

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Dimeric LRR activates Met. (A) Non-reducing (-DTT) and reducing (+DTT) SDS-PAGE of purified recombinant InlB, LRR and LRR-C242A. Molecular weight (Kd). Dimeric LRR is indicated with an asterisk (*). (B) Molecular sizing chromatography of LRR in the absence of reducing agent was performed to fractionate dimeric LRR from monomeric LRR. (C) Sera-starved Hela cells were treated with the indicated ligand preparations for 10 mins at 37°C. Met was immunoprecipitated (IP) with the D0-24 antibody (Ab) and total Met and Met phosphorylation at Y1234,1235 (p-Met) was detected by Western analysis (IB) as indicated.

Figure S2: Met kinase activity is required for receptor internalization in response to LRR. Met^{-/-} mouse kidney cells expressing wild-type (WT) or kinase dead (KinD) Met were treated for 2, 4, 8 min in media containing 2 nM InlB or 10 nM LRR or without ligand (0 mins) at 37°C. The cells were rapidly cooled and the level of residual surface Met was directly determined using a cell ELISA. Values represent the level of surface Met as a percentage of total Met for each time point ± S.E. from 2-3 experiments.

Figure S3: Met internalization is regulated by CHC and Grb2. Representative confocal images from 3 separate experiments using Hela cells transfected with control (Con), clathrin heavy chain (CHC) or Grb2 siRNA following co internalization of Alexa-LRR with Alexa-Tfn or Alexa-Dextran 10,000 for 15 mins at 37°C. Bar = 10 µm.

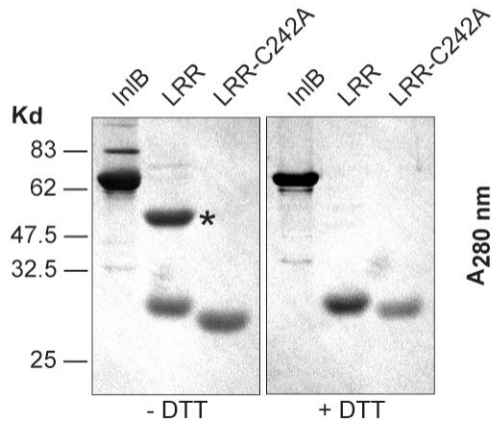
Figure S4: The transit of internalized Met to MVB is delayed in LRR treated cells. Representative confocal images from three separate experiments of Alexa⁴⁸⁸-labeled InlB or LRR in Hela cells stained for endogenous EEA1 or CD63 at the indicated chase times. Arrows indicate areas of colocalization (yellow) and are shown at 3X magnification in the inserts.

Figure S5: LRR-induced tubulogenesis. MDCK cells. MDCK cysts grown in collagen were incubated in media lacking ligand (Con) or supplemented with 2 nM HGF, 2 nM InlB or 10 nM LRR for 7 days as indicated.

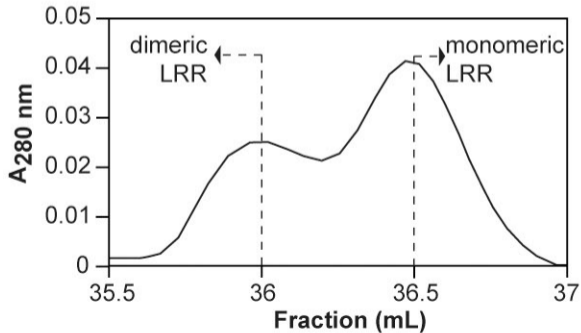
Figure S6: LRR-induced Hela cell scattering. The scattering response of serum-starved Hela cells incubated in media without ligand (Con) or with InlB (2 nM) or LRR (10 nM) for 18 hr was examined by phase-contrast microscopy (20X Magnification). Bars represent the mean ± S.E. Significant differences between LRR- and InlB-treated cells with control cells were detected (*p<0.01, ANOVA) and between LRR and InlB-treated cells (**p<0.01, ANOVA). The results are representative of 3 independent experiments.

Figure S1, Gao et al

A



B



C

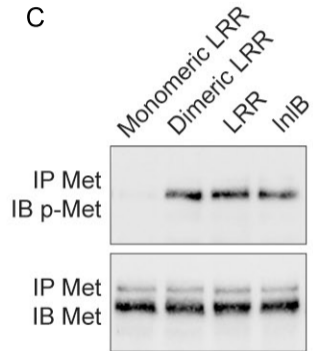


Figure S2, Gao et al

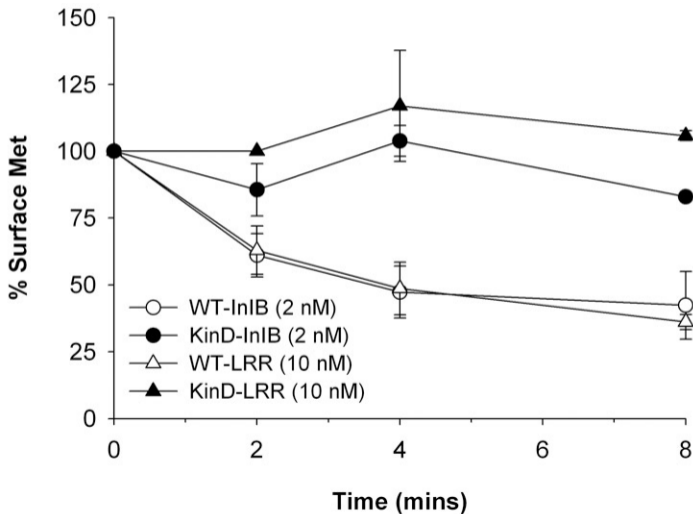


Figure S3, Gao et al

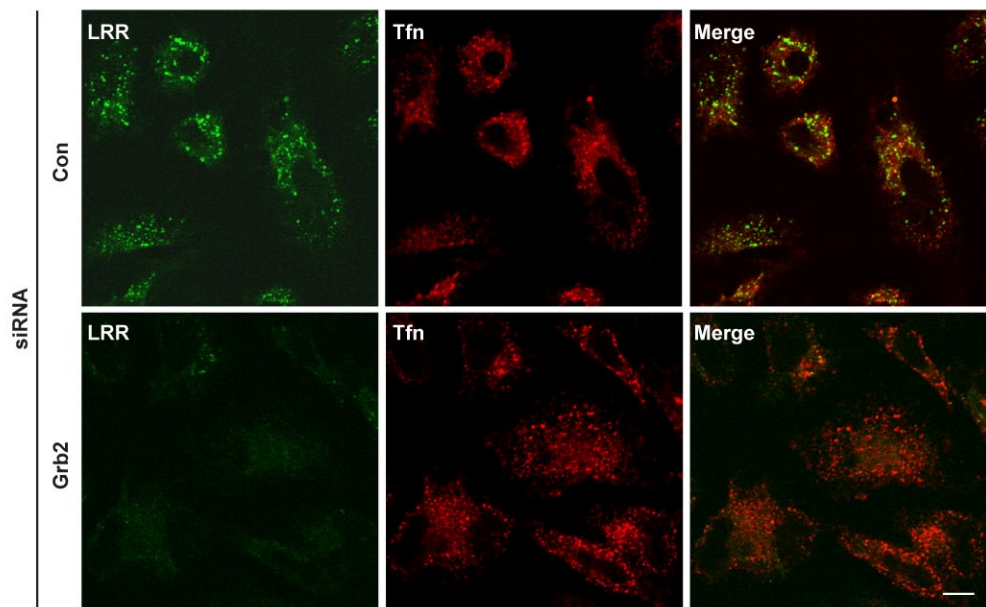
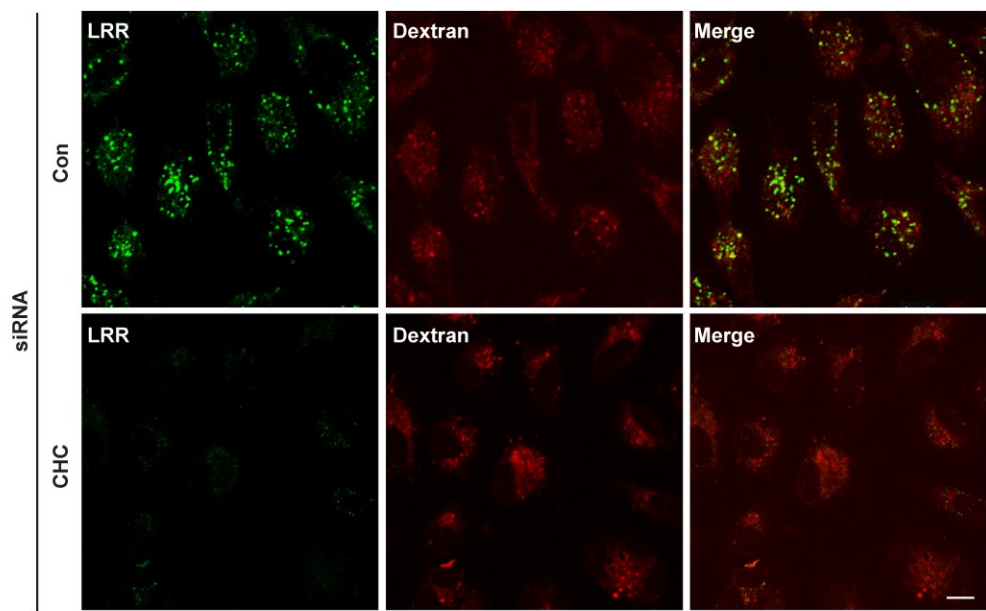


Figure S4, Gao et al

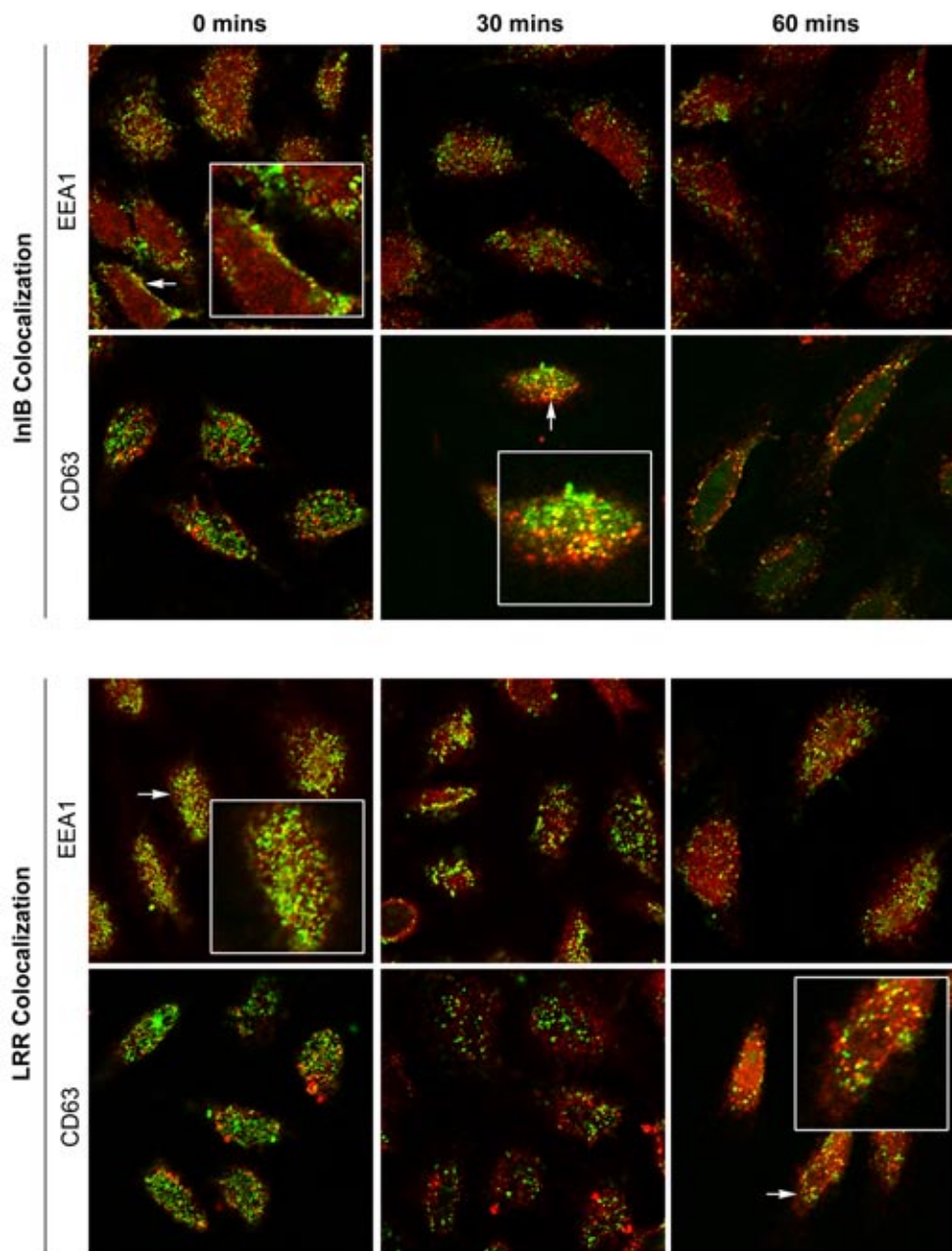


Figure S5, Gao et al

