

1        **Becn1 Deficiency Unlocks Cardiac Lineage Commitment**  
2        **through Convergent Wnt and BMP Signaling Activation**

3        Lilin Xiang<sup>1,2,3,4</sup>, Zhenhua Jia<sup>1,2,4</sup>, Zhangyi Yu<sup>1,2</sup>, Lenan Wang<sup>1,2</sup>, Huanhuan Cai<sup>1,2,3</sup>,  
4        Tong Zhang<sup>1,2,3</sup>, Zhibing Lu<sup>1,2,3,\*</sup>, Li Wang<sup>1,2,\*</sup>

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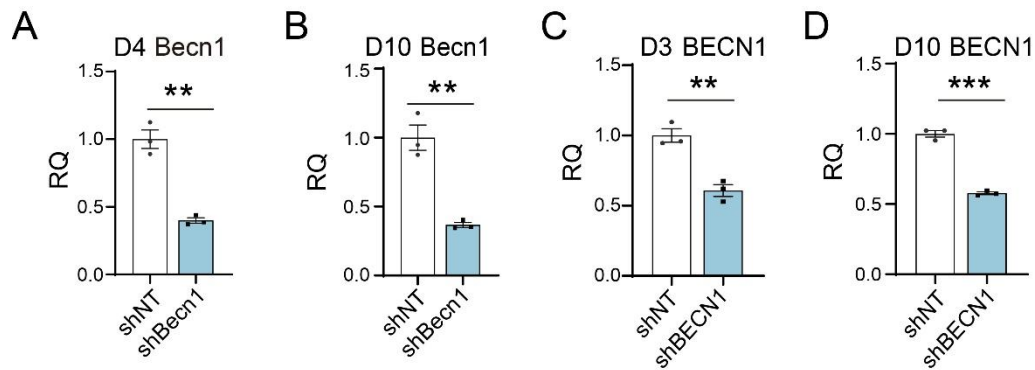
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## Supplementary Figure and Figure Legend

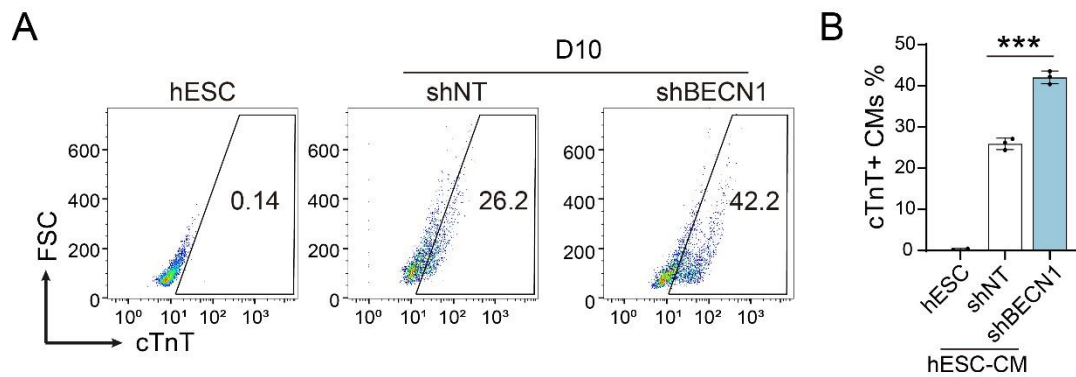


**Figure S1. *Becn1* knockdown effectively continues as cardiomyocytes develop.**

(A-B) RT-qPCR quantification showing *Becn1* mRNA expression at differentiation day 4 (A) and day 10 (B) in cells transduced with lentiviral sh*Becn1* or shNT in cells from mESCs.

(C-D). RT-qPCR quantification showing *BECN1* mRNA expression at differentiation day 3 (C) and day 10 (D) in cells transduced with lentiviral sh*BECN1* or shNT in cells from hESCs.

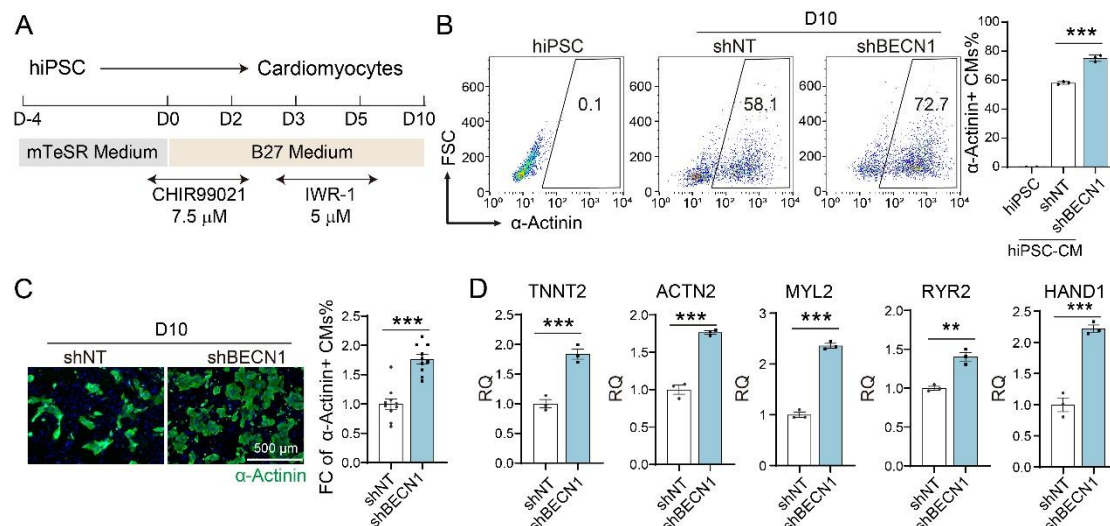
Groups were compared using two-tailed unpaired *t* test. Error bars indicate means  $\pm$  SEM; \**P* < 0.05, \*\**P* < 0.01, and \*\*\**P* < 0.001.



**Figure S2. *BECN1* knockdown promotes human cardiomyocyte differentiation.**

(A-B) Representative flow cytometry plots (A) and quantification (B) of cTnT<sup>+</sup> cells at day 10 of differentiation from hESCs in sh*BECN1* and shNT groups.

Groups were compared using one-way ANOVA with Tukey's multiple comparisons test. Error bars indicate means  $\pm$  SEM; \**P* < 0.05, \*\**P* < 0.01, and \*\*\**P* < 0.001.



**Figure S3. *BECN1* deficiency promotes cardiomyocyte differentiation from human induced pluripotent stem cells (hiPSCs).**

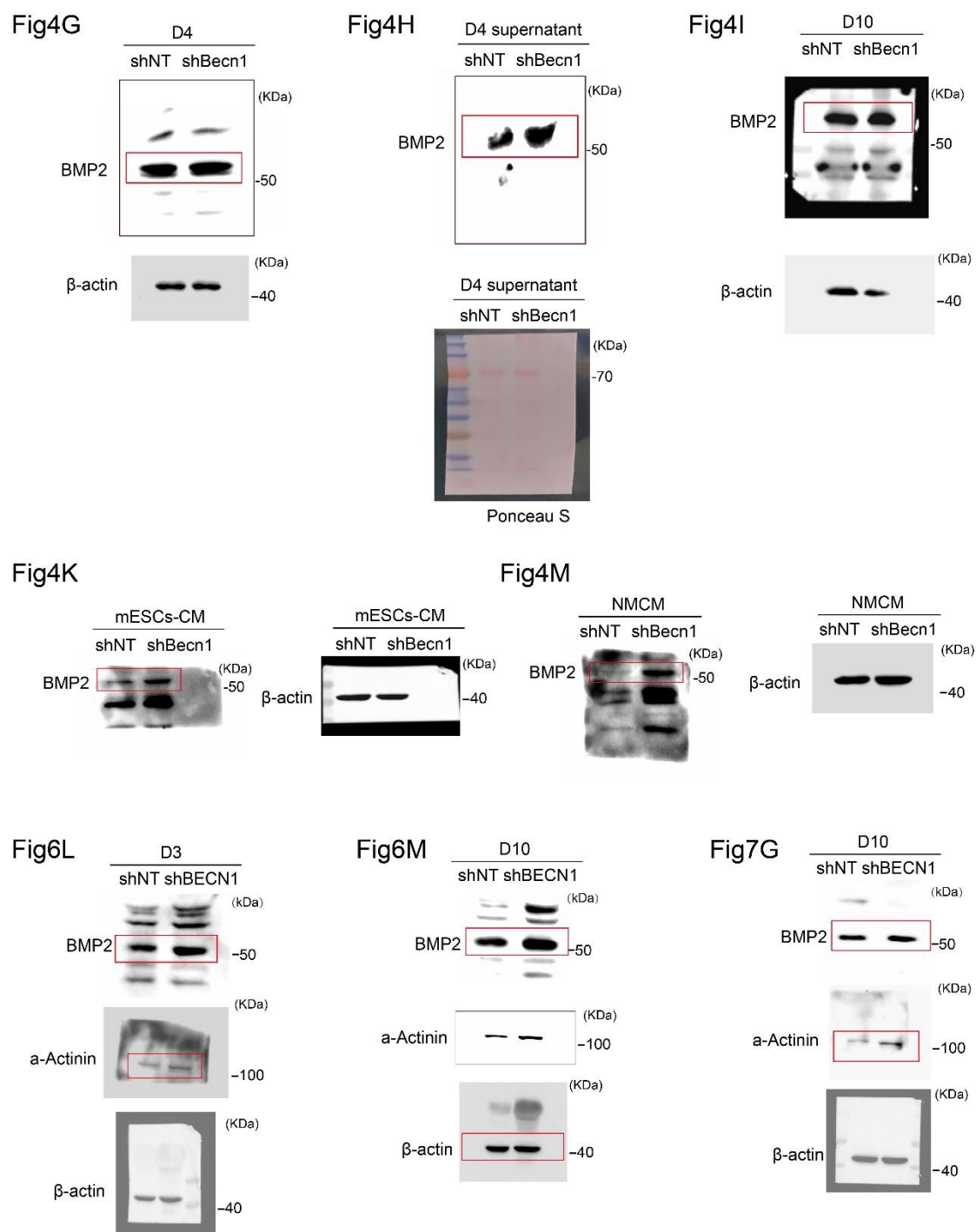
**(A).** Schematic diagram illustrating the protocol to differentiate human induced pluripotent stem cells (hiPSCs) into CMs.

**(B).** Representative flow cytometry plots and quantification of  $\alpha$ -Actinin<sup>+</sup> cells at day 10 of differentiation in shBECN1 and shNT groups.

**(C).** Representative ICC and quantification of  $\alpha$ -Actinin<sup>+</sup> CMs (green) at day 10 of differentiation in shBECN1 or shNT control group. Scale bars, 500  $\mu$ m.

**(D).** RT-qPCR detecting the cardiomyocyte specific marker genes expression at day 10 of differentiation in shBECN1 or shNT control group.

Groups were compared using two-tailed unpaired *t* test or one-way ANOVA with Tukey's multiple comparisons test for multiple groups. Error bars indicate means  $\pm$  SEM; \**P* < 0.05, \*\**P* < 0.01, and \*\*\**P* < 0.001.



**Figure S4. Full-length blots.**

76 **Supplemental Tables**

77 **Table S1| List of reagents and resources used in this study.**

| Reagent and Resource                                                | Source                   | Identifier  |
|---------------------------------------------------------------------|--------------------------|-------------|
| <b>Antibodies</b>                                                   |                          |             |
| Anti-Sarcomeric $\alpha$ -actinin <sup>+</sup> antibody             | Abcam                    | ab68167     |
| Anti-Cardiac Troponin I                                             | Abcam                    | ab56357     |
| Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L)            | Jackson ImmunoResearch   | 711-545-152 |
| Alexa Fluor® 488 AffiniPure™ Donkey Anti-Goat IgG (H+L)             | Jackson ImmunoResearch   | 705-545-147 |
| BMP2 Antibody                                                       | Abcepta                  | AP21447a    |
| beta Actin Antibody                                                 | Santa Cruz Biotechnology | sc-47778    |
| Cy™3 AffiniPure Donkey Anti-Rabbit IgG (H+L)                        | Jackson ImmunoResearch   | 711-165-152 |
| Cardiac Troponin T Antibody, anti-human/mouse/rat, FITC, REAfinity™ | miltenyi                 | 130-119-575 |
| <b>Chemicals, Peptides, and Recombinant proteins</b>                |                          |             |
| GMEM                                                                | Gibco                    | 11710035    |
| IMDM                                                                | Gibco                    | C12440500BT |
| Ham's F12                                                           | Cellgro                  | 10-080-CV   |
| GLUTAMINE 100X                                                      | Gibco                    | 25030081    |
| NEAA                                                                | Gibco                    | 11140050    |
| CHIR99021                                                           | GLPBIO                   | GC16702     |
| PD0325901                                                           | GLPBIO                   | GC10397     |
| Lif                                                                 | Sigma                    | ESG1106     |
| StemPro-34 SFM                                                      | Solarbio                 | A8100       |
| B27                                                                 | BasalMedia               | S441J7      |
| N2 Supplement                                                       | Gibco                    | 17502048    |
| 2-Mercaptoethanol                                                   | Gibco                    | 21985023    |
| Ascorbic acid                                                       | R&D                      | 293-VE      |
| Activin A                                                           | R&D                      | 338-AC      |
| VEGF                                                                | R&D                      | 233-FB      |
| bFGF                                                                | R&D                      | 345-FG      |
| FGF10                                                               | Gibco                    | 25030081    |
| Rhod-3 AM                                                           | Thermo                   | R10145      |
| IWR-1                                                               | MCE                      | HY-12238    |
| mTeSR1                                                              | STEMCELL Technologies    | 85851       |
| B-27 without insulin                                                | Gibco                    | A1895601    |
| CHIR99021                                                           | MCE                      | HY-10182    |

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79 **Table S2| Sequence information for shRNA oligos used in this study.**

| Symbol      | Clone ID       | Oligo sequence         |
|-------------|----------------|------------------------|
| m-shBecn1#1 | TRCN0000087288 | CCACATGTTTACAATACCAAA  |
| m-shBecn1#2 | TRCN0000087290 | GCGGGAGTATAGTGAGTTTAA  |
| h-shBECN1#1 | TRCN0000033549 | CCCGTGGAATGGAATGAGATT  |
| h-shBECN1#2 | TRCN0000033550 | CCGACTTGTTTCCTTACGGAAA |

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81 **Table S3| List of qPCR primers and cloning primers used in this study.**

| qPCR primers-mouse |                           |                         |
|--------------------|---------------------------|-------------------------|
| Genes              | Forword prime             | Reverse prime           |
| Becn1              | ATGGAGGGGTCTAAGGCGTC      | TCCTCTCCTGAGTTAGCCTCT   |
| Nanog              | TCTTCCTGGTCCCCACAGTTT     | GCAAGAATAGTTCTCGGGATGAA |
| Sox2               | GCGGAGTGGAACTTTTGTCC      | CGGGAAGCGTGTACTTATCCTT  |
| Klf4               | GTGCCCCGACTAACCGTTG       | GTCGTTGAACTCCTCGGTCT    |
| Zic3               | TGCTGCCAGTTCAGGCTATG      | GCAGAAGGGGTTTTAGTGGTATC |
| Oct4               | AGAGGATCACCTTGGGGTACA     | CGAAGCGACAGATGGTGGTC    |
| Sall4              | CCCTGGGAACTGCGATGAAG      | TCAGAGAGACTAAAGAACTCGGC |
| Sox17              | GATGCGGGATACGCCAGTG       | CCACCACCTCGCCTTTCAC     |
| Gata4              | CCCTACCCAGCCTACATGG       | ACATATCGAGATTGGGGTGTCT  |
| Sox1               | AAGGAACACCCGGATTACAAGT    | GTTAGCCCAGCCGTTGACAT    |
| Pax6               | TACCAGTGTCTACCAGCCAAT     | TGCACGAGTATGAGGAGGTCT   |
| Hand1              | GGCAGCTACGCACATCATCA      | CCTGGCATCGGGACCATAG     |
| Lefty2             | CAGCCAGAATTTTCGAGAGGT     | CAGTGCGATTGGAGCCATC     |
| Aplnr              | GGTTACAACACTACTATGGGGCTGA | AGCTGAGCGTCTCTTTTCGC    |
| Mesp1              | GTCACTCGGTCCTGGTTTAAAG    | ACGATGGGTCCCACGATTCT    |
| Nkx2.5             | GACAAAGCCGAGACGGATGG      | CTGTCGCTTGCACTTGTAGC    |
| Tbx6               | ATGTACCATCCACGAGAGTTGT    | GGTAGCGGTAACCCTCTGTC    |
| Tnni3              | TCTGCCAACTACCGAGCCTAT     | CTCTTCTGCCTCTCGTTCCAT   |
| Tnnt2              | CAGAGGAGGCCAACGTAGAAG     | CTCCATCGGGGATCTTGGGT    |
| Myl7               | GGCACAACGTGGCTCTTCTAA     | TGCAGATGATCCCATCCCTGT   |
| Ryr2               | ACGGCGACCATCCACAAAG       | AAAGTCTGTTGCCAAATCCTTCT |
| Tbx5               | ATGGCCGATACAGATGAGGG      | TTCGTGGAACCTCAGCCACAG   |

|           |                         |                         |
|-----------|-------------------------|-------------------------|
| Myh7      | ACTGTCAACACTAAGAGGGTCA  | TTGGATGATTTGATCTTCCAGGG |
| Wnt5b     | CTGCTGACTGACGCCAACT     | CCTGATACAACTGACACAGCTTT |
| Myc       | ATGCCCCCTCAACGTGAAC TTC | CGCAACATAGGATGGAGAGCA   |
| Etv2      | CTGGGAGCGGAATTTGGTTTC   | GTAAAGCGGGGTTCCAGTCC    |
| Fzd10     | CATGCCCAACCTGATGGGTC    | GCCACCTGAATTTGAACTGCTC  |
| Axin2     | TGACTCTCCTTCCAGATCCCA   | TGCCCACACTAGGCTGACA     |
| Lef1      | TGTTTATCCCATCACGGGTGG   | CATGGAAGTGTCGCCTGACAG   |
| Ppp3ca    | GTGAAAGCCGTTCCATTTCCA   | GAATCGAAGCACCTCTGTTATT  |
| Rspo3     | ATGCACTTGCGACTGATTTCT   | GCAGCCTTGACTGACATTAGGAT |
| Cer1      | CTCTGGGGAAGGCAGACCTAT   | CCACAAACAGATCCGGCTT     |
| Sfrp1     | CAACGTGGGCTACAAGAAGAT   | GGCCAGTAGAAGCCGAAGAAC   |
| Sfrp2     | CGTGGGCTCTTCTCTTCG      | ATGTTCTGGTACTCGATGCCG   |
| Sfrp4     | AGAAGGTCCATACAGTGGGAAG  | GTTACTGCGACTGGTGCGA     |
| Bmp2      | GGGACCCGCTGTCTTCTAGT    | TCAACTCAAATTCGCTGAGGAC  |
| Bmp4      | TTCCTGGTAACCGAATGCTGA   | CCTGAATCTCGGCGACTTTTT   |
| Bmpr1a    | AACAGCGATGAATGTCTTCGAG  | GTCTGGAGGCTGGATTATGGG   |
| Bmpr2     | TTGGGATAGGTGAGAGTCGAAT  | TGTTTCACAAGATTGATGTCCCC |
| Brachyury | TGCGCTTCAAGGAGCTAACTA   | CCGGTGGTTCTTAGAGCTG     |

#### qPCR primers-human

| Genes   | Forword prime            | Reverse prime           |
|---------|--------------------------|-------------------------|
| BECN1   | GGTGTCTCTCGCAGATTCATC    | TCAGTCTTCGGCTGAGGTTCT   |
| NANOG   | AAGGTCCCGGTCAAGAAACAG    | CTTCTGCGTCACACCATTGC    |
| OCT4    | CTGGGTTGATCCTCGGACCT     | CCATCGGAGTTGCTCTCCA     |
| KLF4    | CAGCTTCACCTATCCGATCCG    | GACTCCCTGCCATAGAGGAGG   |
| SOX2    | TGGACAGTTACGCGCACAT      | CGAGTAGGACATGCTGTAGGT   |
| ZIC3    | GGCGCTCAGTTTCCTAACTAC    | CTGCCGCATATAACGGAAGAA   |
| STAT3   | ACCAGCAGTATAGCCGCTTC     | GCCACAATCCGGGCAATCT     |
| TNNT2   | GGAGGAGTCCAAACCAAAGCC    | TCAAAGTCCACTCTCTCTCCATC |
| ACTN2   | ATGGCCTTGGA CTCTGTGC     | GGTGTTACGATGTCTTCAGC    |
| MYL2    | TTGGGCGAGTGAACGTGAAAA    | CCGAACGTAATCAGCCTTCAG   |
| HAND1   | GAGAGCATTAAACAGCGCATT CG | CGCAGAGTCTTGATCTTGGAGAG |
| RYR2    | CATCGAACACTCCTCTACGGA    | GGACACGCTAACTAAGATGAGGT |
| KCNJ2   | CTGGCTTTTCGTCTGT CATGG   | GCCCACGATTGACTGGAACA    |
| CACNA1C | TGATTCCAACGCCACCAATTC    | GAGGAGTCCATAGGCGATTACT  |

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|       |                        |                         |
|-------|------------------------|-------------------------|
| TBXT  | CTGGGTACTCCCAATGGGG    | GGTTGGAGAATTGTTCCGATGA  |
| EOMES | GTGCCCACGTCTACCTGTG    | CCTGCCCTGTTTCGTAATGAT   |
| HOXB1 | GAGCTTTGCACCGGCCTAT    | CTTCATCCAGTCGAAGGTCCG   |
| ISL1  | TACGGGATCAAATGCGCCAA   | CACACAGCGGAAACACTCGAT   |
| IRX4  | TCTACTGCCCCGGTCTACGAG  | GCAGATCCCGAACCATCCTTG   |
| TBX20 | TTCCAACCATCCGGGTGTC    | TCCATCAGGACTATGTACTTGGC |
| WNT3  | AGGGCACCTCCACCATTG     | GACACTAACACGCCGAAGTCA   |
| WNT3A | AGCTACCCGATCTGGTGTC    | CAAACCTCGATGTCCTCGCTAC  |
| WNT5A | GCCAGTATCAATTCCGACATCG | TCACCGCGTATGTGAAGGC     |
| ETV2  | CTGGAAAGGTACAAGCTCATCC | AACTTCTGGGTGCAGTAACGC   |
| BMP2  | TTCGGCCTGAAACAGAGACC   | CCTGAGTGCCTGCGATACAG    |
| BMP4  | AAAGTCGCCGAGATTGAGG    | GACGGCACTCTTGCTAGGC     |
| BMPR2 | CGGCTGCTTCGCAGAATCA    | TCTTGGGGATCTCCAATGTGAG  |
| SMAD9 | ATGTGATTACTGTCGCGTGT   | GGCGGTAGTGGTAAGGGTTAAT  |

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