Putative Blood Somatic Mutations in PTSD-symptomatic Soldiers: High Impact of Cytoskeletal and Inflammatory Proteins

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Running title: Blood Borne Somatic Mutations in PTSD

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Keywords: Alzheimer's disease, Autism, Blood Biomarkers, Cognition, PTSD

Abstract:

Background: We recently discovered autism/intellectual disability somatic mutations in postmortem brains, presenting higher frequency in Alzheimer's disease subjects, compared with the controls. We further revealed high impact cytoskeletal gene mutations, coupled with potential cytoskeleton-targeted repair mechanisms.

Objective: The current study was aimed at further discerning if somatic mutations in brain diseases are presented only in the most affected tissue (the brain), or if blood samples phenocopy the brain, toward potential diagnostics.

Methods: Variant calling analyses on an RNA-seq database including peripheral blood samples from 85 soldiers (58 controls and 27 with symptoms of posttraumatic stress syndrome - PTSD) was performed.

Results: High (e.g. protein truncating) as well as moderate impact (e.g. single amino acid change) germline and putative somatic mutations in thousands of genes were found. Further crossing the mutated genes with autism, intellectual disability, cytoskeleton, inflammation and DNA repair databases, identified the highest number of cytoskeletal-mutated genes (187 high and 442 moderate impact). Most of the mutated genes were shared and only when crossed with the inflammation database, more putative high impact mutated genes specific to the PTSD-symptom cohorts vs. the controls (14 vs. 13) were revealed, highlighting tumor necrosis factor specifically in the PTSD-symptom cohorts.

Conclusions: With microtubules and neuro-immune interactions playing essential roles in brain neuroprotection and Alzheimer-related neurodegeneration, the current mutation discoveries contribute to mechanistic understanding of PTSD and brain protection, as well as provide future diagnostics toward personalized military deployment strategies and drug design.

Introduction:

Recent studies have associated brain somatic mutations with aging and neurodegeneration. For example, Lodato et al. [1] used single-cell whole-genome sequencing to perform genome-wide somatic single-nucleotide variant (sSNV) identification on single neuronal DNA from the prefrontal cortex and hippocampus of 15 normal individuals (aged 4 months to 82 years), as well as 9 individuals affected by early-onset neurodegeneration due to genetic disorders of DNA repair (Cockayne syndrome and xeroderma pigmentosum). These researchers discovered that the sSNVs increased approximately linearly with age in both areas (with a higher rate in the hippocampus), and were more abundant in neurodegenerative diseases [1]. Similarly, Verheijen et al. [2] discussed somatic mutations, commonly referred as "somatic brain mosaicism" highlighting mutations in post-mitotic neurons of the hypothalamo-neurohypophyseal system, and hypothesized on the implications for Alzheimer's disease (AD), further discussed by Leija-Salazar et al. [3]. Lastly, Rohrback et al. [4] discussed the identification of multiple forms of somatically produced genomic mosaicism (GM). Many of these studies concentrated on single cell analysis, mostly hypothesizing, but not directly analyzing AD postmortem brains.

We have recently hypothesized that *de novo* mutations in genes regulating embryonic development, may instigate AD in the form of brain somatic mutations [5]. Importantly, our hypothesis and results are now further corroborated [6]. A leading gene presenting heterozygous dominant *de novo* autism intellectual disabilities (ID), causing mutations, is activity-dependent neuroprotective protein (ADNP), with intact ADNP protecting against AD-tauopathy [5, 7]. RNA-seq of olfactory bulbs identified a novel ADNP hotspot mutation, c.2187_2188insA. Altogether, 665 mutations in 596 genes, with 441 mutations in AD patients (389 genes, 38% AD-exclusive mutations), and 104 genes presenting disease-causing mutations (OMIM) were discovered [5].

OMIM AD-mutated genes converged on cytoskeletal mechanisms, autism- and ID-causing mutations (about 40% each). Importantly, the number and average frequencies of AD-related mutations per subject were higher in AD subjects compared to controls [5]. These findings were corroborated in other brain areas, and mutation frequencies correlated with the severity of Tau pathology (tauopathy). Interestingly, at the single cell level, most mutations were found in the neuronal support cells, rather than neurons. Furthermore, in cell cultures, ADNP mutations inhibited Tau-microtubule interactions [5]. The drug candidate, ADNP fragment NAP (NAPVSIPQ, containing a SxIP microtubule end binding proteins, EB1,3 binding motif), replaced/enhanced Tau-microtubule interaction in the face of ADNP mutations [5].

While brain somatic mutations present an interesting high-risk factor and a therapeutic target, these mutations are not appropriate for diagnostic measures, as sampling is problematic. However, with the discovery of more somatic mutations in non-neuronal cells as compared to neurons [5], we posited that blood borne cells will also display somatic mutations, accumulating with aging and brain diseases. As such, a study evaluating both AD brain and blood samples showed significant SNV increases with aging, and an almost 5-fold slower accumulation in brain compared to blood [8]. Regardless, the study discovered that low-level brain somatic mutations in the hippocampal formation were associated with dysregulation of Tau hyperphosphorylation [8]. Regarding aging-related accumulation of somatic mutations, Watson et al. [9], using blood sequencing data from ~50,000 individuals, revealed how mutations, genetic drift, and fitness shape the genetic diversity of healthy blood (clonal hematopoiesis), emphasizing that somatic mutations acquired in healthy tissues as we age are potentially major determinants of the aging process.

The advantages of blood biomarkers are considerable. A number of inorganic and organic markers found free in the plasma or within exosomes have shown a solid potential as biomarkers for AD,

including metallic ions, auto-antibodies, cytokines, phospholipids and microRNA-species. In 2012, researchers at the AD Neuroimaging Initiative and Australian Imaging Biomarker and Lifestyle Research Group produced a panel of 27 biomarkers that demonstrated small, but statistically significant changes between healthy individuals and AD patients [10]. These included proteins such as insulin-like growth-factor binding protein 2 (IGF-BP2), and β2 microglobulin (β2M) [10]. We and others also found a reduction in serum ADNP as correlated with reduced cognition and AD [11, 12]. Additionally, lower regulator of G-protein signaling 2 (RGS2) expression levels were discovered in mild cognitive impairment and AD blood samples, compared with controls [13]. These findings suggest ADNP and RGS2 as novel future AD biomarkers toward early AD detection and future disease modifying therapeutics. We also discovered an interaction between circulating pituitary adenylate cyclase-activating polypeptide (PACAP) and ADNP in terms of resilience to stressful conditions [14].

Aging and stress (including PTSD) serve as major risk factors for AD [15, 16], with PTSD possibly even doubling the risk of AD and dementia [16, 17]. The molecular mechanisms for this may include reduced "cognitive reserve", suggested by impaired verbal memory in PTSD [18], as well as brain alterations in the hippocampus [19], anterior cingulate [20], and prefrontal structures [21]. Additionally, PTSD may be associated with independent risk factors for dementia including smoking, hypertension, hyperlipidemia, diabetes, obesity, inflammation, and major depression [21-23]. Interestingly, PTSD and dementia were also suggested to have a bidirectional relationship, with PTSD increasing the risk for late-onset dementia, while dementia increases the risk for delayed-onset PTSD in those who experienced a significant trauma earlier in life [24].

In this respect, a robust multi-omic panel for predicting combat-related PTSD diagnosis in male veteran populations was previously established, with 28 biomarkers including features from DNA

methylation, proteins, miRNAs, metabolites, and other molecular and physiological measurements [25]. This panel was implemented in an independent validation cohort, predicting PTSD diagnosis with 81% accuracy, 85% sensitivity, and 77% specificity, hence indicating that PTSD can potentially be identified using blood-based screening or diagnostic tools [25].

Here, we have analyzed peripheral blood samples from 85 Canadian infantry soldiers and showed mutated genes, associated with AD and autism, in peripheral blood cells of individuals suffering from symptoms of PTSD. These findings may pave the path to new diagnostic measures in molecular neurodegeneration as well as stressful conditions including PTSD.

Materials and Methods:

Design, Measures and Gene Expression Omnibus (GEO) Datamining

The gene expression dataset GSE109409 [26] was identified as containing a complete Next-Generation Sequencing (NGS) transcriptomics, RNA-seq data from peripheral blood samples of 85 male Canadian infantry soldiers (n = 58 participants negative for symptoms of PTSD and n = 27 participants with symptoms of PTSD), with an average age of 29.86±7.4 years, after returning from deployment to Afghanistan.

To control for batch effects, biological and technical confounders, a set of 7 covariates was selected using a greedy step-down regression procedure combined with normalized gene count Principal Component Analysis (PCA). The set included aggregate batch, neutrophil count, white blood cell count, read percentage GC content, percentage of mapped reads, percentage reads not exonic and de-duplicated read percentage. Furthermore, the same number of soldiers had a previous deployment as those whose first deployment was Afghanistan both within and between groups [26].

Specifically, the soldiers took part in the study immediately after their return from deployment and every 4 months following that for up to 1-year [26]. Upon enrollment, soldiers were asked to complete the following series of questionnaires: a demographic information sheet, the Combat Exposure Scale from the Deployment risk and Resilience Inventory (DRRI) [27], and the Posttraumatic Stress Disorder Checklist for military personnel (PCL-M) [28]. The PCL is one of the most widely used self-report measures of PTSD, extensively used in the military [29, 30], and has been repeatedly shown to highly correlate the diagnostic gold standard, namely the Clinical-Administered PTSD Scale (CAPS) [30]. While neither the specific PTSD symptoms, nor any other related psychological disorder (e.g. depression) were mentioned in the original study, grouping the

participants based on a dichotomized PCL score has shown that 58 scored < 34 (Control) and 27 scored ≥ 34 (Symptoms of PTSD) [26]. A cut-off score of 39 was previously found to be optimally efficient at identifying full PTSD [29]. Furthermore, scores between 35 and 49 have been shown to classify as risk for meeting subthreshold PTSD diagnostic criteria [7, 30, 31].

Following form completion, 2.5 ml of blood was collected using the PAXgene blood RNA collection protocol (PreAnalytiX GmbH, QIAGEN or BD) for gene expression and 4 ml of blood was collected for a complete blood count (CBC) [26].

Variant Calling

Variant calling was performed as before [5] according to GATK's best practices pipeline. Namely, trimmed reads were mapped to the human genome (Ensembl's GRCh38) using STAR v2.4.2a [32], with default parameters and twopassMode set to basic. Reads were then deduplicated using Picard. Mapped reads were further processed with GATK's v.3.7 [33] SplitNCigarReads, which was used as a method developed specially for RNA-seq, splits reads into exon segments (getting rid of Ns but maintaining grouping information) and hard-clips any sequences overhanging into the intronic regions. Next, the processed reads were used for variant calling by GATK's HaplotypeCaller with ploidy set to 10 in order to detect also low frequency variants. Variants were filtered with the following values for SNPs and Indels respectively: QD<2.0, FS>30.0, MQ<40.0, MQRankSum<-12.5, ReadPosRankSum<-8.0 and QD<2.0, FS>30.0 and ReadPosRankSum<-20.0. Variants were further filtered against dbSNP build 146 [34, 35], a widely used data source, integrated and referenced by many other databases and projects such as OMIM, Clinvar, 1000 genomes project, to name a few, and includes both pathogenic and non-pathogenic variants. Given the elaborate content of the dbSNP database, combined with the special settings of the current

project, in which low frequency mutations are called, extensive filtering had to be implemented, rather than reliance only on curated databases that may be more accurate than dbSNP, but less elaborate. Any variant that appeared there was discarded. Annotation was done with Ensembl's Variant Effect Predictor v.83 [36] against GRCh38. Only variants that were predicted to have high impact, had average coverage of at least 10 reads in each group and were covered by at least ~90%-95% of the samples in each group, that is by 27 samples in the PTSD-symptom and by 58 samples in the control, were considered in the analysis. Variant analysis and sample description are detailed in Tables S1A-B.

Comparative Databases

Using the Venn diagram tool (https://bioinfogp.cnb.csic.es/tools/venny/), several comparisons were performed with multiple sources including the autism spectrum disorder (ASD, autism) (https://gene.sfari.org/database/human-gene/), intellectual disability (ID) (http://www.ccgenomics.cn/IDGenetics/gene.php?dataset=IDGD_gene_detail), inflammatory response (http://www.informatics.jax.org/go/term/GO:0006954), cytoskeleton (http://www.informatics.jax.org/go/term/GO:0005856), and DNA repair (http://www.informatics.jax.org/go/term/GO:0006281) databases.

Statistics

Results are presented as means ± standard error of the mean (SEM). Data were checked for normal distribution by normality test. Unpaired student's t-test or Mann-Whitney U test analyses were performed. P values smaller than 0.05 were considered significant. All tests were two-tailed. Outlier values were excluded using the GraphPad outlier calculator

(https://www.graphpad.com/quickcalcs/Grubbs1.cfm). All statistical analyses were conducted using either SigmaPlot version 11 for Windows (Systat Software, Inc., Chicago, IL, USA), or GraphPad Prism versions 5 & 6 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

Results:

Individuals in the PTSD-symptom group display a relatively high number of putative high impact mutations, compared with matched controls

When looking at putative high (e.g. protein truncating mutations) and moderate (e.g. a change in amino acid in the protein) impact mutations, a relatively high number of high impact mutation containing genes was found in the PTSD-symptom cohort similar to controls, taking into consideration a saturation effect. Thus, it should be noted that in general, the number of the detected mutations does not increase linearly with the number of samples, as the larger the cohort size is, there would be less new mutations, since most of these already appeared before. For high impact mutations, 1,147 genes were found to display 1,556 mutations in the PTSD-symptom group vs. 1,501 genes displaying 2,107 mutations in the control group (Fig. 1A). For moderate impact mutations, 1,985 genes were found to display 5,645 mutations in the PTSD-symptom group vs. 3,219 genes displaying 9,246 mutations in the control group (Fig. 1B). When looking at the average mutation frequency/number per subject, no significant differences were found between the tested groups, either in the case of high impact mutations, or in the case of moderate impact mutations (Fig. S1).

Cytoskeletal-related genes comprise the largest group of mutated genes among the PTSD-symptom and control cohorts

Based on our previous findings associating AD brain mutations with autism, intellectual disability, inflammatory response, cytoskeleton and DNA repair genes [5], putatively mutated genes in this cohort were crossed with known databases of these gene/protein groups (Supplemental, Figs. S2-21). We have identified the largest number of high impact mutated genes in the cytoskeletal protein

group (187), with 23 genes being specific for PTSD-symptom (Fig. 2, Venn diagram). As our previous analyses regarding gene/protein associations utilized the STRING tool [5], we performed a STRING analysis here, revealing several key mutated protein interactions including *TSC1*, *FMR1*, *GSK3B*, and *EZR*, specific to the PTSD-symptom population. The Tuberous Sclerosis 1 (*TSC1*) gene encodes the growth inhibitory protein hamartin, negatively regulating mammalian target of rapamycin complex 1 (mTORC1) signaling, with mutations in this gene previously associated with Tuberous Sclerosis [37]. The FMRP Translational Regulator 1 (*FMR1*) encodes an RNA-binding protein, and may be involved in mRNA trafficking from the nucleus to the cytoplasm, thus being implicated in Fragile X Syndrome [38]. The Glycogen Synthase Kinase-3 Beta (*GSK3B*) gene product is a serine-threonine kinase, serving as a negative regulator of glucose homeostasis [39]. This protein is involved in various processes including energy metabolism, inflammation, ER-stress, mitochondrial dysfunction, and apoptotic pathways [39]. Mutations in the *GSK3B* gene were linked with Parkinson's disease (PD) and AD [40, 41], with GSKB3 directly linked to tau hyperphosphorylation [42]. The Ezrin (*EZR*) gene encodes a cytoplasmic peripheral membrane protein, playing a key role in cell surface structure adhesion, migration and organization, as well as implicated in different human cancers [43].

When looking at the frequency of moderate impact mutations, a high number of cytoskeletal mutated genes was also discovered (442), including 74 genes specific for the PTSD-symptom group (Fig. 2).

Increased mutations in inflammatory genes in the PTSD-symptom cohort

Most of the high impact mutated genes, in all tested databases were shared (Supplemental, Figs. S2-11). Only the inflammation database showed more high impact putatively mutated genes

specific in the PTSD-symptom cohort as compared to controls (14 vs. 13) (Fig. 3). Further analysis identified the putatively mutated tumor necrosis factor (*TNF*), despite a low coverage of 2/7 in that region, in one person in the PTSD-symptom cohort. Importantly, TNF plays central roles in the immune response [44]. In this respect, other genes that exhibited PTSD-associated mutations are described below. Interleukin 1 Receptor Type 2 (*IL1R2*) encodes a cytokine receptor that belongs to the interleukin 1 receptor family [45]. It should be noted that cytokines in general serve as major mediators of the immune response, controlling different cellular functions including proliferation, differentiation and cell survival/apoptosis, as well as being involved in several pathophysiological processes [46]. Caspase 1 (*CASP1*) and Caspase 4 (*CASP4*) gene products play a central role in the execution-phase of cell apoptosis, with caspase 4 cleaving and activating its own precursor protein, as well as caspase 1 precursor [47, 48].

Moderate impact mutations in pro-inflammatory genes did not show similar trends to the high impact mutations described above (Supplemental Fig. S16-17).

Discussion:

The current paper reveals somatic mutations in the blood of PTSD patients, with suggestive unique patterns/genes, paving the path to future investigations and potential novel biomarkers affecting disease mechanisms.

Interestingly, in terms of gene expression, the study that originally collected the samples and assessed gene expression revealed an increased expression of the low-density lipoprotein receptor-related protein 8 (*LRP8*) as a PTSD-symptom-specific transcript [26]. LRP8 is a cell surface receptor for Reelin (*RELN*) and apolipoprotein E (APOE)-containing ligands, important for brain development, with *APOE4* presenting the highest risk gene for AD [49] (Identifier: ENSP00000303634, LRP8). Additionally, Reelin-mediated atherosclerosis was shown to be promoted by isoforms E2 and E4 of APOE, hence increasing the risk for AD [50]. The other PTSD-symptom gene discovered in the original study is Golgi membrane protein 1 (*GOLM1*) [26], a cellular response protein to viral infection, belonging to the GOLM1/CASC4 family (Identifier: ENSP00000373364, GOLM). These results suggest an association of PTSD with viral infection [51], which may also account, in part, for a potentially increased mutation rate [52]. Interestingly, GOLM1 was previously found to be significantly increased in an aged mouse model of AD [53]. In this respect, gene expression levels are not directly linked with higher mutation rates, with neither *LRP8* nor *GOLM1* found here among the mutated genes. Similarly, we did not find mutations in IGF-BP2, β2 microglobulin (*β2M*) [10] and ADNP, suggested in our introduction to change in blood samples as a consequence of AD, given that serum levels correlate with reduced cognition and AD [11, 12]. Notably, ADNP/NAP (regulating cytoskeletal dynamics) [5] control *Apoe* expression in a sex-dependent manner [54].

Importantly, in the current study several cytoskeleton-related genes were found to carry a high impact mutation, only in the PTSD-symptom cohort, including *TSC1*, *FMR1*, *GSK3B* and *EZR*. Regardless, it should be noted that the current study is a somewhat pioneering study, with many of the mutated genes appearing only in a single or a few individuals, and having a low coverage. This suggests that these mutations should be validated and examined in larger cohorts. Interestingly, *TSC1*, *FMR1*, *GSK3B* and *EZR* are linked with either the neurodegenerative AD or the neurodevelopmental autism spectrum disorder (ASD) [41, 55-61]. In this respect, PTSD may be associated with the risk of developing dementia, specifically of the common AD type [17, 62]. Alterations of hormones, regulating the production and deposition of amyloid beta (Aβ) plaques, a diagnostic feature of AD dementia, have also been suggested to cause PTSD [63, 64]. Additionally, several pathways possibly linking trauma and autism were previously suggested, with ASD potentially serving as a vulnerability marker for PTSD, specifically by increasing the risk for exposure to traumatic events. Then, once PTSD has appeared, it may exacerbate ASD symptoms [65]. Importantly, ASD and PTSD may share underlying common molecular mechanisms, leading to neurological abnormalities associated with both disorders, as well as cognitive and behavioral outcomes such as cognitive rigidity, anger and aggression [65].

Furthermore, the reciprocal neuro-immune interactions, with immune cells/factors affecting brain cells [66, 67] and the brain affecting immune responses [68], are of great interest. These interactions are directly associated with changes in circulating cytokine amounts (e.g. IL-6) as a consequence of trauma [69]. Here, high impact mutations have been discovered in *TNF* (a key player in the immune response), as well as *IL1R2* only in the PTSD-symptom cohorts, and not the control cohorts, thus implicating somatic mutations/immune-genetics in susceptibility to PTSD. Conforming to these findings, previous independent studies looking at blood biomarkers suggested

that individuals suffering from PTSD display increased levels of proinflammatory markers, including interleukin-1β (IL-1β), interleukin-6 (IL-6), TNF-α, and C-reactive protein, compared with healthy controls [70-73]. Additionally, *CASP1* and *CASP4* genes were also found to be mutated solely in the PTSD-symptom cohort. These caspases are involved in the processing and secretion of pro-inflammatory molecules and are often referred as "pro-inflammatory caspases" [47, 48], thereby indicative of a possible inflammatory state in PTSD patients. The connection between a systemic pro-inflammatory state and PTSD was previously emphasized by several studies [74]. For example, increased levels of cytokines, as those observed in PTSD, may cause inflammation, damaging the brain and further increasing the risk of dementia [17, 63]. Interestingly, opposing findings were also reported, with no significant correlations found between inflammatory markers and severity of PTSD symptoms [72].

Future investigations with larger cohorts, deeper coverage and validation methods should further assess the impact of blood as a surrogate source for mutation biomarkers. These future investigations should further investigate similarities of blood and brain-identified cytoskeletal and aging-related mutations, thus enabling the identification of populations at risk. In line with that, a recent study in thousands of civilian and military Europeans identified significant PTSD gene expression associations [75]. Specifically, in the civilian and military cohorts, the Zinc Finger Protein 140 (ZNF140) was predicted to be upregulated in whole blood, and the splicing regulator Small Nuclear Ribonucleoprotein U11/U12 Subunit 35 (SNRNP35) was predicted to be downregulated in the dorsolateral prefrontal cortex, further linked to stress and glucocorticoids [75]. However, this study did not analyze for potential mutations and study limitations should take into consideration potential sequencing bias [76].

Interestingly, when searching PubMed, several RNA-seq gene expression databases obtained from human peripheral blood leukocytes in PTSD cohorts were found. These databases were either limited (GSE83601)[77], did not represent a soldier cohort (GSE97356)[78], or included PTSD patients (rather the symptomatic cases), and cannot be claimed as ethnically different from the Canadian cohort used in our study (GSE64814; RNA-seq from peripheral blood leukocytes of U.S. Marines, N=188, obtained both pre- and post-deployment to conflict zones)[79]. Future studies should target additional populations to provide a further global aspect of the research outcome.

In practical terms, our findings may further suggest the use of preventative treatments, such as drugs targeting the cytoskeletal system, as we proposed before, with NAP [5], and ADNP-regulating peptide hormones including pituitary adenylate cyclase-activating polypeptide (PACAP) [14]. Notably, the previously demonstrated efficacy for NAP in amnestic mild cognitive impairment population [80, 81], coupled with patient stratification-based on similar and extended studies as described above, will facilitate a personalized, precision medicine for PTSD and prodromal AD.

To conclude, our previous discovery of potential brain somatic mutations as driving AD focused on ADNP/NAP targeting microtubule end binding proteins [5]. In this respect, ADNP/NAP take a major regulatory role in neuronal and immunological functions [54, 82-84]. This finding is enhanced by our further discovery of numerous somatic mutated genes revealing preponderance in cytoskeletal/autism/intellectual disability AD-postmortem brain mutations [5], which are associated with synaptic plasticity in the brain [54, 85], as well as the functionality of the immune synapse [86, 87]. Interestingly, original studies, also at the brain ultrastructural level, revealed microtubule reduction in AD and aging that is independent of tau filament formation, focusing on microtubule cytoskeleton in general [88]. Our current findings enhance the applicability of the

previous postmortem brain discoveries, with PTSD-symptomatic patients carrying specific potentially treatable gene mutations, mirroring to some degree brain dysfunctions, and possibly leading toward precision medicine.

Declarations:

Ethics approval and consent to participate: The dataset used in the current study was obtained according to the research protocol, previously accepted under the Human Research Ethics Committee (HREC) of Defense Research and Development Canada (DRDC) - Protocol 2017-019 [26].

Consent for publication: All authors declare their consent for publication.

Availability of data and materials: The dataset supporting the conclusions of this article is available at GEO with the accession number: GSE109409.

Authors' contributions: Shlomo Sragovich performed the data analysis and mining. Michael Gershovits performed the bioinformatics mutational analysis. Professors Jacqueline CK Lam and Victor OK Li contributed to the discussion and background. Professor Illana Gozes led the project, provided funding, analyzed data, and wrote the paper.

Acknowledgements:

Shlomo Sragovich is a former Levi Eshkol Ph.D. fellow, supported by the Israel Ministry of Science and Technology, the Tel Aviv University GRTF, The Naomi Foundation, The Eldee Foundation/Bloomfield Family of Montreal awards for student exchange (Tel Aviv University/McGill University), and The BioInnovation Fellowship & Mentorship by Teva. Professor Illana Gozes the former, Lily and Avraham Gildor Chair for the Investigation of Growth Factors is supported by the following grants: MAFAT, AMN Foundation, ERA-NET neuron ADNPinMED and Drs. Ronith and Armand Stemmer, French Friends of Tel Aviv University).

Conflict of Interest/Disclosure Statement:

Professor Illana Gozes is the Chief Scientific Officer of Coronis Neurosciences. NAP (CP201) use is under patent protection (US patent nos. US7960334, US8618043, and USWO2017130190A1).

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Figure Legends:

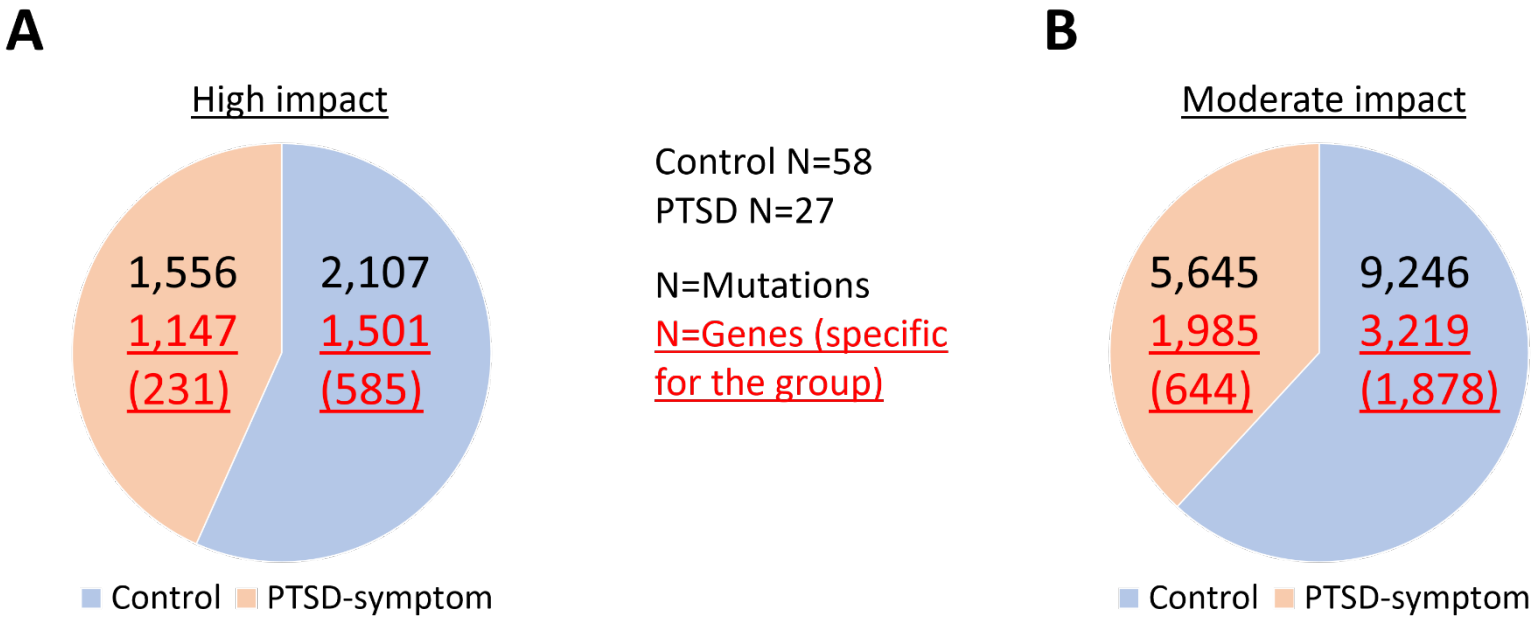


Figure 1. Soldiers display somatic mutations in blood cells.

(A-B) The pie charts represent distribution of high and moderate impact mutations into two groups: control and PTSD-symptom (n = 58 control participants negative for symptoms of PTSD and n = 27 participants positive for PTSD symptoms).

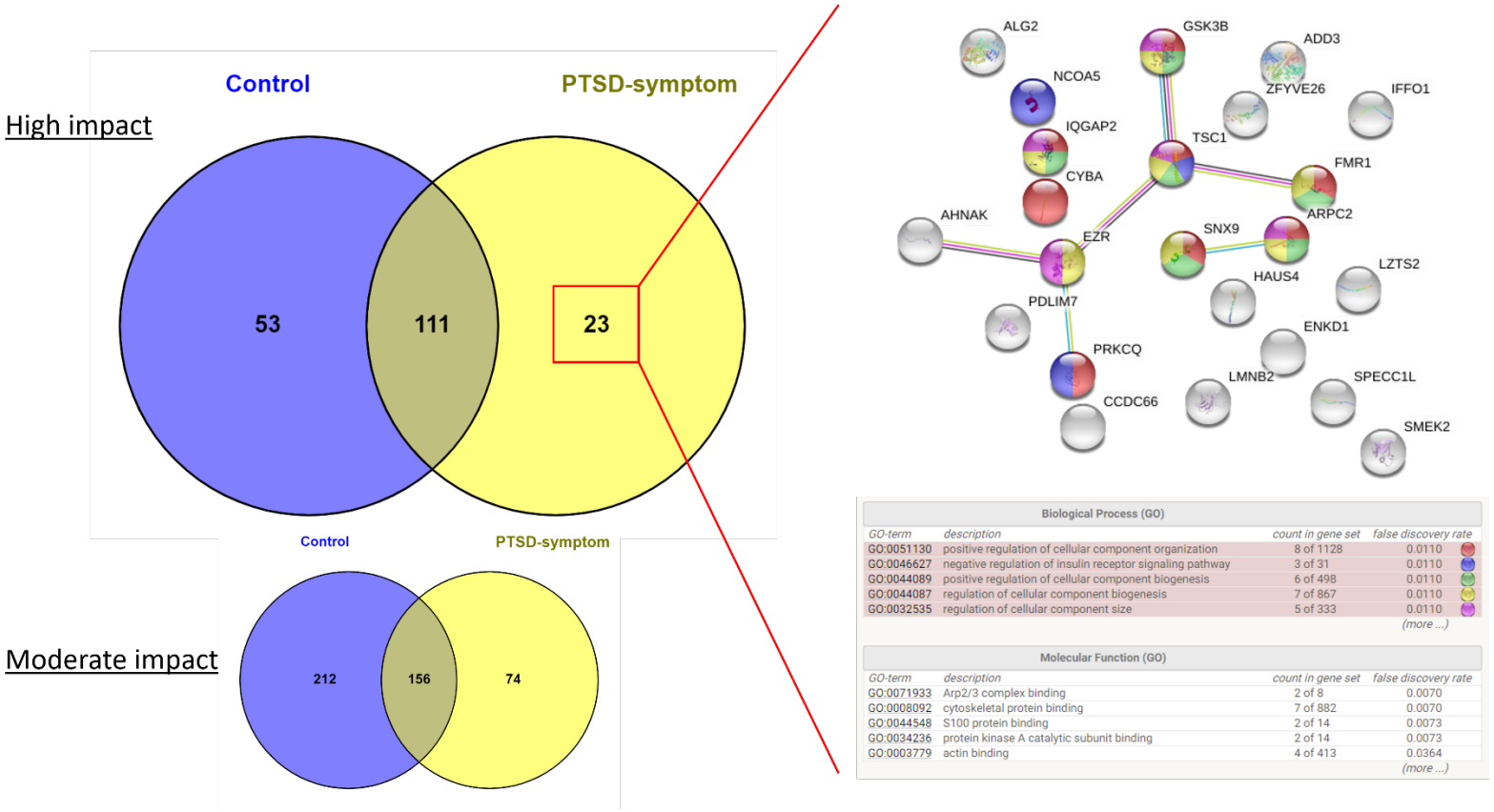
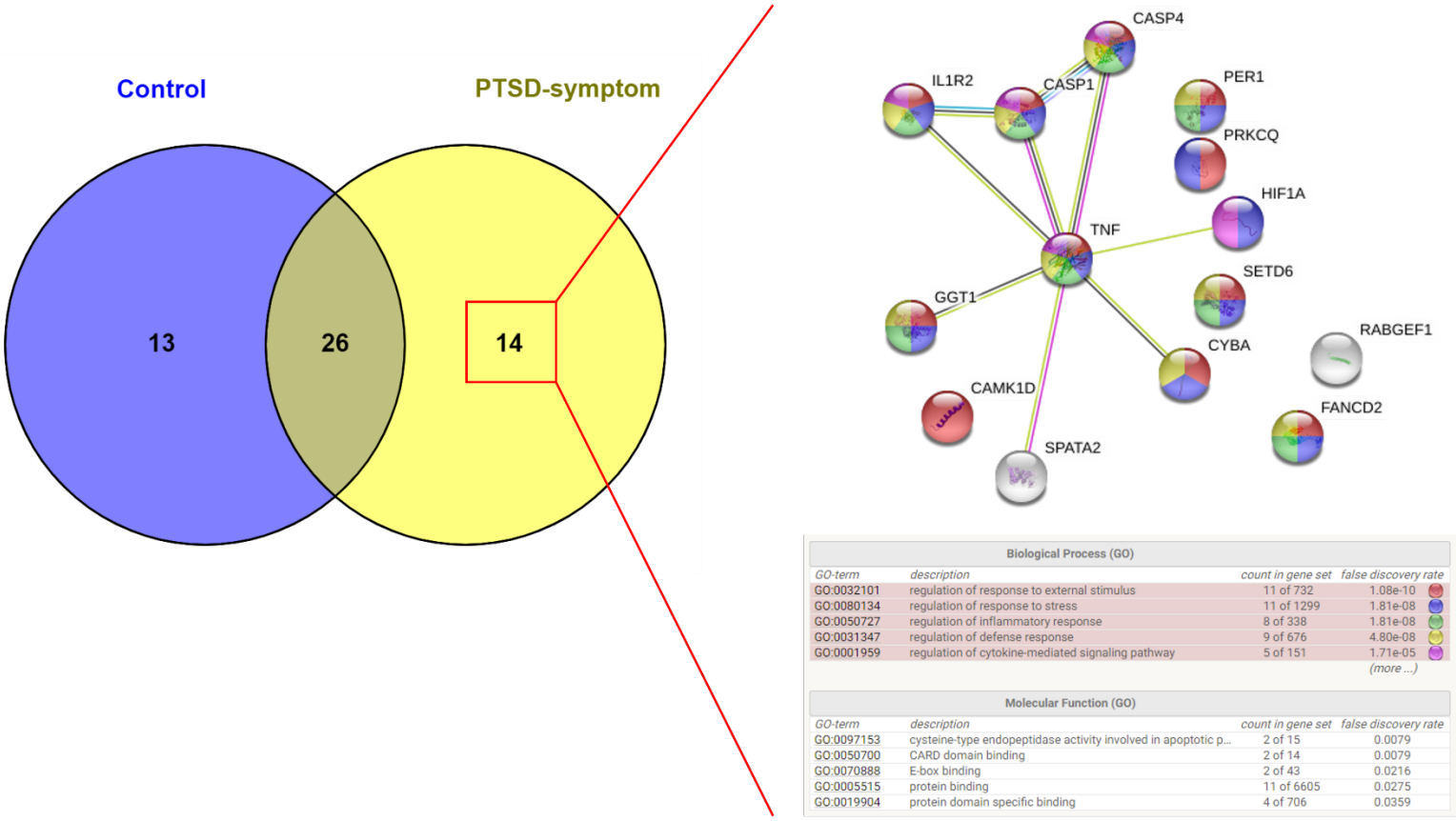


Figure 2. Cytoskeletal-related genes in the PTSD-symptom group are most frequently mutated.

STRING analysis was performed for cytoskeletal-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for putative 23 high impact PTSD-specific genes. Enriched biological processes, molecular functions and pathways are presented for these genes, most frequently mutated, compared with the control group. An additional Venn diagram is presented for moderate impact mutations.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0032101	regulation of response to external stimulus	11 of 732	1.08e-10
GO:0080134	regulation of response to stress	11 of 1299	1.81e-08
GO:0050727	regulation of inflammatory response	8 of 338	1.81e-08
GO:0031347	regulation of defense response	9 of 676	4.80e-08
GO:0001959	regulation of cytokine-mediated signaling pathway	5 of 151	1.71e-05
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0097153	cysteine-type endopeptidase activity involved in apoptotic p...	2 of 15	0.0079
GO:0050700	CARD domain binding	2 of 14	0.0079
GO:0070888	E-box binding	2 of 43	0.0216
GO:0005515	protein binding	11 of 6605	0.0275
GO:0019904	protein domain specific binding	4 of 706	0.0359

Figure 3. Inflammation-related genes in the PTSD-symptom group are increasingly mutated. STRING analysis was performed for inflammation-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for putative 14 high impact PTSD-specific genes. Enriched biological processes, molecular functions and pathways are presented for these genes, most frequently mutated, compared with the control group.

Supplementary Materials:

Putative Blood Somatic Mutations in PTSD-symptomatic Soldiers: High Impact of Cytoskeletal and Inflammatory Proteins

Shlomo Sragovich[1], Michael Gershovits[2] Jacqueline CK Lam[3,4,5], Victor OK Li[3] and Illana Gozes[1*]

Running title: Blood Borne Somatic Mutations in PTSD

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Keywords: ADNP, NAP, PACAP, PTSD, Blood Biomarkers

Supplemental Figures:

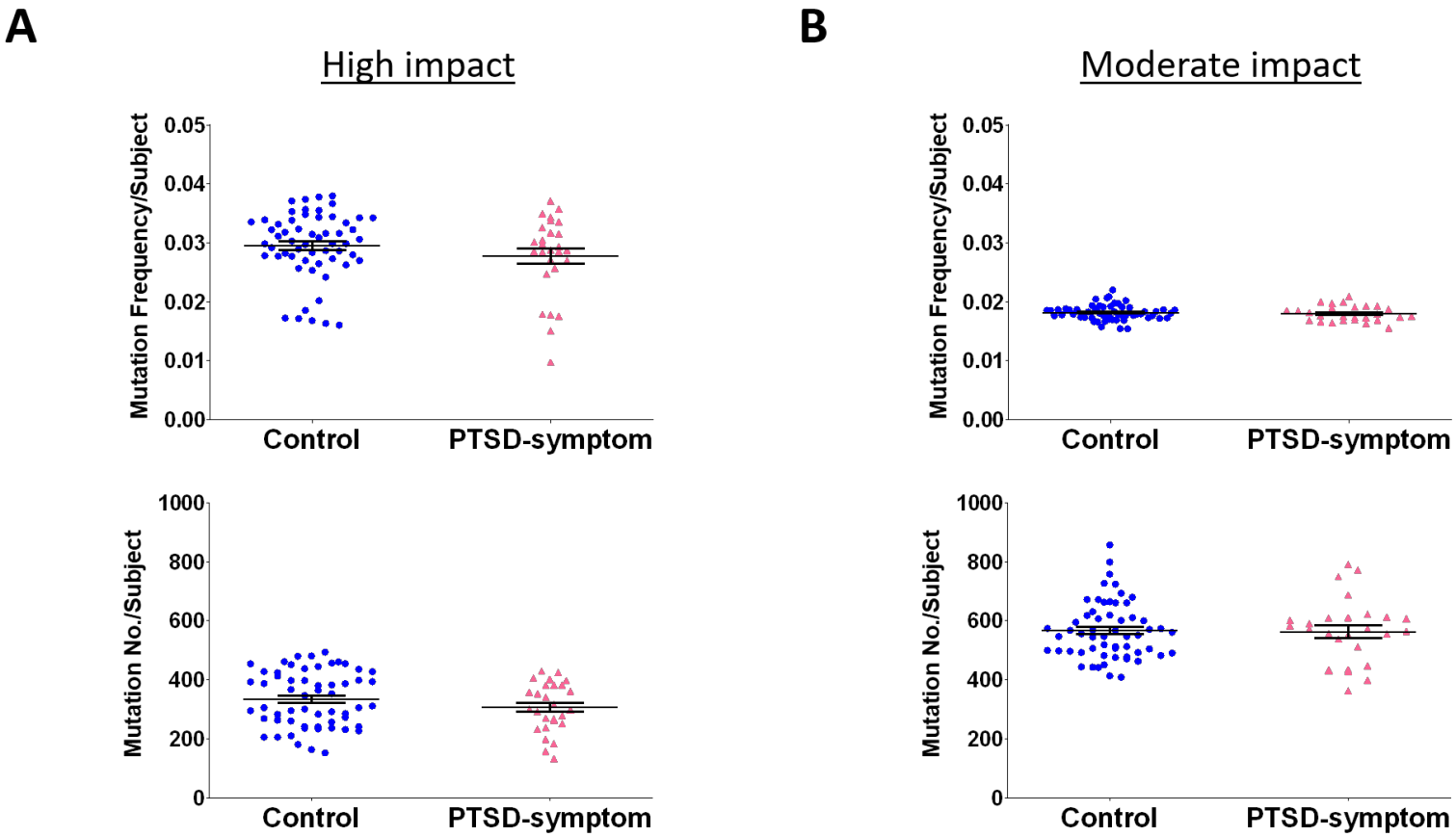
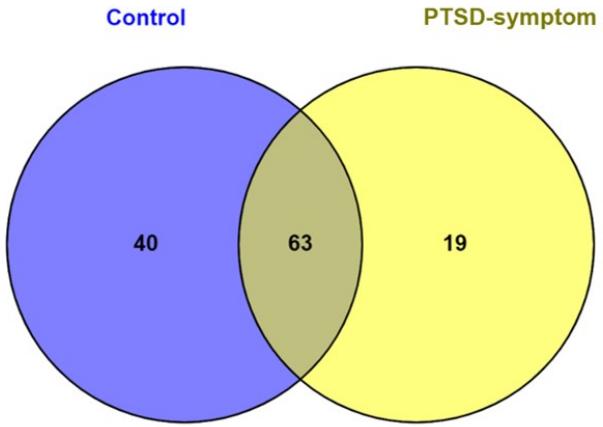


Figure S1. No significant differences were observed in either high or moderate impact mutation frequencies/numbers per subject between control and PTSD-symptom groups.

(A) high impact mutation frequency/number per subject, and **(B)** moderate impact mutation frequency/number per subject for each group in the peripheral blood leukocytes (n = 58 control participants negative for symptoms of PTSD and n = 27 participants positive for PTSD symptoms).



Biological Process (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0051276	chromosome organization	20 of 999	5.56e-08
GO:0006325	chromatin organization	17 of 683	5.56e-08
GO:0016570	histone modification	11 of 347	1.15e-05
GO:0060255	regulation of macromolecule metabolic process	41 of 6072	1.23e-05
GO:0080090	regulation of primary metabolic process	40 of 5982	2.34e-05
			(more ...)

Molecular Function (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0042393	histone binding	8 of 188	7.30e-05
GO:0003682	chromatin binding	11 of 501	0.00011
GO:0032452	histone demethylase activity	4 of 27	0.00033
GO:0018024	histone-lysine N-methyltransferase activity	4 of 40	0.00084
GO:0032454	histone demethylase activity (H3-K9 specific)	3 of 14	0.0012
			(more ...)

KEGG Pathways			
pathway	*description*	*count in gene set*	*false discovery rate*
hsa00310	Lysine degradation	4 of 59	0.0050
hsa05332	Graft-versus-host disease	3 of 36	0.0124
hsa05330	Allograft rejection	3 of 35	0.0124
hsa04940	Type I diabetes mellitus	3 of 40	0.0124
hsa05320	Autoimmune thyroid disease	3 of 49	0.0126
			(more ...)

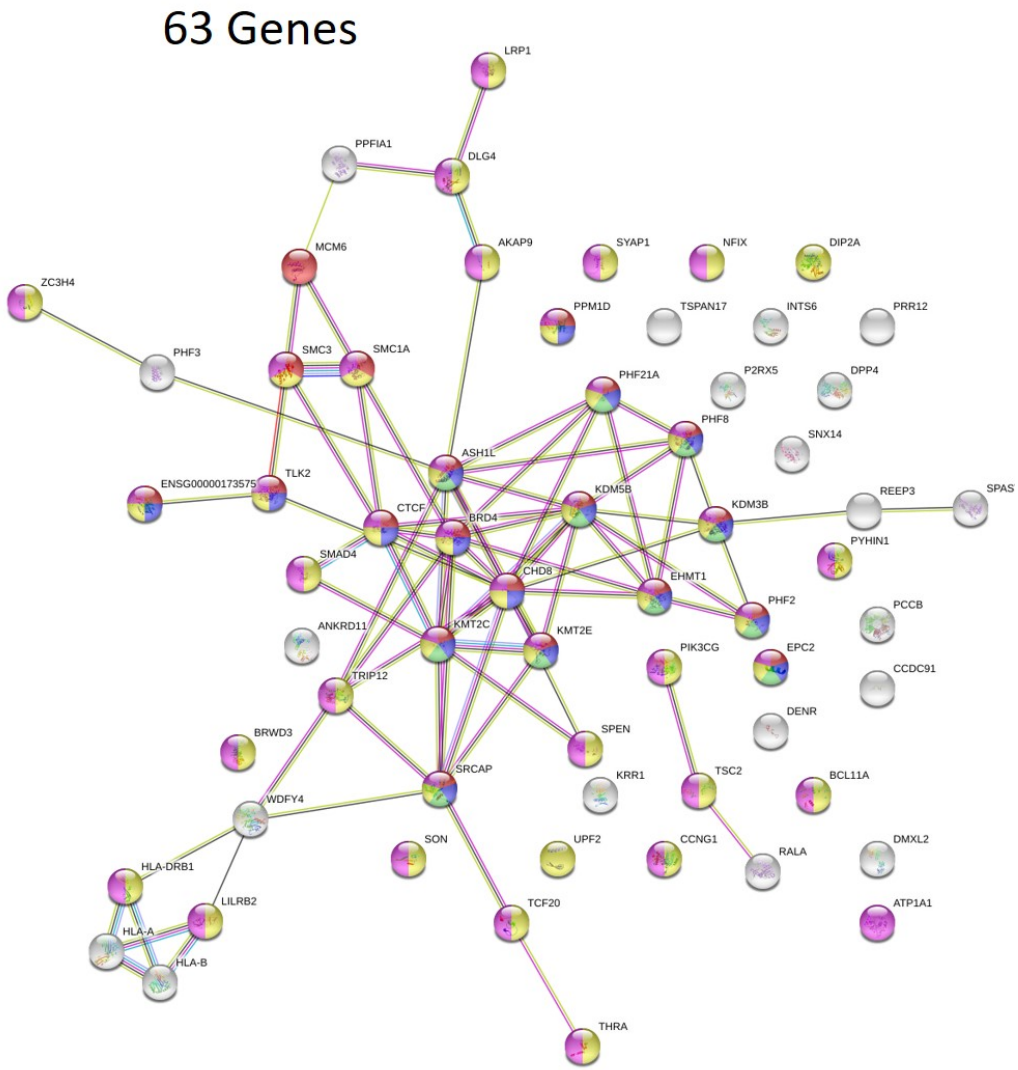
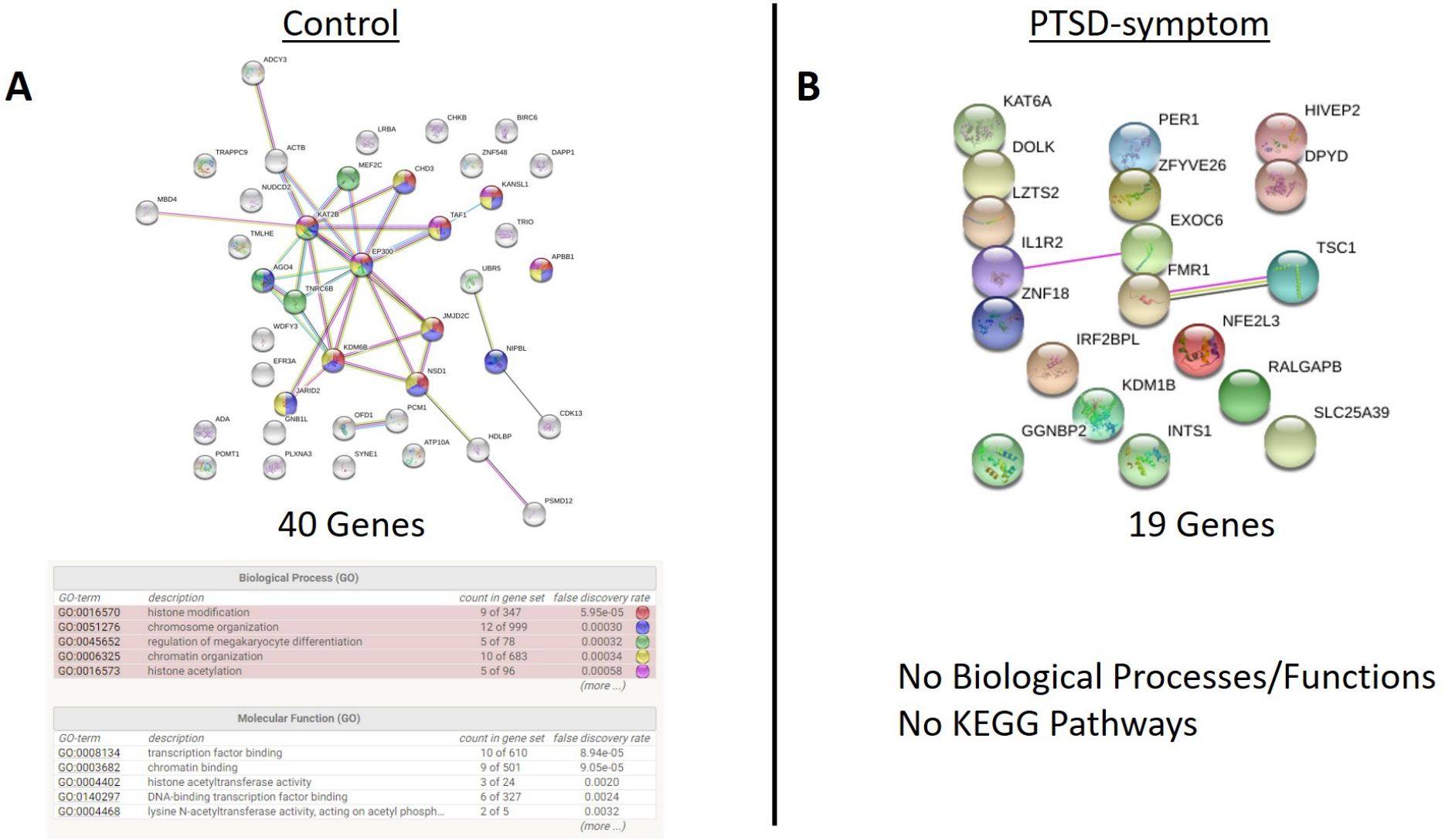


Figure S2. High Impact Shared Autism-related Genes Between Control and PTSD-symptom groups (Crossed with SFARI Database).

STRING analysis was performed for mutated autism-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 63 high impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0016570	histone modification	9 of 347	5.95e-05
GO:0051276	chromosome organization	12 of 999	0.00030
GO:0045652	regulation of megakaryocyte differentiation	5 of 78	0.00032
GO:0006325	chromatin organization	10 of 683	0.00034
GO:0016573	histone acetylation	5 of 96	0.00058

(more ...)

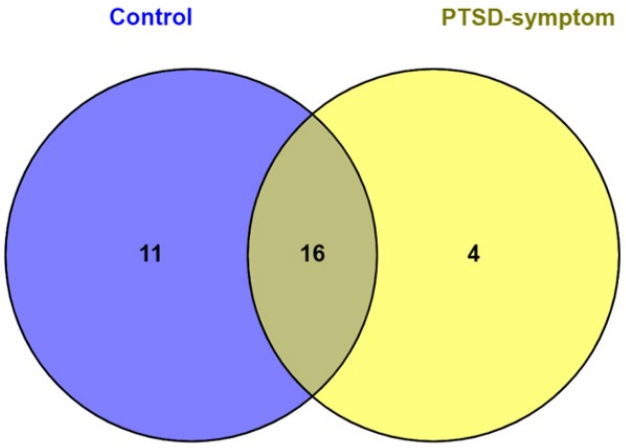
Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0008134	transcription factor binding	10 of 610	8.94e-05
GO:0003682	chromatin binding	9 of 501	9.05e-05
GO:0004402	histone acetyltransferase activity	3 of 24	0.0020
GO:0140297	DNA-binding transcription factor binding	6 of 327	0.0024
GO:0004468	lysine N-acetyltransferase activity, acting on acetyl phosph...	2 of 5	0.0032

(more ...)

Figure S3. Mutated Autism-related Genes in the Control and PTSD-symptom groups (Crossed with SFARI Database).

(A) STRING analysis was performed for 40 high impact autism-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 19 high impact autism-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes (where available).



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0051276	chromosome organization	8 of 999	0.00040
GO:0032876	negative regulation of DNA endoreduplication	2 of 5	0.0051
GO:0006325	chromatin organization	6 of 683	0.0051
GO:0006996	organelle organization	10 of 3131	0.0066
GO:2000113	negative regulation of cellular macromolecule biosynthetic ...	7 of 1348	0.0076

(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0003682	chromatin binding	6 of 501	0.00027
GO:0036033	mediator complex binding	2 of 4	0.00067
GO:0043168	anion binding	8 of 2696	0.0141
GO:0042393	histone binding	3 of 188	0.0141
GO:0035639	purine ribonucleoside triphosphate binding	7 of 1794	0.0141

(more ...)

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa04110	Cell cycle	3 of 123	0.0055
hsa04211	Longevity regulating pathway	2 of 88	0.0481

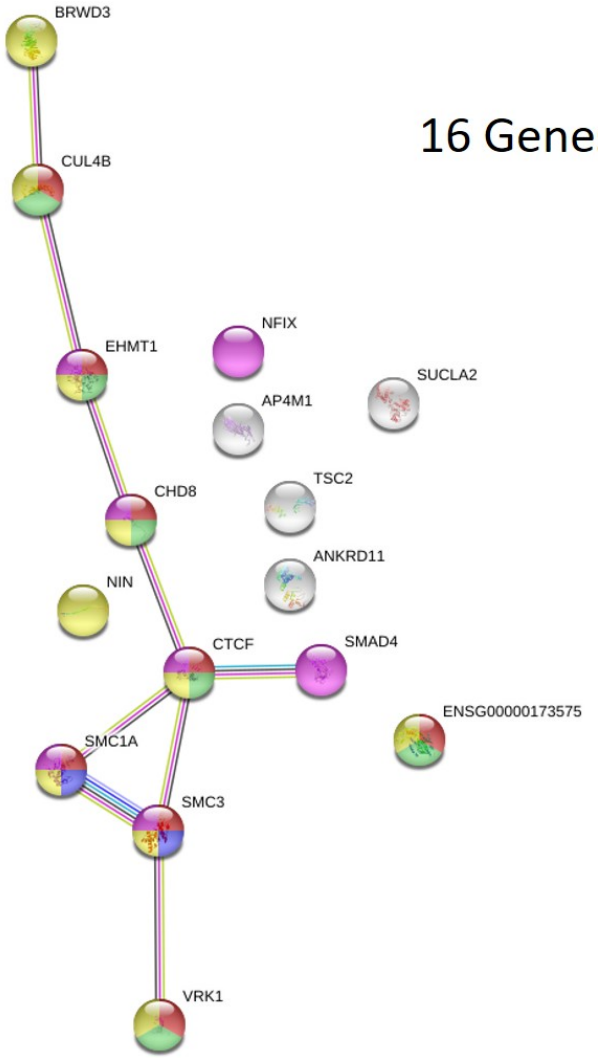


Figure S4. High Impact Shared ID/ASD-related Genes Between Control and PTSD-symptom groups (Crossed with ID_ASD Database).

STRING analysis was performed for mutated ID/ASD-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 16 high impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.

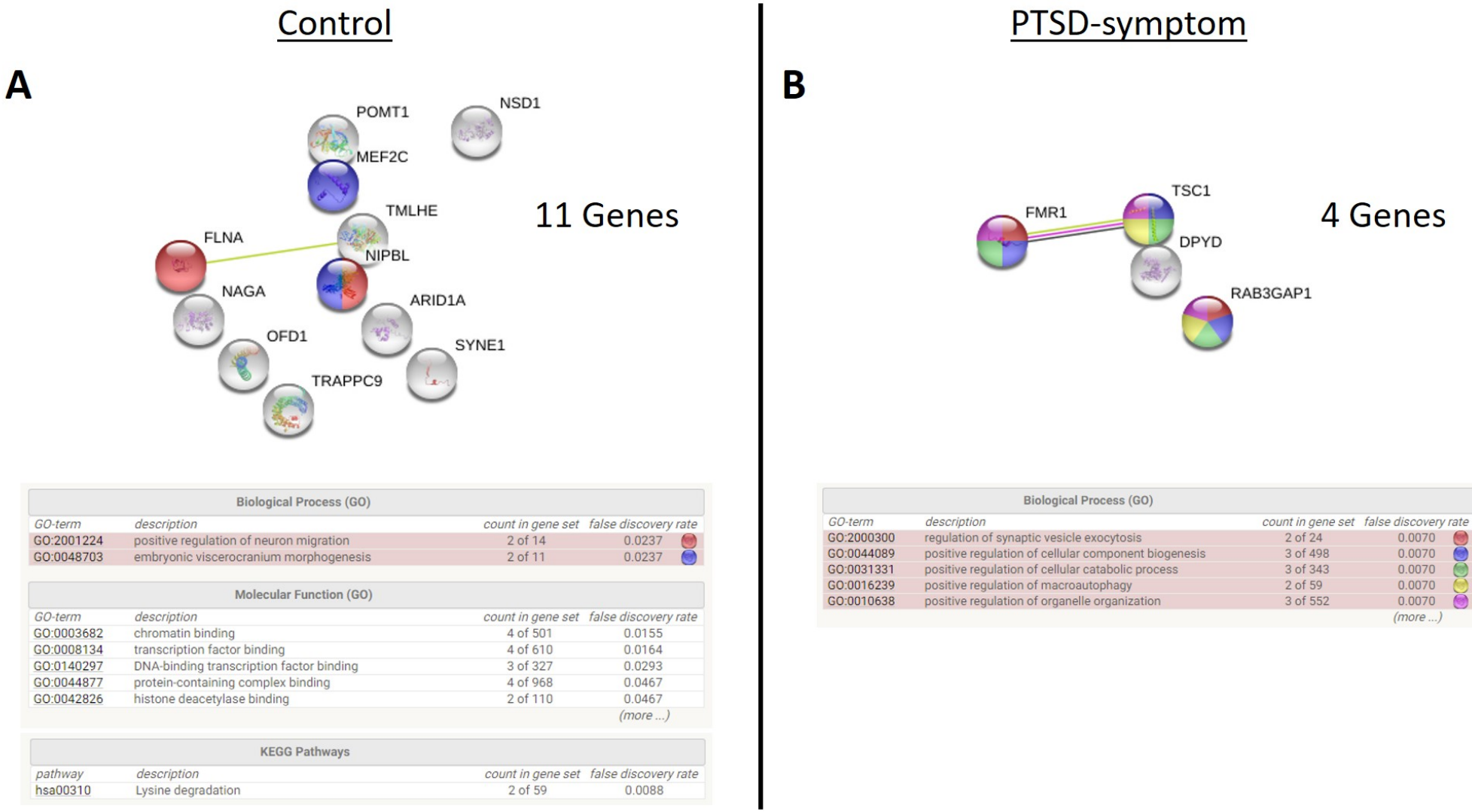
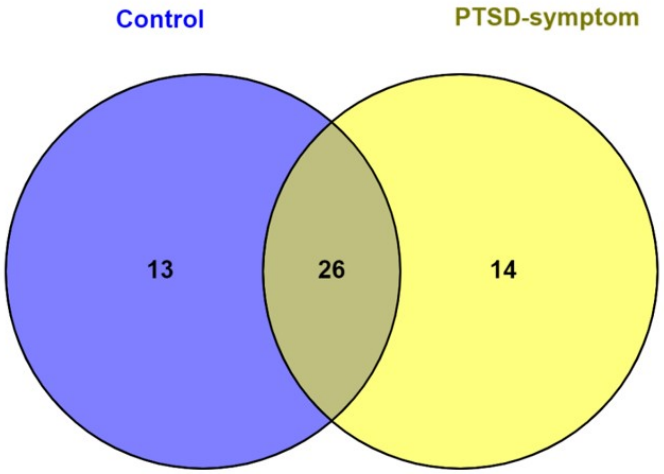


Figure S5. Mutated ID/ASD-related Genes in the Control and PTSD-symptom groups (Crossed with ID_ASD Database).

(A) STRING analysis was performed for 11 high impact ID/ASD-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 4 high impact ID/ASD-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0006954	inflammatory response	13 of 482	9.90e-12
GO:0006952	defense response	17 of 1234	9.90e-12
GO:0031347	regulation of defense response	12 of 676	8.20e-09
GO:0050727	regulation of inflammatory response	9 of 338	1.16e-07
GO:0080134	regulation of response to stress	13 of 1299	5.87e-07
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0097367	carbohydrate derivative binding	12 of 2163	0.0015
GO:0004525	ribonuclease III activity	2 of 3	0.0017
GO:0005041	low-density lipoprotein particle receptor activity	2 of 13	0.0090
GO:0043394	proteoglycan binding	2 of 32	0.0230
GO:0005540	hyaluronic acid binding	2 of 27	0.0230
			(more ...)

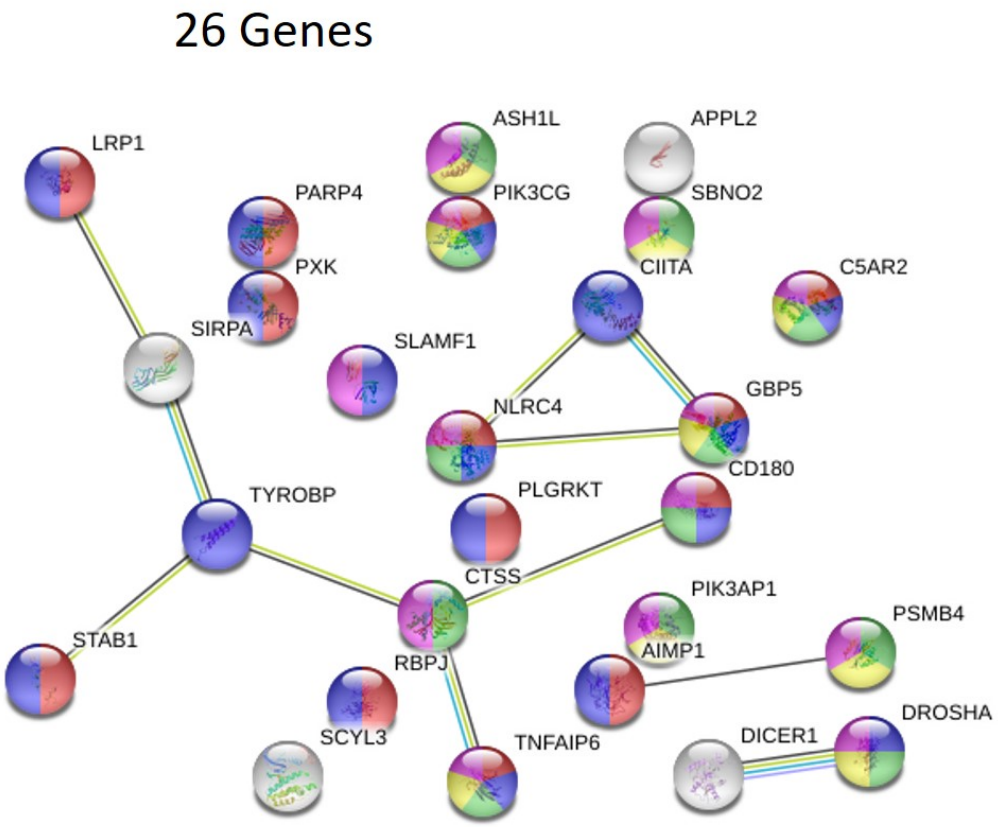


Figure S6. High Impact Shared Inflammation-related Genes Between Control and PTSD-symptom groups (Crossed with Inflammatory Response Database).

STRING analysis was performed for mutated Inflammation-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 26 high impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.

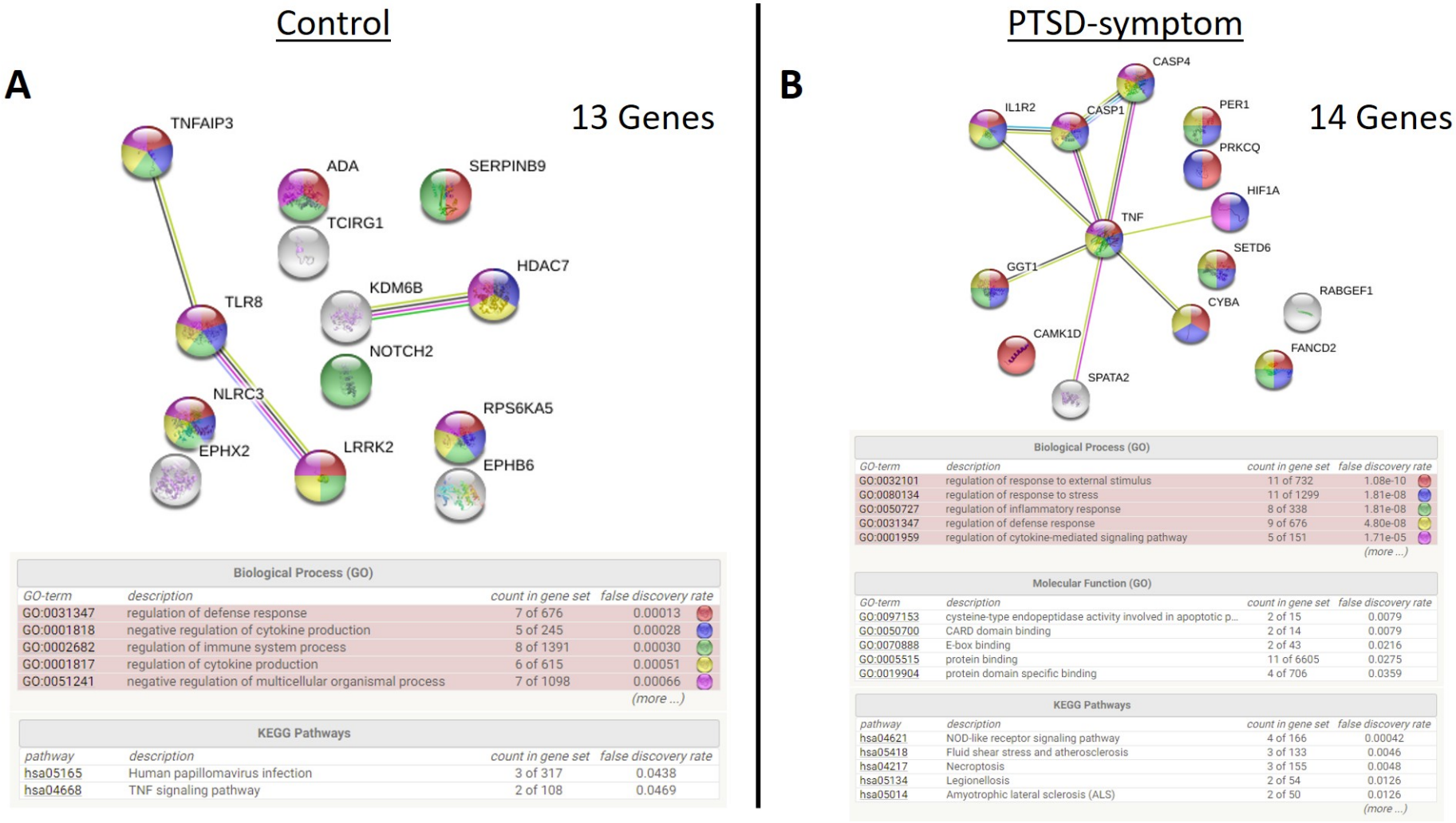
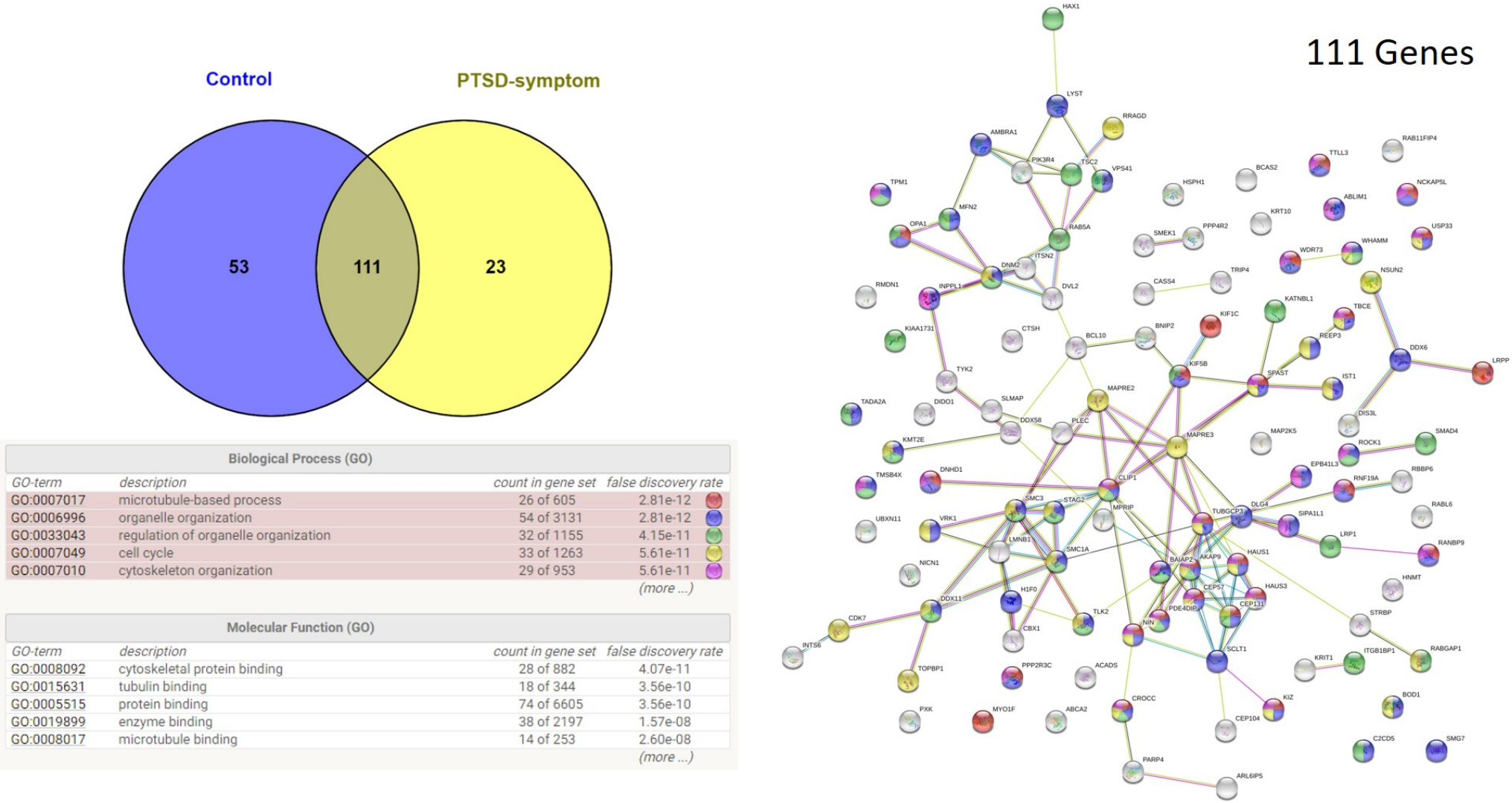


Figure S7. Mutated Inflammation-related Genes in the Control and PTSD-symptom groups (Crossed with Inflammatory Response Database).

(A) STRING analysis was performed for 13 high impact inflammation-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 14 high impact inflammation-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)

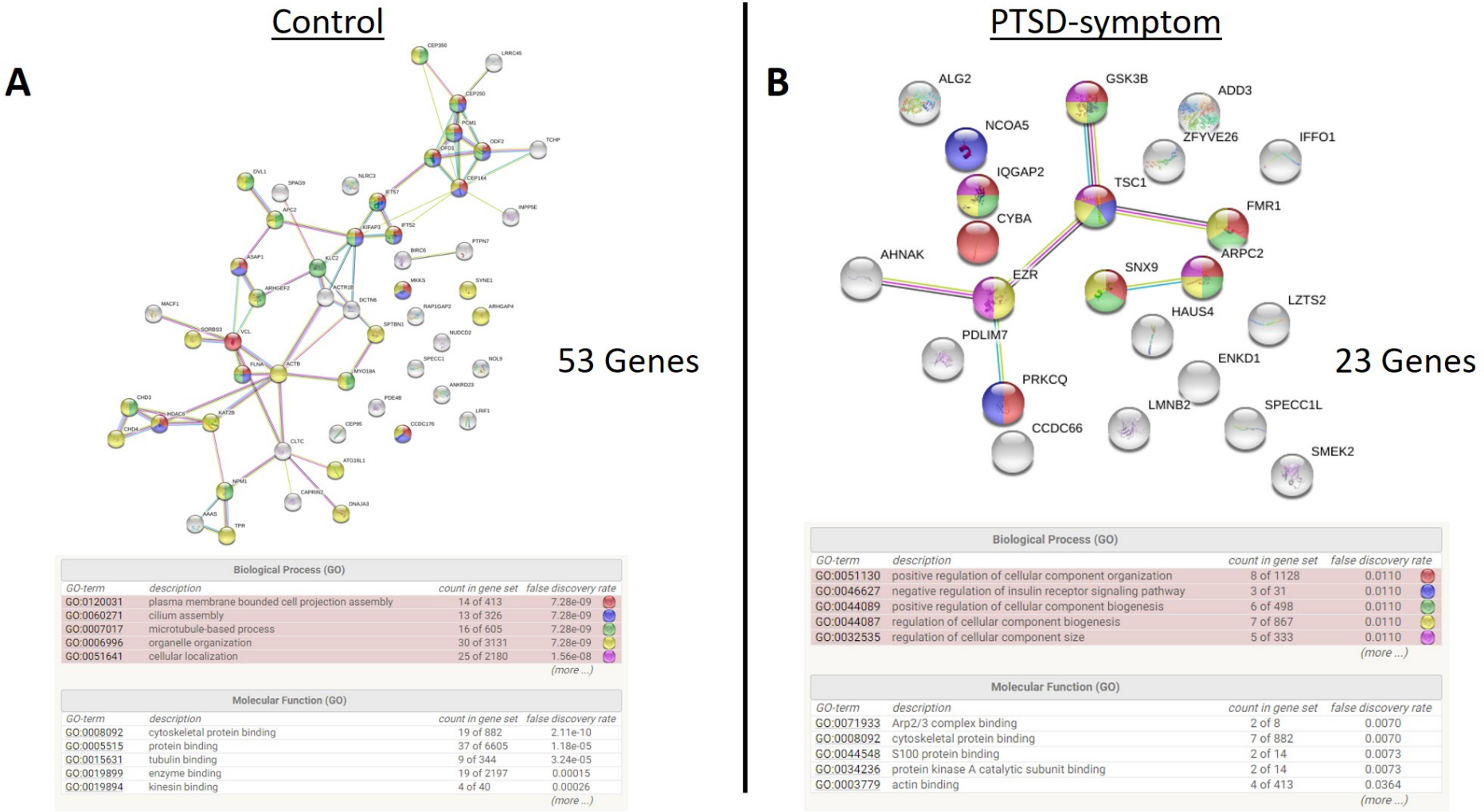
GO-term	description	count in gene set	false discovery rate
GO:0007017	microtubule-based process	26 of 605	2.81e-12
GO:0006996	organelle organization	54 of 3131	2.81e-12
GO:0033043	regulation of organelle organization	32 of 1155	4.15e-11
GO:0007049	cell cycle	33 of 1263	5.61e-11
GO:0007010	cytoskeleton organization	29 of 953	5.61e-11
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0008092	cytoskeletal protein binding	28 of 882	4.07e-11
GO:0015631	tubulin binding	18 of 344	3.56e-10
GO:0005515	protein binding	74 of 6605	3.56e-10
GO:0019899	enzyme binding	38 of 2197	1.57e-08
GO:0008017	microtubule binding	14 of 253	2.60e-08
			(more ...)

Figure S8. High Impact Shared Cytoskeleton-related Genes Between Control and PTSD-symptom groups (Crossed with Cytoskeleton Database).

STRING analysis was performed for mutated cytoskeleton-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 111 high impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0120031	plasma membrane bounded cell projection assembly	14 of 413	7.28e-09
GO:0060271	cilium assembly	13 of 326	7.28e-09
GO:0007017	microtubule-based process	16 of 605	7.28e-09
GO:0006996	organelle organization	30 of 3131	7.28e-09
GO:0051641	cellular localization	25 of 2180	1.56e-08
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0008092	cytoskeletal protein binding	19 of 882	2.11e-10
GO:0005515	protein binding	37 of 6605	1.18e-05
GO:0015631	tubulin binding	9 of 344	3.24e-05
GO:0019899	enzyme binding	19 of 2197	0.00015
GO:0019894	kinesin binding	4 of 40	0.00026
			(more ...)

Biological Process (GO)

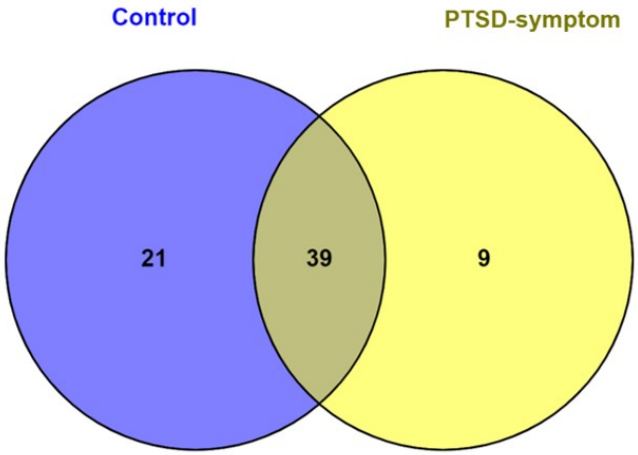
GO-term	description	count in gene set	false discovery rate
GO:0051130	positive regulation of cellular component organization	8 of 1128	0.0110
GO:0046627	negative regulation of insulin receptor signaling pathway	3 of 31	0.0110
GO:0044089	positive regulation of cellular component biogenesis	6 of 498	0.0110
GO:0044087	regulation of cellular component biogenesis	7 of 867	0.0110
GO:0032535	regulation of cellular component size	5 of 333	0.0110
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0071933	Arp2/3 complex binding	2 of 8	0.0070
GO:0008092	cytoskeletal protein binding	7 of 882	0.0070
GO:0044548	S100 protein binding	2 of 14	0.0073
GO:0034236	protein kinase A catalytic subunit binding	2 of 14	0.0073
GO:0003779	actin binding	4 of 413	0.0364
			(more ...)

Figure S9. Mutated Cytoskeleton-related Genes in the Control and PTSD-symptom groups (Crossed with Cytoskeleton Database).

(A) STRING analysis was performed for 53 high impact cytoskeleton-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 23 high impact cytoskeleton-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0006281	DNA repair	33 of 491	1.41e-43
GO:0006974	cellular response to DNA damage stimulus	34 of 749	3.68e-40
GO:0006259	DNA metabolic process	34 of 773	6.96e-40
GO:0090304	nucleic acid metabolic process	37 of 3941	2.18e-21
GO:0044260	cellular macromolecule metabolic process	36 of 6413	1.03e-12
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0008094	DNA-dependent ATPase activity	6 of 66	1.18e-06
GO:0140097	catalytic activity, acting on DNA	7 of 173	4.38e-06
GO:0003677	DNA binding	19 of 2457	4.38e-06
GO:0017111	nucleoside-triphosphatase activity	10 of 778	9.44e-05
GO:0003678	DNA helicase activity	4 of 41	9.44e-05
			(more ...)

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa03420	Nucleotide excision repair	7 of 46	2.62e-10
hsa03430	Mismatch repair	4 of 23	2.95e-06
hsa03460	Fanconi anemia pathway	4 of 51	3.67e-05
hsa03030	DNA replication	3 of 36	0.00041
hsa04110	Cell cycle	4 of 123	0.00058
			(more ...)

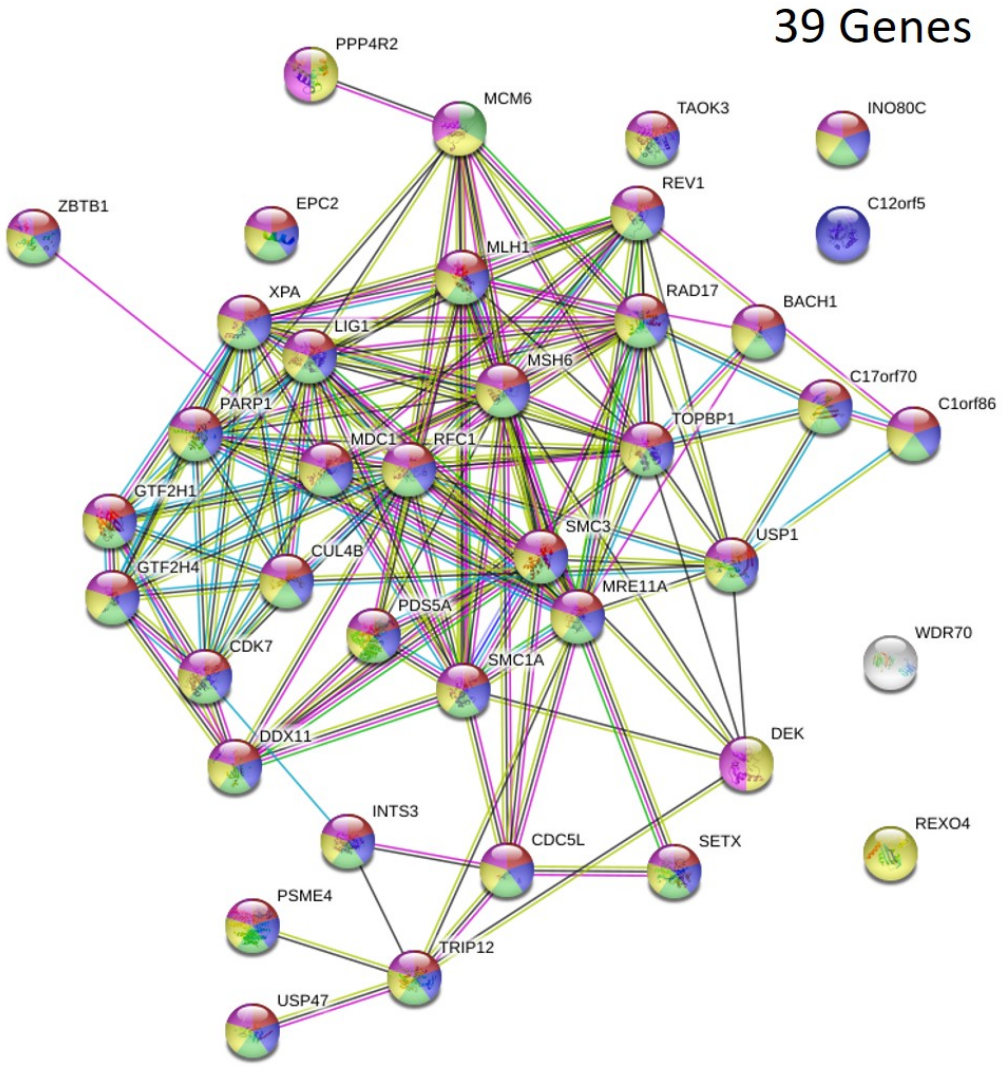
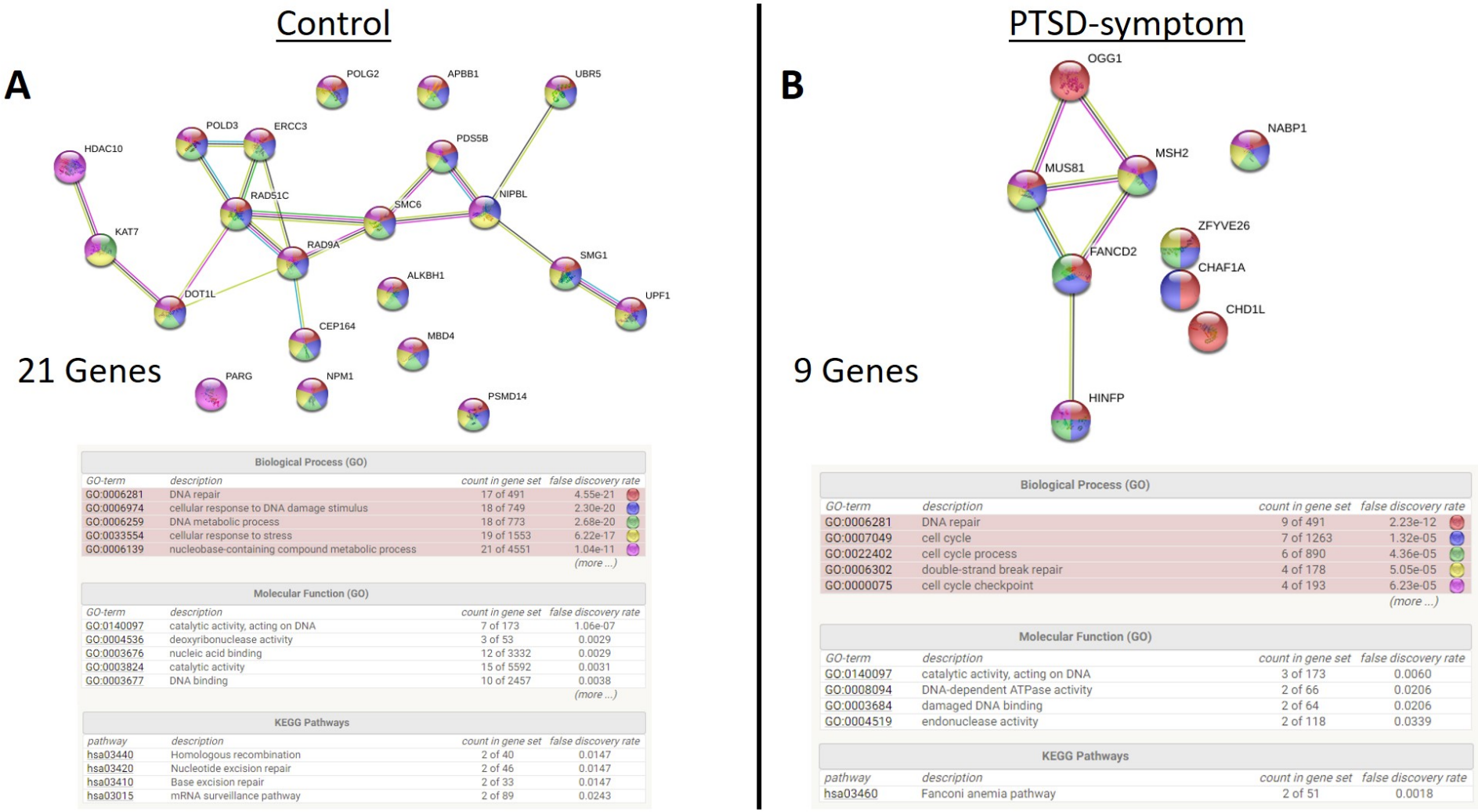


Figure S10. High Impact Shared DNA Repair-related Genes Between Control and PTSD-symptom groups (Crossed with DNA Repair Database).

STRING analysis was performed for mutated DNA repair-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 39 high impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0006281	DNA repair	17 of 491	4.55e-21
GO:0006974	cellular response to DNA damage stimulus	18 of 749	2.30e-20
GO:0006259	DNA metabolic process	18 of 773	2.68e-20
GO:0033554	cellular response to stress	19 of 1553	6.22e-17
GO:0006139	nucleobase-containing compound metabolic process	21 of 4551	1.04e-11

(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0140097	catalytic activity, acting on DNA	7 of 173	1.06e-07
GO:0004536	deoxyribonuclease activity	3 of 53	0.0029
GO:0003676	nucleic acid binding	12 of 3332	0.0029
GO:0003824	catalytic activity	15 of 5592	0.0031
GO:0003677	DNA binding	10 of 2457	0.0038

(more ...)

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa03440	Homologous recombination	2 of 40	0.0147
hsa03420	Nucleotide excision repair	2 of 46	0.0147
hsa03410	Base excision repair	2 of 33	0.0147
hsa03015	mRNA surveillance pathway	2 of 89	0.0243

Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0006281	DNA repair	9 of 491	2.23e-12
GO:0007049	cell cycle	7 of 1263	1.32e-05
GO:0022402	cell cycle process	6 of 890	4.36e-05
GO:0006302	double-strand break repair	4 of 178	5.05e-05
GO:0000075	cell cycle checkpoint	4 of 193	6.23e-05

(more ...)

Molecular Function (GO)

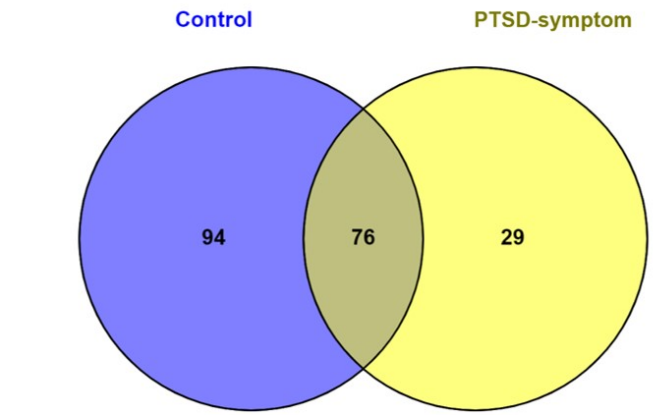
GO-term	description	count in gene set	false discovery rate
GO:0140097	catalytic activity, acting on DNA	3 of 173	0.0060
GO:0008094	DNA-dependent ATPase activity	2 of 66	0.0206
GO:0003684	damaged DNA binding	2 of 64	0.0206
GO:0004519	endonuclease activity	2 of 118	0.0339

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa03460	Fanconi anemia pathway	2 of 51	0.0018

Figure S11. Mutated DNA Repair-related Genes in the Control and PTSD-symptom groups (Crossed with DNA Repair Database).

(A) STRING analysis was performed for 21 high impact DNA repair-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 9 high impact DNA repair-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0051276	chromosome organization	22 of 999	1.63e-08
GO:0016570	histone modification	15 of 347	1.63e-08
GO:0006325	chromatin organization	19 of 683	1.63e-08
GO:0006996	organelle organization	35 of 3131	3.75e-07
GO:0051171	regulation of nitrogen compound metabolic process	45 of 5827	3.87e-05
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0018024	histone-lysine N-methyltransferase activity	5 of 40	0.00030
GO:0042605	peptide antigen binding	4 of 22	0.00041
GO:0019899	enzyme binding	23 of 2197	0.00041
GO:0005515	protein binding	45 of 6605	0.00041
GO:0005488	binding	65 of 11878	0.00041
			(more ...)

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa05332	Graft-versus-host disease	4 of 36	0.00069
hsa05330	Allograft rejection	4 of 35	0.00069
hsa05203	Viral carcinogenesis	7 of 183	0.00069
hsa05168	Herpes simplex infection	7 of 181	0.00069
hsa04940	Type I diabetes mellitus	4 of 40	0.00069
			(more ...)

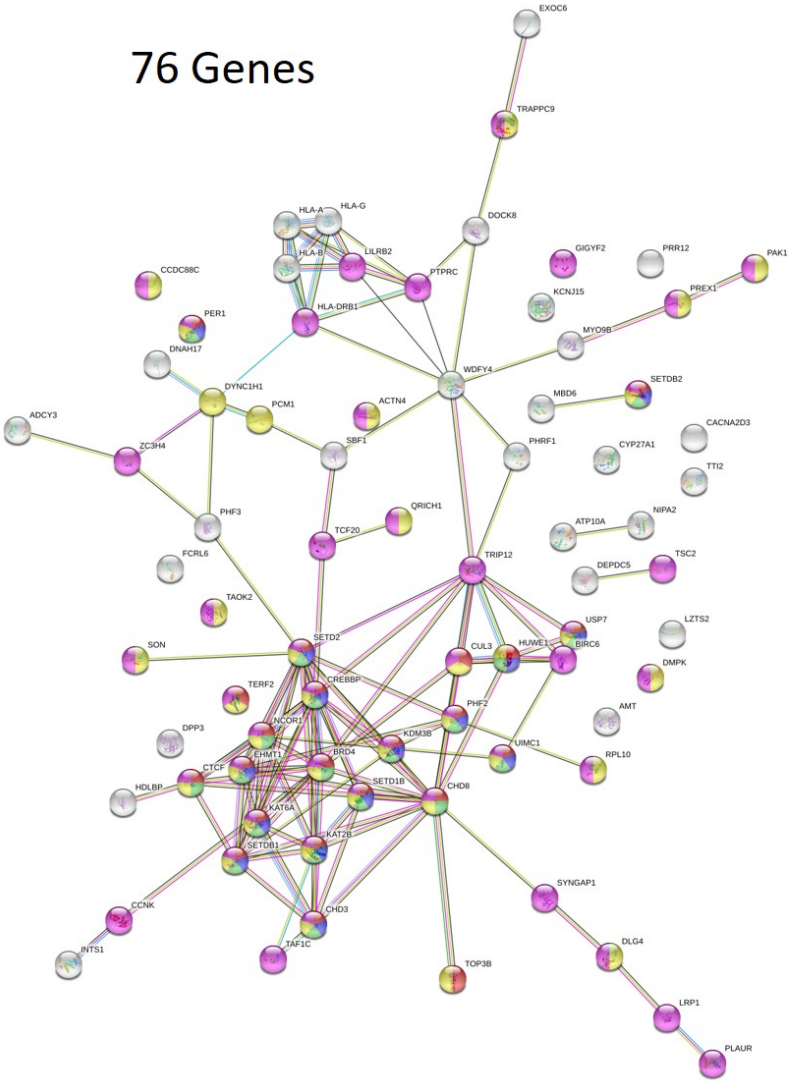


Figure S12. Moderate Impact Shared Autism-related Genes Between Control and PTSD-symptom groups (Crossed with SFARI Database).

STRING analysis was performed for mutated autism-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 76 moderate impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.

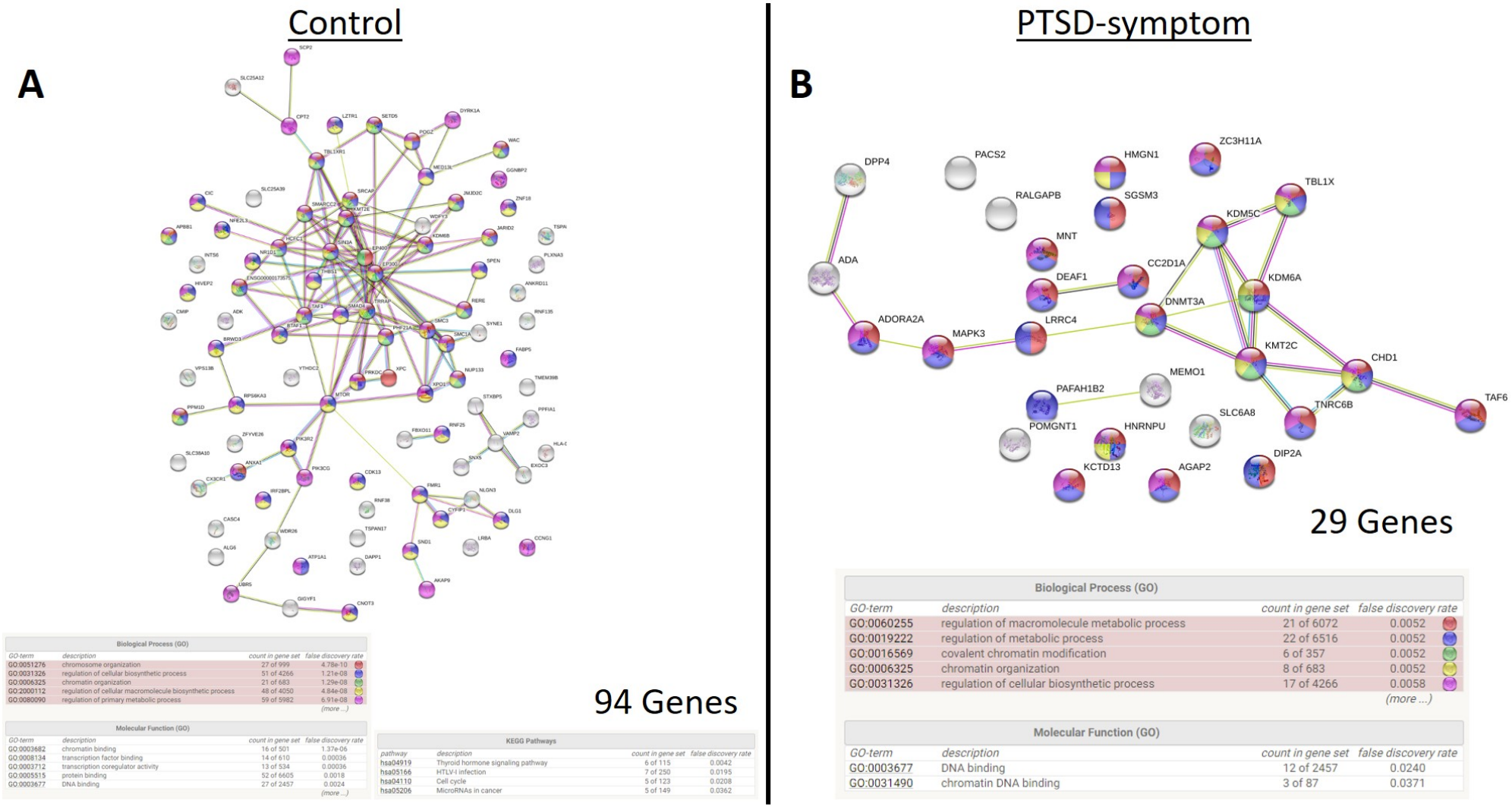
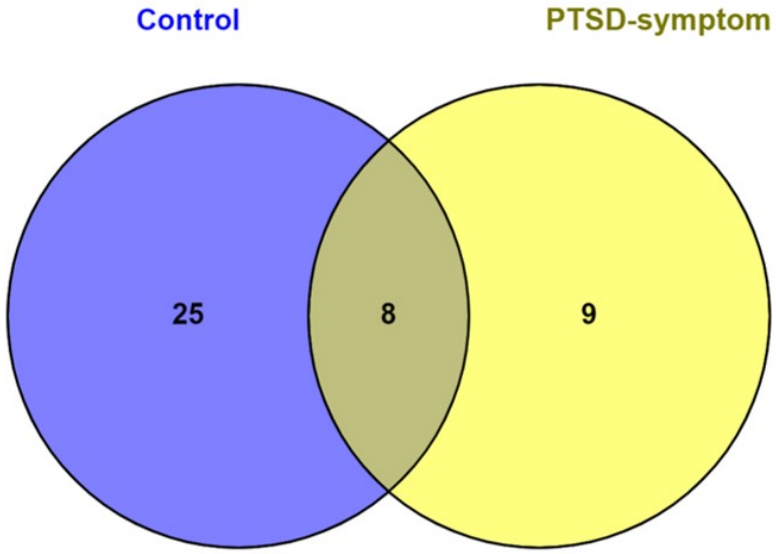
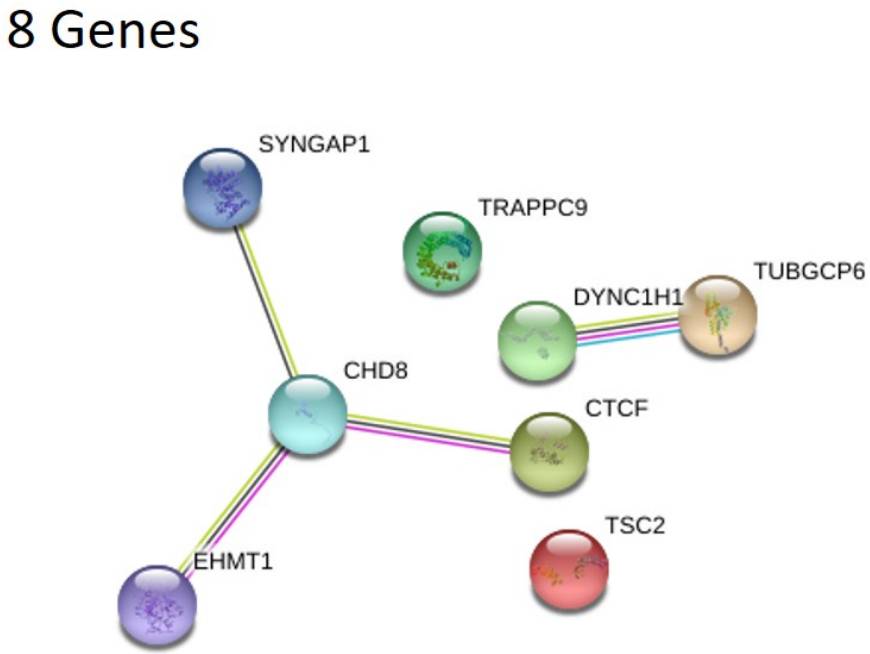


Figure S13. Mutated Autism-related Genes in the Control and PTSD-symptom groups (Crossed with SFARI Database).

(A) STRING analysis was performed for 94 moderate impact autism-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 29 moderate impact autism-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.





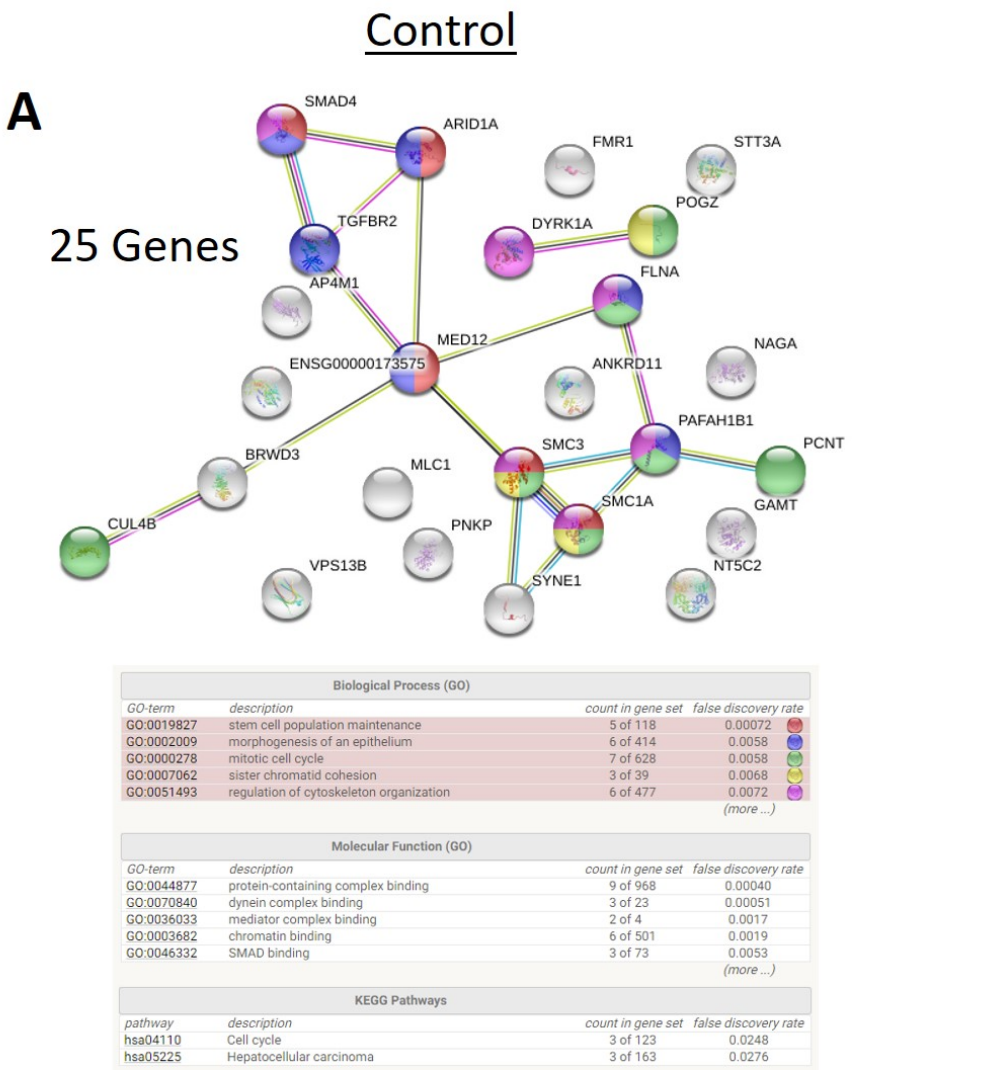
No Biological Processes

Molecular Function (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0002039	p53 binding	2 of 73	0.0428

KEGG Pathways			
pathway	*description*	*count in gene set*	*false discovery rate*
hsa04211	Longevity regulating pathway	2 of 88	0.0109

Figure S14. Moderate Impact Shared ID/ASD-related Genes Between Control and PTSD-symptom groups (Crossed with ID_ASD Database).

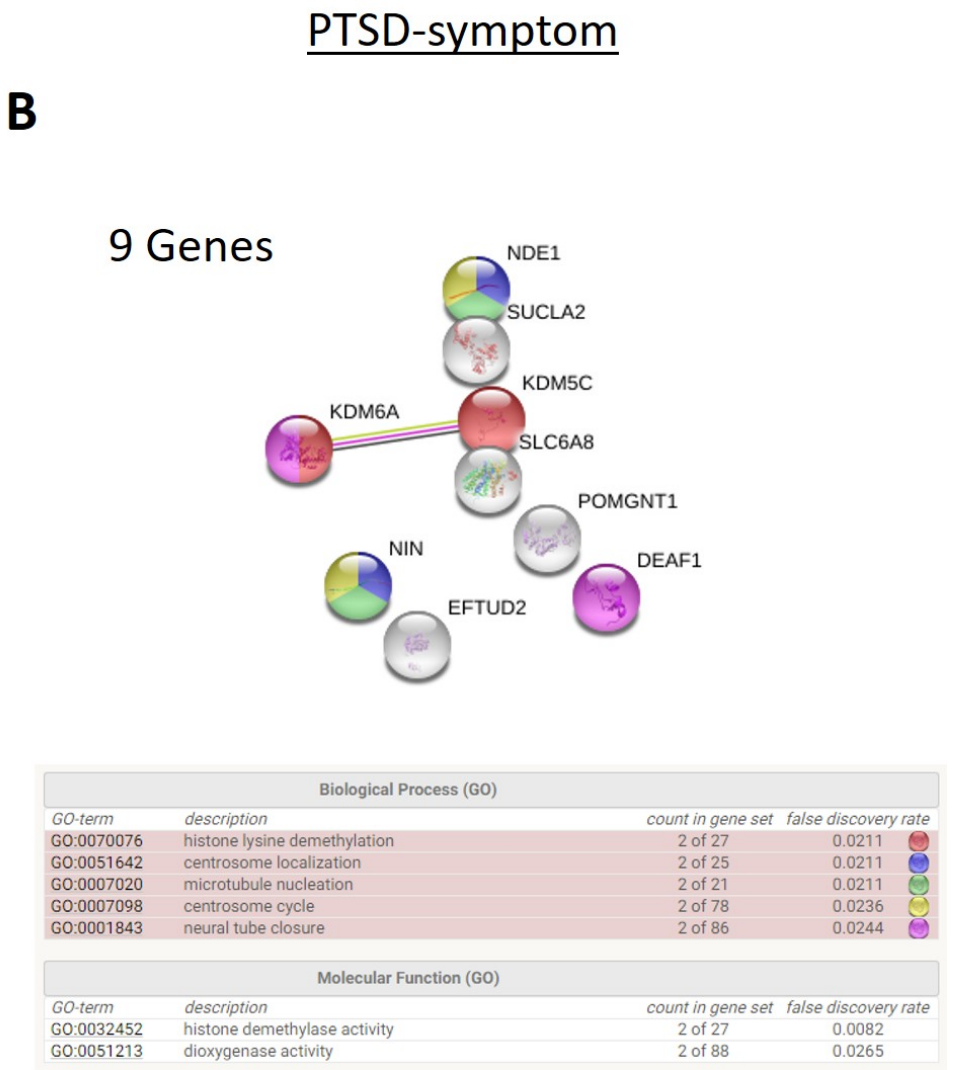
STRING analysis was performed for mutated ID/ASD-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 8 moderate impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.



Biological Process (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0019827	stem cell population maintenance	5 of 118	0.00072
GO:0002009	morphogenesis of an epithelium	6 of 414	0.0058
GO:0000278	mitotic cell cycle	7 of 628	0.0058
GO:0007062	sister chromatid cohesion	3 of 39	0.0068
GO:0051493	regulation of cytoskeleton organization	6 of 477	0.0072
			(more ...)

Molecular Function (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0044877	protein-containing complex binding	9 of 968	0.00040
GO:0070840	dynein complex binding	3 of 23	0.00051
GO:0036033	mediator complex binding	2 of 4	0.0017
GO:0003682	chromatin binding	6 of 501	0.0019
GO:0046332	SMAD binding	3 of 73	0.0053
			(more ...)

KEGG Pathways			
pathway	*description*	*count in gene set*	*false discovery rate*
hsa04110	Cell cycle	3 of 123	0.0248
hsa05225	Hepatocellular carcinoma	3 of 163	0.0276

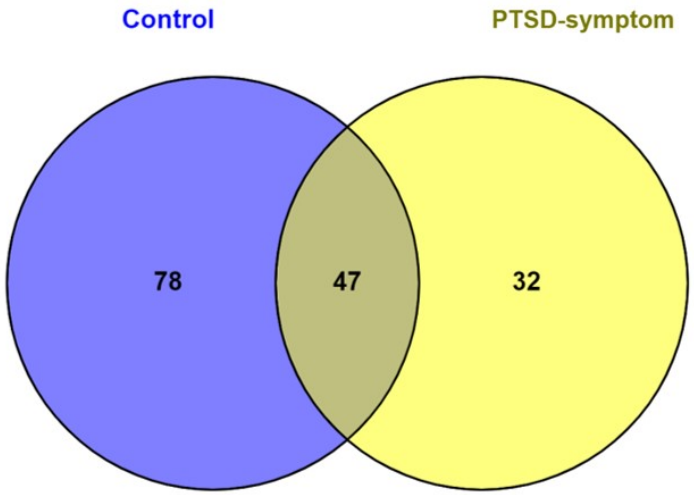


Biological Process (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0070076	histone lysine demethylation	2 of 27	0.0211
GO:0051642	centrosome localization	2 of 25	0.0211
GO:0007020	microtubule nucleation	2 of 21	0.0211
GO:0007098	centrosome cycle	2 of 78	0.0236
GO:0001843	neural tube closure	2 of 86	0.0244

Molecular Function (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0032452	histone demethylase activity	2 of 27	0.0082
GO:0051213	dioxygenase activity	2 of 88	0.0265

Figure S15. Mutated ID/ASD-related Genes in the Control and PTSD-symptom groups (Crossed with ID_ASD Database).

(A) STRING analysis was performed for 25 moderate impact ID/ASD-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 9 moderate impact ID/ASD-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0006954	inflammatory response	28 of 482	9.80e-30
GO:0006952	defense response	31 of 1234	2.35e-23
GO:0032101	regulation of response to external stimulus	23 of 732	6.68e-18
GO:0050727	regulation of inflammatory response	18 of 338	3.54e-17
GO:0031347	regulation of defense response	21 of 676	4.40e-16
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0005515	protein binding	29 of 6605	0.0151
GO:0070851	growth factor receptor binding	4 of 131	0.0414
GO:0005149	interleukin-1 receptor binding	2 of 18	0.0414
GO:0005126	cytokine receptor binding	5 of 272	0.0414
GO:0005102	signaling receptor binding	11 of 1513	0.0414
			(more ...)

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa04621	NOD-like receptor signaling pathway	6 of 166	0.00041
hsa05221	Acute myeloid leukemia	4 of 66	0.0015
hsa04658	Th1 and Th2 cell differentiation	4 of 88	0.0029
hsa05165	Human papillomavirus infection	6 of 317	0.0036
hsa04620	Toll-like receptor signaling pathway	4 of 102	0.0036
			(more ...)

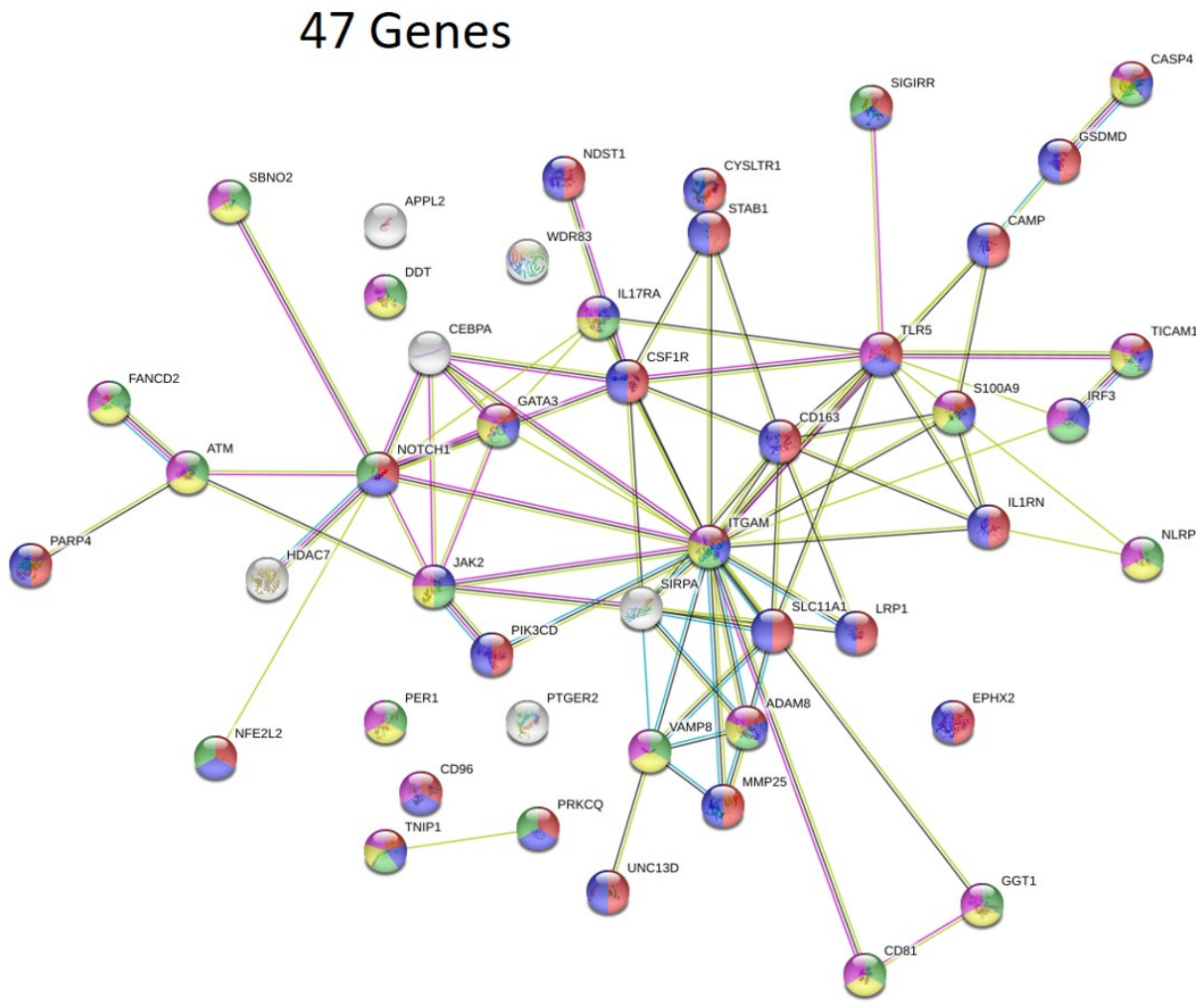


Figure S16. Moderate Impact Shared Inflammation-related Genes Between Control and PTSD-symptom groups (Crossed with Inflammatory Response Database).

STRING analysis was performed for mutated Inflammation-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 47 moderate impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.

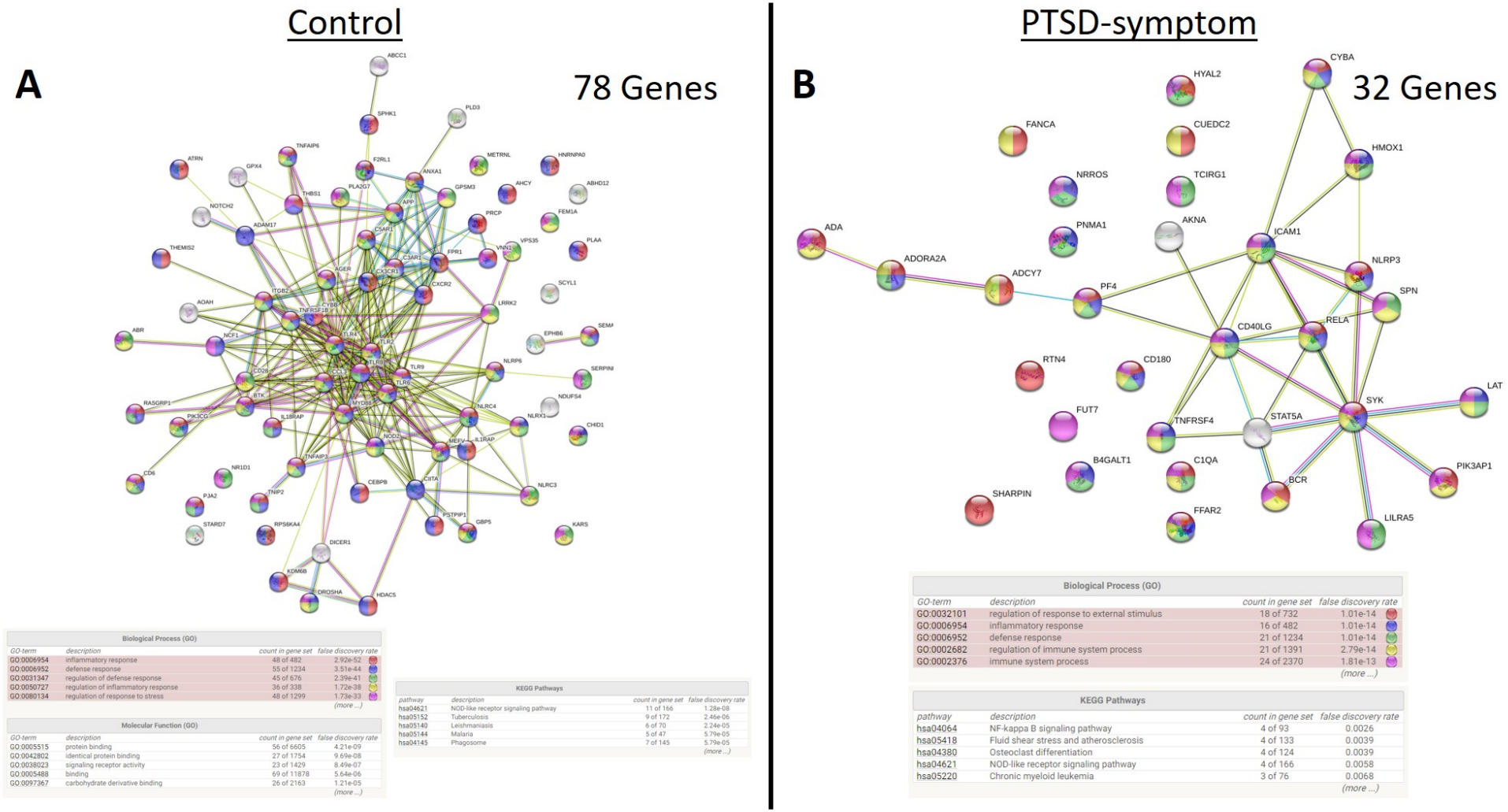
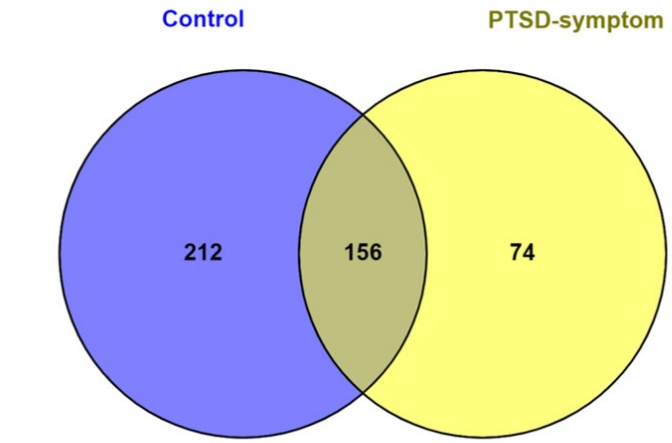


Figure S17. Mutated Inflammation-related Genes in the Control and PTSD-symptom groups (Crossed with Inflammatory Response Database).

(A) STRING analysis was performed for 78 moderate impact inflammation-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 32 moderate impact inflammation-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)

GO-term	description	count in gene set	false discovery rate
GO:0007010	cytoskeleton organization	57 of 953	3.03e-30
GO:0006996	organelle organization	87 of 3131	2.88e-26
GO:0016043	cellular component organization	101 of 5163	2.38e-20
GO:0007017	microtubule-based process	34 of 605	4.00e-16
GO:0030029	actin filament-based process	30 of 493	6.57e-15
			(more ...)

Molecular Function (GO)

GO-term	description	count in gene set	false discovery rate
GO:0008092	cytoskeletal protein binding	56 of 882	1.10e-31
GO:0005515	protein binding	123 of 6605	4.62e-28
GO:0003779	actin binding	34 of 413	1.24e-21
GO:0005488	binding	140 of 11878	7.62e-14
GO:0015631	tubulin binding	24 of 344	1.81e-13
			(more ...)

KEGG Pathways

pathway	description	count in gene set	false discovery rate
hsa05203	Viral carcinogenesis	9 of 183	0.0018
hsa04666	Fc gamma R-mediated phagocytosis	7 of 89	0.0018
hsa04810	Regulation of actin cytoskeleton	9 of 205	0.0028
hsa04140	Autophagy - animal	6 of 125	0.0247
hsa05165	Human papillomavirus infection	9 of 317	0.0377

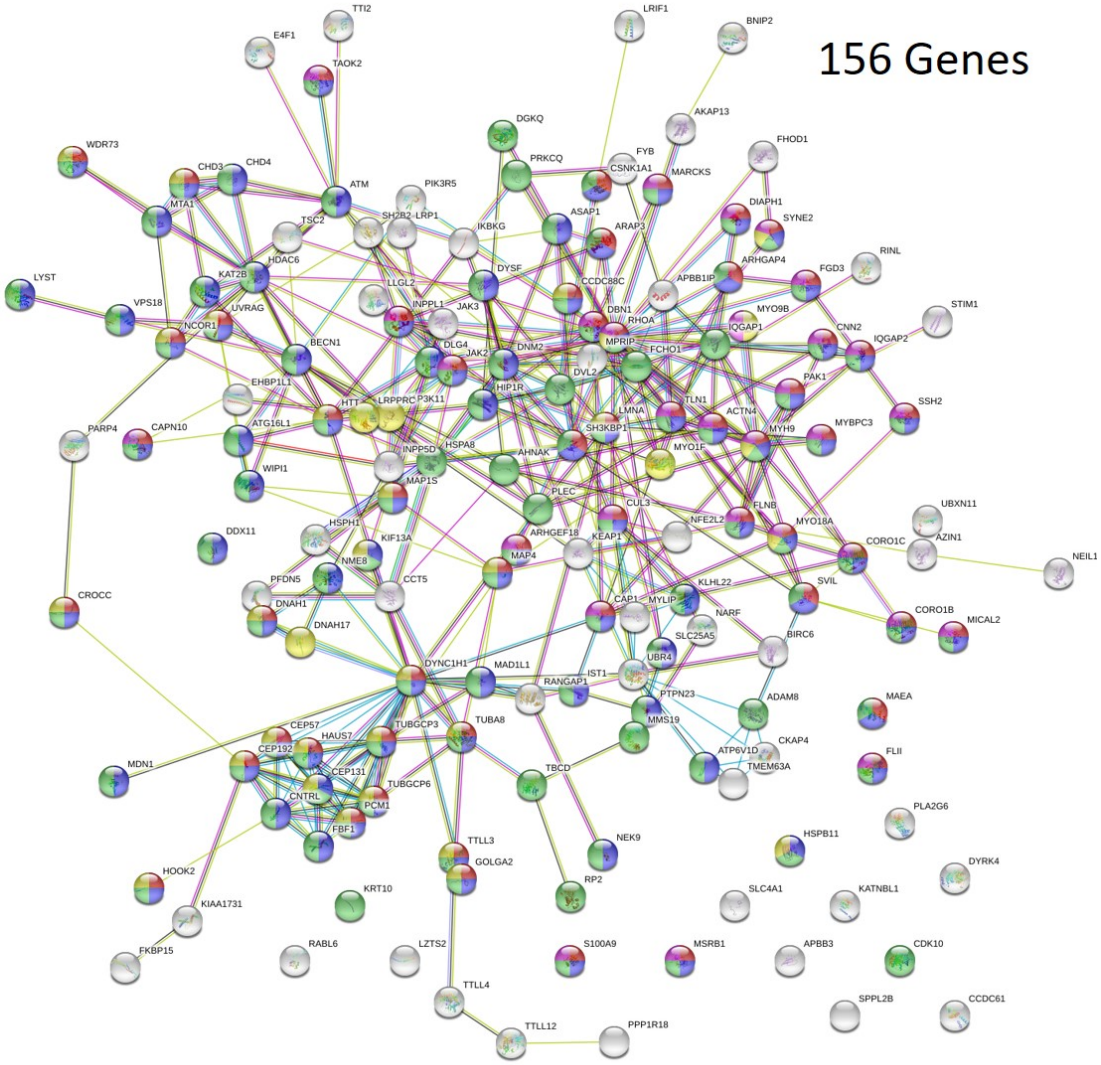


Figure S18. Moderate Impact Shared Cytoskeleton-related Genes Between Control and PTSD-symptom groups (Crossed with Cytoskeleton Database).

STRING analysis was performed for mutated cytoskeleton-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 156 moderate impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.

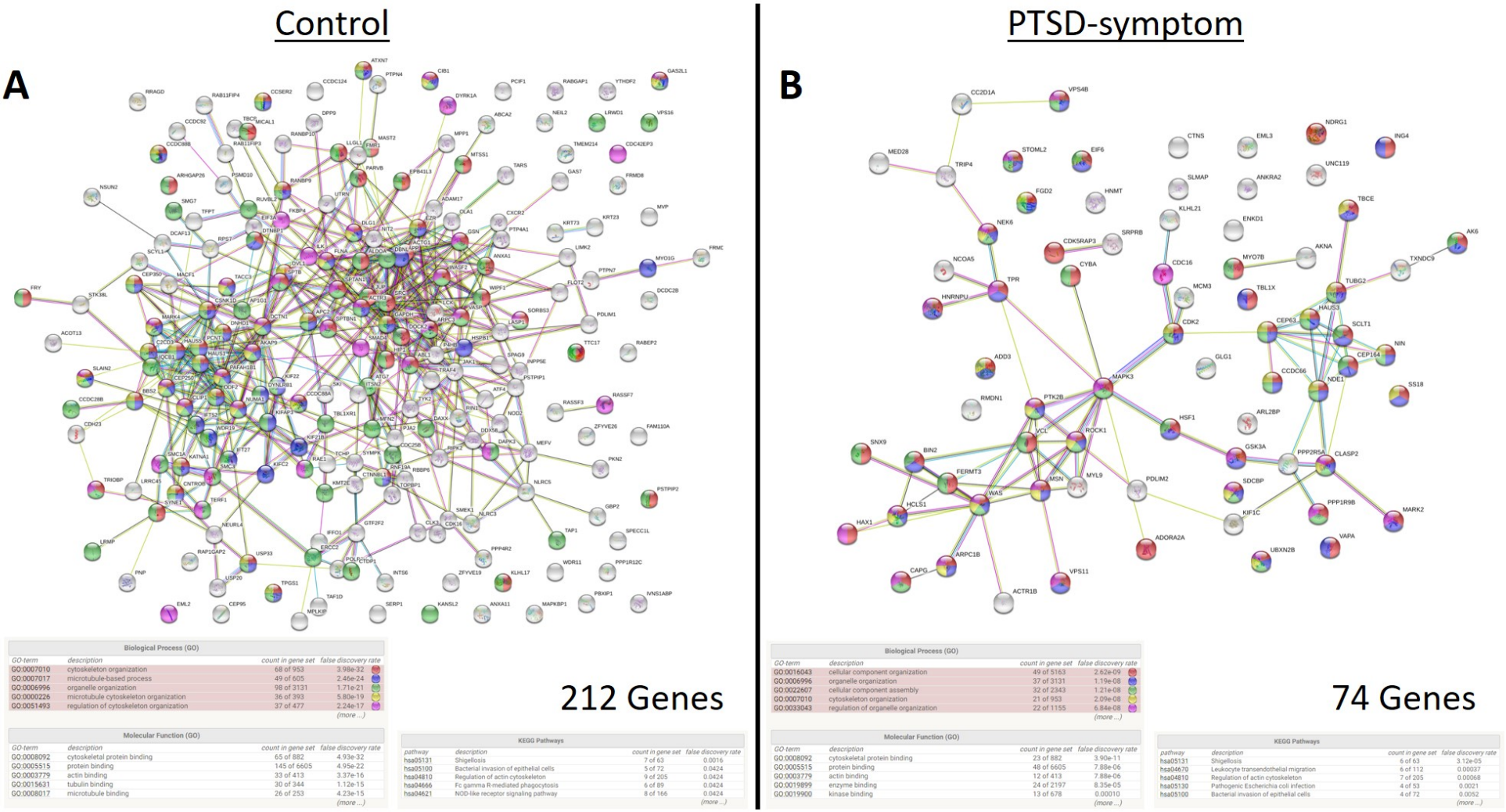
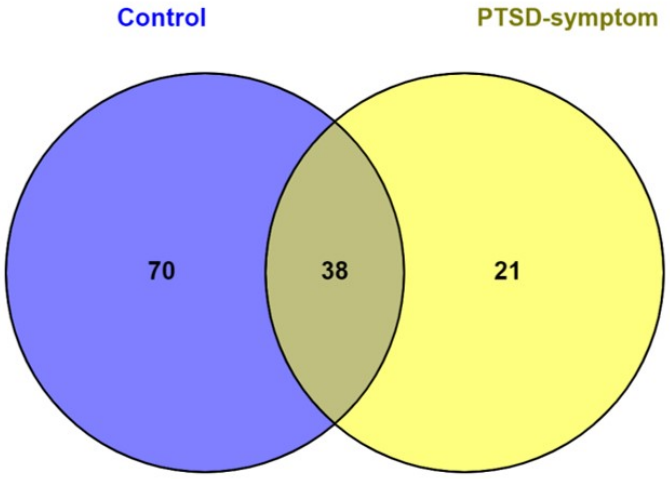


Figure S19. Mutated Cytoskeleton-related Genes in the Control and PTSD-symptom groups (Crossed with Cytoskeleton Database).

(A) STRING analysis was performed for 212 moderate impact cytoskeleton-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 74 moderate impact cytoskeleton-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.



Biological Process (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0006281	DNA repair	32 of 491	5.53e-42
GO:0006974	cellular response to DNA damage stimulus	34 of 749	6.09e-41
GO:0006259	DNA metabolic process	34 of 773	1.15e-40
GO:0090304	nucleic acid metabolic process	36 of 3941	1.26e-20
GO:0006302	double-strand break repair	12 of 178	1.21e-13
			(more ...)

Molecular Function (GO)			
GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0140097	catalytic activity, acting on DNA	11 of 173	8.72e-12
GO:0003677	DNA binding	23 of 2457	5.67e-10
GO:0004518	nuclease activity	7 of 192	5.92e-06
GO:0003684	damaged DNA binding	5 of 64	1.05e-05
GO:0004527	exonuclease activity	5 of 72	1.52e-05
			(more ...)

KEGG Pathways			
pathway	*description*	*count in gene set*	*false discovery rate*
hsa03410	Base excision repair	4 of 33	2.72e-05
hsa03440	Homologous recombination	4 of 40	2.77e-05
hsa03420	Nucleotide excision repair	4 of 46	3.11e-05
hsa03460	Fanconi anemia pathway	4 of 51	3.43e-05
hsa04120	Ubiquitin mediated proteolysis	5 of 134	4.99e-05
			(more ...)

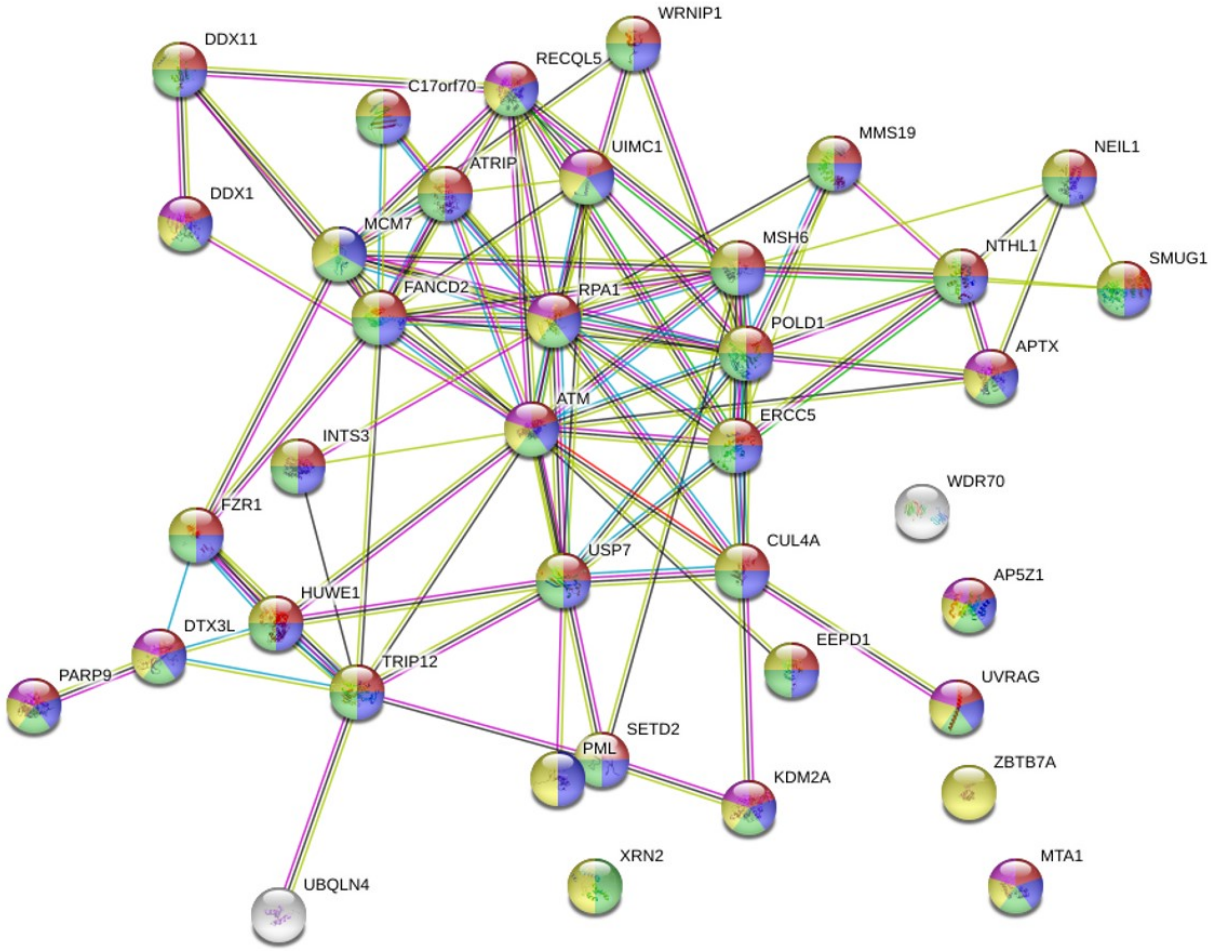


Figure S20. Moderate Impact Shared DNA Repair-related Genes Between Control and PTSD-symptom groups (Crossed with DNA Repair Database).

STRING analysis was performed for mutated DNA repair-related genes in the control and PTSD-symptom groups, as identified by a Venn diagram, and shown for 38 moderate impact genes shared between control and PTSD-symptom groups. Enriched biological processes, molecular functions and pathways are presented for these genes.

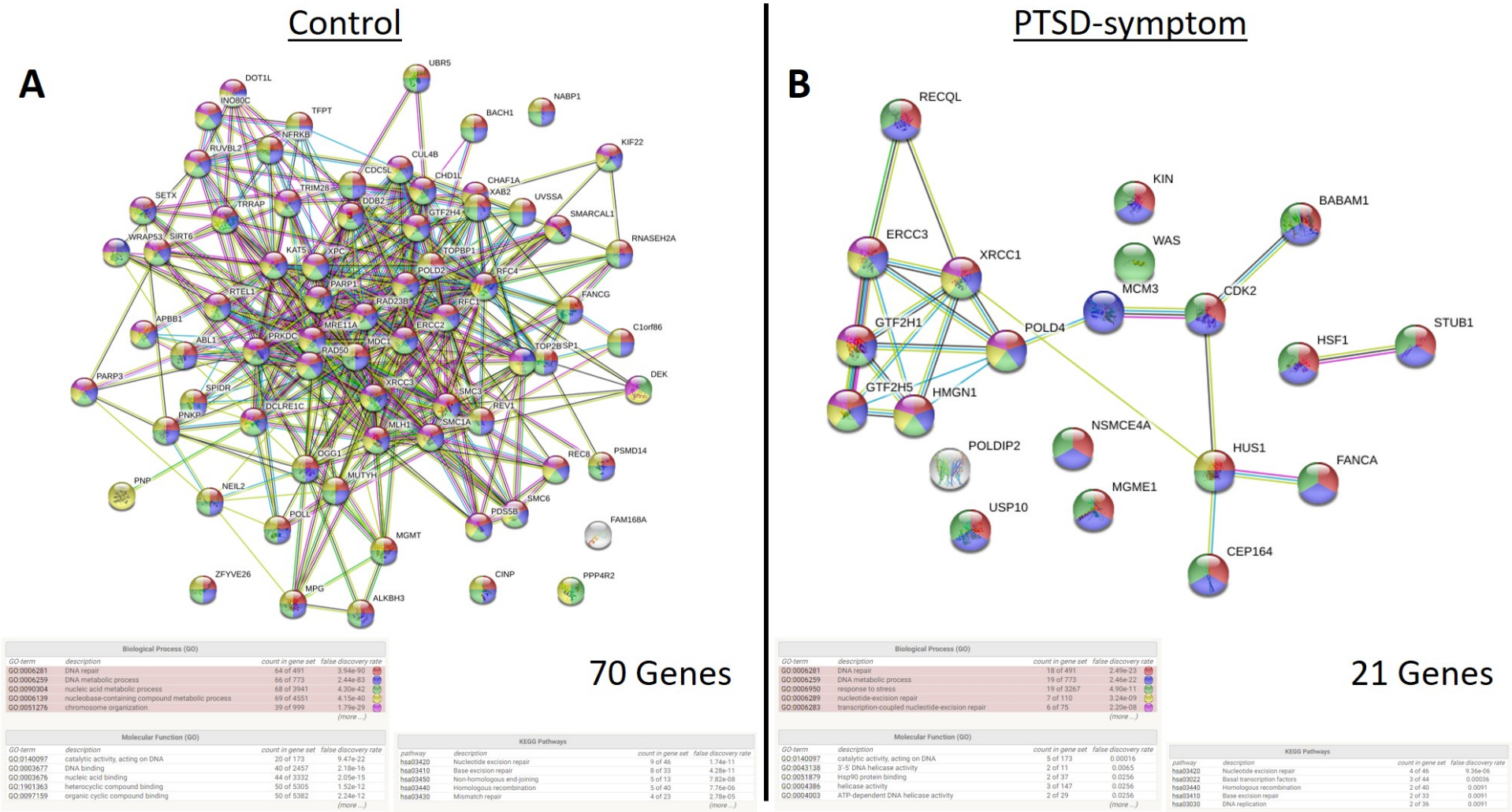


Figure S21. Mutated DNA Repair-related Genes in the Control and PTSD-symptom groups (Crossed with DNA Repair Database).

(A) STRING analysis was performed for 70 moderate impact DNA repair-related mutated genes in the control group, as identified by a Venn diagram. **(B)** STRING analysis was performed for 21 moderate impact DNA repair-related mutated genes in the PTSD-symptom group, as identified by a Venn diagram. For both groups, enriched biological processes, molecular functions and pathways are presented for the genes.