Fig. S1. **Recombinant Sip strains can be reactivated with indole.** Transmission electron microscopy was used to characterize the morphology of recombinant Sip RBs and EBs 24 h post-reactivation with indole. EB and RB morphology of the rescued recombinant Sip isolates was indistinguishable from L2-GFP.

Fig. S2. **Sip mutants form fewer inclusions than L2-GFP in an IFN-γ independent persistence model.** HeLa cell monolayers were incubated in DMEM-10TF for 24h and infected with the L2-GFP and the indicated Sip mutants and recombinant strains. The number of inclusions each strain formed in tryptophan replete conditions 24 hpi and 48 hpi following 24h reactivation with either DMEM-10TF + indole (black bars) or DMEM-10 (gray bars) was measured and used to calculate a ratio. This ratio was compared to L2-GFP. The graph depicts the mean of the results of three experiments performed in triplicate. Error bars indicate SD

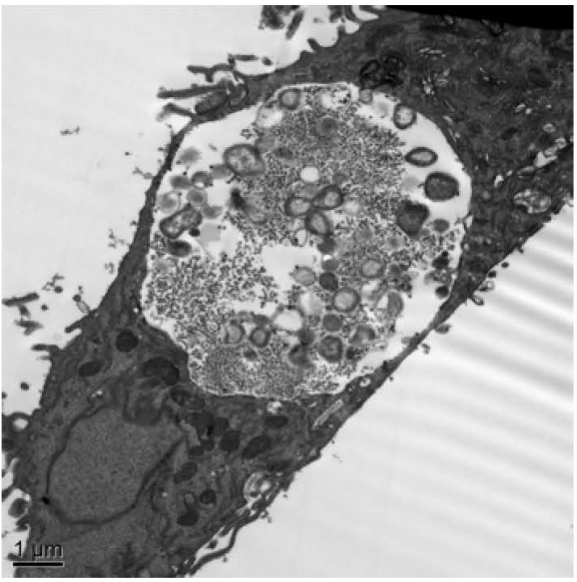
Fig. S3. **CTL0225$^{G77E}$ is sensitive to exogenous leucine.** HeLa cell monolayers were infected with the indicated strains and incubated in DMEM-10 supplemented with increasing concentrations of isoleucine. The cross sectional area of inclusions were measured 44 hpi following fixation, and staining with a chlamydial anti-LPS antibody. At least 1000 inclusions were measured for each strain and condition. The graph depicts the mean of the results of three experiments. Error bars depict SD. ****, $P$<.0001.

Fig. S4. **CTL0225$^{G77E}$ may have altered membrane topology.** TMHMM Server 2.0 analysis predicts that the G77E mutation in CTL0225 would destroy a transmembrane

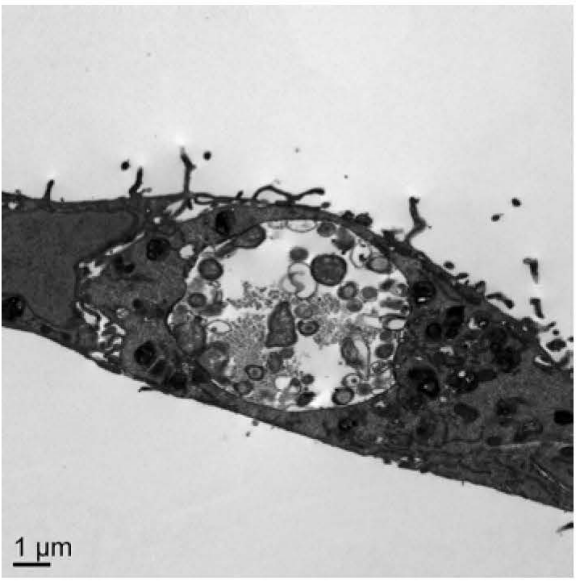
helix and invert the orientation of the C-terminus in the membrane (62). TOPO2 transmembrane display software was used to model the topology of L2-GFP CTL0225 and Sip2 CTL0225$^{G77E}$ (Johns S.J., TOPO2, Transmembrane protein display software, http://www.sacs.ucsf.edu/TOPO2/).

# IFN-γ Treated 24h post indole

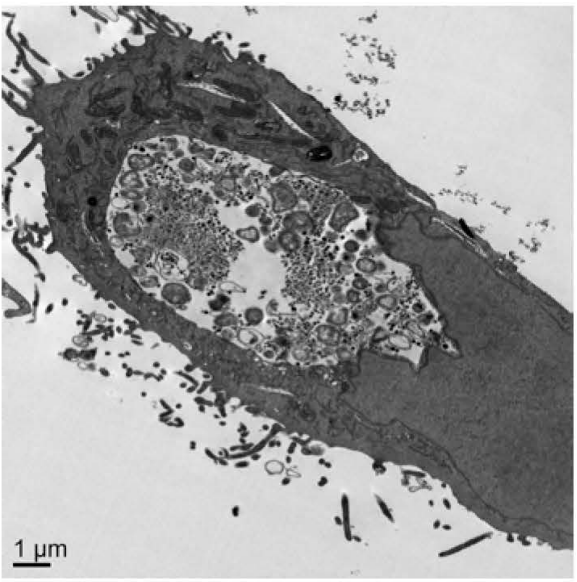
rSip1

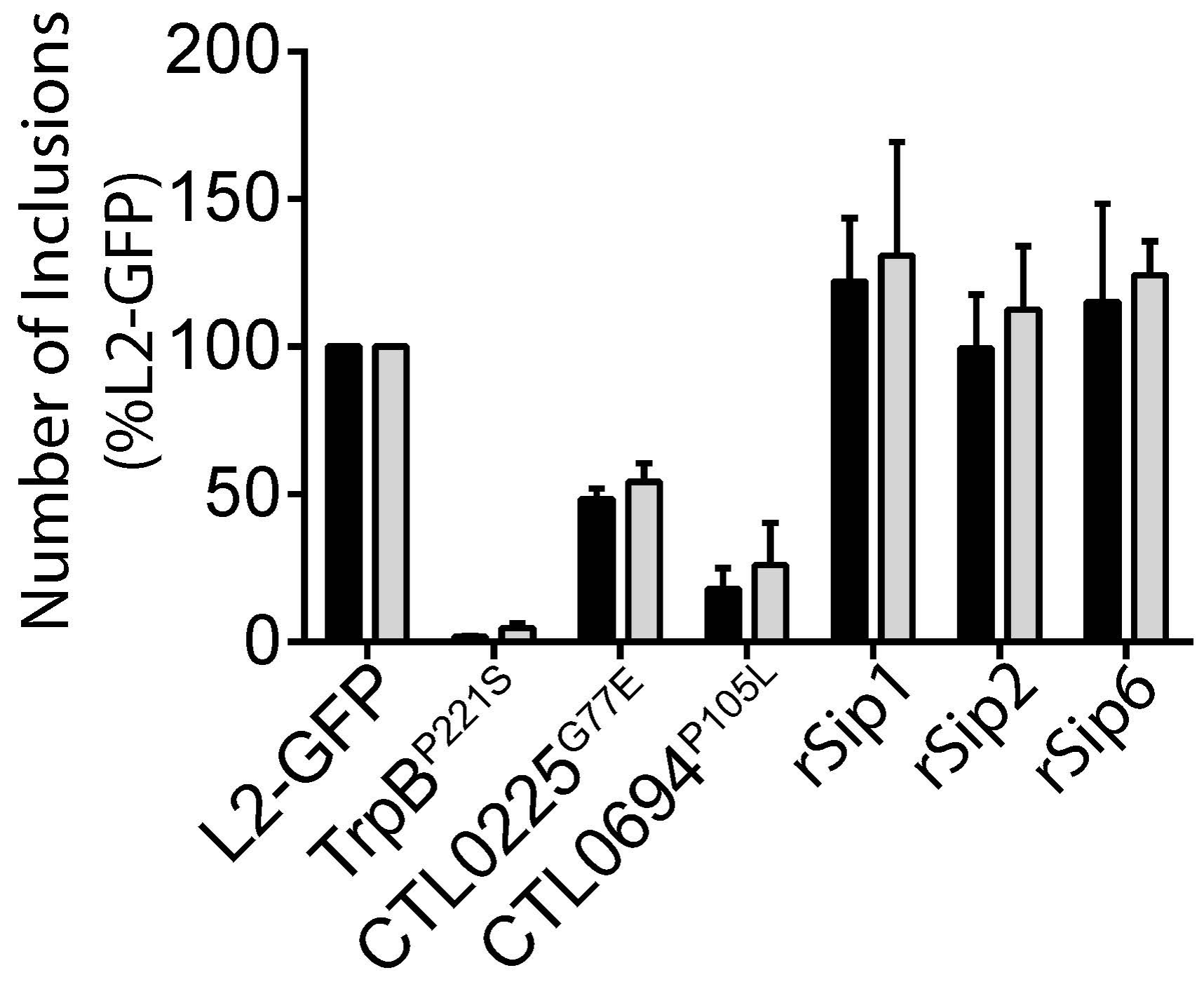


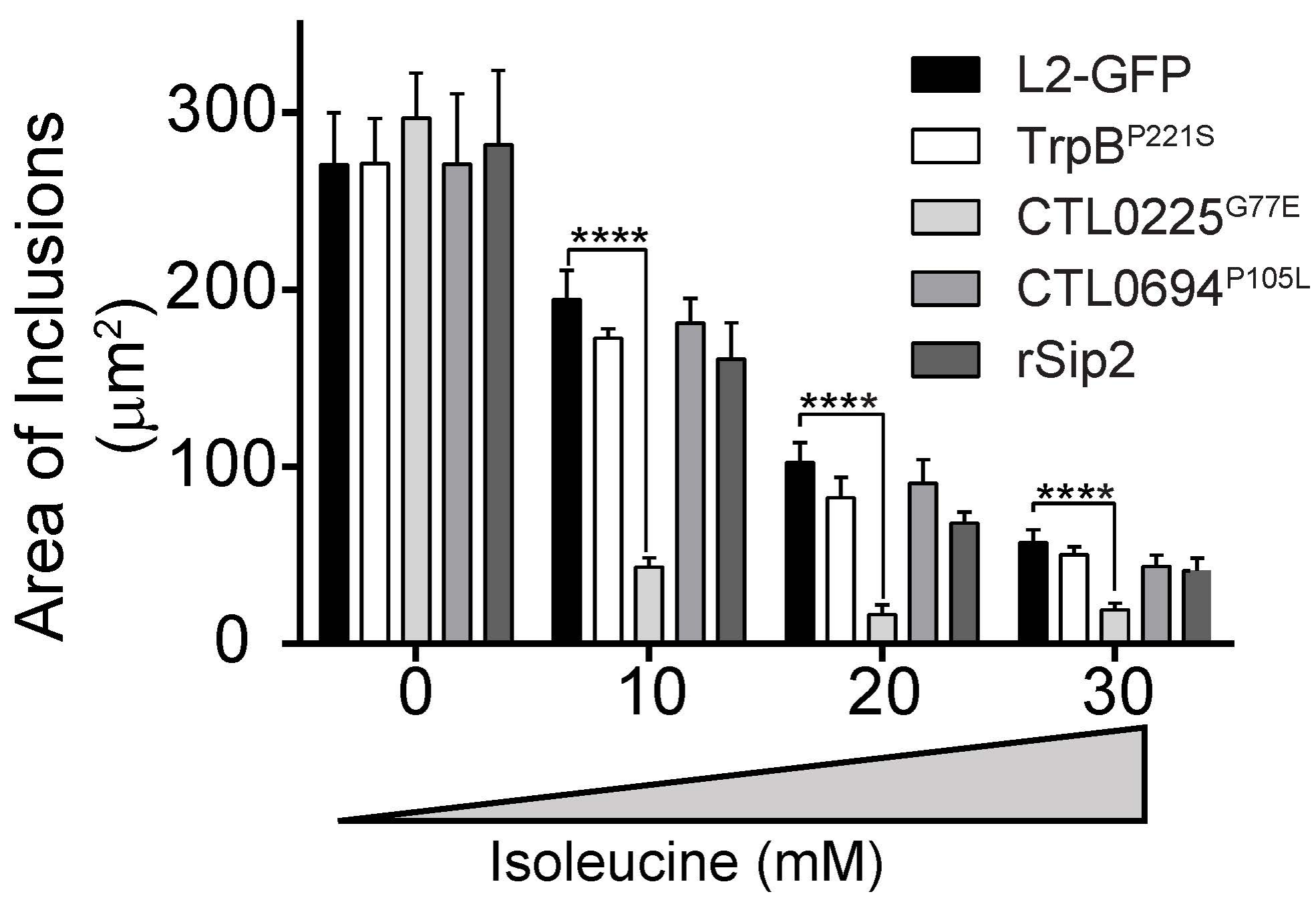

rSip2

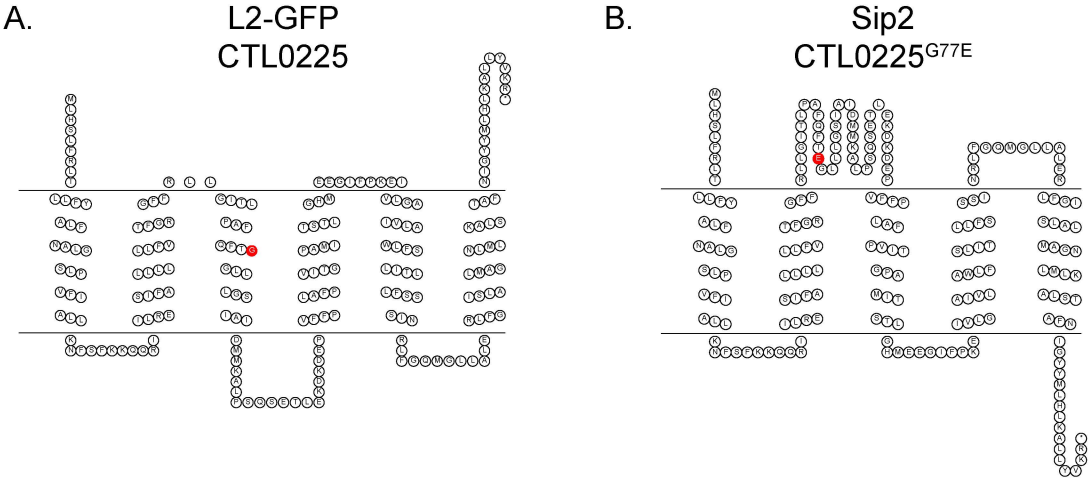



rSip6




Number of Inclusions
(%L2-GFP)
200
150
100
50
0
L2-GFP
TrpB$^{P221S}$
CTL0225$^{G77E}$
CTL0694$^{P105L}$
rSip1
rSip2
rSip6


Area of Inclusions (μm²)
300
200
100
0
****
****
****
0
10
20
30
Isoleucine (mM)
L2-GFP
TrpB^P221S
CTL0225^G77E
CTL0694^P105L
rSip2


A.
L2-GFP
CTL0225
B.
Sip2
CTL0225G77E