

Expanded View Figures

Figure EV1. A CRISPR screen for DUB vulnerabilities in MM identifies OTUD6B.

- A Schematic depiction of the DUB-CRISPR drop out screen workflow.
- B Proliferation analysis of MM1.S-Cas9 cells infected with indicated sgRNAs. The ratio of sgRNA expressing (GFP⁺) cells to uninfected cells was measured by flow cytometry at the indicated time points after infection. Results are normalized to GFP⁺ cells at day 4 ($n = 3$ independent experiments, mean \pm s.d.).
- C Immunoblot analysis verifying OTUD6B knockout in Cas9 expressing MM1.S cells infected with the indicated sgRNAs. GFP⁺ cells were sorted by FACS and harvested 5 days after infection. Whole cell extracts (WCE) were probed with the indicated antibodies. CUL1 served as a loading control.
- D Proliferation analysis of three additional Cas9 expressing MM cell lines lentivirally transduced with the indicated sgRNAs. The ratio of sgRNA expressing (GFP⁺) to uninfected cells was measured by flow cytometry at the indicated time points after infection. Results are normalized to day 4 after infection ($n = 4$ independent experiments, mean \pm s.d.).
- E Immunoblot analysis verifying OTUD6B knockout in Cas9 expressing MM cell lines, which were transduced with indicated sgRNAs. GFP⁺ cells were sorted by FACS 11 days after infection and WCE probed with indicated antibodies.
- F Proliferation analysis of RPMI8226 cells stably expressing OTUD6B isoform 1 from a doxycycline inducible promoter and the indicated shRNAs with or without doxycycline mediated transgene induction. Cells were transduced with pTRIPZ-OTUD6B-IF1 and selected for vector expression using puromycin. Next, cells were infected as indicated with shCtrl or shOTUD6B-3, which binds in the 3'UTR of the gene, and either left untreated (DMSO) or OTUD6B-transgene expression induced by doxycycline addition (DOX, 1 μ g/ml) the day after infection. Cell numbers were assessed by trypan-blue exclusion method 4 days after infection. Cell numbers are depicted as fold of DMSO with mean \pm s.d from three independent experiments.
- G Representative immunoblot analysis of WCE from cells collected at the time of proliferation analysis described in (F) using the indicated antibodies.

Data information: P -values * $P < 0.05$; ** $P < 0.01$; by paired Student's t -test.

Source data are available online for this figure.

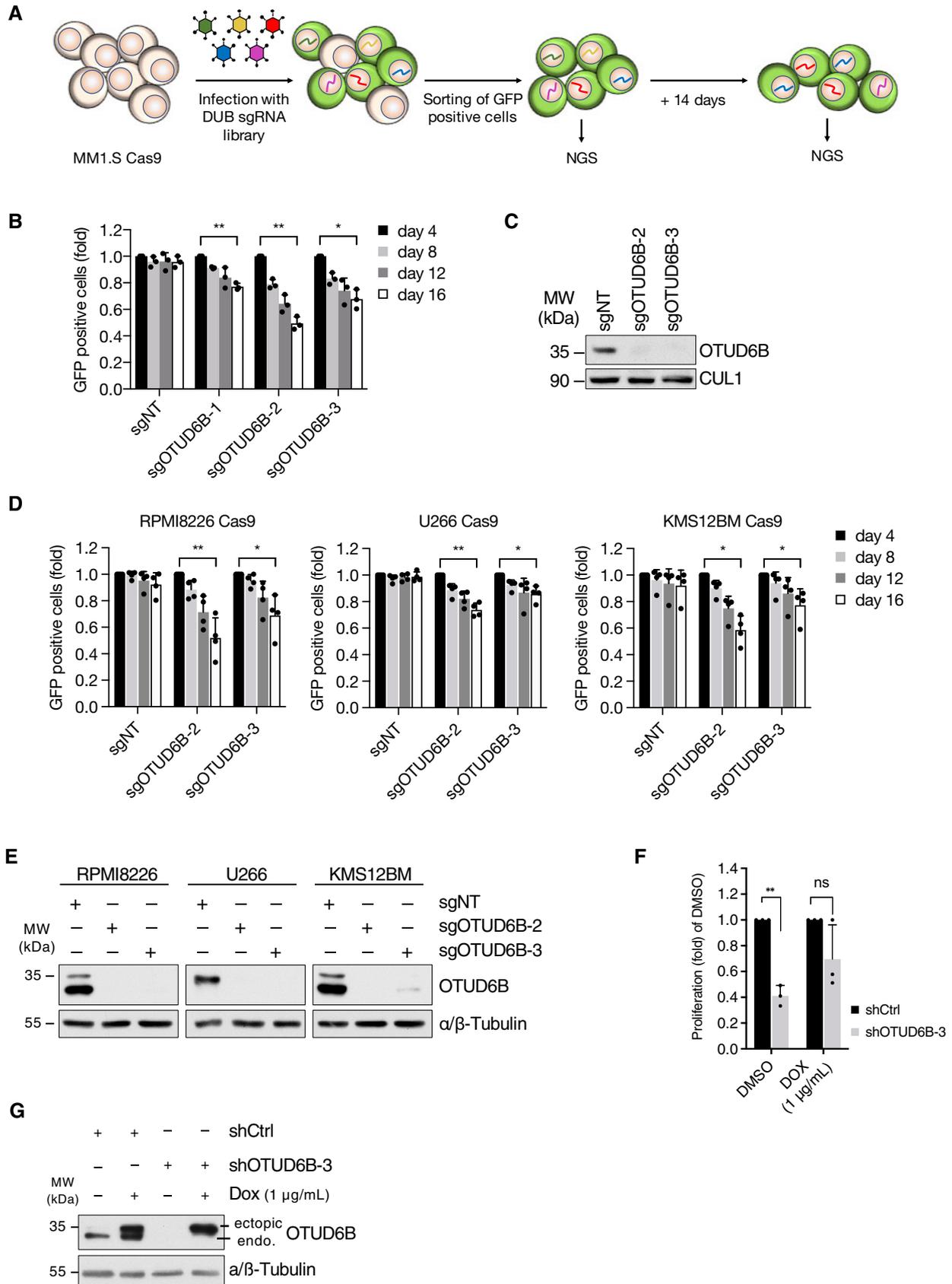


Figure EV1.

Figure EV2. OTUD6B is a vulnerability with cell cycle dependent activity in both MM and LUAC cells.

- A Proliferation analysis of A549-Cas9 cells infected with indicated sgRNAs. The ratio of sgRNA expressing (GFP⁺) to uninfected cells was measured by flow cytometry at the indicated time points after infection. Results are normalized to GFP⁺ cells at day 4 ($n = 3$ independent experiments).
- B Two-dimensional cell cycle analysis of A549 lung adenocarcinoma cells expressing the indicated shRNAs analysed by BrdU/PI staining. Data from one representative experiment are depicted as graphs of BrdU positive cells versus PI/DNA staining.
- C Quantitative cell cycle analysis of A549 cells expressing the indicated shRNAs for 5 days by BrdU/PI flow cytometry as depicted in (B) ($n = 3$ independent experiments).
- D Quantification of OTUD6B activity from three independent experiments as depicted in Fig 1F. Optical densities were measured using the LICOR image studio lite software. Values are depicted as fold of OTUD6B-signal in G1/S arrested cells from three independent experiments.
- E DUB activity assay for OTUD6B in asynchronous, G1/S- or mitotically synchronized RPMI8226 cells using HA-ubiquitin vinyl-sulfone to isolate active forms of DUBs followed by HA-IP. Analysis was performed by immunoblotting using the indicated antibodies.

Data information: Data in (A, C, D) are mean \pm s.d. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; by paired Student's t -test corrected for multiple testing (C). Source data are available online for this figure.

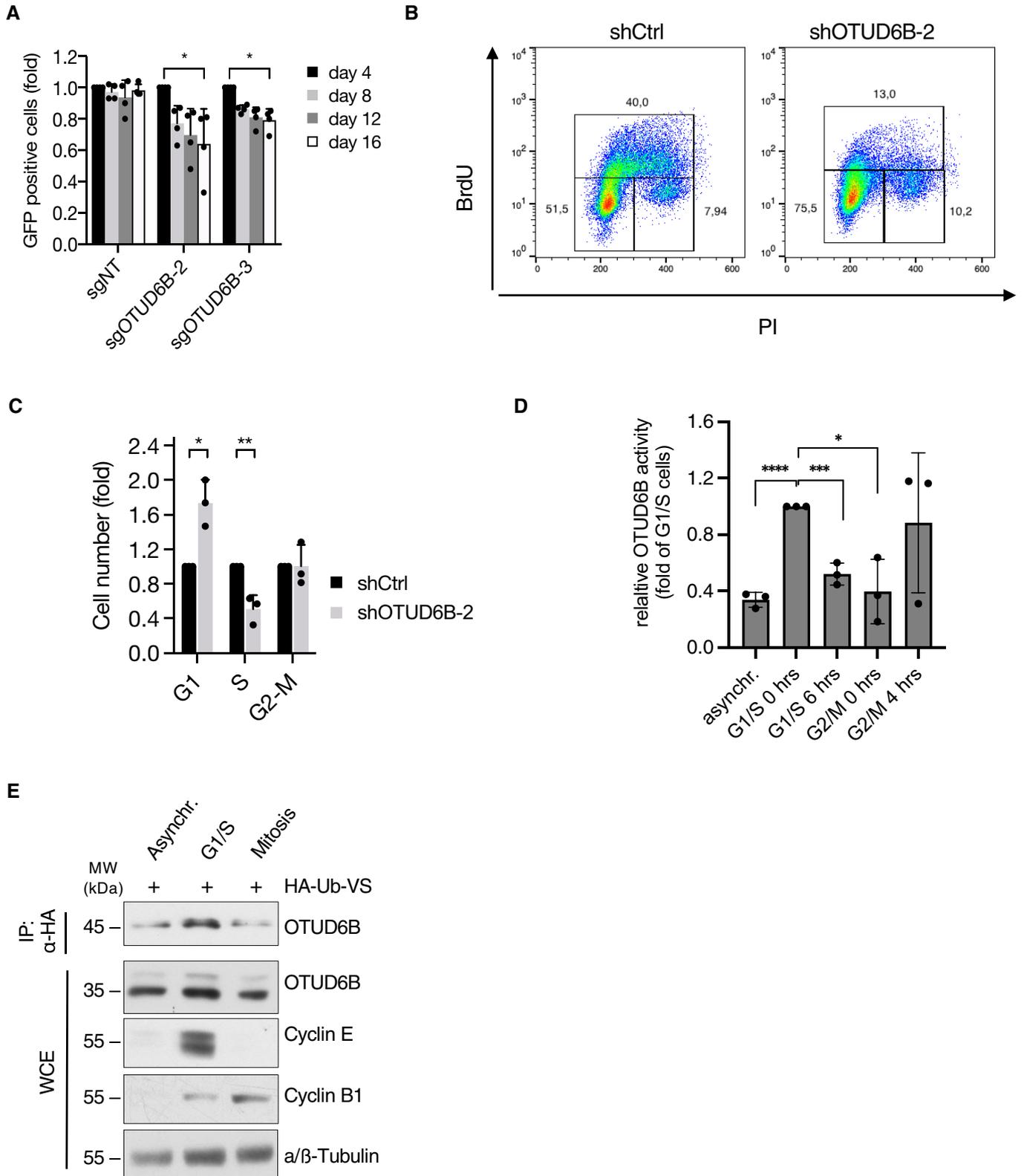


Figure EV2.

Figure EV3. Different Proteome-wide screening approaches identify LIN28B as an interaction partner of OTUD6B.

- A Silver-stain analysis of FLAG-purified OTUD6B. HEK293T were transfected with either FLAG-EV or FLAG-OTUD6B isoform 1 (IF1). FLAG-OTUD6B was immunoprecipitated from WCE using FLAG M2 beads and subsequently eluted by 3x FLAG peptide. 1% of the purification was separated by SDS-PAGE and proteins visualized by silver staining. The arrowhead points to the band at the expected size of OTUD6B.
- B Silver-stained gel of BioID2 purified proteins. HEK293T cells were transfected with MYC-tagged-BioID2 alone or BioID2-OTUD6B-isoform-1 (IF1) or -2 (IF2) fusion proteins, incubated with 50 μ M biotin for 16 h and biotinylated proteins purified for MS-analysis. Non-transfected cells served as a control. The arrowheads point to the bands at the expected sizes of BioID2 alone, BioID2-OTUD6B-IF1 and -IF2.
- C Results of mass spectrometric (MS) based screening for OTUD6B substrates correlating the results from the FLAG- and the Bio-ID-purification (A, B) as shown in Fig 2A. Intensities (LFQs) of co-immunoprecipitated proteins identified by MS were log2 transformed and the differences between OTUD6B and EV analyzed in both purifications. Proteins identified in both FLAG-IP-samples and enriched more than two-fold in both independent screens are listed. OTUD6B (bait) is marked in red and LIN28B in orange.
- D Co-Immunoprecipitation (IP) of FLAG-tagged DUBs and endogenous LIN28B from HEK293T cells. IPs and WCE were analyzed by immunoblotting with the indicated antibodies.
- E Co-IP of endogenous OTUD6B with FLAG-LIN28B.
- F Co-IP of endogenous LIN28B with FLAG-OTUD6B-IF1 and FLAG-OTUD6B-IF2.

Data information: In (D–F) FLAG-tagged proteins were expressed in HEK293T cells, WCE subjected to FLAG-IP and both analysed by immunoblot using the indicated antibodies.

Source data are available online for this figure.

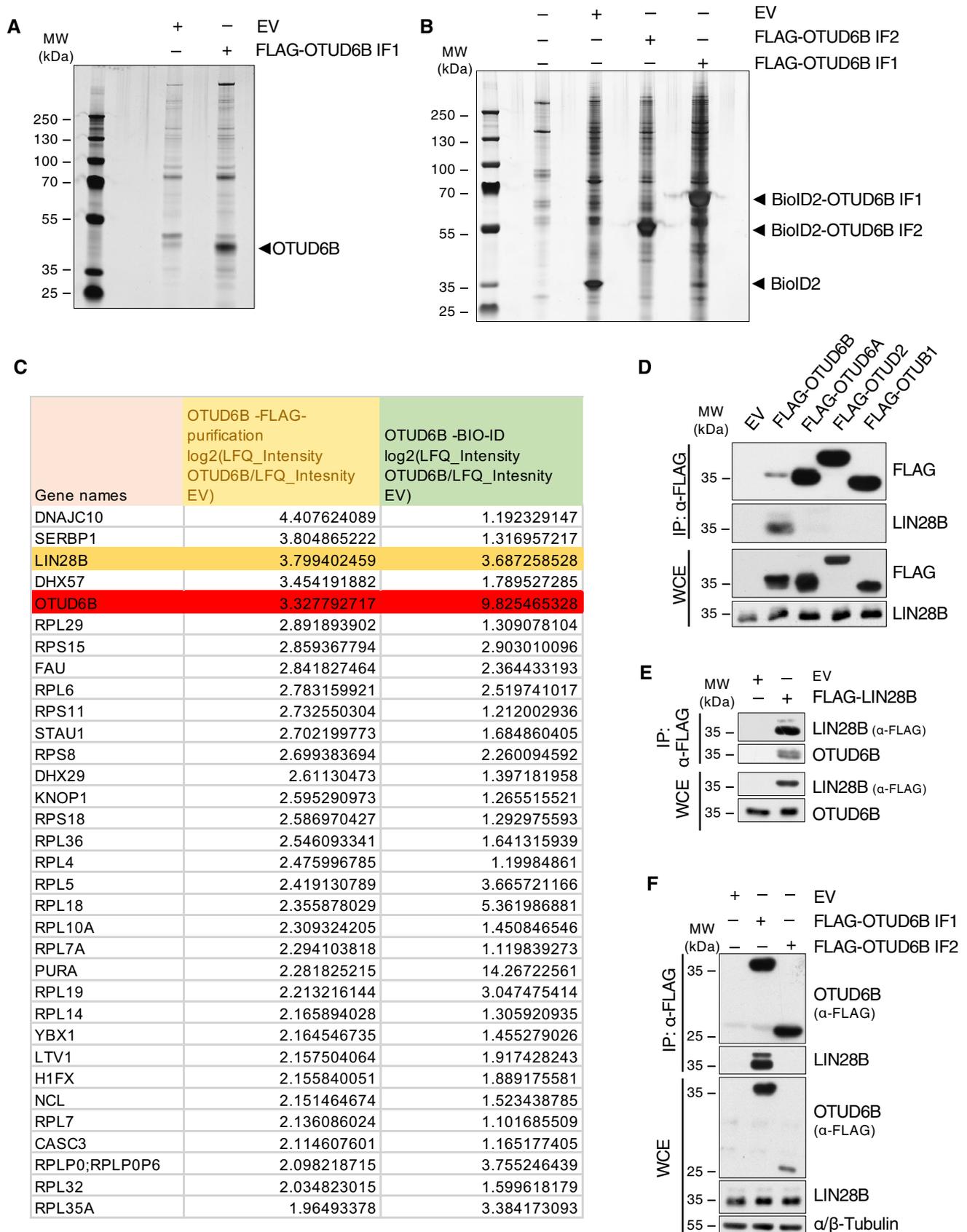


Figure EV3.

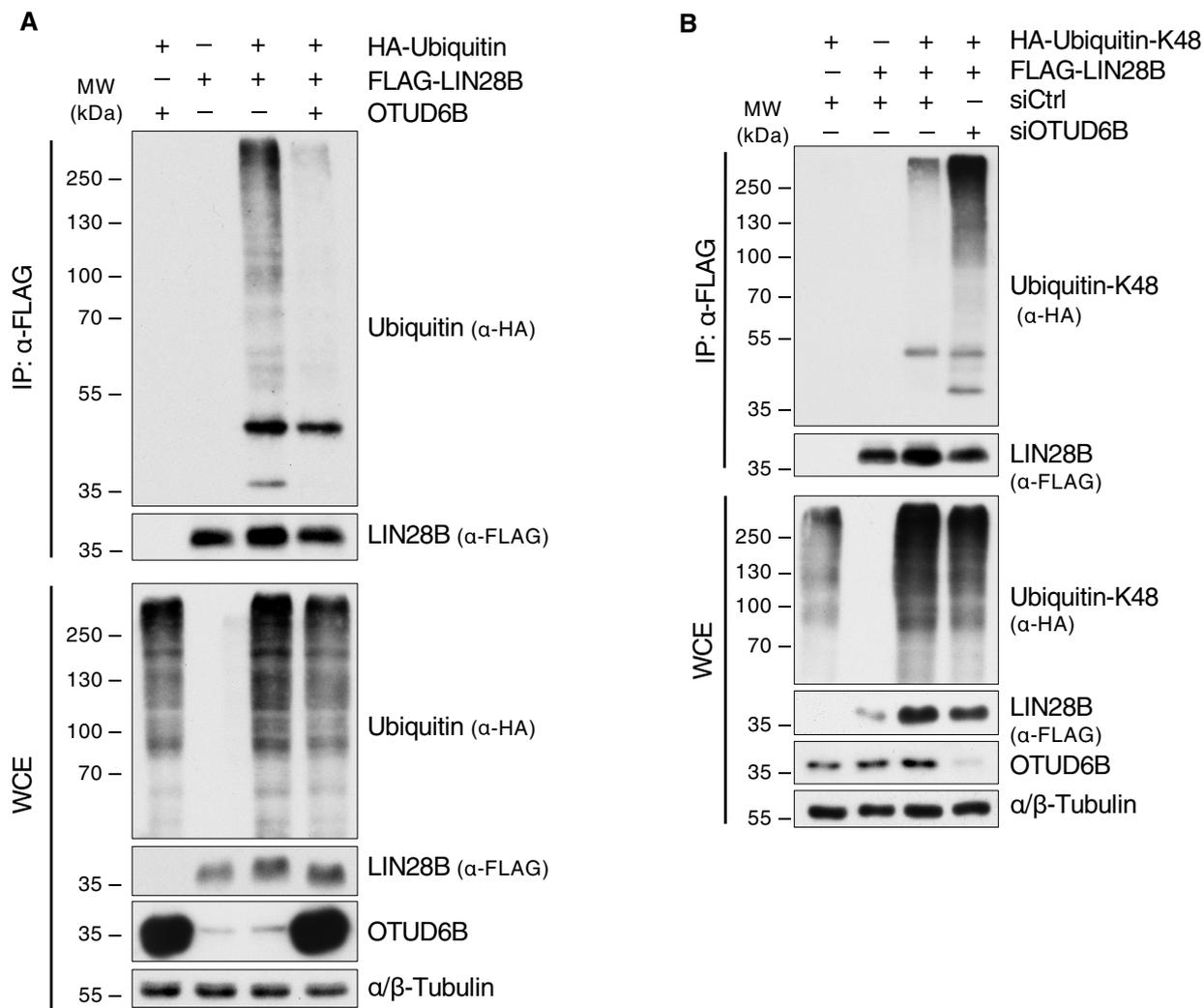


Figure EV4. LIN28B is a K48-specific de-ubiquitylation substrate of OTUD6B.

A *In vivo* ubiquitylation assay of LIN28B in OTUD6B overexpressing cells. HEK293T cells were transfected with indicated combinations of FLAG-LIN28B, HA-Ubiquitin, OTUD6B and EV control and treated with MG132 for 3 h 24 h later. Denatured WCE were subjected to FLAG-IP. WCE and IP were analysed by immunoblotting.

B *In vivo* ubiquitylation analyses of LIN28B in HEK293T cells in which OTUD6B was silenced by siRNA using a K48-only-ubiquitin-mutant. Cells were transfected with the indicated siRNAs and overexpression constructs, then treated with MG132 for 3 h. Lysis and IP was done under denaturing conditions followed by WB analysis.

Source data are available online for this figure.

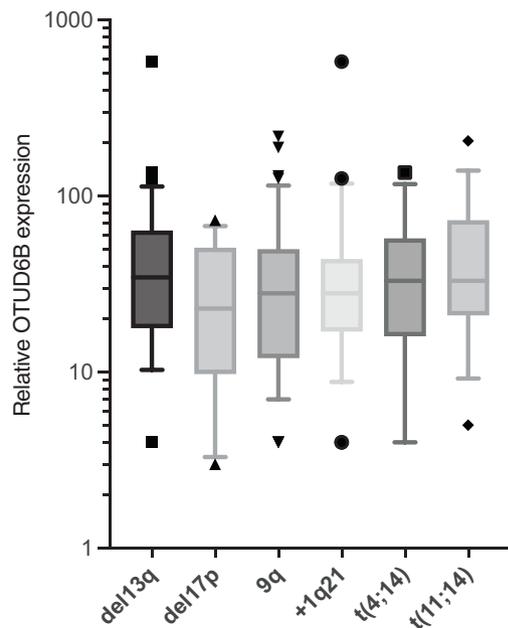


Figure EV5. OTUD6B levels do not correlate with a specific cytogenetic abnormality in MM patients.

Correlation between clinically predictive cytogenetic abnormalities and OTUD6B mRNA expression in MM patient samples at diagnosis. mRNA expression in primary CD138⁺ MM cells was quantified by real-time qPCR ($n = 89$ patients from Fig 6A) and correlated with routinely assessed cytogenetic profiles. Box-and-whisker plots show the upper and lower quartiles (25–75%) with a line at the median, whiskers extend from the 10th to the 90th percentile, and dots correspond values outside the 10th and 90th percentile.

Source data are available online for this figure.