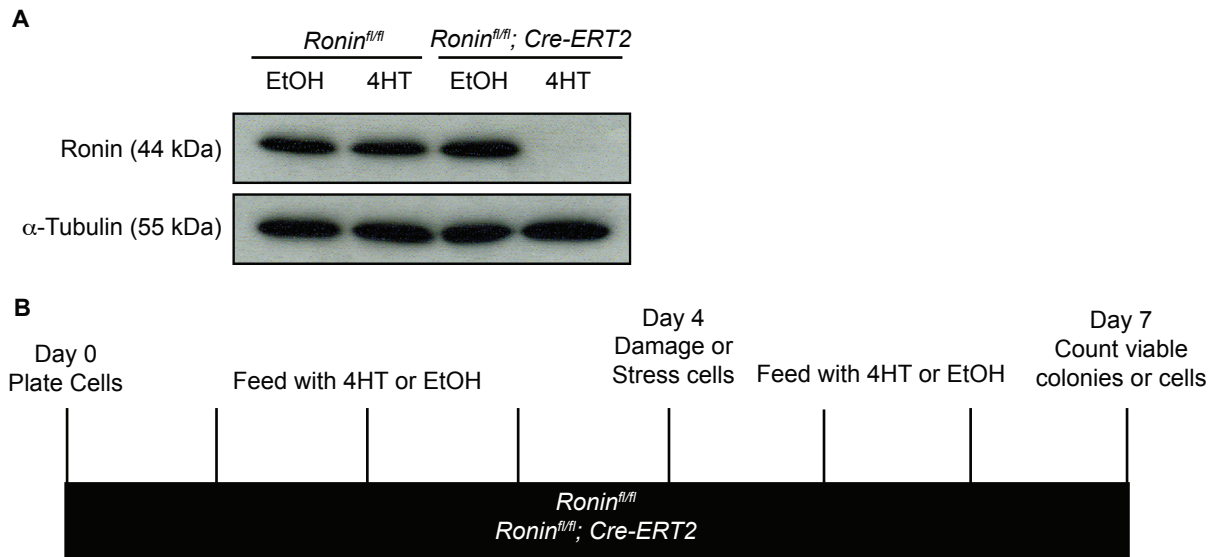


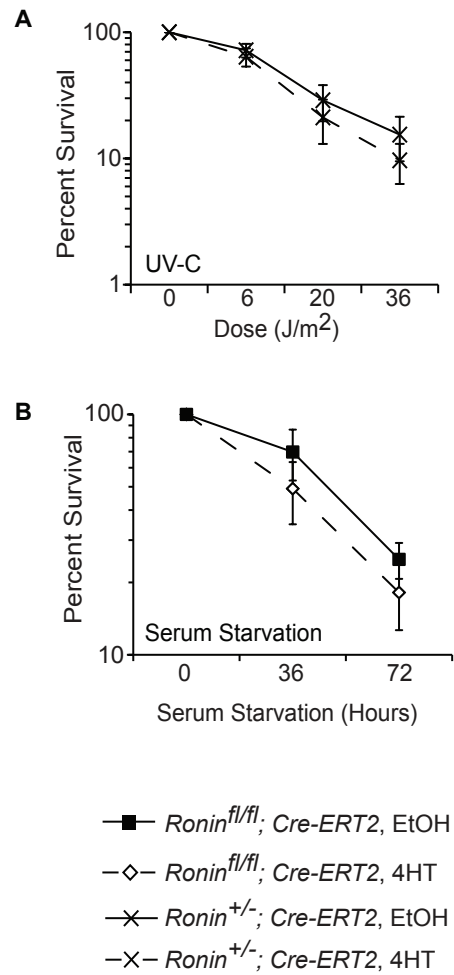
Supplementary Material

Ronin influences the DNA damage response in pluripotent stem cells

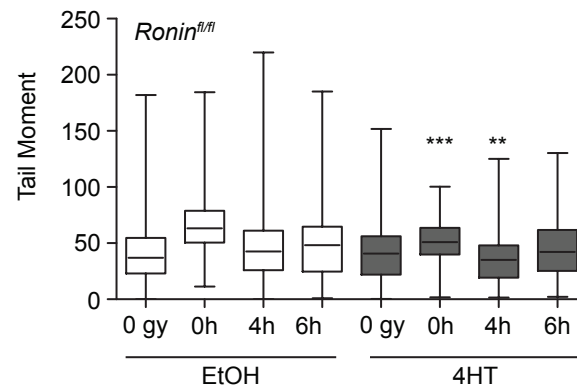
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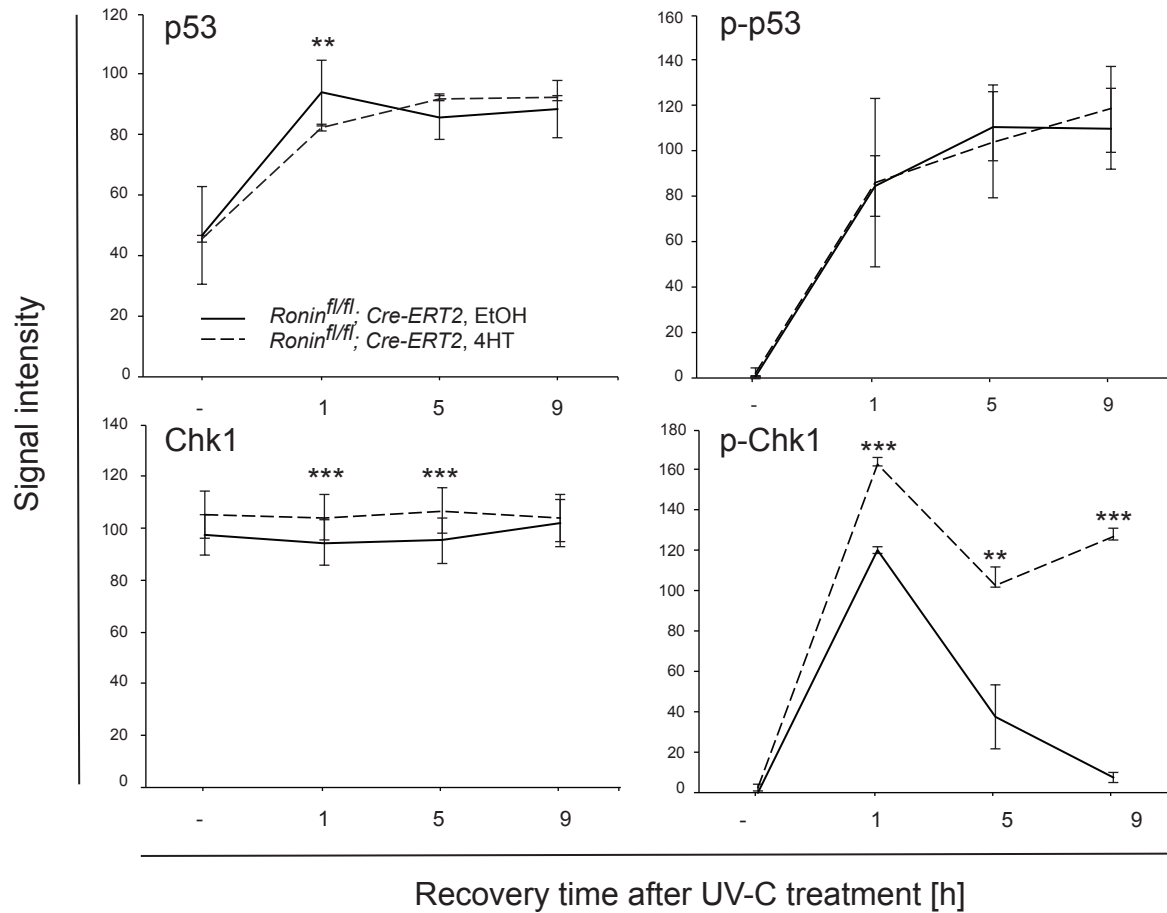
Supplementary Fig. S1. Experimental outline and conditional loss of Ronin upon 4HT treatment. **(A)** *Ronin^{fl/fl}* and *Ronin^{fl/fl}; Cre-ERT2* ESCs were treated for 4 days with 4HT or ethanol and subjected to western blot. **(B)** Experimental timeline for dose response curves and DNA damage assays. *Ronin^{fl/fl}* and *Ronin^{fl/fl}; Cre-ERT2* ESCs were treated with 4HT or vehicle (ethanol) for 4 days prior to DNA-damaging treatment or serum starvation. The cells were subsequently maintained for 3 days prior to determining percent survival. For DNA damage assays, the cells were damaged on day 4 before conducting comet assays, western blots, or flow cytometry.



Supplementary Fig. S2. (A) Activation of Cre recombinase in *Ronin^{+/-}; Cre-ERT2* ESCs does not significantly affect UV-C sensitivity in 4HT treated cells when compared to ethanol treated cells. (B) *Ronin* knockout ESCs do not show increased sensitivity to serum starvation (right). Dose response curves for serum starvation in 4HT-treated and ethanol-treated *Ronin^{fl/fl}; Cre-ERT2* ESCs as controls. Percent survival was determined by counting viable cells by trypan blue exclusion using an automated cell counter. Values represent the mean \pm s.e.m. (n=3).



Supplementary Fig. S3. DNA repair is not impaired after γ -irradiation with 2.3 gy in Control (Ronin^{fl/fl}) cells (lacking Cre-ERT2) when treated with 4HT in comparison with EtOH. Cells were allowed to recover for 0, 4 and 6 h after irradiation and remaining DNA damage was quantified in comet assays. 173 comets were counted for each condition. Shown are the results for one of three independent experiments. ***P<0.001 or **P<0.01 comparing 4HT-treated versus EtOH-treated cells for each time point by Kolmogorov-Smirnov test.



Supplementary Fig. S4. After UV-C induced DNA damage, Ronin loss leads to increased phosphorylation of Chk1, but not of p53. *Ronin^{fl/fl}; Cre-ERT2* ESCs were treated for 4 days with EtOH as control or 4HT to induce *Ronin* knockout, irradiated with 12 J/m² UV-C and allowed to recover for 1, 5, or 9 hours. Protein levels were detected by western blot and signal intensities were quantified with the Image J software. Shown is the mean \pm SD for each time point (n=3). (-, unirradiated control; p-p53, phospho-p53; p-Chk1, phospho-Chk1). Significance was determined by paired, two-tailed t-test. *** $P < 0.001$, ** $P < 0.01$.

Oligo ID	Name	5'-3'-Sequence
BAS254	Rad18_qPCR_F	ATGCTGGGAGCTGTAGTTCTTGTG
BAS255	Rad18_qPCR_R	CTACGCCCTGGAATGTCTCACTTT
BAS258	Wrnip1_qPCR_F	GCCTGTTGTGACGGAGAACTACAT
BAS259	Wrnip1_qPCR_R	TCGTGTCCGGGAAGTGTAGTCCAAA
BAS260	Gtf2h4_qPCR_F	GTCGCAAGGCATCTTGGAATTGT
BAS261	Gtf2h4_qPCR_R	CTTGCGGTGCCTTTAGGGATTTGT
BAS278	RBS_qPCR_F	ACCTTTCGCTTAGGACGAGCTTCA
BAS279	RBS_qPCR_R	ACACCGCGATATCCTGTTCAGAGT
BAS280	NBS_qPCR_F	GAGCACTTGCCTAGCAAGCACAAA
BAS281	NBS_qPCR_R	GAGACCCAAGCAAGTGAGAAGACA
BAS284	Rad18_qRTPCR_F	AGAGAAGTCAGCCCACAACAGACT
BAS285	Rad18_qRTPCR_R	TCACTGTACGGAAAGCTGGCACAA
BAS305	mActb_qRTPCR_F	GACAGGATGCAGAAGGAGATTACTG
BAS306	mActb_qRTPCR_R	TACTTGCGCTCAGGAGGAGCAATG
BAS307	mOct4_qRT-PCR_F	CAGATCACTCACATCGCCAATCAG
BAS308	mOct4_qRT-PCR_R	CTGTAGCCTCATACTCTTCTCGTTG
BAS311	mThap11_qRT-PCR_F	CACTTCTTCAGGTTGGCCAGCAAT
BAS312	mThap11_qRT-PCR_R	ACCGGGTAGCATGGAAAGGAGAAA
BAS329	Wrnip1_qRT-PCR_F	GTTGACTTCTTCAAGCAGAGGCGG
BAS330	Wrnip1_qRT-PCR_R	CAACCAACTCCAAGATCCAGTGAC
BAS331	Gtf2h4_qRT-PCR_F	CATAACAGACCAGATTCCGGCTGTG
BAS332	Gtf2h4_qRT-PCR_R	CTTGCGACAGGAACTGGTTATACAG

Supplementary Table 1. Oligo sequences used in ChIP-PCR and qRT-PCR.

Gene Information	Fold change KO/Control [days of knockout]			Significance			
	Gene ID	1.5	3	6	1.5	3	6
Cbfa2t2h (Cbfa2t2)		1.1190705	0.845767324	0.947253553	0.229503674	0.150781711	0.160580562
Cbfa2t2h (Cbfa2t2)		1.103394828	0.697727241	0.93686578	0.400192622	0.210568251	0.520141331
Cbfa2t2h (Cbfa2t2)		0.893185041	0.746675254	0.847295272	0.539619238	0.481037714	0.229084279
Cbfa2t2h (Cbfa2t2)		1.00861982	1.012056764	1.179095374	0.92119226	0.330638515	0.136753396
Cnot3		1.027300163	1.079991333	1.016854385	0.485531061	0.550581621	0.773914147
Cnot3		0.998308914	0.901209649	1.404196208	0.993397753	0.305634227	0.138619716
Ctcf		1.059746302	1.231055698	1.121702952	0.114507887	0.152067204	0.260485648
Ctcf		1.006982731	1.075305393	0.822268782	0.9600647	0.744960672	0.466062296
Esrrb		1.120213488	0.776682662	0.824615947	0.43443851	0.101676256	0.047819916
Esrrb		1.046848704	0.835224753	0.931174685	0.636818034	0.15484497	0.126561758
Esrrb		1.122824489	0.905527548	0.93501109	0.247243056	0.522555792	0.45836133
Klf4		0.745827506	0.755875282	0.737007796	0.022184083	0.011306939	0.004780177
Med12		0.731789289	0.707181733	0.684802116	0.091065403	0.184502246	0.088070063
Med12		0.934226968	0.901079722	0.675426234	0.06093173	0.567724113	0.077903905
Nanog		0.955684002	1.72214784	1.573735046	0.125184885	0.035432857	0.080177308
Paf1		0.95184773	0.978165729	0.970804355	0.105437425	0.526675881	0.378648697
PAF1		1.01405028	1.302363128	0.952227931	0.75337282	0.133937235	0.305676419
Pou5f1 (Oct4)		0.990759912	1.057564453	1.099939669	0.317184652	0.23244863	0.094456694
Prdm14		0.638943853	0.864634609	0.807418723	0.02753498	0.23826022	0.146882661
Ronin (Thap11)		0.278983934	0.078984524	0.038008024	0.03869025	0.007786696	0.018596079
Sox2		1.093781702	0.955056646	1.061977271	0.512314456	0.378585022	0.005252843
Suz12		0.963281691	0.98205209	0.931389202	0.039953795	0.676976558	0.646435384
Suz12		0.992296766	0.912378362	1.100880343	0.786450973	0.301946493	0.040962463
Suz12		1.158574552	0.961899174	1.04696963	0.459111537	0.588345151	0.560282415
Suz12		1.053080235	0.910743119	1.092731679	0.4252749	0.268583356	0.008184578
Suz12		1.058570537	1.057847885	0.91656667	0.453725675	0.568691899	0.343628619
Suz12		1.049549076	0.984696991	1.061926645	0.232627492	0.685688772	0.15360279
Tcf3		0.993883205	1.254027596	1.258591744	0.017397117	0.028324406	0.028931107
Tcf3		1.093991341	1.335515444	1.305227587	0.065877315	0.048410917	0.049307625
Tex10		0.943242619	1.120772294	0.803581994	0.123635404	0.231571006	0.01041453
Tex10		0.957999815	1.012711205	0.902061578	0.139599359	0.072623682	0.153876677
Trim28		0.987699699	1.144123476	1.046759057	0.035161956	0.143746358	0.076409587
Trim28		0.978046009	1.102087572	1.070640727	0.051764831	0.001020533	0.115248534
Zfx		0.877290856	1.045558119	1.01914338	0.967510721	0.636882058	0.850621919
Zfx		0.909518563	0.930680373	1.018854579	0.804988698	0.245024845	0.435267028
Zfx		0.756514483	1.388840349	0.852014639	0.517963731	0.151220481	0.237053689
Zfx		0.962511347	0.845024695	0.97690258	0.587154489	0.020076073	0.917314147

Supplementary Table 2: Expression changes of selected genes related to pluripotency of ES cells after *Ronin* knockout as determined by microarray. Note that some genes are represented by multiple probes. The significance was determined by two-tailed, paired T-test.