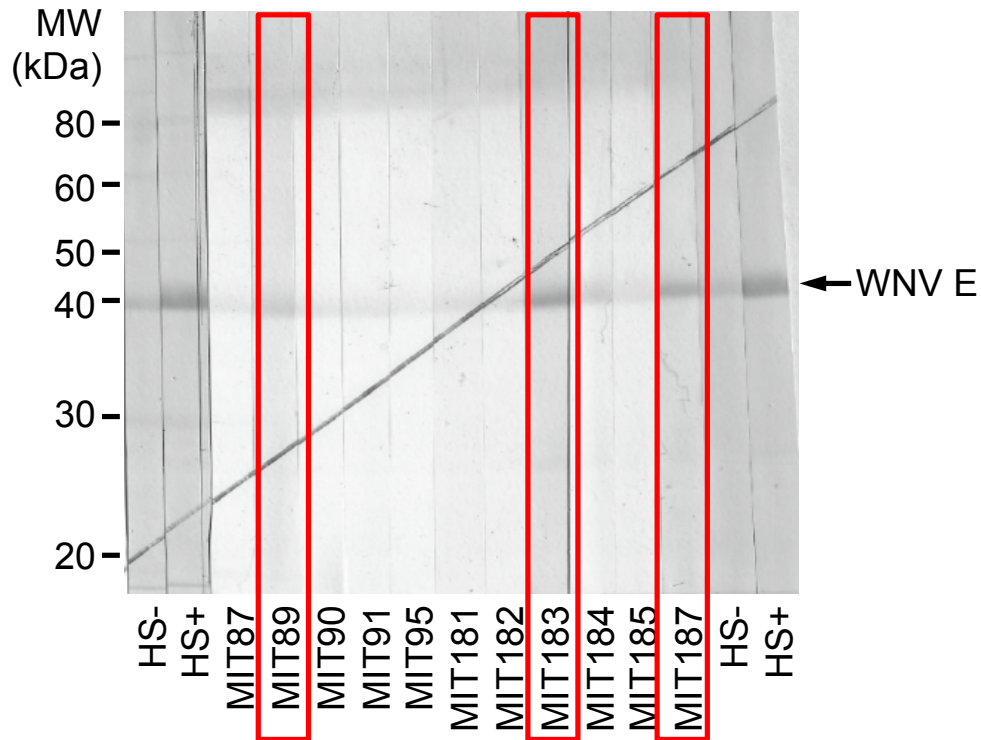


S1: West Nile virus specific antibodies from single-cell analysis.

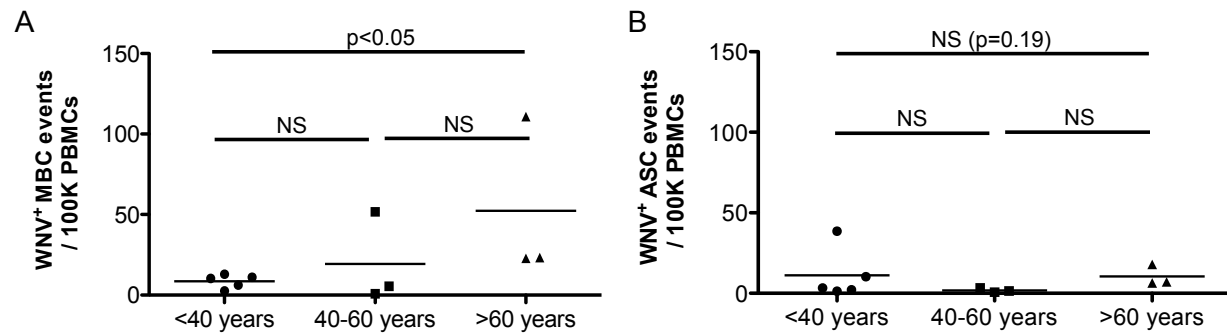
Heavy Chain										Light Chain				
Clone No.	Subject	Well ID	V Region	D Region	J Region	No. of Mismatches	CDR3	CDR3 Length	V Region	J Region	No. of Mismatches	CDR3	CDR3 Length	
MIT 86	PC3	0849 0106	IGHV3-7*03	IGHD3-3*01	IGHJ4*02	0	CARAGGGDRLRLFLWLSKPYFYDYW	25						
	PC3	1363 0301	IGHV2-26*01	IGHD3-22*01	IGHJ4*02	0	CARNSSGGYFYDYW	14						
	PC3	1256 0502				0		0	IGKV2-24*01	IGKJ2*01	0	CMQATQFPPTFYTF	12	
	PC3	1037 0605				0		0	IGKV1D-33*01	IGKJ4*01	0	CQGYDNLPLTF	11	
	PC3	1338 0707				0		0	IGKV1D-33*01	IGKJ4*01	0	CQGYDNLPLTF	11	
MIT 87	PC3	1218 0302				0		0	IGKV1D-33*01	IGKJ4*01	2	CQGYDNLPLTF	11	
	PC3	1404 0501				0		0	IGKV1D-33*01	IGKJ4*01	2	CQGYDNLPLTF	11	
	PC3	2167 0307	IGHV4-59*01	IGHD3-22*01	IGHJ5*02	0	CARGYYDSTRDWFDPW	16		IGKV1-39*01	IGKJ1*01	2	CQGYSTRITF	10
	PC4	0324 0603	IGHV2-26*01	IGHD2-2*02	IGHJ6*02	14	CARPFSEYAAMDVW	16		IGKV3-20*01	IGKJ2*01	5	CQQYSSSYTF	10
	PC4	0235 0501	IGHV2-26*01	IGHD2-2*02	IGHJ6*02	14	CARPFSEYAAMDVW	14						
MIT 88	PC4	0861 0407	IGHV3-64*05	IGHD3-3*01	IGHJ5*02	11	CYKDLTGAIFGVISELAW	0		IGLV2-14*01	IGLJ2*01	9	CSHSTSSSYDVLFF	14
	PC4	1561 0703	IGHV3-64*05	IGHD3-3*01	IGHJ5*02	11	CYKDLTGAIFGVISELAW	19	IGKV1D-12*01	IGKJ4*01	15	CQGYSPPLTF	11	
	MIT 89	PC4	1739 0202	IGHV1-2*02	IGHD3-10*02	IGHJ4*02	15	CARGRGVDMSPLYDNW	17	IGLV2-23*03	IGLJ3*02	16	CCSFAGGGTWWF	12
	MIT 90	PC4	2362 0402	IGHV3-23*04	IGHD2-15*01	IGHJ5*02	17	CGKDRPRCCSDKCNFGPNWFDW	24	IGKV1-5*01	IGKJ2*01	7	CQGFNSPNTF	11
	PC4	1969 0205	IGHV1-69*01	IGHD2-21*02	IGHJ4*02	31	CATNSDWTDFHW	12	IGLV2-23*02	IGLJ2*01	20	CCSYAGSTYNALF	13	
MIT 91	PC1	2344 0206	IGHV1-8*01	IGHD2-2*02	IGHJ6*02	0	CARGPPIVYPAAGVPRMDVW	22						0
	PC1	0429 0602	IGHV1-8*01	IGHD5-5*01	IGHJ5*02	0	CARGLVDITAMVTFDPW	16	IGKV1-9*01	IGKJ5*01	1	CQQLNSYLPTF	12	
	PC1	0908 0104				0		0	IGKV3-20*01	IGKJ1*01	0	CQQYSSSLWTF	11	
	MIT 92.93	PC1	0845 0107	IGHV5-51*03	IGHD3-3*01	IGHJ6*02	0	CARHFTQGYDFWGSYYTYDYGMDVW	25	IGKV3-20*01	IGKJ1*01	1	CQQHDLPLPQLTF	14
	PC1	1944 0207	IGHV3-30-3*01	IGHD1-7*01	IGHJ1*01	12	CATRGNYRASSEYFLHW	12						0
MIT 94	PC1	0667 0101	IGHV5-51*03	IGHD3-16*02	IGHJ6*02	0	CARQLASTFGGVRGMDVW	0						0
	PC2	1048 0207	IGHV1-18*01	IGHD5-12*01	IGHJ5*02	30	CARLHLDQGWIDPW	16	IGKV3-20*01	IGKJ1*01	19	CQQYSSSPWTF	11	
	PC2	1868 0605				0		0	IGKV3-20*01	IGKJ1*01	19	CQQYSSSPWTF	11	
	PC2	0736 0701	IGHV3-30-3*01	IGHD5-5*01	IGHJ4*02	0	CASLGSYSGRADW	13						0
	PC2	0332 0304	IGHV3-33*01	IGHD5-24*01	IGHJ2*01	0	CARWGGRDRGYINWYFDLW	19	IGKV3-11*01	IGKJ5*01	1	CQQRNSPPWTF	12	
MIT 95	PC2	0661 0307	IGHV3-13*01	IGHD3-22*01	IGHJ4*02	0	CARGSSSGSYFDYFW	14						0
	MIT 96	PC2	2037 0106	IGHV1-2*02	IGHD6-19*01	IGHJ6*02	1	CARGPPIYTYDYGMDVW	0	IGLV8-49*01	IGLJ3*02	0	CGADHSGSSNYPPEVWF	18
	MIT 180	R17B	1839 0402	IGHV3-49*04	IGHD2-2*01	IGHJ6*02	9	CTRDLEVAANLAEEYTGMDVW	22	IGKV2-30*01	IGKJ1*01	6	CMQGTWRTF	10
	MIT 181	R17A	0641 0702	IGHV4-61*01	IGHD3-22*01	IGHJ4*02	0	CASLAAGDIHYDRWYFDVW	19	IGKV1-39*01	IGKJ1*01	0	CQGYSTRITF	12
	R17A	0142 0603				0		0	IGLV2-8*02	IGLJ2*01	4	CCSYAGSSNPFVWF	13	
MIT 182	R17A	0751 0301	IGHV3-20*01	IGHD3-3*02	IGHJ4*02	13	CARDEGPAFGYW	13	IGKV1-9*01	IGKJ2*01	6	CQQLNSYPTF	11	
	MIT 183	R17A	1067 0602	IGHV3-64*05	IGHD2-2*03	IGHJ4*02	3	CVKDGQIGYCSSTCRWAAAGTGVDFYW	29	IGKV3-20*01	IGKJ1*01	1	CQQYSSSPWTF	12
	MIT 184	R17A	1627 0203	IGHV1-69*01	IGHD2-15*01	IGHJ6*02	7	CARARSVAATPNDYTYGMDVW	22	IGLV3-15*01	IGLJ3*01	2	CQVWDTSSWTF	12
	R17A	2230 0305				0		0	IGKV3-15*01	IGKJ4*01	2	CQQYNNWPPRAF	12	
	R17A	1939 0506	IGHV1-69*06	IGHD3-22*01	IGHJ3*02	0	CARAGFYDSSSGWGAFDIW	21						0
MIT 185	R17A	2348 0302				0		0	IGKV3-15*01	IGKJ2*01	0	CQQYDNNWPPRTF	13	
	R17A	2461 0304				0		0	IGLV2-23*02	IGLJ1*01	9	CCSYAGSSSFGPVWF	15	
	MIT 186	R11	0907 0101	IGHV4-30-2*01	IGHD2-3*01	IGHJ6*03	34	CALARGLSGDGTSCVAPPYFYMDLW	26	IGKV1-44*01	IGLJ2*01	23	CAAWDVLRLDGVWF	13
	MIT 186	R11	1743 0301	IGHV4-34*01	IGHD1-26*01	IGHJ6*03	38	CVRVMPDLGLARWAEKFNYSLSDGKEYYYMDVW	34	IGKV3-20*01	IGKJ1*01	35	CQQYSSSOWTF	11
	MIT 187	R11	1440 0403	IGHV4-31*03	IGHD3-10*01	IGHJ6*02	17	CARRYRSGGEFFFTVMDVW	22	IGKV2-28*01	IGKJ2*02	12	CMQGLQPTFTF	11
MIT 187	R11	0361 0404				0		0	IGKV3-20*01	IGKJ1*01	8	CQQYSSSOWTF	11	
	R11	0364 0107				0		0	IGKV4-1*01	IGKJ1*01	43	CQGYSTRITF	11	
	R13	2343 0501	IGHV4-4*07	IGHD3-22*01	IGHJ3*02	0	CARDYVAITSPTDFIDW	17	IGLV2-23*02	IGLJ3*02	0	CCSYAGSSSFWWF	13	
	R13	2319 0604	IGHV1-2*02	IGHD3-16*02	IGHJ6*03	5	CARGLRGTTRHHYYTYGMDVW	22	IGLV3-21*03	IGLJ2*01	2	CQVWDTSSDLVWF	13	
	R13	1501 0606				0		0	IGLV3-21*03	IGLJ2*01	2	CQVWDTSSDLVWF	13	
MIT 187	R13	0945 0701				0		0	IGKV4-1*01	IGKJ4*01	1	CQQYSTRITF	11	
	R13	1742 0202	IGHV4-59*01	IGHD3-16*02	IGHJ4*02	30	CARRNYDPSGYHFDYW	16	IGKV3-11*01	IGKJ1*01	0	CQQRNSNWRFTF	11	
	R13	1248 0605				0		0	IGKV4-1*01	IGKJ1*01	9	CQQFYGTTRTF	11	
	R13	0948 0306				0		0	IGKV4-1*01	IGKJ1*01	5	CQQYSTRITF	11	
	R13	1553 0105				0		0	IGLV1-47*01	IGLJ3*02	0	CAAWDLSLGSFVWF	13	
MIT 187	R13	0632 0506				0		0						0
	R13	0355 0604				0		0						0
	R13	1672 0201	IGHV2-5*02	IGHD2-21*01	IGHJ4*02	29	CAHSYATPDYW	12		IGLV2-14*03	IGLJ3*02	0	CSHAGSDTLWF	12
	R13	0123 0304	IGHV4-59*01	IGHD3-3*01	IGHJ6*01	0	CARGDYFNSGYLFDW	17		IGLV2-14*03	IGLJ1*01	0	CSHSTSSSYTHWF	13
	R11_R	2257 0101				0		0	IGKV3-11*01	IGKJ5*01	4	CQQRNSPWTF	11	
MIT 187	R11_R	1421 0706				0		0	IGKV3-20*01	IGKJ4*01	16	CQGHSGYLGTLF	12	
	R11_R	1531 0604				0		0	IGKV3-15*01	IGKJ3*01	9	CQQYNSNWRFTF	11	
	R11_R	1105 0306				0		0	IGKV3-11*01	IGKJ4	23	CQQRDOWPLTF	11	
	R11_R	1853 0303				0		0	IGKV1-12*01	IGKJ1*01	15	CQAGSFPRTF	11	
	R11_R	0715 0607				0		0	IGKV3-20*01	IGKJ1*01	26	CQQYKSLWTF	11	
MIT 187	R11_R	0736 0704				0		0	IGKV3-20*01	IGKJ2*01	21	CQQYSSSPGYTF	13	
	R11_R	0850 0102				0		0	IGKV3-15*01	IGKJ2*04	0	CQQYNNWPPRSF	12	
	R11_R	0651 0605				0		0	IGKV1-33*01	IGKJ4*01	14	CQGYDNLPLTF	11	
	R11_R	0468 0206				0		0	IGKV3-20*01	IGKJ1*01	20	CQYGSAAKTF	11	
	R11_R	0223 0506				0		0	IGKV3-11*01	IGKJ5*01	7	CQQRNSWTF	10	
MIT 187	R11_R	0221 0207				0		0	IGKV1-33*01	IGKJ4*01	11	CQGYDNLPLTF	11	
	R11_R	0419 0703				0		0	IGLV1-40	IGLJ2*01	1	CQSYDSSLGSGVWF	13	
	R11_R	0509 0505				0		0	IGKV3D-15*01	IGKJ1*01	7	CHQYNNWPRTF	11	
	R11_R	0571 0302				0		0	IGKV3-20*01	IGKJ1*01	10	CQQYTSPPWTF	12	
	R11_R	0325 0706				0		0	IGKV3-15*01	IGKJ4*01	7	CQQYNNWPPFTF	12	
MIT 187	R12	0947 0203	IGHV3-30*18	IGHD7-27*01	IGHJ3*02	27	CAKALLGSDSDVLWDDAFHTW	0		IGKV3-15*01	IGKJ1*01	15	CQQYNNWPRTF	11
	R12	0955 0601				0		0	IGKV3-15*01	IGKJ1*01	10	CQQYNNWPKTF	11	
	R12	0145 0507				0		0	IGKV3-15*01	IGKJ1*01	8	CQQYNNWPKTF	11	
	R14	0320 0106	IGHV4-31*03	IGHD2-2*02	IGHJ4*02	11	CARARYTSQSFDSW	0		IGKV3-20*01	IGKJ2*02	5	CQQYSSSPCTF	11
	R14	0371 0104				0		0	IGKV1-5*03	IGKJ1*01	16	CQQYSSSFPYF	12	
MIT 187	R14	1024 0301	IGHV5-51*01	IGHD6-19*01	IGHJ3*02	10	CARFPGPSGWYGFDPW	0		IGKV1-39*01	IGKJ4*01	9	CQGHSTHTF	11
	R14	2459 0405				0		0						0
	R14	2242 0404				0		0						0
	R14	2234 0606	IGHV1-69*01	IGHD2-8*02	IGHJ5*02	17	CARDAHSGYCAGGCGYNNWFDW	0		IGKV4-1*01	IGKJ1*01	0	CQQYSTRITF	11
	R15	1801 0504				0		0	IGKV4-1*01	IGKJ1*01	0	CQQYSTRITF	11	
MIT 187	R15	1931 0301				0		0	IGKV2-28*01	IGKJ1*01	11	CMQALQTLRAF	11	
	R15	1737 0106				0		0	IGKV3-15*01	IGKJ5*01	6	CQQYNNWPLTF	11	
	R15	1814 0703				0		0	IGLV3-25*03	IGLJ2*01	19	CQSDQISGTVWF	12	
	R16	0501 0104				0		0	IGKV3-20*01	IGKJ4*01	8	CQQYKSLPTF	11	
	R16	0521 0703	IGHV3-7*03	IGHD3-3*01	IGHJ5*02	11	CARMFYDFWSGYMDVW	17	IGKV3-20*01	IGKJ4*01	8	CQQYKSLPTF	11	
MIT 187	R16	1134 0405	IGHV3-33*01	IGHD6-12*01	IGHJ6*02	0	CARDQGYTAAGTLYYYYGMDVW	23	IGKV1-17*01	IGKJ1*01	2	CLQHSYSPWTF	11	
	R16	0931 0207	IGHV2-26*01	IGHD3-22*01	IGHJ4*02	0	CARTTYDSSGYPDYW	17	IGKV1-5*03	IGKJ1*01	5	CQQYSSSGTF	11	
	R16	0916 0101	IGHV4-34*01	IGHD2-15*01	IGHJ6	3		0	IGKV1-8*01	IGKJ2	1	CQQYSTRITF	11	
	R16	1235 0205				0		0	IGLV2-8*03	IGLJ3*02	13	CSYAGSDTLF	12	
	R16	1357 0401	IGHV4-39*01	IGHD1-26*01	IGHJ4*02	14	CAROKYSGSYDYW	14	IGLV1-40	IGLJ3*02	7	CQYDSSLGSGGVWF	14	

S2: Paired West Nile virus specific antibody heavy and light chain sequences.

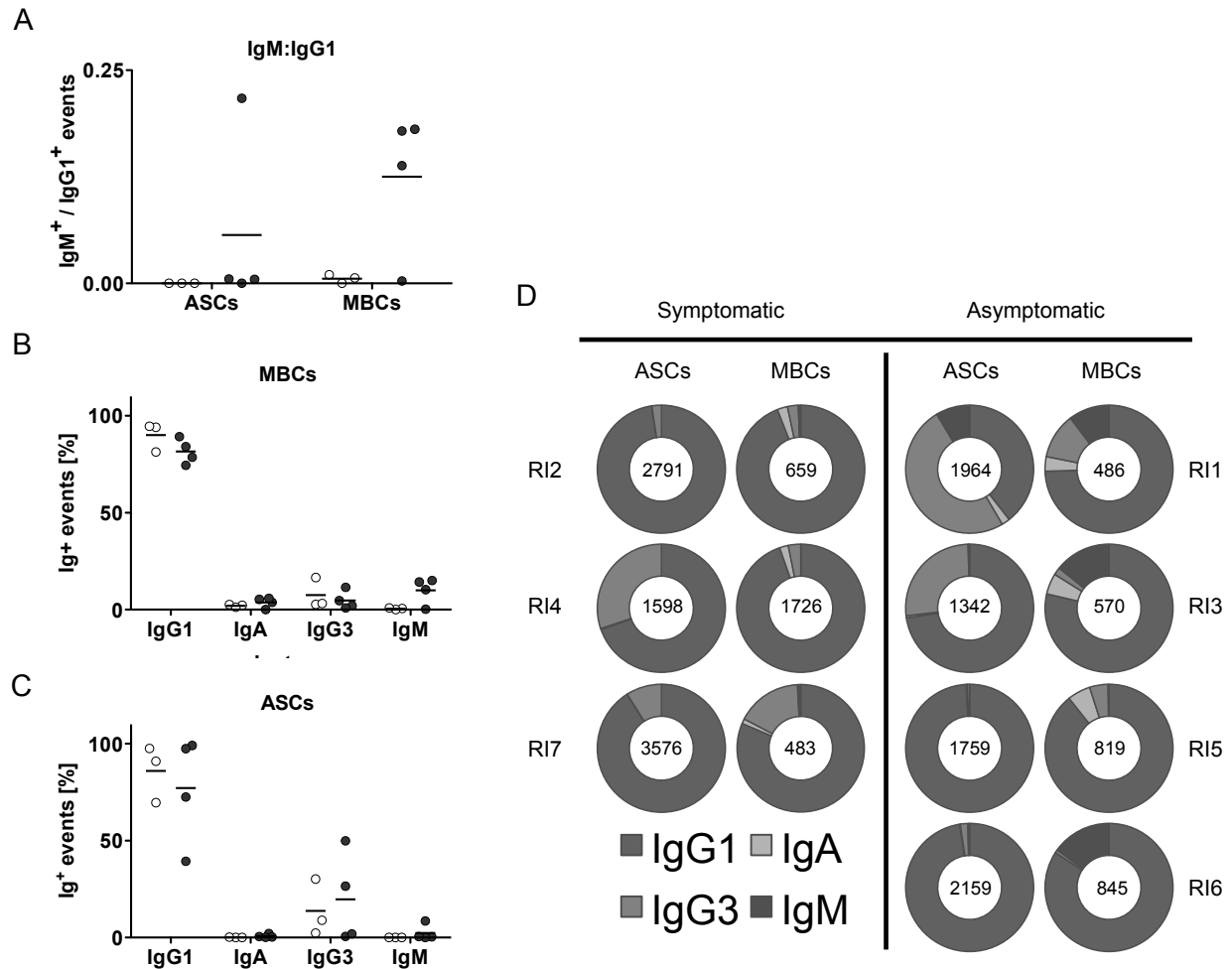
Clone No.	Subject	Heavy Chain					Light Chain					
		V Region	D Region	J Region	No. of Mismatches	CDR3	CDR3 Length	V Region	J Region	No. of Mismatches	CDR3	CDR3 Length
MIT 86	PC3	V2-26*01	D3-22*01	J4*02	0	CARVNSSGYFYDYW	14	V1D-33*01	J4*01	2	CQQYDNLPLTF	11
MIT 87	PC3	V4-59*01	D3-22*01	J5*02	0	CARGYDSTRDWFDPW	16	V1-39*01	J1*01	2	CQGSYSTRTF	10
MIT 88	PC4	V3-64*05	D3-3*01	J5*02	11	CVKDLTGAIFGVISELAW	19	V1D-12*01	J4*01	15	CQAYSPPLTF	11
MIT 89	PC4	V1-2*02	D3-10*02	J4*02	15	CARGRGVDVMSPLYDNW	17	V2-23*03	J3*02	16	CCSFAGSGTWVF	12
MIT 90	PC4	V3-23*04	D2-15*01	J5*02	17	CGKDPRRDCSDIKCNFGPNWFDPW	24	V1-5*01	J2*01	7	CQGFNSYPNTF	11
MIT 91	PC1	V1-8*01	D5-5*01	J5*02	0	CARGLVDTAMVTFDPW	16	V1-9*01	J5*01	1	CQQLNSYLPITF	12
MIT 92	PC1	V5-51*03	D3-3*01	J6*02	0	CARHFTGGYDFWSGYTDTYGMDDW	25	V4-1*01	J1*01	2	CQYYSTPPTF	11
MIT 93	PC1	V5-51*03	D3-3*01	J6*02	0	CARHFTGGYDFWSGYTDTYGMDDW	25	V1-39*01	J1*01	1	CQGSYSTRTF	10
MIT 94	PC2	V1-18*01	D5-12*01	J5*02	30	CARLHYHLDQGWIDPW	16	V3-20*01	J1*01	19	CQQYGSWPPTF	11
MIT 95	PC2	V3-33*01	D5-24*01	J2*01	0	CARWGGRRDGYINWYFDLW	19	V3-11*01	J5*01	1	CQQRSNWPPTF	12
MIT 96	PC2	V1-2*02	D6-19*01	J6*02	1	CARGPPPPYYGYMDWW	16	V9-49*01	J3*02	0	CGADHSGSGNFV*PEIRVF	18
MIT 180	RI7	V4-61*01	D3-22*01	J4*02	0	CASLAGDHYYDRWYFDYW	19	V1-39*01	J4*01	0	CQGSYSTPLTF	12
MIT 181	RI7	V3-20*01	D3-3*02	J4*02	13	CARDEGPGAFGYW	13	V1-9*01	J2*01	6	CQQLNSYPYTF	11
MIT 182	RI7	V3-64*05	D2-2*03	J4*02	3	CVKDDGGYCSSTSCRWAAAGTWGVFDYW	29	V3-20*01	J4*01	1	CQQYGSPPPLTF	12
MIT 183	RI7	V1-69*01	D2-15*01	J6*02	7	CARARSAATPNDYFYGMDDW	22	V3-1*01	J3*01	7	CQAWDSSIAWVF	12
MIT 184	RI7	V3-49*04	D2-2*01	J6*02	9	CTRDLVAAANLAEYGYMDVW	22	V2-30*01	J1*01	6	CMQDTHWRTF	10
MIT 185	RI1	V4-30-2*01	D2-2*01	J6*03	34	CALARGLGSGCTSCWAPYYFMDLW	26	V1-44*01	J2*01	23	CAAWDYSLRGVNF	13
MIT 186	RI1	V4-34*01	D1-28*01	J6*03	38	CVRVMPDLGLARWAEKYNFLSGDAKEYYYMDVW	34	V3-20*01	J1*01	35	CQQYSGSQWTF	11
MIT 187	RI1	V4-31*03	D3-10*01	J6*02	17	CARRYSQRFGFEPTTVDMDVW	22	V2-28*01	J2*02	12	CMOGLQTPPTF	11



S3: Immunoblot assay results. Immunoblot analysis shows binding of MIT89, MIT183, and MIT187 to WNV E protein. Negative controls: HS- serum from healthy donor. Positive control: HS+ serum from WNV positive donor PC1. All controls were diluted by a factor of 1:500.



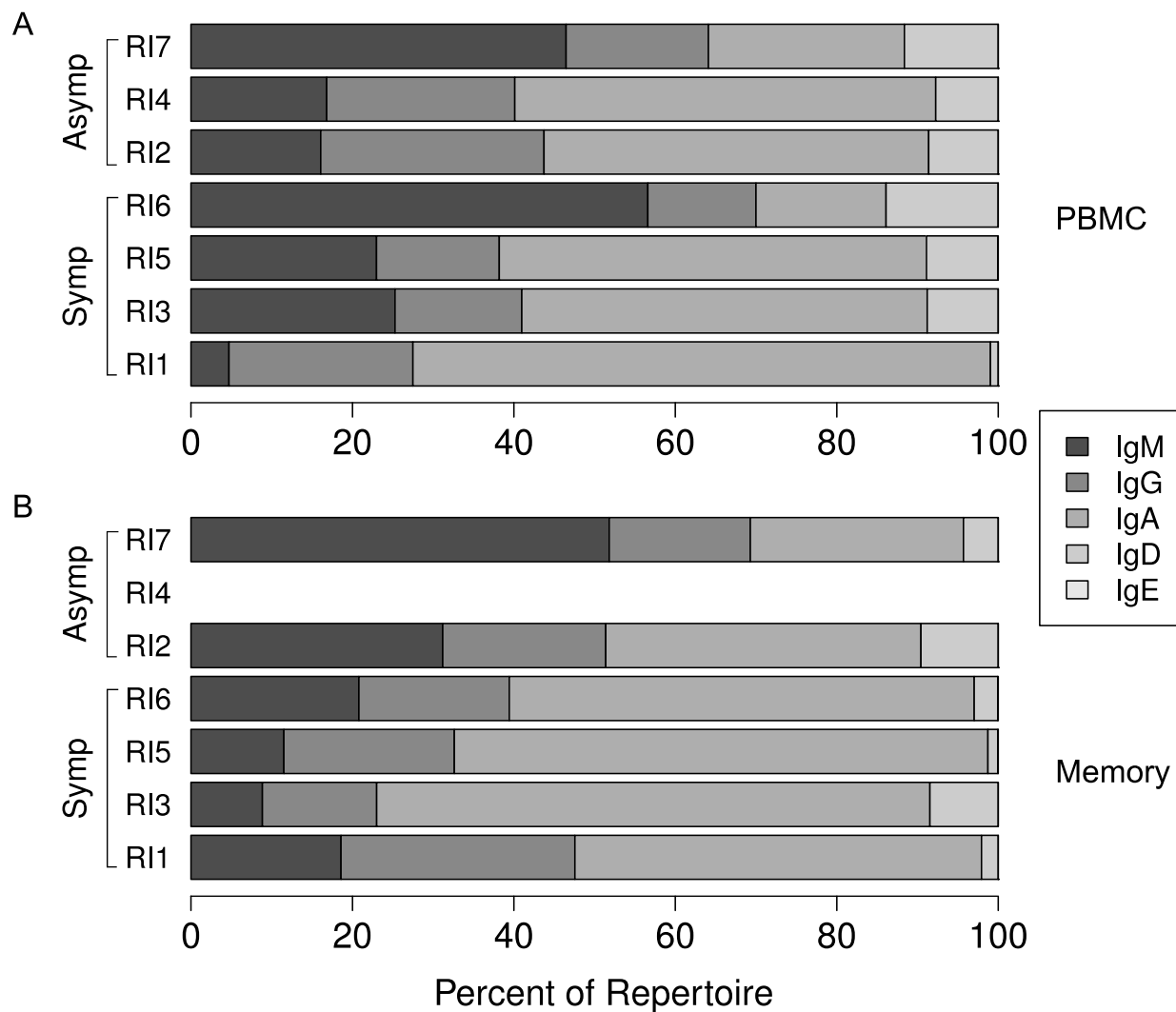
S4: WNV-specific MBC and ASC frequencies by age. A & B) Plot of frequencies of WNV-specific MBCs and ASCs by subject age (<40 vs. 40-60 vs. >60 years). We detected a significant increase in the MBC frequencies for subjects >60 years olds compared to the other age groups ($p<0.05$). No significant differences were detected between the ASC groups.



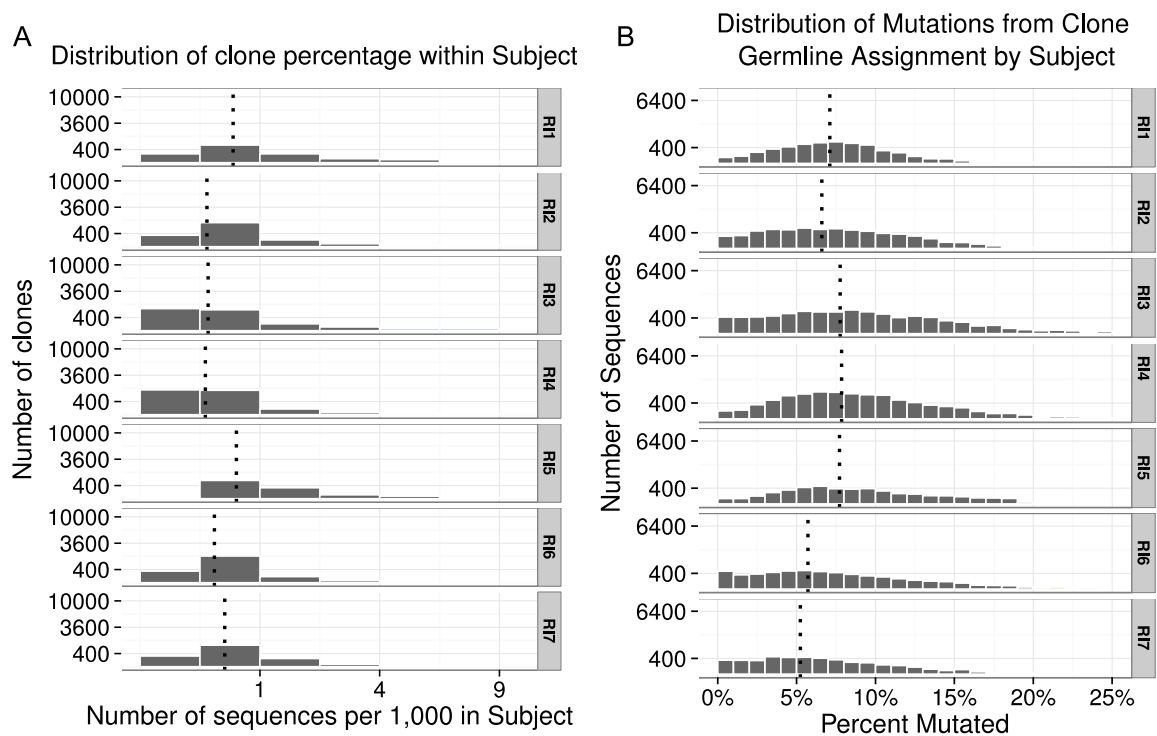
S5: Recently severely infected West Nile virus subjects have increased IgM/IgG1 ratios compared to asymptomatic subjects. A) Plot of ratio of IgM to IgG1 frequencies for ASCs and MBCs as determined by microengraving single-cell analysis. **B&C)** Plot of percentages of isotype distributions of total Ig events for MBCs and ASCs. **D)** Per patient isotype distribution by ASCs and MBCs divided by disease state (symptomatic and asymptomatic). The total number of Ig⁺ events enumerated is indicated in the center of each plot.

S6: Summary of next generation sequencing and bioinformatics for each subject.

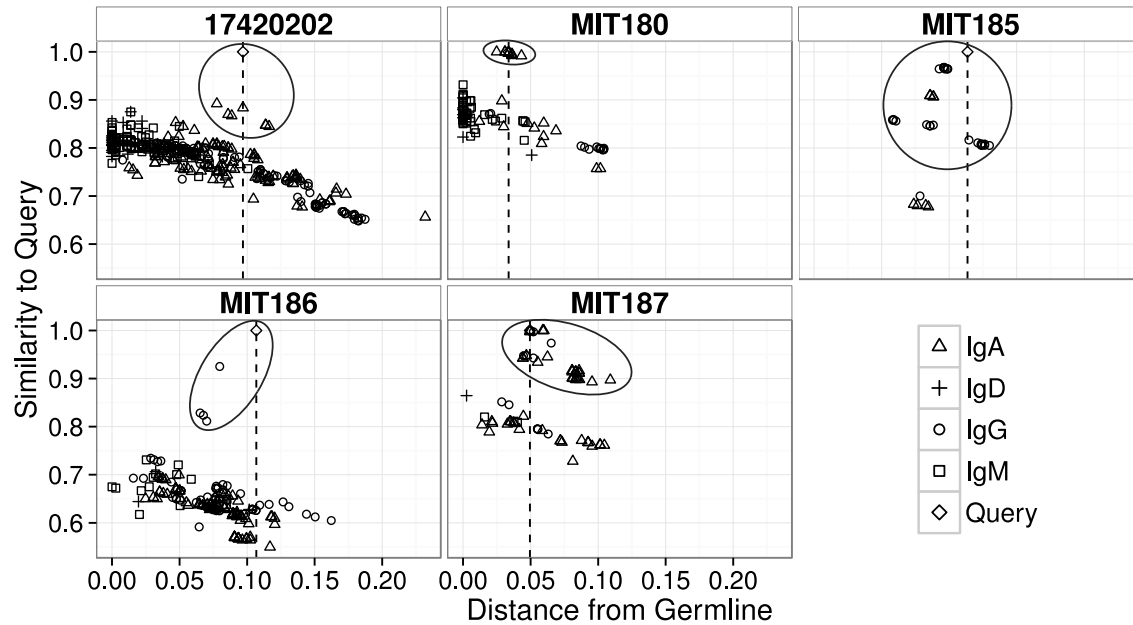
ID	Disease State	Raw Reads	Antibody Sequences	Assigned to clones	WNV+ Antibodies	Number of WNV clones	WNV clone sizes
RI1	Recent infect.	2233926	23788	13056	3	3	5,22,33
RI2	Recent infect.	1900765	75683	52588	1	0	NA
RI3	Recent infect.	1972153	63132	45571	5	1	5
RI4	Recent infect.	1462649	53144	26618	2	0	NA
RI5	Recent infect.	1385024	55486	37869	2	0	NA
RI6	Recent infect.	2491337	79991	58256	10	0	NA
RI7	Recent infect.	1613256	70044	39773	9	1	11
PC1	Post-conv.	1723558	61668	59402	5	0	NA
PC2	Post-conv.	2156022	26078	24996	5	0	NA
PC3	Post-conv.	1893813	143829	135139	2	0	NA
PC4	Post-conv.	1898589	21389	20479	6	0	NA



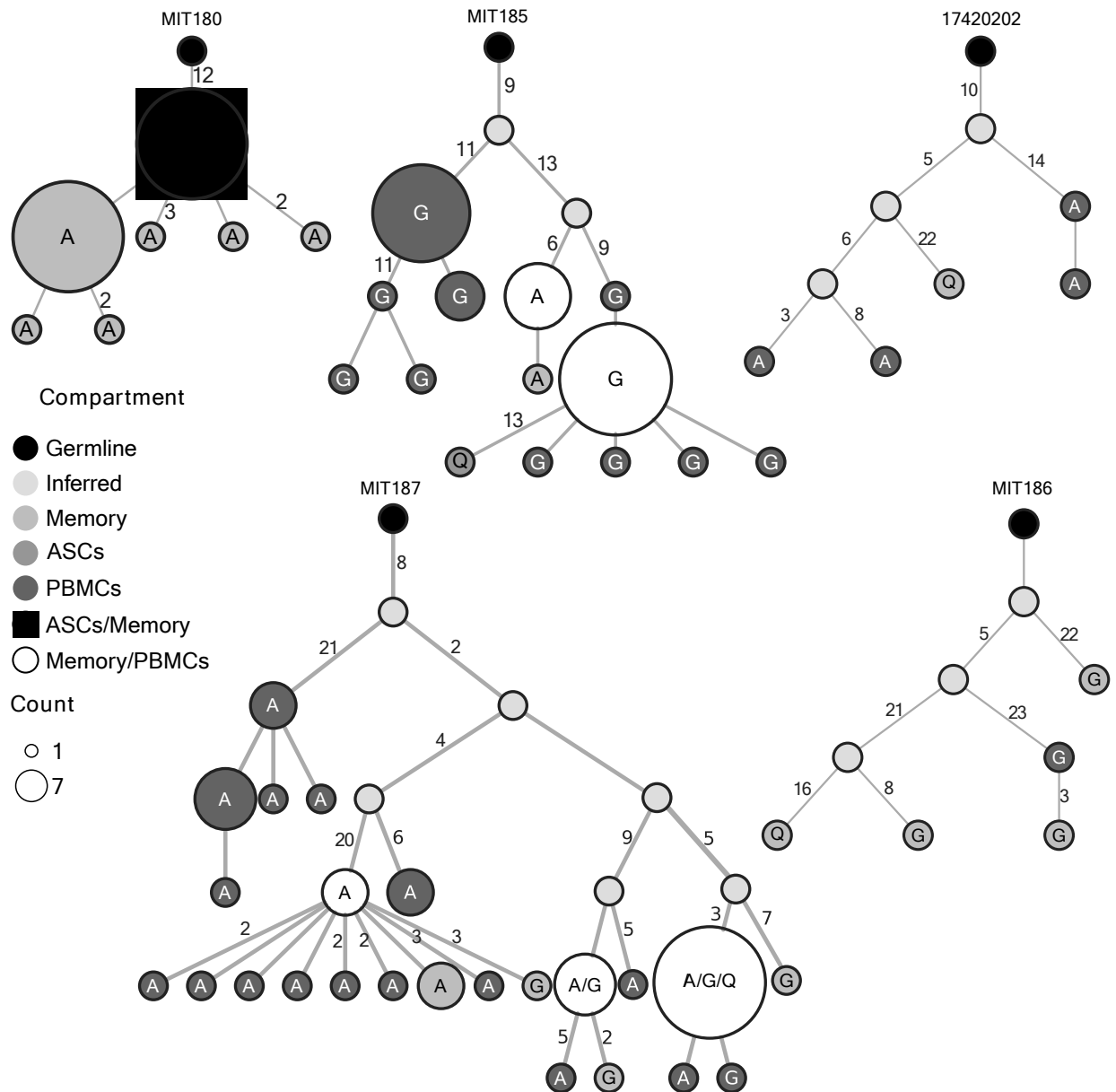
S7: No difference in isotype distribution between asymptomatic and symptomatic recently infected subjects. Distribution of isotypes from PBMCs (upper) and Memory B cells (lower) NGS Ig sequencing by subject.



S8: Distribution of antibody repertoire clone sizes and mutation levels. A) Histogram of clone sizes normalized by sequencing depth in each subject, dotted line shows median of distribution. **B)** Histogram of mutation levels (V segment up to start of CDR3) from the germline sequence of each clone in Ig sequences, dotted line shows median of distribution.



S9: Query plots of West Nile virus-specific clones found in Ig repertoire. Plots of sequence similarity of heavy chain (same V and J segment) identified within the repertoire as a function of distance of the sequence from germline (x-axis) and the corresponding WNV-specific “query” sequence (y-axis). Outlier clusters representing putative WNV-specific clones are circled. The shape of each point indicates the isotype of the Ig sequence. 17420202, MIT180, and MIT187 were identified by automated clonal grouping; MIT185 and MIT186 were identified by manual inspection.



S10: Lineage trees of West Nile virus-specific clones found in Ig repertoire. Maximum parsimony lineage trees of WNV-specific clones. Each node represents a unique sequence, with size correlating to number of duplicate reads observed. Edge lengths correspond to the number of mutation between sequences (unlabeled edges are one mutation). Shading of the node represents the compartment in which the sequence was found. The node label determines the type of sequence observed (A: IgA, G: IgG, Q: WNV-specific query sequence from single-cell screening).