iScience, Volume *24*

Supplemental information

Endoplasmic reticulum stress response and bile acid signatures associate with multi-strain seroresponsiveness during elderly influenza vaccination

Christophe Carre, Glenn Wong, Vipin Narang, Crystal Tan, Joni Chong, Hui Xian Chin, Weili Xu, Yanxia Lu, Michelle Chua, Michael Poidinger, Paul Tambyah, Ma Nyunt, Tze Pin Ng, Daniel Larocque, Catherine Hessler, Nabil Bosco, Laurence Quemeneur, and Anis Larbi

Supplemental Information

Supplemental Figures

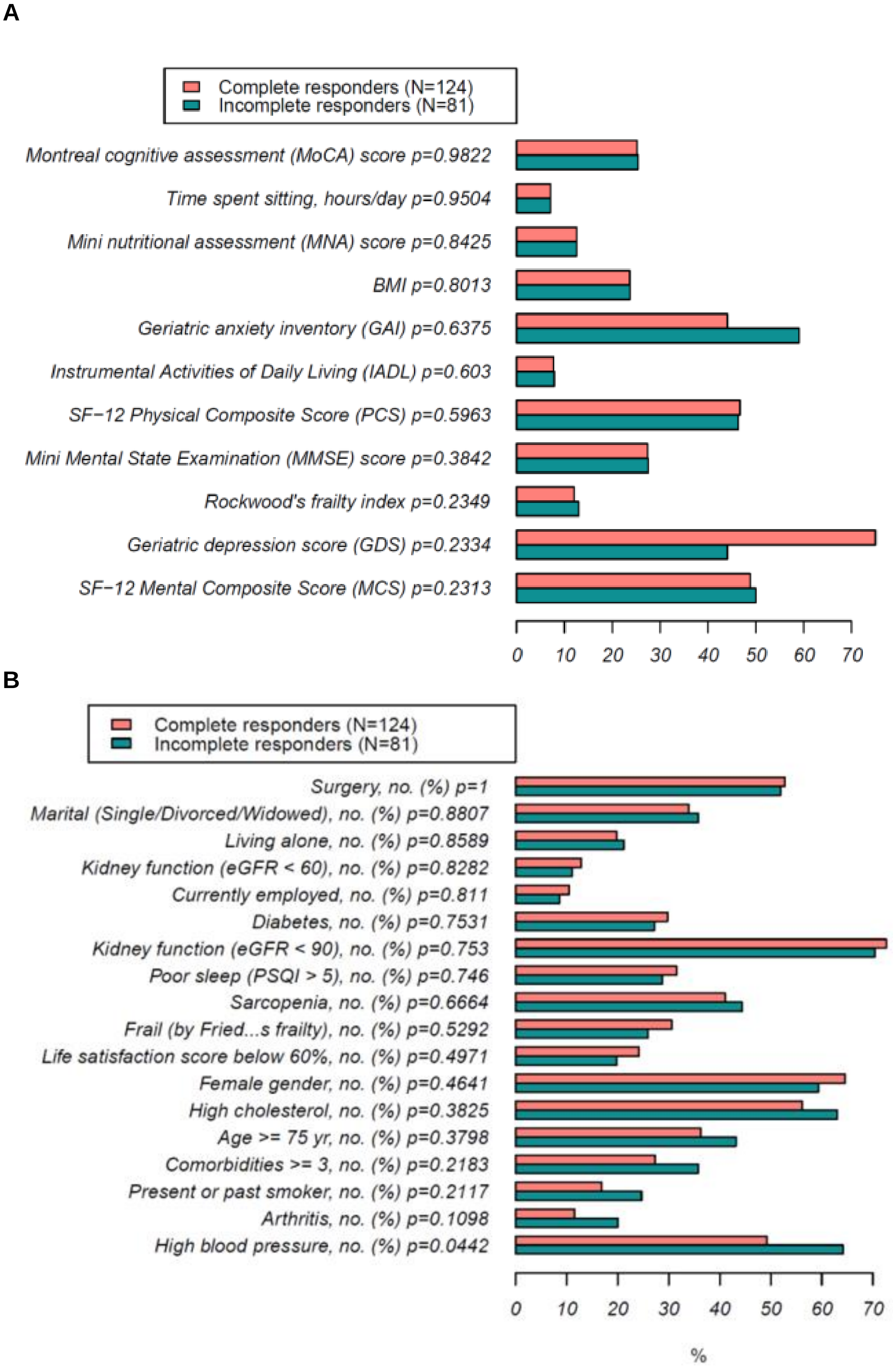

Figure S1. Baseline characteristics of CR and IR elderly subjects (n=205), Related to Figure 1. A. Continuous baseline characteristics and Wilcoxon test for CR vs IR (top) and **B.** Categorical baseline characteristics and Fisher test for CR vs IR (bottom).

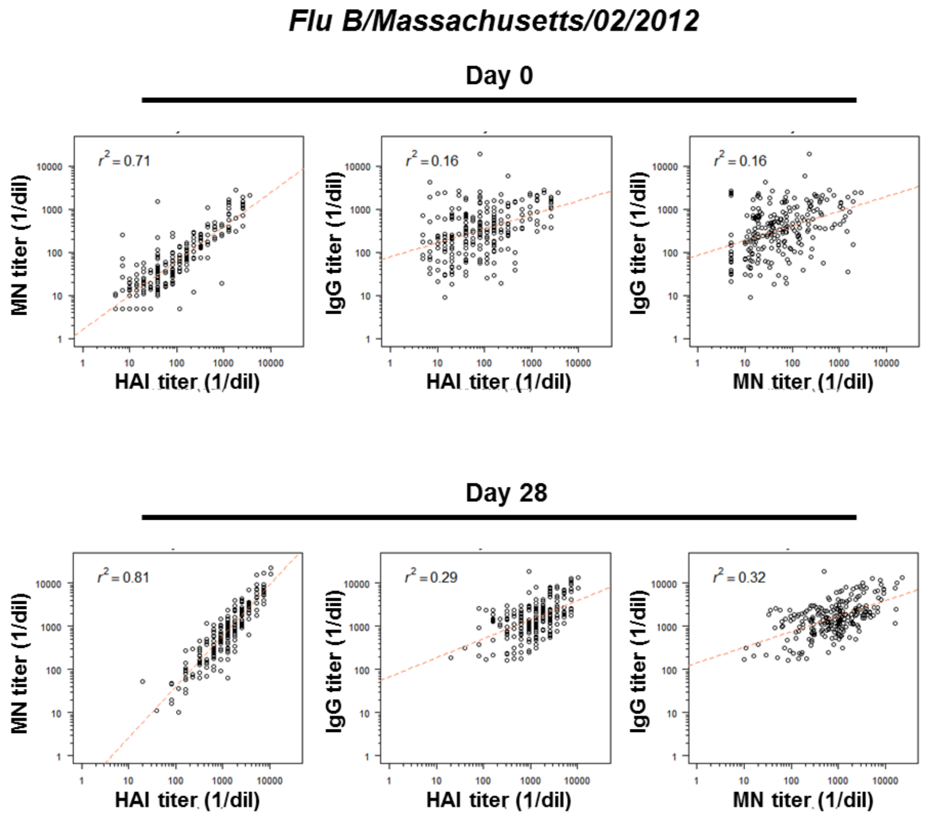

Figure S2. Correlations between HAI, MN and IgG titers at days 0 and 28 for Flu B/Massachusetts/02/2012 vaccine strain, Related to Figure 1. Correlations between MN and HAI titers, IgG and HAI titers; and IgG and MN titers (left to right) at day 0 and day 28 are shown for all three strains.

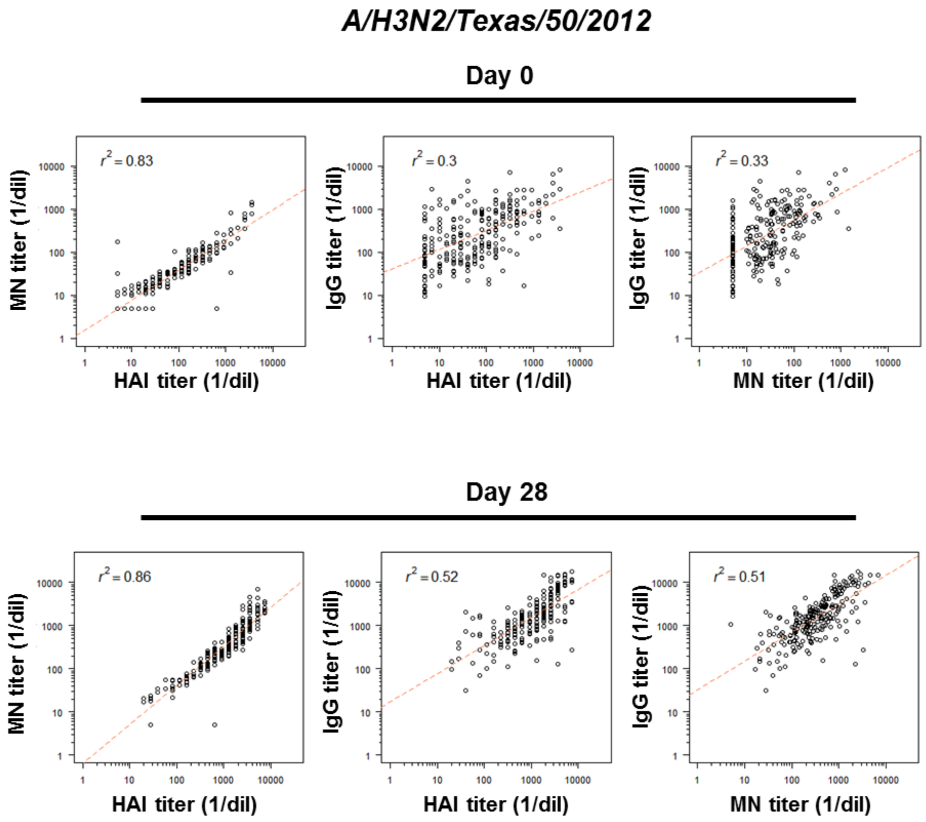

Figure S3. Correlations between HAI, MN and IgG titers at days 0 and 28 for A/H3N2/Texas/50/2012 vaccine strain, Related to Figure 1. Correlations between MN and HAI titers, IgG and HAI titers; and IgG and MN titers (left to right) at day 0 and day 28 are shown for all three strains.

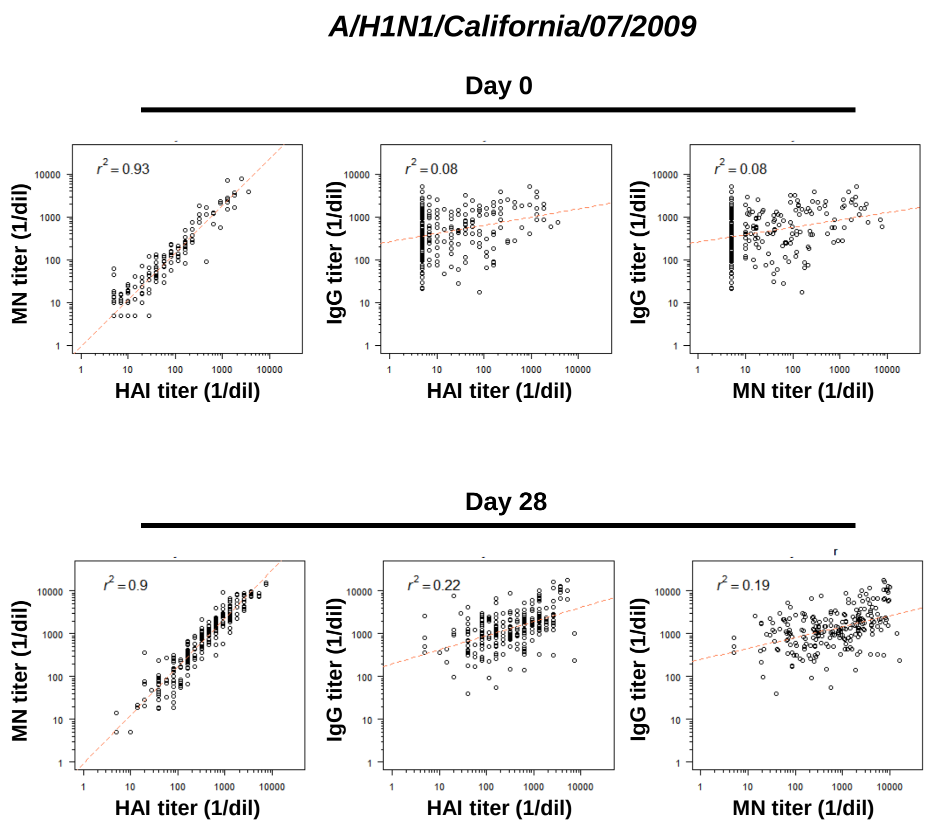

Figure S4. Correlations between HAI, MN and IgG titers at days 0 and 28 for A/H1N1/California/07/2009vaccine strain, Related to Figure 1. Correlations between MN and HAI titers, IgG and HAI titers; and IgG and MN titers (left to right) at day 0 and day 28 are shown for all three strains.

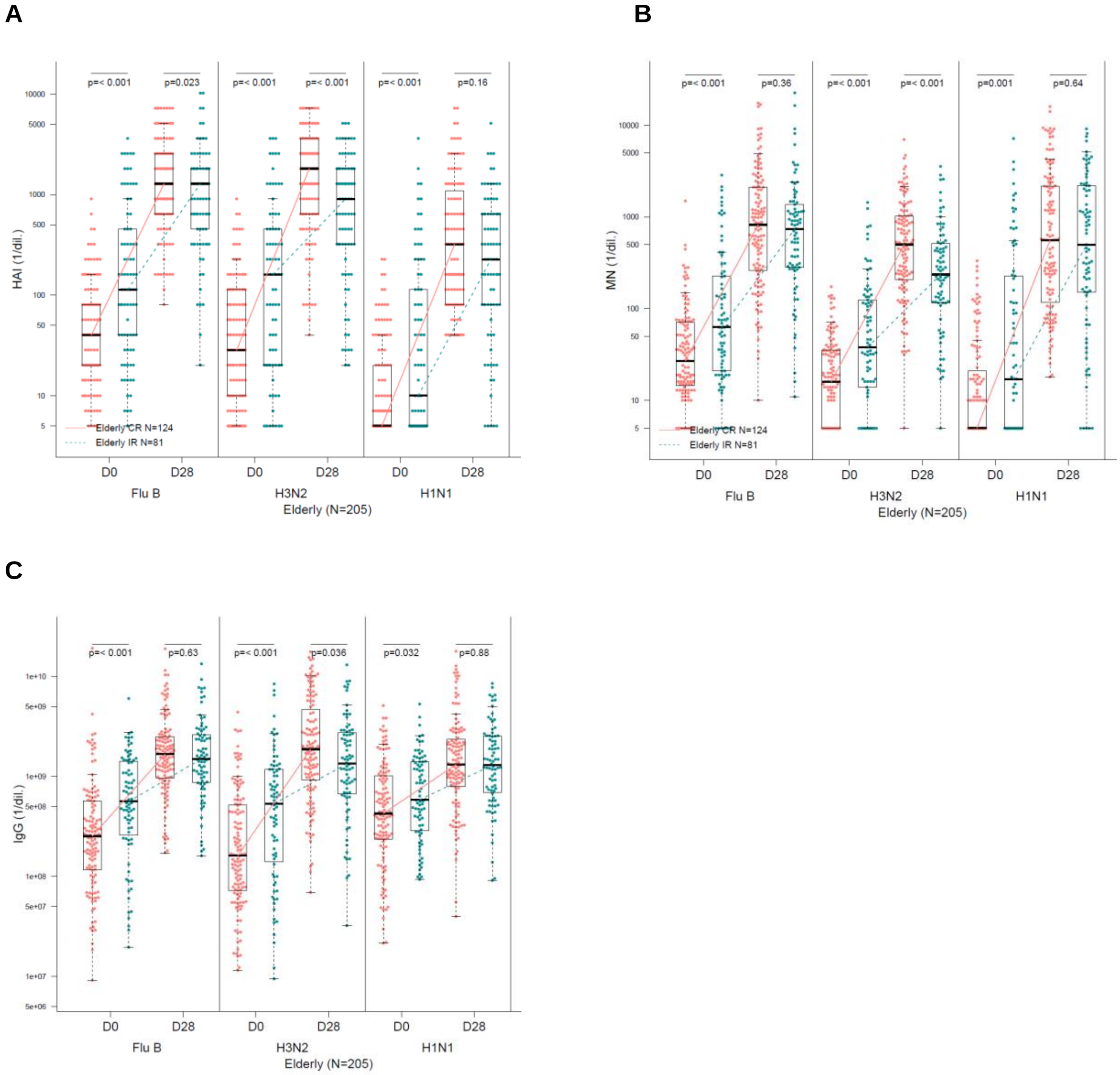

Figure S5. **Humoral response in CR and IR after vaccination in subjects aged >65 years (n=205), Related to Figure 1. A.** HAI titers for elderly CR (salmon) and IR (teal) for all vaccine strains (Wilcoxon test). **B.** MN titers for CR and IR across all vaccine strains. **C.** IgG titers for CR and IR across all vaccine strains.

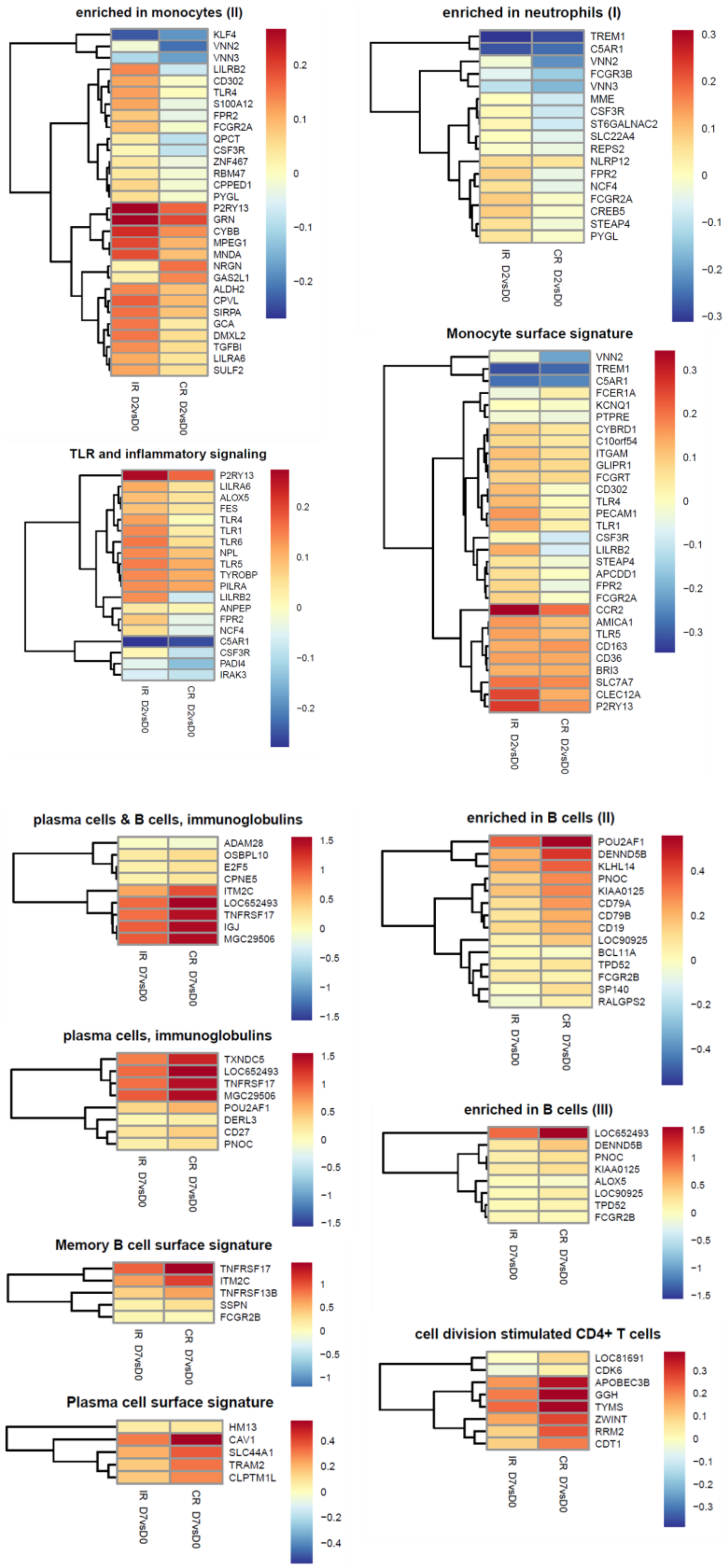

Figure S6. Blood transcriptional modules that were differentially regulated between CR vs IR elderly subjects (n=142), Related to Figure 2. Modulation of individual genes (log2 FC day 2 vs day 0, or day 7 vs day 0) that constitute blood transcriptional modules that significantly differed between CR and IR.

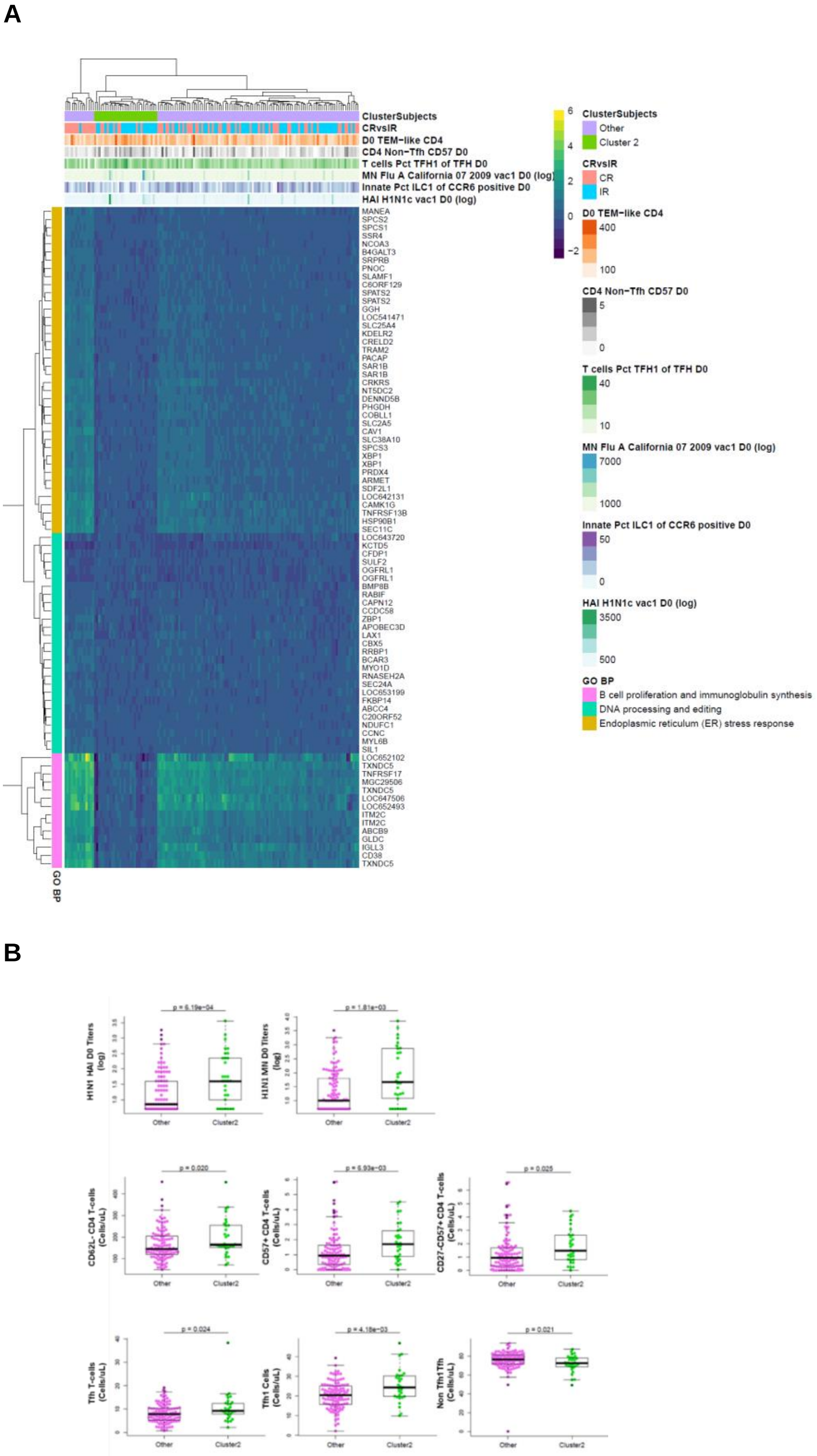

Figure S7. Transcriptomic gene signatures of CR vs IR, Related to Figure 2. A Heatmap and hierarchical clustering of 81 DEGs day 7 vs day 0 in CR vs. IR at FDR 5% (n=142, log2 FC) showing three main GO processes that are differentially regulated in the two groups. A group of subjects with high baseline immune levels is also shown (Cluster 2). **B.** Boxplots of the parameters where Cluster 2 and other subjects most significantly differ. Cluster 2 differed from other participants in terms of baseline H1N1 HAI and MN titers (top panel); percentages of differentiated CD62L-, CD57+ and CD27-CD57+ CD4 T-cells (middle panel) and Tfh1 cells (bottom).

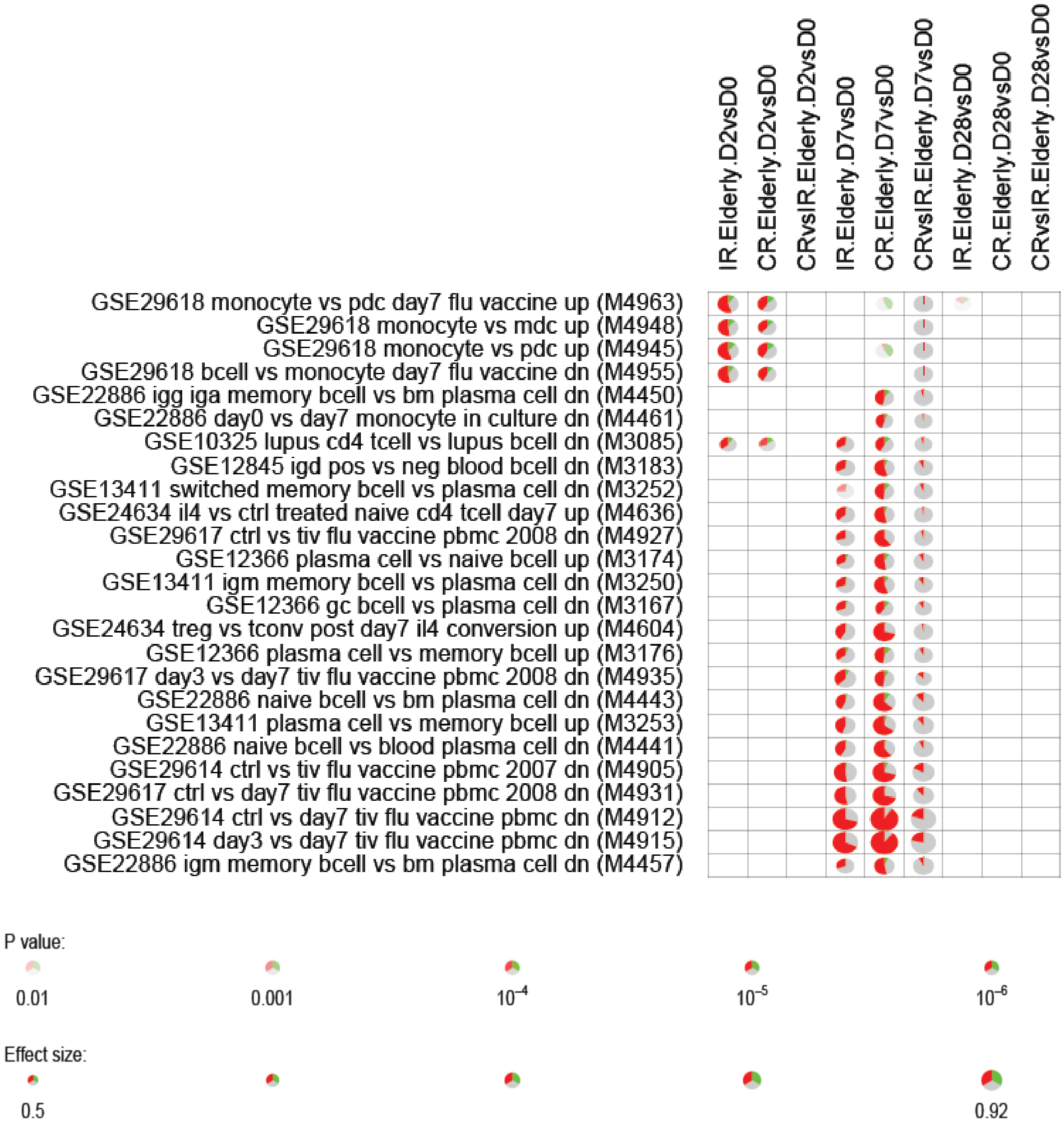

Figure S8. Gene datasets from the C7 Molecule Signature Database that overlap with CR vs IR gene signature (n=142), Related to Figure 2. The top 25 matches among 221 gene sets that significantly overlapped with CR vs IR DEGs across all time point comparisons are shown with their associated NCBI Gene Expression Omnibus ID numbers.

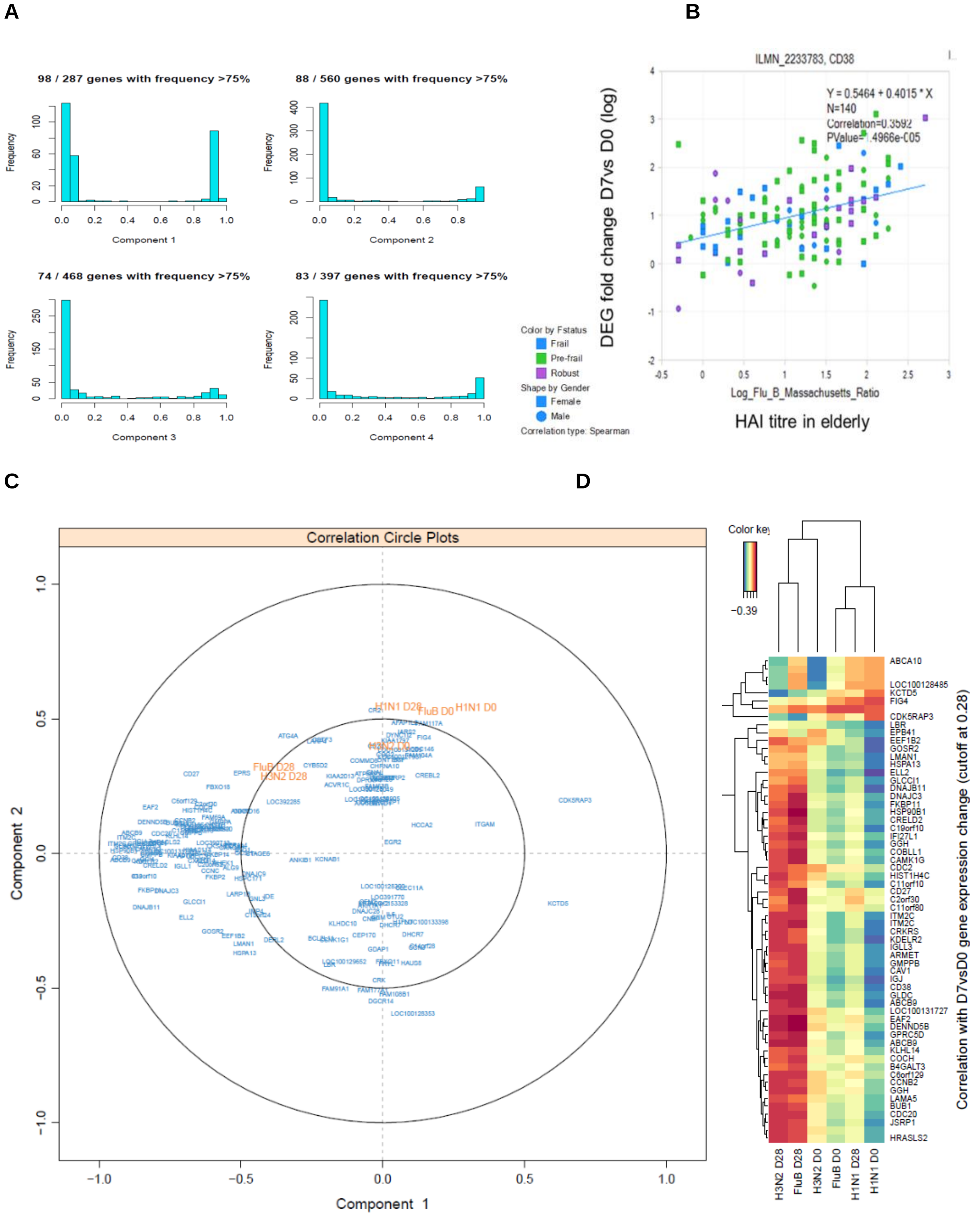

Figure S9. Threshold free validation of the transcriptional signature of the CR vs IR humoral response (n=142), Related to Figure 2. Sparse partial least square regression of day 0 and day 28 (log) titers (Y) against all three strains using day 7 vs day 0 (log2) gene expression (X). **A.** Selection of genes with frequency >75% using 10-fold cross (FC) validation. **B.** Regression between day 28/day 0 Flu B HAI titers and day 7/day 0 CD38 gene expression. **C.** Variable plot of the final model showing the implicated genes and the three strains HAI FC correlations. **D.** Heatmap of the top genes where HAI FC correlated with day 7 vs day 0 gene expression fold changes.

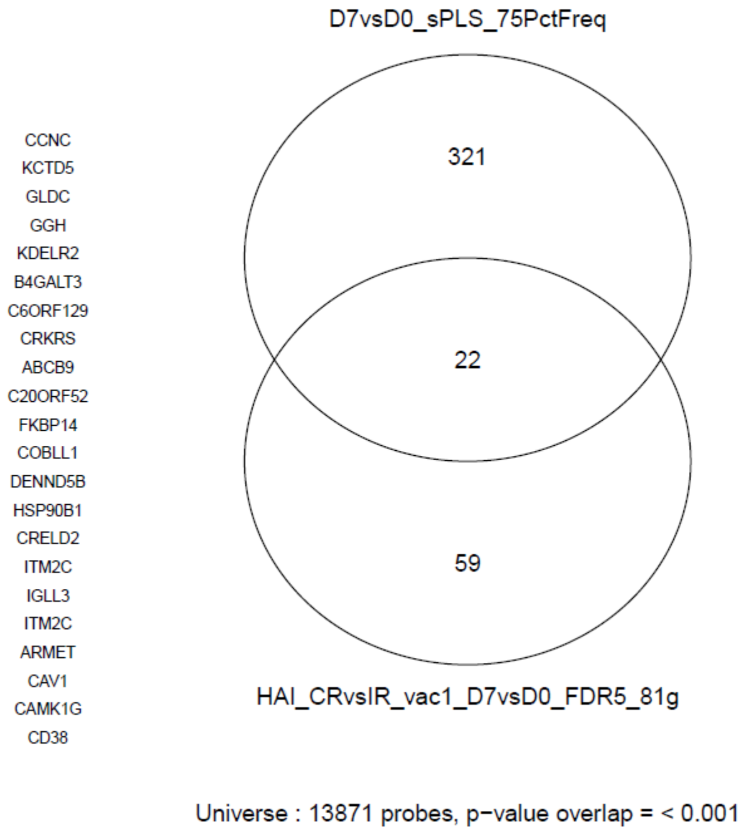

Figure S10. Hypergeometric test of overlap of the DEGs and sparse PLS day 7vsD0 gene probes for CR vs IR, Related to Figure 2.

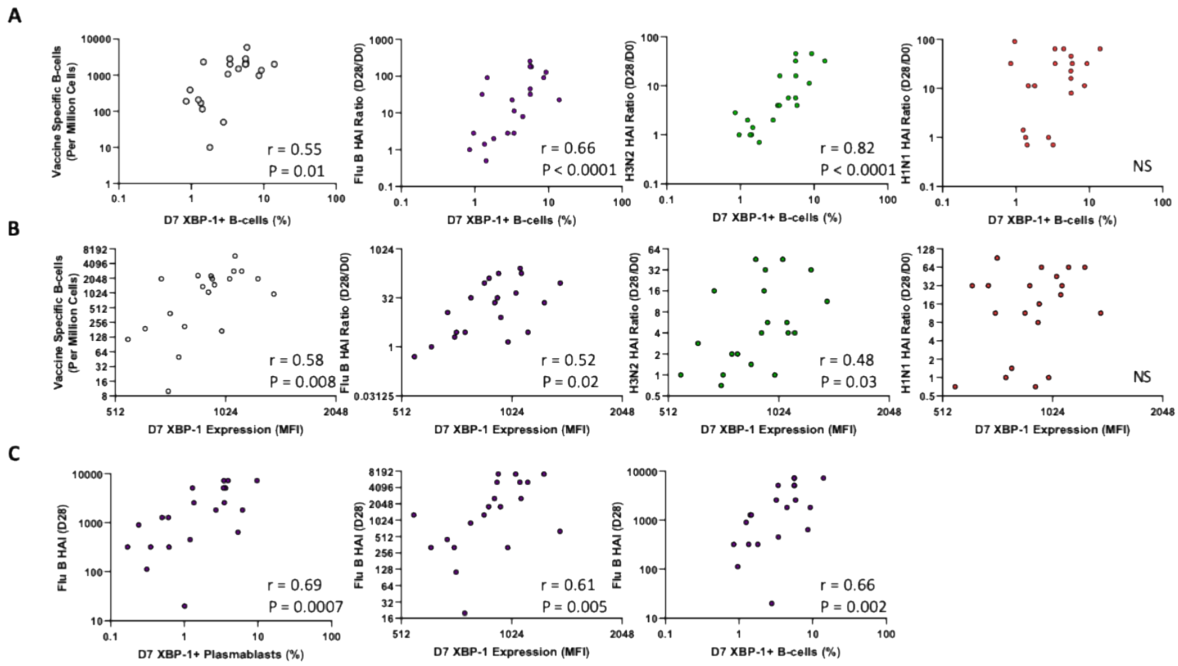

Figure S11. XBP-1 protein expression in B cells and plasmablasts, Related to Figure 4. A. Correlation between XBP-1 B-cell percentage and the vaccine response. **B.** Correlation between XBP-1 expression in plasmablasts (MFI) and the vaccine response. **C.** Correlation between XBP-1 expression in B-cells and plasmablasts with day 28 Flu B titers.

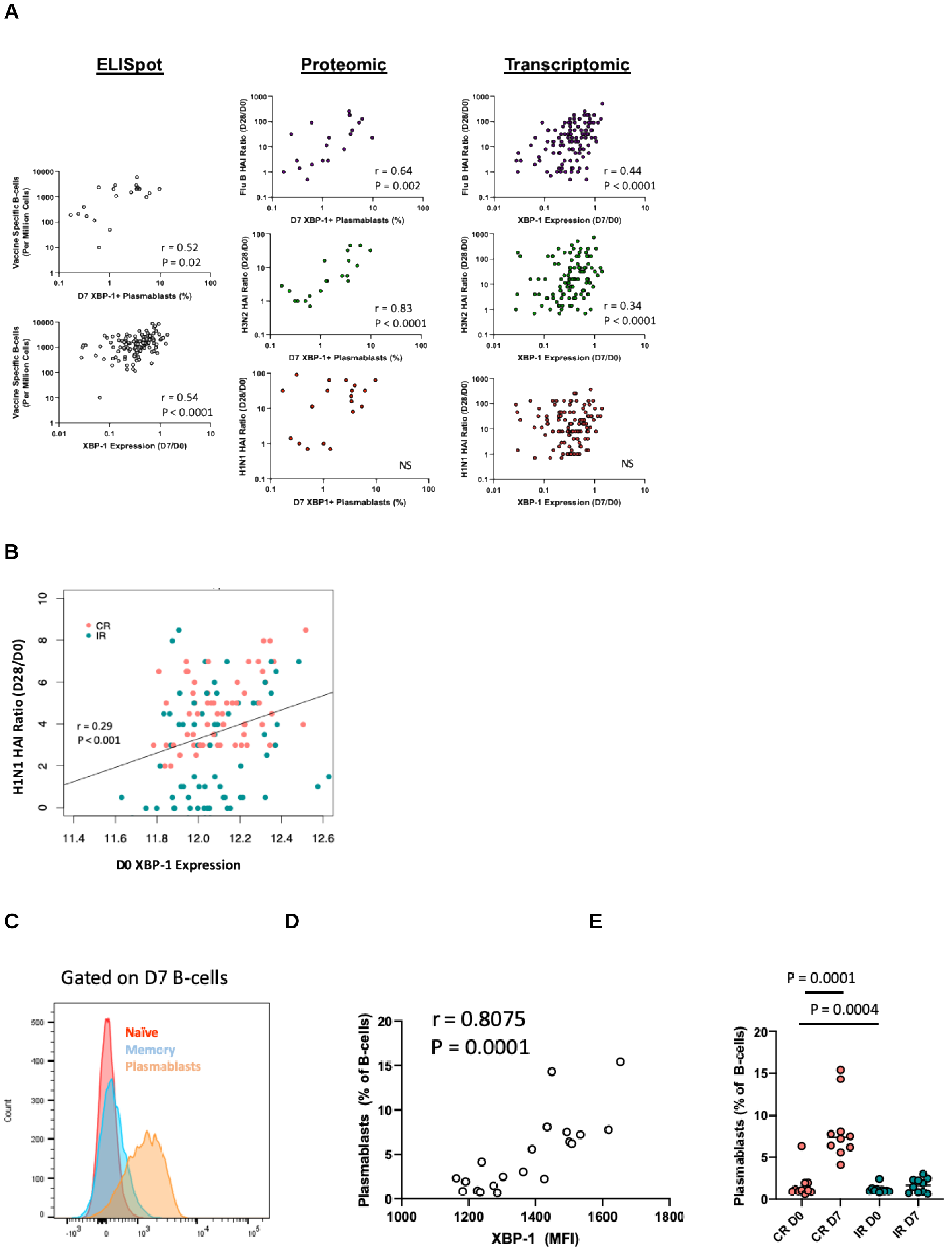

Figure S12. XBP-1 protein and gene expression in plasmablasts, Related to Figure 4. A. The association between XBP-1 proteomic and transcriptomic expression with the vaccine response. **B.** The correlation between day 0 XBP-1 gene expression in PBMCs and the H1N1 HAI day 28/day 0 ratio. **C.** Representative plot of XBP-1 expression in different B-cell subsets (gated on day 7 PBMCs). **D.** The correlation between XBP-1 expression and plasmablast percentages. **E.** The percentage of plasmablasts in CR on day 7. Pairwise comparisons were made by Mann-Whitney U test. Correlation tests were performed using the Spearman Rank Test, or the Pearson's test for part D only.

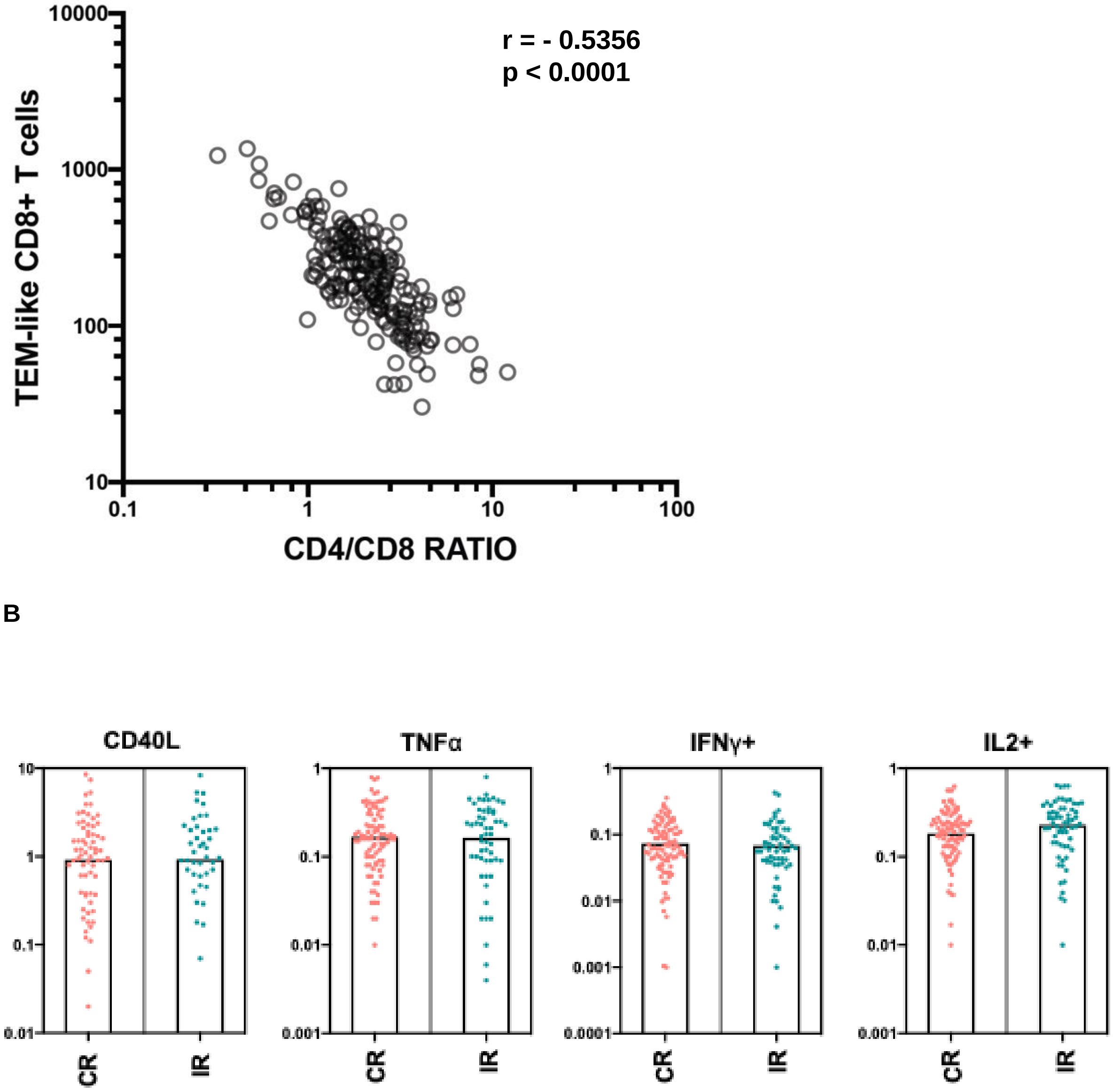

Figure S13. Decoupling of CD4:CD8 ratios with the day 7 B-cell response in IRs, Related to Figure 5. **A.** The correlation between the numbers of CD62L- TEM-like CD8 T cells and the CD4/CD8 ratio. **B.** The magnitudes of cytokine-specific CD4 T-cell responses in CR vs IR after *in vitro* vaccine stimulation.

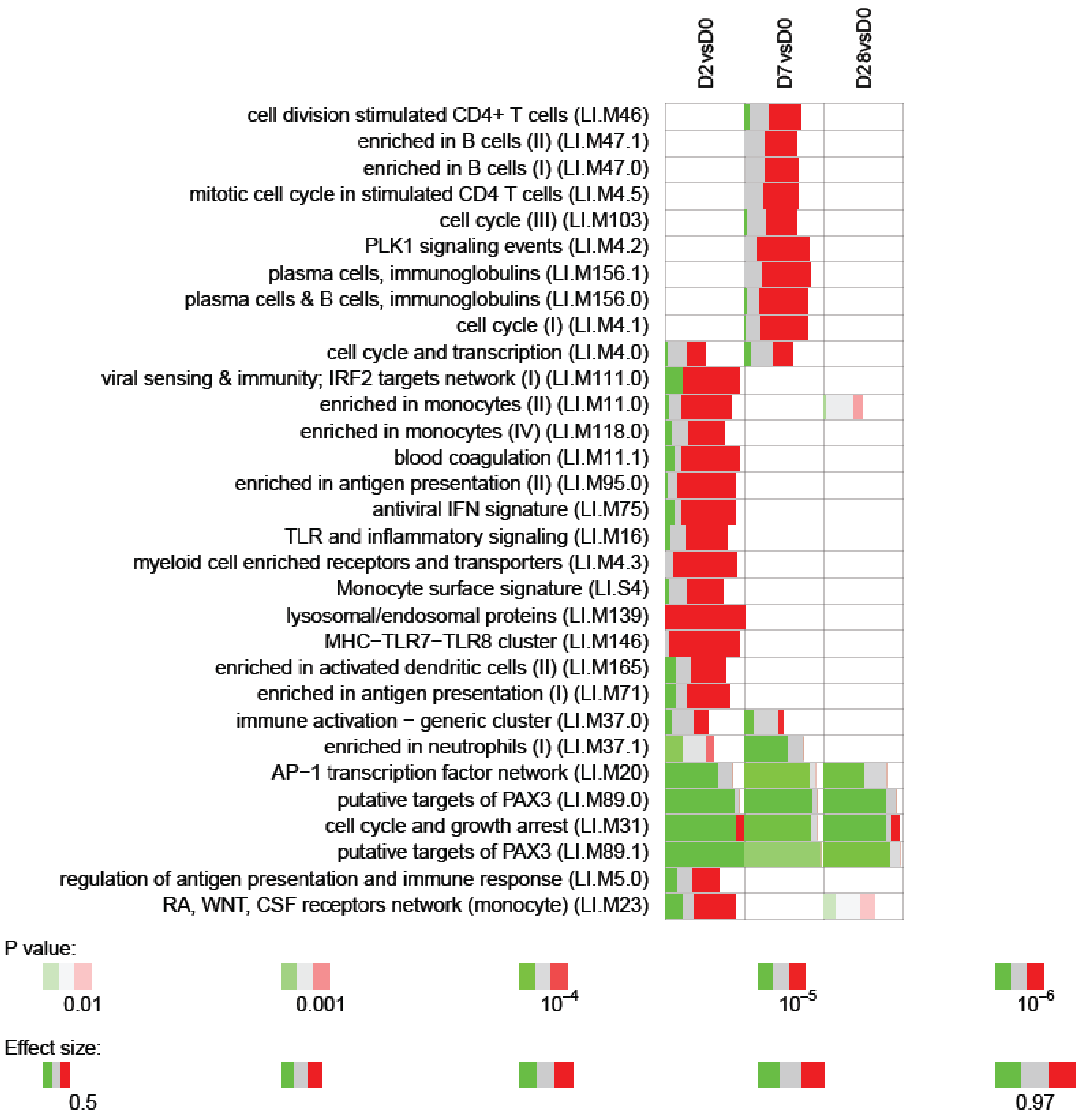

Figure S14. Top blood transcriptional modules impacted by vaccination, Related to Figure 2. The top 30 significant BTMs (among 149) are shown and annotated to show p-values and the proportion of genes that were modulated from baseline at day 2, day 7 or day 28.

Supplemental Tables

Table S1. Summary of HAI results in elderly subjects (n=205), Related to Figure 1

Vaccination 1	Elderly CR (N=124)	Elderly IR (N=81)	Elderly (N=205)	P value	test
Flu B Massachusetts					
GMT day 0 (1/dil.) [95% CI]	42.8 [35, 52.3]	134 [91.4,	67.1 [54.5,	<0.0001	(d)
Seroprotection day 0	61.29%	75.31%	66.83%	0.0483	(a)
GMT day 28 (1/dil.)	1320 [1100,	894 [687,	1130 [969,	0.0226	(d)
Seroprotection day 28	100%	98.77%	99.51%	0.3951	(a)
GMTR day 28/day 0	30.8 [24.8,	6.68 [4.71,	16.8 [13.6,	<0.0001	(d)
Seroconversion or	100%	53.09%	81.46%	<0.0001	(a)
Flu A H3N2 Texas					
GMT day 0 (1/dil.)	32.7 [25.6,	110 [71.2,	52.8 [41.5,	<0.0001	(d)
Seroprotection day 0	46.77%	65.43%	54.15%	0.01	(a)
GMT day 28 (1/dil.)	1380 [1120,	621 [450,	1010 [834,	<0.0001	(d)
Seroprotection day 28	100%	92.59%	97.07%	0.0034	(a)
GMTR day 28/day 0	42.1 [33.6,	5.66 [3.97,	19 [15, 24.1]	<0.0001	(d)
Seroconversion or	100%	48.15%	79.51%	<0.0001	(a)
Flu A H1N1 California					
GMT day 0 (1/dil.)	10.3 [8.55,	27.8 [18.2,	15.3 [12.3,	0.0009	(d)
Seroprotection day 0	19.35%	40.74%	27.80%	0.0013	(a)
GMT day 28 (1/dil.)	313 [244, 402]	194 [134,	259 [209, 320]	0.1585	(d)
Seroprotection day 28	100%	82.72%	93.17%	<0.0001	(a)
GMTR day 28/day 0	30.3 [24.4,	6.98 [4.83,	17 [13.6, 21.1]	<0.0001	(d)
Seroconversion or	100%	50.62%	80.49%	<0.0001	(a)
Three strains					
GM day 0 (1/dil.)	24.3 [21.3,	74.2 [56,	37.8 [32.3,	<0.0001	(d)
N strains with				0.0009	(a)
0	20.97%	7.41%	15.61%		
1	40.32%	29.63%	36.10%		
2	29.03%	37.04%	32.20%		
3	9.68%	25.93%	16.10%		
GM day 28 (1/dil.)	828 [718, 954]	476 [383,	665 [586, 755]	<0.0001	(d)
N strains with				<0.0001	(a)
1	0%	2.47%	0.98%		
2	0%	20.99%	8.29%		
3	100%	76.54%	90.73%		
GMTR day 28/day 0	34 [29.7, 38.9]	6.41 [5.21,	17.6 [15, 20.7]	<0.0001	(d)
N strains with seroconversion or significant				<0.0001	(a)
0	0%	12.35%	4.88%		
1	0%	23.46%	9.27%		
2	0%	64.20%	25.37%		
3	100%	0%	60.49%		

(d) Wilcoxon
(a) Fisher
CR: complete (three strains) HAI responders, IR: incomplete HAI responders
GMT: geometric mean of titers, GMTR: geometric mean of titer individual ratios.
Seroconversion: pre-vaccination HAI titer <1:10 and a postvaccination HAI titer >1:40
Significant increase: pre-vaccination HAI titer >1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer.
Seroprotection HAI antibody titer ≥1:40.

Table S2. Immunogenicity GMT summary in elderly subjects (n=205), Related to Figure 1

Assay	Strain	Timepoint	Elderly CR (N=124)	Elderly IR (N=81)	Elderly (N=205)	p-value	test
HAI	Bm	day 0	42.8 [35, 52.3]	134 [91.4, 196]	67.1 [54.5, 82.6]	<0.0001	(d)
HAI	Bm	day 28	1320 [1100, 1580]	894 [687, 1160]	1130 [969, 1320]	0.0226	(d)
HAI	Bm	day	30.8 [24.8, 38.2]	6.68 [4.71, 9.49]	16.8 [13.6, 20.9]	<0.0001	(d)
HAI	H3N2t	day 0	32.7 [25.6, 41.8]	110 [71.2, 169]	52.8 [41.5, 67.1]	<0.0001	(d)
HAI	H3N2t	day 28	1380 [1120, 1700]	621 [450, 858]	1010 [834, 1210]	<0.0001	(d)
HAI	H3N2t	day	42.1 [33.6, 52.7]	5.66 [3.97, 8.06]	19 [15, 24.1]	<0.0001	(d)
HAI	H1N1c	day 0	10.3 [8.55, 12.4]	27.8 [18.2, 42.4]	15.3 [12.3, 18.9]	0.0009	(d)
HAI	H1N1c	day 28	313 [244, 402]	194 [134, 281]	259 [209, 320]	0.1585	(d)
HAI	H1N1c	day	30.3 [24.4, 37.8]	6.98 [4.83, 10.1]	17 [13.6, 21.1]	<0.0001	(d)
MN	Bm	day 0	31.3 [25.5, 38.4]	77 [53.7, 110]	44.6 [36.6, 54.4]	<0.0001	(d)
MN	Bm	day 28	738 [569, 957]	601 [436, 828]	680 [556, 832]	0.362	(d)
MN	Bm	day	23.6 [18.1, 30.7]	7.81 [5.43, 11.2]	15.2 [12.2, 19.1]	<0.0001	(d)
MN	H3N2t	day 0	16.7 [13.9, 20]	42.2 [30.1, 59.1]	24.1 [20.1, 28.9]	<0.0001	(d)
MN	H3N2t	day 28	440 [353, 549]	220 [164, 294]	334 [279, 401]	0.0002	(d)
MN	H3N2t	day	26.4 [21, 33.1]	5.21 [3.82, 7.09]	13.9 [11.2, 17.2]	<0.0001	(d)
MN	H1N1c	day 0	12.2 [9.81, 15.1]	37.5 [23, 61.2]	19 [14.9, 24.2]	0.0015	(d)
MN	H1N1c	day 28	529 [391, 715]	409 [264, 634]	478 [371, 614]	0.6403	(d)
MN	H1N1c	day	43.5 [33.4, 56.7]	10.9 [7.19, 16.5]	25.2 [19.7, 32.2]	<0.0001	(d)
IgG	Bm	day 0	2.5e+08 [2e+08,	5.01e+08 [3.81e+08,	3.28e+08	<0.0001	(d)
IgG	Bm	day 28	1.6e+09	1.49e+09 [1.22e+09,	1.56e+09	0.6279	(d)
IgG	Bm	day	6.4 [5.23, 7.83]	2.98 [2.32, 3.84]	4.74 [4.02, 5.6]	<0.0001	(d)
IgG	H3N2t	day 0	1.85e+08	3.99e+08 [2.87e+08,	2.5e+08	0.0002	(d)
IgG	H3N2t	day 28	1.84e+09	1.24e+09 [9.56e+08,	1.58e+09	0.0357	(d)
IgG	H3N2t	day	9.97 [7.65, 13]	3.1 [2.31, 4.16]	6.31 [5.1, 7.8]	<0.0001	(d)
IgG	H1N1c	day 0	4.23e+08	6.17e+08 [4.96e+08,	4.9e+08	0.0321	(d)
IgG	H1N1c	day 28	1.33e+09	1.28e+09 [1.03e+09,	1.31e+09	0.8774	(d)
IgG	H1N1c	day	3.15 [2.56, 3.88]	2.08 [1.68, 2.57]	2.68 [2.3, 3.12]	0.0108	(d)

(d) Wilcoxon Test

CR: complete (three strains) HAI responders, IR: incomplete HAI responders
GMT: geometric mean of titers and 95% confidence interval.
Bm : Flu B Massachusetts 02 2012
H3N2t : Flu A (H3N2) Texas 50 2012
H1N1c : Flu A (H1N1) California 07 2009

Table S3. Blood transcriptional modules that were differentially modulated between CR vs IR for at least one time point (n = 142, FDR BH 5%), Related to Figure 2

Day 2 vs Day 0

BTM	Title	AUC IR Elderly	q IR Elderly	AUC CR Elderly	q CR Elderly	AUC CRvsIR Elderly	q CRvsIR Elderly
LI.M11.0	enriched in monocytes (II)	0.8013	0.0000	0.7021	0.0000		
LI.M16	TLR and inflammatory signaling	0.7541	0.0000	0.6421	0.0009		
LI.M37.1	enriched in neutrophils (I)						
LI.S4	Monocyte surface signature	0.7198	0.0000	0.6126	0.0001		
LI.S9	Memory B cell surface signature						
LI.M156.0	plasma cells & B cells, immunoglobulins						
LI.M47.1	enriched in B cells (II)						
LI.M47.2	enriched in B cells (III)						
LI.M4.1	cell cycle (I)						
LI.S3	Plasma cell surface signature						
LI.M4.0	cell cycle and transcription	0.5782	0.0000	0.5391	0.0000		
LI.M46	cell division stimulated CD4+ T cells						
LI.M156.1	plasma cells, immunoglobulins						

Day 7 vs Day 0

BTM	Title	AUC IR Elderly	q IR Elderly	AUC CR Elderly	q CR Elderly	AUC CRvsIR Elderly	q CRvsIR Elderly
LI.M11.0	enriched in monocytes (II)					0.6438	0.0000
LI.M16	TLR and inflammatory signaling						
LI.M37.1	enriched in neutrophils (I)	0.6593	0.0294	0.7565	0.0001		
LI.S4	Monocyte surface signature						
LI.S9	Memory B cell surface signature	0.7470	0.0001	0.6242	0.0001	0.5628	0.0016
LI.M156.0	plasma cells & B cells, immunoglobulins	0.8241	0.0000	0.8000	0.0000	0.6015	0.0001
LI.M47.1	enriched in B cells (II)	0.6065	0.0014	0.6657	0.0000	0.6329	0.0018
LI.M47.2	enriched in B cells (III)	0.6449	0.0099	0.6836	0.0004	0.6440	0.0301
LI.M4.1	cell cycle (I)	0.8043	0.0000	0.8308	0.0000	0.6524	0.0002
LI.S3	Plasma cell surface signature	0.7417	0.0013	0.7274	0.0001	0.6546	0.0063
LI.M4.0	cell cycle and transcription	0.6092	0.0000	0.6720	0.0000	0.6635	0.0000
LI.M46	cell division stimulated CD4+ T cells	0.7574	0.0001	0.8147	0.0000	0.7286	0.0049
LI.M156.1	plasma cells, immunoglobulins	0.9279	0.0000	0.8878	0.0000	0.9240	0.0000

Day 28 vs Day 0

BTM	Title	AUC IR Elderly	q IR Elderly	AUC CR Elderly	q CR Elderly	AUC CRvsIR Elderly	q CRvsIR Elderly
LI.M11.0	enriched in monocytes (II)					0.6173	0.0001
LI.M16	TLR and inflammatory signaling					0.6717	0.0020
LI.M37.1	enriched in neutrophils (I)			0.6961	0.0006	0.6506	0.0238
LI.S4	Monocyte surface signature					0.6048	0.0473
LI.S9	Memory B cell surface signature						
LI.M156.0	plasma cells & B cells, immunoglobulins						
LI.M47.1	enriched in B cells (II)						
LI.M47.2	enriched in B cells (III)						
LI.M4.1	cell cycle (I)						
LI.S3	Plasma cell surface signature						
LI.M4.0	cell cycle and transcription						
LI.M46	cell division stimulated CD4+ T cells						
LI.M156.1	plasma cells, immunoglobulins						

Li et al. BTM with at least one significant adjusted p-value (Benjamini & Hochberg) for day 2 vs day 0, day 7 vs day 0 or day 28 vs day 0, between CR and IR.

Table S4. Upstream regulator Ingenuity Pathway Analysis of differentially expressed genes for day 7 vs day 0 in CR vs IR (n = 142, FDR BH 15%), Related to Figure 2

Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activation State	Activation z-score	P-value of overlap
cyclosporin A		biologic drug		-1.229	1.01E-02
XBP1	1.151	transcription regulator	Activated	4.585	1.12E-16
TCR		complex		0.943	1.21E-03
UCP1		transporter	Activated	2.213	1.31E-02
CD40LG		cytokine		1.741	1.51E-02
1.2-dithiol-3-thione		chemical reagent	Activated	2.423	1.63E-02
IL21		cytokine		1.410	1.75E-02
FBXO32		enzyme		1.633	1.81E-04
estrogen		chemical drug		1.593	1.93E-02
mir-8		microRNA		-1.154	2.11E-02
IL5		cytokine	Activated	2.864	2.34E-05
thapsigargin		chemical toxicant	Activated	2.156	3.01E-02
D-glucose		chemical - endogenous mammalian		-1.904	3.01E-02
CpG oligonucleotide		chemical drug		1.176	3.12E-02
CD38	1.341	enzyme	Activated	2.934	3.12E-05
BRD4		kinase	Activated	2.236	3.52E-04
mibolerone		chemical drug	Activated	2.646	3.87E-04
IL2		cytokine		1.535	4.02E-02
NFE2L2		transcription regulator	Activated	2.621	4.28E-02
ERN1		kinase	Activated	3.079	4.31E-07
KLF3		transcription regulator	Activated	2.000	4.35E-01
CD3		complex		1.258	4.45E-03
ATF6		transcription regulator		1.941	4.45E-04
tunicamycin		chemical - endogenous non-mammalian	Activated	3.193	4.64E-06
TLR9		transmembrane receptor		1.400	4.82E-02
lipopolysaccharide		chemical drug	Activated	3.228	4.93E-02

Table S5. Correlation between bile acid levels and HAI response, Related to Figure 6

Analyte	**r.FluB**	**FluB.p**	**r. H1N1**	**H1N1.p**	**r. H3N2**	**H3N2.p**
alloLCA (ng/mL)	0.151	**0.031**	0.083	0.238	-0.015	0.833
TUDCA (ng/mL)	-0.131	0.063	-0.120	0.088	-0.094	0.180
dehydroLCA (ng/mL)	0.130	0.064	-0.011	0.879	0.059	0.401
CDCA-3Gln (ng/mL)	-0.089	0.204	-0.035	0.619	-0.121	0.086
GCDCA (ng/mL)	-0.087	0.218	-0.067	0.344	-0.092	0.193
apoCA (ng/mL)	-0.085	0.225	0.036	0.607	0.025	0.719
7-DHCA (ng/mL)	-0.066	0.347	-0.138	**0.049**	-0.141	**0.045**
TCA (ng/mL)	-0.002	0.979	-0.021	0.767	-0.064	0.363