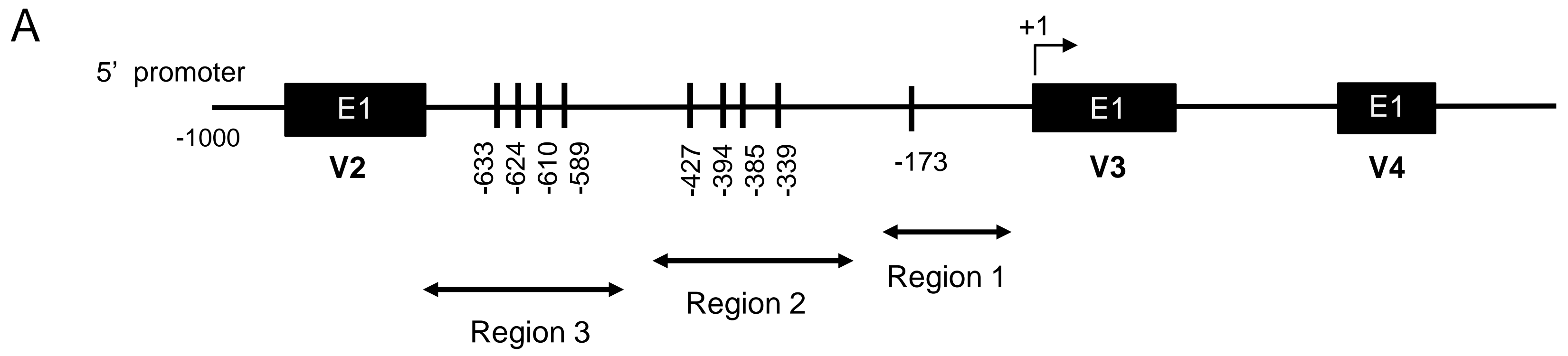
Supplemental Figure 1

A



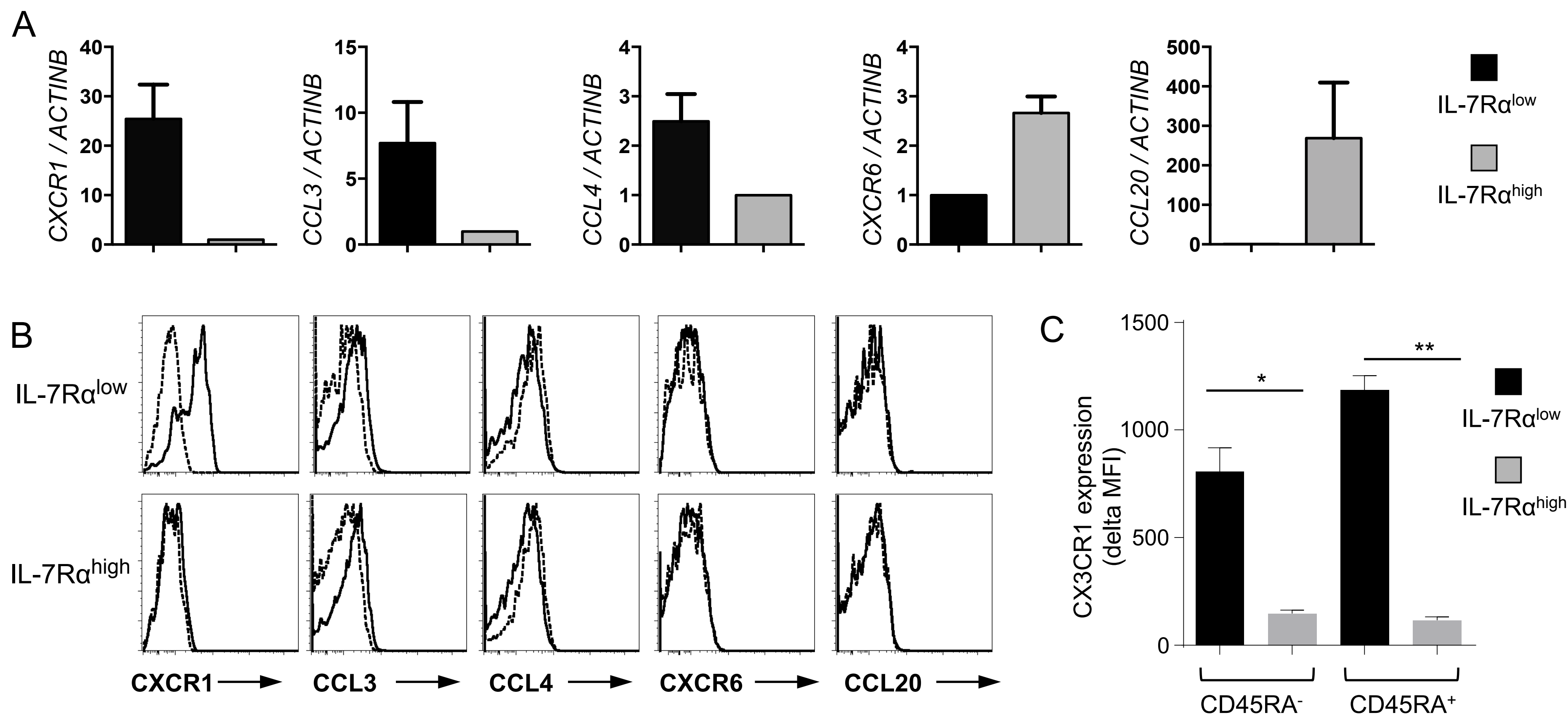

B

Bisulfite-specific PCR primers

| Promoter regions | Forward primer | Reverse primer |
|---|---|---|
| region 1 ( -33/-216) | 5'-ATTAAATGTTTTGGGGTAGAGAGGT-3 | 5'-ACACCTCAAACCCTACCCTATATCTA-3 |
| region 2 (-229/-457) | 5'-TGTTAGGGGTTAGGTAGGTAATGAA-3 | 5'-CCATCAATCAAAACTAAAAACACAA-3 |
| region 3 (-547-770) | 5'-TAAAGAAAGAAAATTGTTGGTGGAT-3 | 5'-AAAACCCCTCTAACCCCTTACTAC-3 |

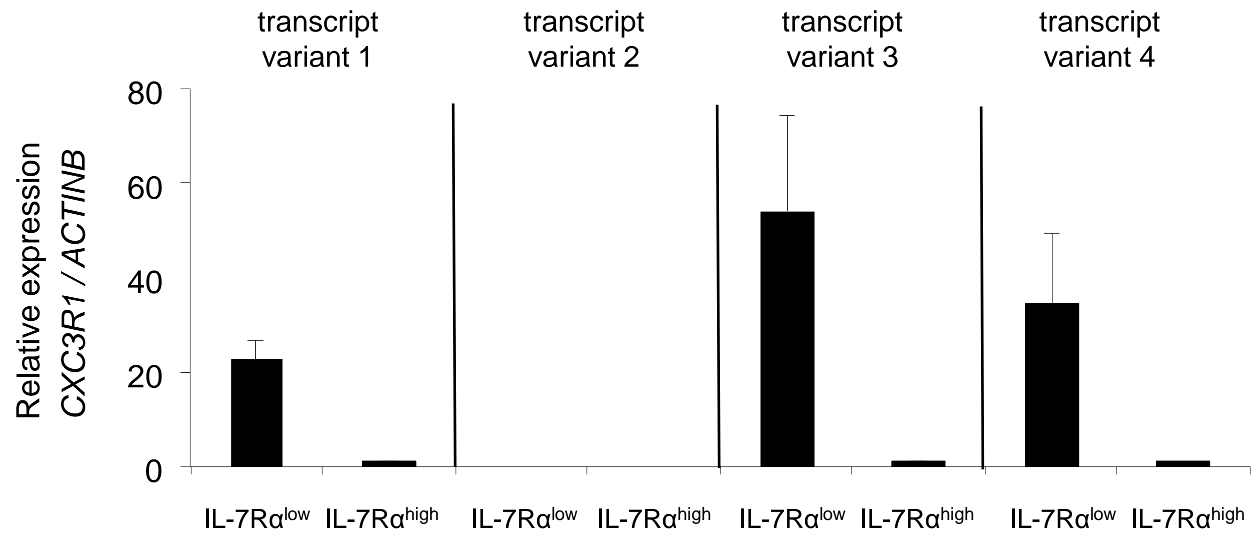
**Supplemental Figure 1**. (A) Illustration of the strategy designing the primers for determining CpG methylation in the *CX3CR1* gene promoter regions by bisulfite-specific PCR. The first exon (E1) of *CX3CR1* variant (V)2, V3 and V4 is indicated in black boxes. The CpG site (-173) included in the original DNA methylation array chip and additional upstream CpG sites that were analyzed are indicated with vertical lines and numbers. The numbers were given in respect to the first nucleotide of the first exon of V3 (+1). Solid arrows (regions 1-3) indicate the positions of the primers used for bisulfite-specific PCR and sequencing. (B) Sequences for bisulfite-specific PCR primers

Supplemental Figure 2



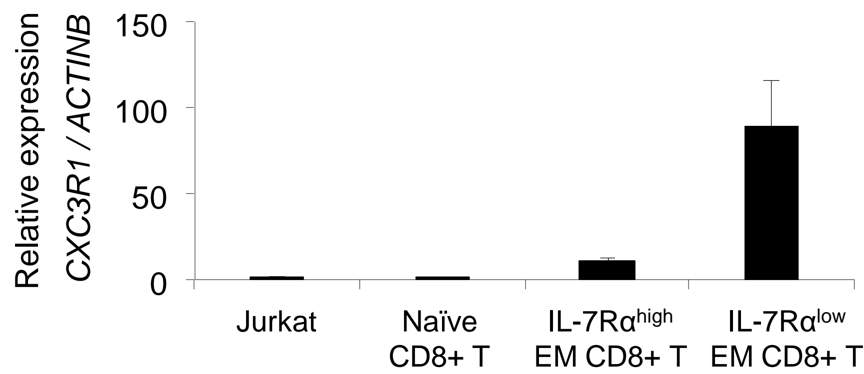

**Supplemental Figure 2.** (A) mRNA expression of chemotaxis-related genes with differential DNA methylation between IL-7Rα$^{low}$ and $^{high}$ effector memory (EM) $CD8^+$ T cells. The gene expression of *CXCR1, CCL3, CCL4, CXCR6* and *CCL20* in FACS-sorted IL-7Rα$^{low}$ and $^{high}$ EM $CD8^+$ T cells was measured by RT-qPCR. (B) Flow cytometric analysis of CXCR1, CCL3, CCL4, CXCR6 and CCL20 in IL-7Rα$^{low}$ and $^{high}$ EM $CD8^+$ T cells in freshly isolated PBMCs from healthy donors. Representative histograms show the expression of CXCR1, CCL3, CCL4, CXCR6 and CCL20. The dashed lines represent isotype-matched control staining. (C) Mean fluorescent intensity (MFI) of CX3CR1 expression by $CCR7^-CD45RA^-$ and $^+$ IL-7Rα$^{low}$ and $^{high}$ EM $CD8^+$ T cells. Delta MFI of CX3CR1 expression was obtained by subtracting MFI of isotype control staining from MFI of CX3CR1 staining. Bars and error bars indicate mean and SEM, respectively (n = 5 (A) and 6 (C) donors). $^*P = 0.0019$, $^{**}P < 0.0001$. Primers used for RT-qPCR are as follows: *CXCR1* (forward (F), 5'-TGGGGACTGTCTATGAATCTG-3'; reverse (RE), 5'-GCAACACCATCCGCCATTTT-3'), *CCL3* (F, 5'-AGTTCTCTGCATCACTTGCTG-3'; RE, 5'-CGGCTTCGCTTGGTTAGGAA-3'), *CCL4* (F, 5'-CGCCTGCTGCTTTTCTTACAC-3'; RE, 5'-CAGACTTGCTTGCTTCTTTTGG-3'), *CXCR6* (F, 5'-TGACGGATGTGTTCCTGGTG-3'; RE, 5'-GCCCAGTAGGCTCTTGCAC-3'), *CCL20* (F, 5'-TGCTGTACCAAGAGTTTGCTC-3'; RE, 5'-CGCACACAGACAACTTTTTCTTT-3).

# Supplemental Figure 3




**Supplemental Figure 3.** The expression of four *CX3CR1* transcript variants in FACS-sorted IL-7R$\alpha^{low}$ and $^{high}$ EM CD8$^+$ T cells from healthy donors (n = 3) as measured by RT-qPCR. All results were normalized to β-*actin* expression. Bars and error bars indicate the mean and SEM, respectively (n = 3 donors). Primer sequences used for RT-qPCR are as follows: variant 1 (forward (F), 5'-GAGGCGTTTAAGTTGGCAGA-3; reverse (RE), 5'-AGACCACGATGTCCCCAATA-3'), variant 2 (F, 5'-GGACTTCTTCCACCATGAGC-3'; RE, 5'- GAAGGCCTCTAGTCGCTGTG-3'), variant 3 F, 5'-GAGAGCTGACTGTGCTGTGC-3; RE, 5'-TAACAGGCCTCAGCCAAATC-3'), variant 4 (F, 5'-CAGATCCAGAGGTTCCCTTG-3; RE, 5'-TAACAGGCCTCAGCCAAATC-3).

Supplemental Figure 4




**Supplemental Figure 4.** RT-qPCR analysis of *CX3CR1* gene expression in Jurkat T cells and FACS-sorted naïve, IL-7R$\alpha^{low}$ and $^{high}$ effector memory (EM) CD8$^{+}$ T cells of healthy individuals. Bars and error bars indicate the mean and SEM, respectively (n = 3 donors).