

Supplemental Figures and Tables

Immune Signatures Following Single Dose Trastuzumab Predict Pathologic Response to Preoperative Trastuzumab and Chemotherapy in HER2-Positive Early Breast Cancer

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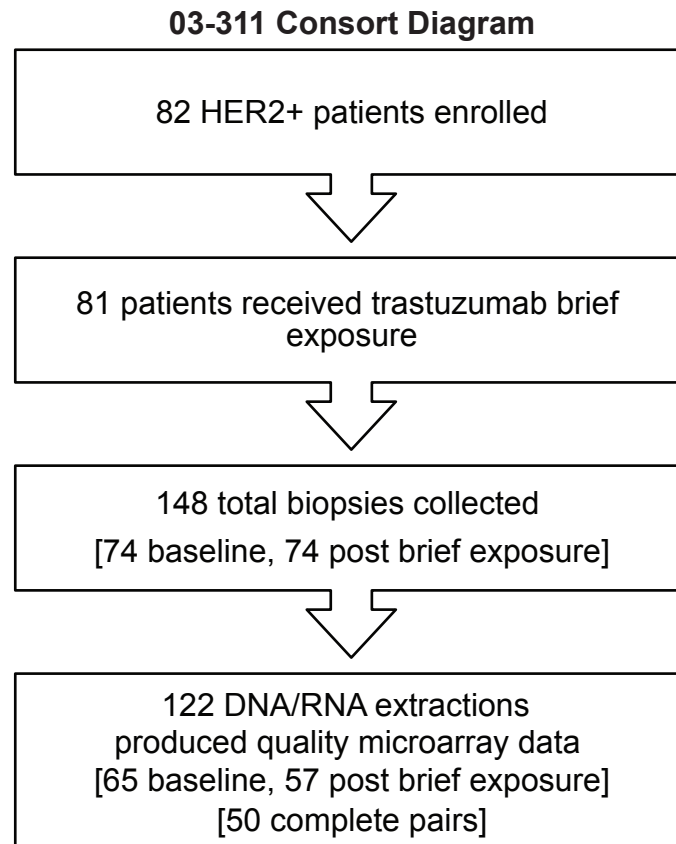
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Supplemental Table 1. Immune Index genes discriminative of response at the post-exposure timepoint in the discovery (03-311) and validation (211B) datasets.

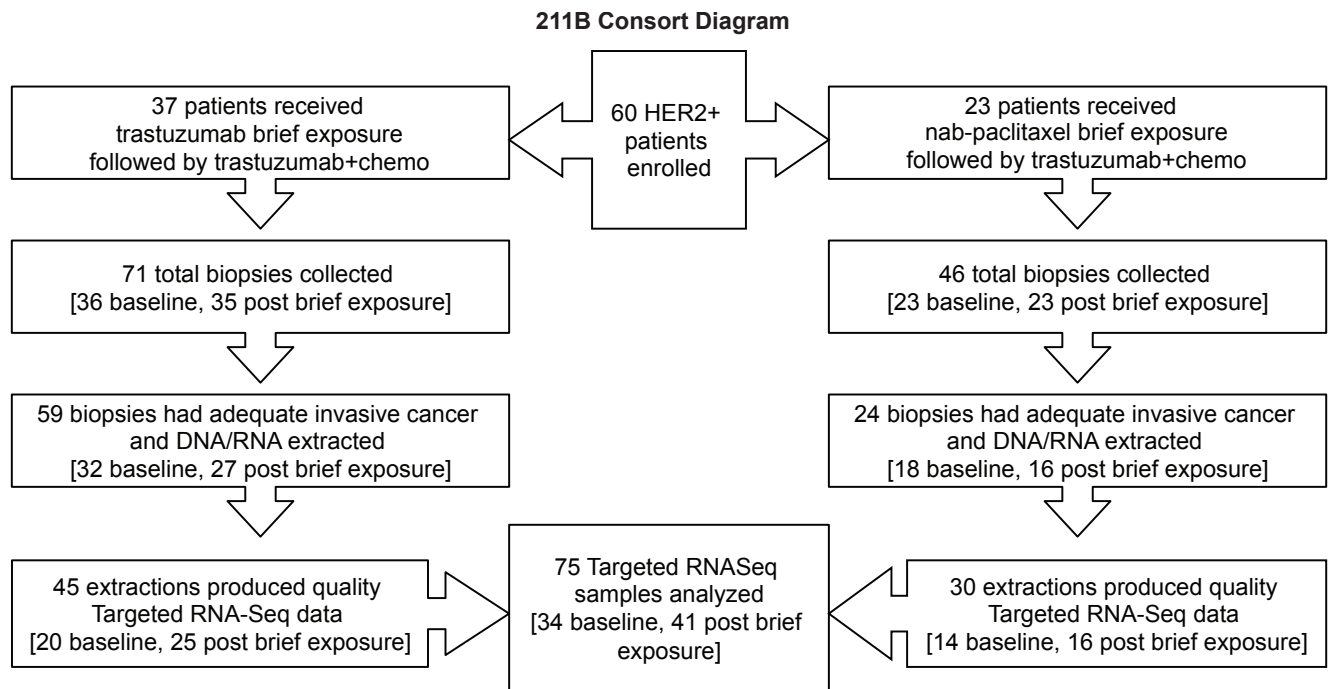
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Supplemental Table 3. CD8+ T-cell cytolytic activity genes discriminative of response at the post-exposure timepoint in the discovery (03-311) and validation (211B) datasets.

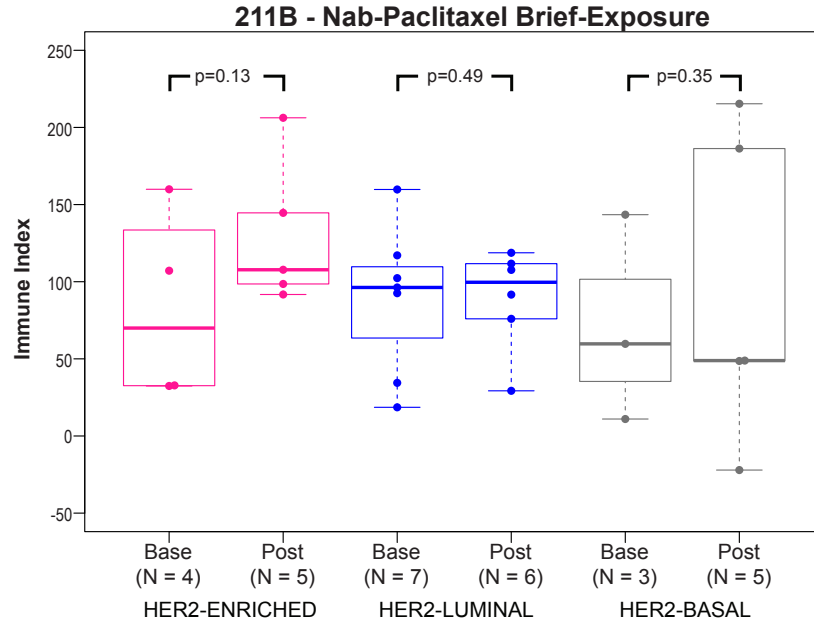
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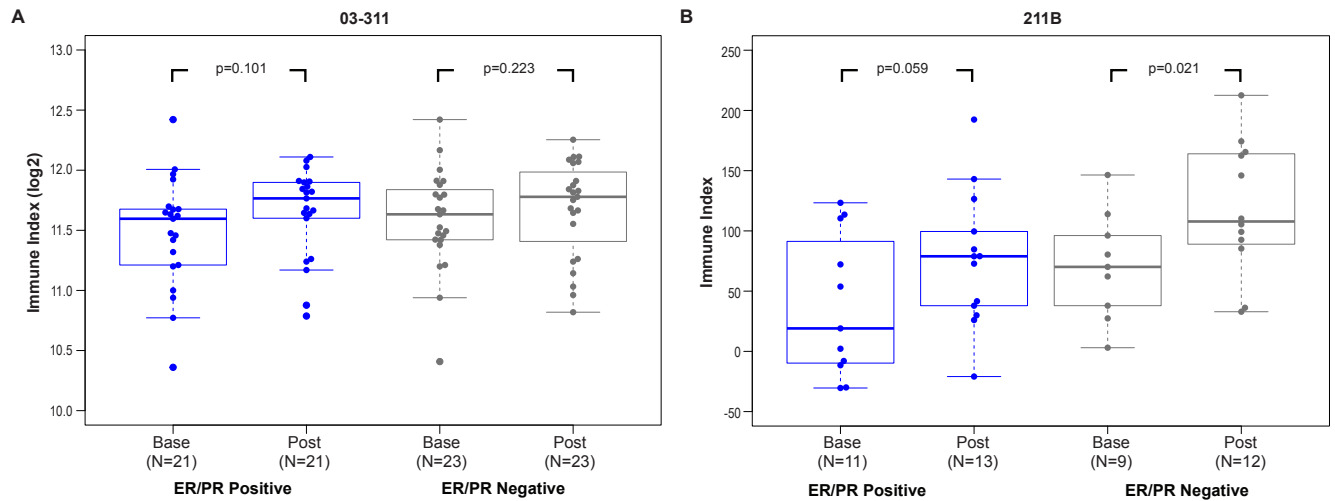
Supplemental Figure 1: Consort Diagram 03-311. The 03-311 dataset was considered the discovery set and contained a total of 100 usable high quality gene expression profiles using Illumina HT12 microarrays. Importantly, this included 50 baseline and 50 matched post brief-exposure samples.



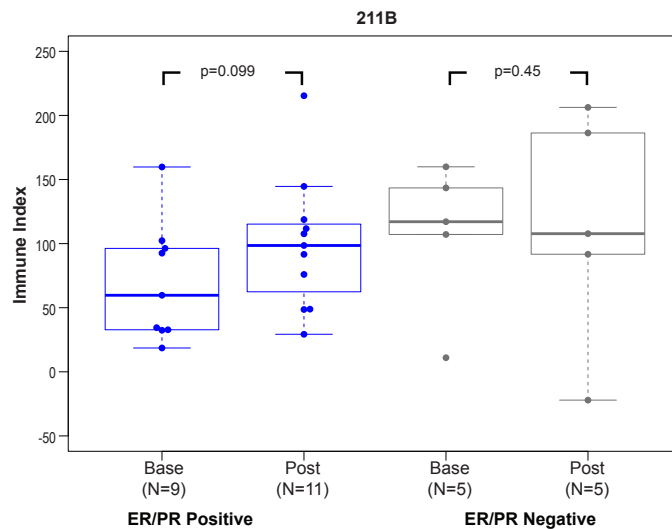
Supplemental Figure 2: Consort Diagram 211B The 211B dataset was considered the validation set and contained 75 samples, including 34 baseline and 41 post brief-exposure samples, that were profiled using a novel Illumina Targeted RNA-Sequencing assay.



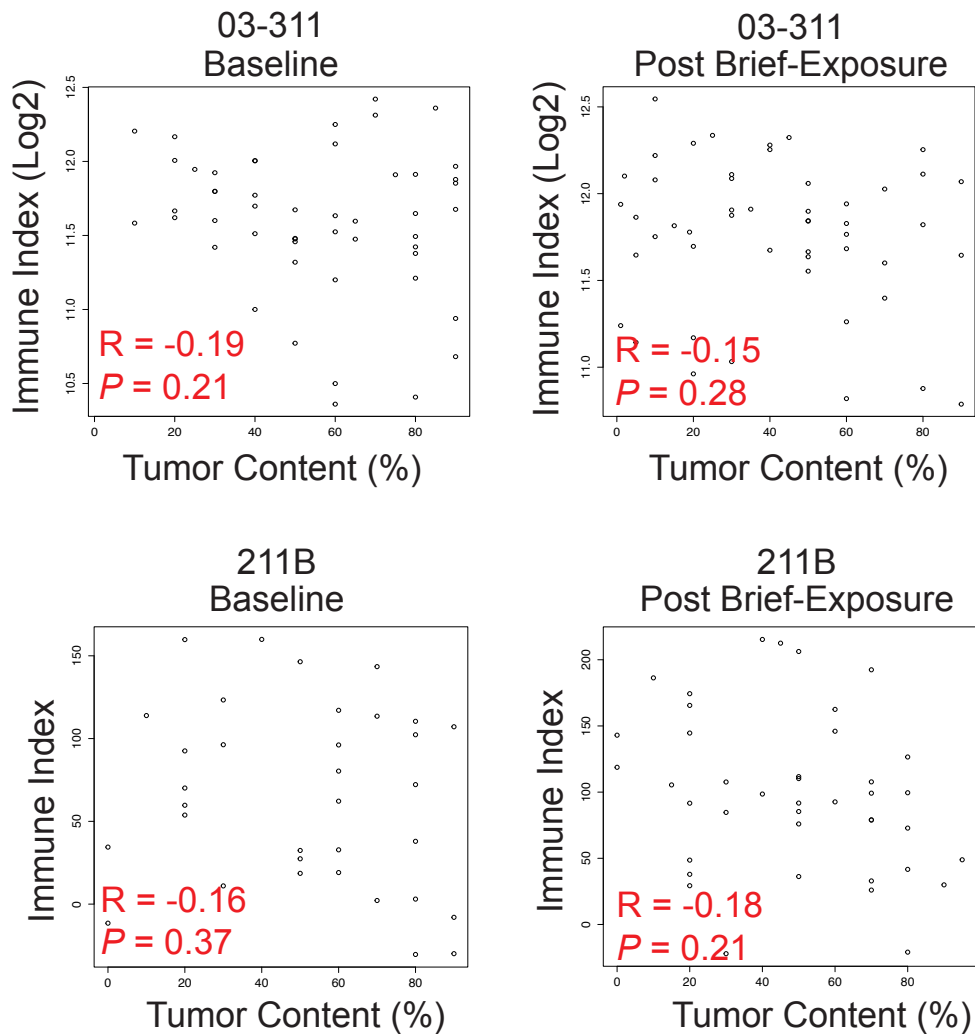
Supplemental Figure 3: Subtype-specific changes in Immune Index upon brief-exposure to nab-paclitaxel in the 211B trial. Plotted are the Immune Index values in individual patient biopsies at baseline and after a single dose of nab-paclitaxel in the 211B cohort across the HER2-enriched, HER2-luminal and HER2-basal subtypes. Significance was calculated using a one-sided non-parametric Wilcoxon test.



Supplemental Figure 4: Evaluation of the Immune Index upon brief-exposure to trastuzumab in the 03-311 and 211B trials according to ER/PR status. Plotted are the Immune Index values in individual patient biopsies at baseline and after a single dose of trastuzumab in the A) 03-311 and B) 211B cohorts across the ER/PR positive and ER/PR negative subtypes. Patient tumors were deemed ER/PR positive if either ER or PR status was positive by IHC. For ER/PR negative tumors, both ER and PR status was negative by IHC. Note that all normal-like samples were excluded from the analyses. Significance was calculated using a one-sided non-parametric Wilcox test.

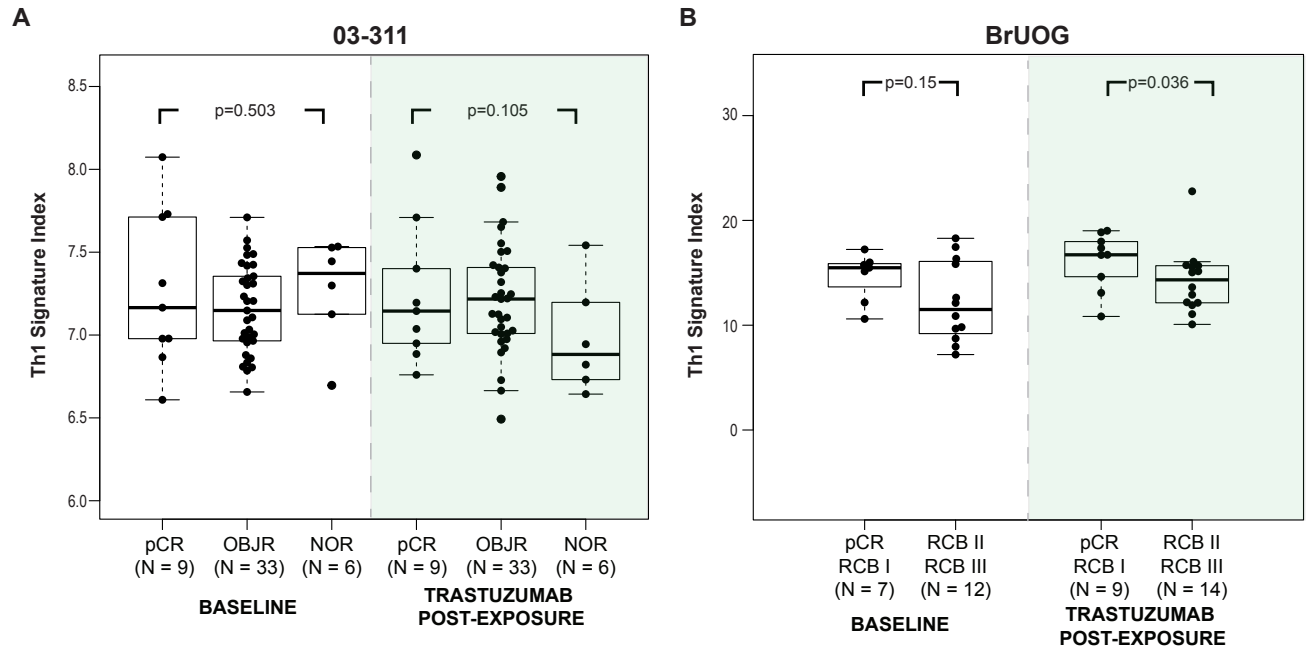


Supplemental Figure 5: Evaluation of the Immune Index upon brief-exposure to nab-paclitaxel in the 211B trial according to ER/PR status. Plotted are the Immune Index values in individual patient biopsies at baseline and after a single dose of nab-paclitaxel 211B cohorts across the ER/PR positive and ER/PR negative subtypes. Patient tumors were deemed ER/PR positive if either ER or PR status was positive by IHC. For ER/PR negative tumors, both ER and PR status was negative by IHC. All normal-like samples were excluded from the analyses. Significance was calculated using a one-sided non-parametric Wilcox test.

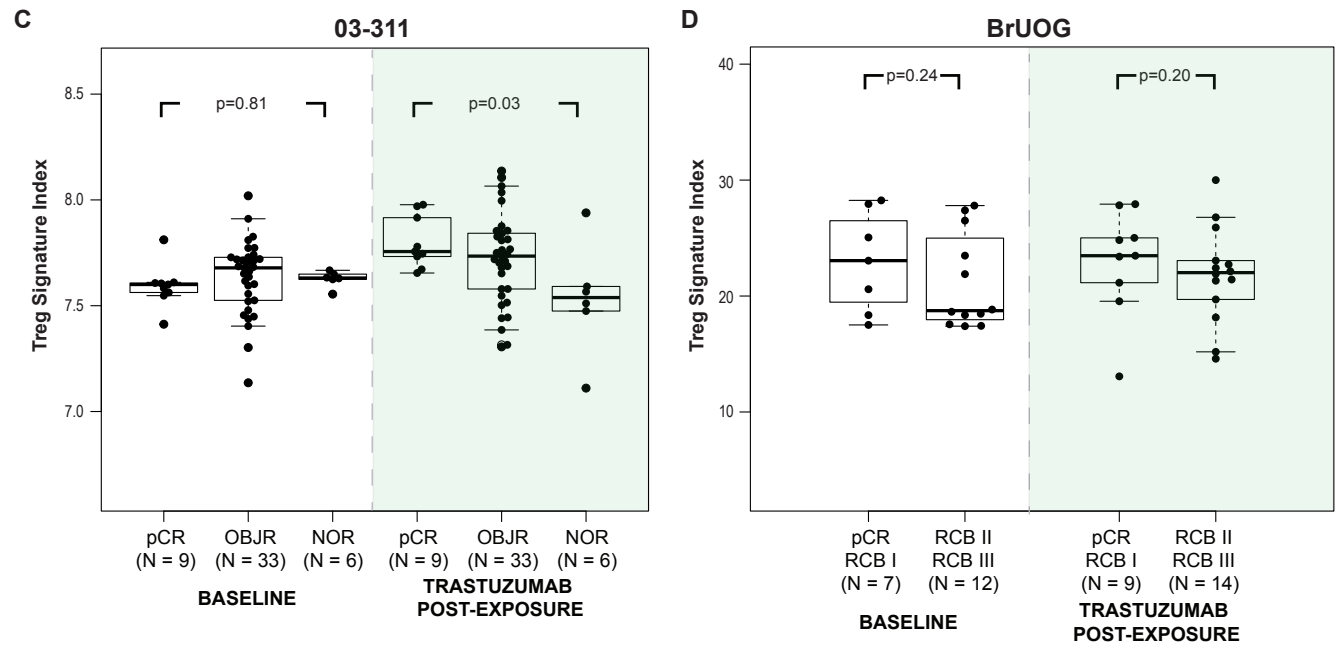


Supplemental Figure 6: Correlation between Immune Index and tumor content A) Plotted are the Immune Index values across a pathologist-inferred tumor content of the biopsy samples in the 03-311 trial at baseline and post brief-exposure timepoints independently. Additional evaluation using linear model and controlling for PAM50 subtypes also revealed a non-significant association ($P = 0.60$) between tumor content and Immune Index. B) The Immune Index is plotted against the pathologist-estimated tumor content in each biopsy sample in the 211B cohort at baseline and post brief-exposure timepoints. As before, an evaluation using linear model and controlling for PAM50 subtypes also revealed a non-significant association ($P = 0.37$) between tumor content and Immune Index at the post brief-exposure timepoint. The Pearson's product moment correlation coefficient and the associated pvalue rejecting the null hypothesis that the correlation is significantly deviating from zero. Notably, no significant correlation was observed between the Immune Index and the tumor content.

CD4+ Th1 Signature



CD4+ Treg Signature



Supplemental Figure 7: Association of CD4+ Th1 and Treg signatures with response The figure shows the 12-gene Th1 signature index for samples at baseline and post brief-exposure to trastuzumab in A) 03-311 and B) 211B cohorts. Similarly, the 8-gene Treg signature index is plotted at baseline and post brief-exposure to trastuzumab in the 03-311 (C) and 211B (D) datasets. Significance was assessed using a one-sided Wilcoxon test to evaluate if higher signature indices were associated with responders. Notably, the Th1 signature was discriminative of response after one dose of trastuzumab in the 211B dataset, whereas the Treg signature was discriminative of response after a single dose of trastuzumab in the 03-311 dataset alone.

Supplemental Tables

Supplemental Table 1. Immune Index genes discriminative of response at the post-exposure timepoint in the discovery (03-311) and validation (211B) datasets. Listed are the individual genes of the 140-gene Immune Index signature that significantly discriminate between responders and non-responders (P-value <0.05 and FDR ≤ 0.25) in either of the two datasets. Significance was estimated using a two-sided Wilcox test followed by multiple testing correction. The difference in median expression level of the individual genes between the responders and non-responders are also included. In the 03-311 cohort, responders corresponded to the pCR samples while non-responders corresponded to the NOR samples. In the 211B cohort, responders included pCR and RCB I samples, while non-responders included RCB II and RCB III samples.

Gene	03-311		211B		Category
	Difference in median expression between pCR and NOR groups	Significance of difference in gene expression between pCR and NOR groups Wilcox P-value (FDR ≤ 0.25)	Difference in median expression between responder (pCR & RCB I) and non-responder (RCB II & RCB III) groups	Significance of difference in gene expression between responder and non-responder groups Wilcox P-value (FDR ≤ 0.25)	
<i>GIMAP4</i>	1.38	0.01	1.37	0.03	Significant in both datasets
<i>ITGAL</i>	1.12	0.05	1.99	0.01	Significant in both datasets
<i>CASP1</i>	0.53	0.00	1.24	0.22	Significant in 03-311 alone
<i>FGR</i>	1.15	0.00	0.74	0.20	Significant in 03-311 alone
<i>IKZF1</i>	0.86	0.00	1.18	0.98	Significant in 03-311 alone
<i>HCLS1</i>	0.82	0.01	1.24	0.34	Significant in 03-311 alone
<i>PTPRE</i>	0.79	0.01	0.60	0.52	Significant in 03-311 alone
<i>SLA</i>	0.54	0.01	0.98	0.60	Significant in 03-311 alone
<i>GBP2</i>	0.81	0.01	1.21	0.18	Significant in 03-311 alone
<i>NCF4</i>	0.56	0.02	1.04	0.16	Significant in 03-311 alone
<i>NCKAP1L</i>	1.10	0.02	0.98	0.48	Significant in 03-311 alone
<i>VAV1</i>	0.45	0.02	0.09	0.69	Significant in 03-311 alone
<i>ARHGEF6</i>	1.33	0.03	0.82	0.18	Significant in 03-311 alone
<i>GMFG</i>	0.77	0.03	0.69	0.56	Significant in 03-311 alone
<i>CD300A</i>	0.65	0.03	0.49	0.69	Significant in 03-311 alone
<i>GZMH</i>	0.46	0.04	10.13	0.20	Significant in 03-311 alone
<i>IL4R</i>	0.63	0.04	0.94	0.28	Significant in 03-311 alone
<i>TPP1</i>	0.28	0.04	0.11	0.44	Significant in 03-311 alone
<i>LILRB1</i>	0.72	0.04	0.07	0.64	Significant in 03-311 alone
<i>SH2B3</i>	0.41	0.04	-0.30	1.00	Significant in 03-311 alone
<i>TRAF3IP3</i>	0.62	0.05	1.54	0.12	Significant in 03-311 alone
<i>TNFRSF1B</i>	0.68	0.05	0.65	0.28	Significant in 03-311 alone
<i>NKG7</i>	0.69	0.05	0.86	0.60	Significant in 03-311 alone
<i>CD52</i>	1.13	0.05	-0.10	0.64	Significant in 03-311 alone
<i>ITK</i>	0.31	0.05	0.18	0.93	Significant in 03-311 alone
<i>ARHGAP15</i>	0.41	0.08	1.06	0.02	Significant in BrUOG alone
<i>PTPRCAP</i>	0.23	0.09	2.11	0.05	Significant in BrUOG alone
<i>LTB</i>	0.60	0.14	1.90	0.05	Significant in BrUOG alone
<i>CST7</i>	0.15	0.17	1.82	0.05	Significant in BrUOG alone
<i>CD247</i>	0.57	0.18	2.79	0.00	Significant in BrUOG alone
<i>CD2</i>	0.61	0.18	3.87	0.01	Significant in BrUOG alone
<i>BIN2</i>	0.03	0.18	2.08	0.04	Significant in BrUOG alone
<i>CD37</i>	0.46	0.18	1.12	0.04	Significant in BrUOG alone
<i>CCR7</i>	0.54	0.22	7.50	0.01	Significant in BrUOG alone
<i>IL2RG</i>	0.05	0.33	1.40	0.01	Significant in BrUOG alone
<i>CTSS</i>	0.04	0.39	0.97	0.03	Significant in BrUOG alone
<i>CORO1A</i>	0.09	0.46	1.91	0.03	Significant in BrUOG alone
<i>IL32</i>	0.00	0.69	1.03	0.02	Significant in BrUOG alone
<i>FLI1</i>	0.05	0.69	0.95	0.03	Significant in BrUOG alone
<i>LYZ</i>	-0.14	0.86	1.29	0.03	Significant in BrUOG alone
<i>CYBB</i>	-0.03	0.95	1.04	0.05	Significant in BrUOG alone

Supplemental Table 2. Immune cell subset signatures discriminative of response at baseline and post-exposure timepoints in the discovery (03-311) and validation (211B) datasets. Listed are the individual immune cell subset signatures along with the corresponding differences in median index values between responders and non-responders at baseline and the post-exposure timepoints. Significance was estimated using a one-sided Wilcox test. In the 03-311 cohort, responders corresponded to the pCR samples while non-responders corresponded to the NOR samples. In the 211B cohort, responders included pCR and RCB I samples, while non-responders included RCB II and RCB III samples.

Immune Signature	03-311				211B				Category
	Baseline		Post Brief-Exposure		Baseline		Post Brief-Exposure		
	Difference in median expression between pCR and NOR groups	Significance of difference in gene expression between pCR and NOR groups (Wilcox P-value)	Difference in median expression between pCR and NOR groups	Significance of difference in gene expression between pCR and NOR groups (Wilcox P-value)	Difference in median expression between responders and non-responder groups	Significance of difference in gene expression between responder and non-responder groups (Wilcox P-value)	Difference in median expression between responders and non-responder groups	Significance of difference in gene expression between responder and non-responder groups (Wilcox P-value)	
<i>B-Cell signature</i>	-0.04	0.62	-0.03	0.13	4.88	0.02	4.07	0.03	Significant only in 211B
<i>T-Cell signature</i>	-0.26	0.54	0.29	0.04	3.55	0.05	2.51	0.01	Significant in both 03-311 and 211B
<i>Macrophage signature</i>	-0.06	0.32	0.36	0.01	1.77	0.10	1.01	0.06	Significant only in 03-311

Supplemental Table 3. CD8+ T-cell cytolytic activity genes discriminative of response at the post-exposure timepoint in the discovery (03-311) and validation (211B) datasets. Listed are the individual genes and the 4-gene cytolytic activity signature known to be upregulated upon activation of CD8+ T-cells. Shown are the comparisons of the expression levels between responders and non-responders at the post-exposure timepoint in either of the two datasets. Significance was estimated using a two-sided Wilcox test. The difference in median expression level of the individual genes between the responders and non-responders are also included. In the 03-311 cohort, responders corresponded to the pCR samples while non-responders corresponded to the NOR samples. In the 211B cohort, responders included pCR and RCB I samples, while non-responders included RCB II and RCB III samples.

Gene	03-311		211B		Category
	Difference in median expression between pCR and NOR groups	Significance of difference in gene expression between pCR and NOR groups (Wilcox P-value)	Difference in median expression between responder (pCR & RCB I) and non-responder (RCB II & RCB III) groups	Significance of difference in gene expression between responder and non-responder groups (Wilcox P-value)	
<i>PRF1</i>	0.41	0.11	1.05	0.40	Not significant in either cohort
<i>GZMK</i>	0.91	0.07	1.96	0.20	Not significant in either cohort
<i>GZMH</i>	0.46	0.04	10.12	0.20	Significant in 03-311 alone
<i>NKG7</i>	0.69	0.05	0.86	0.59	Significant in 03-311 alone
<i>4-Gene Cytolytic Activity</i>	0.54	0.02	3.67	0.14	Significant in 03-311 alone