Figure S1, panels A.1-A.8

Figure S1, panels B.1-B.8

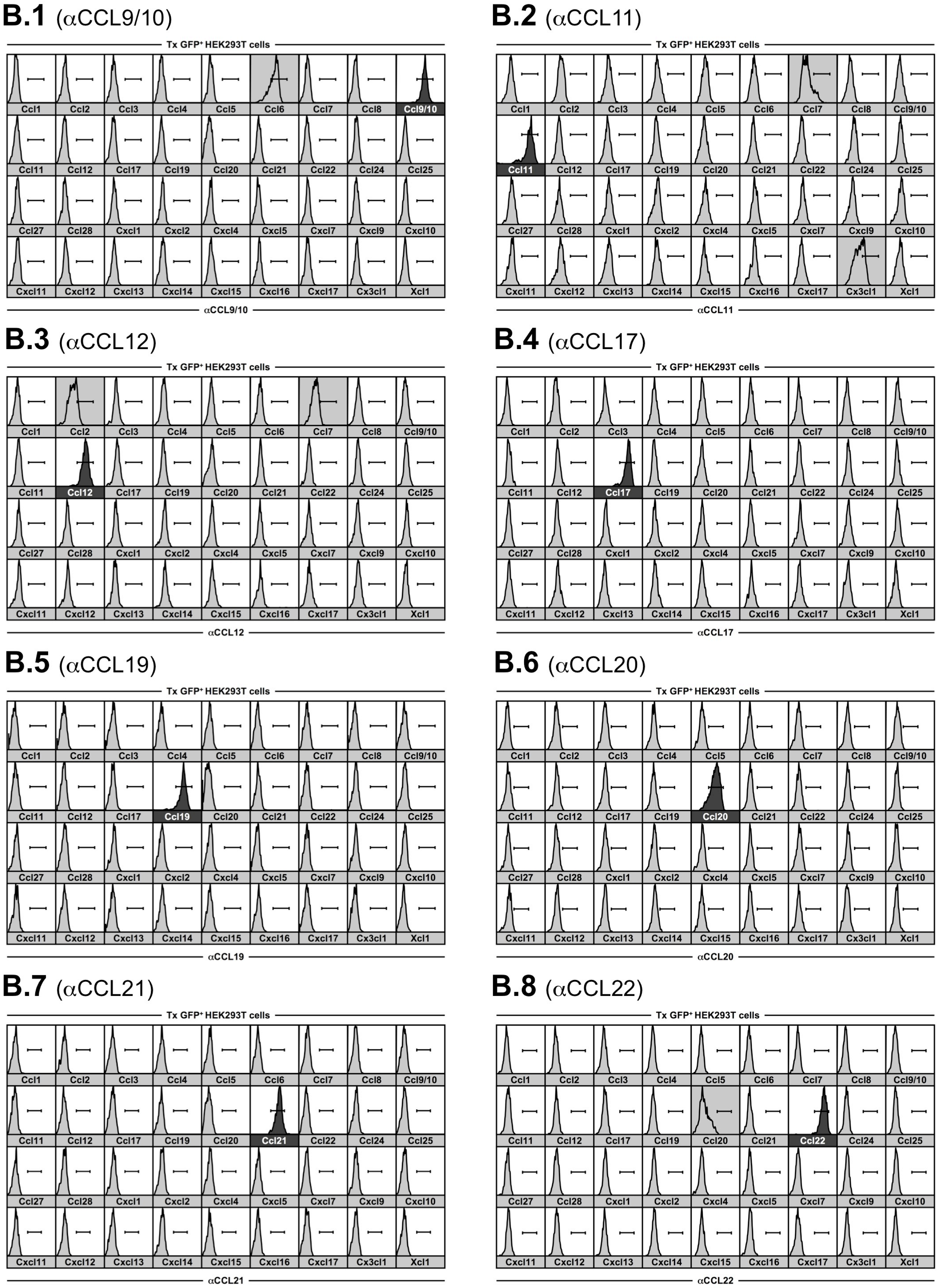


Figure S1, panels C.1-C.8

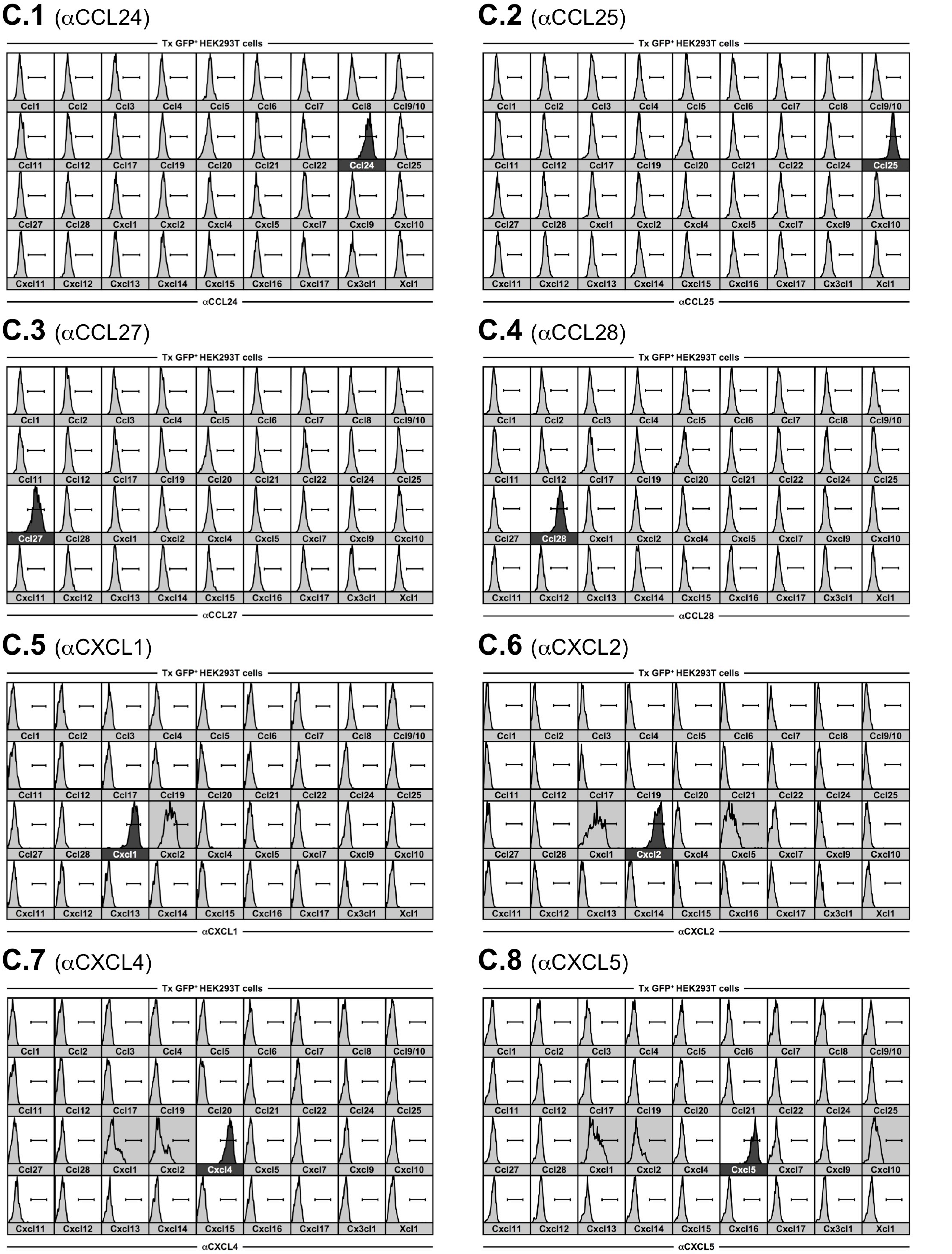
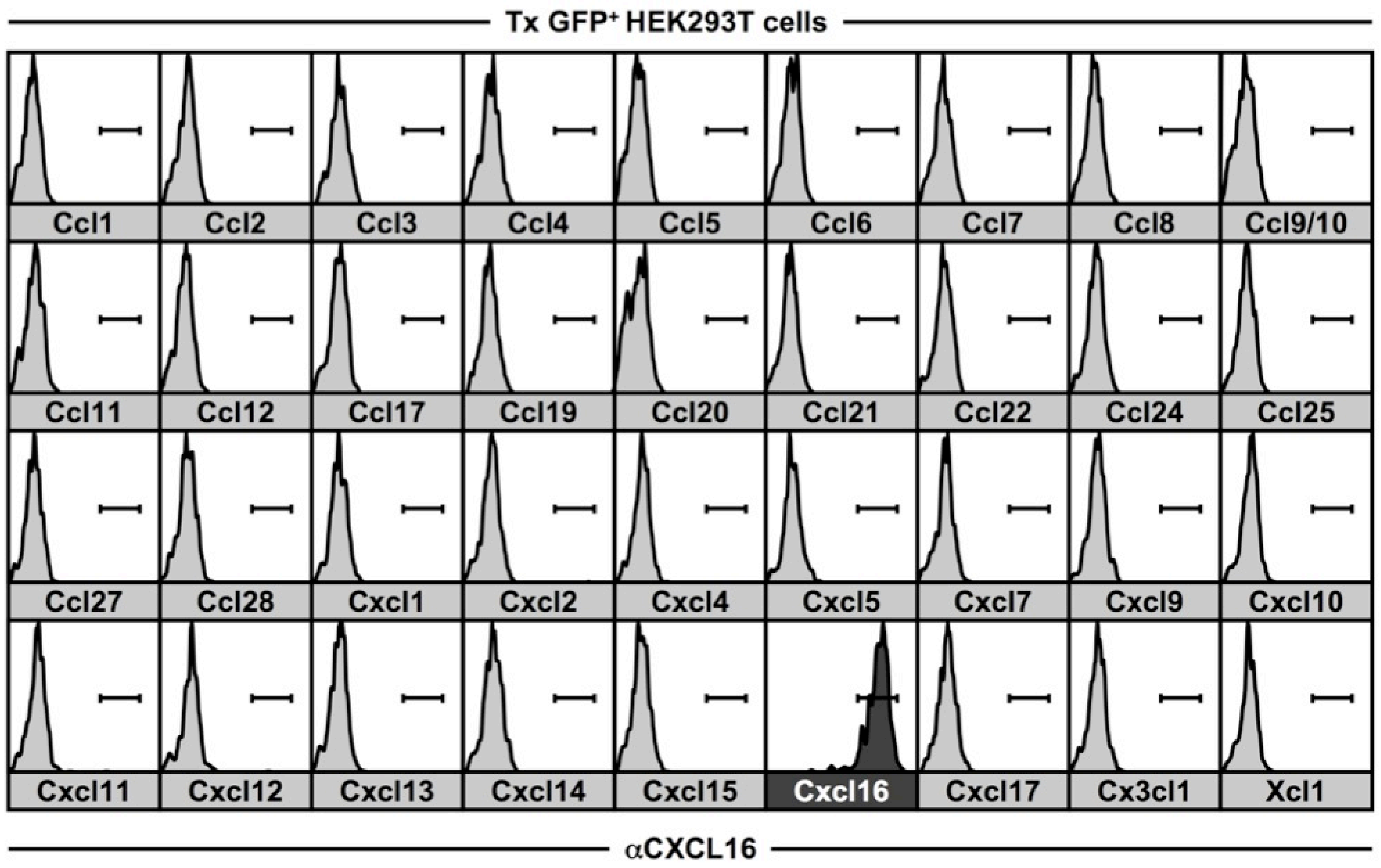


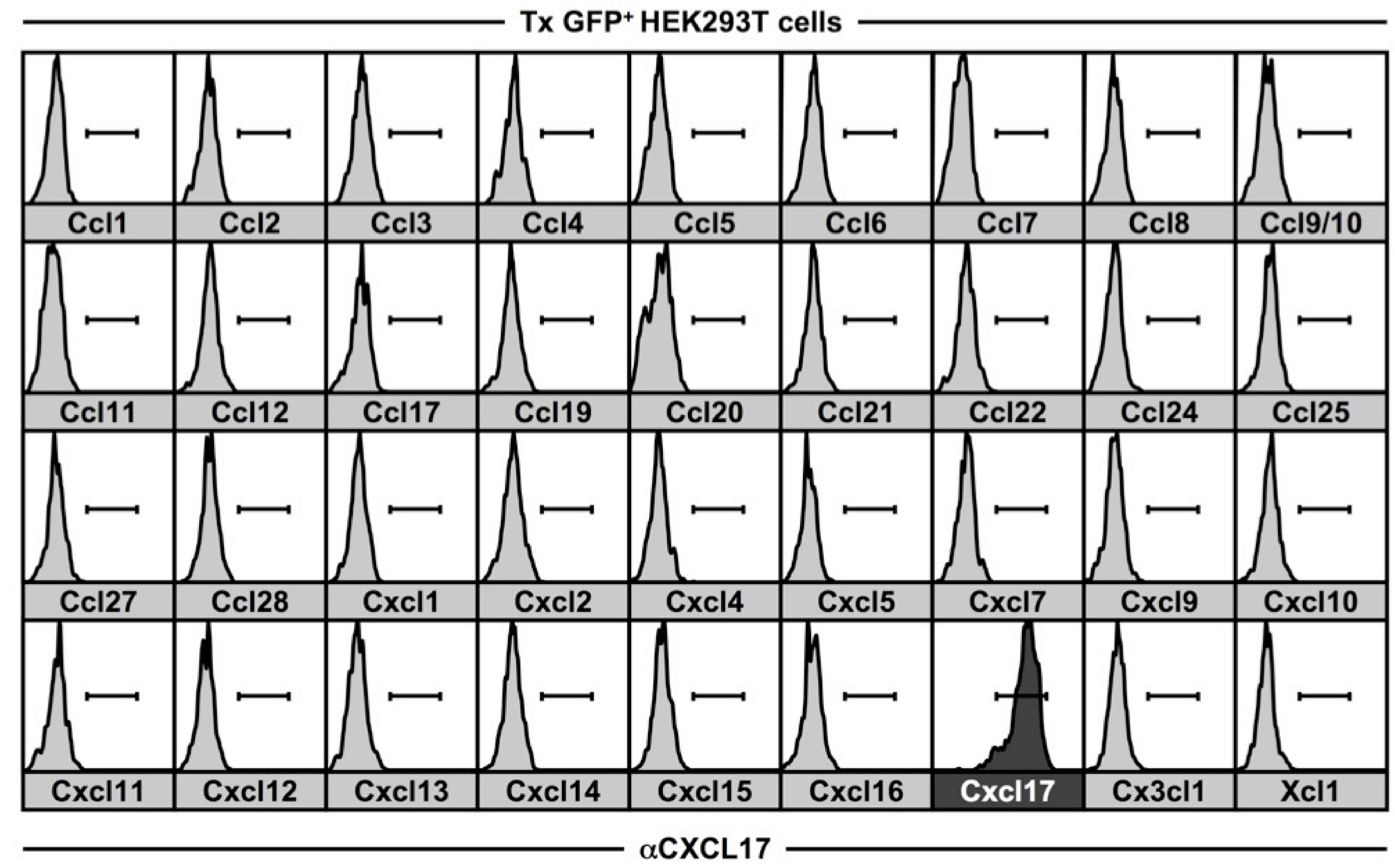
Figure S1, panels D.1-D.8

Figure S1, panels E.1-E.4

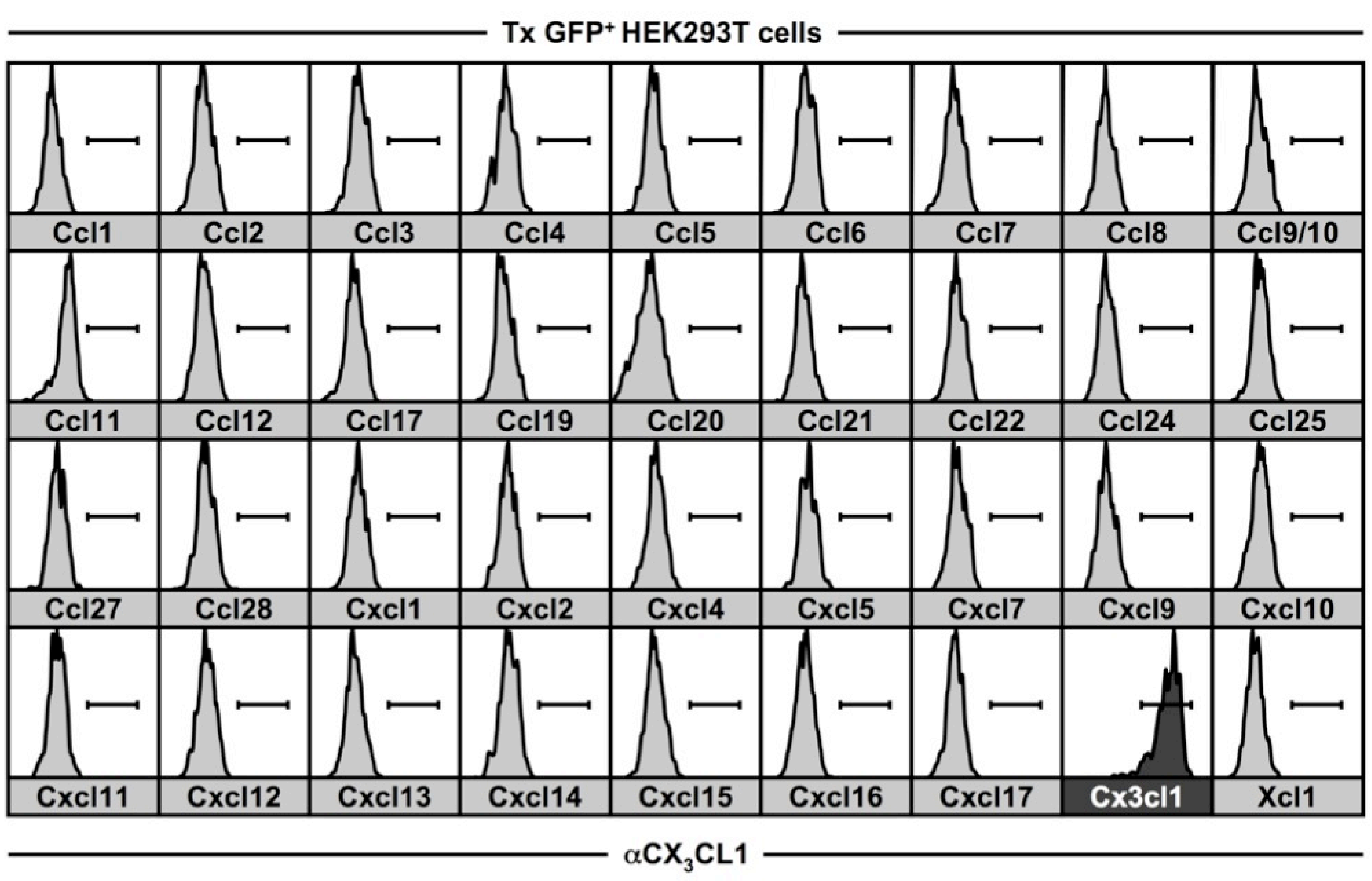
E.1 (αCXCL16)



E.2 (αCXCL17)



E.3 (αCX_3CL1)



E.4 (αXCL1)

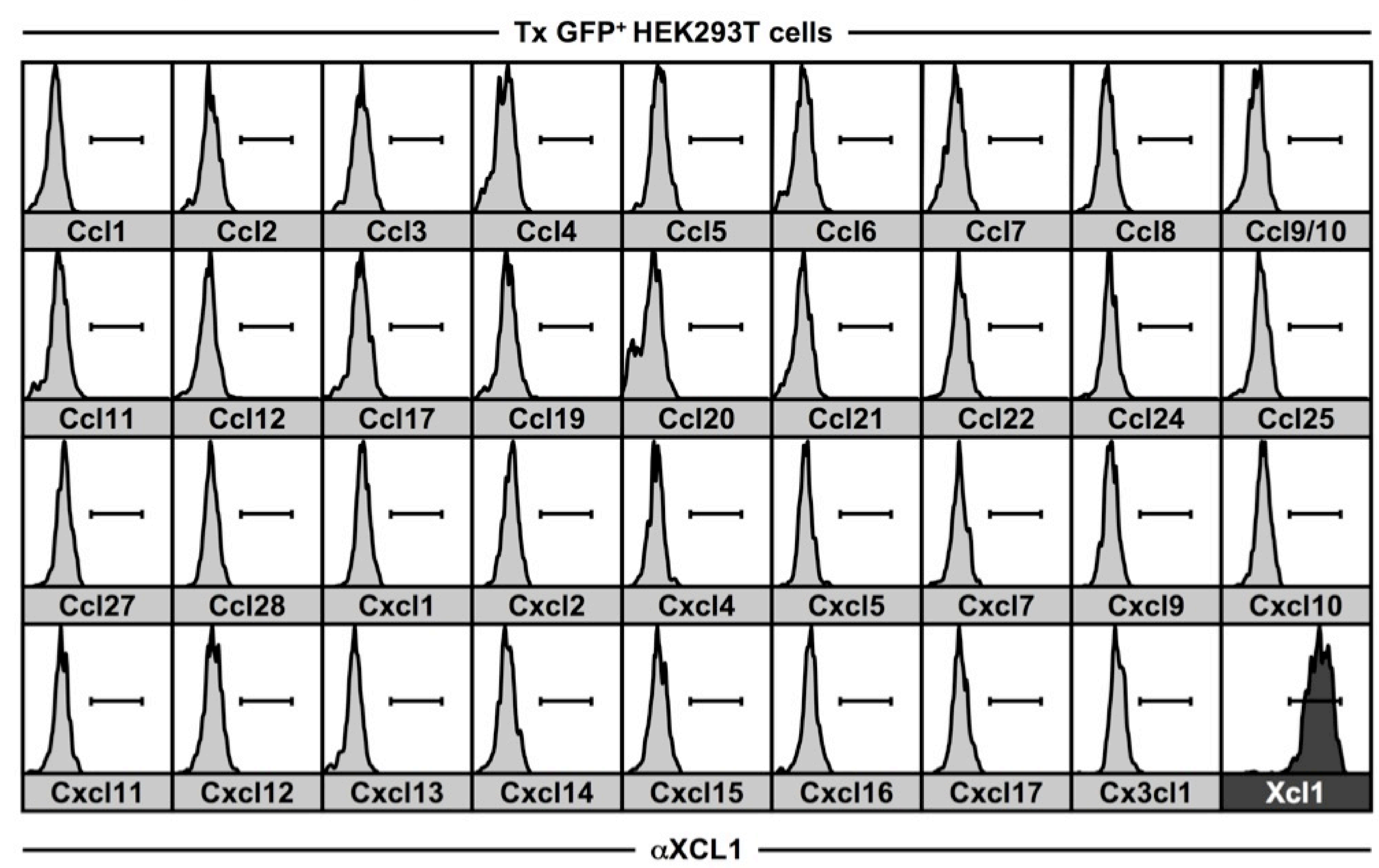


Figure S2

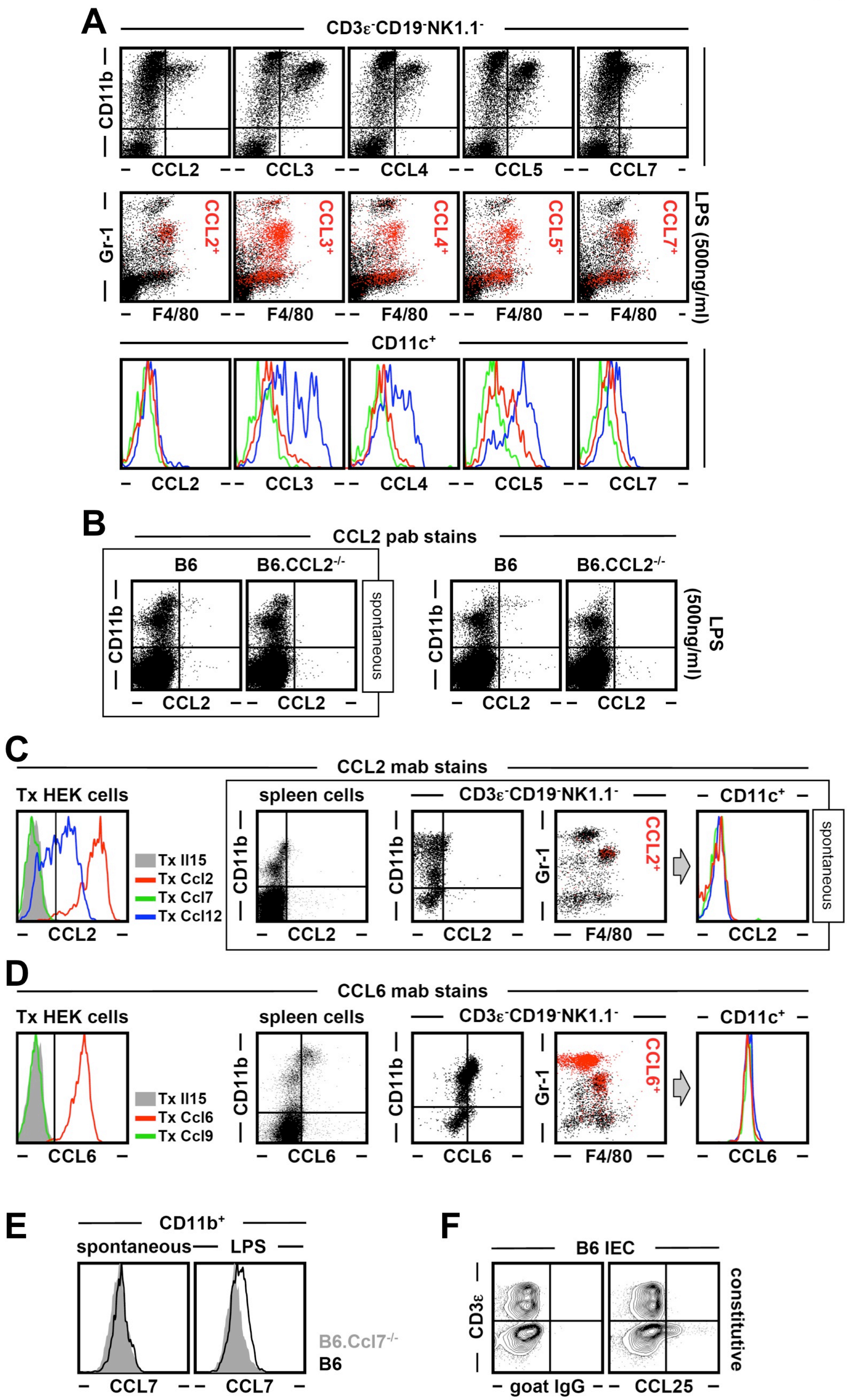
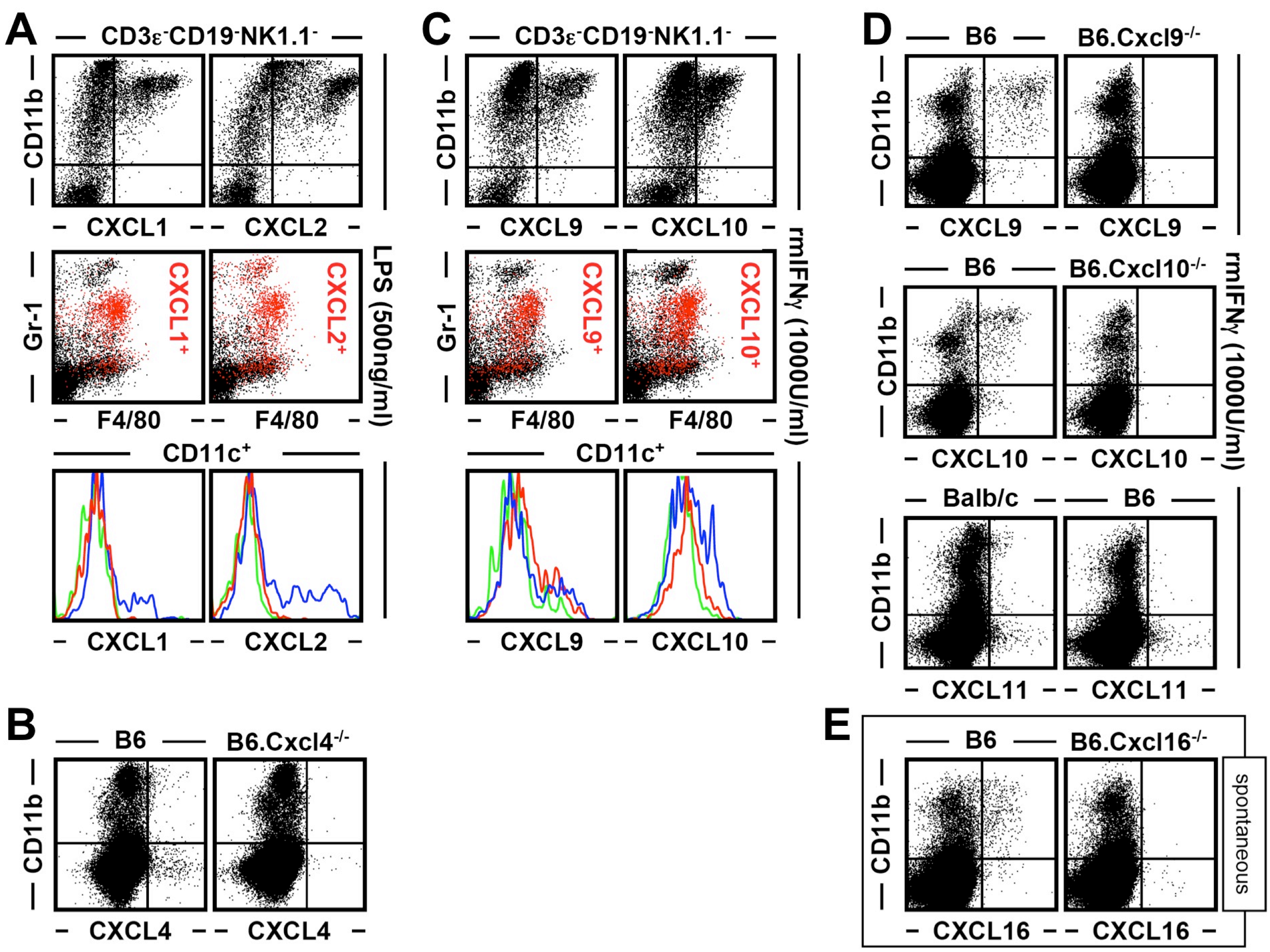

A
CD3ε⁻CD19⁻NK1.1⁻
CD11b
CCL2
CCL3
CCL4
CCL5
CCL7
Gr-1
F4/80
CCL2⁺
CCL3⁺
CCL4⁺
CCL5⁺
CCL7⁺
LPS (500ng/ml)
CD11c⁺
B
CCL2 pab stains
B6
B6.CCL2-/-
spontaneous
C
CCL2 mab stains
Tx HEK cells
Tx Il15
Tx Ccl2
Tx Ccl7
Tx Ccl12
spleen cells
D
CCL6 mab stains
Tx Ccl6
Tx Ccl9
CCL6
CCL6⁺
E
CD11b⁺
LPS
B6.Ccl7-/-
B6
F
B6 IEC
CD3ε
goat IgG
CCL25
constitutive

Figure S3



A
CD3ε⁻CD19⁻NK1.1⁻
CD11b
CXCL1
CXCL2
Gr-1
CXCL1⁺
CXCL2⁺
F4/80
LPS (500ng/ml)
CD11c⁺
B
B6
B6.Cxcl4-/-
CXCL4
C
CXCL9
CXCL10
CXCL9⁺
CXCL10⁺
rmIFNγ (1000U/ml)
D
B6.Cxcl9-/-
B6.Cxcl10-/-
Balb/c
CXCL11
E
B6.Cxcl16-/-
CXCL16
spontaneous

Figure S4

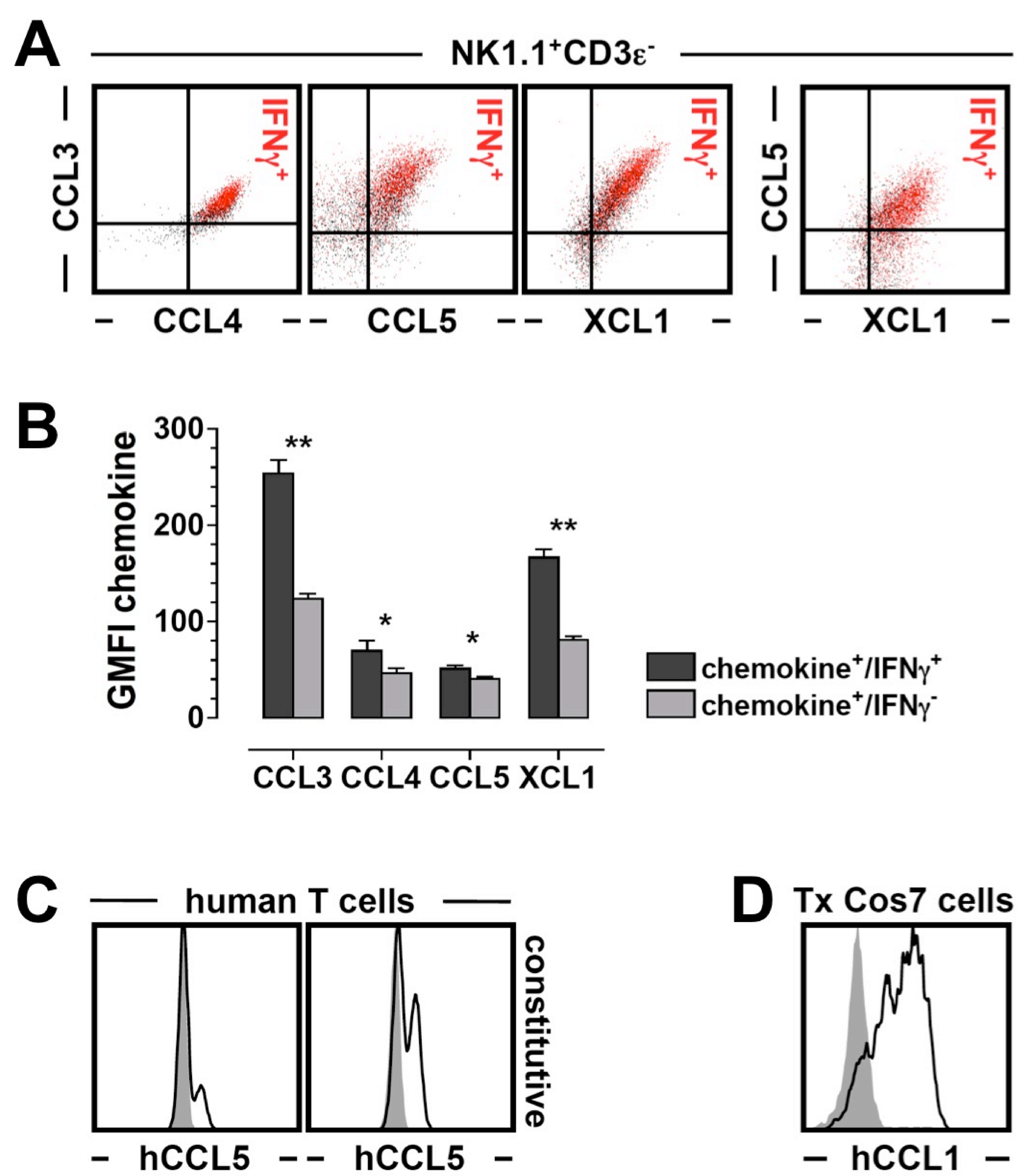
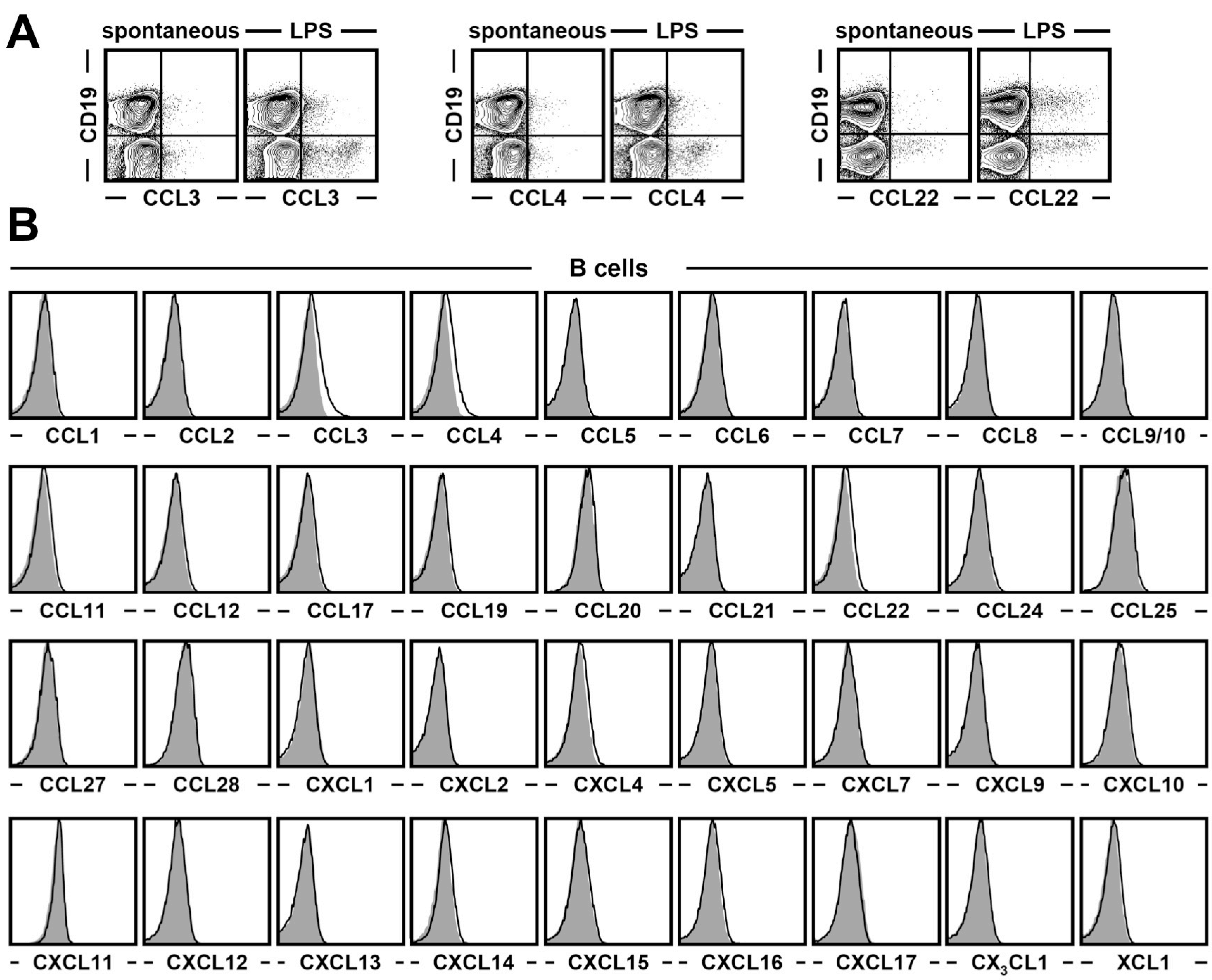

A
NK1.1+CD3ε-
CCL3
IFNγ+
CCL4
CCL5
XCL1
CCL5
XCL1
B
GMFI chemokine
300
200
100
0
**
*
*
**
CCL3 CCL4 CCL5 XCL1
chemokine+/IFNγ+
chemokine+/IFNγ-
C
human T cells
constitutive
hCCL5
hCCL5
D
Tx Cos7 cells
hCCL1

Figure S5


A
spontaneous
LPS
CD19
CCL3
CCL3
spontaneous
LPS
CD19
CCL4
CCL4
spontaneous
LPS
CD19
CCL22
CCL22
B
B cells
CCL1
CCL2
CCL3
CCL4
CCL5
CCL6
CCL7
CCL8
CCL9/10
CCL11
CCL12
CCL17
CCL19
CCL20
CCL21
CCL22
CCL24
CCL25
CCL27
CCL28
CXCL1
CXCL2
CXCL4
CXCL5
CXCL7
CXCL9
CXCL10
CXCL11
CXCL12
CXCL13
CXCL14
CXCL15
CXCL16
CXCL17
CX3CL1
XCL1

SUPPLEMENTARY FIGURE LEGENDS

Figure S1 Specificity controls for polyclonal chemokine antibodies.

HEK cells were transfected with 36 individual pIRES2-AcGFP1 chemokine vectors as shown in ***Figure 1***, stained in parallel with 36 individual chemokine-specific pabs and analyzed by FC. To reduce proteasomal degradation, Cxcl14-transfected HEK cells were cultured in the presence of a protease inhibitor (10μM MG-132). (**A.1**) Reactivity of the CCL1-specific pab with HEK cells transfected with different chemokine vectors as indicated at the bottom of histograms. All histograms are gated on GFP^+ cells, the black solid histogram identifies the panel depicting cognate interactions (αCCL1:CCL1) and the lack of specific staining in all other panels (gray histograms) demonstrates absence of crossreactivity of the αCCL1 pab with other chemokines. (**A.2-E.4**) Experimental procedures and data display for individual chemokine-specific pabs as detailed above. Crossrecognition of non-cognate chemokines by a given pab are highlighted by gray backgrounds (panels A.2, A.3, A.5-7, B.1-3, B.8, C.5-C.8, D.1) and the data are summarized in ***Table 1***. Panel D.7: staining of chemokine transfectants with the αhCXCL14 mab clone 131120. A parallel evaluation of the αhCXCL14 AF866 pab did not reveal apparent crossreactivities (not shown), but this assessment has to take into account the comparatively weak staining of Cxcl14 transfectants (***Figure 1***, insert). Based on our identification of a cross-reactivity cluster involving several gene products of the CXC-GRO group, we also included a comprehensive evaluation of CXCL3-reactivity: αCXCL1/2/5 but no other chemokine-specific pabs or mabs exhibit partial crossreactivity with CXCL3 (not shown & ***Table 1***). All crossreactive pabs were additionally tested on HEK chemokine transfectants prepared with separate plasmid stocks to rule out accidental cross-contamination (not shown).

Figure S2 Antibody specificity controls & constitutive, spontaneous and/or induced expression patterns for selected CC chemokines.

(**A**) Splenocytes obtained from naïve B6 mice were cultured for 5h with BFA plus 500ng/ml LPS and subsequently stained or surface markers and intracellular chemokines. Dot plots are gated on $CD3\varepsilon^-CD19^-NK1.1^-$ cells; histograms depict induced chemokine production by DC subsets within the $CD3\varepsilon^-CD19^-NK1.1^-$ population (red: $CD11c^{++}$DC, blue: $CD11c^+$DC, green: pDC). Data not shown: LPS but not IFNγ stimulation also increases CCL6 and CCL9/10 expression by $CD11b^+$ cell subsets (data are representative for 2-3 independent experiments analyzing 3 mice each). (**B**) Specificity controls for αCCL2 pab stains as determined by spontaneous and LPS-induced CCL2 expression analysis of B6 *vs.* B6.Ccl2$^{-/-}$ mice. (**C**) Characterization of αCCL2 clone 2H5 specificity with indicated HEK transfectants (left panel) and homoestatic CCL2 expression as revealed with the αCCL2 mab. Gating strategies are indicated above the dot plots and the overlaid histograms in the right hand panel demonstrate absence of spontaneous CCL2 production by DC subsets stratified according to CD11b/c expression levels as in panel A. (**D**) Characterization of αCCL6 clone 262016 specificity with indicated HEK transfectants (left panel) and constitutive CCL6 production determined with the αCCL6 mab; gating and data display as in panel C. (**E**) Specificity control for αCCL7 pab stains. Spleen cells from B6 and B6.Ccl7$^{-/-}$ mice were cultured in the absence ("spontaneous") or presence of LPS prior to surface and intracellular CCL7 stains; histograms are gated on CD11b^{+} cells as Mo/MΦ constitute the major population of LPS-induced CCL7 producers (panel A). (**F**) Small intestinal epithelial cells (IEC) were prepared as detailed in Methods, stained and analyzed for constitutive CCL25 expression by FC (representative data for 3 independent analyses of individual mice).

Figure S3 Antibody specificity controls & constitutive, spontaneous and/or induced expression patterns for selected CXC chemokines.

(**A**) LPS-stimulated spleen cells were analyzed for induced CXCL1/2 expression; experimental details and data display as per legend to ***Figure S2A*** (representative data for 2-3 independent experiments analyzing 3 mice each). (**B**) Validation of αCXCL4 pab specificity as determined in direct ex vivo stains of spleen cells from B6.Cxcl4$^{-/-}$ *vs.* wt littermates ("B6"). (**C**) B6 spleen cells were cultured for 5h in the presence of 1000U/ml recombinant mIFNγ and BFA and analyzed for induced CXCL9/10 expression as detailed in legend to ***Figure S2A*** (independent experiments comprising groups of 2-3 mice were performed 4 times). (**D**) Specificity controls for αCXCL9/10/11 pabs. Spleen cells from B6, B6.Cxcl9$^{-/-}$, B6.Cxcl10$^{-/-}$ and Balb/c mice were stimulated with rmIFNγ as above and analyzed for induced CXCL9/10/11 expression. In contrast to the robust CXCL9/10 production, rmIFNγ induces only very modest levels of CXCL11 as evaluated in Balb/c mice (note that regular B6 mice, due to their lack of a functional Cxcl11 gene, are used here as a negative control). (**E**) Analysis of spontaneous CXCL16 synthesis (5h BFA culture of splenocytes) in B6 *vs.* B6.Cxcl16$^{-/-}$ mice confirms the specificity of the αCXCL16 pab. Data not shown: LPS but not IFNγ stimulation also increases CXCL16 expression by CD11b^{+} cell subsets.

Figure S4 Induced chemokine co-expression by murine NK cells & human chemokine control stains.

(**A**) Following a 5h culture with PMA/ionomycin and BFA, B6 spleen cells were stained for surface and intracellular markers using pre-conjugated chemokine-specific pabs and/or the CCL3-specific mab clone 39624 in conjunction with αIFNγ as detailed in Methods. All plots are gated on NK1.1^{+}CD3ε^{-} cells and IFNγ-expressing NK subsets (~40-50% of NK cells) are indicated as red events. Data are compiled from 4 independent experiments evaluating 2-3 mice each. (**B**) Comparison of induced chemokine production (PMA/ionomycin) by IFNγ^{+} *vs.* IFNγ^{-} NK cell populations. The bar diagram displays the GMFI (geometric mean of fluorescence intensity) of indicated chemokine stains in NK subsets demonstrating IFNγ co-expression (black) and NK cells with detectable chemokine but no IFNγ expression (gray) (n=3, one of 3 similar experiments). Asterisks indicate statistical significance as determined by paired t-tests. (**C**) Constitutive CCL5 expression by human T cells. Representative histograms are gated on $CD3^{+}CD56^{-}$ PBMC from 2 healthy volunteers (gray: goat IgG, black: CCL5). (**D**) Cos7 cells were transfected with empty or human Ccl1 containing pIRES2-AcGFP1 vector and stained for expression of CCL1. Histograms are gated on GFP^{+} Cos7 cells (gray: empty vector, black: Ccl1 vector; 24h Tx with BFA for final 5h).

Figure S5 Spontaneous and induced chemokine production by murine B cells.

(A) Spleen cells obtained from naïve B6 mice were cultured for 5h in the presence of BFA ("spontaneous") or 500ng/ml LPS and BFA and stained for CD19 and indicated chemokines. 5% probability contour plots are gated on "live" cells based on FSC/SSC properties; data are representative for at least 3 independent experiments. Similar results were obtained in stimulation cultures containing 5μg/ml LPS (not shown). **(B)** After 5h culture of spleen cells with BFA only (gray histograms) or B cell stimulation with αIgM (10μg/ml) and αCD40 (15μg/ml) abs plus BFA, cells were stained for surface markers and intracellular chemokines. CXCL11 analyses were conducted with spleen cells of Balb/c origin. All histograms are gated on $CD19^{+}CD3\varepsilon^{-}$ cells; data are representative for 2-3 independent experiments analyzing 3 mice each.

SUPPLEMENTARY TABLES

	Gene	Vendor	Clone/ IMAGE ID	Accession #	Insert 3' RE or primer (5'-> 3')	Insert 5' RE or primer (5' -> 3')	Target 3' RE	Target 5' RE
1	Ccl1	OBS	40129729	BC120806	*EcoRI*	*EcoRI*	*EcoRI*	*EcoRI*
2	Ccl2	OBS	40131170	BC145867	CCG CTC GAG CGG ACC ATG CAG GTC CCT G	CCG GAA TTC CGC TAG TTC ACT GTC ACA CTG GTC	*XhoI*	*EcoRI*
3	Ccl3	OBS	40041119	BC111443	CCC CCT CGA GAT CAT GAA GGT CTC CAC CAC	CCC CGA ATT CTC AGG CAT TCA GTT CCA G	*XhoI*	*EcoRI*
4	Ccl4	OBS	8734006	BC119257	CCC CTC GAG ACC ATG AAG CTC TGC GTG TCT GC	CCC CCC GGG TCA GTT CAA CTC CAA GTC ACT C	*XhoI*	*EcoRI*
5	Ccl5	OBS	4925413	BC033508	CCG CTC GAG ACC ATG AAG ATC TCT GCA G	CCG GAA TTC TAG CTC ATC TCC AAA TAG TTG ATG	*XhoI*	*EcoRI*
6	Ccl6	OBS	3492808	BC002073	*EcoRI*	*DraI*	*EcoRI*	*SmaI*
7	Ccl7	OBS	30276350	BC061126	*SalI*	*RsaI*	*XhoI*	*SmaI*
8	Ccl8	OBS	40126422	BC117101	GTG CTC GAG AAC ATG TAC GCA GTG C	CGG AAT TCT CAA GGC TGC AGA ATT TGA GAC TTC	*XhoI*	*EcoRI*
9	Ccl9/10	OBS	40131265	BC145962	*EcoRI*	*EcoRI*	*EcoRI*	*EcoRI*
10	Ccl11	OBS	1527856	BC027521	*EcoRI*	*EcoRV*	*EcoRI*	*SmaI*
11	Ccl12	OBS	1548072	BC027520	*XhoI*	*EcoRV*	*XhoI*	*SmaI*
12	Ccl17	ATCC	3328278	BC028505	CCG AGA TCT CCC ATG AAG ACC TTC ACC	GCG AAT TCG GGA AGG TCA TGG CCT TGG G	*BglII*	*EcoRI*
13	Ccl19	OBS	1349213	BC051472	GTG CTC GAG GCC ATG GCC CCC CGT G	CGG AAT TCT CAA AC ACA GGG CTC CTT CTG GTG C	*XhoI*	*EcoRI*
14	Ccl20	OBS	1380543	BC028504	*EcoRI*	*PvuII*	*EcoRI*	*SmaI*
15	Ccl21b	OBS	3372243	BC038120	GTG CTC GAG ACC ATG GCT CAG ATG ACT CTG	GGG AAT TCT ATC CTC TTG AGG GCT GTG TCT	*XhoI*	*EcoRI*
16	Ccl22	OBS	4192393	BC012658	*EcoRI*	*HpaI*	*EcoRI*	*SmaI*
17	Ccl24	OBS	3472002	BC065389	*EcoRI*	*XmnI*	*EcoRI*	*SmaI*
18	Ccl25	OBS	40126352	BC117033	*BglII*	*EcoRI*	*BglII*	*EcoRI*
20	Ccl27	OBS	3471454	BC028511	GTG CTC GAG ACC ATG ATG GAG GGG CTC	CCG GAA TTC CGG TTA GTT TTG CTG TTG GGG G	*XhoI*	*EcoRI*
22	Ccl28	OBS	4240191	BC055864	*EcoRI*	*PstI*	*EcoRI*	*PstI*
23	Cxcl1	OBS	40130779	BC132502	*EcoRI*	*EcoRI*	*EcoRI*	*EcoRI*
24	Cxcl2	OBS	40055368	BC119511	*SacI*	*XmaI*	*SacI*	*XmaI*
25	Cxcl3	OBS	40126333	BC117014	*EcoRI*	*EcoRI*	*EcoRI*	*EcoRI*
26	Cxcl4	OBS	30280161	BC061111	*SalI*	*PstI*	*XhoI*	*PstI*
27	Cxcl5	OBS	4976854	BC024392	*EcoRI*	*EcoRI*	*EcoRI*	*EcoRI*
28	Cxcl7	OBS	40053104	BC127043	*XhoI*	*BamHI*	*XhoI*	*BamHI*
29	Cxcl9	OBS	3257716	BC003343	*EcoRI*	*RsaI*	*EcoRI*	*SmaI*
30	Cxcl10	OBS	1446589	BC030067	CCG CTC GAG CGG ACC ATG AAC CCA AGT GCT G	CCG GAA TTC CGG TTA AGG AGC CCT TTT AGA CC	*XhoI*	*EcoRI*
31	Cxcl11	OBS	5035983	BC025903	GTG CTC GAG ACC ATG AAC AGG AAG GTC AC	GCC GGA ATT CTC ACA TGT TTT GAC GCC	*XhoI*	*EcoRI*
32	Cxcl12	OBS	6406409	BC046827	CCG CTC GAG ACC ATG GAC GCC AAG GTC	CGC TTC TTT TTT CCT ATC TTT TCT TTT TTC CCC AC	*XhoI*	[3)]
	Cxcl12	OBS	6406409	BC046827	GTG GGG AAA AAA GAA AAG ATA GGA AAA AAG AAG CG	GGA ATT CCC TAG TTT TTC CTT TTC TGG GC	[3)]	*EcoRI*
33	Cxcl13	OBS	4217539	BC012965	CCG CTC GAG CCG ACC ATG AGG CTC AGC ACA GC	CCG GAA TTC CGG TCA GGC AGC TCT TCT CTT AC	*XhoI*	*EcoRI*
34	Cxcl14	OBS	9056321	BC147274	CCC CTC GAG ACC ATG AGG CTC CTG GCG	GGG AAT TCT TAA AGT TCG TCC TTT TCT TCG TAG ACC CTG CG	*XhoI*	*EcoRI*
35	Cxcl15	OBS	30298837	BC061138	CCG CTC GAG CGG ATC CAG ACC AGA GTC AGG C	CCC CCC GGG TTA AAG TTC GTC CTT GGC ATC ACT GCC TGT	*XhoI*	*EcoRI*

36	Cxcl16	OBS	3988542	BC019961	CCG CTC GAG ACC ATG AGG CGG GGC TTT G	GGG AAT TCG GCG CTA GGG TCT TGG TTC	*XhoI*	*EcoRI*
37	Cxcl17	OBS	1547987	BE197231	CCC CTC GAG ACC ATG AAG CTT CTA GCC TCT CC	CCC GAA TTC CTA TAA GGG CAG CGC AAA GCT	*XhoI*	*EcoRI*
39	Cx3cl1	OBS	6490082	BC054838	CCG CTC GAG CGG CCA TGG CTC CCT CG	CGG AAT TCC GTC ACA CTG GCA CCA GGA CG	*XhoI*	*EcoRI*
38	Xcl1	OBS	3974601	BC062249	CCG CTC GAG CCA TGA GAC TTC TCC TCC	CGG GAA TTC CTT ACC CAG TCA GGG TTA TCG C	*XhoI*	*EcoRI*
40	mIL15[1)]				GCT GCT CGA GCT CGG CCA CCA TGT ACA GCA TG	CTG CAG AAT TCC GAG TCA GGA CGT GTT GAT G	*XhoI*	*EcoRI*
41	pIRES2AcGFP1[2)]				AGC AGA GCT GGT TTA GTG AAC CGT [4)]	TAT TCC AAG CGG CTT CGG CCA GTA A [4)]		

Table S1 Overview of chemokine subcloning reagents.

Original clones were obtained from OpenBiosystems (OBS) and American Tissue Culture Consortium (ATCC). [1)]Gift from Dr. T. Sosinowski, [2)]Clontech, [3)]primers for PCR-driven overlap extension (Heckman *et al.*, 2007, PMID:17446874) to correct frame-shift, [4)]sequencing primers to verify proper insert sequence and orientation.

Antigen	Other Name(s)	Antibody clone	Ab species & isotype	Format	Source
Surface & intracellular					
CD3ε		145-2C11	hamster	FITC/PE/PerCP	BDBiosciences/ebioscience
		17A2	$rIgG_{2b}$	eFluor450	ebioscience
CD4		GK1.5	$rIgG_{2b}$	FITC/PE/APC	ebioscience
		RM4-5	$rIgG_{2a}$	FITC/PE/PerCP	BDBiosciences
CD8α		53-6.7	$rIgG_{2a}$	FITC/PE/PerCP/APC/ PE-Cy7/APC-Cy7/PB	BDBiosciences/ebioscience
CD11b	integrin aM	M1/70	$rIgG_{2b}$	FITC/PE/APC/PerCP-Cy5.5	BDBiosciences/ebioscience
CD11c	integrin aX	HL3	hamster	FITC/PE/APC	BDBiosciences
CD16/32	"Fc block"	2.4G2	$rIgG_{2b}$	purified	BDBiosciences
CD19		1D3	$rIgG_{2a}$	PE/PerCP-Cy5.5	ebioscience
CD45		30-F11	$rIgG_{2b}$	FITC/PerCP	BDBiosciences
CD45.2		104	$mIgG_{2a}$	FITC	BDBiosciences
CD45R	B220	RA3-6B2	$rIgG_{2a}$	FITC/PE/APC	ebioscience
CD49b	integrin a2	DX5	rIgM	FITC/PE	ebioscience
CD161c	NK1.1	PK136	$mIgG_{2a}$	FITC/PE/PerCP-Cy5.5	BDBiosciences/ebioscience
CD197	CCR7	4B12	$rIgG_{2a}$	PE	Biolegend
Gr-1	Ly6C/G	RB6-8C5	$rIgG_{2b}$	FITC/Alx488/PE	ebioscience
F4/80		BM8	$rIgG_{2a}$	PE	Invitrogen/Caltag
IFNγ		XMG1.2	$rIgG_{1}$	PE/APC/PE-Cy7	BDBiosciences/ebioscience
hCD3		SK7	$mIgG_{1}$	PerCP	BDBiosciences
hCD56		B159	$mIgG_{1}$	PE/APC	BDBiosciences
hIFNγ		4S.B3	$mIgG_{1}$	Pacific Blue	ebioscience
Isotpe controls					
unkown		R35-95	$rIgG_{2a}$	FITC/PE	BDBiosciences
unknown		A95-1	$rIgG_{2b}$	FITC/PE	BDBiosciences
TNP		A19-3	hamster	PE	BDBiosciences
unknown		G235-2356	hamster	PE	BDBiosciences
KLH		11711	$mIgG_{1}$	PE	RnD Systems
unknown			$mIgG_{1}$	PE	Invitrogen/Caltag
dansyl		27-35	$mIgG_{2b}$	PE/APC	BDBiosciences
unknown		B10	$mIgG_{3}$	PE	Southern Biotech

Table S2 Antibodies for use in FC.

The table lists all abs used for FC other than chemokine-specific abs; for the latter, please see ***Table 1*** (murine chemokine abs) and Methods (human chemokine abs).