**Response to Reviewers**

Our responses to each of the review comments are detailed point-by-point, below.

**Reviewer #1:**

We thank Reviewer #1 for the helpful and positive comments that have enabled us to improve our manuscript. In the revised version of our paper we have incorporated all of the comments and suggestions made, as detailed below.

Comment#1

*The authors should consider mentioning in the title that this is a resting state fmri study using fALFF or investigating the amplitude of low frequency oscillations. This would add significance and appeal to the manuscript.*

We added the information as requested to the title of the revised manuscript.

Comment#2

*I would encourage the authors to highlight the reliability and clinical relevance of both resting fMRI and f/ALFF. Examples of ways to expand the introduction/conclusion would be to mention that:*

*1) Resting fMRI has been shown to be related to cognitive performance. How could this relate to mood regulation/ reactivity for instance?*

*2) In rsfMRI the brain regions are compared to each other to determine if there are synchronized changes of activation over time. Because of their common behavior, these regions are believed to be functionally connected but do not need to be structurally connected.*

*3) Connect the current findings to the concept of default mode network as this network of brain regions becomes active when participants are at wakeful rest and disengage otherwise.*

We thank Reviewer #1 for this helpful suggestion. We have highlighted the reliability and clinical relevance of both resting fMRI and f/ALFF by expanding the Introduction (from line 13 of page 3 to line 7 of page 4) and Discussion (page 17, lines 18–23; page 18, lines 22–24), as suggested.

Added references:

Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 2007; **8**: 700–11.

Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H et al. Resting–state functional connectivity in major depression abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biol Psychiat* 2007; **62**: 429–37.

Kiviniemi V, Jauhiainen J, Tervonen O, Pääkkö E, Oikarinen J, Vainionpää V, Biswal B. Slow vasomotor fluctuation in fMRI of anesthetized child brain. *Magnet Reson Med* 2000; **44**: 373–8.

Mennes M, Zuo XN, Kelly C, Martino A, Zang YF, Biswal B *et al*. Linking inter–individual differences in neural activation and behavior to intrinsic brain dynamics. *Neuroimage* 2011; **54**: 2950–9.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gunsnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U S A* 2001; **98**: 676–82.

Wang L, Hermens DF, Hickie IB Lagopoulos J. A systematic review of resting–state functional–MRI studies in major depression. *J Affect Disorders* 2012; **142**: 6–12.

Yang Z, Jutagir DR, Koyama MS, Craddock RC, Yan CG, Shehzad Z *et al*. Intrinsic brain indices of verbal working memory capacity in children and adolescents. *Dev Cogn Neurosci* 2015; **15**: 67–82.

Comment#3

*–A number of methods have been developed for analyzing rs–fMRI data. Independent component analysis (ICA) and region of interest (ROI)–based FC are the two most common approaches used in rs–fMRI studies. How does the methodology used in this study compare to previous resting fMRI studies in the mood spectrum disorders?*

We thank Reviewer #1 noting this important issue. ICA and ROI-based FC measure temporal correlations between low-frequency oscillations among distant brain regions. However, these two methods do not provide direct information regarding regional brain features. Local brain activity can be assessed by fALFF, as used in the present study.

We have added descriptions of the features of these methods and the interpretation of results obtained via these different methodologies to the Introduction (from line 13 of page 3 to line 7 of page 4) and Discussion (from line 21 of page 15 to line 23 of page 16; page 17, lines 18–23).

Comment#4

*–Previous studies mention that the increased specificity to the gray matter signal for f/ALFF compared to ALFF may suggest favoring the former, but doing so would reduce test–retest reliability. Thus reporting both measures is recommended. Could the authors explain why they did not report ALFF?*

There are three reasons why we did not report ALFF. First, previous studies have revealed superior specificity for the gray matter signal for fALFF compared to ALFF. Second, previous studies have revealed adequate test-retest stability for fALFF and ALFF. Third, we were particularly interested in the brain activity of the DMN because several studies have revealed altered spontaneous neural activity in the DMN in the resting-state in patients with major depression (see page 10, lines 9–18).

However, despite the aforementioned reasons, it is important and useful to report ALFF results. Therefore, we now report same in supplementary tables (supplementary Tables 2 and 3) and we have added further explanation to the Methods (page 11, lines 5–9) and the limitations section of the DISCUSSION (page 20, lines 5–10).

Comment#5

*–could the authors describe the entire imaging protocol, i.e. what kind of sequences, duration of the scanning session.*

We describe the entire imaging protocol as requested in the revised manuscript (page 9, lines 9–14).

Comment#6

*–Did the authors consider correlating fALFF to the MSM score (severity of treatment resistance in the TRD group) to determine if there was a linear/non-linear connection between the two measures.*

We thank Reviewer #1 for this important recommendation. Accordingly, we conducted additional analyses, as described below.

We conducted two statistical analyses to test for associations between fALFF and MSM for brain regions that exhibited statistically different spontaneous neural activity according to TRD > non-TRD and TRD < non-TRD contrasts:

1) Pearson’s correlation coefficient (*r*)

We calculated partial correlations using R (version 3.1.1 for windows) and ppcor, removing the effect of duration of current illness as suggested by Reviewer #2. Statistical significance was set at *P* < 0.05 (two-tailed). **No significant correlations were detected** (inferior frontal gyrus triangular part: *r* = 0.083, *P* = 0.765; middle occipital gyrus: *r* = −0.275, *P* = 0.303; thalamus: *r* = 0.201, *P* = 0.459; supramarginal gyrus: *r* = −0.332, *P* = 0.204; vermis, *r* = 0.143, *P* = 0.521).

2) Maximal information coefficient (MIC)

We calculated MIC, again using R (version 3.1.1 for windows). MIC captures a wide range of linear/non-linear associations (Reshef *et al*., 2011). We obtained *P-*values for a sample size of 20 from the following website: http://www.exploredata.net/Downloads/P-Value-Tables. Statistical significance was set at *P* <0.05. **No significant associations were detected** (inferior frontal gyrus triangular part, MIC = 0.138; middle occipital gyrus, MIC = 0.138; thalamus, MIC = 0.219; supramarginal gyrus, MIC = 0.311; vermis, MIC = 0.311).

We have added these analyses to the revised manuscript (page 12, lines 12–23 and to the Results on page 15, lines 1–9).

Added reference:

Reshef DN, Reshef YA, Finucane HK, Grossman SR, McVean G, Turnbaugh PJ *et al*. Detecting novel associations in large datasets. *Science* 2011; **334**: 1518–24.

Comment#7

*–page 10: "DPARSF": please explain this acronym and provide reference.*

As requested, we now explain the acronym and provide a reference (page 10, lines 7–9).

Comment#8

*–In the discussion the authors should highlight the importance of the reported altered brain circuits in terms of cognitive and emotional function.*

According to the reviewer’s suggestion, we now further discuss and highlight the importance of the reported altered brain circuits in terms of cognitive and emotional function in the Discussion of the revised manuscript (page 16, lines 6–23; page 17, lines 7–12; from line 22 of page 20 to line 3 of page 21).

Added references:

Alexander GE, Delong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci* 1986; **9**: 357–81.

Goldin PR, McRae K, Ramel W, Gross JJ. The neural bases of emotion regulation: reappraisal and suppression of negative emotion. *Biol Psychiatry* 2008; **63**: 577–586.

Ochsner KN, Gross JJ. Cognitive emotion regulation insights from social cognitive and affective neuroscience. *Curr Dir Psychol Sci* 2008; **17**: 153–8.

Price JL, Drevets WC. Neural circuits underlying the pathophysiology of mood disorders. *Trends Cogn Sci* 2012; **16**: 61–71.

Strakowski SM, Adler CM, DelBello MP. Is depression simply a nonspecific response to brain injury? *Curr Psychiatry Rep* 2013; **15**: 1–9.

Comment#9

*–The clinical relevance of these findings in terms of preventive intervention and personalized treatments should be discussed.*

According to the reviewer’s suggestion, we now further discuss the clinical relevance of our findings in terms of preventive intervention and personalized treatments in the Discussion section of the revised manuscript (page 21, lines 3–10).

Added reference:

Vanhaudenhuyse A, Noirhomme Q, Tshibanda LJF, Bruno MA, Boveroux P, Schnakers C *et al*. Default network connectivity reflects the level of consciousness in non–communicative brain–damaged patients. *Brain* 2010; **133**: 161–171.

Comment#10

*–Future research directions in terms of diagnosis, prevention treatments could be discussed too.*

We further discuss future research directions in terms of diagnosis and preventive treatments in the Discussion section of the revised manuscript (page 20, lines 18–21).

Comment#11

*–Minor comment: I noticed a few typos and grammar mistakes. Please proofread before resubmission.*

We thank Reviewer #1 for pointing out that these issues exist in the text. We have proofread the manuscript and corrected the errors.

**Reviewer #2:**

We thank Reviewer #2 for the pertinent comments regarding our study of neural mechanisms of treatment resistance in MDD.

Comment#1

*1. The authors did not report some important clinical features in both TRD and non-TRD group (age of onset, duration of current episode), of which limited the interpretability of the results. The primary result (altered activity in thalamus and correlation to HRSD) might partially reflect different chronicity in non-TRD.*

We apologize for not including sufficient detail regarding clinical features for both TRD and non–TRD groups. We have included all such details in the revised manuscript (page 13, lines 4–6; Table 1).

We compared the age of onset and duration of current episode (month) between TRD and non-TRD groups. The duration of the current episode (in months) was significantly longer in the TRD group.

Because we could not control for the duration of the current episode in the ANOVA, we further conducted a partial correlation analysis between fALFF and HRSD17 change in the non–TRD group. Our primary results hold after adjusting for the duration of the current episode. We have added these details to the Methods (page 12, lines 8–12) and Results (page 14, lines 15–23) sections in the revised manuscript.

Comment#2

*2. I've noticed that patients have moderate severity (mean HRSD-17 scored 15.4 in non–TRD, and 13.6 in TRD), in my experience TRD usually got 17 or higher on HRSD-17. Is there any possibility that some of the subjects are not representative?*

As Reviewer #2 points out, the symptom severity of the TRD patients in the present study might be milder than in a representative sample of patients with TRD. Although the patients recruited for the present study met the established criteria for TRD (Thase and Rush, 1997), it remains possible that some of the subjects were not representative. We extensively discuss this issue in the limitations section of the DISCUSSION in the revised manuscript (page 20, lines 10–13).

Comment#3

*3. Voxel–wise analysis should correct for multiple comparison.*

We used the thresholds recommended by Lieberman (2009) to minimize Type I errors, while also balancing the potential risk of Type II error. However, as pointed out, the inability to correct for multiple comparisons is a concern. Therefore, our findings must be considered preliminary. We note this issue as a limitation of the present study in the Discussion section of the revised manuscript (page 20, lines 13–15).

Comment#4

*4. The author should fully report the correlation analysis between the fALFF in identified clusters and HRSD reduction rate, not only in right thalamus.*

According to the suggestion, we now include the results of the correlation analysis between the fALFF in detected whole clusters and HRSD reduction in the revised manuscript (page 14, lines 15–23).

Comment#5

*Minor: 1. The word "neural activation" usually implies a task–fMRI design, it is not proper in the context of resting–state, please change to "neural activity" throughout the manuscript.*

We thank Reviewer #2 for pointing this out. We have replaced the word “neural activation” with “neural activity” throughout the manuscript.

Comment#6

*2. Discussion, page 16 line 2, "The right IFG is thought to have 3 major functions" is not accurate. Right IFG plays crucial role in those mentioned neuropsychological functions, however they are not completely localized here. Please change the statement.*

We have changed the statement as requested in the revised manuscript (page 17, lines 13–15).

**Reviewer #3:**

We are grateful to Reviewer #3 for the helpful and positive comments regarding our study, and we appreciate the comment As indicated in the responses below, we have incorporated all of the comments and recommendations in revised our manuscript.

Comment#1

*1.The title of this article seems to investigate the association of thalamic hyperactivity with treatment resistant depression. The thalamus was pointed out in the title. However, there are few description of the association between thalamus and depression in Introduction section. The authors should add more literatures and medical evidence relative to this topic. This association should be described in the whole article including the introduction, purpose, results, discussion and conclusion sections.*

Thank you very much for your suggestion. We now describe evidence of associations between the thalamus and depression in the Introduction (lines 9–11 on page 4 and lines 8–11 on page 5), Results (lines 15–23 of page 14), and Discussion (from line 21 of page 15 to line 23 of page 16), as suggested.

Added references:

Alexander GE, Delong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci* 1986; **9**: 357–81.

Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L *et al*. Antidepressant effect on connectivity of the mood–regulating circuit: An FMRI study. *Neuropsychopharmacol* 2005; **30**: 1334–44.

Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H et al. Resting-state functional connectivity in major depression abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biol Psychiat* 2007; **62**: 429–37.

Hamilton JP, Etkin A, Furman DJ, Lemus MG, Johnson RF, Gotlib IH. Functional neuroimaging of major depressive disorder: a meta–analysis and new integration of baseline activation and neural response data. *Am J Psychiat* 2012; **169**: 693–703.

Price JL, Drevets WC. Neural circuits underlying the pathophysiology of mood disorders. *Trends Cogn Sci* 2012; **16**: 61–71.

Strakowski SM, Adler CM, DelBello MP. Is depression simply a nonspecific response to brain injury? *Curr Psychiatry Rep* 2013; **15**: 1–9.

Tadayonnejad R, Yang S, Kumar A, Ajilore O. Clinical, cognitive, and functional connectivity correlations of resting–state intrinsic brain activity alterations in unmedicated depression. *J Affect Disorders* 2015; **172**: 241–50.

Comment#2

*2.The results show that in the right thalamus, mean fALFF values and percent change in HRSD17 scores were negatively correlated (r = −0.52, P = 0.39; Figure 2) in patients with non-TRD. The correlation coefficient, r, is small for a small sample size. In statistics, we could conclude that the correlation has statistically significance if P < 0.05. However, the P value in the results is greater than 0.05. The authored should propose more data to prove the negative correlation between mean fALFF values and percent change in HRSD17 scores in the right thalamus.*

We appreciate you pointing out this issue. The *P*-value listed in the original manuscript was incorrect. As suggested by Reviewer #2, we repeated the correlation analysis, but with clinical variables used as covariates. We list the *P*-values for the partial-correlation analysis regarding the thalamus and another identified clusters as suggested by Reviewer #2 (from lines 15–23 of page 14).

Comment#3

*3.The subjects' age has a wide range from 25 to 75 years old. Is the spontaneous neural activity in brain regions different among age levels?*

Thank you for this suggestion. Although actual range of ages in this study was 25–67, and we conducted an ANOVA that adjusted the effect of age in the original manuscript, we now provide further explanation of why we partialled out the aging effect in the Method (page 12, lines 2–5) and we conducted further analyses, as follows:

1) We conducted a regression analysis for age.

2) We conducted an ANOVA masked by each age contrast. The statistical criterion for significance was set at *Puncorrected* < 0.005, k ≧ 10.

3) Subsequently, none of the brain regions overlapped. This suggests that our analyses successfully removed the age effect.

4) Below, we report the results of 1) and 2) described above.

1) Positive correlations: Postcentral gyrus (Right), vermis 8/cerebellum cluster 2 (bilaterally), cerebellum cluster 1/cluster 2 (left).

2) Negative correlation: Caudate (left), caudate (right), superior temporal gyrus (left), superior temporal gyrus/heschl (right), rolandic operculum (right), superior temporal gyrus (left), parahippocampal (right), middle cingulum (right).

Added reference:

Hu S, Chao HHA, Zhang S, Ide JS, Li CSR. Changes in cerebral morphometry and amplitude of low–frequency fluctuations of BOLD signals during healthy aging: Correlation with inhibitory control. *Brain Struct Funct* 2014; **219**: 983–94.

**Reviewer #4:**

We are grateful to Reviewer #4 for the helpful and positive comments regarding our study, and we welcome the comment that “This paper tackles an important area, namely treatment resistant depression. …Nevertheless, the findings are of interest though they need to be far more speculative than that presented. …By extrapolating the findings in terms of their clinical value and application and how they can be interpreted in the context of extant models, I feel the paper would have some greater value to add”.

Below, we review the specific concerns and recommendation for revision. We have taken all these comments and recommendations into account when revising our manuscript.

Comment#1

*The authors have limited themselves to resting state analyses and, given the complexity of treatment response, it would perhaps have been useful to have coupled this with a task related activation of regions and networks of interest, particularly those involved in adaptive emotional responsivity.*

We now discuss the adaptive emotional circuit and the need for comprehensive research combining resting state neural activity, task related activation, and network analysis to reveal alterations in emotion regulation in patients with TRD in the Discussion (page 17, lines 7–12; page 20, lines 15–18).

Added references:

Ochsner KN, Gross JJ. Cognitive emotion regulation insights from social cognitive and affective neuroscience. *Curr Dir Psychol Sci* 2008; **17**: 153–8.

Goldin PR, McRae K, Ramel W, Gross JJ. The neural bases of emotion regulation: Reappraisal and suppression of negative emotion. *Biol Psychiatry* 2008; **63**: 577–586.

Comment#2

*This may not have been possible, but the authors should allude to the fact that, though this is a novel study, it is perhaps not the most optimal design. Nevertheless, the findings are of interest though they need to be far more speculative than that presented. On the basis of such small numbers, the suggestion that this is a trait is ambitious and over–reaching.*

Thank you very much for your comment. We describe further the design limitations in the Discussion (page 19, lines 17–19; page 20, lines 1–5).

Comment#3

*The fact that those that responded and do not respond have common mechanisms is not a surprise; it is the separation and distinction that is key.*

Thank you for your critical comment. We did not make clear that the key point of this study was to discriminate patients with possible future TRD in the early phase of treatment. We have deleted the description of the common mechanisms between TRD and MDD from the Abstract, from the purpose in the Introduction, and from the summary in the Discussion.

Comment#4

*It would have been useful for the authors to contextualise some of these findings further within recognised models. For example, that by Pizzagalli et al and Ochsner and Gross, perhaps also alluding to research conducted by Strakowski and Phillips. By extrapolating the findings in terms of their clinical value and application and how they can be interpreted in the context of extant models, I feel the paper would have some greater value to add. Otherwise it is a relatively narrow preliminary finding.*

We are grateful for your critical and helpful comment. In the original manuscript, we did not discuss extant models and the importance of the discovery of emotion regulation neural circuits. We describe further research on this topic, including in the context of our results in the Discussion in the revised manuscript (from line 21 of page 15 to line 13 of page 16; page 17, lines 7–12).

Added references:

Alexander GE, Delong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci* 1986; **9**: 357–81.

Goldin PR, McRae K, Ramel W, Gross JJ. The neural bases of emotion regulation: Reappraisal and suppression of negative emotion. *Biol Psychiatry* 2008; **63**: 577–586.

Ochsner KN, Gross JJ. Cognitive emotion regulation insights from social cognitive and affective neuroscience. *Curr Dir Psychol Sci* 2008; **17**: 153–8.

Price JL, Drevets WC. Neural circuits underlying the pathophysiology of mood disorders. *Trends Cogn Sci* 2012; **16**: 61–71.

Strakowski SM, Adler CM, DelBello MP. Is depression simply a nonspecific response to brain injury? *Curr Psychiatry Rep* 2013; **15**: 1–9.