

Figure S1. Shifting associations between the surface phenotype and the Tbet/Eomes expression levels (a) Representative dot plots showing the gating strategy used fpr defining the Tbet/Eomes gating populations, and (b) the gating strategy used for the other markers. Upper row from left to right: the lymphocyte gate (nb. after fixation and permeabilization), the sideward scatter width/height duplet exclusion, the forward scatter width/height duplet exclusion, the gating of alive cells, the gating of CD3+ tetramer+ events, the gating of CD3+CD8+(tetramer+) events. Lower row from left to right: the

gating of the CD45RA/CD27 CD8⁺ T-cell populations, the gating of the CCR7-positive and negative CD8⁺ T-cell populations, the gating of the CD28-positive and negative CD8⁺ T-cell populations, and the expression frequencies of KLRG1, IL-7R α , granzyme K and granzyme B (lower row) (c) The distribution of the Tbet/Eomes expression states per

CD45RA/CCR7/CD28/CD27defined subset in 20 healthy-(left column) and 13 HIV-1infected subjects (right column), mean/SD shown and (c), reciprocally, the distribution of the

CD45RA/CCR7/CD28/CD27defined subsets over the Tbet/Eomes populations in 20 healthy- (left column) and 13 HIV-1-infected subjects (right column), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red. See Figure 1 for statistical analyses.



Figure S2. Virus-specific CD8+ T-cells display distinct CD45RA/CCR7/CD28/CD27 and T-bet/Eomes expression levels Representative dot plots of the different virus-specific CD8+ T-cell populations as stained with tetramers (left column), where CD8 fluorescence intensity (FI) is plotted on the X-axis and tetramer FI on the Y-axis, as well as their CD45RA (Y-axis)/CD27 (X-axis) and T-bet (X-axis)/Eomes (Yaxis) phenotypes presented as an overlay of the respective tetramer+ population in black on the total CD8+ T-cell population in gray. (Second column) The range of CD45RA/CCR7/CD28/CD27 phenotypes found among RSV NP- (n=5), influenza (Flu) MP1- (n=5), EBV EBNA-3a- (n=8), EBV BMLF-1- (n=5), HIV-1 gag- (n=12), HIV-1 nef- (n=11) and hCMV pp65-specific CD8+ T-cells in healthy individuals (n=7) and in HIV-1-infected persons (n=4). (Middle column) The T-bet/Eomes expression states found among the same virus-specific populations, median and IQR shown. (Fourth and fifth columns) the surface marker- and T-bet/Eomes-defined subset distribution of hCMV pp65-specific cells circulating in healthy individuals (n=7) compared to the same populations in HIV-1-infected individuals (n=4), mean/SD shown. See Figure 2 for statistical analyses.



granzyme K

Figure S3. An individual CD45RA/CCR7/CD28/CD27-defined subset comprises cells in different T-bet/Eomes expression states that are linked to functional differences

Representative zebra plots of CD45RA⁻ CCR7⁺CD28⁺CD27⁺ central-memory cells (upper panel) and CD45RA⁻ CCR7⁻ CD28⁻ CD27⁻ effector-type cells (lower panel) plotting granzyme K fluorescence intensity on the X-axis and granzyme B fluorescence intensity on the Y-axis, and the shifts in the expression of these effector molecules per T-bet/Eomes expression state found in these CD45RA/CCR7/CD28/CD27-defined subsets.



Figure S4. Combining the CD45RA/CCR7/CD28/CD27 dimension and the T-bet/Eomes dimension more accurately predicts CD8+ T-cell functional potential

Scatter plots displaying the expression of IL-7Rα (first row), granzyme K (second row), granzyme B (third row) and KLRG1 (last row) per Tbet^{Io}Eomes^{Io} (first column), T-bet^{Int}Eomes^{Io} (second column), T-bet^{Io}Eomes^{hi} (third column), T-bet^{Int}Eomes^{Io} (fourth column), T-bet^{Int}Eomes^{Io} (last column), and per CD45RA/CCR7/CD28/CD27-defined subset plotted on the X-axis in 20 healthy individuals (upper panel) and in 13 HIV-1-infected individuals (lower panel), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red. See Figure 4 for statistical analyses.



Figure S5. Infection history influences the surface markerand T-bet/Eomes-defined subset distribution (A) The

CD45RA/CCR7/CD28/CD27defined subset distribution (left column) and (B) the T-bet/Eomes expression state distribution (right column) over CD8+ T-cells in cord blood samples (first row, n=5), EBV/hCMV seronegative individuals (second row, n=6), EBV seropositive individuals (third row, n=5), hCMV seropositive individuals (fourth row, n=3), EBV/hCMV double-infected individuals (fifth row, n=6) and HIV-1-infected individuals (last row, n=13), mean/SD shown. The two HIV-1-infected patients not coinfected with hCMV are shown in red. See Figure 5 for statistical analyses.



Figure S6. Infection history influences the associations between T-bet/Eomes expression levels and surface phenotype

Scatter plots displaying the relative distribution of the CD45RA/CCR7/CD28/CD27 phenotypes (plotted on the X-axis of each graph) over the different T-bet/Eomes expression states (rows) per study group: cord blood samples (first column, n=5), EBV/hCMV seronegative individuals (second column, n=6), EBV seropositive individuals (third column, n=5), hCMV seropositive individuals (fourth column, n=3), EBV/hCMV double-infected individuals (fifth column, n=6), and HIV-1-infected individuals (last column, n=13), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red.



Figure S7. Viral infection history impacts on the associations between the T-bet/Eomes expression levels and the functional potential

Expression frequencies of IL-7Ra (first row), granzyme K (second row), KLRG1 (third row) and granzyme B (last row) per T-bet^{lo}Eomes^{lo} (first column), T-bet^{int}Eomes^{lo} (second column), T-bet^{lo}Eomes^{lo} (first column), T-bet^{int}Eomes^{lo} (fourth column), T-bet^{lo}Eomes^{lo} (fourth column), T-bet^{lo}Eomes^{lo}



Figure S8. Viral infection history impacts on the associations between CD45RA/CCR7/CD28/CD27 phenotype and the functional potential

Expression frequencies of IL-7Rα (first row), granzyme K (second row), KLRG1 (third row) and granzyme B (last row) per CD45RA/CCR7/CD28/CD27-defined subset (columns) and per study group: cord blood samples, n=5; EBV/hCMV seronegative individuals, n=6; EBV seropositive individuals, n=5, hCMV seropositive individuals, n=3, EBV/hCMV double-infected individuals, n=6 and HIV-1-infected individuals (n=13) plotted on the X-axis of each graph, mean/SD shown. Note that early-like and RA⁻ effector-type populations were absent from cord blood, and that only two cord blood samples comprised sufficient RA⁺ effector-type cells (>20 events) to be included in these analyses. The two HIV-1-infected patients not co-infected with hCMV are shown in red.

		IL7-Rα									granzyme K							KLRG1								granzyme B								
			+	-	-	-	-	-	+		+	-	-	-	-	-	+		+	-	-	-	-	-	+		+	-	-	-	-	-	+	
	Bet	nes	+	+	-	-	-	-	-		+	+	-	-	-	-	-		+	+	-	-	-	-	-		+	+	-	-	-	-	-	
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			+	+	+	-	+	-	-		+	+	+	-	+	-	-		+	+	+	-	+	-	-		+	+	+	-	+	-	-	
	lo	lo	95	93	77		88				0	0	2		6				6	3	9		19				0	0	0		0			
	int	lo	96	94	73		72				0	1	6		5				8	5	27		15				0	0	4		3			
cord blood	lo	hi	84	86							4	16							13	11							0	2						
	int	hi	89								8								19								1							
	hi	hi			28		65		11				33		44		4				31		61		67				4		2		93	
	hi	lo	94	65	52		54		15		8	9	8		15		2		8	8	17		28		84		0	8	5		12		94	
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	lo	lo	88	84	86	84	72	81	68		0	5	29	18	6	4	9		1	16	37	31	22	15	33		0	0	2	3	9	14	29	
	int	lo	92	88	85	74	44	21	22		2	17	61	51	10	7	6		4	29	68	61	55	72	86		0	1	10	18	41	76	86	
hCMV-	lo	hi	77	66	59	72	26				13	73	87	77	43				9	51	80	85	81				0	2	12	13	34			
EBV-	int	hi	82	68	61	66	15	6	22		56	78	85	79	27	22	20		49	66	91	90	88	88	94		3	9	25	28	70	87	75	
	hi	hi		30	38	28	7	3	12			63	62	50	12	7	4	'		63	89	81	88	91	94			47	59	71	88	98	93	
	hi	lo	91	52	56	39	16	6	12		28	47	62	45	5	2	1		25	55	88	80	81	89	89		10	24	50	63	84	97	98	
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	lo	lo	87	78	79	84	72	70	45		1	10	30	14	13	10	9		18	8	24	22	19	13	37		0	0	2	9	12	18	37	
	int	lo	91	81	81	77	32	26	17		7	26	63	45	21	14	10		21	18	58	43	59	50	72		0	1	10	23	61	69	82	
hCMV-	lo	hi	78	54	37	51	13	7	29		27	77	91	86	68	66	44		22	38	65	68	73	68	79		0	2	11	27	26	33	25	
EBV+	int	hi	82	56	46	50	9	6	15		71	85	90	86	52	45	32		50	57	81	77	85	79	92		1	6	22	36	55	75	57	
	hi	hi	58	34	34	30	5	2	11		73	85	78	71	25	18	8		80	69	82	77	87	75	91		27	42	53	54	78	90	83	
	hi	lo	83	45	53	37	13	10	11		43	63	70	52	9	6	2		35	49	81	69	73	70	76		20	31	50	69	92	97	98	
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	lo	lo	83	80	85	80	85	86	68		0	4	16	6	3	3	8		0	12	27	22	14	7	15		0	0	1	1	1	8	15	
	int	lo	87	88	87	79	63	40	20		2	18	51	35	12	11	9		2	23	56	46	38	53	80		0	0	5	11	20	55	79	
hCMV+	lo	hi	73	60	53	76	46				18	83	88	58	64				9	41	69	75	89				0	2	7	9	7			
EBV-	int	hi	78	66	60	73	29	18	24		56	88	90	75	52	37	31		35	63	86	88	87	77	91		1	6	14	17	37	68	52	
	hi	hi	69	41	45	49	17	7	9		89	89	78	73	29	10	6		77	61	86	92	91	84	99		5	39	45	50	68	91	90	
	hi	lo	88	65	65	51	17	5	7		29	68	71	57	8	2	2		21	60	88	81	67	71	88		6	19	40	61	82	99	99	
	lo	lo	86	82	76	78	68	65	34		0	11	39	18	17	12	27		1	22	45	37	33	30	75		0	1	5	6	14	26	64	
	int	lo	90	89	80	71	36	16	16		4	30	66	50	27	14	20		7	37	70	64	71	84	96		1	2	16	27	54	84	93	
hCMV+	lo	hi	77	66	45	58	18	15	26		22	77	92	78	69	57	61		12	52	81	83	86	67	89		7	5	15	20	26	41	41	
EBV+	int	hi	83	71	59	56	16	10	22		71	84	93	85	58	46	51		52	64	90	91	94	89	95		14	10	28	37	59	79	74	
	hi	hi	71	48	43	31	12	5	16		89	88	86	80	40	24	27		90	67	90	88	94	93	96		32	40	58	74	84	95	93	
	hi	lo	87	73	58	35	16	4	10		40	70	74	67	15	5	7		40	66	88	88	88	94	96		13	19	56	75	87	98	99	
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	lo	lo	85	80	80	75	27	14	15		1	11	28	17	35	21	15		2	18	34	42	60	61	72		2	4	13	9	48	67	74	
	int	lo	82	65	70	52	14	5	16		16	43	67	63	27	14	9		14	40	62	66	71	77	79		8	16	32	46	78	92	94	
HIV+	lo	hi	74	46	39	30	12	6	29		53	88	93	87	55	45	31		11	51	72	74	78	73	78		6	16	27	45	39	53	35	
	int	hi	79	33	34	25	7	4	21		72	89	89	85	36	25	19		51	68	80	83	80	77	79		32	41	45	64	62	71	62	
	hi	hi	78	17	22	16	4	2	13		49	79	78	66	24	14	9		74	68	82	84	79	79	80		70	84	75	86	87	92	89	
	hi	lo	66	27	37	26	6	2	9		32	50	64	50	10	4	2		35	64	81	82	75	81	79		73	82	79	89	97	99	100	

Figure S9. Infection history influences the associations between surface phenotype, T-bet/Eomes expression levels and the functional potential

Heat maps indicating the degree of expression of IL-7R α (first column), granzyme K (second column), KLRG1 (third column) and granzyme B (last column) per combined surface marker- and T-bet/Eomes-defined CD8⁺ T-cell subset plotted on the X- and Y-axes, respectively, in cord blood samples (n=5, first row), EBV/hCMV seronegative individuals (n=6, second row), EBV mono-infected individuals (n=5, third row), hCMV mono-infected individuals (n=3, fourth row), EBV/hCMV double-infected individuals (n=6, fifth row) and HIV-1-infected individuals (n=13, last row). The degree of expression intensity is indicated by increments in color intensity and by the mean percentage of positive cells denoted in the boxes. Ns = not significant, * p<0.05, *** P<0.001.