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SUPPLEMENTARY INFORMATION

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Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform

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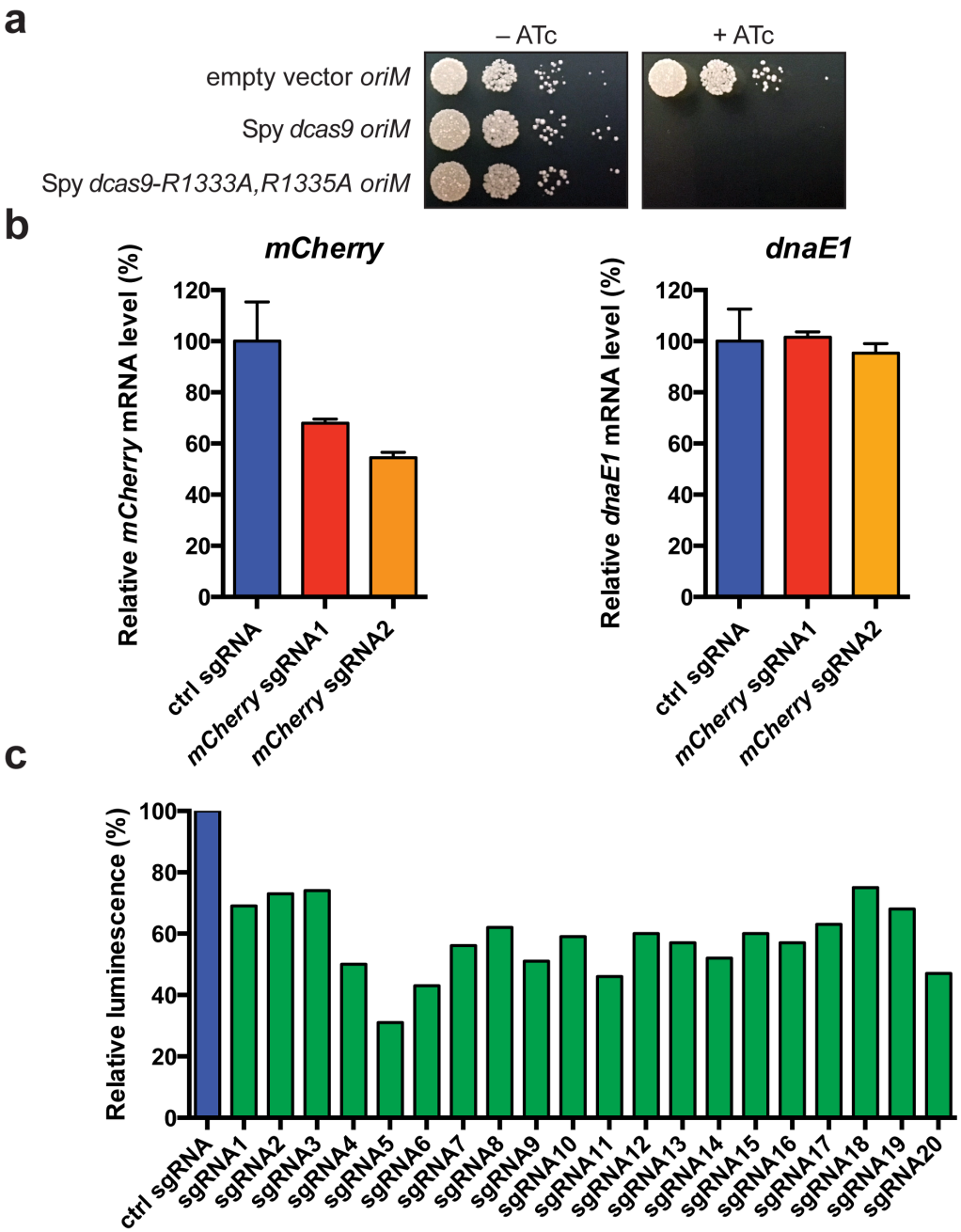
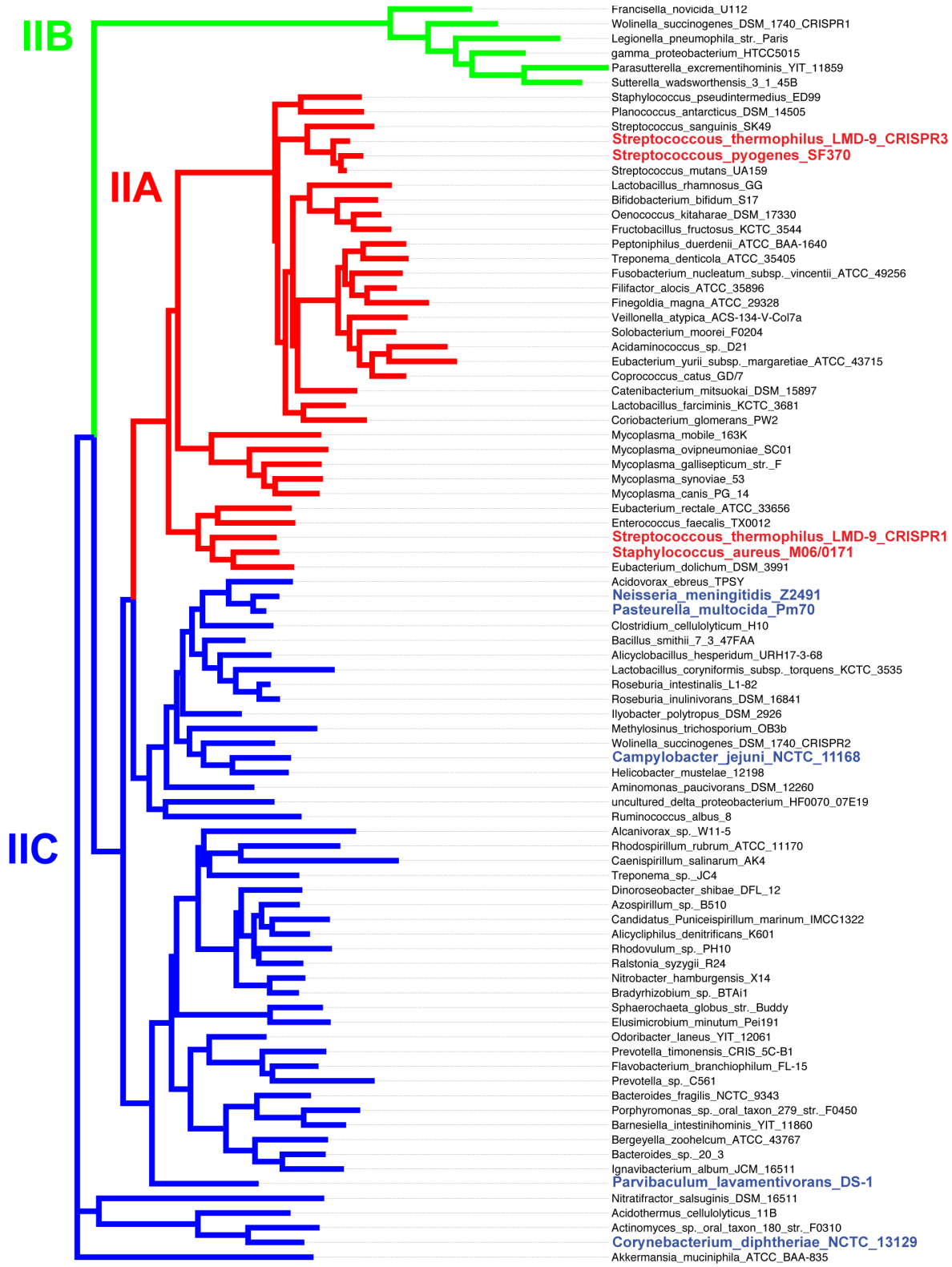
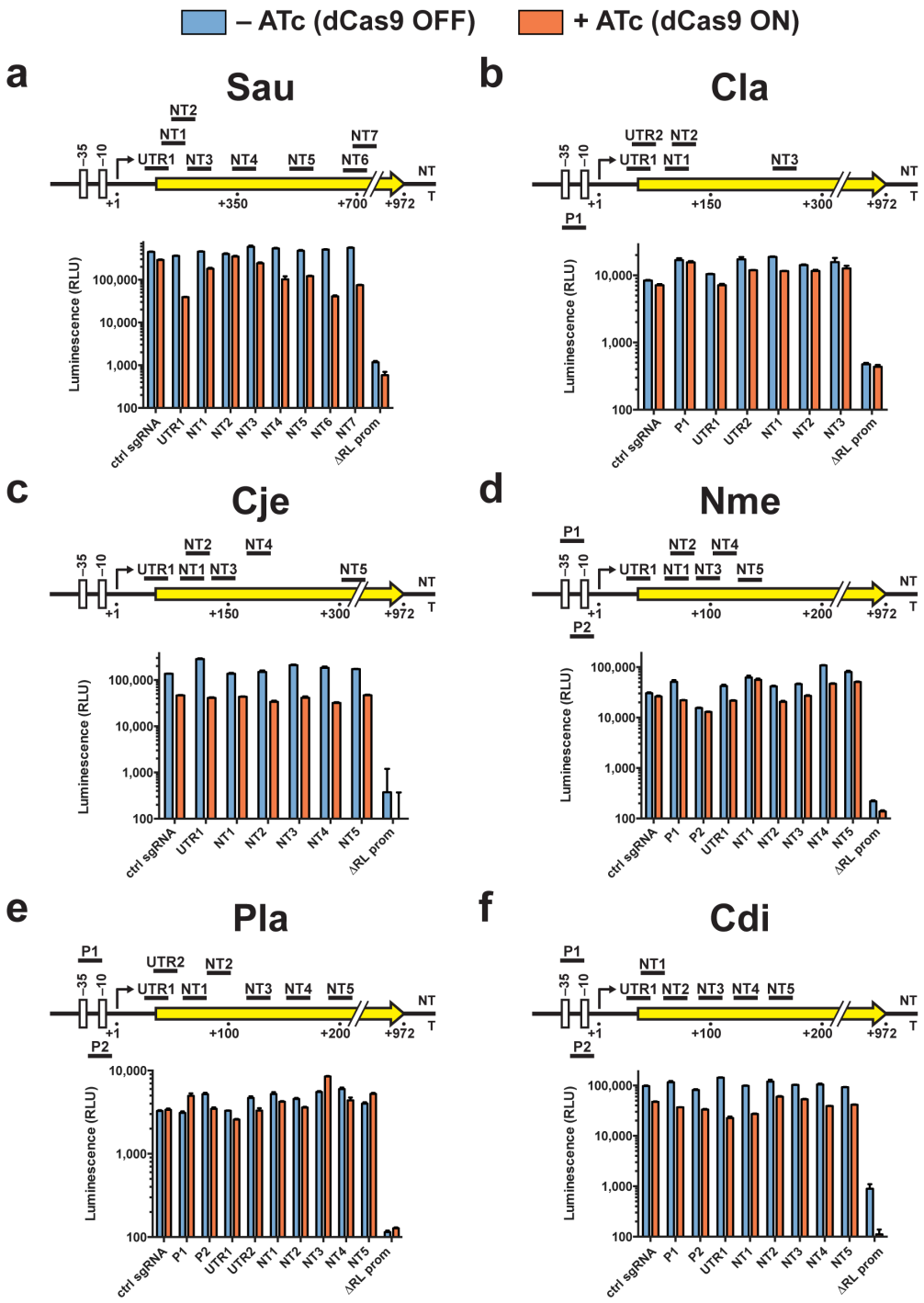


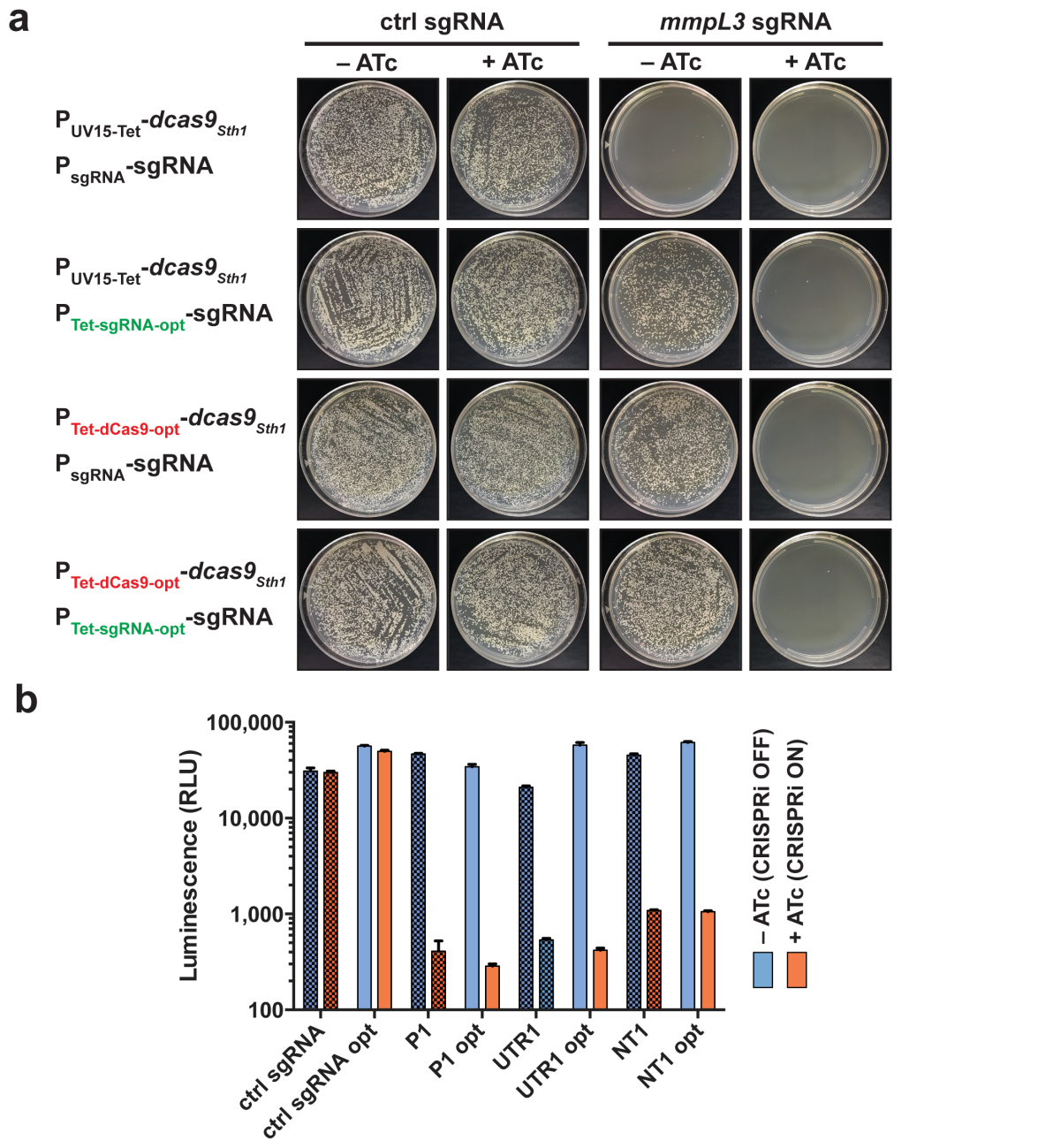
Supplementary Figure 1. First-generation dCas9$_{Spy}$ CRISPRi produces modest gene knockdown in mycobacteria. a, High-level dCas9$_{Spy}$ and the PAM-interacting defective dCas9$_{Spy}$–R1333A,R1335A expression is lethal in *M. smegmatis*. Strains harbor: 1) an episomal plasmid with a constitutively expressed non-targeting control sgRNA and dCas9$_{Spy}$ or dCas9$_{Spy}$–R1333A,R1335A under the control of an ATc-inducible promoter; and 2) an integrated copy of the Tet repressor. Serial dilutions of each culture were spotted on the indicated media (+ATc induces dCas9). Pictures are representative of three independent experiments. **b,** sgRNAs targeting *mCherry* were co-expressed with dCas9$_{Spy}$. Gene knockdown was quantified by qRT-PCR; quantification of a non-targeted gene, *dnaE1*, was performed to show specificity of gene silencing. Error bars are 95% confidence intervals of three technical replicates. **c,** sgRNAs targeting NGG PAMs were co-expressed with dCas9$_{Spy}$ and the effects on the target *Renilla* luciferase were measured by luciferase assay.



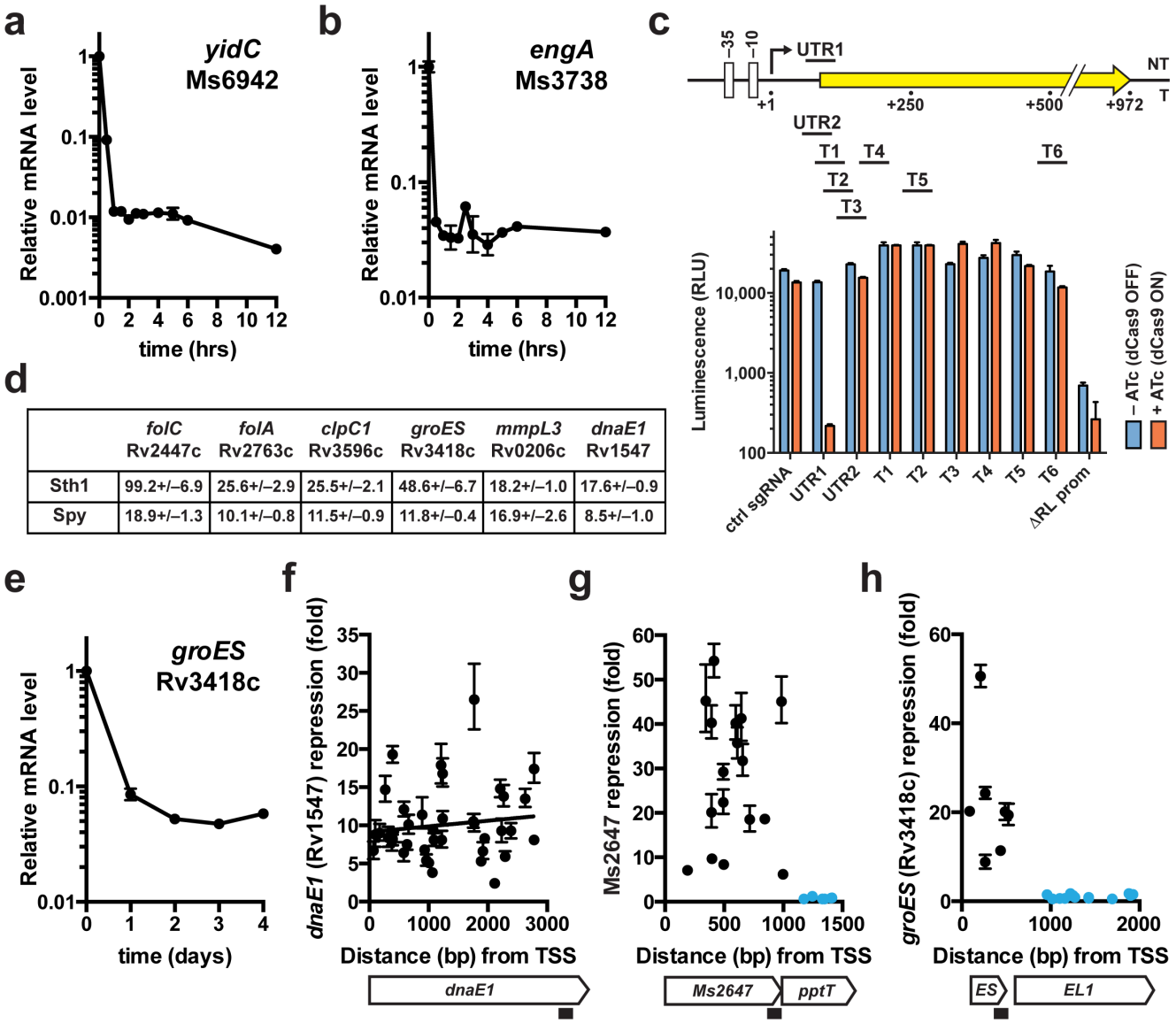
Supplementary Figure 2: Phylogeny of representative Cas9 orthologues. At this phylogenetic resolution, *Streptococcus pasteurianus* and *Campylobacter lari* Cas9 are subsumed into the *Streptococcus thermophilus* LMD9 CRISPR1 and *Campylobacter jejuni* NCTC 11168 clades, respectively, and are not shown.



Supplementary Figure 3: Type IIC Cas9 proteins are ineffective for gene silencing in mycobacteria. sgRNAs targeting NNGRRT PAMs (**a**, $dCas9_{Sau}$), NNGGG PAMs (**b**, $dCas9_{Cla}$), NNNNMCA PAMs (**c**, $dCas9_{Cje}$), NNNNGWW PAMs (**d**, $dCas9_{Nme}$), NNNCAT PAMs (**e**, $dCas9_{Pla}$), and NGG PAMs (**f**, $dCas9_{Cdi}$) were co-expressed with the indicated Cas9 protein and the effects on the *Renilla* target measured by luciferase assay. Shown above the graph is a schematic depicting the *Renilla* target (yellow arrow), transcriptional start site (black arrow), and the relative targeting position of each sgRNA (P = promoter; UTR = 5' untranslated region, NT = *Renilla* non-template strand). The ΔRL promoter sample depicts basal levels of luminescence. Error bars are standard deviations of three technical replicates.



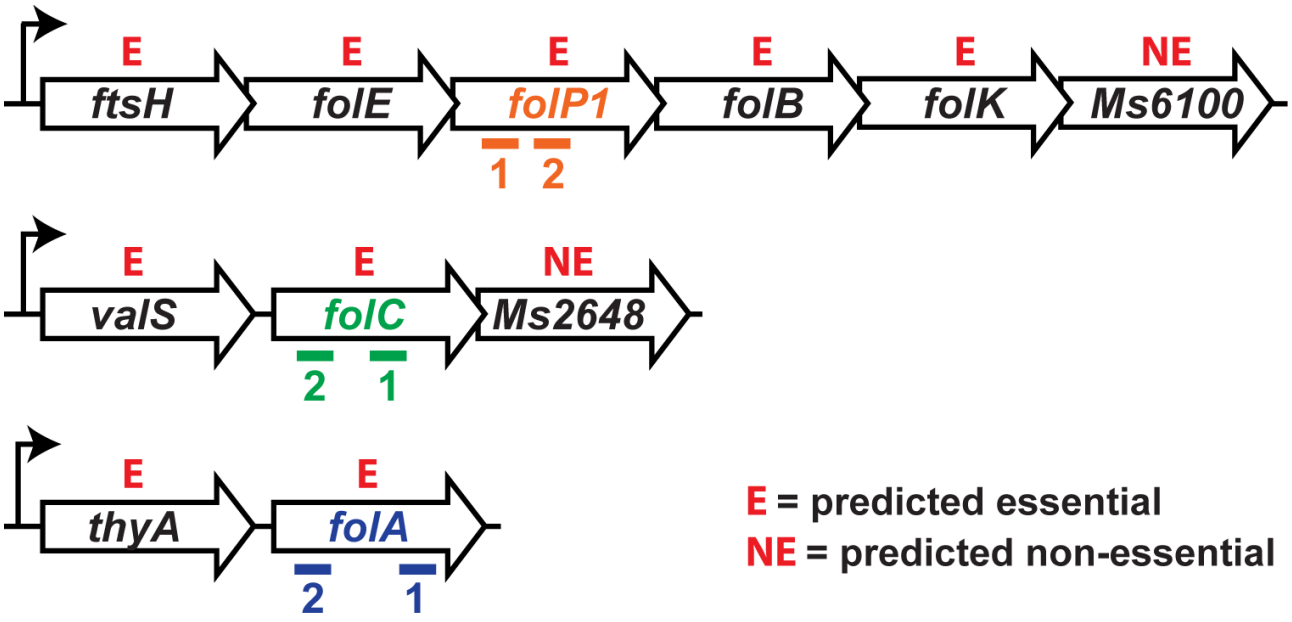
Supplementary Figure 4: Optimization of the *dcas9* and sgRNA promoters. a) Promoter optimization minimizes leaky expression. Plasmids co-expressing $dCas9_{Sth1}$ and a non-genome targeting or *mmpL3* (Ms0250) targeting sgRNA under the control of the indicated promoters were transformed into *M. smegmatis* and plated on the indicated media (+ATc induces CRISPRi). $P_{UV15\text{-}Tet}$: TetR-regulated *dcas9* promoter; $P_{Tet\text{-}dCas9\text{-}opt}$: optimized TetR-regulated *dcas9* promoter; P_{sgRNA}: constitutive promoter; $P_{Tet\text{-}sgRNA\text{-}opt}$: optimized TetR-regulated sgRNA promoter. Pictures are representative of three independent experiments. **b)** Promoter optimization reduces leaky expression without compromising high-level gene knockdown. sgRNAs targeting the *Renilla* reporter were co-expressed with $dCas9_{Sth1}$ (+ATc) and the effects were measured by luciferase assay. Hashed columns depict the original $dCas9_{Sth1}$ system as in Figure 1E; solid columns depict the optimized $dCas9_{Sth1}$ system using the optimized *dcas9* and sgRNA promoters from (**a**). Error bars are standard deviations of three technical replicates.



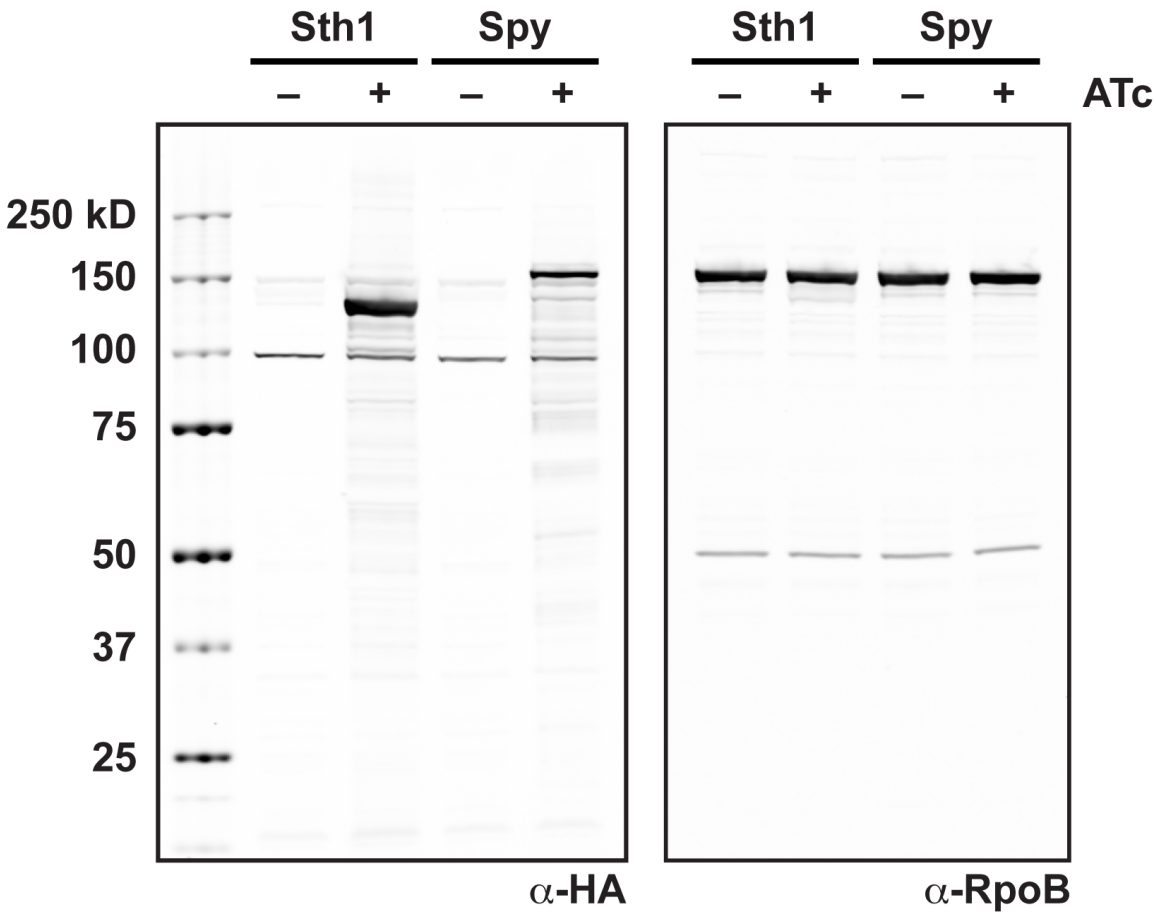
	folC Rv2447c	folA Rv2763c	clpC1 Rv3596c	groES Rv3418c	mmpL3 Rv0206c	dnaE1 Rv1547
Sth1	99.2+/–6.9	25.6+/–2.9	25.5+/–2.1	48.6+/–6.7	18.2+/–1.0	17.6+/–0.9
Spy	18.9+/–1.3	10.1+/–0.8	11.5+/–0.9	11.8+/–0.4	16.9+/–2.6	8.5+/–1.0

Supplementary Figure 5: Further characterization of CRISPRi in mycobacteria. a,b) $dCas9_{Sth1}$-mediated target knockdown is rapid in *M. smegmatis*. sgRNAs targeting *yidC* (Ms6942) or *engA* (Ms3738) were co-expressed with $dCas9_{Sth1}$, beginning at the 0 hour time point. Gene knockdown was quantified by qRT-PCR. **c)** $dCas9_{Sth1}$ blocks transcription elongation in a strand-specific manner. sgRNAs targeting the *Renilla* non-template DNA strand (UTR1; positive control) or template DNA strand (all others) were co-expressed with $dCas9_{Sth1}$ (+ATc) and the effects on the target *Renilla* luciferase measured by luciferase assay. **d)** $dCas9_{Sth1}$ achieves high-level knockdown of endogenous targets in *M. tuberculosis*. sgRNAs targeting the indicated genes were co-expressed with $dCas9_{Spy}$ or $dCas9_{Sth1}$ and gene knockdown was quantified by qRT-PCR (fold knockdown +/– 95% confidence interval is listed). **e)** $dCas9_{Sth1}$-mediated target knockdown is rapid in *M. tuberculosis*. An sgRNA targeting *groES* (Rv3418c) was co-expressed with $dCas9_{Sth1}$, beginning at the 0 day time point. Gene knockdown was quantified by qRT-PCR. **f)** $dCas9_{Sth1}$ achieves robust gene knockdown far from the transcriptional start site (TSS) in *M. tuberculosis*. sgRNAs targeting *dnaE1* (Rv1547) were co-expressed with $dCas9_{Sth1}$ and gene knockdown was quantified by qRT-PCR. The *dnaE1* gene is depicted at scale below the graph; the black bar beneath the *dnaE1* gene marks the site of the qPCR amplicon. **g)** $dCas9_{Sth1}$ mediates a minimal "upstream" polar effect in *M. smegmatis*. sgRNAs (from

Figure 4H) targeting the *pptT* operon (Ms2647-2648) were co-expressed with dCas9$_{Sth1}$. Knockdown of Ms2647 was quantified by qRT-PCR. The first two genes of the *pptT* gene operon are depicted at scale below the graph; the black bar beneath the Ms2647 gene marks the site of the qPCR amplicon. Blue dots mark sgRNAs causing little-to-no upstream polar effect. **h)** dCas9$_{Sth1}$ mediates a minimal "upstream" polar effect in *M. tuberculosis*. sgRNAs (from Figure 4I) targeting the *groES-groEL1* operon (Rv3417c-3418c) were co-expressed with dCas9$_{Sth1}$. Knockdown of *groES* was quantified by qRT-PCR. The *groES-groEL1* operon is depicted at scale below the graph; the black bar beneath the *groES* gene marks the site of the qPCR amplicon. Blue dots mark sgRNAs causing little to no upstream polar effect. Error bars are 95% confidence intervals of three technical replicates (**a,b,d-h**); error bars are standard deviations of three technical replicates (**c**).



Supplementary Figure 6: Putative operon structure of *M. smegmatis* folate biosynthesis genes. Operon predictions from the TB Database or primary data (see Methods) were used to predict syntenic operons in *M. smegmatis*. Line arrows indicate putative transcriptional start sites; colored lines beneath the gene names indicate the sites targeted by sgRNAs used in Figure 5 (1 = sgRNA1, 2 = sgRNA2). *M. smegmatis* gene essentiality predictions are based on essentiality predictions of homologous *M. tuberculosis* genes.



Supplementary Figure 7: Un-cropped western blot from Figure 2C.

Supplementary Table 1: dCas9, sgRNA scaffold, and reporter construct sequences

dCas9 sequences

***Streptococcus pyogenes* dCas9 D10A H840A**

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARR
RYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRK
KLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAI
LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQI
GDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD
QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL
RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFI
ERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV
KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIE
ERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSL
TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQK
GQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAI
VPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLS
ELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLI
ARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF
VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTI
DRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

***Streptococcus thermophilus* CRISPR3 dCas9 D10A H847A**

MTKPYSIGLAIGTNSVGWAVTTDNYKVPSKKMKVLGNTSKKYIKKNLLGVLLFDSGITAEGRRLKRTAR
RRYTRRRNRILYLQEIFSTEMATLDDAFFQRLDDSFLVPDDKRDSKYPIFGNLVEEKAYHDEFPTIYHLR
KYLADSTKKADLRLVYLALAHMIKYRGHFLIEGEFNSKNNDIQKNFQDFLDTYNAIFESDLSLENSKQLE
EIVKDKISKLEKKDRILKLFPGEKNSGIFSEFLKLIVGNQADFRKCFNLDEKASLHFSKESYDEDLETLLG
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DDTKNGYAGYIDGKTNQEDFYVYLKKLLAEFEGADYFLEKIDREDFLRKQRTFDNGSIPYQIHLQEMRAI
LDKQAKFYPFLAKNKERIEKILTFRIPYYVGPLARGNSDFAWSIRKRNEKITPWNFEDVIDKESSAEAFIN
RMTSFDLYLPEEKVLPKHSLLYETFNVYNELTKVRFIAESMRDYQFLDSKQKKDIVRLYFKDKRKVTDK
DIIEYLHAIYGYDGIELKGIEKQFNSSLSTYHDLLNIINDKEFLDDSSNEAIIEEIIHTLTIFEDREMIKQRLSK
FENIFDKSVLKKLSRRHYTGWGKLSAKLINGIRDEKSGNTILDYLIDDGISNRNFMQLIHDDALSFKKKIQ
KAQIIGDEDKGNIKEVVKSLPGSPAIKKGILQSIKIVDELVKVMGGRKPESIVVEMARENQYTNQGKSNS
QQRLKRLEKSLKELGSKILKENIPAKLSKIDNNALQNDRLYLYYLQNGKDMYTGDDLDIDRLSNYDIDAII
PQAFLKDNSIDNKVLVSSASNRGKSDDVPSLEVVKKRKTFWYQLLKSKLISQRKFDNLTKAERGGLSPE
DKAGFIQRQLVETRQITKHVARLLDEKFNNKKDENNRAVRTVKIITLKSTLVSQFRKDFELYKVREINDFH
HAHDAYLNAVVASALLKKYPKLEPEFVYGDYPKYNSFRERKSATEKVYFYSNIMNIFKKSISLADGRVIE
RPLIEVNEETGESVWNKESDLATVRRVLSYPQVNVVKKVEEQNHGLDRGKPKGLFNANLSSKPKPNS
NENLVGAKEYLDPKKYGGYAGISNSFTVLVKGTIEKGAKKKITNVLEFQGISILDRINYRKDKLNFLLEKG
YKDIELIIELPKYSLFELSDGSRRMLASILSTNNKRGEIHKGNQIFLSQKFVKLLYHAKRISNTINENHRKY
VENHKKEFEELFYYILEFNENYVGAKKNGKLLNSAFQSWQNHSIDELCSSFIGPTGSERKGLFELTSRG
SAADFEFLGVKIPRYRDYTPSSLLKDATLIHQSVTGLYETRIDLAKLGEG

***Staphylococcus aureus* dCas9 D10A H557A**
MKRNYILGLAIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQRVKKL
LFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVEEDTGNELSTKEQIS
RNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRR
TYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEY
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AKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLV
PKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKR
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PNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYG
DEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYL
DNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLL
NRIEVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG

***Streptococcus thermophilus* CRISPR1 dCas9 D9A H599A**
MSDLVLGLAIGIGSVGVGILNKVTGEIIHKNSRIFPAAQAENNLVRRTNRQGRRLARRKKHRRVRLNRLF
EESGLITDFTKISINLNPYQLRVKGLTDELSNEELFIALKNMVKHRGISYLDDASDDGNSSVGDYAQIVKE
NSKQLETKTPGQIQLERYQTYGQLRGDFTVEKDGKKHRLINVFPTSAYRSEALRILQTQQEFNPQITDE
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LNDLNNLTVPTETKKLSKEQKNQIINYVKNEKAMGPAKLFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFE
AYRKMKTLETLDIEQMDRETLDKLAYVLTLNTEREGIQEALEHEFADGSFSQKQVDELVQFRKANSSIF
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KIVNAAIKEYGDFDNIVIEMARETNEDDEKKAIQKIQKANKDEKDAAMLKAANQYNGKAELPHSVFHGH
KQLATKIRLWHQQGERCLYTGKTISIHDLINNSNQFEVDAILPLSITFDDSLANKVLVYATANQEKGQRTP
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EHFRAHKIDTKVSVVRGQFTSQLRRHWGIEKTRDTYHHHAVDALIIAASSQLNLWKKQKNTLVSYSEDQ
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FLKYKEEHGYIRKYSKKGNGPEIKSLKYYDSKLGNHIDITPKDSNNKVVLQSVSPWRADVYFNKTTGKY
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MPKQKHYVELKPYDKQKFEGGEALIKVLGNVANSGQCKKGLGKSNISIYKVRTDVLGNQHIIKNEGDKP
KLDF

***Streptococcus pasteurianus* dCas9 D10A H599A**
MTNGKILGLAIGIASVGVGIIEAKTGKVVHANSRLFSAANAENNAERRGFRGSRRLNRRKKHRVKRVRD
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KYELMGLKYSDLSFEKGTGNYHISQEKYDAIKEKEGIGKKSEFKFTLYRNDLILIKDIASGEQEIYRFLSRT
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KLDFKNNKK

***Campylobacter lari* dCas9 D7A H567A**
MRILGFAIGINSIGWAFVENDELKDCGVRIFTKAENPKNKESLALPRRNARSSRRRLKRRKARLIAIKRIL
AKELKLNYKDYVAADGELPKAYEGSLASVYELRYKALTQNLETKDLARVILHIAKHRGYMNKNEKKSND
AKKGKILSALKNNALKLENYQSVGEYFYKEFFQKYKKNTKNFIKIRNTKDNYNNCVLSSDLEKELKLILEK
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SGEIVPTQTINEVLNLILDKGSITYKKFRSCINLHESISFKSLKYDKENAENAKLIDFRKLVEFKKALGVHSL
SRQELDQISTHITLIKDNVKLKTVLEKYNLSNEQINNLLEIEFNDYINLSFKALGMILPLMREGKRYDEACE
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FISRNLNDTRYIATLIAKYTKEYLNFLLLSENENANLKSGEKGSKIHVQTISGMLTSVLRHTWGFDKKDRN
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AIPIYAMDFALGILPNKIVITGKDKNNNPKQWQTIDESYEFCFSLYKNDLILLQKKNMQEPEFAYYNDFSI
STSSICVEKHDNKFENLTSNQKLLFSNAKEGSVKVESLGIQNLKVFEKYIITPLGDKIKADFQPRENISLKT
SKKYGLR

***Campylobacter jejuni* dCas9 D8A H559A**
MARILAFAIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSARKRLARRKARLNHLKH
LIANEFKLNYEDYQSFDESLAKAYKGSLISPYELRFRALNELLSKQDFARVILHIAKRRGYDDIKNSDDKE
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NNHLHHAIDAVIIAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLDKIDEI
FVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNGKIVKNGDMFRVDIFKHKKTN
KFYAVPIYTMDFALKVLPNKAVARSKKGEIKDWILMDENYEFCFSLYKDSLILIQTKDMQEPEFVYYNAF
TSSTVSLIVSKHDNKFETLSKNQKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK

***Neisseria meningitidis* dCas9 D16A H588A**
MAAFKPNPINYILGLAIGIASVGWAMVEIDEDENPICLIDLGVRVFERAEVPKTGDSLAMARRLARSVRR
LTRRRAHRLLRARRLLKREGVLQAADFDENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRG
YLSQRKNEGETADKELGALLKGVADNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKD
LQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKNTYTAER
FIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEAS
TLMEMKAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISF
DKFVQISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVI
NGVVRRYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKLR
LYEQQHGKCLYSGKEINLGRLNEKGYVEIDAALPFSRTWDDSFNNKVLVLGSENQNKGNQTPYEYFN
GKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKG
KKRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTID
KETGEVLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTP
LFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPLTQLKLKDLEKMVNREREPKLYEALKARLEA

HKDDPAKAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEKGDKYY
LVPIYSWQVAKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFGYFASCHRGTG
NINIRIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIRPCRLKKRPPVR

***Pasteurella multocida* dCas9 D13A H580A**
MQTTNLSYILGLALGIASVGWAVVEINENEDPIGLIDVGVRIFERAEVPKTGESLALSRRLARSTRRLIRR
RAHRLLLAKRFLKREGILSTIDLEKGLPNQAWELRVAGLERRLSAIEWGAVLLHLIKHRGYLSKRKNESQ
TNNKELGALLSGVAQNHQLLQSDDYRTPAELALKKFAKEEGHIRNQRGAYTHTFNRLDLLAELNLLFAQ
QHQFGNPHCKEHIQQYMTELLMWQKPALSGEAILKMLGKCTHEKNEFKAAKHTYSAERFVWLTKLNN
LRILEDGAERALNEEERQLLINHPYEKSKLTYAQVRKLLGLSEQAIFKHLRYSKENAESATFMELKAWHA
IRKALENQGLKDTWQDLAKKPDLLDEIGTAFSLYKTDEDIQQYLTNKVPNSVINALLVSLNFDKFIELSLK
SLRKILPLMEQGKRYDQACREIYGHHYGEANQKTSQLLPAIPAQEIRNPVVLRTLSQARKVINAIIRQYG
SPARVHIETGRELGKSFKERREIQKQQEDNRTKRESAVQKFKELFSDFSSEPKSKDILKFRLYEQQHGK
CLYSGKEINIHRLNEKGYVEIDAALPFSRTWDDSFNNKVLVLASENQNKGNQTPYEWLQGKINSERWK
NFVALVLGSQCSAAKKQRLLTQVIDDNKFIDRNLNDTRYIARFLSNYIQENLLLVGKNKKNVFTPNGQIT
ALLRSRWGLIKARENNNRHHALDAIVVACATPSMQQKITRFIRFKEVHPYKIENRYEMVDQESGEIISPH
FPEPWAYFRQEVNIRVFDNHPDTVLKEMLPDRPQANHQFVQPLFVSRAPTRKMSGQGHMETIKSAKR
LAEGISVLRIPLTQLKPNLLENMVNKEREPALYAGLKARLAEFNQDPAKAFATPFYKQGGQQVKAIRVE
QVQKSGVLVRENNGVADNASIVRTDVFIKNNKFFLVPIYTWQVAKGILPNKAIVAHKNEDEWEEMDEGA
KFKFSLFPNDLVELKTKKEYFFGYYIGLDRATGNISLKEHDGEISKGKDGVYRVGVKLALSFEKYQVDEL
GKNRQICRPQQRQPVR

***Parvibaculum lavamentivorans* dCas9 D8A H601A**
MERIFGFAIGTTSIGFSVIDYSSTQSAGNIQRLGVRIFPEARDPDGTPLNQQRRQKRMMRRQLRRRRIR
RKALNETLHEAGFLPAYGSADWPVVMADEPYELRRRGLEEGLSAYEFGRAIYHLAQHRHFKGRELEE
SDTPDPDVDDEKEAANERAATLKALKNEQTTLGAWLARRPPSDRKRGIHAHRNVVAEEFERLWEVQS
KFHPALKSEEMRARISDTIFAQRPVFWRKNTLGECRFMPGEPLCPKGSWLSQQRRMLEKLNNLAIAGG
NARPLDAEERDAILSKLQQQASMSWPGVRSALKALYKQRGEPGAEKSLKFNLELGGESKLLGNALEAK
LADMFGPDWPAHPRKQEIRHAVHERLWAADYGETPDKKRVIILSEKDRKAHREAAANSFVADFGITGE
QAAQLQALKLPTGWEPYSIPALNLFLAELEKGERFGALVNGPDWEGWRRTNFPHRNQPTGEILDKLPS
PASKEERERISQLRNPTVVRTQNELRKVVNNLIGLYGKPDRIRIEVGRDVGKSKREREEIQSGIRRNEK
QRKKATEDLIKNGIANPSRDDVEKWILWKEGQERCPYTGDQIGFNALFREGRYEVEAIWPRSRSFDNS
PRNKTLCRKDVNIEKGNRMPFEAFGHDEDRWSAIQIRLQGMVSAKGGTGMSPGKVKRFLAKTMPEDF
AARQLNDTRYAAKQILAQLKRLWPDMGPEAPVKVEAVTGQVTAQLRKLWTLNNILADDGEKTRADHR
HHAIDALTVACTHPGMTNKLSRYWQLRDDPRAEKPALTPPWDTIRADAEKAVSEIVVSHRVRKKVSGP
LHKETTYGDTGTDIKTKSGTYRQFVTRKKIESLSKGELDEIRDPRIKEIVAAHVAGRGGDPKKAFPPYPC
VSPGGPEIRKVRLTSKQQLNLMAQTGNGYADLGSNHHIAIYRLPDGKADFEIVSLFDASRRLAQRNPIV
QRTRADGASFVMSLAAGEAIMIPEGSKKGIWIVQGVWASGQVVLERDTDADHSTTTRPMPNPILKDDA
KKVSIDPIGRVRPSND

***Corynebacter diphtheria* dCas9 D8A H573A**
MKYHVGIAVGTFSVGLAAIEVDDAGMPIKTLSLVSHIHDSGLDPDEIKSAVTRLASSGIARRTRRLYRRK
RRRLQQLDKFIQRQGWPVIELEDYSDPLYPWKVRAELAASYIADEKERGEKLSVALRHIARHRGWRNP
YAKVSSLYLPDGPSDAFKAIREEIKRASGQPVPETATVGQMVTLCELGTLKLRGEGGVLSARLQQSDY
AREIQEICRMQEIGQELYRKIIDVVFAAESPKGSASSRVGKDPLQPGKNRALKASDAFQRYRIAALIGNL
RVRVDGEKRILSVEEKNLVFDHLVNLTPKKEPEWVTIAEILGIDRGQLIGTATMTDDGERAGARPPTHDT
NRSIVNSRIAPLVDWWKTASALEQHAMVKALSNAEVDDFDSPEGAKVQAFFADLDDDVHAKLDSLHLP
VGRAAYSEDTLVRLTRRMLSDGVDLYTARLQEFGIEPSWTPPTPRIGEPVGNPAVDRVLKTVSRWLES
ATKTWGAPERVIIEHVREGFVTEKRAREMDGDMRRRAARNAKLFQEMQEKLNVQGKPSRADLWRYQ
SVQRQNCQCAYCGSPITFSNSEMDAIVPRAGQGSTNTRENLVAVCHRCNQSKGNTPFAIWAKNTSIE
GVSVKEAVERTRHWVTDTGMRSTDFKKFTKAVVERFQRATMDEEIDARSMESVAWMANELRSRVAQ

HFASHGTTVRVYRGSLTAEARRASGISGKLKFFDGVGKSRLDRRHHAIDAAVIAFTSDYVAETLAVRSN
LKQSQAHRQEAPQWREFTGKDAEHRAAWRVWCQKMEKLSALLTEDLRDDRVVVMSNVRLRLGNGS
AHKETIGKLSKVKLSSQLSVSDIDKASSEALWCALTREPGFDPKEGLPANPERHIRVNGTHVYAGDNIG
LFPVSAGSIALRGGYAELGSSFHHARVYKITSGKKPAFAMLRVYTIDLLPYRNQDLFSVELKPQTMSMR
QAEKKLRDALATGNAEYLGWLVVDDELVVDTSKIATDQVKAVEAELGTIRRWRVDGFFSPSKLRLRPL
QMSKEGIKKESAPELSKIIDRPGWLPAVNKLFSDGNVTVVRRDSLGRVRLESTAHLPVTWKVQ

dCas9 promoters

rrnB-UP element
–35
–10
tet operator
transcriptional start site
ribosome binding site
start codon

$P_{UV15\text{-}Tet}$ (original *dcas9* promoter; TetR-regulated)
AGAAATATTGGATCGTCGGCACCGTCACGGCCGTGGGAGGCGGCACGATCCGCGACGTGATGAT
CGGCCGCATCCCCACGGTGCTGCGCAGTGAGCTCTACGCCATCCCGGCGTTGATCTGTGCGTTC
GCACGCACAGGCCCGGTGTGAGAAGGGTCTCTGACGAGCGGGAGAACTCCCTATCAGTGATAGA
GTTTTGTCCTCCCTATCAGTGATAGATAGGCTCTGGGAGCTGATCGTACTGACTCAGGAGGTATCG
ATATG

$P_{Tet\text{-}dCas9\text{-}opt}$ (optimized *dcas9* promoter; TetR-regulated)
TCTGACCAGGGAAAATAGCCCTCTGACCTGGGGATTTGACTTCCCTATCAGTGATAGAGATAATCT
GGGAGTCCCTATCAGTGATAGAGAAGGCGGTATCGATATG

sgRNA scaffold sequences

crRNA-derived
tracrRNA-derived

Streptococcus pyogenes
5'-N(20-25)GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAA AGUGGCACCGAGUCGGUGCUUUU-3'

***Streptococcus thermophilus* CRISPR3**
5'-(N20-N25)GUUUUAGAGCUGUGAAAACAGCGAGUUAAAAUAAGGCUUAGUCCGUACUCAACUU GAAAAGGUGGCACCGAUUCGGUGUUUU-3'

Staphylococcus aureus
5'-N(20-25)GUUUUAGUACUCUGGAAACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUUAUCU CGUCAACUUGUUGGCGAGAUUUU-3'

***Streptococcus thermophilus* CRISPR1**
5'-(N20-25)GUUUUUGUACUCGAAAGAAGCUACAAAGAUAAGGCUUCAUGCCGAAAUCAACACCCU GUCAUUUUAUGGCAGGGUGUUUU-3'

Streptococcus pasteurianus
5'-(N20-25)GUUUUUGUACUCGAAAGAGCCUACAAAGAUAAGGCUUUAUGCCGAAUUCAAGCACC CCAUGUUUUGACAUGAGGUGCUUUU-3'

Campylobacter lari
5'-(N20-25)GUUUUAGUCUCUGAAAAGAGACUAAAAUAAGUGGUUUUUGGUCAUCCACGCAGGGU UACAAUCCCUUUAAAACCAUUAAAAUUCAAAUAAACUAGGUUGUAUCAACUUAGUUUU-3'

Campylobacter jejuni
5'-(N20-N25)GUUUUAGUCCCUGAAAAGGGACUAAAAUAAAGAGUUUGCGGGACUCUGCGGGGUU ACAAUCCCCUAAAACCGCUUUU-3'

Neisseria meningitidis
5'-(N20-N25)GUUGUAGCUCCCUUUCUCGAAAGAGAACCGUUGCUACAAUAAGGCCGUCUGAAAA GAUGUGCCGCAACGCUCUGCCCCUUAAAGCUUCUGCUUUAAGGGGCUUUU-3'

Pasteurella multocida
5'-(N20-N25)GUUGUAGUUCCCUCUCUGAAAAGAGACGUUGCUACAAUAAGGCUUCUGAAAAGAA UGACCGUAACGCUCUGCCCCUUGUGAUUCUUAAUUGCAAGGGGCAUCGUUUU-3'

Parvibaculum lavamentivorans
5'-(N20-N25)GCUGCGGAUUGCGGGAAAUCGCUUUUCGCAAGCAAAUUGACCCCUUGUGCGGGC UCGGCAUCCCAAGGUCAGCUGCCGGUUAUUAUCGAAAAGGCCCACCGCAAGCAGCGCGUGGGC CUUUU-3'

Corynebacter diphtheria
5'-(N20-N25)ACUGGGGUUCAGGAAACUGAACCUCAGUAAGCAUUGGCUCGUUUCCAAUGUUGAU UGCUCCGCCGGUGCUCCUUAUUUUUAAGGGCGCCGGCUUUU-3'

Synthetic sgRNA promoters

rrnB-UP element
–35
–10
transcriptional start site
tet operator

P_{sgRNA} (constitutive promoter)
TCTGACCAGGGAAAATAGCCCTCTGACCTGGGGATTTGCGATGCGTGTGCTCGGTTGATATAATC
TGGGAG

$P_{Tet\text{-}sgRNA\text{-}opt}$ (optimized sgRNA promoter; TetR-regulated)
TCTGACCAGGGAAAATAGCCCTCTGACCTGGGGATTTGCGATCCCTATCAGTGATAGATATAATCT
GGGAG

Reporter sequences

For all figures excluding Supplemental Figure 1:

The G13 promoter and *Renilla* coding sequence are identical for all dCas9 orthologues tested. The *Renilla* 5'UTR was engineered such that the optimal PAM for each dCas9 protein could be tested with an identical sgRNA targeting sequence (UTR1) for all dCas9 proteins. This results in a unique 8 base-pair sequence (depicted below) within the 5'UTR for each dCas9 orthologue tested.

G13 promoter
5'-GATCGCCACTAGCGCCGCGGTCGGAATCAGCGCACTGGCCGCCGGAGCGGTCAGCTACCTGT
TGCTGCGCCGCTTCGCTTTCTAGCACCGACGTCCCGGCGCCAGCAGCTACCTCTCGAGCAAACG
TTTTGCGCGCCCGAAAAATAAGTCGTTGCAGAACTTTCATGAATTAGGCCTTGCTGCGCCCAGGCT
CCAGTAGTAGAAATGGAGTCA-3'

transcriptional start site

***Renilla* luciferase coding sequence**
5'-ATGGCTTCCAAGGTGTACGACCCCGAGCAACGCAAACGCATGATCACTGGGCCTCAGTGGTGG
GCTCGCTGCAAGCAAATGAACGTGCTGGACTCCTTCATCAACTACTATGATTCCGAGAAGCACGC
CGAGAACGCCGTGATTTTTCTGCATGGTAACGCTGCCTCCAGCTACCTGTGGAGGCACGTCGTGC
CTCACATCGAGCCCGTGGCTAGATGCATCATCCCTGATCTGATTGGAATGGGTAAGTCCGGCAAG
AGCGGGAATGGCTCATATCGCCTCCTGGATCACTACAAGTACCTCACCGCTTGGTTCGAGCTGCT
GAACCTTCCAAAGAAAATCATCTTTGTGGGCCACGACTGGGGGGCTTGTCTGGCCTTTCACTACTC
CTACGAGCACCAAGACAAGATCAAGGCCATCGTCCATGCTGAGAGTGTCGTGGACGTGATCGAGT
CCTGGGACGAGTGGCCTGACATCGAGGAGGATATCGCCCTGATCAAGAGCGAAGAGGGCGAGAA
AATGGTGCTTGAGAATAACTTCTTCGTCGAGACCATGCTCCCAAGCAAGATCATGCGGAAACTGGA
GCCTGAGGAGTTCGCTGCCTACCTGGAGCCATTCAAGGAGAAGGGCGAGGTTAGACGGCCTACC
CTCTCCTGGCCTCGCGAGATCCCTCTCGTTAAGGGAGGCAAGCCCGACGTCGTCCAGATTGTCC
GCAACTACAACGCCTACCTTCGGGCCAGCGACGATCTGCCTAAGATGTTCATCGAGTCCGACCCT
GGGTTCTTTTCCAACGCTATTGTCGAGGGAGCTAAGAAGTTCCCTAACACCGAGTTCGTGAAGGT
GAAGGGCCTCCACTTCAGCCAGGAGGACGCTCCAGATGAAATGGGTAAGTACATCAAGAGCTTCG
TGGAGCGCGTGCTGAAGAACGAGCAGTAA-3'

5'UTR sequences

PAM reverse complement highlighted in red; ribosome binding site highlighted in blue

Streptococcus pyogenes
5'-GCTCTTCAGGACTTGCTCCCCCAGGAGGTATCTCC-3'

***Streptococcus thermophilus* CRISPR3**
5'-GCTCTTCAGGACCAACACCACCAGGAGGTATCTCC-3'

Staphylococcus aureus
5'-GCTCTTCAGGACTGACCCGACCAGGAGGTATCTCC-3'

***Streptococcus thermophilus* CRISPR1**
5'-GCTCTTCAGGACAATTCTCACCAGGAGGTATCTCC-3'

Streptococcus pasteurianus
5'-GCTCTTCAGGACTGTCACGACCAGGAGGTATCTCC-3'

Campylobacter lari
5'-GCTCTTCAGGACATACCCCACCAGGAGGTATCTCC-3'

Campylobacter jejuni
5'-GCTCTTCAGGACCTGTTTCTCCAGGAGGTATCTCC-3'

Neisseria meningitidis
5'-GCTCTTCAGGACAATCCTGACCAGGAGGTATCTCC-3'

Pasteurella multocida
5'-GCTCTTCAGGACTACGATCCCCAGGAGGTATCTCC-3'

Parvibaculum lavamentivorans
5'-GCTCTTCAGGACTCATGCGTCCAGGAGGTATCTCC-3'

Corynebacter diphtheria
5'-GCTCTTCAGGACTATTACCCCCAGGAGGTATCTCC-3'

Supplemental Figure 1 reporter sequences:

P_{hsp60}-5'UTR-mCherry-6His (Giles integrating)
GGTGACCACAACGCGCCCGCTTTGATCGGGGACGTCTGCGGCCGACCATTTACGGGTCTTGTTG
TCGTTGGCGGTCATGGGCCGAACATACTCACCCGGATCGGAGGGCCGAGGACAAGGTCGAACGA
GGGGCATGACCCGGTGCGGGGCTTCTTGCACTCGGCATAGGCGAGTGCTAAGAATAACGTTGGC
ACTCGCGACCGGTGAGTgcTAGGTCGGGACGGTGAGGCCAGGCCCGTCGTCGCAGCGAGTGGCA
GCGAGGACAACTTGAGCCGTCCGTCGCGGGCACTGCGCCCGGCCAGCGTAAGTAGCGGGGTTG
CCGTCACCCGGTGACCCCCGTTTCATCCCCGATCCGGAGGAATCACTTCGCAATGGCCAGCGATA
GCACTGAGAGCGGCTCCACCGAGTCCGTCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGA
GGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGG
CACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTG
TCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTT
GAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTG
GTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCG
GCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCTC
CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAA
GGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTG
CCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGT
GGAACAGTACGAACGCGCCGAGGGCCGCCACTCCGGCTCCCAGGGATCTAGCGGATCCAGTTGT
CATCACCATCACCATCACTGA

P_{G13}-5'UTR-Renilla luciferase (Giles integrating)
GATCGCCACTAGCGCCGCGGTCGGAATCAGCGCACTGGCCGCCGGAGCGGTCAGCTACCTGTTG
CTGCGCCGCTTCGCTTTCTAGCACCGACGTCCCGGCGCCAGCAGCTACCTCTCGAGCAAACGTTT
TGCGCGCCCGAAAAATAAGTCGTTGCAGAACTTTCATGAATTAGGCCTTGCTGCGCCCAGGCTCC
AGTAGTAGAAATGGAGTCACGGCAGCCCGGTGAAGCCGAGGTCGAACCGGAAGAGAAGGTTCGT
CCTCCCGACCCGGGCACCCAGCACGGCCCCCGGAACCCACGCGGAGTCATAGCCGCGATAATG
GCAGAAGTGTTGCGGGCCTGCGTAATTGCGAAATTCAGATGGTGCCGACGGCCCTTTGGGTGGG

GCTGCAGCCAGAAGGGTCGCAAAAGCGCCGAGGCCAACCCACGCAGCCcACAAATGCACGCTTG
GTAACCGAGGATCCAGGAGGTATCTCCATGGCTTCCAAGGTGTACGACCCCGAGCAACGCAAAC
GCATGATCACTGGGCCTCAGTGGTGGGCTCGCTGCAAGCAAATGAACGTGCTGGACTCCTTCATC
AACTACTATGATTCCGAGAAGCACGCCGAGAACGCCGTGATTTTTCTGCATGGTAACGCTGCCTC
CAGCTACCTGTGGAGGCACGTCGTGCCTCACATCGAGCCCGTGGCTAGATGCATCATCCCTGATC
TGATtGGAATGGGTAAGTCCGGCAAGAGCGGGAATGGCTCATATCGCCTCCTGGATCACTACAAG
TACCTCACCGCTTGGTTCGAGCTGCTGAACCTTCCAAAGAAAATCATCTTTGTGGGCCACGACTGG
GGGGCTTGTCTGGCCTTTCACTACTCCTACGAGCACCAAGACAAGATCAAGGCCATCGTCCATGC
TGAGAGTGTCGTGGACGTGATCGAGTCCTGGGACGAGTGGCCTGACATCGAGGAGGATATCGCC
CTGATCAAGAGCGAAGAGGGCGAGAAAATGGTGCTTGAGAATAACTTCTTCGTCGAGACCATGCT
CCCAAGCAAGATCATGCGGAAACTGGAGCCTGAGGAGTTCGCTGCCTACCTGGAGCCATTCAAG
GAGAAGGGCGAGGTTAGACGGCCTACCCTCTCCTGGCCTCGCGAGATCCCTCTCGTTAAGGGAG
GCAAGCCCGACGTCGTCCAGATTGTCCGCAACTACAACGCCTACCTTCGGGCCAGCGACGATCT
GCCTAAGATGTTCATCGAGTCCGACCCTGGGTTCTTTTCCAACGCTATTGTCGAGGGAGCTAAGAA
GTTCCCTAACACCGAGTTCGTGAAGGTGAAGGGCCTCCACTTCAGCCAGGAGGACGCTCCAGAT
GAAATGGGTAAGTACATCAAGAGCTTCGTGGAGCGCGTGCTGAAGAACGAGCAGTAA

Supplementary Table 2: sgRNAs and targets used for Cas9 orthologue testing

Cas9 protein	sgRNA targeting sequence (5'–3')	sgRNA name	PAM (5'–3')	Gene	Figure	Fold KD*
All dCas9 proteins	GGAGACGATTAATGCGTCTCG	ctrl sgRNA	n/a	n/a	All figures	n/a
S. pyogenes	GAAGCCATGGAGATACCTCCTGG	UTR1	GGGAGCAAGT	*Renilla*	1C, 1D	n/a
S. thermophilus #3	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGTGTTGGT	*Renilla*	1C, 1F	n/a
S. aureus	GAAGCCATGGAGATACCTCCTGG	UTR1	TCGGGTCAGT	*Renilla*	1C, S3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGAATTGT	*Renilla*	1C, 1E, 3A, 3B, S4B, S5C	n/a
S. pasteurianus	GAAGCCATGGAGATACCTCCTGG	UTR1	TCGTGACAGT	*Renilla*	1C, 1G	n/a
C. lari	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGGTATGT	*Renilla*	1C, S3B	n/a
C. jejuni	GAAGCCATGGAGATACCTCCTGG	UTR1	AGAAACAGGT	*Renilla*	1C, S3C	n/a
N. meningitidis	GAAGCCATGGAGATACCTCCTGG	UTR1	TCAGGATTGT	*Renilla*	1C, S3D	n/a
P. multocida	GAAGCCATGGAGATACCTCCTGG	UTR1	GGATCGTAGT	*Renilla*	1C	n/a
P. lavamentivorans	GAAGCCATGGAGATACCTCCTGG	UTR1	ACGCATGAGT	*Renilla*	1C, S3E	n/a
C. diphtheria	GAAGCCATGGAGATACCTCCTGG	UTR1	GGGTAATAGT	*Renilla*	1C, S3F	n/a
S. pyogenes	GAATTAGGCCTTGCTGCGCCC	P1	AGGCTCCAGT	*Renilla*	1D	n/a
S. pyogenes	GACTCCATTTCTACTACTGGAGCCT	P2	GGGCGCAGCA	*Renilla*	1D	n/a
S. pyogenes	GATCATGCGTTTGCGTTGCTCG	NT1	GGGTCGTACA	*Renilla*	1D	n/a
S. pyogenes	GGCGTTCTCGGCGTGCTTCT	NT2	CGGAATCATA	*Renilla*	1D	n/a
S. pyogenes	GGTACTTGTAGTGATCCAGG	NT3	AGGCGATATG	*Renilla*	1D	n/a
S. pyogenes	GCAGCTCGAACCAAGCGGTG	NT4	AGGTACTTGT	*Renilla*	1D	n/a
S. pyogenes	GTTCAGCAGCTCGAACCAAG	NT5	CGGTGAGGTA	*Renilla*	1D	n/a
S. thermophilus #1	GCTGCGCCCAGGCTCCAGTA	P1	GTAGAAATGG	*Renilla*	1E, S4B	n/a
S. thermophilus #1	GTAGCTGGAGGCAGCGTTACCAT	NT1	GCAGAAAAAT	*Renilla*	1E, S4B	n/a
S. thermophilus #3	GCCCAGGCTCCAGTAGTAGAAA	P1	TGGAGTCAGC	*Renilla*	1F	n/a
S. thermophilus #3	ACTCCATTTCTACTACTGGAGCCT	P2	GGGCGCAGCA	*Renilla*	1F	n/a
S. thermophilus #3	GCTGACTCCATTTCTACTAC	P3	TGGAGCCTGG	*Renilla*	1F	n/a
S. thermophilus #3	GAAGCCATGGAGATACCTCC	UTR2	TGGTGGTGTT	*Renilla*	1F	n/a
S. thermophilus #3	GATCATGCGTTTGCGTTGCT	NT1	CGGGGTCGTA	*Renilla*	1F	n/a
S. thermophilus #3	GGAATCATAGTAGTTGATGA	NT2	AGGAGTCCAG	*Renilla*	1F	n/a
S. thermophilus #3	GCAGAAAAATCACGGCGTTCT	NT3	CGGCGTGCTT	*Renilla*	1F	n/a
S. pasteurianus	GCAGCGAGCCCACCACTGAGGCC	NT1	CAGTGATCAT	*Renilla*	1G	n/a
S. pasteurianus	GATGCATCTAGCCACGGGCTCG	NT2	ATGTGAGGCA	*Renilla*	1G	n/a
S. pasteurianus	GAACCAAGCGGTGAGGTACTTG	NT3	TAGTGATCCA	*Renilla*	1G	n/a
S. pyogenes	GACACGCCCGAGAGTGTCGGT	sgRNA1	GGGGGCCAAT	*dnaE1* (Ms3178)	2A	6.6
S. pyogenes	GCAGGTGGACAAAAGACCCCG	sgRNA2	AGGACCGTCC	*dnaE1* (Ms3178)	2A	31.2
S. thermophilus #1	GCCGGCCTTGGTCGCCGAGTT	sgRNA1	GTAGAACTCG	*dnaE1* (Ms3178)	2A	20.3
S. thermophilus #1	ATCTCCAACCGCTGCGCCTCGG	sgRNA2	CCAGCATGGG	*dnaE1* (Ms3178)	2A	32.3
S. thermophilus #1	ACGGTCCGGCAGAACCAGGG	sgRNA1	AAAGCAGGCT	*pptT* (Ms2648)	2B, 4H, S5G	38
S. thermophilus #1	GCGACACGGTCCGGCAGAACC	sgRNA2	AGGGAAAGCA	*pptT* (Ms2648)	2B, 4H, S5G	46.8
S. thermophilus #1	GGCCGAGGCGACACGGTCCG	sgRNA3	GCAGAACCAG	*pptT* (Ms2648)	2B, 4H, S5G	12.7
S. thermophilus #1	ACGGCTCGCCCTTGTCGCCCT	sgRNA4	TGAGGATCGG	*pptT* (Ms2648)	2B, 4H, S5G	51.5
S. thermophilus #1	GAGCGTCCCACCACCGCGCC	sgRNA5	GCGGAAGCCC	*pptT* (Ms2648)	2B, 4H, S5G	19.3
S. thermophilus #1	GCGCAGCTCGGCGCGCTCGA	sgRNA6	TGGGAAGCGT	*pptT* (Ms2648)	2B, 4H, S5G	18.5
S. thermophilus #1	ACGTCGCCTCCTTGGCACAGA	sgRNA7	ACAGGATTCG	*pptT* (Ms2648)	2B, 4H, S5G	14.7
S. thermophilus #1	GCCTTGTACGTCGCCTCCTTGGC	sgRNA8	ACAGAACAGG	*pptT* (Ms2648)	2B, 4H, S5G	37.2
S. pyogenes	GACACGGTCCGGCAGAACCA	sgRNA1	GGGAAAGCAG	*pptT* (Ms2648)	2B	n/a
S. pyogenes	GTCGTCGTACACCTCGGCCG	sgRNA2	AGGCGACACG	*pptT* (Ms2648)	2B	n/a
S. pyogenes	GGGCGGGTCGTCGTACACCT	sgRNA3	CGGCCGAGGC	*pptT* (Ms2648)	2B	n/a
S. pyogenes	GGCAGCGGCGAAAGGCCGGG	sgRNA4	CGGGTCGTCG	*pptT* (Ms2648)	2B	n/a
S. pyogenes	AACGGCTCCTCTTCGGGCAG	sgRNA5	CGGCGAAAGG	*pptT* (Ms2648)	2B	n/a
S. pyogenes	GCCACCGACCGCGCGATCAA	sgRNA6	CGGCTCCTCT	*pptT* (Ms2648)	2B	n/a
S. pyogenes	GCACCGTGACGAACTCGTTG	sgRNA7	CGGCGCTTGG	*pptT* (Ms2648)	2B	n/a
S. pyogenes	GCCGACACCGAGCTCGCCCA	sgRNA8	GGGCCTGACG	*pptT* (Ms2648)	2B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGAATTGT	*Renilla*	3A, 3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGCGAATTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGTGAATTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAAAATTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGATAATTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGACAATTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGTATTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGGATTGT	*Renilla*	3A, 3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGCATTGT	*Renilla*	3A, 3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGATTTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGAGTTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGACTTGT	*Renilla*	3A	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGAAATGT	*Renilla*	3A, 3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGAAGTGT	*Renilla*	3A, 3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGAACTGT	*Renilla*	3A, 3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGGAATGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGGAGTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGGACTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGCAATGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGCAGTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGAGCACTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGAAATGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGAAGTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGAACTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGGATTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGGAATGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGGAGTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGGACTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGCATTGT	*Renilla*	3B	n/a

S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGCAATGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGCAGTGT	*Renilla*	3B	n/a
S. thermophilus #1	GAAGCCATGGAGATACCTCCTGG	UTR1	TGGGCACTGT	*Renilla*	3B	n/a
S. thermophilus #1	GCTCTCATCGGACCAGGTGCCG	sgRNA1	TCGGAATCGG	*engA* (Ms3738)	4A	41.5
S. thermophilus #1	GCACCACCGCTTCGCGACGGC	sgRNA2	CCAGGATCCG	*engA* (Ms3738)	4A, S5B	55.3
S. thermophilus #1	GTAATAAATGATGTCCAAGCT	sgRNA1	GAAGAAGTTA	*yidC* (Ms6942)	4B, S5A	226
S. thermophilus #1	ATTGGTGGGGCCCAGCAGGAA	sgRNA2	CGAGAACGCC	*yidC* (Ms6942)	4B	74.5
S. thermophilus #1	GTCTCCGGGCGGATGCCGTC	sgRNA1	GAAGGATTCG	*hisD* (Ms3205)	4C	15.4
S. thermophilus #1	GTGGTGTCGGTGCGACGCTGA	sgRNA2	TCAGCATGCA	*hisD* (Ms3205)	4C	11.1
S. thermophilus #1	ACCTTCTTGTCGTCGGGAGGCG	sgRNA1	TCAGGATGGC	*mmpL3* (Ms0250)	4D	52.8
S. thermophilus #1	GACAGACTGGCTGCCCTCGTC	sgRNA2	GTAGAAACCG	*mmpL3* (Ms0250)	4D	82.5
S. thermophilus #1	GCTTCCAAACCCTCGAGAATGGTG	sgRNA1	ATGGAATCGG	*gyrB* (Ms0005)	4E	34.5
S. thermophilus #1	GCGGACCGCTTCCAAACCCT	sgRNA2	CGAGAATGGT	*gyrB* (Ms0005)	4E	26.5
S. thermophilus #1	GCGTGATCTTCGCGGCGCCGT	sgRNA1	CCAGCATCGA	*dnaE1* (Ms3178)	4F	n/a
S. thermophilus #1	GCCGGCCTTGGTCGCCGAGTT	sgRNA2	GTAGAACTCG	*dnaE1* (Ms3178)	4F	20.3
S. thermophilus #1	ATCTCCAACCGCTGCGCCTCGG	sgRNA3	CCAGCATGGG	*dnaE1* (Ms3178)	4F	32.3
S. thermophilus #1	GCCCAGCACGCTGAGCACCCCGA	n/a	CCAGAAATCG	*folC* (Rv2447c)	4G, S5D	99.2
S. thermophilus #1	GTGTGCCCCATGGTGATCTC	n/a	CCGGAAATGC	*folA* (Rv2763c)	4G, S5D	25.6
S. thermophilus #1	ACCCAGCCACCTCTTGGTCA	n/a	GCGGAAACCA	*pptT* (Rv2794c)	4G	30.4
S. thermophilus #1	GCGTAACGCGGTCAGCATGGC	n/a	CGGGAATTGT	*alr* (Rv3423c)	4G	32.4
S. thermophilus #1	GCCTTCCCCTTCATGGATCAGGCCTA	n/a	AAAGAATGTG	*clpC1* (Rv3596c)	4G, S5D	25.5
S. thermophilus #1	GTCTCGGCCTCGTTGGCCTGCA	n/a	CGAGAATCTT	*groES* (Rv3418c)	4G, S5D, S5E	30.4
S. thermophilus #1	GCCGATTCGAGGTAGTGGTTC	n/a	CCGGAAAACA	*mmpL3* (Rv0206c)	4G, S5D	18.2
S. thermophilus #1	GCCTTTTGGCTGGGGTCACCCC	n/a	ACAGGATGCG	*dnaE1* (Rv1547)	4G, S5D, S5F	12.0
S. thermophilus #1	GTGCAGATCGCTGATCGCCC	n/a	AGAGAACCGG	Ms2647 (*pptT* operon)	4H, S5G	34.5
S. thermophilus #1	GACCTTCGCGAACCGCTTCC	n/a	GCAGCAGGTC	Ms2647 (*pptT* operon)	4H, S5G	8.3
S. thermophilus #1	GCCCGCCCTGCTCGGTCCACAC	n/a	CGGGAACGGG	Ms2647 (*pptT* operon)	4H, S5G	34.6
S. thermophilus #1	AGGAACGAGTAGTCGTACAG	n/a	CAGGAACATC	Ms2647 (*pptT* operon)	4H, S5G	20.7
S. thermophilus #1	GCAGGAACGAGTAGTCGTACA	n/a	GCAGGAACAT	Ms2647 (*pptT* operon)	4H, S5G	31.6
S. thermophilus #1	GGGCAGGAACGAGTAGTCGT	n/a	ACAGCAGGAA	Ms2647 (*pptT* operon)	4H, S5G	12.1
S. thermophilus #1	GCCTTGGACGACGCGCCCTCGGG	n/a	CAGGAACGAG	Ms2647 (*pptT* operon)	4H, S5G	40.8
S. thermophilus #1	GCCTTGGACGACGCGCCCTCGG	n/a	GCAGGAACGA	Ms2647 (*pptT* operon)	4H, S5G	44.4
S. thermophilus #1	GCGTACGGCTCGCACGACAG	n/a	CAGGAACTCG	Ms2647 (*pptT* operon)	4H, S5G	23.2
S. thermophilus #1	GTCGCGTACGGCTCGCACGACA	n/a	GCAGGAACTC	Ms2647 (*pptT* operon)	4H, S5G	28.4
S. thermophilus #1	GCGTCGCGTACGGCTCGCACG	n/a	ACAGCAGGAA	Ms2647 (*pptT* operon)	4H, S5G	8.2
S. thermophilus #1	GACCATCGGGAAGTGGTTGA	n/a	CCAGAACCGT	Ms2647 (*pptT* operon)	4H, S5G	33.6
S. thermophilus #1	GTCGCAGGGTTCGCGGACCAT	n/a	CGGGAAGTGG	Ms2647 (*pptT* operon)	4H, S5G	26.9
S. thermophilus #1	GCACCACAGCGAGAACTCCGG	n/a	ATAGAACATC	Ms2647 (*pptT* operon)	4H, S5G	34.8
S. thermophilus #1	GCCGTGGTGCCGCACCACAG	n/a	CGAGAACTCC	Ms2647 (*pptT* operon)	4H, S5G	32.5
S. thermophilus #1	ATGTGCAGGTGGCCGTAGAC	n/a	CGAGCAGACC	Ms2647 (*pptT* operon)	4H, S5G	16.8
S. thermophilus #1	AGGCGCGTACTTGGGGTCGG	n/a	GCAGGATCTG	Ms2647 (*pptT* operon)	4H, S5G	18.8
S. thermophilus #1	GATGGGAAGCGTGATCGCGT	n/a	CGAGGACGCC	*pptT* (Ms2648)	4H, S5G	13.0
S. thermophilus #1	GCGGTCGGTCCGGGGCGTGCC	n/a	CCGGAACCGT	*groES* (Rv3418c) 5'UTR	4I, S5H	36.3
S. thermophilus #1	GTCTCGGCCTCGTTGGCCTGCA	n/a	CGAGAATCTT	*groES* (Rv3418c)	4I, S5H	48.6
S. thermophilus #1	GCGGCTTCTCCTTGGCGGTGT	n/a	CAGGAATGAC	*groES* (Rv3418c)	4I, S5H	37
S. thermophilus #1	GCGGCTTCTCCTTGGCGGTG	n/a	TCAGGAATGA	*groES* (Rv3418c)	4I, S5H	13.7
S. thermophilus #1	GGCCAGCACGTCGCGTGCCG	n/a	ACAGGATCAG	*groES* (Rv3418c)	4I, S5H	14.2
S. thermophilus #1	GTGAGCACGGGGATCGCCGGG	n/a	GCGGAACACG	*groEL1* (Rv3417c) 5'UTR	4I, S5H	22.6
S. thermophilus #1	GTCCGCTAACGCATGCCGCC	n/a	CCGGAAATCA	*groEL1* (Rv3417c) 5'UTR	4I, S5H	44.2
S. thermophilus #1	GGACACCGGCGTGGCCGATG	n/a	CCAGCAGCGC	*groEL1* (Rv3417c)	4I, S5H	12.4
S. thermophilus #1	GTCACCGATCTGCTCGTCGCG	n/a	CGAGGACACC	*groEL1* (Rv3417c)	4I, S5H	23.8
S. thermophilus #1	ACTCCAACTCGGTGCCCAGCGT	n/a	CGAGGATTCT	*groEL1* (Rv3417c)	4I, S5H	93.9
S. thermophilus #1	GTCGGTAACGAAGTATGCCGA	n/a	CAAGAAGCCC	*groEL1* (Rv3417c)	4I, S5H	48.6
S. thermophilus #1	GCTGATCTTGTCTTGGTGCA	n/a	GCAGGATCAA	*groEL1* (Rv3417c)	4I, S5H	47.5
S. thermophilus #1	GCGAGCTGATCTTGTCTTGGT	n/a	GCAGCAGGAT	*groEL1* (Rv3417c)	4I, S5H	90.5
S. thermophilus #1	ACCTTTTCCAGCAATGGCAACAGAT	n/a	CGGGAAGCGA	*groEL1* (Rv3417c)	4I, S5H	130.4
S. thermophilus #1	ACCCGTTCCTGCAACCTTTT	n/a	CCAGCAATGG	*groEL1* (Rv3417c)	4I, S5H	101.3
S. thermophilus #1	GTCACCACCGCCAGGTCCTC	n/a	AAGGAACGCC	*groEL1* (Rv3417c)	4I, S5H	25.6
S. thermophilus #1	ACCCGTCACCACCGCCAGGTCCT	n/a	CAAGGAACGC	*groEL1* (Rv3417c)	4I, S5H	73.8
S. thermophilus #1	GTGGCGGCACCCACCTTGATG	n/a	ACAGCAACCC	*groEL1* (Rv3417c)	4I, S5H	33.3
S. thermophilus #1	GCTTCGGAGAACACGTCGACAC	n/a	CGAGGACCTC	*groEL1* (Rv3417c)	4I, S5H	51.5
S. thermophilus #1	AACGGCGCGGCAAGGGCTTC	n/a	GGAGAACACG	*groEL1* (Rv3417c)	4I, S5H	8.7
S. thermophilus #1	AAGCCAGCGTTGGCGGCGAT	n/a	CCAGAACAAC	*groEL1* (Rv3417c)	4I, S5H	30.6
S. thermophilus #1	TCAGCGCCGGTAGCTGCAGAA	sgRNA1	CCGGAAACGC	*folA* (Ms2671)	5B	n/a
S. thermophilus #1	GTCGATGAATTTCCCACCGTC	sgRNA1	GGAGAACGAG	*folP1* (Ms6103)	5B	n/a
S. thermophilus #1	GCTGACGGTCGGCCCCGGCGCC	sgRNA1	GAAGAACGCC	*folC* (Ms4629)	5B	n/a
S. thermophilus #1	GAGGTCCTCGGGCAGACGCC	sgRNA2	ATGGAATGGA	*folA* (Ms2671)	5C, 5D	n/a
S. thermophilus #1	GTCGACCGAACGCCAGTGCA	sgRNA2	TGAGGATCCA	*folP1* (Ms6103)	5C, 5D	n/a
S. thermophilus #1	ACGCCTCGACTGCGGCCAGCG	sgRNA2	CGAGGACCGC	*folC* (Ms4629)	5C, 5D	n/a
S. pyogenes	GGCGAGTGCTAAGAATAACGT	sgRNA1	TGGCACTCGC	*mCherry*	S1B	n/a
S. pyogenes	GTAAGAATAACGTTGGCACTCGCGAC	sgRNA2	CGGTGAGTGC	*mCherry*	S1B	n/a
S. pyogenes	GAATTAGGCCTTGCTGCGCCC	sgRNA1	AGGCTCCAGT	*Renilla*	S1C	n/a
S. pyogenes	GTCATGAATTAGGCCTTGCTGCGCCC	sgRNA2	AGGCTCCAGT	*Renilla*	S1C	n/a
S. pyogenes	GACTGGAGCCTGGGCGCAGCA	sgRNA3	AGGCCTAATT	*Renilla*	S1C	n/a
S. pyogenes	GCTACTACTGGAGCCTGGGCGCAGCA	sgRNA4	AGGCCTAATT	*Renilla*	S1C	n/a
S. pyogenes	GCCAGGCTCCAGTAGTAGAAA	sgRNA5	TGGAGTCACG	*Renilla*	S1C	n/a
S. pyogenes	GTGCGCCCAGGCTCCAGTAGTAGAAA	sgRNA6	TGGAGTCACG	*Renilla*	S1C	n/a
S. pyogenes	GCCATTTCTACTACTGGAGCC	sgRNA7	TGGGCGCAGC	*Renilla*	S1C	n/a
S. pyogenes	GTGACTCCATTTCTACTACTGGAGCC	sgRNA8	TGGGCGCAGC	*Renilla*	S1C	n/a
S. pyogenes	GGTTCGACCTCGGCTTCACC	sgRNA9	GGGCTGCCGT	*Renilla*	S1C	n/a
S. pyogenes	GCTTCCGGTTCGACCTCGGCTTCACC	sgRNA10	GGGCTGCCGT	*Renilla*	S1C	n/a
S. pyogenes	GGGAGGACGAACCTTCTCTTC	sgRNA11	CGGTTCGACC	*Renilla*	S1C	n/a
S. pyogenes	GGGTCGGGAGGACGAACCTTCTCTTC	sgRNA12	CGGTTCGACC	*Renilla*	S1C	n/a

S. pyogenes	GTGACTCCGCGTGGGTTCCGG	sgRNA13	GGGCCGTGCT	*Renilla*	S1C	n/a
S. pyogenes	GGGCTATGACTCCGCGTGGGTTCCGG	sgRNA14	GGGCCGTGCT	*Renilla*	S1C	n/a
S. pyogenes	GTCGCGGCTATGACTCCGCGT	sgRNA15	GGGTTCCGGG	*Renilla*	S1C	n/a
S. pyogenes	GCATTATCGCGGCTATGACTCCGCGT	sgRNA16	GGGTTCCGGG	*Renilla*	S1C	n/a
S. pyogenes	GCAACACTTCTGCCATTATCG	sgRNA17	CGGCTATGAC	*Renilla*	S1C	n/a
S. pyogenes	GGCCCGCAACACTTCTGCCATTATCG	sgRNA18	CGGCTATGAC	*Renilla*	S1C	n/a
S. pyogenes	GTGGCTGCAGCCCCACCCAAA	sgRNA19	GGGCCGTCGG	*Renilla*	S1C	n/a
S. pyogenes	GCCTTCTGGCTGCAGCCCCACCCAAA	sgRNA20	GGGCCGTCGG	*Renilla*	S1C	n/a
S. aureus	GGAATCATAGTAGTTGATGA	NT1	AGGAGTCCAG	*Renilla*	S3A	n/a
S. aureus	GGCGTTCTCGGCGTGCTTCT	NT2	CGGAATCATA	*Renilla*	S3A	n/a
S. aureus	GACTTACCCATTCCAATCAGATC	NT3	AGGGATGATG	*Renilla*	S3A	n/a
S. aureus	GCCTTGATCTTGTCTTGGTGCTCGT	NT4	AGGAGTAGTG	*Renilla*	S3A	n/a
S. aureus	GCCGTCTAACCTCGCCCTTCTCC	NT5	TTGAATGGCT	*Renilla*	S3A	n/a
S. aureus	GAGGGATCTCGCGAGGCCAGGA	NT6	GAGGGTAGGC	*Renilla*	S3A	n/a
S. aureus	GGGCTTGCCTCCCTTAACGAG	NT7	AGGGATCTCG	*Renilla*	S3A	n/a
C. lari	ACTCCATTTCTACTACTGGAGC	P1	CTGGGCGCAG	*Renilla*	S3B	n/a
C. lari	GAAGCCATGGAGATACCTCCTG	UTR2	GTGGGGTATG	*Renilla*	S3B	n/a
C. lari	GATCATGCGTTTGCGTTGCT	NT1	CGGGGTCGTA	*Renilla*	S3B	n/a
C. lari	GTGATCATGCGTTTGCGTTGC	NT2	TCGGGGTCGT	*Renilla*	S3B	n/a
C. lari	ATCAGGGATGATGCATCTAGCC	NT3	ACGGGCTCGA	*Renilla*	S3B	n/a
C. jejuni	GCTCGGGGTCGTACACCTTG	NT1	GAAGCCATGG	*Renilla*	S3C	n/a
C. jejuni	GCTTGCAGCGAGCCCACCACTG	NT2	AGGCCCAGTG	*Renilla*	S3C	n/a
C. jejuni	GTTCATTTGCTTGCAGCGAG	NT3	CCCACCACTG	*Renilla*	S3C	n/a
C. jejuni	GCTCGATGTGAGGCACGACGTGC	NT4	CTCCACAGGT	*Renilla*	S3C	n/a
C. jejuni	GCCAGACAAGCCCCCCAGTCGTG	NT5	GCCCACAAAG	*Renilla*	S3C	n/a
N. meningitidis	AGGCCTTGCTGCGCCCAGGC	P1	TCCAGTAGTA	*Renilla*	S3D	n/a
N. meningitidis	GCCTTGCTGCGCCCAGGCTCC	P2	AGTAGTAGAA	*Renilla*	S3D	n/a
N. meningitidis	ATCATGCGTTTGCGTTGCTCGG	NT1	GGTCGTACAC	*Renilla*	S3D	n/a
N. meningitidis	GAGGCCCAGTGATCATGCGT	NT2	TTGCGTTGCT	*Renilla*	S3D	n/a
N. meningitidis	ACCACTGAGGCCCAGTGATC	NT3	ATGCGTTTGC	*Renilla*	S3D	n/a
N. meningitidis	GTAGTTGATGAAGGAGTCCA	NT4	GCACGTTCAT	*Renilla*	S3D	n/a
N. meningitidis	GGCGTGCTTCTCGGAATCAT	NT5	AGTAGTTGAT	*Renilla*	S3D	n/a
P. lavamentivorans	GCCTGGGCGCAGCAAGGCCTA	P1	ATTCATGAAA	*Renilla*	S3E	n/a
P. lavamentivorans	GCATGAGTCCTGAAGAGCTGA	P2	CTCCATTTCT	*Renilla*	S3E	n/a
P. lavamentivorans	GAAATGGAGTCAGCTCTTCAGG	UTR2	ACTCATGCGT	*Renilla*	S3E	n/a
P. lavamentivorans	GCTCGGGGTCGTACACCTTGGA	NT1	AGCCATGGAG	*Renilla*	S3E	n/a
P. lavamentivorans	GCCCACCACTGAGGCCCAGT	NT2	GATCATGCGT	*Renilla*	S3E	n/a
P. lavamentivorans	GTTGATGAAGGAGTCCAGCAC	NT3	GTTCATTTGC	*Renilla*	S3E	n/a
P. lavamentivorans	GTTCTCGGCGTGCTTCTCGG	NT4	AATCATAGTA	*Renilla*	S3E	n/a
P. lavamentivorans	ACAGGTAGCTGGAGGCAGCGT	NT5	TACCATGCAG	*Renilla*	S3E	n/a
C. diphtheria	GAATTAGGCCTTGCTGCGCCC	P1	AGGCTCCAGT	*Renilla*	S3F	n/a
C. diphtheria	GACTCCATTTCTACTACTGGAGCCT	P2	GGGCGCAGCA	*Renilla*	S3F	n/a
C. diphtheria	GCGTTGCTCGGGGTCGTACACCT	NT1	TGGAAGCCAT	*Renilla*	S3F	n/a
C. diphtheria	GATCATGCGTTTGCGTTGCTCG	NT2	GGGTCGTACAC	*Renilla*	S3F	n/a
C. diphtheria	GCTTGCAGCGAGCCCACCACTG	NT3	AGGCCCAGTG	*Renilla*	S3F	n/a
C. diphtheria	GGAATCATAGTAGTTGATGA	NT4	AGGAGTCCAG	*Renilla*	S3F	n/a
C. diphtheria	GGCGTTCTCGGCGTGCTTCT	NT5	CGGAATCATA	*Renilla*	S3F	n/a
S. thermophilus #1	GCGACAGACTGGCTGCCCTCGTC	n/a	GTAGAAACCG	*mmpL3* (Ms0250)	S4A	n/a
S. thermophilus #1	GCTCTTCAGGACATTTCCCA	UTR2	CCAGGAGGTA	*Renilla*	S5C	n/a
S. thermophilus #1	GCTTCCAAGGTGTACGACCC	T1	CGAGCAACGC	*Renilla*	S5C	n/a
S. thermophilus #1	GCCTCAGTGGTGGGCTCGCTG	T2	CAAGCAAATG	*Renilla*	S5C	n/a
S. thermophilus #1	ACTCCTTCATCAACTACTATGATTC	T3	CGAGAAGCAC	*Renilla*	S5C	n/a
S. thermophilus #1	ACTATGATTCCGAGAAGCACGC	T4	CGAGAACGCC	*Renilla*	S5C	n/a
S. thermophilus #1	ATGCATCATCCCTGATCTGA	T5	TTGGAATGGG	*Renilla*	S5C	n/a
S. thermophilus #1	GGGACGAGTGGCCTGACAT	T6	CGAGGAGGAT	*Renilla*	S5C	n/a
S. pyogenes	GGGTCAGGCTCGGATCGATG	n/a	CGGGTCTCCG	*folC* (Rv2447c)	S5D	18.9
S. pyogenes	GCCTGGTCCTCGGGCAAGCGCCA	n/a	GGGGATGTCG	*folA* (Rv2763c)	S5D	10.1
S. pyogenes	GAGCCAGGACGACGACCCTG	n/a	CGGGCACGGT	*clpC1* (Rv3596c)	S5D	11.5
S. pyogenes	ACGAGAATCTTGTCCTCGAG	n/a	TGGCTTGATG	*groES* (Rv3418c)	S5D	11.8
S. pyogenes	GTACACAGTTCGACCCCACC	n/a	AGGCGAACAC	*mmpL3* (Rv0206c)	S5D	16.9
S. pyogenes	GTCCAGCATCGAATACTCGGTG	n/a	TGGTTGTGCA	*dnaE1* (Rv1547)	S5D	8.5
S. thermophilus #1	GCGTGATCTTCGCGGCACCGT	n/a	CCAGCATCGA	*dnaE1* (Rv1547)	S5F	6.7
S. thermophilus #1	ATCCCCAGCCGCTCCACCTCGG	n/a	CGAGCATGGG	*dnaE1* (Rv1547)	S5F	8.8
S. thermophilus #1	ATCCCGGCCTTGGTCGCGGAGTT	n/a	GTAGAACTCG	*dnaE1* (Rv1547)	S5F	9
S. thermophilus #1	GCCATCATCGTCAGGTGCGT	n/a	GTAGGAGCCG	*dnaE1* (Rv1547)	S5F	8.4
S. thermophilus #1	GCTGGCCCTCGAAGGAAGCATG	n/a	CGAGGACAGC	*dnaE1* (Rv1547)	S5F	7.9
S. thermophilus #1	ACTTGCTCAGCTGGCCCTCGAAG	n/a	GAAGCATGCG	*dnaE1* (Rv1547)	S5F	8.1
S. thermophilus #1	ACTTGCTCAGCTGGCCCTCG	n/a	AAGGAAGCAT	*dnaE1* (Rv1547)	S5F	8.2
S. thermophilus #1	ACCACTTGCTCAGCTGGCCCTC	n/a	GAAGGAAGCA	*dnaE1* (Rv1547)	S5F	19.3
S. thermophilus #1	AGCCCGTGGTCCATCAGCTC	n/a	AAGGAAGTAG	*dnaE1* (Rv1547)	S5F	6.4
S. thermophilus #1	GTCAGCCCGTGGTCCATCAGCT	n/a	CAAGGAAGTA	*dnaE1* (Rv1547)	S5F	12.1
S. thermophilus #1	GTTGAGCGCGCGTCCGATCT	n/a	CGAGCAGACC	*dnaE1* (Rv1547)	S5F	7.5
S. thermophilus #1	GCAGTCATTGGTGGCAAGAG	n/a	GCGGAATGTT	*dnaE1* (Rv1547)	S5F	10.1
S. thermophilus #1	GCGCGGTGTCCACACGTCGGC	n/a	GTAGGACTGC	*dnaE1* (Rv1547)	S5F	11.4
S. thermophilus #1	GCCTGGTCATGCCCATCGGGCA	n/a	CCGGAAACAC	*dnaE1* (Rv1547)	S5F	6.8
S. thermophilus #1	GCGTCCACCTCGTGACGCAG	n/a	CCAGGACGCC	*dnaE1* (Rv1547)	S5F	5.4
S. thermophilus #1	GTACCCGTCCGGCGGACCGG	n/a	CCGGAAATCG	*dnaE1* (Rv1547)	S5F	5.1
S. thermophilus #1	AAGTACGATGGGAAACCTTT	n/a	GGAGCAGATG	*dnaE1* (Rv1547)	S5F	3.8
S. thermophilus #1	GCGACGATCAGAAAGTACGA	n/a	TGGGAAACCT	*dnaE1* (Rv1547)	S5F	9.4
S. thermophilus #1	GCTGATCAGGTCGGCGACGA	n/a	TCAGAAAGTA	*dnaE1* (Rv1547)	S5F	8.1
S. thermophilus #1	ACCGCTCGAACAGCAGACCGT	n/a	GTGGAATCGG	*dnaE1* (Rv1547)	S5F	17.9
S. thermophilus #1	GGGGTTGAGGAACCGCTCGA	n/a	ACAGCAGACC	*dnaE1* (Rv1547)	S5F	8.1
S. thermophilus #1	ATCGAGGTGCGCTCGGGGTT	n/a	GAGGAACCGC	*dnaE1* (Rv1547)	S5F	10.9
S. thermophilus #1	GCATCGAGGTGCGCTCGGGGT	n/a	TGAGGAACCG	*dnaE1* (Rv1547)	S5F	16.8
S. thermophilus #1	GCAGGCCCAGGAAGTCCATTT	n/a	TCAGCAGACC	*dnaE1* (Rv1547)	S5F	10.5
S. thermophilus #1	ATCGTCAGGTTCCGCAGGCC	n/a	CAGGAAGTCC	*dnaE1* (Rv1547)	S5F	10.3

S. thermophilus #1	GATCGTCAGGTTCCGCAGGC	n/a	CCAGGAAGTC	*dnaE1* (Rv1547)	S5F	26.5
S. thermophilus #1	GCCCAGGGTGTCGCCGCGGC	n/a	CCAGCAGCTC	*dnaE1* (Rv1547)	S5F	5.3
S. thermophilus #1	GCGCATGGGCCCGCCGTCGAG	n/a	CTGGAACACG	*dnaE1* (Rv1547)	S5F	6.6
S. thermophilus #1	ACCCGGTCGGCTGCATGCGGC	n/a	GCAGCAGGTC	*dnaE1* (Rv1547)	S5F	8.3
S. thermophilus #1	GATGAGGCCGTAGGTCTCGG	n/a	CGAGGATCTC	*dnaE1* (Rv1547)	S5F	2.4
S. thermophilus #1	GTTTCTTCTTGCCCATGGCCTTGC	n/a	GTAGAATGTC	*dnaE1* (Rv1547)	S5F	14.8
S. thermophilus #1	ACCCGTTGGCCTGCATGCCATC	n/a	GGAGAAGCCC	*dnaE1* (Rv1547)	S5F	13.8
S. thermophilus #1	AGCGCCTTGATGGCCGCCGG	n/a	AGAGAACCCG	*dnaE1* (Rv1547)	S5F	5.9
S. thermophilus #1	GAACGCGTAGTCAGCGAACG	n/a	GCAGGATGGT	*dnaE1* (Rv1547)	S5F	9.3
S. thermophilus #1	GCCTTGAGGTAGGCCGTCCA	n/a	GTAGGACACC	*dnaE1* (Rv1547)	S5F	9.3
S. thermophilus #1	GCCCTTGTCGTTGCGGGTTT	n/a	GGAGCAACGA	*dnaE1* (Rv1547)	S5F	13.5
S. thermophilus #1	GTCCACCGCATCGCTGTGCAC	n/a	CAGGAAAAGA	*dnaE1* (Rv1547)	S5F	17.4
S. thermophilus #1	GTCCACCGCATCGCTGTGCA	n/a	CCAGGAAAAG	*dnaE1* (Rv1547)	S5F	8.1

*** Gene knockdown by the indicated sgRNA was measured by qRT-PCR. Fold knockdown relative to a non-genome targeting control sgRNA is shown.**