



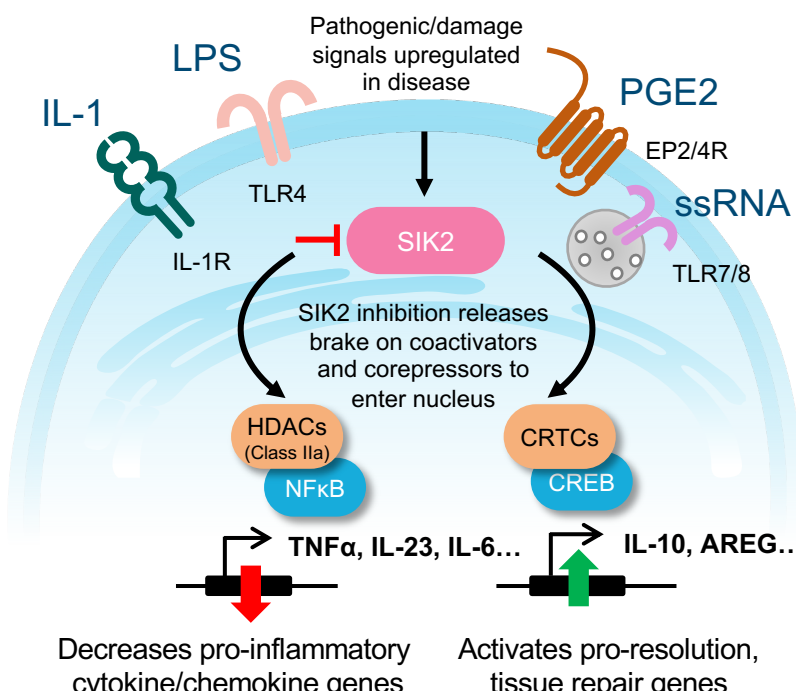
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BACKGROUND

Salt-Inducible Kinase 2 is a Key Signaling Node Relevant to IBD Pathology

- Salt-inducible kinases (SIKs) amplify proinflammatory gene expression in myeloid cells via phosphorylation of transcriptional coregulators.¹
- Genetic loss-of-function (LOF) of SIK2 in mice or use of pan-SIK inhibitors decreases proinflammatory cytokines (TNF α , IL-12/23, IL-6, and IL-1 β) induced by Toll-like receptor (TLR) or IL-1 receptor (IL-1R) agonists.^{1,2}
- SIK2 LOF also enhances production of the anti-inflammatory cytokine IL-10, an effect not observed with SIK1 or SIK3 LOF.²
- Unique positioning downstream of pathogen and damage signals elevated in inflammatory diseases.
- Dual mechanism of action.
 - Suppresses pro-inflammatory cytokines and chemokines; promotes tissue repair and resolution.
- Therapeutic potential where inflammation and damage occurs in mucosa and joint, e.g., IBD, RA.
 - SIK2 inhibitor mechanism blocks signaling during active inflammatory stimuli, analogous to TL1A.



METHODS

- Female BALB/c mice were dosed p.o. with the SIK2-selective inhibitor NTX-147, followed 1 hour later by LPS via i.p. injection or *ex vivo* stimulation, and TNF α and IL-10 were quantified by ELISA.
- Human PBMCs and whole blood from healthy donors were preincubated with NTX-147, stimulated *ex vivo* with LPS, and supernatants analyzed by MSD and/or NULISA inflammation panel.
- Colon explants from ulcerative colitis patients were cultured *ex vivo* for 18 hours with upadacitinib or NTX-147; supernatants assessed with MSD, and biopsy RNA isolated for bulk transcriptomics.

RESULTS

Fig 1: High SIK2 Selective Inhibition to Achieve Desired Pharmacology

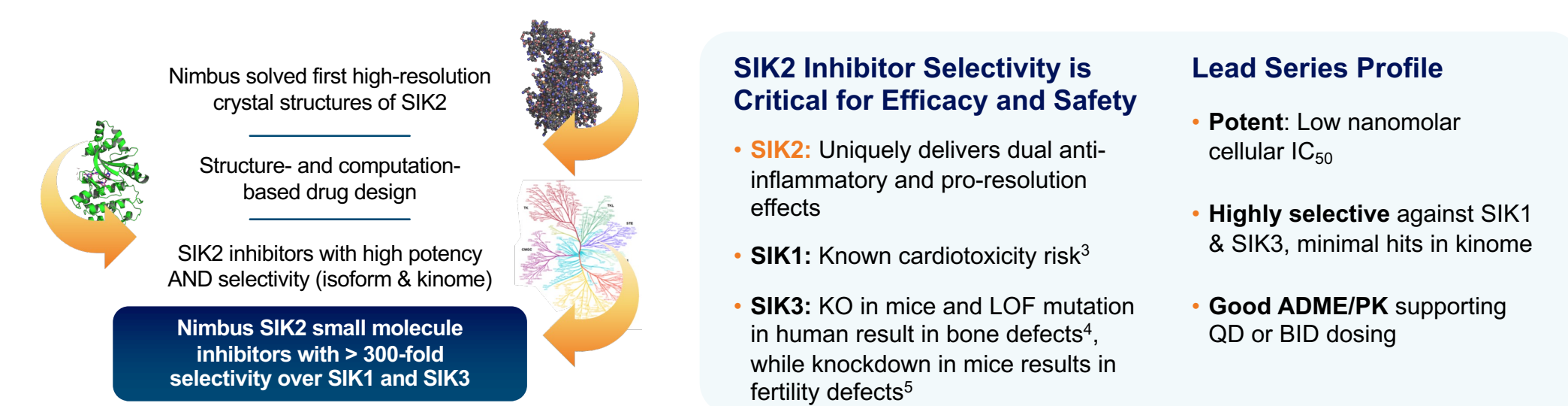
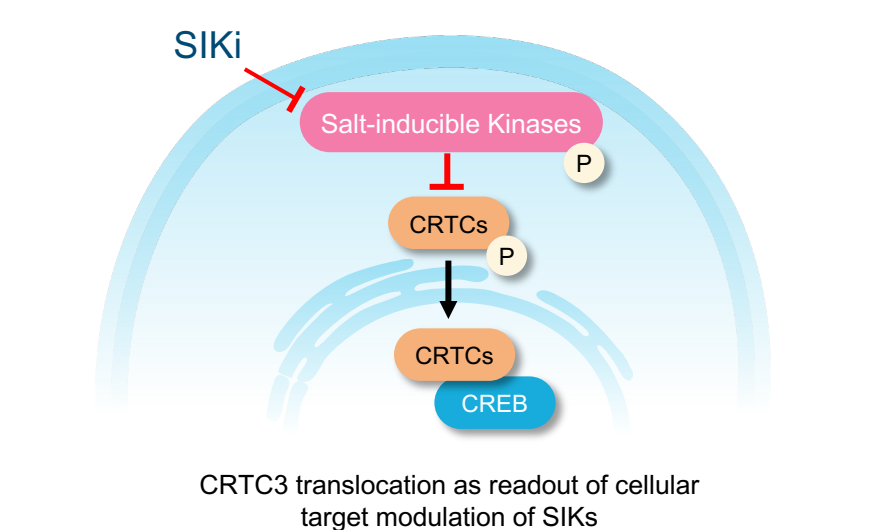


Table 1: SIK2 is a Key Signaling Node Relevant to Disease Biology



Parameter	<i>In vitro</i> Assay	NTX-147
Biochemical Potency (K _{i,app} , μ M)	Human SIK2 ADP-Glo	0.002
	Human SIK1 ADP-Glo	1.76
	Human SIK3 ADP-Glo	0.24
Cellular Potency (μ M)	SIK2-only HEK-293, CRTC3 nuclear translocation (EC ₅₀)	0.0003
	SIK2-only RAW264.7, CRTC3 nuclear translocation (EC ₅₀)	0.0046
	SIK3-only RAW264.7, CRTC3 nuclear translocation (EC ₅₀)	5.13
	Human monocyte-derived macrophage, TNF α by LPS (IC ₅₀)	0.086
Selectivity (Fold)	Selectivity over SIK1 (biochem)	733x
	Selectivity over SIK3 (biochem / cell)	100x / 1115x
Kinome panel	8 of 408 kinases (2%) with >70% inhibition at 1 μ M and 1 mM ATP	

Fig 2: SIK2-Selective Inhibitor Inhibits TNF α and Activates IL-10 Following LPS Challenge in BALB/c Mice

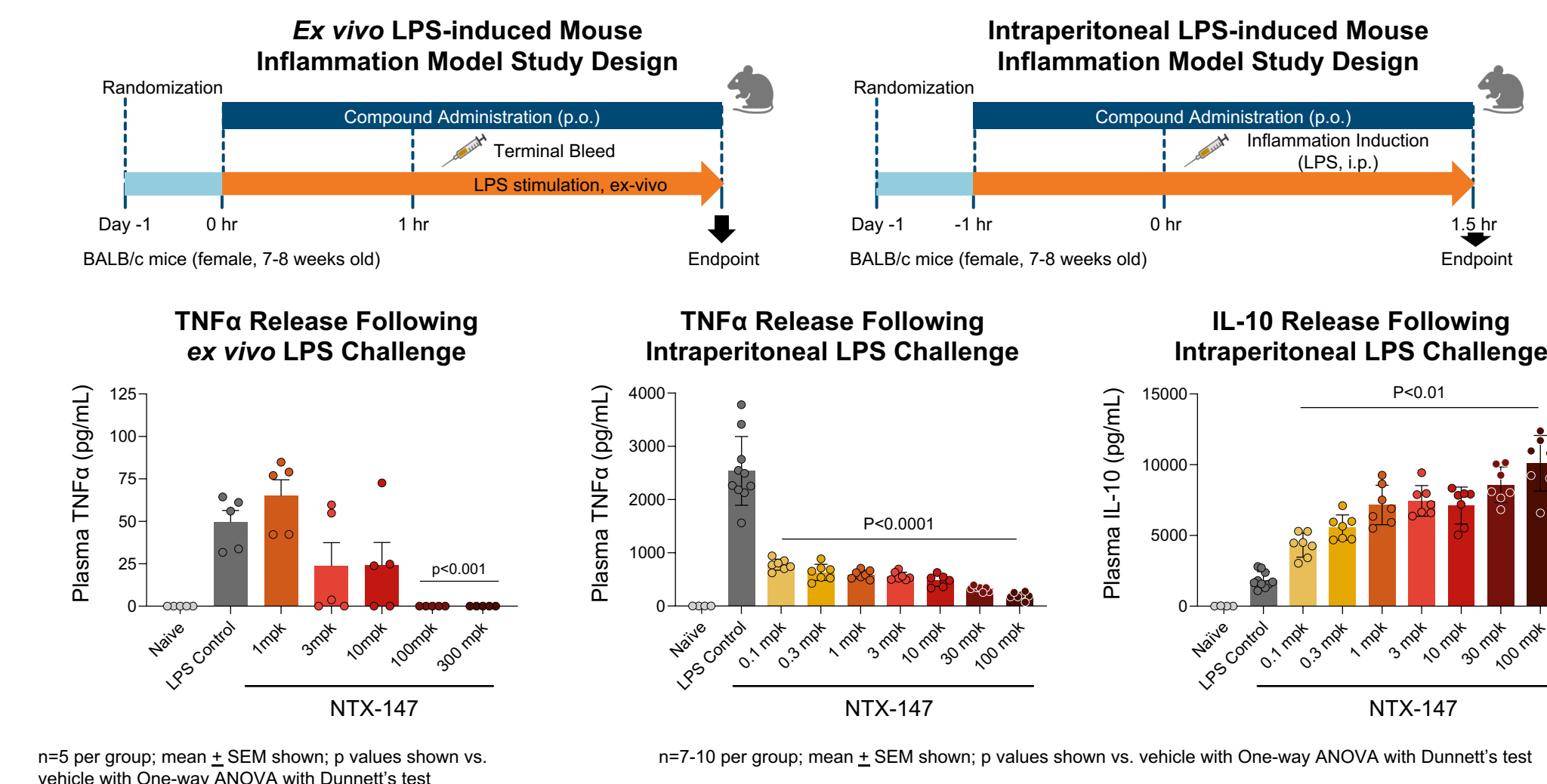


Fig 3: SIK2-Selective Inhibitor Inhibits Pro-inflammatory Cytokines in Human PBMCs ex vivo Challenged with LPS

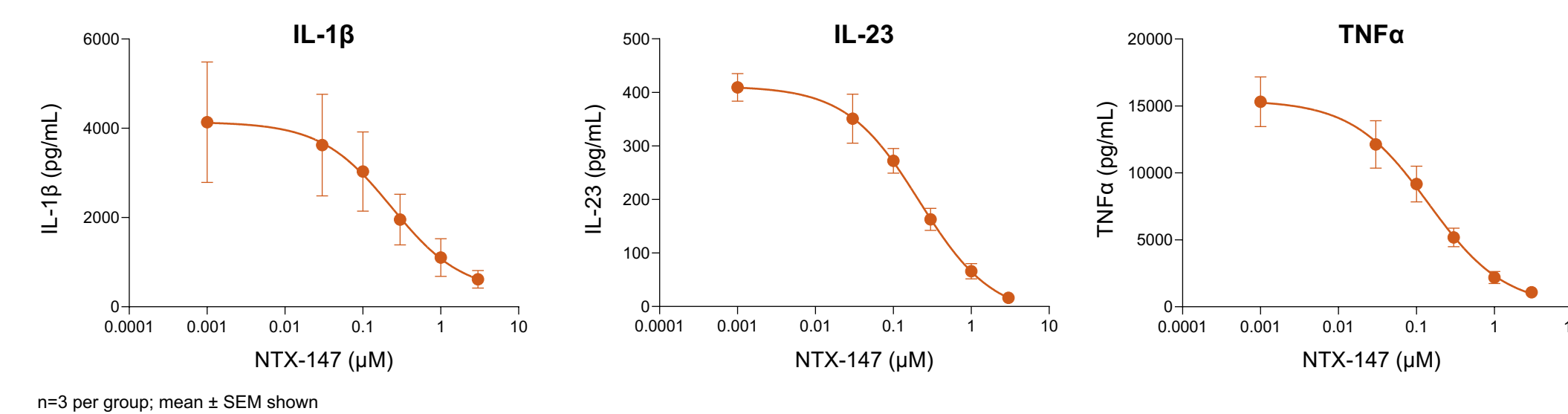


Fig 4: SIK2-Selective Inhibitor Inhibits a Broad Array of Pro-inflammatory Cytokines with Minimal Inter-donor Variability Observed in LPS-stimulated Human Whole Blood

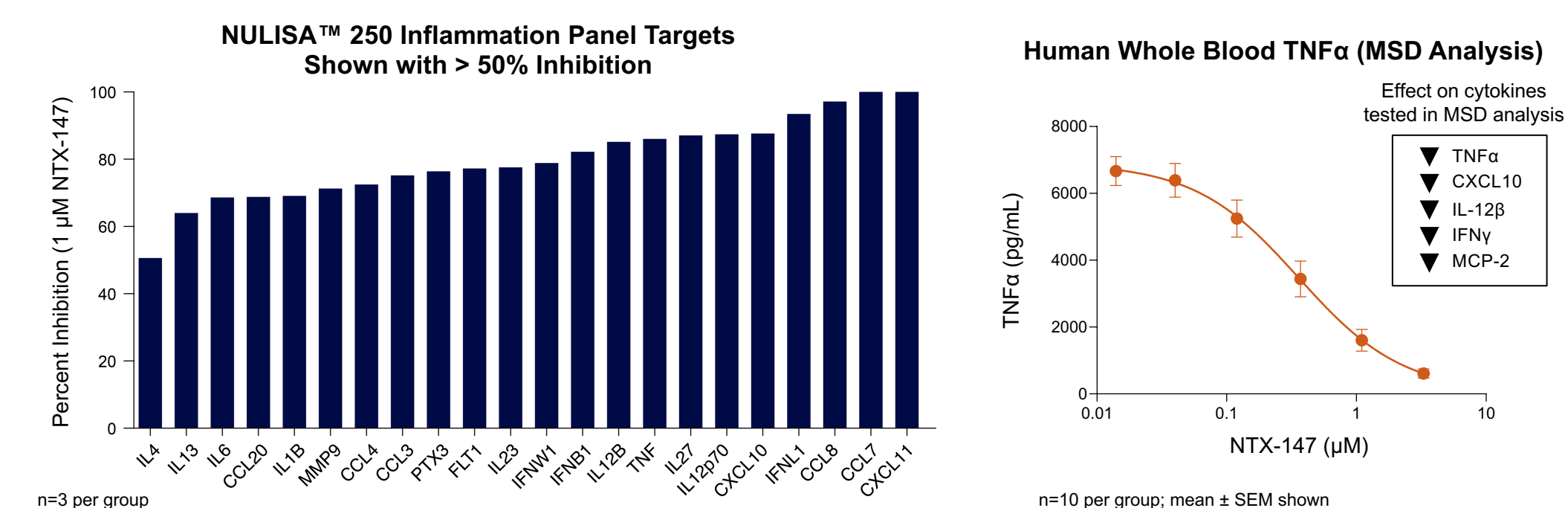


Fig 5: SIK2-Selective Inhibitor Reduces Inflammatory Cytokines In Unstimulated Explants from Ulcerative Colitis Patients

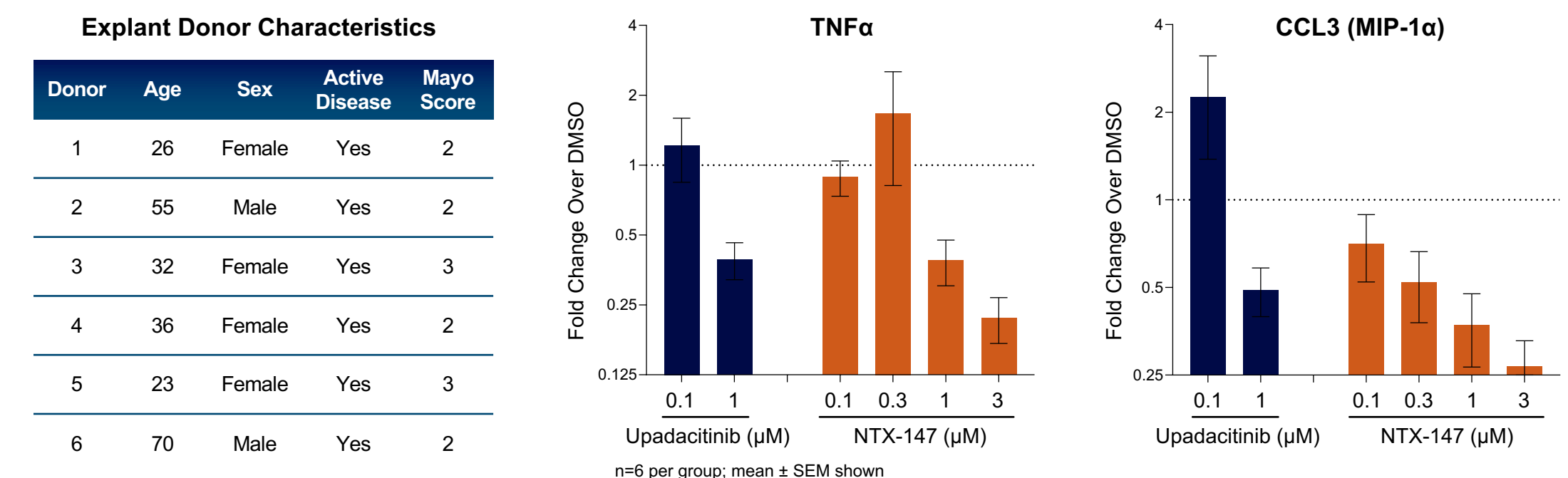
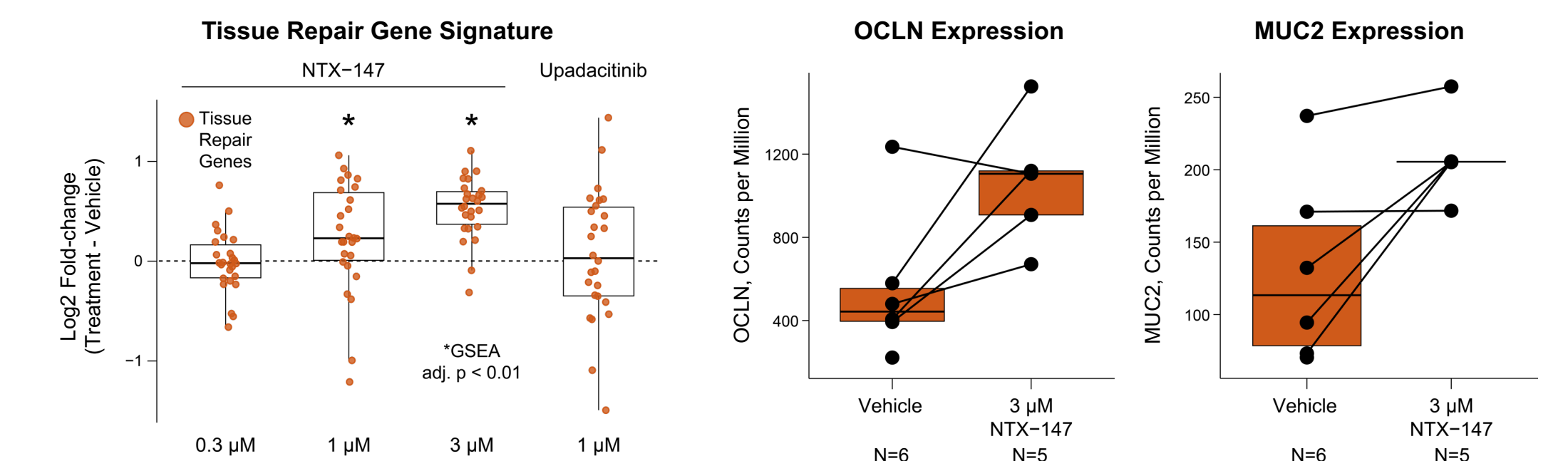


Fig 6: Increased Tissue Repair Gene Expression Observed in Ulcerative Colitis Explants Following SIK2-Selective Inhibitor Treatment



- In ulcerative colitis explants a panel of 26 curated genes involved in tissue repair show a dose-dependent median increase in RNA expression following NTX-147 treatment (GSEA adj. p < 0.01), and no significant shift with 1 μ M upadacitinib treatment.
- NTX-147 treatment of ulcerative colitis explants lead to increased expression of mucosal barrier integrity and intestinal regeneration markers such as Occludin (OCLN) and Mucin-2 (MUC2).

CONCLUSIONS

- The SIK2 selective inhibitor, NTX-147, reduced TNF α and concomitantly increased IL-10 in LPS-challenged mice.
- NTX-147 reduced a broad set of pro-inflammatory cytokines in whole blood collected from healthy human donors stimulated *ex vivo* with LPS.
 - Minimal inter-donor variability was observed supporting the utility of *ex-vivo* LPS challenge in a first-in-human clinical study to demonstrate effect of SIK2 inhibition on pro-inflammatory cytokines.
- In unstimulated ulcerative colitis explants, NTX-147 reduced inflammatory cytokines and upregulated genes associated with healthy colon tissue, including those involved with tissue repair.
- These findings, including the distinct tissue-repair signature relative to upadacitinib, support SIK2 as a potential novel oral therapeutic approach for IBD conferring both anti-inflammatory and tissue reparative benefits for patients.

REFERENCES

1. Lombardi MS, et al. *J Leukoc Biol*. 2016;99(5):711-721. doi:10.1189/jlb.2a0715-307r; 2. Darling NJ, et al. *Biochem J*. 2017;474(4):521-537. doi:10.1042/BCJ20160646 3. Desroy, N. et al. *Med. Chem. Rev.* 2023 58(9): 209-231; 4. Csukasi F, et al. *Sci Transl Med*. 2018;10(459):eaat9356; 5. Hayes, ET, et al. *Endocrinology* 2024; 165(10):1-10

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