Supplementary Information

Nanosensor biodosimetry of mouse blood proteins after exposure to ionizing radiation

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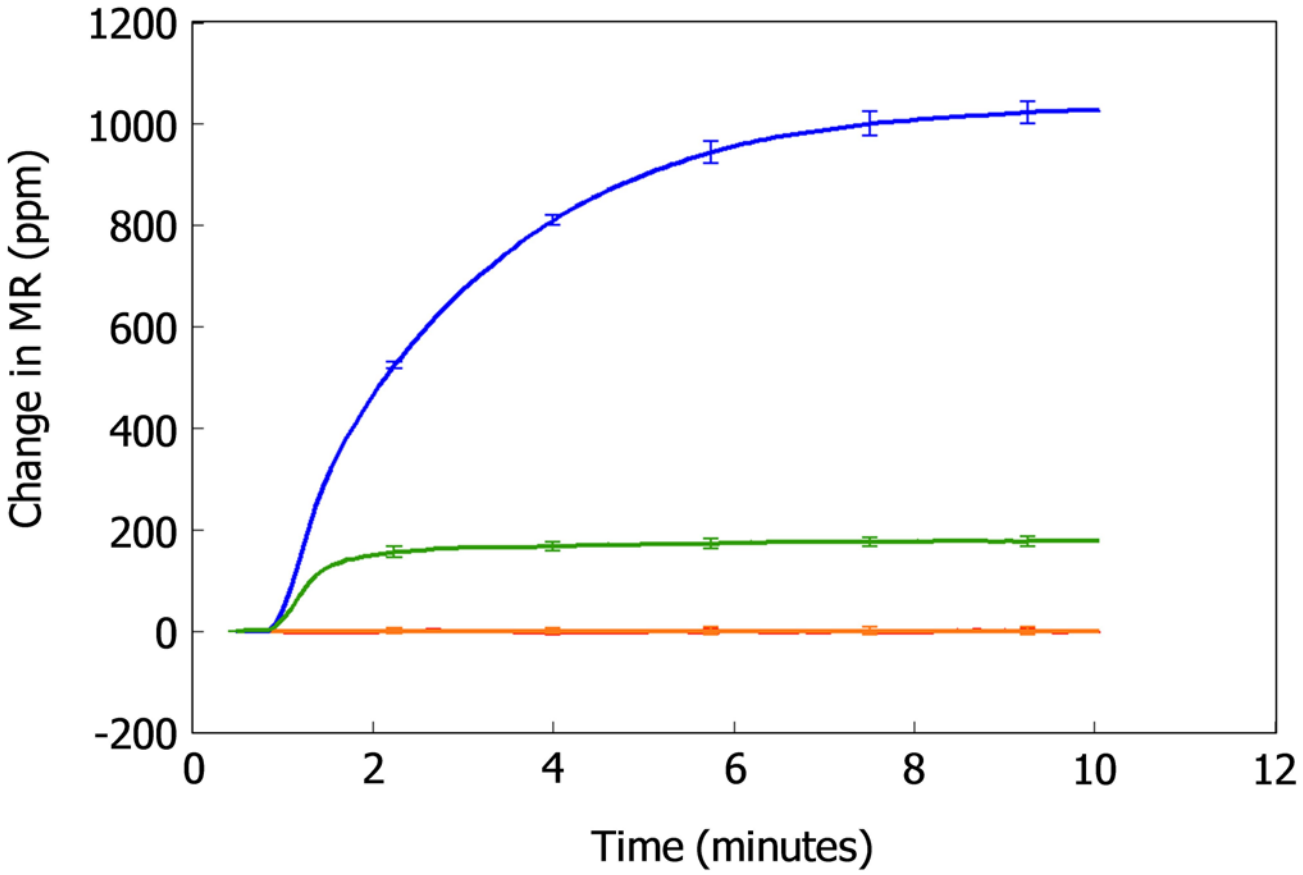

Figure S1 | An example of real-time binding curve showing change in magnetoresistance (MR) in parts per million (ppm) over time for 500 pg/ml Flt3lg (green) compared with biotin-BSA positive control (blue), BSA negative control (orange), and epoxy reference (red). Error bars are ±1 standard deviation.

	sham vs. 0.1 Gy	sham vs. 1 Gy	sham vs. 2 Gy	sham vs. 3 Gy	sham vs. 6 Gy
day 1	0.1263	0.0070	< 0.0001	< 0.0001	< 0.0001
2	0.9048	< 0.0001	< 0.0001	< 0.0001	< 0.0001
3	0.3520	< 0.0001	< 0.0001	< 0.0001	< 0.0001
5	0.1295	0.0006	< 0.0001	< 0.0001	< 0.0001
7	0.5184	0.7518	< 0.0001	< 0.0001	< 0.0001

Table S1 | P values associated with two-tailed Student's t-test for Flt3lg concentrations after comparing sham and irradiated groups. Raw concentrations of Flt3lg were used for the calculation. Here, "sham" group indicates the pre-irradiation samples collected from all mice 8 days before irradiation and the samples collected from sham-irradiated mice. This definition is applicable to Tables S1-S6.

	sham vs. 0.1 Gy	0.1 Gy vs. 1 Gy	1 Gy vs. 2 Gy	2 Gy vs. 3 Gy	3 Gy vs. 6 Gy
day 1	0.1263	0.0122	0.0235	0.446	0.0073
2	0.9048	0.0002	0.0002	< 0.0001	0.1765
3	0.3520	0.0001	0.0008	< 0.0001	0.0973
5	0.1295	0.0053	0.0071	0.0001	0.0009
7	0.5184	0.7672	0.0189	0.0399	< 0.0001

Table S2 | P values associated with two-tailed Student's t-test for Flt3lg concentrations after comparing immediately lower dose groups at the same day. Raw concentrations of Flt3lg were used for the calculation.

The dose discriminant analysis was done to assess how accurately we can classify radiation dose groups at a given day after irradiation based on the concentrations of biomarkers. For example, if all the 6 Gy samples at day 5 after irradiation are correctly classified as such, the sensitivity for 6 Gy is 1. If all of the non-6 Gy samples at day 5 are not classified as 6 Gy, the specificity for 6 Gy is 1. For each cell in Table S3, the DISCRIM procedure in SAS software calculates true positives, false positives, true negatives, and false negatives, and then computes the sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) for the given dose group at a given day post irradiation. All the other radiation dose groups at the same day post irradiation are treated as references. For example, 2 Gy radiation dose group at day 2 has sham, 1, 3, 6 Gy radiation dose groups at day 2 as its references.

Many of the dose groups have excellent sensitivity, specificity, PPV and NPV values. The low PPV and sensitivity values for sham and for 1 Gy at day 7 are due to the fact that the Flt3lg concentrations of the sham and 1 Gy groups are quite similar, resulting in higher number of false positives and false negatives. For example, among the samples classified as 1 Gy at day 7 post irradiation, 80% of them are false positives from 0 or 2 Gy samples. In contrast, the concentration differences at day 7 between sham or 1 Gy groups and 3 or 6 Gy groups are much larger, so the latter groups have much higher PPV and sensitivity values.

	sham	1 Gy	2 Gy	3 Gy	6 Gy
day 1	sensitivity: 1	0.800	0	0.800	0.800
	specificity: 0.909	0.958	0.955	0.917	0.833
	PPV: 0.778	0.800	0	0.667	0.500
	NPV: 1	0.958	0.750	0.957	0.952
2	1	0.800	0.875	0.800	0.400
	0.957	1	0.955	0.880	0.960
	0.875	0.800	0.875	0.571	0.667
	1	0.962	0.955	0.957	0.889

3	0.857	1	0.875	0.800	0.800
	1	0.920	1	0.960	0.960
	1	0.714	1	0.800	0.800
	0.958	1	0.957	0.960	0.960
5	0.857	0.800	0.875	1	1
	0.913	0.960	1	1	1
	0.750	0.800	0.875	1	1
	0.955	0.960	0.957	1	1
7	0.143	0.400	0.500	0.800	1
	0.826	0.680	0.955	0.960	1
	0.200	0.200	0.800	0.800	1
	0.760	0.850	0.840	0.960	1

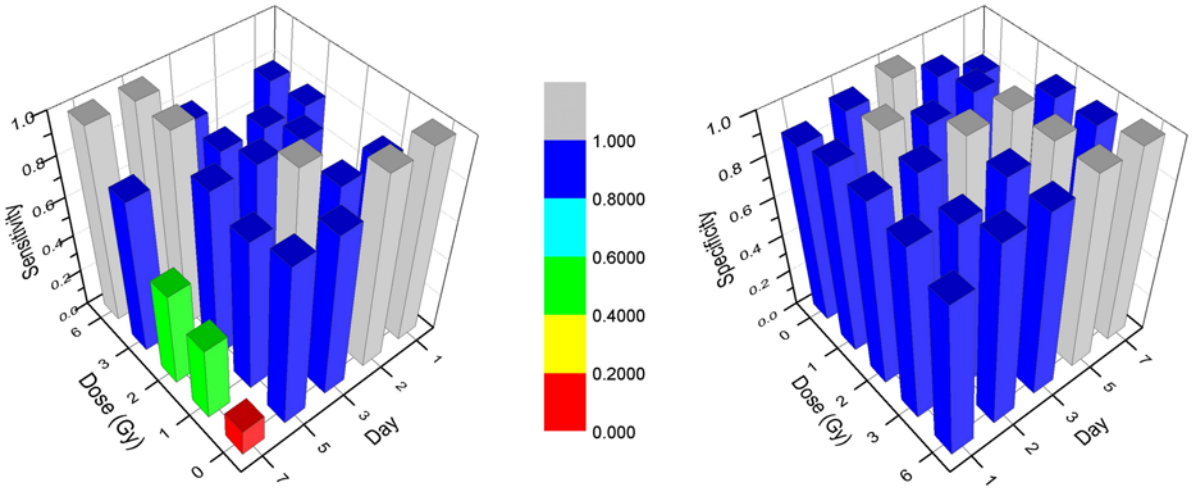

Table S3 | Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) obtained by dose discriminant analysis for classification of radiation dose groups at a given day post irradiation. Flt3lg concentrations in sham, 1, 2, 3, and 6 Gy irradiated samples were used for the analysis. 3-D plots for sensitivity and specificity are shown below the table.

	sham vs. 0.1 Gy	sham vs. 1 Gy	sham vs. 2 Gy	sham vs. 3 Gy	sham vs. 6 Gy
day 1	0.4067	<0.0001	<0.0001	<0.0001	<0.0001
2	0.5588	<0.0001	<0.0001	<0.0001	<0.0001

Table S4 | P values associated with two-tailed Student's t-test for Saa1 concentrations after comparing sham and irradiated groups. Raw concentrations of Saa1 were used for the calculation.

	sham vs. 0.1 Gy	0.1 Gy vs. 1 Gy	1 Gy vs. 2 Gy	2 Gy vs. 3 Gy	3 Gy vs. 6 Gy
day 1	0.4067	0.0011	0.0614	0.9692	0.7122
2	0.5588	0.0352	0.6672	0.2822	0.2439

Table S5 | P values associated with two-tailed Student's t-test for Saa1 concentrations after comparing immediately lower dose groups at the same day. Raw concentrations of Saa1 were used for the calculation.

	sham	1 Gy	2 Gy	3 Gy	6 Gy
day 1	sensitivity: 1	0.800	0.250	0	0.400
	specificity: 1	0.640	0.955	0.960	0.840
	PPV: 1	0.308	0.667	0	0.333
	NPV: 1	0.941	0.778	0.828	0.875
2	1	0.200	0	0.200	0.600
	0.870	0.840	1	0.800	0.760
	0.700	0.200	0	0.167	0.333
	1	0.840	0.733	0.833	0.905

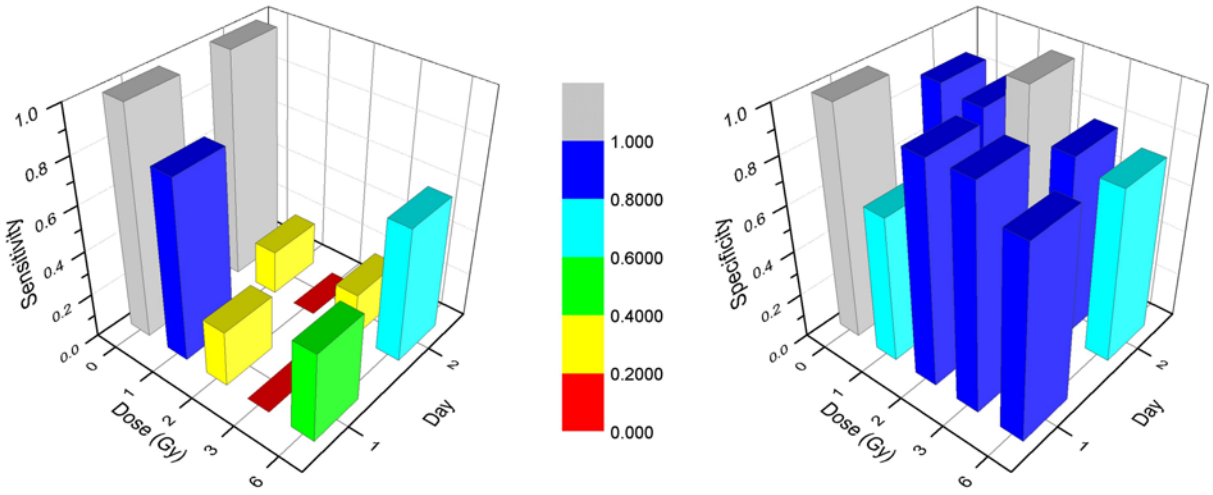

Table S6 | Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) obtained by dose discriminant analysis for classification of radiation dose groups at a given day after irradiation. Saa1 concentrations in sham, 1, 2, 3, and 6 Gy irradiated samples were used for the analysis. 3-D plots for sensitivity and specificity are shown below the table.

Flt3lg concentration (pg/ml) after 0 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
681	0	459	-222	271	-410	441	-241	419	-262	286	-396
569	0	471	-99	442	-128	656	87	500	-69	503	-67
537	0	477	-60	257	-280	291	-246	346	-191	518	-19
436	0	425	-11	292	-144	308	-128	284	-151	293	-142
483	0	430	-54	350	-133	365	-118	300	-184	451	-32
299	0	272	-26	402	103	479	181	413	114	199	-100
357	0	266	-91	435	78	397	40	286	-71	498	140

Flt3lg concentration (pg/ml) after 0.1 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
327	0	385	58	515	188	472	145	385	58	534	206
85	0	151	66	240	156	190	105	185	101	196	112
504	0	368	-136	456	-49	409	-95	339	-165	468	-36
342	0	294	-48	375	33	325	-17	290	-52	338	-4
305	0	279	-26	366	61	283	-22	284	-22	236	-70

Flt3lg concentration (pg/ml) after 1 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
510	0	754	244	850	340	845	335	710	200	335	-175
244	0	544	300	901	657	915	671	707	463	469	225
303	0	714	411	1205	902	1144	841	848	545	456	153
240	0	635	395	1056	815	853	613	625	384	349	108
200	0	298	98	817	617	774	574	335	135	279	79

Flt3lg concentration (pg/ml) after 2 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
510	0	1483	973	1574	1065	1519	1009	1513	1004	1331	822
824	0	1654	831	1577	753	2469	1646	1688	864	1034	210
648	0			1346	698	1744	1096	1670	1022	843	194
894	0	1602	707	1558	663	2196	1301	1564	669	1062	168
288	0	811	522	1366	1077	1828	1539	1271	982	476	187
522	0	619	98	1794	1272	2081	1559	1595	1074	701	180
235	0	906	671	1829	1594	1198	964	396	161	341	107
171	0	228	57	622	451	1772	1601	1117	946	286	115

Flt3lg concentration (pg/ml) after 3 Gy irradiation

Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
215	0	938	723	2893	2679	4539	4325	3437	3222	747	532
454	0	895	442	3600	3146	5135	4682	3078	2624	1212	758
388	0	850	462	2426	2038	5652	5265	5542	5154	1860	1472
512	0	914	401	2972	2460	4148	3636	4315	3802	1237	725
380	0	1407	1027	3263	2883	5081	4701	5118	4738	2010	1630

Flt3lg concentration (pg/ml) after 6 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
284	0	1583	1299	3045	2761	6266	5982	6268	5984	4812	4528
320	0	1150	830	3032	2712	4089	3769	7692	7372	5591	5271
550	0	1718	1168	4163	3612	6271	5721	7666	7116	6270	5720
250	0	1485	1234	3157	2907	6367	6117	9442	9191	6713	6462
543	0	1565	1022	4388	3846	6452	5909	8141	7598	4631	4088

Table S7 | Lists of the measurement data in Figure 5. Each row is the Flt3lg concentration and concentration change data for repeated blood samples from a single animal.

Saa1 concentration (μg/ml) after 0 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
3.6	0	1.9	-1.7	1.3	-2.4	1.6	-2.0	0.8	-2.8	3.7	0.1
2.8	0	3.5	0.7	2.1	-0.8	1.2	-1.6	2.6	-0.3	2.0	-0.8
0.9	0	2.1	1.2	0.7	-0.2	0.8	-0.1	1.1	0.2	2.4	1.5
2.6	0	3.2	0.6	1.9	-0.7	3.1	0.5	2.3	-0.2	3.1	0.5
3.6	0	2.5	-1.1	1.3	-2.3	1.3	-2.3	1.2	-2.4	1.3	-2.3
0.9	0	0.5	-0.4	4.6	3.7	1.9	1.0	4.3	3.5	2.0	1.1
0.8	0	1.1	0.3	1.0	0.2	0.8	0	0.5	-0.3	0.7	-0.1

Saa1 concentration (μg/ml) after 0.1 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
2.0	0	0.4	-1.7	2.2	0.1	1.1	-1.0	2.3	0.3	3.3	1.3
0.8	0	2.6	1.8	1.8	1.0	3.4	2.6	2.5	1.7	2.5	1.7
1.8	0	3.7	1.9	0.9	-0.9	2.5	0.7	1.9	0.1	2.8	1.0
2.0	0	0.8	-1.1	0.8	-1.1	3.1	1.1	1.4	-0.6	2.7	0.8
0.3	0	0.6	0.2	3.9	3.6	0.5	0.1	2.7	2.4	1.9	1.5

Saa1 concentration (μg/ml) after 1 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
7.8	0	47.8	40.0	39.5	31.7	2.9	-5.0	2.4	-5.4	8.0	0.1
15.3	0	69.3	54.1	19.3	4.0	15.3	0	11.7	-3.6	9.8	-5.5
7.0	0	33.1	26.1	21.4	14.4	2.1	-4.9	1.2	-5.8	5.2	-1.8
8.5	0	28.5	20.0	8.3	-0.1	7.7	-0.7	9.6	1.1	9.5	1.0
10.2	0	26.9	16.8	2.3	-7.9	15.0	4.8	7.0	-3.2	14.2	4.1

Saa1 concentration (μg/ml) after 2 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
4.0	0	54.2	50.2	8.0	4.0	2.1	-1.9	9.7	5.7	5.0	1.0
4.0	0	56.9	52.9	4.0	0	1.9	-2.0	1.9	-2.0	1.2	-2.8
4.3	0	53.9	49.5	24.7	20.4	1.5	-2.8	2.5	-1.9	1.3	-3.0
1.4	0	116.8	115.4	9.3	7.9	1.3	-0.1	2.5	1.1	0.9	-0.5
0.2	0	61.2	61.0	19.1	18.9	1.8	1.6	1.3	1.1	0.6	0.3
1.3	0	69.2	67.9	19.3	17.9	1.3	-0.1	1.3	0	1.0	-0.3
3.0	0	153.2	150.2	22.8	19.8	0.4	-2.6	2.3	-0.7	3.5	0.5
2.4	0	35.9	33.5	8.7	6.3	3.2	0.8	1.1	-1.3	1.0	-1.4

Saa1 concentration (μg/ml) after 3 Gy irradiation

Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
1.0	0	22.1	21.1	12.9	11.9	3.9	3.0	6.8	5.9	6.2	5.3
1.7	0	21.9	20.1	10.0	8.2	3.8	2.0	7.1	5.3	3.1	1.3
4.2	0	51.8	47.6	19.8	15.5	7.7	3.5	3.1	-1.1	6.7	2.5
8.7	0	163.3	154.6	23.2	14.5	6.1	-2.5	2.7	-5.9	12.5	3.8
6.8	0	151.0	144.2	50.9	44.0	5.3	-1.5	2.9	-3.9	7.1	0.3

Saa1 concentration (μg/ml) after 6 Gy irradiation											
Day -8		Day 1		Day 2		Day 3		Day 5		Day 7	
raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8	raw concentration	change from day -8
0.9	0	171.0	170.0	26.4	25.5	1.6	0.6	0.8	-0.1	19.9	18.9
1.2	0	51.5	50.3	6.8	5.6	0.6	-0.6	0.8	-0.4	0.6	-0.6
0.5	0	95.3	94.8	12.9	12.3	1.7	1.1	1.1	0.5	0.4	-0.2
0.3	0	56.4	56.2	8.1	7.9	1.2	1.0	0.5	0.2	0.8	0.5
0.3	0	108.3	108.0	11.7	11.3	1.0	0.6	0.5	0.1	0.8	0.4

Table S8 Lists of the measurement data in Figure 6. Each row is the Saa1 concentration and concentration change data for repeated blood samples from a single animal.

DISCRIM data using Flt3lg for 2 Gy exposure groups

	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Remarks
Day -8	5	0	0	0	0	3	sensitivity: 0.625 specificity: 0.821 PPV: 0.417 NPV: 0.914
Day 1	2	0	3	0	0	2	sensitivity: 0 specificity: 0.925 PPV: 0 NPV: 0.841
Day 2	1	0	3	2	2	0	sensitivity: 0.375 specificity: 0.821 PPV: 0.300 NPV: 0.865
Day 3	0	0	1	6	1	0	sensitivity: 0.750 specificity: 0.897 PPV: 0.600 NPV: 0.946
Day 5	1	1	3	2	1	0	sensitivity: 0.125 specificity: 0.897 PPV: 0.200 NPV: 0.833
Day 7	3	2	0	0	1	2	sensitivity: 0.250 specificity: 0.872 PPV: 0.286 NPV: 0.850
Error rate	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Total
	0.3750	1.0000	0.6250	0.2500	0.8750	0.7500	0.6458

DISCRIM data using Flt3lg and Saa1 for 2 Gy exposure groups

	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Remarks
Day -8	5	0	0	0	0	3	sensitivity: 0.625 specificity: 0.846 PPV: 0.455 NPV: 0.917
Day 1	1	5	1	0	0	0	sensitivity: 0.714 specificity: 1 PPV: 1 NPV: 0.952
Day 2	1	0	5	0	2	0	sensitivity: 0.625 specificity: 0.949 PPV: 0.714 NPV: 0.925
Day 3	0	0	0	6	2	0	sensitivity: 0.750 specificity: 0.923 PPV: 0.667 NPV: 0.947
Day 5	1	0	1	3	3	0	sensitivity: 0.375 specificity: 0.821 PPV: 0.300

							NPV: 0.865
Day 7	3	0	0	0	3	2	sensitivity: 0.250 specificity: 0.923 PPV: 0.400 NPV: 0.857
Error rate	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Total
	0.3750	0.2857	0.3750	0.2500	0.6250	0.7500	0.4435

Table S9 | (top) Table showing the number of observations classified into days post radiation exposure and their corresponding sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and error rate estimates for 2 Gy exposure groups using Flt3lg concentrations alone. (bottom) Table showing the number of observations classified into days post radiation exposure and corresponding sensitivity, specificity, PPV, NPV, and error rate estimates for 2 Gy exposure groups using Flt3lg and Saa1 concentrations in combination.

DISCRIM data using Flt3lg for 6 Gy exposure groups

	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Remarks
Day -8	5	0	0	0	0	0	sensitivity: 1 specificity: 1 PPV: 1 NPV: 1
Day 1	0	5	0	0	0	0	sensitivity: 1 specificity: 1 PPV: 1 NPV: 1
Day 2	0	0	5	0	0	0	sensitivity: 1 specificity: 1 PPV: 1 NPV: 1
Day 3	0	0	1	4	0	0	sensitivity: 0.800 specificity: 0.880 PPV: 0.571 NPV: 0.957
Day 5	0	0	0	1	4	0	sensitivity: 0.800 specificity: 1 PPV: 1 NPV: 0.962
Day 7	0	0	1	2	0	2	sensitivity: 0.400 specificity: 1 PPV: 1 NPV: 0.893
Error rate	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Total
	0.0000	0.0000	0.0000	0.2000	0.2000	0.6000	0.1667

DISCRIM data using Flt3lg and Saa1 for 6 Gy exposure groups

	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Remarks
Day -8	5	0	0	0	0	0	sensitivity: 1 specificity: 0.960 PPV: 0.833 NPV: 1
Day 1	1	4	0	0	0	0	sensitivity: 0.800 specificity: 1 PPV: 1 NPV: 0.962
Day 2	0	0	5	0	0	0	sensitivity: 1 specificity: 0.920 PPV: 0.714 NPV: 1
Day 3	0	0	1	4	0	0	sensitivity: 0.800 specificity: 0.880 PPV: 0.571 NPV: 0.957
Day 5	0	0	0	1	4	0	sensitivity: 0.800 specificity: 1 PPV: 1

							NPV: 0.962
Day 7	0	0	1	2	0	2	sensitivity: 0.400 specificity: 1 PPV: 1 NPV: 0.893
Error rate	Day -8	Day 1	Day 2	Day 3	Day 5	Day 7	Total
	0.0000	0.2000	0.0000	0.2000	0.2000	0.6000	0.2000

Table S10 | (top) Table showing the number of observations classified into days post radiation exposure and their corresponding sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and error rate estimates for 6 Gy exposure groups using Flt3lg concentrations alone. (bottom) Table showing the number of observations classified into days post radiation exposure and corresponding sensitivity, specificity, PPV, NPV, and error rate estimates for 6 Gy exposure groups using Flt3lg and Saa1 concentrations in combination.