

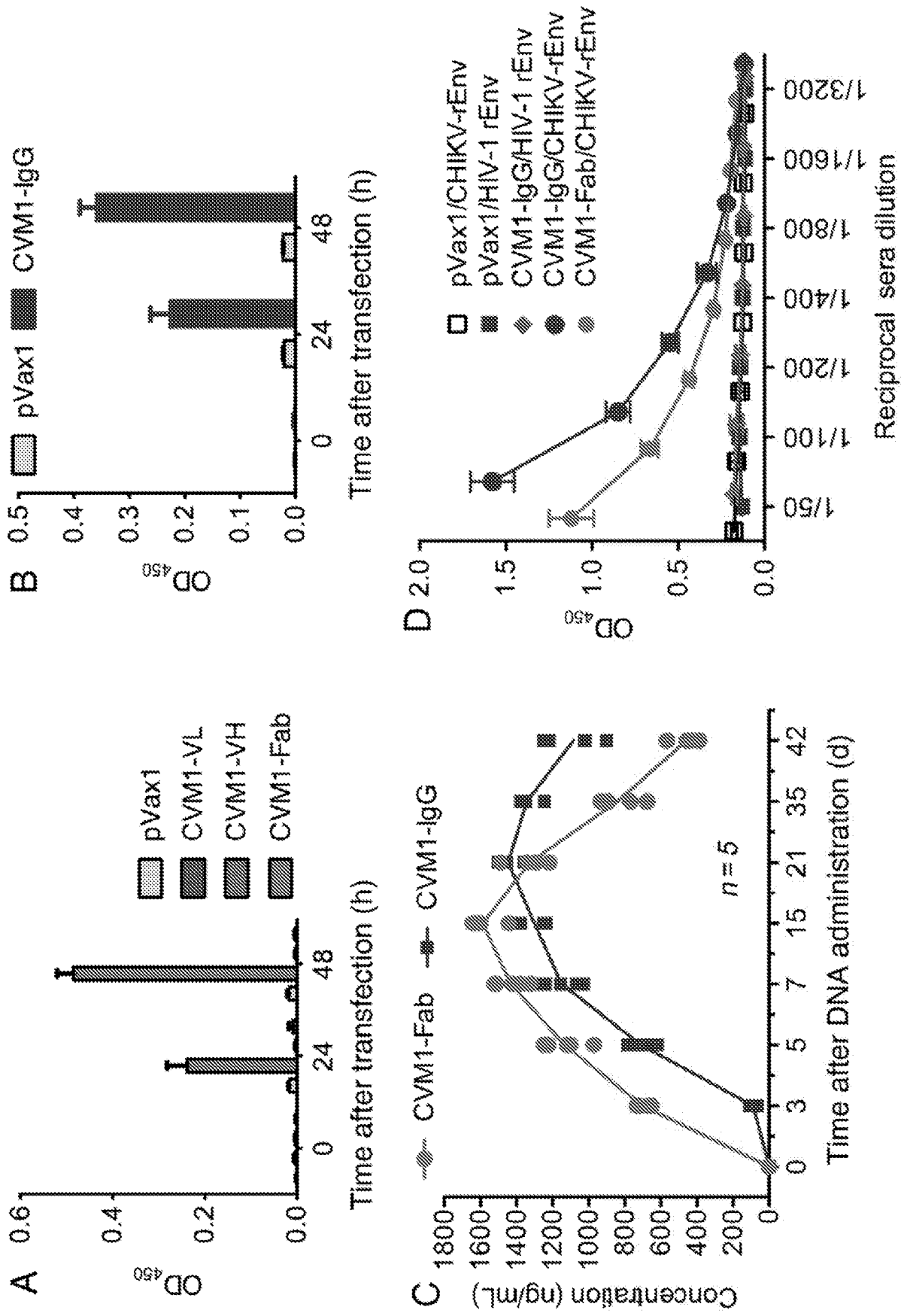


Figure 1A – Figure 1D

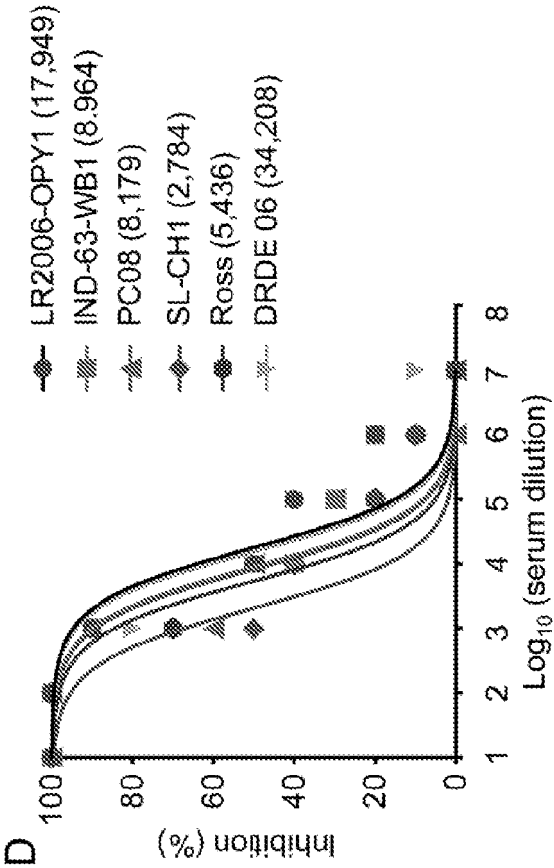
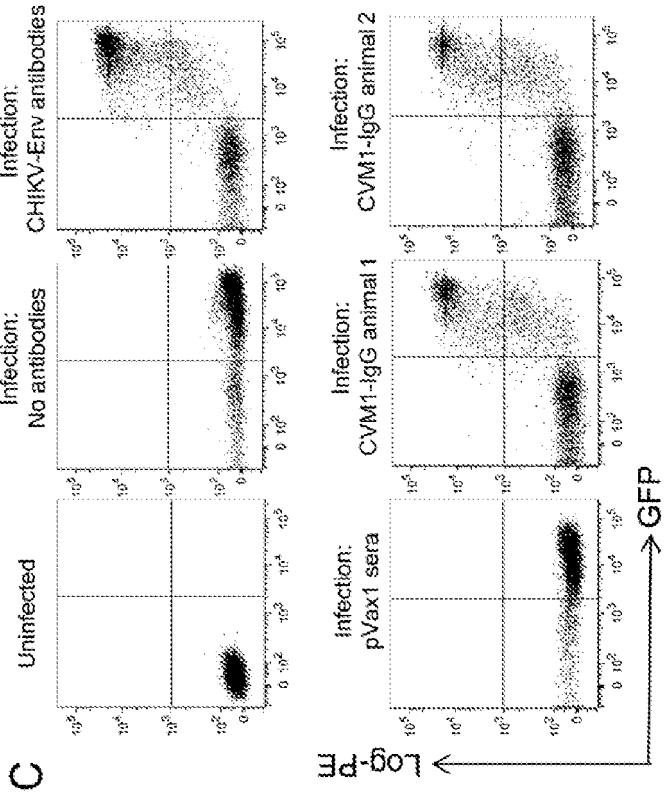
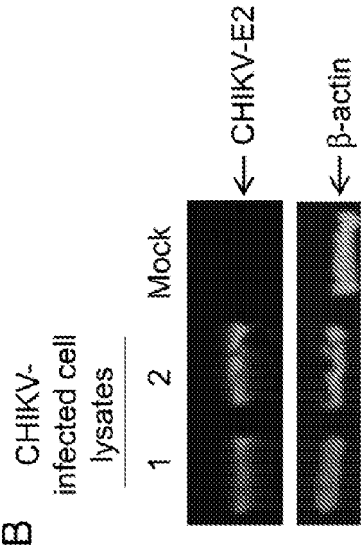
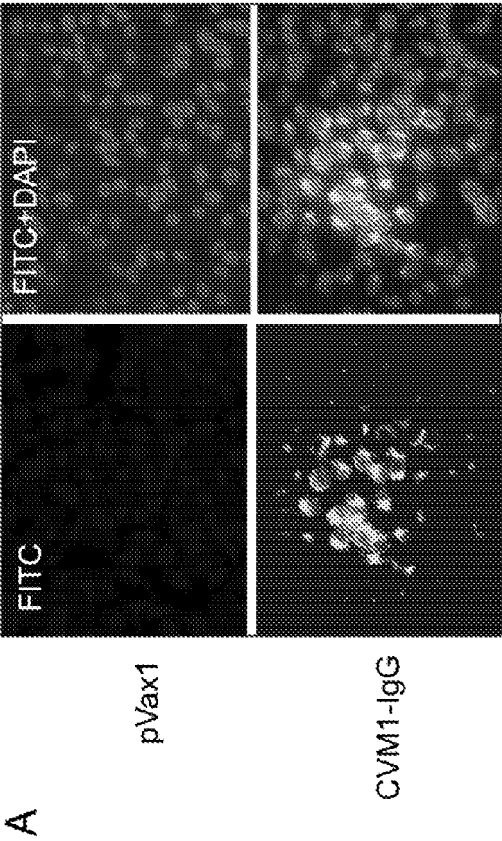


Figure 2A – Figure 2D

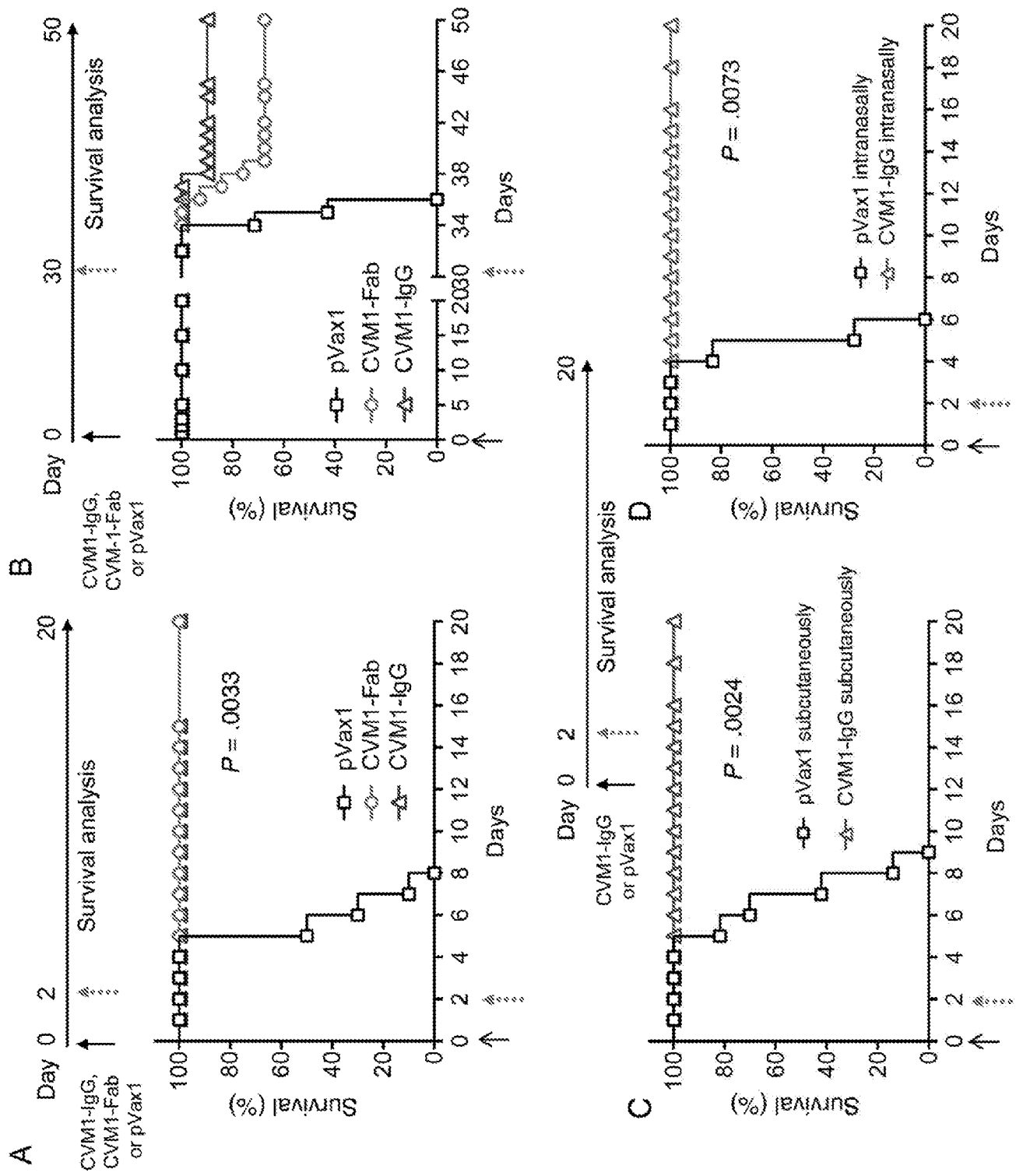


Figure 3A – Figure 3D

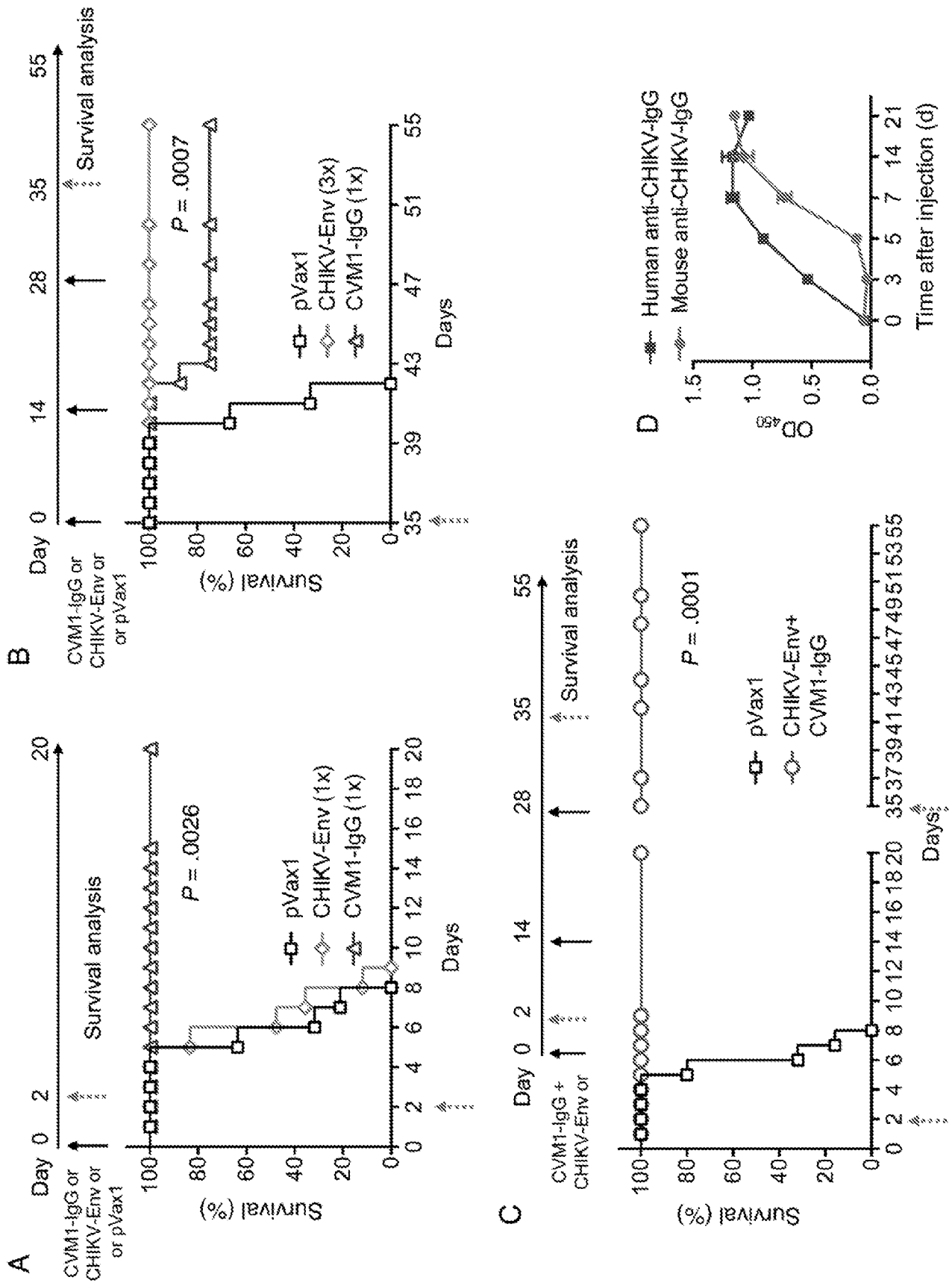


Figure 4A – Figure 4D

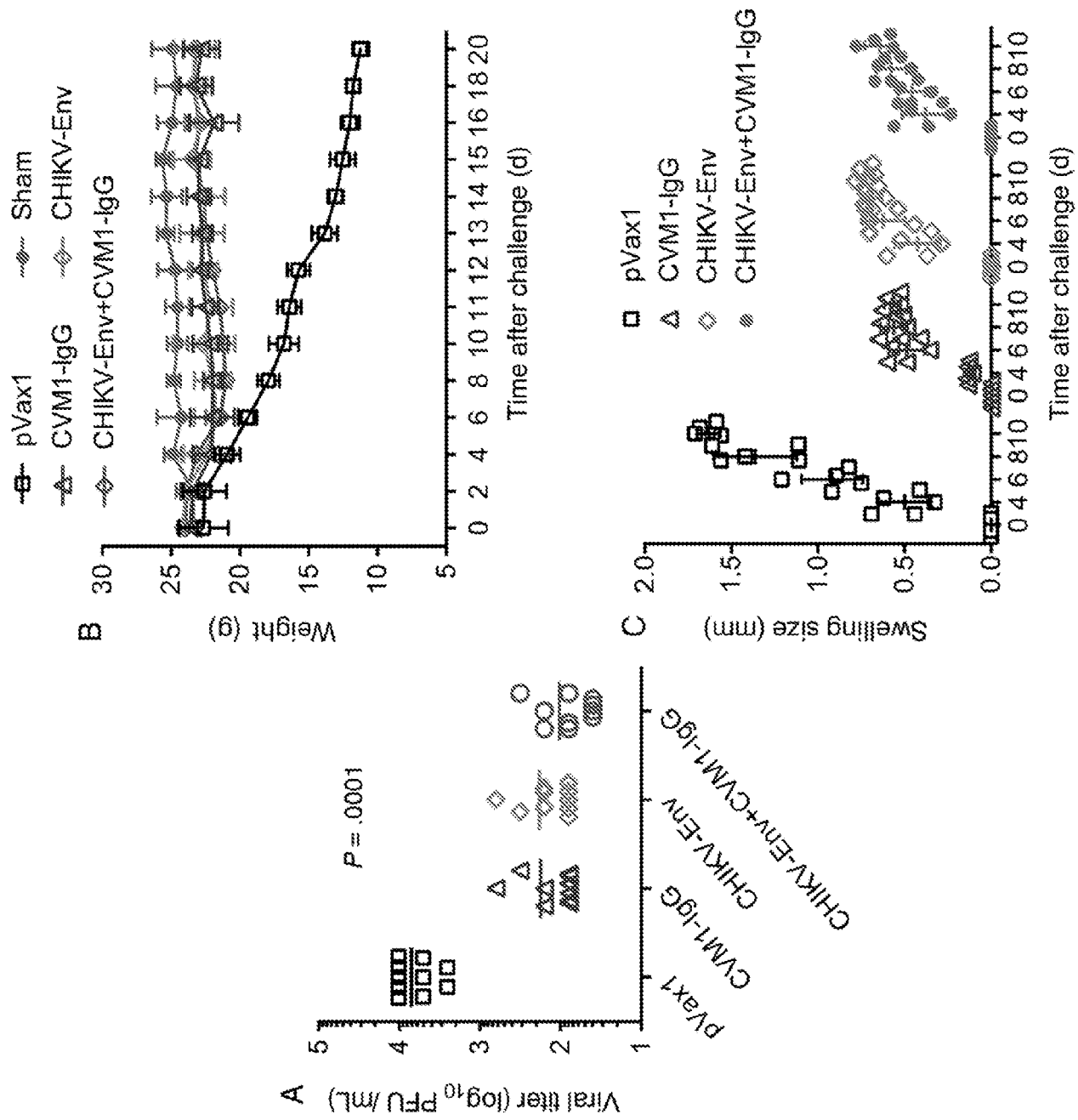


Figure 5A – Figure 5C

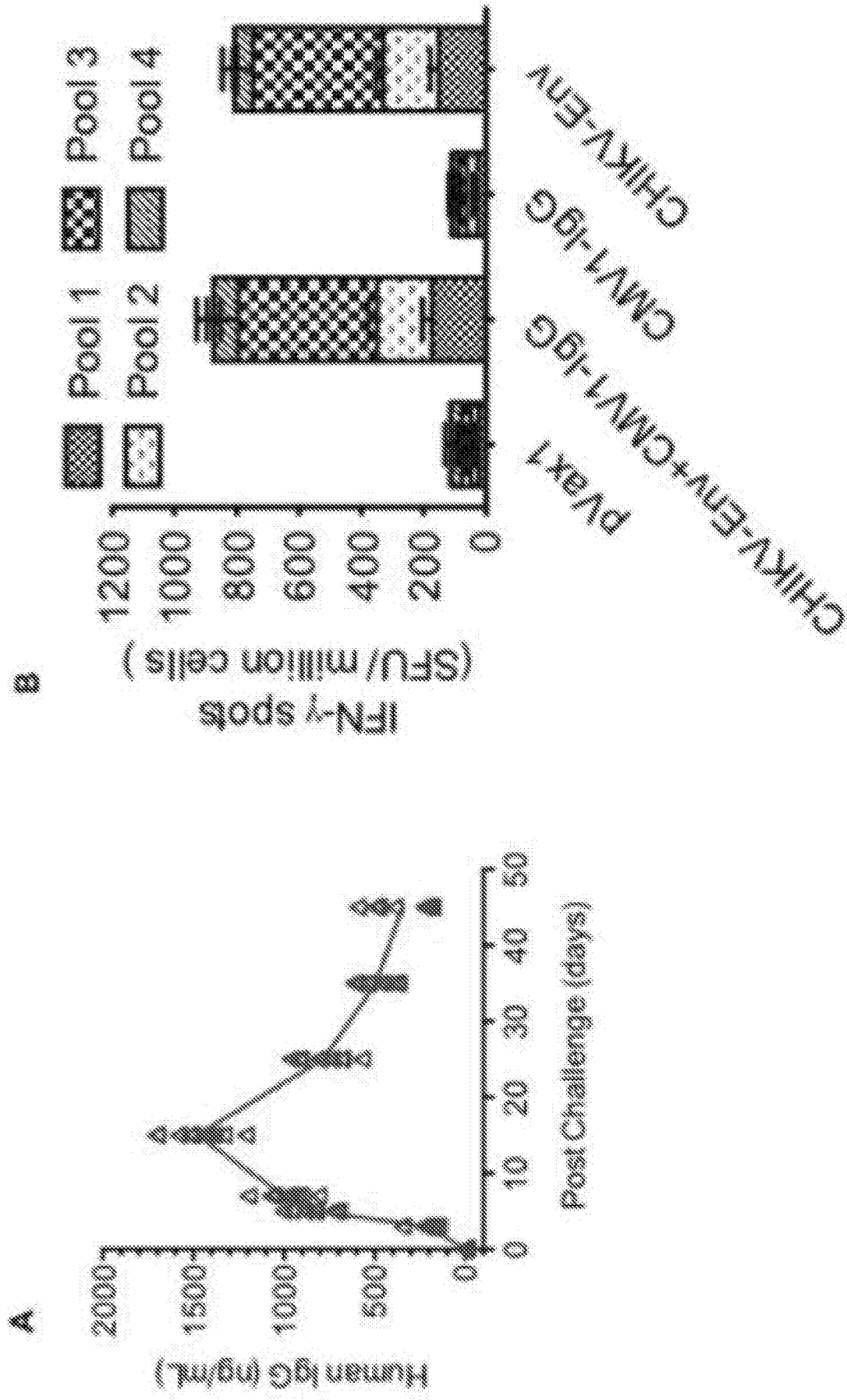


Figure 6A – Figure 6B

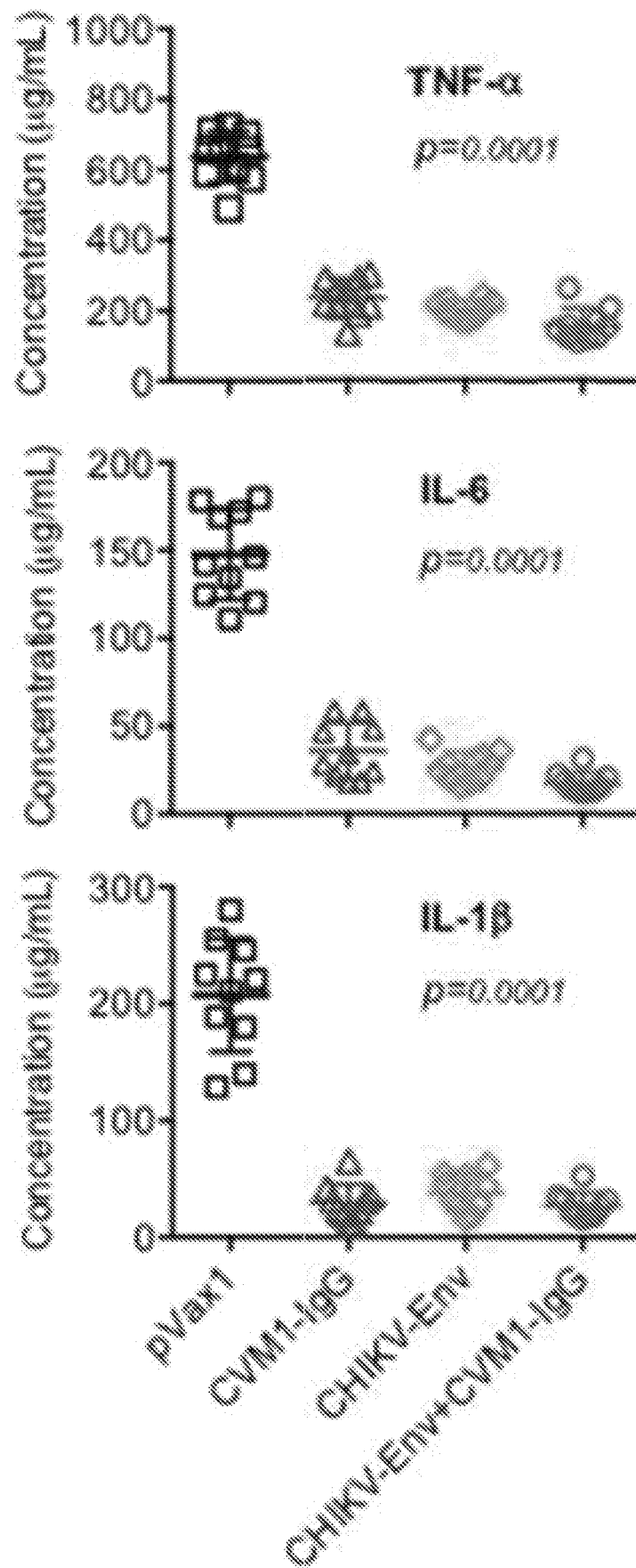


Figure 7

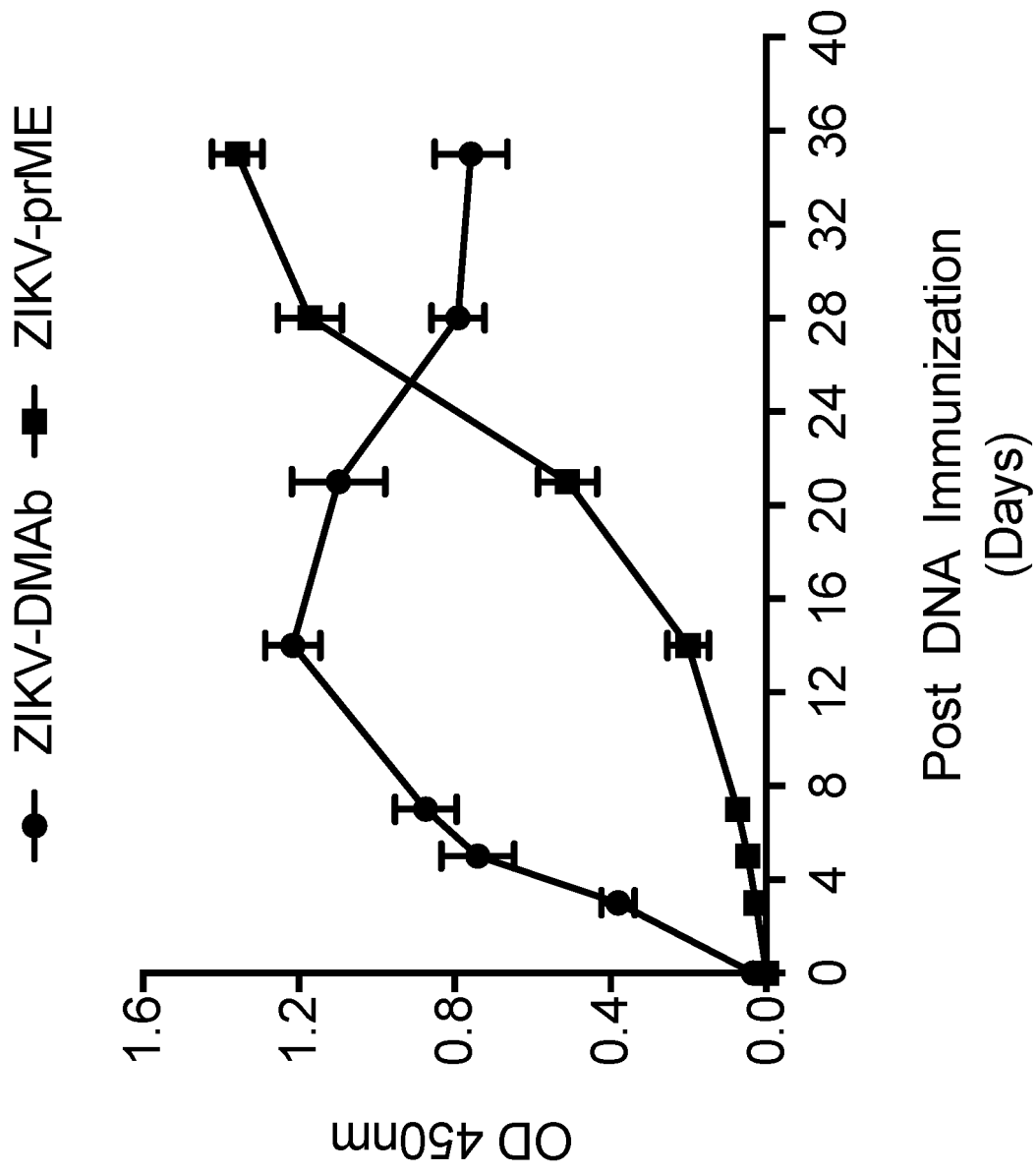


Figure 8

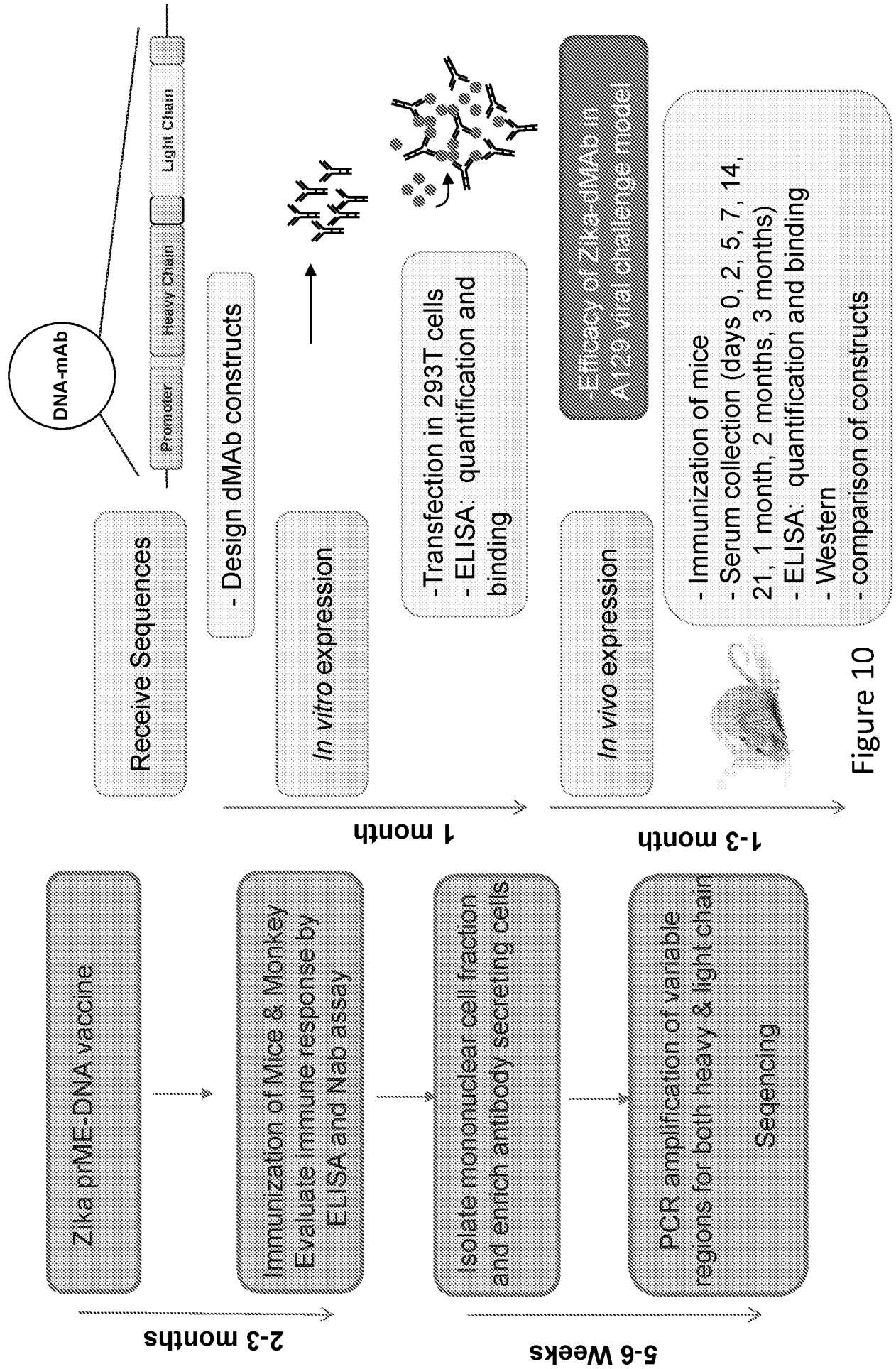


Figure 10

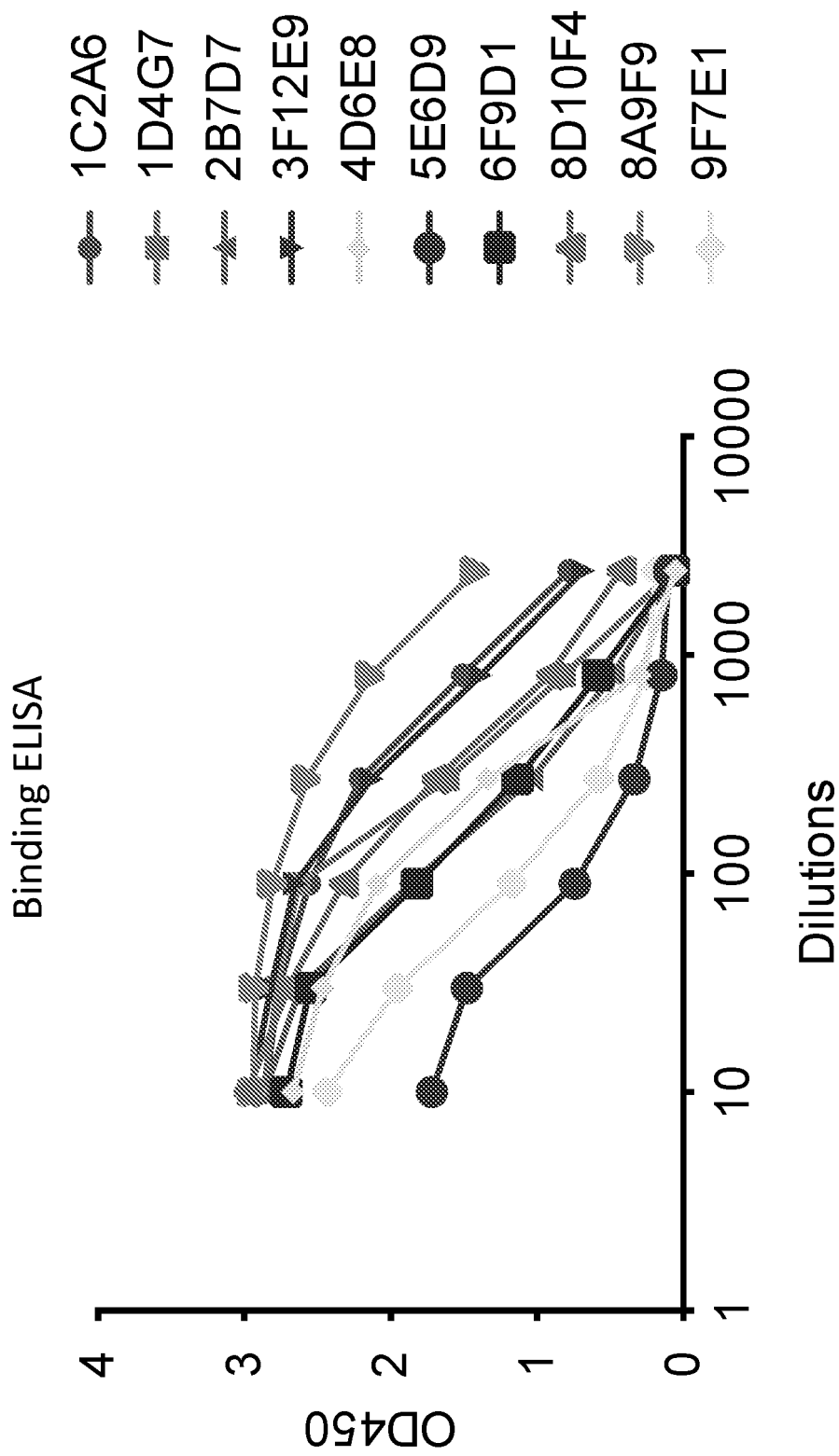


Figure 11

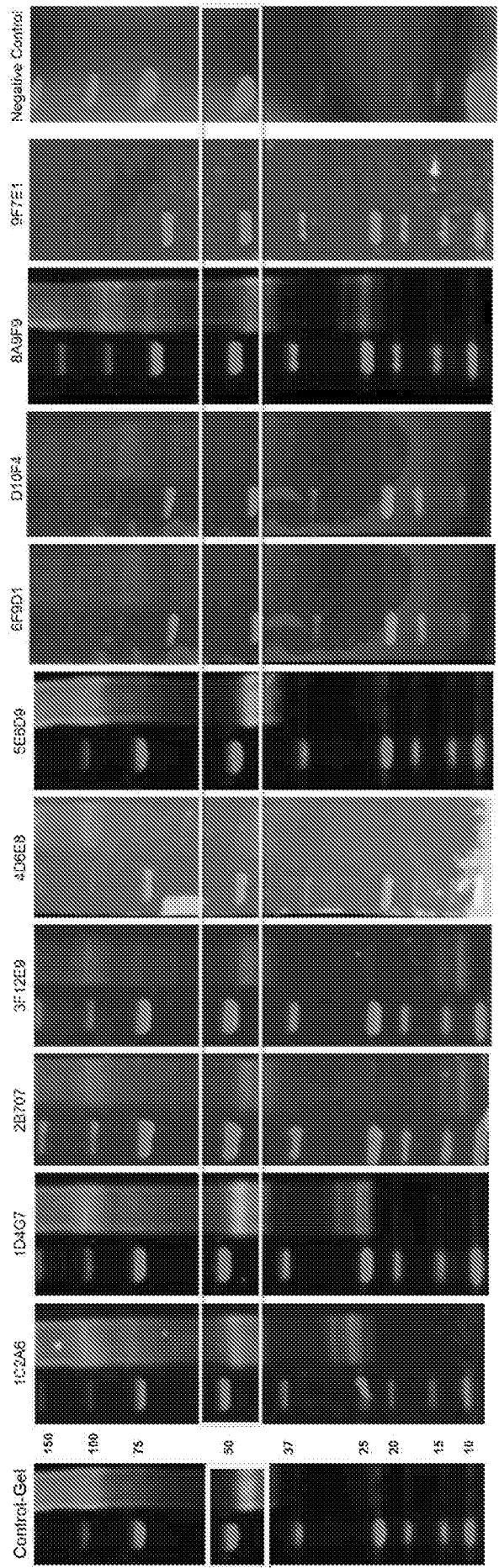
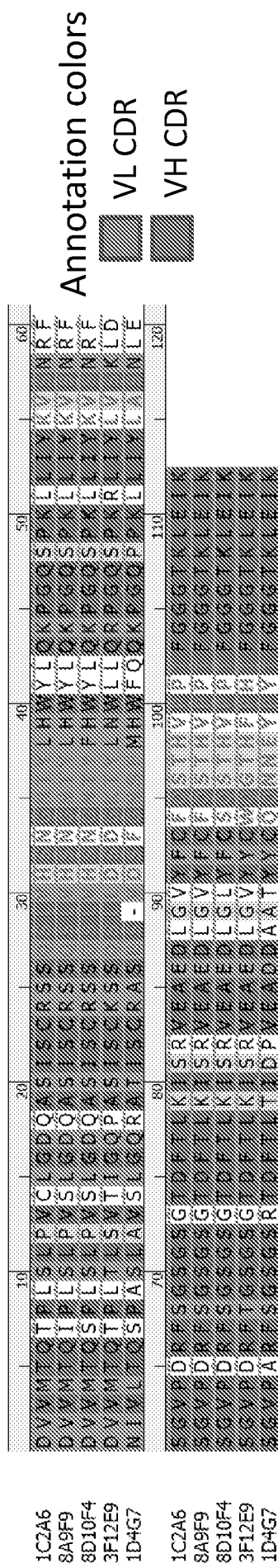


Figure 12



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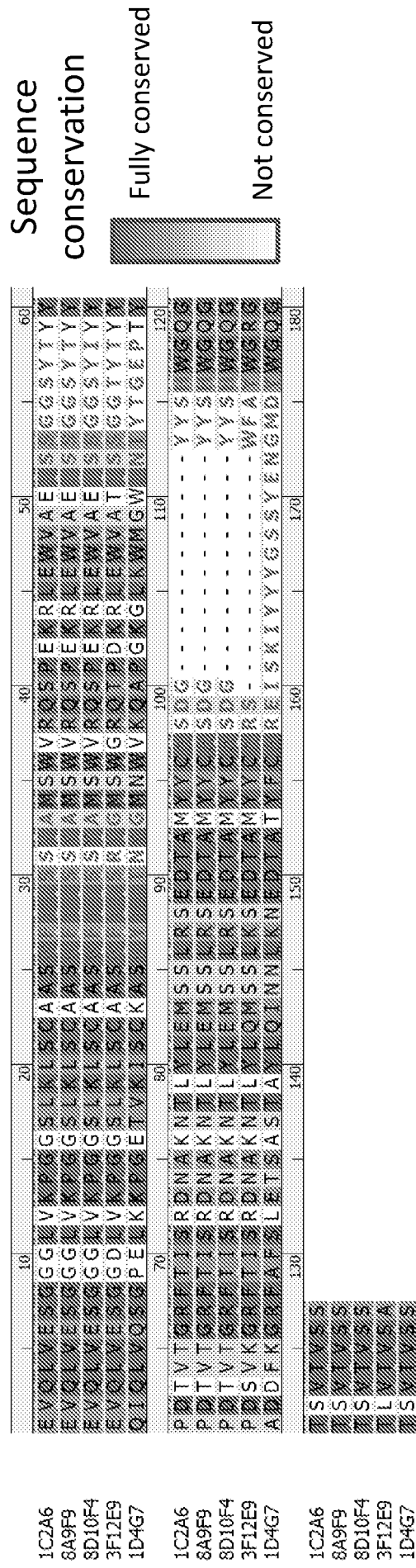


Figure 13

VH identity

	8A9F9_H	1D4G7_H	8D10F4_H	3F12E9_H	1C2A6_H
8A9F9_H	100	43.3	99.1	80.9	100
1D4G7_H	43.3	100	43.3	44.9	43.3
8D10F4_H	99.1	43.3	100	80	99.1
3F12E9_H	80.9	44.9	80	100	80.9
1C2A6_H	100	43.3	99.1	80.9	100

VL identity'

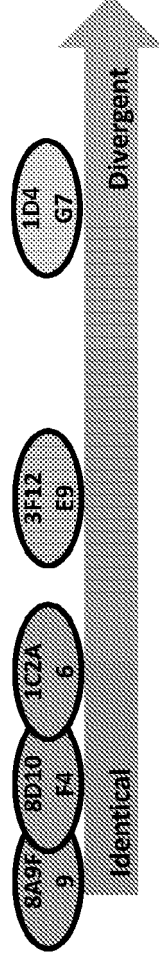
	8A9F9_L	1D4G7_L	8D10F4_L	3F12E9_L	1C2A6_L
8A9F9_L	100	64.3	96.4	76.8	98.2
1D4G7_L	64.3	100	65.2	58.9	63.4
8D10F4_L	96.4	65.2	100	75	95.5
3F12E9_L	76.8	58.9	75	100	77.7
1C2A6_L	98.2	63.4	95.5	77.7	100

Model RMSD

RMSD	8D10F4	1C2A6	8A9F9	3F12E9	1D4G7
8D10F4		2.77	2.77	2.76	2.19
1C2A6	0.302		2.27	2.26	2.19
8A9F9	0.385	0.347		2.26	2.19
3F12E9	0.723	0.752	0.722		2.19
1D4G7	1.364	1.388	1.351	1.434	

RMSD (Å)

residues compared



- The VH and VL chains in 1C2A6, 8D10F4 and 8A9F9 are identical or nearly identical.
- 1C2A6, 8D10F4 and 8A9F9 are likely to bind the same epitope in the same manner. There may be affinity differences, as with any antibody changes.
- 1D4G7 and 3F12E9 represent more divergent Fv's than 1C2A6, 8D10F4 and 8A9F9.
- 3F12E9 is more similar to the set of three mAbs cited above than it is to 1D4G7. It may bind similarly.
- Backbone variation according to RMSD measurement shows the same pattern as sequence identity.

Figure 14

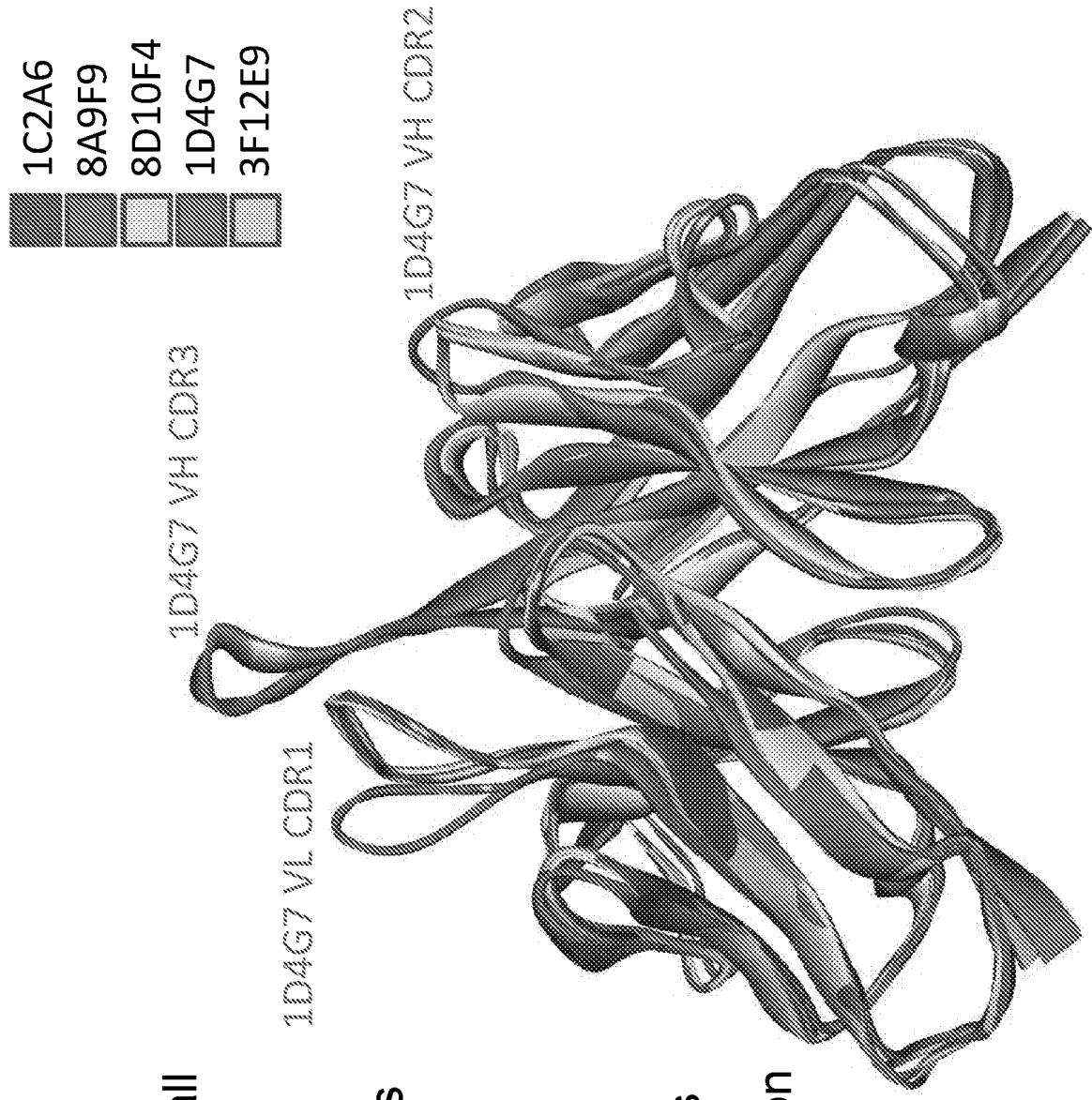
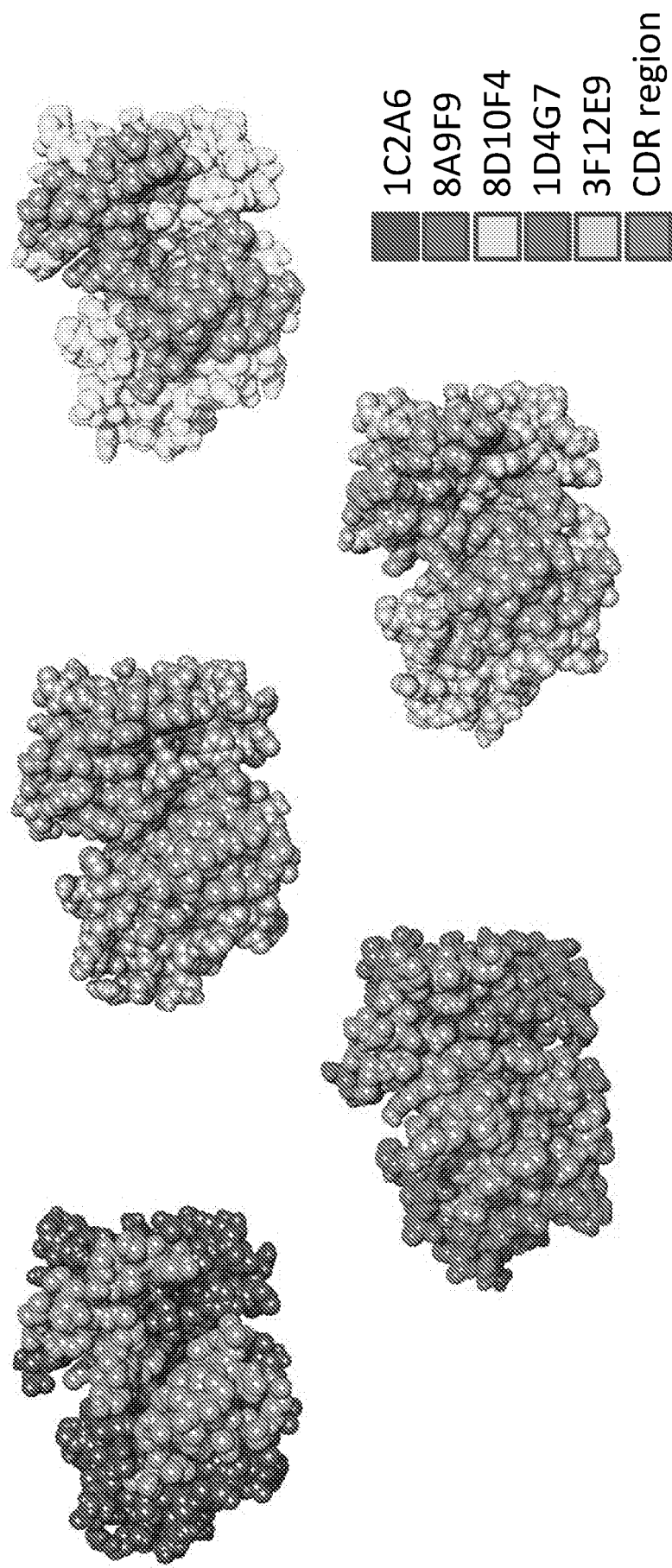


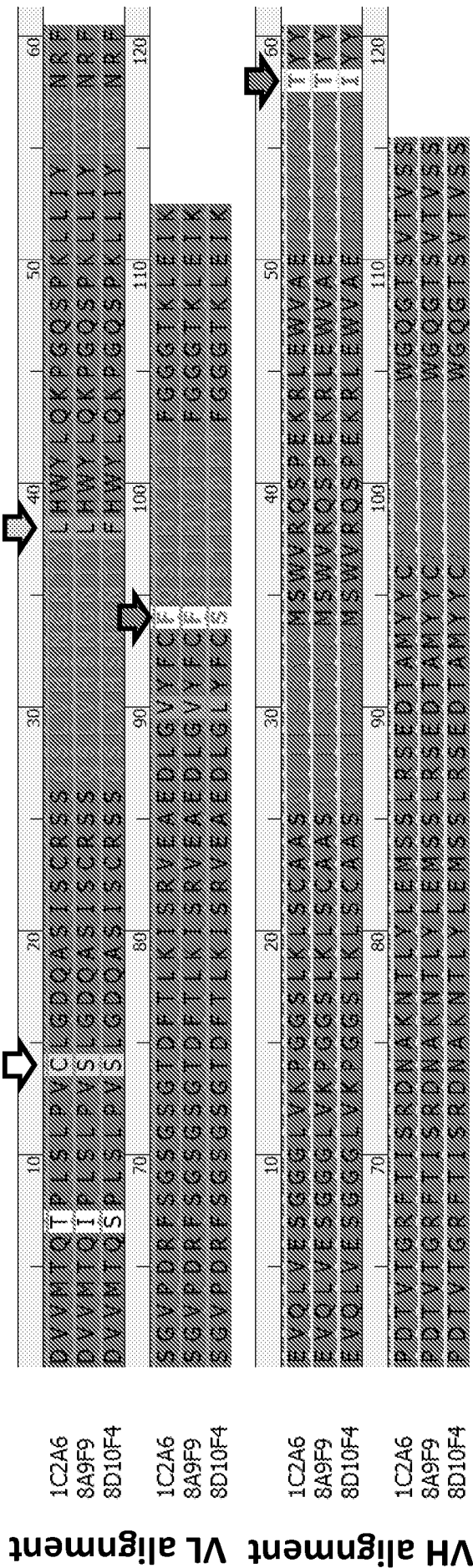
Figure 15

- All Fv models are superimposed in ribbon format to emphasize overall fold.
- The large VH CDR3 of 1D4G7 is clearly visible, as are several other fold differences in other CDR and in framework regions.
- Despite the sequence divergence of 3F12E9, it is still closer in overall sequence and conformation to 1C2A6, 8D10F4 and 8A9F9 than to 1D4G7.

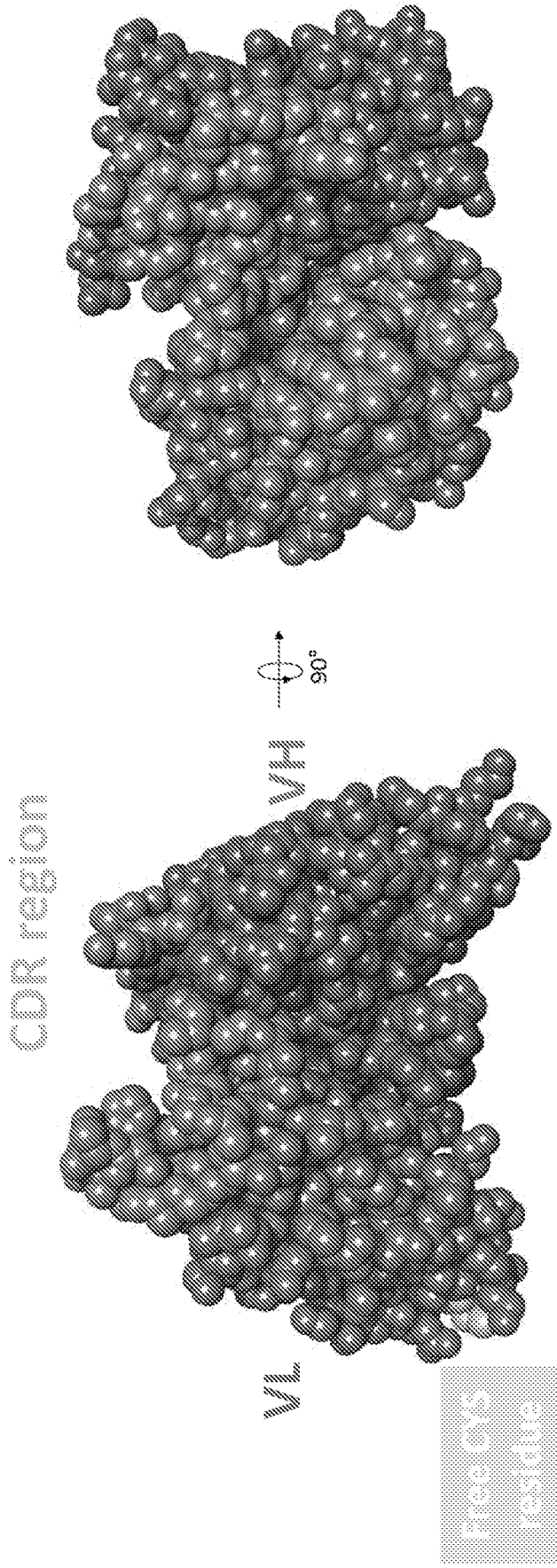


- 1D4G7 lacks a cleft between the VH and VL domains due to its large CDR3 loop.
- Sequence similarities translate to structural similarities, so overall CDR conformations and molecular shapes are conserved according to previously demonstrated clustering.

Figure 16



- 1C2A6 has a free CYS residue distal to the CDRs exposed on the surface (yellow arrow).
- Another potentially relevant difference occurs in VH FR2 region (orange arrow). This residue is not directly involved in CDR conformation but does influence local residue packing.
- Two changes occur within the IMGT-defined CDR regions (red arrows). The VL changes (F, F, S) directly impact the VL-VH interface.



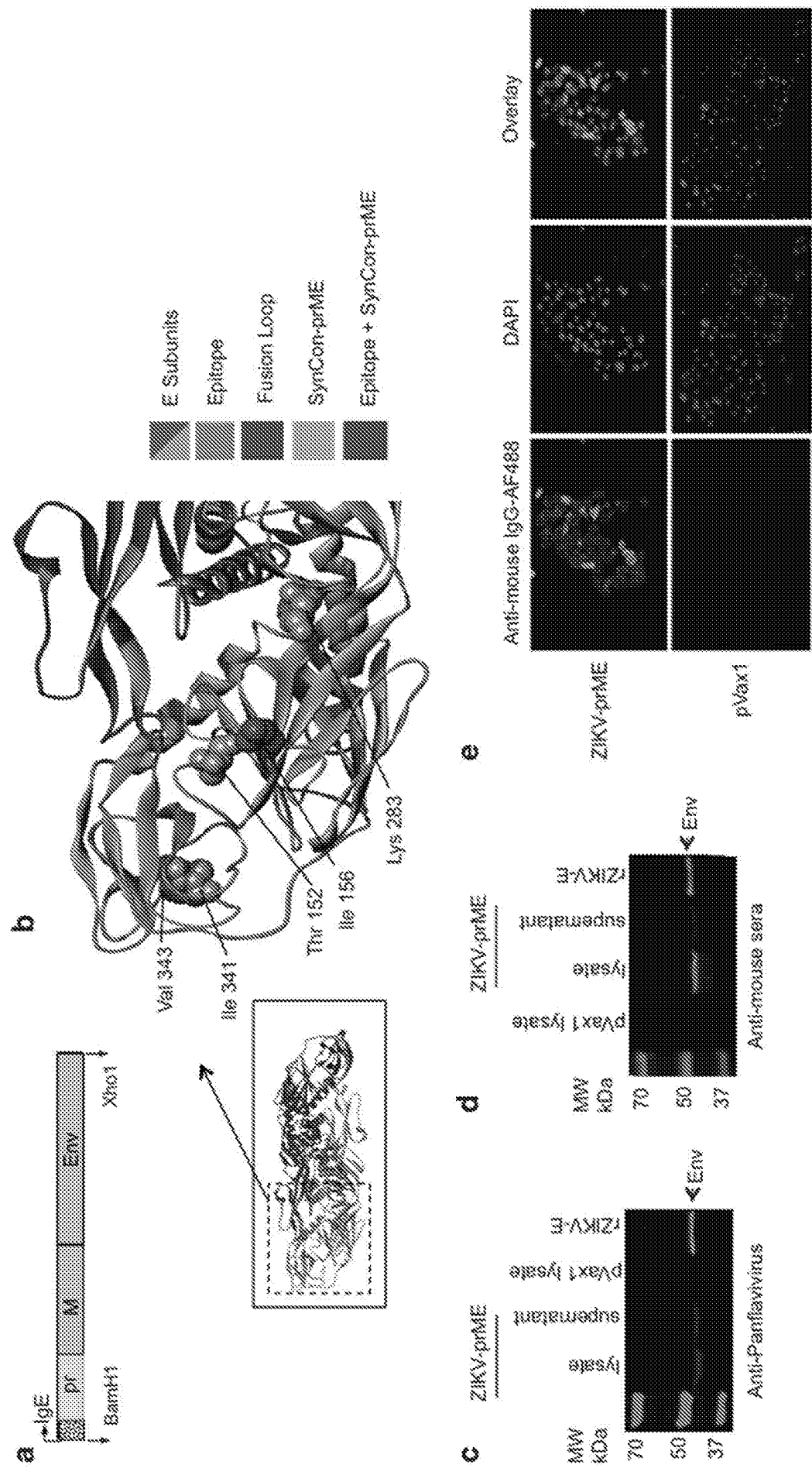
- A free CYS leaves a highly modifiable chemical group exposed on the molecule surface.
- As this mAb is nearly identical to two others (the CYS being unique to 1C2A6), if the other two mAbs show similar neutralizing activity it may be better to go with a mAb that does not have a free CYS.

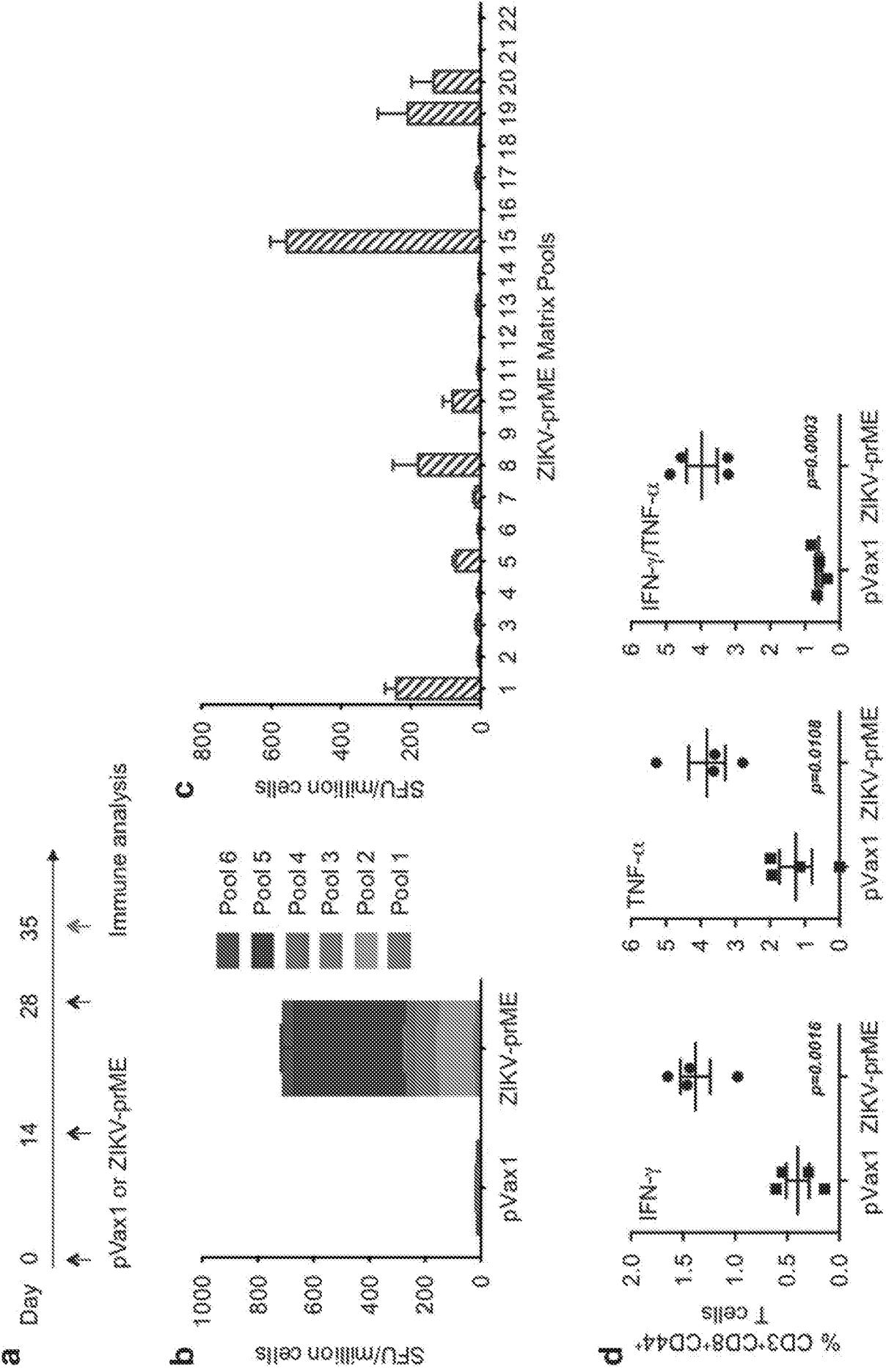
Figure 18

Protein	Developability Index (Fv)	Molecular Charge	Total Pi Interactions	Total Hydrogen Bonds	Ligand Contact Surface Area	Ligand Polar Contact Surface Area	Ligand Nonpolar Contact Surface Area	Receptor Contact Surface Area	Receptor Polar Contact Surface Area	Receptor Nonpolar Contact Surface Area
8D10F4	57.447	2.52	6	4	211.61	85.863	125.75	205	82.854	122.15
1C2A6	57.237	2.73	9	2	186.67	73.108	113.56	182.2	69.19	113.01
8A9F9	60.132	2.94	9	2	186.67	73.108	113.56	182.2	69.19	113.01
3F12F9	60.661	6.71	10	5	224.98	72.912	152.06	223.97	72.732	151.23
1D4G7	70.578	0.84	10	6	323.22	116.31	206.91	331.6	172.51	159.09

- Developability index is highest for 1D4G7, very likely due to the long CDR3 loop which contains multiple nonpolar residues. Based on past experience, though, this alone does not appear to be an issue.
- F→S variation in the interface/CDR region of 8D10F4 is responsible for the reduced number of predicted pi interactions compared to 1C2A6 and 8A9F9.
- Additional features such as potential post-translational modifications can be assessed. These may be less relevant but can be run.

Figure 19





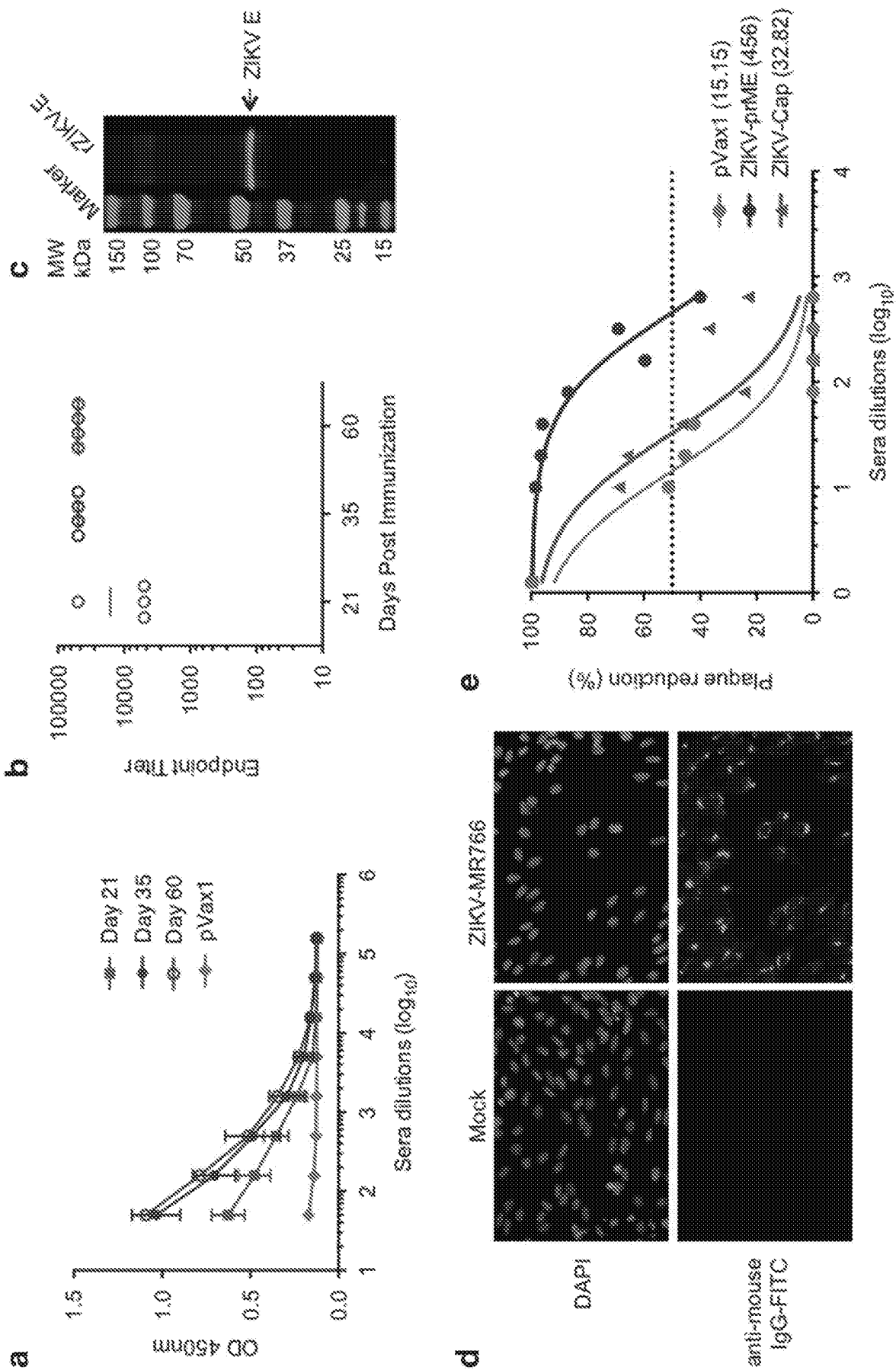


Figure 22A – Figure 22E

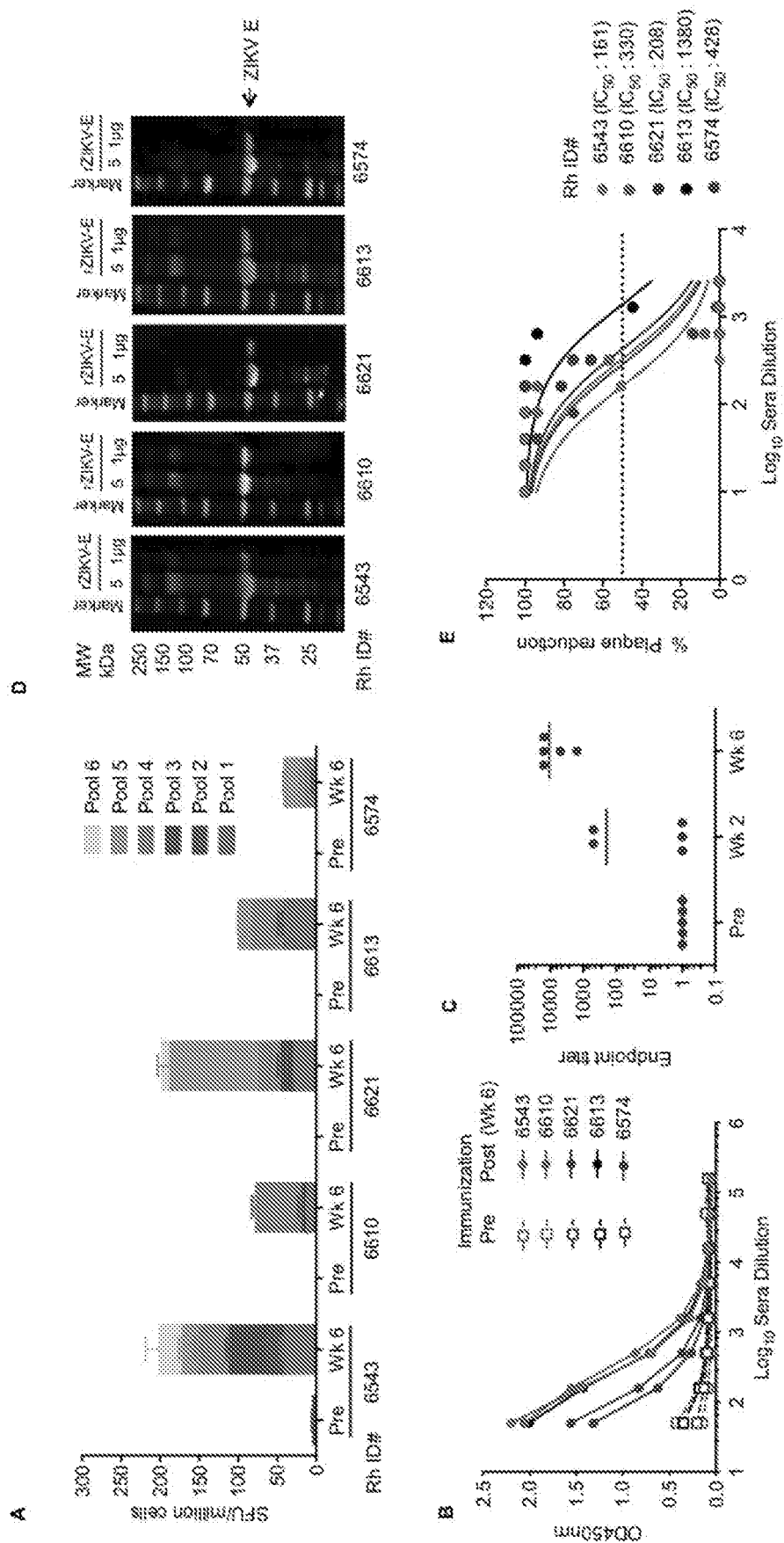


Figure 23A – Figure 23E

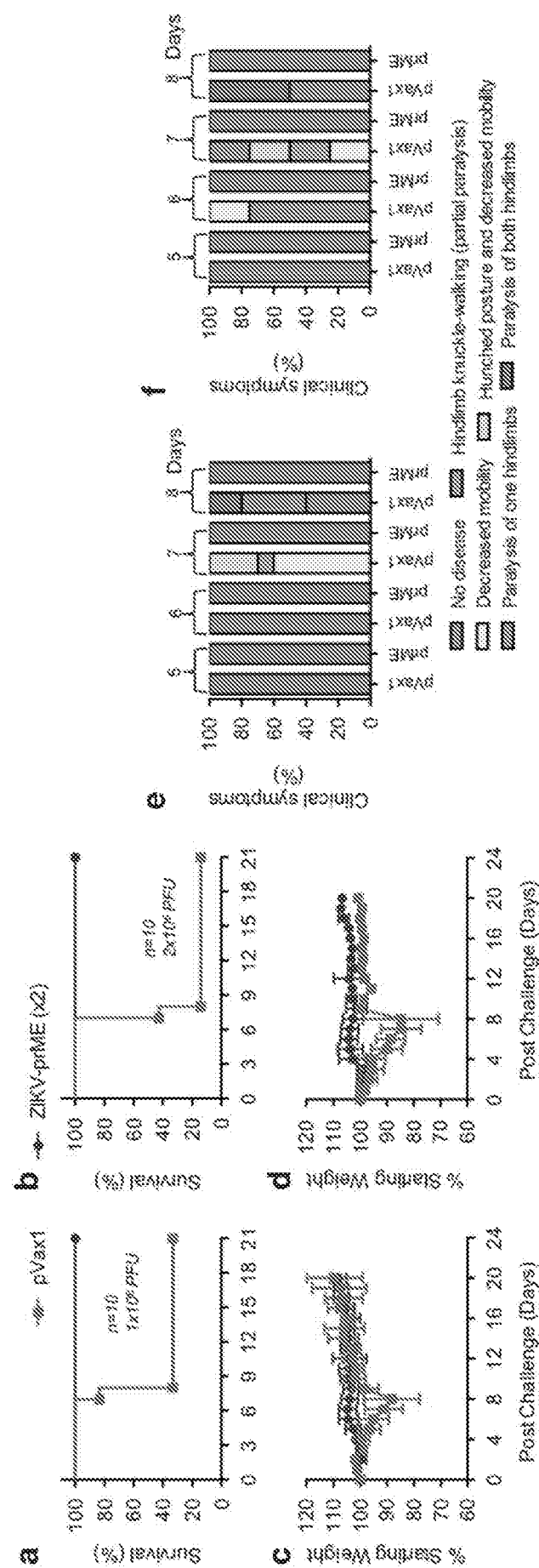


Figure 24A – Figure 24F

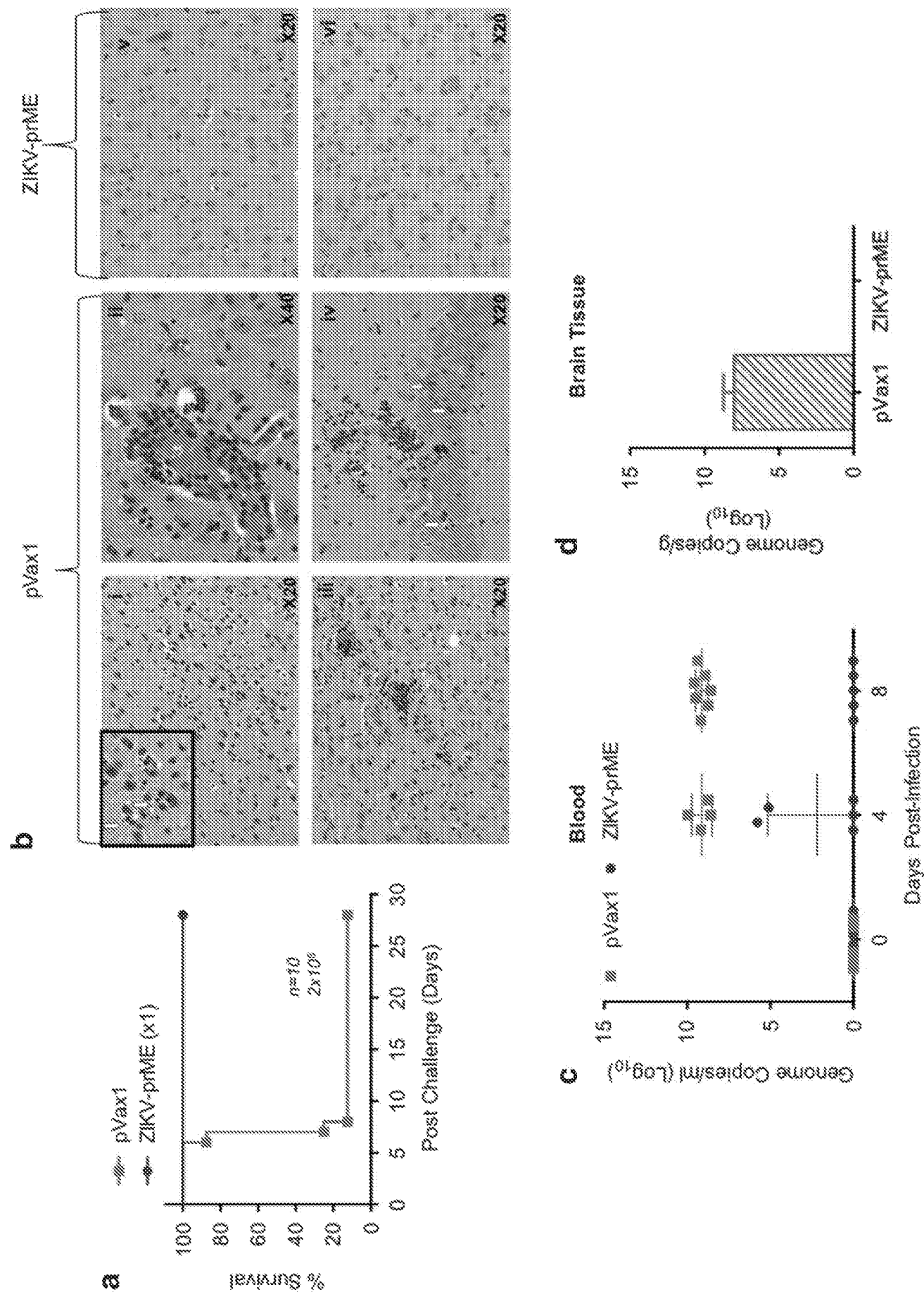


Figure 25A – Figure 25D

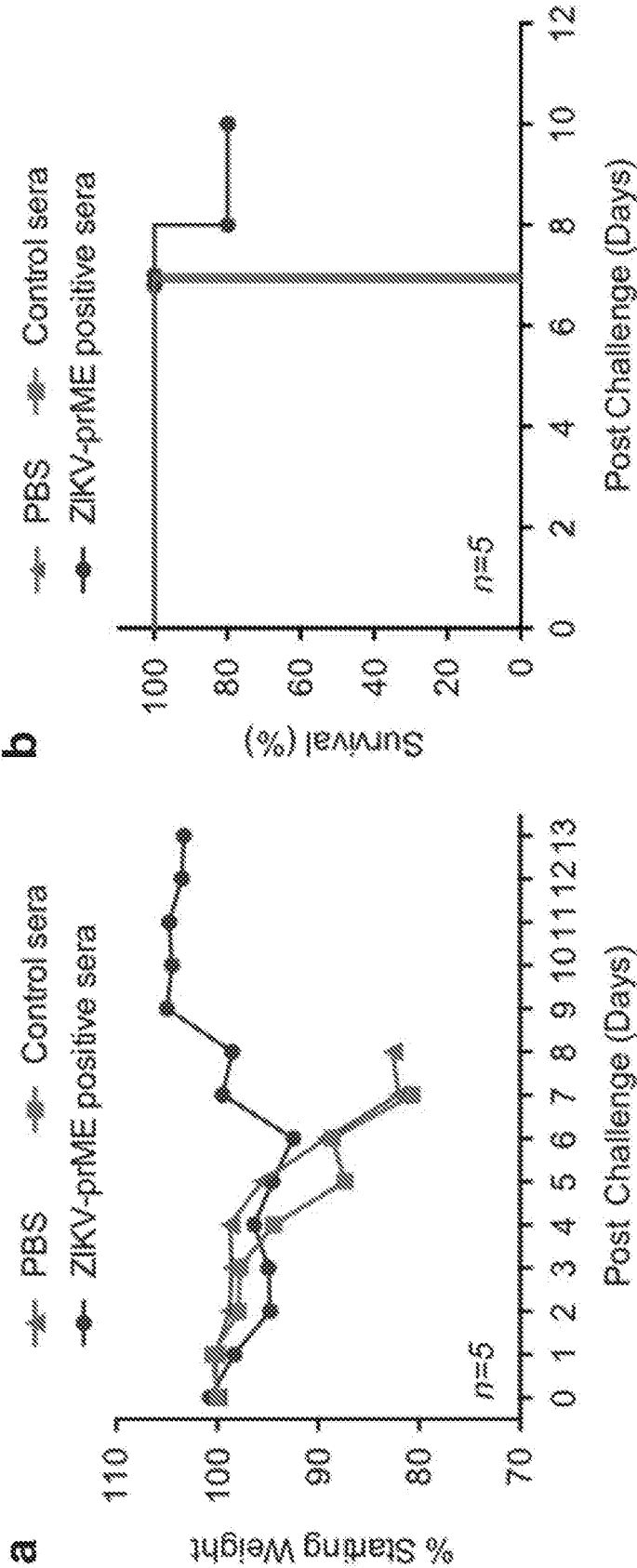


Figure 26A – Figure 26B

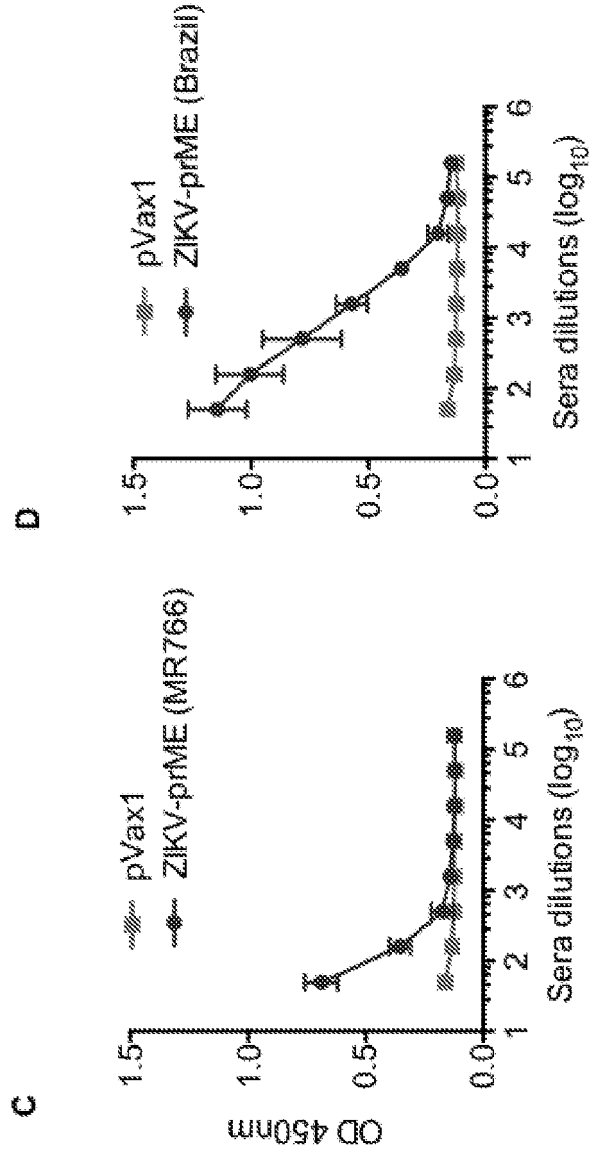
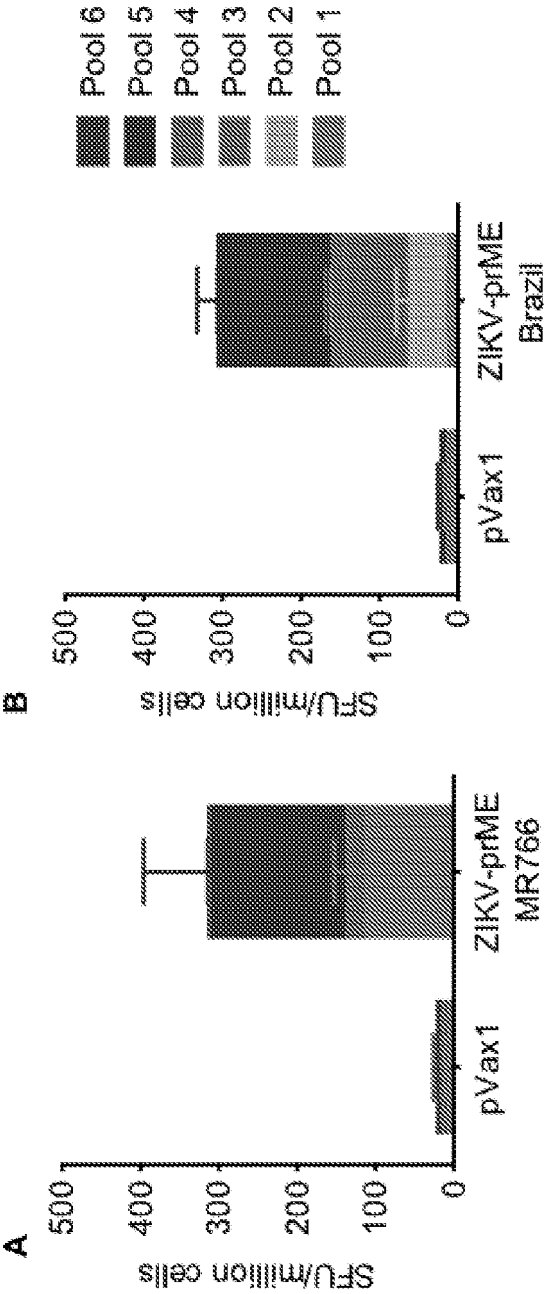
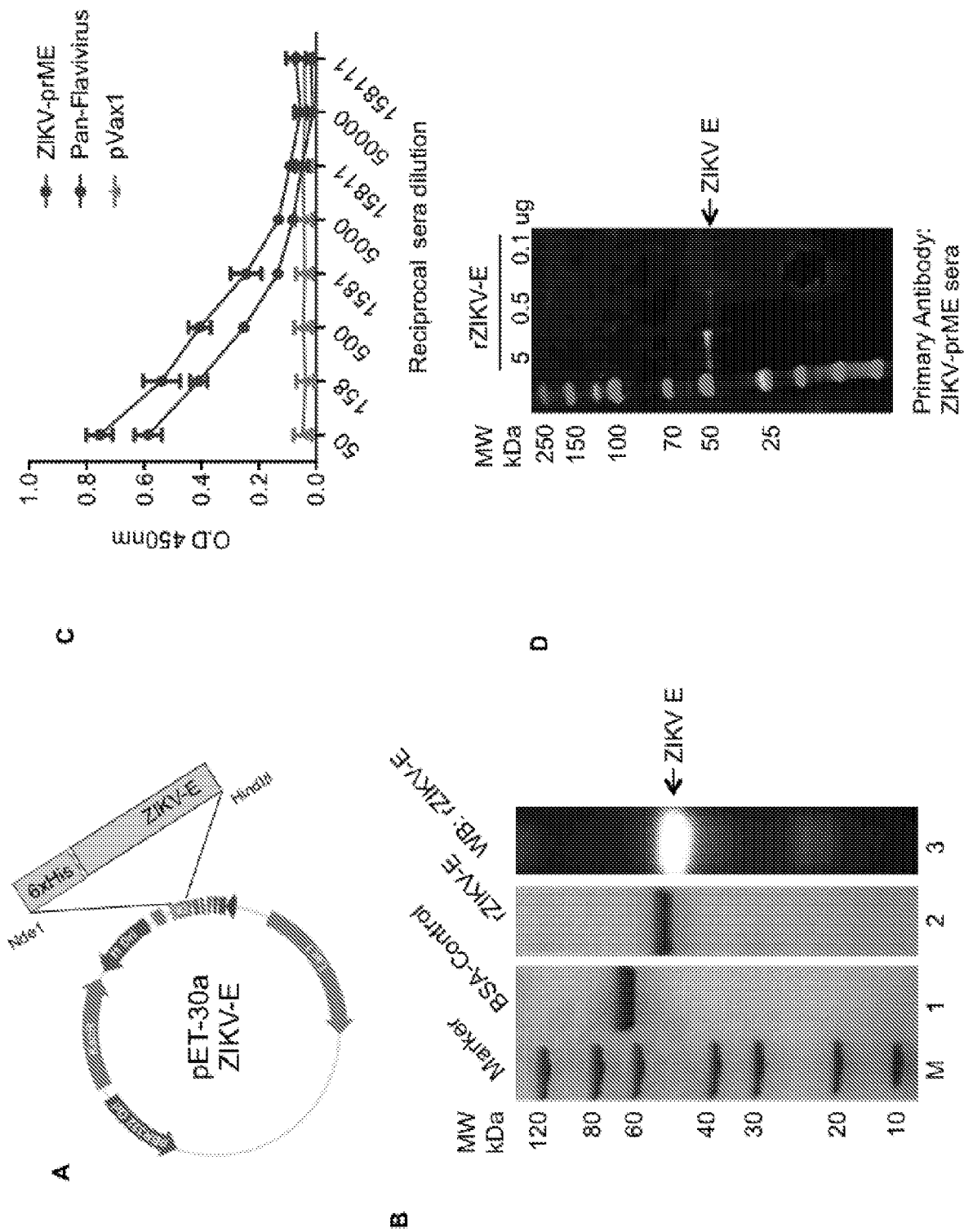


Figure 27A – Figure 27D



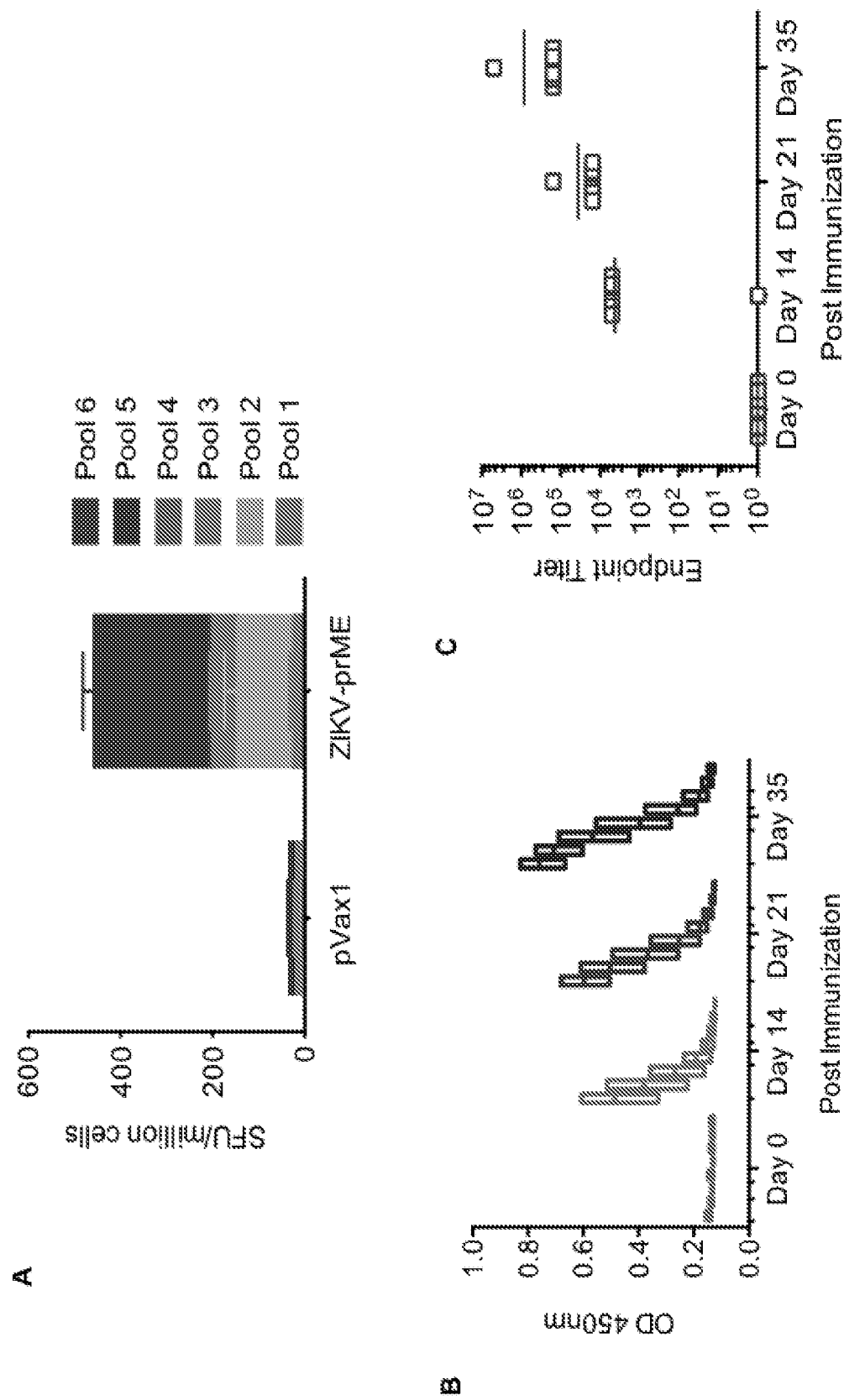


Figure 29A – Figure 29C

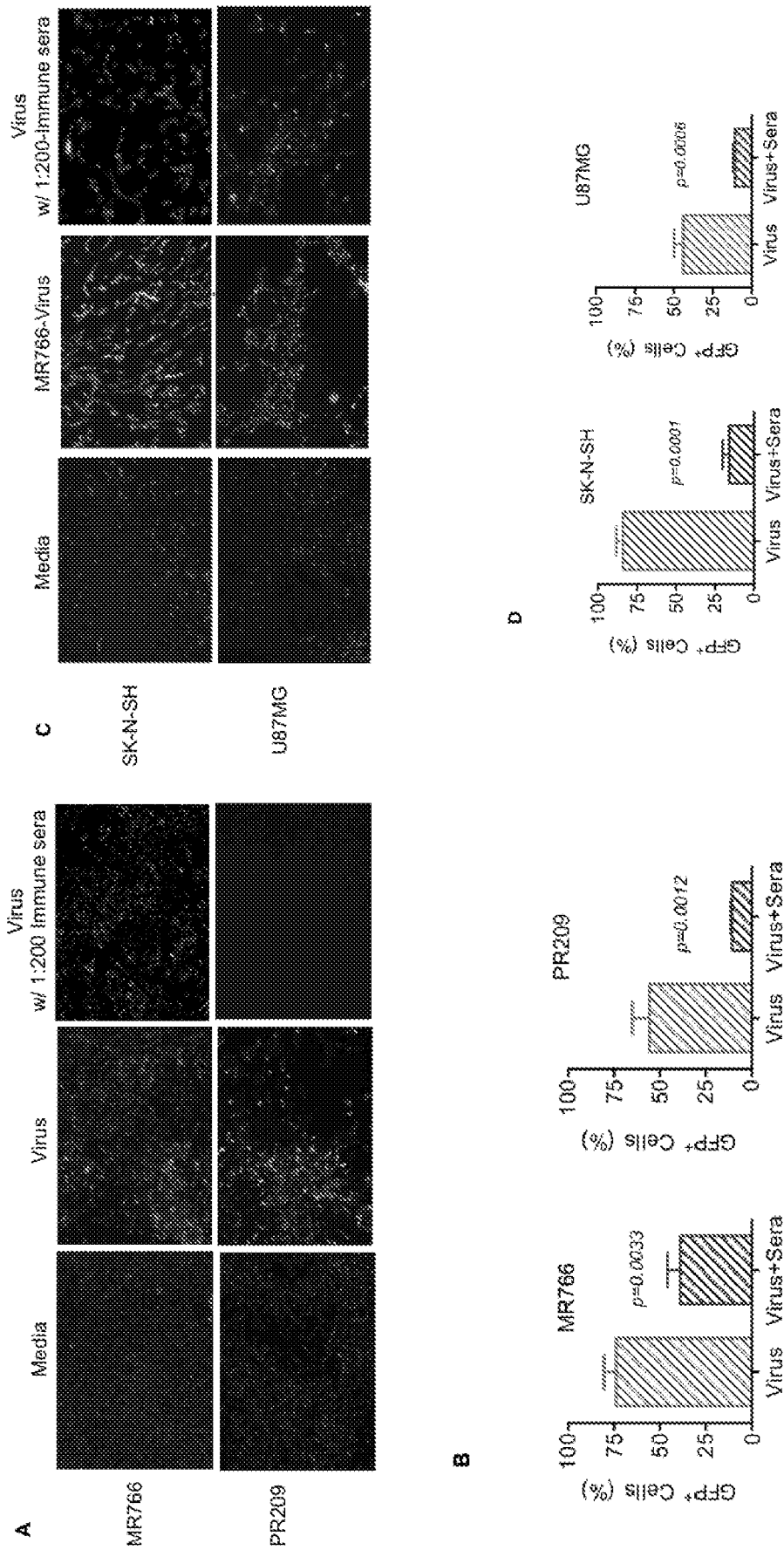


Figure 30A – Figure 30D

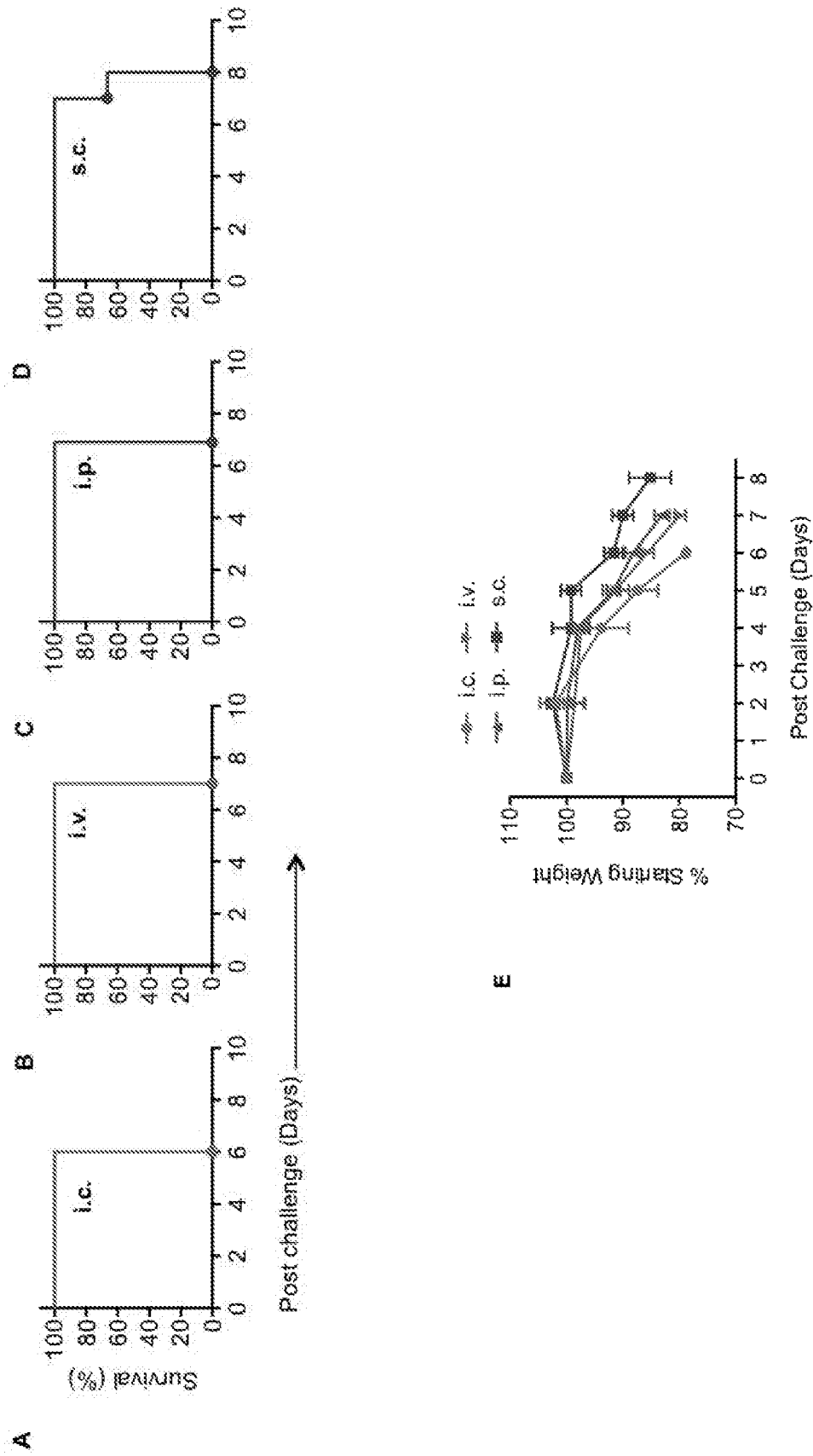


Figure 31A – Figure 31E