

Figure S4. Experimental cell density did not alter the ability of the model to predict NK cell expansion. Experiments with NK cell-enriched splenocytes (45-55% NK cells) were performed combining triplicate wells containing 25,000 NK cells/well (45,000 -56,000 total cells/well). See Figs. 4 and 6. The experiments were repeated with unenriched splenocytes (containing approximately 2% NK cells) with 2000 NK cells/well (100,000 total cells/well). Utilizing parameter estimates of recruitment, proliferation, and death rates from Table 1 (derived from NK cell-enriched splenocytes), predictions of NK cell proliferation from model 3 (solid curve) compared well with experimental results with unenriched splenocytes (filled circles; IL-15 = 25 ng/ml). Therefore, cell density (and other physical constraints within the limited volume of the cell well) did not appear to significantly influence the overall NK cell expansion in our *in vitro* experiments. Furthermore, the estimated values of d_U and d_D derived from experiments with unenriched splenocytes stimulated with 25 ng/ml IL-15 ($0.4 \times 10^{-2} \text{ h}^{-1}$ and $2.8 \times 10^{-2} \text{ h}^{-1}$, respectively) were comparable with values ($d_U 0.7 \times 10^{-2} \text{ h}^{-1}$ and $d_D 2.4 \times 10^{-2} \text{ h}^{-1}$; Table 2) calculated from experiments with enriched NK cells, supporting our conclusion that replicating NK cells have a higher death rate than quiescent NK cells.

