Figure S1

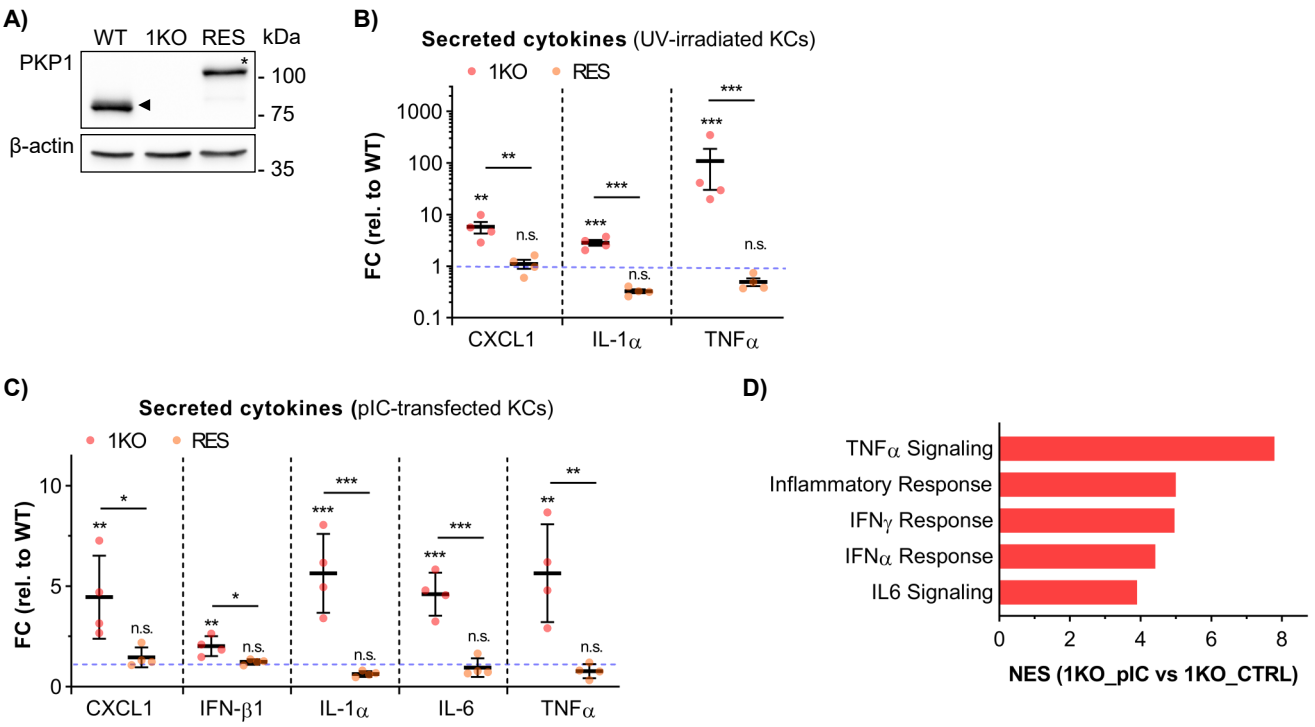

A)
WT
1KO
RES
kDa
PKP1
β-actin
100
75
35
B)
Secreted cytokines (UV-irradiated KCs)
1KO
RES
FC (rel. to WT)
CXCL1
IL-1α
TNFα
n.s.
C)
Secreted cytokines (pIC-transfected KCs)
1KO
RES
FC (rel. to WT)
CXCL1
IFN-β1
IL-1α
IL-6
TNFα
n.s.
D)
TNFα Signaling
Inflammatory Response
IFNγ Response
IFNα Response
IL6 Signaling
NES (1KO_pIC vs 1KO_CTRL)

Figure S2

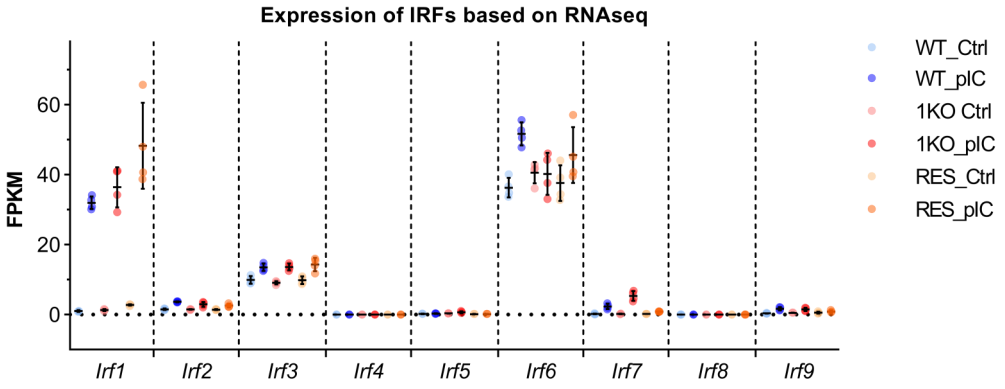
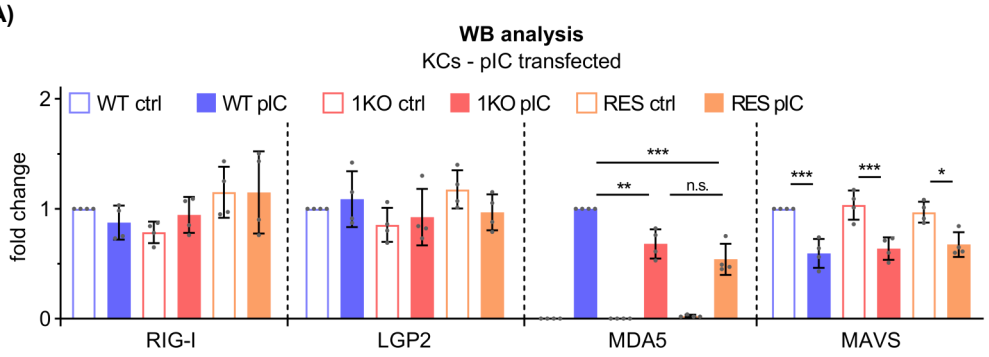

Expression of IRFs based on RNAseq
FPKM
0
20
40
60
Irf1
Irf2
Irf3
Irf4
Irf5
Irf6
Irf7
Irf8
Irf9
WT_Ctrl
WT_pIC
1KO Ctrl
1KO_pIC
RES_Ctrl
RES_pIC

Figure S3

A)


WB analysis
KCs - pIC transfected
WT ctrl
WT pIC
1KO ctrl
1KO pIC
RES ctrl
RES pIC
fold change
RIG-I
LGP2
MDA5
MAVS

B)

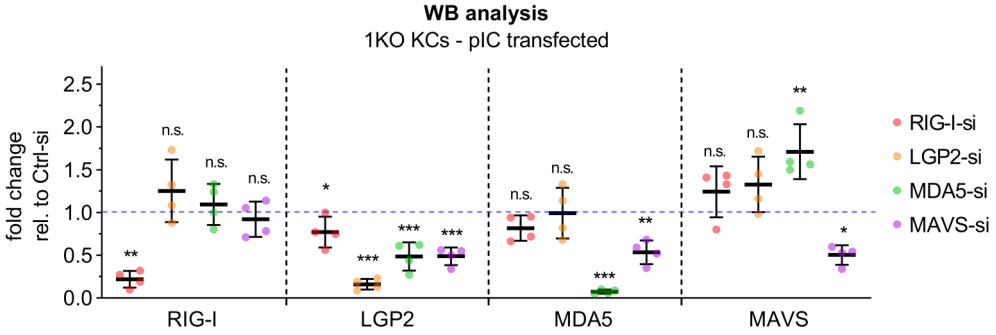

WB analysis
1KO KCs - pIC transfected
fold change
rel. to Ctrl-si
RIG-I
LGP2
MDA5
MAVS
RIG-I-si
LGP2-si
MDA5-si
MAVS-si

Figure S4

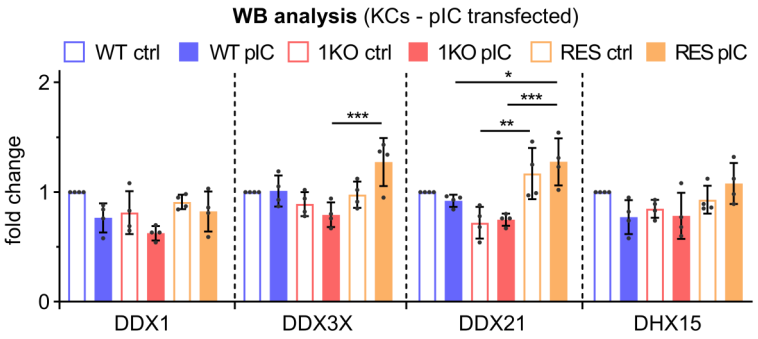

WB analysis (KCs - pIC transfected)
WT ctrl
WT pIC
1KO ctrl
1KO pIC
RES ctrl
RES pIC
fold change
2
1
0
***
*
***
**
DDX1
DDX3X
DDX21
DHX15

Figure S5

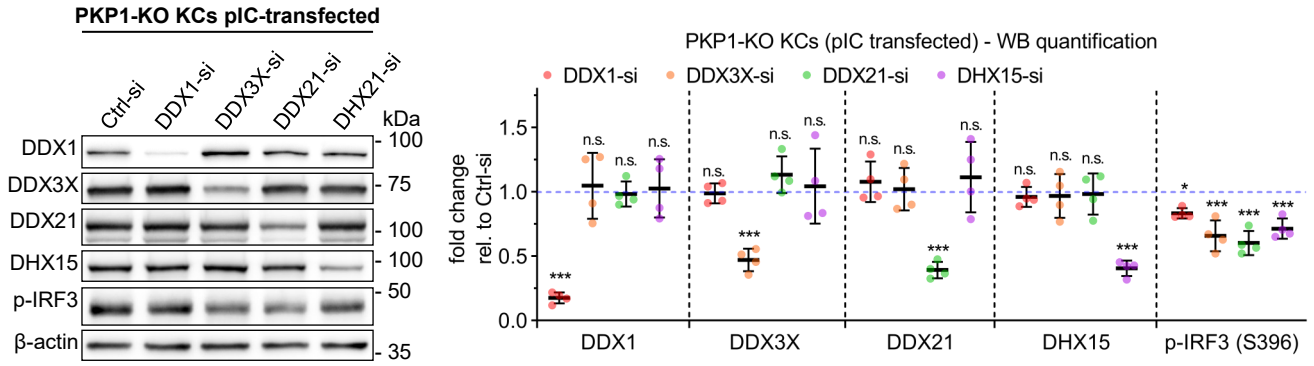
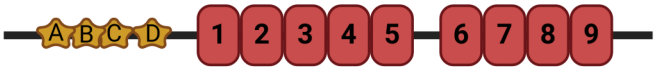

PKP1-KO KCs pIC-transfected
Ctrl-si
DDX1-si
DDX3X-si
DDX21-si
DHX21-si
kDa
DDX1
DDX3X
DDX21
DHX15
p-IRF3
β-actin
100
75
100
100
50
35
PKP1-KO KCs (pIC transfected) - WB quantification
DDX1-si
DDX3X-si
DDX21-si
DHX15-si
fold change rel. to Ctrl-si
1.5
1.0
0.5
0.0
n.s.
***
*
DDX1
DDX3X
DDX21
DHX15
p-IRF3 (S396)

Figure S6

A)

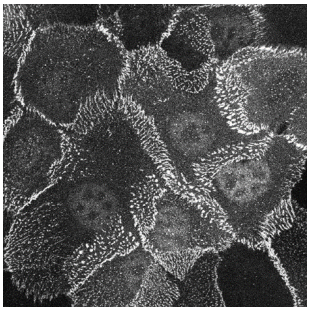
PKP1 - phosphorylation sites



Motive A: RQKS^{54}K$S^{56}S^{57}$Q$S^{59}S^{60}T^{61}$LS^{63}HS^{65}NRGS^{69}
Motive B: DNYNYGT^{82}TS^{84}RSSYYS
Motive C: DNRRF$S^{118}S^{119}$YS^{121}QMENWS^{127}RHYPR
Motive D: RGT^{166}LRKGT^{171}LGS^{174}KGQK$T^{179}T^{180}$QNRYS^{185}FY$S^{188}T^{189}$CS^{191}G

B)

PKP1-WT-EGFP



PKP1-4xA-EGFP

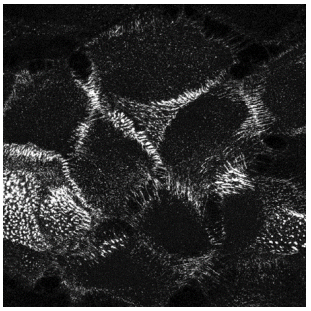


Figure S1: Loss of PKP1 increases secretion of pro-inflammatory cytokines. **(A)** Western blot analysis of WT, PKP1-KO (1KO) and PKP1-rescue (RES) KCs to depict PKP1 expression. Endogenous PKP1 (~ 82 kDa) is indicated by an arrowhead. PKP1-WT-EGFP (~ 136 kDa) expressed in PKP1-KO KCs (RES) is marked with an asterisk. **(B)** WT, PKP1-KO (1KO) and PKP1-rescue (RES) KCs where exposed to UV. After 24h the medium was collected to quantify the amounts of secreted CXCL1, IL-1α, and TNFα using ELISA. Depicted are the mean fold changes (± SD) of secreted cytokines upon UV-treatment relative to WT cells (n = 4). **(C)** WT, PKP1-KO (1KO) and PKP1-rescue (RES) KCs where transfected with pIC. After 24h the medium was collected to quantify the amounts of secreted CXCL1, IFN-β1, IL-1α, IL-6, and TNFα using ELISA. Depicted are the mean fold changes (± SD) of secreted cytokines upon pIC stimulation relative to WT cells (n = 4). **(D)** Normalized enrichment scores (NES) for the top 5 "mouse-ortholog hallmark" gene sets as determined by gene set enrichment analysis (GSEA). GSEA was performed using a gene list ranked by fold change expression between pIC-transfected PKP1-KO (1KO) and untreated 1KO KCs.

Figure S2: Expression of IRF family members in mouse KCs. mRNA level (FPKM, fragments per kilobase per million mapped reads) of IRFs in untreated (Ctrl) and pIC-transfected WT, PKP1-KO (1KO) and PKP1-rescue (RES) KCs based on RNA-seq data.

Figure S3: WB analysis of RLR signaling pathway. **(A)** Quantification of protein amounts derived from Western blot analysis of pIC transfected WT, 1KO and RES KCs shown in Fig. 5B. Depicted are the fold changes (± SD) of β-actin-normalized protein amounts in untreated (ctrl) and pIC transfected KCs relative to untreated (RIG-I, LGP2, MAVS) or pIC- (MDA5) transfected WT KCs (n = 4). **(B)** Quantification of protein amounts derived from Western blot analysis of 1KO KCs transfected with the indicated siPools prior to pIC stimulation. Depicted are the fold changes (± SD) of the respective protein amounts normalized to β-actin and relative to control siPool transfected 1KO KCs (blue dotted line; n = 4, related to Fig. 5C). Statistical significances were

determined by one-way ANOVA with Tukey‘s multiple comparison. n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure S4. Quantification of DExD/H-box helicase expression in pIC transfected WT, 1KO and RES KCs. Quantification of protein amounts from immunoblots of DExD/H-box helicases. β-actin was used as loading control. Depicted are the fold changes (± SD) of the respective protein amounts in untreated and pIC transfected KCs normalized to β-actin and relative to untreated WT KCs (n = 4, related to Fig. 6D). Statistical significances were determined by one-way ANOVA with Tukey‘s multiple comparison. n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure S5. Efficiency of DExD/H-box helicase knock down. Western blot analysis of 1KO KCs transfected with the indicated siPools prior to pIC stimulation. Left: Representative immunoblots of DExD/H-box helicases. β-actin was used as loading control. Right: Quantification of protein amounts. Depicted are the fold changes (± SD) of the respective protein amounts normalized to β-actin and relative to control siPool transfected 1KO KCs (blue dotted line; n = 4). Statistical significances were determined by one-way ANOVA with Tukey‘s multiple comparison. n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure S6. A PKP1 mutant covering several phosphorylation sites localizes to desmosomes. **(A)** Scheme showing the four motives in the head-domain of PKP1 that regulate its localization through phosphorylation. **(B)** Fluorescence images of PKP1-KO KCs either expressing PKP1-WT-EGFP or PKP1-4xA-EGFP incubated for 24h in HCM. Exchange of all serine and threonine residues depicted in (A) to alanine locks PKP1 at cell-cell contacts.

Table S1: List of used siPools (defined pools of 30 selected siRNAs) obtained from siTools Biotech GmbH

Target	NCBI Gene ID
non-targeting control	N/A
murine *Ddx1*	104721
murine *Ddx3x*	13205
murine *Dhx15*	13204
murine *Ddx21*	56200
murine *Rela* (p65)	19697
murine *Irf3*	54131
murine *Mavs*	228607
murine *Ifih1* (MDA5)	71586
murine *Dhx58* (LGP2)	80861
murine *Ddx21*	56200
murine *Rigi* (RIG-I, DDX58)	230073

Table S2: List of used ELISA kits

ELISA-Kit	Source	Identifier
ELISA MAX™ Standard Set Mouse IL-1α	BioLegend	Cat# 433401
ELISA MAX™ Standard Set Mouse IL-6	BioLegend	Cat# 431301
ELISA MAX™ Standard Set Mouse TNF-α	BioLegend	Cat# 430901
Mouse CXCL1/KC DuoSet ELISA	R&D Systems	Cat# DY453-05
Mouse IFN-beta DuoSet ELISA	R&D Systems	Cat# DY8234-05

Table S3: Primary antibodies including dilutions in Western Blot (WB), immunofluorescence (IF) or proximity ligation assay (PLA)

Antibody	Source	Identifier	Dilution
c-Myc (D84C12) Rabbit mAb	Cell Signaling Technology	RRID: AB_1903938	1:1000 (WB)
DDX1 (A-7) Mouse mAb	Santa Cruz Biotechnology	RRID: AB_10650122	1:500 (WB) 1:250 (PLA)
DDX21 Rabbit pAb	Cell Signaling Technology	RRID: n.a. Cat# 75762	1:1000 (WB) 1:500 (PLA)
DDX3 (D19B4) Rabbit mAb	Cell Signaling Technology	RRID: AB_10860416	1:1000 (WB) 1:500 (PLA)
DHX15 (E-6) Mouse mAb	Santa Cruz Biotechnology	RRID: AB_10707990	1:1000 (WB) 1:500 (PLA)
GFP Rabbit pAb	Rockland	RRID: AB_828167	1:2000 (WB)
IRF-3 (D83B9) Rabbit mAb	Cell Signaling Technology	RRID: AB_1904036	1:500 (WB)
LGP2 Rabbit pAb	Thermo Fisher Scientific	RRID: AB_2855046	1:1000 (WB)
MAVS (E8Z7M) Rabbit mAb #83000	Cell Signaling Technology	RRID: AB_2927715	1:2000 (WB)
MDA-5 (D74E4) Rabbit mAb #5321	Cell Signaling Technology	RRID: AB_10694490	1:1000 (WB)
NF-κB p65 (D14E12) XP® Rabbit mAb #8242	Cell Signaling Technology	RRID: AB_10859369	1:2000 (WB) 1:1000 (IF)
Phospho-IRF-3 (Ser396) (D6O1M) Rabbit mAb #29047	Cell Signaling Technology	RRID:AB_2773013	1:500 (WB)
Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb #3033	Cell Signaling Technology	RRID: AB_331284	1:1000 (WB)
PKP1 (10B2) Mouse mAb #sc-33636	Santa Cruz Biotechnology	RRID: AB_2164139	1:1000 (WB)
PKP1 Rabbit (serum)	Hatzfeld Lab	(1,2)	1:1000 (PLA)
RIG-I (D14G6) Rabbit mAb #3743	Cell Signaling Technology	RRID: AB_2269233	1:1000 (WB) 1:500 (PLA)
β-actin Mouse mAb #A2228	Sigma-Aldrich	RRID: AB_476697	1:2000 (WB)

Table S4: List of secondary antibodies including dilutions in immunoblot (IB) and immunofluorescence (IF)

Antibody	Source	Identifier	Dilution
Peroxidase-AffiniPure Donkey Anti-Mouse IgG	Jackson ImmunoResearch Labs	RRID: AB_2340770	1:20,000 (WB)
Peroxidase-AffiniPure Donkey Anti-Rabbit IgG	Jackson ImmunoResearch Labs	RRID: AB_10015282	1:40,000 (WB)
Alexa Fluor® 488 AffiniPure F(ab')$_2$ Fragment Donkey Anti-Rabbit IgG	Jackson ImmunoResearch Labs	RRID: AB_2340619	1:500 (IF)

Table S5: Sequences of primers (FW = forward primer, REV = reverse primer) used in qRT-PCR

Primer	Sequence 5' --> 3'
Eef2-mouse FW	ATGAGGCCGCCATGGGTATTA
Eef2-mouse REV	TAGTTGGGGCCCATGATCCG
Cxcl1-mouse FW	AAGAATGGTCGCGAGGCTTG
Cxcl1-mouse REV	GTGTTGTCAGAAGCCAGCGT
Il1a-mouse FW	GTCAACTCATTGGCGCTTGA
Il1a-mouse REV	TGCAAGTCTCATGAAGTGAGC
Il6-mouse FW	AACGATGATGCACTTGCAGAAA
Il6-mouse REV	TGGTACTCCAGAAGACCAGAG
Tnf-mouse FW	GCCTATGTCTCAGCCTCTTCTC
Tnf-mouse REV	AGGGTCTGGGCCATAGAACTG
Ifnb1-mouse FW	CGTGGGAGATGTCCTCAACT
Ifnb1-mouse REV	CTGAAGATCTCTGCTCGGACC

1. Hatzfeld, M., Haffner, C., Schulze, K. and Vinzens, U. (2000) The function of plakophilin 1 in desmosome assembly and actin filament organization. *J Cell Biol*, **149**, 209-222.
2. Wolf, A., Krause-Gruszczynska, M., Birkenmeier, O., Ostareck-Lederer, A., Huttelmaier, S. and Hatzfeld, M. (2010) Plakophilin 1 stimulates translation by promoting eIF4A1 activity. *J Cell Biol*, **188**, 463-471.