|  |  |  |  |
| --- | --- | --- | --- |
| **Supplementary Table 1: Results for samples obtained ≤12 months prior to therapy (“optimal”) vs. those obtained >12 months from treatment or shortly after starting therapy (“non-optimal”)** | | | |
| **Optimal samples (n = 43)** | | | |
|  | *Responders*  *n = 21* | *Non-responders*  *n = 22* | *p-value* |
| Mutational load (mutations/MB) | 43.5 | 8.1 | <0.001 |
| **Non-optimal samples (n = 22)** | | | |
|  | *Responders*  *n = 11* | *Non-responders*  *n = 11* | *p-value* |
| Mutational load (mutations/MB) | 37.5 | 5.5 | 0.027 |

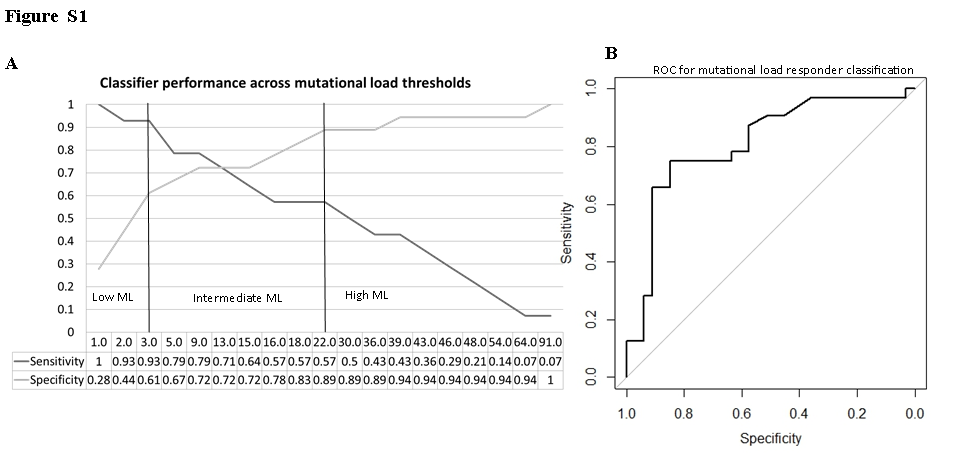
**Supplementary Table 2: Cox proportional hazards model of OS adjusting for baseline variables**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | | Coefficient  () | Standard  Error () | P value | HR | 95% CI |
| Mutational Load Group | |  |  |  |  |  |
|  | Intermediate vs. Low | -0.22 | 0.48 | 0.64 | 0.80 | (0.31, 2.0) |
|  | High vs. Low | -2.39 | 0.65 | 0.0002 | 0.09 | (0.02, 0.32) |
| Age | | -0.01 | 0.02 | 0.54 | 0.99 | (0.95, 1.0) |
| Gender |  |  |  |  |  |  |
|  | M vs. F | 0.49 | 0.48 | 0.30 | 1.64 | (0.64, 4.2) |
| Stage | |  |  |  |  |  |
|  | IVc vs. IIIC - IVb | 0.29 | 0.64 | 0.65 | 1.33 | (0.38, 4.67) |
| Prior ipilimumab | |  |  |  |  |  |
|  | Yes vs. No | 1.35 | 0.47 | 0.004 | 3.85 | (1.53, 9.69) |

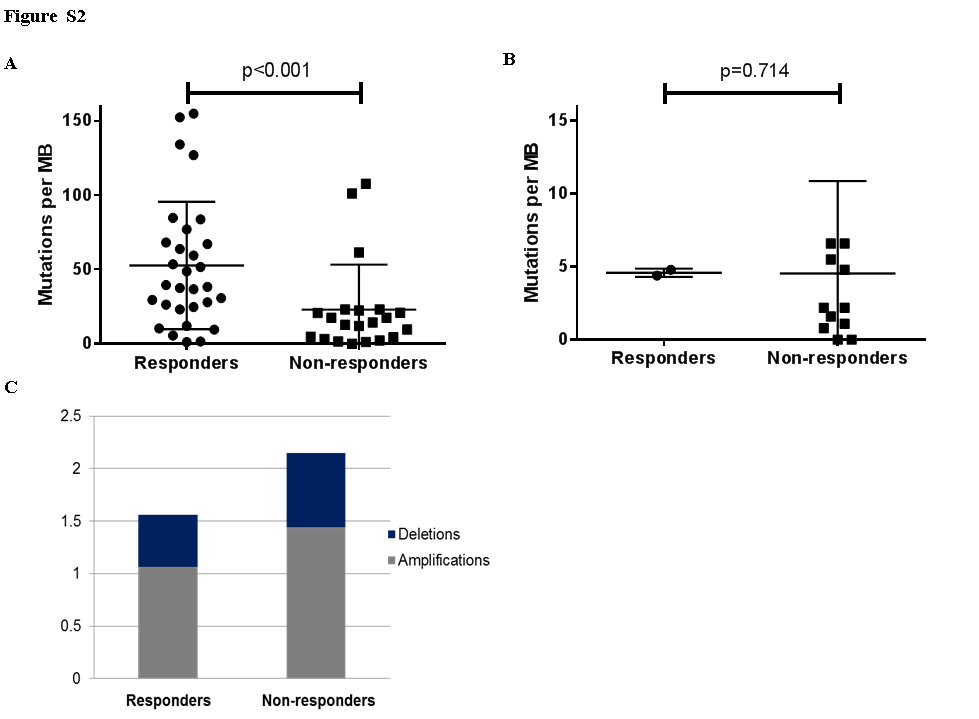
**Supplementary Table 3: Cox proportional hazards model of PFS adjusting for baseline variables**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | | Coefficient  () | Standard  Error () | P value | HR | 95% CI |
| Mutational Load Group | |  |  |  |  |  |
|  | Intermediate vs. Low | -0.10 | 0.40 | 0.80 | 0.90 | (0.41, 1.99) |
|  | High vs. Low | -2.00 | 0.48 | <0.0001 | 0.14 | (0.05, 0.35) |
| Age | | 0.0002 | 0.01 | 0.99 | 1.0 | (0.97, 1.0) |
| Gender |  |  |  |  |  |  |
|  | M vs. F | -0.20 | 0.34 | 0.54 | 0.81 | (0.42, 1.58) |
| Stage | |  |  |  |  |  |
|  | IVc vs. IIIC - IVb | -0.10 | 0.44 | 0.82 | 0.90 | (0.38, 2.16) |
| Prior ipilimumab | |  |  |  |  |  |
|  | Yes vs. No | 0.20 | 0.35 | 0.56 | 1.23 | (0.62, 2.44) |

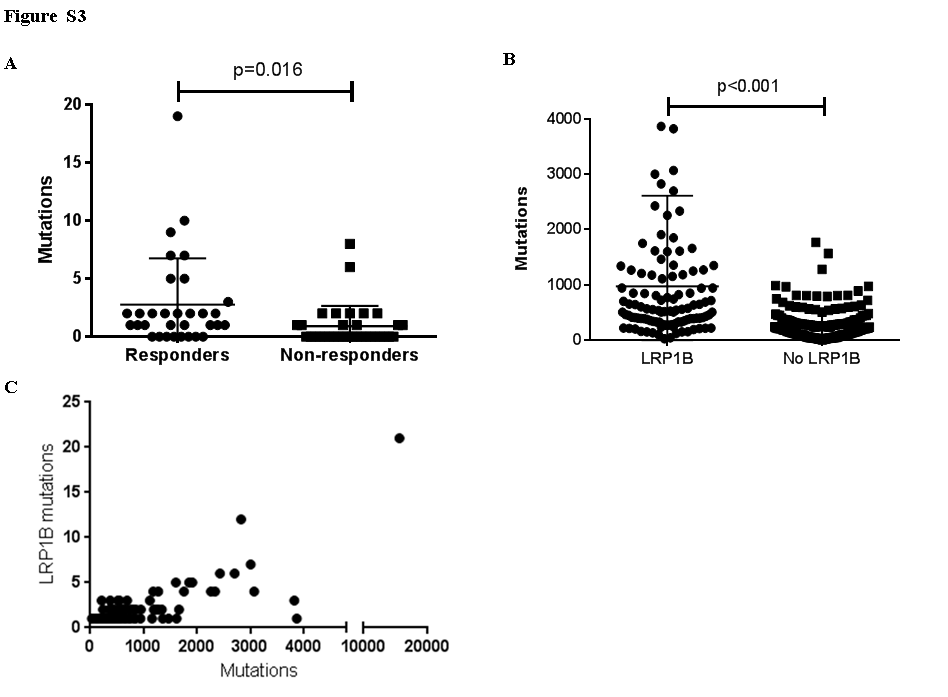
**Supplemental Figures**

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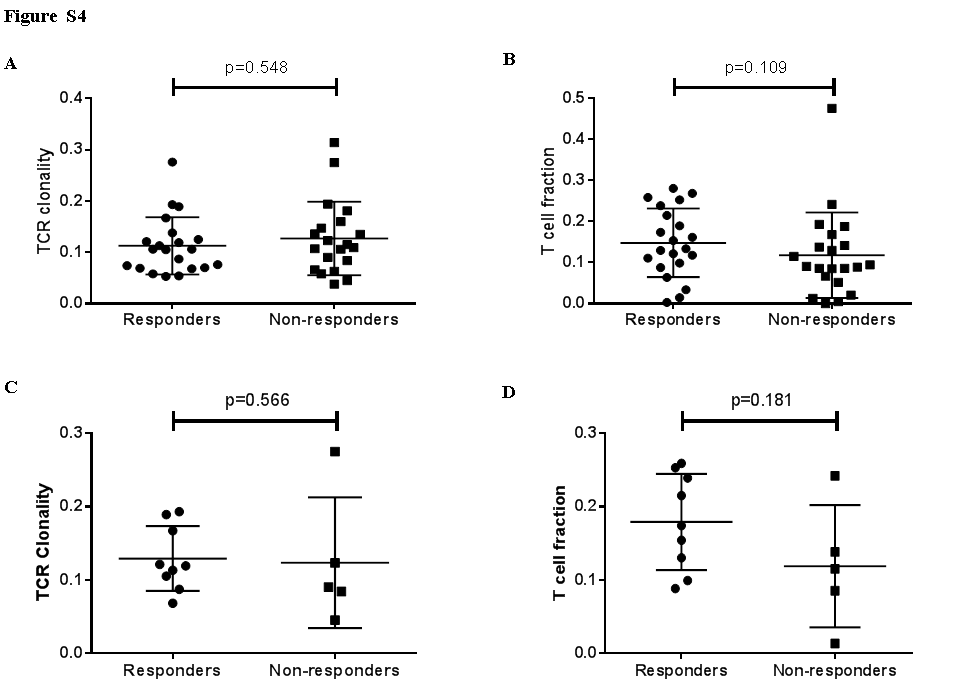
**Figure S1**: (A) Performance of mutational load across a range of potential thresholds. Vertical bars indicate the thresholds selected based on local performance maxima and clinical relevance. (B) Receiver Operating Curve (ROC) for mutational loads cutoffs of 3·3 mutations/MB (low mutational load group) and 23·1 mutations/MB (high mutational load group).



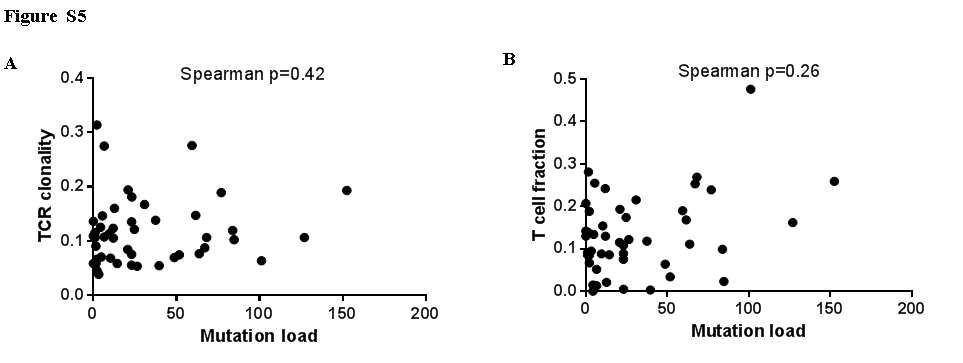
**Figure S2:** (A) Mutational load in tumors with cutaneous/unknown primaries in responders vs. non-responders. (B) Mutational load in tumors with non-cutaneous primaries (acral, mucosal, uveal) in responders vs. non-responders.(C)Gene amplifications and deletions in responders vs. non-responders.



**Figure S3**: (A) *LRP1B* mutations/variants of unknown significance in responders vs. non-responders. (B) Total number of mutations among melanomas with and without *LRP1B* mutations. (C) Association between number of *LRP1B* mutations and total mutations in the melanoma TCGA (The Cancer Genome Atlas).

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**Figure S4**: (A) T cell receptor (TCR) clonality in responders vs. non-responders. (B) T cell fraction in responders vs. non-responders. (C) TCR clonality in responders vs. non-responders in “ideal” samples, defined as those obtained within 4 months of anti-PD-1/PD-L1 treatment without other prior therapies. (D) T cell fraction in these “ideal” samples.



**Figure S5**: Mutation load correlated with (A) T cell receptor (TCR) clonality and (B) T cell fraction.