

DNA damage-induced histone H1 ubiquitylation is mediated by HUWE1 and stimulates the RNF8-RNF168 pathway

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Supplemental figure 1: Isolation of UV-responsive ubiquitylated peptides

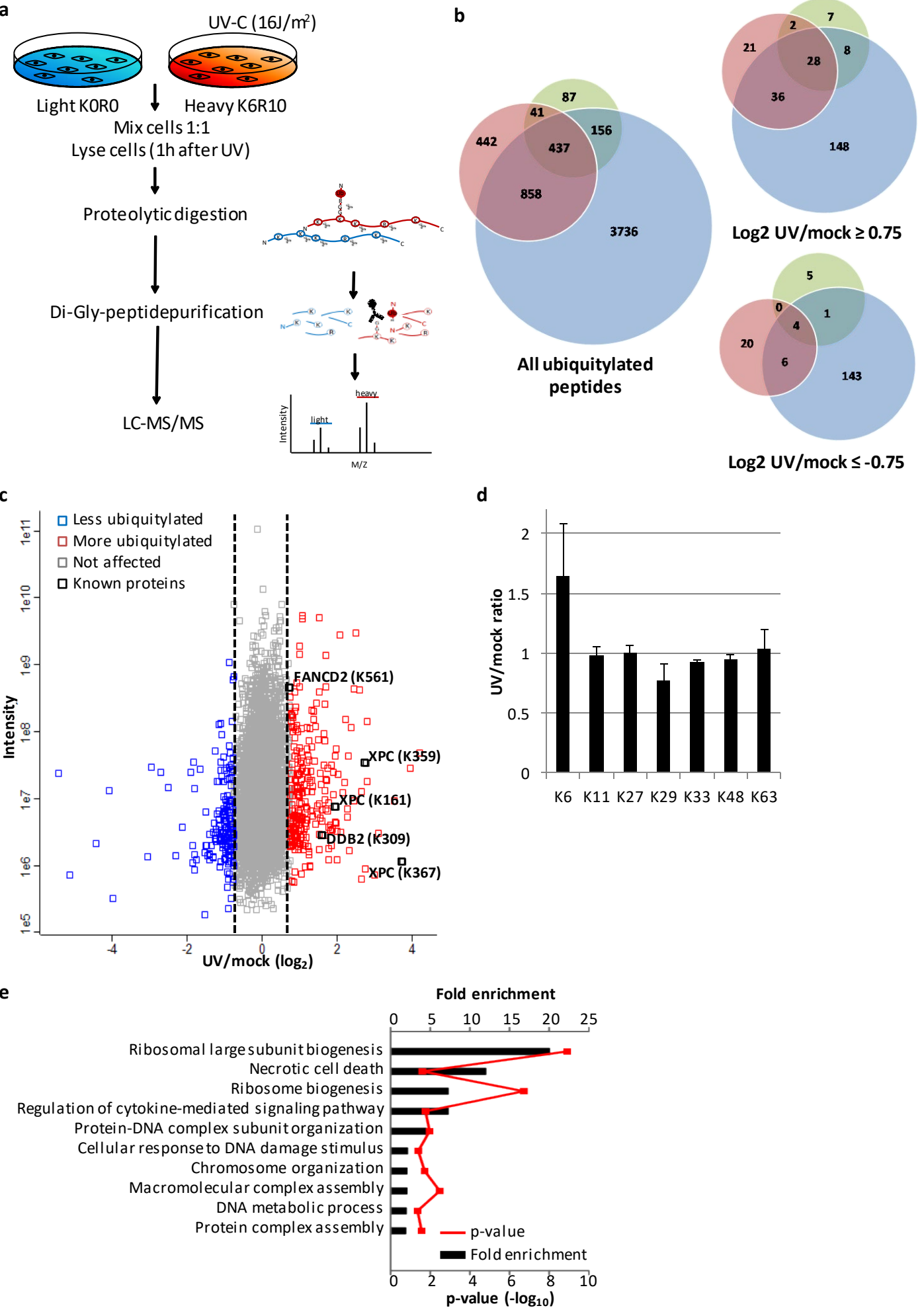
(a) Workflow of SILAC based proteomics approach combined with the isolation of di-Gly peptides to identify UV-responsive ubiquitin sites. Mock treated cells were grown in light (KOR0) medium and UV treated (16J/m^2) cells in heavy (K6R10) medium. One hour after treatments cells were mixed in a 1:1 ratio and lysed in denaturing buffer. Proteins were digested with Lys-C and trypsin after which di-Gly immunopurification was performed. The enriched di-Gly peptides were identified and analysed by LC-MS/MS. **(b)** Experimental overlap of the identified di-Gly peptides in three biological replicates (experiment #1 red, #2 green and #3 blue). Venn diagrams show the total number of di-Gly peptides identified (left), UV-induced di-Gly sites (top right) and UV-reduced sites (bottom right) quantified in each experiment. **(c)** Scatter plot with the $\log_2$ SILAC ratio (UV/mock) plotted against the $\log_{10}$ intensity of the identified di-Gly peptides as quantified by Maxquant analysis from 3 independent experiments. Peptides were considered as UV-responsive when its $\log_2$ SILAC ratio was increased or decreased (dotted lines) more than 0.75. Grey: Non-responsive di-Gly peptides; Red: UV-increased ubiquitylated peptides; Blue: UV-reduced ubiquitylated peptides; Black: Increased di-Gly peptides for known UV-dependent ubiquitylated proteins FANCD2, XPC and DDB2. The amino acid position of the modified lysine is shown between brackets. **(d)** Quantification of the identified di-Gly modified ubiquitin peptides originating from poly-ubiquitin chains, which is a measurement for the abundance of the different ubiquitin chain linkages. Values represent the average of three experiments; error bars indicate SD. **(e)** Functional annotation of UV-induced ubiquitylation sites ($\log_2$ SILAC ratio >0.75) into biological pathways using DAVID bioinformatics resource. Enriched GO-terms (BP4) ($p\text{-value} < 0.01$) are plotted. Bar graph indicates the fold enrichment of biological processes. The fold enrichment defines the ratio between input genes in a pathway over the whole genome in that pathway. The significance ($p\text{-value}$) of the biological pathways was determined by Fisher's exact test and is indicated in red.

Supplemental figure 2: The UV-dependent histone H1 ubiquitylation is dependent on the E3-ligase

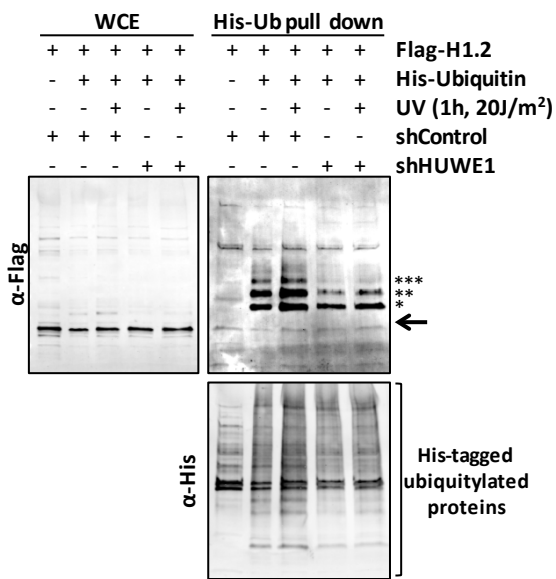
HUWE1. U2OS cells, expressing either shControl or shHUWE1, were transfected with His-Ub and FLAG-H1.2 were UV-C irradiated (20J/m^2). Cells were cultured with doxycycline ($1\mu\text{g/ml}$) for 3 days prior to lysis to induce expression of the shRNA. His-tagged ubiquitylated proteins were isolated 1 hour after UV exposure and analysed by immunoblotting using anti-His and anti-FLAG antibodies. The arrow ($\leftarrow$) indicates the unmodified form of FLAG-H1.2. The asterisks (*) indicate modified forms of FLAG H1.2 and the number of asterisks indicate the expected number of conjugated ubiquitin molecules based on the shift in mass of histone H1.

Supplemental figure 3: HUWE1 mediated histone H1 ubiquitylation stimulates 53BP1 accumulation to sites of DNA damage. **(a)** Immunofluorescence images showing DAPI and EdU signal in cells treated with EdU (20 μ M) for 2 h. Arrows indicate non-S-phase cells. **(b)** Representative images of immunofluorescence experiments to study colocalisation of MDC1 and 53BP1 with γ H2AX in shControl or shHUWE1 expressing cells. Local UV damage was induced (60 J/m²) through a 5 μ M micropore filter. Cells were incubated with EdU (20 μ M) and fixed 2h after irradiation. **(c)** Quantification of fold increase of MDC1 and 53BP1 at sites of local UV damage (LUD). Cells that incorporated high levels of EdU, indicative of S-phase cells, were excluded from analysis. Sites of local UV-induced DNA damage are defined by γ H2AX signal. The fold increase is calculated as the ratio of the fluorescent intensity at site of damage over the fluorescent intensity in the rest of the nucleus. Average of 3 independent experiments, in which at least 25 cells were analysed, is plotted. Error bars represent SEM. P-value (0.019) was calculated with a two-tailed t-test. **(d)** Clonogenic UV-survival experiments in U2OS cells transfected with either siControl, siHUWE1 or siXPC. The percentage of surviving colonies is plotted against the UV-C dose. The number of colonies counted at 0 J/m² is set as 100% survival. The data represent the average of three independent experiments, all done in triplicate, and error bars represent standard error of the mean. **(e)** Western blot showing the knock down efficiency of the siRNAs targeting HUWE1 or XPC. Ku70 is used as a loading control.

Supplemental figure 1

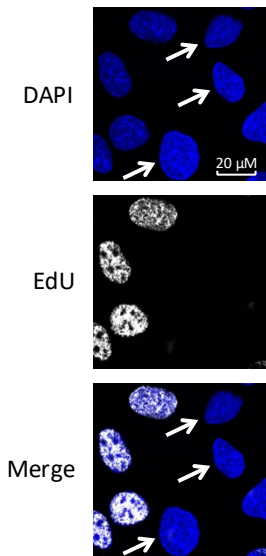


Supplemental figure 2

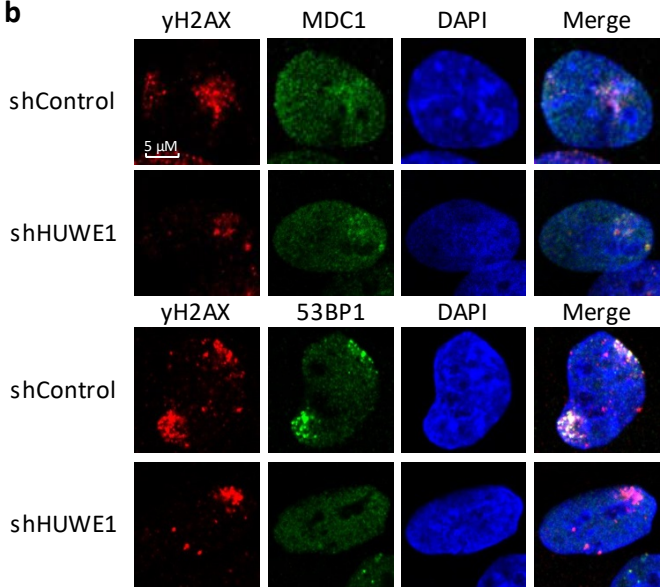


Supplemental figure 3

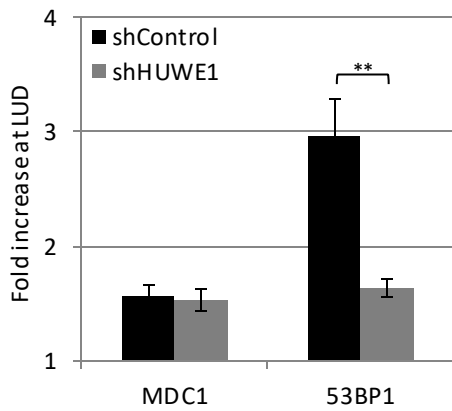
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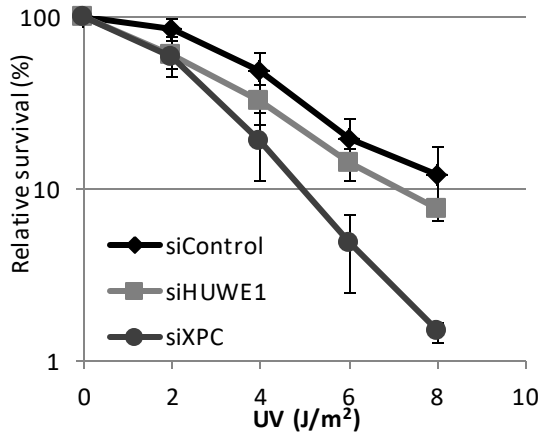
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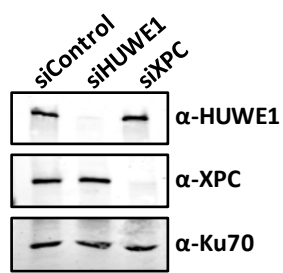
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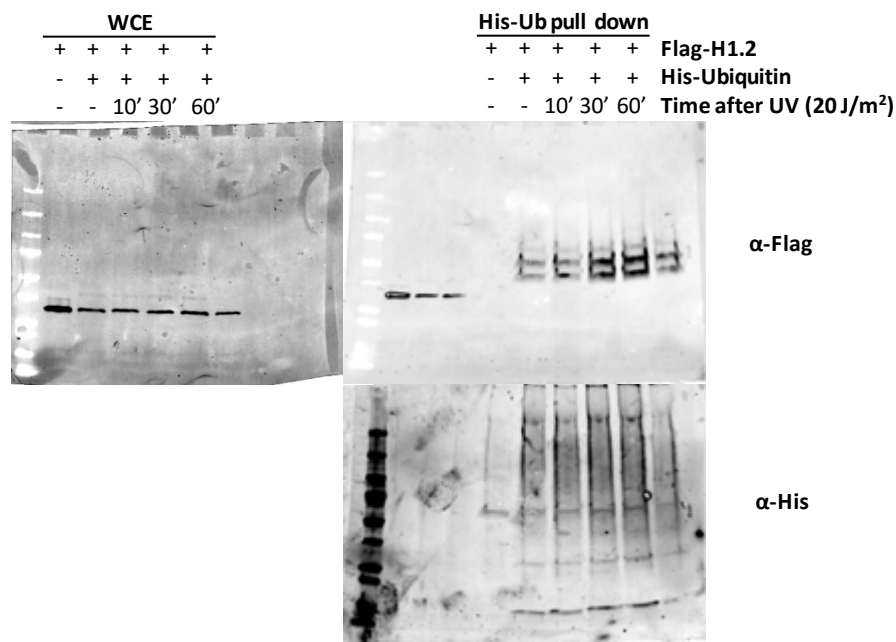
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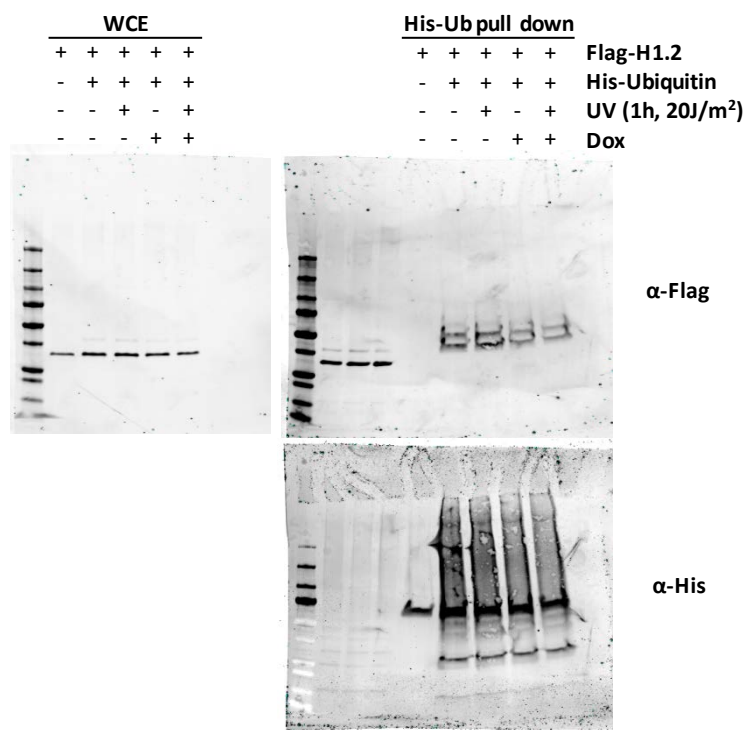
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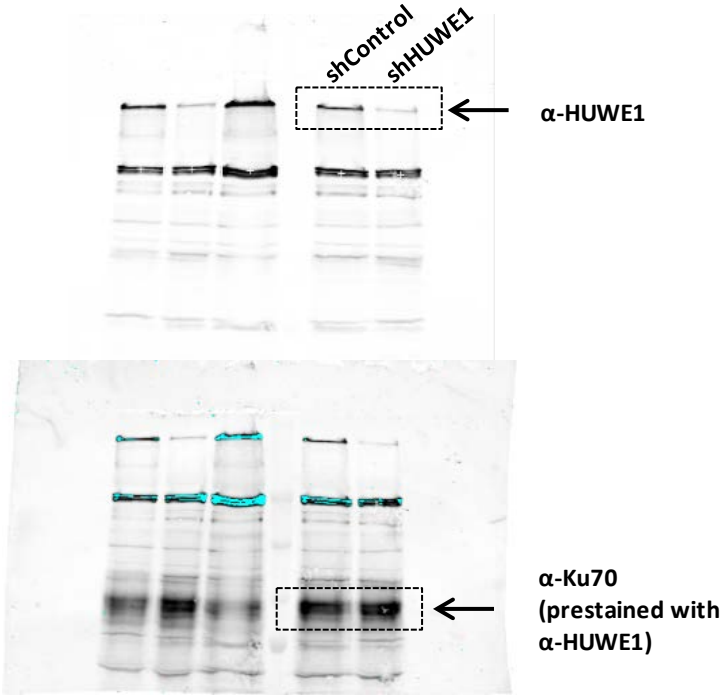
Uncropped westernblots from figure 1d



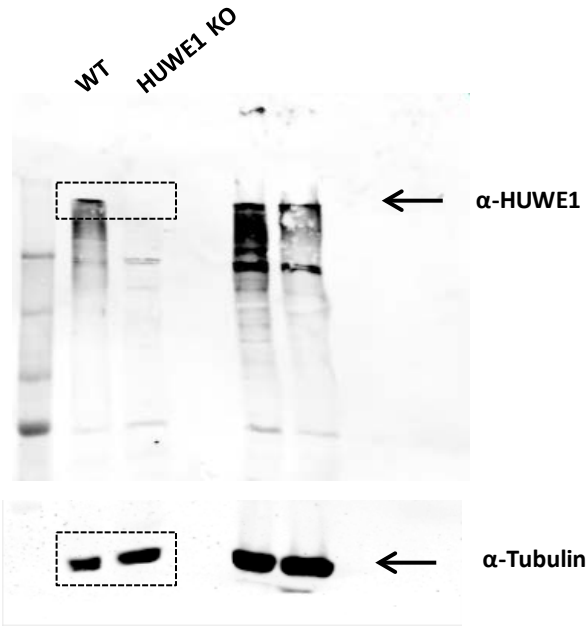
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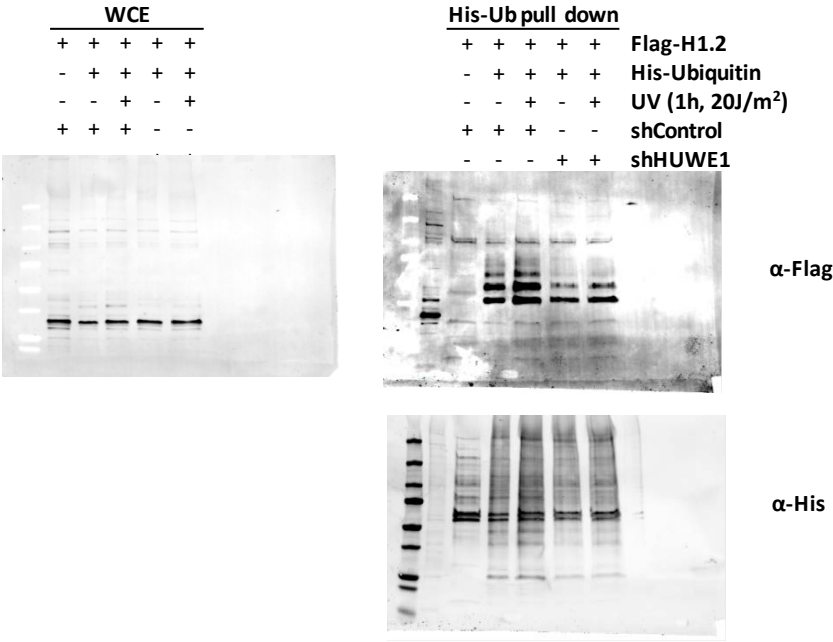
Uncropped westernblots from figure 2d



Uncropped westernblots from figure 2e



Uncropped westernblots from supplemental figure 2



Uncropped westernblots from supplemental figure 3e

