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Supplemental Information

Integrative Tumor and Immune Cell Multi-omic Analyses Predict Response to Immune Checkpoint Blockade in Melanoma

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Supplemental Information

Supplementary Figures

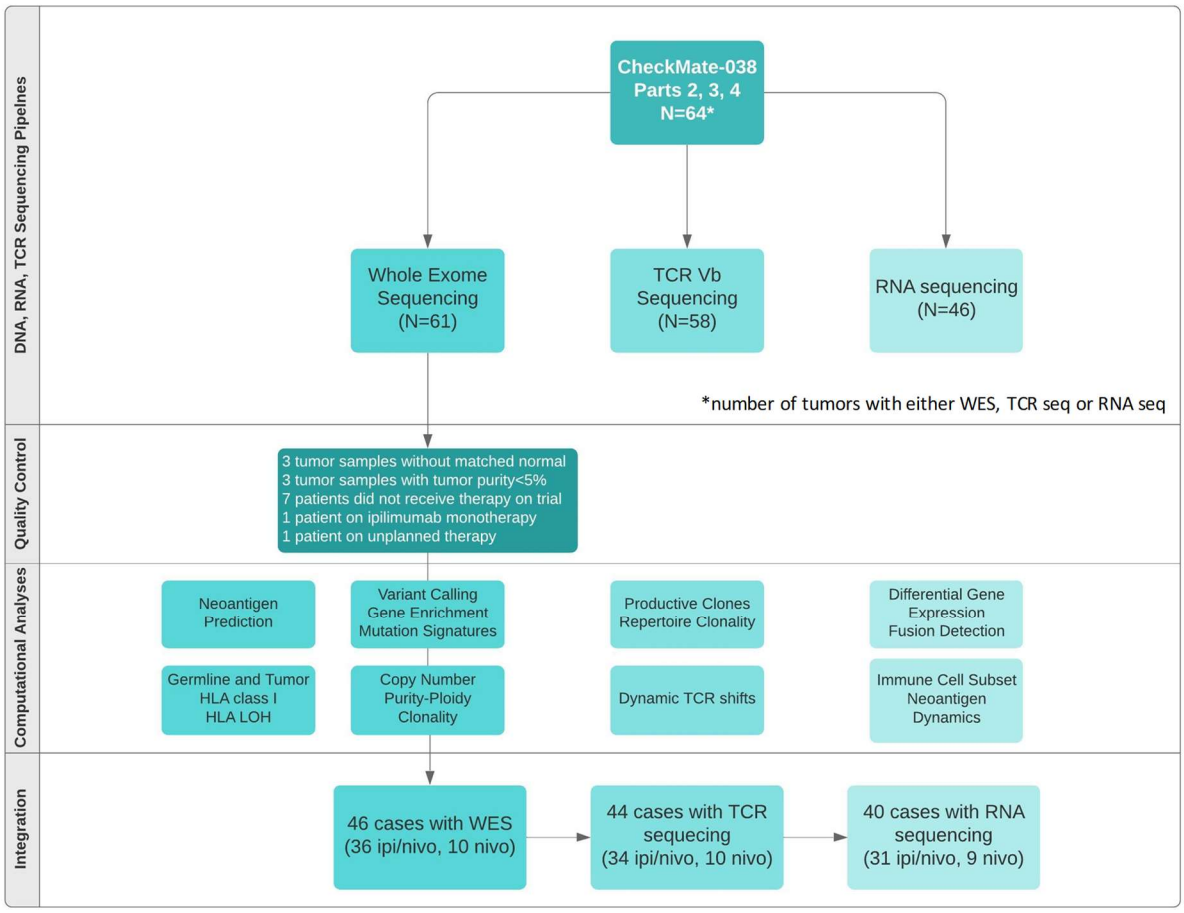
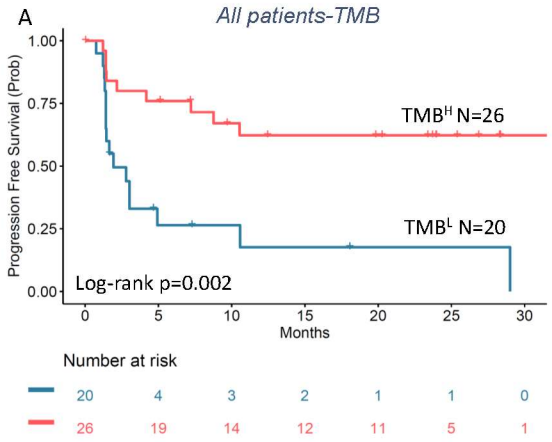
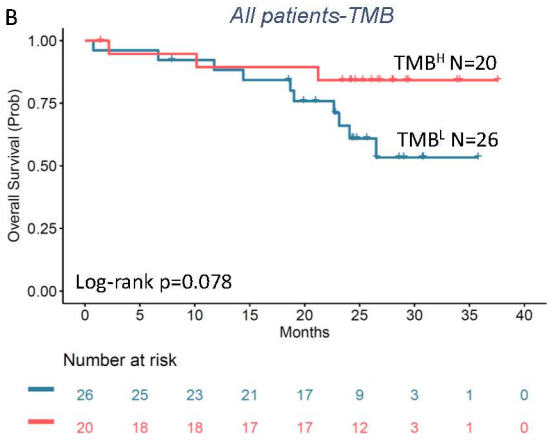


Figure S1. Overview of the whole exome, transcriptome and TCR sequencing analyses. Related to Figures 1-6. CheckMate-038 was a prospective, multi-cohort clinical trial of nivolumab as front-line therapy or after progression on therapy with ipilimumab, or receiving ipilimumab/nivolumab combination. Here, we focused on patients on parts 2, 3 and 4 which included ipilimumab-naïve patients treated either with nivolumab or combination nivolumab/ipilimumab. Sixty four tumors in parts 2, 3 and 4 had evaluable whole exome sequencing, RNA sequencing or TCR sequencing. As an initial quality control step the following cases were excluded: i. 3 tumor samples without matched normal samples, ii. 3 tumors with tumor purity less than 5%, iii. 7 patients that did not ultimately receive therapy on the CheckMate-038 trial, iv. One patient on ipilimumab monotherapy and v. One patient on unplanned therapy. Forty six baseline tumors had evaluable whole exome sequencing data which was processed for variant calling, neoantigen prediction, copy number analyses, purity-ploidy correction, HLA class I germline and somatic status assessment, gene enrichment and mutation signature analyses. Of these, forty four tumors were analyzed by TCR sequencing and forty tumors were also analyzed by RNA sequencing.


A
All patients-TMB
Progression Free Survival (Prob)
1.00
0.75
0.50
0.25
0.00
TMB^H N=26
TMB^L N=20
Log-rank p=0.002
0 5 10 15 20 25 30
Months
Number at risk
20 4 3 2 1 1 0
26 19 14 12 11 5 1


B
All patients-TMB
Overall Survival (Prob)
1.00
0.75
0.50
0.25
0.00
TMB^H N=20
TMB^L N=26
Log-rank p=0.078
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Months
Number at risk
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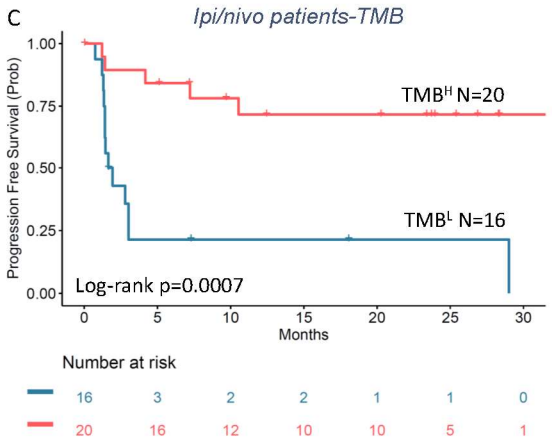
20 18 18 17 17 12 3 1 0


C
Ipi/nivo patients-TMB
Progression Free Survival (Prob)
1.00
0.75
0.50
0.25
0.00
TMB^H N=20
TMB^L N=16
Log-rank p=0.0007
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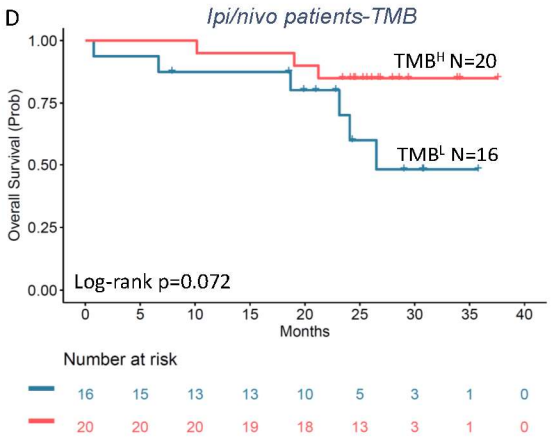
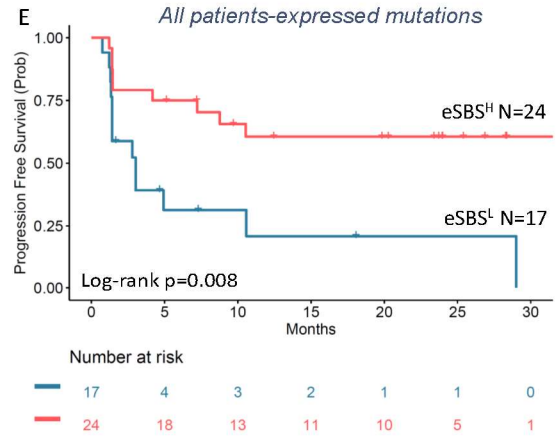
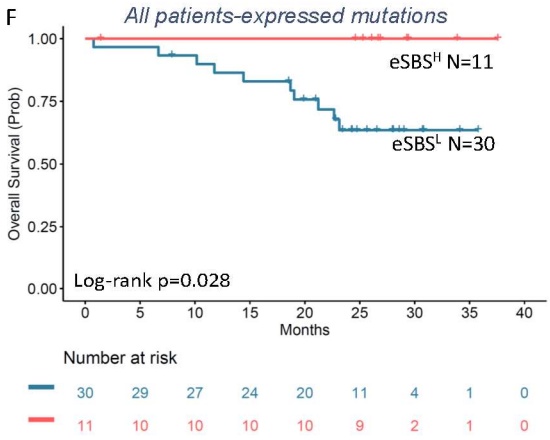
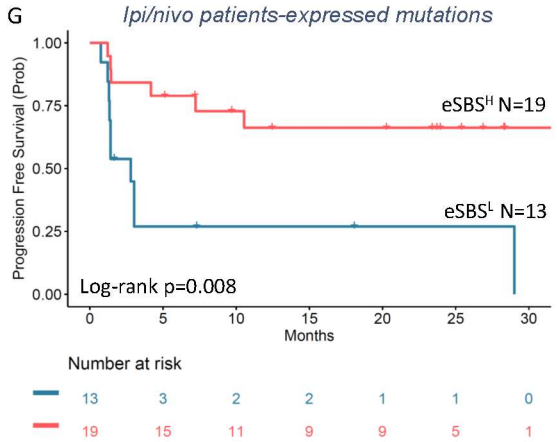
Months
Number at risk
16 3 2 2 1 1 0
20 16 12 10 10 5 1


D
Ipi/nivo patients-TMB
Overall Survival (Prob)
1.00
0.75
0.50
0.25
0.00
TMB^H N=20
TMB^L N=16
Log-rank p=0.072
0 5 10 15 20 25 30 35 40
Months
Number at risk
16 15 13 13 10 5 3 1 0
20 20 20 19 18 13 3 1 0


E
All patients-expressed mutations
Progression Free Survival (Prob)
1.00
0.75
0.50
0.25
0.00
eSBS^H N=24
eSBS^L N=17
Log-rank p=0.008
0 5 10 15 20 25 30
Months
Number at risk
17 4 3 2 1 1 0
24 18 13 11 10 5 1


F
All patients-expressed mutations
Overall Survival (Prob)
1.00
0.75
0.50
0.25
0.00
eSBS^H N=11
eSBS^L N=30
Log-rank p=0.028
0 5 10 15 20 25 30 35 40
Months
Number at risk
30 29 27 24 20 11 4 1 0
11 10 10 10 10 9 2 1 0


G
Ipi/nivo patients-expressed mutations
Progression Free Survival (Prob)
1.00
0.75
0.50
0.25
0.00
eSBS^H N=19
eSBS^L N=13
Log-rank p=0.008
0 5 10 15 20 25 30
Months
Number at risk
13 3 2 2 1 1 0
19 15 11 9 9 5 1

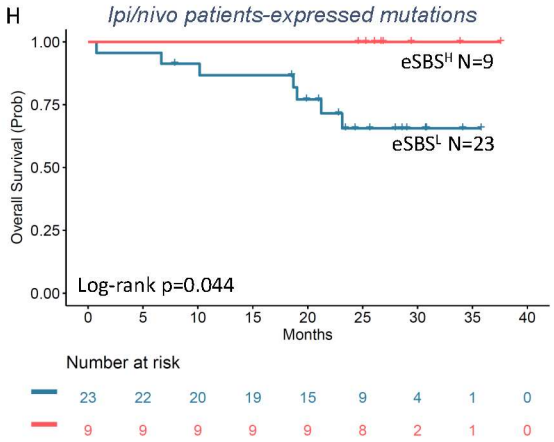

H
Ipi/nivo patients-expressed mutations
Overall Survival (Prob)
1.00
0.75
0.50
0.25
0.00
eSBS^H N=9
eSBS^L N=23
Log-rank p=0.044
0 5 10 15 20 25 30 35 40
Months
Number at risk
23 22 20 19 15 9 4 1 0
9 9 9 9 9 8 2 1 0

Figure S2. Association between TMB and expressed mutation load and clinical outcome. Related to Figure 1. (A) TMB predicted PFS for all patients, such that patients in the TMB-high group had a significantly longer PFS compared to the TMB-low group (median PFS not reached vs 1.938 months, HR=0.263, 95% CI: 0.114-0.609, log rank p=0.02). (B) There was a trend towards longer OS for patients with TMB-high tumors compared to the TMB-low group (median OS not reached for both groups, HR=0.334, 95% CI: 0.092-1.217, log rank p=0.078). (C) In the ipilimumab/nivolumab treatment group, high TMB conferred a favorable prognosis (median PFS for TMB-high and TMB-low patients not reached and 1.791 respectively, HR =0.171, 95% CI: 0.059-0.498, log rank p=0.0007). (D) A trend towards longer OS was observed for patients with TMB-high tumors in the ipilimumab/nivolumab treatment group (median OS not reached and 26.513 months for TMB-high and TMB-low tumors respectively, HR=0.291, 95% CI: 0.072-1.171, log rank p=0.072). (E) Single base substitutions in expressed genes were evaluated as a representation of the expressed mutation load (eSBS). Expressed mutation load predicted PFS for all patients, such that patients in the eSBS-high group had a significantly longer PFS compared to the eSBS-low group (median PFS not reached vs 3.023 months, HR=0.307, 95% CI: 0.129-0.731, log rank p=0.008). (F) Patients with eSBS-high tumors had a longer overall survival compared to the eSBS-low group (median OS not reached for both groups, log rank p=0.028). (G) In the ipilimumab/nivolumab treatment group, high eSBS conferred a favorable prognosis (median PFS for eSBS-high and eSBS-low patients not reached and 2.793 months respectively, HR =0. 245, 95% CI: 0.087-0.693, log rank p=0.008). (H) Patients with eSBS-high tumors had a longer OS compared to eSBS-low patients in the ipilimumab/nivolumab treatment group (median OS not reached for both groups, log rank p=0.044).

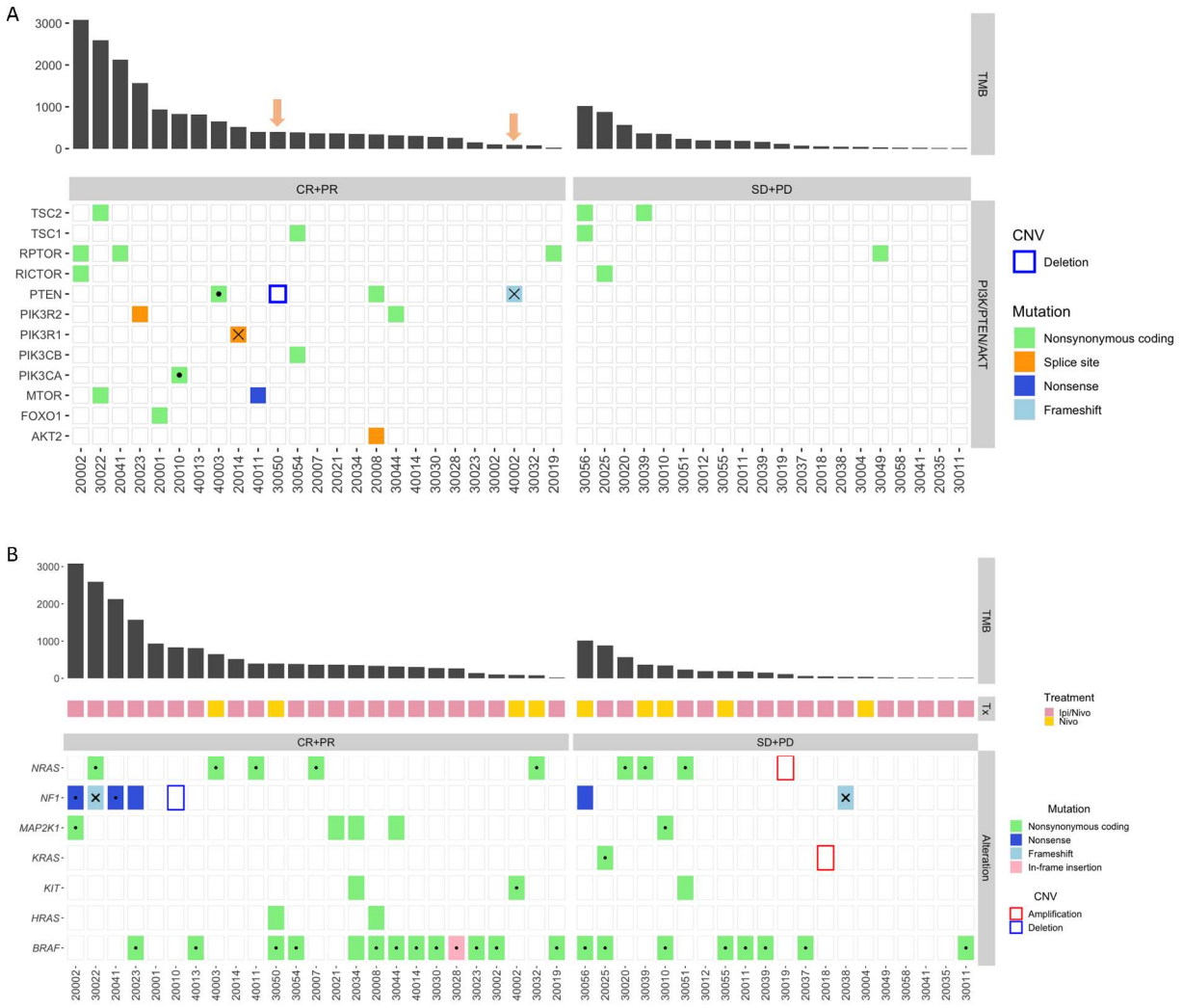


Figure S3. Differential enrichment analysis involving genes in the PI3K/AKT/PTEN and Ras pathways in tumors from responding and non-responding patients. Related to Figure 1. (A) We did not identify any significant differences in genomic alterations in the PI3K/AKT/PTEN pathway in tumors from responders vs. non-responders that could not be explained by the increased TMB of responding patients. Patient 40002, who achieved a radiographic response but had a short overall survival harbored biallelic inactivation of PTEN. In contrast, patient 30050, who achieved a long-term response to therapy, harbored a homozygous deletion in PTEN, suggesting that PTEN inactivation is not always associated with resistance to immune checkpoint blockade. We investigated co-occurrence of sequence and structural mutations in the Ras pathway and single base substitutions were characterized by consequence (missense, frameshift, nonsense, splice site, in-frame) and recurrence (hotspots). Loss of the wild type allele was considered in case of truncating mutations (biallelic inactivation, indicated as an "x"). We did not identify any significant differences in genomic alterations in the Ras pathway in tumors from responders vs non-responders that could not be explained by the increased TMB of responding patients. Dots indicate hotspot mutations.

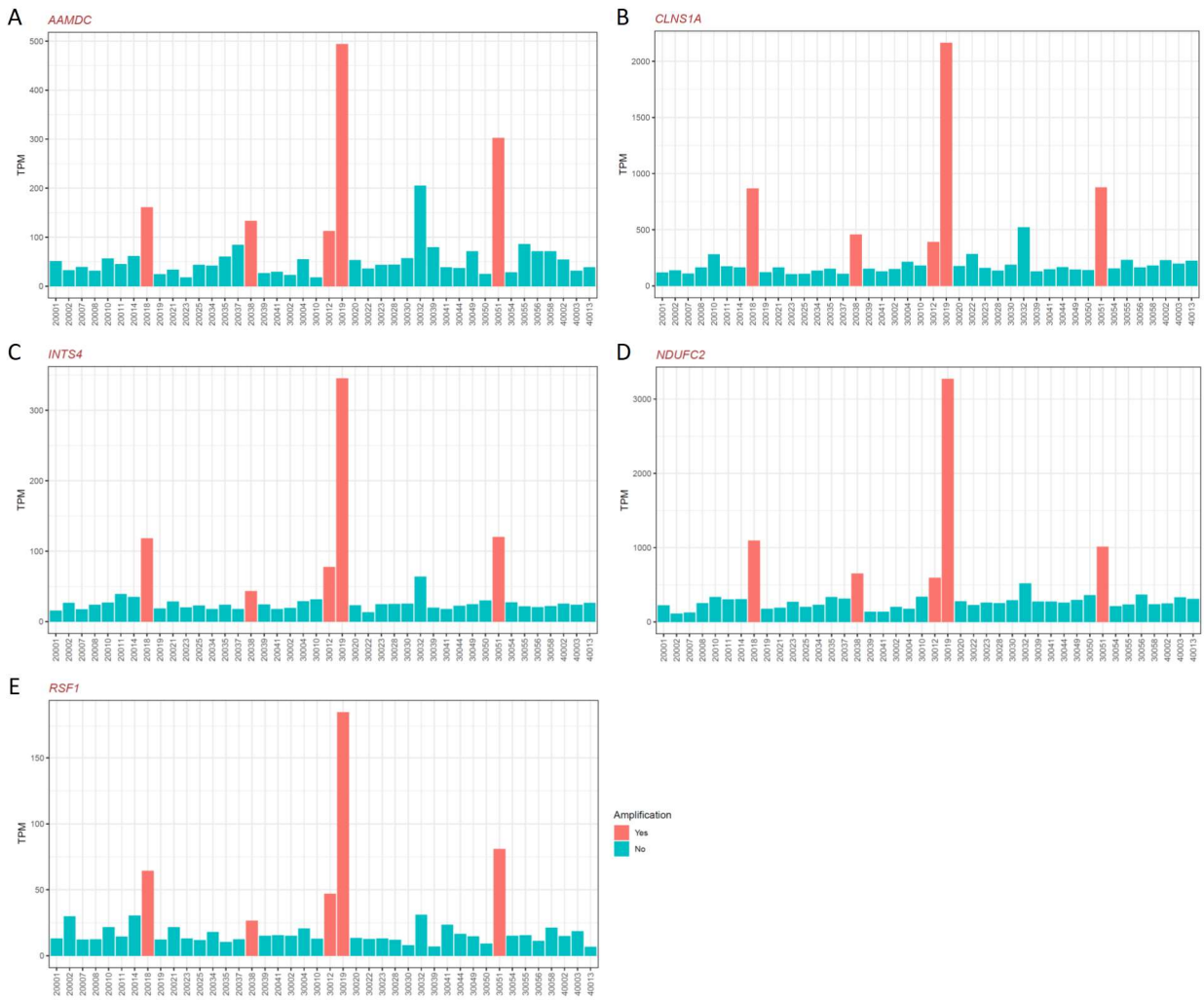


Figure S4. Gene expression of genes in the 11q14.1 locus. Related to Figure 1. Tumors from patients 20018, 20038, 30012, 30019 and 30051 that harbored an amplification in 11q14.1, we are also found to overexpress AAMDC, CLNS1A, INTS4, NDUFC2, and RSF1. Gene expression is shown in transcripts per million (TPM).

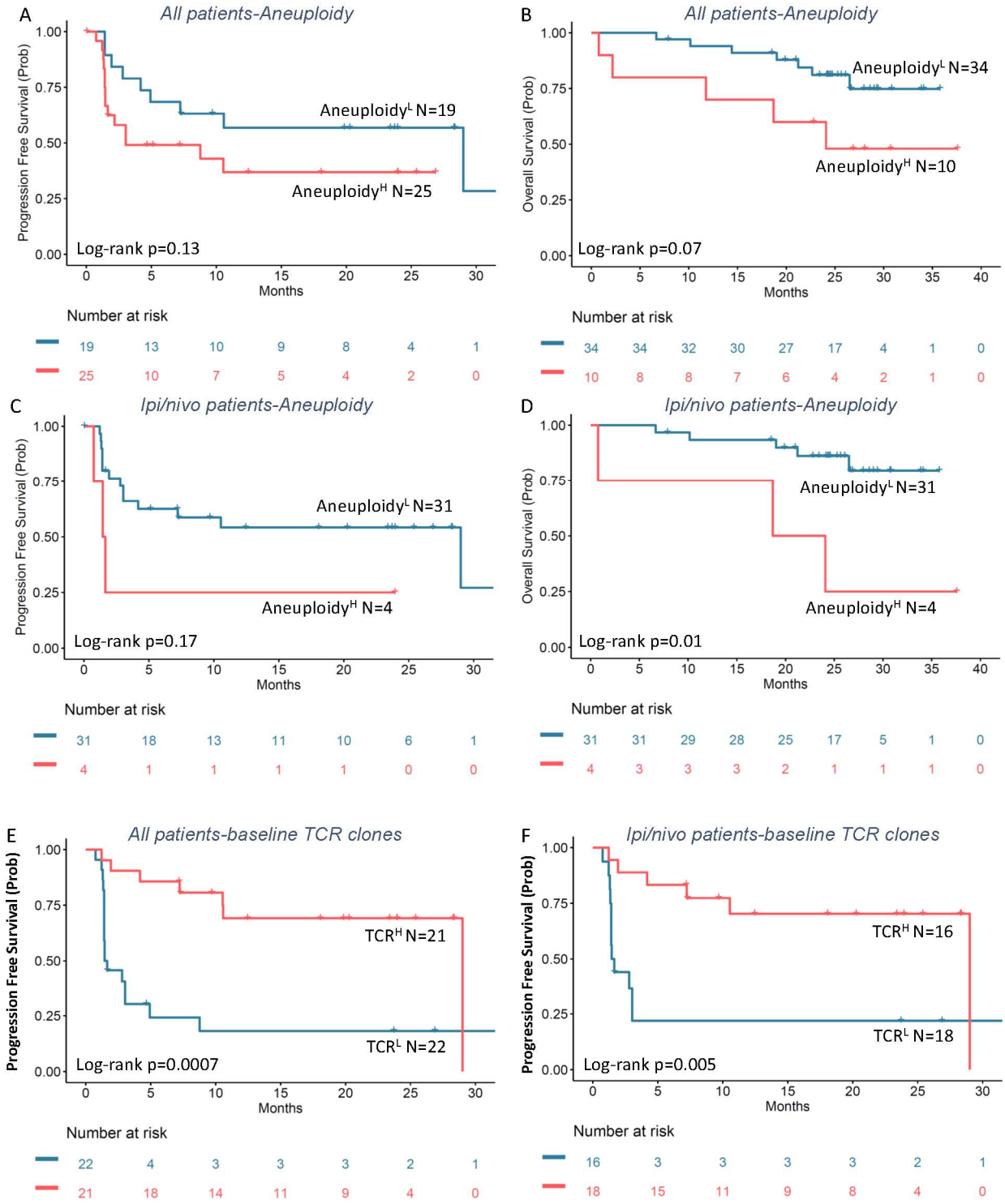


Figure S5. High tumor aneuploidy and low TCR clonotypic density identify patients with inferior outcome to immune checkpoint blockade. Related to Figures 1, 2 and 5. (A) Patients with highly aneuploidy tumors showed a trend towards shorter PFS (median PFS of 3.023 vs 29.01 for aneuploidy-high vs low tumors, HR=1.958, 95% CI: 0.818-4.689, log rank p=0.13). (B) A similar trend was observed for OS (median OS of 24.082 vs not reached for aneuploidy-high vs low tumors, HR=2.899, 95% CI: 0.919-9.152, log rank p=0.13). (C) Patients in the ipilimumab/nivolumab treatment group with highly aneuploidy tumors showed a trend towards shorter PFS (median

PFS of 1.544 vs 29.01 for aneuploidy-high vs low tumors, HR=2.749, 95% CI: 0. 0.773-9.777, log rank p=0.17). (D) Patients with highly aneuploidy tumors in the ipilimumab/nivolumab treatment group had a significantly shorter OS (median OS of 21.388 vs not reached for aneuploidy-high vs low tumors, HR=6.309, 95% CI: 1.5-26.538, log rank p=0.01). (E) An increased number of baseline intratumoral TCR productive clones predicted longer PFS for all patients (median PFS 29.01 vs 1.544 months for patients with TCR-high vs TCR-low tumors respectively, HR=0.215, 95% CI: 0.087-0.532, log rank p=0.0007) and (F) patients in the ipilimumab/nivolumab treatment group (median PFS for TCR-high and TCR-low patients 29.01 and 1.544 months respectively, HR =0.236, 95% CI: 0.086-0.649, log rank p=0.005).

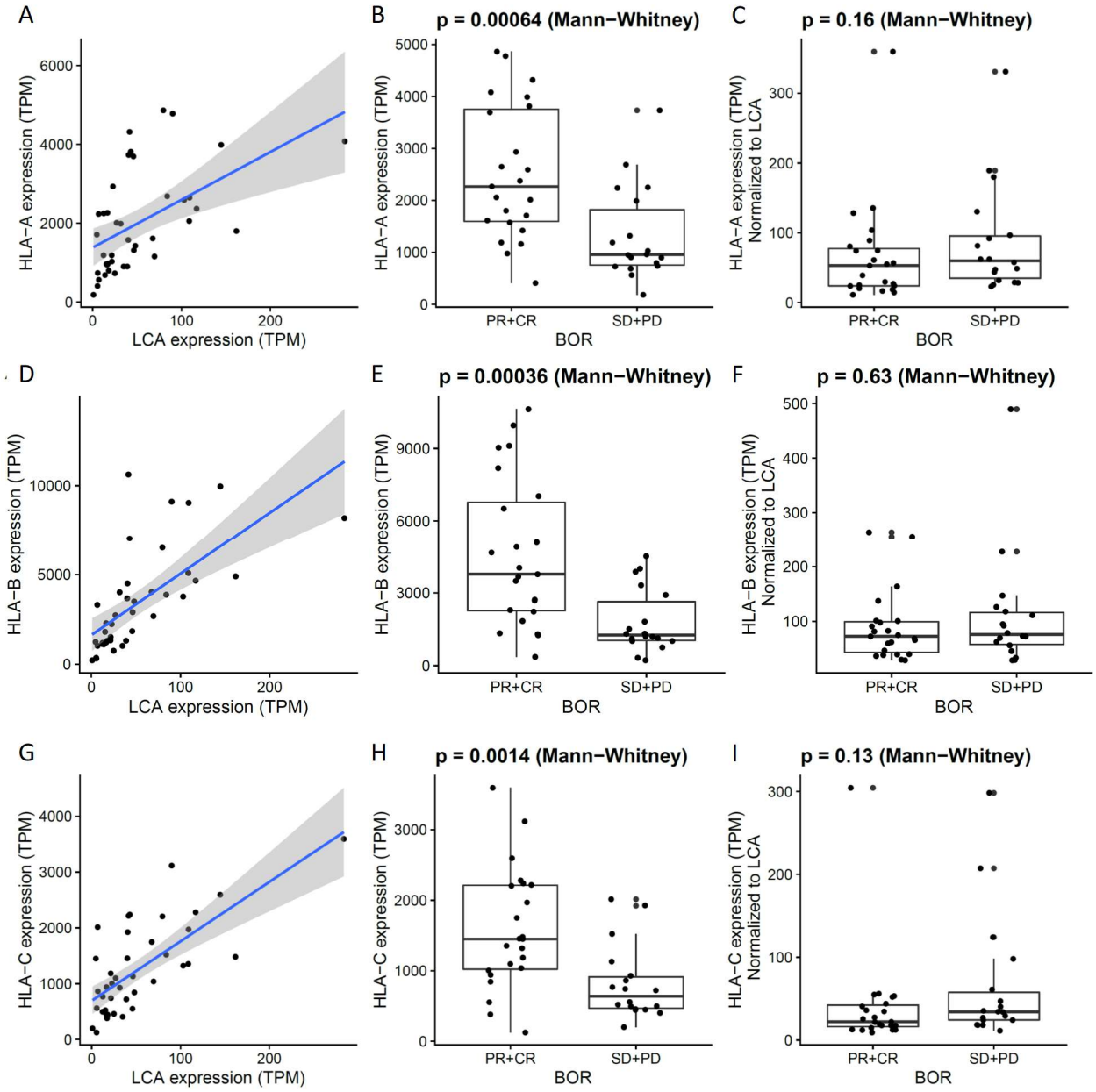


Figure S6. HLA class I genomic diversity in tumors of responding and non-responding patients. Related to Figure 2. A significantly higher expression of HLA-A (B-D), HLA-B (E-G) and HLA-C (H-J) was noted in baseline tumors from responding patients, which reflected a higher density of lymphocytic intratumoral infiltration. When HLA-A, HLA-B and HLA-C expression was normalized by LCA expression, there were no differences between tumors from responding and non-responding patients.

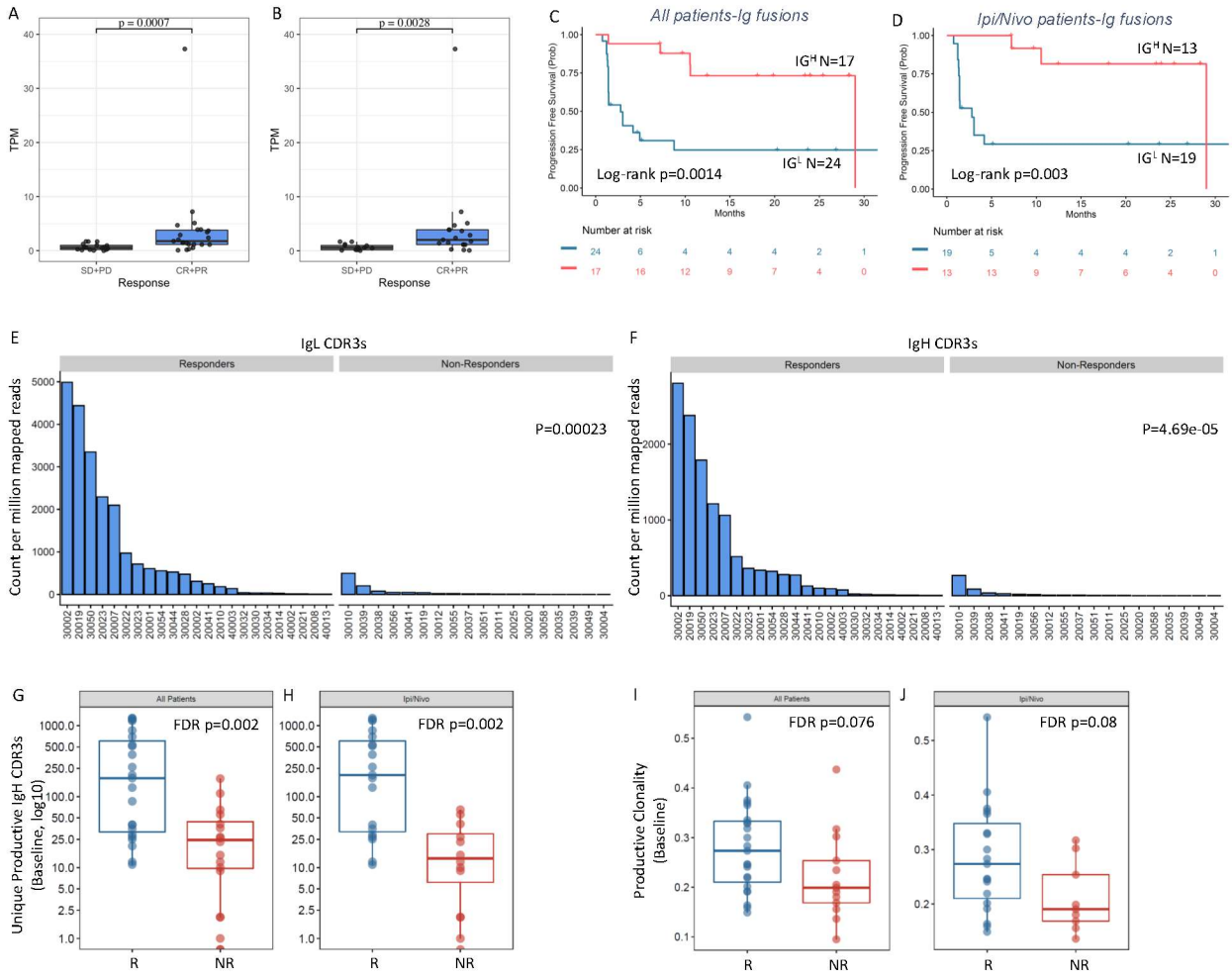


Figure S7. T and B cell features differentiate tumors from responding patients from non-responders. Related to Figures 3, 4 and 5. (A-B) Differential expression of TOX in tumors of responding and non-responding patients. A higher expression of TOX was identified in baseline tumors of responding patients (A; all patients and B; patients in the ipilimumab/nivolumab treatment group). (C-D) High number of immunoglobulin fusions is linked to a progression-free survival benefit on immune checkpoint blockade. (C) An increased number of baseline Immunoglobulin gene fusions, signifying an increased B cell density, predicted longer PFS for all patients (median PFS 29.01 vs 2.793 months for patients with Ig-high vs Ig-low tumors respectively, HR=0.217, 95% CI: 0.078,0.601, -log rank p=0.0014) and (D) patients in the ipilimumab/nivolumab treatment group (median PFS for Ig-high and Ig-low patients 29.01 and 2.793 months respectively, HR=0.180, 95% CI: 0.050-0.645, log rank p=0.003). (E-F) Differences in IgL and IgG abundance in tumors of patients with differential responses to immune checkpoint blockade. IgL and IgH chain CDR3 sequences were assembled from RNAseq data and we identified significantly higher clonal counts for both immunoglobulin light (E) and heavy (F) chains (Mann Whitney p=0.00023 and p=4.69e-05 respectively) in tumors from responding patients. (G-J) Differential IgH CDR3 abundance analysis in baseline tumors. (G-H) A higher IgH CDR3 clonal count was noted in tumors from responding patients for all patients (FDR adjusted p=0.002) and patients in the ipilimumab/nivolumab treatment group (FDR adjusted p=0.002). (I-J) A trend towards a more clonal BCR repertoire was observed for all patients (FDR adjusted p=0.076) and patients in the ipilimumab/nivolumab group (FDR adjusted p=0.08). TPM; transcripts per million.

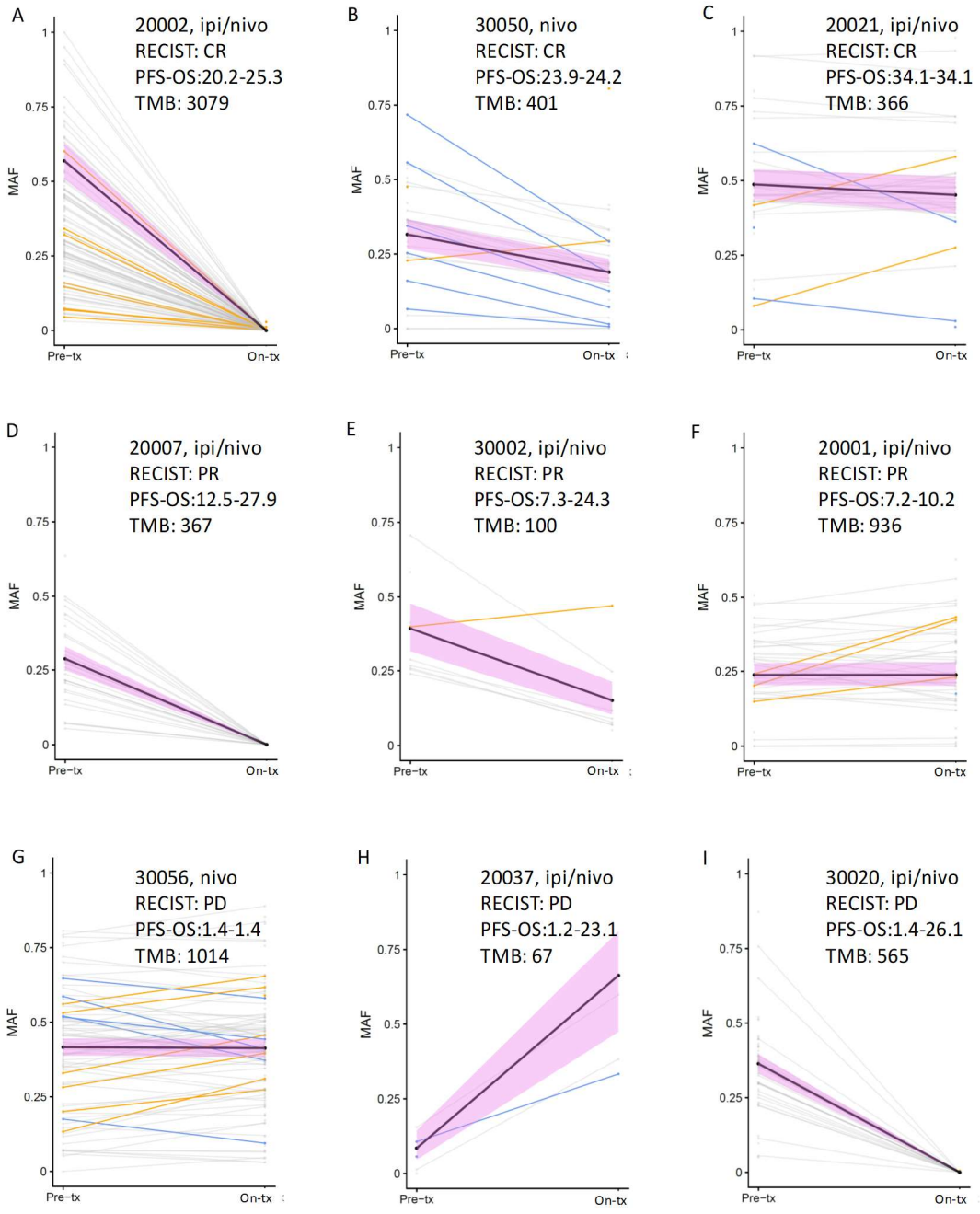


Figure S8. Expressed mutation dynamics during immune checkpoint blockade. Related to Figure 1. A Bayesian generalized linear multilevel mixed-effects model was implemented to interpret changes in mutant transcript levels between pre-treatment and on-treatment tumors. We fit a generalized linear mixed effects model with a fixed effect for the overall mean mutant allele fraction (MAF) pre-therapy and the overall slope describing the change in the average MAF pre- and on-therapy (dark black line with 95% confidence band shaded in purple). Mutations covered by at least 75 reads at both time points are shown and are colored orange if there is >0.99 probability that their slope is positive relative to the overall slope, and blue is there is a >0.99 probability that their slope is negative relative to the overall slope. Panels A, D, E and I correspond to molecular responders with MAFs that uniformly decreased to undetectable levels post-therapy and molecular responses reflected clinical outcomes. Patient 30020 (I) showed a clear pattern of molecular response that was reflected in a long overall survival of 26.1 months, but not captured by early radiographic assessments which showed disease progression at 1.4 months. Responding patient 20021 (C) showed a pattern of expressed mutation retention, most likely due to timing of tumor

biopsy that may have preceded tumor clearance. Panels F, G and H show molecular non-responders, denoted as retention of expressed mutations, with evidence of both positive- and negative- selective pressures of therapy (orange and blue lines respectively) that cannot be explained by overall difference in mean MAFs (black line). Patient 20001 (F) showed a molecular progression pattern that was reflective of a short OS of 10.2 months, despite a radiographic response assessment of partial response.

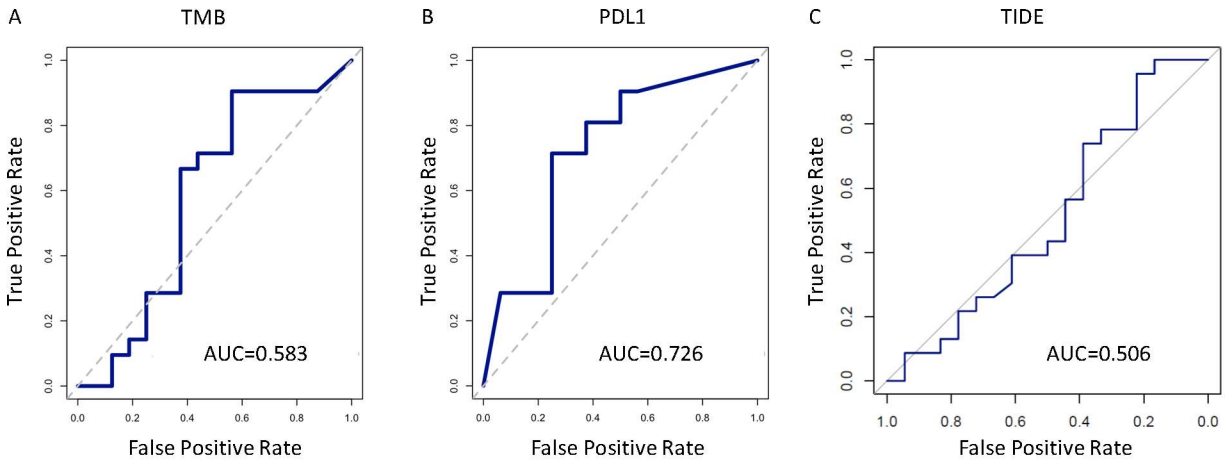


Figure S9. Predictive accuracy of TMB and PD-L1 expression. Related to Figure 6. (A) TMB and (B) PD-L1 expression demonstrated a moderate prediction accuracy with an AUC of 0.583 (95% CI: 0.377-0.79) and 0.726 (95% CI: 0.552-0.9) respectively. (C) The TIDE algorithm was not predictive of outcome in this cohort (AUC=0.506).