Supplementary figures and tables

Analyses of cancer incidence and other morbidities in gamma irradiated B6CF1 mice.
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Supplementary Table S1: Cause of Death (COD) codes for censored mice and sample size for each group.

| Mice Censored                              | # of mice |
|--------------------------------------------|-----------|
| COD – Accidental death                     | 47        |
| COD – Escaped during irradiation           | 8         |
| COD - Discarded                            | 207       |
| COD – Improper irradiation                 | 77        |
| COD - Missing                              | 29        |
| COD – Sacrifice, programmed                | 19        |
| No lethal disease listed                   | 936       |
**Supplementary Table S2**: Description of which mice were removed from our analysis, the corresponding reasoning, and the resulting sample size. N=4330 for control mice and N=7848 for gamma irradiated mice.

| Data removed                             | Reasoning                                                                 | # of mice |
|------------------------------------------|---------------------------------------------------------------------------|-----------|
| -                                        | -                                                                         | 50110     |
| JM11                                     | Not a true data set                                                       | 49,225    |
| JM10                                     | Different species – *Peromyscus leucopus*                                  | 46,835    |
| JM14 mice treated with radioprotectors   | Beyond the scope of our project                                          | 46,635    |
| Breeder mice                             | Held under different conditions                                           | 43,428    |
| JM2 mice                                 | Held under different conditions                                           | 31,843    |
| COD – removal to another experiment      | Mice listed under different experiment, do not want to double count       | 28,153    |
| JM12 mice                                | Controls analysis showed significant difference                           | 27,553    |
| JM3 mice                                 | Controls analysis showed significant difference                           | 24,478    |
| Mice irradiated with 300 fractions       | Controls analysis showed significant difference                           | 23,953    |
| JM8 mice                                 | Experimental design – separate analysis required                         | 22,626    |
| Neutron irradiated mice                  | Different radiation quality - separate analysis                           | 12178     |
Supplementary Figure S1: Cox PH model predicted graphs and table output for controls after filtering

**Table 1**

| Model Group | Independent Variable | Parameter Estimate | Hazard Ratio (95% CI) | P-value |
|-------------|----------------------|--------------------|-----------------------|---------|
| A           | Sex                  | -0.119             | 0.888 (0.83, 0.949)   | 0.001   |
| A           | Expt 7               | 0.025              | 1.025 (0.914, 1.15)   | 0.67    |
| A           | Expt 9               | -0.057             | 0.945 (0.863, 1.035)  | 0.225   |
| A           | Expt 13              | 0.02               | 1.021 (0.932, 1.118)  | 0.659   |
| A           | Expt 14              | -0.009             | 0.991 (0.877, 1.12)   | 0.884   |
| B           | Sex                  | -0.11              | 0.896 (0.839, 0.957)  | 0.001   |
| B           | Fractions            | 0.001              | 1.0007 (1, 1.002)     | 0.17    |
| C           | Sex                  | -0.096             | 0.908 (0.85, 0.96)    | 0.003   |
| C           | First Irradiated     | -0.002             | 0.998 (0.995, 1.001)  | 0.113   |

**Supplementary Figure S1:** Cox proportional hazard (PH) survival curves produced using all data filtered by the criteria in Table 1 that also had a total dose of 0Gy. Age at death was used as a time scale with sex and (A) experiment, (B) number of fractions, or (C) the age first irradiated as independent variables. The predicted outcomes shown are for female mice. Parameter estimates and p-values from each model are shown in D. Kaplan Meier curves with (E) sex, (F) number of fractions, (G) age first irradiated, and (H) experiment group were evaluated to test the proportional hazards assumption of these models.
Supplementary Figure S1: Cox proportional hazard (PH) survival curves produced using all data filtered by the criteria in Table 1 that also had a total dose of 0Gy. Age at death was used as a time scale with sex and (A) experiment, (B) number of fractions, or (C) the age first irradiated as independent variables. Parameter estimates and p-values from each model are shown in D. Kaplan Meier curves with (E) sex, (F) number of fractions, (G) age first irradiated, and (H) experiment group were evaluated to test the proportional hazards assumption of these models.
### Supplementary Table S3: Robustness tests for Cox Proportional Hazards models for controls analysis.

| Model Group | Independent Variable (reference) | Parameter Estimate | Hazard Ratio (95% CI) | P   |
|-------------|----------------------------------|--------------------|-----------------------|-----|
| D           | Sex - stratify                   | NA                 | NA                    | NA  |
| D           | Expt 7 (Expt 4)                  | 0.027              | 1.027 (0.92, 1.15)    | 0.645 |
| D           | Expt 9 (Expt 4)                  | -0.056             | 0.945 (0.86, 1.04)    | 0.230 |
| D           | Expt 13 (Expt 4)                 | 0.0229             | 1.023 (0.93, 1.12)    | 0.621 |
| D           | Expt 14 (Expt 4)                 | -0.010             | 0.990 (0.88, 1.12)    | 0.876 |
| E           | Sex - stratify                   | NA                 | NA                    | NA  |
| E           | Fractions                       | 0.001              | 1.001 (1.00, 1.002)   | 0.158 |
| F           | Sex - stratify                   | NA                 | NA                    | NA  |
| F           | First Irradiated                 | -0.002             | 0.998 (0.995, 1)      | 0.099 |
| G           | Sex                             | -0.114             | 0.892 (0.83, 0.95)    | 0.001 |
| G           | Fractions 1 (Fractions 0)        | -0.083             | 0.921 (0.83, 1.03)    | 0.133 |
| G           | Fractions 24 (Fractions 0)       | -0.014             | 0.986 (0.88, 1.11)    | 0.808 |
| G           | Fractions 60 (Fractions 0)       | -0.004             | 0.996 (0.89, 1.11)    | 0.941 |
| G           | Fractions 120 (Fractions 0)      | -0.005             | 0.995 (0.84, 1.18)    | 0.955 |
| H           | Sex - stratify                   | NA                 | NA                    | NA  |
| H           | Fractions 1 (Fractions 0)        | -0.084             | 0.919 (0.83, 1.024)   | 0.126 |
| H           | Fractions 24 (Fractions 0)       | -0.017             | 0.983 (0.87, 1.11)    | 0.772 |
| H           | Fractions 60 (Fractions 0)       | -0.004             | 0.996 (0.89, 1.11)    | 0.947 |
| H           | Fractions 120 (Fractions 0)      | -0.005             | 0.995 (0.84, 1.19)    | 0.956 |
Supplementary Table S4: Grouping B6CF1 Janus COD codes to match B6C3F1 IES categories

| New code name          | Old code name(s): decoded (coded)                                                                 |
|------------------------|--------------------------------------------------------------------------------------------------|
| Circulatory            | Vascular (TVAS), Heart (THRT), Spleen (TSPL)                                                    |
| Digestive              | Caecum (TCEC), Colon (TCOL), Duodenum (TDUO), Esophagus (TESO), Ileum (TILE), Jejunum (TJEJ), Miscellaneous digestive system (TMID), Pancreas (TPAN), Salivary gland (TSGL), Stomach (TSTO), Tongue (TTGE), Gallbladder (TGBL), Liver (TLIV) |
| Endocrine              | Adrenal (TADR), Pituitary (TPIT), Thyroid (TTRD), Miscellaneous endocrine (TMIE), Hibernating gland (THIB), Miscellaneous glandular (TMIG), Preputial gland (TPPT) |
| Hematopoietic          | Non-thymic lymphoma, generalized (NTYG), Non-thymic lymphoma, localized (NTYL), Thymic lymphoma, generalized (TTYG), Thymic lymphoma, localized (TTYL) |
| Mesothelium            | No match                                                                                         |
| Nervous                | Brain (TBRN), Central nervous system (TCNS), Miscellaneous nervous system (TMIN), Peripheral nervous system (TPNS) |
| Male Reproductive      | Seminal vesicle (TSMV), Testis (TTST), Cowper’s gland (TCGL), Epididymis (TEPI), Prostate (TPST) |
| Female Reproductive    | Ovary (TOVE), Mammary Gland (TMGL), Uterus (TUTE), Vagina (TVAG)                                  |
| Respiratory            | Lung (TADN), Miscellaneous lung (TMIL)                                                            |
| Skeletal               | Bone (TBON)                                                                                       |
| Skin                   | Skin (TSKN)                                                                                       |
| Soft Tissue            | Connective tissue/fibrosarcoma (TCON), Miscellaneous connective tissue (TMIC), Muscle (TMUS)     |
| Special Sense Organ    | Harderian gland (THGL)                                                                            |
| Urinary                | Urinary bladder (TBLA), Kidney (TKID), Miscellaneous urogenital (TMUG)                            |
| Non-neoplastic         | All Non-tumor codes                                                                               |
| Unknown                | Cause of death unknown (CDU)                                                                      |
Supplementary Table S5: Janus experiments used for comparisons with IES chronic irradiation data

|       | Total Dose (Gy) | Fractions | Fractionation schedule | Dose Rate (cGy/min) | Time Irradiated (min) | Sample Size |
|-------|----------------|-----------|------------------------|---------------------|-----------------------|-------------|
| Males |                | Variable  | Variable               | 0                   | 0                     | 1724        |
|       | 9.2            | 24        | 1/week                 | .8516               | 45                    | 200         |
|       | 9.6            | 120       | 5/week                 | .006053             | 1320                  | 80          |
| Females | 0              | Variable  | Variable               | 0                   | 0                     | 2626        |
|       | .43            | 1         | N/A                    | 2.158               | 20                    | 350         |
|       | 7.74           | 24        | 1/week                 | .7164               | 45                    | 367         |
Supplementary Figure S2 – CoxPH model validation for 300 fractions and KM curves showing differences in survival probability based on fraction number for each cause of death

Supplementary Figure S2: Survival probability output from Cox PH model with sex and the number of fractions as a categorical variable control mice (A/B). The predicted outcomes shown (A) are for male mice. Kaplan Meier curve for control mice showing differences in survival probability based on the number of fractions used during sham irradiation treatment (C). Kaplan Meier curves showing how the number of fractions impacts survival in control male mice that died of solid tumors (excluding lung tumors) (D), lung tumors (E), lymphomas (F), non-tumor diseases (G), and CDU (H).
Supplementary Figure S2 – CoxPH model validation for 300 fractions and KM curves showing differences in survival probability based on fraction number of each cause of death

Supplementary Figure S2: Survival probability output from Cox PH model with sex and the number of fractions as a categorical variable control mice (A/B). The predicted outcomes shown (A) are for male mice. Kaplan Meier curve for control mice showing differences in survival probability based on the number of fractions used during sham irradiation treatment (C). Kaplan Meier curves showing how the number of fractions impacts survival in control male mice that died of solid tumors (excluding lung tumors) (D), lung tumors (E), lymphomas (F), non-tumor diseases (G), and CDU (H).
**Supplementary Figure S3: Robustness testing for main model**

CoxPH model output from the base model with the following changes: *(A/B)* including mice irradiated at all ages, *(C/D)* stratifying by sex, *(E/F)* treating fractions as a categorical variable, *(G/H)* including exact fraction number and age first irradiated for control samples, *(I/J)* including mice with total dose received in 300 fractions, *(K/L)* including mice with total dose received in 300 fractions and fractions treated as a categorical variable, and *(M/N)* including an interaction term between total dose and sex. The graphs represent predicted outcomes for female mice first irradiated at 120 days.

| Independent Variable | Estimate | Hazard Ratio (95% CI) | P-value |
|----------------------|----------|-----------------------|---------|
| sexM                 | -0.157   | 0.85 (0.81, 0.90)     | <0.001  |
| Fractions            | 0.002    | 1.002 (1.002, 1.003)  | 0.002   |
| Total Dose           | 0.167    | 1.18 (1.17, 1.19)     | <0.001  |
| First Irradiated     | 0        | 0.9996 (0.9994, 0.9999)| 0.001   |
| Fractions:Total Dose | -0.001   | 0.999 (0.999, 0.999)  | <0.001  |
Supplementary Figure S3: Robustness testing for gamma irradiation main model. CoxPH model output from the base model with the following changes: (A/B) including mice irradiated at all ages, (C/D) stratifying by sex, (E/F) treating fractions as a categorical variable, (G/H) including exact fraction number and age first irradiated for control samples, (I/J) including mice with total dose received in 300 fractions, (K/L) including mice with total dose received in 300 fractions and fractions treated as a categorical variable, and (M/N) including an interaction term between total dose and sex. The graphs represent predicted outcomes for female mice first irradiated at 120 days.
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| Independent Variable         | Estimate | Hazard Ratio (95% CI) | P-value |
|-----------------------------|----------|-----------------------|---------|
| SexM                        | -0.149   | 0.86 (0.83, 0.90)     | <0.001  |
| Total Dose                  | 0.001    | 1.001 (1.001, 1.002)  | <0.001  |
| First Irradiated            | 0.168    | 1.18 (1.18, 1.19)     | <0.001  |
| Fractions:Total Dose        | -0.002   | 0.998 (0.996, 0.9996) | 0.017   |
| Fractions                   | -0.001   | 0.999 (0.9990, 0.9991)| <0.001  |
Supplementary Figure S3: Robustness tests for main model. CoxPH model output from the base model with the following changes: (A/B) including mice irradiated at all ages, (C/D) stratifying by sex, (E/F) treating fractions as a categorical variable, (G/H) including exact fraction number and age first irradiated for control samples, (I/J) including mice with total dose received in 300 fractions, (K/L) including mice with total dose received in 300 fractions and fractions treated as a categorical variable, and (M/N) including an interaction term between total dose and sex. The graphs represent predicted outcomes for female mice first irradiated at 120 days.
Supplementary Figure S3: Robustness tests for main model

| Independent Variable (reference) | Estimate | Hazard Ratio (95% CI) | P-value |
|----------------------------------|----------|-----------------------|---------|
| SexM                             | -0.203   | 0.817 (0.77, 0.86)    | <0.001  |
| Fractions 24                     | -0.008   | 0.992 (0.89, 1.11)    | 0.885   |
| Fractions 60                     | 0.145    | 1.16 (1.04, 1.28)     | 0.0059  |
| Fractions 120                    | 0.270    | 1.31 (1.09, 1.57)     | 0.0035  |
| Fractions 300                    | 0.59     | 1.81 (1.46, 2.25)     | <0.001  |
| Total Dose                       | 0.279    | 1.32 (1.23, 1.42)     | <0.001  |
| First Irradiated                | -0.007   | 0.994 (0.991, 0.996)  | <0.001  |
| Fractions 24: Total Dose        | -0.133   | 0.88 (0.81, 0.94)     | 0.0003  |
| Fractions 60: Total Dose        | -0.194   | 0.82 (0.77, 0.89)     | <0.001  |
| Fractions 120: Total Dose       | -0.189   | 0.83 (0.77, 0.89)     | <0.001  |
| Fractions 300: Total Dose       | -0.227   | 0.80 (0.74, 0.86)     | <0.001  |

Supplementary Figure S3: Robustness testing for gamma irradiation main model. CoxPH model output from the base model with the following changes: (A/B) including mice irradiated at all ages, (C/D) stratifying by sex, (E/F) treating fractions as a categorical variable, (G/H) including exact fraction number and age first irradiated for control samples, (I/J) including mice with total dose received in 300 fractions, (K/L) including mice with total dose received in 300 fractions and fractions treated as a categorical variable, and (M/N) including an interaction term between total dose and sex. The graphs represent predicted outcomes for female mice first irradiated at 120 days.
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Supplementary Figure S4: KM curves showing survival probably vs. time in gamma irradiated and control mice after the filtering shown in Table 1 and removing mice first irradiated after 500 days. KM curves compare (A) sex, (B) number of fractions, (C) age first irradiated, and (D) total dose.

**Supplementary Figure S4:**
Kaplan Meier curves for control and gamma irradiated mice to validate Cox Proportional Hazards model.
Supplementary Figure S5: Cause specific hazards for lung tumors, non-thymic lymphomas, and tumors (excluding lung tumors)

Supplementary Figure S5: Competing risks models for specific causes of death in gamma irradiated mice with age as a time scale and sex, age first irradiated, total dose, fractions, and the interaction between total dose and fractions as independent variables. Survival curves for cause of death being (A) lung tumors, (B) all tumors (excluding lung tumors), and (C) non-thymic lymphomas. The corresponding model output with parameter estimates, hazard ratios with a 95% confidence interval, and p-values are listed in table 2 for lung tumors and all tumors (excluding lung tumors). (D) Model output for non-thymic lymphoma. The graphs represent predicted outcomes for female mice first irradiated at 120 days.
Supplementary Figure S5: Cause specific hazards for lung tumors, non-thymic lymphomas, and tumors (excluding lung tumors)

| Term                  | Estimate | Hazard Ratio (95%)           | P-value |
|-----------------------|----------|-----------------------------|---------|
| SexM                  | -0.33    | 0.72 (0.662, 0.786)         | <0.001  |
| Fractions             | 0.005    | 1.005 (1.003, 1.007)        | <0.001  |
| Total Dose            | 0.129    | 1.14 (1.12, 1.16)           | <0.001  |
| First Irradiated      | -0.005   | 0.995 (0.991, 0.999)        | 0.011   |
| Fractions:Total Dose  | -0.001   | 0.9992 (0.9990, 0.9995)     | <0.001  |

**Supplementary Figure S5:** Competing risks models for specific causes of death in gamma irradiated mice with age as a time scale and sex, age first irradiated, total dose, fractions, and the interaction between total dose and fractions as independent variables. Survival curves for cause of death being (A) lung tumors, (B) all tumors (excluding) lung tumors, and (C) non-thymic lymphomas. The corresponding model output with parameter estimates, hazard ratios with a 95% confidence interval, and p-values are listed in table 2 for lung tumors and all tumors (excluding lung tumors). (D) Model output for non-thymic lymphoma. The graphs represent predicted outcomes for female mice first irradiated at 120 days.
## Supplementary Figure S6: CIF with a dose cutoff of 6Gy

| Variable              | Parameter Estimate | Hazard Ratio (95% CI) | P-value |
|-----------------------|--------------------|-----------------------|---------|
| SexM                  | -0.423             | 0.655 (0.57, 0.75)    | <0.001  |
| Fractions             | 0.043              | 1.044 (0.978, 1.113)  | 0.2     |
| Total Dose            | -1.30E-03          | 0.999 (0.998, 0.999)  | <0.001  |
| First Irradiated      | -3.73E-03          | 0.996 (0.993, 1.000)  | 0.07    |
| Fractions:Total Dose  | 0.001              | 1.001 (0.999, 1.002)  | 0.32    |

### Supplementary Figure S6: CIF regression model output using a total dose cutoff of 6Gy for tumor (no lung) (A), lung tumor (C), lymphoma (E), and non-tumor deaths (G). Predicted output from CIF model for tumor (no lung) (B), lung tumor (D), lymphoma (F), and non-tumor deaths (H). All predicted outputs represent males first irradiated at 120 days under the following conditions: low dose = 0.1Gy, high dose = 6Gy, acute = 1 fraction, fractionated = 60 fractions.
**Supplementary Figure S6: CIF with a dose cutoff of 6Gy**

| Variable                  | Parameter Estimate | Hazard Ratio (95% CI) | P-value |
|---------------------------|--------------------|-----------------------|---------|
| SexM                      | -0.276             | 0.759 (0.69, 0.83)    | <0.001  |
| Fractions                 | -0.023             | 0.977 (0.936, 1.02)   | 0.29    |
| Total Dose                | -2.23E-04          | 1.000 (0.999, 1.00)   | 0.36    |
| First Irradiated          | 6.25E-03           | 1.006 (1.004, 1.01)   | <0.001  |
| Fractions:Total Dose      | -0.001             | 0.999 (0.998, 1)      | 0.014   |

| Variable                  | Parameter Estimate | Hazard Ratio (95% CI) | P-value |
|---------------------------|--------------------|-----------------------|---------|
| SexM                      | -0.342             | 0.71 (0.608, 0.83)    | <0.001  |
| Fractions                 | 0.118              | 1.126 (1.049, 1.207)  | 0.001   |
| Total Dose                | 0.001              | 1.001 (1, 1.001)      | 0.15    |
| First Irradiated          | -0.01              | 0.99 (0.985, 0.995)   | <0.001  |
| Fractions:Total Dose      | 0.001              | 1.001 (0.999, 1.003)  | 0.28    |

**Supplementary Figure S6**: CIF regression model output using a total dose cutoff of 6Gy for tumor (no lung) (A), lung tumor (C), lymphoma (E), and non-tumor deaths (G). Predicted output from CIF model for tumor (no lung) (B), lung tumor (D), lymphoma (F), and non-tumor deaths (H). All predicted outputs represent males first irradiated at 120 days under the following conditions: low dose = 0.1Gy, high dose = 6Gy, acute = 1 fraction, fractionated = 60 fractions.