Supplemental Material

Characterization of the tRNA ligases of pathogenic fungi *Aspergillus fumigatus* and *Coccidioides immitis*

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| Crosslinks   | Hit counts |
|--------------|------------|
|              | DST (6.4 Å) | DSG (7.7 Å) | DSS (11.4 Å) |
| **LIG Domain** |            |            |
| K27–K33      | 18         |            |
| K27–K57      | 10         | 6          |
| K32–K50      | 7          | 8          |
| K32–K155     | 6          |            |
| K50–K57      | 6          |            |
| K57–K87      | 19         | 20         |
| K87–K289     | 6          | 5          |
| K291–K319    | 9          |            |
| K340–K360    | 9          | 8          |
| K360–K365    | 8          | 66         |
| K57–K360     | 7          |            |
| K57–K340     | 6          |            |
| K57–K98      | 5          | 43         |
| K57–K166     | 5          |            |
| K155–K166    | 7          | 17         |
| K242–K340    | 11         |            |
| K242–K360    | 5          | 7          |
| K235–K242    |            | 36         |
| K307–K319    | 11         |            |
| K316–K319    | 20         |            |
| K27–K50      |            |            |
| **KIN Domain** |            |            |
| K442–K453    | 13         | 19         |
| K453–K457    | 11         | 79         |
| K457–K512    | 5          | 5          |
| K482–K512    | 7          | 10         |
| K512–K598    | 10         |            |
| K512–K606    | 9          |            |
| K591–K606    | 10         | 5          |
| K442–K512    | 14         | 9          |
| K442–K457    | 10         | 15         |
| K442–K482    | 10         |            |
| K453–K482    | 15         | 14         |
| K453–K512    | 7          | 5          |
| K457–K482    | 5          | 14         |
| K591–K598    |            | 13         |
| **CPD Domain** |            |            |
| K635–K703    | 21         |            |
| K635–K689    | 6          |            |
| K635–K693    | 5          |            |
| K689–K703    | 43         | 6          |
| K693–K703    | 14         |            |
| K743–K790    | 27         | 8          |
| K632–K703    |            | 16         |
| K635–K707    |            | 11         |

Figure S1. Intra-domain chemical crosslinks of CimTrl1.
| Crosslinks       | DST | DSG | DSS |
|------------------|-----|-----|-----|
| LIG to KIN       |     |     |     |
| K27–K482        | 6   |     |     |
| K27–K591        | 6   |     |     |
| K27–K457        | 5   |     |     |
| K27–K576        | 5   |     |     |
| K27–K598        |     | 5   |     |
| K32–K591        | 6   |     |     |
| K33–K606        |     |     | 9   |
| K57–K442        | 9   | 43  | 5   |
| K155–K606       | 5   |     | 8   |
| K307–K576       | 8   | 6   | 5   |
| K316–K442       | 14  |     |     |
| K316–K576       | 5   |     |     |
| K316–K457       |     | 6   |     |
| K340–K457       | 10  |     |     |
| K340–K482       | 5   |     |     |
| K340–K512       | 5   |     |     |
| K57–K453        | 5   |     |     |
| K87–K442        | 8   |     |     |
| K155–K453       | 6   | 5   |     |
| K155–K457       | 6   | 5   |     |
| K155–K512       | 5   | 5   |     |
| K242–K457       | 5   |     |     |
| K242–K482       | 5   |     |     |
| K291–K442       | 11  |     |     |
| K319–K442       | 5   | 6   |     |
| K322–K442       | 15  |     |     |
| K340–K442       | 9   |     |     |
| K360–K442       | 10  | 5   |     |
| K360–K457       | 6   | 12  |     |
| LIG to CPD      |     |     |     |
| K27–K673        | 5   |     |     |
| K155–K790       | 7   |     |     |
| K155–K673       | 6   | 8   | 9   |
| K242–K790       | 17  | 6   | 5   |
| K242–K632       | 7   |     | 9   |
| K242–K673       | 5   | 10  | 13  |
| K340–K673       | 6   |     |     |
| K360–K673       | 5   | 8   |     |
| K155–K743       | 6   |     |     |
| KIN to CPD      |     |     |     |
| K457–K790       | 6   |     |     |
| K606–K632       | 5   |     |     |
| K606–K635       | 5   | 8   |     |
| K442–K673       | 6   |     |     |
| K457–K821       | 13  |     |     |
| K457–K673       | 5   | 7   |     |
| K482–K673       | 8   |     |     |

Figure S2. Inter-domain chemical crosslinks of CimTrl1.
| Crosslinks | DST (6.4 Å) | DSG (7.7 Å) | DSS (11.4 Å) |
|------------|-------------|-------------|-------------|
| LIG Domain |             |             |             |
| K59–K100  | 6           | 35          | 44          |
| K76–K171  |             |             |             |
| K28–K59   | 11          | 14          | 13          |
| K74–K76   | 8           | 26          | 9           |
| K28–K76   |             |             |             |
| K28–K34   |             |             |             |
| K59–K89   | 18          | 6           | 7           |
| K59–K74   |             |             |             |
| K59–K292  |             |             |             |
| K100–K292 |             |             |             |
| K157–K171 |             |             |             |
| K314–K347 | 20          | 8           | 14          |
| K361–K366 | 5           | 11          | 9           |
| K323–K377 | 14          | 5           | 7           |
| K314–K320 |             |             |             |
| K59–K76   |             |             |             |
| K76–K171  |             |             |             |
| K74–K171  |             |             |             |
| K100–K314 |             |             |             |
| K157–K314 |             |             |             |
| K59–K171  |             |             |             |
| K59–K314  |             |             |             |
| K171–K314 |             |             |             |
| K89–K290  |             |             |             |
| K157–K171 |             |             |             |
| K100–K314 |             |             |             |
| LIG to KIN |             |             |             |
| K314–K439 | 14          | 13          | 13          |
| K34–K439  |             |             |             |
| K59–K439  |             |             |             |
| K100–K439 |             |             |             |
| K28–K524  |             |             |             |
| LIG to CPD |             |             |             |
| K74–K696  |             |             |             |
| K290–K713 |             |             |             |
| KIN to CPD |             |             |             |
| K406–K831 |             |             |             |
| K439–K831 |             |             |             |

Figure S3. Chemical crosslinking of AfuTrl1.