

SUPPLEMENTARY INFORMATION

Supplementary Figure 1. The LPS antagonist RSLA does not affect dsRNA-induced Akt activation and IL-8 production. Human mDCs were coincubated or not with 5 µg/ml RSLA before addition of 100 µg/ml dsRNA or 50 ng/ml LPS and treatment for 40 min (A) or 12 hours (B). In (A) activation of Akt in whole cell lysates was analyzed by immunoblotting with an antibody specific to activated Akt (S473). In (B) levels of IL-8 in supernatants were measured by ELISA.

Supplementary Figure 2. The 34 bp Cy5-labeled dsRNA sequence from human rhinovirus stimulates IFN-β and TNF synthesis in human mDCs and activates an IFN-β reporter gene in TLR3-transfected cells. (A) Human mDCs were treated with 20 µg/ml, 100 µg/ml or 200 µg/ml Cy5-labeled dsRNA for 8 hours. Levels of IFN-β and TNF were measured by ELISA (Levels of IFN-β at 20 µg/ml were not measured). (B) HEK293 cells were transiently cotransfected with plasmids encoding either TLR3YFP-pcDNA3 or pcDNA3 (40 ng) and an IFN-β reporter plasmid (40 ng). After 36 hours cells were left untreated or treated with 20 µg/ml, 100 µg/ml or 200 µg/ml Cy5-labeled dsRNA for 6 hours before measurement of reporter gene activity in cell lysates.

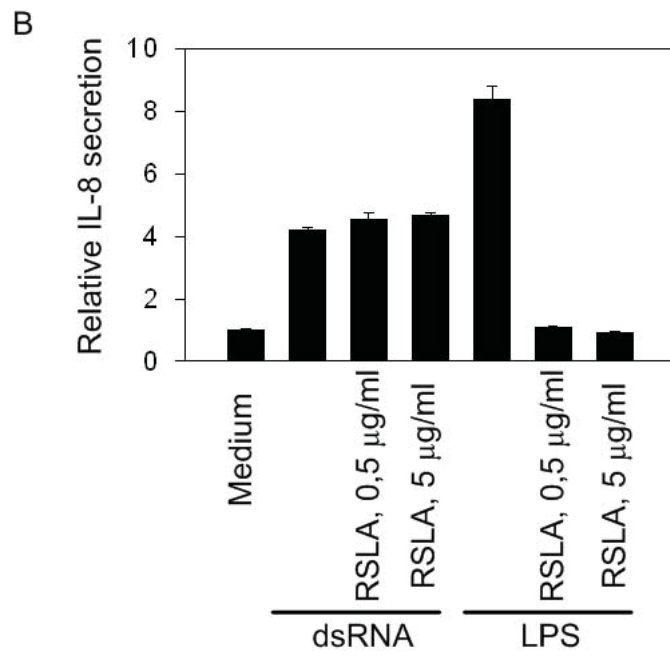
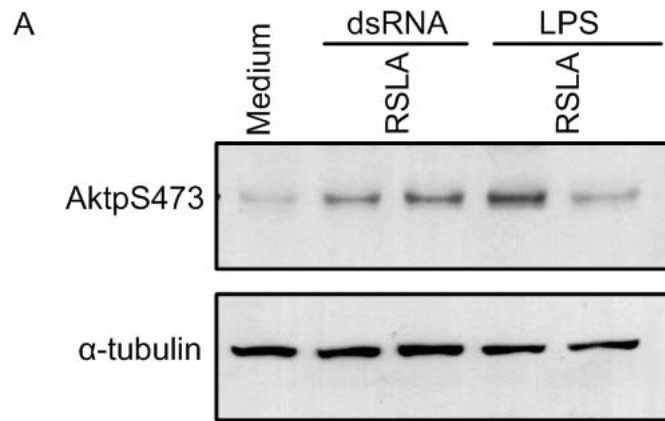
Supplementary Figure 3. DsRNA and CpG DNA follow the same uptake pathway. Confocal images of human mDCs coincubated with Cy5-labeled dsRNA and TexasRed-labeled CpG DNA for indicated time periods.

Supplementary Figure 4. TLR3 colocalizes with TLR9 in the ER. Confocal images of HeLa cells expressing TLR3^{YFP} transiently transfected with CFP-tagged TLR9.

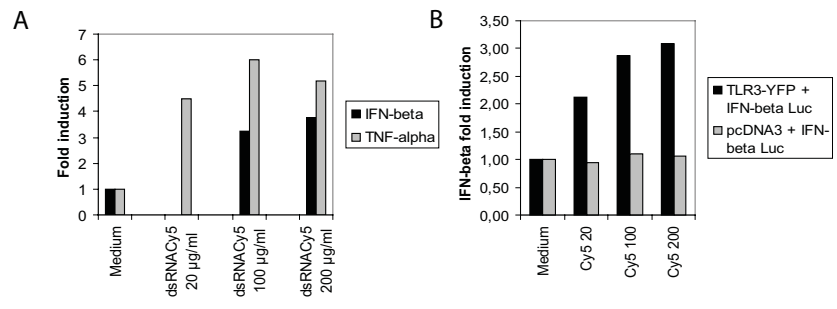
Supplementary Figure 5. TLR4 does not colocalize with a CFP-tagged ER protein marker. (A) Confocal images of HEK293 cells expressing TLR4^{YFP} protein chimera transiently transfected with a CFP-tagged ER protein marker. At the present picture the Golgi apparatus is not included in the optical slice and consequently not shown. (B) Whole cell lysates of HEK E cells, HEK

cells expressing TLR4^{YFP}, HeLa E cells and HeLa cells expressing TLR3^{YFP} were resolved by SDS-PAGE and immunoblotted for GFP and α -tubulin.

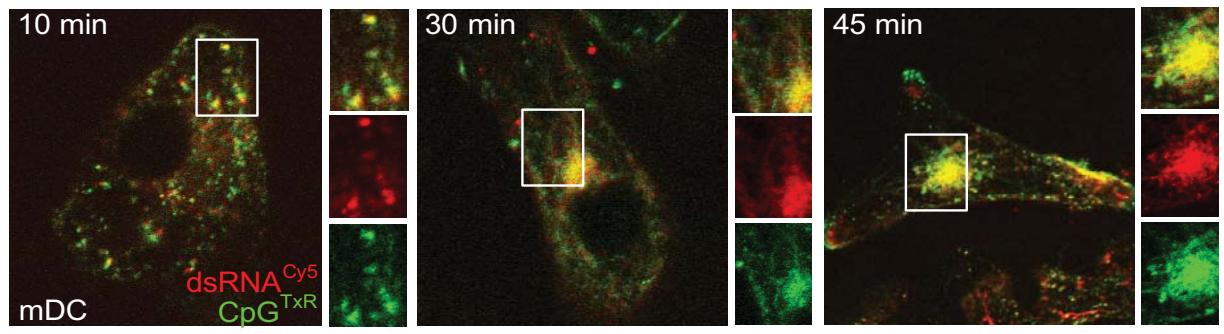
Supplementary Figure 6. TLR3^{YFP} is functional in mediating dsRNA-induced NF- κ B activation and IFN- β production. HEK293 cells were transiently cotransfected with plasmids encoding either TLR3YFP-pcDNA3, TLR3Flag-pCMV, pcDNA3 or pCMV (40 ng), and an IFN- β reporter plasmid (40 ng). After 36 hours cells were treated with 100 μ g/ml dsRNA for 6 hours before measurement of reporter gene activity in cell lysates.



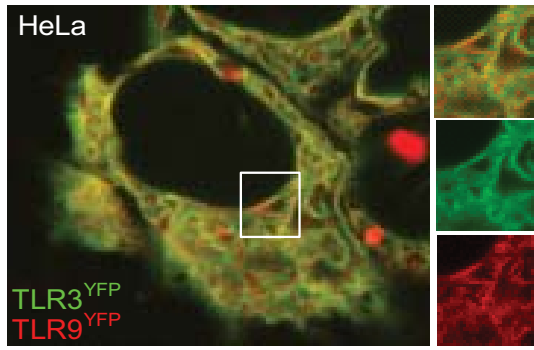
Supplementary Figure 1



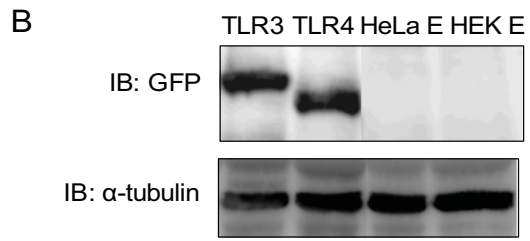
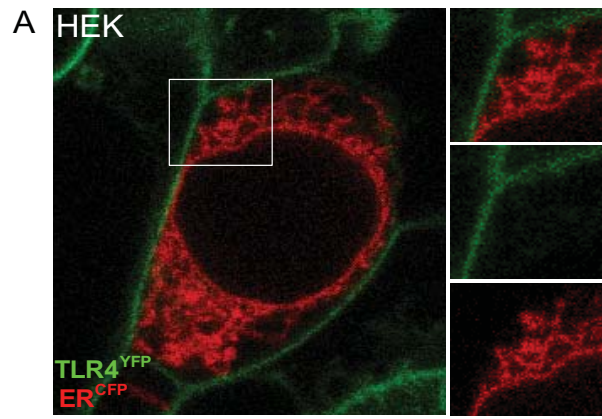
Supplementary figure 2



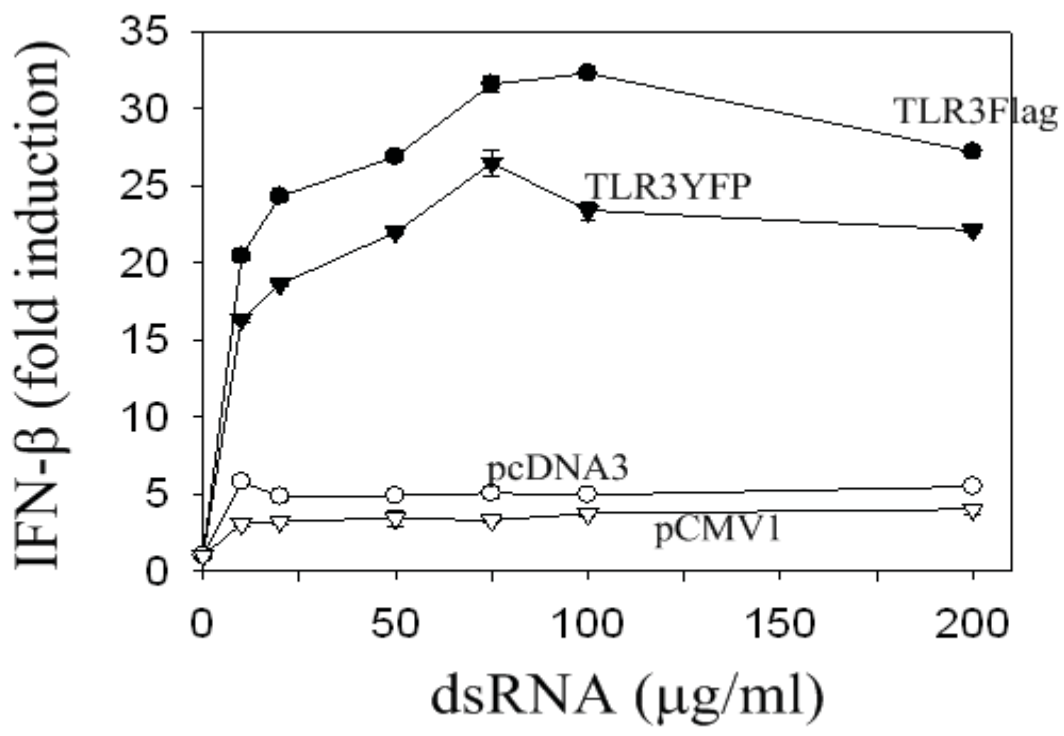
Supplementary figure 3



Supplementary figure 4



Supplementary figure 5



Supplementary figure 6