

Supplementary Materials

Tables

Table S1. Mass spectrometry spectral count estimates of SIRV3 and SMV1 gene products in cell extracts of coinfecting strain REY14A at 3.5 dpi. Acr - anti CRISPR protein homologs; vp - virion protein. gpx denotes a non annotated ORF.

gene product	kDa	spectral counts	gene product	kDa	spectral counts	gene product	kDa	spectral counts
SIRV3								
gp01	6	6	gp16	7	7	gp29_vp	124	264
gp02_Acr	11	24	gp17_vp	18	12	gp30	63	2
gp03	13	80	gp19_vp	14	100	gp31	72	13
gp04	14	1	gp20	6	2	gp32	19	6
gp05_vp	15	3	gp21	39	102	gp33	30	4
gp07	13	14	gp22	8	4	gp34	21	6
gp08	37	1	gp23	9	2	gp35	42	23
gp09	46	50	gp24	13	14	gp36_Acr	11	12
gp10	7	23	gp25	55	2	gp37_Acr	12	10
gp12	15	77	gp26	18	27	gp39	13	4
gp13	51	21	gp27	14	2	gp40	17	21
gp14	25	2	gpx	8	28	gp41	11	35
gp15	9	22	gp28	13	24	gp43	12	7
SMV1								
gp05_vp	86	11	gp11_vp	17	3	gp46	31	nd
gp06_vp	14	2	gp32_vp	11	2			
gp09_vp	224	15	gp36	12	5			

Table S2 Altered expression of antitoxin-toxin gene pairs and IS element ORFs in coinfecting strain REY15A at early and late infection stages. + increased and - reduced transcript levels by ≥ 2 -fold.

host gene ID	amino acids	Orf	early	late	late/early
			log ₂ fold changes		
antitoxin-toxins					
SiRe_0374	80	VapB		+ 2,0	+ 2,8
SiRe_0373	131	VapC			+ 1,6
SiRe_0403	73	VapB		+ 2,0	
SiRe_0402	120	VapC		+ 2,0	
SiRe_0743	81	VapB		+ 1,7	
SiRe_0744	137	VapC		+ 1,9	
SiRe_0888	76	VapB		+ 3,4	
SiRe_2170	73	VapB		+ 1,6	+ 1,5
SiRe_2171	134	VapC		+ 1,6	
SiRe_0458	144	HEPN-domain protein		+ 2,0	
IS elements					
SiRe_0690	401	IS605_OrfB-TpnB	+ 1,6		
SiRe_0692	401	IS200/IS605_OrfB		- 3,2	- 2,7
SiRe_2602	401	IS605_OrfB-TpnB		+ 2,8	
SiRe_0752	92	IS605_OrfB-TpnB	+ 2,7	+ 2,0	
SiRe_0773	63	IS605_OrfB-TpnB		+ 3,0	
SiRe_0856	277	IS110_transposase		+ 1,8	
SiRe_0858	109	IS6_transposase	+ 4,5	+ 3,1	
SiRe_2529	164	IS6_transposase		+ 7,8	
SiRe_2432	249	IS607_transposase		+ 2,8	
SiRe_0449	275	ISC1395_Orf1	+ 2,1	+ 2,4	
SiRe_0463	236	ISC1395_Orf2	+ 3,4	+ 3,1	

Figure Legends

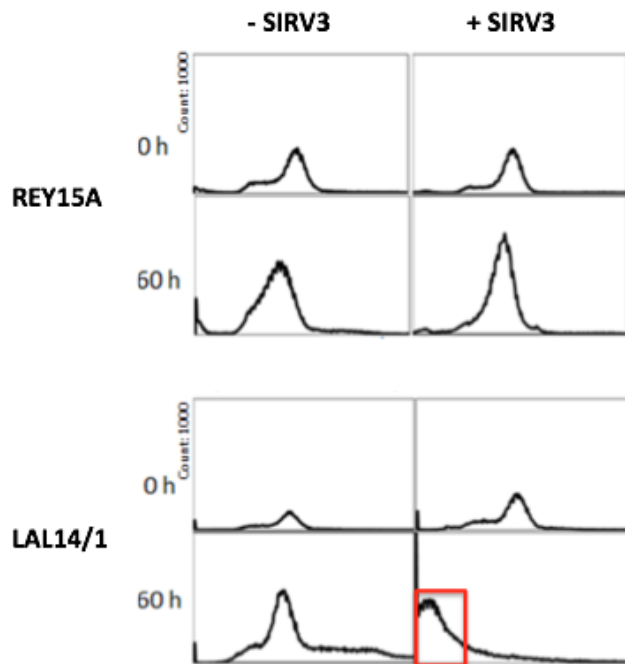


Figure S1. Cellular DNA content of cells that were uninfected (- SIRV3) or infected with SIRV3 (+ SIRV3) in strains REY15A (top) and LAL14/1 (below). The portion of DNA-less cells detected at 60 hpi in LAL14/1 cells is enclosed by the red rectangle.

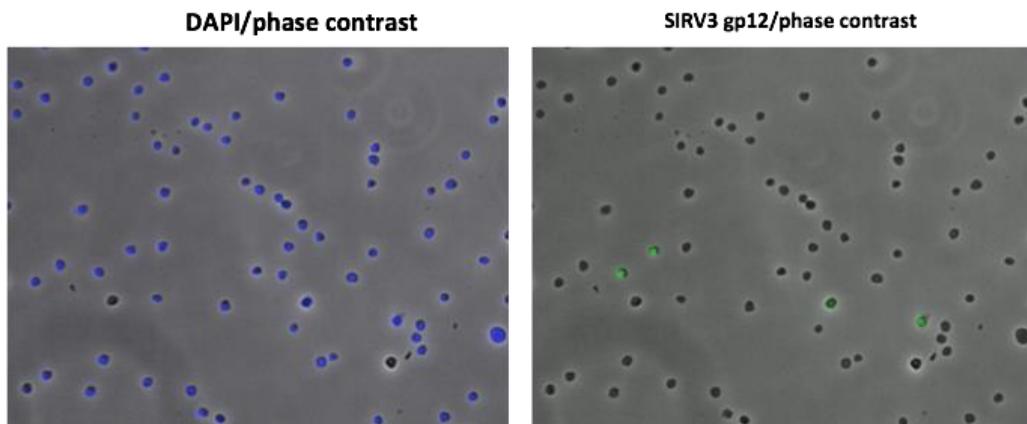


Figure S2. Immunofluorescence microscopy of SIRV3-infected REY15A cells. Left: merging of DNA stained with DAPI (blue) and phase contrast. Right: merging of SIRV3 gp12 staining (green) with phase contrast. The fraction of uninfected cells (Figure 3A) was measured by comparing the number of gp12 positive cells with those of DAPI-stained cells. Approximately 500 cells were analysed per sample.

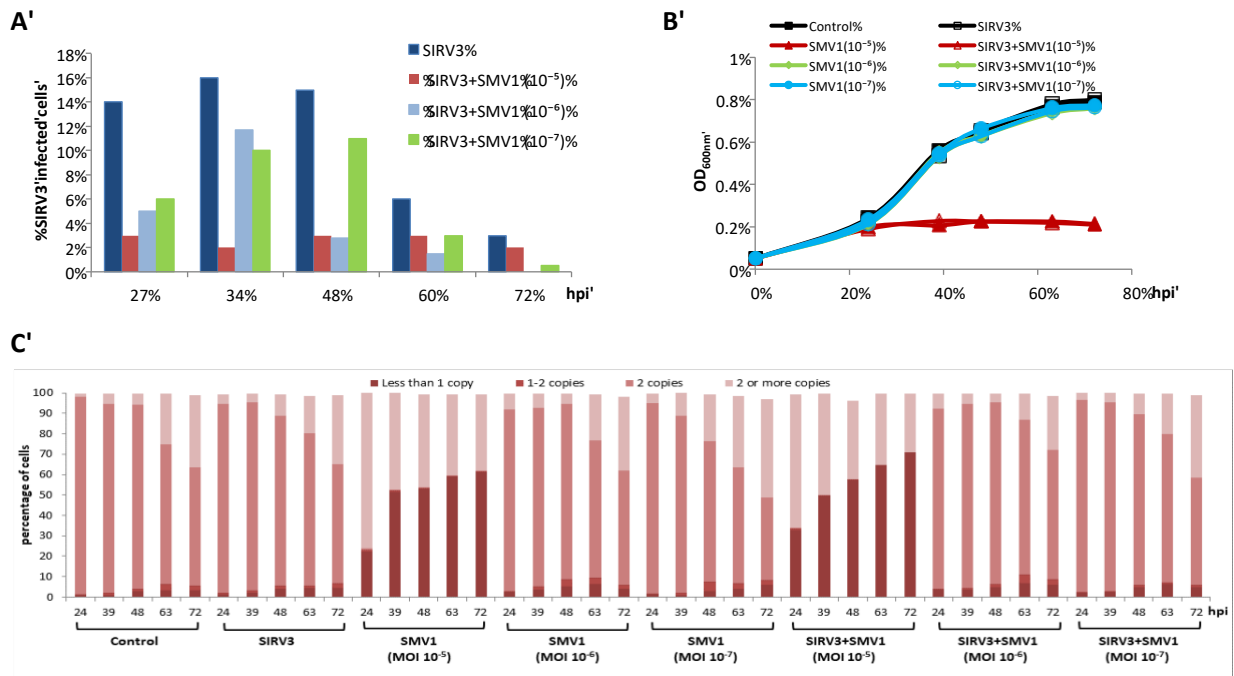


Figure S3. A second dataset for for strain REY15A cells infected by SIRV3 (MOI = 1 always) and SMV1 (MOIs in brackets), singly and together. A. Percentage of SIRV3-containing cells infected by SIRV3 alone, or by both viruses at increasing levels of SMV1. B. Growth curves of strain REY15A cells infected with SIRV3 or coinfecting. C. DNA content of strain REY15A cells infected by single viruses, or the mixture, analysed by flow cytometry.

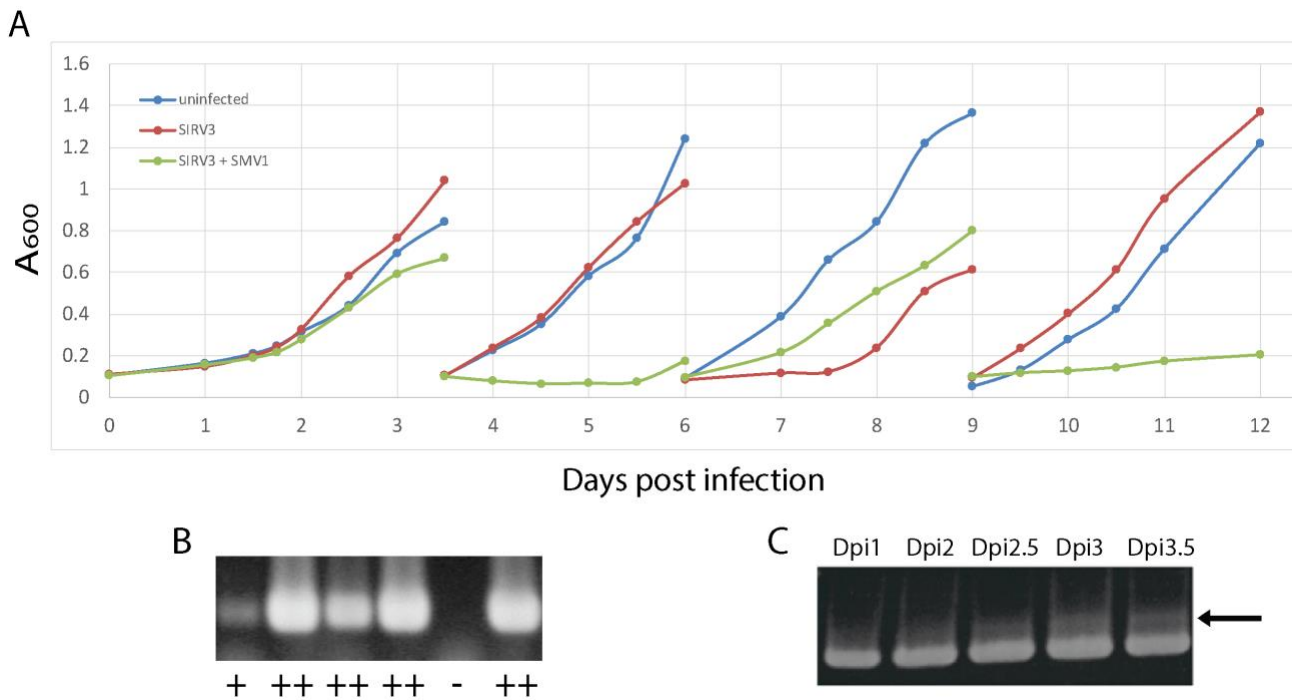


Figure S4. (A) Growth curves of the cultures uninfected, infected with SIRV3 and coinfecting. 50 ml samples were diluted to $A_{600} = 0.05$ with fresh medium in every 3-4 days when uninfected control cultures exceeded $A_{600} = 1$ to prevent cell death due to nutrient deficiency. (B) A gel example illustrating how the relative viral levels presented in Table 3 were estimated visually from the gel band intensities. (C) Example depicting how spacer acquisition was initially detected by PCR amplification of the leader-end CRISPR array. Larger PCR products, indicated by the black arrow, were observed at 3 dpi and beyond.

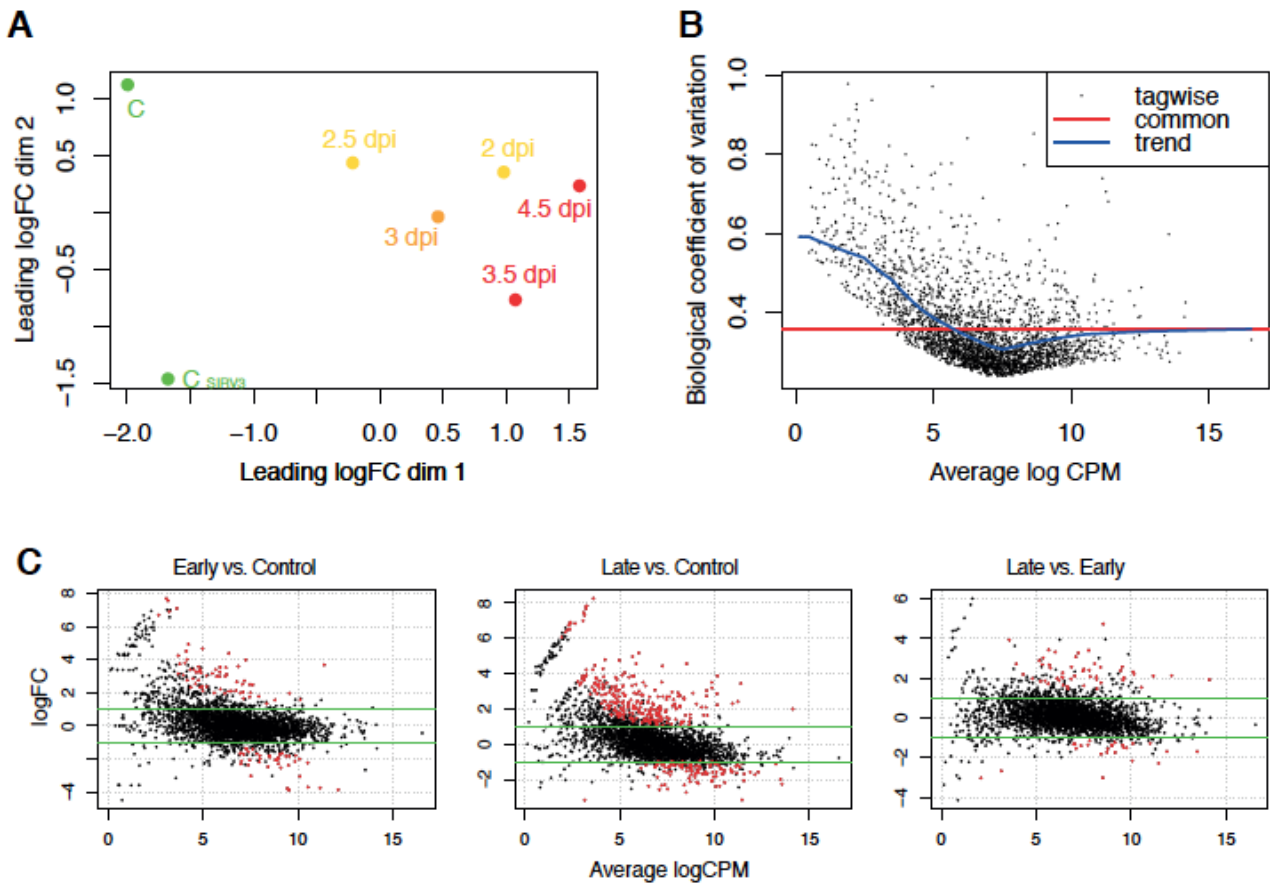


Figure S5. Differential transcription analysis and results overview. A. Multi-dimensional scaling (MDS) plot of the RNA samples. Distances correspond to leading log-fold-changes (logFC) between each pair of RNA samples. Colour codes highlight control (green), early infection (yellow), and late infection (orange and red) groups. Zero-read genes were assigned a value of 0.25 for the \log_2 transformation ($\text{prior.count} = 0.25$, *plotMDS* function from the edgeR package). C and C_{SIRV3} - controls; dpi - days post infection. B. Computed dispersion values for each gene (tagwise), transcription level (trend) and for the entire dataset (common) (*estimateDisp* function). C. Overview of differential transcription results between each group pair (*glmLRT* function); each dot represents a gene, and significantly differentially transcribed genes ($p < 0.05$) are marked in red.