

Figure 1

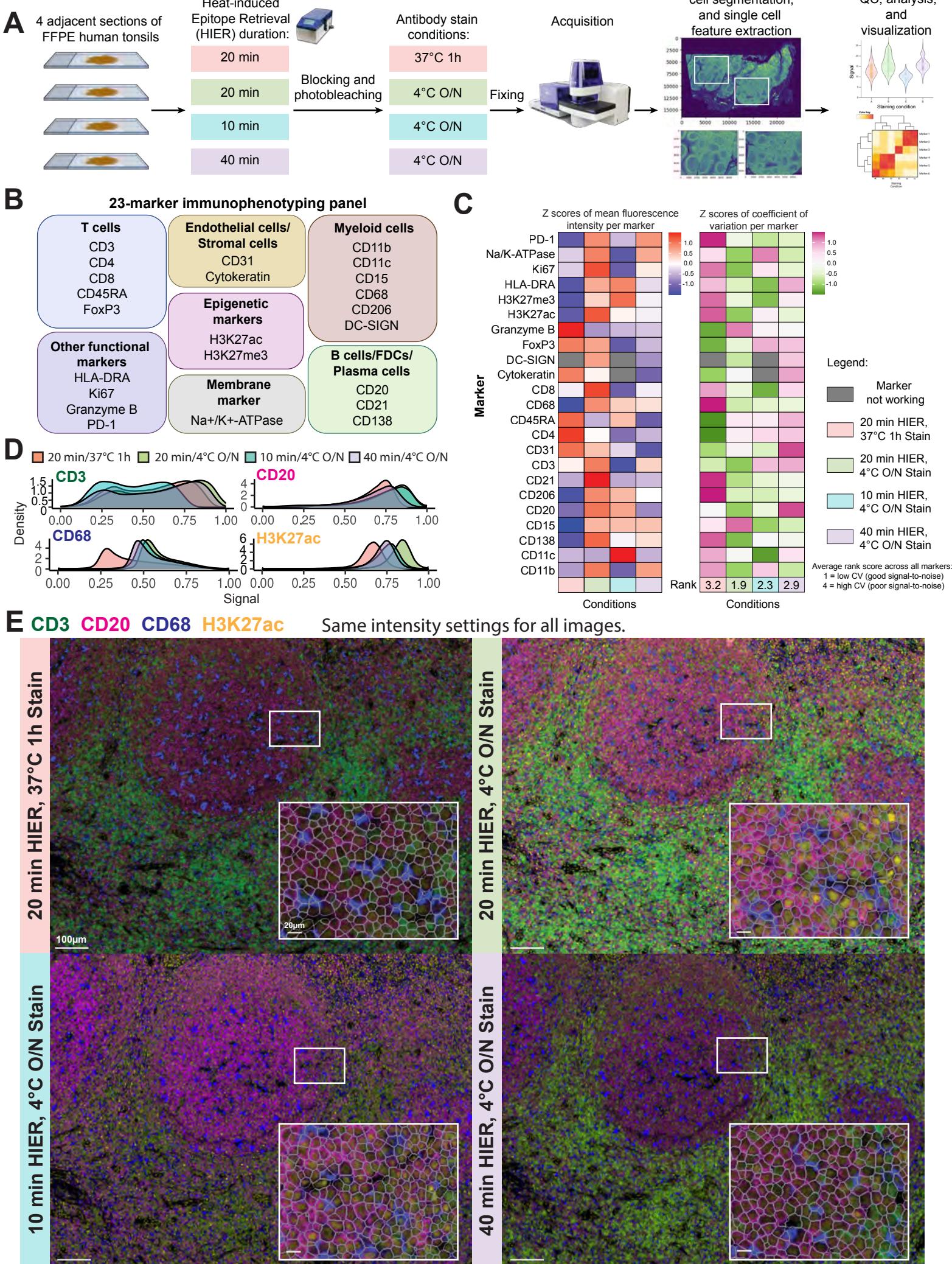
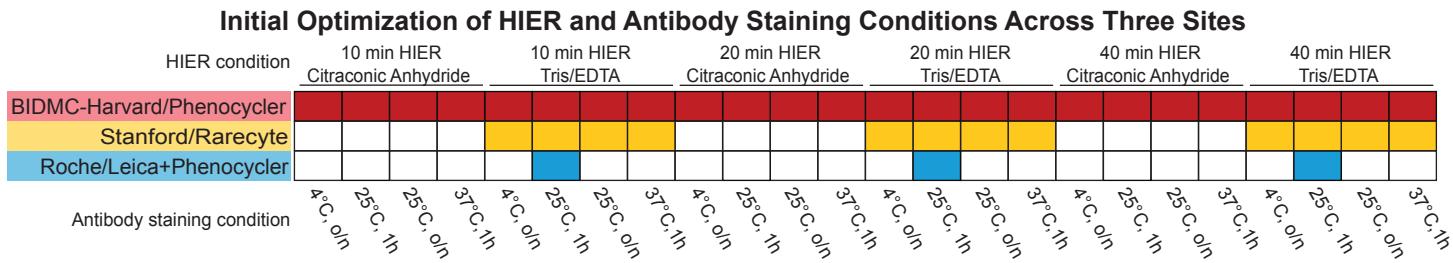


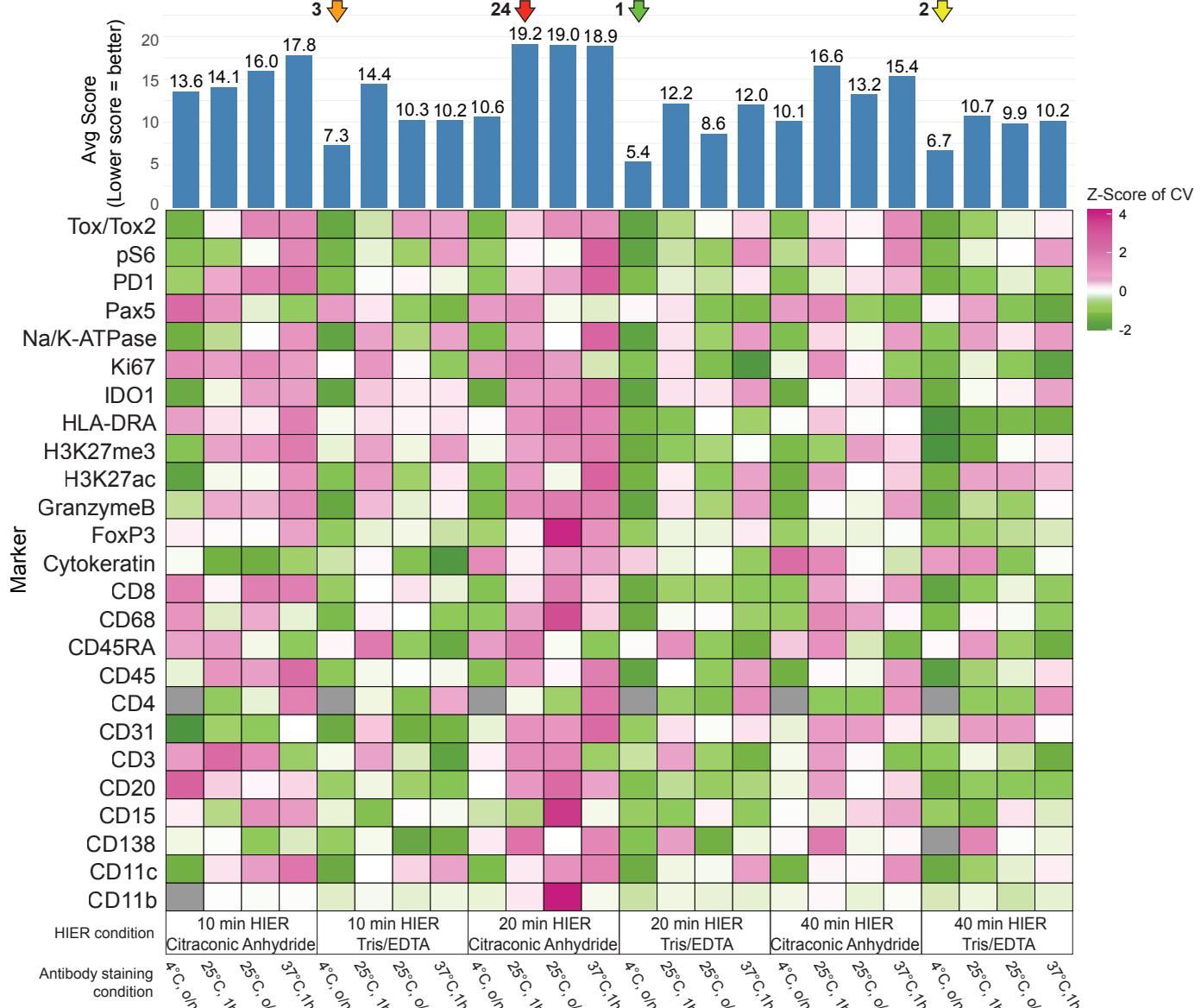
Figure 2

A



B

Comparison of the Coefficient of Variation of Markers across the 24 conditions tested at BIDMC-Harvard



C

Conditions ranked from best to worst

Rank	HIER duration	HIER buffer	Ab Staining	Avg Score
1	20 min	Tris/EDTA	4°C, O/N	5.36
2	40 min	Tris/EDTA	4°C, O/N	6.72
3	10 min	Tris/EDTA	4°C, O/N	7.28
4	20 min	Tris/EDTA	25°C, O/N	8.64
5	40 min	Tris/EDTA	25°C, O/N	9.88
6	40 min	CA	4°C, O/N	10.1
7	40 min	Tris/EDTA	37°C, 1h	10.2
8	10 min	Tris/EDTA	37°C, 1h	10.2
9	10 min	Tris/EDTA	25°C, O/N	10.3
10	20 min	CA	4°C, O/N	10.6
11	40 min	Tris/EDTA	25°C, 1h	10.7
12	20 min	Tris/EDTA	37°C, 1h	12.0
13	20 min	Tris/EDTA	25°C, 1h	12.2
14	40 min	CA	25°C, O/N	13.2
15	10 min	CA	4°C, O/N	13.6
16	10 min	CA	25°C, 1h	14.1
17	10 min	Tris/EDTA	25°C, 1h	14.4
18	40 min	CA	37°C, 1h	15.4
19	10 min	CA	25°C, O/N	16.0
20	40 min	CA	25°C, 1h	16.6
21	10 min	CA	37°C, 1h	17.8
22	20 min	CA	37°C, 1h	18.9
23	20 min	CA	25°C, O/N	19.0
24	20 min	CA	25°C, 1h	19.2

D Condition

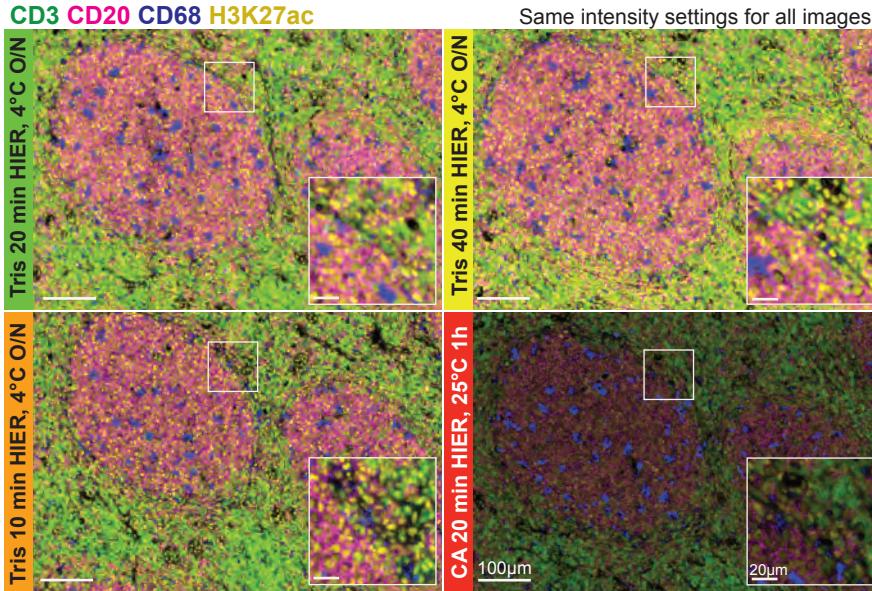
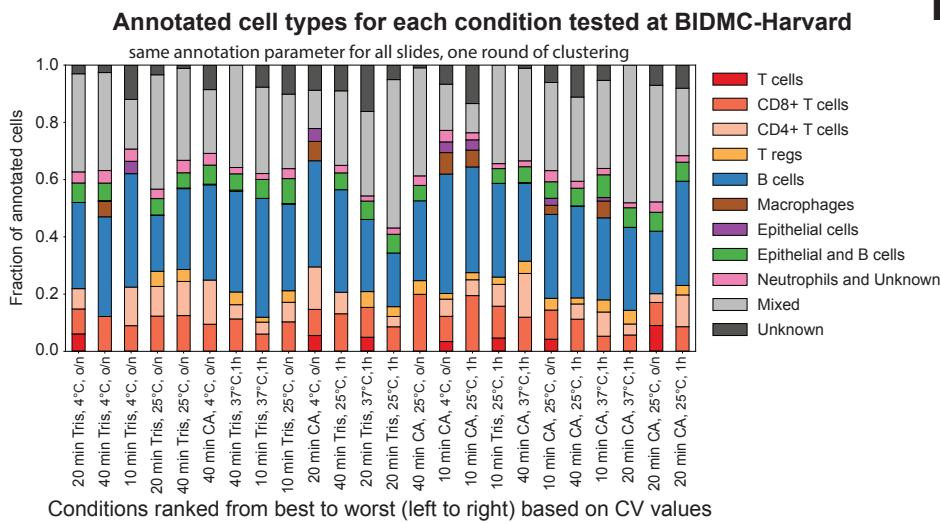


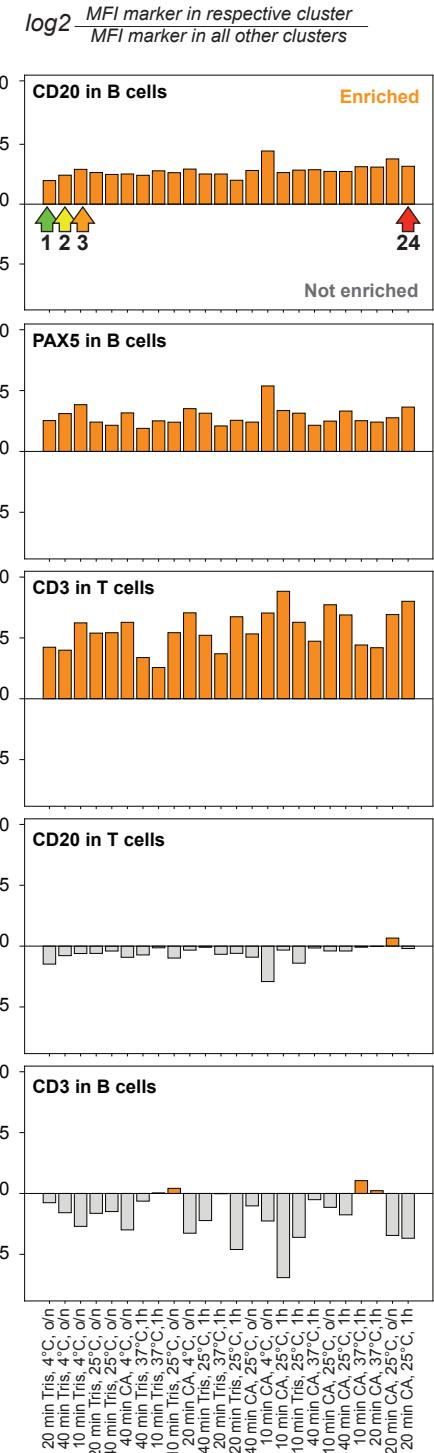
Figure 3

A

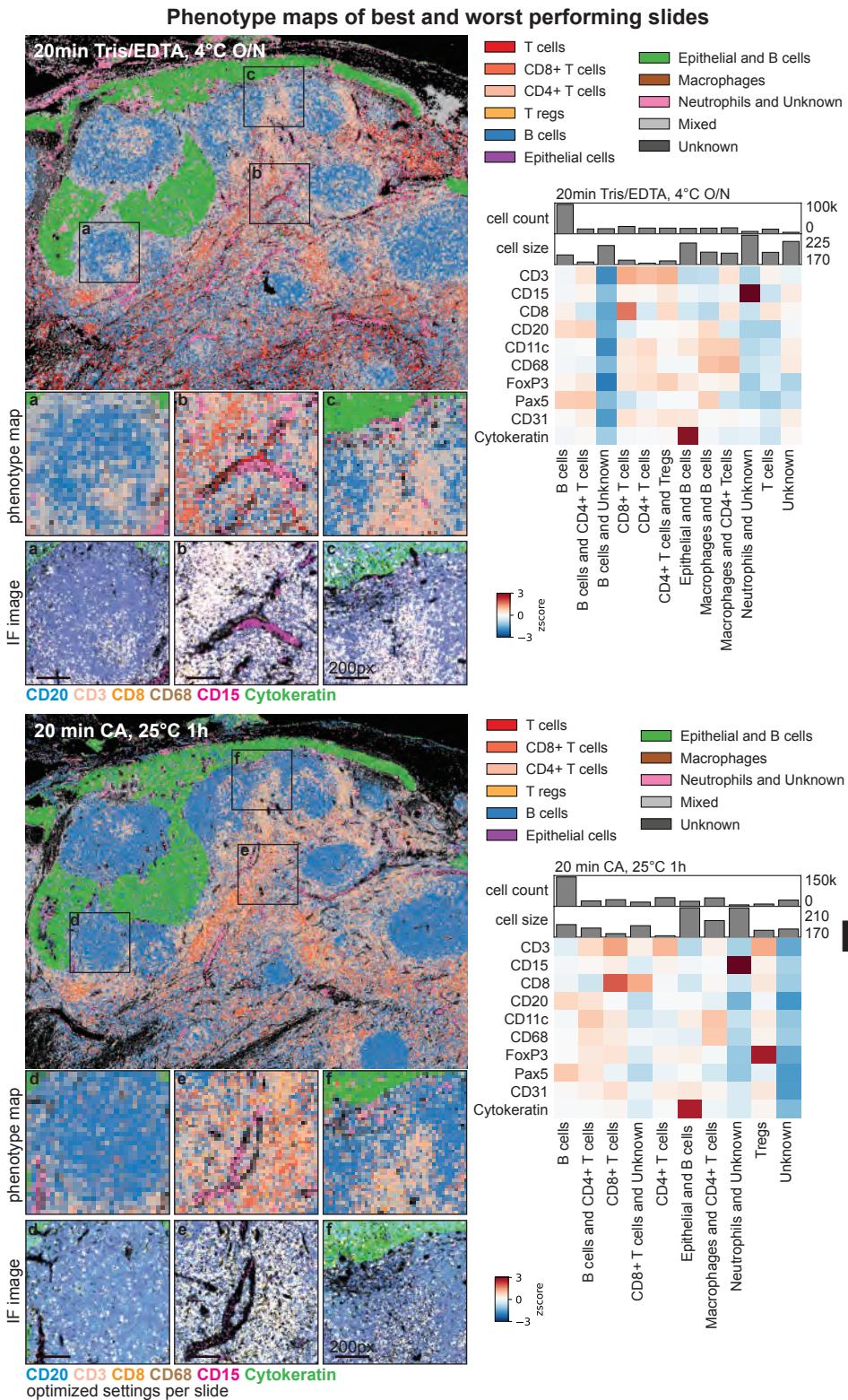


B

Enrichment of marker expression in recovered cell type clusters



C



D

Correlation: CV score vs. Alpha

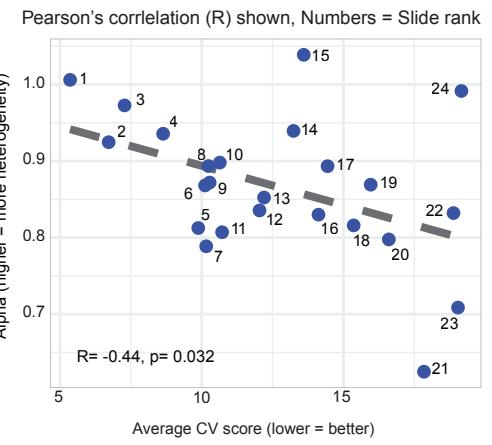


Figure 4

A

Top Three Conditions	Tris 20 min HIER, 4°C O/N Stain (1)
	Tris 40 min HIER, 4°C O/N Stain (2)
	Tris 10 min HIER, 4°C O/N Stain (3)
Worst Condition	CA 20 min HIER, 25°C 1h Stain (24)

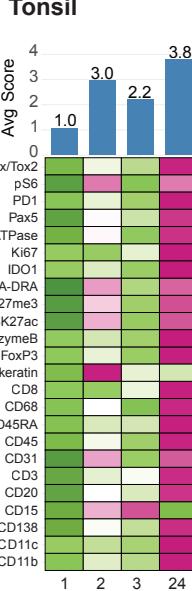
Validation

Site	Lymphoid Tissue	Secondary Tissue	Platform
BIDMC-Harvard	Tonsil	Diffuse large B-cell lymphoma	
Novartis	Tonsil	Lung Cancer	
Roche	Spleen	Intestine	
Stanford University	Lymph Node	Endometrial Cancer	
Stanford University	Tonsil	Oral squamous cell carcinoma	
Stanford University	Lymph Node	Colon and Liver	
A*STAR	Tonsil	Colorectal Cancer	
University of Kentucky	Tonsil	Squamous cell carcinoma of tonsil	

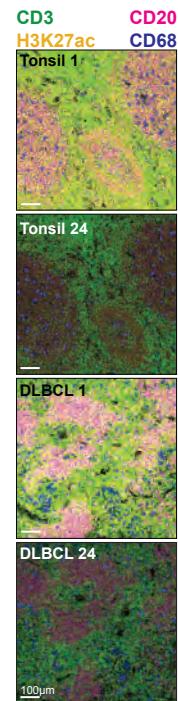
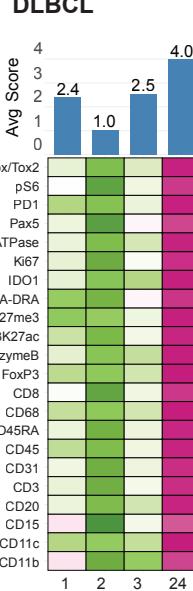
B

Validation at BIDMC-Harvard

Tonsil



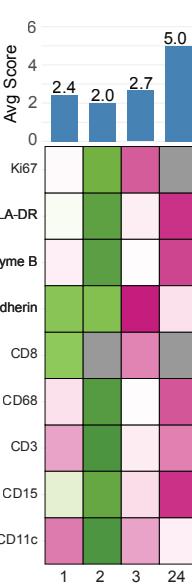
DLBCL



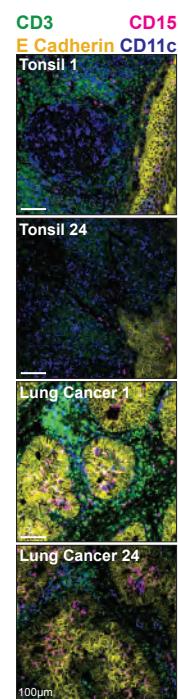
D

Validation at Novartis

Tonsil



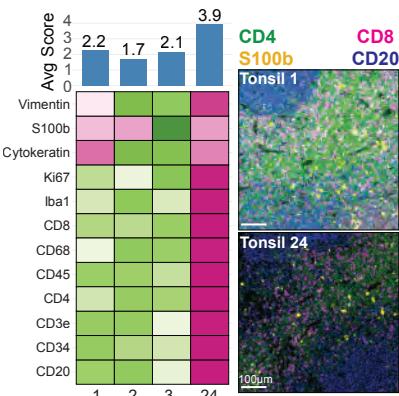
Lung Cancer



C

Validation at Roche

Tonsil



Intestine

