Supplemental Movies and Figures

Movie S1. Internalization of EGF via a clathrin-coated pit.
Internalization of EGF-Alexa488 (green) was analyzed in HER14 cells expressing mRFP-clathrin (red). Movie was collected in TIRF mode for 30 sec at 10 frames/sec. Bar is 1µm.

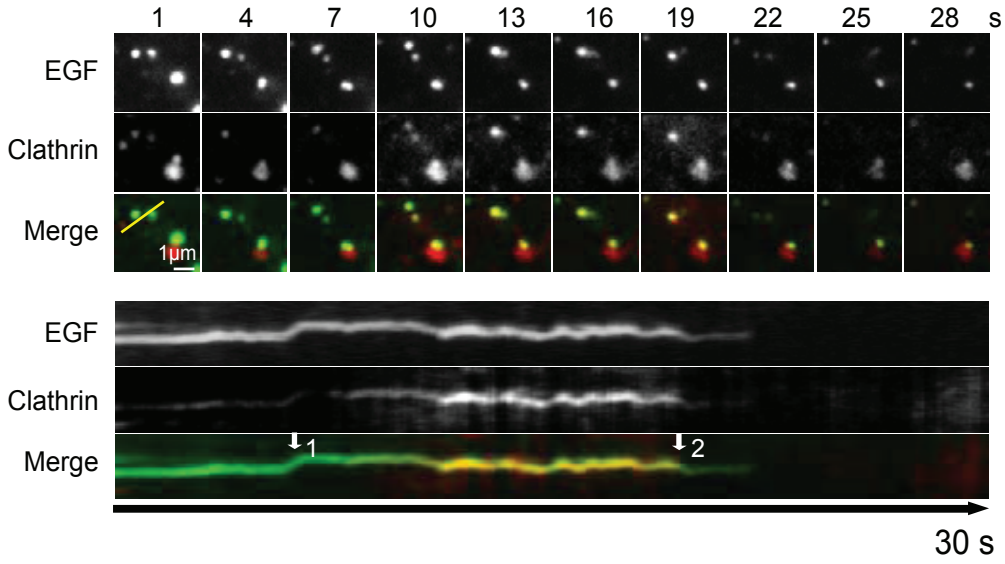

Figure S1. Analysis of EGF internalization via coated pit.
Screen shots of TIRF-M Movie 1 showing internalization of EGF. The bottom panel shows the kymographs of the indicated area (yellow line) generated for both channels separately and merged. Kymographs were generated using MetaMorph 7.7.5 software (Molecular Devices). Arrow 1 indicates colocalization of EGF with clathrin and arrow 2 indicates pinching off from the plasma membrane.

Movie 2. Internalization of Bipar1 via a clathrin-coated pit.
Internalization of Bipar1-Alexa488 (green) was analyzed in HER14 cells expressing mRFP-clathrin (red). Movie was collected in TIRF mode for 50 sec at 10 frames/sec. Bar is 1µm.

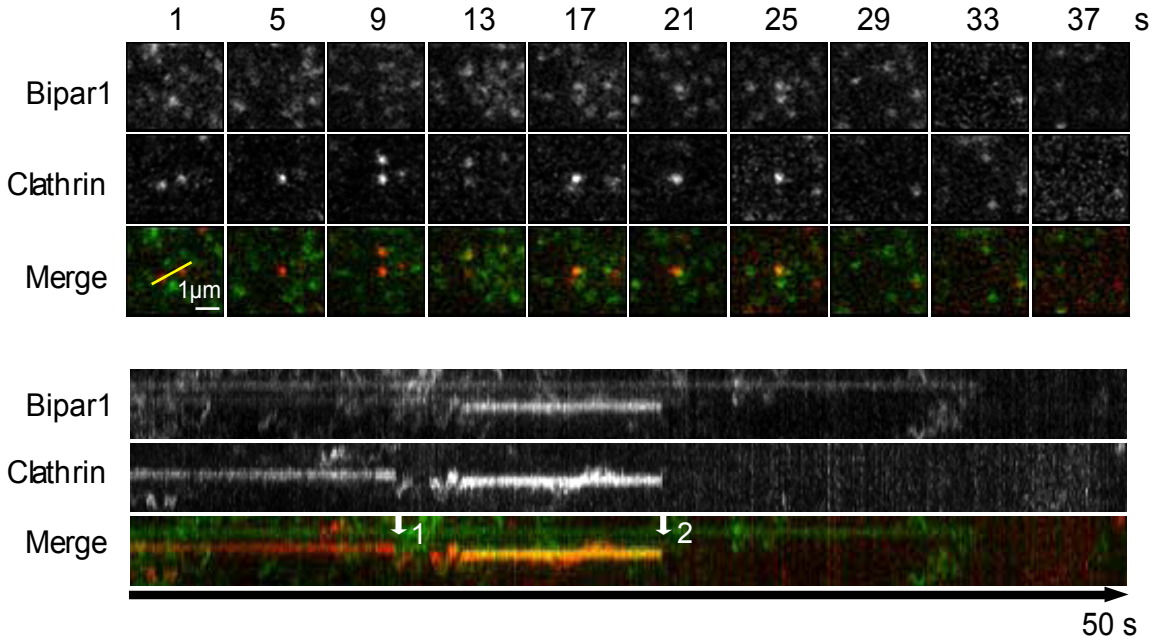

Figure S2. Analysis of Bipar1 internalization via coated pit.
Screen shots of TIRF-M Movie2 showing internalization of Bipar1. The bottom panel shows the kymographs of the indicated area (yellow line) generated for both channels separately and merged. Kymographs were generated using MetaMorph 7.7.5 software (Molecular Devices).

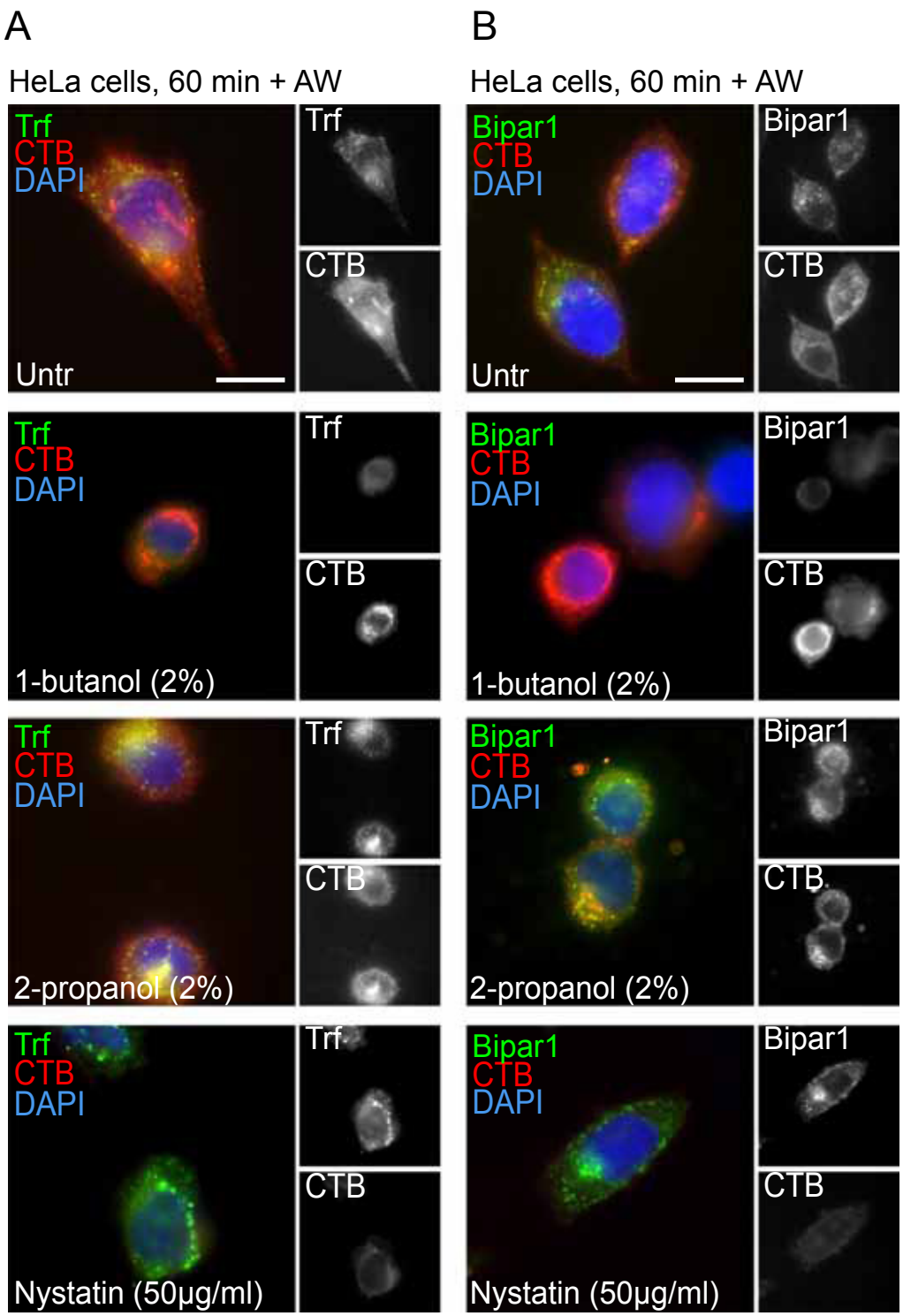

Figure S3. Bipar1 internalization is dependent on PIP2 but independent of caveolin. PIP2 production and thereby clathrin-mediated endocytosis was blocked by pre-treatment with 2% 1-butanol for 2 min. As controls, cells were either left untreated or were treated with 2% 2-propanol. For inhibition of caveolin-mediated endocytosis, 14C cells were pre-treated with 50 µg/ml Nystatin for 2 min. Bipar1-Alexa488 or Transferrin-Alexa488 (Trf) were prebound together with Cholera toxin B-Alexa555 (CTB) to cells on ice and were allowed to internalize in the presence of 1-butanol, 2-propanol or Nystatin for 60 min. Bound ligands were removed by acid wash and cells were fixed in 4% PFA. Bar is 15 µm.

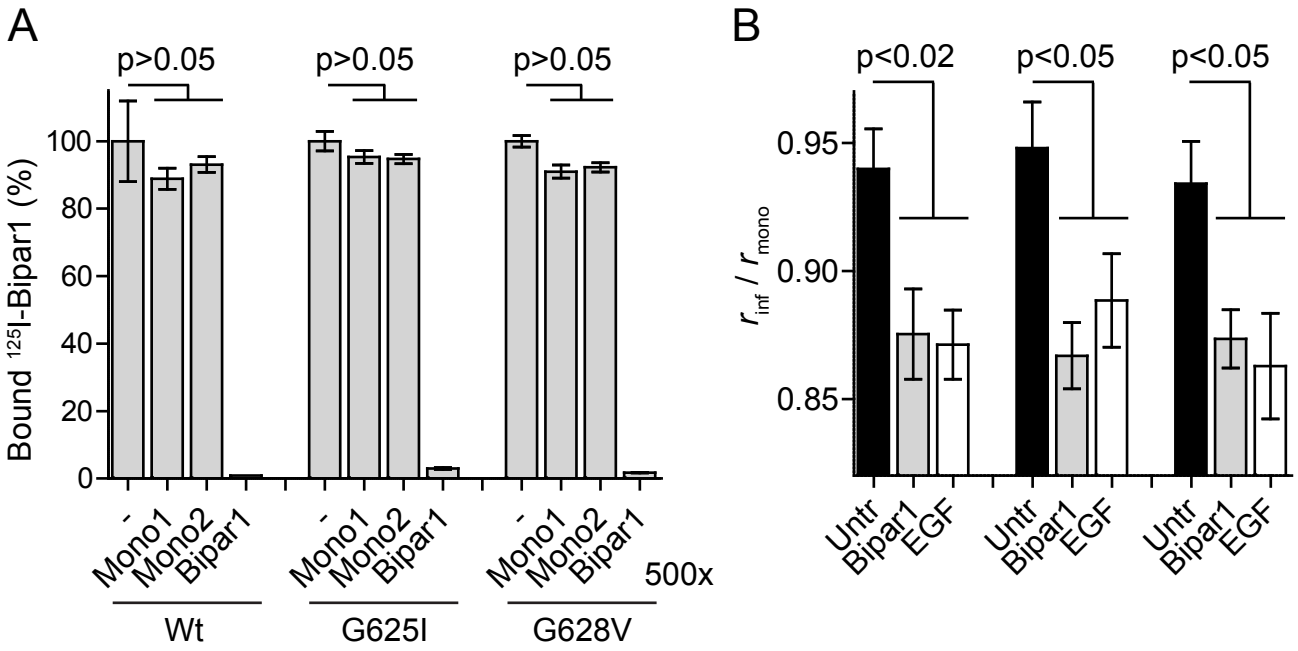

Figure S4. Bipar1 and EGF induces clustering of EGFR TMD mutants.
A. Bipar1 binds to EGFR TMD mutants with both epitope-binding domains. ^{125}I-Bipar1 with or without a 500x excess of unlabeled Mono1, Mono2 or Bipar1 was allowed to bind cells stably expressing EGFR wt or the TMD mutants for 2h on ice. Note that competition is only obtained with excess Bipar1.**B.** Both Bipar1 and EGF induce clustering of EGFR TMD mutants. Cells expressing mGFP-tagged EGFR wt or TMD mutants were incubated with 10 nM of Bipar1 for 20 min and subsequently fixed in 4% PFA. The limiting average anisotropy (r_{inf}) was determined as described in the Materials and methods and was plotted as fraction of the anisotropy value of mGFP (r_{mono}). Error bars represent SEM with n>5.

Tabel S1

	molecules/cell	SD
HER14	2.7E+05	7.3E+03
LALA	1.8E+05	3.9E+04
1-653 LALA	3.2E+05	6.6E+03
K721A LALA	1.3E+05	2.6E+03
9Y LALA	1.0E+05	7.6E+03
G625I	2.8E+05	6.1E+03
1-653 G625I	3.9E+05	8.5E+03
K721A G625I	5.8E+05	1.1E+05
9Y G625I	2.3E+05	1.0E+04
G628V	5.4E+05	3.2E+04
1-653 G628V	4.2E+05	1.6E+04
K721A G628V	5.1E+05	1.3E+04
9Y G628V	2.5E+05	6.9E+03