR

Radiological Dose	Day -7			Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	CCN	sem	n	CCN	sem	n	CCN	sem	n	CCN	sem	n	CCN	sem	n	CCN	sem	n	CCN	sem	n	CCN	sem	n
Control	108.6	0.6	5	106.4	2.0	5																106.2	1.7	5
6 Gy							90.0	1.4	5	83.6	1.4	5	85.5	1.4	5	96.1	2.8	5	95.9	2.4	5	99.7	2.4	5
7 Gy							102.3	1.6	5	86.7	2.0	5	83.7	2.5	5	90.1	1.6	5	95.7	1.1	5	100.0	3.6	5
8 Gy							85.9	2.1	5	48.1	2.6	5	52.4	2.6	5	60.2	2.7	5	59.4	2.8	5	75.7	1.9	5
9 Gy							91.2	2.3	5	44.6	2.6	5	43.3	2.1	5	48.8	2.8	5	53.3	2.0	5	62.1	1.0	5
10 Gy							77.5	0.9	5	22.5	2.4	5	22.2	2.5	5	27.9	1.6	5	24.0	2.2	5	36.1	1.3	5
11 Gy							90.5	2.0	5	22.8	1.8	5	14.6	0.8	5	19.7	2.6	5	24.0	0.8	5	26.8	0.9	5
12 Gy							72.7	1.0	5	9.4	1.4	5	3.6	0.8	5	8.5	0.3	5	8.0	1.5	5	17.5	0.4	5
13 Gy							87.4	3.0	5	5.5	0.4	5	2.9	0.6	5	3.7	0.2	5	8.1	1.4	5	12.5	1.0	4
14 Gy							74.5	1.0	5	2.8	0.1	5	0.8	0.1	5	1.4	0.2	5	2.3	0.3	10			
15 Gy							86.3	2.4	5	1.7	0.2	5	0.3	0.1	5	0.9	0.2	5	1.3	0.1	10			

Table S5.