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Supporting Information for

Sublethal whole-body irradiation induces permanent loss and dysfunction in pathogen-specific circulating memory CD8 T cell populations.

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This PDF file includes:

Figures S1 to S5

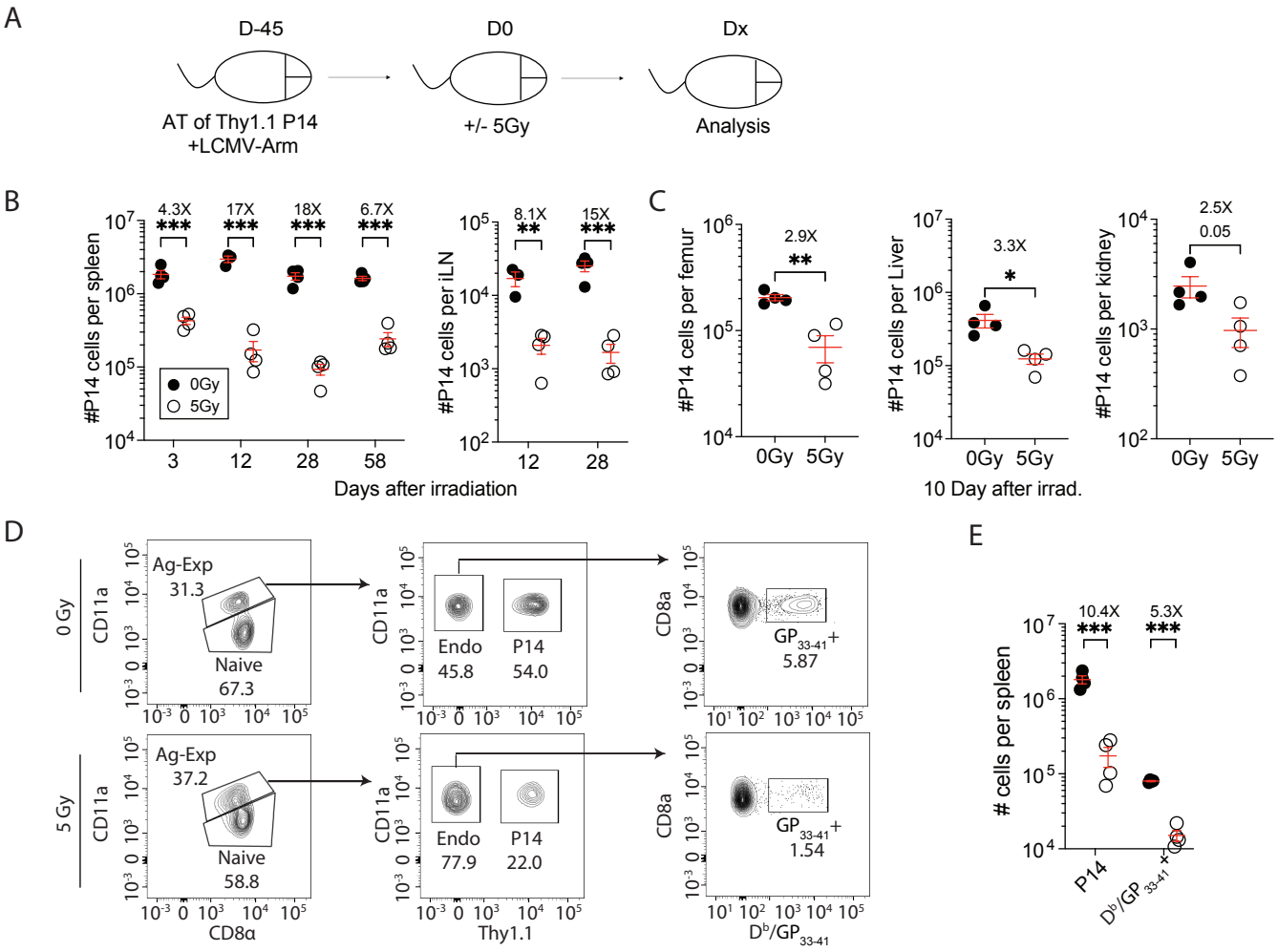

Fig. S1. - Long-lasting decline in number of memory CD8+ T cells following WBI in lymphoid and non-lymphoid organs. A) Experimental Design: 10^4 naive Thy1.1$^+$ P14 CD8+ T cells were adoptively transferred into Thy1.2$^+$ naïve hosts, followed by LCMV-Arm-strong infection to generate memory P14 cells. 45 days later, the memory P14 chimeric mice were either exposed to mock or 5Gy WBI. Analysis was performed on the indicated timepoints after irradiation. **B)** Number of memory P14 cells in spleen, inguinal lymph node at indicated timepoints after WBI. **C)** Number of memory P14 cells in bone marrow, liver, and kidney at D10. **D)** Representative gating for endogenous naïve, antigen-experienced, memory P14, and endogenous GP_{33-41}-specific memory CD8+ T cells in the spleen of mock (top panels) or 5Gy (bottom panels) mice at D28. **E)** Number of memory P14 cells and GP_{33-41}-specific memory CD8+ T cells in the spleen of mock or 5Gy mice at D28. *=p < 0.05, **=p < 0.01, ***=p < 0.001. Error bars represent standard error of the mean.

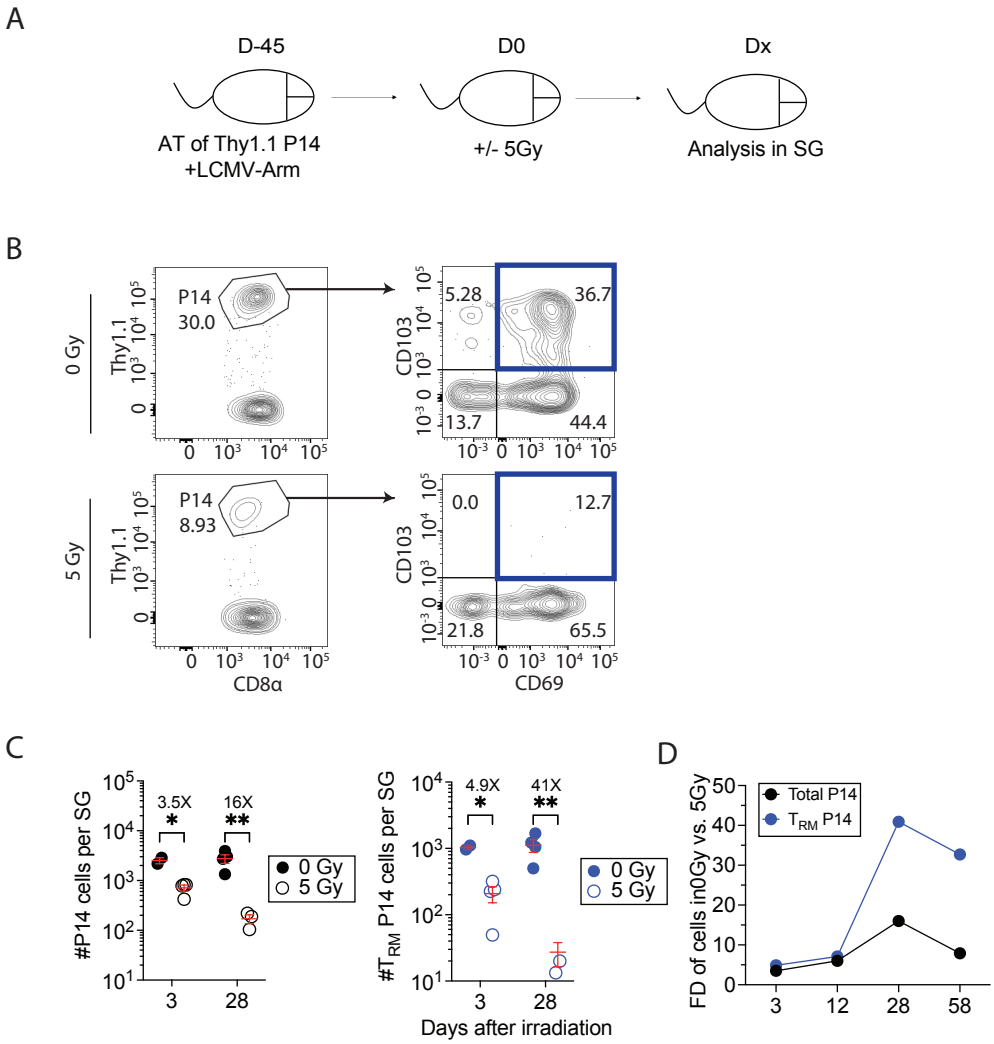

Fig. S2. - Long-lasting decline in number of tissue resident memory CD8+ T cells (T_{RM}) following WBI. A) Experimental Design: 10^4 naive Thy1.1$^+$ P14 CD8+ T cells were adoptively transferred into Thy1.2$^+$ naïve hosts, followed by LCMV-Armstrong infection to generate memory P14 cells. 45 days later, the memory P14 chimeric mice were either exposed to mock or 5Gy WBI. Salivary glands were harvested and analyzed at indicated timepoints. **B)** Representative gating for memory P14 and T_{RM} P14 cells (blue box) in salivary glands (SG) at D28 **C)** Number of memory P14 cells (left) and T_{RM} P14 cells (right) at indicated timepoints in salivary glands after WBI. **D)** fold difference (FD) of the number of total (black) and T_{RM} (blue) P14 cells between 0Gy and 5G hosts in SG following WBI. *=p < 0.05, **=p < 0.01, ***=p < 0.001. Error bars represent standard error of the mean.

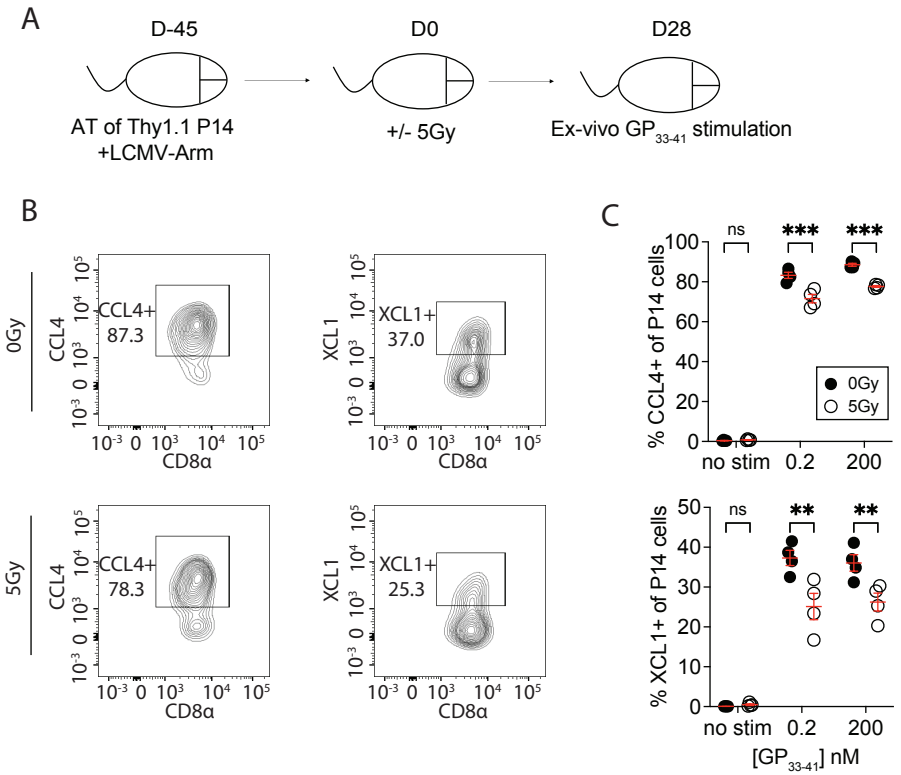

Fig. S3. - WBI reduces the ability of memory CD8 T cells to secrete chemokines following cognate antigen stimulation A) Experimental Design: 10^4 naive Thy1.1$^+$ P14 CD8+ T cells were adoptively transferred into Thy1.2$^+$ naïve hosts, followed by LCMV-Armstrong infection to generate memory P14 cells. 45 days later, the memory P14 chimeric mice were either exposed to mock or 5Gy WBI. At D28, splenocytes from both groups were harvested, and were either left unstimulated or stimulated with different concentrations of GP_{33-41} peptide for 5 hours. **B)** Representative gating of CCL4+ (left) and XCL1+ (right) memory P14 cells from 0Gy (top panels) and 5Gy (bottom panels) after stimmulating with 200 nM of GP_{33-41}. **C)** Frequency of CCL4+ (top) and XCL1+ (bottom) memory P14 cells at D28. *=p < 0.05, **=p < 0.01, ***=p < 0.001. Error bars represent standard error of the mean.

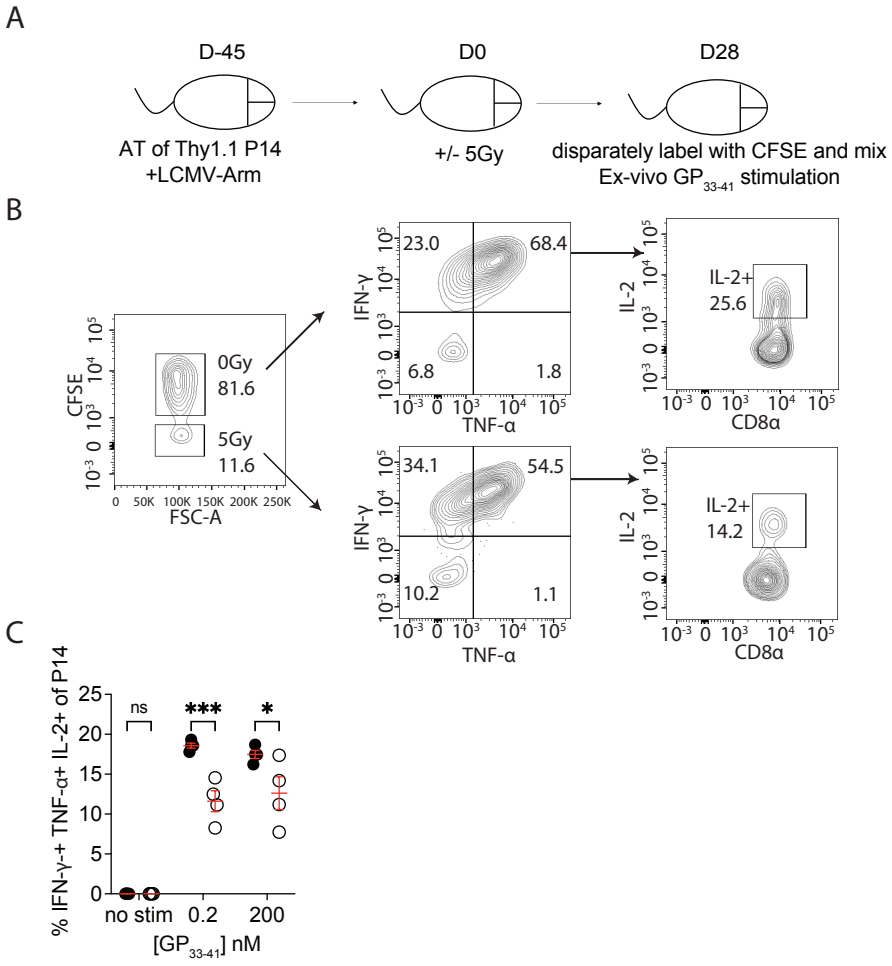

Fig. S4. - WBI-induced impariment in cytokine production following cognate antigen stimulation is T cell-instrinsic A) Experimental Design: 10^4 naive Thy1.1$^+$ P14 CD8+ T cells were adoptively transferred into Thy1.2$^+$ naïve hosts, followed by LCMV-Armstrong infection to generate memory P14 cells. 45 days later, the memory P14 chimeric mice were either exposed to mock or 5Gy WBI. At D28, splenocytes from both groups were harvested, disparately labeled with CFSE, and the two groups were co-incubated either in presence of media alone or different concentrations of GP_{33-41} peptide for 5 hours **B)** Representative gating of IFN-γ, TNF-α, and IL-2-producing memory P14 cells after stimmulating with 200 nM of GP_{33-41}. **C)** Frequency of IFN-γ+TNF-α+IL-2+ memory P14 cells at D28. *=$p < 0.05$, **=$p < 0.01$, ***=$p < 0.001$. Error bars represent standard error of the mean.

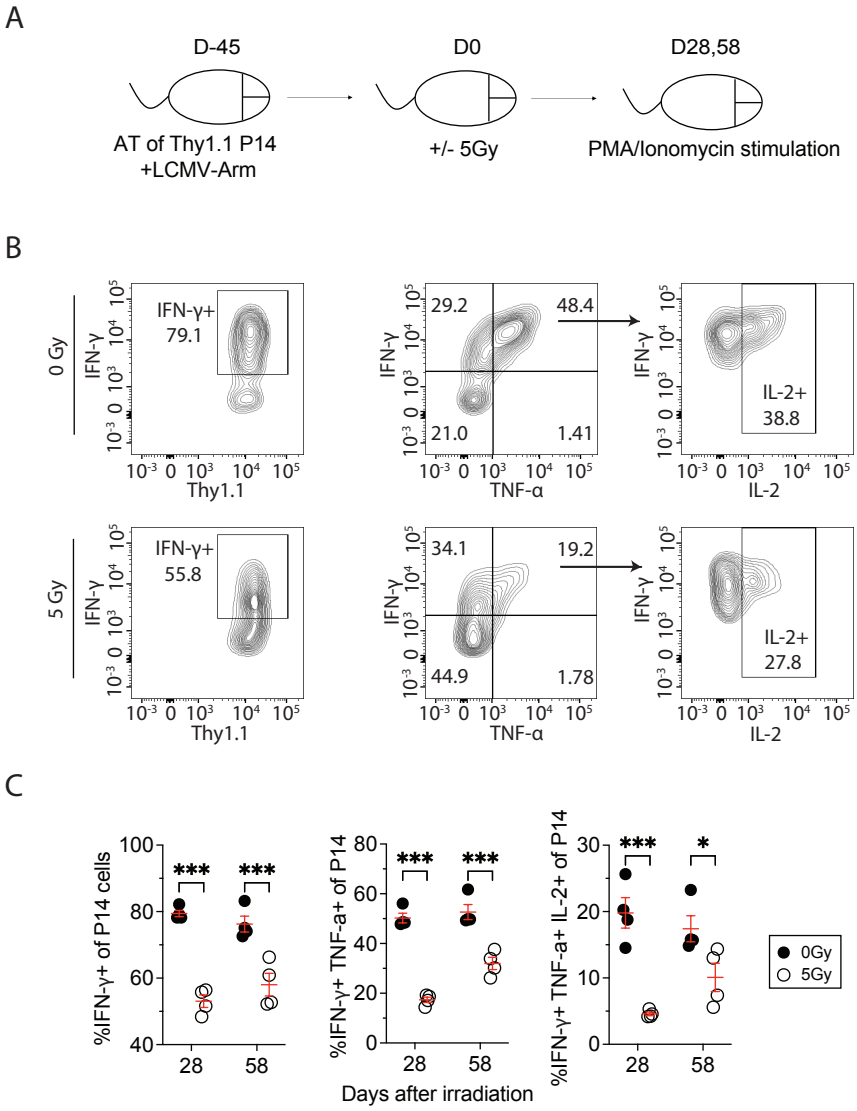

Fig S5. - WBI reduces the ability of memory CD8+ T cells to secrete cytokines following PMA/Ionomycin stimulation. A) Experimental Design: 10^4 naive Thy1.1$^+$ P14 CD8+ T cells were adoptively transferred into Thy1.2$^+$ naïve hosts, followed by LCMV-Armstrong infection to generate memory P14 cells. 45 days later, the memory P14 chimeric mice were either exposed to mock or 5Gy WBI. At D28 and D58, Splenocytes from both groups were harvested, and were either left unstimulated or stimulated with different concentrations of PMA/Ionomycin for 5 hours. **B)** Representative gating of IFN-γ, TNF-α, and IL-2-producing 0Gy (top) and 5Gy (bottom) memory P14 T cells after stimulating with PMA/Ionomycin at D28. **C)** Frequency of IFN-γ+ (left), IFN-γ+ TNF-α+ (middle), and IFN-γ+TNF-α+ IL-2+ (right) memory P14 cells at D12 and D58. *=p < 0.05, **=p < 0.01, ***=p < 0.001. Error bars represent standard error of the mean.