Supplementary Information

Sam68 modulates the promoter specificity of NF-κB and mediates expression of CD25 in activated T cells

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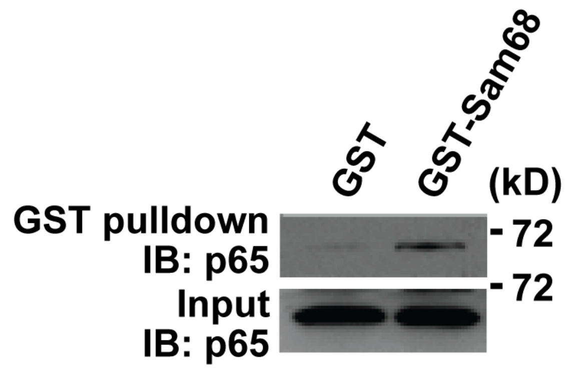
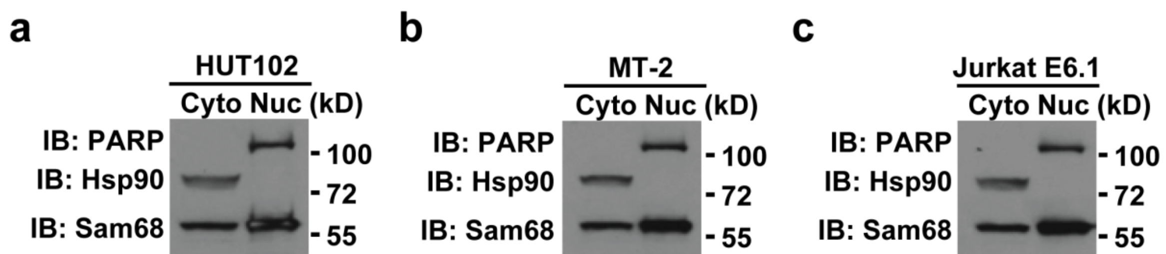
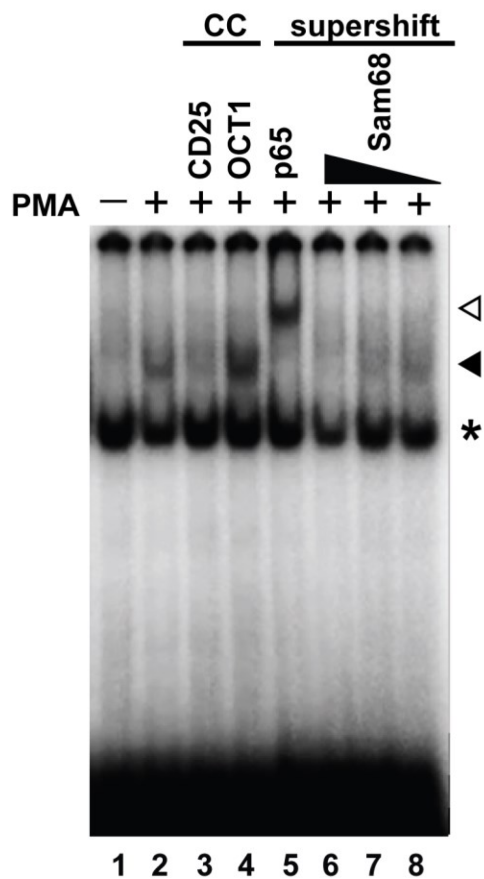


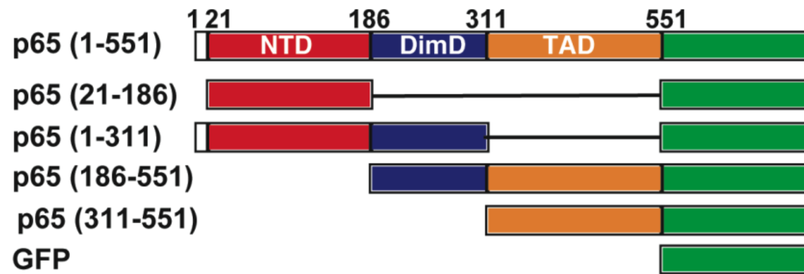
Supplementary Figure S1. The nuclear interaction of Sam68 and p65. Pull-down with recombinant GST or GST-Sam68 proteins with nuclear extracts derived from HUT102 cells, followed by immunoblotted (IB) for p65.



Supplementary Figure S2. Sam68 preferentially localizes in the nucleus. Immunoblot (IB) of the cytosolic (Cyto) and nuclear (Nuc) fractions from HUT102 cells (a), MT-2 cells (b), and Jurkat E6.1 cells (c). Hsp90 and PARP serve as cytosolic and nuclear markers, respectively.

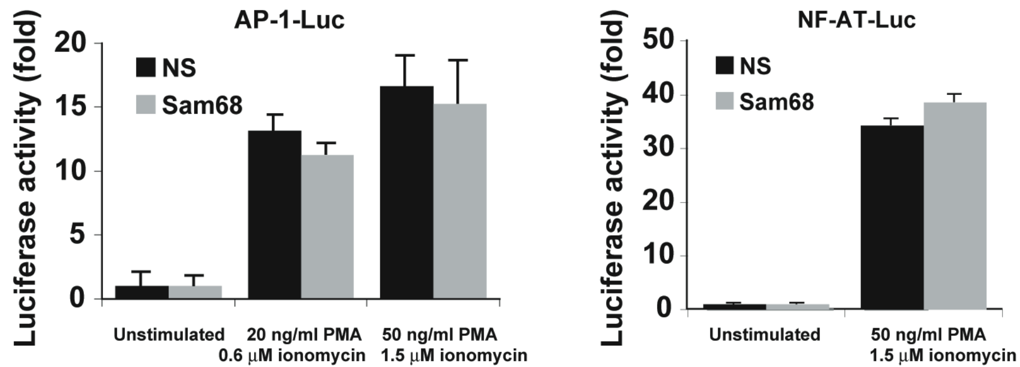


Supplementary Figure S3. Sam68 is an integral component in the *CD25* κB DNA binding complex. Nuclear extracts of Jurkat cells treated with (+) or without (-) PMA (50 ng/ml, 30 min) were analyzed by EMSA with ^{32}P-labeled *CD25* κB oligonucleotides, in some cases with 100-fold unlabeled *CD25* κB or nonspecific OCT1 oligonucleotide competitors (CC). Supershift analysis was conducted with p65 or different doses of Sam68 antibodies (lanes 5-8). *CD25* κB DNA binding complexes in Jurkat cells are labeled with a filled triangle, and the supershifted bands and nonspecific bands are labeled with an open triangle and an asterisk, respectively.

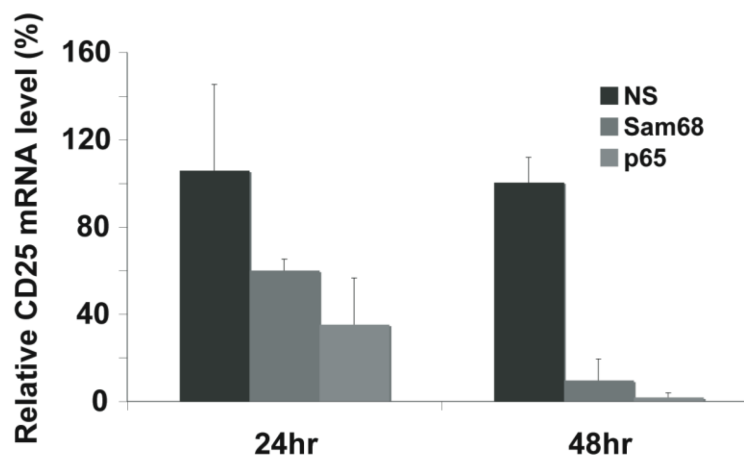


vehicle/p65 truncated proteins	Sam68 interaction
GFP	-
p65 (1-551)	+
p65 (21-186)	-
p65 (1-311)	+
p65 (186-551)	-
p65 (311-551)	-

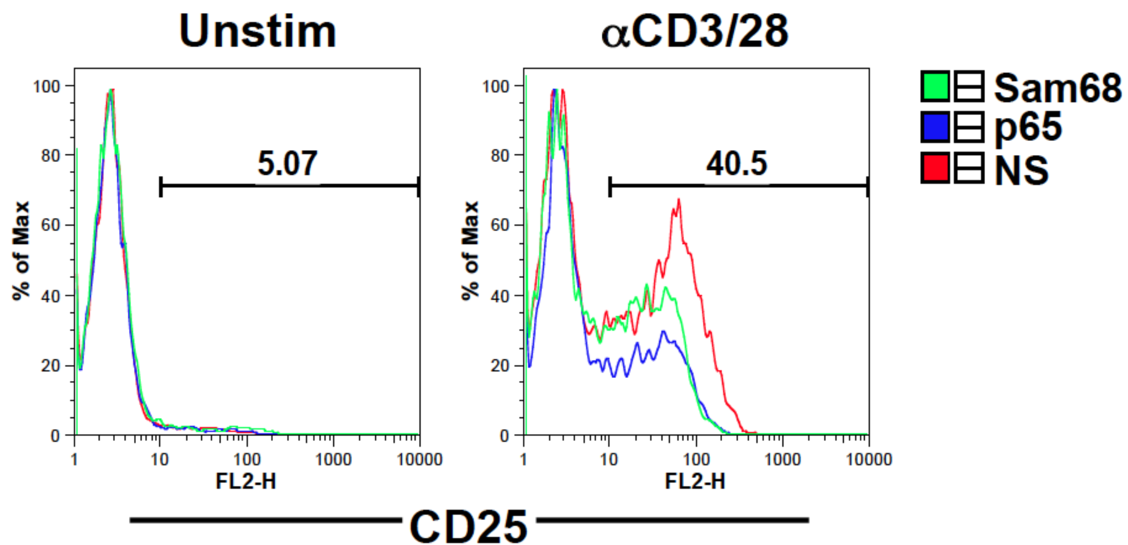
Supplementary Figure S4. Mapping the interaction between p65 and Sam68. Schematic diagram of the truncation mutants of p65 fused with EGFP (*upper*). NTD, N-terminal domain; DimD, dimerization domain; TAD, transcriptional activation domain. EGFP-p65 truncated constructs and myc-Sam68 were transfected into HEK293T cells. The lysates were immunoprecipitated by myc antibody and probed for GFP-p65 truncated proteins. The binding capabilities of p65 truncates to Sam68 were summarized as +, detectable interaction; -, undetectable interaction (*bottom*).



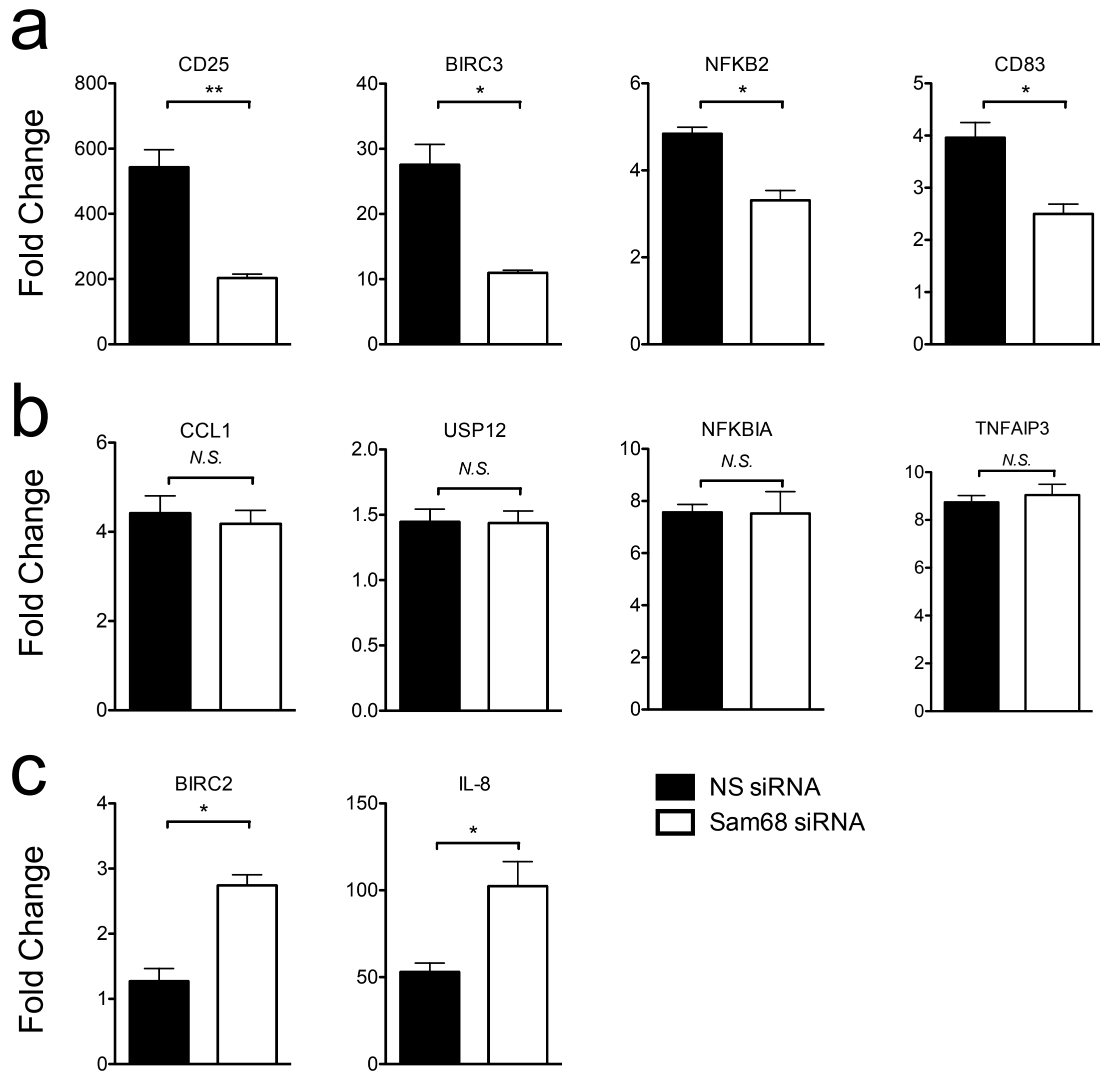
Supplementary Figure S5. The effects of knockdown of Sam68 on T cell receptor stimulation-induced AP-1 and NF-AT expression. Knockdown of Sam68 did not impair T cell receptor stimulation-induced AP-1 or NF-AT signaling in Jurkat T lymphocytes. AP-1 (*left*) and NF-AT (*right*) luciferase assays (mean ± S.D., n = 3) of Jurkat cells transfected with Sam68 or scramble non-specific (NS) siRNAs, and stimulated as indicated.



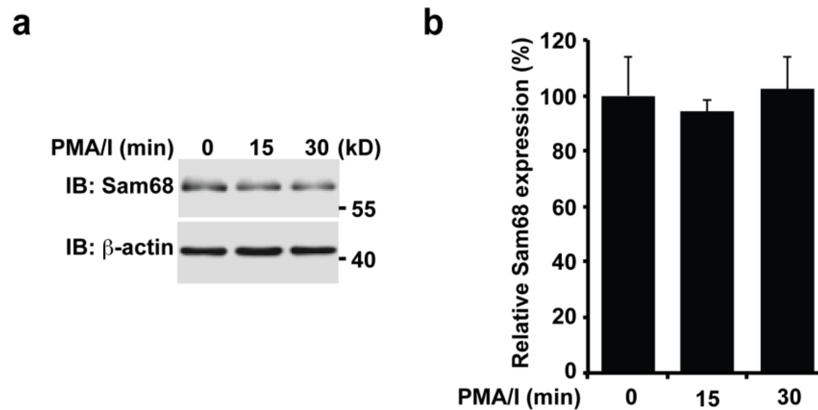
Supplementary Figure S6. The effect of Sam68 knockdown on the *CD25* gene transcription in HUT102 cells. Real-time PCR quantitation (mean ± S.D., n = 3) of mRNA level of *CD25* normalized to *GAPDH* in HUT102 cells after transfected with Sam68, p65 or scramble non-specific (NS) siRNAs for indicated periods. The relative mRNA level (%) was normalized to NS siRNA transfected.



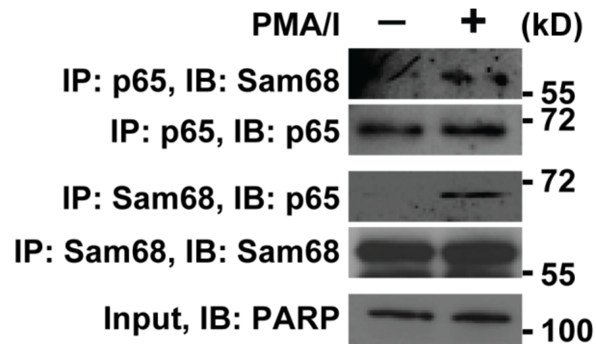
Supplementary Figure S7. Knockdown of Sam68 attenuates the T cell receptor engagement-induced CD25 expression. Human peripheral blood T lymphocytes were transfected with nonspecific (NS), p65, or Sam68 siRNAs and then stimulated with anti-CD3/CD28 antibodies (αCD3/CD28, 1 μg/ml each) or left unstimulated (Unstim). Flow cytometry histograms of T cells with CD25 induction were assessed 12 hr later. The percentage of $CD25^{+}$ cells and of dividing cells in NS silenced cells is shown.



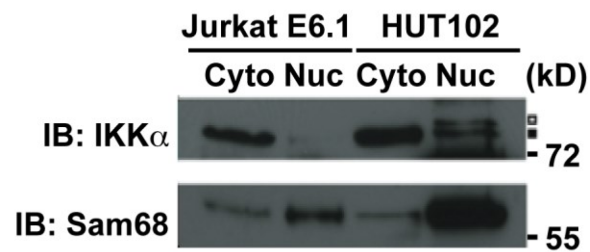
Supplementary Figure S8. The effect of Sam68 knockdown on the induction of selected NF-κB target genes. (**a**-**c**) Real-time PCR quantization of mRNA level of indicated NF-κB target genes normalized to *GAPDH* in Jurkat cells silenced with NS or pooled Sam68 siRNAs and stimulated with or without 50 ng/ml PMA plus 1.5 μM ionomycin for 2 hr. The *SAM68* gene was downregulated by 50%, as measured by Real-time PCR. The induction (fold change) of indicated genes corresponds to a further normalization of stimulated sample to unstimulated sample (mean ± S.D., n = 3). Not statistically significant (*N.S.*), * $P < 0.05$, ** $P < 0.01$, calculated by Student's *t*-test.



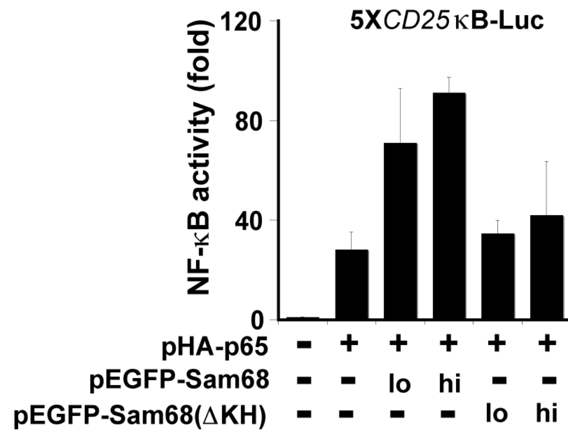
Supplementary Figure S9. T cell receptor stimulation does not induce the expression of Sam68. (**a**) Representative immunoblot (IB) for indicated proteins in the whole cell lysates derived from Jurkat T lymphocytes stimulated with 50 ng/ml PMA plus 1.5 μM ionomycin (PMA/I) for indicated period. (**b**) Relative Sam68 expression in Jurkat T lymphocytes stimulated as in (**a**) (mean ± S.D., n = 3). Densitometry of all bands was performed, and the intensity of each Sam68 band was normalized to corresponding β-actin band. The fold change of Sam68/β-actin was further normalized to the 0-min samples (set as 100%).



Supplementary Figure S10. The association of Sam68 and p65 is enhanced in stimulated Jurkat cells. Immunoprecipitation (IP)/immunoblot (IB), as indicated, in the nuclear extracts from Jurkat T lymphocytes with (+) or without (-) 50 ng/ml PMA plus 1.5 μM ionomycin (PMA/I).



Supplementary Figure S11. Elevated nuclear IKKα in CD25 highly expressing HUT102 cells. Immunoblot (IB) of the cytosolic (Cyto) and nuclear (Nuc) fractions from identical Jurkat or HUT102 cells. Filled and open symbols indicate non-phosphorylated and phosphorylated forms, respectively.



Supplementary Figure S12. The Sam68-p65 interaction is required for induction of CD25 expression. NF-κB luciferase assay (mean ± S.D., n = 3) of Jurkat cells transfected with p65 and low (lo) or high (hi) dose of indicated GFP-fused wild type or mutant Sam68 plasmids together with a luciferase reporter gene driven by 5 × *CD25* κB sites.