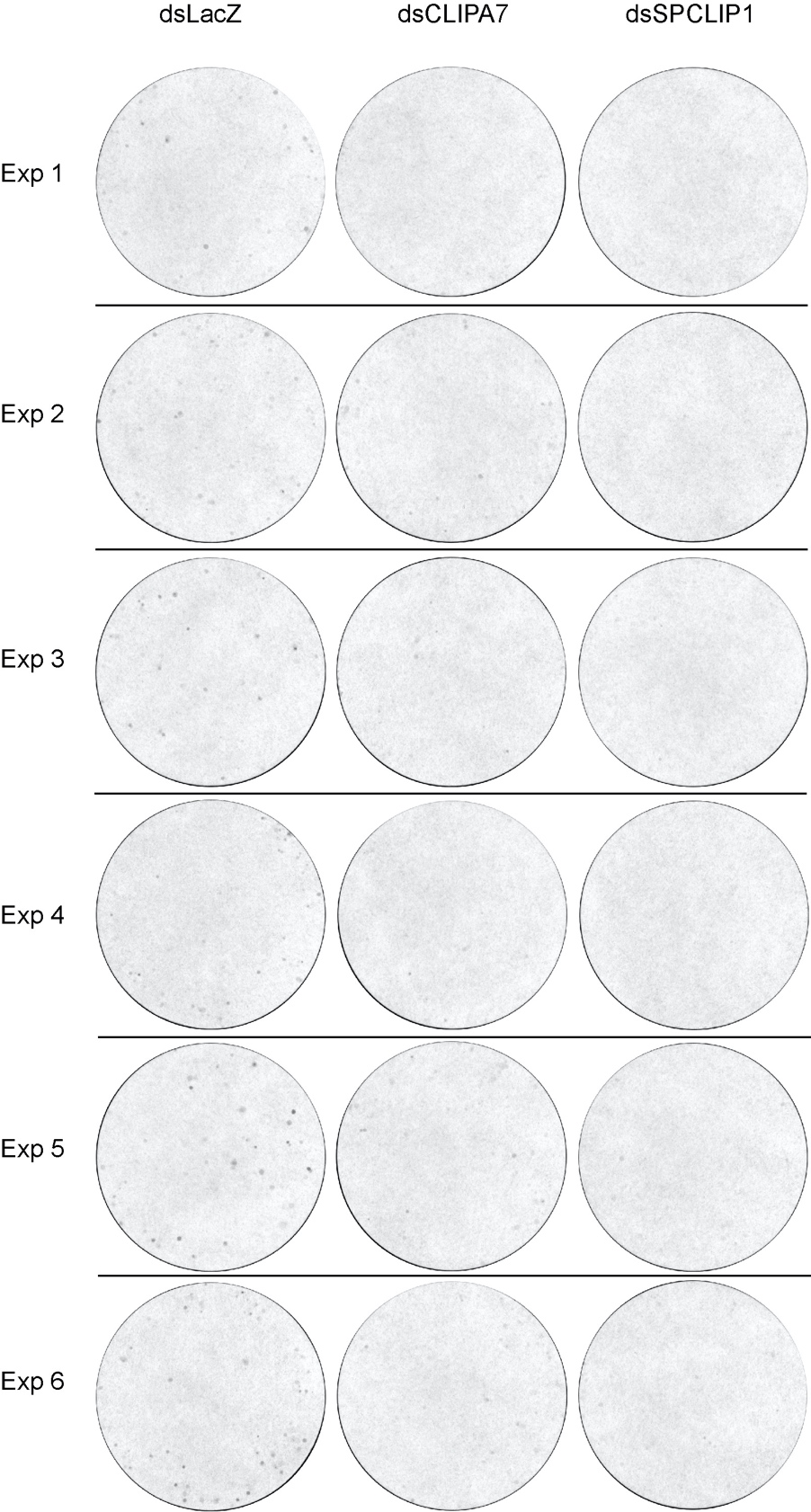
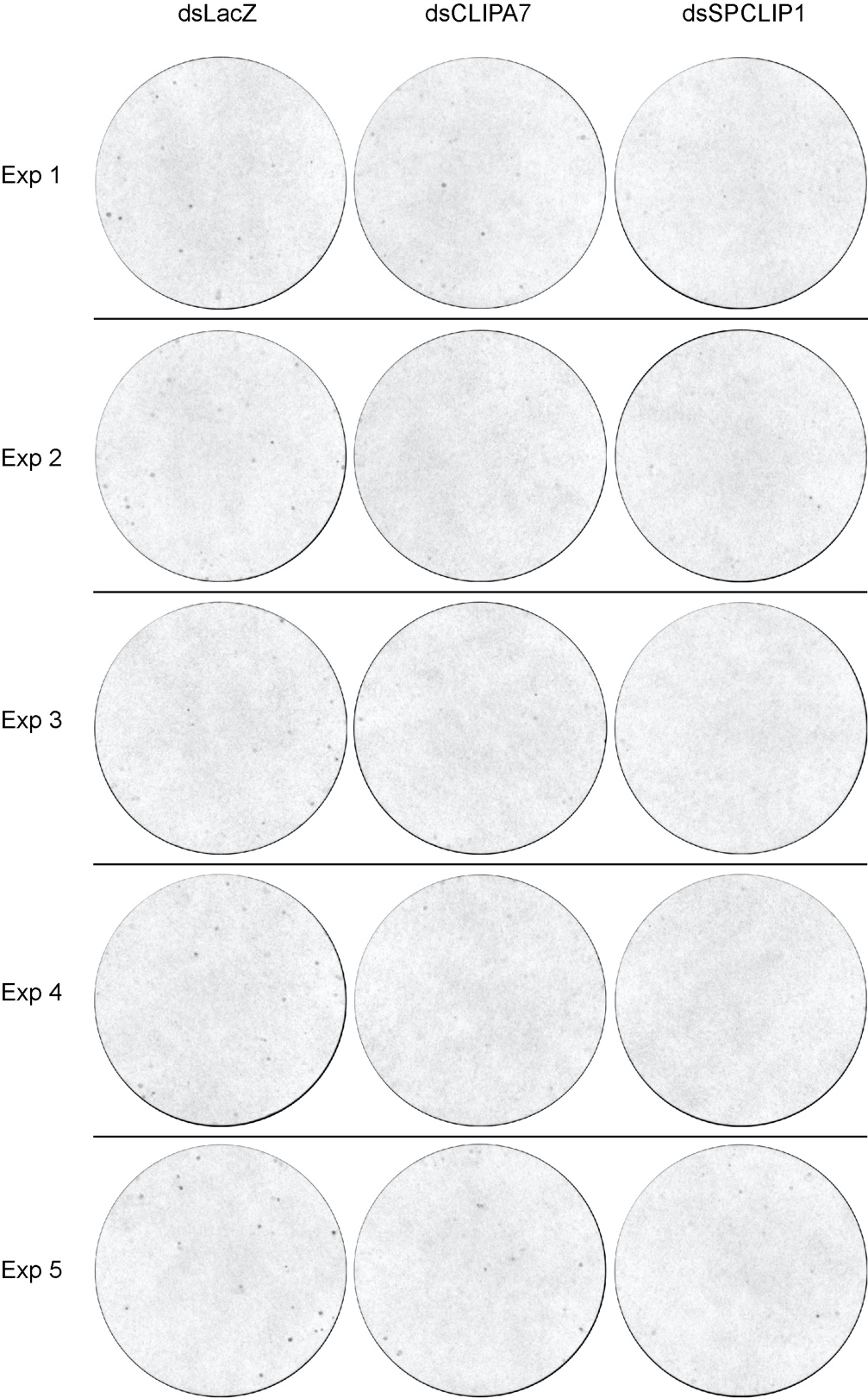
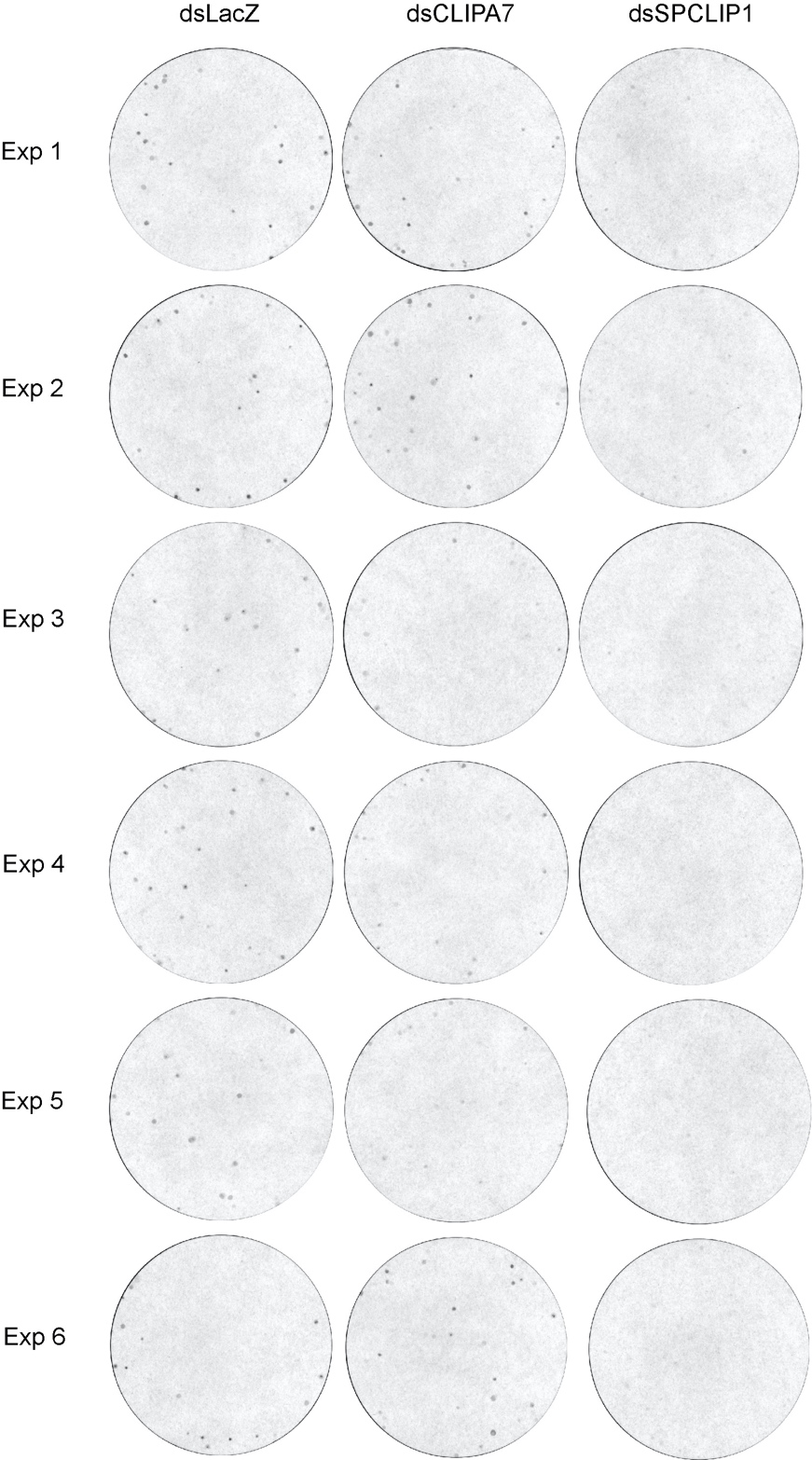
**Online supplementary information**

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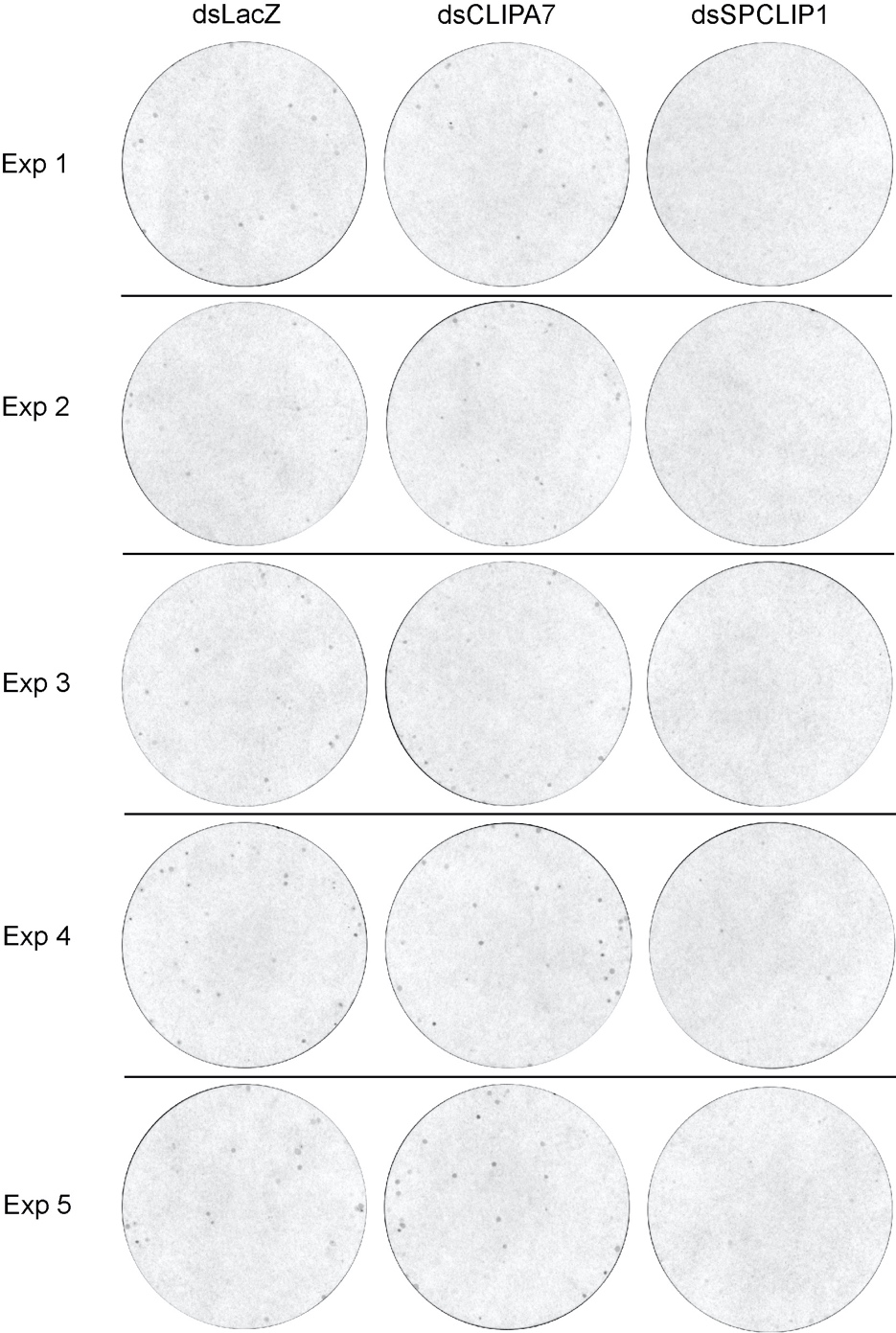
**Online suppl. Fig. 1.** Filters papers showing melanotic excreta at 12 hrs after mosquito injection with *E. coli* (OD600=0.8). Images were acquired on a Bio-Rad Chemidoc MP system and total melanotic spot area was quantified by MelASA (see methods section). Six different experiments (Exp) are shown.



**Online suppl. Fig. 2.** Filters papers showing melanotic excreta at 12 hrs after mosquito injection with *E. coli* (OD600=3). Images were acquired on a Bio-Rad Chemidoc MP system and total melanotic spot area was quantified by MelASA (see methods section). Five different experiments (Exp) are shown.

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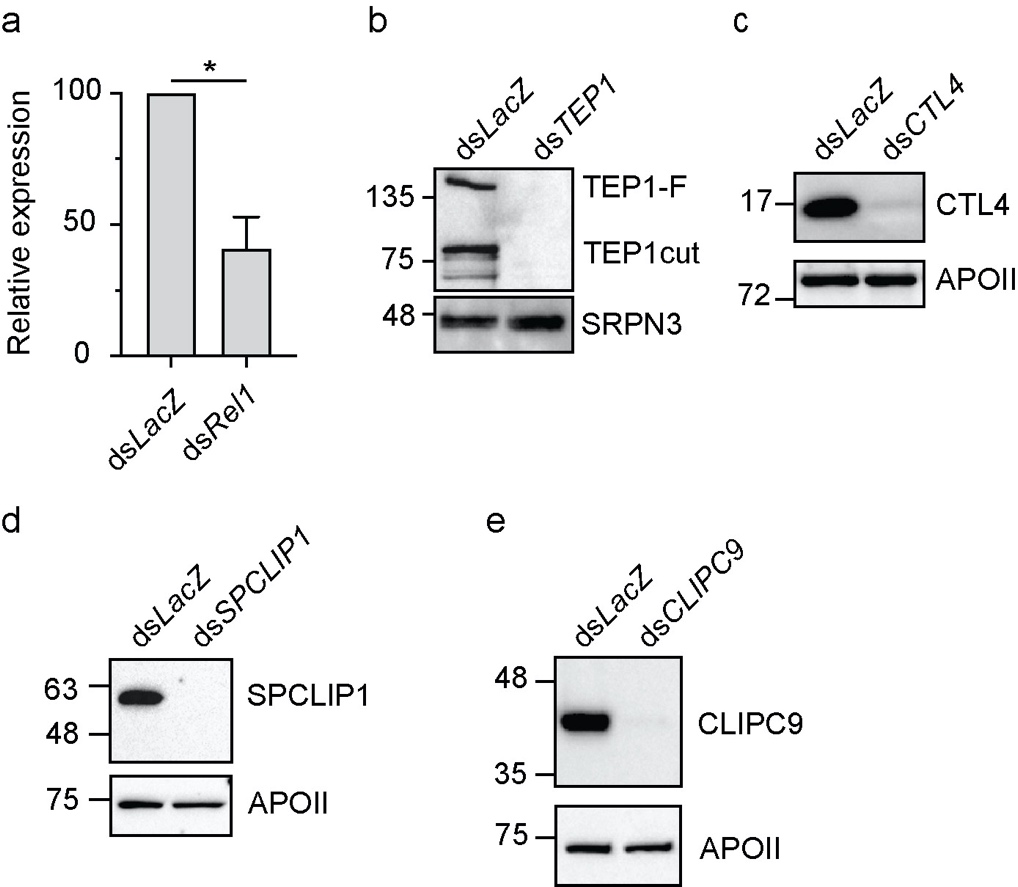
**Online suppl. Fig. 3.** Filters papers showing melanotic excreta at 12 hrs after mosquito injection with *S. aureus* (OD600=0.8). Images were acquired on a Bio-Rad Chemidoc MP system and total melanotic spot area was quantified by MelASA (see methods section). Six different experiments (Exp) are shown.

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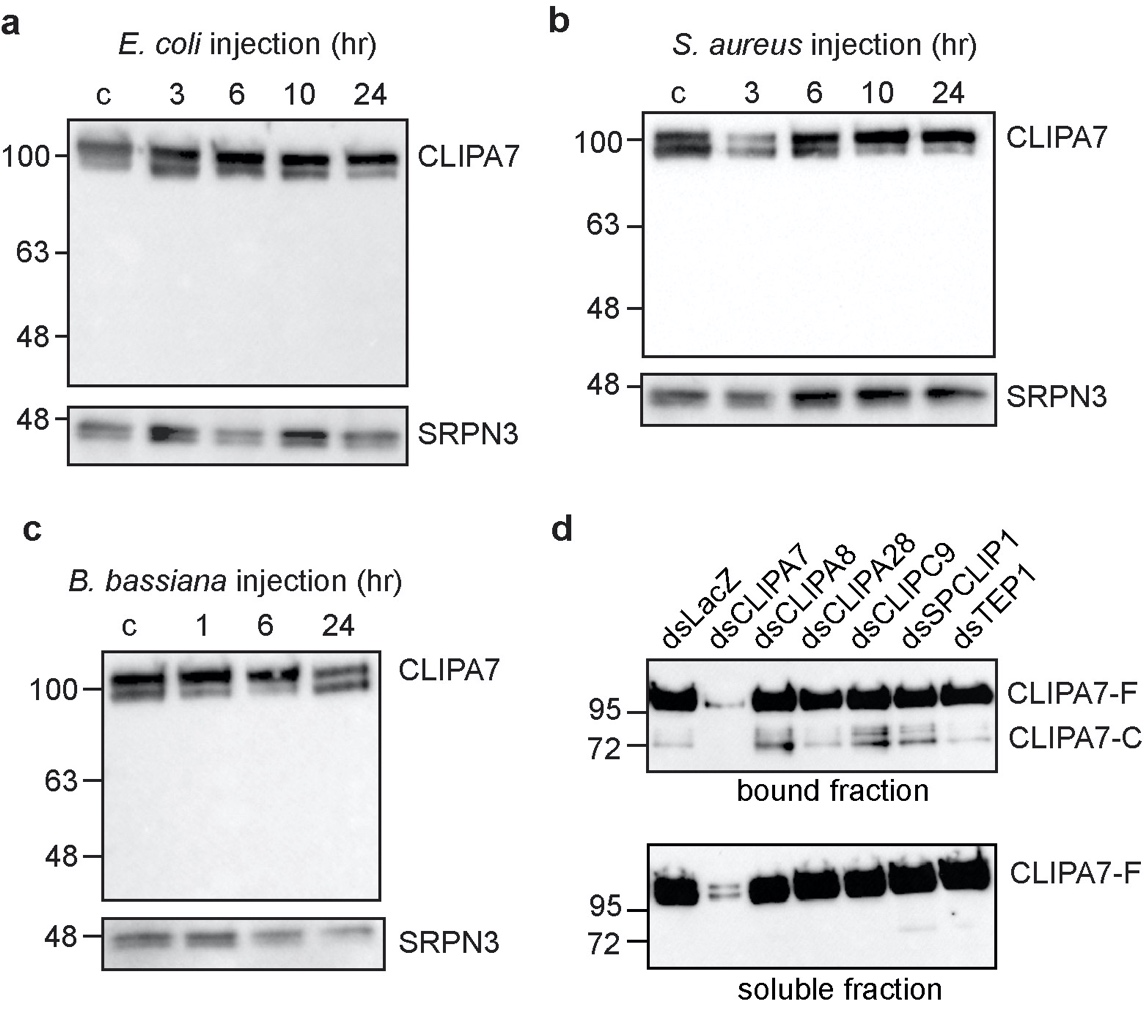
**Online suppl. Fig. 4.** Filter papers showing melanotic excreta at 12 hrs after mosquito injection with *M. luteus* (OD600=2). Images were acquired on a Bio-Rad Chemidoc MP system and total melanotic spot area was quantified by MelASA (see methods section). Five different experiments (Exp) are shown.



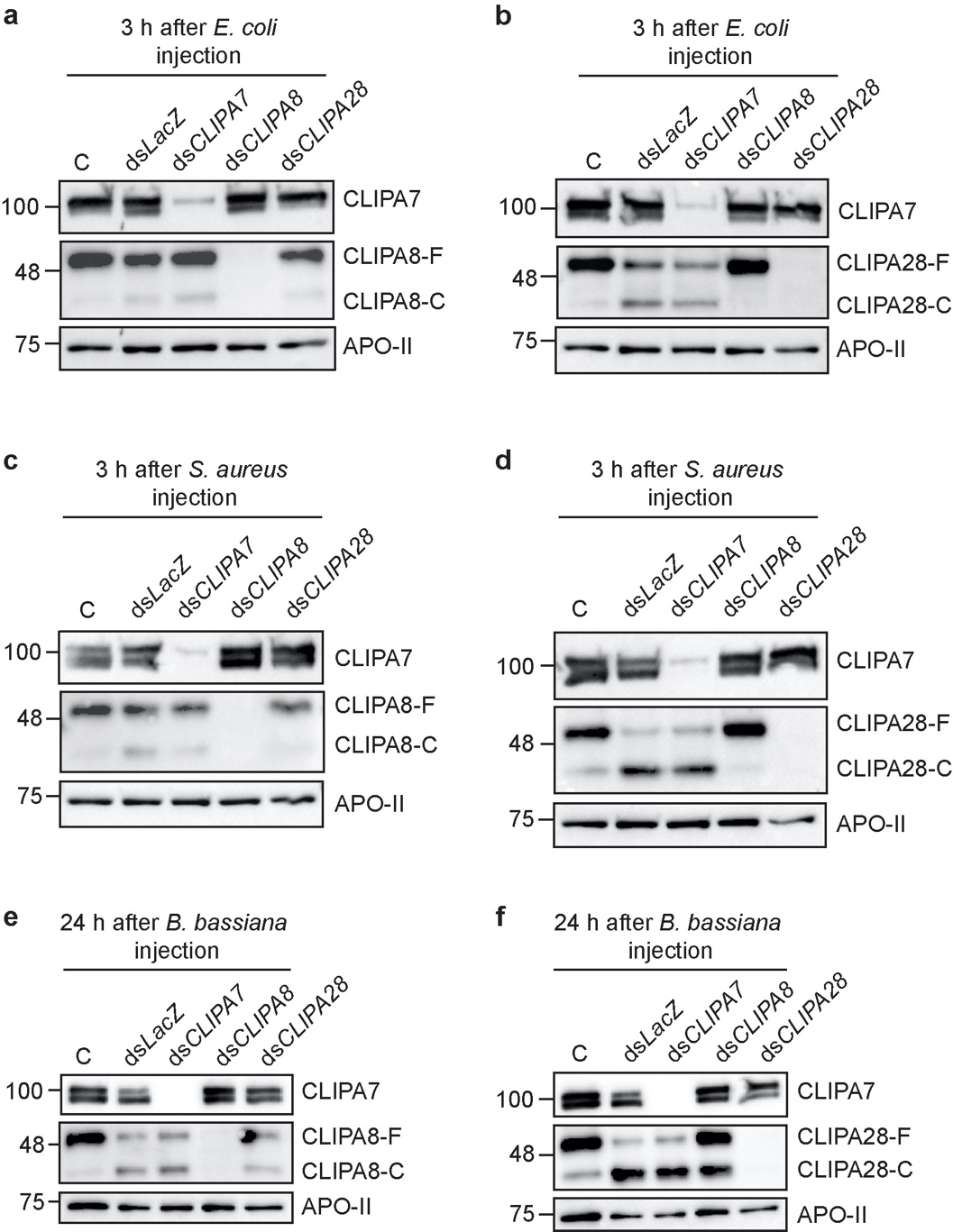
**Online suppl. Fig. 5.** Alignment of the partial coding sequences of CLIPA7 and CLIPA14 corresponding to the protease-like domains.The partial coding sequences (cds) corresponding to the protease-like domains of CLIPA14 (GenBank KY344791) and CLIPA7 (VectorBase AGAP011792) were aligned using MUSCLE sequence alignment tool. The CLIPA7 sequence complementary to the old dsRNA that was previously used to silence CLIPA7 [1] is underlined in red. Highlighted in an opened rectangular box is a contiguous sequence of 34 nucleotides within the ds*CLIPA7* complementary sequence that shares complete sequence identity with a corresponding sequence in *CLIPA14* cds except for one base mismatch (indicated by an asterisk).

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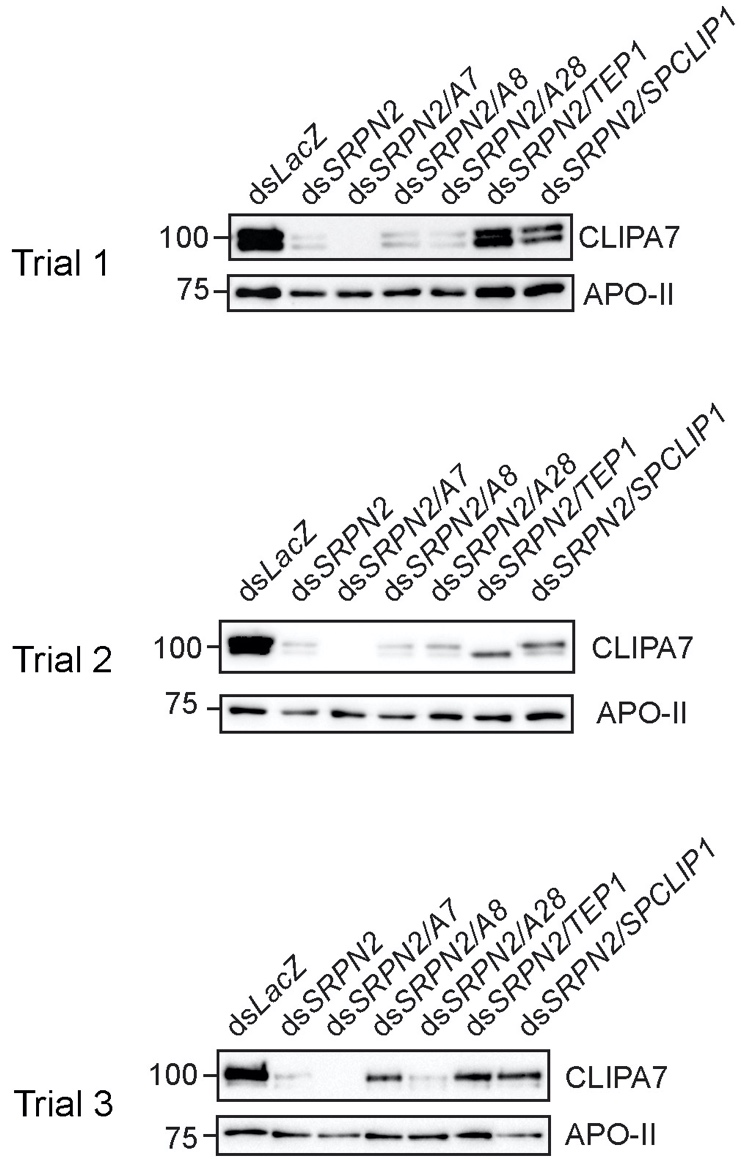
**Online suppl. Fig. 6**. Efficiency of gene silencing by RNAi. Efficiency of gene silencing for the indicated genes was measures by qRT-PCR for **a** Rel1 and by western blot for **b** TEP1, **c** CTL4, **d** SPCLIP1 and **e** CLIPC9. **b**-**e** Hemolymph was extracted from 35 mosquitoes per sample. Protein quantification was performed using Bradford assay and equal amounts of hemolymph proteins were loaded per lane. Membranes were also probed with αApo-II (αApolipophorin II) or αSRPN3 as loading controls.

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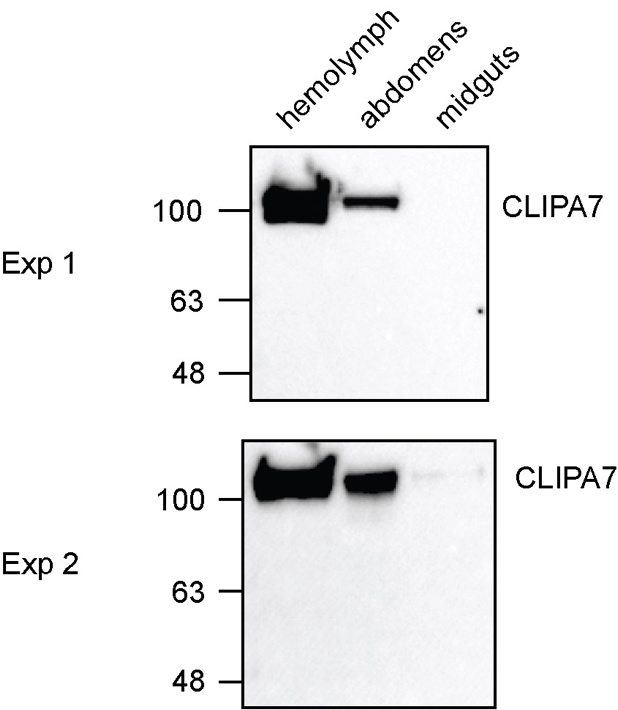
**Online suppl. Fig. 7.** CLIPA7 cleavage is not detected after infections with live bacteria and fungi. **a**-**c** Western blots showing CLIPA7 protein in hemolymph extracts of wildtype mosquitoes at the indicated time points after injection with **a** *E. coli* (OD600=0.8), **b** *S. aureus* (OD600=0.8) and **c** *B. bassiana* (2000 spores/mosquito). In all blots, each lane contained hemolymph extracts from 35 mosquitoes. Membranes were re-probed with αSRPN3 as loading control. **d** Hemolymph containing *E. coli* bioparticles was extracted from the indicated mosquito genotypes at 20 min after bioparticle injection into the hemocoel. Bioparticles were pelleted by centrifugation, and the soluble fractions collected. Bound proteins were extracted from bioparticle pellets with Laemmli protein loading buffer (1x). Shown is a western blot analysis of the soluble and bound fractions.

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**Online suppl. Fig. 8.** CLIPA7 is dispensable for the activation cleavage of CLIPA8 and CLIPA28. **a**-**b** Western blots showing **a** CLIPA8 and **b** CLIPA28 cleavage in hemolymph extracts from the indicated mosquito genotypes at 3 hrs after *E. coli* (OD600=0.8) injection. **c**-**d** Western blots showing **c** CLIPA8 and **d** CLIPA28 cleavage in hemolymph extracts from the indicated mosquito genotypes at 3 hrs after *S. aureus* (OD600=0.8) injection. **e**-**f** Western blots showing **e** CLIPA8 and **f** CLIPA28 cleavage in hemolymph extracts from the indicated mosquito genotypes at 24 hrs after injection of *B. bassiana* (2000 spores/mosquito). In all blots, each lane contained hemolymph extracts from 35 mosquitoes. Membranes were also probed with αCLIPA7, and with αApoII as loading control.

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**Online suppl. Fig. 9.** CLIPA7 consumption in *SRPN2* kd naïve mosquitoes is not rescued by the knockdown of positive regulatory cSPHs nor by TEP1. Three independent western blots showing CLIPA7 protein levels in hemolymph extracts from the indicated naïve mosquito genotypes at day 7 after dsRNA injection. Hemolymph was extracted from 35 mosquitoes per sample. Protein quantification was performed using Bradford assay and equal amounts of hemolymph proteins were loaded per gel lane. Membranes were also probed αApo-II as loading control.

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**Online suppl. Fig. 10.** CLIPA7 protein is detected in hemolymph and abdomen extracts. Hemolymph, abdomen and midgut extracts were prepared from naïve mosquitoes, quantified by Bradford assay to load equal amounts per gel lane. Membranes were probed with αCLIPA7. Two independent experiments are shown.

|  |  |  |
| --- | --- | --- |
| **Online supplementary Table 1: Primers used for dsRNA production** | | |
| Gene | Primers used for dsRNA synthesis (T7 promoter sequence underlined) | Reference |
| *LacZ* | For: 5'-TAATACGACTCACTATAGGGAGAATCCGACGGGTTGTTACT-3'  Rev: 5'-TAATACGACTCACTATAGGGCACCACGCTCATCGATAATTT-3' | [2] |
| *TEP1*  (AGAP010815) | For: 5'-TAATACGACTCACTATAGGGTTTGTGGGCCTTAAAGCGCTG-3'  Rev: 5'- TAATACGACTCACTATAGGGACCACGTAACCGCTCGGTAAG-3' | [3] |
| *CTL4*  (AGAP005335) | For: 5'- TAATACGACTCACTATAGGGGTTAGCAGCATTGGGATTACCCT-3'  Rev: 5'- TAATACGACTCACTATAGGGGAAGTCGCAACCCAGCTCATTGT-3' | [4] |
| *SPCLIP1*  (AGAP028725) | For: 5'- TAATACGACTCACTATAGGGGTCACCGAACACGGCCAAC-3'  Rev: 5'-TAATACGACTCACTATAGGGATCGAAGCTGATCGGATCGGG-3' | [4] |
| *CLIPA7*  (AGAP011792) | For: 5'-TAATACGACTCACTATAGGGGTGCTGGCAGTCCTGGAACT-3’  Rev: 5'-TAATACGACTCACTATAGGGCCACCACCTTGGTTATATCCA-3’ |  |
| *CLIPA8*  (AGAP010731) | For: 5'- TAATACGACTCACTATAGGGAACAACGAACCCGTAGAATATG-3'  Rev: 5'- TAATACGACTCACTATAGGGGGTTAGCGCCTCGATACC-3' | [5] |
| *CLIPA28*  (AGAP010730) | For: 5'-TAATACGACTCACTATAGGGAGACCACCAAGGAACCGTTCCCGCA GCAA-3'  Rev: 5'- TAATACGACTCACTATAGGGAGACCGCAACCGATGCCCCACGAT ACGAT-3' | [6] |
| *SRPN2*  (AGAP006911) | For: 5'- TAATACGACTCACTATAGGGCTGGTCAATGTGATCTACTT-3'  Rev: 5'- TAATACGACTCACTATAGGGATTGTTCCGAGGGTTTCAT-3' |  |
| *CLIPC9*  (AGAP004719) | For: 5'-TAATACGACTCACTATAGGGGGTGCAGTAAGAAGGCCCAT -3'  Rev: 5'-TAATACGACTCACTATAGGGACTGCATGTCCAAGCAATCC -3' | [7] |
| *Rel1*  (AGAP006747) | For : 5'-TAATACGACTCACTATAGGGAGAATCAACAGCACGACGATGAG-3'  Rev : 5'-TAATACGACTCACTATAGGGAGATCGAAAAAGCGCACCTTAATT-3' | [8] |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Online supplementary Table 2: Parasite counts in the indicated genotypes (Pooled trials 1, 2 and 3)** | | | | | | | | | |  |  |
| Gut # | **LacZ** | | **CLIPA7 kd** | | **TEP1 kd** | | **CTL4 kd** | | **CLIPA7/TEP1 dkd** | | **CLIPA7/CTL4 dkd** | |
| live oocyts | Melanized ookinetes | live oocysts | Melanized ookinetes | Live oocysts | Melanized ookinetes | Live oocysts | Melanized ookinetes | Live oocysts | Melanized ookinetes | Live oocysts | Melanized ookinetes |
| 1 | 55 | 0 | 0 | 0 | 1037 | 0 | 0 | 0 | 693 | 0 | 0 | 221 |
| 2 | 63 | 0 | 91 | 0 | 1365 | 0 | 0 | 239 | 1145 | 0 | 3 | 70 |
| 3 | 289 | 0 | 173 | 0 | 1355 | 0 | 0 | 591 | 663 | 0 | 161 | 89 |
| 4 | 6 | 0 | 427 | 11 | 69 | 0 | 7 | 94 | 822 | 0 | 0 | 186 |
| 5 | 249 | 0 | 133 | 0 | 816 | 1 | 1 | 455 | 668 | 0 | 11 | 0 |
| 6 | 230 | 0 | 138 | 0 | 212 | 0 | 6 | 308 | 518 | 0 | 0 | 0 |
| 7 | 25 | 0 | 93 | 8 | 593 | 0 | 0 | 457 | 687 | 0 | 4 | 7 |
| 8 | 80 | 0 | 0 | 0 | 274 | 0 | 0 | 0 | 507 | 0 | 15 | 56 |
| 9 | 253 | 0 | 0 | 0 | 252 | 0 | 6 | 33 | 471 | 0 | 109 | 23 |
| 10 | 1 | 0 | 276 | 2 | 563 | 2 | 2 | 491 | 163 | 0 | 0 | 100 |
| 11 | 636 | 3 | 27 | 0 | 890 | 0 | 37 | 285 | 541 | 3 | 4 | 179 |
| 12 | 24 | 0 | 125 | 0 | 246 | 0 | 0 | 0 | 544 | 0 | 25 | 203 |
| 13 | 850 | 0 | 184 | 0 | 297 | 0 | 1 | 425 | 658 | 0 | 6 | 3 |
| 14 | 17 | 0 | 358 | 0 | 0 | 0 | 2 | 309 | 712 | 1 | 0 | 53 |
| 15 | 26 | 17 | 0 | 0 | 1317 | 0 | 28 | 168 | 1347 | 0 | 6 | 0 |
| 16 | 317 | 0 | 14 | 0 | 800 | 0 | 2 | 20 | 1317 | 1 | 16 | 185 |
| 17 | 273 | 0 | 364 | 0 | 391 | 0 | 8 | 421 | 667 | 0 | 2 | 406 |
| 18 | 20 | 0 | 779 | 0 | 147 | 0 | 0 | 171 | 0 | 0 | 4 | 141 |
| 19 | 100 | 0 | 1356 | 0 | 744 | 0 | 0 | 155 | 14 | 0 | 163 | 4 |
| 20 | 288 | 0 | 38 | 0 | 4 | 0 | 5 | 16 | 898 | 0 | 2 | 940 |
| 21 | 225 | 0 | 119 | 0 | 578 | 0 | 0 | 121 | 759 | 3 | 145 | 241 |
| 22 | 138 | 0 | 266 | 0 | 394 | 0 | 0 | 181 | 1260 | 3 | 20 | 62 |
| 23 | 391 | 0 | 27 | 0 | 515 | 0 | 0 | 624 | 551 | 0 | 7 | 251 |
| 24 | 295 | 0 | 22 | 0 | 724 | 0 | 0 | 0 | 821 | 0 | 28 | 308 |
| 25 | 0 | 0 | 14 | 0 | 250 | 0 | 65 | 147 | 1173 | 0 | 30 | 5 |
| 26 | 89 | 0 | 50 | 13 | 1053 | 0 | 7 | 669 | 8 | 0 | 264 | 6 |
| 27 | 166 | 0 | 325 | 0 | 562 | 0 | 32 | 357 | 1037 | 0 | 0 | 0 |
| 28 | 0 | 0 | 106 | 0 | 495 | 0 | 62 | 179 | 206 | 0 | 30 | 18 |
| 29 | 281 | 0 | 74 | 0 | 16 | 0 | 5 | 465 | 633 | 0 | 55 | 121 |
| 30 | 182 | 0 | 18 | 0 | 609 | 0 | 8 | 12 | 302 | 0 | 147 | 6 |
| 31 | 46 | 0 | 267 | 0 | 11 | 0 | 1 | 131 | 713 | 0 | 151 | 123 |
| 32 | 206 | 0 | 563 | 0 | 774 | 0 | 2 | 11 | 508 | 0 | 0 | 1073 |
| 33 | 236 | 0 | 143 | 0 | 822 | 0 | 2 | 83 | 945 | 0 | 0 | 47 |
| 34 | 32 | 0 | 248 | 9 | 1017 | 0 | 4 | 612 | 346 | 0 | 12 | 367 |
| 35 | 359 | 0 | 441 | 0 | 124 | 0 | 188 | 16 | 32 | 0 | 2 | 43 |
| 36 | 0 | 0 | 47 | 0 | 586 | 0 | 0 | 1105 | 462 | 0 | 0 | 16 |
| 37 | 0 | 0 | 288 | 0 | 462 | 0 | 4 | 14 | 266 | 0 | 16 | 91 |
| 38 | 485 | 0 | 0 | 0 | 298 | 0 | 0 | 460 | 430 | 0 | 16 | 29 |
| 39 | 56 | 0 | 49 | 0 | 102 | 0 | 0 | 1081 | 18 | 0 | 55 | 6 |
| 40 | 26 | 0 | 228 | 0 | 762 | 0 | 9 | 42 | 411 | 0 | 13 | 3 |
| 41 | 48 | 0 | 319 | 0 | 1297 | 3 | 0 | 184 | 57 | 0 | 6 | 138 |
| 42 | 741 | 0 | 64 | 0 | 61 | 0 | 0 | 441 | 0 | 0 | 9 | 33 |
| 43 | 47 | 3 | 378 | 3 | 1179 | 4 | 1 | 254 | 0 | 0 | 115 | 291 |
| 44 | 44 | 2 | 680 | 0 | 0 | 0 | 0 | 305 | 216 | 0 | 107 | 29 |
| 45 | 142 | 0 | 4 | 0 | 623 | 0 | 201 | 19 | 143 | 0 | 12 | 551 |
| 46 | 11 | 0 | 259 | 0 | 635 | 0 | 0 | 651 | 255 | 0 | 42 | 45 |
| 47 | 2 | 1 | 311 | 1 | 230 | 0 | 0 | 691 | 0 | 0 | 3 | 481 |
| 48 | 539 | 0 | 57 | 0 | 221 | 5 | 0 | 34 | 338 | 0 | 3 | 13 |
| 49 | 431 | 0 | 0 | 0 | 177 | 0 | 0 | 309 | 0 | 0 | 1 | 111 |
| 50 | 26 | 2 | 549 | 0 | 561 | 0 | 0 | 313 | 395 | 0 | 67 | 49 |
| 51 | 38 | 0 | 470 | 0 | 623 | 4 | 41 | 0 | 0 | 0 | 77 | 235 |
| 52 | 12 | 19 | 217 | 0 | 345 | 1 | 0 | 336 | 337 | 1 |  |  |
| 53 | 134 | 25 | 325 | 0 | 0 | 0 | 0 | 9 | 282 | 0 |  |  |
| 54 | 138 | 0 | 141 | 3 | 191 | 0 |  |  | 456 | 0 |  |  |
| 55 | 16 | 0 | 101 | 0 | 83 | 0 |  |  | 522 | 4 |  |  |
| 56 | 150 | 0 | 437 | 0 | 120 | 0 |  |  | 441 | 0 |  |  |
| 57 | 27 | 0 | 113 | 9 |  |  |  |  | 0 | 0 |  |  |
| 58 |  |  |  |  |  |  |  |  | 0 | 0 |  |  |
| 59 |  |  |  |  |  |  |  |  | 145 | 0 |  |  |
| 60 |  |  |  |  |  |  |  |  | 274 | 0 |  |  |
| 61 |  |  |  |  |  |  |  |  | 313 | 0 |  |  |
| 62 |  |  |  |  |  |  |  |  | 407 | 1 |  |  |
| 63 |  |  |  |  |  |  |  |  | 307 | 0 |  |  |
| 64 |  |  |  |  |  |  |  |  | 401 | 0 |  |  |
| 65 |  |  |  |  |  |  |  |  | 991 | 0 |  |  |
| 66 |  |  |  |  |  |  |  |  | 309 | 0 |  |  |
| 67 |  |  |  |  |  |  |  |  | 331 | 1 |  |  |
| 68 |  |  |  |  |  |  |  |  | 763 | 0 |  |  |
| 69 |  |  |  |  |  |  |  |  | 0 | 0 |  |  |
| 70 |  |  |  |  |  |  |  |  | 679 | 0 |  |  |
| 71 |  |  |  |  |  |  |  |  | 307 | 0 |  |  |
| 72 |  |  |  |  |  |  |  |  | 617 | 0 |  |  |
| 73 |  |  |  |  |  |  |  |  | 154 | 0 |  |  |
| 74 |  |  |  |  |  |  |  |  | 471 | 0 |  |  |

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