SUPPLEMENTAL MATERIALS AND METHEDS

Production of Recombinant DEK (Continued)

To create the nuclear localization signal mutant DEK (rhNLS-DEK), we mutated the putative nuclear localization signal of human DEK (KAKRTK; 216 t0 221 aa) by introducing mutations to the codons of the lysines K216A (AAG to GCG), K218A (AAG to GCG), and K221 (AAA to ATA) in a GST-tagged DEK expression vector (1). We used the QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) using the conditions recommended by the manufacturer and the primers DEK NLSF 5'- AC AGT TCT GGA ATG GCA AGG GCG GCT GCG CGA ACC ATA TGT CCT GAA ATT CTG TCA G -3' and DEK NLSR 5'-CTGA CAG AAT TTC AGG ACA TAT GGT TCG CGC AGC CGC CCT TGC CAT TCC AGA ACT GT-3' (nucleotide substitutions underlined). The mutations were confirmed by sequencing and the protein expressed in *E. coli* (Clontech, Mountain View, CA, USA). A PCR-based random mutagenesis approach was devised for the creation of rhDBM-DEK. After random mutagenesis via error-prone PCR (performed on the SAP-box containing DEK fragment 85-187) (2, 3), the initial library was created by transformation of the mutated plasmid DNA to *E. coli* BL21 and plating on LB-AMP plates. The resulting mutant libraries were subsequently transferred via replica stamping on LB-AMP plates containing 0.1 mM IPTG for four consecutive days and escape colonies were collected. Surviving colonies were pre-screened via small-scale expression in 96-well plates and subsequent immunoblotting analyses using GST-specific antibodies. Plasmid DNA from colonies expressing a GST-tagged fragment of proper molecular weight were extracted, sequenced and the resulting proteins were expressed in *E. coli* and tested for their DNA binding activity by electrophoretic mobility assay. This yielded a DNA-binding dead DEK mutant carrying 8 specific mutations (H95R, K101M, K111I, K137E, S139R, K150E, L166S, N181D). The cDNA of this fragment was used to assemble a full length DEK in the pGEX-4T1 vector. The mutant NLS protein and the DNA binding dead mutant were purified as previously described for the WT DEK protein (4, 5).

ImageStream Analysis of NFκB p65 Translocation into the Nucleus

Lin$^-$ BM cells from C57BL/6 mice were treated with vehicle control, 100 ng/mL rhIL-8, or 50 nM rmDEK for 15 minutes, 30 minutes, 1 or 16 hours at 37°C. Cells were put on ice, stained for surface markers, and then fixed/permeabilized using BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (BD Biosciences) according to manufacturer's instructions. Cells were then incubated with anti-NFκB p65 antibody (Invitrogen; cat. # PA1-186) for 30 minutes at 4 °C in the dark, followed by a 1:250 AF647 F(ab')2 fragment goat anti-rabbit antibody (Jackson ImmunoResearch). Following staining, cells were washed and refixed using BD Cytofix buffer for 10 minutes. Cells were then washed, resuspended in 20 μM DRAQ5 (BD Biosciences; cat. # 564903), incubated for 10 minutes at room temperature and then analyzed. 60,000-100,000 lineage negative, c-kit positive events were collected using the slow stream setting at a 40X objective for all samples on an ImageStreamX MKII (Amnis/EMD Millipore) using INSPIRE™ ImageStreamX MKII Software and analyzed using IDEAS® Image Data Exploration and Analysis Software Version 4 (Amnis/EMD Millipore). Spectral compensation was digitally performed on a pixel-by-pixel basis prior to data analysis. In focus cells then single, live cells were gated based on their aspect ratio and area of the bright field image. Samples were then gated on the LSK and LK populations and the relationship between the NFκB p65 and DRAQ5 staining was measured using the 'Similarity' feature in the IDEAS® software. The 'Similarity Score' concept is described above. Cells with high 'Similarity Scores' demonstrate greater NFκB p65 translocation into the nucleus and lower 'Similarity Scores' indicate cells with more NFκB p65 in the cytoplasm. Splenocytes from C57BL/6 mice treated with LPS (Sigma Aldrich) for 1 hour at 37°C then stained for NFκB p65 and DRAQ5 as stated above were then gated on CD11b and used as a positive control.

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. (A-C) Bone marrow (BM) engraftment data from F1 recipients that received donor cells from the mouse Lin^- BM hematopoietic stem cell (HSC) expansion assay at a dose of 10000 (A), 25000 (B), and 100000 (C) cells. These data were used to calculate the CRU in the limiting dilution analysis. Data are the mean ± SEM of 5 mice per group. 'p' value is when comparing indicated group to day 0 input group. (D-F) BM engraftment data from NSG recipients that received donor cells from the human $CD34^+$ CB HSC expansion assay at a dose of 500 (D), 2500 (E), and 10000 (F) cells. This data was used to calculate the SRC in the limiting dilution analysis. Data are the mean ± SEM of 5 mice per group. 'p' value is when comparing indicated group to day 0 input group. (A-F) One-way ANOVA with post-hoc Tukey's Multiple Comparison Test was used.

Figure S2. (A-C) *Dek*$^{-/-}$ or littermate WT control mice (n=3-5 mice per group) were given 10 μg rmDEK or vehicle control s.c. once a day for 2 days and then BM was harvested 48 hours post final injection. To determine the percentage of hematopoietic progenitor cells (HPCs) at the time of isolation in cycle, high

specific activity tritiated thymidine kill assays were utilized. * when compared to WT vehicle control. ‡ when compared to *Dek*$^{-/-}$ vehicle control. Represents one of three experiments. (D-F) C57BL/6 mice (n=5 mice per group) were given 10 μg rmDEK, denatured (d)rmDEK or vehicle control s.c. once a day for 2 days and then BM was harvested 24 or 48 hours post final injection. To determine the percentage of HPCs at the time of isolation in cycle, high specific activity tritiated thymidine kill assays were utilized. * when compared to vehicle control. ‡ when compared to drmDEK of the indicated time point. (A-F) Individual mice were plated in triplicate. * or ‡ $p<0.05$, ** or ‡‡ $p<0.01$, *** or ‡‡‡ $p<0.001$ when using One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S3. C57BL/6 mice (n=4 mice per group) were given 10 μg rmDEK or vehicle control s.c. once a day for 2 days and then BM was harvested 72 (A-B), 96 (C-D) and 144 hours (E-F) post final injection. HPC numbers per femur were determined by culture in methylcellulose colony formation assays (A, C, E). To determine the percentage of HPC at the time of isolation in cycle, high specific activity tritiated thymidine kill assays were utilized (B, D, F). (A-F) Results are the mean ± SEM based on 5 individually assessed mice per group with each mouse plated in triplicate. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ when comparing vehicle control treated to rmDEK-treated C57BL/6 mice using One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S4. *Cxcr2*$^{-/-}$ or littermate (WT) mice were injected with 10 μg dialyzed rmDEK or vehicle control once a day for two days subcutaneously. BM was harvested 48 hours after final injection. (A-C) Immunophenotyping of long-term (LT) hematopoietic stem cell (HSC; A), short-term (ST)-HSC (B) and multipotent progenitor (MPP; C) was performed using flow cytometry. (D-F) To determine the percentage of HPC at the time of isolation in cycle, high specific activity tritiated thymidine kill assays were utilized. Results are individually assessed mice with each mouse plated in triplicate. (A-F) Data are

SEM of 4 mice per group. *** $p<0.001$ or p value indicated when compared to vehicle control treated WT mice using One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S5. Representative histograms of C57BL/6 BM LSK $CD150^+$ cells (A) and LK cells (B) that were examined for p38 MAPK phosphorylation at 15, 30, and 60 minutes following vehicle, rhIL-8, or rmDEK treatment by flow cytometry. Grey line peaks are vehicle controls.

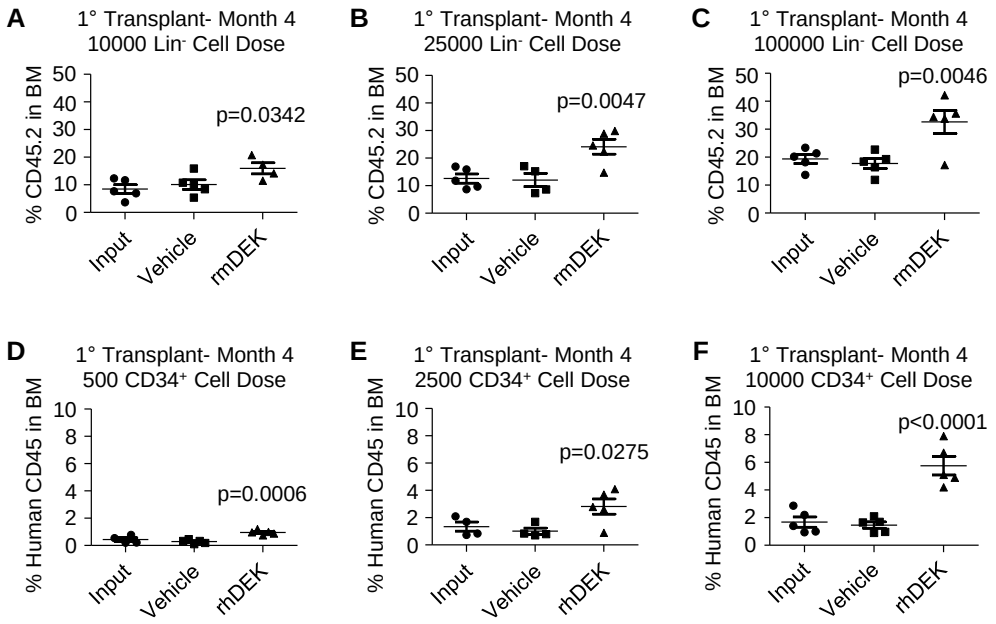
Figure S6. (A) Splenocytes from C57BL/6 mice were treated with LPS for 1 hour. We then quantified the degree of NFkB p65 translocation in the $CDllb^+$ population by calculating the similarity score (s.s.) of NFkB p65 and our nuclear stain using the co-localization feature in IDEAS Image Data Exploration and Analysis Software. Images were taken at a 40X objective on an ImageStream X MKII using INSPIRE ImageStreamX MKII Software. (B-C) C57BL/6 Lin^- BM cells were treated with vehicle control, 100 ng/mL rhIL-8 or 50 nM rmDEK for 15 minutes, 30 minutes, 1 or 16 hours. We then quantified the degree of NFkB p65 translocation in the LSK (B) and LK (C) populations by calculating the s.s. as stated for (A). Each bright detail similarity histogram is a representative image of 2 experimental replicates.

Figure S7. (A-C) LSK $CD150^+$ C57BL/6 BM cells pretreated with vehicle (A), anti-CXCR2 blocking antibody (B) or an HSPG inhibitor (heparin; C) were examined for ERK1/2 phosphorylation 15, 30, or 60 minutes following rmSCF, rhSDF1, rhIL-8 or rmDEK treatment by flow cytometry. Vehicle-treated cells were examined to determine baseline phosphorylation levels. (D-F) LSK $CD150^+$ C57BL/6 BM cells pretreated with vehicle (D), anti-CXCR2 blocking antibody (E) or an HSPG inhibitor (heparin; F) were examined for AKT phosphorylation 15, 30, or 60 minutes following rmSCF, rhSDF1, rhIL-8 or rmDEK treatment by flow cytometry. Vehicle-treated cells were examined to determine baseline phosphorylation levels. (G-H) LSK $CD150^+$ C57BL/6 cells were examined for ERK1/2 (G) and AKT (H) phosphorylation 15, 30, or 60 minutes

following WT rmDEK, GST WT rhDEK, GST rhDBM-DEK or GST rhNLS-DEK by flow cytometry. Vehicle-treated cells were examined to determine baseline phosphorylation levels. (A-H) Data are mean ± standard deviation of triplicate tubes. * $p<0.05$ and ** $p<0.01$ when compared to vehicle control using One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S8. (A) Principal component analysis conducted on experimental replicates (three per experimental group) to assess sample variation. The 2nd principle component clearly separates the DEK-treated from vehicle-treated samples. The 1rst principle component is seen to separate experimental replicates (1 vs. 4), (2 vs. 5), and (3 vs. 6). This variability due to replicate pair was accounted for in the statistical model for differential expression. (B) Volcano plot depicting significance against fold change (FC) between DEK-treated and vehicle-treated lineage-negative BM cells. Genes that satisfied the absolute FC > 1.5 (blue lines) and p-value < 0.01 (top right and top left) criteria were considered differentially expressed. Positive log fold change indicated higher expression in DEK-treated cells, while negative log fold changes indicate higher expression in the vehicle-treated cells.

Capitano et al., Figure S1



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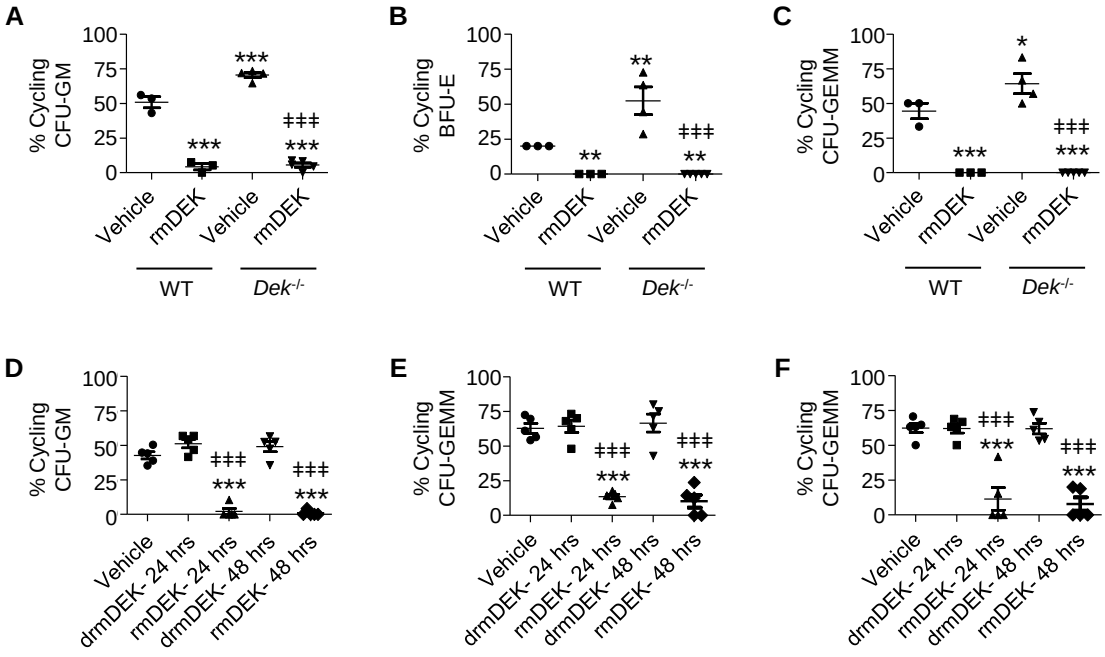

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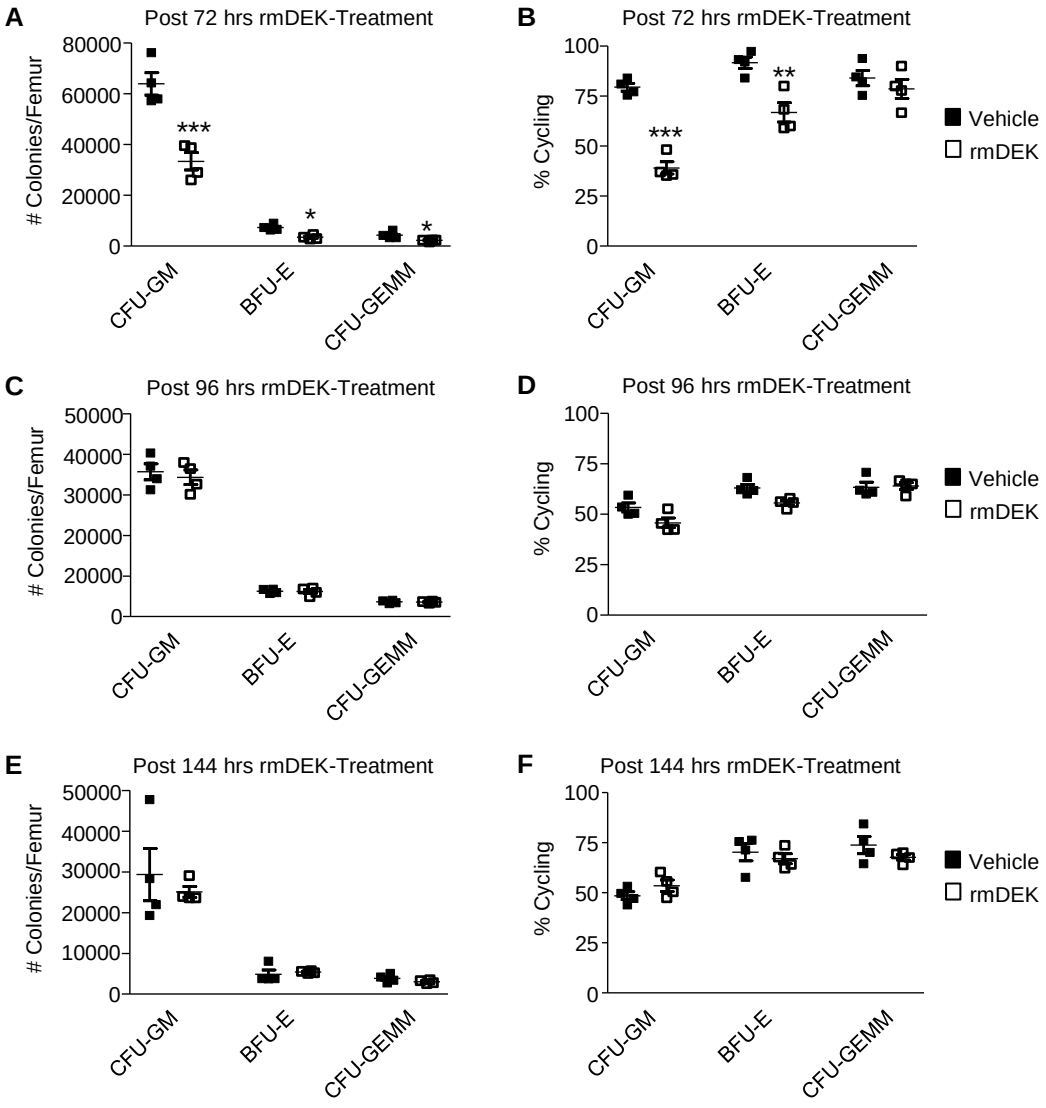

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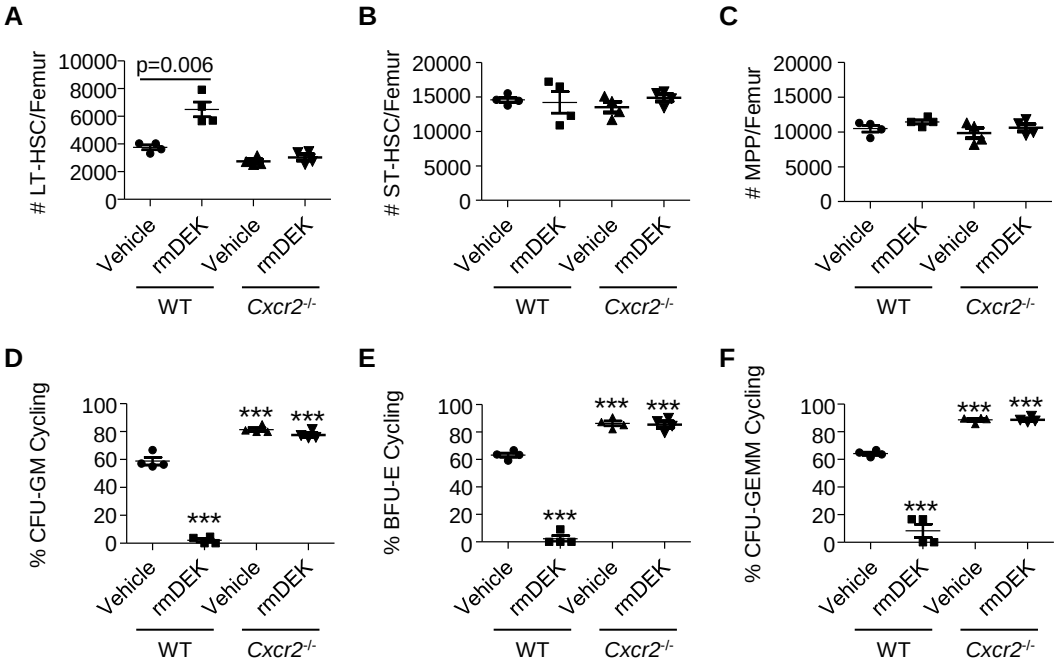

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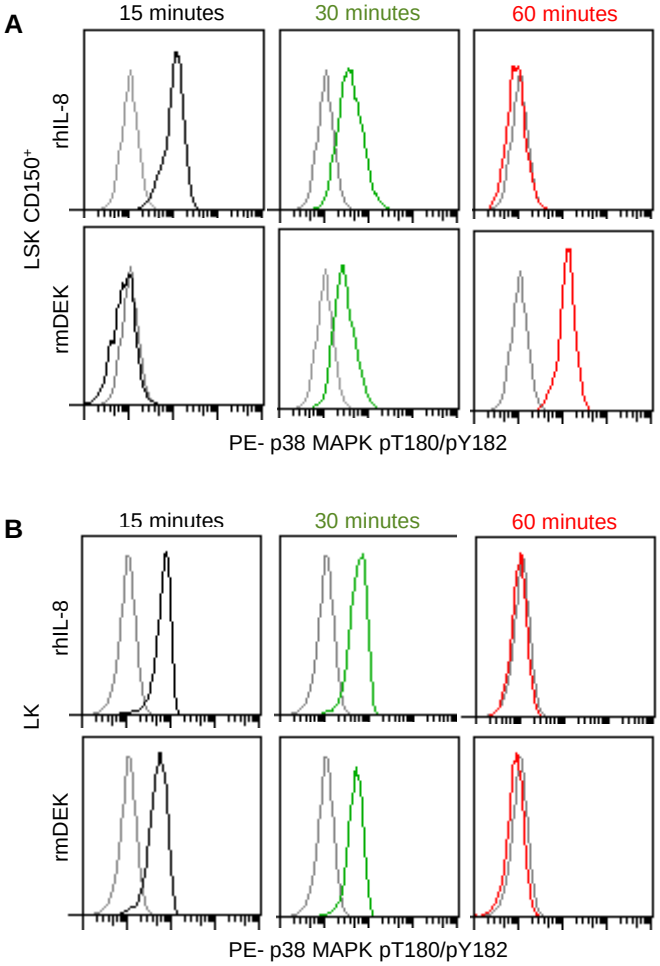

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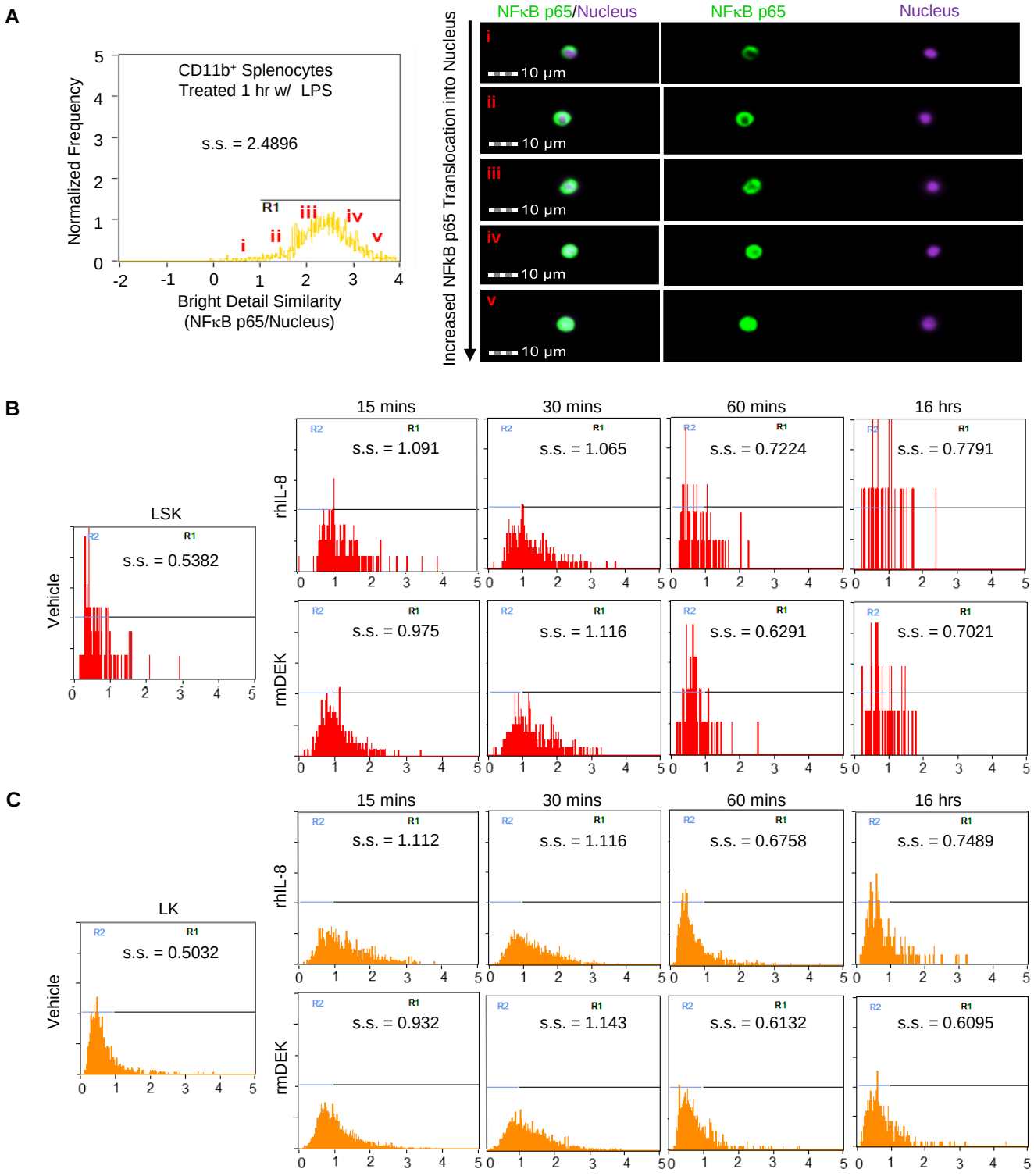

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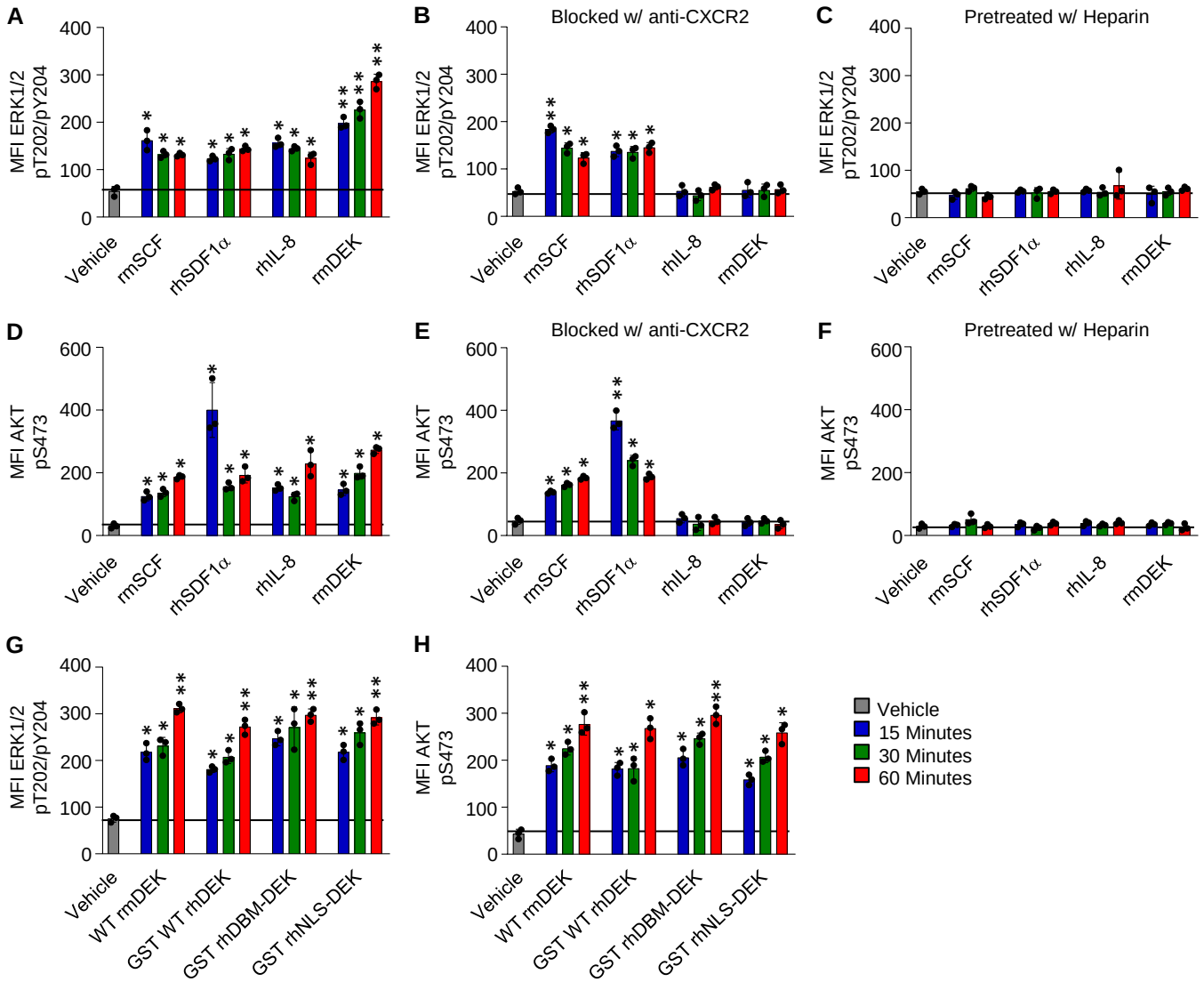
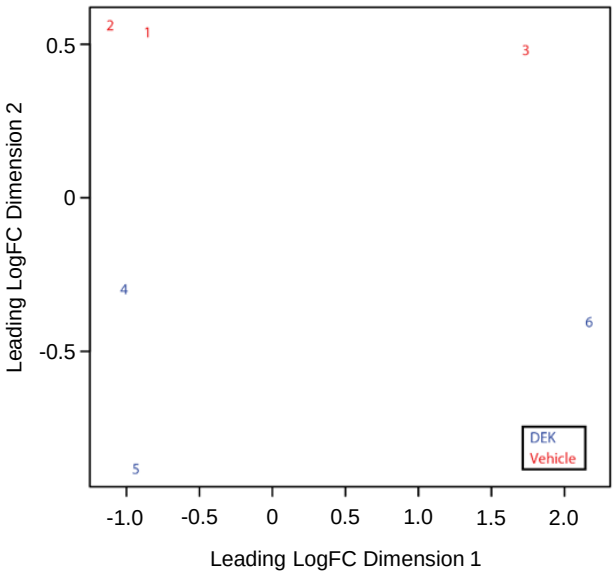


Capitano et al., Figure S8

A



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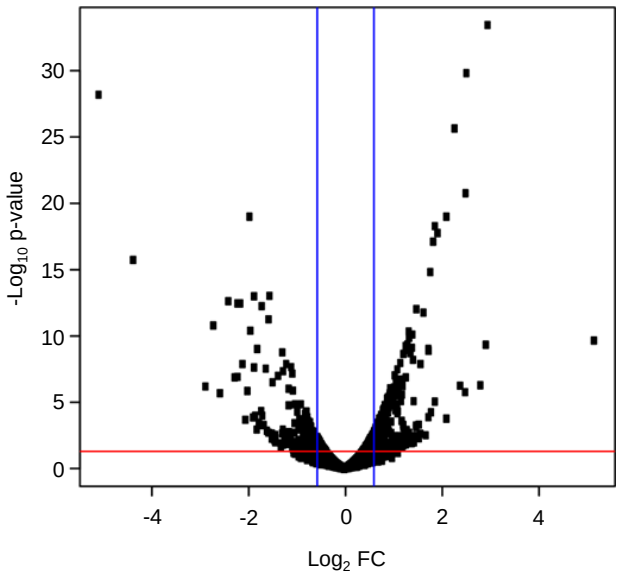

Capitano et al., Table S1

(A) Determination of CRU frequency in vehicle and rmDEK-treated groups.

Group	Number of Lin^- Cells Transplanted	Number of mice with >15% mouse $CD45.2^+$ cell chimerism/total number of mice
Input	10000	0 of 5
	25000	2 of 5
	100000	4 of 5
Vehicle Control	10000	1 of 5
	25000	2 of 4
	100000	4 of 5
rmDEK	10000	2 of 4
	25000	4 of 5
	100000	5 of 5

(B) CRU frequency in vehicle and rmDEK-treated groups

Group	CRU frequency	95% confidence interval	Number of CRUs in $1x10^6$ Lin^- starting cells
Input	1 in 66709	1 in 154211 to 1 in 28857	15
Vehicle	1 in 50878	1 in 116338 to 1 in 22156	20
rmDEK	1 in 14996	1 in 34874 to 1 in 6448	67

(C) Determination of SRC frequency in vehicle and rhDEK-treated groups.

Group	Number of $CD34^+$ Cells Transplanted	Number of mice with >1% human $CD45^+$ cell chimerism/total number of mice
Input	500	0 of 4
	2500	2 of 4
	10000	4 of 5
Vehicle Control	500	0 of 5
	2500	1 of 4
	10000	3 of 5
rhDEK	500	2 of 5
	2500	4 of 5
	10000	5 of 5

(D) SRC frequency in vehicle and rhDEK-treated group.

Group	SRC frequency	95% confidence interval	Number of SRCs in $1x10^6$ $CD34^+$ starting cells
Input	1 in 5612	1 in 13519 to 1 in 2329	178
Vehicle	1 in 10990	1 in 29949 to 1 in 4033	91
rhDEK	1 in 1327	1 in 3220 to 1 in 547	754