

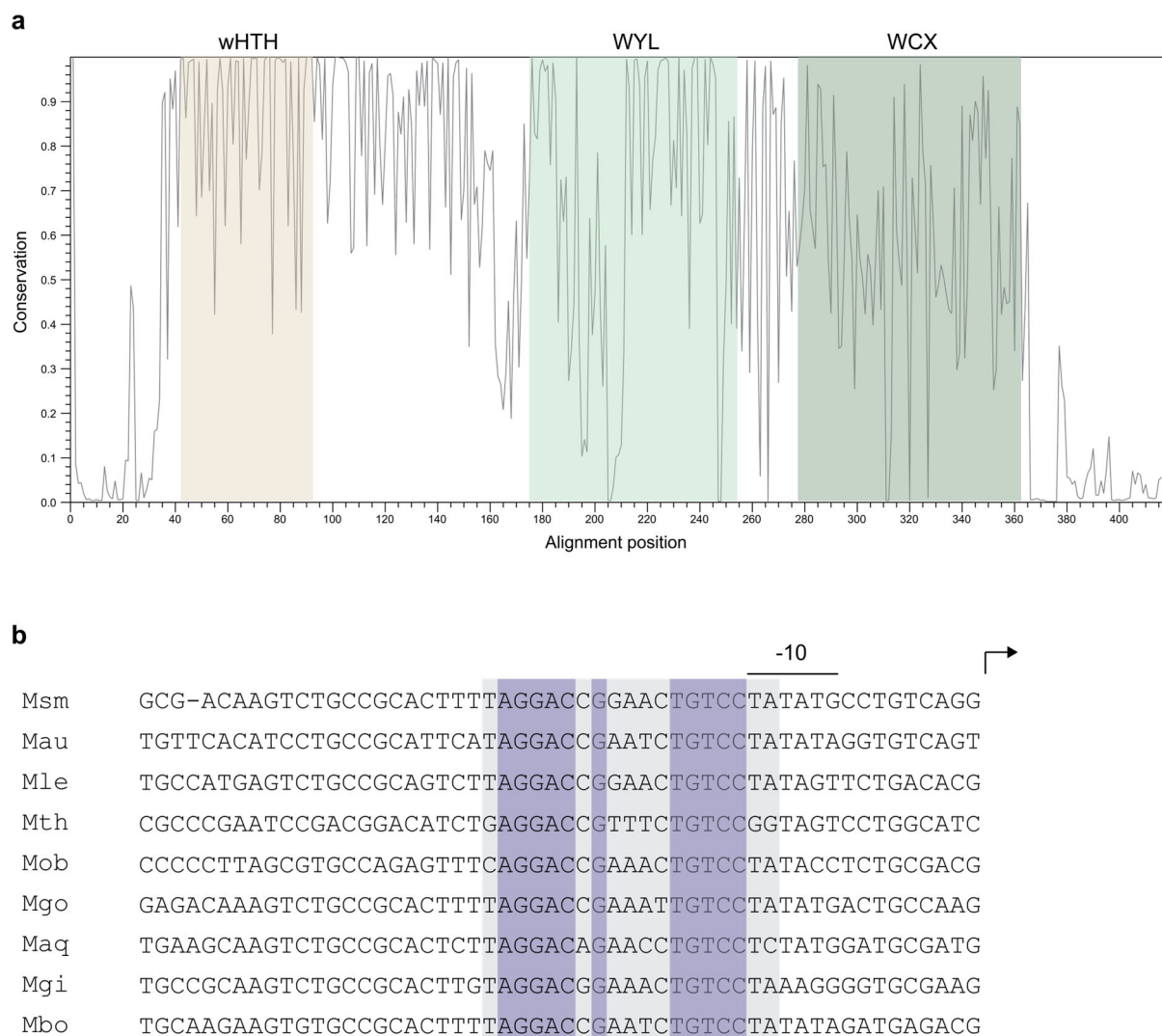
SUPPLEMENTARY FIGURES AND TABLES

Novel WYL domain-containing transcriptional activator acts in response to genotoxic stress in rapidly growing mycobacteria

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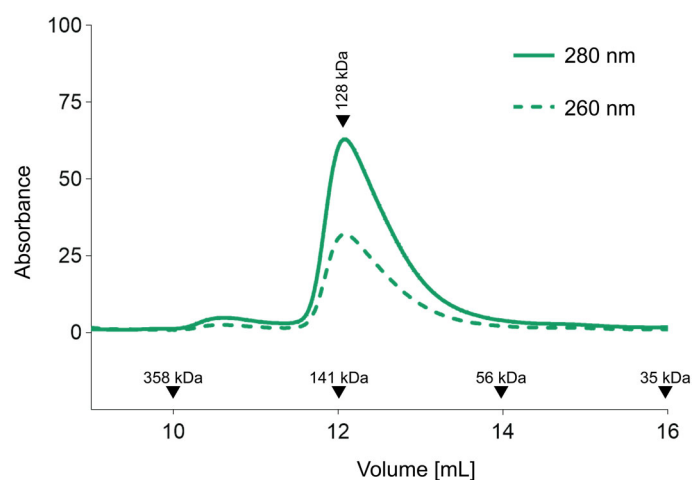
Supplementary Figures

Figure S1



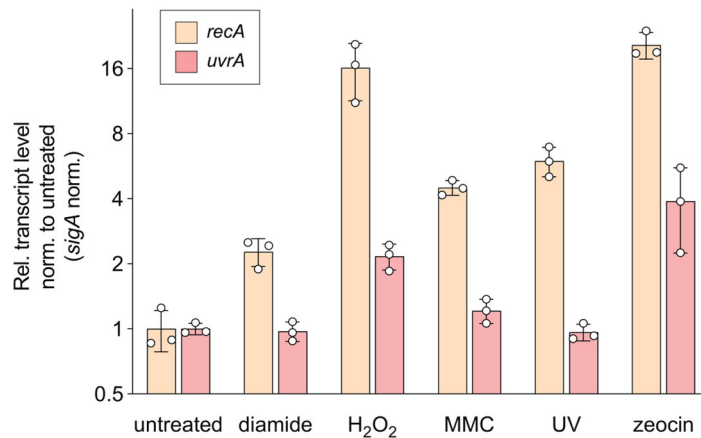
Supplementary Figure 1 Conservation of SiwR orthologs and the intergenic region toward the neighboring *msmeG_1357-56* operon. (a) Sequence conservation across the Top 500 SiwR orthologs shows that the HTH and parts of the WYL domain are highly conserved. The linker region between the HTH and WYL domain displays high conservation as well. The WCX domain exhibits more variability between the orthologs. (b) An alignment of the promoter region of the *msmeG_1357-56* operon from various mycobacteria reveals high sequence conservation and the presence of a 19 bp imperfect palindrome (grey area). Nucleotides conserved across the depicted sequences are highlighted in blue. *Msm* = *M. smegmatis*; *Mau* = *M. aurum*; *Mle* = *M. lehmannii*; *Mth* = *M. thermoresistibile*; *Mob* = *M. obuense*; *Mgo* = *M. goodii*; *Maq* = *M. aquaticum*; *Mgi* = *M. gilvum*; *Mbo* = *M. boenickei*.

Figure S2



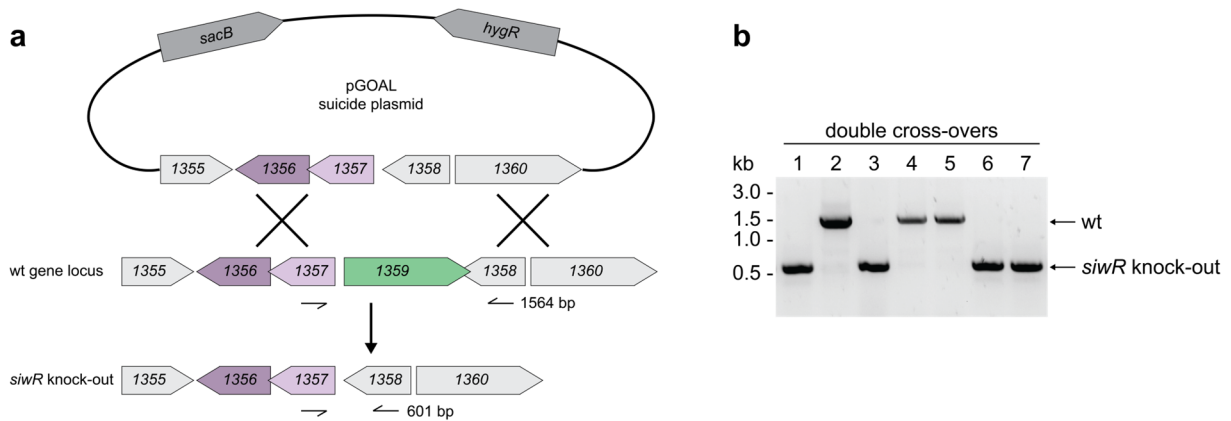
Supplementary Figure 2 SiwR forms homodimers. Analytical size exclusion chromatography (Superdex 200) reveals homodimer formation of recombinantly expressed and purified Sumo-SiwR. The A280/269 absorbance ratio in the elution peak shows the absence of DNA contamination in the sample. The calculated molecular weight for a dimer is about 100 kDa and SiwR elutes at 128 kDa.

Figure S3



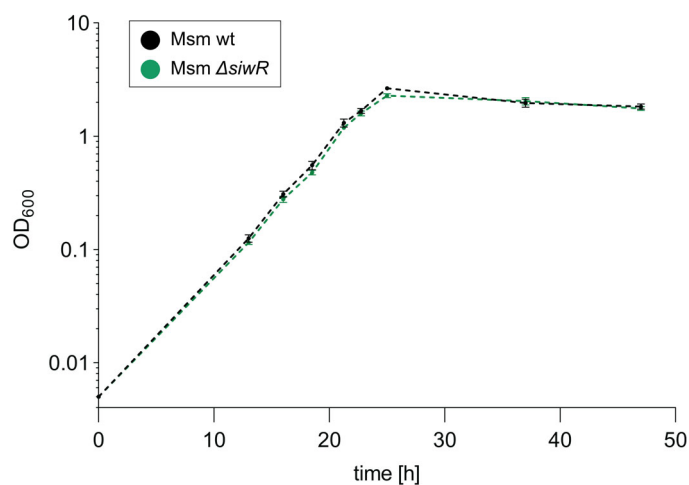
Supplementary Figure 3 Upregulation of PafBC-dependent genes *recA* and *uvrA* in response to different genotoxic stresses. RT-qPCR shows that *recA* and *uvrA* transcript levels increase the most upon treatment with 7 mM H₂O₂ and 5 µg/mL zeocin. Relative transcript levels were calculated by normalizing C_T values of *recA* and *uvrA* against *sigA*. Thus, $2^{-\Delta C_T}$ values were calculated and normalized to untreated cells. Error bars represent the three biological replicates shown as individual data points.

Figure S4



Supplementary Figure 4 Generation of *siwR* knock-out strain. (a) A pGOAL suicide plasmid was used to generate a markerless *siwR* knock-out. Since the *msmeg_1358* stop codon overlaps with the coding region of *siwR* we left this portion of sequence intact. The knock-out was generated by homologous recombination. (b) To verify double crossovers we performed colony PCRs with primers annealing in the *msmeg_1357* and *msmeg_1358* region. In case of wild-type cells the PCR generates a 1564 bp product while successful *siwR* knock-out cells yield a 601 bp product.

Figure S5



Supplementary Figure 5 Growth curves of the *Msm* wild-type and *Msm* $\Delta siwR$ strains. Cells were grown in 7H9 medium supplemented with 0.2% glycerol and 0.05% Tween-80 at 37° C. OD₆₀₀ was measured at the indicated time points. No difference in growth between the parent and the knock-out strain is observed under the tested condition. The error bars represent three independent biological replicates.

Figure S6

a

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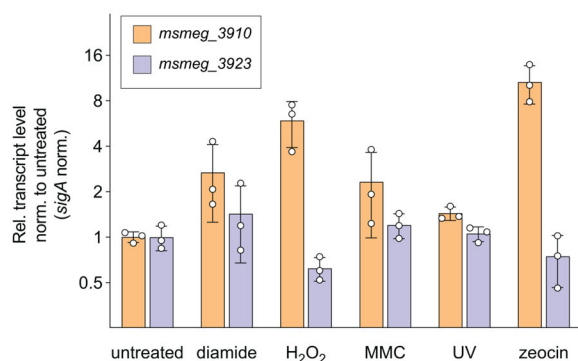
MSMEG_1356 1 -MPGMPPPAADERQTLINFLFQ-QNAFFAVAYGLTDEQARSTP-SVSALSIGGLVKHAAGVQKGWTDRAAYAPDS 73
MSMEG_3923 1 MDRPTPLNADERTTLEGWLDYF-RATLAAKCEGLTDEQVRAASVDPSQMTLSGLVQHAAEVERNWFRVVTGERV 75
MSMEG_1357 1 MD-----I-DLIDQLDFHWHQLRPRLDGLTDEYFWQPV-----PGCWTVHADGSI-----DF-SYPEPQ 55
MSMEG_3910 1 -M-----I-EQIRRFQDLAWALA-DLHLAALDDDDFLWEPA-----PLVWTVHRDECGRWWDW-ADTEPD 57

MSMEG_1356 74 PQP---DPRPMEEQMAEY-----ADQYSMRDDET-----IADLLDALKQAETLRVFAEKDLEAPVPV 129
MSMEG_3923 76 PGIFGSAPHPEGHD-----GGFEISPESS-----YRAAVA IWQDEIGHARANTAERELDD----- 125
MSMEG_1357 56 PTFPTTIAWRLAHVIVGVLAMRNH-SHFGGPPADYQTWPYATDAKTALEQLDDAHRNWITGVRALSDDLQRVGP 130
MSMEG_3910 58 PIPVPTIAWTTWHMIFWWSALAHVRGLTPPDRSSIWSWPGSEDAVARIREL---GEQWSDLLTTLTSGADLAAPATF 130

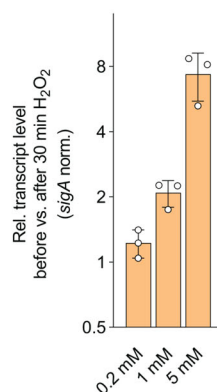
MSMEG_1356 130 PRNVPWFPPADIDHWTVRWVVMHLIEELSRHAGHADIRRESIDRATMYELMAAAEEWPEWDWIRWRPSASAPTA 203
MSMEG_3923 126 ---TSAFM---GTEVSLRWIFVHMI AEYARHCGHADLRERIDGETGV----- 167
MSMEG_1357 131 ---AEGPW---ADHPMSELVLHINREVIHHGAEIACIRDLYAHTNNT-----AEEN----- 175
MSMEG_3910 131 ---PWGEG---SGRTVADTVLWLNVELTKNAEIGQLRMLRAAH----- 168

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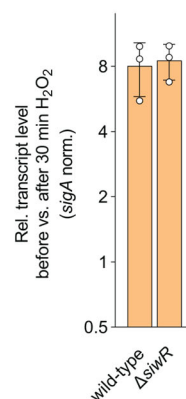
b



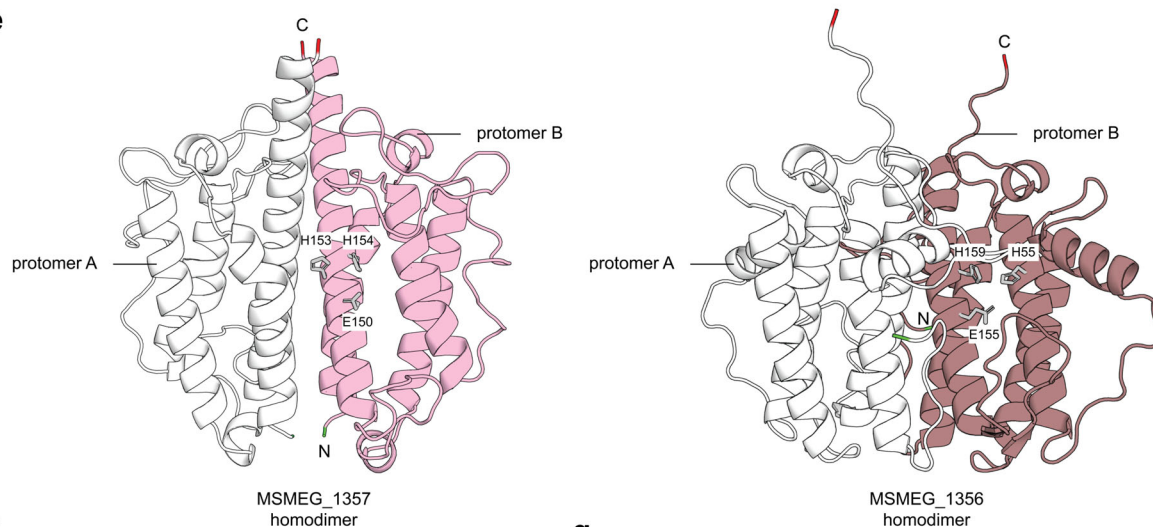
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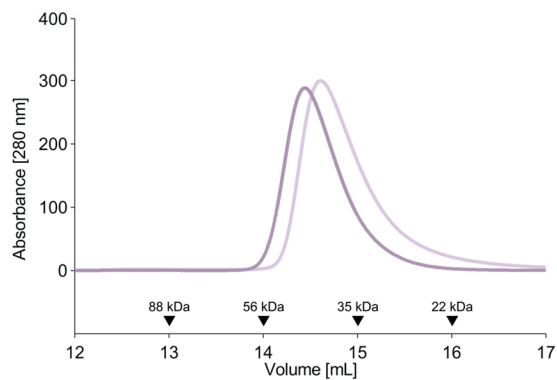
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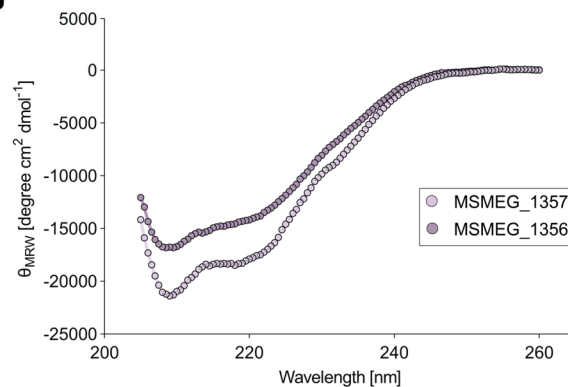
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f

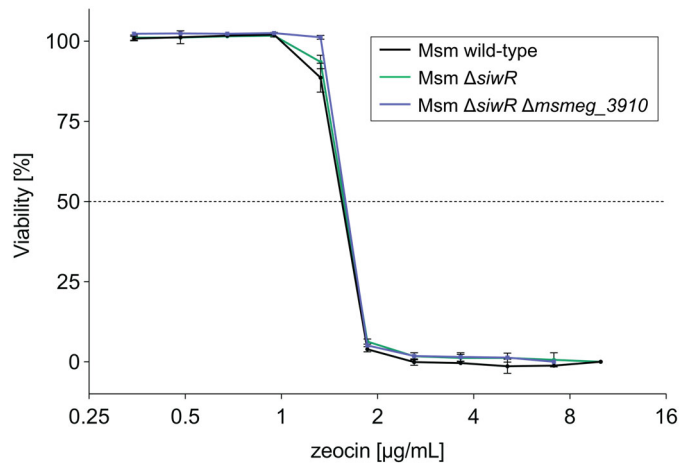


g



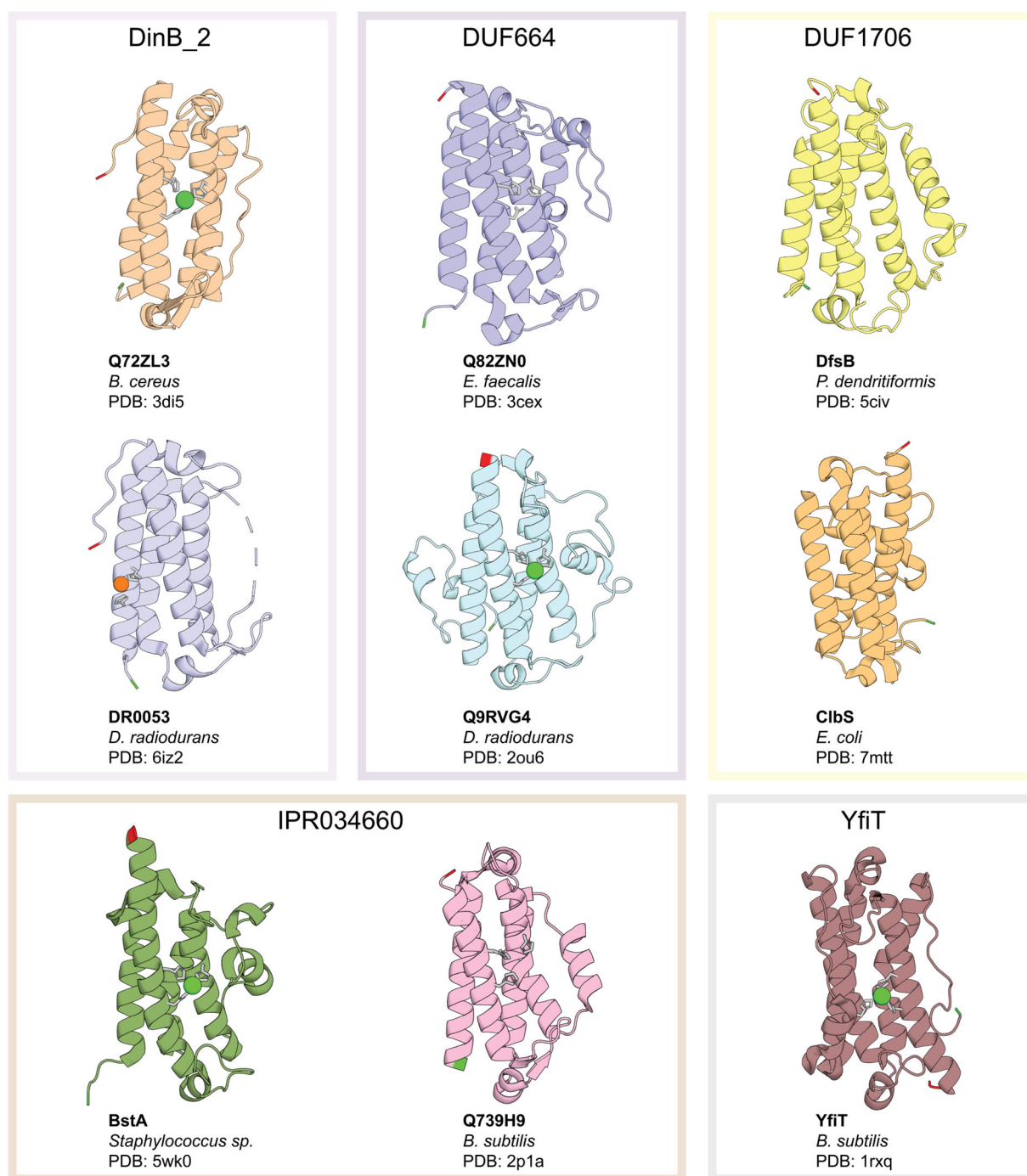
Supplementary Figure 6 Analysis of DinB_2 and DUF664 proteins in Msm. (a) Alignment of the DUF664 protein MSMEG_1356 and its closest homolog in Msm, MSMEG_3923, as well as DinB_2 proteins MSMEG_1357 and its closest homolog MSMEG_3910 in Msm. MSMEG_1356 and MSMEG_3923 share 31% sequence identity and MSMEG_1357 and MSMEG_3910 share 32% sequence identity. Alignment is colored according to sequence identity. (b) RT-qPCR shows that the DinB_2 gene *msmeg_3910* is upregulated upon genotoxic stress while DUF664 gene *msmeg_3923* transcript levels remain unaltered. Relative transcript levels were calculated by normalizing C_T values of *msmeg_3910* and *msmeg_3923* against *sigA*. Thus, $2^{-\Delta C_T}$ values were calculated and normalized to untreated cells. (c) Msm SMR5 wild-type cells were cultured as described before and treated with 0.2, 1 or 5 mM H_2O_2 . RT-qPCR analysis shows that *msmeg_3910* transcript levels increase in a concentration-dependent manner when cells are treated with H_2O_2 . (d) *SiwR* does not regulate *msmeg_3910* as $\Delta siwR$ cells and wild-type cells did not show a difference in *msmeg_3910* upregulation upon H_2O_2 treatment. Müller et al. identified *msmeg_3910* in the PafBC regulon. Relative transcript levels were calculated by normalizing C_T values of *msmeg_3910* against *sigA*. Next, ΔC_T of cells after H_2O_2 treatment were normalized against ΔC_T values of cells before H_2O_2 treatment, resulting in the depicted $2^{-\Delta\Delta C_T}$ values. (e) AlphaFold2 predictions of homodimeric MSMEG_1357 (left) and homodimeric MSMEG_1356 (right) are shown. Both predictions show a four helix bundle typical for the DinB superfamily. Both proteins feature an atypical metal binding triad, and the putative metal binding residues of MSMEG_1357 and MSMEG_1356 are depicted in stick representation (grey). The N-terminus is shown in green and the C-terminus in red. (f) Analytical size exclusion chromatography (Superdex 200) reveals homodimer formation of recombinantly expressed and purified MSMEG_1357 and MSMEG_1356. (g) Circular dichroism of 1 mg/mL MSMEG_1357 and MSMEG_1356 shows that both proteins exhibit mostly alpha-helical secondary structure. Error bars represent the three biological replicates shown as individual data points.

Figure S7



Supplementary Figure 7 A double deletion of *siwR* together with the only other DinB_2 gene in *Msm*, *msmeg_3910*, does not result in decreased viability during zeocin exposure. Resazurin-based viability assay was performed with zeocin in *Msm* SMR5 wild-type, $\Delta siwR$ cells and $\Delta siwR \Delta msmeg_3910$ double knock-out cells. The $\Delta siwR \Delta msmeg_3910$ double knock-out behaves similar to wild-type and $\Delta siwR$ cells. The error bars represent three independent biological replicates.

Figure S8



Supplementary Figure 8 Structural comparison of proteins from the DALI analysis of MSMEG_1357 and MSMEG_1356 AlphaFold2 models. All proteins are shown as monomers. The characteristic four-helix bundle can be observed for all structures solved to date. The N-terminus is depicted in green and the C-terminus in red. Some DinB superfamily protein structures were solved in complex with a metal ion (green = Zn^{2+} , orange = Ni^{2+}) ¹⁻⁴. Putative metal binding residues are shown in stick representation.

Supplementary Tables

Supplementary Table 1. SiwR orthologs can be found in almost all mycobacteria belonging to the genus Mycolicibacterium and mostly in rapid-growing mycobacteria.

Gene ID	Locus Tag	Genome ID	Genome Name	rapid	slow	Genus*
2865517971	Ga0437353_056_262886_263845	2865512620	<i>Mycobacterium aquaticum</i> RW6	x		Mycolicibacterium
2810639375	Ga0316761_10582	2808606769	<i>Mycobacterium asiaticum</i> 1081914.2		x	Mycobacterium
2865745128	Ga0437391_07_8037_9002	2865743993	<i>Mycobacterium boenickei</i> CCUG 47580	x		Mycolicibacterium
2810676650	Ga0317055_1152469	2808606776	<i>Mycobacterium chubuense</i> NCTC 10819	x		Mycolicibacterium
2856260760	Ga0350155_07_162_1112	2856257805	<i>Mycobacterium diernhoferi</i> Bard	x		Mycolicibacterium
2772269269	Ga0225922_155884	2772190613	<i>Mycobacterium dioxanotrophicus</i> PH-06	x		Mycolicibacterium
2865762656	Ga0437393_88_42891_43844	2865757452	<i>Mycobacterium duvalii</i> IP141180004	x		Mycolicibacterium
2815830098	Ga0316778_123304	2814123382	<i>Mycobacterium fortuitum</i> 1089381.4	x		Mycolicibacterium
2932033967	Ga0498898_08_202317_203282	2932029347	<i>Mycobacterium frederiksbergense</i> AC80	x		Mycolicibacterium
2856271497	Ga0337181_079_20121_21089	2856268898	<i>Mycobacterium goodii</i> ST0139456	x		Mycolicibacterium
2813701735	Ga0316775_112310	2811995285	<i>Mycobacterium gordonae</i> 1245695.6		x	Mycobacterium
2862837101	Ga0347309_002_174093_175046	2862836763	<i>Mycobacterium grossiae</i> SCH	x		Mycolicibacterium
2772238408	Ga0165373_105622	2772190608	<i>Mycobacterium holsaticum</i> M7	x		Mycolicibacterium
2823686450	Ga0372454_1286	2823685165	<i>Mycobacterium iranikum</i> AT2.18	x		Mycolicibacterium
2662566349	Ga0113518_169142	2660238483	<i>Mycobacterium lehmannii</i> IS-1744	x		Mycolicibacterium
2918428401	Ga0451163_02_2143413_2144387	2918424375	<i>Mycobacterium lutetiense</i> DSM 46713	x		Mycolicibacterium
2810610378	Ga0316769_103953	2808606764	<i>Mycobacterium mucogenicum</i> 1127319.6	x		Mycolicibacterium
2872608766	Ga0341489_001_336250_337242	2872608444	<i>Mycobacterium neglectum</i> CECT 8778	x		Mycobacterium
2862836272	Ga0342634_191_17983_18939	2862830631	<i>Mycobacterium palauense</i> CECT 8779	x		Mycobacterium
2923208218	Ga0399136_01_1157000_1157959	2923207141	<i>Mycobacterium paragordonae</i> 49061		x	Mycobacterium
2810113079	Ga0316820_115844	2808606628	<i>Mycobacterium peregrinum</i> 852002-10433_SCH5171157	x		Mycolicibacterium
2805940331	Ga0309165_111878	2802429607	<i>Mycobacterium porcinum</i> CSURP1564	x		Mycolicibacterium
2629283683	Ga0077766_103741	2627853864	<i>Mycobacterium senegalense</i> CK1	x		Mycolicibacterium
2810545176	Ga0316807_101472	2808606749	<i>Mycobacterium setense</i> 852014-10208_SCH5295773	x		Mycolicibacterium

2862872879	Ga0335895_257_259796_260764	2862867363	<i>Mycobacterium syngnathidarum</i> 24999	x		Mycolicibacterium
2810532694	Ga0316831_12743	2808606746	<i>Mycobacterium vulneris</i> ACS3670		x	Mycobacterium
2684861730	Ga0124157_11491434	2684622559	<i>Mycolicibacter nonchromogenicus</i> NCK 8460		x	Mycolicibacter
2871778925	Ga0335707_05_1014332_1015288	2871775133	<i>Mycolicibacterium acapulense</i> CSURP1424	x		Mycolicibacterium
2865757098	Ga0437392_261_102161_103114	2865750426	<i>Mycolicibacterium agri</i> CCUG 37673	x		Mycolicibacterium
2856229043	Ga0350748_01_719437_720390	2856228341	<i>Mycolicibacterium aichiense</i> NCTC 10820	x		Mycolicibacterium
2888255061	Ga0439780_01_5165947_5166909	2888250102	<i>Mycolicibacterium anyangense</i> JCM 30275	x		Mycolicibacterium
2888235462	Ga0439569_02_2490170_2491126	2888232652	<i>Mycolicibacterium arabiense</i> JCM 18538	x		Mycolicibacterium
2774726954	Ga0226595_111731	2773857881	<i>Mycolicibacterium aurum liquid</i>	x		Mycolicibacterium
2649755901	Ga0056865_12037	2648501395	<i>Mycolicibacterium austroafricanum</i> DSM 44191	x		Mycolicibacterium
2865529305	Ga0437355_22_196385_197335	2865526555	<i>Mycolicibacterium bacteremicum</i> DSM 45578	x		Mycolicibacterium
2856280505	Ga0347052_06_233072_234037	2856275449	<i>Mycolicibacterium boenickei</i> CIP107829	x		Mycolicibacterium
2726225140	Ga0126509_124338	2724679284	<i>Mycolicibacterium brisbanense</i>	x		Mycolicibacterium
2726233621	Ga0126510_107041	2724679285	<i>Mycolicibacterium canariasense</i>	x		Mycolicibacterium
2807076733	Ga0316717_110137	2806310651	<i>Mycolicibacterium celeriflavum</i> 852002-51296_SCH5728562-a	x		Mycolicibacterium
2867056182	Ga0437590_01_4035433_4036392	2867052265	<i>Mycolicibacterium chitae</i> NCTC 10485	x		Mycolicibacterium
2632775721	Ga0080924_15198	2630968674	<i>Mycolicibacterium chlorophenolicum</i> DSM 43826	x		Mycolicibacterium
2632010368	Ga0080926_11835	2630968491	<i>Mycolicibacterium chubuense</i> DSM 44219	x		Mycolicibacterium
2629182306	Ga0078594_1011462	2627853841	<i>Mycolicibacterium conceptionense</i> D16	x		Mycolicibacterium
2865352701	Ga0437000_01_806399_807349	2865351897	<i>Mycolicibacterium confluentis</i> DSM 44017	x		Mycolicibacterium
2620969673	Ga0055289_13642	2619619211	<i>Mycolicibacterium cosmeticum</i> DSM 44829	x		Mycolicibacterium
2810207619	Ga0316799_117712	2808606658	<i>Mycolicibacterium elephantis</i> 1477680.9	x		Mycolicibacterium
2612471674	Ga0057456_120153	2609460330	<i>Mycolicibacterium farcinogenes</i> DSM 43637		x	Mycolicibacterium
2772243657	Ga0165374_105144	2772190609	<i>Mycolicibacterium flavescens</i> M6	x		Mycolicibacterium
2821253103	Ga0310449_1277	2821251827	<i>Mycolicibacterium fluoranthenorans</i> DSM 44556	x		Mycolicibacterium
2869233756	Ga0351979_64_176123_177091	2869227690	<i>Mycolicibacterium fortuitum acetamidolyticum</i>	x		Mycolicibacterium
2871781718	Ga0350749_08_1157501_1158454	2871780381	<i>Mycolicibacterium gilvum</i> NCTC 10742	x		Mycolicibacterium
2515215201	A3G5DRAFT_01317	2515154012	<i>Mycolicibacterium hassiacum</i> DSM 44199	x		Mycolicibacterium
2888227740	Ga0439836_01_1357012_1357965	2888226484	<i>Mycolicibacterium helvum</i> JCM 30396	x		Mycolicibacterium

2902852786	Ga0439948_02_1848562_1849521	2902850712	<i>Mycolicibacterium hippocampi</i> JCM 30996	x	Mycolicibacterium
2677201906	Ga0129282_106525	2675903240	<i>Mycolicibacterium iranikum</i> H39	x	Mycolicibacterium
2707836928	Ga0124155_15520	2706794658	<i>Mycolicibacterium komaniense</i> GPK 1020	x	Mycolicibacterium
2869211860	Ga0337360_055_234587_235543	2869209271	<i>Mycolicibacterium kyogaense</i> NCTC 11659	x	Mycolicibacterium
2682293914	Ga0104440_1743	2681812963	<i>Mycolicibacterium litorale</i> CGMCC 4.5724	x	Mycolicibacterium
2638082730	Ga0077732_100385	2636415805	<i>Mycolicibacterium llutzerense</i> CLUC14	x	Mycolicibacterium
2618699836	Ga0055290_111005	2617271075	<i>Mycolicibacterium mageritense</i> DSM 44476	x	Mycolicibacterium
2672235888	Ga0124156_105568	2671180307	<i>Mycolicibacterium malmesburii</i> WCM 7299	x	Mycolicibacterium
2807078316	Ga0316815_10012	2806310652	<i>Mycolicibacterium monacense</i> 852013-50142_SCH4511227	x	Mycolicibacterium
2865566250	Ga0437362_161_14936_15895	2865562250	<i>Mycolicibacterium moriokaense</i> CIP105393	x	Mycolicibacterium
2869217203	Ga0350077_172_4571_5533	2869214934	<i>Mycolicibacterium mucogenicum</i> CCH10-A2	x	Mycolicibacterium
2601120661	Ga0056738_04831	2600255158	<i>Mycolicibacterium neoaurum</i> ATCC 25795	x	Mycolicibacterium
2691100840	Ga0124169_113060	2690315792	<i>Mycolicibacterium neworleansense</i> ATCC 49404	x	Mycolicibacterium
2875560324	Ga0398566_01_5175826_5176794	2875555210	<i>Mycolicibacterium nivoides</i> DL90	x	Mycolicibacterium
2726240483	Ga0126511_105634	2724679286	<i>Mycolicibacterium novocastrense</i>	x	Mycolicibacterium
2638242498	Ga0080925_157295	2636415843	<i>Mycolicibacterium obuense</i> DSM 44075	x	Mycolicibacterium
2865576623	Ga0437364_21_49345_50298	2865573456	<i>Mycolicibacterium parafortuitum</i> CCUG 20999	x	Mycolicibacterium
2777979999	Ga0124348_15386	2775507222	<i>Mycolicibacterium peregrinum</i> CSUR P2098	x	Mycolicibacterium
2687181912	Ga0133311_111248	2684623205	<i>Mycolicibacterium phlei</i> CCUG21000	x	Mycolicibacterium
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2888246346	Ga0439622_01_2391708_2392661	2888243993	<i>Mycolicibacterium sediminis</i> JCM 17899	x	Mycolicibacterium
2553039028	NoneDRAFT_01745	2551306423	<i>Mycolicibacterium septicum</i> DSM 44393	x	Mycolicibacterium
2651407372	Ga0078462_105220	2648501822	<i>Mycolicibacterium setense</i> DSM 45070	x	Mycolicibacterium
639739896	MSMEG_1359	639633041	<i>Mycolicibacterium smegmatis</i> MC2 155	x	Mycolicibacterium
2869223170	Ga0365508_10_112402_113355	2869221657	<i>Mycolicibacterium sphagni</i> ATCC 33027	x	Mycolicibacterium
2726245393	Ga0126512_12726	2724679287	<i>Mycolicibacterium thermoresistibile</i>	x	Mycolicibacterium
2869197719	Ga0350752_03_1638106_1639056	2869196048	<i>Mycolicibacterium tokaiense</i> NCTC 10821	x	Mycolicibacterium
2865605184	Ga0437369_33_118322_119308	2865601517	<i>Mycolicibacterium tusciae</i> DSM 44338	x	Mycolicibacterium

2689431429	Ga0131697_111052	2687453599	<i>Mycolicibacterium vaccae</i> 95051	x	Mycolicibacterium
639804748	Mvan_1247	639633044	<i>Mycolicibacterium vanbaalenii</i> PYR-1	x	Mycolicibacterium
2743800569	Ga0132655_124109	2740892518	<i>Mycolicibacterium wolinskyi</i> CDC_01	x	Mycolicibacterium

* According to Gupta et al., *Frontiers in Microbiology*, 2018

Supplementary Table 2. Gene ID numbers of SiwR, MSMEG_1357 and MSMEG_1356 orthologs in other Actinobacteria according to Figure 1C.

Gene ID	Locus Tag	Genome ID	Genome Name	Pfam/Families	Ortholog
2778382424	Ga0197499_2216	2778260946	Terracoccus luteus DSM 44267	pfam08279, pfam13280	SiwR
2778382425	Ga0197499_2217	2778260946	Terracoccus luteus DSM 44267	pfam12867	MSMEG1357
2778382426	Ga0197499_2218	2778260946	Terracoccus luteus DSM 44267	pfam04978	MSMEG1356
2813781592	Ga0163555_103337	2811995306	Corynebacterium variabile NRRL B-4201	pfam08279, pfam13280	SiwR
2813781591	Ga0163555_103336	2811995306	Corynebacterium variabile NRRL B-4201	pfam12867	MSMEG1357
2813781590	Ga0163555_103335	2811995306	Corynebacterium variabile NRRL B-4201	pfam04978	MSMEG1356
2865156176	Ga0437312_08_7555_8520	2865155884	Arthrobacter rhombi B Ar 00.02	pfam08279, pfam13280	SiwR
2865156175	Ga0437312_08_6894_7493	2865155884	Arthrobacter rhombi B Ar 00.02	pfam12867	MSMEG1357
2865156174	Ga0437312_08_6207_6515	2865155884	Arthrobacter rhombi B Ar 00.02	pfam04978	MSMEG1356
2623589907	Ga0070624_4981	2622736626	Micromonospora rhizosphaerae DSM 45431	pfam08279, pfam13280	SiwR
2623589908	Ga0070624_4982	2622736626	Micromonospora rhizosphaerae DSM 45431	pfam12867	MSMEG1357
2623589909	Ga0070624_4983	2622736626	Micromonospora rhizosphaerae DSM 45431	pfam18029	
2547161974	SSPNDRAFT_07978	2547132038	Saccharopolyspora spinosa NRRL 18395	pfam08279, pfam13280	SiwR
2547161973	SSPNDRAFT_07977	2547132038	Saccharopolyspora spinosa NRRL 18395	pfam12867	MSMEG1357
2547161972	SSPNDRAFT_07976	2547132038	Saccharopolyspora spinosa NRRL 18395	pfam18029	
2666353147	Ga0111659_10895	2681813555	Nocardia farcinica DSM 43257	pfam08279, pfam13280	SiwR
2666353146	Ga0111659_10894	2681813555	Nocardia farcinica DSM 43257	pfam12867	MSMEG1357
2666353145	Ga0111659_10893	2681813555	Nocardia farcinica DSM 43257	IPR013813	
2514710500	AK37_22817	2513237387	Rhodococcus pyridinivorans AK37	pfam08279, pfam13280	SiwR
2514710501	AK37_22822	2513237387	Rhodococcus pyridinivorans AK37	pfam12867	MSMEG1357
2514710502	AK37_22827	2513237387	Rhodococcus pyridinivorans AK37	pfam18029	
2875428019	Ga0198576_01_613887_614921	2875427445	Streptomyces hygroscopicus XM201	pfam08279, pfam13280	SiwR
2875428020	Ga0198576_01_615021_615599	2875427445	Streptomyces hygroscopicus XM201	pfam04978	MSMEG1356
2875428021	Ga0198576_01_615697_616737	2875427445	Streptomyces hygroscopicus XM201	pfam00248	

Supplementary Table 3. DinB superfamily proteins in Msm. Families according to Interpro are depicted as well as Mtb homologs.

Accession	Locus TAG	Name	Tax ID	Tax Name	Length	Entry Accession	Matches	F_Interpro	Mtb homolog
A0QS55	MSMEG_1357	DinB_2 domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	175	IPR034660	3..166	DinB_2	-
A0QZ61	MSMEG_3910	DinB_2 domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	168	IPR034660	50..164	DinB_2	-
A0QQV2	MSMEG_0887	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	185	IPR034660	15..183	DUF664	-
A0QS54	MSMEG_1356	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	203	IPR034660	2..174	DUF664	-
A0QZ74	MSMEG_3923	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	167	IPR034660	2..166	DUF664	-
A0QWI8	MSMEG_2957	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	205	IPR034660	1..162	MDMPI	-
A0R422	MSMEG_5677	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	200	IPR034660	1..155	MDMPI	-
A0QUL0	MSMEG_2246	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	220	IPR034660	1..154	MDMPI	-
A0QPB1	MSMEG_0336	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	264	IPR034660	2..152	IPR017517	-
A0R7I0	MSMEG_6923	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	257	IPR034660	2..151	IPR017517	Rv0036c
A0QX68	MSMEG_3192	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	253	IPR034660	3..139	IPR017517	-
A0QQA2	MSMEG_0682	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / mc(2)155)	250	IPR034660	3..136	IPR017517	Rv0332

A0QPL6	MSMEG_0441	Uncharacterized protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	242	IPR034660	25..134	IPR017517	-
A0R355	MSMEG_5349	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	202	IPR034660	3..126	IPR017517	-
A0R749	MSMEG_6779	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	215	IPR034660	3..131	IPR017517	-
A0QQC2	MSMEG_0703	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	207	IPR034660	5..112	IPR017517	Rv1929c
A0R296	MSMEG_5034	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	228	IPR034660	1..134	IPR017517	
A0QWM8	MSMEG_2998	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	178	IPR034660	22..178	none predicted but MDMPI domain	-
A0R798	MSMEG_6832	MDMPI_N domain-containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	207	IPR034660	15..143	IPR017517	Rv3773c
A0QP84	MSMEG_0307	HTH araC/xylS-type domain- containing protein	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	302	IPR034660	146..297	none predicted but MDMPI domain	-
A0R5N0	MSMEG_6249	Hercynine oxygenase	246196	<i>Mycolicibacterium smegmatis</i> (strain ATCC 700084 / <i>mc(2)155</i>)	428	IPR034660	2..141	IPR017806; IPR032890	Rv3703c

Supplementary Table 4. DinB superfamily proteins in Mtb. Families according to Interpro are depicted as well as Msm homologs.

Accession	Locus tag	Name	Tax ID	Tax Name	Length	Entry Accession	Matches	F_Interpro	Msm homolog
O05777	Rv3099c	MDMPI_N domain-containing protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	283	IPR034660	9..166	IPR017517	
O07256	Rv0332	MDMPI_N domain-containing protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	261	IPR034660	13..144	IPR017517	MSMEG_0682
O53480	Rv2036	MDMPI_N domain-containing protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	213	IPR034660	2..132	IPR017517	
O53728	Rv0443	DUF664 domain-containing protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	171	IPR034660	4..171	DUF664	
O53773	Rv0576	HTH arsR-type domain-containing protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	434	IPR034660	243..372	IPR017517	
O69671	Rv3703c	Hercynine oxygenase	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	425	IPR034660	5..133	IPR017806; IPR032890	MSMEG_6249
P71985	Rv1727	MDMPI_N domain-containing protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	189	IPR034660	5..135	IPR017517; IPR017520	
P72040	Rv3773c	TIGR03086 family protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	194	IPR034660	11..138	IPR017517; IPR017520	MSMEG_6832
P95285	Rv1929c	TIGR03085 family protein	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	214	IPR034660	10..120	IPR017517; IPR017519	MSMEG_0703
P9WKS3	Rv0738	Uncharacterized protein Rv0738	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	182	IPR034660	2..126	IPR017517; IPR017520	
P9WM91	Rv0036c	Uncharacterized protein Rv0036c	83332	<i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)	257	IPR034660	1..152	IPR017517; IPR017518	MSMEG_6923

Supplementary Table 5. DALI search results with Z-scores > 10.0 for the alphafold predicted MSMEG_1357 model.

Chain	Z	rmsd	lali	nres	%id	Uniprot	organism	publication	released
3di5-A	13.1	2.3	138	150	15	Q72ZL3	<i>Bacillus cereus</i>	to be published	2008
3cex-B	12.6	3.2	139	170	14	Q82ZN0	<i>Enterococcus faecalis</i> V583	to be published	2008
5civ-A	12.6	2.9	138	169	17	D0EVD3	<i>Paenibacillus dendritiformis</i>	Structures of the DfsB Protein Family Suggest a Cationic, He Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
5cog-B	12.5	2.7	138	169	12	Q03036	<i>Saccharomyces cerevisiae</i>	Structures of the DfsB Protein Family Suggest a Cationic, He Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
2p1a-B	12.5	2.5	135	150	13	Q739H9	<i>Bacillus cereus</i>	to be published	2007
5com-A	12.2	2.5	136	171	12	Q187F5	<i>Clostridioides difficile</i> 630	Structures of the DfsB Protein Family Suggest a Cationic, He Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
2ou6-A	12.2	2.3	133	183	19	Q9RVG4	<i>Deinococcus radiodurans</i>	to be published	2007
2qnl-A	12.1	2.7	134	162	10		<i>Cytophage hutchinsonii</i> ATCC 33406	to be published	2007
3gor-D	12.1	2.8	135	151	11	D0VX25	<i>Geobacillus stearothermophilus</i>	The structure of DinB from <i>Geobacillus stearothermophilus</i> : a representative of a unique four-helix-bundle superfamily (Cooper et al., 2010)	2010
7mtt-A	12.1	2.4	131	169	11	Q0P7K8	<i>Escherichia coli</i>	Structural Basis for the Interactions of the Colibactin Resistance Gene Product CIBS with DNA (Tripathi et al., 2021)	2021
6anr-A	12.1	2.4	132	169	11	Q0P7K8	<i>Escherichia coli</i>	CIBS Is a Cyclopropane Hydrolase That Confers Colibactin Resistance (Tripathi et al., 2017)	2017
7mtl-B	12.0	2.3	128	162	12	Q0P7K8	<i>Escherichia coli</i>	Structural Basis for the Interactions of the Colibactin Resistance Gene Product CIBS with DNA (Tripathi et al., 2021)	2021
5cof-A	11.9	2.5	133	172	8	Q1R1X2	<i>Escherichia coli</i> UT189	Structures of the DfsB Protein Family Suggest a Cationic, He Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
6iz2-A	11.7	2.4	130	145	8	Q9RY97	<i>Deinococcus radiodurans</i>	Crystal structure of the highly radiation-inducible DinB/YfiT superfamily protein DR0053 from <i>Deinococcus radiodurans</i> (Zhang et al., 2019)	2019
2nsf-A	11.7	2.5	127	240	14	Q8NLC1	<i>Corynebacterium glutamicum</i>	Crystal Structures and Site-directed Mutagenesis of a Mycothiol-dependent Enzyme Reveal a Novel Folding and Molecular Basis for Mycothiol-mediated Maleylpyruvate Isomerization (Wang et al., 2007)	2007
4n6c-B	11.5	2.7	136	181	12		<i>Streptococcus pneumoniae</i>	to be published	2013
2yqy-A	11.4	2.7	121	126	13	Q5SLJ0	<i>Thermus thermophilus</i> HB8	Crystal structure of TTHA0303 (TT2238), a four-helix bundle protein with an exposed histidine triad from <i>Thermus thermophilus</i> at 2.0 Å (Nagata et al., 2008)	2008

2nsg-A	11.4	2.4	127	240	14	Q8NLC1	<i>Corynebacterium glutamicum</i>	Crystal Structures and Site-directed Mutagenesis of a Mycothiol-dependent Enzyme Reveal a Novel Folding and Molecular Basis for Mycothiol-mediated Maleylpyruvate Isomerization (Wang et al., 2007)	2007
2hkv-A	11.2	2.6	127	148	11	B1YEV7	<i>Exiguobacterium sibiricum</i> 255-15	to be published	2006
2qe9-B	11.2	2.8	136	162	13	Q7WY73	<i>Bacillus subtilis</i>	to be published	2007
6qki-A	11.1	3.0	134	395	10	G2LET6	<i>Chloroacidobacterium thermophilum B</i>	An Alternative Active Site Architecture for O ₂ Activation in the Ergothioneine Biosynthetic EgtB from Chloracidobacterium thermophilum (Stampfli et al., 2019)	2019
3dka-B	11.1	2.7	127	144	13	O34334	<i>Bacillus subtilis</i>	to be published	2008
6o6l-A	10.9	3.1	134	407	10	G2LET6	<i>Chloroacidobacterium thermophilum B</i>	Crystal Structure of the Ergothioneine Sulfoxide Synthase from Candidatus Chloracidobacterium thermophilum and Structure Guided Engineering To Modulate Its Substrate Selectivity (Naowarojna et al., 2019)	2019
6o6m-B	10.8	3.1	133	406	11	G2LET6	<i>Chloroacidobacterium thermophilum B</i>	Crystal Structure of the Ergothioneine Sulfoxide Synthase from Candidatus Chloracidobacterium thermophilum and Structure Guided Engineering To Modulate Its Substrate Selectivity (Naowarojna et al., 2019)	2019
5wk0-A	10.8	2.9	133	156	16	A0A2C9TMM3	<i>Staphylococcus sp. HMSC055H04</i>	Structure and function of the bacillithiol-S-transferase BstA from Staphylococcus aureus (Francis et al., 2018)	2018
4x8e-B	10.7	4.1	128	426	16	G7CFI3	<i>Mycolicibacterium thermoresistibile</i> ATCC 19527	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
4x8d-B	10.7	4.1	129	427	16	G7CFI3	<i>Mycolicibacterium thermoresistibile</i> ATCC 19527	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
4x8b-B	10.7	4.1	129	426	16	G7CFI3	<i>Mycolicibacterium thermoresistibile</i> ATCC 19527	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
1rxq-D	10.3	3.1	136	175	17	O31562	<i>Bacillus subtilis</i>	YfiT from Bacillus subtilis Is a Probable Metal-Dependent Hydrolase with an Unusual Four-Helix Bundle Topology (Rajan et al., 2004)	2004
2rd9-C	10.2	4.5	141	186	13	Q9KGB8	<i>Alkalihalobacillus halodurans</i> C-125	to be published	2007

Supplementary Table 6. DALI search results with Z-scores > 10.0 for the alphafold predicted MSMEG_1356 model.

Chain	Z	rmsd	lali	nres	%id	Uniprot	organism	publication	released
2ou6-A	14.6	2.5	146	183	23	Q9RVG4	<i>Deinococcus radiodurans</i>	to be published	2007
3cex-A	14.1	2.6	139	170	17	Q82ZN0	<i>Enterococcus faecalis</i> V583	to be published	2008
6qki-A	14	3.9	134	395	10	G2LET6	<i>Chloroacidobacterium thermophilum</i> B	An Alternative Active Site Architecture for O ₂ Activation in the Ergothioneine Biosynthetic EgtB from Chloracidobacterium thermophilum (Stampfli et al., 2019)	2019
5civ-A	13.9	3.4	152	169	9	D0EVD3	<i>Paenibacillus dendritiformis</i>	Structures of the DfsB Protein Family Suggest a Cationic, Helical Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
2yqy-A	13.8	2.2	120	126	13	Q5SLJ0	<i>Thermus thermophilus</i> HB8	Crystal structure of TTHA0303 (TT2238), a four-helix bundle protein with an exposed histidine triad from Thermus thermophilus HB8 at 2.0 Å (Nagata et al., 2008)	2008
6o6l-A	13.7	4	134	407	10	G2LET6	<i>Chloroacidobacterium thermophilum</i> B	Crystal Structure of the Ergothioneine Sulfoxide Synthase from Candidatus Chloracidobacterium thermophilum and Structure-Guided Engineering To Modulate Its Substrate Selectivity (Naowarajna et al., 2019)	2019
4x8b-A	13.6	4.5	138	428	12	G7CFI3	<i>Mycolicibacterium thermoresistibile</i> ATCC 19527	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
5cog-A	13.6	3.1	146	168	10	Q03036	<i>Saccharomyces cerevisiae</i>	Structures of the DfsB Protein Family Suggest a Cationic, Helical Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
1rxq-A	13.4	2.8	145	174	13	O31562	<i>Bacillus subtilis</i>	YfiT from Bacillus subtilis Is a Probable Metal-Dependent Hydrolase with an Unusual Four-Helix Bundle Topology (Rajan et al., 2004)	2004
4n6c-A	13.4	3.3	151	182	7		<i>Streptococcus pneumoniae</i>	to be published	2013
4x8d-A	13.4	6.1	141	429	11	G7CFI3	<i>Mycolicibacterium thermoresistibile</i> ATCC 19527	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
5com-A	13.4	3.1	149	171	7	Q187F5	<i>Clostridioides difficile</i> 630	Structures of the DfsB Protein Family Suggest a Cationic, Helical Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016

6iz2-A	13.3	2.5	136	145	14	Q9RY97	<i>Deinococcus radiodurans</i>	Crystal structure of the highly radiation-inducible DinB/YfiT superfamily protein DR0053 from <i>Deinococcus radiodurans</i> R1 (Zhang et al., 2019)	2019
2nsf-A	13.2	5.4	142	240	9	Q8NLC1	<i>Corynebacterium glutamicum</i>	Crystal Structures and Site-directed Mutagenesis of a Mycothiol-dependent Enzyme Reveal a Novel Folding and Molecular Basis for Mycothiol-mediated Maleylpyruvate Isomerization (Wang et al., 2007)	2007
2nsg-A	12.6	5.4	142	240	8	Q8NLC1	<i>Corynebacterium glutamicum</i>	Crystal Structures and Site-directed Mutagenesis of a Mycothiol-dependent Enzyme Reveal a Novel Folding and Molecular Basis for Mycothiol-mediated Maleylpyruvate Isomerization (Wang et al., 2007)	2007
2p1a-A	12.5	2.7	134	143	12	Q739H9	<i>Bacillus cereus</i>	to be published	2007
2rd9-A	12.5	4.6	149	189	14	Q9KGB8	<i>Alkalihalobacillus halodurans</i> C-125	to be published	2007
5cof-A	12.5	3.3	146	172	11	Q1R1X2	<i>Escherichia coli</i> UT189	Structures of the DfsB Protein Family Suggest a Cationic, Helical Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016
6anr-A	12.5	3.5	146	169	14	Q0P7K8	<i>Escherichia coli</i>	CibS Is a Cyclopropane Hydrolase That Confers Colibactin Resistance (Tripathi et al., 2017)	2017
2hkv-A	12.4	3	139	148	13	B1YEV7	<i>Exiguobacterium sibiricum</i> 255-15	to be published	2006
2qnl-A	12.3	3	130	162	12		<i>Cytophage hutchinsonii</i> ATCC 33406	to be published	2007
2f22-A	12.2	3.1	133	142	14	Q9RC77	<i>Halalkalibacterium halodurans</i>	to be published	2005
2qe9-A	11.9	2.6	135	165	16	Q7WY73	<i>Bacillus subtilis</i>	to be published	2007
3gor-A	11.8	3.1	143	157	8	D0VX25	<i>Geobacillus stearothermophilus</i>	The structure of DinB from <i>Geobacillus stearothermophilus</i> : a representative of a unique four-helix-bundle superfamily (Cooper et al., 2010)	2010
4x8e-A	11.8	5.4	139	428	11	G7CFI3	<i>Mycolicibacterium thermoresistibile</i> ATCC 19527	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
6o6m-A	11.7	4	134	407	10	G2LET6	<i>Chloroacidobacterium thermophilum</i> B	Crystal Structure of the Ergothioneine Sulfoxide Synthase from Candidatus <i>Chloroacidobacterium thermophilum</i> and Structure-Guided Engineering To Modulate Its Substrate Selectivity (Naowarojna et al., 2019)	2019
3di5-A	11.6	2.5	135	150	11	Q72ZL3	<i>Bacillus cereus</i>	to be published	2008

6qkj-A	11.6	4.5	140	417	11	G2LET6	<i>Chloroacidobacterium thermophilum B</i>	Crystal Structure of the Ergothioneine Sulfoxide Synthase from Candidatus Chloracidobacterium thermophilum and Structure-Guided Engineering To Modulate Its Substrate Selectivity (Naowarajna et al., 2019)	2019
3dka-A	11.5	3.6	135	143	13	O34334	<i>Bacillus subtilis</i>	to be published	2006
5wk0-A	10.9	3.1	128	156	11	A0A2C9TMM3	<i>Staphylococcus sp. HMSC055H04</i>	Structure and function of the bacillithiol-S-transferase BstA from Staphylococcus aureus (Francis et al., 2018)	2018
4x8d-A	13.4	6.1	141	429	11	G7CFI3	<i>Mycobacterium thermoresistibile ATCC 19527</i>	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
4x8e-A	11.8	5.4	139	428	11	G7CFI3	<i>Mycobacterium thermoresistibile ATCC 19527</i>	Structure of the Sulfoxide Synthase EgtB from the Ergothioneine Biosynthetic Pathway (Goncharenko et al., 2015)	2015
6o6m-A	11.7	4	134	407	10	G2LET6	<i>Chloroacidobacterium thermophilum B</i>	Crystal Structure of the Ergothioneine Sulfoxide Synthase from Candidatus Chloracidobacterium thermophilum and Structure-Guided Engineering To Modulate Its Substrate Selectivity (Naowarajna et al., 2019)	2019
5cof-A	12.5	3.3	146	172	11	Q1R1X2	<i>Escherichia coli UT189</i>	Structures of the DfsB Protein Family Suggest a Cationic, Helical Sibling Lethal Factor Peptide (Taylor et al., 2016)	2016

Supplementary Table 7. Oligonucleotide primers used in this study.

Sequence (5' → 3')	Purpose
taaaagtgctcatcattggaaaatcggttcagttccgccg	Upstream to msme _g _1359
gcgacaagtgctgccgcacttttagg	msme _g _1359 to upstream
ttttcggcccgaagaacggccctcgaccgtgatgatgg	Downstream to msme _g _1359
gtgcggcagactgtgcTCAGGTGAtcccgagccggtc	msme _g _1359 to downstream
gtgatgatcgccgcgatGTGACCCACCTGACAGGCATATAG	siwR Msm wt int fw
catggtcttttagtctgcagaCCGCGACCGCGGGCAACGAT	siwR Msm wt int rv
GACCACTCGGCCGGTGGTGTC	siwR ΔH _{TH} fw
CTGGGCGCCGGAATGGCGTTG	siwR ΔH _{TH} rv
ACCACCTTCACCGCGCGCCC	siwR ΔW _{YL} fw
GTCGGGTGCCACCGCGGTGTC	siwR ΔW _{YL} rv
CCGCGCCACGTAGGGGTACG	siwR ΔW _{CX} fw
TGATCCCGAGCCGGTCCGGTG	siwR ΔW _{CX} rv
ACTCGCCCCCGTGACGCCGAGCCGC	siwR R38A-R42A fw
GTGGCGCGCGACATCGAGCGCCTGC	siwR R38A-R42A rv
GACGCGATGTCGGATGTCCGTGCGA	siwR R204A-R207A fw
GAGCGCCAGGCTGCGCCAGTCTCTCG	siwR R204A-R207A rv
atgcggccgatcatcac	Integrative plasmid fw
tctgcagactacaaagaccatg	Integrative plasmid rv
GCCACCACCAATCTGTTCTCTGTG	siwR ΔH _{TH} Sumo fw
CTGGGCGCCGGAATGGCGTTG	siwR ΔH _{TH} Sumo rv
AAT GGT TGC GCA TCG CGA GGA	ΔsiwR DCO screen fw
TAC ACC GCC GAC GAC GCC AT	ΔsiwR DCO screen rv
GAC TAC ACC AAG GGC TAC AAG	qPCR sigA fw
TTG ATC ACC TCG ACC ATG TG	qPCR sigA rv
GGTCCAACCGAGCGCCTTGA	qPCR rpoB fw
GGTGCGTGGCTGGAGTTCGA	qPCR rpoB rv
GCC TGC ATC GTC ACT TC	qPCR siwR fw
CCG GTT CAA GCA CCT C	qPCR siwR rv
GCTCCATCGATTTAGCTAC	qPCR msme _g _1357 fw
CCGCCGAAGTGTAATG	qPCR msme _g _1357 rv
GGCCATCAACTCGTACATC	qPCR msme _g _1356 fw
GTGGTCATGCACCTCATC	qPCR msme _g _1356 rv
TGGTGTGGACGGTACAT	qPCR msme _g _3910 fw
TCGACCACCAGAAGATCA	qPCR msme _g _3910 rv
CGCGATCTTCATCAACCA	qPCR recA fw

ACCGAGGCGTAGAACTT	qPCR recA rv
GTGACCCACCCTGACAGGCATATAGGACAGT	EMSA a fw, 5'-FAM
ACTGTCCTATATGCCTGTCAGGGTGGGTAC	EMSA a rv
TCCGGTCCTAAAAGTGCGGCAGACTTGTGCG	EMSA b fw, 5'-FAM
GCGACAAGTCTGCCGCACTTTTAGGACCGGA	EMSA b rv
TGACAGGCATATAGGACAGTTCCGGTCCTAAAAGTGCGGC	EMSA c fw, 5'-FAM
GCCGCACTTTTAGGACCGGAACTGTCCTATATGCCTGTCA	EMSA c rv
GGCATATAGGACAGTTCCGGTCCTAAAAGT	EMSA d fw, 5'-FAM
ACTTTTAGGACCGGAACTGTCCTATATGCC	EMSA d rv
TGACCCACCCTGACAGGCATATAGGACAGTTCCGGTCCTAAAAGT GCGGCAGACTTGTGCG	EMSA msmeg1357-siwR fw
CGACAAGTCTGCCGCACTTTTAGGACCGGAACTGTCCTATATGCC TGTCAGGGTGGGTCA	EMSA msmeg1357-siwR rv, 5'-FAM
GACAGGCATAGCATGGCTTTCGCAAGGCTAAAGTGCGGCAGACT TGTCGC	EMSA mutated palindrome fw, 5'-FAM
GCGACAAGTCTGCCGCACTTTAGCCTTGCGAAAGCCATGCTATG CCTGT	EMSA mutated palindrome rv
GTCAGCAGCCTAACTGGACTGACG	5' tail
GCAGTCAGGTCAATCCGACGACTG	3' tail; FAM
CGUCAGUCCAGU	ssRNA
CGTCAGTCCAGT	ssDNA ligand 5'-FAM
CGTCA5TCCAGT	8-oxo 5'-FAM (5 = oxoG)
CGTCAGTCCAGTATCCGACGACTG	Fork nt; 5'-FAM
CAGTCGTCGGATTGACCTGACTGC	Fork t
aagtgtcatcattggaagGCCGTGCCGTGCGTTCGATG	Upstream to <i>msmeg_1357</i>
GTCACATGGACATCACGGCGTAGCCGGTCAGCG	<i>msmeg_1357</i> to upstream
CGCCGTGATGTCCATGTGACCCACCCTGACAGG	<i>msmeg_1357-56</i> to downstream
gttttcgccccgaagaacgGGCCCGGACTTCGCGCTCG	Downstream to <i>msmeg_1357-56</i>
GACACCGACGATCTCATCGGCG	Δ <i>msmeg_1357-56</i> DCO screen fw
ACGCCCCGCGACGCTTCC	Δ <i>msmeg_1357-56</i> DCO screen rv
tgatgatcgccgcatGCGACAAGTCTGCCGCACTTTTAG	<i>msmeg_1357-56</i> wt int fw
ggtctttagtagctgcagaCCAGCAGTGGCTGGCCGG	<i>msmeg_1357-56</i> wt int rv
AAAAGTGCGGCAGACTTGTGCG	<i>msmeg_1357-56</i> wt int -26 mut fw
GCCTTGGGAACTGTCCTATATGCCTGTC	<i>msmeg_1357-56</i> wt int -26 mut rv
ACC TGA CTG GGA ACC AA	qPCR <i>msmeg_0703</i> fw
GAC TTC GCC ATG GTC ATC	qPCR <i>msmeg_0703</i> rv
ACC ACA TCC GGT GAC TAC	qPCR <i>msmeg_6249</i> fw

CGG ACA CCC GAG AAG AT	<i>qPCR msmeg_6249 rv</i>
TTT CAG CTG TAC GGC ATC	<i>qPCR msmeg_0307 fw</i>
CAG TCC CTT CTC ATC CAT TC	<i>qPCR msmeg_0307 rv</i>
TGG TCG GTG CTC GAA TA	<i>qPCR msmeg_2998 fw</i>
GAA CCT GGG ATC GGT CT	<i>qPCR msmeg_2998 rv</i>
GAAGACCATGTCGATCACC	<i>qPCR msmeg_5034 fw</i>
GCGATGGTTTCGTCGTC	<i>qPCR msmeg_5034 rv</i>
GATTTGTGCGTTTGAAGTC	<i>qPCR msmeg_6832 fw</i>
ATGACCGCTTCGGTACT	<i>qPCR msmeg_6832 rv</i>
GATCTGGCAGGACGAAATAG	<i>qPCR msmeg_3923 fw</i>
GCGATCATGTGGACGAAA	<i>qPCR msmeg_3923 rv</i>
CGAGGAAGTGTCGTCTA	<i>qPCR msmeg_0887 fw</i>
CGAGGAAGTGTCGTCTA	<i>qPCR msmeg_0887 rv</i>
CGGTGGTGAGACATGGA	<i>qPCR msmeg_0336 fw</i>
TGACGACGAGGACGAAAT	<i>qPCR msmeg_0336 rv</i>
CGTTGCTGAAGTTCCATCT	<i>qPCR msmeg_2246 fw</i>
GTAGGCGCCGAGATAGT	<i>qPCR msmeg_2246 rv</i>
TCACGACGTTTCGAGTCA	<i>qPCR msmeg_2957 fw</i>
GTCGGGTTGGTTGAAGATG	<i>qPCR msmeg_2957 rv</i>
ACCCGATCATCAAGGTCT	<i>qPCR msmeg_5677 fw</i>
CTGTGCACGACGACTTC	<i>qPCR msmeg_5677 rv</i>
GTGAGCGCCAACTGAT	<i>qPCR msmeg_6932 fw</i>
GCTGCAGCATGATTGCC	<i>qPCR msmeg_6932 rv</i>
GAAGTGCAGGCTGGA	<i>qPCR msmeg_3192 fw</i>
AACGCCTCGTAGGTCTG	<i>qPCR msmeg_3192 rv</i>
CGTTGCGTCAGCTTCTC	<i>qPCR msmeg_0682 fw</i>
CGGTTTACCGTCACGAAC	<i>qPCR msmeg_0682 rv</i>
GACCTCTACCTCGGATTGT	<i>qPCR msmeg_0441 fw</i>
ACGTTGAGTGCTTGCTG	<i>qPCR msmeg_0441 rv</i>
CTCCTGGACACCCTCAA	<i>qPCR msmeg_5349 fw</i>
CGGAATCCCGACCTGAT	<i>qPCR msmeg_5349 rv</i>
CTCACACCGGAACAGTTG	<i>qPCR msmeg_6779 fw</i>
CGGAAACCGTCCAAGAAA	<i>qPCR msmeg_6779 rv</i>
aagtgtcatcattgaaaaAGGACCTGTACGGCCTGCCC	<i>msmeg_3910 to upstream</i>
CGAGCGTCAGTGAGCCTCGATCACGGCGCGCGCAT	<i>upstream to msmeg_3910</i>
CGCGCCGTGATCGAGGCTCACTGACGCTCGGGTGT	<i>msmeg_3910 to downstream</i>
gttttcgccccgaagaacgGTGACGGGCGCGATTATTCG	<i>downstream to msmeg_3910</i>

CGAGGTGTGGATGAGCCCGCTC

Δmsmeg_3910 DCO screening fw

GCTCGTCGAGCAGTACGCGTCG

Δmsmeg_3910 DCO screening rv

Supplementary References

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