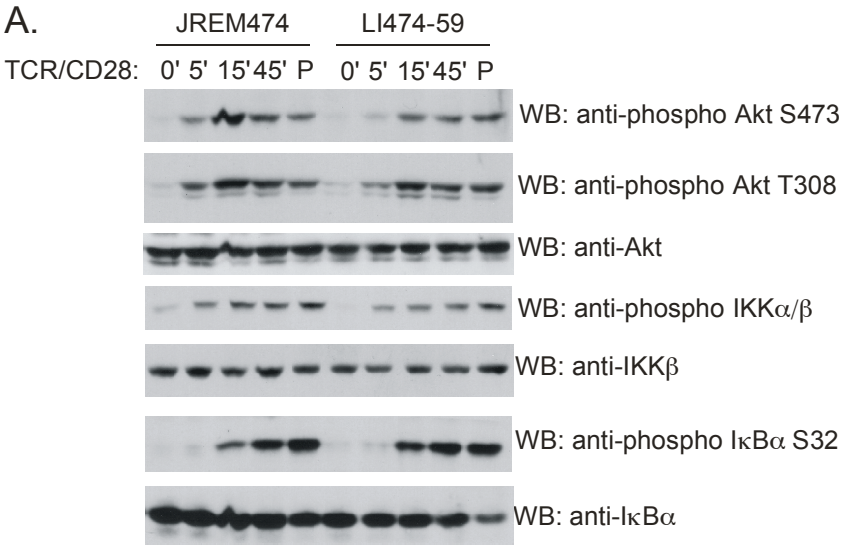
Online Supplemental Material

Supplemental Figure 1. Analysis of NF-κB signaling pathways in the library rescued LI474-59 cell line.

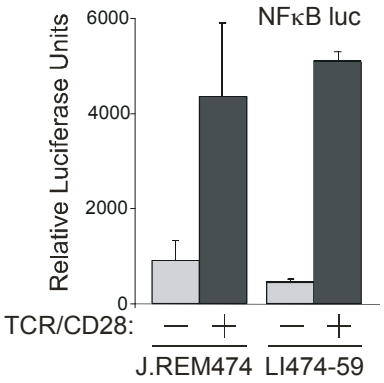
(A) J.REM 474 and LI474-59 were stimulated with TCR/CD28 for either 0, 5, 15 or 45 minutes, or with PMA for 10 minutes, as previously described (Shapiro et al., 2005). Whole cell extracts were examined by Western blot for phospho-Akt (T308, #4056), phospho-Akt (S473, #9271), Akt (#9272), phospho-IKKα/β (#2697), IKKβ (#2684), IκBα S32 (#9241), and IκBα (#9242). All antibodies were purchased from Cell Signaling Technologies.

(B) J.REM 474 and LI474-59 were transfected with an NF-κB luciferase reporter, as previously described (Shapiro et al., 1996; Shapiro et al., 1997). Briefly, 5 x 10^6 J.REM 474 or LI474-59 cells were washed once, and resuspended in 0.4 ml of serum-free RPMI. 10 μg of an NF-κB luciferase reporter was added. Electroporation was performed using a Gene Pulser II (BioRad) at 250 volts, 950 μF. Cells were resuspended in 10 mls of RPMI with 5% FCS (Gibco BRL). The following day, live cells were counted by trypan blue exclusion (Bio-Whittaker), and 1 x 10^5 cells per sample were stimulated as denoted in the figures. Cells were left unstimulated or stimulated with antibodies to TCR (C305, 1:1000 final dilution) and CD28 (Caltag, 1 μg/ml) for 7 hours. Luciferase assays were performed as previously described (Shapiro et al., 1996). The results shown are the average relative luciferase units from triplicate transfections. Error bars reflect +/- SEM.

A.


JREM474
LI474-59
TCR/CD28: 0' 5' 15' 45' P 0' 5' 15' 45' P
WB: anti-phospho Akt S473
WB: anti-phospho Akt T308
WB: anti-Akt
WB: anti-phospho IKKα/β
WB: anti-IKKβ
WB: anti-phospho IκBα S32
WB: anti-IκBα

B.


NFκB luc
Relative Luciferase Units
6000
4000
2000
0
TCR/CD28: — + — +
J.REM474
LI474-59