

**Table S1A**

| <b>Bacterial strains</b>   | <b>Description</b>   |
|----------------------------|--|
| SM10 $\lambda$ pir         | thi thr leu tonA lacY supE recA : : RP4-2Tc : :Mu ( $\lambda$ pirR6K)            |
| C6706                      | Wild-type, El Tor biotype, Inaba serotype (Peru isolate, 1991)                   |
| C6706 $\Delta$ lacZ        | C6706, $\Delta$ lacZ (YangM et al., <i>PNAS</i> , <b>110</b> , 2348 (Feb, 2013)) |
| C6706 $\Delta$ dncV        | C6706, $\Delta$ dncV (this study)  |
| C6706 $\Delta$ V-cGAP1     | C6706, $\Delta$ VCA0681 (this study)   |
| C6706 $\Delta$ V-cGAP2     | C6706, $\Delta$ VCA0210 (this study)   |
| C6706 $\Delta$ V-cGAP3     | C6706, $\Delta$ VCA0931 (this study)   |
| C6706 $\Delta$ V-cGAP1/2   | C6706, $\Delta$ VCA0681/0210 (this study)  |
| C6706 $\Delta$ V-cGAP1/3   | C6706, $\Delta$ VCA0681/0931 (this study)  |
| C6706 $\Delta$ V-cGAP1/2/3 | C6706, $\Delta$ VCA0681/0210/0931 (this study)                                   |

**Table S1B**

| Gene name                                       | Vector  | Tag |           | Forward Primer (5'-3')                      | Reverse Primer (5'-3')               | Length |
|---|---------|-----|-----------|---|--------------------------------------|--------|
| <b>Plasmids for Eukaryotic Screening System</b> |         |     |           |   |                                      |        |
| VC0179  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGGTGAGAATGACTTGG AAC        | AGTCTCTAGAGCCACTTACCATTGTGCTGC       | FL     |
| VCA0956   | pCMV7.1 | F   | NotI/KpnI | ACAGTGGCGGCCCGCGGTGATGACA ACTGAAGAT TTC     | AGTCGGTACCTTAGAGCGGCATGACTCGATTGCG   | FL     |
| VC1710  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGAGTGTGAATGTATCAACCG        | AGTCTCTAGA ACTCGCTAAATAAGCGAGCAG     | FL     |
| VC0137  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGTTGGTTCGATGTTTATGGGC       | AGTCTCTAGAAATTAATCGATTGATGTCCTGG     | FL     |
| VCA0101   | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGGCTAAGGAAAAGACTGTG         | AGTCTCTAGATATTTTGCTTGTGGCAAAGTTTAATG | FL     |
| VC1211  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGTTGAGCCTCTTAGCATGC         | AGTCTCTAGATTGCGCCTCAACTGGGACTG       | FL     |
| VC1592  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGAATCACATCCACCCCTAC         | AGTCTCTAGATAACGCAGACATACGTGGAG       | FL     |
| VC1851  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGTTGAAGTACTCGTATGTCGC       | AGTCTCTAGAGGTTTGTACACCAAGAAGATCG     | FL     |
| VC2340  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGCAGATGCAACCCAAAGTC         | AGTCTCTAGAGTGGGTGATTCCCTGGTCTTG      | FL     |
| VC1348  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGGCAACCGCCAATATCGC          | AGTCTCTAGAGCCTGACGCTTGTGACCCAG       | FL     |
| VC0694  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGGATCTCATCCTCTCATTAC        | AGTCTCTAGAGTAAGTGCCTTGCTGTGGAATG     | FL     |
| VC1295  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGTTGACCATTGGGGTCTATCAC      | AGTCTCTAGATGCGGCATCTTTAAAGTGTGCTG    | FL     |
| VC2497  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGGTGGCAAGCATTAAAATCACG      | AGTCTCTAGACTCTTCGCTGTCAAAGAAGTATG    | FL     |
| VC1152  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCTTGATCGTGTATTTTATGAGTC        | AGTCTCTAGAGATGCCACTGGCACTGATCAC      | FL     |
| VC0653  | pCMV7.1 | F   | NotI/SalI | ACAGTGGCGGCCCGCATGCCTGCTCAAACCTCATCTC       | AGTCGTCGACTTAGAGGGATTTGCGATGGCGGC    | FL     |
| VCA0536   | pCMV7.1 | F   | NotI/SalI | ACAGTGGCGGCCCGCATGGCGTGCCTGCTCAAAAGTCTTAC   | AGTCGTCGACTTAGTTAGCATTGCTACCTTAG     | FL     |
| VCA1083   | pCMV7.1 | F   | NotI/SalI | ACAGTGGCGGCCCGGTTGGGCAAACAGCAGTGGAAAAC      | AGTCGTCGACTTACTTATCAACAATGAAGCC      | FL     |
| VC0398  | pCMV14  | F   | NotI/XbaI | ACAGTGGCGGCCGCATGGTGGCACAAATGAGGTATACA<br>C | AGTCTCTAGATTTGGTTCGCCATCGATTACG      | FL     |
| VC0515  | pCMV7.1 | F   | NotI/SalI | ACAGTGGCGGCCCGCATGGATAGTTTCATAGCAAAC        | AGTCGTCGACCTATTTAACTTCTTTCATTAAAGTTC | FL     |
| VCA0895   | pCMV7.1 | F   | NotI/SalI | ACAGTGGCGGCCCGGTTGCAACCTAGCCAAGATGAAC       | AGTCGTCGACCTAGTAATCTTCCCGTAAATACG    | FL     |

|                             |         |   |            |   |  |                 |
|-----------------------------|---------|---|------------|---|--|-----------------|
| VCA0802                     | pCMV7.1 | F | NotI/SalI  | ACAGTGGCGGCCGCGGTGACGACTTTGAATAAACTC            | AGTCGTCGACTTATTTTTCCAATTCATAAC           | FL              |
| VC2600                      | pCMV7.1 | F | NotI/SalI  | ACAGTGGCGGCCGCGTTGCTGTCCAACCAATCCCATTG          | AGTCGTCGACTTAGCGCAAGAATTGCCGCATC         | FL              |
| VCA0510                     | pCMV14  | F | NotI/BamHI | ACAGTGGCGGCCGCATGTTGACGCATTATCAAACCTGAA<br>ATAG | AGTCGGATCCCTGAATATCTAACTTATTGAG          | FL              |
| VC1687                      | pCMV14  | F | NotI/XbaI  | AAAAAAGCGGCCGCATGATTTTAGTCGTTGGTCATAAAA<br>AC   | GTAAGTCTAGAAAGCCAGTTCGGTTTGTAGCCA        | FL              |
| VC1081                      | pCMV14  | F | EcoRI/XbaI | GTAAGAATTCATGACAGTAGAAGCGATGAAAAG               | GTAATCTAGAAGCTGGAAAAAATTGAGTTTCC         | FL              |
| VC1086                      | pCMV14  | F | EcoRI/XbaI | GATTGAATTCATGCAAAGCAACCGTGAAATTC                | GTTTTCTAGATGGAGATAGTTTGTATGGAGATAG       | FL              |
| VC1652                      | pCMV7.1 | F | NotI/SalI  | ACAGTGGCGGCCGCGATGAAAATAATGATAGTAGAAGA<br>TG    | AGTCGTCGACTTATTTAATGTTACAAAACGCACC       | FL              |
| VC0658                      | pCMV14  | F | NotI/XbaI  | AAAAAAGCGGCCGCATGACCAAATTCGCAAGATTC             | GTTTTCTAGATTTTGTAAACTGTTGTGCGCAAC        | FL              |
| VC1641                      | pCMV7.1 | F | NotI/SalI  | ACAGTGGCGGCCGCGATGCATATTATTCCTGTGGCG            | AGTCGTCGACTCACGCTTCGCTGAACTCAGGATTG      | FL              |
| VC0072                      | pCMV14  | F | NotI/XbaI  | ACAGTGGCGGCCGCATGTTGGAGTCTGACTTGAGCCG           | AGTCTCTAGACGCTTCTCTTTGAGTTGCTC           | FL              |
| VC1934                      | pCMV14  | F | EcoRI/XbaI | GATCGAATTCATGGCAGATCAGCAGTATAGAAAATC            | GTTTTCTAGACTCATAAATTTGCGTGGATAAC         | FL              |
| VCA0165                     | pCMV14  | F | NotI/XbaI  | ACAGTGGCGGCCGCATGTTTCTCGAACCCCTACTC             | AGTCTCTAGACATTTCTGCCTGTATGCAGTCG         | FL              |
| VCA0080                     | pCMV14  | F | NotI/XbaI  | AAAAAAGCGGCCGCATGACCAAGACTCAACTGATAAC           | ATATTCTAGAGGCTACATTCGTTTCTTTTCTC         | FL              |
| VC2750                      | pCMV14  | F | NotI/XbaI  | AAAAAAGCGGCCGCATGAGATTAGAGAAGGGTATGCCT          | ATATTCTAGAACTTTGCTTCATGTTAACCAG          | FL              |
| VCA0785                     | pCMV14  | F | EcoRI/XbaI | GATTGAATTCATGGCACCGATCCTTTCACAC                 | GGGTCTAGACAGAGCGTGGCTTTCGAGCTTG          | FL              |
| VCA0681                     | pCMV14  | F | EcoRI/XbaI | GATCGAATTCATGAGATGGTCAGAAATAGGCTGC              | GTTTTCTAGATTGCGTTGGGCTTCCGCTATC          | FL              |
| VCA0681-M1                  | pCMV14  | F | EcoRI/XbaI | GCGCTGGGTTTGATCGCTGCCTGTGGCGTGGCTCAG            | CTGAGCCACGCCACAGGCAGCGATCAAACCCAGCG<br>C | H74A/D7<br>5A   |
| VCA0681-M1/<br>WT           | pCMV14  | F | EcoRI/XbaI | GCGCTGGGTTTGATCCATGACTGTGGCGTGGCTCAG            | CTGAGCCACGCCACAGTCATGGATCAAACCCAGCG<br>C | A74H/A7<br>5D   |
| VCA0681-M2                  | pCMV14  | F | EcoRI/XbaI | CTGACAGGACTGGTTGCCGCTATCGGCAAGCTGCAC            | GTGCAGCTTGCCGATAGCGGCAACCAGTCCTGTGCTCAG  | H288A/D<br>289A |
| VCA0681-M2/<br>WT           | pCMV14  | F | EcoRI/XbaI | CTGACAGGACTGGTTCACGATATCGGCAAGCTGCAC            | GTGCAGCTTGCCGATATCGTGAACCAGTCCTGTGCTCAG  | A288H/A<br>289D |
| VCA0210                     | pCMV14  | F | NotI/XbaI  | ACAGTGGCGGCCGCATGTTGAAGTGGTTTAAATATGGA<br>G     | AGTCTCTAGAGTCAGGCAGCGAAGCACGGATAG        | FL              |
| VCA0931                     | pCMV14  | F | NotI/XbaI  | ACAGTGGCGGCCGCATGAGTGTGCACAAAATACC              | AGTCTCTAGAGGTCTTATCTGAAGAGTGTGG          | FL              |
| <b>Recombinant Proteins</b> |         |   |            |   |  |                 |

|                   |        |     |           |  |   |                   |
|-------------------|--------|-----|-----------|--|---|-------------------|
| VC1710            | pET21b | His | Sall/NotI | AGTCGTCGACATGAGTGTGAATGTATCAACCG             | ACAGTGGCGGCCGCACTCGCTAAATAAGCGAGCAG         | FL                |
| VC2340            | pET21b | His | Sall/NotI | AGTCGTCGACATGCAGATGCAACCCAAAGTC              | ACAGTGGCGGCCGCGTGGGTGATTCCCTGGTCTTG         | FL                |
| VC1348            | pET21b | His | Sall/NotI | AGTCGTCGACATGGCAACCGCCAATATCGC               | ACAGTGGCGGCCGCGCCTGACGCTTGTTGACCCAG         | FL                |
| VC1295            | pET21b | His | Sall/NotI | AGTCGTCGACCGAATGATGAAAGAGATCCTC              | ACAGTGGCGGCCGCTGCGGCATCTTTAAAGTGTGCT<br>G   | CT(226-4<br>58aa) |
| VC2497            | pET21b | His | NdeI/XhoI | AGTCCATATGGTGGCAAGCATTAAAATCACG              | AGTCCTCGAGCTCTTCGCTGTCAAAGAAGTATG           | FL                |
| VCA0895           | pET21b | His | Sall/NotI | AGTCGTCGACAGTTATTACAGCTCTCAACAG              | ACAGTGGCGGCCGCGTAATCTTCCCGTAAATACGC         | CT(50-98<br>2aa)  |
| VCA0802           | pET21b | His | Sall/NotI | AGTCGTCGACATGCATCGCCCAATGAACCTGCA            | ACAGTGGCGGCCGCTTTTCCAATTCATAACTTTTT<br>G    | CT(201-6<br>57aa) |
| VC1081            | pET21b | His | Sall/NotI | AGTCGTCGACATGACAGTAGAAGCGATGAAAG             | ACAGTGGCGGCCGCGAGCTGGAAAAAATTGAGTTTC<br>C   | FL                |
| VCA0681           | pET21b | His | Sall/NotI | AGTCGTCGACATGAGATGGTCAGAAATAGGC              | ACAGTGGCGGCCGCTTCGGTTGGGCTTCCGCTATC         | FL                |
| VCA0681-M1        | pET21b | His | Sall/NotI | GCGCTGGGTTTGATCGCTGCCTGTGGCGTGGCTCAG         | CTGAGCCACGCCACAGGCAGCGATCAAACCCAGCG<br>C    | H74A/D7<br>5A     |
| VCA0681-M1/<br>WT | pET21b | His | Sall/NotI | GCGCTGGGTTTGATCCATGACTGTGGCGTGGCTCAG         | CTGAGCCACGCCACAGTCATGGATCAAACCCAGCG<br>C    | A74H/A7<br>5D     |
| VCA0681-M2        | pET21b | His | Sall/NotI | CTGACAGGACTGGTTCGCCGTATCGGCAAGCTGCAC         | GTGCAGCTTGCCGATAGCGGCAACCAGTCCTGTGCTCAG     | H288A/D<br>289A   |
| VCA0681-M2/<br>WT | pET21b | His | Sall/NotI | CTGACAGGACTGGTTCACGATATCGGCAAGCTGCAC         | GTGCAGCTTGCCGATATCGTGAACCAGTCCTGTGCTCAG     | A288H/A<br>289D   |
| 0681-Y/A          | pET21b | His | Sall/NotI | GTTTAGATGGTTCGGGTGCCCAAGAGGACTCCAAG          | CTTGAGTCTCTTGGGGCACCCGAACCATCTAAAC          | Y350A             |
| 0681-HHE/AA<br>A  | pET21b | His | Sall/NotI | GAGTGGGCAGGAAACGCCGCTGCGCGTTTAGATGGTTC       | GAACCATCTAAACGCGCAGCGGCGTTTCTGCCAC<br>TC    | HHE/AA<br>A       |
| 0681-GK/AA        | pET21b | His | Sall/NotI | CTGGTTCACGATATCGCCGCGCTGCACACCCAGAAG         | CTTCTGGGGTGTGCAGCGCGGCGATATCGTGAACCA<br>G   | G291A/K<br>292A   |
| 0681-DG/AA        | pET21b | His | Sall/NotI | CACCATGAGCGTTTAGCTGCTCGGGTTACCCAAGAG         | CTCTTGGGTAACCCGAAGCAGCTAAACGCTCATGGT<br>G   | D346A/G<br>347A   |
| VCA0210           | pET21b | His | Sall/NotI | AGTCGTCGACATGTTGAAGTGGTTTAAATATGGAG          | ACAGTGGCGGCCGCGTCAGGCAGCGAAGCACGGAT<br>AG   | FL                |
| VCA0210M          | pET21b | His | Sall/NotI | CAAAAATGCCTCACCTTTGGCTGCTGTTGGCAAAGTCGC<br>C | GGCGACTTGCCAACAGCAGCCAAAGGTGAGGCAT<br>TTTTG | H382A/D<br>383A   |
| VCA0931           | pET21b | His | Sall/NotI | AGTCGTCGACATGAGTGTTCACAAAATACC               | ACAGTGGCGGCCGCGTCTTATCTGAAGAGTGTGG          | FL                |
| VCA0931M          | pET21b | His | Sall/NotI | CGTGCGGCACTGCTGGCTGCTATTGGTAAGCTTGGTG        | CACCAAGCTTACCAATAGCAGCCAGCAGTGCAGCGCA<br>CG | H317A/D<br>318A   |

| DNA manipulations in <i>V. cholerae</i>    |         |  |            |  |   |  |
|--|---------|--|------------|--|---|--|
| VC0179                                     | pBAD24  |  | EcoRI/XbaI | GGAATTCGTGAGAATGACTTGGAAC                                    | GCTCTAGATCAGCCACTTACCATTGT                                      | FL   |
| VC0179                                     | pBAD33  |  | KpnI/PstI  | GGGGTACCCGTGAGAATGACTTGGAAC                                  | AACTGCAGATCAGCCACTTACCATTGT                                     | FL   |
| VCA0681                                    | pMalc2x |  | NdeI/XbaI  | GGAATTCATATGAGATGGTCAGAAATAGG                                | GCTCTAGATTATGCCCATTCGGTTG                                       | FL   |
| VCA0681-M1                                 | pMalc2x |  | NdeI/XbaI  | GGAATTCATATGAGATGGTCAGAAATAGG                                | GCTCTAGATTATGCCCATTCGGTTG                                       | H74A/D75A  |
| VCA0681-M2                                 | pMalc2x |  | NdeI/XbaI  | GGAATTCATATGAGATGGTCAGAAATAGG                                | GCTCTAGATTATGCCCATTCGGTTG                                       | H288A/D289A  |
| VCA0210                                    | pMalc2x |  | NdeI/XbaI  | GGAATAGCATATGAAGTGGTTTAAATATGGA                              | GCTCTAGATCCTAGTCAGGCAGCGAAG                                     | FL   |
| VCA0210                                    | pMalc2x |  | NdeI/XbaI  | GGAATAGCATATGAAGTGGTTTAAATATGGA                              | GCTCTAGATCCTAGTCAGGCAGCGAAG                                     | H382A/D383A  |
| VCA0931                                    | pMalc2x |  | NdeI/XbaI  | GCTCTAGATTGTTAGTCTTATCTGAA                                   | GGAATAGCATATGAGTGTGCACAAAATA                                    | FL   |
| VCA0931                                    | pMalc2x |  | NdeI/XbaI  | GCTCTAGATTGTTAGTCTTATCTGAA                                   | GGAATAGCATATGAGTGTGCACAAAATA                                    | H317A/D318A  |
| VC0179                                     | pWM91   |  | BamHI/XhoI | CGGGATCCAGTTTGAATGGCGGTGGT<br>/CACTTTCAGCCTCACTCTCCTTAAGATT  | GGAGAGTGAGGCTGAAAGTGAAGCAGGAAT<br>/CCGCTCGAGATTTCACACGTCTGGTCGC | Red labeled primers indicate the up and down flanking sequences for bridge PCR |
| VCA0681                                    | pWM91   |  | BamHI/XhoI | CGGGATCCGCGAAGATGCGGACTGT<br>/TGAGATGGTCCCGAATGGGCATAAAAAATC | GCCCATTCGGGACCATCTCATTGCTCATCC<br>/CCGCTCGAGAGCGTAAGCCTGTGGGAA  |  |
| VCA0210                                    | pWM91   |  | BamHI/XhoI | CGGGATCCCAGTACGCGCATTCTTC<br>/CCTAGTCAGGACCACTTCAACCTCTTTAG  | TTGAAGTGGTCTGACTAGGACAGCGACAA<br>/CCGCTCGAGATCTGGAGCAGCAAGTCG   |  |
| VCA0931                                    | pWM91   |  | BamHI/XhoI | CGGGATCCACGCCAATCACACCATTCA<br>/TGAGTGTGCAGATAAGACCTAACAAGGC | GGTCTTATCTGCAACACTCATTATTTGGG<br>/CCGCTCGAGTTTTGCCAATCAGAGTAC   |  |
| VC0179-seq                                 |         |  |            | CGGTAGGCAGTTAGCT   | TAGTAGCCTTTGCGTC  |  |
| VCA0681-seq                                |         |  |            | GTCGCTCAATTGGCT  | TATTGTGCGATGGCTG  |  |
| VCA0210-seq                                |         |  |            | CCAGCCACACATATTC   | GATCACATTACCCCAAGC  |  |
| VCA0931-seq                                |         |  |            | GGACATCAATGCCACATC   | TTTGCTTGAGCTGCGAGT  |  |
| <b>quantitative real-time RT-PCR</b>       |         |  |            |  |   |  |
| recA (Microbiology, 153, 2964 (Sep, 2007)) |         |  |            | GTGCTGTGGATGTCATCGTTGTTG                                     | CCACCACTTCTCGCCTTCTTTGA   |  |

|         |                          |                     |  |
|---------|--------------------------|---------------------|--|
| VCA0681 | GCAAGCGGTGGACAATC        | GTGGAGCGAAGAGGAGTG  |  |
| VCA0210 | CGATGATGATGAGCAGATG      | GTAACGCACCAAGTCTAAG |  |
| VCA0931 | GCCATTCTGCTTCGTCTAACTTAC | TGGTCATAGCGAGCGTGTG |  |
|         |                          |                     |  |