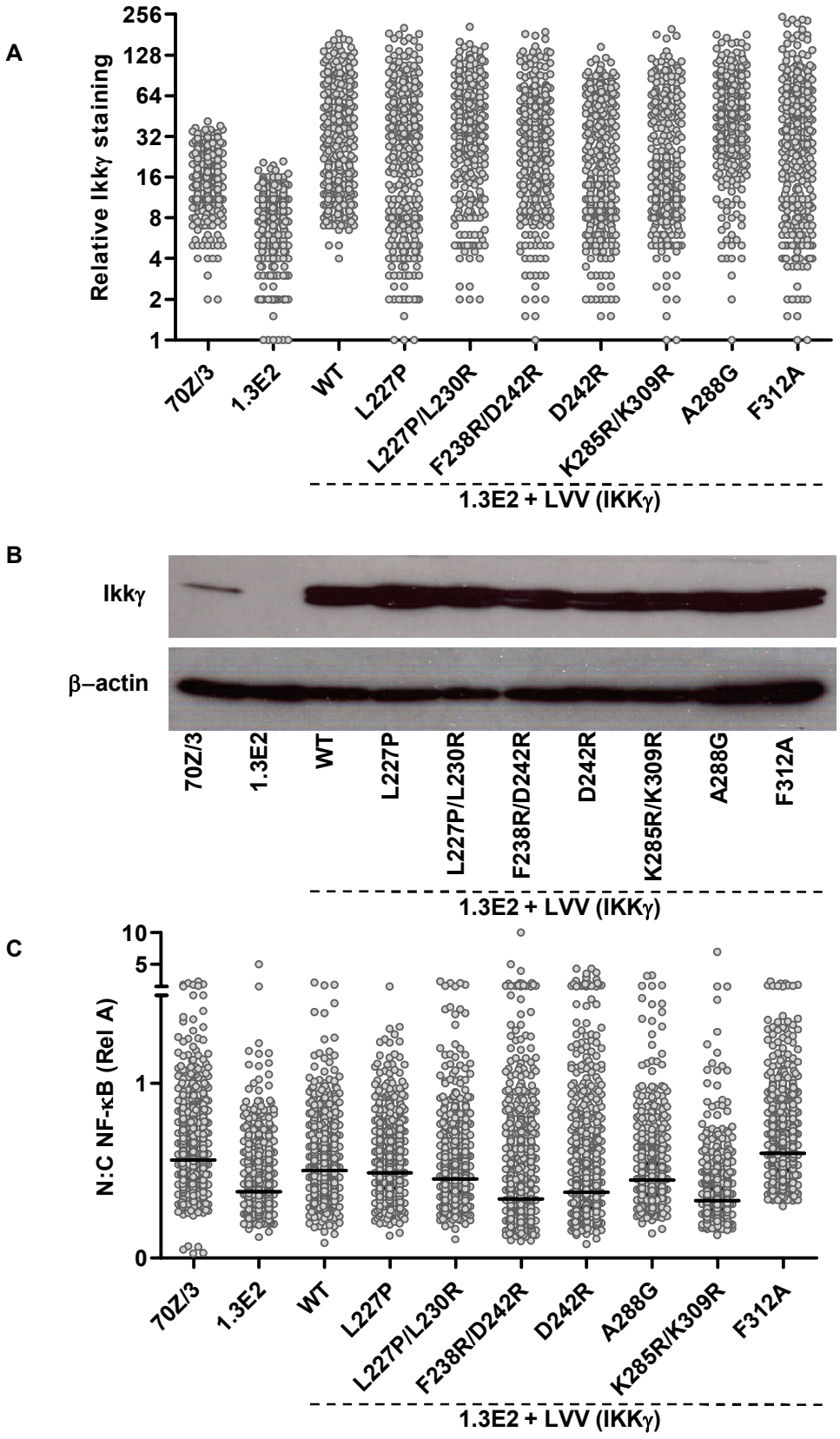
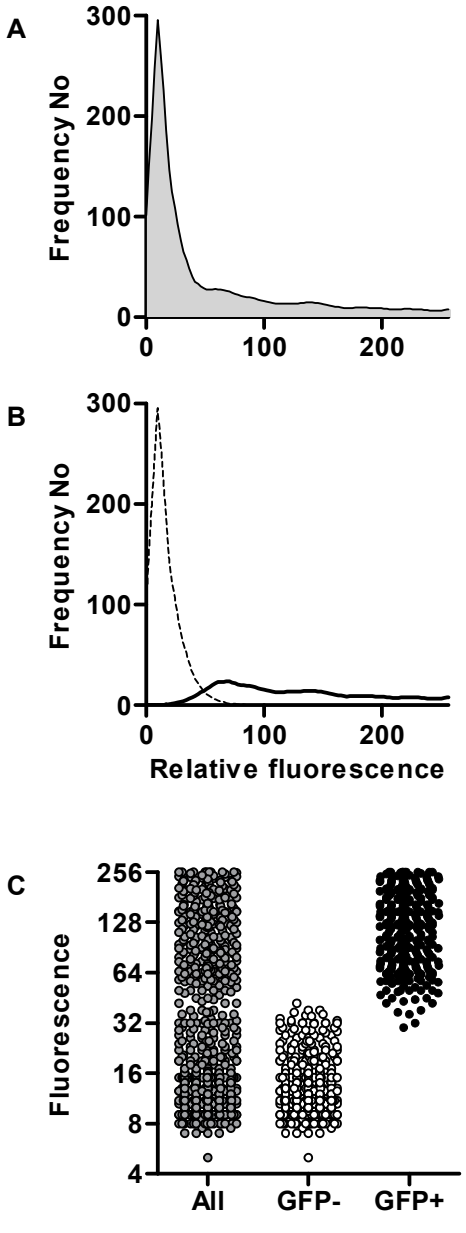
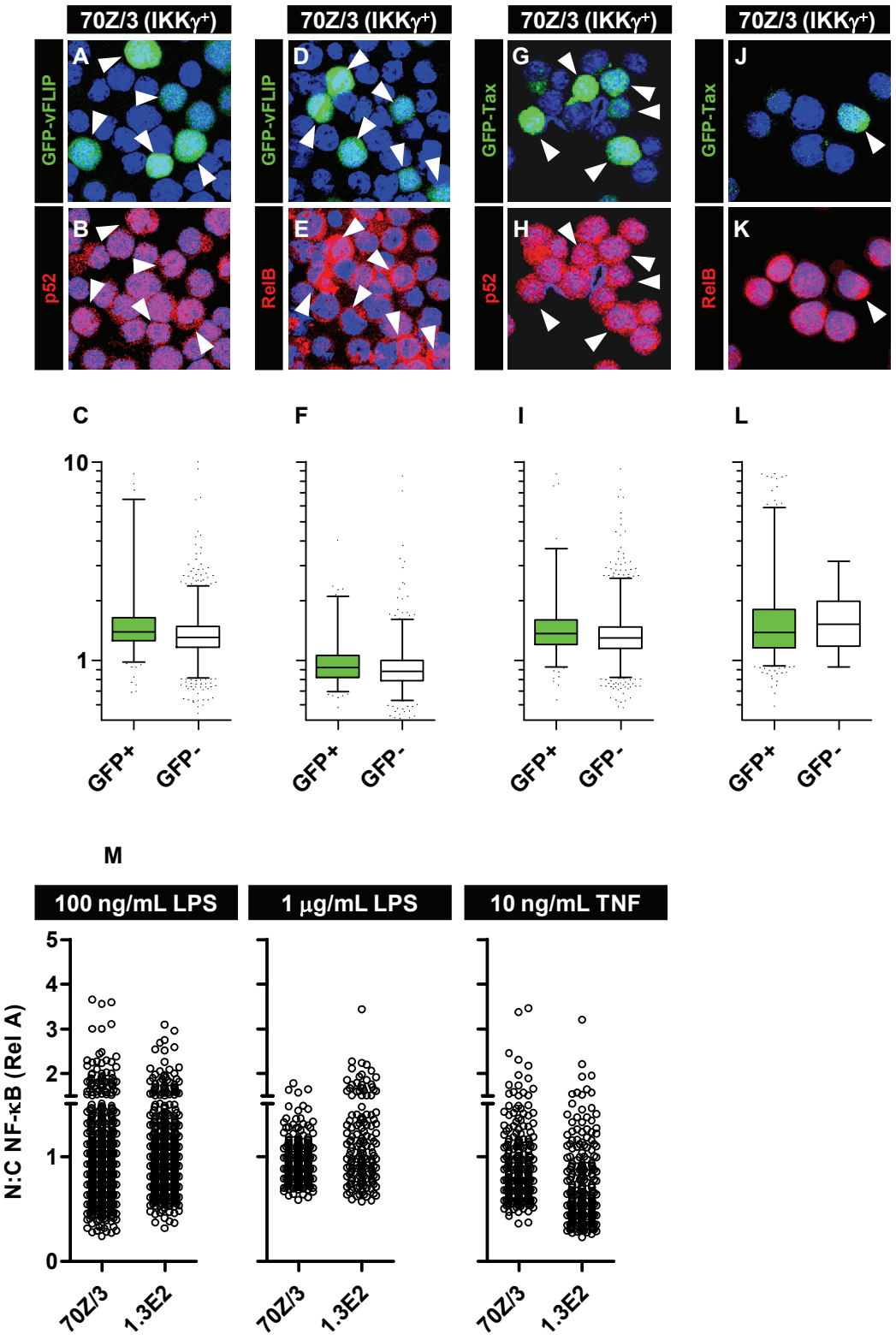
Supplementary Figure 1



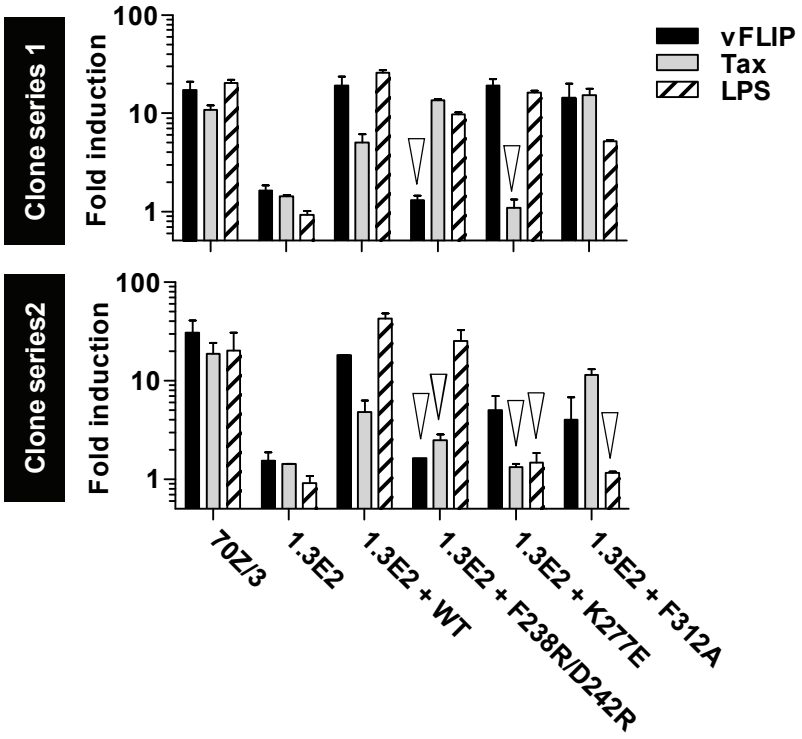
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



SUPPLEMENTARY FIGURE 1

Quantification of IKKγ expression by immunostaining with a polyclonal antibody confirms deficiency of IKKγ in 1.3E2 in comparison to 70Z/3 cells, and shows comparable distribution of expression levels, 2-4 fold greater than that of 70Z/3 cells, in 1.3E2 cells transduced with lentiviral vectors encoding WT IKKγ or the IKKγ mutants indicated **(A)**, and reflected in IKKγ levels detected by Western blotting **(B)**. Overexpression of IKKγ in complemented 1.3E2 cells did not cause increased NF-κB Rel A nuclear:cytoplasmic ratios in comparison to WT cells **(C)**. In scatter plots data points represent measurements from individual cells from one experiment representative of multiple replicate experiments. Median values and interquartile ranges are shown in C.

SUPPLEMENTARY FIGURE 2

Frequency distribution of GFP positive cells in 70Z/3 cells transduced with lentiviral vector encoding vFLIP and GFP **(A)** shows mixed GFP^+ and GFP^- cells that can be segregated by image analysis software **(B,C)**.

SUPPLEMENTARY FIGURE 3

In 70Z/3 cells with WT IKKγ, LVV transduction encoding vFLIP/GFP or Tax/GFP was not associated with differential nuclear translocation of components of the alternative NF-κB activation pathway. p52 demonstrated a predominantly nuclear localization **(A-C, G-I)** and Rel B predominantly cytoplasmic localization **(D-F, J-L)**, that was comparable in GFP^+ and GFP^- cells. Box and whisker plots show median, interquartile range and 5-95^{th} centiles of at least 500 cells. **(M)** Nuclear:cytoplasmic ratios of Rel A staining after 1 hour stimulation with LPS (at the concentrations

indicated) was not significantly different in 70Z/3 (WT IKKγ) cells compared to 1.3E2 (KO IKKγ) cells, and 1 hour TNFα stimulation was associated with only modest increase in Rel A nuclear translocation. Scatter plots data points represent measurements from individual cells from one experiment representative of multiple replicate experiments.

SUPPLEMENTARY FIGURE 4

Activation of the classical NF-κB pathway was assessed in WT 70Z/3 cells, IKKγ deficient 1.3E2 cells and 1.3E2 cells complemented with WT or selected mutants of IKKγ, following transduction with LVVs expressing vFLIP or Tax, or stimulation with LPS (5 μg/mL) by luciferase assay (Bright-Glo™ Luciferase Assay System, Promega) in two separate series of cell clones encoding the NF-κB luciferase reporter gene. Bars represent mean ±SD of 3-4 separate experiments. **(▽)** denotes cells which show attenuated activation of NF-κB in comparison to 1.3E2 cells complemented with WT IKKγ ($p<0.05$, Mann-Whitney U rank test).