Fig S1 Optimization of drug treatments. Viability of wt *M. smegmatis* in liquid cultures (A) and on plates (B) containing second line antituberculotics. Relative viability is calculated upon CFU after 8 hours of drug treatment or CFU of exponentially growing wt *M. smegmatis* cultures on plates containing the respective antibiotic at varying concentrations.
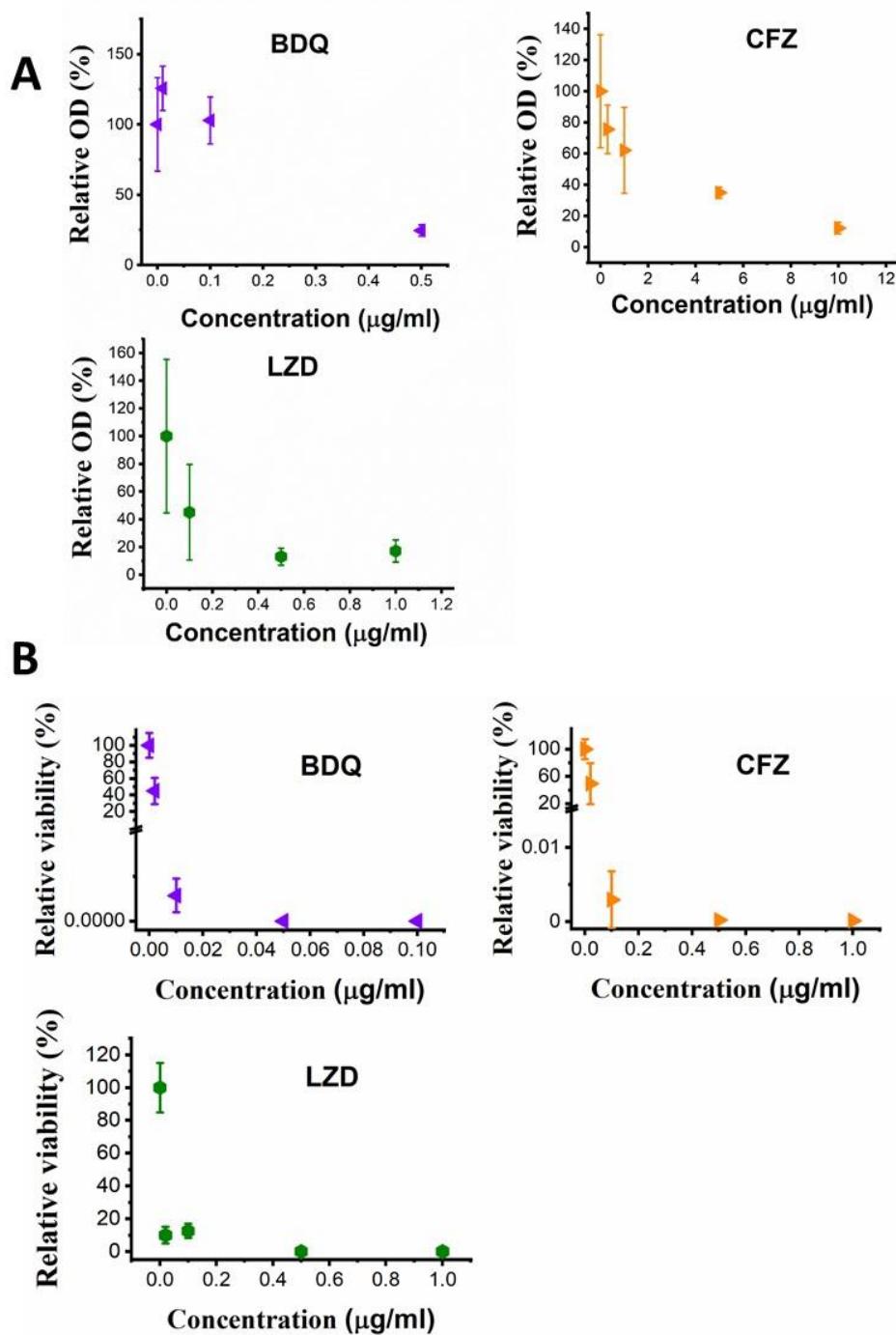
Table S1: Average normalized gene expressions. SEM= standard error of the mean. For raw data, see 10.6084/m9.figshare.25218191

Target	Treatment	REFERENCE Fold change	+/- SEM	SigA/Ffh p
AdnA	CFZ	8.60	0.42	0.19
	NT	1.00	0.05	
AhpC	CFZ	1.21	0.10	0.77
	NT	1.00	0.10	
AlkA	CFZ	1.33	0.20	0.82
	NT	1.00	0.17	
Dcd:dut	CFZ	0.31	0.02	0.26
	NT	1.00	0.91	
DinB1	CFZ	0.74	0.11	0.59
	NT	1.00	0.54	
DinB2	CFZ	7.18	0.66	0.00
	NT	1.00	0.05	
DNA ligase	CFZ	1.00	0.06	0.97
	NT	1.00	0.12	
DnaE2	CFZ	2.99	0.38	0.12
	NT	1.00	0.08	
Dut	CFZ	0.51	0.04	0.73
	NT	1.00	0.48	
End	CFZ	2.71	0.17	0.22
	NT	1.00	0.09	
KatG1	CFZ	2.93	0.21	0.17
	NT	1.00	0.10	
LexA	CFZ	0.93	0.08	0.97
	NT	1.00	0.13	
Mfd	CFZ	1.47	0.15	0.38
	NT	1.00	0.13	
Mpg	CFZ	1.31	0.23	0.13
	NT	1.00	0.36	
MutM1	CFZ	2.21	0.13	0.49
	NT	1.00	0.06	
MutT1	CFZ	1.03	0.09	0.81
	NT	1.00	0.14	
MutT2	CFZ	0.22	0.03	0.07
	NT	1.00	0.60	
MutT3	CFZ	0.88	0.27	0.96
	NT	1.00	0.44	
MutT4	CFZ	0.32	0.02	0.20
	NT	1.00	0.46	
MutY	CFZ	1.67	0.13	0.80
	NT	1.00	0.26	
Nei1	CFZ	0.29	0.06	0.38

	NT	**1.00**	**1.14**	
Nei2	**CFZ**	**1.15**	**0.16**	**0.87**
	NT	**1.00**	**0.24**	
NucS	**CFZ**	**0.33**	**0.03**	**0.59**
	NT	**1.00**	**0.80**	
Ogt	**CFZ**	**0.63**	**0.07**	**0.45**
	NT	**1.00**	**0.40**	
PolA	**CFZ**	**1.42**	**0.10**	**0.72**
	NT	**1.00**	**0.24**	
RecA	**CFZ**	**0.66**	**0.04**	**0.36**
	NT	**1.00**	**0.23**	
RecX	**CFZ**	**0.61**	**0.05**	**0.80**
	NT	**1.00**	**0.50**	
TagA	**CFZ**	**5.12**	**0.45**	**0.07**
	NT	**1.00**	**0.06**	
ThyA	**CFZ**	**0.63**	**0.04**	**0.67**
	NT	**1.00**	**0.21**	
ThyX	**CFZ**	**1.01**	**0.09**	**0.95**
	NT	**1.00**	**0.10**	
UdgB	**CFZ**	**1.49**	**0.15**	**0.10**
	NT	**1.00**	**0.21**	
UdgX	**CFZ**	**1.63**	**0.11**	**0.78**
	NT	**1.00**	**0.15**	
Ung	**CFZ**	**1.03**	**0.19**	**0.93**
	NT	**1.00**	**0.35**	
UvrA	**CFZ**	**1.14**	**0.13**	**0.76**
	NT	**1.00**	**0.12**	
UvrB	**CFZ**	**25.94**	**1.40**	**0.01**
	NT	**1.00**	**0.05**	
UvrC	**CFZ**	**1.33**	**0.19**	**0.77**
	NT	**1.00**	**0.23**	
UvrD	**CFZ**	**1.46**	**0.09**	**0.39**
	NT	**1.00**	**0.08**	
XthA	**CFZ**	**0.42**	**0.05**	**0.24**
	NT	**1.00**	**0.63**	
AdnA	**BDQ**	**0.82**	**0.17**	**0.59**
	NT	**1.00**	**0.23**	
AhpC	**BDQ**	**0.79**	**0.03**	**0.83**
	NT	**1.00**	**0.14**	
AlkA	**BDQ**	**0.39**	**0.05**	**0.21**
	NT	**1.00**	**0.22**	
Dcd:dut	**BDQ**	**0.55**	**0.03**	**0.58**
	NT	**1.00**	**0.22**	
DinB1	**BDQ**	**0.45**	**0.18**	**0.08**
	NT	**1.00**	**1.13**	
DinB2	**BDQ**	**0.68**	**0.04**	**0.31**
	NT	**1.00**	**0.53**	

DNA ligase	BDQ	0.49	0.03	0.11
	NT	1.00	0.13	
DnaE2	BDQ	0.17	0.02	0.00
	NT	1.00	2.28	
Dut	BDQ	0.39	0.03	0.10
	NT	1.00	0.45	
End	BDQ	1.01	0.07	0.99
	NT	1.00	0.11	
KatG1	BDQ	0.19	0.02	0.27
	NT	1.00	0.16	
LexA	BDQ	0.58	0.05	0.17
	NT	1.00	0.53	
Mfd	BDQ	0.89	0.07	0.86
	NT	1.00	0.25	
Mpg	BDQ	0.29	0.05	0.01
	NT	1.00	1.03	
MutM1	BDQ	0.45	0.03	0.07
	NT	1.00	0.35	
MutT1	BDQ	0.24	0.02	0.11
	NT	1.00	0.20	
MutT2	BDQ	0.16	0.01	0.26
	NT	1.00	1.79	
MutT3	BDQ	0.46	0.02	0.33
	NT	1.00	0.29	
MutT4	BDQ	0.28	0.02	0.41
	NT	1.00	0.33	
MutY	BDQ	0.84	0.38	0.86
	NT	1.00	0.70	
Nei1	BDQ	0.50	0.09	0.58
	NT	1.00	0.34	
Nei2	BDQ	0.72	0.06	0.73
	NT	1.00	0.13	
NucS	BDQ	0.49	0.10	0.09
	NT	1.00	0.60	
Ogt	BDQ	0.55	0.04	0.59
	NT	1.00	0.21	
PolA	BDQ	1.05	0.06	0.96
	NT	1.00	0.10	
RecA	BDQ	0.60	0.03	0.08
	NT	1.00	0.16	
RecX	BDQ	0.37	0.02	0.04
	NT	1.00	0.47	
TagA	BDQ	0.75	0.09	0.63
	NT	1.00	0.23	
ThyA	BDQ	0.20	0.01	0.22

	NT	**1.00**	**0.47**	
ThyX	**BDQ**	**0.59**	**0.03**	**0.64**
	NT	**1.00**	**0.14**	
UdgB	**BDQ**	**1.29**	**0.05**	**0.03**
	NT	**1.00**	**0.25**	
UdgX	**BDQ**	**0.26**	**0.03**	**0.01**
	NT	**1.00**	**0.15**	
Ung	**BDQ**	**4.76**	**2.50**	**0.29**
	NT	**1.00**	**0.06**	
UvrA	**BDQ**	**1.89**	**0.11**	**0.47**
	NT	**1.00**	**0.09**	
UvrB	**BDQ**	**0.90**	**0.05**	**0.82**
	NT	**1.00**	**NA**	
UvrC	**BDQ**	**0.18**	**0.02**	**0.17**
	NT	**1.00**	**0.29**	
UvrD	**BDQ**	**0.38**	**0.03**	**0.03**
	NT	**1.00**	**0.19**	
XthA	**BDQ**	**0.58**	**0.04**	**0.59**
	NT	**1.00**	**0.42**	
AdnA	**LZD**	**0.82**	**0.07**	**0.28**
	NT	**1.00**	**0.11**	
AhpC	**LZD**	**0.16**	**0.00**	**0.18**
	NT	**1.00**	**0.24**	
AlkA	**LZD**	**3.55**	**0.25**	**0.05**
	NT	**1.00**	**0.05**	
Dcd:dut	**LZD**	**0.65**	**0.05**	**0.61**
	NT	**1.00**	**0.10**	
DinB1	**LZD**	**1.16**	**0.22**	**0.88**
	NT	**1.00**	**0.24**	
DinB2	**LZD**	**2.97**	**0.28**	**0.08**
	NT	**1.00**	**0.05**	
DNA ligase	**LZD**	**0.77**	**0.03**	**0.36**
	NT	**1.00**	**0.19**	
DnaE2	**LZD**	**0.19**	**0.02**	**0.03**
	NT	**1.00**	**0.39**	
Dut	**LZD**	**2.19**	**0.11**	**0.29**
	NT	**1.00**	**0.04**	
End	**LZD**	**0.77**	**0.04**	**0.40**
	NT	**1.00**	**0.11**	
KatG1	**LZD**	**0.15**	**0.01**	**0.14**
	NT	**1.00**	**0.81**	
LexA	**LZD**	**1.12**	**0.13**	**0.85**
	NT	**1.00**	**0.08**	
Mfd	**LZD**	**0.37**	**0.03**	**0.04**
	NT	**1.00**	**0.12**	
Mpg	**LZD**	**1.15**	**0.11**	**0.81**
	NT	**1.00**	**0.13**	

MutM1	LZD	1.02	0.04	0.78
	NT	1.00	0.05	
MutT1	LZD	0.32	0.02	0.17
	NT	1.00	0.11	
MutT2	LZD	0.53	0.03	0.47
	NT	1.00	0.17	
MutT3	LZD	0.93	0.06	0.92
	NT	1.00	0.08	
MutT4	LZD	1.37	0.14	0.69
	NT	1.00	0.05	
MutY	LZD	0.58	0.05	0.08
	NT	1.00	0.27	
Nei1	LZD	0.31	0.03	0.30
	NT	1.00	0.20	
Nei2	LZD	0.14	0.01	0.20
	NT	1.00	0.42	
NucS	LZD	1.45	0.11	0.53
	NT	1.00	0.08	
Ogt	LZD	6.41	0.32	0.08
	NT	1.00	0.05	
PolA	LZD	0.42	0.01	0.11
	NT	1.00	0.13	
RecA	LZD	0.46	0.03	0.09
	NT	1.00	0.07	
RecX	LZD	0.57	0.03	0.50
	NT	1.00	0.15	
TagA	LZD	0.47	0.02	0.35
	NT	1.00	0.14	
ThyA	LZD	0.12	0.01	0.12
	NT	1.00	0.43	
ThyX	LZD	0.55	0.03	0.60
	NT	1.00	0.08	
UdgB	LZD	0.72	0.06	0.78
	NT	1.00	0.12	
UdgX	LZD	0.21	0.01	0.01
	NT	1.00	0.12	
Ung	LZD	2.05	0.14	0.58
	NT	1.00	0.04	
UvrA	LZD	0.57	0.03	0.37
	NT	1.00	0.06	
UvrB	LZD	0.64	0.03	0.49
	NT	1.00	0.12	
UvrC	LZD	0.44	0.03	0.29
	NT	1.00	0.13	
UvrD	LZD	0.50	0.02	0.02
	NT	1.00	0.10	
XthA	LZD	0.86	0.06	0.50

		NT	1.00	0.09	

Table S2: Cellular parameters of BDQ and CFZ treated *M. smegmatis* cells analyzed by microscopic measurements

Strain	N	Cell length (μm)	SD	With (μm)	SD	Cell volume (μm3)
BDQ	117	3.78	1.26	1.13	0.11	3.80
control	96	6.36	2.46	0.84	0.20	3.56
CFZ	100	3.90	1.23	1.10	0.10	3.71
control	116	6.32	2.01	0.90	0.08	4.02

Table S3: Results and statistical analysis of dNTP measurements

Treatment	dNTP	Sample	uM	+/-SEM	Fold change	+/- SE	p
BDQ	dGTP	Treated	33.45	2.70	0.23	0.02	0.12
		Non-treated	147.96	55.06			
	dCTP	Treated	27.33	nd	0.67	nd	nd
		Non-treated	40.50	7.13			
	dTTP	Treated	35.72	8.30	0.70	0.16	0.29
		Non-treated	51.13	10.81			
	dATP	Treated	13.57	nd	0.35	0.25	0.19
		Non-treated	39.33	21.46			
CFZ	dGTP	Treated	1.21	0.25	0.01	0.00	0.02
		Non-treated	91.13	3.55			
	dCTP	Treated	13.28	3.08	0.37	0.09	0.08
		Non-treated	35.75	7.50			
	dTTP	Treated	33.69	9.07	0.38	0.10	0.18
		Non-treated	88.79	27.66			
	dATP	Treated	11.11	2.48	0.15	0.03	0.08
		Non-treated	74.68	22.24			

Table S4: WGS samples deposited at ENA webserver, with PRJEB72651 project number under an umbrella project

Treatment	Sample	Sample description	ENA filename	Experiment length
Clofazimine	CLFA	5 different lineages of clofazimine treated *Mycobacterium smegmatis*	CLFA.rg.bam	60 days
	CLFB	5 different lineages of clofazimine treated *Mycobacterium smegmatis*	CLFB.rg.bam	60 days
	CLFC	6 different lineages of clofazimine treated *Mycobacterium smegmatis*	CLFC.rg.bam	60 days
Bedaquiline	BedA	5 different lineages of bedaquiline treated *Mycobacterium smegmatis*	BedA.rg.bam	60 days
	BedB	6 different lineages of bedaquiline treated *Mycobacterium smegmatis*	BedB.rg.bam	60 days
	BedC	5 different lineages of bedaquiline treated *Mycobacterium smegmatis*	BedC.rg.bam	60 days
	BedD	5 different lineages of bedaquiline treated *Mycobacterium smegmatis*	BedD.rg.bam	60 days
Linezolid	LzdA	5 different lineages of linezolid treated *Mycobacterium smegmatis*	LzdA.rg.bam	60 days
	LzdB	6 different lineages of linezolid treated *Mycobacterium smegmatis*	LzdB.rg.bam	60 days
	LzdC	5 different lineages of linezolid treated *Mycobacterium smegmatis*	LzdC.rg.bam	60 days

Table S5 Primer sequences and primer efficiencies used for qPCR

Target	Msm Gene ID	Sequence (5'⮕3')	Efficiency (%)
AdnA_for	MSMEG_1941	CGCAGTCCTACCGTTGC	81.6
AdnA_rev		CTTCGGCGTGTGTGGAG	
AhpC_for	MSMEG_4753	CCGACAAGCCCGAGAAG	103
AlhpC_rev		GGAACGTGGACCGGATG	
AlkA_for	MSMEG_4925	GCCTCCATCCGTCAGTTC	83.5
AlkaA_rev		GCCGAACAATCCCTCGTAG	
Dcd:dut_for	MSMEG_0678	CGGTTGGAGGGCAAGTC	98.2
Dcd:dut_rev		CACAGCTGCCCGATCTTC	
DinB1_for	MSMEG_3172	TCACGGTCAAGCTCAAGAAG	83.2
DinB1_rev		AACCCAACGCCCACAAG	
DinB2_for	MSMEG_6443	CCAGTTACGAGGCCAAGG	81.2
DinB2_rev		GGGTGGTGTCGTGGAAG	
DNA ligase_for	MSMEG_2362	GGAGGTCAAACGCAAGGG	91.1
DNA ligase_rev		CAACTCGGTTCCGCACTC	
DnaE2_for	MSMEG_1633	GCCTCGCTGGTGTTCTAC	87.7
DnaE2_rev		AGGCTGGCATTCACGTC	
Dut_for	MSMEG_2765	ATACCGCACGGAATGGTC	97.1
Dut_rev		GTCTGCGGATCCAGGTTG	
End_for	MSMEG_1383	AGCGCATCAAGTCCATCAC	83

End_rev		GCCGTCTCGCAGATCAC	
Ffh_for	MSMEG_2430	GAGCTCATCGGCATCCTC	86.2
Ffh_rev		GGGCTGTGACCCTTGTC	
KatG1_for	MSMEG_6384	GCCCATCGGAGAAGCTC	96
KatG1_rev		CCTCGTCCAGCGGATTG	
LexA_for	MSMEG_2740	GTTCCTGCTCAAGGTCGTC	105
LexA_rev		CATGAGCCACACCTGACC	
Mfd_for	MSMEG_5423	GAGCTCACCCGGTTCAC	89
Mfd_rev		CTTGGTGGCGGTCTTCTC	
Mpg_for	MSMEG_3759	GTGCGGAACTCGGTGATG	89.6
Mpg_rev		GCTGTGCACTGTCGACTC	
MutM1_for	MSMEG_2419	CAGGCGCGGAAAGTACC	93
MutM1_rev		CGCTGATCGACGAAGCTC	
MutT1_for	MSMEG_2390	GGTGGACAAGCTCGTATGG	107
MutT1_rev		GCGATCGTCACCCTTGTAG	
MutT2_for	MSMEG_5148	GGCTGTGGGAACTTCCTG	86.7
MutT2_rev		TGTCATGGCGTCGTTGAG	
MutT3_for	MSMEG_0790	CGTACACGACGGTGATCG	92.1
MutT3_rev		GTAGGCGCTGCCAACTC	
MutT4_for	MSMEG_6927	CAGGCGGTCTGGTCATC	87.1
MutT4_rev		TGGATCCCGGTCTCCTC	
MutY_for	MSMEG_6083	GGGAAAGCTCGGCTACC	86.4
MutY_rev		GGAACGCTCGCCTGATAG	
Nei1_for	MSMEG_4683	CATCGGCGCTCAGTACG	99.7
Nei1_rev		CGGTCTGCGTGACTTGG	
Nei2_for	MSMEG_1756	GCGGTACCGACATGGAC	92.1
Nei2_rev		GAAACACAACTCGTTGCAGTAG	
NucS_for	MSMEG_4923	CGCGCTACCTGGAACTG	99.7
NucS_rev		GTACTCGTCGCTGTCCATTC	
Ogt_for	MSMEG_4928	AGATCCCGTACGGACAGAC	92.6
Ogt_rev		CCCATAACCCGTGAGACTTC	
PolA_for	MSMEG_3839	GAGCTCACCCGGTTCAC	83.9
PolA_rev		CTTGGTGGCGGTCTTCTC	
ProC_for	MSMEG_0943	GCCCGGCGTACTTCTTC	73.3
ProC_rev		CGGCGTTCACCTGATCC	
RecA_for	MSMEG_2723	CGCGTCAAGGTCGTCAAG	90.1
RecA_rev		ACCCTCGTAGGTGAACCAG	
RecX_for	MSMEG_2724	CTCGAAACCCAGCTGACC	89.9
RecX_rev		GAGCTCGACAGCCAAGG	
SigA_for	MSMEG_2758	CATCTGCTGGAGGCGAAC	87.7
SigA_rev		CTTGTAGCCCTTGGTGTAGTC	
TagA_for	MSMEG_5082	GACTACCACGACACCGAATG	91.4
TagA_rev		GGATCGAACCCGTGGAAC	
ThyA_for	MSMEG_2670	GTCGGGTGAGCACATCG	92.8
ThyA_rev		GGCGACGTAGAACTGGAAG	
ThyX_for	MSMEG_2683	CGTACAGCTGATCGCCAAG	94.2
ThyX_rev		CGTTGGTCGCGGTCTTC	

UdgB_for	MSMEG_5031	ACGTTGACCACCGCATAC	97.7
UdgB_rev		TTCGTTCCCGATCTGCTTG	
UdgX_for	MSMEG_0265	CCCGGTGACAAAGAGGAC	91.8
UdgX_rev		CTTGTGGATGCGTCGTTTG	
Ung_for	MSMEG_2399	CCGTGGCAGATCAGGTG	89.2
Ung_rev		TGTCGGGTAGGGATCCTG	
UvrA_for	MSMEG_3808	CCTGGGCATCCGCAAAG	94.6
UvrA_rev		CCCATCTCCTCACCGAGAC	
UvrB_for	MSMEG_3816	GAGAAGGACAGCTCGATCAAC	96.6
UvrB_rev		CTGACCCACCTGCAACTC	
UvrC_for	MSMEG_3078	TCGATTTCTGCGACTTCCTG	88.1
UvrC_rev		CCACGGCCTGTTTCTCC	
UvrD_for	MSMEG_5534	GGGAGGACGGCATGTTC	86.3
UvrD_rev		CGCGATTCCGGGTTGAG	
XthA_for	MSMEG_0829	CCTACCACGGGCTCAAC	80.7
XthA_rev		GCACGTAGAGGCTCCATAC	

Fig S2: geNorm analysis based reference gene stability used in the qPCR measurements outlined in the methods section.
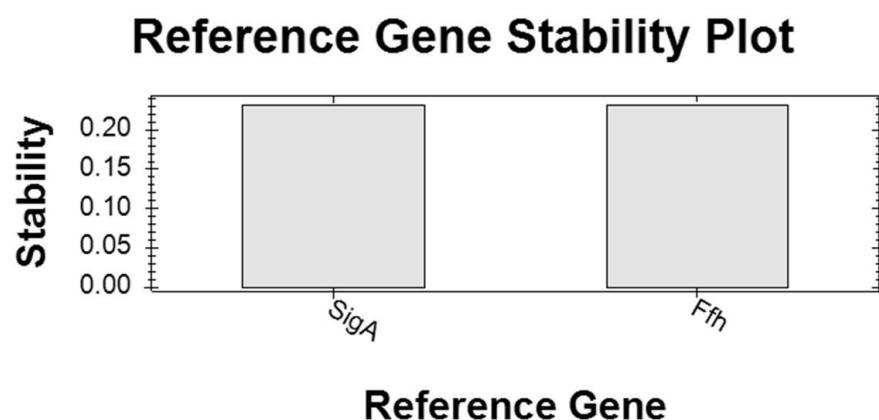
Table S6: Primer sequences for dNTP measurements

Name	Sequence (5'→3')
NDP-1 primer	CCGCCTCCACCGCC
FAM-dTTP probe	**6-FAM/**AGGACCGAG**/ZEN/**GCAAGAGCGAGCGA**/IBFQ**
FAM-dATP probe	**6-FAM/**TGGTCCGTG**/ZEN/**GCTTGTGCGTGCGT**/IBFQ**
FAM-dGTP probe	**6-FAM/**ACCATTCAC**/ZEN/**CTCACACTCACTCC**/IBFQ**
FAM-dCTP probe	**6-FAM/**AGGATTGAG**/ZEN/**GTAAGAGTGAGTGG**/IBFQ**
dTTP-DT1 template	TCGCTCGCTCTTGCCTCGGTCCTTT**A**TTTGGCGGTGGAGGCGG
dATP-DT1 template	ACGCACGCACAAGCCACGGACCAAA**T**AAAGGCGGTGGAGGCGG
dCTP-DT1 template	CCACTCACTCTTACCTCAATCCTTT**G**TTTGGCGGTGGAGGCGG
dGTP-DT2 template	GGAGTGAGTGTGAGGTGAATGGTTT**C**TTT**C**TTTGGCGGTGGAGGCGG