Supplementary Figures and Tables

Supplementary Figure 1: Development of neutralizing antibodies against heterologous viruses. (a) IC50 titers of sera from SIVsmE660-FL14 (Tier 1A) infected macaques against SIVsmE543-3. (b) IC50 titers of sera from H807-16w-6 (Tier 2) infected macaques against SIVsmE543-3. (c) IC50 titers of sera from H807-24w-4 (Tier 3) infected macaques against SIVsmE543-3. (d) IC50 titers of sera from SIVsmE660-FL14 (Tier 1A) infected macaques against SIVmac239. (e) IC50 titers of sera from H807-16w-6 (Tier 2) infected macaques against SIVmac239. (f) IC50 titers of sera from H807-24w-4 (Tier 3) infected macaques against SIVmac239.

Supplementary Figure 2: Different sequence divergence rates of viral Env in macaques infected with the three clones. Viral Env sequences in each macaque at sequential time points post infection were sequenced by Illumina MiSeq deep sequencing. Sequences were split into 20 non-overlapping but contiguous loci composed of 40 codons based on the read length. Sequence divergence in each macaque at specific time points was calculated by aligning the NGS reads against the inoculum sequences. Divergence rates were calculated by applying smoothed spline regressions with divergence and sample week acting as the response and predictor, respectively. (A) Total rates of sequence divergence at the most divergent regions of Env over all times sampled. (B) Divergence rates of selected regions of Env at early, intermediate and late points of infection.

Supplementary Figure 3: Longitudinal Env divergence based on non-synonymous mutations. Sequences were split into 20 non-overlapping but contiguous loci composed of 40 codons based on the read length. Sequence divergence in each macaque at specific time points was calculated by aligning the NGS reads against the inoculum sequences. The divergence rate at Env regions C1 (34-73), V1 (113-154), V2 (154-194), V4 (405-440) and gp41 tail (801-840) are shown.

Supplementary Figure 4: Longitudinal Env diversity based on non-synonymous mutations. Sequence diversity (the average hamming distance between haplotypes) in each macaque at specific time points at Env regions C1 (34-73), V1 (113-154), V2 (154-194), V4 (405-440) and gp41 tail (801-840) are shown.
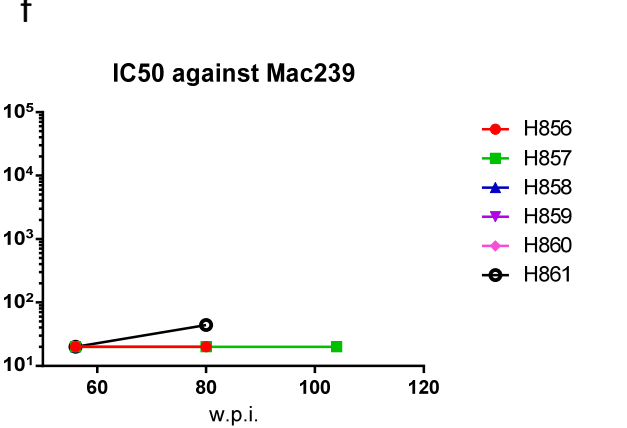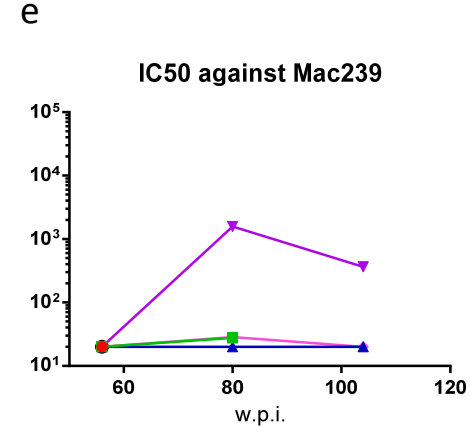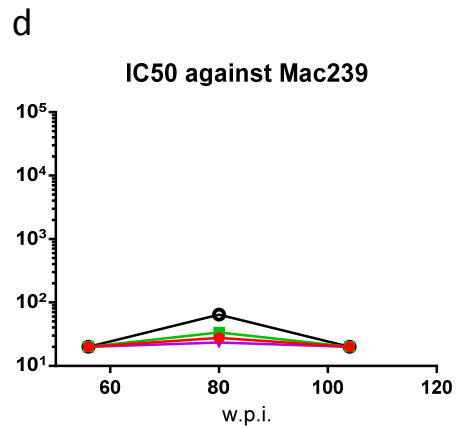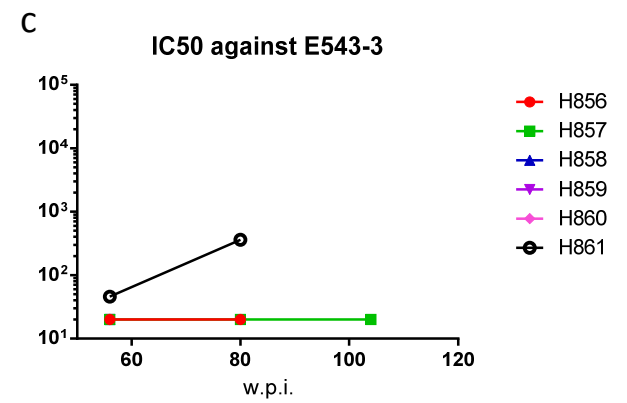
Supplementary Table 1. Neutralization sensitivity screen of SIVsmE660 clones

Supplementary Table 2. MHC genotype and gender of macaques

a

IC50 against E543-3

H844
H845
H846
H847
H848
H849

w.p.i.

b

IC50 against E543-3

H850
H851
H852
H853
H854
H855

w.p.i.

c

IC50 against E543-3

H856
H857
H858
H859
H860
H861

w.p.i.

d

IC50 against Mac239

H844
H845
H846
H847
H848
H849

w.p.i.

e

IC50 against Mac239

H850
H851
H852
H853
H854
H855

w.p.i.

f

IC50 against Mac239
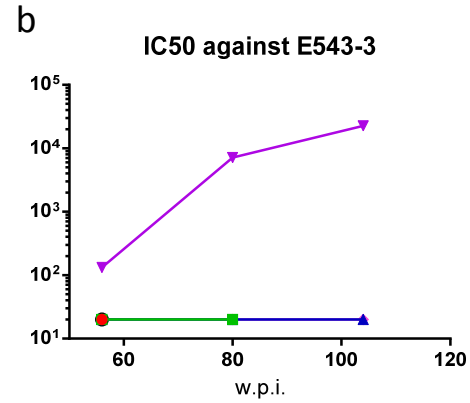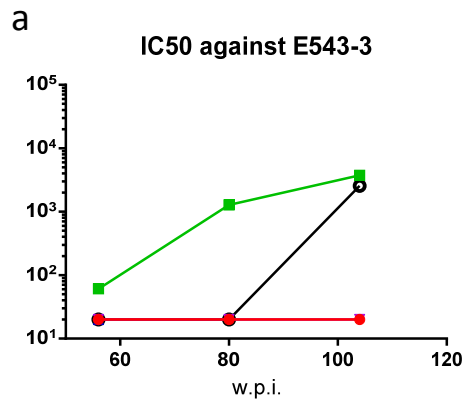
H856
H857
H858
H859
H860
H861

w.p.i.

Supplementary Fig. 1
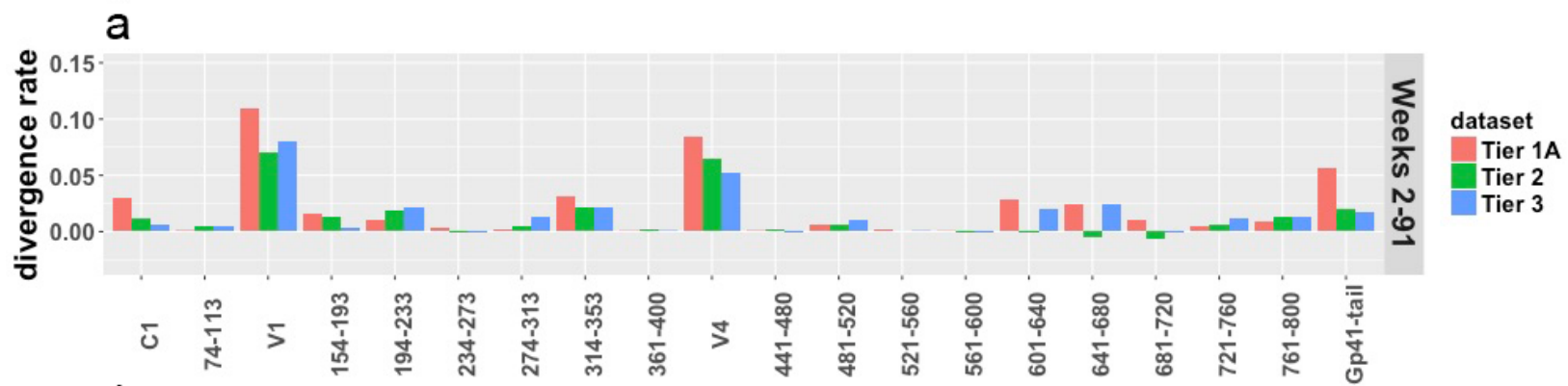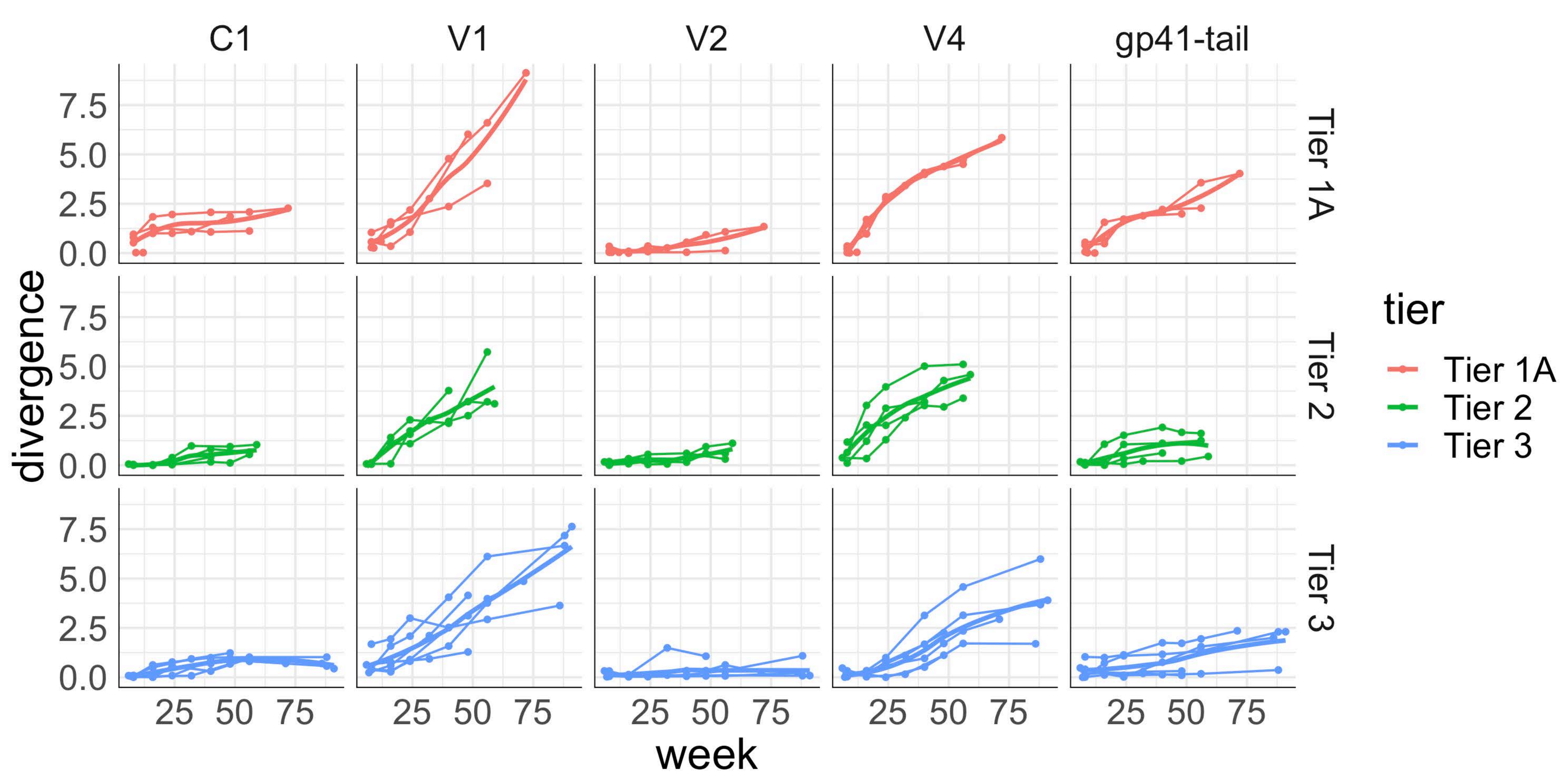
Supplementary Table S1. Neutralization sensitivity screen of SIVsmE660 clones

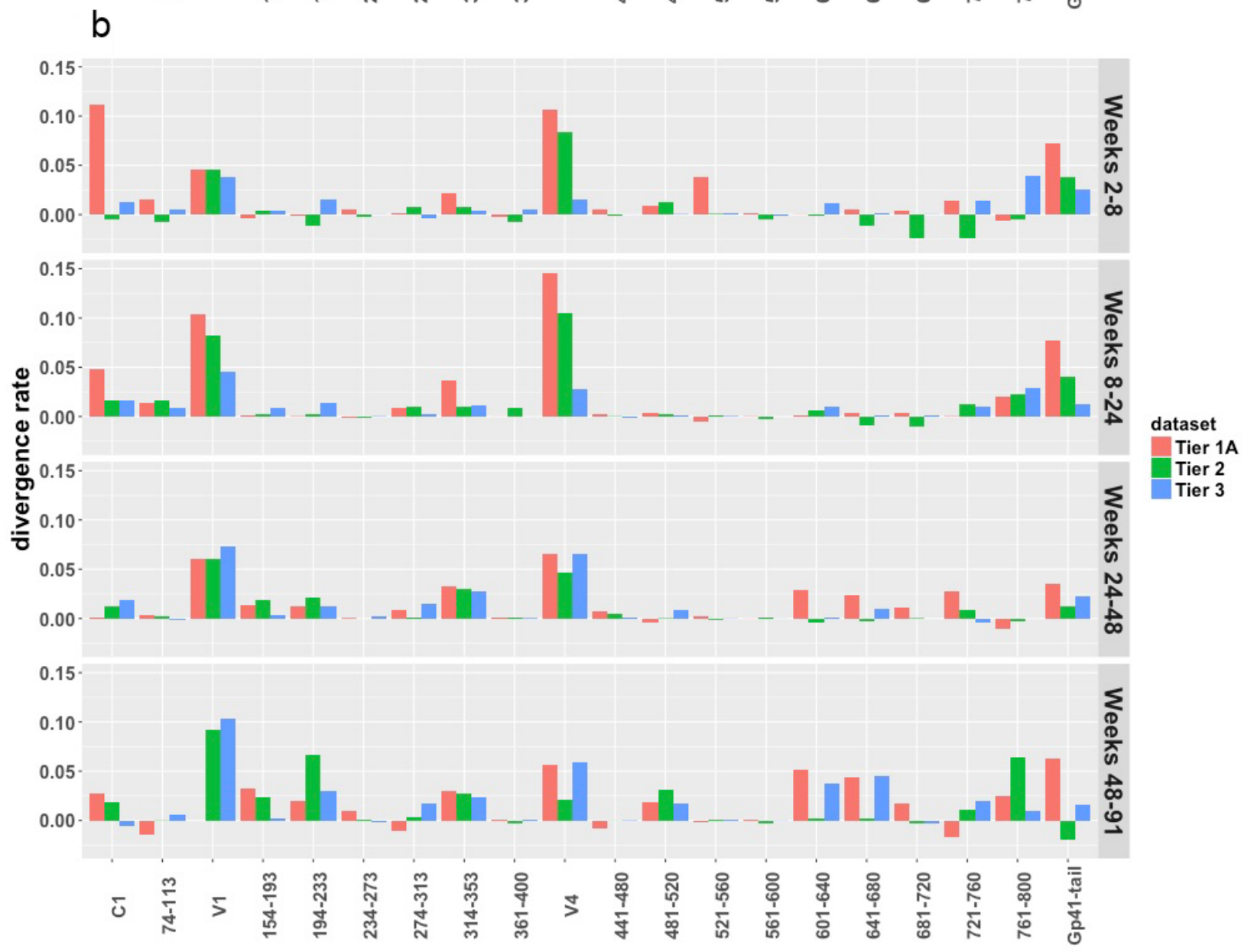| Viruses:	GenBank	SIVsmE660 infected RM					SIVmac251 infected RM	SIVsmm G932 infected RM	SIVagm infected AGM
		Rh422	RhB1	RhB2	RhB4	RhB5	pool sera	pool sera	pool sera
SIVsmE660-FL6/H805.16w.8	JQ864119	>43740	>43740	>43740	>43740	>43740	>43740	>43740	>43740
SIVsmE660-FL6/H806.24w.2	JQ864135	>43740	>43740	>43740	>43740	>43740	>43740	>43740	206
SIVsmE660-FL6/H806.24w.5	JQ864132	>43740	>43740	>43740	>43740	>43740	>43740	>43740	202
SIVsmE660-FL14/H807.4w.6	JQ864148	>43740	>43740	>43740	>43740	>43740	>43740	>43740	2935
SIVsmE660-FL14/H807.16w.9	JQ864163	>43740	>43740	>43740	>43740	>43740	>43740	>43740	1960
SIVsmE660-FL14/H808.24w.5	JQ864175	>43740	>43740	>43740	>43740	>43740	>43740	>43740	1743
SIVsmE660/BR-CG7G.IR1	JX648292	365716	>2343750	>2343750	>2343750	>2343750	>2343750	>2343750	3200
SIVmac251.6	Not available	513727	741907	453567	319258	623600	610	86	467
SIVsmE660-FL6/H805.24w.2	JQ864123	>43740	>43740	>43740	>43740	>43740	45	63	93
SIVsmE660-300-16	KC595653	>43740	>43740	>43740	>43740	>43740	20	27	49
SIVsmE660/CR54-PK-2A5	FJ578939	>43740	>43740	>43740	>43740	>43740	76	273	1933
SIVsmE660/BR-CG7V.IR1	JX648291	>43740	>43740	>43740	>43740	>43740	106	136	168
SIVsmE660-FL6/H806.24w.3	JQ864138	>43740	>43740	>43740	>43740	>43740	52	<20	<20
SIVsmE660-FL14/H807.24w.5	JQ864169	>43740	>43740	>43740	>43740	>43740	54	49	75
SIVsmE660-FL6/H805.16w.5	JQ864117	>43740	>43740	>43740	>43740	>43740	33	<20	<20
SIVsmE660-FL14/H807.16w.6	JQ864161	22	13190	>43740	>43740	>43740	22	33	42
SIVsmE660-FL6/H805.24w.3	JQ864124	<20	114	>43740	>43740	>43740	62	>43740	>43740
SIVsmE660-FL14/H807.8w.7	JQ864153	<20	50	>43740	>43740	>43740	23	38	96
SIVsmE660-FL14/H807.16w.3	JQ864158	<20	37	1140	93	>43740	<20	<20	24
SIVsmE660-FL14/H808.24w.1	JQ864176	<20	<20	>43740	>43740	>43740	79	88	114
SIVsmE660-FL6/H805.24w.8	JQ864129	34	30	863	47	817	<20	<20	<20
SIVsmE660-FL14/H807.24w.4	JQ864168	<20	<20	283	48	377	<20	<20	<20
SIVsmE660-FL14/H807.24w.7	JQ864170	<20	<20	399	81	6080	25	22	44
SIVsmE660-FL14/H808.24w.10	JQ864180	27	33	1997	51	1018	20	<20	35

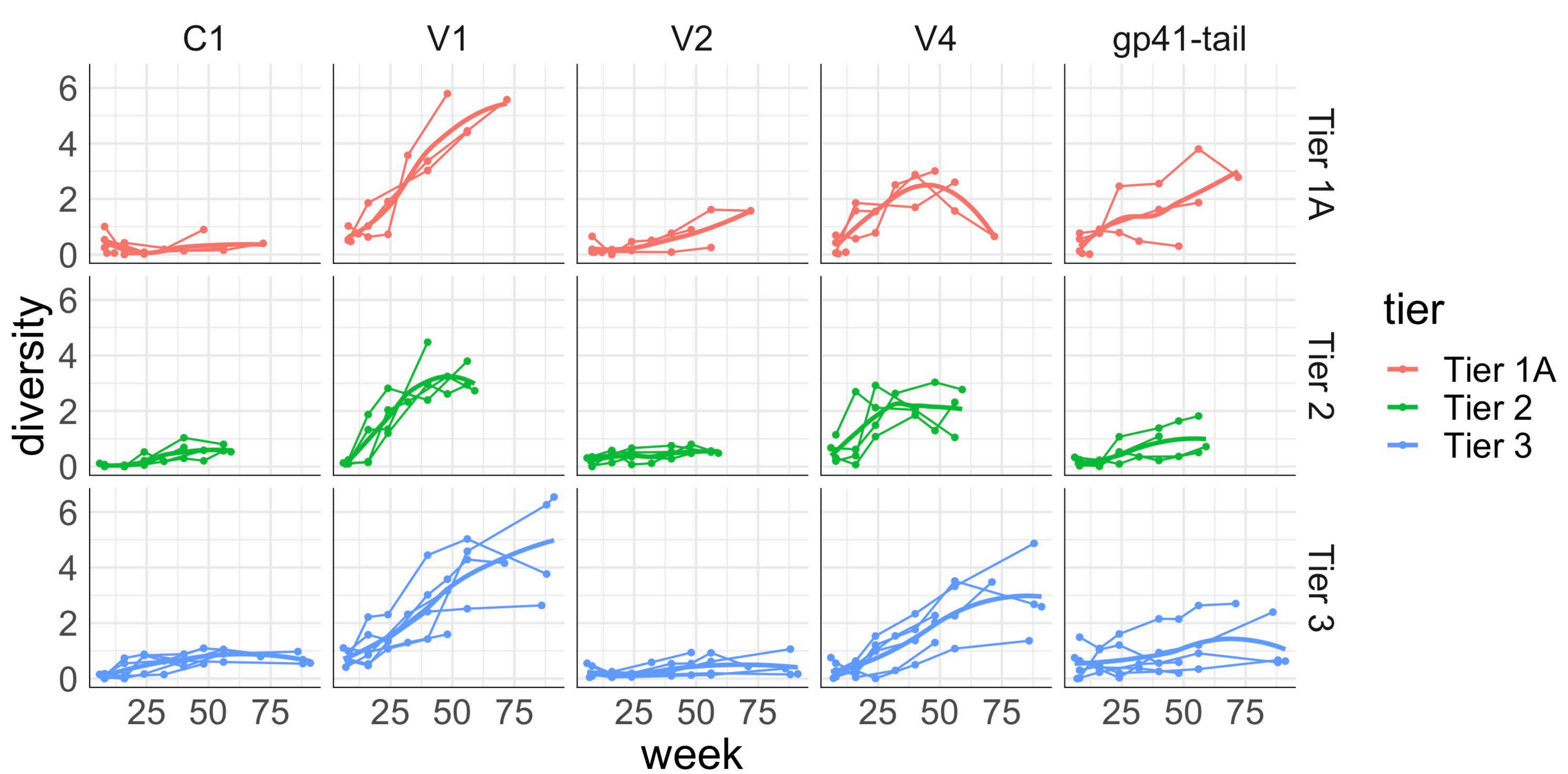**Supplementary Table S2. MHC genotype and gender of macaques**

Inoculation	Animal ID	MHC	M/F
SIVsmE660-FL14	Rh844	-	M
Tier 1A	Rh845	A02,B01	M
	Rh846	A02	M
	Rh847	**B08**	M
	Rh848	B01	M
	Rh849	-	F
SIVsmE660/H807-16w-6	Rh850	-	M
Tier 2	Rh851	A02,B01	M
	Rh852	B01	M
	Rh853	A02	M
	Rh854	-	F
	Rh855	A02	F
SIVsmE660/H807-24w-4	Rh856	-	M
Tier 3	Rh857	A02	M
	Rh858	B01	M
	Rh859	A08	M
	Rh860	A02,B01	F
	Rh861	**A01**	F