

Supplementary Figure 1

Relocation of GFP-Cbl upon EGF stimulation. (A-D) CHO cells transiently expressing EGFR and GFP-Cbl were observed in the absence of EGF (A), or the cells were stimulated with 1 µg/ml Texas Red-EGF (B and C) or 100 ng/ml EGF (D) for the indicated time periods. In D, myc-Rab5(Q79L) was also expressed and the image is the localization of GFP-Cbl. Bars, 20 µm.

Supplementary Figure 2

Localization of FLAG-E2s upon EGF stimulation. CHO cells were transiently transfected with EGFR, GFP-Cbl and the indicated FLAG-E2 plasmids and stimulated with EGF for 15 min. The cells were stained with the anti-FLAG antibody. Bars, 20 µm.

Supplementary Figure 3

Control experiments for the knockdown of E2s. HeLa cells were depleted of Ubc4/5 or UbcH7 to examine ubiquitination and degradation of EGFR as described in MATERIALS AND METHODS. (A) The receptor degradation at 30 min was retarded by the depletion of Ubc4/5 but not UbcH7. (B) Effects of other dsRNAs (4/5 #2 and H7 #2) were tested in parallel with the experiment shown in A. For 4/5 #2, sense sequence 5'-GAUCACAGUGGUCGCCUGCTT-3' and antisense sequence 5'-GCAGGCGACCACUGUGAUCTT-3' were used according to the previous report (*J. Biol. Chem.*, 2004, 279, 42169-42181). For H7 #2, sense sequence 5'-AAAUGUGGGAUGAAAAACUUCTT-3' and antisense sequence 5'-GAAGUUUUUCAUCCCACAUUUTT-3' were used according to the previous report (*Mol. Cell Biol.*, 2004, 24, 8716-8726). Compared to the original dsRNA, 4/5 #2 was less effective: it decreased the Ubc4/5 protein level and EGFR ubiquitination to 41 and 75%, respectively. The 4/5 #2 dsRNA retarded the EGFR degradation, but not so severely as the original dsRNA did: at 60 min, 59% of the receptor was remaining in

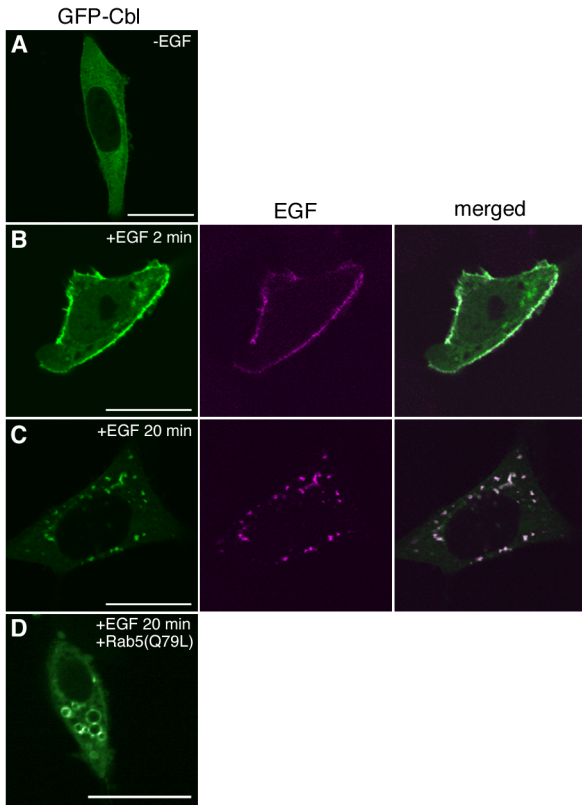
cells treated with 4/5 #2 and 70% with the original dsRNA (shown in A). Thus, the effects of the 4/5 #2 dsRNA were all modest, arguing against the possibility that the effects of the original dsRNA were nonspecific. Although the 7 #2 dsRNA depleted UbcH7 less effectively, the protein level was decreased to 24%. Nonetheless, this dsRNA did not attenuate the EGFR ubiquitination at all. The receptor degradation was slightly delayed by the 7 #2 dsRNA, but the effect was only marginal compared with that of the Ubc4/5 knockdown. (C) After depletion of either Ubc4/5 or UbcH7 using the original dsRNA, the cells were stimulated for 5 min with 20 ng/ml EGF. The depletion of Ubc4/5, but not UbcH7, attenuated the receptor ubiquitination.

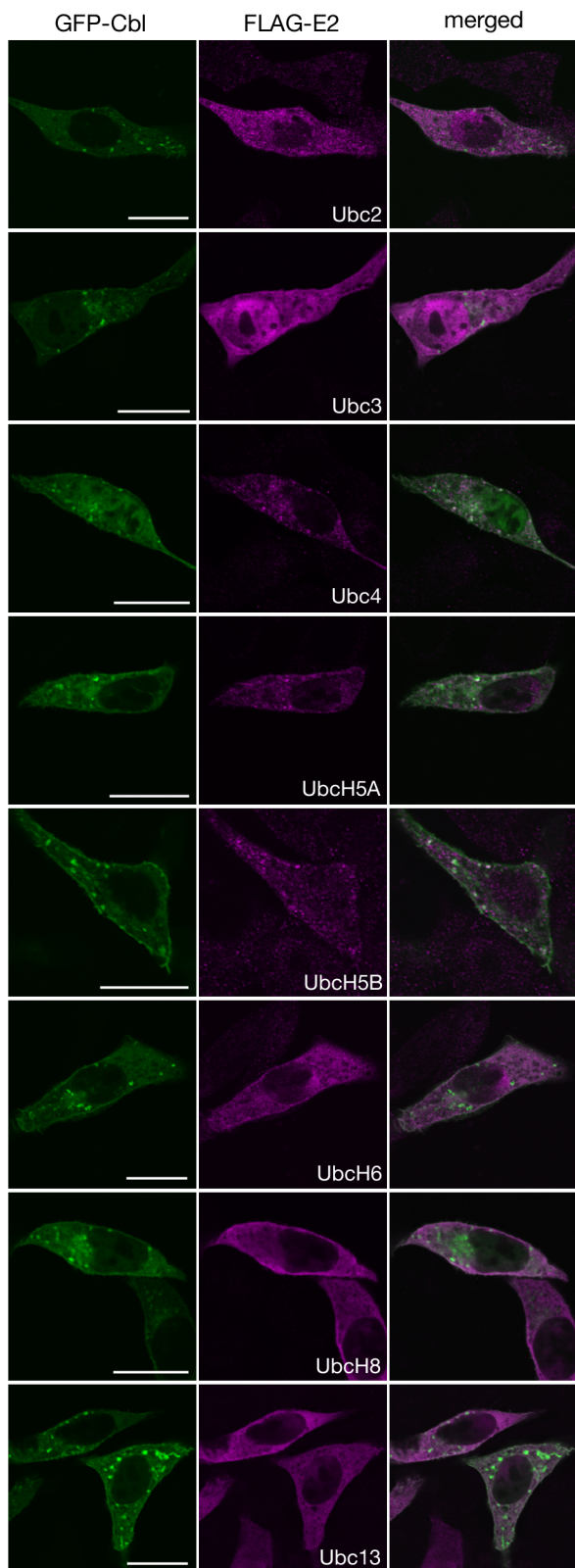
Supplementary Figure 4

Non-linear reactivity of the polyubiquitin-specific antibody FK1. (A) The samples in Figure 5 were serially diluted and the signal intensities were quantitated. (B) The samples in Figure 6C at 10 min of EGF stimulation were serially diluted and the signal intensities were quantitated.

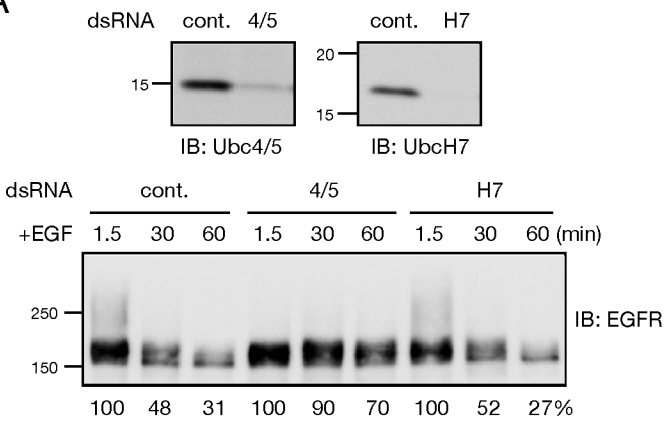
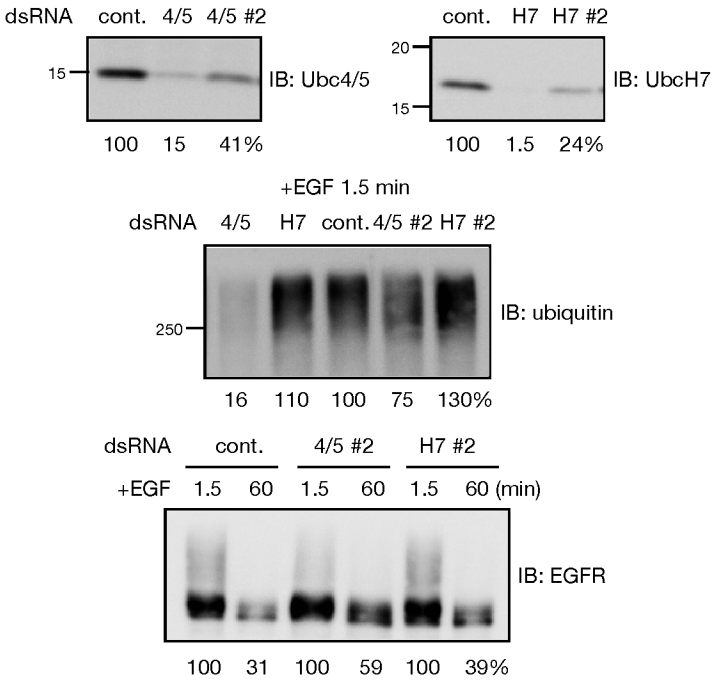
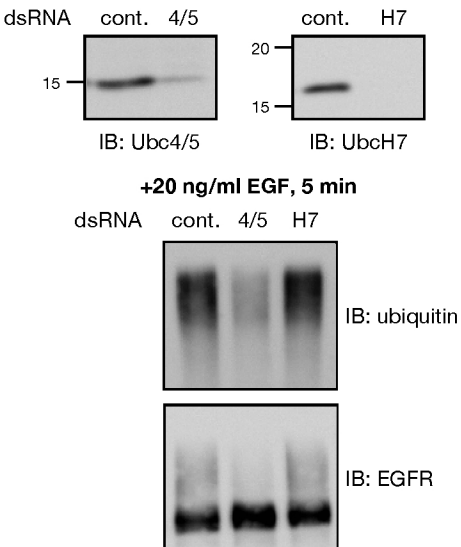
Supplementary Figure 5

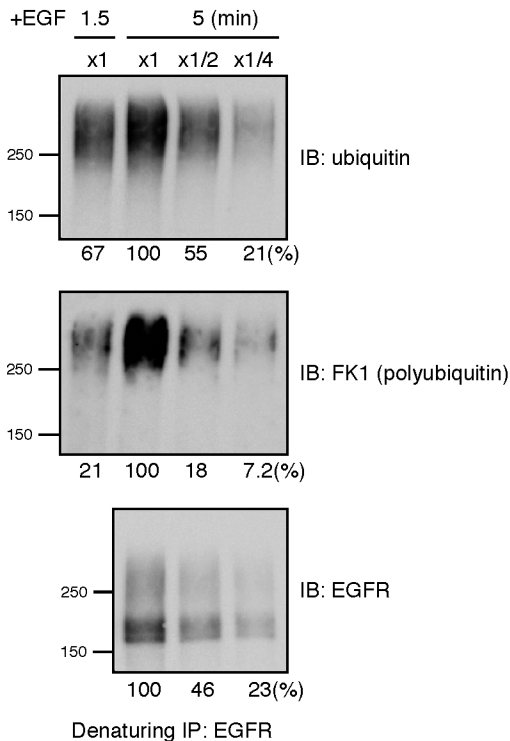
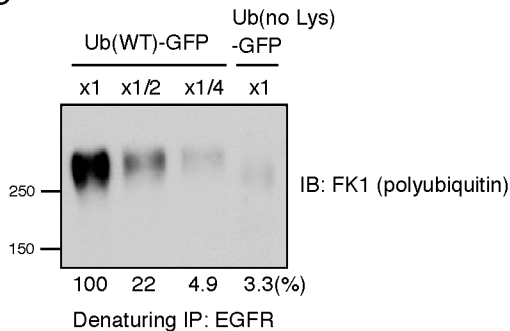
Effects of AG1478 on HEK293T cells transiently expressing EGFR and HA-Cbl. After 10 min of EGF stimulation, AG1478 was added to the medium at a final concentration of 20 nM, and the cells were further incubated for 10 min. The immunoprecipitation and immunoblotting were done as described in Figure 7. HA-tagged and endogenous c-Cbl are not distinguishable from their migration on the gel.

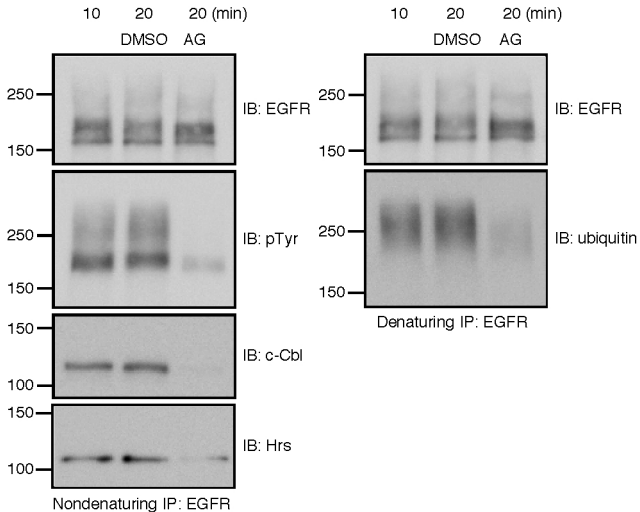
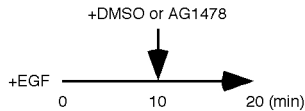




Umeybayashi *et al.*, Supplementary Figure 2

A**B****C**

A**B**



Umehayashi *et al.*, Supplementary Figure 5