

**Table S2** Temperature-sensitive mutants in a lethal insertion cluster identified by linker-scanning mutagenesis

| Insertion (first mutation)<br>or Mutation | Sequence <sup>a</sup>   | Growth |      |      |
|---|-------------------------|--------|------|------|
|   |                         | 25°C   | 30°C | 37°C |
| CGRRQ (Y465C)                             | TLRQ <b>CGRRQ</b> YDSS  | ++     | -    | -    |
| CGRKY (D466C)                             | LRQY <b>CGRKY</b> DSSI  | +++    | +    | -    |
| CGRND (S467C)                             | RQYDC <b>GRNDSSIQ</b>   | +++    | +    | -    |
| CGRNS (S468C)                             | QYDSC <b>GRNSSION</b> Q | +++    | ++   | -    |
| MRPQS (I469M)                             | YDSS <b>MRPQS</b> IQLN  | +++    | +++  | -    |
| Wild-type                                 | TLRQYDSSIQNL            | +++    | +++  | +++  |
| Y465C                                     | TLRQCDSSIQNL            | +++    | +++  | +++  |
| I469Q                                     | TLRQYDSSQQNL            | +++    | +++  | +++  |
| Y465C I469Q <sup>b</sup>                  | TLRQCDSSQQNL            | +++    | +++  | -    |
| S467A S468A                               | TLRQYD <b>AAI</b> QNL   | +++    | +++  | +++  |
| S467D S468D                               | TLRQYD <b>DDI</b> QNL   | +++    | +++  | +++  |
| D466G                                     | TLRQY <b>GSS</b> IQLN   | +++    | +++  | +++  |

<sup>a</sup>Insertions and mutations are in bold text<sup>b</sup>*ndc80-121* allele