

**Supplementary Figure S1** Dlg1 is critical for T cell receptor microcluster (TCR-MC) formation.

A and B. Microclusters of ZAP-70–YFP (green) and phosphorylated Y323 p38 (red)

were imaged in immunostained Jurkat cells stably expressing ZAP-70–YFP. Cells

were pretreated with control or DLG1 specific siRNA, then dropped onto coverslips

coated with anti-CD3 (stimulated) or anti-CD45 (unstimulated) and visualized after 5

min. Scale bars, 10  $\mu$ m. Data are representative of three experiments

C and D. ZAP70-YFP and P-Y323 p38 intensities were quantified using Image J in

immunostained Jurkat cells stably expressing ZAP-70–YFP. Cells were pretreated

with control or DLG1-specific siRNA, then dropped onto coverslips coated with anti-

CD3 (stimulated) or anti-CD45 (unstimulated). Data are representative of 8 fields from

3 independent experiments.

E. Intracellular IL-2 production in human CD4+ and CD8+ T cells upon anti-CD3 +anti-CD28

stimulation for 18 hours.

**Supplementary Figure S2** Sequence analysis of DLG1 KO clones

A. IGV snapshot of DLG1 KO clones

B. Allele analysis of Clonal populations

**Supplementary Figure S3** Characteristic analysis of DLG1 KO cells

A. Immunoblot of DLG1 from wildtype and DLG1 KO Jurkat cell

B. TCR- $\zeta$  expression in wildtype and DLG1 KO Jurkat cell

C. Microcluster of phosphorylated Y493 ZAP-70 (red) and expression of DLG1 (blue)

In immunostained wildtype or DLG1 KO Jurkat cells. Cells were dropped onto

coverslips coated with anti-CD3 (stimulated) and visualized 5 min post stimulation.

**Supplementary Figure S4** SHP2 knockdown fails to rescue the inhibition of T cell

signaling conferred by DLG1 deficiency

Immunoblot analysis of lysates from wildtype and DLG1 KO Jurkat cells. Cells

were pretreated with control or SHP2 specific siRNA, then activated with soluble anti-

CD3 (monoclonal antibody OKT3) and antibody to mouse immunoglobulin G (IgG),

followed by probing for phosphorylated and total proteins.

Figure S1

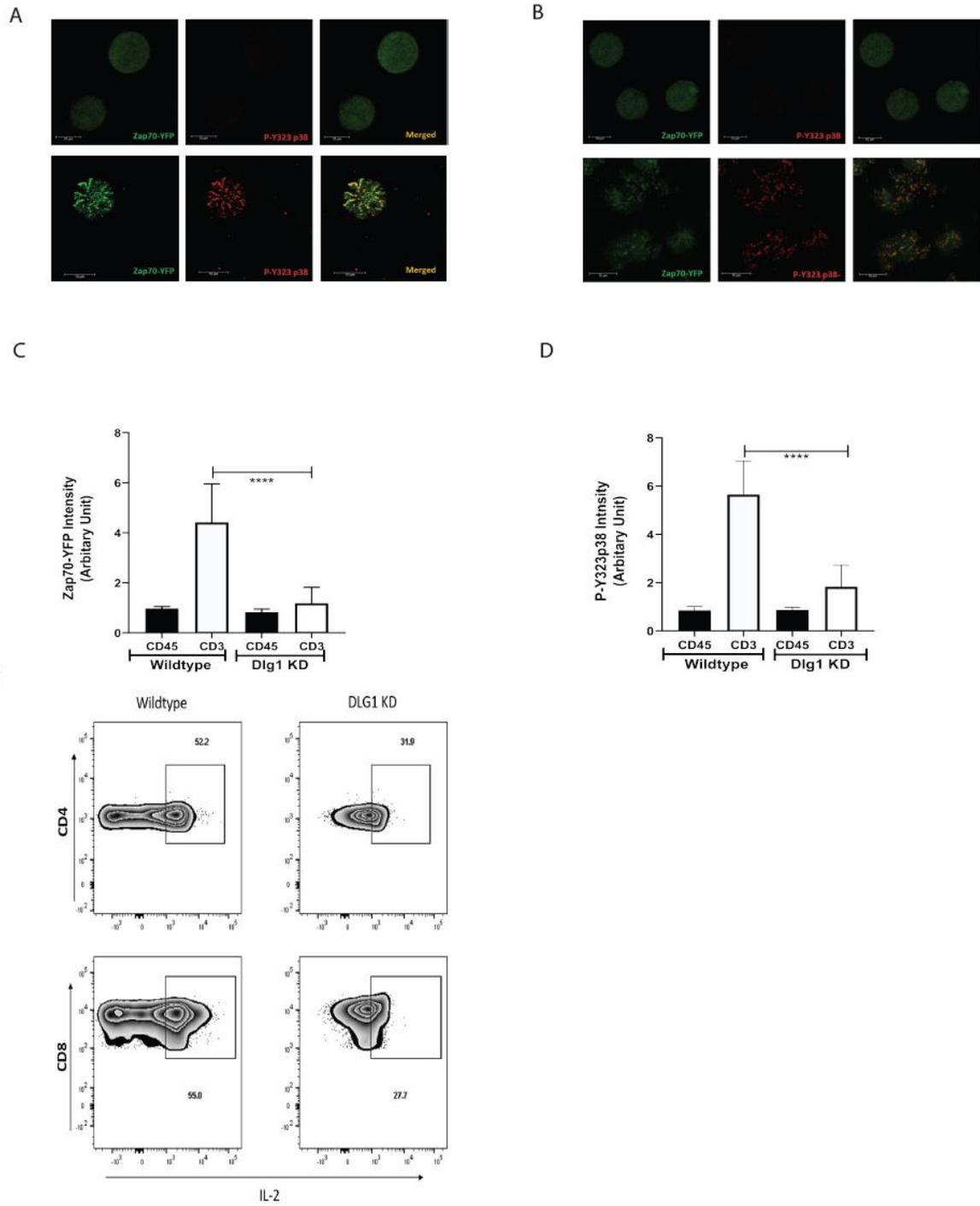
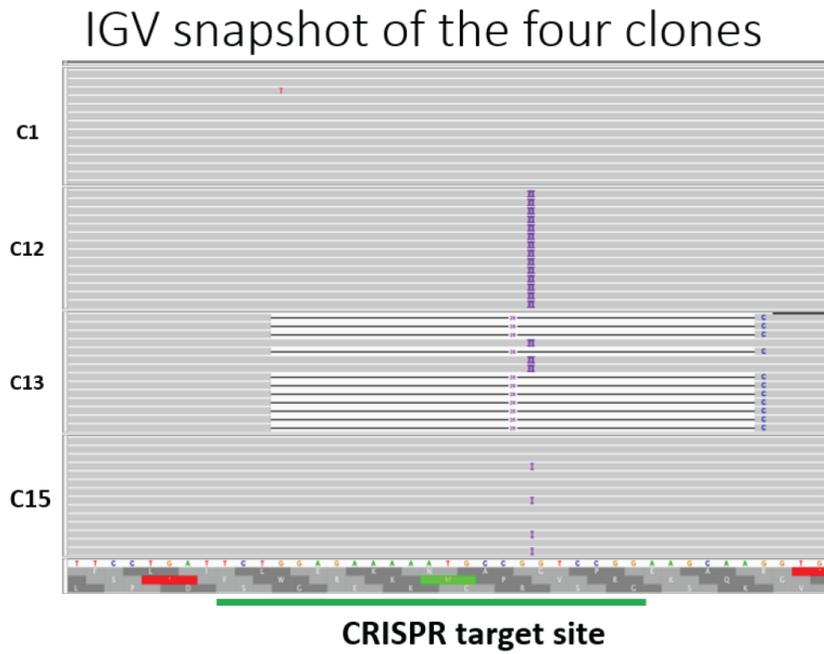


Figure S2

A



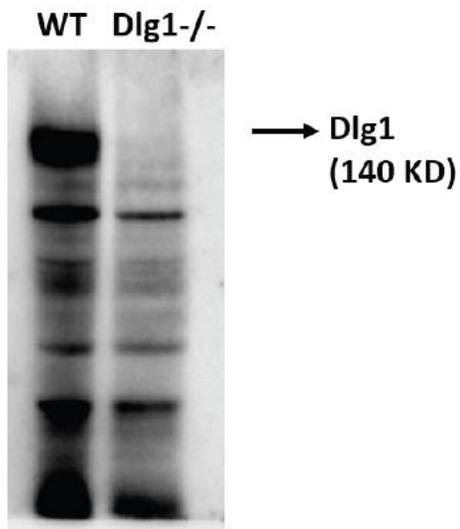
B

<b>Clone</b>	<div style="border: 1px solid black; border-radius: 10px; background-color: #e0f2f1; padding: 5px; display: inline-block;">Coding Exon 1</div>
	<u>TCTTCCTGATTCTGGAGAAAAATGCCGGTCCGGAAGCAAGGTGAGAGTTTATTCCGTA</u> ACT
<b>C1</b>	<b>Allele 1</b> TCTTCCTGATTCTGGAGAAAAATGCCGGTCCGGAAGCAAGGTGAGAGTTTATTCCGTA <b>Allele 2</b> TCTTCCTGATTCTGGAGAAAAATGCCGGTCCGGAAGCAAGGTGAGAGTTTATTCCGTA
<b>C13</b>	<b>Allele 1</b> TCTTCCTGATTCT-----CGTGAGAGTTTATTCCGTA <b>Allele 2</b> TCTTCCTGATTCTGGAGAAAAATGCCG <b>TC</b> GTCCGGAAGCAAGGTGAGAGTTTATTCCGTA
<b>C12</b>	<b>Allele 1</b> TCTTCCTGATTCTGGAGAAAAATGCCG <b>CTC</b> GTCCGGAAGCAAGGTGAGAGTTTATTCCGTA <b>Allele 2</b> TCTTCCTGATTCTGGAGAAAAATGCCG <b>CTC</b> GTCCGGAAGCAAGGTGAGAGTTTATTCCGTA
<b>C15</b>	<b>Allele 1</b> TCTTCCTGATTCTGGAGAAAAATGCCG <b>T</b> GTCCGGAAGCAAGGTGAGAGTTTATTCCGTA <b>Allele 2</b> TCTTCCTGATTCTGGAGAAAAATGCCGGTCCGGAAGCAAGGTGAGAGTTTATTCCGTA

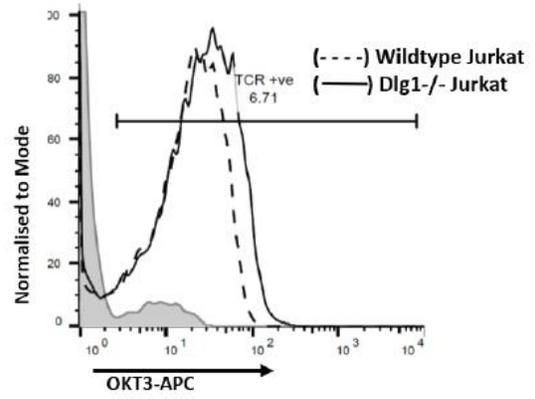
sgRNA target site underlined

Figure S3

A



B



C

