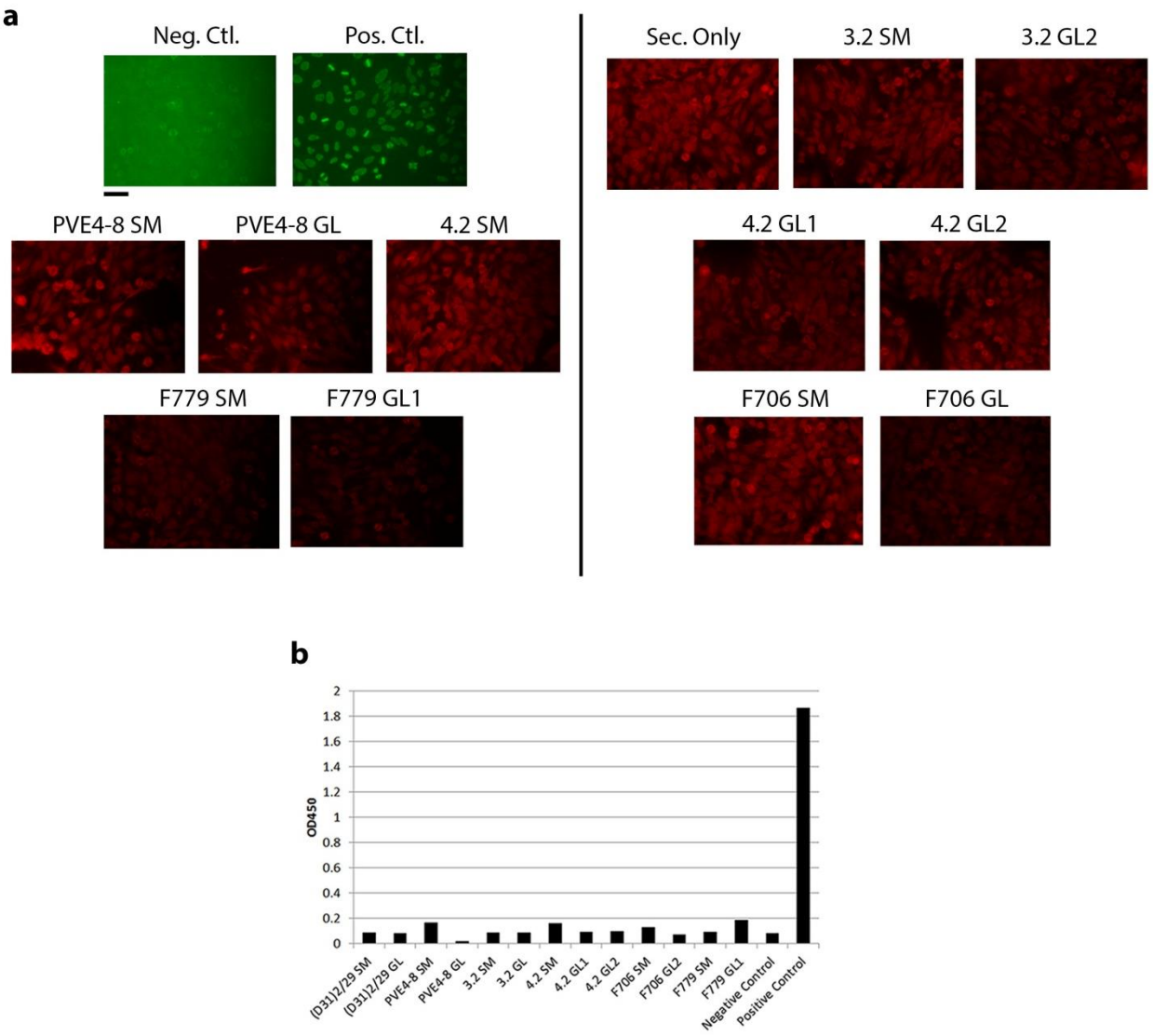
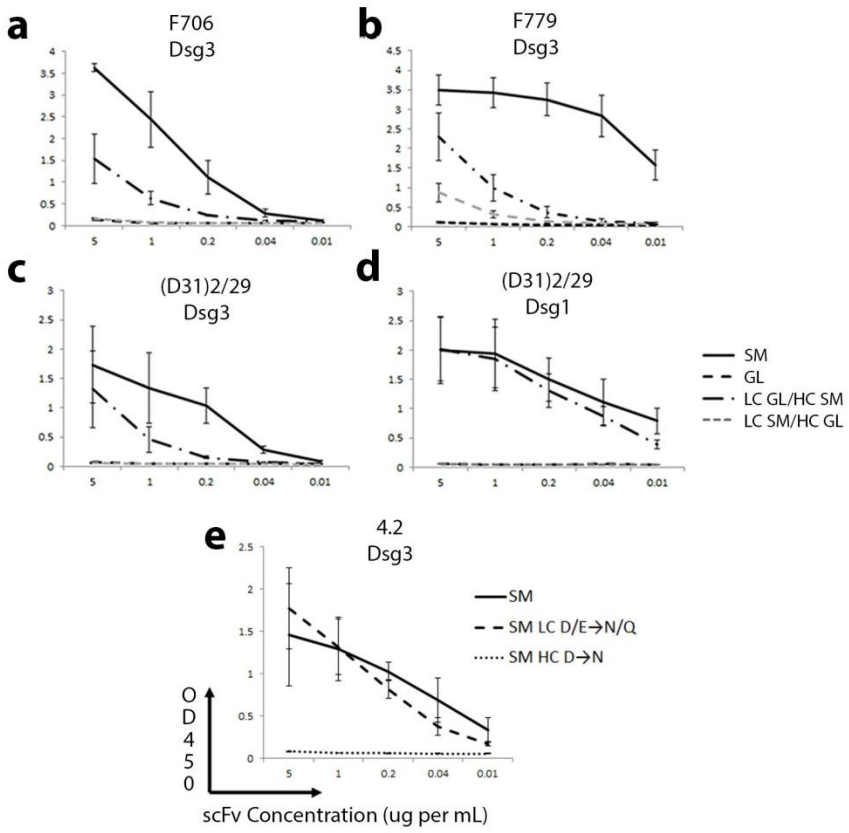
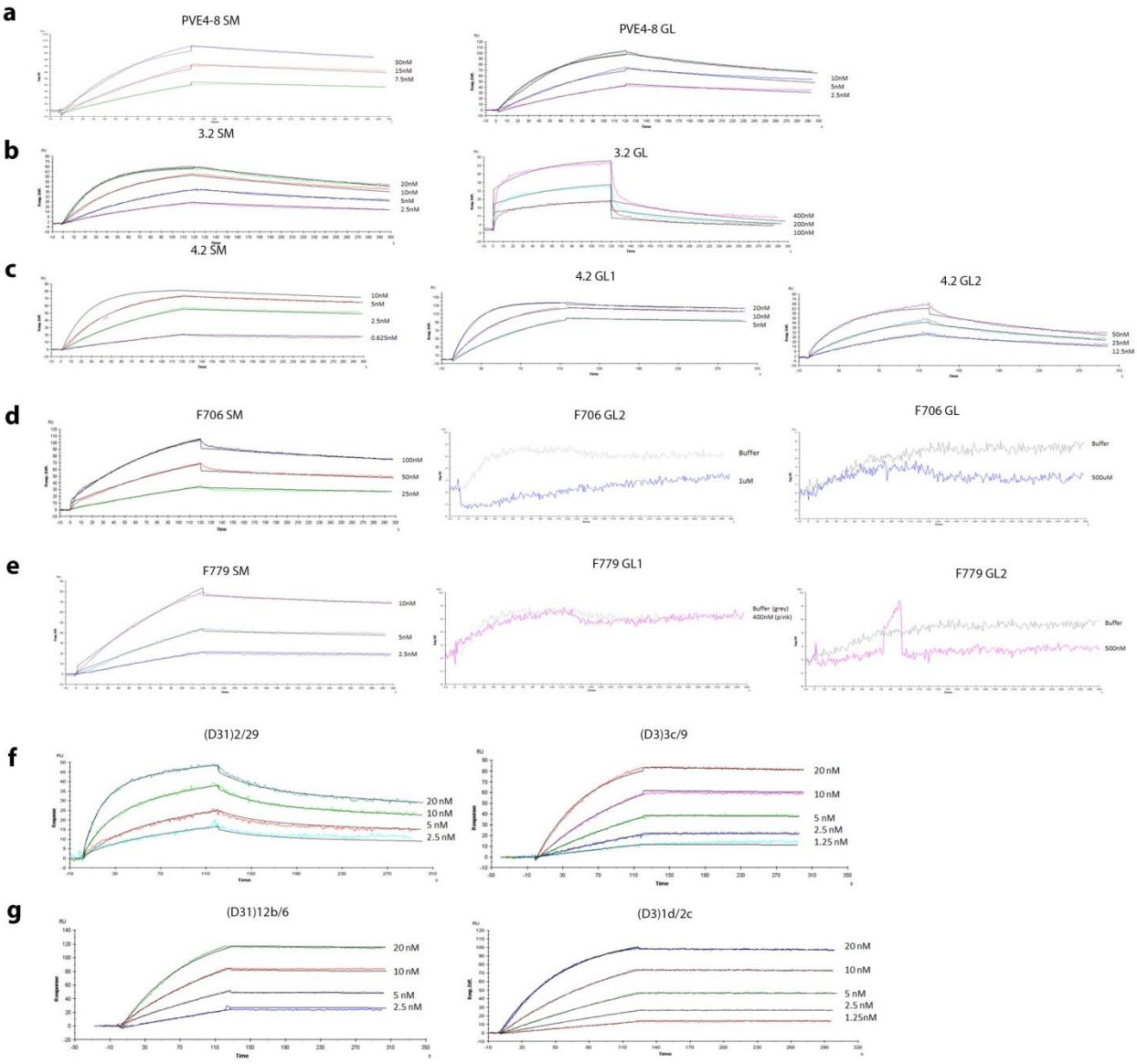


Supplementary Figure 1. PV mAbs do not demonstrate Hep 2 polyreactivity.

SM and GL mAbs were tested for reactivity to Hep2 cells by immunofluorescence **(a)** and ELISA **(b)** at a concentration of 2-5 ug/mL. Negative (Neg.) and positive (Pos.) controls (Ctl.) from commercial kits are displayed and were developed with anti- human immunoglobulin conjugated antibodies. Sec. only indicates staining of cells with anti-hemagglutinin conjugated antibody only. Scale bar, 20 μM. Data are representative of 1-2 experiments tested at multiple concentrations.



Supplementary Figure 2. Determinants of Dsg3 autoreactivity are predominantly encoded within the heavy chain. **(a)** F706 somatically mutated (SM) binds Dsg3 while F706 germline-reverted (GL) does not. Reversion of mutations in only the heavy chain (F706 LC SM/HC GL) does not bind Dsg3, whereas reversion of mutations in the light chain maintains Dsg3 autoreactivity (F706 LC GL/HC SM). **(b)** Reversion of somatic mutations in F779 indicates that the loss of mutations in the heavy chain affects relative binding affinity more than loss of mutations in the light chain. (D31)2/29, a previously characterized pathogenic scFv mAb, retains binding to **(c)** Dsg3 and **(d)** Dsg1 if mutations in the light chain, but not heavy chain, are reverted to their germline sequences. **(e)** Mutation of only acidic amino acid residues in the 4.2 HC CDRs (4.2 SM HC D→N) abolished Dsg3 binding by ELISA, whereas mutation of acidic amino acid residues in the LC CDRs did not significantly affect Dsg3 binding (4.2 SM LC D/E→N/Q). Errors bars indicate s.e.m. Data are representative of 3 experiments.



Supplementary Figure 3. Surface plasmon resonance curves for anti-Dsg3 mAbs **(a-g).** For most mAbs, the kinetic data conformed to a 1:1 Langmuir binding model. (D31)2/29 only fit a conformational change model. Some mAbs (such as 3.2GL) that have high chi-squared values or significant bulk change added to the fit may interact with Dsg3 in a more complex fashion than the 1:1 model shown (conformational change or heterogeneous ligand models did not improve the fit). Data are representative of 1-2 independent experiments, each testing multiple antibody concentrations.

Supplementary Figure 4. VH1-46 is the closest human homolog of mouse VH1-53, used by the pathogenic mouse anti-Dsg3 mAb AK23. Homology between human VH1-46 and murine VH1-53 germline sequences and AK23 mAb sequence is shown; red=negatively charged, green=positively charged, blue=hydrophobic, black=hydrophilic. CDR1 sequence is boxed in blue; CDR2 sequence is boxed in green. Asterisks indicate replacement mutations occurring in AK23 relative to the VH1-53 germline sequence; only one replacement mutation is observed in the CDRs.

Supplementary Table 1. CDR sequences of anti-Dsg3 somatically mutated (SM), germline-reverted (GL), and point-mutated mAbs.

Clone	CDR1 LC	CDR2 LC	CDR3 LC	VL gene	JL gene	CDR1 HC	CDR2 HC	CDR3 HC	VH gene	DH gene	JH gene
PVE4-8 SM	SSDVGGYNY	EVN	SSYAGSNNLV			GYTFTAYY	INPSGGIA	ARDRQGFDLDV			
PVE4-8 GL	SSDVGGYNY	EVS	SSYAGSNNLV	IGLV2-8*01	IGLJ2*01	GYTFTSYY	INPSGGST	ARDRQGFDLDV	IGHV1-46*01	IGHD3-22*01	IGHJ6*02
PVE4-8 GL HC D→N	SSDVGGYNY	EVS	SSYAGSNNLV			GYTFTSYY	INPSGGST	ARNRQGFNLNV			
3.2 SM	SSDIGRYNF	EIY	SSYVGNNDLV			GYTFTSYY	INPSGGIA	ARDLGGFDFDY			
3.2 GL	SSDVGGYNY	EVS	SSYVGNNDLV	IGLV2-8*01	IGLJ7*01	GYTFTSYY	INPSGGST	ARDLGGFDFDY	IGHV1-46*01	IGHD5-12*01	IGHJ4*02
4.2 SM	SSDVGGYNY	EVS	SSYAGSNNWV			GYIFTSHY	INPSGGKT	ARDQSLGMDV			
4.2 GL1	SSDVGGYNY	EVS	SSYTSSNNWV	IGLV2-14*01	IGLJ3*02	GYTFTSYY	INPSGGST	ARDQRLGMDV	IGHV1-46*01	IGHD6-25*01	IGHJ6*02
4.2 GL1 HC D→N	SSDVGGYNY	EVS	SSYTSSNNWV			GYTFTSYY	INPSGGST	ARNQRLGMNV			
4.2 GL2	SSDVGGYNY	EVS	SSYTSSNNWV	IGLV2-14*01	IGLJ3*02	GYTFTSYY	INPSGGST	ARDHSLGMDV	IGHV1-46*01	IGHD3-22*01	IGHJ6*02
4.2 GL2 HC D→N	SSDVGGYNY	EVS	SSYTSSNNWV			GYTFTSYY	INPSGGST	ARNHSLGMNV			
4.2 SM LC D/E → N/Q	SSNVGGYNY	QVS	SSYAGSNNWV			GYIFTSHY	INPSGGKT	ARDQSLGMDV			
4.2 SM HC D → Q	SSDVGGYNY	EVS	SSYAGSNNWV			GYIFTSHY	INPSGGKT	ARNQSLGMNV			
F706 SM	ETLVHSDGNTY	KIS	TQSTDFPWT			GYTFTSYY	IDSRGGST	ARGVGTLDH			
F706 GL2	QSLVHSDGNTY	KIS	TQSTDFPWT	IGKV2-24*01	IGLJ1*01	GYTFTSYY	INPSGGST	ARGVGTLDH	IGHV1-46*01	IGHD2-21*02	IGHJ4*02
F706 GL2 + D/E	ESLVHSDGNTY	KIS	TQSTDFPWT			GYTFTSYY	IDPSGGST	ARGVGTLDH			
F706 GL	QSLVHSDGNTY	KIS	MQATQFPWT			GYTFTSYY	INPSGGST	ARVVVTLDY			
F779 SM	ETLVHSDGNTY	KIS	MQATEFPYT			GNTFTTYS	IDPSGGST	ARSIESISGRTLGY			
F779 GL1	QSLVHSDGNTY	KIS	MQATEFPYT	IGKV2-24*01	IGLJ2*01	GYTFTSYY	INPSGGST	ARSIESISGRTLGY	IGHV1-46*01	IGHD6-19*01	IGHJ4*02
F779 GL1 + D/E	ESLVHSDGNTY	KIS	MQATEFPYT			GYTFTSYY	IDPSGGST	ARSIESISGRTLGY			
F779 SM D/E → N/Q	QTLVHSDGNTY	KIS	MQATEFPYT			GNTFTTYS	INPSGGST	ARSIESISGRTLGY			
F779 GL2	QSLVHSDGNTY	KIS	MQATQFPYT			GYTFTSYY	INPSGGST	ARSIEYSSGWTLGY			
F779 GL2 + D/E	ESLVHSDGNTY	KIS	MQATEFPYT			GYTFTSYY	IDPSGGST	ARSIEYSSGWTLGY			
(D3)1d/2c SM	SSNIAGNT	YND	ATWDEDVNGWV			GGTFDKYA	IIPMLGAP	ARDKAAYYESGYYYIGDF			
(D3)1d/2c GL	SSNIGSNT	SNN	AAWDDSLNGWV	IGLV1-44*01	IGLJ3*02	GGTFSSYA	IIPIFGTA	ARDKAAYYESGYYYYFDY	IGHV1-69*06	IGHD3-22*01	IGHJ4*02
(D31)2/29 SM	KLGDKY	QDR	QAWDSSTAV			GGTFGNYA	IIPTLDLL	ARGGDYSGWYNFDY			
(D31)2/29 GL	KLGDKY	QDS	QAWDSSTAV	IGLV3-1*01	IGLJ3*02	GGTFSSYA	IIPILGIA	ARGGDYSGWYNFDY	IGHV1-69*06/09	IGHD6-19*01	IGHJ4*02
(D3)3c/9 SM	SSYVGIFNL	EGD	YSYVAGSDLWV			GLPFNSYW	INQDGNEK	ASGGVVDFDH			
(D3)3c/9 GL	SSDVGSYNL	EGS	CSYAGSSTLWV	IGLV2-23*01/03	IGLJ3*02	GFTFSSYW	IKQDGSEK	ARDGVDYFDY	IGHV3-07*03	IGHD2-15*01	IGHJ4*02
(D31)12b/6 SM	SSHIGSNY	SND	AAWDDGQGGV			GGSISSNHW	IYHNGST	ARGWHRTGFRGYPSHWYFDL			
(D31)12b/6 LC SM/HC GL	SSHIGSNY	SND	AAWDDGQGGV			GGSISSSNW	IYHSGST	AREWHRTGYSGYPSYWYFDL	IGHV4-04*02	IGHD5-12*01	IGHJ2*01
VH5a SM	SSNIRNNY	DDN	GTWDSSQSVGV			GPNFSNYW	IDPFDGYT	ARINYYDGSGHHSDADYM			
VH5a LC GL/HC SM	SSNIGNNY	DNN	GTWDSSLSAGV	IGLV1-51*01	IGLJ3*02	GPNFSNYW	IDPFDGYT	ARINYYDGSGHHSDADYM			
VH5a LC SM/HC GL	SSNIRNNY	DDN	GTWDSSQSVGV			GYSFTSYW	IDPSDSYT	ARINYYDSSGYYSDAFDI	IGHV5a*01	IGHD3-22*01	IGHJ3*02

Supplementary Table 2. 3.2 epitope specificity for EC1 and EC3 is predominantly determined by the heavy chain. Epitope mapping of a second mAb that utilizes the identical heavy chain as 3.2, but paired with a different light chain. Data are representative of 2 independent experiments.

	CDR1	CDR2	CDR3	V gene	J gene	EC1	EC2	EC3	EC4	EC5
3.2 LC 1	SSDIGRYNF	EIY	SSYVGNNDLV	IGLV2-8*01	IGLJ7*01	X		X		
3.2 LC 2	SSDVGRYDL	EVT	CSYAGRYTLL	IGLV2-23*01	IGLJ3*01	X		X		

Supplementary Table 3. BASELINe test of significance for antigen-driven selection. Negative and positive selection are indicated by – and + symbols before the p values. BASELINe sigma value is a measure of the strength of negative or positive selection and allows comparison of selection strength between different Abs. VH1-46 mAbs do not show statistically significant evidence of positive antigen-driven selection in the CDRs, although two VH1-46 mAbs demonstrate significant evidence of negative selection against replacement mutations in the FWRs ($p<0.05$, highlighted in dark gray). Multiple other clonal lineages also demonstrate statistically significant evidence of negative selection against replacement mutations in the FWRs. VH1-69 clonal lineage 1 demonstrates statistically significant evidence of positive antigen-driven selection in the CDRs. Increasing sigma values within the VH1-69 clonal lineage 2 CDRs, with a trend toward significance ($0.05<p<0.1$, highlighted in light gray), suggest the presence of increasing antigen-driven positive selection pressure on these clones. Statistical significance is determined by a binomial test.

ID	Observed Mutations				Total Mutations	Expected Mutation Frequencies				Expected Mutations				BASELINEe Selection Analysis			
	CDR		FWR			CDR		FWR		CDR		FWR		CDR		FWR	
	R	S	R	S		R	S	R	S	R	S	R	S	Σ	P-Value	Σ	P-Value
IGHV1-46*01 Clonal Lineage 1																	
3.2	2	4	4	1	11	0.183	0.056	0.579	0.182	2.013	0.616	6.369	2.002	-0.57	-0.234	-1.1	-0.0429
IGHV1-46*01 Clonal Lineage 2																	
4.2	4	2	9	1	16	0.183	0.056	0.579	0.182	2.928	0.896	9.264	2.912	0.524	0.233	0.129	0.435
IGHV1-46*01/03 Clonal Lineage 3																	
F706	5	2	5	8	20	0.172	0.053	0.584	0.191	3.44	1.06	11.68	3.82	-0.307	-0.284	-1.53	-0.00132
IGHV1-46*01/03 Clonal Lineage 4																	
F779	4	0	7	5	16	0.172	0.053	0.584	0.191	2.752	0.848	9.344	3.056	0.14	0.408	-0.56	-0.158
IGHV1-46*01 Clonal Lineage 5																	
PVE4-8	4	2	6	3	15	0.183	0.056	0.579	0.182	2.745	0.84	8.685	2.73	0.0538	0.462	-0.719	-0.108
IGHV1-69*06 Clonal Lineage 1																	
(D31)2/29	10	1	8	4	23	0.178	0.059	0.564	0.2	4.094	1.357	12.972	4.6	1.03	0.0224	-0.338	-0.263
IGHV1-69*06 Clonal Lineage 2																	
(D3)1i/4d	9	1	9	7	26	0.178	0.059	0.564	0.2	4.628	1.534	14.664	5.2	0.485	0.151	-0.668	-0.0793
(D3)1d/2c	8	1	9	4	22	0.178	0.059	0.564	0.2	3.916	1.298	12.408	4.4	0.814	0.0659	-0.226	-0.328
(D3)1g/2e	9	1	8	7	25	0.178	0.059	0.564	0.2	4.45	1.475	14.1	5	0.485	0.151	-0.78	-0.0545
(D3)1b/3a	8	1	8	5	22	0.178	0.059	0.564	0.2	3.916	1.298	12.408	4.4	0.644	0.107	-0.508	-0.164
(D3)1c/2a	9	1	8	5	23	0.178	0.059	0.564	0.2	4.094	1.357	12.972	4.6	0.756	0.0666	-0.508	-0.164
(D3)1h/2b	8	1	9	4	22	0.178	0.059	0.564	0.2	3.916	1.298	12.408	4.4	0.814	0.0659	-0.226	-0.328
(D3)1f/4c	8	1	8	6	23	0.178	0.059	0.564	0.2	4.094	1.357	12.972	4.6	0.499	0.159	-0.653	-0.0965
(D3)1e/2d	8	1	6	6	21	0.178	0.059	0.564	0.2	3.738	1.239	11.844	4.2	0.499	0.159	-0.925	-0.0423
(D3)1a/4a	9	1	8	5	23	0.178	0.059	0.564	0.2	4.094	1.357	12.972	4.6	0.756	0.0666	-0.508	-0.164
IGHV3-7*03 Clonal Lineage																	
(D3)3c/9	5	2	8	7	22	0.213	0.051	0.55	0.186	4.686	1.122	12.1	4.092	-0.45	-0.201	-0.955	-0.0219
(D3)3a/9	5	2	7	6	20	0.213	0.051	0.55	0.186	4.26	1.02	11	3.72	-0.338	-0.271	-0.969	-0.0268
(D3)3b/8	5	2	6	5	18	0.213	0.051	0.55	0.186	3.834	0.918	9.9	3.348	-0.212	-0.356	-0.988	-0.0329
IGHV4-4*02 Clonal Lineage																	
(D31)12b/6	3	1	9	4	17	0.235	0.042	0.52	0.203	3.995	0.714	8.84	3.451	-0.431	-0.268	-0.201	-0.345
(D31)12a/5	5	0	11	4	20	0.235	0.042	0.52	0.203	4.7	0.84	10.4	4.06	0.246	0.352	0.195	0.375
(D31)12c/7	5	0	9	3	17	0.235	0.042	0.52	0.203	3.995	0.714	8.84	3.451	0.509	0.231	0.264	0.349
IGHV5-a*01 Clonal Lineage																	
VH5a	9	1	18	9	37	0.197	0.058	0.592	0.154	7.289	2.146	21.904	5.698	-0.0308	-0.474	-0.463	-0.116
IGHV3-30*04 Clonal Lineage																	
(D3)4/30	2	0	2	4	8	0.227	0.05	0.534	0.188	1.816	0.4	4.272	1.504	-0.578	-0.239	-1.43	-0.0334

Supplementary Table 4. VH1-46 single nucleotide variants (SNVs). 35 previously reported VH1-46 SNVs (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes) are shown, along with observed minor allele frequency, the majority codon sequence (observed in the ancestral VH1-46 allele *01), the variant sequence, and whether the nucleotide variant is silent or non-silent in regard to the amino acid sequence. CDR1 residues are boxed in blue; CDR2 residues are boxed in green. None of the CDR1-2 SNVs were identified in our 4 PV patients, suggesting that VH1-46 polymorphisms likely did not contribute to PV susceptibility in these patients.

SNV reference	minor allele frequency	majority codon	variant	silent	non-silent	AA change
rs374571144	-	GTG	GTT	x		
rs370958214	-	CAG	CAA	x		
rs184383009	0.001	CTG	TTG	x		
rs191958250	-	GTG	ATG		x	V->M
rs375773401	-	GGG	GCG		x	G->A
rs61732934	-	GCT	GGT/GTT		x	A->G/A->V
rs188566927	0.001	AAG	CAG		x	K->Q
rs370633142	-	AAG	AAC		x	K->N
rs377180003	-	GGG	GGA	x		
rs372749973	-	GCC	ACC		x	A->T
rs61747196	0.002	GTT	ATT		x	V->I
rs61995748	0.002	AAG	ATG		x	K->M
rs192285778	-	GCA	TCA		x	A->S
rs187613260	0.0005	TAC	GAC		x	Y->D
rs377014204	-	TAC	TAT	x		
rs374579537	-	AGC	AGG		x	S->R
rs182132309	0.001	TAC	ATC		x	Y->I
rs369870124	-	TAT	TTT		x	Y->F
rs376685264	-	ATG	GTG		x	M->V
rs144704015	0.002	ATG	ATA		x	M->I
rs367562305	-	CCT	TCT		x	P->S
rs376171941	-	GAG	GAA	x		
rs371609422	-	CCT	CTT		x	P->L
rs190309173	-	GGT	AGT		x	G->S
rs185595166	0.002	AGC	ACC		x	S->T
rs181189514	-	ACA	GCA		x	T->G
rs368402773	-	TAC	AAC		x	Y->N
rs149338091	0.001	AAG	AGG		x	K->R
rs55801711	0.008	TTC	TTG		x	F->L
rs371133633	-	GGC	GAC		x	G->D
rs368616898	-	ACC	ATC		x	T->I
rs147211698	0.003	ACG	ATG		x	T->M
rs370428883	-	ACG	ACA	x		
rs376062317	-	GTG	ATG		x	V->M
rs11848941	0.156	GCG	GCA/GCT	x		

Supplementary Methods

3’ Light Chain scFv Long Linker Primer Sequences

3.2
5’-
CTCTCTAGAGGAACCACCGCCGAGGACGGTCAGCTGGGTGCCTCCGCCAAAGACCAAATCGT
TGTTGCCCACATAGGAACTGCAGTAATAATCAGCCTCATC

4.2
5’-
CTCTCTAGAGGAACCACCACCTAGGACGGTCAGCTTGGTCCCTCCGCCGAACACCCAATTGTT
GCTGCCTGCATATGAGCTGCAGTAATAATCAGCCTC

PVE4-8
5’-
CTCTCTAGAGGAACCACCACCTAGGACGGTCACCTTGGTGCCTCCGCCGAATACGAGATTGTT
GCTGCCTGCATATGAGCTGCAGTAATAATCAGCCTC

F706
5’-
CTCTCTAGAGGAACCACCGCCTTTGATTTCCACCTTGGTCCCTTGGCCGAACGTCCAAGGGAA
ATCTGTAGATTGCGTGCAGTAATAAACCCCGACATC

F779
5’-
CTCTCTAGAGGAACCACCGCCTTTGATCTCCAGCTTGGTCCCCTGGCCAAAAGTGTACGGAAA
TTCTGTAGCTTGCATGCAGTAATAAACCCCGACATC

(D31)2/29
CTCTCTAGAGGAACCACCGCCTAGGACGGTCACCTTGGTCCCTCCGCCGAACACCGCAGTGCT
GCTGTCCCACGCCTGACAGTAATAGTCAGCCTCATC