SUPPLEMENTARY MATERIALS AND METHODS

Memory B cell assay. An alternative mitogen cocktail was used essentially as previously described (1). Briefly, PBMCs were cultured at $1X10^6$ cells per mL of R10 supplemented with 50 μM of beta-mercaptomethanol (Sigma) containing R848 (1 μg/mL, Invivogen) and human-IL2 (10 μg/mL, Biolegend) for 3 days. Total and influenza vaccine-specific IgG secreting cells were quantified by ELISPOT assay.

Desmoglein-specific ELISA. Anti-Dsg1 and anti-Dsg3 serum autoantibody titers were determined with an ELISA test (MESACUP DSG1 & DSG3 ELISA test system, MBL International Corporation) using patient sera sampled during routine clinical visits. 1:100 diluted sera samples were tested according to manufacturer's instructions. Results are reported as Units/mL of sera, as determined by negative and positive calibrators supplied by the manufacturer.

Statistical analysis. Seroprotection was analyzed using a Fisher exact test. *P* values less than 0.05 were considered statistically significant.

REFERENCES

1. Pinna D, et al. Clonal dissection of the human memory B-cell repertoire following infection and vaccination. *Eur J Immunol.* 2009;39(5):1260-70.

Supplementary Figure 1

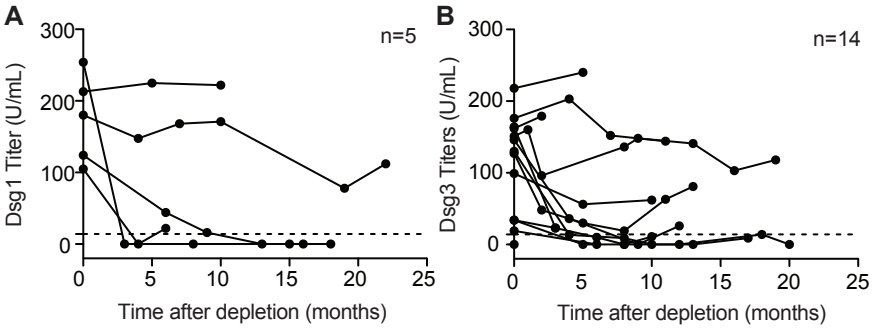

FIGURE S1. Anti-desmoglein autoantibody titers decrease after Rituximab treatment. (A) Anti-desmoglein-1 antibody titers (major target in PF) in 5 PF patients and (B) anti-desmoglein-3 antibody titers (major target in PV) in 14 PV patients were determined by ELISA. Dotted lines represent the value at which titers were considered to be positive, as recommended by the manufacturer (Dsg1 = 18 U/mL, Dsg3 = 19 U/mL). Samples were tested during routine clinical visits. Generally, serum titers decreased after Rituximab treatment, although several patients sustained high levels of serum autoantibody titers. However, all patients showed improved clinical symptoms in response to Rituximab treatment.

Supplementary Figure 2

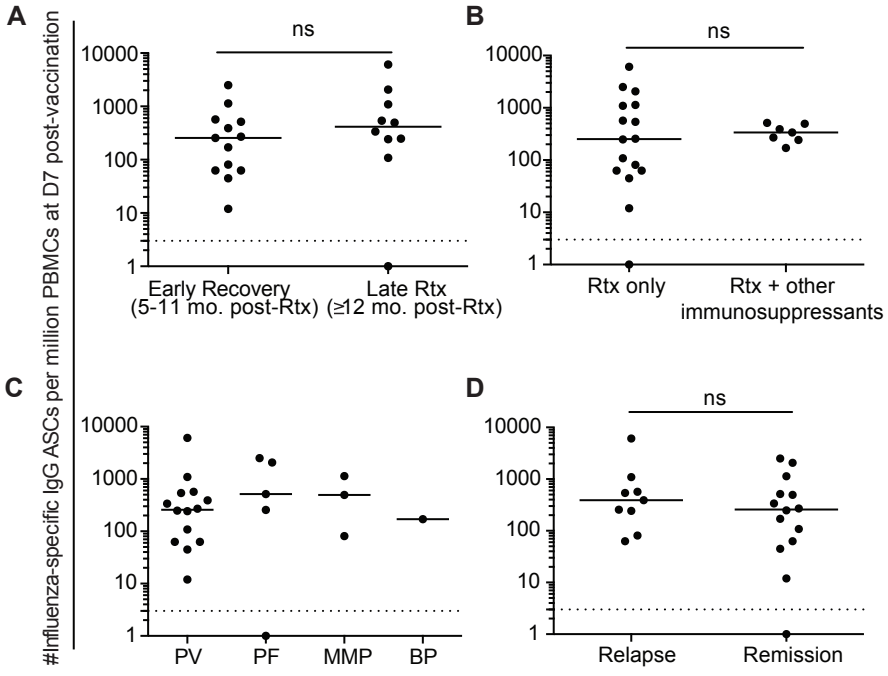

Figure S2. Impact of AIBD disease and treatment on plasmablast responses to influenza vaccine. (A) There was no difference in magnitudes of plasmablast responses to influenza comparing patients who were early in recovery from Rituximab treatment (5-11 month post-Rtx) to patients late in recovery (≥12 months post-Rtx (B) No significant difference was observed when comparing patients who received the vaccine while treated with Rituximab as a stand-alone therapy compared to patients who were prescribed additional immunosuppressants. (C) There appears to be no major differences in plasmablast responses based on various types of AIBD disease. (D) Responses were also compared between patients who experienced disease and patients who stayed in remission (follow-up time: 1-2 years). No significant different was observed. Mann-Whitney U test was used to compare patients where appropriate.

Supplementary Figure 3

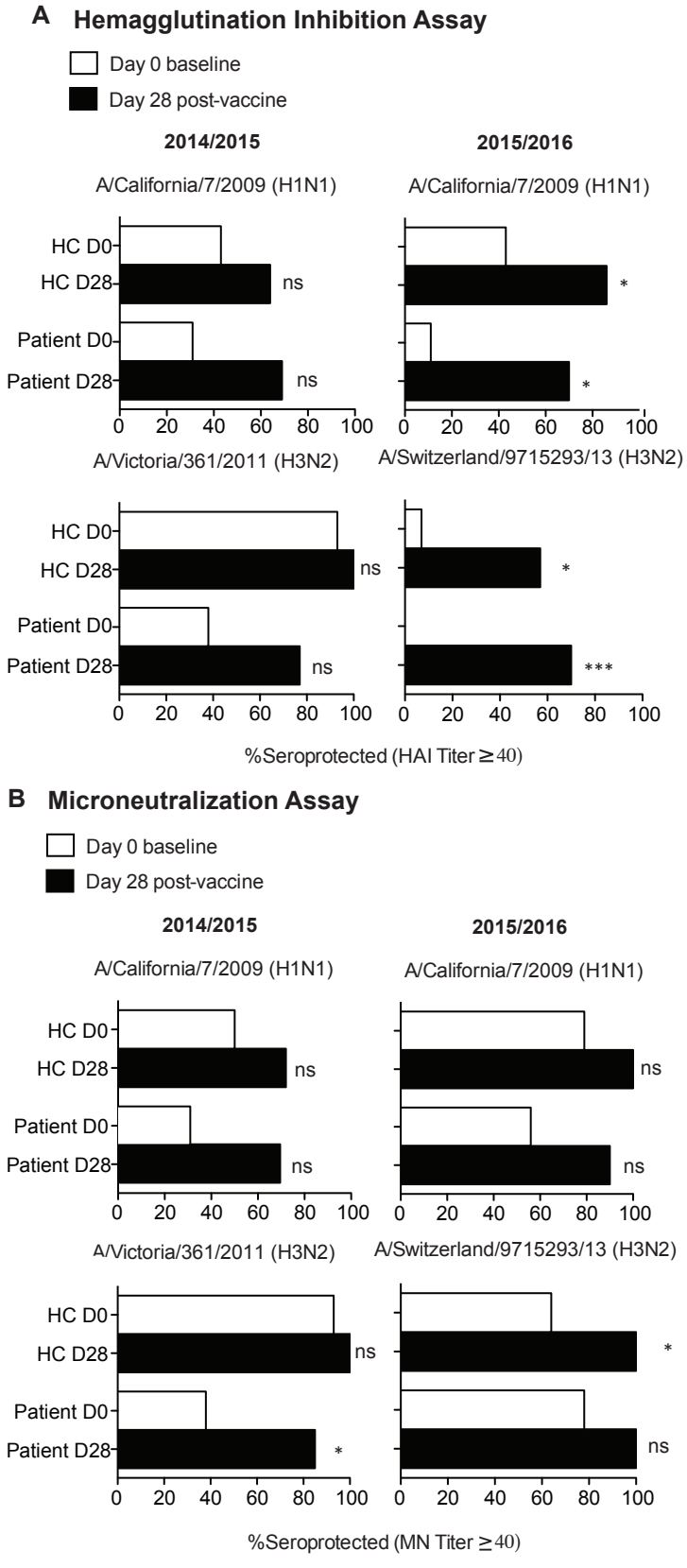

Figure S3. Comparable frequencies of seroprotection in patients and healthy controls. (A) Percentage of seroprotected individuals, as determined by HAI titers ≥40, after one dose of vaccination. (B) Percentage of seroprotected individuals, as determined by MN titers ≥40. A Fisher exact test was used to compare seroprotection at Day 0 to Day 28 post-vaccination. *** = P<0.005; * = P≤0.05; ns = not significant.

Supplementary Figure 4

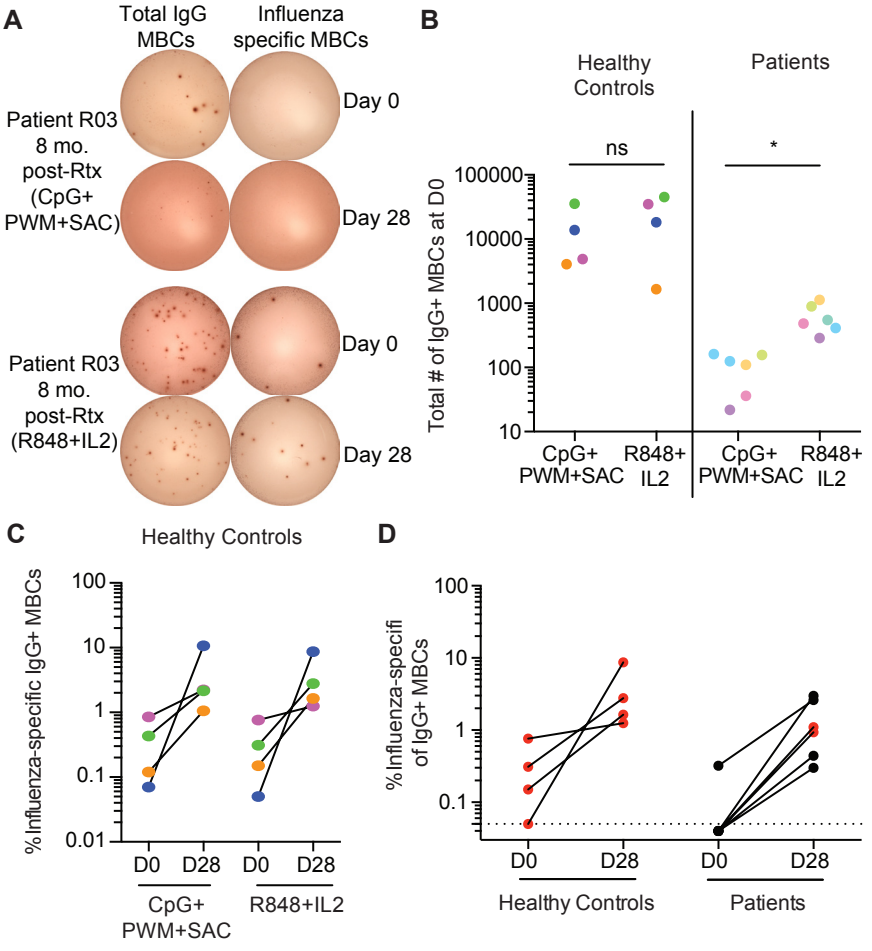

Figure S4. R848+IL2 mitogen cocktail is an effective alternative to assess antigen-specific memory B cells. (A) Representative ELISPOT data of a patient who failed stimulation using a mitogen cocktail composed of CpG, PWM, and SAC, but responded sufficiently to R848 with IL2. (B) Total numbers of IgG+ memory B cells detected at Day 0 using the different mitogen cocktails. Healthy controls, with high numbers of memory B cells, showed comparable levels of stimulation regardless of mitogens used. However, patients with low number of memory B cells who failed stimulation with CpG/PWM/SAC had significantly increased stimulation with R848/IL2 mitogens. Each color circle represents one individual vaccinee. Wilcoxon-paired T-test was used to compare groups. * = P≤ 0.05. (C) The frequency of antigen-specific MBCs measured in healthy controls did not differ when using either method of stimulation. (D) Frequency of vaccine-specific IgG memory B cells at day 0 and day 28 post-vaccination from vaccinees stimulated using the R848/IL2 mitogen cocktail. Black circles represent data from 2014/15 influenza season; red circles represent data from 2015/16 influenza season.

TABLE SI. Characteristics of subjects used for plasmablast repertoire analysis

	Patients *n*=8	Healthy controls *n*=5
Gender (M/F)	3/5	2/3
Age in years, median (range)	54 (28-74)	42 (26-67)
Ethnicity		
Caucasian	2	4
African American	5	1
Other	1	0
Received influenza vaccination during 2011-2013	4*	2
Enrollment		
2014/15 flu season	6	3
2015/16 flu season	2	2
Time since Rituximab dose in months, median (range)	12 (5-19)	
5-11 months post-Rituximab (early)	5	
At least 12 months post-Rituximab (late)	3	
No. of previous Rituximab cycles		
0	6	
1	2	
Other medications at time of vaccination		
None	5	
Cellcept	2	
Azathioprine	1	

*One patient was unable to recall vaccination history