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Supplementary Materials for

Multicohort analysis reveals baseline transcriptional predictors of influenza vaccination responses

HIPC-CHI Signatures Project Team* and HIPC-I Consortium

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This PDF file includes:

SUPPLEMENTARY FIGURES

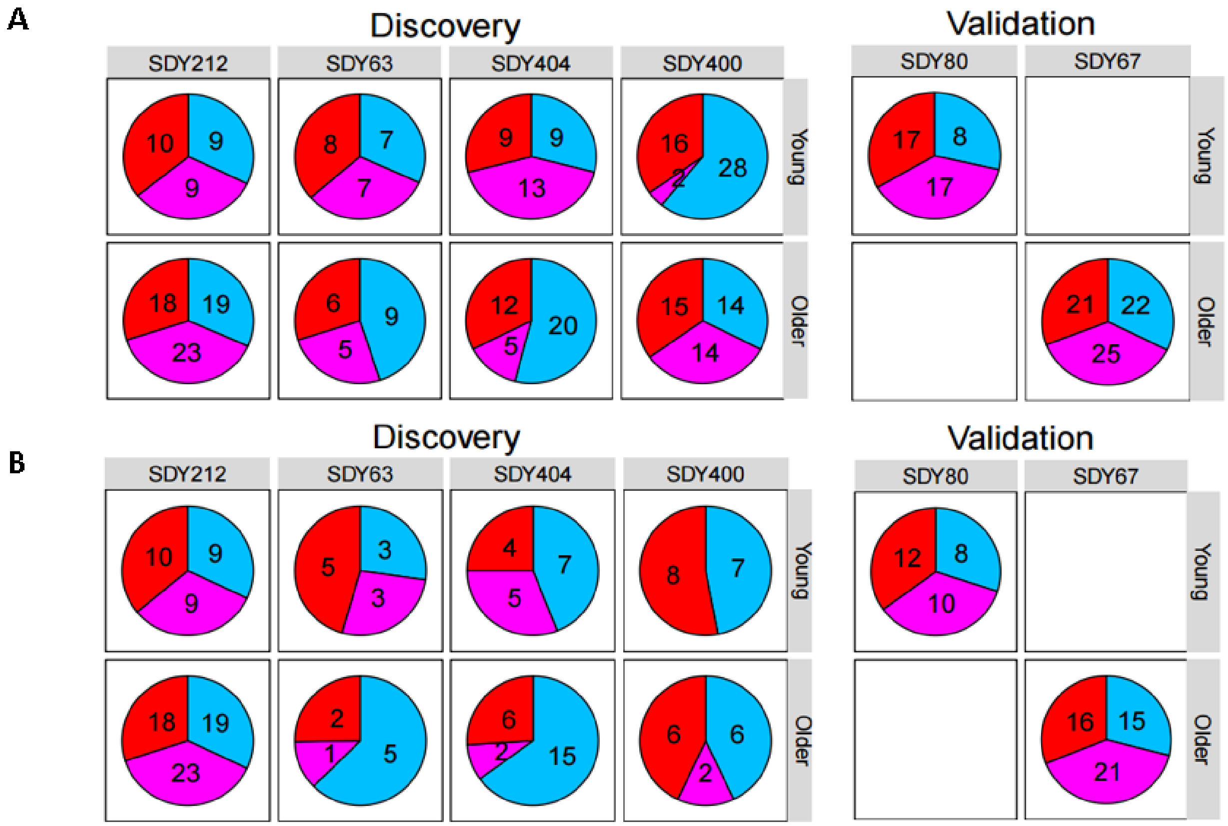

Fig. S1. Distribution of low (blue), moderate (purple), and high (red) responders in the discovery and validation cohorts. (A) All participants included in either the Young or Older groups (*i.e*, in the unshaded areas from Fig 2). Note that these numbers also exclude the smaller batch from SDY67, which was not used in this study. (B) The subset of participants where transcriptional profiling data were available and used in the analysis. Note the enrichment of high and low responders in those with transcriptional profiling data (see Fig 2 for details.)

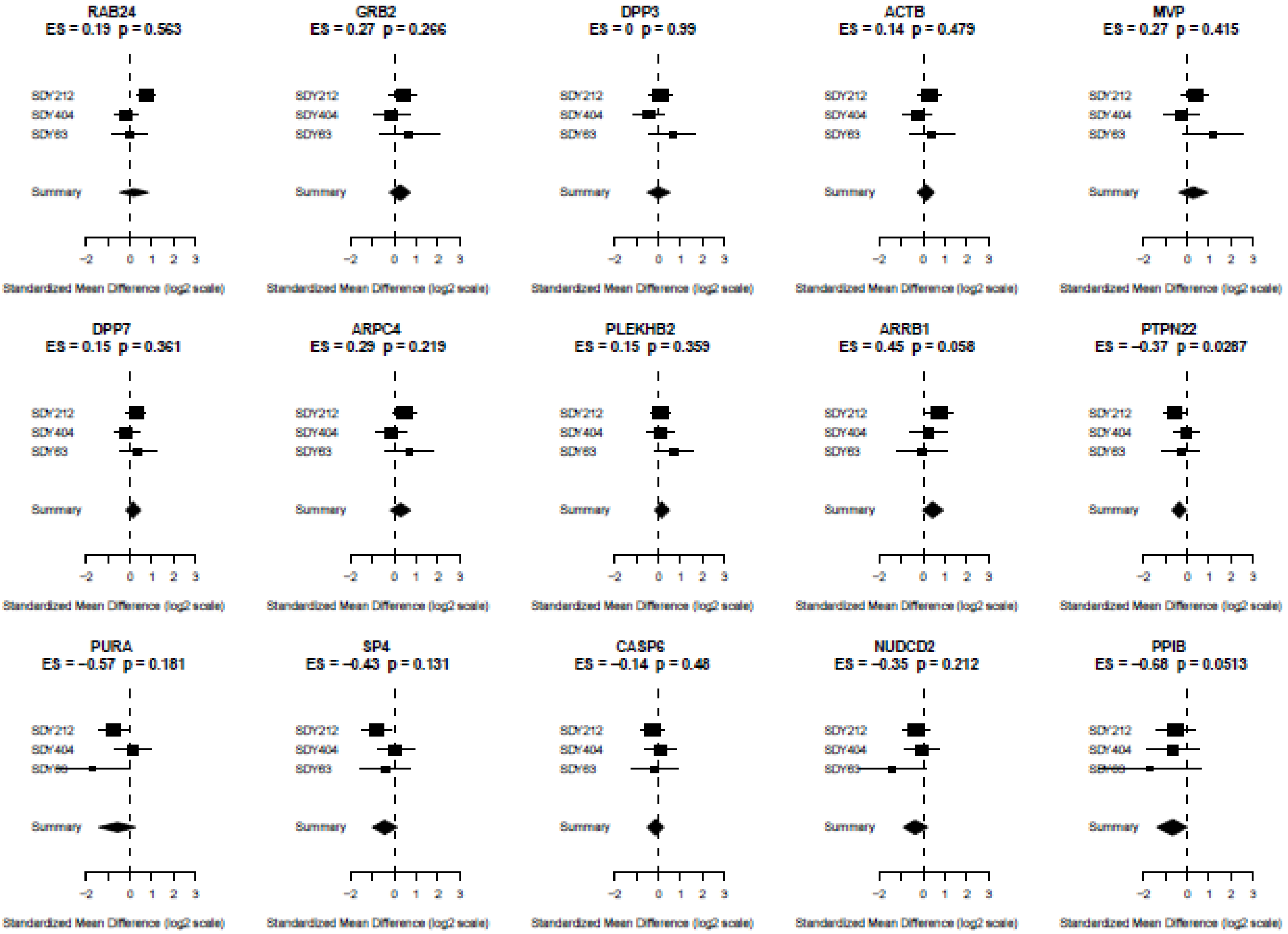

Fig. S2. Genes that predict vaccination response in young individuals when comparing moderate responders versus low responders. The x axes correspond to standardized mean difference, referred to as effect size (ES), between moderate and low responders, computed as Hedges' g, in log2 scale. The size of the rectangles is inversely proportional to the standard error of mean (SEM) in the individual cohort. Whiskers represent the 95% confidence interval. The diamonds represent overall, mean difference for a given gene combined across the discovery cohorts. The width of the diamonds represents the 95% confidence interval of overall mean difference.

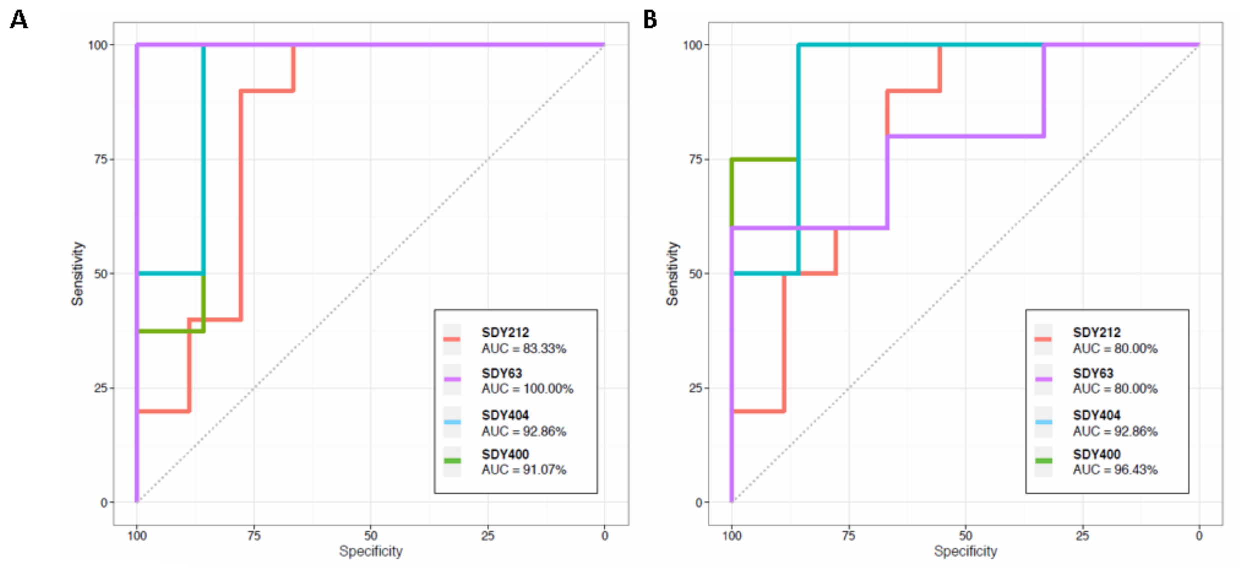

Fig. S3. Performance of genes significantly different in young high versus low responders. (A) The nine genes (*RAB24*, *GRB2*, *DPP3*, *ACTB*, *MVP*, *DPP7*, *ARPC4*, *PLEKHB2*, and *ARRB1*) with significantly increased expression, and (B) The six genes (*PTPN22*, *PURA*, *SP4*, *CASP6*, *NUDCD2*, and *PPIB*) with significantly reduced expression in young high responders. Each ROC curve summarizes the ability to classify individual participants as low vs. high responders within the indicated discovery cohort.

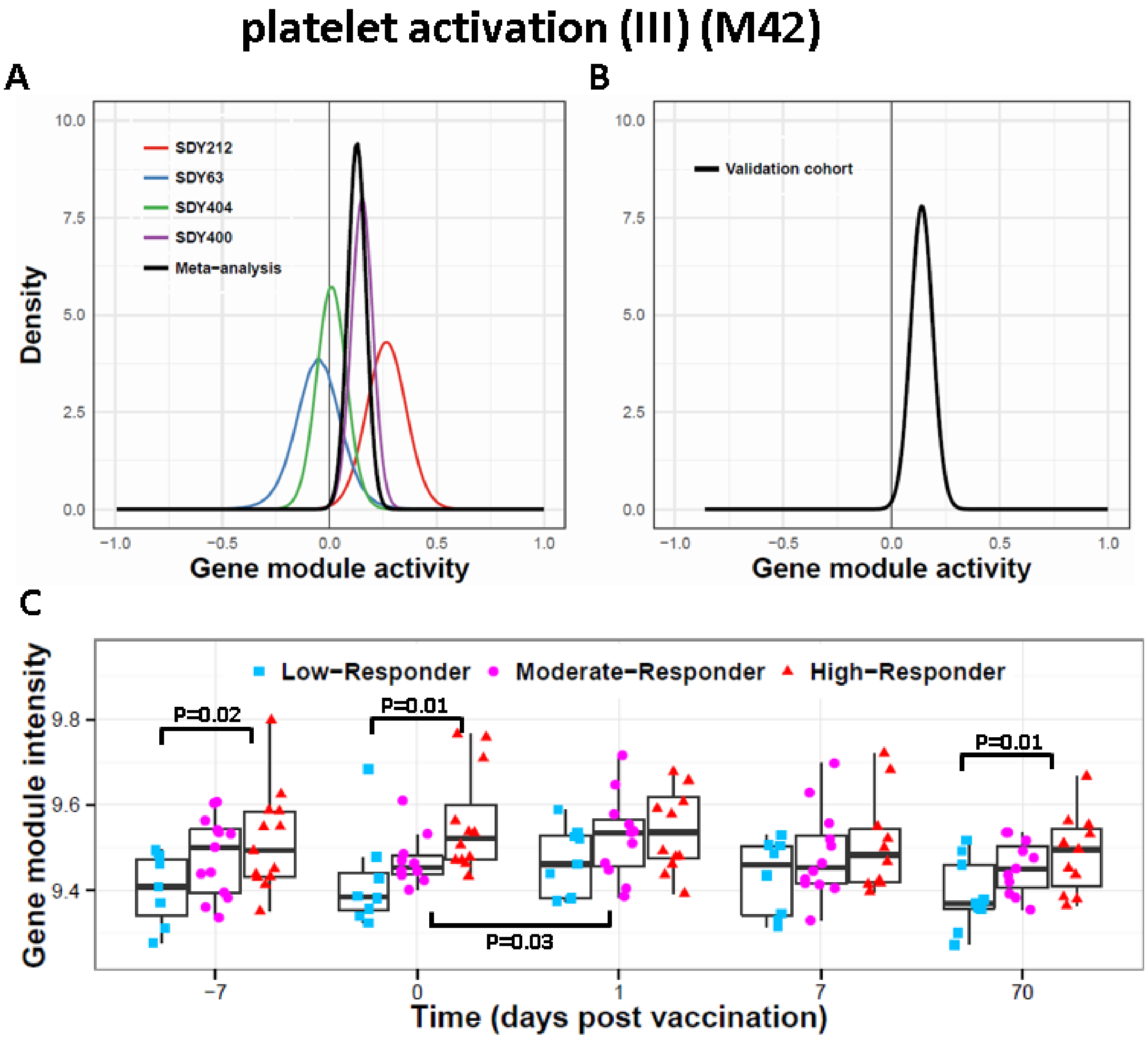

Fig. S4. Baseline activity of the platelet activation (III) (M42) gene module is associated with influenza vaccination responses in young individuals. See Fig 7 for details.

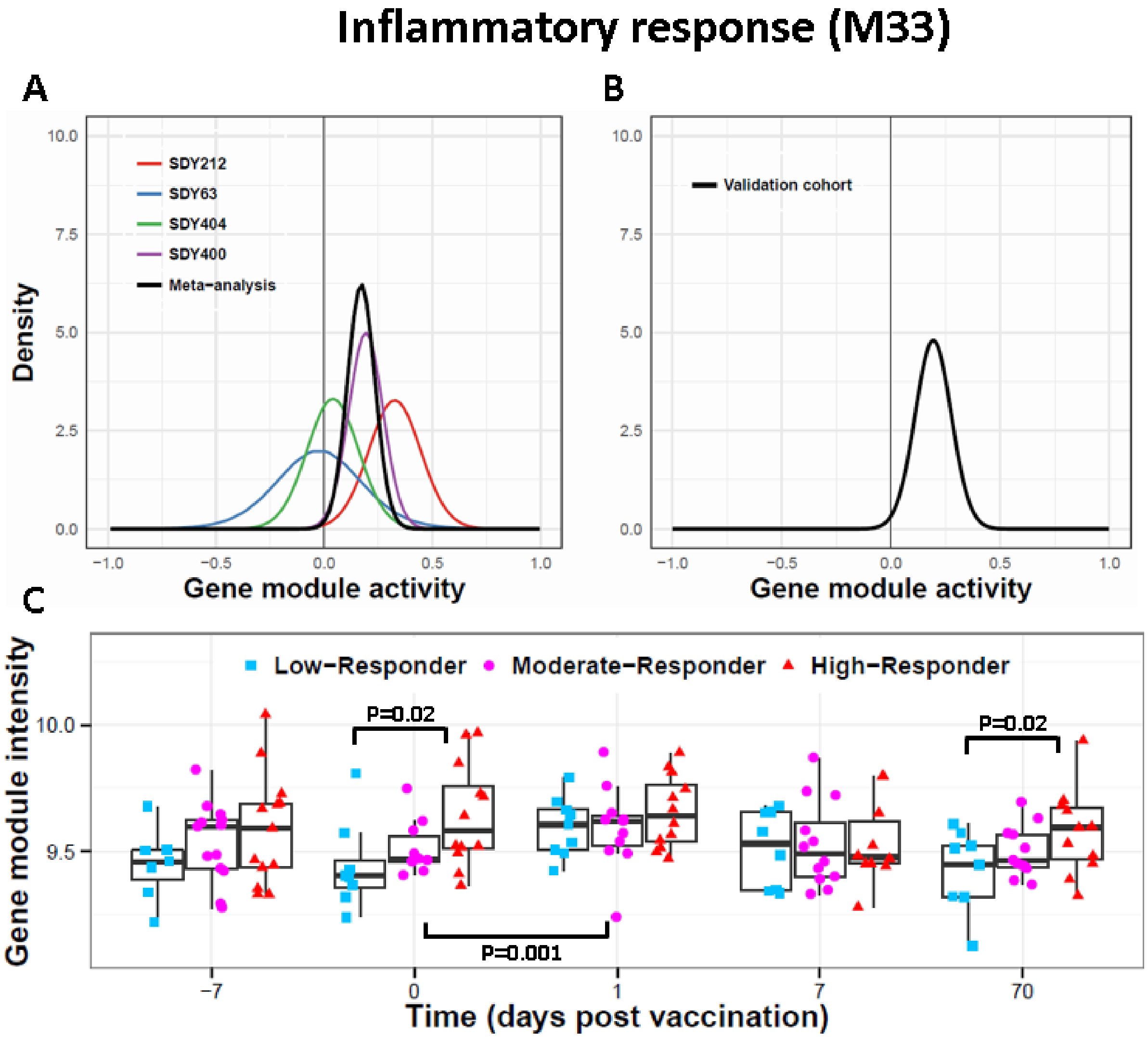

Fig. S5. Baseline activity of the inflammatory response (M33) gene module is associated with influenza vaccination responses in young individuals. See Fig 7 for details.

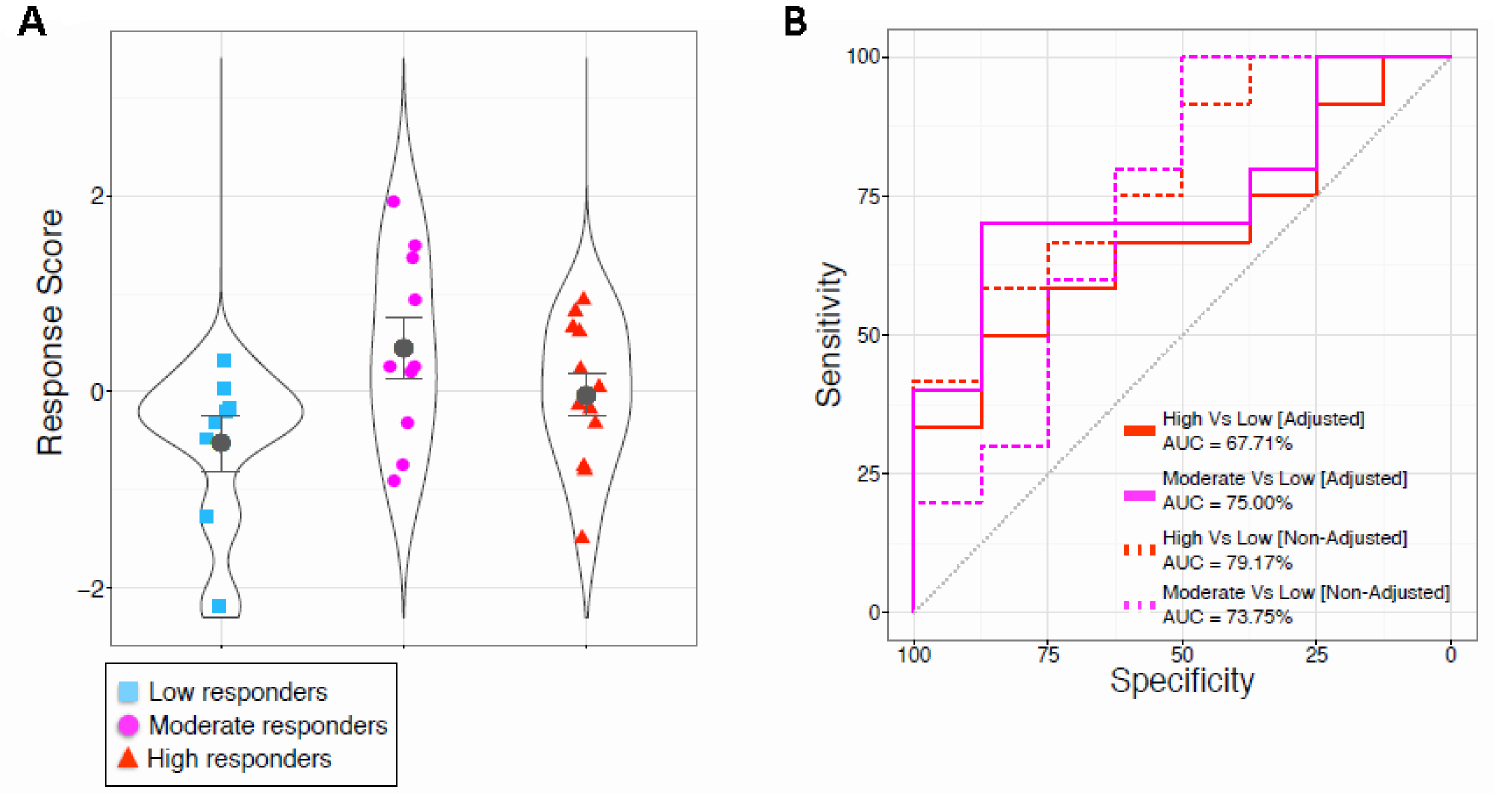

Fig. S6. Validation of *GRB2, ACTB, MVP, DPP7, ARPC4, PLEKHB2*, and *ARRB1* as predictors of influenza vaccination response in the validation cohort (SDY80) after correction for cell subset proportions. Note that two genes (*RAB24* and *DPP3*) identified as increased in high versus low responders in the discovery cohort were not measured in the validation cohort.

Table S1. **Characteristics of the discovery and validation cohorts for (A) young and (B) older participants.**

A. Young participants

Cohort	Gender	# Participants	Min Age	Max Age	Median Age
SDY63	Female	17	21	29	26
SDY63	Male	5	23	27	24
SDY80	Female	27	21	35	23
SDY80	Male	19	21	34	23
SDY212	Female	14	21	30	25.5
SDY212	Male	16	21	31	25
SDY400	Female	28	22	30	26
SDY400	Male	20	21	30	26
SDY404	Female	18	22	30	27
SDY404	Male	14	22	30	24.5

B. Older participants

Cohort	Gender	# Participants	Min Age	Max Age	Median Age
SDY63	Female	11	71	78	74
SDY63	Male	9	71	79	75
SDY67	Female	45	60	73	67
SDY67	Male	24	60	73	67
SDY212	Female	40	61	90	80
SDY212	Male	21	62	90	78
SDY400	Female	28	65	88	72
SDY400	Male	17	65	85	70
SDY404	Female	22	66	92	75
SDY404	Male	15	69	93	73

Table S2. Gene module activities that are associated with vaccination response in the discovery cohorts for older participants.

Gene Module	Discovery Cohorts			Validation Cohort		
	P value	FDR	Gene Module Activity	P value	FDR	Gene Module Activity
lysosomal/endosomal proteins (M139)	0.008	0.473	-0.145	0.955	0.963	0.010
enriched in antigen presentation (II) (M95.0)	0.009	0.473	-0.117	0.963	0.963	0.003

Table S3. Validation of gene modules that are associated with vaccination response in KEGG and Reactome and the modules defined in Obermoser *et al.* for young participants.

Gene Module	P value	FDR	Gene Module Activity
REACTOME_INFLAMMASOMES	0.000	0.001	-0.014
REACTOME_THE_NLRP3_INFLAMMASOME	0.000	0.001	0.015
REACTOME_PLATELET_SENSITIZATION_BY_LDL	0.000	0.001	0.013
Obermoser_M7.1_Inflammation	0.000	0.002	0.081
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.000	0.004	0.034
Obermoser_M4.6_Inflammation	0.001	0.008	0.084
Obermoser_M4.13_Inflammation	0.001	0.008	0.108
Obermoser_M4.2_Inflammation	0.001	0.008	0.096
REACTOME_ANTIGEN_ACTIVATES_B_CELL_RECEPTOR_LEADING_TO_GENERATION_OF_SECOND_MESSENGERS	0.001	0.008	0.011
Obermoser_M5.7_Inflammation	0.001	0.009	0.066
Obermoser_M3.2_Inflammation	0.004	0.022	0.110
REACTOME_FACTORS_INVOLVED_IN_MEGAKARYOCYTE_DEVELOPMENT_AND_PLATELET_PRODUCTION	0.006	0.030	0.061
REACTOME_SIGNALING_BY_THE_B_CELL_RECEPTOR_BCR	0.011	0.047	-0.002
Obermoser_M5.1_Inflammation	0.013	0.052	0.059
REACTOME_PLATELET_HOMEOSTASIS	0.023	0.078	0.065
Obermoser_M4.10_B cell	0.064	0.167	0.086
REACTOME_DOWNSTREAM_SIGNALING_EVENTS_OF_B_CELL_RECEPTOR_BCR	0.075	0.191	0.026
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	0.138	0.283	-0.003
REACTOME_ACTIVATION_OF_NF_KAPPAB_IN_B_CELLS	0.139	0.285	0.034
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2_	0.167	0.319	0.017
REACTOME_PLATELET_CALCIUM_HOMEOSTASIS	0.252	0.420	0.085
REACTOME_PLATELET_ADHESION_TO_EXPOSED_COLLAGEN	0.334	0.505	-0.011
REACTOME_PLATELET_AGGREGATION_PLUG_FORMATION	0.591	0.729	0.023
Obermoser_M1.1_Platelets	0.952	0.965	0.043