Alloreactive and leukemia-reactive T cells are preferentially derived from naïve precursors in healthy donors: implications for immunotherapy with memory T cells

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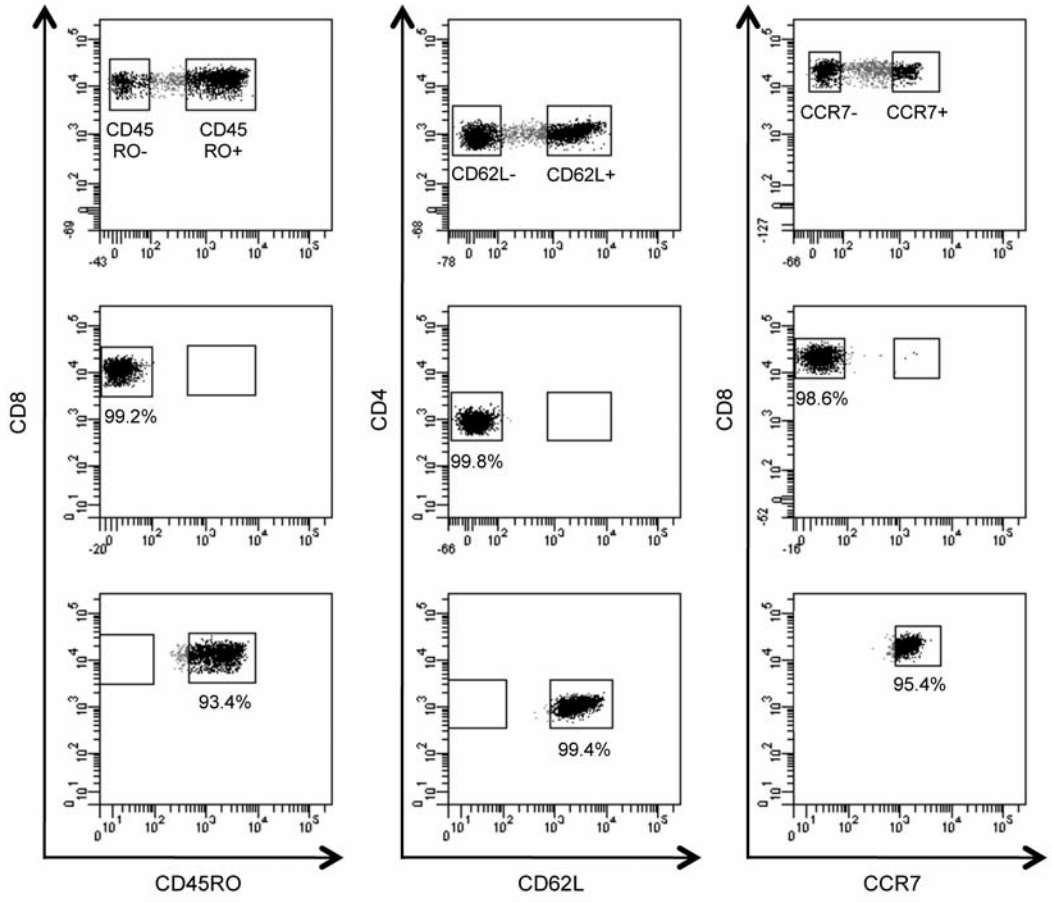
Online Supplementary Table S1. HLA types of healthy donors and cells used for *in vitro* stimulation.

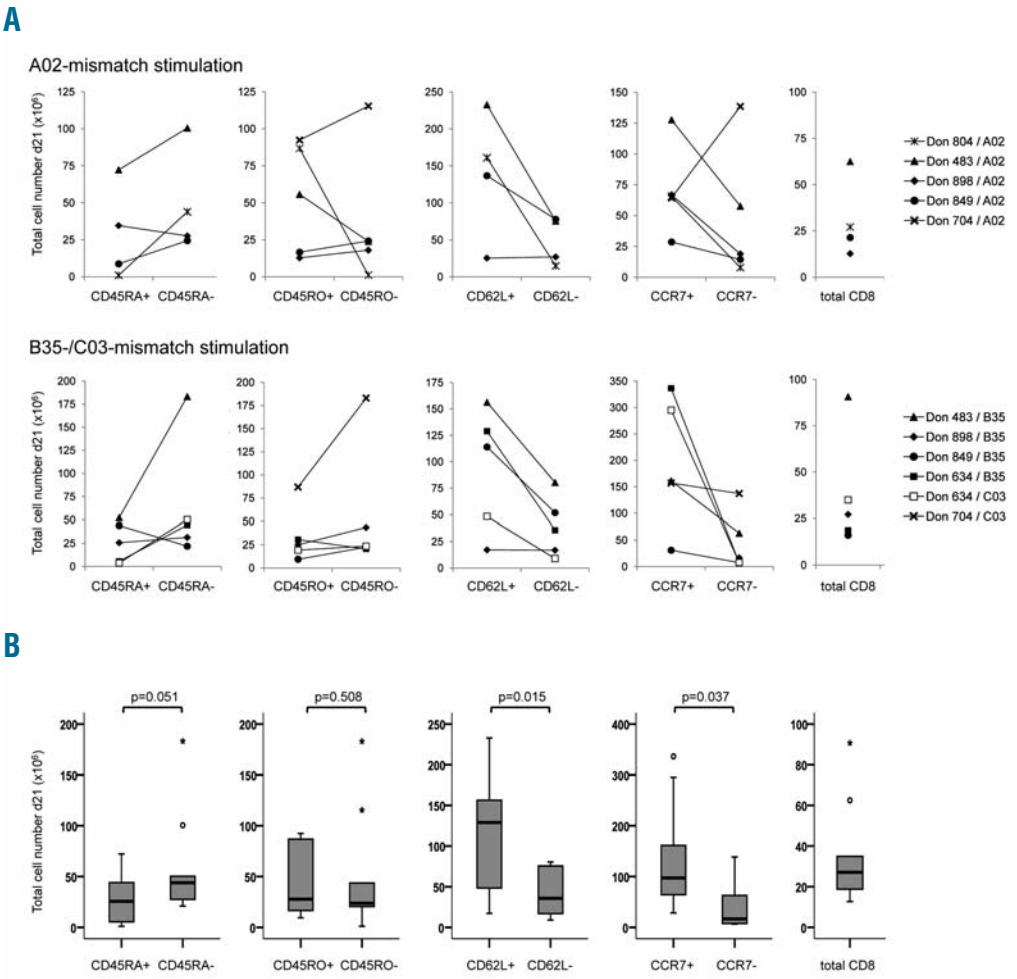
				HLA class I-mismatched K562 transfectant cells			HLA class I-matched AML blasts
	HLA-A*	HLA-B*	HLA-C*	A*02:01	B*35:03	C*03:03	
Don 804	03 / 30	13 / 18	06 / 07	×	n.d.	n.d.	n.d.
Don 483	32 / 68	27 / 64	02 / 08	×	×	n.d.	n.d.
Don 898	11 / 24	27 / 51	03 / 04	×	×	n.d.	n.d.
Don 849	01 / 33	18 / 58	03 / 07	×	×	n.d.	n.d.
Don 634	01 / 24	08 / 27	02 / 07	n.d.	×	×	n.d.
Don 704	03 / 32	44 / 50	05 / 06	×	n.d.	×	n.d.
SIB 369	01:01 / 24:02	18:01 / 38:01	07:02 / 12:03	×	n.d.	n.d.	MZ369-AML
Don 069	01:01 / 30:01	08:01 / 13:02	06:02 / 07:01	×	n.d.	n.d.	MZ653-AML
Don 940	02:01	15:01 / 15:17	03:04 / 07:01	n.d.	×	n.d.	MZ987-AML

			HLA class II-mismatched K562 transfectant cells	
	HLA-DRB1*	HLA-DQB1*	DRB1*07:01	DQB1*06:02
Don 053	10:01 / 11:04	03:01 / 05:01	×	n.d.
Don 073	03:01 / 11:01	02:01 / 03:01	×	×
Don 372	11:01 / 13:01	03:01 / 06:03	×	×
Don 079	04:01 / 15:01	03:02 / 06:02	×	n.d.
Don 454	04:01 / 14:54	03:02 / 05:03	×	×
Don 225	01:01 / 11:01	03:01 / 05:01	n.d.	×

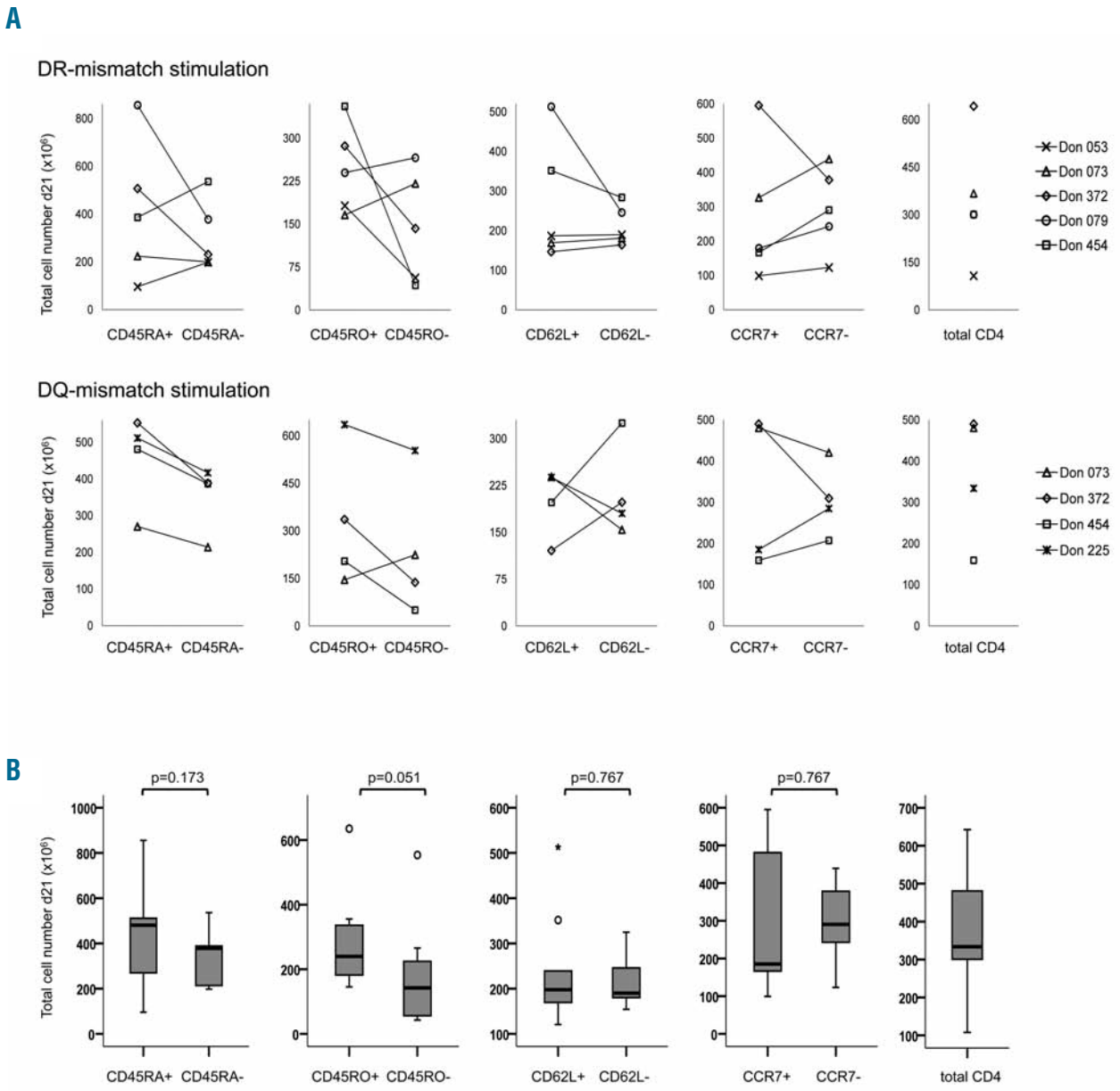
Don, unrelated donor; SIB, sibling donor; AML, acute myeloid leukemia; n.d., not determined



Online Supplementary Figure S1. Gating strategy for cell sorting. After gating of CD3 CD8 T cells or CD3 CD4 T cells, the gates for a single T-cell differentiation marker were set according to strong or absent expression of this marker (>0.5 log difference in fluorescence intensity). The sorting gates and resulting fractions for CD62L subsets in CD4 T cells of donor 372, as well as those for CD45RO and CCR7 subsets in CD8 T cells of donor 804 are shown.

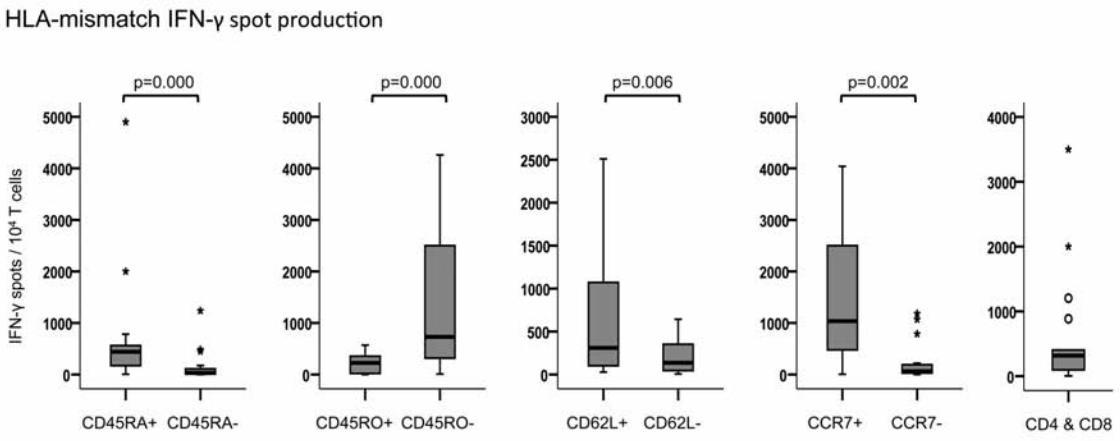


Online Supplementary Figure S2. Alloproliferation to HLA class I alleles in CD8 T-cell subsets. Cell counts of MLR cultures were determined weekly. (A) Total numbers of MLR responder cells at d21 if cultures were initiated at d0 with $1x10^6$ cells of sorted naïve-enriched, memory-enriched or entire CD8 T cells, respectively. Data from six healthy donors screened against mismatched HLA-A*02:01 (upper panel), HLA-B*35:03 or HLA-C*03:03 (both lower panel) are shown. (B) Box plots and *P* values of data presented in (A); for explanation see legend to Figure 2C. Data shown in Figures 2 and 3 are derived from the same MLR experiments.

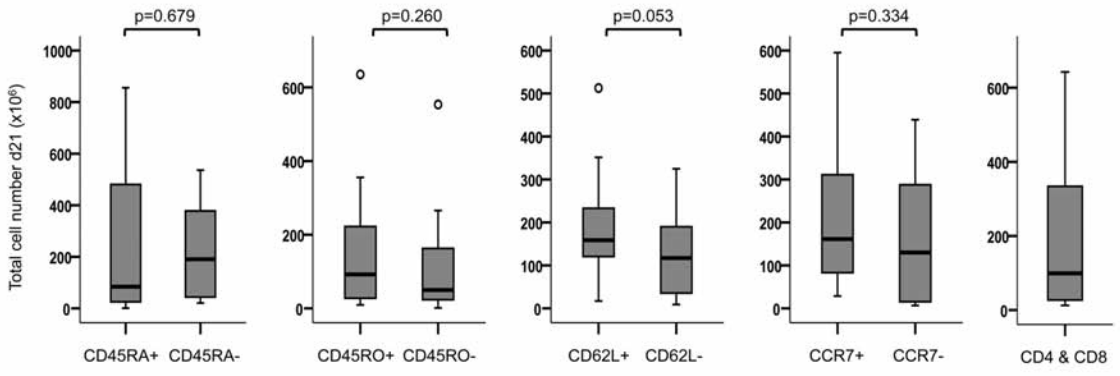


Online Supplementary Figure S3. Allo-HLA class II proliferation of CD4 T-cell subsets. Cell counts of MLR cultures were determined weekly. (A) Total numbers of MLR responder cells at d21 in cultures initiated at d0 with $1x10^6$ cells of sorted naïve-enriched, memory-enriched or entire CD4 T cells. Data from six healthy donors screened against mismatched HLA-DRB1*07:01 (upper panel) and HLA-DQB1*06:02 (lower panel) are shown. (B) Box plots and *P* values of data presented in (A); for explanation see the legend to Figure 2C. Data shown in Figure 4 and *Online Supplementary Figure S3* are derived from the same MLR experiments.

A



B



Online Supplementary Figure S4. Allo-HLA responses of naïve and memory enriched subsets in entire CD8 and CD4 T cells. Subset data from CD8 and CD4 T cells (Figures 2 and 4, IFN-γ spot production; *Online Supplementary Figures S2 and S3*, proliferation) were combined with regard to allorecognition (A) and alloproliferation (B), and were then analyzed for significant differences. Median (line), 25th to 75th percentile (box), minimum and maximum values (error bars) are indicated.