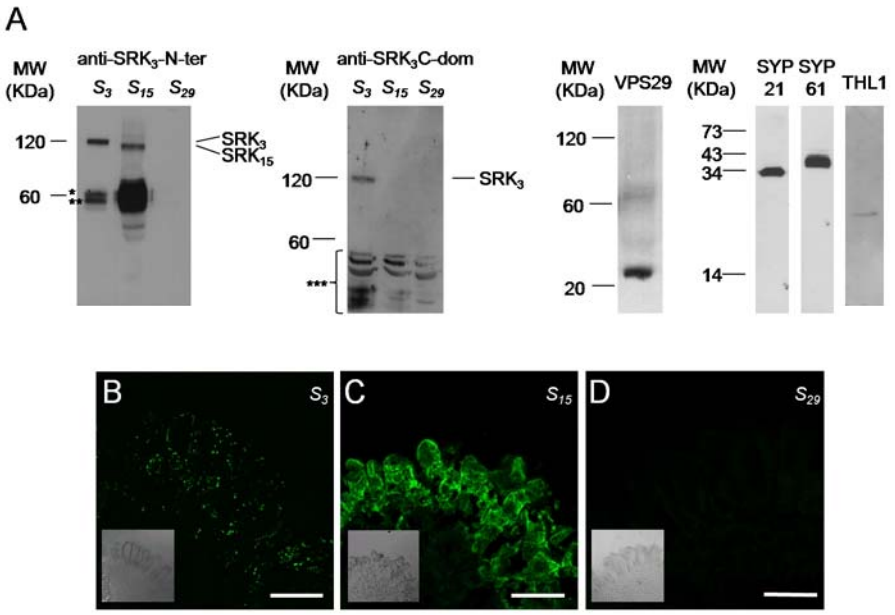
Supplemental Data. Ivanov and Gaude. (2009). Endocytosis and endosomal regulation of the *S*-receptor kinase during the self-incompatibility response in *Brassica oleracea*



Supplemental Figure 1. Specificity test for antibodies on *Brassica oleracea* stigma proteins

A: Specificities of anti-SRK_3-N-ter (mAb85-36-71), anti-SRK_3C-dom, anti-VPS29, anti-SYP21, anti-SYP61 and anti-THL1 antibodies were tested on western blot against total S_3 stigma proteins. Anti-SRK_3 antibodies were additionally tested against extracts from the S_{15}- and S_{29}-haplotypes as positive and negative controls.

Anti-SRK_3-N-ter antibody is raised against SRK_3 and in S_3 haplotype recognizes the full-length receptor as well as four different glycosylated forms of its splice variant $eSRK_3$: two weak ones at 65.2 and 56.7 kDa and two abundant at 62.8 (*) and 59.5 kDa (**). In S_{15} haplotype, the antibody recognizes additionally the *S*-locus Glycoprotein (SLG), an abundant protein related to self-incompatibility with high sequence similarity to SRK. Detection of SLG_{15} results in strong signal around 60 kDa.

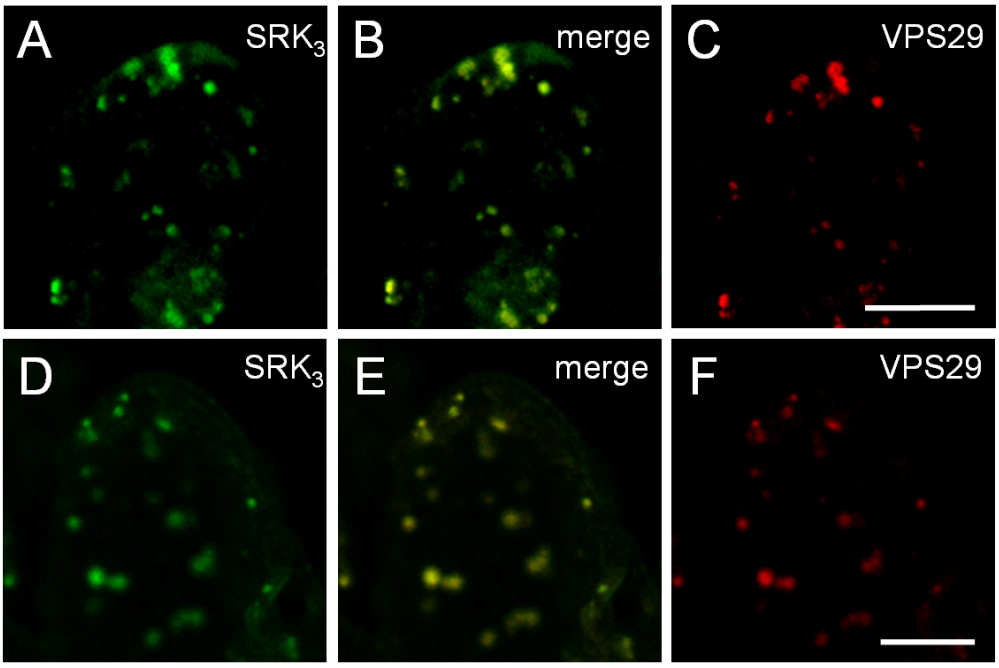
Anti-SRK_3C-dom antibody is raised against the very C-terminus of SRK_3 and does not recognize SRK from any other known haplotype. Additional cross-reactivity can be seen on western blot, which is not haplotype specific (***). Immunocytochemical experiments show that the cross-reactive proteins are localized in the cell wall (see Figure 4K-M in the main text)

B-D: Specificity of anti-SRK_3-N-ter for immunolocalization was tested against 10µm-thick sections from S_3, S_{15} and S_{29} stigmas. The inserts show the brightfield images.

B: Immunolabeling on S_3-sections using anti-SRK_3-N-ter antibody. Bar 50µm

C: Immunolabeling on S_{15}-sections using anti-SRK_3-N-ter antibody. The highly abundant SLG_{15} protein cross-reacts with the antibody, which results in a strong immunofluorescence signal (used as a positive control). Bar 50µm

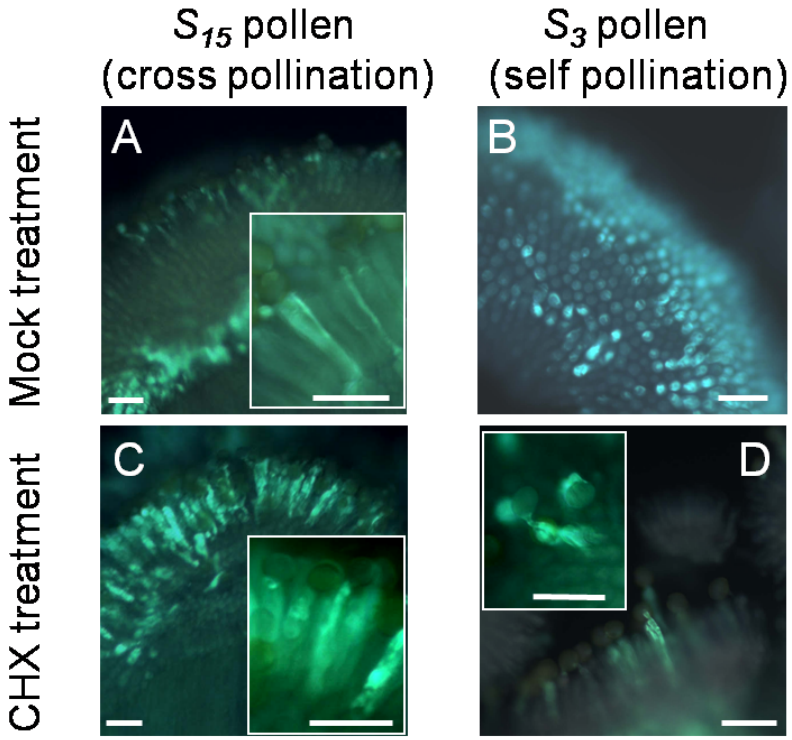
D: Immunolabeling on S_{29}-sections using anti-SRK_3-N-ter antibody. The antibody does not cross react to any protein in S_{29}-haplotype (used as a negative control). Bar 50µm



Supplemental Figure 2. SRK_3 colocalizes with sorting endosome markers after pollination

A-C: Colocalization between SRK_3 (A) and sorting endosome marker VPS29 (C) 50 minutes after self-pollination. Merged image is presented in (B). Images shown are representative of three independent experiments Bar: 10μm

D-F: Colocalization between SRK_3 (D) and sorting endosome marker VPS29 (F) 50 minutes after cross-pollination. Merged image is presented in (E). Images shown are representative of five independent experiments. Bar: 10μm



Supplemental Figure 3. Effect of Cycloheximide (CHX) treatment on self-incompatibility.

CHX-pretreated S_3-stigmas were pollinated with S_{15}- (A and C) or S_3- (B and D) pollen grains (cross- or self-pollination, respectively). CHX treatment breaks the self-incompatibility response.

A: S_3 mock-treated stigma accepts growth of compatible S_{15}-pollen grains.

B: S_3 mock-treated stigma rejects S_3-pollen grains. The grains fail to adhere on the papilla cell surface and are washed away during the fixation procedure.

C: CHX-treated S_3 stigma accepts growth of compatible S_{15}-pollen grains.

D: CHX-treated S_3 stigma accepts growth of otherwise incompatible S_3-pollen grains. Bars:100μm