Supporting Information

Emonet *et al.* 10.1073/pnas.0900088106

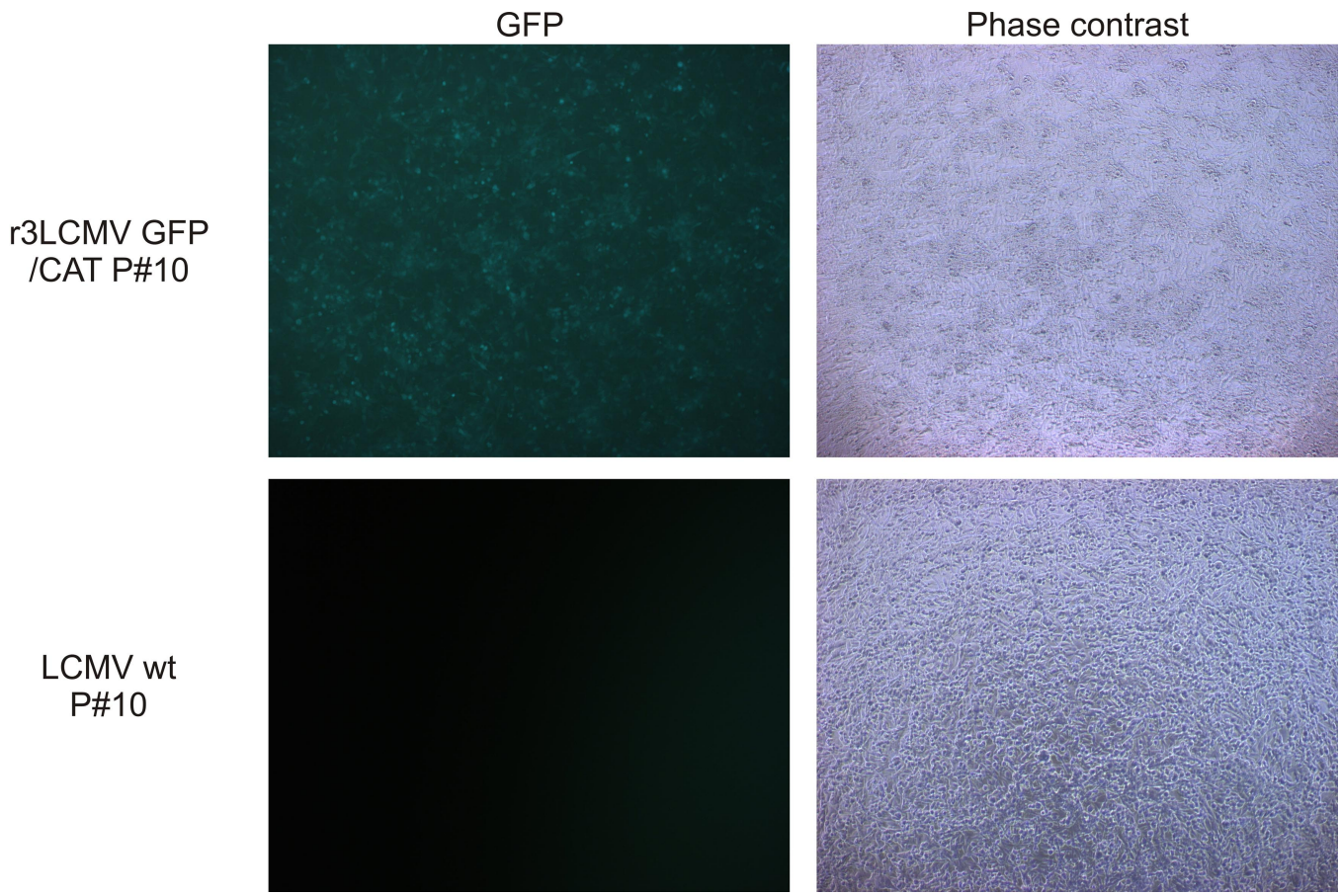

Fig. S1. Stable GFP expression by trisegmented recombinant lymphocytic choriomeningitis virus (r3LCMV) GFP/CAT during serial passages in cultured cells. BHK-21 cells were infected [multiplicity of infection (moi) = 0.1] with r3LCMV GFP/CAT from passage 9 and at 48 h after infection (p.i.). GFP expression was examined by epifluorescence microscopy using a 5× objective.

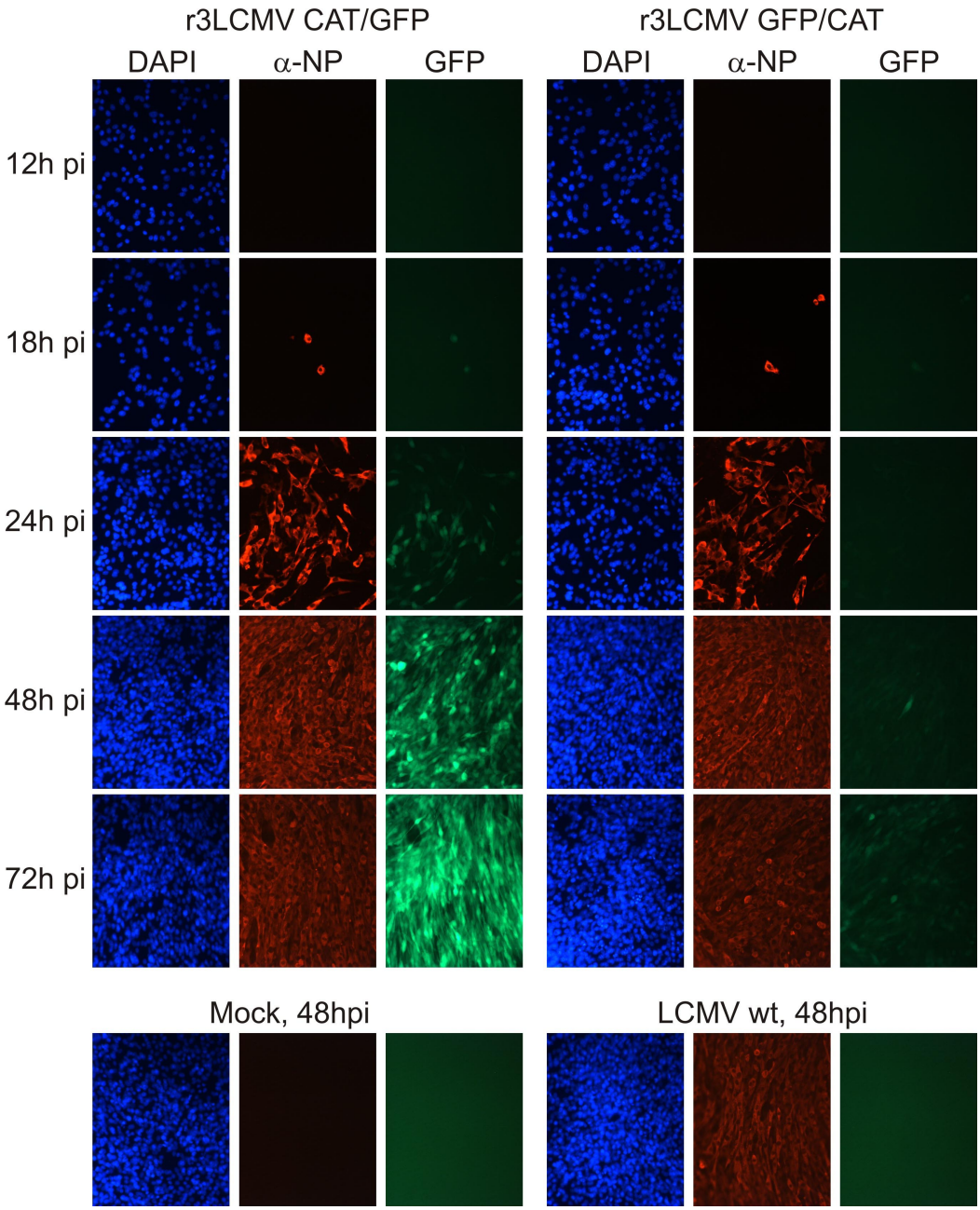

Fig. S2. Effect of genome location on GFP expression by r3LCMV. BHK-21 cells were infected (moi = 0.01) with either r3LCMV GFP/CAT or r3LCMV CAT/GFP and at different time points. Cells were fixed, and expression of viral antigen was detected by immunofluorescence with an anti-LCMV antibody (red). GFP expression (green) was detected directly. Cell nuclei were visualized by using DAPI staining (blue). Images were taken by using an epifluorescence microscope at 20× magnification with the same exposure conditions.

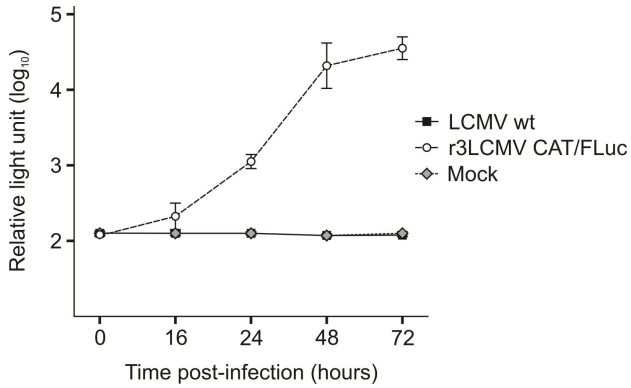

Fig. S3. Firefly luciferase (FLuc) activity of cells infected by r3LCMV CAT/FLuc. BHK-21 cells (96-well plate) were infected (moi = 0.1) with either LCMV WT or r3LCMV CAT/Fluc, and at different time points cell lysates were prepared and FLuc activity was determined.

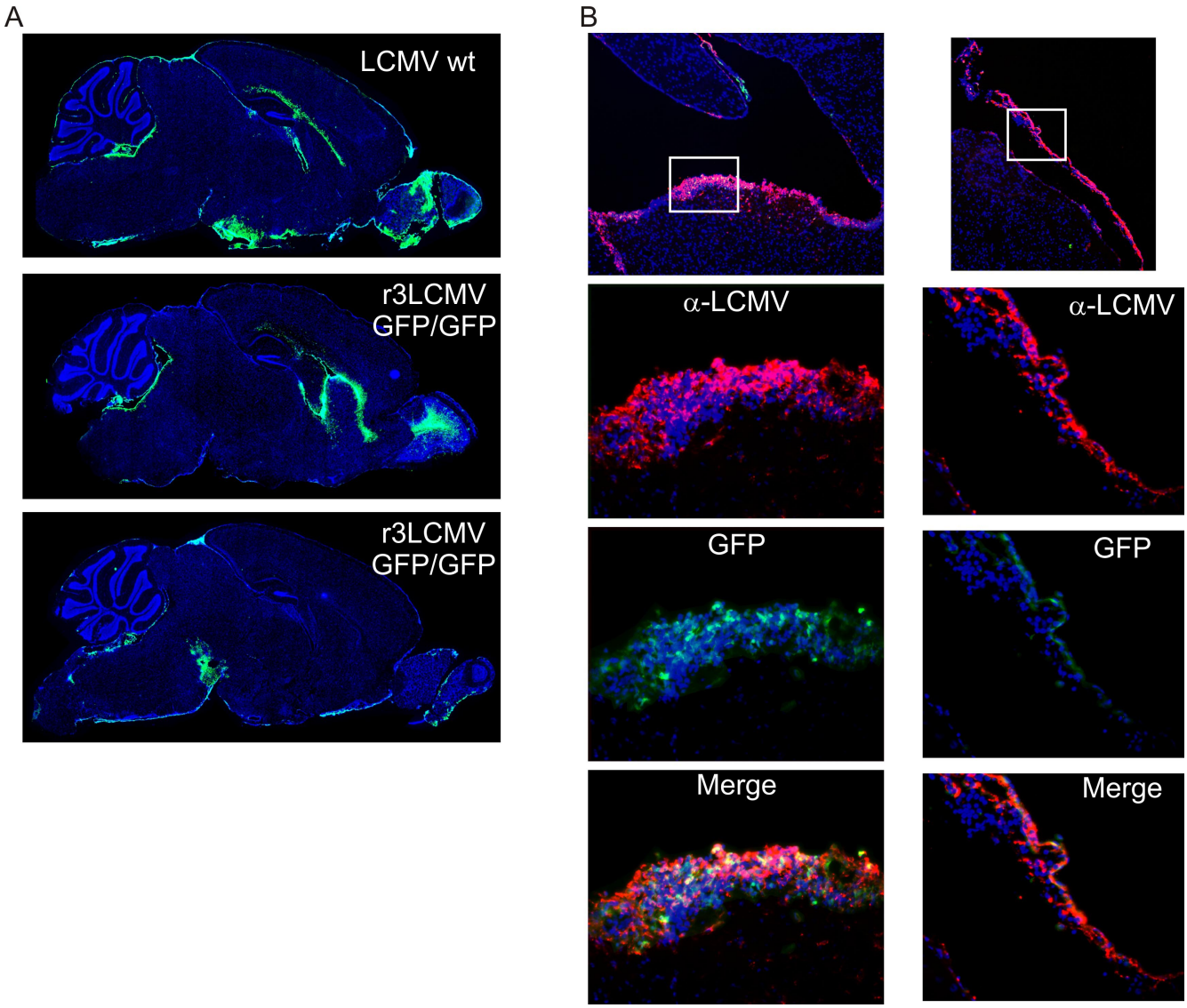

Fig. S4. Tropism and GFP expression of r3LCMV GFP/GFP in mouse brain. Mice were infected with LCMV wild-type (WT) or r3LCMV GFP/GFP [10^5 focus-forming units (ffu), intracerebrally (i.c.)]. At days 6 (*A*) or 5 (*B*) p.i., brains were collected, and 6-μm sagittal sections were analyzed by immunohistochemistry with an antibody against LCMV. (*A*) Two-color organ reconstructions to visualize the distribution of LCMV antigen (green) and cell nuclei (blue) were generated by using an epifluorescence microscope and a 5× objective for one LCMV WT- and two r3LCMV GFP/GFP-infected mice. (*B*) Details of a r3LCMV GFP/GFP-infected brain showing the direct GFP expression (no amplification, green) and the LCMV-infected cells (red) in the ventricle (*Left*) and meninges (*Right*) areas. (*Top*) Images were obtained with a 5× objective. (*Bottom three*) Higher-resolution images were obtained with a 20× objective. Areas shown at higher resolution correspond to those indicated by the white squares.

Table S1. Mutation accumulated in GPC and GFP genes during serial passages of r3LCMV GFP/CAT in cultured cells

Gene	Passage no.	Clone no.	Nucleotide substitutions	Mutations
GPC	2	1	0/1456	/
		2	1/1456	A262G (M88V)
		3	0/1456	/
		4	4/1456	T201C (silent); A262G (M88V); C1149T (silent); A1404G (silent)
	10	1	0/1456	/
		2	0/1456	/
		3	0/1456	/
GFP	2	1	1/679	A278G (Y93C)
		2	1/679	C117G (silent)
		3	2/679	A593G (D198G); A668G (E223G)
		4	0/679	/
	10	1	0/679	/
		2	1/679	A449G (K150R)
		3	0/679	/
		4	1/679	C117T (silent)
		5	1/679	G73A (G25S)

Clones of GPC and GFP genes were sequenced at passages 2 and 10 and compared with the sequences of the corresponding pol-I S vector used to rescue the virus. For each individual clone sequenced, the total number of nucleotide substitutions and location and type of mutation are indicated. Amino acids changes are shown in parentheses. A slash is indicated when no mutation was found.