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Original Article

The interaction of *p53* and DNA repair gene mutations and their impact on tumor mutation burden and immune response in human malignancies

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Abstract: *P53* suppresses tumorigenesis through multiple cellular functions/mechanisms, including genomic stability surveillance. Recently, it has also be reported for its role in cancer immune response modulation. Deficiency in DNA repair pathways lead to the accumulation of genomic alterations and tumor mutation burden and in consequence resulting in the activation of immune response. We investigated the interaction of *p53* and DNA repair gene mutations and their impact on tumor mutation burden and immune response in human malignancies by mining cBioPortal data of a range of human cancers. We found that in the majority of human cancers, *p53* mutations are equally distributed between DNA repair gene mutation positive and negative cases and in a number of human cancers, *p53* and DNA repair gene mutations have a tendency of co-occurrence. Only in colorectal cancer, there is a tendency of 'mutual exclusivity' of mutations in *p53* and DNA repair genes. In most tumors, *p53* and DNA repair gene mutations have synergistic/additive effect in increasing tumor mutation burden, but not in colorectal cancer where they are mutually exclusive. The impact of *p53* and DNA repair gene mutations and their interaction on tumor microenvironment immune cells are complex and tumor type specific and not always correlated with tumor mutation burden. In colorectal cancers, these two types of mutations resulted in similar immune cell subpopulation changes and in tumors where the mutations have a tendency of co-occurrence, *p53* showed dominant roles on immune response, although they can also counter-act each other for their effect on certain immune cell subtypes.

Keywords: Gene mutation, P53, DNA repair gene, tumor mutation burden, tumor infiltrating immune cell population

Introduction

p53 plays a critical role in suppressing tumor development and is inactivated by gene mutations and/or deletions in half of human cancers [1, 2]. The well-established mechanisms of *p53* tumor suppression are induction of cell apoptosis, cell cycle arrest and cell senescence [1, 2]. However, combined loss of cell cycle suspension, apoptosis and senescence did not result in spontaneous tumorigenesis as observed upon loss of *p53* [3-5], indicating that there are other critical molecular/cellular mechanisms that *p53* activates to suppress tumorigenesis, such as metabolic [3] and immune response [6-12] pathways. Exploring the known defective molecular pathways in *p53* mutated cancer cells has led to novel forms of tumor therapy strategies [2, 13, 14], thus further illustration of the role, underlying mechanisms and interacting molecular pathways of *p53* in tumorigenesis would improve cancer therapeutic approaches for *p53* mutated tumors.

It has been reported recently that DNA repair pathways were critical mediators of p53-dependent tumor suppression [15]. DNA repair processes are critical for cells to maintain genomic stability. Deficiency in DNA repair processes, frequently caused by DNA repair gene (DRG) mutations, leads to genomic instability

and consequently accumulation of genomic alterations [16]. There are several DNA repair pathways, including mismatch repair, base-excision repair, nucleotide-excision repair, translation synthesis, homologous recombination, non-homologous end joining, the Fanconi anemia and the O6-methylguanine DNA methyltransferase pathways [16]. The DNA repair system is a very complex network, including many genes and cellular pathways that affect genomic changes and they can be defined more or less strictly based on if genes are directly or indirectly involved in DNA damage repair. The DRG database created and maintained by R. Wood and M. Lowery, providing a valuable reference of DRGs, has listed more than 200 genes in over 14 DNA damage repair/response pathways (https://www.mdanderson.org/documents/Labs/Wood-Laboratory/human-dna-repair-genes.html) and the number is expected to further increase [17, 18]. Among them, deficiency in mismatch repair (MMR) pathways leads to microsatellite instability and consequently increased tumor mutation burden (TMB) and neoantigen load in tumor cells, which can predict anti-PD-1/PD-L1 immunotherapy response better than the predicting value of PD-L1 expression level [19].

In the paper published by Janic *et al*. [15], the authors demonstrated in mouse models that DNA repair processes are critical mediators of p53-dependent tumor suppression as knockdown of p53 target genes implicated in DNA repair, including *MLH1, MSH2, RNF144B, CAV1* and *DDIT4*, accelerated MYC-driven lymphoma development to a similar extent as knockdown of *p53*, although not all DRGs had equal effect in tumorigenesis. To translate this research finding from mouse models into human cancers, they analyzed leukemia, lymphoma and colorectal cancer data in the cBioPortal data [20, 21], and reported that *p53* and DRG mutations were mutually exclusive in those human malignancies [15]. This may provide new insight into *p53* tumor suppression mechanisms and would help with the development of novel therapeutic approaches. We analyzed cBioPortal data in a broad range of human cancers to fully assess the association of mutations in *p53* and these p53 target DRGs [15], as well as their impact alone and in combination on TMB and non-silence mutations, which potentially generate neoantigens. As both DRG mutation induced TMB/neoantigen load and *p53* mutation are associated with tumor infiltrating lymphocytes (TILs), we also investigated their potential effect on tumor microenvironment (TME) immune cells, in particular the interaction of these two types of mutations. Since out of the DNA repair pathways, deficiency in MMR pathway, which increases tumor mutation burden (TMB) and neoantigen load, predicts anti-PD-1/PD-L1 immunotherapy response well [19], we also investigated the interaction of *p53* mutation with mutations of DRGs including all the MMR genes in addition to the p53 target DRGs.

Materials and methods

Data mining using cBioPortal online tools

The cBioPortal [20, 21] online data mining was performed at the website https://www.cbioportal.org by selecting the dataset(s) of relevant cancer types. To enable the comparison of our results to the previously published results [15], we attempted to use the data sets of colorectal cancers and hematological malignancies as previously analyzed that the combined colorectal adenocarcinoma analysis included data from Colorectal Adenocarcinoma (DFCI, Cell Reports 2016), Colorectal Adenocarcinoma (Genentech, Nature 2012), Colorectal Adenocarcinoma (TCGA, Firehose Legacy), Colorectal Adenocarcinoma Triplets (MSKCC, Genome Biol 2014) and Metastatic Colorectal Cancer (MSKCC, Cancer Cell 2018) and the combined hematological malignancies analysis included data from Acute Lymphoblastic Leukemia (St Jude, Nat Genet 2015), Acute Myeloid Leukemia (TCGA, Firehose Legacy), Chronic Lymphocytic Leukemia (Broad, Cell 2013), Chronic Lymphocytic Leukemia (IUOPA, Nature 2015), Cutaneous T Cell Lymphoma (Columbia U, Nat Genet 2015), Diffuse Large B-Cell Lymphoma (Broad, PNAS 2012), Hypodiploid Acute Lymphoid Leukemia (St Jude, Nat Genet 2013), Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (TCGA, Firehose Legacy), Mantle Cell Lymphoma (IDIBIPS, PNAS 2013), Multiple Myeloma (Broad, Cancer Cell 2014) and Primary Central Nervous System Lymphoma (Mayo Clinic, Clin Cancer Res 2015), and followed the same analysis approach.

For the further TCGA data analysis, we excluded amplification of *p53* or DRGs, as amplifications are unlikely to cause loss of function of

these genes. All other genomic alterations, including in-frame mutation (putative driver and unknown significance), missense mutation (putative driver and unknown significance), truncating mutation (putative driver and unknown significance), germline mutation and deep deletions to *p53* or the relevant DRGs were counted. Correlation analysis of genomic alterations between *p53* and the 10 p53 target DRGs (*MLH1, MSH2, PMS2, RNF144B, CAV1, DDIT4, FANCC, POLK, ERCC5, MGMT*) [15] was firstly performed in the TCGA provisional dataset available in April 2019 by submitting each of these genes for querying. We then performed correlation analysis of genomic alterations both between *p53* and the 10 p53 target DRGs and between *p53* and DRGs including MMR genes in addition to the 10 p53 target DRGs (*MLH1, MSH2, PMS2, RNF144B, CAV1, DDIT4, FANCC, POLK, ERCC5, MGMT, MSH6, MSH3, MLH3, PMS1, MSH4, MSH5, EPCAM, PMS2P3* and *HFM1*) in the TCGA Pan-Cancer dataset available in June 2021 by submitting each of these genes for querying. The number of p53 target DRG (10_DRGs) mutation positive cases were calculated by including samples with mutations in any of the ten p53 targeted DRGs. The number of all the MMR and p53 target DRG (19_DRGs) mutation positive cases were calculated by including samples with mutations in any of the 19 DRGs.

Heatmaps were displayed by clicking on "OncoPrint". The correlations between genomic alterations of *p53* and these DRGs were generated by clicking on "Mutual Exclusivity".

We used TCGA abbreviations for tumor type names, except colorectal cancer (COADREAD) which contains COAD and READ, glioma which contains GBM and LGG, and renal cancer which contains all TCGA renal tumor subtypes including KICH, KIRC and KIRP, due to the similarity between them and/or the limited number of tumor subtype samples for statistical analysis.

Correlation analysis

The "alterations_across_samples.tsv" file for the dataset(s) of each cancer type was downloaded from cBioPortal website [20, 21]. Correlation analysis was run using Fisher exact test with one-tail according to cBioPortal [20, 21]. Tendency of co-occurrence or mutual exclusivity was determined by odd ratio (OR) or Log2_OR [20, 21]. Heatmap was plotted using ggplot2 package. All these analyses were run in R 3.6.3 statistic software.

Analysis of tumor mutation load and tumor microenvironment immune cell changes among p53 and DRG mutation only, double positive and double negative groups

The TCGA Pan-Cancer mutation load, immune cells, leucocyte fractions, leucocyte proportion of tumor stromal fraction and TIL regional fraction data were acquired from Thorsson V et al. study (https://gdc.cancer.gov/about-data/publications/panimmune) [9]. The details were shown as follows: 1) The mutation load, including silence and non-silence mutation data, were obtained from *mutation-load_updated.txt* file. Then duplicated data demonstrating zero value were excluded. The tumor mutation burden (TMB) was calculated by sum of the silence and non-silence mutation data. 2) The TCGA immune cells (CIBERSORT) data were acquired from *TCGA.Kallisto.fullIDs.cibersort.relative.tsv* file. Then data from normal samples were deleted. 3) The total tissue and stromal leucocyte fractions data and TIL regional fraction data were acquired from supplementary file (*Table S1. PanImmune Feature Matrix of Immune Characteristics*) of Thorsson *et. al* study [9]. 4) The stromal leucocyte proportion = leucocyte fractions/stromal fraction, as described in the method parts of Thorsson *et. al* study [9].

TCGA Pan-Cancer *p53* and DRGs genetic alteration data, including mutation, deletion and fusion, were download from cBioPortal [20, 21], as was mentioned previously. According to *p53* and DRG mutation status, four patient groups were generated (single type mutations, both types of mutations and none of them).

Afterwards, boxplots were plotted using ggpubr package and wilcoxon test was run in R 3.6.3 statistic software.

Results

The co-occurrence of mutations in p53 target DRGs and p53 is more common than mutual exclusivity in human cancers

Our reanalysis using the same approach and similar cBioPortal data as previously reported [15] produced similar distribution patterns

Table 1. Correlation of mutations in *p53* and the 10 p53 target DNA repair genes together in human tumors based on TCGA-Provision datasets from cBioPortal [20, 21]

TCGA Provisional Dataset	p53 mutation		Log2_OR	Tendency	*p* value
	DRG mutation cases	DRG wildtype cases			
Colorectal cancer*	36.0% (552/938)	58.8% (41/114)	-1.348	Mu-ex	2.82×10^{-6}
SKCM	41.5% (33/246)	13.4% (17/41)	2.193	Co-oc	6.65×10^{-5}
Glioma#	81.0% (306/773)	39.6% (17/21)	2.697	Co-oc	1.65×10^{-4}
BRCA	51.6% (275/901)	30.5% (32/62)	1.280	Co-oc	6.72×10^{-4}
ACC	54.5% (12/77)	15.6% (6/11)	2.700	Co-oc	0.008
CHOL	25.0% (5/31)	16.1% (1/4)	0.794	-	0.546
HNSC	74.5% (328/457)	71.8% (35/47)	0.198	-	0.420
Haematological malignancy*	3.8% (114/1348)	8.5% (1/26)	-1.197	-	0.349
LIHC	35.0% (113/346)	32.7% (7/20)	0.151	-	0.501
LUAD	59.5% (84/193)	43.5% (22/37)	0.928	-	0.055
LUSC	73.3% (123/148)	83.1% (22/30)	-0.839	-	0.158
OV	88.2% (244/277)	88.1% (30/34)	0.021	-	0.621
PRAD	20.0% (76/432)	17.6% (12/60)	0.228	-	0.381
STAD	55.9% (159/334)	47.6% (33/59)	0.482	-	0.150
UCEC	27.3% (59/209)	28.2% (9/33)	-0.069	-	0.546

*Combined study as shown in method part; #TCGA, Cell 2016.

of *p53* mutation in relation to these DRG mutations in hematological malignancies and colorectal cancers [15]. However, we only found an inverse correlation of *p53* and these DRG mutations in colorectal cancers (Figure S1A), but not hematological malignancies (Figure S1B). In hematological malignancies, where the frequencies of mutations in both *p53* and DRGs are very low (each DRG mutation rate is < 1%), the chance of these two types of mutations co-existing in the same patient is expected to be rare. Hence, neither in the original publication (data showed in Figure S19 of the publication) [15] nor in our analysis (Figure S1B), is mutual exclusivity of mutations in *p53* and these 10 p53 target DRGs statistically significant (all P>0.4).

To further determine if mutual exclusivity of *p53* and these p53 target DRG mutations commonly exist in human cancers, we further analyzed the TCGA data for other cancers using the same analysis approach. As amplifications are unlikely to cause loss of function of p53 or DRGs, in the further correlation analysis we excluded amplification of these genes. We also performed the correlation analysis between mutations of *p53* and any of these 10 p53 target DRGs in combination to increase the statistical power compared to individual DRGs. With this combination, we still only found a significant inverse correlation between these two types of mutations in colorectal cancers, but not in hematological malignancies (**Table 1**; Figure S2).

In our further analysis of other human cancers, we found that *p53* mutations are equally distributed between DRG mutation positive and negative cases in many human cancer types, including prostate, ovarian, liver, head and neck, stomach and endometrial cancers (**Table 1**; Figure S2). Only in LUSC is there a trend (P=0.158) of inverse correlation between *p53* and any of these DRG mutations with *MLH1* mutation being significantly (P=0.006) inversely correlated with *p53* mutation prior to multiple testing correction. Most importantly, in breast cancer (BRCA), skin cutaneous melanoma (SKCM), adrenocortical carcinoma (ACC), and glioma, we found that mutations in *p53* and DRGs are closely associated with each other and these DRG mutations have a significant ($P=6.72\times10^{-4}$, 6.65×10^{-5}, 8×10^{-3} and 1.65×10^{-4}, respectively) tendency of co-occurrence with *p53* mutation (**Table 1**; Figure S2).

As recently more cancer samples have been sequenced and included more cancer types in the TCGA Pan-Cancer study, we further analyzed the TCGA Pan-Cancer data in cBioPortal to investigate association of *p53* mutation with

Table 2. Correlation of mutations in *p53* and the 10 p53 target DNA repair genes together in human tumors based on TCGA-Pan-Cancer datasets from cBioPortal [20, 21]

TCGA Pan-Cancer Dataset	p53 mutation		Log2_OR	Tendency	*p* value
	DRG mutation cases	DRG wildtype cases			
Colorectal cancer	48.5% (32/66)	61.1% (281/460)	-0.738	Mu-ex	0.035
SKCM	32.6% (28/86)	14.1% (39/277)	1.559	Co-oc	0.000196
GBM	77.8% (7/9)	32.0% (118/369)	2.896	Co-oc	0.007
LGG	75.0% (15/20)	47.7% (234/491)	1.720	Co-oc	0.014
Glioma	75.9% (22/29)	40.9% (352/860)	2.181	Co-oc	0.000185
BRCA	53.1% (34/64)	34.0% (317/932)	1.137	Co-oc	0.002
ACC	55.6% (5/9)	16.3% (13/80)	2.688	Co-oc	0.015
BLCA	64.5% (40/62)	48.3% (166/344)	0.963	Co-oc	0.013
SARC	78.6% (11/14)	44.4% (106/239)	2.202	Co-oc	0.012
CESC	19.0% (4/21)	8.6% (22/257)	1.330	-	0.119
ESCA	94.4% (17/18)	86.6% (142/164)	1.397	-	0.302
HNSC	67.5% (27/40)	71.5% (326/456)	-0.272	-	0.355
Renal cancer	6.7% (2/30)	5.6% (37/663)	0.273	-	0.514
LAML	0.0% (0/5)	9.2% (17/185)	<-3	-	0.623
DLBC	0.0% (0/3)	14.7% (5/34)	<-3	-	0.638
LIHC	31.6% (6/19)	32.3% (108/334)	-0.05	-	0.583
LUAD	60.0% (36/60)	51.0% (228/447)	0.527	-	0.12
LUSC	82.1% (46/56)	86.7% (358/413)	-0.501	-	0.231
OV	88.4% (38/43)	92.1% (327/355)	-0.62	-	0.277
PAAD	66.7% (4/6)	61.5% (104/169)	0.322	-	0.580
PRAD	20.5% (8/39)	15.8% (71/450)	0.462	-	0.283
STAD	54.2% (32/59)	48.5% (182/375)	0.33	-	0.25
MESO	66.7% (2/3)	15.2% (12/79)	>3	-	0.074
PCPG	0.0% (0/2)	1.9% (3/159)	<-3	-	0.963
TGCT	0.0% (0/5)	1.4% (2/139)	<-3	-	0.932
THYM	33.3% (1/3)	3.3% (4/120)	>3	-	0.118
THCA	0.0% (0/7)	0.6% (3/475)	<-3	-	0.957
UCEC	31.2% (34/109)	39.8% (159/400)	-0.541	-	0.063
UCS	100.0% (3/3)	90.6% (48/53)	>3	-	0.751
UVM	0.0% (0/1)	0.0% (0/79)	>3	-	1.000

-: Tendency not clear or no tendency; Co-oc: co-occurrence; Mu-ex: mutual exclusivity.

DRG mutations and the potential of additional cancer types, in which mutations in *p53* and DRGs are mutually exclusive. Consistent with the above analysis, *p53* mutations are equally distributed between DRG mutation positive and negative cases in many human cancer types (**Table 2**; Figure S3). Most importantly, in addition to BRCA, SKCM, ACC, and glioma, we also found in BLCA and SARC that mutations in *p53* and DRGs are closely associated with each other and these DRG mutations have a significant (P=0.013 and 0.012 respectively) tendency of co-occurrence with *p53* mutation. In THYM, there is also a trend (P=0.118) of co-occurrence of these two types of mutations (**Table 2**; Figure S3). Mutual exclusivity of mutations in *p53* and DRGs was still only observed in colorectal cancer, although in UCEC, there was a trend (P=0.063) of reverse correlation between *p53* and any of these DRG mutations, with *PMS2* mutation being significantly (P=0.030) reversely correlated with *p53* mutation prior to multiple testing correction (**Table 2**; Figure S3).

DRG and p53 mutations increase TMB in most of human cancers and they commonly have synergistic/additive effect

To investigate if *p53* mutation has a similar effect as DRG mutations and their combined effect, we analyzed the consequence of TMB

changes in association with *p53* and DRG mutations. We analyzed both total TMB (calculated using both silence and non-silence mutation) which reflects the deficiency in certain DRGs, and non-silence mutations which potentially produce neoantigens. We firstly analyzed the 10 p53 target DRGs [15]. Surprisingly, we found that while DRG mutation induced dramatic (9 fold) increase of TMB ($P=2.18\times10^{-13}$) and non-silence mutations ($P=7.91\times10^{-13}$) in colorectal cancers, *p53* mutation alone did not increase but instead slightly decreased TMB (P=0.003) and decreased non-silence mutations (P=0.005) induced by DRG mutations, suggesting that *p53* did not play the same role as DRGs to prevent the accumulation of genome-wide mutations in colorectal cancer cell genome (**Figure 1A** and **1B**). Dramatic (>10 fold) increase of TMB and non-silence mutations by DRG mutations, while slightly (low fold change) but significant decrease of TMB (P=0.004) and non-silence mutation load (P=0.007) by *p53* mutation was also seen in uterine endometrial cancers (UCEC). However, in UCEC, *p53* mutation did not prevent the increase of TMB and non-silence mutation load induced by DRG mutations in the cases with both DRG and *p53* mutations (**Figure 1A** and **1B**). The other cancer types where DRGs potentially dramatically (>10 fold) increased TMB and non-silence mutation load were BRCA and PAAD, although the *p*-values were >0.05 (P=0.058 and P=0.08 respectively) in PAAD due to limited number of cases with only DRG mutations. In these two types of cancers, *p53* mutation also significantly increased both TMB and non-silence mutation load (**Figure 1A** and **1B**).

Equally interestingly, in most of the cancers where *p53* and DRG mutations are not mutually exclusive, such as CESC, HNSC, LIHC, LUAD, LUSC, OV, PAAD, Renal cancer, and even in most of cancers where *p53* and DRG mutations showed strong co-occurrence (ACC, BLCA, BRCA and SKCM), both *p53* and DRG mutations increased TMB and non-silence mutations, although the increase is not statistically significant in the *p53* mutation alone group of ACC for TMB (P=0.057) and in the DRG mutation alone group for both TMB and non-silence mutation load in LIHC (P=0.12 and 0.137 respectively), PAAD (P=0.058 and 0.08 respectively) and SKCM (P=0.063 and 0.058 respectively). Moreover, except BLCA, BRCA, LUSC, PAAD and SARC, *p53* and DRG mutations have synergistic or additive effect in causing global genomic mutations including both TMB and non-silence mutations.

In ESCA and gliomas, neither DRGs or *p53* mutations significantly affected TMB or non-silence mutations, but in gliomas with both types of mutations there was a trend of many fold increase in both TMB (P=0.185) and non-silence mutation load (P=0.132). In PRAD and SARC, mutations of *p53* but not DRGs increased TMB or non-silence mutations, and in PRAD with both types of mutations there was a trend to dramatically increase (>10 fold) both TMB (P=0.037) and non-silence mutation load (P=0.066).

Overall, the effect of *p53* and DRGs mutations on TMB and non-silence mutations varies in different tumor types, although in most cancers each of them promotes the accumulation of global genomic mutations, including mutations that may generate neoantigens and they work together to further increase TMB and non-silence mutation load (**Figure 1A** and **1B**).

We also analyzed the effect of mutations of *p53* and of the 19 MMR and P53 target DRGs on TMB and non-silence mutations. The results are similar to the above by analyzing the 10 p53 target DRG [15] mutation analysis in relation to *p53* mutation, except that *p53* and DRG double mutations induced an increase of TMB in CESC, glioma and renal cancer and the increase of non-silence mutations in CESC, glioma and PRAD became significant (P<0.05); while DRG mutation alone induced an increase of TMB and non-silence mutations, both become statistically significant (P<0.05) in glioma, LIHC, PAAD, PRAD and SKCM, due to increased number of cases with such mutations. However, in OV DRG mutation alone group, the increase of TMB (P=0.062) and non-silence mutation load (P=0.055) were no longer statistically significant, and in ACC *p53* mutation alone group, the increase of non-silence mutation load did not remain statistically significant (P=0.063) (Figure S4).

Different effects of DRG and p53 mutations on TME immune cells and the distinguished DRG and p53 mutation interaction patterns between cancers where they are mutually exclusive and co-occur

We further investigated different immune cell components in the tumor microenvironment

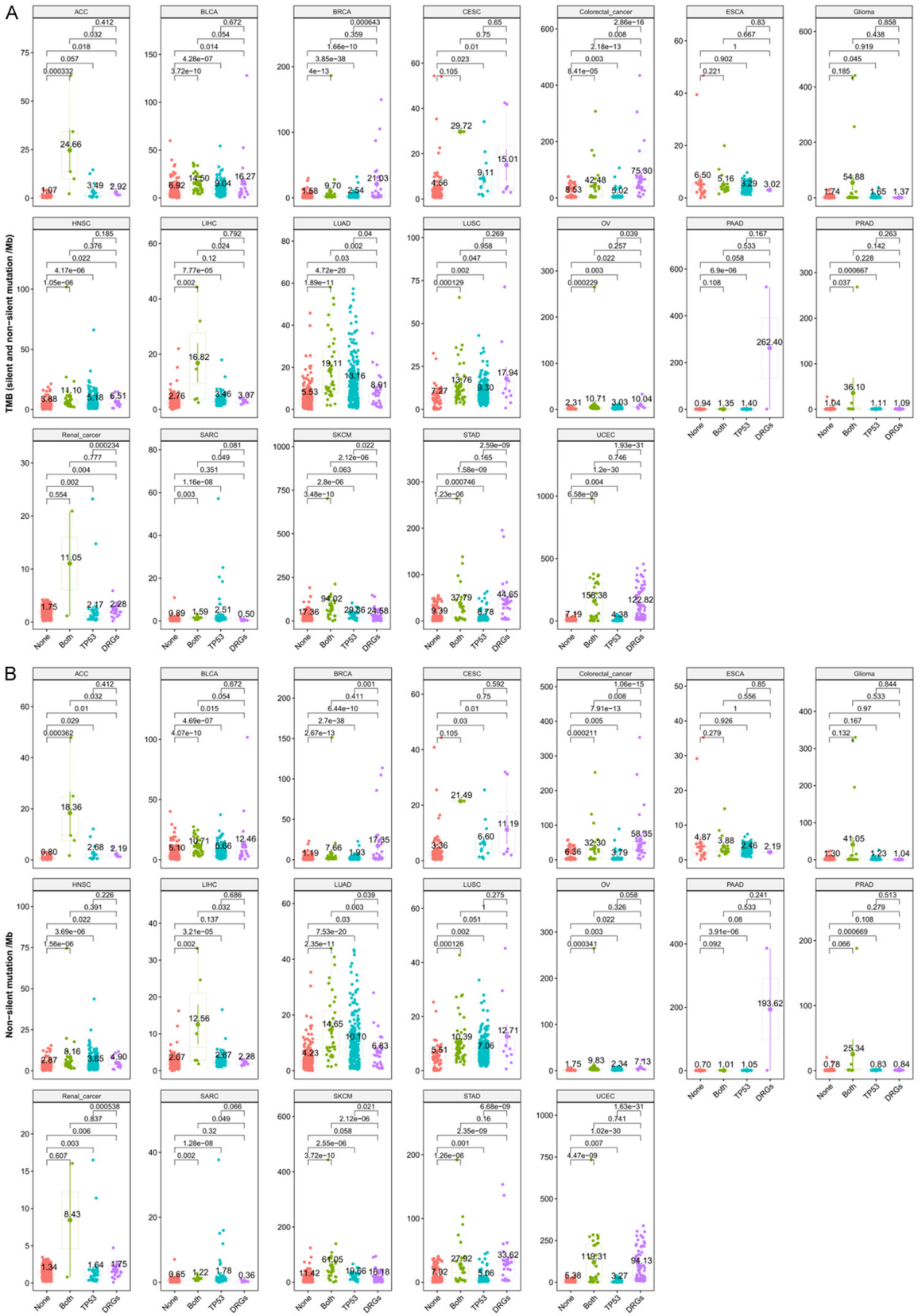

Figure 1. Boxplot of global mutations among four groups of samples based on *p53* and the 10 DRG (p53 target) mutation status in each type of cancer with wilcoxon test. A. TMB (Silent and non-silent mutations/MB). B. Non-silent mutations/MB. MB: megabase; None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

based on CIBERSORT deconvolution analysis of RNA-seq data in the tumor types where mutations of *p53* and the 10 p53 target DRGs are either mutually exclusive (colorectal cancers) or co-occur (ACC, BRCA, BLCA, glioma and SKCM) for the impact of mutations of *p53* and p53 target DRGs on them.

In colorectal cancers, mutation of DRGs alone correlated (significantly or with a trend) with increase of activated memory CD4+ T cells (P=0.0053), activated natural killer cells (NKs) (P=0.044), M1 ($P=1\times10^{-6}$), mono- (P=0.044) and total (P=0.014) macrophages, resting mast cells (P=0.18) and neutrophils (P=0.003); and decrease of plasma cells (P=0.0058), Tregs (P=0.037) and eosinophils (P=0.084) (**Figure 2**). Mutation of *p53* alone correlated (significantly or with a trend) with increase of activated NKs (P=0.062), M0 (P=0.00032), M1 (P=0.028), mono- (P=0.0027) and total (P=0.003) macrophages, resting mast cells (P=0.0022) and total B cells (P=0.018); and decrease of activated mast cells ($P=1.2\times10^{-5}$), eosinophils (P=0.034), neutrophils (P=0.042), and total mast cells (P=0.0037) (**Figure 2**).

Mutations of both types correlated (significantly or with a trend) with increase of activated memory CD4+ T cells (P=0.057), activated NKs (P=0.041), M1 ($P=1.1\times10^{-5}$), mono- (P=0.18) and total (P=0.13) macrophages and resting mast cells (P=0.065); and decrease of resting memory CD4+ T cells (P=0.19), activated dendritic cells (DCs) (P=0.061), monocytes (P=0.28) and eosinophils (P=0.093) (**Figure 2**).

While *p53* and DRG mutations differentially correlated with the changes of certain types of tumor microenvironment immune cells, both of them had the same effect on most of the altered immune cells, including the increase of activated NKs, M1, mono- and total macrophages, resting mast cells; and decrease of eosinophils, and the effect remained the same when both mutations occurred. Interestingly, here we observed that both *p53* and DRG mutations were generally associated with an increase of anti-tumor immune response [22, 23], although they correlated with TMB differentially.

In ACC, where tumor immune cell infiltration is low, mutation of DRGs alone correlated (significantly or with a trend) with an increase of eosinophils (P=0.071) and total mast cells (P=0.25); and decrease of resting (P=0.11) and total (P=0.029) NKs (Figure S5). Mutation of *p53* alone was correlated (significantly or with a trend) with the increase of naïve CD4+ T cells (P=0.11) and M0 macrophage (P=0.097), and decrease of resting NKs (P=0.15), monocytes (P=0.07), resting (P=0.064) and total (P=0.01) mast cells (Figure S5).

Mutations of both types correlated (only with a trend but not statistically significant) with an increase of memory (P=0.12) and total (P=0.073) B cells and M0 macrophage (P=0.064); and decrease of activated NK (P=0.054), monocytes (P=0.17) and M2 macrophages (P=0.10). The only TME immune cells potentially similarly affected (decreased) by *p53* and DRG mutations were resting NKs, but in cases with both *p53* and DRG mutations, this decreased effect disappeared, indicating the two mutations may decrease resting NKs through different mechanisms which counteract each other when they occur at the same time. For total mast cells, the two types of mutations had opposite effects, which is neutralized in cases with both types of mutation. The potential increase of eosinophils and decrease of total NK by DRG mutation and increase of naïve CD4+ T cells and decrease of resting mast cells by *p53* mutation were also diminished by the co-occurrence of the other type of mutation.

In BLCA, mutation of DRGs alone correlated (significantly or with a trend) with an increase of plasma cells (P=0.0015), CD8+ T cells (P=0.065), activated (P=0.044) and total (P=0.27) NK cells, M1 macrophages (P=0.017), resting DCs (P=0.16) and total lymphocytes (P=0.067); and a decrease of memory B cells (P=0.065), naïve CD4+ T cells (P=0.29), resting NK cells (P=0.12), M0 macrophages (P=0.11), resting (P=0.12) and total (P=0.11) mast cells and eosinophils (P=0.037) (Figure S6). Mutation of *p53* alone was correlated (significantly or with a trend) with the increase of plasma cells (P=0.062), CD8+ T cells (P=0.08), activated memory CD4+ T cells (P=0.044), resting (P=0.066) and total (P=0.031) NK cells, M0 (P=0.11), mono- (P=0.005), M1 ($P=7.56\times10^{-6}$) and M2 (P=0.191) macrophages and activated mast cells (P=0.14); and a decrease in memory (P=0.0065) and total

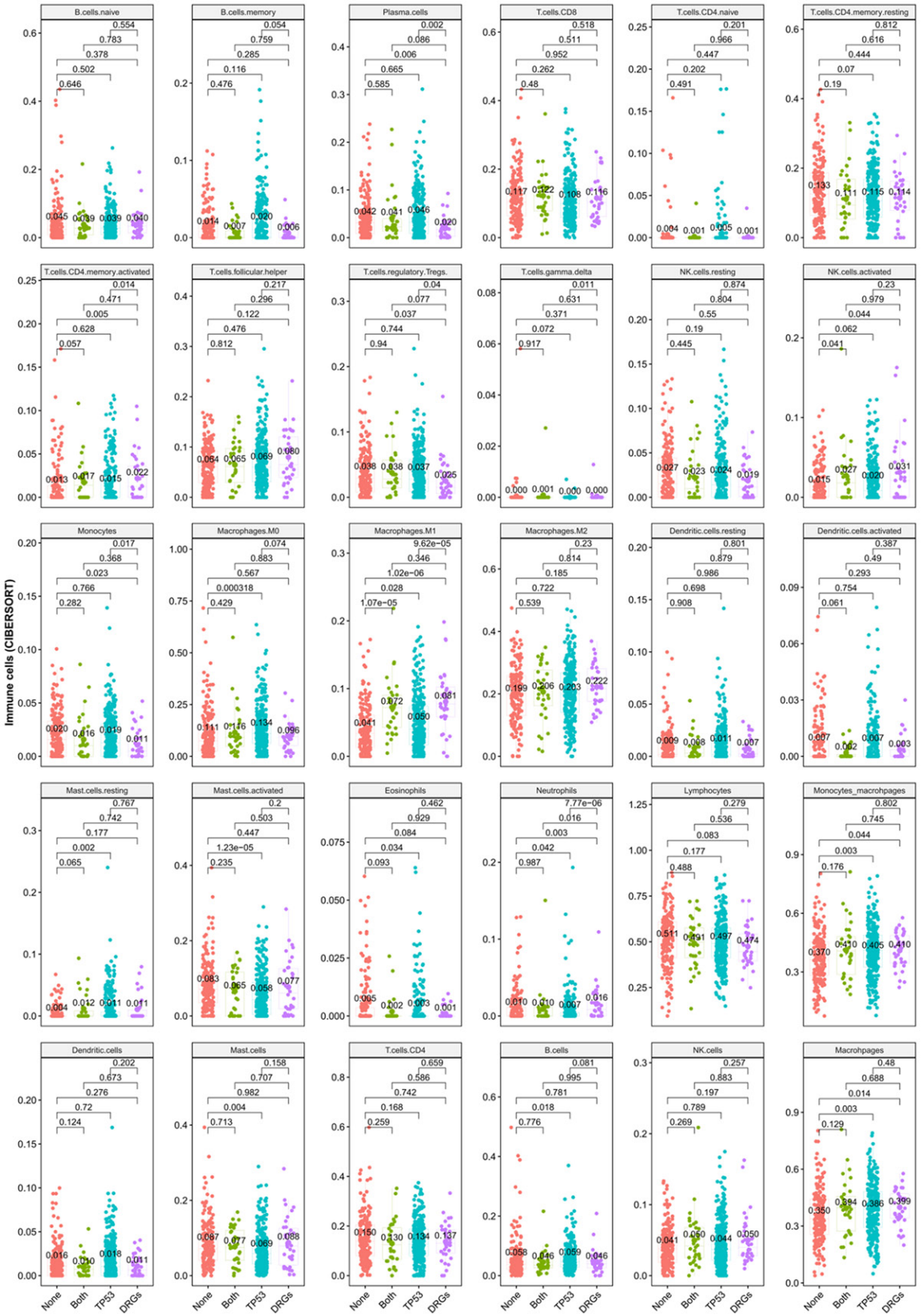

Figure 2. Boxplot of immune cells (CIBERSORT) among four groups of cancers based on *p53* and the 10 DRG (p53 target) mutation status with wilcoxon test in colorectal cancer. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

(P=0.00073) B cells, naïve CD4+ T cells (P= 0.27), Tregs (P=0.21), monocytes (P=0.016), resting (P=0.0035) and total (P=0.067) mast cells, total eosinophils (P=0.064) and lymphocytes (P=0.16) (Figure S6).

Mutations of both types correlated (significantly or with a trend) with an increase of resting (P=0.033) and activated (P=0.043) memory CD4+ T cells, M0 (P=0.013) and M1 (P=0.092), M2 (P=0.13), mono- (P=0.044) and total (P= 0.016) macrophages, resting (P=0.23) and total (P=0.19) NK cells, activated mast cells (P=0.064) and CD4+ T cells (P=0.097); and a decrease of naïve CD4+ T cells (P=0.053), monocytes (P=0.0059), resting (P=0.088) and total DCs (P=0.093), resting (P=0.0085) and total (P=0.21) mast cells and eosinophils (P=0.024) (Figure S6).

Mutations of *p53* and DRGs individually resulted in the same effect on a number of immune cell types, including the increase of plasma cells, CD8+ T cells, total NK cells and M1 macrophages and a decrease of memory B cells, naïve CD4+ T cells, resting and total mast cells and eosinophils. However, the effects were either not additive (for increasing total NK cells and decreasing resting and total mast cells and eosinophils) or even reduced (for increasing plasma cells, CD8+ T cells and M1 macrophages and decreasing memory B cells) in cases with both types of mutations compared to their individual effects in cases with only one type of mutation (Figure S6). Only for naïve CD4+ T cells, where the individual decreasing effects of both *p53* and DRG mutations were not significant (less than 1/3 and P=0.27 and 0.29 respectively), the decrease was much more apparent (60% with P=0.053) in cases with both types of mutations. For resting memory CD4+ T cells, while neither *p53* nor DRG mutations had an effect, a significant influence (increasing the cell number) was also only found in cases with both types of mutations. Similar effect was also observed for total DCs cells. *P53* and DRG mutations displayed an opposing effect on resting NK, M0 macrophages and total lymphocytes with *p53* effect dominant in cases with both types of mutations (Figure S6).

In BRCA, mutation of DRGs alone correlated (significantly or with a trend) with an increase of activated memory CD4+ T cells (P=0.28), M1 (P=0.04), M2 (P=0.075), mono- (P=0.031) and total (p=0.04) macrophages and total NKs (P=0.16); and decrease of naïve (P=0.032), memory (P=0.077) and total (P=0.0013) B cells, resting (P=0.087), activated (P=0.17) and total (P=0.041) mast cells (Figure S7). Mutation of *p53* alone was correlated (significantly or with a trend) with the increase of activated memory CD4+ T cells ($P=2\times10^{-13}$), follicular T helper cells ($P=4.2\times10^{-7}$), Tregs ($P=7.7\times10^{-7}$), resting NK (P=0.025), M0 ($P=3.3\times10^{-15}$) and M1 ($P=9.06\times10^{-20}$), mono- (P=0.00073) and total ($P=1.4\times10^{-4}$) macrophages, activated dendritic cells (P=0.021) and activated mast cells (P=0.06); and decrease of CD8+ T cells (P=0.073), monocytes ($P=3\times10^{-5}$), M2 macrophages (7.7×10^{-6}), resting dendritic cells (P=0.029), resting ($P=9.65\times10^{-35}$) and total ($P=3.45\times10^{-36}$) mast cells (Figure S7).

Mutations of both types correlated (significantly or with a trend) with an increase of activated memory CD4+ T cells ($P=3.8\times10^{-6}$), follicular T-helper cells ($P=1.2\times10^{-5}$), Tregs (P= 0.0043), M0 (P=0.0062) and M1 ($P=5.3\times10^{-6}$) macrophages, activated dendritic cells (P= 0.14) and activated mast cells (P=0.12); and decrease M2 macrophages (P=0.0023), resting ($P=4.9\times10^{-10}$) and total ($P=1.5\times10^{-10}$) mast cells (Figure S7). Although the mutations of *p53* and DRGs resulted in the same effect on a number of immune cell types, including the increase of activated memory CD4+ T cells, M1, mono- and total macrophages, and decrease of resting and total mast cells, the effect was generally stronger for *p53* mutations. These two mutation types also have their own effect on a few types of tumor microenvironment immune cells, and opposite effects on M2 macrophages and activated mast cells. In cases with both mutations, the effect of *p53* mutation played a dominant role and all the immune cell alterations were increased/ decreased with similar levels as observed in cases with *p53* mutation alone.

In glioma, where neither *p53* nor DRG mutations significantly affects TMB and tumor infiltrating immune cells are generally rare, *p53* mutation significantly influenced most of the TME immune cell types and DRG mutations also potentially affected a number of immune cell subtypes, although the impact may be lim-

ited due to limited cases of gliomas with DRG mutations (Figure S8). Mutation of DRGs alone had a trend (but none of them statistically significant, potentially due to limited number of samples) of correlation with increase of memory B cells (P=0.13), naïve CD4+ T cells (P=0.24) and activated mast cells (P=0.21); and decrease of gamma delta T cells (P=0.19), M0 macrophage (P=0.24) and neutrophils (P=0.29) (Figure S8). Mutation of *p53* alone was correlated (significantly or with a trend) with the increase of resting memory CD4+ T cells (P=0.055), activated NKs (P=0.052), monocyte ($P=1.4\times10^{-8}$), M2 (P=0.008) and mono- ($P=1.4\times10^{-5}$) macrophages, activated DCs (P=0.046), activated (P=0.003) and total (P=0.057) mast cells and eosinophils (P=0.0017); and decrease of memory (P=0.0086) and total (P=0.0022) B cells, CD8+ T cells ($P=1.1\times10^{-10}$), naïve CD4+ T cells (P=0.048), follicular T-helper ($P=2.3\times10^{-5}$), Tregs ($P=1.6\times10^{-4}$), resting (P=0.0047) and total (P=0.019) NKs, M0 ($P=5.3\times10^{-4}$) and M1 ($P=3\times10^{-4}$) macrophages, neutrophils (P=0.032) and total lymphocytes ($P=1.1\times10^{-11}$) (Figure S8).

Mutations of both types correlated (significantly or with a trend) with increase of M2 (P=0.011), mono- (P=0.0027) and total (P=0.01) macrophages and activated mast cells (P=0.25); and decrease of memory (P=0.10) and total (P=0.014) B cells, CD8+ T cells (P=0.087), follicular T-helper (P=0.046), Tregs (P=0.085), neutrophils (P=0.046) and total lymphocytes (P=0.002) (Figure S8). In general, *p53* mutation had strong impact on TME immune cells, which was not mediated by its effect on TMB, and suppressed anti-tumor immune response, while DRG mutations had limited impact on tumor infiltrating immune cells. The two types of mutations had similar effects on M0 macrophage, activated mast cells and neutrophils, but opposite effects on memory and total B cells and naïve CD4+ T cells, where the effect of *p53* mutation was dominant. For total macrophages, although each of the two types of mutations alone did not have significant effects, together they increased macrophage infiltration.

In SKCM, the impact of both *p53* and DRG mutations on tumor microenvironment immune cells were limited. Mutation of DRGs alone correlated (significantly or with a trend) with increase of naïve B cells (P=0.16), Tregs (P=0.014), M1 macrophages (P=0.19), resting (P=0.021) and total (P=0.044) DCs; and decrease of resting NKs (P=0.072) and activated mast cells (P=0.017) (Figure S9). Mutation of *p53* alone was correlated (significantly or with a trend) with the increase of naïve (P=0.24) and total (P=0.14) B cells, M1 macrophages (P=0.025) and CD4+ T cells (P=0.13); and decrease of gamma delta T cells (P=0.09), M0 macrophage (P=0.15) and activated mast cells (P=0.0067) (Figure S9). Mutations of both types had a trend (but none of them statistically significant) of correlation with a slight increase of resting CD4+ memory T cells (P=0.18) and decrease of Tregs (P=0.18) (Figure S9). While the mutations of *p53* and DRG potentially posed the same effect on increasing naïve B cells and M1 macrophages and decreasing activated mast cells, the effects were not apparent or disappeared in cases with both types of mutations. Most of the genetic effects on immune cells were exclusive to only one type of mutation and the co-occurrence of the other mutations diminished the effect, such as, DRG mutations increased Tregs, resting and total DCs and decreased resting NKs, and *p53* mutation increased CD4+ T cells as well as decreased gamma delta T cells and M0 macrophages.

Overall, in different tumor types, *p53* and DRG mutations were associated with changes of different types of immune cells, which were not always correlated to TMB. There were a few interacting patterns between *p53* and DRG mutations which were clearly different between tumor types with these mutations being either mutually exclusive (colorectal) or co-occurring. In colorectal cancers we observed generally the same effect of *p53* and DRG mutations, which remained in both mutation cases without apparent synergistic/additive effect, supporting redundant roles. In tumor types where these two types of mutations tend to co-occur, either one type of mutation counteracts the effect of the other (in ACC, BLCA and SKCM, *p53* significantly increased TMB and worked synergistically with DRG mutations) or *p53* mutation had a dominant effect on immune cell changes (in BRCA and glioma) in the patient groups with both types of mutations.

The impact of DRG and p53 mutations on overall TME immune cells in human cancers

Next, we investigated the impact of these DRG and *p53* mutations and their interaction on overall total proportion of TME immune cell changes. As we have analyzed above individual immune cell type changes for p53 target DRGs, and the results are similar to the 19 MMR and p53 target DRGs, we focused our analysis here on the influence of all the 19 MMR and p53 target DRGs on immune cell infiltration. We firstly analyzed leucocyte fraction in the tumor tissue and found that in most of the tumor types, mutations of the 19 DRGs and *p53* did not correlate with significant cancer tissue infiltrating leucocyte fraction changes. Mutations of the 19 DRGs alone significantly increased leucocyte fraction in colorectal cancer and UCEC, while a significant decrease was observed in HNSC (**Figure 3**). *p53* mutation alone significantly increased leucocyte fraction in BRCA and OV, but significantly decreased it in ESCA, HNSC, renal cancer, LUSC and STAD (**Figure 3**). In the *p53* mutation alone group, although TMB did not correlate clearly with tumor infiltrating leucocyte fraction changes across the tumor types, in TCGA database the two squamous cancer types (HNSC and LUSC) are significantly associated with decreased leucocyte fraction. These data suggest that *p53* mutation may affect leucocyte fraction in a cell type or tumor content specific manner, but not through *p53* mutation associated TMB or neoantigen changes. In cases with both DRG and *p53* mutations, the effect on leucocyte fractions by DRGs in UCEC and HNSC and *p53* in BRCA, HNSC, LUSC, OV and STAD was not reduced by the co-existence of the other type of mutations. In colorectal cancer and SKCM, DRG mutation induced leucocyte fraction increase was reduced by the occurrence of *p53* mutation, and in ESCA *p53* mutation induced leucocyte fraction decrease was reduced by the occurrence of DRG mutations (**Figure 3**).

Analyzing leucocyte fractions only in the stromal component, the significant increase/decrease effect of DRG and *p53* mutations alone and in combination observed in total tissue remained for BRCA, and colorectal cancers. In colorectal cancers, although DRG mutations significantly associated with increased stromal leucocyte fraction, it remained significant in the group with both types of mutations despite that *p53* induced significant decrease of stromal leucocyte fraction. In ESCA, LUSC, OV, renal cancer and SKCM, the effect of DRG and *p53* mutations disappeared (no longer remained significant). The *p53* mutation alone was significantly associated with decreased stromal leucocyte fraction in colorectal cancers, glioma, HNSC and STAD, and increased stromal leucocyte fraction in BRCA (Figure S10).

Tumor infiltrating lymphocytes (TILs) play a major role in anti-tumor immune response. Our further data analysis showed that the impact of DRG and *p53* mutations on TILs were different from the overall leucocyte fractions. While we found that DRG mutation alone also only significantly increased TILs in certain tumor types and never decreased TIL in any tumor type, the TIL increase was only statistically significant in BRCA (P=0.002) and UCEC ($P=1.78\times10^{-4}$). *p53* mutation alone increased TIL in a number of cancer types, including BLCA (P=0.001), BRCA ($P=1.09\times10^{-13}$) and SKCM (P=0.025). In BLCA (P=0.002), BRCA ($P=6.32\times10^{-9}$) and UCEC (P=0.043), TILs remained significantly increased in cases with both DRG and *p53* mutations. Consistent with the interacting effect of DRG and *p53* mutations on certain immune cell subtype and the total leucocyte proportion in the tumour tissues, DRG mutations reduced the TIL increase effect of *p53* mutation in SKCM (**Figure 4**).

Discussion

In this study we analyzed the cBioPortal/TCGA data for the distribution association (co-occurrence or mutual exclusivity) of *p53* and DRG mutations; and the differences of TMB, non-silence mutation load and microenvironment immune cells in four patient groups of *p53* and DRG mutation status (single type mutations, both types of mutations and neither mutation) in a large number of human cancer types with sufficient number of cases of whole genome DNA and RNA sequencing data. We found that mutual exclusivity of *p53* and DRG mutations is rare in human cancers and both types of mutations were associated with increased TMB and the co-occurrence of these two types of mutations has synergistic/additive

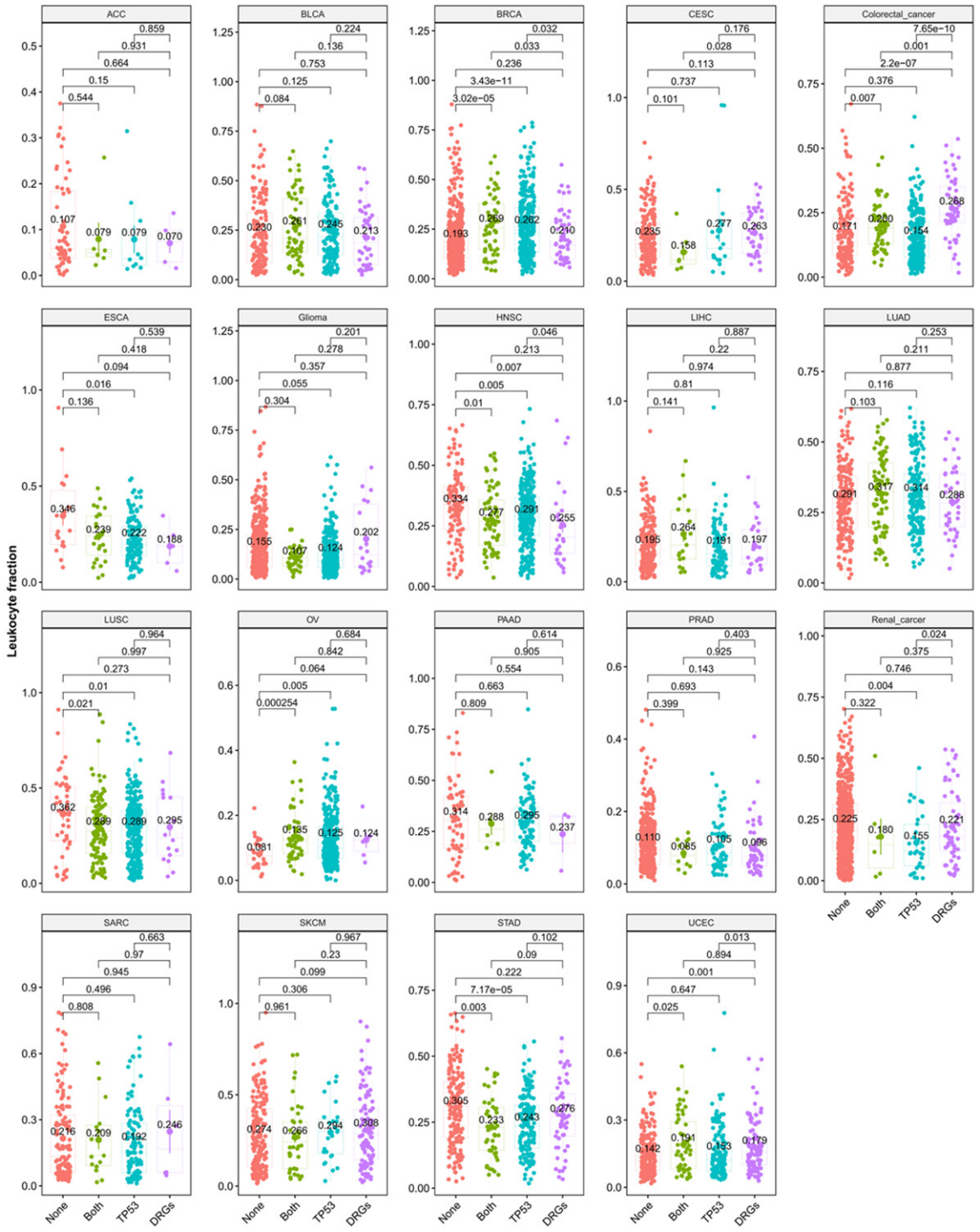

Figure 3. Boxplot of leucocyte fraction among four groups of samples based on *p53* and the 19 DRG (MMRs and p53 target) mutation status in each type of cancer with wilcoxon test. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 19 DRG mutations but not *p53* mutation.

effect. Although *p53* mutation is associated with the increase of TILs in several cancer types, the impact of *p53* and DRG mutations on TME immune cells and the interaction between the two types of mutations are cancer type and immune cell type specific.

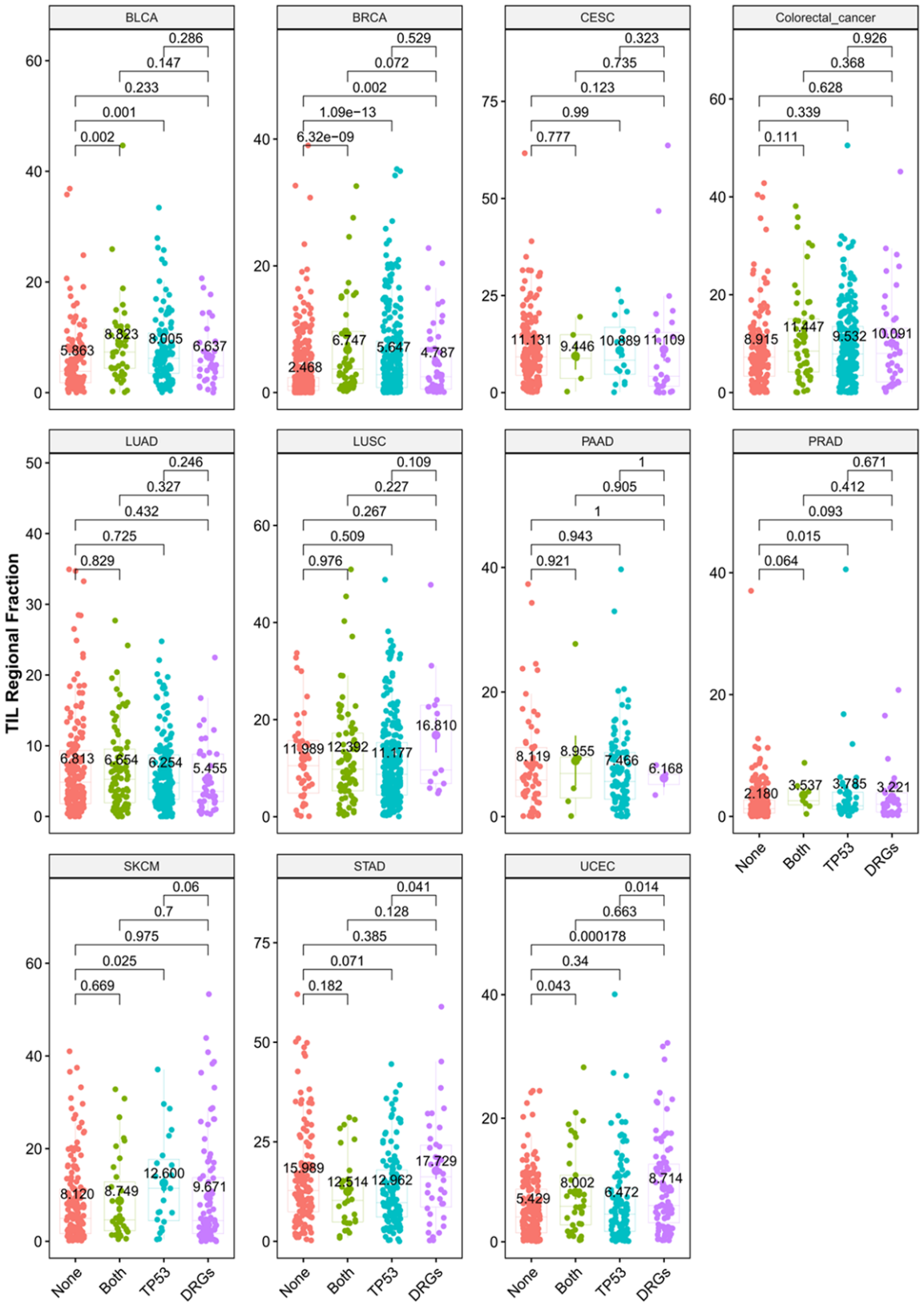

Figure 4. Boxplot of TIL regional fraction among four groups of samples based on *p53* and the 19 DRG (MMRs and p53 target) mutation status in each type of cancer with wilcoxon test. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 19 DRG mutations but not *p53* mutation.

Our initial distribution analysis in human malignancies revealed that different correlation patterns of *p53* and DRG mutations existed depending on tumor types. The random distribution of *p53* and DRG mutations in most of human malignancies and the tendency of co-occurrence of them in a number of tumors suggest that DNA repair processes are unlikely to be the mediators of p53-dependent tumor suppression as previously reported [15] in most of human tumors. On the contrary, the tendency of co-occurrence of these two types of mutations may indicate that cooperation between them promote tumorigenesis in certain types of human tumors. For example, as *p53* can activate cell senescence, apoptosis, cell cycle arrest and anti-tumor immunity [2, 6-11], loss of *p53* function by its mutation enables cancer cells with DRG mutations and the resulted high neoantigens to escape the p53-mediated tumor suppressor role.

The general consequence of DRG mutations is the increase of TMB [24]. As *p53* has a genomic stability surveillance role [1], *p53* mutation also has the potential to increase TMB and may be mediated through the activation of DRG genes in colorectal cancers, where *p53* and DRG mutations are mutually exclusive due to the functional redundancy of the two types of mutations in inducing TMB [15]. Surprisingly, *p53* has no effect in increasing TMB in colorectal cancers and it significantly reduced DRG mutation induced TMB rates in cases with both types of mutations. Therefore, the data further supports that the tumor suppressive role of *p53* may not be mediated by DRG genes through their function of DNA repairing. Our data analysis also confirmed the general expectation that TMB and non-silent mutations were highly correlated across different cancers, although there were some slightly different results between TMB and non-silent mutation analysis in certain tumour types. However, although none of them significantly affected TIL abundance and they affected total TME immune cells differentially as they did on TMB and non-silence mutation load, our data showed that the *p53* and DRG mutations had similar effects on most of the immune cell subtypes in colorectal cancers, and the effect seems redundant (no additive effect in cases with both types of mutations). This also suggests that DRGs may also modulate anti-tumor immune response through molecular pathways/mechanisms separating from their well-established function in DNA damage repair and prevention of the accumulation of TMB, which may be shared with *p53* in regulating certain types of immune cells. It has recently been reported that DRG mutations predict immune checkpoint inhibitor response beyond TMB although the mechanisms are not clear yet [25]. Further investigation is warranted.

To further investigate the role of *p53* and DRG mutations in human cancers, we investigated the impact of *p53* and DRG mutations alone or together on TMB in other human tumors. If their role in increasing TMB are redundant, we will not see synergistic/additive effect of increasing TMB in cases with both types of mutations. As expected, we observed the increase of TMB in cases with DRG mutations alone for most tumor types with sufficient cases with DRG mutations, except ESCA, glioma and SARC. While *p53* mutation also increased TMB in most of the cancer types, in the majority of these tumors *p53* and DRG mutations had synergistic/additive effect of increasing TMB. Therefore, the data further support that in the majority of human cancers, the tumor suppressive role of *p53* may not be mediated by DRG genes through their function of DNA repair. Although in certain human cancers some DRGs may mediate the tumor suppressive role of p53, it is not a common *p53* pathway of action in human tumorigenesis.

One important finding of this study is the cooperation of *p53* and DRG mutations to synergistically/additively increase TMB in many human tumor types, which may be explained by the cooperation of the well-established effects of DRG mutations in TMB induction and *p53* mutations in permitting the survival of high TMB tumor cells. In these cancers, the function of p53, either by the traditional role in suppressing [2] or *p53* promoted anti-tumor immune response [6-11], may prevent tumor cells from accumulation of genomic mutation independent from DRGs. In breast cancer, where both *p53* and DRG mutations increased TMB and non-silence mutation load without additive effect, the two types of mutations showed strong co-occurrence. The counteractive effect of these two types of mutation on

many TME immune cell types may explain the advantage of their co-occurrence during BRCA development and/or progression, by avoiding immune surveillance induced by TMB and neoantigen, which are caused by the mutations. Further investigation and understanding of the counter-active effect on immune response may open important novel therapeutic strategies. It will also be interesting in further investigating why DRG mutation failed to increase TMB in glioma.

It has now been well established that the host immune response plays a critical role in tumorigenesis and cancer cell evolution [26]. As non-silence mutations have the potential to cause neoantigens and induce anti-tumor immune response, both *p53* and DRG mutations have the potential to induce anti-tumor immune response, either through the induction of neoantigens or via the immune activation role of *p53*, independent from increasing neoantigens [6, 7, 11, 12]. Interestingly, we found that in colorectal cancer where *p53* and DRG mutations were mutually exclusive, although these two types of mutations did not have the same effect on TMB, they had similar effect on TME immune cell population changes without apparent additive effect. This finding suggests that certain undiscovered novel cellular pathways irrelevant to DNA damage repair may be shared by *p53* and DRGs. It has been reported that *p53* mutation increases cancer promoting inflammation through the activation of NF-kB [7]. Further mechanistic investigations are warranted.

Although our results of the general impact of *p53* mutation on TILs is consistent with the previous study without considering the interaction of *p53* and DRG mutations [9], we observed a different effect of *p53* and DRG mutations on TME immune cells in a tumor type specific manner. Different effects of *p53* mutations and TMB on TME immune cells in various human tumors have been observed in previous studies [7, 10, 12]. In certain tumor types, *p53* mutation is associated with increased anti-tumor immune response [10, 27], which may selectively kill cancer cells with increased TMB and non-silence mutation load.

Importantly, we observed various interacting patterns between these two types of mutations, including synergistic/additive effect, counteracting effect and effects generated only when both mutations occur together while neither of them showed effects individually, depending on tumor types and immune cell subtypes. Mismatch repair deficiency and microsatellite instability have been developed as biomarkers to predict anti-PD-1/PL-L1 immunotherapy response and it was the first time that the FDA approved a cancer treatment based solely on the genetic profile irrespective of the tumor type [28-30]. However, not all cancers with mismatch repair deficiency/microsatellite instability respond to anti-PD-1/PL-L1 immunotherapy and the reasons are not clear yet [29, 30]. We showed here that in several cancers including ACC, BRCA, glioma and SKAM, *p53* mutation induced immune response either counteracted or dominated DRG mutation induced immune response, suggesting that *p53* mutation status may be a critical factor to consider when using mismatch repair deficiency or microsatellite instability as a predictive biomarker for anti-PD-1/PL-L1 immunotherapy of certain cancers.

In certain tumors, we observed different effects of *p53* and TMB mutations on immune cells from the previous study investigating the impact of *p53* mutation and TMB [10]. This may be explained by the difference in grouping of tumor samples for data analysis and certain varying effects of DRG mutations and TMB on immune cells. This also further supports the importance of considering the interacting genes needed for a molecular change on immune response in a tumor type specific manner, which may be critical for the design/selection of therapeutic strategies including immunotherapy. Based on our findings of *p53* and DRG mutations in influencing TME immune cells, further mechanistic investigations of the functions of *p53* and DRGs and their interaction in individual tumor types are encouraged.

In summary, we analyzed many human tumors in TCGA for the distribution association (co-occurrence or mutual exclusivity) of *p53* and DRG mutations and their impact alone and in combination on TMB, potential neoantigen generating non-silence mutation load and tumor microenvironment immune cell changes. We found that in most cancer types, both *p53* and DRG mutations are associated individually with

increased TMB and their role in general has a synergistic/additive effect instead of redundant in cases where the two types of mutations co-occur. The impact of *p53* and DRG mutations and their interaction on TMB and tumor microenvironment immune cells are complex and in a cancer type and immune cell subtype specific manor. *p53* mutation can induce TME immune cell changes through multiple molecular pathways. While DRG mutations may induce TME immune cell changes mainly through increasing TMB and non-silence mutation load, novel function of DRG may exist. This study provides new insights into the interaction of *p53* and DRG mutations in tumorigenesis and their impact on TMB and immune response. The difference in association of *p53* and DRG mutation patterns and their role in tumorigenesis and cancer immune response suggest that different therapeutic strategies should be developed accordingly.

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Disclosure of conflict of interest

None.

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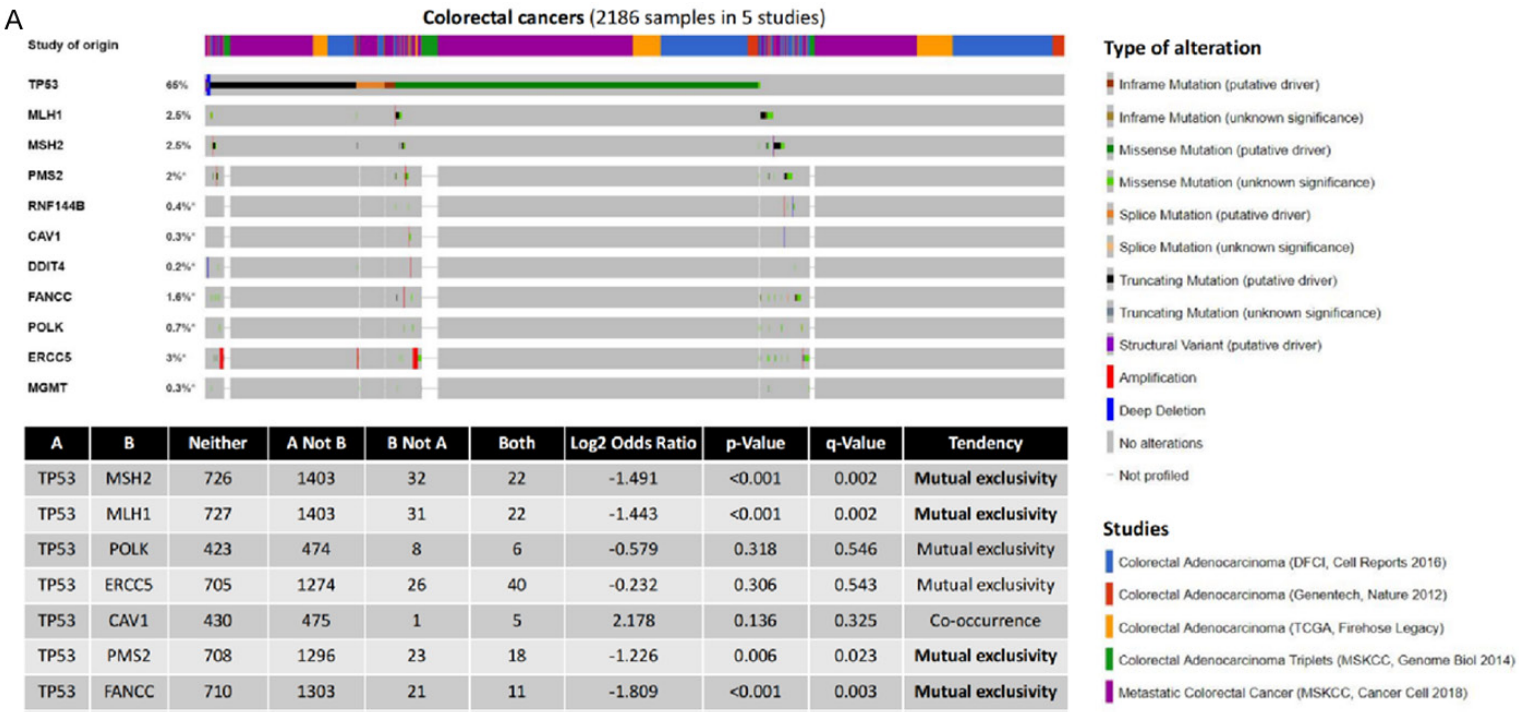
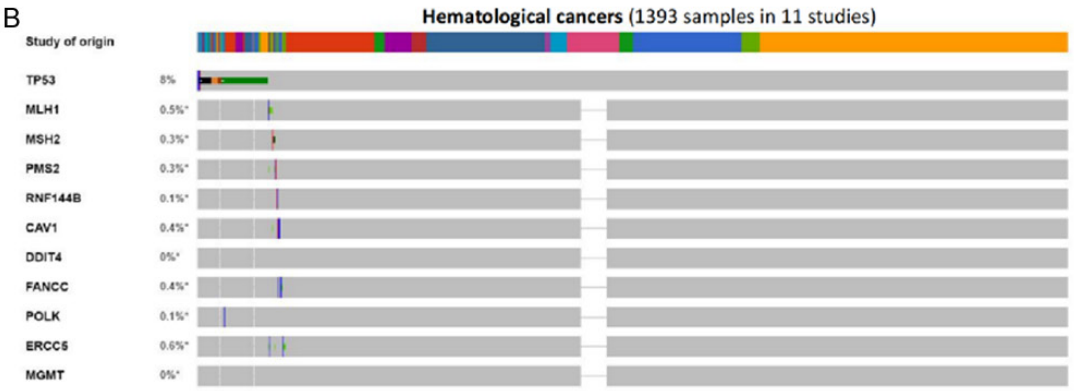
A



A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
TP53	MSH2	726	1403	32	22	-1.491	<0.001	0.002	**Mutual exclusivity**
TP53	MLH1	727	1403	31	22	-1.443	<0.001	0.002	**Mutual exclusivity**
TP53	POLK	423	474	8	6	-0.579	0.318	0.546	Mutual exclusivity
TP53	ERCC5	705	1274	26	40	-0.232	0.306	0.543	Mutual exclusivity
TP53	CAV1	430	475	1	5	2.178	0.136	0.325	Co-occurrence
TP53	PMS2	708	1296	23	18	-1.226	0.006	0.023	**Mutual exclusivity**
TP53	FANCC	710	1303	21	11	-1.809	<0.001	0.003	**Mutual exclusivity**
TP53	RNF144B	424	478	7	2	-1.98	0.065	0.171	Mutual exclusivity
TP53	DDIT4	430	476	1	4	1.853	0.222	0.452	Co-occurrence
TP53	MGMT	428	476	3	4	0.262	0.559	0.931	Co-occurrence

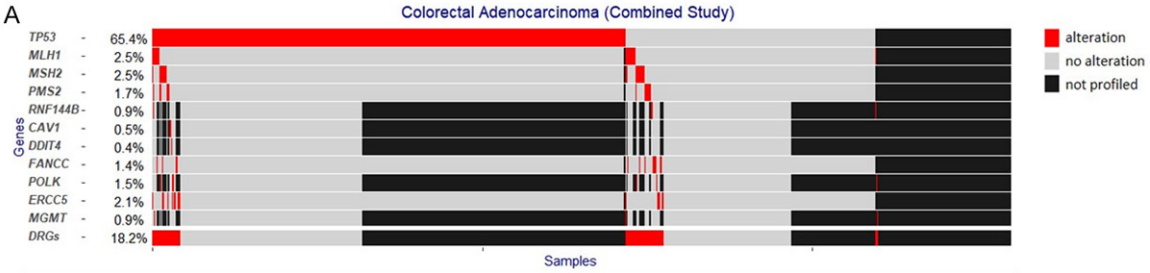
B



A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
TP53	POLK	1224	110	0	1	>3	0.083	0.572	Co-occurrence
TP53	ERCC5	1216	111	8	0	<-3	0.498	1.000	Mutual exclusivity
TP53	MLH1	1217	111	7	0	<-3	0.544	1.000	Mutual exclusivity
TP53	CAV1	1219	111	5	0	<-3	0.647	1.000	Mutual exclusivity
TP53	MSH2	1220	111	4	0	<-3	0.706	1.000	Mutual exclusivity
TP53	PMS2	1220	111	4	0	<-3	0.706	1.000	Mutual exclusivity
TP53	FANCC	1220	111	4	0	<-3	0.706	1.000	Mutual exclusivity
TP53	RNF144B	1222	111	2	0	<-3	0.841	1.000	Mutual exclusivity
TP53	DDIT4	1224	111	0	0	>3	1.000	1.000	Co-occurrence
TP53	MGMT	1224	111	0	0	>3	1.000	1.000	Co-occurrence

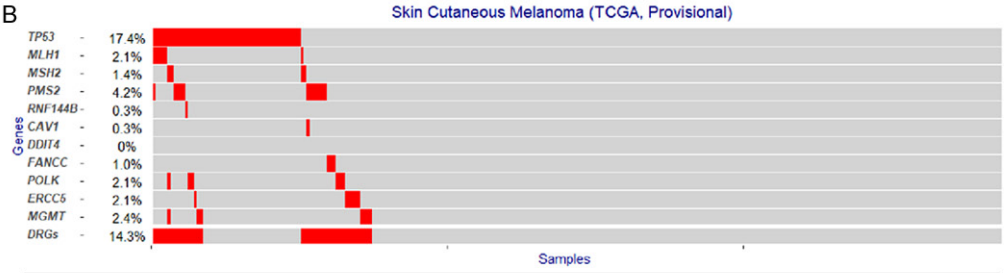
Figure S1. *P53* and the 10 p53 target DNA repair gene mutation distribution in human malignancies analyzed in the same way as in the publication by Janic *et al*. [15] based on cBioPortal [20, 21] data. A. Colorectal cancer (combined study); B. Hematological malignancies (combined study).

A



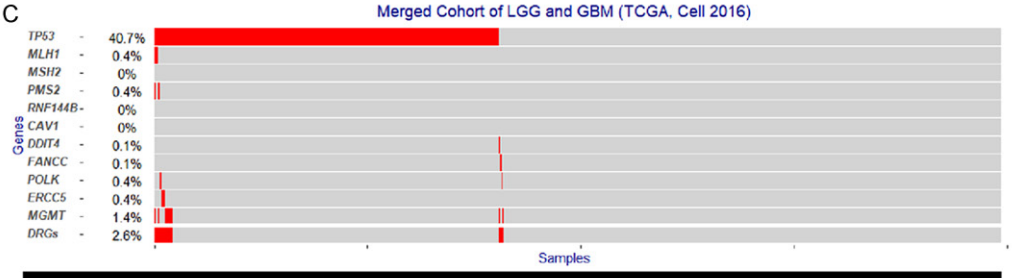
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.Value	q.value
TP53	*MLH1*	728	1405	31	22	−1.443	**Mu-ex**	**2.93E-04**	0.001
TP53	*MSH2*	728	1405	31	23	−1.379	**Mu-ex**	**4.59E-04**	0.002
TP53	*PMS2*	736	1412	23	15	−1.557	**Mu-ex**	**9.31E-04**	0.002
TP53	*RNF144B*	453	591	6	2	−1.969	-	0.075	0.126
TP53	*CAV1*	458	589	1	4	1.637	-	0.277	0.346
TP53	*DDIT4*	458	590	1	3	1.22	-	0.413	0.459
TP53	*FANCC*	738	1417	21	10	−2.012	**Mu-ex**	**1.65E-04**	0.001
TP53	*POLK*	451	587	8	6	−0.795	-	0.224	0.320
TP53	*ERCC5*	734	1408	25	19	−1.336	**Mu-ex**	**0.002**	0.004
TP53	*MGMT*	456	589	3	4	0.046	-	0.637	0.637
TP53	DRGs	386	552	117	84	−0.994	**Mu-ex**	**8.00E-06**	

B



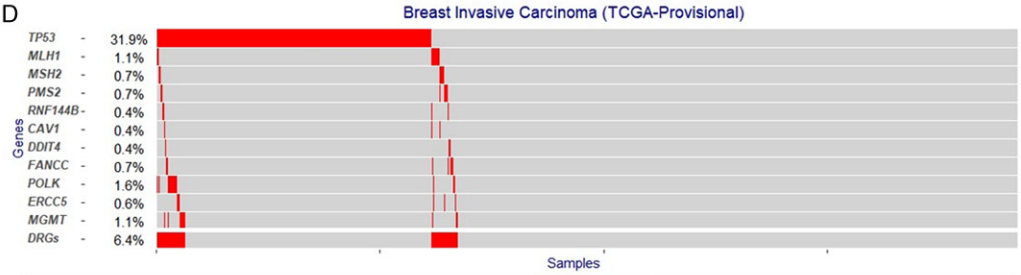
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	236	45	1	5	4.713	**Co-oc**	**7.03e-04**	0.006
TP53	*MSH2*	235	48	2	2	2.292	-	0.141	0.254
TP53	*PMS2*	230	45	7	5	1.868	**Co-oc**	**0.040**	0.179
TP53	*RNF144B*	237	49	0	1	Inf	-	0.174	0.261
TP53	*CAV1*	236	50	1	0	-Inf	-	0.826	0.826
TP53	*DDIT4*								
TP53	*FANCC*	234	50	3	0	-Inf	-	0.562	0.722
TP53	*POLK*	234	47	3	3	2.316	-	0.068	0.203
TP53	*ERCC5*	232	49	5	1	-0.079	-	0.719	0.809
TP53	*MGMT*	233	47	4	3	1.895	-	0.104	0.234
TP53	DRGs	213	33	24	17	2.193	**Co-oc**	**6.65e-05**	

C



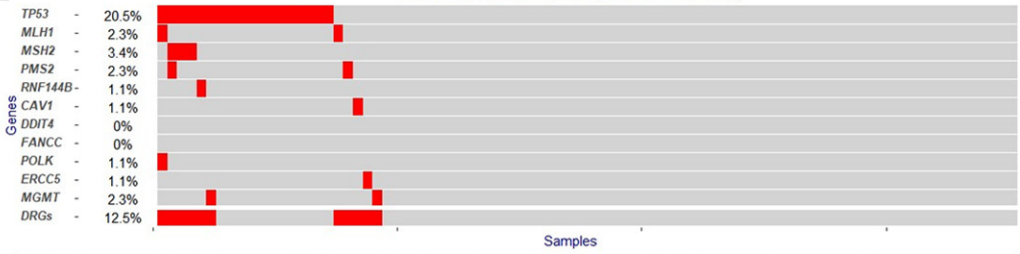
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	471	320	0	3	Inf	-	0.067	0.117
TP53	*MSH2*								
TP53	*PMS2*	471	320	0	3	Inf	-	0.067	0.117
TP53	*RNF144B*								
TP53	*CAV1*								
TP53	*DDIT4*	470	323	1	0	-Inf	-	0.593	0.593
TP53	*FANCC*	470	323	1	0	-Inf	-	0.593	0.593
TP53	*POLK*	470	321	1	2	1.55	-	0.362	0.506
TP53	*ERCC5*	471	320	0	3	Inf	-	0.067	0.117
TP53	*MGMT*	469	314	2	9	2.749	**Co-oc**	**0.006**	0.045
TP53	DRGs	467	306	4	17	2.697	**Co-oc**	**1.65e-04**	

D



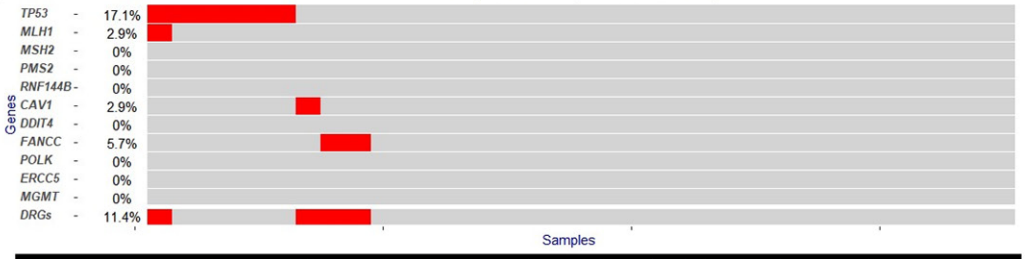
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	647	305	9	2	-1.085	-	0.265	0.618
TP53	*MSH2*	651	305	5	2	-0.228	-	0.604	0.618
TP53	*PMS2*	651	305	5	2	-0.228	-	0.604	0.618
TP53	*RNF144B*	654	305	2	2	1.1	-	0.382	0.618
TP53	*CAV1*	653	306	3	1	-0.491	-	0.618	0.618
TP53	*DDIT4*	654	305	2	2	1.1	-	0.382	0.618
TP53	*FANCC*	651	305	5	2	-0.228	-	0.604	0.618
TP53	*POLK*	653	295	3	12	3.146	**Co-oc**	**1.57e-04**	0.002
TP53	*ERCC5*	653	304	3	3	1.103	-	0.291	0.618
TP53	*MGMT*	653	299	3	8	2.542	**Co-oc**	**0.006**	0.031
TP53	DRGs	626	275	30	32	1.28	**Co-oc**	**6.72e-04**	

E



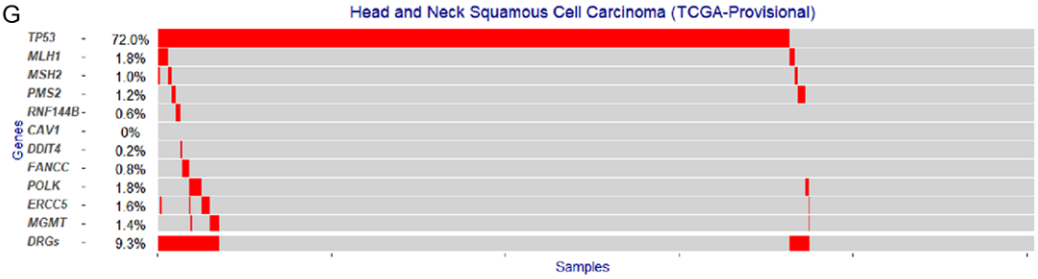
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	69	17	1	1	2.021	-	0.369	0.492
TP53	*MSH2*	70	15	0	3	Inf	**Co-oc**	**0.007**	0.059
TP53	*PMS2*	69	17	1	1	2.021	-	0.369	0.492
TP53	*RNF144B*	70	17	0	1	Inf	-	0.205	0.492
TP53	*CAV1*	69	18	1	0	-Inf	-	0.795	0.795
TP53	*DDIT4*								
TP53	*FANCC*								
TP53	*POLK*	70	17	0	1	Inf	-	0.205	0.492
TP53	*ERCC5*	69	18	1	0	-Inf	-	0.795	0.795
TP53	*MGMT*	69	17	1	1	2.021	-	0.369	0.492
TP53	DRGs	65	12	5	6	2.7	**Co-oc**	**0.008**	

F



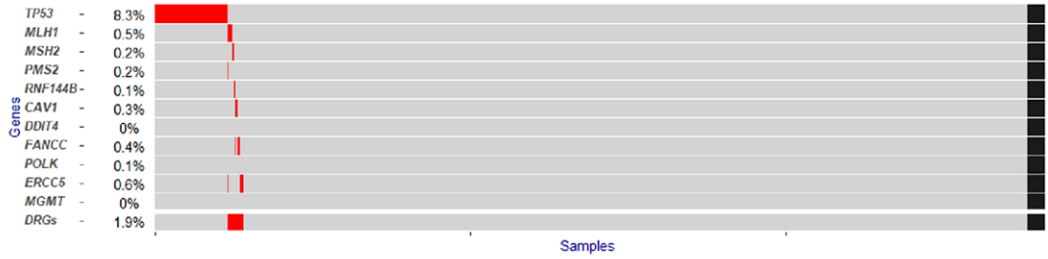
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.Value
TP53	*MLH1*	29	5	0	1	Inf	-	0.171	0.514
TP53	*MSH2*								
TP53	*PMS2*								
TP53	*RNF144B*								
TP53	*CAV1*	28	6	1	0	-Inf	-	0.829	0.829
TP53	*DDIT4*								
TP53	*FANCC*	27	6	2	0	-Inf	-	0.682	0.829
TP53	*POLK*								
TP53	*ERCC5*								
TP53	*MGMT*								
TP53	DRGs	26	5	3	1	0.794	-	0.546	

G



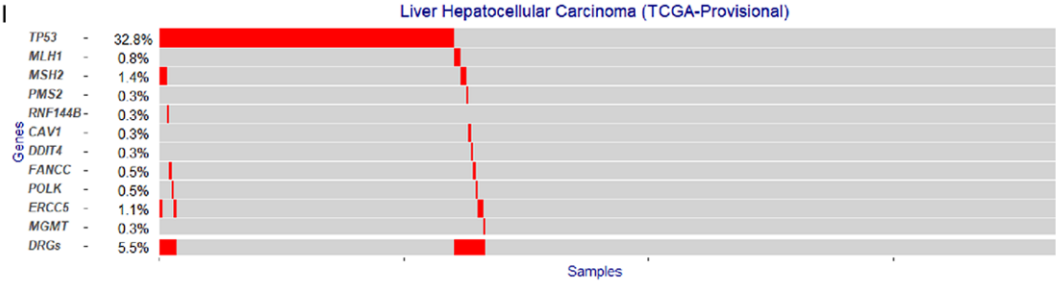
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	138	357	3	6	-0.371	-	0.483	0.582
TP53	*MSH2*	139	360	2	3	-0.788	-	0.430	0.582
TP53	*PMS2*	137	361	4	2	-2.398	-	0.054	0.490
TP53	*RNF144B*	141	360	0	3	Inf	-	0.373	0.582
TP53	*CAV1*								
TP53	*DDIT4*	141	362	0	1	Inf	-	0.720	0.720
TP53	*FANCC*	141	359	0	4	Inf	-	0.268	0.582
TP53	*POLK*	139	356	2	7	0.451	-	0.517	0.582
TP53	*ERCC5*	140	356	1	7	1.461	-	0.295	0.582
TP53	*MGMT*	140	357	1	6	1.234	-	0.372	0.582
TP53	DRGs	129	328	12	35	0.198	-	0.420	

H



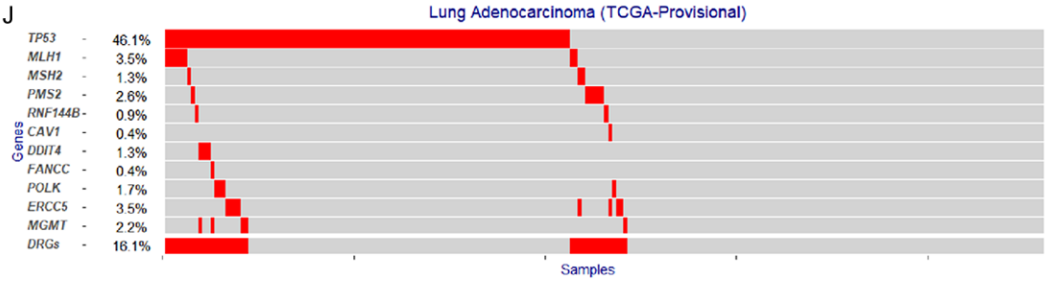
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	1261	115	7	0	-Inf	-	0.544	0.881
TP53	*MSH2*	1265	115	3	0	-Inf	-	0.771	0.881
TP53	*PMS2*	1265	115	3	0	-Inf	-	0.771	0.881
TP53	*RNF144B*	1267	115	1	0	-Inf	-	0.917	0.917
TP53	*CAV1*	1264	115	4	0	-Inf	-	0.706	0.881
TP53	*DDIT4*								
TP53	*FANCC*	1263	115	5	0	-Inf	-	0.647	0.881
TP53	*POLK*	1268	114	0	1	Inf	-	0.083	0.665
TP53	*ERCC5*	1260	115	8	0	-Inf	-	0.498	0.881
TP53	*MGMT*								
TP53	DRGs	1243	114	25	1	-1.197	-	0.349	

I



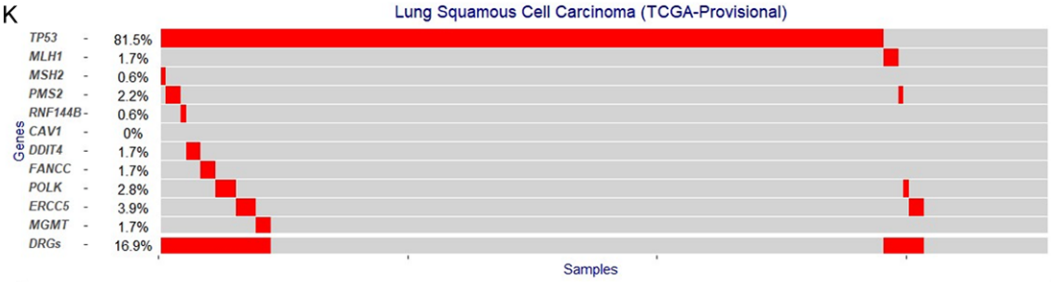
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	243	120	3	0	-Inf	-	0.302	0.672
TP53	*MSH2*	244	117	2	3	1.645	-	0.200	0.672
TP53	*PMS2*	245	120	1	0	-Inf	-	0.672	0.672
TP53	*RNF144B*	246	119	0	1	Inf	-	0.328	0.672
TP53	*CAV1*	245	120	1	0	-Inf	-	0.672	0.672
TP53	*DDIT4*	245	120	1	0	-Inf	-	0.672	0.672
TP53	*FANCC*	245	119	1	1	1.042	-	0.549	0.672
TP53	*POLK*	245	119	1	1	1.042	-	0.549	0.672
TP53	*ERCC5*	244	118	2	2	1.048	-	0.398	0.672
TP53	*MGMT*	245	120	1	0	-Inf	-	0.672	0.672
TP53	DRGs	233	113	13	7	0.151	-	0.501	

J



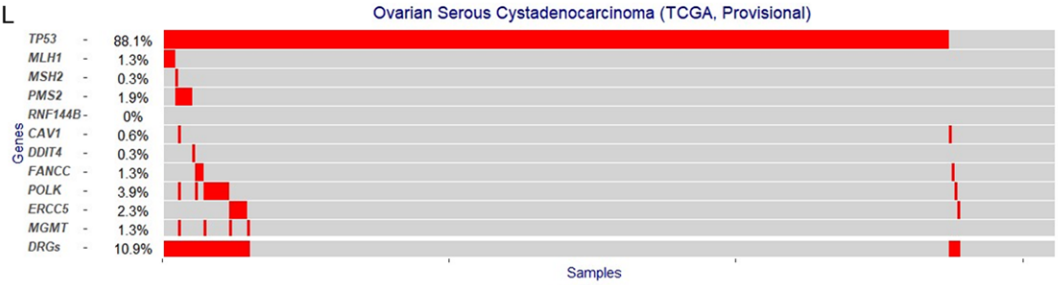
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	122	100	2	6	1.872	-	0.095	0.368
TP53	*MSH2*	122	105	2	1	-0.784	-	0.559	0.621
TP53	*PMS2*	119	105	5	1	-2.141	-	0.147	0.368
TP53	*RNF144B*	123	105	1	1	0.228	-	0.710	0.710
TP53	*CAV1*	123	106	1	0	-Inf	-	0.539	0.621
TP53	*DDIT4*	124	103	0	3	Inf	-	0.096	0.368
TP53	*FANCC*	124	105	0	1	Inf	-	0.461	0.621
TP53	*POLK*	123	103	1	3	1.841	-	0.254	0.509
TP53	*ERCC5*	120	102	4	4	0.234	-	0.549	0.621
TP53	*MGMT*	123	102	1	4	2.270	-	0.140	0.368
TP53	DRGs	109	84	15	22	0.928	-	0.055	

K



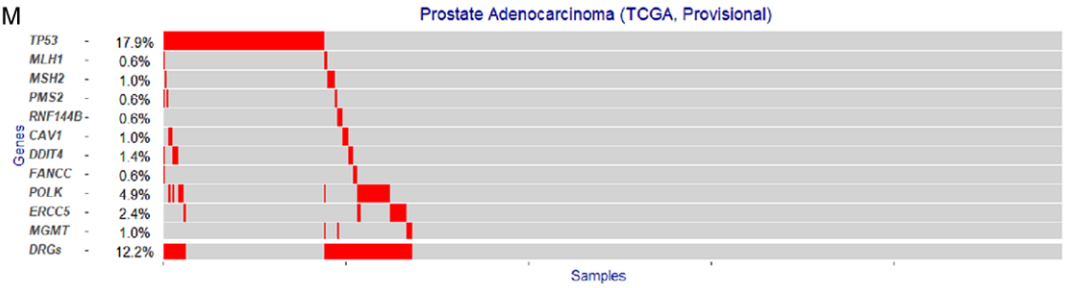
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	30	145	3	0	-Inf	**Mu-ex**	0.006	0.053
TP53	*MSH2*	33	144	0	1	Inf	-	0.815	0.815
TP53	*PMS2*	32	142	1	3	-0.565	-	0.563	0.815
TP53	*RNF144B*	33	144	0	1	Inf	-	0.815	0.815
TP53	*CAV1*								
TP53	*DDIT4*	33	142	0	3	Inf	-	0.538	0.815
TP53	*FANCC*	33	142	0	3	Inf	-	0.538	0.815
TP53	*POLK*	32	141	1	4	-0.140	-	0.646	0.815
TP53	*ERCC5*	30	141	3	4	-1.818	-	0.120	0.539
TP53	*MGMT*	33	142	0	3	Inf	-	0.538	0.815
TP53	DRGs	25	123	8	22	-0.839	-	0.158	

L



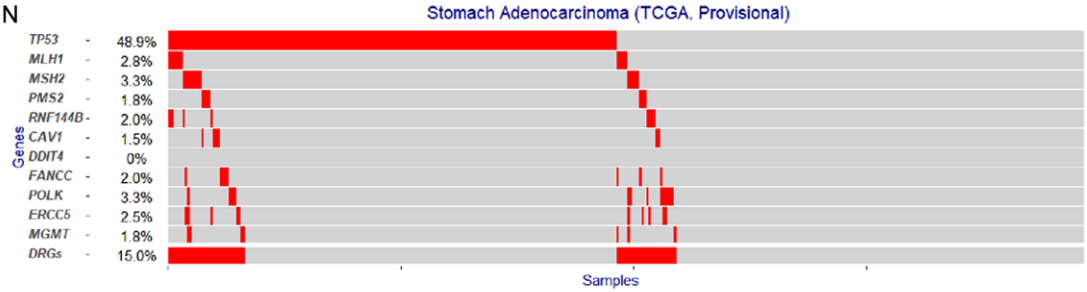
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	37	270	0	4	Inf	-	0.601	0.773
TP53	*MSH2*	37	273	0	1	Inf	-	0.881	0.881
TP53	*PMS2*	37	268	0	6	Inf	-	0.465	0.773
TP53	*RNF144B*								
TP53	*CAV1*	36	273	1	1	-2.923	-	0.224	0.773
TP53	*DDIT4*	37	273	0	1	Inf	-	0.881	0.881
TP53	*FANCC*	36	271	1	3	-1.327	-	0.399	0.773
TP53	*POLK*	36	263	1	11	0.590	-	0.571	0.773
TP53	*ERCC5*	36	268	1	6	-0.311	-	0.592	0.773
TP53	*MGMT*	37	270	0	4	Inf	-	0.601	0.773
TP53	DRGs	33	244	4	30	0.021	-	0.621	

M



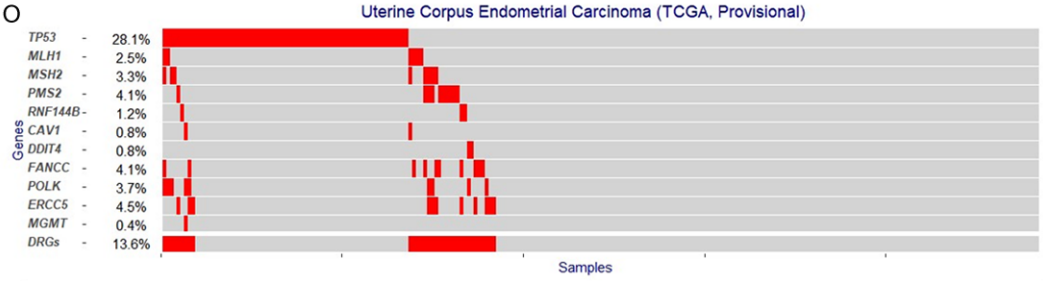
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	402	87	2	1	1.208	-	0.447	0.559
TP53	*MSH2*	400	87	4	1	0.201	-	0.628	0.628
TP53	*PMS2*	403	86	1	2	3.228	-	0.084	0.420
TP53	*RNF144B*	401	88	3	0	-Inf	-	0.553	0.614
TP53	*CAV1*	401	86	3	2	1.636	-	0.220	0.559
TP53	*DDIT4*	401	84	3	4	2.670	**Co-oc**	**0.022**	0.218
TP53	*FANCC*	402	87	2	1	1.208	-	0.447	0.559
TP53	*POLK*	385	83	19	5	0.288	-	0.434	0.559
TP53	*ERCC5*	393	87	11	1	-1.284	-	0.336	0.559
TP53	*MGMT*	399	88	5	0	-Inf	-	0.372	0.559
TP53	DRGs	356	76	48	12	0.228	-	0.381	

N



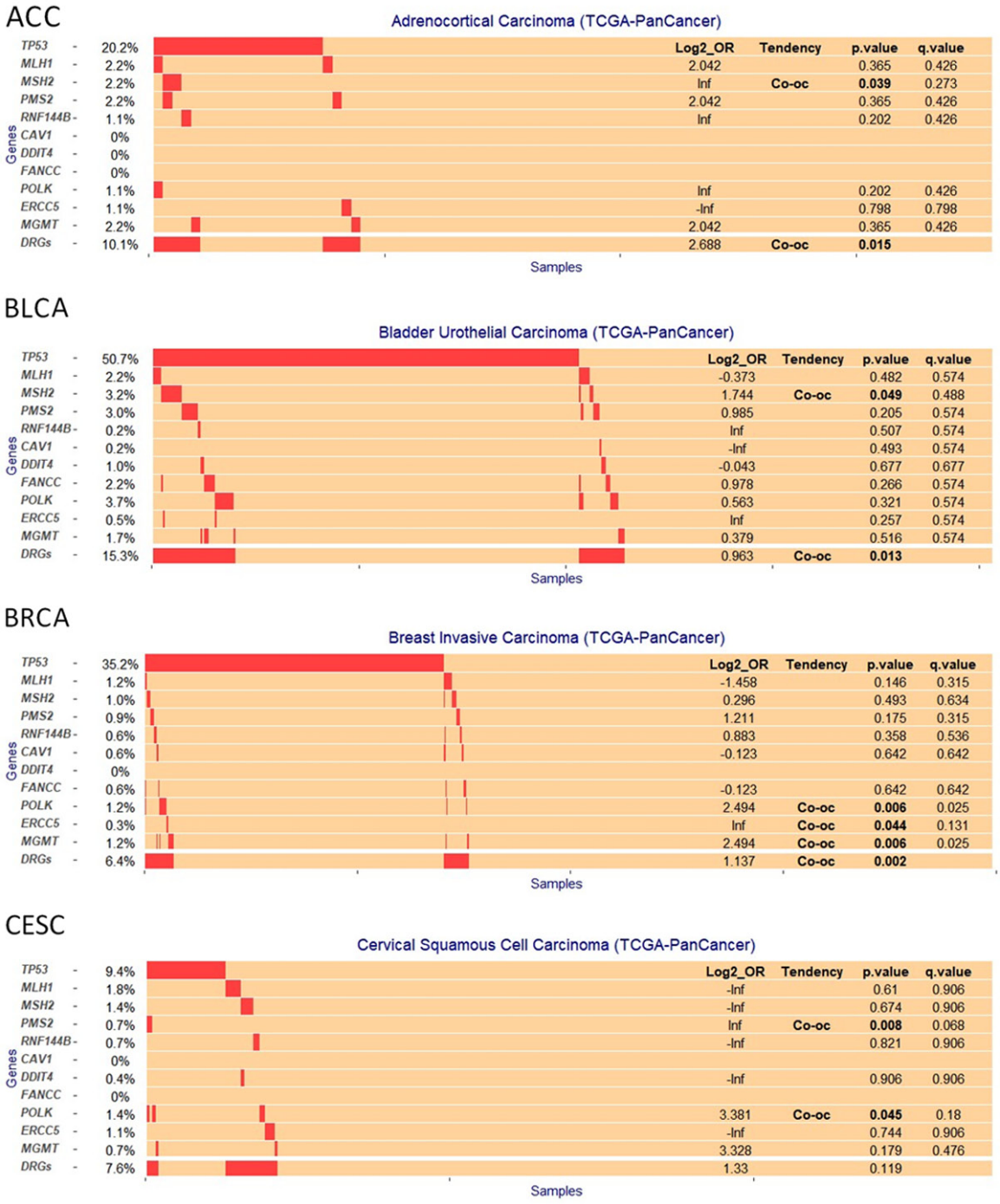
Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	196	186	5	6	0.339	-	0.469	0.610
TP53	*MSH2*	196	184	5	8	0.769	-	0.259	0.610
TP53	*PMS2*	198	188	3	4	0.490	-	0.475	0.610
TP53	*RNF144B*	197	188	4	4	0.067	-	0.613	0.613
TP53	*CAV1*	199	188	2	4	1.082	-	0.321	0.610
TP53	*DDIT4*								
TP53	*FANCC*	198	187	3	5	0.819	-	0.337	0.610
TP53	*POLK*	192	188	9	4	-1.140	-	0.148	0.610
TP53	*ERCC5*	196	187	5	5	0.068	-	0.596	0.613
TP53	*MGMT*	198	188	3	4	0.490	-	0.475	0.610
TP53	DRGs	175	159	26	33	0.482	-	0.150	

O



Gene_A	Gene_B	Neither	A_not_B	B_not_A	Both	Log2_OR	Tendency	p.value	q.value
TP53	*MLH1*	170	66	4	2	0.365	-	0.540	0.630
TP53	*MSH2*	169	65	5	3	0.642	-	0.400	0.630
TP53	*PMS2*	165	67	9	1	-1.870	-	0.175	0.630
TP53	*RNF144B*	172	67	2	1	0.360	-	0.630	0.630
TP53	*CAV1*	173	67	1	1	1.369	-	0.484	0.630
TP53	*DDIT4*	172	68	2	0	-Inf	-	0.516	0.630
TP53	*FANCC*	166	66	8	2	-0.669	-	0.432	0.630
TP53	*POLK*	170	63	4	5	1.754	-	0.073	0.630
TP53	*ERCC5*	166	65	8	3	-0.062	-	0.626	0.630
TP53	*MGMT*	174	67	0	1	Inf	-	0.281	0.630
TP53	DRGs	150	59	24	9	-0.069	-	0.546	

Figure S2. Distribution of *p53* and the 10 p53 target DNA repair gene mutations (excluding amplification) in human malignancies based on TCGA-Provision datasets from cBioPortal [20, 21] data. A. Colorectal cancer (combined study); B. Skin cutaneous melanoma; C. Glioma; D. Breast invasive carcinoma; E. Adrenocorticalcarcinoma; F. Cholangiocarcinoma; G. Head and neck squamous cell carcinoma; H. Hematological malignancies (combined study); I. Liver hepatocellular carcinoma; J. Lung adenocarcinoma; K. Lung squamous cell carcinoma; L. Ovarian serous cystadenocarcinoma; M. Prostate adenocarcinoma; N. Stomach adenocarcinoma; O. Uterine corpus endometrial carcinoma. DRGs: mutation in any of the 10 p53 target DNA repair genes in combination; e-n: $\times 10^{-n}$; OR: odd ratio; Mu-ex: mutual exclusivity; Co-oc: co-occurrence; Inf: Infinity.



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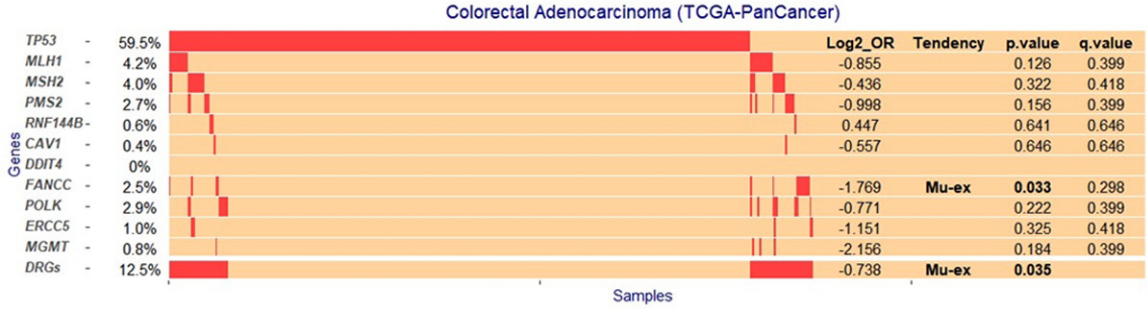

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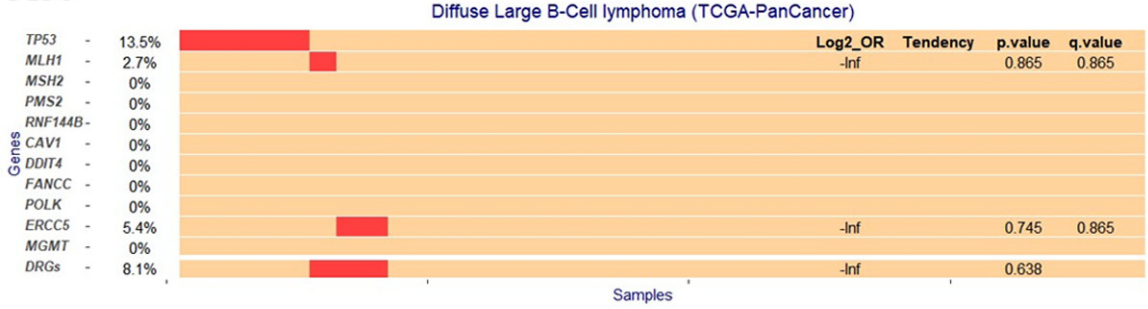

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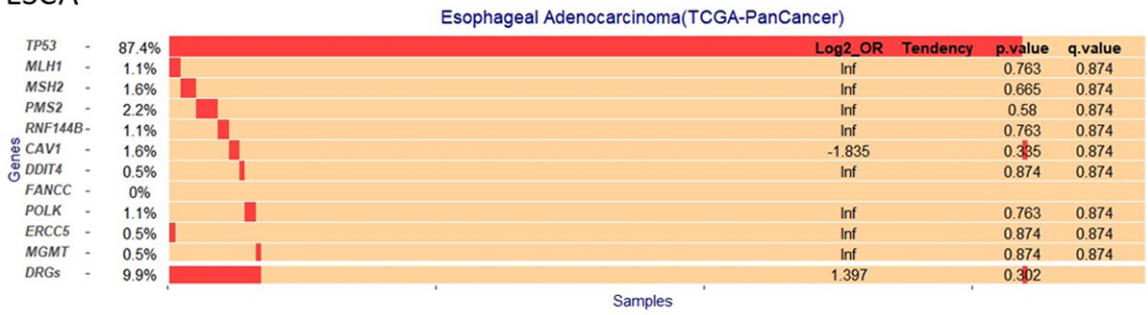

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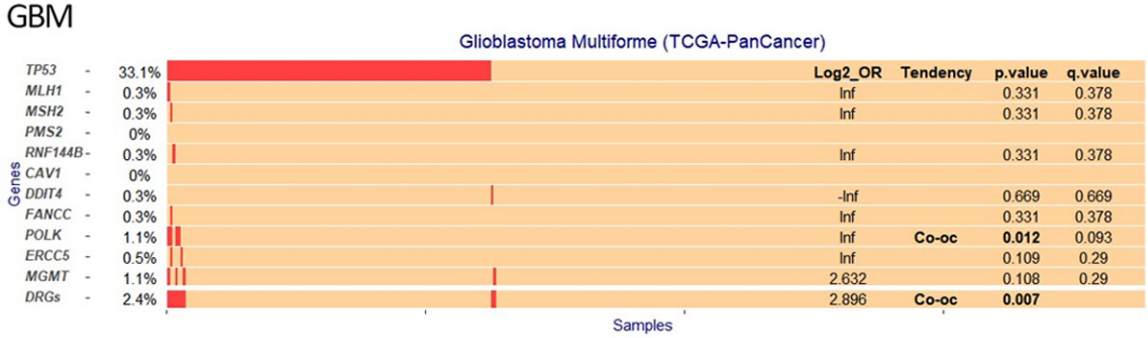

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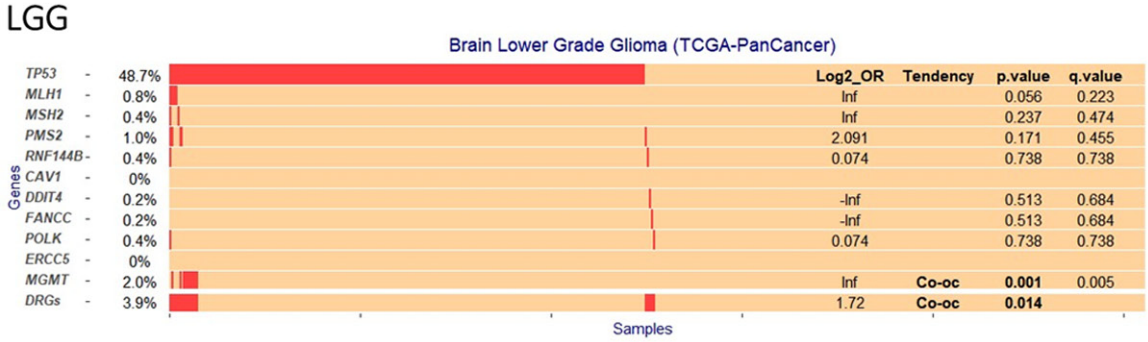

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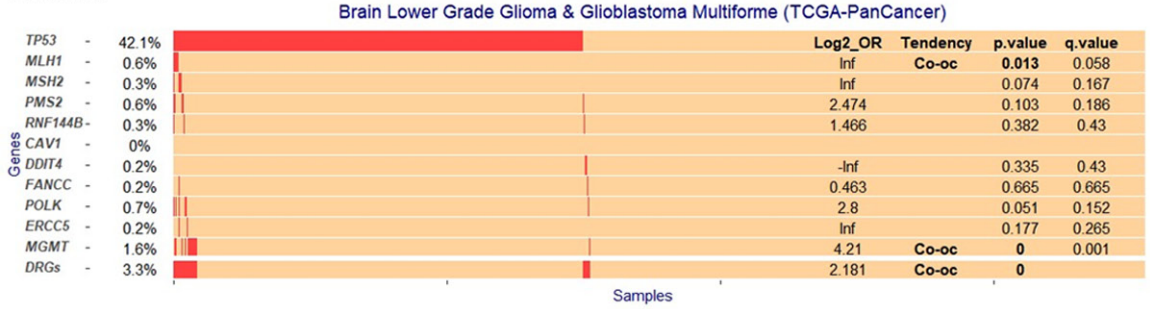

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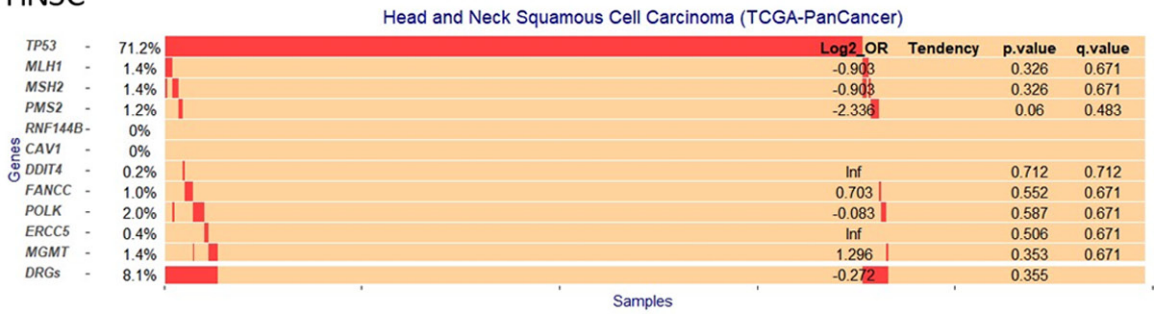

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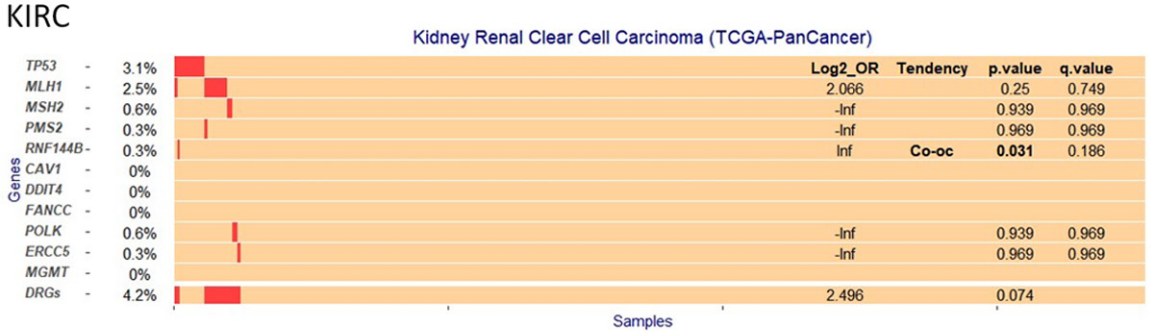

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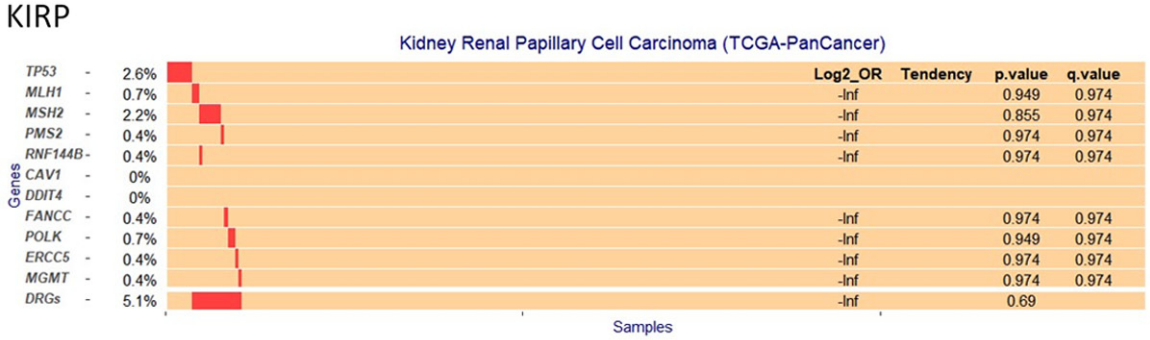

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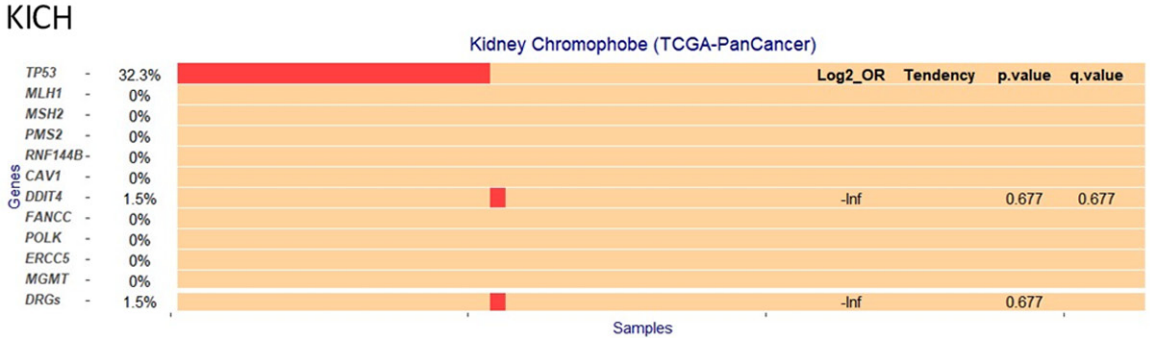
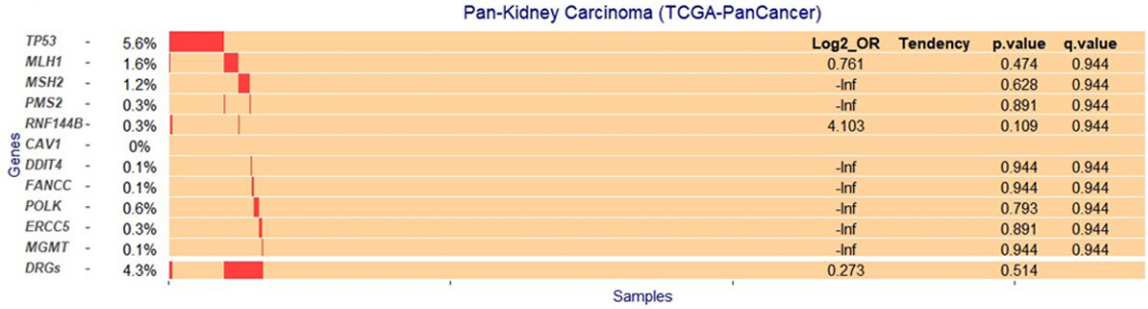


Renal cancer



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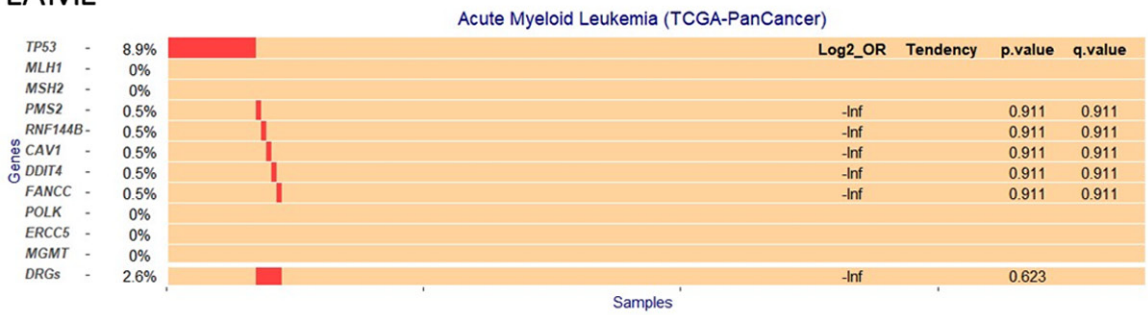

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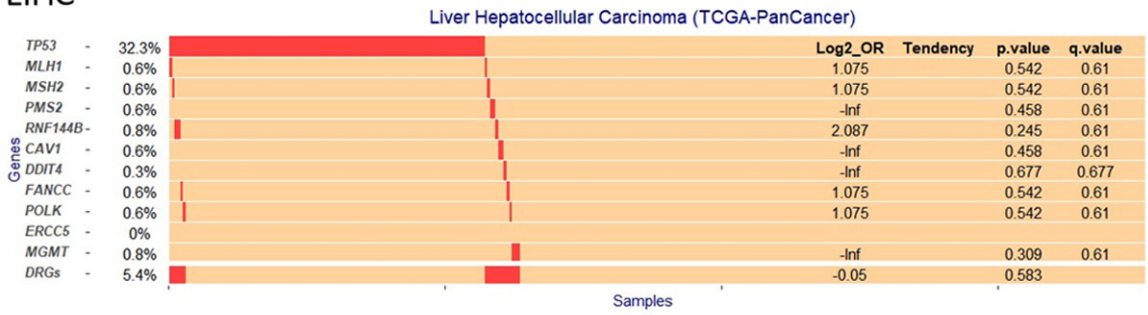

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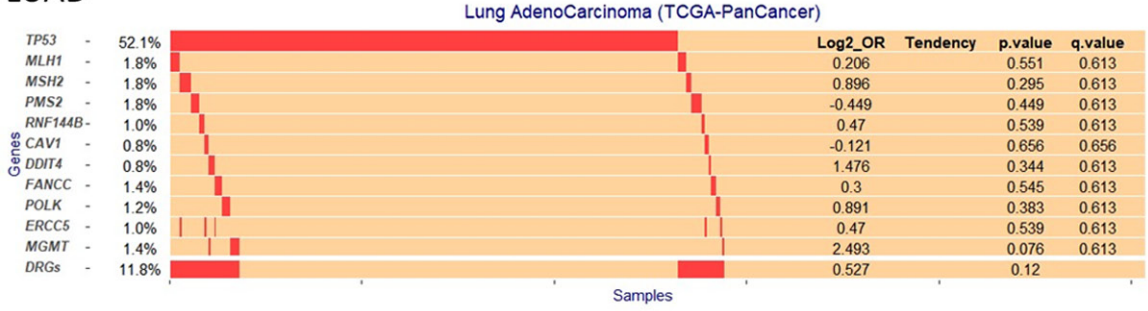

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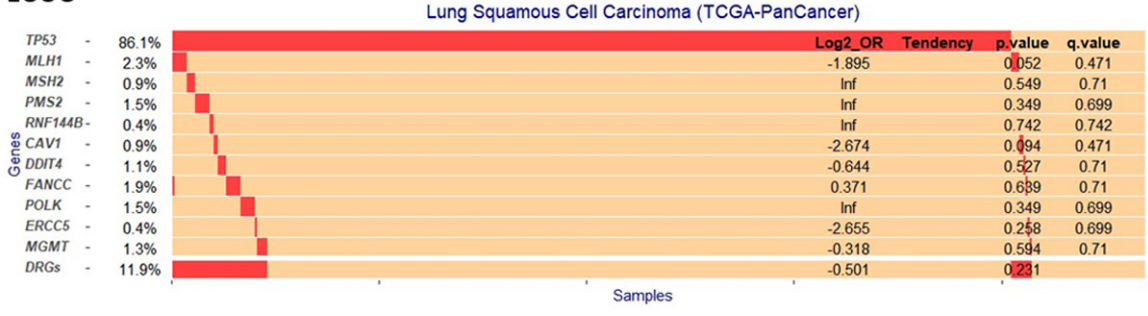

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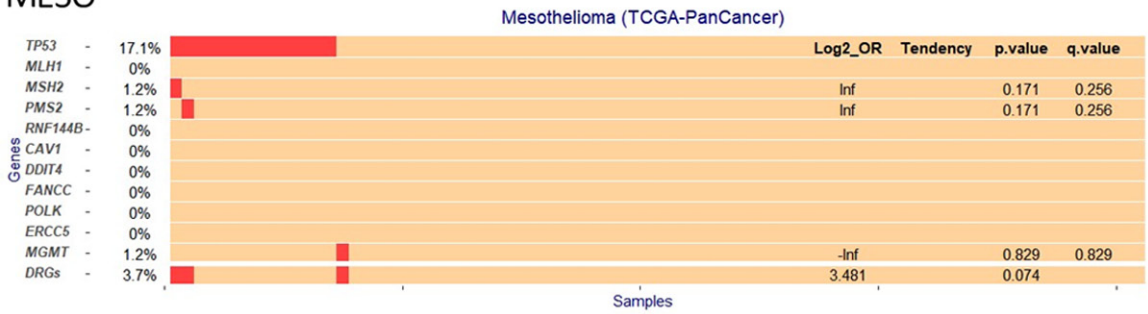

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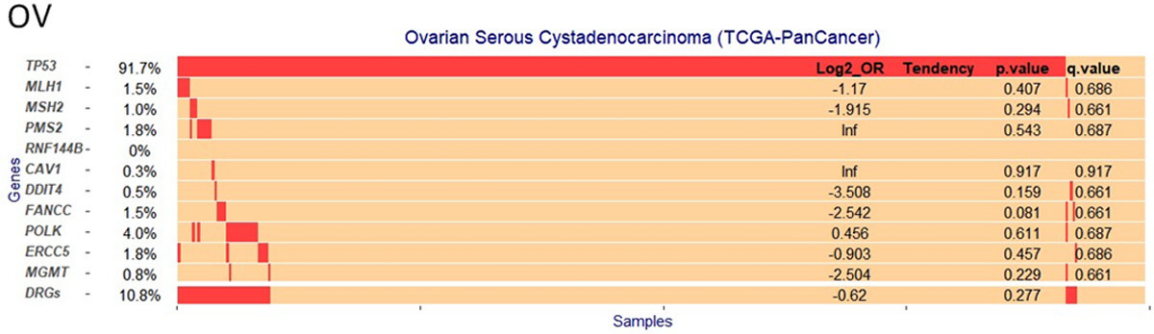

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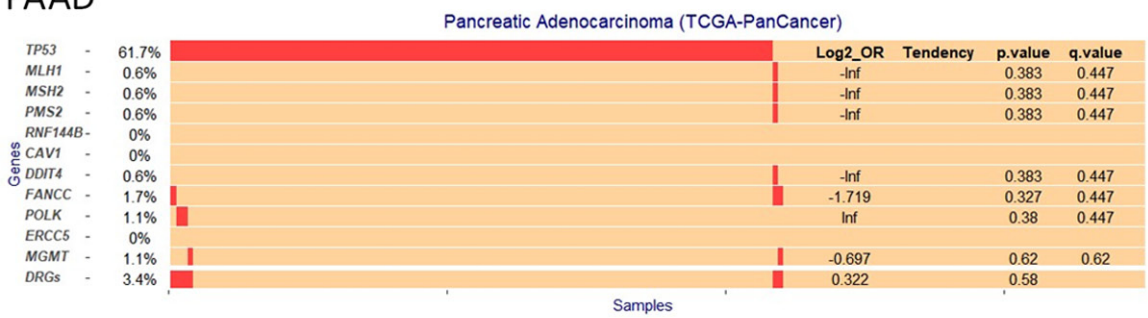

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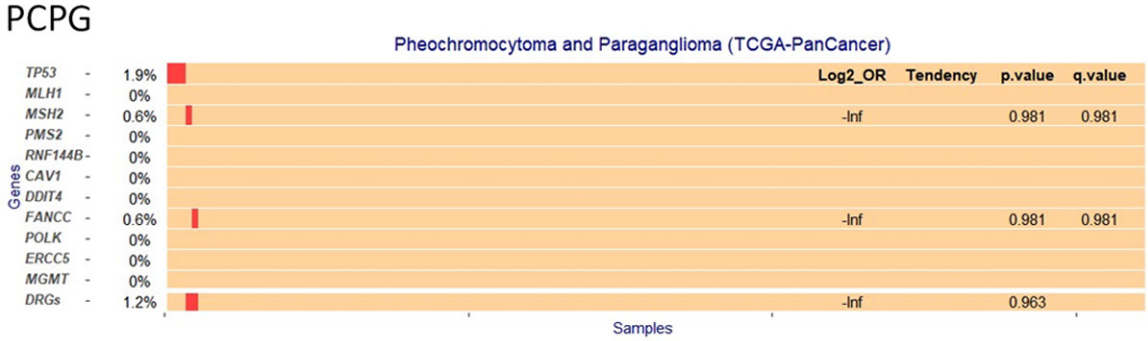

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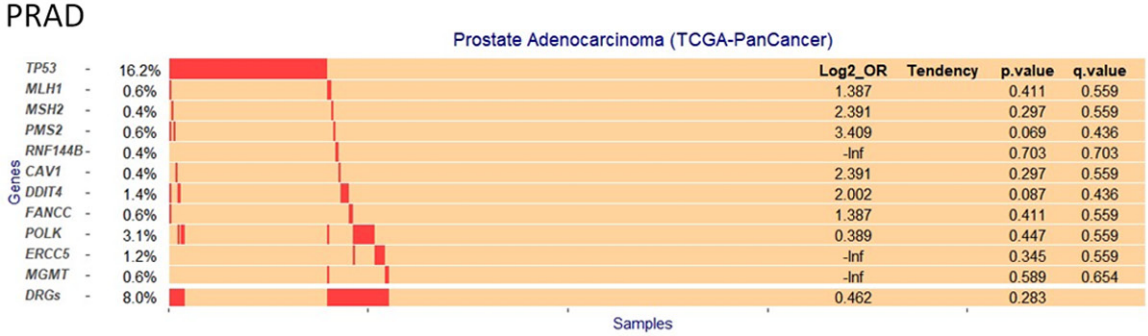

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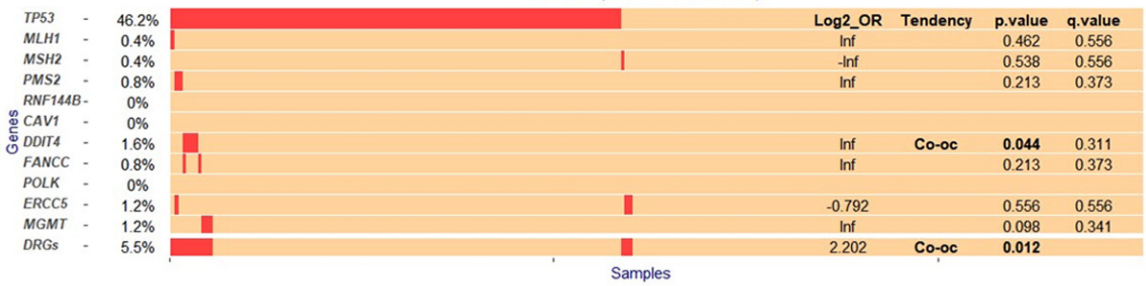

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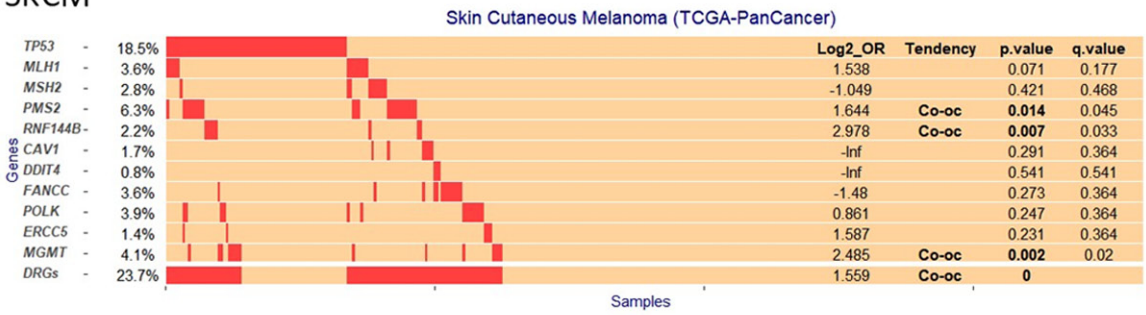

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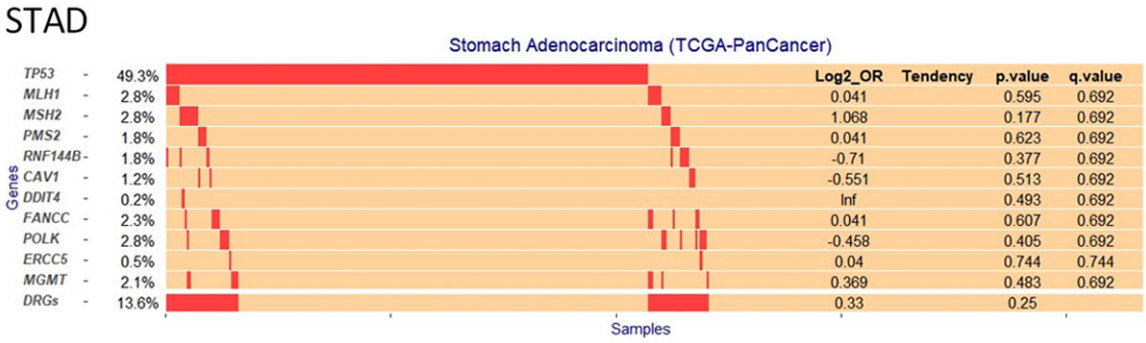

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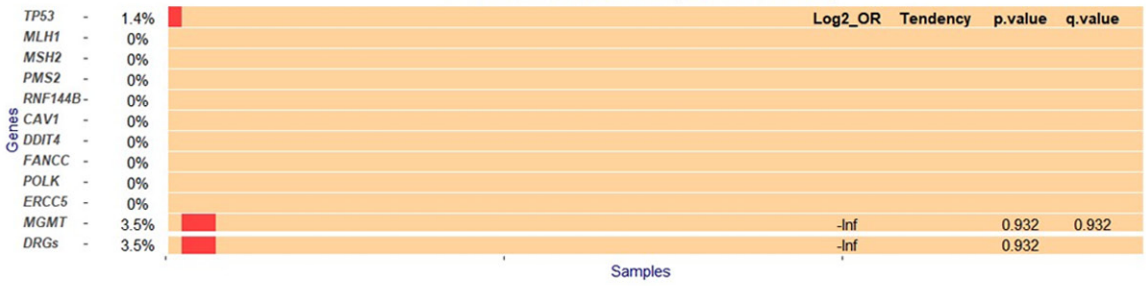

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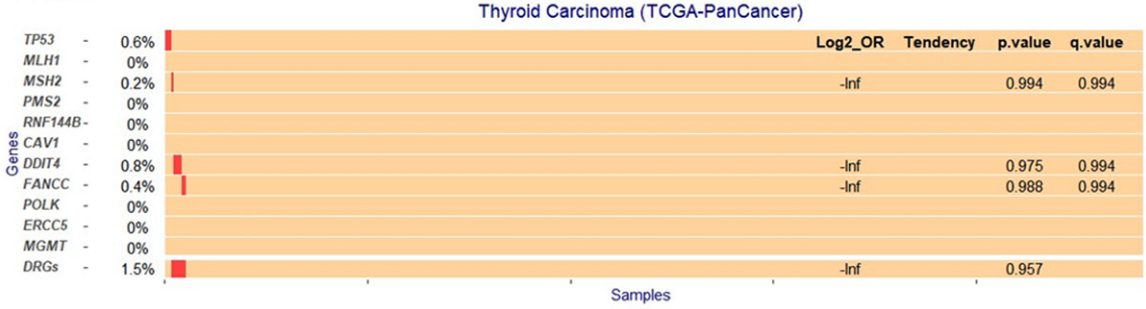

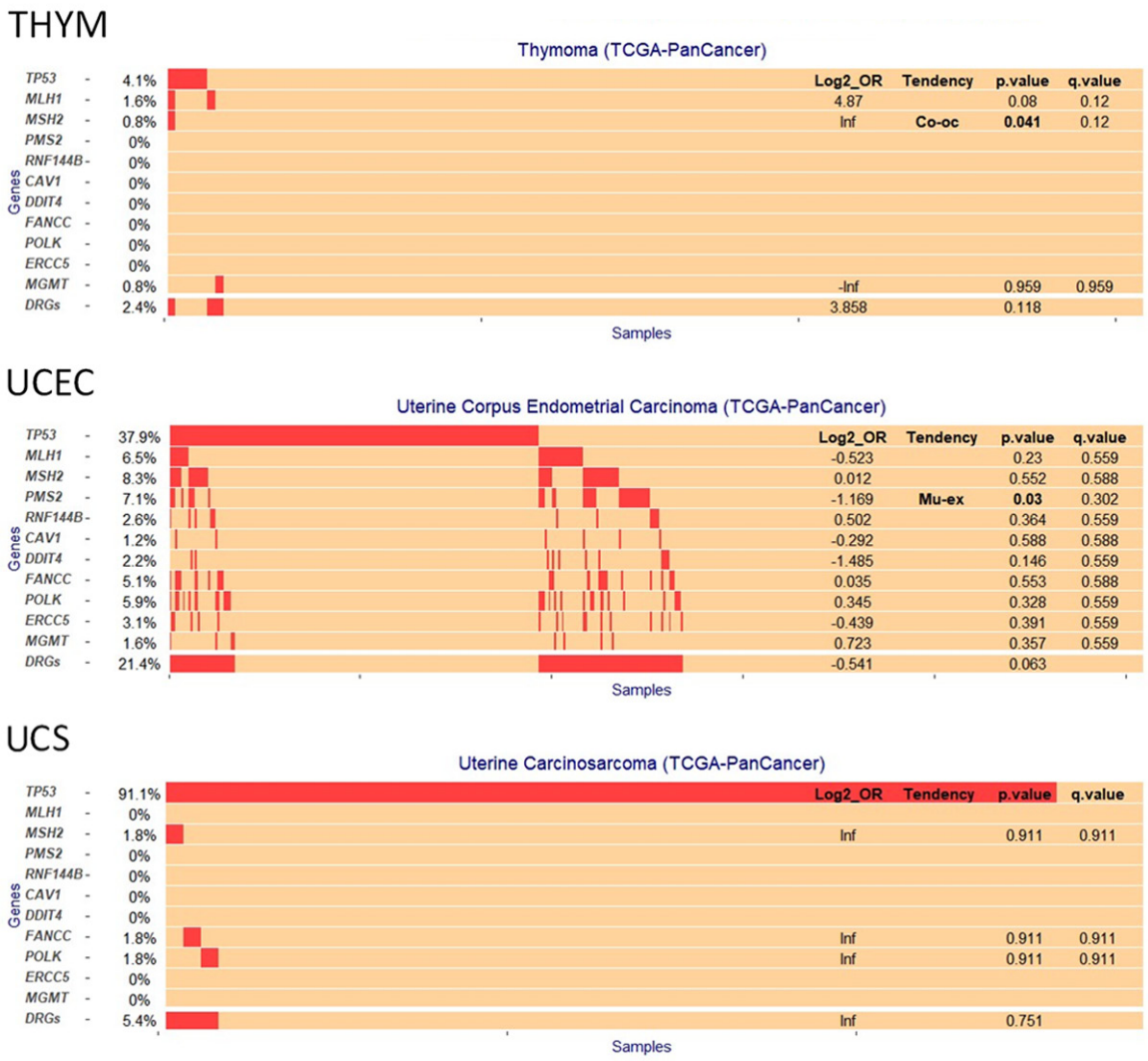

Figure S3. Distribution of *p53* and the 10 p53 target DNA repair gene mutations (excluding amplification) in human malignancies based on TCGA-Pan-Cancer datasets from cBioPortal [20, 21] data; DRGs; mutation in any of the 10 p53 target DNA repair genes in combination.

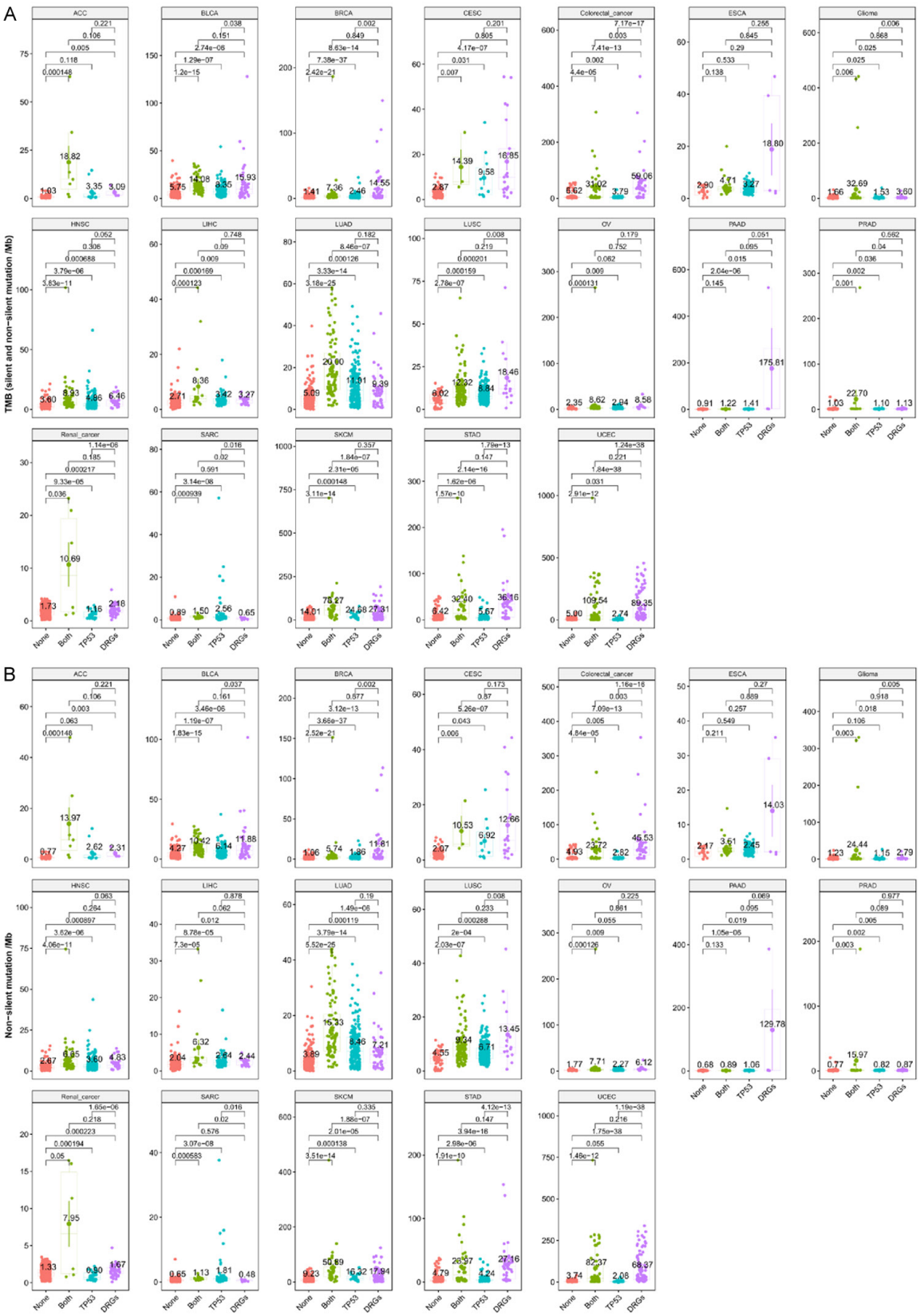

Figure S4. Boxplot of global mutations among four group of samples based on *p53* and the 19 DRG (MMRs and p53 target) mutation status in each type of cancer with wilcoxon test. A. TMB (Silent and non-silent mutations/MB), B. Non-silent mutations/MB. MB: megabase. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 19 DRG mutations but not *p53* mutation.

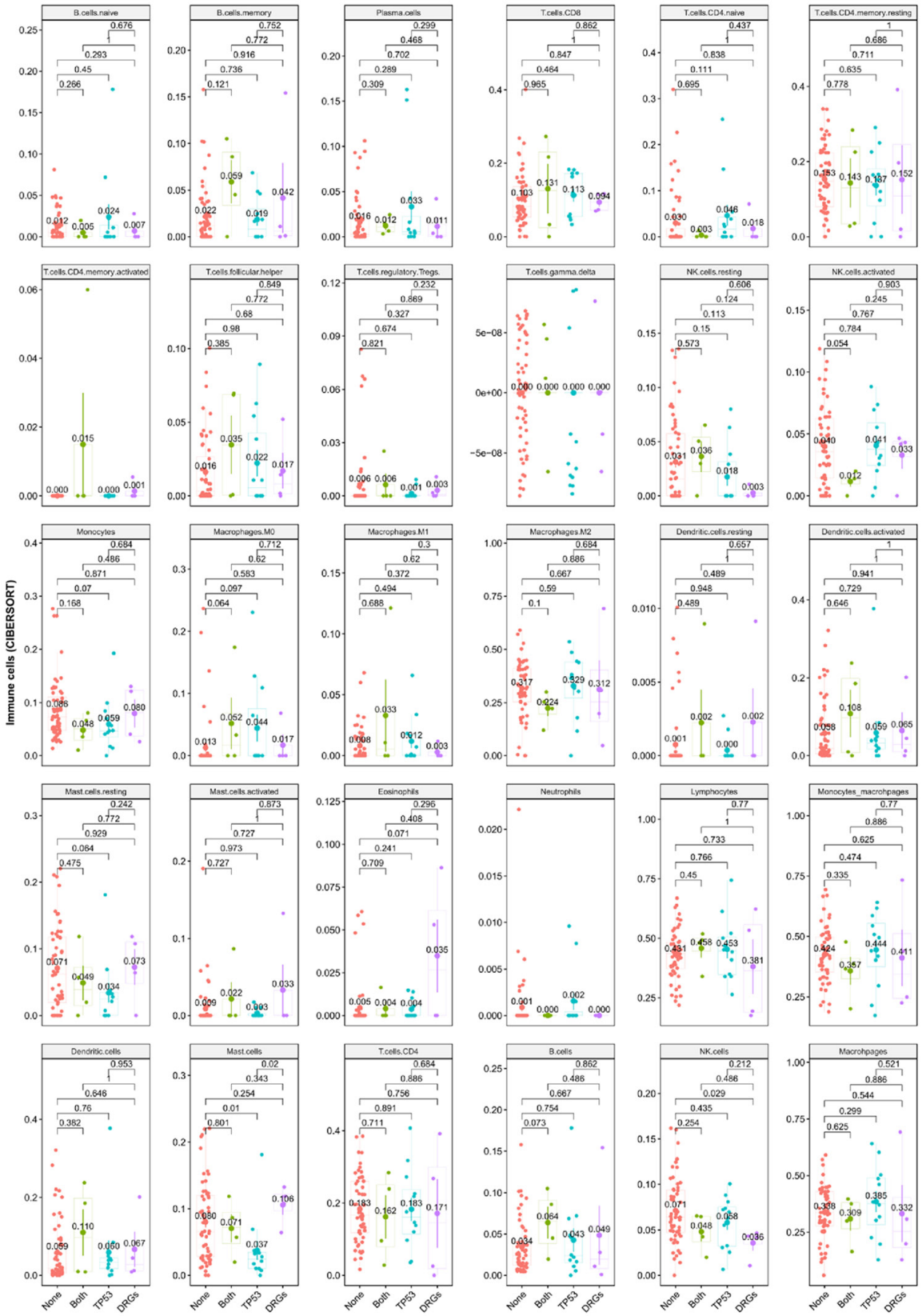

Figure S5. Boxplot of immune cells (CIBERSORT) among four groups of cancers based on *p53* and the 10 DRG (p53 target) mutation status with wilcoxon test in ACC. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

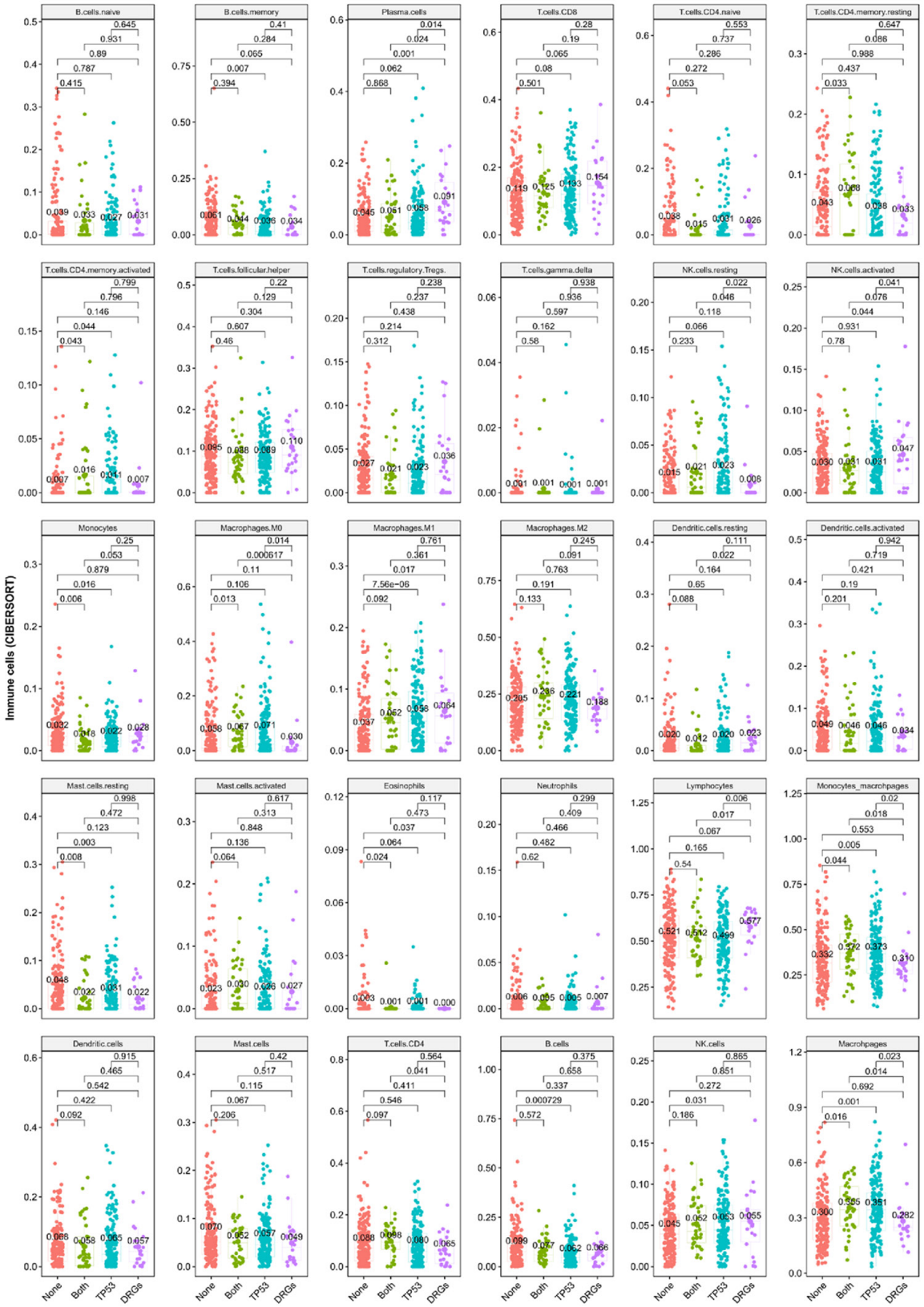

Figure S6. Boxplot of immune cells (CIBERSORT) among four groups of cancers based on *p53* and the 10 DRG (p53 target) mutation status with wilcoxon test in BLCA. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

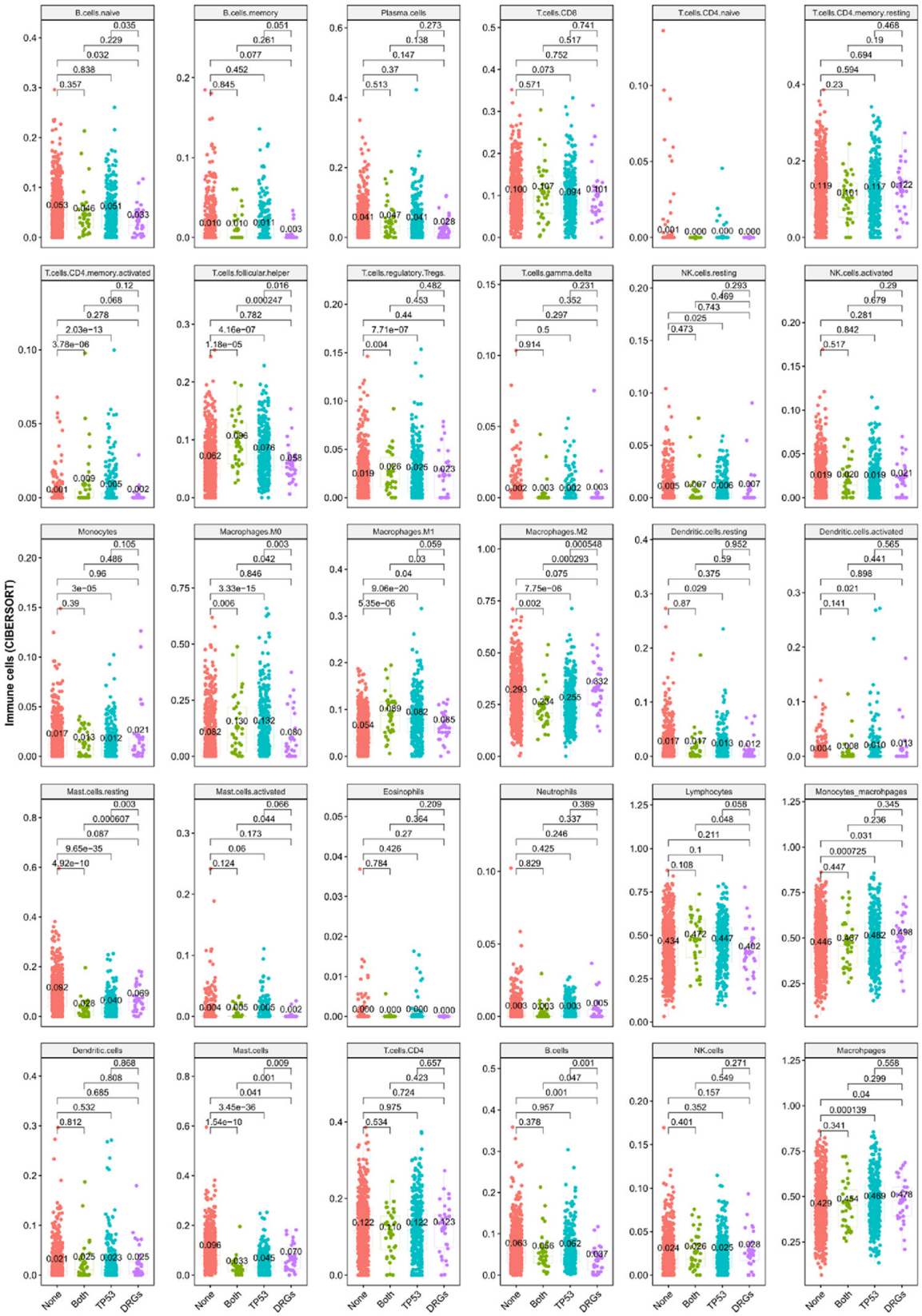

Figure S7. Boxplot of immune cells (CIBERSORT) among four groups of cancers based on *p53* and the 10 DRG (p53 target) mutation status with wilcoxon test in BRCA. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

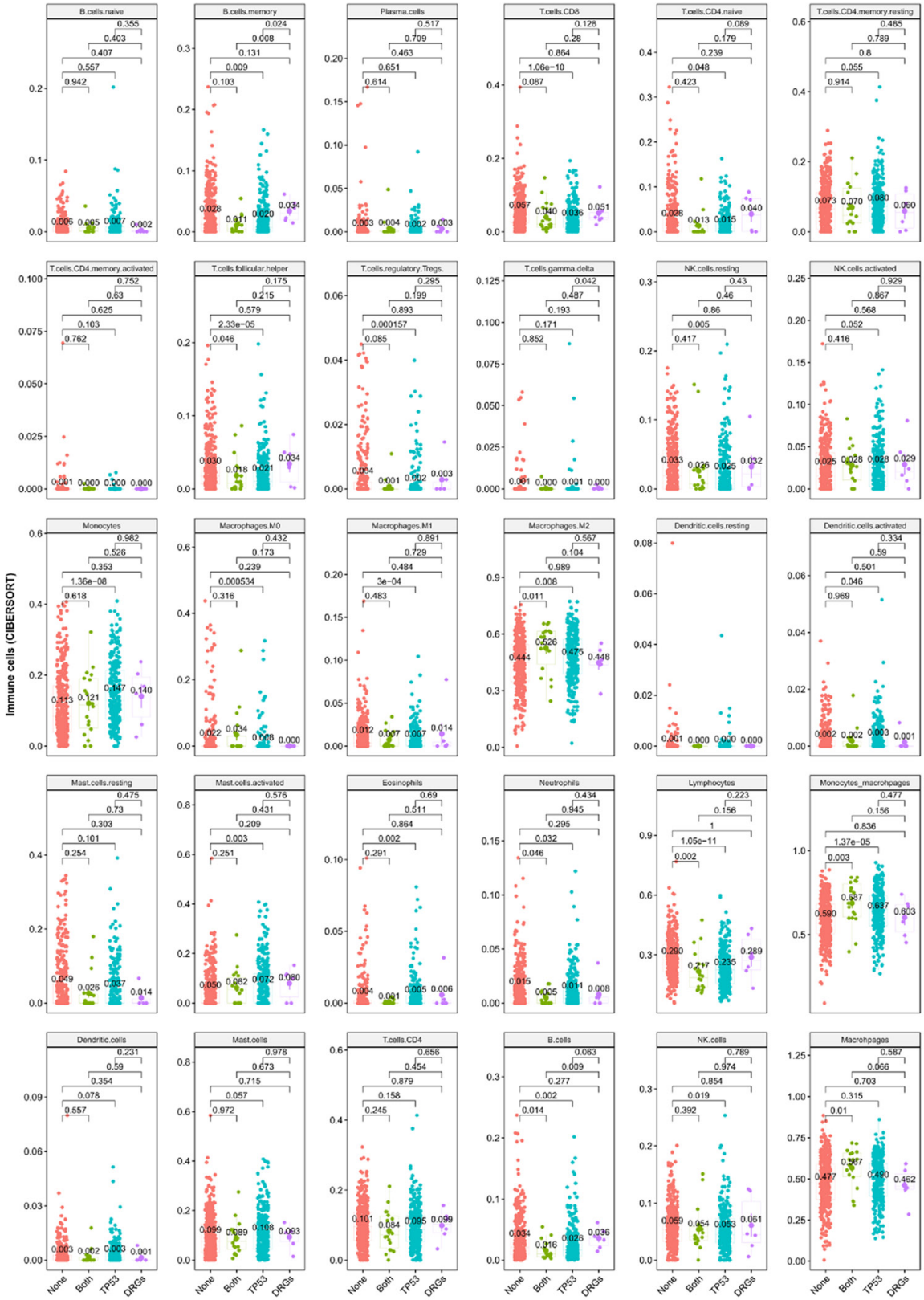

Figure S8. Boxplot of immune cells (CIBERSORT) among four group of cancers based on *p53* and the 10 DRG (p53 target) mutation status with wilcoxon test in glioma. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

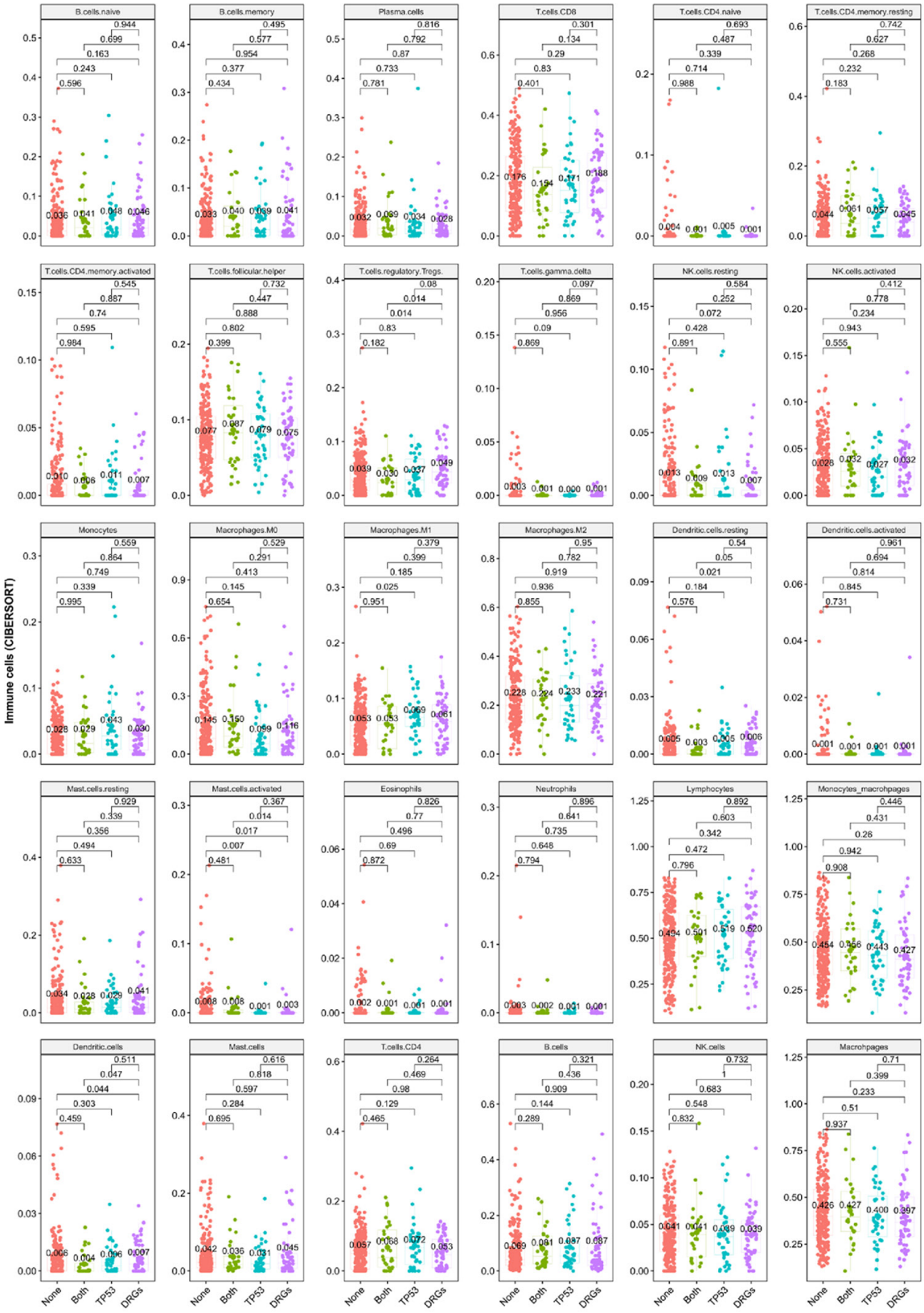

Figure S9. Boxplot of immune cells (CIBERSORT) among four group of cancers based on *p53* and the 10 DRG (p53 target) mutation status with wilcoxon test in SKCM. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 10 DRG mutations but not *p53* mutation.

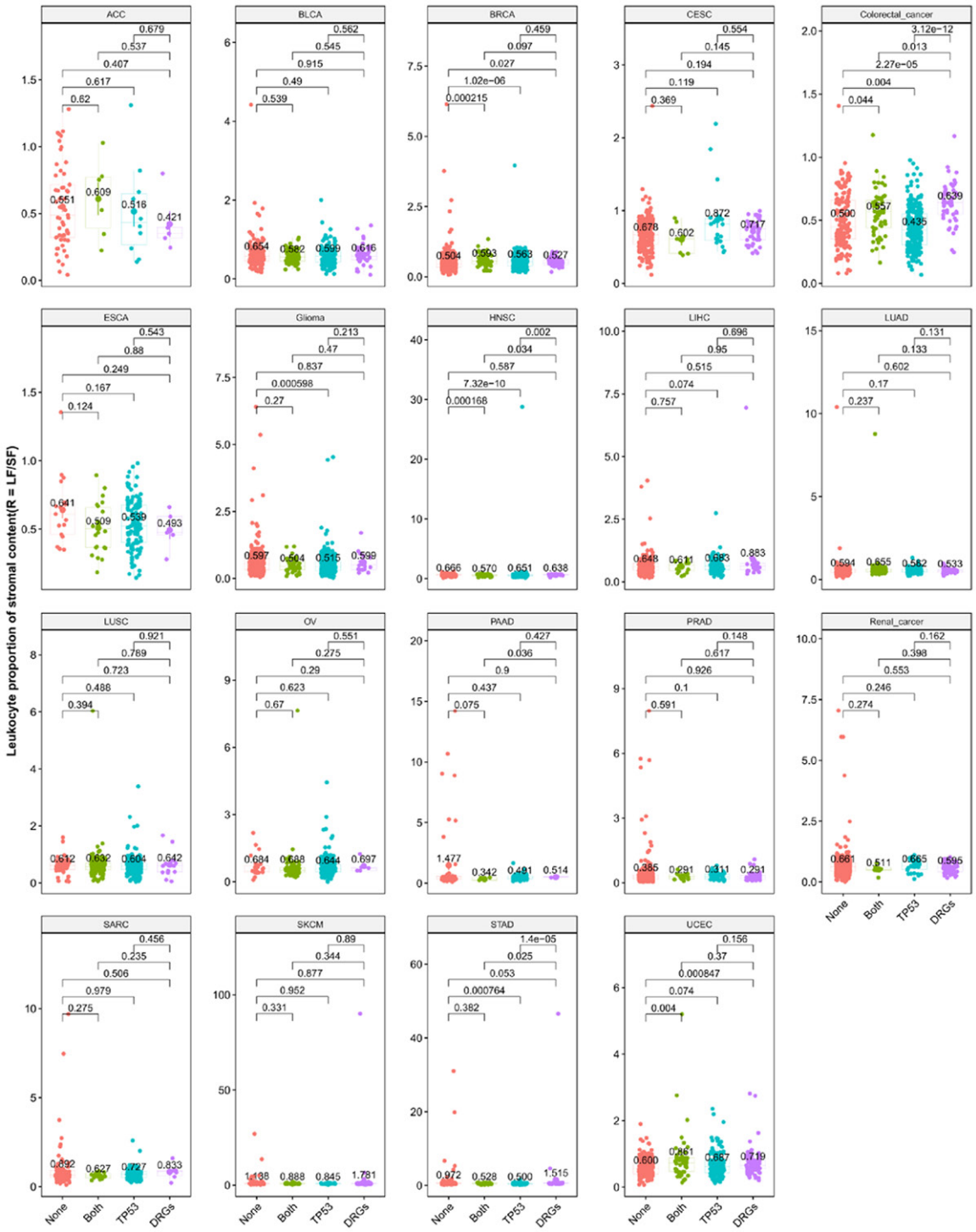

Figure S10. Boxplot of leucocyte proportion of tumor stromal fraction among four groups of samples based on *p53* and the 19 DRG (MMRs and P53 target) mutation status in each type of cancer with wilcoxon test. None: without any mutation in both type of genes; Both: with mutations in both type of genes; TP53: with *p53* mutation but not DRG mutation; DRGs: with any of the 19 DRG mutations but not *p53* mutation.