

Table S1: *P. chrysogenum* strains used in this work

Strain	Characteristics and genotype	Source
P2niaD18	<i>niaD</i> ⁻	(1)
ΔPcku70 ^b	<i>Pcku70Δ::FRT; niaD</i> ⁻	(2)
ΔPcvelA ^b	<i>Pcku70Δ::FRT; PcvelAΔ::FRT; niaD</i> ⁻	(3)
PcVelA-ChIP (T16.2)	<i>Pcku70Δ::FRT; PcvelAΔ::FRT; P_{gpd}::PcvelA::EGFP::T_{trpC}; PtrpC::nat1; niaD</i> ⁻	This study
ΔPcllMAFRT1 ^a	<i>Pcku70Δ::FRT; PcllMAΔ::FRT::Pxyl::Pcflp::PtrpC::nat1::FRT; niaD</i> ⁻	This study
ΔPcllMAFRT2 ^b	<i>Pcku70Δ::FRT; PcllMAΔ::FRT; niaD</i> ⁻	This study
P2niaD18::PcllMA	<i>Pgpd::PcllMA::T_{trpC}; PtrpC::nat1; niaD</i> ⁻	This study
ΔPcllMAFRT2::PcllMA	<i>Pcku70Δ::FRT; PcllMAΔ::FRT; PPcllMA::PcllMA::T_{PcllMA}; PtrpC::nat1; niaD</i> ⁻	This study
ΔPcvelAΔPcllMAFRT1	<i>Pcku70Δ::FRT; PcvelAΔ::FRT; PcllMAΔ::FRT::Pxyl::Pcflp::PtrpC::nat1::FRT; niaD</i> ⁻	This study
BiFC-N/N	<i>Pgpd::eyfpC::T_{trpC}; Pgpd::eyfpN::T_{trpC}; PtrpC::nat1; niaD</i> ⁻	This study
BiFC-N/PcLlMA	<i>Pgpd::eyfpC::T_{trpC}; Pgpd::PcllMA::eyfpC::T_{trpC}; PtrpC::nat1; niaD</i> ⁻	This study
BiFC-PcVelA/N	<i>Pgpd::PcvelA::eyfpN::T_{trpC}; Pgpd::eyfpN::T_{trpC}; PtrpC::nat1; niaD</i> ⁻	This study
BiFC-PcVelA/PcLlMA	<i>Pgpd::PcvelA::eyfpN::T_{trpC}; Pgpd::PcllMA::eyfpC::T_{trpC}; PtrpC::nat1; niaD</i> ⁻	This study

^a strains that still carry a resistance marker from the flipper knockout construct

^b strains without any resistance markers due to FLP/FRT marker recycling.

1. **Hoff B, Pöggeler S, Kück U.** 2008. Eighty years after its discovery, Fleming's *Penicillium* strain discloses the secret of its sex. *Eukaryot Cell* **7**:465-470.
2. **Kopke K, Hoff B, Kück U.** 2010. Application of the *Saccharomyces cerevisiae* FLP/FRT recombination system in filamentous fungi for marker recycling and construction of knockout strains devoid of heterologous genes. *Appl Environ Microbiol* **76**:4664-4674.
3. **Kopke K, Hoff B, Bloemendal S, Katschorowski A, Kamerewerd J, Kück U.** 2013. Members of the *Penicillium chrysogenum* velvet complex play functionally opposing roles in the regulation of penicillin biosynthesis and conidiation. *Eukaryot Cell* **12**:299-310.