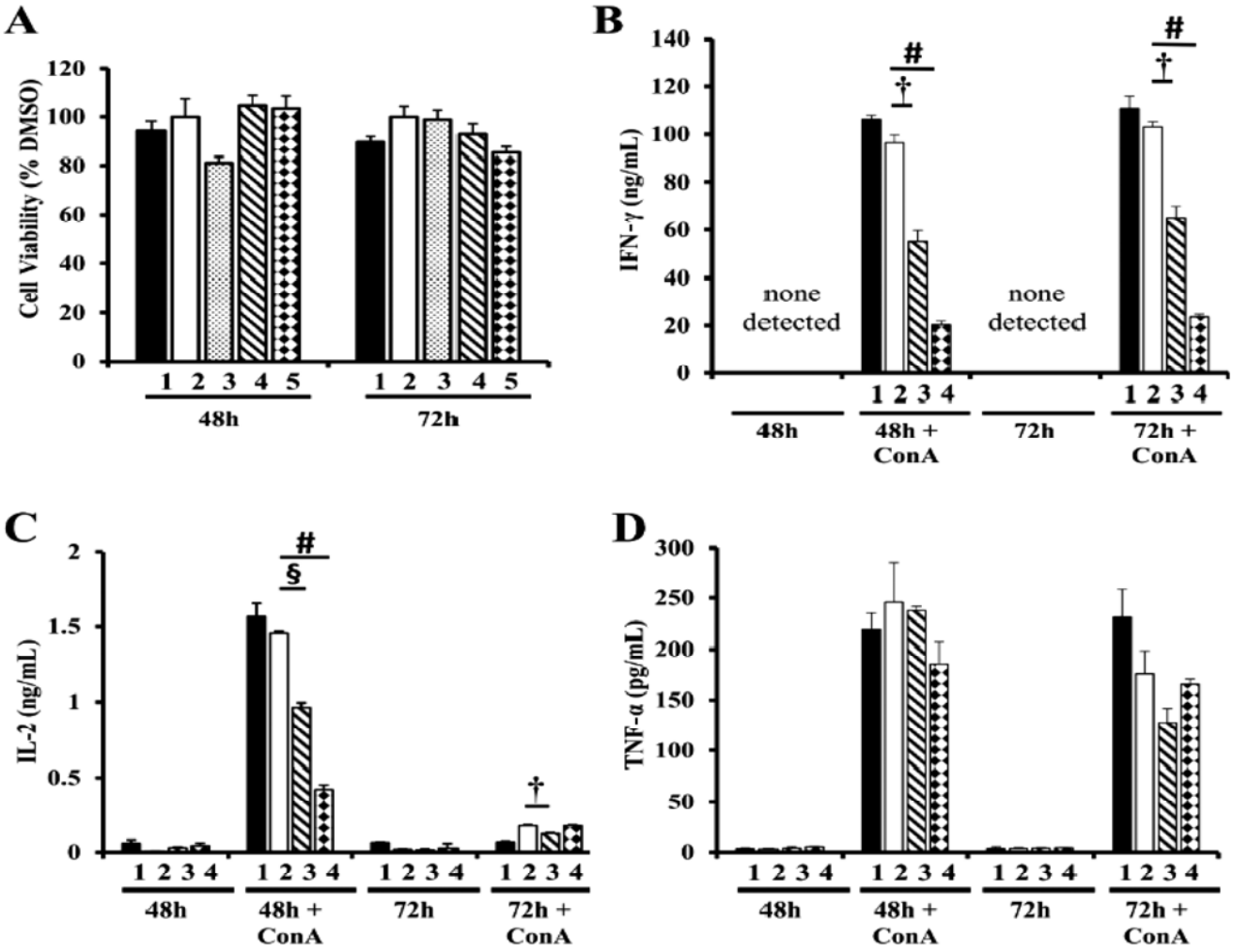
SUPPLEMENTARY DATA

Effects of FKGK18 on mixed splenocytes and macrophage viability and cytokine production. To examine the impact of iPLA$_2$β inactivation by FKGK18 on immune function, we first examined cytokine production from mixed splenocytes. Cell Viability (**Suppl. Fig. 1*A***) was not compromised following exposure to FKGK18 for 72 h, in comparison with vehicle-treated cells, suggesting absence of cytotoxicity. As expected, cytokine production from splenocytes was negligible under basal conditions and was induced by the non-specific mitogen concanavalin A (1) at 48 and 72 h (**Suppl. Figs. 1*B*-*D*)**. However, in the presence of FKGK18, production of IFN-γ (**Suppl. Fig. 1*B***) and IL-2 (**Suppl. Fig. 1*C***) was significantly reduced, while that of TNF-α (**Suppl. Fig. 1*D***) was unaffected. These data suggest that iPLA$_2$β activation can modulate immune cell responses.

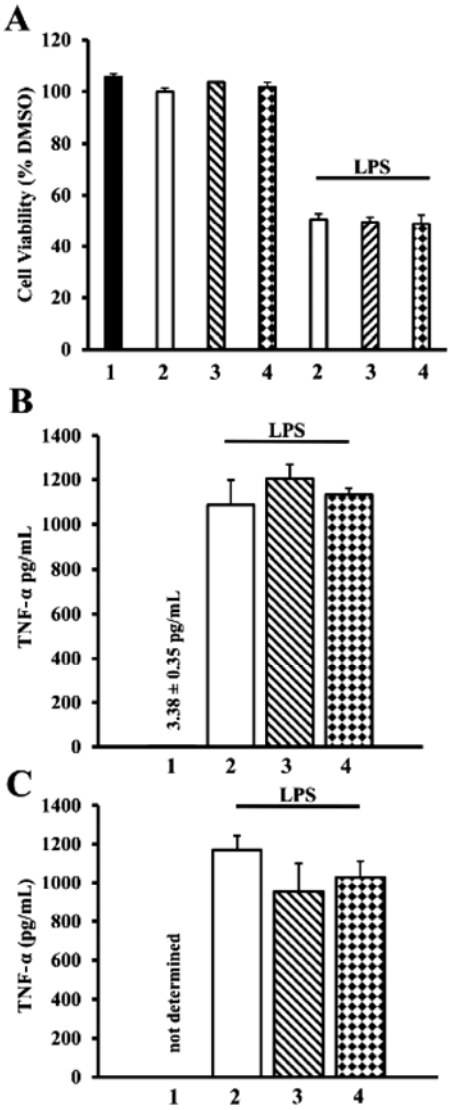
Similar analyses were done with macrophages and their viability was also unaffected by FKGK18 (**Suppl. Fig. 2*A***) and as expected, the classical activator of macrophages, LPS (2) decreased viability and increased TNF-α production. In contrast to its effect on CD4$^+$ T-cells, FKGK18 did not affect LPS-stimulated TNF-α generation from either bone marrow-derived (**Suppl. Fig. 2*B***) or peritoneal (**Suppl. Fig. 2*C***) macrophages.



SUPPLEMENTARY DATA

METHODS. *Splenocyte cytokine production assay*. Spleens obtained from 8-12 week-old NOD mice were individually homogenized into single-cell suspensions and red blood cells lysed. Splenocytes were seeded in 96-well plates (5 x 10^5 cells/well) and challenged with concanavalin A (2.5 μg/mL, Sigma-Aldrich, St. Louis, MO). The concentrations of cytokines at 48 and 72 h in the supernatant were measured by ELISA (IL-2, IFN-γ, BD Biosciences; TNF-α, R&D Systems, Minneapolis, MN).

LEGEND. *Viability of and cytokine production by splenocytes in the absence and presence of FKGK18*. Splenocytes were prepared from 8-12 week-old NOD mice, plated in 96-well plates (5 x 10^5 cells/well) and cultured in media alone (± DMSO) or one containing concanavalin A (2.5 μg/mL). ***A*:** *Cell Viability*. MTT assay was used to assess number of viable cells, as described in **Fig. 5**, and absorbance, relative to DMSO, are presented. (**1** = -DMSO, **2** = +DMSO, **3** = 10^{-7} mol/L FKGK18, **4** = 10^{-6} mol/L FKGK18, **5** = 10^{-5} mol/L FKGK18; n = 6 under each condition) ***B-D:** Cytokine production.* IFN-γ (***B***), IL-2 (***C***), and TNF-α (***D***) in the media at 48 and 72 h were measured by ELISA and presented as mean ± SEM ng/mL and pg/mL. **1** = -DMSO, **2** = +DMSO, **3** = 10^{-6} mol/L FKGK18, and **4** = 10^{-5} mol/L FKGK18. ($^{\dagger\#\S}$Significantly different from +DMSO group, $p < 0.0005, 0.0001, 0.001$, respectively, n = 3 under each condition.)



SUPPLEMENTARY DATA

METHODS. ***Macrophage stimulation assays.*** Bone marrow-derived and peritoneal macrophages were obtained, as described (3). Macrophages (5 x 10^5/well) were seeded in 96-well plates with media ± 10 μg/mL lipopolysaccharide, and incubated (37ºC, 72 h) ± DMSO or FKGK18 (10^{-7} - 10^{-5} mol/L). The concentrations of cytokines at 72 h in the supernatant were measured by ELISA (TNF-α, R&D Systems, Minneapolis, MN).

LEGEND. ***Viability of and cytokine production by macrophages in the absence and presence of FKGK18***. Bone marrow-derived and peritoneal macrophages were prepared from 8-12 week-old NOD mice. Survival was assessed by MTT assay and cytokine generation by measurement of media contents of TNF-α by ELISA in the absence and presence of FKGK18 for 72 h. ***A*****:** *Cell viability*. MTT absorbance values for bone-derived macrophages ± LPS ± FKGK18 (n = 6 under each condition). ***B-C*****:** *TNF-α production by bone-derived (**B**) and peritoneal (**C**) macrophages*. TNF-α in the media at 72 h was measured by ELISA and presented as pg/ml (nd = not determined, n =3 under each condition). All data are mean ± SEM where **1** = -DMSO, **2** = +DMSO, **3** = 10^{-6} mol/L FKGK18, and **4** = 10^{-5} mol/L FKGK18.

1. Dutton RW. Inhibitory and stimulatory effects of concanavalin A on the response of mouse spleen cell suspensions to antigen. I. Characterization of the inhibitory cell activity. The Journal of experimental medicine 1972;136:1445-1460.
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3. Zhang X, Goncalves R, Mosser DM. The isolation and characterization of murine macrophages. Current protocols in immunology / edited by John E Coligan [et al] 2008;Chapter 14:Unit 14 11.