

Supplemental Figure S8. DUB3 depletion increases ubiquitylation of ITCH.

HEK293T cells were transfected to express Myc-tagged ITCH along with ubiquitin and siRNA targeting DUB3 or a scrambled control siRNA. Transfected cells were treated with 5μ M of MG132 and 5μ M of Lactacystin overnight before being subjected to immunoprecipitation with anti-Myc. Blots were probed with antibodies against DUB3, actin, and the Myc epitope to detect Myc-tagged ITCH and with antibodies specific to ubiquitin linked at lysine residues K48 and K63.