

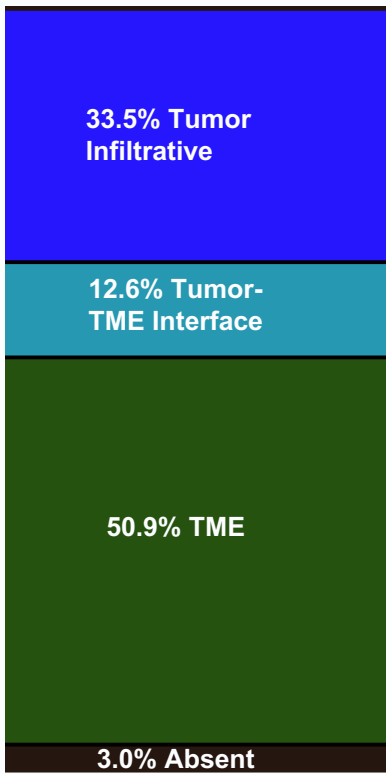
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Supplemental information

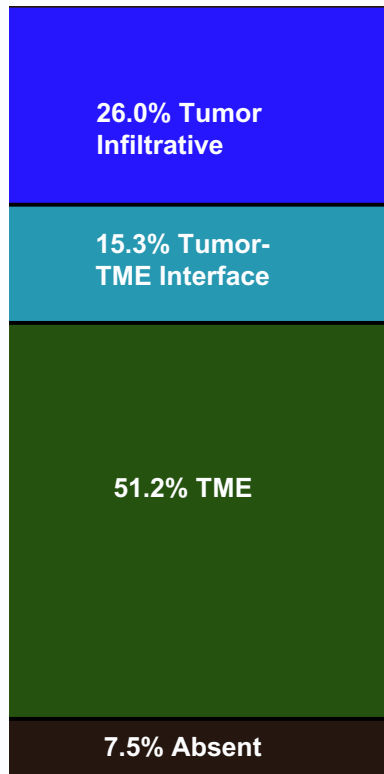
**Derivation of CD8⁺ T cell infiltration
potentiators in non-small-cell lung cancer
through tumor microenvironment analysis**

Michael J. Topper, Valsamo Anagnostou, Kristen A. Marrone, Victor E. Velculescu, Peter A. Jones, Julie R. Brahmer, Stephen B. Baylin, and Galen H. Hostetter

A.



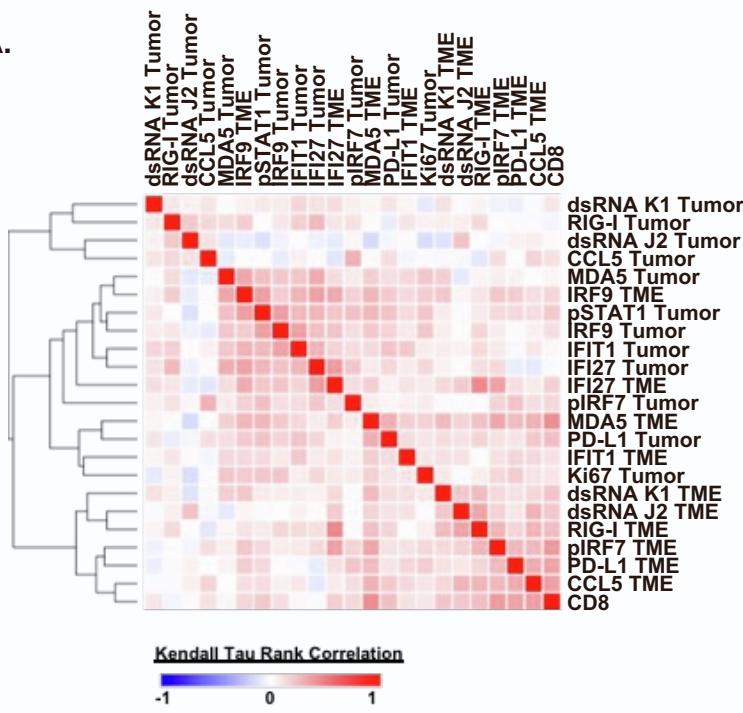
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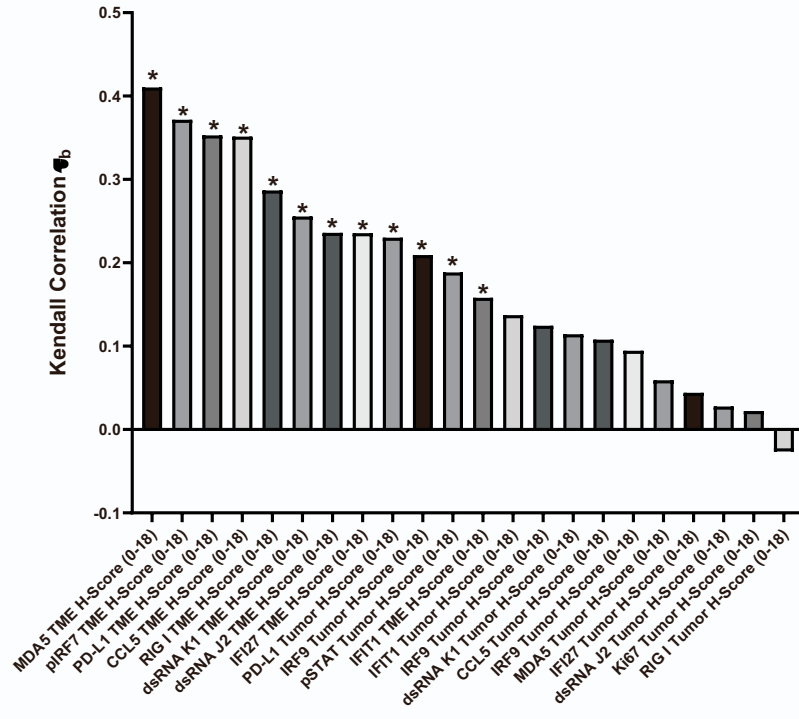
Supplemental Figure 1: CD8⁺ T cell localization status across LUAD and LUSC TMAs, Related to Figure 3.

A) CD8⁺ T cell infiltration status LUAD (n=128). **B)** CD8⁺ T cell infiltration status LUSC (n=92).

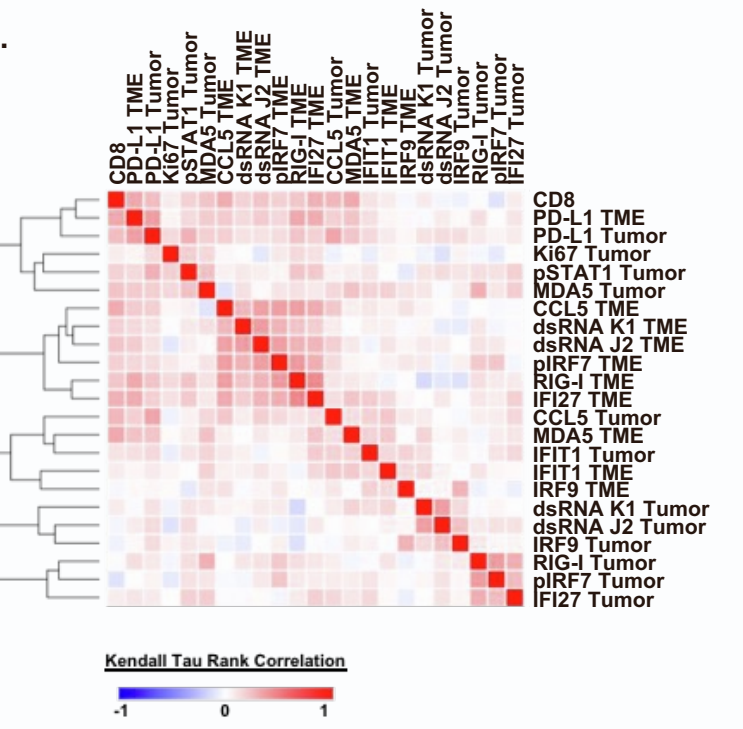
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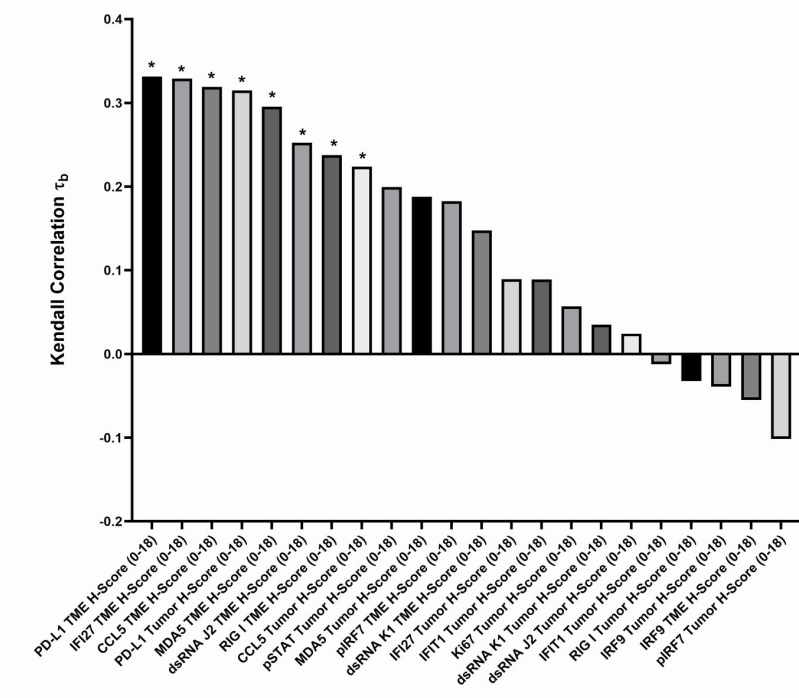
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C.



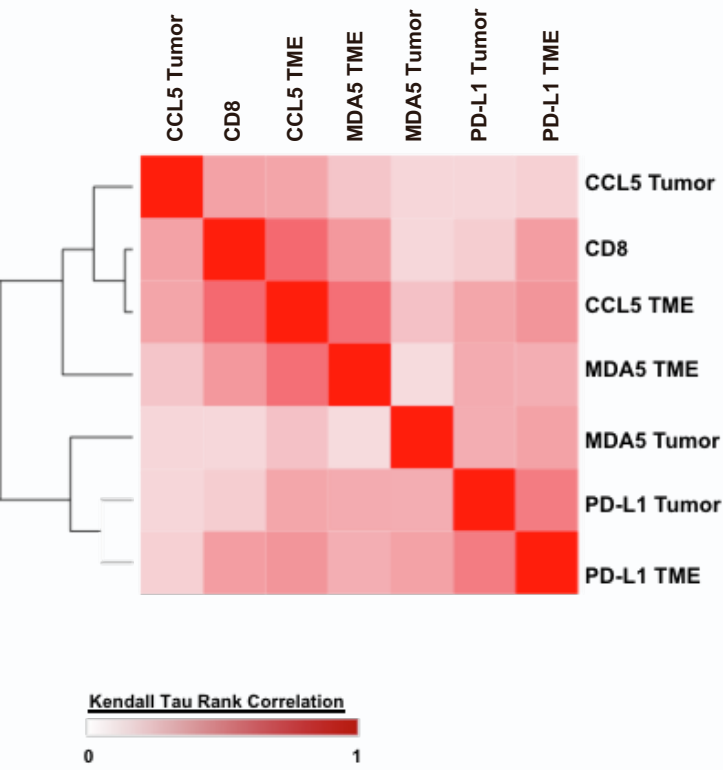
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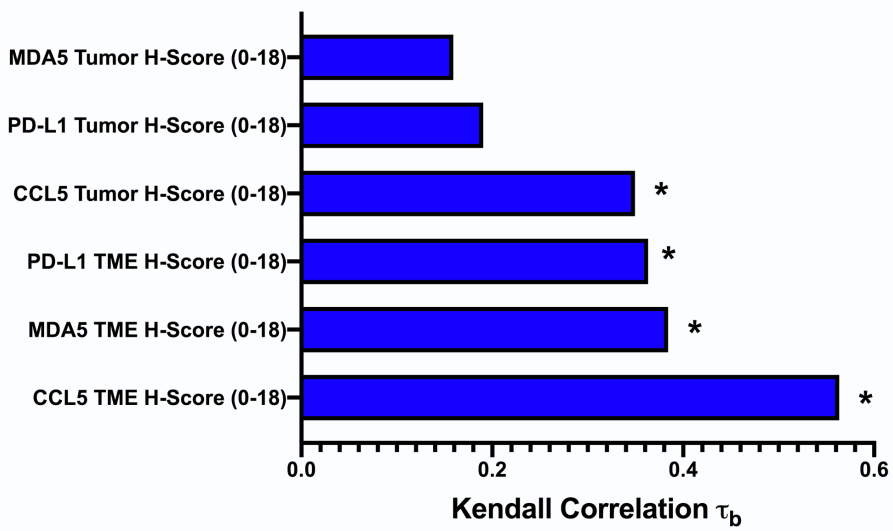
Supplemental Figure 2: IHC-based correlation analyses of CD8⁺ vs immune and viral defense markers in LUAD TMAs, Related to Figure 3.

A) LUAD H-score-based Kendall tau-b concordance similarity matrix, one minus Kendall tau-b concordance-based clustering (n=128). **B)** LUAD H-score-based Kendall tau-b concordance bar plot for panel markers vs CD8 α H-score, * indicates adjusted p-value < 0.05 after FDR 0.05 multiple comparisons correction two-stage linear step-up procedure of Benjamini, Kriegerm and Yekutieli. **C)** LUSC H-score-based Kendall tau-b concordance similarity matrix, one minus Kendall tau-b concordance-based clustering (n=92). **D).** LUSC H-score-based Kendall tau-b concordance bar plot for panel markers vs CD8 α H-score, * indicates an adjusted p-value < 0.05 after FDR 0.05 multiple comparisons correction two-stage linear step-up procedure of Benjamini, Kriegerm and Yekutieli.

A.

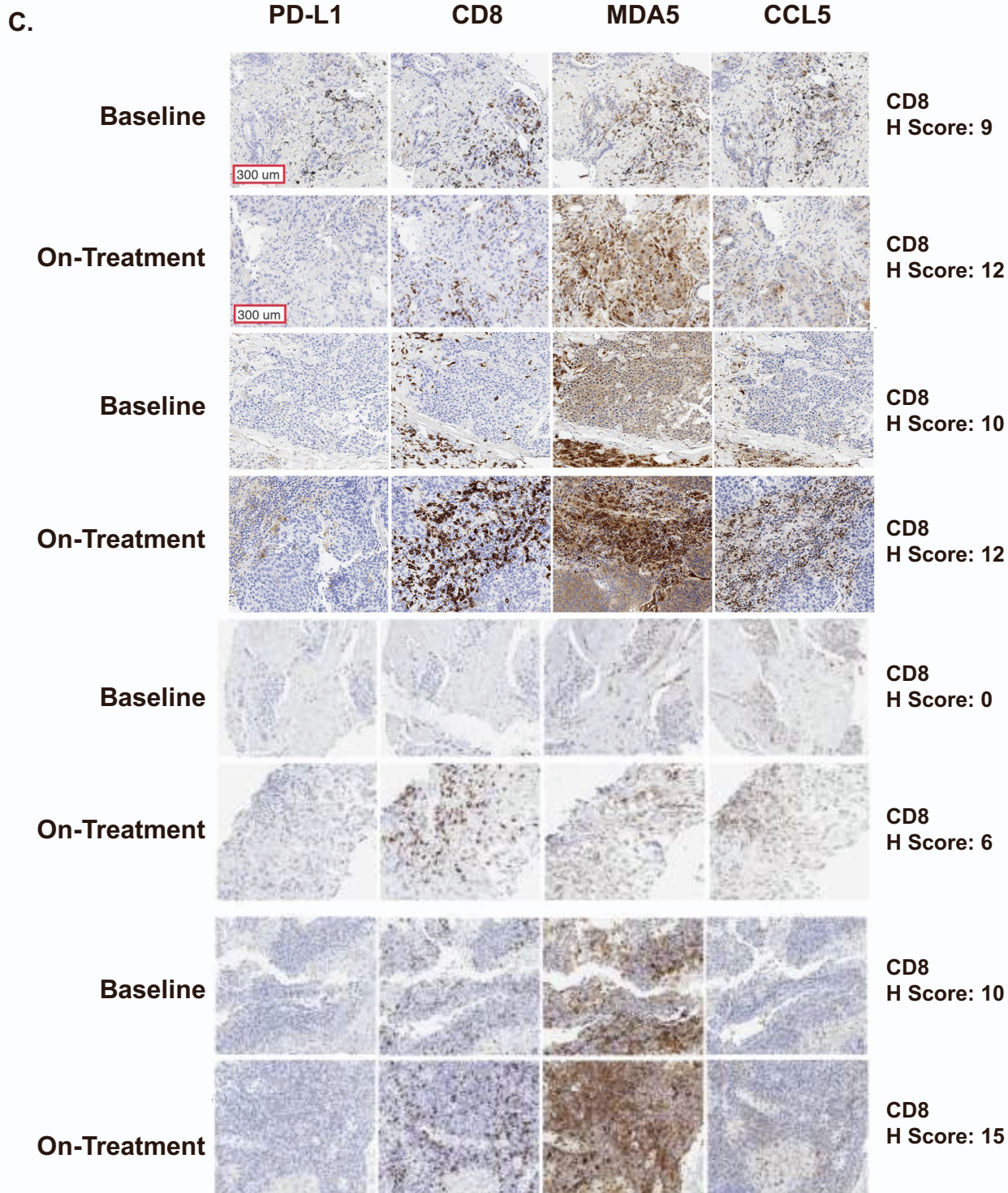
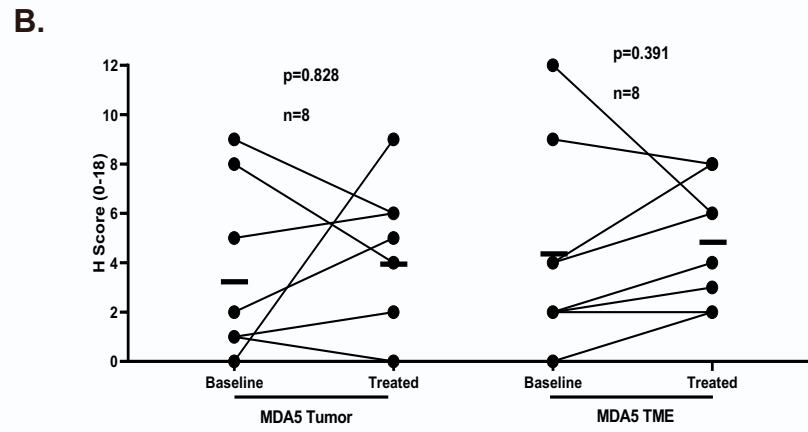
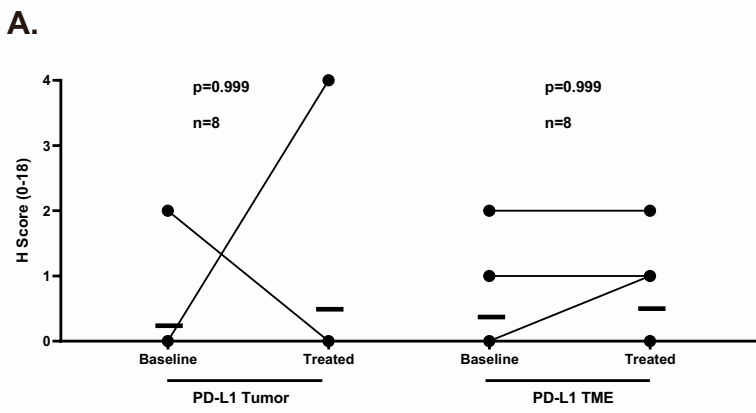


B.



Supplemental Figure 3: IHC-based correlation analyses in NSCLC tumor biopsy validation cohort, Related to Figure 5.

A) H-score-based Kendall tau-b concordance similarity matrix, one minus Kendall tau-b concordance-based clustering (n=63). **B)** H-score-based Kendall tau-b concordance bar plot for panel markers vs CD8 α H-score. * indicates an adjusted p-value < 0.05 after FDR 0.05 multiple comparisons correction two-stage linear step-up procedure of Benjamini, Kriegerm, and Yekutieli (n=63).



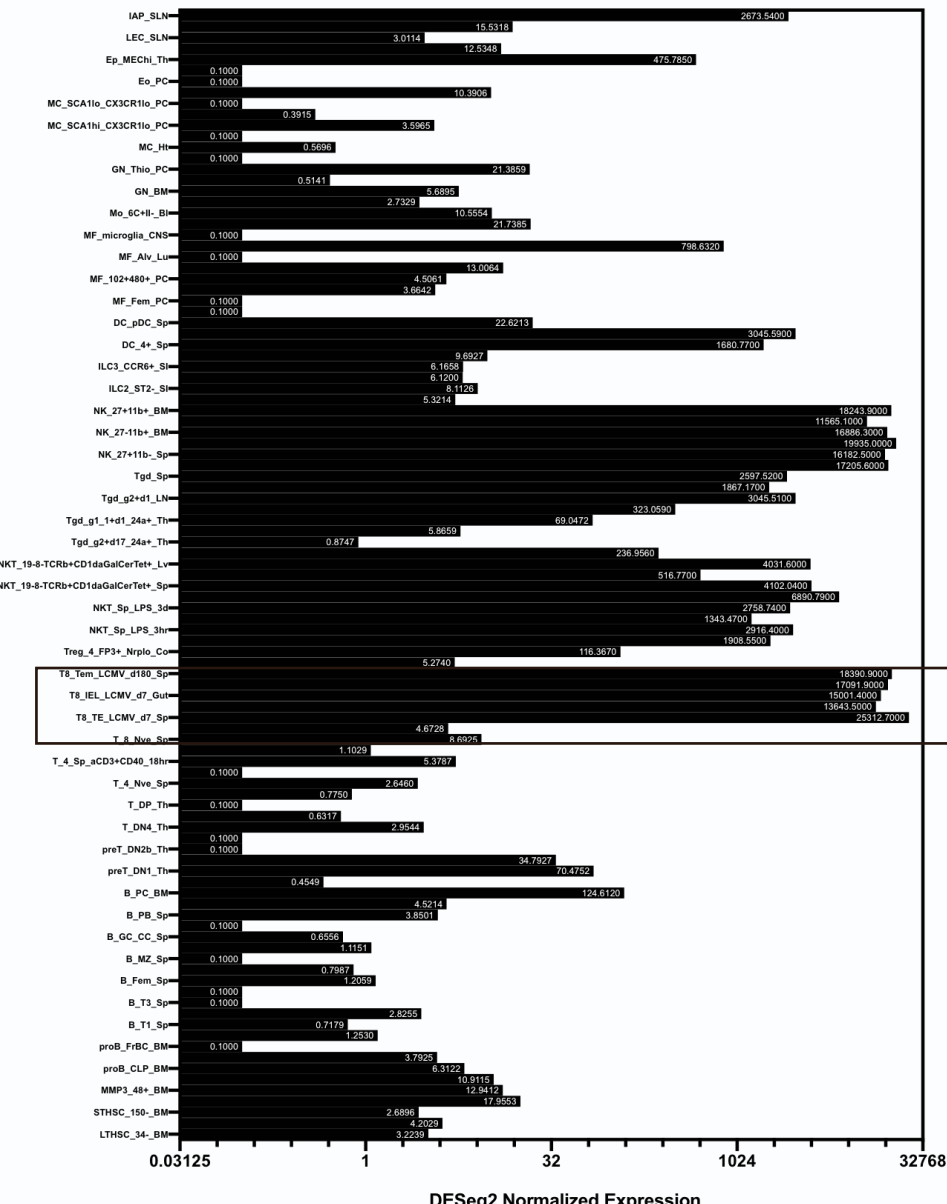
Supplemental Figure 4: Analysis of paired biopsies reveals epigenetic therapy-induced augmentation of both tumor accumulation and tumor infiltration of CD8⁺ T cells, Related to Figures 6 and 7.

A). PD-L1 H-score comparison baseline vs on-treatment (n=8 paired samples). Statistical significance by Wilcoxon Signed Ranked Test, mean and individual sample values are plotted. **B).** MDA5 H-score comparison baseline vs on-treatment (n=8 paired samples). Statistical significance by Wilcoxon Signed Ranked Test, mean and individual sample values are plotted. **C).** Expanded panel and larger field of single-plex IHC sections depicted in Figure 6 for CD8 and CCL5 alone and multiplex sections depicted in Figure 7 (Top 2 panels), and additional sections (bottom 2 panels) of clinical trial biopsies to show a prioritized 4-marker IHC panel. Representative scale bar provided in top left image of figure panel.

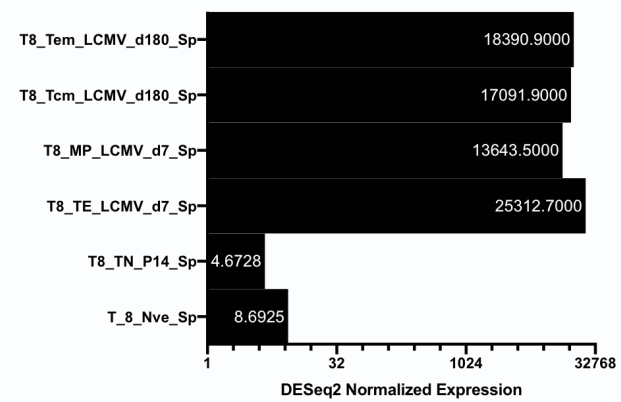
A.

Subject	Pre & Post Epigenetic Priming/ treatment	H-Score CD8 (GH)	H-Score CCL5 tumor (GH)	H-Score CCL5 stroma (GH)	Aperio Algorithm, Multiplex Analysis	Total nuclei, hematoxylin (PC blue)	Percent magenta (CCL5) (PC red)	Percent DAB (CD8) (PC green)	Percent CCL5/CD8; Colocalized (PC yellow)	Total Stained Area (mm ²)	Total Analysis Area (mm ²)
1	Pre	9	4	3	CD8 CCL5	97.0991	0.136663	2.65393	0.11026	0.795647	2.80635
1	Post	12	8	9	CD8 CCL5	90.5775	0.437535	7.95653	1.02843	1.55649	6.43151
2	Pre	1	1	1	CD8 CCL5	98.5633	0.528358	0.883917	2.44E-02	0.69122	3.58895
2	Post	2	1	2	CD8 CCL5	97.6687	0.323731	1.92605	8.15E-02	0.427516	2.74249
3	Pre	10	2	4	CD8 CCL5	82.9066	2.42173	11.5155	3.15615	0.767931	3.4669
3	Post	12	0	8	CD8 CCL5	94.7671	0.965191	3.08314	1.18461	3.80609	8.46867
4	Pre	10	1	2	CD8 CCL5	93.4705	1.03781	4.65892	0.832773	1.7724	4.74249
4	Post	15	8	3	CD8 CCL5	87.8979	2.38935	7.66203	2.05071	0.953868	2.21517
5	Pre	0	0	0	CD8 CCL5	97.426	0.548876	1.86805	0.15712	7.28E-02	0.480357
5	Post	4	4	3	CD8 CCL5	98.289	1.0778	0.512253	0.120989	1.20311	6.72521
6	Pre	4	5	2	CD8 CCL5	92.3779	1.35268	4.96858	1.30087	0.197838	1.31737
6	Post	6	4	3	CD8 CCL5	88.9628	7.91251	2.92017	0.204489	0.754542	4.04813
7	Pre	0	6	1	CD8 CCL5	97.9027	1.64234	0.387532	6.74E-02	0.45974	3.62186
7	Post	6	1	2	CD8 CCL5	93.2436	0.406604	4.85804	1.49174	0.156371	0.958558
8	Pre	1	3	1	CD8 CCL5	97.0351	2.88159	6.98E-02	1.36E-02	0.143639	0.639019
8	Post	0	0	1	CD8 CCL5	97.1685	2.15412	0.472727	0.204648	1.4812	7.05401
9	Pre	8	0	1	CD8 CCL5	92.6226	4.17441	1.44607	1.75688	72.5127	268.409
9	Post	3	6	9	CD8 CCL5	97.4823	0.187465	2.02331	0.306932	2.17162	7.97423
10	Pre	12	2	10	CD8 CCL5	27.6166	52.5501	6.57707	13.2562	1.90027	3.83065
10	Post	12	8	10	CD8 CCL5	38.973	34.7689	10.0566	16.2015	3.1248	7.04703
11	Pre	10	6	2	CD8 CCL5	81.9337	7.28E-02	14.0342	3.95929	0.603883	1.98084
11	Post	15	8	12	CD8 CCL5	70.0331	3.07642	18.5512	8.33931	1.95878	7.71406
12	Pre	12	3	3	CD8 CCL5	45.7451	43.8879	4.06869	6.29825	0.533791	1.33104
12	Post	15	8	3	CD8 CCL5	41.8109	33.3529	9.93727	14.8989	0.849029	2.42247

B.



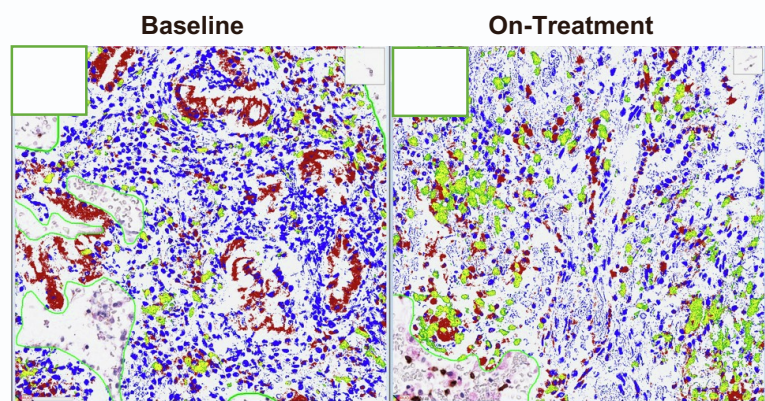
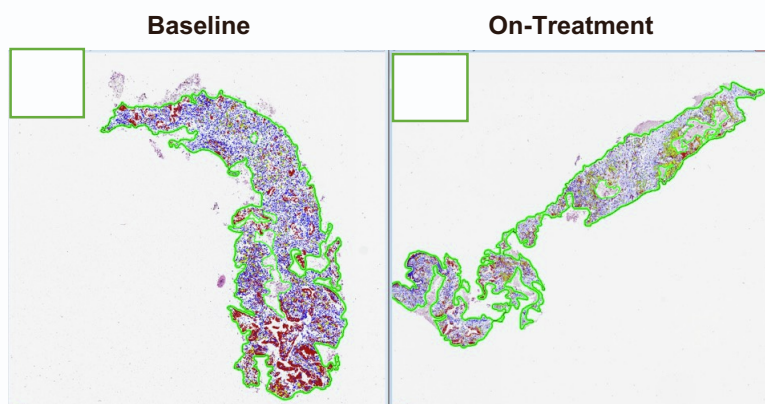
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Supplemental Figure 5: CD8 and CCL5 correlative behavior in NSCLC clinical trial samples, Related to Figure 7

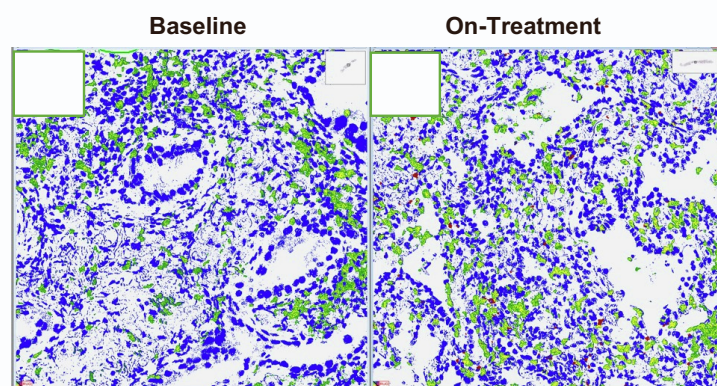
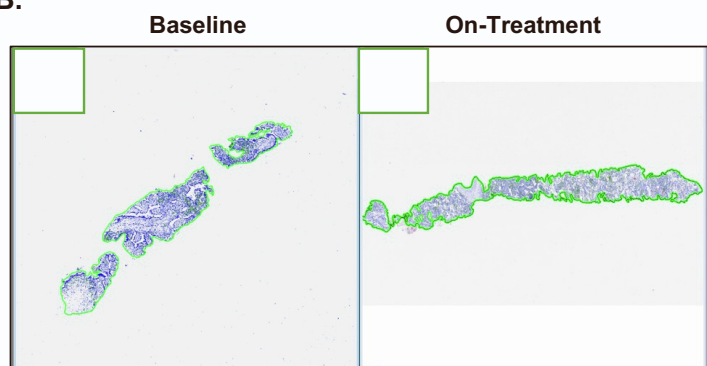
A). Baseline and on-treatment summary table for CD8 and CCL5 H-score and Aperio quantitation of multiplex IHC, provided subject IDs are arbitrary and provided to assist in matching samples. **B).** DESeq2 normalized CCL5 expression across immune populations as deposited into ImmGen database x-axis: CCL5 DESeq2 normalized expression RNA-seq data, y-axis: ImmGen annotated immune populations. **C).** DESeq2 normalized CCL5 expression across CD8 T cell experimental populations. CD8 T cell-specific data from experimental murine models as provided by the Immgen database. X-axis: CCL5 DESeq2 normalized expression RNA-seq data, y-axis: Spleen-derived CD8⁺ T cell populations.

A.



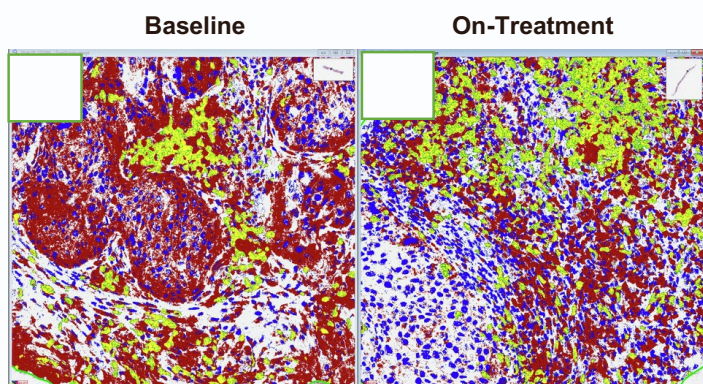
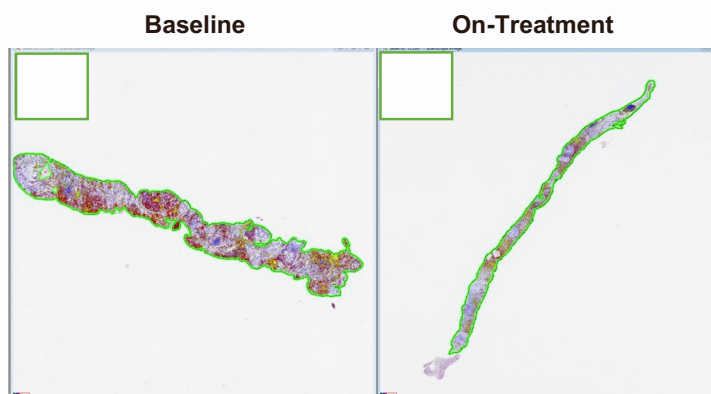
Pre & Post Epigenetic Priming/ treatment	H-Score CD8 (GH)	H-Score CCL5 tumor (GH)	H-Score CCL5 stroma (GH)	Aperio Algorithm, Multiplex Analysis	Total nuclei, hematoxylin (PC blue)	Percent magenta (CCL5) (PC red)	Percent DAB (CD8) (PC green)	Percent CCL5/CD8; Colocalized (PC yellow)	Total Stained Area (mm ²)	Total Analysis Area (mm ²)
Pre	12	3	3	CD8 CCL5	45.7451	43.8879	4.06869	6.29825	0.533791	1.33104
Post	15	8	3	CD8 CCL5	41.8109	33.3529	9.93727	14.8989	0.849029	2.42247

B.



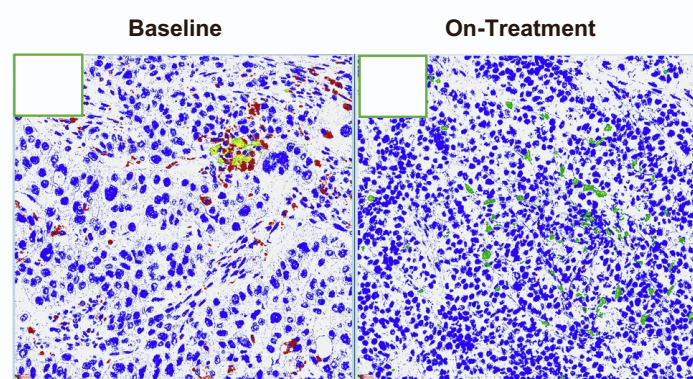
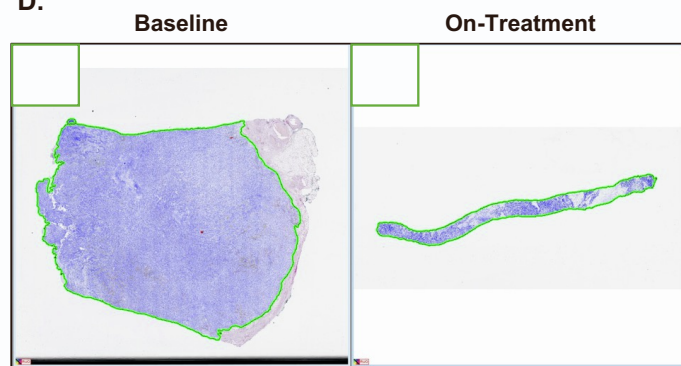
Pre & Post Epigenetic Priming/ treatment	H-Score CD8 (GH)	H-Score CCL5 tumor (GH)	H-Score CCL5 stroma (GH)	Aperio Algorithm, Multiplex Analysis	Total nuclei, hematoxylin (PC blue)	Percent magenta (CCL5) (PC red)	Percent DAB (CD8) (PC green)	Percent CCL5/CD8; Colocalized (PC yellow)	Total Stained Area (mm ²)	Total Analysis Area (mm ²)
Pre	10	6	2	CD8 CCL5	81.9337	7.28E-02	14.0342	3.95929	0.603883	1.98084
Post	15	8	12	CD8 CCL5	70.0331	3.07642	18.5512	8.33931	1.95878	7.71406

C.



Pre & Post Epigenetic Priming/ treatment	H-Score CD8 (GH)	H-Score CCL5 tumor (GH)	H-Score CCL5 stroma (GH)	Aperio Algorithm, Multiplex Analysis	Total nuclei, hematoxylin (PC blue)	Percent magenta (CCL5) (PC red)	Percent DAB (CD8) (PC green)	Percent CCL5/CD8; Colocalized (PC yellow)	Total Stained Area (mm ²)	Total Analysis Area (mm ²)
Pre	12	2	10	CD8 CCL5	27.6166	52.5501	6.57707	13.2562	1.90027	3.83065
Post	12	8	10	CD8 CCL5	38.973	34.7689	10.0566	16.2015	3.1248	7.04703

D.

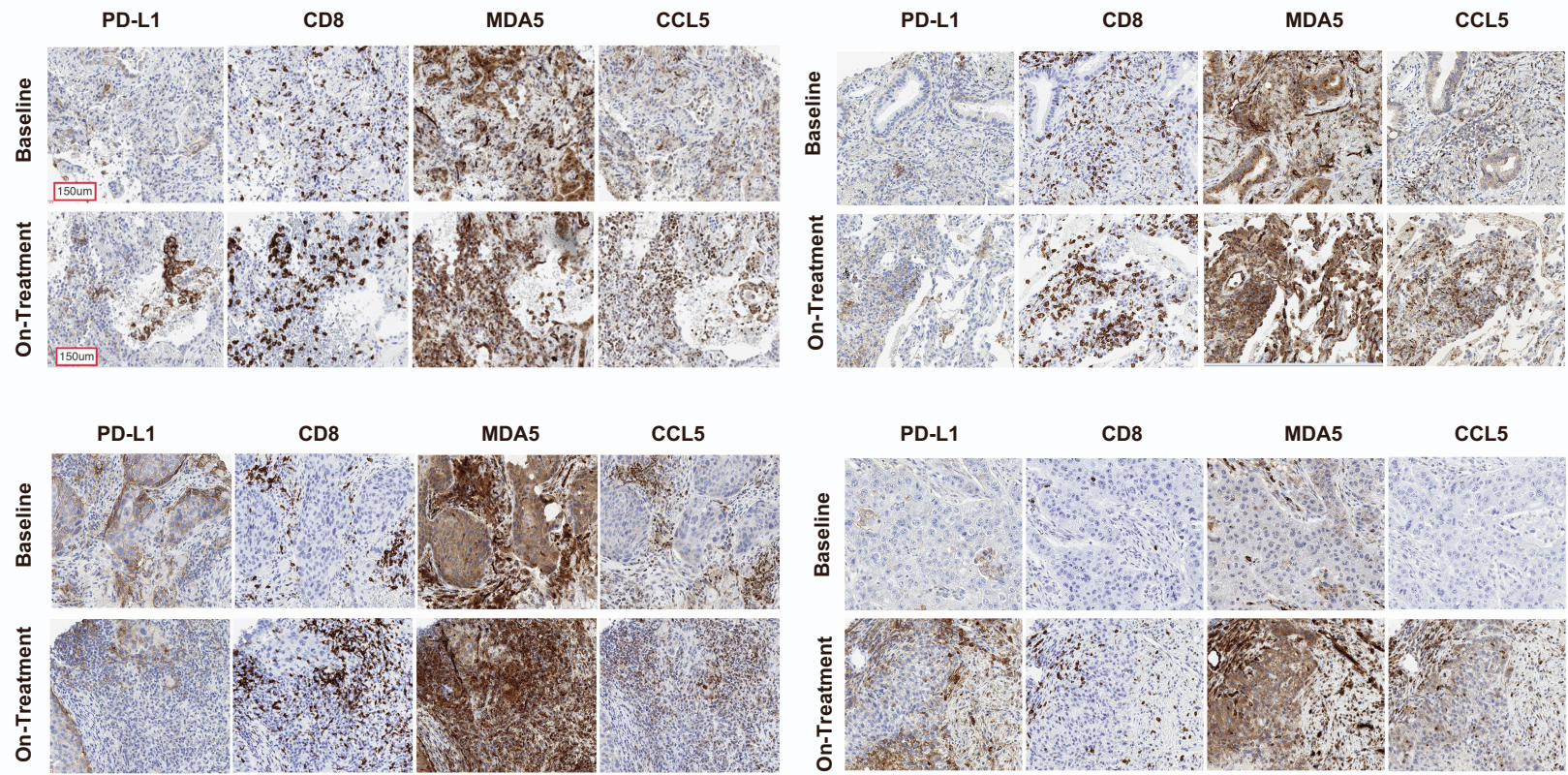


Pre & Post Epigenetic Priming/ treatment	H-Score CD8 (GH)	H-Score CCL5 tumor (GH)	H-Score CCL5 stroma (GH)	Aperio Algorithm, Multiplex Analysis	Total nuclei, hematoxylin (PC blue)	Percent magenta (CCL5) (PC red)	Percent DAB (CD8) (PC green)	Percent CCL5/CD8; Colocalized (PC yellow)	Total Stained Area (mm ²)	Total Analysis Area (mm ²)
Pre	8	0	1	CD8 CCL5	92.6226	4.17441	1.44607	1.75686	72.5127	268.409
Post	3	6	9	CD8 CCL5	97.4823	0.187465	2.02331	0.306932	2.17162	7.97423

Supplemental Figure 6: Concurrent epigenetic immunotherapy in advanced NSCLC clinical trial specimens case studies, Related to Figure 8.

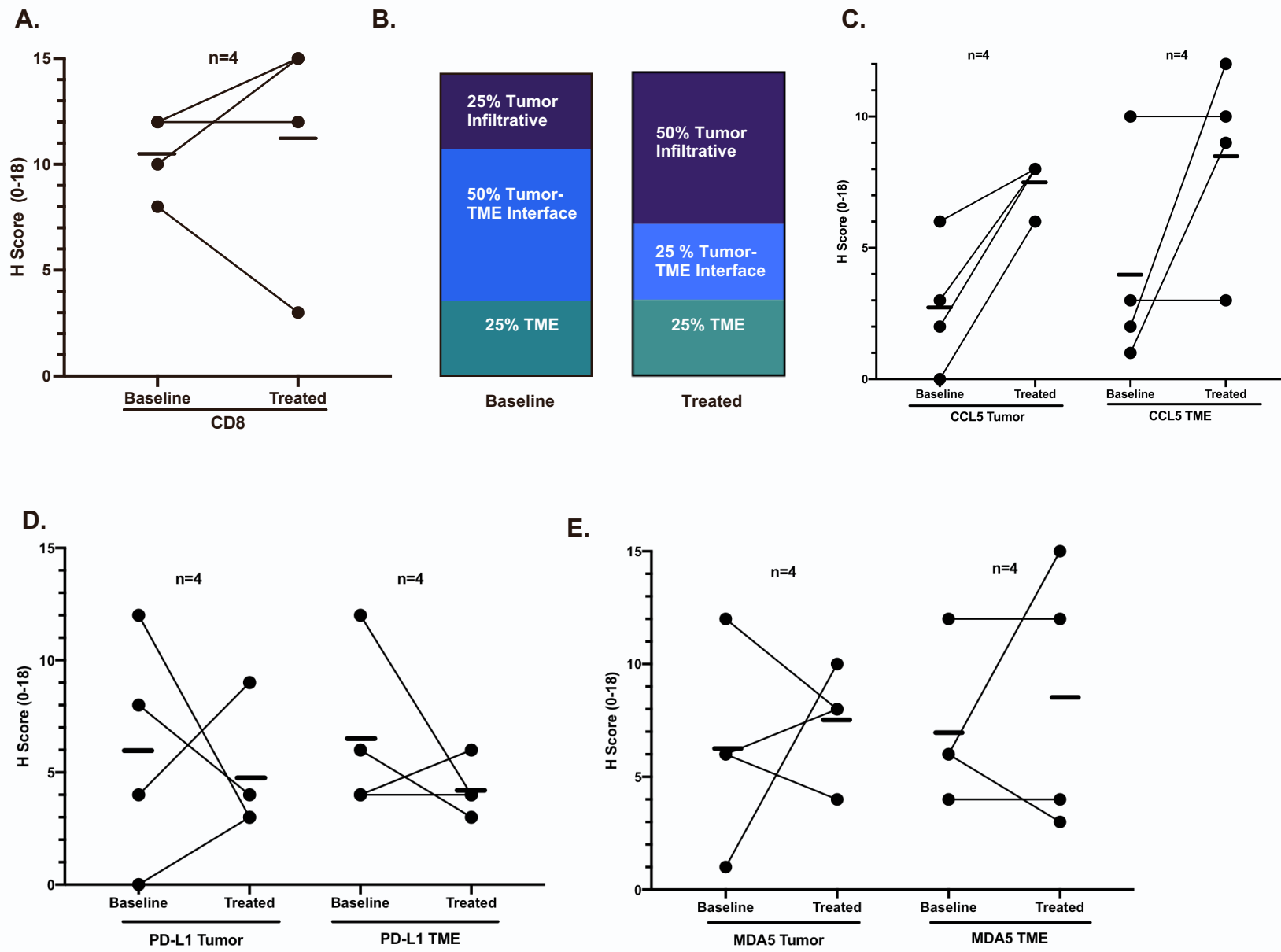
A-D). CD8 α and CCL5 multiplex IHC, representative whole sections and enhanced image size for case studies in Figure 8. Top panel: whole section, bottom panel: Region of interest, 200x magnification, left panel: Baseline, right panel: on-treatment combination epigenetic treatment (Azacitidine + Entinostat). CD8 α + CCL5 multiplex IHC pseudo-colored (green= CD8 α , red=CCL5, and yellow=CD8 α +CCL5 co-localization).

A.



Supplemental Figure 7: Single-plex IHC, concurrent epigenetic immunotherapy in advanced NSCLC clinical trial specimens case studies, Related to Figure 8.

A). Larger field and expanded panel single-plex IHC sections corresponding to sections presented as case studies in Figure 8. Clinical trial biopsy to show the prioritized 4-marker IHC panel. All IHC stains were scored by modified H-score with a primary focus on CD8 cells and tumor infiltrative pattern. 200x magnification.



Supplemental Figure 8: Single-plex summary IHC Concurrent epigenetic immunotherapy in advanced NSCLC clinical trial specimens case studies, Related to Figure 8.

A). CD8 α H-score comparison baseline vs on-treatment (n=4 paired samples), mean and individual sample values are plotted. **B).** CD8⁺ infiltration status baseline vs. on-treatment (n=4 paired samples). **C).** CCL5 H-score comparison baseline vs on-treatment (n=4 paired samples), mean and individual sample values are plotted. **D).** PD-L1 H-score comparison baseline vs on-treatment (n=4 paired samples), mean and individual sample values are plotted. **E).** MDA5 H-score comparison baseline vs on-treatment (n=4 paired samples), mean and individual sample values are plotted.