Priority Communication

Biological Psychiatry

Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles

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ABSTRACT

BACKGROUND: Examining transcriptional regulation by antidepressants in key neural circuits implicated in depression and understanding the relation to transcriptional mechanisms of susceptibility and natural resilience may help in the search for new therapeutic agents. Given the heterogeneity of treatment response in human populations, examining both treatment response and nonresponse is critical.

METHODS: We compared the effects of a conventional monoamine-based tricyclic antidepressant, imipramine, and a rapidly acting, non–monoamine-based antidepressant, ketamine, in mice subjected to chronic social defeat stress, a validated depression model, and used RNA sequencing to analyze transcriptional profiles associated with susceptibility, resilience, and antidepressant response and nonresponse in the prefrontal cortex (PFC), nucleus accumbens, hippocampus, and amygdala.

RESULTS: We identified similar numbers of responders and nonresponders after ketamine or imipramine treatment. Ketamine induced more expression changes in the hippocampus; imipramine induced more expression changes in the nucleus accumbens and amygdala. Transcriptional profiles in treatment responders were most similar in the PFC. Nonresponse reflected both the lack of response-associated gene expression changes and unique gene regulation. In responders, both drugs reversed susceptibility-associated transcriptional changes and induced resilience-associated transcription in the PFC.

CONCLUSIONS: We generated a uniquely large resource of gene expression data in four interconnected limbic brain regions implicated in depression and its treatment with imipramine or ketamine. Our analyses highlight the PFC as a key site of common transcriptional regulation by antidepressant drugs and in both reversing susceptibility– and inducing resilience–associated molecular adaptations. In addition, we found region-specific effects of each drug, suggesting both common and unique effects of imipramine versus ketamine.

Keywords: Depression, Imipramine, Ketamine, Resilience, RNA-seq, Susceptibility

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Depression is a complex and heterogeneous disorder and a leading cause of disability worldwide, yet existing pharmacotherapies have limited efficacy (1). Virtually all drugs used to treat depression today target the same basic mechanisms identified more than 60 years ago, inducing full remission in fewer than 50% of affected individuals (2). Earlier treatments, such as tricyclic antidepressants (e.g., imipramine), target multiple neurotransmitter systems. Specifically, imipramine inhibits reuptake of serotonin and norepinephrine (thought to mediate its therapeutic actions) and influences numerous monoaminergic and cholinergic receptors. More recently developed antidepressants have greater selectivity at inhibiting serotonin and/or norepinephrine transporters but have roughly the same intrinsic efficacy as older tricyclic medications. Moreover, the therapeutic actions of both tricyclics and more selective reuptake inhibitors require weeks or months of treatment. Although the initial target of these drugs is known, the slowly developing drug-induced adaptations that mediate antidepressant outcomes remain unknown (3,4). There is a great unmet need to develop more effective and more rapidly acting treatments for depression, ideally guided by an improved understanding of the pathophysiology of the syndrome.

Several groups have shown that ketamine, a dissociative anesthetic, induces rapid antidepressant effects in approximately 50% of patients who are resistant to available tricyclic and

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reuptake inhibitor antidepressants (5,6). Although ketamine's mechanism of action as a noncompetitive *N*-methyl-D-aspartate glutamate receptor antagonist has been studied with regard to its anesthetic and recreational use at high doses, the functional and molecular underpinnings of ketamine's antidepressant action at lower doses are a matter of ongoing study, with several attractive models of altered synaptic and structural changes proposed (7–9). Unbiased genomewide transcriptional profiling may shed new light on the molecular mechanisms targeted by both established and experimental pharmacotherapies, thereby facilitating the development of novel antidepressant treatments.

A key challenge in understanding the mechanism of action of existing pharmacotherapies for depression is to identify the brain regions in which antidepressant treatments exert their effects. Neuroimaging studies of depressed patients, and findings in animal models, show that depression is a circuit-level disorder in which several functionally interconnected brain regions are affected (10–13). One involved circuit is the highly studied corticomesolimbic reward system consisting of several limbic brain regions, including the nucleus accumbens (NAC), prefrontal cortex (PFC), hippocampus (HIP), and amygdala (AMY). The NAC integrates information from glutamatergic inputs from the PFC, AMY, and HIP, among other regions (14). Structural, functional, and transcriptional changes in each of these brain regions have been reported in both rodent depression models and depressed humans (12,15–25). Thus, examining how antidepressant drugs regulate transcriptional profiles in these functionally interconnected brain regions may offer important mechanistic insights into their therapeutic actions.

In studying the mechanism of action of antidepressant drugs, it is important to address both the individual receiving the treatment and the heterogeneity of treatment response. Antidepressants do not elevate mood in nondepressed individuals, suggesting that unique responses may occur in depressed patients. Likewise, analyzing drug-induced transcriptional changes in both responders and nonresponders may be particularly informative in distinguishing drug-induced therapeutic changes from off-target effects. A key question is whether the lack of response reflects simply the lack of drug-induced therapeutic changes or induction of aberrant transcriptional programs that mask antidepressant actions.

Here, we compared imipramine and ketamine action in mice subjected to chronic social defeat stress (CSDS), an ethologically validated model of depression and social stress-related disorders (26,27). Chronic, but not acute, administration of imipramine or other standard antidepressants has been shown to reverse a range of behavioral abnormalities in roughly 60% of mice (26,28). Recently, single doses of ketamine were shown to induce roughly equivalent treatment responses (29). We used RNA-sequencing (RNA-seq) to characterize transcriptomic responses genomewide to either chronic imipramine or acute ketamine within the limbic circuitry noted above: NAC, PFC, HIP, and AMY. Our findings demonstrate fundamental differences in the molecular and brain region targets of these two medications in responders and nonresponders, results that have important implications for antidepressant drug discovery efforts.

METHODS AND MATERIALS

More information is available in the Supplement.

CSDS, Behavioral Testing, and Drug Treatment

An established CSDS protocol was used to induce depressive-like behaviors in mice (26,27). C57BL/6J mice were subjected to 10 daily, 5-minute defeats by a novel CD1 aggressor, and social avoidance behavior was assessed in a two-stage social interaction (SI) test 24 hours after the final defeat. In the first 2.5-minute test (no target), the experimental mouse was allowed to freely explore an arena containing a plexiglass and wire mesh enclosure centered against one wall of the arena. In the second 2.5-minute test (target), the experimental mouse was returned to the arena with a novel CD1 mouse enclosed in the plexiglass wire mesh cage. Time spent in the interaction zone (IZ) surrounding the enclosure was measured. Resilient mice spent more time in the IZ in target than no target, and total time in the IZ in target was >60 seconds. Susceptible mice spent less time in the IZ with target than with no target, and total time in the IZ in target was <60 seconds.

Susceptible mice were treated with either saline, ketamine, or imipramine. Twenty-four hours after the final injection, mice were subjected to a second SI test (SI2). Mice were defined as responders to imipramine or ketamine treatment if they spent more time in the IZ in target after antidepressant treatment and had an increase of >20 seconds in the IZ in target from SI1 to SI2. Mice were defined as nonresponders if they spent less time in the IZ in target after treatment or had an increase of <10 seconds in the IZ in target from SI1 to SI2. Saline-treated resilient and susceptible animals were included in transcriptome-wide analyses if they continued to meet the SI1 criteria in SI2. All control animals were included in downstream analysis.

RNA Isolation, Library Preparation, and RNA Sequencing

Mice were killed 2 days after SI2, and NAC, PFC, HIP, and AMY tissues were rapidly dissected and frozen on dry ice. Tissue from two mice were pooled for each sample for three to five biological replicates for each brain region and phenotype. RNA isolation, quantitative real-time polymerase chain reaction, and data analyses were performed as described (12). Libraries were prepared using the TruSeq RNA Sample Prep Kit version 2 protocol (Illumina, San Diego, CA) and sequenced with 50 base pair paired-end reads (Supplement).

Statistical and Bioinformatic Data Analysis

Differential Expression Analyses. Pairwise differential expression comparisons were performed using Voom Limma (30) and a nominal significance threshold of fold change >1.3 and $p < .05$ (Supplement).

Enrichment Analyses. Enrichment between gene lists was analyzed using the GeneOverlap R package (www.bioconductor.org/packages/release/bioc/html/GeneOverlap.html).

RESULTS

Differential Expression Signatures of Susceptibility Versus Resilience to CSDS and Treatment Response Versus Nonresponse

C57BL/6J mice were exposed to CSDS and (Figure 1A, C) 24 hours after the final defeat underwent initial SI testing (SI1) to screen for susceptibility versus resilience (Figure 1D–F). Previous work has established that CSDS induces two phenotypes: mice that are susceptible to stress (approximately 67%) exhibiting profound and enduring social avoidance, and a resilient population (approximately 33%) that continue to show a preference for SI similar to control mice (27). The mechanisms underlying such different responses to stress among inbred mice raised under identical conditions remain unknown. Our data showed a similar split with 55 susceptible animals and 22 resilient animals (Supplemental Figure S1). Figure 1D–F shows group averages for animals included in downstream sequencing analysis (highlighted in Supplemental Figure S1).

Control and resilient mice were treated with saline for 14 days (control = 10, resilient = 8; Figure 1B). Groups of susceptible mice were treated chronically (14 days) with saline, 20 mg/kg imipramine, or saline followed by acute treatment with 10 mg/kg ketamine (saline = 6 mice, imipramine = 14 mice, ketamine = 12 mice; Figure 1B). After treatment, all mice were retested in SI2. Repeated-measures analysis of variance of time in the IZ found a phenotype × test type (no target, target) interaction in both SI1 and SI2 (Figure 1D; SI1: $F_{2,21} = 26.09$, $p < .0001$; SI2: $F_{2,21} = 13.31$, $p = .0002$). Susceptible mice spent significantly less time in the IZ when target was present than when the enclosure was empty (Bonferroni post hoc SI1: $t_5 = 5.596$, $p = .0025$; SI2: $t_5 = 6.427$, $p = .0014$), and in the presence of the social target both control and resilient mice spent more time in the IZ than susceptible mice in both SI1 and SI2 (SI1: $t_{13} = 6.910$, $p < .0001$; SI2: $t_{13} = 5.882$, $p < .0001$) or control (SI1: $t_{15} = 7.392$, $p < .0001$; SI2: $t_{15} = 4.372$, $p = .0005$). Mice in which imipramine or ketamine treatment increased SI (responders) and mice in which drug treatment did not alter SI (nonresponders) were identified and included in downstream sequencing analysis (post hoc analysis imipramine responders vs. nonresponders with target present, SI2: $t_{13} = 6.590$, $p < .0001$; ketamine responders vs. nonresponders with target present, SI2: $t_{11} = 4.049$, $p = .0019$; Figure 1E, F). Each treatment reversed SI deficits in approximately 50% of susceptible mice—similar to previous studies (26,28,29). To generate circuit-wide transcriptional profiles we used RNA-seq to analyze the NAC, PFC, HIP, and AMY from seven groups of mice—control, susceptible, and resilient saline-treated mice as well as ketamine and imipramine responders and nonresponders (control = 10 mice; resilient, imipramine nonresponders = 8 mice per group; susceptible, ketamine responders, ketamine nonresponders, imipramine responders = 6 mice per group).

In each brain region, we profiled differential gene expression in susceptible-saline mice versus control-saline mice (SUS-SAL vs. CON-SAL) and resilient-saline mice versus control-saline mice (RES-SAL vs. CON-SAL). We also directly compared RES-SAL versus SUS-SAL mice to identify transcriptional changes associated uniquely with either condition. In addition, we examined differential gene expression in ketamine and imipramine responders (SUS-KET-RESP, SUS-IMI-RESP) and nonresponders (SUS-KET-NON, SUS-IMI-NON) relative to SUS-SAL and RES-SAL to examine how treatment response and nonresponse relate to natural processes of susceptibility and resilience to chronic stress. Figure 1G summarizes the number of upregulated and downregulated differentially expressed genes (DEGs) in each comparison (see Supplemental Tables S1–S4 for full differential lists). The largest number of DEGs in SUS-KET-RESP compared with SUS-SAL mice were observed in HIP and AMY. In contrast, more DEGs were detected in NAC and AMY in SUS-IMI-RESP mice compared with SUS-SAL mice. Intriguingly, the largest number of DEGs across all comparisons was observed in NAC and AMY comparing SUS-IMI-RESP mice with RES-SAL mice, and a large number of DEGs were observed across all brain regions in comparing SUS-KET-RESP mice with RES-SAL mice. These initial observations raise interesting questions about how transcriptional profiles associated with ketamine or imipramine response relate to natural processes of resilience and susceptibility.

Comparison of Ketamine and Imipramine Treatment Response and Nonresponse

To identify similarities between transcriptional profiles associated with effective treatment response to either ketamine or imipramine, we plotted union heatmaps of $\log_2$ fold change of all significant DEGs in either SUS-KET-RESP versus SUS-SAL or SUS-IMI-RESP versus SUS-SAL in each brain region (Figure 2A). Visual inspection reveals the greatest similarity between treatment responses in upregulated and downregulated DEGs in the PFC, the region in which the fewest DEGs were detected for both drug responses (Figure 1G). In contrast, transcriptional profiles were most distinct in the AMY (Figure 2A), where many more DEGs were associated with imipramine response than ketamine response (Figure 1G). These observations were confirmed by Fisher's exact tests of enrichment, which identified a highly significant overlap of DEGs upregulated (29.96 times, $p = 4 \times 10^{-26}$) and downregulated (10.07 times, $p = 3 \times 10^{-10}$) in both SUS-KET-RESP versus SUS-SAL and SUS-IMI-RESP versus SUS-SAL in the PFC (Figure 2B and Supplemental Table S5A). In contrast, the overlap of ketamine and imipramine response was not significant for DEGs downregulated in the AMY (1.09 times, $p > .05$), with only modest overlap observed for DEGs upregulated in this region (1.74 times, $p = 2 \times 10^{-03}$) (Figure 2B).

Contrasting with the large number of DEGs and limited overlap between ketamine and imipramine response across brain regions, relatively fewer DEGs associated with nonresponse to the two drugs (Figure 1G), and there was a much greater similarity in nonresponse transcriptional profiles across all brain regions (Figure 2C). Fisher's exact tests identified the largest overlap in DEGs upregulated in both SUS-KET-NON versus SUS-SAL and SUS-IMI-NON versus SUS-SAL in the PFC (69.92 times, $p = 1 \times 10^{-38}$) and in the NAC (34.22 times, $p = 4 \times 10^{-33}$), with substantial overlap observed across DEGs in all brain regions (Figure 2D and Supplemental Table S5A). The increased overlap in DEGs of nonresponse to ketamine and imipramine points to greater specificity in transcriptional profiles associated with a therapeutic-like response

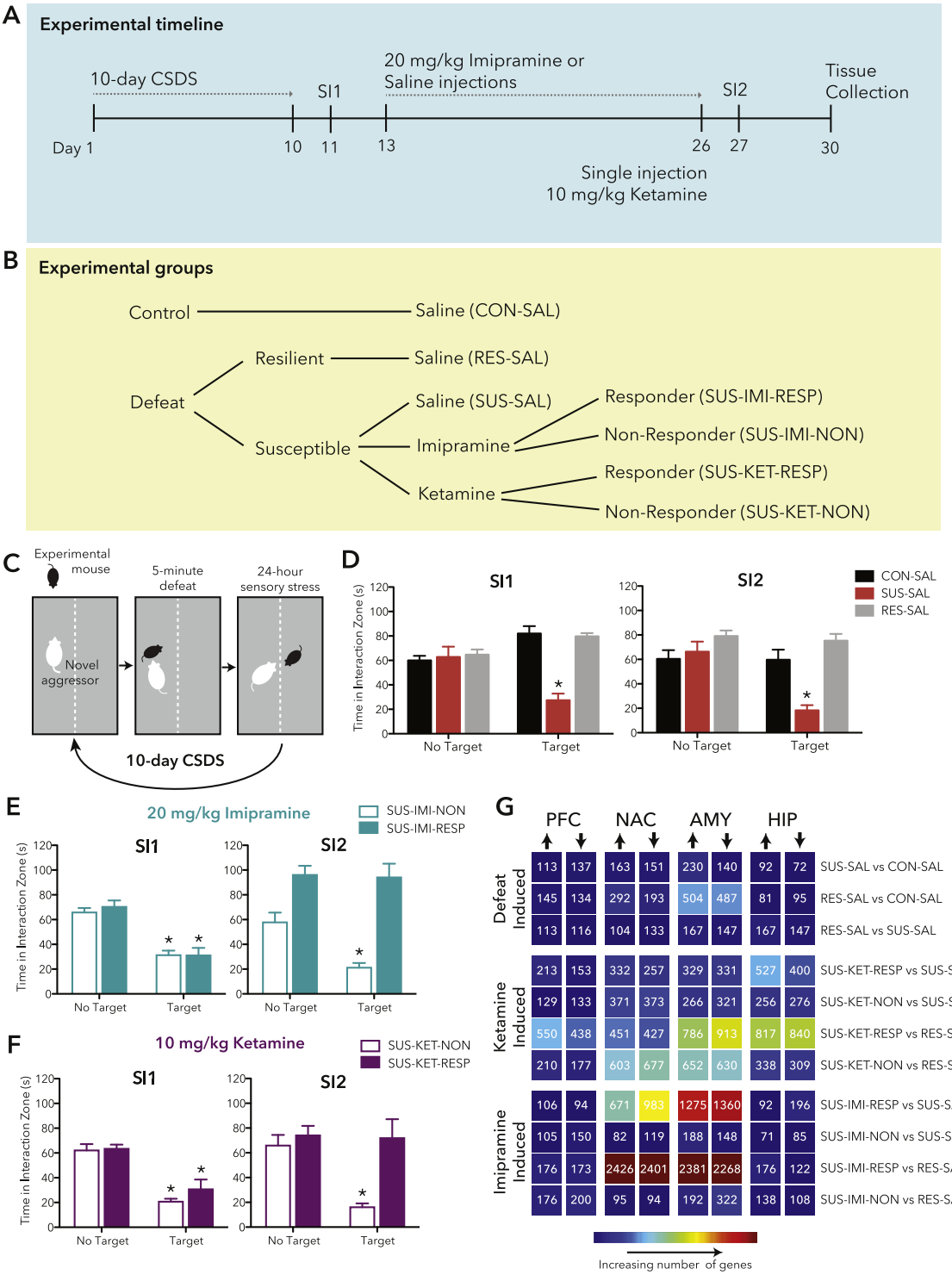

Figure 1. Study overview. **(A)** Schematic outlining study design and experimental manipulations. **(B)** Social interaction (SI) data 24 hours after chronic social defeat stress (CSDS) and again after drug treatment. **(C)** The number of differentially expressed genes (DEGs) in each pairwise comparison ($p < .05$) is displayed in the matrix with warmer colors indicating increasing numbers of DEGs. Time spent in the interaction zone in the absence (No Target) or presence (Target) of a novel mouse 24 hours after CSDS (SI1) and 24 hours after 14 daily injections (SI2) in **(D)** saline (SAL)-treated control (CON), susceptible (SUS) and resilient (RES) mice, **(E)** imipramine (IMI)-treated susceptible responders (RESP) and nonresponders (NON) and **(F)** ketamine (KET)-treated susceptible RESP and NON. **(G)** Table summarizes number of differentially expressed genes ($p < .05$, fold change > 1.3; DEGs) in each pairwise comparison in each brain region with warmer colors representing increasing numbers of DEGs and text indicating exact number. AMY, amygdala; HIP, hippocampus; NAC, nucleus accumbens; PFC, prefrontal cortex.

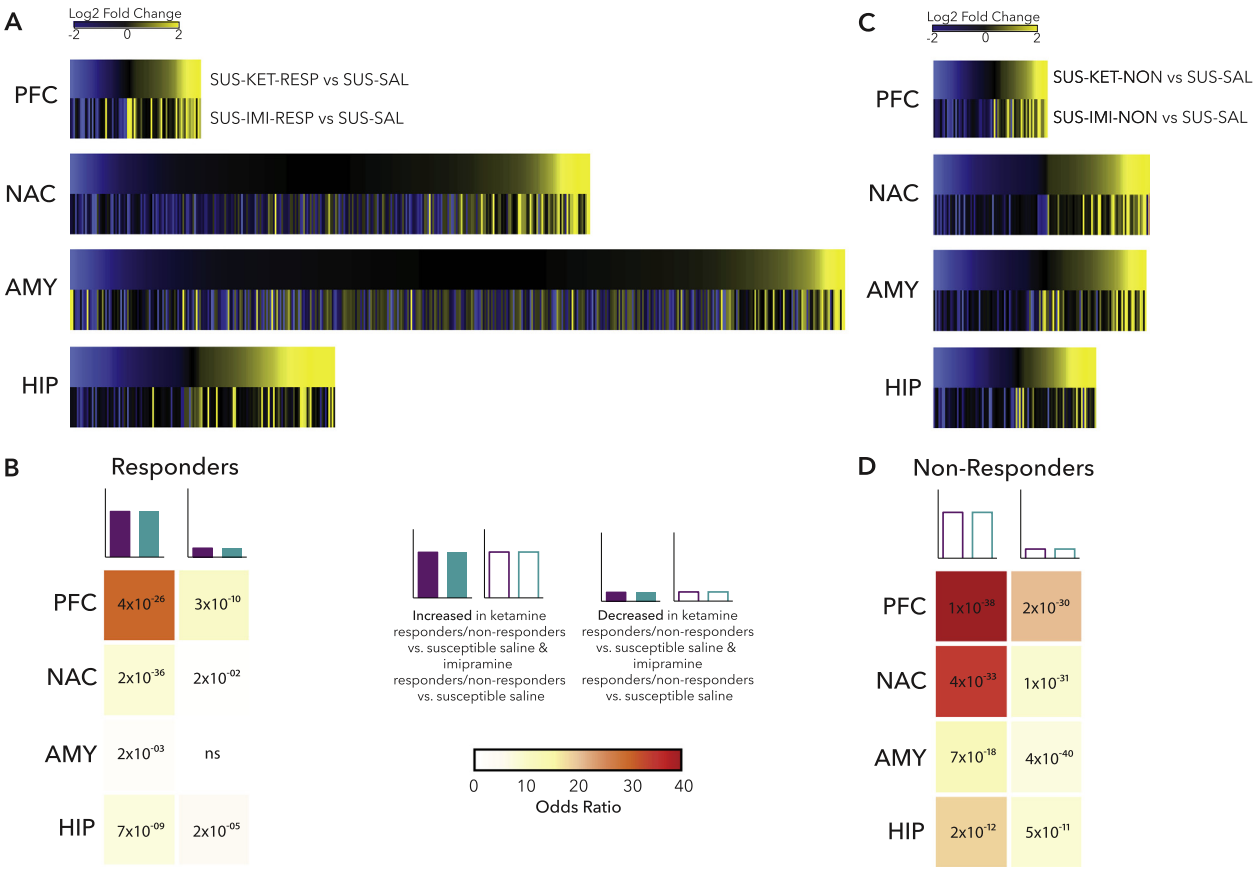

Figure 2. Characterization of treatment response and nonresponse. **(A)** Heatmaps show the union of ketamine response (SUS-KET-RESP vs. SUS-SAL) and imipramine response (SUS-IMI-RESP vs. SUS-SAL) differentially expressed genes (DEGs) rank ordered by $\log_2$ fold change of ketamine response and scaled by relative number of DEGs. **(B)** Table of *p* value (text) and odds ratio (warmer colors indicating increasing odds ratio) for Fisher's exact test for enrichment of ketamine response DEGs in imipramine response DEGs. **(C)** Heatmaps show the union of ketamine nonresponse (SUS-KET-NON vs. SUS-SAL) and imipramine nonresponse (SUS-IMI-NON vs. SUS-SAL) DEGs rank ordered by $\log_2$ fold change of ketamine nonresponse and scaled by relative number of DEGs. **(D)** Table of *p* value (text) and odds ratio (warmer colors indicating increasing odds ratio) for Fisher's exact test for enrichment of ketamine nonresponse DEGs in imipramine nonresponse DEGs. $^*p < .05$. AMY, amygdala; HIP, hippocampus; NAC, nucleus accumbens; ns, nonsignificant; PFC, prefrontal cortex.

to each drug than nonresponse, which may reflect that, in part, nonresponders to either drug remain more similar to susceptible mice.

Characteristics of DEGs in Treatment Responders and Nonresponders

Direct comparison of ketamine responders with nonresponders and of imipramine responders with nonresponders revealed that, although nonresponse is associated with the lack of some transcriptional changes seen in responders, nonresponse is also associated with transcriptional regulation unique to this condition (Figure 3A–D). In imipramine-treated mice, AMY had the largest number of genes uniquely associated with response and the largest number of genes uniquely associated with nonresponse (Figure 3C). Gene ontology analysis revealed that genes regulated in AMY in imipramine responders enriched for biological processes, including oxidative phosphorylation (5.65 times, $p = 3.91 \times 10^{-17}$) and synaptic transmission (2.14 times, $p = 2.111 \times 10^{-13}$), whereas genes regulated in nonresponders were modestly enriched in biological processes such as immune response (2.35 times, $p = 1.56 \times 10^{-07}$) and response to wounding (2.73 times, $p = 2.81 \times 10^{-07}$; Figure 3E). In ketamine-treated mice, HIP had the largest number of genes uniquely associated with response, whereas NAC had the largest number of genes associated with nonresponse (Figure 3F). Gene ontology analysis revealed that genes regulated in HIP in ketamine responders enriched for biological processes of ion transport (2.72 times, $p = 8.24 \times 10^{-17}$) and circulation (3.78 times, $p = 5.05 \times 10^{-11}$). Genes regulated in nonresponders in NAC strongly enriched for biological processes, including cell-cell signaling (2.76 times, $p = 2.56 \times 10^{-15}$), transmission of nerve impulse (2.98 times, $p = 1.65 \times 10^{-10}$), and circulation (3.49 times, $p = 2.10 \times 10^{-07}$).

Treatment Response Is Associated With Induction of Transcriptional Profiles of Resilience

A growing body of literature demonstrates that resilience to CSDS or other forms of stress reflects active processes and

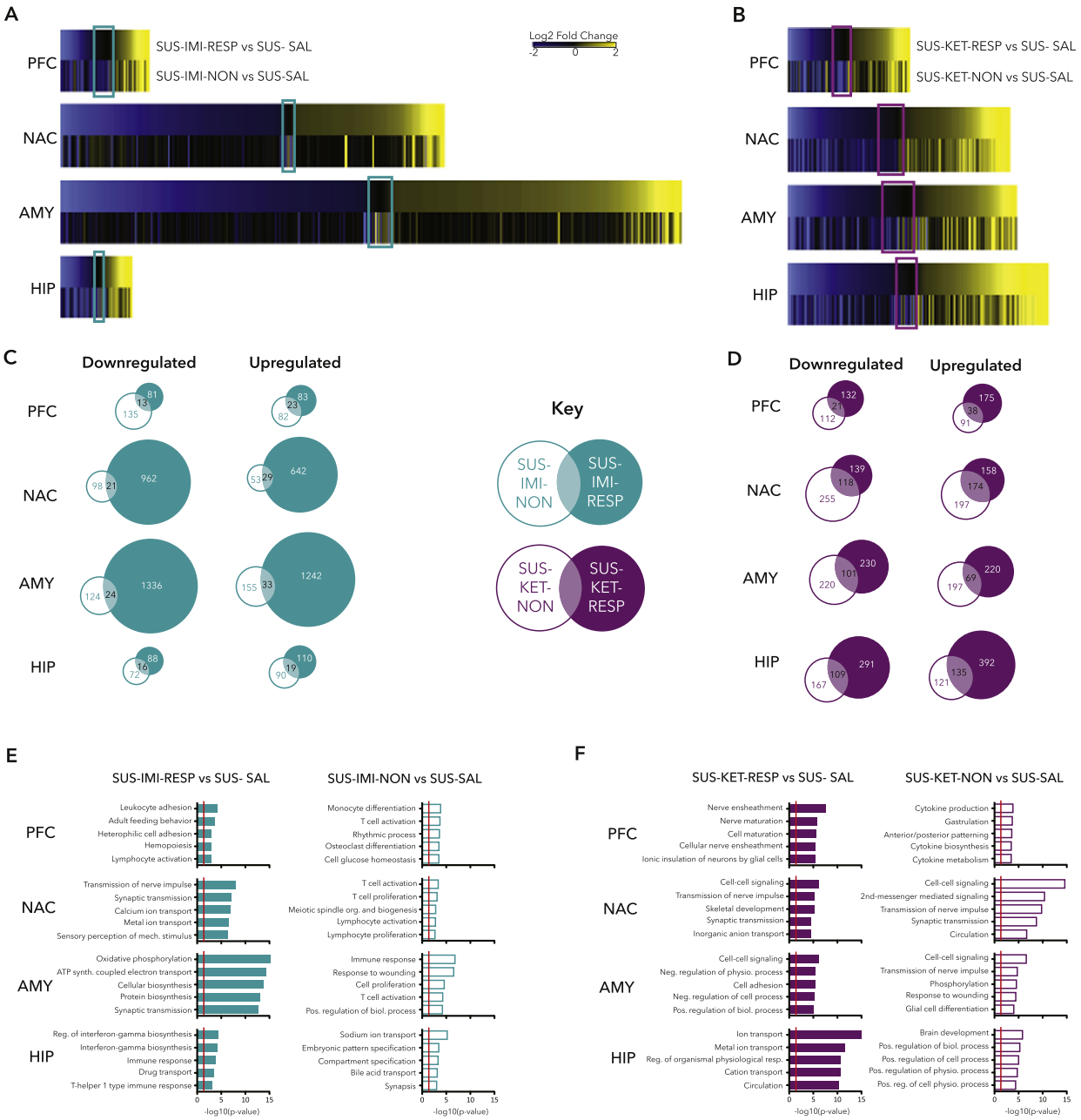

Figure 3. Comparison of treatment response and nonresponse. **(A)** Heatmaps show the union of ketamine response differently expressed genes (DEGs) (SUS-KET-RESP vs. SUS-SAL) and ketamine nonresponse DEGs (SUS-KET-NON vs. SUS-SAL) in each brain region, rank ordered by $\log_2$ fold change of ketamine response and scaled by relative number of DEGs. **(B)** Heatmaps show the union of imipramine response DEGs (SUS-IMI-RESP vs. SUS-SAL) and imipramine nonresponse DEGs (SUS-IMI-NON vs. SUS-SAL) in each brain region, rank ordered by $\log_2$ fold change of imipramine response and scaled by relative number of DEGs. Colored rectangles highlight DEGs significantly regulated exclusively in nonresponders and not in responders. **(C)** Venn diagrams represent the number of common and unique downregulated (left panel) and upregulated (right panel) DEGs in imipramine responders and nonresponders in each brain region. **(D)** Venn diagrams represent the number of common and unique downregulated (left panel) and upregulated (right panel) DEGs in ketamine responders and nonresponders in each brain region. **(E)** Bar graphs show biological pathways enriched in DEGs in imipramine responders (left panel) and nonresponders (right panel). **(F)** Bar graphs show biological pathways enriched in DEGs in ketamine responders (left panel) and nonresponders (right panel). Red line indicates $p = .05$. AMY, amygdala; HIP, hippocampus; NAC, nucleus accumbens; PFC, prefrontal cortex.

not merely the absence of susceptibility (27,31–34). We therefore examined the possibility that ketamine and imipramine induce—in responders—transcriptional profiles associated with natural resilience. Plotting union heatmaps of $\log_2$ fold change of all significant DEGs regulated in either RES-SAL versus SUS-SAL or SUS-KET-RESP versus SUS-SAL and

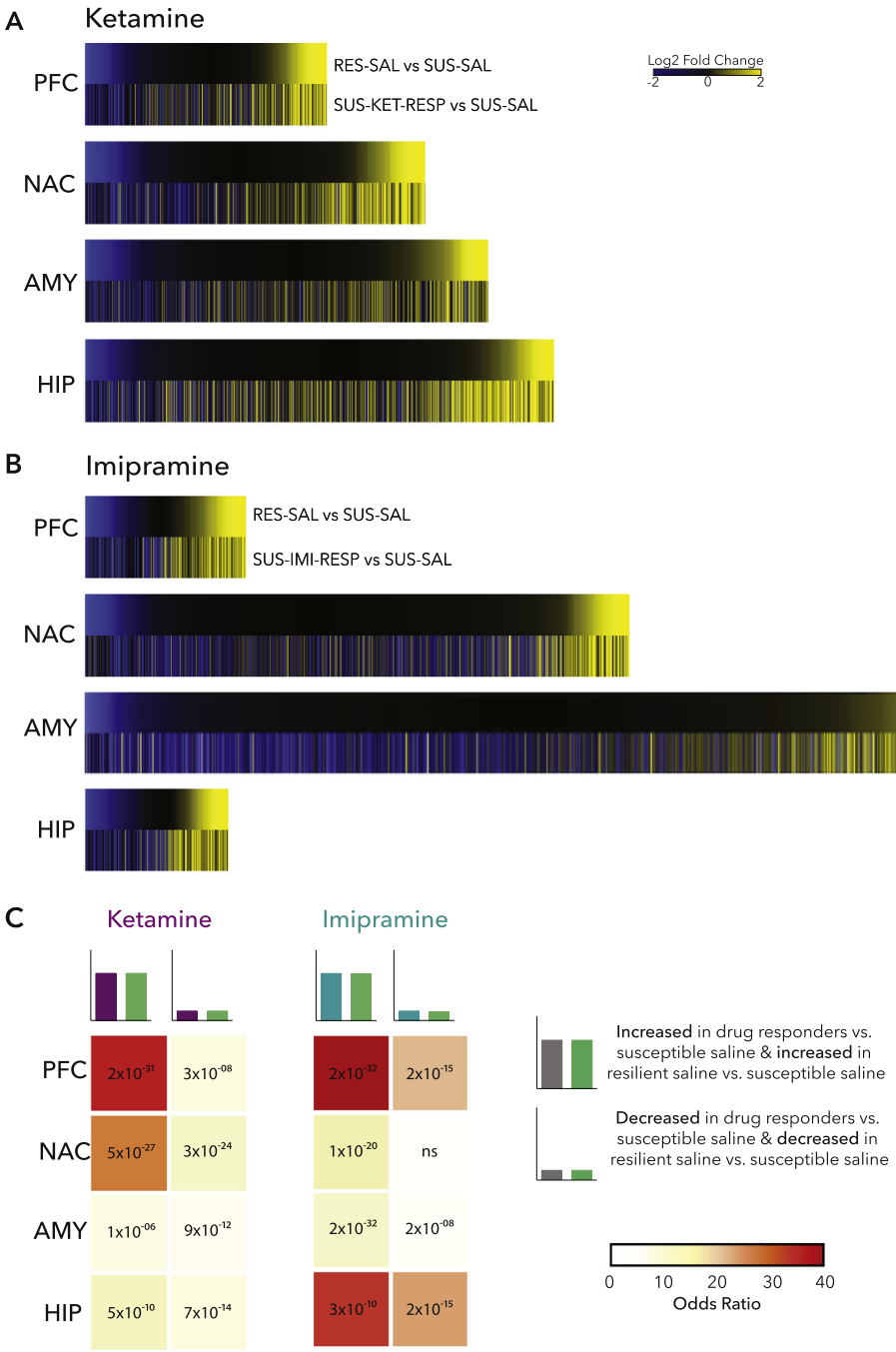

Figure 4. Induction of resilience differentially expressed genes (DEGs) with treatment response. **(A)** Heatmaps show the union of ketamine response (SUS-KET-RESP vs. SUS-SAL) and resilience (RES-SAL vs. SUS-SAL) DEGs in each brain region rank ordered by $\log_2$ fold change of RES and scaled by relative number of DEGs. **(B)** Heatmaps show the union of imipramine response (SUS-IMI-RESP vs. SUS-SAL) and resilience (RES-SAL vs. SUS-SAL) DEGs in each brain region rank ordered by $\log_2$ fold change of resilience and scaled by relative number of DEGs. **(C)** Table of *p* value (text) and odds ratio (warmer colors indicating increasing odds ratio) for Fisher's exact test for enrichment of ketamine response and imipramine response DEGs with resilience DEGs. AMY, amygdala; HIP, hippocampus; NAC, nucleus accumbens; ns, nonsignificant; PFC, prefrontal cortex.

separately RES-SAL versus SUS-SAL or SUS-IMI-RESP versus SUS-SAL in each brain region (Figure 4A, B) revealed considerable overlap across all brain regions with both drug responses, although the magnitude of overlap differed by brain region and drug. In ketamine responders, DEGs upregulated in the PFC (36.05 times, $p = 2 \times 10^{-31}$), or those upregulated in the NAC (24.36 times, $p = 5 \times 10^{-27}$), showed the largest amount of overlap with resilient-specific DEGs (Figure 4C and Supplemental Table S5B). [Note that SUS-SAL, not CON-SAL, was used as the reference condition for these analyses because many gene expression changes seen in resilience also occur in susceptibility (27), and we sought to isolate those associated uniquely with resilience.]

Similarly, the largest overlap between resilient-specific DEGs and imipramine response DEGs occurred in PFC (64.31 times, $p = 2 \times 10^{-32}$). Although less overlap was seen in NAC between resilient-specific and imipramine response DEGs (12.39 times, $p = 1 \times 10^{-20}$) than with ketamine response, there was a large overlap in upregulated HIP DEGs (33.24 times, $p = 3 \times 10^{-10}$).

Taken together, these findings suggest that both ketamine and imipramine exert antidepressant effects in part through regulating expression of genes associated with natural resilience. However, interestingly, although both drugs induce resilient-specific transcriptional profiles in PFC, this effect of ketamine is also seen in NAC, whereas the effect of imipramine is also seen in HIP, indicating important circuit-level specificity of these two different antidepressant drugs.

Treatment Response Is Also Associated With Reversal of Transcriptional Profiles of Susceptibility

We next examined the possibility that antidepressant-like effects of ketamine and imipramine reverse transcriptional profiles associated with susceptibility. Plotting $\log_2$ fold change of all significant DEGs regulated in either SUS-SAL versus CON-SAL or SUS-KET-RESP versus SUS-SAL in each brain region in union heatmaps revealed substantial opposing regulation between ketamine response and susceptibility in all brain regions (Figure 5A). As with induction of resilient-specific DEGs, the largest overlap was observed in NAC (31.28 times, $p = 1 \times 10^{-78}$) and PFC ($p = 6 \times 10^{-20}$) with genes increased in drug responders overlapping with genes decreased in susceptibility (Figure 5C and Supplemental Table S5C).

The union of $\log_2$ fold change of all significant DEGs regulated in either SUS-SAL versus CON-SAL or SUS-IMI-NON versus SUS-SAL revealed more region-specific reversal of susceptible-specific transcriptional profiles by imipramine than for ketamine (Figure 5B). Genes decreased in drug responders also overlapped considerably with genes increased in susceptibility in PFC (18.93 times, 19.35 times, $p = 6 \times 10^{-20}$). Again, as observed with induction of resilient-specific DEGs by imipramine, the strongest effects in reversing susceptibility occurred in PFC and HIP. Genes increased in drug responders were greatly enriched among genes decreased in susceptibility in both PFC (44.80 times, $p = 4 \times 10^{-34}$) and HIP (47.77 times, $p = 2 \times 10^{-18}$). Genes decreased in drug responders also strongly overlapped with genes increased in susceptibility in PFC (35.45 times, $p = 2 \times 10^{-19}$). In contrast, only limited opposing regulation was observed in NAC (7.60 times, $p = 8 \times 10^{-24}$) and no significant regulation in AMY (1.36 times, $p > .05$) (Figure 5C and Supplemental Table S5C).

DISCUSSION

We generated a uniquely large resource of genome-wide gene expression data (publicly available in Gene Expression Omnibus [http://www.ncbi.nlm.nih.gov/geo/]) in four interconnected limbic brain regions implicated in depression and its treatment to extend our understanding of transcriptional mechanisms of antidepressant response versus nonresponse with a conventional monoamine-based tricyclic antidepressant (imipramine) and a rapidly acting, non–monoamine-based antidepressant (ketamine). Importantly, our study examines drug responses in stressed mice exhibiting behavioral susceptibility, as opposed to naive mice, and encompasses the heterogeneity of response—responders versus nonresponders—to antidepressant treatment. By independently analyzing transcriptional profiles in brains of responders (mice in which treatment reversed the deleterious behavioral effects of stress) and nonresponders (mice in which treatment failed to induce this positive behavioral change), our data differentiate between transcriptional regulation associated with the therapeutic-like effects of each drug and off-target drug effects that either do not contribute to or may even antagonize antidepressant actions. Our data show that, at the transcriptional level, treatment response is characterized by both the reversal of some susceptibility-associated changes in gene expression and the induction of some resilience-specific gene regulation. Likewise, our data reveal that nonresponse is more than simply the lack of response to treatment, but additionally reflects some aberrant regulation of gene expression. These features of treatment response and nonresponse differ for ketamine versus imipramine, with clear differences observed across the four brain regions examined. Interestingly, the brain regions associated with the greatest number of DEGs with drug response were distinct from the regions in which the most statistically significant enrichment of proresilience and antisusceptible transcriptional profiles was detected. One interpretation of this observation is that antidepressant response encompasses unique transcriptional regulation above and beyond both the normalization of aberrant transcription associated with susceptibility and induction of transcriptional programs of resilience.

Our findings suggest that antidepressant effects can be achieved through different circuit-level mechanisms. The greatest similarity between ketamine and imipramine responders was observed in PFC. Interestingly, the most overlap in ketamine and imipramine nonresponders was also found in PFC. This suggests that PFC is a key locus of stress-induced transcriptional changes relevant to depression and a common site of action for these two very distinct antidepressant drugs. Both drugs exerted robust proresilience effects in PFC, inducing patterns of gene expression significantly enriched for resilient-specific DEGs. Imipramine and, to a lesser extent, ketamine also powerfully reversed susceptibility in PFC, inducing patterns of gene expression that strongly opposed susceptible DEGs. These transcriptional-level findings with imipramine are consistent with an earlier report in which we also showed similarities in genome-wide patterns of phosphorylated cyclic adenosine monophosphate response element binding protein and in repressive histone methylation in NAC of resilient versus imipramine-treated mice after CSDS (32). At the functional level, the importance of normalization of disrupted PFC activity is well established in mouse models and humans (7,12,15,18,35). Altered activity in PFC is observed in human depression, in which deep brain stimulation induces an antidepressant response in treatment-resistant depression and normalizes depression-associated metabolic changes (35). In addition, successful antidepressant response with either pharmacological or cognitive-behavioral treatments is associated with regulation of PFC activity (18,36–38).

Beyond similarities in PFC, our findings identify interesting circuit-level differences in the sites of action of ketamine and imipramine. We identified NAC as a key site of action for both the proresilience and antisusceptibility effects of ketamine, although NAC appeared far less important than HIP in the case of imipramine. Antidepressant response was most

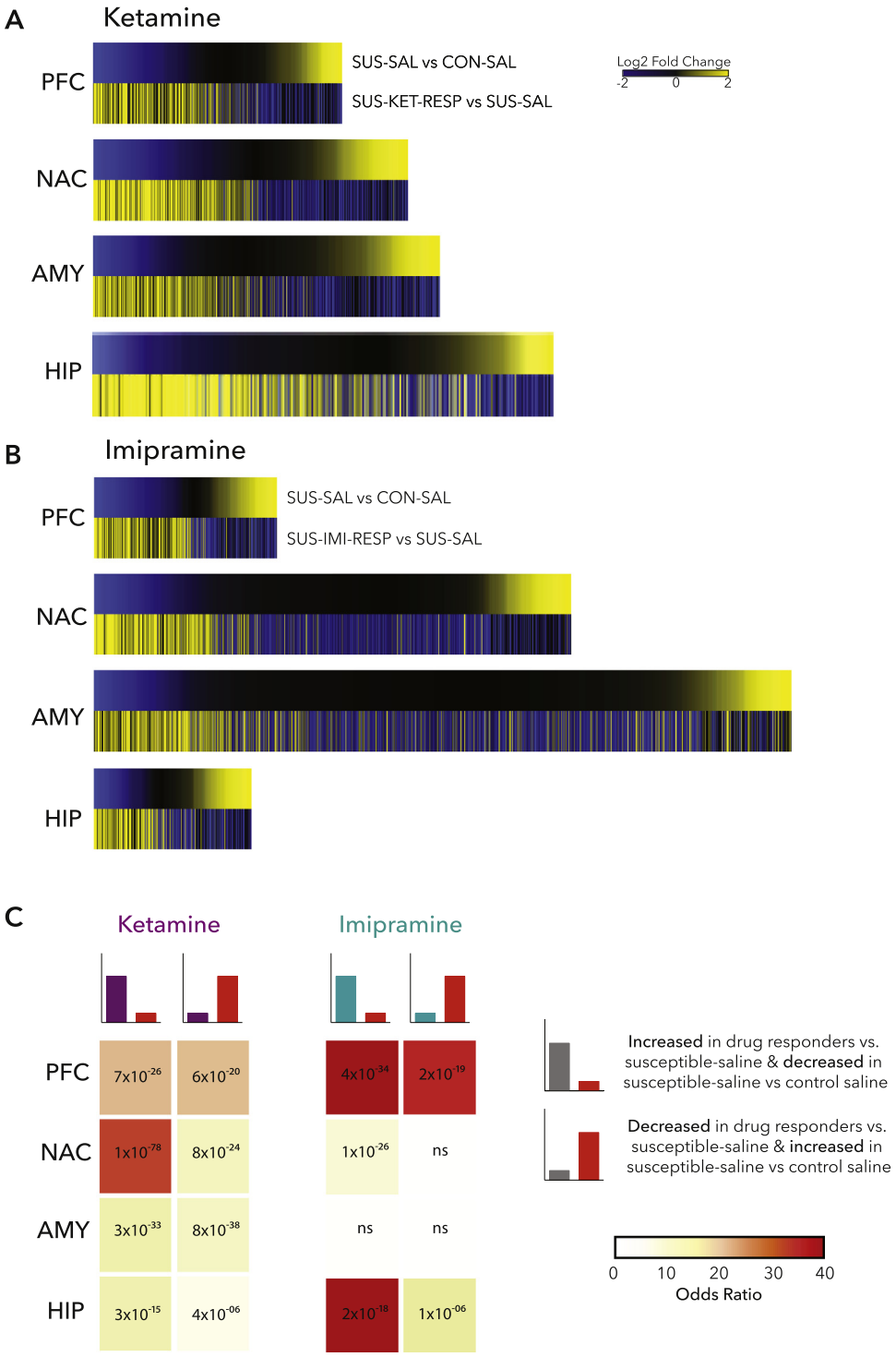

Figure 5. Reversal of susceptibility differentially expressed genes (DEGs) with treatment response (RESP). **(A)** Heatmaps show the union of ketamine response (SUS-KET-RESP vs. SUS-SAL) and susceptibility (SUS-SAL vs. CON-SAL) DEGs in each brain region rank ordered by $\log_2$ fold change of SUS and scaled by relative number of DEGs. **(B)** Heatmaps show the union of imipramine response (SUS-IMI-RESP vs. SUS-SAL) and susceptibility (SUS-SAL vs. CON-SAL) DEGs in each brain region rank ordered by $\log_2$ fold change of susceptibility and scaled by relative number of DEGs. **(C)** Table of *p* value (text) and odds ratio (warmer colors indicating increasing odds ratio) for Fisher's exact test for enrichment of ketamine response and imipramine response DEGs with susceptible DEGs. AMY, amygdala; HIP, hippocampus; NAC, nucleus accumbens; ns, nonsignificant; PFC, prefrontal cortex.

disparate in AMY, with many more genes regulated in imipramine responders than ketamine responders. Interestingly, although imipramine response was associated with a large number of genes in AMY, these DEGs were not highly enriched for either proresilience or antisusceptible DEG signatures, suggesting that the antidepressant effects of imipramine do not relate to natural processes of susceptibility and resilience in this brain region. Together, our findings identify PFC as a common, and potentially essential, target of antidepressant drugs in addition to which more

drug-specific antidepressant effects are mediated by other brain regions in this functionally interconnected circuit.

The rich datasets presented in this study provide an invaluable resource to aid the development of new antidepressant compounds that selectively target transcriptional changes associated with stress susceptibility, resilience, or treatment responsiveness. Of interest, a number of genes identified in key signatures of reversal of susceptibility or induction of resilience in association with drug response have been previously studied in depression. In PFC, ketamine normalized the reduced levels of *Dusp1* associated with susceptibility. Dual specificity phosphatase 1 (DUSP1) is a regulator of mitogen-activated protein kinase signaling that was found to be increased in the postmortem HIP of depressed humans and is regulated by antidepressant treatment in mice (39). Ketamine also reversed the reduction of *Arc* observed in PFC of susceptible mice, which we have implicated in depression in previous studies (12,15). *Fgf23*, a member of the fibroblast growth factor family of genes known to regulate affective behavior, was induced in PFC by both ketamine and imipramine, similar to effects in resilient mice (40,41). Likewise, in NAC, among the susceptibility genes reversed by ketamine was another family member, *Fgf3*. Interestingly, ketamine also reversed decreased levels of *Htr1b*, which encodes the serotonin 1b receptor, seen in NAC of susceptible mice. Single nucleotide polymorphisms in *HTR1B* have been reported to regulate response to serotonin-selective reuptake inhibitor antidepressants, and manipulation of this gene in mouse models regulates emotional behavior (42,43). In HIP, imipramine induced expression of *Ctla4* similar to effects in resilient mice. Polymorphisms in *CTLA4*, a member of the immunoglobulin superfamily expressed on helper T cells, have been reported in both Korean and Han Chinese populations in association with depression (44,45). Finally, our discovery of unique changes that occur in nonresponders raises the interesting possibility that a subset of these changes may oppose therapeutic efficacy, something that now warrants direct examination. If this proves to be true, it might be possible to enhance therapeutic efficacy of available antidepressant treatments by developing ways of opposing these nonresponse associated transcriptional changes.

As the impact of depression on humanity increases, we need to develop a far broader range of antidepressant treatments with more rapid onset of action that also effectively treat patients failed by existing medications. Ketamine is an extremely promising new agent; however, it is not fully efficacious in all treatment-resistant individuals. In addition, its antidepressant effects are transient, and the safety of long-term ketamine treatment needs to be established. The genome-wide transcriptome mapping used here offers a template of several strategies to identify—in unbiased ways—novel drug targets that alone or in combination can be exploited to develop improved treatments for depression.

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