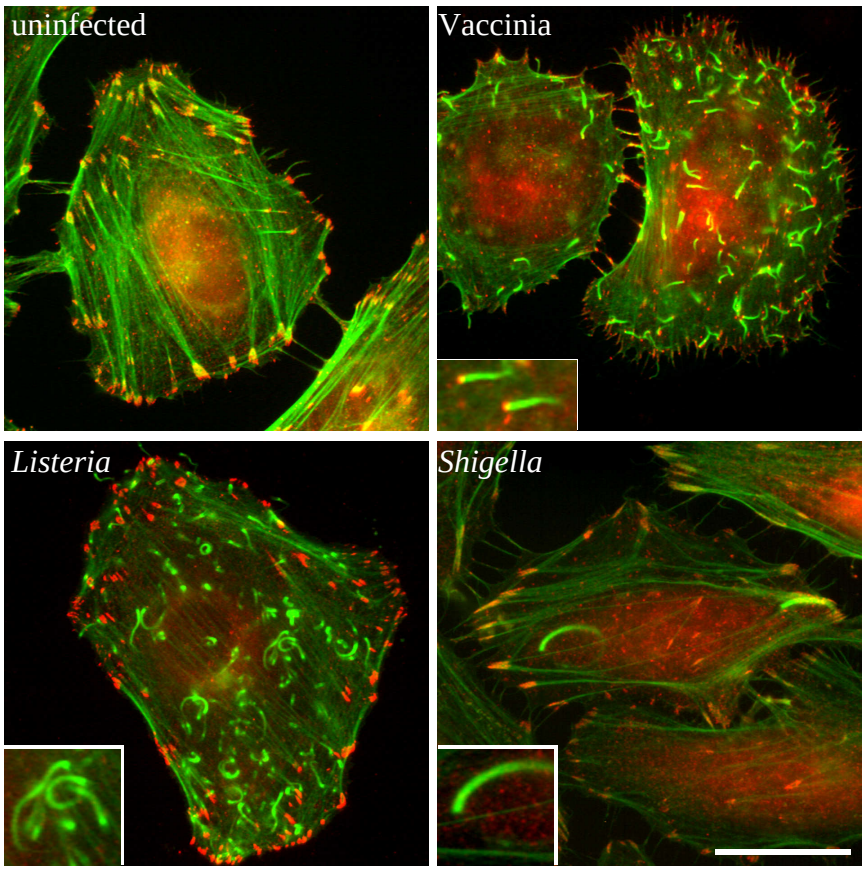


Figure 11: Localization of phosphotyrosine epitopes (red) in uninfected and infected HeLa cells. A phosphotyrosine signal is observed on vaccinia but not *Listeria* or *Shigella* actin tails . The actin cytoskeleton is visualized in green. Insets show enlarged actin tails. Scale bar: 20 μm.

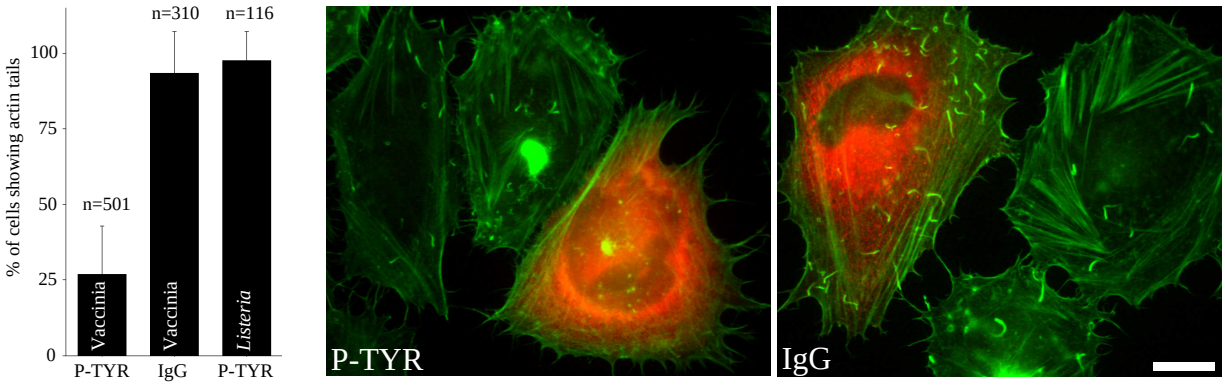
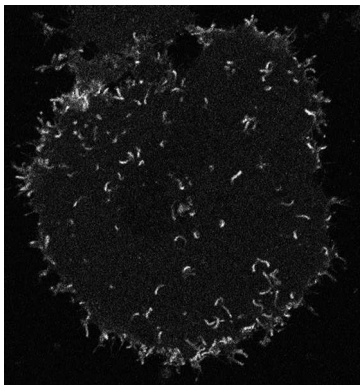


Figure 12: Micro-injection of anti-phosphotyrosine antibody into cells infected with vaccinia virus severely inhibits viral actin tail assembly. In 7 independent experiments a total of 501 vaccinia infected cells were injected (graph on left). In 5 independent control experiments micro-injection of IgG antibody did not show any effect on viral actin tail assembly. Micro-injection of anti-phosphotyrosine antibodies into cells infected with *Listeria* (n=116 in 7 experiments) did not inhibit bacterial actin tail formation. Error bars denote standard deviation from the mean. Example cells are shown with the actin cytoskeleton visualized in green and co-injected dextran in red to reveal the injected cells. In the cell injected with phosphotyrosine antibodies no actin tails are visible, while the cell injected with IgG as a control shows numerous actin tails. Scale bar: 20 μm.



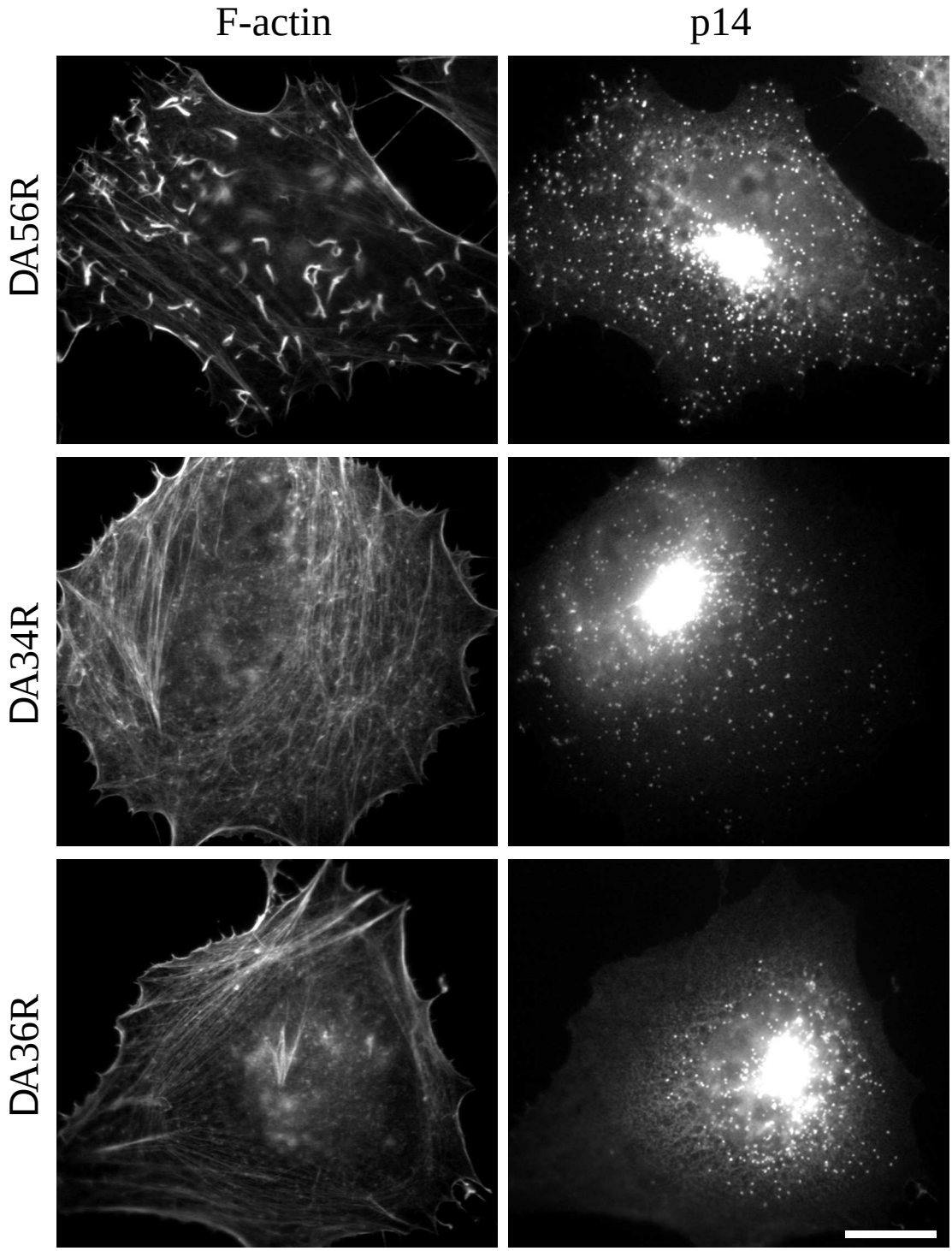
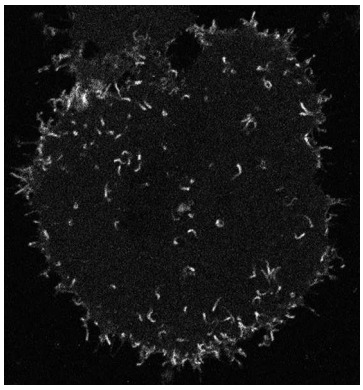


Figure 13: Role of IEV proteins in actin tail formation. Immunofluorescence analysis of different recombinant vaccinia viruses lacking the genes for the IEV specific proteins A56R, A34R or A36R. Actin is visualized with phalloidin and virus particles are labelled by an antibody against the viral protein p14 (A27L). Cells were fixed 10 hours post infection. Note that viruses can disperse throughout the cell in the absence of actin tail formation. Viruses lacking the genes B5R or F13L also do not make actin tails at this time after infection (Figure 14 and Cudmore et al., 1995). Scale bar: 10 ㎜.



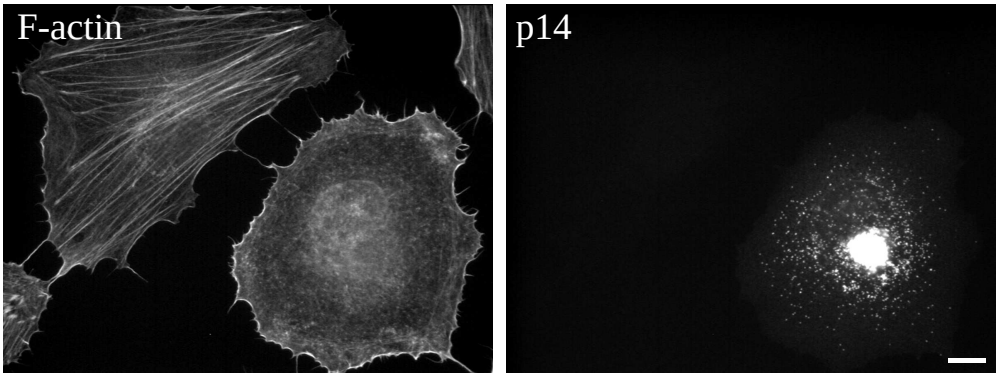


Figure 14: Infection for 8 hours with vaccinia strains that do not make actin tails still induces disassembly of actin stress fibers. A cell infected with a recombinant vaccinia virus lacking the IEV specific protein B5R is shown next to an uninfected cell. Virus particles are labelled with an antibody against the IMV specific protein p14. As IEVs are wrapped IMVs, p14 labels both virus forms. Scale bar: 10 μm.

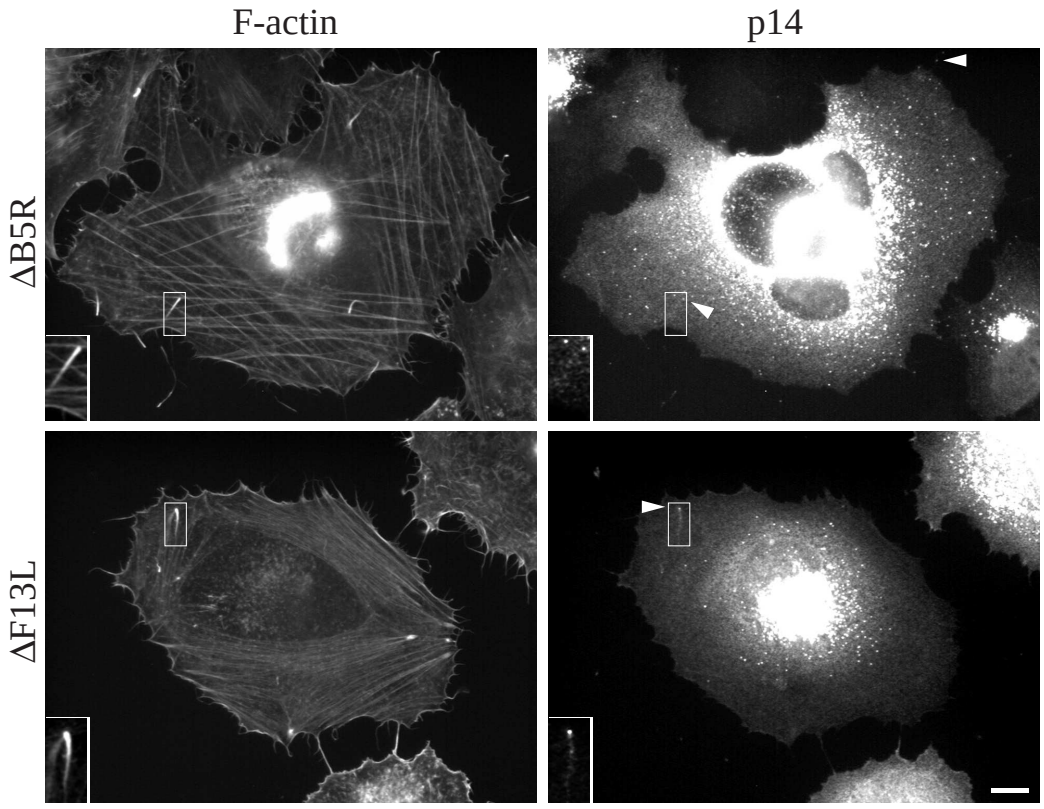
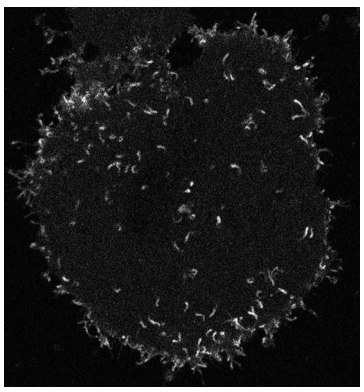


Figure 15: Vaccinia strains lacking the IEV specific proteins B5R or F13L are able to form actin tails at late stages of infection. Cells were fixed 18 hours post infection, stained for actin and virus particles. The viral staining has been over-exposed to visualize single viral particles on the tip of actin tails (arrowheads, see also enlargements of the boxed areas as insets). Scale bar: 10 μm.



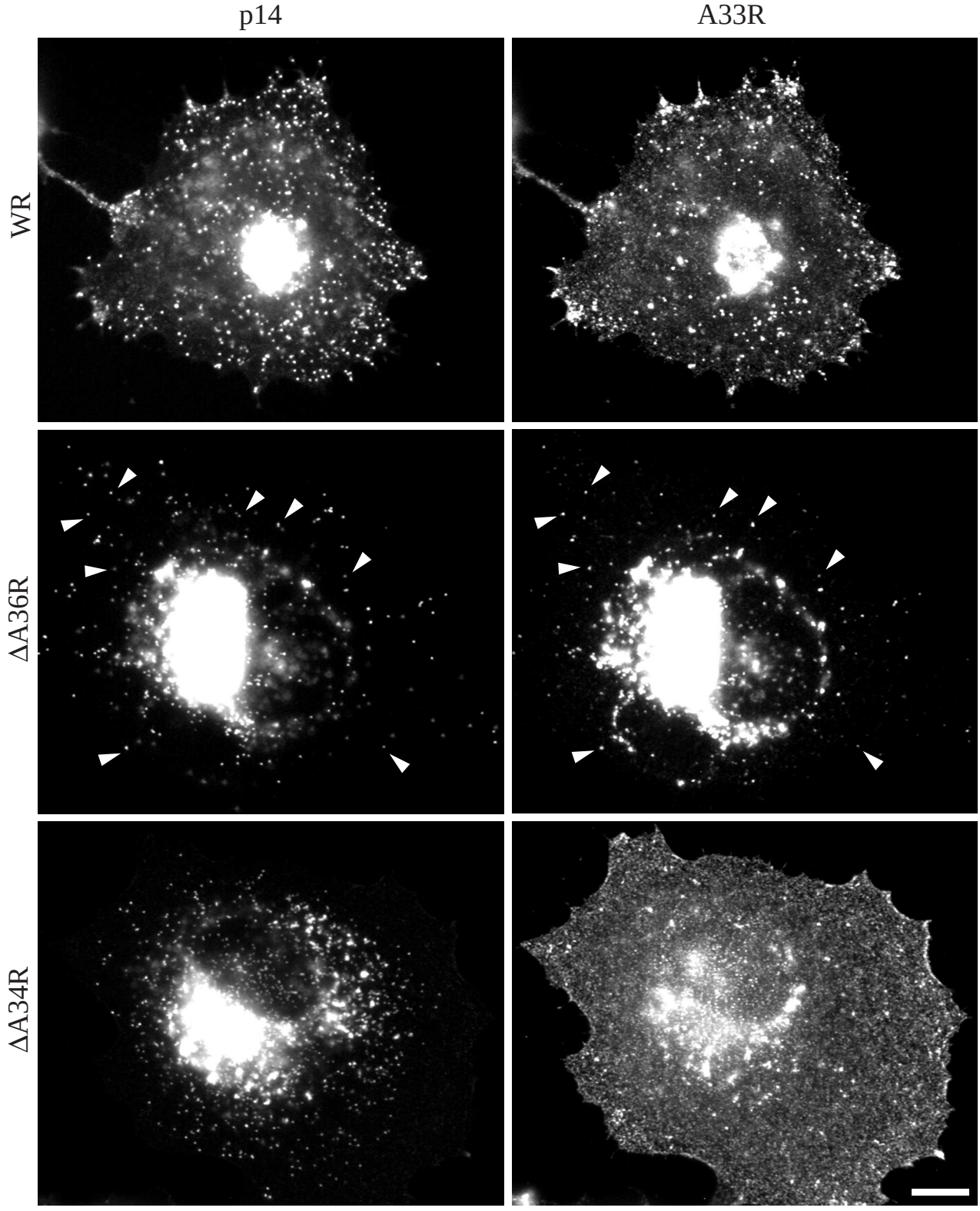
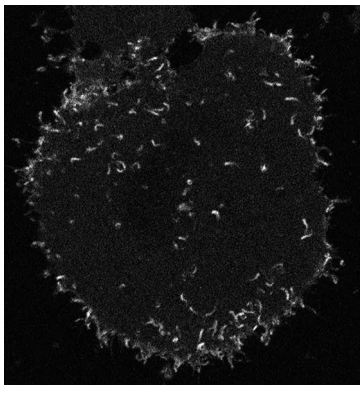


Figure 16: Vaccinia virus strain ΔA34R does not make IEVs. Immunostaining with antibodies staining both IMVs and IEVs (anti-p14) or specifically only IEVs (anti-A33R) reveals that viruses lacking the A36R gene are still able to form IEVs. In contrast, viruses lacking the A34R gene are not making IEVs. Arrowheads indicate IEVs as found by co-localisation of p14 and A33R labelling. Note that A33R is dispersed in cells infected with ΔA34R. Scale bar: 10 μm.



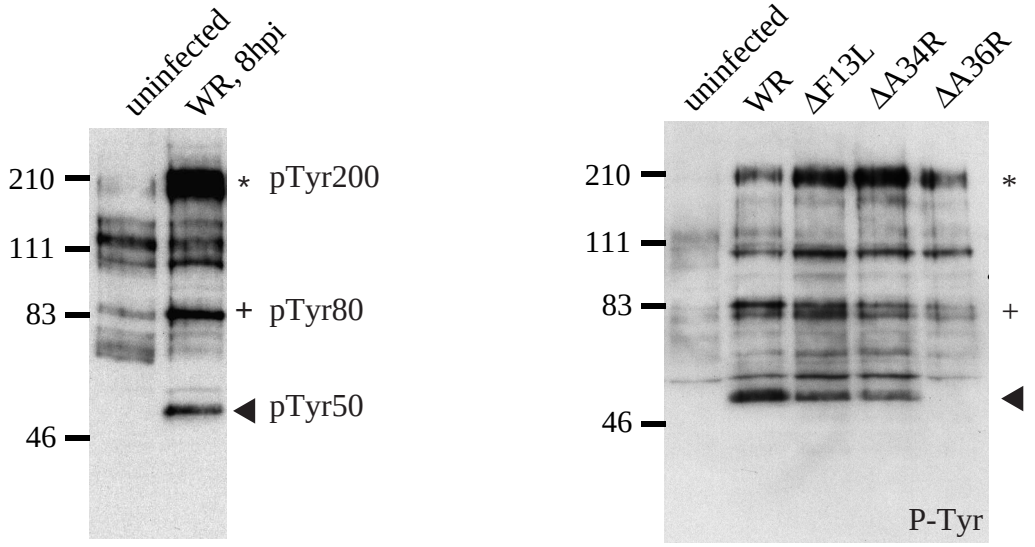


Figure 17: Vaccinia infection consistently induces tyrosine phosphorylation of three proteins as judged by western analysis (left). Western analysis of cells infected with recombinant vaccinia strains reveals that pTyr50 is absent from ΔA36R infected cells indicating that A36R might be phosphorylated. A 110 kDa band is only observed in some experiments. Molecular weight markers are indicated in kDa.

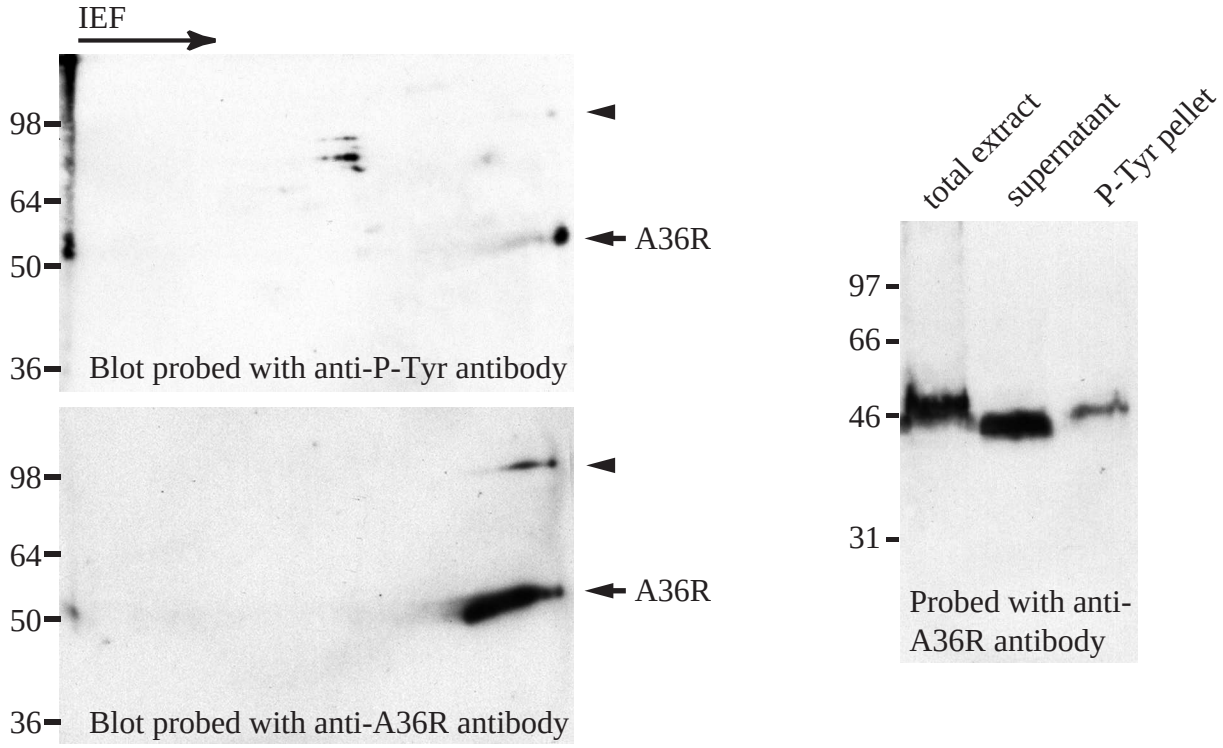
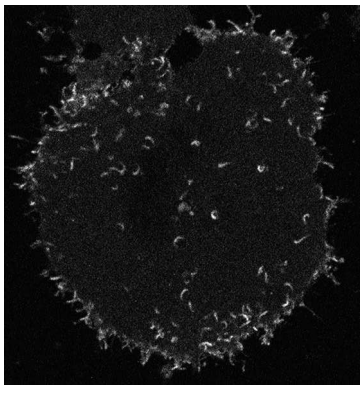


Figure 18: A36R is phosphorylated on tyrosine. Western analysis of 2-dimensional gel electrophoresis shows that a small fraction of A36R is phosphorylated on tyrosine (left). Arrowheads indicate a higher mass form of A36R. Immunoprecipitation with anti-phosphotyrosine antibody pellets A36R (right). Extracts were prepared from cells 8 hours post infection.



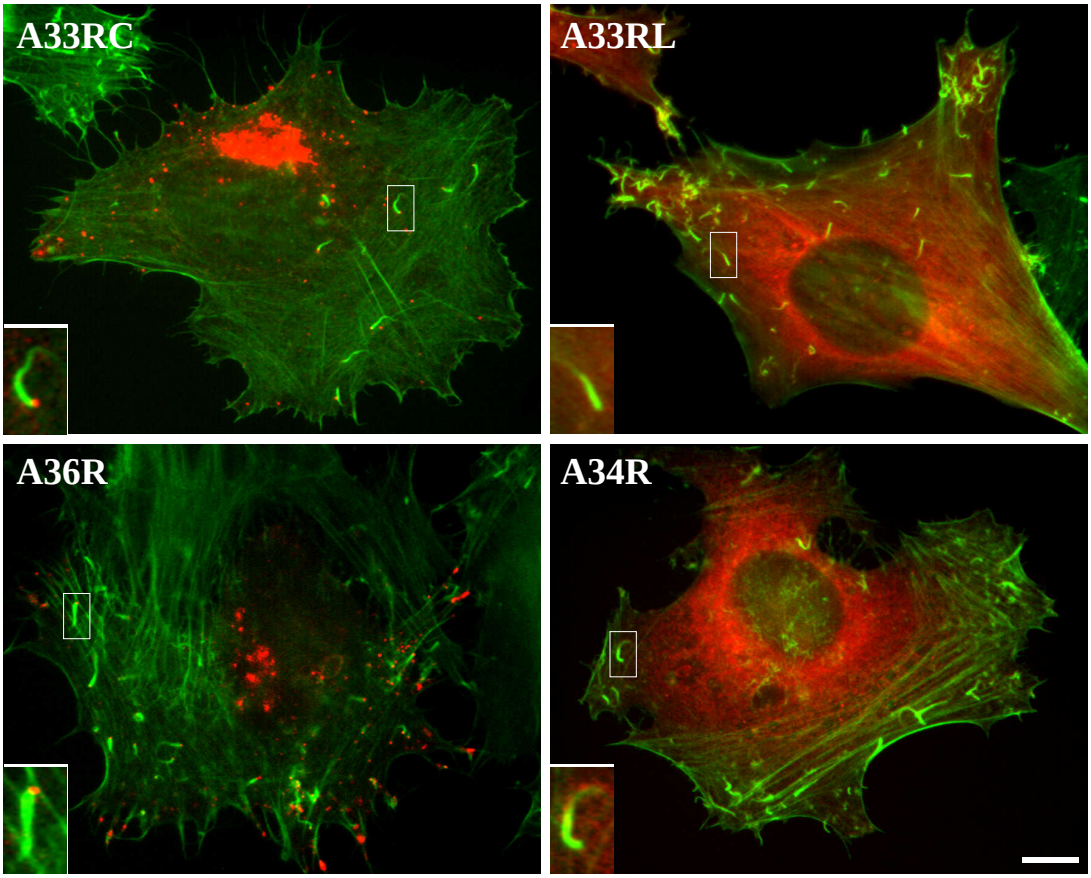


Figure 19: Micro-injection of antibodies raised against IEV specific proteins reveals that A36R has a large cytoplasmic domain. Injected antibodies against the cytoplasmic domain of A33R localize to IEV on the tip of actin tails (A33RC) while antibodies raised against the lumenal part fail to localize (A33RL). Antibodies against A36R localize to IEV in a similar fashion as antibodies against the cytoplasmic domain of A33R. Insets show enlarged actin tails. Scale bar: 10 μm.

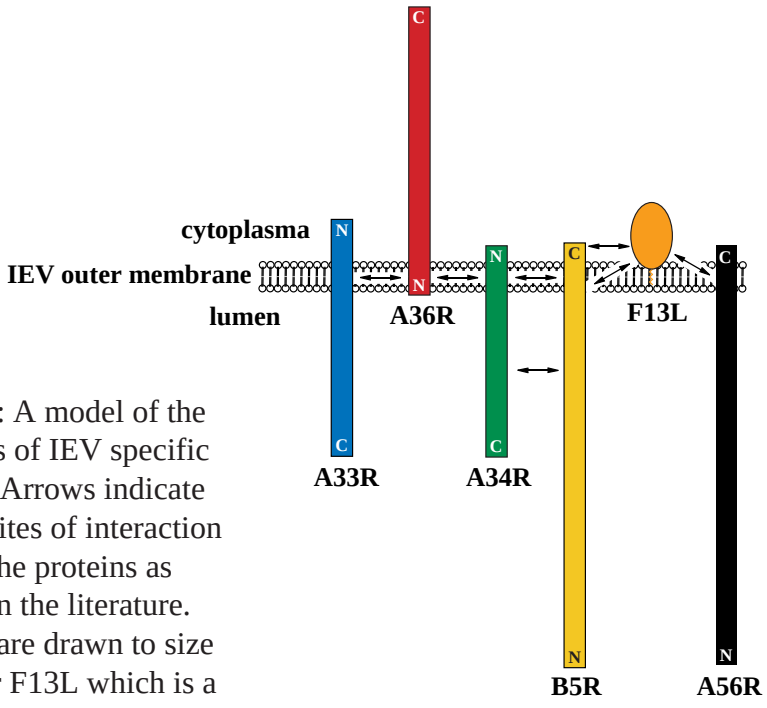


Figure 20: A model of the topologies of IEV specific proteins. Arrows indicate possible sites of interaction between the proteins as reported in the literature. Domains are drawn to size except for F13L which is a membrane associated protein. Compare this model with the one presented in figure 2.