Legends to Supplementary Figures.

Figure S1. Specificity of the IKKβ inhibitor BI605906. (A) The specificity of BI605906 was examined against a panel of over 100 kinases by the National Centre for Protein Kinase Profiling, MRC Protein phosphorylation Unit, University of Dundee (http//www.kinase-screen.mrc.ac.uk). The results for each protein kinase tested are presented as the percentage activity remaining in the presence of the inhibitor as compared to control incubations in which the inhibitor was omitted. The assayed were performed in duplicate and the average value is shown. The protein kinases were arranged left to right in order of increasing potency of inhibition. (B) Purified IKKβ (filled circles) and IKKα (open circles) were assayed for 30 min at 0.1 mM ATP using the peptide LDDRHDSGLDSMKDEEY as substrate in the presence or absence of the indicated concentrations of BI605906. Data are presented as the percentage activity remaining in the presence of the inhibitor as compared to control incubations in which the inhibitor was omitted. The error bars show the average of duplicate determinations (C) Murine embryonic fibroblasts (MEFs) obtained from IKKα-/- (upper three panels) or IKKβ-/- mice (lower three panels) were incubated for 1 h with the indicated concentrations of BI605906, then stimulated for 10 min with 5 ng/ml of murine IL-1α. Following cell lysis, aliquots of the extracts were subjected to SDS-PAGE followed by immunoblotting with the antibodies indicated. The blot shown is representative of two independent experiments that were carried out.

Figure S2. The IKKβ inhibitor PS1145 blocks type 1 interferon production in Gen2.2 cells. (A) Gen 2.2 cells were incubated for 1 h in the absence (ND, no drug) or presence of the indicated doses of PS1145 and then stimulated for 12 h with 1 μM CpG type B, 1 μM CpG type A and for 5 h with 1 μg/ml CL097. The concentration of IFNα or IFNβ in the cell culture medium was measured by ELISA. The experiment was performed in 96 well plates with two wells being used for each concentration of inhibitor studied and the results being averaged. Similar results were obtained in two other independent experiments. Error bars show the variation in duplicate determinations. (B) As in (A), except that IL-6 in the cell culture medium was measured after stimulation with CpG type B (left hand panel) or CL097 (right hand panel). Similar results were obtained two other independent experiments.

Figure S3. IKKβ inhibitors do not affect cell viability. Gen2.2 cells were incubated for 12 h in the absence (ND, no drug) or presence of the indicated concentrations of (A) BI605906 or (B) PS1145. The cells were then fixed and the percentage of live cells measured by flow cytometry. The results are presented as the percentage of live cells compared to cells incubated for the same time in the absence of any drug. The experiment was performed in 96 well plates with two wells being used for each concentration of inhibitor studied and the results being averaged. Similar results were obtained in two other independent experiments.

Figure S4. Specificities of the protein kinase inhibitors used in this study. The specificities of (A) INCB18424 and (B) NG-25 were examined against a panel of over 100 protein kinases by the National Centre for Protein Kinase Profiling as in Fig S1A. The results for each protein kinase tested are presented as the percentage activity remaining in the presence of the inhibitor as compared to control incubations in which the inhibitor was omitted. The results obtained from duplicate determinations were averaged. The protein kinases were arranged left to right in order of increasing potency of inhibition. (C) The chemical structure of NG-25.

Figure S5. TAK1 inhibitors block type 1 interferon production in Gen2.2 cells in response to CpG A without affecting cell viability.

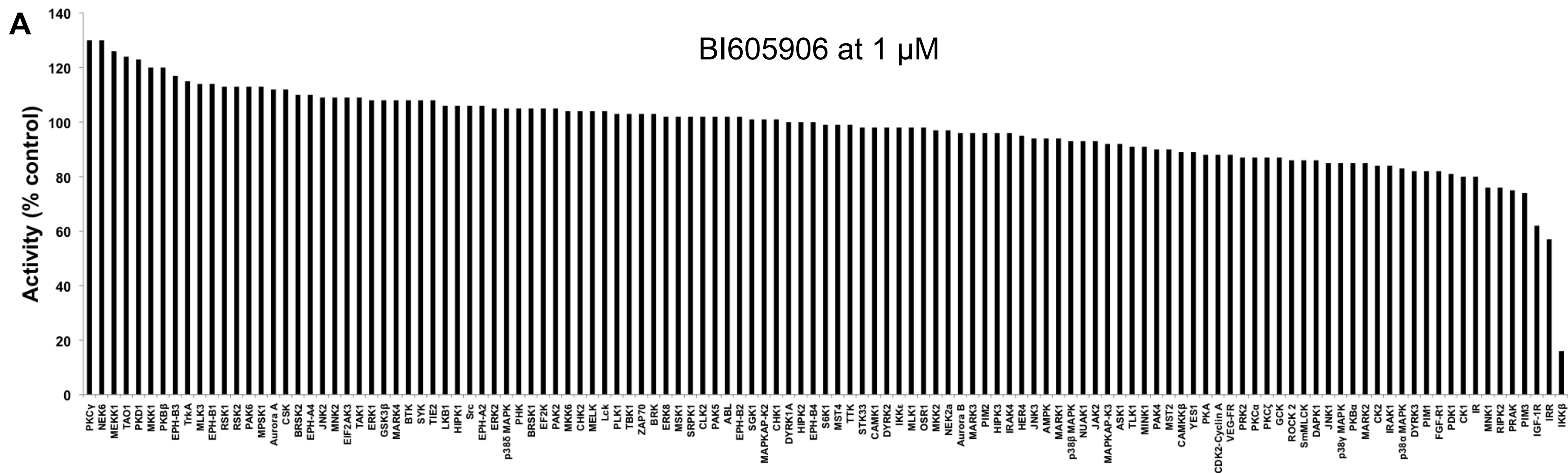
(A) Gen2.2 cells were incubated for 1 h with the indicated concentrations of (5z)-7-oxozeaenol (left hand panel) or NG25 (right hand panel) and then stimulated for 12 h with 1 μM CpG type A. The concentration of IFNα in the cell culture medium was measured by ELISA. The experiment was performed in 96 well plates with two wells being used for each concentration of inhibitor studied and the results being averaged. Similar results were obtained in two other independent experiments. Error bars show the variation in duplicate determinations. (B) As in Fig S3, except that the percentage of live cells was measured in samples treated for 12 h with indicated doses of (5z)-7-oxozeaenol (left) or NG25 (right). Similar results were obtained in two other independent experiments.

Figure S6. Identification of the site on IRF7 phosphorylated by IKKβ in vitro. (A) Wild-type IRF7 (1 μM) was incubated with IKKβ (4 U/ml) or TBK1 (4 U/ml) prior to initiating the protein kinase reactions with Mg-[γ^{32}p]ATP. The reactions were terminated in SDS and subjected to SDS-PAGE, and the gel was autoradiographed. (B) ^{32}P-labeled IRF7 obtained by

phosphorylation with IKKβ was digested with trypsin and subjected to chromatography on a Vydac C18 column. ^{32}P radioactivity in arbitrary units (au) is shown by the full line, and the acetonitrile gradient is indicated by the diagonal line. (C) Solid phase sequencing of fraction 1 generated after phosphorylation with IKKβ. Peptides were subjected to solid phase sequencing to identify the cycles of Edman degradation at which ^{32}P radioactivity (filled bars) was released from the phosphopeptides present in this fraction.

Figure S1

A



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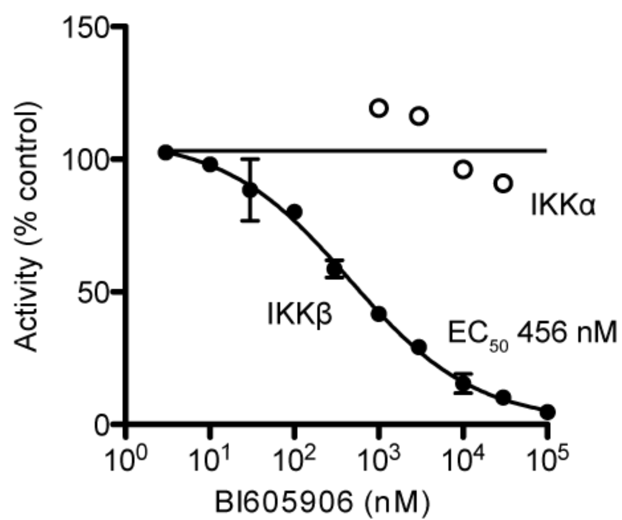

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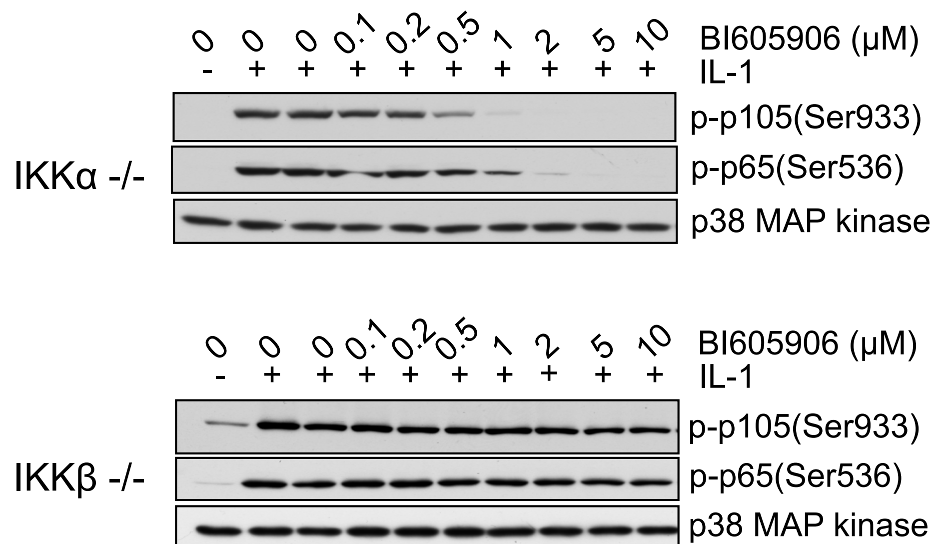


Figure S2

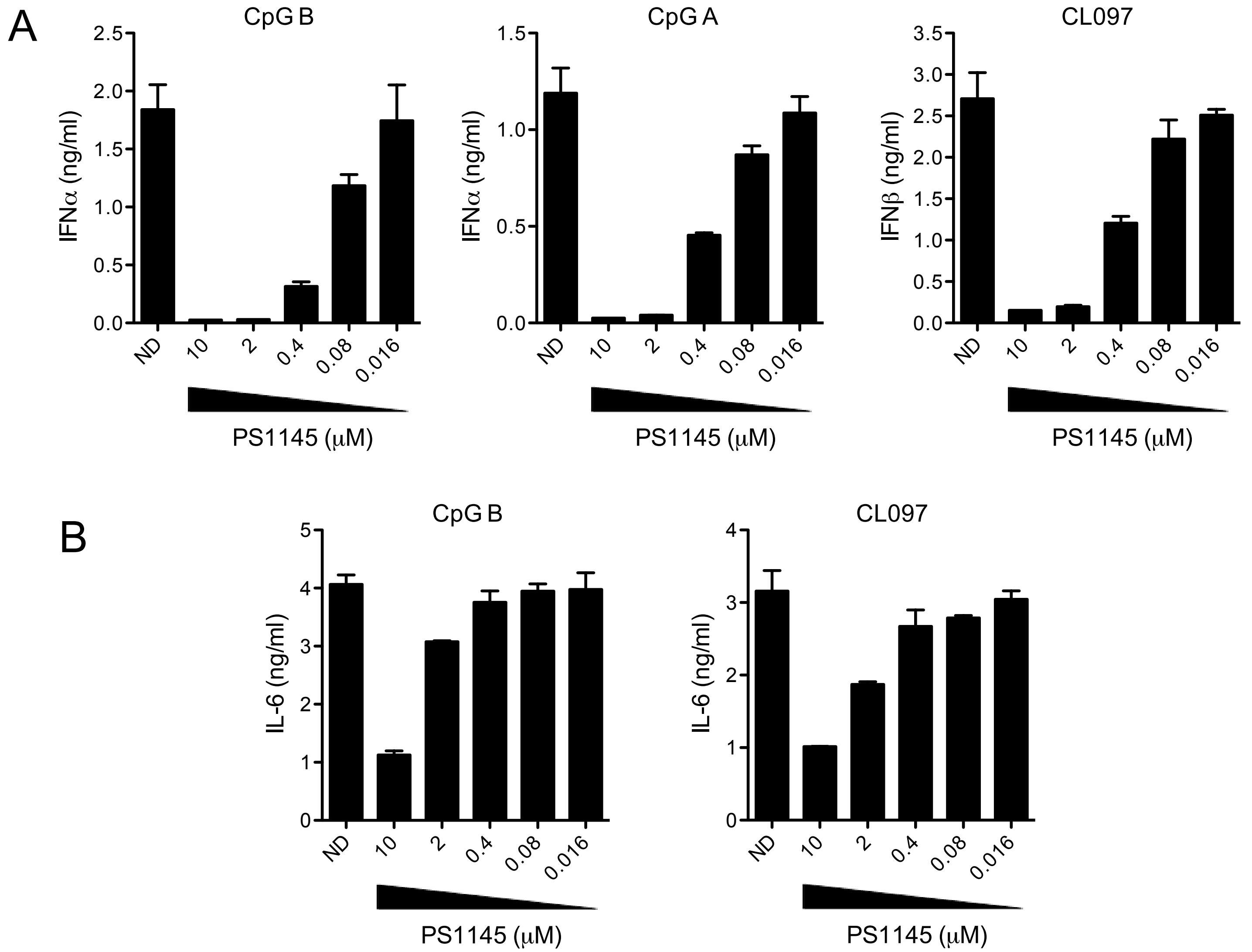
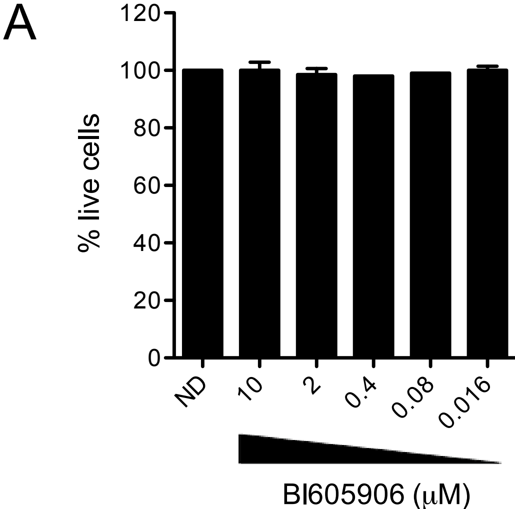


Figure S3



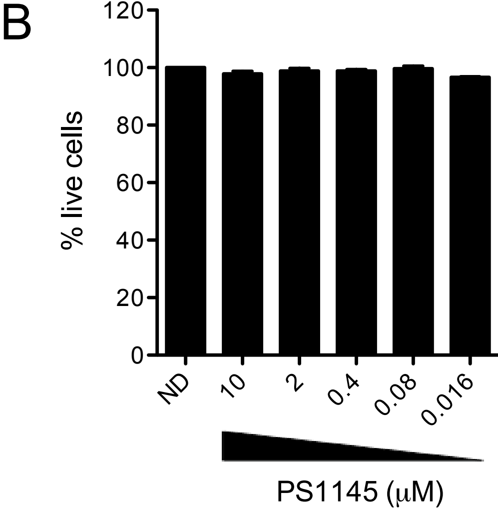
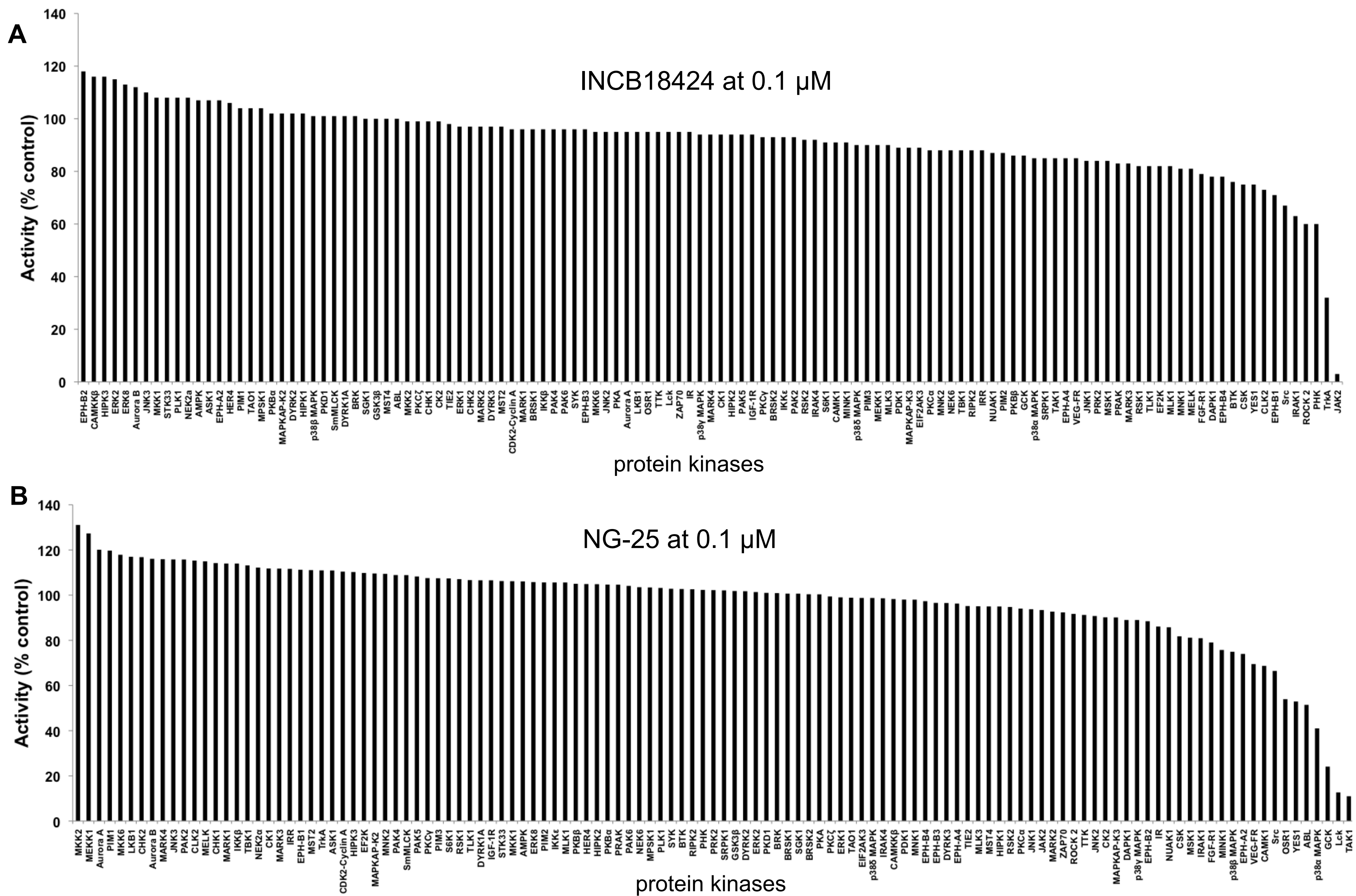


Figure S4



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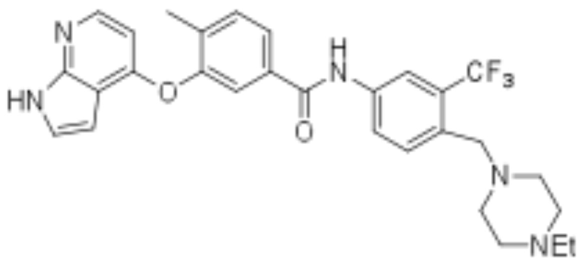

CC1=CC=C(C(NC2=CC=C(CN3CCN(CC)CC3)C(C(F)(F)F)=C2)=O)C=C1OC4=C5C(NC=C5)=NC=C4

Figure S5

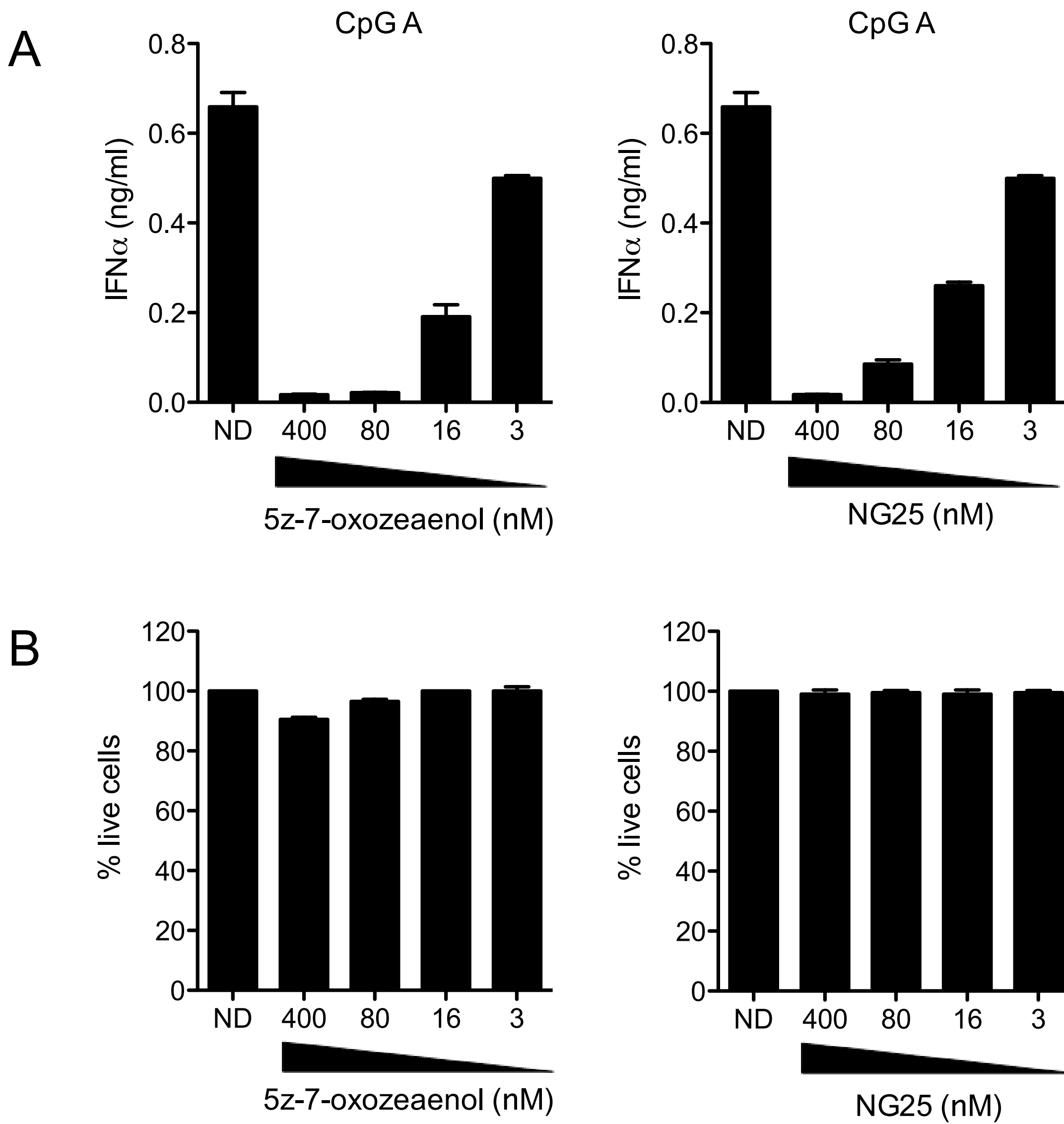

Figure S6