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Supplemental Information

Chemerin triggers migration of a CD8 T cell subset with natural killer cell functions

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Supplemental Information

Supplemental Figures

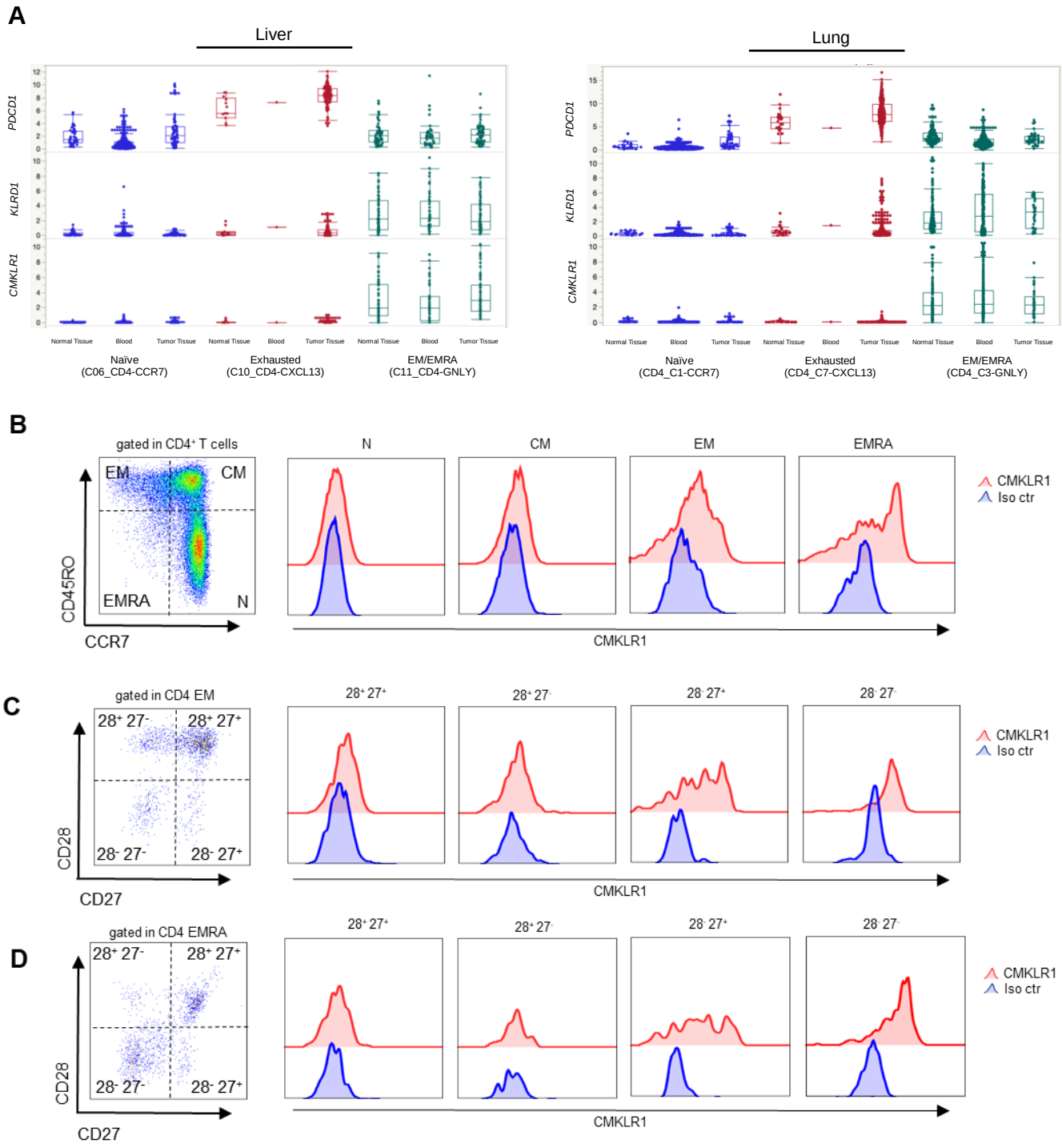

Figure S1. scRNA-seq and flow cytometry analyses reveal CD4 subsets expressing CMKLR1. (A)

Analysis of publicly available single cell gene expression data from human CD4+ T cells (Liver[10]; Lung[11]).

Boxplots showing expression of *PDCD1, KLRD1*, and *CMKLR1* in naïve (C06_CD4-CCR7), exhausted (C10_CD4-CXCL13), and effector memory or effector memory RA (EM/EMRA, C11_CD4-GNLY) CD4+ T cells from human blood, tumor tissue or normal tissue of liver and lung cancer patients at single cell resolution. **(B-D)** Flow cytometry of CMKLR1 immunostaining in subsets of **(B)** CD4 effector memory (EM, CD45RO+ CCR7-), effector memory RA (EMRA, CD45RO- CCR7-), central memory (CM, CD45RO+ CCR7+), or naïve (N, CD45RO- CCR7+) **(C-D)** CD28- CD27- (28- 27-), CD28- CD27+ (28- 27+), CD28+ CD27- (28+ 27-), or CD28+ CD27+ (28- 27-) CD4 EM **(C)** or EMRA **(D)**. (Left) Shown are representative gating strategy and histograms for CMKLR1 in each CD4 subsets. Representative dot plots from n= 2 experiments with 5 donors total.

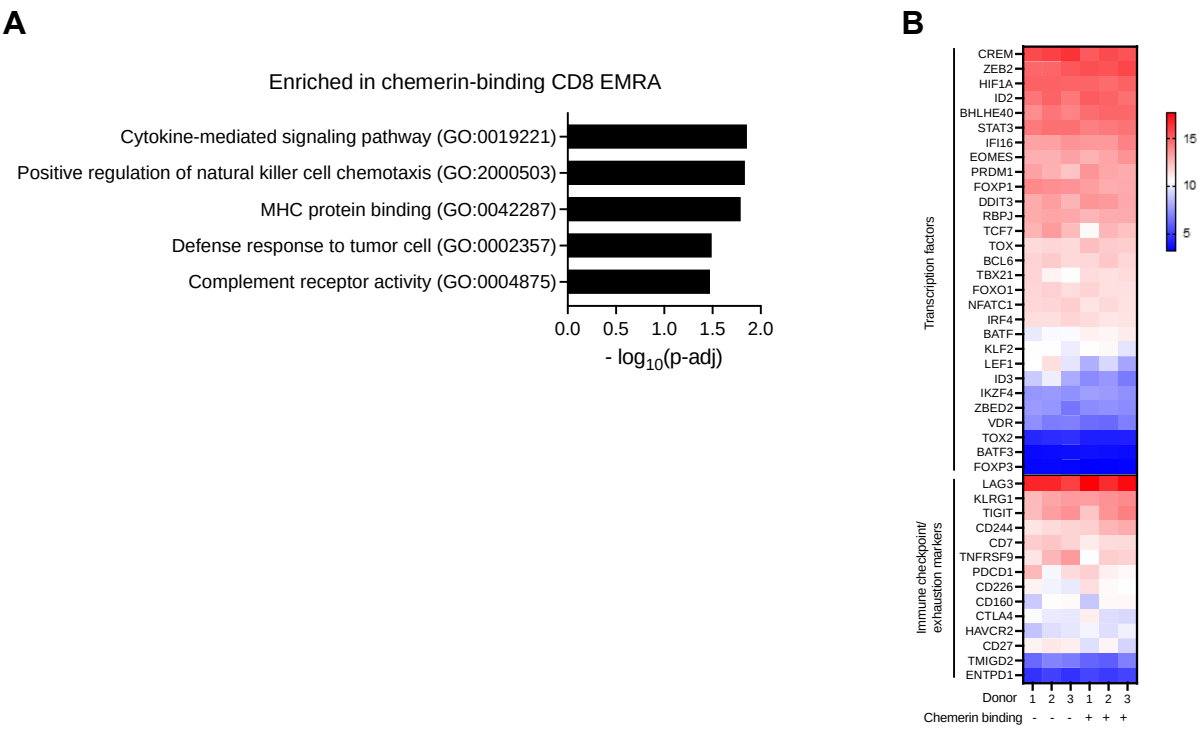

Figure S2. RNAseq analysis of human CD8 EMRA T cells. (**A**) Gene set enrichment analysis (EnrichR) was performed to identify GO terms enriched in chemerin-binding CD8 EMRA subset relative to chemerin non-binders. Significantly enriched GO term-defined functional properties are listed (adjusted P-value <0.05). (**B**) Heat map of selected genes encoding transcription factors and markers of T cell immune checkpoints/exhaustion. Gene expression counts were rlog-transformed for visualization and are colored from blue (low expression) to red (high expression).

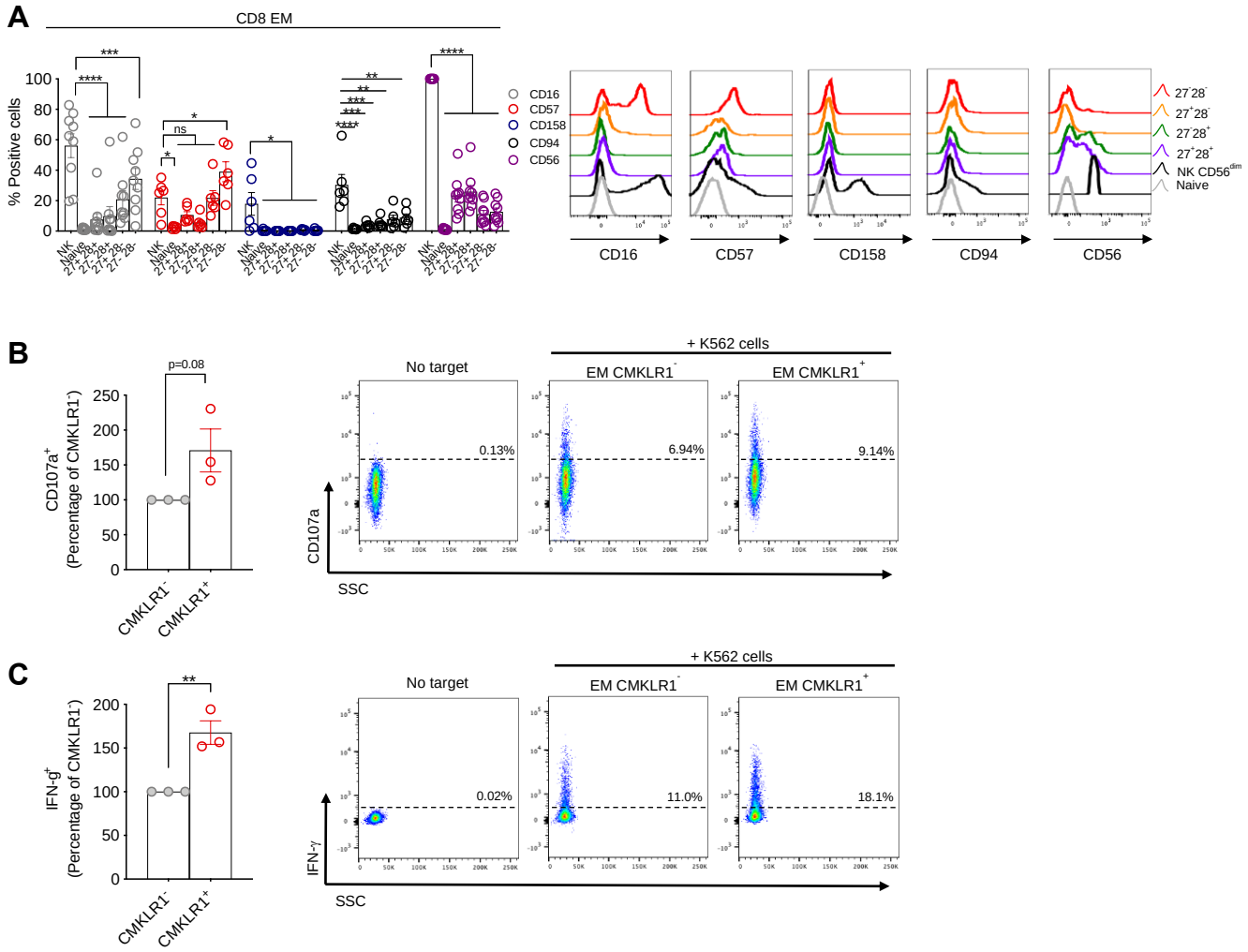

Figure S3. CMKLR1-expressing CD8 EM display phenotypic and cytotoxic features of NK cells (A) Flow cytometry of CD16, CD57, CD156, CD94, and CD56 immunostaining in subsets of NK cells, CD8 T naïve and EM divided into CD27/CD28 subsets as shown in Figure 1. (Left) For each subset, the percentage of positive cells for each marker is shown. Shown are pooled data (means ± s.e.m.) from n= 3 experiments with 6-9 donors total. (Right) Representative histogram overlays are shown. **(B-C)** Cell surface expression of CD107a **(B)** and intracellular IFN-g **(C)** in cell-sorted populations of CD8 EM T cells positive (CMKLR1+) or negative (CMKLR1-) for CMKLR1 cocultured with K562 cells at an effector to target ratio (E:T) of 2:1. Shown are pooled data (means ± s.e.m.) from n = 3 experiments. For each

experiment, the percentage of CD107a or IFN-g positive cells is set to 100 for the CMKLR1- group, and the data for the CMKLR1+ group is shown as a percentage of the CMKLR1- control group. Groups were compared by two-way ANOVA with Dunnett's multiple comparisons test **(A)** or two-tailed Student's t-test **(B-C).** *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001, ns: not significant.

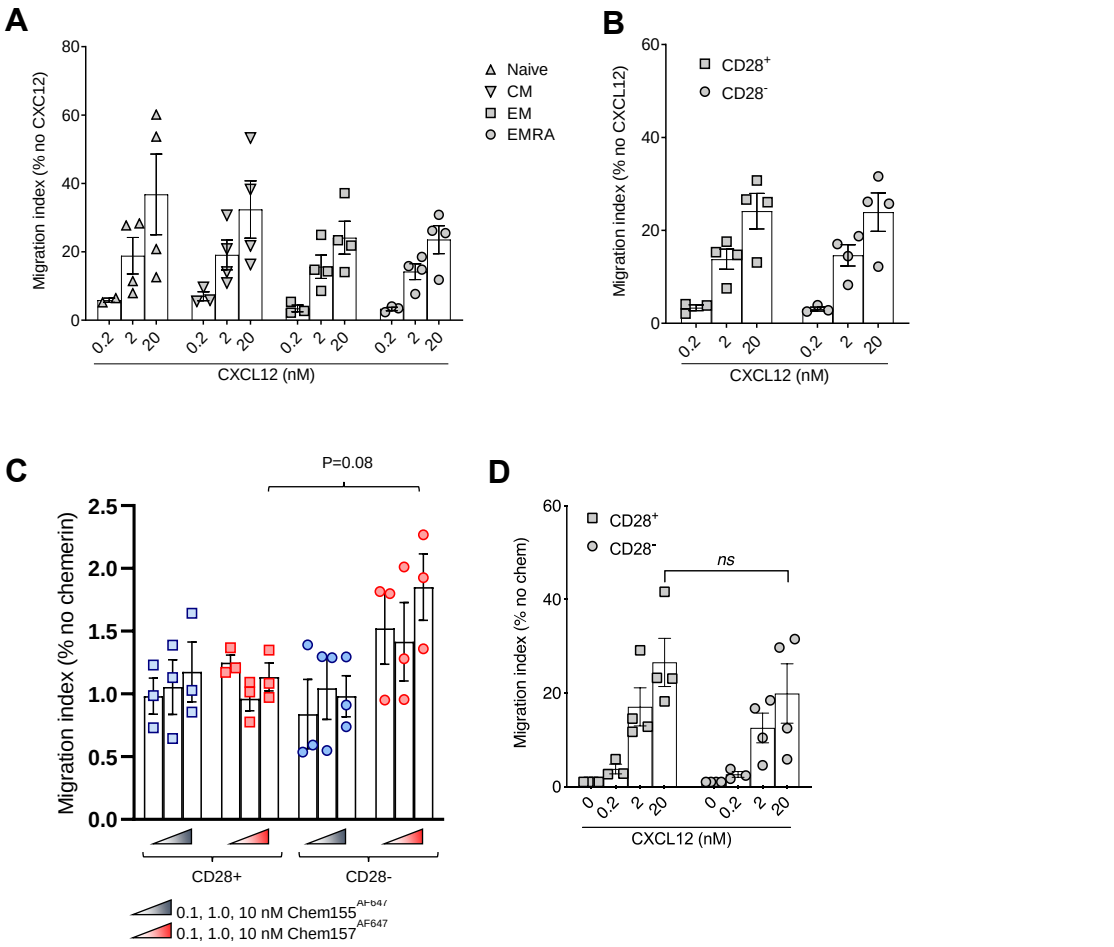
A
Migration index (% no CXC12)
Naive
CM
EM
EMRA
CXCL12 (nM)
B
Migration index (% no CXCL12)
CD28+
CD28-
CXCL12 (nM)
C
P=0.08
Migration index (% no chemerin)
CD28+
CD28-
0.1, 1.0, 10 nM Chem155AF647
0.1, 1.0, 10 nM Chem157AF647
D
Migration index (% no chem)
CD28+
CD28-
ns
CXCL12 (nM)
E
CD28- EM
Chem155
Chem157
CXCL12
0.01nM
0.1nM
1nM
10nM
MFI (% max)
Time (s)
F
CD28- EM
Chem155AF647
Chem157AF647
CXCL12
1 nM
ns
***
MFI (% max)
Time (s)
G
CD28+ EM
Chem155
Chem157
CXCL12
0.01nM
0.1nM
1nM
10nM
MFI (% max)
Time (s)
H
CD28+ EM
Chem155AF647
Chem157AF647
CXCL12
1 nM
ns
****
Time (s)

Figure S4. Active chemerin triggers chemotaxis and alpha 4 integrin activation in CMKLR1+ CD8 EM T cells. Migration of CD8 subsets **(A)** or CD8 EMRA CD28- or CD28+ subsets **(B)** in response to human CXCL12**.** Migration of CD8 EM CD28- or CD28+ subsets in response to human active chemerin (Chem157^{AF647} or dead chemerin (Chem155^{AF647}) (**C**), or human CXCL12 **(D).** For (**A-D**), shown are pooled data from two experiments (means ± s.e.m.) with n=3 donors in total. For each donor and subset, results are shown as a percentage of the absolute migrating cell number in absence of CXCL12 or chemerin in the bottom chamber. Groups were compared by one-way ANOVA with Šídák's multiple comparisons test. Flow cytometry analyses of LDV-FITC binding to CD28- **(E-F)** or CD28+**(G-H)** EM upon stimulation with human Chem155^{AF647}, Chem157^{AF647}, or CXCL12. For each sample, the median fluorescence intensity (MFI) of the LDV-FITC staining was calculated per ten seconds bins, and data expressed as a percentage of the maximal MFI recorded upon Manganese stimulation, taken as a positive control. Shown are pooled data (means ± s.e.m.) from four experiments with n=6 donors in total. Groups were compared by two-way ANOVA with Šídák's or Tukey's multiple comparisons test. ***P ≤ 0.001; ****P ≤ 0.0001, ns: not significant.