

**Activation and molecular mechanism of a cryptic oviedomycin biosynthetic gene cluster via the disruption of a global regulatory gene--*adpA* in *Streptomyces ansochromogenes***

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**Supplementary Method**

*Bioassays for anticancer activity*

**Supplementary Table 1: Examples of OvmZW-like proteins from different strains**

| <b>Strains</b>                      | <b>Identity with OvmZ</b> | <b>Identity with OvmW</b> | <b>Cluster type</b>  |
|-------------------------------------|---------------------------|---------------------------|----------------------|
| <i>Streptomyces alni</i>            | 40%                       | 79%                       | T2pks                |
| <i>Streptomyces fradiae</i>         | 40%                       | 62%                       | T1pks                |
| <i>Streptomyces fulvissimus</i>     | 47%                       | 78%                       | T2pks                |
| <i>Streptomyces</i> sp. Ag109_O5-10 | 49%                       | 81%                       | T2pks                |
| <i>Streptomyces</i> sp. CCM_MD2014  | 45%                       | 75%                       | T2pks                |
| <i>Streptomyces</i> sp. DvalAA-19   | 49%                       | 77%                       | T2pks                |
| <i>Amycolatopsis halophila</i>      | 42%                       | 72%                       | Other                |
| <i>Kitasatospora</i> sp. HKI 714    | 34%                       | 65%                       | Phenazine            |
| <i>Nocardia crassostreae</i>        | 40%                       | 74%                       | Terpene              |
| <i>Nocardia vulneris</i>            | 35%                       | 71%                       | Lantipeptide-Terpene |

Supplementary Table 2: Primers used in this study

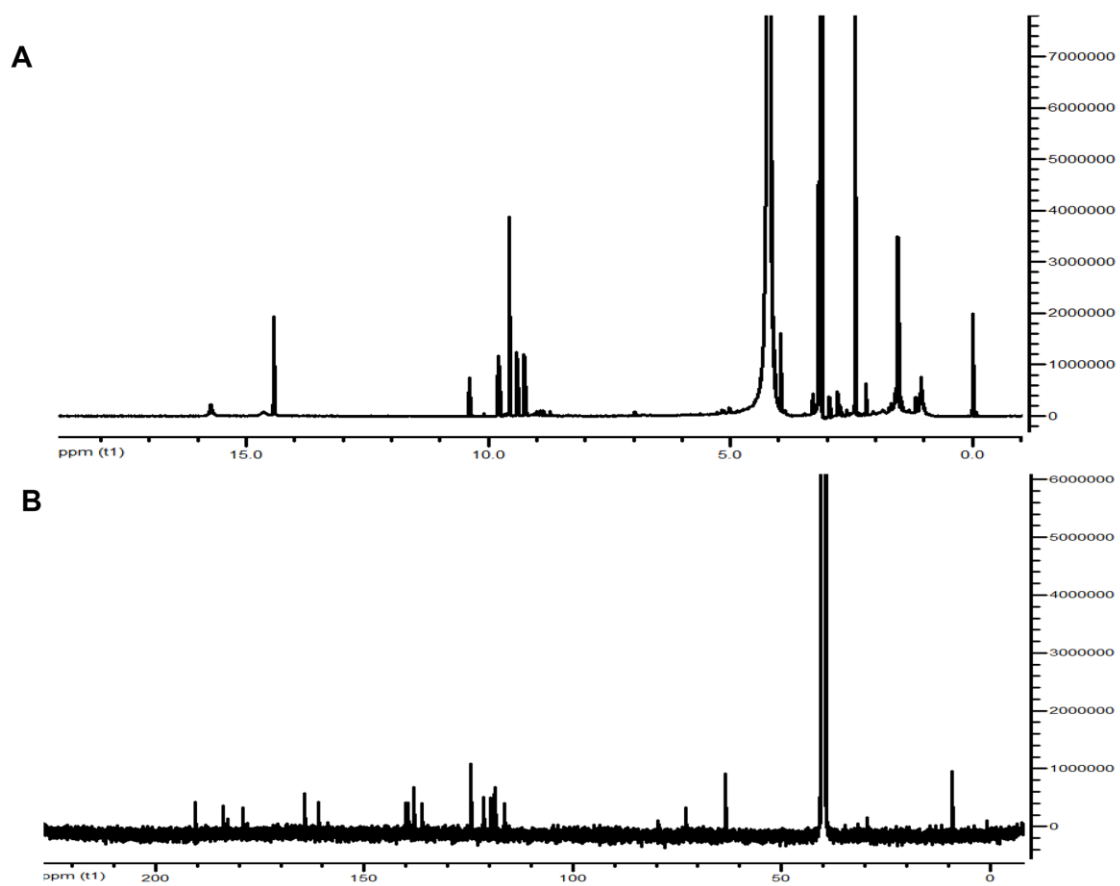
| Primers             | Sequence (5'-3')  | Purpose  |
|---------------------|---|--|
| UZF                 | AAAAAGCTTAACACCGACCTGAGGGCACA                                 | <i>ovmZ</i> disruption                           |
| UZR                 | AAACATATGGTTTGTCTGGACCCGTCGTT                                 | <i>ovmZ</i> disruption                           |
| DZF                 | AAACATATGAGGAGGCCGCCGATGA                                     | <i>ovmZ</i> disruption                           |
| DZR                 | AAATCTAGATCGGTGGTGGACGAACA                                    | <i>ovmZ</i> disruption                           |
| UWF                 | AAAGAATTCACGCTGCTGCTCACGGTGGT                                 | <i>ovmW</i> disruption                           |
| UWR                 | AAAAAGCTTTGACGACCGCTCACGGTCAG                                 | <i>ovmW</i> disruption                           |
| DWF                 | AAAAAGCTTGAGTTCGGTGGGCACGAAGC                                 | <i>ovmW</i> disruption                           |
| DWR                 | AAATCTAGACGAAACCGCGTGCATGTCCAT                                | <i>ovmW</i> disruption                           |
| URF                 | AAAAAGCTTGATGATGTTTCGGCATCGCGA                                | <i>ovmR</i> disruption                           |
| URR                 | AAATCTAGACGAAACCGCGTGCATGTCCAT                                | <i>ovmR</i> disruption                           |
| DRF                 | AAATCTAGAATCGGCCTGGAGGAGTGCATCG                               | <i>ovmR</i> disruption                           |
| DRR                 | AAAGAATTCGACGTCGTAGCGGATGTCCA                                 | <i>ovmR</i> disruption                           |
| UYF                 | AAAGAATTCACGCTGCTGCTCACGGTGGT                                 | <i>ovmY</i> disruption                           |
| UYR                 | AAAAAGCTTGCTTCCGGTCACTGAAGTCA                                 | <i>ovmY</i> disruption                           |
| DYF                 | AAAAAGCTTGATGATGTTTCGGCATCGCGA                                | <i>ovmY</i> disruption                           |
| DYR                 | AAATCTAGACGAAACCGCGTGCATGTCCAT                                | <i>ovmY</i> disruption                           |
| UOrf3F              | AAAGAATTCCTCGCCAACGTCGTGGACGAC                                | <i>orf3</i> disruption                           |
| UOrf3R              | AAAAAGCTTGCTAGATGGGACGGTTCGCAC                                | <i>orf3</i> disruption                           |
| DOrf3F              | AAAAAGCTTGATCTCCACCGTGGCGAGGT                                 | <i>orf3</i> disruption                           |
| DOrf3R              | AAATCTAGACACCGGTCACCAGGATCATCC                                | <i>orf3</i> disruption                           |
| UOrf4F              | AAATCTAGAAGGCGATCCGTGACCTGGGT                                 | <i>orf4</i> disruption                           |
| UOrf4R              | AAAGAATTCGACCTGGAGGGAGCAGTCCACA                               | <i>orf4</i> disruption                           |
| DOrf4F              | AAAGAATTCGACCTGCTTCATGATCACGT                                 | <i>orf4</i> disruption                           |
| DOrf4R              | AAAAAGCTTAGCATCATCGTAAGAACGT                                  | <i>orf4</i> disruption                           |
| ad1-F               | TCGTCGCGGTGCTGCTGTT   | <i>adpA</i> disruption                           |
| ad1-R               | CCACCGGGGAGCGGAAGC  | <i>adpA</i> disruption                           |
| ad2-F               | GGAATTCTGTCTCGGCGTTGTGAT                                      | <i>adpA</i> disruption                           |
| ad2-R               | GGGGTACCGGCTCCTCGGTTGTGT                                      | <i>adpA</i> disruption                           |
| hrdBpF              | GTCGACTCTAGACCGCCTTCCGCCG                                     | <i>hrdB</i> promoter for over-expression         |
| hrdBpR              | GAACAACCTCTCGGAACGTTGA  | <i>hrdB</i> promoter for over-expression         |
| ovmZF               | ATGGCCGGCCAGCCACGACCCAA                                       | <i>ovmZ</i> over-expression                      |
| ovmZR               | AAAGAATTCATCGGGCGGCCTCCTC                                     | <i>ovmZ</i> over-expression                      |
| ovmWF               | ATGAGCCGTACGCCACGGCGGCGCTT                                    | <i>ovmW</i> over-expression                      |
| ovmWR               | AAAGAATTC TCAGCCGAGGGCCTCGGA                                  | <i>ovmW</i> over-expression                      |
| ovmRF               | ATGGACATGCACGCGGTTTC  | <i>ovmR</i> over-expression                      |
| ovmRR               | AAAGAATTCGTTTACATGACGCGGTGGT                                  | <i>ovmR</i> over-expression                      |
| hrdBpF <sup>7</sup> | GACTCTAGAGGATCCGCGGCCGCGCGCATC<br>GAGCACTGACCGCCTTCCGC        | <i>hrdB</i> promoter for heterologous expression |
| hrdBpR <sup>7</sup> | GGTGTCTCCTTGGAACGTTGAAAAACGGCTT<br>CCGGC                      | <i>hrdB</i> promoter for heterologous expression |
| F1F                 | GCCGGAAGCCGTTTTTCAACGTTCCAAGGAG<br>ACACCATGGACGCTGCCCTCGTCGAC | <i>ovm</i> heterologous expression               |
| F1R                 | GGTGCCCTGCTCGGCCAGCAGCCGGGCCA                                 | <i>ovm</i> heterologous expression               |
| F2F                 | TGGCCCGGCTGCTGGCCGAGCAGGGGCACC                                | <i>ovm</i> heterologous expression               |

SUPPLEMENTAL DATA

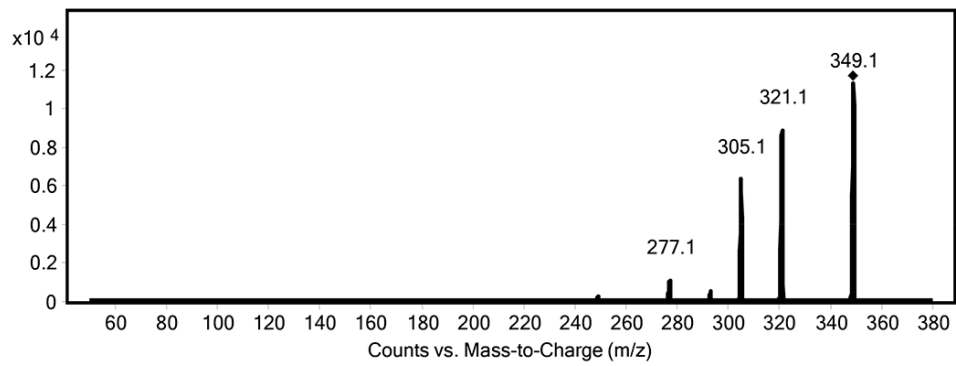
|                             |   |   |
|-----------------------------|---|---|
| F2R                         | CCCGGGACCGCGGGACGGCCGTGCGGACCG                          | <i>ovm</i> heterologous expression                                    |
| F3F                         | CGGTCCGCACGGCCGTCCCGCGGTCCCGGG                          | <i>ovm</i> heterologous expression                                    |
| F3R                         | AAACAGCTATGACATGATTACGAATTCGATT<br>CCCGTCAGCCGGTGATCTCG | <i>ovm</i> heterologous expression                                    |
| ovmOIPF                     | TCCTGCCGGACTCCGCGCACGT                                  | Probe <i>ovmOI</i> for EMSA   |
| ovmOIPR                     | CCGCGCCTCCTTTCTGTTGC                                    | Probe <i>ovmOI</i> for EMSA   |
| OIF                         | GGTCATCGTGGGAGCAGG                                      | Probe for EMSA as a negative control                                  |
| OIR                         | CCTTGACGCTGAAGTGGG                                      | Probe for EMSA as a negative control                                  |
| P <sub><i>ovmOI</i></sub> F | AAACATATGTCCTGCCGGACTCCGCGCACGT                         | Promoter <i>ovmOI</i> for <i>gusA</i> reporter plasmid construct      |
| P <sub><i>ovmOI</i></sub> R | CCGCGCCTCCTTTCTGTTGC                                    | Promoter <i>ovmOI</i> for <i>gusA</i> reporter plasmid construct      |
| <i>gusA</i> F               | ATGACCGGTCTGCGGCCCGTCGAA                                | <i>gusA</i> reporter plasmid construct                                |
| <i>gusA</i> R               | AAAGCGGCCGCCTCACTGCTTCCCGCCCTGC<br>T                    | <i>gusA</i> reporter plasmid construct                                |
| tfdF                        | AAACATATGGACTCACCCGTCGTCGCGCGCT                         | <i>gusA</i> reporter plasmid construct                                |
| <i>gusA</i> 2R              | AAAAGTAGTCTCACTGCTTCCCGCCCTGCT                          | <i>gusA</i> reporter plasmid construct                                |
| ovmZPF                      | TTTGTCGGACCCGTCGTT                                      | Probe <i>ovmZ</i> for EMSA  |
| ovmZPR                      | TCCTTCGGCCGAAACCGCGT                                    | Probe <i>ovmZ</i> for EMSA  |
| T7ter                       | AAAGAATTCGCTAGTTATTGCTCAGCGGTG                          | <i>ovmZ</i> and <i>ovmW</i> over-expression with His <sub>6</sub> tag |
| M13F                        | GGCCGCTCTAGAAGTAGTGGATCC                                | Probe P <sub><i>ovmZ</i></sub> for DNaseI footprinting                |
| M13R                        | GGTCGACGGTATCGATAAGCTTG                                 | Probe P <sub><i>ovmZ</i></sub> for DNaseI footprinting                |
| <i>pks1</i> -F              | CACCGCCTCCGTCTACGAAGCACA                                | <i>pks1</i> RT-PCR  |
| <i>pks1</i> -R              | GGCGCATCCGGCATCCCTCCAT                                  | <i>pks1</i> RT-PCR  |
| <i>pks2</i> -F              | CGGGCAGATGTACCACGAC                                     | <i>pks2</i> RT-PCR  |
| <i>pks2</i> -R              | CACCAGCGAGGAGGAGCAG                                     | <i>pks2</i> RT-PCR  |
| <i>pks3</i> -F              | CTGCGAGTTCGGCGGCTAC                                     | <i>pks3</i> RT-PCR  |
| <i>pks3</i> -R              | TCGGCGGTACGACCTGCTT                                     | <i>pks3</i> RT-PCR  |
| <i>pks4</i> -F              | GGTGCTGGCCCTGATACGC                                     | <i>pks4</i> RT-PCR  |
| <i>pks4</i> -R              | GTGCCCAGGTTGGACTTGA                                     | <i>pks4</i> RT-PCR  |
| <i>pks5</i> -F              | AAGGACCCGTCGTCACCG                                      | <i>pks5</i> RT-PCR  |
| <i>pks5</i> -R              | CCGTCCAGCAGCAGCAT                                       | <i>pks5</i> RT-PCR  |
| <i>pks6</i> -F              | TGTGCAAGCCCTCGGTGTC                                     | <i>pks6</i> RT-PCR  |
| <i>pks6</i> -R              | CACGTCGATGTCGCTGGTG                                     | <i>pks6</i> RT-PCR  |
| <i>pks7</i> -F              | AGGGCTCCGCCTTCTTCGT                                     | <i>pks7</i> RT-PCR  |
| <i>pks7</i> -R              | CCGGTCGTTCTGCTTGGTG                                     | <i>pks7</i> RT-PCR  |
| <i>pks8</i> -F              | CGGTGGTCAACTCCCTCTTC                                    | <i>pks8</i> RT-PCR  |
| <i>pks8</i> -R              | TCGGTCGTCCCAGTCGTAG                                     | <i>pks8</i> RT-PCR  |
| <i>nrps1</i> -F             | CATCGACAGCCAGGTGAAGC                                    | <i>nrps1</i> RT-PCR   |
| <i>nrps1</i> -R             | GTAGGCGGGCAGGTAGGAG                                     | <i>nrps1</i> RT-PCR   |
| <i>nrps2</i> -F             | CGGTGAGTGCATCGACATCC                                    | <i>nrps2</i> RT-PCR   |
| <i>nrps2</i> -R             | CCGTAGGCGTCCACGAGAAT                                    | <i>nrps2</i> RT-PCR   |
| <i>nrps3</i> -F             | GGACACCTACGGGCTCACC                                     | <i>nrps3</i> RT-PCR   |
| <i>nrps3</i> -R             | CACAGCACCAGACGCAAGG                                     | <i>nrps3</i> RT-PCR   |

## SUPPLEMENTAL DATA

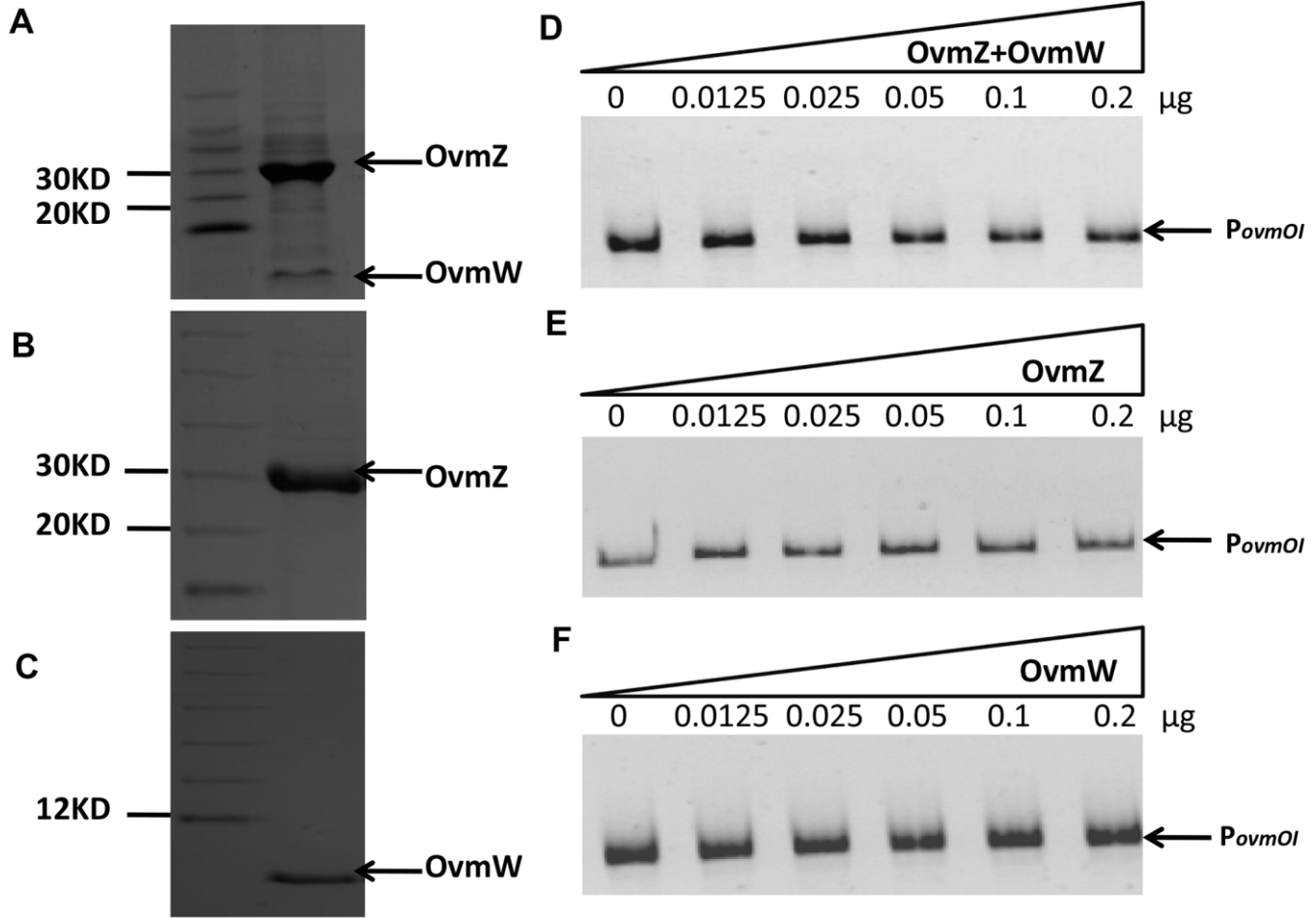
|                     |                       |                         |
|---------------------|-----------------------|-------------------------|
| <i>nrps4</i> -F     | CTACCACGCCGAGACCACC   | <i>nrps4</i> RT-PCR     |
| <i>nrps4</i> -R     | CTGCTGAAGTCCCGCCAAG   | <i>nrps4</i> RT-PCR     |
| <i>nrps5</i> -F     | CGGTGGTGAACGCCGAGTA   | <i>nrps5</i> RT-PCR     |
| <i>nrps5</i> -R     | CCGAACAGGGTCAGCAGGA   | <i>nrps5</i> RT-PCR     |
| <i>pks-nrps1</i> -F | GGGGCCTGATCGGCTTCTT   | <i>pks-nrps1</i> RT-PCR |
| <i>pks-nrps1</i> -R | GCACCTCGGCGTGTTCAT    | <i>pks-nrps1</i> RT-PCR |
| <i>pks-nrps2</i> -F | CGGGAGAAGGTCGGCTACA   | <i>pks-nrps2</i> RT-PCR |
| <i>pks-nrps2</i> -R | GGGTTGGGACGGGAGAAGT   | <i>pks-nrps2</i> RT-PCR |
| <i>sanO</i> -F      | CGGCGCTGGAGGAACGTAC   | <i>sanO</i> RT-PCR      |
| <i>sanO</i> -R      | GGGTGTAGAGGCCGATGCT   | <i>sanO</i> RT-PCR      |
| qRT-ZF              | CGCGGAGCTTTACGAAGAAT  | <i>ovmZ</i> Q-PCR       |
| qRT-ZR              | CTGCGTTTTCCGTCCGTATC  | <i>ovmZ</i> Q-PCR       |
| qRT-WF              | TTCGTGCCACGGAACT      | <i>ovmW</i> Q-PCR       |
| qRT-WR              | GATGAGTTCGTCCAGGTCGT  | <i>ovmW</i> Q-PCR       |
| qRT-RF              | CAGCAGGCGTTCCTCAA     | <i>ovmR</i> Q-PCR       |
| qRT-RR              | GAAGACGCCCTCGTAGATG   | <i>ovmR</i> Q-PCR       |
| qRT-orf3F           | ATGCGAACGACGGAGGAG    | <i>orf3</i> Q-PCR       |
| qRT-orf3R           | ACGATCTGCAGCCGTACC    | <i>orf3</i> Q-PCR       |
| qRT-orf4F           | AACTCTGCCAGCGGATTC    | <i>orf4</i> Q-PCR       |
| qRT-orf4R           | AGCAGCTCGATCTCCCA     | <i>orf4</i> Q-PCR       |
| qRT-OIF             | GCATCTCGCTGCCGCTGA    | <i>ovmOI</i> Q-PCR      |
| qRT-OIR             | CGTCAACCTGGGCTGGAAGC  | <i>ovmOI</i> Q-PCR      |
| qRT-PF              | AGGGCTCCGCCTTCTTCGT   | <i>ovmP</i> Q-PCR       |
| qRT-PR              | TCATGTGGTACGCGTTGGA   | <i>ovmP</i> Q-PCR       |
| qRT-SF              | GGCCACTCAGGAATTCACC   | <i>ovmS</i> Q-PCR       |
| qRT-SR              | AACTCGGAGTCGAGGAAGT   | <i>ovmS</i> Q-PCR       |
| qRT-TF              | GGACGTGCTGGTGAACAA    | <i>ovmT</i> Q-PCR       |
| qRT-TR              | CTGTTGAGGTTGGTGTTCGAT | <i>ovmT</i> Q-PCR       |



**Supplementary Figure 1. NMR analysis of the new bioactive compound. A,  $^1\text{H}$  NMR. B,  $^{13}\text{C}$  NMR.**



**Supplementary Figure 2. MS/MS analysis of oviedomycin generated by heterologous expression in *S. coelicolor* M1146.**



**Supplementary Figure 3. EMSAs to determine the potential targets of OvmZW.** A-C, SDS-PAGE of purified OvmZ, OvmW and OvmZW. Samples were separated by 12% SDS-PAGE and stained with Coomassie brilliant blue R-250. D-F, EMSAs of OvmZ, OvmW and OvmZW with the probe  $P_{ovmOI}$  containing the promoter of *ovmOI*. Each lane contains 20 ng DNA probes.



A

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                *           20           *           40           *           60           *           80           *           100
San : --MAGQPRPNDGSDKQCFQFKKRGNGMRQALPLS---ETSEEQPSADALIPTRILAELEECGRMIAHTPYSS--LRQVTVGSFAT-GISTNEKAMAIKTE
Sal : -----VQKCGSVPHGTRSRRT-AAP--GLRLCTS-GYHDLKANLLELPEIYDDCESVDHPRRNP--TLQRVSGSRQSTGIRIDEAATARGG
Sfr : -----METQSLCPAADCPGSGHPARARPCPAGT--GPRICAA--CRDRRLRDLGLPRLMEDGGERIAPSRPA--VAERISGGRSRQGIRIDEAATLRARD
Sfu : -----MHKHPELSAGHIGRDCAALC---GRICRM--CVERLRGELTLLGLYRESDHAIPTGPAR--MRERVSGSR-TVGMVVDERTVEMRTE
Ssp-A : -----MEPERLTDHVSRDCCRRC---GRICVR--CLDRIDGGRLTLLDLYRESDQIVVGPAPV--LRERVSGSRATVGVVDERTLALRAR
Ssp-C : MPSCETATDRGISMDERERATEEHTSRDCAQRC---GRICAL--CLDRTGRELRTLLTLKKESETIA--AAPTTALRQVSGSRGSGVGVVDERTVSLRSG
Ssp-D : -----MHQYSELSVGHVGRDCATLC---GSVCRQ--CVERLRGELRNLDDLYRESDHAIAPAHAR--LRQVSGSR-AVGVVDERTVAMRTE
Aha : -----MLTTETIGPHAG--GSLPEPCALPACHRRFPVDEWRICQT--GAAEVSATVRSIAEDIDECADMLDRRPGW--LSERVSGSR-RIGISSETAMTVRGG
Ksp-H : -----MEFHSCP RNH--GREVPAAA---GSVLCGP--CIRQCEHTLRALPEI--HRECLHFIQ--TEPRRRNPTRVSGSRRRDRNPS--AIDARNN
Ncr : -----MSEALSEALCENPGCGGA---AT--DGAFVCAEDLSRFCEHTLSRPLVHRCGERTRGLPRAH--GGRRE--GVHADVPEARAE
Nvu : -----MSEALCENPTCRSAVAIETLRSTPPADDVVRVCPDLSRFFETLRQLPVLHARQGETLQGERNRRMAPAR--GGLRQ--GVHDKDALVEARTE

                *           120           *           140           *           160           *           180           *           200
San : TQNTLGSWRLVVDRCGTAG--PSQEGPPLVSTLIRHSGWLAHPHAGDFABEIAALARSARRIAGGSAHR--VDLGRQLRSGCAGLRAVVHDGD--D
Sal : IIGFLASWRLVADBRAVRK--PVRRQPEEMADFTVAHLDWLLAHATAADFABEISEITHRRARRSAYTQFALR--MDLCCOIHSGCNAAMSTTPS--ARDGR
Sfr : MLRVLASWRLVAVABHGPGAG--PDRREHATAAPFVRRIDELVAHPHAGCEGAEVRRVDDAARSVAER--REVVR--RELGFQVACCGEPEVRAEAD--ADAPV
Sfu : TADVLASWRLVVEBRTKGLPGCD--VESLVAFTDRDEVENIACHHPAASFDEVRRLQLRLGALFAVRG--MPLGACVEPECTGTLAVVGGGGSA
Ssp-A : AAEMYALWRLVVDERAGAR--PADRGVPAVVGFTRGQLSFACHHPGALDDEIAELTDDFGHLIGGCVVRR--FALGACTRPPCACTLVGV--LPADGGD
Ssp-C : VTETLSWRLVLDERGDAAFGAKERGLARLVRFVGGQLEVFADHHPAGVEFTBEVTELLADLDLDFGSGVSR--FPLGFQPRPCEGTLFVGV--RPADR--D
Ssp-D : TAEVLASWRLVVEBRGARGLRDCC--VESLVEPFCGQLEWLAGHPAVSFDLVEVRGLLRIGSALGAFASG--RPLGACVEPECTGTLAVVREAGEVS
Aha : IVALLASWRLVVAEAGVPS--AGSADVTMAYVDEHLWLAHPHVVDDFADEITTLADARRVLDTEVTKV--VHLGVCAEPICDRTVAATVY--AAPGE
Ksp-H : IVALLASWRFVAERLGTTA---VIRSVPHTRFTDRHLDNLTQCPAADEFAEITEDLRLELLNAID--EAGDRAVPTACVVDGCPGTIDASPQHNGGR
Ncr : IMALVLSWRLVVDARPPRRP--RRTVPIVVEPFLAQQLWLAHPHIVDAIBEFVAVHGLAQGAVEGCF--DR--IEVGRCDRHGCGQFVVAHLGGSGATS
Nvu : VLALASWRLVVDARPPRRP--RRDSTLVDFLQYDGLAHPHIVDAMEBFAEVHALAQQVIDEVE--NQ--IEVGLCDRPGCHQKVAQLATAGPGD

                *           220           *           240           *           260
San : ATPGVSCDAGHALPQOWLLVAHRMRTASRGERVTRRHGDGRAGVQADRAGVREEAAR
Sal : RTRE-VSCTAGHSWQHHOWLLSRQIQQTRQFARGTAGAAAQSERPEDAA-----
Sfr : TAHT-VRCGAGHSVPEHOWLLDRRAA-----
Sfu : AAPGVSCDAGHPLPERQWLLVAGQLARWSE-----
Ssp-A : RVPDQVACDAGHALPERQWLLVANRAREAVTT-----
Ssp-C : RVPSSHVSCDAGHPVPERQWLLLAGRTEKERA-----
Ssp-D : PGPDRVACDAGHPLPERQWLLVAGRLARGSV-----
Aha : KPRRPVSCSAGHPLPERQWLLSRWLRTAADESLGAAA-----
Ksp-H : TS---VCCSAGHSWQIHOWLLNQRVGHRRRMSQRAESVA-----
Ncr : RL---VSCAAGHSWQHHOWLLNQRVGHRRRMSQRAESVA-----
Nvu : SL---VRCSSAGHSWQHHOWLLQRRVSRPQRHAVRRGEAVA-----
    
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B

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                *           20           *           40           *           60           *           80           *
San : -----MSRTPRR--RVEVTELAALAAGVSEATIRKWSRGKITRNGSPRRQYDIDELIA--VADRSEALG-----
Sal : -----MNPPRRNRSEIVPTQLAALAAGVSEATIRKWSRGKITRNGSPGRAQYDIDELLERVAAKTESAAVKE-----
Sfr : -----MAAAPRPRTIETRLAAVAAAGVSEATIRKWSRGKITRNGRGRAEYDIDELI--LARRAGAG-----
Sfu : -----VVGVTIRRRRTVSTELAALAMGVSEATIRKWSRGKITRNGRKRAEYDIDELIT--RERGAPLSAGGQQAPGL-----
Ssp-A : MTGTGDGGGTVPAAAKPRTVPTELAALAMGVQATIRKWSRGKITRNGGPRRAEYDIDELFA--VRRGETE-----
Ssp-C : ----VSGFAERTGGTARRRTVPTELAALAMGVQATIRKWSRGKITRNGGPRRAEYDIDELI--TRRDARAPKGPSPA-----
Ssp-D : -----VTTSRRTVSTELAALAMGVSEATIRKWSRGKITRNGRKRAEYDIDELI--TEGGAPLPVGRSGAVELAKGSGAGRGALDRDC-----
Aha : -----MTGARRRRTVPTCLAALAMGVSEATIRKWSRGKITRNGKRRAEYDIDELFE--AESVQLT-----
Ksp-H : -----VPTNVAALAAAGVSEATIRKWSRGKITRNGTGRRAEYDIDELI--TEALRRS-----
Ncr : --MTTPPLGRRRTPPPGRRTVPTELAALAAVRRKETIRKWSRGKITRNGTGRRAEYDIDELI--RGAPDPSIQ-----
Nvu : -----MSMRLADRRTVPTELAALAAGVKCEATIRKWSRGKITRNGRGRAEYDIDELIFV--LGDPEPVR-----
    
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**Supplementary Figure 4. Alignment of OvmZ and OvmW-like regulators from different strains.** A, Alignment of OvmZ-like regulators from different strains. B, Alignment of OvmW-like regulators from different strains. San: *Streptomyces ansochromogenes*, Sal: *Streptomyces alni*, Sfr: *Streptomyces fradiae*, Sfu: *Streptomyces fulvissimus*, Ssp-A: *Streptomyces* sp. Ag109\_05-10, Ssp-C: *Streptomyces* sp. CCM\_MD2014, Ssp-D: *Streptomyces* sp. DvalAA-19, Aha: *Amycolatopsis halophila*, Ksp-H: *Kitasatospora* sp. HKI 714, Ncr: *Nocardia crassostreae*, Nvu: *Nocardia vulneris*.

**Supplementary Method**

**Bioassays for anticancer activity:** Human lung carcinoma cell line A549, human hepatocellular carcinoma cell line HepG2 and human breast adenocarcinoma cell line MCF-7 were purchased from the Cell Bank of Chinese Academy of Sciences, Shanghai, China, and were cultured and propagated according to the procedures provided by the supplier. Confluent cells grown in 75 cm<sup>2</sup> flasks were collected and seeded in a 96-well plate and cultured for 24 h at 37 °C in a humid incubator containing 5% CO<sub>2</sub>. Then the cells were exposed to different concentrations of oviedomycin for 72 h. 20 microliters of 5 mg/ml thiazolyl blue tetrazolium bromide (MTT) in phosphate buffered saline was added into each well followed by 4 h incubation. The supernatant was removed and 150 µl dimethyl sulfate was added to dissolve the reduced formazan, and the absorption at 570 nm was recorded on a microplate reader. The assays were performed in four replicates for IC<sub>50</sub> calculation based on the fraction of viable cells.