Foci kinetics after IR (2 Gy)

**UBE2K (E2 family 1*)**

- **FK2:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **53BP1:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **γH2AX:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

**UBE2A, -B (E2 family 2)**

- **FK2:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **53BP1:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **γH2AX:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

**UBE2G1, -G2 (E2 family 3-1)**

- **FK2:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **53BP1:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **γH2AX:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

**UBE2R1, -R2 (E2 family 3-2)**

- **FK2:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **53BP1:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **γH2AX:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

**UBE2D1-D4 (E2 family 4-1)**

- **FK2:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **53BP1:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

- **γH2AX:**
  - Time after IR (2 Gy):
    - 30 min
    - 2 h
    - 8 h
    - 24 h

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* E2 families according to Michelle et al., 2009

** siD3 depletes all UBE2Ds

** siD2 depletes UBE2D2 and -D3 (and some effect on -D1)
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**UBE2E1-E3** (E2 family 4-2)

**UBE2U** (E2 family 4-3)

**UBE2J1, -J2** (E2 family 5)

**UBE2H** (E2 family 6*) and **AKTIP**

**UBE2F, -M** (E2 family 8*)

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*E2 family 7: UBE2I (SUMO E2)
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**UBE2N, -NL (E2 family 9-1)**

**UBE2T (E2 family 9-2)**

**UBE2V1-V2 (E2 family 10)**

**UBE2S (E2 family 11)**

**UBE2C (E2 family 12)**

*Inefficient depletion of UBE2N*
Foci kinetics after IR (2 Gy) page 4/5

c-4  

**UBE2W** (E2 family 13)

![Graph showing Foci/cell (IR induction) against Time after IR (2 Gy) for different siRNA concentrations.]

**BIRC6, UBE2O, -Z** (E2 family 14)

![Graph showing Foci/cell (IR induction) against Time after IR (2 Gy) for different siRNA concentrations.]

**UBE2L3, -L6** (E2 family 15)

![Graph showing Foci/cell (IR induction) against Time after IR (2 Gy) for different siRNA concentrations.]

**TSG101** (E2 family 16) and **UEV3**

![Graph showing Foci/cell (IR induction) against Time after IR (2 Gy) for different siRNA concentrations.]

**UBE2Q1-Q2** (E2 family 17)

![Graph showing Foci/cell (IR induction) against Time after IR (2 Gy) for different siRNA concentrations.]

siRNA:
- CTRL
- RNF8/168
- W
- O
- Z
- L3
- L6
- TSG101
- UEV3
- Q1
- Q2
IRIF screen results for module 2: Automated HT/HC confocal microscopy quantification of the kinetics of FK2, 53BP1 and gH2AX IRIF after IR-treatment (2 Gy) in siE2 treated cells. Data are arranged according to phylogenetic families as reported previously\(^1\). siCTRL and siRNF8/168 (co-depletion of RNF8 and RNF168)-treated cells were used as a negative and positive control, respectively. Note: siCTRL and siRNF8/168 curves are identical in each graph being part of the same experimental pipeline. Data represent 96-microplate well averages ± range of, on average, n=704 cells per siRNA condition based on one screening experiment.

Reference

**DDR signalling page 1/4**

- **siUBE2D1**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h
- **siUBE2D2**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h

- **siUBE2G1**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h
- **siUBE2G2**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h

- **siUBE2A**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h
- **siUBE2B**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h

- **siUBE2D3**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h
- **siUBE2D4**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h

- **siUBE2Q1**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h
- **siUBE2Q2**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h

- **siUBE2K**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h
- **siUBE2C**
  - Non-treated
  - IR 10 Gy 2 h
  - CPT 1 μM 1 h

*siD3 depletes all UBE2Ds
siD2 depletes UBE2D2 and -D3 (and some effect on -D1)
Whole cell extract immunoblots representing one screening experiment. U2OS cells were treated with the indicated siE2 pools at the indicated time points following IR or treatment with camptothecin (CPT) and probed with the indicated antibodies. Black arrowheads indicate CHK1-specific bands. Positive controls used in this module were siCtIP, siBRCA1 and MG132 treatment (1 hour, 20 μM).

*siD3 depletes all UBE2Ds
siD2 depletes UBE2D2 and -D3 (and some effect on -D1)

**Inefficient depletion of UBE2N