

Supplementary Data

NLRP1 inflammasome promotes senescence and the senescence-associated secretory phenotype

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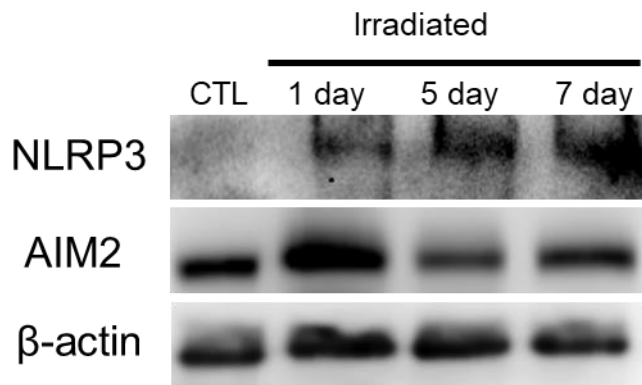
Running Title: NLRP1-inflammasome modulates senescence

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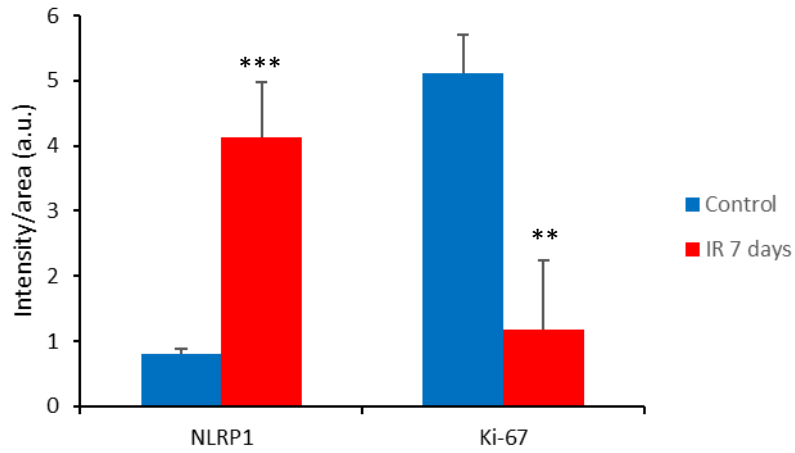
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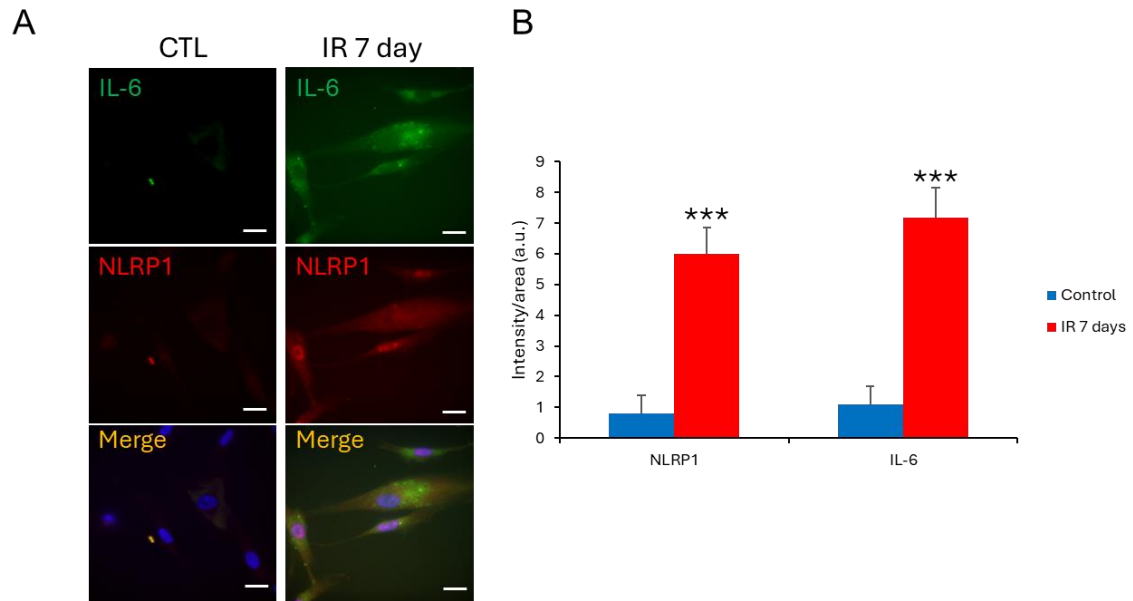
Keywords: NLRP1-inflammasome, senescence, SASP, aging



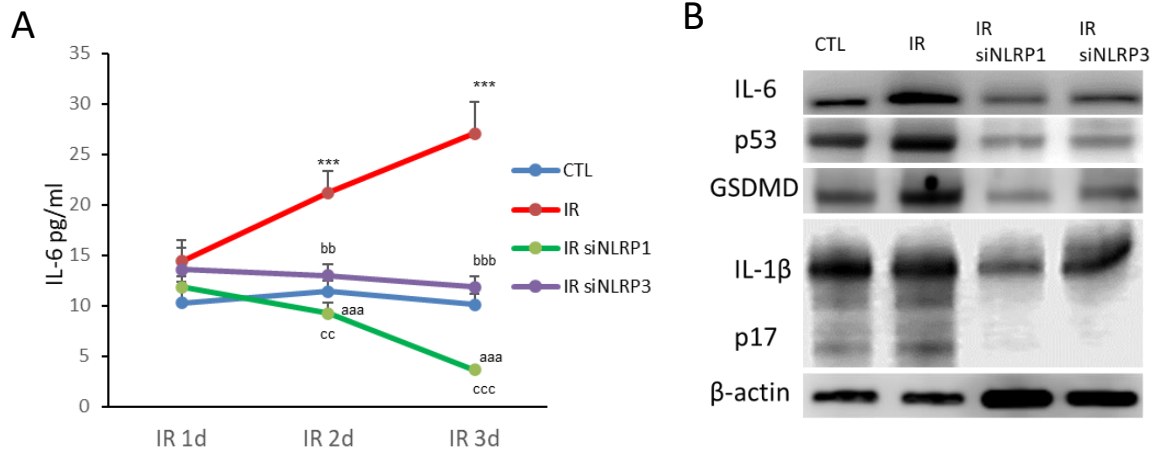
Supplementary Figure 1. NLRP3 expression is induced in a sustained manner during senescence induced by irradiation. At day 1, 5 and 7 NLRP3 and AIM2 expression was analysed by immunoblotting after irradiation. n = 4 independent experiments.



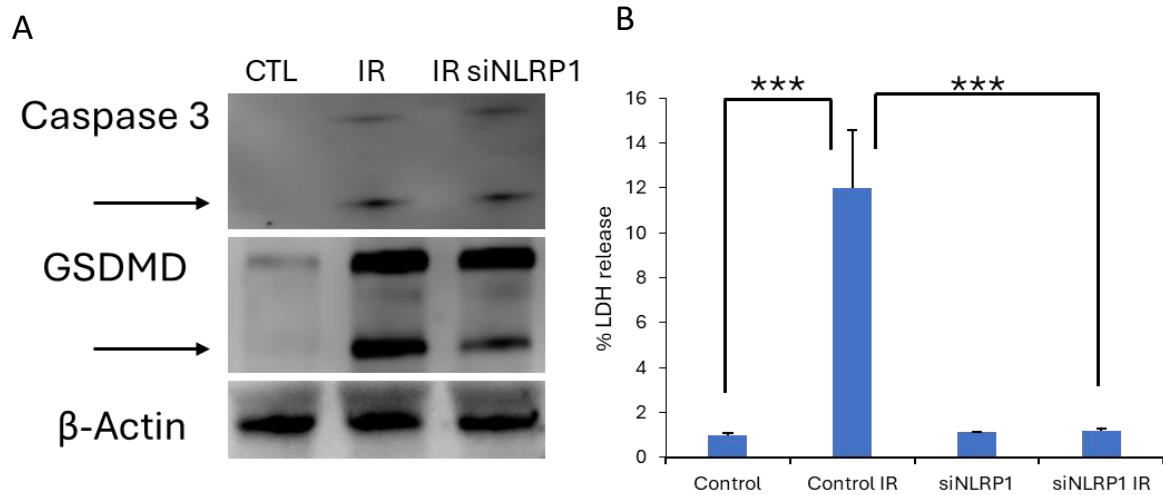
Supplementary Figure 2. Quantification of NLRP1 and Ki-67 fluorescence intensity levels from control and irradiated cells after 7 days. All data are presented as means \pm SEM, n = 4 independent experiments; ***P < 0.001; **P < 0.01 irradiated vs control.



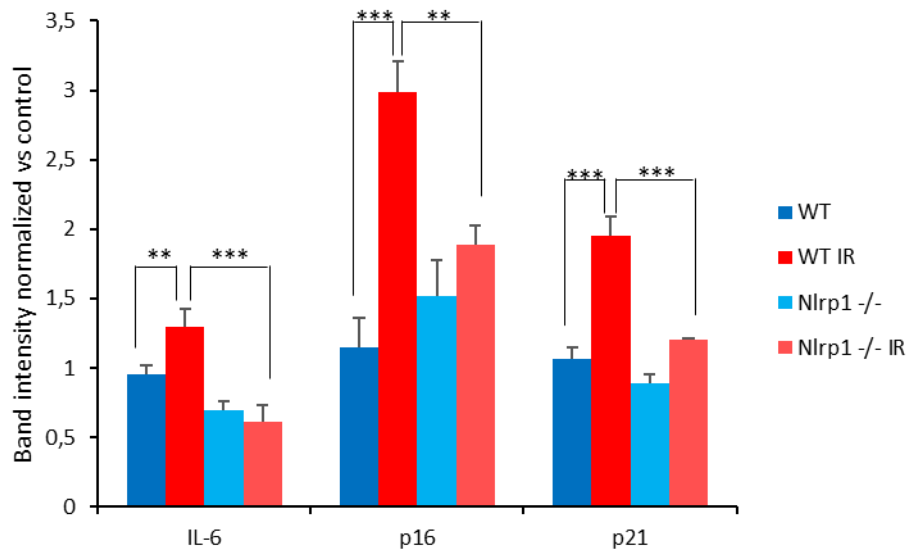
Supplementary Figure 3. Colocalization of NLRP1 and IL-6 SASP marker. A. Representative images of IL-6 and NLRP1 immunofluorescence. Scale bar = 50 μm . B. Intensity quantification of NLRP1 and IL-6 fluorescence levels from control and irradiated cells after 7 days. All data are presented as means \pm SEM, $n = 4$ independent experiments; *** $P < 0.001$; irradiated vs control.



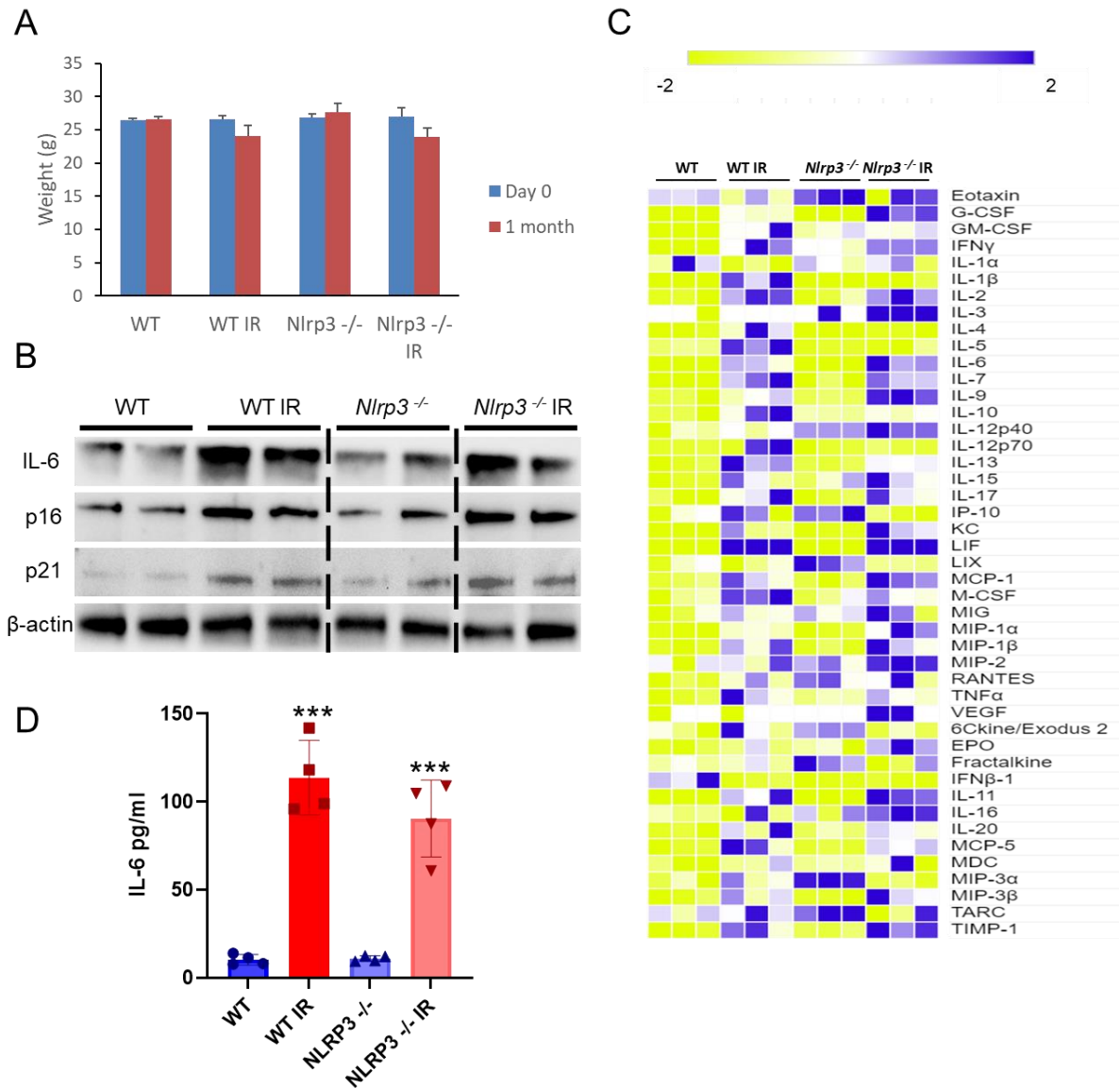
Supplementary Figure 4. NLRP1 induces a more significant senescence effect than NLRP3. (A) IL-6 release from cell IR, IR+siNLRP1 or IR+siNLRP3 cells and (B) protein expression. All data are presented as means \pm SEM, $n = 4$ independent experiments; *** $P < 0.001$ irradiated vs control. ^{aaa} $P < 0.001$, IR+siRNA NLRP1 vs IR cells; ^{bb} $P < 0.005$, ^{bbb} $P < 0.001$, IR+siRNA NLRP3 vs IR cells; ^{cc} $P < 0.005$, ^{ccc} $P < 0.001$, IR+siRNA NLRP1 vs IR+siRNA NLRP3 cells.



Supplementary Figure 5. Irradiation induces pyroptosis. A. Analysis of caspase-3 and Gasdermin D (GSDMD) after irradiation. B. Lactate dehydrogenase (LDH) release after irradiation. All data are presented as means \pm SEM, n = 3 independent experiments; ***P < 0.001.



Supplementary Figure 6. Densitometric analysis of Figure 3D. Data are presented as means \pm SEM, n = 3 independent experiments; ***P < 0.001; **P < 0.01.



Supplementary Figure 7. NLRP3 deletion does not prevent senescence and SASP. (A) Effect of IR on the bodyweight of WT and NLRP3 knockout (KO) mice. (B) Protein expression in livers from IR and non IR-WT and NLRP3 KO mice of senescence protein markers (IL-6, p16 and p21). (C) Heat map depicting expression of 44 mouse cytokines in serum at 5 weeks following from IR of WT and NLRP3 KO mice. (D) IL-6 release from healthy fibroblasts was assessed after 24 and 48 hr of incubation with media containing serum from IR and non IR WT and NLRP3 KO mice. All data are presented as means \pm SEM, n = 6-8 mice per group; ***P < 0.001 irradiated vs control.