

Discovery and Characterization of a Potent and Selective Inhibitor of STAT6 for the Treatment of Allergic Diseases

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BACKGROUND

- STAT6 is a transcription factor downstream of IL-4 and IL-13 signaling that acts as a key driver of type 2 inflammation, which is associated with atopic dermatitis and asthma.
- STAT6 gain-of-function variants can result in severe atopic dermatitis¹ and conversely, STAT6 loss-of-function has been demonstrated to protect against type 2 high asthma².
- To date, there are no FDA approved oral therapies selectively targeting the IL-4 and IL-13 pathway.
- STAT6 inhibitors have the potential to become an oral alternative to dupilumab by blocking IL-4 and IL-13 signaling and reducing type 2 inflammation.
- EPS-3903 is an oral, small molecule allosteric inhibitor that potently and selectively inhibits STAT6, with the potential to offer an oral therapeutic option for treating allergic diseases including atopic dermatitis and asthma.

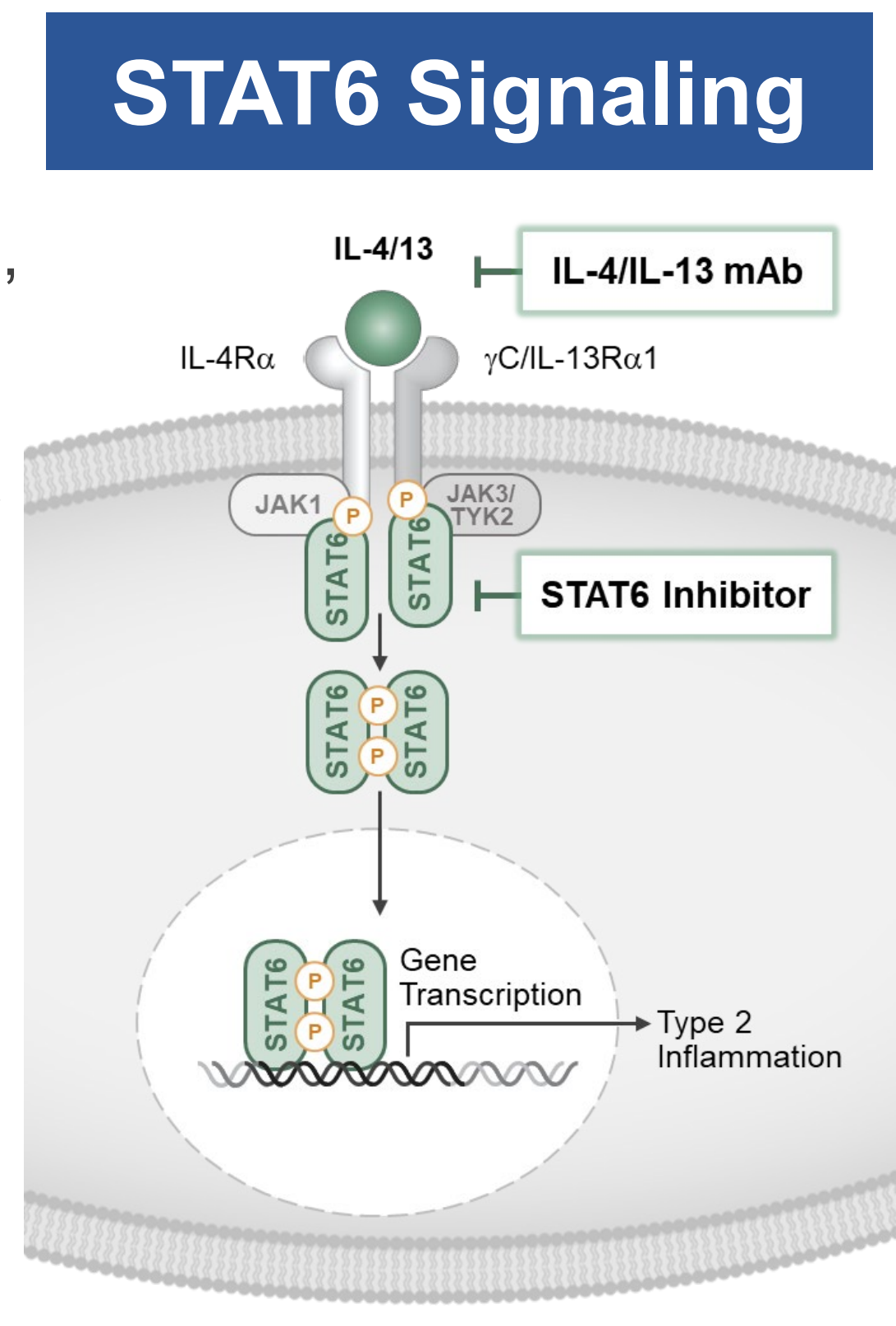


Figure 1. Inhibition of STAT6 in the IL-4 and IL-13 Signaling Pathway

¹PMID 36216080
²PMID 39423878

METHODS

- Binding affinity was determined with SPR and SH2 competition assays.
- Selectivity against STATs was assessed in PBMC assays with the indicated cytokines.
- Potency of EPS-3903 and upadacitinib were evaluated by measuring STAT6 phosphorylation (Y641) and TARC secretion in IL-4 stimulated PBMC, periostin release in IL-13 stimulated primary bronchial cells and ATP levels in IL-4 stimulated TF-1 proliferation assays.
- Effects of EPS-3903 on phosphorylated STAT6 nuclear translocation were measured in human alveolar A549 cells staining for phosphorylated (Y641) STAT6 and nuclei with DAPI.
- For *in vivo/ex vivo* studies, mice were orally dosed with EPS-3903. Whole blood was collected after 24 hours for IL-4 stimulation and pSTAT6 was measured.

CONCLUSION

EPS-3903 is a selective allosteric inhibitor of STAT6 that potently blocks STAT6 activation and downstream Th2 biomarker production. EPS-3903 suppresses STAT6 activation *in vivo* by >90% for 24 hours. These promising results of EPS-3903 demonstrate the potential for an oral alternative to dupilumab and other biologics for treating allergic diseases including asthma and atopic dermatitis.

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Disclosure

All authors are either current or former employees of Enanta Pharmaceuticals, Inc. and received salary and stock compensation.

RESULTS

EPS-3903 is a potent and selective STAT6 inhibitor

| Assay EC ₅₀ , nM | STAT6i EPS-3903 | JAKi Upadacitinib |
|--------------------------------|--------------------|----------------------|
| STAT6 (SPR)* | 13 | N/A |
| STAT6* | 0.4 | N/A |
| IL-4 pSTAT6 | 4 | 10 |
| Type I IFN pSTAT1 | >5000 | 1 |
| Type I IFN pSTAT2 | >5000 | 8 |
| IL-6 pSTAT3 | >5000 | 24 |
| IL-12 pSTAT4 | >5000 | 195 |
| IL-2 pSTAT5 | >5000 | 16 |

Table 1. *K_d value. Cellular assays performed in peripheral blood mononuclear cell. K_d, dissociation constant. EC₅₀, half-maximal effective concentration. SPR, surface plasmon resonance. pSTAT, phosphorylated STAT. IFN, interferon.

EPS-3903 suppresses Th2 biomarker TARC, blocks pruritogen periostin, and prevents cell proliferation

| Assay EC ₅₀ , nM | STAT6i EPS-3903 | JAKi Upadacitinib |
|--------------------------------|--------------------|----------------------|
| PBMC IL-4 pSTAT6 | 4 | 10 |
| PBMC IL-4 TARC | 11 | 17 |
| BSMC IL-13 Periostin | 3 | 11 |
| TF-1 IL-4 Proliferation | 8 | 16 |

Table 2. EC₅₀, half-maximal effective concentration. PBMC, peripheral blood mononuclear cell. pSTAT, phosphorylated STAT. TARC, thymus and activation-regulated chemokine. BSMC, bronchial smooth muscle cell. TF-1, human erythroleukemia cells.

EPS-3903 prevents STAT6 activation, suppresses TARC and STAT6-mediated proliferation

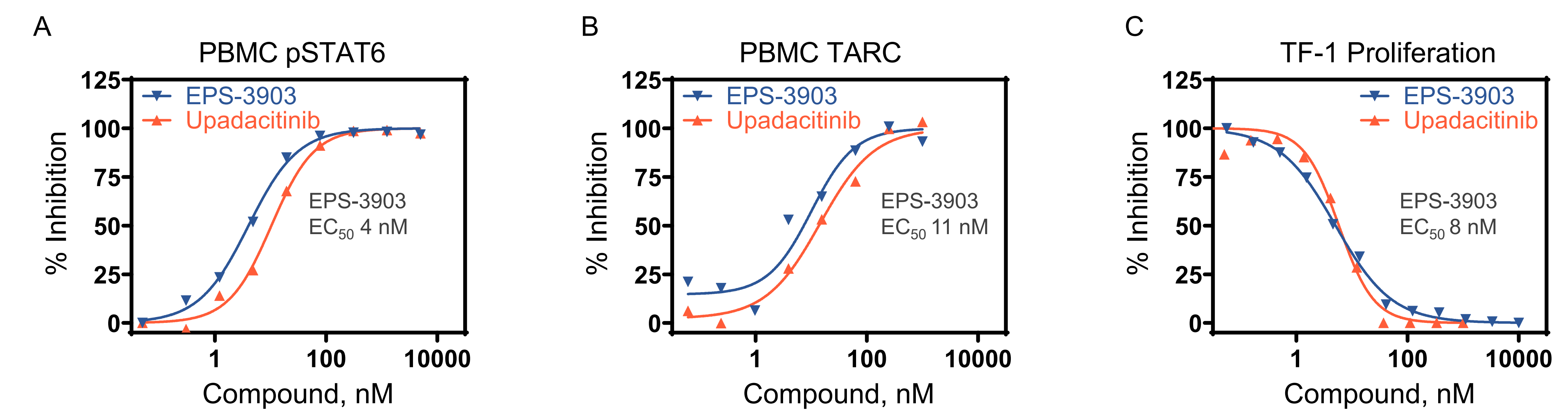


Figure 2. EPS-3903 potency demonstrated in IL-4 induced (A) STAT6 phosphorylation in PBMC, (B) TARC secretion in PBMC, and (C) cell proliferation in TF-1 cells.

EPS-3903 impedes pSTAT6 nuclear translocation with low nanomolar inhibition

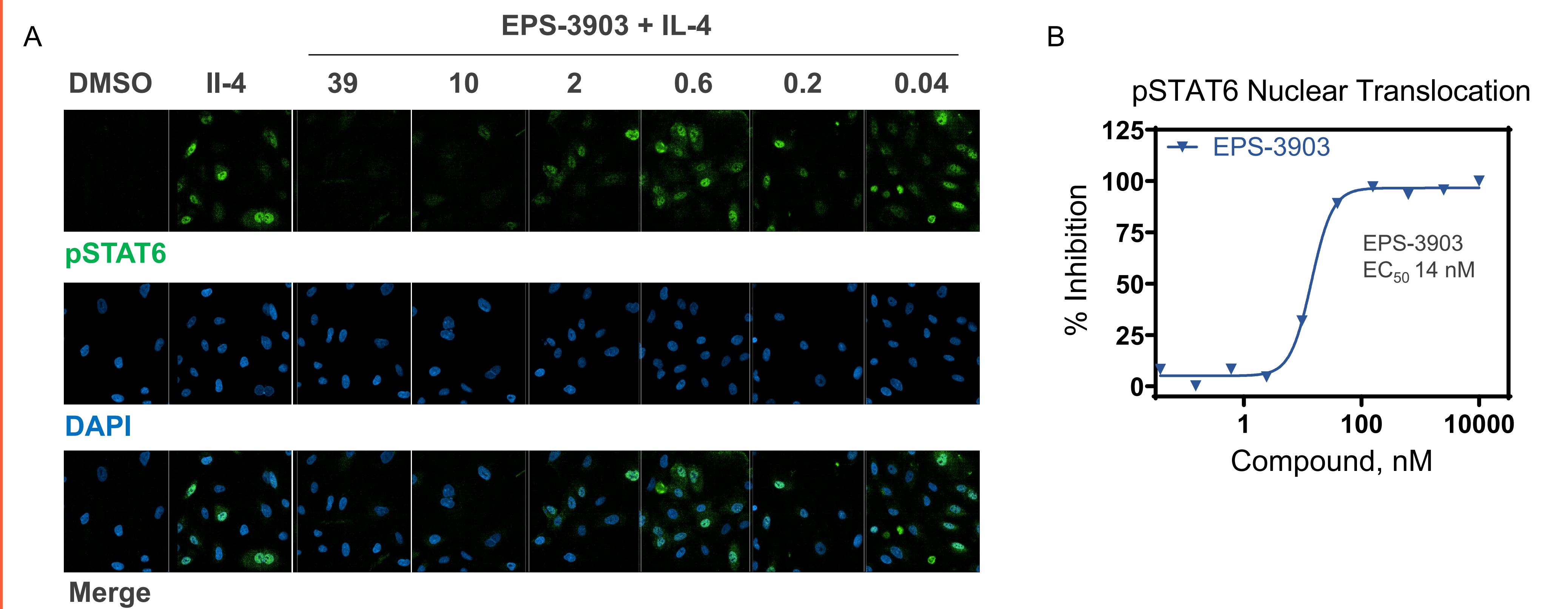


Figure 3. EPS-3903 prevents pSTAT6 nuclear translocation in human A549 alveolar cells. (A) Immunofluorescence (IF) images of A549 cells. (B) EC₅₀ analysis of IF images in panel (A).

Oral dosing of EPS-3903 suppresses pSTAT6 by >90% at 24 hours

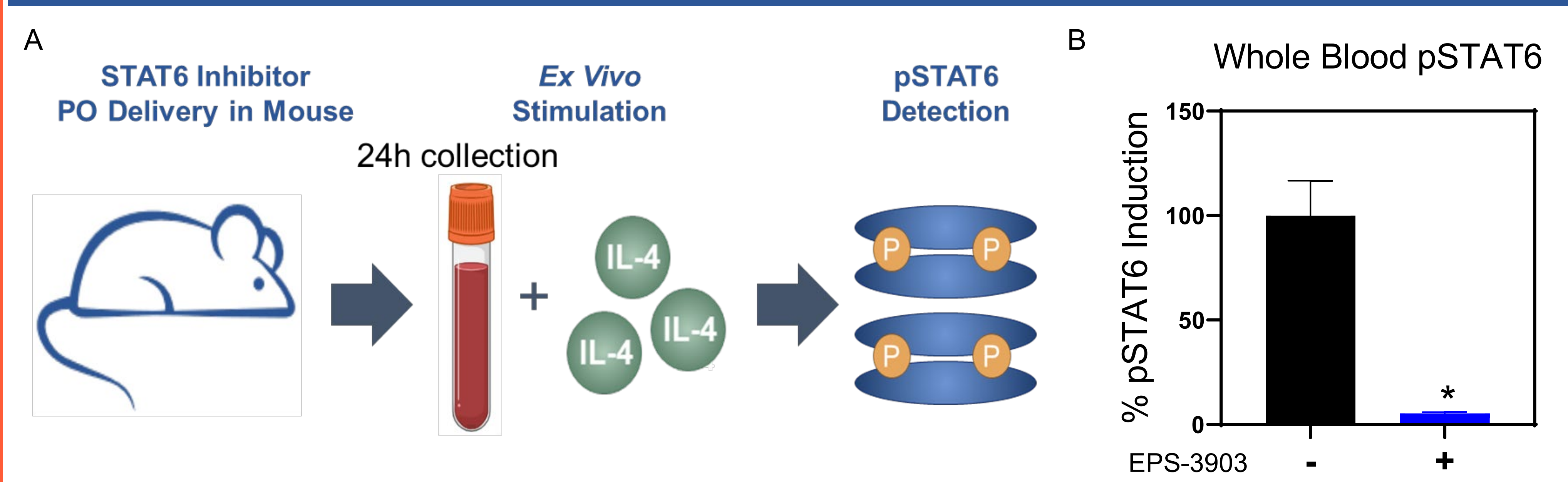


Figure 4. *Ex Vivo* pSTAT6 in IL-4 stimulated mouse whole blood. (A) Mice were orally dosed with EPS-3903, followed by *ex-vivo* stimulation of whole blood with IL-4 and pSTAT6 detection. (B) Inhibition of pSTAT6 induction sustained 24 hours post oral dose.