TARGETING POLO-LIKE KINASE 4 (PLK4) TRIGGERS POLYPLOIDY AND APOPTOSIS IN *TP53*-MUTANT ACUTE MYELOID LEUKEMIA AND RESULTS IN IMPROVED SURVIVAL

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Background

• *TP53* mutations in acute myeloid leukemia (AML) are associated with complex karyotype and high risk of relapse (Döhner et al., 2017; Giacomelli et al., 2018). The mechanisms responsible for response and relapse in *TP53*-mutant AML remain unclear and investigating novel mechanisms is critical to develop more effective therapies.

• In order to shed light on the defective p53 signaling pathways underlying *TP53* mutant AML, we performed RNA-sequencing (RNA-seq) on bulk mononuclear cells or FACS-sorted leukemic stem cells (LSCs) using samples collected from *TP53*-mutant or *TP53*-wt high-risk AML patients.

• We identified a key regulator of centriole biogenesis: Polo-like kinase 4 (PLK4) as a potential target highly expressed in *TP53*-mutant AML samples.

• Previous publications showed that PLK4 is transcriptionally repressed by p53 and induces apoptosis upon RNAi silencing (Fischer et al., 2014; Li et al., 2005). Here we show that *TP53*-mutant AML samples lack the p53-dependent PLK4 repression and have higher levels of PLK4 compared to *TP53*-wt AML.

• **Gap of knowledge:** The mechanisms responsible for response and relapse in *TP53*-mutant AML remain unclear and there are no effective treatments against *TP53*-mut AML.

• We hypothesized that targeting PLK4 will trigger mitotic defects, and activate apoptosis in *TP53*-mut leukemia cells, making it a potential treatment approach for *TP53*-mut AML.
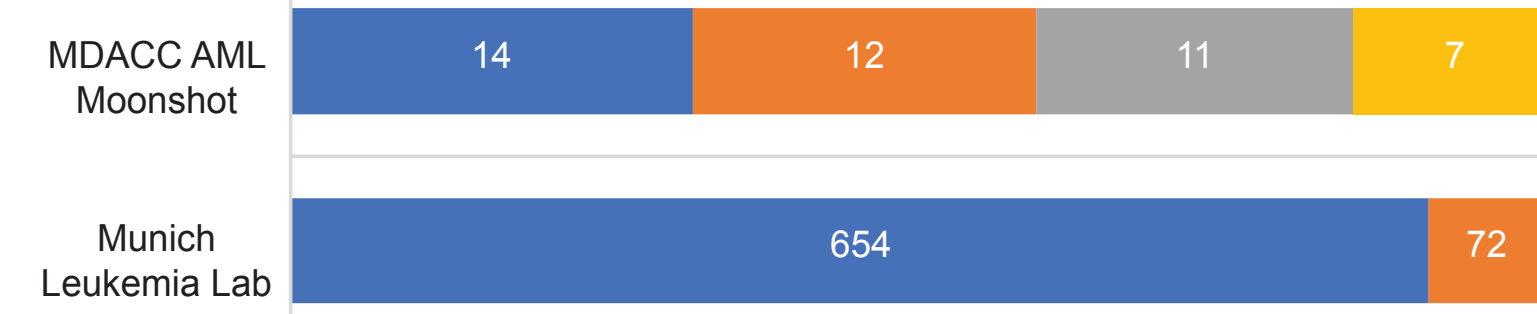
Materials and Methods

RNA sequencing datasets:

MD Anderson AML Moonshot RNA seq dataset:

Total = 44 AML samples as following:
TP53-mut samples = 19 (bulk = 12, LSC = 7)
TP53-wt samples = 25 (bulk =14, LSC =11)

Munich Leukemia Laboratory (MLL) RNA-seq dataset:

Total = 726 AML samples as following:
TP53-mut samples = 72, *TP53*-wt samples = 654.

Cell lines: MOLM13 cell lines from Dr. S. Boettcher

Statistical analysis: Variables were compared using the Wilcoxon rank-sum test for pairwise comparisons. The Kaplan–Meier method was used to estimate the probability of OS, and compared by the log-rank test. Univariate and multivariate Cox proportional hazards models were used to assess the association between *TP53* status and PLK4 expression. Analyses were performed using R version 4.0.3.

Results

TP53-mut and PLK4 overexpression are associated with poor overall survival (OS) in AML
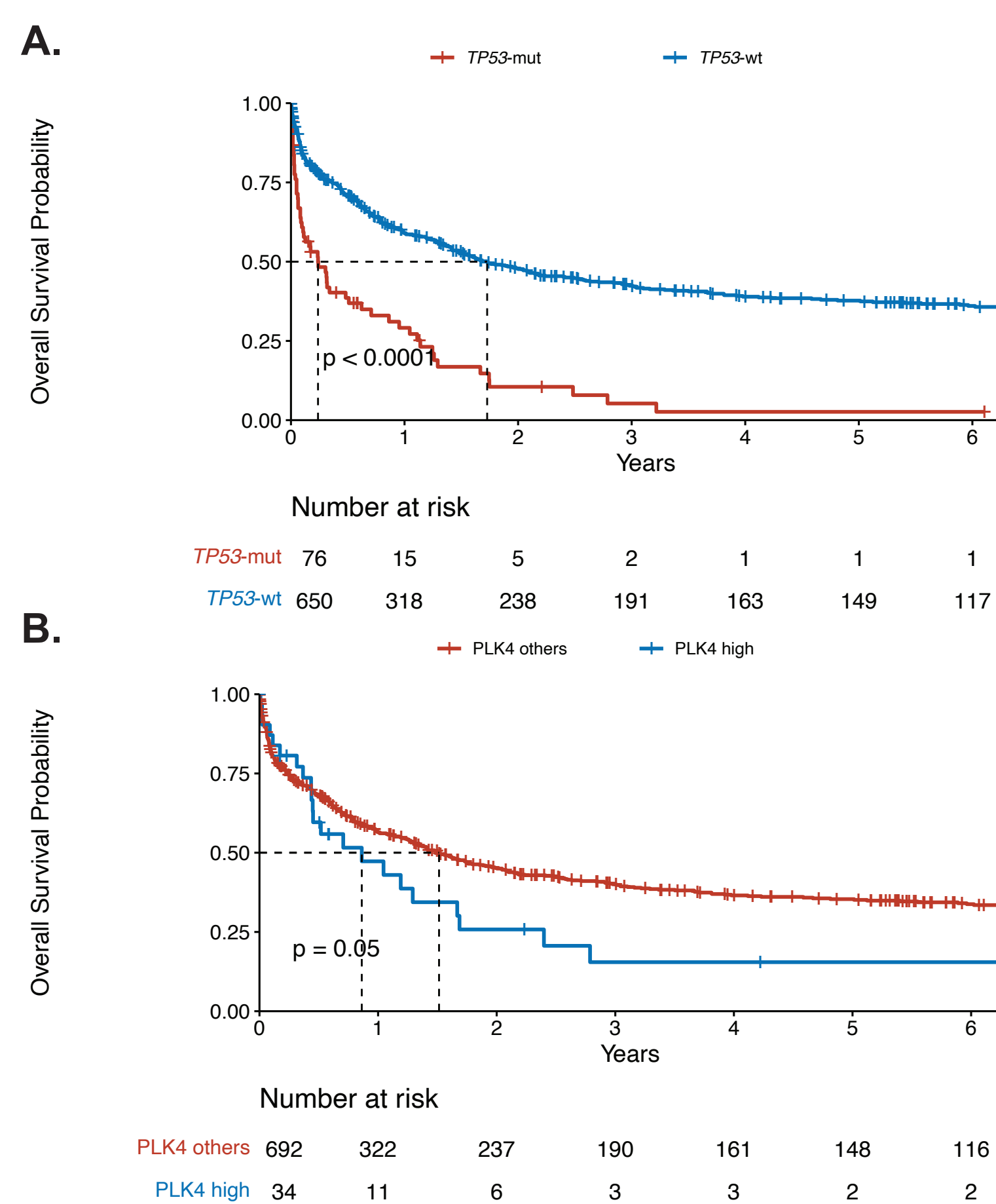

Figure 1: Survival analysis performed based on A) *TP53* status. B) PLK4 expression levels.

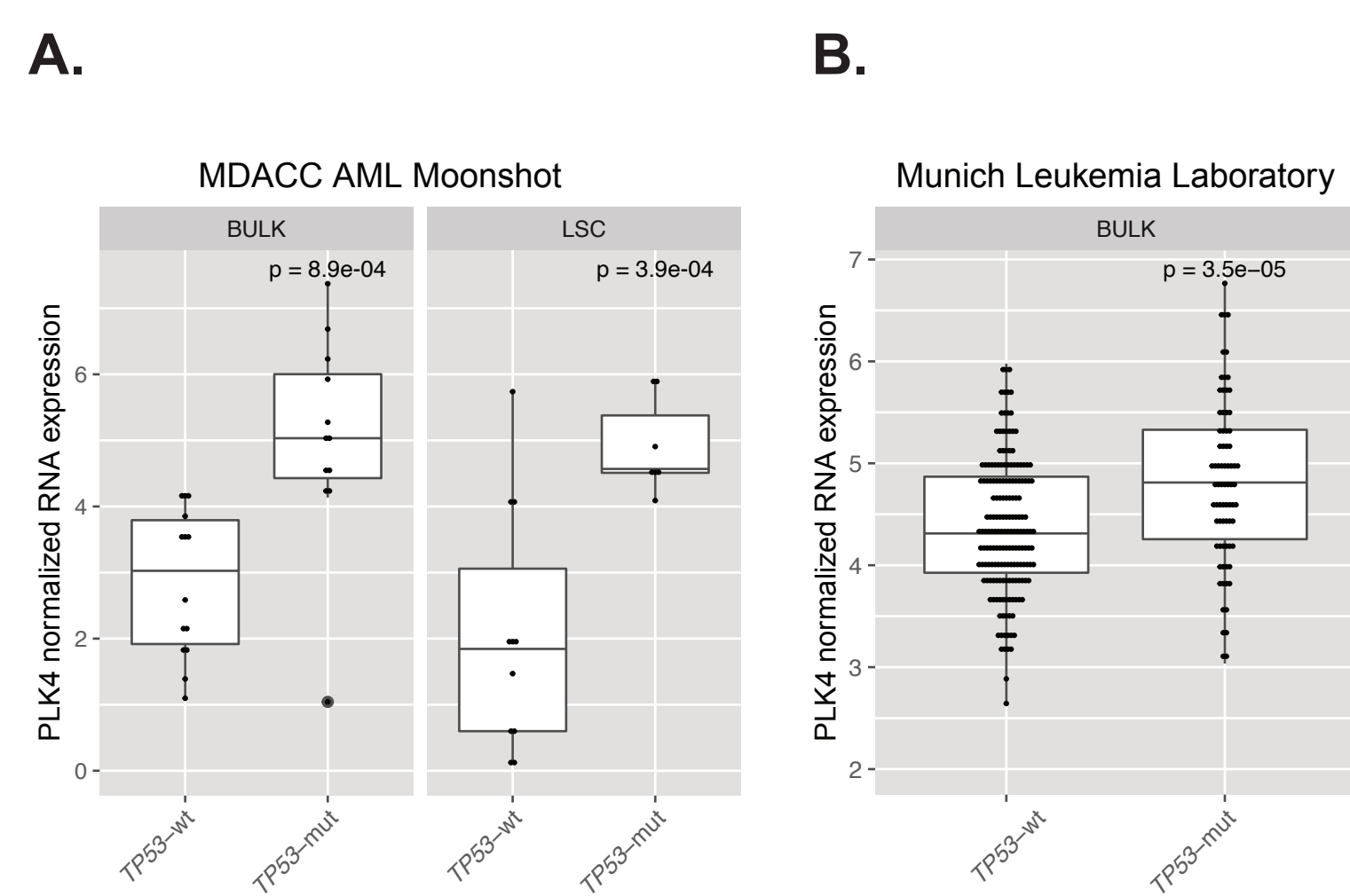### PLK4 is overexpressed in primary samples from *TP53*-mut AML patients

Figure 2: RNA-seq datasets show a significant increase in PLK4 expression in *TP53*-mut AML.
A) MDACC Moonshot (bulk n=26, LSC n=18).
B) Munich Leukemia Laboratory (bulk n = 726).

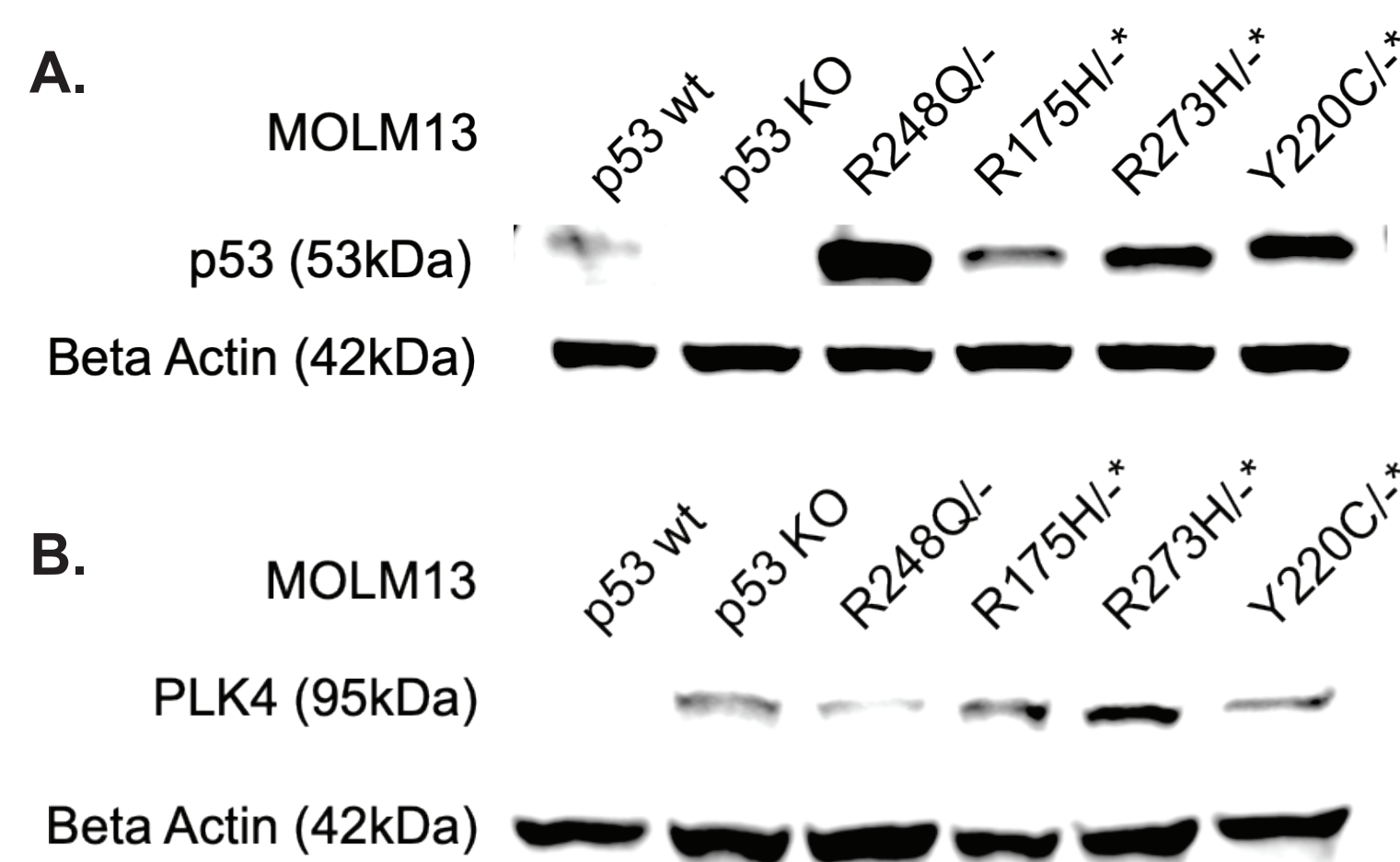### PLK4 is overexpressed in *TP53*-mut AML MOLM13 cell lines

Figure 3: Western blot showing p53 (fig A) and PLK4 (fig B) protein levels in *TP53*-wt and *TP53*-mut MOLM13 cell lines

PLK4 inhibition results in higher polyploidy in *TP53*-mut vs *TP53*-wt MOLM13
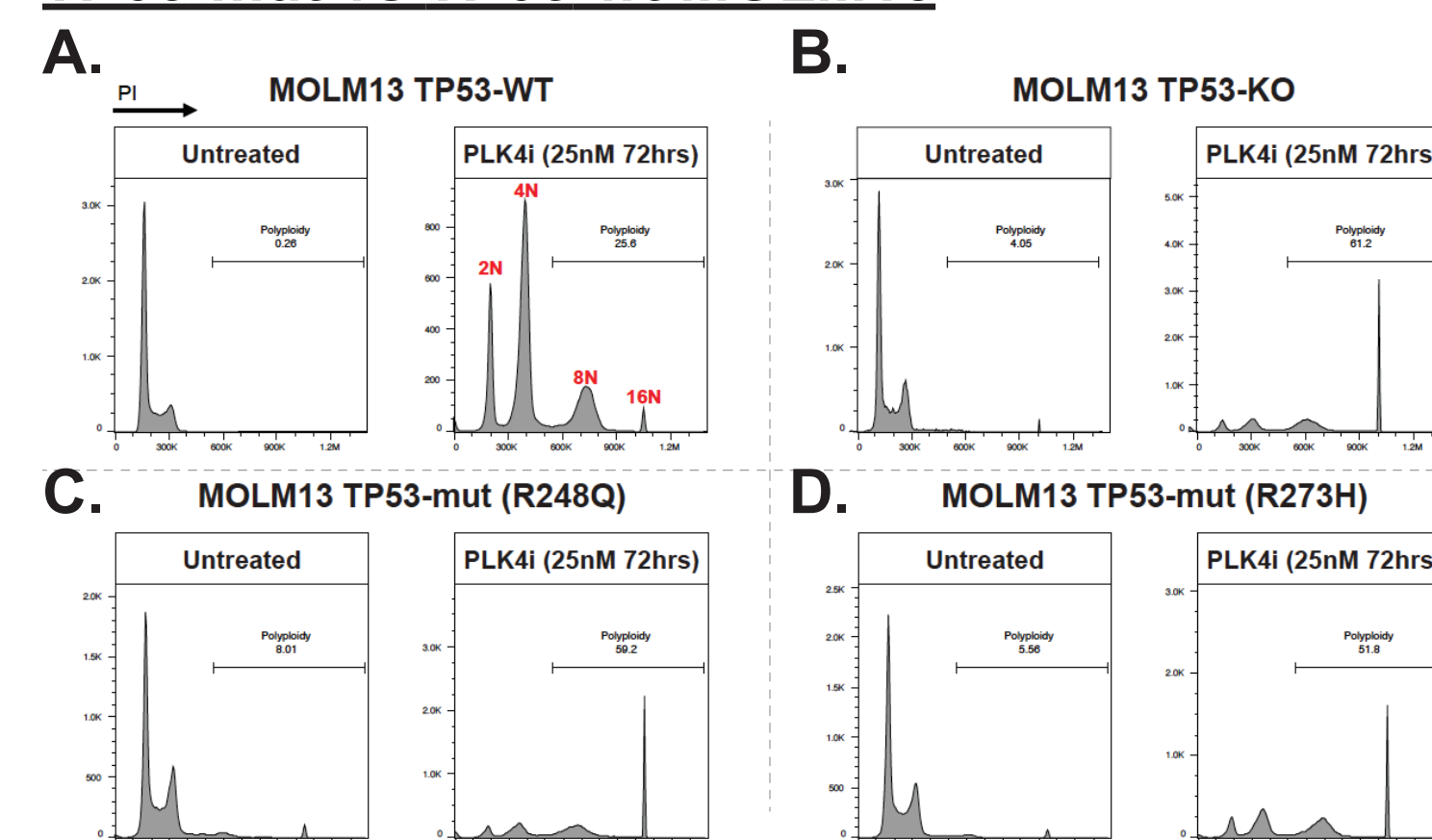

Figure 4: *TP53*-wt and *TP53*-mut MOLM13 cell lines were treated with 25nM CFI-400945 for 72 hours. Ploidy status shown in A) *TP53*-wt, B) *TP53*-ko, C) *TP53*-R248Q, and D) *TP53*-R273H.

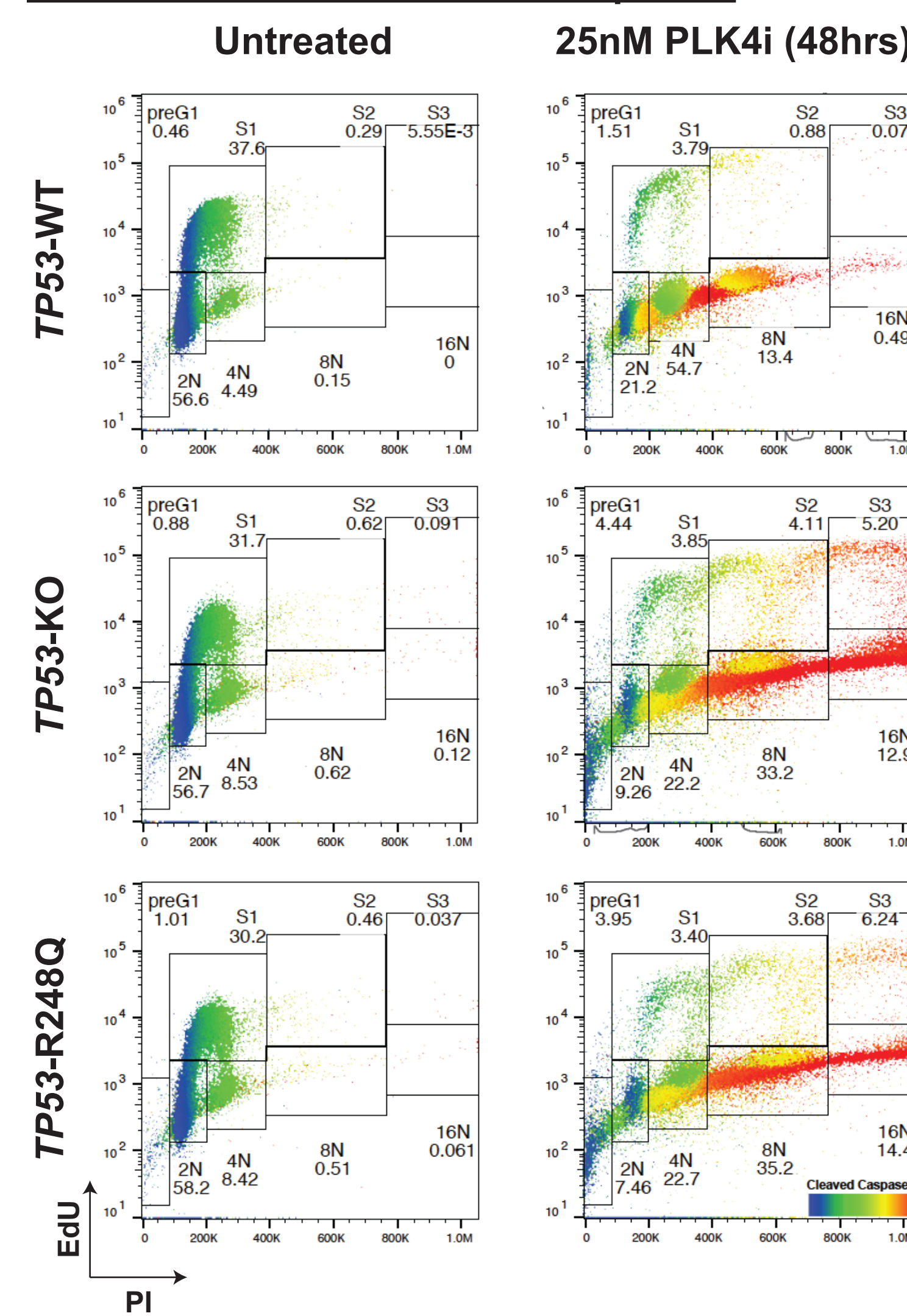### Polyploid *TP53*-mut MOLM13 cells have increased levels of cleaved Caspase-3

Figure 5: Monitoring DNA content with the Click-iT EdU labeling. Pseudocolor plots showing the levels PI and Edu levels in *TP53*-wt, *TP53*-KO, and *TP53*-R234Q MOLM13 cell lines. Left column shows untreated samples. Right column shows samples treated with 25nM CFI-400945 for 48 hours. Color axis represents the levels of cleaved Caspase-3.
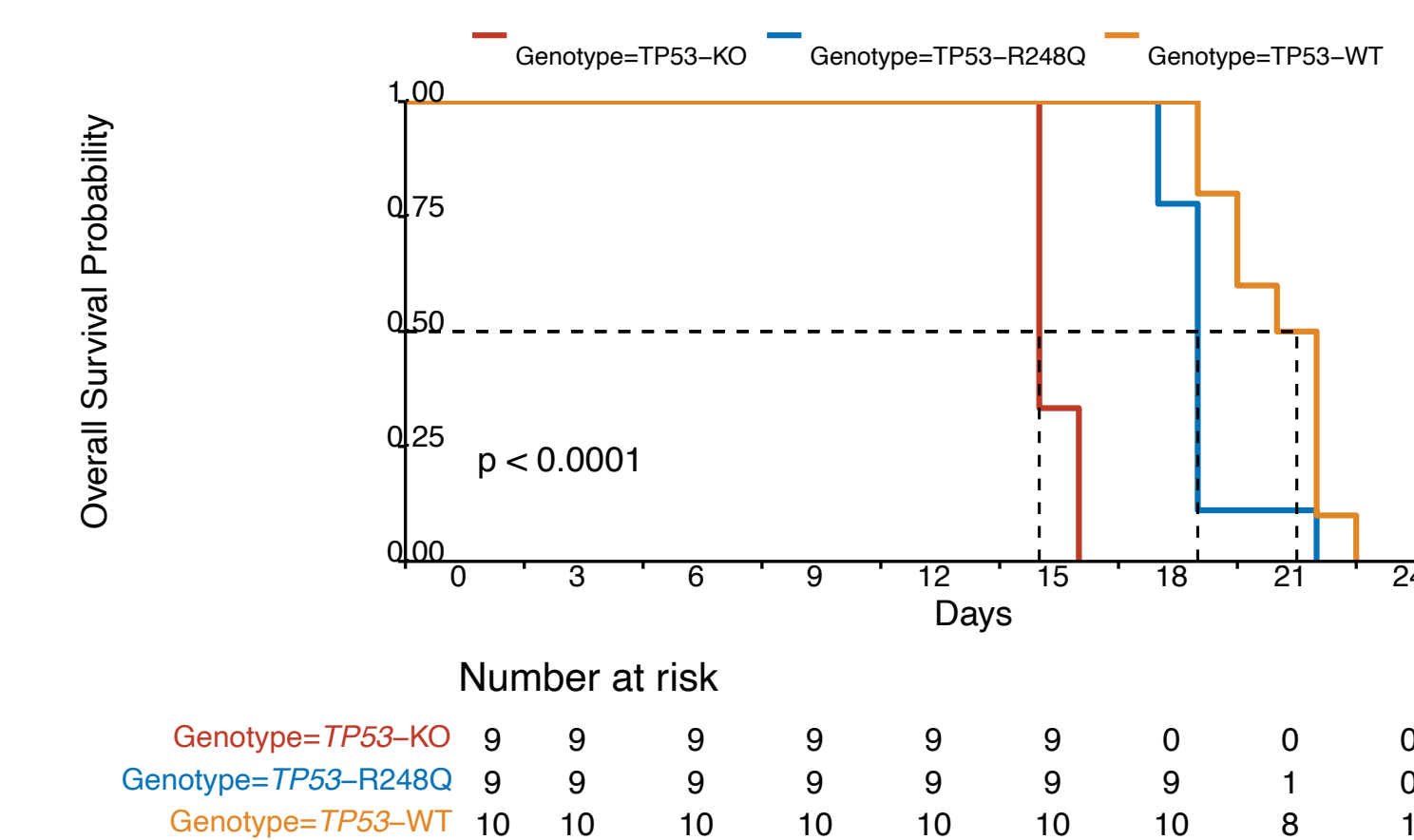
TP53-mut PDX have worst OS

Figure 6: Survival analysis performed on three different PDX models.

PLK4i leads to improved survival outcomes in *TP53*-wt, *TP53*-mut, and *TP53*-ko AML models
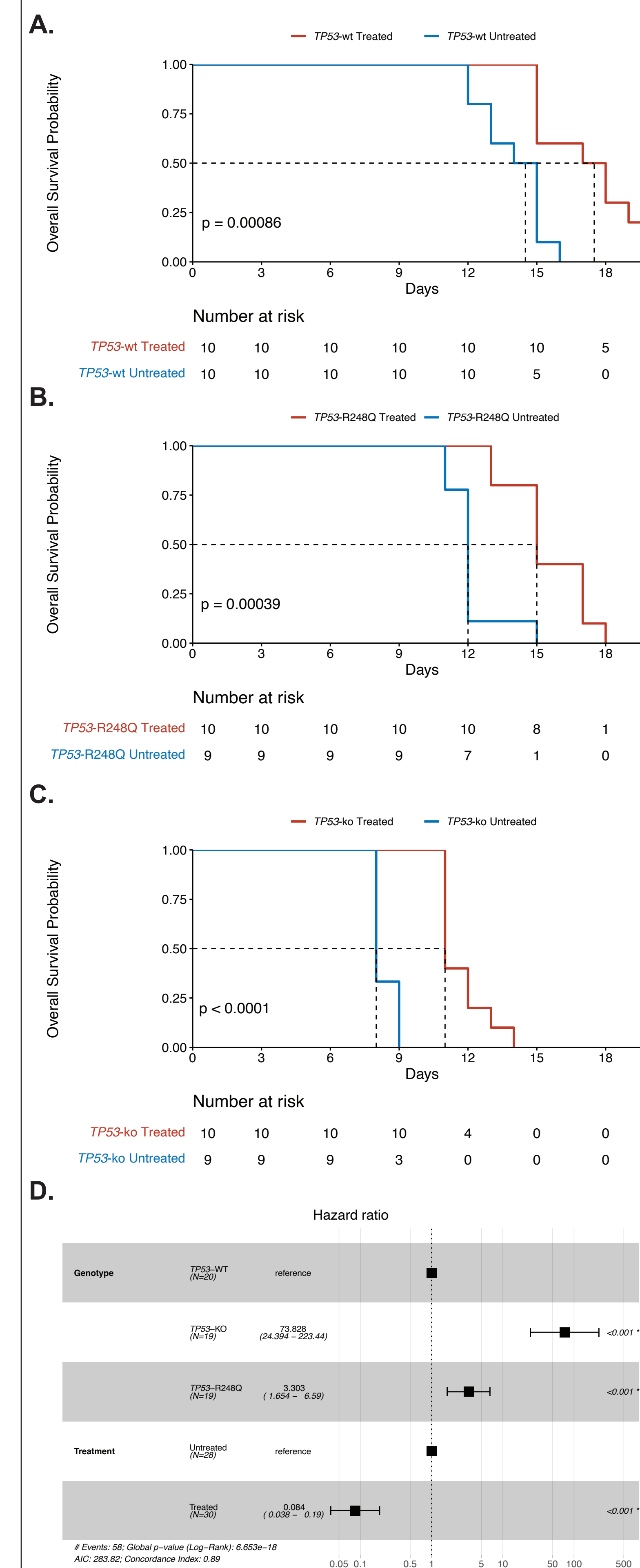

Figure 7: OS in untreated & treated A) *TP53*-wt, B) *TP53*-R248Q, and C) *TP53*-ko PDX models. D) Hazard ratio plotted based on treatement & genotype.

Conclusions

• *TP53*-mutant AML has an overexpression of PLK4, which is a central regulator for centriole duplication.

• Targeting PLK4 results in increased levels of poly-ploidy in *TP53*-mut AML vs *TP53*-wt AML.

• PLK4 inhibition upregulates cleaved Caspase-3 in polyploid cells, and results in significantly higher apoptosis in *TP53*-mut MOLM13 cell lines in comparison to *TP53*-wt MOLM13 cell line.

• A clinical trial is ongoing testing the efficacy of PLK4 inhibition (CFI-400945) in AML (ID: NCT04730258, TWT-202).