

Supplementary information for:

Utilizing human TLR selective ligands in a humanized immune system mouse model to investigate human TLR4 signaling

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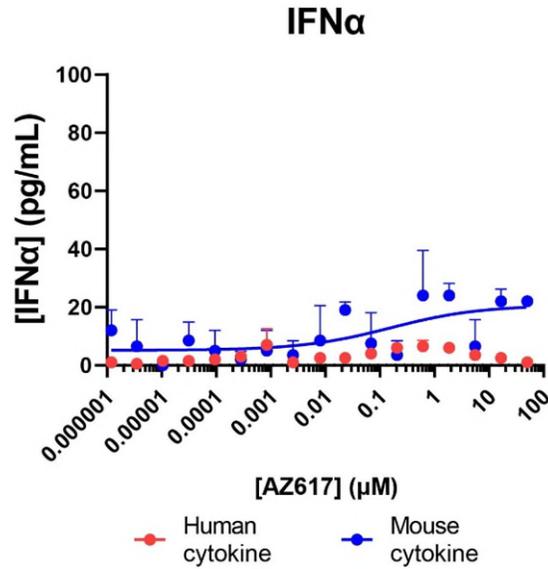
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Use	Marker	Clone	Fluorochrome	Vendor	Catalog #
Cell frequencies from <i>in vivo</i> experiments	huHLA-DR	G46-6	BUV395	BD Biosciences	564040
	huCD16	3G8	BV421	Biolegend	302038
	huCD20	2H7	BV510	Biolegend	302340
	huCD19	H1B19	BV711	Biolegend	302246
	huCD14	M5E2	FITC	Biolegend	301804
	huCD11c	3.9	PE-Cy7	Biolegend	301608
	huCD3	SK7	AF700	Biolegend	344822
	huCD11b	M1/70	PE Dazzle 594	Biolegend	101256
	huCD45	HI30	BV785	Biolegend	304048
Engraftment checks from longitudinal experiment	muCD45	30-F11	BV605	Biolegend	103155
	muCD45	30-F11	AF647	Biolegend	103124
	huCD45	2D1	PE	Biolegend	368510
	huCD14	63D3	BV421	Biolegend	367143

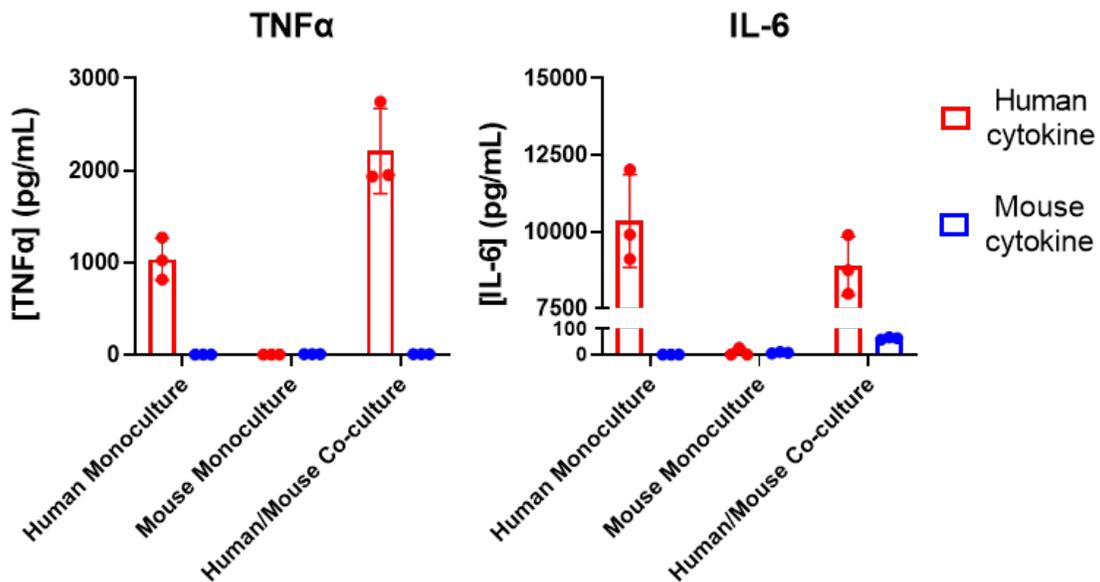
**Table S1: Staining antibodies used for flow cytometry.** Clones and fluorochromes used for assessment of cell frequencies in *in vivo* efficacy experiments as well as for engraftment assessment of human leukocyte and myeloid cells in longitudinal experiment.

Donor ID	Age	Sex	Demographic	Smoker	Current medications	Health comments
CC00863	51	M	Caucasian	No	Lisinopril	None
CC00935	37	F	Caucasian	No	Levothyroxine	None
RM10158	58	F	Caucasian	No	None	None
90265	37	F	Caucasian	No	Lexapro	None

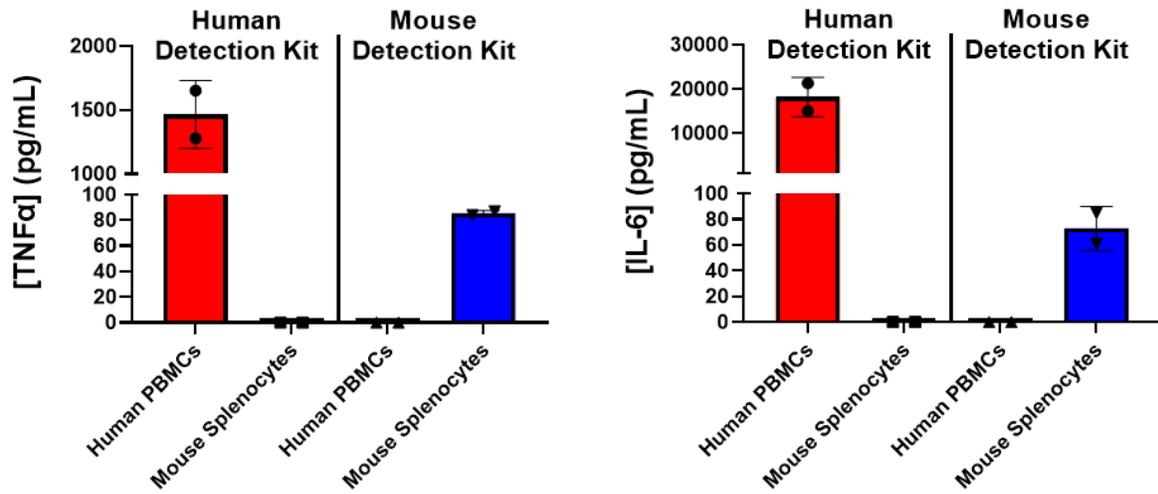
**Table S2: Demographic information for human PBMC donors.**



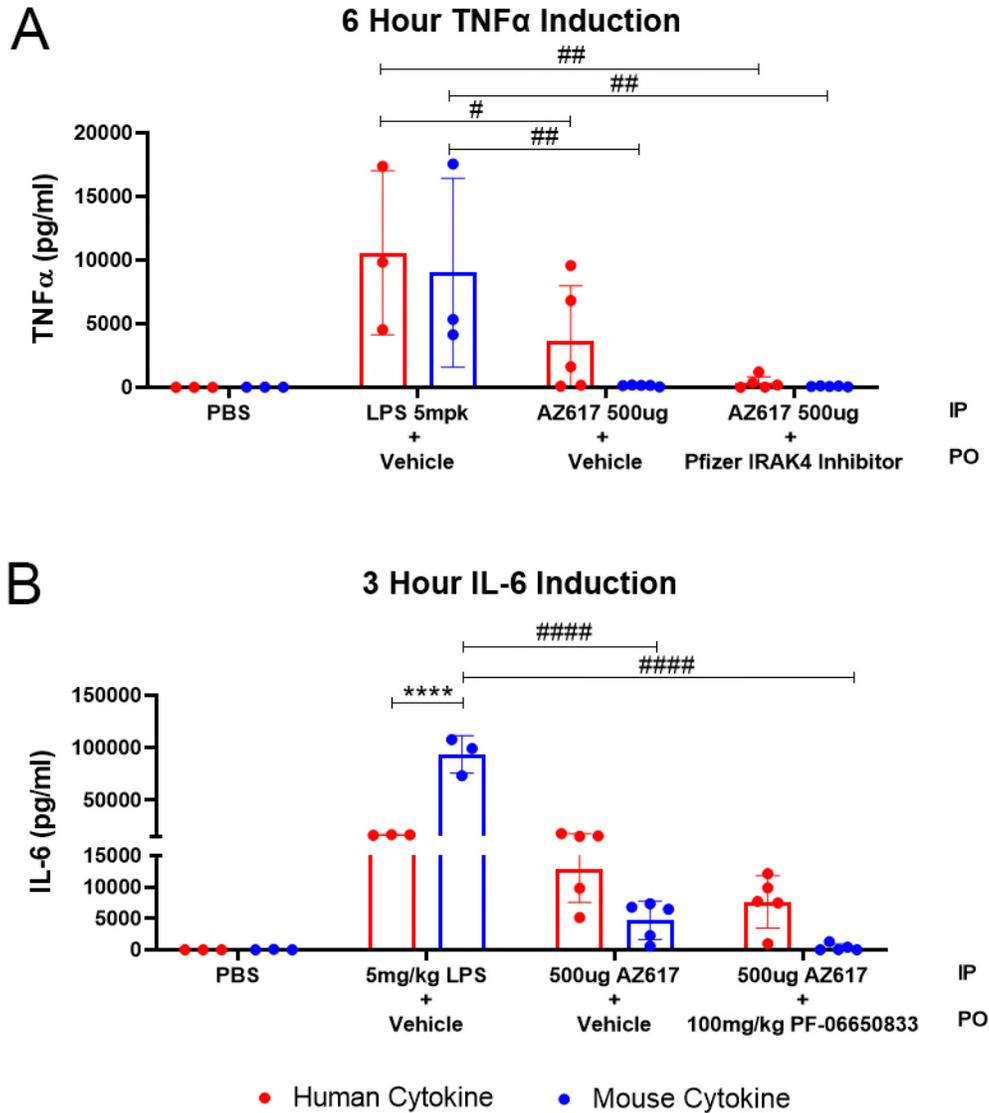
**Figure S1. AZ617 does not induce IFN $\alpha$ .** Neither human nor C57/B6 mouse cells secreted IFN $\alpha$  in response to AZ617 titration. Representative graphs shown from 4 independent experiments. Error bars represent standard error of the mean (SEM).



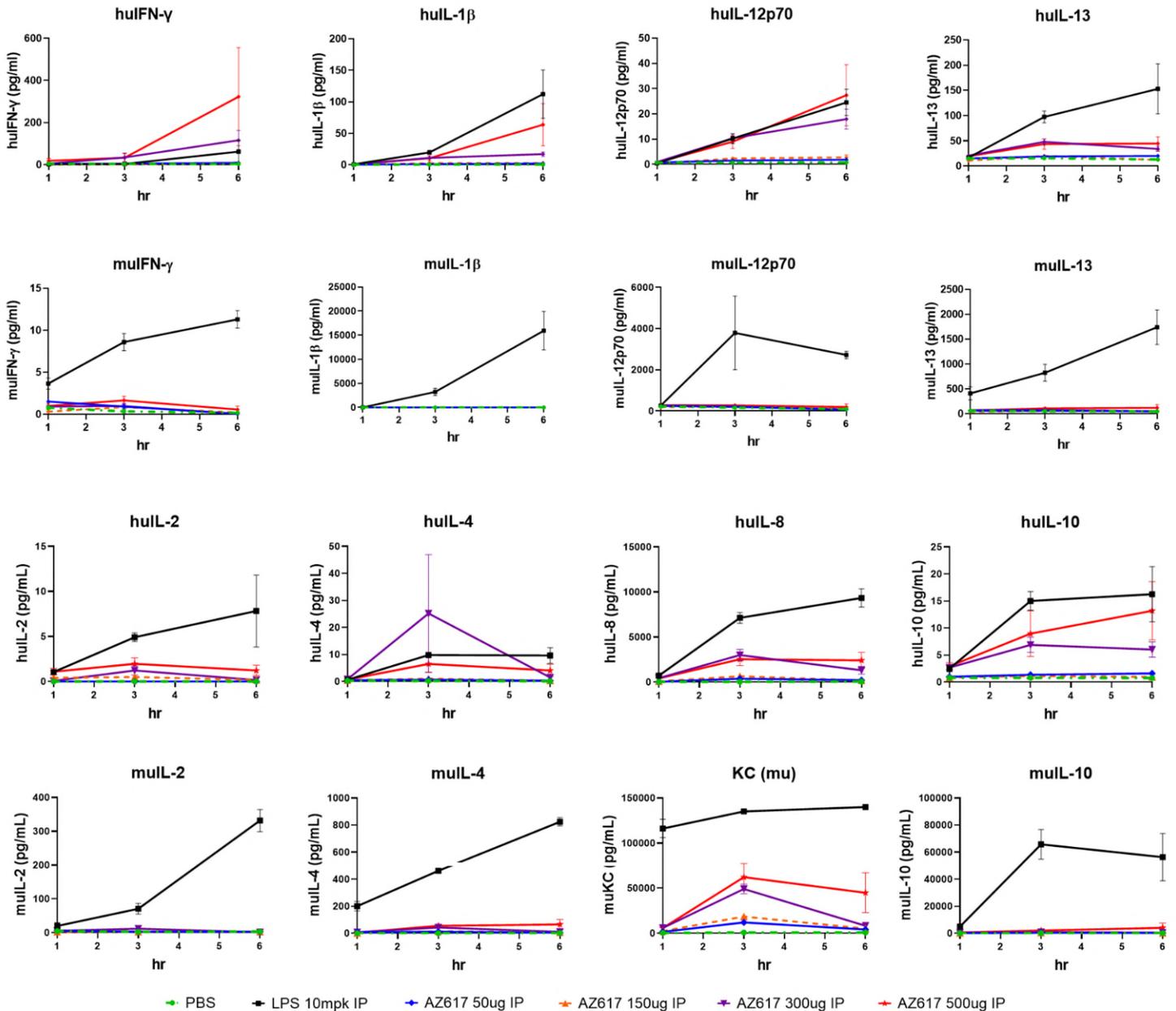
**Figure S2. Human PBMCs or mouse splenocytes do not cross-talk *in vitro* upon AZ617 stimulation.** Human PBMCs or naïve C57/B6 mouse splenocytes are incubated in either monoculture, or co-culture with equal cell inputs, with AZ617 for 24 hours prior to supernatant analysis for species-specific TNF $\alpha$  or IL-6 concentrations. Representative graph of 2 independent experiments. Error bars represent standard error of the mean (SEM).



**Figure S3. Human and mouse TNF $\alpha$  and IL-6 detection kits do not cross-react.** Human PBMCs and C57/B6 mouse splenocytes were incubated in monoculture with 1ng/mL LPS for 6 hours. Following incubation, supernatants were assayed for TNF $\alpha$  (A) or IL-6 (B) using Meso Scale Discovery (MSD) singleplex kits. Data is representative of 3 independent experiments.



**Figure S4. Comparison of mouse and human TNF $\alpha$  and IL-6 outside of ideal time points.** **A.** At 6 hours post-challenge, the significant differences between human and mouse TNF $\alpha$  after AZ617 challenge, as well as with IRAK4 inhibitor, was lost. **B.** At 3 hours post-challenge, human and mouse IL-6 looked similar to levels observed after 6h; induction was not different between species, and IRAK4 inhibition did not reduce circulating levels of IL-6. Statistics were as follows: human vs. mouse by two-way ANOVA and Sidak's multiple comparisons, where \* $p < 0.05$  and \*\*\*\* $p < 0.0001$ . Human/human or mouse/mouse by two-way ANOVA and Tukey's multiple comparisons, where # $p < 0.05$  and ##### $p < 0.0001$ . Representative data out of two experiments,  $n = 3-9$ /group.



**Figure S5. Profiles of additional human and mouse cytokines after AZ617 challenge.** Slight increases in human IL12p70, IFN $\gamma$  and IL-1 $\beta$  were observed at 500 $\mu$ g, but overall values were generally low. The greatest induction was observed in human IL-8. Of note, mouse KC (equivalent to human IL-8) was induced to greater levels than hIL-8, but neither species showed induction with AZ617 over levels generated with LPS.