

Supplementary table S1A. Expression of selected signature genes in young adult and aged iNKT cells according to sequencing data (numbers of RNA reads). **iNKT1=red**; **iNKT2=green**; **iNKT17=blue**. *: signature genes not known so far.

Gene	Reads young adult iNKT cells			Reads aged iNKT cells		
	iNKT1	iNKT2	iNKT17	iNKT1	iNKT2p	iNKT17
<i>Tbx21</i>	1,579 1,560	80 100	18 14	2,168 1,920	104 38	15 19
<i>Slamf7</i>	1,579 988	0 0	4 1	1,568 1,672	11 12	0 0
<i>Fgl2</i>	6,433 7,365	19 0	2 2	5,995 5,664	4 18	2 11
<i>Klra3</i>	1,900 1,956	46 76	28 11	1,785 2,117	47 25	0 0
<i>Nkg7</i>	2,454 3,436	3 24	8 16	2,194 2,155	11 3	3 0
<i>Ccl5</i>	1,593 1,504	1 0	0 0	1,247 1,370	1 0	0 0
<i>Itga1</i>	1,748 1,036	2 0	3 1	3,119 2,895	13 19	2 0
<i>Ly6c2</i>	540 553	0 0	2 0	1,075 1,207	0 0	0 0
<i>Ifng</i>	427 507	8 8	1 0	305 322	0 0	1 0
<i>Il6ra</i>	1 0	376 615	68 148	1 3	1,427 1,505	91 82
<i>Ccr7</i>	25 100	654 611	124 165	9 0	461 444	32 75
<i>Il4</i>	10 44	263 307	75 82	6 6	116 136	49 68
<i>Nrp2*</i>	72 59	578 622	31 18	100 143	899 1,171	0 4
<i>Slamf6</i>	167 157	1,016 999	388 382	96 79	648 689	95 109
<i>Sdc1</i>	0 0	75 78	3,542 3,379	4 0	532 540	4,508 5,057
<i>Rorc</i>	223 118	73 81	2,834 2,579	23 60	478 454	2,851 2,770
<i>Il23r</i>	0 0	77 72	4,004 3,574	0 0	480 607	4,889 5,159
<i>Blk</i>	6 0	85 65	6,079 5,063	1 0	504 526	6,623 7,185
<i>Il1r1</i>	2 0	77 87	3,745 3,280	0 0	367 534	3,423 3,183
<i>Mmp25</i>	0 0	9 63	2,212 2,351	1 0	403 422	3,513 3,927
<i>Socs3</i>	94 223	112 82	1,196 1,287	140 115	193 240	1,276 1,265
<i>Coro2b</i>	4 0	58 142	1,197 1,521	2 1	220 319	1,724 1,953
<i>Stab2</i>	1 0	7 0	1,254 931	0 0	51 84	1,078 1,089
<i>Serpinb1a</i>	5 4	5 0	741 429	4 1	87 135	869 975
<i>Ascl1</i>	0 0	16 15	852 792	0 0	94 52	619 766
<i>Ankrd63*</i>	5 11	182 250	1,210 1,388	1 3	215 151	1,214 1,045

Supplementary table S1B. Expression of selected genes differently expressed in young adult versus aged iNKT cells according to sequencing data (numbers of RNA reads). **iNKT1=red**; **iNKT2=green**; **iNKT17=blue**: Numbers>50 reads are colored accordingly when a regulation (ratio reads) >4 between young adult and aged cells was observed. Genes mentioned in the text are in bold. Genes in red shaded box: Genes possibly related to precursor iNKT cells that display down-regulated expression in aged iNKT1 cells.

Gene	Reads young adult iNKT cells			Reads aged iNKT cells		
	iNKT1	iNKT2	iNKT17	iNKT1	iNKT2p	iNKT17
<i>Gzma</i>	4,106 4,140	11 0	6 17	297 265	1 5	2 1
<i>Gzmb</i>	2,842 2,421	5 0	21 28	1,016 1,008	0 0	3 0
<i>Myb</i>	1,267 949	1,693 1,660	2,217 1,942	161 106	1,454 1,534	1,097 817
<i>Ezh2</i>	837 583	397 239	313 256	99 151	299 276	88 85
<i>Stmn1</i>	789 600	496 451	285 249	108 75	220 187	39 36
<i>Ccr9</i>	587 640	427 330	76 128	94 94	703 723	9 31
<i>Sox4</i>	502 313	494 657	589 550	67 59	574 632	75 99
<i>Tjp1</i>	128 109	0 5	11 11	1,004 1,058	4 2	7 27
<i>Gas7</i>	153 80	0 0	0 1	516 534	2 8	0 0
<i>Dntt</i>	1,708 1,854	171 174	124 256	124 170	696 604	17 41
<i>Cd8b1</i>	1,350 1,012	184 264	114 238	156 197	843 915	32 105
<i>Ctsl</i>	1,742 1,282	241 228	171 217	157 145	689 746	49 80
<i>Themis</i>	981 999	541 452	117 154	108 136	889 1,182	22 30
<i>Slpr1</i>	102 113	459 261	68 57	233 242	98 162	10 5
<i>Igf1r</i>	33 2	160 342	2,647 3,552	73 62	2,440 3,025	6,483 6,629
<i>Rad50</i>	280 137	133 179	304 401	513 507	625 639	803 711
<i>Prg4</i>	10 0	312 487	73 93	93 82	1,688 1,296	379 490
<i>Pcsk1</i>	9 0	416 260	459 523	0 3	25 78	43 48
<i>Igfbp4</i>	130 148	2,072 1,280	810 638	7 6	458 712	26 34
<i>Flt4</i>	0 0	0 0	88 95	1 0	66 54	1,008 954
<i>Il2ra</i>	195 48	14 23	346 391	88 85	281 275	1,842 1,871

Supplementary table S2A. Expression of replication-dependent histone genes in young and aged iNKT cells. Given are the read numbers for the 15 most highly expressed histone genes in young adult BALB/c mice. The degree of down regulation in aged iNKT subtypes was determined by the ratios of values obtained for the corresponding population. For example for *Hist1h1c* expression by iNKT1 cells: $1y/o = (17,084+19016) : (5442+5172) = 3,40$. Genes depicted in red: the degree of downregulation is high for iNKT1 (>14) and higher than average also for iNKT17 (>5). For the latter the degree of down regulation is rather uniform: $2,27 \pm 0,48$ (\pm SD).

Gene	Reads young adult iNKT cells			Reads aged iNKT cells (+ ratio regulation)					
	iNKT1	iNKT2	iNKT17	iNKT1	1y/o	iNKT2p	2y/o	iNKT17	17y/o
<i>Hist1h1c</i>	17,084 19,016	9,744 5,022	7,386 4,939	5,442 5,172	3,40	4,029 4,093	1,82	4,047 3,834	1,56
<i>Hist1h1d</i>	8,927 12,178	4,752 2,524	2,305 1,832	674 776	14,6	1,513 1,380	2,52	350 460	5,11
<i>Hist2h2bb</i>	8,082 11,692	3,932 2,295	3,234 2,457	1,533 1,937	5,70	1,224 1,252	2,51	1,733 2,025	1,51
<i>Hist1h1e</i>	5,095 4,223	3,193 2,334	2,581 2,116	1,409 1,403	3,31	1,334 1,206	2,18	1,146 1,098	2,09
<i>Hist1h4d</i>	4,143 6,971	2,927 2,155	1,524 1,686	1,391 1,303	4,13	1,053 839	2,69	913 696	2,00
<i>Hist2h2ac</i>	3,688 6,231	2,327 1,290	1,631 1,208	849 789	6,06	839 686	2,37	1,024 779	1,57
<i>Hist1h1b</i>	3,353 4,234	1,084 428	418 391	86 98	41,2	343 240	2,59	19 35	15,0
<i>Hist1h1a</i>	3,337 4,475	803 464	409 508	112 112	34,9	262 317	2,19	30 92	7,52
<i>Hist1h2be</i>	2,617 3,629	1,193 620	423 484	540 615	5,41	456 427	2,05	207 310	1,75
<i>Hist1h2bn</i>	2,347 2,990	589 326	263 233	68 86	34,7	175 261	2,10	13 69	6,05
<i>Hist4h4</i>	2,228 3,886	2,495 1,646	1,409 1,129	888 765	3,70	763 601	3,04	756 483	2,05
<i>Hist1h2ak</i>	2,223 2,400	623 450	292 362	132 127	17,9	301 372	1,59	54 112	3,94
<i>Hist1h2af</i>	2,090 2,500	416 284	214 217	67 85	30,2	239 311	1,27	25 21	9,37
<i>Hist1h3b</i>	1,535 2,324	424 222	168 191	52 68	32,2	145 155	2,15	10 27	9,70
<i>Hist1h4h</i>	1,439 2,021	1,014 755	569 615	330 295	5,54	324 266	3,00	269 261	2,23

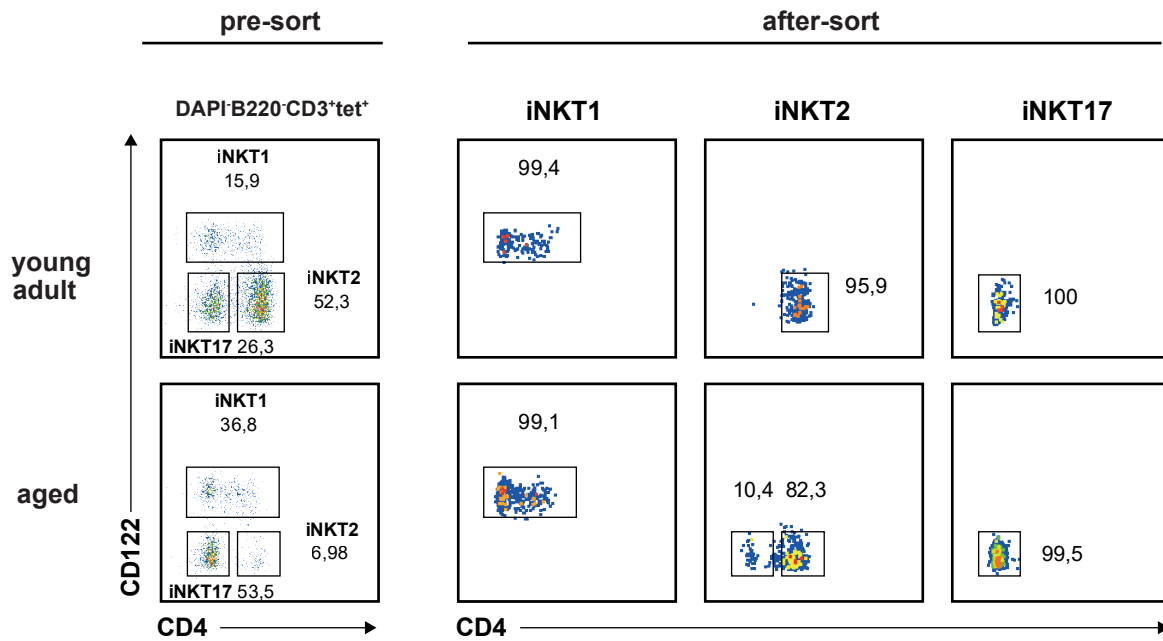
Supplementary Table S2B. Expression of replication-independent histone genes in young and aged iNKT cells. Setup of the table as in S2A.

Gene	Reads young adult iNKT cells			Reads aged iNKT cells (+ ratio regulation)					
	iNKT1	iNKT2	iNKT17	iNKT1	1y/o	iNKT2p	2y/o	iNKT17	17y/o
<i>H2afx</i>	1,818 1,656	693 524	437 420	284 242	6,60	388 292	1,79	211 189	2,14
<i>H2afy</i>	1,688 970	1,096 1,159	1,263 1,025	1,310 1,282	1,02	1,026 1,011	1,11	820 727	1,48
<i>H2afz</i>	1,145 1,355	747 448	708 574	573 519	2,29	383 396	1,53	484 361	1,52
<i>H2afj</i>	742 1,238	816 796	542 462	624 552	1,68	431 498	1,74	405 339	1,35
<i>H2afv</i>	452 361	305 303	279 331	219 256	1,71	203 181	1,58	242 261	1,21
<i>H3f3b</i>	2,899 3,433	2,741 2,030	2,391 1,849	1,597 1,789	1,87	1,565 2,091	1,30	1,328 1,486	1,51
<i>H3f3a</i>	312 524	575 408	422 410	256 273	1,58	298 295	1,66	312 237	1,52

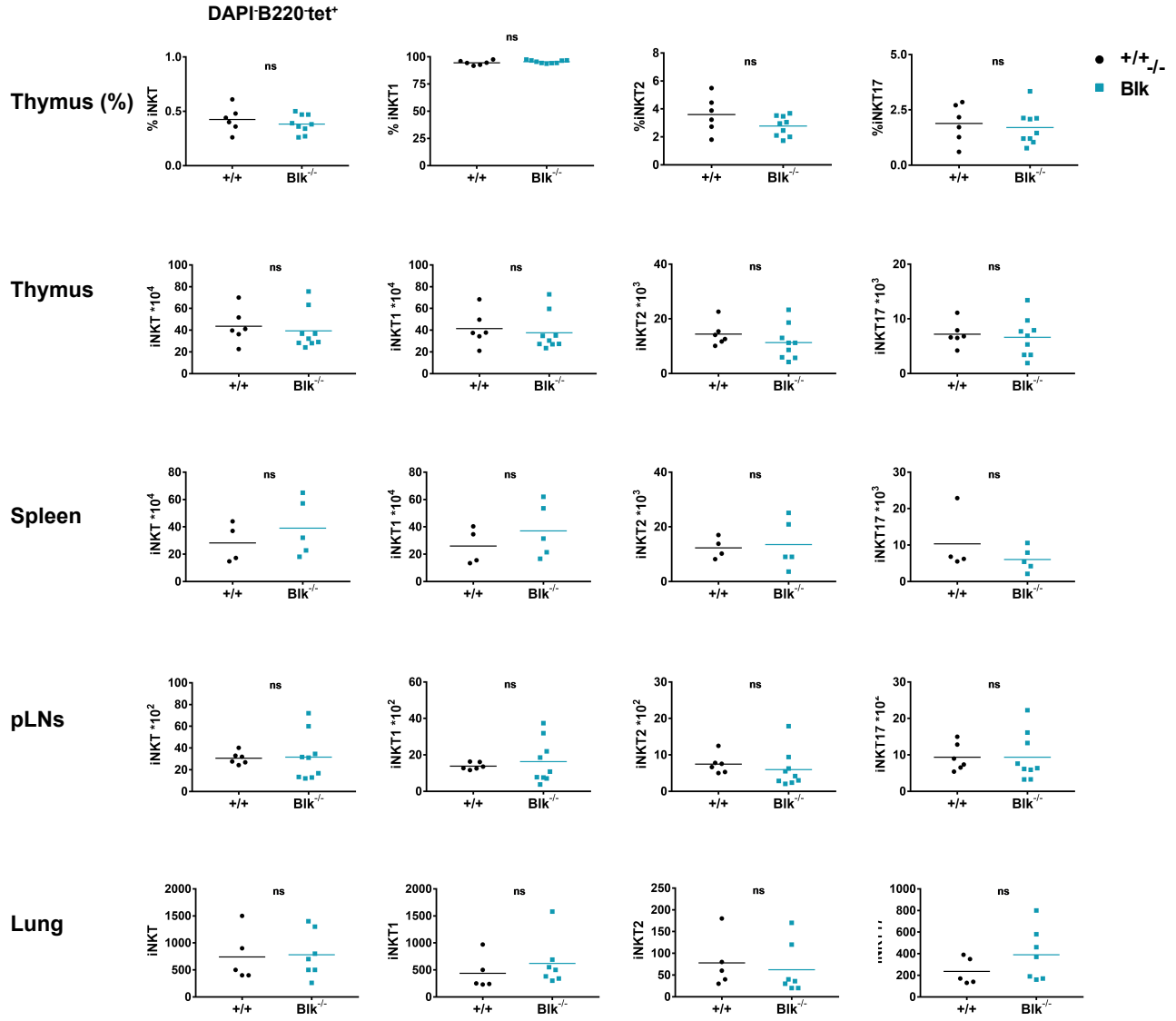
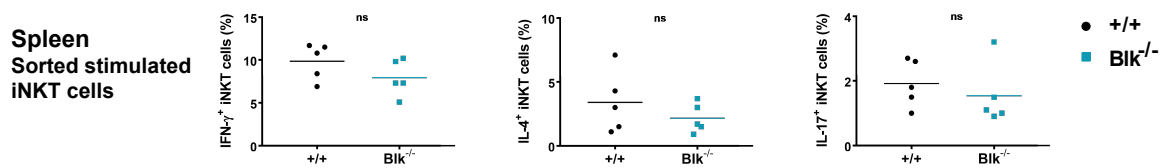
Supplementary table S3. Expression of selected genes in young adult and aged iNKT cells according to sequencing data (RNA reads). Data source as in Fig. 2.

Gene	Reads young adult iNKT cells			Reads aged iNKT cells		
	iNKT1	iNKT2	iNKT17	iNKT1	iNKT2p	iNKT17
<i>Scart2¹</i>	186 135	602 685	4,665 3,741	149 125	625 846	5,247 4,909
<i>Aqp3</i>	104 128	58 65	1,389 1,090	100 111	179 281	1,595 1,592
<i>Camk2d</i>	289 298	148 159	234 251	341 381	234 306	269 277
<i>Gpr183</i>	1,227 1,741	437 418	1,435 1,187	1,547 1,765	569 802	1,677 1,644
<i>Sox13</i>	16 4	532 1,100	1,260 2,583	13 15	1,483 1,268	2,766 3,074
<i>Fasl</i>	869 1,092	36 20	9 7	884 878	34 25	7 35
<i>Tnfsf10</i>	1,776 1,281	413 410	244 249	1,363 1,361	389 363	263 278

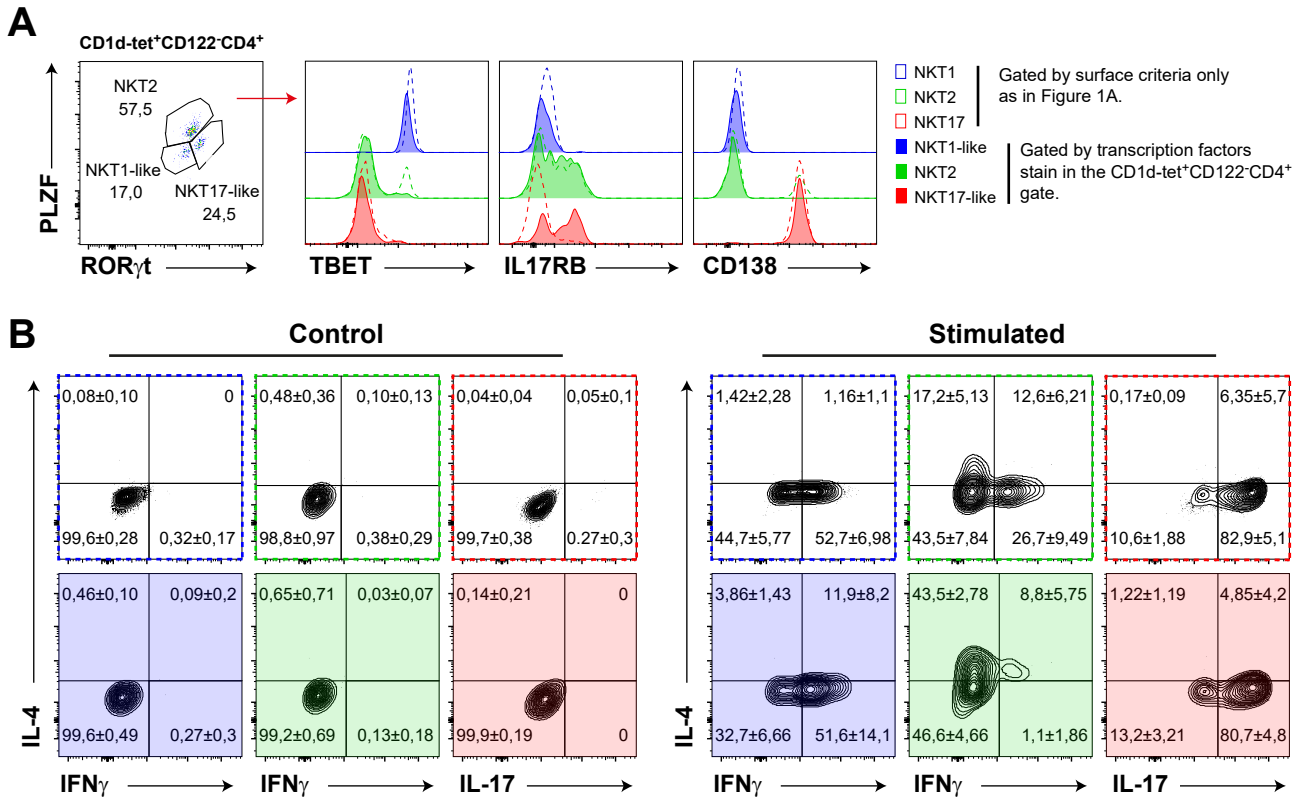
1) Scart2 = 5830411N06Rik



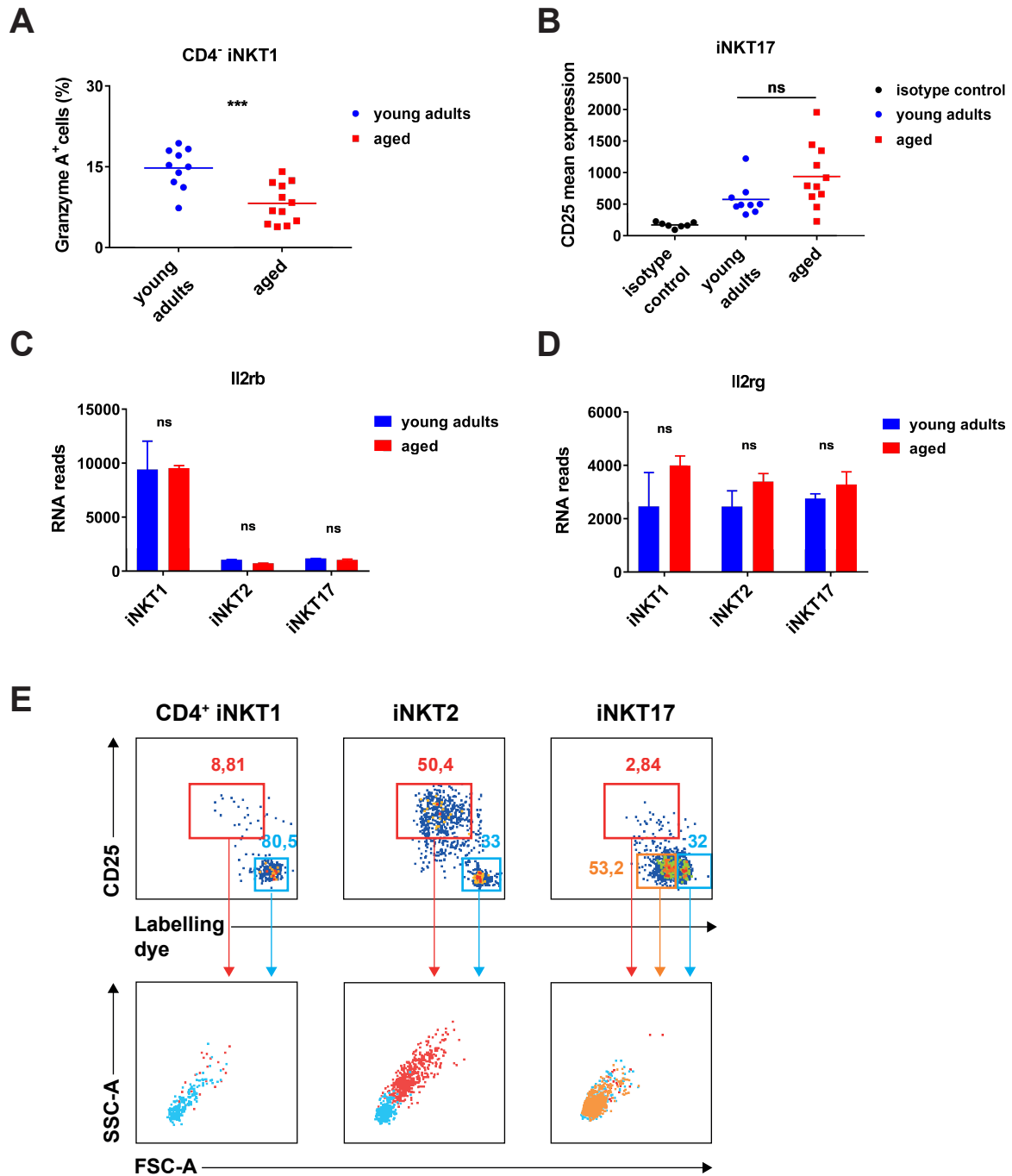
Supplementary Figure 1. Sorting strategy of iNKT subsets from young adult and aged mice. The plots illustrate the gating strategy (pre-sort) for live cell sorts (one out of two performed) to obtain iNKT subpopulations as also shown in Fig. 1B but using a flow cytometry sorter. In each experiment thymic cells were pooled from either 3 young adult (upper panel) or 3 aged (lower panel) BALB/c mice. After-sort: Small aliquots of sorted subsets were re-analyzed to check for purity that is indicated by the numbers accompanying the gates. Note that in aged iNKT2 cells an impurity of approximately 10% iNKT17 cells was found.

A**B**

Supplementary Figure 2. *Blk* deficiency does not have an impact on iNKT cell frequencies and cytokine production. (A) iNKT cell frequencies and cell numbers in BL6 wt and *Blk*^{-/-} mice as determined by flow cytometry. Gating as shown in fig. 1B. Data were summarized from at least 3 independent experiments. Each dot represents data from one animal. (B) IFN- γ , IL-4 and IL-17 production of iNKT cells from wt and *Blk*^{-/-} BL6. Total splenic iNKT cells were sorted and stimulated with anti-CD3 and anti-CD28 for 16h as described in Experimental Procedures. Data are collected from 5 independent experiments (dots) and statistically evaluated with unpaired t test. ns: not significant.



Supplementary Figure 3. Characterization of aged iNKT2p cells. (A) Thymi from 35 week old female BALB/c mice were investigated by flow cytometry. Gating for surface markers was done as shown in Figure 1A. The representative plot (left panel) depicts the three main subsets in aged iNKT2p cells based on additional stain for PLZF and ROR γ t (see also Figure 1B). The histograms (middle part) show the expression of TBET, IL17RB and CD138 (syndecan) by either iNKT1 (CD122⁺CD4⁺), iNKT2 (CD122⁺CD4⁺) and iNKT17 (CD122⁺CD4⁺) cells based only on gating by surface markers (dashed lines) or by the subsets of aged iNKT2p cells in the CD122⁺CD4⁺ gate but now gated additionally according to their expression of PLZF and ROR (filled and colored). (B) Production of cytokines as indicated following PMA/ionomycin stimulation. Open boxes (framed by dashed lines) correspond to the cytokine production profile of cells gated by surface markers only (as in A) or by the cells dissected further into the iNKT2p subpopulations as defined by PLZF/ROR γ t expression. Data are shown for 5 mice (thymi) analyzed (mean \pm SD).



Supplementary Figure 4. (A) Granzyme A⁺ cell frequencies among thymic CD4⁻iNKT1 cells from young adult and aged BL6 mice using the same analytic strategy as for BALB/c cells (Fig. 3I). Data are collected from 5 independent experiments. Each dot represents data from one animal. (B) Mean expression of CD25 in thymic iNKT17 cells from young adult and aged BL6 mice using the same gating strategy as for BALB/c cells (Fig. 4B). Data are collected from 5 independent experiments. Each dot represents data from one animal. (C-D) *Il2rb* and *Il2rg* RNA reads (mean ± sd), same source as in Fig.2. (E) Alternative presentation of levels of CD25 and cell tracker dye (upper panel) in the iNKT cell subsets displayed in Fig 4E as well as forward/side scatter of the color-gated populations (lower panel) on day 3 after stimulation with IL2. Unpaired t test was performed in (A). One-way ANOVA followed by Tukey's multiple comparisons test were performed in (B). Two-way ANOVA followed by Sidak's multiple comparisons test were performed in (C) and (D). ***p < 0.001, ns=not significant.