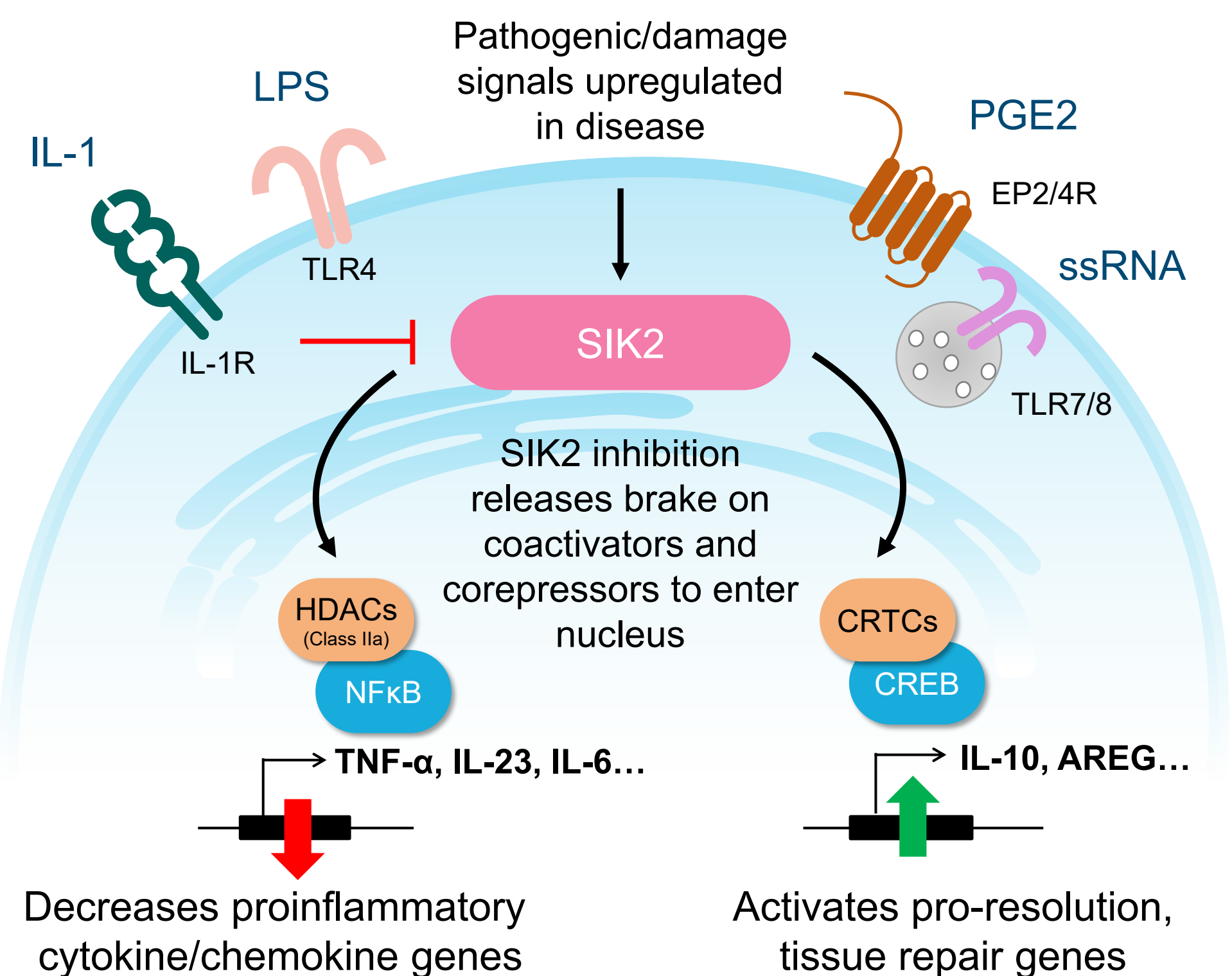




## INTRODUCTION

- Salt-inducible kinases (SIKs) amplify proinflammatory gene expression in myeloid cells via phosphorylation of transcriptional coregulators.<sup>1</sup>
- SIK2 shows the highest kinase activity in myeloid cells.<sup>2</sup>
- Genetic loss-of-function (LOF) of SIK2 in mice or use of pan-SIK inhibitors decreases proinflammatory cytokines (TNF $\alpha$ , IL-12/23, IL-6, and IL-1 $\beta$ ) induced by Toll-like receptor (TLR) or IL-1 receptor (IL-1R) agonists.<sup>1,2</sup>
- SIK2 LOF also enhances production of the anti-inflammatory cytokine IL-10, an effect not observed with SIK1 or SIK3 LOF.<sup>2</sup>

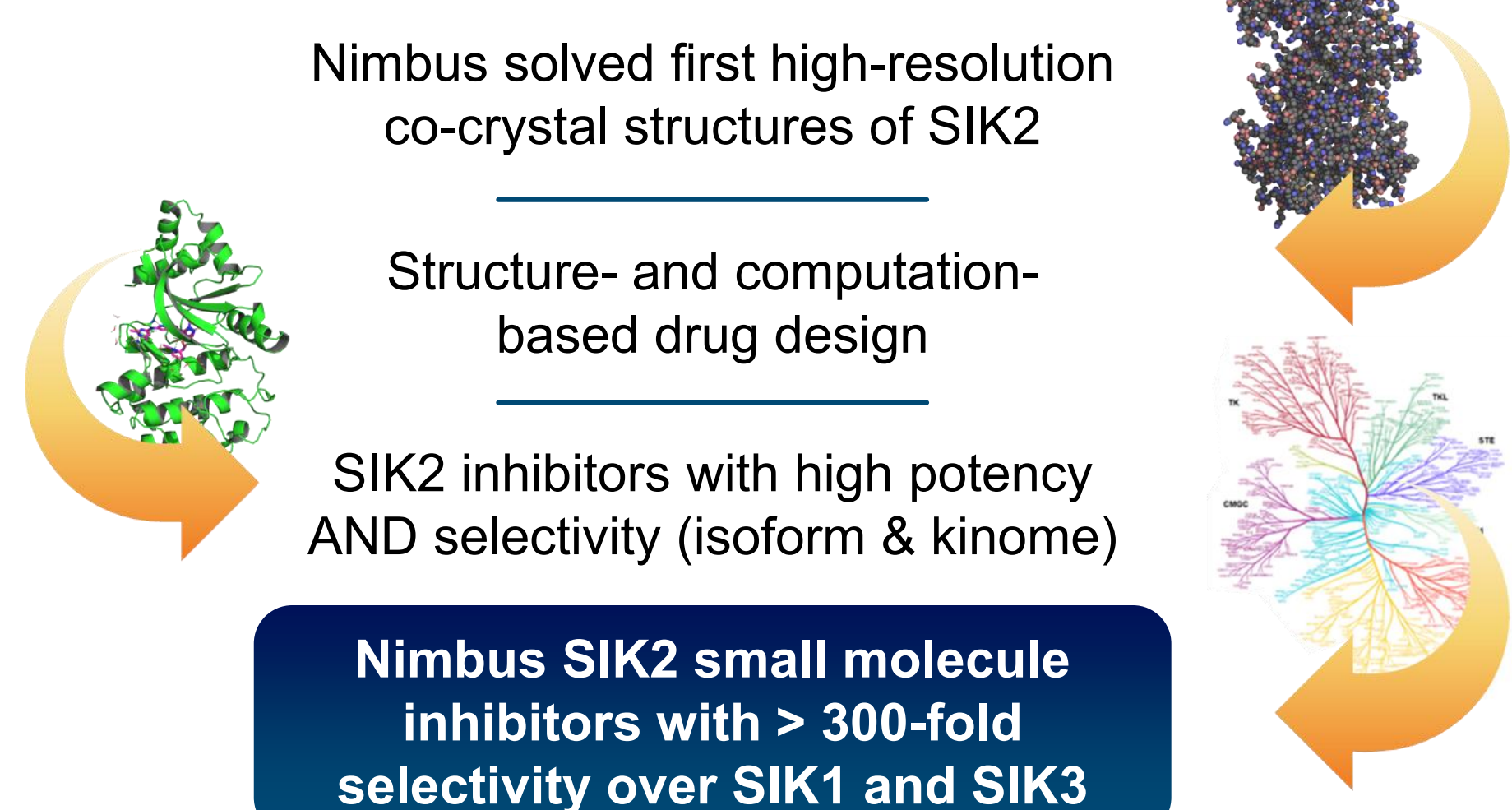


## METHODS

Using structure-based drug design, we developed highly SIK2-selective inhibitors with nanomolar cellular potency, favorable *in vitro* ADME and off-target profiles, and suitable PK properties to support *in vivo* evaluation. SIK2 inhibitors were assessed *in vitro* using differentiated mouse and human myeloid cells and *in vivo* in LPS challenged mice.

## RESULTS

**Fig 1: High SIK2 Selectivity and Potency to Achieve Desired Pharmacology and Safety**



### SIK2 Inhibitor Selectivity is Critical for Efficacy and Safety

- SIK2:** Uniquely delivers dual anti-inflammatory and pro-resolution effects
- SIK1:** Known cardiotoxicity risks<sup>3</sup>
- SIK3:** LOF in mice and humans results in bone defects<sup>4</sup> while knockdown in mice results in fertility defects<sup>5</sup>

### Lead Series Profile

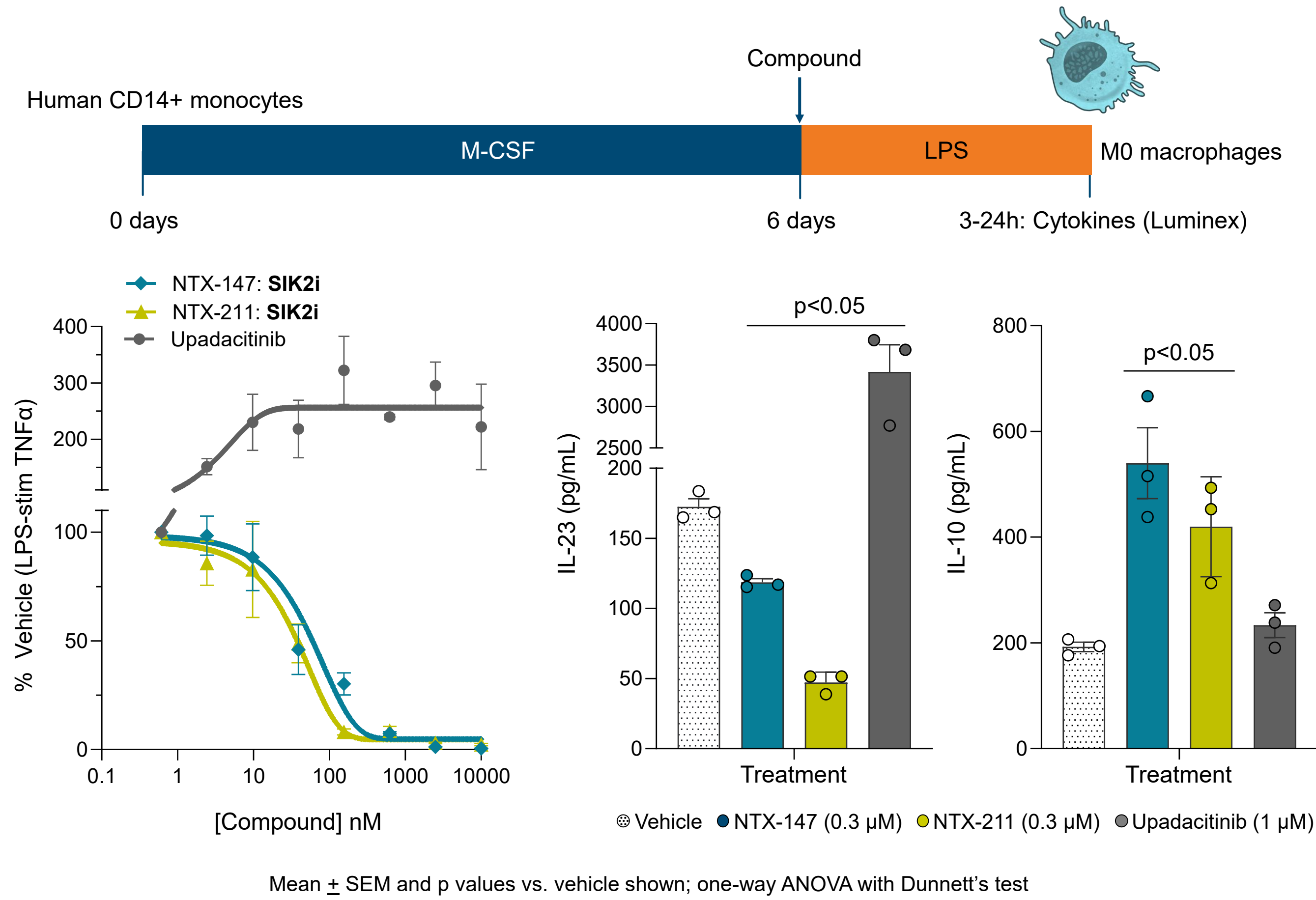
- Potent:** Low nanomolar cellular IC<sub>50</sub>
- Highly selective** against SIK1 & SIK3, minimal hits in kinome
- Good ADME/PK** supporting QD or BID dosing

**Table 1: Highly Potent and Selective SIK2 Inhibitors, from Two Distinct Chemical Series, Exemplified by NTX-147 and NTX-211**

Parameter	<i>In vitro</i> Assay	NTX-147 (SIK2i)	NTX-211 (SIK2i)	NTX-250 (SIK2/1i)	NTX-902 (SIK3i)	NTX-539 (SIK2/3i)
Potency (K <sub>i</sub> <sub>app</sub> , $\mu$ M; 1mM ATP)	Human SIK2 ADP-Glo	0.002	0.001	0.001	19.62	0.709
	Human SIK1 ADP-Glo	1.76	2.87	0.038	47.29	25.64
	Human SIK3 ADP-Glo	0.240	0.114	0.380	0.115	0.404
Cellular Potency ( $\mu$ M)	RAW264.7 SIK2-only, CRTCS nuclear translocation (EC <sub>50</sub> ) <sup>*</sup>	0.005	0.003	0.002	>1.17	1.38
	RAW264.7 SIK3-only, CRTCS nuclear translocation (EC <sub>50</sub> ) <sup>*</sup>	5.13	0.606	1.75	0.517	1.82
SIK Selectivity (Fold)	Selectivity over SIK1 (biochem)	733x	2870x	38x	411x	36x
	Selectivity over SIK3 (biochem/cell)	100x / 1115x	114x / 202x	380x / 946x	-	0.6x / 1.3x
Kinase Inhibition	Kinome Panel (hits >70% inhibition; at 1 $\mu$ M and 1 mM ATP)	8 of 408 (2%)	10 of 409 (2%)	2 of 408 (0.5%)	0 of 407 (0%)	0 of 402 (0%)

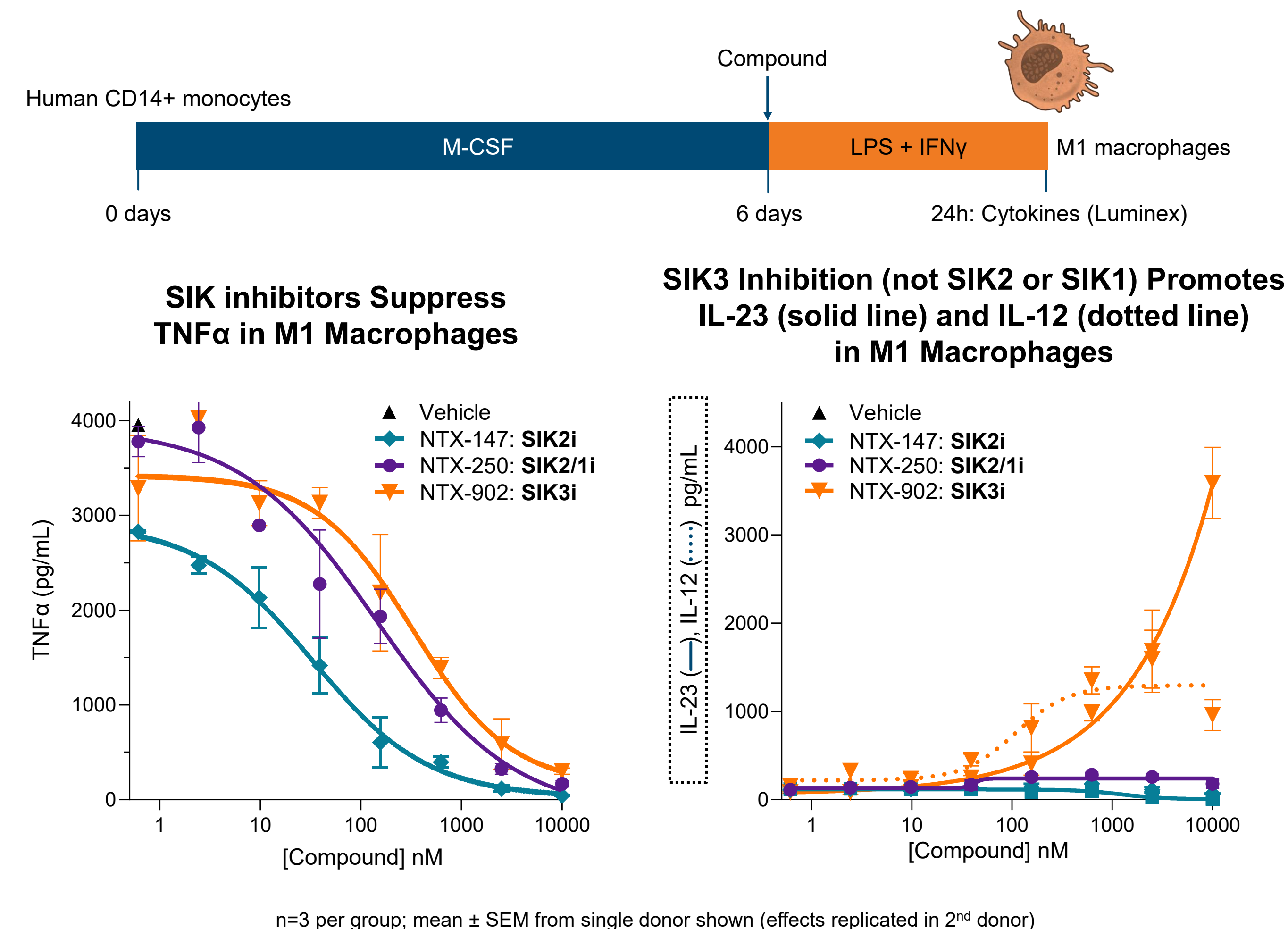
<sup>\*</sup>Cell lines engineered to express only SIK2 or SIK3, with SIK1 and/or SIK2/SIK3 knocked out

**Fig 2: SIK2-Selective Inhibitors Promote Anti-inflammatory and Pro-resolution Profiles in Human Macrophages: A More Favorable Profile vs. JAK1 Inhibition in Myeloid Cells**

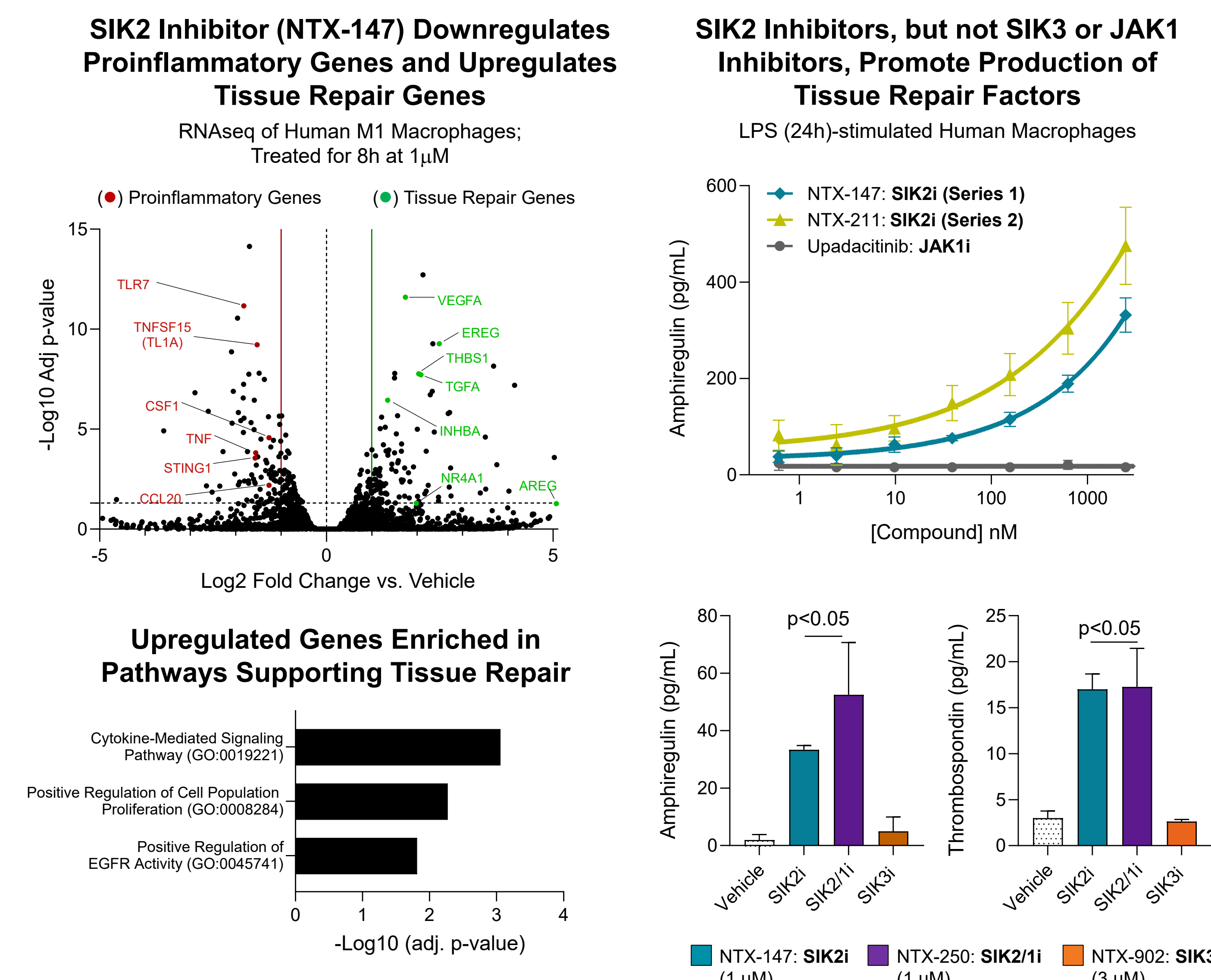


- SIK2 inhibitors decrease TNF $\alpha$ , IL-23, IL-6 (not shown) and increase IL-10 in LPS-stim human macrophages.
- Upadacitinib stimulated TNF $\alpha$  and IL-23 with no effect on IL-10.

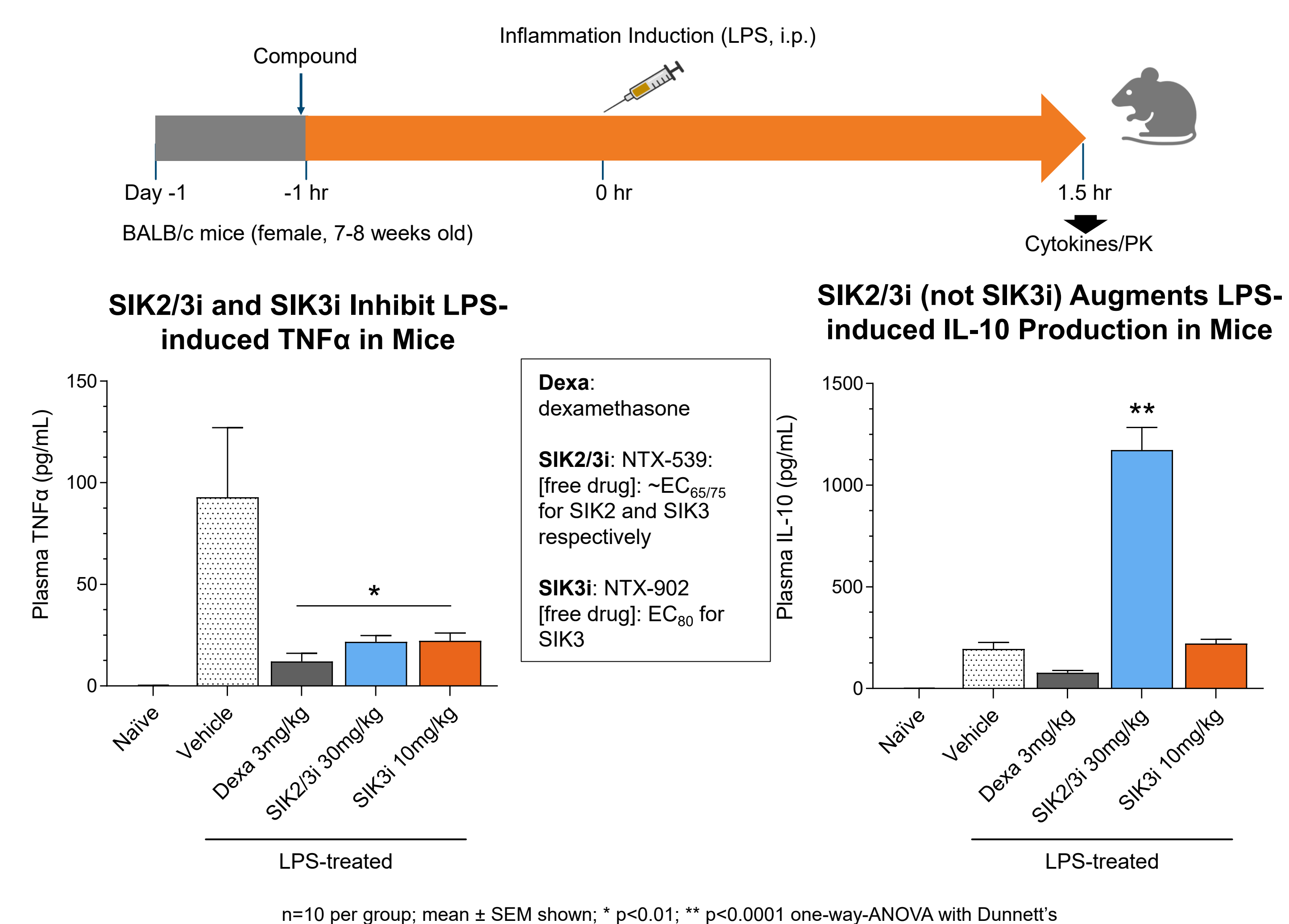
**Fig 3: SIK2-Selective and SIK3-Selective Inhibitors Exhibit Differential Pharmacology in Proinflammatory Macrophages**



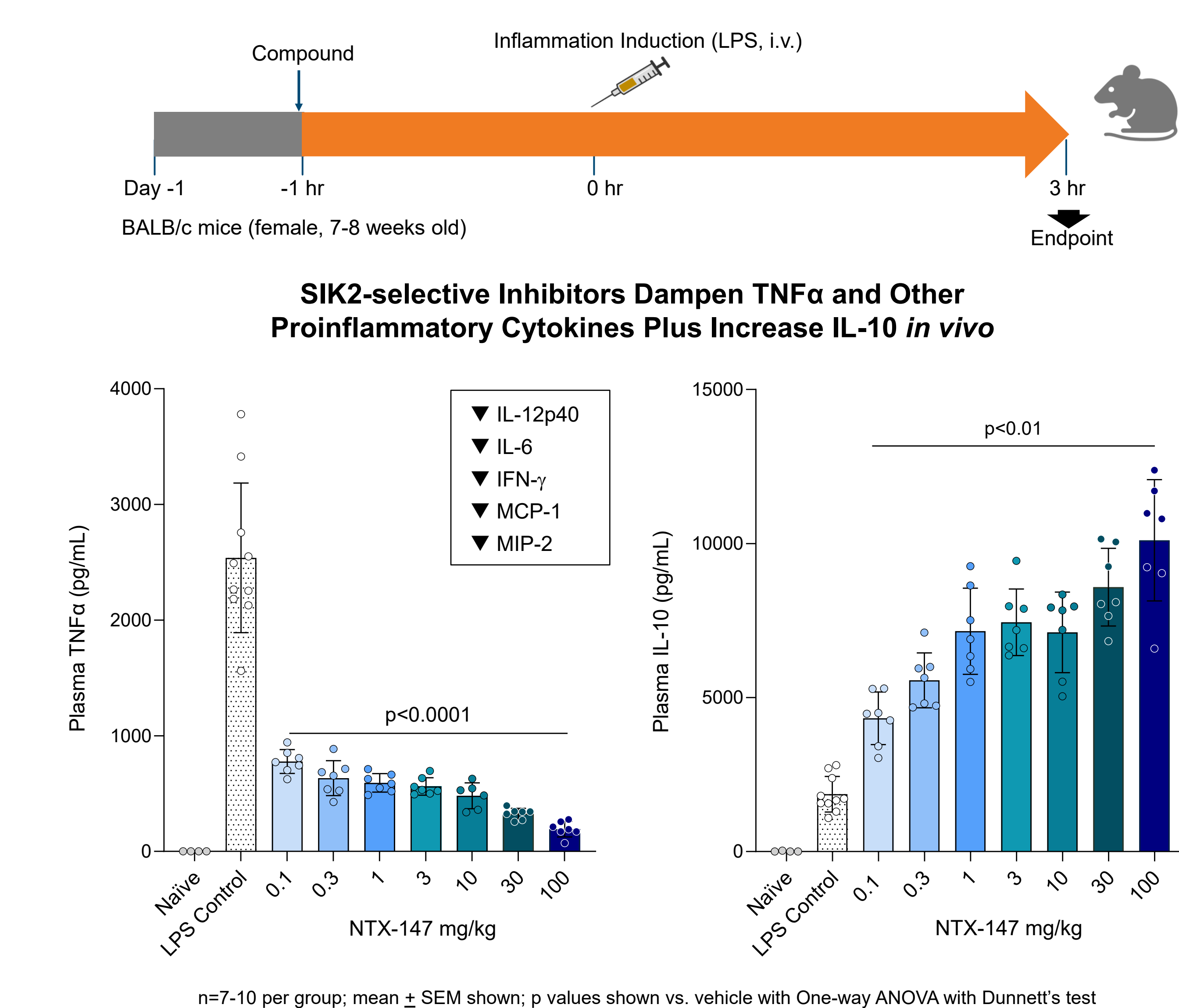
**Fig 4: SIK2 Inhibitors Downregulate Proinflammatory Cytokines While Upregulating Tissue Repair Factors in Human Macrophages; Effects not Observed with JAK1 or SIK3 Inhibitors**



**Fig 5: SIK2 Inhibition (not SIK3) is Required for the Dual Mechanism-of-action of TNF $\alpha$  Lowering and IL-10 Upregulation in Mice**



**Fig 6: SIK2-Selective Inhibitors Dose-Dependently Lower Multiple Proinflammatory Cytokines with Concomitant IL-10 Upregulation**



## CONCLUSIONS

- We identified highly potent and selective SIK2 inhibitors which demonstrate a novel dual mechanism of action in myeloid cells and with *in vivo* LPS challenge:
  - Downregulation of multiple proinflammatory cytokines,
  - Elevation of pro-resolution and tissue repair factors,
  - No effect on phagocytosis in pro-resolving (M2c) macrophages (data not shown).
- This profile was not observed with SIK3 or JAK1 inhibitors, where elevation of proinflammatory cytokines was also observed. These proinflammatory effects have been noted by others.<sup>6,7,8</sup>
- The cellular and *in vivo* profile of SIK2 inhibitors translates to robust efficacy and protection of the mucosal barrier in mouse colitis models as presented by Nimbus at this year's ECCO conference.<sup>9,10</sup>
- Therefore, selective SIK2 inhibitors may offer a differentiated oral therapeutic approach for inflammatory bowel disease and other chronic inflammatory diseases where:
  - Pathogenic and damage signals drive innate immunity,
  - Mucosal or tissue healing are critical to achieve remission.

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