LSD1 splicing is regulated by the long non-coding RNA *MALAT1* and orchestrates stress resilience in mammals

Arteda Paplekaj,[1,†] Elena Romito,[1,†] Chiara Forastieri,[1,†] Emanuela Toffolo,[1] Sabrina Briguglio,[1] Paride Pelucchi,[2] Ettore Mosca,[2] Alice Chiodi,[2] Marika Viatore,[1,3] Maria P. Bonasoni.,[4] Anna L. Santunione,[5] Filippo Pirani,[6] Eleonora Maggioni,[7] Paolo Brambilla,[8,9] Elena Battaglioli[1,‡] and Francesco Rusconi[1,‡]

[†,‡]**These authors contributed equally to this work**

Abstract

Psychiatric disorders often arise from the interaction between genetic predisposition and chronic psychosocial stress, yet the molecular programs determining resilience versus susceptibility remain incompletely understood. Building on evidence that the transcriptional corepressor LSD1 links environmental stress to neuronal gene regulation, we investigated whether isoform-specific regulation of LSD1 splicing contributes to stress adaptation.

Using a mouse model of chronic social defeat stress, we analyzed LSD1 microexon E8a splicing in the hippocampus of resilient and susceptible animals. RNA-seq was performed after the last stress session to capture genome-wide transcriptional responses during the window of LSD1 splicing regulation. Comparative analyses with published LSD1 knockdown, LSD1 ChIP-seq and chronic stress datasets were conducted. Hippocampal samples from suicide victims were analyzed to assess translational relevance.

Analysis of LSD1 splicing dynamics revealed that resilient mice, but not susceptible animals, retained the ability to reiterate acute stress-induced exon E8a skipping after repeated stress exposure, preserving the capacity to upregulate the enzymatically active ubLSD1 isoform in the hippocampus. In susceptible mice this inducible splicing response was absent. Mechanistically, splicing regulation involved the long non-coding RNA MALAT1, which controls the neurospecific splicing factor nSR100, a regulator of LSD1 exon E8a inclusion. Reduced MALAT1 expression in susceptible mice coincided with marked overactivation of stress-responsive genes revealed by RNA-seq. Approximately 15% (86 of 595) of genes deregulated in susceptible versus resilient hippocampi overlapped with transcripts modulated by LSD1 knockdown in an independent neuronal system. Of these, 25

were direct LSD1 ChIP-seq targets. ESR1 emerged as a regionally divergent upstream regulator associated with susceptibility.

The MALAT1–nSR100–LSD1 axis represents a regulatory pathway modulating stress adaptation. Downregulation of ubLSD1 and MALAT1 in the hippocampus of suicide victims recapitulates the molecular phenotype observed in stress-susceptible mice, linking disruption of this pathway to pathological behavioral outcomes.

Authors affiliations:

1 University of Milan, Department of Medical Biotechnology and Translational Medicine. Segrate 20054 (MI), Italy

2National Research Council, Institute of Biomedical Technologies. Segrate 20054 (MI), Italy

3 IRCCS Humanitas Research Hospital, Department of Immunology and Inflammation. Rozzano 20089 (MI), Italy

4 Azienda USL-IRCCS di Reggio Emilia, Unit of Pathology, Reggio Emilia 42123, Italy

5 University of Modena and Reggio Emilia, Biomedical, Metabolic and Neural Sciences. Institute of Legal Medicine, Modena, 41125, Italy

6 University of Bologna, Department of Medical and Surgical Sciences. Unit of Legal Medicine. Bologna 40126, Italy

7 Politecnico di Milano, Department of Electronics Information and Bioengineering. Milano 20133, Italy

8 Department of Neurosciences and Mental Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Milano 20122, Italy

9 Department of Pathophysiology and Transplantation, University of Milan. Milano 20122, Italy

Correspondence to: Francesco Rusconi

Department of Medical Biotechnology and Translational Medicine, University of Milan, Via F.lli Cervi 93, Segrate 20054, Milano, Italy

E-mail: Francesco.rusconi@unimi.it

Correspondence may also be addressed to: Elena Battaglioli

E-mail: Elena.battaglioli@unimi.it

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Introduction

Stress-related affective disorders, including depression, anxiety and post-traumatic stress disorder (PTSD), represent a significant global burden, yet their underlying molecular and circuital determinants remain poorly understood[1-5]. Chronic psychosocial stress is a key environmental factor contributing to psychiatric vulnerability[1,6,7]. Individual genome x environment (GxE)-relevant differences in stress susceptibility indeed play a crucial role in determining clinical outcomes[8]. While some individuals exhibit resilience to chronic stress, others develop maladaptive responses leading to increased psychiatric risk[9-13]. Unraveling the (epi)genetic basis of stress susceptibility and resilience is therefore essential for developing targeted therapeutic interventions[6,14].

Among candidate molecular players, Lysine-Specific Demethylase 1 (LSD1/*KDM1A*) has emerged as a key epigenetic regulator of neuronal plasticity in response to stress[15-17]. LSD1 exists in two main isoforms generated through alternative splicing of the 12-nucleotide microexon E8a[18]. The ubiquitously expressed ubLSD1 isoform, which skips microexon E8a, is fully enzymatically competent, functions as a histone H3K4me1/2 demethylase, and represses neuroplasticity-related gene transcription in the brain in complex with CoREST and HDAC2[19-22]. In line with this framework, reduced expression or activity of histone deacetylases, particularly HDAC2, has been recently reported in schizophrenia[23] and bipolar disorder[24], pointing to impaired epigenetic repression as a feature of psychiatric vulnerability. Conversely, the neuron-restricted neuronal LSD1 (nLSD1), which includes exon E8a, represents a hypomorphic variant that retains H3K4me1/2 demethylase activity when assembled within appropriate multiprotein complexes, albeit with reduced catalytic efficiency compared to ubLSD1[16,25-28]. Notably, inclusion of exon E8a introduces a regulatory phosphosite (the threonine within the E8a-encoded tetrapeptide DTVK), that impacts corepressor recruitment[26] further constraining nLSD1 activity and supporting its proposed role as a dominant-negative-like regulator in neuronal chromatin contexts[15,16]. In neurons, balanced expression of ubLSD1 and nLSD1 is required for LTP-like neuroplastic processes impacting learning and memory[16,17,29]. Previous studies have shown that LSD1 exon E8a splicing is transiently suppressed in response to acute stress (increasing ubLSD1 and

reducing nLSD1) contributing to a post-traumatic window of transcriptional repression with physiological implications in homeostatic adaptation[15,29-32]. As said, this splicing modulation is short-lived, and the LSD1 isoforms level returns to resting conditions within 24 hours from stress cessation[30].

In mammals, alternative splicing plays a pivotal role in shaping the functional landscape of neuronal circuits, allowing for dynamic modulation of gene expression in response to environmental challenges[18,33-35] and is particularly relevant within the glutamatergic system, where activity-dependent splicing events contribute to synaptic plasticity and homeostasis[33,36]. A growing body of evidence supports the view that LSD1 alternative splicing in neurons is governed by a restricted set of neuro-specific splicing factors, operating in a coordinated and hierarchically organized manner[37,38]. At the top of this hierarchy is nSR100 (*SRRM4*), which plays a necessary and sufficient role in promoting inclusion of the LSD1 microexon E8a[37] and is robustly implicated in autism spectrum disorders[18,39-41]. NOVA1, a key regulator of synaptic function[36,42], physically interacts with nSR100 to fine-tune E8a inclusion[37], while concurrently diversifying the neuronal transcriptome and modulating excitability and seizure susceptibility[36]. More recently in evolution, RbFOX1[43,44] also acquired control over LSD1 splicing through a higher primate- and human-specific point mutation (AA>AG) in intron 8a–9, which created a novel 3' splice acceptor site (3'SS)[38]. This site preferentially promotes nonsense-mediated decay of the neurospecific isoform nLSD1, thereby modulating the ubLSD1/nLSD1 ratio. Notably, multiple genetic and genome-wide association studies implicate RbFOX1 in psychiatric disorders such as depression[45], autism[46], and others[46-48], further linking LSD1 splicing control to neuropsychiatric vulnerability.

Together, these splicing factors converge on LSD1 as a *core* target, suggesting that its alternative splicing is not only a downstream target but also a critical regulatory node at the intersection of specialized and broader neuronal splicing programs, ultimately shaping neural plasticity and adaptive responses[38,49]. Despite this central role, how this regulatory module—particularly nSR100—responds to neuronal activity[41] or environmental stress to modulate LSD1 splicing is only partially understood. Emerging evidence points to long non-coding RNAs (lncRNAs) as upstream regulators of activity-dependent splicing and stress-responsive gene expression[50-53], offering a potential mechanism for dynamic modulation of factors like nSR100. Among them, Metastasis-Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) stands out for its ability to buffer splicing factor activity through high-affinity double-stranded RNA structures[51,52], placing it in a key position to influence LSD1 splicing in response to neuronal cues[51,54]. Interestingly, also *MALAT1* has been previously implicated in psychiatric disorders, in particular schizophrenia[50,55].

This study aims to identify transcriptomic signatures and regulatory mechanisms that differentiate stress susceptibility from resilience, focusing on LSD1 alternative splicing as a key experience-dependent modulator of neuronal transcriptional responses. We employed the Chronic Social Defeat Stress (CSDS) paradigm in mice[13,56], and identified an alternative splicing-mediated selective upregulation of the ubLSD1 isoform in resilient (RES) animals. Mechanistically, we show that this effect is driven by a previously unrecognized interaction between *Malat1* and nSR100, which controls its availability at the LSD1 pre-mRNA[37]. Consistent with this model, *Malat1* is markedly downregulated in the hippocampus of susceptible (SUS) mice. Our findings are consistent with a model in which the *MALAT1*–nSR100–LSD1 axis participates in the adaptive modulation of stress-induced gene expression. Indeed, among genes deregulated in SUS animals, a significant proportion is controlled by LSD1. Notably, both *MALAT1* and ubLSD1 are also consistently reduced in the hippocampus of postmortem suicide victims, suggesting that the *MALAT1*–nSR100–LSD1 axis may also represent a convergent molecular signature of psychiatric vulnerability.

Materials and methods

Male C57BL/6N and CD-1 mice were maintained under SPF conditions. All procedures were approved by the Italian Ministry of Health and conducted in accordance with national and EU guidelines. Animals were exposed to a modified Chronic Social Defeat Stress (CSDS) paradigm or to an acute version (ASDS). Stress susceptibility and resilience were determined using the Social Interaction Test and calculating a Social Ratio (SR), based on comparison with control distributions.

Human Neural Progenitor Cells (hNPCs) and neuroblastoma cell models were used for molecular and functional assays, including minigene splicing assay, depolarization, and perturbation of nuclear speckles. Protein interactions were analyzed by immunoprecipitation and Western-Blot, while RNA–protein interactions were assessed by UV-CLIP. For in vivo manipulation of *MALAT1*, antisense oligonucleotides (ASO or SCRA-*MALAT1*[57]) were stereotaxically delivered to the hippocampus, and behavioral and molecular endpoints were assessed at defined time points.

Gene expression was quantified by qPCR and transcriptome-wide RNA sequencing. RNA-seq data were aligned to the mouse reference genome and analyzed using established normalization and differential expression pipelines. Differentially expressed genes were identified based on fold-change and multiple-testing–corrected significance thresholds.

Upstream regulator analysis was performed QIAGEN's Ingenuity® Pathway Analysis. LSD1 potential target genes were defined by integration with published ChIP-seq datasets.

Human post-mortem hippocampal samples were analyzed in accordance with ethical approvals and quality control criteria defined according to post-mortem interval (PMI) (Supplementary Table 1).

Statistical analyses were performed using appropriate parametric or non-parametric tests as indicated in the figure legends.

Full experimental protocols, sequencing parameters, bioinformatic settings, and statistical details are provided in the Supplementary Materials and Methods.

Results

Activity-dependent LSD1 splicing in the hippocampus predicts stress resilience

Because alternative splicing emerged as a dynamic component of the LSD1 stress response[15,16,29,30], we first sought to identify upstream regulators of this modulation. We examined hippocampal and in the medial prefrontal cortex (mPFC) transcript levels 2 or 7 hours after a single social defeat session, focusing on neuronal activity–sensitive splicing regulators. As expected, acute stress induced an increase in UbLSD1 (one-way ANOVA, SDS 2 h vs CTR, $p = 0.0074$) together with a complementary decrease in nLSD1 isoforms (one-way ANOVA, SDS 2 h vs CTR, $p = 0.0012$) in the hippocampus (Fig. 1A) and, notably, also in the mPFC (Fig. S1A), while total LSD1 levels remained stable. Within this early time window, the *master regulator* of LSD1 exon E8a inclusion, the serine/arginine-rich (SR) protein nSR100[37], was not transcriptionally modulated within the hippocampus (Fig. 1B), but its level appared to be decreased in the mPFC (Fig. S1B).

To explain LSD1 splicing modulation in the frame of invariant levels of nSR100 in the hippocampus, we hypothesized that its activity, rather than levels, could be sensitive to stress. We interrogated the long non-coding RNA *Malat1*, a plausible upstream candidate for influencing LSD1 splicing through a modification of nSR100 activity. *Malat1* plays indeed, an established role in splicing regulation within nuclear speckles, where it modulates the availability of SR proteins such as SRSF1[52]. Consistently with a putative LSD1 splicing regulatory role, *Malat1* displayed a transient upregulation at 2 hours after stress onset (one-way ANOVA, SDS 2 h vs CTR, $p = 0.0001$), returning to baseline by 7 hours in the

hippocampus (Fig. 1C). *Malat1* transactivation upon acute stress mirrors that of the immediate early genes such as *Egr1*, *Npas4*, and *cFos* (Fig. 1D), sharing with them core molecular mechanisms of activity-dependent response[58].

Notably, also in the mPFC, *Malat1* upregulation peaked 2 hours after stress onset (Fig. S1C), a timing again compatible with the early kinetics of ubLSD1 induction. In contrast, nSR100 levels—unchanged in the hippocampus—decreased in the mPFC only at a later time point (7 hours) (Fig. S1A–C), a delay that is unlikely to drive initial LSD1 isoform switching but is compatible with a role in the longer-term maintenance of ubLSD1 upregulation and nLSD1 downregulation in the mPFC relative to the hippocampus. Together, these observations support the hypothesis that nSR100 can regulate LSD1 exon skipping either through modulation of its activity/availability—potentially mediated by *Malat1*—or through changes in its expression levels.

We thus asked whether the acute LSD1 splicing program is preserved or disrupted under chronic stress conditions, able to induce **allostatic load**[59] and predisposing susceptible individuals to **long-term maladaptive outcomes**. We investigated **LSD1 splicing modulation** under **chronic Social Defeat Stress (CSDS)**. To maximize allostatic pressure on LSD1 stress-response mechanisms while maintaining construct validity, we employed a modified version of this stress paradigm[56]. Unlike continuous stress paradigms, we alternated 7-hour social defeat sessions with 17-hour homecage recovery periods over ten days (Fig. 2A). Each stress exposure induced a transient LSD1 splicing response—marked by increased ubLSD1 and decreased nLSD1—peaking 2–7 hours after stress onset and fully reverting to baseline upon homecage return, thus preventing cumulative stabilization of splicing changes across the paradigm[30]. On day 11, mice underwent Social Interaction Test. Time spent in the interaction zone and in the avoidance corners was recorded (Fig. 2B and C), and a Social Ratio (SR) was calculated as interaction time divided by the sum of interaction and avoidance to assess social *approach-avoidance* behavior[60]. Animals were stratified into resilient (RES) or susceptible (SUS) groups based on SR values. This index allowed a continuous and integrative behavioral readout of social response. Mice showing SR in the range of CTR were classified as RES ($mean_{CTR} \pm 2SD$)[61], all the others were classified as SUS (in Fig. 2D). We further characterized the outcomes of our modified CSDS protocol, assessing locomotor (LOC) and anxiety-like open field (OF) behavior[62] between SUS and RES (Fig. S1D and E). On day 12, mice underwent a final stress session and were sacrificed at the end of the 7-hour sensory interaction. This time point was specifically chosen to replicate the temporal window used in acute stress paradigms, enabling direct comparison of LSD1 isoform dynamics[15,29,30] across acute and chronic conditions. We performed isoform-specific qRT-PCR to quantify ubLSD1, nLSD1, and total LSD1 mRNA levels in the

hippocampus and the mPFC of CTR and CSDS mice. RES mice exhibited a significant upregulation of ubLSD1 compared to both CTR and SUS groups (Fig. 2E; ubLSD1 One-way ANOVA: RES *vs* CTR, $p<0,0001$; RES *vs* SUS, $p=0,021$), accompanied by a complementary downregulation of nLSD1 (Fig. 2E; nLSD1 One-way ANOVA: RES *vs* CTR, $p<0,0001$), recapitulating the acute stress response pattern previously described[15,29,30] and (Fig. 1A). Notably, SUS mice failed to upregulate ubLSD1, maintaining levels comparable to CTR (Fig. 2E; ubLSD1 One-way ANOVA: SUS vs CTR, $p=0,115$), yet exhibited an unexpected reduction in nLSD1, which cannot be explained by a splicing process (Fig. 2E; nLSD1 One-way ANOVA: SUS vs CTR, $p<0,0001$). To clarify this apparent inconsistency and to test whether isoform-specific transcript degradation could contribute to the observed phenotype, we implemented an additional qPCR assay using primers positioned at the 3' end of the LSD1 transcript, deliberately downstream of the alternative splicing region. Because mRNA decay can proceed through 3'→5' exonucleolytic pathways, reduced signal at the 3' end provides a more sensitive readout of ongoing transcript destabilization. Using conventional qPCR with primers positioned at the 5' region of the transcript (Fig. 2E; 5' primers), total LSD1 mRNA levels appeared unchanged across experimental conditions. In contrast, repeating the analysis with primers annealing at the 3' end revealed a selective reduction of total LSD1 transcripts in SUS mice compared with both CTR and RES animals (Fig. 2E; 3' primers; one-way ANOVA, SUS vs CTR, $p = 0.035$). These data indicate reduced LSD1 transcript stability in SUS mice and support the existence of isoform-selective degradation mechanisms, likely preferentially affecting the nLSD1 transcript in this group.

Despite clear RES-restricted splicing-dependent regulation at the transcript level, Western-Blot analysis using available pan-LSD1 antibodies did not reveal differences in total LSD1 protein expression in the chronically stressed hippocampus, reflecting the combined signal of the two co-migrating isoforms (Fig. S2A–B). Also SUS-restricted transcript degradation though sugnificant, was probably too modest to be detectable at the protein level.

As a further perspective linking ubLSD1 upregulation with resilience, we show that its hippocampal levels predicted resilience or susceptibility with ~75% accuracy; specifically, stressed mice with ubLSD1 expression above the average threshold, exhibited significantly increased social interaction (Fig. 2F; ubLSD1 One-way ANOVA: High vs Low, $p=0,046$) and decreased avoidance behavior (Fig. 2F; ubLSD1 One-way ANOVA: High vs Low, $p=0,0004$). In contrast, nLSD1 levels showed no predictive value, as RES and SUS mice were evenly distributed across the expression range (Fig. S2C), further suggesting ubLSD1 level as a novel modifier of stress resilience. Since ubLSD1—the exon E8a–skipped isoform—is selectively upregulated in RES mice (Fig. 2E) but not in SUS animals and given that *Malat1* could influence E8a inclusion through the modulation of nSR100 availability, we asked whether *Malat1* expression might also differ between the two groups. We therefore

quantified *Malat1* levels in the hippocampus of RES and SUS mice. *Malat1* was significantly reduced in SUS animals compared to both RES and CTR groups (Fig. 2G; one-way ANOVA: SUS vs CTR p=0,002; SUS vs RES p<0,0001), despite unchanged nSR100 transcript levels.

In mPFC, the modulation of LSD1 splicing isoforms appeared attenuated. No significant ubLSD1 upregulation was observed in either RES or SUS mice compared to controls in the mPFC (Fig. S2D). By contrast, total LSD1 transcript levels were reduced in both RES and SUS animals relative to CTR, indicating that, as observed in the hippocampus of SUS mice, nLSD1 downregulation in the mPFC primarily reflects transcript degradation rather than regulated alternative splicing. In line, *Malat1* expression in the mPFC remained stable across groups (Fig. S2E).

These findings indicate that Malat1 regulation diverges between RES and SUS mice under chronic stress, and suggest that reduced *Malat1* expression in SUS hippocampi, may contribute to an altered splicing environment, consistent with the failure to upregulate ubLSD1 in these animals.

MALAT1 modulates activity-dependent nSR100 function via physical interaction

Analysis of human (Chr11:65497933–65497938; Chr11:65498492–65498497) and mouse (Chr19:5849025–5849030; Chr19:5847665–5847670) *MALAT1* transcripts revealed the presence of two conserved nSR100-binding motifs, consistent with the possibility of a direct *MALAT1*–nSR100 interaction. This observation provides an initial clue that *MALAT1* may influence LSD1 exon E8a regulation through effects on nSR100 availability or activity. We therefore reanalyzed a published PAR-iCLIP dataset generated by Raj and colleagues[63], which identified nSR100-bound transcripts in human HEK293T and mouse N2a cells. Although the original study focused on protein-coding RNAs and did not explore lncRNAs, our independent inspection of the dataset using the UCSC-GenomeBrowser revealed a high density of reads mapping to the *MALAT1* locus in both species (Fig. 3A–B, upper panels), strongly suggesting direct binding. Notably, *MALAT1*-associated reads were even more abundant than those mapping to the *REST* transcript—the top candidate identified by the original study (Fig. 3A–B, lower panels). To further characterize nSR100–*MALAT1* interaction in a cellular paradigm of neuronal activation, we generated a Flag-tagged human nSR100 construct for immunoprecipitation assays. The tagged protein was expressed in N2a cells and validated by Western blot and immunoprecipitation (Fig. S3A). It also proved functionally active, as its overexpression increased exon E8a inclusion in ΔPAL transcripts (Fig. S3A). To confirm the suitability of our simplified in vitro system for studying activity-dependent processes, we assessed its responsiveness to depolarizing stimuli. Treatment of

N2a cells with KCl, though undifferentiated, induced detectable *c-Fos* and *Malat1* transactivation (Fig. S3C), confirming that this model *quasi* recapitulates key features of activity-induced transcription. RNA-CLIP assays performed using anti-FLAG antibodies revealed a trend toward MALAT1 enrichment in nSR100-bound RNA, which became statistically significant upon neuronal depolarization (Fig. 3C; two-way ANOVA, FLAG–nSR100 vs FLAG–CTR under KCl, $p = 0.012$). Given the high abundance of MALAT1, specificity was stringently controlled by including parallel CLIP assays with pre-immune IgG and anti-FLAG immunoprecipitation in cells transfected with the empty vector. MALAT1 levels quantified by RT–qPCR were normalized to the amount recovered in the IgG control, and data are expressed as fold enrichment over IgG, thereby accounting for non-specific RNA capture. Thus, neuronal stimulation enhances the physical interplay between *Malat1* and nSR100, suggesting *Malat1* as an activity-responsive upstream regulator of nSR100 function.

MALAT1 acts as an upstream modulator of the nSR100–LSD1 splicing axis

During the perinatal window, exon E8a inclusion transiently increases in the mammalian brain to support developmental synaptogenesis[64]. In contrast, *Malat1* expression follows an antagonistic developmental trajectory from embryonic day 10 (E10) to postnatal day 10 (PN10) in both the mouse hippocampus and mPFC, consistent with a negative regulatory relationship (Fig. S3D).

To determine whether *MALAT1* functionally influences LSD1 exon E8a splicing via nSR100, we conducted a minigene splicing assay in human SH-SY5Y neuroblastoma cells, selected for their human origin and transfectability. Although not fully neuronal, this system allows a controlled analysis of nSR100 splicing regulation[37]. We used a human exon E8a splicing reporter minigene (ΔPAL), in which E8a inclusion in minigene-derived transcripts is entirely dependent on nSR100[37]. Indeed, point mutations in the UGCUGC motif located in the upstream intron of E8a abolish nSR100 binding and fully prevent exon inclusion. Using this system, we found that h*MALAT1* overexpression significantly increased the proportion of minigene-derived transcripts lacking exon E8a, as reflected by the enrichment of the exon 8–9 junction (characterizing ubLSD1) relative to control cells (Fig. 3D; unpaired t-test *MALAT1* vs CTR $p<0.0001$). These results indicate that *Malat1* can influence E8a inclusion in a negative nSR100-dependent manner.

To examine the activity-dependent relationship between *MALAT1* and LSD1 exon E8a splicing, we used differentiated human neural progenitor cells (hNPCs), which—unlike N2a and SH-SY5Y cells—acquire neuron-like inclusion of LSD1 exon E8a and display

homeostatic splicing adjustments upon depolarization[33,65]. A 3-hour KCl stimulation induced *MALAT1* upregulation (Fig. S3E; Two-way ANOVA: CTR vs KCl in DMSO p = 0.027) together with a shift in LSD1 isoforms, increasing ubLSD1 (Fig. S3F; p = 0.022) and reducing nLSD1 (Fig. S3F; p = 0.007), without affecting total LSD1 mRNA. This trend parallels in vivo acute-stress response, where *MALAT1* and LSD1 splicing change with similar timing. To gain preliminary functional insight into *MALAT1* involvement, we used tubercidin, which disrupts nuclear speckles via *MALAT1* downregulation[52,66]. Tubercidin prevented activity-induced *MALAT1* upregulation and abolished the associated LSD1 splicing shift (Fig. S3E–F), providing correlative support for *MALAT1* involvement and motivating more specific loss-of-function approaches. To reduce *Malat1* expression in vivo, we first validated an antisense oligonucleotide targeting *Malat1* (already characterized in a human cell line[57]) in mouse N2a cells, where *MALAT1*-ASO reduced lncRNA levels by approximately 70%, with no effect observed for the scrambled control (SCRA) (Fig. S3G). We then stereotaxically delivered the *MALAT1*-ASO into the hippocampus. Mice received bilateral injections followed by a 7-day recovery period to allow effective knockdown. At day 7, animals were subjected to a single session of acute social defeat stress (SDS) and sacrificed 7 hours later, matching the temporal window in which ubLSD1 induction peaks after stress. ASO robustly reduced *Malat1* levels relative to scrambled (SCRA) controls (Fig. 3E; one-way ANOVA SCRA vs ASO p < 0.0001), confirming efficient hippocampal knockdown. Importantly, *Malat1* reduction did not alter basal ubLSD1 levels in unstressed mice (SCRA-CTR vs ASO-CTR, n.s.), indicating that *Malat1* does not influence steady-state LSD1 isoform ratios. However, *Malat1* knockdown significantly attenuated the stress-induced increase in ubLSD1 observed in scrambled controls (Fig. 3F). ubLSD1 induction remained detectable, though clearly diminished in ASO-treated mice, suggesting that *Malat1* contributes to the extent, rather than the occurrence, of the splicing shift. Together, these results indicate that *Malat1* acts in vivo to tune the activity-dependent regulation of LSD1 exon E8a, specifically influencing the magnitude of its induction under stress.

Convergent dysregulation of *MALAT1* and LSD1 splicing in the hippocampus of suicide victims

After functionally validating the *MALAT1*–nSR100–LSD1 module in mice, we investigated whether this axis shows signs of dysregulation in the human hippocampus in association with severe psychiatric vulnerability, focusing on individuals who died by suicide. While the relationship between suicide and stress susceptibility is multifaceted, accumulating evidence supports the view that suicide frequently represents the final outcome of prolonged exposure to psychosocial stress and the failure of adaptive coping

mechanisms[67,68]. In this context, suicide possibly represents a biological convergence point, in other words, a unifying endpoint across distinct psychiatric diagnoses. This approach allows a molecular dissection of stress susceptibility while reducing confounding effects related to nosological boundaries. We collected hippocampal samples from 9 SD male victims and 14 controls (CTR) (Suppl. Table 1) and assessed *MALAT1* and ubLSD1 levels in human postmortem specimens. In designing the study, we ensured a well-balanced representation of age ranges across diagnostic groups to enable robust cross-sectional comparisons. Both control and SD subjects showed a similar age-related increase in ubLSD1 levels, consistent with previous findings[29], supporting the validity of this comparative approach (Fig. 4A, left). Nonetheless, despite following a comparable developmental trajectory, SD individuals consistently exhibited lower absolute ubLSD1 levels (Fig. 4A, right; Unpaired t-test: SD vs CTR, p=0,025). In the same samples, we also assessed LSD1 protein levels (note that the two different isoforms ubLSD1 and nLSD1 show the same electrophoretic mobility). Unexpectedly, we observed a reduction in SD individuals compared to controls (Fig. 4B; Mann–Whitney test: SD vs CTR p=0,058), that was not evidenced in the mouse model (S2A-B). Decreased LSD1 protein expression level in the hippocampus of SD suggests a further level of LSD1 involvement as a disease-relevant gene that could be primate- or human-specific consistently with other primate-restricted LSD1 regulation mechnisms already described elsewhere[38].

We then performed a linear regression analysis of *MALAT1* expression across the age spectrum in both CTR and SD groups, which revealed no age-related modulation (Fig. 4C, left). Remarkably, *MALAT1* levels were significantly reduced in the hippocampus of SD individuals compared to controls (Fig. 4C; unpaired t-test: SD vs CTR, p=0,041).

In females, where statistical power was limited, we nonetheless observed a significant downregulation of ubLSD1 transcripts in SD individuals compared to controls (Fig. S4A; unpaired t-test: SD vs CTR, p=0,033). Both *MALAT1* expression (Fig. S5B) and LSD1 protein levels (Fig. S5C) displayed a downward trend. Altogether, these findings reveal a hippocampal vulnerability associated with a disruption of *MALAT1*–LSD1 regulation, prompting us to return to the mouse model to chart, through transcriptomic profiling, whether the molecular programs defining susceptibility may partly converge onto this disrupted regulatory module.

Runaway transcription in stress susceptibility reflects impaired LSD1 repression

To determine how the molecular divergence between RES and SUS mice relates to the regulatory failure of the *MALAT1*–LSD1 module, we performed bulk hippocampal RNA-seq in CSDS-stratified and control animals to search for a possible contribution of LSD1 in distinguishing adaptive and maladaptive trajectories. The experiment was indeed timed to coincide with the peak of ubLSD1 induction in resilient animals, after the last stress session (Fig. 2E), maximizing the chance of capturing inherent downstream transcritpional effects. The first two principal components of the PCA clearly separated SUS mice from both RES and CTR groups (Fig. 5A), further validating our modified behavioral stratification at the transcriptional level. **An initial differential expression analysis [FDR < 0.25 and |log2(FC)| > 1.5], confirmed a marked transcriptional asymmetry in SUS mice, with a strong predominance of upregulated genes in both SUS vs CTR (97 out of 102 DEGs) and SUS vs RES (84 out of 86 DEGs) comparisons (Fig. 5B and Suppl. Table 2). This analysis provided a high-confidence core set of SUS-biased transcripts.** In contrast, RES mice showed a more balanced transcriptional response (28 upregulated and 16 downregulated genes), supporting the idea that resilience is not defined by a mere attenuation of stress-induced responses, rather by a qualitatively distinct program of regulation[69] **(Fig. 5B and Suppl. Table 2)**. To further characterize this divergence, we examined the expression of the DEGs in every sample (Fig. 5C) and found three covariant clusters: group A, comprising genes downregulated in both RES and SUS compared to CTR; group B—the most prominent—highlighting genes selectively upregulated in SUS mice and group C, including genes broadly upregulated in response to stress across both groups. These patterns defined a transcriptional signature of susceptibility and were consistent with a model of widespread transcriptional disinhibition. In contrast, a tempered expression profile in RES mice suggests the activation of buffering or repressive mechanisms. Among SUS-upregulated genes, we identified multiple candidates pointing to synaptic (*Sema3b*)[70], inflammatory (*A2m*)[71], neurohormonal (*Ttr*)[72], and vascular plasticity (*F5, Col8a2*)[73], highlighting the multifaceted nature of stress susceptibility. Conversely, RES-upregulated transcripts such as *Dusp1* and *Npas4* aligned with known resilience pathways[74].

To test whether SUS transcriptional changes overlapped with LSD1-dependent regulation, we used the published dataset from Agarwal et al., [75] which defines the transcriptional profile elicited by LSD1 (*Kdm1a*) knockdown (LSD1-KD) in primary mouse cortical neurons. Their discovery that LSD1-KD primarily affects activity-driven gene expression made this dataset particularly well aligned with our stress-induced SUS vs RES transcriptional framework. Remarkably, when we intersected our extended list of SUS vs RES DEGs (595

genes; $p < 0.05$, $|FC| > 0.25$) with the LSD1-KD dataset[75], 86 genes (≈14%) were found to be differentially expressed in both systems ($p = 10^{-10}$) (Fig. 5D). This substantial overlap, including genes that are either directly or indirectly regulated by LSD1, indicates that a sizeable component of the SUS-associated transcriptional drift is recapitulated by a perturbation of LSD1 levels in an independent mouse neuronal system. Interestingly, 25 genes (Fig. S5A, gene list) emerged at the three way intersection among *our* SUS vs RES DEGs, the *Iwase's lab* LSD1-KD responsive genes[75], and *Rosenfeld's lab* LSD1 ChIP-seq peaks in primary neurons[17]. These 25 genes out of the 86 that show convergent regulation in SUS mice and upon LSD1 depletion ($p = 0.045$), (i) discriminate stress susceptibility in vivo, (ii) respond to LSD1 loss of function in an orthogonal neuronal system, and (iii) are directly bound by LSD1 (Fig. S5A). Among these genes we underline *Tmem132d, Rarb* and *Myo* that were also graphically analyzed to show LSD1 occupancy (Fig. S5B). *Tmem132d*[76,77] was reportedly described as a psychiatric risk gene[78] mainly associated to anxiety and panic disoder, whose mRNA is increased in clinical cases; *Rarb*, another psychiatric risk gene[79] encodes the retinoic acid receptor beta, highly expressed in the hippocampus and cortex and already reported to functionally interact with LSD1[80]. *Rarb* modulates synapse shape through actin interaction and its expression is altered in depression and shizophrenia[81]; *Myo10* plays distinct and important roles in the development and function of synapses. The encoded protein, molecular motor myosin-X, localizes at the filopodia tips and regulates the structure of dendritic spines[82]. While not a classical psychiatric risk gene, *Myo10* has been implicated in processes central to stress-related circuit remodeling and has been associated with psychiatric vulnerability in selected genetic studies[83]. Collectively, these findings delineate a convergent LSD1-dependent transcriptional program that discriminates stress susceptibility from resilience across three integrated axes: cytoskeletal and dendritic spine remodeling, regulation of the extracellular matrix and perineuronal nets, and state-dependent transcriptional control. Together, these data implicate impaired engagement of the *MALAT1*–LSD1 module as a key determinant of maladaptive hippocampal transcriptional trajectories.

Upstream regulators and cross-regional transcriptional dynamics in stress susceptibility

To gain further insight into upstream regulatory mechanisms, potentially shedding light on deregulated molecular processes, we performed Qiagen Ingenuity® Pathway Analysis (IPA) on our DEGs (Fig. 5E). The analysis highlighted *ESR1, OTX2, COLQ, and IKBKB* as influential upstream regulators of SUS-restricted transcriptional drift. *ESR1*, encoding the estrogen receptor alpha (ER-α), emerged as the top upstream regulator. Notably, ER-α is known to

physically and functionally interact with LSD1, acting together with CoREST and HDACs as a core epigenetic regulator of ER-α–dependent transcription[84-87]. Consistently, up to 58% of ER-α–enriched promoters also display LSD1 binding across multiple cellular systems[86], supporting a model in which ER-α serves as a recruitment platform for LSD1 at specific genomic loci. Within this framework, the LSD1-dependent regulation of Rarb—itself a modulator of ER-α signaling[88]—may contribute to shaping *ESR1*-regulated transcriptional programs under stress.

Remarkably, ESR1 was independently identified as a key transcriptional driver of chronic stress responses in two independent RNA-seq datasets: one from the Nucleus Accumbens (NAc) of CSDS-stratified SUS and RES mice, analyzed 48 hours post-stress[89,90], and another from the hippocampus of adult rats previously exposed to adolescent chronic stress[91]. Again, in both studies, *ESR1* was the most significantly enriched upstream regulator. However, the direction of *ESR1* signaling modulation appeared area-dependent: it was upregulated in the hippocampus of our SUS mice and in the hippocampus of rats re-exposed to acute stress[91], but downregulated in the NAc of SUS mice[89].

Having underlined a significant role for *ESR1* as a potent regulator of chronic stress-induced transcriptional programs, we proceeded with a cross-regional comparison of all our hippocampal DEGs with transcriptional alterations reported in the inherent multi-region RNA-seq dataset published by Bagot et al.[90]. This allowed us to broaden the perspective from ESR1-controlled gene regulation to unbiased view of coordinated—or discordant—transcriptional dynamics under chronic stress. Notably, the differential expression profile of SUS vs RES mice in our hippocampal dataset showed again a striking correlation (Pearson's r=0,93) with that of the ventral hippocampus (vHIP) from the Bagot's dataset[90] at 48 hours post-stress (Fig. S5C, left panel), reinforcing the robustness of all these results. Encouraged by this concordance, we extended the analysis to other brain regions. Remarkably, the transcriptional profile of SUS vs RES in our hippocampus dataset was strongly anti-correlated with that of the nucleus accumbens (NAc) from the same study (Pearson's r = –0.81; Fig. S5C, right panel). Together with previous observations of coordinated vulnerability-related transcription between PFC and vHIP, and resilience-associated coupling between NAc and PFC[90], our data suggest that stress susceptibility may not solely reflect within-region gene expression programs, but also arise from antagonistic cross-regional transcriptional dynamics, particularly between the vHIP and NAc.

Discussion

Our study identifies alternative splicing of the LSD1 microexon E8a[15,16] in the mammalian hippocampus as a novel molecular discriminator of environmental stress resilience versus susceptibility. Across chronic social defeat stress[13], a defining feature of RES animals is the selective persistence of ubLSD1 upregulation, which emerges as a reproducible biomarker of the resilient state. By contrast, SUS mice consistently fail to engage this splicing response, drifting toward an amplified transcription of neuroplasticity-related genes. Notably, ubLSD1 lacks microexon E8a and represents the fully enzymatically competent, transcriptionally repressive isoform[25-28]. Accordingly, the sustained induction of ubLSD1 provides a mechanistic route contributing to resilience-relevant transcriptional control. We next asked what upstream signal could enforce ubLSD1/nLSD1 isoform switch over time. Our data converge on an activity-coupled mechanism centered on the lncRNA *MALAT1*[52,92]. We here show that, under acute stress, neuronal activation increases *MALAT1* and promotes its physical interaction with nSR100, thereby limiting nSR100-driven E8a inclusion and biasing splicing toward ubLSD1[37]. Critically, our results indicate that resilience requires this acute program[29,30] to be reiterated and stabilized during chronic stress. In SUS mice, *Malat1* levels are reduced compared with both CTR and RES animals, a deficit that is likely to propagate into an insufficient activity-dependent *Malat1* response, preventing effective nSR100 sequestration and ultimately compromising the ubLSD1 shift. In contrast, *Malat1* expression is preserved in RES animals, enabling repeated nSR100 buffering and sustained ubLSD1 availability within the post-stress window in which ubLSD1 physiologically peaks. Consistently, a similar downregulation of ubLSD1 and *MALAT1* is observed in postmortem hippocampal samples from suicide subjects, placing the *MALAT1*–LSD1 axis among the few molecular correlates that converge across species and levels of clinical severity, also molecularly supporting the idea that suicide may represent an extreme endpoint along a continuum of stress-related vulnerability[67,93-95]. Notably, *Malat1* has been deleted in mice proving its dispensability in the context of neurodevelopment[92]. These aspects are consistent with its role in fine-tuning the genome x environment (GxE) interaction warranting inherent investigation.

Failure of *MALAT1*-LSD1 axis maps cleanly onto the global transcriptional phenotype of the hippocampus. RNA-seq revealed a striking asymmetry in SUS animals, dominated by transcript upregulation and a paucity of downregulated genes, consistent with a state of insufficient transcriptional restraint and culminating in a hippocampal runaway transcription signature. Notably, this signature converges with recent independent human evidence indicating reduced availability or expression of class I histone deacetylases across stress-related brain disorders including schizophrenia[23] and bipolar disorder[24], but also

Alzheimer's disease[96], as revealed by postmortem analyses and in vivo *epigenetic imaging studies*. Together, these findings support the notion that impaired transcriptional restraint—rather than excessive repression—may represent a shared molecular denominator of vulnerability across psychiatric and neurodegenerative conditions. RES mice, in contrast, exhibited limited perturbation, compatible with an actively contained transcriptome. Importantly, while LSD1 dysregulation could in principle be epiphenomenal, three independent lines of evidence argue that it is functionally positioned to shape precisely this kind of activity-linked transcriptional imbalance. First, LSD1-knock down (LSD1-KD) in mouse neurons has been shown to preferentially derepress[75]—while a genetically doubled ubLSD1 dosage represses[15,17]—activity-regulated gene programs, converging with LSD1 acting as a gatekeeper of activity-driven transcription. Second, intersecting our CSDS hippocampal dataset with the Agarwal et al. LSD1-KD[75], revealed a substantial overlap of differentially expressed genes despite the different biological contexts (in vivo hippocampal tissue versus cultured cortical neurons), unravelling that a *LSD1-dependent module* is engaged in susceptibility. Within the higher-order physiology of the stress system, ESR1 has emerged as a convergent upstream driver of susceptibility-linked transcriptional programs[89,91,97,98]. Consistently, our IPA analysis of differentially expressed genes in SUS versus RES mice identified ESR1 as the principal upstream regulator of the observed transcriptomic changes. Notably, ESR1 downstream targets are repeatedly and robustly shifted in SUS mice across independent investigations[89,91,97,98], including the present study. However, in none of these datasets is ESR1 itself detectably deregulated. This apparent paradox provides a third, independent line of evidence supporting a *causal role for LSD1 in the resilience-oriented regulation of the stressed transcriptome*. The identification of LSD1 as a biomarker of chronic stress response offers a mechanistic resolution to the ESR1 paradox namely, how ESR1 can remain unchanged in its expression levels while actively reshaping its downstream transcriptional programs in response to stress. Indeed, LSD1 is a well-established ESR1 coregulator: ESR1 recruits LSD1 to chromatin, while ESR1-dependent transcription is, in turn, broadly shaped by LSD1-containing chromatin complexes[84-86,99]. Together, these findings delineate a *MALAT1*–nSR100–LSD1 axis that couples environmental stress response to splicing-dependent repression, whose failure yields runaway transcription and vulnerability, and whose preservation enables the resilient state.

An important aspect emerging from our data concerns the regional specificity of the *MALAT1*-mediated splicing mechanism. While acute stress engages a *MALAT1*–nSR100 response in both hippocampus and mPFC, chronic stress reveals a clear divergence between these regions. In the hippocampus, the splicing-mediated control of LSD1 is selectively preserved in RES animals, enabling sustained ubLSD1 upregulation at a defined

post-stress time window. In contrast, the mPFC progressively loses differential control under chronic stress: ubLSD1 is not induced in either RES or SUS animals, and *MALAT1* itself does not display stress-dependent modulation. Thus, the *MALAT1*–nSR100–LSD1 splicing axis that characterizes resilience is not globally engaged across stress-related brain regions but rather becomes selectively consolidated in the hippocampus.

A critical distinction emerging from our data concerns the biological meaning of nLSD1 reduction in resilient versus susceptible animals. While nLSD1 decreases in both phenotypes, the underlying mechanisms and functional consequences differ markedly. In RES mice, nLSD1 reduction accompanies a reciprocal, *MALAT1*-dependent shift toward ubLSD1, consistent with regulated alternative splicing and enhanced transcriptional restraint. In SUS mice, by contrast, nLSD1 loss occurs in the absence of compensatory ubLSD1 induction and is accompanied by a net reduction in total LSD1 transcripts, arguing against a regulated splicing switch and instead pointing to the engagement of parallel mechanisms selectively destabilizing the nLSD1 isoform. Support for the plausibility of such mechanisms comes from a primate-specific regulatory event recently described by our group[38], in which alternative splicing generates a 5' extension of exon E9 that introduces a premature stop codon and preferentially targets the exon E8a–including transcript to nonsense-mediated decay. Notably, although mechanistically distinct, this pathway also originates from alternative splicing within the same regulatory region of the LSD1 gene, underscoring the exceptional regulatory density of the E8–E9 intronic interval. This process is controlled by the splicing factor RbFOX1, a gene repeatedly implicated by large-scale genetic studies in psychiatric vulnerability, hyperexcitability, aggression, and epilepsy[38,45-47,100]. Together, these observations highlight the centrality of LSD1 splicing biology in psychiatric drift, emphasize the contribution of primate-specific regulatory layers to stress vulnerability, and point to impaired LSD1-dependent control of hippocampal excitability as a convergent mechanism linking stress, transcriptional dysregulation, and neuropsychiatric disease. In this framework, a modest yet reproducible induction of ubLSD1 in resilient animals delineates more than a molecular adjustment: it outlines a navigational map toward a distinct transcriptional state that moves beyond descriptive gene ontology classifications of susceptibility and resilience. The convergence on a compact set of 25 LSD1-associated genes—many of which have already been independently implicated in psychiatric vulnerability—defines a concentrated and biologically coherent substrate that merits focused future investigation, including from a therapeutic perspective. An important open question emerging from this work concerns the mechanisms governing MALAT1 regulation itself, which may represent a critical upstream determinant of stress-adaptive versus maladaptive transcriptional trajectories.

Data availability

Data are available upon request. Sequencing data can be found at ArrayExpress under the accession number E-MTAB-15221.

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Competing interests

The authors report no competing interest.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Acute Social Defeat Stress (ASDS) engages LSD1 splicing and *Malat1* activity-dependent transcription. On top a schematic representation of the primer design for LSD1 isoform. qPCR analysis in the mouse hippocampus following a single session of Social Defeat Stress (SDS) of (A) ubLSD1, nLSD1, and total LSD1, (B) *nSR100*, (C) *Malat1*, and (D) Immediate Early Genes (IEGs; *Egr1, Npas4,* and *c-Fos)* all expression normalized to RPSA. Data are presented as mean ± SEM. Statistical significance: p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****); one-way ANOVA for multiple-group comparisons.

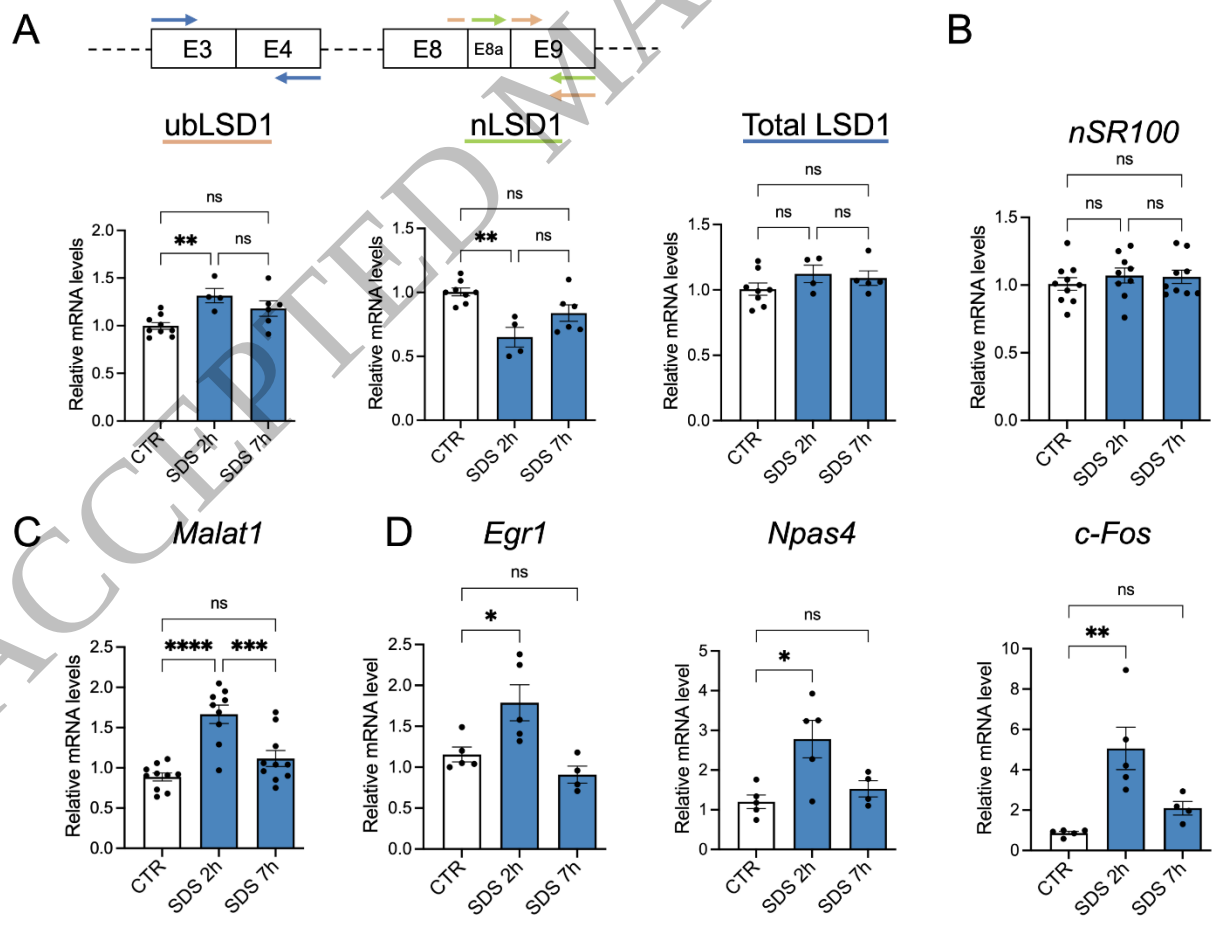

Figure 2 Chronic Social Defeat Stress (CSDS) paradigm and LSD1 splicing regulation in stress susceptibility and resilience. (A) CSDS experimental timeline. Schematic representation of the CSDS protocol, including alternating stress sessions (red) and home-cage recovery periods (blue). (B) Schematic representation of the Social Interaction Test (SIT) arena (40 × 40 × 40 cm). The social target is placed in a wire-mesh enclosure (10 × 10 × 7 cm). Two behavioral areas are defined: the interaction zone (green; 24 × 9 cm) surrounding the social target and the avoidance corners (red; 9 × 9 cm) opposite to it. (C) SIT behavioral results showing time spent in the interaction zone (left) and avoidance corners (right) during the SIT, comparing CSDS mice (n = 30) with controls (CTR, n = 20). (D) Social Ratio (SR), defined as the ratio between total interaction time and the sum of interaction and avoidance times; identifying 15 resilient (RES) and 15 susceptible (SUS) animals within the CSDS group. (E) Hippocampal expression of LSD1 isoforms in defeated mice. From left to right: qPCR relative quantification of ubLSD1, nLSD1, and total LSD1 at both the 5' and 3' ends of the Lsd1 transcript, normalized to RPSA, in SUS (n = 15) and RES (n = 15) mice compared with undefeated controls (CTR, n = 15). On the top: primer location on LSD1 transcript is shown. (F) High ubLSD1 levels predict resilient behavior. Interaction and avoidance time of defeated mice (SUS and RES) and controls, with animals stratified into High and Low groups based on ubLSD1 expression levels. (G) qPCR relative quantification of *Malat1* and *nSR100* expression normalized to RPSA. Data are presented as mean ± SEM. Statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****); Student's t-test for pairwise comparisons; one-way ANOVA for multiple-group comparisons.

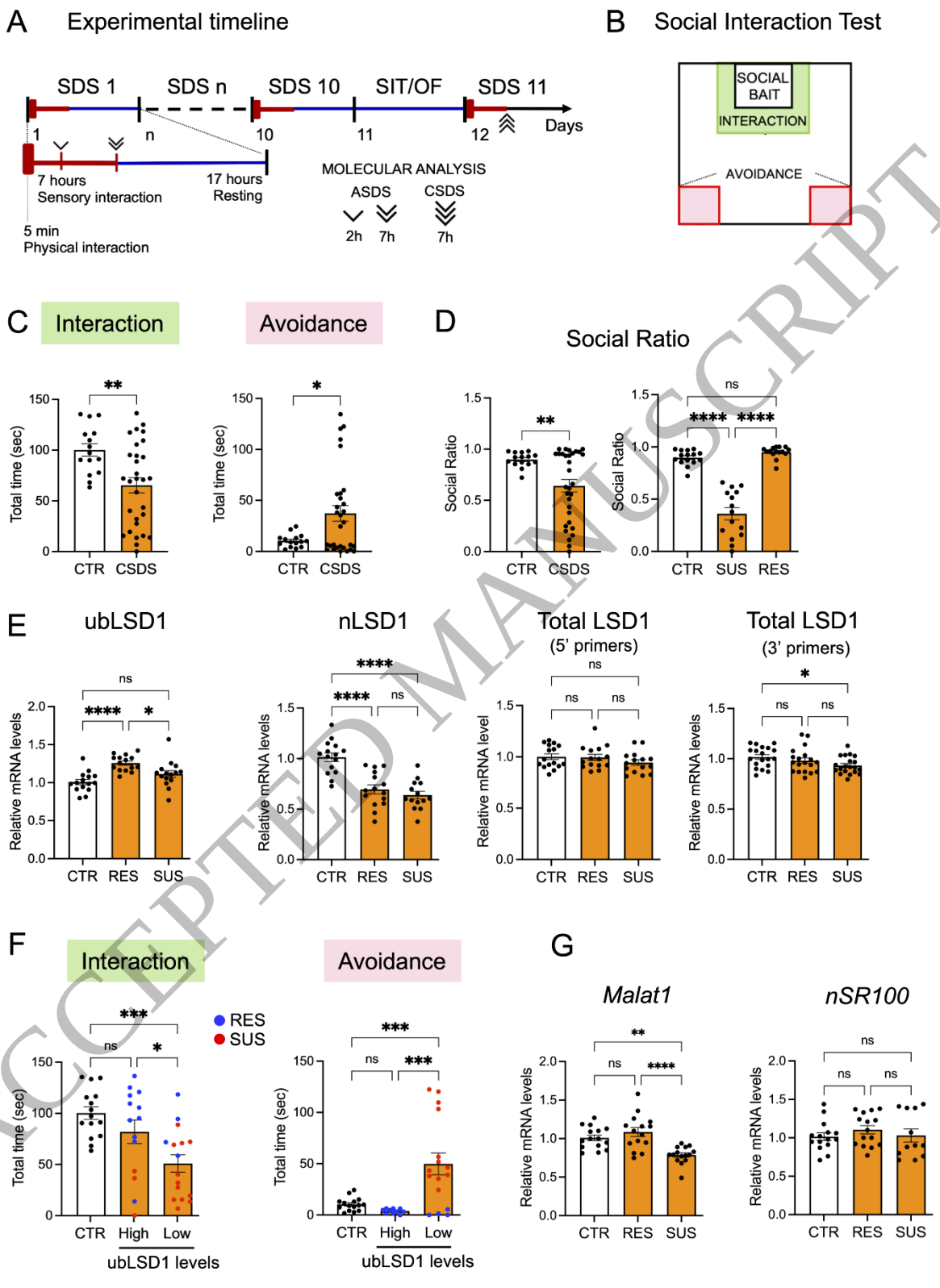

Figure 3 *Malat1* and *nSR100* interplay in LSD1 splicing regulation and neuronal activation. Re-analysis of the PAR-iCLIP dataset[63] in (A) human HEK293T cells and (B) mouse N2a cells. Upper panels show reads mapping to *MALAT1* genomic sequences; lower panels show reads mapping to *REST/NRSF* genomic sequences, confirming *REST* as a primary nSR100 RNA target, as reported in the original study[63]. (C) RNA-CLIP experiment performed by UV crosslinking followed by Flag-M2 immunoprecipitation of nSR100-bound RNAs, revealing *Malat1* enrichment under basal conditions and after KCl stimulation. (D) LSD1 exon E8a minigene reporter assay in SH-SY5Y cells following *MALAT1* overexpression. The percentage of exon E8a skipping, resulting in exon 8–9 junctions, is reported. (E) Generation of a mouse *Malat1* knockdown (*Malat1*-KD) model using *Malat1*-targeting antisense oligonucleotides (ASO) or scrambled control (SCRA), followed by Acute Social Defeat Stress (ASDS; red or blue) or no-stress conditions (outlined red or blue). Animals were sacrificed 7 h after stress exposure for molecular analyses. qPCR analysis of *Malat1* expression in the hippocampus across experimental groups to validate ASO efficacy at the injection site. (F) rqfRT-PCR analysis expressed as percentage of ubLSD1 in injected hippocampi to assess stress-induced splicing dynamics. Data are presented as mean ± SEM. Statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****); Student's t-test for pairwise comparisons; two-way ANOVA for multiple-group comparisons.

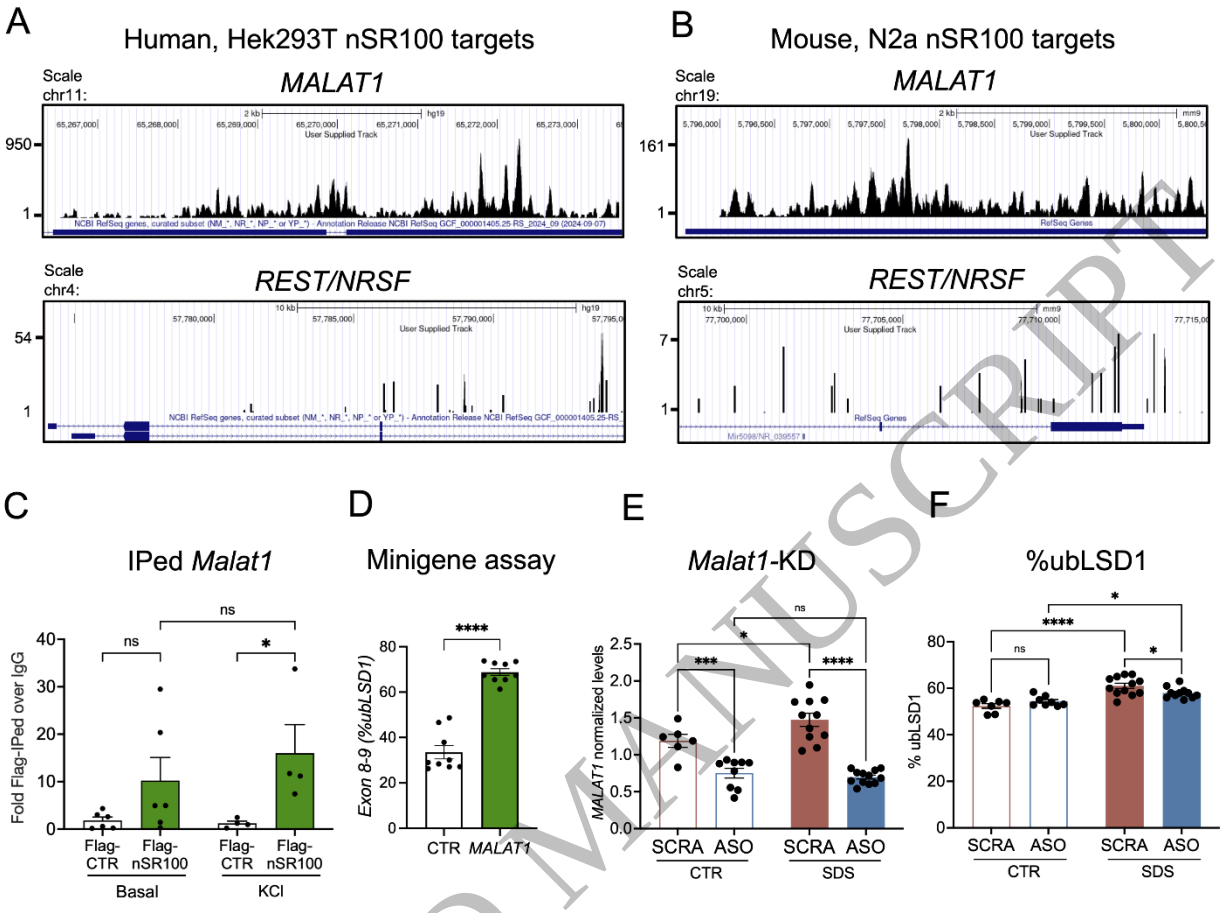

Figure 4 LSD1 alternative splicing and *MALAT1* expression in postmortem hippocampal samples from male suicide victims and controls. LSD1 alternative splicing and MALAT1 expression were analyzed in postmortem hippocampal samples from male suicide victims (SD) and matched controls (CTR). (A) LSD1 splicing trajectory across aging. Left: linear regression analysis of ubLSD1 levels (rqfRT-PCR, expressed as percentage of ubLSD1) in CTR (green) and SD (red) showing age-dependent ubLSD1 regulation. Right: levels ubLSD1 measured by rqfRT-PCR in all SD versus CTR samples. (B) Western blot analysis of total LSD1 protein levels. Left: quantitative comparison between SD and CTR samples. Right: representative SDS–PAGE gel. (C) *MALAT1* expression across aging. Left: linear regression analysis of *MALAT1* expression levels in CTR (green) and SD (red) showing no age-related expression regulation. Right: relative *MALAT1* mRNA levels in CTR versus SD individuals normalized to *RPL13*. Data are presented as mean ± SEM. $p < 0.05$ (*); Student's t-test for pairwise comparisons.

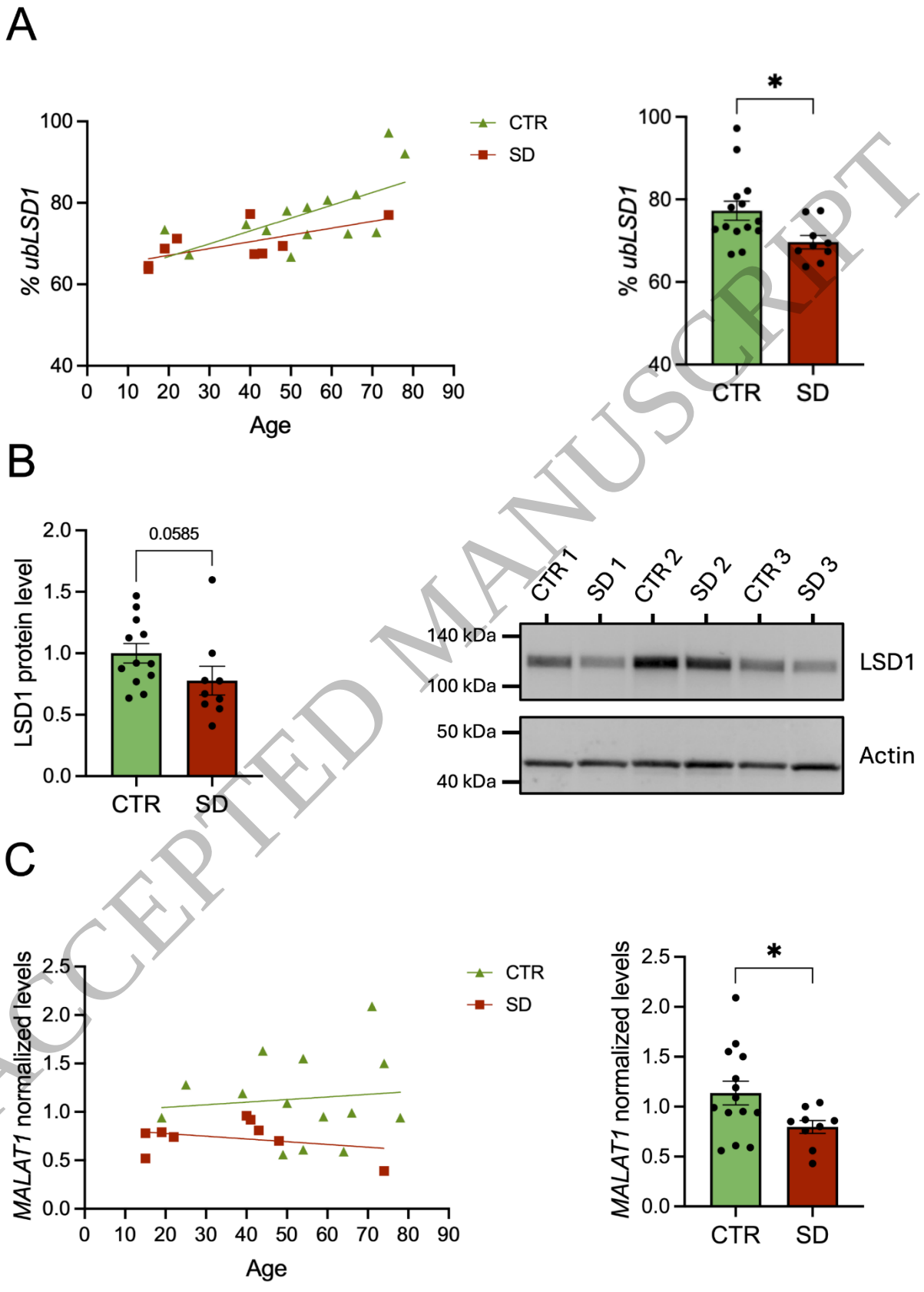

Figure 5 Transcriptomic profiling of stress susceptibility and resilience depicts LSD1 contribution to resilience. (A) Principal component analysis (PCA) showing the first two principal components (PC1 and PC2) of control (CTR), resilient (RES), and susceptible (SUS) samples. (B) Volcano plot depicting log2 fold changes (x-axis) versus false discovery rate (FDR, q-value; y-axis) in RES vs CTR, SUS vs CTR and SUS vs RES. Significantly up- and downregulated genes (FDR < 0.25 and |log2FC| > log2(1.5)) are indicated in black. (C) Heatmap of differentially expressed genes (DEGs). Z-scored gene expression values are shown for all samples across the three comparisons on the right, with genes grouped according to shared expression patterns (group A, group B and group C). (D) Upper panel: Venn diagram showing the overlap between genes differentially expressed in the SUS versus RES comparison (595 genes; $p < 0.05$, $|FC| > 0.25$) and genes reported as differentially expressed in an LSD1 knockdown model[75]. Lower panel: Venn diagram illustrating the intersection among SUS versus RES DEGs, LSD1 knockdown–responsive genes, and LSD1-bound genes identified by ChIP-seq[17]. Overrepresentation analysis was performed using a hypergeometric test. (E) Ingenuity Pathway Analysis (IPA) heatmap of top upstream regulators identified in the DEG dataset (RES vs CTR, RES vs CTR and SUS vs RES), highlighting regulators involved in stress adaptation and neuronal plasticity, ranked by activation Z-score in the SUS versus RES comparison.