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Science. 2016 November 11; 354(6313): 765–768. doi:10.1126/science.aaf7532.**The DNA Sensing Aim2 Inflammasome Controls Radiation Induced Cell death and Tissue Injury****Bo Hu^{1,*}, Chengcheng Jin^{1,*}, Hua-Bing Li^{1,*}, Jiyu Tong^{1,2}, Xinshou Ouyang³, Naniye Malli Cetinbas⁴, Shu Zhu¹, Till Strowig^{1,α}, Fred C. Lam⁴, Chen Zhao⁵, Jorge Henao-Mejia^{1,β}, Omer Yilmaz⁴, Katherine A. Fitzgerald⁶, Stephanie C. Eisenbarth^{1,7}, Eran Elinav^{1,γ}, and Richard A. Flavell^{1,8,†}**¹Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520²Biomedical Translational Research Institute, Jinan University, Guangzhou 510632, China³Section of Digestive Diseases, Yale University, New Haven, CT 06520⁴Koch Institute for Integrative Cancer Biology, Massachusetts Institute of Technology, 500 Main Street, Cambridge, MA 02139⁵Hematology Oncology Fellowship Program, National Institutes of Health (NIH), Bethesda, MD 20892⁶Division of Infectious Diseases and Immunology, Program in Innate Immunity, University of Massachusetts Medical School, Worcester, MA 01605⁷Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06520⁸Howard Hughes Medical Institute, Chevy Chase, MD 20815-6789, USA**Summary**

Acute exposure to ionizing radiation induces massive cell death and severe damage to tissues containing actively proliferating cells, including bone marrow and the gastrointestinal tract. However, the cellular and molecular mechanisms underlying this pathology remain controversial. Herein, we show that mice deficient in the double-strand DNA (dsDNA) sensor AIM2 are protected from both subtotal body irradiation-induced gastrointestinal syndrome and total body irradiation-induced hematopoietic failure. AIM2 mediates the caspase-1-dependent death of intestinal epithelial cells and bone marrow cells in response to dsDNA breaks caused by ionizing radiation and chemotherapeutic agents. Mechanistically, we found that AIM2 senses radiation-induced DNA damage in the nucleus to mediate inflammasome activation and cell death. Our results suggest that AIM2 may be a new therapeutic target for ionizing radiation exposure.

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Whole-body exposure to 2 Gy or higher radiation can induce hematopoietic syndrome, which might lead to death from infection or hemorrhage within 30 days (1). Higher doses of radiation cause severe damage to the GI tract, resulting in diarrhea, malabsorption and lethality within 10 days(1). However the cellular targets of GI syndrome and the mechanism of radiation-induced cell death remain controversial. Different forms of cell death have been implicated in radiation-induced GI syndrome, including apoptosis (2) or mitotic catastrophe (3) of intestinal epithelial cells (IEC), and apoptosis of endothelial cells in the intestinal vasculature (4). Although GI toxicity is a common complication in cancer patients undergoing radiation or chemotherapy with DNA-damaging agents, there are currently no effective medical treatments to prevent or ameliorate GI syndrome. It is therefore important to gain a better understanding of the underlying mechanisms of cell death and tissue injury in response to double-strand DNA damage.

Several innate pattern recognition receptors (PRR), including NLRP1, NLRP3, NLRC4, NLRP6 and Absent in melanoma 2 (AIM2) can drive the assembly of multi-protein complexes named inflammasomes to govern caspase-1 activation (5). Inflammasomes are critical regulators of intestinal tissue homeostasis through modulating intestinal microbial ecology, inflammation and tissue repair(6). However, the role of inflammasomes in radiation-induced intestinal damage is unknown.

To investigate this, we used an established mouse model of radiation-induced small intestine syndrome in which mice are exposed to a lethal dose of subtotal body irradiation (SBI) with their limbs and head shielded to avoid hematopoietic syndrome(3). In this model, most wildtype (WT) mice died from severe intestinal damage within 10 days of radiation exposure. Notably, mice lacking caspase-1 were resistant to SBI induced lethality (Figure 1A), suggesting that inflammasome pathways play a critical role in controlling intestinal radio-sensitivity. This commonly used caspase-1 knockout strain (7) was recently also found to be deficient in caspase-11 (herein referred to as *Casp1(11)^{-/-}*). Caspase-11 mediates non-canonical inflammasome activation in response to various gram-negative bacterial infections, whereas caspase-1 is critical for the canonical inflammasome pathway downstream of several intracellular PRRs including NLRP3 and AIM2(8). We therefore repeated the experiment and found that mice lacking only caspase-1 or the adaptor protein ASC were also protected from SBI-induced lethality, indicating that a caspase-1 dependent, ASC-dependent canonical inflammasome pathway regulates intestinal radiosensitivity (Fig. 1, B and C).

AIM2 is an innate immune sensor that mediates assembly and activation of inflammasome in response to double-stranded DNA(9, 10). We found that AIM2-deficient mice were protected from SBI induced lethality and intestinal damage (Fig. 1D). In accordance with previous observations (11–13), WT mice exhibited severe loss of crypts 3.5 days after SBI, whereas crypts of Aim2-deficient mice largely maintained their integrity (Fig. 1E). In contrast, no difference in survival from the GI syndrome was observed for mice lacking other inflammasome sensors including NLRP3 or NLRC4, indicating the specific role of the AIM2 inflammasome in controlling SBI-induced intestinal damage (Fig. S1, A and B).

Upon binding to dsDNA via its HIN200 domain, AIM2 recruits the adaptor protein ASC through its pyrin domain and assembles into an inflammasome to activate caspase-1, and thus maturation and secretion of pro-inflammatory cytokines interleukin (IL)-1 β and IL-18. In addition, activation of the AIM2 inflammasome in macrophages can induce caspase-1 dependent cell death known as pyroptosis(5, 9). To elucidate the pathway downstream of the AIM2 inflammasome which might mediate intestinal radiosensitivity, we studied *Ill1b*^{-/-}, *Ill1r1*^{-/-} and *Ill18*^{-/-} mice and found them to be equally susceptible to SBI-induced GI syndrome as WT controls (Fig. S1, C–E). Therefore, AIM2 does not act through cytokine production to regulate intestinal damage in response to radiation. As IEC death plays a critical role in SBI induced GI syndrome(3), we next examined the contribution of caspase-1-mediated cell death in this model. We selectively deleted caspase-1 in IECs by crossing mice carrying floxed caspase-1 alleles with mice expressing Cre under the control of Villin promoter (Villin-Cre). IEC-specific caspase-1 deletion protected mice from SBI induced GI syndrome (Fig. 2A), suggesting that caspase-1-mediated pyroptosis of IECs is critical in controlling SBI sensitivity downstream of the AIM2 inflammasome.. Consistently, fewer TUNEL+ cells were observed in the crypts of the jejunum of *Casp1(11)*^{-/-} and *Aim2*^{-/-} mice 24 hours after SBI (Fig. 2, B and C). In addition, the levels of cleaved caspase-3 and caspase-7 were not decreased in intestines of *Aim2*^{-/-} mice compared to WT (Fig. S2, A and B), indicating the reduction in TUNEL+ cells in *Aim2*^{-/-} mice was caused by abrogation of caspase-1 mediated pyroptosis (Fig. S2 C), not caspase-3/7 dependent apoptosis. AIM2 was suggested to inhibit AKT activation to suppress colorectal tumorigenesis(14, 15). However, we did not observe any elevation of AKT activity in *Aim2*^{-/-} mice after radiation, implying that AKT might not be involved in AIM2 inflammasome signaling in response to radiation (Fig. S2 D). Mechanistically, loss of clonogenic (stem/progenitor) cells in the crypts has been suggested to be responsible for radiation-induced intestinal damage(16). We performed the microcolony formation assay *in vivo* and found that AIM2-deficiency significantly enhanced crypt survival and regeneration in response to a range of radiation doses as assessed by histological analysis of haematoxylin-and-eosin (H&E) staining (Fig. S3, A and B) as well as BrdU incorporation (Fig. S3C). Furthermore, intestinal organoids derived from AIM2-deficient crypts were more resistant to radiation (Fig. S3, D and E). Taken together, our data suggest that the AIM2 inflammasome mediated pyroptosis of clonogenic cells in the intestinal crypts plays a critical role in radiation-induced GI syndrome.

Importantly, mice deficient in inflammasome components including ASC, caspase-1(11) (17), as well as AIM2(15, 18), can develop altered intestinal microbiota composition. Therefore, we cohoused WT controls used in these studies with the individual knockout strains at least two weeks before radiation in all of our experiments to equilibrate their gut microbiota (Fig. 1, Fig. 2 and Fig. S1), and thereby rule out the contribution of dysbiosis in regulating radiation-induced intestinal injury. Taken together, our data demonstrated that the AIM2 inflammasome acts intrinsically in IECs to control intestinal radiosensitivity through caspase-1 mediated pyroptosis.

In addition to gastrointestinal toxicity (GI syndrome), acute irradiation can also induce bone marrow toxicity (hematopoietic syndrome) depending on the dose and route of radiation exposure(1, 19, 20). To further investigate whether the AIM2 inflammasome also contributes

to radiosensitivity in the bone marrow compartment, we subjected mice to a lower dose (7 Gy) of total body irradiation (TBI). In this model, about 50% of WT mice died from hematopoietic syndrome starting around 2 weeks post irradiation; remarkably, *Aim2*^{-/-} and *Casp1*^{-/-} mice were resistant to TBI and survived beyond a month (Fig. 3, A and B; Fig. S4A).

To investigate the cellular mechanism of AIM2-mediated radio-sensitivity, we used primary bone marrow derived macrophages (BMDMs). In WT cells, ionizing radiation activated the AIM2 inflammasome, as directly evidenced by the cleavage of caspase-1 to yield the p10 subunit and secretion of mature IL-1 β , which were absent from *Casp1*^{-/-} and *Aim2*^{-/-} BMDMs (Fig. 3, C and D; Fig. S4B). Consistent with our *in vivo* findings, we observed a dose-dependent increase of cell death in response to ionizing radiation in WT BMDMs, which was abrogated in AIM2-deficient and caspase-1 deficient BMDMs (Fig. 3, E–G). As radiation-induced uric acid release from dead/damaged cells was previously suggested to activate caspase-1 in spleen cells(21), we harvested conditioned medium from irradiated bone marrow cells and found it did not affect survival of either un-irradiated WT or *Aim2*^{-/-} bone marrow cells (Fig. S4C). Our data suggest that the AIM2 inflammasome acts in a cell-autonomous manner in response to radiation, independently of soluble factors released from dead/injured cells. Interestingly, WT and *Aim2*^{-/-} BMDMs were equally sensitive to UV radiation which causes single-strand DNA breaks (Fig. 3H); however, *Aim2*^{-/-} BMDMs showed significantly higher resistance to the commonly used chemotherapeutic agents doxorubicin and etoposide, which kill malignant cells by introducing DNA double-strand breaks (DSBs) (Fig. 3, I and J). In line with the *in vitro* data, *Aim2*^{-/-} mice were also less sensitive to intestinal damage and lethality of high-dose doxorubicin treatment (Fig. S4, D and E). Altogether, these findings suggested that the AIM2 inflammasome is specifically involved in mediating cell death in response to DSBs such as those caused by ionizing radiation and chemotherapeutic agents.

To further explore the molecular mechanism by which the AIM2 inflammasome responds to ionizing radiation, we reconstituted the AIM2 inflammasome in human embryonic kidney (HEK) 293T cells by overexpressing Flag-tagged AIM2, caspase-1 and ASC. Radiation induced the formation of AIM2-positive specks, a classical marker for inflammasome assembly, and resulted in cell death (Fig. S5, A and B). In addition, we generated a Flag-tagged AIM2 mouse in which a Flag tag was knocked into the C-terminus of AIM2 protein using the CRISPR/cas9 based genome-editing system (herein referred to as AIM2-Flag mice, Fig. S5C), because highly specific antibodies against endogenous murine AIM2 are not available(14). As we found an important role of AIM2 in regulating cell death and tissue injury in immune cells and the small intestine, we first verified the steady-state expression of endogenous AIM2 protein in the spleen and small intestine using the AIM2-Flag mice (Fig. S5D). Next, we analyzed irradiated primary macrophages from AIM2-Flag mice by immunofluorescence (IF) microscopy. Endogenous AIM2 showed very diffuse and weak staining before radiation exposure, but formed puncta in the nucleus upon radiation exposure (Fig. 4, A and B; Fig. S5E). Moreover, considerable colocalization of AIM2-containing specks and gamma-H2AX-positive foci was observed in the nucleus, suggesting that AIM2 is recruited to sites of dsDNA breaks (Fig. 4C; Fig. S5F). In support of our IF experimental observations, a strong interaction between Flag-tagged AIM2 and gamma-H2AX was

detected by co-immunoprecipitation in irradiated cells (Fig. 4D). To further investigate the molecular mechanism of AIM2 inflammasome assembly, we also generated an ASC-HA knockin mouse to tag endogenous ASC protein using a similar strategy (Fig. S5G and H), and we crossed it to the AIM2-Flag mice to study the interaction between AIM2 and ASC. Importantly, using AIM2-Flag/ASC-HA double knockin mice, we found that radiation induced AIM2 and ASC co-localization and speck formation in the nuclei following radiation exposure, and the speckles containing both AIM2 and ASC later accumulated in the peri-nuclear region (Fig. 4E). Together with the data showing robust caspase-1 processing in irradiated macrophages (Fig. 3C), these results provide strong evidence for the assembly and activation of an inflammasome in response to radiation. AIM2 was previously known as a cytoplasmic DNA sensor(9, 10). Although its nuclear localization has been implicated in certain cell lines, the biological importance of nuclear AIM2 was not understood, and the sub-cellular distribution of endogenous AIM2 is unclear (22–24). Our results suggest that the recruitment of AIM2 to chromatin sites of radiation-induced DNA damage may be involved in mediating inflammasome activation and cell death.

Our present study demonstrates an unexpected role for AIM2 in sensing ionizing radiation-induced DNA damage in the nucleus. AIM2 acts thereby through the inflammasome pathway to trigger caspase-1 mediated cell death in intestinal epithelial cells and bone marrow cells. We show here that deficiency in the AIM2 inflammasome protects mice from radiation-induced small intestine syndrome as well as hematopoietic failure. While the relative contribution of pyroptosis and other forms of cell death such as apoptosis to radiation-induced tissue damage merits further investigation, our findings may have important implications for development of therapy against radiation induced GI/hematopoietic toxicity. Drugs that block the activity of the AIM2 inflammasome may be effective to treat patients exposed to ionizing radiation such as in radiation exposure via nuclear reactors or cancer patients suffering from hematopoietic or GI toxicity as a consequence of radiotherapy or chemotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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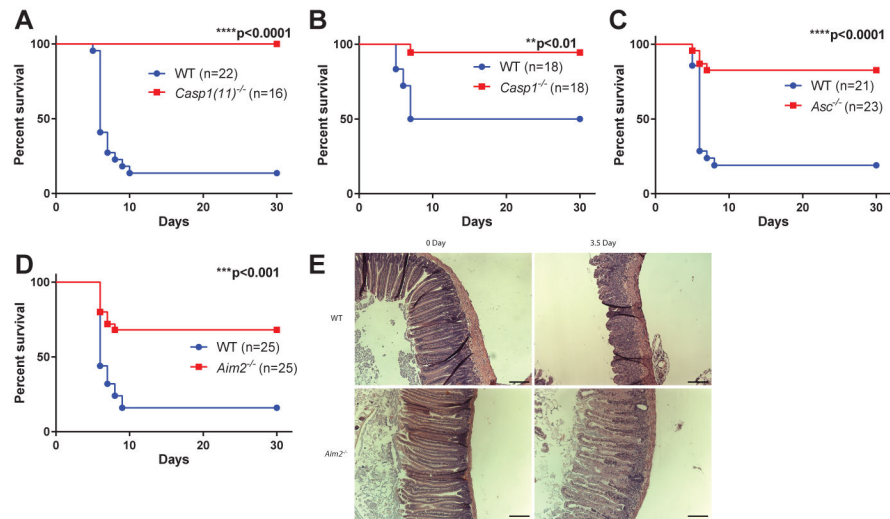


Figure 1. AIM2 inflammasome deficiency protects mice from SBI induced small intestine syndrome

(A–D) WT mice were cohoused with *Casp1(11)^{-/-}*, *Casp1^{-/-}*, *Asc^{-/-}* or *Aim2^{-/-}* mice for two weeks and then exposed to 14.2 Gy of subtotal-body irradiation (SBI). Kaplan-Meier survival analysis of *Casp1(11)^{-/-}* mice (A), *Casp1^{-/-}* mice (B), *Asc^{-/-}* mice (C), *Aim2^{-/-}* (D) mice and their cohoused WT mice was performed. Each figure represents the pooled data from two to three independent experiments. The total number of mice in each group and the p value by log-rank comparison are indicated on the plots. (E) Representative pictures of H&E staining of the jejunum from *Aim2^{-/-}* mice and their cohoused WT mice at day 0 and day 3.5 after 14.2 Gy of SBI. Scale bar=100 μ m.

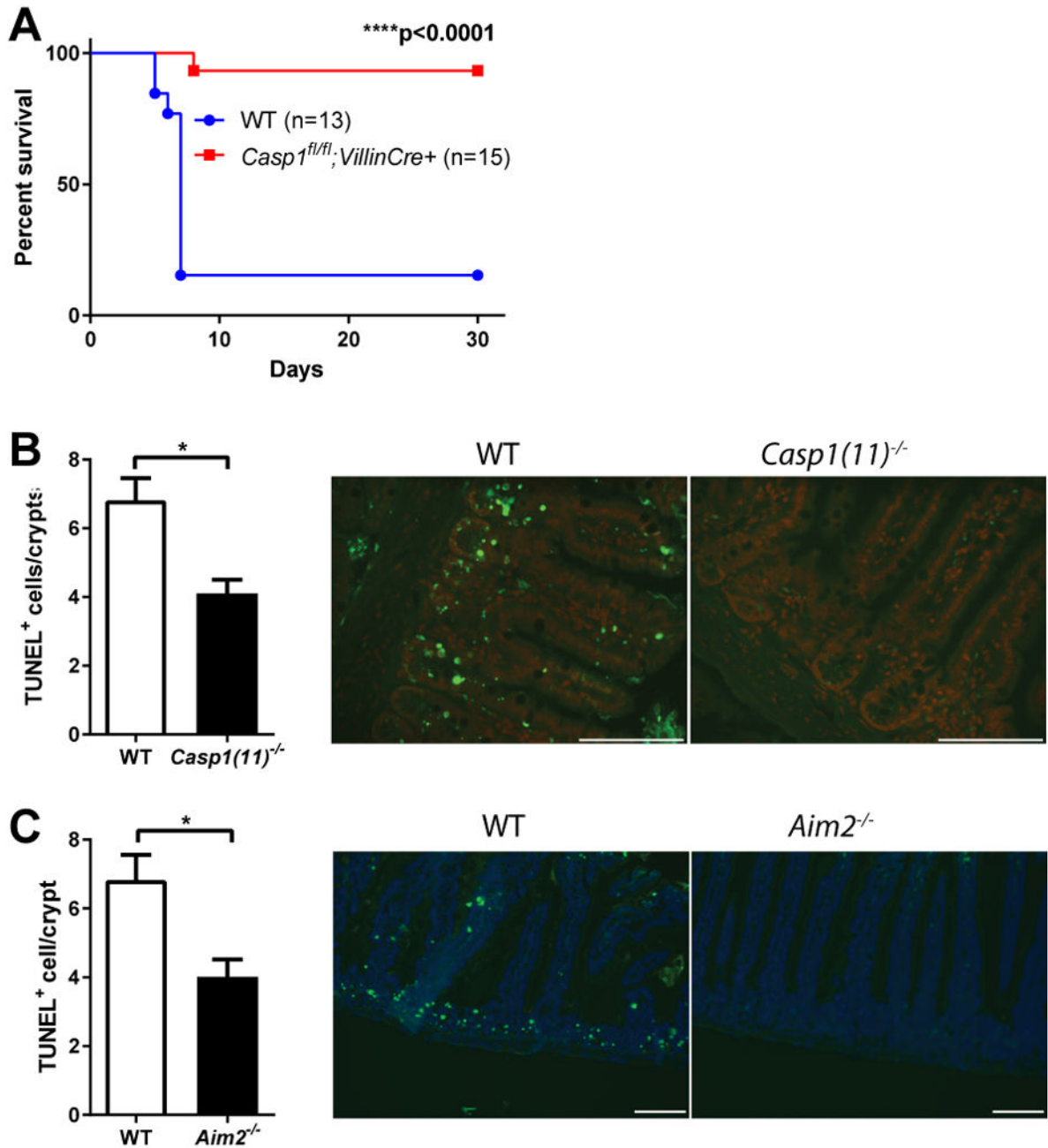


Figure 2. AIM2 inflammasome regulates intestinal radio-sensitivity through caspase-1 mediated epithelial cell death

(A) WT mice were cohoused with *Casp1^{fl/fl}; VillinCre+* mice for two weeks and then exposed to 14.2 Gy of SBI. Kaplan-Meier survival analysis of *Casp1^{fl/fl}; VillinCre+* mice and their cohoused WT mice was performed on pooled data from two independent experiments. By log-rank comparison, ****P<0.0001. (B, C) Small intestines were harvested from *Casp1(11)^{-/-}*, *Aim2^{-/-}* and their cohoused WT mice 24 hours post 14.2 Gy SBI, and cell death was analyzed by TUNEL staining. Epithelial cells stained positively for TUNEL showed green fluorescence. Nuclei were stained with PI (red) or DAPI (blue).

Scale bar=100 μ m. Numbers of TUNEL-positive cells per crypts were quantified (n=3–5 mouse/group, at least 20 crypts of each mouse were counted) and representative pictures were shown. Results are expressed as mean \pm SEM, *P<0.05 by Student's t test.

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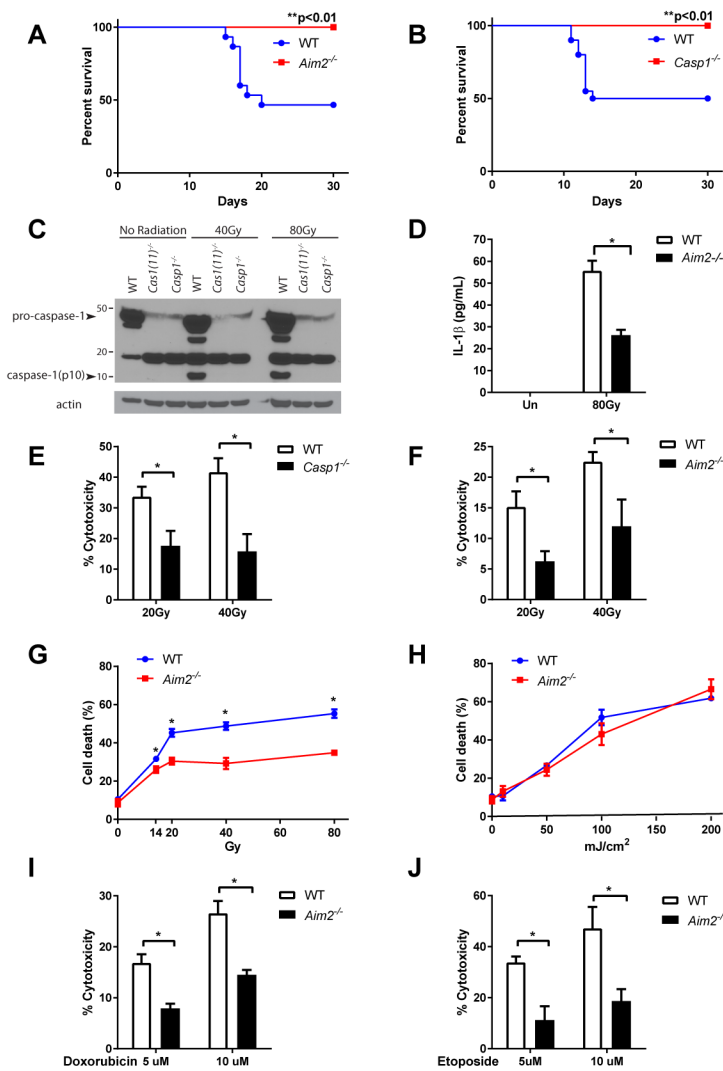


Figure 3. AIM2 inflammasome mediates radio-sensitivity of hematopoietic cells in response to dsDNA damage

(A, B) Kaplan-Meier survival analysis was performed on *Aim2*^{-/-} mice (A), *Casp1*^{-/-} mice (B) and their WT controls exposed to 7 Gy of total-body irradiation (TBI). Each figure represents the pooled data from two independent experiments. N=10–20 mice/group. (C) Caspase-1 activation in WT and *Casp1*^{-/-} BMDMs 4 hours after exposure to indicated doses of radiation was assayed by immunoblotting of the cleaved form of caspase-1 (p10 subunit). (D) Supernatant was collected from un-irradiated or 80 Gy-irradiated LPS-primed WT and *Aim2*^{-/-} BMDMs, and IL-1 β concentration was measured by ELISA. (E, F, I, J) WT, *Casp1*^{-/-} or *Aim2*^{-/-} BMDMs were treated with different doses of ionizing radiation or drugs inducing dsDNA breaks, and cell death was measured by the amount of lactate dehydrogenase (LDH) released into the supernatant. (G, H) WT and *Aim2*^{-/-} BMDMs were treated with different doses of ionizing radiation or UV radiation, and cell death was quantified by trypan blue staining. Determinations were performed in triplicate and expressed as the mean \pm SEM. * P<0.05 by Student's t test.

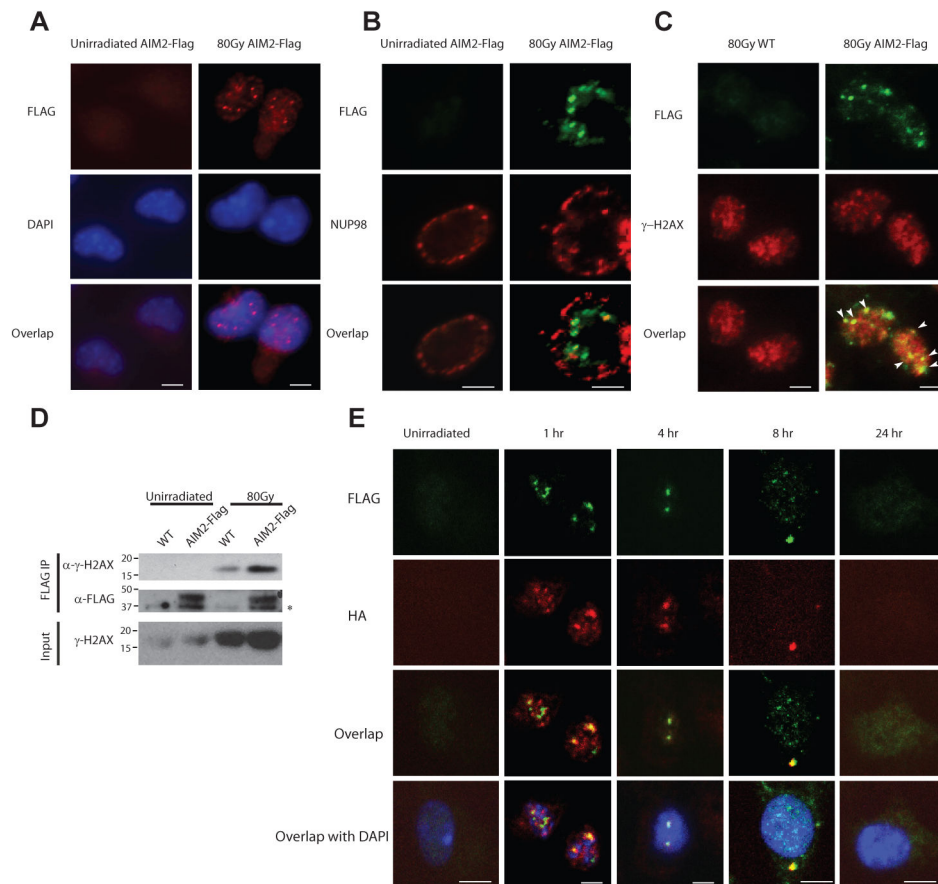


Figure 4. Ionizing radiation induces the formation of AIM2 specks in the nucleus

Primary macrophages from AIM2-Flag mice (A, B, C, D) or AIM2-Flag/ASC-HA double knock-in mice (E) were left un-irradiated or exposed to 80 Gy ionizing radiation. For immunofluorescence microscopy, cells were fixed at 4 hours (A, B, C) or at indicated time points (E) after radiation. AIM2 was stained with anti-Flag antibody (red in A; green in B, C, E), and co-stained with nuclear envelope protein NUP98 (red, B) or gamma-H2AX (red, C) or ASC (using anti-HA antibody, red, E). Cell nuclei were visualized by DAPI (blue) in A and E. Co-localization of AIM2-Flag specks and gamma-H2AX foci was indicated by white arrowheads in C. Scale bar=5 μ m. Figures represent results from three independent experiments and at least 100 cells were analyzed for each condition. (D) Co-immunoprecipitation (co-IP) of gamma-H2AX with AIM2-Flag in irradiated macrophages using anti-Flag M2 agarose beads. The immunoprecipitates (Flag IP) or the total lysates were analyzed by immunoblotting with antibodies against gamma-H2AX(Ser139) or the Flag tag. Samples from untagged WT mice were used as controls to determine the specificity of immunoblots. IB, immunoblotting. Non-specific band is indicated with an asterisk. Data represent two independent experiments.