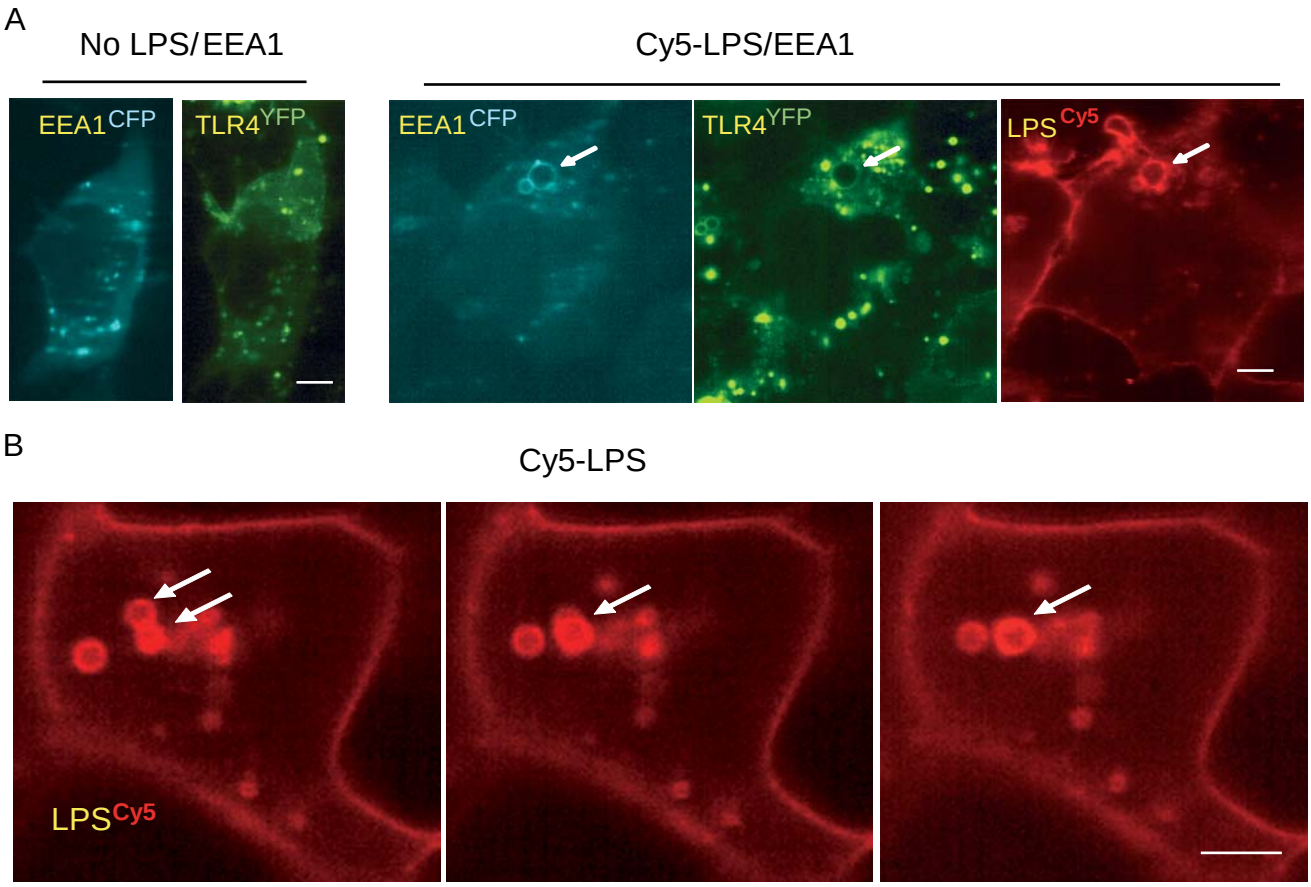
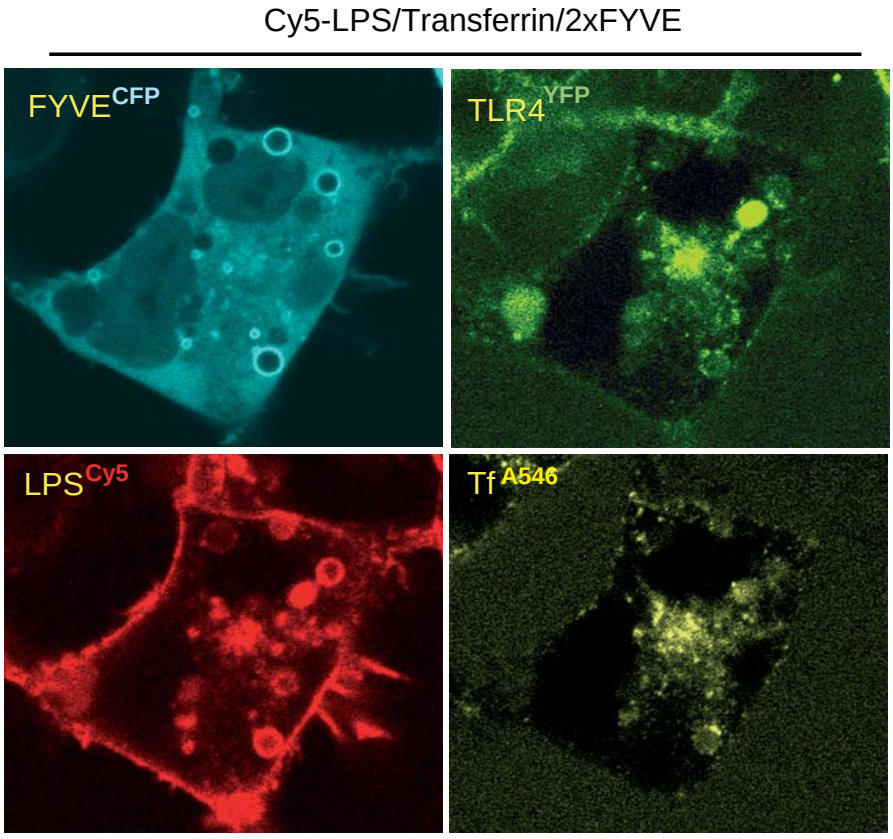
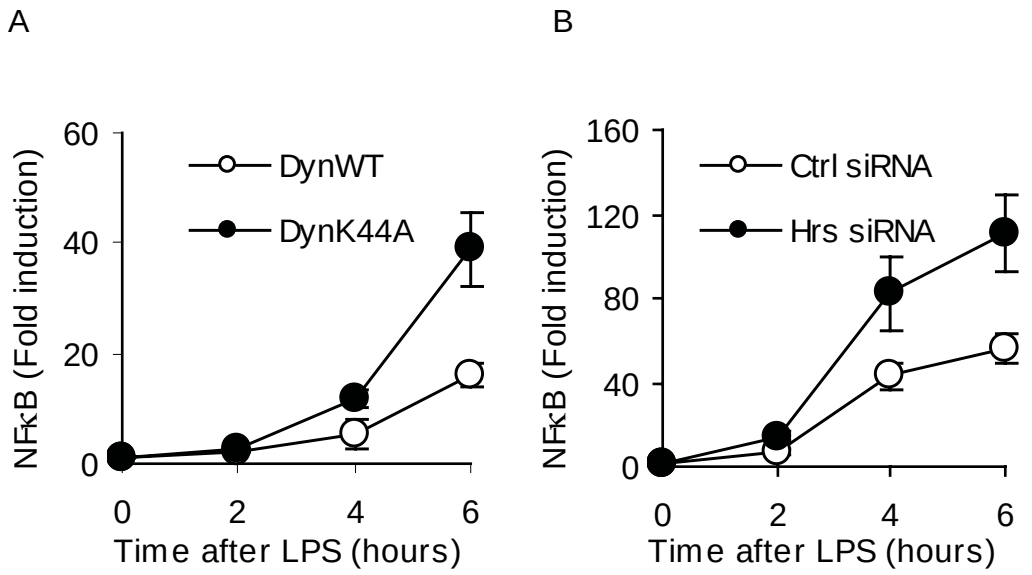


Supplementary Information, Figure 1.
Addition of LPS results in enlarged early/sorting endosomes.
HEK293 cells stably expressing $TLR4^{YFP}$/MD-2 were transfected with CD14 and $EEA1^{CFP}$ (A), and stimulated by adding LPS^{Cy5} (250 ng/ml). The left panel shows the confocal images of a cell without stimulation and the rigth panel a cell after 30 minutes of LPS^{Cy5} stimulation. Enlarged EEA1 positive endosomes (arrow) were not observed in unstimulated cells. HEK293 cells stably expressing TLR4YFP/MD-2 were transfected with CD14 and incubated with LPS^{Cy5}(B). LPS positive endosomes engaged in a fusion event are shown (arrows). The fusion of the two equally sized large LPS positive endosomes gives rise to an endosome with a 25% increased diameter. Time interval between each image capture is 5 s. These three images are taken from the Supplementary Movie 1. Bar = 5µm

Methods: LPS (0111:B4) from Escherichia coli was purchased from Invivogen. Cy5-labelling of LPS was performed as previously described (Latz et al., 2002). The EEA1 in pECFP-C1 (Clontech) ($EEA1^{CFP}$) encodes the C-terminal part of EEA1 with amino acid residues 1257-1411 representing the minimal region of EEA1 able of early endosomal targeting (Stenmark et al., 1996). Images of live cells were captured at 37ºC using an Axiovert 200M microscope with a heated stage equipped with a LSM 5 LIVE laser scanning microscope and a 100x/1.45 NA oil alpha Plan-Fluar objective. CFP-, -YFP and Cy5-flouresence were obtained using the 405 nm-, 488 nm- and 633 nm-diode lasers, respectively.



Supplementary Information, Figure 2.
LPS is internalized on early/sorting endosomes containing TLR4. HEK293 cells expressing $TLR4^{YFP}$/MD-2 co-transfected with CD14 and a marker for early/sorting endosomes, $2xFYVE^{CFP}$. Confocal images of a cell 45 minutes after simultaneously addition of Alexa546-labeled transferrin Tf^{A546} (5µg/ml) and LPS^{Cy5} (250 ng/ml) addition. Confocal images presented are the full captures of the four separate channels presented in Figure 2e. Bar=5 µm.



Supplementary Information, Figure 3.
Time kinetics of NFκB-activation with DynK44A and Hrs siRNA and controls.
HEK293 cells expressing TLR4^{YFP}/MD-2 were transfected with Elam-luc together with wild type dynamin (DynWT) or a dominant negative dynamin (DynK44A) (A), or treated with 40 nM Hrs siRNA or control siRNA for 48 hours before being transfected with Elam-luc together with CD14 (B). LPS-induced NFκB-activation was monitored after 0, 2, 4 and 6 hours of stimulation with LPS (10 ng/ml).

Methods: SiRNA duplexes targeting the coding region of Hrs and a non-interfering scrambled RNA duplex have previously been described (Bache et al., 2003). The siRNA duplexes used were synthesised by Dharmacon. HEK293 cells expressing TLR4^{YFP}/MD-2 were transfected with 40 nM siRNA for 48 hours using Oligofectamine (Invitrogen) according to the manufacturer's protocol. For the introduction of plasmid DNA into the siRNA transfected cells, the cells were replated and transfected for 24 hours using the GeneJuice transfection reagent.

Supplementary Information, Movie 1

Movie clip of a fusion event between two LPS positive endosomes. HEK293 cells stably expressing $TLR4^{YFP}$/MD-2 were transfected with CD14 and incubated with 300ng/ml LPS^{Cy5}. The movie was captured approximately 45 min after addition of LPS. The real time length of the movie is 245 s. Images of live cells were captured at 37 °C using an Axiovert 200M microscope with a heated stage equipped with a LSM 5 LIVE laser scanning microscope and a 100x/1.45 NA oil alpha Plan-Fluar objective. Cy5-flouresence was obtained using a 633 nm- diode laser.