

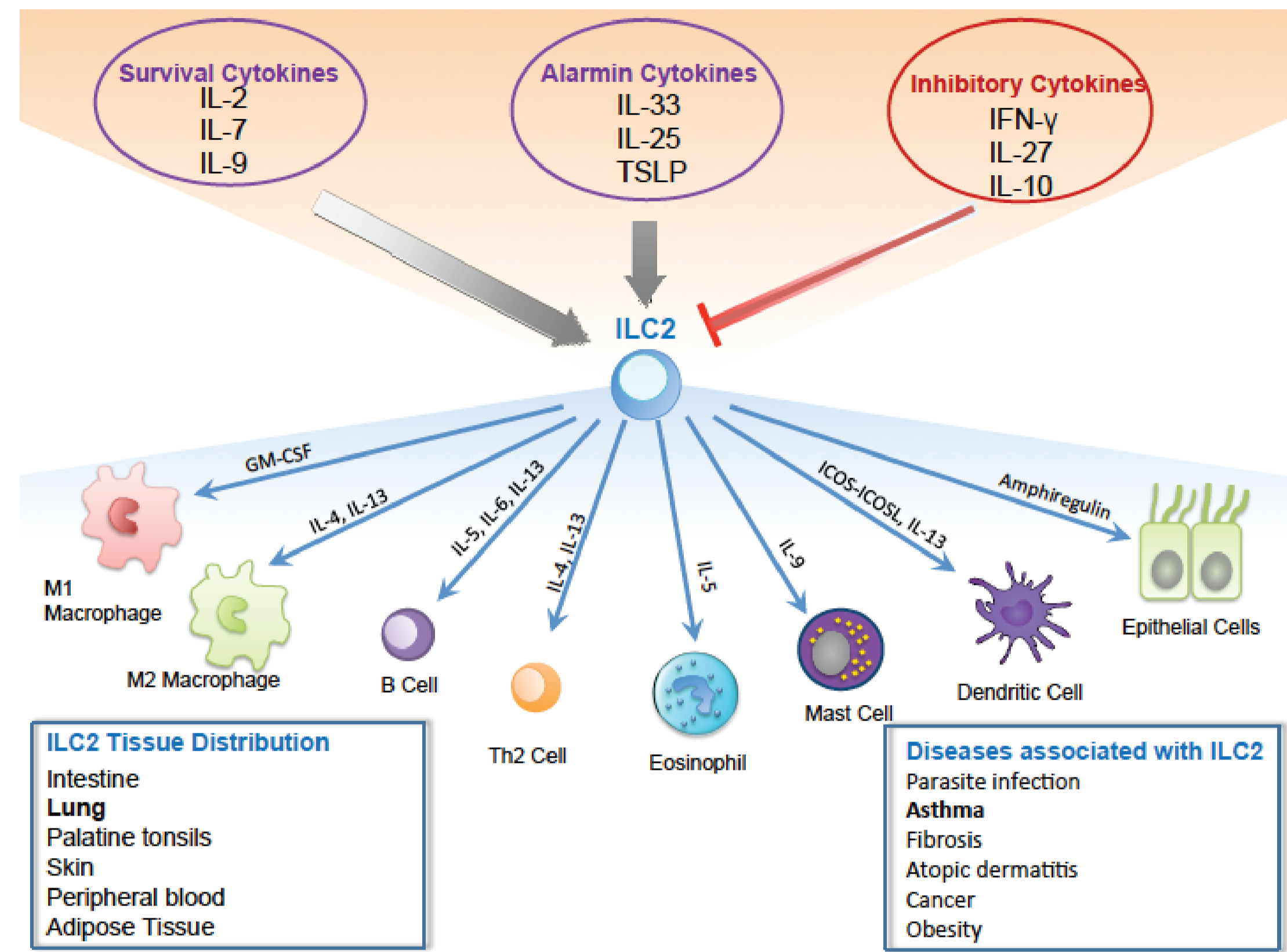
# Magnetic Enrichment of Mouse ILC2s from the Lung

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## Introduction

Figure 1. Group 2 Innate Lymphoid Cells (ILC2s)



- ILC2s are widely distributed throughout the body but are extremely rare within a given tissue.
- To study ILC2 function, researchers often require purified ILC2s.
- Currently, the only method to isolate ILC2s is by Fluorescence Activated Cell Sorting (FACS).
- However, sorting of a small population is time consuming, and it is difficult to obtain high purity or high numbers of ILC2s.
- Therefore, there is a need for a more efficient way to purify ILC2s.

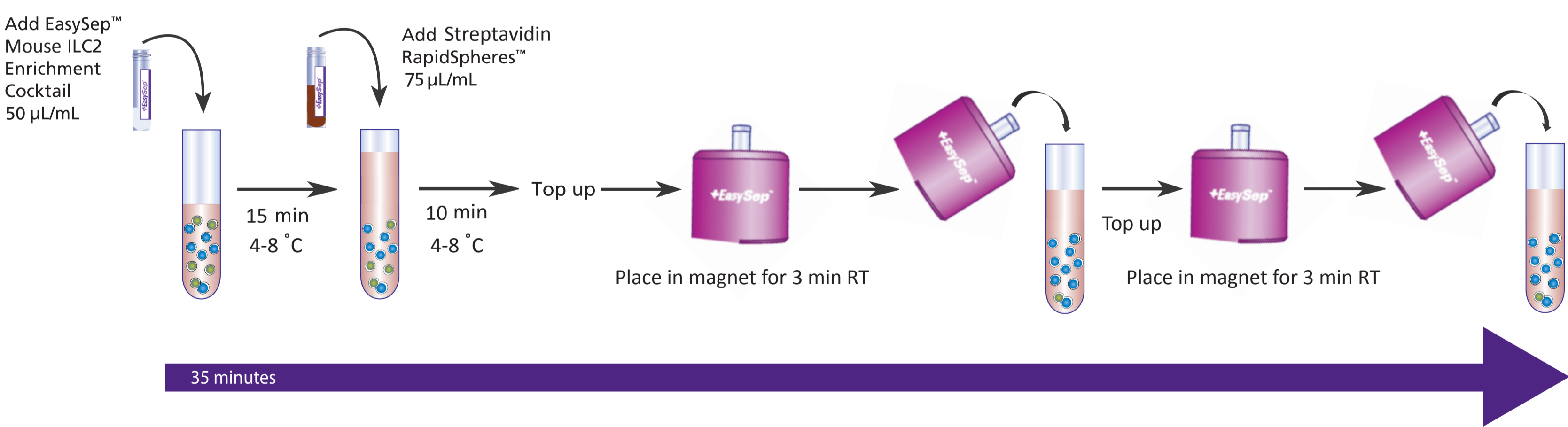
**Goal: To develop a method to enrich for ILC2s from mouse lungs**

## Methods

### Sample

Lungs were isolated from naïve (n = 15) or IL-33 treated (n = 8) C57BL/6J mice. To obtain a single-cell suspension, the lungs were digested in RPMI media containing Collagenase/ Hyaluronidase (Catalog #07912), DNase (Catalog #07900). For each experiment, multiple lungs were pooled (unenriched sample) and a portion of the pooled sample was then enriched using EasySep™ Mouse ILC2 Enrichment kit (Figure 2, enriched samples).

Figure 2. Enrichment of ILC2s by negative selection



### Assessment of ILC2s by flow cytometry

ILC2s were defined as CD45<sup>+</sup> lineage<sup>-</sup> ICOS<sup>+</sup> CD90<sup>+</sup> ST2<sup>+</sup>. The lineage cocktail consists of antibodies targeting CD3, CD4, CD11b, CD11c, CD19, NK1.1, Gr-1, TCR β, TCR γδ and Ter119.

### FACS sorting of ILC2s and functional analysis

Sort time and the number of ILC2s recovered from unenriched and enriched lung samples were compared. Sorted ILC2s were stimulated in the presence of a cytokine cocktail *in vitro*, and their ability to produce IL-5 and IL-13 were measured by ELISA.

## Results

Figure 3. Gating strategy for ILC2s from mouse lungs.  
Data shown is from a naïve mouse lung.

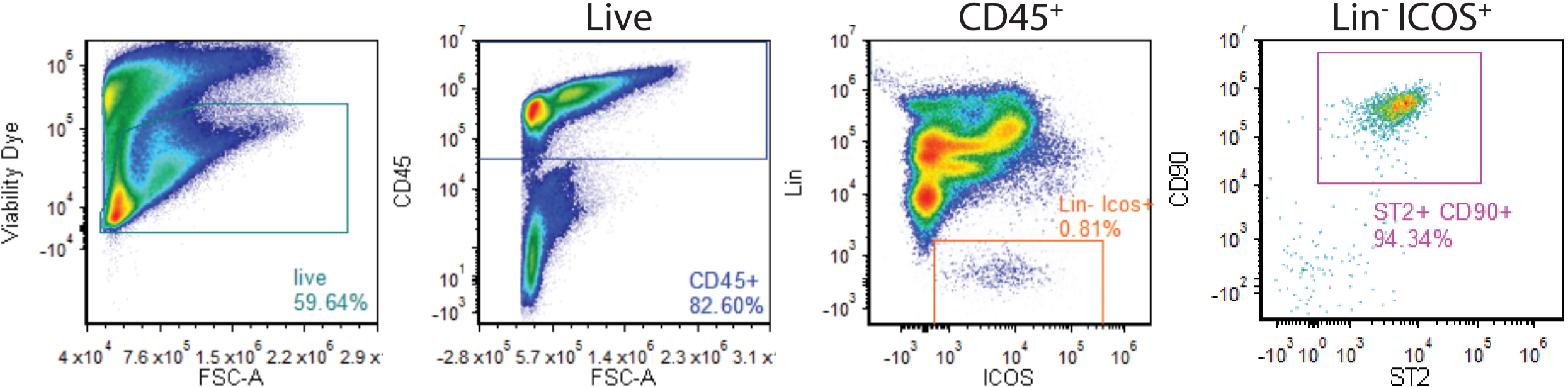
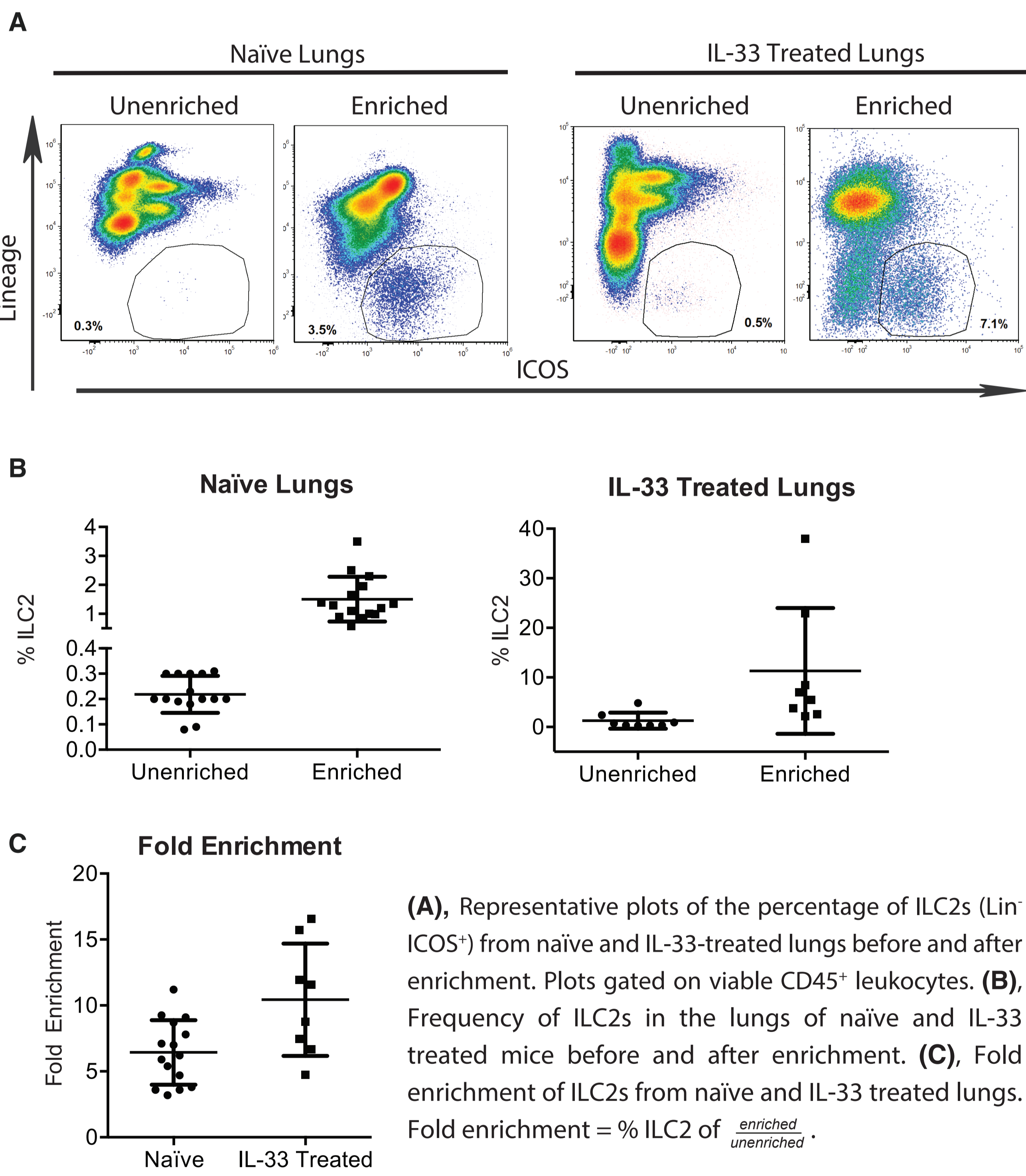


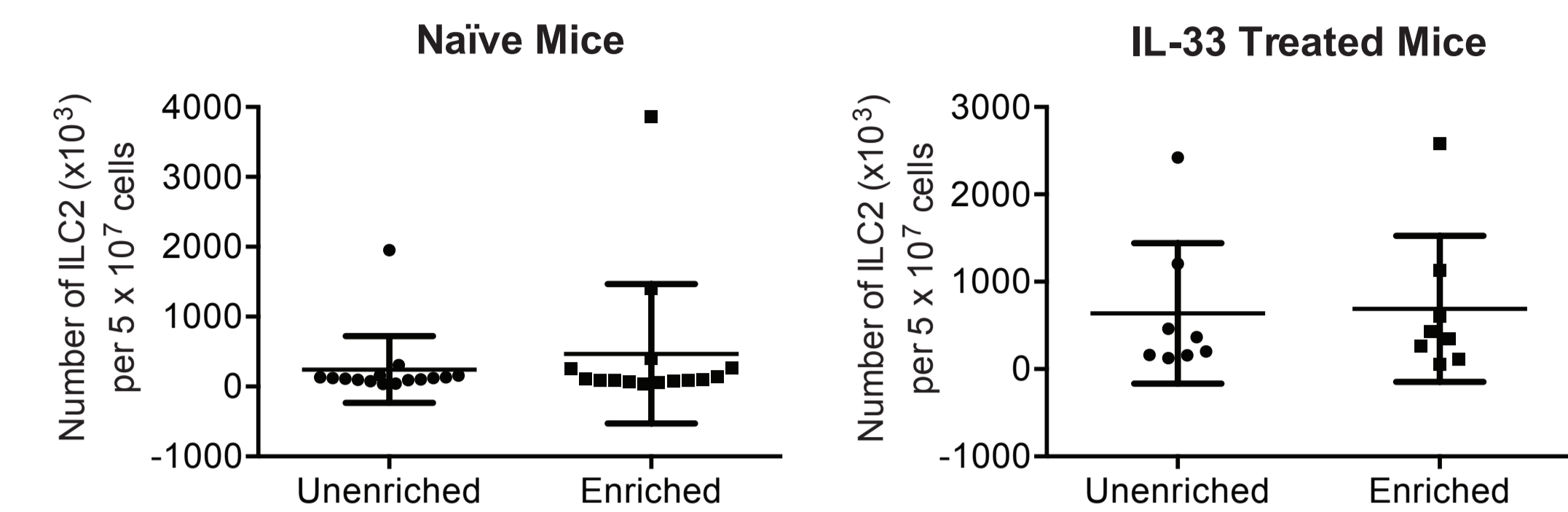
Figure 4. EasySep™ enrichment increases the frequency of ILC2s



## Conclusions

- EasySep™ provides an easy and rapid way to enrich mouse ILC2s from both naïve and IL-33 treated lungs.
- In naïve mice, lung ILC2s were enriched 3 - 11 fold (n = 15). In IL-33 treated mice, lung ILC2s were enriched 5 - 17 fold (n = 8)
- ILC2 enrichment reduces sorting time and increases ILC2 recovery from both the naïve and IL-33 treated lungs.
- Isolated cells are functional as evidenced by IL-5 and IL-13 production upon activation.

Figure 5. No loss of ILC2s following EasySep™ enrichment



Recovery of ILC2s from naïve and IL-33 treated lungs was determined by the number of ILC2s that could be obtained from 5 x 10<sup>7</sup> lung cells with or without enrichment.

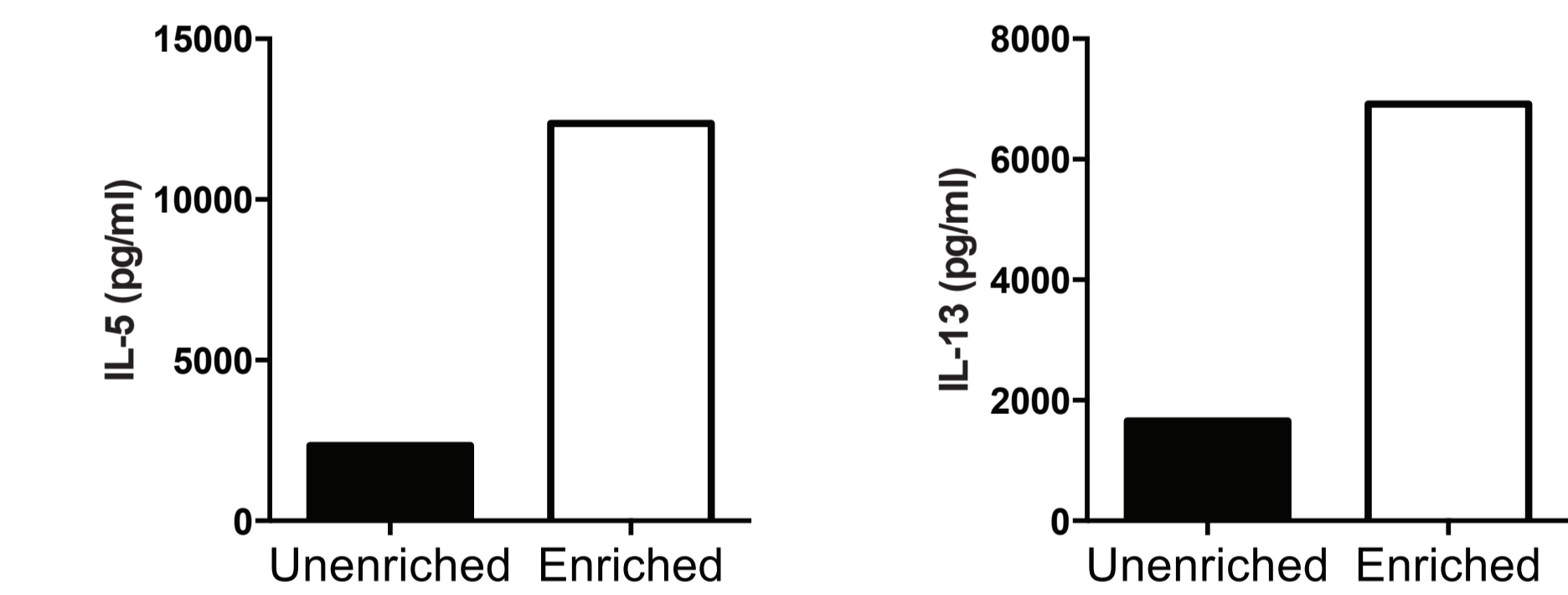
Table 1. Enrichment of ILC2s using EasySep™ reduces sorting time and yields higher ILC2 recovery

A	Sample	Number of cells sorted	Sort Time	Number of ILC2s obtained
	Unenriched	5 x 10 <sup>7</sup>	45 min	3,500
B	Enriched	5 x 10 <sup>7</sup>	20 min	8,000

B	Sample	Number of cells sorted	Sort Time	Number of ILC2s obtained
	Unenriched	3 x 10 <sup>7</sup>	35 min	16,000
B	Enriched	3 x 10 <sup>7</sup>	12 min	30,000

Representative example of the number of ILC2s obtained when FACS sorted from unenriched and enriched samples from naïve (A) and IL-33 treated lungs (B).

Figure 6. EasySep™ enriched ILC2s are functional as assessed by IL-5 and IL-13 production



ILC2s from unenriched and EasySep™-enriched mouse lung samples were sorted by FACS. ILC2s were cultured with a cytokine cocktail, and cell culture supernatants were analyzed for IL-5 and IL-13 expression by ELISA.