Figure 1. Overview of the experimental and analytic design of the study. We hypothesize that a classifier based on the pathway-level transcriptional responses that differ between symptomatic and asymptomatic responses to HRV infection in healthy patients can predict which asthmatic patients will have exacerbations over a 1-year follow-up period, based on those patients' transcriptomic responses to an in vitro HRV stimulation assay. Panel A illustrates the development of a classifier using innovative features. Both shared and unique genetic and nongenetic variables influence transcript expression and healthy vs disease state within an individual. Exposure to a relevant stimulus (here, HRV infection) reveals relevant pathway gene sets whose genome and environmentally informed responses (G × E response pathways) can be used to predict individual prognosis. Panel B describes the development of classifier from the training set using data from PBMCs of healthy volunteers exposed to HRV in vivo. For each patient, paired microarrays analyzing gene expression before and after HRV exposure were compared using N-of-1-pathways analysis to identify significant Gene Ontology biological process (GO-BP) features describing each response. Responses in asymptomatic patients were then compared to responses in symptomatic patients to develop the classifier. Panel C describes the laboratory stimulation assay of asthmatic patients' PBMCs in this study. As in the training set, paired microarrays for each patient were used to determine the response using N-of-1-pathways. The classifier developed in panel B was then applied to individual responses and used to predict recurrent exacerbation.

Figure 2. Metrics derived from responsive pathways discriminate asymptomatic from symptomatic subjects in the training set of in vivo HRV-stimulation data. In panel A, principal component analysis was conducted using responsive gene sets derived from each subject in the training set (Figure 1B and Materials and Methods section). The scatter plot on the left illustrates the bivariate relationship of the first and third principal components for each patient's ternary-represented N-of-1-pathways scores derived from paired samples of PBMCs collected at baseline and after HRV exposure. Each point represents a subject in the training set through a linear combination of pathway gene set–level scores that explain the maximal variation in the data (see Materials and Methods section for details on the ternary representation and PCA construction). The first and third principal components show 2 clusters emerging that separate asymptomatic from symptomatic individuals. Thus, N-of-1-pathways scores are associated with the phenotype of interest. On the right, side-by-side box plots display the first principal component scores among asymptomatic and symptomatic subjects, and this component alone significantly dichotomizes the 2 phenotypes (Mann-Whitney U test, $P = .0069$). Panel B lists the 20 Gene Ontology biological processes used as features in our classifier, organized according to broad biological function categories.

Figure 3. Fully specified classifier derived from responsive pathways in training set performs accurately in independent asthmatic validation/virogram set to predict exacerbation status. Panel A shows receiver operating curve (ROC) of the G × E classifier conducted in the validation set (overall accuracy, 74%; sensitivity, 75%; specificity, 73%). In panel B, the star plots illustrate the level of response to HRV stimulation for each pathway in that patient. The classifier is designed using 20 pathways, with each radial line representing the score of a pathway. The area above and below the gray zone represents upregulation and downregulation, respectively, of any given pathway (see Materials and Methods section for complete details). In panel C, each star plot represents a single subject, with label

appearing above (eg, Subject 16 = SUB16). The star plot is located in the quadrant of the contingency table that represents the performance of the G × E classifier on predicting the clinical progression from a specific asthmatic patient's data. This classifier prediction applied to the HRV-stimulation assay recapitulated the clinical progression in 17 out of the 23 asthmatic patients; 6 were misclassified: 3 false positives (SUBM1, SUB09, SUB23) and 3 false negatives (SUB2, SUB23, and SUBM2). One can straightforwardly identify that innate immunity (mauve) is upregulated in every asthmatic patient and does not contribute to the classification in the validation set. On the other hand, observed upregulation in acquired immunity (orange) pathways could be used to correctly classify 19 subjects.

Figure 1

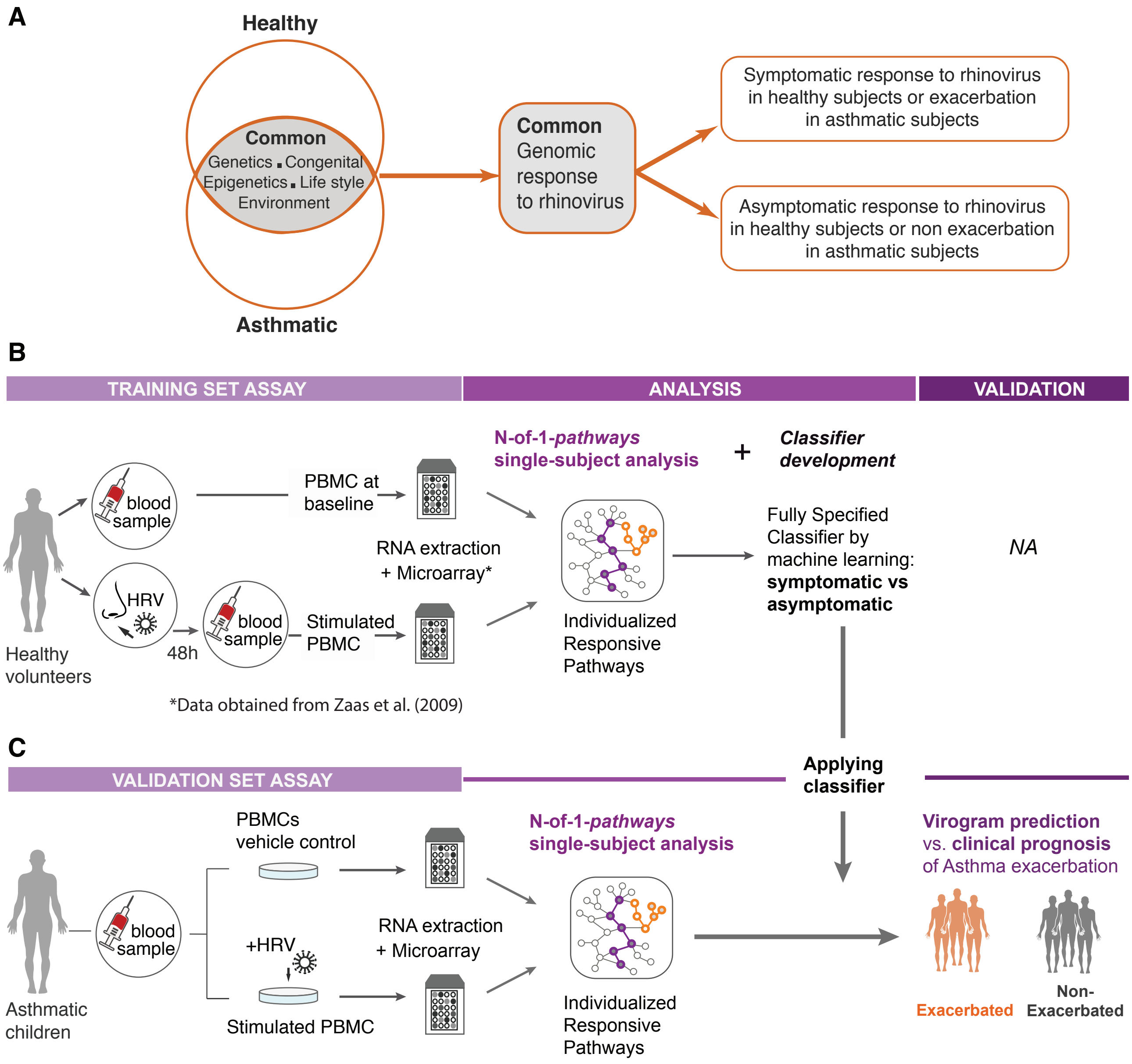
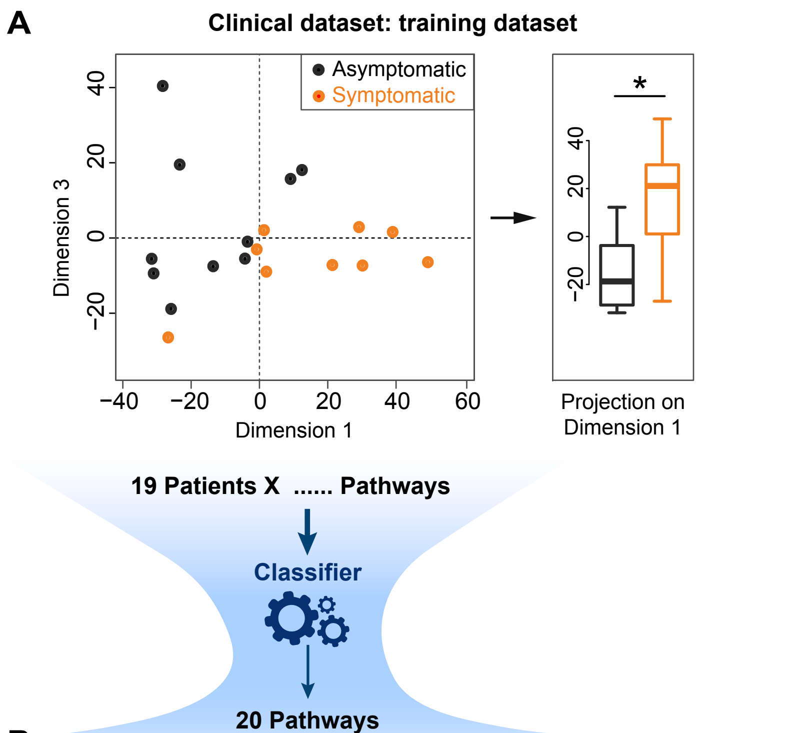

A
Healthy
Common
Genetics
Congenital
Epigenetics
Life style
Environment
Asthmatic
Common
Genomic
response
to rhinovirus
Symptomatic response to rhinovirus
in healthy subjects or exacerbation
in asthmatic subjects
Asymptomatic response to rhinovirus
in healthy subjects or non exacerbation
in asthmatic subjects
B
TRAINING SET ASSAY
ANALYSIS
VALIDATION
N-of-1-pathways
single-subject analysis
+
Classifier
development
blood
sample
PBMC at
baseline
RNA extraction
+ Microarray*
HRV
48h
blood
sample
Stimulated
PBMC
Healthy
volunteers
Individualized
Responsive
Pathways
Fully Specified
Classifier by
machine learning:
symptomatic vs
asymptomatic
NA
*Data obtained from Zaas et al. (2009)
C
VALIDATION SET ASSAY
Applying
classifier
PBMCs
vehicle control
N-of-1-pathways
single-subject analysis
Virogram prediction
vs. clinical prognosis
of Asthma exacerbation
blood
sample
+HRV
RNA extraction
+ Microarray
Asthmatic
children
Stimulated PBMC
Individualized
Responsive
Pathways
Exacerbated
Non-
Exacerbated

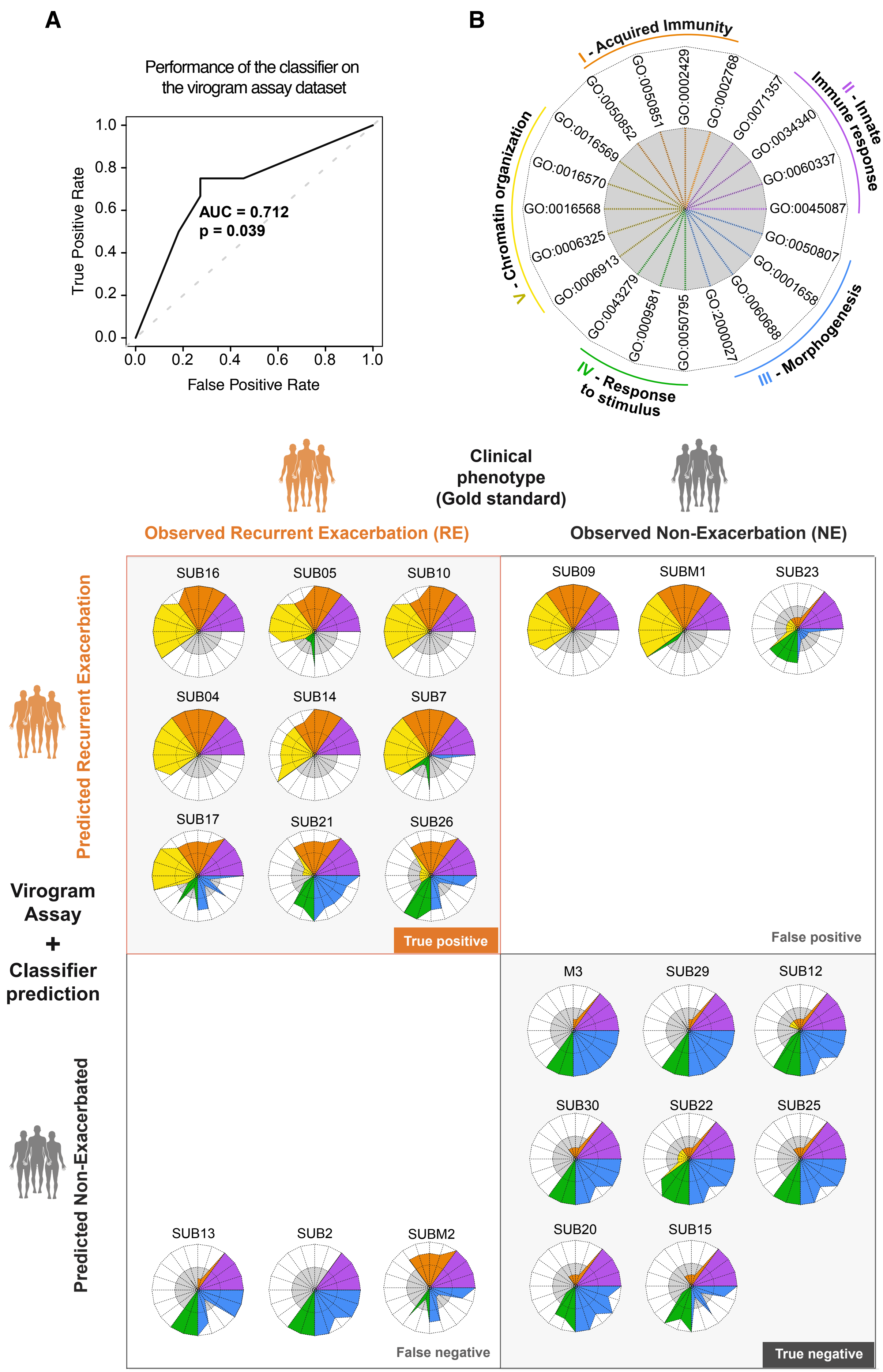
Figure 2



B

I- Acquired immunity	GO:0002768	immune response-regulating cell surface receptor ..
	GO:0002429	immune response-activating cell surface receptor ..
	GO:0050851	antigen receptor-mediated signaling pathway
	GO:0050852	T cell receptor signaling pathway
II- Innate immune response	GO:0045087	innate immune response
	GO:0060337	type I interferon-mediated signaling pathway
	GO:0034340	response to type I interferon
	GO:0071357	cellular response to type I interferon
III- Morphogenesis	GO:0050807	regulation of synapse organization
	GO:0001658	branching involved in ureteric bud morphogenesis
	GO:0060688	regulation of morphogenesis of a branching structure
	GO:2000027	regulation of organ morphogenesis
IV- Response to stimulus	GO:0043279	response to alkaloid
	GO:0050795	regulation of behavior
	GO:0009581	detection of external stimulus
V- Chromatin organization	GO:0016569	covalent chromatin modification
	GO:0016570	histone modification
	GO:0016568	chromatin modification
	GO:0006325	chromatin organization
	GO:0006913	nucleocytoplasmic transport

Figure 3


A
Performance of the classifier on the virogram assay dataset
True Positive Rate
False Positive Rate
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
AUC = 0.712
p = 0.039
B
I - Acquired Immunity
II - Innate Immune response
III - Morphogenesis
IV - Response to stimulus
V - Chromatin organization
GO:0002429
GO:0002768
GO:0071357
GO:0034340
GO:0060337
GO:0045087
GO:0050807
GO:0001658
GO:0060688
GO:2000027
GO:0050795
GO:0009581
GO:0043279
GO:0006913
GO:0006325
GO:0016568
GO:0016570
GO:0016569
GO:0050852
GO:0050851
Clinical phenotype (Gold standard)
Observed Recurrent Exacerbation (RE)
Observed Non-Exacerbation (NE)
Virogram Assay + Classifier prediction
Predicted Recurrent Exacerbation
Predicted Non-Exacerbated
SUB16
SUB05
SUB10
SUB04
SUB14
SUB7
SUB17
SUB21
SUB26
True positive
SUB09
SUBM1
SUB23
False positive
SUB13
SUB2
SUBM2
False negative
M3
SUB29
SUB12
SUB30
SUB22
SUB25
SUB20
SUB15
True negative