Supplemental Figure Legends

Supplemental Figure 1. The SUMOylation pathway is deregulated in PDAC.

A Gene expression analysis (z-transformed; GSE 62452) reveals a significant enrichment of core SUMO pathway components within PDAC patients vs. healthy control tissue.

B Unweighted GSEA demonstrates significant enrichment of established SUMOylation-associated gene sets in PDAC patients vs. healthy controls (GO PROTEIN SUMOYLATION; GO SUMO TRANSFERASE ACTIVITY).

C Unweighted GSEA of *SUMO1, SUMO2,* or *SUMO3* mRNA high (>75th percentile) and low (<25th percentile) expressing PDACs (TCGA-PAAD dataset). q-value is depicted.

D Unweighted GSEA of a publicly available dataset (GSE36924) indicates significant enrichment of MYC hallmark target genes within the SUMO^{high} group vs. SUMO^{low} group.

E SUMO-high group, depicted in blue, reveals a worse overall survival (p=0.011) and progression-free-survival (p=0.005).

Supplemental Figure 2. MYC is connected to the core Sumo pathway in murine PDAC.

A *Myc* mRNA expression of murine PDAC cell lines (PRJEB23787 dataset) (n=38) were correlated with the mRNA expression of the core Sumo pathway genes *Sumo1*, *Sumo2*, *Sumo3*, *Ube2i*, *Sae1*, and *Uba2/Sae2*. The color-coded Pearson correlation coefficient r is provided. *** p<0.001, ** p<0.01

B GSEA of a publicly available data set (GSE77328) reveals loss of SUMO transcriptional signature in KRAS/p53mut-driven PDAC under OmoMYC-mediated suppression of MYC compared to MYC active controls.

Supplemental Figure 3. Specificity and potency of SUMOi

A Six human PDAC cells were treated for 72 hours with the indicated concentrations of ML-792. Viability was measured with MTT assays. Vehicle treated controls were arbitrarily set to 1.

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B To test the potency and selectivity of ML-93 in cells, HCT-116 cells were treated for 4h with increasing concentrations of ML-93, and 1 μM of ML-792 as a positive control, and assayed by Western blot hybridization for inhibition of formation of UBC9-SUMO thioester conjugates (UBC9 is the E2 enzyme in the SUMO pathway), UBC12-NEDD8 thioester conjugates (UBC12 is the E2 enzyme in the NAE pathway), and UBC10-Ub thioester conjugates (UBC10 is an E2 enzyme in the UAE pathway), as well as inhibition of global SUMOylation.

Supplemental Figure 4. Characterization of the SUMOi.

A, B Clonogenic growth of the depicted six human PDAC cell lines treated with increasing ML-93 doses. **A** representative experiment. **B** quantification of clonogenic growth of at least three independent experiment. ANOVA *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

C, D ML-93 induces cell cycle alteration, polyploidization, and apoptosis in **C**: PSN1 and **D**: PaTu-8988T cells as demonstrated by PI cell cycle flow cytometry analyses. Shown are the proportions of cells in the sub G1-, G1-, and G2/M- phase 48 hours after ML-93 treatment (50 nM). All biological replicates (PSN1: n=3; PaTu-8988T: n=5) were performed as technical triplicates. Shown is the mean +/- SD and statistical significance was assessed using Student's t-test.

Supplemental Figure 5. On target activity and pathways regulated by ML-93

A ML-93 long-term effects on specific target SUMOylation (144 hours). ML-93 treatment with the indicated doses stabilizes the endogenous non-SUMOylated form of RanGap1 in PaTu-8988T cells.

B RNA-seq expression profiles of PaTu-8988T cells treated with 500nM ML-93 for 24h or left as vehicle treated controls were analyzed by GSEA. NES: normalized enrichment score, p: nominal p-value, FDR: false discovery rate q-value.

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C Venn diagrams depict the overlap in both downregulated as well as upregulated genes under SUMOylation inhibition with ML-93. RNA-seq in BxPc3, PaTU-8988T, and PSN1 was performed 24h after the ML-93 treatment.

D Genes significantly regulated upon ML-93 treatment (log FC +/- 0.58, FDR<0.05) were analyzed with the Molecular Signature Database. Venn diagrams of pathways associated with up and downregulated genes.

E Common HALLMARK signature associated with ML-93 up- or down-regulated genes (corresponding to D). The color-coded FDR q-value is shown for each line and signature.

F Global proteomic analysis of human PDAC cell lines PaTu-8988T and PSN1 was performed after treatment with 500 nM ML-93 for 48h. Depicted are proteins, which are exclusively expressed in control or ML-93 treated cells as well as an overlap of both cell lines. Changes in the proteome were visualized by plotting the difference of the log2 mean protein intensities between the ML-93 treatment and DMSO control against the negative logarithmized p-values. Proteins with 2-fold change and a p-value < 0.05 are considered high-confidence hits (designated in red as significantly regulated). Measurements were made each in triplicates.

Supplemental Figure 6. SUMO inhibitor activity in PDAC cells and association to MYC

A Human conventional PDAC cell lines with quantified MYC protein expression were analyzed by GSEA. High expression: MYC protein expression >66th percentile; Low expression: MYC protein expression <66th percentile (according to Fig. 3C). NES: normalized enrichment score, p: nominal p-value, FDR: false discovery rate q-value.

B, C mRNAs from a RNA-seq expression dataset of 38 murine PDAC cell lines were correlated with the GI_{50} values for ML-93 and ML-792. The Pearson correlation coefficient was used as a rank to run a pre-ranked GSEA. B, Venn diagram of HALLMARK signatures associated with GI_{50} negative correlated genes. The common signatures for ML-93 and ML-792 are depicted. C, Enrichment blots for both HALLMARK

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MYC signatures for both SUMO inhibitors. NES: normalized enrichment score, p: nominal p-value, FDR: false discovery rate q-value.

Supplemental Figure 7. SUMO inhibitor activity in PDAC cells and association to MYC

A Murine PPT-8024, PPT-5671, PPT-53631, and PPT-S559 PDAC cell lines were transduced with an MYC^{ER} vector or an empty control vector (PPT-53631). MYC western blot to demonstrate expression of MYC^{ER}. actin: loading control.

B The indicated murine cell lines were treated with 4-OHT (600 nM) for 8 hours or were left as vehicle treated control. qPCR analysis for *Myc*, *Odc1*, *Cad, Sae1,* and *Uba2/Sae2* mRNA expression. *Gapdh* mRNA was used for normalization. Shown is the mean +/- SD of three independent experiments performed as technical triplicates.

C, D Murine PPT-8024, PPT-5671, PPT-53631 PDAC cell lines were transduced with an MYC^{ER} vector or an empty control vector (PPT-53631). **C**, Cell were treated with 4-Hydroxytamoxifen (4-OHT) (600 nM) or vehicle control and increasing doses of ML-93 as indicated. **C**, Viability was measured after 72 hours with MTT assays and dose-response curves are depicted. Each experiment represents at least three biological replicates performed as triplicates. Shown is the mean +/- SD. * t-test $p<0.05$. **D**, Shows representative experiments of clonogenic assays (upper panel) and the corresponding quantification (lower panel). Each experiment represents at least three biological replicates performed as duplicates. Shown is the mean +/- SD. t-test *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

E Cells described in A-D were treated with 4-Hydroxytamoxifen (600 nM) or vehicle control and increasing doses of ML-792 as indicated. Shown are representative experiments of clonogenic assays.

F Human IMIM-PC1^{MYCER} cells were treated with 4-Hydroxytamoxifen (500 nM) or vehicle control and increasing doses of ML-93 or ML-792 as indicated. Viability was measured after 72 hours with MTT assays and dose-response curves are depicted. Each

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experiment was performed: ML-93 n=4, ML-792 n=2; experiments were done as triplicates. Shown is the mean +/- SD. * t-test p<0.05.

G PPT-S559^{MYCER} cells were treated with 4-OHT (600 nM) for 8 hours or were left as vehicle treated control. qPCR analysis for *Myc*, *Odc1*, *Cad, Sae1, and Sae2* mRNA expression. *Gapdh* mRNA was used for normalization. Shown is the mean +/- SD of three independent experiments performed as technical triplicates.

H,I Murine PPT-S559^{MYCER} cells were transduced with either *Ube2i* shRNA or an empty vector control, both co-expressing GFP. **H**, to assess *Ube2i* knockdown efficacy, viable GFP+ cells were FACS-sorted and *Ube2i* mRNA expression was quantified and normalized to *Ubiquitin*. Two independent experiments were analyzed. **I**, Cells were treated with 4-Hydroxytamoxifen (4-OHT) (600 nM) or vehicle control and viability of GFP-positive cells was measured after 72 hours with DAPI staining. GFP/DAPI double positive cells were assessed by flow cytometry. The relative fold change was measured as the relative difference in the fraction of GFP/DAPI double positive cells in vehicle control versus 4-OHT treated cells. The experiment represents three biological replicates. Shown is the mean +/- SD. Unpaired t-test *p<0.05.

Supplemental Figure 8. *In vivo* testing of ML-93

A ML-93 treatment schedule used for the *in vivo* toxicity analysis in C57BL/6 mice.

B Body weight of control (n=6) and ML-93 (n=6) treated mice at day 1 and day 8.

C Blood cell parameters, hemoglobin, white blood cells, and thrombocytes of control and ML-93 treated mice at day 8.

D ML-93 treatment schedule to determine on-target efficacy of ML-93 in protein lysates of splenocytes.

E SUMO2/3 western blot in splenocyte lysates of four control and four ML-93 treated mice. actin: loading control.

F, G Immunodeficient mice were treated with 50mg/kg ML-93 intravenously on d1,2 and d8,9 (**F**) and tumor size of PSN1 (control n=3 mice; ML-93 n=5 mice), HuPDAC7

(control n=5 mice; ML-93 n=5 mice), BxPc3 (control n=3 mice; ML-93 n=3 mice), and IMIM-PC1 (control n=3 mice; ML-93 n=3 mice)-derived tumors was measured over time (**G**).

H-J Immunodeficient mice were treated with 50mg/kg ML-93 intravenously on d1,2 and tumor tissue for IHC was prepared at d3 (**H**). Percent Ki67 (**I**) and cleaved caspase3 (**J**) of two control mice and two ML-93 treated mice were compared. * p value of an unpaired t-test <0.05.

Supplemental Figure 9. SUMOi activity in primary human PDAC models.

A Patient-derived organoids were treated with increasing doses of ML-93 for five days and cellular viability was assessed using ATP levels as a readout for viability. All replicates were performed as technical triplicates.

B mRNA expression based on RNA-seq of the four analyzed human PDAC organoid lines for the indicated transcripts. mRNA expression Z-score is color coded.

C Quantification of MYC protein expression in the depicted three primary-dispersed human PDAC cell lines. Each circle represents one biological replicate. Shown is the mean +/- SD.

D The growth inhibitory concentration 50% (GI_{50}) was determined for ML-93 and ML-792 in the depicted human primary dispersed PDAC lines. ATP (CellTiter-Glo®) was used as a surrogate to determine the dose-response of a seven-point dilution (ML-93: 0-1000 nM; ML-792: 0-5000 nM). Cells were treated for 72 hours. Assay was performed with at least three biological replicates conducted as technical triplicates. Shown is the mean +/- SD.

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