RADAR: Annotation and prioritization of variants in the post-transcriptional regulome for RNA-binding proteins

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# Abstract

RNA Binding proteins (RBP) play key roles in post-transcriptional regulation. Their binding sites cover more nucleotides than coding exons, but most current methods ignore RBP-mediated regulation. Here, we integrate the entire ENCODE eCLIP experiments to deeply annotate the RBP regulome. We propose a variant impact scoring framework RADAR, which uses conservation, structure, network, and motif information to provide a baseline impact score. Then RADAR further incorporates user-specific inputs to highlight disease-specific variants. Results on somatic and germline variants demonstrate that RADAR can successfully pinpoint deleterious variants, such as splicing-disruptive ones that cannot be highlighted by current prioritization methods.

# Keywords

RNA binding protein, post-transcriptional regulation, variant prioritization, variant functional impact

# Background

Dysregulation of gene expression is a hallmark of many diseases, including cancer[1]. In recent years, the accumulation of transcription-level functional characterization data, such as transcriptional factor binding, chromatin accessibility, histone modification, and methylation, has brought great success to annotating and pinpointing deleterious variants. However, beyond transcriptional processing, genes also experience various delicately controlled steps, including the conversion of premature RNA to mature RNA, and then the transportation, translation, and degradation of RNA in the cell. Dysregulation in any one of these steps can alter the final fate of gene products and result in abnormal phenotypes[2-4]. Furthermore, the post-transcriptional regulome covers an even larger amount of the genome than coding exons and demonstrates significantly higher cross-population and cross-species conservation. Unfortunately, the variant impact in the post-transcriptional regulome has been barely investigated, partially due to the lack of large-scale functional mapping.

RNA binding proteins (RBPs) have been reported to play essential roles in both co- and post-transcriptional regulation[5-7]. RBPs bind to thousands of genes in the cell through multiple processes, including splicing, cleavage and polyadenylation, editing, localization, stability, and translation[8-12]. Recently, scientists have made efforts to complete these post- or co-transcriptional regulomes by synthesizing public RBP binding profiles[13-16], which have greatly expanded our understanding of RBP regulation. Since 2016, the Encyclopedia of DNA Elements (ENCODE) consortium started to release data from various types of assays on matched cell types to map the functional elements in post-transcriptional regulome. For instance, ENCODE has released large-scale enhanced crosslinking and immunoprecipitation (eCLIP) experiments for hundreds of RBPs[17]. This methodology provides high-quality RBP binding profiles with strict quality control and uniform peak calling to accurately catalog the RBP binding sites at a single nucleotide resolution. Simultaneously, ENCODE performed expression quantification by RNA-Seq after knocking down various RBPs. Finally, ENCODE has quantitatively assessed the context and structural binding specificity of many RBPs by Bind-n-Seq experiments[18].

In this study, we aimed to construct a comprehensive RBP regulome and a scoring framework to annotate and prioritize variants within it. We collected the full catalog of 318 eCLIP (for 112 RBPs), 76 Bind-n-Seq, and 472 RNA-Seq experiments after RBP knockdown from ENCODE to construct a comprehensive post-transcriptional regulome. By combining polymorphism data from large sequencing cohorts, like the 1,000 Genomes Project, we demonstrated that RBPs showed increased cross-population conservations in both coding and noncoding regions. This strongly indicates the purifying selection of the RBP regulome. Furthermore, we developed a scoring scheme, named RADAR (***R***N***A*** Bin***D***ing Protein regulome ***A***nnotation and p***R***ioritization), to investigate variant impacts in such regions. RADAR first combines RBP binding, conservation, network, and motif features with polymorphism data to quantify variant impact described by a baseline score. Then, it allows tissue- or disease-specific inputs, such as patient expression or somatic mutation profiles and other prior information, to further highlight relevant variants (Fig. 1). By applying RADAR to both somatic and germline variants from disease genomes, we demonstrate that it can pinpoint disease-associated variants missed by other methods. In summary, RADAR provides an effective approach to analyzing genetic variants in the RBP regulome, and can be leveraged to expand our understanding of post-transcriptional regulation. To this end, we have implemented the RADAR annotation and prioritization scheme into software for community use (radar.gersteinlab.org).

# Results

## Defining the RBP regulome using eCLIP data

We used the binding profiles of 112 distinct RBPs from ENCODE to fully explore the human RBP regulome (Supplementary Table S1), which has been previously under-investigated. Many of these RBPs are known to play key roles in post-transcriptional regulation, including splicing, RNA localization, transportation, decay, and translation (Supplementary Fig. S1).

Our definition of the RBP regulome covers 52.6 Mbp of the human genome after duplicate and blacklist removal (Fig. 2A). It is 1.5 and 5.9 times the size of the whole exome and lincRNAs, respectively. In addition, only 53.1% of the RBP regulome has transcription-level annotations, such as transcription binding sites, open chromatin regions, and enhancers. Unlike the transcription regulome, which has many distal elements, 55.1% of the RBP regulome is in the immediate neighborhood of the exome regions, such as coding exons, 3’ or 5’ untranslated regions (UTRs), and nearby introns (Fig. 2B; see methods section for more details). Furthermore, we observed significantly higher cross-species conservation score in the peak regions versus the non-peak regions in almost all annotation categories, providing additional evidence of regulatory roles of RBPs (Fig. 2C). In summary, the large size of the regulome, the limited overlap with previous annotations, and the elevated conservation level highlight the necessity of computational efforts to annotate and prioritize the RBP regulome.

## Using universal features for baseline RADAR score

To annotate and prioritize variants in RBP binding sites, we built a baseline score framework for RADAR that includes three components: (1) sequence and structure conservation; (2) network centrality; and (3) nucleotide impact from motif analysis.

### Sequence and structure conservation in the RBP regulome

Cross-species sequence comparisons have been widely used to discover regions with biological functions [19, 20]. For example, GERP score maps the human genome to other species to identify nucleotide-level evolutional constraints[21, 22]. Therefore, we used the GERP score in our RADAR baseline framework to detect potentially deleterious mutations in the RBP regulome (see methods section for more details).

Since the enrichment of rare variants indicates a purifying selection in functional regions in the human genome[19, 23, 24], here we inferred the conservation of RBP binding sites by integrating population-level polymorphism data from large cohorts (i.e. the 1,000 Genomes Project)[25, 26]. GC percentage may confound such inference by introducing read coverage variations, which is a sensitive parameter in the downstream variant calling process[27, 28]. Therefore, we calculated the fraction of rare variants, defined as those with derived allele frequencies (DAFs) less than 0.5%, within each RBP binding site. Then we compared them with those from regions with similar GC content as the background (see methods section for more details). In total, 88.4% of the RBPs (99 out of 112) showed elevated rare variant fraction in coding regions after GC correction (Fig. 3A). Similarly, in the noncoding part of the binding sites, 93.8% of RBPs (105 out of 112) exhibited an enrichment of rare variants. This observation convincingly demonstrates the accuracy of our RBP regulome definition (Supplementary Table S3).

Some well-known disease-causing RBPs demonstrate the largest enrichment of rare variants. For example, the oncogene XRN2, which binds to the 3’ end of transcripts to degrade aberrantly transcribed isoforms, showed significant enrichment of rare variants in its binding sites[29]. Specifically, it demonstrates 12.7% and 10.3% more rare variants in coding and noncoding regions, respectively (adjusted P values are 1.89$×$10-9 and 2.85$×$10-118 for one-sided binomial tests)[30]. Hence, we used the enrichment of rare variants to infer the selection pressure in RBP binding sites and adjust the baseline variant scores in such regulator regions (see methods for more details).

RNA secondary structures have been reported to affect every step of protein expression and RNA stability[31]. We incorporated structural features predicted by Evofold, which uses a phylogenetic stochastic context-free grammar to identify functional RNAs in the human genome that are deeply conserved across species[32]. We found that the RBP binding sites demonstrated significantly higher conversation after intersecting with conserved structural regions defined by Evofold. Thus, we used the Evofold regions in our baseline scoring system.

## Incorporating network information

### Highlighting variants in binding hubs

It has been reported that genes within network hubs demonstrate higher cross-population conservation —a sign of strong purifying selection[23, 24, 33]. We hypothesized that RBP binding hubs could show similar characteristics because once mutated they might introduce larger regulation alterations. To test this, we separated the regulome based on the number of associated RBPs. Most regulome regions (62%) were associated with only one RBP (Fig. 3B and Supplementary Fig. 4). As the number of RBPs increased, we observed a clear trend of larger rare variant enrichment. For instance, noncoding regions with at least five or 10 RBPs exhibited 2.2% or 13.4% more rare variants, respectively (top 5% and 1%, Fig 3C). This observation supports our hypothesis that the RNA regulome hubs are under stronger selection pressure and, therefore, should be given higher priority when evaluating functional impact of mutations.

### Emphasizing genes differentially expressed after RBP knockdown

RNA-seq expression profiling before and after shRNA mediated RBP depletion from ENCODE can help to infer the gene expression changes introduced by RBP knockdown. Variants with disruptive effects on RBP binding may affect or even completely remove the RBP binding and hence affect gene expressions in a similar way. Therefore, we extracted the differentially expressed genes from RNA-Seq before and after shRNA-mediated RBP depletion. Then, we up-weighted all variants that were located near the differentially expressed genes and simultaneously disrupted the binding of the corresponding RBPs (schematic in Supplementary Fig. S9).

### Using motif analysis to determine nucleotide impact

Mutations that change the RBP binding affinity may alter RBP regulation via motif disruption. We quantified the difference of position weight matrix (PWM) scores of the mutant allele against the reference allele. RADAR consists of two sources of motifs. First, we used the motifs identified from RNA Bind-n-Seq experiments from ENCODE because it has been reported that many RBP binding events *in vivo* can be captured by binding preferences *in vitro*. Second, we used the *de novo* motifs discovered directly from binding peaks using the default settings in DREME (see details in methods). For each variant, we quantified the nucleotide effect using the highest motif score from these two sources.

## Incorporating user-specific features to reweight variant impact

Variant Prioritization can be improved if informative priors can be appropriately incorporated into the scoring system. Therefore, our RADAR framework allows various types of user-input to help identify disease-relevant variants. Specifically, we adopted a top-down scheme to incorporate regulator, element, and variant level information to up-weight factors that are possibly associated with disease of interest.

### Highlighting key regulators through expression profiles

Key regulators are often associated with disease progression, so variants that affect such regulation should be prioritized[34]. RADAR tried to find such key regulators by combining the RBP regulatory network information with expression profiles. Specifically, we first constructed the RBP network from eCLIP binding peaks and defined the gene differential expression status from disease and normal cell types. Then for each RBP, we quantified its regulation power by associating its network connectivity with aggregated disease-to-normal differential expressions from many samples. We applied this approach on 18 cancer types from TCGA and the regulation powers are given in Fig. 4. We found that among many of the RBPs with larger regulation power have been reported as cancer-associated genes (with \* in Fig. 4B, Table S8). Interestingly, the regulatory power of two key genes PPIL4 and SUB1 were found to be significantly associated with patient survival (Fig. 4C).

In our RADAR framework, we further highlight variants that are associated with RBPs with high regulation power in their corresponding cancer types by adding extra point to their disease-specific scores (see more details in methods). We can easily extend such analysis for other diseases by incorporating differential expression profiles from others cohorts such as GTEx[35, 36].

### Up-weighting key elements from either prior knowledge or mutational profiles

RADAR reconsiders the functional impact difference among RBP peaks either by their associated genes. For example, users can input a prioritized gene list, such as well-documented risk genes for a disease of interest. Alternatively, genes that undergo significant expression or epigenetic changes are mostly cell-type-specific and can be used to highlight more relevant variants. Our RADAR framework will up-weight all the RBP peaks that are close to these genes defined by users.

In addition, RADAR can incorporate somatic variant recurrence, which has been widely used to discover key disease regions, to reweight different RBP peaks. Peaks with more somatic mutations than expected are often considered to be disease-driving[37-39]. Here, we first defined a local background somatic mutation rate from a large cohort of cancer patients to evaluate the mutation burden in each RBP peak (see more details in methods). Variants that are associated with burdened elements are given higher priority in our scoring scheme.

## Prioritizing variants with a RADAR weighted scoring scheme

By integrating the pre-built and user-specific data context described above, our entropy-based scoring scheme evaluated the functional impacts of variants that are specific to post-transcriptional regulation (Fig. 1 and Table 1). First, RADAR added up the (adjusted) score of variants for all pre-built features, which include sequence and structure conservation, network, and motif information. Then, depending on user inputs, RADAR further up-weighted variants with mutations in the key RBP binding sites, nearby genes of interest, or within prioritized regulatory elements.

Table 1. Features used by RADAR

|  |  |  |  |
| --- | --- | --- | --- |
| **Category** | **Feature** | **Source** | **Scoring Scheme** |
| **Universal** | Cross-species conservation | eCLIP | Adjusted-entropy |
| Cross-population conservation | Gerp | Sigmoid Function |
| Structural conservation | Evofold | Entropy |
| Binding hub | eCLIP | Adjusted-entropy |
| RBP-gene association | shRNA RNA-seq | Entropy |
| Motif disruption | Bind-n-Seq | Adjusted-entropy |
| DREME |
| **User-specific** | RBP regulatory power | Expression | Entropy |
| Key genes | Prior knowledge | Entropy |
| Mutation Recurrence | Mutation profiles | Fix Value |

## Applying RADAR to pathological germline variants

We calculated baseline RADAR scores on all pathological variants from HGMD (version 2015). We used the 1,000 Genomes variants as the background to calibrate the distribution of scores. As expected, the HGMD variants scored significantly higher than somatic mutations (Supplementary Fig. S10). For example, the mean RADAR score for HGMD variants is 0.589, while it is only 0.025 for 1,000 genomes variants (P value <2.2$×$10-16 for two sided Wilcoxon test). We further compared the baseline RADAR scores of HGMD and 1,000 genomes variants within the RBP regulome to remove potential bias since HGMD variants may be more likely to be within or nearby to exons. We still observed significantly higher baseline RADAR score in the HGMD ones (1.871 vs. 1.337, P value <2.2$×$10-16 for one sided Wilcoxon test).

We further compared RADAR scores of HGMD variants with other methods (Supplementary Table S10). In total, 720 HGMD variants were explained by our methods but could not be highlighted by other methods (see details in methods). Many of these variants are located nearby the splice junctions. An example of such a variant is shown in Fig. 5; this variant is located 4 base pairs away from splice junction in BRCA1. eCLIP experiments showed strong binding evidence in 5 RBPs (Fig. 5). Specifically, the T to C mutation strongly disrupts the binding motif of PRPF8, increasing the possibility of splicing alteration effects. Our finding is not highlighed in previous methods for variant prioritization, such as Funseq and CADD.

## Applying RADAR baseline score to somatic variants in cancer

We next aimed to leverage our scheme to evaluate the deleteriousness of somatic variants from public datasets. Due to the lack of a gold standard, we evaluated our results from two perspectives. First, we reasoned that since hundreds of cancer-associated genes are known to play essential roles through various pathways38,39, variants associated with these genes are likely to have the highest functional impact21. To test this hypothesis, we first linked each variant with a gene by the shortest distance according to the Gencode v19 annotation. We tested four cancer types, breast, liver, lung, and prostate cancer, and found in all cases that variants associated with cancer-related genes showed significant enrichment, with a larger RNA level functional impact (Supplementary Fig. S11). For example, we found a 4.58- and 8.75-fold increase in high-impact variants at a threshold level of 3 and 4, respectively, in breast cancer patients (P < 2.2$×$10-16, one-sided Wilcoxon).

In our second approach, we hypothesized that variant recurrence could be a sign of functionality and may indicate an association with cancer21-23. Thus, we compared the variants’ score distribution from RBP binding peaks with or without recurrence. Specifically, we separated the peaks of variants from more than one sample from those that were mutated in only one sample, and then compared the baseline RADAR scores. We found that in most cancer types, elements with recurrent variants were associated with a larger fraction of high-impact mutations. For example, in breast cancer recurrent elements demonstrated a 1.67-, and 2.57-fold more of high-impact variants with RADAR greater than 3.0 and 4.0, respectively, resulting in a P value of 2.2$×$10-16 in one-sided Wilcoxon test. We observed similar trend in other cancer types. Results of other cancer types are given in Supplementary Fig. S11.

## A case study on breast cancer patients

We applied our method on a set of breast cancer somatic variants from 963 patients released by Alexandrov *et al*[42]. We used COSMIC gene list, expression and mutational profiles as additional features. In total, we found that around 3% of the 687,517 variants could alter post-transcriptional regulation to some degree. We incorporated the above disease-specific features and demonstrated how they could help to reweight the variant scoring process on a coding variant in Fig. 6. This variant is located within an RBP binding ultra-hot region and showed high sequence conservations (7% more rare variants in its RBP peaks). It also demonstrated strong motif disruption effect (PPIG in Fig 6). All such features resulted in a baseline RADAR score of 3.67, which is ranked 296 out of all variants. Also, it only showed moderate Funseq2 (3) and CADD scores (7.46). However, we found that it is located in the exon region of the well-known tumor suppressor TP53 (orange track in Fig.6), and its binding peaks demonstrated more than expected somatic mutations (purple in Fig. 6). Besides, 3 out of the 6 RBPs binding there showed high regulation power in breast cancer (green in Fig. 6). Hence, these additional features boost its overall RADAR score to 6.67, which is ranked 38 out of all variants).

We also found many variants demonstrate high overall RADAR scores but only show moderate transcriptional functional impact score, such as Funseq2 and CADD scores. For example, 108 and 52 variants are ranked within top 1% of overall RADAR scores but cannot be highlighted by other methods in coding and noncoding regions respectively (Supplementary Table S10 and Table S11). Many of such variants are located in RBP binding hubs, underwent strong purifying selection, demonstrated strong motif disruptiveness, and being regulated by important RBPs that are associated with breast cancer from multiple sources of evidence. We believe the discovery of such events demonstrates the value of RADAR as an important supplement to the existing transcriptional-level function annotation and prioritization tools.

# Discussion

In this study, we integrated the full catalog of eCLIP, Bind-n-Seq, and shRNA RNA-Seq experiments from ENCODE to build an RNA regulome for post-transcriptional regulation. Although DNA-level regulation takes up a larger part of the genome, our defined RBP regulome is remarkably larger than one may think and covered up to 52.6 Mbp of the genome (Fig. 2A). In fact, the regulome is larger than the size of the whole exome and only showed limited overlap with previous transcription-level annotations. Furthermore, we found that the RBP regulome demonstrated a noticeably higher conservation from two aspects: higher cross-species conservation in almost all annotation categories (Fig. 2C) and higher cross-population conservation by showing significant enrichment in rare variants (Fig. 3A). These two sources of evidence support the notion that the RBP regulome is under strong purifying selection and carries out essential biological functions. In addition, these results signify the necessity of computational tools to annotate and prioritize variants in the RBP regulome, which are under-investigated.

By integrating a variety of regulator-, element-, and nucleotide-level features, we propose an entropy-based scoring frame, RADAR, to investigate the impact of somatic and germline variants. The variant prioritization framework of RADAR contains two parts. First, by incorporating eCLIP, Bind-n-Seq, shRNA RNA-seq experiments with conservation and structural features, we built a pre-defined data context to quantify the baseline variant impact score. This approach is suitable for multiple-disease analysis or cases where no other prior information can be used. We applied this RADAR baseline score to HGMD pathological variants and highlighted many candidates that cannot be highlighted by other methods. Besides, our RADAR framework provided detailed explanations of the underlying disease-causing mechanism (Fig. 5). In addition to the baseline score, RADAR also allows user-specific inputs such as prior knowledge, patient expression and mutation profiles for a re-weighting process to highlight relevant variants in a disease-specific manner. As an example, we applied the RADAR disease-specific scores to variants from several cancer types and showed that RADAR could identify relevant variants in key cancer-associated genes (Fig. 6).

# Conclusions

In summary, we have shown that RADAR is a useful tool for annotating and prioritizing post-transcriptional regulome for RBPs, which has not been covered by most of the current variant impact interpretation tools. Our method provides additional layers of information to the current gene regulomes. Importantly, the RADAR scoring scheme can be used in conjunction with existing transcriptional-level variant impact evaluation tools, such as Funseq[23, 24], to quantify variant impacts. Given the fast-expanding collection of RBP binding profiles from additional cell types, we envision that our RADAR framework can better tackle the functional consequence of mutations from both somatic and germline genomes.

# Methods

## eCLIP Data Processing and Quality Control

We collected 318 eCLIP experiments of 112 unique RBPs from the ENCODE data portal (encodeprojects.org, released and processed by July 2017). eCLIP data was processed through the ENCODE 3 uniform data processing pipeline and peaks with score 1,000 were used in our analysis. We then removed peaks overlapped with blacklisted regions. We further separated the peaks into coding regions and the noncoding regions in our analysis to infer the selection pressure.

## Cross-population conservation inference

For each RBP, we used germline variants from the 1,000 Genomes Project to infer the selection pressure of its binding sites by evaluating the enrichment of rare variants. Specifically, for any RBP, suppose its binding peaks contain $n\_{r}$ rare variants (DAF<= 0.005) and $n\_{c}$ common variants$.$ The percentage of rare variants is defined as

$ρ=\frac{n\_{r}}{n\_{r}+n\_{c}}$ (1)

In order to correct for potential GC bias, we first binned the genome into 500 base pair bins and grouped them according to their GC percentage. For RBP with GC percentage $g$, we only selected the bin group with closest GC to calculate the background rare variant percentage $ρ\_{b}^{g}$. As a result, the adjusted enrichment of rare variants was defined as

$ρ\_{adj}={ρ}/{ρ\_{b}^{g}}$ (2)

Regions with $ρ\_{adj} $ larger than 1 suggested a purifying selection.

## RBP Network information

We first separated the RBP peaks into coding and noncoding regions and then calculated the number of RBPs binding at each nucleotide. Then we grouped the regulome by the number of binding RBPs and calculated the GC-corrected enrichment of rare variants $ρ\_{adj}$ for each group in coding and noncoding regions by equation (2). We found that the cross-population conservation of the binding sites increases as the number of binding RBPs grows. In our framework, we selected regions with at least the top 5 and 1 percent of binding RBPs as the hot and ultra-hot regions to add an extra score.

Then we also tried to set up RBP regulatory network directly from the RBP peaks. We used the full set of protein coding genes in Gencode v19, and then extracted their 3’UTR regions. For any protein coding gene, a RBP is supposed to regulate this gene if this RBP has a binding peak intersecting the 3’UTR region. For any RBP, $x\_{i}=1$ if it regulates gene $i$. $x\_{i}=0$ otherwise.

## Motif Analysis

We used the changes of PWMs introduced by a variant to quantify the motif disruptiveness effect through motiftools (<https://github.com/hoondy/MotifTools>). Specifically, we defined the D-score as in equation (3) to represent the difference between sequence specificities in reference to an alternative sequence.

$Dscore= motifscore\_{ref}-motifscore\_{alt}=-10×log\_{10}\left(\frac{P\_{ref}}{P\_{alt}}\right)$ (3),

where $P\_{ref}$ and $P\_{alt}$ are the PWM scores from the reference and alternative allele. To quantify a motif breaking event, we require that the P value for the reference allele is at least $5×10^{-4}$. There are two motif sources in our analysis. First, we identified RBP motifs using DREME software (Version 4.12.0) directly from RBP peaks. Then, we also incorporated motifs from RNA Bind-N-Seq[18] (RBNS) to characterize sequence and structural specificities of RBPs. For each variant that affected multiple RBP binding profiles, we used the max score.

## Baseline Variant Scoring

We considered 6 different features in the RADAR baseline scoring scheme (details in Table 1). For any discrete entropy based feature $i$ listed in Table 1, we first calculated the percent of SNPs from 1,000 Genomes that are falling into this feature category and denote it as $f\_{i}$. Then the entropy related weight is used to evaluate the functional impact.

$S\_{i}=1+f\_{i}×log\_{2}f\_{i}+(1-f\_{i})×log\_{2}(1-f\_{i})$ (4)

For the motif analysis, for any motif breaking event with D-score $d\_{t}$, $f\_{i} $should be the percent of SNPs with equal or larger D-scores among all the 1,000 Genomes SNPs.

$f\_{i}=\frac{\#(d\geq d\_{t})}{n}$ (5)

For any feature that used the adjusted entropy based score, we further used the GC corrected rare variant enrichment $ρ\_{adj}$ of the RBP as a coefficient to reweight the score S. For the mutation recurrence features in Table1, we gave an extra 1 to any variant in the highly-mutated binding peaks. Finally, the overall RADAR score is $S=\sum\_{i}^{}S\_{i}$

## RBP Regulatory Power from Linear Regression

RADAR allows inputs in addition to the pre-built context to calculate the disease-specific variant score. In this paper, we used the TCGA expression profiles as an example on the cancer variant prioritization. Specifically, we downloaded expression profiles of 19 cancer patients of 24 types from TCGA. In order to get a robust differential expression analysis, we excluded 6 cancer types that have less than 10 normal expression profiles and used DESeq2[43] to find tumor-to-normal differentially expressed genes (corrected P value from DESeq2 <0.05). Let $y\_{i}^{k}$ represent the differential expression status of gene $i$ of the $k^{th}$ cancer type.

We inferred the regulatory power of each RBP by through a regression approach of the above differential expression and RBP network connectivity as $\vec{y}^{k}=β\_{0}+$ $β\_{1}\vec{x}^{k}$, where $\vec{x}^{k}$ is the binary connectivity vector for all genes defined above. We used the absolute value of $β\_{1}$ to indicate the regulation power of each RBP. The full table of regulation powers in all 18 cancer types were given in Table S7. Interestingly, we found that for the RBPs with larger regulatory power are those tends to be known to associate with cancer, as listed in Table S8.

For RBPs with high regulation powers from aggregated expression analysis, we also performed a patient-wise regulation power inference, where the differential expression is determined as the difference between individual normalized tumor and normal expressions. Then, we tried to associate this individual regulation power with disease prognosis. We downloaded the patient survival data from TCGA and performed survival analysis using the survival package in R (version 2.4.1-3).

## Recurrence in Somatic Mutations

We prioritized variants in RBP binding sites are with more-than-expected somatic mutations. In order to evaluate the somatic mutation burden, we first separated the genome into 1Mbp bins and calculated a local background mutation rate in each window. Then for each eCLIP peak, we counted the number of somatic mutations, and compared it to the nearest local 1Mbp context using a one-sided binomial test. If a specific RBP binding site was enriched for somatic mutations, the variant falling in that site was given a higher priority.

# Accessibility Availability of data and materials

We have made this RNA variant annotation and prioritization tool available as an open-source software at [radar.gersteinlab.org](http://radar.gersteinlab.org/). The website contains details on usage, examples, resources, and dependencies. We also provided a genome-wide pre-built RADAR baseline score for every base pair on the genome (hg19 version of genome). Users can directly query the annotation and functional impact score from radar.gersteinlab.org. Also, we released all pre-built data context and genome-wide baseline scores at radar.gersteinlab.org.

# List of abbreviations

RBP: RNA binding Protein; ENCODE: the Encyclopedia of DNA Elements; PWM: position weight matrix; eCLIP: enhanced crosslinking and immunoprecipitation; RADAR: ***R***N***A*** Bin***D***ing Protein regulome ***A***nnotation and p***R***ioritization; UTR: untranslated regions; DAF: derived allele frequency; RBNS: RNA Bind-N-Seq.

# Competing interests

The authors declare that they have no competing interests

# Authors’ contributions

JZ, JL and MG conceived the study and wrote the manuscript. JZ, JL, DL, LL, JF wrote the framework and performed the method evaluation. DL developed the website. LL and MR carried out studies associating RNA structure. DL and SL participated in motif analysis. All authors have read and approved the final manuscript.

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# Figure Legends and Tables

## Figure 1. RADAR workflow

There are two RADAR score components: (1) it first defines baseline component using pre-built data context including sequence and structural conservation, network and motif information; (2) it then incorporates user-specific input to further highlight disease-specific variants.

## Figure 2. RBP regulome and cross-species conservation.

(A): length of RBP binding sites vs. other coding/noncoding annotation categories; (B) Fraction of RBPs falling into each annotation category and boxplot of PhastCons scores in peak (blue) vs. nonpeak regions (white).

## Figure 3. Cross-population conservation of RBP peaks and binding hubs.

(A-B): Rare variant percentage in coding/noncoding regions. Blue dot represent RBP peaks and yellow dot represent genome average. Shaded lines are the 95% confidence interval of rare variant percentage of the RBP peaks; (C): example of RBP binding hubs. Red/orange dots denote positions with top 1 and 5 RBPs binding; (D): corrected rare variant percentage at positions with different binding RBPs.

## Figure 4. Regulation Power inference of RBPs

(A): schematic of RBP regulation power calculation; (B): Heatmap of RBP regulation power in various cancer types; (C): RBPs associated with patient survival.

## Figure 5. Baseline RADAR score on germline and somatic variants

(A) RADAR scores in HGMD and 1000 genomes variants that are inside the RBP regulome; (B) example of BRCA1 intron HGMD variant highlighted by RADAR baseline score. This variant breaks the motif of the splicing factor PRPF8 (red); (C) enrichment of high RADAR baseline score variants associated with COSMIC genes in breast cancer; (D) enrichment of high RADAR baseline score variants associated with recurrent RBP peaks in breast cancer;

## Figure 6. Example of breast cancer somatic variant with high overall RADAR score

We selected an exonic variant with high RADAR score on chromosome 17 as an example. It was within an RBP binding hub with a high Gerp score and broke the motif of PPIG, resulting in a high baseline RADAR score (light blue track). It also has several tissue-specific features, like within the well-known cancer-associated gene TP53 (orange track) and its associated binding peaks were significantly burdened (purple). Besides, by adding expression profiles from TCGA, we found that 3 out of the 6 RBPs binding there demonstrated high regulation powers and drove tumor-specific expression pattern. All these external pieces of evidence further boost this variant’s tissue-specific score.