Expanded View Figures

Figure EV1. Aurora kinase inhibition regulates transcriptional pathways that promote inflammation and immune recognition.

Primary MEF were treated with DMSO or Aurora kinase inhibitor (ZM, 2 μM) for 72 h, followed by RNA isolation and 3′ mRNA-sequencing.

A TRRUST analysis of significant DEGs in ZM vs. DMSO treated cells displaying transcription factors predicted to participate in their regulation. Adjusted $P \leq 0.05$ and cut-off values $\log_2 FC \geq 1$ or $\log_2 FC \leq -1$.

B Gene set enrichment analysis (GSEA) analysis of transcripts differentially regulated between ZM and DMSO treatment.

C Curated heatmap for significant DEGs involved in the GO-term "immune system process" (GO: 0002376) that are enriched in ZM vs. DMSO treated MEF. Adjusted $P \leq 0.05$ and cut-off values $\log_2 FC \geq 1$ or $\log_2 FC \leq -1$.

D qRT-PCR analysis of selected pro-inflammatory cytokines and chemokines induced in ZM-treated MEF, plotted as fold-change expression relative to untreated cells. *Hprt1*, *β-actin* and *Gapdh* were used as house-keeping gene expression controls. $n = 3$ biological replicates; data represent mean values ± SEM. The statistical significance was determined using two-tailed *t*-test; $P \leq 0.1$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), $P \leq 0.0001$ (****).

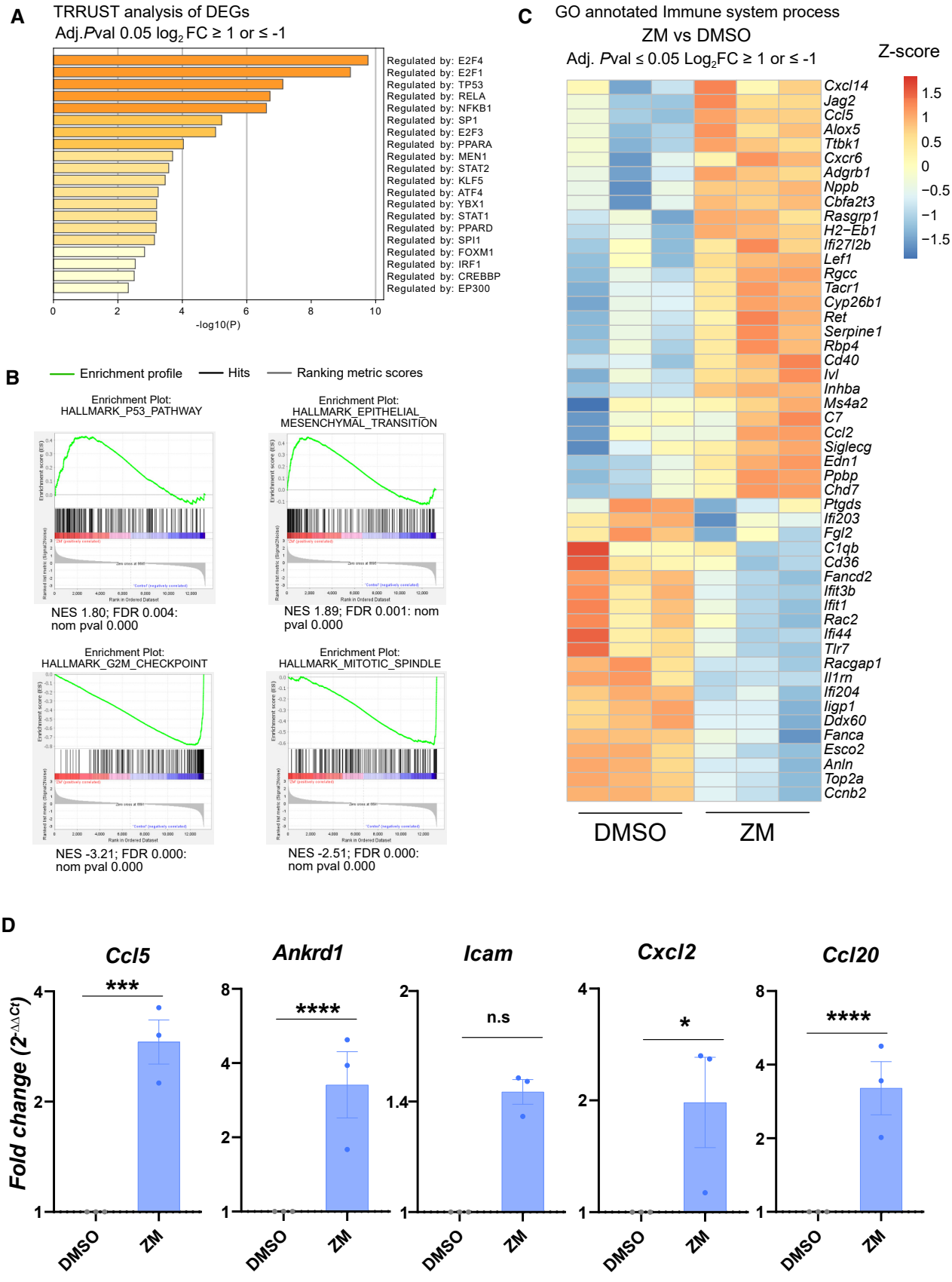

Figure EV1.

Figure EV2. Cytokinesis failure induces p65 nuclear translocation and NF-κB activation via PIDD1.

A Flow cytometric DNA content analysis of single-thymidine synchronized A549 cells, released in DMSO or ZM.

B Quantification of the percentage of polyploid cells of samples shown in (A). Red dashed line indicates the beginning of mitotic exit in DMSO control cells.

C Nuclear translocation of p65/RELA was quantified over time and is represented as percentage of cells displaying nuclear p65 signal ($n \geq 50$ cells per condition). Bars represent means ± SEM of two technical replicates.

D Representative immunofluorescence images of synchronized A549 cells released in ZM and analyzed at different time points by IF. Cells were incubated with TNF (50 ng/ml) for 10 min, as a positive control. Scale bars: 10 μm.

E Flow cytometric analysis of DNA content in RPE1 cells of the indicated genotypes after induction of cytokinesis failure with ZM (2 μM) or DHCB (4 μM) compared to DMSO. Two independent experiments were performed.

F, G (F) Western blot analysis confirming lack of PIDD1 expression and (G) impaired MDM2 processing in RPE1 cells lacking *PIDD1* after induction of cytokinesis failure using ZM (2 μM) for 48 h.

H–J (H) Luciferase reporter assay of NF-κB activity in U2OS single cell clone lacking PIDD1 or (I) A549 single cell clones generated with two different guide RNAs targeting *PIDD1* or (J) lacking caspase-2. $n \geq 3$ independent biological experiments, mean ± SEM.

K, L (K) Live-cell imaging analysis of NF-κB EGFP-reporter activity in CRIPSR-edited polyclonal PIDD1-competent or –deficient A549$^{\kappa B}$ cells or (L) RPE1 cells treated with TNF (20 ng/ml). $n \geq 3$ independent experiments; bars represent mean values ± SEM.

Data information: The statistical significance was determined using two-way ANOVA; $P \leq 0.0\,1$ (**), $P \leq 0.001$ (***), $P \leq 0.0001$ (****).

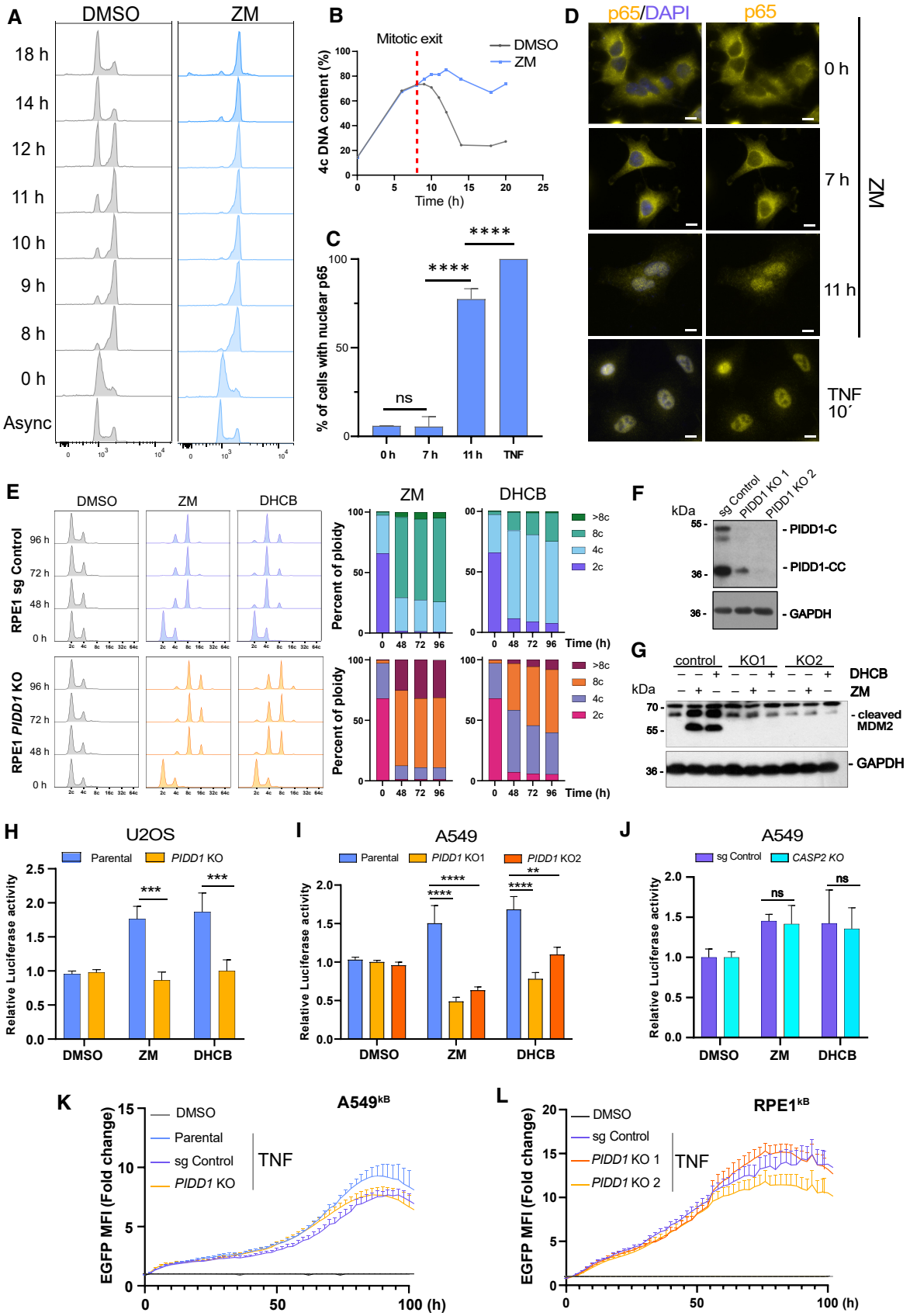

Figure EV2.

Figure EV3. Assessment of impact of DNA-damage and cGAS/STING signaling on NF-kB activation induced by extra centrosomes.

A Enumeration of centriole numbers in A549 cells after centrinone pretreatment (24 h) followed by induction of cytokinesis failure using ZM (2 μM) for additional 48 h by immunofluorescence imaging and γ-Tubulin staining. A minimum of 50 cells per condition were analyzed in two independent experiments. Data are presented as mean values.

B, C (B) Immunoblot analysis of PIDD1 pathway competence, monitored by MDM2 processing and assessed in the indicated (C) CRISPR-edited polyclonal U2OS^{PLK4} cells after induction of MYC-PLK4 expression using Dox (1 μg/ml, 48 h).

D A549^{PLK4} reporter cells lacking or expressing PIDD1 were treated for the indicated times with ZM or Dox and analyzed for MDM2 processing by western blot analysis.

E Centrosome number was quantified based on γ-Tubulin staining in the indicated genotypes of U2OS cells treated as indicated. A minimum of 50 cells per condition were analyzed in two independent experiments. Data are presented as mean values.

F DNA damage levels were assessed in U2OS^{PLK4} cells exposed to Dox treatment (1 μg/ml) for the indicated times. Nuclear γH2AX levels were determined by immunofluorescence and plotted as mean intensity per nucleus. Etoposide treatment (10 μg/ml, 24 h) was used as positive control. Top, representative immunofluorescence images. $n \geq 200$ cells per condition were analyzed. Data represented as mean values ± SEM. The statistical significance was determined using one-way ANOVA; $P \leq 0.0001$ (****). Scale bar: 10 μm.

G Representative immunofluorescence images of nuclear γH2AX foci in A549^{PLK4} cells forming upon the indicated treatments. Scale bar: 10 μm.

H Quantification of DNA damage levels in A549^{PLK4} cells determined by the number of γH2AX nuclear foci per cell upon Dox or ZM treatment. $n \geq 200$ cells per condition, $n = 3$ independent experiments; mean ± SEM. The statistical significance was determined using one-way ANOVA.

I Immunoblot analysis of STING endogenous protein levels in different cancer cell lines.

J STING levels were assessed by immunoblot in parental and CRISPR-edited polyclonal STING-deficient A549 derivatives.

K Life cell imaging analysis of NF-κB EGFP-reporter activity in polyclonal CRISPR-edited STING-competent or -deficient A549^{κB} cells after ZM treatment. $n = 5$ independent experiments. Data represent mean values ± SEM.

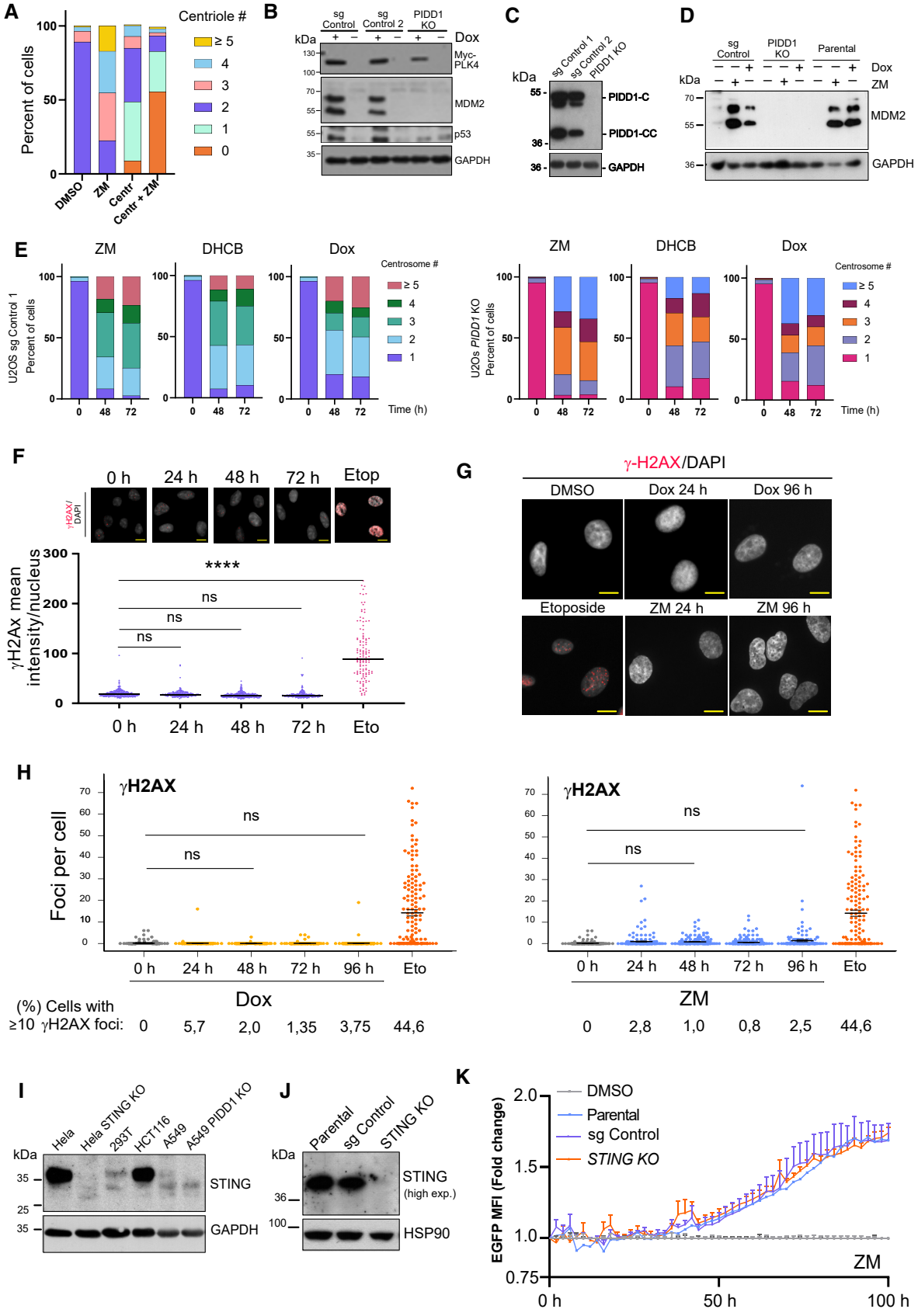

Figure EV3.

Figure EV4. Extra centrosomes induce sterile inflammation in a PIDD1-dependent manner.

A Volcano plot of DEGs in *Pidd1*$^{-/-}$ primary MEF induced by ZM vs. DMSO treatment for 72 h. Adjusted *P* value $\leq$ 0.01 and $\log_2$ FC $\geq$ 1 or $\log_2$ FC ≤ -1.

B GO-term analysis for significant DEGs for ZM vs. DMSO stimulated primary *Pidd1*$^{-/-}$ MEF. FDR $\leq$ 0.05, adjusted $P \leq$ 0.01 and cut-off values $\log_2$ FC $\geq$ 1 or $\log_2$ FC ≤ -1.

C, D (C) Representative histograms of DNA content analysis in primary MEF after induction of cytokinesis failure with ZM (2 μM) for the indicated times, quantified in (D). Two independent experiments were performed.

E–G (E) Primary MEF derived from TET-PLK4 mice were treated with Dox for 48 h and processed for immunofluorescence detection of extra centrosomes using γ-tubulin, quantified in (F), or RNA extraction for qRT-PCR analyses of NF-κB target genes (G). Scale bars: 10 μm. *β-actin* was used as housekeeping control. $n = 5$ biological replicates; mean ± SEM. The statistical significance was determined using two-way ANOVA; $P \leq 0.1$ (*), $P \leq 0.001$ (***), $P \leq 0.0001$ (****).

H Flow cytometric analysis of F4/80 and CD11b in BMDM on day 7 after isolation.

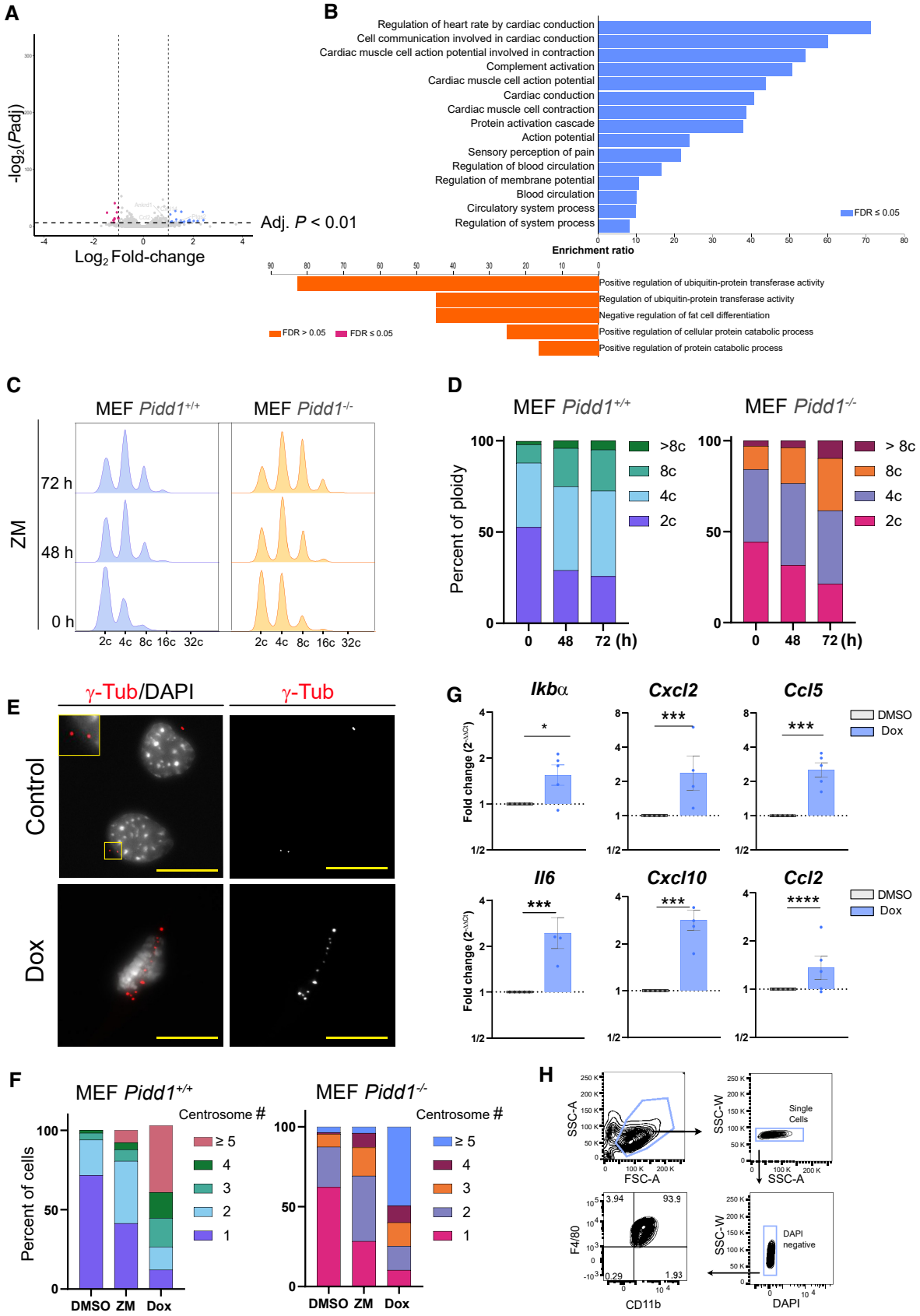

Figure EV4.

Figure EV5. PIDD1 is necessary to activate NK cells targeting polyploid cells.

A Experimental design of NK cell killing assays.

B Evaluation of centriole counts based on IF analysis and γ-tubulin staining. More than 50 cells were evaluated per condition in two independent experiments. Data are presented as means values.

C Evaluation of reporter activity in A549 cells of the indicated genotypes proficient or deficient in NF-κB activation, due to loss of RELA/B or overexpression of the IkBα super-repressor (SR) after treatment with ZM, the canonical NF-κB pathway agonist, TNF or the non-canonical NF-κB pathway agonist TWEAK. $n = 3$ independent experiments; mean ± SEM.

D Confluence curves from $A549^{EGFP}$ derivatives of the indicated genotypes, pretreated with DHCB (4 μM) to induce cytokinesis defects and co-cultured with NK-92 cells. $n \geq 3$ independent experiments; mean ± SEM.

E Conditioned medium from ZM pretreated A549 cells of the indicated genotypes was applied to human monocyte–derived macrophages for 24 h followed by RNA isolation and qRT-qPCR analysis of macrophage activation markers. Expression values are plotted as the fold-change relative to macrophages stimulated with conditioned medium from untreated A549 cells. *HPRT1 expression* was used for normalization. Bars represent mean ± SEM of $n \geq 4$ independent experiments. The statistical significance was determined using two-way ANOVA; $P \leq 0.1$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***).

F Proliferation curves of different $A549^{EGFP}$ derivatives, pretreated with ZM (2 μM) or DHCB (4 μM), for 72 h in the absence of NK cells. Time zero is defined as the moment of the drug wash out. Live-cell imaging of target cells without NK cells was performed in parallel to killing experiments using the same conditions as described in the methods section. $n = 5$ independent experiments; mean ± SEM.

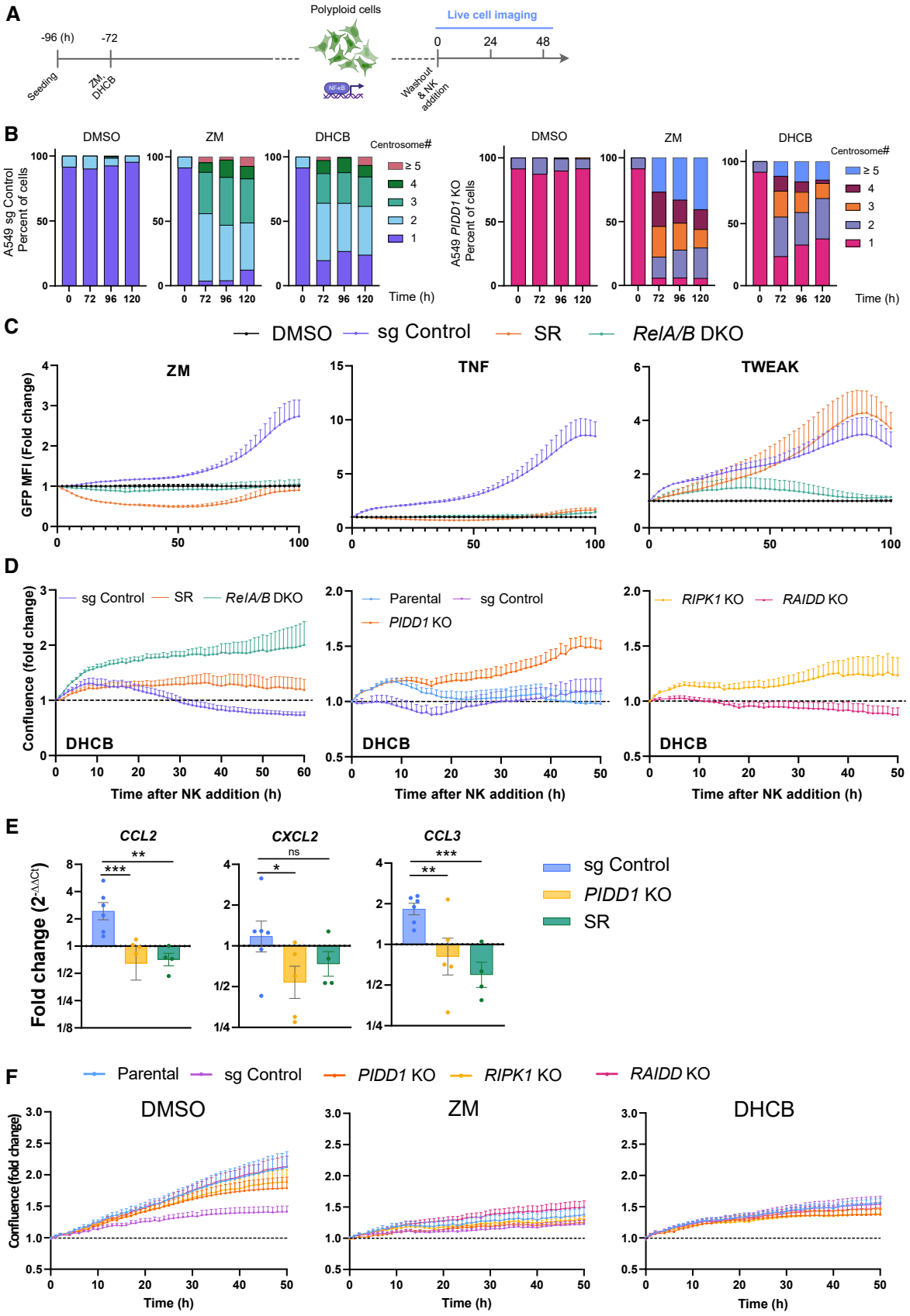

Figure EV5.