# Supporting information

# CRISPR interference of genes involved in nucleotide biosynthesis improves production of a single-domain antibody

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# Supplementary Table 1

**Table S1**. Primers used in the study.

| Nr. | Primer sequence 5'- 3' | Description |
|---|---|---|
| jl37 | **TTTACCTTCGTCACCCCATT**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purA sgRNA** into pSLQ1236 |
| jl38 | **AATGGGGTGACGAAGGTAAA**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purA sgRNA** into pSLQ1236 |
| jl39 | **TCCATCGACAGGGGAAACGG**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purB sgRNA** into pSLQ1236 |
| jl40 | **CCGTTTCCCCTGTCGATGGA**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **purB sgRNA** into pSLQ1236 |
| jl41 | **CGGGTTTTCCGTGCTGTATA**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purC sgRNA** into pSLQ1236 |
| jl42 | **TATACAGCACGGAAAACCCG**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purC sgRNA** into pSLQ1236 |
| jl43 | **CGGCGACTGGGCCGCTTTCC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purD sgRNA** into pSLQ1236 |
| jl44 | **GGAAAGCGGCCCAGTCGCCG**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purD sgRNA** into pSLQ1236 |
| jl45 | **GACACGCGCCGGATTATTGC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purE sgRNA** into pSLQ1236 |
| jl46 | **GCAATAATCCGGCGCGTGTC**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purE sgRNA** into pSLQ1236 |
| jl47 | **TCATAAATCGACTGGTTAAC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purF sgRNA** into pSLQ1236 |
| jl48 | **GTTAACCAGTCGATTTATGA**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **purF sgRNA** into pSLQ1236 |
| jl49 | **AAACACTGAGCAGAGCGCGG**GTTTTAGAGCTA GAAATAGCAAGTTAAAATAAGGC | Forward insert of **purH sgRNA** into pSLQ1236 |
| jl50 | **CCGCGCTCTGCTCAGTGTTT**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **purH sgRNA** into pSLQ1236 |
| jl51 | **GGCCTAACTGCCCGTTACCG**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purK sgRNA** into pSLQ1236 |

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|---|---|---|
| jl52 | **CGGTAACGGGCAGTTAGGCC**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purK sgRNA** into pSLQ1236 |
| jl53 | **ATTCGGAATGCCGACAGTGC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purL sgRNA** into pSLQ1236 |
| jl54 | **GCACTGTCGGCATTCCGAAT**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purL sgRNA** into pSLQ1236 |
| jl55 | **GGCATCTTTGTAGCTAAGAG**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purM sgRNA** into pSLQ1236 |
| jl56 | **CTCTTAGCTACAAAGATGCC**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **purM sgRNA** into pSLQ1236 |
| jl57 | **CTGTAAATTACTTCCGTTGC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **purN sgRNA** into pSLQ1236 |
| jl58 | **GCAACGGAAGTAATTTACAG**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **purN sgRNA** into pSLQ1236 |
| jl59 | **GAGAACCGAAGTCCAGAATG**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **guaA sgRNA** into pSLQ1236 |
| jl60 | **CATTCTGGACTTCGGTTCTC**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **guaA sgRNA** into pSLQ1236 |
| jl61 | **CGGTAGAGTGAGCAGGAACG**GTTTTAGAGCTA GAAATAGCAAGTTAAAATAAGGC | Forward insert of **guaB sgRNA** into pSLQ1236 |
| jl62 | **CGTTCCTGCTCACTCTACCG**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **guaB sgRNA** into pSLQ1236 |
| jl63 | **ATGATATGTTTCTGATATAG**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrB sgRNA** into pSLQ1236 |
| jl64 | **CTATATCAGAAACATATCAT**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **pyrB sgRNA** into pSLQ1236 |
| jl65 | **GCGGCGGATCTTTAATACCT**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrC sgRNA** into pSLQ1236 |
| jl66 | **AGGTATTAAAGATCCGCCGC**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **pyrC sgRNA** into pSLQ1236 |
| jl67 | **AAAAGGGCTTTACGAACGAA**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrD sgRNA** into pSLQ1236 |
| jl68 | **TTCGTTCGTAAAGCCCTTTT**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **pyrD sgRNA** into pSLQ1236 |

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|---|---|---|
| jl69 | **TAAGCGCAAATTCAATAAAC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrE sgRNA** into pSLQ1236 |
| jl70 | **GTTTATTGAATTTGCGCTTA**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **pyrE sgRNA** into pSLQ1236 |
| jl71 | **AGGAGAATTCGTAACAGCGC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrF sgRNA** into pSLQ1236 |
| jl72 | **GCGCTGTTACGAATTCTCCT**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **pyrF sgRNA** into pSLQ1236 |
| jl73 | **GGCAATGCCTTTACCCAGAG**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrG sgRNA** into pSLQ1236 |
| jl74 | **CTCTGGGTAAAGGCATTGCC**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **pyrG sgRNA** into pSLQ1236 |
| jl75 | **TTTATAGACGGGTTTTGCAT**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **pyrH sgRNA** into pSLQ1236 |
| jl76 | **ATGCAAAACCCGTCTATAAA**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **pyrH sgRNA** into pSLQ1236 |
| jl77 | **GGCCATCAATGGTAATAACC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **cmk sgRNA** into pSLQ1236 |
| jl78 | **GGTTATTACCATTGATGGCC**ACTAGTCTTTTCTC TATCACTGATAGGGA | Reverse insert of **cmk sgRNA** into pSLQ1236 |
| jl79 | **ACGTTTTTTGCTACCGCGTT**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGC | Forward insert of **ndk sgRNA** into pSLQ1236 |
| jl80 | **AACGCGGTAGCAAAAAACGT**ACTAGTCTTTTCT CTATCACTGATAGGGA | Reverse insert of **ndk sgRNA** into pSLQ1236 |
| jl130 | ACATGTTC**U**TTCCTGCGTTATCCC | Forward primer for amplifying psdAb-TIR1/2 to change origin of replication to CloDF13 |
| jl131 | ATCTTTTC**U**ACGGGGTCTGACGC | Reverse primer for amplifying psdAb-TIR1/2 to change origin of replication to CloDF13 |
| jl154 | AGAAAAGA**U**CAAATAGCTAGCTCACTCGG | Forward primer for amplifying pCDF-Duet CloDf13 origin |
| jl155 | AGAACATG**U**AAATCTAGAGCGGTTCAGTAG | Reverse primer for amplifying pCDF-Duet CloDf13 origin |

# Supplementary Table 2

**Table S2.** Strains and plasmids used in the study. The name used to refer to a specific strain or plasmid in the main text is written in bold.

| Strain | Description | Reference/source |
|---|---|---|
| *E. coli* NEB 5-alpha | *fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*; cloning strain | New England Biolabs |
| *E. coli* K12 MG1655 | F- lambda- *ilvG- rfb-50 rph-1* | Lab collection |
| SIJ19 | **MG1655-gfp**: MG1655 Bb_J23100 *gfp* ::FRT | (Bonde et al., 2016) |
| *E.coli* MG1655 (DE3) | **MG1655-DE3**: MG1655 λ*(DE3)* | (Mundhada et al., 2016) |
| JL100 | **MG1655-DE3-dCas9**: MG1655 λ*(DE3)* attB-186(O): tetR-Ptet-dCas9 | This study |
| JL86 | MG1655-gfp with psgRNA-purA and pdCas9 | This study |
| JL87 | MG1655-gfp with psgRNA-purB and pdCas9 | This study |
| JL88 | MG1655-gfp with psgRNA-purC and pdCas9 | This study |
| JL89 | MG1655-gfp with psgRNA-purD and pdCas9 | This study |
| JL90 | MG1655-gfp with psgRNA-purE and pdCas9 | This study |
| JL91 | MG1655-gfp with psgRNA-purF and pdCas9 | This study |
| JL92 | MG1655-gfp with psgRNA-purH and pdCas9 | This study |
| JL93 | MG1655-gfp with psgRNA-purK and pdCas9 | This study |
| JL94 | MG1655-gfp with psgRNA-purL and pdCas9 | This study |
| JL95 | MG1655-gfp with psgRNA-purM and pdCas9 | This study |
| JL96 | MG1655-gfp with psgRNA-purN and pdCas9 | This study |
| JL97 | MG1655-gfp with psgRNA-guaA and pdCas9 | This study |
| JL98 | MG1655-gfp with psgRNA-guaB and pdCas9 | This study |
| JL99 | MG1655-gfp with psgRNA-pyrB and pdCas9 | This study |
| JL101 | MG1655-gfp with psgRNA-pyrD and pdCas9 | This study |
| JL102 | MG1655-gfp with psgRNA-pyrE and pdCas9 | This study |
| JL103 | MG1655-gfp with psgRNA-pyrF and pdCas9 | This study |
| JL104 | MG1655-gfp with psgRNA-pyrG and pdCas9 | This study |
| JL105 | MG1655-gfp with psgRNA-pyrH and pdCas9 | This study |

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|---|---|---|
| JL214 | MG1655-DE3-dCas9 with psgRNA-pyrF and psdAb-TIR1 | This study |
| JL215 | MG1655-DE3-dCas9 with psgRNA-pyrG and psdAb-TIR1 | This study |
| JL216 | MG1655-DE3-dCas9 with psgRNA-cmk and psdAb-TIR1 | This study |
| JL217 | MG1655-DE3-dCas9 with psgRNA-blank and psdAb-TIR1 | This study |
| JL218 | MG1655-DE3-dCas9 with psgRNA-pyrF and psdAb-TIR2 | This study |
| JL219 | MG1655-DE3-dCas9 with psgRNA-pyrG and psdAb-TIR2 | This study |
| JL220 | MG1655-DE3-dCas9 with psgRNA-cmk and psdAb-TIR2 | This study |
| JL221 | MG1655-DE3-dCas9 with psgRNA-blank and psdAb-TIR2 | This study |

| **Plasmid** | **Description** | **Reference/source** |
|---|---|---|
| pCDF-Duet | Cloning and expression vector; $Spec_R$ | Novagen |
| pdCas9-bacteria | **pdCas9**: p15A vector with aTc-inducible expression of dCas9; $Chlor_R$ | (Larson et al., 2013) |
| pSLQ1236 | **pSLQ1236**: pColE1 vector with aTc-inducible expression of sgRNA; $Amp_R$ | (Larson et al., 2013) |
| pOSIP KO | Cloning-integration plasmid, integrates at the phage 186 site; $Kan_R$ | (St-Pierre et al., 2013) |
| pOSIP KO-dCas9 | Plasmid for genomic integration of aTc-inducible dCas9; $Kan_R$ | (Li et al., 2016) |
| pE-FLP | Plasmid for expressing FLP recombinase to excise the integration plasmid backbone; $Amp_R$ | (St-Pierre et al., 2013) |
| pET28a-Nanobody®-$TIR_{SynEvo1}$ | pBR322/ROP vector with Nanobody® expressed from the T7 promoter. Synthetically evolved translation initiation region (TIR) for optimized expression; $Kan_R$ | (Rennig et al., 2018) |
| pET28a-Nanobody®-$TIR_{SynEvo2}$ | pBR322/ROP vector with Nanobody® expressed from the T7 promoter. Synthetically evolved TIR for optimized expression; $Kan_R$ | (Rennig et al., 2018) |
| psgRNA-blank | **control**: pSLQ1236 without sgRNA sequence; $Amp_R$ | (Li et al., 2016) |
| pJL45 | **purA**: pSLQ1236 with sgRNA targeting purA; $Amp_R$ | This study |
| pJL46 | **purB**: pSLQ1236 with sgRNA targeting purB, $Amp_R$ | This study |
| pJL51 | **purC**: pSLQ1236 with sgRNA targeting purC; $Amp_R$ | This study |
| pJL52 | **purD**: pSLQ1236 with sgRNA targeting purD; $Amp_R$ | This study |
| pJL53 | **purE**: pSLQ1236 with sgRNA targeting purE; $Amp_R$ | This study |

| | | |
|---|---|---|
| pJL54 | **purF**: pSLQ1236 with sgRNA targeting purF; $Amp^R$ | This study |
| pJL55 | **purH**: pSLQ1236 with sgRNA targeting purH; $Amp^R$ | This study |
| pJL56 | **purK**: pSLQ1236 with sgRNA targeting purK; $Amp^R$ | This study |
| pJL57 | **purL**: pSLQ1236 with sgRNA targeting purL; $Amp^R$ | This study |
| pJL58 | **purM**: pSLQ1236 with sgRNA targeting purM; $Amp^R$ | This study |
| pJL59 | **purN**: pSLQ1236 with sgRNA targeting purN; $Amp^R$ | This study |
| pJL60 | **guaA**: pSLQ1236 with sgRNA targeting guaA; $Amp^{R;}$ | This study |
| pJL61 | **guaB**: pSLQ1236 with sgRNA targeting guaB; $Amp^R$ | This study |
| pJL62 | **pyrB**: pSLQ1236 with sgRNA targeting pyrB; $Amp^R$ | This study |
| pJL64 | **pyrD**: pSLQ1236 with sgRNA targeting pyrD; $Amp^R$ | This study |
| pJL65 | **pyrE**: pSLQ1236 with sgRNA targeting pyrE; $Amp^R$ | This study |
| pJL66 | **pyrF**: pSLQ1236 with sgRNA targeting pyrF; $Amp^R$ | This study |
| pJL67 | **pyrG**: pSLQ1236 with sgRNA targeting pyrG; $Amp^R$ | This study |
| pJL68 | **pyrH**: pSLQ1236 with sgRNA targeting pyrH; $Amp^R$ | This study |
| pJL69 | **cmk**: pSLQ1236 with sgRNA targeting cmk; $Amp^R$ | This study |
| pJL70 | **ndk**: pSLQ1236 with sgRNA targeting ndk; $Amp^R$ | This study |
| pJL97 | **psdAb-TIR1**: pET28a-Nanobody®-$TIR_{SynEvo1}$ plasmid with origin of replication changed to ClodF13; $Kan^R$ | This study |
| pJL98 | **psdAb-TIR2**: pET28a-Nanobody®-$TIR_{SynEvo1}$ plasmid with origin of replication changed to ClodF13; $Kan^R$ | This study |

# Supplementary Figure 1

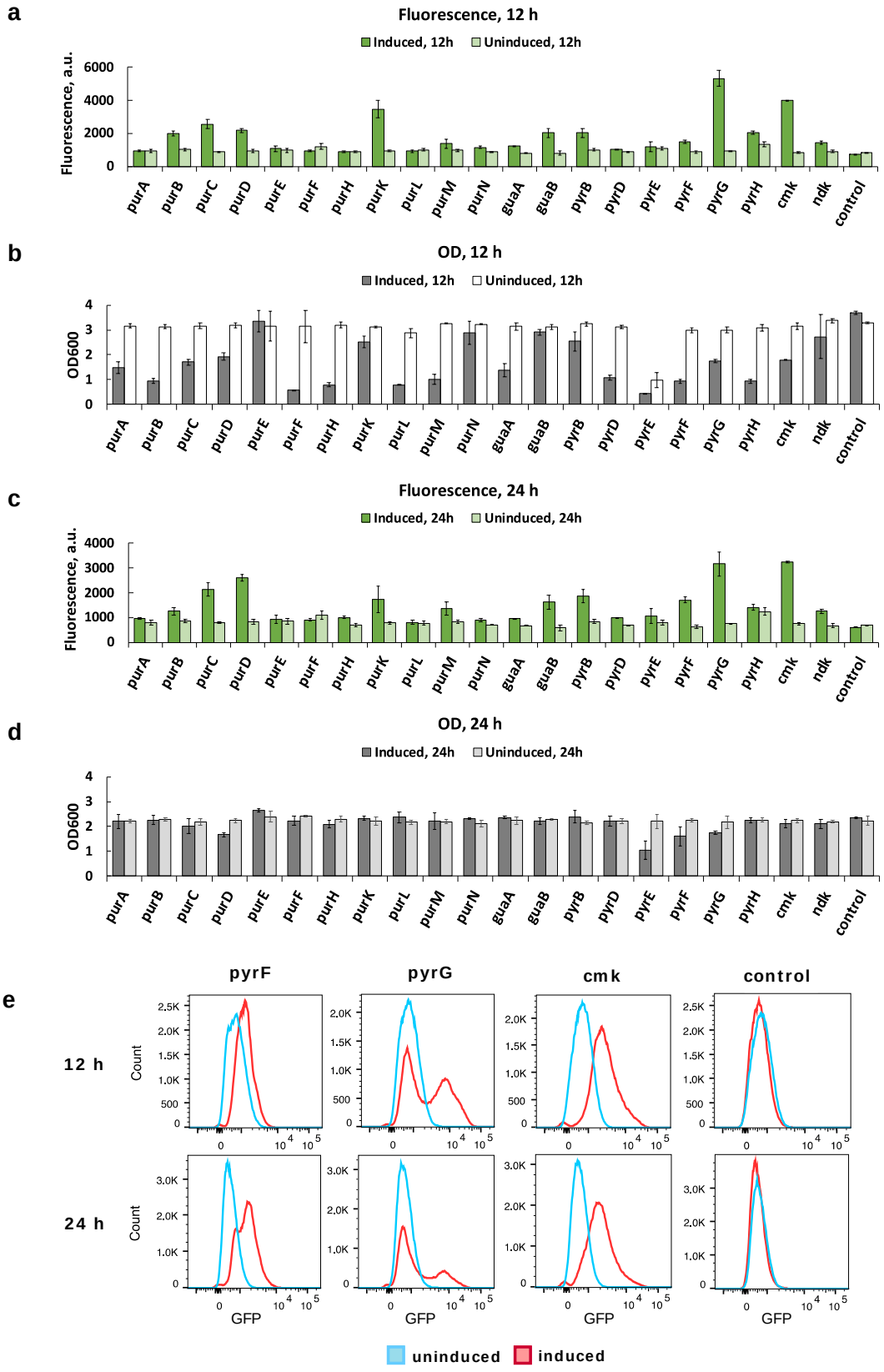

**Figure S1.** Screening the purine and pyrimidine biosynthesis pathway for growth decoupling targets. (a) GFP fluorescence in CRISPRi-induced and uninduced strains after 12 h of growth. (b) OD of CRISPRi-induced and uninduced strains after 12 h of growth. (c) GFP fluorescence in CRISPRi-induced and uninduced strains after 24 h of growth. (d) OD of CRISPRi-induced and uninduced strains after 24 h of growth. (e) Histograms over 12 h and 24 h GFP fluorescence in CRISPRi-induced and not induced cultures harboring sgRNA plasmids targeting *pyrF*, *pyrG*, *cmk* or an empty control vector. The OD and fluorescence were calculated as the average of three biological replicates. Standard deviations between replicates are shown as error bars. CRISPRi-induced strains are shown in dark green (fluorescence in bar plots), dark grey (OD) or red (fluorescence in histograms), and uninduced strains are shown in bright green (fluorescence in bar plots), bright grey (OD) or blue (fluorescence in histograms).

Supplementary Figure 2

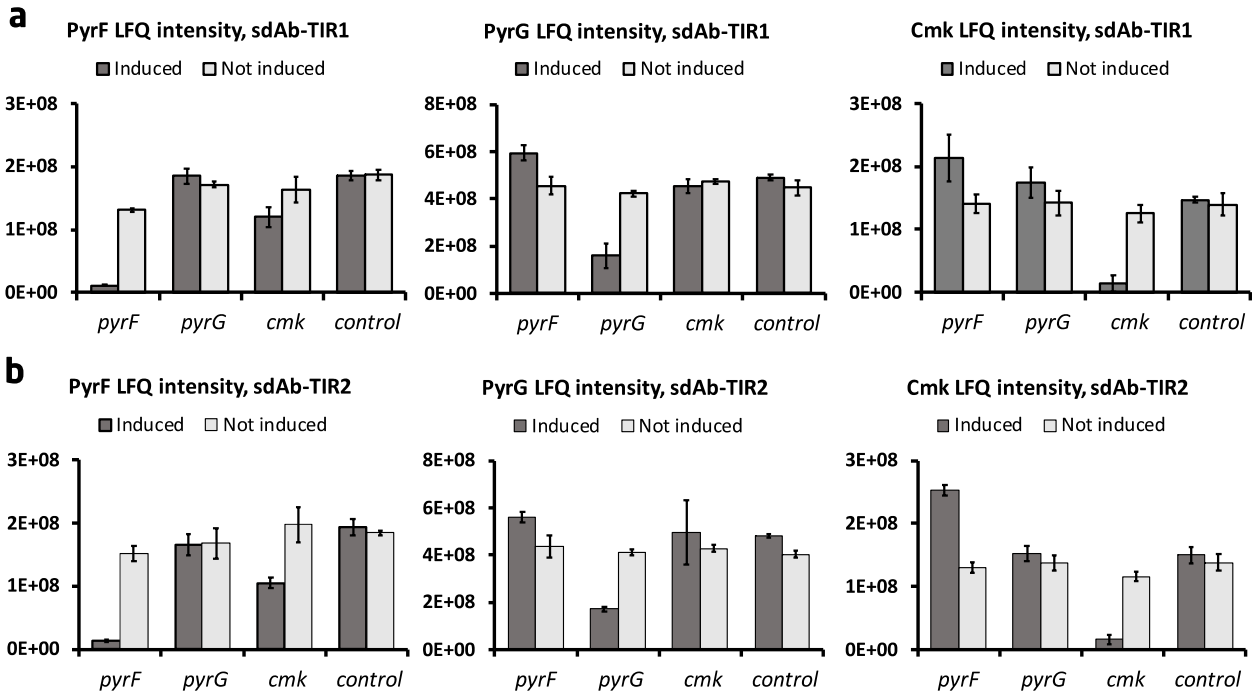


**Figure S2.** Protein levels of PyrF, PyrG and Cmk in sdAb production strains with or without induction of the CRISPRi growth decoupling system. (a) Protein levels of PyrF (panel 1), PyrG (panel 2) and Cmk (panel 3) in strains harboring the psdAb-TIR1 plasmid and sgRNA plasmids targeting *pyrF*, *pyrG*, *cmk* or an empty control vector. (a) Protein levels of PyrF (panel 1), PyrG (panel 2) and Cmk (panel 3) in strains harboring the psdAb-TIR2 plasmid and sgRNA plasmids targeting *pyrF*, *pyrG*, *cmk* or an empty control vector. CRISPRi-induced strains are shown in dark grey and uninduced strains are shown in bright grey. Protein LFQ intensities are normalized to OD and were calculated as the average of three biological replicates. Standard deviations between replicates are shown as error bars. The proteome data set can be found in Supplementary File S1.

Supplementary Figure 3

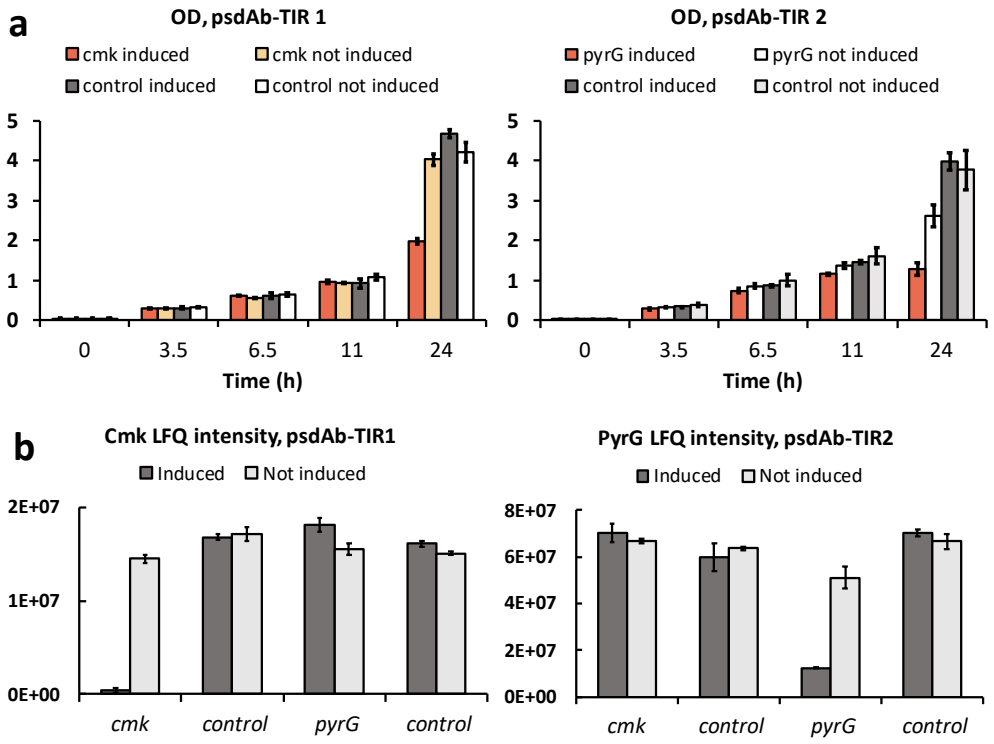


**Figure S3.** Growth and protein levels during production of sdAb in shake flask fermentation. (a) OD of CRISPRi-induced and not induced strains harboring psdAb-TIR1 and an sgRNA plasmid targeting *cmk* or an empty control vector (panel 1), and psdAb-TIR2 and an sgRNA plasmid targeting *pyrG* or an empty control vector (panel 2). CRISPRi-induced strains are shown in dark orange (*cmk* and *pyrG*) or dark grey (control), and uninduced strains are shown in bright orange (*cmk* and *pyrG*) or bright grey (control). (b) Protein levels of Cmk in strains harboring the psdAb-TIR1 plasmid and sgRNA plasmids targeting *cmk* or an empty control vector (panel 1). Protein levels of PyrG in strains harboring the psdAb-TIR2 plasmid and sgRNA plasmids targeting *pyrG* or an empty control vector (panel 2). CRISPRi-induced strains are shown in dark grey and uninduced strains are shown in bright grey. LFQ intensities are normalized to OD. The OD and protein LFQ intensities were calculated as the average of three biological replicates. Standard deviations between replicates are shown as error bars. The proteome data set can be found in Supplementary File S1.