

**Figure 1.**

## Figure 2

(A)

(B)

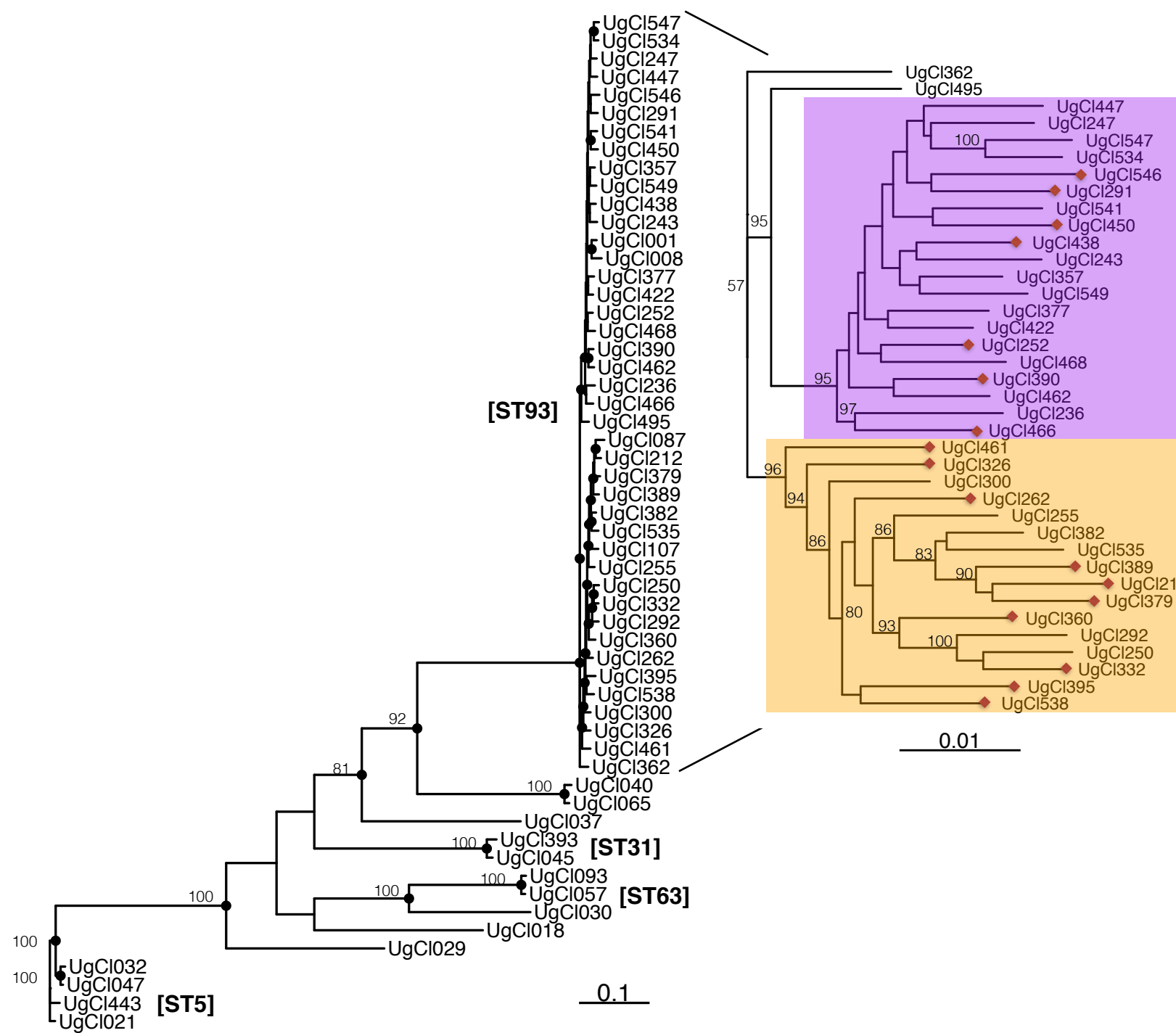
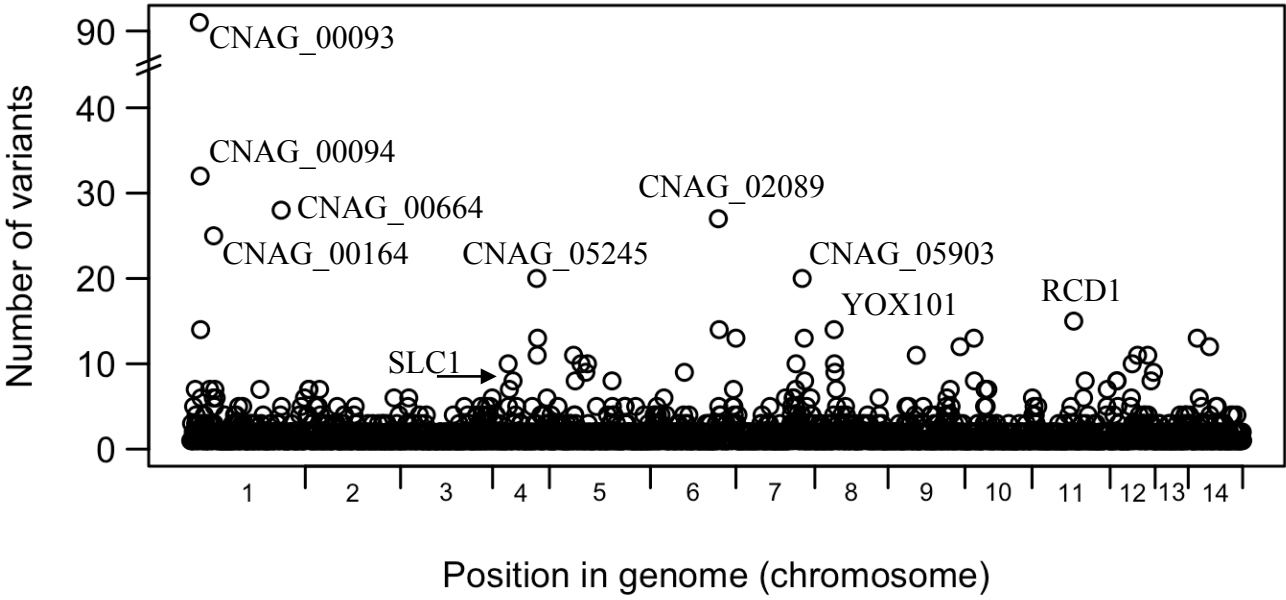


Figure 3.



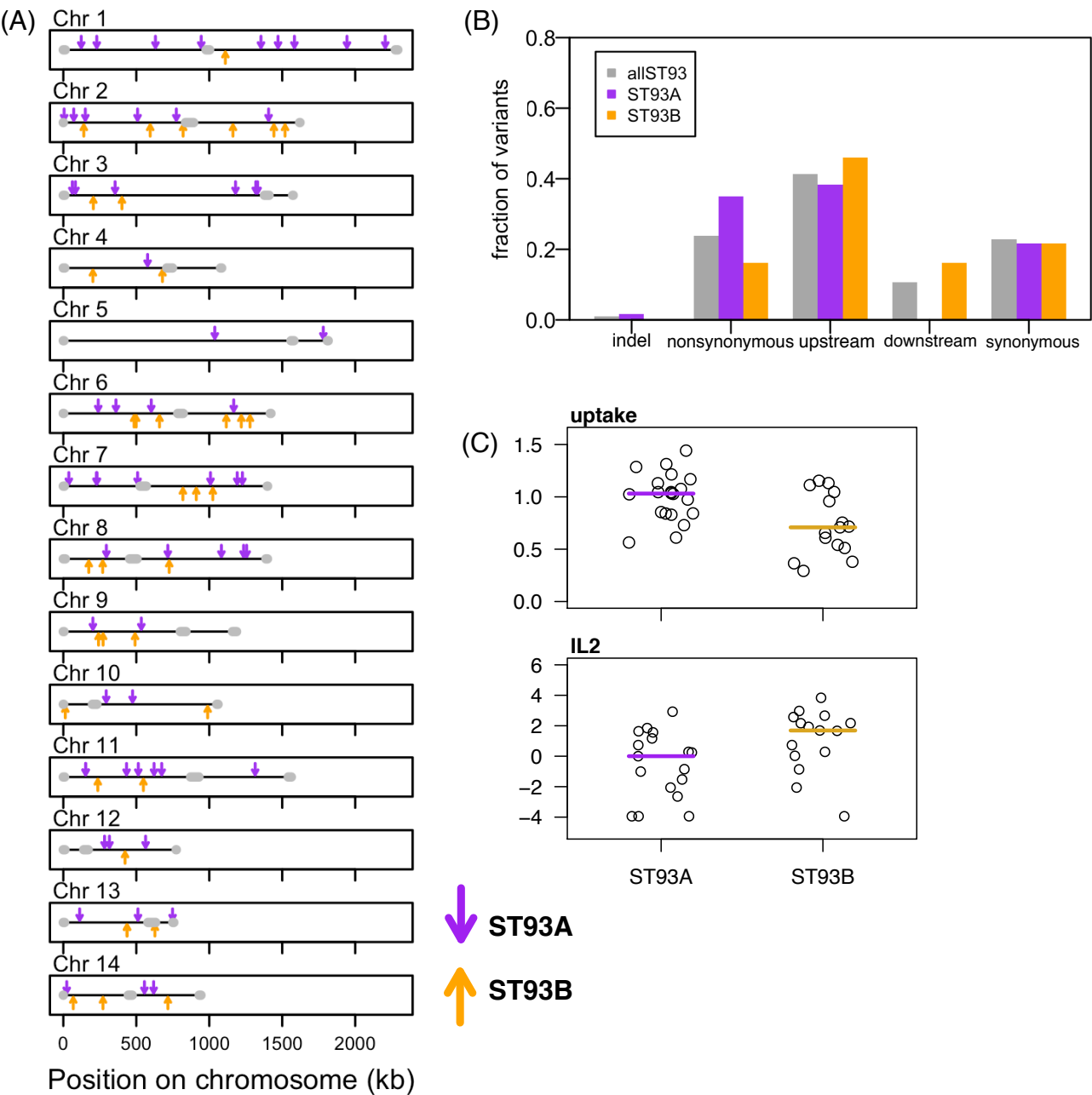


Figure 4

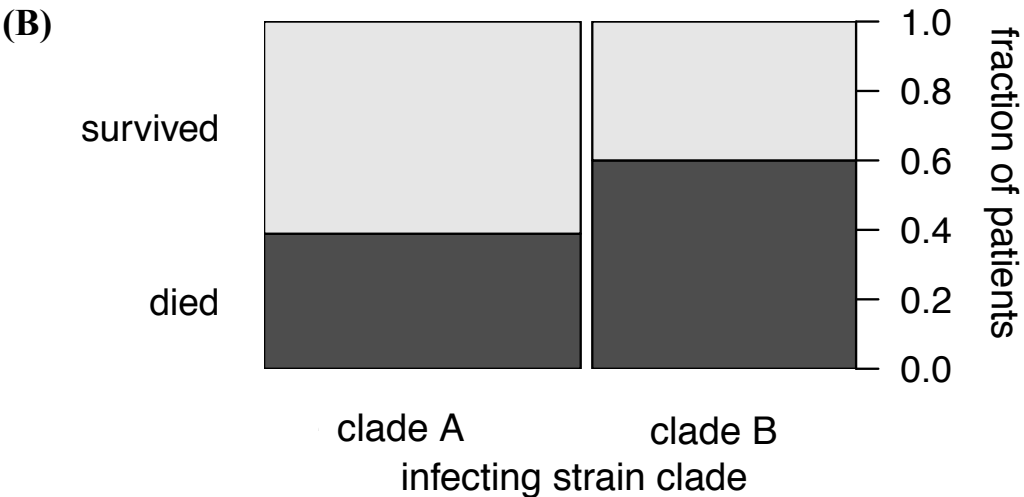
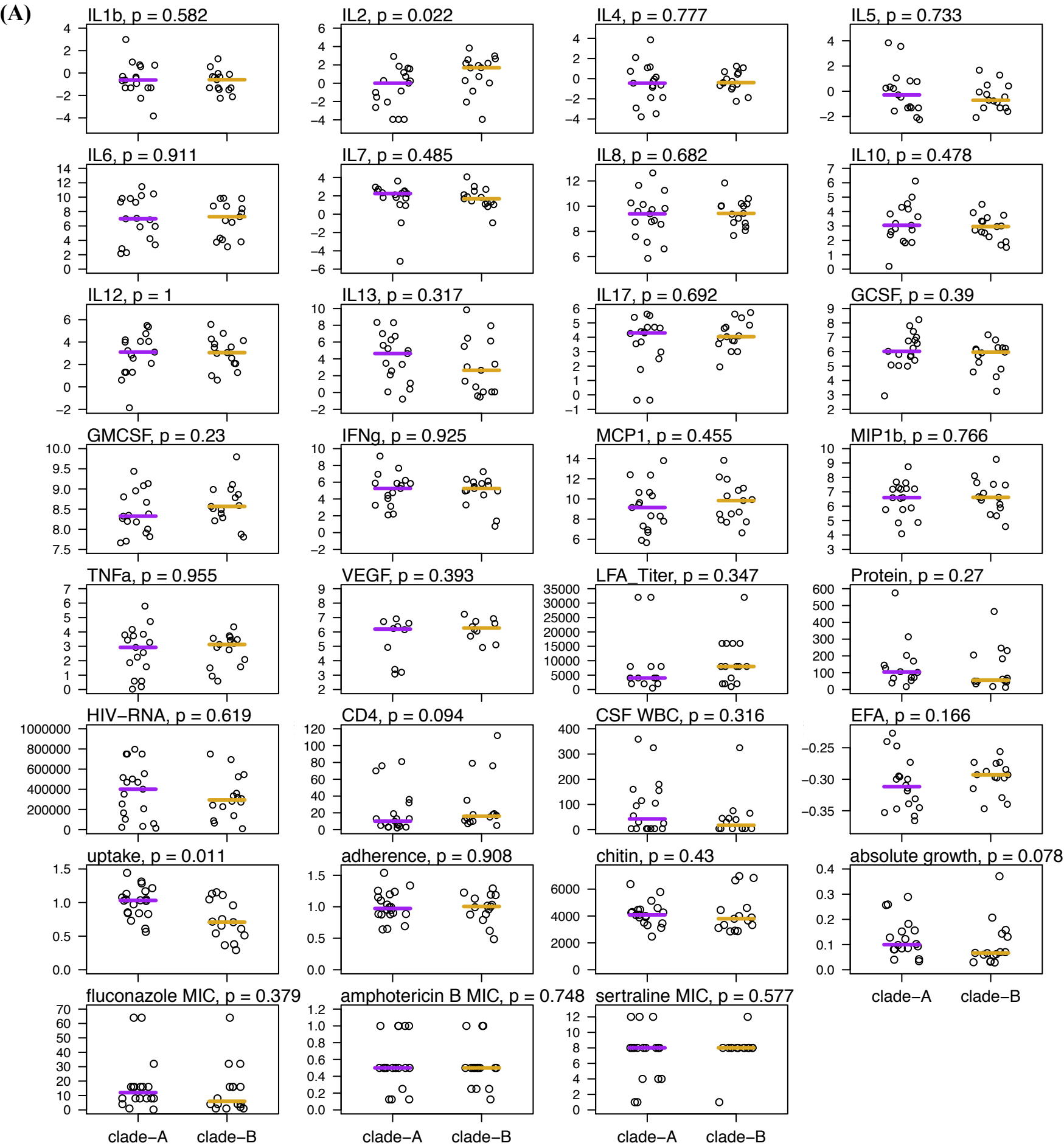
**Table 1:** Quantitative infections phenotypes measured from patients enrolled in the COAT trial (mortality, clinical, and immune response) and disease-associated phenotypes measured *in vitro*.

Class	Phenotype (n strains)
Mortality	Survival days (38)
Clinical	CD4 (38)
Clinical	CSF WBC (35)
Clinical	protein (31)
Clinical	HIV-viral (35)
Clinical	EFA (37)
Clinical	LFA titer (30)
Immune response	IL1-b (36)
Immune response	IL2 (36)
Immune response	IL4 (36)
Immune response	IL-5 (36)
Immune response	IL-6 (36)
Immune response	IL-7 (36)
Immune response	IL-8 (36)
Immune response	IL-10 (36)
Immune response	IL-12 (36)
Immune response	IL-13 (36)
Immune response	IL-17 (36)
Immune response	GCSF (36)
Immune response	GMCSF (36)
Immune response	IFNg (36)
Immune response	MCP1 (36)
Immune response	TNF $\alpha$ (36)
Immune response	MIP1b (36)
<i>in vitro</i>	macrophage uptake (38)
<i>in vitro</i>	Macrophage adherence (38)
<i>in vitro</i>	chitin (37)
<i>in vitro</i>	absolute growth at 30 ° (37)
<i>in vitro</i>	fluconazole MIC (37)
<i>in vitro</i>	amphoterecin B MIC (37)
<i>in vitro</i>	sertraline MIC (37)

**Table S5:** Phenotypes measured from patients enrolled in the COAT trial (clinical and cytokines) and *in vitro*.

Class	Phenotype	Lines with data missing
Clinical	LFA titer	UgCI390, UgCI447, UgCI450, UgCI461, UgCI462, UgCI466, UgCI468, UgCI495 UgCI541
Clinical	protein	UgCI447, UgCI450, UgCI461, UgCI462, UgCI466, UgCI468, UgCI495 UgCI541
Clinical	HIV-viral	UgCI360, UgCI395, UgCI447, UgCI547
Clinical	EFA	UgCI332, UgCI447
Clinical	CD4	UgCI447
Clinical	CSF WBC	UgCI360, UgCI395, UgCI447, UgCI547
Clinical	Survival	UgCI447
Cytokines	IL1-b	UgCI447, UgCI461, UgCI541
Cytokines	IL2	UgCI447, UgCI461, UgCI541
Cytokines	IL4	UgCI447, UgCI461, UgCI541
Cytokines	IL-5	UgCI447, UgCI461, UgCI541
Cytokines	IL-6	UgCI447, UgCI461, UgCI541
Cytokines	IL-7	UgCI447, UgCI461, UgCI541
Cytokines	IL-8	UgCI447, UgCI461, UgCI541
Cytokines	IL-10	UgCI447, UgCI461, UgCI541
Cytokines	IL-12	UgCI447, UgCI461, UgCI541
Cytokines	IL-13	UgCI447, UgCI461, UgCI541
Cytokines	IL-17	UgCI447, UgCI461, UgCI541
Cytokines	GCSF	UgCI447, UgCI461, UgCI541
Cytokines	GMCSF	UgCI447, UgCI461, UgCI541
Cytokines	IFNg	UgCI447, UgCI461, UgCI541
Cytokines	<b>MCP1</b>	UgCI447, UgCI461, UgCI541
Cytokines	<b>TNFα</b>	UgCI447, UgCI461, UgCI541
Cytokines	MIP1b	UgCI447, UgCI461, UgCI541
<i>in vitro</i>	uptake	UgCI212
<i>in vitro</i>	adherence	UgCI212
<i>in vitro</i>	chitin	UgCI212, UgCI549
<i>in vitro</i>	absolute growth	UgCI357, UgCI422
<i>in vitro</i>	fluconazole MIC	UgCI357, UgCI422
<i>in vitro</i>	amphoterecin B MIC	UgCI357, UgCI422
<i>in vitro</i>	sertraline MIC	UgCI357, UgCI422

**Figure S1. Clade-specific differences in phenotype.**  
 (A) cytokines, (B) clinical, (C) survival, (D) *in vitro* phenotypes. Coloured bars indicate the median value for illustrative purposes.



**Table S3.**Wilcoxon rank-sum results testing clade association with infection phenotypes.

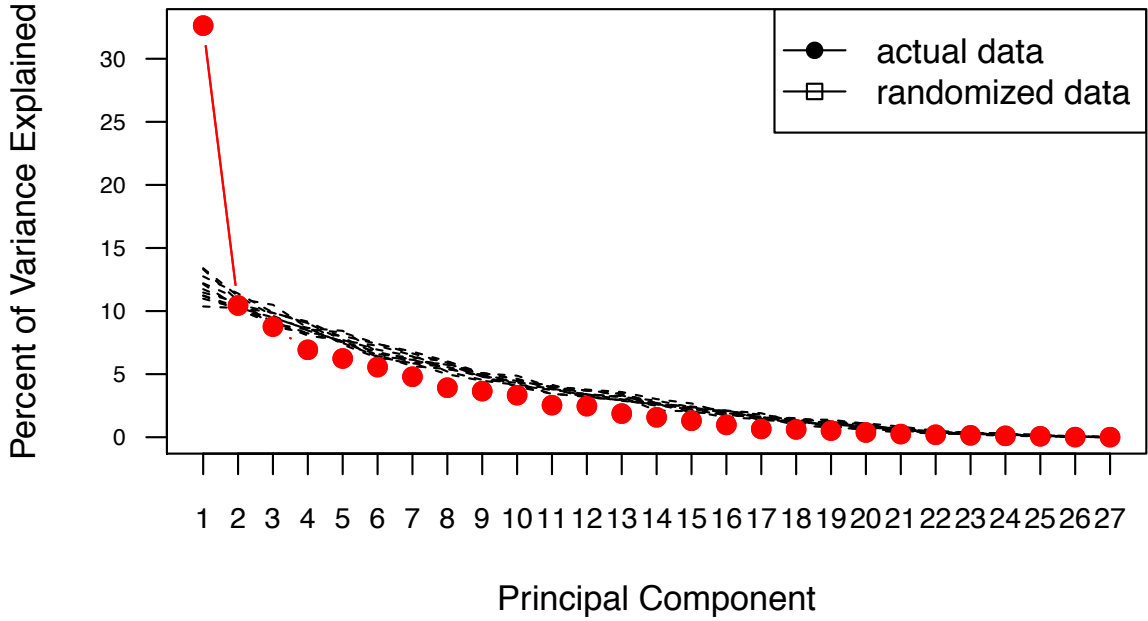
phenotype	W	pvalue
IL1b	142.5	0.582
IL2	66.5	0.022
IL4	119.5	0.777
IL5	137	0.733
IL6	131	0.911
IL7	146.5	0.485
IL8	116	0.682
IL10	147	0.478
IL12	127	1
IL13	154.5	0.317
IL17	116.5	0.692
GCSF	151	0.39
GMCSF	95	0.23
IFNg	124.5	0.925
MCP1	107	0.455
MIP1b	119	0.766
TNFa	129.5	0.955
VEGF	38	0.393
HIV-RNA	167.5	0.619
CD4	101	0.094
CSF WBC	152	0.316
EFA	96	0.166
uptake	226	0.011
adherence	154	0.908
chitin	166	0.43
Absolute.Growth	195.5	0.078
Fluconazole MIC	169.5	0.379
AmphotericinB MIC	153	0.748
Sertraline MIC	130.5	0.577



**Figure S2. PCA analysis.**

(A) Each dashed line represents one of 20 randomization trials. (B) There was no association between PC1 or PC2 and clade.

**(A)**



**(B)**

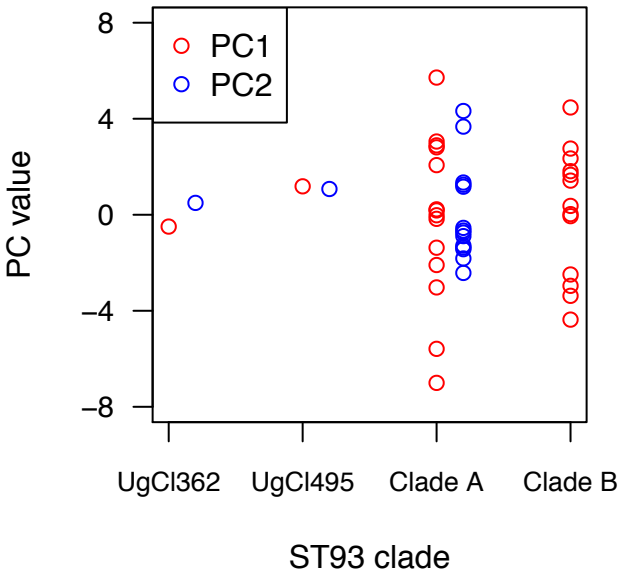


Table 2. Significant variants from logistic regression analysis against measured phenotypes.

gene	chr	# variants	effect	# phenotypes	phenotypes
CNAG_00014	1	3	ns	3	GCSF, GMCSF
CNAG_00363	1	2	ns	2	IL2
CNAG_07950	1	3	upstream	9	IL4,IL6,IL8,GMCSF,IFNg,FLC, efa, hivrna
CNAG_06704	2	1	upstream	2	IL2,LFA_Titer
CNAG_02798	3	1	upstream	3	protein, CD4, AMP
CNAG_05185	4	2	upstream	3	Survival, uptake
CNAG_06876	5	1	downstream	3	IFNg, MIP1b, TNFa
CNAG_01371	5	1	upstream	2	MCP1,hivrna
CNAG_01241	5	3	upstream	14	IL2,IL4,IL5,IL7,IL12,IL13,IL17,GCSF,GMCSF,TNFa,chitin
CNAG_02475	6	3	upstream	4	IL7,growth
CNAG_02176	6	12	downstream; ns; upstream	21	IL1b, IL2, IL10, IL13,MCP1,MIP1b, IL12, TNFa, survival, chitin, AMP, hivrna, SERT
CNAG_02177	6	1	upstream	3	IL1b,IL6,IL10
CNAG_02112	6	3	upstream	3	AMP
CNAG_06525	7	2	ns ; upstream	4	IL5,IL6,IL8,IL10
CNAG_12610	7	1	upstream	2	MCP1,uptake
CNAG_06574	7	12	upstream	19	IL1b,IL2,IL13,TNFa,MIP1b,GCSF,GMCSF,survival,efa,growth,hivrna,uptake, CD4, protein
CNAG_05913	7	2	upstream	6	MIP1b,adherence; IL13,IL17
CNAG_05937	7	3	upstream	4	uptake,SERT
CNAG_07703	7	1	ns	2	IL6,IL8
CNAG_06968	8	1	indel	2	IL12,IL17
CNAG_04100	9	3	upstream	6	adherence,FLC,SERT,growth,efa
CNAG_04102	9	1	downstream	2	GMCSF,efa
CNAG_04179	9	1	upstream	2	efa,SERT
CNAG_04373	9	2	upstream	3	IL8,efa; Survival
CNAG_04535	9	1	upstream	3	IL17,GCSF,LFA_Titer
CNAG_07837	10	3	upstream; downstream	3	IL2,csf_wbc,CD4
CNAG_04922	10	7	upstream	7	IL2,adherence
CNAG_08006	11	2	upstream	11	IL4,IL5,IL6,MIP1b,TNFa,adherence,chitin,IFNg,MCP1
CNAG_01802	11	3	upstream	3	csf_wbc; IL2; IL7
CNAG_07026	12	7	upstream	19	IL1b,IL13,Survival,efa,IL7
CNAG_05987	12	6	nonyonymous; indel; upstream	8	IL2 chitin,efa,adherence
CNAG_06169	12	10	downstream	23	IL6,IL8,IL10,IL12,IL13,GCSF,GMCSF,MIP1b,growth,hivrna,CSF WBC,chitin
CNAG_06256	13	2	upstream	3	IFNg,TNFa
CNAG_13108	13	3	upstream	6	IL13,GCSF
CNAG_06332	13	3	upstream	3	adherence; efa
CNAG_06422	13	2	upstream	2	IL2
CNAG_06490	13	1	indel	3	protein,hivrna,cd4
CNAG_05450	14	1	ns	6	IL6,IL7,IL12,IL13,GCSF,MIP1b
CNAG_05661	14	6	upstream	17	IL1b,IL6,IL8,GMCSF,IFNg,MCP1,MIP1b,uptake,FLC,adherence,hivrna
CNAG_05663	14	3	downsream	5	IL1b,IL13,TNFa,Survival
CNAG_05662	14	16	downstream	34	IL2,IL5,IL8,IL12,IL13,IL17,GMCSF,MCP1,TNFa,MIP1b,adherence,AMP,Survival,FLC,growth,SERT,uptake
CNAG_13204	14	4	upstream	4	GMCSF; IL13

Table 3: Significant variants from logistic regression analysis against PCA1 and PCA2.

<b>gene</b>	<b>chr</b>	<b>pos</b>	<b>effect</b>	<b>PCA1 <i>p value</i></b>	<b>PCA2 <i>p value</i></b>
CNAG_07950	1	975212	upstream	0.047	0.141
CNAG_01241	5	836697	upstream	0.040	0.505
CNAG_01241	5	836899	upstream	0.025	0.290
CNAG_02176	6	988733	STOP_GAINED	0.047	0.749
CNAG_02176	6	989490	ns	0.834	0.030
CNAG_02176	6	989960	ns	0.967	0.039
CNAG_07703	7	1341024	ns	0.031	0.289
CNAG_07727	8	818838	upstream	0.036	0.726
CNAG_08006	11	804710	UTR-5	0.048	0.312
CNAG_05987	12	19741	upstream	0.355	0.031
CNAG_06169	12	503321	UTR-3	0.048	0.795
CNAG_05450	14	342562	ns	0.024	0.142
CNAG_05661	14	908850	upstream	0.042	0.928
CNAG_05663	14	910328	downstream	0.042	0.120
CNAG_05662	14	911099	downstream	0.045	0.143
CNAG_05662	14	911129	downstream	0.048	0.046

Figure 5.

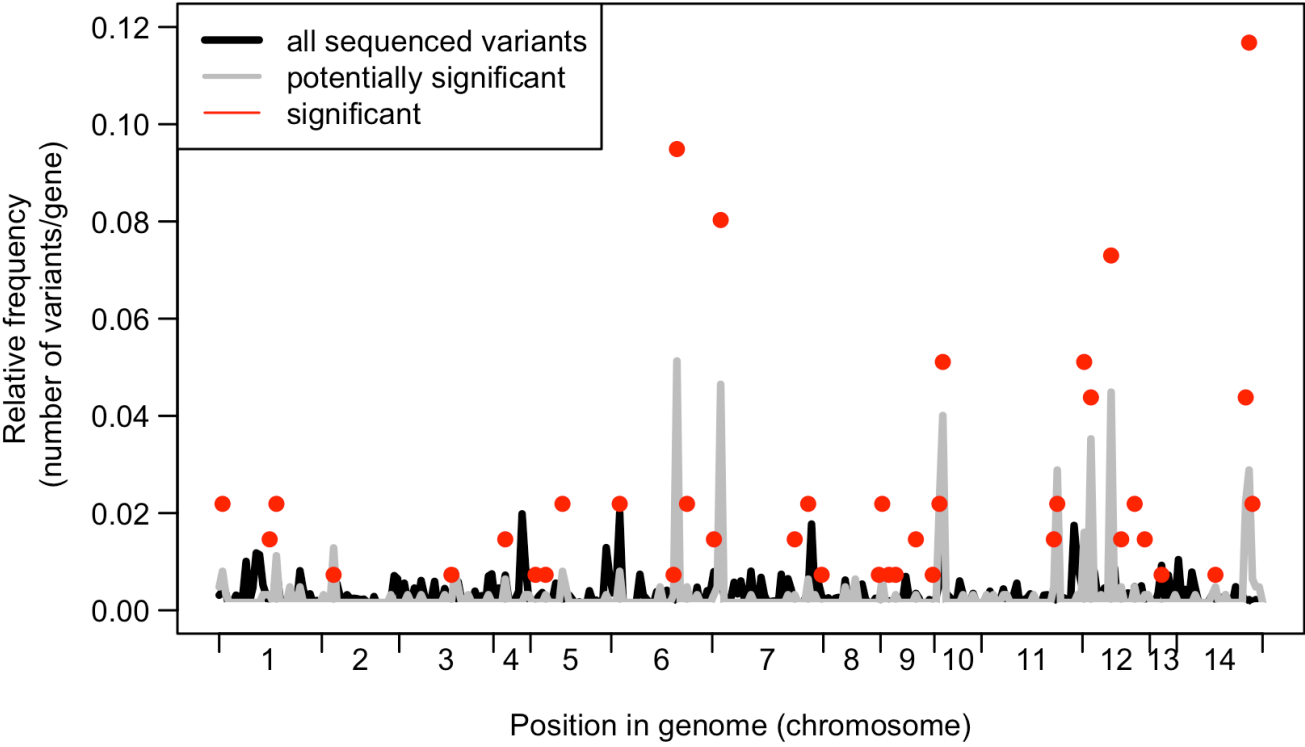


Figure 6.

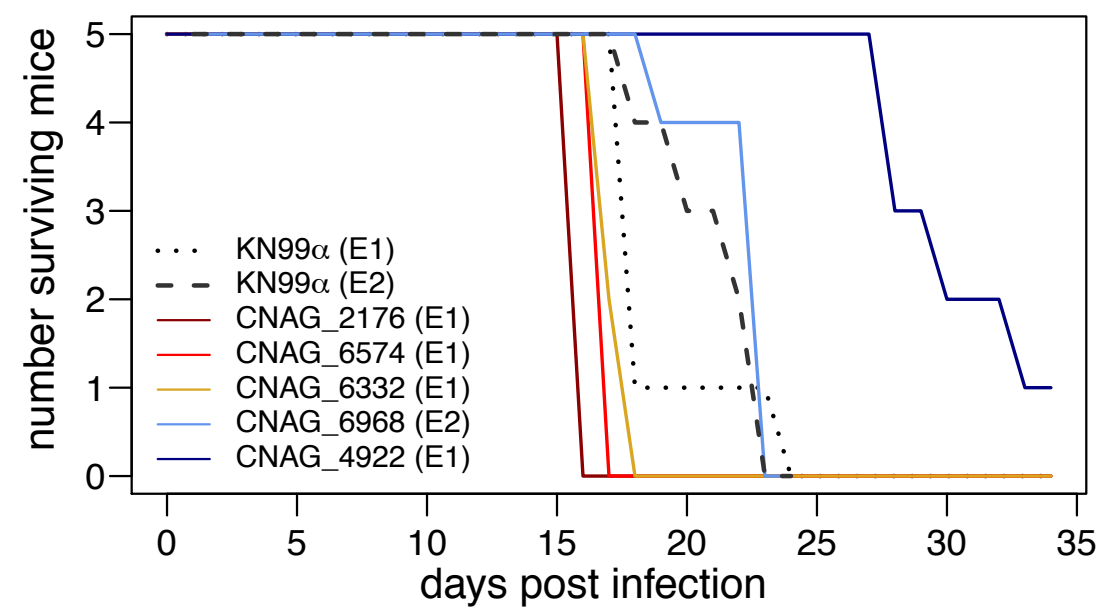
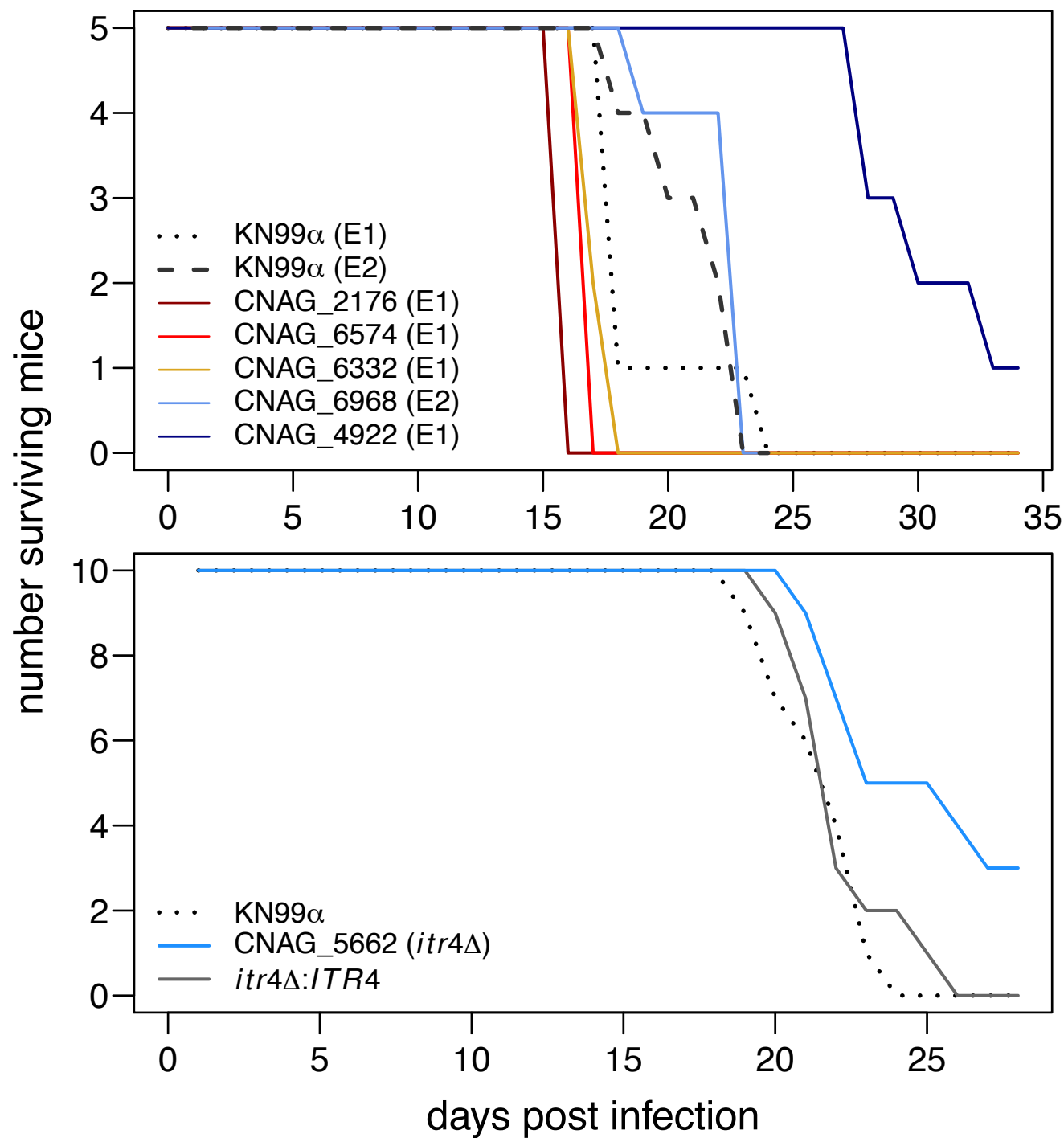
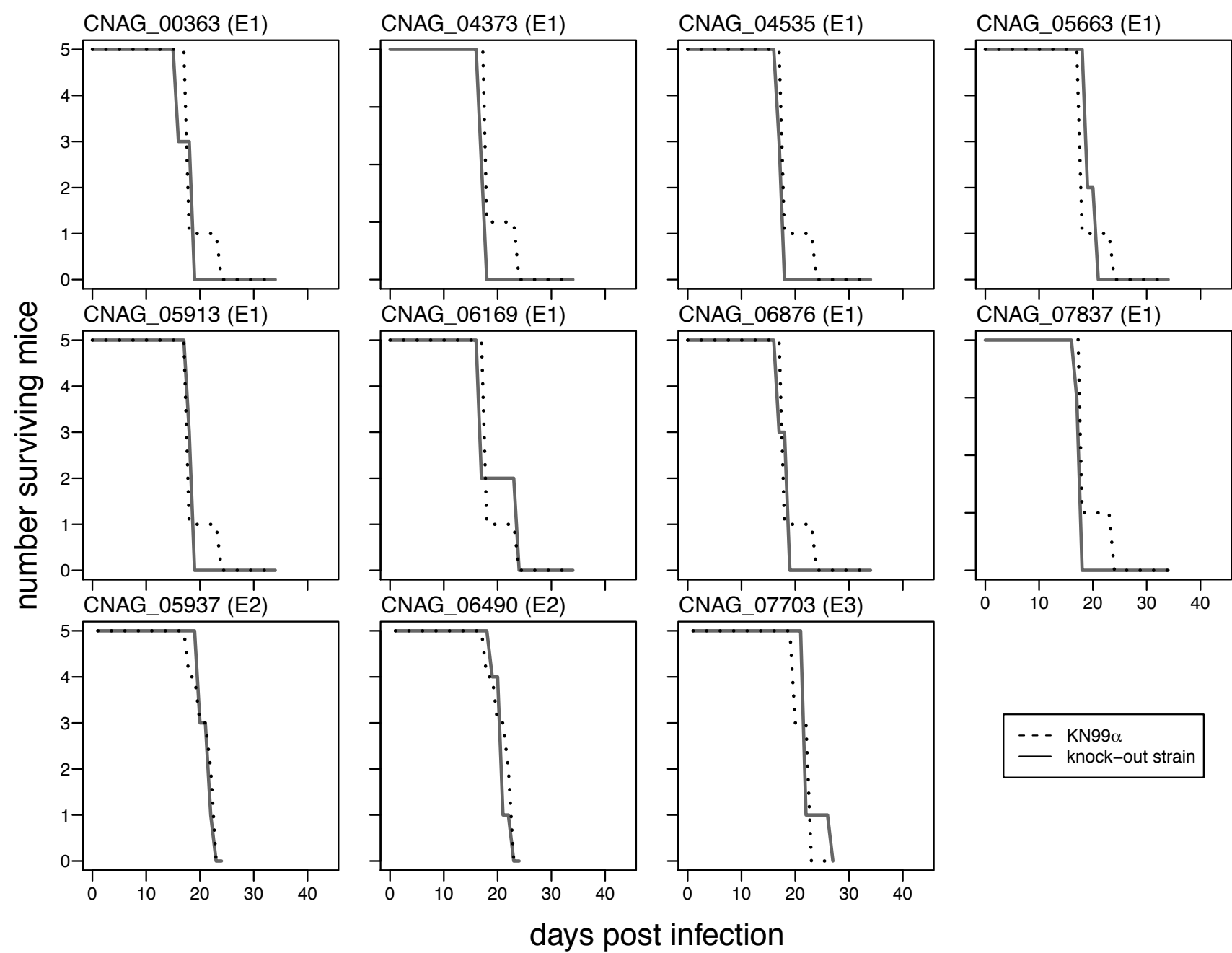


Figure 6. Knockout strain virulence in mice. Six tested knockout strains had a significant virulence influence compared to KN99. A) Five strains were identified in experiments 1 (E1) and 2 (E2). B) The knockout of CNAG\_05662 (ITR4) was confirmed to have reduced virulence compared to the control KN99 $\alpha$  strain and the ITR4 knockout strain with the ITR4 complement.



**Figure S3. Knockout strain virulence in mice.**

Eleven tested knockout strains did not have a significant influence on virulence compared to the control KN99 $\alpha$  strain. The KN99 $\alpha$  curves differ slightly depending on the experiment; knockout strains are always compared against KN99 $\alpha$  from the same experiment.



**Table 3.** Survival comparison of each knockout strain against KN99 $\alpha$ . Chisq and p values from the G-rho family log-rank test.

Strain	Chisq	pvalue
CNAG_00363 (tco6 $\Delta$ )	0.05	0.82
CNAG_02176	9.0	0.003
CNAG_04373	3.07	0.08
CNAG_04535	2.79	0.095
CNAG_04922	9.97	0.002
CNAG_05662 (itr4 $\Delta$ )	6.22	0.013
CNAG_05663	0.61	0.43
CNAG_05913	0.073	0.79
CNAG_05937	0.089	0.77
CNAG_06169	0.13	0.72
CNAG_06332	4.05	0.044
CNAG_06574 (app1 $\Delta$ )	9.00	0.0027
CNAG_06704	5.83	0.016
CNAG_06878	0.054	0.82
CNAG_06490	1.02	0.31
CNAG_06968	7.0	0.0082
CNAG_07703	0.054	0.80
CNAG_07838	1.8	0.18
itr4 $\Delta$ :ITR4	0.51	0.47