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Supplemental Data

UV as an Amplifier Rather

Than Inducer of NF-κB Activity

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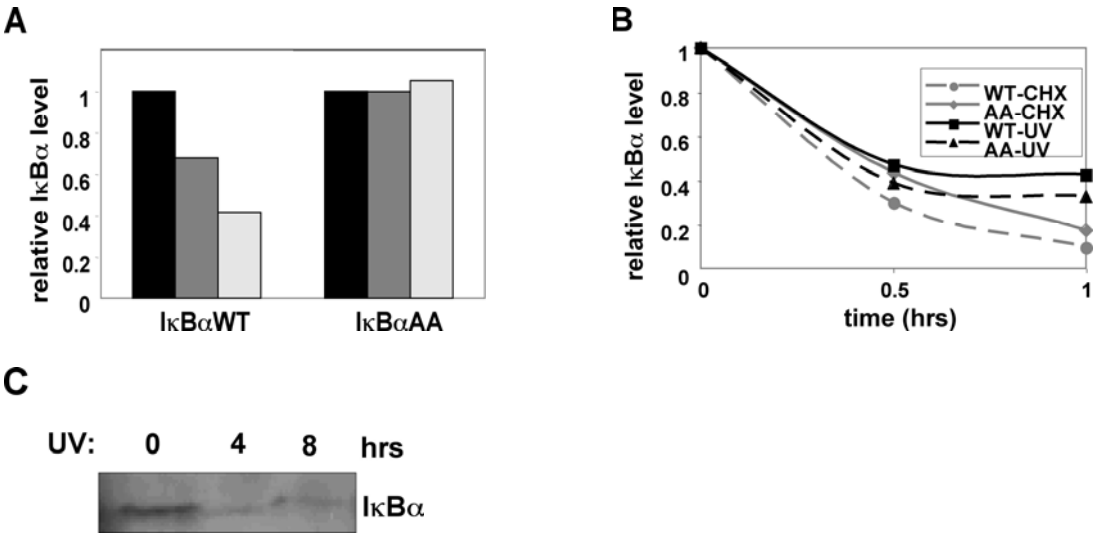

Figure S1

(**A**) Quantitation of the gels shown in Figure 2F. *iκbα*$^{-/-}$ cells transduced with wild-type IκBα (IκBαWT, on left) or with Ser32/36Ala IκBα (IκBαAA, on right) were left untreated (black bars), treated with 60 J/m^2 UVC for 8 hours (dark gray bars), or TNF for 10 minutes as a control (light gray bars), and whole cell extracts subject to immunoprecipitation with anti-p65 antibody and immunoblot for IκBα. Levels of IκBα were normalized to the respective p65 levels and the ratio for untreated lanes were normalized to 1.0.

(**B**) Quantitation of the gels shown in Figure 2G. *nfκb*$^{-/-}$ cells transduced with either IκBαWT or IκBαAA were treated with CHX or UV and whole cell extracts subject to immunoblot for IκBα.

(**C**) Immunoblot for IκBα of whole cell extracts from *nfkb*$^{-/-}$ (*rela*$^{-/-}$*nfκb1*$^{-/-}$*crel*$^{-/-}$) treated with 40 J/m^2 UVC for the indicated times.

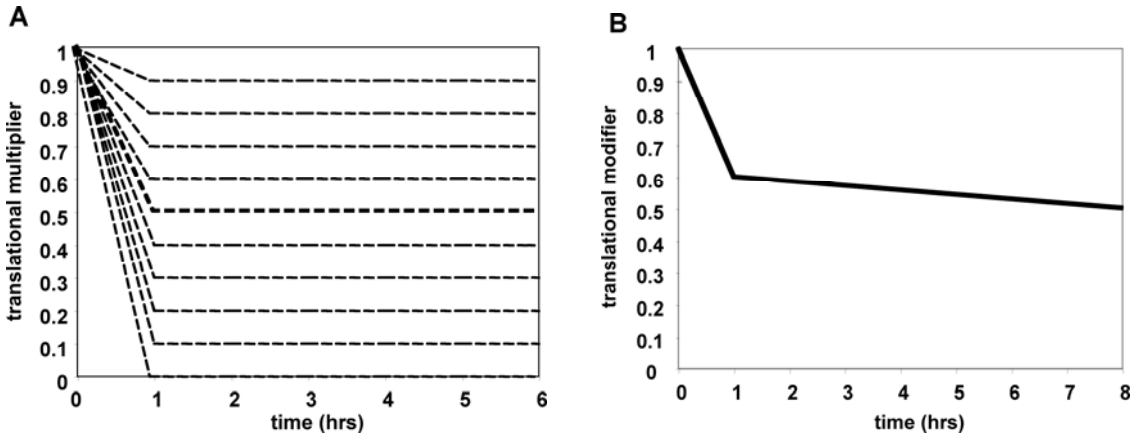

Figure S2
To simulate UV-induced translational inhibition (Figures 3, 4, and 7), all protein synthesis parameters in the model were multiplied by a translational multiplier that linearly decreased from time zero to one hour from the modifier 1.0 to 0.5 (to make it 50% inhibition of protein synthesis, data based on results in Figure 1C). After one hour, the protein synthesis rates were held over time at 50% inhibition.
For the dose response curves in Figures 1, 5, and 6 the same tool was applied but the translational multiplier varied depending on the degree of translational inhibition indicated on the curve (dashed lines).

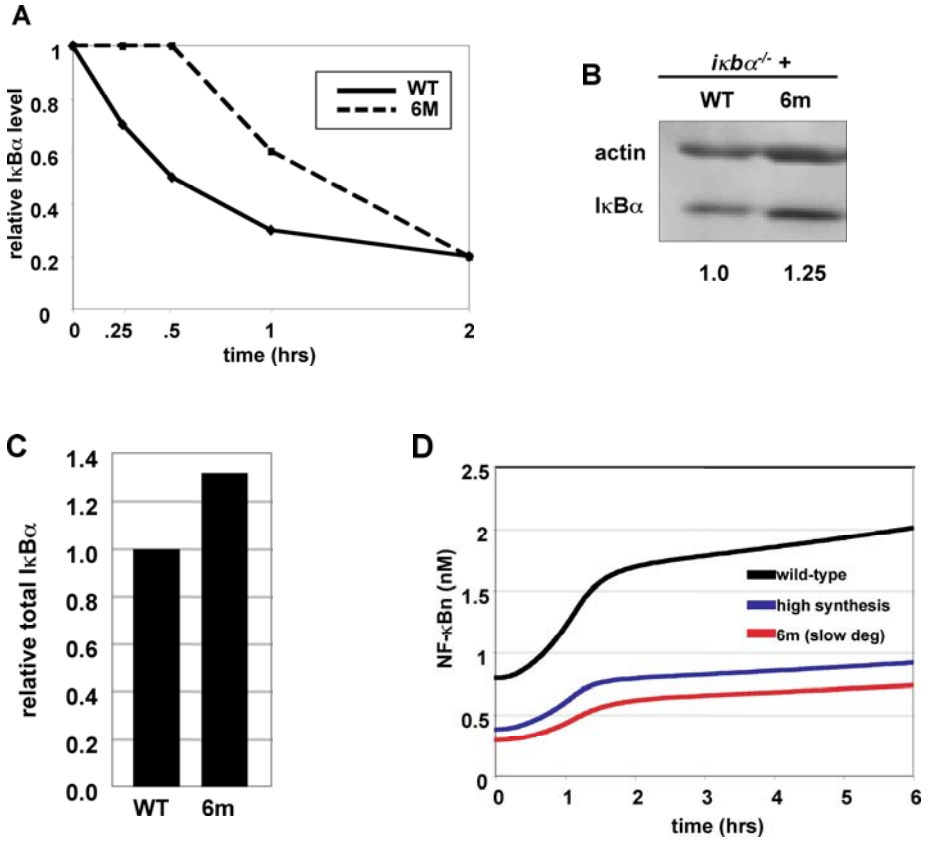

Figure S3

(**A)** Quantitation of Western blot gels shown in Figure 4B of *nfκb*$^{-/-}$ cells transduced with either IκBαWT or IκBα6m treated with CHX. Band intensities were normalized to the untreated lane for each cell line.

(**B)** Immunoblot and quatitation of steady-state IκBα levels in *iκbα*$^{-/-}$ cells trasduced with either IκBαWT or IκBα6m. Levels of IκBα were normalized to their respective actin levels.

(**C)** Computational simulation of steady-state total cellular IκBα levels. In the "6m" model, the IKK-independent degradation rate of free IκBα was decreased 2-fold to mimic the effect of the 6m IκBα mutant.

(**D**) Computational simulations of NF-κB activation by UV-induced translational inhibition (40-50% translational inhibition, described in Supplemental Figure 2B). For "6m (slow deg)", red line, the IKK-independent free IκBα degradation rate was decreased 2-fold. For "high synthesis", blue line, IκBα degradation rates were as in the wild-type model, but the IκBα translation rate (mRNA to protein) was increased 1.9-fold, a degree to which the total cellular IκBα steady-state levels match the levels in the 6m model (1.3-fold higher than in the wild-type model).

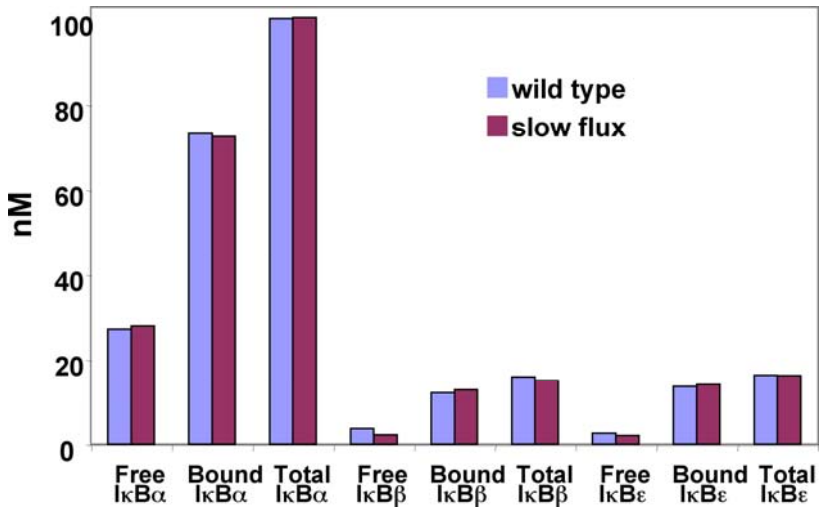

Figure S4

Graph showing the calculated equilibrium levels of IκBα, IκBβ, and IκBε in the wild-type and slow flux models.

The specific alterations to parameters in the slow flux model as compared to the wild-type model are as follows: The IKK-independent degradation rate for free IκBα was divided by 2000, and for IκBβ and IκBε they were divided by 3000 (all to make the IKK-independent degradation rate for free IκB equal to the respective IKK-dependent rate for bound IκB). The IκBα translation rate for IκBα was multiplied by 0.0752, the for IκBβ it was multiplied by 0.0177, and for IκBε it was multiplied by 0.0283.

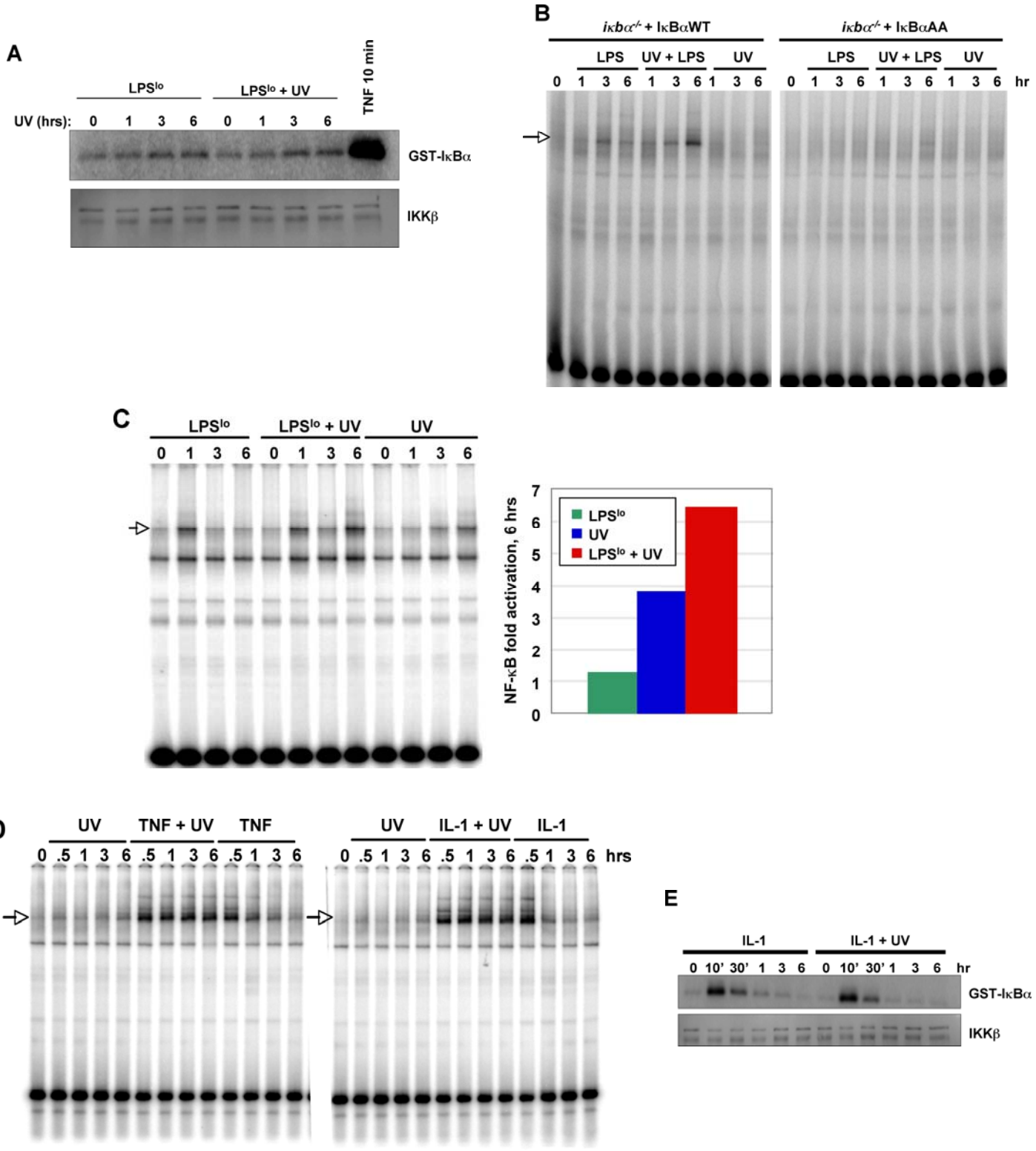
A
LPS^lo
LPS^lo + UV
TNF 10 min
UV (hrs): 0 1 3 6 0 1 3 6
GST-IκBα
IKKβ
B
iκbα-/- + IκBαWT
iκbα-/- + IκBαAA
LPS
UV + LPS
UV
0 1 3 6 1 3 6 1 3 6
0 1 3 6 1 3 6 1 3 6
hr
C
LPS^lo
LPS^lo + UV
UV
0 1 3 6 0 1 3 6 0 1 3 6
NF-κB fold activation, 6 hrs
0 1 2 3 4 5 6 7
LPS^lo
UV
LPS^lo + UV
D
UV
TNF + UV
TNF
UV
IL-1 + UV
IL-1
0 .5 1 3 6 .5 1 3 6 .5 1 3 6
0 .5 1 3 6 .5 1 3 6 .5 1 3 6
hrs
E
IL-1
IL-1 + UV
0 10' 30' 1 3 6 0 10' 30' 1 3 6
hr
GST-IκBα
IKKβ

Figure S5

(A) *In vitro* IKK kinase assay of cytoplasmic extracts from wild-type cells treated with 1 ng/mL LPS ("LPS^{lo}") or co-treated with 1 ng/mL LPS and 40 J/m^2 UVC ("LPS^{lo} + UV"). Immunoblot for IKKβ is shown as a IP-efficiency and loading control. Quantitation from this gel was used as an IKK input for the computational simulations in Figure 7A.

(B) EMSA of nuclear extracts from *iκbα*$^{-/-}$ cells transduced with either wild-type IκBα (IκBαWT) or Ser32/36Ala IκBα (IκBαAA) treated with 1 ng/mL only, 40 J/m^2 UVC only, or co-treated with LPS and UVC for the indicated times.

(C) EMSA of nuclear extracts from mouse keratinocytes treated with 1 ng/mL LPS or co-treated with 1 ng/mL LPS and 40 J/m^2 UVC. A graph showing quantitation of the NF-κB fold-induction (y-axis) at the 6-hour time point for each treatment regime is shown to the left of the gel.

(D) EMSA of wild-type cells treated with either 1 ng/mL IL-1, 40 J/m^2 UVC, 1 ng/mL TNF, co-treated with 1 ng/mL IL-1 and 40 J/m^2 UVC, or co-treated with 1 ng/mL TNF and 40 J/m^2 UVC for the indicated times

(E) *In vitro* IKK kinase assay of cytoplsmic extracts of wild-type cells treated with 1 ng/mL IL-1 or co-treated with 1 ng/mL IL-1 and 40 J/m^2 UVC. Immunoblot for IKKβ is shown as a IP-efficiency and loading control.

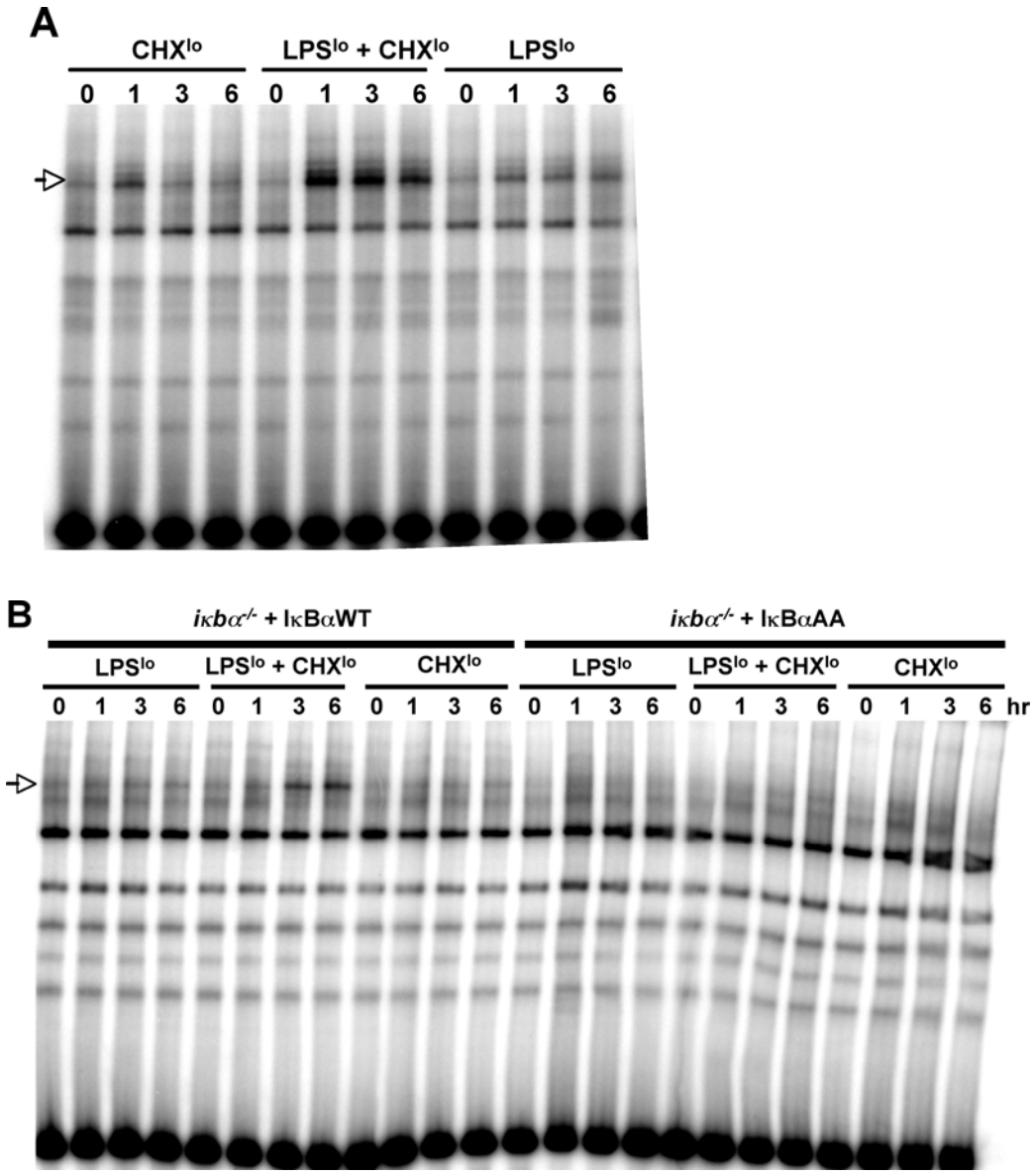

Figure S6

(**A**) Combined low dose CHX and low dose LPS hyper activate NF-κB. Nuclear extracts from wild-type cells treated with 0.1 μg/mL CHX ("CHX^{lo}"), 1 ng/mL LPS ("LPS^{lo}"), or co-treated with CHX and LPS for the indicated times were subject to EMSA.

(**B**) Synergistic NF-κB activation by CHX and LPS requires IKK-mediated phosphoylation of IκBα. Nuclear extracts from *iκbα*$^{-/-}$ cells transduced with either wild-type or Ser32/36Ala (IκBαAA) treated with 0.1 μg/mL CHX ("CHX^{lo}"), 1 ng/mL LPS ("LPS^{lo}"), or co-treated with CHX and LPS for the indicated times were subject to EMSA.

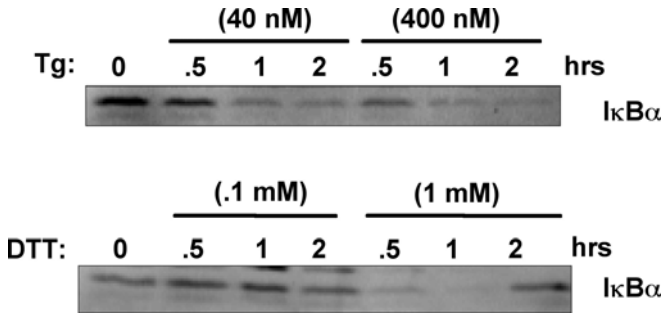

Figure S7
ER stress induced-translational inhibition depletes the pool of free IκBα. Immunoblot of whole cell extracts for IκBα from *nfκb*$^{-/-}$ MEFs transduced with wild-type IκBα treated with 40 or 400 nM thapsigargin or 0.1 mM or 1 mM DTT for the indicated time.