Letter to the Editor

Pan-cancer analysis of *KEAP1* mutations as biomarkers for immunotherapy outcomes

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Immune checkpoint inhibitors (ICIs) that target programmed cell death protein 1 (PD-1) and its ligand (PD-L1), or cytotoxic T lymphocyte antigen-4 (CTLA-4) elicit durable antitumor responses in multiple cancer types. Yet, only a minority of patients could derive clinical benefit (1). Understanding genomic correlates of response to ICIs could lay a foundation for the development of novel biomarkers and treatment to further enhance the therapeutic benefit (2,3). Previous studies demonstrated that genetic alterations of *KEAP1* would dysregulate oxidative stress pathway, resulting in oncogenesis and drug- and radio-resistance in different cancers (4,5). Recently, some exploratory analyses with limited samples showed the association between *KEAP1* mutations and clinical benefit to ICI. However, a comprehensive analysis of *KEAP1* mutation frequency and their predictive significance for ICI treatment outcome in diverse cancers has not yet been investigated. Therefore, we conducted this pan-cancer analysis by using online database to systematically characterize the prevalence and predictive value of *KEAP1* mutations across multiple cancer types.

All included patients and sequencing data were identified from the cBioPortal online database (https://www.cbioportal.org) (6). *KEAP1* mutations were defined as all kinds of nonsynonymous mutations including missense, frame-shift, splice site, nonstop, nonsense, and translation start site changes. To evaluate the difference of tumor mutation burden (TMB) level between *KEAP1* mutant and wild type groups, a subset generated from MSK-IMPACT cohort was selected to avoid the selection bias and ensure the TMB could be comparable (7). The six immune infiltrates abundances including B cells, $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, macrophages and neutrophils were estimated by using a web server for comprehensive analysis of tumor-infiltrating immune cells, named TIMER (Tumor Immune Estimation Resource, https://cistrome.shinyapps.io/timer/) (8). Kaplan-Meier curves with log-rank tests were used to determine the survival difference.

We summarized all the relevant data in *Figure 1*. The prevalence of *KEAP1* mutations in 40,167 patients with distinct cancer types was 2.7% (*Figure 1A*), with patients with non-small cell lung cancer (NSCLC) having the highest levels of *KEAP1* mutations (15.8%). Most of the alterations were missense mutations. The prevalence and spectrum of *KEAP1* mutations were slightly different in early-stage (441/10,967, TCGA cohort; *Figure S1A*) versus advanced-stage cancers (388/10,945, MSK-IMPACT cohort; *Figure S1B*). In the MSK-IMPACT cohort (7), TMB of patients with *KEAP1* mutations was significantly higher than in those without the mutations (10 *vs.* 4 mutations/Mb, $P<0.0001$; *Figure 1B*). This was validated in the ICI-treated cohort (*Figure 1C*) (9). Notably, cancers with *KEAP1* missense mutations had the highest TMB level (*Figure S2*). Together, these findings reveal a high prevalence of *KEAP1* mutations and its close relationship with TMB level across cancer types, suggesting that *KEAP1* mutations should be considered as biomarkers when conducting ICI treatment.

Next, we surveyed the relationship between *KEAP1* mutations and overall survival (OS) in both whole group and ICI-treated cohort. We firstly found that patients with *KEAP1* mutations showed a significantly shorter OS

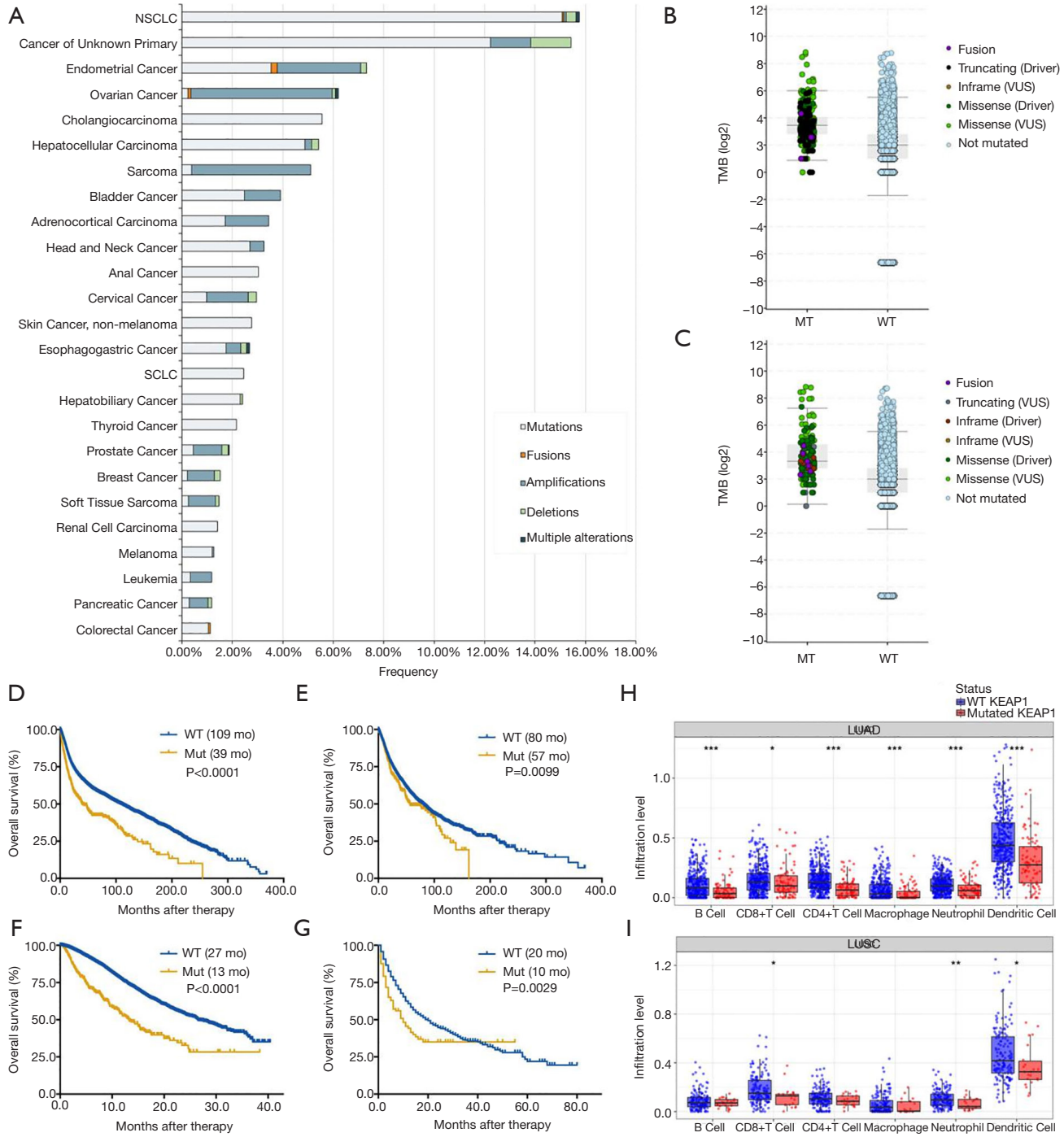

Figure 1 Pan-cancer analysis of *KEAP1* mutations as biomarkers for immunotherapy outcomes. (A) Prevalence of *KEAP1* mutations in different cancer types; (B) the association between TMB and *KEAP1* mutations in MSK-IMPACT cohort; (C) the association between TMB and *KEAP1* alterations in immune checkpoint inhibitors treatment cohort; (D) prognostic value of *KEAP1* mutations in all cancers; (E) prognostic value of *KEAP1* mutations in early-stage cancers (TCGA cohort); (F) prognostic value of *KEAP1* mutations in advanced-stage cancers (MSK-IMPACT cohort); (G) predictive value of *KEAP1* mutations in patients received ICI therapy; (H) the association between *KEAP1* mutations and six immune infiltrates in lung adenocarcinoma; (I) the association between *KEAP1* mutations and six immune infiltrates in lung squamous cell carcinoma. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; Mut, mutation; WT, wild type; TMB, tumor mutation burden; ICI, immune checkpoint inhibitor.

(39 *vs.* 109 months, P<0.0001; *Figure 1D*) than those without in whole populations. The prognostic value of *KEAP1* mutations was also found in early-stage (P=0.0099; *Figure 1E*) and advanced-stage cancers (P<0.0001; *Figure 1F*). Although *KEAP1* mutations were associated with marginally significantly shorter disease-free survival (DFS, 97 *vs.* 158 months, P=0.0677; *Figure S3A*), it was associated with markedly inferior DFS in early-stage cancers (P=0.0009; *Figure S3B*). In the ICI treatment cohort (9), we identified 1,661 patients with different cancers receiving ICI therapy and 99 of them with *KEAP1* mutations. Patients with *KEAP1* mutations also had a substantially inferior OS of 10 *vs.* 20 months in the wild-type group (P=0.0029; *Figure 1G*). Further investigation showed that only *KEAP1* mutations could not predict OS in patients with microsatellite-stable (MSS) solid tumors (14 *vs.* 21 months, P=0.5619; *Figure S3C*).

To unravel the potential mechanism of the predictive value of *KEAP1* mutations for ICI treatment, we then investigated the association between *KEAP1* mutations and immune landscape across multiple cancer types. We observed that these mutations were associated with significantly lower $CD8^+$ T cells infiltrations in most of the cancer types including endometrial cancer, breast cancer, bladder cancer, colorectal cancer, lung adenocarcinoma (*Figure 1H*), lung squamous cell carcinoma (*Figure 1I*) and so on. Notably, patients with *KEAP1*-mutant lung adenocarcinoma had dramatically lower $CD8^+$ T cells, neutrophils and dendritic cells infiltrations than those without, which was consistent with our recent publication (10). Of note, copy number variations (especially deep deletion or arm-level deletion) of *KEAP1* were associated with substantially lower immune infiltrates in most cancer types including lung adenocarcinoma (*Figure S4A*) and lung squamous cell carcinoma (*Figure S4B*).

To our knowledge, this study firstly reported a high frequency of *KEAP1* mutations in diverse cancers including lung cancer, endometrial cancer, hepatocellular carcinoma, head and neck cancer, bladder cancer, colorectal cancer, esophagogastric cancer, etc. and negative prognostic value of *KEAP1* mutations for patients with different types of cancer. We also observed that *KEAP1* mutations were a negative predictive biomarker and might be utilized to predict a survival benefit from ICI treatment across multiple cancers. Although *KEAP1* mutations were correlated with significantly higher TMB level, they were also associated with significantly lower immune infiltrates especially $CD8^+$ T cells, suggesting that tumor with these mutations could promote establishment of a cold-tumor immune microenvironment. Considering the high prevalence of *KEAP1* mutations, there is an urgent need for the development of rational and novel therapeutic. We are planning to initiate a prospective study to investigate the efficacy of PD-1 antibody plus vascular endothelial growth factor receptor tyrosine kinase inhibitors for patients with solid cancer and *KEAP1* mutations. Collectively, our findings highlight the important value of *KEAP1* alterations as pan-cancer predictive biomarkers for ICI treatment.

Acknowledgment

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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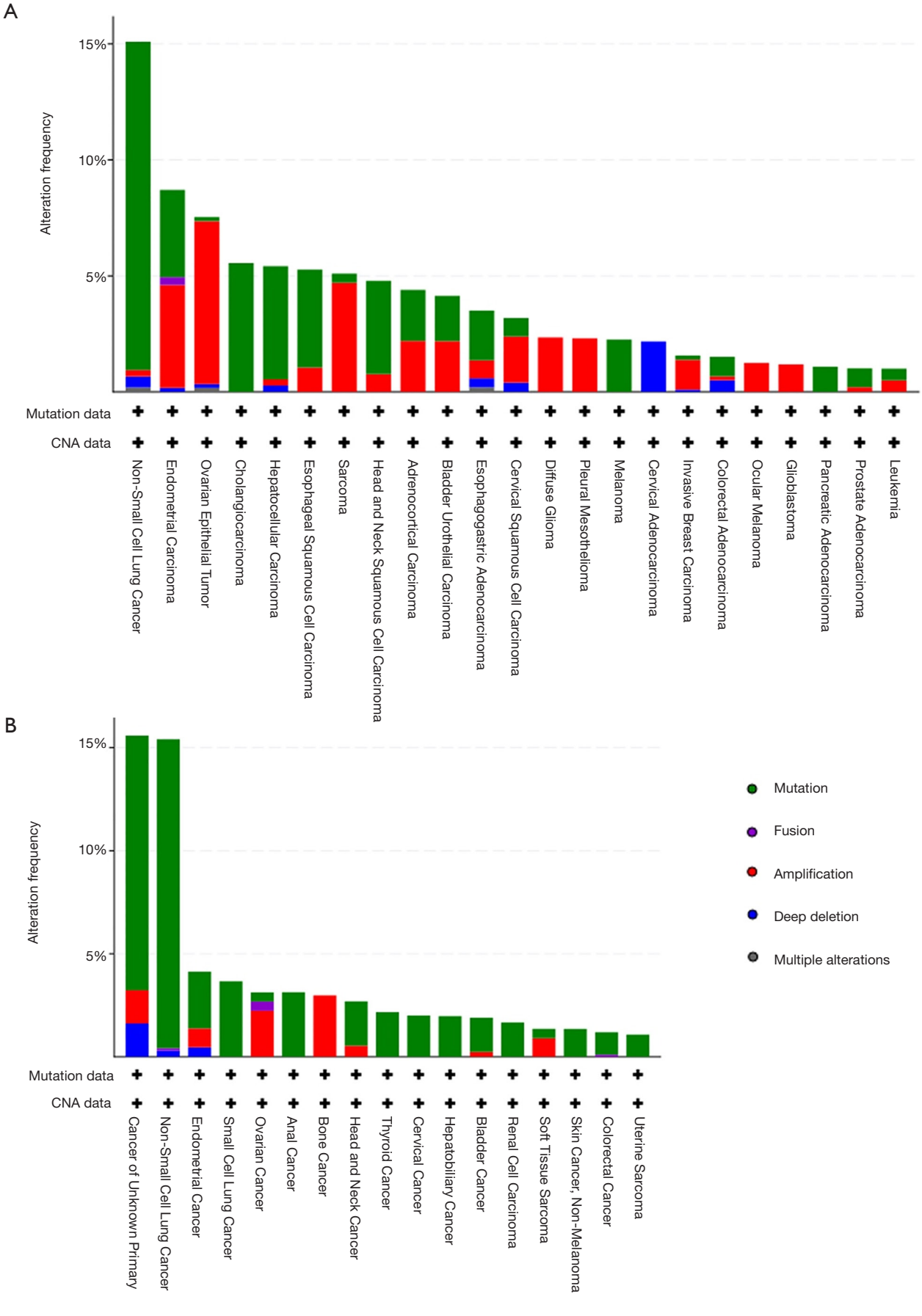

Figure S1 The frequency of *KEAP1* mutations in early-stage cancer (A) and advanced-stage cancer (B).

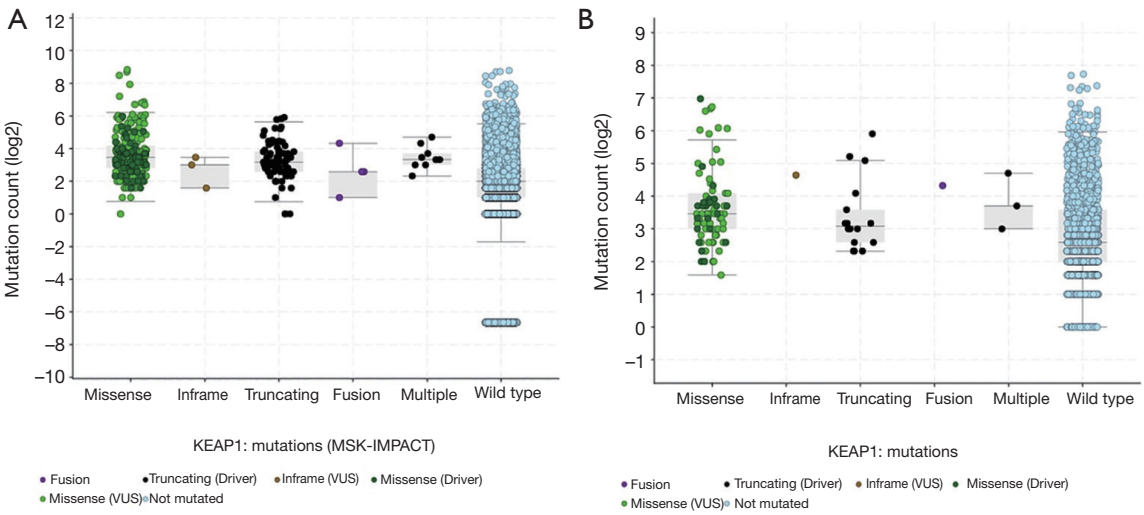

Figure S2 The association between TMB and *KEAP1* mutations subtypes in MSK-IMPACT cohort (A) and immune checkpoint inhibitors treatment cohort (B). TMB, tumor mutation burden.

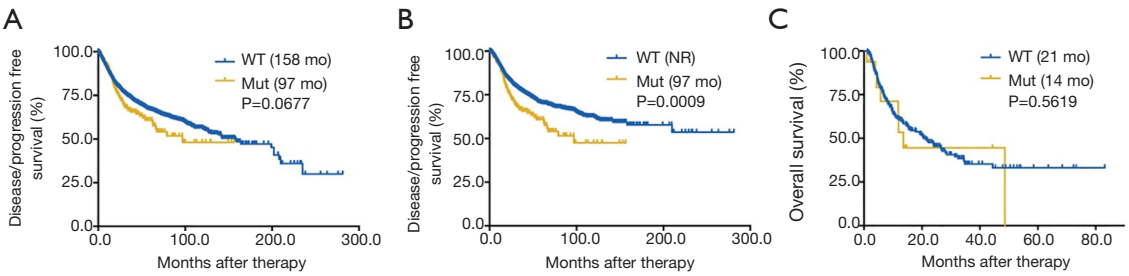

Figure S3 Predictive value of *KEAP1* mutations in all cancers (A), in TCGA cohort (B) and in patients with MSS solid tumors (C). MSS, microsatellite-stable.

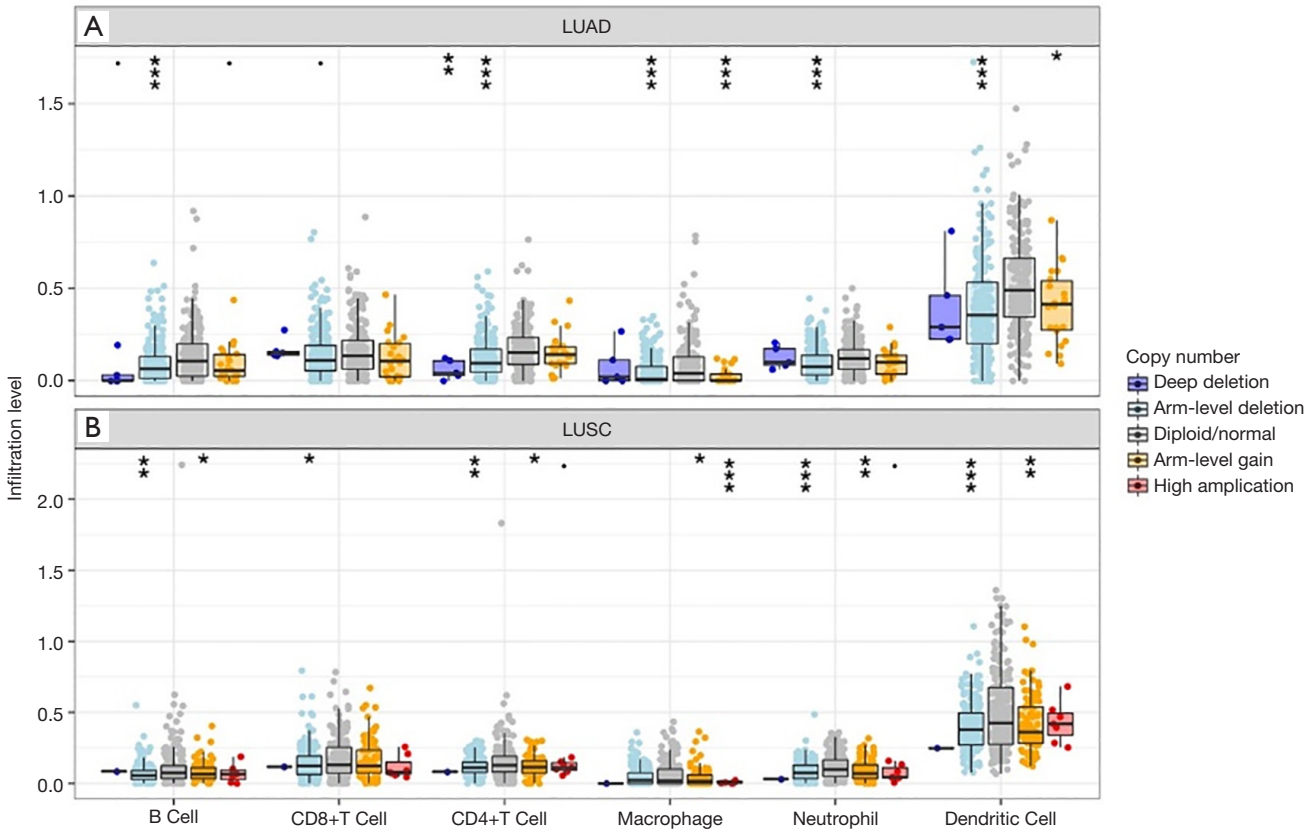

Figure S4 The association between *KEAP1* copy number variations and six immune infiltrates in lung adenocarcinoma (A) and lung squamous cell carcinoma (B).