

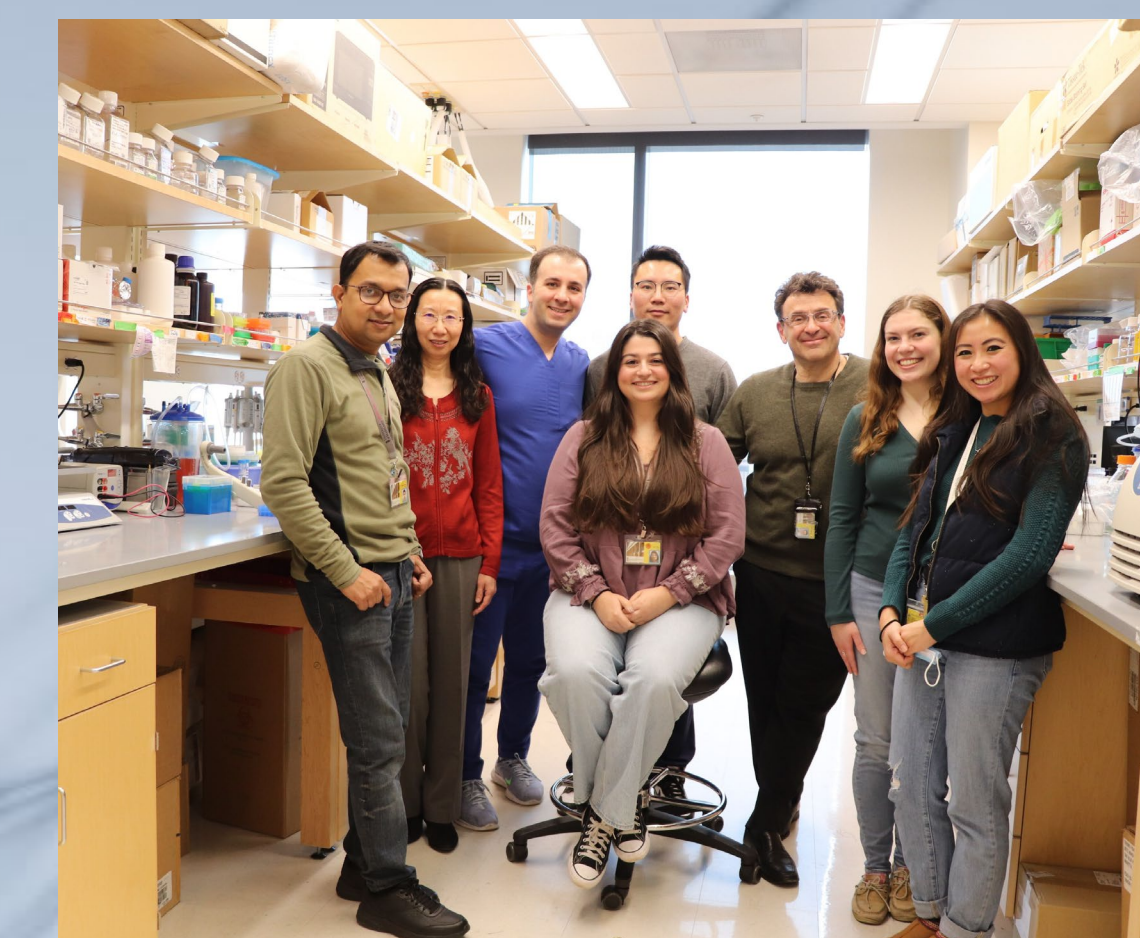
# IL-5 and IL-5Ra Act as Checkpoint Inhibitors in Regulating Neutrophil-Mediated Cytotoxicity after Lung Surgery

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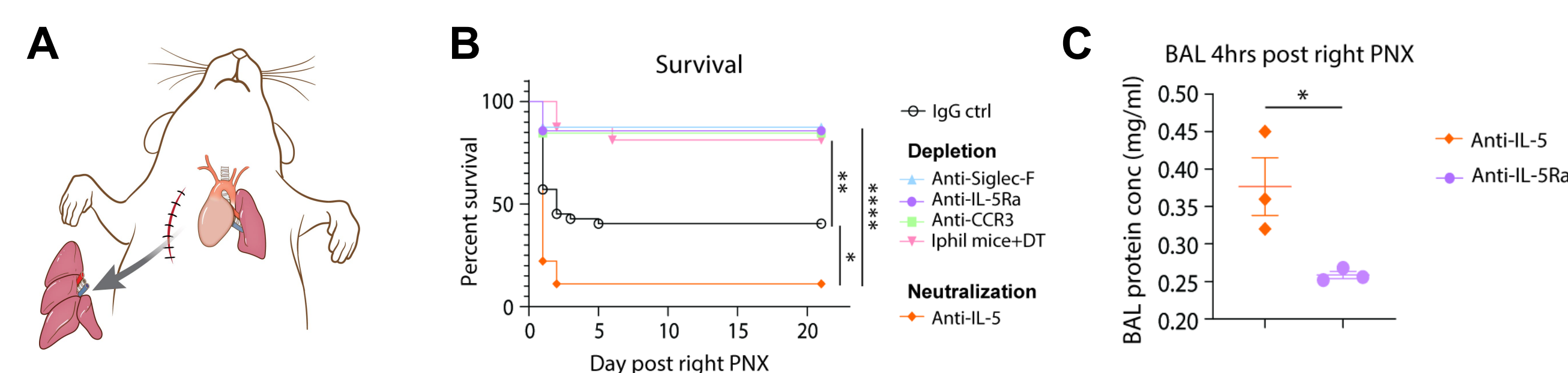


## Abstract

Pulmonary resection triggers complex immune responses that can lead to postoperative lung injury (PLI). While our previous work implicated eosinophils in postoperative mortality, the roles of interleukin-5 (IL-5) and its receptor IL-5Ra—therapeutic targets in eosinophilic disorders—remain unclear in this setting. In a murine model of PLI, we unexpectedly found that neutralization of IL-5 worsened survival. Further analysis revealed that a subset of neutrophils expressing IL-5Ra exhibited cytotoxic features and contributed to tissue damage. We identified granulocyte colony-stimulating factor (G-CSF) as a key upstream driver of IL-5Ra<sup>+</sup> neutrophil activation, whose cytotoxicity was suppressed in the presence of IL-5. These findings uncover a previously unrecognized IL-5–G-CSF axis that regulates postoperative inflammation and highlight new therapeutic opportunities to mitigate lung injury following surgery.

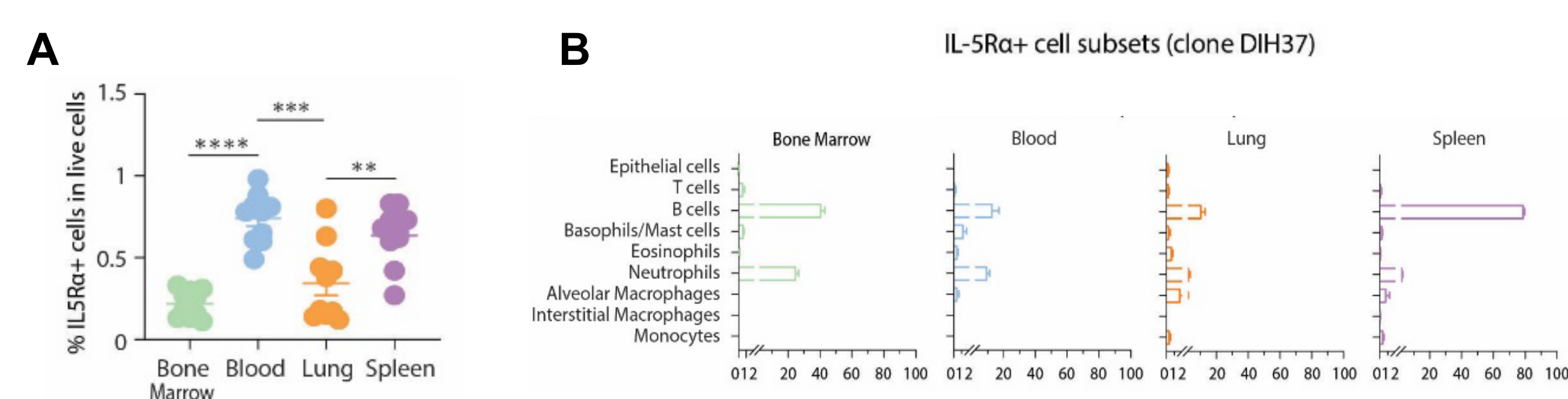
## Methods/Experiments

### IL-5 neutralization exacerbates post-resection mortality and lung injury



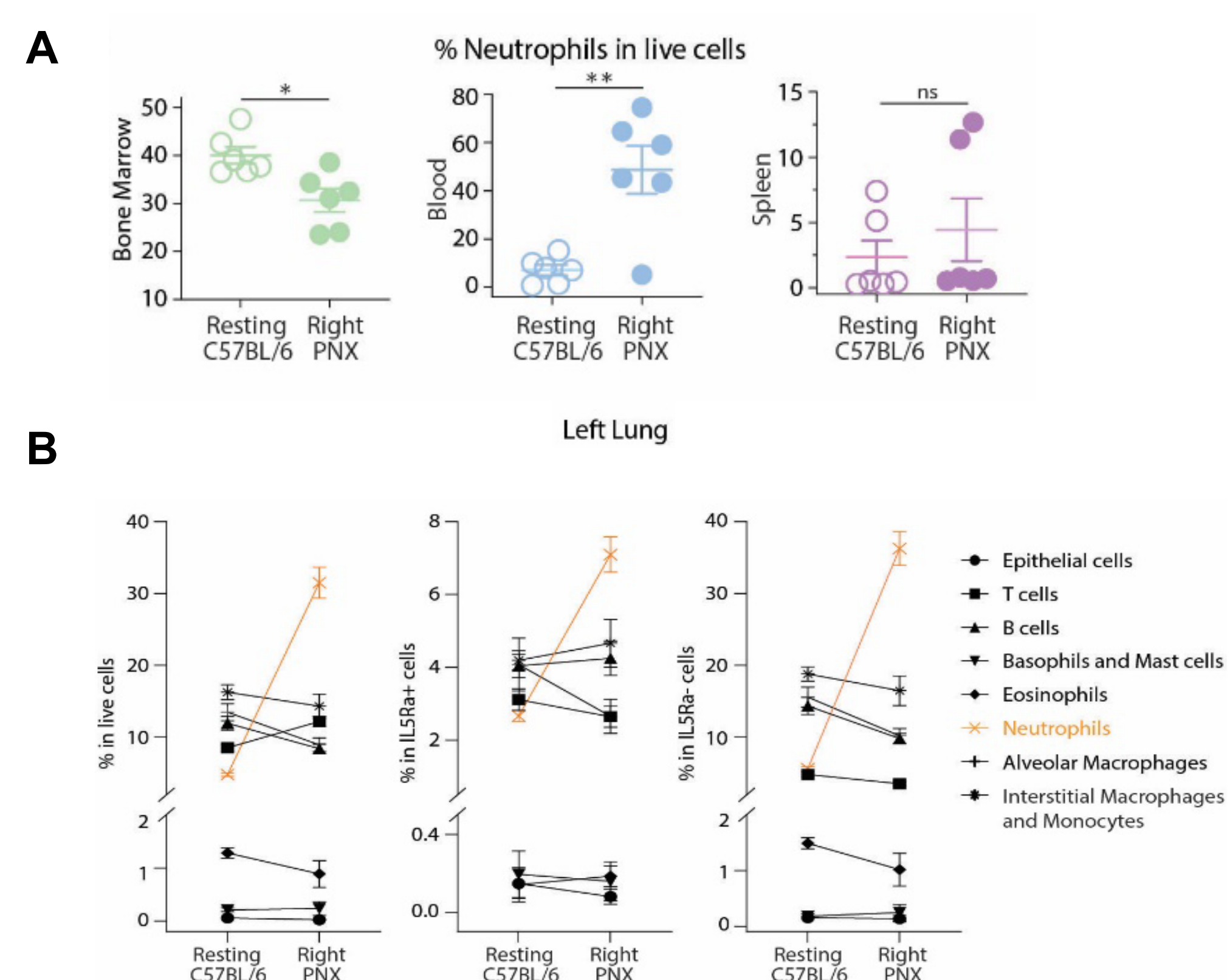
**Figure 1:** (A) Representative schematic of right pneumonectomy in a mouse. (B) Survival curve of mice following right pneumonectomy with different eosinophils depletion strategies. (C) Total protein concentration in bronchoalveolar lavage fluid (BALF) measured by spectrophotometry (NanoDrop).

### Neutrophils express IL-5Ra in WT C57BL/6 mice

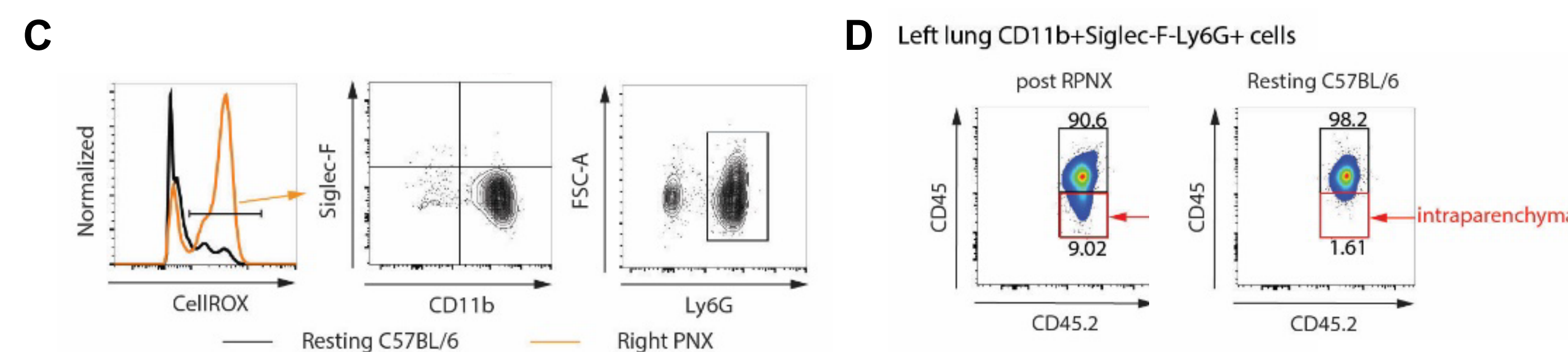


**Figure 2:** (A) IL-5Ra expressing cells in resting mice. (B) subsets of IL-5Ra+ cells.

### Neutrophils migrate to residual lung and mediate tissue damage

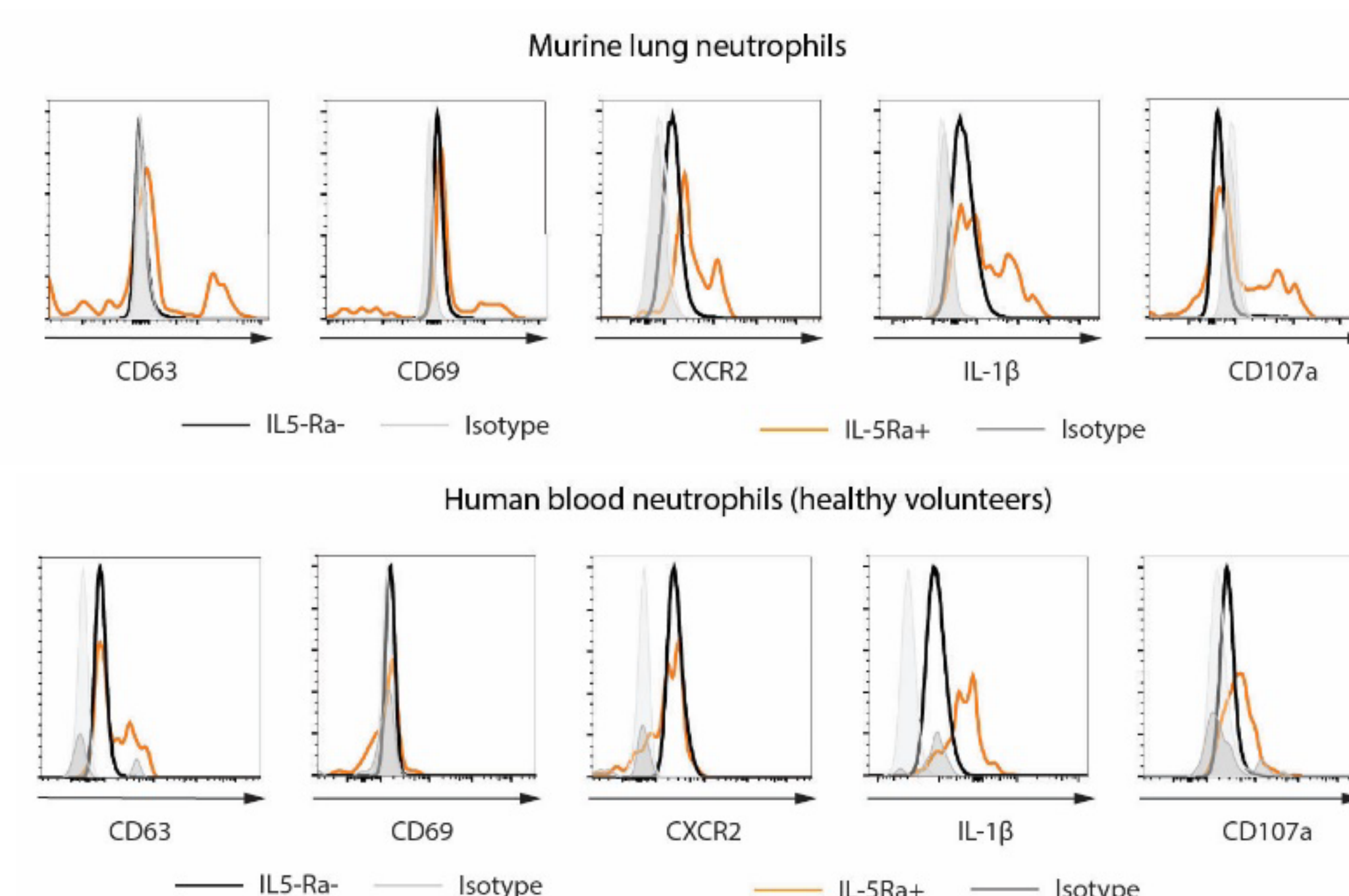


## Key Findings



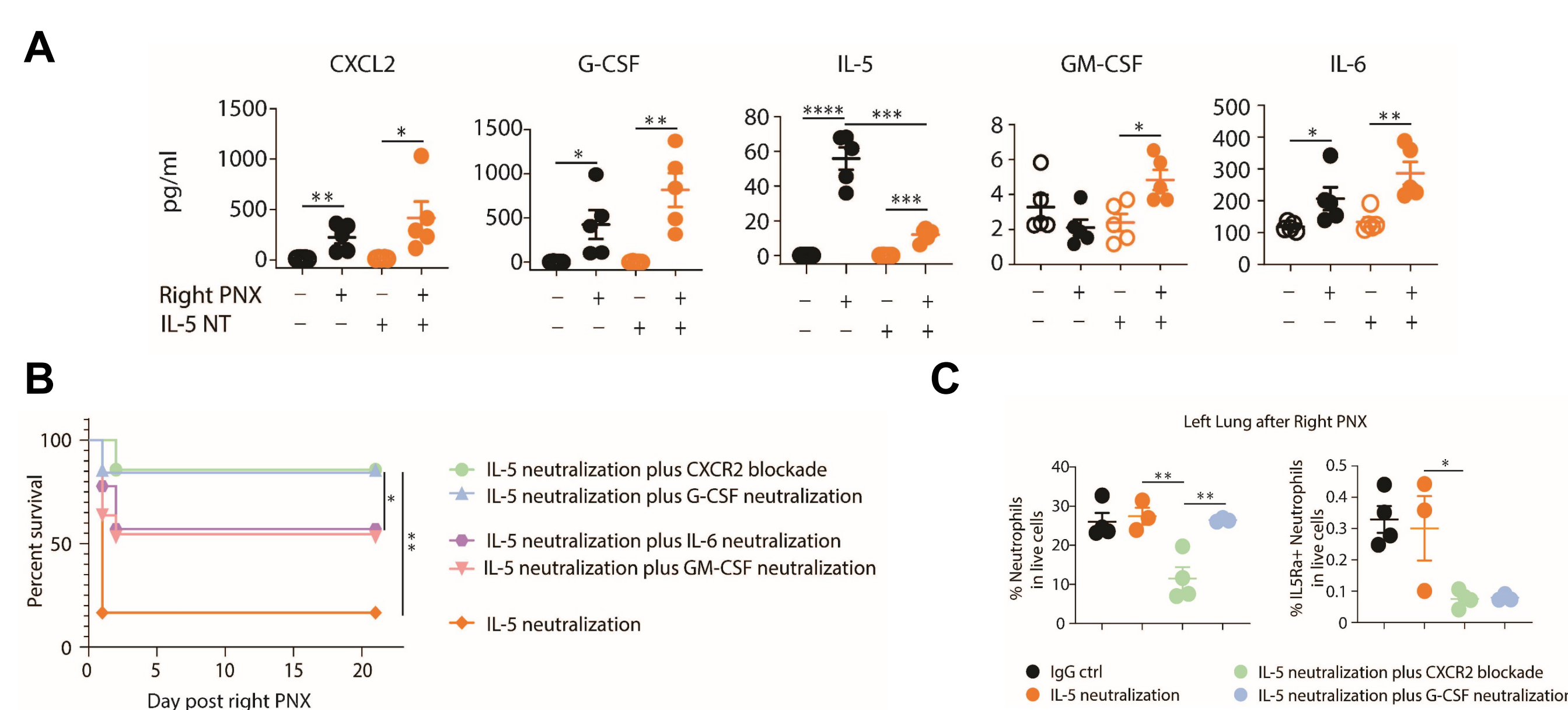
**Figure 3:** (A) Neutrophils dynamic post right pneumonectomy. (B) leucocytes dynamic post right pneumonectomy. (C) Cellrox producer detection by flow cytometry. (D) intravascular injection of CD45 shows parenchymal infiltrated neutrophils.

### IL-5Ra+ neutrophils display increased cytotoxic activity



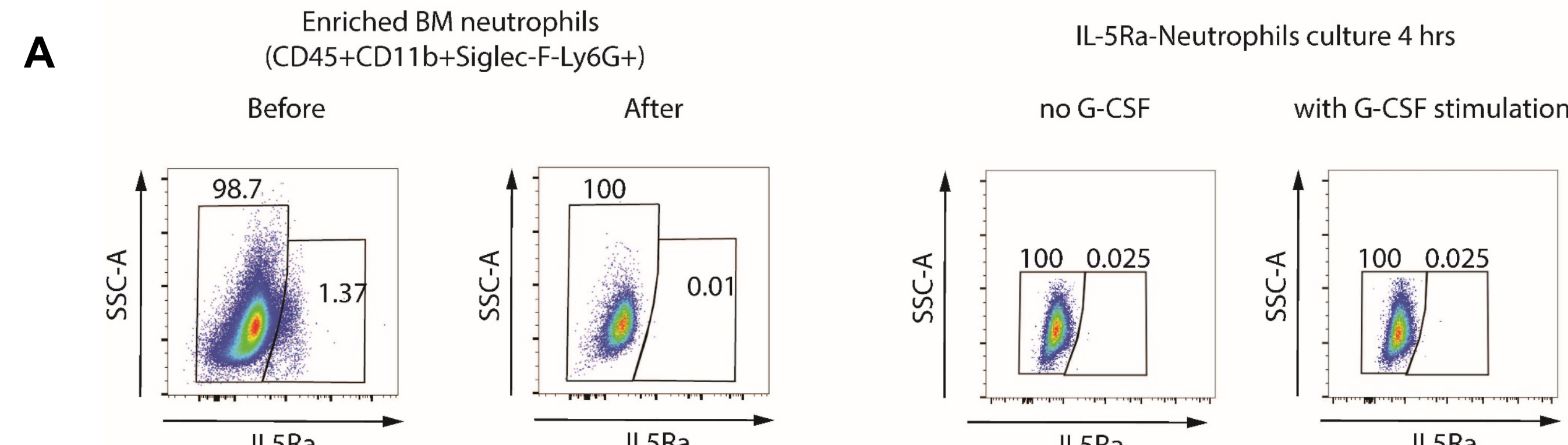
**Figure 4:** Histograms show expression of activation and degranulation markers in IL-5Ra<sup>+</sup> vs IL-5Ra<sup>-</sup> neutrophils.

### G-CSF is essential for IL-5Ra+ Neutrophil survival and activation

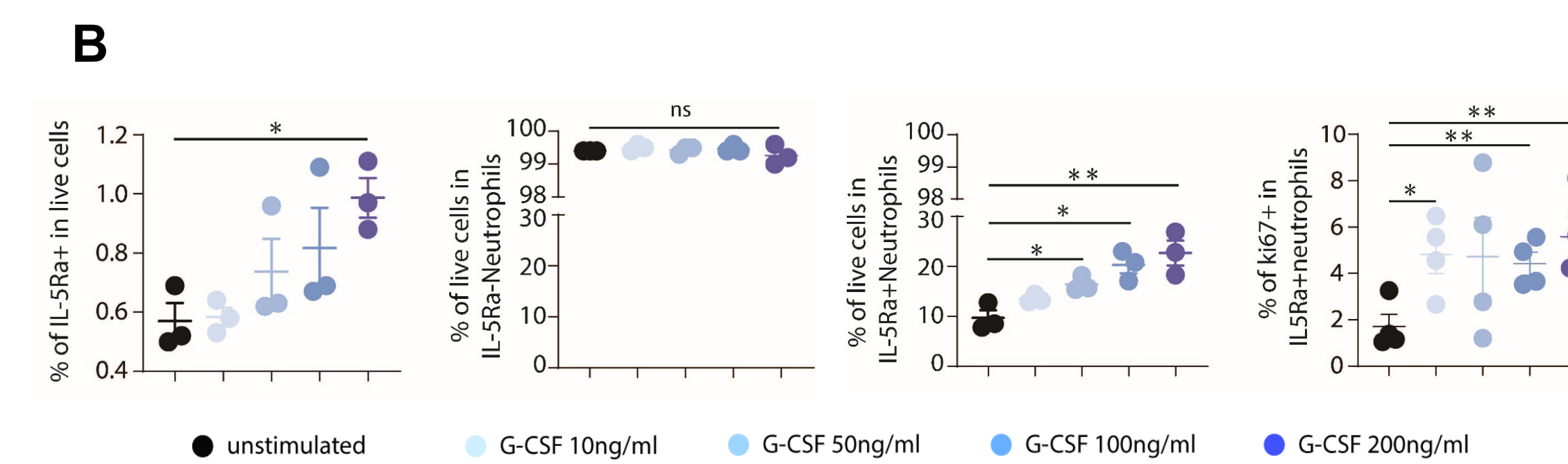


**Figure 5:** (A) lung cytokines ELISA. (B) survival curve of mice post right pneumonectomy. (C) Neutrophils and IL-5Ra<sup>+</sup> neutrophils in the deficient of either CXCR2 or G-CSF with IL-5.

### G-CSF maintains IL-5Ra expression on neutrophils

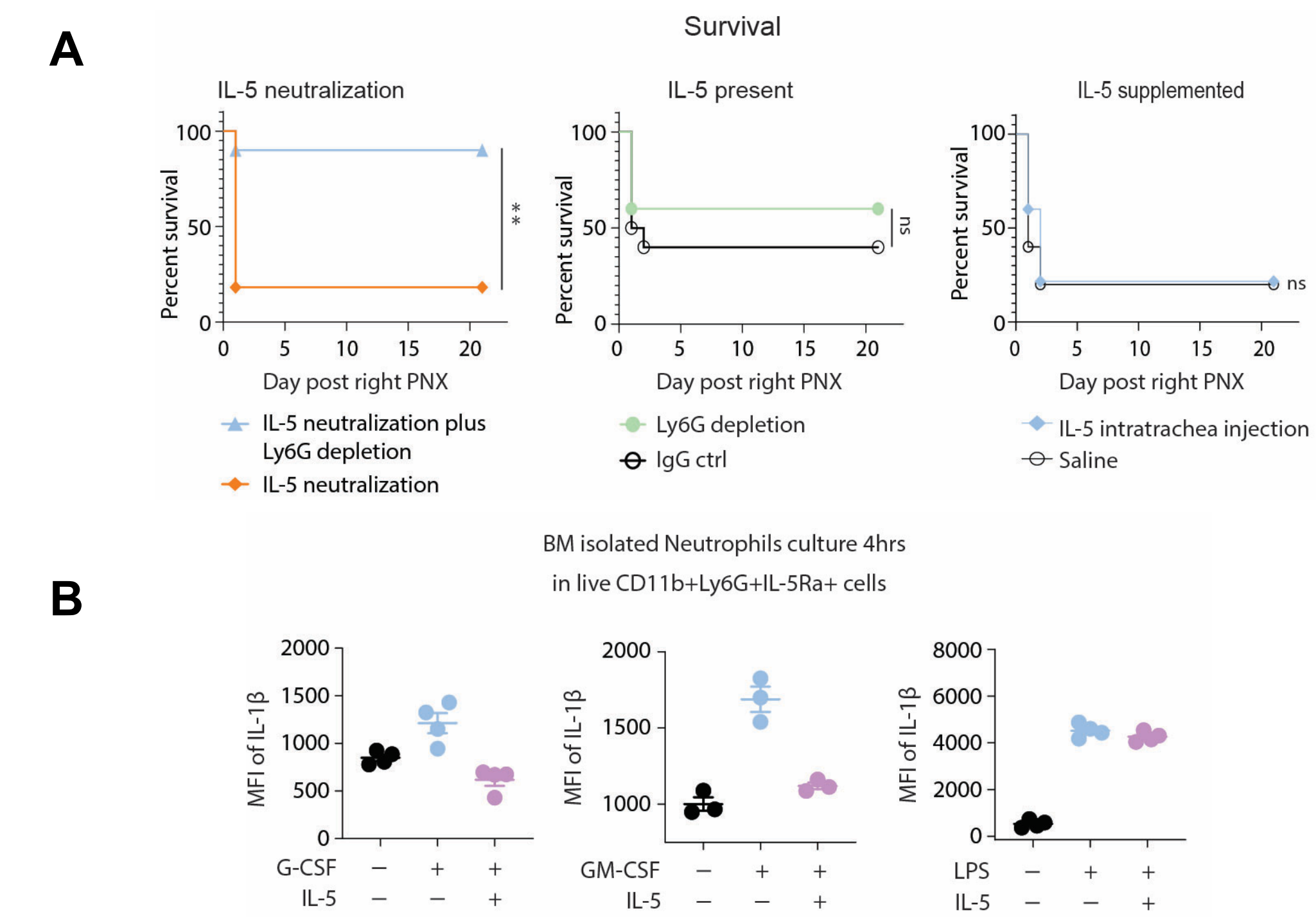


## Results



**Figure 6:** (A) in vitro experiment of isolated IL-5Ra<sup>-</sup> neutrophils culture with G-CSF. (B) IL-5Ra<sup>+</sup> neutrophils culture with different concentrations of G-CSF.

### IL-5 inhibits G-CSF-driven IL-5Ra+neutrophil cytotoxicity



**Figure 7:** (A) survival curve of IL-5 neutralization, IL-5 present, and IL-5 supplemented post right pneumonectomy. (B) Activation marker IL-1β expression in IL-5Ra<sup>+</sup> neutrophils with G-CSF and/or IL-5.

## Conclusions

- IL-5 neutralization exacerbates post-resection mortality and lung injury
- IL-5Ra<sup>+</sup> Neutrophils act as cytotoxic cells
- G-CSF maintains IL-5Ra<sup>+</sup> Neutrophils survival and proliferation
- IL-5 and IL-5Ra signaling limit neutrophil cytotoxicity and protect against postoperative lung injury

## References

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