Supplementary Material

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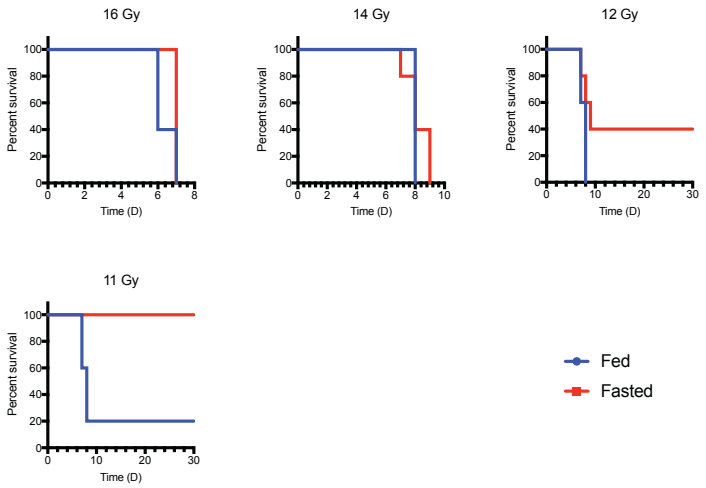

Figure S1: Optimization of total abdominal radiation (TA-XRT) maximum tolerated dose (MTD) for pre-radiation fasted mice. C57Bl/6J mice were allowed to feed *ad libitum* or were fasted for 24 h. Total abdominal radiation (TA-XRT; 11-16 Gy) was administered (day 1). Mice were returned to single-housed cages with food. Survival was monitored daily up to study endpoint.

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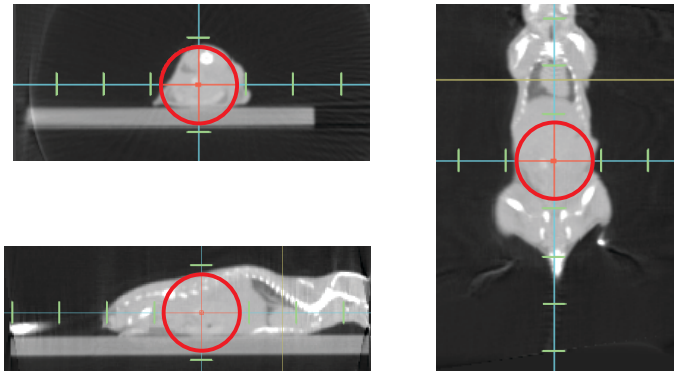

Figure S2: Illustration of sub-xyphoid 25-mm cone radiation field. Red circle denotes radiated area. Ticks are 12.5 mm from each other.

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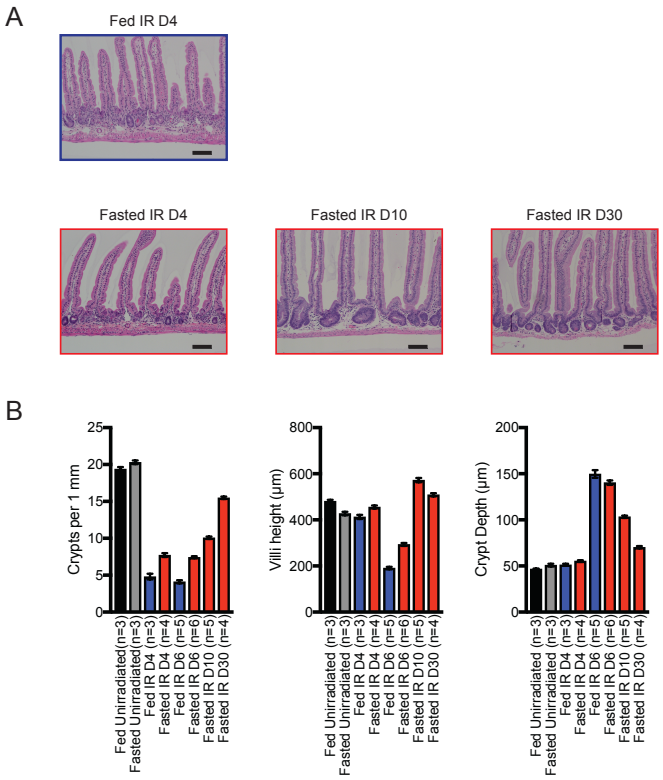

Figure S3: Fasting induced protection of small intestinal stem cells allows for intestinal epithelium recovery after radiation. Mice were treated as described in Fig. 2 and their SI were harvested at various time points following radiation (D4, D10, D30). (A) Representative images of H & E-stained jejunum. Scale bars, 100 µm. Magnification, 10x. (B) Quantification of H & E data. Crypt depth and villi height (n = 50 per mouse) were measured and average value per treatment group plotted. Number of crypts per length of SI (n = 30 fields per mouse) was quantified for each sample and average number of crypts per millimeter of SI length plotted.

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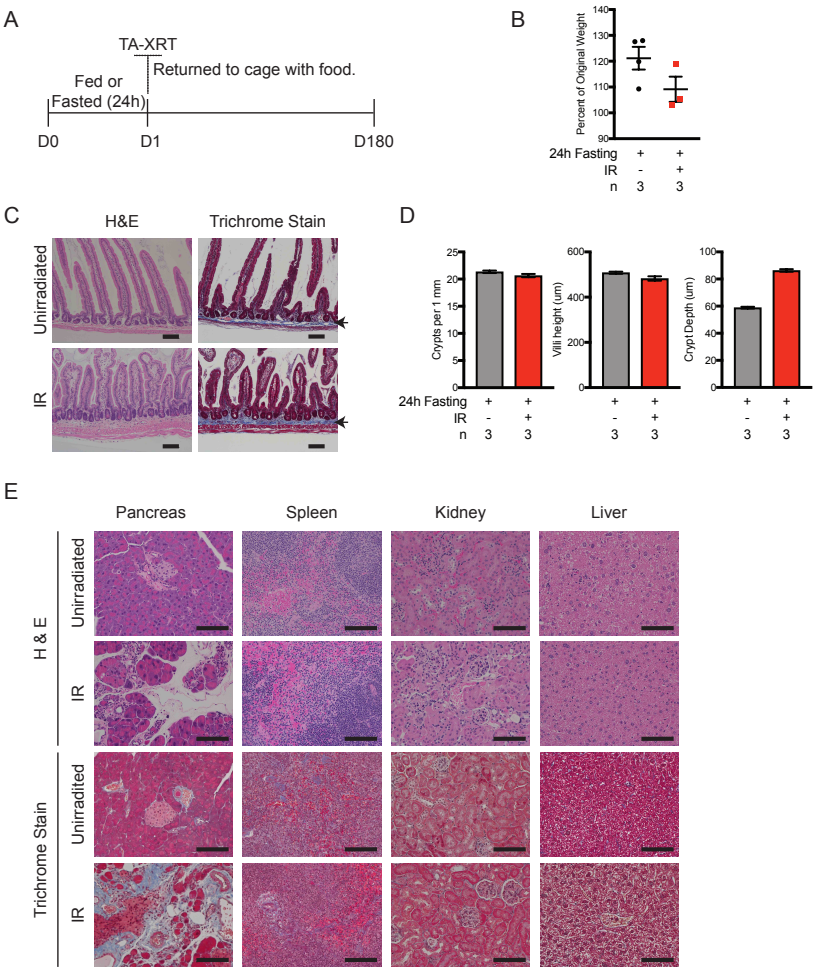

Figure S4: Long term effects of high dose radiation. (A) C57Bl/6J mice were treated as shown. (B) Body weight was recorded at time of euthanasia. (C) Representative images of H & E and Trichrome-stained SI. Arrows denote intestinal submucosa.Scale bars, 100 µm. Magnification, 10x. (D) Crypt depth and villi height (n = 50 per mouse) were measured and average value per treatment group plotted. Number of crypts per length of SI as quantified (n = 30 fields per mouse) and average number of crypts per millimeter of SI length plotted. (E) Representative images of H & E stained and Trichrome stained abdominal organs within the radiation field. Scale bars, 100 µm. Magnification, 20x.

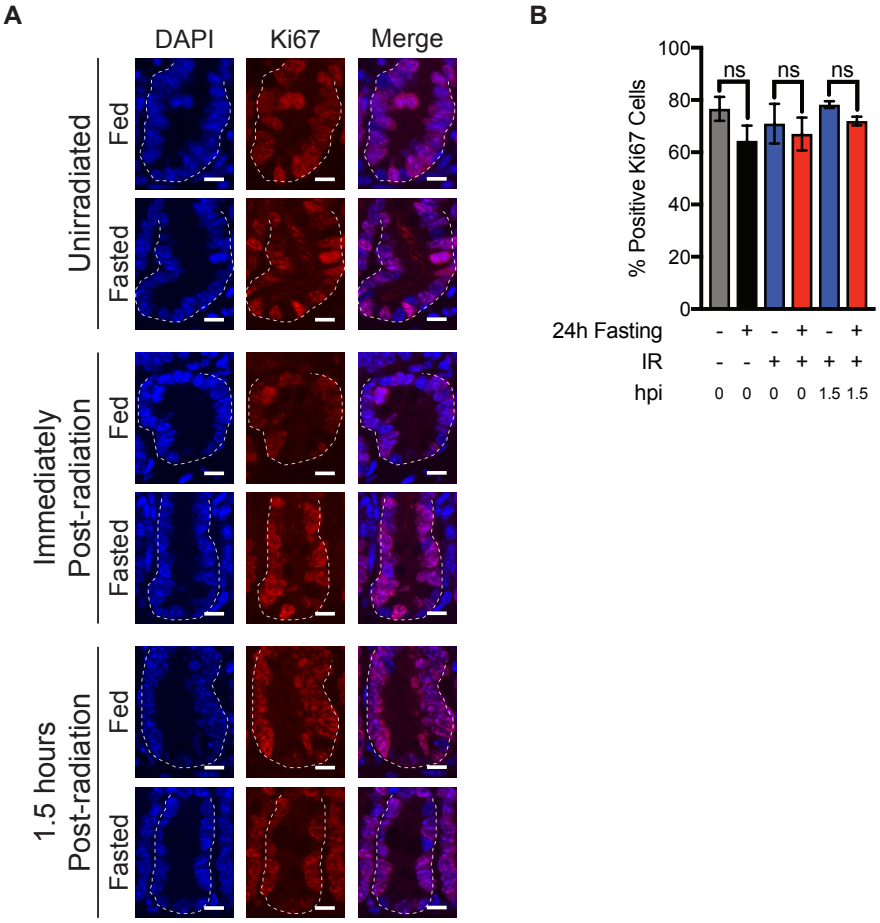

Figure S5: Twenty-four hour fasting did not reduce the number of crypt epithelial cells staining positive for Ki67 relative to fed controls. (A) C57Bl/6J mice were allowed to feed *ad libitum* or were fasted for 24h. Unirradiated SI tissues were harvested at this time. Other cohorts were radiated with TA-XRT (11.5 Gy) and SI tissues harvested either immediately or 1.5 h after radiation (hpi= hours post irradiation). Tissues were analyzed for Ki67 by immunofluorescence staining. Representative crypt images shown. Scale bars, 10 μm. Magnification, 20x. (B) Ki67 positive cells per crypt were quantified (30 per mouse, n=3-4 mice per treatment group) and average per treatment plotted. ns= not significant by student's t-test. Error bars are ± SEM.

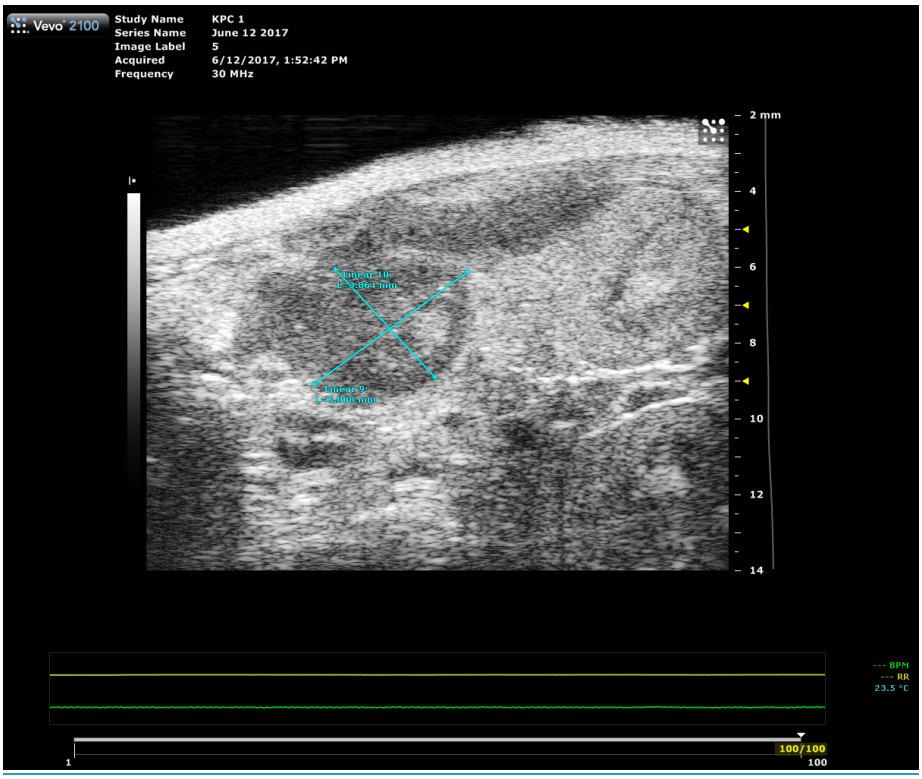

Figure S6: Diagnostic ultrasounds of pancreatic tumor. Representative ultrasound image of orthotopically-implanted pancreatic tumor. Blue lines denote tumor diameter measurements.

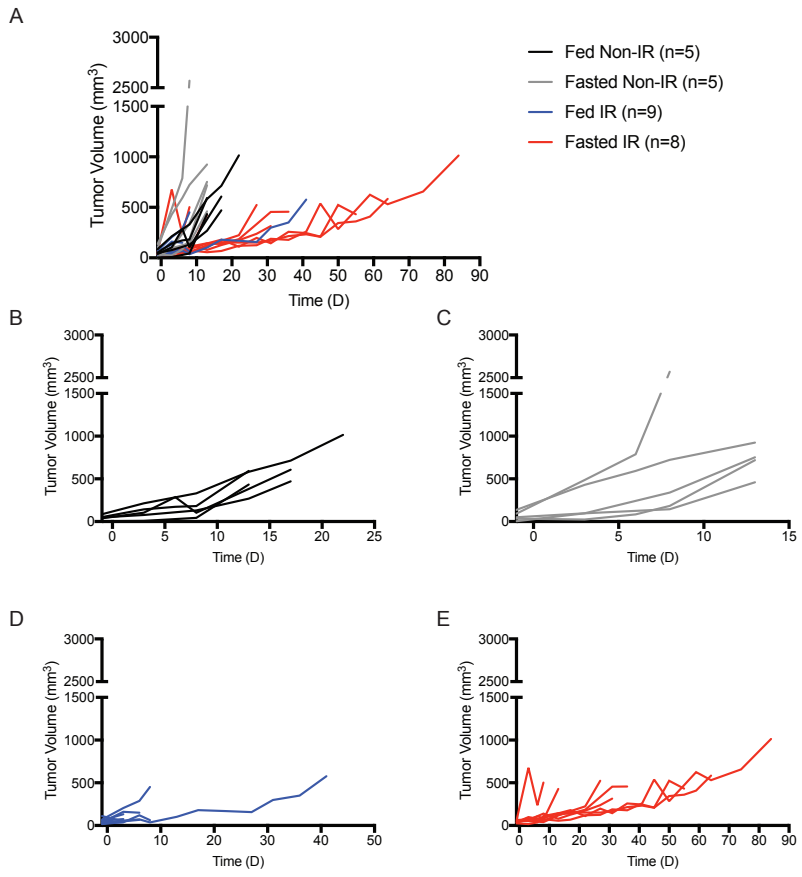

Figure S7: Individual tumor growth curves. KPC cells (2 x 10^5) were orthotopically injected into 12-week-old C57Bl/6J mice. Two weeks later, tumors were measured using ultrasound and mice were randomized into four treatment groups. Mice were allowed to feed or were fasted for 24 h. Total abdominal radiation (TA-XRT; 12 Gy) was administered (day 1). Access to food was restored immediately after treatment. Ultrasound tumor measurements were taken every 4-5 days until death. Tumor growth curves for individual mice are shown.

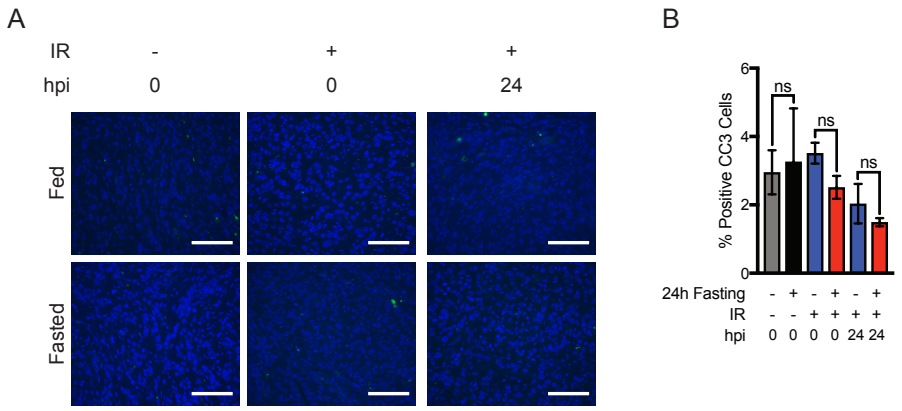

Figure S8: (A)Pancreatic tumor-bearing mice were treated as indicated (hpi = hours post irradiation) and tumors were analyzed for cleaved caspase-3 (CC3) by immunofluorescence staining. Representative images shown. (B) Positive cells per field were quantified (5 fields per mouse, n = 3 mice per treatment) and average per treatment plotted. Scale bars, 100 μm. Magnification, 40x. ns= not significant by Tukey post-test of a two-way ANOVA. Error bars are ± SEM.

Fed/ Fasted	IR/ Unirradiated	Day of Death	Recorded cause of death
Fed	Unirradiated	16	Euthanized due to excessive tumor burden
Fed	Unirradiated	13	Euthanized due to excessive tumor burden
Fed	Unirradiated	13	Euthanized due to excessive tumor burden
Fed	Unirradiated	22	Euthanized due to excessive tumor burden
Fed	Unirradiated	17	Unknown
Fasted	Unirradiated	8	Euthanized due to excessive tumor burden
Fasted	Unirradiated	13	Euthanized due to excessive tumor burden
Fasted	Unirradiated	13	Euthanized due to excessive tumor burden
Fasted	Unirradiated	13	Euthanized due to excessive tumor burden
Fasted	Unirradiated	13	Euthanized due to excessive tumor burden
Fed	IR	7	Euthanized due to radiation toxicity
Fed	IR	8	Euthanized due to radiation toxicity
Fed	IR	7	Euthanized due to radiation toxicity
Fed	IR	9	Euthanized due to radiation toxicity
Fed	IR	41	Euthanized due to excessive tumor burden
Fed	IR	7	Euthanized due to radiation toxicity
Fed	IR	7	Euthanized due to radiation toxicity
Fed	IR	6	Euthanized due to radiation toxicity
Fed	IR	6	Euthanized due to radiation toxicity
Fasted	IR	64	Euthanized due to general decline of health
Fasted	IR	84	Euthanized due to general decline of health
Fasted	IR	36	Euthanized due to excessive tumor burden
Fasted	IR	55	Unknown
Fasted	IR	8	Euthanized due to radiation toxicity
Fasted	IR	31	Euthanized due to excessive tumor burden
Fasted	IR	13	Euthanized due to excessive tumor burden
Fasted	IR	50	Euthanized due to general decline of health

Table S1: Recorded cause of death in tumor-bearing mice.

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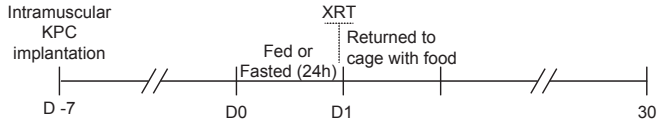

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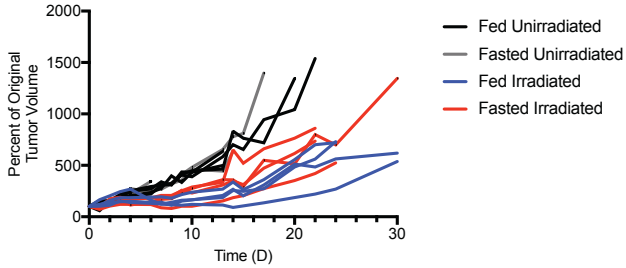

Figure S9: Pre-radiation fasting does not confer protection to KPC tumors. (A) Overall schema and timeline. To test the effect of fasting on tumor response to radiation, KPC pancreatic cancer–derived (K8484) cells syngeneic with C57Bl/6J (1×10^6) were injected into the hind leg of 8-week-old C57Bl/6J mice. Mice were randomized to one of four treatment groups as indicated and treatment began 6 days after implantation. Mice were either fed or fasted for 24 h (day 0) and then not irradiated or subjected to irradiation of the hind leg with a single dose of 16 Gy radiation (day 1). Animals were returned to single-housed cages with food. (B) Tumors were measured with calipers daily for the first 2 weeks and every other day thereafter. Mice were euthanized when tumors reached 15 mm in any dimension. Individual tumor growth curves were plotted.

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