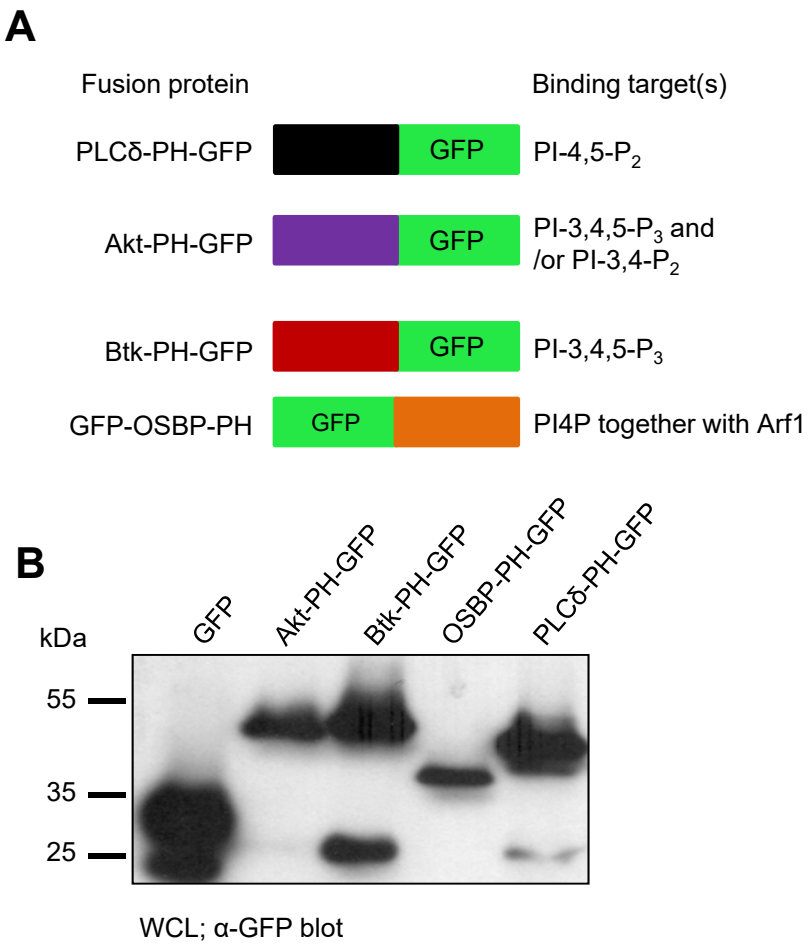
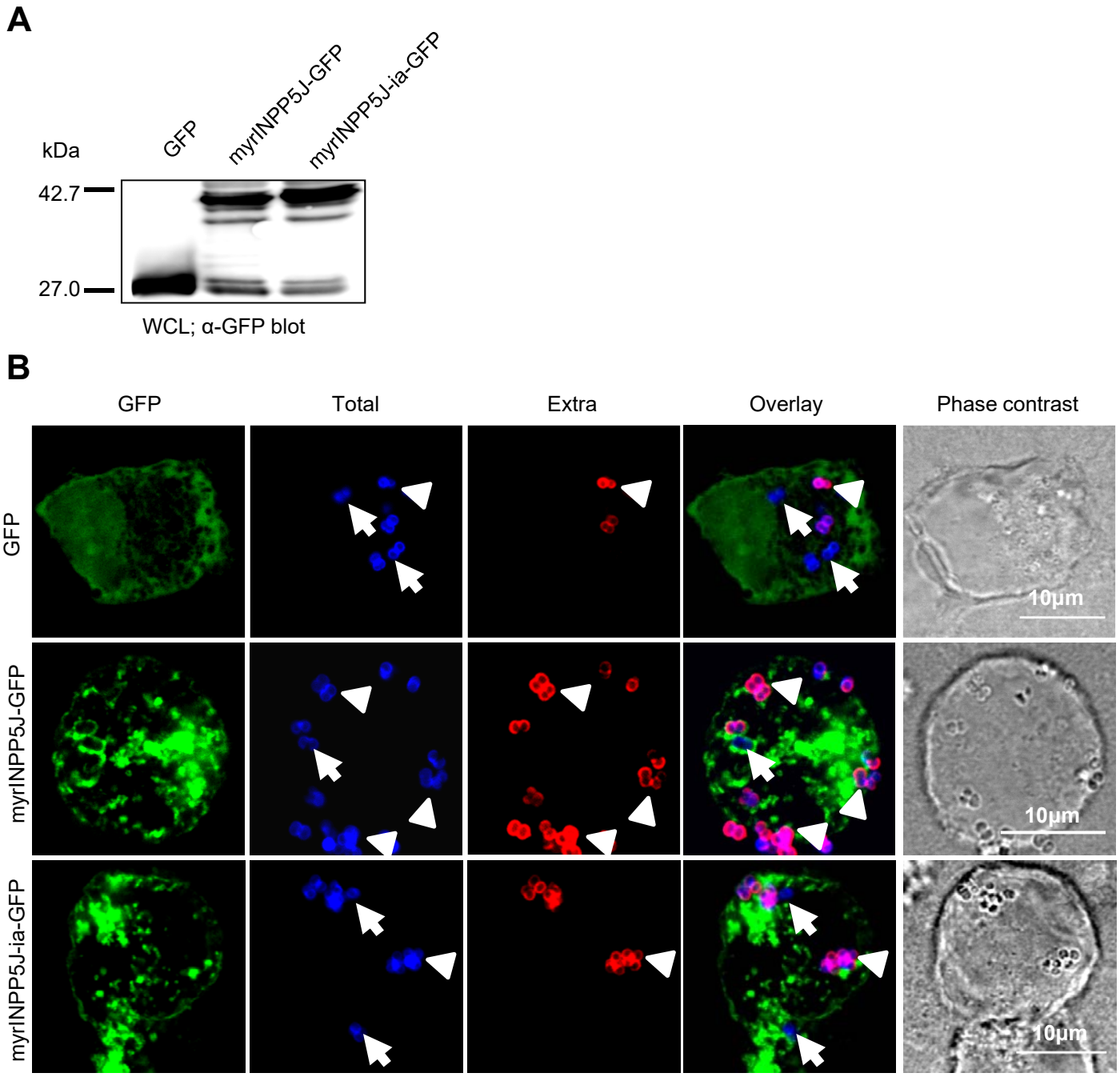
Shi et al., Suppl. Fig. S1

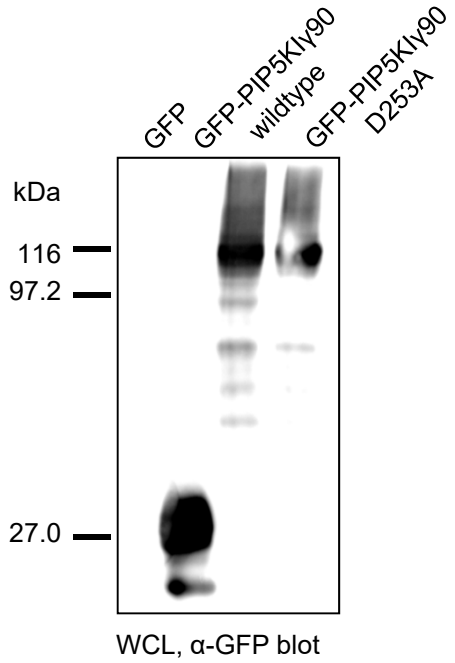


Suppl. Fig. S1. PH domains and their binding phosphoinositides. (A) Schematic depiction of PH domain-GFP fusion proteins and their known specificity for phosphoinositides. (B) 293 cells were transfected with vectors encoding the indicated PH-GFP fusion proteins and expression of the GFP-fusion proteins was analyzed by Western blotting of whole cell lysates (WCL) with polyclonal α-GFP antibody.



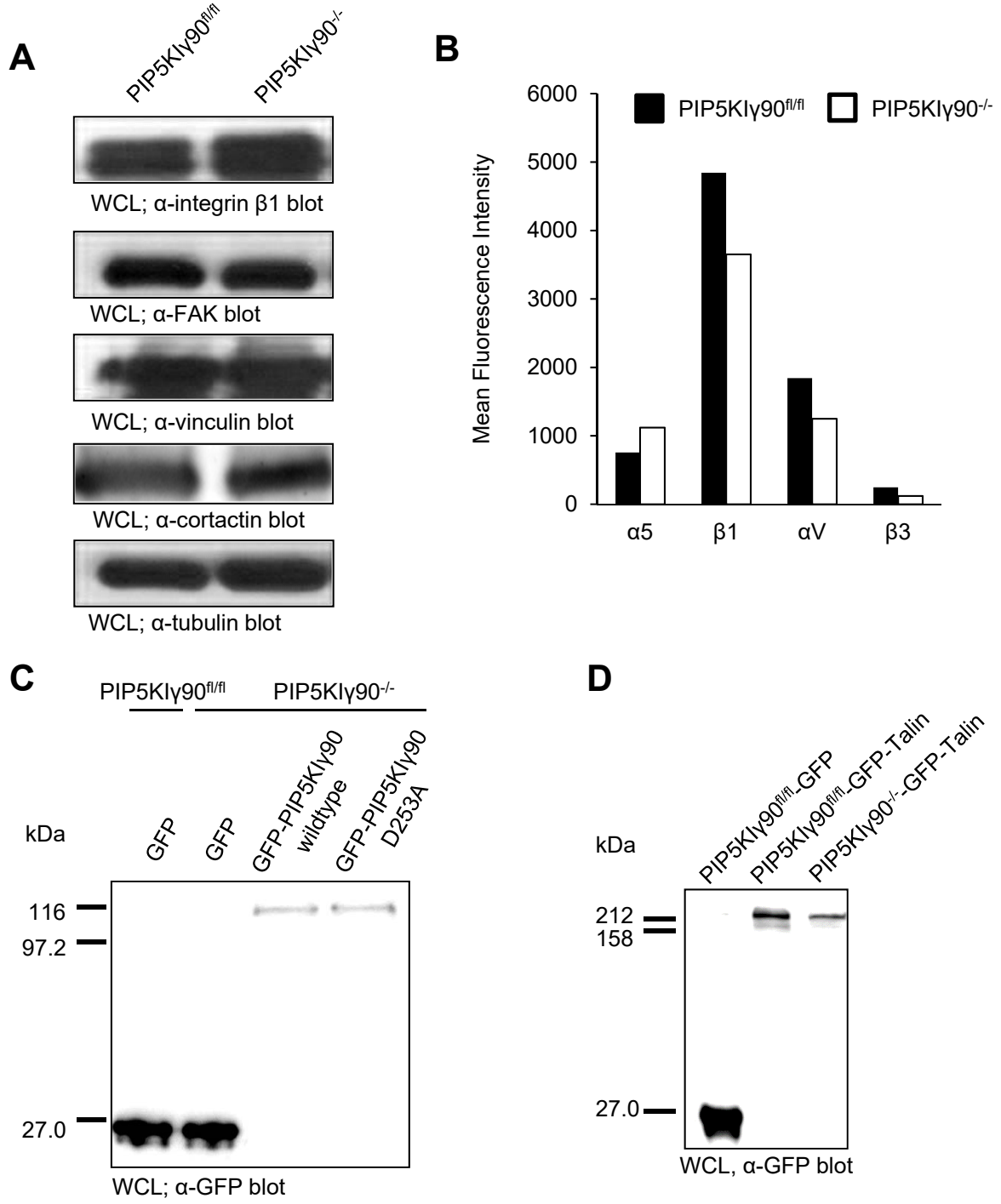
Suppl. Fig. S2 Overexpression of a PI-3,4-bisphosphate-directed phosphatase interferes with internalization of *S. aureus*.

(A) 293 cells were transfected with vector encoding GFP, the membrane targeted phosphatase domain of INPPJ5 (myrINPP5J-GFP), or the inactive phosphatase (myrINPP5J-ia-GFP), respectively. Expression of the proteins was analyzed in whole cell lysates (WCL) by Western blotting using anti-GFP antibody. (B) Cells transfected as in (A) were seeded on poly-L-lysine coated coverslips 24h after transfection. Next day, cells were infected with pacific-blue stained and biotin-labeled *S. aureus* for 2h. After fixation, extracellular bacteria were further stained with streptavidin-AlexaFluor647. Extracellular bacteria were marked by blue and red staining (examples indicated by arrowheads), whereas intracellular bacteria stained only blue (marked by arrows). Bars, 10 µm.



Suppl. Fig. S3. Expression of wildtype or kinase-inactive PIP5KIγ90-GFP in 293T cells.
Constructs encoding GFP, GFP-tagged PIP5KIγ90 wild type, or GFP-tagged inactive PIP5KIγ90 D253A were transfected into 203 cells and the expression of the proteins was verified by Western blotting of whole cell lysates (WCL) using monoclonal anti-GFP antibodies.

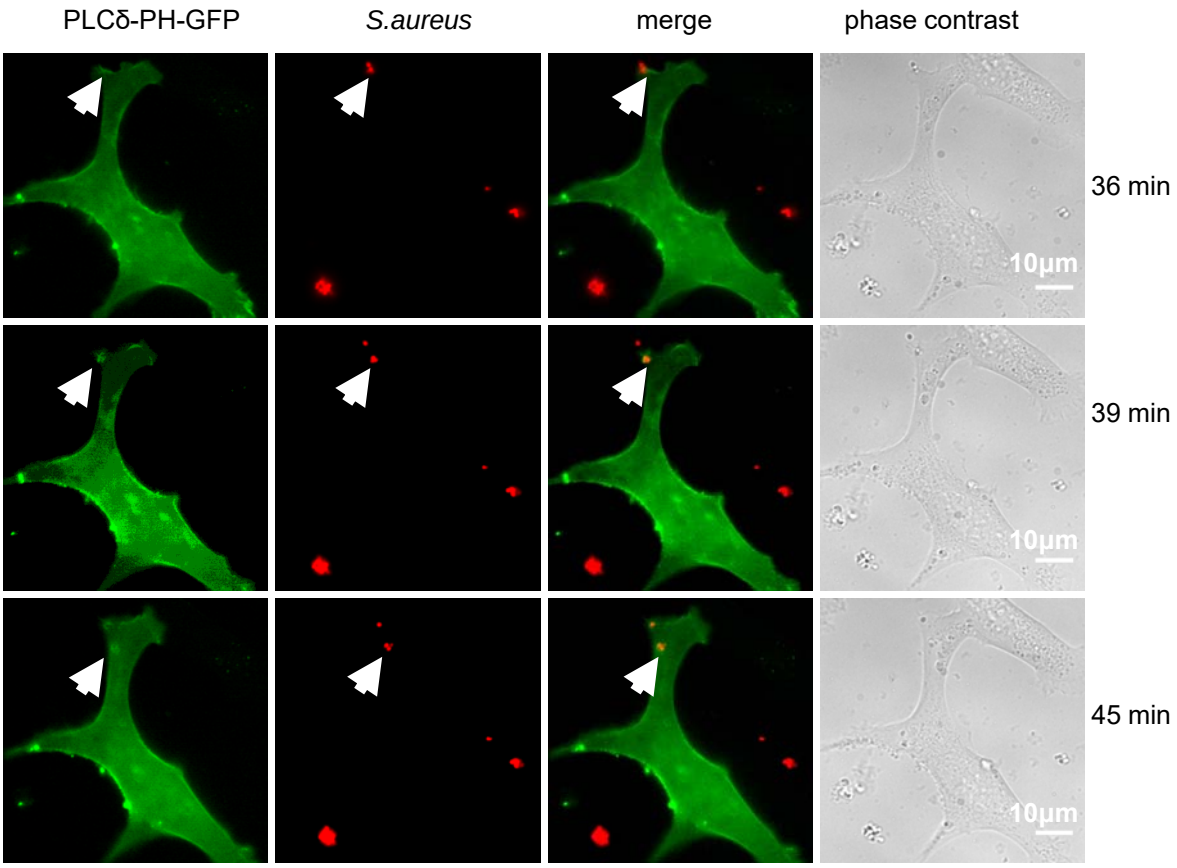
Shi et al., Suppl. Fig. S4



Suppl. Fig. S4 Levels of focal adhesion proteins and surface level of integrins in PIP5KIγ90-/- and PIP5KIγ90fl/fl cells, re-expression of PIP5KIγ90 and expression of GFP-Talin in PIP5KIγ90-/- fibroblasts.

The levels of integrin β1, FAK, vinculin, cortactin, or tubulin in whole cell lysates (WCLs) from PIP5KIγ90$^{fl/fl}$ or PIP5KIγ90$^{-/-}$ cells were analyzed by Western blotting. (B) PIP5KIγ90$^{fl/fl}$ or PIP5KIγ90$^{-/-}$ cells were stained for surface located integrin α5, β1, αV, or β3 and examined by flow cytometry. Bars depict mean fluorescence intensity from three independent experiments. (C) GFP expression in transfected PIP5KIγ90$^{fl/fl}$ cells as well as the expression of GFP, GFP-tagged PIP5KIγ90 wild type or GFP-tagged inactive PIP5KIγ90 D253A in transfected PIP5KIγ90$^{-/-}$ cells was verified by Western blotting using a monoclonal anti-GFP antibody. (D) PIP5KIγ90$^{fl/fl}$ and PIP5KIγ90$^{-/-}$ cells were transfected with constructs encoding GFP or GFP-Talin, respectively. Expression of transfected proteins was detected by Western Blot as in (C).

Shi et al., Suppl. Fig. S5



Supppl. Fig. S5. Still frames taken from live cell movies showing PLCδ-PH-GFP recruitment.

Live cell imaging of PIP5KIγ90$^{fl/fl}$ cells transfected with a vector encoding PLCδ-PH-GFP upon infection with *S. aureus*. 5×10^4 transfected cells were seeded on poly-L-lysine coated live cell imaging dishes. Next day, cells were infected with Rhodamine-labeled *S. aureus* at MOI100 and observed by widefield microscopy. Sequential frames were taken from a live cell imaging movie. The times on the right hand side indicate the elapsed time after addition of the bacteria. The arrow indicates recruitment of PLCδ-PH-GFP to the *S.aureus*-host cell contact site.

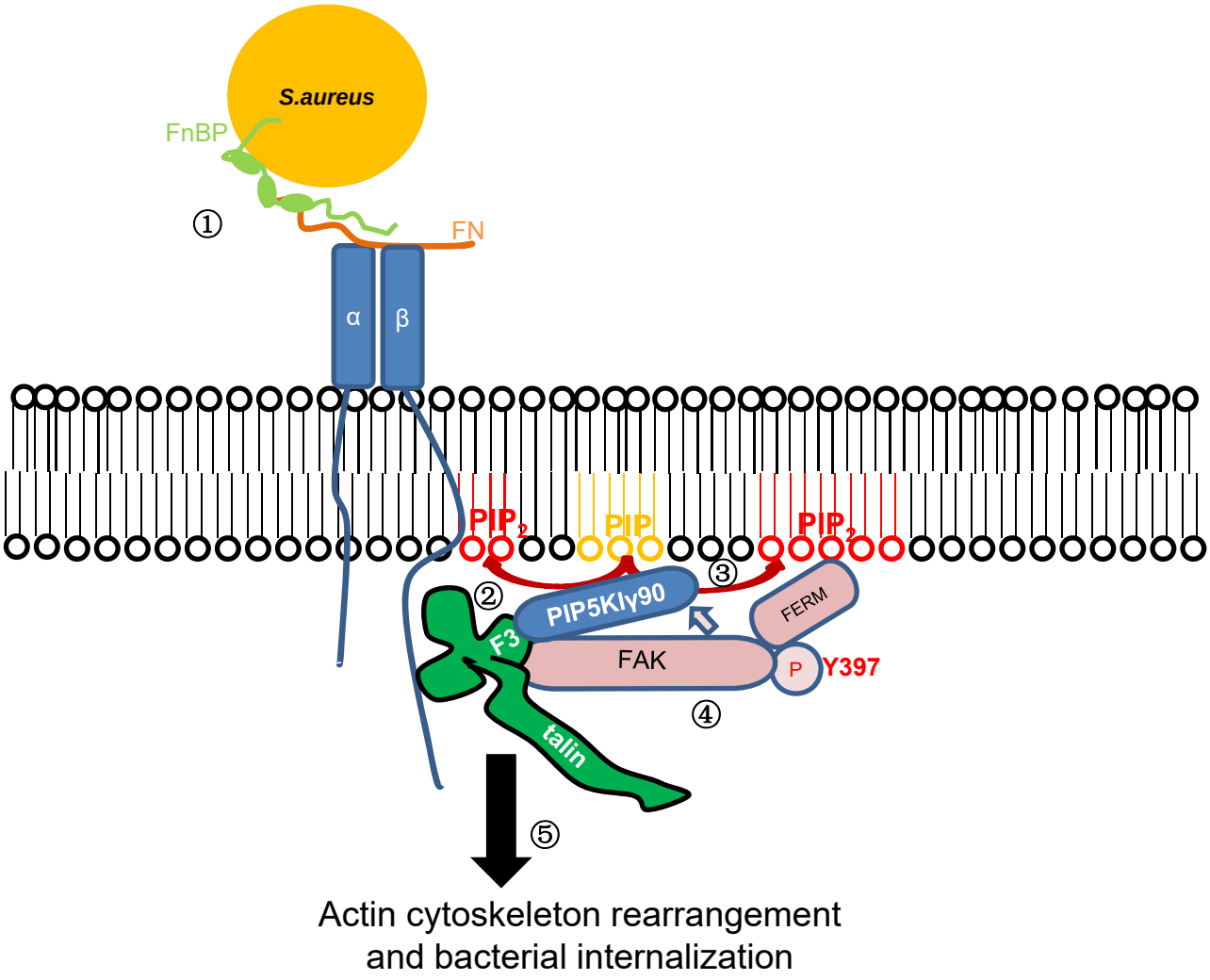


Fig. S6 Schematic view of molecular events during FnBP-Fn-integrin-mediated uptake of *S. aureus*.
(1) Contact of fibronectin-coated *S. aureus* with host cell integrins triggers association of the talin FERM domain F3 lobe (2) with the cytoplasmic domain of the integrin β subunit. The PIP5K1γ90 isoform binds talin and generates local phosphatidylinositol-4,5-bisphosphate (PIP_2), in turn leading to conformational changes in talin-bound focal adhesion kinase (FAK) (3). PIP_2-assisted re-orientation of the FAK-FERM domain increases FAK enzymatic activity and autophosphorylation at residue Y397 (4). Known downstream effectors of active FAK include c-Src and cortactin, which orchestrate cytoskeletal rearrangements during bacterial internalization (5).