Supporting Information

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SI Materials and Methods

Flow Cytometry of Bone Marrow, Spleen, and Thymus. Cell suspensions from bone marrow, spleen, and thymus were made using standard methods. Cells were resuspended at 5×10^5 cells/mL in BD Pharmingen FACS staining buffer, blocked with anti-mouse CD16/32 (BD Pharmingen), stained with the appropriate mixture of antibodies (see below), and fixed with BD Cytofix all according to manufacturer instructions. Final cell pellets were resuspended in 0.5 mL staining buffer and analyzed using a BD FACS Calibur and BD CellQuest Pro software.

Antibody Mixtures for Bone Marrow.

i) Rat anti-mouse IgMb-FITC (Pharmingen; #553520), rat anti-mouse IgMa-PE (Pharmingen; #553517), and anti-mouse CD45R(B220)-APC (Pharmingen; #553092);
ii) Rat anti-mouse CD43(S7)-PE (Pharmingen; #553271) and anti-mouse CD45R(B220)-APC (Pharmingen; #553092);
iii) Rat anti-mouse CD24(HSA)-PE (Pharmingen; #553262) and anti-mouse CD45R(B220)-APC (Pharmingen; #553092); and
iv) Rat anti-mouse BP-1-PE (Pharmingen; #53735) and anti-mouse CD45R(B220)-APC (Pharmingen; #553092).

Antibody Mixtures for Spleen and Inguinal Lymph Node.

i) Rat anti-mouse IgMb-FITC (Pharmingen; #553520), rat anti-mouse IgMa-PE (Pharmingen; #553517), and rat anti-mouse CD45R(B220)-APC (Pharmingen; #553092);
ii) Rat anti-mouse Ig, λ_1, λ_2, λ_3 light chain-FITC (Pharmingen; #553434), rat anti-mouse Ig κ light chain-PE (Pharmingen; #559940), and rat anti-mouse CD45R(B220)-APC (Pharmingen; #553092);
iii) Rat anti-mouse Ly6 G/C-FITC (Pharmingen; #553127), rat anti-mouse CD49b(DX5)-PE (Pharmingen; #553858), and rat anti-mouse CD11b-APC (Pharmingen; #553312); and
iv) Rat anti-mouse CD4(L3T4)-FITC (Pharmingen; #553729), rat anti-mouse CD45R(B220)-PE (Pharmingen; #553090), and rat anti-mouse CD8a-APC (Pharmingen; #553035).

All monoclonal antibodies were prepared in a mass dilution/mixture. All antibodies were added to a final concentration of 0.5 mg/10^5 cells.

RT-PCR Analysis of Human Variable Regions from Splenocytes and Hybridoma Cells. Total RNA was extracted from 1×10^7 to 2×10^7 splenocytes or about 10^4–10^5 hybridoma cells using TRIzol (Invitrogen) or Qiagen RNeasy Mini Kit (Qiagen) and primed with mouse constant region-specific primers using the SuperScript III One-Step RT-PCR system (Invitrogen).

Reactions were carried out with 2–5 μL of RNA from each sample using the aforementioned 3′ constant specific primers paired with pooled leader primers for each family of human variable regions for both the heavy chain and kappa light chain, separately. Volumes of reagents and primers and RT-PCR/PCR conditions were performed according to the manufacturer's instructions. Primers sequences were based on multiple sources (1) (Ig primer sets; Novagen) and are listed below. Where appropriate, nested 2° PCR reactions were carried out with pooled family-specific framework primers and the same mouse 3′ Ig constant-specific primer used in the primary reaction. Aliquots (5 μL) from each reaction were analyzed by agarose electrophoresis, and reaction products were purified from agarose using the Montage Gel Extraction Kit (Millipore). Purified products were cloned using the TOPO TA Cloning System (Invitrogen) and transformed into DH10β *Escherichia coli* cells by electroporation. Individual clones were selected from each transformation reaction and grown in 2 mL Luria broth cultures with antibiotic selection overnight at 37 °C. Plasmid DNA was purified from bacterial cultures by a kit-based approach (Qiagen).

Sequence Analysis and Identification of V-D-J/V-J Recombination. Plasmid DNA IgH and IgK clones were sequenced with either T7 or M13 reverse primers on the ABI 3100 Genetic Analyzer (Applied Biosystems). Raw sequence data were imported into Sequencher (v4.5, Gene Codes). Each sequence was assembled into contigs and aligned to human Ig sequences using the IMGT V-Quest (2) search function. Sequences were compared with germ-line sequences for somatic hypermutation and recombination junction analysis.

Primer Sequences. Human IgH leader primers (5′-3′): VHL-1 (TCACCATGGACTGSACCTGGA), VHL-2 (CCATGGACACACTTTGYTCCAC), VHL-3 (TCACCATGGAGTTTGGGCTGAGC), VHL-4 (AGAACATGAAACAYCTGTGGTTCTT), VHL-5 (ATGGGGTCAACCGCCATCCT), VHL-6 (ACAATGTCTGTCTCCTTCCTCAT).

Human IgH framework primers (5′-3′): VHF-1 (CAGGTSCAGCTGGTRCAGTC), VHF-2 (CAGRTCACCTTGAAGGAGTC), VHF-3 (SAGGTGCAGCTGGTGGAGTC), VHF-4 (CAGGTGCAGCTGCAGGAGTC), VHF-5 (GARGTGCAGCTGGTGCAGTC), VHF-6 (CAGGTACAGCTGCAGCAGTC).

Human IgK leader primers (5′-3′): VKL-1 (CACAGCATGGACATGAGRGTCCYY), VKL-2 (CTTCTCACMATGAGGSTCCYT), VKL-3 (GASGGAACCATGGAARCCCCA), VKL-4 (CAGGGGCAGCAAGATGGTGTT), VKL-5 (CAGGGCCAAGGTTATGGGGTC), VKL-6 (CAAGGCAGGAAGATGTTGCCA), VKL-7 (CAGCTCTCAGAGATAGGGTCC).

Human IgK framework primers (5′-3′): VKF-1 (CATAAGATCTCGMCATCCRGWTGACCCAGT), VKF-2 (CACCAGATCTCGATRTTGTGATGACYCAG), VKF-3 (CACCAGATCTCGAAATWGTGWTGACRCAGTCT), VKF-4 (ACCAGATCTCGACATCGTGATGACCCAGT), VKF-5 (GGATAGATCTTACCAGGGCAGAAACGACAC), VKF-6 (GCCTAGATCTGTGAAATTGTGCTGACTCA), VKF-7 (CTCCAGATCTCAATGGGGACATTGTGCTGA).

IgH mouse constant primers (5′-3′): MuIgM3′-1 (CCCAAGCTTACGAGGGGGAAGACATTTGGGAA), MuIgG3′-2 (CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG), MuIgD3′-1 (CCCAAGCTTTGAGAGGAGGAACATGTCAGGTT), MuIgA3′-1 (CCCAAGCTTGTCAGTGGGTAGATGGTGGGATT), MuIgE3′-1 (CCCAAGCTTAAGGGGTAGAGCTGAGGGTTCCT).

IgK mouse constant primer (5′-3′): MuIgKVL3′-1 (CCCAAGCTTACTGGATGGTGGGAAGATGGA).

Immunizaton and Hybridoma Development. Seven *VelocImmune* mice and five WT littermates were immunized with the extracellular domain of the IL6 receptor using standard adjuvant, and the animals were boosted every three weeks a total of two to three times. Three days before fusion, two mice received a final prefusion boost of 5 μg protein via i.p. and i.v. injections. Splenocytes were harvested and fused to P3 × 63Ag8.653 myeloma cells in an electrofusion chamber according to manufacture suggested protocol (Cyto Pulse Sciences). Ten days after culture,

hybridomas were screened for antigen specificity using antigen-coated ELISA (3).

After the above-described mice received the third antigen boost and titers were measured by ELISA, two mice were selected from the WT and *VelocImmune* cohorts and splenocytes were isolated. The splenocytes were fused with P3 × 63Ag8.653 myeloma cells to form hybridomas and grown under hypoxanthine/aminopterin/thymidine selection. Media harvested from the antigen positive wells were used to determine the antibody affinity of binding to antigen using a solution competition ELISA. Of a total of 671 hybridomas, 236 were found to express antigen-specific antibodies, with 49 of the 236 hybridomas able to block IL6 from binding to the receptor in an in vitro bioassay.

Serum Titer Determination. To monitor animal antiantigen serum response, serum samples were collected 10 d after each boost, and the titers were determined using antigen-specific ELISA. Briefly, Nunc MaxiSorp 96-well plates were coated with 2 μg/mL receptor protein overnight at 4 °C and blocked with BSA (Sigma). Serum samples in a serial threefold dilutions were allowed to bind to the plates for 1 h at room temperature. The plates were then washed with PBS containing 0.05% Tween-20, and the bound IgGs were detected using HRP-conjugated goat anti-mouse Fcg (Jackson Immuno Research Laboratories) for total IgG titer, or biotin-labeled isotype-specific or light chain-specific polyclonal antibodies purchased from SouthernBiotech for isotype specific titers, respectively. If biotin antibody was used, following plate wash, HRP-conjugated streptavidin (Pierce) was added. All plates were developed using colorimetric substrates such as BD OptEIA (BD Biosciences Pharmingen). After the reaction was stopped with 1 M phosphoric acid, optical absorptions at 450 nm were recorded, and the data were analyzed using Prism software from GraphPad. Dilutions required to obtain twofold background signal were defined as titer.

Affinity Determination of Antibody Binding to Antigen in Solution. To determine antibody-binding affinity to the antigen, a high-throughput ELISA-based solution competition assay was designed. Briefly, the antibodies in conditioned medium were premixed with serial dilutions of antigen protein ranging from 0 to 10 mg/mL. The solutions of the antibody and antigen mixture were then incubated for 2–4 h at room temperature to reach binding equilibriums. The amounts of free antibody in the mixtures were then measured using a quantitative sandwich ELISA as follows. Ninety-six-well Maxisorb plates (VWR) were coated with 1 μg/mL of the IL6 receptor human Fc recombinant protein in PBS solution overnight at 4 °C, followed by BSA nonspecific blocking. The antibody-antigen mixture solutions were then transferred to these plates, followed by 1-h incubation. The plates were then washed with washing buffer, and the plate-bound antibodies were detected with an HRP-conjugated goat anti-mouse IgG polyclonal antibody reagent (Jackson ImmunoResearch Laboratory) and developed using colorimetric substrates such as BD OptEIA (BD Biosciences). After the reaction was stopped with 1 M phosphoric acid, optical absorptions at 450 nm were recorded, and the data were analyzed using Prism software from GraphPad. The dependency of the signals on the concentrations of antigen in solution was analyzed with a four-parameter fit analysis and reported as IC_{50}, which is the antigen concentration required to achieve 50% reduction of the signal from the antibody samples without the presence of antigen in solution.

1. Wang X, Stollar BD (2000) Human immunoglobulin variable region gene analysis by single cell RT-PCR. *J Immunol Methods* 244(1-2):217–225.
2. Brochet X, Lefranc MP, Giudicelli V (2008) IMGT/V-QUEST: The highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. *Nucleic Acids Res* 36(Web Server issue):W503–W508.
3. Harlow E, Lane D (1988) in *Antibodies: A Laboratory Manual* (Cold Spring Harbor Press, New York).

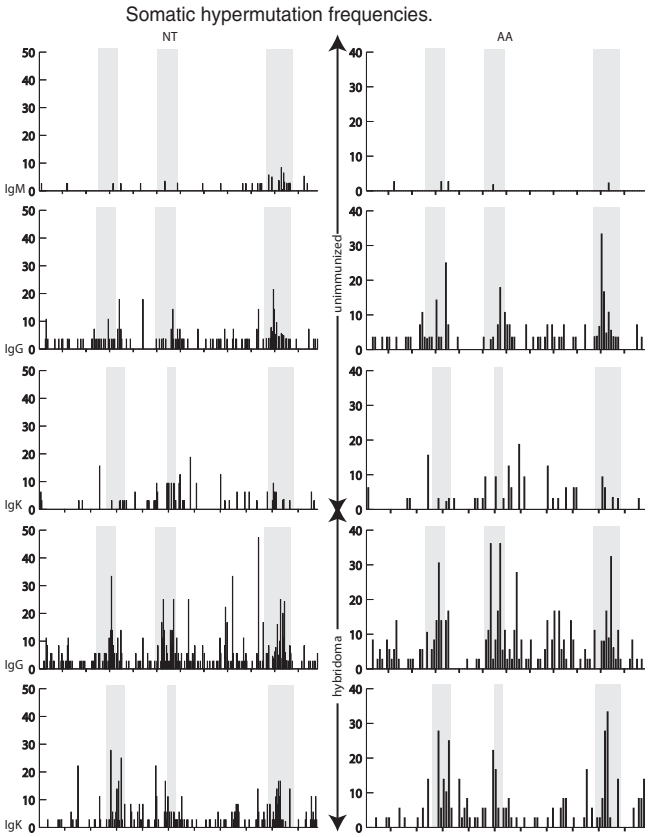

Fig. S1. Somatic hypermutation frequencies. Hypermutation frequencies were scored (after alignment to matching germ-line sequences) as percent of sequences changed at each nucleotide (NT) or amino acid (AA) position among sets of 38 (unimmunized IgM), 28 (unimmunized IgG), 32 (unimmunized IgK from IgG), 36 (IgG from hybridomas) or 36 (IgK from IgG hybridomas) sequences. Shading indicates CDRs.

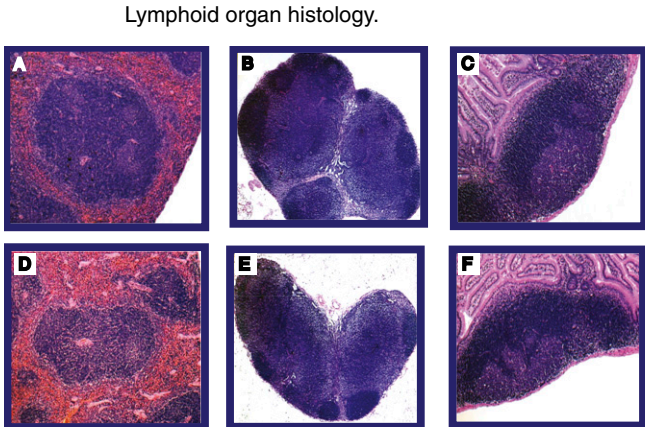

Fig. S2. Lymphoid organ histology. Spleen (*A* and *D*), inguinal lymph node (*B* and *E*), and Peyer's patch (*C* and *F*) from WT (*A*–*C*) or *VelocImmune* (*D*–*F*) mice were stained with H&E. Representative sections are shown.

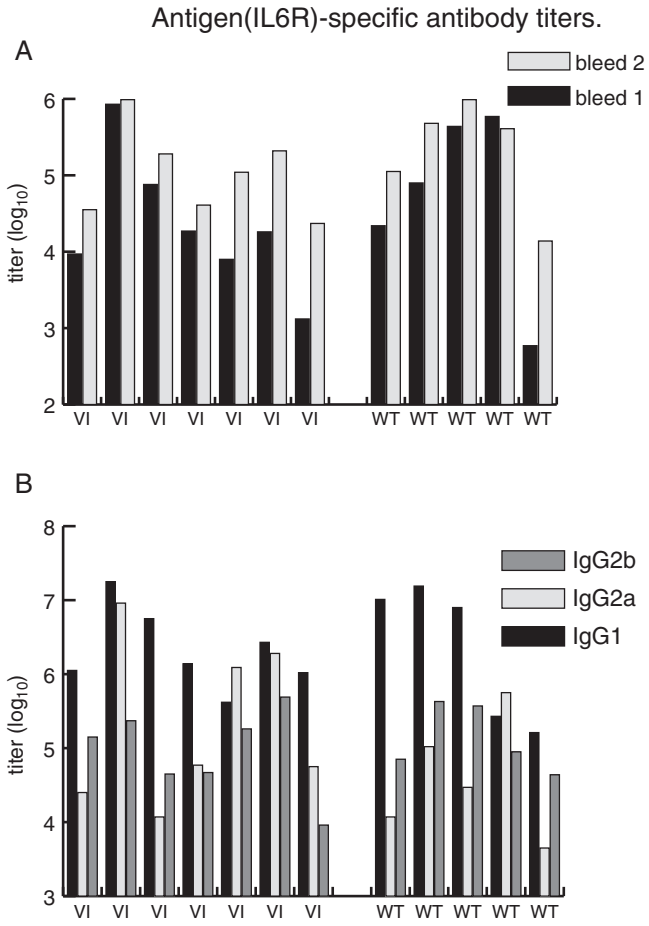

Fig. S3. Antigen-specific antibody titers. Antigen specific IgG titers against IL6R (*A*) were determined by ELISA of serum from seven *VelocImmune* (VI) and five WT mice after two (bleed 1) or three (bleed 2) rounds of immunization with IL6R ectodomain protein. IgG isotype-specific titers (*B*) were determined from the same mice.

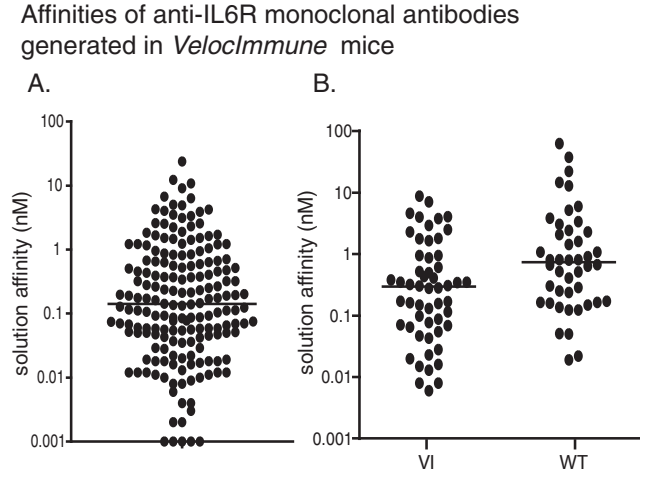

Fig. S4. Affinities of anti-IL6R monoclonal antibodies generated in *VelocImmune* mice. The affinity distribution of antigen-specific monoclonals (*A*) from *VelocImmune* mice and antigen-specific blocking monoclonals (*B*) from *VelocImmune* (VI) and WT mice.

Table S1. Representation of gene segments in a set of RT-PCR–amplified cDNAs from unimmunized *VelocImmune* 3 mice

V_H	No.	D_H	No.	V_K	No.
7–81*	0	1–1	7	2–40	1
3–74*	0	2–2	8	1–39	34
3–73	1	3–3	9	1–37	2
3–72	2	4–4	4	1–33	35
2–70	2	5–5	6	2–30	8
1–69	3	5–18	6	2–29	2
3–66	1	6–6	29	2–28	7
3–64	1	1–7	30	1–27	5
4–61	1	2–8	4	2–24	7
4–59	10	3–9	8	6–21†	3
1–58‡	0	3–10	10	3–20	10
3–53‡	0	4–11	4	1–17	13
5–51	5	5–12	5	1–16	10
3–49	2	6–13	17	3–15	13
3–48	7	1–14	2	1–12	13
1–46	1	2–15	3	3–11	13
1–45	0	3–16	4	1–9	11
3–43	10	4–17	3	1–8	1
4–39	4	6–19	8	3–7†	0
3–38†	0	1–20	3	1–6	6
3–35†	0	2–21	1	1–5	7
4–34	8	3–22	5	5–2‡	0
3–33	14	4–23	2	4–1	21
4–31	4	5–24	2		
3–30	13	6–25	2		
4–28‡	0	1–26	17	J_K	No.
2–26	0	7–27	7	1	50
1–24	3			2	37
3–23	18			3	28
3–21	0	J_H	No.	4	64
3–20	0	1	2	5	22
1–18	4	2	8		
1–17P	1	3	26		
3–16†	0	4	95		
3–15	13	5	11		
3–13	6	6	58		
3–11	5				
3–9	31				
1–8	7				
3–7	11				
2–5	1				
1–3	0				
1–2	6				
6–1	9				

Number of times each germ-line segment was occurred in a sampling of antibody sequences from *VelocImmune* mice.

*Missing or pseudogene.

†Defective RSS.

‡Detected in subsequent sequencing of antigen-specific mAbs.

Table S2. Some representative VH CDR3 sequences demonstrating TdT additions

V_H		N	D_H	N	J_H	
			D7-27			
			CTAACTGGGGA			
1-10	...GAGAGA	GA	GGGA	C	TACATT...	4
1-16	...TGCGAG	TCT	GGGGA	TCGAG	GACTAC...	4
1-8	...CGAGAG	GA	GGA	TCTGG	CTTTGA...	4
16-17	...TGCGAG	TCT	GGGGA	TCGAG	GACTAC...	4
11-40	...CGAGAG	—	GGGA	CCCCTC	CTTTGA...	4
			D3-3			
			GTATTACGATTTTTGGAGTGGTTATTATACC			
1-13	...CGAGAG	GG	TTTGGAGTGGTT	GAT	GCTTTT...	3
1-3	...GAGAGA	TAG	TTTTTGGAGTGGTTAT	CCC	GACTAC...	4
16-11	...GAGAGA	TAG	TTTTTGGAGTGGTTAT	CCC	GACTAC...	4
11-37	...CGAGAG	GG	TTTGGAGTGGTT	GAT	GCTTTT...	3
			D1-7			
			GGTATAACTGGAACTAC			
1-17	...GAGAGA	TC	ATAACTGGGACTAC	—	TACTTT...	4
1-6	...GAGAGA	TCAGGG	TAACTGGAACTAC	GCCCC	CTTTGA...	4
16-13	...GGGAGA	AA	ATAACTGGAACT	CTGAT	GCTTTT...	3
16-20	...GAGAGA	CC	TAACTGGAACTAC	TGGG	GACTAC...	4
11-11	...GAGAGA	TCAGGG	TAACTGGAACTAC	GCCCCCC	TTGACT...	4
11-20	...GTGCGA	—	ATAACTGG	TCTGG	CTACTG...	4
11-23	...GAGAGA	—	CTGGAACT	T	CTGGGG...	4

Sequences of 16 randomly selected *VelocImmune* antibodies around the V-D-J (CDR3) junction, demonstrating junctional diversity. Text in red represents the identity of the germline segments and, in the case of the D-segments, their gerline sequences.