

Supplemental material

Supporting tables

Table S1. Sequences of all oligonucleotides used in this work.

Primer for plasmid constructions

5'3'-sequence

P1	ACCACGCATGCTTCGGTATACGACGGTTGGCAATCAATGTC
P2	TAGGAACTTCCCATACATATGTTCTGCCGGTCGCAGTATGG
P3	AGGAACTTCGCGGCCGCGGCCGCCATACTCTCCCTGGGCTTTC
P4	AATACAGCGGCCGTTGTTAACGGCACTGATATTGCGCAGTC
P5	AGGAACTTCGCGGCCGTTTAAACTGTAGTTCGCGTTTCGGGAC
P6	AATACAGCGGCCGTTATTTAAATGCCATCACATGGTTGGATAG
P7	ACCACGCATGCTTCGGTATACGAAGGCGTGGATATTTACCG
P8	TAGGAACTTCCCATACATATGTTTTGATTTGTGAGTGGTGG
P9	GCGGCCGCTCACAGTATTGAATAATG
P10	GTAAACCCAAGAACTGGAGTGAGGTC
P11	CCATGGGAATGCCACACGGCTTCGAC
P12	CCATGGCCCCGCTTCTTCCCCTTC
P13	GTAACGCCAGGGTTTTCCCAGTCACGACGATAGCCGGCGCCAACCCTGGGCTTT
P14	CTACATGAGCATGCCCTGCCCTGATGTCAAGTGGGATTCCCTGGACGACC
P15	TCAGGGGCAGGGCATGCTCATGTAG
P16	AACTGATATTGAAGGAGCATTTTTT
P17	AAAAAATGTCCTTCAATATCAGTTTTTGTGACGTCCTCGATGTTCTG
P18	GCGGATAACAATTTACACAGGAAACAGCATGTAACGCTCGGCGAAACTGACA C

Primer for gene amplifications

5'3'-sequence

P19	GCTATTTTCATCTTCCCAGATCTG
P20	GGTGTCGTATCTGGGCATATGC
P21	CTCCATGGGAATGTACGCCGTCGAGGATAGACAT
P22	AGCCATGGTTACTCCGTCCTTGCGAATGGGGAT
P23	ATGCCACACGGCTTCGACAAGC
P24	CCATGGTCACCGCTTCTTCCCCT
P25	ATGTTGAACAACACCAGTTCTGAC
P26	TCATTGTGGCGTTCATTCCGGGAATC

Primer for the verification of knockout and recombination events

5'3'-sequence

P27	GGATGAACAACACTGGCAGCTGGG
P28	GTCCCGAGATGTCTGAGCTCC
P29	CCGTCTACTGTTATGCCTTACA
P30	GGGCTTTGTTGAGGTAAGTTATT
P31	TGCACTTCATTATAGATGTCGACGC
P32	GACCGTCTTTCGAGAGATCGGAG
P33	CTACGGAGTACGGAGTACTC
P34	ATCAGTGCCAGCTGTCTTCG
P35	GCGCTCTACATGAGCATGCC
P36	CAACTGATATTGAAGGAGCATTT
P37	TCATGAGATGCCTGCAAGCAATTCG
P38	ACCTTCTTAAGTTCGCCCTTCCCTCC
P39	GTGCGTTGACGTTGGTGACCTCCAG

Primer for cDNA synthesis

5'3'-sequence

- P40** GATTGGGGGTGGGTCAAC
P41 ATTACCGGTGGATACGCCAATC

Primer for yeast two-hybrid analysis/BiFC

5'3'-sequence

- P42** CCCGGGAATGGCCAACAGACCATCTCT
P43 GAGCTCTTATGTACTCGACGAAGGAGCTT
P44 AAGCTGATCTCAGAGGAGGACCTGCATATGGCCAACAGACCATCTCTCATGC
P45 CCTTGTCGGCATCCTTGGCGTCCTTGATCTCTTCGTATAGGTGGAAGCTC
P46 CTCAAATACCTGTCGAACGAGGTGGTTACCCCAAGCCTCCTATGAGTTCC
P47 GTTATGCTAGTTATGCGGCCGCTGCAGTTATGTACTCGACGGAGGAGCTTTC
P48 CACAGAATTCATGTACGCCGTCGAGGATAGACATCAT
P49 GGTGGATCCTTAATAGTCGCCTTCATCATCATCGT
P50 CAGAATTCATGCCACACGGCTTCGACAAGCT
P51 GTGGATCCTCACCGCCTCTTCTTCCCCTTC
P52 CGAATGAATTCATGTTGAACAACACCAGTTCTGA
P53 CGATTCTCGAGTCATTGTGGCGTTCATTTCGGGGAAT
P54 CCAGATTACGCTCATATGGCCATGGAGATGTTTACGAACGGGGATTCC
P55 GGAGCGGGTAGATTCCTTTCCGATAGCCATGGTACATTC
P56 GAATGTACCATGGCTATCGGAAAGGAATCTACCCGCTCC
P57 GCGGGGTTTTTCAGTATCTACGATTCATTTATTCCTCGACTGGTTTTTCG
P58 CTCAGAGGAGGACCTGCATATGGCCATGTTTACGAACGGGGATTTC
P59 GAGGCCCAAGGGGTTATGCTAGTTATTTATTCCTCGACTGGTTTTTCG
P60 CATGGAGCTCAGCGGCCGCATGTTGAACAACACCAGTTCTGACTTTG
P61 CAGGCCGGGCGGGCGGCCCTTGTGGCGTTCATTTCGGGGAATC

Table S2. Percentage identities and similarities (amino acids) of the velvet domains and full length velvet proteins from *P. chrysogenum* and *A. nidulans*

	Velvet domain		Full length protein	
	Identity	Similarity	Identity	Similarity
PcVelA/VeA	68 %	79 %	45 %	56 %
PcVelB/VelB	80 %	86 %	63 %	68 %
PcVelC/VelC	58 %	68 %	38 %	48 %
PcVosA/VosA	54 %	61 %	50 %	60 %

Supporting Figures

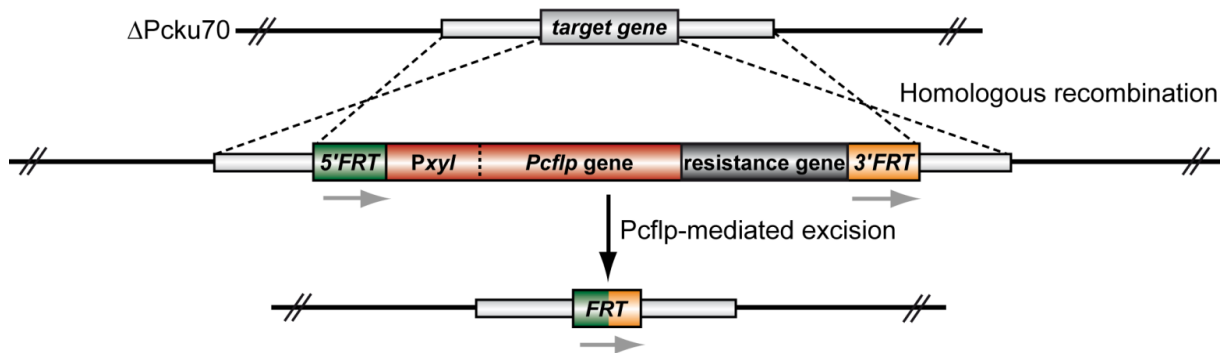


Fig. S1. Use of the flipper cassette to generate knockout strains by homologous recombination. As a resistance marker either *nat1* or *ble* was used (specified in the text). *Pxyl*: xylose-inducible promoter; *FRT*: Flippase recognition target; *Pcflp*: site-specific recombinase gene.

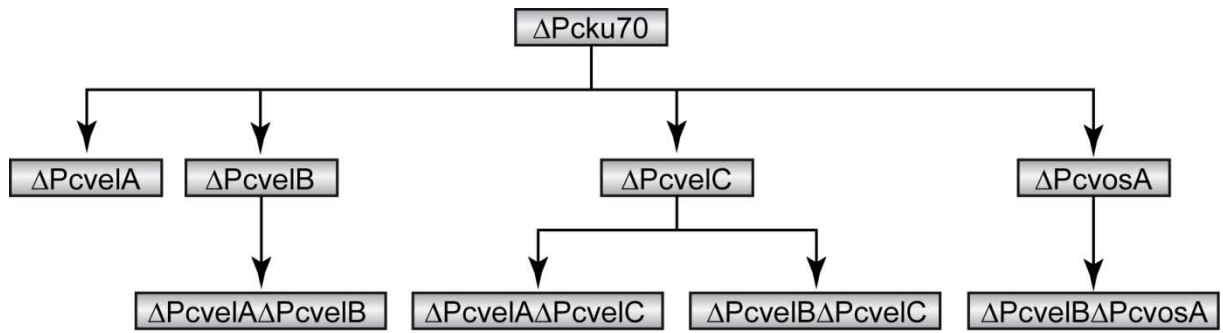


Fig. S2. Genealogy of all knockout strains used in this study. As reference, $\Delta Pcku70$ was used for homologous recombination.

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PcVelA : QLTAKLSVMQOPEERARAGGAKSSADRRF---VDPPPVELRIFESDPANDTQKTDITFAYNANFFLYATLETARPIAHGRVGGP----- : 83
VeA : -----MQQPKARAGGQESKSHTDRRF---VDPPPVELNIFESDPHDDSNKTDITFVYNANFFLFATLEPERPIATGKLMTN----- : 75
PcVelB : GRSSLSIQVVOQPEERARMGGEEDKDRRPITPEPCRLIVRDAQTKKEDINEITDSFYVLTVDLWNADGTSEVNLVKH----- : 77
VelB : GRISLSIQVVOQPEERARMGGEEDKDRRPITPEPCRLIVKDAQTKQKQVINDLSDFSYVVMADLWNADGTSEVNLVKH----- : 77
PcVelC : --RHLHTRQOPLAARAGGAEEDRRRPVDEPPIVQILLTDFDSSNCEBDRDLLQDPRFTVGCLLFPVSPSLPYAEPTEAHSRERDQERDQDRDGNDRERERE : 100
VelC : --RHLHTRQOPLAARAGGAEEDRRRPVDEPPIVQILLTDFDSSNCEBDRSILQDPRFTVGCLLFPVVRNPSYIIPGSGSGAGSSDNTNSNDKSRHESMSSYSQ : 100
PcVosA : SSDLDLIRQOPEERARVAGGKEKKEERVPVA----- : 31
VosA : SDDDELIVRQOPEERARVAGGKEKKEERVREDEKRELTWGDQFAERKPVDPPIVQIIRVREEGTYLAHLYDAQEDAPASIP----- : 76

PcVelA : -----QSCPVLKGVFVAGVAYLDRPSQ-----AGYFIIPDLSVRHSGRYRISSHHY : 129
VeA : -----QGSPVLKGVFVAGVAYLDKPNR-----AGYFIIPDLSVRNNGSYRFSHHEF : 121
PcVelB : -----VTGGPGGMFTRNLKCSLSAFAFRLTDPDKKI-----GWVFLQDLSVRTEGIFRLKMSFV : 132
VelB : -----VTPGPGGMFTRNLKCSLSAFAFRLYDTEFKI-----GWVFLQDLSVRTEGIFRLKMSFV : 132
PcVelC : HERERERERETDGVARTDDNFSTPLLSGKAFMSFFVVDADPPNSAPAHPSISDPHISNPPHHVYNHAASR--LHQPATFFIADLSIRSAGLYRLOQRHM : 200
VelC : LGINLEDSHATG-----QSTPLLSGKAFVSPFFVDEEPPNTAPAHPSSTDDSTYDASPRVTVTHFRNRRLPKPPACFFIADLSVRTAGLYRLOQRHM : 194
PcVosA : -----PSTALACTLVSLHRLKDVNDSD-----GGFFVIGDLSVRTEGIFRLKMSHF : 78
VosA : -----PSTALACTLVSLHRLKDVNDSD-----GGFFVIGDLSVRTEGIFRLKMSHF : 123

PcVelA : EEIKDAKDADKSTLPLNQIPLSATSCKGIPQAFHFRLEWKSVEFTVYSARKKFPGLATSTLSLRIIADQGCRRVIRRDV : 210
VeA : EQIKDPKDATEG--TQPMSPVPVPGKLSS---PQEFHFRLEWISNPFIVYSARKKFPGLTSTPISRMIAEQGCRRVIRRDV : 197
PcVelB : DVGTSTETSNG-----APVINHGTAPEVLSVSEPEQVFSARKKFPGVIESTLQSKCFADQGIKTPIRKDG : 197
VelB : NVGKSVSDLPQS-----DIAEVINKGTAPILASTFSEPEQVFSARKKFPGVIESTPLSKVFANQGIKTPIRKDG : 200
PcVelC : NWGSVEDTGQS-----MPIIAQAWSDEBRVYPAKDFPGMRDSSILAEGLKELCFVELKTRGH : 257
VelC : NWGSIEDTGQS-----MPIIAEWSDEBRVYPAKDFPGMRDSSILAEGLKELCFVELKTRGH : 251
PcVosA : EMRKDR-----WSYIKTVISERTVSPPEPFGMESTHLERSFADQGVKIRIRKEP : 130
VosA : EMRKEDEELTG-----NSSVRTDWEVFKSIVSERTVSPPEPFGMAESTHLERSFADQGVKIRIRKEP : 187

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Fig. S3. Amino acid sequence comparison of the conserved velvet domain of PcVelA (aa 27-236), PcVelB (aa 49-125, 208-327), PcVelC (aa 220-476), and PcVosA (aa 6-135) as shown in Fig. 1. For comparison, the sequence of the corresponding *A. nidulans* homologs VeA (aa 1-197), VelB (aa 56-132, aa 226-348), VelC (aa 252-502), and VosA (aa 17-203) are given.

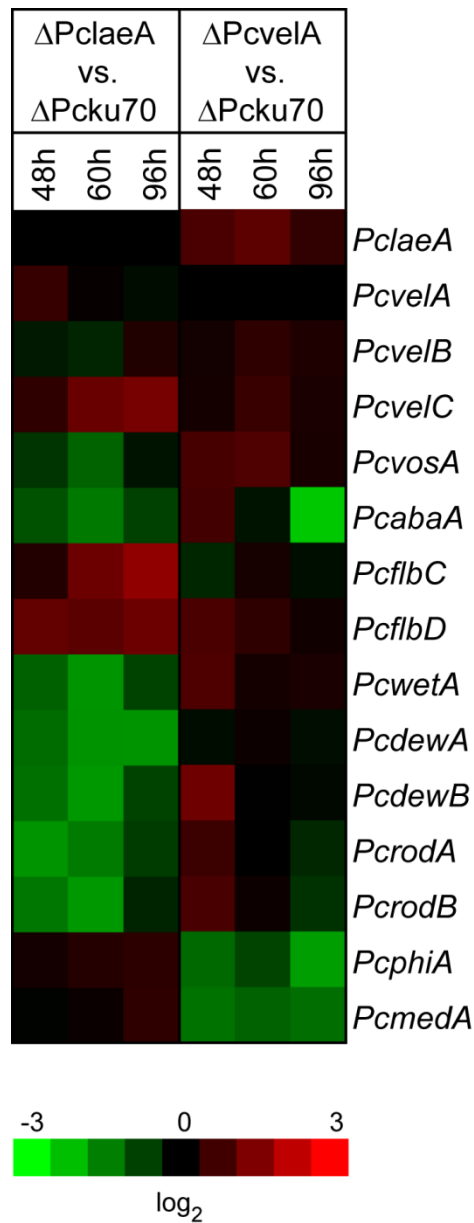


Fig. S4. Microarray analyses of selected *PclaeA*- and *PcvelA*-dependent genes. Heatmaps of genes from the velvet complex and developmental genes are shown. Red indicates genes that are upregulated in the corresponding mutant compared to the reference strain $\Delta Pcku70$; green marks those that are downregulated compared to the reference strain. All genes and their products are mentioned in the text. The color of each square represents a \log_2 -fold change in expression at the given time point.

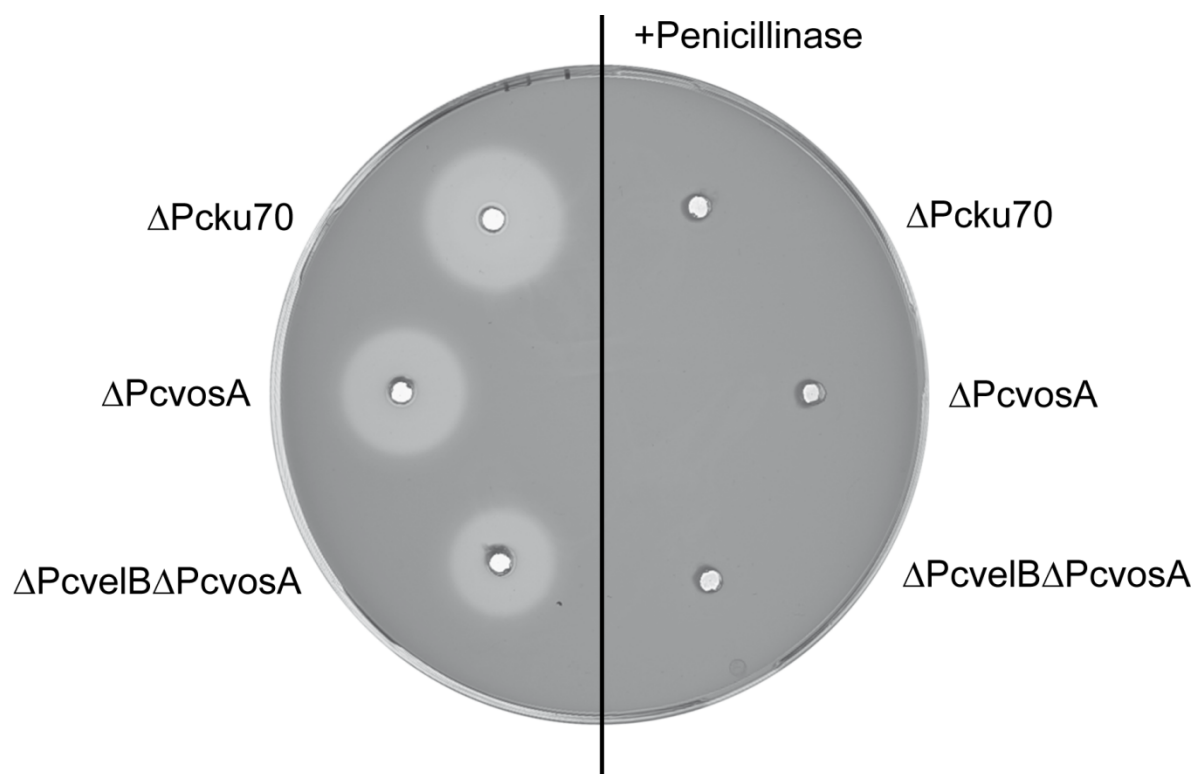


Fig. S5. Penicillinase assay. To confirm that growth inhibition of the indicator organism *S. aureus* was due to penicillin production, penicillinase was added to the supernatant of selected strains.

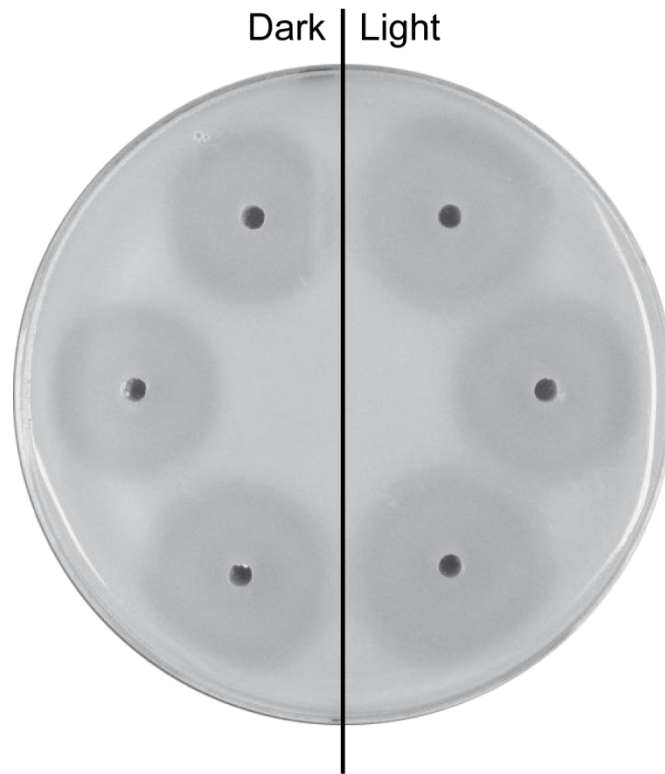


Fig. S6. Penicillin production under light and dark conditions. The reference strain P2niaD18 was grown in triplicate for 72 h in shaking culture with either constant light or constant dark conditions. A halo assay indicates the amount of penicillin produced under both conditions.