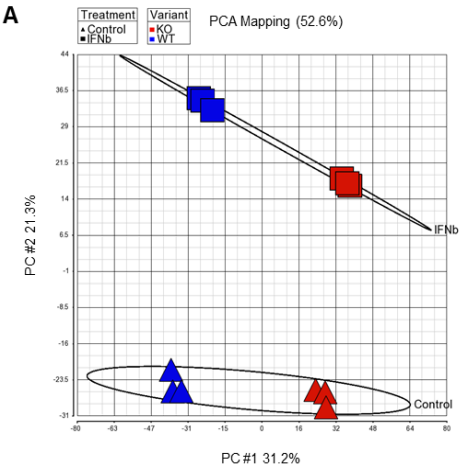
SUPPLEMENTAL FIGURES

A



B

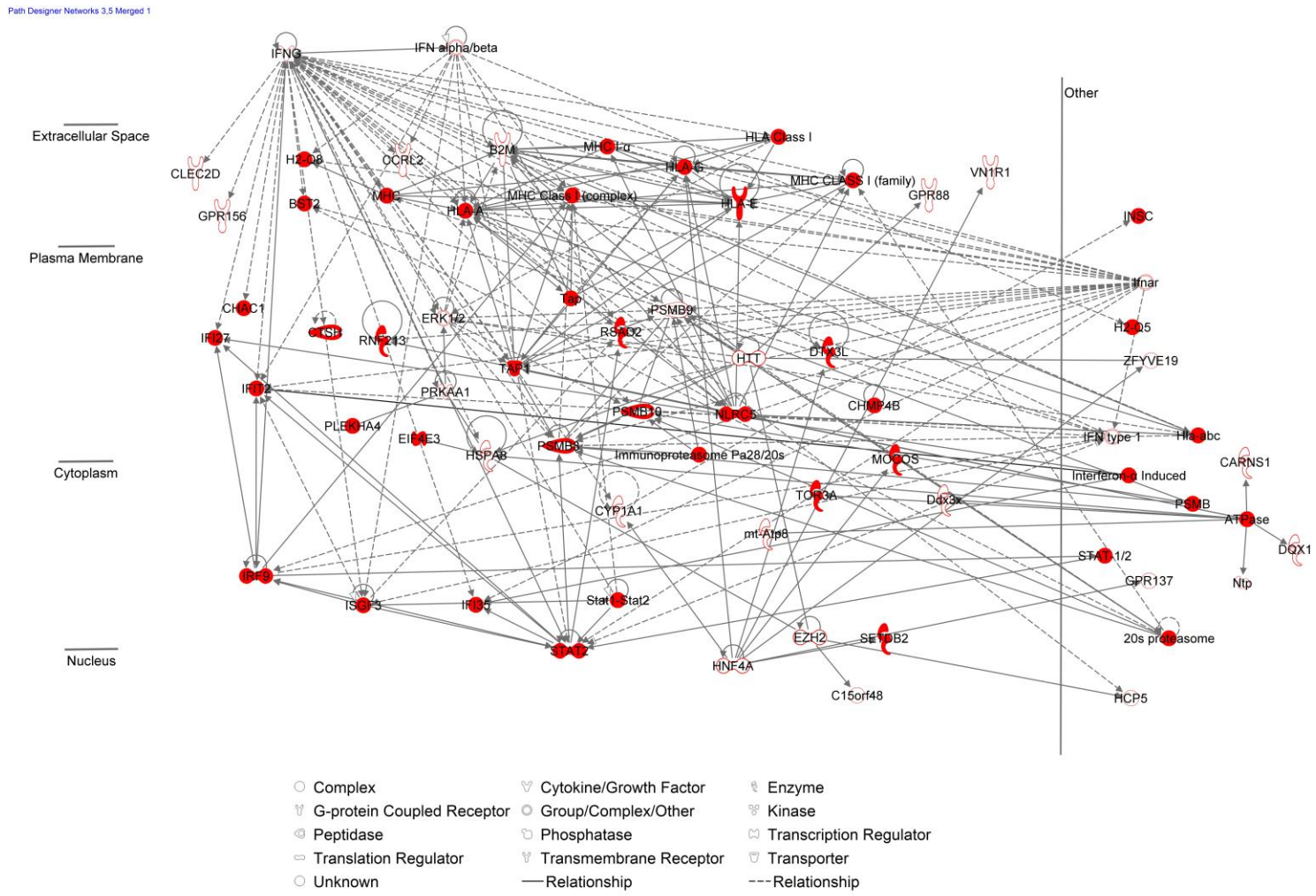
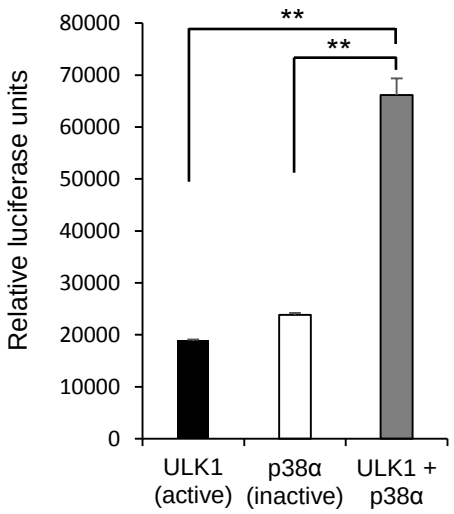


Figure S1, Related to Figure 2. Gene networks and associations for Ulk1/2-dependent IFN-inducible genes. (A) Principal component analysis (PCA) plot of microarray data generated from transcriptomes of Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs. The PCA shows reproducibility of all samples and clear separation between experimental groups. Three biological replicates cluster together, for control (untreated) and mouse IFNβ-treated (IFNβ) Ulk1/2$^{+/+}$ (WT) and Ulk1/2$^{-/-}$ (KO) MEFs. (B) IPA Gene network graphical representation of the molecular relationships between genes and the gene products among the 215 genes the expression of which is either defective or significantly decreased in Ulk1/2$^{-/-}$ MEFs after mouse IFNβ treatment. Genes or gene products are represented as nodes, and biological relationships between two nodes are represented as an edge (line). All edges are supported by canonical information stored in the Ingenuity® knowledge database. The intensity of the node color indicates the degree of expression after IFNβ treatment. Nodes are displayed using various shapes that represent the functional class of the gene product.



<u>Figure S2, related to Figure 5.</u> p38α MAPK is a substrate for ULK1 kinase *in vitro*. ULK1 kinase activity was measured using an ADP-Glo kinase assay. Kinase reactions were carried out using human ULK1 recombinant active protein (ULK1 active) and human p38α MAPK recombinant inactive protein (p38α inactive) alone or in combination (ULK1+p38α). Data are expressed as relative luciferase units, which are proportional to the ADP concentration produced after each kinase reaction. Bar graphs show means ± SE of three independent experiments, each performed in technical triplicates. Statistical analysis was performed using ANOVA followed by Tukey's test (**$p < 0.01$). Note: Human p38α MAPK recombinant inactive protein is unable to induce autophosphorylation or phosphorylation of other proteins.

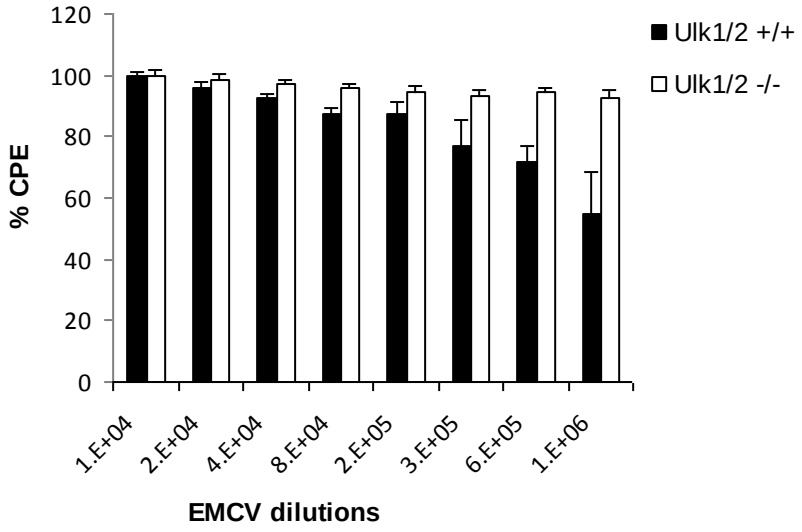


Figure S3, Related to Figure 6. Ulk1/2$^{-/-}$ MEFs are more sensitive to viral infection compared to Ulk1/2$^{+/+}$ MEFs. Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs were challenged with increasing dilutions of encephalomyocarditis virus (EMCV). EMCV-induced cytopathic effects (CPE) were determined 24 hours later. The data are expressed as percent of CPE, and bar graphs represent means ± SD.

SUPPLEMENTAL TABLES

Table S1, related to Figure 2. List of ISGs induced in both Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs after IFNβ treatment. Fold change and *p* values after FDR between treated and untreated samples for each cell line are presented. The genes for which differences in fold change between the Ulk1/2$^{-/-}$ (IFNβ vs. untreated) and Ulk1/2$^{+/+}$ (IFNβ vs. untreated) groups are greater than 2 are highlighted in red. The genes for which differences in fold change between the Ulk1/2$^{-/-}$ (IFNβ vs. untreated) and Ulk1/2$^{+/+}$ (IFNβ vs. untreated) groups are lower than -2 are highlighted in green. Provided as an Excel file.

Table S2, Related to Figure 2. List of ISGs induced only in Ulk1/2$^{+/+}$ MEFs after IFNβ treatment. Fold change and *p* values after FDR between treated and untreated samples are presented. Provided as an Excel file.

Table S3, Related to Figure 2. List of ISGs induced only in Ulk1/2$^{-/-}$ MEFs after IFNβ treatment. Fold change and *p* values after FDR between treated and untreated samples are presented.Provided as an Excel file.

Table S4, Related to Figure 2. Gene ontology analysis of common genes induced in Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs after IFNβ treatment. Genes identified in Table S1 as presenting a higher induction after IFNβ stimulation in Ulk1/2$^{+/+}$ cells when compared to Ulk1/2$^{-/-}$ cells are highlighted in red. Genes identified in Table S1 as presenting a lower induction after IFNβ stimulation in Ulk1/2$^{+/+}$ cells when compared to Ulk1/2$^{-/-}$ cells are highlighted in green. Provided as an Excel file.

Table S5, Related to Figure 2. Gene ontology analysis of genes induced only in Ulk1/2$^{+/+}$ MEFs after IFNβ treatment. Provided as an Excel file.

Table S6, Related to Figure 2. Gene ontology analysis of genes induced only in Ulk1/2$^{-/-}$ MEFs after IFNβ treatment. Provided as an Excel file.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Materials

Chloroquine (CQ) and Bafilomycin A1 (BFA) were purchased from Sigma-Aldrich. The plasmid 27629 pcDNA6.2-myc ULK1 WT and the plasmid 27630 pcDNA6.2-myc ULK1 k46I were purchased from Addgene (Egan et al., 2011). The pcDNA6.2/V5/GW/CAT control empty vector was purchased from Invitrogen. The ISRE-luciferase construct containing the WT ISG15 ISRE was provided by Dr. Richard Pine (Public Health Research Institute, New York) (Uddin et al., 1999). The 8X GAS construct was provided by Dr. Christopher Glass (University of California, San Diego) (Horvai et al., 1997). Control siRNA-B and human ULK1 siRNA were from Santa Cruz Biotechnology. Control non-targeting siRNA, and ON-TARGETplus ATG5 siRNA (SMARTpool L-004374-00-0005) were from Dharmacon. Recombinant human IFNα was from Hoffman-La Roche Inc. and recombinant human and mouse IFNβ were from BiogenIdec. Antibodies against phospho-ULK1 (Ser757), phospho-ULK1 (Ser555), ULK1, phospho-p38 MAPK (Thr180/Tyr182), p38 MAPK, phospho-STAT1 (Ser727), phospho-STAT1 (Tyr701), and ATG5 were from Cell Signaling. The anti-STAT1 antibody was from Santa Cruz Biotechnology. The antibody against GAPDH was purchased from Millipore.

Cell starvation, transfection, treatment, and lysis for immunoblotting analyses

U937, U266, Akt1/2$^{+/+}$, and Akt1/2$^{-/-}$ cells were starved prior to IFNβ treatment. For starvation, U937 and U266 cells were cultured overnight in RPMI 1640 medium supplemented with 0.5% FBS and 2% FBS, respectively, and the Akt MEFs were incubated overnight in DMEM medium supplemented with 0.5% FBS. Peripheral blood from a patient with Polycythemia Vera was collected after obtaining consent approved by the Institutional Review Board of Northwestern University, and PBMCs were isolated following Histopaque density gradient separation (Sigma). PBMCs were cultured overnight in IMDM medium (Gibco) supplemented with 20% FBS,

followed by 2 hours of starvation in IMDM medium supplemented with 1% FBS prior to IFNβ treatment. Ulk1/2$^{-/-}$ MEFs were transfected with pcDNA6.2, ULK1 WT, or ULK1 k46I plasmids using Amaxa Biosystems MEF 2 Nucleofector Kit (Lonza) per the manufacturer's instructions. For immunoblotting analyses, cells were treated with 10^4 IU/ml of mouse or human IFNβ for the indicated times. Cells were lysed in phosphorylation lysis buffer (1M Hepes pH 7.3, 5M NaCl, 1M MgCl2, 0.5M EDTA pH 8.0, 100μM sodium fluoride, 100μM sodium pyrophosphate, 0.5% Triton X-100, and 10% glycerol) supplemented with protease and phosphatase inhibitors. For lysis of PBMCs, 100μM of DTT was added to the phosphorylation lysis buffer.

Luciferase Reporter Assays

Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs were co-transfected with a β-galactosidase expression vector and either an ISRE-luciferase construct or a luciferase reporter gene containing eight GAS elements linked to a minimal prolactin promoter (8X GAS) by using the SuperFect transfection reagent (QIAGEN) per the manufacturer's instructions. Forty-eight hours after transfection, triplicate cultures were left untreated or were treated with mouse IFNβ ($5x10^3$ IU/ml) for 6 hours. The cells were then lysed, and luciferase activity was measured using Luciferase Assay System (Promega) per the manufacturer's instructions. Luciferase activities were normalized for β-galactosidase activity for each sample.

Microarray analysis, data normalization, and selection of differentially expressed genes

Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs were plated in triplicate and were then left untreated or were treated for 6 hours with 2.5 x 10^3 IU/ml of mouse IFNβ. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN) per the manufacturer's instructions. The expression analysis was performed at the Genomics Core Facility at Northwestern University. Briefly, samples with RNA integrity numbers (RIN) greater than 9.20 were used for labeling of 150ng of total RNA using the Illumina TotalPrep RNA amplification kit (Life Technologies). 1.5μg of labeled cRNA was hybridized to

MouseWG-6 v2.0 Expression BeadChips that represent more than 45,000 mouse gene transcripts, for 18 hours. After staining, the chips were scanned with the Illumina iScan (Illumina) according to the manufacturer's protocol. Raw data were imported to GenomeStudio (Illumina), quantile normalized, and the average signal intensities were analyzed in Partek Genomic Suite v.6.6 (Partek, Inc.) after $\log_2$ transformation. Qualitative principal component analysis (PCA) did not reveal any outlier samples or artifacts on the microarray. Differential expression between the experimental groups, Ulk1/2$^{-/-}$ *versus* Ulk1/2$^{-/-}$ + IFNβ, and Ulk1/2$^{+/+}$ *versus* Ulk1/2$^{+/+}$ + IFNβ, was assessed by using two-way analysis of variance (ANOVA) and Method of Moments together with a false-discovery rate (FDR) correction of the *p value* below 0.05 (and with -1.5 > Fold Change > 1.5) (Reiner et al., 2003). Fisher's Least Significant Difference (LSD) was used as the contrast method to compare the experimental groups. Expression of *Cxcl10*, *Eif2ak2*, *Irgm2*, *Gch1*, *Ifit3*, *Oasl2*, *Irf7*, *Irf9*, and *Isg54 (Ifit2)* genes found to be differentially expressed between Ulk1/2$^{-/-}$ *versus* Ulk1/2$^{-/-}$ + IFNβ, and Ulk1/2$^{+/+}$ *versus* Ulk1/2$^{+/+}$ + IFNβ groups was confirmed by quantitative RT-PCR.

Bioinformatics, Database Search, and Gene Enrichment Analysis

Hierarchical clustering and Venn diagram were performed using Partek Genomic Suite v.6.6. For construction of gene expression networks, the entire microarray datasets and associated p-values were imported into gene networks module of Ingenuity Pathway Analysis® 7.0 software (IPA, Ingenuity Systems). Data generated were used to build an integrative model linking gene networks and physiological events. List of genes (GenBank accession numbers) generated from the microarray results were submitted to the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.7. (http://david.abcc.ncifcrf.gov). The genes were classified into functional categories using biological process, cellular component, and molecular function of Gene Ontology terms (GO; http://www.geneontology.org/index.shtml). Analysis of the association of the genes with physiological or biochemical pathways was performed using the

Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html). To identify significantly overrepresented biological categories and KEGG pathways within the lists of differentially expressed genes, the threshold for the enrichment analysis was set at $p \leq 0.05$. For details see Lisowski et al., 2011 and 2013.

Quantitative RT-PCR

Ulk1/2$^{+/+}$ and Ulk1/2$^{-/-}$ MEFs were left untreated or were treated for 6 hours with 5x10^3 IU/ml of mouse IFNβ. U937 cells were transfected with control or ULK1 siRNA using Amaxa Biosystems Nucleofector Kit C (Lonza) following the manufacturer's instructions and 24 hours later cells were left untreated or treated for 6 hours with 5x10^3 IU/ml of human IFNβ. To determine whether inhibition of autophagy would affect ISG expression, Ulk1/2$^{+/+}$ MEFs were pre-incubated for 1 hour with 6μM of CQ or 100nM of BFA, as indicated, followed by six hours of co-treatment with 5x10^3 IU/ml of mouse IFNβ. As controls, cells were left untreated or were treated with each compound used alone. Ulk1/2$^{+/+}$ MEFs were transfected with control or ATG5 siRNA using Amaxa Biosystems MEF 2 Nucleofector Kit (Lonza) and 24 hours later were left untreated or were treated with 5x10^3 IU/ml of mouse IFNβ for 6 hours. Additionally, blood samples were collected from patients with MPNs and controls evaluated at Albert Einstein School of Medicine (Bronx, NY) under an Institutional Review Board approved study. RNA was isolated from neutrophils isolated from peripheral blood via gradient density separation (Lympholyte-poly) (Nischal et al., 2013). Total RNA was isolated using the RNeasy Mini Kit (QIAGEN) per the manufacturer's instructions. 2μg of total cellular mRNA was reverse-transcribed into cDNA using the Omniscript RT kit (QIAGEN) and oligo(dT)$_{12\text{-}18}$ primers (Life Technologies). Quantitative RT-PCR was carried out using an ABI7900 sequence detection system (Applied Biosystems) using commercially-available FAM-labeled probes and primers (Applied Biosystems) to determine mouse *Cxcl10*, *Eif2ak2*, *Irgm2*, *Gch1*, *Ifit3*, *Oasl2*, *Irf7*, *Irf9*, *Isg54, Isg15, and human ISG15, ISG54,* and *ULK1* mRNA expression. Mouse *Gapdh* and human *GAPDH* were used for

normalization. The mRNA amplification was calculated as previously (Kaur et al., 2007), and the data were plotted as the increase of fold change as compared with control samples.

ADP-Glo Kinase Assay

Kinase reactions between recombinant human ULK1 active protein (100ng) (SignalChem) and MAPK14 (p38α MAPK) recombinant human inactive protein (5μg) (Life Technologies) were performed using DTT, ATP, and 5x kinase buffer (SignalChem) following the manufacturer's instructions. As controls, the same kinase reactions were carried out, but using each recombinant protein alone. The ADP formed from the kinase reactions was measured using the ADP-Glo Kinase Assay Kit (Promega) following the manufacturer's instructions.

Immunoprecipitations and *in vitro* kinase assays

U937 cells were starved overnight followed by human IFNβ treatment (10^4 IU/ml) for 5, 10, and 30 minutes as indicated, and lysed in NP-40 buffer (20mM HEPES pH7.4, 180mM KCl, 0.2mM EGTA, 1.5mM $MgCl_2$, 10% glycerol, 0.1% NP-40) supplemented with protease and phosphatase inhibitors. 200μg of protein (total cell lysates) from each sample were used for immunoprecipitation of ULK1 using ULK1 rabbit monoclonal antibody (1:100) (Cell signaling), followed by incubation with protein G Sepharose 4 Fast Flow beads (GE Healthcare Life Sciences). As a control, the same procedure was followed, but using non-immune Rabbit IgG antibody (BD Pharmingen), instead of ULK1 antibody. The beads were washed three times with NP-40 buffer and twice with kinase buffer (20mM HEPES pH 7.4, 1mM EGTA, 0.4mM EDTA, 5mM $MgCl_2$, 1mM DTT) prior to the kinase reaction. *In vitro* kinase reactions to detect ULK1 kinase activity were performed for 30min at 30°C as in previous studies (Kroczynska et al., 2009). MAPK14 (p38α MAPK) recombinant human inactive protein (5μg) (Life Technologies) was used as an exogenous substrate.

Hematopoietic Cell Progenitor Assays

In the experiments to assess the effects of ULK1 silencing on growth of leukemic CFU-L progenitors, U937 cells were transfected with either control or ULK1 siRNA using Amaxa Biosystems Nucleofector Kit C (Lonza) per the manufacturer's instructions. Clonogenic assays in methylcellulose (Stemcell Technologies) in the absence or presence of human IFNβ (25 IU/ml) were then performed. Leukemic CFU-L colonies were scored as previously (Kaur et al., 2014). Human normal bone marrow $CD34^+$ cells (Stemcell Technologies) were transfected with either control or ULK1 siRNA using TKO transfection reagent (Mirus) per the manufacturer's instructions. Clonogenic assays in methylcellulose (Stemcell Technologies) in the absence or presence of human IFNα (10^3 IU/ml) were then performed. Erythroid (BFU-E) or myeloid (CFU-GM) colonies were scored as in previous studies (Mayer et al., 2001; Kroczynska et al., 2012). Peripheral blood from patients with Polycythemia Vera was collected after obtaining consent approved by the Institutional Review Board of Northwestern University, and PBMCs were isolated following Histopaque density gradient separation (Sigma). PBMCs were transfected with control or ULK1 siRNA using TKO transfection reagent (Mirus) per the manufacturer's instructions. Hematopoietic progenitor colony formation for human erythroid precursors (burst-forming unit erythroid (BFU-E)) was then determined in clonogenic assays in methylcellulose (Stemcell Technologies) in the absence or presence of human IFNβ (30 IU/ml). Hematopoietic colony formations were scored as in previous studies (Mayer et al., 2001; Joshi et al., 2009; Mehrotra et al., 2013; Kaur et al., 2014).

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