Description of Additional Supplementary Files

Supplementary Data 1. Enterobacterial strains and primers used in this study.

Supplementary Data 2. Variant calling results. Sheet SNPs_pOXA-48 includes SNPs detected by Snippy using as reference the sequence of pOXA-48_K8, the most frequent variant that was introduced into pOXA-48-naive strains by conjugation, mapping the RNA-Seq reads. Sheet All_SNPs includes all SNPs detected by Snippy in all strains and replicates using as reference the closed references genomes of the pOXA-48-carrying strains. Sheet SNPs_log2FC includes SNPs detected by Snippy not present in all replicates and conditions for each sample and the significant log_2FoldChanges of the affected genes or intergenic regions (note: Snippy does not annotate mutations in intergenic regions; annotations were completed manually for this sheet).

Supplementary Data 3. Transcripts Per Million (TPM) values. TPM values were calculated for each pOXA-48-carrying strain (sheets <strain>), including all genes of the genome, and were later filtered to obtain chromosomal and pOXA-48 TPM values. Then, pOXA-48 TPM values were normalized by the median chromosomal TPM expression of the respective strains, and the median TPM per gene between replicates was calculated for plotting (sheet pOXA-48_norm_TPM).

Supplementary Data 4. Differential expression analysis results (first RNA-Seq dataset). Sheet All_chromosome_DEGs includes a summary of significant differentially expressed chromosomal genes (CDSs) between strains. The summary is a presence/absence table where 0 means the gene was not DE in the specific strain, and values >0 or <0 indicate the log_2FoldChanges of the significant DEGs. Genes were matched between strains by RefSeq ID, Gene and Product, which means that if an ortholog gene was annotated with different RefSeq IDs, Gene and/or Product between strains, the information of that gene is split in different rows. Also, hypothetical proteins were excluded because they were not differentiable by RefSeq ID, Gene and Product. The Count column is the number of strains that show DE of a specific gene. Rows of the pirin and isochorismatase family proteins and their putative LysR transcriptional regulator are highlighted (note that they are separated in different rows for *Klebsiella* spp. and *C. freundii* due to different RefSeq ID). Sheet Protein_counts_chr shows the total counts of chromosomal genes with matching Product names. Sheet All_plasmids_DEGs includes a summary table of significant differentially expressed plasmid genes (CDSs), excluding pOXA-48. The presence/absence table was created as in All_chromosome_DEGs. Sheets <strain>_chr include the DESeq2 result tables of significant chromosomal DEGs (columns baseMean, Log2FoldChange, lfcSE, stat, pvalue, padj). Columns H to K-M include the raw counts (featureCounts output) of reads mapping to features. The GO column includes GO annotations of biological processes, molecular functions and cellular components. Sheets <strain>_plas include the DESeq2 result tables of significant plasmid DEGs, excluding pOXA-48. Columns are as in <strain>_chr.

Supplementary Data 5. Gene Set Enrichment Analysis (GSEA) results from clusterProfiler. Sheets GSEA_chr_* correspond to GSEA results of chromosomal genes, while sheets GSEA_plas_* correspond to GSEA results of plasmid genes other than pOXA-48. BP: Biological Process; MF: Molecular Function; CC: Cellular Component.

Supplementary Data 6. Number of differentially expressed genes (DEGs) by each Biological Process (BP). Sheet Summarized contains a list of all BP annotated across strains and the number (count) of total DEGs up- or downregulated for that BP. BP are colored according to higher parental GO annotations as in the main manuscript. Sheet By_strain is as Summarized, but the number of DEGs is shown by strain.

Supplementary Data 7. RefSeq database of Proteobacteria. Each <class>- sheet contains information of the Gamma-, Beta-, Alpha-, Epsilon-, Delta- and Zetaproteobacteria strains used in this work, including the given species code and whether the *lysR-pfp-ifp* cluster is present or absent. Sheets Summary_<class> includes a summary of strains per species encoding and not encoding the *lysR-pfp-ifp* cluster.

Supplementary Data 8. GC content and Codon Adaptation Index (CAI) of representative strains encoding the operon system. Sheet GC_content includes the GC content of the operon system (from the start of the *lysR* gene to the end of the *ifp* gene) and the chromosome of the selected strains. The difference in GC content between the operon system and the chromosome is also indicated. Sheet CAI includes the CAI values of the genes of the operon system, and the median CAI of genes encoding for ribosomal proteins (used as reference to compute the codon usage table) and of all chromosomal genes.

Supplementary Data 9. BLASTp of the protein sequence of the pOXA-48-encoded LysR against the reference genomes of the pOXA-48-carrying strains. Each sheet includes the BLASTp results for each strain. Hits are ordered from best to worst. Bold indicates the hit of the pOXA-48-encoded LysR against itself. Gray background indicates the hit to the LysR that putatively regulates the *pfp-ifp* operon.

Supplementary Data 10. Differential expression analysis results (second RNA-Seq dataset). Sheet All_DEGs includes a summary of significant differentially expressed genes (CDSs) between strains, comparing the strains carrying pOXA-48 against pOXA-48Δ*lysR* carriers. The summary is a presence/absence table were 0 means the gene was not DE in the specific strain, and values >0 or <0 indicate the $\log_2$FoldChanges of the significant DEGs. Genes were matched between strains by Chr, RefSeq ID, Gene and Product, which means that if an ortholog gene was annotated with different RefSeq IDs, Gene and/or Product between strains, the information of that gene is split in different rows. Also, hypothetical proteins were excluded because they were not differentiable by RefSeq ID, Gene and Product. The Count column is the number of strains that show DE of a specific gene. Rows of the pirin and isochorismatase family proteins and their putative LysR transcriptional regulator are highlighted (note that they are separated in different rows for *Klebsiella* spp. and *C. freundii* due to different RefSeq ID). Sheets <strain> include the DESeq2 result tables of significant chromosomal DEGs (colums baseMean, Log2FoldChange, lfcSE, stat, pvalue, padj). Columns H to M include the raw counts (featureCounts output) of reads mapping to features. Sheet DEGs_LysRs includes the DEGs putatively regulated by chromosomal LysRs, used to plot Fig 5.

Supplementary Data 11. Statistical analyses results. Sheet GAM_model_selection contains fit statistics for each generalized additive model (GAM) constructed with different basis functions (k) values. Sheet GAM_results includes the GAM results for CF13 and KPN15, for the formula AUC ~ s(concentration, k=5, by = genotype_drug) + genotype * drug. Sheet Stats_pTet-LysR_expression

includes the ANOVA and post-hoc Tukey's tests for the experiments expressing LysR_pOXA-48 in trans (Fig 7A). Sheet Stats_CRISPRi_curves includes the ANOVA and post-hoc Tukey's tests for the CRISPRi experiments of KPN15 (Fig 7C).

Supplementary Data 12. BLASTp of the protein sequence of the pOXA-48-encoded LysR against the RefSeq database. Sheet BLASTp_filtered includes the BLASTp results filtered by >90% identity and full alignment length (303 aa). Sheet Plasmid_Incs includes the number of plasmid Inc groups that encode a LysR with a high percentage of identity with the pOXA-48-encoded LysR. The grouping of the LysRs is indicated: 100% identity or <100% (and >96%) identity with LysR_pOXA-48. Sheet pOXA-48_plasmids includes the pOXA-48 plasmids that encode for the LysR_pOXA-48 with >96% identity. Sheet dNdS_pOXA-48 includes the results from the analysis of the mutations affecting the different genes of pOXA-48. P values correspond to the permutation test analyzing whether the genes are enriched or decreased in mutations.