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

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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).

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

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siUBE2NL	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h	siAKTIP	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h
siUBE2Z	siCTRL siUBE2NL siUBE2Z	siCTRL siUBE2NL siUBE2Z	siCTRL siUBE2NL siUBE2Z	siTSG101	siCTRL siAKTIP siTSG101	siCTRL siAKTIP siTSG101	siCTRL siAKTIP siTSG101
				pS824 KAP1			
	-	-		KAP1			
			199700 House 19970	pS345 CHK1			Renter and an encourse
				CHK1			
				Cyclin A			
	17-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-			pS4/S8 RPA2		······································	
				RPA2			835
				γΗ2ΑΧ	-		
siUBE2R1	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 Ι	siUBE2L3	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h
siUBE2R2	IL 12R1 12R2	2R1	E2R1	siUBE2L6	L 2L3 2L6	L 2L6	L 2L6
	siCTF siUBE siUBE	siCTF siUBE siUBE	siCTF siUBE		siCTR siUBE siUBE	siUBE	siUBE
		100 al 1900 8-00	a annua	pS824 KAP1		name and anoth	-
			, anness interior pa	KAP1			-
				pS345 CHK1			
				CHK1		-	
				Cyclin A			
	10 10 10			pS4/S8 RPA2		a the first series	
				RPA2			
				γΗ2ΑΧ			
siUBE2N*	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h	siUBE2V1	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h
siUBE2N	IZN*	IS N*	SN*	siUBE2V2	2V1 2V2	2V1 2V2	2V1
	siUBE	siUBE	siCTR siUBE		siCTR	siCTR siUBE	siCTR siUBE
				pS824 KAP1			
	_			KAP1	1		
		-	www.windo	pS345 CHK1			
				CHK1			
	~			Cyclin A			
	-	1		pS4/S8 RPA2		and and have	
			==-	RPA2			===
	-			γΗ2ΑΧ			

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siUBE2H	Non-treated	IR 10 Gy 2 h	CPT 1 µM 1 h	siUBE2T	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h
siUBE2U	siCTRL siUBE2H siUBE2U	siCTRL siUBE2H siUBE2U	siCTRL siUBE2H siUBE2U	siUBE2W	siCTRL siUBE2T siUBE2W	siCTRL siUBE2T siUBE2W	siCTRL siUBE2T siUBE2W
					影響的	10.0 10.0 10.0	THE REAL PROPERTY.
				J KAP1			
		, and must see	were there along	pS345 CHK1		ganta mont toron	gasge process conver-
				CHK1			
				Cyclin A			
	1000	Station and		pS4/S8 RPA2	time and with	and some set	
	-			RPA2			
				γH2AX			
siUBE2E1 siUBE2E2	siCTRL siUBE2E1 siUBE2E2	IR 10 Gy 2 h siUBE2E1 siUBE2E2 siUBE2E2	sictrl siubezet siubezez siubezez	siUBE2E3 siUEV3	siCTRL siUBE2E3 siUEV3	IL 10 Gy 2 h siUBE2E3 siUEV3	siCTRL siUBE2E3 siUEV3 siUEV3
				JpS824 KAP1			
				pS345 CHK1			
				CHK1			
				Cyclin A			
				pS4/S8 RPA2	14 44 44	- 112 - 112 - 112 	
				RPA2			
				η γΗ2ΑΧ			
siUBE2J1	Non-treated	IR 10 Gy 2 h	CPT 1 µM 1 h	siUBE20	Non-treated	IR 10 Gy 2 h	CPT 1 μM 1 h
SIUBEZJZ	siCTRL siUBE2J1 siUBE2J2	siCTRL siUBE2J1 siUBE2J2	siUBE2J1 siUBE2J2	InS824 KAP1	siCTRL siUBE2O siBIRC6	siCTRL siUBE2O siBIRC6	siCTRL siUBE2O siBIRC6
				KAP1		-	
				pS345 CHK1	-		
	-		-	Cyclin A			
				pS4/S8 RPA2			
				RPA2			
				γH2AX			

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*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 μ M).