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Supplemental Information

Tissue origin of cytotoxic natural killer cells dictates their differential roles in mouse digit tip regeneration and progenitor cell survival Nadjib Dastagir, Zachery Beal, and James Godwin

Supplementary Information

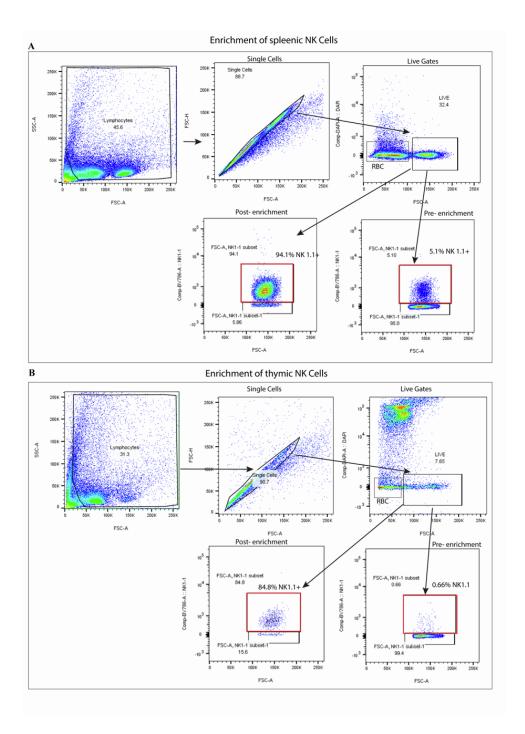


Figure S1. Isolation of untouched NK cells from spleen and thymus by using the NK cell Isolation kit "MojoSort™" by BioLegend. Sample gating strategy for calculating purity of isolated NK cells. After gating on live single cell lymphocytes using forward and side scatter profiles and DAPI dye exclusion, the NK cell percentages were determined using NK 1.1 antibody staining. A. High levels of NK cell purity were obtained from spleen tissue (enriched from 5.1% to 94%). B. High levels of NK cell purity were obtained from Thymic tissue (enriched from 0.66% to 84.8%). Contaminating cells non-immune.

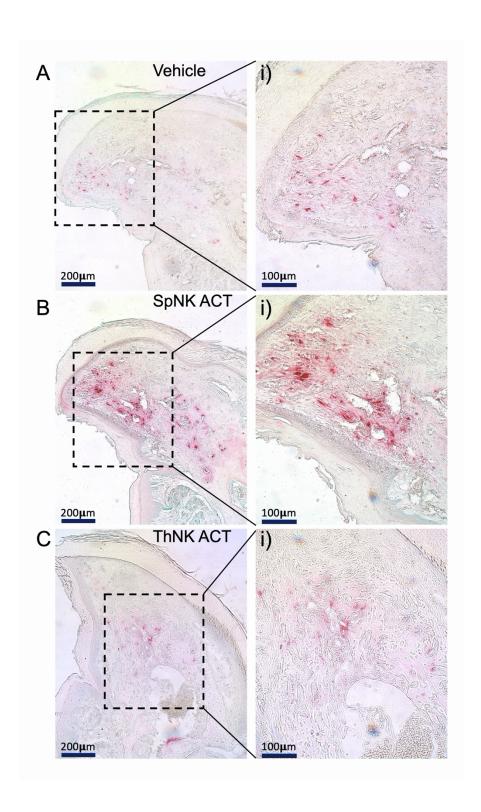


Figure S2. ThNK ACT into NSG mice causes a reduction in the number of osteoclasts at 12DPA compared to vehicle treated animals where SpNK ACT causes osteoclast induction.

Representative TRAP+ osteoclast staining on paraffin embedded sections. Images taken at 10x and 20x. Scale bar as shown.

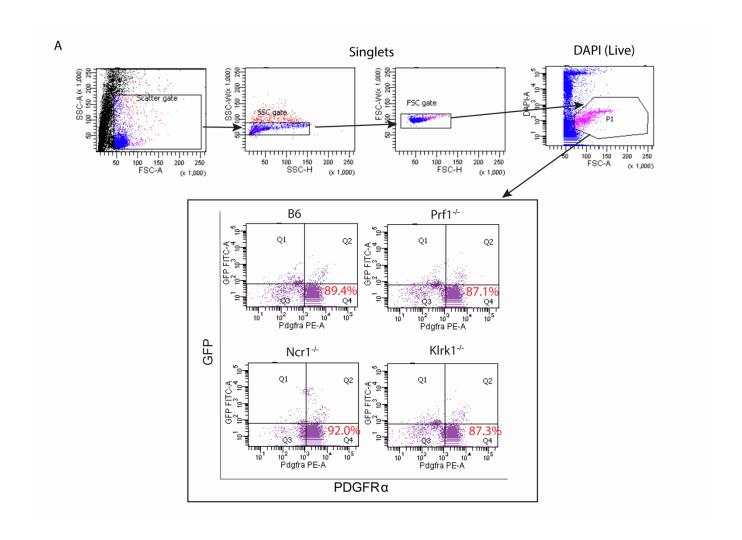


Figure S3. Fluorescence-activated cell sorting (FACS) -BD FACSymphonyTM S6- was used to sort for PDFGRα positive cells. Single cells were identified by plotting forward side scatter-area- vs forward scatter-height. Single cells were then separated from dead cells by DAPI. $PDGFRα^+$ positive cells from B6, $Prf^{-/-}$, Ncr 1^{gfp/gfp} and Klrk --- were sorted before use in autologous NK cytotoxicity assays. Sorting was performed on 20 pooled digits per genotype (N=5 mice) and purified cells used in cytotoxicity assays.