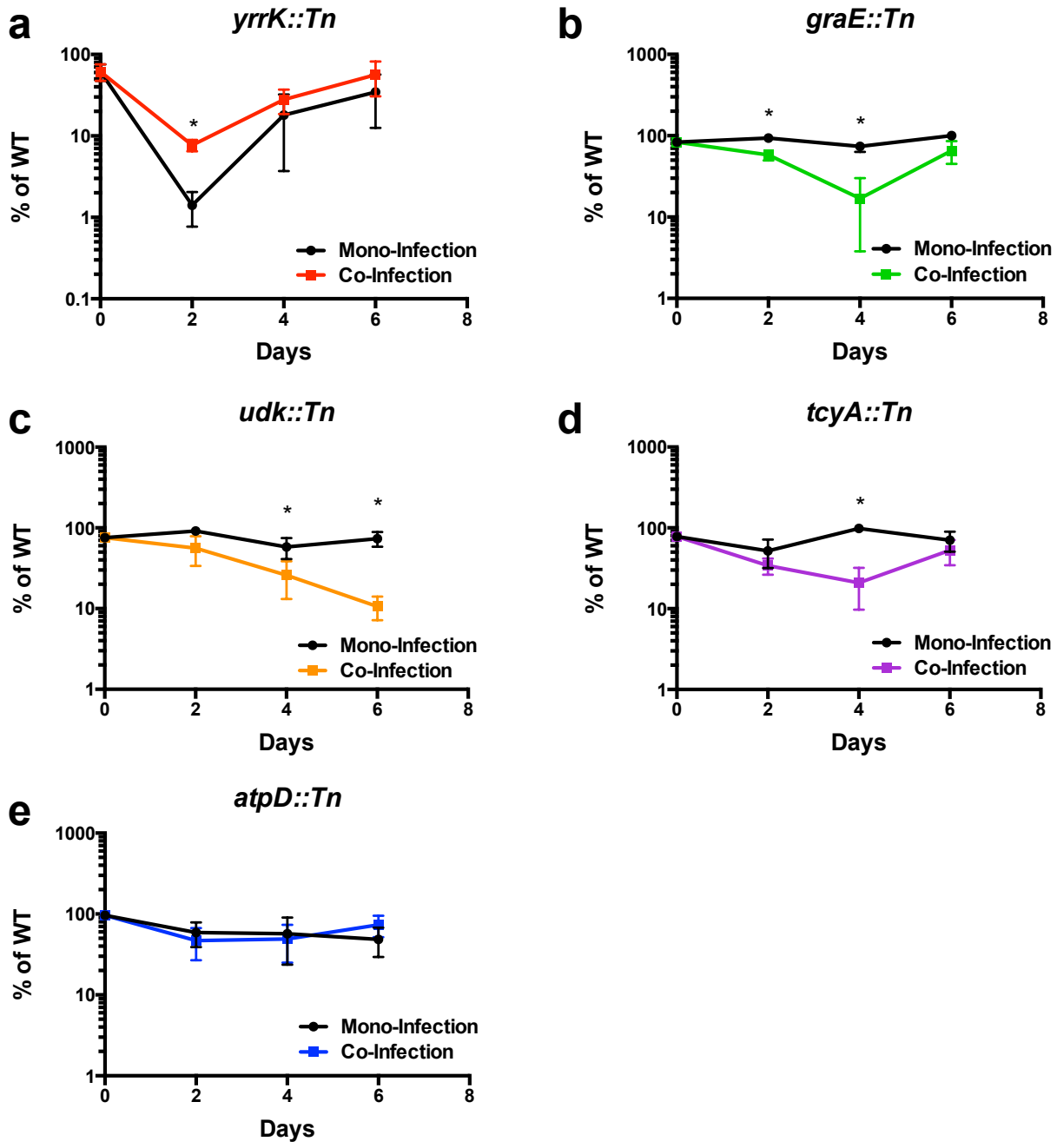
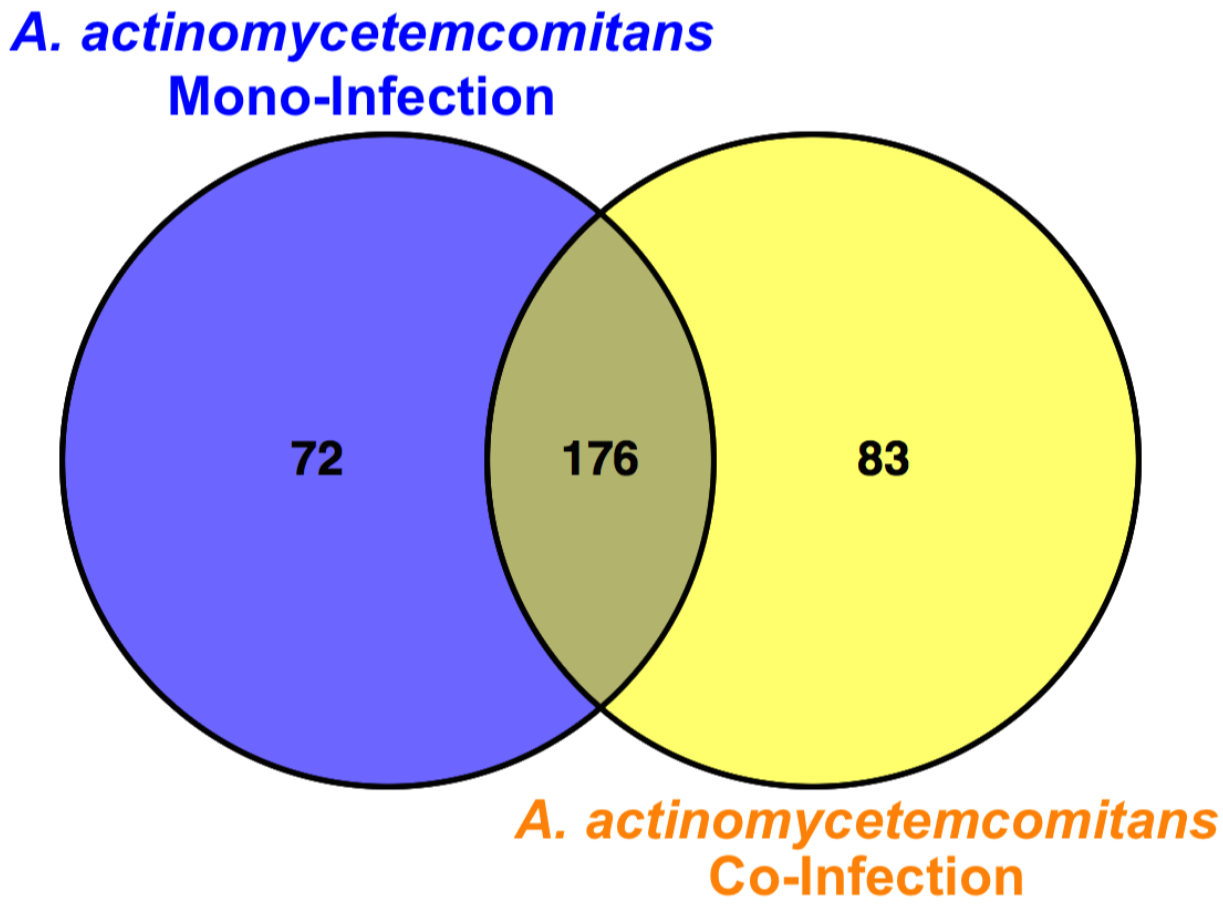
Co-infecting microbes dramatically alter pathogen gene essentiality during polymicrobial infection

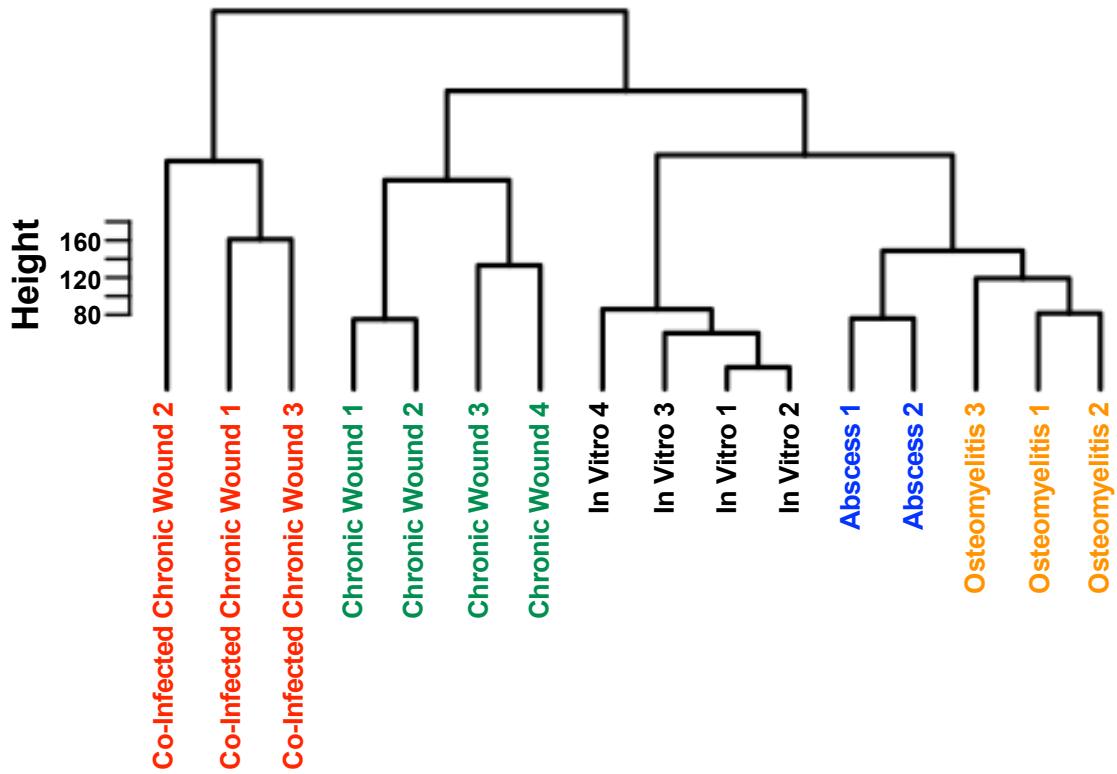
Supplementary Information



Supplementary Figure 1. Some *S. aureus* CoDE genes are not *in vivo* specific. Comparison of wildtype *S. aureus* and *S. aureus* CoDE gene mutant cell numbers in an *in vitro* chronic wound model during mono-culture and *P. aeruginosa* co-culture. *graE::TnMariner* (b, green), *udk* (c, orange), and *tcyA::TnMariner* (d, purple) were predicted to be required in co-culture but not mono-culture, while while *yrrK::TnMariner* (a, red) and *atpD::TnMariner* (e, blue) were predicted to be required in mono-infection but not in co-infection. All mutants are reported as % wildtype cell number (*S. aureus* HG003) in the same condition. Statistical analysis was performed by arcsine transforming the data prior to analysis with a Student's *t*-test (* = $p < 0.05$). For each condition, 4 biological replicates were used. Error bars represent the standard error of the mean (SEM).



Supplementary Figure 2. Identification of CoDE genes in *A. actinomycetemcomitans* during co-infection with *S. gordonii*. Venn diagram of the *A. actinomycetemcomitans in vivo* essential genome in mono-culture (blue, n=2) and co-culture (yellow, n=2) infection with *S. gordonii*. 72 CoDE genes were identified that were essential in mono-culture infection but non-essential in co-culture, and 83 CoDE genes were uniquely essential in co-infection. Data are the result of two replicates per condition.



Supplementary Figure 3. Experimental replicates cluster by condition. Hierarchal clustering (Ward method) of the average normalized read counts per gene in each of the replicates (numbered and color coded) for all experimental conditions. Height indicates the Euclidean distance between clusters.

Supplementary Table 1. **The essential genes for *S. aureus* and *A. actinomycetemcomitans***.

Supplementary Table 2. Strains used in this study.

Strain or Plasmid	Description	Source or Reference
S. aureus		
HG003 Tn Library	HG003 transposon library	Valentino *et al.*[1]
HG003	*S. aureus* wildtype strain derived from NCTC8325	Herbert *et al*.[2]
CI46	JE2 SAUSA300_1573::ΦNΣ	Fey *et al.*[3]
CI54	HG003 SAUSA300_1573::ΦNΣ	This work
CI58	JE2 SAUSA300_0113:: ΦNΣ	Fey *et al.*[3]
CI61	HG003 SAUSA300_0113:: ΦNΣ	This work
CI59	JE2 SAUSA300_0773::ΦNΣ	Fey *et al.*[3]
CI62	HG003 SAUSA300_0773::ΦNΣ	This work
CI67	JE2 SAUSA300_1455:: ΦNΣ	Fey *et al.*[3]
CI71	HG003 SAUSA300_1455::ΦNΣ	This work
CI66	JE2 SAUSA300_1568::ΦNΣ	Fey *et al*.[3]
CI72	HG003 SAUSA300_1568::ΦNΣ	This work
CI68	JE2 SAUSA300_2359::ΦNΣ	Fey *et al.*[3]
CI73	HG003 SAUSA300_2359::ΦNΣ	This work
CI69	JE2 SAUSA300_2058::ΦNΣ	Fey *et al.*[3]
CI74	HG003 SAUSA300_2058::ΦNΣ	This work
Other bacteria		
PAO1 Tn Library	PAO1 transposon Library	Jacobs *et al.*[4]
Aa624 Tn Library	High density transposon library in *A. actinomycetemcomitans* strain 624	This work
S. gordonii Challis DL 1.1	*Streptococcus gordonii* (ATCC® 49818)	ATCC

Supplementary Table 3. Primers used in this study.

Primer	Sequence
olj376	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGGGGGGGGGGGG
PCR1-Ba-Bio	TACAATAAGGATAAATTTGAATGGTACCATAAACGACCG
PCR2-Ba	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC GATCTNNNNNCGTCTAGAGACCGGGGACTTATCA
mariner-1	ACTCACTATAGGAGGGCGGGAATCATTTGAAGGTTGGTAC
mariner-2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC GATCTNNNNNGTGTCAGACCGGGGACTTATCAG
Barcoded Primer	CAAGCAGAAGACGGCATACGAGATXXXXXXGTGACTGGAGTTCAGACGTGTG
Tnp-F-SalI	TTAGTCGACATAAGGAGGCACTCACCATGGAA
Tnp-R-NotI	TTAGCGGCCGCGAAAATTCGTTTTTATTATTCAACATAGTTCCC
KanR-UP-F	CTACCAAGACGAAGAGGATGAAGGATTGCCTTGAATATATTGACAA
KanR-DN-R	CCCCTTTTCATATATTATATAGTCAGTACTAAAACAATTCATCCAGTA
mar-UP-R	CTTCATCCTCTTCGTCTTGGTAG
mar-IR-NotI	GCGGCCGCTAACAGGTTGGCTGATAAGTCCC
mar-DN-F	GACTATATAATATATGAAAAGGGG
CBI7	CGACTATGAAGAACTTCCAACGC
CBI8	CCCATTCTTCATCAGTTTCGATTGG
CBI9	GCCTTTTACTTCCTGAATAAATCTTTC
CBI10	GTTGCTCGTGCATTTAGATG
CBI11	GGGTGTAAGGTAGGTTGTTA
CBI12	CCACACCTGTTTAGAATGTGG
CBI13	GAGCATGTTTAATTGTAATTTTGATGGG
CBI14	GCCACATTCTGTGCATGCTA
CBI16	GGGTCAATCGAGAATATCGTC
CBI21	GAGCGAATGACAAGGATTG
CBI22	CGTCCATATGAAGCATGTG
CBI23	GGTAACAGCTTAGACGCG
CBI24	CTCTACAACTTCAGGACGC
CBI46	GATGTTGCATGGTTATCACGCTGG
CBI47	GGTGCCAATGTCCATTGGTTGTC
CBI48	CGGAACAATATTAGACGCAAAAGCAAGTG
CBI49	CCACTCATGACACCTATCTCACCAG

*xxxxxx= One of the 6 bp TruSeq barcode sequences, available at

Supplementary Table 4. Sequencing data for each replicate in this study.

Supplementary Table 5. A list of all of the 'TA' dinucleotide positions in the *S. aureus* NCTC8325 reference genome.

Supplementary Table 6. A list of all of the 'TA' dinucleotide positions in the *A. actinomycetemcomitans* strain 624 reference genome.

Supplementary Table 7. Homologues in *S. aureus* strain NCTC8325 and *S. aureus* strain USA300_FPR3757 for the transposon mutants used in this study.

Supplementary References:

1 Valentino, M. D. *et al.* Genes contributing to *Staphylococcus aureus* fitness in abscess- and infection-related ecologies. *MBio* **5**, e01729-01714, doi:10.1128/mBio.01729-14 (2014).

2 Herbert, S. *et al.* Repair of global regulators in *Staphylococcus aureus* 8325 and comparative analysis with other clinical isolates. *Infect Immun* **78**, 2877-2889, doi:10.1128/IAI.00088-10 (2010).

3 Fey, P. D. *et al.* A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. *MBio* **4**, e00537-00512, doi:10.1128/mBio.00537-12 (2013).

4 Jacobs, M. A. *et al.* Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 14339-14344, doi:10.1073/pnas.2036282100 (2003).