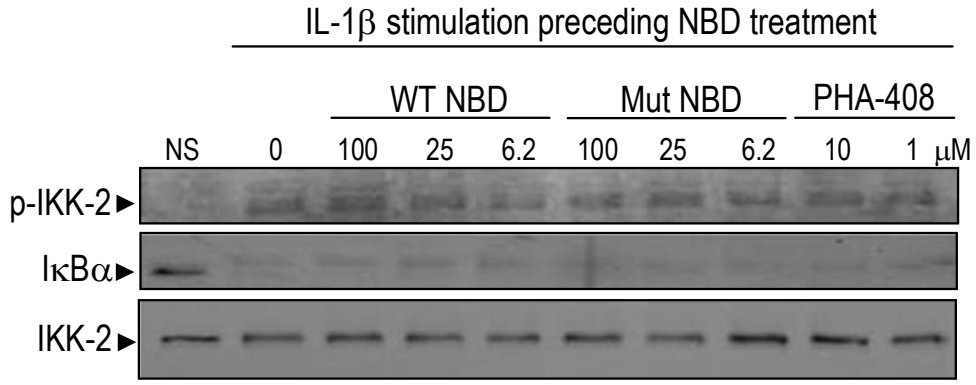
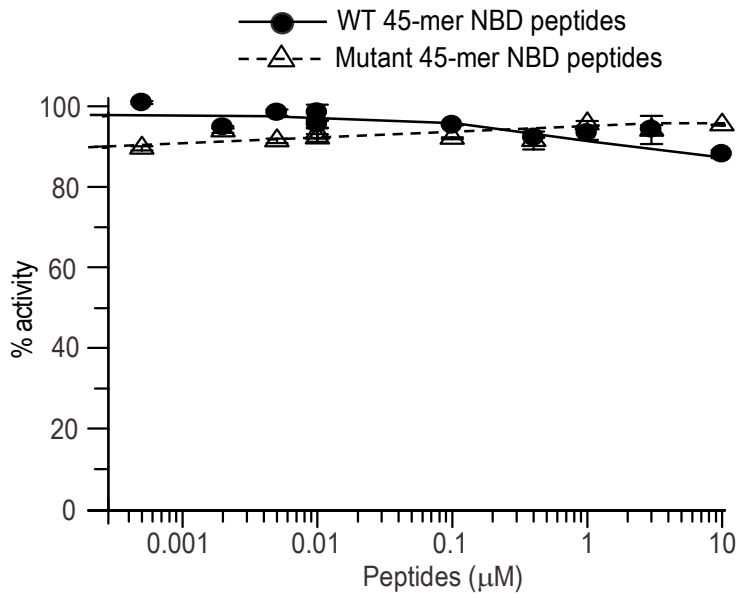
Supplementary Figure S1



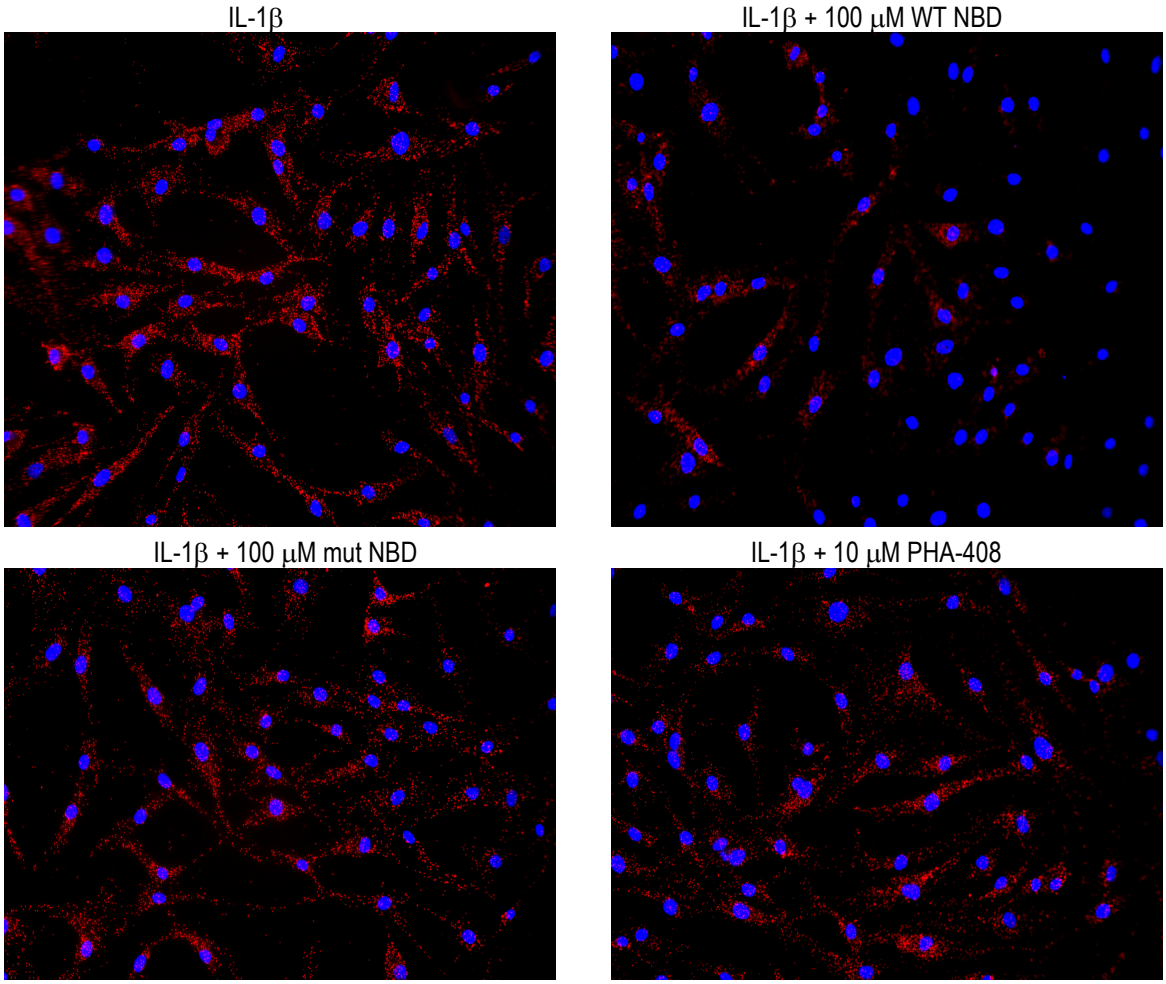
Effects of NBD peptides added to lysates from IL-1β treated-cells. RASF were stimulated for 10 min with 1 ng/ml IL-1β. Cell lysates were incubated for 1 h with NBD peptides or PHA-408 then subjected to Western blot analysis. NS, nonstimulation. NBD peptides used were IKK-2 11-mer.

Supplementary Figure S2



Negative control for the Alphascreen assay. GST-FLAG fusion construct was used to monitor unspecific binding of NBD containing peptides.

Supplementary Figure S3



Lack of PHA-408 effects on NEMO-IKK-2 interactions in RASF. RASF cells were incubated with the indicated concentrations of WT or mut NBD peptides or PHA-408 for 30 min before stimulation with 1 ng/ml IL-1β for 15 min. NEMO-IKK2 interactions were visualized at 20x magnification (A) using PLA as detailed under "Experimental Procedures". NBD peptides used were IKK-2 11-mer.

Supplementary Table 1

Peptides	AlphaScreen IC_{50}	FRET IC_{50}	Fluorescence Polarization IC_{50}	Biacore K_d
WT NBP (45-mer)	15- 47 nM	1 - 7 nM	15 - 30 nM	76.2 nM
Mut NBP (45-mer)	4,7 μM	>30 μM	>1 μM	ND
WT NBP (11-mer)	> 100 μM	> 100 μM	> 10 μM	> 100 μM

Comparison of the potency of NBD containing peptides in various biochemical assays. The ability of NBD peptides to inhibit the binding of FLAG-full length IKK-2 and GST-NEMO-(2-200) (Alphascreen), biotinylated IKK-1/2 chimeric 44-mer peptide and NEMO-(2-200) (FRET), TAMRA-IKK-2 45-mer peptide and NEMO-(2-200) (fluorescence polarization), IKK-2 45-mer peptide and biotinylated- NEMO-(2-200) (biacore) was measured. ND, not determined.