

In vitro replication of varicella-zoster virus in human retinal pigment epithelial cells



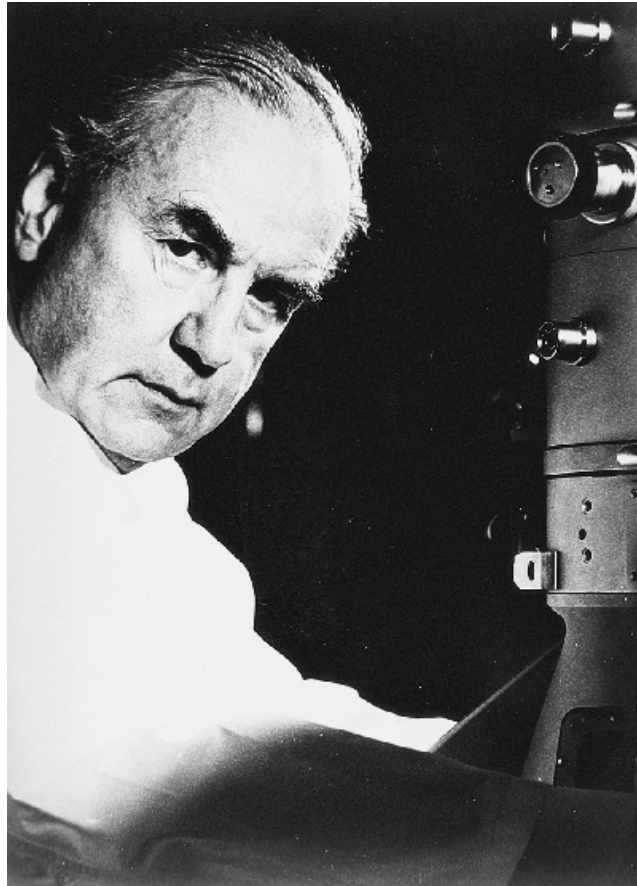
1900 - 2009

BERNHARD NOCHT INSTITUTE FOR TROPICAL MEDICINE

FREE AND HANSEATIC CITY OF HAMBURG

JONAS SCHMIDT-CHANASIT

Helmut Ruska and the visualisation of viruses



Helmut Ruska 1908 - 1973

Helmut Ruska: Über das Virus der Varicellen und des Zoster. (1943)
Klin Wochenschr 22: 703-04.

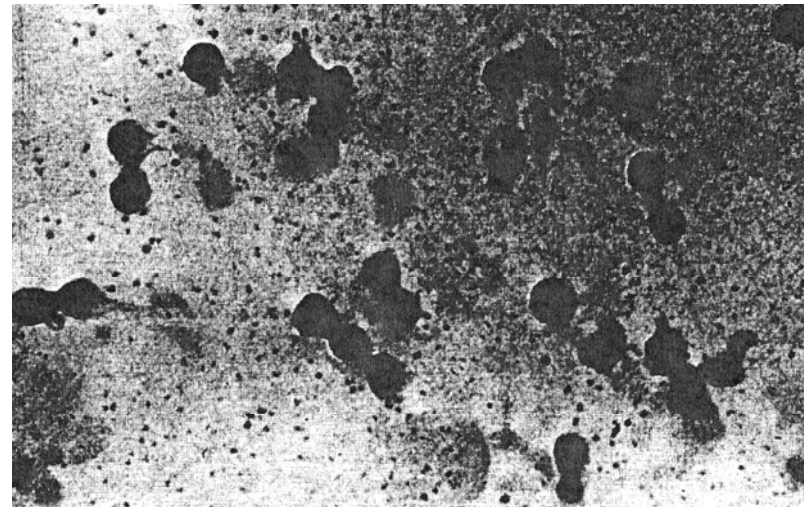


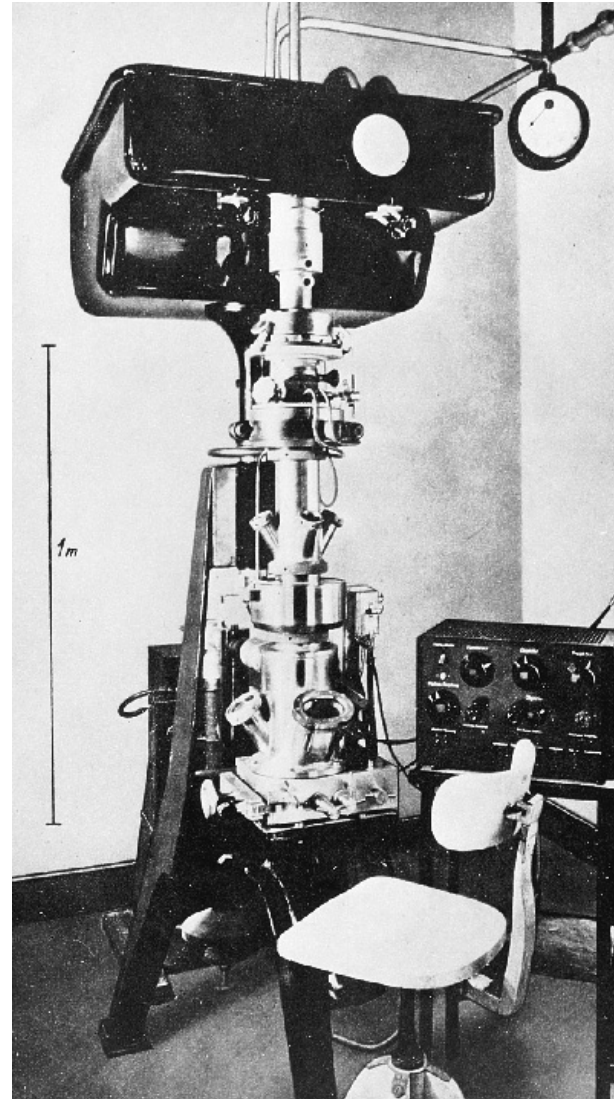
Abb. 1. 1403/43. Virus der Varicellen. Abb. 30000:1.

The Nobel Prize in Physics 1986

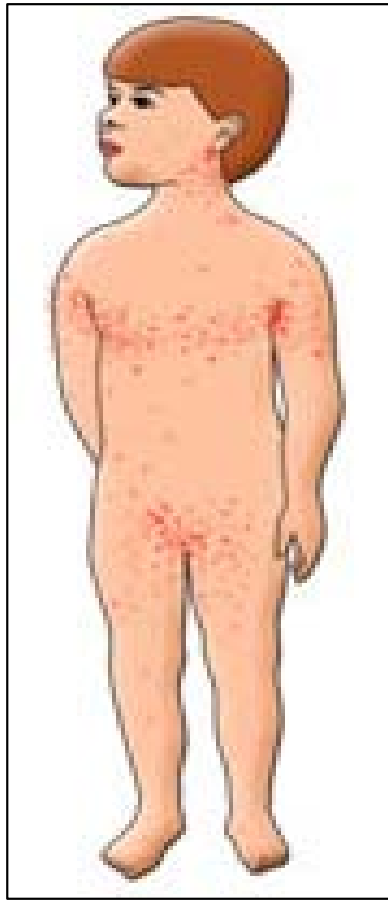
"for his fundamental work in electron optics, and for the design of the first electron microscope"



Ernst Ruska 1906 - 1988



Varicella-zoster virus (VZV) is the causative agent of chicken pox and shingles



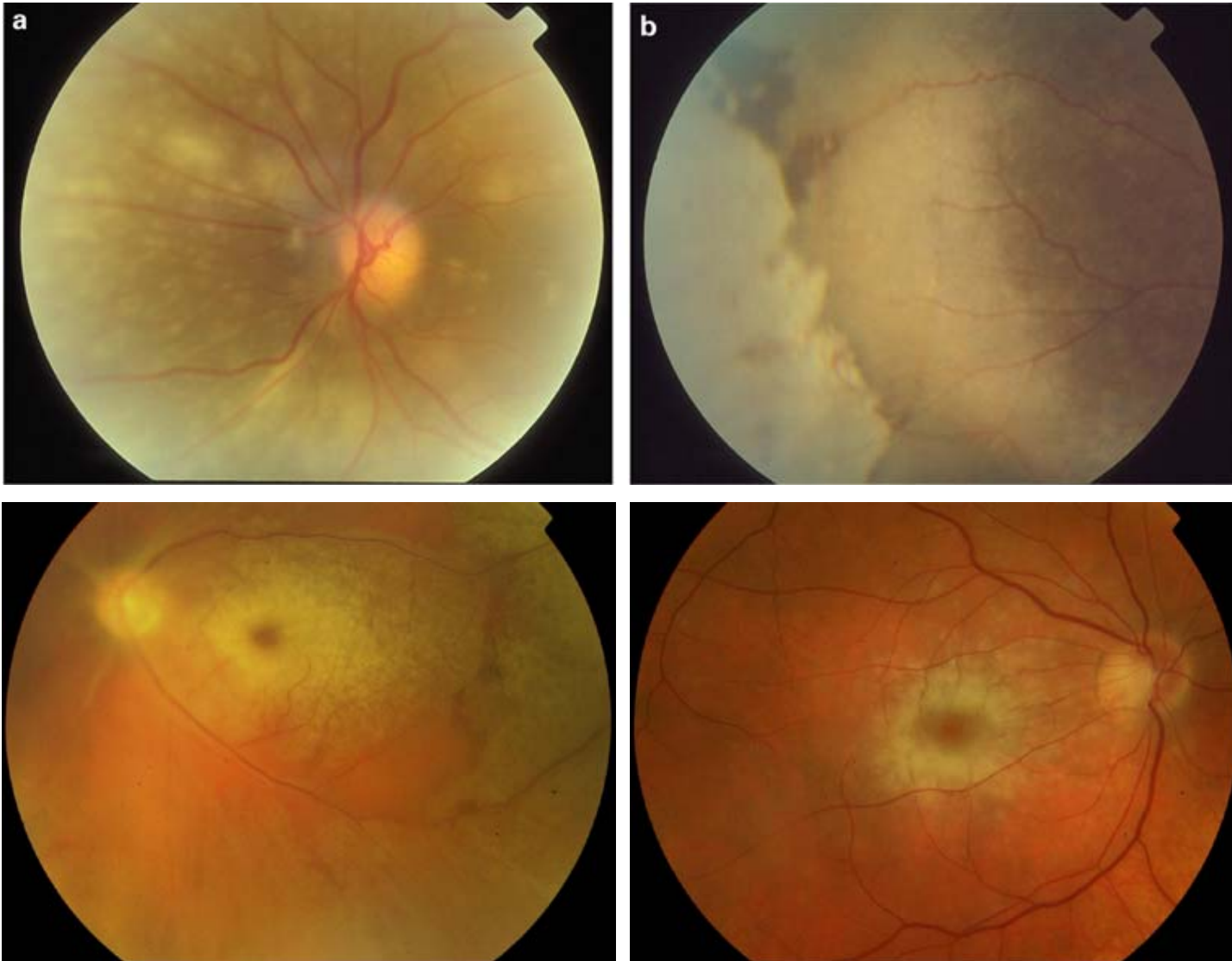
chicken pox (varicella)

VZV reactivation from latency →

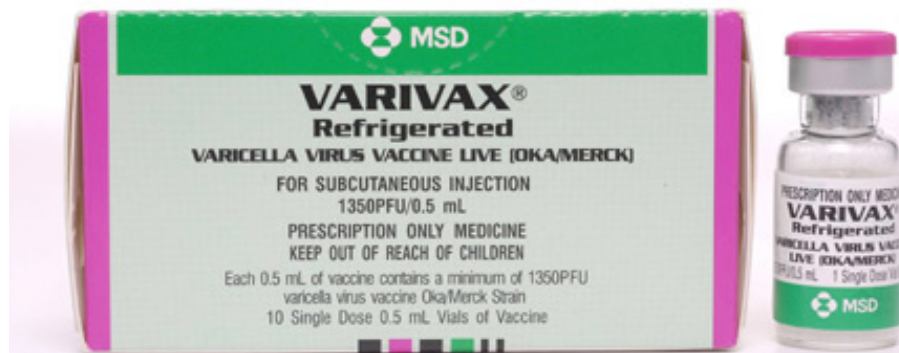


shingles (zoster)

VZV and progressive outer retinal necrosis (PORN)



The live vaccine enhance humoral and cell-mediated immunity to VZV



First full-genome analysis of VZV in 1986

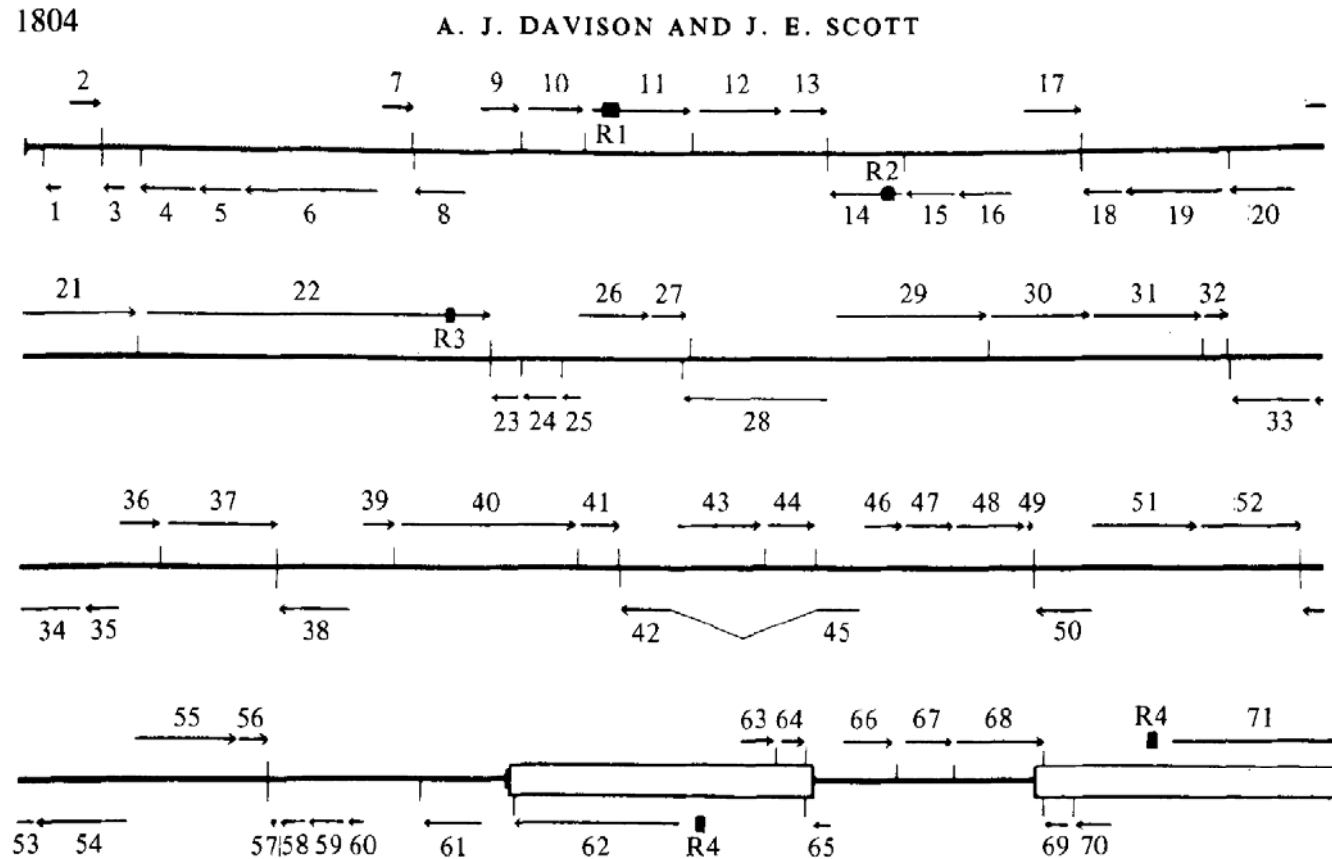
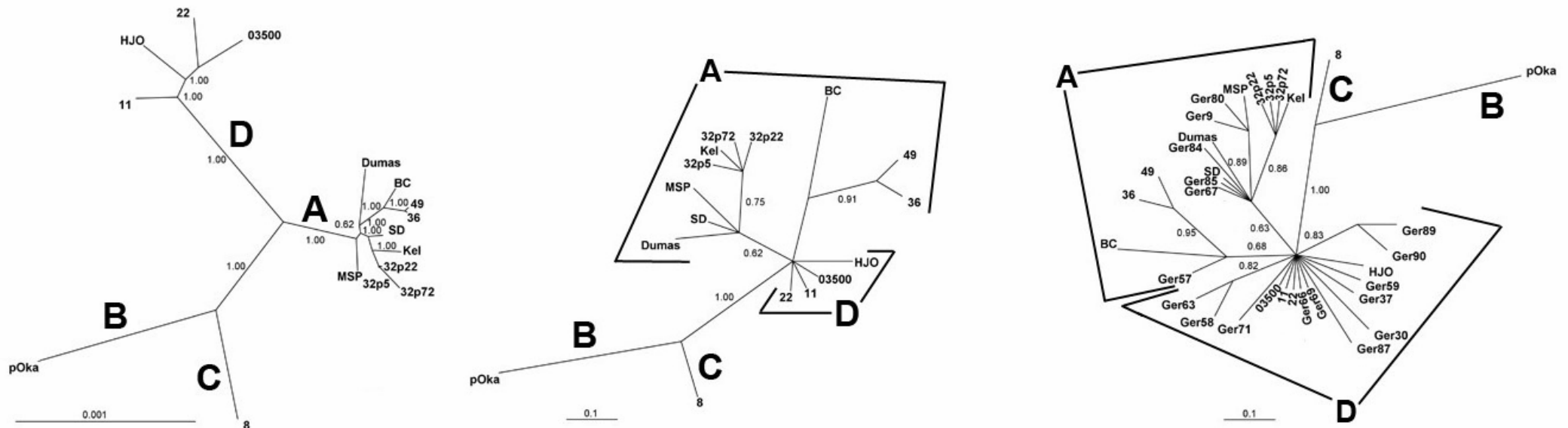


Fig. 2. Summary of the VZV gene layout. The genome is represented in four sections, heavy horizontal lines indicating the unique regions (U_L and U_S), the two large open rectangles depicting the inverted repeats flanking U_S (TR_S and IR_S), and the two heavy short vertical lines denoting the inverted repeats flanking U_L (TR_L and IR_L). ORFs 1 to 71 are illustrated as arrows against the appropriate strand, and correspond to the protein-coding regions shown in Table 1 and Fig. 1. Regions of overlap between ORFs shown in Fig. 1 are not included. Light vertical lines indicate potential polyadenylation sites in the appropriate strand. The locations of the four reiterations (R1 to R4) are denoted by filled rectangles; R1, R2 and R3 are in the coding regions of ORFs 11, 14 and 22, respectively.

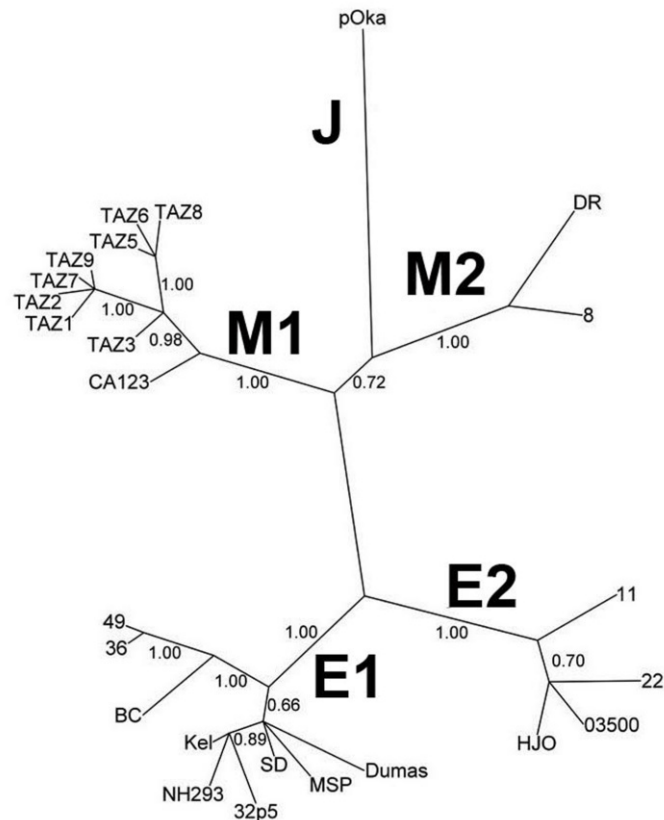
The new genotyping scheme produced identical clusters in phylogenetic analyses compared to full-genome sequences

(A) Full genome ~125 kbp (B) ORF51-58 1990 bp stretch (C) ORF51-58 1990 bp stretch



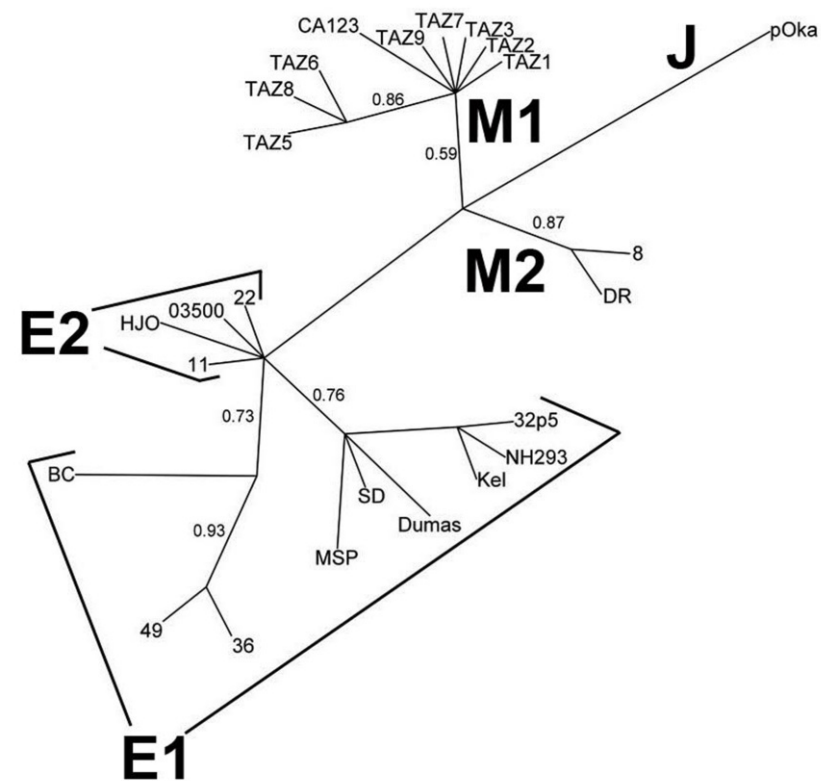
Genotype M1 VZV wild-type strains circulating in Tanzania

(A) 16392 bp stretch



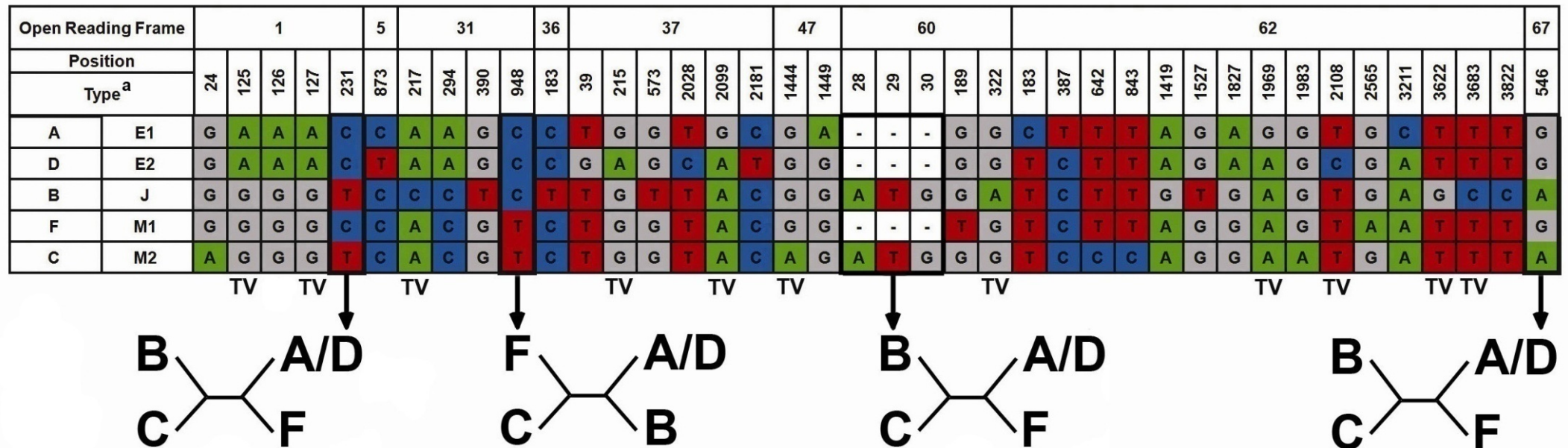
0.1

(B) 1990 bp stretch

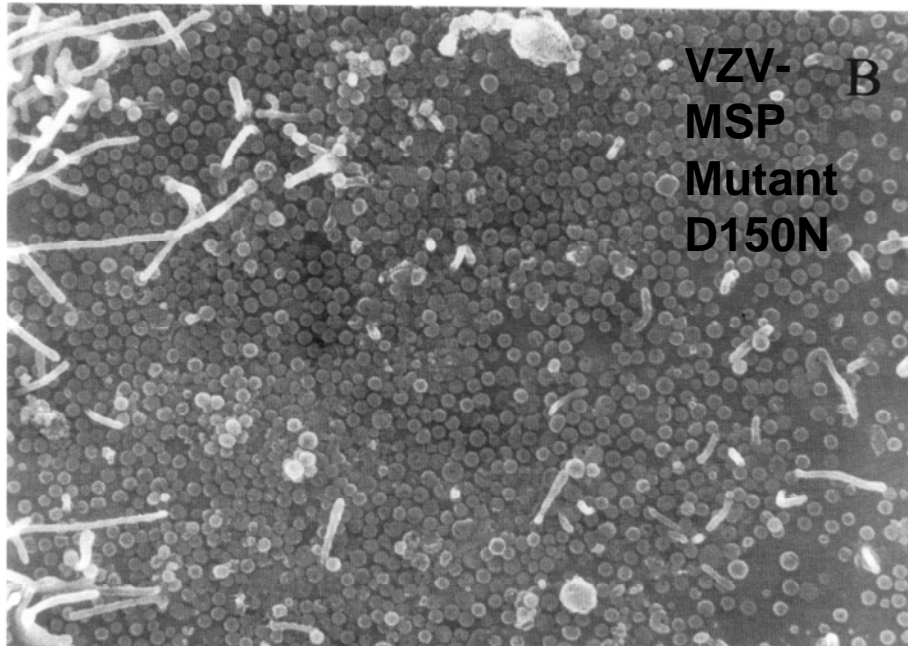
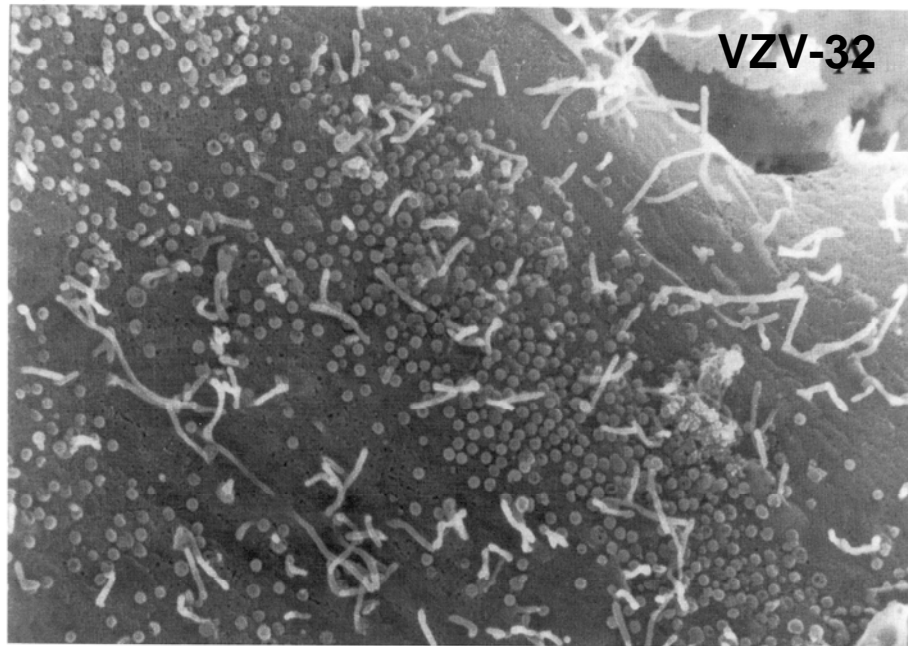


0.1

Sequence analysis suggests recombination events between different VZV genotypes within ORFs 1, 31, 60, and 67



VZV glycoprotein E gene mutations and genotypes



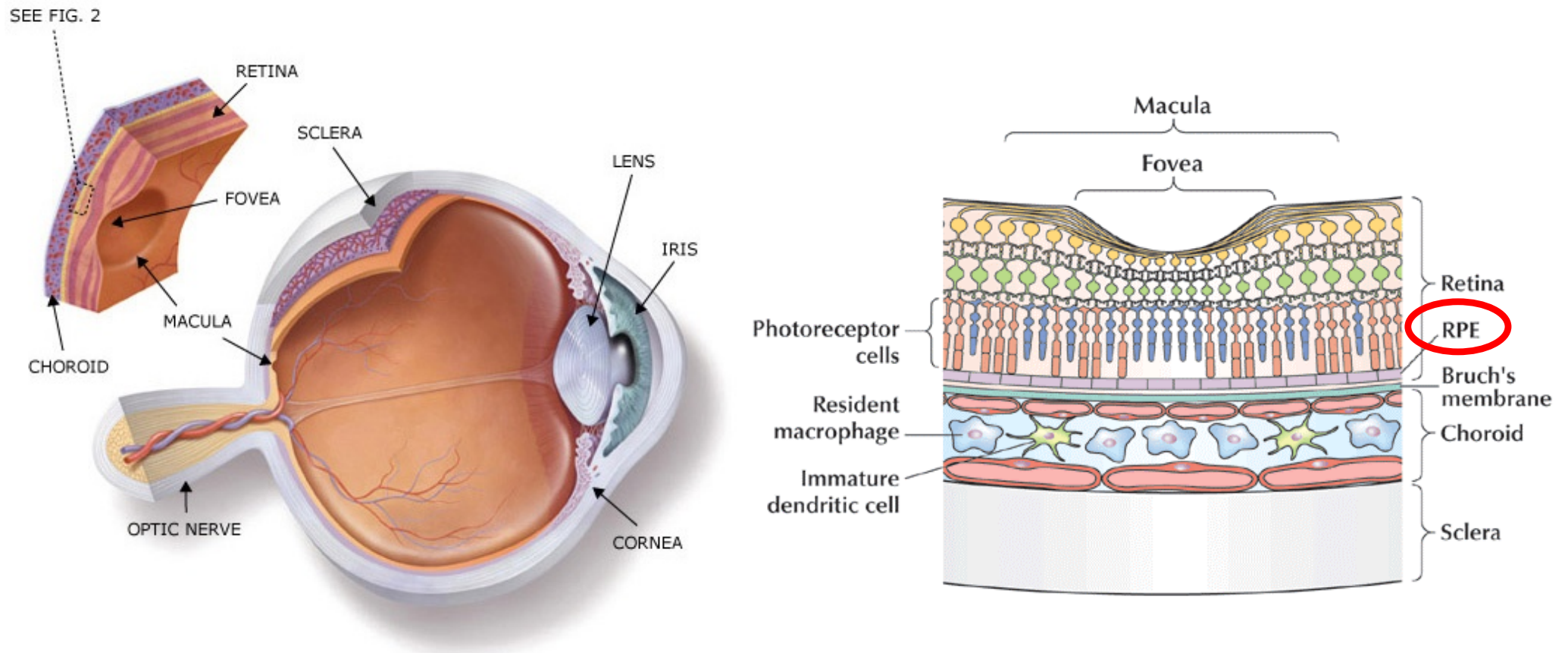
VZV strain	Genotype	gE gene mutation(s) ^b
Ger1*	A	
Ger4*	A	
Ger8*	A	
Ger53*	A	<i>C119T</i> , T660C, <i>C1606A</i>
Ger54*	A	<i>C119T</i> , C393A , T660C, <i>C1606A</i> , C1626T
Ger70*	A	
Ger80*	A	
Ger81*	A	C513T
Ger84*	A	
Ger97	A	
Ger99	A	
Ger100	A	
Ger102	A	
Ger103	A	
Ger107	A	G1284A
Ger110	A	
Ger111	A	
Ger112	A	
Ger113	A	
Ger114	A	
Ger117	A	
Ger118	A	
Ger119	A	
Ger120	A	
Ger121	A	
Ger29*	D	
Ger31*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger33*	D	
Ger45*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger47*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger55*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger66*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger69*	D	
Ger78*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger82*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger88*	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger89*	D	<i>C119T</i> , T660C, <i>C1606A</i> , C1752T
Ger94	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger95	D	<i>C119T</i> , T660C, <i>C1606A</i> , T1683G
Ger101	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger104	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger105	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger108	D	<i>C119T</i> , T660C, <i>C1606A</i>
Ger115	D	<i>C119T</i> , T660C, <i>C1606A</i> , T1683G
Ger116	D	<i>C119T</i> , T660C, <i>C1606A</i>

P < 0.0001

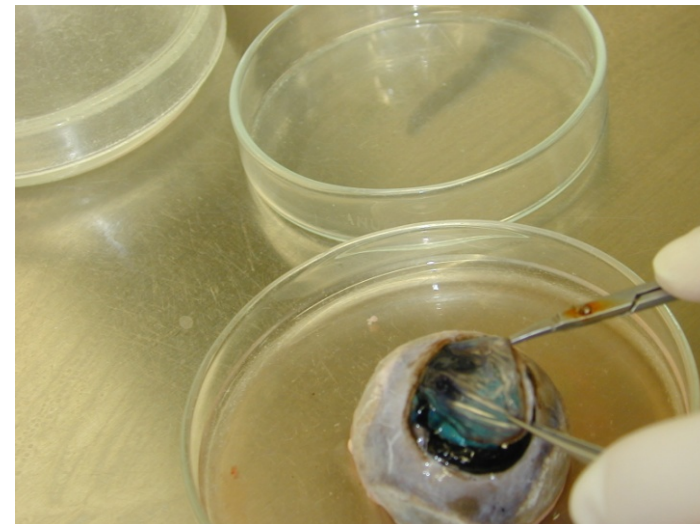
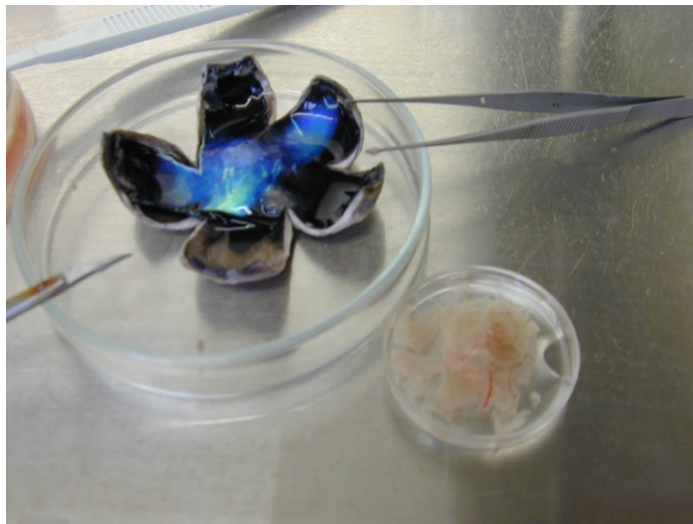
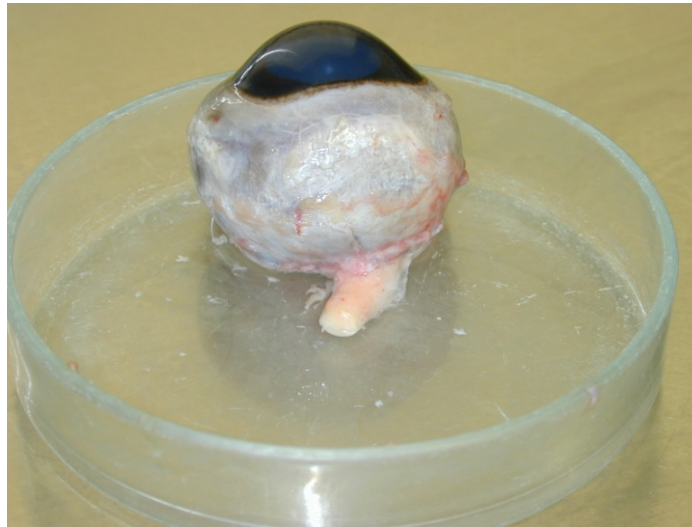
Objective

Reproductive *in vitro* infection system for generating high-titred and cryostable cell-free VZV stocks

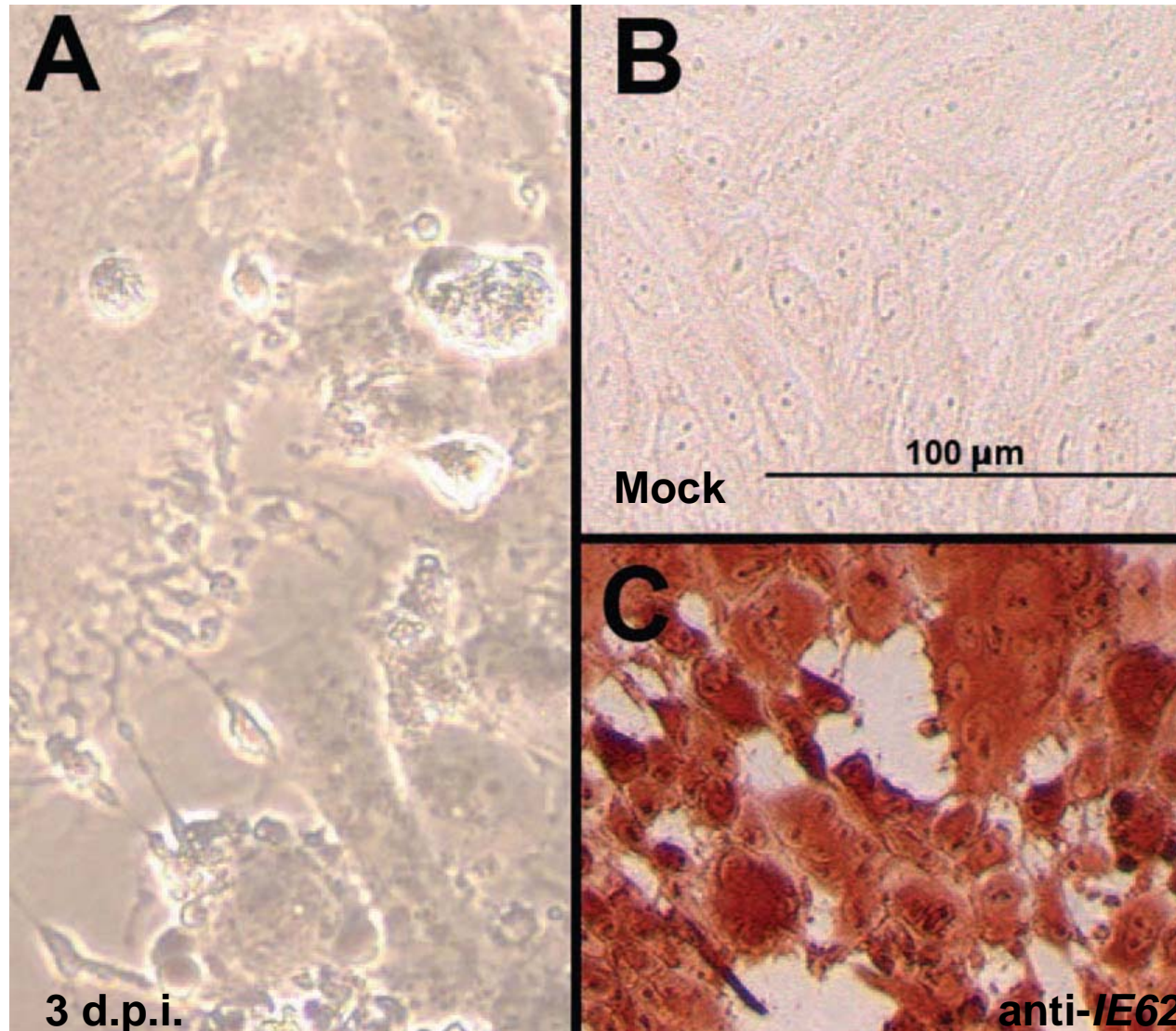
The retinal pigment epithelium (RPE) is involved in the phagocytosis of the outer segment of photoreceptor cells



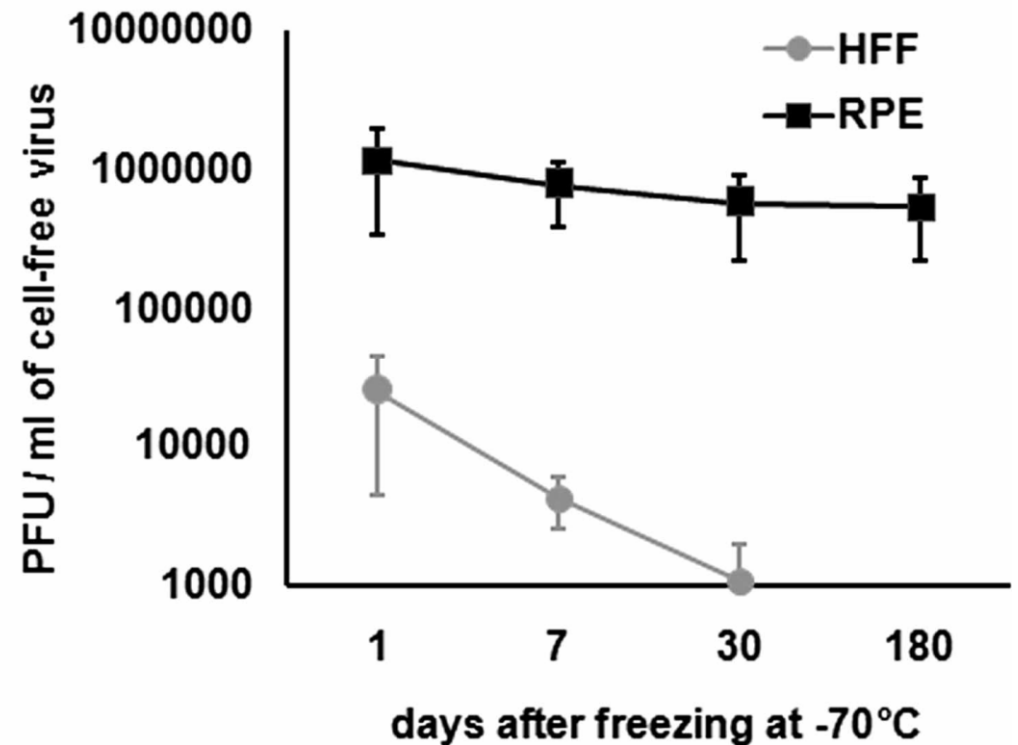
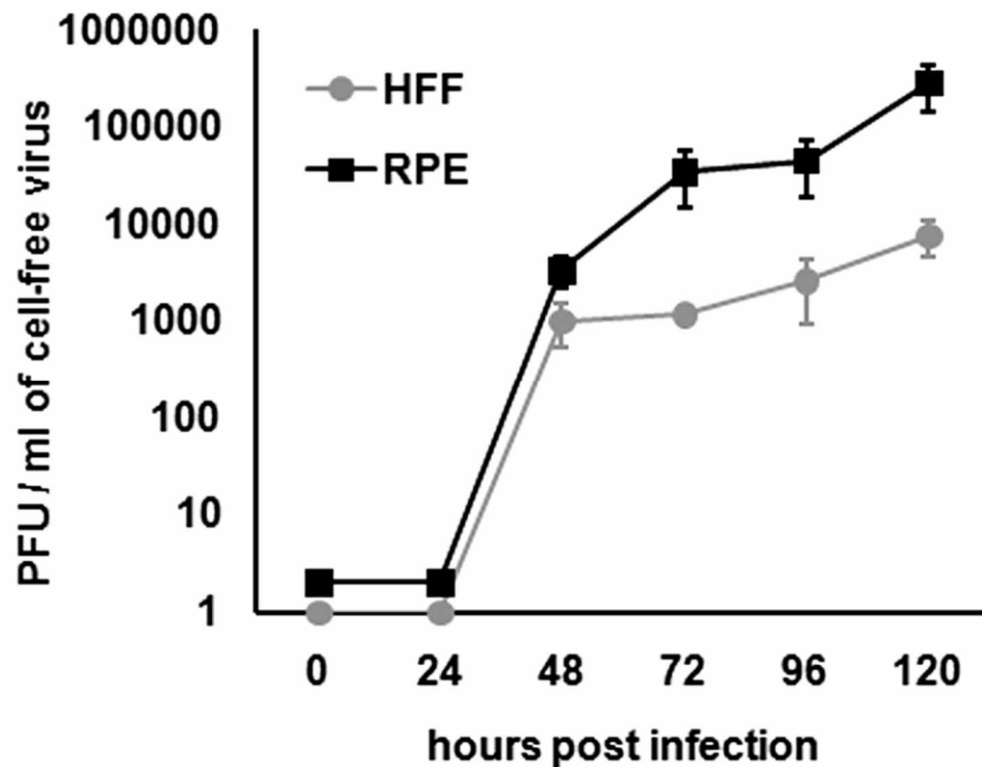
RPE cells were isolated from freshly enucleated bulbi for corneal transplantation



RPE cells are susceptible to productive VZV infection

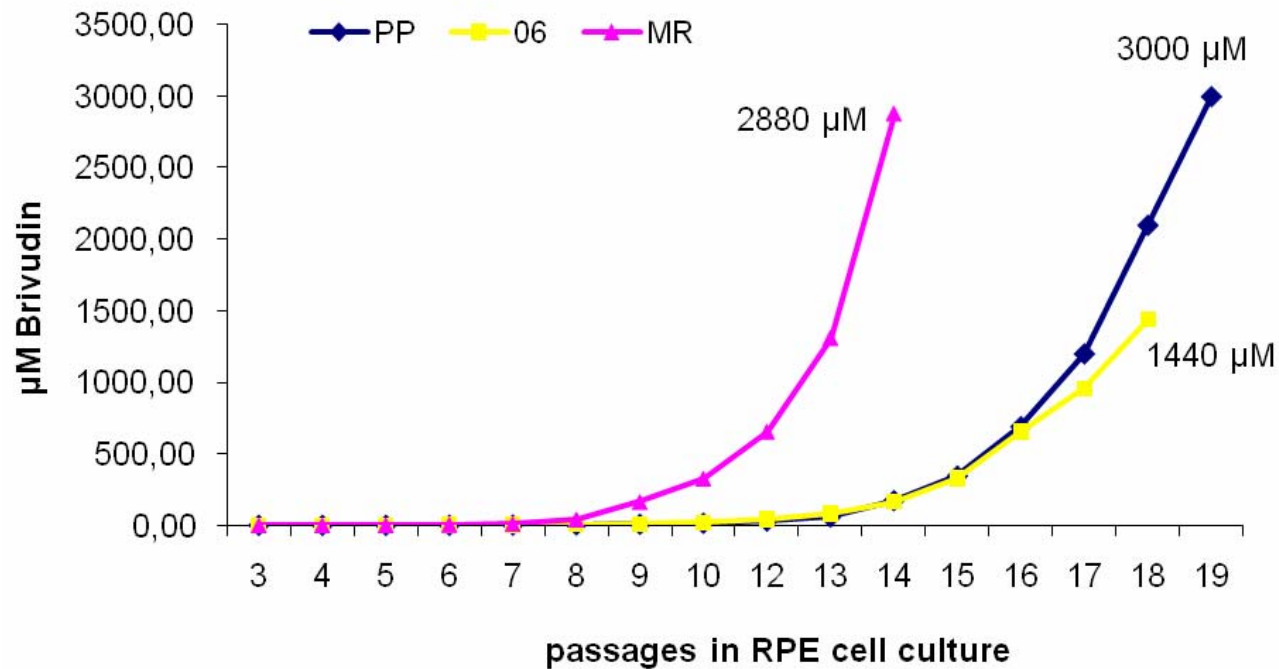


VZV infection of RPE cells is suitable to obtain high-titred and cryostable cell-free VZV

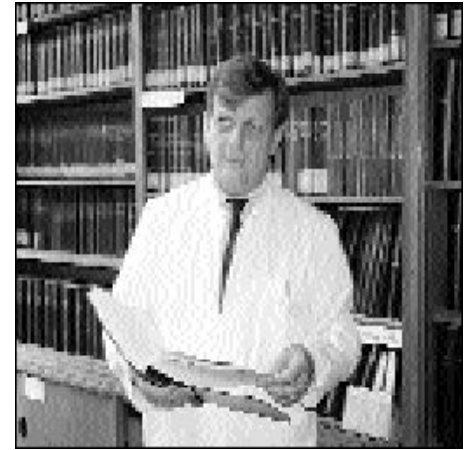


OUTLOOK:

Phenotypic and genetic characterization of varicella-zoster virus mutants resistant to acyclovir, brivudin and foscarnet



Acknowledgement



Paul and Ursula Klein Foundation

Dr. Walter and Luise Freundlich Foundation