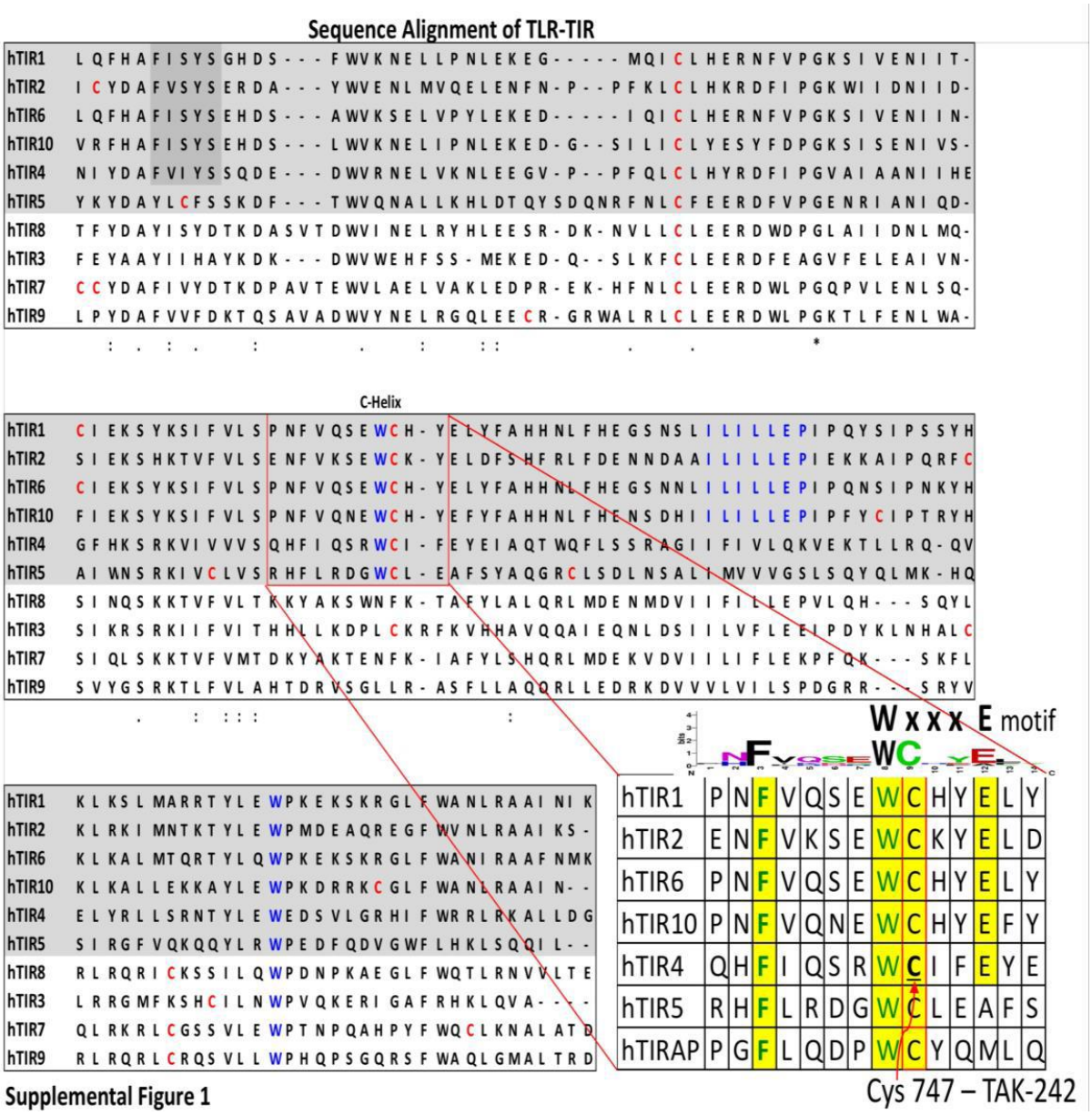
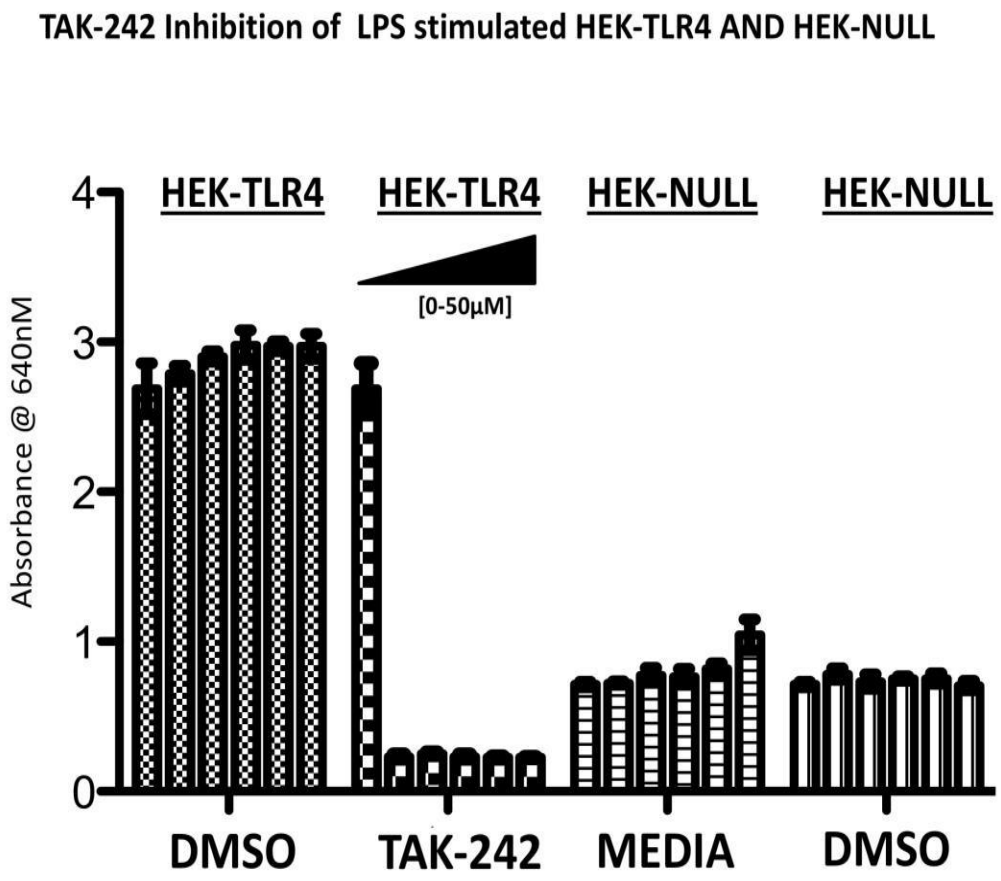
Supplemental Material

Supplemental Figure 1. Clustal W sequence alignment of TLR TIR domains. Box inset identifies sequence conservation of C helix Cys WxxxE motif as well as catalytic E (Glu) observed in bacterial and NADase TIR proteins. TAK-242 targets TLR4-TIR Cys 747.30

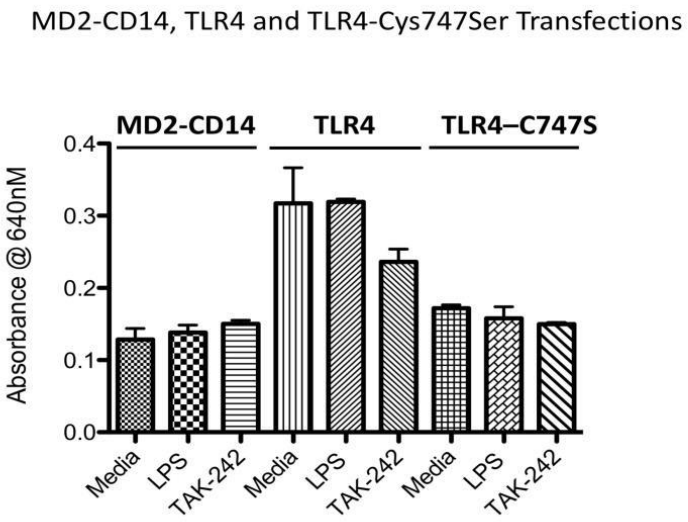


Supplemental Figure 1

Supplemental Figure 2. TAK-242 inhibition of LPS-stimulated HEK-TLR4 cells. Increasing amounts of TAK-242 (0-50 μM) inhibits LPS activation of HEK-TLR4 cells in comparison with HEK null cells, media and vehicle control (DMSO).



Supplemental Figure 3. LPS stimulation of HEK-MD2-CD14 BlueTM cells alone or transfected with WT TLR4 or mutant TLR4 Cys747Ser. TAK-242 (0-50 μM) inhibits LPS activation of HEK-TLR4 cells in comparison to vehicle control (DMSO 0-0.2%) and HEK null cells. Left to right - LPS responses of HEK MD2-CD14-blue cells (stably expressing MD2 and CD14 but lacking TLR4) transfected with 100 ng of TLR4 WT or mutant TLR4 C747S and then stimulated with 10 ng/mL LPS in the presence of TAK-242 (50 μM) or vehicle control (DMSO 0.2%).



Supplemental Figure 4. Model of cytoplasmic TLR4 TIR dimer interactions. Model of TLR4- TIR dimer showing TAK-242 (ball and stick) binding target C747 (yellow). Conserved WxxxE motif, including catalytic glutamic acid (E) residue, observed in bacterial and NADase TIR proteins are highlighted and illustrated as ball and stick. Surface representations of potential substrate binding pocket shown in grey.