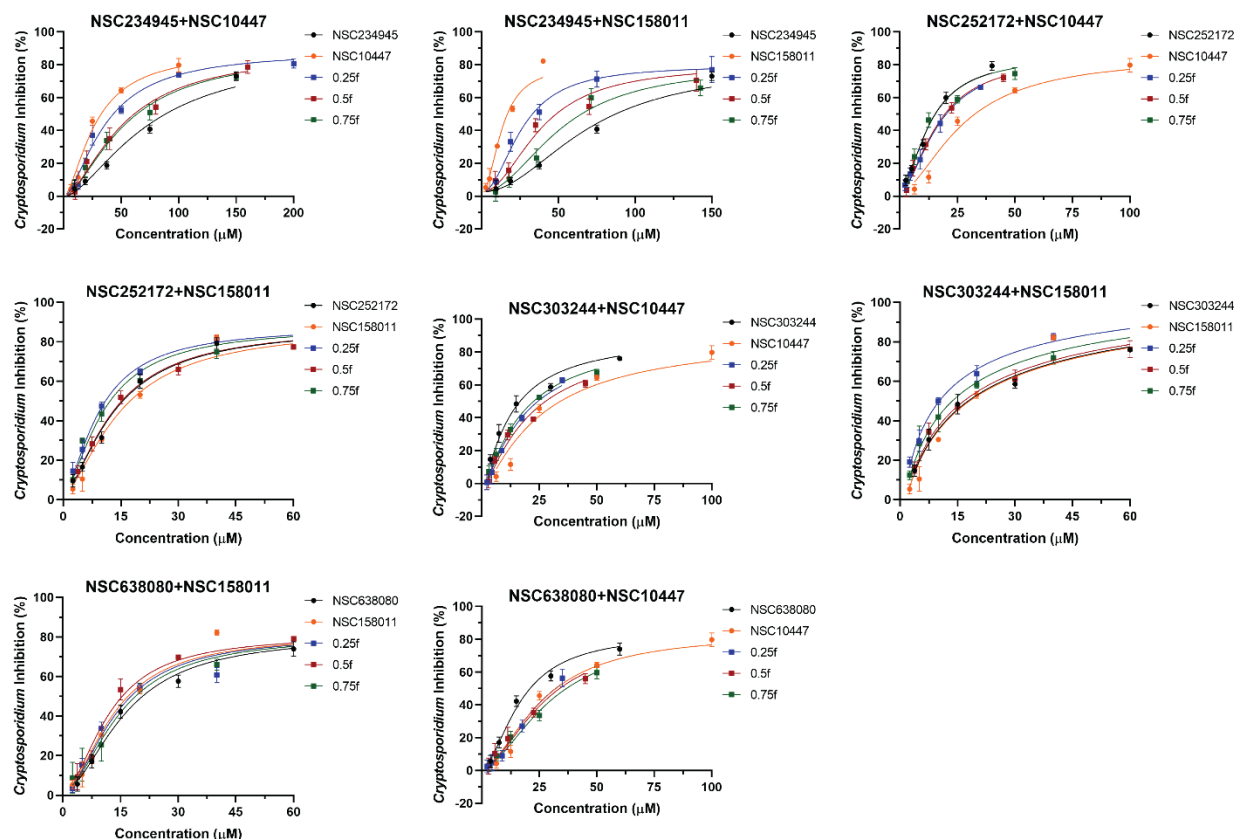
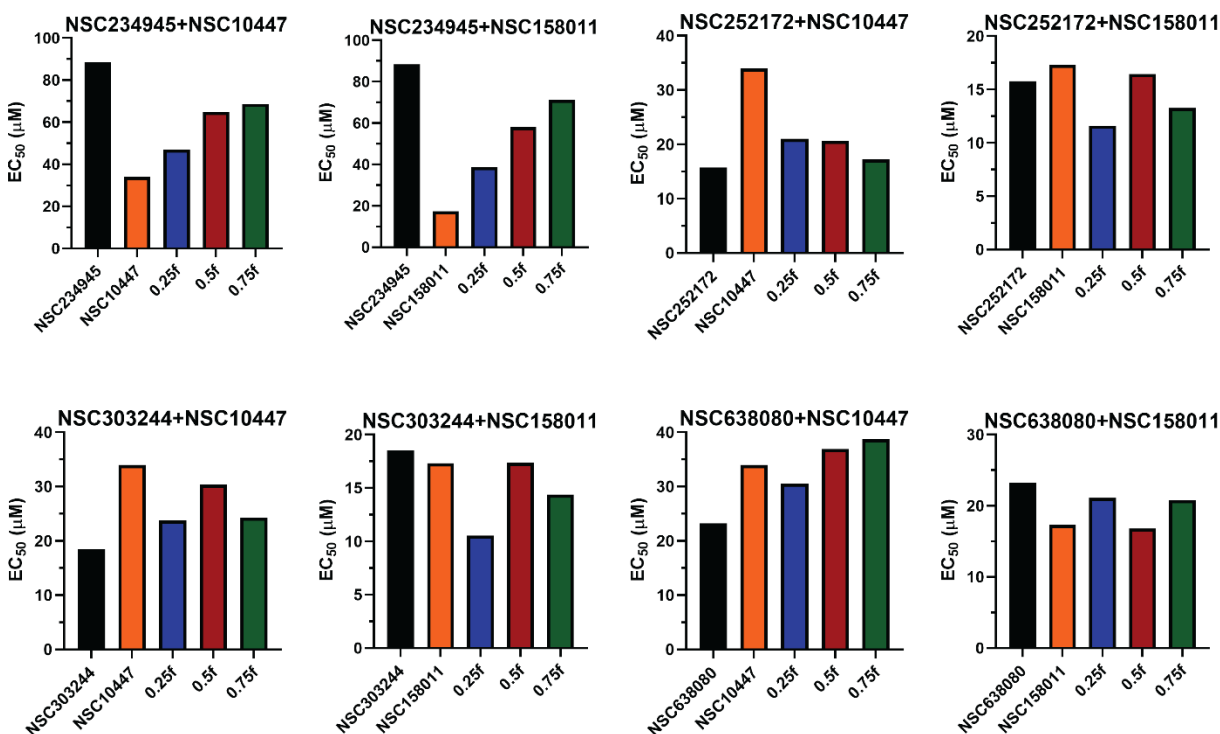




**FIG S1** Curve-shift analyses of individual CpPyK- and CpLDH-inhibitors and their combinations at different combination ratios. CpPyK- and CpLDH-inhibitors were combined in concentration ratios determined by 3 values of mixture factor ( $f$ ) to prepare 3 different compound combinations ( $0.25f$ ,  $0.5f$ , and  $0.75f$ ). Concentration-response data of each compound mixture and individual compounds acquired from the *in vitro* parasite growth inhibition assays were plotted in GraphPad PRISM® v8. A leftward shift of curves for combinations compared with individual compound curves indicates synergism and a rightward shift indicates antagonism. Data are presented as mean  $\pm$  standard deviation of the mean (SD) of three independent experiments.



**FIG S2** Comparison of anti-*Cryptosporidium* EC<sub>50</sub> values of individual CpPyK- and CpLDH-inhibitors and their combinations at different combination ratios. CpPyK- and CpLDH-inhibitors were combined in concentration ratios determined by 3 values of mixture factor ( $f$ ) to prepare 3 different combinations (0.25 $f$ , 0.5 $f$ , and 0.75 $f$ ). Concentration-response data points of each compound mixture and individual compounds obtained from the *in vitro* *C. parvum* growth inhibition assays were analyzed by the non-linear regression equation in GraphPad PRISM® v8 software to calculate EC<sub>50</sub> values. The EC<sub>50</sub> of the individual drugs were compared to the resulting EC<sub>50</sub> after combination treatments. The data shown represent the mean EC<sub>50</sub> values of three independent experiments.

TABLE S1 Mean percent cytotoxicity of combinations of CpPyK- and CpLDH-inhibitors in HCT-8 cells.

| 0.25f      |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC234945  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | 0.4                           | 4.5  | -4.9 | 0.0  | 4.7   | 2.7 |
| 12.5       | 12.5     | -0.4                          | 1.4  | 1.5  | 0.8  | 1.1   | 0.6 |
| 25         | 25       | 10.5                          | -0.6 | 7.1  | 5.7  | 5.7   | 3.3 |
| 50         | 50       | 10.9                          | 11.8 | 1.6  | 8.1  | 5.6   | 3.2 |
| 100        | 100      | 17.0                          | 19.4 | 23.4 | 19.9 | 3.3   | 1.9 |
| 200        | 200      | 40.7                          | 40.5 | 46.1 | 42.4 | 3.2   | 1.8 |

| 0.5f       |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC234945  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | -0.5                          | 0.7  | -0.1 | 0.0  | 0.6   | 0.4 |
| 15         | 5        | 5.6                           | -5.1 | 3.3  | 1.2  | 5.6   | 3.3 |
| 30         | 10       | 4.9                           | 9.8  | 3.2  | 6.0  | 3.4   | 2.0 |
| 60         | 20       | 5.1                           | 9.6  | 15.4 | 10.0 | 5.2   | 3.0 |
| 120        | 40       | 18.6                          | 21.5 | 13.4 | 17.8 | 4.1   | 2.4 |
| 240        | 80       | 24.1                          | 37.8 | 28.5 | 30.2 | 7.0   | 4.0 |

| 0.75f      |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC234945  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | -1.4                          | 5.6  | -4.2 | 0.0  | 5.1   | 2.9 |
| 16.875     | 1.875    | 2.1                           | -1.2 | 5.2  | 2.1  | 3.2   | 1.8 |
| 33.75      | 3.75     | 6.3                           | 4.4  | 2.5  | 4.4  | 1.9   | 1.1 |
| 67.5       | 7.5      | 8.4                           | 0.5  | 11.3 | 6.7  | 5.6   | 3.2 |
| 135        | 15       | 15.3                          | 11.4 | 5.9  | 10.9 | 4.7   | 2.7 |
| 270        | 30       | 37.4                          | 21.1 | 33.6 | 30.7 | 8.5   | 4.9 |

| 0.25f      |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |           | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC234945  | NSC158011 |                               |      |      |      |       |     |
| 0          | 0         | -5.7                          | 2.6  | 3.1  | 0.0  | 4.9   | 2.9 |
| 12.5       | 6.25      | -5.1                          | 0.1  | 2.3  | -0.9 | 3.8   | 2.2 |
| 25         | 12.5      | 1.6                           | 6.0  | 0.1  | 2.6  | 3.1   | 1.8 |
| 50         | 25        | 3.7                           | 16.5 | 8.8  | 9.6  | 6.5   | 3.7 |
| 100        | 50        | 17.7                          | 27.6 | 13.5 | 19.6 | 7.2   | 4.2 |
| 200        | 100       | 34.7                          | 40.9 | 34.0 | 36.6 | 3.8   | 2.2 |

| 0.5f       |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |           | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC234945  | NSC158011 |                               |      |      |      |       |     |
| 0          | 0         | -5.0                          | 5.7  | -0.7 | 0.0  | 5.4   | 3.1 |
| 15         | 2.5       | -2.4                          | 8.1  | 1.0  | 2.2  | 5.3   | 3.1 |
| 30         | 5         | 4.8                           | 6.5  | 0.4  | 3.9  | 3.1   | 1.8 |
| 60         | 10        | 11.3                          | 5.9  | 12.8 | 10.0 | 3.6   | 2.1 |
| 120        | 20        | 21.2                          | 12.6 | 17.0 | 16.9 | 4.3   | 2.5 |
| 240        | 40        | 36.4                          | 33.7 | 27.6 | 32.6 | 4.5   | 2.6 |

| 0.75f      |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |           | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC234945  | NSC158011 |                               |      |      |      |       |     |
| 0          | 0         | 2.8                           | 2.2  | -5.1 | 0.0  | 4.4   | 2.5 |
| 16.875     | 0.9375    | 4.6                           | 3.7  | -5.7 | 0.9  | 5.7   | 3.3 |
| 33.75      | 1.875     | 7.6                           | 4.2  | 1.6  | 4.5  | 3.0   | 1.7 |
| 67.5       | 3.75      | 5.3                           | 6.3  | 10.0 | 7.2  | 2.5   | 1.5 |
| 135        | 7.5       | 10.5                          | 17.8 | 6.7  | 11.6 | 5.6   | 3.2 |
| 270        | 15        | 29.6                          | 25.3 | 21.0 | 25.3 | 4.3   | 2.5 |

| 0.25f      |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC252172  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | 4.7                           | -3.5 | -1.2 | 0.0  | 4.2   | 2.4 |
| 1.25       | 7.5      | 1.1                           | 13.8 | 4.9  | 6.6  | 6.5   | 3.8 |
| 2.5        | 15       | 12.8                          | 12.1 | 16.9 | 13.9 | 2.6   | 1.5 |
| 5          | 30       | 20.4                          | 14.0 | 23.6 | 19.3 | 4.9   | 2.8 |
| 10         | 60       | 28.5                          | 22.9 | 34.3 | 28.6 | 5.7   | 3.3 |
| 20         | 120      | 54.4                          | 47.4 | 49.7 | 50.5 | 3.6   | 2.1 |

| 0.5f       |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC252172  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | -5.2                          | -2.8 | 8.0  | 0.0  | 7.0   | 4.1 |
| 3.75       | 7.5      | 13.0                          | 2.7  | -2.8 | 4.3  | 8.0   | 4.6 |
| 7.5        | 15       | 13.3                          | 16.0 | 4.2  | 11.2 | 6.2   | 3.6 |
| 15         | 30       | 14.6                          | 24.3 | 20.4 | 19.8 | 4.9   | 2.8 |
| 30         | 60       | 29.8                          | 27.0 | 38.2 | 31.7 | 5.8   | 3.4 |
| 60         | 120      | 63.0                          | 57.3 | 60.1 | 60.1 | 2.8   | 1.6 |

| 0.75f      |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC252172  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | 8.8                           | -8.3 | -0.5 | 0.0  | 8.5   | 4.9 |
| 7.5        | 5        | 12.8                          | 8.1  | -3.9 | 5.7  | 8.6   | 5.0 |
| 15         | 10       | 13.0                          | 17.0 | 2.1  | 10.7 | 7.7   | 4.5 |
| 30         | 20       | 18.7                          | 13.7 | 23.3 | 18.6 | 4.8   | 2.8 |
| 60         | 40       | 40.6                          | 34.5 | 27.3 | 34.1 | 6.7   | 3.9 |
| 120        | 80       | 54.0                          | 59.6 | 60.3 | 58.0 | 3.4   | 2.0 |

| 0.25f      |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |           | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC252172  | NSC158011 |                               |      |      |      |       |     |
| 0          | 0         | 2.2                           | 0.8  | -3.0 | 0.0  | 2.7   | 1.5 |
| 2.5        | 7.5       | 6.1                           | 5.0  | -1.0 | 3.4  | 3.8   | 2.2 |
| 5          | 15        | 8.5                           | 14.2 | 6.9  | 9.9  | 3.8   | 2.2 |
| 10         | 30        | 23.1                          | 12.4 | 14.6 | 16.7 | 5.7   | 3.3 |
| 20         | 60        | 36.4                          | 30.1 | 26.0 | 30.8 | 5.3   | 3.0 |
| 40         | 120       | 48.6                          | 39.9 | 40.5 | 43.0 | 4.9   | 2.8 |

| 0.5f       |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |           | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC252172  | NSC158011 |                               |      |      |      |       |     |
| 0          | 0         | 1.2                           | 5.0  | -6.2 | 0.0  | 5.7   | 3.3 |
| 7.5        | 7.5       | -2.1                          | 2.7  | 6.5  | 2.4  | 4.3   | 2.5 |
| 15         | 15        | 16.5                          | 6.2  | 11.9 | 11.6 | 5.1   | 3.0 |
| 30         | 30        | 25.6                          | 12.8 | 19.3 | 19.2 | 6.4   | 3.7 |
| 60         | 60        | 38.6                          | 43.7 | 50.9 | 44.4 | 6.2   | 3.6 |
| 120        | 120       | 61.2                          | 63.1 | 56.0 | 60.1 | 3.7   | 2.1 |

| 0.75f      |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |           | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC252172  | NSC158011 |                               |      |      |      |       |     |
| 0          | 0         | -0.7                          | 0.5  | 0.2  | 0.0  | 0.6   | 0.3 |
| 7.5        | 2.5       | -2.4                          | 6.5  | 3.5  | 2.6  | 4.5   | 2.6 |
| 15         | 5         | 6.5                           | 15.1 | 4.9  | 8.8  | 5.5   | 3.2 |
| 30         | 10        | 14.3                          | 16.7 | 24.4 | 18.5 | 5.3   | 3.0 |
| 60         | 20        | 33.1                          | 42.2 | 34.6 | 36.6 | 4.9   | 2.8 |
| 120        | 40        | 57.6                          | 56.1 | 51.5 | 55.1 | 3.2   | 1.9 |

| 0.25f      |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC303244  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | -10.4                         | 7.0  | 3.4  | 0.0  | 9.2   | 5.3 |
| 1.25       | 7.5      | -0.2                          | -6.5 | 11.6 | 1.6  | 9.2   | 5.3 |
| 2.5        | 15       | -1.2                          | 8.3  | 12.0 | 6.4  | 6.8   | 3.9 |
| 5          | 30       | 19.1                          | 3.9  | 11.4 | 11.5 | 7.6   | 4.4 |
| 10         | 60       | 18.9                          | 22.1 | 29.0 | 23.4 | 5.2   | 3.0 |
| 20         | 120      | 37.3                          | 43.5 | 36.9 | 39.2 | 3.7   | 2.1 |

| 0.5f       |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC303244  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | -8.4                          | 1.9  | 6.5  | 0.0  | 7.6   | 4.4 |
| 3.75       | 7.5      | 8.5                           | 8.3  | -1.7 | 5.0  | 5.8   | 3.4 |
| 7.5        | 15       | -0.4                          | 14.4 | 11.4 | 8.4  | 7.8   | 4.5 |
| 15         | 30       | 22.5                          | 20.0 | 6.1  | 16.2 | 8.8   | 5.1 |
| 30         | 60       | 27.9                          | 23.1 | 33.0 | 28.0 | 5.0   | 2.9 |
| 60         | 120      | 45.0                          | 54.8 | 47.4 | 49.1 | 5.1   | 3.0 |

| 0.75f      |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (μM) |          | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| NSC303244  | NSC10447 |                               |      |      |      |       |     |
| 0          | 0        | 6.9                           | -2.1 | -4.8 | 0.0  | 6.1   | 3.5 |
| 7.5        | 5        | 0.0                           | -3.5 | 11.1 | 2.5  | 7.7   | 4.4 |
| 15         | 10       | 11.2                          | 6.3  | 1.5  | 6.3  | 4.9   | 2.8 |
| 30         | 20       | 21.7                          | 12.4 | 17.6 | 17.2 | 4.7   | 2.7 |
| 60         | 40       | 22.0                          | 27.9 | 24.7 | 24.9 | 3.0   | 1.7 |
| 120        | 80       | 34.1                          | 42.6 | 41.4 | 39.4 | 4.6   | 2.6 |

| 0.25f      |           |                               |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC303244  | NSC158011 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0         | -1.9                          | -0.3 | 2.2  | 0.0  | 2.1   | 1.2 |
| 2.5        | 7.5       | -4.0                          | 7.7  | 5.9  | 3.2  | 6.3   | 3.7 |
| 5          | 15        | 3.4                           | 6.9  | 1.2  | 3.8  | 2.9   | 1.7 |
| 10         | 30        | 5.6                           | 13.8 | 9.5  | 9.6  | 4.1   | 2.4 |
| 20         | 60        | 25.7                          | 21.2 | 16.4 | 21.1 | 4.6   | 2.7 |
| 40         | 120       | 27.0                          | 34.6 | 26.4 | 29.4 | 4.6   | 2.7 |

| 0.5f       |           |                               |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC303244  | NSC158011 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0         | 0.3                           | -1.1 | 0.8  | 0.0  | 1.0   | 0.6 |
| 7.5        | 7.5       | 6.7                           | 9.0  | 2.1  | 5.9  | 3.5   | 2.0 |
| 15         | 15        | 7.6                           | 16.3 | 9.6  | 11.1 | 4.6   | 2.6 |
| 30         | 30        | 10.2                          | 18.6 | 18.4 | 15.7 | 4.8   | 2.8 |
| 60         | 60        | 34.1                          | 31.8 | 39.9 | 35.3 | 4.2   | 2.4 |
| 120        | 120       | 45.7                          | 48.9 | 53.6 | 49.4 | 4.0   | 2.3 |

| 0.75f      |           |                               |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC303244  | NSC158011 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0         | 3.7                           | 1.0  | -4.7 | 0.0  | 4.3   | 2.5 |
| 7.5        | 2.5       | 9.1                           | 3.7  | -0.8 | 4.0  | 5.0   | 2.9 |
| 15         | 5         | 11.3                          | 7.6  | -0.4 | 6.2  | 6.0   | 3.4 |
| 30         | 10        | 15.2                          | 13.7 | 6.0  | 11.6 | 4.9   | 2.9 |
| 60         | 20        | 19.2                          | 20.0 | 27.7 | 22.3 | 4.7   | 2.7 |
| 120        | 40        | 29.3                          | 34.1 | 39.2 | 34.2 | 4.9   | 2.9 |

| 0.25f      |          |                               |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC638080  | NSC10447 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0        | -5.0                          | 6.8  | -1.8 | 0.0  | 6.1   | 3.5 |
| 1.25       | 7.5      | 2.8                           | 10.3 | -1.1 | 4.0  | 5.8   | 3.3 |
| 2.5        | 15       | 1.2                           | 9.9  | 11.5 | 7.5  | 5.6   | 3.2 |
| 5          | 30       | 14.5                          | 12.4 | 20.9 | 15.9 | 4.4   | 2.6 |
| 10         | 60       | 27.4                          | 27.4 | 24.9 | 26.5 | 1.4   | 0.8 |
| 20         | 120      | 43.7                          | 49.6 | 42.2 | 45.1 | 3.9   | 2.3 |

| 0.5f       |          |                               |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC638080  | NSC10447 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0        | -2.5                          | -2.6 | 5.1  | 0.0  | 4.5   | 2.6 |
| 3.75       | 7.5      | 0.1                           | 7.6  | 9.2  | 5.6  | 4.9   | 2.8 |
| 7.5        | 15       | 10.1                          | 8.4  | 15.0 | 11.2 | 3.5   | 2.0 |
| 15         | 30       | 13.1                          | 20.7 | 21.9 | 18.5 | 4.8   | 2.8 |
| 30         | 60       | 28.0                          | 26.6 | 35.4 | 30.0 | 4.7   | 2.7 |
| 60         | 120      | 48.4                          | 46.0 | 52.9 | 49.1 | 3.5   | 2.0 |

| 0.75f      |          |                               |      |      |      |       |     |
|------------|----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |          | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC638080  | NSC10447 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0        | 5.7                           | -5.0 | -0.7 | 0.0  | 5.4   | 3.1 |
| 7.5        | 5        | 2.5                           | -2.3 | 4.8  | 1.7  | 3.6   | 2.1 |
| 15         | 10       | 6.2                           | -0.6 | 9.4  | 5.0  | 5.1   | 2.9 |
| 30         | 20       | 24.4                          | 17.3 | 16.9 | 19.5 | 4.2   | 2.4 |
| 60         | 40       | 21.7                          | 24.7 | 27.8 | 24.8 | 3.1   | 1.8 |
| 120        | 80       | 39.7                          | 39.0 | 38.5 | 39.1 | 0.6   | 0.3 |

| 0.25f      |           |                               |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC638080  | NSC158011 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0         | -3.2                          | 3.3  | -0.1 | 0.0  | 3.3   | 1.9 |
| 2.5        | 7.5       | 1.1                           | 0.2  | 8.6  | 3.3  | 4.6   | 2.7 |
| 5          | 15        | 14.3                          | 2.5  | 9.4  | 8.7  | 5.9   | 3.4 |
| 10         | 30        | 20.8                          | 10.3 | 5.8  | 12.3 | 7.7   | 4.4 |
| 20         | 60        | 18.9                          | 22.4 | 28.4 | 23.2 | 4.8   | 2.8 |
| 40         | 120       | 29.3                          | 34.5 | 40.4 | 34.7 | 5.6   | 3.2 |

| 0.5f       |           |                               |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC638080  | NSC158011 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0         | -2.5                          | 2.5  | -0.1 | 0.0  | 2.5   | 1.4 |
| 7.5        | 7.5       | 1.3                           | 7.4  | 1.9  | 3.5  | 3.4   | 1.9 |
| 15         | 15        | 12.8                          | 11.0 | 21.0 | 14.9 | 5.3   | 3.1 |
| 30         | 30        | 16.0                          | 24.6 | 19.0 | 19.9 | 4.4   | 2.5 |
| 60         | 60        | 45.7                          | 42.8 | 45.4 | 44.6 | 1.6   | 0.9 |
| 120        | 120       | 60.5                          | 60.3 | 55.7 | 58.9 | 2.7   | 1.6 |

| 0.75f      |           |                               |      |      |      |       |     |
|------------|-----------|-------------------------------|------|------|------|-------|-----|
| Conc. (µM) |           | Mean Percent Cytotoxicity (%) |      |      |      |       |     |
| NSC638080  | NSC158011 | 1                             | 2    | 3    | AVG  | STDEV | SEM |
| 0          | 0         | -3.2                          | -0.1 | 3.3  | 0.0  | 3.2   | 1.9 |
| 7.5        | 2.5       | -5.1                          | 6.0  | 3.0  | 1.3  | 5.7   | 3.3 |
| 15         | 5         | 2.9                           | 5.2  | 13.0 | 7.0  | 5.3   | 3.0 |
| 30         | 10        | 12.1                          | 16.4 | 14.6 | 14.4 | 2.1   | 1.2 |
| 60         | 20        | 25.2                          | 26.8 | 14.7 | 22.2 | 6.6   | 3.8 |
| 120        | 40        | 33.2                          | 35.3 | 38.8 | 35.8 | 2.8   | 1.6 |

**TABLE S2** Dose-reduction index (DRI) values of compound mixtures at the parasite inhibition effective concentration (EC) of 50%, 75%, and 90% (EC<sub>50</sub>, EC<sub>75</sub>, and EC<sub>90</sub>, respectively).

| Compound mixture<br>(CpPyKi + CpLDHi) | Mixture<br>factor ( <i>f</i> ) | DRI value of CpPyKi and CpLDHi at |        |                  |        |                  |        |
|---------------------------------------|--------------------------------|-----------------------------------|--------|------------------|--------|------------------|--------|
|                                       |                                | EC <sub>50</sub>                  |        | EC <sub>75</sub> |        | EC <sub>90</sub> |        |
|                                       |                                | CpPyKi                            | CpLDHi | CpPyKi           | CpLDHi | CpPyKi           | CpLDHi |
| NSC234945 + NSC10447                  | 0.25                           | 3.29                              | 1.39   | 1.79             | 2.27   | 1.44             | 5.47   |
|                                       | 0.50                           | 3.15                              | 1.20   | 1.95             | 2.24   | 1.30             | 4.47   |
|                                       | 0.75                           | 3.01                              | 1.04   | 2.13             | 2.20   | 1.17             | 3.64   |
| NSC234945 + NSC158011                 | 0.25                           | 3.06                              | 1.17   | 1.71             | 1.96   | 1.25             | 4.30   |
|                                       | 0.50                           | 2.74                              | 0.98   | 1.44             | 1.53   | 1.35             | 4.31   |
|                                       | 0.75                           | 2.45                              | 0.81   | 1.20             | 1.20   | 1.45             | 4.32   |
| NSC252172 + NSC10447                  | 0.25                           | 5.09                              | 2.05   | 2.22             | 2.69   | 1.43             | 5.20   |
|                                       | 0.50                           | 4.64                              | 1.57   | 2.42             | 2.46   | 1.31             | 4.00   |
|                                       | 0.75                           | 4.22                              | 1.21   | 2.63             | 2.25   | 1.20             | 3.08   |
| NSC252172 + NSC158011                 | 0.25                           | 5.31                              | 1.93   | 1.80             | 1.96   | 1.50             | 4.91   |
|                                       | 0.50                           | 4.77                              | 1.51   | 1.52             | 1.44   | 1.30             | 3.69   |
|                                       | 0.75                           | 4.28                              | 1.17   | 1.28             | 1.05   | 1.12             | 2.77   |
| NSC303244 + NSC10447                  | 0.25                           | 5.96                              | 1.98   | 2.06             | 2.05   | 1.28             | 3.82   |
|                                       | 0.50                           | 5.03                              | 2.15   | 3.01             | 1.94   | 1.51             | 2.91   |
|                                       | 0.75                           | 4.87                              | 2.35   | 3.39             | 1.83   | 1.77             | 2.22   |
| NSC303244 + NSC158011                 | 0.25                           | 7.29                              | 2.18   | 2.14             | 1.91   | 1.72             | 4.62   |
|                                       | 0.50                           | 7.77                              | 1.55   | 2.05             | 1.23   | 1.73             | 3.12   |
|                                       | 0.75                           | 8.28                              | 1.11   | 1.96             | 0.79   | 1.75             | 2.11   |
| NSC638080 + NSC10447                  | 0.25                           | 4.96                              | 1.27   | 2.10             | 1.61   | 1.08             | 2.50   |
|                                       | 0.50                           | 5.05                              | 1.13   | 2.03             | 1.36   | 1.07             | 2.15   |
|                                       | 0.75                           | 5.14                              | 1.00   | 1.97             | 1.15   | 1.06             | 1.86   |
| NSC638080 + NSC158011                 | 0.25                           | 4.60                              | 1.06   | 2.64             | 1.83   | 1.53             | 3.17   |
|                                       | 0.50                           | 4.55                              | 0.95   | 2.61             | 1.63   | 1.28             | 2.40   |
|                                       | 0.75                           | 4.50                              | 0.84   | 2.59             | 1.45   | 1.08             | 1.82   |

**TABLE S3** Effect of oral administration of CpPyK- and CpLDH-inhibitors on the physical indicators of health in mice.

| Treatment | Dose (mg/kg) | Day | Average Body Weight (g) | Average Score* |           |         |              |
|-----------|--------------|-----|-------------------------|----------------|-----------|---------|--------------|
|           |              |     |                         | Activity       | Skin/ Fur | Posture | Mental State |
| NSC252172 | 50           | 1   | 23.7                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 24.4                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 24.7                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 24.9                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 24.8                    | 4              | 4         | 4       | 4            |
|           | 75           | 1   | 24.4                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 24.6                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 24.5                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 24.7                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 24.9                    | 4              | 4         | 4       | 4            |
|           | 100          | 1   | 24.6                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 24.6                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 25.1                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 25.1                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 25.3                    | 4              | 4         | 4       | 4            |
| NSC303244 | 50           | 1   | 21.9                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 21.6                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 22.2                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 22.4                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 22.4                    | 4              | 4         | 4       | 4            |
|           | 75           | 1   | 23.7                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 23.6                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 23.5                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 23.2                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 22.7                    | 3              | 3         | 4       | 4            |
|           | 100          | 1   | 23.1                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 22.9                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 22.7                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 22                      | 3              | 3         | 4       | 3            |
|           |              | 5   | 20.9                    | 3              | 2         | 3       | 3            |
| NSC158011 | 75           | 1   | 22                      | 4              | 4         | 4       | 4            |
|           |              | 2   | 22.2                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 22.2                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 22.1                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 22.4                    | 4              | 4         | 4       | 4            |
|           | 150          | 1   | 22.4                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 22.6                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 22.8                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 22.9                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 23.4                    | 4              | 4         | 4       | 4            |
|           | 200          | 1   | 22.9                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 22.7                    | 4              | 4         | 4       | 4            |
|           |              | 3   | 22.9                    | 4              | 4         | 4       | 4            |
|           |              | 4   | 23.2                    | 4              | 4         | 4       | 4            |
|           |              | 5   | 23.3                    | 4              | 4         | 4       | 4            |
| NSC10447  | 100          | 1   | 22.7                    | 4              | 4         | 4       | 4            |
|           |              | 2   | 22.9                    | 4              | 4         | 4       | 4            |

|                                |     |   |      |   |   |   |   |
|--------------------------------|-----|---|------|---|---|---|---|
|                                |     | 3 | 22.8 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 22.9 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 23   | 4 | 4 | 4 | 4 |
|                                | 200 | 1 | 24.4 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 24.9 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 24.9 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 25   | 4 | 4 | 4 | 4 |
|                                |     | 5 | 25.3 | 4 | 4 | 4 | 4 |
|                                | 400 | 1 | 22.6 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 23   | 4 | 4 | 4 | 4 |
|                                |     | 3 | 23.2 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 23   | 4 | 4 | 4 | 4 |
|                                |     | 5 | 23   | 4 | 4 | 4 | 4 |
| NSC252172 + NSC10447<br>(1:2)  | 75  | 1 | 22.9 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 23.3 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 23.3 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 23.5 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 23.9 | 4 | 4 | 4 | 4 |
|                                | 150 | 1 | 24.6 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 24.5 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 24.4 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 24.5 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 24.4 | 4 | 4 | 4 | 4 |
|                                | 300 | 1 | 22.4 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 22.7 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 22.5 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 22.6 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 22.7 | 4 | 4 | 4 | 4 |
| NSC252172 + NSC158011<br>(1:3) | 50  | 1 | 23.9 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 23.9 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 24.1 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 23.9 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 24   | 4 | 4 | 4 | 4 |
|                                | 100 | 1 | 24.7 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 24.9 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 24.7 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 25   | 4 | 4 | 4 | 4 |
|                                |     | 5 | 24.9 | 4 | 4 | 4 | 4 |
|                                | 200 | 1 | 23.5 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 23.8 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 23.7 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 23.9 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 24.1 | 4 | 4 | 4 | 4 |
| NSC303244 + NSC158011<br>(1:3) | 50  | 1 | 25.1 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 25.4 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 25.6 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 25.5 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 25.5 | 4 | 4 | 4 | 4 |
|                                | 100 | 1 | 23.4 | 4 | 4 | 4 | 4 |
|                                |     | 2 | 23.6 | 4 | 4 | 4 | 4 |
|                                |     | 3 | 23.8 | 4 | 4 | 4 | 4 |
|                                |     | 4 | 23.7 | 4 | 4 | 4 | 4 |
|                                |     | 5 | 23.9 | 4 | 4 | 4 | 4 |

|     |   |      |   |   |   |   |
|-----|---|------|---|---|---|---|
| 200 | 1 | 24   | 4 | 4 | 4 | 4 |
|     | 2 | 23.8 | 4 | 4 | 4 | 4 |
|     | 3 | 24   | 4 | 4 | 4 | 4 |
|     | 4 | 23.9 | 4 | 4 | 4 | 4 |
|     | 5 | 23.9 | 4 | 4 | 4 | 4 |

\* See Table S4 for the scoring rubric.



**TABLE S4** Criteria used for scoring changes in physical activity, fur condition, body posture, and mental state due to compound toxicity in mice.

| <b>Score</b> | <b>Physical activity</b> | <b>Skin/ Fur</b>         | <b>Posture</b>   | <b>Mental State</b> |
|--------------|--------------------------|--------------------------|------------------|---------------------|
| 1            | immobile                 | sparse                   | lying flat       | lifeless/ trembling |
| 2            | bradykinesia             | rough/ lackluster        | hunched          | depressed           |
| 3            | slightly sluggish        | little rough/ lackluster | slightly hunched | agitated            |
| 4            | fully active             | smooth/ glossy           | normal standing  | good spirit         |