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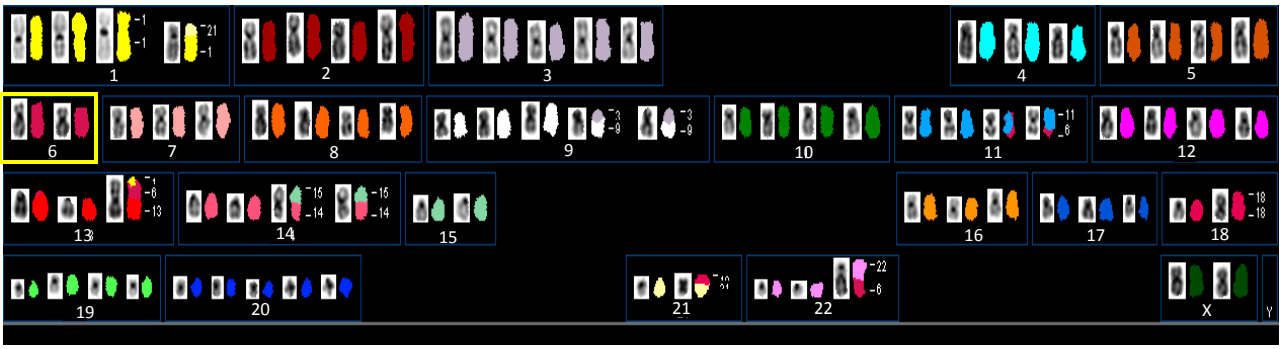
	Normal	SK-MEL 5	SK-MEL 19	SK-MEL 29	SK-MEL 94	SK-MEL 103	SK-MEL 147	SK-MEL 173	G-361	Malme 3M	UACC 62	UACC 257	WM 1366	MM 96L	MM 200	MM 540	MM 576	SK-MEL 28
DEK	-/+	+++	+++	+++	++	+	++	+	+	++	+	++	++	+++	+	+++	+++	+
NRAS	wt	wt	wt	ND	wt	**Q61R**	**Q61R**	wt	wt	wt	wt	wt	**Q61R**	wt	wt	wt	wt	wt
BRAF	wt	**V600E**	**V600E**	**V600E**	**V600E**	wt	wt	wt	**V600E**	**V600E**	**V600E**	wt	wt	**V600E**	**V600E**	**V600E**	**V600E**	**V600E**
p53	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	ND	wt	wt	**S366P**	**L350P**	**L145R**
p14	+	ND	+	+	+	-	-	-	-	-	-	+	+	wt	wt	wt	wt	+
p16	+	-	-	-	+	+	+	-	-	-	-	-	-	-	wt	-	+	+
PTEN	+	+	+	+	-	-	-	-/+	-	+	-	-	-	+	-	-/+	-	-

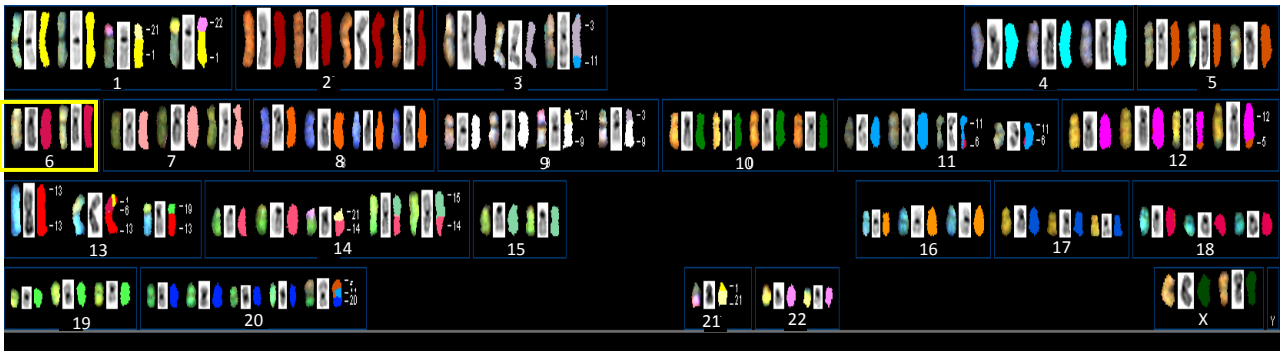
Khodadoust et al. Supplementary Figure 1

A

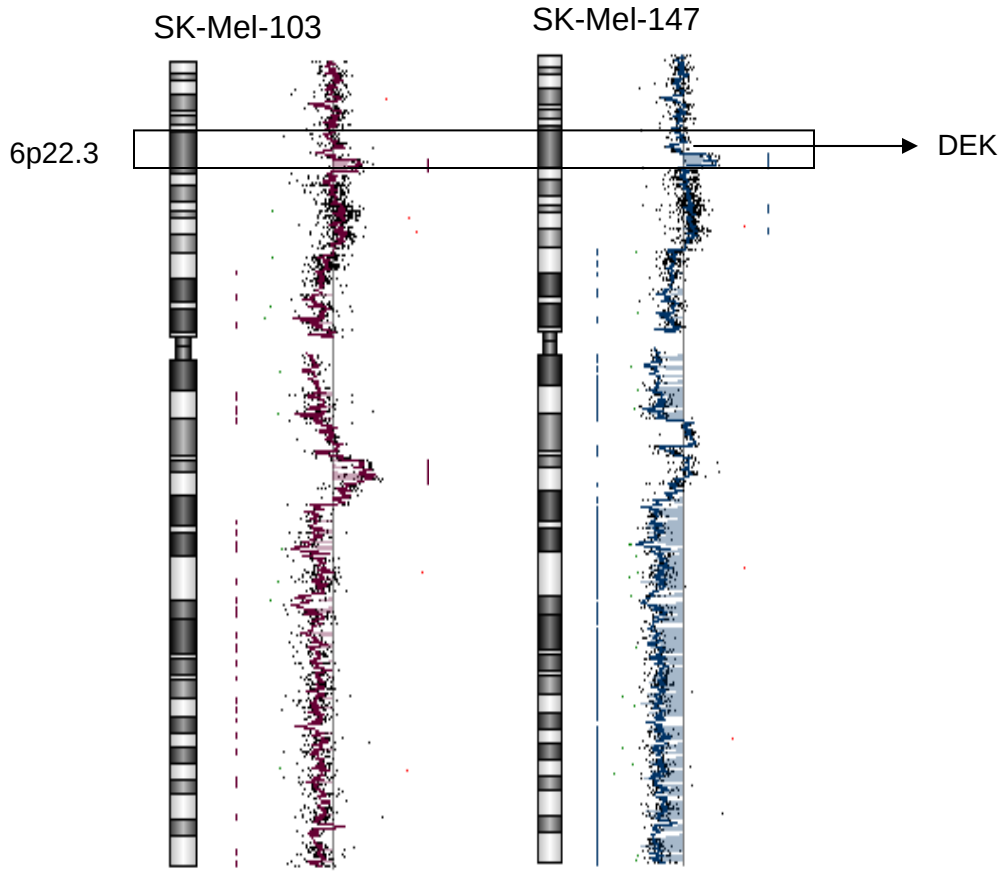
SK-Mel-103



SK-Mel-147

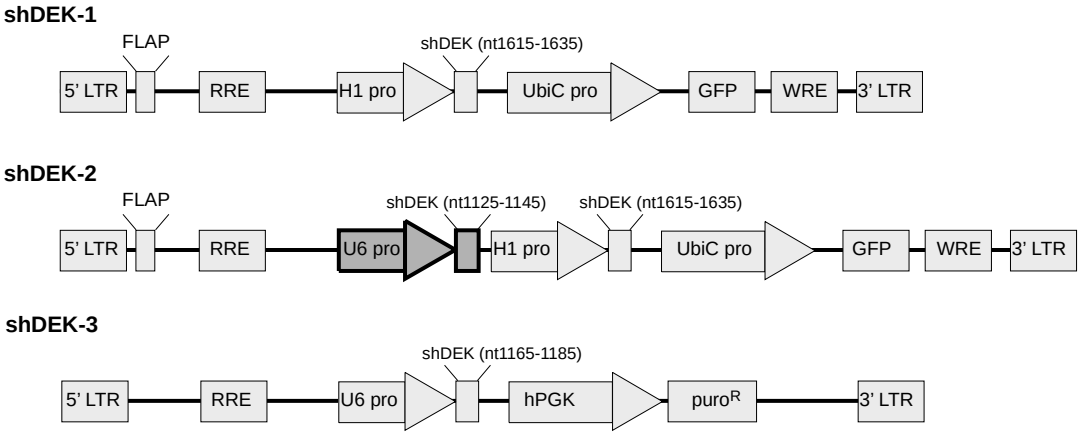


B



Khodadoust et al. Supplementary Figure 2

A



SUPPLEMENTARY INFORMATION

Extract preparation and protein immunoblots. Adherent and non-adherent cells were collected. Total cell lysates were obtained by Laemmli extraction, separated by SDS-PAGE, and transferred to Immobilon-P membranes (Millipore, Bedford, MA). Protein concentrations were determined using the BCA Protein Assay (Pierce, Rockford, IL). Antibodies to BCL-x_L and monoclonal antibodies to DEK were obtained from BD Biosciences (Franklin Lake, NJ), β-actin from Sigma Chemical (St. Louis, MO), p53 from Novacastra Laboratories (Newcastle upon Tyne, UK), BCL-2 from DAKO Diagnostics (Glostrup, Denmark), MCL-1 and cytochrome *c* from Santa Cruz Biotechnology (Santa Cruz, CA), caspase-3 and phospho-p53-ser^{15} from Cell Signaling Technologies (Beverly, MA). Polyclonal antibodies to DEK were produced as described (39). Unless otherwise specified, DEK was detected in immunoblots using the polyclonal anti-DEK antibodies. Detection was performed using ECL Western blotting substrate from Pierce (Rockford, IL). Protein expression was quantified using Scion image densitometry software (Scion Corporation, Frederick, MD).

Tissue microarrays (TMAs). Two TMAs comprising a total of 238 unique surgically removed lesions (each spotted in triplicate) were stained with monoclonal DEK-specific antibodies (BD Pharmingen, dilution 1:400). TMAs included primary, metastatic (single and multiple site, multiple morphologies; lesions were greater or equal to 1 mm in depth), dysplastic and benign nevi and as a control normal skin. DEK-specific staining was scored blinded using a score system: 3 (high), 2 (medium), 1 (low) or 0 (no DEK-expression).

Spectral karyotype (SKY). Metaphase chromosomes from melanoma cell lines were prepared after treatment with colcemid for 2 h, followed by incubation in 0.075 M KCl for 20 min at 37°C. Chromosomes were subsequently fixed in 3:1 methanol/acetic acid. Metaphases were dropped on slides, dried overnight, and stained with DAPI before microscopic examination. Slides were hybridized to Applied Spectral Imaging SKY probes (Migdal Ha'Emek, Israel). Metaphase images were acquired with an SD300 Spectra Cube (Applied Spectral Imaging) mounted on a Zeiss Axioplan microscope using a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT). Up to 20 metaphase cells were captured and analyzed for each case whenever possible.

Array comparative genomic hybridization (CGH). Genomic DNA from melanoma cell lines was extracted using DNeasy Blood & Tissue kit (QIAGEN, Valencia, CA, USA) and processed for analysis in Human Genome CGH 4x44k microarrays (Agilent Technologies, Palo Alto, CA, USA), essentially as previously described. The Human Genomic female DNA from

Promega was used as the reference DNA for all hybridizations. Slides were scanned using an Agilent 2565AA DNAMicroarray Scanner (Agilent Technologies).

Lentiviral shRNA constructs. Construction of lentiviral shRNA vectors targeting BCL-2, BCL-x_L, and MCL-1 has been described previously (8). The H1-LV vector was used to produce shRNAs targeting DEK. The shDEK-1 vector used the human H1 promoter to drive expression of an shRNA targeting a sequence in the 3' untranslated region of the human DEK transcript (nucleotides 1615-35, GenBank NM_003472.2). The shDEK-2 vector was constructed by inserting a second Pol III promoter and shRNA immediately 5' of the H1 promoter. This insertion contained the murine U6 promoter expressing a second shRNA targeting a coding region of the DEK transcript (nucleotides 1118-1141, GenBank NM_003472.2). A third lentiviral vector targeting DEK expression based on the PLKO.1 vector, PLKO.1-DEK832, was purchased from the Sigma Chemicals (St. Louis, MO). This vector targets a third region of the human DEK transcript (nucleotides 860-879, GenBank NM_003472.2) distinct from the shRNA targets listed above. Lentivirus production and infection was performed as described previously (8). When cells were sequentially infected with two lentiviral vectors, the second infection was performed 72 hours after the initial infection. Transduction efficiency of H1-LV derived lentiviral vectors was estimated by detection of GFP expression (driven by the human ubiquitin c promoter) by fluorescence microscopy or flow cytometry and was routinely greater than 95%. Cells transduced with the PLKO.1-DEK832 vector were selected for 3 days in 2μg/mL puromycin.

MCL-1 and BCL-x_L expression lentiviruses. The coding region of human MCL-1 mRNA was amplified by RT-PCR from SK-MEL-19 cells. An N-terminal FLAG-tag was cloned in frame with the coding region of the cDNA, and this construct was inserted under control of the CMV promoter into the FG12-eGFP lentiviral vector. Complete cloning strategy is available from the authors upon request. The BCL-x_L expression lentivirus was a generous gift from Colin Duckett.

FIGURE LEGENDS

Supplementary Table 1. Genetic background of the human metastatic melanoma lines used in this study. *BRAF* and *NRAS* mutational status was determined by direct sequencing of PCR-amplified genomic fragments of exons 15 and 3 respectively. *p53* mutational status was determined by direct sequencing of exons 2-10. DEK, and PTEN protein levels were determined by immunoblotting. Expression is categorized into +, ++ and +++ for levels comparable, 2 to 4 fold induced or <4-fold induced, with respect to

control melanocytes. Untedectable protein levels are indicated as -. ND, stands for Non Detectable.

Supplementary Figure 1. DEK overexpression even in melanoma cell lines with no amplification or focal gains at 6p22.3. The SK-Mel-103 and -147 melanoma cell lines expressing moderately high levels of DEK protein (Fig. 1A) were analyzed by SKY (*A*) and CGH (*B*). Both lines were found to have multiple genomic alterations, but contained a normal copy number of chromosome 6 (*A*). *B*. CGH showed focal gains at 6p23 (but not at the DEK locus in 6p22.3). These results, together with the frequent DEK overexpression determined by TMAs (Fig. 1D), indicate that DEK is a frequent target of pro-tumorigenic events that extend beyond simple chromosomal amplification.

Supplementary Figure 2. Knockdown of DEK expression by shRNAs. Shown is a schematic of the lentiviral shRNA vectors used in this study. A modified H1-LV lentiviral vector that expressed two distinct shRNAs targeting DEK was contructed by insertion of a second shRNA sequence under the control of the murine U6 promoter *(shDEK-2).* A third vector *(shDEK-3)* targeting a distinct third region of the DEK transcript and based on the pLKO.1 vector was used to ensure that effects were not due to non-specific effects of the vector or particular shRNAs.