**Supplementary Methods:**

**Cell cycle analysis**

Cells were plated and incubated at 37ºC in complete DMEM medium. 24 hours later, they were treated with 0,2 $\mu$M MLN4924 for 1, 2 or 24 hrs, as indicated. After collecting the cells with trypsin, they were resuspended in 1 ml PBS and washed twice in 1 ml PBS. Then, cells were fixed in 5 ml 70% ethanol and incubated at 4ºC for 2 hrs, washed twice with PBS, resuspended in 0,2 $\mu$l of 1 mg/ml of propidium iodide and RNAse 0,2 mg/ml and incubated a 37ºC for 30 minutes. At least 10000 cells were counted from each sample.

**Supplementary legends**

Figure S1: An example of cells positive (solid arrows) or negative (empty arrows) for RPA foci.

Figure S2: Cell cycle distribution of U2OS cells treated with DMSO or MLN4924 for the indicated length of time. Cell cycle distribution was determined by FACS.

Figure S3: Protein depletions. A, Protein extracts were blotted with an anti-NEDD8 antibody to analyze the overall neddylation status in the cells. The signal of the whole lane, normalized to the loading control and relative to the transfection with the empty plasmid was calculated. The average and standard deviation of three independent experiments is shown. B, CtIP levels upon shRNA-mediated downregulation of CtIP. C, siRNA-dependent depletion of RNF111 using two alternative siRNAs compared with control. D, same as C, but depletion of UBE2M. An asterisk marks an unspecific band.

Figure S4: Effect of NEDD8 overexpression on the NHEJ/HR balance. Cells harboring the SSR system were transfected with HA-NEDD8 or an empty HA vector. The NHEJ/HR ratio was calculated as described and normalized to the cells with the empty vector.

**Supplementary tables**

**Table S1: Inhibitors and concentrations used in the SSR screening**

| Inhibitor | Concentration | Supplier | Reference |
|---|---|---|---|
| **Thrichostatin A (TSA)** | 1.3 μM | Sigma | T8552 |
| **Aphidicolin** | 1 μM | Sigma | A0781 |
| **Mirin** | 25 μM | Tocris | 3190 |
| **PARPi** | 1 μM | AstraZeneca | Olaparib |
| **MNL4924** | 1 μM | Vitro | I-502-01M |

**Table S2: siRNAs used in this study**

| Target gene | Description | Supplier | Reference |
|---|---|---|---|
| **Control** | ON-Targetplus Non-targeting pool | Dharmacon | D-001810-10-20 |
| **RNF111-1** | GCGCUUCCAUUAACAAUUC | Dharmacon | J-007002-07-0010 |
| **RNF111-2** | GAGUUGAGAUGAUUAAUAG | Dharmacon | J-007002-08-0010 |
| **hUBE2M-1** | CAGAGGUCCUGCAGAACAA | Sigma | **(7)** |
| **hUBE2M-2** | GGGCUUCUACAAGAGUGGGAAGUUU | Sigma | **(14)** |

**Table S3: shRNAs used in this study**

| Target gene | Description | Supplier | Reference |
|---|---|---|---|
| **Scramble** | pLKO.1-puro Non-Target shRNA Control Plasmid DNA | Sigma | SHC016 |
| **CtIP** | TRCN0000005403 | Sigma | NM_0022894.1-3008s1c1 |

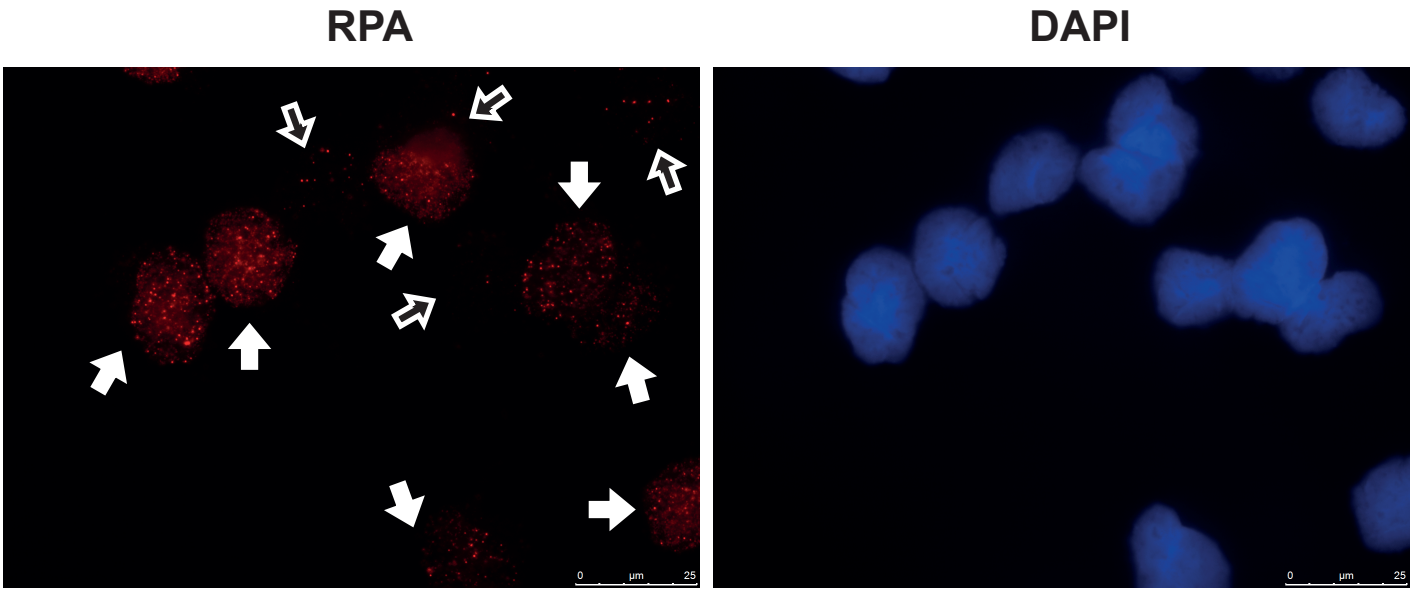
**Table S4: PrimaryAntibodies used in this study.** IF, immunofluorescence. WB Western blotting. IP, Immunoprecipitation. SMART, Single Molecule Analysis of Resection Tracks

| Target protein | Application | Supplier | Reference |
|---|---|---|---|
| **NEDD8** | IP | GeneTex | GTX61205 |
| **RNF111** | WB | Abnova | H00054778-M05 |
| **BRCA1** | IP | Santa Cruz | sc-642 |
| **BRCA1** | IP, WB | Santa Cruz | sc-6954 |
| **HSP70** | WB | Santa Cruz | sc-24 |
| **Tubulin** | WB | Sigma | T9026 |
| **RPA32** | IF | Abcam | ab2175 |
| **RIF1** | IF | Bethyl Laboratories | A300-568A |
| **Non related Rabbit IgG** | IP | Sigma | I8140 |

| | | | |
|---|---|---|---|
| **Non related Mouse IgG** | IP | Sigma | I8765 |
| **CtIP** | WB | R.Baer | |
| **UBE2M** | WB | Bostom Biochem | A-655 |
| **BrdU** | SMART | Sigma | B5002-100MG |

**Table S5: Secondary antibodies used in this study.** IF, immunofluorescence. WB Western blotting.

| Antibody | Source | Application |
|---|---|---|
| **Alexa Fluor 594 goat anti-mouse** | Invitrogen | IF |
| **Alexa Fluor 488 goat anti-rabbit** | Invitrogen | IF |
| **Alexa Fluor 647 goat anti-mouse IgG** | Invitrogen | IF |
| **Alexa Fluor 568 goat anti-rabbit** | Invitrogen | IF |
| **IR-Dye 680 RD goat anti-mouse IgG (H+L)** | Li-Cor | WB |
| **IR-Dye 800CW goat anti-rabbit IgG (H+L)** | Li-Cor | WB |

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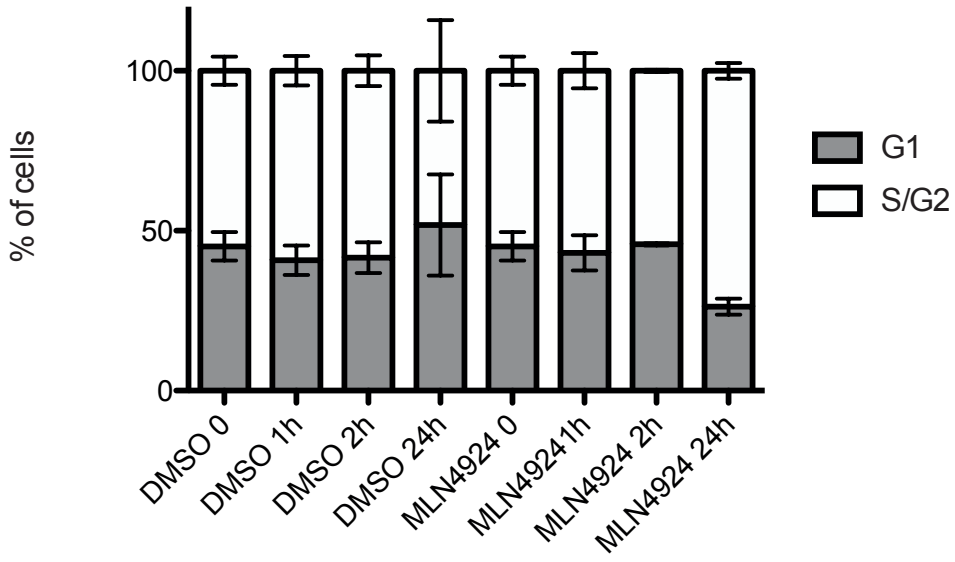


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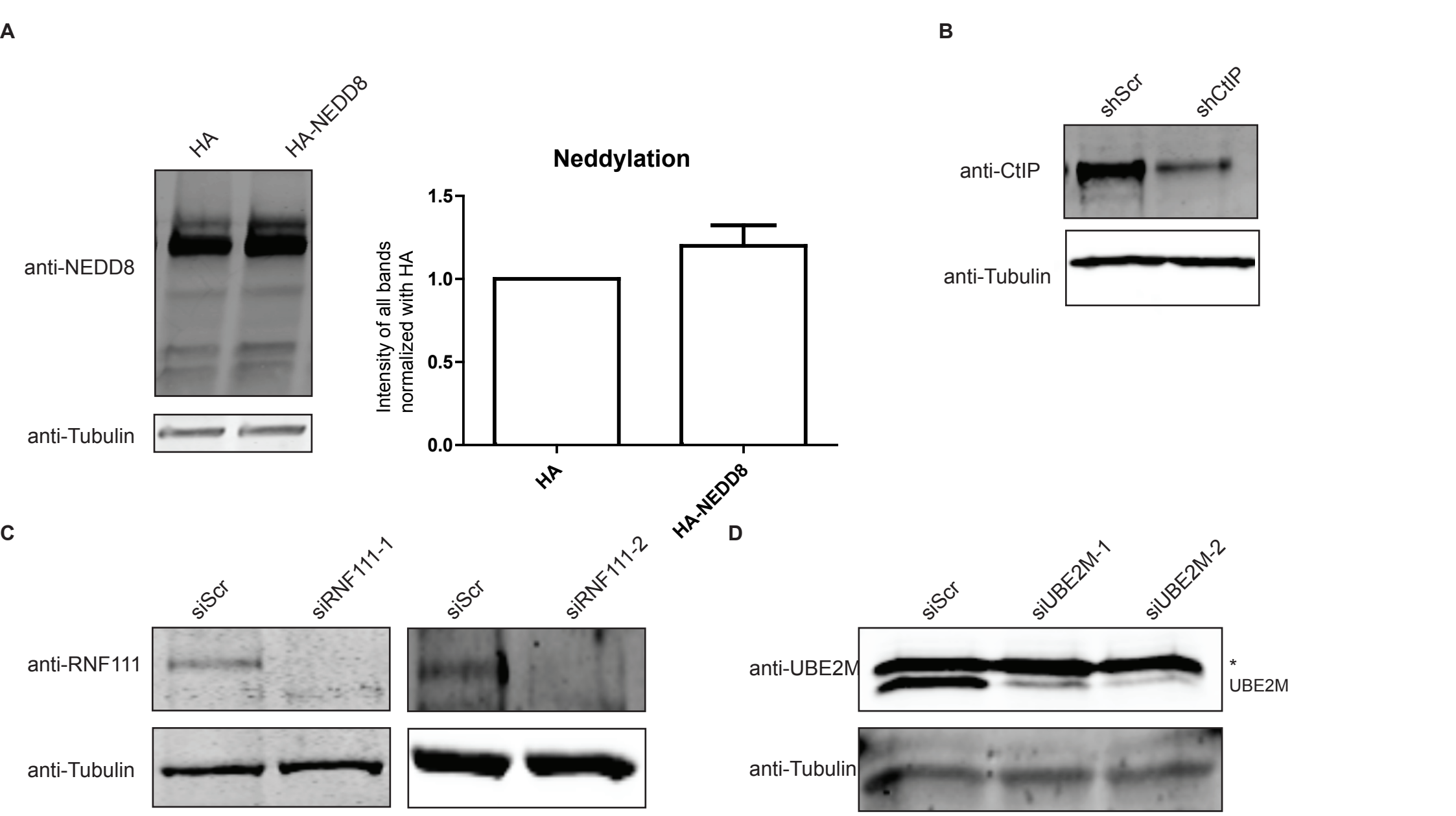
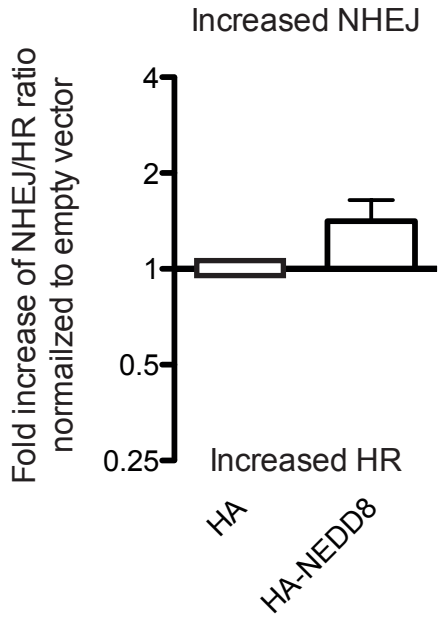



Jimeno et al Figure S3

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