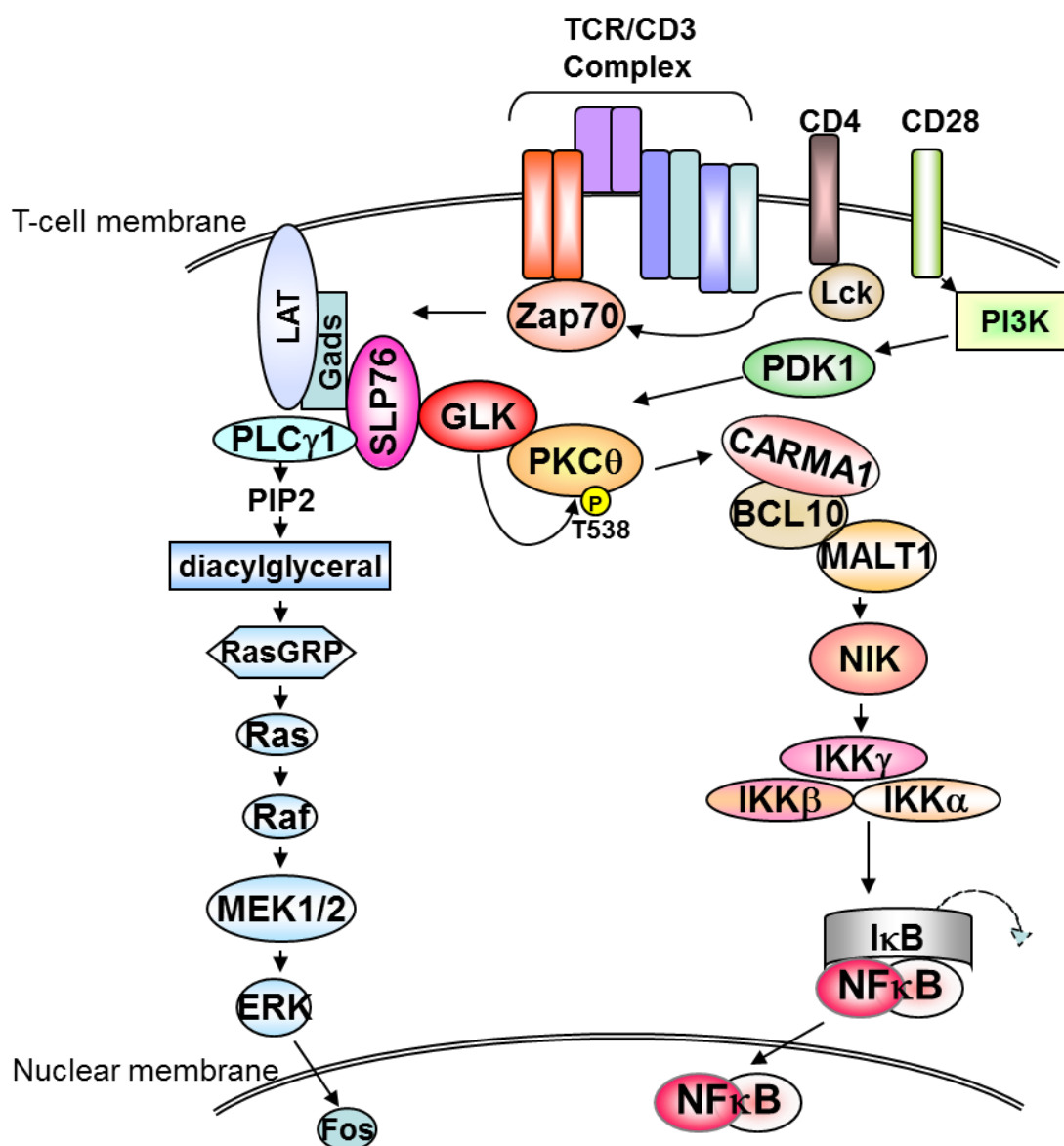
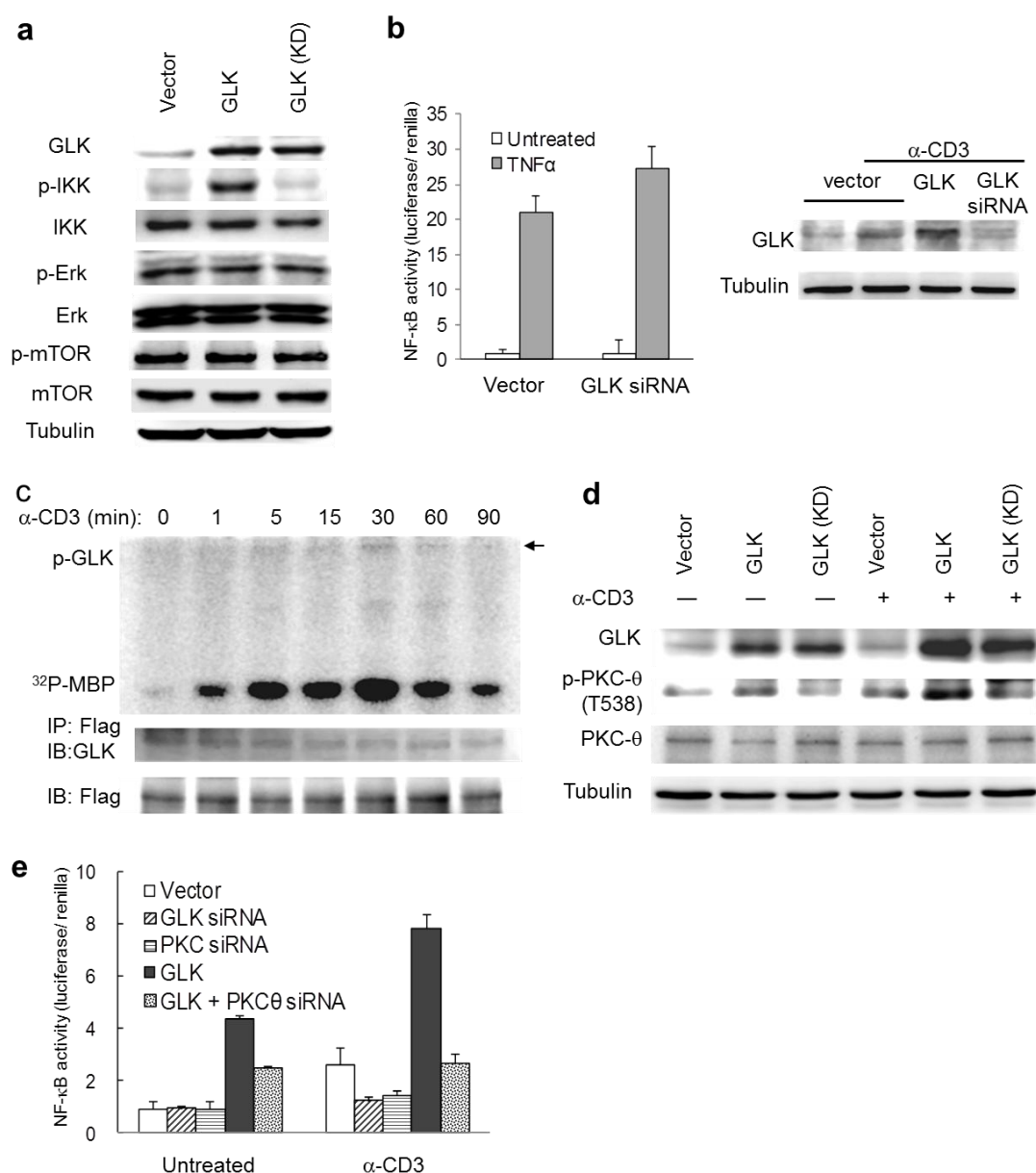


# Supplementary Figures:

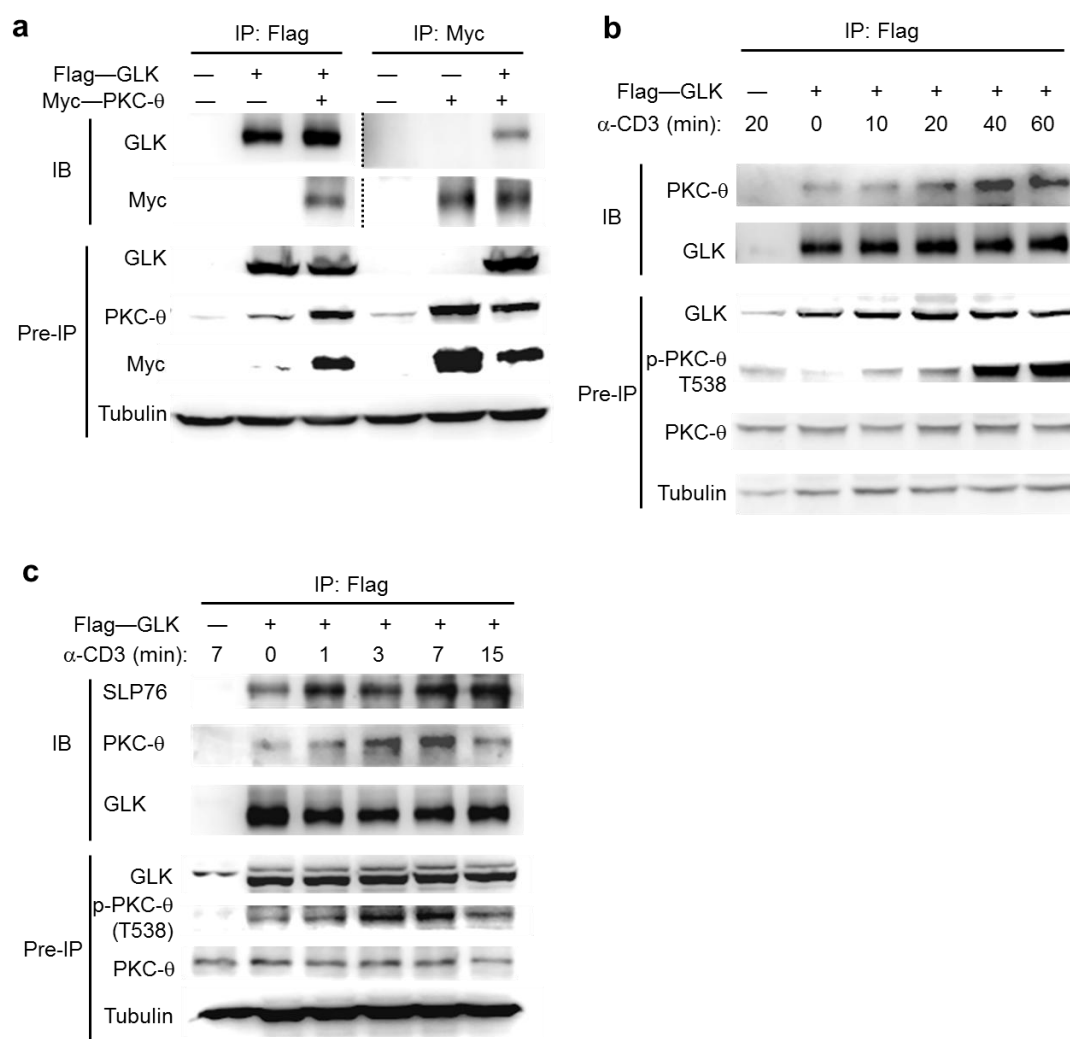


**Supplementary Figure 1. Diagram of GLK-induced PKC-θ/NF-κB activation during TCR signaling.** After TCR ligation, activated Lck is recruited to TCR complex and phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) of CD3, resulting in Zap70 recruitment and activation. Zap70 activation induces the assembly of the proximal SLP-76 signaling complex. SLP-76 directly interacts with GLK and is required for GLK kinase activation. The activated GLK directly interacts with and phosphorylates PKC-θ at T538, resulting in PKC-θ membrane translocation and kinase activation. The activated PKC-θ binds to and phosphorylates the signaling scaffold protein CARMA1, resulting in the activation of CARMA1 and the subsequent assembly of CARMA1-BCL10-MALT1 (CBM) complex. The CBM complex in turn induces the activation of the IKK/NF-κB signaling cascade.

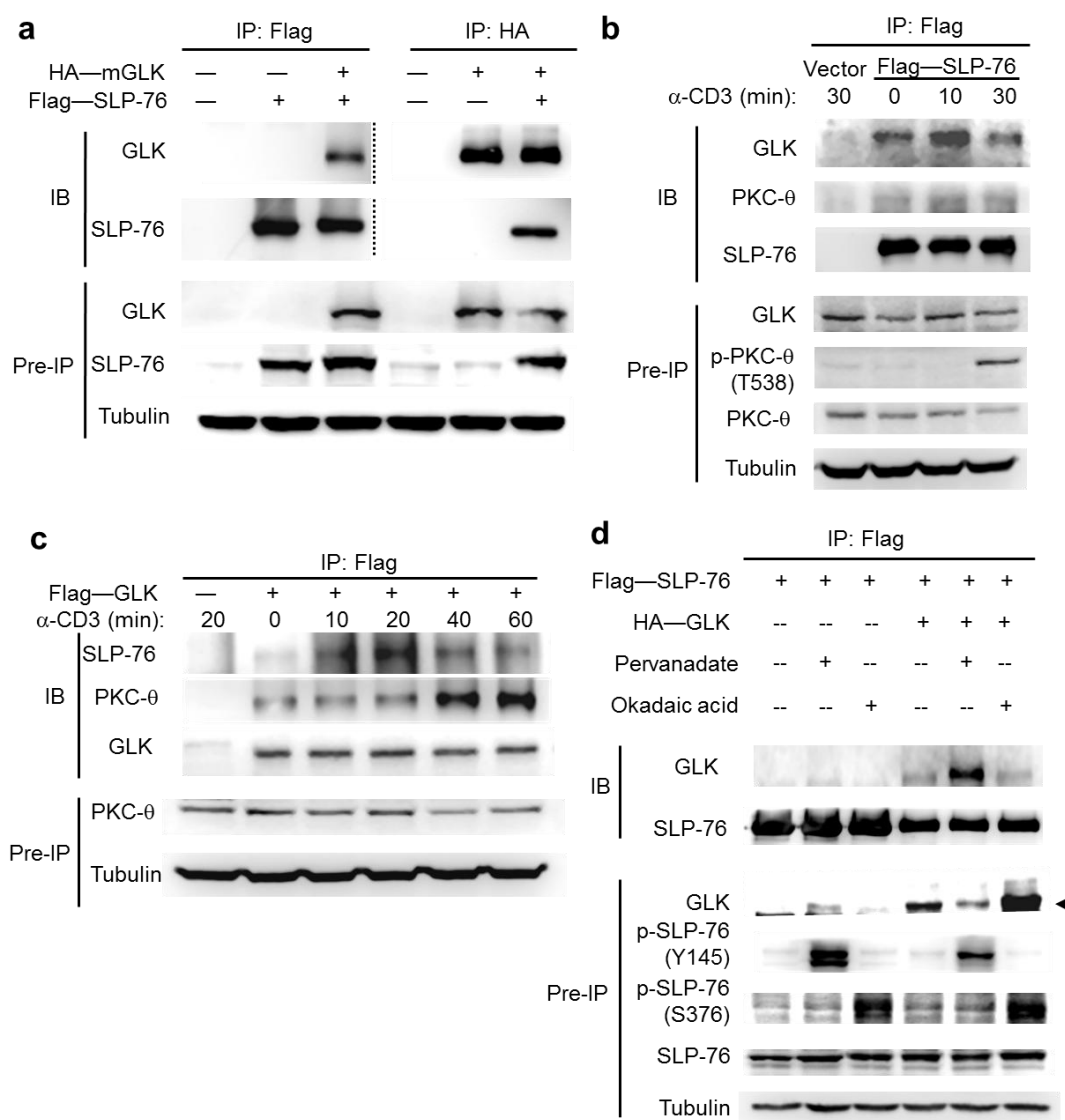


**Supplementary Figure 2. GLK induces NF-κB activation in T cells upon TCR stimulation.** (a) Immunoblot analyses of GLK, p-IKK, IKK, p-Erk, Erk, p-mTOR, mTOR and tubulin in the lysates of vector, GLK, or GLK(KD) mutant-expressing Jurkat T cells stimulated with anti-CD3 antibodies for 15 min. (b) NF-κB reporter assays of Jurkat cells transfected with empty vector or GLK siRNA following TNF-α stimulation (left). Immunoblot analyses of GLK and tubulin in lysates of Jurkat T cells transfected with empty vector, plasmid encoding GLK or GLK siRNA following anti-CD3 stimulation. (c) *In vitro* kinase assays of GLK isolated from Flag-GLK-expressing J-TAg T cells stimulated with anti-CD3 antibodies. Arrow, GLK autophosphorylation. IP, immunoprecipitation. IB, immunoblot. (d) Immunoblot

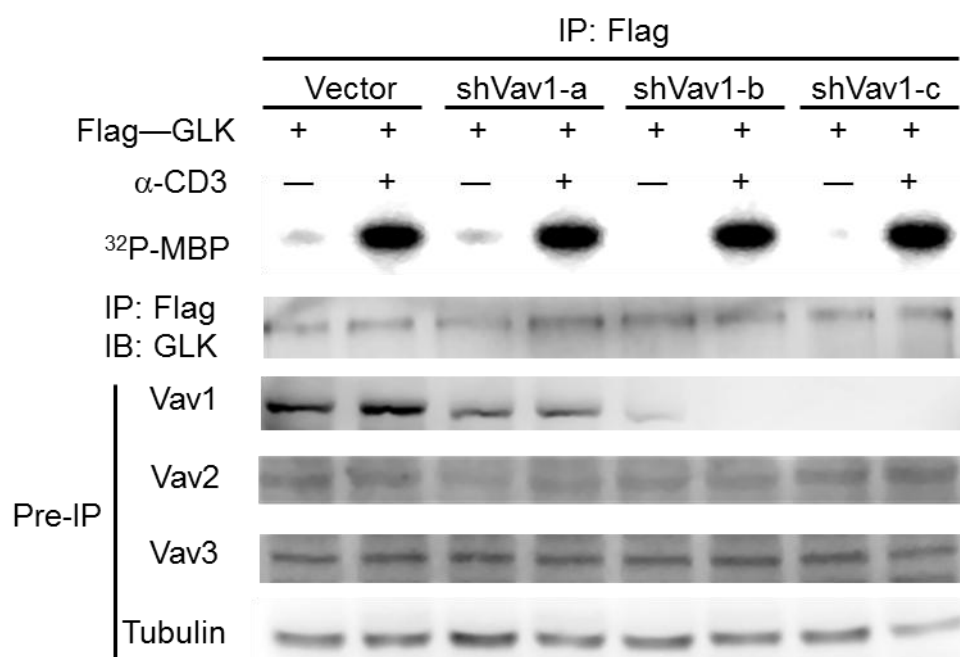
analyses of GLK, p-PKC- $\theta$ , PKC- $\theta$  and tubulin in lysates of J-TAg T cells transfected with plasmid encoding GLK or the GLK(KD) mutant following CD3 stimulation for 30 min. (e) NF- $\kappa$ B reporter assays of Jurkat T cells transfected with indicated empty vector, plasmid encoding GLK, GLK or PKC- $\theta$  siRNA alone, or plasmids encoding GLK plus PKC- $\theta$  siRNA. Cells were stimulated with or without anti-CD3 antibodies for 2 h. Data are representative of three independent experiments. \*,  $P$  value < 0.05; \*\*,  $P$  value < 0.001. Error bars in panels (b) and (e) are standard deviations (s.d.) of triplicate samples. Data are representative of at least three independent experiments.



**Supplementary Figure 3. GLK interacts with PKC-θ.** (a) Co-immunoprecipitation (IP) and immunoblot (IB) analyses of GLK and PKC-θ in lysates of HEK293T cells transfected with empty vector or plasmid encoding GLK plus with or without plasmid encoding PKC-θ. (b,c) Co-immunoprecipitations of the Flag-GLK and endogenous PKC-θ in lysates of Flag-GLK-expressing J-Tag (b) or EL4 (c) T cells stimulated with 5 µg/ml anti-CD3 antibodies (upper panel). Immunoblot analyses of GLK, p-PKC-θ, PKC-θ and tubulin in pre-immunoprecipitation samples (lower panel). Data are representative of at least three independent experiments.

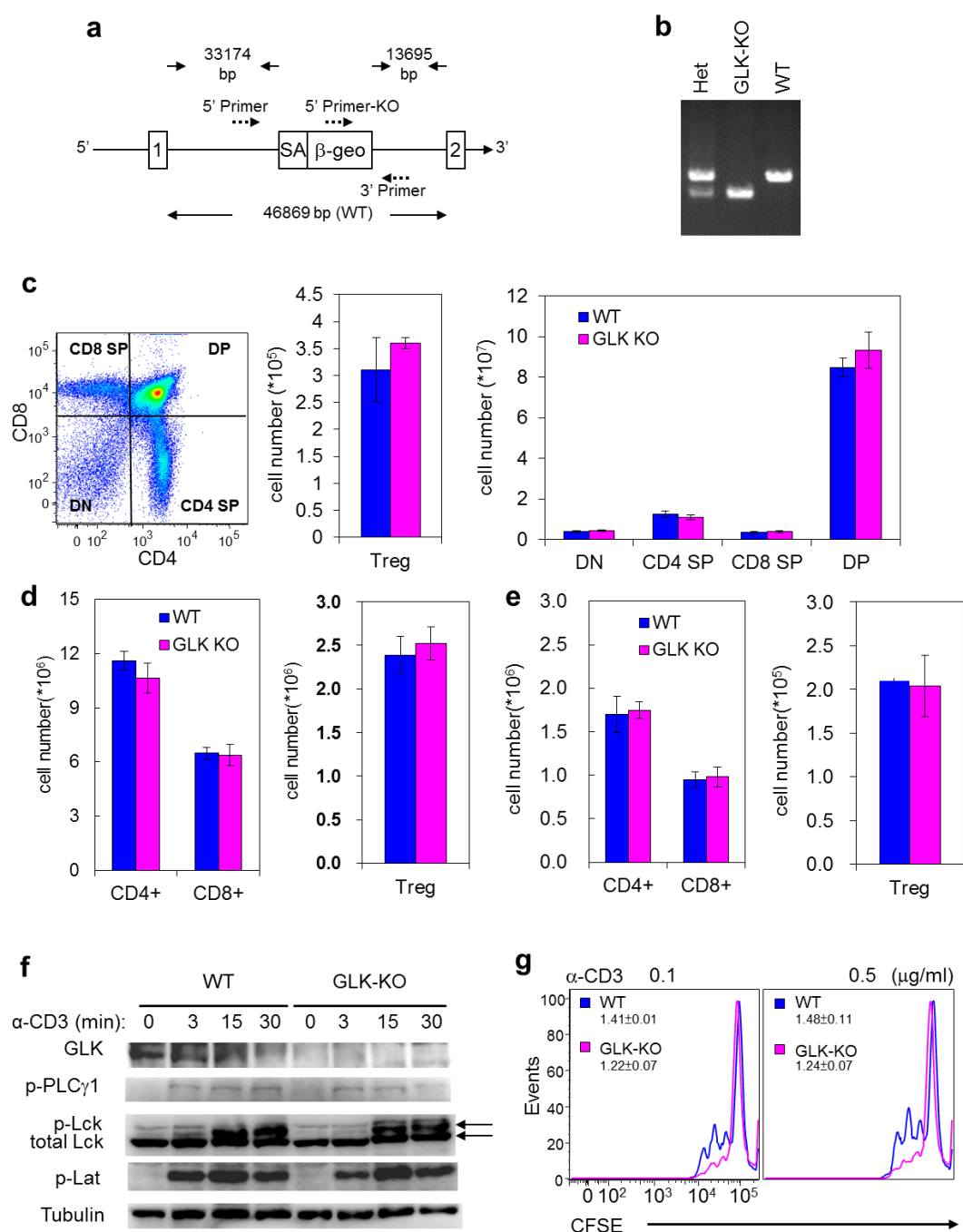


**Supplementary Figure 4. The interaction between GLK and SLP-76 is mediated through tyrosine phosphorylation.** (a) Co-immunoprecipitations (IP) and immunoblot (IB) analyses of GLK and SLP-76 in lysates of HEK293T cells transfected with empty vector or plasmid encoding SLP-76 plus with plasmid encoding GLK. (b) Co-immunoprecipitations of SLP-76 and GLK in lysates of Flag-SLP-76-overexpressed J-TAG T cells stimulated with anti-CD3 antibodies. (c) Co-immunoprecipitations of anti-CD3-induced SLP-76/GLK/PKC $\theta$  interaction in J-TAG T cells transfected with Flag-GLK. (d) Co-immunoprecipitations of GLK and SLP-76 in lysates of HEK293T cells transfected with plasmid encoding SLP-76 plus empty vector or plasmid encoding GLK. Cells were pre-treated with/without the tyrosine phosphatase inhibitor pervanadate or the serine/threonine phosphatase inhibitor okadaic acid. Data are representative of at least three independent experiments.



**Supplementary Figure 5. Vav1 is not required for TCR-induced GLK activation.**

*In vitro* kinase assays of Flag-GLK isolated from the Vav1 shRNA-knocked down Jurkat T cells (shVav1 a-c) stimulated for 30 min with or without anti-CD3 antibodies. MBP was used as the substrate. Data are representative of at least three independent experiments.

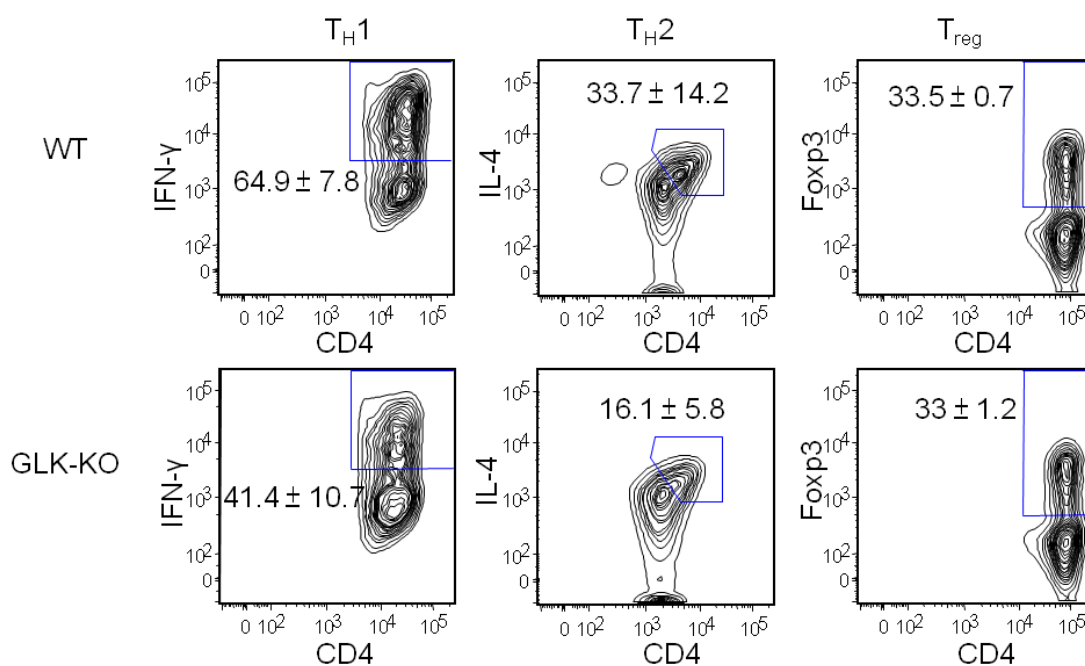


**Supplementary Figure 6. T cell development is normal in GLK-deficient mice. (a)**

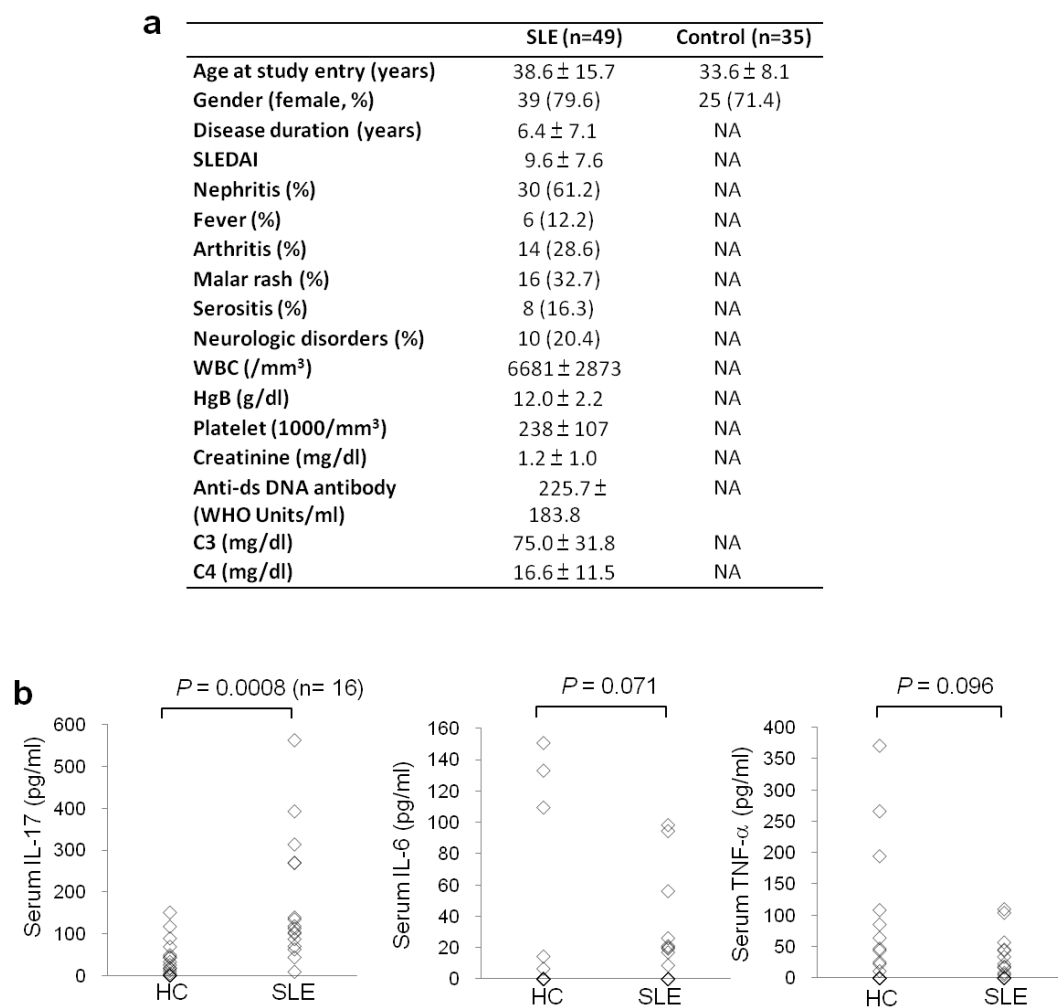
The structure of the gene-trap vector. β-geo, a fusion with β-galactosidase and neomycin phosphotransferase genes; SA, splicing acceptor; the box with numbers, the exon of GLK; dotted arrow, the primers for PCR. **(b)** PCR analyses of *GLK* wild-type and mutant allele in the genomic DNA from mouse tails. The PCR products of the higher band (1400 bp) indicate wild-type (WT) allele, and the lower band (1000 bp) indicates *GLK* mutant allele. **(c–e)** Flow cytometry analyses of T lymphocytes from the thymus (c), spleen (d), and lymph nodes (e) of wild-type and *GLK*-deficient (*GLK*-KO) mice. Data are presented as mean ± s.e.m. **(f)** Immunoblot analyses of

GLK, p-PLC $\gamma$ 1, p-Lck, Lck and tubulin in lysates of mouse T cells stimulated with anti-CD3-biotin and streptavidin. **(g)** CFSE dilution assays of T cell proliferation in purified T cells. Proliferation indexes (mean  $\pm$  s.e.m) analyzed by FlowJo software are also shown. Data are representative of at least three independent experiments **(b-g)**.





**Supplementary Figure 7. Differentiation of  $T_H1$  and  $T_H2$  is impaired by GLK deficiency.** Flow cytometry of IFN- $\gamma$ -producing, IL-4-producing and Foxp3-positive  $CD4^+$  T cells. Data are presented as mean  $\pm$  s.e.m. Data are representative of at least three independent experiments.



**Supplementary Figure 8. Serum IL-17 levels are increased in SLE patients. (a)** Profile of SLE patients and paired healthy control. Data are presented as mean ± s.d. **(b)** ELISA assays of IL-17, TNF-α, and IL-6 in the sera of SLE patients and healthy controls (HC).