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	GTTAACCCAAGAACTGGAGTGAGGTC
P11	CCATGGGAATGCCACACGGCTTCGAC
P12	CCATGGCCCGCCTCTTCTTCCCCTTC
P13	GTAACGCCAGGGTTTTCCCAGTCACGACGATAGCCGGCGCCAACCCTGGGCTTT
P14	CTACATGAGCATGCCCTGCCCCTGATGTCAGTGGGATTCCCTGGACGACC
P15	TCAGGGGCAGGGCATGCTCATGTAG
P16	AACTGATATTGAAGGAGCATTTTTT
P17	AAAAAATGCTCCTTCAATATCAGTTTTTGCTTGACGTCCTCGATGTTCTG
P18	GCGGATAACAATTTCACACAGGAAACAGCATGTAACGCTCGGCGAAACTGACA C

Primer for gene amplifications

	5´3´-sequence
P19	GCTATTTCATCTTCCCAGATCTG
P20	GGTGTCGTATCTGGGCATATGC
P21	CTCCATGGGAATGTACGCCGTCGAGGATAGACAT
P22	AGCCATGGTTACTCCGTCCTTGCGAATGGGGAT
P23	ATGCCACACGGCTTCGACAAGC
P24	CCATGGTCACCGCCTCTTCTTCCCCT
P25	ATGTTGAACAACACCAGTTCTGAC
P26	TCATTGTGGCGTTCCATTCGGGGAATC

Primer for the verification of knockout and recombination events

	5´3´-sequence
P27	GGATGAACAACTGGCAGCTGGG
P28	GTCCCGAGATGTCTGAGCTCC
P29	CCGTCTACTGTTATGCCTTACA
P30	GGGCTTTGTTGAGGTAAGTTATT
P31	TGCACTTCATTATAGATGTCGACGC
P32	GACCGTCTTTCGAGAGATCGGAG
P33	CTACGGAGTACGGAGTACTC
P34	ATCAGTGCCAGCTGTCTTCG
P35	GCGCTCTACATGAGCATGCC
P36	CAACTGATATTGAAGGAGCATTT
P37	TCATGAGATGCCTGCAAGCAATTCG
P38	ACCTTCTTAAGTTCGCCCTTCCTCC
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	GGTTGGATCCTTAATAGTCGCCTTCATCATCATCGT
P50	CAGAATTCATGCCACACGGCTTCGACAAGCT
P51	GTGGATCCTCACCGCCTCTTCTTCCCCTTC
P52	CGAATGAATTCATGTTGAACAACACCAGTTCTGA
P53	CGATTCTCGAGTCATTGTGGCGTTCCATTCGGGGAAT
P54	CCAGATTACGCTCATATGGCCATGGAGATGTTTACGAACGGGGATTCC
P55	GGAGCGGGTAGATTCCTTTCCGATAGCCATGGTACATTC
P56	GAATGTACCATGGCTATCGGAAAGGAATCTACCCGCTCC
P57	GCGGGGTTTTTCAGTATCTACGATTCATTTATTCCTCGACTGGTTTTCG
P58	CTCAGAGGAGGACCTGCATATGGCCATGTTTACGAACGGGGATTC
P59	GAGGCCCCAAGGGGTTATGCTAGTTATTTATTCCTCGACTGGTTTTCG
P60	CATGGAGCTCAGCGGCCGCATGTTGAACAACACCAGTTCTGACTTTG
P61	CAGGCCGGGCGGCGGCCGCCTTGTGGCGTTCCATTCGGGGAATC

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

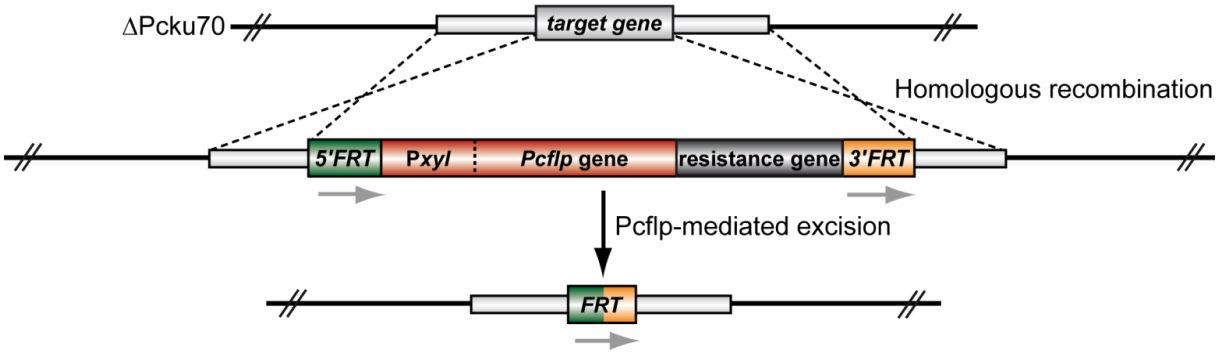

Fig. S1. Use of the flipper cassette to generate knockout strains by homologous recombination. As a resistance marker either *nat1* or *ble* was used (specified in the text). *Pxyl*: xylose-inducible promoter; *FRT*: Flippase recognition target; *Pcflp*: site-specific recombinase gene.

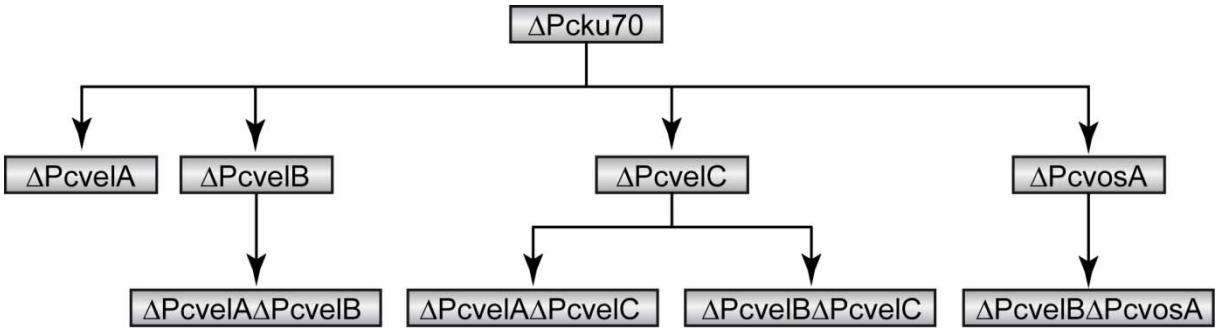

Fig. S2. Genealogy of all knockout strains used in this study. As reference, ΔPcku70 was used for homologous recombination.

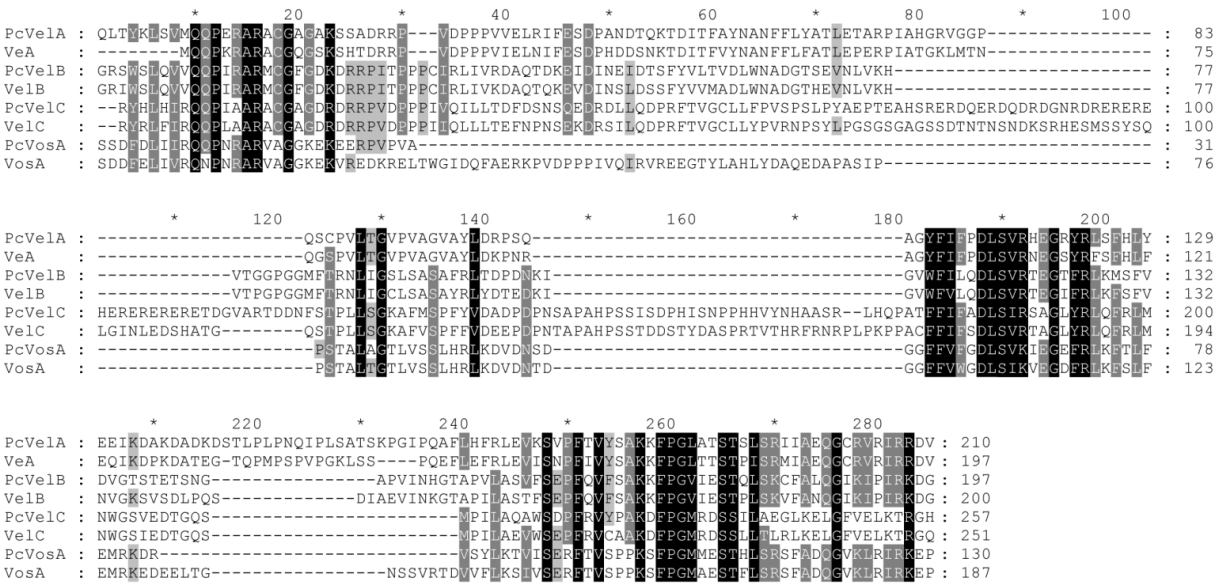

Fig. S3. Amino acid sequence comparison of the conserved velvet domain of PcVelA (aa 27-236), PcVelB (aa 49-125, 208-327), PcVelC (aa 220-476), and PcVosA (aa 6-135) as shown in Fig. 1. For comparison, the sequence of the corresponding *A. nidulans* homologs VeA (aa 1-197), VelB (aa 56-132, aa 226-348),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

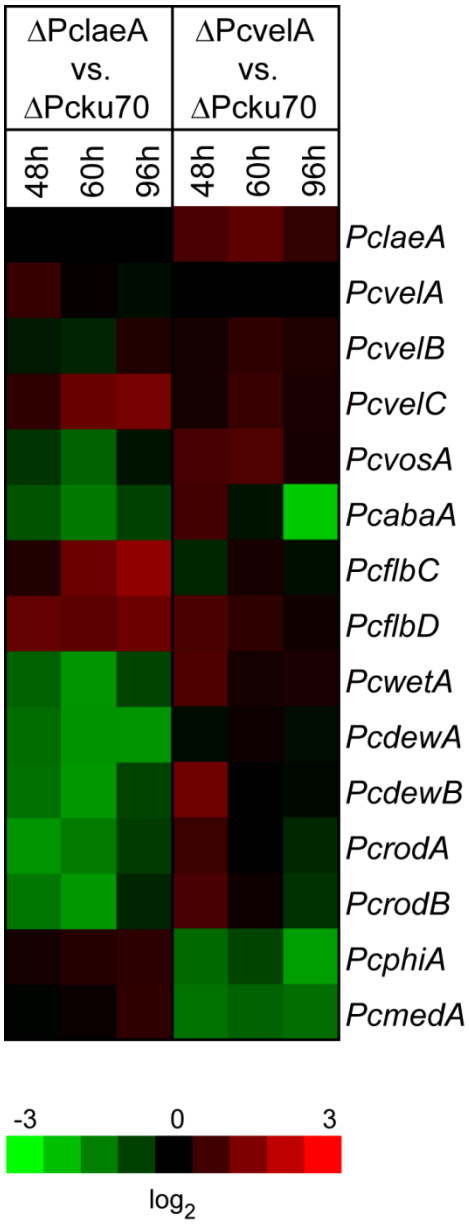

Fig. S4. Microarray analyses of selected *PclaeA*- and *PcvelA*-dependent genes. Heatmaps of genes from the velvet complex and developmental genes are shown. Red indicates genes that are upregulated in the corresponding mutant compared to the reference strain ΔPcku70; green marks those that are downregulated compared to the reference strain. All genes and their products are mentioned in the text. The color of each square represents a log2-fold change in expression at the given time point.

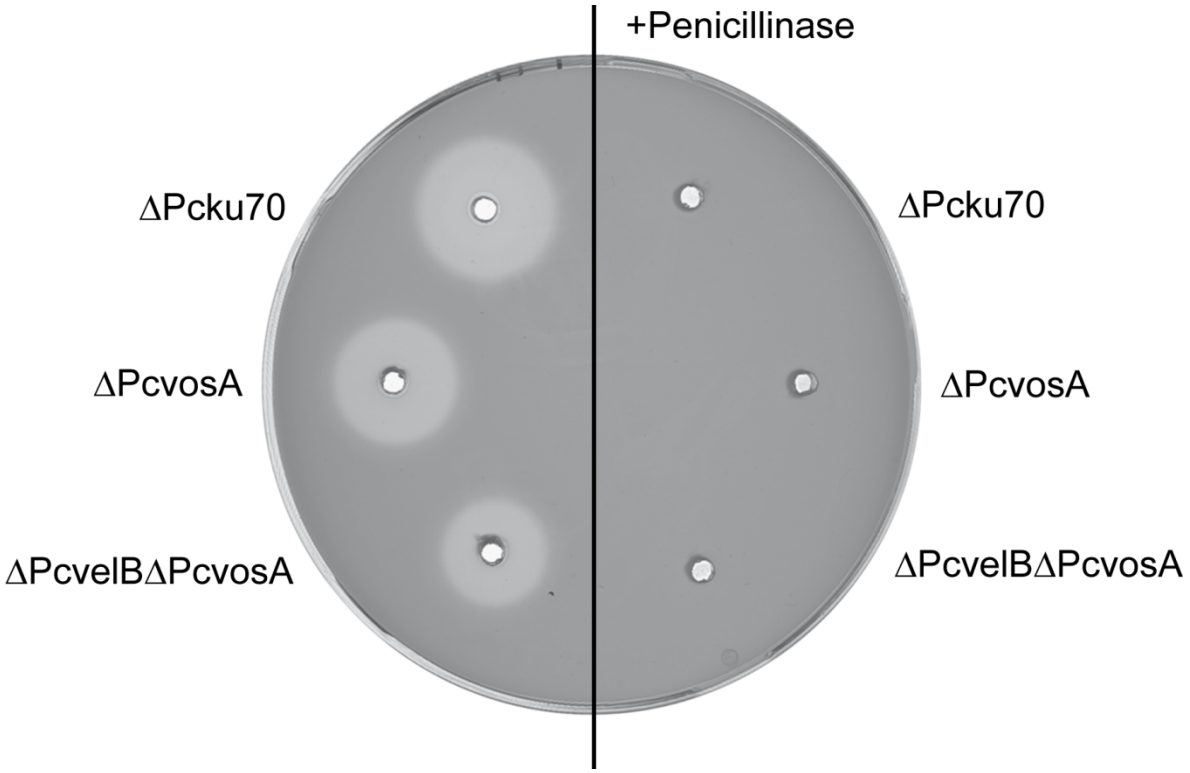

Fig. S5. Penicillinase assay. To confirm that growth inhibition of the indicator organism *S. aureus* was due to penicillin production, penicillinase was added to the supernatant of selected strains.

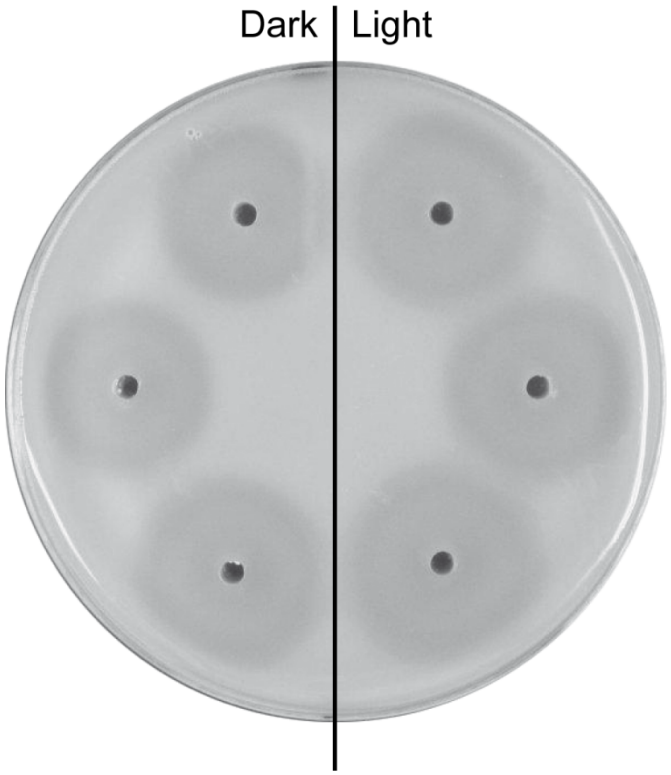

Fig. S6. Penicillin production under light and dark conditions. The reference strain P2niaD18 was grown in triplicate for 72 h in shaking culture with either constant light or constant dark conditions. A halo assay indicates the amount of penicillin produced under both conditions.