Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Redox-Related Transcript Patterns in Skeletal Muscle From a Rat Intensive-Care Model: An Exploratory Re-analysis

Amjed Majid Rashid Alrashedi

amjad93majid11@gmail.com

Kashan State University

Ali Sultan Khazaal Nazal

Kashan State University

Munaf M. Naji

Kufa University

Abdulmohaimen Hasan Kadhim

Kashan State University

Short Report

Keywords: skeletal muscle, oxidative stress, gene expression profiling, intensive care, passive loading, microarray

Posted Date: November 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11183550/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Skeletal-muscle unloading during intensive-care treatment is associated with muscle wasting and oxidative injury. We performed an exploratory secondary analysis of a public rat-muscle microarray dataset to describe expression patterns and variability involving redox-related genes in an intensive-care model that included anesthesia, ventilation, neuromuscular blockade, and unilateral passive loading.

Methods

We examined 46 Affymetrix Rat Gene 1.0 ST arrays from 23 female Sprague-Dawley rats in GEO series GSE37944. The reported preprocessing used robust multi-array average normalization and array-level quality control. We summarized a selected redox/xenobiotic-response panel, explored correlations among panel genes, and performed Gene Ontology over-representation analysis of the 500 most variable transcript features.

Results

Among the selected targets, *Txn1, Cat, Gpx3, Sod1, Sod2*, and *Prdx1* had the highest reported mean normalized intensities. The exploratory correlation network contained 32 nodes and 48 edges; *Sod1* had the largest reported degree. The most variable-feature set was enriched for terms involving muscle contraction, muscle-cell differentiation and development, and response to nutrient.

Conclusions

The analysis describes redox-related expression and transcriptomic variability in GSE37944 but does not, on the reported outputs, establish that passive loading changed the expression of individual genes. These results should be treated as hypothesis-generating. A validated paired loaded-versus-unloaded differential-expression analysis is needed before attributing the observed patterns to loading.

Introduction

Intensive-care unit (ICU)-acquired muscle weakness is associated with prolonged ventilation, delayed rehabilitation, and loss of skeletal-muscle mass and force. Mechanical silencing, critical illness, and prolonged ventilation can act together to alter muscle structure and redox balance [1, 2]. Animal models have therefore been used to examine how passive mechanical loading might preserve muscle during deep sedation and neuromuscular blockade. Muscle phenotype reflects interactions among mechanical input, neural activation, metabolic demand, and other stress signals. Redox responses may be adaptive or damaging depending on timing and context; transcript abundance alone cannot establish oxidative

injury or antioxidant capacity. This makes it important to distinguish descriptive gene profiles from a statistical estimate of the loading effect.

The original study associated with GEO series GSE37944 reported that unilateral passive loading attenuated loss of muscle mass and force in a rat ICU model and was accompanied by lower oxidative damage and less myosin loss [1]. Nevertheless, oxidative stress is not a single-direction process: unloading can disturb antioxidant capacity, while interventions targeting mitochondrial reactive oxygen species have not consistently rescued muscle atrophy across models [3, 4]. Isoflurane and mechanical ventilation may also influence redox-related pathways, complicating attribution of molecular patterns to a single exposure [5, 9].

Here, we re-examined GSE37944 with an exploratory focus on selected redox/xenobiotic-response transcripts, their across-sample correlation structure, and biological processes associated with highly variable transcript features. The purpose of this study is to describe patterns in the deposited data, not to infer causal effects of anesthesia or passive loading without a clearly reported condition-level contrast. This scope is important because the experimental model combines several exposures and includes samples collected at different time points. A pooled profile can reveal which genes and pathways contribute to transcriptomic variability, but it cannot by itself separate effects of passive loading from time-dependent adaptation, anesthesia, ventilation, neuromuscular blockade, or inter-animal differences. We therefore emphasize description.

Methods

Data source and experimental model

GSE37944 contains 46 Affymetrix Rat Gene 1.0 ST array samples from gastrocnemius muscle in 23 female Sprague-Dawley rats subjected to an experimental ICU model [1]. The model included anesthesia, mechanical ventilation, neuromuscular blockade, and unilateral passive mechanical loading; sample collection covered experimental durations ranging from hours to 14 days. The present work is a secondary analysis of the public data, not a new animal experiment.

Preprocessing and quality control

The prior analysis record reports that raw CEL files were processed in R (v4.5.1) using the oligo package and the platform-specific design/annotation resources. Background adjustment, quantile normalization, and probe summarization were performed with robust multi-array average (RMA), yielding log2-transformed normalized intensities. Array quality was assessed using intensity distributions, RNA-degradation plots, sample distances, and principal component analysis (PCA). No arrays were reported as excluded; the submitted analysis described comparable normalized intensity distributions and no samples flagged as outliers by array-level metrics. Sample-level PCA and hierarchical clustering were used to describe overall structure, not to assign biological meaning to clusters without independent

evidence. Because the data span different collection times, any broad separation among arrays could reflect temporal or animal-level effects as well as the intervention.

Redox panel, correlation network, and pathway analysis

A selected panel of redox-defense and xenobiotic-response targets included antioxidant enzymes and regulators such as *Sod1, Sod2, Cat, Gpx*-family genes, *Prdx*-family genes, *Txn1, Txnrd1, Nfe2l2, Keap1, Hmox1*, and glutathione-transferase genes. The wider panel also included selected metabolic and phase II conjugation targets. Mean normalized intensity, standard deviation, and the reported coefficient of variation were summarized descriptively across the available arrays (Supplementary Table S1). The panel is a targeted set of candidate transcripts, not a comprehensive assay of reactive oxygen species, antioxidant capacity, protein oxidation, or enzyme activity.

For exploratory network analysis, pairwise Pearson correlations were computed across the selected panel and edges were retained at $|r| \geq 0.60$. Selected network metrics, including node degree and strength, and Louvain community labels were summarized; these statistics describe covariance in the pooled dataset and do not establish regulatory direction or causation (Supplementary Table S2). For pathway analysis, the 500 most variable transcript features were subjected to Gene Ontology Biological Process over-representation analysis. Adjusted p-values were calculated using the Benjamini-Hochberg procedure (Supplementary Table S3).

Statistical interpretation

The present research treats panel-expression summaries, network topology, and enrichment results as exploratory. The current findings do not assert condition-specific gene regulation; such a claim requires a correctly specified paired analysis with effect sizes and multiplicity-adjusted p-values reported for the loaded-versus-unloaded comparison.

Results

Global transcriptomic structure

After RMA normalization, PCA indicated that PC1 and PC2 accounted for 22.2% and 9.7% of total variance, respectively (Fig. 1A). Hierarchical clustering based on Euclidean distance and Ward's minimum-variance method showed sample substructure (Fig. 1B). The source analysis reported that no array exceeded the predefined outlier thresholds in the array-level quality plots. The highest reported mean silhouette score among the tested cluster counts was 0.2615 at $K = 2$, indicating modest rather than strong separation. These unsupervised patterns describe overall sample variation and should not be interpreted as evidence that loading groups differ.

Correlation structure and variable-gene enrichment

The exploratory network contained 32 target nodes and 48 edges at the prespecified absolute-correlation threshold. *Sod1* had the largest reported degree (9; strength 6.68); *Prdx1, Txn1*, and *Gsta1* each had degree 8. The reported community structure grouped *Sod1, Prdx1, Cat, Txnrd1*, and *Gclm* with antioxidant-related nodes, while another group included *Keap1, Nfe2l2, Txn1, Ppara, Sod2*, and *Ugt1a1* (Supplementary Table S2). These groupings depend on the selected panel and threshold. The associations may reflect shared variation across animals, time points, or experimental exposures, and should not be taken as evidence of direct molecular interaction.

The 500 most variable transcript features included *Emb, Ankrd1*, and *Ostn* among the highest-variance examples. Enrichment analysis identified striated muscle contraction (28 genes; fold enrichment 7.58; adjusted $p = 3.20 \times 10^{-13}$), muscle-cell differentiation (39 genes; fold enrichment 4.35; adjusted $p = 2.55 \times 10^{-11}$), response to nutrient (33 genes; fold enrichment 5.05; adjusted $p = 2.55 \times 10^{-11}$), and muscle-cell development (28 genes; fold enrichment 6.07; adjusted $p = 2.55 \times 10^{-11}$) among the leading terms (Fig. 2; Supplementary Table S3).

Discussion

This secondary analysis characterizes expression variability in GSE37944, a dataset originally generated to investigate passive mechanical loading in an ICU-like rat model. The original study reported preservation of muscle mass and force with passive loading and linked this benefit to lower oxidative damage and reduced myosin loss [1]. The present analysis does not independently reproduce those intervention effects at the transcript level; instead, it summarizes pooled expression distributions, gene-gene correlations, and enrichment among variable features.

Several redox-related targets had relatively high normalized intensities in the selected panel, and a correlation network connected antioxidant and glutathione-associated genes. This pattern is biologically plausible because skeletal muscle relies on superoxide dismutases, catalase, peroxidases, and thiol-based systems to maintain redox homeostasis, and disuse-related redox imbalance has been described in experimental muscle studies [3, 10]. However, normalized abundance alone cannot show that a gene is induced by oxidative stress or suppressed by loading. Transcript levels may not parallel protein abundance, enzyme activity, or oxidative damage, and the present analysis did not measure these endpoints. Likewise, correlations may arise from shared cellular composition, time after intervention, or other experimental covariates rather than direct regulation.

The enrichment of muscle contraction, differentiation, and development terms among highly variable features is also consistent with the muscle origin of the dataset and with the time-dependent remodeling described in the original report [1, 2]. Other enriched terms included striated muscle-cell differentiation, muscle-organ development, skeletal-muscle adaptation, and muscle contraction. These categories overlap biologically and may share genes, so the number of significant ontology terms should not be

mistaken for the number of independent mechanisms discovered. Enrichment results also depend on the selected background universe, feature-to-gene annotation, and the threshold used to define the variable list. These findings should therefore be interpreted cautiously: a variability-ranked gene list asks which transcripts differ most across all arrays, not which transcripts respond specifically to passive loading. The same distinction applies to the network analysis, whose topology is descriptive rather than causal.

The renin-angiotensin system (RAS) is a plausible candidate pathway for future study because angiotensin signaling has been associated with skeletal-muscle wasting in experimental settings [6, 7]. Nevertheless, the outputs reported here do not demonstrate that RAS components are down-modulated in loaded muscle. Such a statement would require a direct matched-limb contrast, effect estimates, uncertainty measures, and multiple-testing correction. The same caution applies to proposed antioxidant or anesthetic-related mechanisms: these remain hypotheses rather than conclusions of the present exploratory analysis. In particular, exposure to isoflurane cannot be isolated from the other components of this model with the available design, and published work showing anesthetic-associated stress signaling in other tissues is background context rather than direct proof of a muscle-specific response [9].

A next analysis should use the paired design explicitly, include rat identity and relevant duration/batch terms where appropriate, and report $\log_2$ fold changes with confidence intervals and false-discovery-rate-adjusted p-values for loaded versus unloaded tissue. Where duration differs between animals, the analysis should assess whether the loading contrast is consistent across time or interacts with duration. Sensitivity analyses across experimental duration, examination of sample-label pairing, and an independent validation step would help distinguish reproducible loading-associated responses from time-dependent or animal-level variation. Transcript-level findings could then be interpreted alongside the functional and oxidative-damage measurements reported in the original experiment. This distinction also matters for translation: although passive or assisted motion is used in sedated and ventilated patients, the available clinical literature notes uncertainty around early effects and intervention replicability [8]. A careful molecular contrast is needed before this re-analysis can suggest specific targets for clinical intervention.

Limitations

The limitations of this study were composed of: this secondary analysis used an existing microarray dataset without new measurements or independent validation. Microarrays provide less transcript and isoform resolution than RNA sequencing. The cohort included 23 female rats, with variation in experimental duration and no non-anesthetized control; generalizability and separation of anesthesia from other exposures are limited. The supplied outputs also lack a verifiable paired loaded-versus-unloaded differential-expression table, so gene-specific loading effects cannot be concluded. Pooled correlations and enrichment are exploratory and may reflect time, animal, or cell-composition differences.

Conclusion

This exploratory re-analysis describes redox-related expression patterns and muscle-associated pathways among variable transcript features in GSE37944. The findings can prioritize targets for a formal paired analysis, but they do not establish that passive loading altered specific genes or RAS signaling. A transparent loaded-versus-unloaded differential-expression analysis with effect sizes, adjusted p-values, and independent validation is required before making intervention-specific molecular claims.

Declarations

Ethics approval and consent to participate

Not applicable; this study re-analyzed publicly available data from a previously published animal experiment.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

Funding

No external funding was received.

Author Contribution

A.M.R. Alrashedi designed the study, conducted the primary investigation, project administrator, and wrote the original manuscript. A.S.K. Nazal performed the formal analysis and validated the data. M.M. Naji contributed to data visualization and provided essential resources. A.H. Khadim supervised the project and assisted in reviewing and editing the manuscript. All authors reviewed the final manuscript and approved it for publication.

Acknowledgements

None.

Data Availability

The raw microarray data are publicly available in the Gene Expression Omnibus under accession GSE37944. Summary tables for the exploratory analyses described here are provided in the supplementary file.

References

1. Renaud G, Llano-Diez M, Ravara B, Gorza L, Feng H-Z, Jin J-P, et al. Sparing of muscle mass and function by passive loading in an experimental intensive care unit model. J Physiol. 2013;591:1385–402. https://doi.org/10.1113/jphysiol.2012.248724.
2. Llano-Diez M, Renaud G, Andersson M, Marrero HG, Cacciani N, Engquist H, et al. Mechanisms underlying ICU muscle wasting and effects of passive mechanical loading. Crit Care. 2012;16:R209. https://doi.org/10.1186/cc11841.
3. Lawler JM, Song W, Demaree SR. Hindlimb unloading increases oxidative stress and disrupts antioxidant capacity in skeletal muscle. Free Radic Biol Med. 2003;35:9–16. https://doi.org/10.1016/S0891-5849(03)00186-2.
4. Eshima H, Siripoksup P, Mahmassani ZS, Johnson JM, Ferrara PJ, Verkerke ARP, et al. Neutralizing mitochondrial ROS does not rescue muscle atrophy induced by hindlimb unloading in female mice. J Appl Physiol. 2020;129:124–32. https://doi.org/10.1152/japplphysiol.00456.2019.
5. Falk DJ, Kavazis AN, Whidden MA, Smuder AJ, McClung JM, Hudson MB, et al. Mechanical ventilation-induced oxidative stress in the diaphragm: role of heme oxygenase-1. Chest. 2011;139:816–24. https://doi.org/10.1378/chest.09-2787.
6. Powers SK, Morton AB, Hyatt H, Hinkley MJ. The renin-angiotensin system and skeletal muscle. Exerc Sport Sci Rev. 2018;46:205–14. https://doi.org/10.1249/JES.0000000000000158.
7. Takayama S, Inoue K, Ogura Y, Hoshino S, Sugaya T, Ohata K, et al. Angiotensin II type 1a receptor deficiency alleviates muscle atrophy after denervation. Sci Rep. 2023;13:519. https://doi.org/10.1038/s41598-023-27737-7.
8. Vollenweider R, Manettas AI, Häni N, de Bruin ED, Knols RH. Passive motion of the lower extremities in sedated and ventilated patients in the ICU: a systematic review of early effects and replicability of interventions. PLoS ONE. 2022;17:e0267255. https://doi.org/10.1371/journal.pone.0267255.
9. Hoetzel A, Leitz D, Schmidt R, Tritschler E, Bauer I, Loop T, et al. Mechanism of hepatic heme oxygenase-1 induction by isoflurane. Anesthesiology. 2006;104:101–9. https://doi.org/10.1097/00000542-200601000-00016.

10. Pellegrino MA, Desaphy JF, Brocca L, Pierno S, Camerino DC, Bottinelli R. Redox homeostasis, oxidative stress and disuse muscle atrophy. J Physiol. 2011;589:2147–60. https://doi.org/10.1113/jphysiol.2010.203232.

Figures

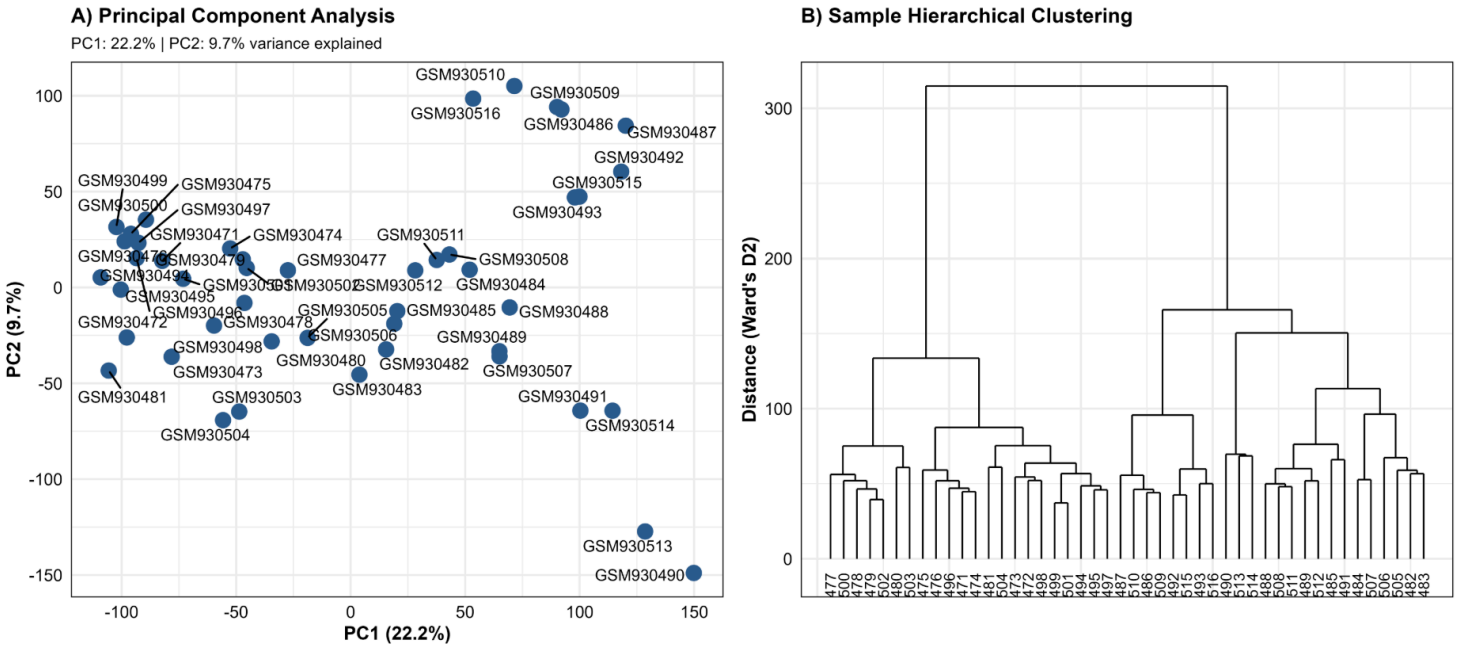


Figure 1

Global transcriptomic structure of GSE37944 muscle samples. (A) Principal component analysis of 46 arrays; PC1 and PC2 account for 22.2% and 9.7% of variance. (B) Hierarchical clustering using Euclidean distances and Ward's minimum-variance method. These unsupervised results describe sample structure and are not tests of a loading effect.

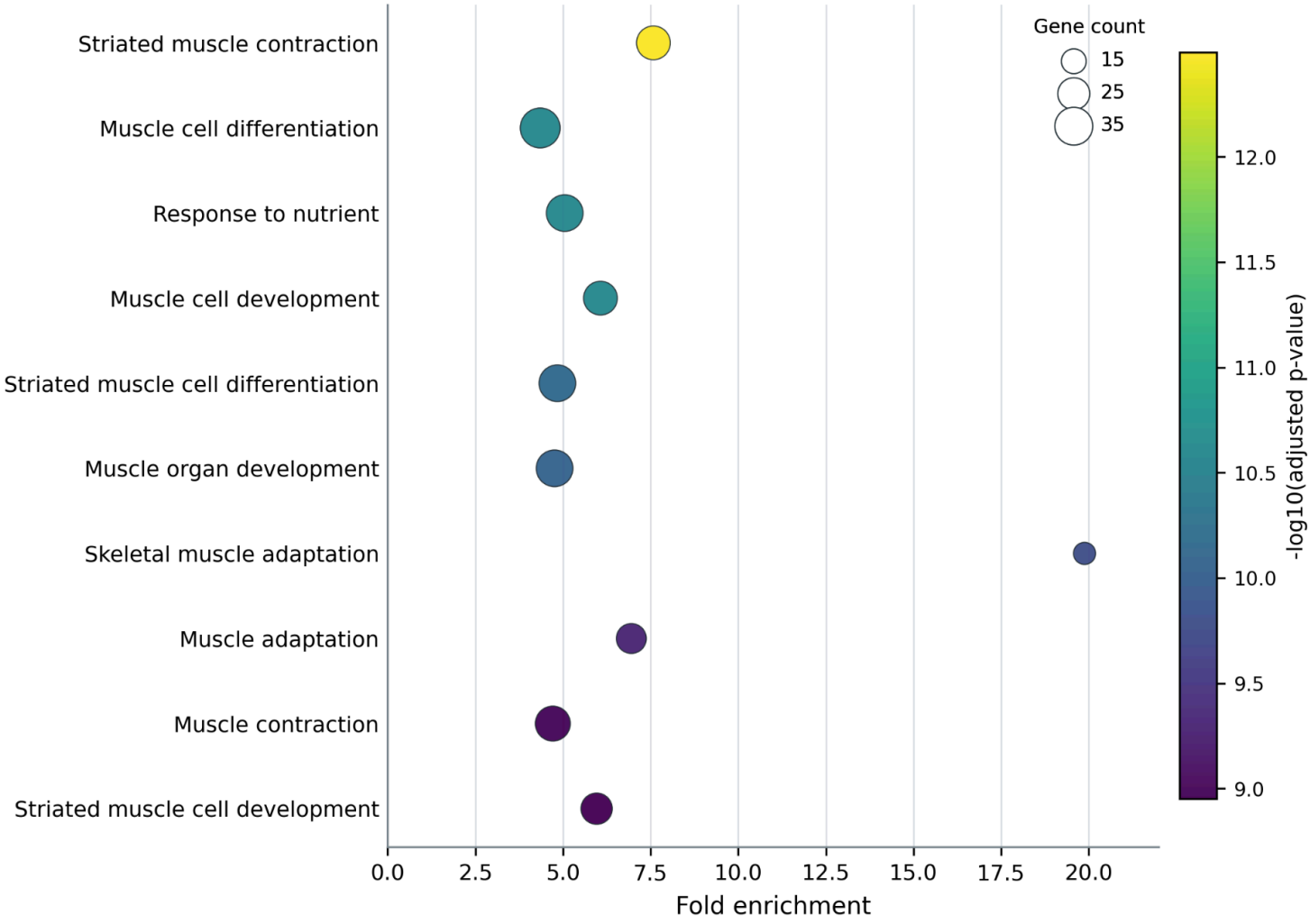


Figure 2

Gene Ontology Biological Process enrichment among the 500 most variable transcript features. Dot size represents gene count and color represents the adjusted p-value. Because features were selected by variability, the plot does not represent a loaded-versus-unloaded differential-expression analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarytableS1.docx
- supplementarytableS2.docx
- supplementarytableS3.docx