

SUPPLEMENTARY FIGURES AND TABLES

Title: Single-cell analysis reveals shared and stage-specific features of the human skin response in primary and secondary syphilis lesions

Authors

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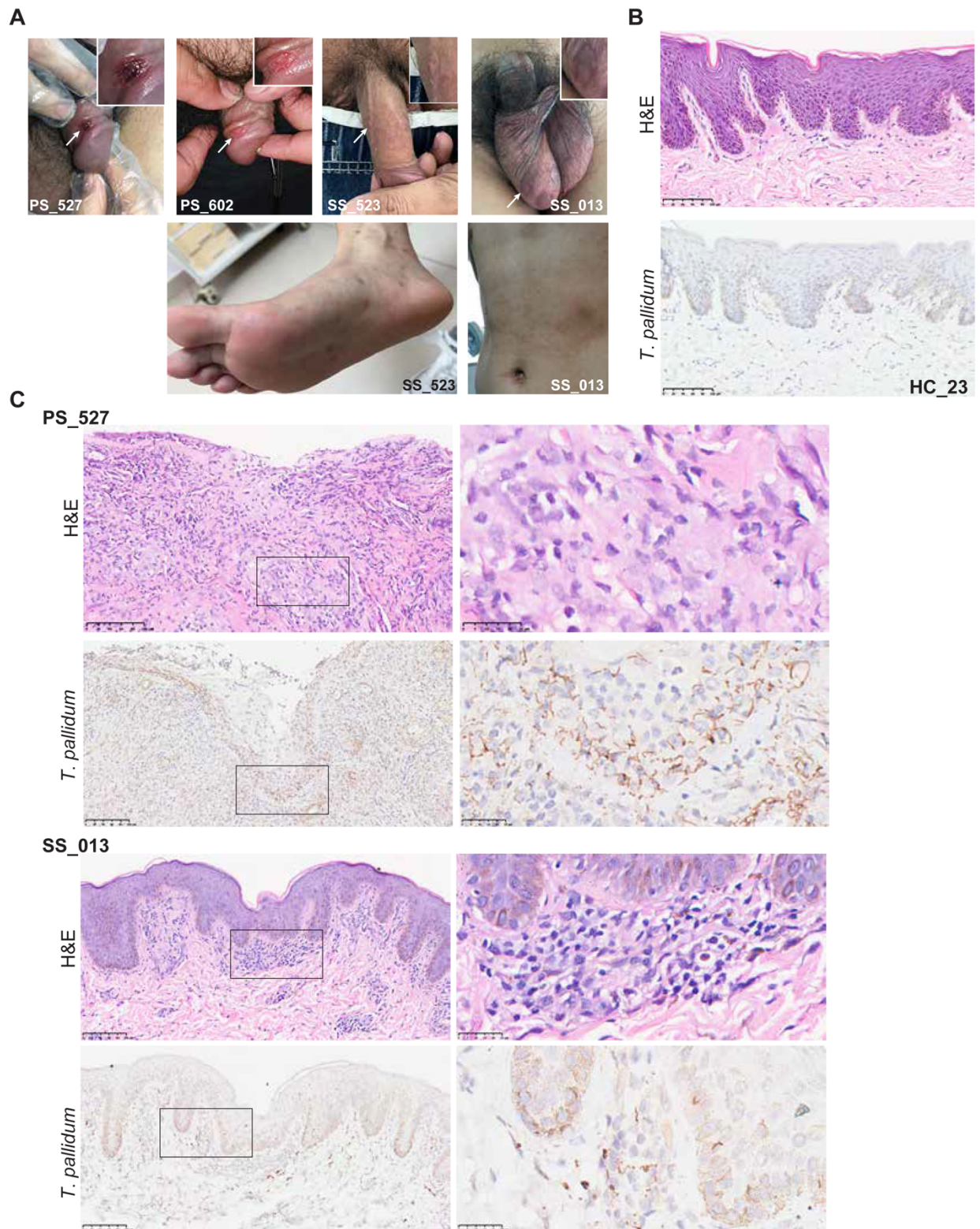
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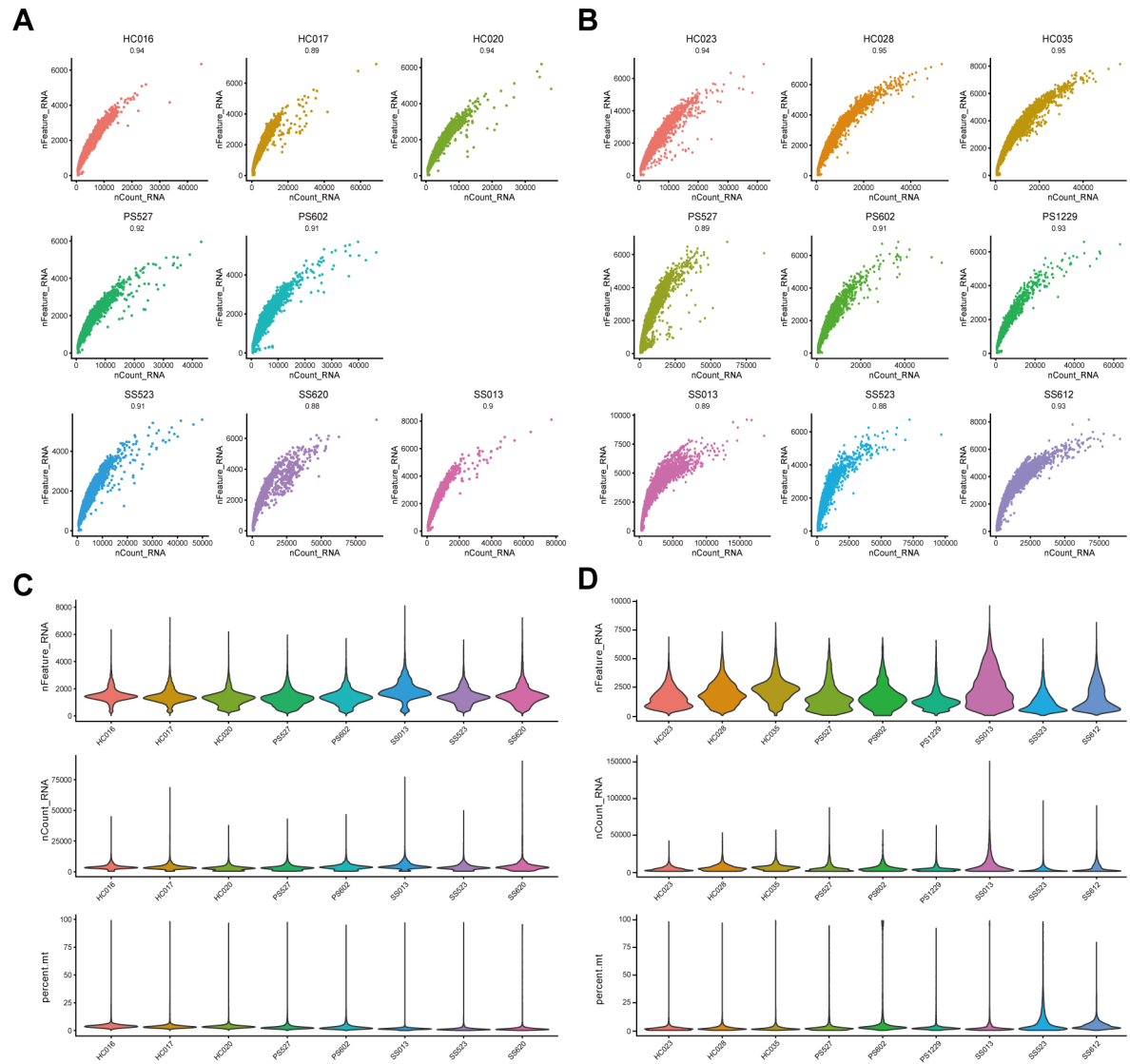
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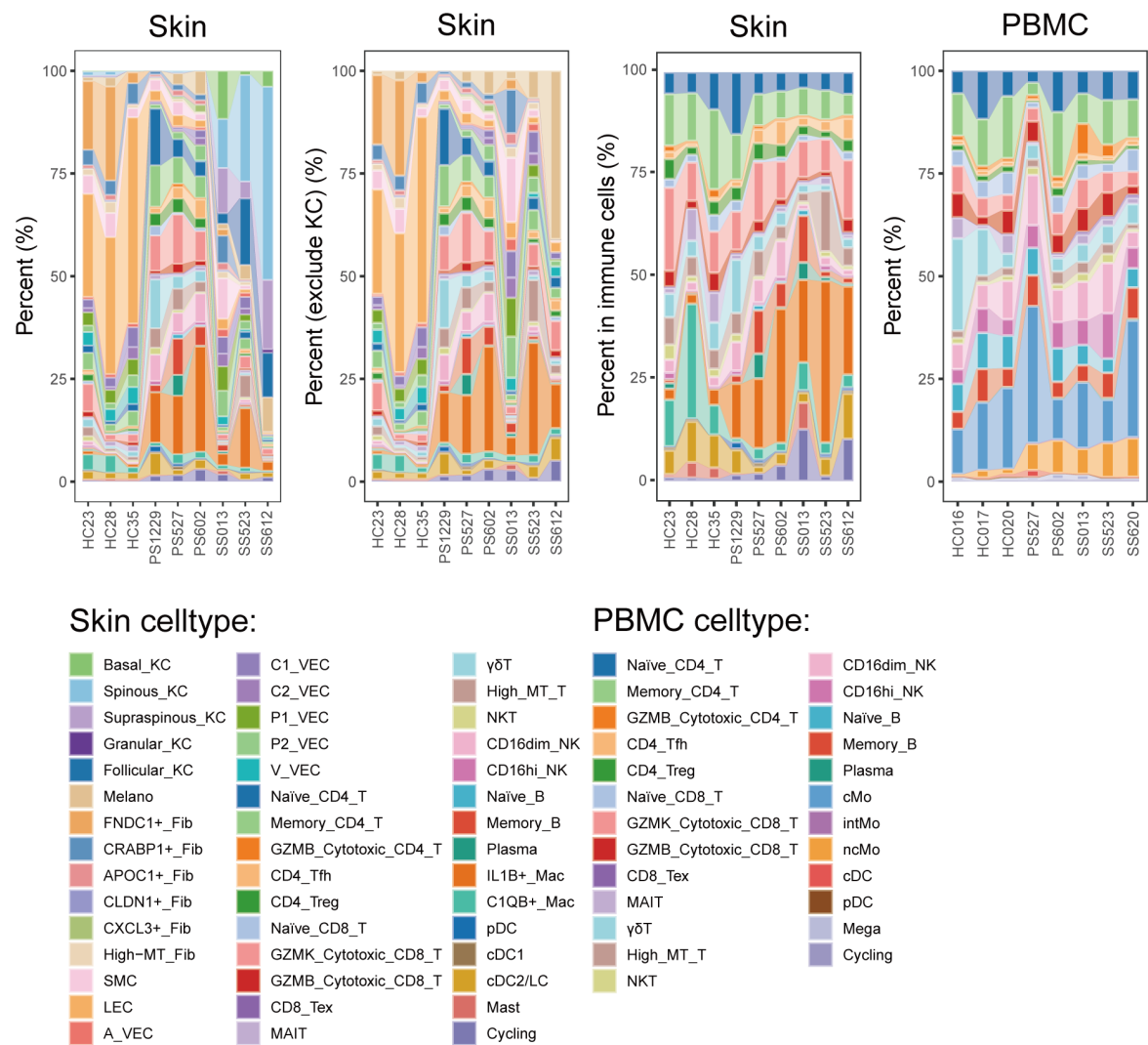
Supplementary Fig. 1. Clinical presentation and histopathologic characterization of early syphilis patients. (A) Clinical morphology of syphilis lesions. Primary syphilis patients (PS_527 and PS_602) presented with classic chancres (ulcers) on the

penile coronal sulcus. Secondary syphilis patient SS_523 exhibited papules on the penis (top) and maculopapular rashes on the feet (bottom). Patient SS_013 presented with erythematous plaques on the scrotum (top) and a generalized maculopapular rash on the trunk (bottom). White arrowheads denote the specific sites of specimen collection for scRNA-seq analysis. **(B)** Representative Hematoxylin and Eosin (H&E) and *TPA* immunohistochemical (IHC) staining of HC foreskin. Scale bars indicate 100µm. **(C)** Histopathologic features of primary and secondary syphilis. Representative case PS_527 (Primary): Epidermal thinning with basal vacuolar degeneration. Dermis shows pan-dermal vascular proliferation with endothelial swelling and dense perivascular infiltrates of lymphocytes, plasma cells, and histiocytes. Mild collagen fiber thickening is present. Representative case SS_013 (Secondary): Irregular acanthosis with basal vacuolar degeneration. Dermis shows prominent superficial to mid-dermal vascular proliferation with endothelial swelling and perivascular infiltrates of lymphocytes, plasma cells, and histiocytes. Spirochetes were clearly visible by IHC. Scale bars indicate 100µm in the 20X zoom image on the left and 25µm in the 80X zoom image inset on the right.

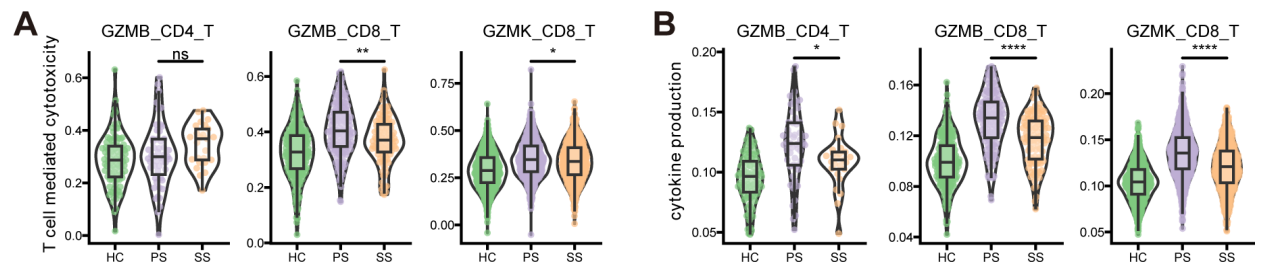


Supplementary Fig. 2. Quality control of all cells acquired by scRNA-seq of immune cell subpopulations. (A-B) Scatterplots showing quality control metrics for PBMC (A) and skin (B) samples. Each plot displays the relationship between total UMI counts (nCount_RNA) and the number of detected genes (nFeature_RNA), reflecting transcriptional complexity and library quality. (C-D) Violin plots depicting the distribution of nFeature_RNA, nCount_RNA, and mitochondrial transcript percentages (percent.mt) across major cell populations in PBMC (C) and skin (D) samples. UMI, unique molecular identifier; PBMC, peripheral blood mononuclear cell.

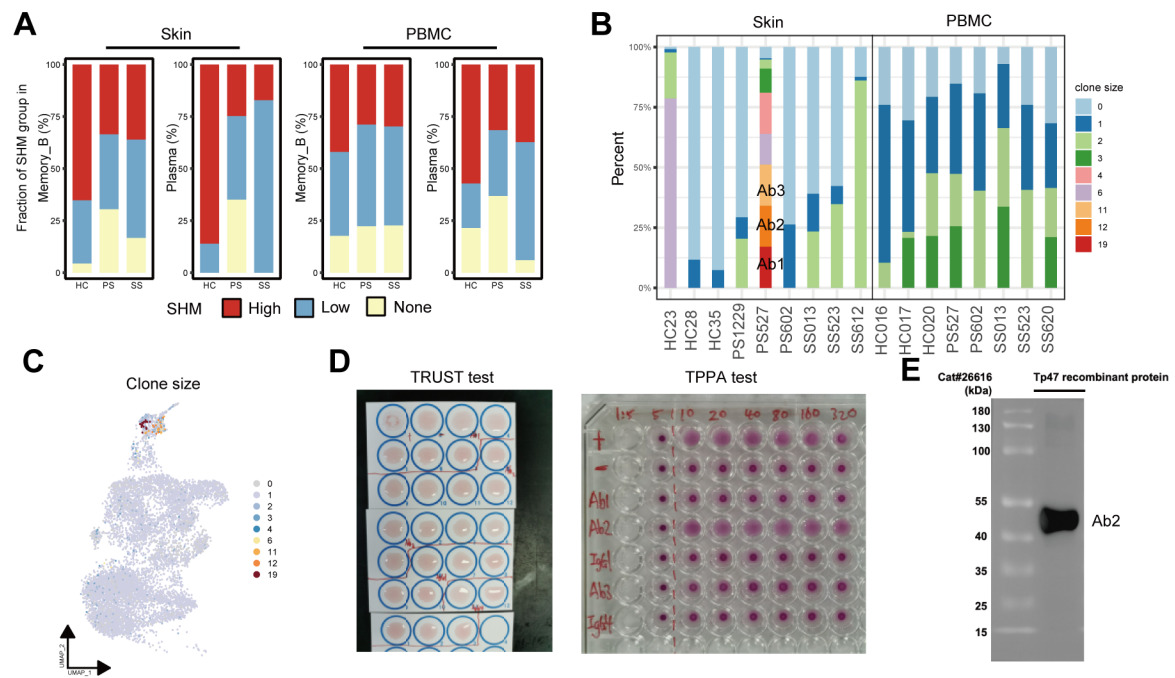
H) UMAP plots depicting finer subclustering of dendritic cells, monocytes, and macrophages (**F**), lymphocyte subsets (**G**), and B cell populations (**H**), along with relevant marker expression profiles. UMAP, Uniform Manifold Approximation and Projection.



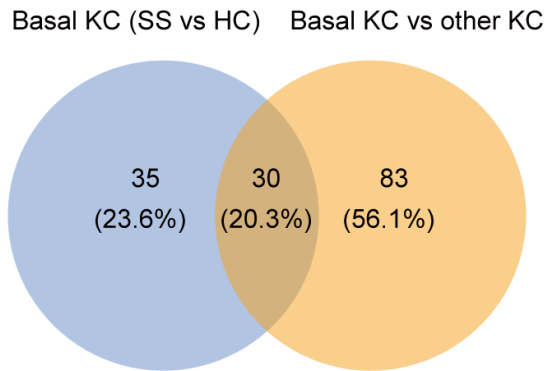
Supplementary Fig. 4. Proportion of all cell subpopulations in skin and PBMC across samples. The relative abundance of each annotated cell subset in individual skin and PBMC samples.



Supplementary Fig. 5. Cytotoxic T cells display stronger cytotoxic and inflammatory signatures in PS lesions than in SS lesions. (A and B) AddModuleScore of T cell mediated cytotoxicity (**A**) or cytokine production (**B**) for individual cytotoxic T cells from skin. Statistical comparisons were performed using the Wilcoxon rank-sum test. ns, not significant; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$.



Supplementary Fig. 6. Reduced somatic hypermutation and limited clonal expansion of B cells in syphilis lesions and blood. (A) Proportion of B cells with high (>0.03), low ($0-0.03$), or no somatic hypermutation (SHM) in skin and PBMC samples. (B) Distribution of BCR clone sizes across samples. Three monoclonal antibodies (Ab1-Ab3) were generated from the dominant BCR clones in PS327, with sizes of 19, 12, and 11 cells, respectively. (C) UMAP visualization of BCR clone sizes across all samples. (D) TRUST and TPPA reactivity of Ab1-Ab3 compared with corresponding isotype controls. (E) Immunoblot demonstrating binding of monoclonal antibody 2 (Ab2) to recombinant Tp47 protein. SHM, somatic hypermutation; TRUST, *Treponema pallidum* rapid test; TPPA, *Treponema pallidum* particle agglutination assay.



Supplementary Fig. 7. Basal keratinocytes in secondary syphilis dermal lesions exhibit aberrant inflammatory and proliferative signaling. Venn diagram showing annotated pathways from DEGs comparing SS Basal KCs versus HC (blue), and Basal KCs versus other KC subsets (orange). DEG, differentially expressed gene; KC, keratinocyte; HC, healthy control; SS, secondary syphilis.

SUPPLEMENTARY TABLES

Supplementary Table 1. Clinical characteristics of study participants with skin and blood samples analyzed by scRNA-seq.

No.	Stage	Age	Sex	PBMC	Skin	Site of skin	Tissue for scRNA-seq	TRUST	TPPA	Darkfield microscopy
HC_23y	HC	23	M	No	Yes	foreskin	epidermis mix dermis (1:5)	-	-	NP
HC_28y	HC	28	M	No	Yes	foreskin	epidermis mix dermis (1:5)	-	-	NP
HC_35y	HC	35	M	No	Yes	foreskin	epidermis mix dermis (1:5)	-	-	NP
HC_016	HC	24	M	Yes	No	NA	NA	-	-	NP
HC_017	HC	28	M	Yes	No	NA	NA	-	-	NP
HC_020	HC	24	M	Yes	No	NA	NA	-	-	NP
PS_527	PS	35	M	Yes	Yes	Penis	whole skin	+	1:640	+
PS_602	PS	47	M	Yes	Yes	Penis	whole skin	+	1:640	+
PS_1229	PS	30	M	No	Yes	Penis	whole skin	+	1:640	+
SS_523	SS	42	M	Yes	Yes	Scrotum	epidermis & dermis	1:128	>1:1280	NP
SS_612	SS	35	M	No	Yes	Foot	epidermis mix dermis	1:128	>1:1280	NP
SS_013	SS	31	M	Yes	Yes	Penis	epidermis mix dermis (1:5)	1:32	>1:1280	NP
SS_620	SS	29	F	Yes	No	NA	NA	1:16	>1:1280	NP

HC: healthy control; PS: primary syphilis; SS: secondary syphilis; M: male; F: Female; NA: Not Applicable ; +: reactive test; -: non-reactive test; NP: not performed

Supplementary Table 2. Clinical characteristics of study participants with skin samples analyzed by bulk RNA-seq.

No.	Stage	Age	Sex	TRUST	TPPA	Paired with non-lesion
PS_1	PS	29	M	1:8	+	Yes
PS_2	PS	19	M	1:8	1:8	Yes
PS_3	PS	25	M	1:8	+	Yes
SS_1	SS	70	M	1:64	+	Yes
SS_2	SS	24	M	1:32	+	Yes
SS_3	SS	25	M	1:16	>1:1280	Yes

PS: primary syphilis; SS: secondary syphilis; M: male; +: reactive test.

Supplementary Table 3. Clinical characteristics of study participants with blood samples analyzed by flow cytometry.

No. Patients	Stage	Age	Sex	TRUST	TPPA
HC_1	HC	26	M	-	-
HC_2	HC	38	M	-	-
HC_3	HC	25	M	-	-
HC_4	HC	30	F	-	-
HC_5	HC	25	F	-	-
HC_6	HC	28	F	-	-
SS_1	SS	70	M	1:64	+
SS_2	SS	29	F	1:64	+
SS_3	SS	14	M	1:64	>1:1280
SS_4	SS	27	M	1:32	+
SS_5	SS	25	M	1:16	>1:1280
SS_6	SS	32	M	1:32	>1:1280

HC: healthy control; SS: secondary syphilis; M: male; F: Female; +: reactive test; -: non-reactive test.

Supplementary Table 4. Cell numbers of each cell type in each donor

All cells																				
	pbmc-HC016	pbmc-HC017	pbmc-HC020	pbmc-PS527	pbmc-PS602	pbmc-SS013	pbmc-SS523	pbmc-SS620	skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP	PBMC total	Skin total	Total
KC	0	0	0	0	0	0	0	0	447	174	21	10	42	8	2519	3435	15259	0	21915	21915
Melano	0	0	0	0	0	0	0	0	72	223	28	2	110	107	87	239	1565	0	2433	2433
Fib	0	0	0	0	0	0	0	0	1817	3248	1098	73	184	96	831	115	67	0	7529	7529
SMC	0	0	0	0	0	0	0	0	337	582	269	89	131	29	651	64	9	0	2161	2161
LEC	0	0	0	0	0	0	0	0	1876	3316	6037	85	107	33	169	58	95	0	11776	11776
VEC	0	0	0	0	0	0	0	0	969	1189	2520	69	138	173	1598	664	328	0	7648	7648
NK/NKT/gdT	5685	3474	3335	2781	2739	3352	4343	1914	1196	393	920	1189	1190	447	160	698	722	27623	6915	34538
T	2374	3224	2792	713	2903	1369	2130	2039	678	401	850	1104	750	307	212	669	480	17544	5451	22995
B	1305	1819	1763	1189	1225	730	1139	1090	64	7	2	91	528	91	127	83	70	10260	1063	11323
pDC	10	10	9	9	6	9	28	10	15	4	1	33	138	16	34	32	12	91	285	376
mDC	11	22	55	120	12	15	26	37	289	279	237	378	435	241	160	546	363	298	2928	3226
Mono	1228	1689	2163	2703	976	1145	1227	2470	52	74	91	239	121	186	24	359	114	13601	1260	14861
Mono-Mac	62	169	151	457	538	364	718	661	206	269	127	65	223	145	99	295	222	3120	1651	4771
Mega	82	39	49	73	97	84	14	16	0	0	0	0	0	0	0	0	0	454	0	454
Mast	0	0	0	0	0	0	0	0	24	53	57	12	30	2	17	2	27	0	224	224
Cycling	21	4	25	17	41	22	50	48	20	11	13	47	61	44	112	31	194	228	533	761
																Total: 146991 (PBMCs: 73219; skin: 73772)				
Immune cells																				
	pbmc-HC016	pbmc-HC017	pbmc-HC020	pbmc-PS527	pbmc-PS602	pbmc-SS013	pbmc-SS523	pbmc-SS620	skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP	PBMC total	Skin total	Total
NK	1072	1241	1237	1409	1110	1044	2260	757	350	113	220	311	323	162	25	78	92	10130	1674	11804
NKT	1862	1026	1350	1002	1308	1875	1677	642	581	184	423	417	637	198	87	333	378	10742	3238	13980
NKT/gdT	2783	1231	718	404	322	457	494	501	162	103	234	419	124	59	23	55	63	6910	1242	8152
CD4_T	1956	2788	2478	593	2569	1136	1860	1617	713	361	821	1031	825	315	157	707	398	14997	5328	20325
CD8_T	396	413	473	106	341	220	200	454	64	25	64	107	28	19	16	25	27	2603	375	2978
B	1294	1805	1604	1191	1215	712	1075	1064	13	9	4	67	423	96	117	43	35	9960	807	10767
Plasma	3	1	39	2	18	6	42	27	54	2	1	7	230	5	44	30	18	138	391	529
pDC	10	20	37	5	4	7	17	1	6	1	1	50	28	6	2	25	15	101	134	235
mDC	7	11	55	110	8	5	27	24	222	223	146	224	99	47	25	82	196	247	1264	1511
Mono	1286	1867	2266	3148	1499	1516	1954	3130	51	53	81	238	102	124	10	148	78	16666	885	17551
Mono-Mac	4	3	4	12	2	6	1	5	275	345	228	230	589	400	254	987	459	37	3767	3804
Mega	81	39	52	61	99	83	14	14	0	0	0	0	0	0	0	0	0	443	0	443

Mast	3	1	4	2	1	1	4	1	26	56	60	10	7	4	60	3	4	17	230	247
Mix (KC)	0	0	0	0	0	0	0	0	7	5	2	0	0	0	13	168	247	0	442	442
																Total: 92768 (PBMCs: 72991; skin: 19777)				
Myeloid cells																				
									skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP		Skin total	
IL1B+_Mac									65	35	88	413	576	483	185	972	411		3228	
C1QB+_Mac									284	411	165	29	94	31	63	69	60		1206	
pDC									5	1	1	48	28	5	2	19	14		123	
cDC1									19	9	1	10	31	2	3	7	14		96	
cDC2/LC									145	146	182	179	53	41	22	105	211		1084	
Mast									19	55	60	8	2	3	60	1	0		208	
Mix_1 (LEC)									18	8	6	47	9	3	7	3	0		101	
Mix_2 (KC)									6	7	4	2	21	3	9	64	27		143	
Mix_3 (T)									19	6	9	16	11	10	0	5	15		91	
	pbmc-HC016	pbmc-HC017	pbmc-HC020	pbmc-PS527	pbmc-PS602	pbmc-SS013	pbmc-SS523	pbmc-SS620										PBMC total		
cMo	1126	1644	1755	2588	742	1039	926	2291										12111		
intMo	5	9	4	18	18	13	18	18										103		
ncMo	52	179	114	497	598	403	745	757										3345		
cDC	23	35	60	131	13	8	17	30										317		
pDC	10	20	26	8	4	7	17	2										94		
Mix_1 (T)	91	14	343	27	134	60	275	57										1001		
Mix_2 (B)	3	1	64	8	5	5	5	6										97		
																Total: 23348 (PBMCs: 17068; skin: 6280)				
NK & T cells																				
	pbmc-HC016	pbmc-HC017	pbmc-HC020	pbmc-PS527	pbmc-PS602	pbmc-SS013	pbmc-SS523	pbmc-SS620	skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP	PBMC total	Skin total	Total
Naive_CD4_T	565	1176	550	222	753	362	636	561	130	74	206	466	179	68	35	109	101	4825	1368	6193
Memory_CD4_T	1065	1146	1350	239	1184	478	973	748	315	172	444	341	260	99	62	176	99	7183	1968	9151
GZMB_Cytotoxic_CD4_T	119	107	79	78	92	490	264	50	36	9	47	10	33	8	4	7	14	1279	168	1447
CD4_Tfh	109	123	92	26	112	90	88	105	44	12	19	45	115	86	19	62	99	745	501	1246
CD4_Treg	142	139	134	27	154	75	65	73	122	28	77	103	137	45	21	39	56	809	628	1437
Naive_CD8_T	385	402	485	109	310	227	185	438	51	27	91	80	19	14	13	10	39	2541	344	2885
GZMK_Cytotoxic_CD8_T	686	465	345	256	393	467	449	287	505	137	230	290	487	137	81	195	274	3348	2336	5684
GZMB_Cytotoxic_CD8_T	606	202	507	388	341	369	514	165	96	29	103	24	91	22	8	29	62	3092	464	3556
CD8_Tex	13	9	18	4	27	50	59	65	8	0	6	10	13	6	3	4	11	245	61	306
MAIT	514	88	412	48	63	150	147	118	34	114	167	38	19	18	6	46	18	1540	460	2000

gdT	2315	1137	307	278	245	285	398	394	144	58	151	402	122	50	18	37	43	5359	1025	6384
High_MT_T	184	153	173	152	233	181	219	102	166	70	108	160	212	48	20	365	84	1397	1233	2630
NKT	146	68	108	144	96	129	197	51	90	20	43	57	26	6	6	33	17	939	298	1237
CD16dim_NK	636	579	839	951	596	606	1096	306	79	32	54	218	191	131	12	52	27	5609	796	6405
CD16hi_NK	364	594	353	429	480	400	989	406	40	3	13	31	21	14	0	29	10	4015	161	4176
PPBP_T	220	311	504	163	571	373	212	102	10	1	3	10	12	1	0	5	4	2456	46	2502
																Total: 57239 (PBMCs: 45382; skin: 11857)				
B cells																				
	pbmc-HC016	pbmc-HC017	pbmc-HC020	pbmc-PS527	pbmc-PS602	pbmc-SS013	pbmc-SS523	pbmc-SS620	skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP	PBMC total	Skin total	Total
Naive_B	698	879	714	519	615	322	314	381	1	0	0	2	43	7	13	4	0	4442	70	4512
Memory_B	435	809	406	584	295	270	548	616	22	6	1	58	359	88	105	36	28	3963	703	4666
Plasma	4	11	18	2	17	7	37	27	36	2	1	4	206	4	39	25	13	123	330	453
PPBP_Naive_B	33	82	110	66	154	42	30	22	0	0	0	0	0	0	0	0	0	539	0	539
Mix (NKT)	127	25	395	22	152	77	188	45	8	3	3	10	45	2	4	8	12	1031	95	1126
																Total: 11296 (PBMCs: 10098; skin: 1198)				
VECs																				
									skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP		Skin total	
A									46	85	109	6	14	13	124	68	1		466	
C1									156	228	579	18	22	36	269	190	64		1562	
C2									79	70	350	4	21	32	205	98	0		859	
P1									240	321	474	1	4	2	393	109	12		1556	
P2									118	146	343	34	69	69	414	137	55		1385	
V									247	291	500	2	1	5	135	39	97		1317	
Profiler									2	1	1	0	3	12	4	0	2		25	
Mix_1 (LEC)									37	14	128	2	1	2	20	8	21		233	
Mix_2 (KC)									5	9	2	0	0	0	17	7	68		108	
Mix_3 (Imm)									32	15	17	2	3	1	10	7	8		95	
Mix_4 (Fib)									7	9	17	0	0	1	7	1	0		42	
																Total: 7648				
Fibroblasts																				
									skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP		Skin total	
FNDC1+ Fib									1251	2275	322	0	0	1	102	2	3		3956	
CRABP1+ Fib									279	346	611	28	57	26	442	2	1		1792	
APOC1+ Fib									61	127	20	18	54	20	113	20	0		433	
CLDN1+ Fib									5	43	2	7	7	1	28	12	1		106	

CXCL3+ Fib									0	0	0	0	24	34	0	0	0		58	
High-MT Fib									119	270	96	18	30	8	106	61	11		719	
Mix_1 (VEC)									33	104	26	0	0	0	16	1	6		186	
Mix_2 (T)									54	49	14	1	7	1	6	2	4		138	
Mix_3 (KC)									10	20	2	1	2	4	10	15	32		96	
Mix_4 (Myel)									5	14	5	0	3	1	8	0	9		45	
																Total: 7529				
KCs																				
									skin-HC23	skin-HC28	skin-HC35	skin-PS1229	skin-PS527	skin-PS602	skin-SS013	skin-SS523	skin-SS612 BP		Skin total	
Basal									14	11	2	0	0	0	776	68	718		1589	
Spinous									74	99	12	1	20	1	788	1751	8734		11480	
Supraspinous									26	39	2	0	5	0	728	280	3133		4213	
Granular									0	0	0	0	0	0	3	0	162		165	
Follicular									2	8	0	0	0	0	172	1092	2008		3282	
Mix_1 (Fib)									329	17	5	9	7	6	48	38	437		896	
Mix_2 (NKT)									2	0	0	0	10	1	4	206	67		290	
																Total: 21915				