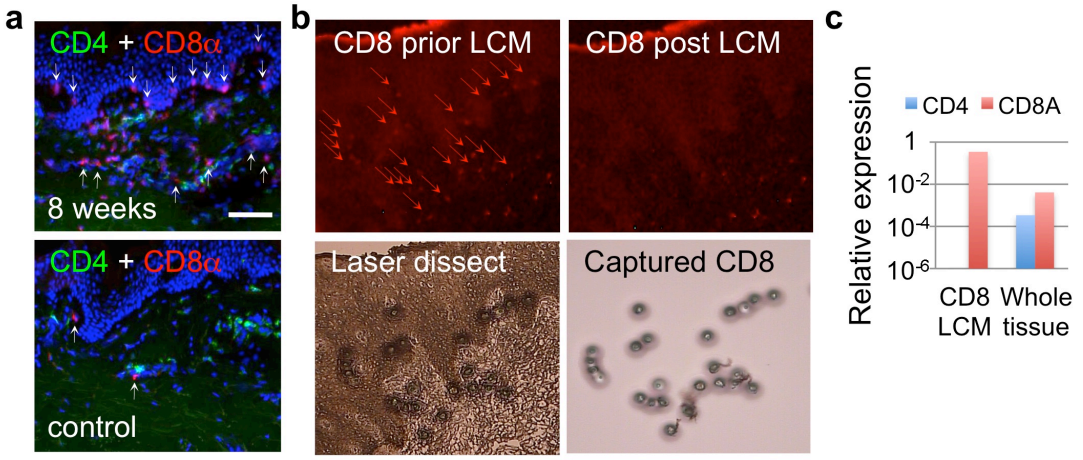
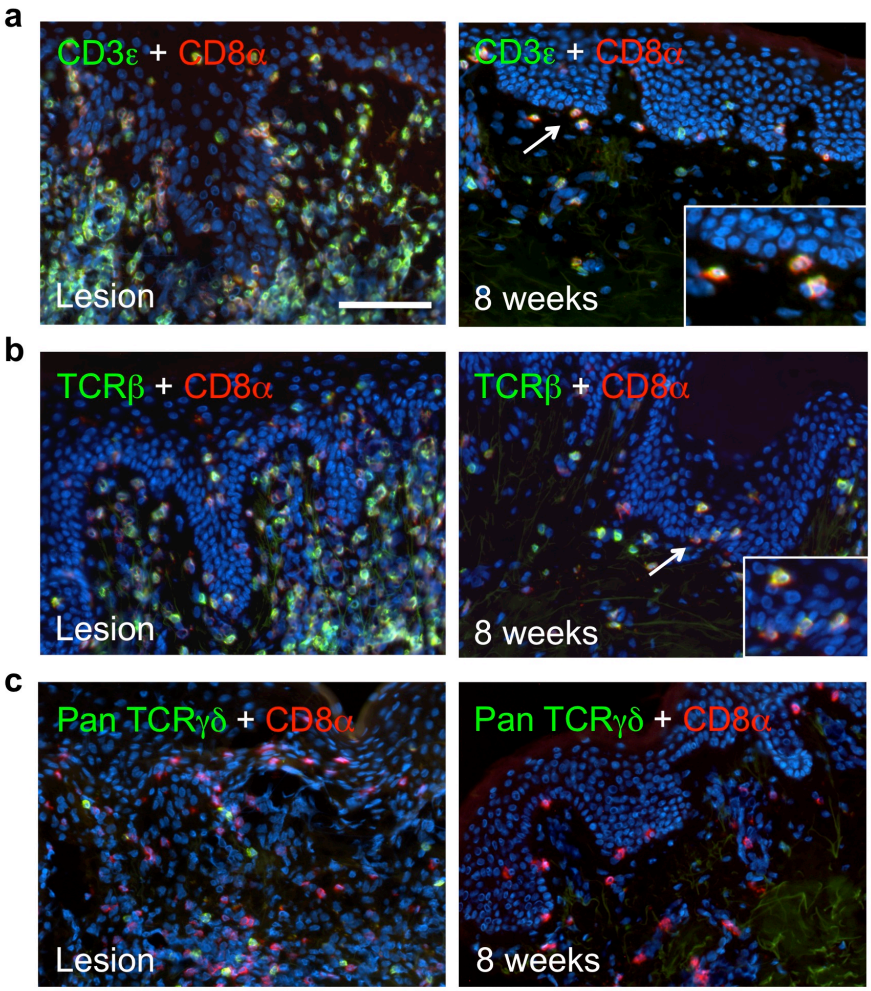


Supplementary Figure 1

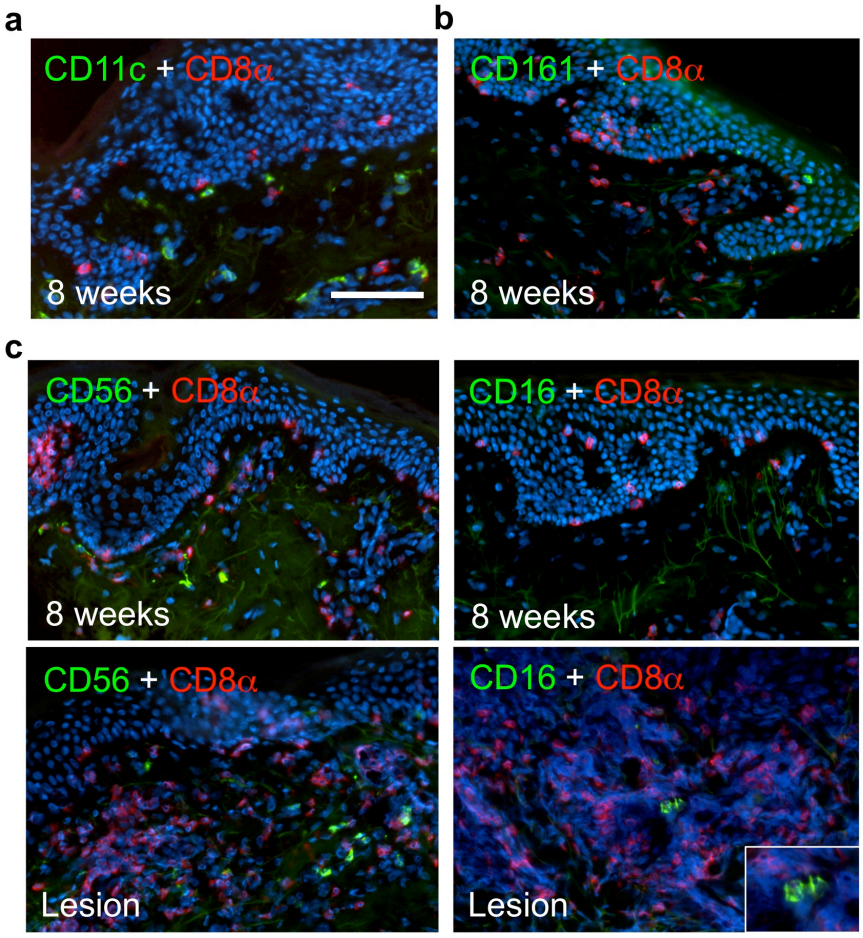
Laser capture microdissection of individual $CD8^+$ T cells in genital tissue. **a,** $CD8^+$ T cells (red), but not $CD4^+$ T cells (green) persisted at the dermal-epidermal junction (DEJ) in skin tissue obtained 8 weeks post-healing of a genital herpes lesion (top); matching control tissue biopsied at the same time in the contralateral genital site (bottom). Arrows denote $CD8^+$ T cells localized at DEJ (DEJ CD8, top, downward arrows), near blood vessel in the dermis (BV CD8, top, upward arrows) from a biopsy tissue 8 weeks after lesion completely healed, and $CD8^+$ T cells in matching control tissue (bottom, upward arrows). **b,** Laser capture microdissection of $CD8^+$ T cells at a single cell level. The micrographs show fluorescence labeled $CD8^+$ T cells before (upper left) and after (upper right) laser dissection. The pattern of imprinted laser markers (lower left) matches with individually captured $CD8^+$ T cells (lower right). Arrows denote selection of $CD8^+$ T cells for microdissection. **c,** Purity of captured $CD8^+$ T cells. TaqMan PCR analysis shows both *CD4* and *CD8A* genes are expressed in whole tissue sections. Only *CD8A* is expressed in captured $CD8^+$ T cells. Expression normalized to *ACTB*. Bar: 100 μm.



Supplementary Figure 2

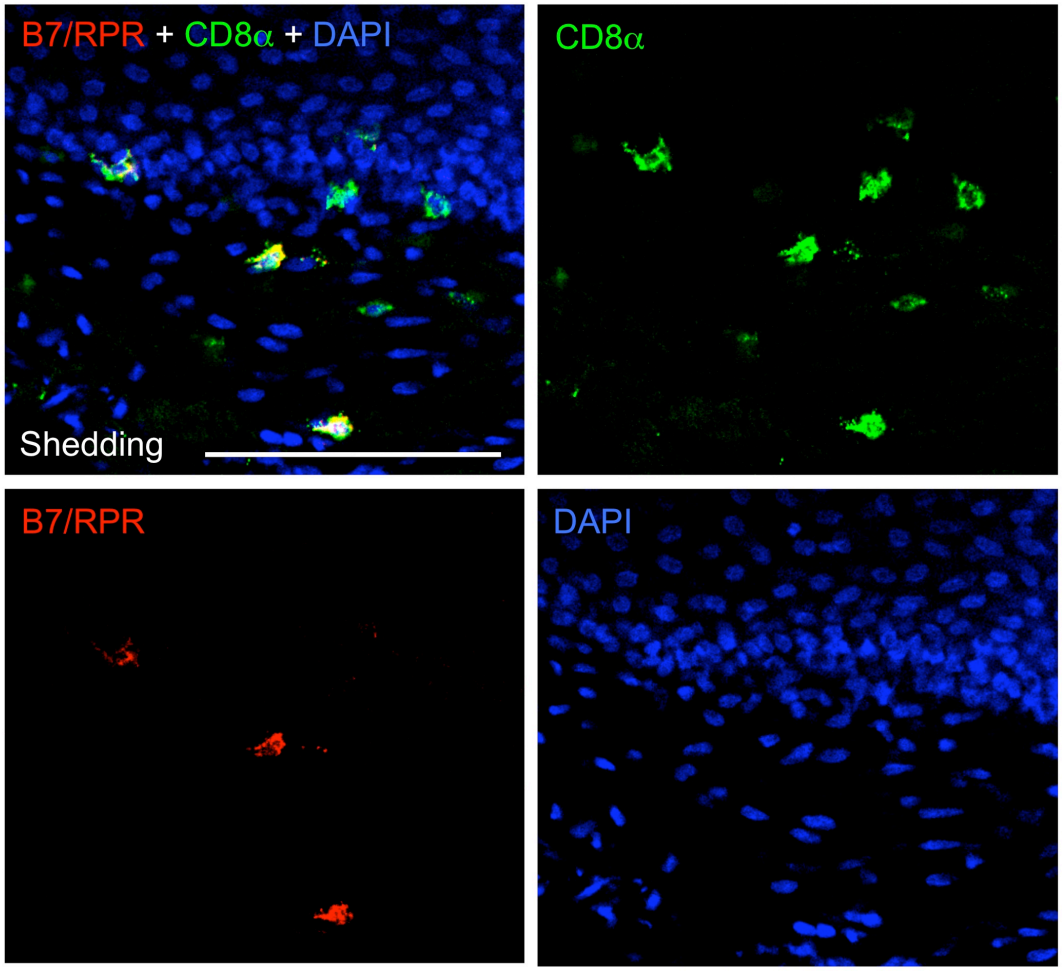
DEJ CD8 cells are TCRαβ T cells, not TCRγδ T cells.

a&b, Dual Immunofluorescence staining indicates DEJ CD8 cells co-expressing CD3ε (yellow) (**a**) and TCRβ chain (yellow) (**b**) in both active lesion and 8 week post-healed tissue. Arrows denote enlarged area. **c,** DEJ CD8 cells express no TCRγδ receptor during both active lesion and 8 week post-healed tissue. A few TCRγδ T cells infiltrate in the dermis of genital skin during active herpes lesions. Bar: 100 μm.



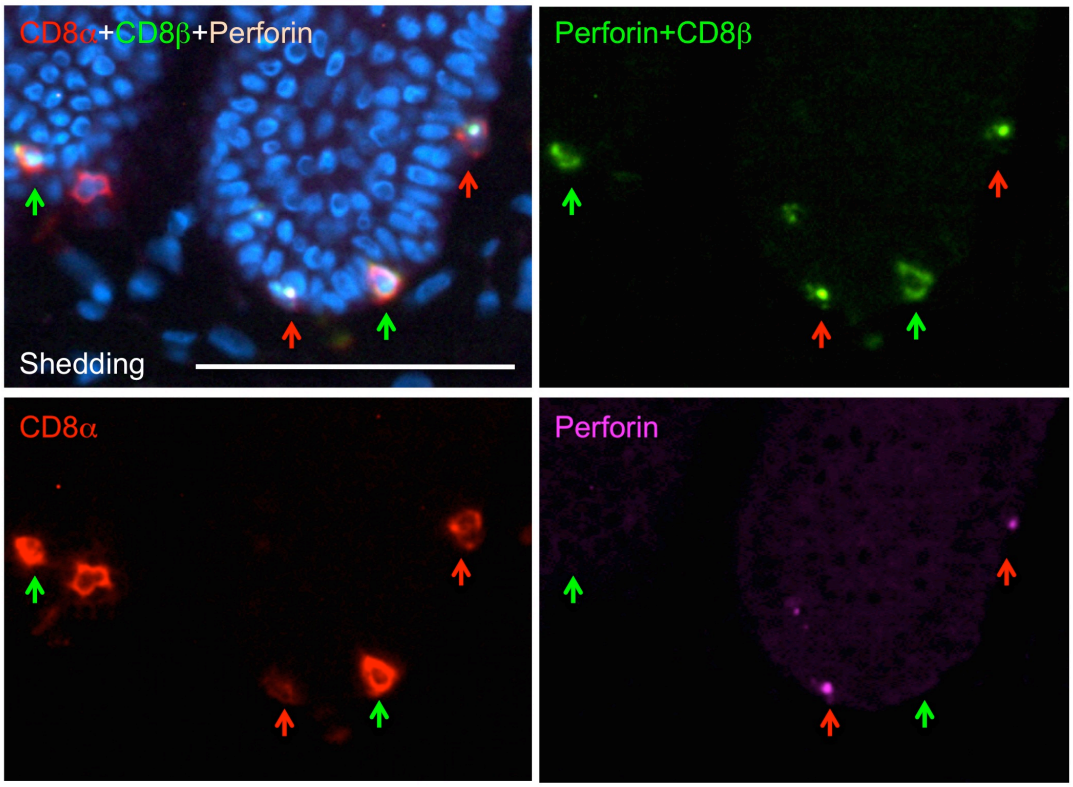
Supplementary Figure 3

DEJ CD8 cells do not co-express markers specific for dendritic cells (DC), natural killer (NK) cells, nor nonconventional T cells. Dual immunofluorescence staining indicates that DEJ CD8 cells persisting in 8 weeks post-healed tissue express no CD11c, a marker for conventional DC (**a**); CD161, a marker for NK T and MAIT cells (**b**); nor NK cell markers for NK cells, CD56 and CD16 (**c**). NK cells positive for CD16 or CD56 are present in active lesions (**c**). Bar: 100 μm.



Supplementary Figure 4

In situ detection of HSV-specific $CD8^+$ T cells in tissue undergoing asymptomatic reactivation. Biopsy tissue obtained at 8 week post-healing time point from a subject undergoing asymptomatic HSV-2 reactivation was dual stained with Qdot 655 conjugated peptide-MHC complex, B*0702/RPR, *UL49* aa 49-57 (red), anti-CD8α antibody (green), and counter stained with DAPI (blue). A projection of a 30-μm stack shows HSV-specific $CD8^+$ T cells localized at the DEJ and upper dermis. Bar: 100 μm.



Supplementary Figure 5

DEJ CD8αα^{+} T cells express perforin granules during asymptomatic shedding. Biopsy tissue was obtained at 8 weeks post-healing time point from a subject undergoing asymptomatic HSV-2 reactivation. Tissue was immunofluorescence stained using antibodies specific to CD8α (red), CD8β (green), Perforin (both green and pink) and counter stained with DAPI (blue). Perforin granules (both green and pink dot) are detected in DEJ CD8αα (CD8α^{+} β^{-} T cells, red only) as indicated by red arrows. CD8α^{+}β^{+} T cells (red and green) that do not express perforin granule as indicated by green arrows. Bar: 100 μm.

Supplementary Table 1 CDR3 sequences and cognate V and J gene usage of dominant DEJ CD8 clones. List shows the top six most abundant DEJ CD8 clones in each biopsy.

Biopsy Stage	Amino Acid Sequence	V Gene	J Gene	Normalized Frequency
Newly Healed 03/2005	CASRKTGIPSEQYF	TRBV6-2/TRBV6-3	TRBJ2-7	0.435
	CASSFRDGHYEQYF	(undefined)	TRBJ2-7	0.208
	CASSPDGQGGYTF	TRBV19	TRBJ1-2	0.154
	CASSYQARASWGYEQYF	(undefined)	TRBJ2-3	0.059
	CASSLLAGHTGELFF	TRBV12-3/TRBV12-4	TRBJ2-2	0.031
	CASRLDRGTLTDTQYF	TRBV12-3/TRBV12-4	TRBJ2-3	0.027
2 wph* 04/2005	CASSLLAGHTGELFF	TRBV12-3/TRBV12-4	TRBJ2-2	0.401
	CATRRTSGRYNEQFF	TRBV5-1	TRBJ2-1	0.212
	CASSHGYEQYF	TRBV14	TRBJ2-7	0.185
	CASSAGSLVYNEQFF	TRBV7-3	TRBJ2-1	0.141
	CASSPTRNTGELFF	TRBV11-2	TRBJ2-7	0.013
	CASRKTGIPSEQYF	TRBV6-2/TRBV6-3	TRBJ2-2	0.012
4 wph* 04/2006	CAISEPTSSSVTGELFF	TRBV10-3	TRBJ2-2	0.431
	CASSFDLGGRSNQPQHF	(undefined)	TRBJ1-5	0.388
	CASSHGYEQYF	TRBV14	TRBJ2-7	0.047
	CATSRDRYYGYTF	TRBV15	TRBJ1-2	0.038
	CASSPGRDWGSEQYF	TRBV7-9	TRBJ2-7	0.020
	CACGDSLYEQYF	(undefined)	TRBJ2-7	0.014
Healing Lesion 10/2006	CASSLLAGHTGELFF	TRBV12-3/TRBV12-4	TRBJ2-2	0.291
	CASSMRVQPQHF	TRBV19	TRBJ1-5	0.260
	CASSQVTASTDTQYF	TRBV23-1	TRBJ2-3	0.173
	CASSQEGGQGPSGANVLTF	TRBV3-1	TRBJ2-6	0.062
	CASSEASGSTDTQYF	TRBV6-4	TRBJ2-3	0.045
	CASSIDPGEGKLFF	TRBV19	TRBJ1-4	0.017
4 wph* 12/2006	CASSDRDTGELFF	TRBV6-4	TRBJ2-2	0.474
	CASSLEPGQGANEQFF	(undefined)	TRBJ2-1	0.202
	CASRKTGIPSEQYF	TRBV6-2/TRBV6-3	TRBJ2-7	0.063
	CASSLLKGGNYEQYF	TRBV7-9	TRBJ2-3	0.052
	CASRLDRGTLTDTQYF	TRBV12-3/TRBV12-4	TRBJ2-3	0.041
	CASSDRRDEQYF	TRBV7-6	TRBJ2-7	0.022
8 wph* 10/2007	CASSLLAGHTGELFF	TRBV12-3/TRBV12-4	TRBJ2-2	0.457
	CASSQEGGQGPSGANVLTF	TRBV3-1	TRBJ2-6	0.380
	CASRKTGIPSEQYF	TRBV6-2/TRBV6-3	TRBJ2-7	0.048
	CATASAYEQYF	TRBV24-1	TRBJ2-7	0.045
	CASSQEGGQGPSGANVLFF	TRBV3-1	TRBJ1-4	0.023
	CASSPGTGTGANVLTF	(undefined)	TRBJ2-6	0.021

* weeks post-healing.

Supplementary Table 2 CDR3 sequences and cognate V and J gene usage of DEJ CD8 clones commonly shared between multiple biopsies.

Amino Acid Sequence	V Gene	J Gene
CASSLLAGHTGELFF	TRBV12-3/TRBV12-4	TRBJ2-2
CASSQEGGQGPSGANVLTF	TRBV3-1	TRBJ2-6
CAISEPTSSSVTGELFF	TRBV10-3	TRBJ2-2
CASRKTGIPSEQYF	TRBV6-2/TRBV6-3	TRBJ2-7
CASSHGYEQYF	TRBV14	TRBJ2-7
CASSFRDGHYEQYF	(undefined)	TRBJ2-7
CATASAYEQYF	TRBV24-1	TRBJ2-7
CASRLDRGTLTDTQYF	TRBV12-3/TRBV12-4	TRBJ2-3
CASSPGTGTGANVLTF	(undefined)	TRBJ2-6
CASSLDVVGIEAFF	TRBV11-2	TRBJ1-1
CASSDTYNSPLHF	TRBV6-1	TRBJ1-6
CASSLRGLAGGPGTDTQYF	TRBV7-3	TRBJ2-3

Supplementary Table 3 Identification of $CD8^+$ T cell clones shared among all three T cell populations obtained from the same subject: lesion infiltrating T cells, PBMC-derived $CD8\alpha\alpha^+$ T cells and HSV-2 reactive $CD8^+$ T cells isolated directly *ex vivo* from PBMC. Listed are CDR3 sequences and cognate V and J gene usage of 15 shared clonotypes.

Amino Acid	**V Gene**	**J Gene**
CASSAGLEAFF	TRBV6-2/TRBV6-3	TRBJ1-1
CASKSGASNEQFF	TRBV19	TRBJ2-1
CASSFRGTGELFF	TRBV28	TRBJ2-2
CSAGGRVATGAWEQFF	TRBV20-1	TRBJ2-1
CASSFWGNPVDEQFF	TRBV7-2	TRBJ2-1
CASRLDRGTLTDTQYF	TRBV12-3/TRBV12-4	TRBJ2-3
CASAGTSGVQETQYF	TRBV19	TRBJ2-5
CASSDTGPGNSPLHF	TRBV7-9	TRBJ1-6
CASSLWGSGPAFF	TRBV7-2	TRBJ1-1
CAITGGDTYEQYF	TRBV10-3	TRBJ2-7
CASSLGQAYEQYF	TRBV7-8	TRBJ2-7
CARASMGTEAFF	TRBV2	TRBJ1-1
CAWNPIGEGTEAFF	TRBV30	TRBJ1-1
CASRPPGAQDTQYF	TRBV7-9	TRBJ2-3
CASGGRGSSYEQYF	TRBV10-2	TRBJ2-7

Supplementary Table 4 Antibody resources.

Antibody	Clone	Manufacturer
CD3ε, purified	UCH-T1	Santa Cruz
CD4, purified	RPA-T4	Biolegend
CD8α, purified	RPA-T8	BD Pharmingen
CD8α AF647	RPA-T8	BD Pharmingen
CD8β, purified	F-5	Santa Cruz
CD11c, purified	B-ly6	BD Pharmingen
CD16, purified	3G8	Biolegend
CD56, purified	MEM-188	Biolegend
HSV-2, purified	Polyclonal	Dako
Perforin, purified	dG9	eBioscience
Pan TCRγδ, purified	5A6.E91	Pierce antibodies
TCRβ, purified	8A3	Pierce antibodies

Supplementary Table 5 Applied Biosystems primer/probe sets used for Taqman PCR assays.

Gene	**Assay ID**
ACTB	Hs03023880_g1
CD4	Hs00181217_m1
CD8A	Hs00233520_m1
CD8B	Hs00174762_m1
CCR7	Hs01013469_m1
GZMA	Hs00989184_m1
GZMB	Hs00188051_m1
IFNG	Hs00989291_m1
PRF1	Hs00169473_m1
S1PR1	Hs01922614_s1
TNFA	Hs00174128_m1