Supplementary information Figures and Tables for

Rapid expansion and international spread of $M1_{UK}$ in the post-pandemic UK upsurge of *Streptococcus pyogenes*

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Supplementary Table 1 – Signature SNPs of three emergent $M1_{UK}$ clades identified during iGAS upsurge

	Location	Locus	Gene	Product	Genetic change Synonymous (S) Non-synonymous (NS)	Protein change	Allele in $M1_{UK}$ Clade of interest	Allele in remaining $M1_{UK}$	Allele in $M1_{global}$
$M1_{UK}$ Clade 1 (123 strains)	359672	M5005_Spy0353*	x	putative membrane spanning protein	STOP 28C>T	Gln10*	T	C	C
	365403	M5005_Spy0360	x	NAD-dependent oxidoreductase	S 444C>T	Asp148Asp	T	C	C
$M1_{UK}$ Clade 2 (166 strains)	1514811[Ψ]	Intergenic region			Intergenic	Intergenic	T	C	C
	1681178[β]	M5005_Spy1718	*sic1.01*	streptococcal inhibitor of complement	NS 770A>T	Gln257Leu	A	T	T
	711809	M5005_Spy0709	*pyrC*	dihydroorotase	NS.509A>C	Asn170Thr	C	A	A
	727058	M5005_Spy0723	*hflX*	GTP-binding protein	S.813C>T	Thr271Thr	T	C	C
	47867	M5005_Spy0029	*purD*	phosphoribosylamine—glycine ligase	S 840C>T	Ile280Ile	T	C	C
	1122436	M5005_Spy1146		DNA polymerase III, delta subunit	NS.643G>A	Asp215Asn	T	C	C
$M1_{UK}$ Clade 3 (285 strains)	899440	M5005_Spy0913	*xerD*[Ω]	recombinase	NS791C>T	Ala264Val	A	G	G
	1732547	M5005_Spy1771	*hutU*	urocanate hydratase	NS .98G>T	Arg33Ile	T	G	G
	1493521	M5005_Spy1534	*secA*	protein translocase subunit	NS.1331A>G	Asp444Gly	C	T	T

SNP annotation made using snippy and MGAS5005 (CP000017.2) as a reference.
[†]MGAS5005 (NC_007297) has a different annotation in this location: Intergenic region upstream of M5005_Spy0353;
[‡]One strain in remaining $M1_{UK}$ group had missing data;
[§]One strain in remaining $M1_{UK}$ group and one strain in the $M1_{global}$ group had a large deletion in *sic*.
[Ω]MGAS5005 (NC_007297) has a different annotation in this location, *xerS*.
[X]no assigned gene name

Supplementary Table 2 - SNP variation observed within different $M1_{UK}$ and $M1_{global}$ groups.

	Groups	No. of isolates analysed	No. of SNPs (mean)	No. of SNPs (median)	Interquartile range	Max no. of SNPs	Min no. of SNPs
$M1_{global}$	$M1_{global\ 2013\text{-}2021}$	143	32	34	19.8-50.0	79	0
	$M1_{global2022\text{-}2023}$	46	45	55	38.0-51.7	73	0
	All $M1_{global}$	189	45	34	22.0-50.5	73	0
$M1_{UK}$	$M1_{UK2013\text{-}2021}$	551	15	16	5.9-24.0	39	0
	$M1_{UK2022\text{-}2023}$	1001	22	22	17.9-25.4	50	0
	All $M1_{UK}$	1552	17	17	18.2-24.4	50	0
	$M1_{UK}$ Clade1	123	2	2	1.2-3.0	10	0
	$M1_{UK}$Clade2	166	3	3	2.3-3.7	13	0
	$M1_{UK}$Clade3	285	4	3	2.9-4.8	11	0

Supplementary Table 3 – Clinical source of invasive disease *emm1* strains used in this study (2013 to 2023)

	Overall 2013-2023			2013-2021			2022-2023		
Specimens from invasive disease	**$M1_{UK}$**	**$M1_{global}$**	**Inter†**	**$M1_{UK}$**	**$M1_{global}$**	**Inter†**	**$M1_{UK}$**	**$M1_{global}$**	**Inter†**
Blood	1069	124	36	356	95	16	713	29	20
Lower Respiratory Tract* (pleural samples)	108 (78)	10 (5)	7 (6)	24 (11)	4 (2)	1 (1)	84 (67)	6 (3)	6 (5)
Invasive others¶	194	29	12	69	21	6	125	8	6
Swab samples‡	181	26	19	102	23	6	79	3	13
Total	1552	189	74	551	143	29	1001	46	45

†Inter category includes intermediate strains ($M1_{13SNPs}$, $M1_{23SNPs}$, $M1_{22SNPs}$, $M1_{19SNPs}$) and derivatives of $M1_{UK}$ ($M1_{26SNPs}$)
*Samples from lower respiratory tract include pleural. Subset of isolates from pleural samples shown in parentheses
¶Invasive others include samples from joints (n=22); central nervous system (n=10); viscera (n=13); tissue or fluid (n=137); information not provided (n=39); others (14)
‡Swab samples include skin, wound, vagina, upper respiratory tract

Supplementary Table 4 – *emm1* isolates from lower respiratory tract samples and pleural samples: association with *emm1* sub lineages over time.

Lineages	Proportion of each lineage from lower respiratory tract samples			Proportion of each lineage from pleural samples*		
	Pre-upsurge 2013-2021	Upsurge 2022/2023	Overall 2013-2023	Pre-upsurge 2013-2021	Upsurge 2022-2023	Overall 2013-2023
$M1_{global}$	2.7% (4/143)	13.0% (6/46)	5.2% (10/189)$^{\Psi}$	1.4% (2/143)	6.5% (3/46)	2.6% (5/189)§
$M1_{UK}$	4.3% (24/551)	8.3% (84/1001)	6.9 % (108/1552)	2.0% (11/551)	6.7% (67/1001)	5.0% (78/1552)
$M1_{UK}$ separated by new clades						
$M1_{UK}$ Clade1 (123 strains)	nil	7.3% (9/123)	7.3% (9/123)	nil	6.5% (8/123)	6.5% (8/123)§
$M1_{UK}$ Clade2 (166 strains)	nil	7.8% (13/166)	7.8% (13/166)	nil	6.6% (11/166)	6.6% (11/166)§¶
$M1_{UK}$ Clade3 (285 strains)	nil	10.2% (29/285)	10.2% (29/285)$^{\Psi}$	nil	8.4% (24/285)	8.4% (24/285)§¶
$M1_{UK[excluding\ clades\ 1,2,3]}$ (978 strains)	nil	7.7% (33/427)	5.8% (57/978)$^{\Psi}$	nil	5.6% (24/427)	3.5% (35/978)¶

*****Isolates from pleural samples are a subset of lower respiratory tract isolates, collected from pleural empyema.

§Difference between new $M1_{UK}$ clades and $M1_{global}$ empyema isolates significant ($M1_{uk}$ clade1 vs $M1_{global}$ p=0.047; $M1_{uk}$ clade2 vs $M1_{global}$ p=0.036; $M1_{uk}$ clade3 vs $M1_{global}$ p=0.005).

¶Difference between new $M1_{UK}$ clades and $M1_{UK[excluding\ clades\ 1,2,3]}$ empyema significant ($M1_{uk}$ clade2 vs $M1_{UK[excluding\ clades\ 1,2,3]}$ p=0.032; $M1_{uk}$ clade3 vs $M1_{UK\ [excluding\ clades\ 1,2,3]}$ p<0.001)

$^{\Psi}$Difference between $M1_{uk}$clade3 and $M1_{global}$ significant (p= 0.029); difference between $M1_{uk}$ clade3 and $M1_{UK[excluding\ clades\ 1,2,3]}$ significant (p=0.005).

Statistical comparison between proportions was undertaken using a one-tailed proportion test between pairs of values using either $M1_{global}$ as the comparator or using $M1_{UK[excluding\ clades\ 1,2,3]}$ as the comparator. No adjustment for multiple comparisons was made.

Supplementary Table 5 – Differences in phage content in 1815 *emm1* invasive strains

Phage	Phage change	No. isolates affected $^{\Omega}$	Proportion of $M1_{global}$ strains	Proportion of $M1_{UK}$ strains	Superantigen change	DNAse change
Φ5005.1	Partial deletion	11	2/189	9/1552	*speA* absent	no change
Φ5005.2	Partial deletion*	10	8/189	2/1552	no change	*spd3* present
Φ5005.2	Partial deletion*	55	6/189	49/1552	no change	*spd4* present/*spd3* absent
Φ5005.2	Present recombinant[#]	1	0/189	1/1552	no change	*spd3* present
Φ5005.3	Complete absence	45$^{\Delta}$	1/189	43/1552	no change	*sda2* absent
ΦSP1380.vir	Present	4	1/189	3/1552	*speC* and *ssa* present	*spd1* present
Φ370.1	Present	172$^{\Psi}$	30/189	138/1552	*speC* present	*spd1* present

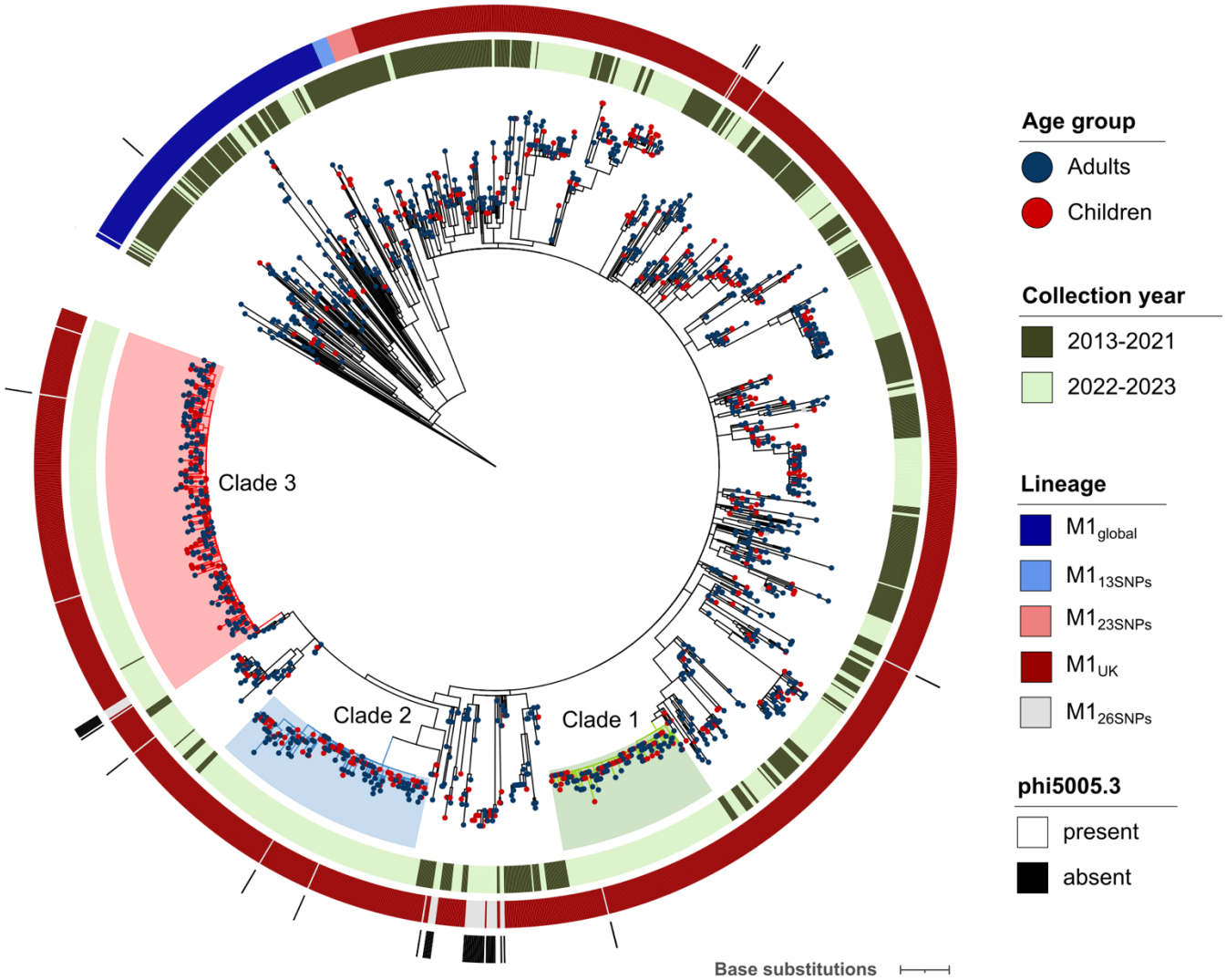
* Different versions of partially deleted phage.

[#] Recombinant version of phi5005.2 phage.

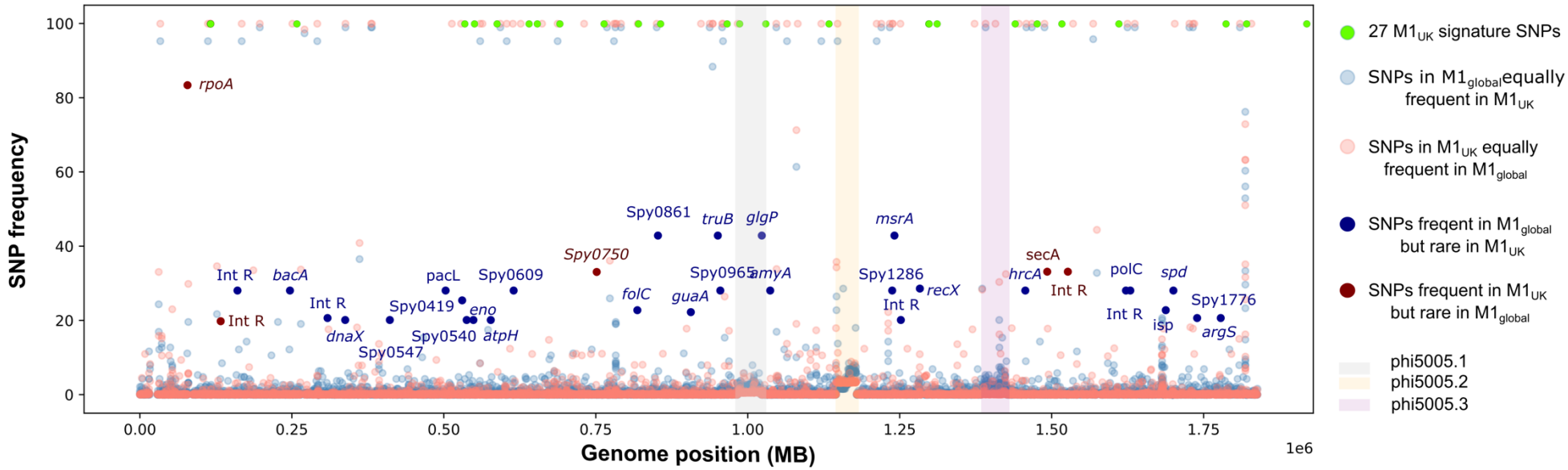
Δ Includes one intermediate strain ($M1_{22SNPs}$) derived from $M1_{23SNPs}$ that also lost Φ5005.3 phage.

Ψ Includes one intermediate strain ($M1_{22SNPs}$, derived from $M1_{23SNPs}$) and three $M1_{26SNPs}$ strains with Φ370.1 phage.

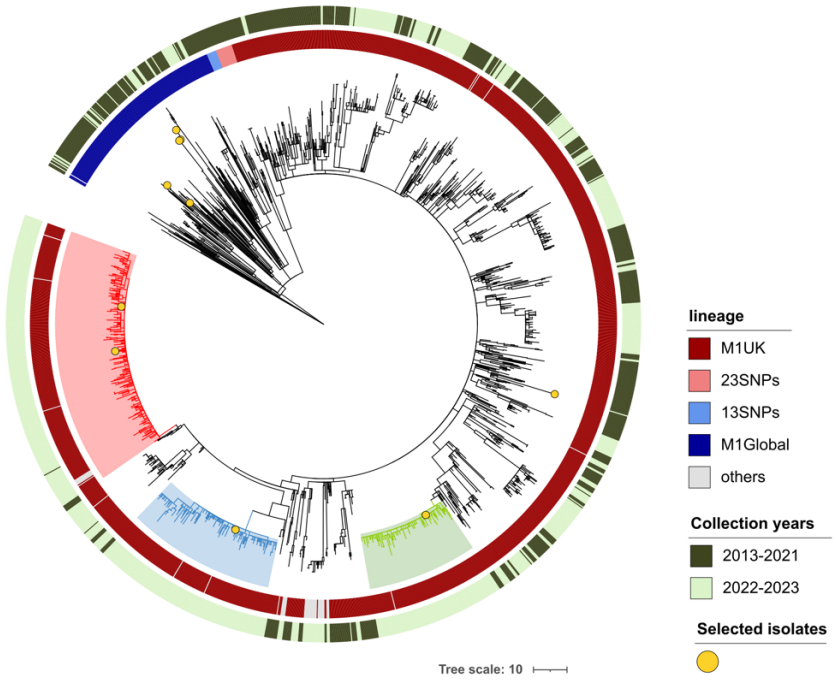
Ω Denominator includes all 1815 invasive isolates, 74 of which were intermediate strains.



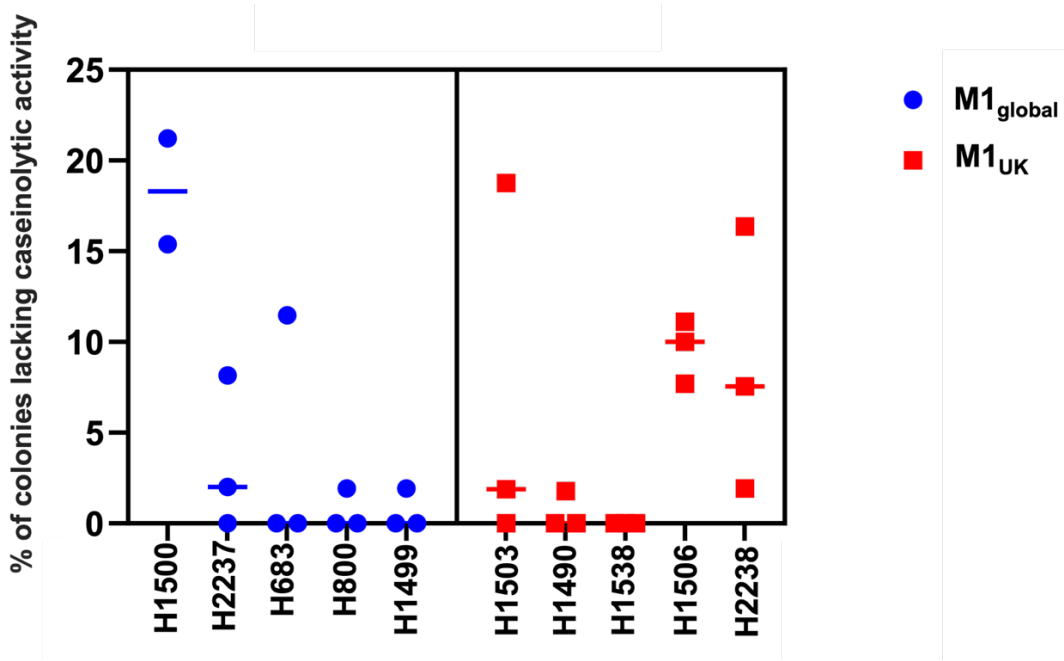
Supplementary Figure 1 - *emm1* phylogenetic tree showing isolates from iGAS infections in children (< 15 years) intermixed with isolates from iGAS infections in adults (2013 to 2023). Maximum likelihood phylogenetic tree constructed from 278 core SNPs (excluding recombination regions) extracted after mapping 1815 *emm1* isolated to the MGAS5005 reference genome. The tree was drawn in a circular layout and rooted on outgroup genome NCTC8198. Bars in concentric circles (from inside to outside) are coloured by collection years (pre-upsurge 2013-2021 and upsurge 2022-2023); *emm*1 lineages; and presence-absence of the phi5005.3 phage. The branch tips represent age group (adult or child<15y). Source data are provided as a Source Data file Supplementary Figure 1.



Supplementary Figure 2 – Differences between lineages in SNP distribution across 1815 *emm1* genomes mapped against MGAS5005 (CP000017.2)**.** The percentage of strains with a specific SNP mutation are indicated. $M1_{UK}$ signature SNPs are coloured in green; SNPs present in both lineages are coloured with a pale colour; SNPs occurring with high frequency (present in >20%) in one of the lineages but rare in the other lineage (frequency < 0.5%) are indicated with darker colours. Int R- indicates Intergenic region. Data processed using python v 3.11.6. script. Resolution of individual SNPs is limited due to Figure scale hence some individual SNPs and position of individual SNPs relative to phages may overlap. Source data are provided as a Source Data file Supplementary Figure 2.

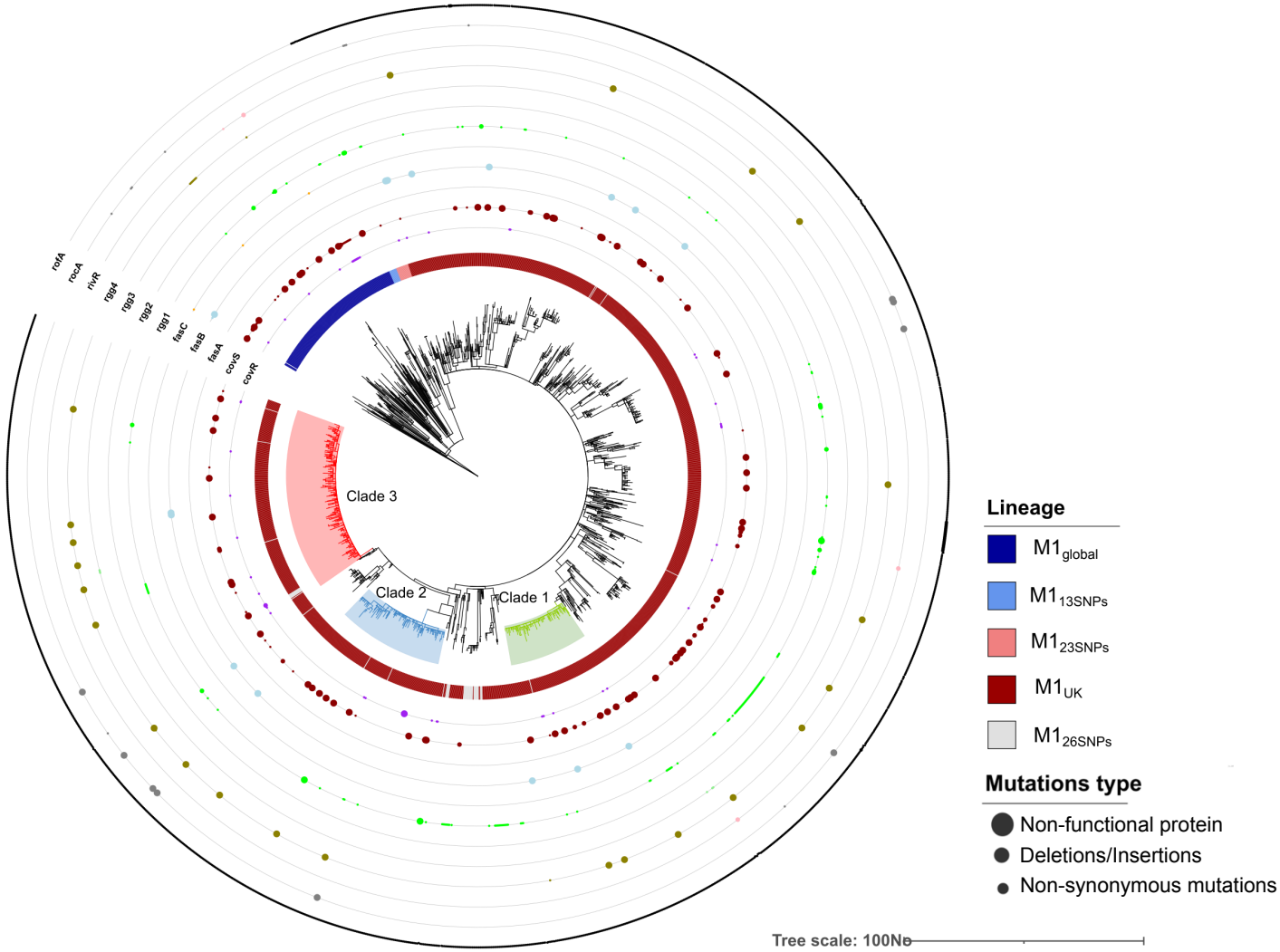


A.

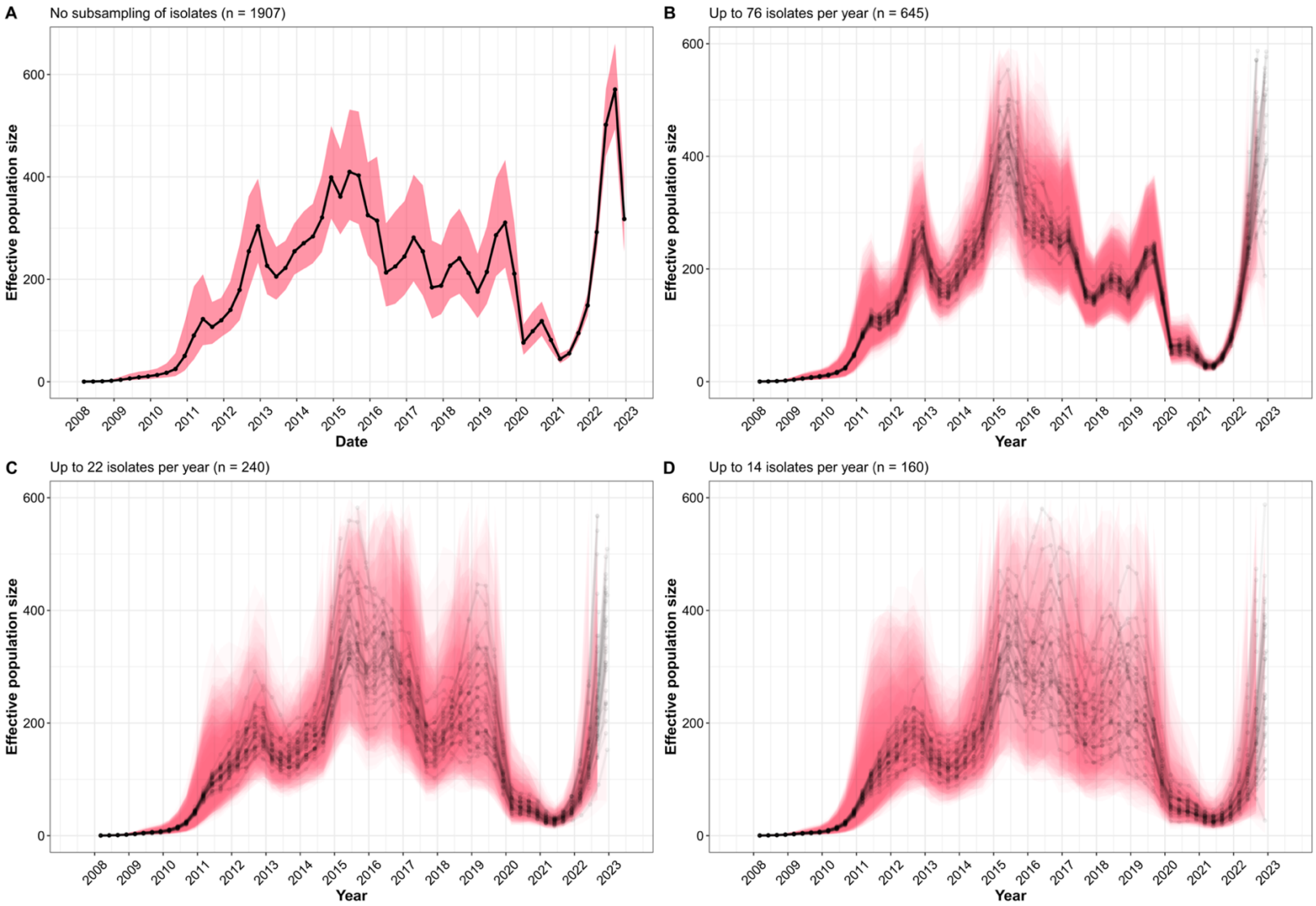


B.

Supplementary Figure 3 Frequency of change in caseinolytic activity after *in vivo* passage of panel of $M1_{global}$ and $M1_{UK}$ strains.
Ten *S. pyogenes emm*1 strains dating from 2022, that lacked any pre-existing *covS* or *covR* mutation, were selected as representatives of each lineage (5 strains from each of $M1_{global}$ and $M1_{UK}$) as indicated within the *emm*1 phylogenetic tree (A). *S. pyogenes* strains were then used to infect mice intramuscularly, three mice per strain. 50 colonies from each spleen were tested for SpeB activity using a casein digestion assay as a surrogate marker of *covS* mutation. Graph (B) shows percentage of isolates with reduced caseinolytic (SpeB) activity. $M1_{global}$ and $M1_{UK}$ isolates were not significantly different (two-tailed nested t-test, p=0.98). All isolates were previously sequenced and from 2022 *S. pyogenes* non-invasive *emm*1 isolates. $M1_{global}$ strains (H1500, ERS1020045; H2237, ERS1020385; H683, ERS1020523; H800, ERS1020472; H1499, ERS1020620); $M1_{UK}$ (H1503, ERS1020090; H1490, ERS1020174; H1538, ERS1020508; H1506, ERS1020714; H2238, ERS1463088). Source data are provided as a Source Data file Supplementary Figure 3.



Supplementary Figure 4 – Non-synonymous mutations in regulatory genes of *emm1* strains: distribution within the phylogenetic tree. Main phylogenetic tree is central, showing $M1_{global}$, $M1_{UK}$ and associated subclades. Individual regulators are indicated by outer concentric circles. Coloured bubbles indicate a mutation in an individual regulator. Large protein modifications (e.g. truncations) are highlighted with large bubbles; indels and insertions are indicated by medium sized bubbles; non-synonymous mutations are highlighted with small bubbles. All $M1_{UK}$ isolates have 3 missense mutations in RofA (outside ring). Source data are provided as a Source Data file Supplementary Figure 4.



Supplementary Figure 5. Estimated effective population size (N_e) of M1$_{UK}$ in the UK through time. Panels A-D indicate effect of altering number of isolates considered per year. A) no limit on isolates; B) up to 76 isolates per year; C) up to 22 isolates per year; D) up to 14 isolates per year. The black line and pink shading within each time interval indicate the mean and 95% confidence interval of N_e, respectively, estimated from each subtree. Source data are provided as a Source Data file Supplementary Figure 5.