Enhancers are critical genomic elements that define cellular and functional identity through the spatial and temporal regulation of gene expression. Recent studies suggest that key genes regulating cell type-specific functions reside in enhancer-dense genomic regions (i.e., super enhancers, stretch enhancers). Here we report that enhancer RNAs (eRNAs) identified by global nuclear run-on sequencing are extensively transcribed within super enhancers and are dynamically regulated in response to cellular signaling. Using Toll-like receptor 4 (TLR4) signaling in macrophages as a model system, we find that transcription of super enhancer-associated eRNAs is dynamically induced at most of the key genes driving innate immunity and inflammation. Unexpectedly, genes repressed by TLR4 signaling are also associated with super enhancer domains and accompanied by massive repression of eRNA transcription. Furthermore, we find each super enhancer acts as a single regulatory unit within which eRNA and genomic transcripts are coordinately regulated. The key regulatory activity of these domains is further supported by the finding that super enhancer-associated transcription factor binding is twice as likely to be conserved between human and mouse than typical enhancer sites. Our study suggests that transcriptional activities at super enhancers are critical components to understand the dynamic gene regulatory network.

super enhancers | enhancer RNAs | transcription factors | GRO-Seq | macrophage

Significance

Super enhancers (SEs) are enhancer-dense regions found near genes that play key roles in determining cellular identity. Using global nuclear run-on sequencing (GRO-Seq), we find extensive regulation of enhancer RNAs (eRNAs) within SEs in response to lipopolysaccharide (LPS) treatment in macrophages. Both activation and repression of gene expression are associated with SEs and eRNA transcription dynamics. Furthermore, we find that each SE acts as a single regulatory unit within which eRNA and genomic transcripts are coordinately regulated. We also find that transcription factor (TF) composition within an SE determines regulatory properties of each SE and associated eRNAs. We propose that signal-dependent SEs and their eRNA function as molecular rheostats integrating the binding profiles of key regulators to produce dynamic profiles of gene expression.
the phenomena remain to be explored, the study provides insight into how SEs play critical roles in inflammatory responses. It has also been strongly implicated that eRNAs and enhancer functions are involved in the regulation of inflammatory transcription networks (22, 34–37); however, it remains to be solved how, and if at all, SE-associated eRNAs (seRNAs) contribute to the regulatory landscape. Using global nuclear run-on sequencing (GRO-Seq) to map the location and orientation of all active RNA polymerases genome-wide (38), we found eRNAs extensively transcribed within the macrophage SE subset. We found that SEs, although only 3% of the enhancer network, are strongly enriched near genes that are either induced or repressed in response to TLR4 signaling, suggesting that they may play a key role in both signal response and developmental transcriptional programs. The key regulatory activity of these domains is further supported by the finding that SE-associated TF binding is twice as likely to be conserved between human and mouse than TE sites. Notably, we found that each SE acts as a single regulatory unit within which transcription of eRNAs and their genic transcripts are coordinately regulated. Induction or repression of transcription within each SE appears to be largely dictated by the cumulative binding of specific TFs. Collectively, these results suggest that each SE and its associated eRNAs function as a type of molecular rheostat with a high dynamic range to control gene expression.

Results and Discussion

Detecting Highly Active Transcription at SEs. To investigate the transcriptional response of macrophages to TLR4 signaling, we generated nascent RNA transcriptomes using GRO-Seq from mouse primary macrophages in response to lipopolysaccharide (LPS) at 0, 20, 60, and 180 min. We defined de novo transcription units to characterize the macrophage response in an unbiased manner. The transcripts were classified into different groups based on their location relative to known gene annotations (Fig. 1A). Nearly 27% of transcripts were classified as eRNAs, found in intergenic regions, closely associated with TFs and epigenetic modifications. We observed that eRNAs were typically clustered at key genes within enhancer-dense regions, resembling sites that were recently described as SEs (12–14). To explore the relationship between eRNAs and SEs more closely, we analyzed ChIP-Seq data for key macrophage TFs, PU.1, C/EBPA, NFκB p65, and JUNB, and SE unit (blue box) at Cebpa locus. (C) Analyses of average GRO-Seq reads per bp per ChIP-Seq peak within either SEs or TEs. (D) Comparison of TF (PU.1 and C/EBPA) binding conservation at SEs or TEs between human and mouse. (E) Genome browser view of Spi1 (PU.1) gene loci where PU.1 and C/EBPA binding are conserved between human and mouse.

intergenic enhancers overlap with an eRNA (30.6%), whereas nearly all SEs contain eRNAs (93.3%) within intergenic regions. This implies that the frequency and presence of eRNAs can be used to mark SEs and these SEs are likely to be an assembly of very active and functional enhancers. Furthermore, the transcriptional activity at individual intergenic TFBSSs was much higher in SEs than at sites in TEs, with about a 3.3-fold increase in the area under the curve (Fig. 1C). The fact that SEs tend to cluster at lineage-defining gene loci (12, 13) suggests that regulatory elements in these regions may be conserved between species. To address this hypothesis, we compared binding of PU.1 and C/EBPA, using ChIP-Seq data from human and mouse macrophages. Interestingly, mouse PU.1 and C/EBPA binding peaks that are located in SEs are more likely to bind conserved regions in human homologs compared with the peaks at TEs (~20-fold enrichment) (Fig. 1D). As an example, PU.1- and C/EBPA-bound peaks near the Spti (PU.1) gene locus showed more conserved binding at the defined SE regions between human and mouse compared to other binding sites (Fig. 1E), indicating the possibility that this conservation of SEs is linked to their conserved roles in gene regulation in different species.
Signal-Dependent SEs. LPS treatment provides the opportunity to screen for “facultative” or “signal-dependent” SEs that can be “gained” or “lost” during macrophage activation (34, 36). Indeed, within minutes of LPS addition, 80 new SEs materialize, whereas 277 SEs recede within minutes of LPS addition (Fig. S2). These transitory SEs reflect gained or lost seRNAs that directly center over regions of previously identified TFs (Fig. 3A and B). Interestingly, genes that gain SEs appear to be involved in immune processes and inflammatory responses (Fig. 3B), whereas the genes that lose SEs upon LPS stimulation tend to have more general functions in cellular metabolism and nuclear organization (e.g., chromatin assembly/disassembly, nucleosome organization) (Fig. 3C). This suggests that SEs are involved in regulating not only cellular identity at the basal level by marking the subset of lineage-restricted genes but also functional identity by concurrently robustly activating immune response genes and repressing cellular maintenance genes. It is possible that LPS maximizes inflammatory responses by redistributing TFs that are required to transcribe genes involved in normal cellular metabolism in a resting condition to SEs that mark functionally relevant proinflammatory genes. Thus, the decrease in transcriptional levels might be due to either a loss or redistribution of these TFs or, as previously shown in other systems, a sequestering of TFs (14, 29, 30, 33). Further studies are needed to address this issue. It is notable that genes that respond within 20 min of LPS stimulation are marked by nearby SEs and show a clear bias toward activation (Fig. 2C and D). Thus, new seRNA transcripts appear to be the immediate early products of TLR4 signaling. In exploring the mechanism of this rapid transcriptional response, we found that this SE subset has higher levels of paused Pol II at basal levels compared with genes without SEs. Furthermore, genes with LPS-induced SEs show enhanced release of Pol II into the gene body and increased recruitment of Pol II at their promoters (Fig. 3D). This suggests that SEs mark those genes selected to rapidly respond to LPS stimulation. It is possible that the presence of signal-dependent SE subsets allows scalable communication, to allow precise temporal control of activated and repressed gene expression networks.

Coordinate Regulation of SEs and seRNAs. Inspection of several LPS-regulated genes revealed that the seRNAs within a given SE were often coordinately regulated with the target gene. For...
Elongating RNA Pol II has also shown the involvement of NF-κB in inducing gene expression (31). Regarding TLR4 activation, a key question is how molecular determinants that direct these opposing events? Interestingly, ChIP-Seq for NF-κB within the SE domain located in the upstream of Igf1 are coordinately repressed (Fig. 4A). Surprisingly, the same concerted trend was observed at LPS-repressed loci, such that all eRNAs are either induced or repressed by LPS in each SE relative to a randomized control. Strikingly, we found a clear reduction in transcription with LPS in the presence of JQ1 (Fig. S6), suggesting the involvement of BRD4 in seRNA transcription, at least in the SE subset examined in our system. Additionally, it is possible that these specific subsets of SEs and seRNAs serve as targets for anti-inflammatory pathways by modulating the TF Composition in SEs Determines Regulatory Properties. With regard to TLR4 activation, a key question is “What are the molecular determinates that direct these opposing events?” TFs activated by TLR4 signaling, such as NF-κB, are known to play a key role in inducing gene expression (31–33). A recent study has also shown the involvement of NF-κB in SE formation (33). Interestingly, ChIP-Seq for NF-κB (p65) shows the binding in both induced and repressed SEs, which is at odds with its assumed function as an activator (41). Closer inspection revealed that key inflammatory genes show more intense p65 binding within the SE relative to repressed loci. To assess whether SE regulation may be related to the quantity of binding at multiple sites within the regulatory domain, we calculated the ChIP-Seq read densities of NF-κB relative to other key master TFs that are used to define SEs and ranked them based on the ratio of NF-κB to these key TFs. We then compared this ratio to the fraction of eRNAs repressed within each SE (Fig. 5A). Interestingly, SEs with strong p65 binding relative to other factors, such as Fpr1/2, exhibited high fractions of eRNA induction, whereas SEs with low p65 binding, such as Igf1, exhibited eRNA reduction (Fig. 5A), indicating that TF composition in SEs determines regulatory properties, in both eRNA activation and repression (Fig. 5B). Because it is possible that a subset of SEs requires redistribution of transcriptional cofactors such as BRD4 (33), we examined a series of seRNAs by quantitative RT-PCR in response to LPS treatment in the presence and absence of JQ1, a BRD4 inhibitor. The results show a clear reduction in transcription with LPS in the presence of JQ1 (Fig. S6), suggesting the involvement of BRD4 in seRNA transcription, at least in the SE subset examined in our system. Additionally, it is possible that these specific subsets of SEs and seRNAs serve as targets for anti-inflammatory pathways by modulating the...
composition of TFs and attenuating their seRNA expression, leading to reduced expression of key inflammatory genes.

Conclusion
Collectively, using macrophage activation as a model, we describe a relationship between SEs, seRNAs, and TF binding that reveals a role for SE domains and the roles of transcriptional events in those sites. In our study, we showed that SEs and seRNAs, although spread over long genomic regions, function as a single regulatory unit, which allows coordinated genomic transcriptional regulation in the context of gene activation and gene repression. Previous studies have focused on the role of SEs in maintaining cellular identity (12, 13, 16, 39). However, Brown et al. (33) and our current study further investigate the regulatory dynamics of SEs upon inflammatory stimuli in endothelial cells and macrophages. Interestingly, both studies identify signal-regulated loss or gain of SE formation. Whereas Brown et al. focus on BRD4 as an enhancer output that directs SE formation and transcriptional changes of proinflammatory genes (33), our study uses eRNA production and regulation as a readout to determine enhancer activities in addition to TF binding. One of the advantages of using eRNA synthesis as a readout for determining SE is that it can allow more unbiased information of active loci throughout the genome. We also demonstrated that SEs are likely to be more conserved among TFs compared with TEs and can mediate dynamic signaling events such as macrophage activation. Interestingly, both activating and repressive properties of SEs and seRNAs are largely dictated by the composition of TFs within SEs. It is possible that this may not only be limited to LPS but may also apply to any signaling pathway that triggers changes in the transcriptome. For instance, different TLR ligands can regulate different compositions of IRFs in SEs, resulting in a distinctive set of gene regulations based on the stimuli. As SEs provide insights into how inflammatory responses are driven, SEs might also play a critical role in developing endotoxin tolerance in macrophages. It is possible that low-dose LPS priming is sufficient to fully induce eRNA synthesis as well as key TF binding within SEs, masking further activation of inflammatory responses in macrophages at a high-dose LPS response. Although further mechanistic understanding is required, we propose that proinflammatory SEs may be driven by the binding of specific enhancer factors and may regulate distinct sets of SEs function as molecular sensors and rheostats integrating the binding profiles of key regulators to produce dynamic profiles of gene expression in a signal-dependent fashion.

Materials and Methods
Cell Culture and Treatments. All primary macrophages were differentiated from bone marrow isolated from an isogenic C57 background as described previously (42). The primary macrophages were plated for experiments in cell culture and treated with LPS. GRO-Seq libraries were sequenced on an Illumina HiSeq2500 sequencer to a depth of over 50 million reads per library.

Generation of GRO-Seq Libraries. GRO-Seq library generation was carried out as described previously (20) from two biological replicates of mouse primary macrophages treated with LPS. GRO-Seq libraries were sequenced on an Illumina Hiseq2500 sequencer to a depth of over 50 million reads per library.

Genomic Data Analysis and Visualization. GRO-Seq analysis. GRO-Seq sequencing reads were mapped to the mouse genome (mm9, National Center for Biotechnology Information build 37) using Bowtie2 with default parameters (v2.1.0) (43). Only reads that mapped to a single unique location were considered for further analysis. Genome browser BedGraph tracks and read density histograms were generated using HOMER (44). De novo transcript identification was performed using HOMER (findPeaks program using the “style groseq” option), which looks for regions of contiguous GRO-Seq read coverage (45). Discovered transcripts were annotated as genic (overlapping known RefSeq genes, sense direction), antisense (overlapping known genes in the antisense direction), repetitive (5′ end of transcript overlapping University of California Santa Cruz (UCSC) annotated repeatmasker region), promoter antisense (5′ end emanates from promoter within 500 bp in the opposite direction from a gene), or eRNA (intergenic transcript not belonging to either of the above categories). eRNA read density or transcript expression was determined using HOMER to count reads within transcript boundaries (exon + intron) for either previously annotated or de novo identified transcripts. eRNA levels at individual enhancer locations were calculated as the total GRO-Seq signal within ±500 bp relative to the peak center. Only intergenic enhancers that at least 3 kb upstream of the TSS or greater than 10 kb downstream of the transcript end were used for these calculations to minimize signal from transcriptional read-through from genic transcripts.

ChIP-Seq analysis. The macrophage ChIP-Seq data used in this study came from the following Gene Expression Omnibus data accessions: GSE46494 (PU.1 and CEBPA), GSE16723 (NF-κB p65), GSE38379 (JUNB), and GSE31621 (human PU.1 and CEBPB). All ChIP-Seq data were analyzed from raw sequencing reads and aligned to either the mouse or human genomes (mm9/hg19) using Bowtie2. Genome browser tracks, ChIP-Seq peak finding (“style factor”), and ChIP-Seq read density calculations were all performed using HOMER. To compare PU.1 and CEBP recruitment between human and mouse macrophages, the mapped coordinates of human ChIP-Seq reads and peak positions were converted to the mm9 genome using the UCSC LiftOver tool.

Identification of SEs. SEs were identified in a highly similar manner to the method outlined in Whyte et al. (13). First, master TFs of macrophage development and activation were identified using HOMER (46). We sampled 10 million reads from each sequencing experiment and merging the results into a single metaexperiment for untreated and LPS-treated conditions separately. Input/IGG experiments were merged in the same manner. SEs were then found by running HOMER findPeaks using the “style super” option. This process first identifies traditional ChIP-Seq peaks by treating the metaexperiment and metaput as normal ChIP-Seq experiments and then merges individual peaks found in different regions. The “enhancer score” for each region is defined by the number of metaexperiment reads minus the metaput reads normalized for sequencing depth. All regions are then sorted by their score and plotted by their relative rank and score (0–1). Enhancer regions past which the slope of the line reaches 1 are considered SEs, and all remaining enhancers are considered as “typical enhancers.” Discovery of these SEs was further validated by comparing SEs found using HOMER (33) with SEs found using HOMER with a macrophage H4K12ac ChIP-Seq dataset (33). Differences were mostly attributed to differential identification of key peaks that might allow a given SE region to expand across 12.5-kb regions to increase signal. Independently, the functional quality of differential SE calls was assessed by measuring eRNA production from intergenic TFBs that were specific to Brown et al. or HOMER SEs.

The binding index of a given TF within an SE is defined as the log2 ratio of reads for the given factor in the SE relative to the total reads of the metaexperiment in the SE. In this sense, if a factor is highly bound within the SE relative to other TFs, the binding index will be high, whereas if there is minimal binding of the factor in the SE relative to other factors, the binding index will be low. Gene ontology analysis was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID). The release of RNA Pol II from proximal promoter pausing was quantified by calculating the density of Pol II ChIP-Seq reads within 200 bp of the TSS relative to the gene body (200 bp – 3 kb relative to the TSS).

Coordinate regulation within SEs. To detect coregulated eRNAs within SEs, individual peak regions within each SE (defined by applying peak finding to the “metaexperiment” representing the merge of master regulators) were analyzed for changes in GRO-Seq read density (±500 bp from peak center). Only peaks located in intergenic regions >3 kb from the TSS and >10 kb from transcript end locations were considered. Only peaks with detectable transcription (>10 GRO-Seq reads in either control or treated conditions) were considered. Peaks were assigned to their overlapping SE or assigned to a random SE as a control. Each SE was considered in terms of the fraction of peaks with up-regulated or down-regulated eRNA expression and then plotted as a contourplot in R using a 2D Gaussian kernel density estimator. Regulated eRNAs were defined by >twofold.

ACKNOWLEDGMENTS. We thank L. Ong and C. Brondos for administrative assistance. N.H. was supported by a Pioneer Postdoctoral Endowment Fellowship. R.M.E. is supported by National Institutes of Health Grants DK057978, DK090662, HL088093, HL105278 and ES010337; a Stand Up to Cancer Dream Team Translational Cancer Research Grant; Program of the Entertainment Industry Foundation Grant SU2C-AACR-DT0509; the Glenn Foundation for Medical Research; the Leona M. and Harry B. Helmsley Charitable Trust; Ipsen/Biomeasure; California Institute for Regenerative Medicine; and The Elionion Medical Foundation. R.M.E. is an investigator of the Howard Hughes Medical Institute at the Salk Institute and March of Dimes Chair in Molecular and Developmental Biology.
1. Bulger M, Groudine M (2011) Functional and mechanistic diversity of distal tran-
2. Ong CT, Corces VG (2011) Enhancer function: New insights into the regulation of
5. Hsieh CL, et al. (2014) Enhancer RNAs participate in androgen receptor-driven loop-
9. Rada-Iglesias A, et al. (2011) A unique chromatin signature uncovers early de-
10. Wang KC, et al. (2011) A long noncoding RNA maintains active chromatin to co-
13. Whyte WA, et al. (2013) Master transcription factors and mediator establish super-
14. Siersbæk R, et al. (2014) Transcription factor cooperativity in early adipogenic hot-
15. Parker SC, et al.; NISC Comparative Sequencing Program; National Institutes of Health
Intramural Sequencing Center Comparative Sequencing Program Authors; NISC
Comparative Sequencing Program Authors (2013) Chromatin stretch enhancer states
drive cell-specific gene regulation and harbor human disease risk variants. Proc Natl
Acad Sci USA 110(44):17921–17926.
18. Hah N, et al. (2011) A rapid, extensive, and transient transcriptional response to es-
Opin Genet Dev 25:38–42.
22. Lam MT, Li W, Rosenfeld MG, Glass CK (2014) Enhancer RNAs and regulated tran-
23. Melo CA, et al. (2013) eRNAs are required for p53-dependent enhancer activity and
24. Mouavi K, Zare H, Koulmis N, Sartorelli V (2014) The emerging roles of eRNAs in
redistribution support a squelching mechanism for E2-repressed genes. Mol Endo-
crinol 28(9):1522–1533.
30. Step SE, et al. (2014) Anti-diabetic rosiglitazone remodels the adipocyte tran-
scriptionome by redistributing transcription to PPARγ-driven enhancers. Genes Dev
28(9):1018–1028.
33. Brown JD, et al. (2014) NF-kB directs dynamic super enhancer formation in in-
34. Kaikkonen MU, et al. (2013) Remodeling of the enhancer landscape during macro-
phage activation is coupled to enhancer transcription. Mol Cell 51(3):310–325.
36. Ibott NE, et al. (2014) Long non-coding RNAs and enhancer RNAs regulate the lipo-
5:3979.
37. Daniel B, et al. (2014) The active enhancer network operated by liganded RXR sup-
42. Barish GD, et al. (2010) Bcl-6 and NF-kappaB cistromes mediate opposing regulation
Supporting Information

Sah et al. 10.1073/pnas.1424028112

SI Materials and Methods

Cell Culture and Treatments. The primary macrophages were pre-treated with JQ1 (500 nM) or vehicle (DMSO) for 1 h before treatment with 100 ng/mL of LPS (Sigma-Aldrich) for 1 h.

Quantitative RT-PCR. Total RNA was isolated using TRIzol according to the manufacturer’s instructions (Invitrogen) and reverse-transcribed to cDNA using iScript reagent (Bio-Rad). Quantitative RT-PCR was performed using SsoAdvanced SYBR Green reagent on the CFX384 system (BioRad).

Quantitative RT-PCR Primer Sequences. The following primer sequences were used: Ccl5-gene, forward 5′-ACCACTCC-CTGCTGCTTT-3′, reverse 5′-AGCACTTGCTGCTGGT-3′; Ccl5-seRNA1, forward 5′-GGAACTGTGCTTGGGTCA-3′, reverse 5′-CTCACACTGCACCACCT-3′; Ccl5-seRNA2, forward 5′-AACCGAAGAGCAGCTTTA-3′, reverse 5′-TTTGGTGTGGCCTCTAGT-3′; Ccl5-seRNA3, forward 5′-GGCCATCACTTGGGATTT-3′, reverse 5′-CTCGTGTGTGCCATGTG-3′; Irf1-gene, forward 5′-CTCTGTCTTTTCCCTCCAG-3′, reverse 5′-CTCTAGCCAGGTTCTCATCC-3′; and Irf1-seRNA, forward 5′-CACGTAT-CCAGGTGATTGA-3′, reverse 5′-CCAAGAGGCAGACTTTT-3′.

Fig. S1. Comparison of SE callings. (A) Venn diagram representations of SE comparisons between SEs that are called using H3K27Ac ChIP-Seq data and those that are called using key macrophage TFs used in this study in both vehicle and LPS treatment conditions. (B and C) Comparison between SEs found in Brown et al. (1) and SEs found using HOMER in the same mouse macrophage H4K12ac ChIP-Seq dataset. (D) Assessment of the functional quality of differential SE calls by measuring the eRNA production from intergenic TFBSs that were specific to Brown et al. or HOMER SEs.

Fig. S2. Relationship between the presence of eRNAs and SEs. Pie chart representations of the relationship between the number of eRNAs near (±50 kb from TSS) a gene and the presence of an SE (blue). The size of the pie reflects the number of genes in each class.

Fig. S3. Changes in Pol II levels in an actively down-regulated seRNA by LPS. A genome browser representation of a comparison between GRO-Seq and Pol II ChIP-Seq of actively down-regulated seRNAs and gene transcription at the Igf11 locus.
Fig. S4. Comparison of seRNA regulation by LPS and KLA. (A) Genome browser representations of GRO-Seq data generated with KLA stimulation in thioglycollate-induced peritoneal macrophages (ThioMac) used in Kaikkonen et al. (1) versus LPS treatment in bone marrow-derived macrophages (BMDM) in our study. (B) Correlation of seRNA regulations between KLA and LPS stimulation. (C) Contour graph representations of the fraction of eRNAs that are either repressed or induced upon KLA stimulation within an SE.


Fig. S5. Changes in SEs upon LPS treatment in macrophages. A Venn diagram representation of the number of SEs in vehicle- or LPS-treated macrophages.

Fig. S6. The effect of JQ1 and LPS in seRNAs and gene expression in macrophages. Quantitative RT-PCR analyses of expression changes in seRNAs and their associated genes in VEH or LPS (100 ng/mL) ± JQ1 (500 nM)-treated macrophages. Each experiment was performed in biological triplicates, and error bars indicate SEM.
Table S1. The number of eRNAs near (± 50 kb from TSS) a gene and the presence of an SE

<table>
<thead>
<tr>
<th>No. of eRNAs</th>
<th>Total no. of genes</th>
<th>No. of genes without SEs</th>
<th>No. of genes with SEs</th>
<th>Fraction of genes with SEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22,489</td>
<td>21,342</td>
<td>1,147</td>
<td>0.051</td>
</tr>
<tr>
<td>1</td>
<td>4,338</td>
<td>3,804</td>
<td>534</td>
<td>0.123</td>
</tr>
<tr>
<td>2 and 3</td>
<td>4,041</td>
<td>3,164</td>
<td>877</td>
<td>0.217</td>
</tr>
<tr>
<td>4 and 6</td>
<td>1,373</td>
<td>870</td>
<td>503</td>
<td>0.366</td>
</tr>
<tr>
<td>&gt;7</td>
<td>172</td>
<td>68</td>
<td>104</td>
<td>0.604</td>
</tr>
</tbody>
</table>

Other Supporting Information Files

Dataset S1 (XLSX)