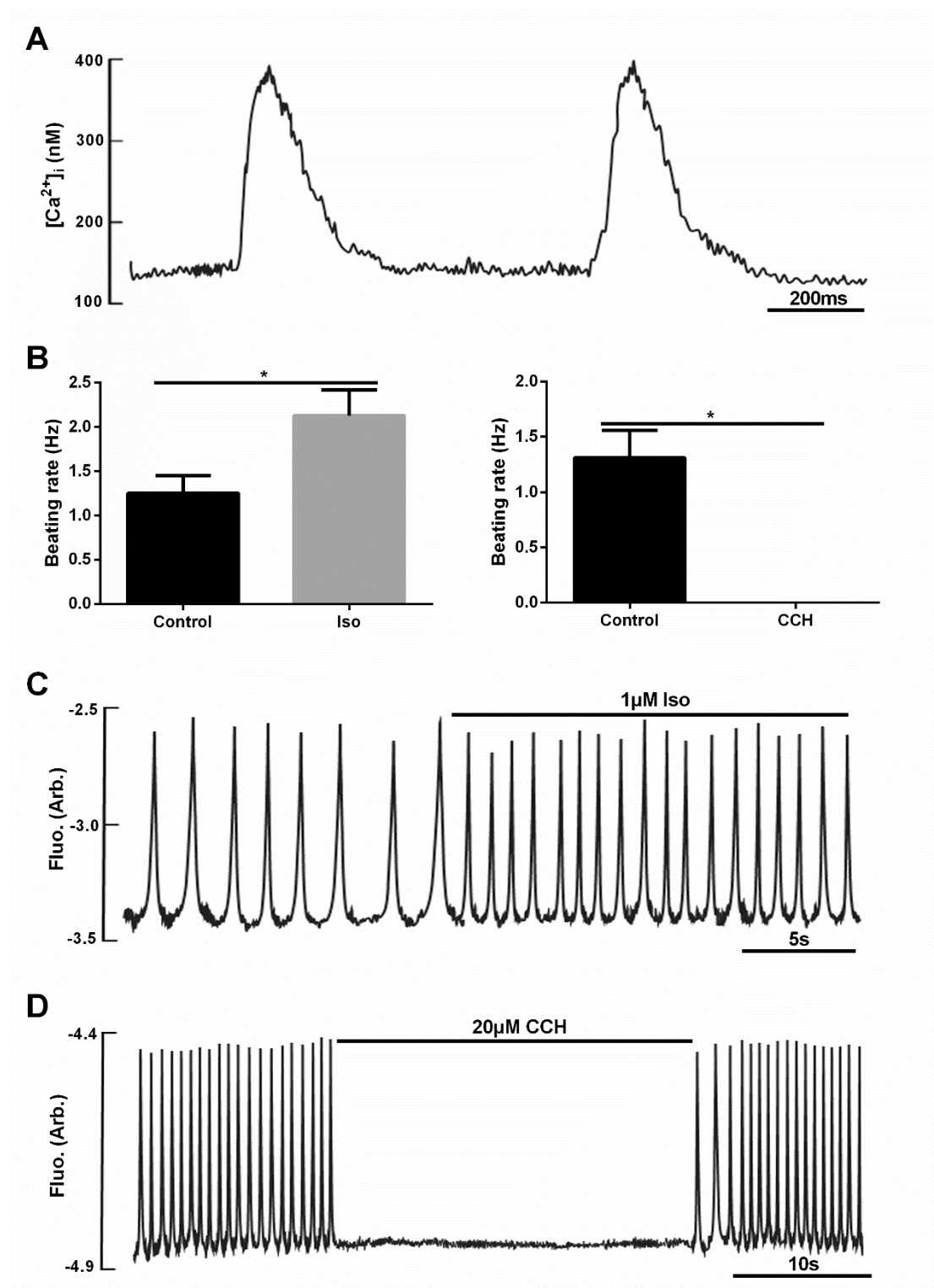**Supplementary figure 1 Contracting cardiomyocytes generated by EB methods exhibit typical calcium movement and sensitive response to both cardiomyocyte agonist and antagonist**



(A) Spontaneously contracting cardiomyocytes differentiated were recorded for the characteristic calcium transients ($[Ca^{2+}]_i$), and the representative curve was showed. (B) The effects of 1mM isoproterenol (Iso) (E and F) and 25mM carbachol (CCH) on the beating rates of enabled cardiomyocytes . (C, D) Calcium transients were compared before and after treatment 1mM Iso (C) and 25mM CCH) (G). Data are mean ± s.d. * P<0.05 compared with control.

**Supplementary table 1 Primers used for qRT-PCR analysis**

| KLF4 | SOX1 |
|---|---|
| Forward: 5'- GATGAACTGACCAGGCACTA-3'; | Forward: 5'-ATACCCCCAAAATGCATCAA-3'; |
| Reverse: 5'- GTGGGTCATATCCACTGTCT-3'. | Reverse: 5'-GGAAACGGGCTTTTCTCTCT-3'. |
| **4-Oct** | **PAX6** |
| Forward: 5'-TCAGTGATGCTGTTGATCAGG-3' | Forward: 5'-GTTCCCTGTCCTGTGGACTC-3'; |
| Reverse: 5'-GCTATCTACTGTGTGTCCCAGTC-3' | Reverse: 5'-ACCGCCCTTGGTTAAAGTCT-3'. |
| **Nanog** | **Notch1** |
| Forward: 5'-AAACCAGTGGTTGAAGACTAGCAA-3'; | Forward: 5'-TGCCAGACCAACATCAAC-3'; |
| Reverse: 5'-GGTGCTGAGCCCTTCTGAATC-3'. | Reverse: 5'-CTCATAGTCCTCGGATTGC-3'. |
| **Sox2** | **GAPDH** |
| Forward: 5'- CCGTTTTCGTGGTCTTGTTT-3'. | Forward: 5'-GGTGGTCTCCTCTGACTTCAACA-3'; |
| Reverse: 5'- TCAACCTGCATGGACATTTT-3'. | Reverse: 5'-GTGGTCGTTGAGGGCAATG-3'. |
| **FOXA2** | **NKX2.5** |
| Forward: 5'- GGCCCAGTCACGAACAAAGC-3'; | Forward: 5'-CAAGTGCTCTCCTGCTTTCC-3'; |
| Reverse: 5'-CCCAAAGTCTCCACTCAGCCTC-3'. | Reverse: 5'-GGCTTTGTCCAGCTCCACT-3'. |
| **SOX17** | **GATA4** |
| Forward: 5'-AAGAAACCCTAAACACAAACAGCG-3'; | Forward: 5'-TCTCACTATGGGCACAGCAG-3'; |
| Reverse: 5'-TTTGTGGGAAGTGGGATCAAGAC -3'. | Reverse: 5'-GCGATGTCTGAGTGACAGGA-3'. |
| **AFP** | **cTnT** |
| Forward: 5'-CCCGAACTTTCCAAGCCATA-3'; | Forward: 5'-CAGAGCGGAAAAGTGGGAAGA-3'; |
| Reverse: 5'-TACATGGGCCACATCCAGG-3'. | Reverse: 5'-TCGTTGATCCTGTTTCGGAGA-3'. |
| **MESP1** | **α-MHC** |
| Forward: 5'-TGTACGCAGAAACAGCATCC-3'; | Forward: 5'-CGCATCAAGGAGCTCACC-3'; |
| Reverse: 5'-TTGTCCCCTCCACTCTTCAG-3'. | Reverse: 5'-CCTGCAGCCGCATTAAGT-3'. |
| **Brachyury** | **β-MHC** |
| Forward: 5'-GCTTCAAGGAGCTAACTAACGAG-3'; | Forward: 5'-TATCGATGACCTGGAGCTGA-3'; |
| Reverse: 5'- CCAGCAAGAAAGAGTACATGGC-3'. | Reverse: 5'-AGTATTGACCTTGTCTTCCTC-3'. |
| **Mixl1** | **SRF** |
| Forward: 5'-GGAGCTCGTCTTCCGACAGA-3'; | Forward: 5'-AGTGCAGGCCATTCAAGT-3'; |
| Reverse: 5'-TTGAGGATAAGGGCTGAAATGAC-3'. | Reverse: 5'-ACGGATGACGTCATGATGGTG-3'. |