

SUPPLEMENTAL MATERIAL

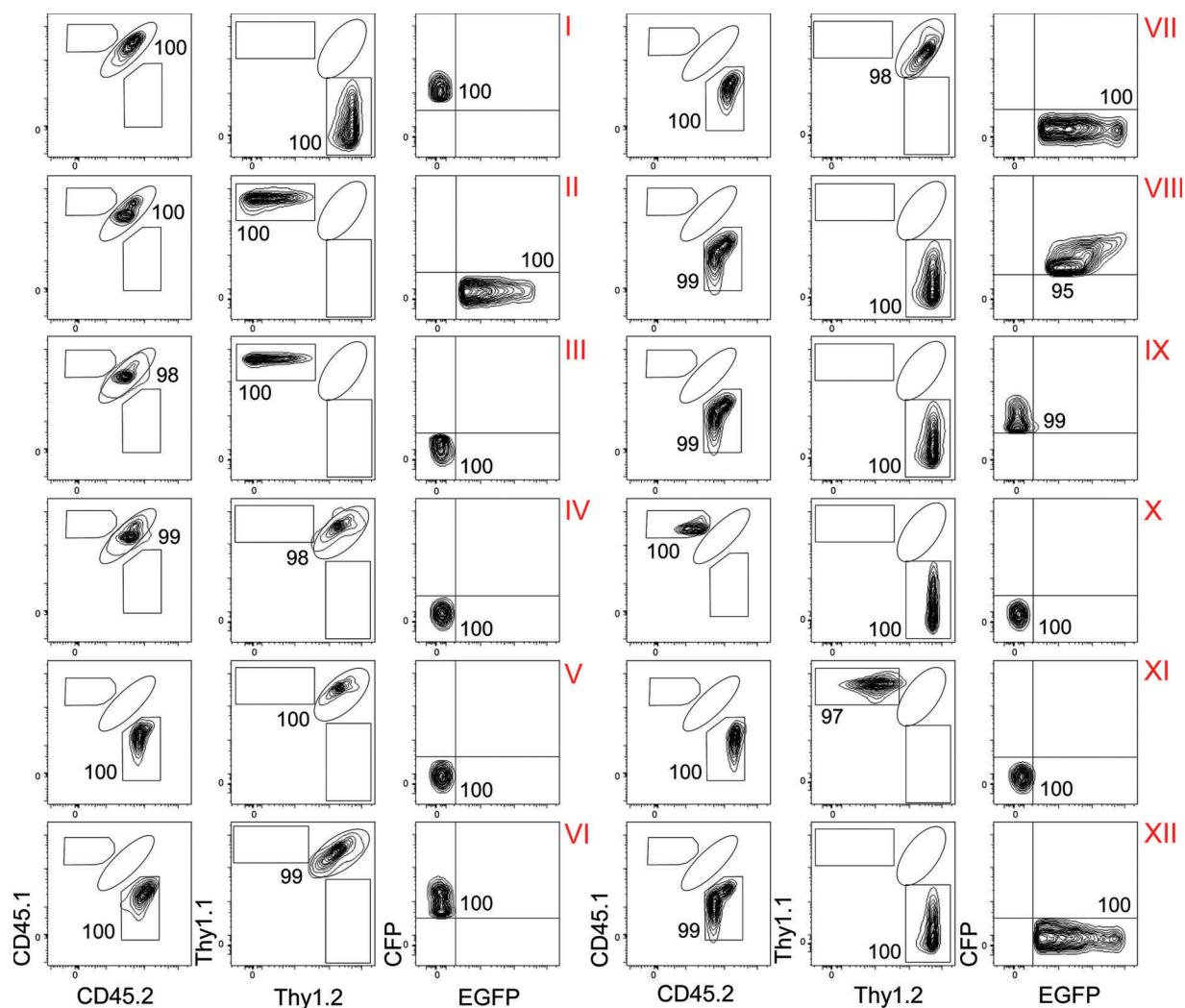
Ziętara et al., <http://www.jem.org/cgi/content/full/jem.20142143/DC1>

Figure S1. Phenotype of multicongenic progenitors in the thymus. Thymus of DKO recipient mice 21 d after injection and representative plots of individual tags from multicongenic progenitor library. Age of recipient mice used in individual experiments is indicated in the Materials and methods section. Donor mice were 7–10 wk old. The experiment was performed 4 times, with $n = 30$ (DKO).

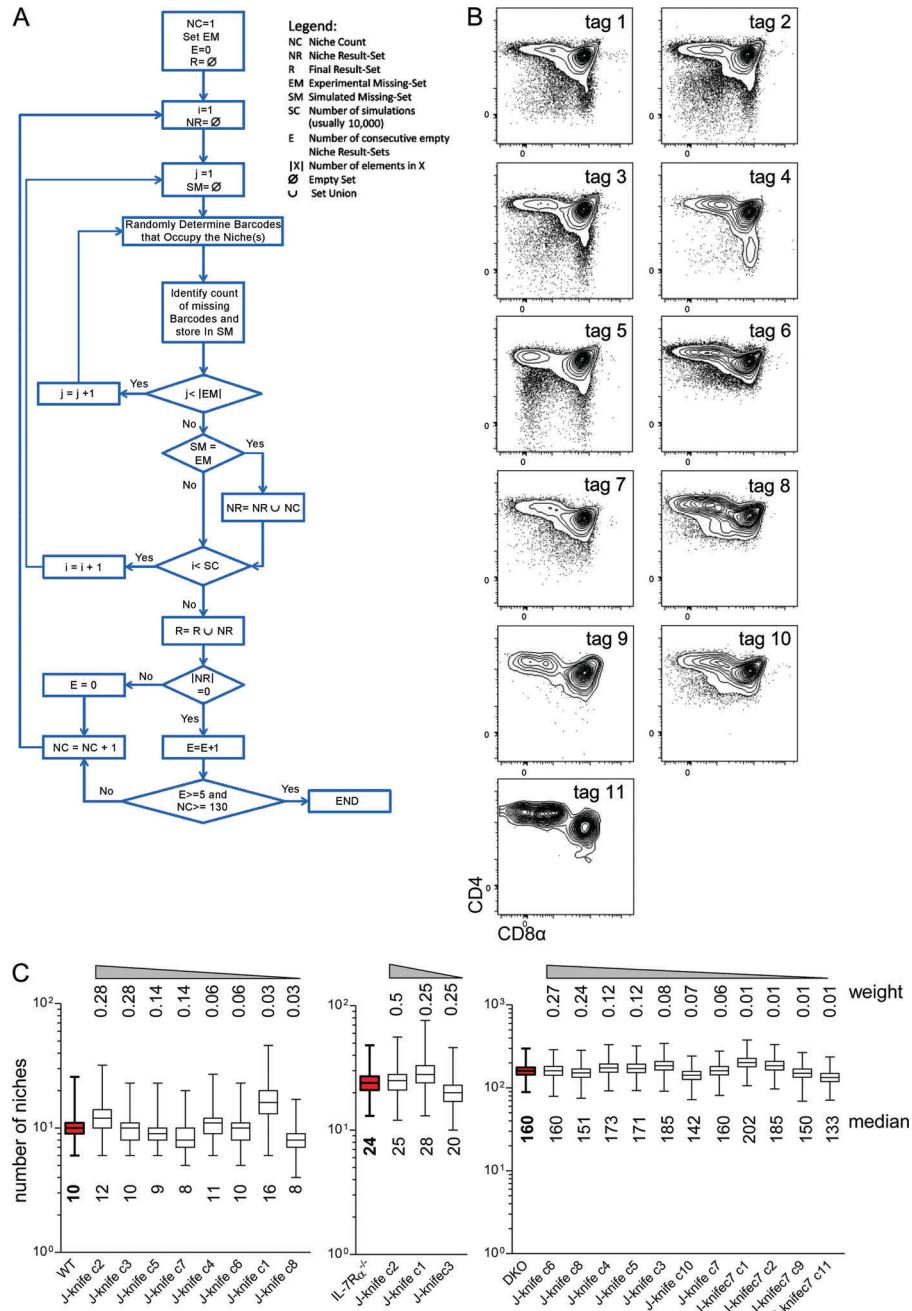
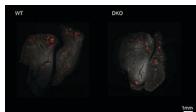


Figure S2. Quantification of thymus colonization using Monte-Carlo simulation. (A) Block diagram scheme for the algorithm used to quantify TSPNs. (B) Representative plots of individual tags of multicongenic progenitors injected i.v. into DKO recipients and their i.t. development measured by surface expression of CD4 and CD8 α (data from experiment I, Table S1). (C) Delete-d Jackknife estimation of variance performed to estimate the sensitivity of Monte Carlo simulation to experimental errors. A set of subsampled results was generated from the original result by removing a predefined number of single measurements. This yields a set of $\binom{n}{k} = \frac{n!}{(n-k)!k!}$ results, where n is the group size and k the count of elements that were removed.

Then the simulation was performed on each set member and the results were collected. As several results are obtained for each original result the estimation of variance is possible. This procedure was performed for each group while removing 25% of single experiments (rounded to the nearest integer) and running the simulation for every unique combination of missing clones (as single experiments lead often to the same number of missing clones, there are significantly fewer unique combinations than the total derived by the above formula). The result of such simulation was then weighted according to the prevalence of the corresponding combination.



Video 1. LSFM analysis of WT (left) and DKO (right) thymi. Optical cross sections through whole thymi are shown. Medulla staining (UEA-1, red) and background autofluorescence (white) are depicted. z-step: 10 μ m. Number of optical sections: 300 (WT) and 350 (DKO). Representative movie of five mice/genotype from two independent experiments.

Table S1, available as an Excel file, shows a combination of alleles used to generate multicongenic library of BM precursors. Multicongenic library of BM-derived progenitors was generated by intercrossing of indicated mouse strains. F1 animals with appropriate combination of surface/fluorescent phenotype, as determined by flow cytometry, were used for experiments; Exp (I) $n = 5$ (WT) and 6 (DKO); Exp (II) $n = 6$ (WT) and 4 (IL-7R $\alpha^{-/-}$); Exp (III) $n = 10$ (WT) and 11 (DKO); Exp (IV) $n = 7$ (WT_{irr}) and 7 (DKO); Exp (V) $n = 3$ (IL-7R $\alpha^{-/-}$ _{irr}); Exp (VI) $n = 4$ (IL-7R $\alpha^{-/-}$ _{irr}).

Table S2, available as an Excel file, shows a phenotype and sequence of injections used to determine cellular feedback which allows for entry of new progenitors from BM. Multicongenic F1 mice were generated as indicated in Table S1 and their surface/fluorescent phenotype was determined by flow cytometry before injection. BM-derived progenitors (10^6 lin $^-$ BM cells/recipient mouse per single injection) from these animals were injected, at 3 d intervals for 21 d; Exp (I) $n = 7$ (WT) and 7 (DKO); Exp (II) $n = 12$ (WT) and 12 (DKO).