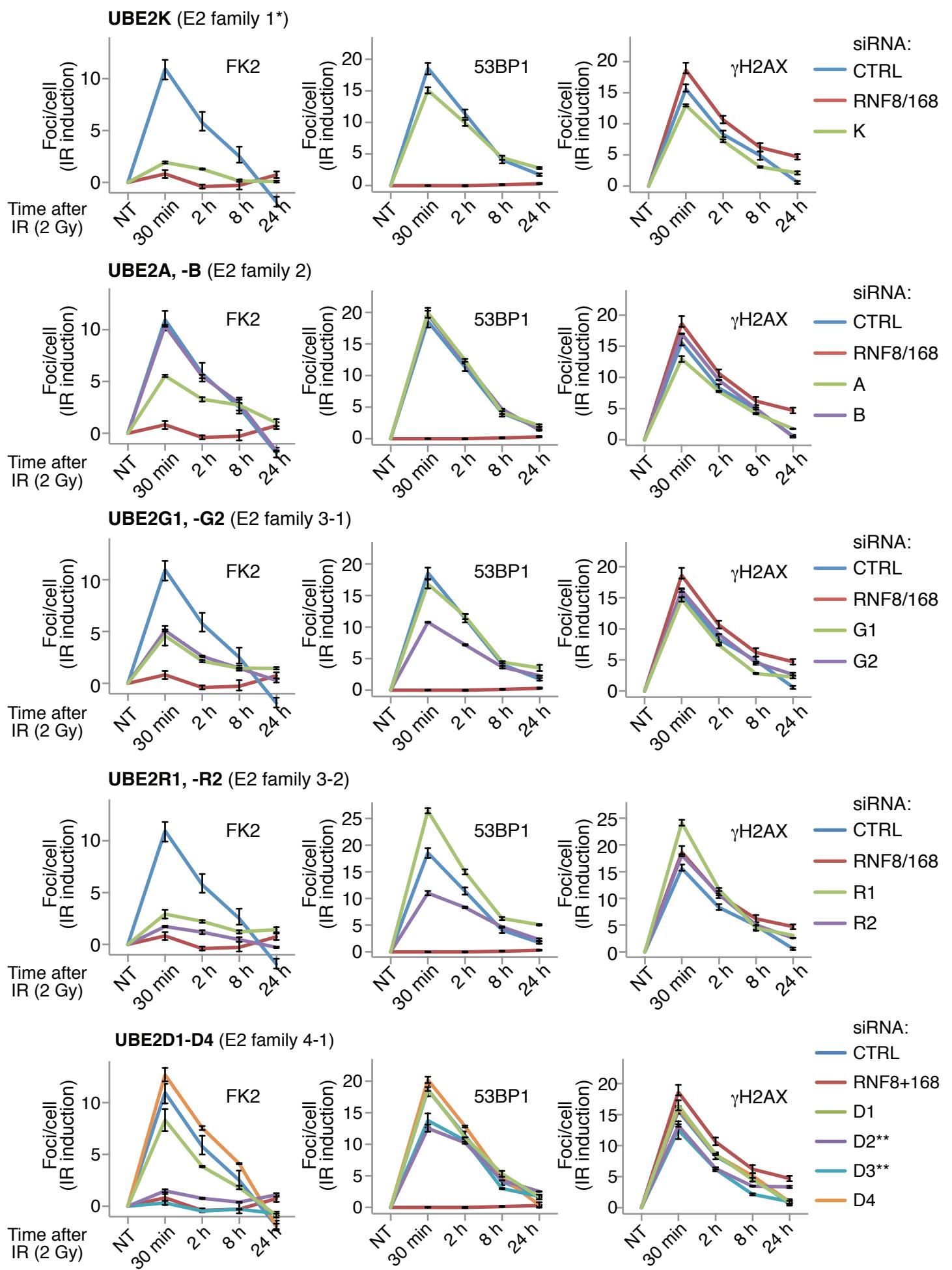


Foci kinetics after IR (2 Gy) page 1/5

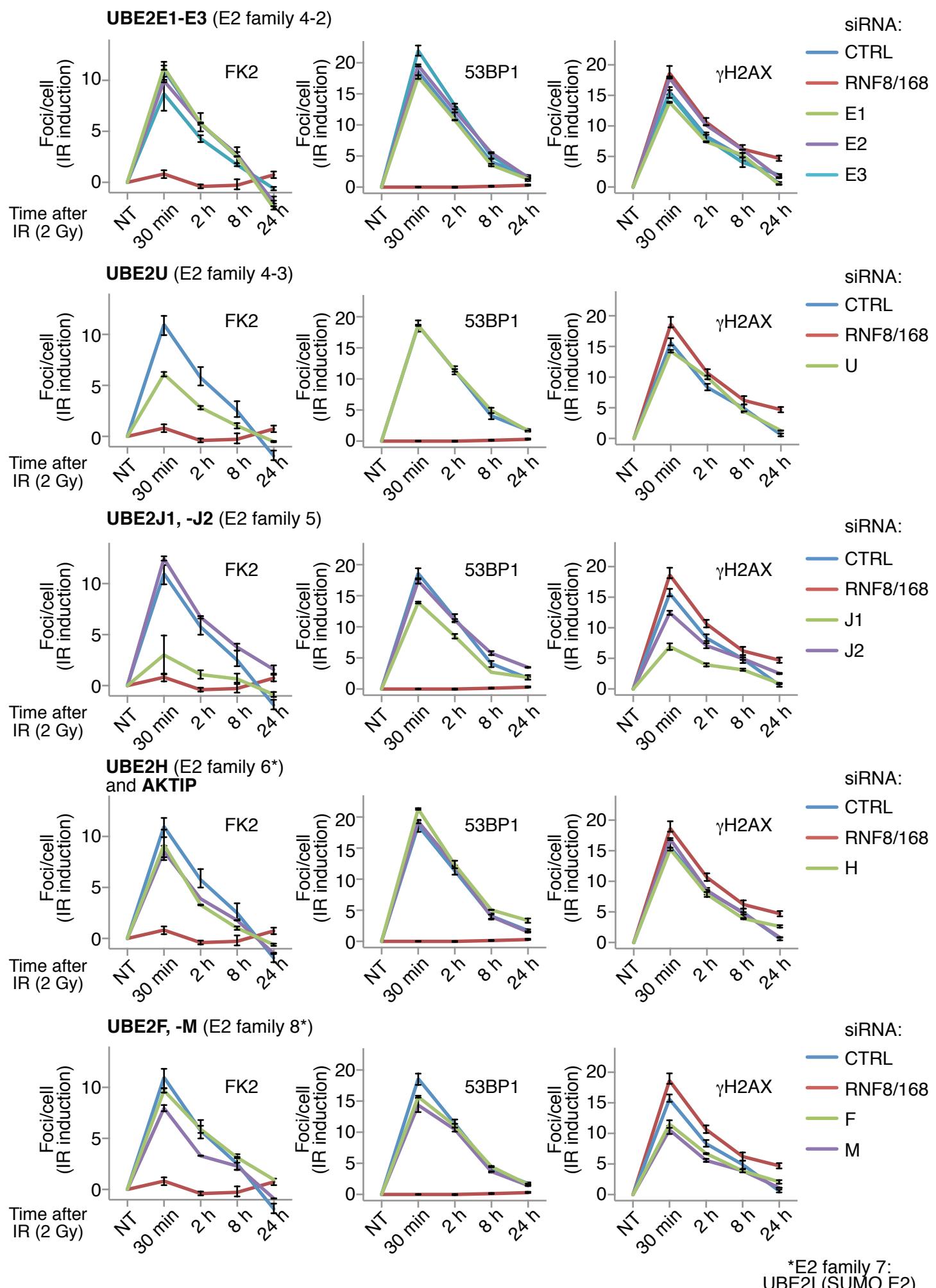


* E2 families according to Michelle et al., 2009

** siD3 depletes all UBE2Ds

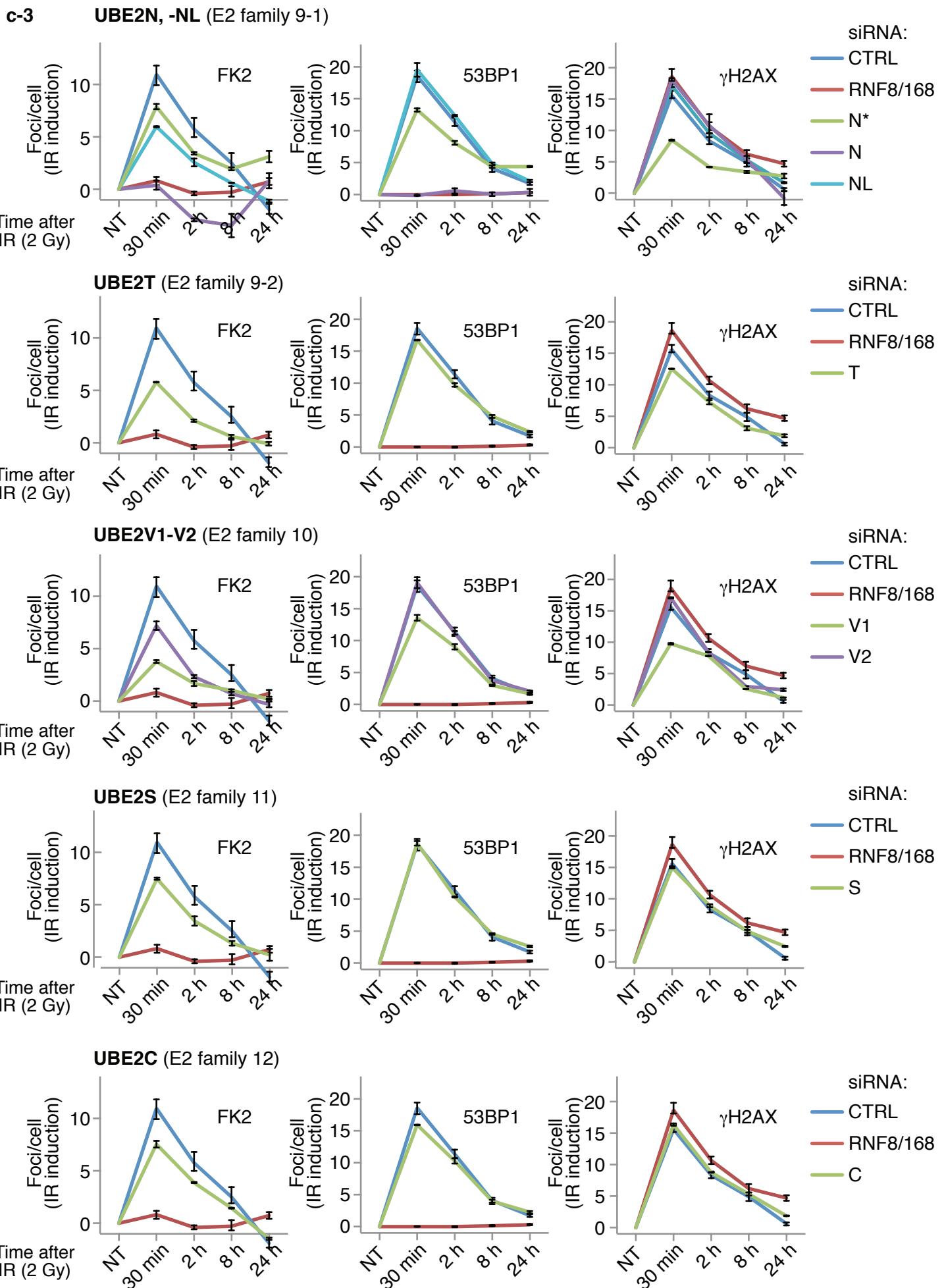
siD2 depletes UBE2D2 and -D3 (and some effect on -D1)

Foci kinetics after IR (2 Gy) page 2/5



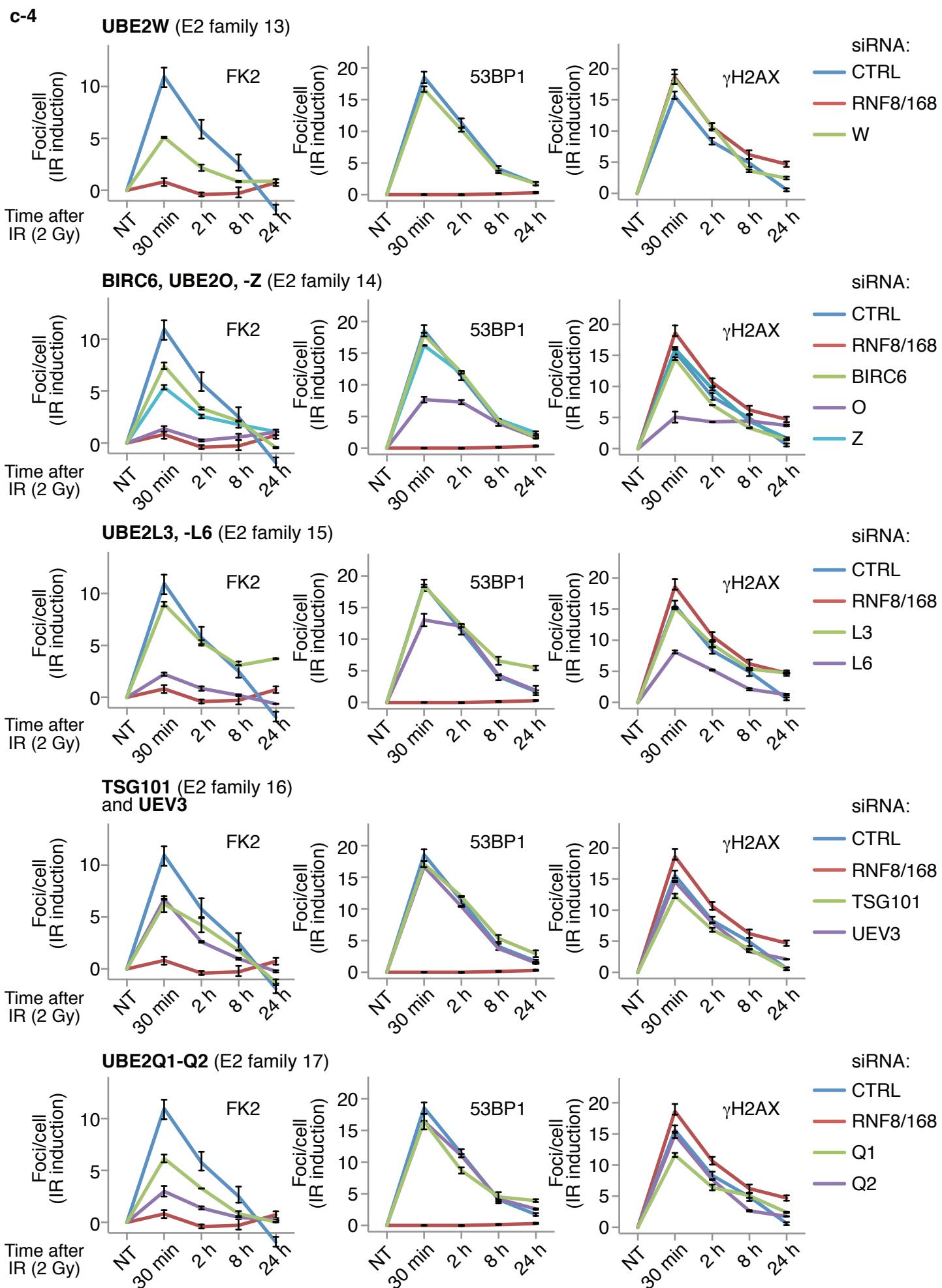
*E2 family 7:
UBE2I (SUMO E2)

Foci kinetics after IR (2 Gy) page 3/5



*Inefficient depletion of UBE2N

Foci kinetics after IR (2 Gy) page 4/5

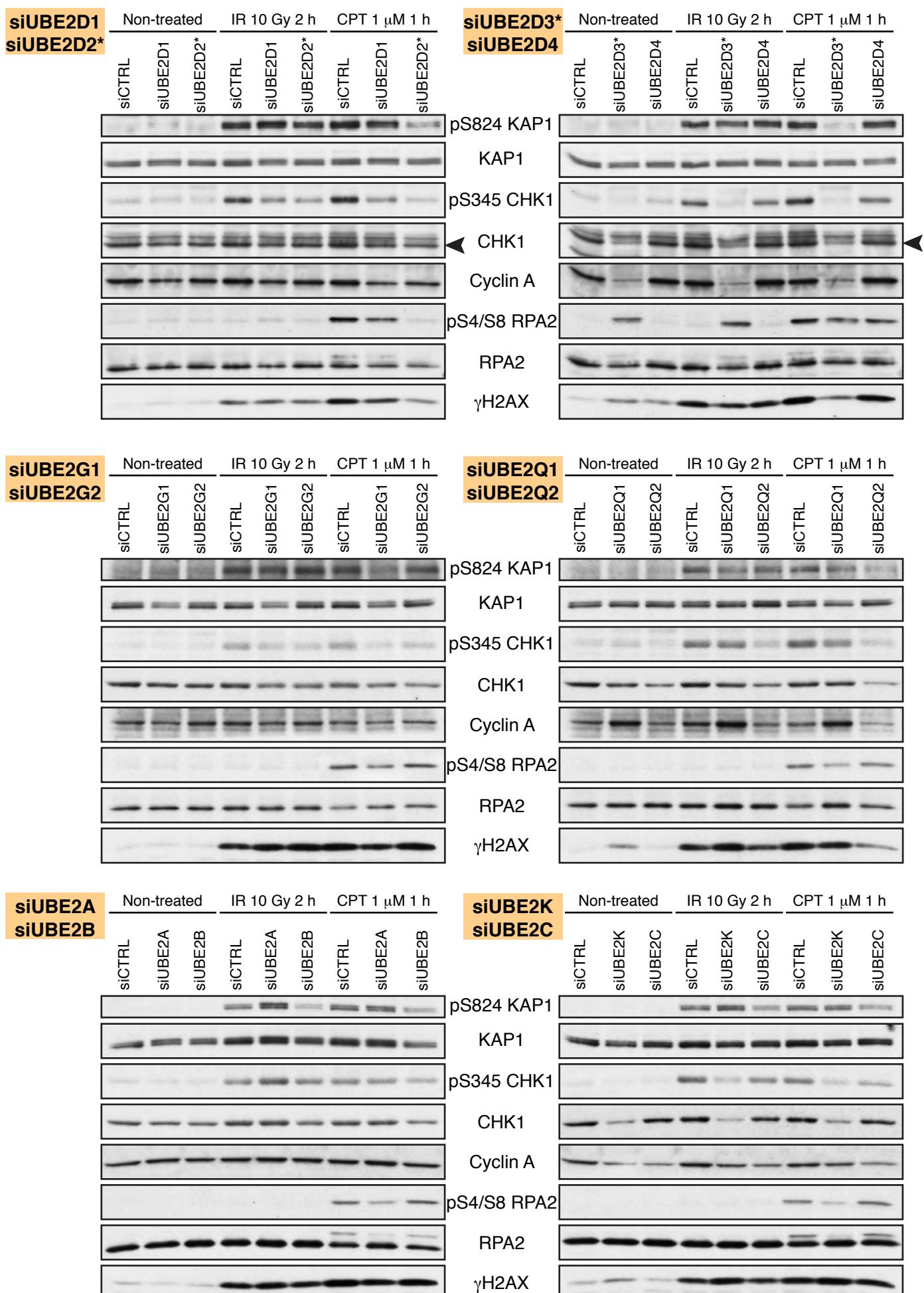


Foci kinetics after IR (2 Gy) page 5/5

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 previously¹. 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 \pm range of, on average, n=704 cells per siRNA condition based on one screening experiment.

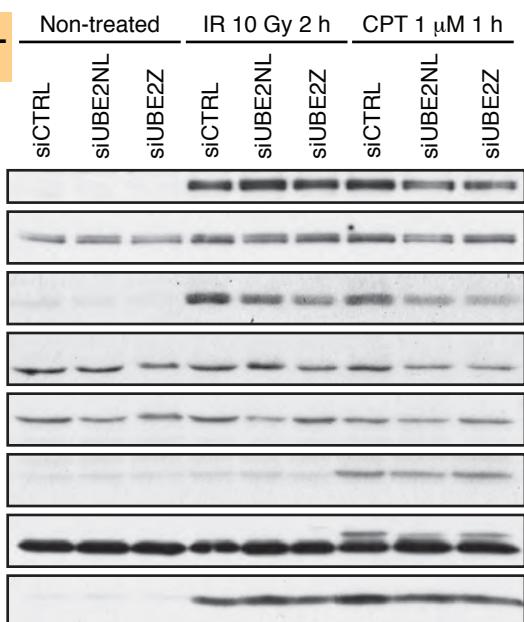
Reference

1. Michelle, C., Vourc'h, P., Mignon, L. & Andres, C. R. What was the set of ubiquitin and ubiquitin-like conjugating enzymes in the eukaryote common ancestor? *J. Mol. Evol.* **68**, 616–28 (2009).

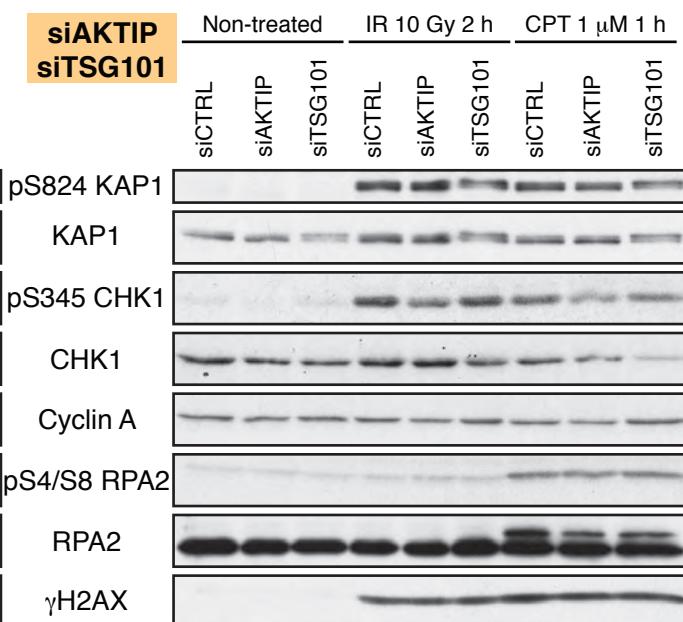


*siD3 depletes all UBE2Ds
siD2 depletes UBE2D2 and -D3
(and some effect on -D1)

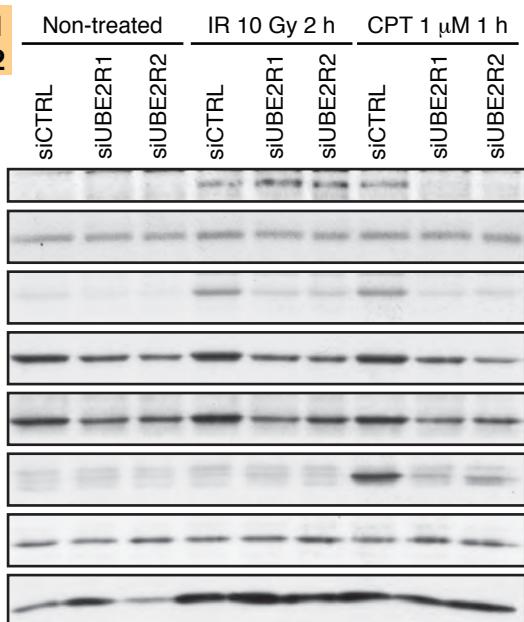
**siUBE2NL
siUBE2Z**



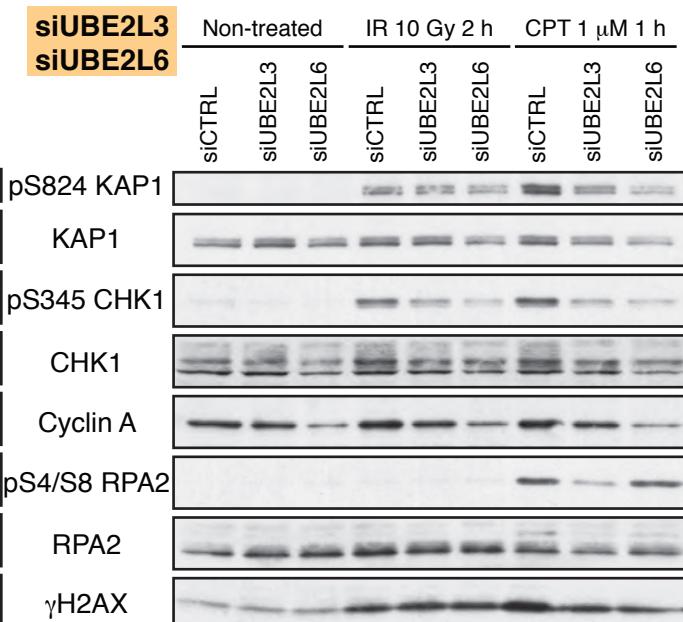
**siAKTIP
siTSG101**



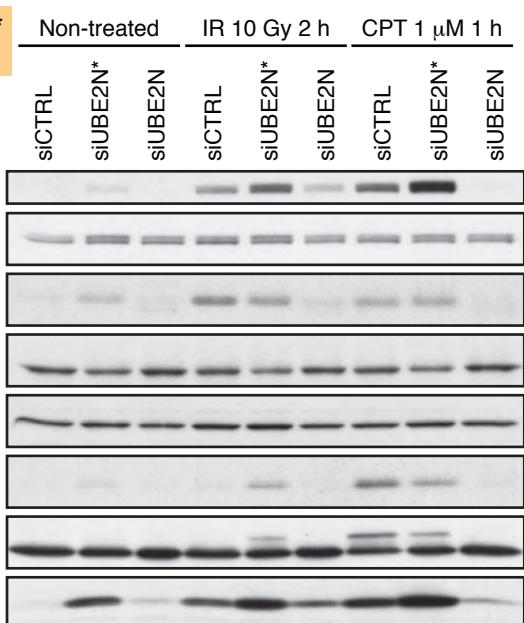
**siUBE2R1
siUBE2R2**



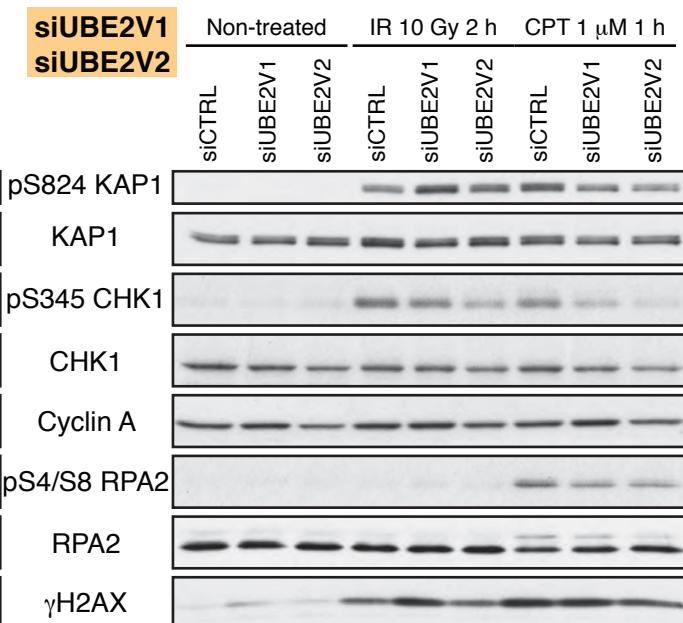
**siUBE2L3
siUBE2L6**



**siUBE2N*
siUBE2N**

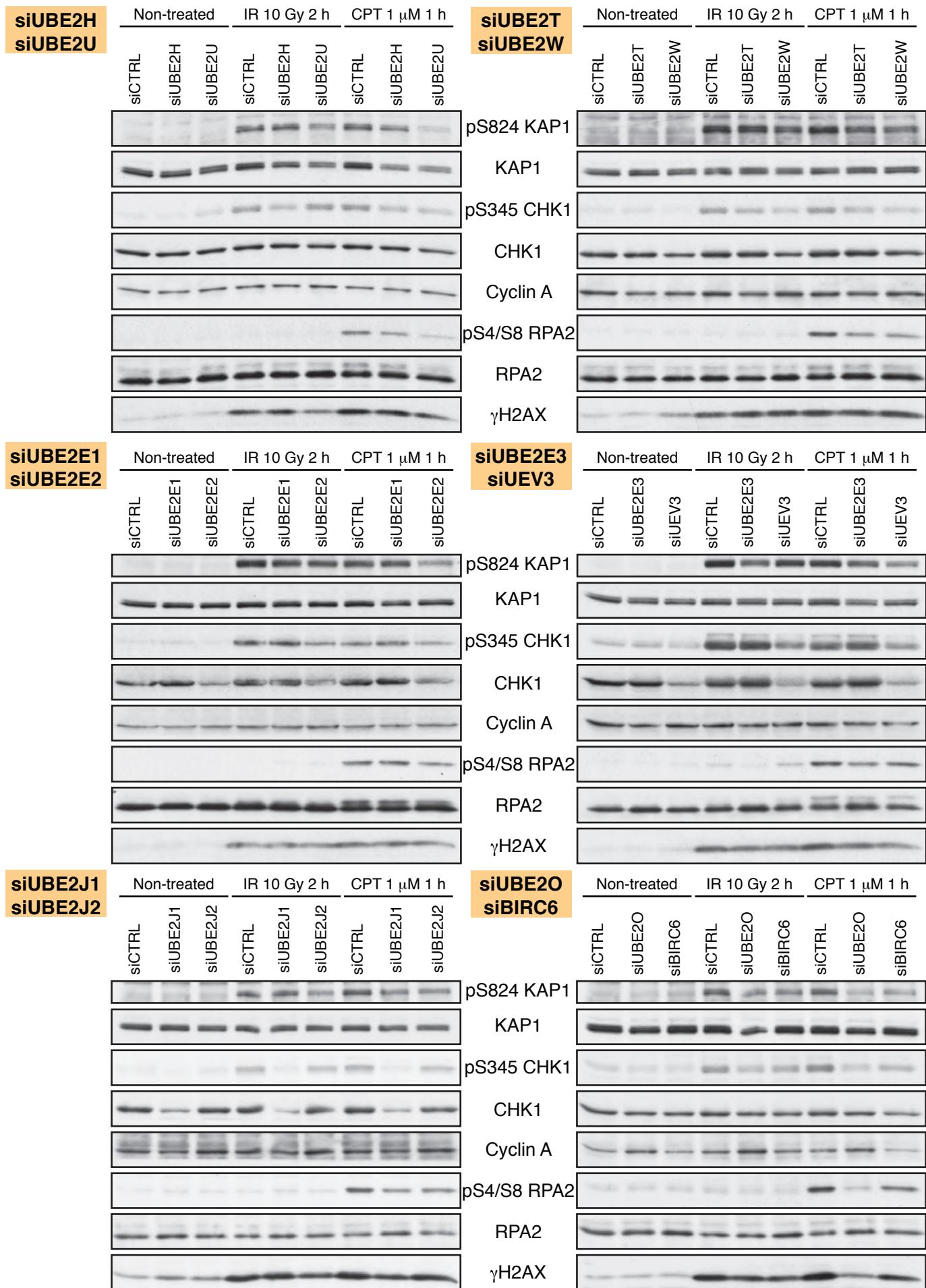


**siUBE2V1
siUBE2V2**

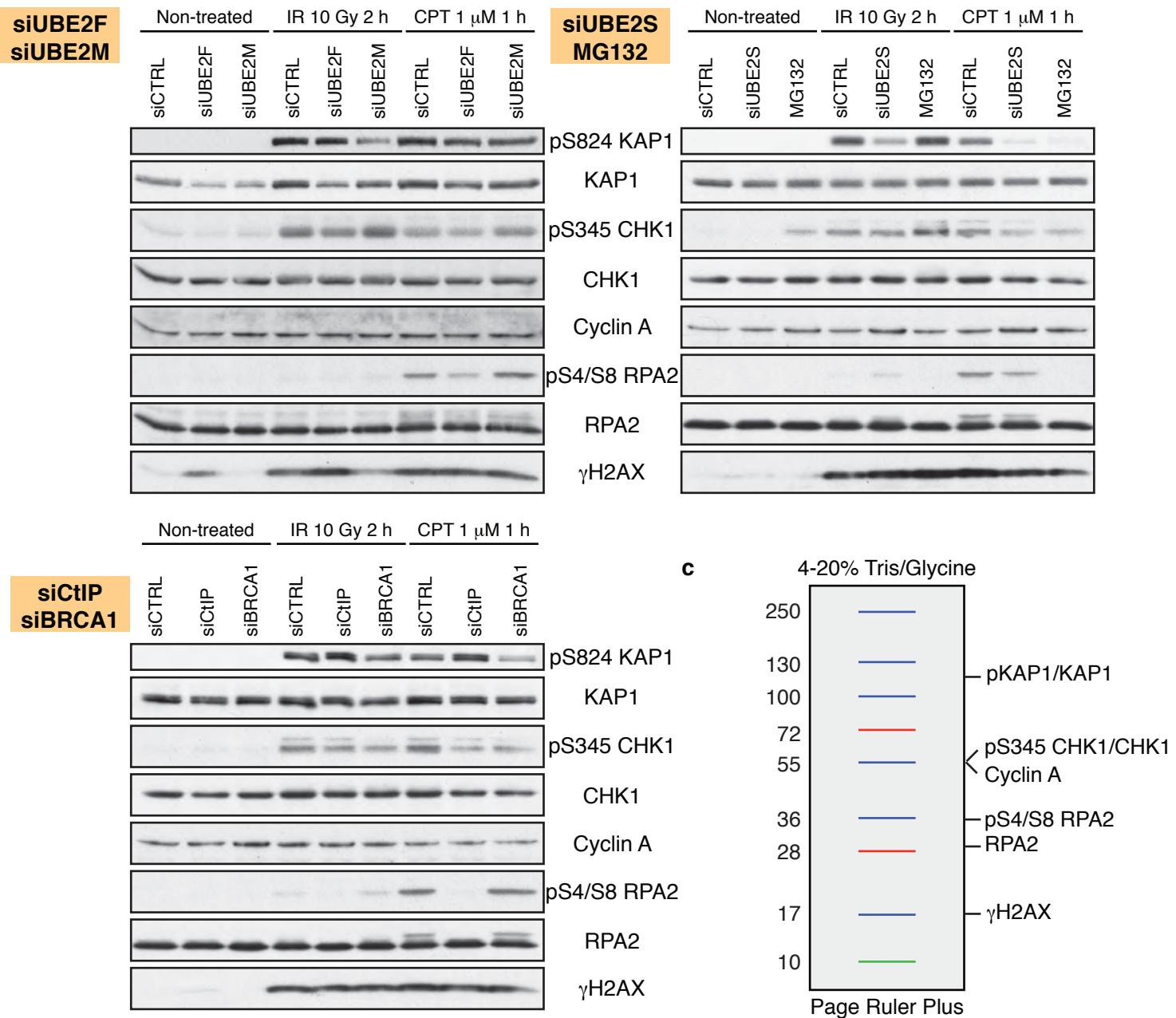


*Inefficient depletion of UBE2N

DDR signalling page 3/4



DDR signalling page 4/4



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

**Inefficient depletion of UBE2N

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 μ M).