Ubiquitin ligase SMURF2 enhances epidermal growth factor receptor stability and tyrosine-kinase inhibitor resistance

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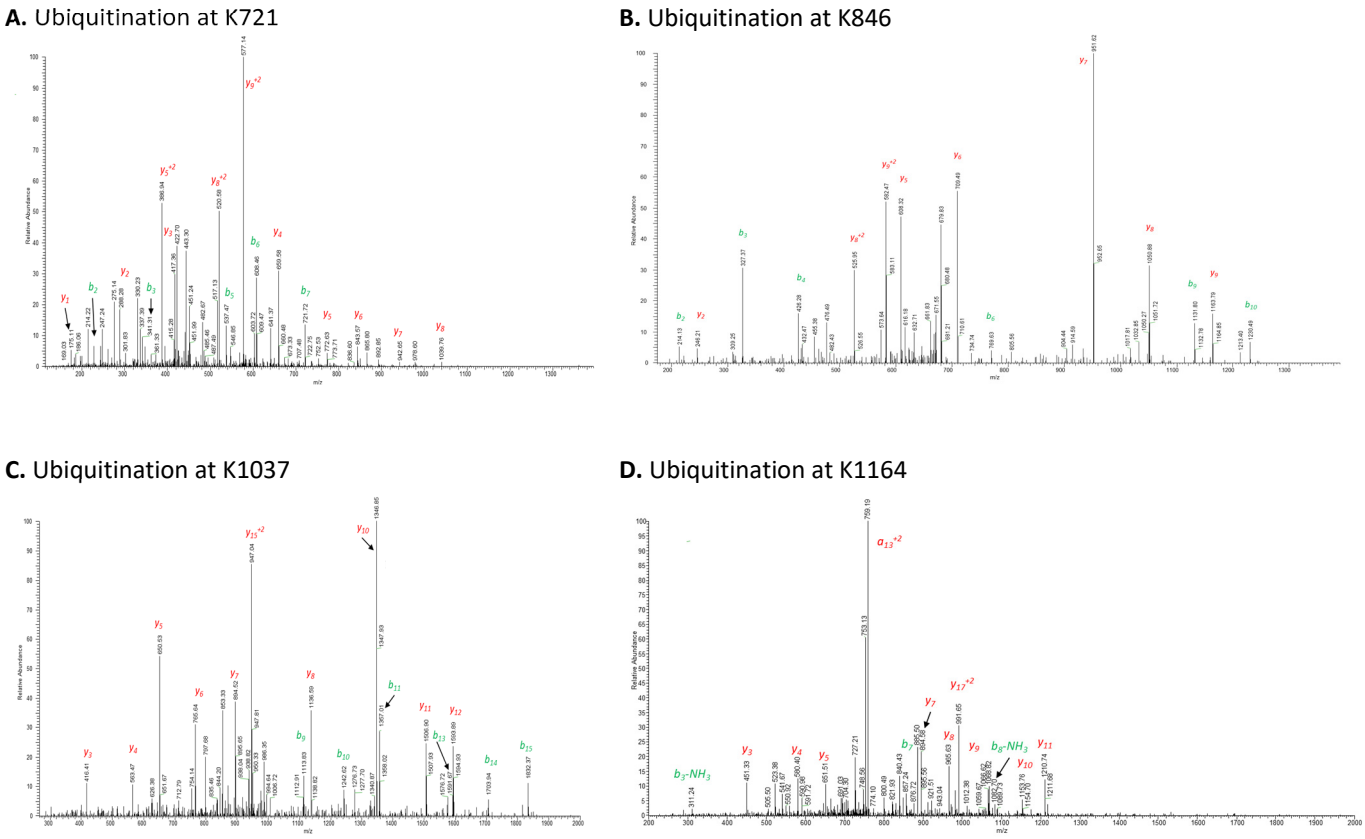
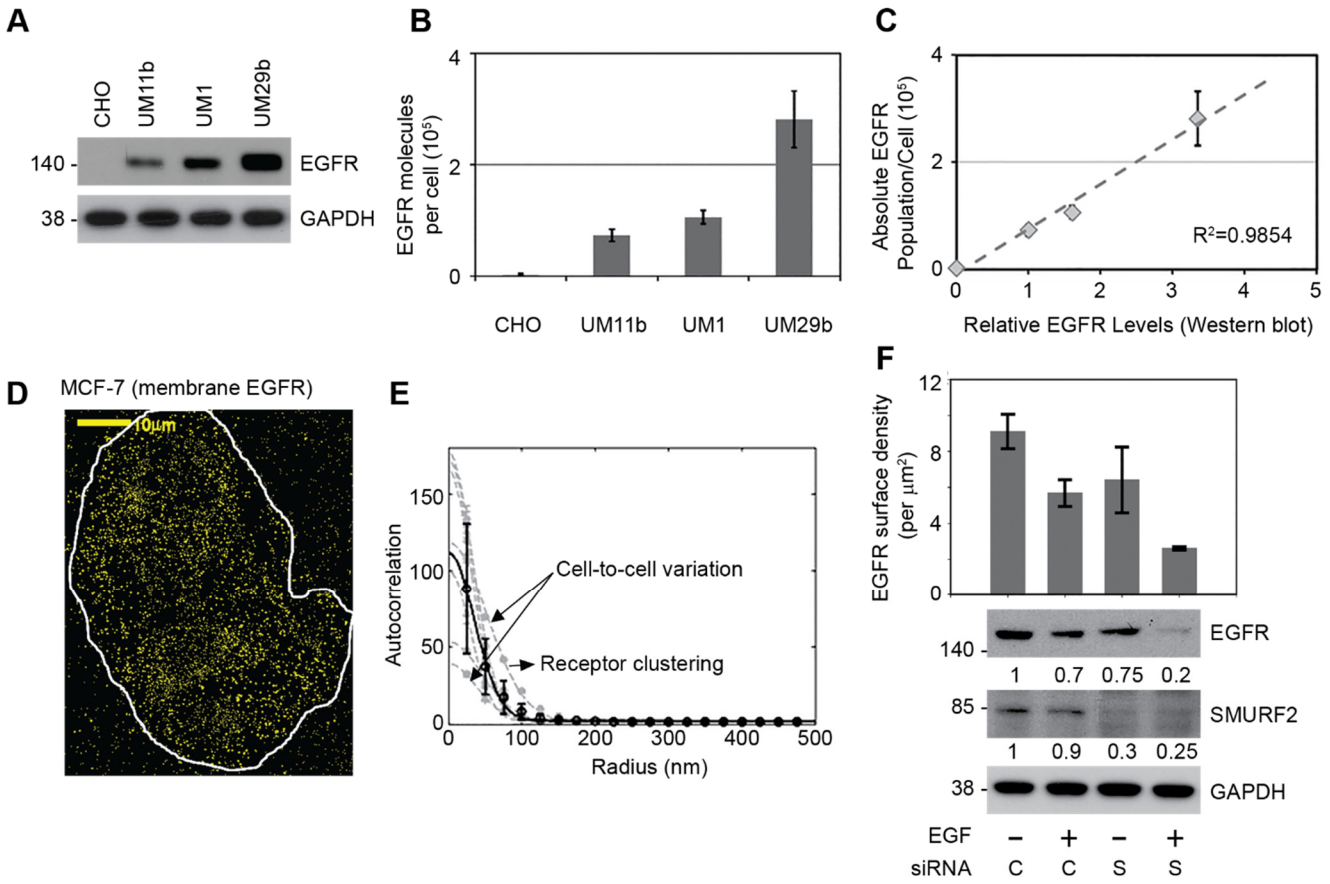
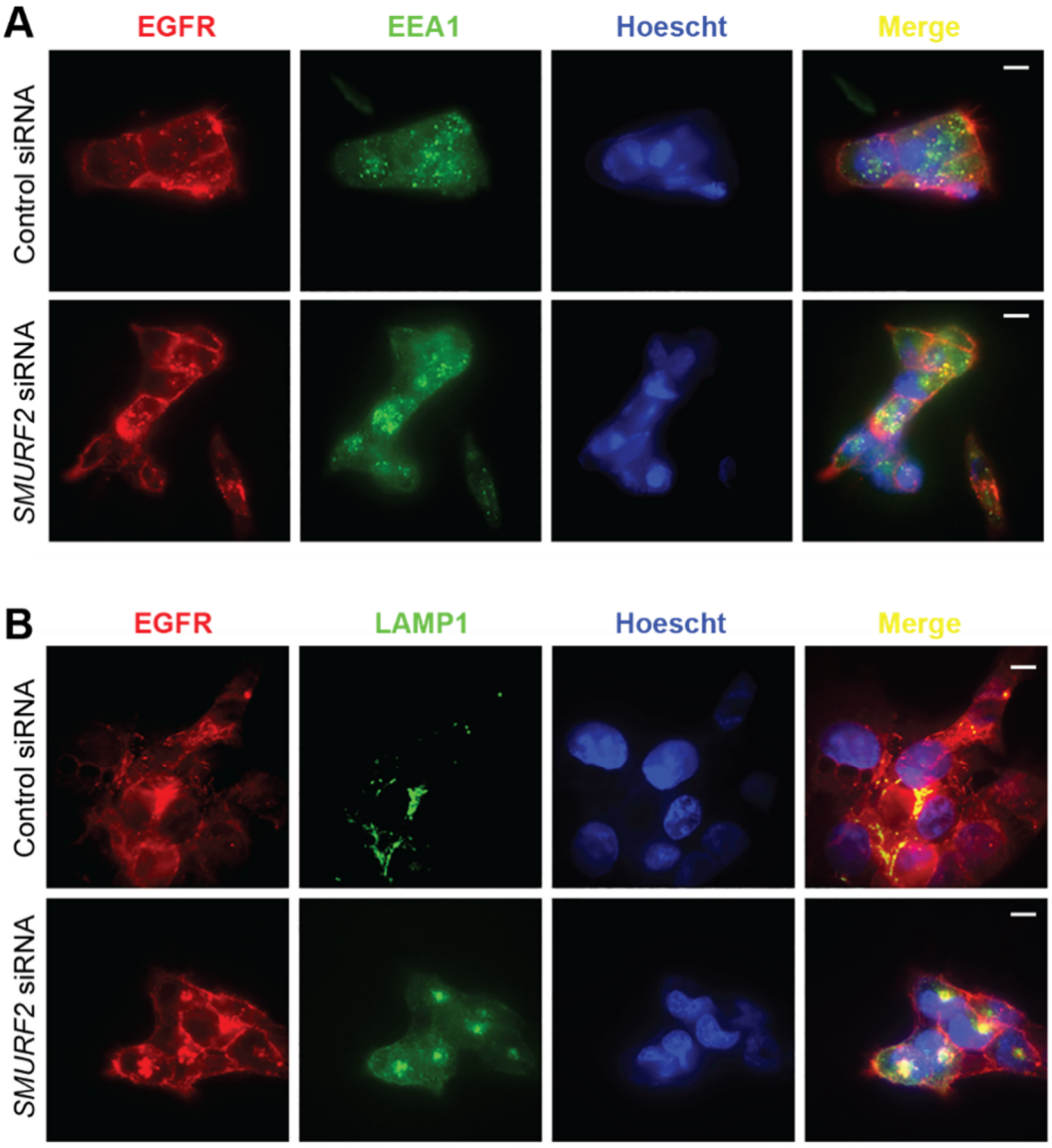
Supporting Figures 1-3



Supporting Figure 1. MS/MS spectrum of a peptide identifying ubiquitination of at K721, K846, K1037, K1164 sites in L858R/790M EGFR. Peptides isolated upon in-gel digestion were resolved on a reverse phase column, and collision induced dissociation spectra were obtained using an Orbitrap XL mass spectrometer. MS/MS corresponding to ^{714}VKIPVAIK*ELR^{724} (in **A**), ^{842}NVLVK*TPQHVK^{852} (in **B**), ^{1029}NGLQSC$_{cam}$PIK*EDSFLQR^{1044} (in **C**), and ^{1156}EAKPNGIFK*GSTAENAEYLR^{1075} (in **D**) of L858R/T790M EGFR are shown above. Observed b- and y-ions are indicated. Modified Lys (K) is denoted with *. C_{cam} = Carboxymethylated Cys.



Supporting Figure 2. Validation of EGFR quantification in the membrane using super-resolution imaging. **(A)** Quantification of EGFR in CHO, UMSCC-11b, UMSCC-1 and UMSCC-29b cells using immunoblot analysis. (**B**) Measurements of EGFR surface population in above mentioned cell lines using super-resolution imaging. Density measured through super-resolution image is multiplied with surface area of the cell to give population per cell. (**C**) EGFR quantification using two methodologies showing strong correlation (R^2=0.9854). (**D**) Representative reconstructed TIRF image of MCF-7 cells stained for EGFR using super-resolution imaging. Scale bar, 10 μm. (**E**) Auto-correlation functions were tabulated from images of 12 MCF-7 cells and results are summarized showing reasons of variation arise due to cell-to-cell variation and receptor clustering. (**F**) Top panel, quantification of EGFR membrane density in UMSCC-1 cells using TIRFM showing effects of EGF treatment in the presence and absence of SMURF2. Bottom panel, showing efficiency of SMURF2 siRNA (S) compared to control (C) on EGFR and SMURF2 steady state levels in the presence and absence of EGF (10 ng/ml for the last 6 hours).



Supporting Figure 3. Increased localization of EGFR in the endosomes and lysosomes following SMURF2 loss. UMSCC-1 cells were either transfected with control or SMURF2 siRNA. 48 hours following transfection, cells were fixed and stained either with EGFR and EEA1 (in **panel A**), or EGFR and LAMP1 (**panel B**) antibodies. Representative merged images are showing colocalization. Scale bar, 10 μm.