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Dear Editor,

We would like to submit our manuscript, "The origin, distribution, and genetic interactions of *KRAS* alleles across cancer types," to be considered for publication as an *Analysis* paper in *Nature Genetics*. Mutational activation of *KRAS* is a frequent somatic event in colorectal adenocarcinomas (COAD), lung adenocarcinomas (LUAD), multiple myelomas (MM), and pancreatic adenocarcinomas (PAAD). In addition to its high frequency in these cancers, *KRAS* mutations are associated with poor clinical outcome, making it a significant barrier in oncology. Since most mutant forms of K-RAS cannot be targeted directly, therapeutic strategies focus on pathways that function downstream of the constitutively active protein to mediate its oncogenicity. This approach requires a detailed understanding of K-RAS signaling and function. However, we have recently shown that K-RAS function is highly dependent upon which allele is expressed, which tissue the mutation occurs in, and which cooperating mutations are present (Poulin et al. *Cancer Discovery*, 9:738–755, 2019 and Brubaker et al. *Cell Systems* 9: 258-270, 2019).

The foundational hypothesis of this study is that each mutant *KRAS* allele will function within a distinct genetic network because its own oncogenic function is highly context dependent. Moreover, variation in the genetic landscape associated with each *KRAS* allele will lead to distinct genetic dependencies. We addressed this hypothesis by analyzing mutations in thousands of tumor samples from COAD, LUAD, MM, and PAAD to identify *KRAS* allele-specific genetic interactions. We then integrated this analysis with the results of genome-wide CRISPR-Cas9 knockout screens from the Dependency Mapping (DepMap) project. The results of the analysis revealed several key conclusions about the *KRAS* oncogene:

1. Using mutational signatures to elucidate the latent mutational processes impacting individual cancers, we demonstrated that the frequencies of *KRAS* alleles – which vary depending on the cancer type – were not solely determined by the active mutagenic forces. This suggested that much of the tissue-specific selection for *KRAS* alleles is driven by the distinct biological properties of the mutant protein.

2. Consistent with the distinct functions of mutant K-RAS proteins, we identified dozens of genes in each cancer type that were more of less likely to comutate with each *KRAS* allele relative to other mutants. These genetic interactions were tissue-specific, demonstrating that the *KRAS* genetic network is unique in each cancer type. The implication of this finding is that the clinical and epidemiological features associated with *KRAS* mutants might, instead, be due to the mutations that commonly segregate with specific *KRAS* alleles.

3. The unique features of mutant *KRAS* alleles were further demonstrated by their differential dependencies on other genes in the genome. These dependencies clustered in genes that participate

in cellular processes that were specifically required by cancer cells expressing particular *KRAS* alleles, again in a tissue-specific manner. A major implication of this finding is that cancers expressing different mutant forms of K-RAS are likely to require distinct therapeutic approaches.

4. In some cases, the genetic dependencies associated with *KRAS* alleles could be attributed to comutation partners, rather than to *KRAS* itself. By extension, the complex genetic network surrounding each mutant form of *KRAS* influences the outcomes of genetic screens for dependencies and will likely influence the design and implementation of *KRAS* allele-specific therapeutic approaches.

This analysis presents a unique viewpoint of the highly complex genetic network of *KRAS*. Moreover, as highlighted in our paper, we believe that this study establishes a paradigm in cancer genetics, as all oncogenes likely exhibit allele-specific and tissue-specific functions and genetic interactions. For this reason, this study will appeal to the broad and diverse audience of *Nature Genetics*.

We would like to recommend that the following reviewers would be appropriate for our paper:
1. Ronald DePinho (MD Anderson), RDePinho@mdanderson.org
2. David Solit (MSKCC), solitd@mskcc.org
3. Wafik El-Deiry (Brown), wafik_el-deiry@brown.edu

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to *Nature Genetics*. We look forward to constructive comments from the reviewers.

Sincerely,

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Peter Park
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