In brief, we were concerned about the bold statement for a causal effect for oxygen in lung carcinogenesis on the basis of an ecological investigation, albeit an elegant one. Although you attempted to control for as many confounders as you could there are limits on what can be attained via this type of investigation. We recommend that you revise along the lines indicated in the reviewers' critiques and submit your paper to a specialty journal in the domain of cancer epidemiology and prevention.
We thank the reviewers and the editors at *eLife* for their comments. We revised the manuscript to suggest rather than to boldly state oxygen-driven tumorigenesis. We have thoroughly addressed the points of the reviewers and believe the manuscript has improved as a result.

We thank the editors at *PeerJ* for examining the transferred reviews and our corresponding revisions and responses, while considering our revised article, "Lung cancer incidence decreases with elevation: evidence for oxygen as an inhaled carcinogen" for publication in *PeerJ*.

Please find our responses to the comments below.

<u>Responses to the comments of Reviewer #1:</u>
This research investigated the ecologic relationship between elevation and lung cancer incidence in the Western U.S.; strong inverse associations were reported and atmospheric oxygen was presented as the underlying biologic hypothesis. The statistical framework of this paper was well-developed and thorough. However, the authors should consider tempering their conclusions on oxygen as an inhaled carcinogen throughout the tone of the article since in this ecological analysis, oxygen was not measured. The comments below pertain mostly to clarifying concepts and methodology in epidemiology as an ecological study design was employed.
We appreciate the reviewer's detailed suggestions and comments that follow. We have tempered our language regarding oxygen as an inhaled carcinogen throughout the abstract, introduction, and discussion. We have also made all clarifying and organizational changes as suggested.

<u>Abstract</u>
1)Line 12 'concrete epidemiological support' for oxygen-driven tumorigenesis is an overstatement based solely on the results of this study.
We have removed 'concrete' and modified the sentence to specify that oxygen-driven tumorigenesis is a suggestion. We have also tempered the abstract as a whole.

<u>Introduction</u>
1)Page 3: Line 44-46 The word "control" has a very precise meaning in epidemiology, and the term "negative control" is not used. The use of this terminology is unclear.

Discussion of controls has been replaced with a straightforward explanation of the matter.

2)Page 3: Line 47 to 54
The strategy underlying the literature review is unclear. Four studies are cited (Amsel 1982; Hayes 2010; Cohen 1995; van Pelt 2003). However, Hayes 2010 is a review article that the authors use to represent 7 additional studies; Cohen 1995 and Amsel 1982 is among the 7 'additional studies.' The authors should consider reviewing the original studies.
We revised this section to present the studies with greater strategy and purpose.

Also, the authors cite the two articles by Cohen 1995 and van Pelt 2003. For thoroughness in summarizing the literature, the authors should present the original findings by Cohen 1995, the reassessment of the data by van Pelt 2003, as well as the counter-argument presented by the original authors in Cohen, 2004.
The radon debate has been moved to the Discussion, where we now mention Cohen 1995 and the followup correspondences, Van Pelt 2003 and Cohen 2004.

In addition, studies conducted by Mason and Miller 1974, Hart 2013, Hart 2011 and Ezzati 2012 may be of relevance to this review.
We now cite Mason 1974. While we mention Ezzati 2012 in the Discussion, we do not include it in the Introduction as it neither focused primarily on cancer nor found an association with elevation. We found that the Hart studies did not add additional clarity and were limited in their experimental design and statistical methodology.

3)Page 3: Line 57 It is unclear what the authors mean by "extraneous variation."
"Extraneous variation" has been replaced with "potential confounding". The sentence structure has also been modified to improve clarity.

<u>Methods</u>
1)The Methods are not presented clearly. Consider reorganizing the Methods in the following sections:
A.Study population i.e. County filtering
B.Data collection and preparation
C.Exposure of interest i.e. Population-weighted mean elevation
D.Outcome of interest i.e .Cancer incidence
E.Confounders/Covariates
We have modified the organization of the Methods sections to more closely match the reviewer's suggested layout.

2)Page 9-10: Line 232-233 The use of the term outliers seems inappropriate - do the authors mean that they initially considered these counties but for these counties exposure/outcome values were outliers and thus excluded? This should be explained.

We documented in greater detail the reasoning and methodology behind these exclusions, and added Figure S1 to visualize and describe the threshold selection criteria further.

3)Why was sex not included as a covariate for colorectal cancer?
We revised our analysis to include 'percent male' as a covariate for lung and colorectal cancer.

4)Page 11: Line 270-271 It is unclear why a one-tailed t-test was used; please justify? Furthermore, in Table 2 a one-tailed test was used and in Figure 2 (Figure supplement 1) a 2-tailed p-value is reported; please justify.
A one-tailed t-test is used to test whether the elevation coefficient is negative. The one-tailed test is appropriate given the directionality of our hypothesis: cancer incidence decreases with rising elevation. The supplementary table reports the best subset regression coefficients. The two-tailed p-value of included predictors is included for the curious reader. Covariates were included to account for potential confounding influences rather than to test for a hypothesized directionality of effect. Therefore, the two-tailed test is appropriate for covariates. For consistency and comparison, we also reported the elevation p-value from a two-tailed test in this table.

a. In Table 2: It is unclear which model the one-tailed p-value is referring to.
The p-value here is evaluating elevation-coefficient negativity in the optimal best subset models. This is reflected in the title of Table 2: "Summary of the optimal best subset model for each cancer."

5)Page 12: Line 307 It may be more appropriate to refer to this as uncontrolled confounding, which is the standard term used in epidemiology
We adopted the terminology 'uncontrolled confounding' in place of "omitted variable bias" throughout the manuscript.

6)Page 13: Line 326 For collection periods that spanned multiple years - were covariates averaged over time?
All variables were averaged over the entire collection period reported in Table 1. We have added a sentence clearly stating this in the "Data collection & preparation" subsection of the Methods. Averaging was performed by the source databases for all variables except diabetes and smoking.

7)Page 13: Line 335-337
a.Why do the N's differ for other cancers for each site in Table 1?
The counties with missing cancer incidence varied by cancer site. The method for calculating 'other cancer' for each site relies on incidence of the corresponding cancer. Therefore, different missing values were introduced for different cancers.

b.Dividing by half to obtain this 'other cancer variable' for sex-specific analysis seems inappropriate; you should obtain sex-specific incidences to calculate this variable.
We updated the 'other cancer' calculation to use sex-specific all-sites incidence for breast and prostate cancer. For breast cancer, this revision resulted in the addition of income in the optimal best subset model and a reduction in the elevation effect size in both the lasso and best subset models. Changes elsewhere were minimal.

<u>Discussion</u>
1)Page 9 Line 184-185 It is unclear which result the authors are referring to in this statement
This sentence was redundant with the following section of the discussion and was removed.

2)Page 9 Paragraph on Confounding effect of elevation
a.This paragraph should be moved before Limitations and future directions
The paragraph has been moved as suggested.

b.This paragraph is confusing. It tries to make varying points on elevation as a possible confounder in other environmental risk factor-lung cancer relationship. But additionally adds that lung cancer should not be used as a proxy for smoking. These are distinct points that should be separated.
We reorganized this section into three paragraphs to more clearly separate the distinct points.

c.Page 9 Line 209-210 This sentence is an overstatement of the results of this study. The authors cannot 'wholly attribute' the findings of previous papers on elevation.
We were attempting to convey that in our dataset radon and UVB associations disappeared when accounting for elevation (please see the "Radon and UVB associations with lung cancer confounded by elevation" subsection of the Results). We added "in our analysis" to the end of the sentence to avoid miscommunication.

<u>Minor Comments</u>
<u>Introduction</u>
1)Page 3: Line 63-64 "We compared elevation's association with lung cancer versus its association with breast, colon and prostate cancer for oxygen-independent elevation effects." This sentence is a bit confusing consider rewording.
We reworded the sentence to improve readability.

<u>Methods</u>
1)Page 10: Line 251 Consider specifying that it is the exposure that has been converted to z-scores.

We clarified that cancer incidence and all predictors were standardized. The only regression-relevant variable that was not converted to z-scores was the observation weighting by population.

2)Page 11: What are the environmental variables? Only 5 are listed in Table 1 (Should particulate and radon should also be labeled 'env' in Table 1?)
The 'env' tag was mistakenly omitted for particulate and radon. We fixed the omission and reordered the table rows for greater clarity.

3)Page 13: Line 327 Define FIPS
We added (Federal Information Processing Standards).

4)Page 13-14: Line 339-344 Provide references for all surveys
BRFSS, NHIS, and ACS are long-running government programs without singular publications describing their existence, which makes their specific citation impractical. Moreover, we believe it is more appropriate to cite State Cancer Profiles, which is the direct source of the data we used. State Cancer Profiles compiled and organized the data from these surveys and thoroughly documents the specific contributions of each.

Results
1)Page 4: Line 86-87 Incomplete sentence
This appears to have been an accidental deletion. We returned the sentence to its original and correct form.

Responses to the comments of Reviewer #2:
Overall comments
I thought this to be a well-written paper that examines the influence of a novel risk factor on the incidence of lung cancer. Though well written the study design (cross-sectional), despite their assertions to the contrary, is not well suited to investigating the possible role of elevation as an etiological factor for lung cancer. The methods do not adequately describe the types of data used in the analyses including where the data are drawn from, nor the other variables that were incorporated into the analyses (smoking, radon, etc). I feel strongly this paper should be rejected. This type of study design should not be applied to attempt to make a determination about causality for a novel risk factor.
We apologize for our poor communication of the Methods. In retrospect, misordering was likely responsible for much of the confusion. In addition to extensive restructuring, we have expanded upon and clarified several sections of the Methods.

Starting with our hypothesis of oxygen-driven tumorigenesis, we aimed to conduct the strongest analysis possible given the data available. This happened to be a cross-sectional and ecological analysis. We reformed any statements that unintentionally implied causality throughout the manuscript. However, given the

impracticality and cost associated with more controlled experimental designs, we believe our analysis is the proper starting point for a broader investigation of oxygen-driven tumorigenesis. Notably, as discussed by Pearce 2000, many insights into cancer etiology were originally put forth and supported by ecological analyses.

<u>More specific comments</u>:
<u>Abstract</u>:
1. Bearing in mind most individuals interested in the paper read only the abstract (and not the full paper) the abstract should clearly describe the methods used.
We restructured the abstract to improve clarity and included additional methodological information.

This abstract make no mention of the data used to derive the incidence rates, nor the time frame.
We have added the source and time frame to the abstract.

These mention that there is a 25.2% decrease in lung cancer incidence but the author has not way of determine what change in altitude produces this decrease in risk.
We have rewritten this sentence, so the change in elevation is explicitly stated.

No mention is made of the other environmental factors used in the analyses, nor how they were collected. Given that smoking is estimated to cause ~ 90% of lung cancers this is fairly important to address.
To provide contextual clarity, we now mention, in the abstract, two of the environmental correlates used in the elevation replacement analysis. Additionally, we now mention the elevation effect size in relation to smoking indicating smoking is a member of the considered covariates.

There is no way of telling whether risk estimates were adjusted for differences in age - which is critical.
We updated the abstract to specify that cancer incidence was age-adjusted (to the 2000 census population).

The authors indicate their findings in the abstract before telling the reader what methods were used. This is akin to telling a reader the ending of the story at the beginning of a book - it does not follow the standard scientific approach.
We reorganized the abstract with the reviewer's concern in mind.

<u>Introduction</u>
1. The strengths and limitations of the previous epidemiological studies are not well described.
We have entirely rewritten this section of the Introduction to improve readability and present the background literature more clearly and completely.

2. Some comment is made on the inverse associations between radon and lung cancer, with the statement that Van Pelt attributes some of this correlation to the confounding influence of elevation- dependent oxygen. While this may be true, this does not mean that Van Pelt was correct, and a number of others have implicated ecological fallacy as a driver of inverse associations between radon and lung cancer rates at a county level. So much so, that this example is taught in many introductory epidemiological. The authors need to acknowledge this.
We have modified the discussion of past epidemiological studies in the Introduction to focus only on elevation-based investigations. Cohen 1995 is no longer mentioned here. Our discussion of Cohen and the inverse radon-lung cancer association has been moved to the Discussion under the "Confounding effects of elevation" subsection. There, we discuss ecological fallacy in the context of radon association and cite Lagarde 1999, a study devoted to the topic.

3. The authors skip from an Introductory section straight to the Result section. There is no methods and materials section. Therefore the reader is never really provided the details needed to understand the nature of the data used in this study. What data were used to derive cancer incidence rates? How complete were they? What about smoking, radon, and other risk factors like age and sex? What calendar period was involved? Air pollution is a recognized risk factor for lung cancer, was some attempt made to model these effects which surely vary by altitude?
The Methods now precede the Results. In regards to these questions, Table 1 provides a condensed reference and the "Data collection & preparation" subsection of the Methods provides exhaustive detail. Our code and data are fully available for readers who would like to investigate the data further.

4. Results section, the authors indicate they dropped from 414 counties to 260 counties after quality control. The reader needs to be told what these quality control efforts entailed. The authors described that they avoided redundant factors and covariates but the reader is never told what the list of covariates were, nor how redundant factors were dropped.
The "County filtering" subsection of the methods now discusses the quality control steps in greater detail. During the preselection phase, when multiple variables risked redundancy, we chose data sources with the best "coverage, precision, collection period, and accessibility",variables with the most relevance to cancer risk, and important demographic health factors. These decisions required subjective evaluations of variable quality, relevance, and non-redundancy. However, we feel that the process succeeded in pre-selecting a diverse set of relevant high-quality covariates.

<u>Discussion</u>
1. In my view, there is no way that ecological fallacy can be dismissed with this study design.
The reviewer correctly identifies the concern of ecological fallacy. Although it is impossible to definitively rule out ecological fallacy, we hope our study motivates

individual-level analyses, which were outside our means. In the revised manuscript, we have tempered and removed any references to causality. Nonetheless, we believe many aspects of the observed association are strongly inconsistent with ecological fallacy (please see the "Limitations & future directions" subsection of the Discussion).

<u>Materials and methods</u>

1. It is not common, nor in my view desirable, to describe the methods after the results and discussion. In all my years of reviewing papers, I have never seen this
The Methods now precede the Results.

2. Page 10, some mention is made of the risk factors considered, but no attempt to explain where the data were obtained from.
The "Data collection & preparation" subsection now appears earlier in the Methods.

3. Unclear how stable the smoking rates were for the areas given no description of the denominators in the BRFSS and NHIS data.
We chose the NCI Small Area Estimates of smoking prevalence since it provided the most comprehensive, widely-adopted, and bias-adjusted estimates we found. We added the citation to the methods paper (Raghunathan 2007) describing the data derivation. Additionally, we now include the 95% confidence interval bounds for smoking prevalence in our dataset. Given the complexity of the model-based estimation approach, these NCI-reported confidence intervals may provide a better estimate of measurement uncertainty than the number of survey respondents. For counties passing quality control, the weighted mean of smoking confidence intervals was 9.4%, compared to a mean smoking prevalence of 47%.

4. No description of the quality of radon data, just a reference is provided.
We added a citation to Apte 1998 which provides a more detailed explanation of the radon data and added information on the goodness-of-fit of the model that produced radon estimates.

<u>Figure 4</u>:

1. it would be worth adjusting comparisons of lung cancer incidence by sex.
We have added 'percent male' as a covariate for both lung and colorectal cancer.

It is interesting to note that for UTAH, a state of low smoking prevalence due to large number of Mormons, there is virtually no association between elevation and lung cancer risk.
The state-specific models adjusted for smoking prevalence. Given the large uncertainty regarding state-specific elevation coefficients, it is difficult to assess whether the elevation association is meaningfully weaker for Utah. For example, the 95% confidence interval for Utah's elevation coefficient, overlaps the confidence interval from the best subset model.

The figure needs to describe adjustment factors included.
We believe the figure does indicate which covariates were included. For the stratification by smoking, elevation was the sole predictor. Thus we specified that a "bivariate regression was fit". For the stratification by state, smoking was included as a covariate, and we specified that "lung cancer was regressed against elevation and smoking for each state."

<u>Responses to the comments of Reviewer #3:</u>
This is a very important and high quality epidemiological paper and deserves publication.

The inclusion of only the highest altitude (Western US) counties puzzles me. The larger range of altitude obtained by including counties at all altitudes would seem to make for a greater chance of finding an oxygen association. The overt choice to include counties only within a narrow range of altitudes (i.e., higher altitudes) needs to be explained. The 2003 study by Van Pelt clearly showed the inverse relation between lung cancer and altitude for 1601 counties.
Previously, we selected states in the contiguous United States with peak elevation exceeding 3000 m. For clarity, we now select states in the contiguous United States with elevation spans exceeding 3000 m. This happens to select the same 11 states (which compose the Western United States) as the peak elevation threshold . We consider all counties in these 11 states that passed quality control. We focused on a more homogenous and yet still elevation-varying region compared to Van Pelt to minimize the risk of confounding.

The abstract claims a "25.2 point decrease". The paper uses the term "point" several times. "Point" is not a valid unit of measure. It should be clearly defined in the context of cancer incidence.
We removed the use of "point" as a unit of measure and replaced with clearly defined units and phrasing (i.e. cases per 100,000 individuals).

At line 184: "Notably, we found that elevation's large and unanticipated impact on lung cancer had confounded reported lung cancer associations [23, 24]." This effect was anticipated by Van Pelt (your ref 25) who reached almost identical conclusions.
We have rewritten the relevant sections of the Introduction and Discussion to provide a clearer explanation of Van Pelt's important contributions and limitations.

<u>Minor Comments</u>
at line 86-87 there needs to be a grammar adjustment.
The grammatical error has been corrected.

Finally, we would like to thank the reviewers for their comments, which have helped us to improve and expand upon our original submission. We hope that you will find our revised manuscript suitable for publication in *PeerJ*.

Thank you,
Kamen Simeonov & Daniel Himmelstein