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Multi-omics co-localization with genome-wide association studies reveals context-specific mechanisms of asthma risk variants

Marcus M. Soliai^{1,2*}, Atsushi Kato³, Catherine T. Stanhope², James E. Norton³, Katherine A. Naughton², Aiko I. Klinger³, Robert C. Kern⁴, Bruce K. Tan⁴, Robert P. Schleimer³, Dan L. Nicolae^{1,2,5,6}, Jayant M. Pinto⁷, Carole Ober^{1,2*}

¹Committee on Genetics, Genomics and Systems Biology, University of Chicago, Chicago, IL, United States of America

²Departments of Human Genetics, University of Chicago, Chicago, IL, United States of America

³Departments of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, United States of America

⁴Department of Otolaryngology-Head and Neck Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL, United States of America

⁵Department of Medicine, University of Chicago, Chicago, IL, United States of America

⁶Department of Statistics, University of Chicago, Chicago, IL, United States of America

⁷Department of Surgery, University of Chicago, Chicago, IL, United States of America

*Corresponding authors

E-mail: msoliai@uchicago.edu (MS); c-ober@bsd.uchicago.edu (CO)

32 **Abstract**

33 Genome-wide association studies (GWASs) have identified thousands of variants associated with
34 asthma and other complex diseases. However, the functional effects of most of these variants are
35 unknown. Moreover, GWASs do not provide context-specific information on cell types or
36 environmental factors that affect specific disease risks and outcomes. To address these
37 limitations, we used cultured upper airway (sinonasal) epithelial cell models to assess
38 transcriptional and epigenetic responses to a virus (rhinovirus [RV]) and a bacterium
39 (*Staphylococcus aureus* [SA]) and provide context-specific functional annotations to variants
40 discovered in GWASs of asthma. Using genome-wide genetic, gene expression, and DNA
41 methylation data in RV-, SA- and vehicle-treated cells from 115 individuals, we mapped *cis*
42 expression and methylation quantitative trait loci (*cis*-eQTLs and *cis*-meQTLs, respectively) in
43 each condition. Co-localization analyses of these airway epithelial cell molecular QTLs with
44 asthma GWAS variants revealed potential molecular disease mechanisms of asthma for GWAS
45 variants, including QTLs at the *TLSP* locus that were common both to exposure conditions and
46 childhood onset and adult onset asthma and at the 17q12-21 asthma locus that were specific to
47 both RV exposure and childhood onset asthma, consistent with clinical and epidemiological
48 studies of these loci. Overall, our study provides information on functional effects of asthma risk
49 variants in airway epithelial cells and provides insight into disease-relevant microbial exposures
50 that modulate genetic effects on transcriptional and epigenetic responses in cells and on risk for
51 asthma in GWAS.

52

53 **Author Summary**

54 Both genetic and environmental factors influence asthma. Genome-wide association studies have
55 identified thousands of genetic variants associated with asthma but do not provide information
56 on their functional effects, tissue specificity, or environmental context. To address these
57 limitations, we used an upper airway epithelial cell model to study responses to microbes that
58 potentially influence airway disease inception and/or symptoms, and to understand the functional
59 relevance of asthma risk variants. To this end, we mapped genetic variation associated with gene
60 expression and DNA methylation in cells exposed to a virus (rhinovirus) or a bacterium
61 (*Staphylococcus aureus*) compared to vehicle controls and tested for co-localization of these
62 molecular traits with variants associated with adult onset and childhood onset asthma in GWAS.
63 We report putative disease mechanisms of asthma and associated genes and DNA methylation
64 sites in airway epithelial cells exposed to disease-promoting risk factors.

65 **Introduction**

66 Over the past decade, genome-wide association studies (GWASs) have identified many
67 thousands of variants at hundreds of loci containing susceptibility genes for asthma and other
68 complex diseases [1]. Notably, over 90% of the single nucleotide polymorphisms (SNPs)
69 identified in GWAS reside in non-coding regions of the genome that are enriched for chromatin
70 signatures suggestive of enhancers [2] and for expression quantitative trait loci (eQTLs) [2-4].
71 These features of SNPs associated in GWAS indicate that they most likely affect underlying
72 disease pathophysiology through their effects on gene regulation. However, identifying the
73 causal variants and their target genes for associated loci has been challenging, and the functions
74 of most associated SNPs remain unknown. Furthermore, the significance threshold ($p < 5 \times 10^{-8}$)
75 required to control the false discovery rate in GWASs likely excludes many true associations that
76 do not reach this stringent threshold. We and others have suggested that SNPs with small p-

77 values that do not meet genome-wide significant thresholds, i.e., the mid-hanging fruit [5], may
78 be environment- or context-specific associations that are missed in GWAS that typically do not
79 control for either [6, 7]. Additionally, databases such as GTEx, ENCODE, and ROADMAP are
80 used to annotate GWAS SNPs and predict molecular mechanisms through which risk variants
81 affect disease phenotypes [4, 8, 9]. Although these resources have provided important insights
82 into the interpretation of GWAS results, they do not include all cell types relevant to all diseases
83 or information on environmental exposures that influence disease outcomes. As a result,
84 annotations of asthma GWAS variants have been largely limited to studies in cell lines, blood
85 (immune) cells, and whole lung tissue [10-12].

86 *In vitro* cell models provide an opportunity to address these limitations by identifying and
87 characterizing genetic and molecular responses to environmental exposures in cells from disease-
88 relevant tissues, and identifying genotypes that modify response to environmental risk [13, 14].
89 Joint analysis of datasets (e.g. eQTLs and GWAS) identifies variants associated with both
90 disease risk and molecular traits as candidate causal variants that contribute to mechanisms of
91 disease pathophysiology. A multi-trait co-localization (*moloc*) method [15] was recently
92 developed to integrate summary data from GWAS and multiple molecular QTL datasets to
93 identify regulatory drivers of complex phenotypes and to provide a more comprehensive analysis
94 in multi-trait studies.

95 Here, we report the results of a multi-omics co-localization study to identify condition-
96 specific regulatory effects of asthma risk variants in an epithelial cell model of microbial
97 response. Because airway epithelium plays a crucial role in response to inhaled exposures, we
98 used an *in vitro* upper airway (sinonasal) epithelial cell model of transcriptional and epigenetic
99 responses to two microbial exposures that are associated with asthma, RV and SA. RV

100 respiratory infections are a major cause of asthma inception in young children [16] and asthma
101 exacerbations in older children and adults [17, 18], and colonization of the nose with SA is more
102 common among asthmatics and associated with increased asthma symptoms and exacerbations in
103 children and young adults [19], underscoring the importance of these microbes as contextual
104 influences in asthma pathophysiology. Using co-localizations of airway epithelial cell molecular
105 QTLs with asthma GWAS risk variants, we characterized the effects of regulatory variation on
106 gene expression and DNA methylation levels in each treatment condition and used this
107 information to annotate and assign condition-specific regulatory effects at asthma GWAS risk
108 loci. Our integrative multi-omics approach revealed potential environment-specific mechanisms
109 of asthma pathogenesis, and further support a key role of airway epithelium in the pathogenesis
110 of childhood onset asthma.

111

112 **Results**

113 **Genome-wide identification of *cis*-eQTLs in cultured airway epithelial cells**

114 To identify genetic variation influencing gene expression under different conditions, we
115 performed eQTL mapping in cultured airway epithelial cells treated with two common microbial
116 exposures (RV and SA), and their corresponding vehicles from 115 individuals (S1 Fig).
117 Because the vehicles for RV and SA differed (bronchial epithelial basal medium [BEBM] for
118 RVveh and Dulbecco's phosphate buffered saline [dPBS] for SAveh) and cells were cultured for
119 different lengths of time (48 hours for RV and 24 hours for SA), we considered each vehicle as a
120 separate treatment condition and refer to them as RVveh and SAveh, respectively. We defined
121 eQTLs within a *cis*-window of 1 Mb from either side of the transcriptional start site (TSS) of
122 each autosomal gene and used a false discovery rate (FDR) of 10%. Analyses were performed

123 separately for each of the four conditions, testing for associations with 6,665,553 imputed SNPs
124 and 11,231 and 11,421 autosomal genes (from RNA-seq) for the RV and SA experiments,
125 respectively (see Materials and Methods). The numbers of SNPs associated with gene expression
126 (eQTLs), SNPs that are eQTLs for at least one gene (eSNPs), and genes with at least one eQTL
127 (eGenes) in each condition are summarized in Fig 1.

128

129 **Genome-wide identification of *cis*-meQTLs in cultured airway epithelial cells**

130 In parallel to eQTL mapping, we performed methylation quantitative trait loci (meQTL)
131 mapping in the same cells used for gene expression studies. We defined meQTLs within a *cis*-
132 window of 10 Kb from either side of each CpG site on the Illumina EPIC array, using an FDR of
133 10%. We performed this analysis separately for each condition, testing for associations with the
134 same imputed SNP set as that used for eQTL mapping and interrogated 792,392 and 749,125
135 autosomal CpGs for the RV and SA experiments, respectively. A summary of the number of
136 SNPs associated with methylation levels at one or more CpG sites (meQTLs), SNPs that are
137 meQTLs for at least one CpG (meSNPs), and CpG sites with at least one meQTL (meCpGs) are
138 shown in Fig 1.

139

140 **Estimating shared and condition-specific molecular QTL effects**

141 We first explored the impact of culture conditions (RV+RVveh vs SA+SAveh) and treatments
142 (RV and/or SA vs RVveh and/or SAveh, respectively) on eQTLs and meQTL effects using an
143 empirical Bayes method, **multivariate adaptive shrinkage** (*mash*) [20]. This is accomplished in
144 two general steps; we first identified existing patterns within the observed dataset, including
145 correlations among effects, sparsity, and sharing, and then we used these learned patterns to

146 make improved effect estimates and significance measurements for a given set of data from
147 multiple conditions. Compared to direct comparisons between conditions, *mash* increases power,
148 improves effect-size estimates, and provides better quantitative assessments of effect size
149 heterogeneity of molecular QTLs, thereby allowing for greater confidence in effect sharing and
150 estimates of condition-specificity. Additionally, as a confidence measurement of the direction of
151 each effect (molecular QTL), *mash* provides a ‘local false sign rate’ (lfsr) that is the probability
152 that the estimated effect has the incorrect sign [21], rather than the expected proportion of Type I
153 errors as would be assessed using FDR thresholds.

154 To identify condition-specific eQTLs, we analyzed the effect estimates for 337,699
155 eQTLs and observed broad sharing of eQTLs across these treatment conditions (see Materials
156 and Methods). Pairwise comparisons showed that between 63-89% of eQTLs were shared in at
157 least two conditions (lfsr < 0.05; Fig 1A and S2 Fig). The RV and RVveh cells showed the most,
158 and SA and SAveh cells the second most, eQTL effect sharing (89% and 72%, respectively),
159 while lower and similar amounts of sharing were observed between the other pairwise
160 comparisons (63-71%). In contrast, only 0.98 to 9.20% of all eQTLs were specific to one
161 condition, with the largest number detected in the SA-treated cells and the least in the RV-treated
162 cells (Fig 1A). The RV and SA culture conditions (RV+RVveh vs SA+SAveh) had relatively
163 large impacts on eQTL effects, with 10.6% and 20.6% of the eQTLs specific to culture
164 conditions, respectively. Another 10.6% of the eQTLs (35,874) were specific to the microbial-
165 treated cells only (RV+SA vs RVveh+SAveh), potentially representing genetic variants that
166 modify responses to microbes in airway epithelial cells. Examples of treatment-specific eQTLs
167 are shown in Fig 2B.

168 Condition-specific meQTLs were identified among the 1,669,925 meQTLs. A pair-wise
169 analysis revealed that between 73-79% of meQTLs were shared in two or more of the four
170 conditions ($lfsr < 0.05$; Fig 2C and S2 Fig), with 73% of these shared among culture conditions
171 (RV vs RVveh or SA vs SAveh), many more than those observed for eQTLs. In contrast, only
172 3.0 and 5.2% of meQTLs were treatment-specific (RV+SA or RVveh+SAveh), respectively (Fig
173 2D), many fewer than observed for eQTLs. Examples of treatment-specific meQTLs are shown
174 in Fig 2D.

175 In total, using *mash* we identified between 3,295 - 30,994 eQTLs associated with 102 -
176 582 eGenes ($lfsr < 0.05$) that were unique to a culture condition, and between 84,602 - 48,415
177 meQTLs associated with 7,636 - 12,817 meCpGs that were unique to a culture condition. This
178 approach allowed us to assign QTL effects sharing and potential condition- and/or treatment-
179 specific effects with greater confidence than by pairwise comparisons using FDR thresholds
180 [20].

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182 **Molecular QTL co-localizations with adult onset and childhood onset asthma loci**

183 Integrating molecular QTLs with GWAS data is a powerful way to identify functional variants
184 that may ultimately influence disease risk. This approach can provide functional insights into
185 known disease-associated variants as well to facilitate prioritizing variants with small p-values
186 that do not reach criteria for genome-wide significance in GWAS. One such approach is through
187 co-localization in which we directly test whether a genetic variant is underlying associations
188 between two or more traits (e.g., gene expression and asthma), providing clues to causal disease
189 pathways. We hypothesized that integrating molecular QTLs from microbial-exposed epithelial
190 cells with results of GWASs for adult onset and childhood onset asthma would reveal genetic

191 variants that are associated with both disease risk and gene expression and/or DNA methylation
192 patterns. This could reveal potential genetic and epigenetic mechanisms of childhood onset
193 and/or adult onset asthma that are modulated by disease-relevant microbial exposures in airway
194 epithelial cells, an asthma-relevant tissue that is the target of these microbes.

195 To test this hypothesis, we extracted summary statistics from the largest GWASs of adult
196 onset and childhood onset asthma to date [12], and tested each for co-localization with genetic
197 variants associated with gene expression, DNA methylation, and asthma, using *moloc*, a
198 Bayesian statistical strategy that allows the integration and co-localization of more than two
199 molecular traits [15]. We performed a separate co-localization test in each of the four conditions
200 using variants from the GWASs of adult onset and childhood onset asthma separately. These
201 analyses provide three possible configurations in which a variant is shared between the GWAS
202 and QTL traits: eQTL-GWAS pairs, meQTL-GWAS pairs, eQTL-meQTL-GWAS triplets.

203 Using this approach, we found evidence for 72 unique multiple trait co-localizations (76
204 total) associated with eQTLs for 11 genes (*ACO2*, *ERBB2*, *FGFR4*, *FLG*, *FLG-AS1*, *FRK*,
205 *GSTO2*, *IRF5*, *ORMDL3*, *PMM1*, *POLI*) and meQTLs for 31 CpG sites (PPA>70%) for adult
206 onset or childhood onset asthma (Table 1; S1 Table). Among the 72 unique co-localizations, 14
207 were eQTL-meQTL-GWAS triplets associated with six genes (*ACO2*, *ERBB2*, *FRK*, *GSTO2*,
208 *IRF5*, *PMM1*) and 11 CpG sites. There were also 25 eQTL-GWAS pairs associated with seven
209 genes (*ACO2*, *FGFR4*, *FLG*, *ORMDL3*, *PMM1*, *POLI*) and 33 meQTL-GWAS co-localized
210 pairs associated with 24 CpG sites. The majority of the co-localizations (88%) were identified in
211 just one treatment condition (PPA>70%). Twenty-eight of the co-localizations were identified
212 only in the microbial-exposed cells (RV and/or SA) and 41 were identified only in the vehicle
213 exposed cells (RVveh and/or SAveh). These 69 treatment-specific colocalizations represent

214 potential response mechanisms to microbial infection that contribute to asthma risk. The
215 remaining seven co-localizations were identified in combinations of microbe and vehicle
216 exposed cells. Notably, over 72% of the SNPs associated with these co-localizations did not
217 reach genome-wide significance ($p\text{-value}=5\times 10^{-8}$) in their respective GWASs ($p\text{-value}_{\text{range}}=$
218 1.03×10^{-7} to 4.63×10^{-4}), and all were identified only in the childhood onset asthma GWAS. These
219 analyses therefore provided functional inferences both for variants that were significant in the
220 GWASs at known asthma loci and for variants that did not meet strict criteria for significance in
221 the GWASs, thereby facilitating prioritization of variants among the mid-hanging fruit.

222 Of the 76 co-localizations, only six were at adult onset asthma loci and all six were
223 meQTL-GWAS pairs. The remaining 70 were at childhood onset asthma loci and 14 were eQTL-
224 meQTL-GWAS triplets, 25 were eQTL-GWAS pairs, and 31 meQTL-GWAS pairs) (Table 1; S1
225 Table). Four meQTL-GWAS pairs were co-localized in both GWASs and two were specific to
226 adult onset asthma. In contrast, 66 co-localizations were specific to childhood onset asthma, and
227 were associated with 11 genes (*ACO2*, *ERBB2*, *FGFR4*, *FLG*, *FLG-AS1*, *FRK*, *GSTO2*, *IRF5*,
228 *ORMDL3*, *PMM1*, *POL1*) and 27 CpG sites. The larger number of co-localizations for childhood
229 onset asthma relative to adult onset asthma is consistent with the previous observation that genes
230 at the childhood onset asthma loci were most highly expressed in skin, an epithelial cell type
231 [12].

232

Table 1. Number of QTL-GWAS pairs or triplets with evidence of co-localization

| GWAS | eQTL-meQTL-GWAS | eQTL-GWAS | meQTL-GWAS |
|-------------------------------|-----------------|-----------|------------|
| Adult onset asthma | 0 | 0 | 6 |
| Childhood onset asthma | 14 | 25 | 31 |
| Adult or childhood onset GWAS | 14 | 25 | 35 |

PPA ≥ 0.70

233

234 **meCpGs associated with co-localized risk variants correlate with gene expression**

235 Methylation at CpG dinucleotides plays critical roles in the regulation of various cellular
236 processes [22] and can potentially mediate the effects of environmental exposures on gene
237 expression [23]. Many complex diseases, including asthma, have been associated with DNA
238 methylation patterns, supporting the important role of epigenetics in disease processes [24-26]. A
239 mechanism through which DNA methylation can affect phenotypic outcomes is by influencing
240 gene expression [27]. Because of the large number of meSNPs that co-localized with asthma risk
241 variants, we next asked whether methylation levels at the CpG sites with co-localized meSNPs
242 were also associated with the expression of nearby genes. To address this question, we defined
243 nearby genes as those in which the meCpG was in the 5'UTR, gene body, 3'UTR or within 1500
244 bp of its transcriptional start site (TSS). Using these criteria, 21 of the 33 unique meQTL-GWAS
245 pairs were assigned to 12 unique genes (*EEFSEC*, *FLG-AS1*, *FRK*, *GSTO2*, *IRF1*, *IRF5*,
246 *MAP3K14*, *NEK6*, *POLR3H*, *SLC22A5*, *SMARCE1*, *TSLP*). We then tested for correlation
247 between methylation and gene expression for the 22 gene-CpG pairs for each treatment condition
248 using Spearman rank order correlation. Sixteen gene-CpG correlations ($\rho_{\text{bsolute}} = 0.26 - 0.68$)
249 with five genes (*FLG-AS1*, *FRK*, *GSTO2*, *IRF5*, *SLC22A5*) and eight meCpGs were significant
250 (FDR ≤ 0.05 ; Table 2; S3 Fig; S2 Table).

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Table 2. Correlation of methylation levels and expression of the nearest gene for 8 meQTL-GWAS co-localized pairs (Spearman’s rank order correlation). See text for details on gene assignments. Five gene-CpG pairs were correlated in multiple conditions (gray shaded rows).

| Gene | CpG | Spearman p-value | FDR | Spearman ρ | Treatment |
|----------------|------------|------------------|----------|-----------------|-----------|
| <i>FLG-ASI</i> | cg26320663 | 2.03E-03 | 2.03E-02 | -0.32 | rv |
| | | 6.46E-03 | 3.71E-02 | -0.28 | vehrv |
| <i>FLG-ASI</i> | cg23107878 | 6.49E-03 | 3.71E-02 | -0.28 | rv |
| <i>FRK</i> | cg20254830 | 2.70E-06 | 4.32E-05 | 0.45 | sa |
| <i>GSTO2</i> | cg23659134 | 0.00E+00 | 0.00E+00 | -0.66 | sa |
| | | 0.00E+00 | 0.00E+00 | -0.68 | vehsa |
| | | 2.53E-05 | 3.37E-04 | -0.42 | vehrv |
| | | 7.81E-03 | 3.95E-02 | -0.27 | rv |
| <i>GSTO2</i> | cg07488549 | 2.94E-07 | 7.84E-06 | 0.49 | vehsa |
| | | 5.73E-07 | 1.15E-05 | 0.48 | sa |
| <i>IRF5</i> | cg26616347 | 4.08E-03 | 3.26E-02 | -0.29 | vehrv |
| | | 4.67E-03 | 3.40E-02 | -0.28 | vehsa |
| | | 7.90E-03 | 3.95E-02 | -0.26 | sa |
| <i>SLC22A5</i> | cg04774966 | 7.34E-05 | 8.39E-04 | -0.39 | vehsa |
| | | 3.08E-03 | 2.74E-02 | -0.29 | sa |
| <i>SLC22A5</i> | cg26647941 | 5.88E-03 | 3.71E-02 | -0.27 | sa |

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259 Correlations between DNA methylation and gene expression were identified in multiple
260 treatment conditions and after exposure to RV or SA only. For example, significant correlations
261 between DNA methylation and *GSTO2* on chromosome 10 occur in all treatment conditions
262 ($\rho_{\text{absolute}} = 0.27 - 0.68$), suggesting that the correlation signals are robust across treatments (Fig
263 3A). In contrast, we observed potential treatment-specific effects on correlations between an
264 meCpG 241 bp upstream of *IRF5* and expression of this gene on chromosome 7 (Fig 3B), for
265 which correlation was observed in all conditions except after RV exposure ($\rho = 0.11$). In
266 contrast, correlation between DNA methylation levels one meCpG and *SLC22A5* on
267 chromosome 5 were only observed after SA treatment (S3 Fig).

268 Taken together these data suggest that meCpGs in airway epithelial cells that co-localize
269 with asthma GWAS variants influence the expression of nearby genes, and that these effects can
270 be reduced or enhanced by microbial exposures. These observations suggest that environment-
271 specific epigenetic responses at meCpGs contribute to asthma pathogenesis.

272

273 **meCpGs at *TSLP* co-localize with an asthma risk variant**

274 To more deeply characterize associations between an meQTL-GWAS pair and better understand
275 shared disease mechanisms between adult onset and childhood onset asthma, we focused on the
276 four meQTL-GWAS pairs that co-localized in both the adult onset and childhood onset asthma
277 GWASs (S3 Table). These pairs all included an intergenic SNP (rs1837253) located 5.7 kb
278 upstream from the TSS of the *TSLP* gene on 5q22, encoding an epithelial cell cytokine that plays
279 a key role in the inflammatory response in asthma and other allergic diseases [28]. rs1837253 co-
280 localized with four meCpGs (cg15557878, cg10931190, cg15089387, cg25328184) in both the
281 adult onset ($p_{\text{GWAS}} = 2.77 \times 10^{-13}$) and childhood onset ($p_{\text{GWAS}} = 2.33 \times 10^{-27}$) asthma GWASs. The
282 four meCpGs are located in the first (untranslated) exon (5' UTR) of the *TSLP* gene (Fig 4), a
283 region characterized as a promoter in keratinocyte primary cells (NHEK; ROADMAP). In fact,
284 rs1837253 was the lead SNP within previous asthma GWASs (e.g. [12, 29]). The rs1837253-C
285 asthma risk allele is associated with decreased methylation at each of the four meCpGs (Fig 4).
286 Because the meQTL for each of the four meCpGs were observed in each treatment condition, we
287 suggest that the first untranslated exon of *TSLP* is epigenetically poised for genotype-specific
288 expression in epithelial cells.

289 Although this is the first study to annotate the asthma-associated rs1837253 as an
290 meQTL, previous studies have shown *TSLP* to be a methylation-sensitive gene and that

291 hypomethylation at its promoter is associated with atopic dermatitis (AD) and prenatal tobacco
292 smoke exposure [30, 31]. Another study showed that increased excretion of TSLP in primary
293 cultured nasal epithelial cells after exposure to polyI:C (a dsRNA surrogate used to simulate viral
294 infection) was associated with the CC genotype [32]. Our co-localization studies further suggest
295 that the C allele is associated both with hypomethylation at CpG sites in an untranslated exon of
296 *TSLP*, and with adult onset and childhood onset asthma. Furthermore, the lack of LD of SNPs
297 with rs1837253 (\pm 50 kb) in European ($r^2 < 0.12$) and African American ($r^2 < 0.12$)
298 1000Genomes reference panels suggests that this SNP may indeed be causal for variation in
299 DNA methylation levels at *TSLP* and its association with asthma.

300

301 **Multi-trait co-localizations of molecular QTLs and asthma risk at the 17q12-21 asthma**

302 **locus**

303 Identifying regulatory effects at GWAS risk loci through co-localization with QTLs can help to
304 understand molecular mechanisms of disease, including those that are modulated by
305 environmental exposures. To further explore this, we focused on the co-localizations of eQTLs
306 and meQTLs with asthma GWAS loci at 17q12-21 (17q), the most significant and most
307 replicated locus for childhood onset asthma (reviewed in [33]). This locus is characterized by
308 high LD across a 206.5 kb region encoding at least 10 genes (including *ORMDL3* and *GSDMB*).
309 SNPs extending both proximal (including *PGAP3* and *ERBB2*) and distal (including *GSDMA*) to
310 the core region show less LD with those in the core region but have been implicated as
311 potentially independent asthma risk loci. Previous studies have shown that SNPs at this extended
312 locus are eQTLs for at least four genes (*ORMDL3*, *GSDMB*, *GSDMA*, *PGAP3*) in blood cells

313 and/or lung cells [33] and that genetic risk at this locus is mediated through early life wheezing
314 illness [34], particularly RV-associated wheezing [35].

315 We identified six co-localizations at the extended 17q locus of molecular QTLs with
316 childhood onset asthma GWAS SNPs (Fig 5A). As expected at this childhood onset locus [36,
317 37], we did not find co-localizations with variants from the adult onset asthma GWAS. Among
318 the co-localizations, one eQTL-GWAS pair was with rs12603332 on the 17q core haplotype and
319 expression of *ORMDL3* (PPA>0.70; S4 Fig). The co-localized SNP (rs12603332) is in LD
320 ($r^2>0.65$; 1000 Genomes European reference panel) with other previously reported asthma-
321 associated GWAS SNPs in this region, including some that were reported as eQTLs for
322 *ORMDL3* and *GSDMB*, primarily in immune cells. However, in our model none of the SNPs
323 were eQTLs for *GSDMB*, which is typically co-regulated with *ORMDL3*, at least in blood cells
324 [33]. In contrast to other studies, the asthma-associated allele (rs12603332-??) was associated
325 with decreased expression of *ORMDL3* in our cell model. Moreover, the co-localizations with
326 rs12603332 were only significant in vehicle treated cells, suggesting that exposure to RV or SA
327 weakens the effects of rs12603332 genotype on *ORMDL3* expression in epithelial cells.

328 We also detected four meQTL-GWAS pairs among the six co-localizations at 17q that
329 were associated with three meCpGs (cg24910161, cg21230266, cg17401724) and four SNPs at
330 the distal end of (rs3902025, rs4239225, rs3859191) and beyond (rs66826786) the extended 17q
331 haplotype where there is a breakdown of LD with SNPs in the core region. Two of these CpGs
332 were each located 27 bp upstream (cg24910161) and in an intron (cg21230266) of *GSDMA* in
333 regions characterized by ROADMAP as enhancers in NHEK cells. SNPs in modest to perfect
334 LD ($r^2_{\text{range}}=0.46 - 1.00$; 1000 Genomes European panel) with these co-localizations (rs3902025,
335 rs4239225, rs3859191) were described in previous studies as independent GWAS signals for

336 asthma (rs3894194) or as an eQTL for *GSDMA* (rs3859192) [4, 38, 39]. The meQTL-GWAS co-
337 localization associated with cg24910161 was specific to the SAveh treatment, while those
338 associated with cg21230266 were present in three conditions (RV, SA, and SAveh).

339 The one eQTL-meQTL-GWAS triplet in this region was associated with expression of
340 *ERBB2* (Fig 5B), more than 361 Kb proximal to the co-localized asthma risk variant in an intron
341 of *MED2* (rs66826786) and to the co-localized meCpG (cg17401724), distal to rs66826786 and
342 8.6 kb downstream of *MED2*. This is beyond the extended 17q locus as previously defined [33]
343 in a region characterized by ROADMAP as both an enhancer and transcriptional start site (TSS)
344 in NHEKs. Although the meQTL associated with this triplet was present in all four conditions,
345 the eQTL for *ERBB2* is observed only after exposure to RV. The asthma risk allele at
346 rs66826786-C was associated with both decreased *ERBB2* transcript in RV-treated cells and
347 decreased DNA methylation of cg17401724 in all conditions (Fig 5). The 8.6 kb distance
348 between the promoter of *ERBB2* and its eSNP (rs66826786) is highly suggestive of a long-range
349 interaction between *ERBB2* and the region harboring cg17401724 and rs66826786. The fact that
350 the eQTL is observed only after RV infection, further suggests that infection with RV triggers
351 this long-range interaction in airway epithelial cells, likely via chromatin looping between these
352 loci. The fact that the meQTL for cg17401724 is observed in all conditions suggests an
353 epigenetically poised chromatin state at the distal end of the locus that directly affects
354 transcription of *ERBB2* at the proximal end of the locus after exposure to RV, and possibly to
355 other viral exposures.

356

357

358 **Mendelian randomization of multi-trait co-localized triplets**

359 Co-localization analyses reveal genetic variants that are associated with asthma and molecular
360 traits (gene expression and/or DNA methylation) but the question of causality between the
361 molecular traits remains unanswered. To infer causal relationships between DNA methylation
362 and gene expression, we performed Mendelian randomization (MR), a method in which genetic
363 variation associated with modifiable exposure patterns (i.e. DNA methylation) can be used as an
364 instrumental variable to estimate directionality of effects between correlated traits (i.e. DNA
365 methylation and gene expression) [40]. Specifically, we applied a 2-stage least squares
366 regression (2SLS) to estimate the causal effects of DNA methylation (exposure) on gene
367 expression (outcome) using the QTL SNP as the genetic instrument for each of the 14 co-
368 localized triplets in each of the four conditions (see Materials and Methods). In this way, we are
369 able to estimate whether the asthma risk variant has an effect on gene expression levels,
370 mediated by DNA methylation.

371 Using MR, we detected a causal relationship between methylation and gene expression
372 for each of the 14 triplets, indicating that DNA methylation at the meCpG mediates the genotype
373 effects (eQTL) on gene expression (FDR < 0.10; Table 3). Specifically, for 12 of the triplets, we
374 detected a causal relationship between methylation and gene expression in all four treatment
375 conditions. These triplets were associated with four genes (*ACO2*, *GSTO2*, *IRF5*, *PMM1*) and
376 nine meCpGs. For one triplet, the association with *FRK* was significant in the SA and SAveh
377 treatments only, suggesting the methylation effects on this gene is specific to the culture
378 conditions used for the SA experiment (SA+SAveh). For the remaining triplet, meCpG effects on
379 *ERBB2* gene expression was only detected in RV-treated cells (FDR < 0.02, $lfsr_{RV} = 1.6 \times 10^{-4}$),
380 suggesting a long-range interaction after exposure to RV, as discussed above.

Table 3. Mendelian randomization results for 14 co-localized eQTL-meQTL-GWAS triplets

| Condition-Specificity | Gene | CpG | rsID | SNP Position | P-value | | | | FDR | | | |
|------------------------------|------------|------------|-------------|--------------|----------|----------|----------|----------|----------|----------|----------|----------|
| | | | | | RV | RVveh | SA | SAveh | RV | RVveh | SA | SAveh |
| All Conditions | ACO2 | cg19274703 | rs132905 | 22:41799106 | 2.00E-03 | 1.00E-03 | 0.00E+00 | 4.00E-03 | 3.61E-03 | 2.07E-03 | 0.00E+00 | 6.59E-03 |
| | ACO2 | cg07830128 | rs4822038 | 22:41958495 | 2.80E-02 | 2.00E-03 | 0.00E+00 | 2.00E-03 | 3.34E-02 | 3.61E-03 | 0.00E+00 | 3.61E-03 |
| | ACO2 | cg10386501 | rs5758461 | 22:42162189 | 7.00E-02 | 0.00E+00 | 0.00E+00 | 7.00E-03 | 7.69E-02 | 0.00E+00 | 0.00E+00 | 1.03E-02 |
| | GSTO2 | cg23659134 | rs156697 | 10:106039185 | 2.50E-02 | 3.00E-03 | 0.00E+00 | 0.00E+00 | 3.04E-02 | 5.25E-03 | 0.00E+00 | 0.00E+00 |
| | GSTO2 | cg07488549 | rs276210 | 10:106046403 | 1.30E-02 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.69E-02 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | IRF5 | cg26616347 | rs3778754 | 7:128575552 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| | PMM1 | cg04809988 | rs12483998 | 22:41935362 | 6.00E-03 | 4.20E-02 | 1.00E-03 | 0.00E+00 | 9.33E-03 | 4.70E-02 | 2.07E-03 | 0.00E+00 |
| | PMM1 | cg02738086 | rs9607812 | 22:41941243 | 5.00E-03 | 3.80E-02 | 1.00E-03 | 0.00E+00 | 8.00E-03 | 4.34E-02 | 2.07E-03 | 0.00E+00 |
| | PMM1 | cg07830128 | rs4822038 | 22:41958495 | 1.10E-02 | 1.00E-02 | 1.00E-03 | 0.00E+00 | 1.50E-02 | 1.40E-02 | 2.07E-03 | 0.00E+00 |
| | PMM1 | cg07830128 | rs9607819 | 22:41958862 | 7.00E-03 | 4.00E-03 | 0.00E+00 | 1.00E-03 | 1.03E-02 | 6.59E-03 | 0.00E+00 | 2.07E-03 |
| | PMM1 | cg12016267 | rs715498 | 22:42148467 | 1.30E-02 | 2.00E-03 | 0.00E+00 | 0.00E+00 | 1.69E-02 | 3.61E-03 | 0.00E+00 | 0.00E+00 |
| PMM1 | cg10386501 | rs5758461 | 22:42162189 | 2.90E-02 | 1.50E-02 | 9.00E-03 | 0.00E+00 | 3.38E-02 | 1.91E-02 | 1.29E-02 | 0.00E+00 | |
| Treatment (microbe)-specific | ERBB2 | cg17401724 | rs66826786 | 17:38206092 | 0.00E+00 | 8.05E-01 | 1.44E-01 | 2.36E-01 | 0.00E+00 | 8.05E-01 | 1.52E-01 | 2.45E-01 |
| Culture condition-specific | FRK | cg20254830 | rs10456902 | 6:116333742 | 8.04E-01 | 1.43E-01 | 0.00E+00 | 2.40E-02 | 8.05E-01 | 1.52E-01 | 0.00E+00 | 2.99E-02 |

383 Overall, the MR results provide additional, orthogonal evidence for co-localized triplets
384 and novel evidence for causal inference with respect to the co-localized traits (DNA methylation,
385 gene expression). These results reinforce arguments for epigenetic mechanisms of disease that
386 occur not only independent of exposures but also for epigenetic mechanisms that modify gene
387 expression in response to environmental exposures.

388

389 **Discussion**

390 One of the major challenges of genetic research is to uncover molecular mechanisms of disease
391 and to understand how genetic and environmental factors interact to influence these mechanisms
392 and individuals' risk for disease. Genome-wide association studies have identified thousands of
393 SNPs associated with complex diseases; however, the functions of non-coding SNPs identified in
394 GWASs, and therefore the molecular mechanisms in which they result in disease, is difficult to
395 infer from GWAS alone. Furthermore, other important contributors to disease pathophysiology
396 are not readily informed by GWAS, including epigenetic, environmental, and tissue- or cell type-
397 specific effects. Cell models address these limitations and advance our understanding of disease
398 pathobiology through experimental testing of disease mechanisms in controlled environments. In
399 this multi-omics study, we leveraged an airway epithelial cell model of microbial response to
400 identify functional variants that may have context-specific effects on transcriptional and
401 epigenetic responses and participate in molecular mechanisms that lead to a disease with an
402 underlying airway epithelial etiology. SNPs that were molecular QTLs in these models were co-
403 localized with adult onset and childhood onset asthma GWAS SNPs to identify 72 unique co-
404 localizations in at least one treatment condition. Integrating this information using Mendelian

405 randomization provided inferences into causality and insight into the molecular basis of
406 childhood onset asthma.

407 It is notable that we identified only six co-localizations with adult onset asthma GWAS
408 SNPs, compared to 70 with childhood onset asthma GWAS SNPs. None of the colocalizations in
409 the adult onset GWAS included an eQTL compared to 39 childhood onset co-localizations with
410 eQTLs, and only four meQTL-GWAS pairs were shared between adult and childhood onset
411 asthma, despite the fact the participants in our cell culture studies were all adults. Moreover,
412 even though there were 2.5-times the number of loci associated with childhood onset asthma
413 compared to adult onset asthma in the GWASs [12], we identified more than 11-times more co-
414 localizations using the childhood onset compared to the adult onset GWAS results (70 vs. 6,
415 respectively). These observations likely reflect the more important role of gene regulation and
416 dysregulation in airway epithelium in the etiology of childhood onset asthma compared to adult
417 onset asthma [41]. Focusing on other tissues (e.g., lung tissue) or cell types (e.g., immune cells)
418 might yield more co-localizations with adult onset GWAS SNPs or more shared co-localizations
419 between adult onset and childhood onset asthma.

420 Our study provides mechanistic evidence for associations between GWAS SNPs and
421 asthma at two important asthma loci: the *TSLP* locus at 5q22.1 and the 17q12-21 locus. Co-
422 localizations of the asthma associated SNP rs1837253 with DNA methylation levels in the *TSLP*
423 gene suggest an epigenetic mechanism of disease that contributes to both adult and childhood
424 onset asthma. Associations of this SNP with asthma have been highly replicated in GWASs and
425 *TSLP* is recognized as an important regulator in asthma pathogenesis through its broad effects on
426 innate and adaptive immune cells promoting Th2 inflammation [42]. Our data further show that
427 the effect of rs1837253 genotype on risk for both adult and childhood onset asthma is mediated

428 through DNA methylation levels at CpG sites in the untranslated first exon of the *TSLP* gene in
429 airway epithelial cells. Finally, the lack of LD with other SNPs in a 100 kb window suggests that
430 rs1837253 may indeed be the causal asthma SNP at this important locus.

431 Mendelian randomization of the childhood onset asthma 17q locus eQTL-meQTL-
432 GWAS triplets revealed a novel epigenetic mechanism through which a SNP at the 17q locus was
433 associated with expression of *ERBB2* only after exposure to RV. This eQTL was mediated
434 through differential methylation that was present in all treatment conditions. Previous studies
435 have shown that variation at the 17q core locus confers risk to asthma only among children who
436 experience wheezing illness in early life [36, 37], particularly with RV-associated wheezing [35].
437 Our study further connects RV infection and genotype at this locus but implicates for the first
438 time interaction effects between genetic and methylation variation at the distal end of the locus
439 and the expression of *ERBB2* at the proximal end of the locus only in RV infected epithelial
440 cells. The SNP that is the eQTL for *ERBB2* in RV infected epithelial cells was also associated
441 with asthma in the childhood onset asthma GWAS ($p_{\text{GWAS}} = 6.43 \times 10^{-26}$), directly connecting the
442 eQTL for *ERBB2* in RV-treated cells to asthma risk. The asthma associated allele, rs66826786-
443 C, which was associated with decreased expression of *ERBB2* in our study (Fig 5B), is consistent
444 with results of a study of 155 asthma cases and controls that reported an inverse correlation
445 between *ERBB2* expression in *ex vivo* lower airway epithelial cells and asthma severity [43].
446 These combined data suggest that the low expression of *ERBB2* associated with asthma severity
447 may be modulated by RV, the most common trigger of asthma exacerbations, via epigenetic
448 mechanisms involving DNA methylation and long-range chromatin looping between the
449 proximal and distal ends of this important locus. Our findings further highlight the importance of

450 RV exposure at this prominent asthma risk locus and provide mechanistic evidence for this
451 genotype-exposure interaction.

452 Many associations in GWASs have small p-values that do not reach genome-wide
453 significance ($p < 5 \times 10^{-8}$) but may be true signals. Distinguishing true positive from false signals
454 for variants among these mid-hanging fruit can be challenging. Cell culture models provide a
455 way to identify functional variants that regulate gene expression and/or epigenetic marks,
456 establishing a framework for distinguishing true from false positive associations. In our study,
457 over 72% of the co-localizations (52 total co-localizations; 22 eQTL-GWAS pairs; 17 meQTL-
458 GWAS pairs; 13 triplets) were with a GWAS SNP that did not meet genome-wide significance
459 in the GWAS for childhood onset asthma (GWAS p-value range 1.0×10^{-7} - 4.6×10^{-4} ; S1 Table).
460 These co-localizations were associated with eight eGenes (*ACO2*, *FGFR4*, *FRK*, *GSTO2*, *IRF5*,
461 *PMML1*, *POL1*) and 20 meCpGs. Notably, the majority of the co-localized triplets (13 of 14) were
462 associated with SNPs that did not reach genome-wide significance, perhaps because the variants
463 have exposure-specific or endotype-specific effects that are heterogeneous among subjects
464 included in asthma GWASs. Annotating SNPs among the mid-hanging fruit for functionality
465 provides a more complete picture of the genetic architecture of asthma and a paradigm for
466 selecting loci for further studies.

467 Our study has several limitations. First, the sample sizes for the eQTL and meQTL
468 studies were smaller than the minimum recommended by *moloc* ($n_{\min}=300$) [15]. In such cases,
469 *moloc* can miss true co-localizations in QTL datasets. For example, an eQTL-GWAS pair with
470 supporting evidence may, in reality, be an eQTL-meQTL-GWAS triplet. As a result, the eQTL-
471 GWAS and meQTL-GWAS pairs that we identified could be eQTL-meQTL-GWAS triplets that
472 we were not powered to detect, or we may have missed other co-localizations entirely.

473 Nonetheless, the 72 unique co-localization detected in our study are likely to be real although
474 replication studies in larger samples will increase the confidence in our findings. Second, we
475 focused our studies on one cell type (upper airway sinonasal epithelium), four exposures (RV,
476 SA, RVveh, SAveh), and one epigenetic mark (DNA methylation). It is likely that many of the
477 co-localizations are not specific to airway epithelium or to these four conditions, and that
478 additional epigenetic marks, such as those associated with chromatin accessibility, are involved
479 in these or other co-localizations. Studies in other cell types and evaluation of additional
480 exposures and epigenetic marks in larger sample sizes will be necessary to validate the cell- and
481 condition-specific colocalizations identified here and extend these studies to additional
482 conditions and molecular traits. Finally, characterizing chromatin conformational changes in
483 airway epithelial cells before and after exposure to RV will allow a direct assessment of the
484 chromatin looping at 17q that may occur in response to viral infection and potentially identify
485 other context-specific interactions.

486 In summary, we identified *cis*-eQTLs and *cis*-meQTLs in an airway epithelial cell model
487 of microbial response to RV and SA and integrated these data with asthma GWASs to assign
488 potential molecular mechanisms for variants associated with asthma in two large GWASs. By
489 combining co-localization analysis with Mendelian randomization, we provide robust statistical
490 evidence of epigenetic mechanisms that contribute to childhood onset asthma, at least one of
491 which is modulated by exposure to RV. We demonstrate that a multi-omics approach using
492 disease-relevant cell types and exposures allows prioritization of disease-associated variants and
493 provides insight into potential epigenetic mechanisms of asthma pathogenesis.

494

495

496 **Materials and Methods**

497 **Ethics statement**

498 Study participants were recruited between March 2012 and August 2015. Nasal specimens were
499 collected as part of routine endoscopic endonasal surgeries. Informed written consent was
500 obtained from each study participant and randomly generated ID codes were assigned to all
501 samples thereby preserving the participant's anonymity and privacy. This study was approved by
502 the institutional review boards of Northwestern University Feinberg School of Medicine and the
503 University of Chicago.

504

505 **Sample collection and composition**

506 Sinonasal epithelial cells were obtained by brushing the uncinat process collected at elective
507 surgery at Northwestern University from 68 males, 47 females, ages 18 – 73 years old (mean age
508 44), and self-reported ethnicities as Caucasian (67%), Black (16%), Hispanic (9%), and “other”
509 (8%). Blood samples for genotyping were collected from study participants. A summary of the
510 study design is shown in S1 Fig.

511

512 **Upper airway epithelial cell culture and microbial treatments**

513 After isolation, nasal airway epithelial cells were cultured in bronchial epithelial cell growth
514 medium (Lonza, BEGM BulletKit, catalog number CC-3170) to near confluence, then frozen at -
515 80°C and stored in Liquid Nitrogen. Cells were subsequently thawed and cultured in collagen-
516 coated (PureCol, INAMED BioMaterials, catalog number 5,409, 3 mg/mL, 1:15 dilution) tissue
517 culture plates (6 wells of 2x 12 well plates) using BEGM overnight at 37°C and 5% CO₂. In
518 preparation for rhinovirus (HRV-16; RV) RV infection/stimulation, plates at 50-60% confluency

519 were incubated overnight in BEGM without hydrocortisone (HC) followed by a two-hour RV
520 infection at a multiplicity of infection (MOI) of 2 and vehicle treatment (Bronchial epithelial cell
521 basal medium (BEBM) + Gentamicin/Amphotericin) at 33°C (low speed rocking, ~15 RPM).
522 RV- and vehicle-treated cells were washed and then were cultured at 33°C for 46 hours (48 hours
523 total) in BEGM without HC. Prior to heat-killed *Staphylococcus aureus* (SA, Life Technologies,
524 catalog number S-2859)-stimulation, cells were cultured to near 100% confluency in BEGM and
525 were further incubated without HC overnight at 37°C. Cells were then stimulated with SA ($5e^8$
526 particles/mL) and vehicle control (sterile 1x dPBS) for 24 hours at 37 °C in 5% CO₂.

527

528 **Genotyping and imputation**

529 DNA was extracted from whole blood or sinus tissue (if no blood was available) with the
530 Macherey-Nagel NucleoSpin Blood L or NucleoSpin Tissue L Extraction kits, respectively, and
531 quantified with the NanoDrop ND1000. Genotyping of all study participants was performed
532 using the Illumina Infinium HumanCore Exome+Custom Array (550,224 SNPs). After quality
533 control (QC) (excluding SNPs with HWE < 0.0001 by race/ethnicity, call rate < 0.95, MAF <
534 0.05 and individuals with genotype call rates < 0.05), 529,993 markers for 115 individuals were
535 available for analysis. Ancestral principal component analysis (PCA) was performed using 676
536 ancestral informative markers included on the array that overlap with the HapMap release 3 (S5
537 Fig).

538 Phasing and imputation were performed using the ShapeIt2 [44] and Impute2 [45]
539 software packages, respectively. Variants were imputed in 5 Mb windows across the genome
540 against the 1000 Genomes Phase 3 haplotypes (Build 37; October 2014). Individuals were
541 categorized into two groups based on the k-means clustering of ancestral PCs, using the

542 kmeans() function in R; individuals were grouped as European or African American based on
543 how they related to the HapMap reference panel and means clustering of their ancestral PCs (S5
544 Fig). After imputation, both groups were merged and QC was performed with gtool [46]. X and
545 Y chromosome-linked SNPs and SNPs that did not meet the QC criteria (info score < 0.8, MAF
546 < 0.05, missingness > 0.05 and a probability score < 0.9) were excluded from analyses.
547 Probability scores were converted to dosages for 6,665,552 of the remaining sites used in
548 downstream analyses.

549

550 **RNA extraction and sequencing**

551 Following RV and SA treatments, RNA from cells underwent extraction and purification using
552 the QIAGEN AllPrep DNA/RNA Kit. RNA quality and quantity were measured at the
553 University of Chicago Functional Genomics Core using the Agilent RNA 6000 Pico assay and
554 the Agilent 2100 Bioanalyzer. RNA integrity numbers (RIN) were greater than 7.7 for all
555 samples. cDNA libraries were constructed using the Illumina TruSeq RNA Library Prep Kit v2
556 and sequenced on the Illumina HiSeq 2500 System (50 bp, single-end); RNA sequencing was
557 completed at the University of Chicago Genomics Core. Subsequently, we checked for potential
558 sample contamination and sample swaps using the publicly available software VerifyBamID
559 (<http://genome.sph.umich.edu/wiki/VerifyBamID>) [47] for cells from all 115 individuals
560 included in each treatment condition. We did not detect any cross-contamination between
561 samples but we did identify one sample swap between individuals, which we subsequently
562 corrected.

563 Sequences were mapped to the human reference genome (hg19) and reads per gene were
564 quantified using the Spliced Transcripts Alignment to a Reference (STAR) [48] software. X,Y,

565 and mitochondrial chromosome genes, and low count data (genes < 1CPM) were removed prior
566 to normalization via the trimmed mean of M-values method (TMM) and variance modeling
567 (voom) [49]; samples contain > 8M mapped reads. Principle components analysis (PCA)
568 identified biological and technical sources of variation in the voom-normalized RNA-seq reads.
569 We identified contributors to batch and other technical effects in the RV experiment (days in
570 liquid nitrogen, experimental culture days, cell culture batches, RNA concentration, RNA
571 fragment length, technician, sequencing pool) and SA experiment (day in liquid nitrogen,
572 experimental culture days, cell culture batches, RNA concentration, RIN score, cDNA library
573 concentration). Sex was a significant variable in the PCA for the RV experiment. Additionally,
574 unknown sources of variation were predicted with the Surrogate Variable Analysis (SVA) [50]
575 package in R where 15 and 21 surrogate variables (SVs) were estimated for the RV and SA
576 experiments, respectively. Voom-normalized RNA-seq data were then adjusted for technical
577 effects, SVs, sex, and ancestral PCs (1-3) using the function removeBatchEffect() from the R
578 package limma [51].

579

580 **DNA extraction and methylation profiling**

581 Following RV and SA treatments, DNA was extracted from cells and purified using the
582 QIAGEN AllPrep DNA/RNA Kit. DNA methylation profiles for cells from each treatment were
583 measured on the Illumina Infinium MethylationEPIC BeadChip at the University of Chicago
584 Functional Genomics Core. Methylation data were preprocessed using the minfi package [52].
585 Probes located on sex chromosomes and with detection p-values greater than 0.01 in more than
586 10% of samples were removed from the analysis; samples with more than 5% missing probes
587 were also removed. A preprocessing control normalization function was applied to correct for

588 raw probe values or background and a Subset-quantile Within Array Normalization (SWAN)
589 [53] was used to correct for technical differences between the Infinium type I and type II probes.
590 Additionally, we removed cross-reactive probes and probes within two nucleotides of a SNP
591 with an MAF greater than 0.05 using the function `rmSNPandCH()` from the R package `DMRcate`
592 [54].

593 PCA identified technical and biological sources of variation in the normalized DNA
594 methylation datasets. We identified contributors to batch and technical effects in the RV
595 experiment (array, cell harvest date) and SA experiment (day in liquid nitrogen, array, cell
596 harvest date, DNA concentration). Sex, age, and smoking were significant variables in the PCA
597 across each treatment condition. Unknown sources of variation were predicted with the `SVA`
598 package where 37 SVs were estimated for both the RV and SA experiments. SWAN and
599 quantile-normalized M-values were then adjusted for batch and technical effects, SVs, sex, age,
600 smoking and steroid use (for the SA dataset) using the function `removeBatchEffect()` in R.

601

602 **eQTL and meQTL analyses**

603 Prior to e/meQTL analysis, voom-transformed gene expression values and normalized
604 methylation M-values were adjusted for potential batch, technical, and biological variables as
605 described above. Linear regression between the permuted genotypes and molecular phenotypes
606 (gene expression and methylation residuals) from each treatment condition was performed with
607 the `FastQTL` [55] software package within *cis*-window sizes of 1 Mb and 10 Kb for eQTL and
608 meQTL analyses, respectively. Nominal passes were conducted for each eQTL and meQTL
609 analysis within `FastQTL`, and an FDR threshold of 0.10 was applied to adjust for multiple testing
610 within each experimental dataset with the `p.adjust()` function in R.

611

612 **Multivariate adaptive shrinkage analysis (mash)**

613 An Empirical Bayes method of multivariate adaptive shrinkage was applied separately to the
614 eQTL and meQTL data sets as implemented in the R statistical package, *mashr*
615 (<https://github.com/stephenslab/mashr>) [20], to produce improved estimates of QTL effects and
616 corresponding significance values in each treatment condition. *Mashr* implements this in two
617 general steps: 1) identification of pattern sharing, sparsity, and correlation among QTL effects,
618 and 2) integration of these learned patterns to produce improved effects estimates and measures
619 of significance for eQTLs or meQTLs in each treatment condition. To fit the *mash* model, we
620 identified eQTLs and meQTLs at an FDR < 20% in at least one treatment condition to generate a
621 list of covariance matrices, constructed to represent patterns of effects in the data which included
622 both ‘data-driven’ and ‘canonical’ estimates (see [20]). The instructions found in the *mashr* data-
623 driven vignette were followed to run *mash*.

624

625 **Co-localization analysis**

626 To estimate the posterior probability that a variant was contributing to the signal of a genetic
627 variant was also associated with asthma, gene expression, and/or DNA methylation, we applied a
628 Bayesian statistical framework implemented in the R package *multiple-trait-coloc* (*moloc*) [15].
629 Summary data from adult onset and childhood onset asthma GWAS from [12], along with eQTL
630 and meQTL summary data from cells within each treatment condition (described above), were
631 included in the *moloc* analysis. Each co-localization analysis included summary data from a
632 GWAS and epithelial cell eQTLs and meQTLs from corresponding treatment conditions.
633 Because a genome-wide co-localization analysis was computationally untenable, genomic

634 regions for co-localization were defined using the GWAS Analysis of Regulatory of Functional
635 Information Enrichment with LD correction (GARFIELD) package implemented in R [56] .
636 First, we analyzed the enrichment pattern of e/meSNPs from all four treatment conditions in
637 adult onset and childhood onset GWASs using the default package settings. Second, we
638 extracted variants driving the enrichment signals at a GWAS p-value threshold of 1×10^{-4} .
639 Regions were defined as 2 Mb windows centered around these variants. Only regions with at
640 least 10 SNPs in common between all three datasets or ‘traits’ (GWAS, eQTL, and meQTL)
641 were assessed by moloc and 15 ‘configurations’ of possible variant sharing was computed across
642 these three traits (see [15] for more details). PPAs $\geq 70\%$ were considered as evidence for co-
643 localization. Prior probabilities of 1×10^{-4} , 1×10^{-6} , and 1×10^{-7} were chosen for the association of
644 one, two, or three traits, respectively, as recommended by the authors of moloc.

645

646 **Mendelian randomization**

647 Mendelian Randomization was performed using the `ivreg2` function in R ([https://www.r-
648 bloggers.com/an-ivreg2-function-for-r/](https://www.r-bloggers.com/an-ivreg2-function-for-r/)) which applies 2SLS regression, as implemented in [24].
649 We co-localized triplets (eQTL-meQTL-GWAS) to assess the causal effects of DNA
650 methylation on gene expression, using genotypes as the instrumental variable. P-values were
651 adjusted using the FDR method in the `p.adjust()` function in R. An FDR of less than 0.05 was
652 considered to be significant.

653

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656 and library preparation, and study subjects for their participation.

657

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866 **Fig 1. Summary of eQTL and meQTL mapping results.** Venn diagrams of overlapping
867 eQTLs (A) and meQTLs (B) in each condition (FDR<0.10). (C) Summary of eQTL and meQTL
868 mapping results for each treatment condition.

869

870 **Fig 2. Molecular QTL effects sharing across treatment conditions (lfsr<0.05).** Heatmaps
871 showing pairwise comparison of eQTL (A) and meQTL (B) pairwise sharing between treatment
872 conditions. (C) Examples of RV- (left) and SA-specific (right) eQTLs. (D) Examples of vehicle-
873 (left) and SA-specific (right) meQTLs.

874

875 **Fig 3. Spearman correlation plots showing correlations between co-localized meCpGs and**
876 **nearby genes in each treatment condition.** Scatterplots showing the correlation of DNA
877 methylation levels and gene expression in each treatment condition. (A) cg23659134 and
878 *GSTO2*. (B) cg26616347 and *IRF5* expression. In all panels, the y-axis shows methylation levels
879 and x-axis shows gene expression levels. See text for details.

880

881 **Fig 4. Co-localization of rs1837253 with DNA methylation levels of four meCpGs at *TSLP*.**
882 rs1837253 (red vertical bar) is associated with DNA methylation levels at four meCpGs (yellow
883 vertical bars). Box plots show DNA methylation levels (y-axes) for each meCpGs by rs1837253
884 genotype (x-axes) in each treatment condition.

885

886 **Fig 5. Co-localizations at the 17q asthma susceptibility locus.** (A) e/meSNPs and CpGs
887 included in each of the six co-localizations are shown by vertical colored lines. Solid lines
888 indicate the location of the SNPs; dashed lines show the location of CpGs. SNP-CpG co-

889 localizations are indicated by vertical lines of the same color. A single eQTL-GWAS pair
890 (rs12603332) for *ORDML3* is shown in orange. The three meQTL-GWAS pairs are shown in
891 green (rs66826786; cg1740124), purple (rs4239225; cg21230266), and turquoise (rs2859191;
892 cg21230266); cg21230266, shown in purple and turquoise, co-localizes with both rs4239225 and
893 rs2859191. The SNP (rs66828786) and meCpG (cg17401724) associated with the triplet
894 containing *ERBB2* is shown in red. (B) Box plots for eQTLs (top row) and meQTLs (bottom
895 row) associated with the eQTL-meQTL-GWAS triplet. The SNP in this triplet is associated with
896 *ERBB2* expression only in the RV-treated cells, but with DNA methylation levels (cg17401724)
897 in all treatments.

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905 **Supporting information**

906 **S1 Fig. Overview of the e/meQTL and colocalization studies in upper airway epithelial cells**

907 **treated with RV and SA.** (A) Step-wise experimental design to identify treatment-specific
908 e/meQTLs in airway epithelial cells from 115 individuals: 1. Nasal epithelial cells collected from
909 study participants were cultured and treated with either RV or SA for 48 and 24 hours,
910 respectively. 2. Gene expression and DNA methylation measured in nasal epithelial cells from
911 each treatment condition. 3. Genotype profiling to identify genetic variation influencing gene
912 expression and DNA methylation to RV- and SA-treatment. 4. QC and analyses including
913 e/meQTL mapping, multi-trait co-localization analysis, and Mendelian randomization. (B) Venn
914 diagram of asthma and atopy status for study subjects. (C) Breakdown of the number of subjects
915 for each experiment (RV and SA) and molecular QTL mapping.

916

917 **S2 Fig. Summary of molecular QTL effects sharing across treatment conditions (lfsr<0.05).**

918 (A) Number of eQTLs and corresponding eGenes and eSNPs shared between treatment
919 conditions. (B) Number of meQTLs and corresponding meCpGs and meSNPs shared between
920 treatment conditions.

921

922 **S3 Fig. Scatterplots showing the Spearman correlation of DNA methylation levels for** 923 **meCpGs of co-localized meQTLs and gene expression of nearby genes for each treatment**

924 **condition.** Examples of treatment and culture effects on DNA methylation and gene expression
925 correlations. (A) Correlations for DNA methylation and *FRK* gene expression suggest culture-
926 specific effects. (B and C) Correlations for DNA methylation and *GSTO2* do not show
927 preference to any treatment condition. Although the meCpGs (cg23659134, cg07488549) that

928 are correlated with *GSTO2* gene expression are both located intergenically and within the same
929 intron as *GSTO2*, the direction of their effects on *GSTO2* expression are in opposing directions.
930 (D-F) DNA methylation and gene expression correlations suggestive of RV-specific effects in
931 which the correlation is reduced after treatment with RV.

932

933 **S4 Fig. Association of *ORMDL3* expression and genotype.** Box plots of an eQTL for
934 *ORMDL3* and rs12603332 for RV, RVveh, SA, and SAveh treatment conditions (A-D,
935 respectively).

936

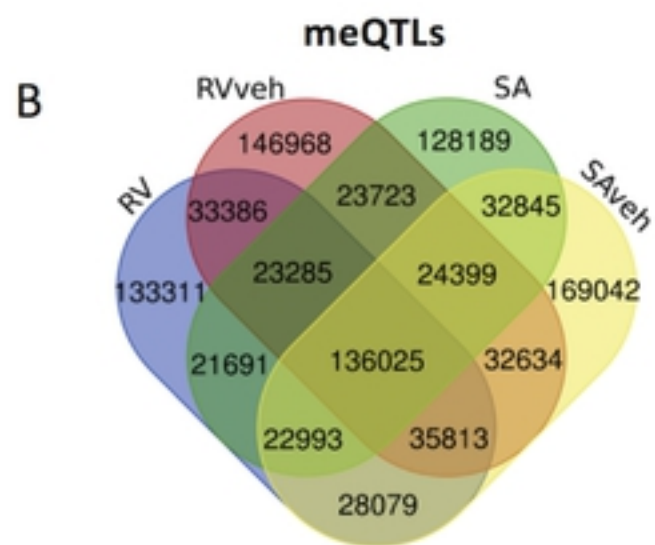
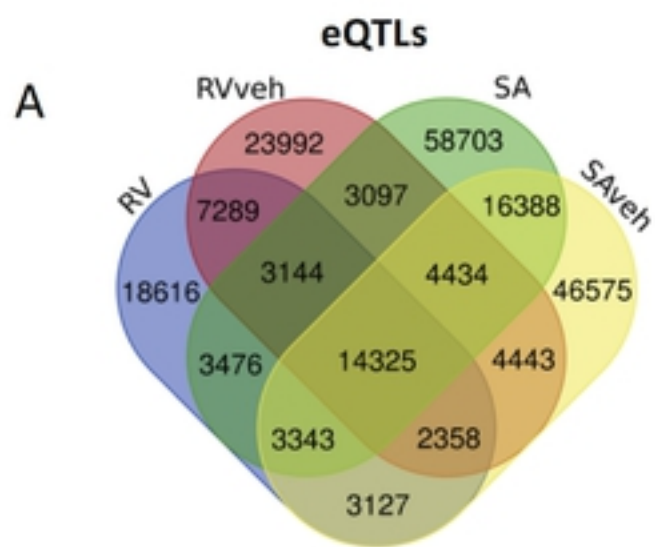
937 **S5 Fig. PCA and k-means clustering of genotypes.** (A) PCA plot of study participant's
938 genotypes (circles) projected on HapMap genotypes (squares). (B) Scree plot of k-means
939 clustering of ancestral PCs in which the within groups sum of squares (y-axis) is plotted against
940 the number of potential group clusters (x-axis); using the 'elbow criterion', it is determined that
941 two clusters are best representative of how many clusters we can group the study samples. (C)
942 PCA plot of study participants grouped into two cluster for genotype imputation, European (red),
943 and African American (Blue), according to the k-means clustering criterion.

944

945 **S1 Table.** *moloc* results indicating molecular QTL-GWAS pars and triplets.

946 **S2 Table.** Gene expression and meCpG Spearman correlations.

947 **S3 Table.** Shared meQTL-GWAS pairs between adult onset and childhood onset asthma.



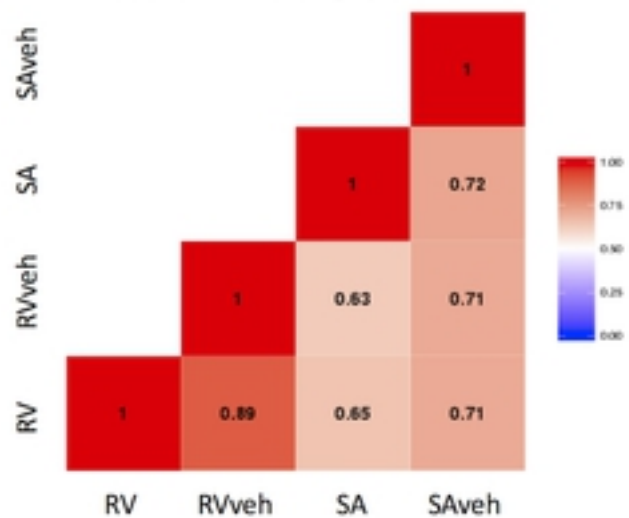
C

eQTL and meQTL mapping results (FDR<0.10)

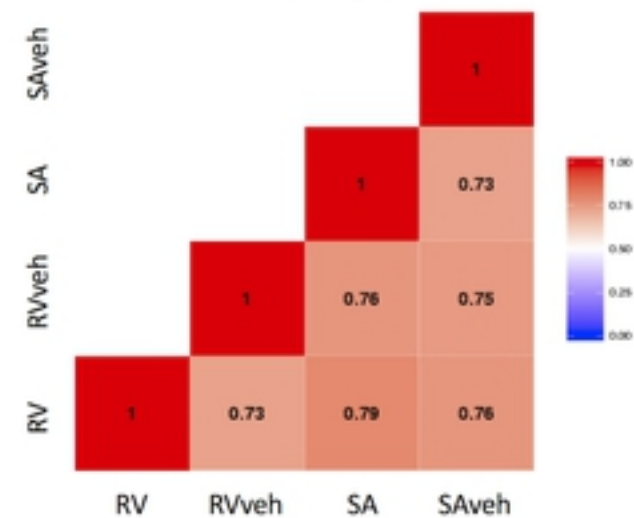
| | RV | RVveh | SA | SAveh |
|---------------|---------|---------|---------|---------|
| eQTLs | 55,678 | 63,082 | 106,910 | 94,993 |
| eSNPs | 52,519 | 59,689 | 97,627 | 87,971 |
| eGenes | 1,637 | 1,873 | 2,599 | 2,406 |
| meQTLs | 434,483 | 456,233 | 413,150 | 481,830 |
| meSNPs | 306,850 | 320,174 | 293,276 | 336,901 |
| meCpGs | 40,789 | 42,038 | 38,501 | 44,840 |

Fig 1

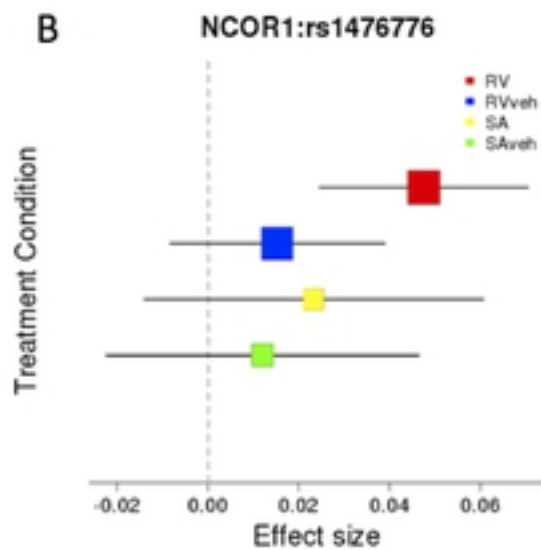
A eQTL Pairwise Sharing by Treatment



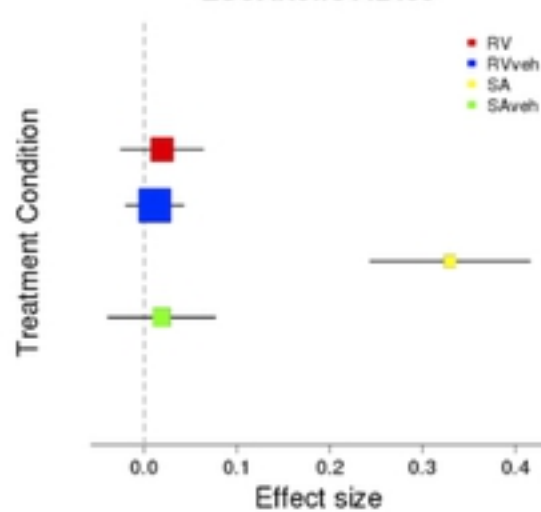
C meQTL Pairwise Sharing by Treatment



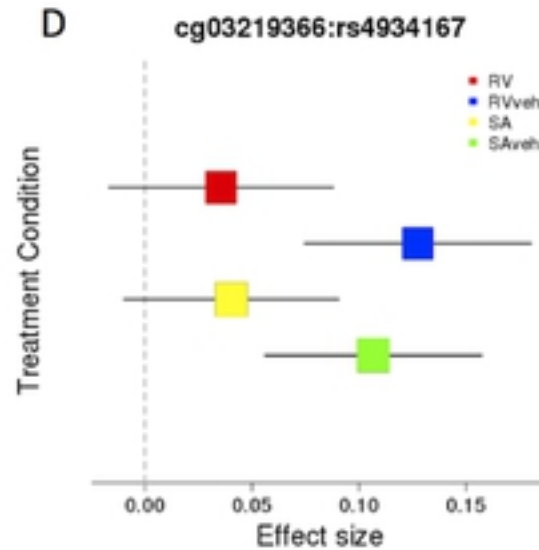
B



ZSCAN9:rs442439



D



cg11318571:rs10886413

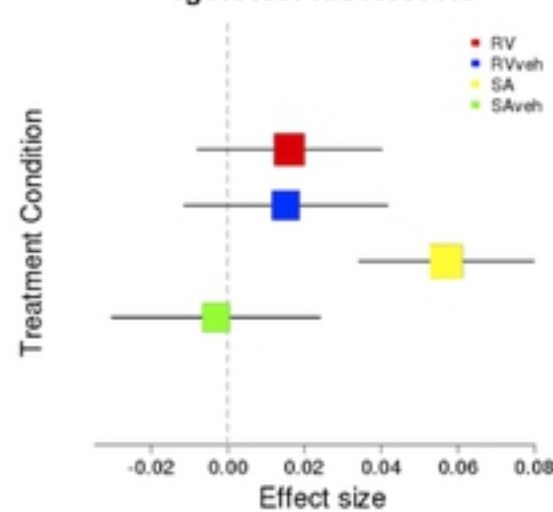


Fig 2

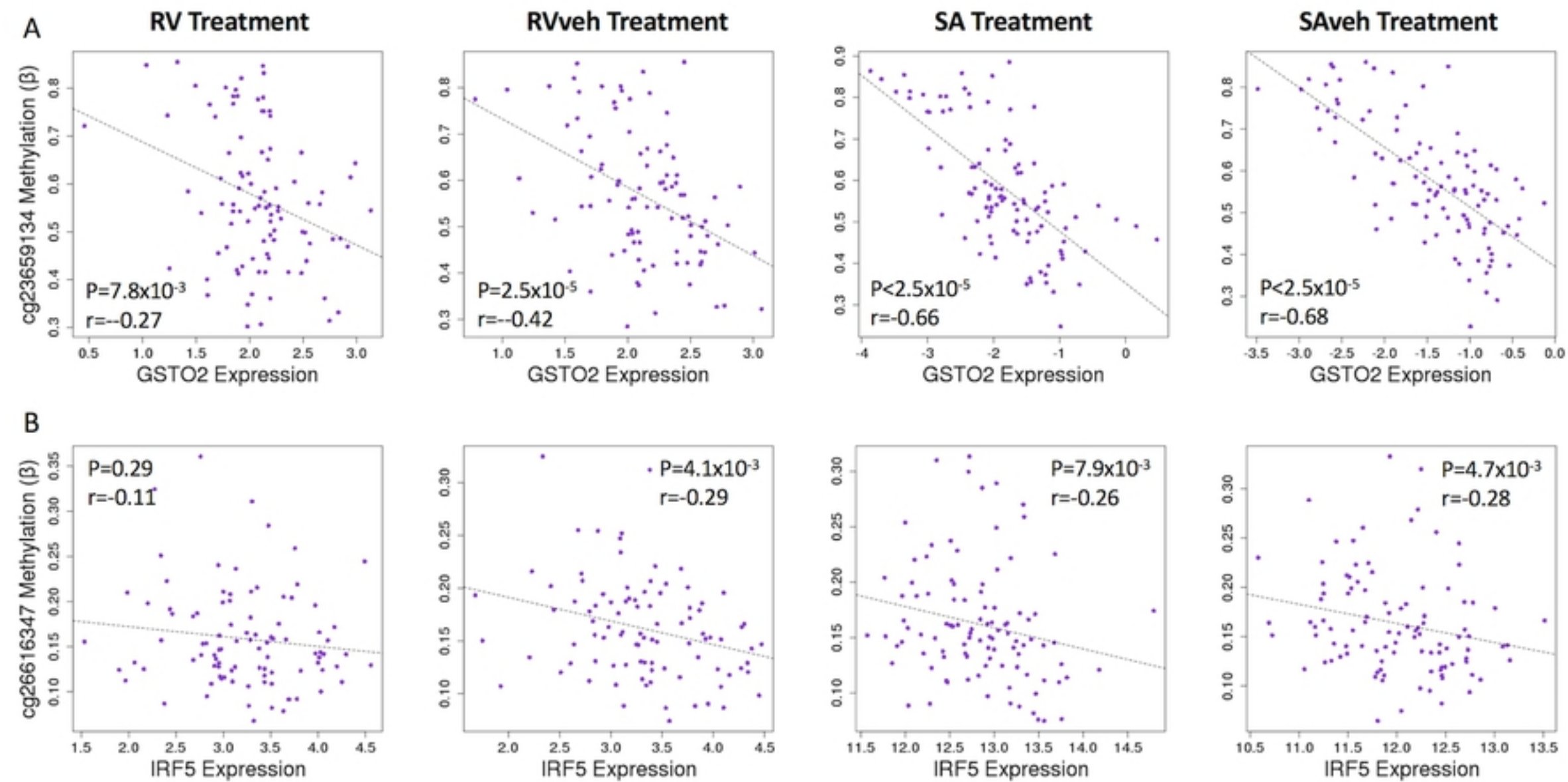
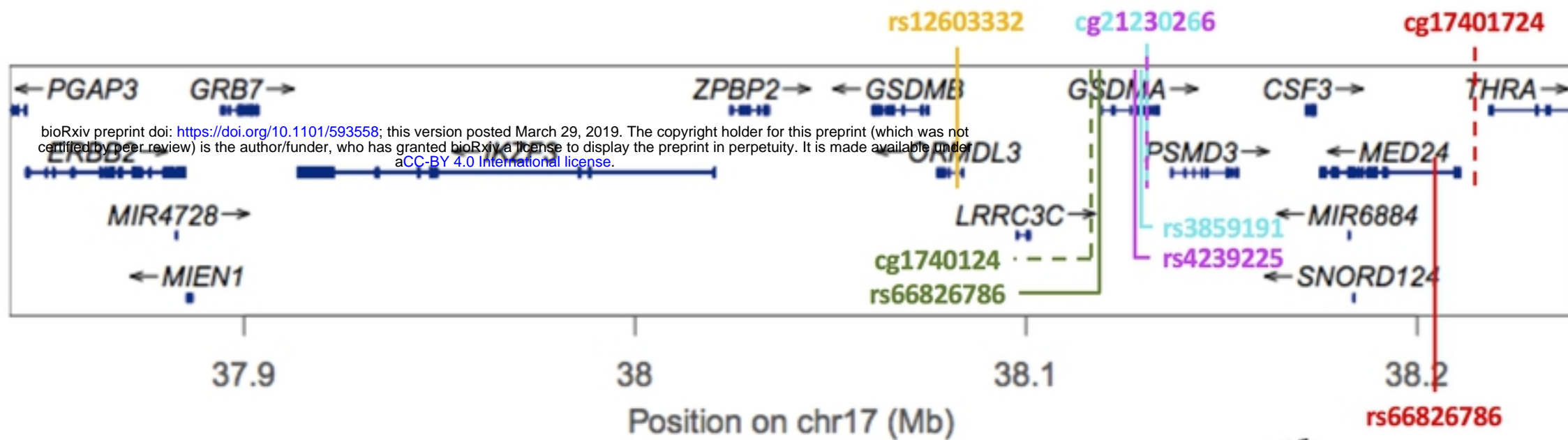


Fig 3

A



B

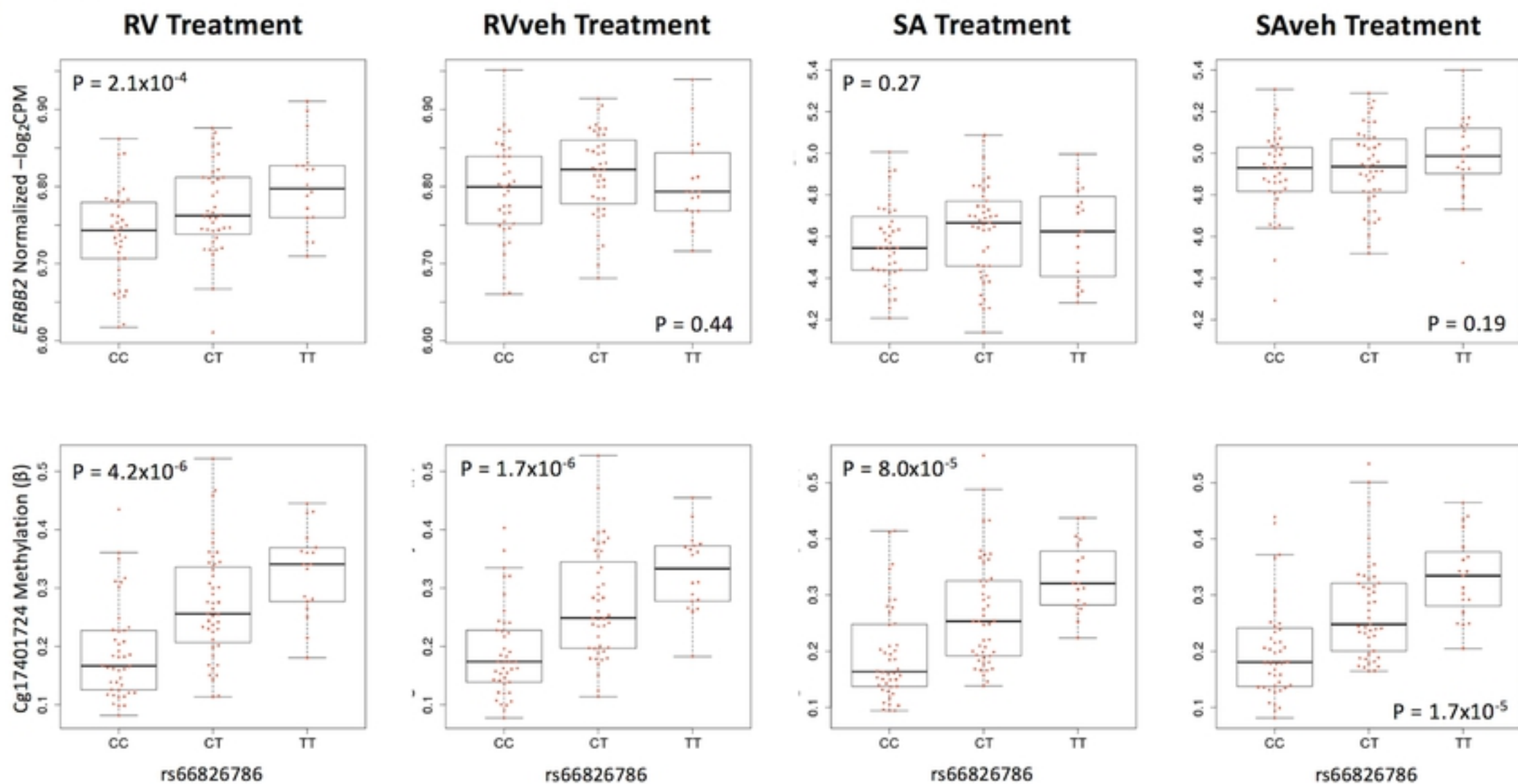


Fig 5