Supporting Information

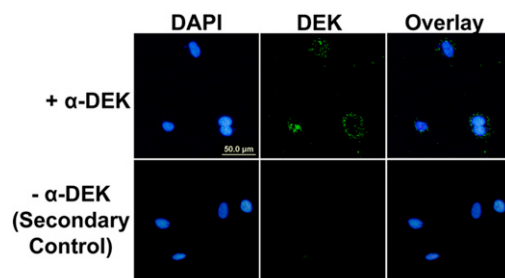## Saha et al. 10.1073/pnas.1220751110

Fig. S1. Secondary antibody controls for (DEK)-positive foci show no nonspecific staining. HeLa cells incubated with recombinant histidine (His)-DEK for 1 h at 37 °C were subsequently fixed and subjected to treatment with or without a monoclonal α-DEK antibody. Cells were then stained with a FITC-conjugated goat anti-mouse antibody (Molecular Probes/Invitrogen) and visualized by laser-scanning confocal microscopy (Olympus FV-500).
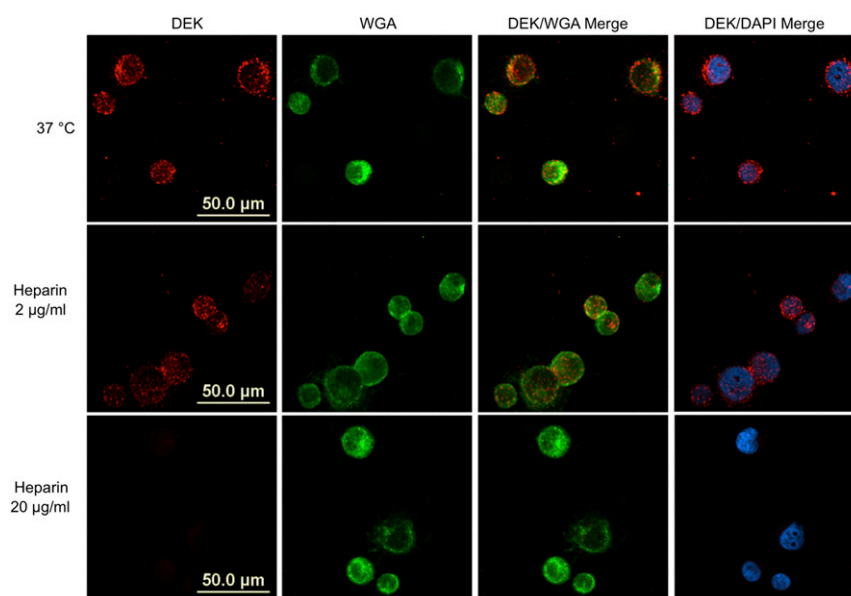

Fig. S2. Soluble heparin inhibits DEK internalization in a dose-dependent fashion. HeLa DEK-knockdown (DEK-KD) cells were left untreated or were pre-treated with either 2 μg/mL or 20 μg/mL soluble heparin. Cells were washed and then incubated with recombinant His-DEK and either PBS or 2 μg/mL or 20 μg/mL soluble heparin at 37 °C. Cells were stained with wheat germ agglutinin (WGA; green), a monoclonal DEK antibody (red), and DAPI (blue). Images were obtained by confocal microscopy.
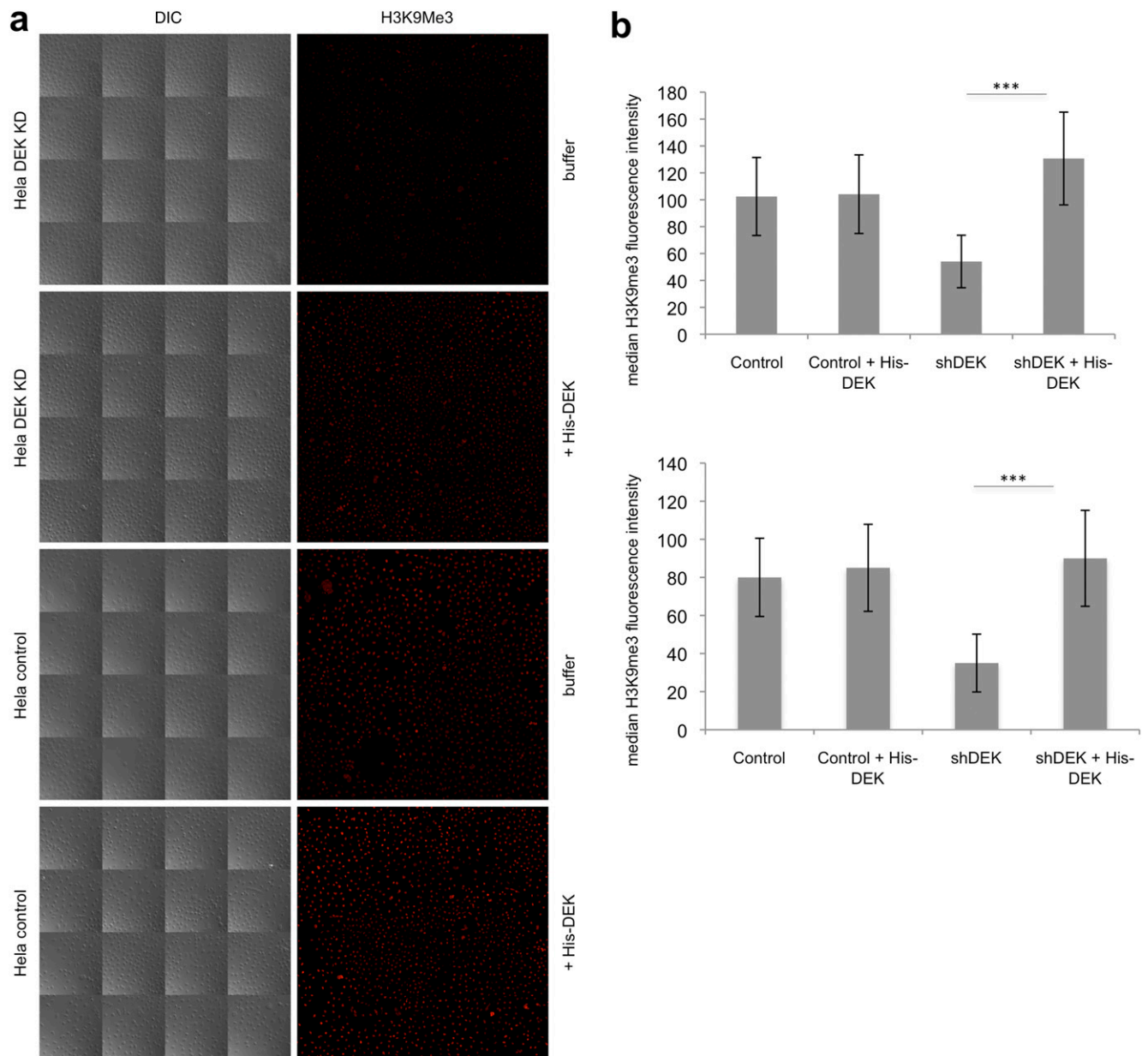
Fig. S3. Depiction of the KNIME workflow. Shown is the image processing workflow created with the alpha version of the Konstanz Information Miner (KNIME) Image Processing plug-in. KNIME is a user-friendly and comprehensive open-source data integration, processing, analysis, and exploration platform designed to handle large amounts of heterogeneous data. The workflow is structured as follows. Both channels, DEK antibody (red), and DAPI (blue) of the image were converted and normalized, such that the intensity values were between 0 and 255. A maximum intensity projection was applied, followed by standard smoothing with a 6×6 Gauss kernel. Yen's thresholding method was used to separate the cells from the background. Because of undersegmentation, cell clumps occur; these were split under the assumption that a single cell has a roughly convex shape. Finally, cells smaller than 350 pixels were filtered, and the average intensity of each cell was computed, such that for each individual cell, a value between 0 (dark) and 255 (bright) was available and could be used to apply a standard, unpaired t test.

Fig. S4. Representative tile scans used for Histone H3 tri-methylated at lysine 9 (H3K9Me3) intensity analysis with KNIME. (*A*) Tile scans for a representative biological replicate. Differential interference contrast (DIC) images and H3K9Me3 intensities are depicted for DEK-KD and control cells, either untreated or treated with His-DEK for 48 h. (*B*) Analysis of H3K9Me3 intensities using KNIME as shown in Fig. 4C. Shown is the analysis of two additional biological replicates, performed on two separate occasions. ***$P = 0.001$ in both experiments.
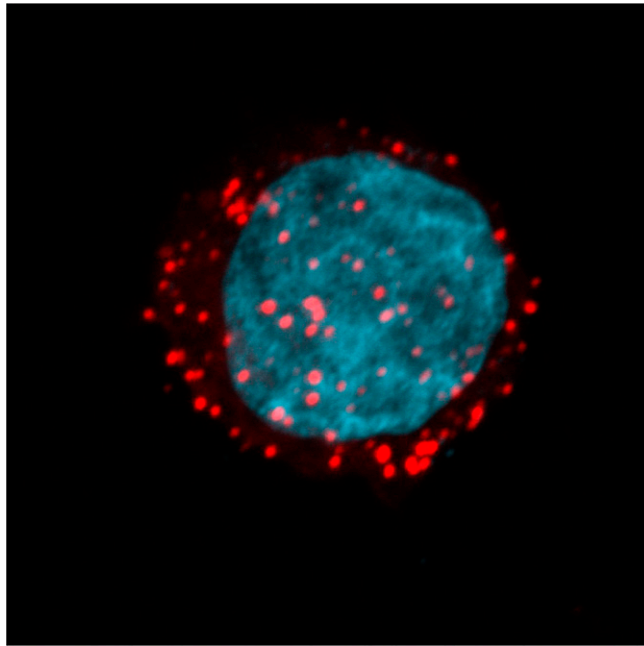
Movie S1. A 3D representation of DEK internalization. Stacks of z-slices were compiled into a video of HeLa DEK-KD S3 cells incubated with His-tagged recombinant DEK (20 µg/1 × 10^7 cells) for 2 h and stained with DAPI (blue) and a monoclonal DEK antibody (red).

Movie S1