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Supplementary Materials for

Cbl Controls EGFR Fate by Regulating Early Endosome Fusion

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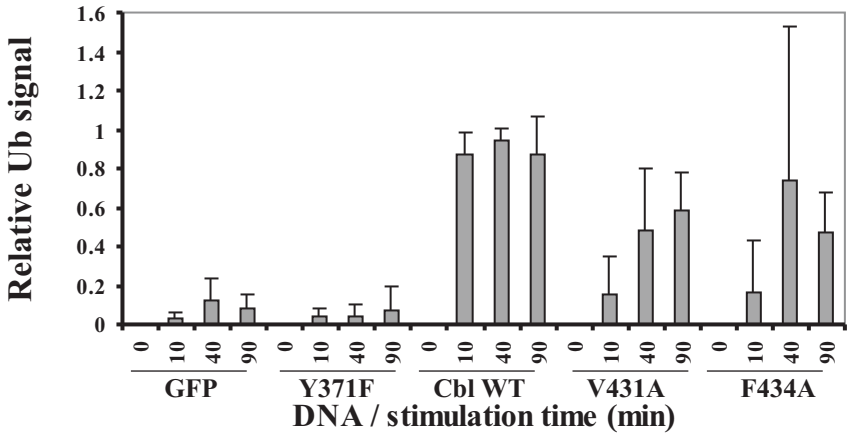
This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/2/102/ra86/DC1)

Movies S1 to S3 (.mov format)

A



B

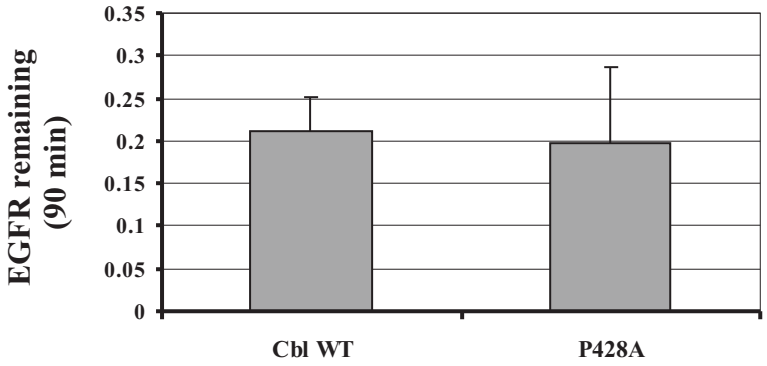


Fig. S1. Quantitation of the ubiquitination and degradation of EGFR. (**A**) The abundance of protein detected by Western blotting in Fig. 1D was quantified with Image J software. The results shown are from three independent experiments, with error bars indicating the SD. (**B**) Cells that were transfected with plasmids encoding GFP-Cbl WT or GFP-Cbl P428A were processed as described in the legend for Fig. 1D. The abundance of EGFR as determined by Western blotting analysis of three independent experiments were quantified with Image J, with error bars indicating the SD.

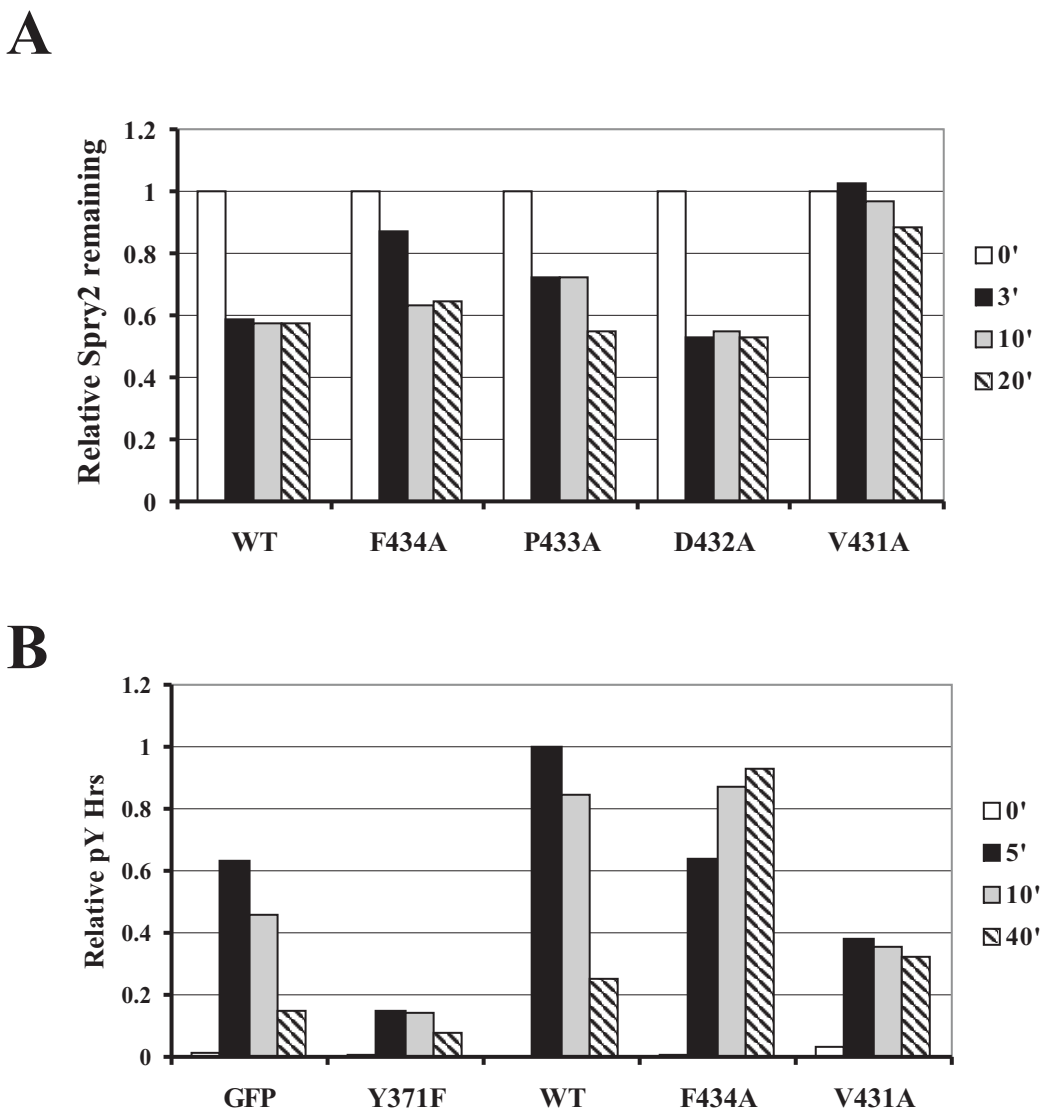


Fig. S2. Quantitation of data from Fig. 2. (**A**) Quantitation of the data set shown in the bottom panel of Fig. 2A was performed with Image J software. (**B**) Quantitation of the data set shown in the top panel of Fig. 2B was performed with Image J software. The results shown are representative of those obtained in three independent experiments.

Movie Descriptions

Movie S1. Rapid fusion of endosomes in cells transfected with a plasmid expressing GFP-tagged WT Cbl. COS-7 cells transfected with a plasmid expressing GFP-Cbl were stimulated with EGF in OptiMEM. Acidified compartments, presumably lysosomes, were visualized with LysoTracker. Image collection was begun approximately 15 min after the addition of EGF and LysoTracker. Images were collected every 30 s for a total of 40 min. Still images from this movie are shown in Fig. 4A.

Movie S2. Delayed fusion of endosomes in cells transfected with a plasmid expressing GFP-tagged Cbl F434A. COS-7 cells transfected with a plasmid expressing GFP-Cbl F434A were imaged as described for movie S1. As a result of delayed fusion, pairs and clusters of docked vesicles persisted over the 40-min collection period. Still images from this movie are shown in Fig. 4A.

Movie S3. Impaired trafficking and fusion of endosomes in cells containing GFP-Cbl V431A. COS-7 cells transfected with a plasmid expressing GFP-Cbl V431A were incubated on ice with EGF (1:1 mixture of Alexa Fluor 647–labeled EGF and unlabeled EGF) in OptiMEM at 100 ng/ml. Image collection was begun approximately 10 min after excess ligand was removed and the cells were warmed to 37°C. For the movie, images were collected every 30 s for a total of 30 min. The V431A-induced impairment of the trafficking of EGFR and of endosome fusion is reflected by the persistence of small puncta that contained GFP and EGF at the cell periphery. This phenotype is consistent with a defect in the degradation of hSprouty2 and in the internalization of EGFR. Still images from this movie are shown in Fig. 4A.