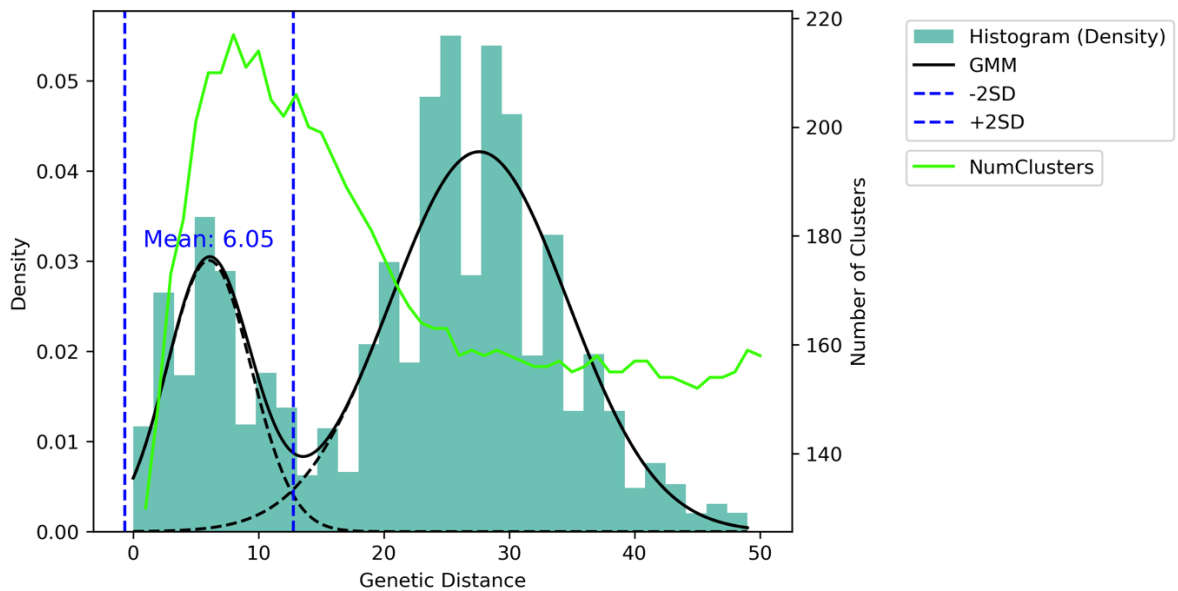
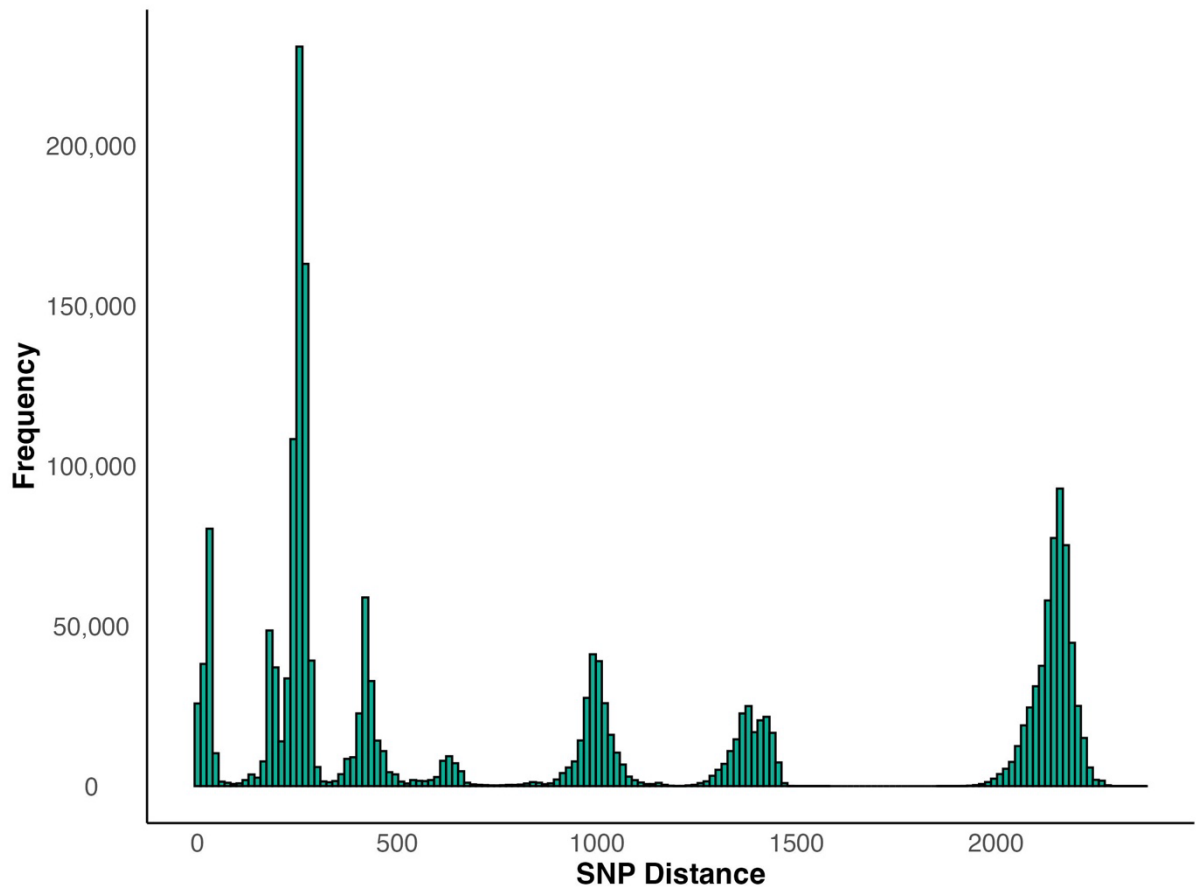


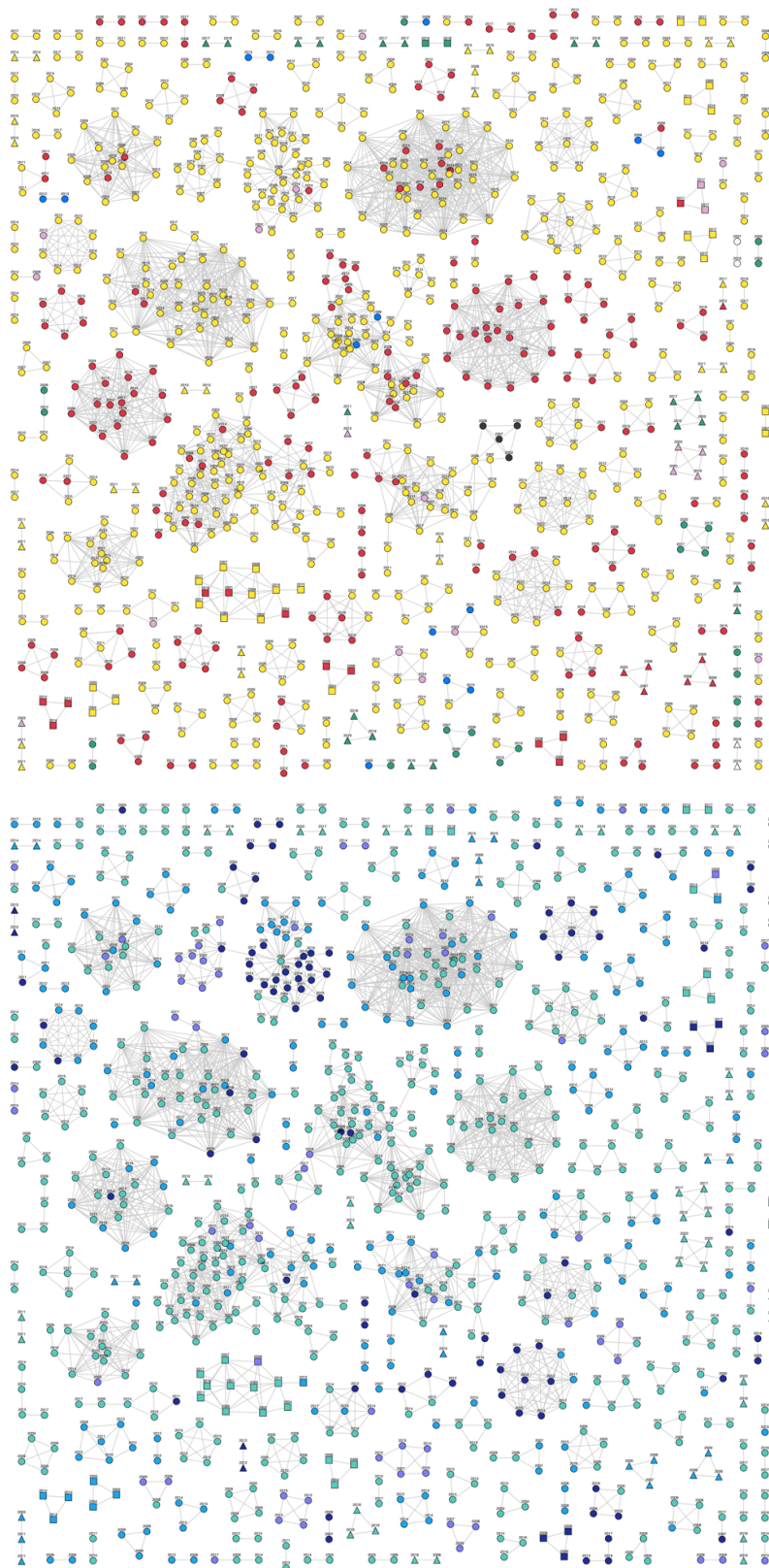
## Supplementary Information



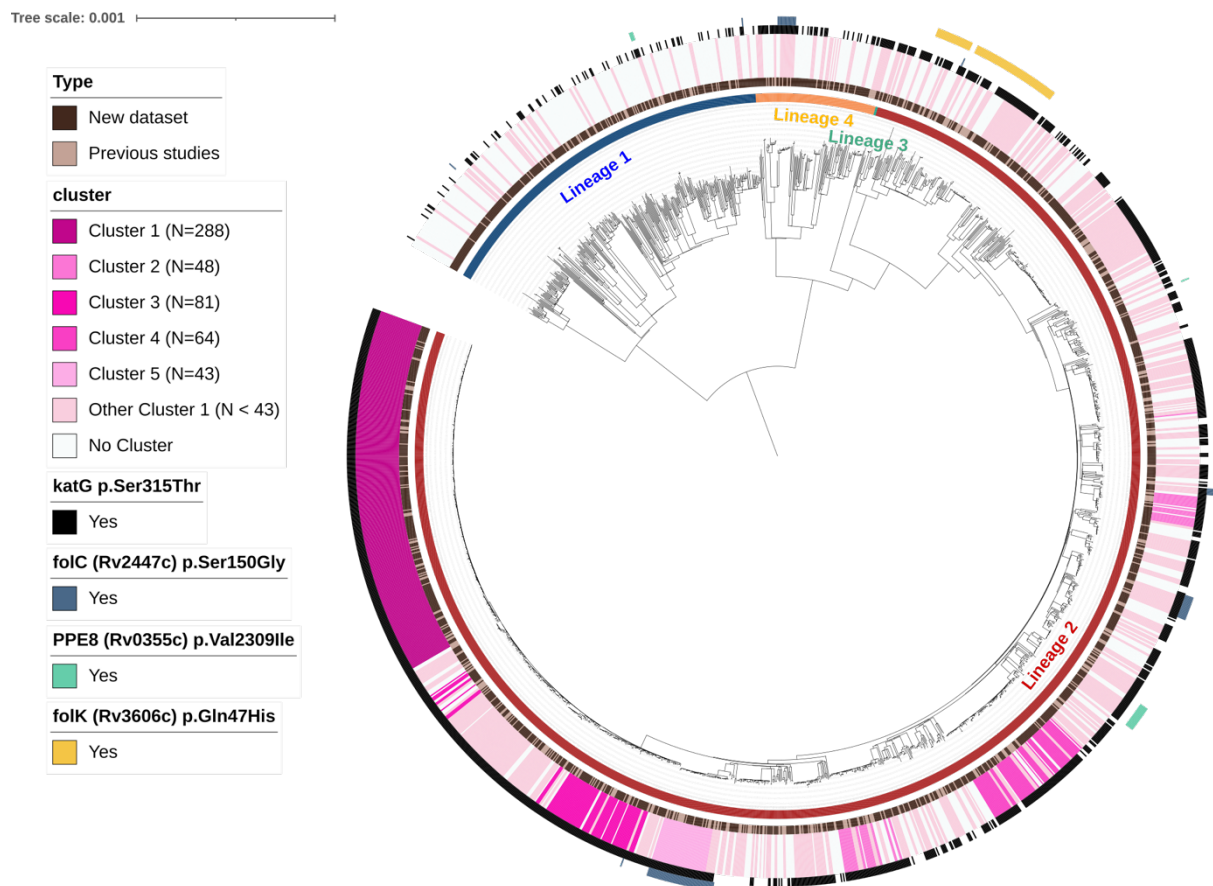
**Figure S1. Principal component analysis (n=2,005)**



**Figure S2. (top). Histogram of SNP Distances. (bottom). Distribution of Pairwise SNP Distances Less Than 50.** GMM components are indicated. Dashed lines mark the boundaries separating the two components. The Gaussian Mixture Model (GMM) was used to estimate the means and standard deviations (SD) for these distributions. For the first component, the mean  $\pm$  2 SD was calculated as  $6.05 \pm 6.71$ . Utilising the mean plus 2 SD, we established a cutoff for clustering in the main analysis, resulting in a cutoff value of approximately 13 (estimated from 12.76).



**Figure S3. Transmission clusters were identified using an SNP distance cut-off value of  $\leq 13$ , excluding the largest cluster (288 isolates). (A) Clusters coloured by genotypic drug resistance profile results and (B) clusters coloured by region of Thailand. The shape of each node represents different lineages.**



**Figure S4. Phylogenetic Tree of 2,005 Mtb Isolates from Thailand, 1994–2020.** The scale bar indicates 0.01 substitutions per site SNP. The tree displays data types and the presence or absence of the *katG* Ser315Thr, *folC* Ser150Gly, *ppe8* Val2309Ile and *folK* Gin47His mutations, within each cluster.

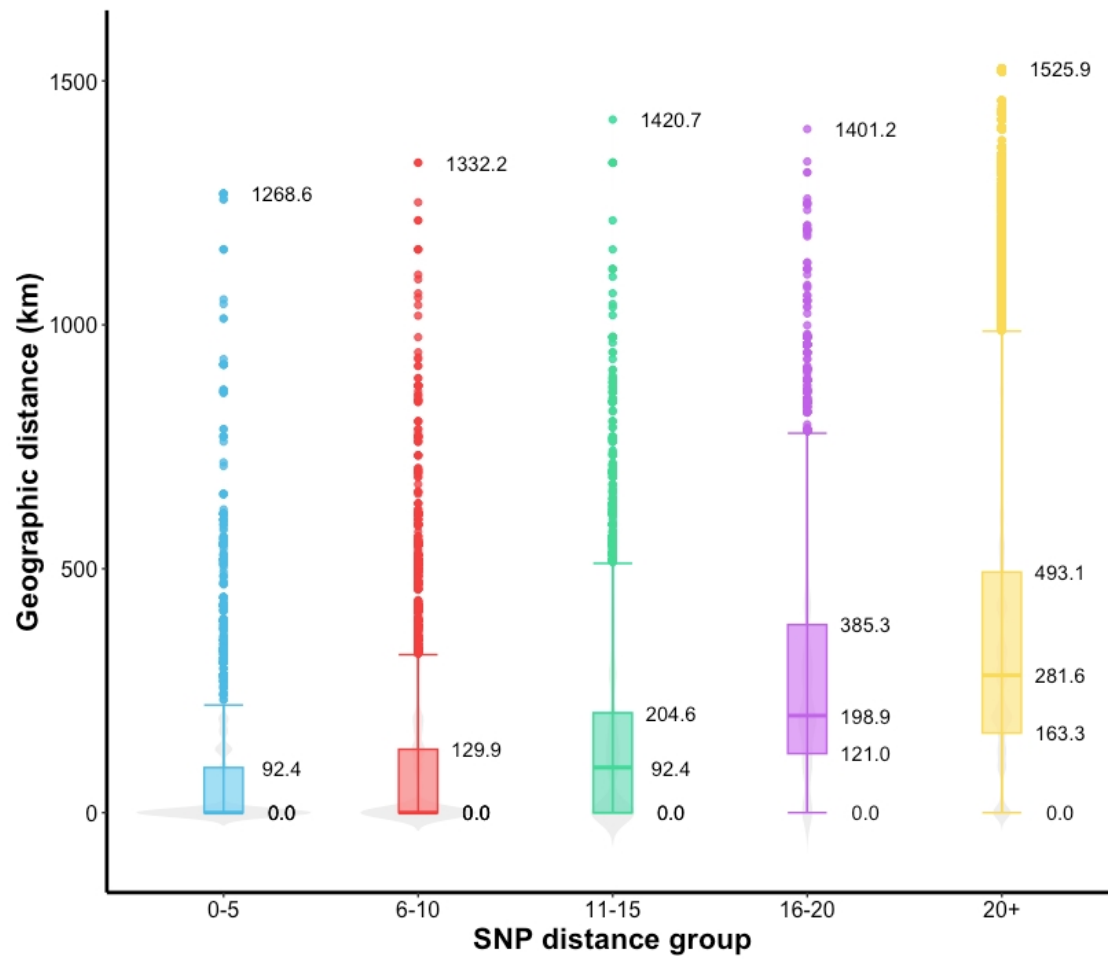
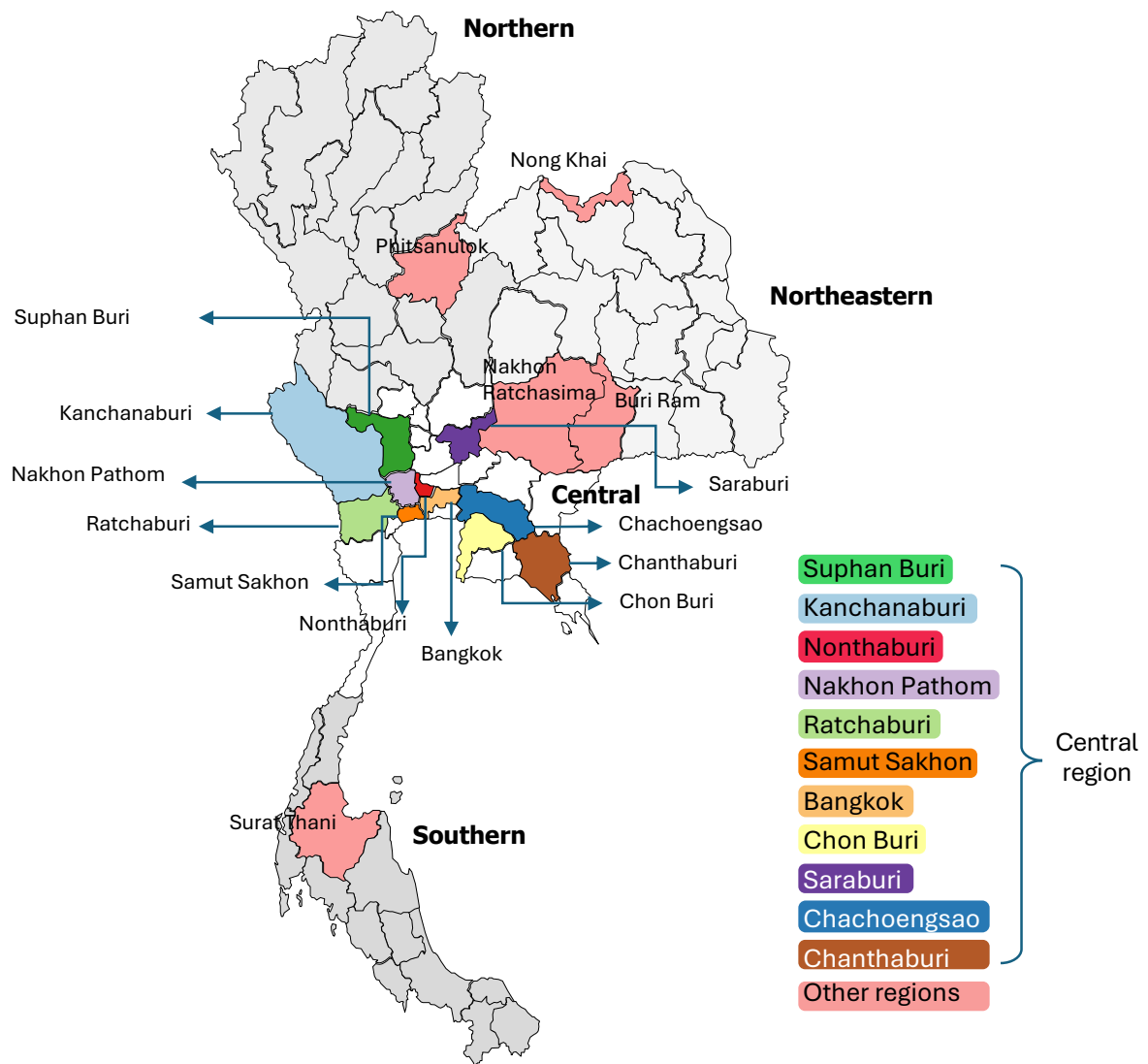
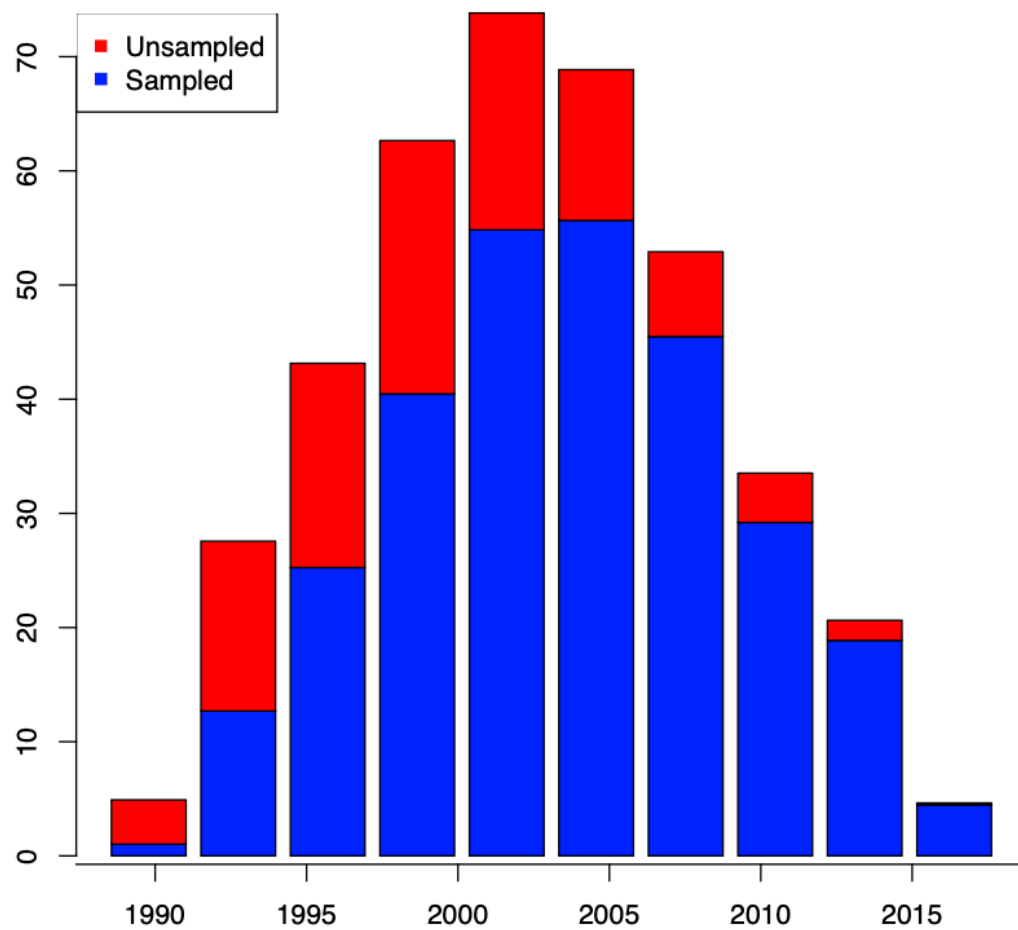


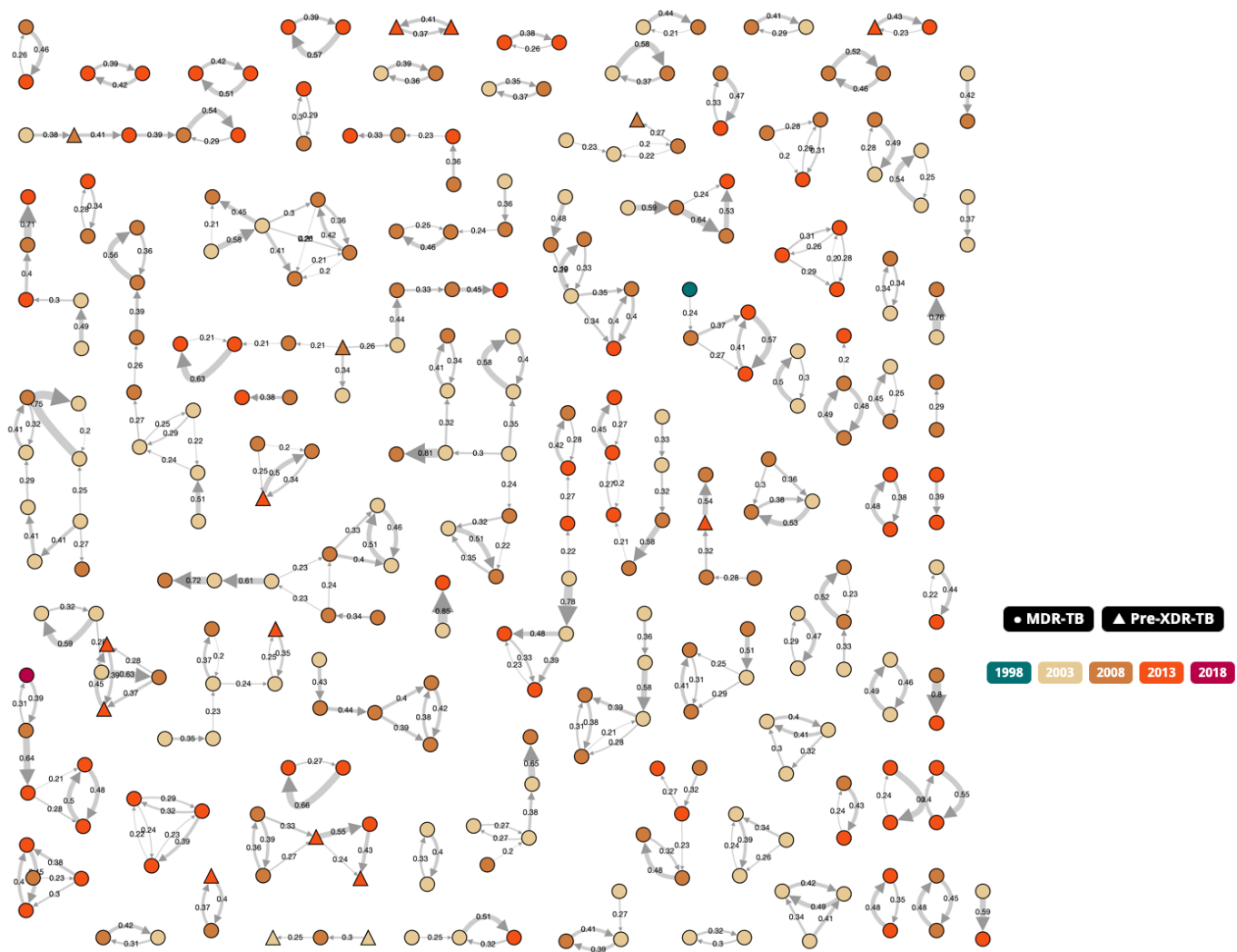
Figure S5. The boxplot displays the relationship between SNP and geographic distance.



**Figure S6. Distribution of the clusters of Mtb isolates in the largest cluster (SNP distance  $\leq 13$ , N = 288 isolates), Thailand**



**Figure S7. Histogram of sampled (n=288) and unsampled TB cases by year based on the combined posterior transmission tree for the largest cluster**



**Figure S8.** The transmission cluster graph depicts relationships between Mtb cases with a transmission probability greater than 0.2. Arrows indicate the direction of transmission, with arrow size reflecting the likelihood of one host infecting another. Node colors correspond to the year of sample collection: 1998 (1998-2002), 2003 (2003-2007), 2008 (2008-2012), 2013 (2013-2017), and 2018 (2018-2019).

**Table S2. List of drug resistance mutations**

| Drugs | Gene name    | Changes (Count)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AMK   | <i>eis</i>   | c.-14C>T (8)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|       | <i>rrs</i>   | n.1401A>G (99), n.1484G>T (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| BDQ   | <i>mmpR5</i> | Ser68Gly (1), Leu117Arg (5), Arg156* (2), c.198dupG (5), c.466dupC (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| CAP   | <i>rrs</i>   | n.1401A>G (99), n.1484G>T (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|       | <i>tlyA</i>  | c.363_364dupGC (1), c.52_53dupCG (3)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| CFZ   | <i>mmpR5</i> | Ser68Gly (1), Leu117Arg (5), Arg156* (2), c.198dupG (5), c.466dupC (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Cs    | <i>alr</i>   | Leu113Arg (3), Met343Thr (2)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|       | <i>ald</i>   | c.436_437dupGC (4), c.464delG (3)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| DLM   | <i>fbiC</i>  | c.989_990dupGC (1),<br>c.2565_*55delGGCCTAGCCCCGGCGACGATGCCGGGTCGCGGGATGCGGCCCCGTT<br>GAGGAGCGGGCAATCT (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| EMB   | <i>embA</i>  | c.-16C>T (15), c.-16C>G (3), c.-12C>T (47), c.-11C>A (15), Asp4Asn (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|       | <i>embB</i>  | Phe285Leu (1), Met306Val (261), Met306Ile (233), Met306Leu (21),<br>Met306Thr (1), Tyr319Ser (32), Asp328Tyr (32), Asp328Gly (3), Tyr334His (2),<br>Ser347Ile (1), Asp354Ala (20), Val369Ala (1), Pro404Ser (2), Glu405Asp (3),<br>Gly406Asp (320), Gly406Ala (28), Gly406Cys (18), Gly406Ser (8), Gln497Arg<br>(78), Gln497Pro (6), Gln497Lys (6), Glu504Asp (1), Arg507Lys (1), Ala659Thr<br>(8), Met1000Arg (1), His1002Arg (5), Asp1024Asn (31)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| ETO   | <i>ethA</i>  | c.-152_766del (2), Met1 (Start loss) (1), His22Pro (1), c.32dupG (1), Trp45* (3),<br>Trp69* (1), Thr88Ile (3), c.110delA (8), c.139_293del (1), c.140delT (4),<br>c.163_506del (1), c.168_681del (1), Glu223Lys (3), Trp228* (1), Thr232Ala (3),<br>Tyr235* (1), Cys253* (3), Gln269* (1), c.280delA (1), Trp289* (1), Arg292* (1),<br>Lys309* (1), Ile338Ser (1), c.341delA (10), Thr342Lys (1), Gln347* (2), Gln359*<br>(5), Gln360* (1), c.391delT (1), Leu397Arg (3), c.413_419dupGCGGCTA (1),<br>c.439delT (1), Glu445* (5), Trp455* (1), c.456delC (4), c.481_482delAT (1),<br>c.489dupT (3),<br>c.489_531delTCCGCAGCACTGGCCCCGAGGACCTCGACTACGACGCTAAGAAC (3),<br>c.551dupG (1), c.594delC (1), c.608_1137del (1), c.628dupA (1),<br>c.639_640delGT (534), c.672_673dupGC (1), c.704_707delACAC (4),<br>c.741_742insC (3), c.752dupG (3), c.754_755dupGC (2), c.771dupA (1),<br>c.815delT (2), c.1031delT (1), c.1034delA (3), c.1047delT (1), c.1054delG (5),<br>c.1080_1159delAGTGGACATCACACGACGATGGCCTACAAGGGCATGATGCTTTC<br>CGGCATCCCCAACATGGCCTACACGGTTGGCTACA (1), c.1123delT (1),<br>c.1203delT (1), c.1290delC (1), c.1307dupC (1), c.1341delC (2),<br>c.1355_1356dupGT (1), c.1361delC (1), c.1392dupC (1), c.1407delG (1),<br>c.1431dupT (1), c.24_25insT (1) |
| ETO   | <i>ethR</i>  | Phe110Leu (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|       | <i>inhA</i>  | c.-779G>T (2), c.-777C>T (182), c.-770T>C (15), c.-770T>A (4), c.-154G>A (16),<br>Ile21Thr (4), Ser94Ala (29), Ile194Thr (7)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| INH   | <i>ahpC</i>  | c.-81C>T (4), c.-74G>A (1), c.-54C>T (3), c.-52C>T (6), c.-48G>A (9), Pro2Ser (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|       | <i>inhA</i>  | c.-779G>T (2), c.-777C>T (182), c.-770T>C (15), c.-770T>A (4), c.-154G>A (16),<br>Ile21Val (5), Ile21Thr (4), Gly40Trp (1), Ser94Ala (29), Ile194Thr (7)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|       | <i>katG</i>  | c.-7239_992del (1), c.-5295_*5855del (1), c.-2345_1109del (1), Val1Leu (1),<br>c.40_47delGGAGCCGC (1), Asp94Gly (2), Ala110Val (1), Gly121Val (1),<br>Gln127Pro (1), Asn138Ser (2), Asn138His (1), Ser140Asn (1), c.152delA (1),<br>Met176Ile (1), Met257Ile (3), Gly279Asp (4), Gln295Pro (1), Asp311Gly (1),<br>Ser315Thr (1435), Ser315Asn (25), Ser315Arg (3), Ser315Ile (2), Ser315Gly (2),<br>Thr326Met (2), Tyr337Phe (1), Gln352* (1), Leu378Pro (3), Thr380Ile (1),                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

| Drugs | Gene name   | Changes (Count)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-------|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|       |             | Ala424Gly (21), c.603_*4483del (2), Trp728Cys (1), c.1060dupA (1), c.1752dupT (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| KAN   | <i>eis</i>  | c.-14C>T (8), c.-12C>T (2), c.-10G>A (17), c.-8C>A (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|       | <i>rrs</i>  | n.1401A>G (99), n.1484G>T (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| LFX   | <i>gyrA</i> | Gly88Ala (5), Ala90Val (88), Ser91Pro (18), Asp94Gly (121), Asp94Ala (39), Asp94Asn (20), Asp94His (11), Asp94Tyr (11), Asp94Val (3)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|       | <i>gyrB</i> | Arg446Cys (3), Arg446His (1), Ser447Phe (1), Asp461Ala (2), Gly470Cys (2), Asp494Ala (8), Asn499Asp (1), Glu501Asp (4), Ala504Val (4)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| MFX   | <i>gyrA</i> | Gly88Ala (5), Ala90Val (88), Ser91Pro (18), Asp94Gly (121), Asp94Ala (39), Asp94Asn (20), Asp94His (11), Asp94Tyr (11), Asp94Val (3)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|       | <i>gyrB</i> | Arg446Cys (3), Arg446His (1), Ser447Phe (1), Asp461Ala (2), Gly470Cys (2), Asp494Ala (8), Asn499Asp (1), Glu501Asp (4), Ala504Val (4)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| PAS   | <i>folC</i> | Thr20Pro (1), Glu40Gly (29), Ile43Thr (20), Ile43Ser (2), Arg49Trp (10), Ser150Gly (91), Glu153Ala (11), Glu153Gly (2)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|       | <i>thyA</i> | c.-6493_*3719del (2), c.-6333_*2084del (1), c.-4473_*2082del (1), c.-4473_*2083del (1), c.-4188_*2026del (1), c.-2787_*2084del (3), c.-2608_*2083del (3), c.-2608_*2082del (1), c.-1233_*1246del (2), c.-683_*84del (1), c.-522_*3884del (1), Thr22Ala (23), Trp83* (5), Gly91Arg (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|       | <i>thyX</i> | c.-16C>T (77)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Pa    | <i>fbiC</i> | c.989_990dupGC (1), c.2565_*55delGGCCTAGCCCCGGCGACGATGCCGGGTCGCGGGATGCGGCCCCGTT GAGGAGCGGGGCAATCT (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| PZA   | <i>pncA</i> | c.2565_*55delGGCCTAGCCCCGGCGACGATGCCGGGTCGCGGGATGCGGCCCCGTT GAGGAGCGGGGCAATCT (1), c.-2134_*13301del (1), c.-1908_*14025del (1), c.-1832_*2695del (1), c.-1644_*7del (1), c.-1103_*3980del (1), c.-1092_*625del (2), c.-1081_314del (1), c.-1072_*3285del (2), c.-642_*3550del (2), c.-438_*27692del (1), c.-388_*2261del (1), c.-12T>C (3), c.-11A>G (25), c.-11A>C (19), Met1 (Start loss) (12), Ala3Glu (1), Leu4Ser (3), Leu4Trp (3), Ile5Ser (2), Ile6Thr (2), Val7Gly (2), Asp8Ala (5), Val9Gly (1), Gln10Pro (12), Gln10* (2), Gln10Lys (1), Asp12Ala (6), Asp12Asn (4), Phe13Leu (1), Cys14Tyr (6), Cys14Trp (1), Cys14Gly (1), Gly17Asp (1), Ser18* (1), Leu19Arg (3), Leu19Pro (3), Val21Gly (2), Leu27Pro (8), Ala28Asp (1), Ile31Thr (290), Ile31Ser (2), Leu35Pro (2), Glu37* (1), Tyr41* (2), Val44Gly (1), Ala46Val (2), Ala46Glu (1), Thr47Pro (11), Thr47Ala (1), c.47delG (1), Lys48* (1), c.48delT (1), Asp49Gly (5), Asp49Glu (1), c.50delG (1), His51Tyr (2), His51Pro (2), His51Arg (2), Pro54Ser (3), Pro54Leu (1), His57Arg (1), Ser59Pro (1), Thr61Pro (14), Pro62Leu (4), Asp63Gly (2), Asp63Ala (1), Ser66Pro (1), Ser67Pro (7), Trp68Arg (7), Trp68Cys (4), Trp68* (3), Trp68Gly (1), Pro69Arg (2), His71Arg (4), His71Asp (2), His71Tyr (2), Cys72Tyr (3), Cys72Arg (2), Thr76Pro (8), Thr76Ile (4), c.80_81insTT (1), c.80_89delTTGGCCCGCGC (1), His82Asp (1), Ile90Ser (31), Ile90Asn (1), Phe94Leu (8), Phe94Ser (2), Lys96Glu (6), Lys96Thr (5), Lys96Arg (4), Lys96Asn (1), Gly97Ser (3), Gly97Asp (2), Gly97Cys (2), Gly97Arg (1), Thr100Pro (18), Tyr103* (4), Tyr103His (4), Ser104Arg (9), Gly105Val (6), Gly105Asp (3), Phe106Leu (2), Phe106Ser (1), Gly108Arg (8), Trp119Arg (3), Trp119Cys (1), Trp119Ser (1), Trp119Gly (1), Glu127* (4), Val128Gly (1), Val131Gly (1), Gly132Ser (1), Gly132Ala (1), Ile133Thr (2), Ala134Val (1), Thr135Pro (6), Asp136Gly (3), His137Pro (1), Cys138Tyr (2), Cys138Ser (1), Cys138Arg (1), Val139Gly (13), Val139Ala (5), c.139delA (3), Val139Leu (1), Gln141Pro (13), Gln141* (2), Thr142Ala (5), Thr142Lys (2), Thr142Pro (1), Thr142Met (1), Ala143Pro (1), Ala146Val (2), Leu151Ser (5), Leu151* (1), |

| Drugs | Gene name   | Changes (Count)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|       |             | c.152_161delACATCGACCC (1), Arg154Gly (2), c.154dupA (1), Val155Gly (2), Leu156Pro (1), Leu159Pro (1), Leu159Arg (1), Thr160Pro (5), Val163Ala (7), Ser164Pro (1), c.166_167dupGA (1), c.171_172insA (1), Leu172Pro (2), Leu172Arg (2), Glu174* (2), Met175Thr (1), Val180Ala (1), Glu181* (2), Leu182Ser (1), c.220_229delAGCGGTACTC (1), c.237_240dupGGAC (1), c.244dupC (2), c.249delC (4), c.287dupA (1), c.294dupC (2), c.295delT (1), c.296delA (1), c.321dupA (1), c.354delTinsGA (1), c.364_365dupCA (1), c.377_389delATGAGGTCGATGT (1), c.386_394delATGTGGTCG (4), c.390_391dupGG (5), c.391dupG (10), c.393dupC (1), c.407dupA (5), c.407_408insC (2), c.416_417delTG (2), c.429delC (1), c.446delA (2), c.449dupG (6), c.459delC (4), c.470_471insA (1), c.486_487insA (1), c.493_496dupGCCG (1), c.519_520dupGG (2), c.559T>C (1)                                                                                                                                                                                                                                                                                                                                                                                                               |
| RIF   | <i>rpoB</i> | Val170Phe (19), Ala286Val (3), Thr400Ala (10), Phe424Leu (1), Gln429His (1), Leu430Pro (6), Leu430Arg (3), Leu430Val (1), Gln432Pro (10), Gln432Lys (7), Gln432Glu (5), Gln432Leu (1), Met434Val (2), Asp435Val (115), Asp435Tyr (26), Asp435Phe (11), Asp435Glu (6), Asp435Ala (5), Asp435Gly (4), Asp435Asn (1), Asp435Cys (1), Asp435Ile (1), Asp435Leu (1), Asp435Ser (1), Asn437His (4), Asn437Asp (2), Pro439Ser (1), Ser441Leu (21), Ser441Gln (2), Ser441Met (1), Leu443Trp (1), Thr444Pro (1), His445Tyr (209), His445Asp (107), His445Arg (56), His445Leu (33), His445Pro (6), His445Gln (5), His445Gly (4), His445Cys (4), His445Asn (3), His445Thr (1), His445Val (1), His445Ser (1), Lys446Gln (1), Arg448Gln (2), Ser450Leu (924), Ser450Trp (18), Ser450Phe (4), Ser450Tyr (3), Ser450Gln (1), Ser450Cys (1), Ala451Gly (1), Leu452Pro (71), Pro454His (2), Glu460Gly (7), Ile491Phe (13), Arg552Cys (3), Glu761Asp (2), c.1295_1300delAATTCA (2), c.1296_1304delATTCATGGA (5), c.1297_1299dupTTC (7), c.1297_1305dupTTCATGGAC (3), c.1299_1305delCATGGACinsT (2), c.1300_1305delATGGAC (5), c.1301_1303dupTGG (1), c.1303_1305delGAC (3), c.1303_1308delGACCAG (1), c.1306_1311delCAGAAC (1), c.1312_1314delAAC (17), c.1335_1337delCAA (1) |
|       | <i>rpoC</i> | Gly332Arg (8), Phe452Ser (1), Ile491Thr (10), Leu527Val (8)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| STM   | <i>gid</i>  | c.-834_*101del (1), c.-559_171del (1), Tyr22* (2), Leu26* (1), Gly28* (1), c.31_248del (7), c.52_59dupCTTGCTCG (1), Ser70Asn (1), Gly73Ala (22), Pro75Arg (1), Leu79Ser (7), c.87delC (2), c.87dupC (1), Glu92* (1), c.102delG (30), c.102dupG (1), c.115delC (9), Gln125* (1), Gln127* (1), Glu173* (3), Arg213* (1), c.254delA (2), c.294delC (1), c.311dupT (1), c.340_343delATCG (2), c.346dupC (1), c.351delG (18), c.351dupG (3), c.371dupT (2), c.373delC (2), c.386delG (3), c.477delG (1), c.518dupA (1), c.541delT (1), c.570delA (3), c.575dupG (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|       | <i>rpsL</i> | Lys43Arg (931), Lys88Arg (74), Lys88Met (3), Lys88Thr (2), Lys88Gln (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|       | <i>rrs</i>  | n.513C>T (2), n.514A>C (34), n.514A>T (3), n.517C>T (21), n.878G>A (3), n.888G>A (1), n.905C>A (6), n.905C>G (1), n.906A>G (6), n.907A>C (5), n.907A>T (2)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

AMK Amikacin; BDQ Bedaquiline; CAP Capreomycin; CFZ Clofazimine; Cs Cycloserine; DLM Delamanid; EMB Ethambutol; ETO Ethionamide; INH Isoniazid; KAN Kanamycin; LFX Levofloxacin; MFX Moxifloxacin; PAS Para-aminosalicylic acid; PZA Pyrazinamide; RIF Rifampicin; STM Streptomycin

**Table S3. Phenotypic data (laboratory DSTs)**

| Characteristics               |            | Phenotypic     |        |       | Genotypic (N=2,005) |       |
|-------------------------------|------------|----------------|--------|-------|---------------------|-------|
|                               |            | Max.<br>N=1813 | Number | %     | Number              | %     |
| Drug resistance               | Sensitive  | -              | 59     | 2.94  | 233                 | 11.62 |
|                               | RR TB      | -              | 4      | 0.20  | 39                  | 1.95  |
|                               | HR TB      | -              | 19     | 0.95  | 53                  | 2.64  |
|                               | MDR TB     | -              | 1522   | 75.91 | 1349                | 67.28 |
|                               | Pre-XDR TB | -              | 201    | 10.02 | 293                 | 14.61 |
|                               | XDR TB     | -              | 3      | 0.16  | 5                   | 0.25  |
|                               | Other      | -              | 11     | 0.55  | 33                  | 1.65  |
|                               | Not Done   | -              | 186    | 9.28  | -                   | -     |
| Anti-TB drug*<br>(Resistance) | INH        | 1811           | 1732   | 95.64 | 1686                | 84.09 |
|                               | RIF        | 1813           | 1720   | 94.87 | 1693                | 84.44 |
|                               | EMB        | 1653           | 727    | 43.98 | 1095                | 54.61 |
|                               | STM        | 1801           | 1113   | 61.8  | 1212                | 60.45 |
|                               | PZA        | ND             | -      | -     | 812                 | 40.50 |
|                               | LFX        | 1602           | 199    | 12.42 | 305                 | 15.21 |
|                               | MFx        | 1101           | 95     | 8.63  | 305                 | 15.21 |
|                               | AMK        | 1172           | 81     | 6.91  | 108                 | 5.39  |
|                               | KAN        | 1687           | 127    | 7.53  | 128                 | 6.38  |
|                               | ETO        | ND             | -      | -     | 875                 | 43.64 |
|                               | BDQ        | ND             | -      | -     | 14                  | 0.70  |
|                               | LZD        | 1097           | 4      | 0.36  | 0                   | 0     |

RR-TB, Rifampicin-Resistant; HR-TB, isoniazid-monoresistant; MDR-TB, Multidrug-Resistant; Pre-XDR TB, Pre-Extensively Drug-Resistant; XDR-TB, Extensively Drug-Resistant; INH, Isoniazid; RIF, Rifampicin; EMB, Ethambutol; STM, Streptomycin; PZA, Pyrazinamide; LFX, Levofloxacin; MFx, Moxifloxacin; AMK, Amikacin, KAN, Kanamycin; ETO, Ethionamide; PAS, Para-aminosalicylic acid; CAP, Capreomycin; \* The number of isolates that underwent phenotypic DST varied for each drug.

**Table S4. Performance Comparison of laboratory phenotypic DST and WGS-based genotypic resistance**

| Drug | Phenotypic Resistance<br>N (%) |             | Phenotypic Sensitive<br>N (%) |                  | ND             | Concordance<br>rate (%) | Sens<br>(%) | Spec<br>(%) | PPV<br>(%) | NPV<br>(%) |
|------|--------------------------------|-------------|-------------------------------|------------------|----------------|-------------------------|-------------|-------------|------------|------------|
|      | Geno R                         | Geno S      | Geno R                        | Geno S           |                |                         |             |             |            |            |
| INH  | 1,665<br>(83.04)               | 67 (3.34)   | 4<br>(0.20)                   | 75 (3.74)        | 194<br>(9.68)  | 96.08                   | 96.13       | 94.94       | 99.76      | 52.82      |
| RIF  | 1,668<br>(83.19)               | 52 (2.59)   | 5<br>(0.25)                   | 88 (4.39)        | 192<br>(9.58)  | 96.86                   | 96.98       | 94.62       | 99.70      | 62.86      |
| STM  | 1,056<br>(52.67)               | 57 (2.84)   | 128<br>(6.38)                 | 560 (27.93)      | 204<br>(10.17) | 89.73                   | 94.88       | 81.4        | 89.19      | 90.76      |
| EMB  | 627<br>(31.27)                 | 100 (4.99)  | 367<br>(18.30)                | 559 (27.88)      | 352<br>(17.56) | 71.75                   | 86.24       | 60.37       | 63.08      | 84.83      |
| AMK  | 59 (2.94)                      | 22 (1.10)   | 7<br>(0.35)                   | 1,084<br>(54.06) | 833<br>(41.55) | 97.53                   | 72.84       | 99.36       | 89.39      | 98.01      |
| KAN  | 100 (4.99)                     | 27 (1.35)   | 27<br>(1.35)                  | 1,533<br>(76.46) | 318<br>(15.86) | 96.80                   | 78.74       | 98.27       | 78.74      | 98.27      |
| LFX  | 178 (8.88)                     | 21 (1.05)   | 109<br>(5.44)                 | 1,294<br>(64.54) | 403<br>(20.10) | 91.89                   | 89.45       | 92.23       | 62.02      | 98.4       |
| MFX  | 87 (4.34)                      | 8<br>(0.40) | 117<br>(5.84)                 | 889 (44.34)      | 904<br>(45.09) | 88.65                   | 91.58       | 88.37       | 42.65      | 99.11      |

Geno Genotypic; R Resistance; S Sensitive; ND not determined; Spec specificity; Sens sensitivity; PPV Positive Predictive Value; NPV Negative Predictive Value; INH Isoniazid; RIF Rifampicin; EMB Ethambutol; STM Streptomycin; LFX Levofloxacin; MFX Moxifloxacin; AMK Amikacin, KAN Kanamycin

**Table S5. High-confidence mutations predicted to be related to resistance to TB drugs.**

| Drug | Gene name   | Change                   | Our study (N=2,005) |   |           |     | Global (N = 50,723) <sup>1</sup> |    |           |     |
|------|-------------|--------------------------|---------------------|---|-----------|-----|----------------------------------|----|-----------|-----|
|      |             |                          | Phenotypic DST      |   | DST group |     | Phenotypic DST                   |    | DST group |     |
|      |             |                          | R                   | S | MDR       | Sen | R                                | S  | MDR       | Sen |
| INH  | <i>katG</i> | Pro232Ser                | 3                   |   |           |     | 8                                |    | 5         |     |
|      | <i>katG</i> | Gly285Ala                | 2                   | 1 |           |     |                                  | 1  |           |     |
|      | <i>katG</i> | Gly121Ala                | 2                   |   | 1         |     | 1                                | 1  |           | 1   |
|      | <i>katG</i> | Leu707Phe                | 2                   | 1 |           |     |                                  | 1  |           |     |
|      | <i>katG</i> | Trp300Arg                | 1                   |   |           |     | 2                                |    |           | 1   |
|      | <i>katG</i> | Trp341Gly                | 2                   |   | 1         |     |                                  |    |           |     |
|      | <i>katG</i> | Phe483Ser                | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | Leu430Pro                | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | Leu333Pro                | 1                   |   |           | 1   | 2                                |    |           |     |
|      | <i>katG</i> | Trp412Ser                | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | Arg385Pro                | 1                   |   |           |     | 2                                |    | 3         |     |
|      | <i>katG</i> | Asp142Gly                | 1                   |   |           |     | 10                               |    | 1         | 4   |
|      | <i>katG</i> | Ala550Thr                | 1                   |   |           | 1   |                                  |    |           |     |
|      | <i>katG</i> | Ala245Glu                | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | c.-9A>T                  | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | c.-7G>C                  | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | Tyr64Cys                 | 1                   |   |           |     | 1                                |    | 1         |     |
|      | <i>katG</i> | Gln679Pro                | 1                   |   |           |     |                                  |    |           |     |
|      | <i>katG</i> | Leu662Pro                | 1                   |   | 1         |     |                                  |    |           |     |
|      | <i>katG</i> | Leu427Pro                | 1                   |   | 1         |     |                                  |    |           | 1   |
|      | <i>katG</i> | Gly699Glu                | 1                   |   | 1         |     | 5                                |    |           |     |
|      | <i>katG</i> | c.-1286_444del           | 2                   |   | 2         |     |                                  |    |           |     |
|      | <i>ahpC</i> | c.-72C>T                 | 1                   |   |           |     | 36                               | 3  | 23        | 2   |
|      | <i>ahpC</i> | c.-51G>A                 | 2                   |   | 1         |     | 51                               | 8  | 19        | 7   |
| RIF  | <i>rpoB</i> | c.1290_1298delGAGC CAATT | 1                   |   |           |     |                                  |    |           |     |
|      | <i>rpoB</i> | c.1292_1300delGCCAA TTCA | 2                   |   |           | 1   |                                  |    |           |     |
|      | <i>rpoB</i> | Val496Met                | 1                   |   | 1         |     | 16                               |    | 9         |     |
|      | <i>rpoB</i> | Lys37Arg                 | 34                  |   | 33        |     | 9                                | 2  | 9         | 1   |
|      | <i>rpoB</i> | Met655Thr                | 11                  | 0 | 10        | 1   | 1                                | 49 | 1         | 47  |
|      | <i>rpoC</i> | Gly1198Ser               | 93                  | 1 | 65        | 2   | 21                               | 18 | 13        | 15  |
|      | <i>rpoC</i> | Lys445Arg                | 14                  |   | 13        |     | 54                               |    | 37        |     |
| EMB  | <i>embA</i> | c.-43G>C                 | 4                   | 2 | 6         |     | 90                               | 23 | 97        |     |
|      | <i>embA</i> | Leu832Phe                | 2                   |   | 2         |     |                                  | 1  |           |     |
|      | <i>embA</i> | c.-16C>A                 | 13                  | 5 | 17        |     | 48                               | 15 | 58        | 1   |
|      | <i>embA</i> | c.-15C>G                 | 3                   |   | 1         |     | 19                               | 12 | 20        |     |
|      | <i>embA</i> | Arg537Trp                |                     |   | 1         |     |                                  |    |           |     |
|      | <i>embA</i> | Gly814Glu                | 3                   | 1 | 4         |     |                                  |    | 1         |     |
|      | <i>embA</i> | Val613Gly                | 1                   |   | 1         |     |                                  |    |           |     |
|      | <i>embB</i> | Gln497His                | 7                   | 1 | 8         |     | 17                               | 5  | 18        |     |
|      | <i>embB</i> | Ile303Leu                | 1                   | 1 | 2         |     | 1                                |    | 1         |     |
|      | <i>embB</i> | Gln445Arg                | 1                   |   |           |     | 54                               |    | 23        |     |
|      | <i>embB</i> | Asn399Thr                | 1                   |   | 1         |     | 3                                | 2  | 5         |     |
|      | <i>embB</i> | Asn318Asp                | 1                   |   | 1         |     | 1                                | 1  | 3         |     |
|      | <i>embB</i> | Asn296Tyr                | 1                   | 1 | 2         |     | 2                                |    | 1         |     |

| Drug | Gene name   | Change    | Our study (N=2,005) |    |           |     | Global (N = 50,723) <sup>1</sup> |     |           |     |
|------|-------------|-----------|---------------------|----|-----------|-----|----------------------------------|-----|-----------|-----|
|      |             |           | Phenotypic DST      |    | DST group |     | Phenotypic DST                   |     | DST group |     |
|      |             |           | R                   | S  | MDR       | Sen | R                                | S   | MDR       | Sen |
|      | <i>embB</i> | Ala81Thr  | 1                   |    |           |     |                                  |     |           |     |
|      | <i>embB</i> | Gly443Cys | 2                   |    | 2         |     |                                  |     |           |     |
|      | <i>embC</i> | Ala177Thr | 1                   |    | 1         |     |                                  |     |           |     |
| STM  | <i>gid</i>  | Val112Gly | 1                   |    | 1         |     |                                  | 1   | 3         | 1   |
|      | <i>gid</i>  | Ala138Val | 1                   | 1  | 1         |     | 12                               | 8   | 26        | 28  |
|      | <i>gid</i>  | Arg21Pro  | 1                   | 3  | 4         |     | 2                                | 1   | 7         | 2   |
|      | <i>gid</i>  | Leu91Arg  | 1                   |    |           |     | 1                                |     | 4         | 5   |
|      | <i>gid</i>  | Leu90Pro  | 1                   | 1  | 1         |     |                                  | 1   | 1         |     |
|      | <i>gid</i>  | Leu16Pro  | 1                   | 1  | 2         |     | 1                                |     | 3         | 2   |
|      | <i>gid</i>  | Ile81Thr  | 1                   | 1  | 2         |     | 1                                | 1   | 4         | 9   |
|      | <i>gid</i>  | Gly157Glu | 1                   |    | 1         |     | 1                                |     | 6         | 1   |
|      | <i>gid</i>  | Cys52Trp  | 1                   |    | 1         |     |                                  | 2   |           | 2   |
|      | <i>gid</i>  | Cys52Phe  | 1                   | 1  | 2         |     | 3                                |     | 3         | 2   |
|      | <i>gid</i>  | Arg64Trp  | 1                   |    |           | 1   | 1                                |     |           | 2   |
|      | <i>rpsL</i> | Lys43Asn  | 4                   |    |           |     |                                  |     |           |     |
|      | <i>rpsL</i> | Arg86Gln  | 3                   |    | 3         |     |                                  |     | 4         | 2   |
|      | <i>rrs</i>  | n.16T>C   | 4                   |    | 4         |     | 1                                |     | 2         |     |
|      | <i>rrs</i>  | n.1401A>G | 73                  | 18 | 23        |     | 541                              | 126 | 790       |     |
|      | <i>rrs</i>  | n.13A>G   | 3                   |    |           |     | 2                                |     |           |     |
|      | <i>rrs</i>  | n.23T>G   | 2                   | 2  | 1         |     |                                  |     | 2         |     |
|      | <i>rrs</i>  | n.908A>C  | 2                   |    | 2         |     | 11                               | 1   | 17        | 1   |
|      | <i>rrs</i>  | n.908A>T  | 1                   |    |           |     | 1                                |     | 1         |     |

R Resistance; S Sensitive; MDR multidrug resistant; INH Isoniazid; RIF Rifampicin; EMB Ethambutol; STM Streptomycin; <sup>1</sup> The global and Thailand percentage of anti-TB drug resistance mutations identified by TB-Profiler database

**Table S6. Discrepancies between laboratory phenotypic DSTs and WGS-based genotypic resistance profiles**

|                      |            | Phenotypic DST categories |        |        |         |             |         |       | Total |
|----------------------|------------|---------------------------|--------|--------|---------|-------------|---------|-------|-------|
|                      |            | Sens                      | RR- TB | HR- TB | MDR- TB | Pre-XDR- TB | XDR- TB | Other |       |
| Genotypic resistance | Sensitive  | 51                        | 0      | 2      | 18      | 4           | 0       | 4     | 233   |
|                      | RR -TB     | 1                         | 5      | 0      | 30      | 1           | 0       | 1     | 39    |
|                      | HR-TB      | 2                         | 0      | 17     | 21      | 3           | 0       | 0     | 53    |
|                      | MDR-TB     | 0                         | 0      | 0      | 1331    | 9           | 3       | 1     | 1349  |
|                      | Pre-XDR-TB | 1                         | 0      | 0      | 111     | 178         | 0       | 2     | 293   |
|                      | XDR-TB     | 0                         | 0      | 0      | 3       | 2           | 0       | 0     | 5     |
|                      | Other      | 4                         | 0      | 0      | 6       | 1           | 0       | 7     | 33    |
| Total                |            | 59                        | 5      | 19     | 1520    | 198         | 3       | 15    | 2005  |

ND not determined; Sens sensitive; RR-TB Rifampicin-Resistant; HR-TB isoniazid-monoresistant; MDR-TB Multidrug-Resistant; Pre-XDR-TB Pre-Extensively Drug-Resistant; XDR-TB Extensively Drug-Resistant

**Table S7. Anti-TB Drug Resistance Rates and Gene Changes (Resistance Rate < 50%; Total > 10 Mtb isolates)**

| Drug | Gene name   | Changes   | Phenotypic DSTs (N) |     | Total (N) | Resistance rate (%) |
|------|-------------|-----------|---------------------|-----|-----------|---------------------|
|      |             |           | R                   | S   |           |                     |
| EMB  | <i>embB</i> | Gly406Asp | 128                 | 156 | 284       | 45                  |
| KAN  | <i>eis</i>  | c.-10G>A  | 0                   | 17  | 17        | 0                   |
| LFX  | <i>gyrA</i> | Asp94Ala  | 15                  | 20  | 35        | 43                  |
|      | <i>gyrA</i> | Ser91Pro  | 8                   | 10  | 18        | 44                  |
| MFX  | <i>gyrA</i> | Ala90Val  | 14                  | 40  | 54        | 26                  |
|      | <i>gyrA</i> | Asp94Ala  | 4                   | 22  | 26        | 15                  |
|      | <i>gyrA</i> | Ser91Pro  | 5                   | 10  | 15        | 33                  |
| STM  | <i>gid</i>  | c.102delG | 9                   | 12  | 21        | 43                  |
|      | <i>gid</i>  | c.351delG | 4                   | 11  | 15        | 27                  |

EMB, Ethambutol; STM, Streptomycin; LFX, Levofloxacin; MFX, Moxifloxacin; AMK, Amikacin, KAN, Kanamycin

**Table S8. Summary of Percentages of Lineage, genotypic drug resistance (DR) categories, and Region for Clusters with  $\geq 10$  Isolates (SNP Cut-off of 13)**

| Cluster No.     |           | 1     | 2     | 3    | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14      | 15      | 16    |
|-----------------|-----------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|-------|
| No. of isolates |           | 288   | 81    | 64   | 48    | 43    | 42    | 36    | 22    | 19    | 18    | 16    | 12    | 11    | 11      | 10      | 10    |
| Region (%)      | Central   | 96.9  | 75.3  | 85.9 | 18.8  | 44.2  | 61.9  | 38.9  | 100   | 57.9  | 83.3  | 50    | 83.3  | 63.6  | 9.1     | 90      | 50    |
|                 | Northeast | 1.4   | 17.3  | 4.7  | 18.8  | 44.2  | 23.8  | 41.7  | -     | 36.8  | 11.1  | 37.5  | 8.3   | 9.1   | 18.2    | -       | 20    |
|                 | Northern  | 1     | 4.9   | 4.7  | 18.8  | 11.6  | 4.8   | 11.1  | -     | -     | 5.6   | 12.5  | 8.3   | 9.1   | -       | 10      | -     |
|                 | Southern  | 0.7   | 2.5   | 4.7  | 43.8  | -     | 9.5   | 8.3   | -     | 5.3   | -     | -     | -     | 18.2  | 72.7    | -       | 30    |
| Sub-lineage     |           | 2.2.1 | 2.2.1 | 2.1  | 2.1.1 | 2.2.1 | 2.2.1 | 2.2.1 | 2.2.1 | 2.2.1 | 2.2.1 | 2.2.1 | 4.2.2 | 2.2.1 | 2.2.1.1 | 2.2.1.1 | 2.2.1 |
| Drug Res. (%)   | HR-TB     | -     | -     | -    | 4.2   | -     | -     | 2.8   | -     | -     | -     | -     | -     | -     | -       | -       | -     |
|                 | MDR-TB    | 93.8  | 82.7  | 68.8 | 93.8  | 81.4  | 95.2  | 69.4  | -     | -     | 100   | 87.5  | 75    | 100   | 72.7    | 100     | 100   |
|                 | Pre-XDR   | 6.3   | 17.3  | 28.1 | 2.1   | 18.6  | 4.8   | 16.7  | 100   | 100   | -     | 12.5  | 25    | -     | 27.3    | -       | -     |
|                 | RR-TB     | -     | -     | 3.1  | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -       | -       | -     |
|                 | XDR-TB    | -     | -     | -    | -     | -     | -     | 11.1  | -     | -     | -     | -     | -     | -     | -       | -       | -     |
| Collection Year | 1999      | -     | -     | 1.6  | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -       | -       | -     |
|                 | 2000      | -     | -     | 1.6  | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -       | -       | -     |
|                 | 2001      | -     | 1.2   | 0.0  | 2.1   | -     | -     | -     | -     | -     | -     | -     | 8.3   | -     | -       | -       | 10.0  |
|                 | 2002      | 0.3   | 1.2   | 1.6  | 8.3   | -     | -     | -     | -     | -     | 5.6   | -     | 8.3   | -     | -       | -       | 0.0   |
|                 | 2003      | 2.8   | 3.7   | 7.8  | 0.0   | -     | 4.8   | 2.8   | -     | -     | 0.0   | 6.3   | 0.0   | -     | -       | -       | 0.0   |
|                 | 2004      | 5.6   | 1.2   | 3.1  | 0.0   | -     | 0.0   | 0.0   | -     | 10.5  | 0.0   | 0.0   | 8.3   | -     | -       | -       | 0.0   |
|                 | 2005      | 6.6   | 4.9   | 7.8  | 6.3   | -     | 0.0   | 2.8   | 9.1   | 0.0   | 5.6   | 0.0   | 0.0   | 9.1   | -       | -       | 0.0   |
|                 | 2006      | 7.3   | 6.2   | 4.7  | 6.3   | 7.0   | 0.0   | 2.8   | 4.5   | 5.3   | 5.6   | 0.0   | 8.3   | 0.0   | -       | -       | 0.0   |
|                 | 2007      | 12.8  | 9.9   | 3.1  | 6.3   | 2.3   | 0.0   | 8.3   | 13.6  | 0.0   | 0.0   | 6.3   | 8.3   | 0.0   | -       | -       | 10.0  |
|                 | 2008      | 6.6   | 7.4   | 9.4  | 8.3   | 2.3   | 0.0   | 11.1  | 22.7  | 0.0   | 0.0   | 12.5  | 8.3   | 9.1   | -       | 10.0    | 0.0   |
|                 | 2009      | 11.1  | 3.7   | 7.8  | 4.2   | 0.0   | 9.5   | 5.6   | 9.1   | 10.5  | 0.0   | 12.5  | 8.3   | 18.2  | -       | 0.0     | 0.0   |
|                 | 2010      | 6.3   | 2.5   | 9.4  | 8.3   | 0.0   | 9.5   | 2.8   | 0.0   | 0.0   | 11.1  | 0.0   | 0.0   | 0.0   | -       | 10.0    | 0.0   |
|                 | 2011      | 8.3   | 3.7   | 6.3  | 2.1   | 4.7   | 0.0   | 16.7  | 4.5   | 0.0   | 5.6   | 6.3   | 8.3   | 0.0   | -       | 10.0    | 0.0   |
|                 | 2012      | 3.1   | 7.4   | 4.7  | 2.1   | 4.7   | 14.3  | 8.3   | 22.7  | 5.3   | 11.1  | 12.5  | 0.0   | 9.1   | 27.3    | 10.0    | 20.0  |
|                 | 2013      | 3.1   | 4.9   | 1.6  | 0.0   | 4.7   | 0.0   | 2.8   | 0.0   | 5.3   | 11.1  | 6.3   | 0.0   | 18.2  | 9.1     | 0.0     | 10.0  |
|                 | 2014      | 8.7   | 8.6   | 7.8  | 10.4  | 27.9  | 11.9  | 11.1  | 4.5   | 26.3  | 11.1  | 12.5  | 0.0   | 27.3  | 18.2    | 10.0    | 30.0  |
|                 | 2015      | 5.2   | 17.3  | 10.9 | 14.6  | 18.6  | 14.3  | 2.8   | 0.0   | 10.5  | 11.1  | 6.3   | 8.3   | 0.0   | 18.2    | 30.0    | 10.0  |
|                 | 2016      | 3.1   | 2.5   | 4.7  | 12.5  | 14.0  | 14.3  | 11.1  | 4.5   | 15.8  | 11.1  | 0.0   | 16.7  | 0.0   | 9.1     | 0.0     | 0.0   |
|                 | 2017      | 8.7   | 13.6  | 6.3  | 6.3   | 14.0  | 21.4  | 11.1  | 4.5   | 10.5  | 11.1  | 18.8  | 8.3   | 9.1   | 18.2    | 20.0    | 10.0  |
|                 | 2019      | 0.3   | -     | -    | 0.0   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -       | -       | -     |

|             |   |   |   |     |   |   |   |   |   |   |   |   |   |   |   |   |
|-------------|---|---|---|-----|---|---|---|---|---|---|---|---|---|---|---|---|
| <b>2020</b> | - | - | - | 2.1 | - | - | - | - | - | - | - | - | - | - | - | - |
|-------------|---|---|---|-----|---|---|---|---|---|---|---|---|---|---|---|---|

- represents a zero count; RR-TB Rifampicin-Resistant; HR-TB isoniazid-monoresistant; MDR-TB Multidrug-Resistant; Pre-XDR Pre-Extensively Drug-Resistant; XDR-TB Extensively Drug-Resistant; L Lineage

**Table S9. Logistic Regression Analysis of Factors Associated with Clustered isolates**

| Variable                            | OR    | LCL  | UCL    | P-value |
|-------------------------------------|-------|------|--------|---------|
| Lineage L4 vs. L1                   | 2.03  | 1.23 | 3.36   | *       |
| Lineage L2 vs. L1                   | 7.41  | 5.34 | 10.29  | ***     |
| HR-TB vs. Sensitive                 | 2.96  | 1.47 | 5.98   | *       |
| MDR-TB vs. Sensitive                | 5.82  | 3.93 | 8.61   | ***     |
| Pre-XDR-TB vs. Sensitive            | 10.98 | 6.72 | 17.94  | ***     |
| XDR-TB vs. Sensitive                | 11.30 | 1.00 | 126.89 | *       |
| Central vs. Northern<br>region      | 0.33  | 0.23 | 0.49   | ***     |
| Northeastern vs.<br>Northern region | 0.50  | 0.36 | 0.73   | ***     |

\*  $P < 0.05$ ; \*\*\*,  $P < 0.001$ ; L Lineage; OR odds ratio; LCL 95% lower confidence limit; UCL 95% upper confidence limit; RR-TB rifampicin resistant; HR-TB, isoniazid-monoresistant; MDR-TB Multidrug-Resistant; Pre-XDR-TB Pre-Extensively Drug-Resistant; XDR-TB Extensively Drug-Resistant

**Table S10. SNPs Associated with Clustered isolates (GEMMA, beta P-value < 0.001)**

| Gene                              | Ref | Alt | Position | Protein Change | beta  | OR   | LCL  | UCL   | P   | Gene function                                                                                  |
|-----------------------------------|-----|-----|----------|----------------|-------|------|------|-------|-----|------------------------------------------------------------------------------------------------|
| <i>katG</i><br>( <i>Rv1908c</i> ) | C   | G   | 2155168  | Ser315Thr      | 0.09  | 7.94 | 6.39 | 9.87  | *** | a catalase-<br>peroxidase,<br>converts INH to an<br>active form                                |
| <i>folC</i><br>( <i>Rv2447c</i> ) | T   | C   | 2747151  | Ser150Gly      | 0.19  | 6.50 | 3.13 | 13.51 | *** | folate biosynthesis,<br>involved in the<br>activation of Para-<br>aminosalicylic acid<br>(PAS) |
| <i>ppe8</i><br>( <i>Rv0355c</i> ) | C   | T   | 427755   | Val2309Ile     | 0.22  | 2.81 | 1.05 | 8.28  | *   | adaptation to host<br>defence<br>mechanisms                                                    |
| <i>folK</i><br>( <i>Rv3606c</i> ) | C   | G   | 4048607  | Gln47His       | -0.30 | 0.65 | 0.43 | 0.99  | *   | dihydrofolate<br>biosynthesis<br>pathway                                                       |

\* P < 0.05; \*\*\* P < 0.00001; OR odds ratio; LCL 95% lower confidence limit; UCL 95% upper confidence limit

Table S11. Association between drug resistance, strain types, and Mtb genotype clusters ( $\leq 5$  and 13 SNPs difference).

| Variable           | Cut-off 5          |                       | OR<br>(95% CI)            | Cut-off 13          |                      | OR<br>(95% CI)             |
|--------------------|--------------------|-----------------------|---------------------------|---------------------|----------------------|----------------------------|
|                    | Cluster<br>n=888   | Non-Cluster<br>n=1117 |                           | Cluster<br>n=1265   | Non-Cluster<br>n=740 |                            |
| DR                 |                    |                       |                           |                     |                      |                            |
| Sensitive          | 22 (9.44)          | 211 (90.56)           | 0.11 (0.07-0.17)*         | 43 (18.45)          | 190 (81.55)          | 0.10 (0.07-0.14)*          |
| RR-TB              | 6 (15.38)          | 33 (84.62)            | 0.22 (0.09-0.54)*         | 14 (35.90)          | 25 (64.10)           | 0.32 (0.17-0.62)*          |
| HR-TB              | 13 (24.53)         | 40 (75.47)            | 0.40 (0.21-0.75)          | 22 (41.51)          | 31 (58.49)           | 0.40 (0.23-0.70)*          |
| <b>MDR-TB</b>      | <b>664 (49.22)</b> | <b>685 (50.78)</b>    | <b>1.87 (1.54-2.27)*</b>  | <b>936 (69.38)</b>  | <b>413 (30.62)</b>   | <b>2.25 (1.86-2.73)*</b>   |
| <b>Pre-XDR</b>     | <b>175 (59.73)</b> | <b>118 (40.27)</b>    | <b>2.08 (1.61-2.67)*</b>  | <b>242 (82.59)</b>  | <b>51 (17.41)</b>    | <b>2.78 (2.33-4.39)*</b>   |
| XDR-TB             | 4 (80.00)          | 1 (20.00)             | 5.05 (0.56-45.26)         | 4 (80.00)           | 1 (20.00)            | 2.34 (0.26-21.01)          |
| Other              | 4 (12.12)          | 29 (87.88)            | 0.17 (0.06-0.48)*         | 4 (12.12)           | 29 (87.88)           | 0.08 (0.03-0.22)*          |
| Main lineage       |                    |                       |                           |                     |                      |                            |
| L1                 | 41 (12.65)         | 283 (87.35)           | 0.14 (0.10-0.20)*         | 62 (19.14)          | 262 (80.86)          | 0.09 (0.07-0.13)*          |
| <b>L2</b>          | <b>823 (52.45)</b> | <b>746 (47.55)</b>    | <b>6.30 (4.75-8.34)*</b>  | <b>1158 (73.80)</b> | <b>411 (26.20)</b>   | <b>8.66 (6.78-11.07)*</b>  |
| L4                 | 24 (21.82)         | 86 (78.18)            | 0.33 (0.21-0.53)*         | 45 (40.91)          | 65 (59.09)           | 0.38 (0.26-0.57)*          |
| Comparison lineage |                    |                       |                           |                     |                      |                            |
| <b>L2 vs. L1</b>   | -                  | -                     | <b>7.61 (5.41-10.72)*</b> | -                   | -                    | <b>11.91 (8.83-16.05)*</b> |
| <b>L2 vs. L4</b>   | -                  | -                     | <b>3.95 (2.49-6.28)*</b>  | -                   | -                    | <b>4.07 (2.74-6.05)*</b>   |
| <b>L4 vs. L1</b>   | -                  | -                     | <b>1.93 (1.10-3.37)*</b>  | -                   | -                    | <b>2.93 (1.83-4.68)*</b>   |

\*p-value < 0.001; OR odds ratio; L Lineage; Non-Cluster = non-clustered isolates; CI confidence interval