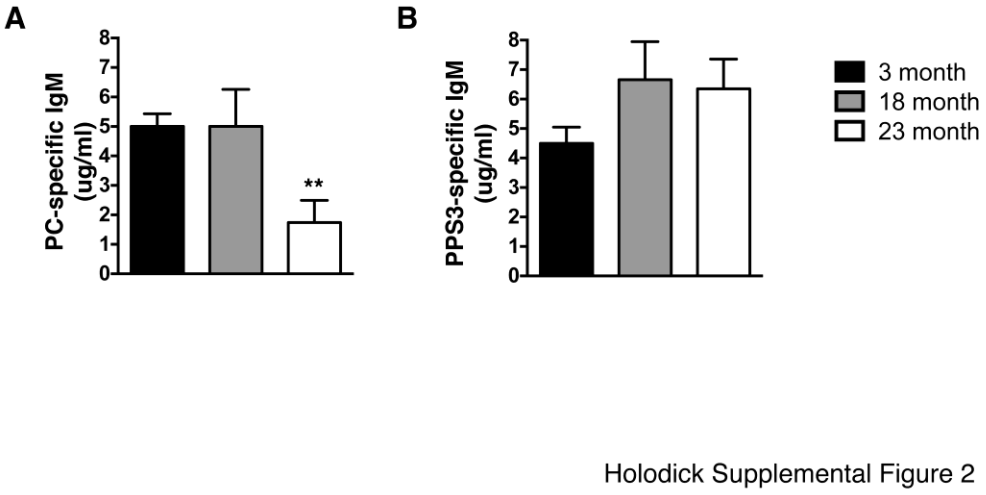
Supplemental Data

Sample	IgM (ug/ml)	IgG (ug/ml)	IgM (ug)	IgG (ug)	Day of Death
3mo #1	231	51	70	15	D8
3mo #2	255	14	70	4	D14
3mo #3	270	0	70	0	D14
3mo #4	273	0	70	0	D8
3mo #5	310	0	70	0	D4
3mo #6	355	0	70	0	D6
3mo #7	421	0	70	0	D5
3mo #8	375	0	70	0	D5
3mo #9	388	0	70	0	D5
3mo #10	402	0	70	0	D6
3mo #11	348	38	70	0	D7
3mo #12	486	0	70	0	D14
3mo #13	466	0	70	0	D4
3mo #14	326	0	70	0	D14
3mo #15	332	0	70	0	D4
3mo #16	386	0	70	0	D14

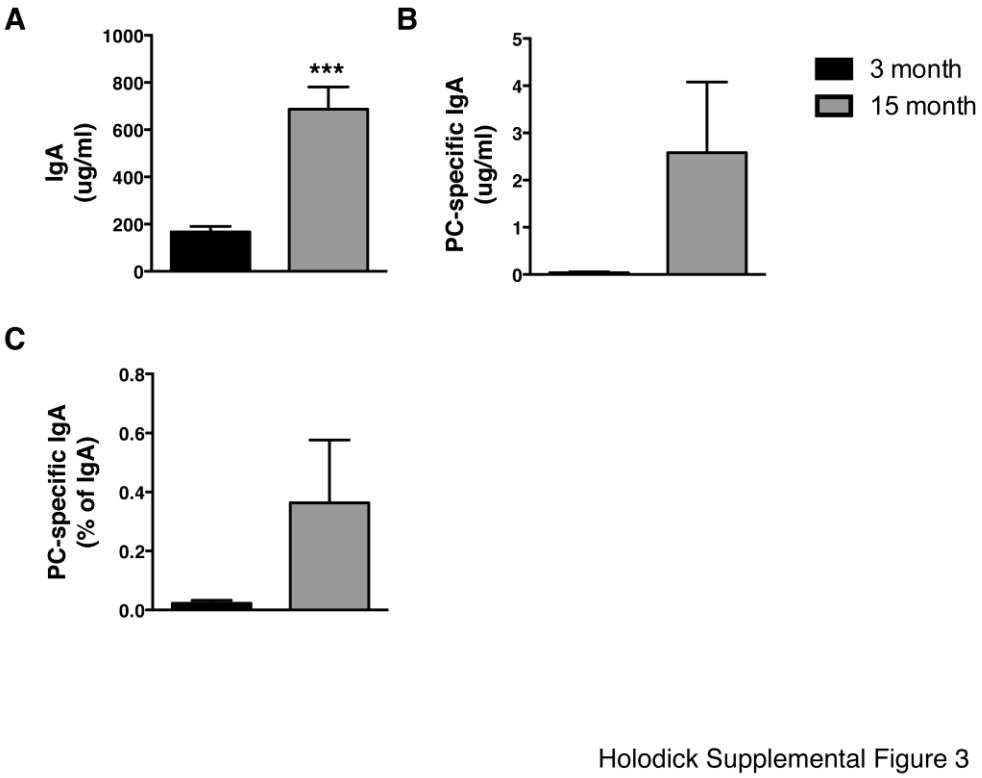
Sample	IgM (ug/ml)	IgG (ug/ml)	IgM (ug)	IgG (ug)	Day of Death
18mo #1	292	0	70	0	D4
18mo #2	60	0	60	0	D5
18mo #3	387	0	70	0	D5
18mo #4	397	0	70	0	D4
18mo #5	639	0	70	0	D4
23mo #1	195	22	70	8	D6
23mo #2	366	668	70	128	D9
23mo #3	350	0	70	0	D3
23mo #4	225	496	70	154	D5
23mo #5	287	290	70	71	D4
23mo #6	155	0	70	0	D5
24mo #1	181	0	70	0	D4
24mo #2	388	43	70	8	D3
24mo #3	178	0	70	0	D3
24mo #4	399	411	70	72	D3
24mo #5	231	37	70	11	D4

Holodick Suppl Figure 1

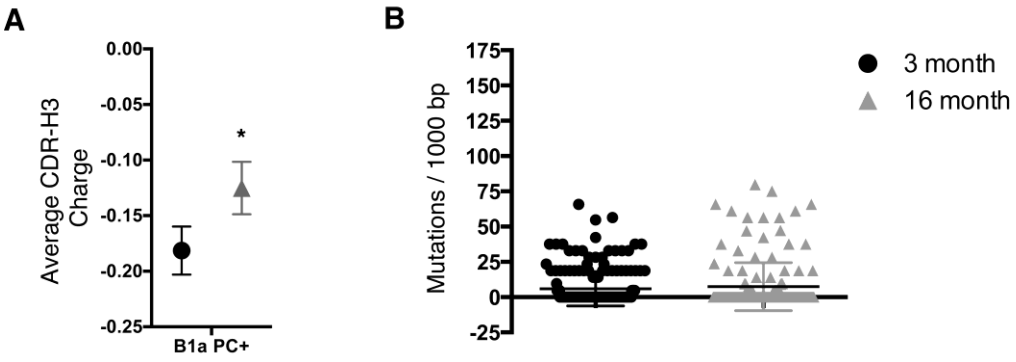
Supplemental Figure 1, Related to Figure 1: Ig characteristics from serum samples and corresponding day of death for recipients used in the pneumococcal infection experiment. All serum samples were obtained from 3, 18, or 23-24-month old male BALB/c-ByJ mice at time of euthanasia. The amount of IgM and IgG post protein G depletion is shown (μg/ml). The amount (μg) of serum IgM and remaining IgG (if present) injected into each animal is shown. The day post injection the animal succumbed to infection is also indicated.



Supplemental Figure 2, Related to Figure 2: Serum levels of anti-PC and anti-PPS3 specific IgM from aged and young mice. Serum was collected from 3 (n=9), 18 (n=5), 23-month (n=6) old BALB/c-ByJ mice at time of euthanasia. Serum samples were analyzed for **(A)** PC-specific IgM, and **(B)** PPS3-specific IgM. Black bars represent 3-month old mice, grey bars represent 18-month old mice, and white bars represent 23-month old mice. Values are displayed as the mean (±SEM) of individual mouse serum samples. Statistics were performed using unpaired, two-tailed student's t-test. PC-specific IgM: 3-month vs. 23-month p=0.001.



Supplemental Figure 3: Serum levels of total IgA and anti-PC specific IgA from aged and young mice. Serum was collected from 3 (n=5) and 15-month (n=3) old BALB/c-ByJ mice at time of euthanasia. Serum samples were analyzed for **(A)** total IgA, and **(B)** PC-specific IgA. **(C)** The total amount of PC-specific IgA was normalized as a percent of total IgA in each sample. Black bars represent 3-month old mice and grey bars represent 15-month old mice. Values are displayed as the mean (±SEM) of individual mouse serum samples. Statistics were performed using unpaired, two-tailed student's t-test. Total IgA: 3-month vs. 15-month p=0.0004. PC-specific IgA: 3-month vs. 15-month p=0.06.



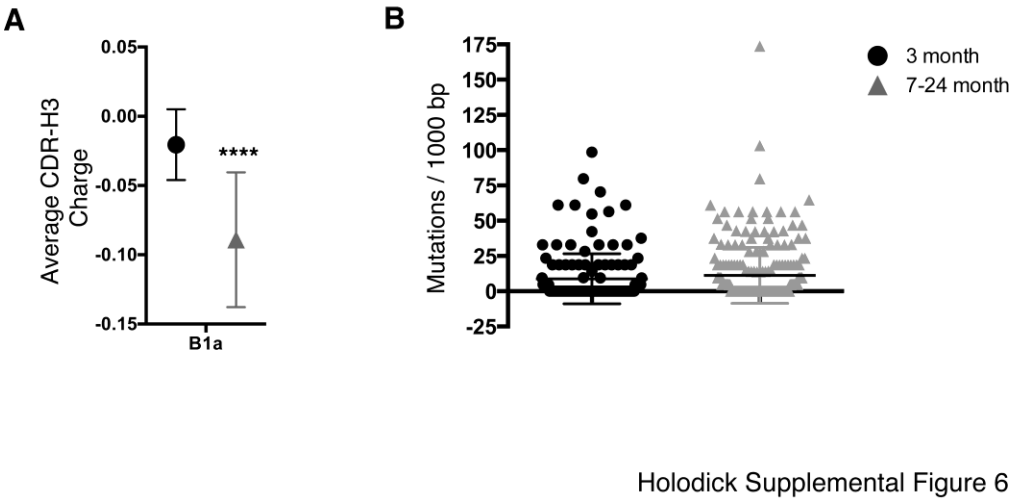
Holodick Supplemental Figure 4

Supplemental Figure 4, Related to Figure 3: Hydrophobicity and mutational analysis of PC+ peritoneal B-1a cell IgM from 3- and 16-month old mice. PC+ peritoneal B-1a cells were single cell sorted from 3 and 16-month old (as indicated) male BALB/c-ByJ mice. The VH region was amplified and sequenced as detailed in the Materials and Methods section. **(A)** The average charge of the CDR-H3 loop region of IgM from peritoneal B-1a cells from 3-month (black circles) and 16-month old mice (grey triangles) is shown (p=0.04, Mann-Whiteny test, two-tailed). **(B)** The average mutational rate (mutations per 1000 base pairs) is 5.9 in IgM from 3-month old mice and 7.4 in IgM from 16-month old mice. Values are displayed as the mean (±SEM).

Age of BALB/c-ByJ (months)	Number of N-Additions at both junctions in IgM from peritoneal B-1a cells (% of sequences)				Total number of sequences
	0 at both	0 at V-D ≥1 at D-J	≥1 at both	0 at D-J ≥1 at V-D	
3	46	8	23	22	157
7-12	27	21	35	18	197
18-24	32	14	34	20	76
7-24	28	19	34	18	273

Holodick Supplemental Figure 5

Supplemental Figure 5, Related to Figure 4: N-region addition analysis of IgM from peritoneal B-1a cells obtained from aged and young adult mice. Peritoneal B-1a cells were single cell sorted from 3, 7, 9, 10, 12, 18, 23, and 24-month old BALB/c-ByJ mice. The VH region was amplified and sequenced as detailed in the Materials and Methods section. The percent of sequences with zero N-additions at both junctions, one or more N-additions at both junctions, zero N-additions at V-D and 1 or more at D-J junctions, or zero N-additions at D-J and 1 or more at V-D junctions is shown. Chi-square analysis of the junctions (0 at both, 0 at V-D, ≥1 at both, and 0 at D-J) with 2 populations (4 x 2 Chi-square analysis): 3-month vs 7-12month $p<0.0001$; 3-month vs 18-24month $p=0.06$; 3-month vs 7-24month $p<0.0001$.



Supplemental Figure 6, Related to Figure 4: Hydrophobicity and mutational analysis of peritoneal B-1a cell IgM from 3- and 7-24-month old mice. B-1a cells were single cell sorted from 3, 7, 9, 10, 12, 18, 23, and 24-month old BALB/c-ByJ mice. The V_H region was amplified and sequenced as detailed in the Materials and Methods section. **(A)** The average charge of the CDR-H3 loop region of IgM from peritoneal B-1a cells from 3-month (black circles) and 7-24 month old mice (grey triangles) is shown ($p<0.0001$, Mann-Whiteny test, two-tailed). **(B)** The average mutational rate (mutations per 1000 base pairs) is 8.8 in IgM from 3-month old mice and 11.2 in IgM from 7-24-month old mice ($p=0.01$, Mann-Whiteny test, two-tailed). Values are displayed as the mean (±SEM).

Accession Numbers for 3-month peritoneal B-1a cells

KU927047	KU927096	KU927145	KU927194
KU927048	KU927097	KU927146	KU927195
KU927049	KU927098	KU927147	KU927196
KU927050	KU927099	KU927148	KU927197
KU927051	KU927100	KU927149	KU927198
KU927052	KU927101	KU927150	KU927199
KU927053	KU927102	KU927151	KU927200
KU927054	KU927103	KU927152	KU927201
KU927055	KU927104	KU927153	KU927202
KU927056	KU927105	KU927154	KU927203
KU927057	KU927106	KU927155	KU927204
KU927058	KU927107	KU927156	KU927205
KU927059	KU927108	KU927157	KU927206
KU927060	KU927109	KU927158	
KU927061	KU927110	KU927159	
KU927062	KU927111	KU927160	
KU927063	KU927112	KU927161	
KU927064	KU927113	KU927162	
KU927065	KU927114	KU927163	
KU927066	KU927115	KU927164	
KU927067	KU927116	KU927165	
KU927068	KU927117	KU927166	
KU927069	KU927118	KU927167	
KU927070	KU927119	KU927168	
KU927071	KU927120	KU927169	
KU927072	KU927121	KU927170	
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KU927075	KU927124	KU927173	
KU927076	KU927125	KU927174	
KU927077	KU927126	KU927175	
KU927078	KU927127	KU927176	
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Accession Numbers for 7-24-month peritoneal B-1a cells

KU927207	KU927256	KU927305	KU927354	KU927403	KU927452
KU927208	KU927257	KU927306	KU927355	KU927404	KU927453
KU927209	KU927258	KU927307	KU927356	KU927405	KU927454
KU927210	KU927259	KU927308	KU927357	KU927406	KU927455
KU927211	KU927260	KU927309	KU927358	KU927407	KU927456
KU927212	KU927261	KU927310	KU927359	KU927408	KU927457
KU927213	KU927262	KU927311	KU927360	KU927409	KU927458
KU927214	KU927263	KU927312	KU927361	KU927410	KU927459
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KU927216	KU927265	KU927314	KU927363	KU927412	KU927461
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KU927226	KU927275	KU927324	KU927373	KU927422	KU927471
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Supplemental Figure 7. GenBank accession numbers for B-1a Cell sequence data. Sequencing data was deposited into NCBI's Genbank (http://www.ncbi.nlm.nih.gov/genbank/). The accession numbers for the sequences are listed.