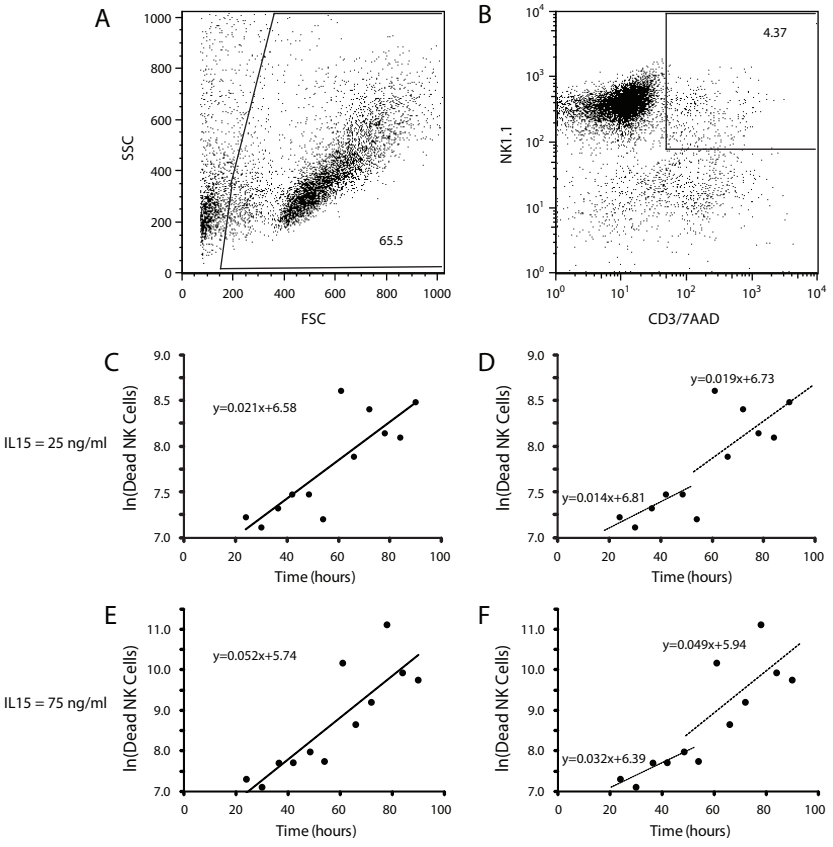
Figure S2. Alternative estimates of NK cell death rates based on direct enumeration of 7-aminoactinomycin (7-AAD)+ NK cells. NK cell-enriched splenocytes were incubated in specific concentrations of IL-15 for various lengths of time. Prior to flow cytometer analysis, 7-AAD was added to the NK cells for 10 minutes. *A*, Cell fragments were excluded from analysis of the lymphocytes on the side scatter vs. forward scatter plot. *B*, Dead NK cells were identified as $NK1.1^+$ $7\text{-}AAD^+$ lymphocytes. (FL3 also contained $CD3^+$ splenocytes; however, dual positive NK1.1+CD3+ splenocytes were negligible after NK cell negative selection as well as in parallel staining of NK cell-enriched splenocytes without the addition of 7-AAD). *C and E,* Dead NK cells were enumerated at various time-points using calibration beads and plotted on a semi-log scale. The slope (determined by linear regression) provided an estimate of the overall death rate and is shown for NK cells stimulated with IL-15 concentrations of 25 ng/ml (*C*) or 75 ng/ml (*E*). *D and F*, The dead cell data for NK cells stimulated with IL-15 concentrations of 25 ng/ml (*D*) or 75 ng/ml (*F*) was divided into early (<48 h) and late (>48 h) subsets (based on Fig. 3F), and slopes for each group were calculated to allow death rate comparisons with d_U and d_D. Data shown are representative of three independent experiments. *G,* Comparison of NK cell death rates estimated by mathematical analysis or direct enumeration of 7-aminoactinomycin+ NK cells. The parameters d, d_U and d_D represent overall death rate, death rate of compartment U cells, and death rate of compartment D cells, respectively. Model estimates represent values obtained through mathematical analysis (see Results section). Average values and standard deviations are shown for 3 independent experiments at each IL-15 concentration.



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		IL-15 = 25 ng/ml	IL-15 = 25 ng/ml	IL-15 = 75 ng/ml	IL-15 = 75 ng/ml
		Model Estimate	7-AAD measurement	Model Estimate	7-AAD measurement
$d \times 10^{-2}$	h^{-1}		1.9 ± 0.67		3.6 ± 1.6
$d_U \times 10^{-2}$	h^{-1}	0.69 ± 0.49	1.2 ± 0.59	1.2 ± 0.39	2.1 ± 2.0
$d_D \times 10^{-2}$	h^{-1}	2.4 ± 1.6	2.1 ± 0.95	4.0 ± 0.72	3.3 ± 1.8