

## The RNA helicases DDX19A/B modulate selinexor sensitivity by regulating MCL1 mRNA nuclear export in leukemia cells

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## Abstract

Selinexor, a first-in-class exportin1 (XPO1) inhibitor, is an attractive anti-tumor agent because of its unique mechanisms of action; however, its dose-dependent toxicity and lack of biomarkers preclude its wide use in clinical applications. To identify key molecules/pathways regulating selinexor sensitivity, we performed genome-wide CRISPR/Cas9 dropout screens using two B-ALL lines. We identified, for the first time, that paralogous DDX19A and DDX19B RNA helicases modulate selinexor sensitivity by regulating *MCL1* mRNA nuclear export. While single depletion of either *DDX19A* or *DDX19B* barely altered MCL1 protein levels, depletion of both significantly attenuated *MCL1* mRNA nuclear export, reducing MCL1 protein levels. Importantly, combining selinexor treatment with depletion of either *DDX19A* or *DDX19B* markedly induced intrinsic apoptosis of leukemia cells, an effect rescued by MCL1 overexpression. Analysis of Depmap datasets indicated that a subset of T-ALL lines expresses minimal *DDX19B* mRNA levels. Moreover, we found that either selinexor treatment or *DDX19A* depletion effectively induced apoptosis of T-ALL lines expressing low DDX19B levels. We conclude that XPO1

40 and DDX19A/B coordinately regulate cellular MCL1 levels and propose that  
41 DDX19A/B could serve as biomarkers for selinexor treatment. Moreover,  
42 pharmacological targeting of DDX19 paralogs may represent a potential strategy to  
43 induce intrinsic apoptosis in leukemia cells.

## INTRODUCTION

XPO1, also known as exportin 1, is a member of the importin superfamily of nuclear transport receptors, which functions in nucleocytoplasmic transport of proteins containing a nuclear export signal (NES) <sup>1-4</sup>. XPO1/cargo complexes are transferred from the nucleus to the cytoplasm through the nuclear pore complex (NPC) and then dissociate in the cytoplasm in a Ran-dependent manner <sup>5</sup>. XPO1 reportedly transfers at least 221 NES-containing proteins: among those relevant to cancer cell survival are TP53, APC, BRCA1/2, BIRC5 and NPM1 <sup>1</sup>. XPO1 also regulates RNA metabolism by modulating localization of proteins functioning in mRNA transport, translation initiation and ribosomal RNA (rRNA) biogenesis <sup>1-3, 6</sup>.

Selinexor, formerly known as KPT-330, is an orally bioavailable small molecule that covalently binds to the NES-binding groove of human XPO1, thereby competitively inhibiting XPO1/cargo interactions <sup>1, 7, 8</sup>. Since XPO1 exports multiple NES-containing proteins, such as p53, RB1, p27 and IκB, selinexor likely exerts anti-tumor activity through various mechanisms, such as enabling nuclear retention of tumor suppressor proteins, cell cycle regulators or signaling molecules <sup>1</sup>. While

selinexor is currently FDA-approved for treatment of multiple myeloma (MM) and diffuse large B-cell lymphoma (DLBCL)<sup>9, 10</sup>, its dose-dependent toxicity and lack of biomarkers preclude its wide use in clinical applications<sup>1</sup>.

To define molecules/pathways relevant to selinexor sensitivity and to identify potential biomarkers, we performed genome-wide CRISPR/Cas9 functional screens with or without selinexor using B-ALL lines. Here we show, for the first time, that *DDX19* (DEAD-box helicase 19) paralogs play a critical role in *MCL1* mRNA nuclear export and thereby modulate selinexor sensitivity in leukemia cells.

## RESULTS

### Genome-wide CRISPR-Cas9 screens identify *DDX19A* and *DDX19B* as modulators of selinexor sensitivity

To identify molecules/pathways relevant to selinexor sensitivity, we performed genome-wide CRISPR/Cas9 dropout screens with or without selinexor using two B-ALL lines: NALM-6 and KOPN49. sgRNA-library transduced cells were maintained for 7 days post-transduction and then divided into groups treated with either DMSO or selinexor. For the latter, we used selinexor at dosages allowing 50% cell viability after a 10-day culture period (100nM, NALM-6; 40nM, KOPN49) (**Fig. 1a** and **Supplemental Fig. 1**). Changes in sgRNA abundance occurring over the culture period were assessed using the MAGeCK MLE program <sup>11</sup> (**Fig. 1b** and **Supplemental Table 1**). Genes with sgRNA abundance significantly altered by selinexor treatment were also analyzed using the DrugZ program <sup>12</sup> (**Fig. 1c**). Notably, unlike the case in *TP53*-mutated KOPN49 cells, sgRNAs targeting *TP53* were among the most enriched in NALM-6 cells, which harbor wild-type (WT) *TP53* (**Fig. 1b, c**).

Genes functioning in RNA metabolism were most enriched in NALM-6 and KOPN49 lines and represent potential selinexor synthetic lethal genes (**Supplemental Fig. 2**). Specifically, sgRNAs targeting either *DDX19A* or *DDX19B*, each encoding a putative RNA helicase implicated in RNA export<sup>13</sup> were significantly depleted in the presence of selinexor in both lines (**Fig. 1b, c**). To validate these results, we depleted NALM-6 and KOPN49 cells of either *DDX19A* or *DDX19B* via lentivirus-based Crimson-tagged sgRNA and assessed effects on cell growth over 6 days of treatment with or without selinexor. As expected, cells expressing sgRNA targeting either *DDX19A* or *DDX19B*, but not those transduced with an empty vector, showed a proliferative disadvantage relative to control cells in the presence of selinexor (**Fig. 1d**). We validated these findings by conducting dose-response analysis of selinexor effects on NALM-6 and KOPN49 cell viability: in both cases, depletion of either *DDX19A* or *DDX19B* significantly enhanced cell sensitivity to selinexor (**Fig. 1e**).

Since *DDX19A* and *DDX19B* are adjacent, paralogous genes located on the long arm of chromosome 16<sup>14</sup>, we hypothesized that they function redundantly

to prevent cell death. To confirm this, we depleted each gene individually via lentivirus-based Crimson-tagged sgRNA and assessed effects on cell growth over 6 days. Cells expressing sgRNA targeting either *DDX19A* or *DDX19B* showed a moderate proliferative disadvantage relative to non-transduced cells (**Fig. 1f**). By contrast, in both NALM-6 and KOPN49 cells lacking endogenous *DDX19B*, additional *DDX19A* deletion promoted cell death (**Fig. 1g**), confirming a critical and redundant role of DDX19A and DDX19B in cell fitness.

#### **DDX19A/DDX19B depletion induces intrinsic apoptosis of leukemia cells by reducing MCL1 protein levels**

To gain mechanistic insight into how DDX19A/B regulate cell fitness, we established *DDX19A/B* double knockout (DKO) NALM-6 cells also stably expressing HA-FKBP12<sup>F36V</sup>-tagged DDX19A (NALM-6<sup>19A\_FK</sup> *DDX19B* KO), enabling us to induce DDX19A protein degradation by treating cells with dTAG<sup>V</sup>-1 ligand <sup>15</sup>. Based on Annexin V positivity, apoptosis was evident at 4 hours, which was the earliest time point assessed, following dTAG<sup>V</sup>-1 treatment and became more pronounced at later

time points (**Fig. 2a**). These effects were absent in cells treated with negative control ligand dTAG<sup>V</sup>-1-NEG, a dTAG<sup>V</sup>-1 diastereomer that cannot bind VHL <sup>15</sup> (**Fig. 2a**). As expected, DDX19A protein expression was significantly reduced as early as 2h after dTAG<sup>V</sup>-1 treatment (**Fig. 2b**). Given rapid induction of apoptosis seen after DDX19A depletion in NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells, we concluded that DDX19A/B regulate factors directly functioning in the apoptosis pathway. Therefore, we examined levels of anti-apoptotic proteins in these cells with or without dTAG<sup>V</sup>-1 treatment. Strikingly, levels of MCL1 protein, but not those of the anti-apoptotic proteins BCL2 and BCL2L1 (also known as BCL-XL), were significantly reduced by dTAG<sup>V</sup>-1 treatment, accompanied by an increase in cleaved Caspase-3, a marker of intrinsic apoptosis <sup>16</sup> (**Fig. 2c**). During intrinsic apoptosis, BAX and/or BAK1 are released from anti-apoptotic proteins, self-assemble, and then form pores that permeabilize the mitochondrial outer membrane. Subsequently, cytochrome c is released from the mitochondria, activating the caspase “death pathway” <sup>17</sup>. Importantly, DDX19A/B depletion did not promote intrinsic apoptosis in cells lacking both *BAX* and *BAK1*, although these cells showed reduced MCL1 protein levels (**Fig. 2c**). Furthermore,

apoptosis was not evident in DDX19A/B-deficient cells overexpressing MCL1 after 6 hours of dTAG<sup>V</sup>-1 treatment (**Fig. 2d**), suggesting that apoptosis promoted by DDX19A/B depletion primarily depends on MCL1 levels.

### **DDX19A/B regulate nucleocytoplasmic transport of *MCL1* mRNA without altering *MCL1* mRNA or protein stability**

To determine how DDX19A/B regulate MCL1 protein levels, we first asked whether DDX19A/B depletion alters *MCL1* mRNA stability. To do so, we assessed the time course of changes in *MCL1* mRNA levels in the presence of Actinomycin D, which inhibits transcription, in NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells treated with either dTAG<sup>V</sup>-1 or negative ligand. We observed comparable *MCL1* mRNA levels at each time point, regardless of DDX19A status (**Fig. 3a**). Furthermore, the time course of changes in MCL1 protein levels was comparable in dTAG<sup>V</sup>-1 and negative ligand-treated cells following treatment with Cycloheximide, which inhibits de novo protein synthesis (**Fig. 3b**). These data indicate that DDX19A/B do not regulate stability of MCL1 mRNA or protein.

We next assessed whether DDX19A/B regulate nucleocytoplasmic transfer of *MCL1* mRNA. To do so, we performed RNA-FISH in NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells, with or without dTAG<sup>V</sup>-1 treatment. When we used oligo d(T) probes, which hybridize to polyA-tailed mRNAs, FISH signals were diffuse in the nucleus and cytoplasm of negative ligand-treated cells but exhibited a patchy and predominantly nuclear pattern in cells treated 3h with dTAG<sup>V</sup>-1. In fact, fluorescent signal intensities in the nucleus relative to those seen in the cytoplasm were markedly enhanced following dTAG<sup>V</sup>-1 treatment (**Fig. 3c**). Similarly, FISH signals specific to *MCL1* mRNA were also predominantly nuclear in cells treated with dTAG<sup>V</sup>-1 (**Fig. 3d**), strongly suggesting that DDX19A/B regulate nucleocytoplasmic transport of polyA-tailed mRNA, including that of *MCL1*. We next performed nuclear/cytoplasmic fractionation of NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells, with or without dTAG<sup>V</sup>-1 treatment, followed by quantitative RT-PCR of *MCL1* mRNA. As expected, *MCL1* mRNA levels in the nucleus relative to those in the cytoplasm significantly increased following dTAG<sup>V</sup>-1 treatment (**Supplemental Figs. 3a and 3b**).

**Synthetic lethal interaction of selinexor and a single *DDX19* paralog underlies decreases in MCL1 levels**

We next asked whether synthetic lethal interaction between selinexor and *DDX19A* or *DDX19B* accounts for changes in MCL1 levels. To do so, we assessed levels of MCL1 and cleaved Caspase-3 proteins in selinexor-treated NALM-6 and KOPN49 cells transduced with control empty vector or *DDX19A* or *DDX19B* sgRNAs. MCL1 levels were barely changed by 10h selinexor treatment in empty vector-expressing cells (**Fig. 4a**), nor were they changed by *DDX19A* or *DDX19B* depletion in the absence of selinexor treatment (**Fig. 4a**). By contrast, combining selinexor treatment and depletion of either *DDX19A* or *DDX19B* significantly reduced MCL1 levels and increased levels of cleaved Caspase-3 (**Fig. 4a**). We next overexpressed MCL1 or empty vector in NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells, and treated them with either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). We then treated all 4 groups with selinexor (250nM) for 6 hours and monitored each for cleaved Caspase-3 levels by western blot. Interestingly, in selinexor-treated empty vector (control) cells, cleaved Caspase-3 expression was induced in both dTAG<sup>V</sup>-1- and negative ligand-treated

cells; however, Caspase-3 was not induced in selinexor-treated MCL1-overexpressing cells, either with or without dTAG<sup>V</sup>-1 treatment (**Fig. 4c**). Of note, MCL1 protein levels began to decrease at around 24 hours following dTAG<sup>V</sup>-1/selinexor treatment, and over time, cleaved Caspase-3 levels increased in these cells (**Fig. 4c**). Furthermore, MCL1 overexpression failed to rescue the growth disadvantage observed in selinexor-treated cells lacking either *DDX19A* or *DDX19B* over 6 days (**Supplemental Fig. 4**).

#### **DDX19A/B levels could serve as a biomarker for selinexor sensitivity**

Given the critical and apparently redundant role of DDX19A/B in intrinsic apoptosis, we hypothesized that tumors expressing low levels of either DDX19A or DDX19B would be particularly sensitive to selinexor. To test this, we assessed *DDX19A* and *DDX19B* mRNA levels in 159 lymphoid cell lines using DepMap datasets (DepMap Public 23Q4: <https://depmap.org/portal/>) and identified a subset of T-ALL lines (5 of 13 lines) that express *DDX19B* mRNA at markedly low levels (**Fig. 5a**). We validated these findings by analyzing DDX19A and DDX19B protein levels in Western blots of

197 seven T-ALL (MOLT-3, RPMI-8402, JURKAT, PEER, HPB-ALL, KE-37 and PF-382)  
198 lines (**Fig. 5b**). As predicted, T-ALL cells expressing low DDX19B levels were  
199 profoundly more sensitive to selinexor treatment than those expressing high  
200 DDX19B levels (**Fig. 5c**). Furthermore, *DDX19A* depletion, but not that of *DDX19B*,  
201 significantly attenuated fitness of MOLT-3 cells (**Fig. 5d**), an outcome in stark  
202 contrast to effects seen in NALM-6 or KOPN49 cells (**Fig. 1f**). To validate these  
203 findings, we depleted *DDX19A* via lentivirus-based Crimson-tagged sgRNA in  
204 MOLT-3 cells stably expressing HA-FKBP12<sup>F36V</sup>-tagged DDX19B (MOLT-3<sup>19B\_FK</sup>)  
205 and assessed cell growth over 6 days in the presence or absence of dTAG<sup>V</sup>-1  
206 treatment. *DDX19A* depletion significantly attenuated cell proliferation when  
207 exogenous DDX19B protein was depleted via dTAG<sup>V</sup>-1 treatment (**Fig. 5e**). We next  
208 established *DDX19A* KO MOLT-3 cells stably expressing HA-FKBP12<sup>F36V</sup>-tagged  
209 DDX19A (MOLT-3<sup>19A\_FK</sup>) and induced DDX19A depletion via dTAG<sup>V</sup>-1 treatment. As  
210 expected, DDX19A depletion significantly reduced MCL1 levels and induced intrinsic  
211 apoptosis in a BAX/BAK1-dependent manner (**Fig. 5f**). dTAG<sup>V</sup>-1-induced apoptosis  
212 was rescued by MCL1 overexpression, based on increased cleaved Caspase-3

213 levels (**Fig. 5g**).

214 Unlike results observed in NALM-6 and KOPN49 cells (**Fig. 4c**), selinexor  
215 treatment alone effectively decreased MCL1 levels and induced apoptosis in MOLT-  
216 3 cells (**Fig. 5h**). Notably, we observed comparable levels of cleaved Caspase-3  
217 upon selinexor treatment in MOLT-3 cells regardless of *TP53* status (**Fig. 5i**), and  
218 these results were validated by dose response curves: *TP53*-depleted MOLT-3 cells  
219 were only slightly less sensitive to selinexor (**Fig. 5j**). Importantly, overexpression of  
220 either DDX19A or DDX19B in MOLT-3 cells greatly attenuated effects of selinexor  
221 on cell viability, indicating that DDX19A/B dosage critically modulates selinexor  
222 sensitivity in these cells (**Fig. 5k**).

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## DISCUSSION

Selinexor, an XPO1 inhibitor, is the first-in-class selective inhibitor of nuclear export (SINE) and FDA-approved for treatment of DLBCL and MM. Selinexor exerts potent anti-tumor activity by inhibiting nuclear export of cancer-relevant proteins, including tumor suppressors, cell cycle regulators and factors functioning in the DNA damage response <sup>1-4</sup>. Selinexor also modulates RNA metabolism, as XPO1 regulates nuclear export of a series of RNA binding proteins <sup>1</sup>.

As a novel class of cancer drug, selinexor is promising due to its unique mechanism of action; in fact, selinexor is currently being evaluated either as monotherapy or a component of combination therapy to treat multiple cancers <sup>1-4</sup>. Notably, eltanexor, a second-generation XPO1 inhibitor, was recently granted orphan drug designation by the FDA to treat myelodysplastic syndromes (MDS). Despite its potent anti-tumor activity and novel mechanisms of action, dose-dependent toxicity and lack of biomarkers currently preclude wide usage of selinexor in oncology.

In eukaryotic cells, transport of macromolecules from the nucleus to the cytoplasm occurs Ran-dependently through the NPC, while small molecules

passively diffuse out of the nucleus <sup>1,3</sup>. Nuclear export of mRNA is a complex process orchestrated by sequential addition and/or subtraction of mRNA-binding proteins as mRNA is transcribed and undergoes nuclear processing, leading to assembly of an export-competent messenger ribonucleoprotein particle (mRNP) that contains mature mRNA and RNA export proteins, such as the NXF1 (nuclear RNA export factor 1) and NXT1 (nuclear transport factor 2 like export factor 1) <sup>18</sup>. Most mRNAs use the NXF1/NXT1 dimer for export through NPCs, while a subset of endogenous RNA species, such as ribosomal RNA (rRNA), small nuclear RNAs (snRNAs) and tRNAs, as well as viral RNAs, use XPO1 via RNA-binding proteins containing a NES <sup>18</sup>. After mRNPs transit through the NPC, cargo mRNA is disassembled from RNA-binding proteins. DDX19A/B proteins are reportedly found on cytoplasmic filaments of the NPC, and their helicase activity is required for mRNP disassembly, to conclude mRNA nucleocytoplasmic transport and allow translation to occur <sup>14, 18, 19</sup>.

We found that simultaneous depletion of both DDX19A and DDX19B significantly altered nuclear export of poly-A tailed mRNA, including *MCL1* mRNA, while these effects were absent following depletion of either DDX19A or DDX19B

alone (**Fig. 3c, d**), suggesting redundancy of these proteins. Furthermore, selinexor treatment combined with depletion of either *DDX19A* or *DDX19B*, but not selinexor treatment alone, markedly reduced MCL1 protein levels in leukemia cells (**Fig. 4a**). While we were preparing this manuscript, others also demonstrated synergistic effects of selinexor treatment with depletion of either *DDX19A* or *DDX19B* using CRISPR/Cas9 dropout screens of multiple myeloma (MM) and diffuse large B-cell lymphoma (DLBCL) cell lines. Mechanisms underlying these effects have not been identified <sup>20</sup>.

Importantly, MCL1 overexpression rescued intrinsic apoptosis promoted by *DDX19A/B* depletion (**Fig. 2c**), indicating that nuclear retention of *MCL1* mRNA is the primary cause of cell death caused by *DDX19A/B* depletion, despite the broad impact on nuclear export of polyA-tailed mRNA seen following *DDX19A/B* loss (**Fig. 3c**). Relative to many proteins, the half-life of MCL1 protein is quite short <sup>21</sup>; thus cells may be exceptionally vulnerable to decreases in levels of de novo MCL1 protein synthesis caused by *DDX19A/B* depletion. Of note, MCL1 overexpression failed to rescue the growth disadvantage seen in *DDX19A*- or *DDX19B*-depleted cells treated

with selinexor over 6 days (**Fig. 4c** and **Supplemental Figure 4**). Three factors, either alone or working in concert, could account for this observation. First, nuclear export of mRNA transcribed from exogenous, overexpressed *MCL1* might have been attenuated, as was endogenous *MCL1* mRNA, following selinexor treatment combined with either *DDX19A* or *DDX19B* depletion. Second, selinexor reportedly reduces MCL1 protein levels independently of DDX19A/B by regulating their stability and/or translation <sup>6, 22-25</sup>. Third, nuclear import/export of XPO1 and/or DDX19A/B targets relevant to cell death/proliferation (mRNAs and/or proteins) other than MCL1 might be compromised at later time points, collectively leading to attenuated cell growth regardless of MCL1 levels.

MCL1 protein does not contain a NES, and therefore it is unlikely that XPO1 directly regulates its cellular localization. Alternatively, XPO1 may regulate nuclear export of *MCL1* mRNAs through eIF4E (eukaryotic translation initiation factor 4E) <sup>26-28</sup>, a NES-containing protein. In fact, *MCL1* mRNA has been identified a target of phosphorylated eIF4E during oncogenesis <sup>22, 23</sup>. Others also report that selinexor perturbs MCL1 protein levels and/or their function by suppressing mTOR signaling

<sup>6</sup>, inhibiting MCL1/Bim interaction<sup>24</sup> or reducing overall *MCL1* mRNA levels<sup>25</sup>. Thus, the synthetic lethal interaction reported here may be due to synergy of these effects with decreased levels of cytoplasmic *MCL1* mRNA levels due to *DDX19* depletion, as observed here (**Fig. 6**).

We also report here that a subset of T-ALL lines expresses very low *DDX19B* levels (**Figs. 5a** and **5b**), and that all of these lines are sensitive to selinexor (**Fig. 5c**), a result consistent with the observed synthetic interaction between selinexor treatment and *DDX19* paralog dosage. Furthermore, selinexor induces intrinsic apoptosis regardless of *TP53* status (**Figs. 5i** and **5j**). These results could have clinical significance as they suggest that *DDX19A/B* expression levels could serve as a biomarker for selinexor sensitivity, independent of *TP53* status.

In summary, our study reveals, for the first time, that *DDX19* paralog dosage is critical for selinexor sensitivity by regulating *MCL1* mRNA nuclear export (**Fig. 6**). We propose that pharmacological inhibition of *DDX19A/B*, which are 96% homologous at the amino acid level, could serve as a novel class of SINE that specifically interferes with mRNA nuclear export.

## **METHODS**

### **Cell culture**

ALL cell lines were cultured in RPMI 1640 medium (FUJIFILM Wako Pure Chemical Corporation) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich or NICHIREI BIOSCIENCES INC.) and 100 U/mL penicillin-streptomycin (Nacalai Tesque Inc.) at 37°C with 5% CO<sub>2</sub>. NALM-6, RPMI-8402, and KE-37 cells were purchased from the DSMZ, while MOLT-3 cells were obtained from the Japanese Cancer Research Resources Bank (JCRB). JURKAT, PEER, and HPB-ALL cells were obtained from the RIKEN BRC. PF-382 and KOPN49 cells were described previously<sup>29, 30</sup>.

### **BaEV lentiviral transduction**

Lentiviral transduction was performed as described<sup>31</sup>. Briefly, HEK293T cells (#ACC 635, DSMZ) were cultured in DMEM (FUJIFILM Wako Pure Chemical Corporation) supplemented with 10% FBS and 1% penicillin streptomycin in 10 cm dishes. Cells were transfected with 1 mL OptiMem (Life Technologies) containing 8.6 µg psPAX2

(Addgene), 7.0 µg BaEVTR <sup>32</sup> and 8.6 µg lentiviral vectors using 60 µg linear polyethylenimine (Polysciences), and medium was changed 8 hours later. Lentiviral supernatants were harvested at 48 and 72 hours post-transfection and concentrated using a Lenti-X Concentrator according to the manufacturer's protocol (Takara Bio USA). After removing supernatants, pellets were resuspended in 200 µL OptiMem and stored at -80 °C until use. For infection, cells were plated in 12-well plates (1-3 × 10<sup>6</sup> cells per well) and incubated 2 hours with virus concentrates in the presence of 8 µg/mL polybrene (Santa Cruz Biotechnology), followed by 2 hours of spin-infection at 700 xg and 37 °C. Selection with either Blasticidin or Puromycin (FUJIFILM Wako Pure Chemical Corporation) began the following day.

### **CRISPR/Cas9 dropout screen and next-generation sequencing**

Cas9-expressing ALL lines were established as reported <sup>31</sup>. The Brunello library was purchased from Addgene (plasmid #73179). Plasmid pool preparation and sgRNA library transduction were performed as described <sup>31, 33</sup>. sgRNA-transduced cells were then selected 2 days in 2 µg/mL puromycin starting the day after transduction.

At least  $4 \times 10^7$  cells were harvested on the third day after transduction to obtain input DNA, ensuring representation of >500 selected cells per guide RNA. Those cells were maintained for 7 days post-transduction and then divided into groups treated with either 0.1% DMSO or selinexor (100nM, NALM-6 cells; 40nM, KOPN49 cells). After 10 more days of incubation, at least  $4 \times 10^7$  cells were harvested, and genomic DNA was extracted from collected cell pellets using a QIAamp DNA Blood Midi Kit or QIAamp DNA Blood Maxi Kit (QIAGEN). Library preparation was performed as described<sup>31, 33</sup>. sgRNA inserts were PCR-amplified from 110 µg of genomic DNA using Herculase 2 fusion DNA polymerase (Agilent). Resulting PCR products were purified and sequenced on a NextSeq500 sequencer (Illumina) to assess potential changes in sgRNA abundance between initial and final cell populations, and between cell populations treated with or without selinexor (**Supplemental Table 1**).

#### **CRISPR/Cas9-mediated gene knockout**

sgRNA sequence oligos derived from the Brunello library for *DDX19A*, *DDX19B*,

353 *BAX*, *BAK1* or *TP53* were cloned into either lentiGuide-Puro (Addgene), lentiGuide-  
354 Crimson (Addgene plasmid #70683), lentiGuide-humanized Kusabira Orange 1  
355 (hKO1) or lentiGuide-BFP<sup>16</sup>. sgRNA sequences were as follows: *DDX19A*:  
356 GCCATCAGACAGATACCCCC; *DDX19B*: TACGGCTCAGCAAGCATCAG; *BAX*:  
357 GTTTCATCCAGGATCGAGCA; *BAK1*: ACGGCAGCTCGCCATCATCG; *TP53*:  
358 CCATTGTTCAATATCGTCCG. Lentivirus transduction was performed as  
359 described above.

360

## 361 **Immunoblotting**

362 Cells were harvested and washed once with PBS. For whole cell lysates cells were  
363 lysed in 2 or 4× Laemmli sample buffer (Bio-Rad Laboratories, Inc.) diluted with  
364 water containing 2.5% 2-mercaptoethanol (FUJIFILM Wako Pure Chemical  
365 Corporation), followed by sonication and heat denaturation. Lysates were separated  
366 by SDS-PAGE and transferred to PVDF membranes, which were then blocked with  
367 5% nonfat milk in TBS plus 0.5% Tween-20 (Nacalai Tesque Inc.). The following  
368 antibodies were used for Western blotting: *DDX19A* (AV36455, Sigma-Aldrich),

DDX19B (SAB4500242, Sigma-Aldrich), BAX (ab32503, abcam), BAK1 [#12105, Cell Signaling Technology (CST)], BCL2 (sc-7382, Santa Cruz Biotechnology), MCL1 (#94296, CST), BCL2L1 (#2764, CST), MYC (#626802, BioLegend), XPO1 (sc-74454, Santa Cruz Biotechnology), cleaved Caspase-3 (#9661, CST), TP53 (sc-126, Santa Cruz Biotechnology), GAPDH (#5174, CST) and LMNB1 (ab16048, abcam). To analyze multiple proteins blotted onto one membrane, we divided the membrane into horizontal strips based on target protein molecular weight and stained strips separately with appropriate antibodies. Proteins of similar molecular weight were blotted onto separate membranes. GAPDH served as a loading control.

#### **Cell viability and apoptosis assays**

Cell viability and apoptosis were assessed as described<sup>16, 31</sup>. Annexin V Binding Buffer (BD Biosciences), FITC-Annexin V antibody (BioLegend) and either Propidium Iodide (PI) (BioLegend) or DAPI (Thermo Scientific) were used to detect apoptotic cells by FACS.

### **Cell competition assay**

Cells were transduced with lentivirus vectors encoding sgRNAs and a Crimson reporter protein. Transduced cells were co-cultured with non-transduced cells and treated with either selinexor or DMSO vehicle. Proportions of transduced cells were monitored over time by FACS.

### **mRNA FISH assay**

Cells were treated 3 hours with either dTAG<sup>V</sup>-1 or negative ligand and then cytopun onto glass slides using a Thermo Scientific Cytospin 4 Centrifuge (Thermo Scientific). Cells were then fixed 15 minutes with 4% PFA and permeabilized 5 minutes with 100% methanol, followed by overnight hybridization at 37°C with FAM-labelled oligos targeting either polyA-tailed mRNA or *MCL1* mRNA. To make FAM-labeled probes, we designed six oligonucleotides for *MCL1* mRNA and one for polyA-tailed mRNA. Their sequences are following:

*MCL1* #1: (AAGAACTCCACAAACCCATCCCAGCCTCTTTGTTTAACTA);

*MCL1* #2: (TCGTTTTTGTATGTCCAGTTTCCGAAGCATGCCTTGGAAGG);

401 *MCL1* #3: (ACAATCCTGCCCCAGTTTGTACGCCGTCGCTGAAAACAT);

402 *MCL1* #4: (AAACCAGCTCCTACTCCAGCAACACCTGCAAAAGCCAGCA);

403 *MCL1* #5: (CCCCACAGTAGAGGTTGAGTCCGATTACCGCGTTTCTTTT);

404 *MCL1* #6: (TGGTGTTATTACCAGATTCCCCGACCAACTCCAGCAGCGG);

405 FAM-dT(30): TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT.

406 Hybridization buffer consisted of 10% formamide (Sigma-Aldrich), 10% dextran

407 sulfate (Sigma-Aldrich), and 0.1 mg/mL herring sperm DNA (Sigma-Aldrich) in 2x

408 SSC buffer (NIPPON GENE CO., LTD.). Post-hybridization, cells were washed twice

409 with 2x SSC buffer and once with 0.2x SSC buffer, mounted with DAPI/mounting

410 solution (Thermo Scientific) and sealed with nail polish. For each condition, cells in

411 multiple fields were randomly imaged using an LSM 700 confocal microscope (Carl

412 Zeiss). Quantification of nuclear and cytoplasmic all polyA-tailed mRNA or *MCL1*

413 mRNA fluorescence signals was performed using ImageJ software

414 (<https://imagej.nih.gov/ij/>). Nuclear regions were defined by DAPI positivity, and

415 cytoplasmic based on cell contour and DAPI staining.

416

417 **Actinomycin D chase assay**

418 Cells were plated into 12-well plates ( $1 \times 10^6$  cells per well) with medium  
419 supplemented with 5  $\mu\text{g/mL}$  Actinomycin D and treated with either dTAG<sup>V</sup>-1 (500nM)  
420 or negative ligand (500nM). RNA was extracted using the RNeasy Mini Kit (Qiagen)  
421 according to the manufacturer's instructions. RNA was reverse transcribed using the  
422 ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO Co., Ltd.). q-  
423 RT-PCR was performed with THUNDERBIRD® SYBR® qPCR Mix (TOYOBO CO.,  
424 LTD.) using the Mx3000P qPCR System (Agilent Technologies, Inc.). Expression  
425 levels of target genes relative to those of *GAPDH* were calculated using the  
426 comparative CT method. Primer sequences used for q-RT-PCR were as follows:

427 *MCL1* Fw: TGCTTCGGAAACTGGACATCA;

428 *MCL1* Rv: TAGCCACAAAGGCACCAAAAAG;

429 *GAPDH* Fw: GGAGCGAGATCCCTCCAAAAT;

430 *GAPDH* Rv: GGCTGTTGTCATACTTCTCATGG.

431

432 **Cycloheximide chase assay**

433 Cycloheximide chase assays were performed as described <sup>34</sup>. Cells were plated into  
434 12-well plates ( $1 \times 10^6$  cells per well) with medium supplemented with 100 µg/mL  
435 Cycloheximide and treated with either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM).  
436 Cells were harvested at specified time intervals following Cycloheximide treatment,  
437 and protein levels were assessed by Western blots.

438

#### 439 **Nuclear/cytoplasmic fractionation**

440  $4.0 \times 10^6$  cells were treated 3 hours with either dTAG<sup>V</sup>-1 (500 nM) or dTAG<sup>V</sup>-1-NEG  
441 (500 nM), washed with ice-cold PBS, centrifuged, and subjected to cytosolic and  
442 nuclear fractionation using 400 µL of ice-cold Buffer A (25 mM Tris-HCl, 50 mM NaCl,  
443 0.5% Triton X-100) with gentle pipetting. 200 µL was used for each protein (for  
444 Western blotting) and RNA (for RT-q-PCR) analysis. For both applications, solutions  
445 were centrifuged at 600g for 5 min at 4°C, and supernatants were used as the  
446 cytoplasmic fraction, while pellets were resuspended in 100 µL of Buffer B (25 mM  
447 Tris-HCl, 700 mM NaCl) and served as the nuclear fraction. RNA samples were  
448 prepared using QIAzol and a miRNeasy Mini Kit (QIAGEN) following the

manufacturer's specifications, and their quality and quality were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific) and Qubit RNA Broad Range Assay Kits (Thermo Scientific).

### **Plasmids and reagents**

Plasmids used in this study were as follows: psPAX2 (Plasmid #12260, Addgene), BaEV-TR (kind gifts from Dr. Els Verhoeyen), lentiCas9-Blast (Plasmid #52962, Addgene), lentiGuide-Puro (kind gifts from Dr. Dan Bauer), lentiGuide-Crimson (Plasmid #70683, Addgene), lentiGuide-hKO1, lentiGuide-BFP, CSII-EF-MCS-IRES2-TagBFP, CSII-EF-MCL1-IRES2-TagBFP and Lenti\_EFS\_CdTAG\_PuroR<sup>16</sup>. Lenti\_EFS\_CdTAG\_HA-DDX19A\_PuroR and Lenti\_EFS\_CdTAG\_HA-DDX19B\_PuroR were generated using the Lenti\_EFS\_CdTAG\_PuroR vector. Details of plasmids will be provided upon request. The following compounds were used: dTAG<sup>V</sup>-1 and dTAG<sup>V</sup>-1-NEG (Tocris Bioscience); selinexor (S7252, Selleck Biotech); Actinomycin-D (A9415, Sigma-Aldrich) and Cycloheximide (#601105, Cayman Chemical).

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## **AUTHOR CONTRIBUTIONS**

620 T.T., Y.S., T.Y., and T.M. designed CRISPR-Cas9 screen experiments. T.T., Y.S., K.S.,  
621 T.Y., H.I., T.S., K. Akashi and T.M. reviewed CRISPR screen data. T.T., K.S., Y.S.,  
622 T.Y., K.S., S.H., H.I. and F.N. executed the CRISPR-Cas9 and cell biology  
623 experiments. K. Akahane and T. I. provided KOPN49 cells. T.T. and T.M. wrote the  
624 manuscript with help from all authors.

625

## 626 **Conflict-of-interest disclosure**

627 The authors declare that they have no conflicts of interest.

628

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633

## 634 **Figure legends**

635 **Fig. 1 Genome-wide CRISPR-Cas9 screens identify *DDX19* paralogs as key**  
636 **modulators of selinexor sensitivity. a** Schematic showing protocol used for the  
637 CRISPR-Cas9 screen in the presence or absence of selinexor. Cas9-expressing  
638 NALM-6 and KOPN49 cells were transduced with the Brunello library, selected in  
639 puromycin, and cultured 7 days, followed by 10 days of treatment with either DMSO  
640 or selinexor (100nM, NALM-6 cells; 40nM, KOPN49 cells). sgRNA abundance before  
641 and after culture was assessed as described <sup>33</sup>. **b** Dot graphs representing  $\beta$ -scores  
642 of 19,114 genes in the presence or absence of selinexor. Genes whose  $\beta$ -scores in  
643 both cell lines reflected a statistically significant change upon selinexor treatment  
644 (FDR <0.1) are depicted in red. **c** Genes whose sgRNA abundance was significantly  
645 altered by selinexor treatment (FDR <0.1) were assessed in both lines using the  
646 DrugZ program and are depicted in red. **d** Competition assays: NALM-6 and  
647 KOPN49 cells were transduced via CRISPR/Cas9 gene targeting with a lentivirus  
648 vector encoding sgRNA targeting *DDX19A* or *DDX19B* plus a Crimson cassette.  
649 Cells were treated with DMSO or selinexor (100nM), and proportions of Crimson-

650 positive cells were assessed over 6 days by FACS. Empty vector served as control.

651 Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD.

652 *P* values were calculated using an unpaired *t* test. n.s.: not significant, \**P*<0.0001. **e**

653 Cells expressing sgRNA targeting either *DDX19A* or *DDX19B* were treated with

654 selinexor at indicated concentrations and counted 48 hours later. Cells transduced

655 with empty vector served as controls. Counts at each dose were normalized to those

656 of control cells not treated with selinexor. Each condition was assessed in triplicate,

657 and data are represented as means  $\pm$  SD. **f, g** Competition assays: NALM-6 and

658 KOPN49 cells lacking *DDX19B* (*DDX19A* WT, *DDX19B* KO) were generated via

659 CRISPR/Cas9 gene targeting. Cells were transduced with a lentivirus vector

660 encoding sgRNAs targeting either *DDX19A* or *DDX19B* plus a Crimson cassette.

661 Proportions of Crimson-positive cells were assessed over 6 days by FACS. Empty

662 vector served as control. Proportions of Crimson-positive cells normalized to those

663 at day 0 (two days after transduction) are shown. Empty vector (EV) served as a

664 control. Each condition was assessed in triplicate, and data are represented as

665 means  $\pm$  SD. *P* values were calculated using an unpaired *t* test or one-way ANOVA

with Dunnett's post-test for multiple comparisons. n.s.: not significant, \* $P < 0.01$ ,  
\*\* $P < 0.0001$ .

**Fig. 2 Co-depletion of *DDX19A* and *DDX19B* decreases MCL1 protein levels**

**and induces intrinsic apoptosis. a** Annexin V assay using *DDX19A/DDX19B*

double KO (DKO) NALM-6 cells overexpressing HA-FKBP12<sup>F36V</sup>-tagged codon-

optimized *DDX19A* (NALM-6<sup>19A\_FK</sup> *DDX19B* KO). Cells were treated with either

dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM) for indicated times, and proportions of

apoptotic cells were assessed by Annexin V assay. Each condition was assessed in

triplicate, and data are represented as means  $\pm$  SD. P values were calculated using

an unpaired t-test. n.s.: not significant. **b** NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells were

treated with dTAG<sup>V</sup>-1 (500nM) for indicated times, and protein samples were

prepared. Protein levels of *DDX19A*, *DDX19B*, MCL1, and cleaved Caspase-3 (c-

Casp3) were assessed by Western blots. GAPDH served as a loading control. Since

the sizes of *DDX19A* (478 aa) and *DDX19B* (479 aa) proteins are very similar, we

assessed those protein levels independently using the same cell lysates. exo:

exogenous; endo: endogenous. **c** NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells, either wild-type or doubly-mutant in *BAX/BAK1* (*BAX/BAK1* DKO), were treated 7 hours with either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). Indicated proteins were assessed by Western blots. GAPDH served as a loading control. **d** NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells transduced with either empty vector or an *MCL1* expression vector were each treated 6 hours with either dTAG<sup>V</sup>-1 (500nM) or dTAG<sup>V</sup>-1-NEG (500nM), and indicated proteins were assessed by Western blots. GAPDH served as a loading control.

**Fig. 3 DDX19 paralogs regulate nucleocytoplasmic transport of *MCL1* mRNA.**

**a** *MCL1* mRNA stability was assessed via Actinomycin-D chase assay using NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells. *MCL1* mRNA levels were assessed by real-time q-RT-PCR following either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM) treatment in the presence of Actinomycin-D (5 µg/mL). *P* values were calculated using an unpaired *t* test. n.s.: not significant. **b** *MCL1* protein stability was assessed via a Cycloheximide chase assay. NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells were treated with Cycloheximide (100

698  $\mu\text{g/mL}$ ) in the presence of dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). Protein  
699 lysates were obtained at indicated time points, and protein levels were assessed by  
700 Western blots. MYC, a protein with a short half-life, served as a control for  
701 Cycloheximide treatment. GAPDH served as a loading control. *exo*: exogenous;  
702 *endo*: endogenous. **c** RNA-FISH for polyA-tailed mRNA was performed following 3h  
703 of treatment with either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). DAPI was  
704 used for a nuclear stain. Nuclear and cytoplasmic fluorescent signal intensities were  
705 measured, and their ratios were calculated for each cell. A total of 97 and 99 cells  
706 were examined for dTAG<sup>V</sup>-1-NEG and dTAG<sup>V</sup>-1 treated groups, respectively. *P*  
707 values were calculated using an unpaired *t* test. \**P* < 0.0001. **d** RNA-FISH for *MCL1*  
708 mRNA was performed as described in (c). A total of 81 and 105 cells were examined  
709 for dTAG<sup>V</sup>-1-NEG and dTAG<sup>V</sup>-1 treated groups, respectively. *P* values were  
710 calculated using an unpaired *t* test. \**P* < 0.0001.

711  
712 **Fig. 4 Selinexor treatment synergizes with loss of either *DDX19A* or *DDX19B***  
713 **to induce intrinsic apoptosis by reducing *MCL1* levels. a** NALM-6 or KOPN49

cells transduced with empty vector or sgRNA targeting either *DDX19A* or *DDX19B* were treated 10 hours with selinexor at indicated concentrations. Levels of indicated proteins were assessed by Western blots. GAPDH served as a loading control. Given the similar size of *DDX19A* (478 aa) and *DDX19B* (479 aa) proteins, we assessed their protein levels independently using the same cell lysates. **b** NALM-6<sup>19A\_FK</sup> *DDX19B* KO cells transduced with either empty vector or an MCL1 expression vector were treated 6 hours with selinexor (250nM) in the presence of either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). Indicated protein levels were assessed by Western blots. GAPDH served as a loading control. *exo*: exogenous; *endo*: endogenous. **c** Indicated protein levels were assessed at various time points up to 48 hours.

**Fig. 5 DDX19A/B levels could serve as a biomarker for selinexor sensitivity, independent of TP53 status. a** Depmap datasets showing mRNA levels of *DDX19A* and *DDX19B* among lymphoid cell lines (n=159). X- and Y-axes represent expression levels of *DDX19B* and *DDX19A*, respectively. **b** Western blots showing

730 levels of DDX19A and DDX19B protein in seven T-ALL lines. GAPDH served as a  
731 loading control. Although *DDX19B* mRNA levels in JURKAT cells were reportedly  
732 high in the DepMap dataset (a), DDX19B protein was barely detectable by Western  
733 blot in our JURKAT cells. **c** Cells were treated 48 hours with Selinexor at various  
734 concentrations, and dose response curves were generated. Each condition was  
735 assessed in triplicate, and data are represented as means  $\pm$  SD. **d** MOLT-3 cells  
736 were transduced with empty vector or with lentivirus vectors encoding sgRNAs  
737 targeting either *DDX19A* or *DDX19B*, plus a Crimson cassette. Competition assays  
738 were performed as described in Fig. 1f. Each condition was assessed in triplicate,  
739 and data are represented as means  $\pm$  SD. *P* values were calculated using one-way  
740 ANOVA with Dunnett's post-test for multiple comparisons. \**P*<0.001, \*\**P*<0.0001. **e**  
741 MOLT-3 cells overexpressing HA-FKBP12<sup>F36V</sup>-tagged codon-optimized DDX19B  
742 (MOLT3<sup>19B\_FK</sup>) were established. Cells were then transduced with a lentivirus vector  
743 encoding sgRNA targeting *DDX19A* and a Crimson cassette and cultured 6 days  
744 with either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). Proportions of Crimson-  
745 positive (*DDX19A*-deleted) cell fractions at indicated time-points were assessed by

746 FACS, as described in Fig. 1f. Each condition was assessed in triplicate, and data  
747 are represented as means  $\pm$  SD. *P* values were calculated using an unpaired *t* test.  
748 \**P*<0.0001. **f** *DDX19A* KO MOLT-3 cells overexpressing HA-FKBP12<sup>F36V</sup>-tagged  
749 codon-optimized *DDX19A* (MOLT3<sup>19A\_FK</sup>) or comparable cells lacking both *BAX* and  
750 *BAK1* (*BAX/BAK1* DKO) were treated 5 hours with either dTAG<sup>V</sup>-1 (500nM) or  
751 negative ligand (500nM). Indicated proteins were assessed by Western blots.  
752 GAPDH served as a loading control. exo: exogenous; endo: endogenous. **g** MOLT-  
753 3<sup>19A\_FK</sup> cells transduced with empty vector or with an MCL1 expression vector were  
754 treated 4 hours with either dTAG<sup>V</sup>-1 (500nM) or negative ligand (500nM). Indicated  
755 proteins were assessed by Western blots. GAPDH served as a loading control. **h**  
756 MOLT-3 cells were treated 10 hours with selinexor at indicated concentrations, and  
757 XPO1, *DDX19A*, MCL1 and cleaved Caspase-3 proteins were assessed by Western  
758 blots. GAPDH served as a loading control. **i** Wild-type MOLT-3 cells or those lacking  
759 *TP53* (*TP53* KO) were treated 24 hours with selinexor (1 $\mu$ M), and levels of cleaved-  
760 Caspase-3 and *TP53* were assessed by western blots. GAPDH served as a loading  
761 control. **j** *TP53*-WT and -KO MOLT3 cells were treated 48 hours with selinexor at

various concentrations, and dose response curves were generated. Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD. **k** MOLT-3 cells either wild-type or overexpressing either DDX19A or DDX19B were treated 48 hours with selinexor at various concentrations, and dose response curves were generated. Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD.

**Fig. 6 Proposed model of the synthetic lethal interaction between selinexor treatment and loss of a *DDX19* paralog.** During mRNA nuclear export, mature mRNA and mRNA-binding proteins (RBPs) assemble into an export-competent messenger ribonucleoprotein particle (mRNP). RBPs required for mRNP formation and nuclear export include NXF1 (nuclear RNA export factor 1), NXT1 (nuclear transport factor 2-like export factor 1), and the TREX (TRanscription-EXport) complex<sup>18</sup>. DDX19A/DDX19B helicase activity is essential for disassembling mRNPs, stopping mRNA nucleocytoplasmic transport, and initiating translation (left panel). In the absence of both *DDX19A* and *DDX19B* (double knockout: DKO), polyA-tailed mRNAs, including *MCL1* mRNA, accumulate in the nucleus due to

778 impaired export to the cytoplasm, reducing both MCL1 protein levels and its anti-  
779 apoptotic activity (middle panel). When either *DDX19A* or *DDX19B* is depleted,  
780 *MCL1* mRNA nuclear export is partially attenuated, but MCL1 protein levels are  
781 maintained at levels sufficient for cell survival. Selinexor reportedly perturbs MCL1  
782 protein levels and/or function via multiple mechanisms, including attenuating protein  
783 translation and/or stability<sup>6, 24-28</sup>. Thus, the synthetic lethal interaction reported may  
784 be due to synergy between those effects and decreases in cytoplasmic *MCL1* mRNA  
785 levels observed here following loss of a *DDX19* paralog.

## **Supplemental Figure Legends**

### **Supplemental Figure 1. Analysis of cell growth in the presence of selinexor.**

NALM-6 and KOPN49 cells were cultured 9-10 days with indicated concentrations of selinexor, and growth curves were generated. Results of two independent experiments are shown. Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD.

### **Supplemental Figure 2. Signature/Pathway analysis of genes identified as relevant to selinexor sensitivity.**

Bar graph showing the top 20 biological processes /pathways significantly enriched among genes whose sgRNAs were significantly depleted in the presence of selinexor.

### **Supplemental Figure 3. Assessment of nuclear *MCL1* mRNA levels by RT-**

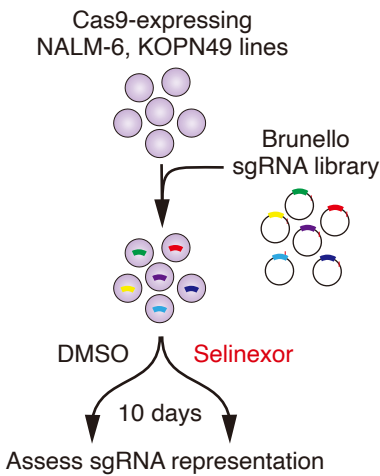
**qPCR. a** Nuclear (N) and cytoplasmic (C) fractions were validated by Western blots.

Lamin B1 (LMNB1) served as a nuclear marker and GAPDH as a cytoplasmic marker. W: whole cell lysate. **b** RT-qPCR for *MCL1* mRNA was also performed using nuclear and cytoplasmic mRNA following dTAG<sup>V</sup>-1 or negative ligand treatment, and *MCL1* mRNA values were normalized to cell number to compare their relative levels in each fraction between experimental conditions. Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD.

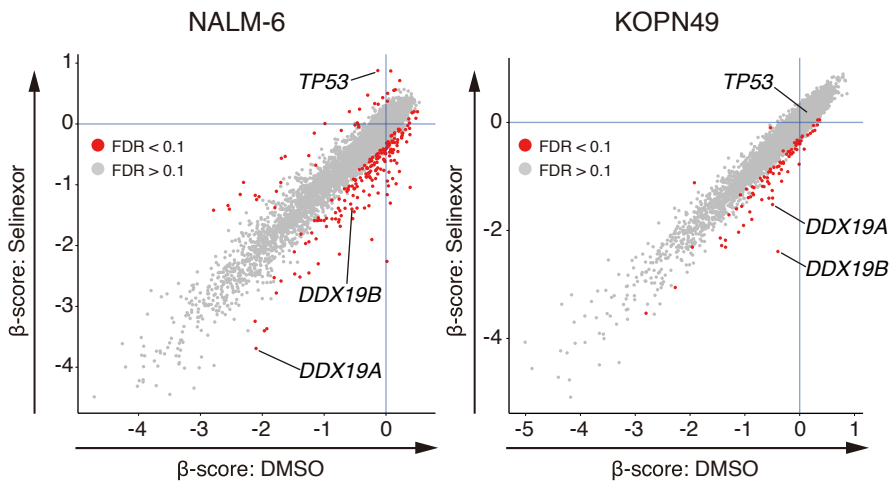
**Supplemental Figure 4. Growth curves of MCL1-overexpressing cells over an extended period.** Control and MCL1-overexpressing NALM-6 and KOPN49 cells were transduced with a lentivirus vector encoding sgRNA targeting *DDX19A* or *DDX19B* plus a Crimson cassette via CRISPR/Cas9 gene targeting. Cells were treated with DMSO or selinexor (100nM), and proportions of Crimson-positive cells were assessed over 6 days by FACS. Empty vector served as control. Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD.

Figure 1

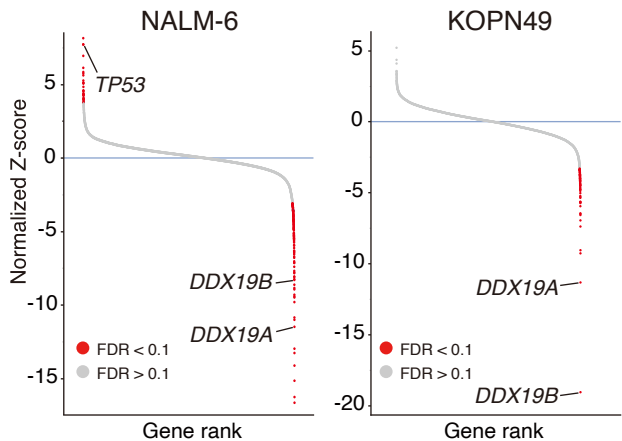
**a**



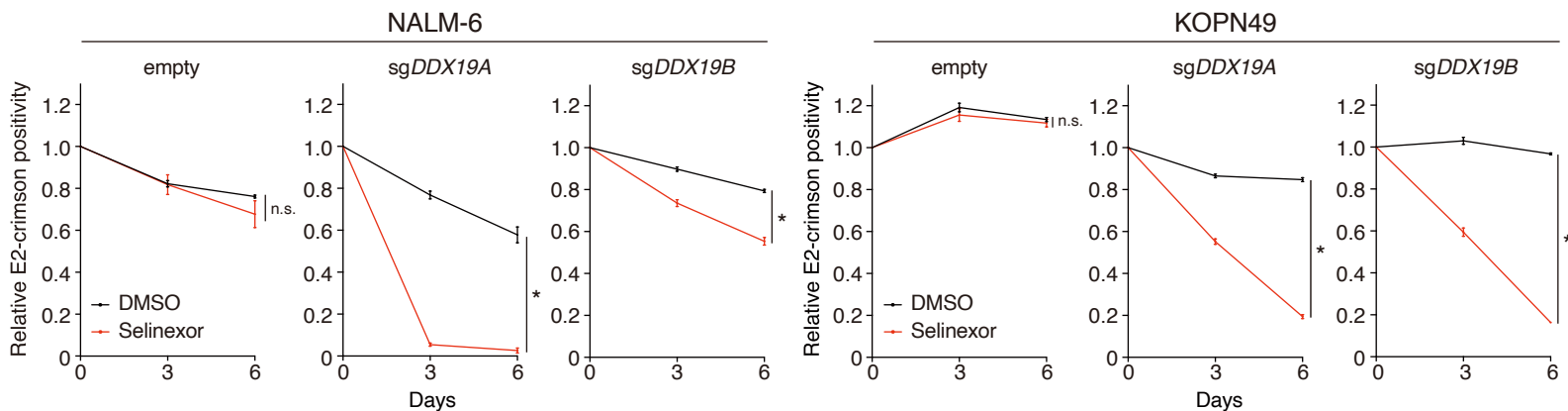
**b**



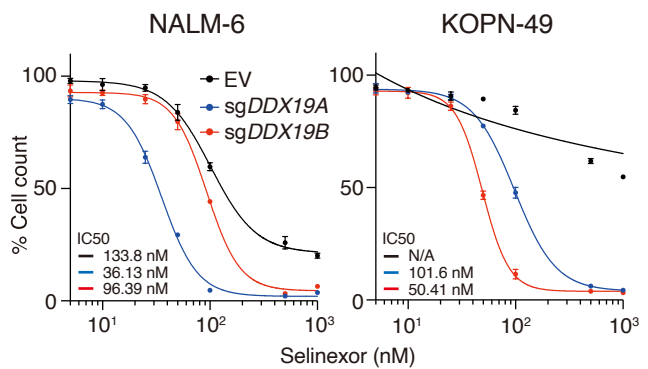
**c**



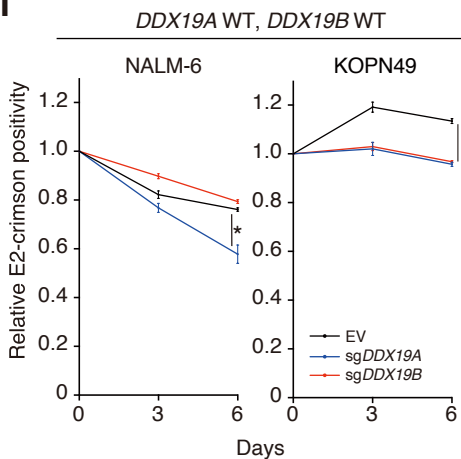
**d**



**e**



**f**



**g**

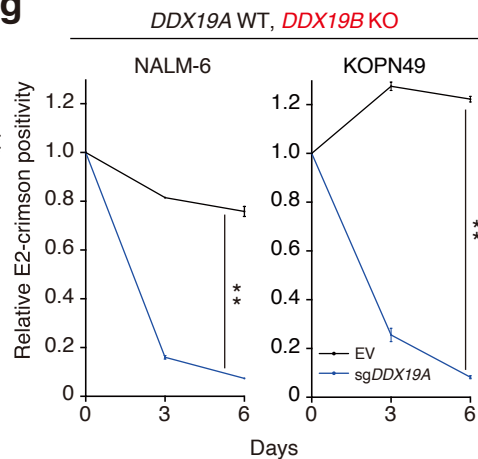


Figure 2

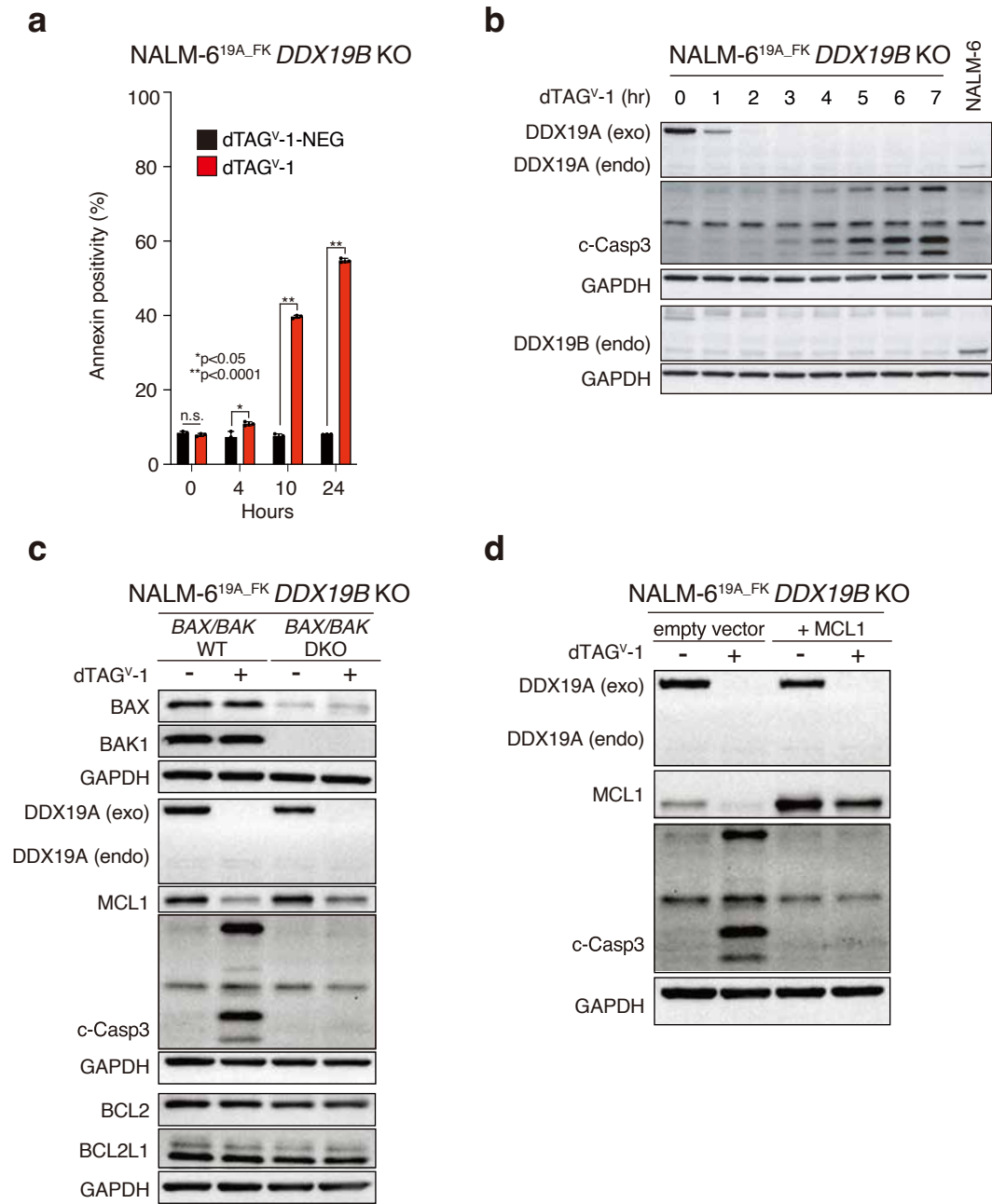


Figure 3

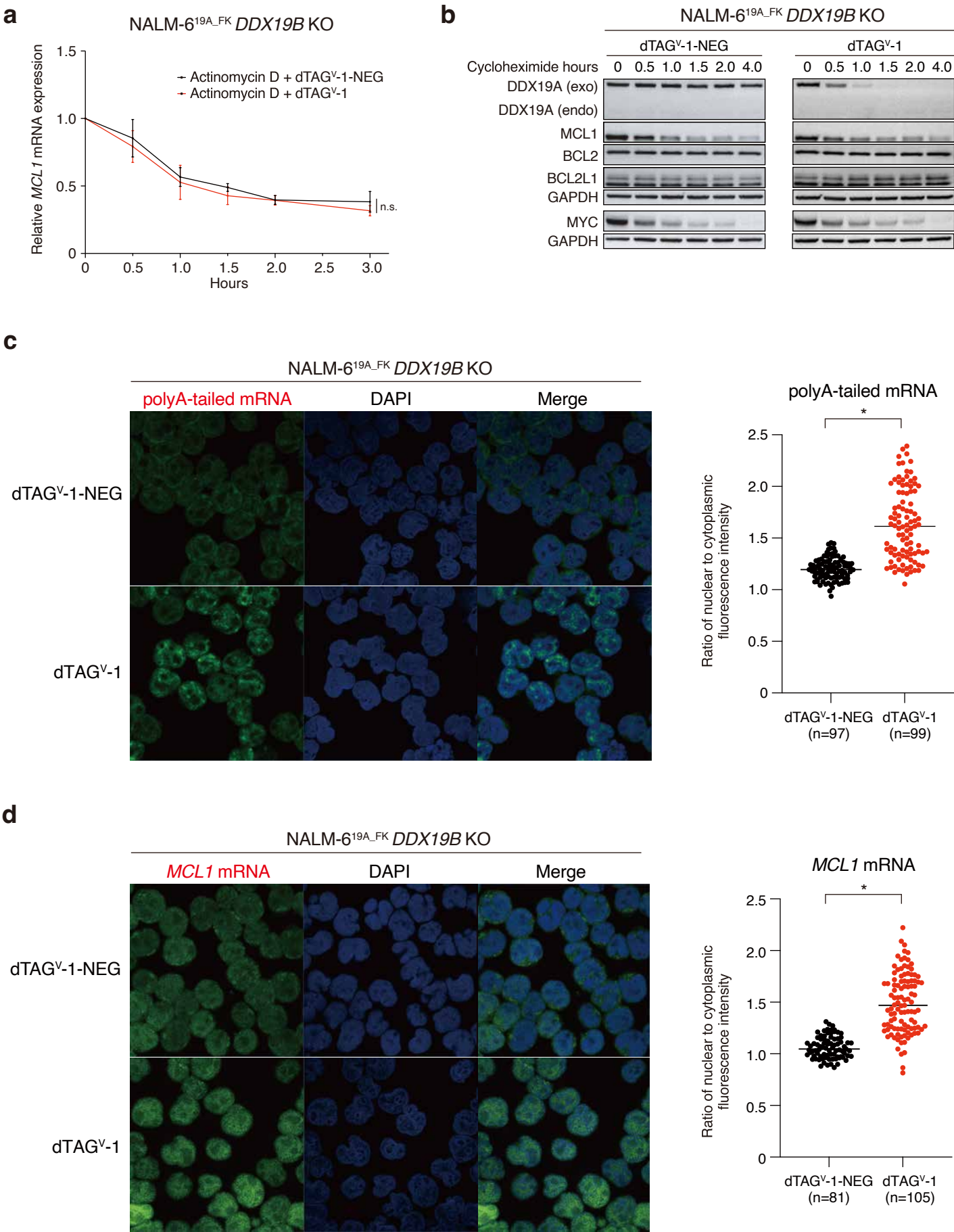


Figure 4

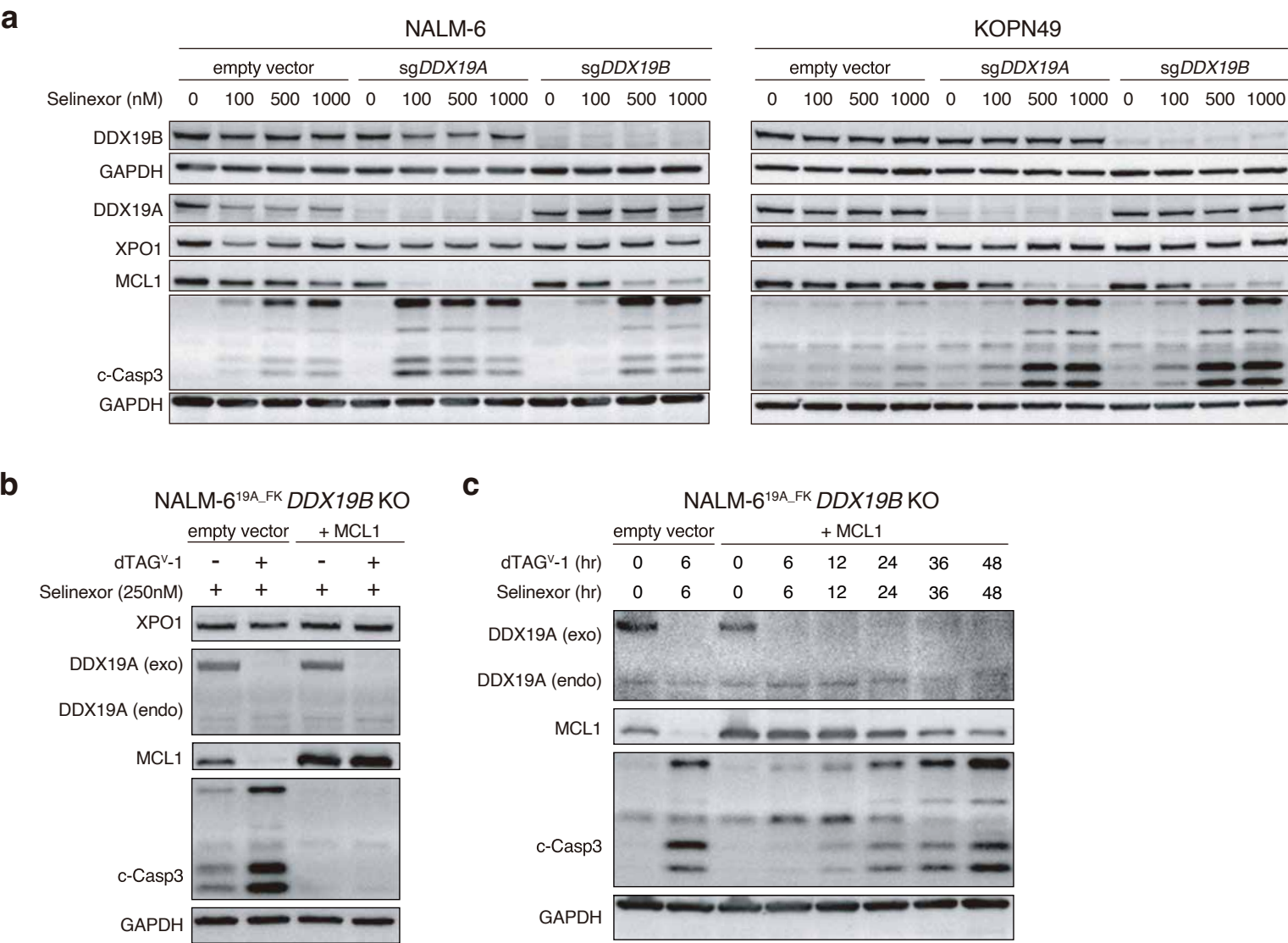


Figure 5

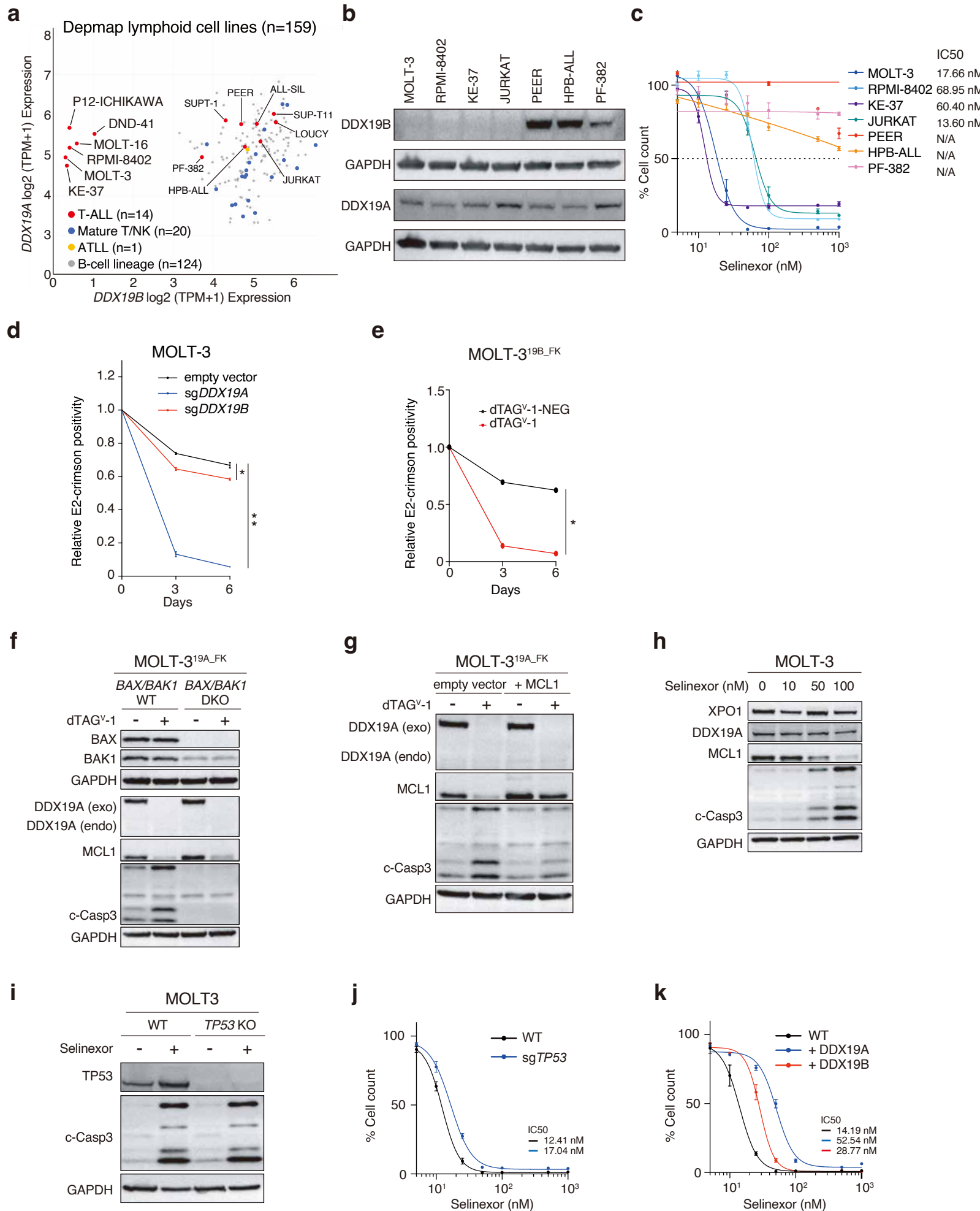
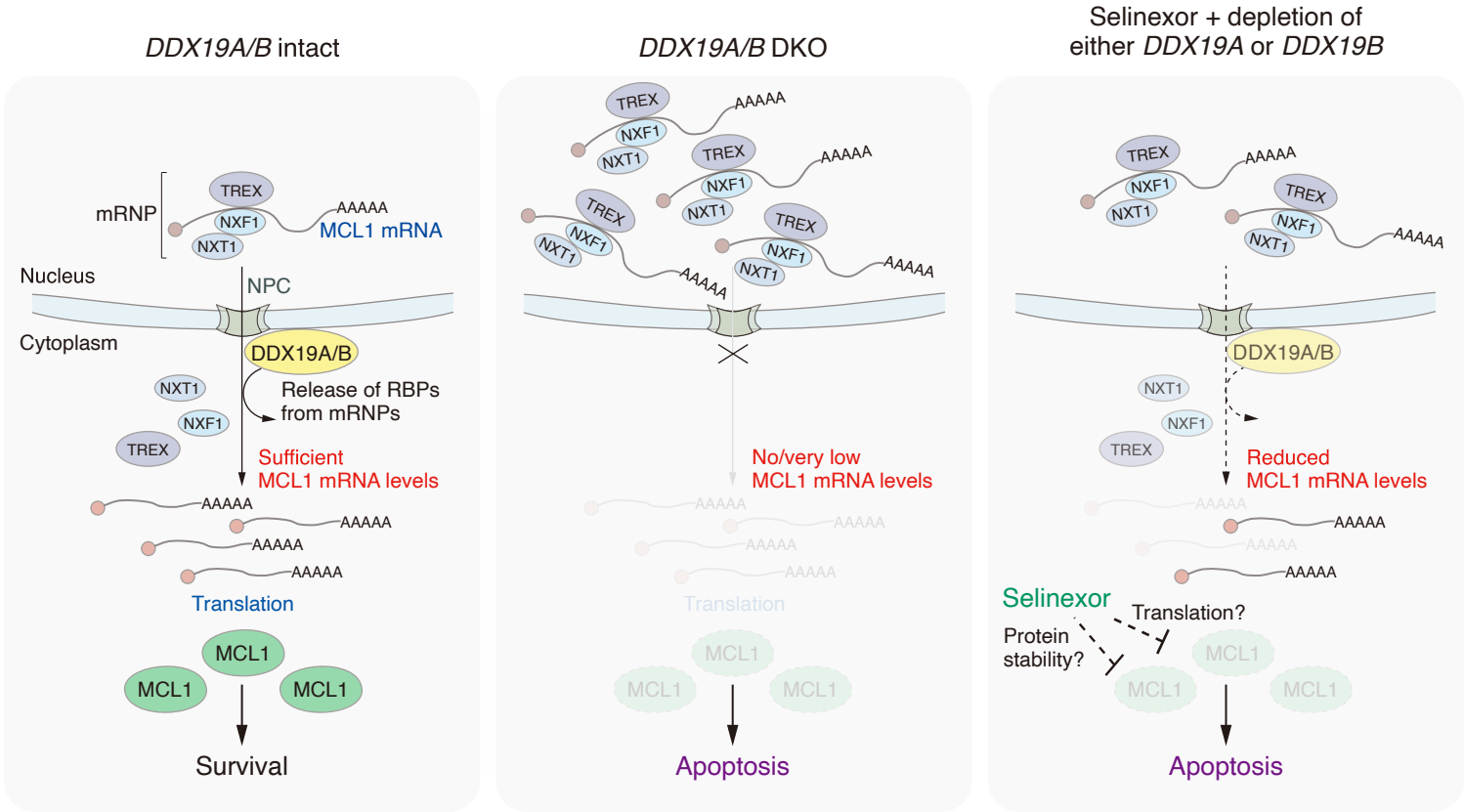
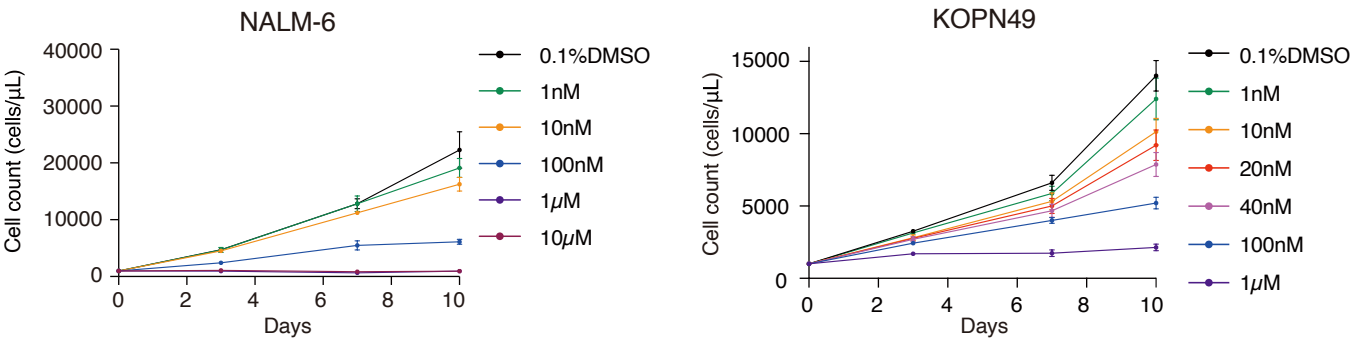


Figure 6

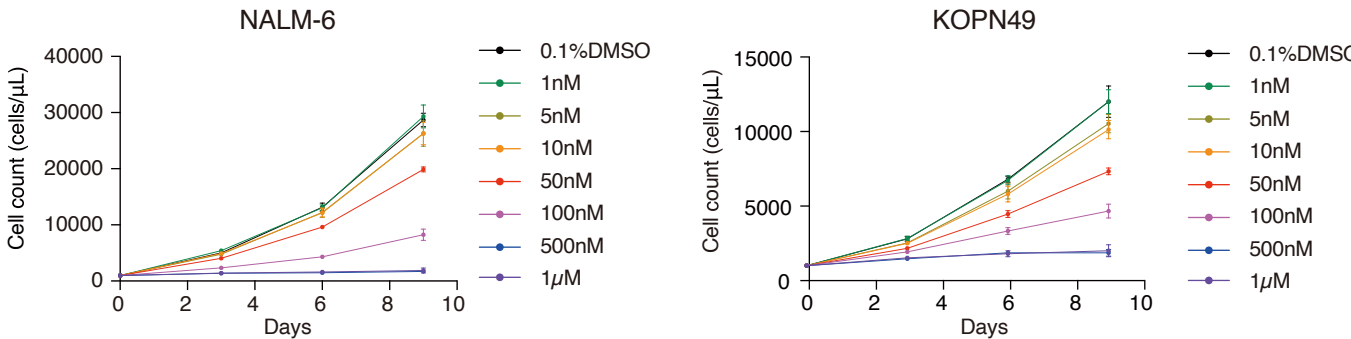


# Supplemental Figure 1

Experiment #1



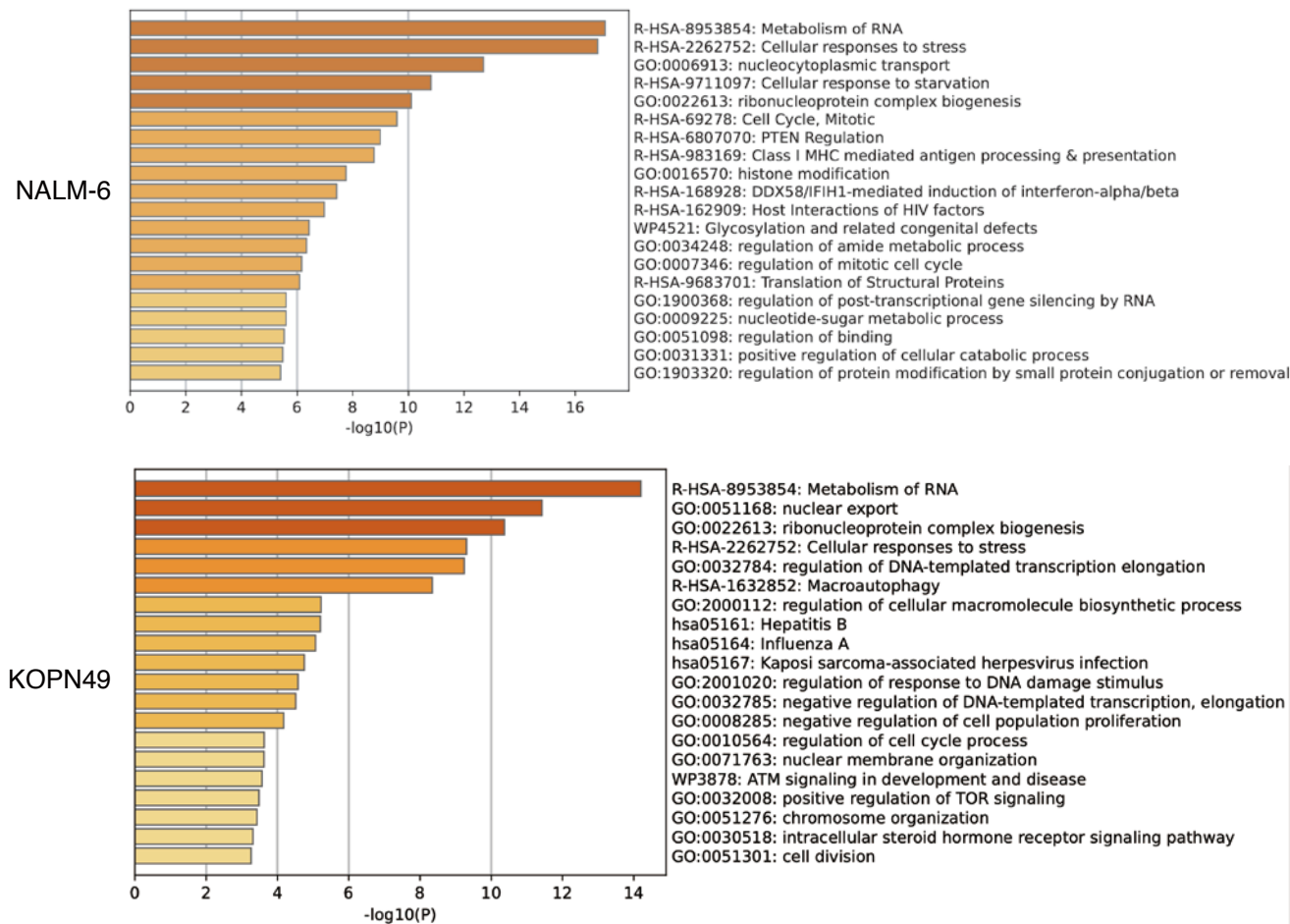
Experiment #2



**Supplemental Figure 1. Analysis of cell growth in the presence of selinexor.** NALM-6 and KOPN49 cells were cultured 9-10 days with indicated concentrations of selinexor, and growth curves were generated. Results of two independent experiments are shown. Each condition was assessed in triplicate, and data are represented as means ± SD.

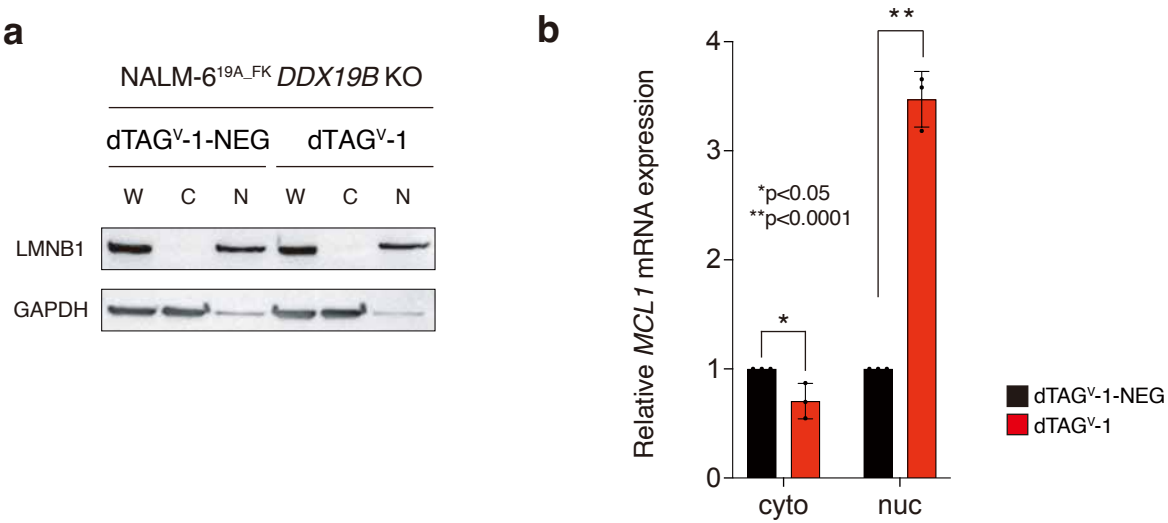
Supplemental Figure 2

Genes significantly depleted in the presence of selinexor



**Supplemental Figure 2. Signature/Pathway analysis of genes identified as relevant to selinexor sensitivity.** Bar graph showing the top 20 biological processes /pathways significantly enriched among genes whose sgRNAs were significantly depleted in the presence of selinexor.

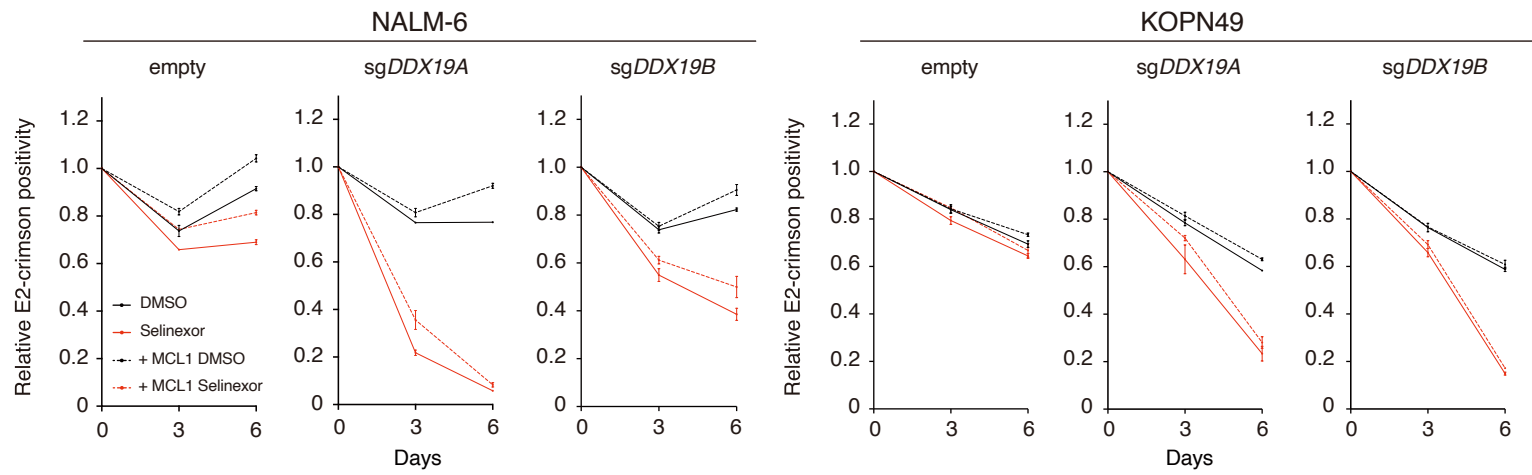
Supplemental Figure 3



**Supplemental Figure 3. Assessment of nuclear *MCL1* mRNA levels by RT-qPCR.**

**a** Nuclear (N) and cytoplasmic (C) fractions were validated by Western blots. Lamin B1 (LMNB1) served as a nuclear marker and GAPDH as a cytoplasmic marker. W: whole cell lysate. **b** RT-qPCR for *MCL1* mRNA was also performed using nuclear and cytoplasmic mRNA following dTAG<sup>V</sup>-1 or negative ligand treatment, and *MCL1* mRNA values were normalized to cell number to compare their relative levels in each fraction between experimental conditions. Each condition was assessed in triplicate, and data are represented as means ± SD.

Supplemental Figure 4



**Supplemental Figure 4. Growth curves of MCL1-overexpressing cells over an extended period.**

Control and MCL1-overexpressing NALM-6 and KOPN49 cells were transduced with a lentivirus vector encoding sgRNA targeting *DDX19A* or *DDX19B* plus a Crimson cassette via CRISPR/Cas9 gene targeting. Cells were treated with DMSO or selinexor (100nM), and proportions of Crimson-positive cells were assessed over 6 days by FACS. Empty vector served as control. Each condition was assessed in triplicate, and data are represented as means  $\pm$  SD.