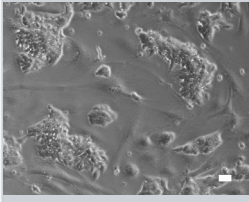
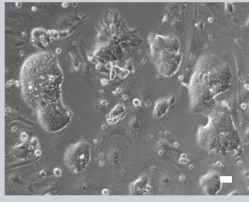
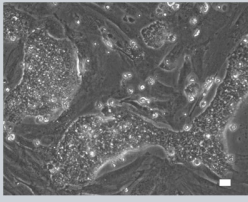
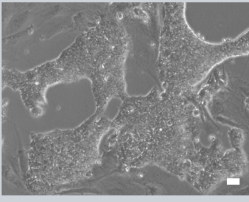
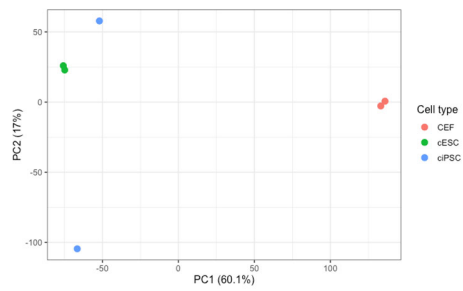


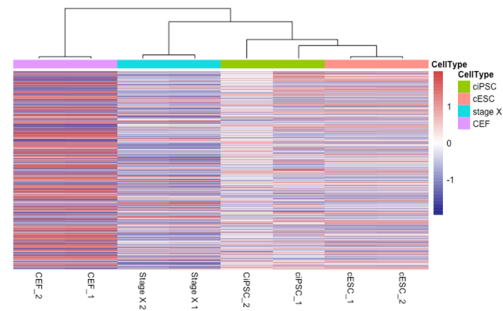
| Condition    | Condition A                                                                       | Condition B                                                                       | Condition C                                                                        | Condition D<br>(AC medium)                                                          |
|--------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Basal Medium | N2B27                                                                             | N2B27                                                                             | E4                                                                                 | E4                                                                                  |
| Go6983       | ✓                                                                                 | ✓                                                                                 | ✓                                                                                  | ✓                                                                                   |
| IWR-1        | ✓                                                                                 | ✓                                                                                 | ✓                                                                                  | ✓                                                                                   |
| SB431542     | ✓                                                                                 | ✓                                                                                 | ✓                                                                                  |                                                                                     |
| cLIF         | ✓                                                                                 | ✓                                                                                 | ✓                                                                                  | ✓                                                                                   |
| CP-673451    |                                                                                   | ✓                                                                                 | ✓                                                                                  | ✓                                                                                   |
| Morphology   |  |  |  |  |

**Supplementary Figure S1.** (A–D) Optimization the medium for chicken iPSCs reprogramming. Summary of culture conditions used during optimization of chicken pluripotent stem cell culture and reprogramming. And representative phase-contrast images of colonies under different culture conditions. Scale bars, 50  $\mu$ m.

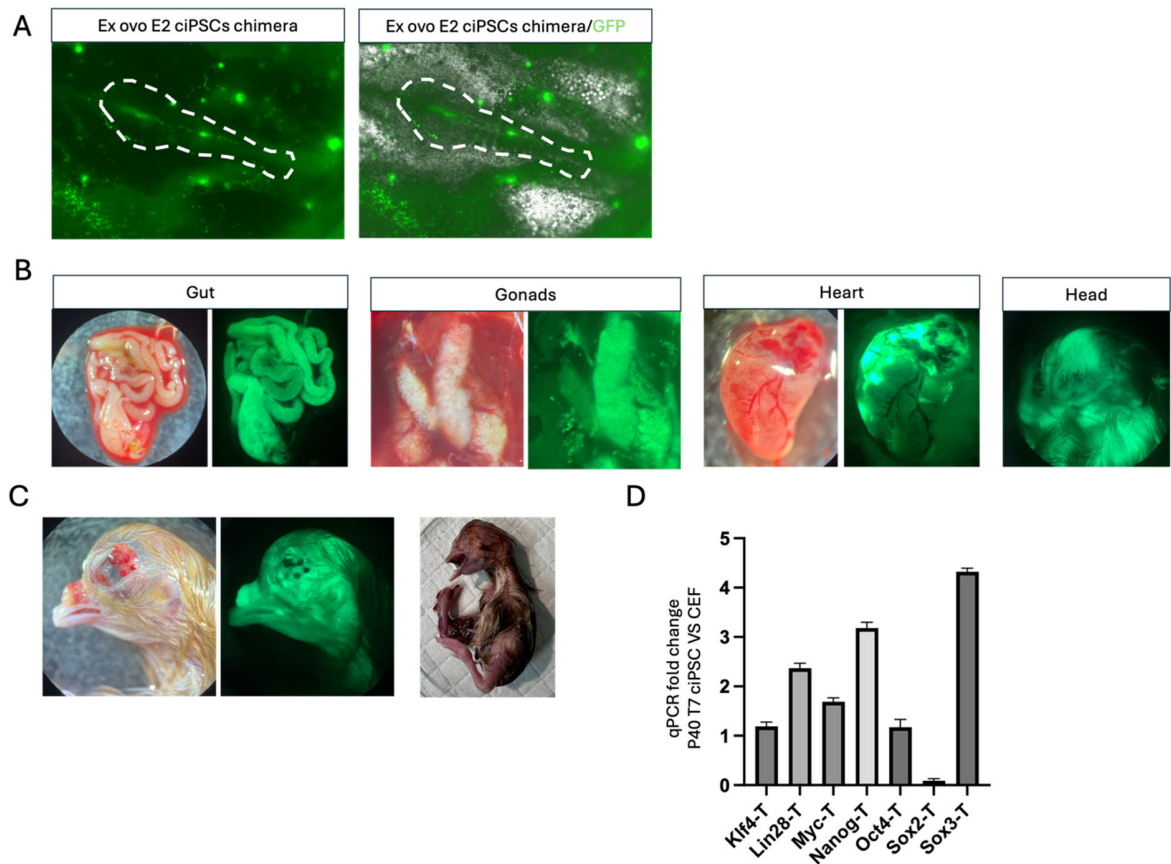
A



B



**Supplementary Figure S2.** Bulk RNA-seq analysis of T7 ciPSCs. (A) Principal component analysis (PCA) of RNA-seq data from CEFs, chicken ESCs, and chicken iPSCs; (B) Heatmap of the top 1,000 interquartile range (IQR) genes comparing EGK.X embryos, CEFs, chicken ESCs, and chicken iPSCs.



**Supplementary Figure S3.** T7 ciPSCs chimera contribution under ex ovo and in ovo. (A) Representative images of implantation of GFP-positive iPSCs onto ex ovo EGK.X chicken embryo; (B) Images showing isolation and plating of gonads from duck–chicken interspecies chimeras. White arrows indicate GFP-positive PGC-like cells; black arrows indicate GFP-negative PGC-like cells. Scale bar, 50  $\mu$ m; (C) Representative images of E19 chick dead with tumor on the head (left) and E20 chick dead with tumors on the leg and mouth; (D) qRT-PCR analysis of retroviral transgene expression in P40 chicken iPSCs. Data represent mean  $\pm$  s.d. of three biological replicates.

|                  | Forward                     | Reverse                    |
|------------------|-----------------------------|----------------------------|
| Chicken Primers  |                             |                            |
| RS17(House keep) | ACACCCGTCTGGGCAACGAC        | CCCGCTGGATGCGCTTCATC       |
| Oct4             | AGCACAGGAGAGGGGTTG          | CAACTACAGCAGGCTCAAAGG      |
| Nanog            | CTCCGCTGCCTCTTTTGC          | CTGGGCTACAAATAGGGTATTTCTTG |
| SOX3             | ACTGTGAACGATGTTTTGACATATCAG | GGAAGAAATCCGGTAAACAAAACAAA |
| Sox2             | CCCCTGTGGTTACCTCTTCCTCC     | TGCCGTTAATGGCCGTGCC        |
| KLF4             | ACTCTGGGTTCGCTCTTC          | CCCACTCTTACCCCGTACTC       |
| CMYC             | GATACTTTGGGCATAAGGGATGATG   | CTGGGGACAACCTCTATTTGGAATTC |
| Lin28B           | GACCATGCGAGCGAAATTGG        | CCTTTACTCGCCCCTGCTTC       |
| Nestin           | GCAGAGCCAGAGCGCACCAA        | CGTGTCACCCCGACTCGGAC       |
| Pax6             | AACTCCATCAGCTCCAATGG        | AGGGCTTCGATTTGCTCTTG       |
| BMP4             | TCCACCATGAAGAGCACCTG        | TGAGGTTGAAGACGAAGCGG       |
| TBXT             | AGGTCAAGCTCACCAACAAG        | AGGAATGGCTGGTGATCATC       |
| MyoD             | TCTCGCTCCCTCGAGAAAAA        | AGTGCGATTTCCCGAGACAG       |
| Gata4            | TTCGACAGCCCCATGCTGCAC       | AAATTGATGTTGGCATGCCGGG     |
| Oct4-T           | GACGTGGTGAGAGTGTGGTT        | CGTACATCACTCCCTCGCTC       |
| Sox3-T           | GGGCCAGAGGATTGACACTT        | ATGTCGTAGCGGTGCATCTG       |
| Klf4-T           | CACACATGCGATTACGCTGG        | GCGAATTTCCATCCGCATCC       |
| cMyc-T           | GCGCTGGACTCTATCTGCAT        | TGGGTATGGAAACACCACGG       |
| Nanog-T          | CACCCATCTCACCCCACTTTT       | ACTTCTGGCTCTGAAACCGC       |
| Sox2-T           | TGGTCAAGACGGAATCCAGC        | GATCATGTCCCGAAGGTCCC       |
| Lin28-T          | CTCAGGGAAAGCCTGCCTAC        | GGCGGCCGCTCATTCC           |
| Duck Primers     |                             |                            |
| GAPDH            | GAGGGTAGTGAAGGCTGCTG        | CATCAAAGGTGGAGGAATGG       |
| Nanog            | GGTTTCAGAACCAACGGATG        | GTGGGGGTCTATCCAGGTA        |
| Oct4             | TGGGCACTCTCTATGGGAAGA       | ATTGAGCCAACGCTGTAGCA       |
| Klf4             | GAGAACCCACACAGGTGAGAAA      | GGATCGGGCAAACCTCCATC       |
| Sox2             | CACAACCTCCGAGATCAGCAA       | TATAATCCGGGTGCTCCTTC       |
| Nestin           | GGCGACCTACAGACCCAC          | ACAGCCCAAAAATCCGGGGT       |
| Gata4            | GCACGCCAACATCGAATTTTT       | CATCTTGTGGTAGAGCCCGC       |
| MyoD             | AAG GCG TGC AAG AGG AAG AC  | TGG TTG GGG TTG GTG GA     |
| TBXT             | ACAACCTCGCCAACAGCCTAT       | GACTGGAGCCAGTAGCTGTG       |

**Supplementary Table S1.** Primer sequences in qRT-PCR.