

1 Title: **Unveiling the autoreactome: Proteome-wide immunological fingerprints**
2 **reveal the promise of plasma cell depleting therapy**
3

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49 **Abstract:** The prevalence and burden of autoimmune and autoantibody mediated disease is
50 increasing worldwide, yet most disease etiologies remain unclear. Despite numerous new
51 targeted immunomodulatory therapies, comprehensive approaches to apply and evaluate the
52 effects of these treatments longitudinally are lacking. Here, we leverage advances in
53 programmable-phage immunoprecipitation (PhIP-Seq) methodology to explore the modulation,
54 or lack thereof, of proteome-wide autoantibody profiles in both health and disease. We
55 demonstrate that each individual, regardless of disease state, possesses a distinct set of
56 autoreactivities constituting a unique immunological fingerprint, or "autoreactome", that is
57 remarkably stable over years. In addition to uncovering important new biology, the autoreactome
58 can be used to better evaluate the relative effectiveness of various therapies in altering
59 autoantibody repertoires. We find that therapies targeting B-Cell Maturation Antigen (BCMA)
60 profoundly alter an individual's autoreactome, while anti-CD19 and CD-20 therapies have
61 minimal effects, strongly suggesting a rationale for BCMA or other plasma cell targeted
62 therapies in autoantibody mediated diseases.

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80 **Main:**

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82 **Introduction:**

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84 Autoantibodies have been identified in a wide range of autoimmune diseases¹⁻⁴. In many cases
85 these autoantibodies are directly pathogenic⁵⁻¹⁰, while in others they amplify or support T cell
86 driven pathologies^{6,11}. Numerous technologies now allow for the detection of autoantibodies to
87 many proteins simultaneously¹²⁻¹⁵, and in the case of phage immunoprecipitation and sequencing
88 (PhIP-Seq), the entire human proteome¹⁶, using relatively small amounts of plasma or serum.
89 Using these new tools, a wide spectrum of novel autoantibodies have been discovered to be
90 associated with various disease states^{11,17-21}, including, but not limited to, paraneoplastic
91 encephalitis, lipodystrophy, inborn genetic disorders, and multisystem inflammatory syndrome in
92 children (MIS-C)^{11,16-23}. To accurately identify shared autoreactive profiles among individuals
93 that also share a disease state, we and others have found that serum samples from large numbers
94 of healthy individuals are required, due to the presence of highly diverse autoreactivities present
95 in every individual¹⁷. However, it remains unclear whether individual autoreactive signatures are
96 stable or variable with time or with immunosuppressive treatment. Here, we leveraged a custom
97 proteome-wide PhIP-Seq autoantibody discovery platform to comprehensively profile the
98 autoreactive repertoire in healthy individuals, and discovered that each individual harbors a
99 unique, distinctive, and highly reproducible set of autoreactivities we term the “autoreactome”.
100 Using longitudinal samples from an additional cohort of healthy individuals, we determined that
101 an individuals’ autoreactome, once formed, remains minimally changed over the course of years.

102

103 Extending these findings, we explored the impact of B cell depletion therapies upon the
104 autoreactome. Various surface markers are expressed and then downregulated over the course of
105 B cell development and maturation^{24,25}. A subset of these surface markers are targeted by B cell
106 depleting therapies which are used to treat suspected autoantibody mediated diseases, yet a
107 comprehensive evaluation of the effects of these treatments on autoantibodies remains lacking.
108 The most commonly used agent, rituximab, targets CD20, which is not expressed by antibody
109 secreting cells²⁶. Recently, chimeric antigen receptor T cell (CAR-T) therapy targeting CD19
110 positive cells was shown to be safe and effective in the treatment of refractory systemic lupus
111 erythematosus (SLE)²⁷. CD19 is expressed on naive and memory B cells, with reduced
112 expression on plasmablasts and plasma cells^{28,29}. CAR-T cells targeting B cell maturation
113 antigen (BCMA), which is expressed primarily by antibody secreting plasma cells^{30,31}, are
114 approved for the treatment of multiple myeloma^{32,33}. Here, we examined the impact of three
115 major B cell depleting therapies, rituximab (anti-CD20), anti-CD19 CAR-T cells, and anti-
116 BCMA CAR-T cells by PhIP-Seq and validate our findings using orthogonal assays. Our
117 findings demonstrate profound impact of anti-BCMA targeted therapies and minimal impact of
118 therapies targeting CD19 or CD20 on individuals’ autoantibody signatures.

119

120 **Healthy individuals harbor a unique set of autoreactivities: the “autoreactome”**

121 The search for disease causing or associated autoantibodies is confounded by the fact that
122 autoreactive antibodies are present in all healthy individuals. We have previously shown that
123 large numbers of healthy samples are required to control for this natural confounder and avoid
124 false positive associations¹⁷. To better understand the variation of the autoreactive antibodies
125 between and within healthy individuals, we obtained serum from 79 pre-COVID healthy blood

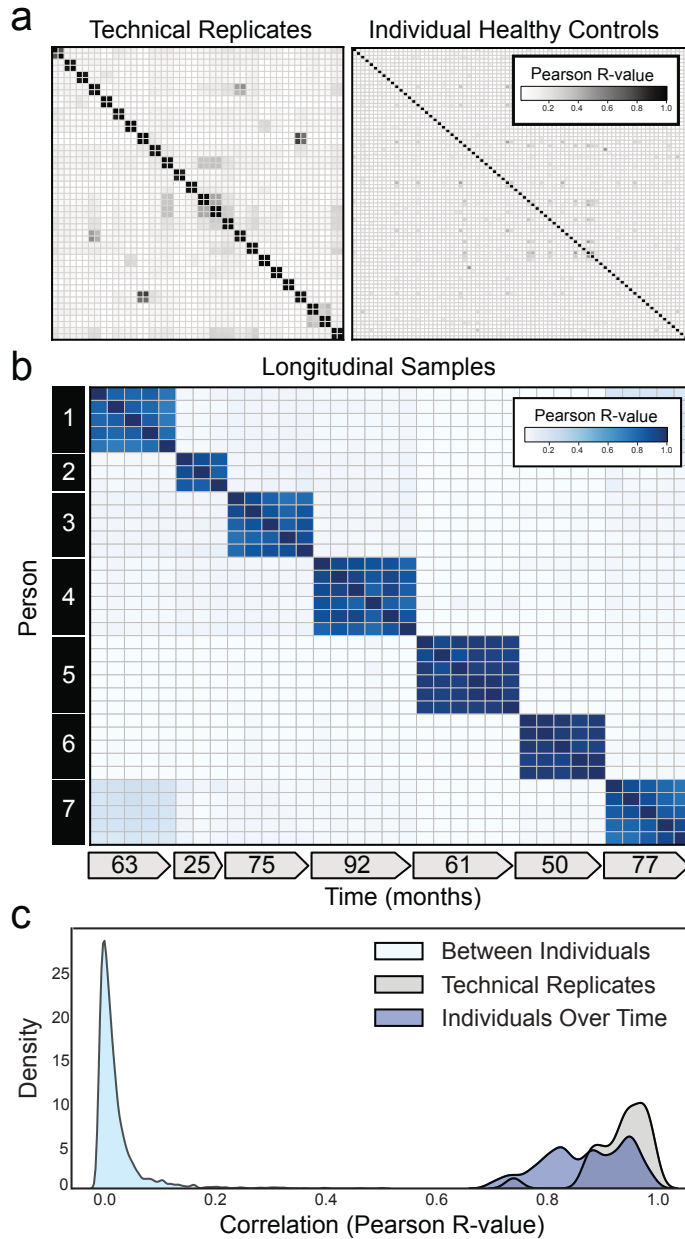
126 donors (“Healthy” demographics in Extended Data Table 1) and used our customized, previously
127 described 768,000 element phage immunoprecipitation and sequencing platform(PhIP-Seq)^{11,17-}
128 ^{21,34} to determine the proteome-wide set of autoreactivities present within each individual
129 (hereafter referred to as the “autoreactome”).

130
131 To determine inter- and intra-individual similarity by PhIP-Seq requires high reproducibility as
132 measured by technical replicate. Over the past several years, we have refined our PhIP-Seq
133 protocols to maximize reproducibility. The master version of this protocol is available at:
134 [https://www.protocols.io/view/derisi-lab-phage-immunoprecipitation-sequencing-ph-](https://www.protocols.io/view/derisi-lab-phage-immunoprecipitation-sequencing-ph-czw7x7hn?step=14.1)
135 [czw7x7hn?step=14.1](https://www.protocols.io/view/derisi-lab-phage-immunoprecipitation-sequencing-ph-czw7x7hn?step=14.1). PhIP-Seq protocol performance was evaluated by identifying the PhIP-Seq
136 enrichment similarity of 24 sets of technical duplicates using serum from healthy controls. The
137 raw signal (reads per 100k; rpk) of each phage-presented peptide which was immunoprecipitated
138 within a given sample was calculated, and then used to compare each individual sample.
139 Technical replicates showed high reproducibility (Pearson R-coefficient median =0.946; first
140 quartile (Q1) = 0.907, third quartile (Q3) =0.974) (Fig. 1a: Left Panel).

141
142 Next, the similarity of individual autoreactomes was compared between each of the 79 healthy
143 individuals in this study. For each sample, the PhIP-Seq enrichment was compared to each of the
144 78 other samples. We found that individual autoreactomes were distinctive, with very little
145 similarity to others (Pearson R-coefficient median = 0.021; Q1=0.018, Q3=0.023)(Fig. 1a: Right
146 Panel).

147
148 **The autoreactome is longitudinally stable**
149 While individual autoreactomes appear distinct, it remained an open question whether these
150 profiles were stable over time within a given individual. To address this question, we performed
151 PhIP-Seq on longitudinal serum samples from 7 distinct healthy individuals collected over a
152 median of 63 months (35 samples total; Q1=55.5 months, Q3=76 months) (“Longitudinal”
153 demographics in Extended Data Table 1). None of these individuals were being treated with
154 immunomodulatory agents at any point during sample collection, however one had basal cell
155 carcinoma and another had a history of Sjögren’s disease (clinical details in Extended Data Table
156 2). We compared the complete PhIP-Seq enrichment profile for each sample to all other samples.
157 (Fig. 1b). These results clearly revealed that the intraindividual autoreactome profiles were
158 highly correlated (Pearson R-coefficient median=0.883; Q1 =0.817, Q3=0.940). Conversely, the
159 autoreactome of longitudinal samples within an individual was significantly more similar to each
160 other than to the autoreactomes between different individuals (Mann-Whitney U p-value=4.91e⁻
161 ⁹⁵). Additionally, the distribution of intraindividual correlations overlapped considerably with the
162 distribution from technical replicates (104 of 146 (71.2%) longitudinal sample R-values fall
163 within 2 standard deviations of the mean of technical replicate R-values), indicating that in most
164 cases longitudinal autoreactomes are as similar to one another as technical replicates (Fig. 1c).
165 These results suggest that the dominant humoral determinants of autoreactivity within an
166 individual are not subject to large variation by this assay, at least within the median 5-year time
167 scale of this analysis.

168
169 **Figure 1. Individuals harbor a unique set of longitudinally stable autoreactivities**



170
171 **(a)** Correlation matrices showing Pearson correlation coefficients of complete PhIP-Seq signal in
172 healthy individuals. Left: 48 samples representing 24 individuals in technical replicate. Right: 79
173 distinct individuals. **(b)** Correlation matrix showing Pearson R-values of complete PhIP-Seq
174 signal in 7 distinct individuals each of whom have serial samples over at least 3 years. **(c)** Kernel
175 density estimate plot showing distribution of Pearson R correlation coefficients among technical
176 replicates, individuals over time, and between different individuals.

177
178 **The autoreactome is driven by rare autoreactivities and minimally altered by IVIG**
179 We and others have used PhIP-Seq to investigate autoimmune disease determinants; however,
180 many patients with immune disorders or deficiencies are treated with intravenous
181 immunoglobulin (IVIG). IVIG is pooled from thousands of donors³⁵ and therefore may contain
182 relatively common autoantibodies, which has the potential to confound autoantibody assays³⁶. To

183 investigate the effect of IVIG on intraindividual PhIP-Seq performance, we examined a cohort of
184 189 samples from patients with myasthenia gravis, an autoantibody-mediated autoimmune
185 disease. Among these 189 samples we identified 4 paired sets of samples in which the first
186 collection was in a patient naïve to any immunomodulatory treatments, the second sample was
187 within 6 weeks of IVIG treatment (2 within days, 1 within 3 weeks, 1 within 6 weeks), and no
188 additional immunomodulatory treatments had been given except for steroids and in one case
189 azathioprine (“IVIG” demographics in Extended Data Table 1; sample details in Extended Data
190 Table 3).

191
192 PhIP-Seq was performed on these samples, and the mean correlation before and after IVIG was
193 0.815 (relative to 0.87 in longitudinal samples from individuals over time without any
194 intervention)(Extended Data Fig. 1a,b). To further determine whether there were directional
195 differences in the levels of PhIP-Seq detected autoantibodies following IVIG treatment, the sum
196 of the top 10 differentially enriched autoantibodies (see “Methods) derived from each individual
197 before and after IVIG administration was compared. No significant difference was observed
198 (two-sided paired-samples Wilcoxon test p-value=0.625) (Extended Data Fig. 1c) before and
199 after IVIG.

200
201 **Rituximab treatment has minimal effect on the autoreactome**
202 Depletion of CD20 positive B cells with rituximab is a common treatment in autoimmunity and
203 presumed autoantibody mediated diseases^{37–39}. To determine the extent to which rituximab
204 treatment alters the autoreactome, we examined our cohort of 189 samples from patients with
205 myasthenia gravis to identify pairs of pre- and post-rituximab treatment samples. Like other
206 autoantibody mediated diseases, treatment of myasthenia gravis can include multiple concurrent
207 therapies which could potentially alter the autoantibody profile of an individual. To be
208 conservative, we excluded all patients who had received any immunomodulation (including
209 IVIG and plasma exchange) other than rituximab, steroids, or azathioprine, and for whom a pre-
210 treatment sample was not available. Using these stringent criteria, 35 longitudinal samples were
211 identified from 7 individuals and analyzed by PhIP-Seq (“Rituximab” demographics in Extended
212 Data Table 1; sample clinical details in Extended Data Table 4).

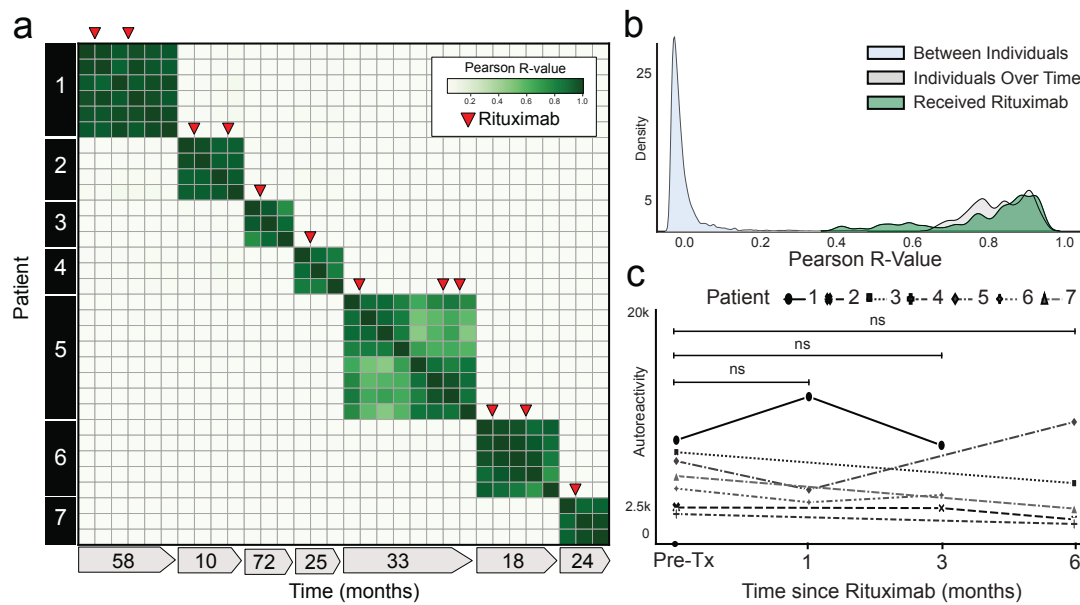
213
214 The PhIP-Seq enrichment profile for each sample from a given individual who received
215 rituximab was compared. Despite rituximab therapy, the autoreactome remained overall stable
216 within each individual over time (Pearson R-coefficient mean of 0.887; Q1=0.782,
217 Q3=0.940)(Fig. 2a). The overall distribution of correlation coefficients from individuals over
218 time who received rituximab was similar to individuals who did not receive rituximab (108 of
219 136 (79.4%) within 2 standard deviations of the mean of R-values from longitudinal samples
220 without interventions) and was not significantly different (Mann-Whitney U test p-
221 value=0.66)(Fig. 2b). While rituximab is known to transiently reduce certain antibodies, these
222 results suggest the overall profile of autoreactivity post rituximab treatment remains essentially
223 unchanged.

224
225 To determine whether there were decreases in subsets of PhIP-Seq enriched autoreactivities
226 following rituximab treatment, as opposed to the complete profile, the sum of the top 10
227 differentially enriched protein targets (see “Methods”) in each individual were calculated at the
228 time of initial sample collection, and then tracked longitudinally following the first dose of

229 rituximab. To avoid overlapping timelines, datapoints following an additional round of rituximab
230 therapy were removed from this analysis. There was no significant difference in the overall
231 autoreactivity at 1, 3, or 6 months post-rituximab therapy (one-way paired samples Wilcoxon test
232 p-value=0.625 at 1 month, 0.125 at 3 months, and 0.3125 at 6 months) (Fig. 2c). While PhIP-Seq
233 enrichment does not report on absolute immunoglobulin levels, the levels of the disease-causing
234 autoantibody (either anti-AChR or anti-MuSK antibodies) in myasthenia gravis were measured
235 independently by a clinical radioimmunoassay (RIA; either Athena Diagnostic or Mayo Clinic
236 Laboratory) in 6 of the 7 patients at the same timepoints. Although autoantibody levels
237 minimally decreased in 3 patients, and moderately decreased in the other 3 patients following
238 rituximab treatment, they never fell below the established positive cutoff for the assay (Extended
239 Data Fig. 2), suggesting that rituximab therapy was unable to quantitatively remove the
240 pathogenic autoantibodies.

241

242 **Figure 2. Rituximab treatment does not significantly alter the autoreactome**



243
244 **(a)** Correlation matrix showing Pearson R-values of complete PhIP-Seq signal in 7 distinct
245 individuals with myasthenia gravis each of whom were either rituximab naive or had not
246 received rituximab for more than 6 months prior to first sample collection. Red arrows represent
247 administration of rituximab. **(b)** Kernel density estimate plot showing distribution of Pearson R-
248 value correlation coefficients among longitudinal samples from individuals receiving rituximab
249 relative to longitudinal samples from individuals received no intervention. **(c)** Lineplots showing
250 the autoreactivity (sum of top 10 PhIP-Seq Z-scores relative to the 79 healthy controls) for each
251 patient over the first 6 months following initial rituximab dose. One-way paired samples
252 Wilcoxon test p-value=0.625 at 1 month, 0.125 at 3 months, and 0.3125 at 6 months.

253

254 **CD19+ B cells are not required to maintain the autoreactome**

255 To evaluate the impact of CD19 positive B cell depletion on the autoreactome, PhIP-Seq was
256 performed on samples prior to, and approximately 6-months following, anti-CD19 CAR-T
257 therapy in 14 individuals being treated for lymphoma (“CD19 CAR-T” demographics in
258 Extended Data Table 1) who achieved and remained in remission, indicating successful depletion

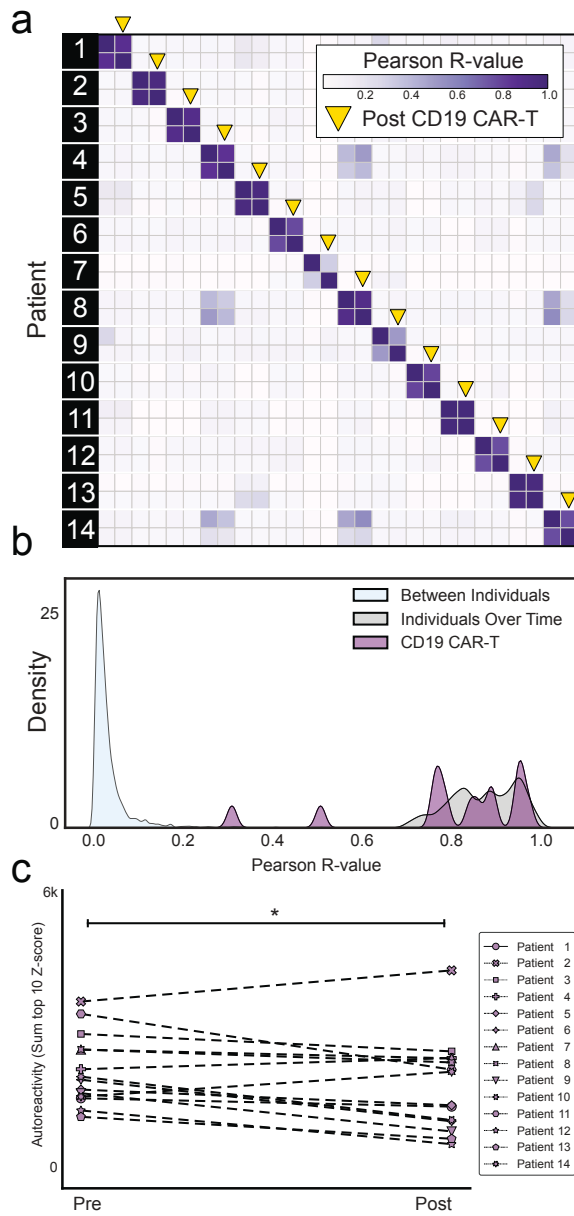
259 of the targeted cells. In 13 of the 14 patients, circulating CD19 cells were either persistently
260 absent (defined as less than 10 CD19+ B cells per microliter) or became absent following
261 treatment. In the remaining patient, only a sample prior to therapy was available, and CD19 B
262 cells were already absent, indicating that they likely remained absent following additional
263 targeted CD19 depleting therapy (Extended Data Fig. 3). None of the individuals had received an
264 allogeneic hematopoietic cell transplant (HCT) in the preceding year (though two had received
265 an autologous HCT), and 12 of the 14 patients were free of any additional B cell depleting
266 therapies for the 6 months prior to initial sample collection. Intravenous immunoglobulin (IVIG)
267 is often administered to patients receiving B cell depleting therapy, and 5 of the 14 patients
268 received IVIG ("CD19" sample clinical details in Extended Data Table 5).

269
270 The complete PhIP-Seq enrichment profile within each individual before and after CD19 CAR-T
271 therapy was once again compared. Despite depletion or persistently absent CD19 B cells in the
272 setting of active CD19 CAR-T therapy, the autoreactome remained remarkably stable over time
273 (Pearson R-coefficient median = 0.850; Q1=0.770, Q3=0.921) (Fig. 3a).

274
275 The overall distribution of correlation coefficients in the CD19 CAR-T therapy group was not
276 significantly different from intraindividual variation (Mann Whitney U p-value=0.284) The
277 distribution of correlation values in 12 of the 14 (85.7%) patients fell within the distribution (2
278 standard deviations from the mean) of longitudinal samples from healthy individuals who
279 received no interventions (Fig. 3b).

280
281 Using the same analysis approach as described for rituximab, we assessed differential PhIP-Seq
282 enrichments using the sum of top 10 most enriched protein targets before and after CD19 CAR-T
283 treatment (see "Methods"). Of the 14 individuals evaluated, 11 had a decrease in enrichment
284 values while the remaining 3 had an increase, and levels overall were significantly decreased
285 (one-sided paired samples Wilcoxon test p-value=0.021) (Fig. 3c). However, the size of the
286 effect was minimal (median percent decrease in autoreactivity of 11.9%). These results suggest
287 that similar to rituximab, sufficient immunoglobulin producing cells remain after treatment such
288 that the pattern of autoreactivity by PhIP-Seq remains largely unaltered.

289
290 **Figure 3. CD19 CAR-T therapy has minimal effect on the autoreactome 6 months after**
291 **treatment**



292
293 **(a)** Correlation matrix showing Pearson R-values of complete PhIP-Seq signal in 14 distinct
294 individuals before and after anti-CD19 CAR-T therapy. Yellow arrows represent the 6-month
295 post-treatment timepoint. **(b)** Kernel density estimate plot showing distribution of correlation
296 coefficients within each individual before and after therapy relative to the distribution among
297 untreated individuals over time and between different individuals. **(c)** Lineplots showing the
298 autoreactivity (sum of top 10 PhIP-Seq Z-scores relative to the 79 healthy controls) for each
299 patient before and after treatment. One-sided paired samples Wilcoxon test p -value=0.021.

300
301 To orthogonally validate the PhIP-Seq results, serum from 9 of these 14 patients were assayed
302 using a previously described, multiplexed micro-bead assay consisting of 55 known protein
303 autoantigens, each of which was covalently bound to microbeads with distinct barcodes⁴⁰. The
304 list of autoantigens included proteins targeted in connective tissue diseases such as SLE,

305 scleroderma, and myositis, as well as secreted proteins such as cytokines, chemokines and
306 growth factors. Additionally, antibody signal to 21 viral antigens was tested to determine
307 whether antibodies targeting both self-proteins and viral proteins respond similarly in the
308 absence of CD19⁺ B cells (see Extended Data Table 6 for a list of antigens). Because IVIG
309 contains autoantibodies that confound measurements in bead-based assays (our unpublished
310 observations), we excluded all patients who had received IVIG within 8 weeks of the initial
311 blood draw (pre CAR-T), or had received interim IVIG between the pre CAR-T and post CAR-T
312 (6 months after) blood draw. Nine of our 14 patients met these stringent criteria and were
313 included in these experiments.

314
315 As expected, a minority of autoantigens were recognized by serum IgG autoantibodies, including
316 3 intracellular proteins (thyroperoxidase, TPO; bactericidal permeability inducing protein, BPI;
317 and pyruvate dehydrogenase complex (PDC)) and 13 secreted proteins. Although levels of
318 fourteen of these 16 autoantibodies had decreased signal overall (sum of normalized MFIs; see
319 “Methods”) following anti-CD19 treatment, this decrease was only statistically significant in one
320 case (Extended Data Fig. 4a). Among the 17 antiviral antibodies with meaningful signal, 12 were
321 lower following CD19 therapy, but none were statistically significant (Extended Data Fig. 4b).
322 These data, generated with an orthogonal platform using full-length proteins as targets confirms
323 that the autoreactome, as well as IgG responses to viruses, remain largely stable over time
324 following anti-CD19 CAR-T therapy.

325
326 **The autoreactome is profoundly altered following depletion of BCMA positive B cells**
327 While CD19 is known to be expressed on a subset of antibody secreting plasma cells²⁹, BCMA is
328 a marker expressed on all plasma cells³¹, making it an attractive target for broad autoantibody
329 depletion. To assess the impact of BCMA CAR-T on the autoreactome, we performed PhIP-Seq
330 on serum samples from 9 individuals before, and approximately 6-months following, successful
331 treatment with BCMA targeted CAR-T therapy (“BCMA” demographics in Extended Data Table
332 1). All 9 individuals had confirmed depletion of plasma cells in bone marrow following anti-
333 BCMA CAR-T treatment (Extended Data Fig. 5). Each individual was being treated for multiple
334 myeloma, none had received a HCT in the previous year, and 6 of the 9 had not received any
335 additional B cell depleting therapy in the prior year. All post-treatment samples were collected at
336 least 56 days from the last dose of IVIG, and 3 of the 9 patients never received interim IVIG
337 (“BCMA” sample clinical details in Extended Data Table 5).

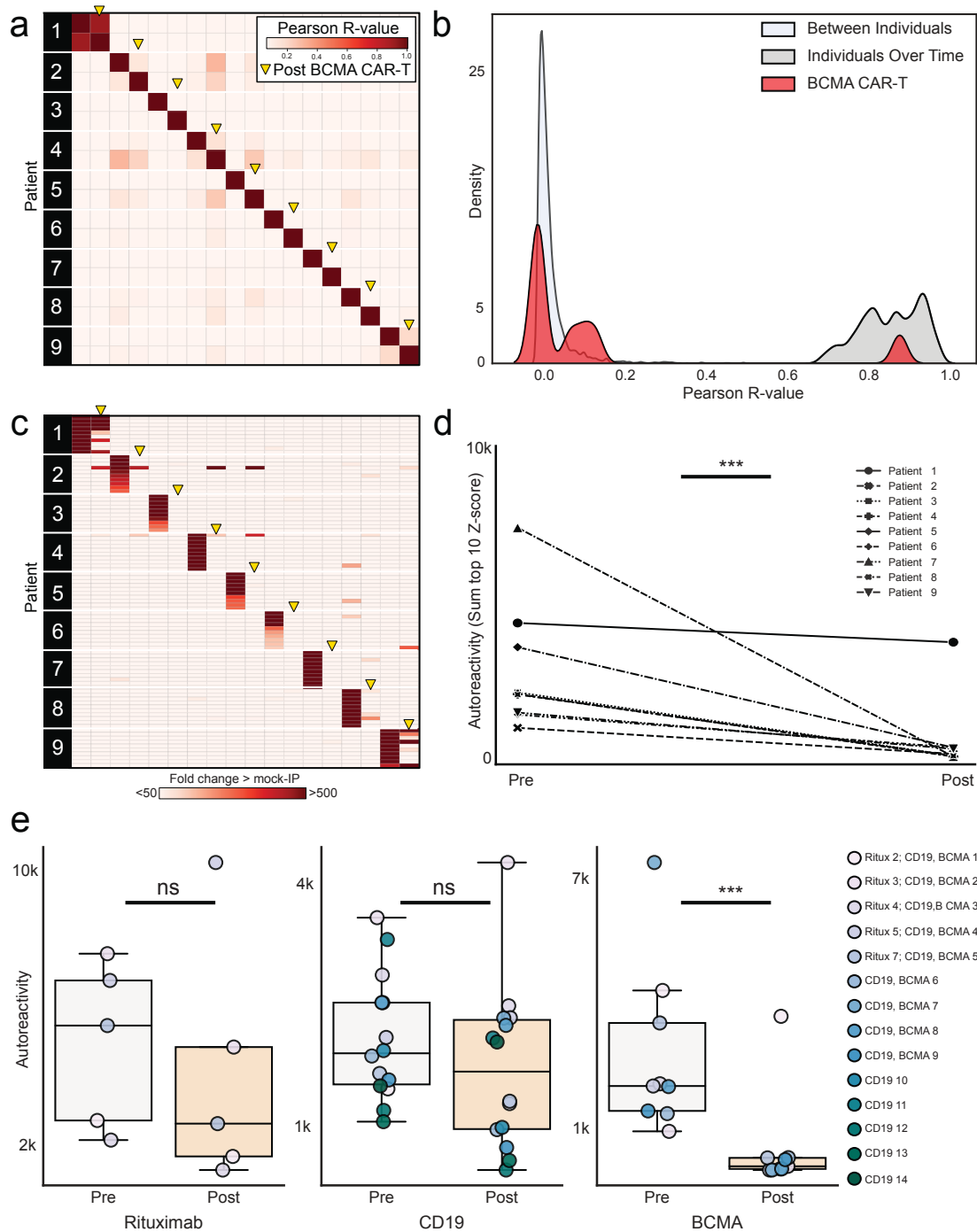
338
339 The complete PhIP-Seq enrichment profile obtained for each individual before and after anti-
340 BCMA CAR-T therapy was compared. In contrast to CD19 and CD20 targeting therapies, the
341 autoreactome was essentially devoid of any similarity following BCMA targeted therapy
342 (Pearson R-value median = 0.006; Q1=0.002, Q3=0.130) for 8 of the 9 individuals (Fig. 4a). The
343 autoreactome of one individual remained unaltered (R-value = 0.894). This individual was
344 subsequently found to have relapsed around the time of sample acquisition, indicating potential
345 failure of the CAR-T treatment. The overall distribution of correlation coefficients in the BCMA
346 CAR-T therapy group was significantly different from alterations in healthy individuals over
347 time without interventions (Mann Whitney U p-value=0.000012)(Fig. 4b). The samples from the
348 individual with disease relapse were the only set whose autoreactome remained within 2 standard
349 deviations from the mean of individuals over time without treatment. Remarkably, of the
350 remaining 8 individuals, 7 of them had autoreactome correlation values following anti-BCMA

351 CAR-T therapy that fell within 2 standard deviations of the mean of samples taken from entirely
352 different individuals. The observed complete “reset” of the autoreactome in these patients
353 suggest that successful treatment with anti-BCMA CAR-T cells sufficiently removes a lifetime
354 of accumulated antibody producing plasma cells.

355
356 While the overall autoreactive profile in sera following anti-BCMA CAR-T treatment is
357 markedly altered, we also examined changes to the most differentially enriched protein targets
358 from pre-treatment samples. Patients with multiple myeloma have a monoclonal expansion of a
359 single plasma cell which secretes paraprotein antibody. Because paraprotein is potentially
360 autoreactive, and because it is known to dramatically decrease following BCMA CAR-T
361 treatment in multiple myeloma, we removed the top two enriched protein targets from each
362 individual prior to analysis to minimize the chance that our results were being confounded by
363 changes in paraprotein level. The autoreactivity levels for the top 3-12 proteins decreased in
364 every patient follow anti-BCMA CAR-T therapy, and the overall change was statistically
365 significant (one-sided paired samples Wilcoxon test p-value=0.0019) (Fig. 4c and 4d). Unlike
366 anti-CD19 CAR-T treatment, in which the size of the decrease was minimal, there was a 95.6
367 percent decrease in PhIP-Seq enrichment following anti-BCMA CAR-T treatment (Fig. 4e). To
368 ensure these findings were similar if the top 10 protein targets were used (and potential
369 confounding by paraprotein is not accounted for), the same analysis was performed without the
370 removal of the top 2 autoreactivities, with similar findings (Extended Data Fig. 6).

371

372 **Figure 4. Anti-BCMA CAR-T therapy significantly alters the autoreactome**



373
374 **(a)** Correlation matrix showing Pearson R-values of complete PhIP-Seq signal in 9 distinct
375 individuals before and after anti-BCMA CAR-T therapy. Yellow arrows represent 6 month post-
376 treatment time point. **(b)** Kernel density estimate plot showing distribution of correlation
377 coefficients within each individual before and after BCMA-targeted therapy relative to the
378 distribution among untreated individuals over time, and the distribution between different
379 individuals. **(c)** Heatmap showing top 10 autoreactivities (sum of top 10 PhIP-Seq Z-scores
380 relative to the 79 healthy controls) in each individual before and after treatment. **(d)** Lineplots

381 showing the autoreactivity (sum of top 3 through 12 PhIP-Seq Z-scores relative to the 79 healthy
382 controls therefore accounting for potential paraprotein confounding) for each patient before and
383 after treatment. One-sided paired samples Wilcoxon test p-value=0.0019. (e) Boxplots showing
384 the relative distributions of autoreactivity before and after treatment with rituximab (sum of top
385 10), anti-CD19 CAR-T (sum of top 10), and anti-BCMA CAR-T (sum of top 3 through 12
386 autoreactivities). Rituximab treatment cohort Mann-Whitney U p-value=0.42 with a median
387 percent decrease of 52.3%; anti-CD19 CAR-T treatment cohort Mann-Whitney U p-value=0.206
388 with a median percent decrease of 11.9%; rituximab treatment cohort Mann-Whitney U p-
389 value=0.003 with a median percent decrease of 95.6%.

390
391 To orthogonally validate the PhIP-Seq results and further explore the effects of plasma cell
392 depletion on antiviral antibodies, we used the same multiplexed bead-based arrays described
393 earlier to characterize IgG binding to 55 autoantigens and 21 viral proteins. However, because
394 IVIG is routinely given following BCMA CAR-T therapy, only 3 of the original 9 patients were
395 IVIG free, so samples from 4 additional patients meeting the stringent criteria outlined
396 previously were used (demographics and sample details in Extended Data Tables 7 and 8).

397
398 Of the same 16 measurable autoantigens analyzed in the anti-CD19 CAR-T treatment cohort, 13
399 had overall decreased IgG binding (sum of normalized MFIs; see “Methods”) following anti-
400 BCMA CAR-T treatment. In contrast to the CD19 CAR-T cohort in which there was only one
401 autoantibody with a statistically significant decrease, levels of 9 of the 16 measured
402 autoantibodies significantly decreased following anti-BCMA CAR-T therapy (Extended Data
403 Figure 7a). Also, unlike the CD19 CAR-T cohort in which levels of none of the anti-viral
404 antibodies significantly decreased, all 17 of the measured antiviral antibodies decreased
405 following BCMA therapy, and 8 of these decreases were statistically significant (Extended Data
406 Figure 7b).

407 408 **Discussion**

409 Adaptive immune responses targeting self rather than foreign proteins are a hallmark of
410 autoimmune disease. Previous work has noted the existence of circulating self-reactive
411 antibodies in healthy individuals. This work has focused on “natural autoantibodies”, which are
412 thought to arise without antigen stimulation and to consist primarily of unmutated, polyreactive,
413 often IgM-class antibodies^{41,42}. Despite considerable interest, the role of these antibodies in
414 health and disease remains unclear, though there is evidence of variable and context-dependent
415 roles; the autoantibodies can contribute to, or protect against, inflammatory and autoimmune
416 disease^{43–53}. The natural autoantibody repertoire is thought to be relatively shared amongst
417 individuals, and to develop during the first few years of life^{42,54–56}. Though natural
418 autoantibodies may target autoantigens present in autoimmune disease, substantial evidence
419 exists that the specific epitope differs from the disease-associated autoantibody, which may in
420 part explain why the healthy individuals studied here are clinically immune tolerant^{57,58}.

421
422 Here, through the targeted application of a custom proteome-wide autoantibody detection
423 technology, we find that the repertoire of auto-reactive immunoglobins present in each healthy
424 individual is highly distinctive. This “autoreactome” is described by the complex set of
425 thousands of peptides reproducibly enriched by immunoprecipitation, and apparently unique to
426 each individual. We observe that the autoreactome profile of individuals is remarkably stable,

427 even over years of time, and thus may be considered a “serological fingerprint.” In contrast to
428 natural autoantibodies, the autoreactome is characterized by uniqueness in each individual, rather
429 than a common, shared set of autoreactivities. Interestingly, the stability and uniqueness of the
430 autoreactome suggests that our approach may be utilized to de-convolute the origin of an
431 unknown patient serum sample in repositories where multiple samples have been collected at
432 different timepoints from the same patient.

433
434 During a humoral immune response, plasma cells of varied lifespans are generated, with short-
435 lived plasmablasts providing rapid antibody production in the acute phase and long-lived plasma
436 cells sustaining durable antibody titers. While foreign antigen-specific long-lived plasma cells
437 are known to persist for decades in humans⁵⁹⁻⁶¹, the cellular phenotype of pathogenic plasma
438 cells remains controversial. Since individual autoantibodies decline following therapeutic B cell
439 ablation, despite a lack of CD19 and CD20 expression on plasma cells^{27,62,63}, the prevailing
440 model holds that the continuous generation of short-lived plasma cells from memory B cell
441 precursors underlies the autoantibody repertoire⁶⁴. In contrast with this view, we show that not
442 only is the broader intraindividual autoreactome stable over time, but that this autoantibody
443 repertoire is resistant to therapeutic B cell ablation with rituximab (anti-CD20) and anti-CD19
444 CAR-T cells. Given the expanded options for targeting B cells and plasma cells in systemic
445 autoimmunity, this enhanced understanding of autoreactome biology has important clinical
446 implications. Indeed, by using the autoreactome as a proxy for the autoantibody repertoire within
447 an individual, we show here that depletion of plasma cells via anti-BCMA CAR-T therapy
448 drastically alters the autoantibody repertoires. In most cases, the autoreactome of a sample after
449 treatment with BCMA CAR-T cells appears as similar to an entirely different person as to
450 themselves prior to treatment.

451
452 It has been shown that rituximab reduces the level of certain autoantibodies⁶⁵, and has clinical
453 efficacy in many autoimmune disease settings, which may relate more to the antigen presentation
454 function of CD20-positive B cells as proposed by others⁶⁶. Additionally, CD19 CAR-T therapy
455 has recently been shown to be effective in the treatment of refractory SLE. Because both anti-
456 CD19 CAR-T and anti-BCMA CAR-T are primarily used in the treatment of hematologic
457 malignancy, our cohorts received chemotherapy prior to CAR-T therapy, and therefore had low
458 CD19 and plasma cells numbers even prior to the initiation of therapy. Future studies will be
459 required to further understand the relationship of the pre- and post-CAR-T treatment
460 autoreactomes, and its relationship to clinical efficacy, in the specific context of autoimmune
461 diseases. Our broad based approach to screen autoantibodies at a proteome wide level allows a
462 more comprehensive and unbiased assessment of the effect of therapies on the specificity of the
463 autoreactome. We posit that this approach could greatly complement emerging data on the use of
464 CAR-T cell therapies that are being utilized against severe autoimmune diseases.

465
466 The prevalence and burden of autoimmune disease continues to rise^{67,68}, yet the precise
467 mechanisms for the development of autoreactivity remain unclear. There is mounting evidence
468 that combinations of genetic and environmental risk factors, including viral infections,
469 predispose individuals to disease. In addition to the emerging therapies already discussed, other
470 new therapies exist which can halt or prevent the progression of certain autoimmune diseases,
471 including type 1 diabetes (T1DM)⁶⁹. By defining the autoreactome and describing the expected
472 longitudinal changes over time, we introduce a new tool which can be applied for additional

473 basic and translational studies in autoimmunity. Future work will be needed to explore the
474 autoreactome to determine at what age it develops, whether there are shared features of
475 autoreactive epitopes, what drives the development of the autoreactivities, and what biological
476 role these autoreactive antibodies may have in health and disease. Beyond the already discussed
477 application for evaluating the relative effectiveness of various emerging therapies, there are
478 additional potential future applications of this work by tracking longitudinal autoreactome
479 changes in individuals prior to, and following, the development of specific autoimmune diseases.
480 This may allow for the identification of autoreactive signatures which precede the development
481 of symptoms, and thereby produce novel biomarkers to identify patients in whom autoimmune
482 disease could be prevented.

483

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485

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638 **Methods**

639 **Patients**

640 The “Healthy Control” cohort consists of plasma from 79 self-reported healthy, pre-COVID-19
641 individuals which were obtained as deidentified samples from the New York Blood Center.
642 These samples were part of retention tubes collected at the time of blood donations from
643 volunteer donors who provided informed consent for their samples to be used for research.
644

645 For the bead-based protein arrays, serum and/or plasma was used for internal controls and
646 validations, as has been previously described, as deidentified samples⁷⁰. Negative controls
647 included a pediatric sample from an infant between 6 and 12 months of age which was a gift
648 from the Pulendran lab, as well as samples from 3 healthy subjects with known CMV-
649 seronegative status confirmed by ELISA from the Stanford Blood Center. Positive controls
650 include plasma samples derived from participants with autoimmune or other types of diseases
651 with known reactivity patterns (e.g. thyroid peroxidase [TPO], pyruvate dehydrogenase complex
652 [PDC-E2], fibroblast growth factor 7 [FGF7], proteinase 3, IL-11, CXCL-13) which were
653 purchased from ImmunoVision or were obtained from Stanford Autoimmune Diseases Biobank
654 and Oklahoma Medical Research Foundation (Oklahoma City, Oklahoma, USA; a gift of Judith
655 James, Oklahoma Medical Research Foundation). A total of 12 positive control patient samples
656 were used. All 16 of these patients (4 negative controls and 12 positive controls) provided
657 informed consent for their samples to be used for research.
658

659 Longitudinal plasma samples were obtained from a set of 7 community dwelling, cognitively
660 unimpaired, pre-COVID-19, healthy older adults recruited through the Brain Aging Network for
661 Cognitive Health (BRANCH) at the University of California, San Francisco. All participants are
662 screened at baseline and enrollment criteria excluded individuals with severe psychiatric illness,
663 neurologic disorders (e.g., epilepsy, multiple sclerosis), and medical conditions that could impact
664 cognition (e.g., recent substance use disorders, active chemotherapy). Following a
665 comprehensive neurobehavioral evaluation, participants were classified cognitively normal per
666 consensus conference with board-certified neurologists and neuropsychologists. Inclusion in the
667 current study was contingent on no known autoimmune conditions at baseline or follow up visits,
668 either active or inactive at the time of plasma collection. Study procedures were approved by the
669 UCSF Committee on Human Research and all participants provided written informed consent
670 (IRB #10-02076).
671

672 For the myasthenia gravis patient cohort (used to evaluate the effects of IVIG and rituximab),
673 deidentified serum samples from myasthenia gravis patients, with laboratory confirmed AChR or
674 MuSK autoantibody serostatus, were retrieved from a biorepository established at the Yale
675 School of Medicine, Myasthenia Gravis Clinic (EXPLORE-MG Registry, NCT03792659) under
676 the approval of Yale University’s Institutional Review Board.
677

678 The CAR-T cell cohorts consisted of adults aged ≥ 18 years with relapsed or refractory CD19+ or
679 BCMA+ hematologic malignancies who received lymphodepleting chemotherapy with
680 cyclophosphamide and fludarabine followed by anti-CD19 or anti-BCMA-CAR-T cells at Fred
681 Hutchinson Cancer Center (Fred Hutch). Serum and PBMCs were prospectively collected once

682 prior to lymphodepleting chemotherapy and at approximately 6-12 months after CAR-T cell
683 infusion among individuals who achieved durable remissions and received no subsequent anti-
684 tumor therapies. This work was approved by the Fred Hutch institutional review board (Protocol
685 10080), and all participants provided informed consent in accordance with the Declaration of
686 Helsinki.

687

688 **PhIP-Seq**

689 All PhIP-Seq was performed similar to our previously published multichannel protocol, with
690 minor adjustments as outlined in our new protocol: [https://www.protocols.io/view/derisi-lab-
691 phage-immunoprecipitation-sequencing-ph-czw7x7hn?step=14.1](https://www.protocols.io/view/derisi-lab-phage-immunoprecipitation-sequencing-ph-czw7x7hn?step=14.1)

692

693 As previously described¹¹, our human peptidome library consists of a custom-designed phage
694 library of 731,724 unique T7 bacteriophage each presenting a different 49 amino-acid peptide on
695 its surface. Collectively these peptides tile the entire human proteome including all known
696 isoforms (as of 2016) with 25 amino-acid overlaps. 1 milliliter of phage library was incubated
697 with 1 microliter of human serum overnight at 4C and immunoprecipitated with 25 microliters of
698 1:1 mixed protein A and protein G magnetic beads (Thermo Fisher, Waltham, MA, #10008D and
699 #10009D). These beads were then washed, and the remaining phage-antibody complexes were
700 eluted in 1 milliliter of E.Coli (BLT5403, EMD Millipore, Burlington, MA) at 0.5-0.7 OD and
701 amplified by growing in 37C incubator. This new phage library was then re-incubated with the
702 same individual's serum and the previously described protocol repeated. DNA was then
703 extracted from the final phage-library, barcoded, and PCR-amplified and Illumina adaptors
704 added. Next-Generation Sequencing was then performed using an Illumina sequencer (Illumina,
705 San Diego, CA) to a read depth of approximately 1 million per sample.

706

707 **Bead-based protein array**

708 A 76-plex protein array comprising a 55-plex autoantigen protein subarray and a 21-plex viral
709 protein subarray was constructed as previously described, with modifications⁴⁰. In brief, antigens
710 (Extended Data Table 6) were conjugated to uniquely barcoded carboxylated magnetic beads
711 (MagPlex-C, Luminex Corp). The beads were stored in aliquots at -80°C after conjugation and
712 thawed on the day of the experiment. 45 µl of diluted serum or plasma sample (1:100 in PBS-
713 1%BSA) was transferred into the 384-well plate (Greiner BioOne) containing 5 µl of bead array
714 per well. Samples were incubated for 60 min on a shaker at room temperature, and then at 4C
715 overnight. The following day beads were washed with 4 × 60 µl PBS-Tween on a plate washer
716 (EL406, Biotek) and then incubated with 50 µl of 1:1000 diluted R-phycoerythrin (R-PE)
717 conjugated Fc-γ-specific goat anti-human IgG F(ab')₂ fragment (Jackson ImmunoResearch 109-
718 116-170) for 30 min. The plate was washed with 4 × 60 µl PBS-Tween and re-suspended in 50
719 µl PBS-Tween prior to analysis using a FlexMap3D™ instrument (Luminex Corp.) and
720 measuring MFI with at least 50 beads per barcode for each sample. Most of the individual
721 antigens that had been conjugated to beads were qualified prior to mixing using antibodies-
722 directed against epitope tags, monoclonal antibodies specific for the antigen, or prototype human
723 plasma samples derived from participants with autoimmune diseases with known reactivity
724 patterns (e.g., Scl-70+ systemic sclerosis; SSA+, which is associated with lupus and Sjögren's
725 syndrome; ribonucleoprotein (RNP)+ sera, which is associated with lupus and mixed connective
726 tissue disease; and APS1 sera which are reactive with multiple cytokines; data not shown).

727 Binding events were displayed as Median Fluorescence Intensity (MFI). MFI values were
728 normalized by subtracting bare-bead MFI values. Replicate MFI values were averaged.

729

730 **Enumeration of B cells and plasma cells**

731 B cells were quantified using a research flow cytometry panel (Representative gates shown in
732 Extended Data Fig. 8). Briefly, peripheral blood mononuclear cells (PBMCs) were incubated on
733 ice for 30 minutes with fluorescently labeled antibodies (in fluorescence-activated single cell
734 sorting [FACS] buffer containing Dulbecco's phosphate-buffered saline [DPBS] and
735 1% newborn calf serum [Life Technologies]) before its analysis on a FACSymphony A5 (BD
736 Bioscience) flow cytometer. The following antibodies were included for cell labeling: fixable
737 viability stain 700 (BD cat#564997), anti-CD19 BV421 (HIB19, BD), anti-CD45 BV510 (HI30,
738 BD), anti-CD3 BV605 (UCHT1, BioLegend), anti-CD16 BV711 (3G8, BD), anti-CD14 BV711
739 (MOP-9, BD). FlowJoTM Software version 10.7.1 was used for analyses with flow cytometry
740 proportions multiplied by absolute lymphocyte counts to calculate total CD19⁺ B cell numbers.
741 Plasma cells in the bone marrow were quantified based on detection of CD138⁺ plasma cells in
742 clinically obtained bone marrow core biopsies by immunohistochemistry in the University of
743 Washington Hematopathology Laboratory.

744

745 **Analysis of PhIP-Seq**

746 As previously described¹¹, next generation sequencing reads from fastq files were aligned at the
747 level of amino acids using RAPSearch2. All results represent the average of technical replicates,
748 except for the 79 individual healthy controls in whom only 24 of the individuals (48 samples)
749 were performed in technical replicate. All human peptidome analysis was performed at the gene-
750 level, in which all reads for all peptides mapping to the same gene were summed, and 0.5 reads
751 were added to each gene to allow inclusion of genes with zero reads in mathematical analyses.
752 Within each individual sample, reads were normalized by converting to the percentage of total
753 reads. To normalize each sample against background non-specific binding, a fold-change (FC)
754 over mock-IP was calculated by dividing the sample read percentage for each gene by the mean
755 read-percentage of the same gene for the AG bead only controls. This FC signal was then used
756 for side-by-side comparison between samples and cohorts. FC values were also used to calculate
757 z-scores for specific cohorts relative to defined controls.

758

759 **Analysis of multiplexed bead arrays**

760 Antibody reactivity to each target for each sample was measured in mean fluorescent intensity
761 (MFI). Data is only shown for targets which have an MFI of at least 1000 MFI units at any time
762 point for any patient. To adjust for background binding to beads only, each sample was
763 additionally run on unconjugated beads, and bare bead MFI was subtracted to create an adjusted
764 MFI for each target antigen within the same corresponding sample. To account for nonspecific
765 binding from human serum, a sample from an infant control known to lack antibodies to any of
766 the viral or human targets was run in duplicate. The mean of the adjusted MFI's for the control
767 duplicates was calculated for each target, and further subtracted from each corresponding target
768 from each sample. A normalization function was then performed such that all sample values for
769 each target antigen ranged from zero to one, referred to as "Normalized MFI".

770

771 **Statistical methods**

772 All statistical analysis was performed in Python using the Scipy Stats package. To calculate the
773 overall similarity of PhIP-Seq detected autoreactive profiles between different samples, a
774 Pearson correlation coefficient was calculated using all FC over mock-IP values (unless
775 specifically stated otherwise) for each autoantigen in each sample relative to each autoantigen in
776 each other sample. For comparisons of distributions of PhIP-Seq signal (either autoreactivity
777 scores or Pearson R-values) between two groups, a non-parametric Mann-Whitney U test was
778 utilized. To determine whether specific interventions (IVIG, rituximab, anti-CD19 CAR-T, or
779 anti-BCMA CAR-T) directionally decreased PhIP-Seq detected autoreactivities, first an
780 “autoreactivity” score was calculated by summing the top 10 autoantigen z-scores (relative to the
781 79 healthy controls) in each pre-treatment sample. The sum of the z-scores from these same 10
782 autoantigens were again calculated following the intervention for each post-treatment sample
783 from the same individual. These autoreactivity scores from before and after a treatment were
784 paired within each individual, and used as input for a one-sided paired-samples Wilcoxon test.
785 To determine whether anti-CD19 CAR-T treatment, or anti-BCMA CAR-T treatment altered
786 Luminex detected antibody levels from the bead-based protein arrays, the pre-treatment and
787 post-treatment normalized MFI values were compared using a two-sided paired-samples
788 Wilcoxon test for each target antigen. P-values: ns > 0.05, * < 0.05, ** < 0.01, *** < 0.005, ****
789 < 0.001.

790

791 **Data Availability**

792 All PhIP-Seq data will be made publicly available upon publication of this manuscript via a
793 Dryad digital repository; DOI: 10.5061/dryad.w3r2280z6

794

795 **Methods References**

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797 C. L. Dekker, M. M. Davis, P. J. Utz, P. Khatri, A. J. Kuo, Single-Cell Chromatin Modification
798 Profiling Reveals Increased Epigenetic Variations with Aging. *Cell*. **173**, 1385-1397.e14 (2018).

799

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806 **Author Contributions**

807 Conceptualization: A.B., H.L., M.R.W., J.A.H., S.W.J., M.S.A., J.L.D. Methodology: A.B., J.A.,
808 A.F.K., H.K., E.R., C.Y.W., A.S., P.J.U., A.R., M.K., M.S.A., J.L.D. Performed or contributed
809 to experiments: A.B., D.J.L.Y., A.R., M.K., J.B., C.M.B., B.O. Formal analysis: A.B., J.L.D.
810 Patient sample and clinical data acquisition: K.Z., K.D.D., V.E.D., E.Q.G., B.C., D.J.G, J.G.,
811 C.J.T., J.A.H., G.M., R.J.W, K.C.O. Clinical data curation: K.Z., K.D.D., K.B.C., J.H.K.,
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814 Supervision: P.J.U., J.A.H, S.W.J., M.S.A., J.L.D.

815

816 **Competing Interest Declaration**

817

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835

836 **Potential Conflicts**

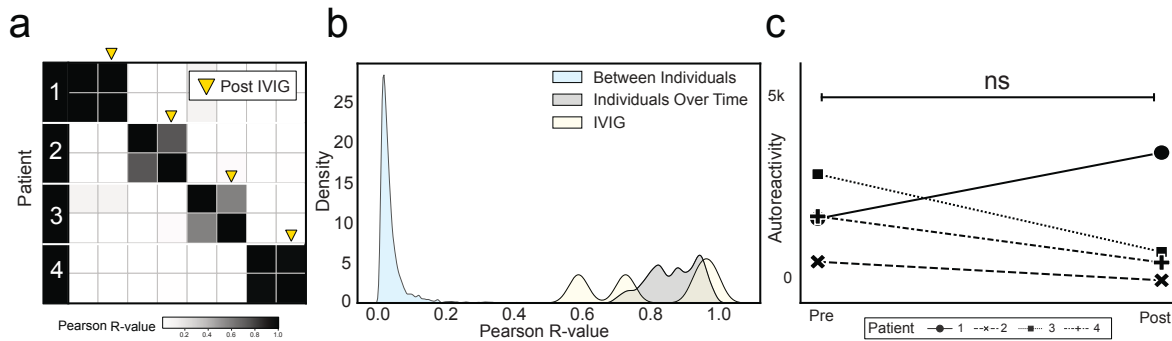
837 J.D.R. reports being a founder and paid consultant for Delve Bio, Inc., and a paid consultant for
838 the Public Health Company and Allen & Co. M.A.S. receives unrelated research funding from
839 the NIH, the CDC, Cepheid and Merck and unrelated Honoria from UpToDate, Inc. M.R.W.
840 reports being a founder and paid consultant for Delve Bio, Inc. and receives unrelated research
841 grant funding from Roche/Genentech and Novartis, and received speaking honoraria from
842 Genentech, Takeda, WebMD, and Novartis. C.J.T. reports being on the Scientific Advisory
843 Boards for Caribou Biosciences, T-CURX, Myeloid Therapeutics, ArsenalBio, Cargo
844 Therapeutics, Celgene/BMS Cell Therapy; is a member a DSMB member of Kyverna; is on Ad
845 hoc advisory boards/consulting (last 12 months) for Nektar Therapeutics, Legend Biotech,
846 Prescient Therapeutics, Century Therapeutics, IGM Biosciences, Abbvie; has Stock options in
847 Eureka Therapeutics, Caribou Biosciences, Myeloid Therapeutics, ArsenalBio, Cargo
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869
870 **Correspondence and requests for materials should be addressed to Mark S. Anderson**
871 **(mark.anderson@ucsf.edu) and Joseph L. DeRisi (joe@derisilab.ucsf.edu).**
872

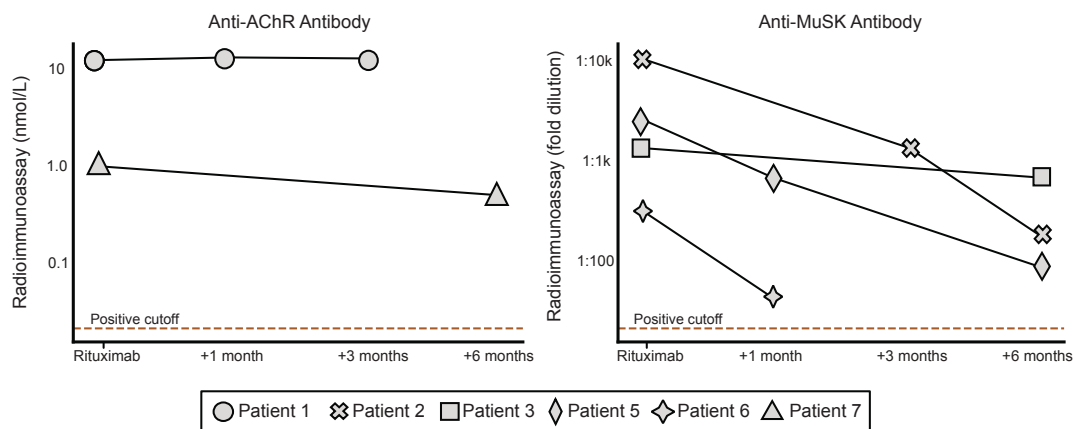
873 Extended Data Figures and Tables

874
875 **Extended Data Figure 1. Administration of IVIG minimally alters the autoreactome**



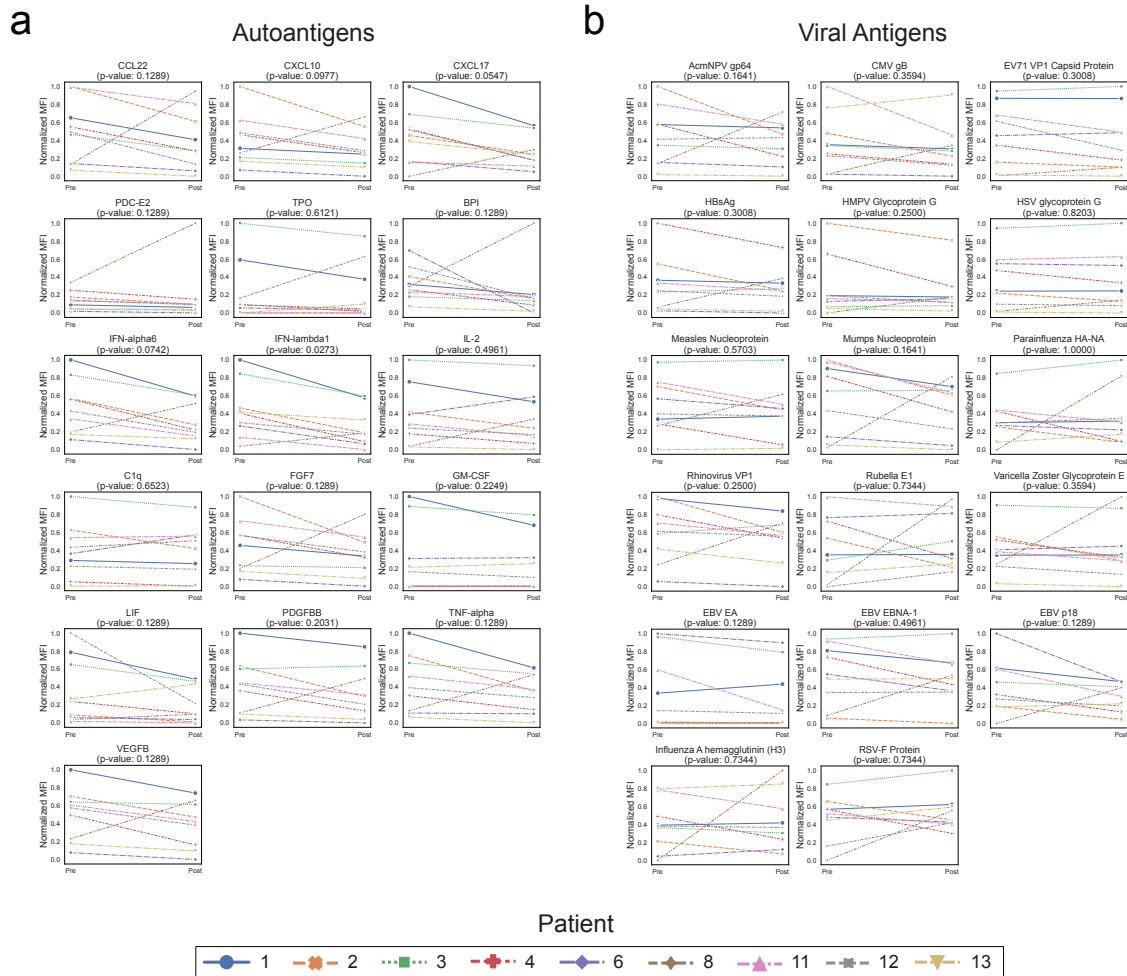
876
877 **(a)** Correlation matrices showing Pearson correlation coefficients of complete PhIP-Seq signal
878 before and after IVIG administration. **(b)** Kernel density estimate plot showing distribution of
879 Pearson R correlation coefficients from before and after IVIG administration relative to
880 longitudinal samples from individuals over time, and between different individuals. **(c)** Lineplots
881 showing the autoreactivity (sum of top 10 PhIP-Seq Z-scores relative to the 79 healthy controls)
882 for each patient before and after IVIG administration. Paired-samples Wilcoxon test p-value
883 0.625.

884
885 **Extended Data Figure 2. Changes in disease-causing autoantibody levels following**
886 **rituximab**



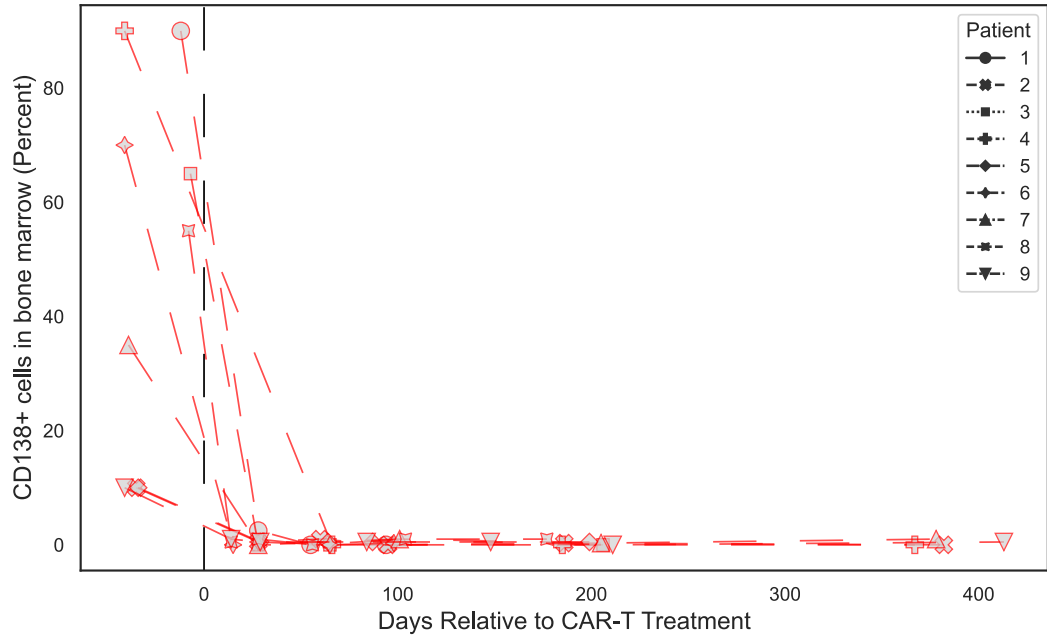
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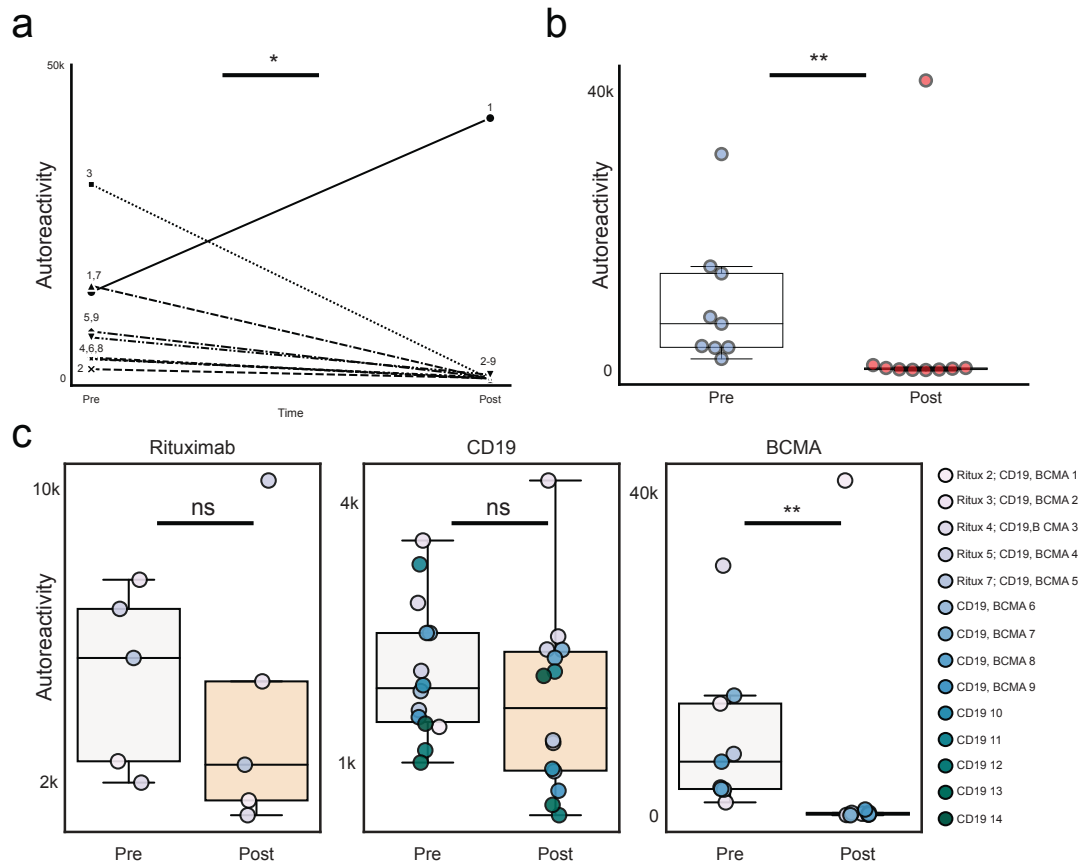
903
 904 **(a)** Multiplex bead-based protein array detected normalized MFI values (see “Methods”) for each
 905 of the 16 autoantigens with meaningful signal (see “Methods”) in each of the patients before and
 906 approximately 6 months after treatment with anti-CD19 CAR-T cells. P-values shown are from a
 907 paired-samples Wilcoxon test. **(b)** Multiplex bead-based protein array detected normalized MFI
 908 values (see “Methods”) for each of the 17 viral antigens with meaningful signal (see “Methods”)
 909 in each of the patients before and after treatment with anti-CD19 CAR-T cells. P-values shown
 910 are from a paired-samples Wilcoxon test.

911
 912
 913 **Extended Data Figure 5: Changes in bone marrow plasma cell percentage following anti-**
 914 **BCMA CAR-T cell therapy**



915
916 Lineplots showing the levels of plasma cells in the bone marrow measured by flow cytometry at
917 various timepoints relative to anti-BCMA CAR-T treatment, in each patient.
918

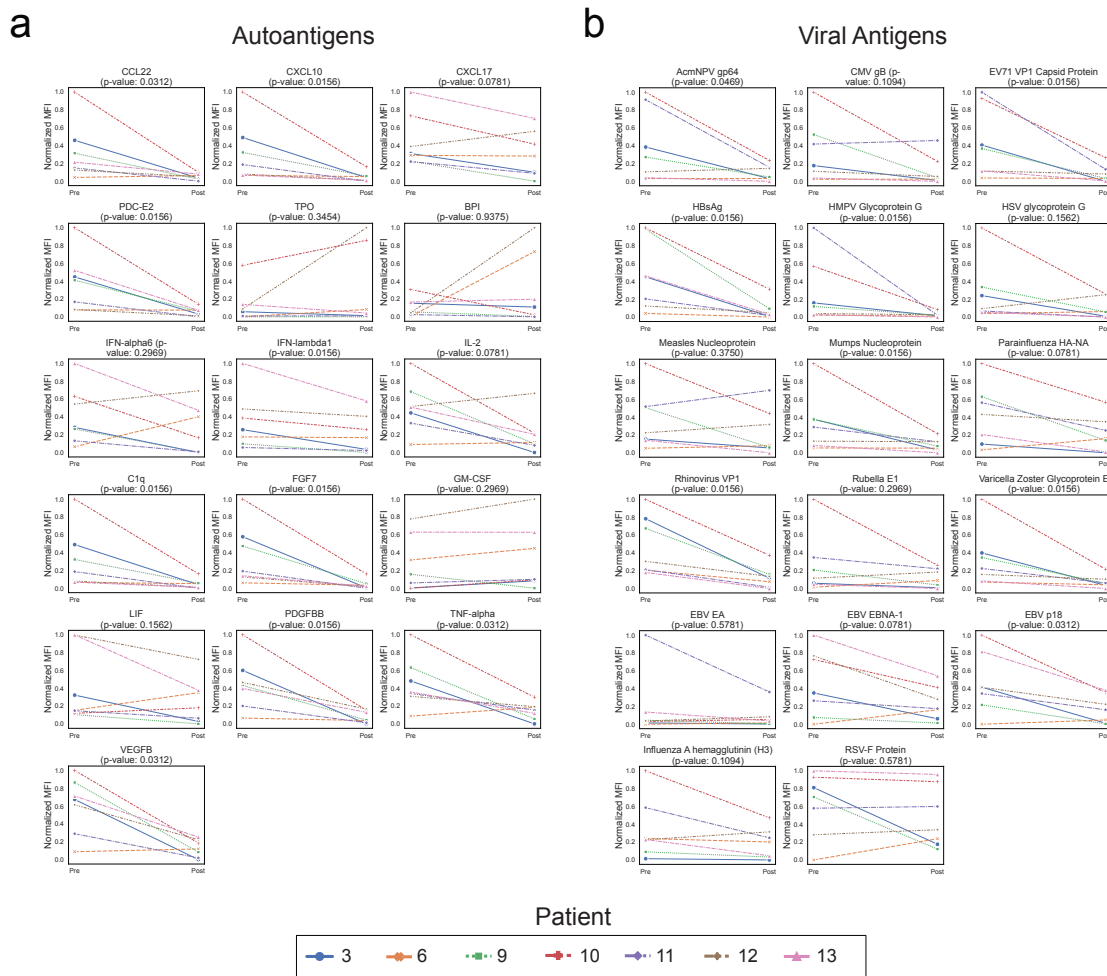
919 **Extended Data Figure 6: Changes in PhIP-Seq detected autoreactivities following anti-BCMA CAR-T cell therapy without controlling for potential confounding by paraprotein**
920



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922 **(a)** Lineplots showing the autoreactivity (sum of top 10 PhIP-Seq Z-scores relative to the 79
 923 healthy controls) for each patient before and after treatment. One-sided paired samples Wilcoxon
 924 test p-value=0.049. **(b)** Swarmplots showing the relative distributions of autoreactivity (sum of
 925 top 10 PhIP-Seq Z-scores relative to the 79 healthy controls) before and after treatment with anti-
 926 BCMA CAR-T. Mann-Whitney U p-value=0.006. **(c)** Boxplots showing the relative distributions
 927 of autoreactivity (sum of top 10 PhIP-Seq Z-scores relative to the 79 healthy controls) before and
 928 after treatment with rituximab, anti-CD19 CAR-T, and anti-BCMA CAR-T. rituximab treatment
 929 cohort Mann-Whitney U p-value=0.42 with a median percent decrease of 52.3%; anti-CD19
 930 CAR-T treatment cohort Mann-Whitney U p-value=0.206 with a median percent decrease of
 931 11.9%; anti-BCMA CAR-T treatment cohort Mann-Whitney U p-value=0.006 with a median
 932 percent decrease of 97.2%.

933
 934 **Extended Data Figure 7: Changes in multiplex bead-based protein array detected antibody**
 935 **levels following BCMA therapy**

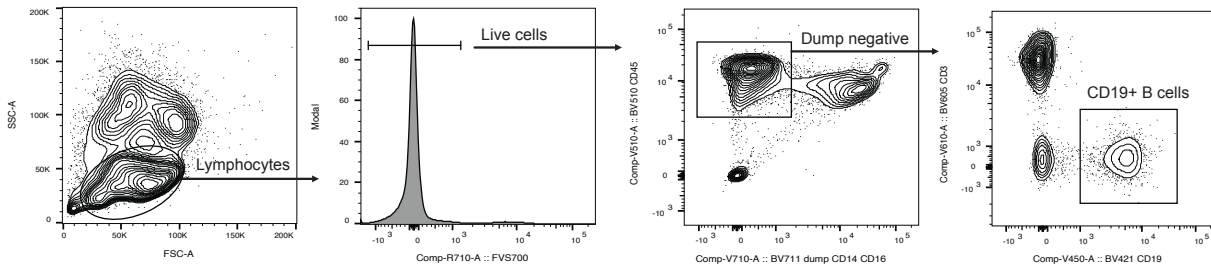


936 **(a)** Multiplex bead-based protein array detected normalized MFI values (see “Methods”) for each
 937 of the 16 autoantigens with meaningful signal (see “Methods”) in each of the patients before and
 938 after treatment with anti-BCMA CAR-T cells. P-values shown are from a paired-samples
 939 Wilcoxon test. **(b)** Multiplex bead-based protein array detected normalized MFI values (see
 940 “Methods”) for each of the 17 viral antigens with meaningful signal (see “Methods”) in each of
 941

942 the patients before and after treatment with anti-BCMA CAR-T cells. P-values shown are from a
 943 paired-samples Wilcoxon test.

944

945 **Extended Data Figure 8: Flow cytometry gating strategy for identification of CD19⁺ B cells**



946

947 Representative flow cytometry gating for the identification and enumeration of CD19⁺ B-cells.

948

949 **Extended Data Table 1: PHIP-Seq Cohort Demographics**

	Healthy	Longitudinal	IVIG	Rituximab	CD19 CAR-T	BCMA CAR-T
Number	79	7	4	7	14	9
Male sex (%)	41 (51.9)	2 (28.6)	1 (25)	2 (28.6)	9(64.3)	4 (44.4)
Median age in years (IQR)	48 (36-55.5)	74 (71.5-76.5)	Ranges*	Ranges**	58 (47-64)	65 (60-70)
Race and Ethnicity (%)						
White	63 (77.7)	7 (100)	3 (75)	6 (85.7)	14 (100)	8 (88.9)
Black	1 (1.2)	0	0	1 (14.3)	0	0
Hispanic/Latinx	10 (12.3)	0	0	0	0	0
Asian/Pacific Islander	3 (3.7)	0	1 (25)	0	0	0
Multiple Races	3 (3.7)	0	0	0	0	0
Unknown/Other	1 (1.2)	0	0	0	0	1 (11.1)

950 * Age ranges at time of first sample collection: 26-30 years, n=1; 31-35 years, n=1; 35-40 years,
 951 n=1; 51-55 years, n=1.

952 **Age ranges at time of first sample collection: <20 year old, n=1; 21-25 years, n=1; 35-40
 953 years, n=1; 61-65 years, n=2, 66-70 years, n=2.

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955 **Extended Data Table 2: Healthy Longitudinal Patient Clinical Details**

ID	Duration	Autoimmunity	Cancer	Immunomodulation
Patient 1	63 months	None	None	None

Patient 2	25 months	None	Basal cell carcinoma	None
Patient 3	75 months	None	None	None
Patient 4	92 months	None	None	None
Patient 5	61 months	Sjögren’s Disease	None	None
Patient 6	50 months	None	None	None
Patient 7	77 months	None	None	None

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Extended Data Table 3: IVIG Sample Details

ID	Sample Timing	Immunomodulation	Last IVIG Dose
Patient 1	Initial	None	Naïve
	6 weeks later	Prednisone/Imuran	3 weeks prior
Patient 2	Initial	None	Naïve
	14 weeks later	Prednisone	1 day prior
Patient 3	Initial	Prednisone	Naïve
	7 weeks later	Prednisone	2 days prior
Patient 4	Initial	None	Naïve
	21 weeks later	Prednisone	5 weeks prior

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Extended Data Table 4: Rituximab Sample Details

ID	Autoantibody	Sample	Immunomodulation	Rituximab
Patient 1	AchR			
		1	Prednisone	Naïve
		2	Prednisone	1.1
		3	Prednisone	3.9
		4	Prednisone	3.4
		5	Prednisone	6.1
Patient 2	MuSK	6	Prednisone	24.6
		1	Prednisone	Naïve
		2	Prednisone	2.3
		3	Prednisone	5.1
Patient 3	MuSK	4	Prednisone	4.8
		1	Prednisone	6.1
		2	Prednisone	5.1

		3	None	68.9
Patient 4	MuSK			
		1	None	8
		2	None	7.9
		3	None	24.7
Patient 5	MuSK			
		1	None	17.8
		2	None	1.3
		3	None	8.6
		4	None	13.9
		5	None	18.8
		6	None	21
		7	None	5.7
		8	None	1.6
Patient 6	MuSK			
		1	Prednisone	Naïve
		2	Prednisone, Imuran	1.7
		3	Prednisone, Imuran	4.6
		4	Prednisone, Imuran	4.7
		5	Prednisone, Imuran	8.5
Patient 7	AChR			
		1	Prednisone, Imuran	5.7
		2	Prednisone, Imuran	7
		3	None	24.1

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Extended Data Table 5. CAR-T Sample Details

CAR-T	Patient	Malignancy	Post-CAR-T Sample Timing (weeks)	Prior therapy (months pre-CAR-T)	Concurrent treatment (days prior to second sample)
BCMA					
	Patient 1	MM	51	IVIg, 6	IVIg, 117
	Patient 2	MM	27	IVIg, 4	IVIg, 56
	Patient 3	MM	30	None	None
	Patient 4	MM	61	None	IVIg, 68
	Patient 5	MM	29	BCMA, 3	IVIg, 112
	Patient 6	MM	29	Daratumumab, 3	IVIg, 117
	Patient 7	MM	30	Daratumumab, 5	IVIg, 47
	Patient 8	MM	22	None	None

	Patient 9	MM	22	None	None
CD19					
	Patient 1	Lymphoma	33	Polatuzumab, 1	None
	Patient 2	Lymphoma	28	Autologous HCT, 7	None
	Patient 3	Lymphoma	32	None	None
	Patient 4	Lymphoma	36	None	None
	Patient 5	Lymphoma	27	Ibrutinib, 1	IVIG, 121
	Patient 6	Lymphoma	30	None	None
	Patient 7	Lymphoma	29	None	IVIG, 59
	Patient 8	Lymphoma	26	None	None
	Patient 9	Lymphoma	30	None	IVIG, 28
	Patient 10	Lymphoma	35	Autologous HCT, 8	None
	Patient 11	Lymphoma	30	None	IVIG, 28
	Patient 12	Lymphoma	21	None	None
	Patient 13	Lymphoma	27	None	None
	Patient 14	Lymphoma	27	None	IVIG, 155

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Table Legend: MM=multiple myeloma; Sample timing refers to number of weeks between CAR-T infusion and the post-infusion sample being drawn; Prior therapy lists additional immunomodulatory therapies given in the year prior to initial sample collection, and the number of months between administration of the therapy and collection of the initial sample, excluding the pre-CAR-T conditioning chemotherapy which consists of cyclophosphamide and fludarabine for every patient; Concurrent treatment lists the number of days prior to the second sample being collected when treatment was administered, only IVIG is listed because no other B cell or antibody modulating therapies were given.

Extended Data Table 6: Multiplex Bead-based Protein Array Antigen Panel

Antigen	Antigen Vendor	Antigen Catalog #
IL-1A	Peprtech	200-01A
IL-2	Peprtech	200-02
IL-4	Peprtech	200-04
IL-6	Peprtech	200-06
IL-7	Prospec	CYT-214
IL-11	Peprtech	200-11
IL-15	Peprtech	200-15
IL-17A	Peprtech	200-17
IL-17F	Peprtech	200-25
IL-21	Peprtech	200-21
IL-22	Peprtech	200-22
IL-23	Peprtech	200-23
IL-31	Peprtech	200-31

Influenza A HA (H1)	Kim Lab - Stanford	N/A
Haemophilus Influenzae B	Creative Diagnostics	DAGHIB002
Influenza A HA (H3)	Kim Lab - Stanford	N/A
RSV-F Protein	Sino	11049-V08B
EBV p18	Prospec	EBV-273
EBV EA-D	MyBioSource	MBS319448
HAV VP1	Creative Diagnostics	DAG2415
EBV EBNA-1	Abcam	ab138345
EBV EA	Prospec	EBV-272
HBsAg	MyBioSource	MBS142509
Varicella Zoster Glycoprotein E	MyBioSource	MBS553197
EV71 VP1 Capsid Protein	BioMART	Custom Request
Mumps Nucleoprotein	Prospec	MMP-001
Rubella E1	Prospec	RUB-291
Measles Nucleoprotein	MyBioSource	MBS319759
AcmNPV gp64	Sino Biological	40496-V08B
Rhinovirus VP1	MyBioSource	MBS1220686
Parainfluenza Hemagglutinin-neuraminidase	Sino Biological	40629-V07B
HSV glycoprotein G	MyBioSource	MBS145445
CMV gB	Prospec	CMV-211
HMPV Glycoprotein G	Sino Biological	40791-V08H
Anti-Human IgG Fc fragment Specific	Jackson	109-005-008
Anti-Human IgG F(ab') fragment specific	Jackson	109-005-006
Anti-Human IgG (H+L)	Jackson	109-005-003
Human IgG from serum	Sigma	I4506
PDC-E2	Diarect	A17901
TPO	Diarect	A12101
TG	Diarect	A12201
CXCL10	Peprtech	300-12
CXCL9	Peprtech	300-26

MIP-1alpha (CCL3)	Peprotech	300-08
CXCL13	Peprotech	300-47
CXCL16	Peprotech	300-55
CXCL5	Peprotech	300-22
CCL21	Peprotech	300-35A
CXCL8	Peprotech	200-08
CCL22	Peprotech	300-36
CCL19	Peprotech	300-29B
CCL25	Peprotech	300-45
CXCL17	Prospec	CHM-024
IFN-alpha1	Prospec	CYT-291
IFN-alpha2	R&D	11101-2
IFN-alpha6	Origene	TP760329
IFN-alpha7	Prospec	CYT-196
IFN-alpha8	Sino	10347-H08H
IFN-alpha10	Sino	10349-H08H
IFN-beta	Peprotech	300-02BC
IFN-epsilon	R&D	9667-ME-025/CF
IFN-omega	Peprotech	300-02J
IFN-gamma	Peprotech	300-02
IFN-lambda1	Peprotech	300-02L
IFN-lambda2	Peprotech	300-02K
C3	Complement Tech	A113
C1q	EMD	204876
FGF7	Peprotech	100-19
GM-CSF	Peprotech	300-03
LIF	Peprotech	300-05
PDGFBB	Peprotech	100-14B
VEGFB	Peprotech	100-20B
TNF-alpha	Peprotech	300-01A
IL1RA	Peprotech	200-01RA
ACE2	Sino biological	10108-H05H
Proteinase 3	Diarect	A18601
BPI	Sigma	SRP6307
IFN-lambda3	R&D	5259-IL-025/CF
IL-33	Peprotech	200-33
CCL26	Peprotech	300-48

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Extended Data Table 7: Additional BCMA Patient Demographics

Patient	Cohort	Age Range (years)	Sex	Race/Ethnicity
Patient 10	BCMA	51-55	Female	White
Patient 11	BCMA	61-65	Male	White
Patient 12	BCMA	51-55	Male	Not reported
Patient 13	BCMA	56-60	Male	White

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Extended Data Table 8: Additional BCMA Sample Details

CAR-T	Patient	Malignancy	Post-CAR-T Sample Timing (weeks)	Prior therapy (months pre-CAR-T)	Concurrent treatment (days prior to second sample)
BCMA					
	Patient 10	MM	27	None	None
	Patient 11	MM	24	None	None
	Patient 12	MM	27	None	None
	Patient 13	MM	22	Elotuzumab, 4	None

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Table Legend: MM=multiple myeloma; Sample timing refers to number of weeks between CAR-T infusion and the post-infusion sample being drawn; Prior therapy lists additional immunomodulatory therapies given in the year prior to initial sample collection, and the number of months between administration of the therapy and collection of the initial sample, excluding the pre-CAR-T conditioning chemotherapy which consists of cyclophosphamide and fludarabine for every patient.