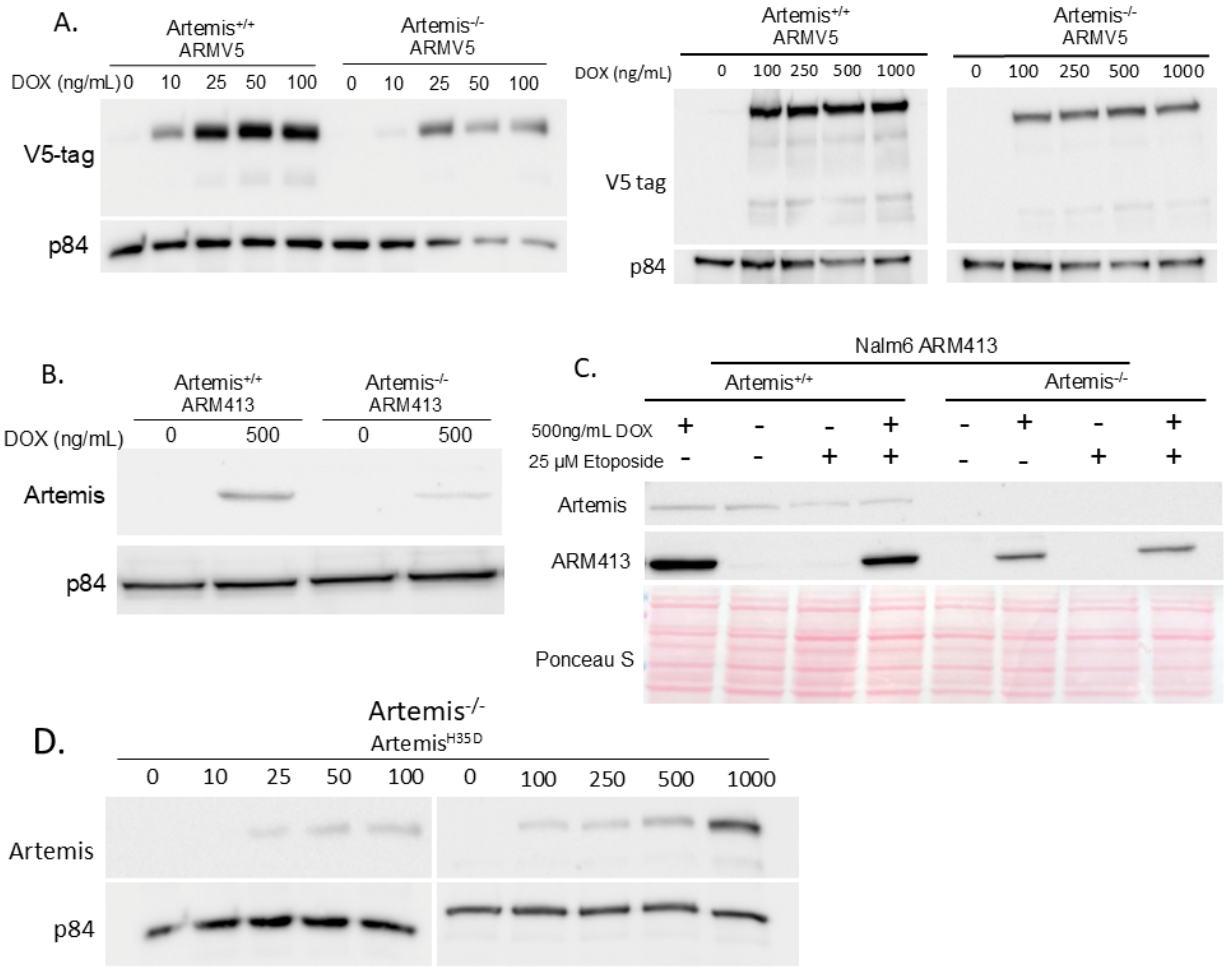


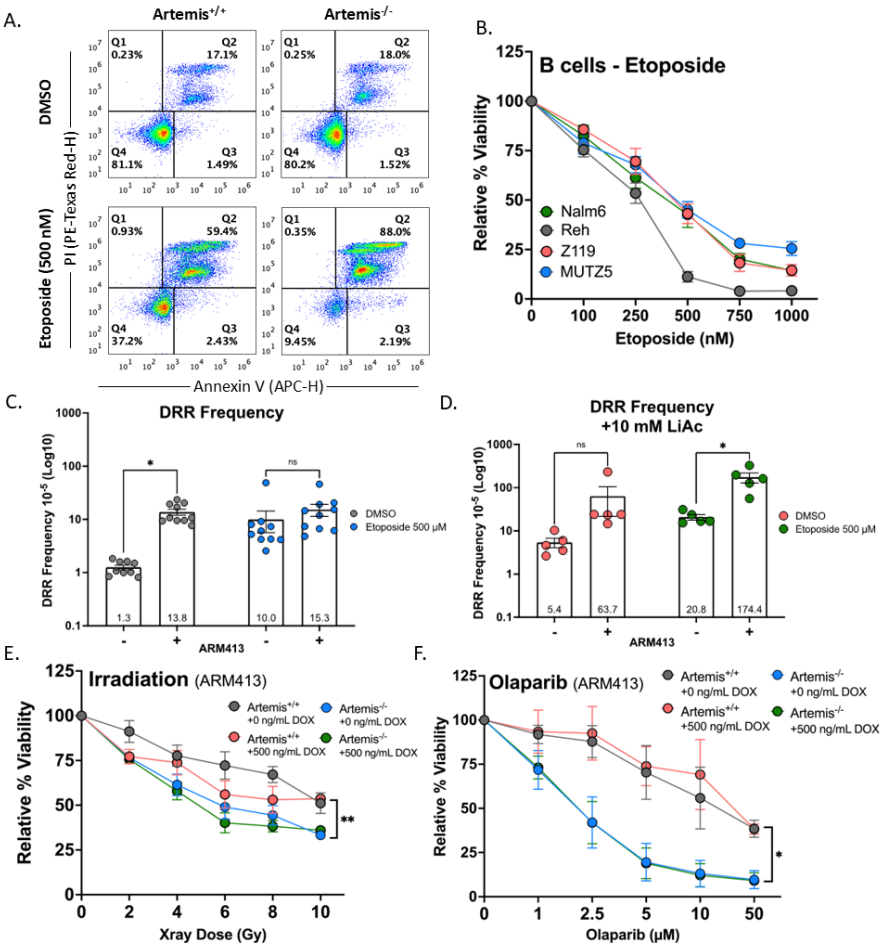
Supplemental Figure 1. Expression of Artemis variants in Nalm6 human pre-B cells.

(A) Western blot with Artemis$^{+/+}$ and Artemis$^{-/-}$ Nalm6 transduced to express ARMV5 with a Dox-inducible promoter treated with indicated concentrations of Dox overnight.

(B) As in (A) but transduced with the ARM413 cassette.

(C) As in (B), but comparing cells treated with or without etoposide for 1 hour and depicting endogenous Artemis as well as expressed ARM413.

(D) As in (A) but transduced with the Artemis^{H35D} cassette in Artemis$^{-/-}$ only.



Supplemental Figure 2. Effect of Top2 poisons with or without ARM413.

(A) Density plots of Artemis$^{+/+}$ and Artemis$^{-/-}$ treated with DMSO or Etoposide (500 nM) for 24h from flow cytometry acquisition. Cells are gated on APC-H (for Annexin V positive cells) and PE-Texas Red (for PI positive cells) in quadrants. Viable cell percentage is indicated as the double negative population in Q4.

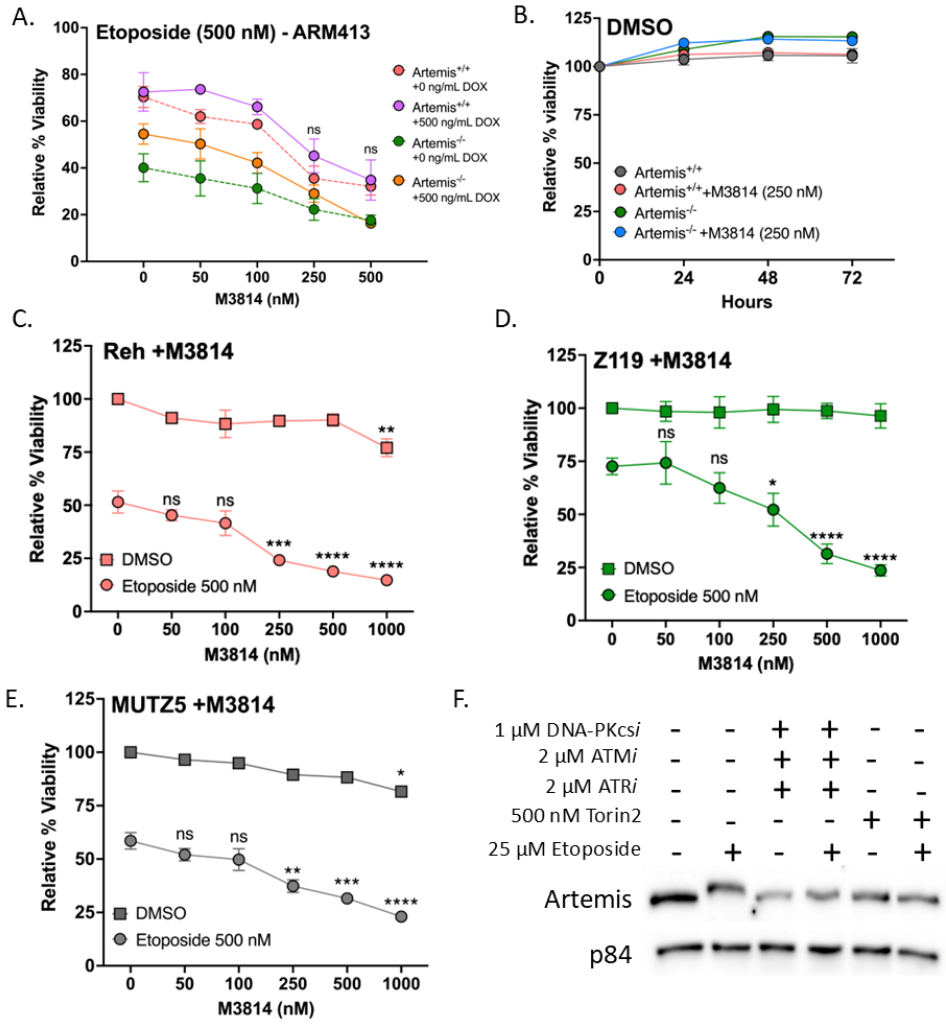
(B) Line graph comparing viability of Nalm6, Reh, Z119, and MUTZ5 cells measured using CellTiter Glo after increasing doses of Etoposide over 48h.

(C) Bar plots showing change in recombination frequency representing DSB frequency in yeast after treatment with etoposide and expression of ARM413 (2way ANOVA; * p-value = 0.0168, ns=not significant).

(D) As in (C), but with the addition of lithium acetate for increased permeability of etoposide (* p-value = 0.0172).

(E) Line graph comparing viability of Nalm6 ARM413 cells measured using CellTiter Glo after exposure to increasing doses of radiation and recovery for 24h. Values are normalized to non-irradiated values (paired t-test; ** p-value = 0.0061).

(F) As in (A) but treated with increasing doses of Olaparib over 72H (paired t-test; * p-value = 0.0102)



Supplemental Figure 3. M3814 does not affect viability alone while PIKK inhibition blocks Artemis phosphorylation.

(A) Line graph comparing viability measured using CellTiter Glo after overnight Dox treatment to express ARM413. Values are normalized to DMSO. Dashed lines indicate data presented in Figure 2A for direct comparison (2way ANOVA; ns=not significant).

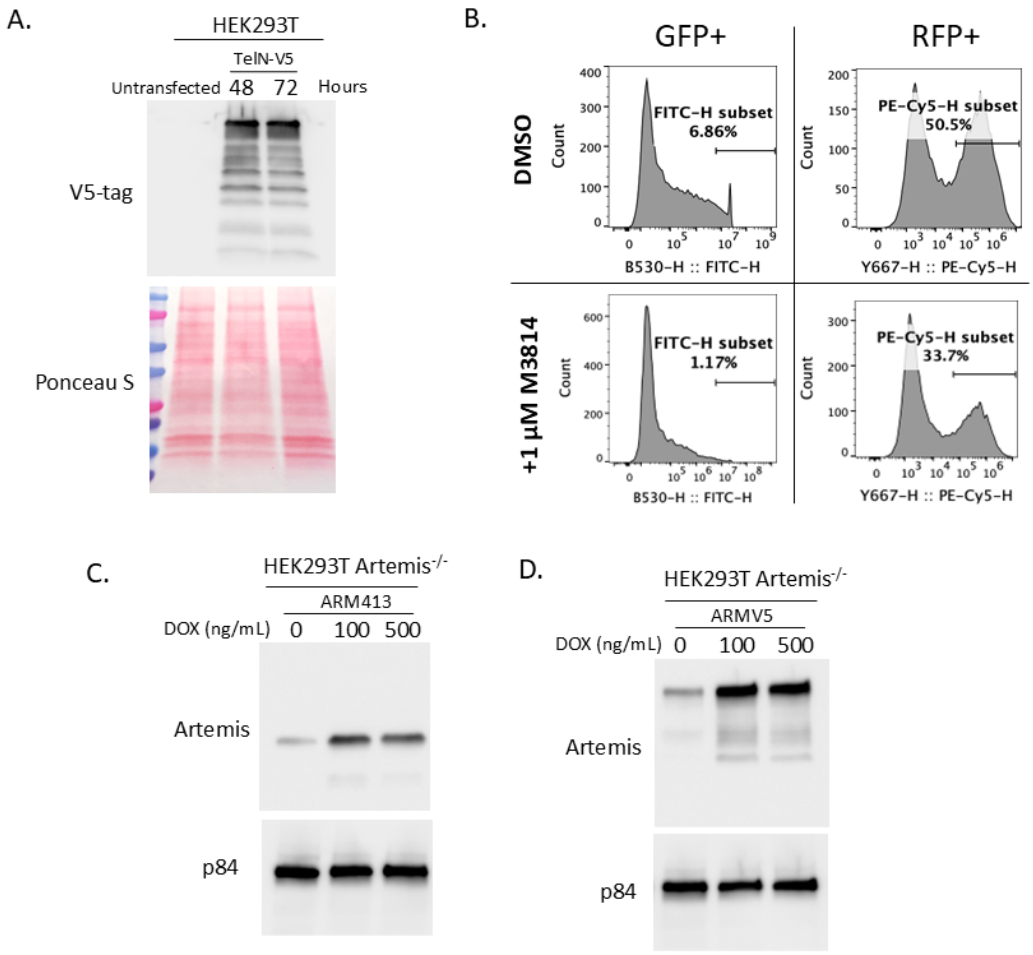
(B) Line graph of a time course showing decreased viability over indicated time points after DMSO treatment with or without M3814.

(C) Line graph comparing viability of Reh with M3814 treatment measured using CellTiter Glo with or without etoposide. Values are normalized to 0 nM M3814 (2way ANOVA; **=p value ≤ 0.01, ***=p value ≤ 0.0005, ****=p value ≤ 0.0001).

(D) As in (C) but with Z119 (2way ANOVA; *=p value ≤ 0.05)

(E) As in (D) but with MUTZ5.

(F) Western blot depicting Artemis phosphorylation after 3h treatment with described inhibitors and 1h treatment with etoposide. p84 is used as a loading control.



Supplemental Figure 4. Expression of Artemis variants in HEK293T and Top2 accumulation in Nalm6 Artemis$^{-/-}$ cells treated with mitoxantrone.

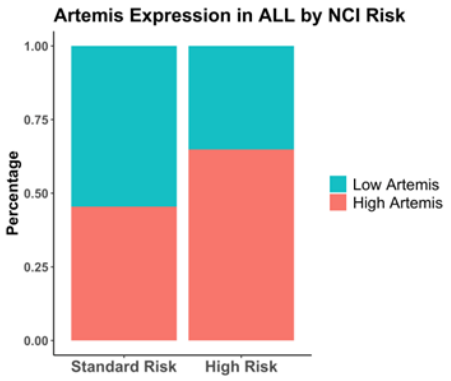
(A) Western blot showing TelN-V5 expression 48h and 72h post-transfection in HEK293T. Ponceau S is shown as a loading control.

(B) Histograms representing gating strategy in FlowJo for HEK293T cells after treatment with DMSO or 1 µM M3814.

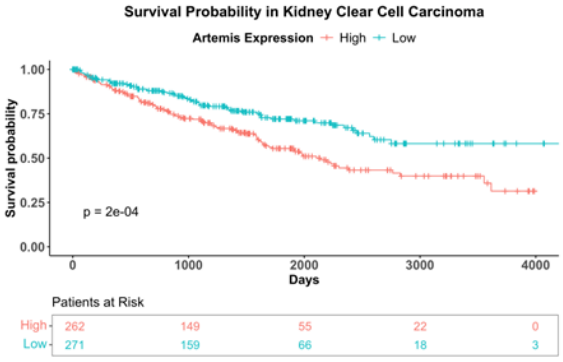
(C) Western blots depicting Artemis variant expression 8h post-transfection of plasmids into HEK293T Artemis$^{-/-}$. p84 is used as a loading control.

(D) As in (C), with ARMV5.

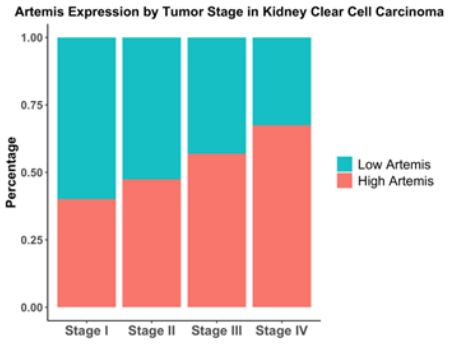
A.


Artemis Expression in ALL by NCI Risk
Percentage
1.00
0.75
0.50
0.25
0.00
Standard Risk
High Risk
Low Artemis
High Artemis

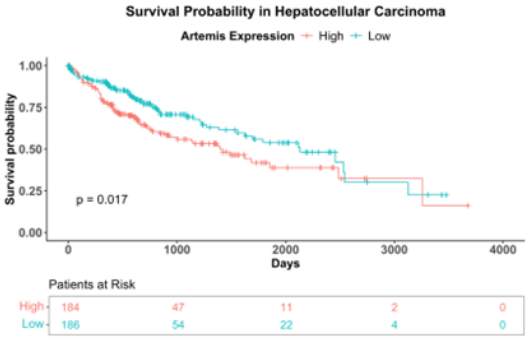
B.


Survival Probability in Kidney Clear Cell Carcinoma
Artemis Expression High Low
Survival probability
p = 2e-04
Days
Patients at Risk
High 262 149 55 22 0
Low 271 159 66 18 3

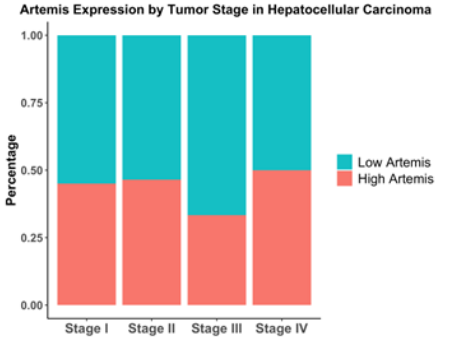
C.


Artemis Expression by Tumor Stage in Kidney Clear Cell Carcinoma
Percentage
Stage I
Stage II
Stage III
Stage IV
Low Artemis
High Artemis

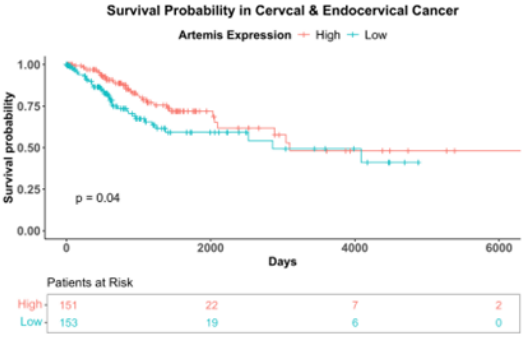
D.


Survival Probability in Hepatocellular Carcinoma
Artemis Expression High Low
Survival probability
p = 0.017
Days
Patients at Risk
High 184 47 11 2 0
Low 186 54 22 4 0

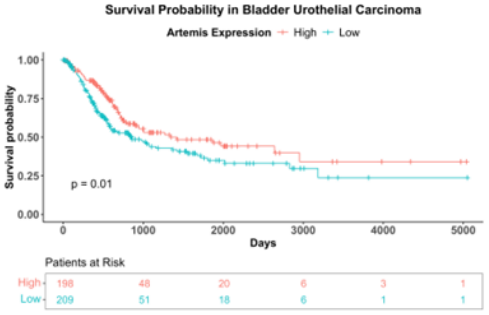
E.


Artemis Expression by Tumor Stage in Hepatocellular Carcinoma
Percentage
Stage I
Stage II
Stage III
Stage IV
Low Artemis
High Artemis

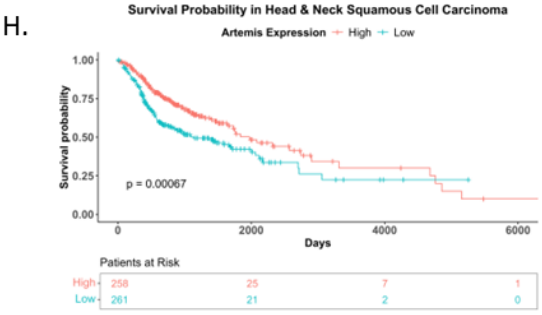
F.


Survival Probability in Cervcal & Endocervical Cancer
Artemis Expression High Low
Survival probability
p = 0.04
Days
Patients at Risk
High 151 22 7 2
Low 153 19 6 0

G.


Survival Probability in Bladder Urothelial Carcinoma
Artemis Expression High Low
Survival probability
p = 0.01
Days
Patients at Risk
High 198 48 20 6 3 1
Low 209 51 18 6 1 1

H.


Survival Probability in Head & Neck Squamous Cell Carcinoma
Artemis Expression High Low
Survival probability
p = 0.00067
Days
Patients at Risk
High 258 25 7 1
Low 261 21 2 0

Supplemental Figure 5. High Artemis expression has variable correlation with cancer prognosis in several cancer types.

(A) Bar Chart comparing the percentage of patients within each NCI Risk group with high/low Artemis expression using the DFCI ALL database (n=10-24 patients per risk group).

(B) Kaplan-Meier survival curve of Kidney Clear Cell Carcinoma patients based on high/low Artemis expression using TCGA (n=533).

(C) Bar Chart comparing the percentage of patients with high/low Artemis expression within each kidney clear cell carcinoma tumor stage using TCGA (n=27-160 patients per stage).

(D) Kaplan-Meier survival curve of Hepatocellular Carcinoma patients based on high/low Artemis expression using TCGA (n=370).

(E) Bar Chart comparing the percentage of patients with high/low Artemis expression within each hepatocellular carcinoma tumor stage using TCGA (n=1-94 patients per stage).

(F) Kaplan-Meier survival curve of Cervical & Endocervical Cancer patients based on high/low Artemis expression using TCGA (n=304).

(G) Kaplan-Meier survival curve of Bladder Urothelial Carcinoma patients based on high/low Artemis expression using TCGA (n=407).

(H) Kaplan-Meier survival curve of Head & Neck Squamous Cell Carcinoma patients based on high/low Artemis expression using TCGA (n=519).

Table S1. Resources and Materials Used in this Work

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Top2A (D10G9) XP®; rabbit monoclonal	Cell Signaling Technologies	Cat# 12286; RRID:AB_2797871
Anti-Artemis (D708V); rabbit monoclonal	Cell Signaling Technologies	Cat# 13381; RRID:AB_2798197
Anti-p84; mouse monoclonal	GeneTex	Cat# GTX70220 RRID:AB_372637
Anti-V5-tag (D3H8Q); rabbit monoclonal	Cell Signaling Technologies	Cat# 13202; RRID:AB_2687461
Anti-mouse IgG (H+L); secondary HRP-linked	Thermo Fisher Scientific	Cat# 31430; RRID:AB_228307
Anti-rabbit IgG; secondary HRP-linked	Millipore	Cat# 12-348; RRID:AB_390191
Anti-phospho-CHK1 (Ser 345); rabbit monoclonal	Cell Signaling	Cat# 2348T; RRID:AB_331212
Anti-CHK1 (G-4); mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-8408; RRID:AB_627257
Chemicals, peptides, and recombinant proteins		
Immobilon Western Chemiluminescent HRP Substrate	Millipore	Cat# WBKLS0050
Glutamax	Gibco	Cat# 35050-061
RPMI 1640 Medium, no glutamine	Thermo Fisher Scientific	Cat# 21870092
Corning® 96-well White/Clear Flat Bottom Microplate	Corning	Cat# 3632
G418	Gold Biotechnology	Cat# G-418-10
Ciprofloxacin Hydrochloride	Fisher Scientific	Cat# NC1265500
DMEM/High glucose, L-glutamine, sodium pyruvate	Fisher Scientific	Cat# SH30243.FS
TRIzol	Thermo Fisher Scientific	Cat# 15596018
DNAzol Reagent	Thermo Fisher Scientific	Cat# 10503027
Critical commercial assays		
Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific	Cat# 23227
CellTiter Glo 2.0	Promega	Cat# G9242
NanoDrop One Spectrophotometer	Thermo Fisher Scientific	Cat# ND-ONE-W
Maxima H Minus cDNA Synthesis Master Mix	Thermo Fisher Scientific	Cat# M1661
SYBR Green Master Mix	Applied Biosystems	Cat# 100029283

Deposited Data		
GEO (GSE 181157)	Tran et. al., 2022	
Experimental models: Cell lines		
Nalm6	ATCC	CRL-3273
Nalm6 Artemis$^{-/-}$	Kurosawa, et. al., 2008	
Reh	ATCC	CRL-8286
Z119	ATCC	CRL-3394
MUTZ5	DSMZ	ACC-490
U2OS	ATCC	HTB-96
HEK293T	ATCC	CRL-3216
HEK293T Artemis$^{-/-}$	Meek, 2020[43]	N/A
Nalm6 Artemis$^{+/+}$ ARM413	This Study	N/A
Nalm6 Artemis$^{-/-}$ ARM413	This Study	N/A
Nalm6 Artemis$^{+/+}$ ARMV5	This Study	N/A
Nalm6 Artemis$^{-/-}$ ARMV5	This Study	N/A
Nalm6 Artemis$^{-/-}$ ARMV5	This Study	N/A
Nalm6 Artemis$^{-/-}$ Artemis^{H35D}	This Study	N/A
Recombinant DNA		
TelN-V5	Meek, 2020	N/A
TelN/Crim	Meek, 2020	
pINDUCER20-ARMV5	This Study	N/A
pINDUCER20-ARM413	This Study	N/A
pINDUCER20-Artemis^{H35D}	This Study	N/A
Oligonucleotides		
RT-qPCR Primers		
DCLRE1C Forward 5'-GAGCTAGAACAGTTCACCGAGAC-3'	Integrated DNA Technologies, Inc	
DCLRE1C Reverse 5'-CAGGCTGCTTTTCTGATACTGCA -3'	Integrated DNA Technologies, Inc	
Software and algorithms		
Image Lab, version 6.1.0 build 7	Bio-Rad Laboratories	https://www.bio-rad.com/en-us/product/image-lab-software?ID=KRE6P5E8Z
R (version 4.4.0)	R Core Team	https://www.R-project.org/
ggplot2 (version 3.5.1)	Wickham et. al., 2024[66]	https://cran.r-project.org/package=ggplot2

survival (version 3.5-7)	Therneau et. al., 2024[67]	https://CRAN.R-project.org/package=survival
survminer (version 0.4.9)	Kassambra et. al., 2024[68]	https://cran.r-project.org/package=survminer