

Immune repertoire profiling uncovers pervasive T-cell clonal expansions in benign prostatic hyperplasia

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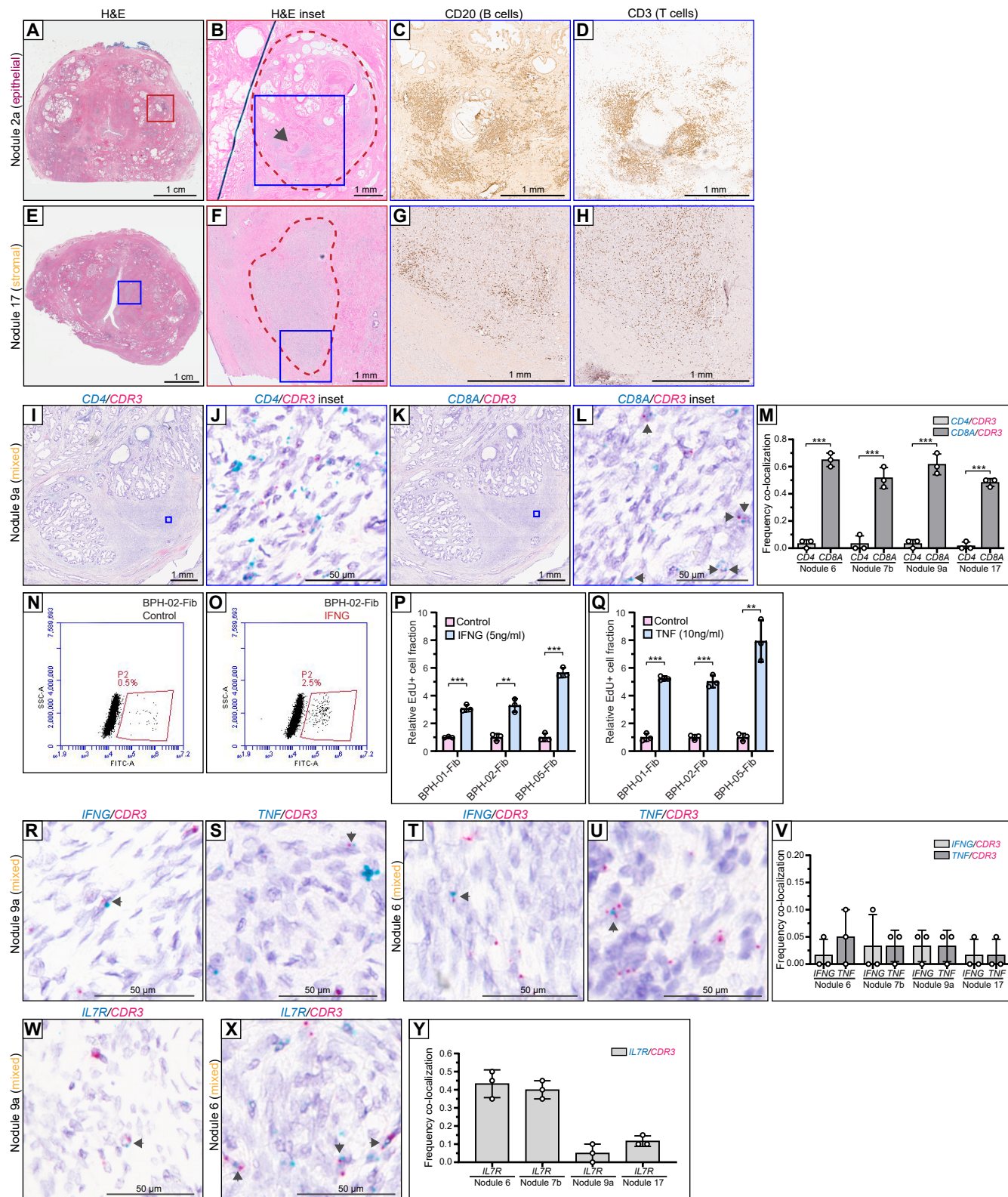
SUPPLEMENTAL TABLES, FIGURES, AND METHODS

Supplemental Table 1. Human BPH and blood specimens					
Patient ID	Samples ^a	Sex	Age (yrs)	Prostate size (cc) ^b	International Prostate Symptom Score (IPSS) ^c
1	1a (stromal), 1b (stromal), 1c (epithelial), 1d (PZ)	M	68	91	17
2	2a (epithelial), 2b (epithelial), 2c (PZ)	M	63	38	5
3	3a (epithelial), 3b (PZ)	M	69	19	17
4	4a (stromal), 4b (stromal-mixed), 4c (epithelial)	M	65	39	3
5	5 (epithelial)	M	69	60	16
6	6 (stromal-mixed)	M	57	41	2
7	7a (epithelial), 7b (stromal-mixed)	M	71	51	7
8	8 (stromal)	M	57	30	1
9	9a (stromal-mixed), 9b (epithelial), 9c (PZ)	M	61	107	na
10	10a (epithelial), 10b (PZ)	M	67	53	4
11	11a (stromal), 11b (stromal)	M	69	69	0
12	12 (stromal)	M	62	31	1
13	13a (stromal), 13b (epithelial)	M	66	45	1
14	14a (stromal), 14b (epithelial)	M	52	42	14
15	15 (stromal)	M	67	82	18
16	16 (stromal)	M	70	66	28
17	17 (stromal)	M	66	53	4
18	b1 (blood)	M	64	na	22
19	b2 (blood)	M	66	72	2
20	b3 (blood)	M	64	41	3
21	b4 (blood)	M	68	53	5
22	b5 (blood)	M	74	na	na
23	b6 (blood)	M	53	47	4
24	b7 (blood)	M	64	46	na
25	b8 (blood)	M	57	61	5
26	b9 (blood)	M	66	43	na
27	b10 (blood)	M	68	49	6
28	b11 (blood)	M	66	44	4
29	b12 (blood)	M	63	53	6
^a PZ, peripheral zone					
^b Determined from pre-op prostate MRI					
^c Mild (0-7); Moderate (8-19); Severe (20-35)					
na, not available					

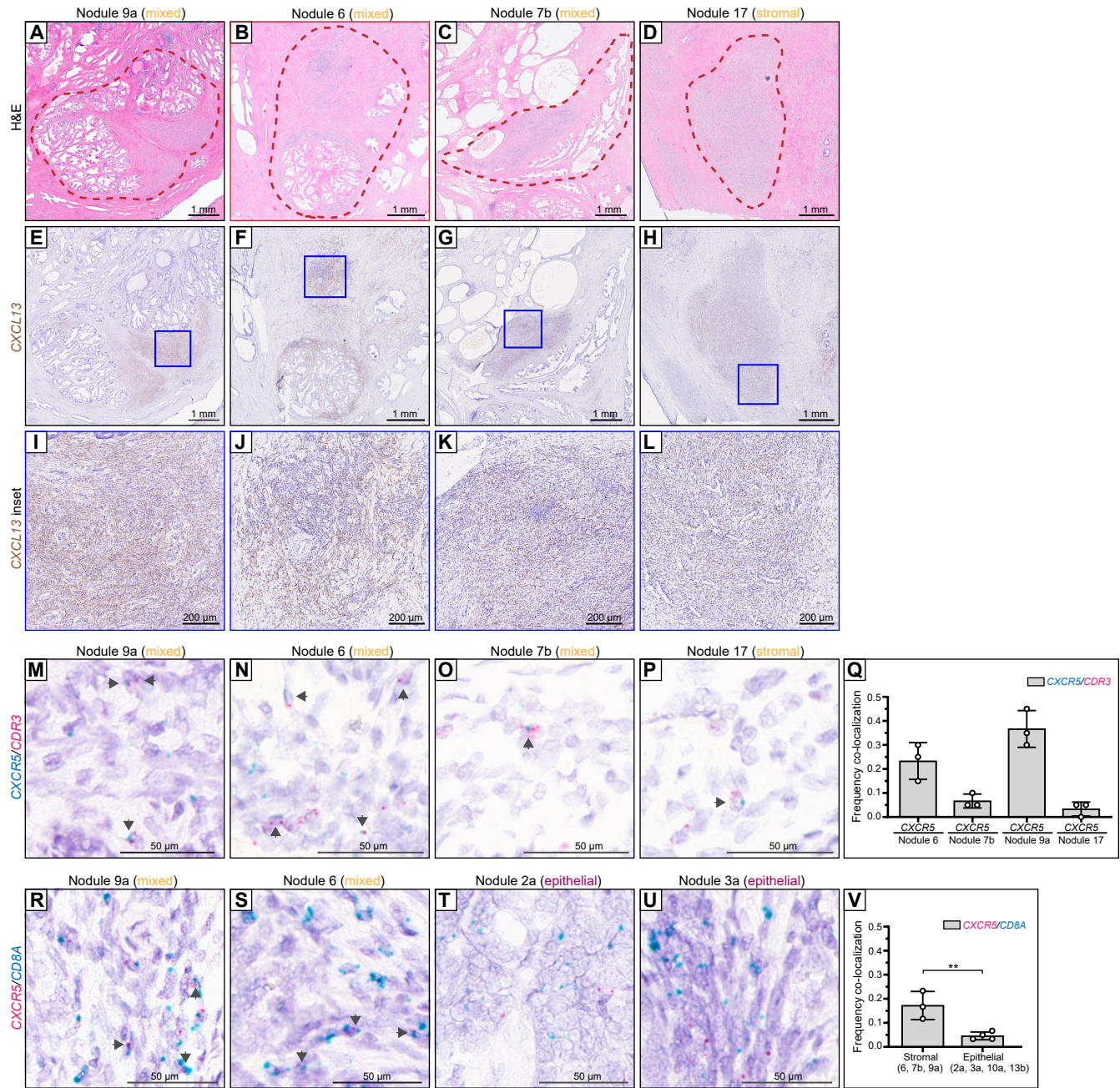
Supplemental Table 2. Top-10 TCR clonotype CDR3 sequences and their predicted antigen matches

Sample	Type	TRB V-gene	TRB J-gene	% Reads ^a	TRB CDR3 (nt)	TRB CDR3 (aa)	Predicted antigen match(es) ^b	CDR3 BaseScope
17	Stromal	Vb10-1	Jb2-2	33.894306	GCCAGCAGTTGGTTTCGCGGGGAGCTGTTT	ASSWFRGELF	0	X
17	Stromal	Vb7-8	Jb2-7	21.045582	GCCAGCAGCATGGGACAGGCTACGAGCAGTAC	ASSMQAYEQY	EBV, SARS-CoV-2, CMV	
11a	Stromal	Vb4-3	Jb1-6	19.107285	GCCAGCAGCCAGGGCTTACAGCACCCCTCCAC	ASSQGLQPLH	0	
11b	Stromal	Vb4-3	Jb1-6	19.002089	GCCAGCAGCCAGGGCTTACAGCACCCCTCCAC	ASSQGLQPLH	0	
7b	Mixed	Vb11-2	Jb1-2	15.623991	GCCAGCAGCTGGGGGGAGGGTCCCACTATGGCTACACC	ASSWGGGSHYGYT	EBV, InfA, CMV	X
1b	Stromal	Vb14	Jb1-2	14.901462	GCCAGCAGCCAAGATCGAAGGGGGGGCTACACC	ASSQDRRGYYT	0	
6	Mixed	Vb2	Jb1-5	14.765678	GCCAGCAGCGGATAGACAGGGTTGGGTATATAGCAATCAGCCCCAGCAT	ASSGLDRVGVSNQPOH	0	X
8	Stromal	Vb2	Jb1-1	12.437969	GCCAGCAAGGGGACAGGGAATGAACCTGAAGCTTTC	ASKGDREMNTEAF	0	
4a	Stromal	Vb28	Jb1-2	12.230172	GCCAGCAACACCTAGTACGGTTTATGGCTACACC	ASNTLGTVYGYT	0	
9a	Mixed	Vb4-3	Jb1-3	11.047346	GCCAGCAGCCCTGGGTCTAATCTCGAAACCATATAT	ASSPGNSGNITVY	0	X
4c	Epithelial	Vb28	Jb1-2	9.7343972	GCCAGCAACACCTAGTACGGTTTATGGCTACACC	ASNTLGTVYGYT	0	
16	Stromal	Vb14	Jb1-2	9.6046035	GCCAGCAGCCAAGATCCTGGAGGGGTTTACGGCTACACC	ASSQDPGGVYGYT	0	
8	Stromal	Vb25-1	Jb1-2	9.1920875	GCCAGCAGTTTACGGGTTTGGCTACACC	ASSSGFGYT	SARS-CoV-2, InfA, CMV	
17	Stromal	Vb28	Jb2-7	8.0353452	GCCAGCAGTCCCTTACGATTGAGAACGAGCAGTAC	ASSPLTIENEQY	0	
4b	Mixed	Vb28	Jb1-2	7.612171	GCCAGCAACACCTAGTACGGTTTATGGCTACACC	ASNTLGTVYGYT	0	
7a	Epithelial	Vb11-2	Jb1-2	7.4628112	GCCAGCAGCTGGGGGGAGGGTCCCACTATGGCTACACC	ASSWGGGSHYGYT	EBV, InfA, CMV	
1a	Stromal	Vb7-8	Jb1-2	7.2506456	GCCAGCAGCTTAGCGTCAAGTGGCGGGGGCTACACC	ASSLASSGRGYT	0	
1b	Stromal	Vb7-8	Jb1-2	6.1998324	GCCAGCAGCTTAGCGTCAAGTGGCGGGGGCTACACC	ASSLASSGRGYT	0	
12	Stromal	Vb14	Jb1-1	5.9936986	GCCAGCAGCCAGTGCACAGGATTGGAGCTTTC	ASSQSTRIGAF	0	
13a	Stromal	Vb2	Jb2-4	5.1453433	GCCAGCAGTTGCCTCTGGCAGTCAAAACATTCAGTAC	ASSPLAVKNQY	0	
15	Stromal	Vb7-2	Jb2-1	4.5744907	GCCAGCAGCTTAGGCTATAGCGGGAGTAGCCCTCAATGAGCAGTTC	ASSLGYSGSSPYNEQF	0	
13a	Stromal	Vb3-1	Jb2-1	3.961858	GCCAGCAGCCCGGACCAACCAATTCTACAATGAGCAGTTC	ASSPGPSQFYNEQF	0	
16	Stromal	Vb19	Jb2-7	3.6173567	GCCAGTAGTACCCGGGGAGGGCGGCACTACGAGCAGTAC	ASSTRGGAANYEQY	0	
4a	Stromal	Vb15	Jb1-1	3.4887263	GCCACCTATGCTACCCGGACAGGGGCACTGAAGCTTTC	ATYAYDRGTEAF	0	
9a	Mixed	Vb25-1	Jb1-4	3.354096	GCCAGCAGTGTACAGGGGAATGAAAACTGTTT	ASSVQGNELF	0	
17	Stromal	Vb7-8	Jb2-7	3.288665	GCCAGCAGCATGGGACAGGCTACGAGCAGTAC	ASSMQAYEQY	EBV, SARS-CoV-2, CMV	
4a	Stromal	Vb4-1	Jb2-1	3.1079718	GCCAGCATCGGGGGGCGCGGAGAAATGAGCAGTTC	ASIGGPGENEQF	0	
14a	Stromal	Vb11-2	Jb2-6	3.0449459	GCCAGCAGCTCCCTCTCTCCGGGGGACAGGGCTCTGGGGCCAAGCTCCTGACT	ASSLPPSGRGSANVLT	0	
1c	Epithelial	Vb14	Jb1-2	2.9738664	GCCAGCAGCCAAGATCGAAGGGGGGGCTACACC	ASSQDRRGYYT	0	
17	Stromal	Vb6-4	Jb1-1	2.5522157	GCCAGCAGGCCACAGGGCTGGAACACTGAAGCTTTC	ASRPQGWNTTEAF	0	
8	Stromal	Vb18	Jb2-2	2.5392783	GCCAGCTCAGCGTTACCGGAGGATAGCAATGAACCCGGGGAGCTGTTT	ASSPLPGYEMNTGELF	0	
13a	Stromal	Vb4-1	Jb2-7	2.5022261	GCCAGCAGCCAAGGGATTCCGAGGAGGCTCTACGAGCAGTAC	ASSQRDSAGASYEQY	0	
9a	Mixed	Vb5-1	Jb2-7	2.2273157	GCCAGCAGCTTGAAGGAGCAGGCGAGCTCTACGAGCAGTAC	ASSLEGQASSYEQY	InfA, CMV, EBV, MART-1, SARS-CoV-2	
2a	Epithelial	Vb13	Jb1-1	2.1936274	GCCAGCAGCCAAGGAGGACGGGACAGGGGGAGAGGACTGAAGCTTTC	ASSHRTGQERTEAF	0	
16	Stromal	Vb4-2	Jb2-1	2.1192116	GCCAGCAGCCAAGGAGGGGAGCTACGCTACAATGAGCAGTTC	ASSQEGGTIAYNEQF	0	
2b	Epithelial	Vb13	Jb1-1	1.949884	GCCAGCAGCCAAGGAGGACGGGACAGGGGGAGAGGACTGAAGCTTTC	ASSHRTGQERTEAF	0	
13a	Stromal	Vb4-3	Jb2-2	1.8935765	GCCAGCAGCCGACTAGGCGGACGGGGGAGCTGTTT	ASSPTSGTAGELF	0	
12	Stromal	Vb25-1	Jb2-7	1.8227244	GCCAGCAGTGAATCGGTTCTCTACGAGCAGTAC	ASSSEGSYEQY	SARS-CoV1, SARS-CoV-2	
9a	Mixed	Vb4-1	Jb1-2	1.779403	GCCAGCAGACCGACGGGGGCTATAGTCACGGCTACACC	ASRPRTGGYSHGYT	0	
13a	Stromal	Vb2	Jb2-1	1.7515583	GCCAGCAGTGAACCGGGGACTAGTACAATGAGCAGTTC	ASSPEPTSSNEQF	0	
9a	Mixed	Vb9	Jb2-2	1.702418	GCCAGCAGCGTAGGGGCTCCCGGGAGCTGTTT	ASSVGAPGELF	0	
12	Stromal	Vb13	Jb2-7	1.6824201	GCCAGCAGCCCGGGGACGGGCACTACGAGCAGTAC	ASTPQGNVEQY	0	
14a	Stromal	Vb10-3	Jb2-3	1.6662339	GCCATCAGTGAGTCCGACGGGGATACGAGTAT	AISEDGDTQY	0	
1a	Stromal	Vb14	Jb2-1	1.6253682	GCCAGCAGCCCCAGGGGACTAGTCTTTGGGAATGAGCAGTTC	ASSSPTGLVFNEQF	0	
14b	Epithelial	Vb10-3	Jb2-3	1.6094015	GCCATCAGTGAGTCCGACGGGGATACGAGTAT	AISEDGDTQY	0	
12	Stromal	Vb25-1	Jb1-1	1.5999606	GCCAGCAGTGATCCCGGACAGGGTTTGCCTGAAGCTTTC	ASSDPQQLPEAF	0	
12	Stromal	Vb2	Jb2-1	1.5864225	GCCAGCAGTGTGGTTCTCGGCAATGAGCAGTTC	ASSVLGNEQF	0	
16	Stromal	Vb2	Jb1-5	1.5834825	GCCAGCAGCCAGGACAGGACCGGCTTATAGCAATCAGCCCCAGCAT	ASSQDRDRLYSNQPOH	0	
13b	Epithelial	Vb3-1	Jb2-1	1.5605993	GCCAGCAGCCCCGACCAAGCAATTCTACAATGAGCAGTTC	ASSPGPSQFYNEQF	0	
1a	Stromal	Vb14	Jb1-2	1.5450071	GCCAGCAGCCAAGATCGAAGGGGGGGCTACACC	ASSQDRRGYYT	0	
1c	Epithelial	Vb7-8	Jb1-2	1.5196231	GCCAGCAGCTTAGCGTCAAGTGGCGGGGCTACACC	ASSLASSGRGYT	0	
7b	Mixed	Vb2	Jb1-5	1.5100597	GCCAGCAGTGAAGGCGCAGTAAACGACCAATCAGCCCCAGCAT	ASSGAVTSNQPOH	0	
11a	Stromal	Vb4-1	Jb2-2	1.5011213	GCCAGCAGCTATCGGCATCTACCGGGAGCTGTTT	ASSLASTGELF	EBV, Yellow fever virus, SARS-CoV-2	
12	Stromal	Vb14	Jb1-2	1.4744252	GCCAGCAGCCAACAGGGGGCGGCTACACC	ASSQTGGGYT	0	
14b	Epithelial	Vb11-2	Jb2-6	1.4321283	GCCAGCAGCTCCCTCTCTCCGGGGGACAGGGCTCTGGGGCCAAGCTCCTGACT	ASSLPPSGRGSANVLT	0	
4b	Mixed	Vb4-1	Jb2-1	1.4007116	GCCAGCATCGGGGGGCGCGGAGAAATGAGCAGTTC	ASIGGPGENEQF	0	
4a	Stromal	Vb5-1	Jb2-1	1.2773043	GCCAGCAGCTTAGAGGGGGACTCTACAATGAGCAGTTC	ASSLGGDSYNEQF	0	
1b	Stromal	Vb14	Jb2-1	1.2740879	GCCAGCAGCCCCACGGGACTAGTCTTTGGGAATGAGCAGTTC	ASSPTGLVFNEQF	0	
14a	Stromal	Vb9	Jb2-3	1.2609167	GCCAGCAGCGTCTGGGAGGGTTCGGGAGATACGAGTAT	ASSVVEGSDTQY	0	
17	Stromal	Vb10-1	Jb2-1	1.2573659	GCCAGCAACATCTTAGGGGGGAGGGGCTCTACAATGAGCAGTTC	ASSNLRGRGSYNEQF	0	
10a	Epithelial	Vb5-1	Jb2-7	1.2051173	GCCAGCAGCCCGACGGGTACTACGAGCAGTAC	ASSPDGYEQY	0	
1a	Stromal	Vb7-8	Jb1-2	1.1931032	GCCAGCAGCTTAGCGTCAAGTGGGCGGGGCTACACC	ASSLASSGRGYT	0	
10a	Epithelial	Vb2	Jb1-2	1.1902742	GCCAGCAGTCTTGACAGGACTACATGGGCTACACC	ASSPGQDPHYGYT	0	
17	Stromal	Vb14	Jb2-1	1.1647585	GCCAGCAGCCAATTCTCGAGGGGGCCGAGACCGTACGAGTTC	ASSQFARGDRREQF	0	
1b	Stromal	Vb18	Jb1-2	1.119382	GCCAGCTCAGCTCTCTTTATGGCTACACC	ASSPLLYGYT	0	
4c	Epithelial	Vb2	Jb1-2	1.0427369	GCCAGCAGTTACATTACAGGGGGCGCAATGGCTACACC	ASSYITGGNGYNT	0	
1b	Stromal	Vb12-4	none	1.0359004	GCCAGCAGACACAGGGGTCGGCTTTC	ASRAQGSF	0	
1b	Stromal	Vb12-4	Jb2-7	1.0130423	GCCAGCAGTTTTTGCCCCCGGTACGAGCAGTAC	ASSFAPRYEQY	0	
13a	Stromal	Vb14	Jb1-2	1.0110346	GCCAGCAGCGCCCGGACAGGGGGCGCATAGGCTATGGCTACACC	ASSARDRGRIGYGYT	0	
9a	Mixed	Vb14	Jb2-2	1.0025545	GCCAGCAGCCCTTGAAGGGGACACGGGGAGCTGTTT	ASSPLERDTGELF	0	
9b	Epithelial	Vb4-3	Jb1-3	0.9833776	GCCAGCAGCCCTGGGTCTAATCTCTGGAACACCATATAT	ASSPGNSGNITVY	0	
1b	Stromal	Vb7-8	Jb1-2	0.9736205	GCCAGCAGCTTAGCGTCAAGTGGGCGGGGCTACACC	ASSLASSGRGYT	0	
9b	Epithelial	Vb11-2	Jb2-2	0.9191228	GCCAGCAGGGGAGGAACCTCGAACACCGGGGAGCTGTTT	ASRRGTSNTGELF	0	
9b	Epithelial	Vb5-1	Jb1-2	0.9135534	GCCAGCAGCTTTAATTAGGACGGGGCTCTATGGCTACACC	ASSFNLGRGLYGYT	0	
4c	Epithelial	Vb14	Jb2-7	0.9086603	GCCAGCAGCCTTTTACCGACAGCCGAGCAGTAC	ASSLLPTAEQY	0	
4b	Mixed	Vb5-1	Jb2-1	0.8996532	GCCAGCAGCTTAGGAGGGGACTCTACAATGAGCAGTTC	ASSLGGDSYNEQF	0	
1a	Stromal	Vb12-4	Jb2-7	0.8807138	GCCAGCAGTTTTTGCCCCCGGTACGAGCAGTAC	ASSFAPRYEQY	0	
9b	Epithelial	Vb11-2	Jb1-2	0.8632491	GCCAGCAGCATCAGACAGGGGGGATGGCTACACC	ASSIQTGGNGYNT	0	
9b	Epithelial	Vb14	Jb2-2	0.8436933	GCCAGCAGCCAACAGGGGACGACACCGGGGAGCTGTTT	ASSPTGNTGELF	0	
2b	Epithelial	Vb15	Jb1-2	0.8399847	GCCACCAGCAGAGAGGAAGGGATGGCTACACC	ATSRREGDGYT	CMV	
6	Mixed	Vb4-3	Jb2-2	0.8149273	GCCAGCAGCCAAGGATACCCGAGCGGGGTACACGGGGAGCTGTTT	ASSQGIPIRAGYTGELF	0	
9b	Epithelial	Vb14	Jb1-1	0.8129627	GCCAGCAGCCAAGCTTGGGTTAGGGGCTGAAGCTTTC	ASSQAWVGAEAF	0	
1c	Epithelial	Vb18	Jb1-2	0.7992228	GCCAGCTCAGCTCTCTTTATGGCTACACC	ASSPLLYGYT	0	
5	Epithelial	Vb27	Jb1-2	0.7850234	GCCAGCAGTATAAGTGGAGCTAATGAGCTACACC	ASSISGANVYGYT	0	
7b	Mixed	Vb19	Jb1-5	0.7676875	GCCAGTAGTACCCGGGAGCTCAATCAGCCCCAGCAT	ASSTGDSNQPOH	CMV, EBV, SARS-CoV-2	
2a	Epithelial	Vb9	Jb1-1	0.7028538	GCCAGCAGCTCCGACAGGGGAAAGCTTTC	ASTSRQKAF	0	
7b	Mixed	Vb30	Jb2-1	0.6860456	CCTTGAGCCCCACTGTAAAGCAATGAGCAGTTC	AWSPVTSNEQF	0	
1c	Epithelial	Vb12-4	none	0.6745734	GCCAGCAGACGACAGGGGTCGGCTTTC	ASRAQGSF	0	
10a	Epithelial	Vb15	Jb1-2	0.6728866	GCCACAGTTATGGCAGGGGGCGCTATGGCTACACC	ATSYRGAYGYT	0	
10a	Epithelial	Vb2	Jb1-2	0.6516822	GCCAGCAGTGGACAGGGCTTAAAGATGGCTACACC	ASSGQAVKDYGT	0	
14b	Epithelial	Vb30	none	0.6383826	GCCTGGAGGGGAACTTCCAC	AWRGNFT	0	
9a	Mixed	Vb14	Jb2-7	0.6071316	GCCAGCAGCCAGGACTTACTACGAGCAGTAC	ASSQDFTYEQY	CMV	
7a	Epithelial	Vb14	Jb1-6	0.5994748	GCCAGCAGCCAGTTACTATCACCCCTCCAC	ASSQLLSPLH	0	
13b	Epithelial	Vb28	Jb1-5	0.5843586	GCCAGCAGAGTTTCGGGGGAGGGGAATCAGCCCCAGCAT	ASRVRGKGNQPOH	0	

10a	Epithelial	Vb7-9	Jb2-2	0.5817077	GCCAGCAGCTCGCTACCGGGGAGCTGTTT	ASSSPTGELF	EBV	
7b	Mixed	Vb14	Jb2-7	0.5474441	GCCAGCAGCGAAACCGGGGAGGGCGGAGCAGTAC	ASSETGGRGGEQY	0	
13b	Epithelial	Vb14	Jb1-2	0.5435536	GCCAGCAGCGCCCGGACAGGGGGCGCATAGGCTATGGCTACACC	ASSARDRGRIGYGT	0	
13b	Epithelial	Vb2	Jb2-4	0.5289254	GCCAGCAGTTTCGCTCTGGCAGTCAAAACATTACGTAC	ASSSPLAVKNIQY	0	
4c	Epithelial	Vb28	Jb2-6	0.5268335	GCCAGCAGTTTATTTCTGGGGAGTCTCCGCTCTGGGGCCAACGTCCTGACT	ASSLFRGSLRSGANVLT	0	
7b	Mixed	Vb12-5	Jb1-1	0.5221288	GCTAGTGCCCTGGGAATGGGGAACACTGAAGCTTTC	ASALGMGNTEAF	0	
7a	Epithelial	Vb14	Jb1-6	0.5215245	GCCAGCAGCCGACACGATTACTCTATAATTACCCCTCCAC	ASSRHDYSYNSPLH	0	
13b	Epithelial	Vb15	Jb2-2	0.5196865	GCCACCAGCCGACTAGCGGGAATACGAACACCGGGGAGCTGTTT	ATSPTSNTNTGELF	0	
2a	Epithelial	Vb18	Jb2-2	0.5169401	GCCAGCTCATATCGACGGGACACGGGGAGCTGTTT	ASSYRRDTGELF	0	
14b	Epithelial	Vb4-3	Jb2-1	0.4969625	GCCAGCAGCCCTCTAGCGATTTCTACAATGAGCAGTTC	ASSPLAISYNEQF	SARS-CoV-2	
15	Stromal	Vb14	Jb2-1	0.4873967	GCCAGCAGCCAAGATATGGGGCCACCTCAGAATGAGCAGTTC	ASSQDMGPPQNEQF	0	
1c	Epithelial	Vb18	Jb1-2	0.4772118	GCCAGCTCAGCGCAGGAGGGGGGCTACACC	ASSRQEGGYT	0	
6	Mixed	Vb18	Jb1-5	0.4654113	GCCAGCTCACCACTCCCTACGGGACAGGGGGCGAGATTAGGTCAGCCCCAGCAT	ASSPLPTGGGARLGQPQH	0	
13b	Epithelial	Vb5-1	Jb1-6	0.4650232	GCCAGCAGCTCAGGGGGTACTCTATAATTACCCCTCCAC	ASSSGGYSYNSPLH	0	
4c	Epithelial	Vb4-1	Jb2-1	0.4583379	GCCAGCATCGGGGGGCCCGGAGAAATGAGCAGTTC	ASIGGPGENEQF	0	
2a	Epithelial	Vb6-1	Jb1-2	0.4361081	GCCAGCAGCTTACAGGTTAACTATGGCTACACC	ASSLQVNYGYT	SARS-CoV-2	
10a	Epithelial	Vb10-3	Jb1-1	0.4353972	GCCATCAGTGATTCGACAGGGAATTCGGTGAGGGAGCTTTC	AISDSTGNSGEGAF	0	
6	Mixed	Vb12-4	Jb1-5	0.4221031	GCCAGCGCCCGAGGTGGAAATCAGCCCCAGCAT	ASARGGNQPQH	0	
15	Stromal	Vb7-2	Jb2-1	0.4057213	GCCAGCAGCTTAGGCTATAGCGGGAGTAGCCCTACAATGAGCAGTTC	ASSLGYSGSSPYNEQF	0	
3a	Epithelial	Vb11-2	Jb1-6	0.3921512	GCCAGCAGCCCTGGACAAGGGAATTACCCCTCCAC	ASSPGQGN SPLH	CMV, SARS-CoV-2	
2b	Epithelial	Vb9	Jb1-1	0.3895908	GCCAGCACCTCCCGCAGGGGAAAGCTTTC	ASTSRQ GKAF	0	
3a	Epithelial	Vb3-1	Jb1-2	0.3867247	GCCAGCAGCCAAGAATTTGGCTACACC	ASSQEFGYT	0	
7a	Epithelial	Vb6-4	Jb2-7	0.3749037	GCCAGCAGTGACTCAGCGGGAGACAGGGGAACCTCTACGAGCAGTAC	ASSDSRGRQNSYEQY	0	
7a	Epithelial	Vb14	Jb1-2	0.36284	GCCAGCAGCCAAGTCTTAGGGACACCGGCTACACC	ASSQVLGTSGYT	0	
3a	Epithelial	Vb15	Jb1-1	0.3400573	GCCACGAGACAGGGAGAAGACTTGATCACTGAAGCTTTC	ATRQGEDLITEAF	0	
2b	Epithelial	Vb18	Jb2-7	0.3303961	GCCAGCTCAGGTCCGGACAGGGGGTCTACGAGCAGTAC	ASSRSGQGVYEQY	0	
2b	Epithelial	Vb6-1	Jb1-2	0.3178494	GCCAGCAGCTTACAGGTTAACTATGGCTACACC	ASSLQVNYGYT	SARS-CoV-2	
5	Epithelial	Vb12-4	Jb1-5	0.2106007	GCCAGCACCAACGACGGGGCAGGTAGGAGCTAGCCCCAGCAT	ASTTTGQVGAQPQH	0	
5	Epithelial	Vb7-8	Jb2-7	0.1891623	GCCAGCAGCATCGGACAGGCCCTACGAGCAGTAC	ASSIGQAYEQY	EBV	
^a Ranked by % reads								
^b Predicted matches from TCRmatch (IEDB database)								



Supplemental Figure 1. Clonally-expanded T cells in BPH stromal-rich nodules are CD8+ (cytotoxic) T cells. (A-H) Representative BPH (A-D) epithelial nodule (#2a) and (E-H) stromal nodule (#17) showing (A, E) H&E-stained transverse section through prostate with red square marking transition zone location of selected nodule; (B, F) Magnified view with nodule border (and region microdissected for DNA) demarcated by dashed red line. Blue square indicates further-magnified region shown for IHC (brown-staining) of (C, G) CD20 (B cells) and (D, H) CD3 (T cells). (I-L) Two-color RISH of representative nodule (#9a, mixed) shows expression of (I-J) CD4 (blue) and (K-L) CD8A (blue) versus TCR clonotype-specific CDR3 sequence (red); arrows identify cells co-expressing CD8A and CDR3. (M) Bar graph quantifying co-localization frequency of CD4/CDR3 and CD8A/CDR3 in 4 different stromal/mixed nodules. Mean $\pm$ 1 SD shown. ***, P-value < 0.001 (2-sided Student's t-test). (N-O) Representative flow cytometry dot plots of (N) control and (O) IFNG-induced cell proliferation of quiescent primary BPH fibroblasts (BPH-02-Fib), assayed by EdU incorporation (FITC channel). (P, Q) Bar graphs quantifying cell proliferation induced by (P) IFNG and (Q) TNF across 3 different quiescent primary BPH fibroblast samples. Mean $\pm$ 1 SD shown. **, P-value < 0.01 ***, P-value < 0.001 (2-sided Student's t-test). Each experiment was conducted twice. (R-U) Two-color RISH of representative nodules (#9a and #6, mixed) shows expression of (R, T) IFNG (blue) and (S, U) TNF (blue) versus TCR clonotype-specific CDR3 sequence (red); arrows identify cells co-expressing IFN or TNF together with CDR3. (V) Bar graph quantifying co-localization frequency of IFNG/CDR3 and TNF/CDR3 in 4 different stromal/mixed nodules. Mean $\pm$ 1 SD shown. (W-X) Two-color RISH of representative nodules (#9a and #6, mixed) shows expression of IL7R (blue) versus TCR clonotype-specific CDR3 sequence (red); arrows identify cells co-expressing IL7R and CDR3. (Y) Bar graph quantifying co-localization frequency of IL7R/CDR3 in 4 different stromal/mixed nodules. Mean $\pm$ 1 SD shown.



Supplemental Figure 2. Clonally-expanded T cells in BPH stromal-rich nodules express CXCL13 receptor, CXCR5. (A-L) RISH assays of *CXCL13* expression (brown staining) for four representative stromal/mixed nodules. (A-D) H&E stain with the nodule border (and region microdissected for DNA) outlined by a dashed red line. (E-H) *CXCL13* RISH assays and (I-L) corresponding insets with magnified view. Note, the H&E images shown here in panels A and D are reproduced from Figure 1B and Supplemental Figure 1F. (M-P) Two-color RISH of the above nodules shows expression of *CXCR5* (blue) versus TCR clonotype-specific *CDR3* sequence (red); arrows identify cells co-expressing *CXCR5* and *CDR3*. (Q) Bar graph quantifying co-localization frequency of *CXCR5/CDR3* in the 4 stromal/mixed nodules. Mean $\pm$ 1 SD shown. (R-U) Two-color RISH of representative (R-S) stromal/mixed (9a and 6, mixed) and (T-U) epithelial (2a, 3a) nodules shows expression of *CXCR5* (blue) versus *CD8A* (red); arrows identify cells co-expressing *CXCR5* and *CD8A*. (V) Bar graph quantifying co-localization frequency of *CXCR5/CD8A* in 3 stromal/mixed versus 4 epithelial nodules. Mean $\pm$ 1 SD shown. **, P-value < 0.01 (2-sided Student's t-test).

SUPPLEMENTAL METHODS

Sex as a biological variable

Our study examined male subjects only, because BPH is a disease exclusive to men.

Patient samples

All human specimens were obtained with Institutional Review Board approval and patient informed consent. Human prostate specimens were from radical prostatectomies done for prostate cancer treatment at the Stanford Hospital between 2017 and 2021. Study samples were selected from whole-mount cases where benign prostatic hyperplasia (BPH) transition zone (TZ) was distant from prostate cancer, and where BPH nodules – stromal, epithelial, or mixed (i.e., stromal nodules with substantial epithelial component) – contained microscopically-evident lymphocytes. The proportion of lymphocytes that were T cells ranged from approximately 40-60% (by immunohistochemistry). In a subset of representative cases, regions of lymphocyte-rich, normal prostate peripheral zone (PZ) were also sampled. Human blood specimens, matched for patient age and prostate size, were collected pre-operatively from patients undergoing radical prostatectomy done for prostate cancer treatment during 2023. Patient age, prostate size, and International Prostate Symptom Score (IPSS) are listed in Supplemental Table 1. Human primary BPH stromal fibroblast cultures (1, 2) were generated from surplus prostate tissue of cysto-prostatectomy cases (done for bladder cancer). Briefly, fresh BPH TZ tissue (with evident BPH nodules) was minced, then tissue disaggregated to single cells by sequential treatment with collagenase/hyaluronidase, trypsin, and dispase, following STEMCELL Technology's Prostate Tissue Dissociation protocol (3). Filtered single cells were then plated and passaged in a fibroblast-selective media, complete Stromal Cell Growth Media (Lonza), supplemented with

10nM metribolone (synthetic androgen) (Perkin Elmer). By the first passage, all cultured cells exhibited classic fibroblast spindle and stellate morphology.

BPH nodule microdissection and DNA isolation

BPH nodules were microdissected using the Pinpoint Slide DNA Isolation System (Zymo Research). Briefly, a dissecting microscope assisted the demarcation of individual BPH nodules on 10µM FFPE sections (guided by flanking H&E-stained slides). Pinpoint solution (a tissue adhesive) was applied by pipette tip to 10-15 serial 10µM sections, then the tissue detached and genomic DNA extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen), and quantified by NanoDrop One. For blood samples, genomic DNA was isolated from buffy coats using the DNeasy Blood & Tissue Kit (Qiagen).

Immune Repertoire Profiling

Each sample containing 100 ng of genomic DNA underwent lymphocyte repertoire analysis using the LymphoTrack *IGH* (FR1/FR2/FR3) and *TRB* Assays (Invivoscribe) in a CLIA-certified molecular pathology laboratory (4). Barcoded gene-specific PCR primers were utilized to amplify the framework 2 of *IGH* and *TRB* repertoires flanking the BCR and TCR VDJ segments followed by deep-sequenced on a MiSeq (Illumina). Data processing, including de-multiplexing, read mapping (V(D)J gene assignment), CDR3 identification, and clonotype frequency (percent total reads) determination, was performed using LymphoTrack v2.4.3 software (Invivoscribe). BPH nodules were sequenced to an average read count of 90K (*IGH* FR2) and 130K (*TRB*). BPH nodule immune repertoires were estimated on average to represent approximately 3,800 different B-cell clonotypes and 1,900 different T-cell clonotypes, as determined by unique CDR3

sequences. Clonality/oligoclonality was operationally defined as one or more clonotype with $\geq 5\%$ frequency. The Shannon equitability index (5) was calculated as described (4). TRB CDR3 epitope/antigen matches were predicted from TCRmatch (6) using the IEDB database (of infectious disease, allergy, autoimmunity, and transplantation antigens) (7) with default settings.

RNA in situ Hybridization and Immunohistochemistry

RNA in situ Hybridization (RISH) was done using manual BaseScope Duplex Assay (*CD4*, *CD8A*, *CXCR5*, *IL7R*, and *CDR3* regions), and RNAscope 2.5 HD Assay-Brown (*CXCL13*) according to the manufacturer (ACDBio). Chromogenic probes were Hs-CD4-C1, Hs-CD8A-C1, Hs-CXCR5-C1, Hs-CXCR5-C2, Hs-IL7R-C1, Hs-CXCL13-C1, and custom-designed TCR clonotype-specific CDR3(-C2) probes (Supplemental Table 2). Slides were scanned on an Aperio AT2 scanner. Co-localization was scored as red and blue signal spots either abutting or else clearly originating from the same cell. Co-localization was quantified by counting the number of co-localized signals per 20 *CDR3*⁺ cells, in each of three representative high-power (40X) fields. Immunohistochemistry was done using Tris-EDTA antigen retrieval, with ImmPRESS HRP Detection Kit (Vector Laboratories). Primary antibodies were CD20 (L26, 1:400) (Sigma), and CD3 (MRQ-39, 1:800) (Sigma).

Prostate cell culture experiments

Early-passage human primary BPH stromal fibroblasts were plated in 6-well plate wells (100K cells/well) in complete Stromal Cell Growth Media (SCGM; Lonza) (containing 5% FBS). The following day, the media was exchanged for low-serum SCGM (0-0.5% FBS) for 48 hrs to drive replicative quiescence (verified as $< 1\%$ EdU incorporation). Cells were then treated for 48 hrs

with physiological concentrations of recombinant human IFNG (PeproTech) (5ng/ml) or recombinant human TNF (10ng/ml) (PeproTech). DNA synthesis was then assayed by EdU incorporation using the Click-iT Plus EdU Flow Cytometry Assay Kit (Life Technologies). For each well, 10,000 cells were analyzed on an Accuri C6 flow cytometer (BD Biosciences). Each condition was assayed in biological triplicate, and each experiment was done twice.

Data availability

Raw data (Fastq files) for BCR and TCR repertoire analysis are available at the NCBI Sequence Read Archive (SRA) (accession PRJNA1218273). Supporting data values associated with the manuscript figures are available as supplemental material, in the Excel file “Supporting_data_values”.

Statistics

Two-sided Student’s t-test and one-way ANOVA (with Tukey’s multiple comparisons test) were done to assess statistical significance, where $P < 0.05$ was considered significant. Graphs and charts were created using GraphPad Prism 10.0.

Study approval

The study was approved by the Stanford University Institutional Review Board (IRB #30642). Written informed consent was received from participants prior to including in the study.

AUTHOR CONTRIBUTIONS

ASP, CAK, BMZ, RBW, JDB and JRP conceived and planned the studies; ASP, JC, CH, RLPG, and JRP performed experiments; ASP, CH, AJP, BMZ and JRP analyzed data; ASP and JRP wrote the manuscript.

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