# RESPONSE LETTER

## Reviewer #1 (Josh's review)

### -- Ref 1.0 Clarifying terminology–--

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| Reviewer Comment | It is reassuring that the authors used published definitions. In that case, please insert the appropriate references to the literature where the terms are first introduced if that hasn't been done already. |
| Author Response | We thank the reviewer for suggestion. In the updated manuscript, we have already used references at appropriate position. |
| Excerpt From Revised Manuscript | |

### -- Ref 1.1 – Background model--

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| Reviewer Comment | The authors have addressed the issue of including additional covariates as best as I can envision at this point. |
| Author Response | We thank the reviewer for recognizing the robustness of our updated background model. |
| Excerpt From Revised Manuscript | |

### -- Ref 1.3 –Response variable--

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| Reviewer Comment | The authors have now clarified their response variable more clearly. |
| Author Response | We thank the reviewer for confirming our edits to be clear. |
| Excerpt From Revised Manuscript | |

### -- Ref 1.4 – Choice of random effects model --

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| Reviewer Comment | The authors have documented the prior literature and appropriateness of the application of their chosen random effects model. |
| Author Response | Reviewer's earlier suggestion was very helpful in this regard. We thank him for mentioning this earlier. |
| Excerpt From Revised Manuscript | |

## -- Ref 1.5 – Related to LoF spectrum –

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| Reviewer Comment | The authors have addressed this point by making clarifying changes to the text and Figure 3. |
| Author Response | We thank the reviewer for going through the updated text and confirming this. |
| Excerpt From Revised Manuscript | |

## -- Ref 1.6 – Analysis of samples w/o driving mutations –

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| Reviewer Comment | Could the authors coordinate with Nuria's group that did the patient specific analysis for this work? They correlate the increase in putative passengers in those samples that lack known drivers. Nuria's group attempted to expand the set of drivers by assessing the expected number of passengers in each sample, calculating an "excess" and then re-analyzing whether variants found in those samples might be reconsidered as drivers. If the authors haven't done so already, I'd suggest they use this new list of samples lacking known drivers for this correlative analysis. |
| Author Response | As suggested, our updated results are based on the updated version of driver mutation list curated by Nuria's group. |
| Excerpt From Revised Manuscript | |

## -- Ref 1.7 – Overlap of results w/ driver group for coding genes --

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| Reviewer Comment | Overlap of results w/ driver group for coding genes: Did the authors restrict the analysis to coding regions as suggested by Gaddy? I can't tell from the response. |
| Author Response | Yes, we did this analysis for both coding and noncoding driver elements as suggested. However, we note that despite being interesting we downplay this result as we restrict our analysis only for PCAWG samples without known driver mutations. |
| Excerpt From Revised Manuscript | |

## -- Ref 1.8 – Definition of impact assessment for SVs --

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| Reviewer Comment | The authors point us to the supplement, section 4.2, for their description of how they assessed the impact of SVs. I found this section very confusing and needing a rewrite for clarity. The method is a machine-learning (random forest) based approach that takes in a set of features and predicts the impact of an SV. However, I could not find a clear description of the prediction labels anywhere in the section that would help me understand the gold standard their method is trying to predict. What is the overall target of the prediction? Is it the presence/absence of an SV at all? This needs to be explicitly stated somewhere. Also, the set of features is not listed but instead a windowing approach is defined to account for different SV length sizes. The authors should tabulate the features used for the model. Also, its not clear to me how the 1000 genomes data is being used for this and the rationale on why any of it should be included in the training since one might expect germline events to be generated under very different processes distinct from somatic events? Finally, its not clear what the SV impact score (SVIS) reflects. It is some probability that an SV would be seen in a particular window given the features of that window? If so, how would that correspond to an impact? Would genomic regions with little impact have **more** SV potential since their alterations have relatively smaller functional effect? |
| Author Response | We thank the reviewer for pointing this out. We have updated the supplement to further clarify this section and provide more details on the features utilized for the prediction. The overall goal of prediction to assign a prioritization score to each SV based on how divergent it's features are from a common benign SV (based on 1KG SV dataset). |
| Excerpt From Revised Manuscript | |

# Reviewer #2 (Peter's comments)

## -- Ref 2.0 Original & random functional impact distribution–--

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| Reviewer Comment | I would like to see what Figure 1a looks like for totally neutral simulated mutations – it is difficult to know how much the three peaks are explicable by the background mutational process. |
| Author Response | In past, we have done this analysis and found significant differences. However, member of steering committee pointed out these differences could be attributed to imprecision in background model. With the updated background model, the overall differences are not significant for majority of cancer cohorts. However, we restrict this comparison to specific regions of the genome then for few cohorts we do observed some differences. |
| Excerpt From Revised Manuscript | |

## -- Ref 2.1 – BLUP prediction on somatic SNVs in normal tissues--

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| Reviewer Comment | In the additive model, the question remains as to whether the small excess predictive signal derived from the putative passengers in true cancer samples relates to unmodelled factors influencing mutation distribution or to selection on non-coding mutations. One potential way to assess this would be to apply the BLUP predictor to sets of genome-wide somatic mutations in normal tissues (rather than cancers). The two datasets that I am aware of for this are our one whole genome from normal skin and the 45 genomes in liver, small and large bowel organoids from Ruben van Boxtel (published in Nature, 2016). If selection drives the signal in the additive model, then these should cluster with the simulated samples; if it's unaccounted mutational processes then they should cluster with the cancer samples (albeit the mutational landscape of the normal samples is less rich than the cancers). |
| Author Response | We thank the reviewer for giving this suggestion. Although this suggestion is intriguing and we decided to follow-up on this. However, as the reviewer mentions the number of samples/mutations in both studies are very low to perform the BLUP analysis. |
| Excerpt From Revised Manuscript | |

## -- Ref 2.2 – Influence of aneuploidy on additive variance--

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| Reviewer Comment | The finding of the lower predictive capacity when samples with CNAs are removed is an interesting one – what does arm-level or whole chromosome aneuploidy do to the predictive model? In other words, 1q is commonly gained across many many tumours – and correspondingly has a higher mutation burden overall compared to other regions. Would this lead to apparent discriminative power in the additive model for variants on 1q? One could test this by looking at the genomic distribution of BLUP estimates of the coefficients – at individual-gene level, these should be somewhat correlated, but decay rapidly to be minimal at the chromosome arm-level |
| Author Response | We thank the reviewer for this suggestion. The current model doesn't explore the influence of aneuploidy on predictability. In the future iteration of this model, we do intend to explore this question in detail. |
| Excerpt From Revised Manuscript | |

# Reviewer #3 (Gaddy's review)

## -- Ref 3.0 use predicted impact score–--

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| Reviewer Comment | Replace impact score with "predicted impact score" throughout the text. |
| Author Response | We thank the reviewer for suggesting this change. We have updated the text to reflect this change. |
| Excerpt From Revised Manuscript | |

## -- Ref 3.1 reorganizing text–--

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| Reviewer Comment | Reorder sections that you start with mutational processes and clonal vs. sub-clonal. |
| Author Response | |
| Excerpt From Revised Manuscript | |

## -- Ref 3.2 Clarify what is considered drivers–--

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| Reviewer Comment | Clarify what you consider a driver: (i) Drivers discovered by the Driver paper, (ii) Events in known cancer genes (e.g. any event in NF1), (iii) Events called as drivers in the Panorama paper. |
| Author Response | For majority of our analysis we have used driver events as defined in Nuria's paper. For additive variance analysis, we have done additional analysis on driver elements discovered by the driver paper as well. |
| Excerpt From Revised Manuscript | |

## -- Ref 3.3 Additive variance beyond TERT promoter–--

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| Reviewer Comment | See how much of the missing variance is explained beyond the TERT promoter mutations. |
| Author Response | |
| Excerpt From Revised Manuscript | |

## -- Ref 3.0 Survival and signature–--

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| Reviewer Comment | Are survival differences account for different signatures and subtypes of disease? |
| Author Response | Reviewer makes a good point. We have updated the text to point out this caveat in the survival analysis section. |
| Excerpt From Revised Manuscript | "Finally, we note the potential role of unmeasured patient clinical characteristics or tumor molecular subtypes in partially influencing these correlations." |

## -- Ref 3.2 – TADs and partial SV depletion--

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| Reviewer Comment | Regarding depletion of partial SVs, can this be related to TADs in both cases? |
| Author Response | |
| Excerpt From Revised Manuscript | |

## -- Ref 3.3 – LoF spectrum--

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| Reviewer Comment | Regarding LoF spectrum, can it be fully explained by the prevalence of signatures in each cohort?<br>If yes, you may want to write a shorter section saying that "STOP codons are distributed as expected by the mutations signatures. |
| Author Response | *STL suggest that we use some LS's figures (expected v.s. observed) in the supplement.* |
| Excerpt From Revised Manuscript | |

## -- Ref 3.4 – Impact score distribution --

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| Reviewer Comment | Are putative passengers only non-coding? Fig 1a is non-coding |
| Author Response | Putative passengers are both coding and non-coding. Since the majority of putative passengers are non-coding, we highlight the impact score distribution for them. |
| Excerpt From Revised Manuscript | |

## -- Ref 3.5 – Signature and early vs late mutations --

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| Reviewer Comment | Is there a difference in signatures between early and late mutations? If yes, can it explain the differences in fraction of impact categories? |
| Author Response | We thank the reviewer for this helpful comment. We note that PCAWG11 group compared signature profile for early and late subclone mutations and concluded that "mutational processes act at a rather constant rate during tumor progression". We clarify this point in the updated text. |
| Excerpt From Revised Manuscript | "We note that different signatures between and early and late subclone mutations have limited contribution to the observed variations[18]." |