

Supplemental figure legends.

Supplemental Figure. 1. Mutated HA-MC4R I-316S is retained in the ER. A large fraction of HA-MC4R-I316S co-localizes with calnexin in the ER. N2A cells were transiently transfected with wt-HA-MC4R and mutant HA-MC4R-I316S. Cells were stained with primary rabbit polyclonal antibodies against calnexin and rat monoclonal antibodies against the HA tag. Secondary staining was carried out by using Cy3-conjugated anti-rat and FITC-conjugated anti-rabbit antibodies.

Supplemental Figure. 2. Mutated HA-MC4R-I316S is more ubiquitinated than wt-HA-MC4R. N2A cells were transiently transfected with Flag-ubiquitin or co-transfected with Flag-ubiquitin and wt HA-MC4R or mutated HA-MC4R I316S. Immunoprecipitations from cell lysates were carried out with anti-HA-antibodies. Immunoprecipitated samples and cell lysates were analyzed by Western blot by using mouse monoclonal anti-Flag antibodies.

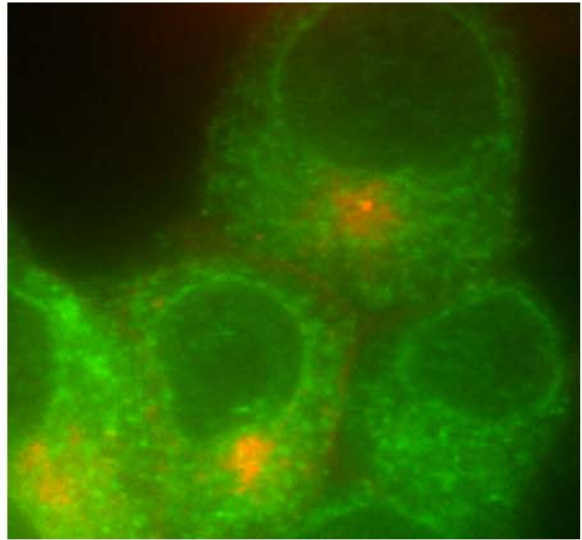
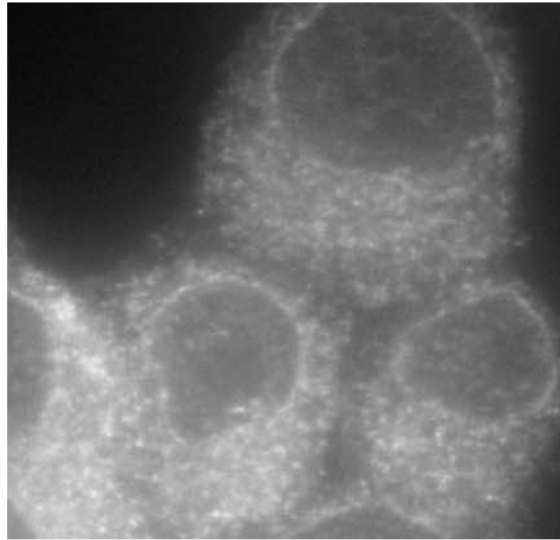
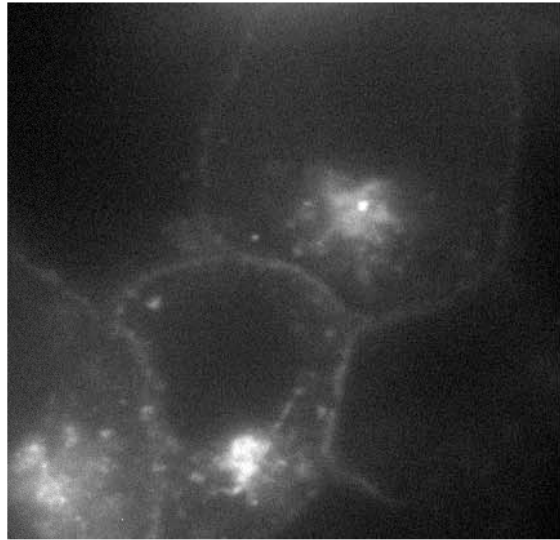
HA-MC4R

Cy3

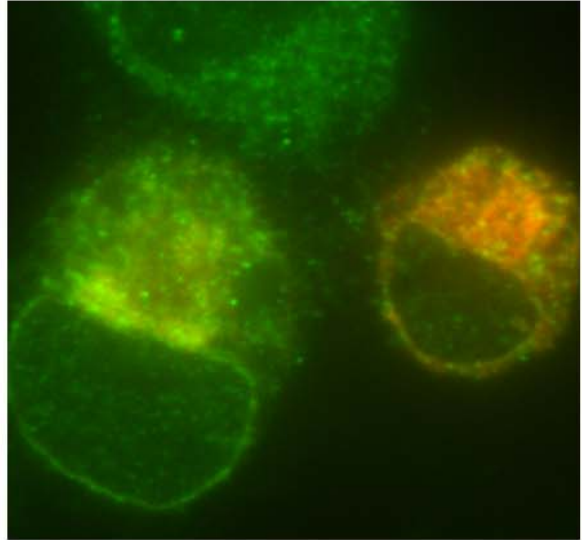
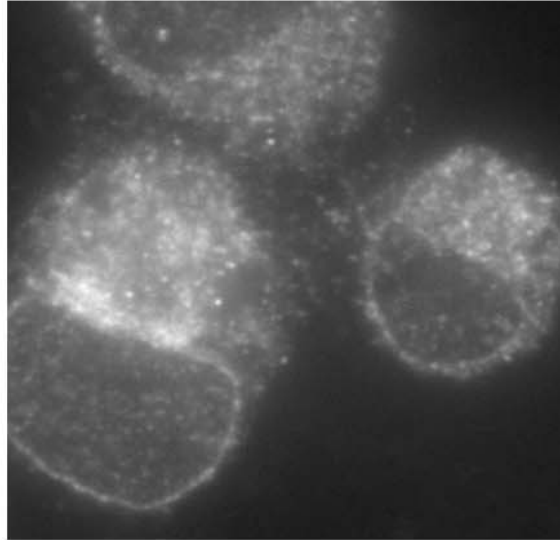
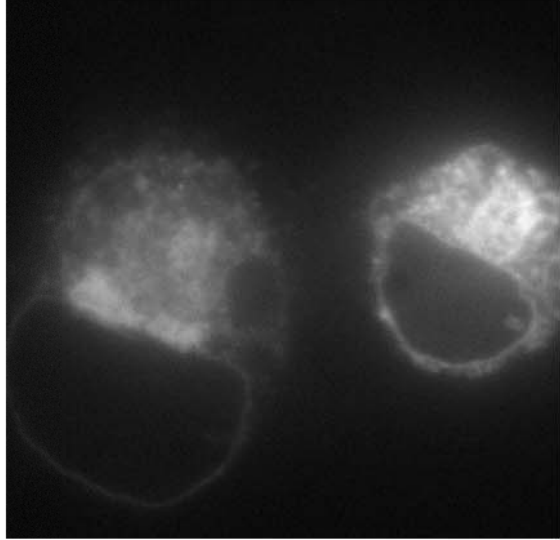
calnexin

merge

wt



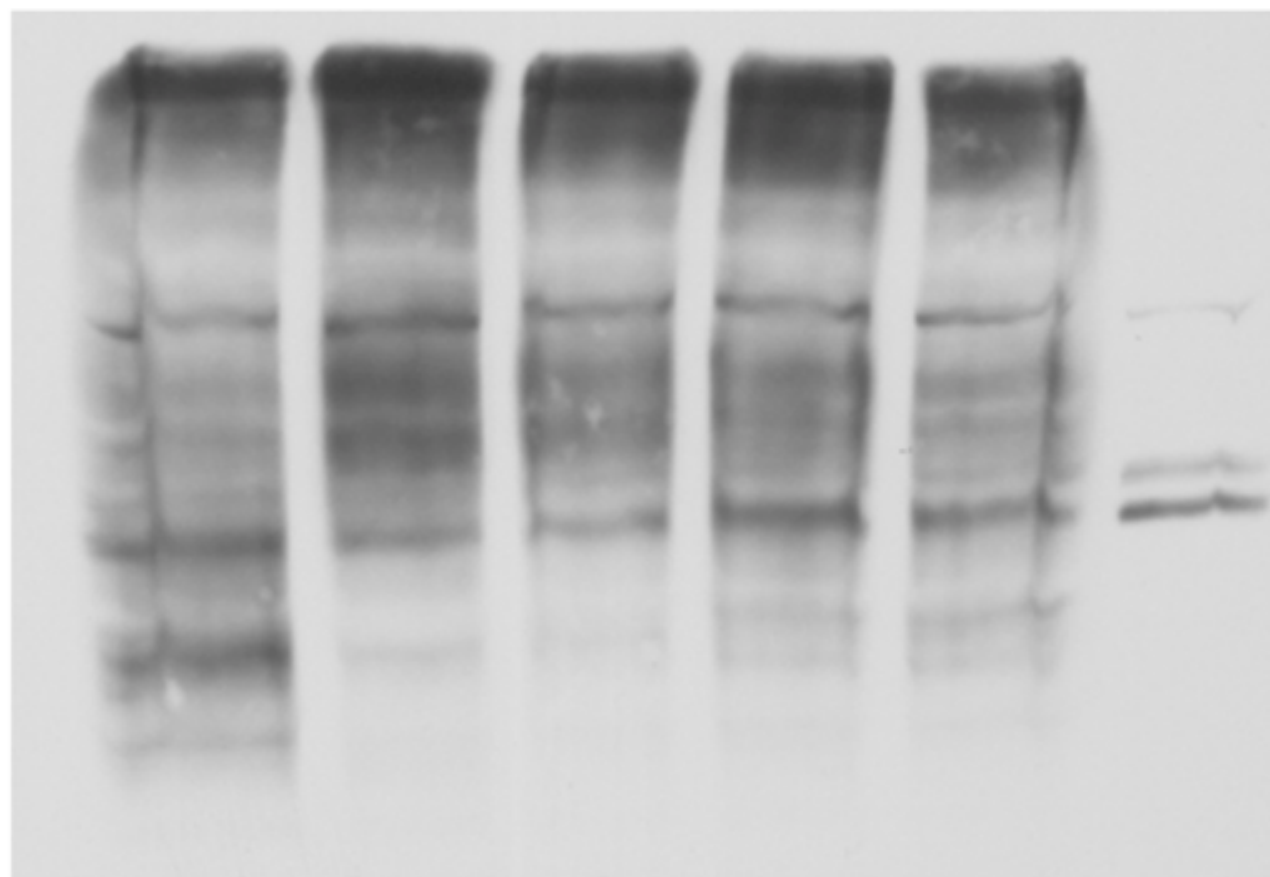
I316S



cell lysates

WB= anti-HA-POD

9% gel



-250kD

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*

*-98kD

-64kD

wt

P78L

R165W

I316S

I317T

control