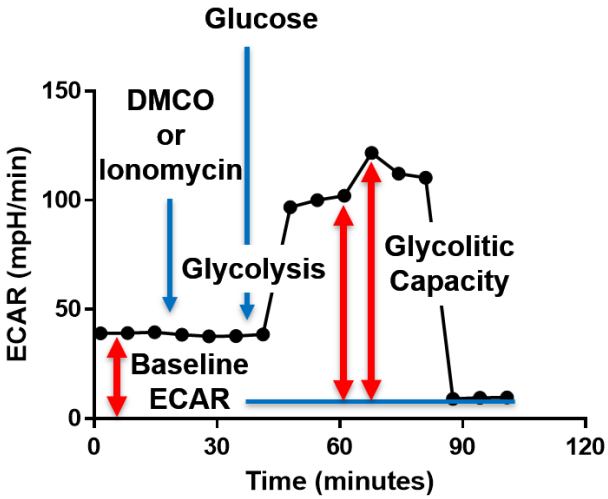
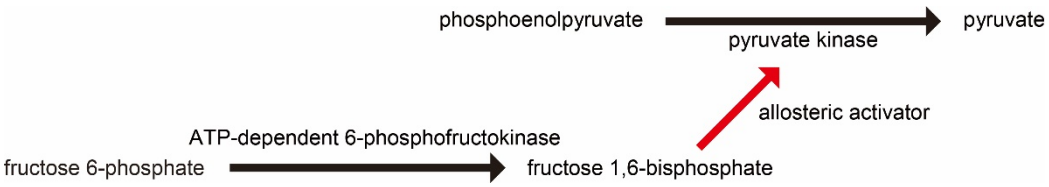
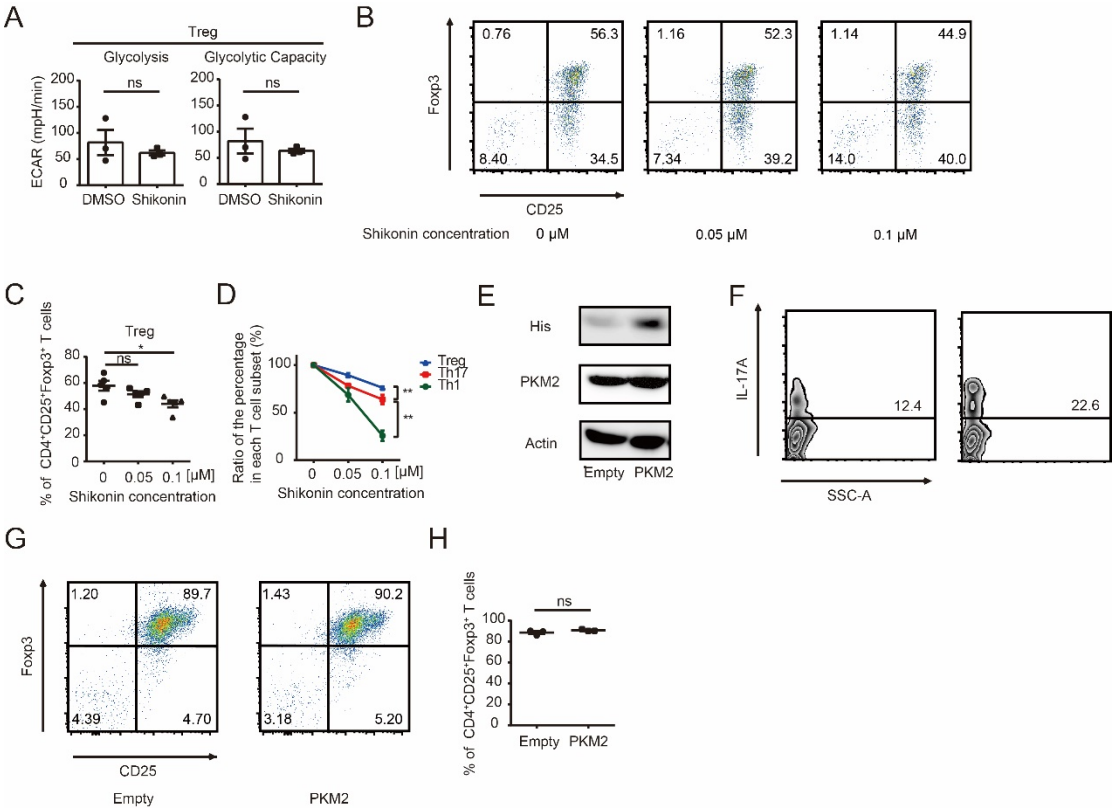
Supplemental Figures



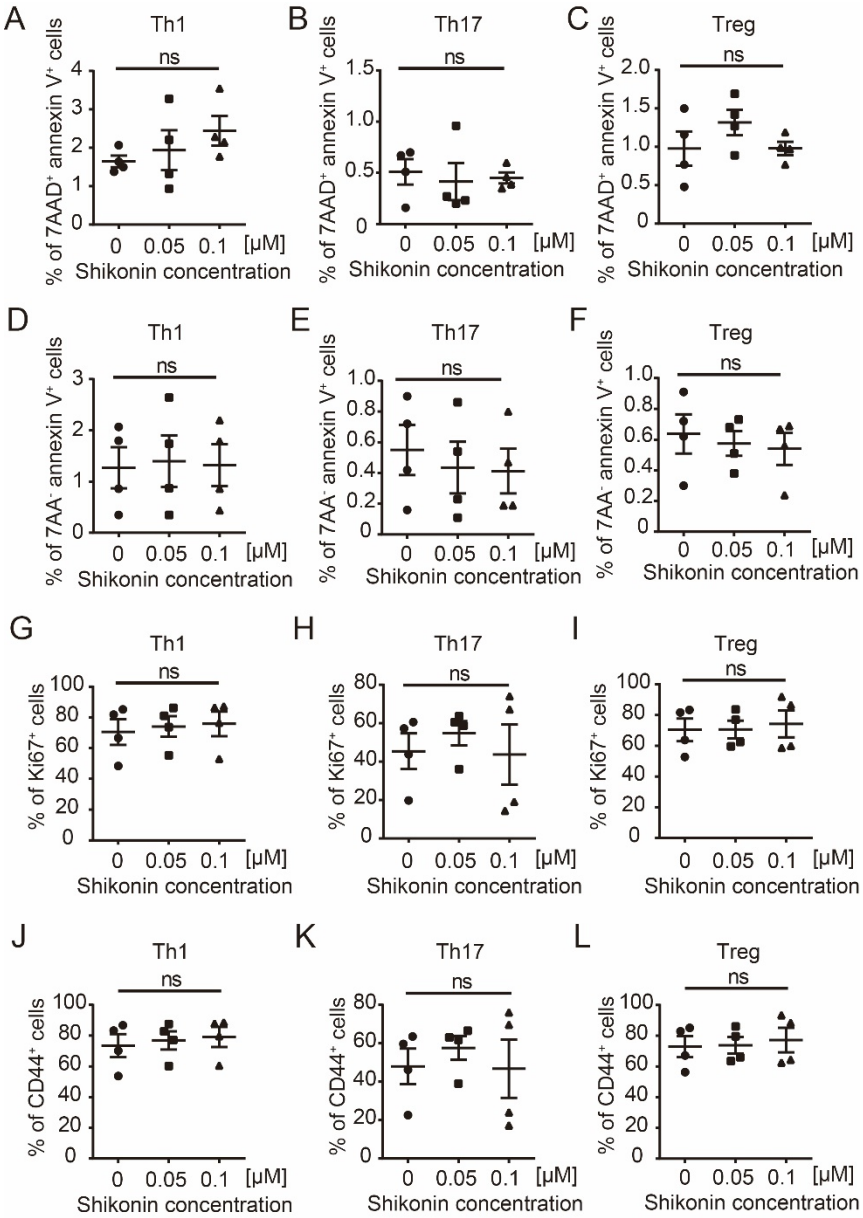
Supplemental Figure 1. Schematic representation of the experiments performed to measure glycolysis and glycolytic capacity after the injection of dimethyl sulfoxide (DMSO) or ionomycin. After the injection of DMSO or ionomycin, glycolysis stress test was performed according to the manufacturer's instructions. 2-DG, 2-deoxy-glucose. ECAR, extracellular acidification rate.



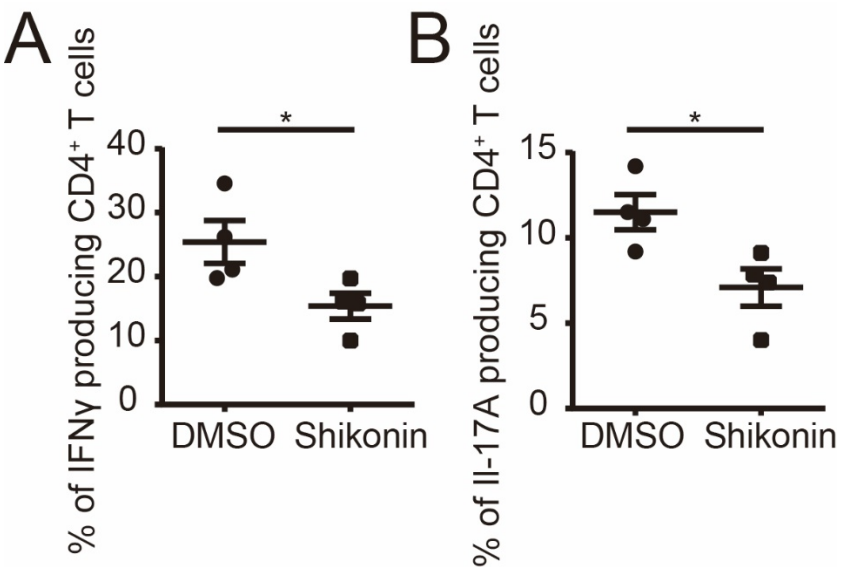
Supplemental Figure 2. Schema of the relationship between pyruvate kinase and fructose 1,6-bisphosphate. Fructose 1,6-bisphosphate, which is converted from fructose 6-phosphate by ATP-dependent 6-phosphofructokinase, is an allosteric activator of pyruvate kinase.



Supplemental Figure 3. PKM2 Promotes *in vitro* Th1 and Th17 Differentiation. (**A**-**C**) Naïve $CD4^+$ T cells were cultured for 3 days under Treg-polarizing conditions and various concentrations of shikonin were added on Day 0. (**A**) Extracellular acidification rate (ECAR) in Treg cells with or without shikonin was measured on day 3. Cumulative data are shown (mean ± SEM); n = 3. (**B**-**D**) Representative flow plots of $CD4^+CD25^+Foxp3^+$ T cells (**B**) are shown. (**C**) Cumulative data of flow plots of $CD4^+CD25^+Foxp3^+$ T cells are shown (mean ± SEM); n = 5. (**D**) Cumulative data of the ratios of the percentage in each T cell subset with shikonin/those without shikonin are shown (mean ± SEM); n = 5. (**E**-**H**) Naïve $CD4^+$ T cells were cultured for 3 days under Th17- (**E** and **F**) or Treg- (**G** and **H**) polarizing conditions and were transfected with empty or mouse PKM2 expression vector on day 1. (**E**) His, PKM2 and Actin protein expression on day 3 was accessed by Western blotting. Representative blots are shown. Data are representative of three experiments. (**F**) Representative flow plots of IL-17A producing $CD4^+$ T cells are shown. (**G**) Representative flow plots of $CD4^+CD25^+Foxp3^+$ T cells are shown. (**H**) Cumulative data of flow plots of $CD4^+CD25^+Foxp3^+$ T cells are shown (mean ± SEM); n = 3. $*P < 0.05$.$**P < 0.01$. ns, not significant. t-tests (two-tailed) or one-way ANOVA with Bonfferoni's multiple comparisons tests.



Supplemental Figure 4. PKM2 inhibitor does not affect cell viability and T cell activation. (**A**-**L**) Naïve $CD4^+$ T cells were cultured for 3 days under Th1-, Th17-, and Treg-polarizing conditions and various concentrations of shikonin were added on Day 0. (**A**-**C**) Cumulative data of the ratios of $7AAD^+$ annexin V^+ cells on day 3 are shown (mean ± SEM); n = 4. (**D**-**F**) Cumulative data of the ratios of $7AAD^-$ annexin V^+ cells on day 3 are shown (mean ± SEM); n = 4. (**G**-**I**) Cumulative data of the ratios of $Ki67^+$ cells on day 3 are shown (mean ± SEM); n = 4. (**J**-**L**) Cumulative data of the ratios of $CD44^+$ cells on day 3 are shown (mean ± SEM); n = 4. ns, not significant. one-way ANOVA with Bonfferoni's multiple comparisons tests.



Supplemental Figure 5. PKM2 inhibitor-treated $CD4^+$ T cells cause decreased disease activity in an adoptive cell transfer EAE model. (**A**-**B**) $CD4^+$ T cells from 2D2 mice were cultured with MOG_{35-55}, mitomycin-treated splenocytes, and rIL-12 for 48 hours and DMSO or shikonin were added on day 0. (**A**-**B**) The percentages of IFNγ (**A**) and IL-17A (**B**) producing cells. Cumulative data are shown (mean ± SEM); n = 4. **P* < 0.05.