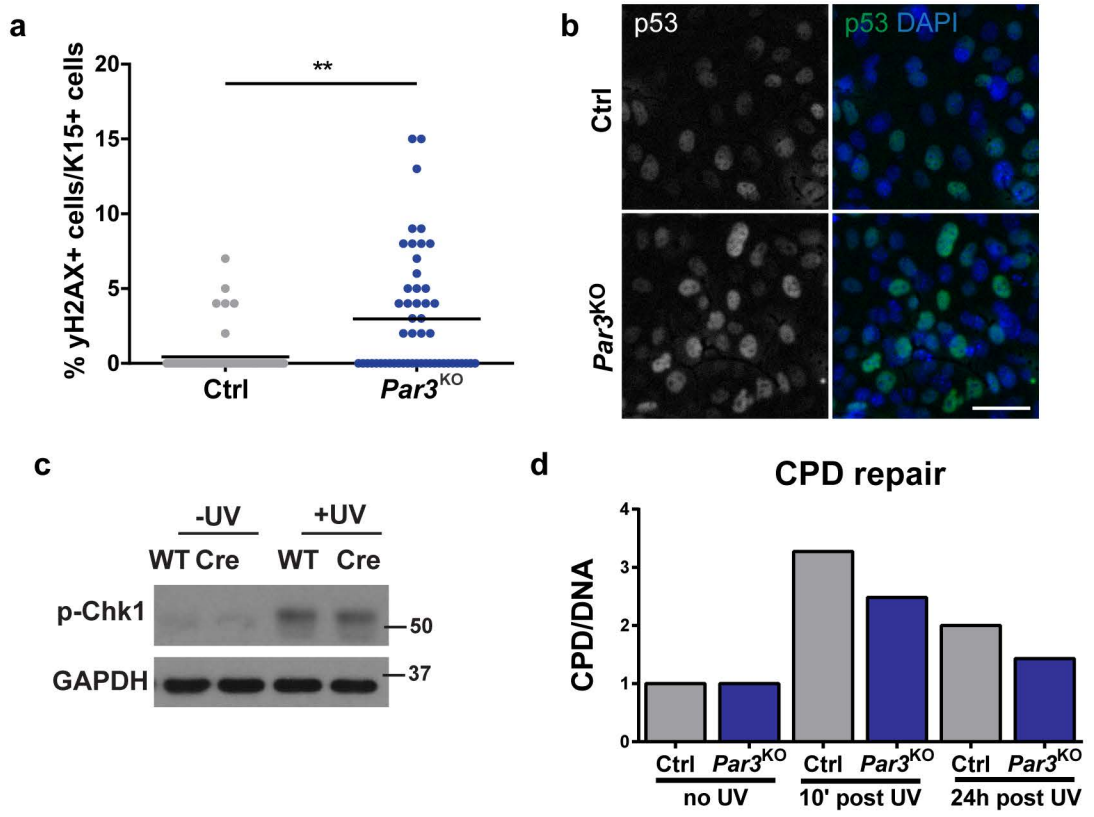
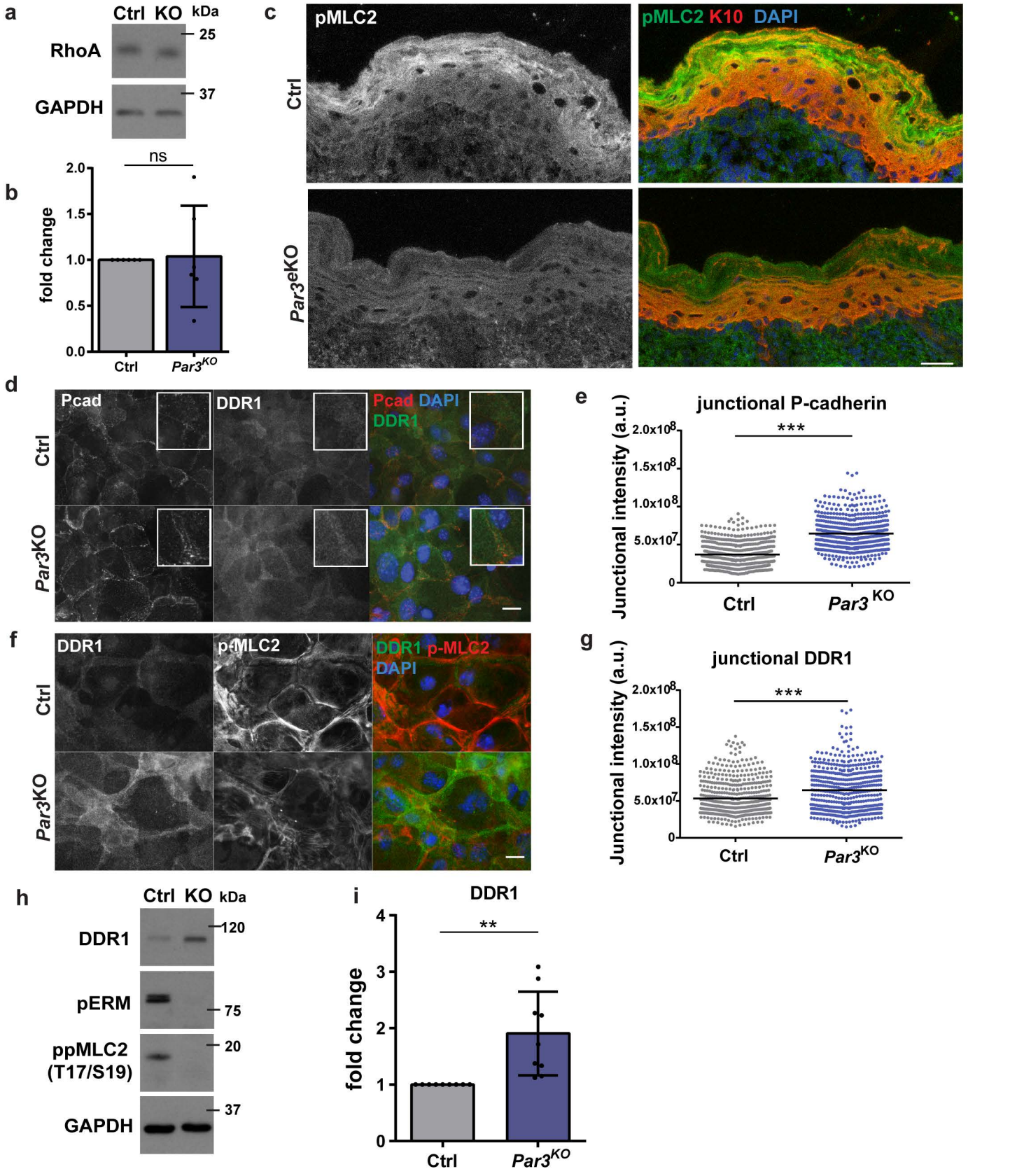
Supplementary Information Dias Gomes, Letzian et al.

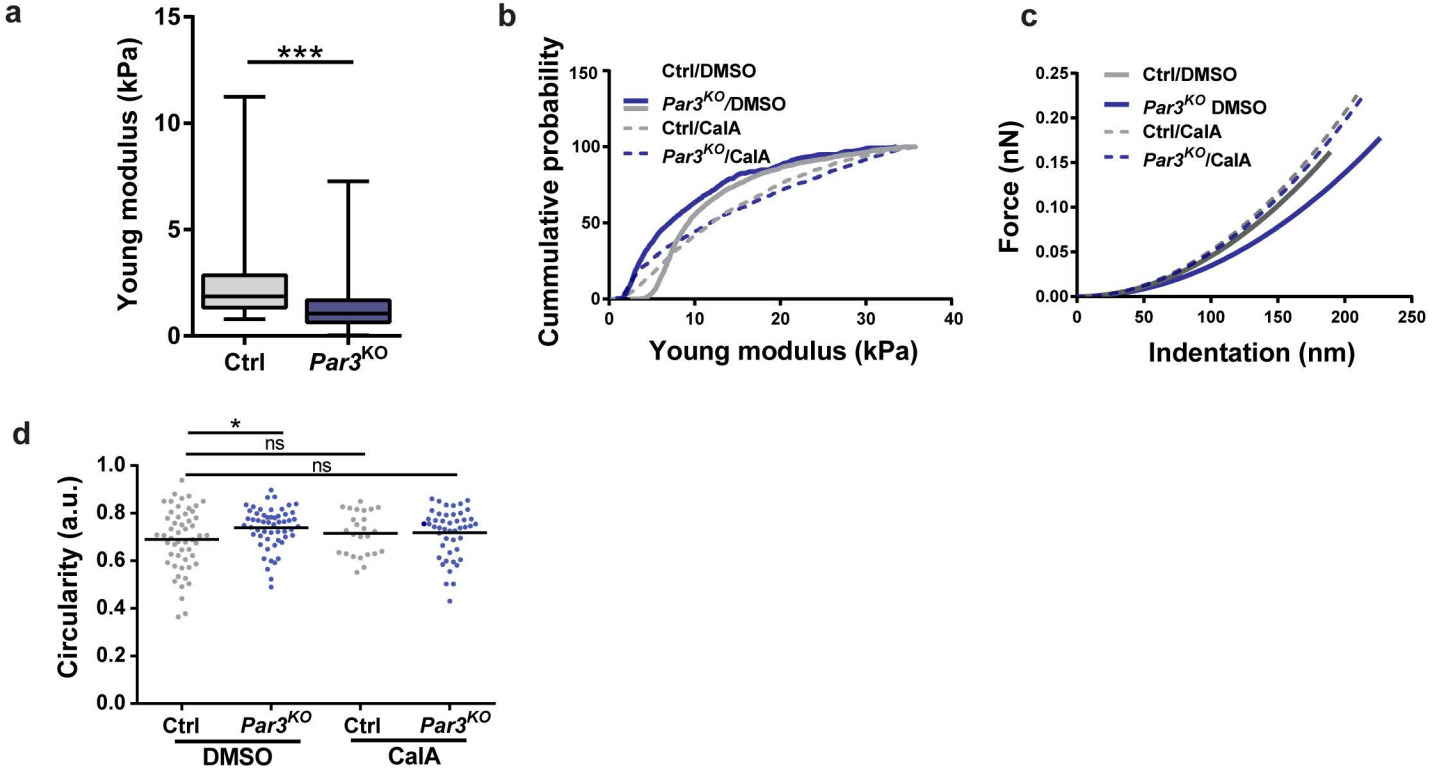
Inventory:



Supplementary Figure 1, related to Figure 1.
Loss of Par3 leads to increased DNA damage in hair follicle stem cells and ectopic activation of DNA damage responses.
(a) Percentage of γH2AX-positive cells per Keratin15 positive bulge cells from 3 Par3A knockout (Par3eKO: K14Cre/+;Par3fl/fl) and control mice (K14Cre/+) (n=3; **, p=0.0014, mean number of γH2AX-positive cells per K15+ bulge cells, students t-test).
(b) p53 immunofluorescence micrographs of primary Par3KO and control keratinocytes. DAPI was used to counterstain nuclei. Scale bar: 50μm. Micrographs were subjected to rolling ball background subtraction, radius=50μm.
(c) Immunoblot analysis of pChk1 in control (K14Cre/+) and wildtype keratinocytes either non-treated or UV-B treated (100mJ/cm2).
(d) DNA repair assay. Cyclobutane pyrimidine dimer (CPD) signal intensity from slot blot experiment normalized to total DNA. DNA was isolated from Par3KO and control keratinocytes lysed at indicated time-points after UV-B irradiation (3mJ/cm2).



Supplementary Figure 2, related to Figure 3.
Par3 inactivation reduces MLC phosphorylation in the epidermis and results in increase of P-cadherin and DDR1.
(a) Immunoblot analysis of RhoA in control and Par3KO keratinocytes. GAPDH was used as loading control.
(b) Quantification of (a). RhoA levels were first normalized to GAPDH before all values were normalized to controls; (n=6; ns: p=0.6039; mean+SD, paired two-tailed-test).
(c) Micrographs of P0 murine epidermis immunostained for pMLC2 (Ser19) and Keratin10 (K10), Scale bar: 50µm.
(d) Immunofluorescence micrographs of P-cadherin and DDR1 in primary murine keratinocytes. Scale bar: 25µm.
(e) Quantification of P-cadherin immunoreactivity at keratinocyte cell-cell junctions (intensity in arbitrary units, n=450 cells pooled from three independent experiments; ***, p=0.0002, two-tailed Mann–Whitney U test).
(f) Immunofluorescence micrographs of primary keratinocytes stained for DDR1 and pMLC2 (Ser19). Scale bar: 25µm.
(g) Quantification of DDR1 immunoreactivity at keratinocyte cell-cell junctions (intensity in arbitrary units, n=450 cells pooled from three independent experiments; ***, p=0.0002, two-tailed Mann–Whitney U test).
(h) Immunoblot analysis of DDR1 in control and Par3KO keratinocytes. GAPDH served as loading control.
(i) Quantification of (h). DDR1 protein levels were first normalized to GAPDH before all values were normalized to controls (n=9, paired student t-test, **, p=0.0063, mean+SD, two-tailed t-test). Abbreviations used: Ctrl, control; ns, non-significant.



Supplementary Figure 3, related to Figure 4.
Altered viscoelastic properties of Par3-deficient primary keratinocytes.

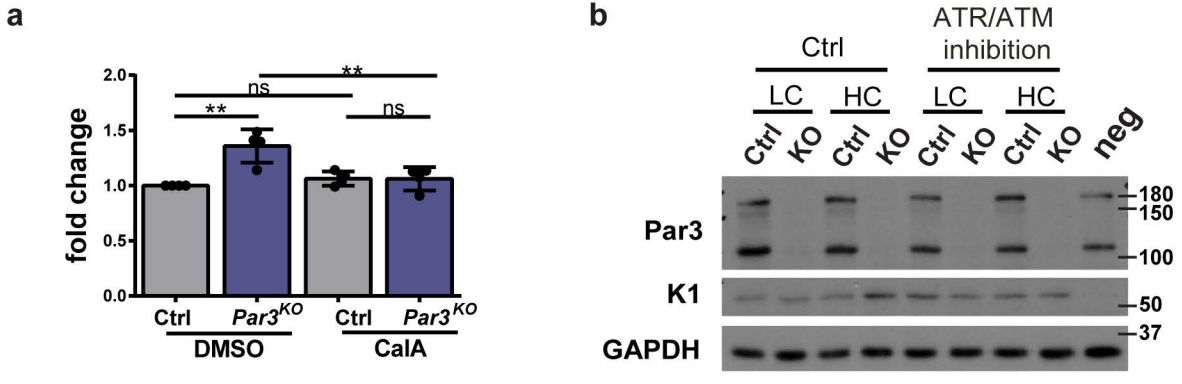
(a) Young Modulus box-plot based on force indentation spectroscopy with a spherical tip (n>500 measurements, pooled from three independent experiments ***, p=0.0001, Mann-Whitney U test, box plots show minimum, 25% percentile, median, 75% percentile and maximum).

(b) Cumulative probability of Young Moduli extracted from Hertzian fits from force spectroscopy indentations (n=2000, pooled across three independent experiments).

(c) Indentation force plots showing mean Hertzian fits (n=8 representative force indents per condition).

(d) Mitotic spindle circularity measurements in Ctrl and Par3KO keratinocytes upon DMSO or CalA treatment (n>25, pooled from four independent experiments, *, p=0.0432 Ctrl/DMSO vs. Par3KO/DMSO, ns, p=0.6274 Ctrl/DMSO vs. Ctrl/CalA, ns, p=0.4082 Ctrl/DMSO vs. Par3KO/CalA, two-tailed one-way ANOVA).

Abbreviations used: Ctrl, control; ns, non-significant; CalA, calyculin A.



Supplementary Figure 4, related to Figure 5.
Increased differentiation in Par3-deficient keratinocytes can be prevented by restoring actomyosin contractility or inhibiting DNA damage responses.

(a) Differentiation assay. Differentiation was induced by switch to 1.8mM extracellular calcium ions in the culture medium (HC). Quantification of the epidermal differentiation marker Involucrin. Protein levels were first normalized to GAPDH (loading control) before all values were normalized to controls. (n=5; ns: p=0.4254, **, p=0.0022; mean+SD, two-way ANOVA Dunn's comparison test).

(b) Immunoblot analysis of differentiation marker Keratin1. Differentiation was induced by switch to 1.8mM extracellular calcium ions in the culture medium (HC). Inhibiting DNA damage responses using ATR/ATM inhibitors suppresses ectopic differentiation upon Par3 loss. GAPDH was used as loading control.

Abbreviations used: Ctrl, control; ns, non-significant; CalA, calyculin A; neg, negative control; ATM, Ataxia telangiectasia mutated; ATR, ATM and RAD3-related.

Supplementary Table 1 Dias Gomes, Letzian et al.

Primary antibodies (supplier)	Catalog nr.	Clone/Ref.	Lot nr.	Species	Dilution
GAPDH (Millipore)	MAB374	6C5	2322571	mouse	WB 1:18.000
Keratin-1 (Covance)	PRB-165P	Polyclonal	N/A	rabbit	WB 1:1000
p53 (Leica)	NCL-L-p53-CM5p	Polyclonal	6044554	rabbit	IF 1:200
p53 (Cell Signaling)	2524	1C12	10	mouse	1:500
Par3 (Millipore)	07-330	Polyclonal	2615671	rabbit	IF 1: 400
pMLC2 (Ser19) (Cell Signaling)	3675	Polyclonal/ 09/2016	5	mouse	IF 1:300
ppMLC2 (Thr17Ser19) (Cell Signaling)	3674	Polyclonal/ 10/2017	5	rabbit	WB 1:1000 IF 1:300
MLC2	3672	Polyclonal/ 09/2017	4	rabbit	WB 1:1000
pEzrin (Thr567)/Radixin (Thr567)/Moesin (Thr558) (Cell Signaling)	3726	48G2/ 01/2018	5	rabbit	IF 1:300
pEzrin (Thr567)/Radixin (Thr567)/Moesin (Thr558) (Cell Signaling)	3141	T558/ 04/2018	15	rabbit	WB 1:1000
pATR (Ser428) (Cell Signaling)	2853	Polyclonal 02/2014	5	rabbit	WB 1:1000
pChk1 (Ser345) (Cell Signaling)	2348	133D3/ 09/2015	15	rabbit	WB 1:1000
Chk1 (Cell Signaling)	2360	2G1D5/ 10/2015	3	mouse	WB 1:1000
γH2AX (S139) (Cell Signaling)	9718	20E3/ 07/2017	13	rabbit	IF 1:500
CPD (Cosmo Bio)	NM-DND-001	TDM-2	TM-C-08	mouse	SB: 1:10000
RhoA (Cell Signaling)	2117T	67B9	5	rabbit	WB1:1000
Involucrin (Covance)	PRB 140C-200	Polyclonal	N/A	rabbit	WB 1:1000
DDR1 (Cell Signaling)	5583	D1G6/ 09/2017	3	rabbit	WB 1:1000 IF1:800
Alpha-Tubulin (Sigma)	T6793	B-5-1-2	034M4837	mouse	IF 1:1000
Pericentrin (BioLegend)	923701	Poly 19237	B200667	rabbit	IF 1:300
Secondary antibodies for immunofluorescence analyses					
AlexaFluor 488 α-mouse (Invitrogen)	A21202	Polyclonal	1741782	donkey	IF 1:500
AlexaFluor 568 α-rabbit (Invitrogen)	A11036	Polyclonal	1832035	goat	IF 1:500
AlexaFluor 568 α-rat (Invitrogen)	A11077	Polyclonal	870966	goat	IF 1:500
AlexaFluor 594 anti-mouse (Invitrogen)	A21203	Polyclonal	1165596	donkey	IF 1:500
AlexaFluor 488 α-rabbit (Invitrogen)	A21206	Polyclonal	1910751	donkey	IF 1:500
AlexaFluor 594 α-rabbit (Invitrogen)	A21207	Polyclonal	N/A	donkey	IF 1:500
AlexaFluor 488 α-guinea pig (Invitrogen)	A11073	Polyclonal	1458631	goat	IF 1:500
Secondary antibodies for immunoblot analyses					
HRP α-rabbit (GE Healthcare)	NA9340V	Polyclonal	9720820	donkey	WB 1:4000
HRP α-mouse (GE Healthcare)	NA931V	Polyclonal	11076057	sheep	WB 1:4000
Other Reagents					
DAPI (Roth)	D1306	N/A	N/A	N/A	1mg/ml (1:500)
iFISH probes: Tlk2 (11qE1) / Aurka (2qH3) Mouse probe (Leica)	KBI-30501	N/A	59325	DNA probe	1:1