

**Supplemental Fig. 1. Comet Assays.** U2OS and SAOS-2 cells were infected with Ad-GFP and AdDEKsh adenoviruses, collected on day 3 post-infection and subjected to alkaline comet assay analysis for determination of DNA damage. AdDEKsh-infected cells show increased DNA damage overall, but do not reach statistical significance.



**Supplemental Fig. 2. MEF colony viability assays.** Dek knockout MEFs exhibit defects in cellular growth when subjected to the stress of clonogenic outgrowth in the presence or absence of etoposide. One thousand cells were plated per 60mm dish, and the following day were either left untreated or were treated with  $4\mu$ M etoposide (E) for 6 hours, washed, and allowed to recover for five days. Colonies that formed were fixed with 2% paraformaldehyde in PBS and stained with ethylene blue and counted. The degree of Dek knockout cell colony formation was similar to that of etoposide-treated wild type cells, and no colonies were visible with those same cells after exposure to etoposide.

## Supplemental Figure 3

DEK_HUMAN/1-375	5 1 M S - A S A P A A E G E G T P T Q P A S E K E P E M P G P R E E S E E E E D E D D E E E E E	E E K E K S L I V E G K R E K K K V E R L 66
DEK_MOUSE/1-380	1 M S A A A A P A A E G E D A P V P P S S E K E P E M P G P R E E S E E E E E D D E D D D E E D E E	E E K E K S L I V E G K R E K K K V E R L 70
DEK_HUMAN/1-375 DEK_MOUSE/1-380	5 67 TMQVSSLQREPFTIAQGKGQKLCEIERIHFFLSKKKTDELRNLHKLLYN 71 TMQVSSLQREPFTVTQGKGQKLCEIERIHFFLSKKKPDELRNLHKLLYN	R
DEK_HUMAN/1-375	137 K G S V Q Y K K K E EMLKK F R NAMLK S I C E V L D L E R S G V N S E L V K R I L N F L M H	P K P S G K P L P K S K K T C S K G S K K 206
DEK_MOUSE/1-380	141 K G S T Q Y K K K E EMLKK F R NAMLK S I C E V L D L E R S G V N S E L V K R I L N F L M H	P K P S G K P L P K S K K S S S K G S K K 210
DEK_HUMAN/1-375	207 ERNSSGMARKAKRTKCPEILSDESSSDEDEKKNKEESSDDEDKKNKEESS	Р Р К К Т А К R Е К Р К Q К А Т S К S К К 275
DEK_MOUSE/1-380	211 ERNSSGTTRKSKQTKCPEILSDESSSDEDEKKNKEESSEDE EKESEEEQ	Р Р К К Т S К К Е К А К Q К А Т A К S К К 280
DEK_HUMAN/1-375	276 SVK SANVKKADS STTKKNQ N SSKK E SE SEDS SDDEPLIKKLKKPPTDEE	L K E T I K K L L A S A N L E E V T M K Q 345
DEK_MOUSE/1-380	281 SVK SANVKKADS STTKKNQ K SSKK E SE SEDS SDDEPLIKKLKKPPTDEE	L K E T V K K L L A D A N L E E V T M K Q 350
DEK_HUMAN/1-375	346 ICKKVYENYPTYDLTERKDFIKTTVKELIS	375
DEK_MOUSE/1-380	351 ICKEVYENYPAYDLTERKDFIKTTVKELIS	380

**Supplemental Fig. 3. DEK protein sequence alignment.** Human and mouse DEK share 89 percent sequence identity.



**Supplemental Fig. 4. MEF DNA damage blots.** Wild type and Dek knockout MEFs were left untreated or treated with 25µM etoposide for the time points indicated, and whole cell protein lysates were collected. The samples were subjected to western blot analysis with pSMC1 (Ser957), total SMC1, pDNA-PKcs (Ser2056), and total DNA-PKcs specific antibodies. Western blots depicted in (A) are from different parts of the same immunoblot. \*The pDNA-PK (Ser2056) and total DNA-PK bands run with identical mobility. Specificity of the Abcam pDNA-PK (Ser2056) antibody for murine protein has not yet been demonstrated.