ADDITIONAL FILE 1: Supplemental Information

belonging to the manuscript

A novel locus for mycelial aggregation forms a gateway to improved *Streptomyces* cell factories

by

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Running title: Morphological engineering of *Streptomyces*
Figure S1. Phenotypes of disruption mutants of *S. lividans* in submerged cultures.

SLI_2849, Sli_5273, Sli_6469, Sli_3391, Sli_6143, Sli_6089 and Sli_6232 were disrupted by a transposon insertion. The region of Sli_3306a-Sli_3306 was removed by homologous recombination replacement. J1681 (*S. coelicolor* Δ*bldA*) was published previously [1].

Cultures were grown in baffled shake flasks in TSBS for 48 h. Scale bar, 200 μm.
Figure S2: Identification of suppressor mutations in *S. lividans* PM01 and PM02. SLI_3391 and SLI_6143 were identified by SNP analysis as the major changes during evolution of PM02 from PM01. Note that mutation of SLI_3391 enhanced dispersed growth of PM01, giving a phenotype similar to that observed for PM02. Scale bar, 100 µm.
### Table S1. Bacterial strains.

| Strain        | Description and genotype | Reference |
|---------------|--------------------------|-----------|
| *Streptomyces lividans 66 (1326)* | SLP2+ SLP3+ | [2]       |
| PM01          | Evolved from *S. lividans* 66 | [3]       |
| PM02          | Evolved from PM01          | [4]       |
| J1681         | J1501 \( \Delta \)bldA    | [1]       |
| GAD01         | *S. lividans* 66 \( \Delta \)SLI\_3306a::aacC4 Apr\( ^R \) | This study |
| GAD02         | *S. lividans* 66 \( \Delta \)SLI\_3306a\( ^\text{clean} \) | This study |
| GAD03         | *S. coelicolor* M145 \( \Delta \)SCO2962::aacC4 Apr\( ^R \) | This study |
| GAD04         | *S. lividans* 66 \( \Delta \)SLI\_3306a-$\text{Sli\_3306}$::aacC4 Apr\( ^R \) | This study |
| GAD05         | *S. lividans* 66 \( \Delta \)SLI\_3306a-SLI\_3306\( ^\text{clean} \) | This study |

IFD, in-frame deletion mutant; Apr\( ^R \) apramycin resistant.

### Table S2. Transposon-mediated gene-replacement cosmids.

Cosmid nomenclature refers to the *Streptomyces coelicolor* genome database (strepdb.streptomyces.org.uk). The genomic location of the insertion of the apramycin cassette is given for the *S. coelicolor* genome.

| Cosmid name | target gene | Cosmids location in genome | Start gene | position relative to start |
|-------------|-------------|----------------------------|------------|----------------------------|
| SCI7.2.C04  | SCO1907     | 2043368                    | 2044163    | 795                        |
| C121.1.E05  | SCO2513     | 2709849                    | 2709485    | 364                        |
| E34.2.E04   | SCO3043     | 3331409                    | 3331178    | 231                        |
| 2SCK36.1.F01| SCO4998     | 5437068                    | 5437222    | 154                        |
| SC5B8.1.F05 | SCO5821     | 6369817                    | 6369367    | 450                        |
| SC2E9.1.F02 | SCO5871     | 6426513                    | 6426319    | 194                        |
| 7H1.2.H01   | SCO5952     | 6521053                    | 6520547    | 506                        |
| SC9B1.2.C03 | SCO6076     | 6670727                    | 6670057    | 670                        |
Table S3. Plasmids and constructs.

| Plasmid or construct | Description | Reference |
|----------------------|-------------|-----------|
| pWHM3                | Cloning vector, colE1 replicon, pSG5 replicon, Thio<sup>R</sup>, Amp<sup>R</sup> | [5] |
| pSET152              | Complementation vector, oriT RK2, pUC18 replicon, Apra<sup>R</sup> | [6] |
| pUWLcre              | pUWLoriT derivative with creA gene under ermE* promoter, Thio<sup>R</sup> | [7] |
| pMAT1                | pWHM3 containing flanking regions of S. coelicolor SCO2963 and SCO2962 with a aac(3)IV-loxP Xbal inserted between them in pWHM3 EcoRI-HindIII | this work |
| pMAT2                | pWHM3 containing flanking regions of S. coelicolor SCO2963 with a aac(3)IV-loxP Xbal inserted between them in pWHM3 EcoRI-HindIII | this work |
| pMAT3                | Cosmid StE59 derivative in which the matB coding sequence was replaced by the aac(3)IV resistance cassette | this work |
| pMAT4                | pSET152 containing SCO2963 with the 500bp upstream (promoter) region | this work |

Table S4. Oligonucleotides.

| Name                  | Primer Sequence ▲ |
|-----------------------|------------------|
| matB_+2190            | AGTCCTCTAGAAGCCGGTCGGATGACCACC |
| matB_+3610            | AGTCAGCTTCCTTGTTGTAACCCTGGCAACCCG |
| matA_+1326            | AGTGAATTCAAGCCGGTGAGAGCACCCCTGGATG |
| matA_+43              | AGCTTCAGAGAGCACCCCTGGATG |
| matA_2809             | AGCTAACGGTGAGATCAGAGGGTGTTCTCCGGGGATCCGTCGACC |
| matA_+1466            | AGCTGCTAGACCCGGAGAACCCCTGGATG |
| matA_-54              | TTCTTGGCCGAGACGGGTGATG |
| matB_+1528            | TTCTTGGCCGAGACGGGTGATG |
| pmatA_-537            | AGCTGAATTCAAGCCGGTGAGAGCACCCCTGGATG |
| pmatA_+1485           | ATCTTCAGAGAGCACCCCTGGATG |
| matB_FW_REDIRECT      | CCGGGGTGCGCCGGTGGCAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGAGCGA ▲Restriction sites are underlined. TCTAGA, XbaI; AAGCTT, HindIII; GAATTC, EcoRI.
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