LETTER TO THE EDITOR

Latexin deletion protects against radiation-induced hematopoietic damages via selective activation of Bcl-2 prosurvival pathway

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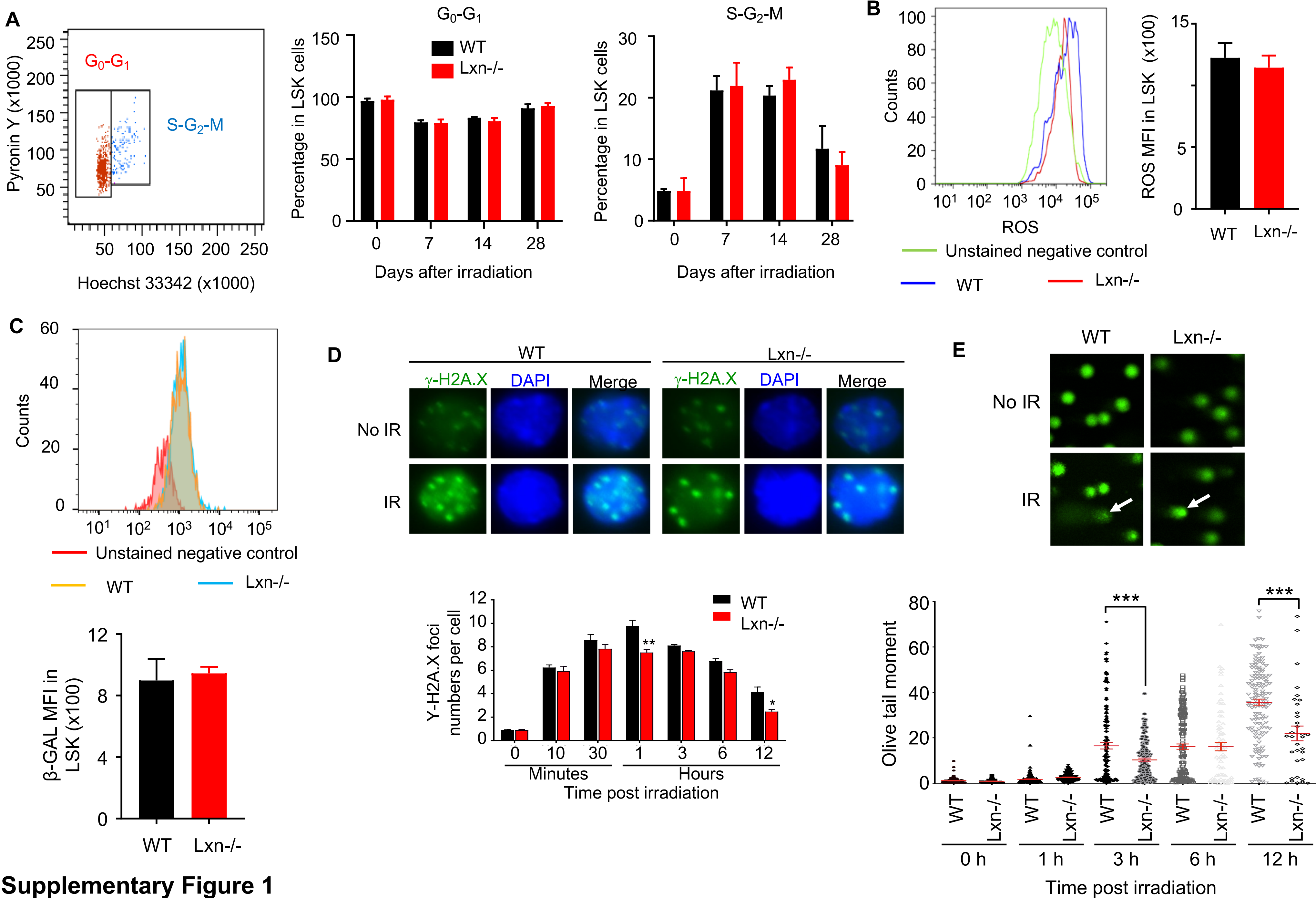
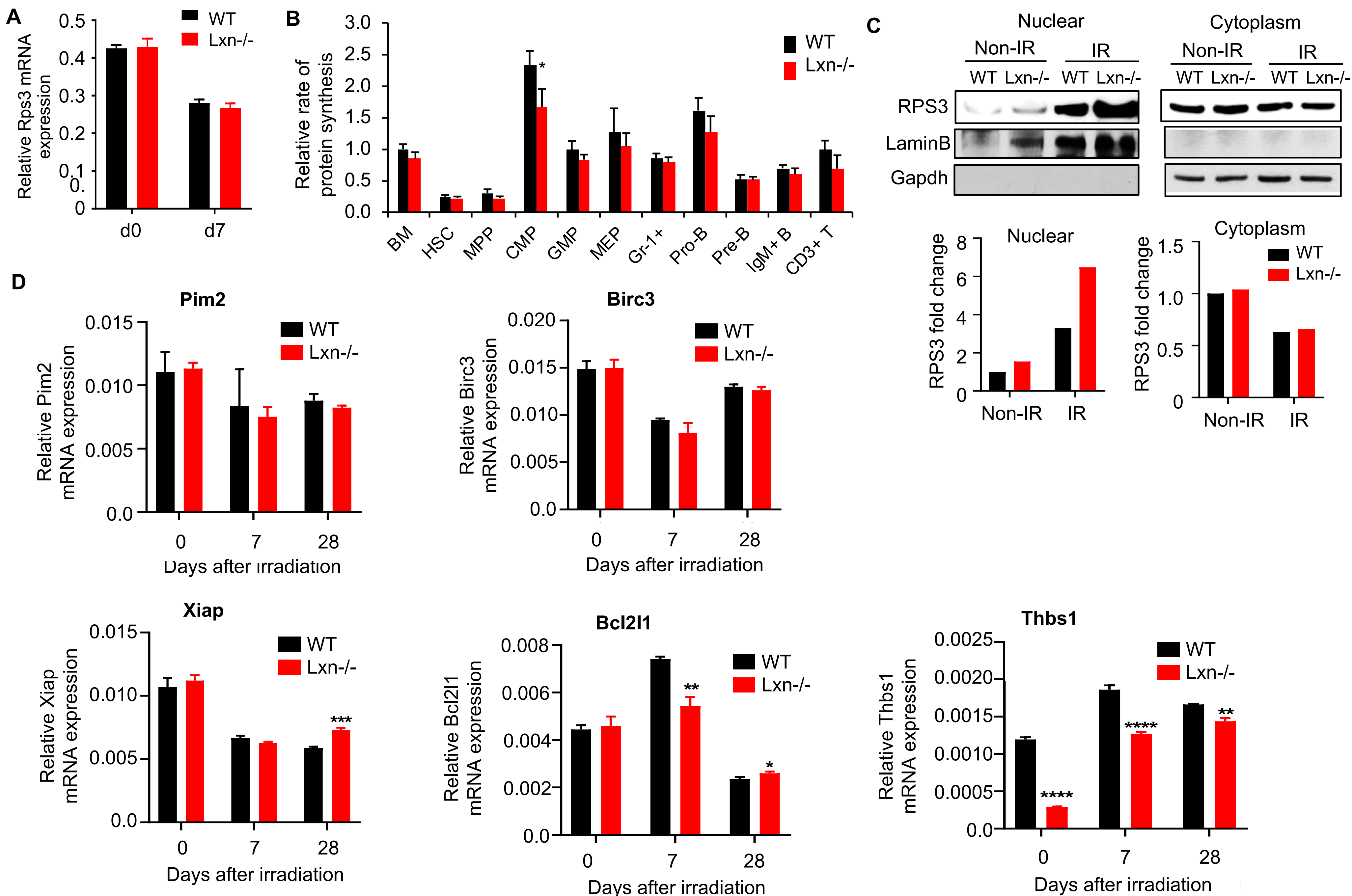


Supplementary Figure 1



Supplementary Figure 2

Supplementary Figure 1. Lxn-/- HSPCs don't change in the cell cycle, ROS and senescence with maintenance of their genomic integrity upon radiation.

A. Flow cytometry cell cycle analysis and quantification of LSK cells showed no difference for the percentage of G_0-G_1 and S-G_2-M phase between WT and Lxn-/- groups at day 0, 7, 28 after 6.5Gy TBI. **B.** Flow cytometry analysis of ROS level in LSK cells showed no difference between WT and Lxn-/- groups at day 7 after 6.5Gy TBI. ROS was measured using total ROS assay kit (ThermoFisher) by following manufactory protocol. **C.** Flow cytometry analysis and quantification of β-Gal expression in LSK cells showed no difference between WT and Lxn-/- groups at day 7 after 6.5Gy TBI. Senescence was measured suing CellEvent™ senescence green flow cytometry assay kit (ThermoFisher) by following manufactory protocol. N=5 mice/group. **D**. The number of γ-H2A.X foci was significantly less in Lxn-/- LSK cells than WT LSK cells after in vitro 0.5Gy IR. The representative immunostaining of γ-H2A.X foci in a single LSK cell (top panel) and quantification of γ-H2A.X foci number per cell (bottom panel) are shown. **E.** The comet tail length was significantly less in Lxn-/- LSK cells than in WT LSK cells after in vitro 0.5Gy IR. The representative immunostaining of the comet tail in a single LSK cell (top panel) and quantification of tail length per cell (bottom panel) are shown. LSK cells were sorted from a pool of at least 5 mice, and irradiated in vitro with 0.5Gy dose. N= 50-70 cells per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure 2. Rps3 in Lxn-/- cells is specifically involved in the NF-kB prosurvival signaling pathway.

A. No difference for Rps3 mRNA expression in LSK cells between WT and Lxn-/- mice before or at day 7 after 6.5Gy TBI. LSK cells were sorted and pooled from 5 mice per group. **B.** Different subsets of BM HSCs and HPCs showed no difference in the protein synthesis rate measured by OP-Puro incorporation in vivo assay. The data are normalized to WT unfractionated BM cells. N= 4 mice per group. **C.** Lxn deletion promotes RPS3 nuclear translocation after IR (6.5Gy). Representative Western blot of RPS3 (top panel) in the nuclear and cytoplasm extracts of Lin^- cells from WT and $Lxn^{-/-}$ mice and the band quantification (bottom panel) are shown. Lamin B is used as the nuclear protein internal control and Gapdh as the cytoplasm protein internal control. **D.** Dynamic expression changes of survival-related genes in LSK cells of WT and Lxn-/- mice subjected to 6.5Gy TBI. The genes tested are Pim2(Pim-2 Proto-Oncogene, Serine/Threonine Kinase), Birc3(baculoviral IAP repeat containing 3), Xiap(X-linked inhibitor of apoptosis), Bcl2L1(BCL2 Like 1) and Thbs1(Thrombospondin 1). N=5 mice in each group. Two independent

real-time PCR was performed and three replicates in each experiment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p <0.0001$.