SUPPLEMENTARY INFORMATION

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TABLE S1: Primers used in this study

Name	Sequence	Product Size
Myco_16S_F1	GGCGTGCTTAACACATGCA	230
Myco_16S_R1	5'P-ctggcgtcatagctgtttcctgtgtgaCCGGCTACCCGTCGTC	

[a] 5'P indicated the 5' phosphate modification on primers that is used for specific digestion of the antisense strand by λ-exonuclease.

[b] Lowercase letters indicate a linker included on reverse primers to allow dual use of multiplex analytes in an unrelated assay.

TABLE S2: **Oligonucleotides used in this study**[a]

Name[b]	**Sequence**	**[Oligonucleotide]**
Fluorogenic substrate (F-sub)	AAGGT(T-FAM)TCCTCguCCCTGGGCA-(BHQ)	200 nM
Dz_a_Mabs	TGCCCAGGGAGGCTAGCT*agtgtgtggtcctatccggt*	15 nM
Dz_b_Mabs	aaaagctttgcaccactcaccatgaACAACGAGAGGAAACCTT	15 nM
Dz_a_Mavi	TGCCCAGGGAGGCTAGCT*gtcttgaggtcctatccggtat*	15 nM
Dz_b_Mavi	tccaccagaagacatgcACAACGAGAGGAAACCTT	15 nM
Dz_a_Mint	TGCCCAGGGAGGCTAGCT*atgcgcctaaaggtcctat*	15 nM
Dz_b_Mint	aaaagctttccacctaaagacACAACGAGAGGAAACCTT	15 nM
Dz_a_Mfor	TGCCCAGGGAGGCTAGCT*agcgcgtggtcatattcggtattag*	15 nM
Dz_b_Mfor	aaaagctttccaccacacaccatgaACAACGAGAGGAAACCTT	15 nM
Dz_a_Mkan	TGCCCAGGGAGGCTAGCT*agtggtcctatccggt*	15 nM
Dz_b_Mkan	gctttccaccacaaggcatgcgccaACAACGAGAGGAAACCTT	3 nM
Dz_a_Mgor	TGCCCAGGGAGGCTAGCT*atgtgtcctgtggtcctattcggta*	15 nM
Dz_b_Mgor	aaaagctttccaccacaggacACAACGAGAGGAAACCTT	15 nM
Dz_a_Mtb	TGCCCAGGGAGGCTAGCT*ggtcctatccggtattagaccc*	150 nM
Dz_b_Mtb	cacaagacatgcatcccgtACAACGAGAGGAAACCTT	150 nM
Mabs	*ctaataccggataggaccacacact*tcatggtgagtggtgcaaagctttt	1 nM
Mavi	*ctaataccggataggacctcaagac*gcatgtcttctggtggaaagctttt	1 nM
Mint	*ctaataccggataggacctttaggc*gcatgtctttaggtggaaagctttt	1 nM
Mfor	*ctaataccgaatatgaccacgcgct*tcatggtgtgtggtggaaagctttt	1 nM
Mkan	*ctaataccggataggaccacttggc*gcatgccttgtggtggaaagctttt	1 nM
Mgor	*ctaataccgaataggaccacaggac*acatgtcctgtggtggaaagctttt	1 nM
Mtb	*actgggtctaataccggataggacc*acgggatgcatgtcttgtggtggaa	1 nM
IPDz substrate	GGGTAGGGCGGGTTGGGTTCguCCATGAGCAACTCGCCC	1000 nM
vDz_a_Mabs	gcaccactcaccatgACAACGAGAACCCAACC	30 nM
vDz_b_Mabs	GTTGCTCATGGAGGCTAGCT*aagtgtgtggtcctatcc*	100 nM
vDz_a_Mavi	ccaccagaagacatgACAACGAGAACCCAACC	30 nM
vDz_b_Mavi	GTTGCTCATGGAGGCTAGCT*cgtcttgaggtcctatc*	100 nM
vDz_a_Mint	ctttccacctaaagacatgACAACGAGAACCCAACC	30 nM
vDz_b_Mint	GTTGCTCATGGAGGCTAGCT*cgcctaaaggtcctatc*	100 nM
vDz_a_Mfor	ctttccaccacaccatgACAACGAGAACCCAACC	30 nM
vDz_b_Mfor	GTTGCTCATGGAGGCTAGCT*aagcgcgtggtcatattc*	100 nM
vDz_a_Mkan	ccaccacaaggcatgACAACGAGAACCCAACC	30 nM
vDz_b_Mkan	GTTGCTCATGGAGGCTAGCT*cgccaagtggtcctat*	100 nM
vDz_a_Mgor	aagctttccaccACAACGAGAACCCAACC	30 nM
vDz_b_Mgor	GTTGCTCATGGAGGCTAGCT*aggacatgtgtcctgtggt*	100 nM
vDz_a_Mtb	ctttccaccacaagacatgACAACGAGAACCCAACC	30 nM
vDz_b_Mtb	GTTGCTCATGGAGGCTAGCT*catcccgtggtcctatcc*	100 nM
vMabs	ctaatacc*ggataggaccacacactt*catggtgagtggtgcaaagctttt	100 nM
vMavi	ctaatacc*gataggacctcaagacg*catgtcttctggtggaaagctttt	100 nM
vMint	ctaatacc*gataggacctttaggcg*catgtctttaggtggaaagctttt	100 nM
vMfor	ctaatacc*gaatatgaccacgcgctt*catggtgtgtggtggaaagctttt	100 nM
vMkan	ctaataccgg*ataggaccacttggcg*catgccttgtggtggaaagctttt	100 nM
Mgor	ctaataccgaatagg*accacaggacacatgtcct*gtggtggaaagctttt	100 nM
Mtb	ctaatacc*ggataggaccacgggatg*catgtcttgtggtggaaagcgctt	100 nM

[a] Formatting: the portion of the Dz sensor complementary to the 5' end of the analyte is in italics; the portion of the sensor complementary to the 3' end of the analyte and analyte sequences are in lowercase; sequences complementary to F-sub or IPDz are UPPERCASE; parts of Dz catalytic core are UNDERLINED. The portions of synthetic DNA analytes not complementary to vBiDz are in lowercase grey. The fragment of IPDz responsible for signal generation is in UPPERCASE grey

[b] All oligonucleotides which begin with the gene name are synthetic DNA analytes.

Figure S1. Sequence Alignment of mycobacterial 16S rRNA sequences. The purple and orange sequences represent the forward and reverse primer sites, respectively. The blue and green sequences represent the binding site for Dz_a and Dz_b, respectively.

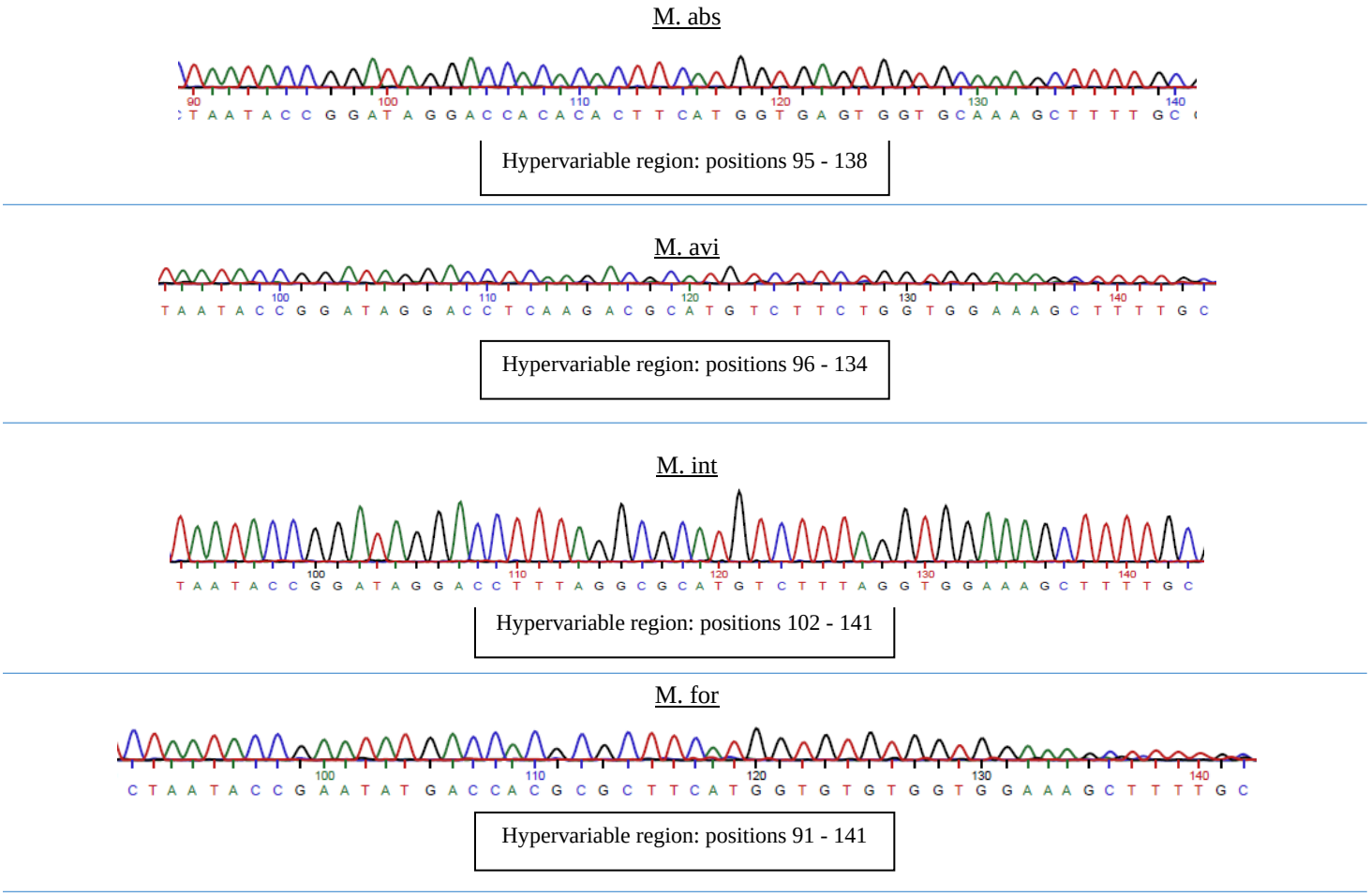

Fig S2. Representative raw sequencing data for validation of NTM species identification. Similar sequencing reads for all 16S species-specific hypervariable regions targeted by Dz sensors were generated for each NTM sample analyzed.

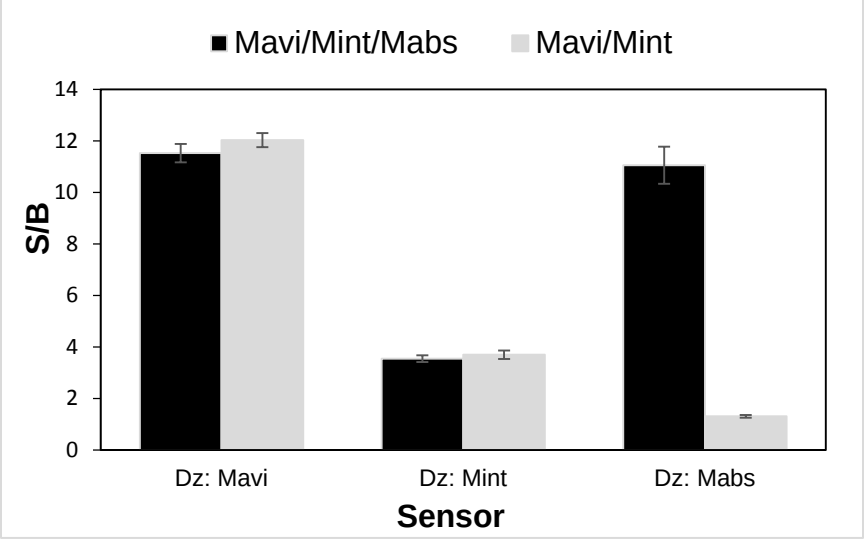

Fig S3: Detection of mixed synthetic analytes. Synthetic analytes of the 16s rRNA region for the indicated strains were mixed in equimolar amounts (1nM each) and detected with BiDz sensors: M. avium, M. abscessus, and M. intracellulare. The data shown is the average of four independent measurements. S/B ratio indicates the ratio of the specific Signal (samples containing analytes divided by Background (consisting of Dz master mix of sensors and substrates plus water) Error bars indicate standard deviation.

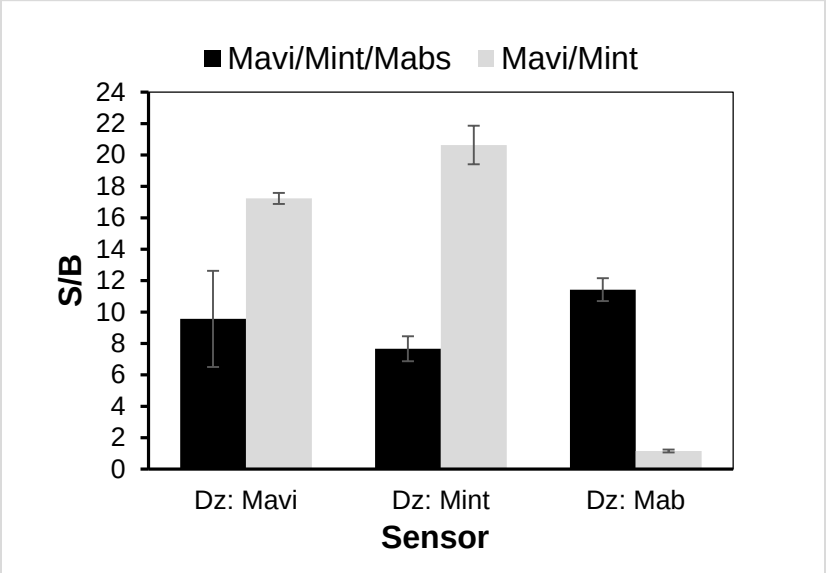

Fig S4: Detection of PCR analytes from mixed DNA templates. 16S rRNA PCR products amplified from mixtures of NTM chromosomal DNAs detected with BiDz assay. S/B ratio was calculated using the average of four independent measurements. S/B ratio indicates the ratio of the specific Signal (samples containing analytes divided by Background (consisting of Dz master mix of sensors and substrates plus water) Error bars indicate standard deviation.

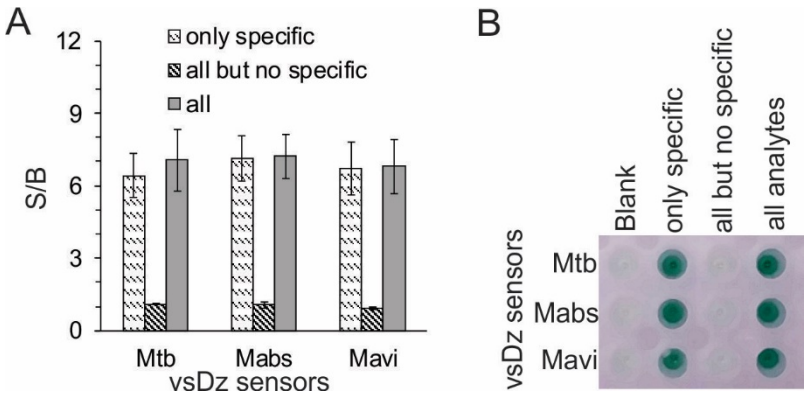

Fig S5: Performance of vBiDz sensors using individual NTM-mimicking synthetic analytes or their mixtures. A. Signal-to-background ratio (S/B) for the samples containing *Mtb*-, *Mab*s- or *Mavi*-specific vBiDz sensor in the presence of only specific analyte, the mixture of all NTM analytes but the specific one, or the mixture of all NTM anhalytes including the specific one. The S/B values were calculated as absorbance at 420 nm for the samples divided by the absorbance of the blank (no analyte added).The data is averaged from three independent experiments with a standard deviation as an error bar. **B.** Images of the samples corresponding to panel A in a well-plate.

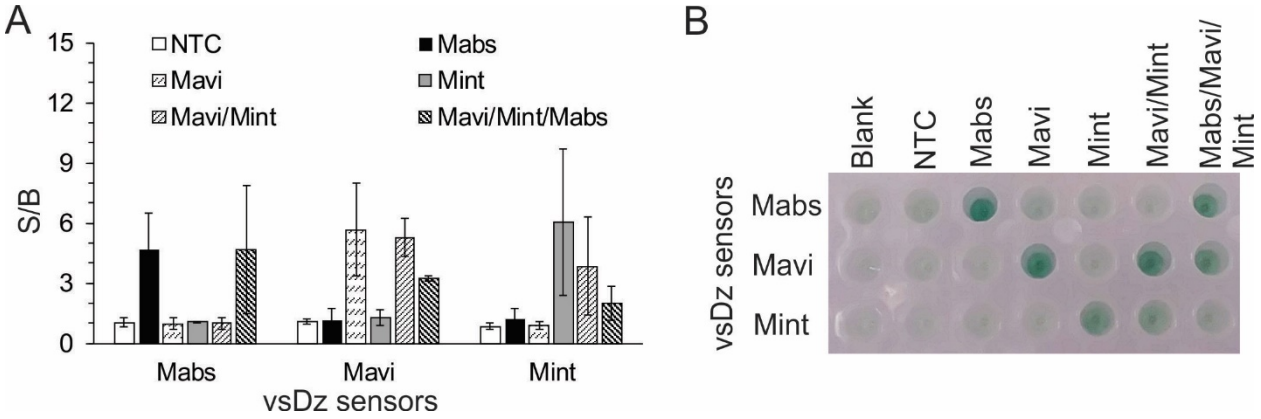

Fig S6: Performance of vBiDz sensors using fragments of indicated NTM DNA after their PCR amplification (individual or their mixtures). A. Signal-to-background ratio (S/B) for the samples containing *Mabs*-, *Mavi*- or *Mint*-specific vBiDz sensor in the presence of 10% PCR amplicon obtaining using either individual DNA from Mabs, Mavi, or Mint; or their mixtures (Mavi/Mint; Mavi/Mint/Mabs). As a negative control, the sample containing the sensor in the presence of 10% no-target control (NTC) of the PCR reaction were used. The S/B values were calculated as absorbance at 420 nm for the samples divided by the absorbance of the blank (no analyte added). The data is averaged from three independent experiments with a standard deviation as an error bar. **B.** Images of the samples corresponding to panel A in a well-plate.

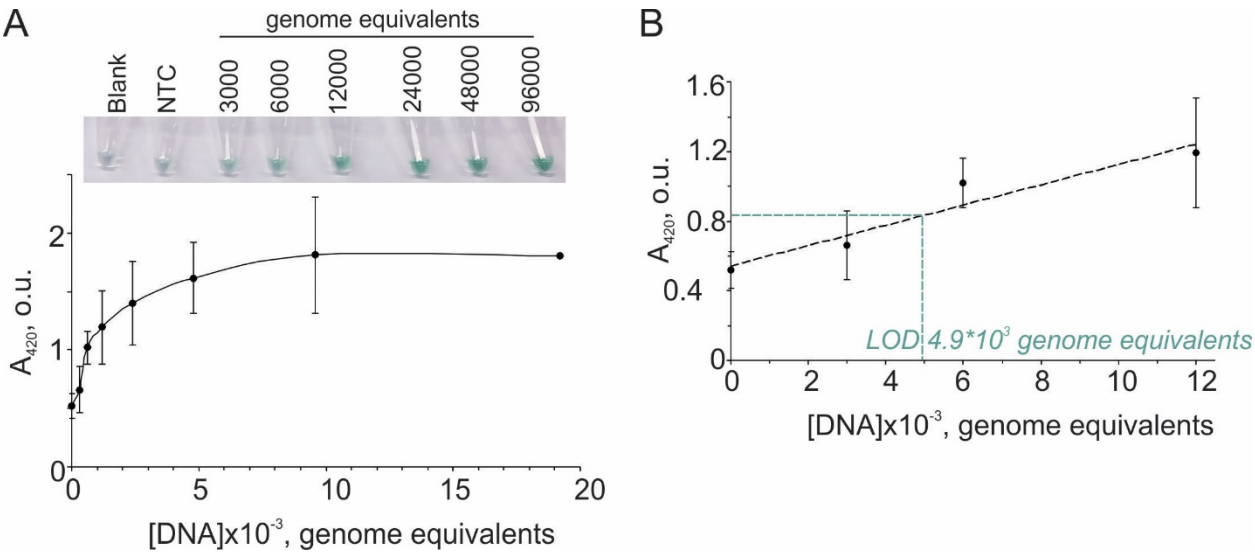

Fig S7: Dependence of the signal for *Mtb*-specific vBiDz sensor on the target concentration. A. Absorbance at 420 nm for the samples containing the sensor in the presence of 10% PCR product obtained using different starting concentrations of *Mtb* DNA. Inset: images of the tubes containing the blank sample and the samples corresponding to the data points on the graph. **B.** Calculation of the limit of detection (LOD) using PCR products obtained with 0-12000 *Mtb* genome equivalents in the aliquot (10%) used for the analysis with the vBi-Dz sensor.