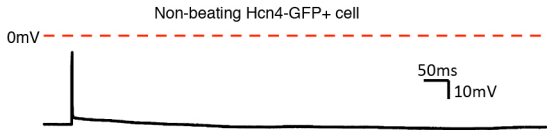


Figure S1. Electrical activity of a reprogrammed Hcn4-GFP$^+$ cell.
A representative action potential tracing of a non-beating Hcn4-GFP$^+$ cell following 4F transduction is shown. Action potentials from a total of 6 cells were recorded with electrophysiological stimulation.

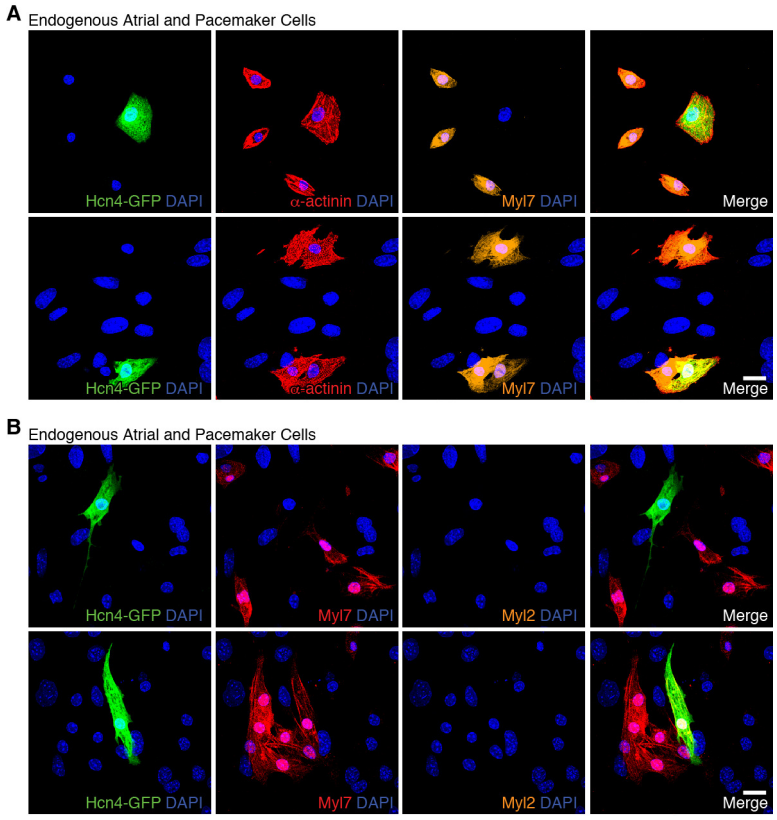


Figure S2. Myl7 expression in Hcn4-GFP$^+$ atrial cells.
A. Immunofluorescence staining of neonatal atrial cells isolated from Hcn4-GFP reporter mice for α-actinin (red), Hcn4-GFP (green), and Myl7 (orange). Hcn4-GFP$^+$ atrial cells in top panel did not express Myl7, while Hcn4-GFP$^+$ atrial cells in bottom panel expressed Myl7. **B**. Immunofluorescence staining of atrial cells isolated from Hcn4-GFP reporter mice for Myl7 (red), Hcn4-GFP (green), and Myl2 (orange). Hcn4-GFP$^+$ atrial cells in top panel showed very weak Myl7 expression, while Hcn4-GFP$^+$ atrial cells in bottom panel showed the comparable level of Myl7 expression to neighboring Hcn4-GFP$^-$ cells. None of atrial cells expressed Myl2. Scale bar: 20 μm

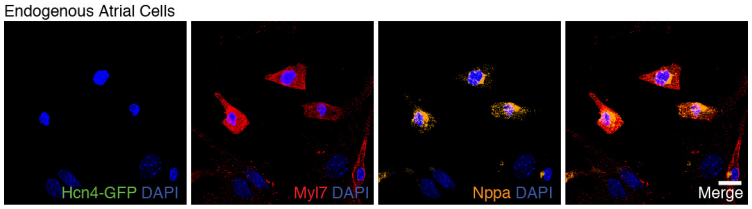


Figure S3. Co-expression of Myl7 and Nppa in atrial myocytes.
Immunofluorescence staining of neonatal atrial cells isolated from Hcn4-GFP reporter mice for Hcn4-GFP (green), Myl7 (red), and Nppa (orange). Scale bar: 20 μm

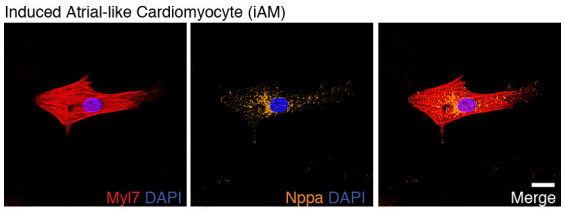


Figure S4. Co-induction of atrial markers, Myl7 and Nppa, by GHMT.
Immunofluorescence staining of GHMT-transduced WT MEFs for Myl7 (red) and Nppa (orange). Scale bar: 20 μm

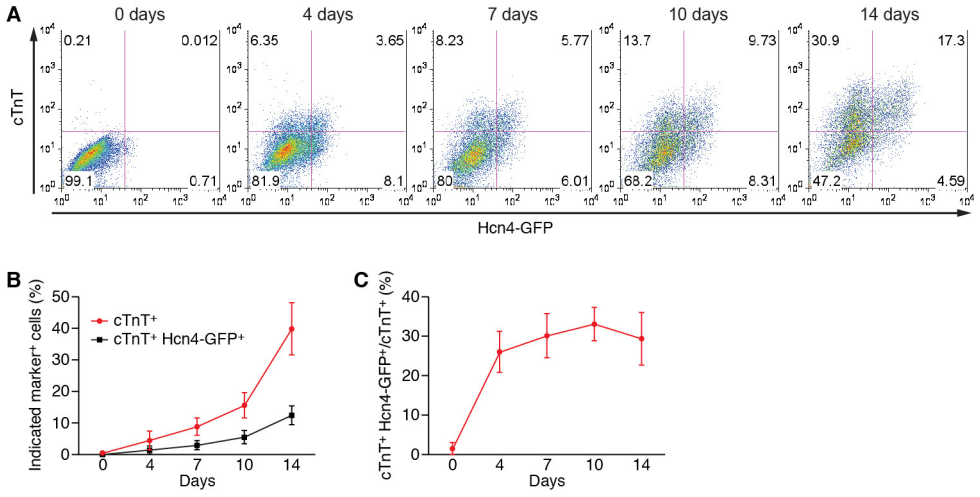


Figure S5. Time course of cTnT and Hcn4-GFP expression.

A. A representative flow cytometry plot for analyses of $cTnT^+$ and $Hcn4\text{-}GFP^+$ cells after the indicated number of days following transduction with GHMT retroviruses. GHMT were transduced into MEFs isolated from Hcn4-GFP reporter mice. The expression of pan-cardiac (cTnT) and pacemaker (Hcn4-GFP) markers were analyzed by flow cytometry at the indicated time points. The numbers in the right upper quadrant of each plot indicate the percentage of double positive cells for cTnT and Hcn4-GFP. **B**. A summary graph of the time course for single positive cells ($cTnT^+$) and double positive cells ($cTnT^+Hcn4\text{-}GFP^+$). Data from three independent experiments are presented as mean±s.d. **C**. A graph showing the time course of the fraction of double positive cells ($cTnT^+Hcn4\text{-}GFP^+$) among all pan-cardiac marker (cTnT) expressing cells.

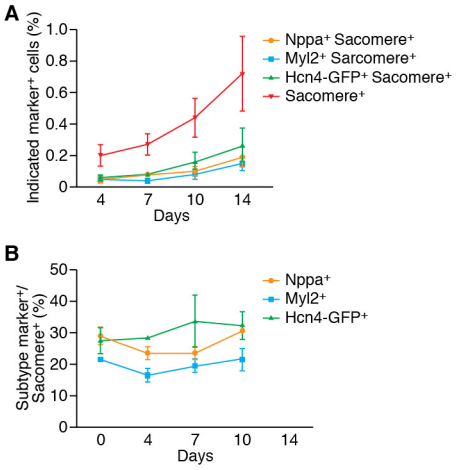


Figure S6. Time course of CM subtype reprogramming.
A. A summary graph of the time course for subtype specific marker expression with sarcomere development. GHMT were transduced into MEFs isolated from Hcn4-GFP reporter mice. The cells demonstrating sarcomere structures with or without subtype specific markers were counted on a confocal microscope after multiplex immunostaining. Data from three independent experiments are presented as mean±s.d. **B**. A graph showing the time course of subtype-specific marker positive cells as a fraction of all cells that developed sarcomere structures.

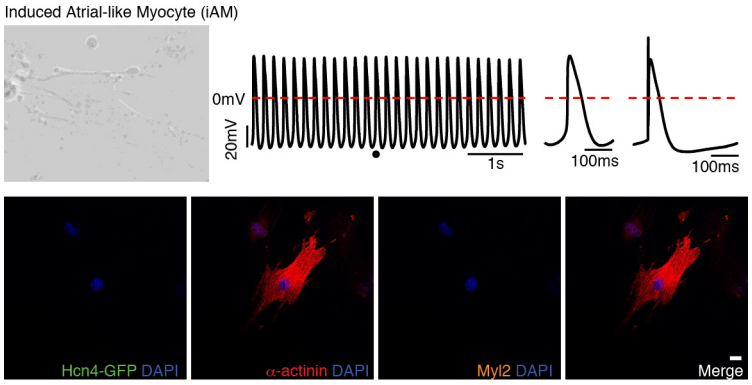


Figure S7. Confirmation of induction of atrial phenotype defined by action potential recordings with multiplex immunostaining.
DIC image was taken immediately before patch clamping (top left). Action potential of single beating iCLM (supplemental movie 10) was recorded 2-3 weeks after transduction of GHMT into Hcn4-GFP MEFs (top second panel from the left: action potential tracing, top third panel from the left: enlarged action potential at dotted point of tracing, top right: action potential with electrical stimulation). Immediately after patch clamping, multiplex immunofluorescence staining for Hcn4-GFP (green), α-actinin (red) and Myl2 (orange) was performed on the same cell from which action potential was recorded. The immunofluorescence staining was most consistent with iAM (Hcn4-GFP$^-$/α-actinin$^+$/Myl2$^-$) (bottom). Scale bar: 20 μm

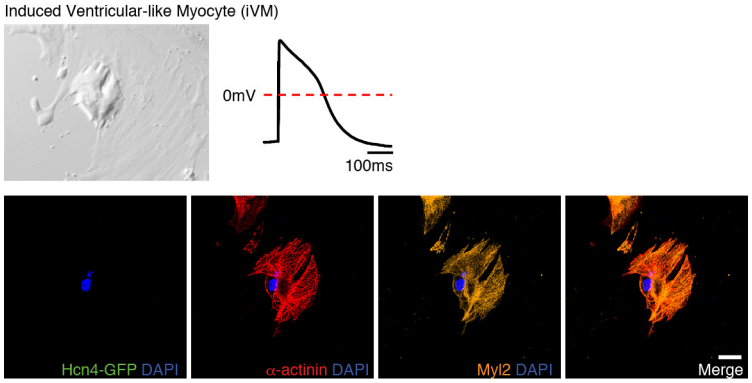


Figure S8. Confirmation of induction of ventricular phenotype defined by action potential recordings with multiplex immunostaining.
DIC image was taken immediately before patch clamping (top left). Action potential of single beating iCLM (supplemental movie 11) was recorded 2-3 weeks after transduction of GHMT into Hcn4-GFP MEFs. Due to the lack of spontaneous action potential, electrical stimulation was required to record the action potential of this cell (top right). Immediately after patch clamping, multiplex immunofluorescence staining for Hcn4-GFP (green), α-actinin (red) and Myl2 (orange) was performed on the same cell from which action potential was recorded. The immunofluorescence staining indicates iVM (Hcn4-GFP$^-$/α-actinin$^+$/Myl2$^+$) (bottom). Scale bar: 20 μm

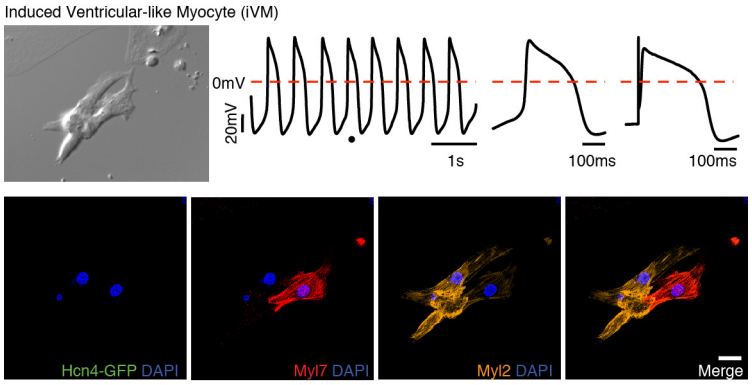


Figure S9. Confirmation of induction of ventricular phenotype defined by action potential recordings with multiplex immunostaining.
DIC image was taken immediately before patch clamping (top left). Action potential of single beating iCLM (located at upper left in DIC image and supplemental movie 12) was recorded 2-3 weeks after transduction of GHMT into Hcn4-GFP MEFs (top second from the left: action potential tracing, top third from the left: enlarged action potential at dotted point of tracing, top right: action potential with electrical stimulation). Immediately after patch clamping, multiplex immunofluorescence staining for Hcn4-GFP (green), Myl7 (red) and Myl2 (orange) was performed on the same cell from which action potential was recorded. The patch clamped cell's immunostaining (upper left) indicates iVM (Hcn4-GFP$^-$/Myl7$^-$/Myl2$^+$), while the cell attached to the patch clamped one (lower right) was most consistent with an intermediate phenotype between iAM and iVM (Hcn4-GFP$^-$/Myl7$^+$/Myl2$^+$). Scale bar: 20 μm

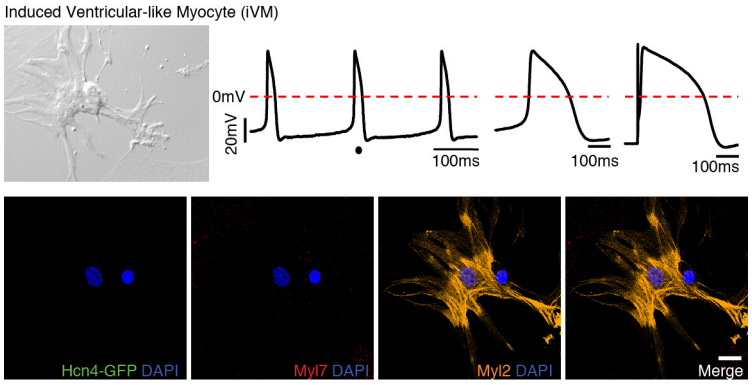


Figure S10. Confirmation of induction of ventricular phenotype defined by action potential recordings with multiplex immunostaining.
DIC image was taken immediately before patch clamping (top left). Action potential of single beating iCLM (supplemental movie 13) was recorded 2-3 weeks after transduction of GHMT into Hcn4-GFP MEFs (top second from the left: action potential tracing, top third from the left: enlarged action potential at dotted point of tracing, top right: action potential with electrical stimulation). Immediately after patch clamping, multiplex immunofluorescence staining for Hcn4-GFP (green), Myl7 (red) and Myl2 (orange) was performed on the same cell from which action potential was recorded. The patch clamped cell's immunostaining indicates iVM (Hcn4-GFP$^-$/Myl7$^-$/Myl2$^+$) (bottom). Scale bar: 20 μm

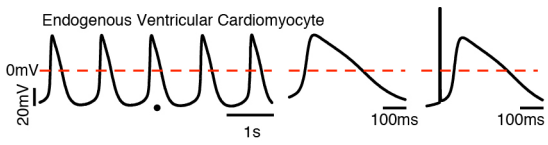


Figure S11. Action potential recording of ventricular myocytes isolated from Hcn4-GFP mouse.
Representative action potential tracing of endogenous Hcn4-GFP$^-$ neonatal ventricular myocytes isolated for Hcn4-GFP reporter mice (left). The dot indicates the location of tracing which was zoomed in the middle panel. The right panel showed the action potential with electrical stimulation.

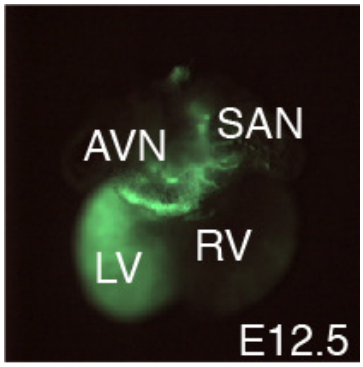


Figure S12. Fluorescent image of Hcn4-GFP E12.5 heart showing expression in the first heart field.

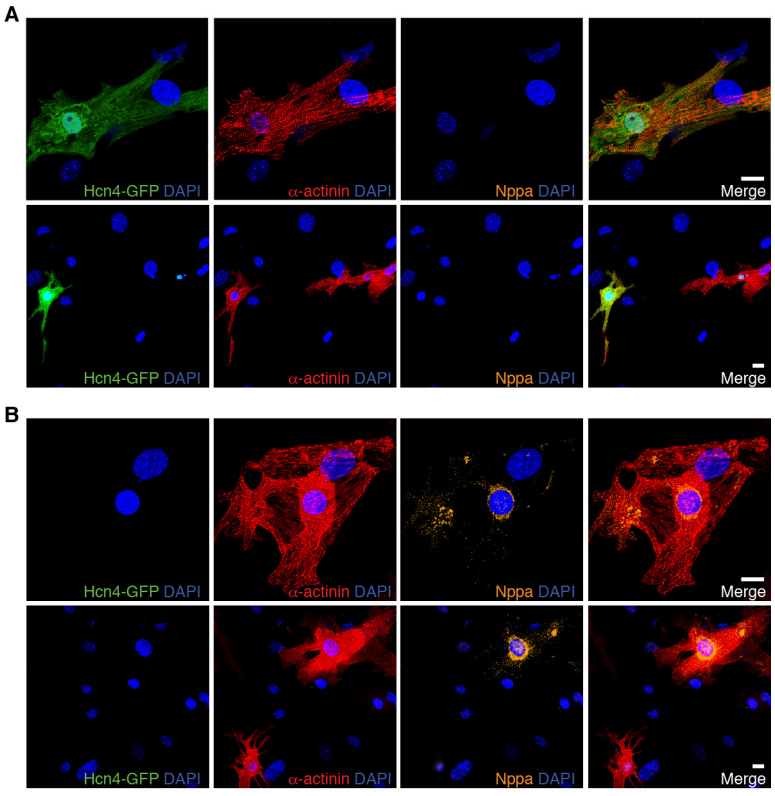


Figure S13. Induction of pacemaker and Nppa$^+$ atrial phenotypes by GHMT in adult cardiac fibroblasts.
Multiplex immunofluorescence staining of GHMT-transduced Hcn4-GFP adult cardiac fibroblasts for Hcn4-GFP (green), α-actinin (red), and Nppa (orange) showed induction of iPM (Hcn4-GFP$^+$/α-actinin$^+$/Nppa$^-$)(A) and iAM (Hcn4-GFP$^-$/α-actinin$^+$/ Nppa$^+$) (B). Scale bar: 20 μm

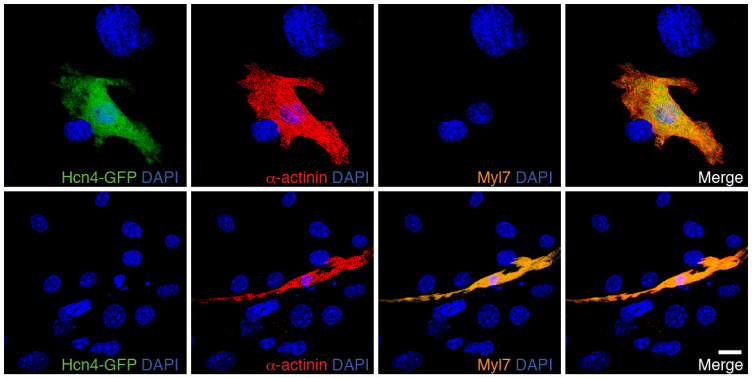


Figure S14. Induction of pacemaker and Myl7^{+} atrial phenotypes by GHMT in adult cardiac fibroblasts.
Multiplex immunofluorescence staining of GHMT-transduced Hcn4-GFP adult cardiac fibroblasts for Hcn4-GFP (green), α-actinin (red), and Myl7 (orange) showed induction of iPM (Hcn4-GFP^{+}/α-actinin^{+}/Myl7^{-})(top) and iAM (α-actinin^{+}/Hcn4-GFP^{-}/Myl7^{+}) (bottom). Scale bar: 20 µm

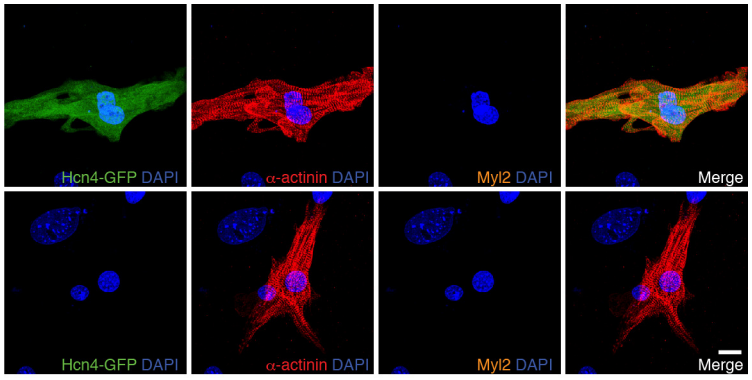


Figure S15. No induction of ventricular phenotype by GHMT in adult cardiac fibroblasts.
Multiplex immunofluorescence staining of GHMT-transduced Hcn4-GFP adult cardiac fibroblasts for Hcn4-GFP (green), α-actinin (red) and Myl2 (orange) showed induction of iPM (top), but ventricular phenotype (Hcn4-GFP^{-}/α-actinin^{+}/ Myl2^{+}) was not observed (bottom). Scale bar: 20 µm

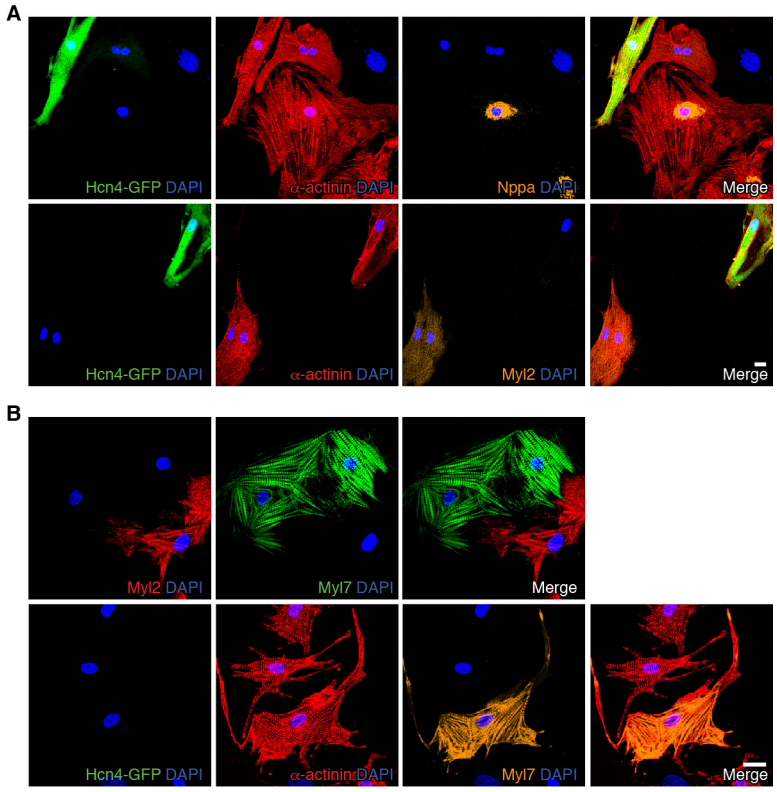
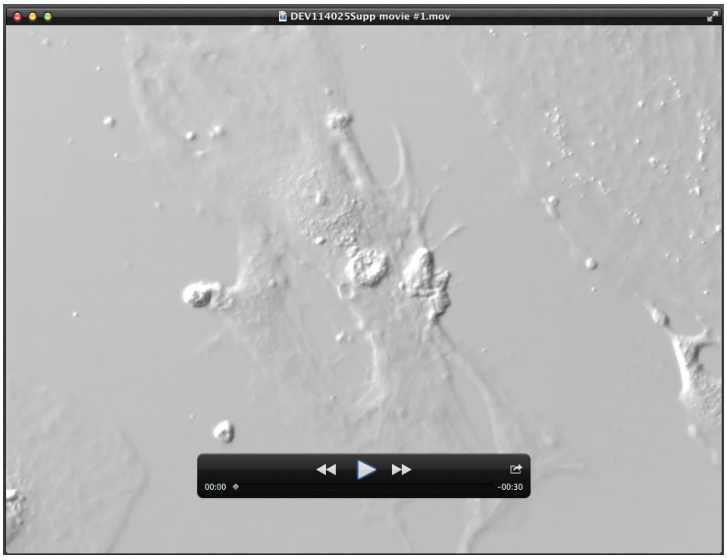


Figure S16. Induction of multiple adjacent cardiac subtypes by GHMT.
A. Multiplex immunofluorescence staining of GHMT-transduced Hcn4-GFP MEFs for Hcn4-GFP (green), α-actinin (red), and Nppa (top) or Myl2 (bottom) (orange). iPM (Hcn4-GFP^{+}/α-actinin^{+}/Nppa^{-} (top) or Hcn4-GFP^{+}/α-actinin^{+}/Myl2^{-} (bottom)) is adjacent to iAM (Hcn4-GFP^{-}/α-actinin^{+}/Nppa^{+}) (top) and iVM (Hcn4-GFP^{-}/α-actinin^{+}/Myl2^{+}) (bottom). **B.** Immunofluorescence staining of GHMT-transduced WT MEFs for Myl2 (red) and Myl7 (green) demonstrated that iAMs (Myl2^{-}/Myl7^{+}) are adjacent to iVMs (Myl2^{+}/Myl7^{-}) (top). Multiplex immunofluorescence staining of GHMT-transduced Hcn4-GFP MEFs for Hcn4-GFP (green), α-actinin (red), and Myl7 (orange) showed that iAM (Hcn4-GFP^{-}/α-actinin^{+}/Myl7^{+}) is adjacent to possible iVMs or unidentified iCLMs (Hcn4-GFP^{-}/α-actinin^{+}/Myl7^{-}).

Table S1. Action potential characteristics for reprogrammed cells presented in indicated figures.

	Maximum diastolic Potential (mV)	AP amplitude (pA)	APD 50 (ms)	APD 90 (ms)	Vmax (V/s)
Fig7A	-40.34	65.96	208.23	539.8	2.73
Fig7B	-64.08	111.08	126.39	189.1	109.89
Fig7C	-51.29	94.77	178.08	455.1	16.16
Fig S7	-47.99	75.52	50.34	104.1	39.06
Fig S8	-71.04	112.79	145.11	254.3	119.02
Fig S9	-56.24	102.51	244.33	288.9	8.25
Fig S10	-32.11	67.21	149.49	295.3	5.19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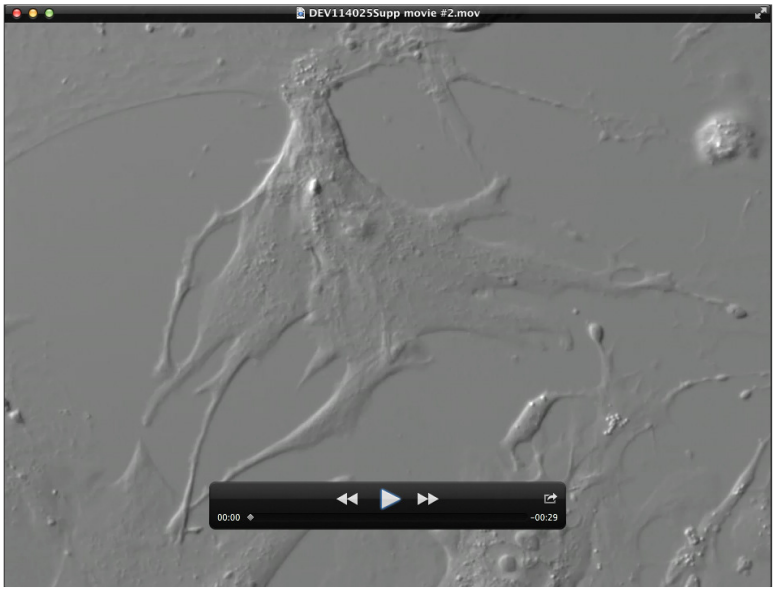
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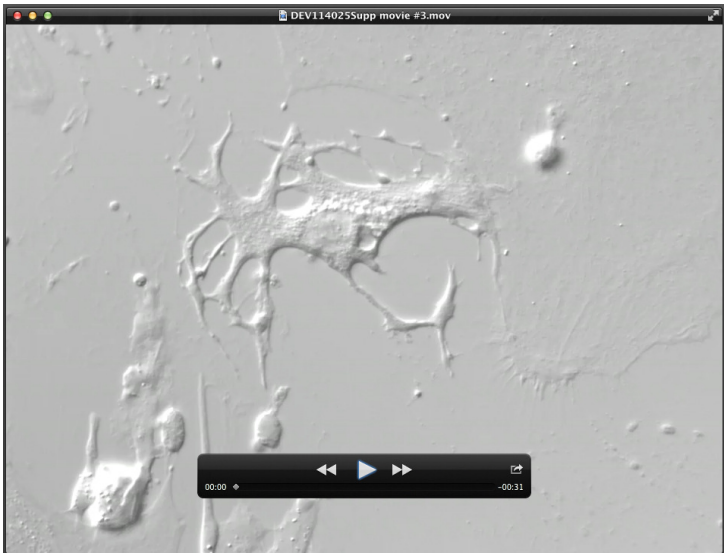
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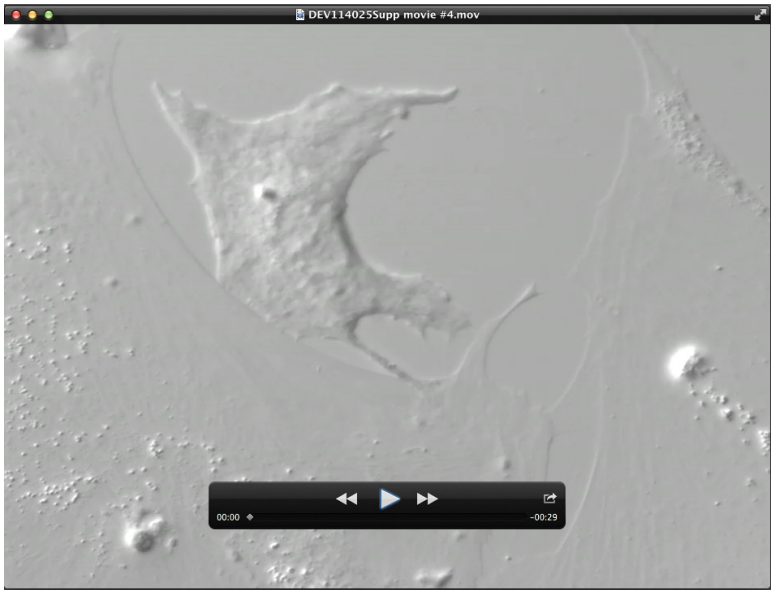
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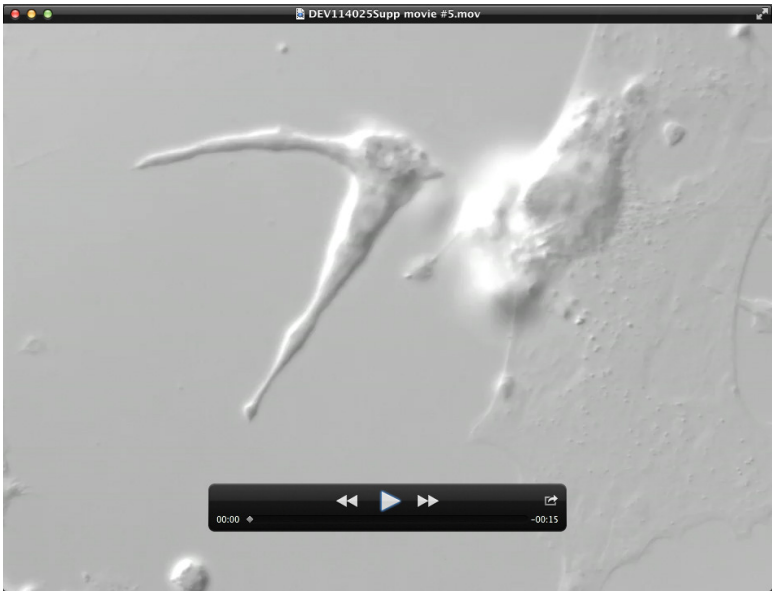
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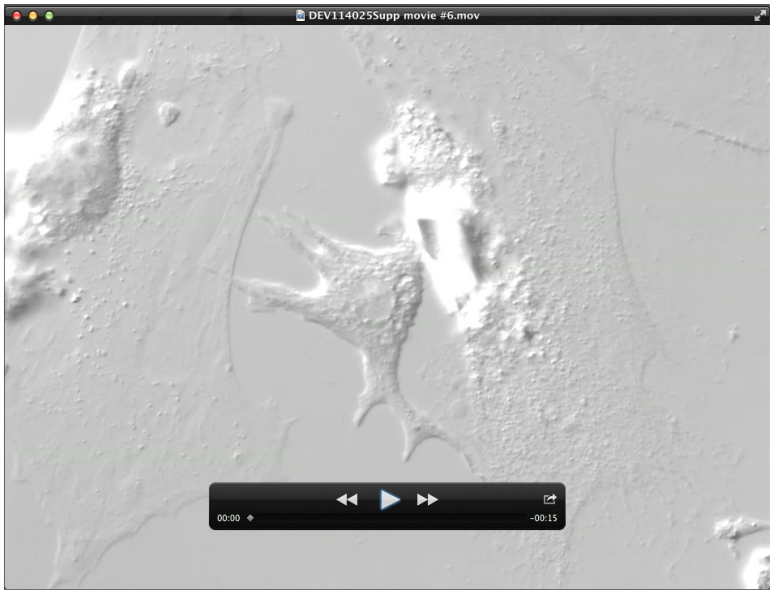
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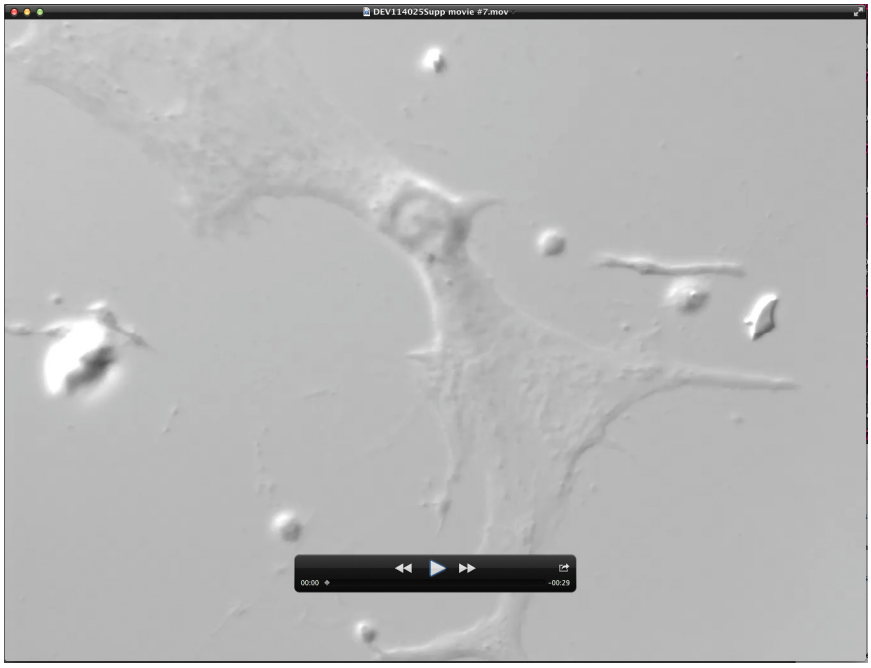
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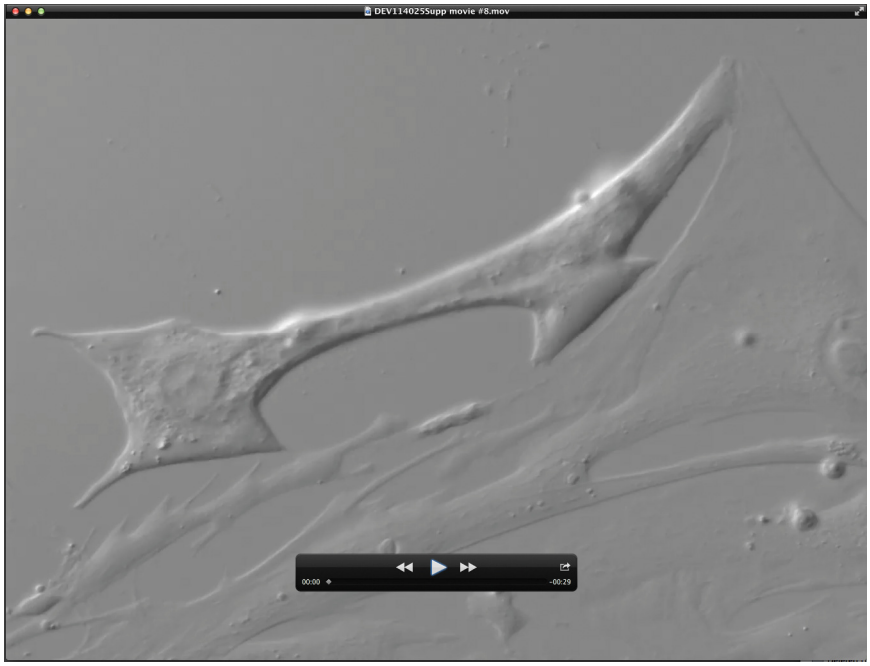
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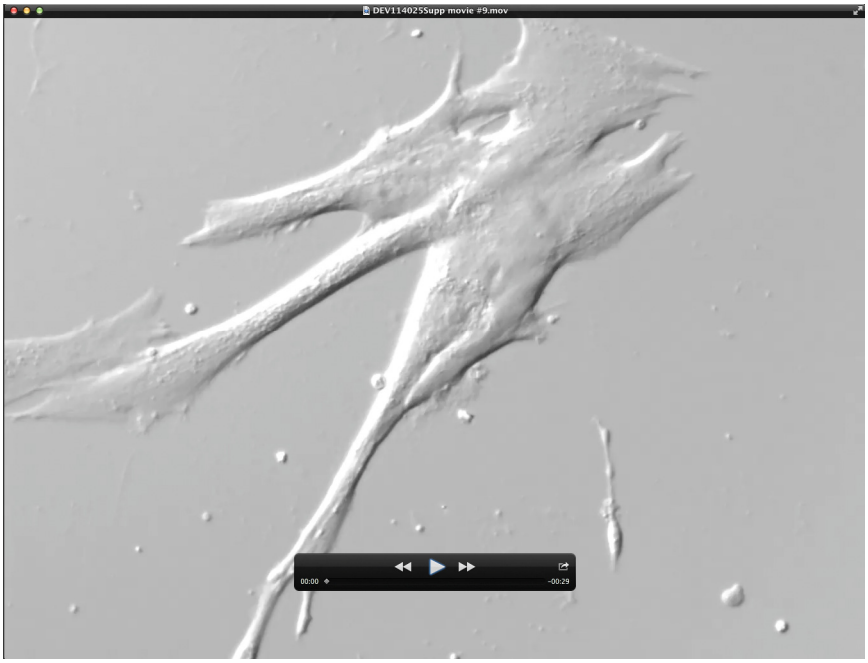
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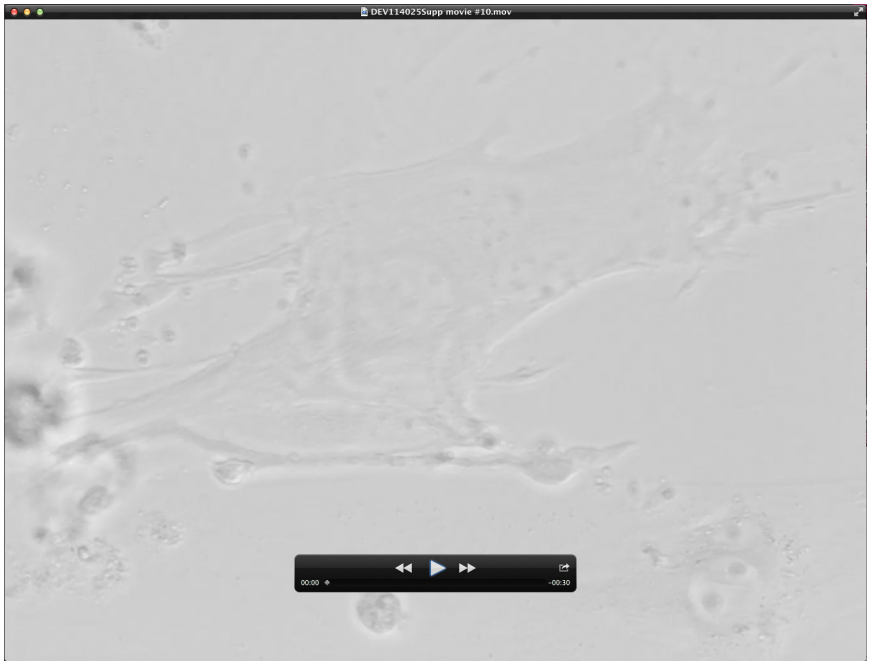
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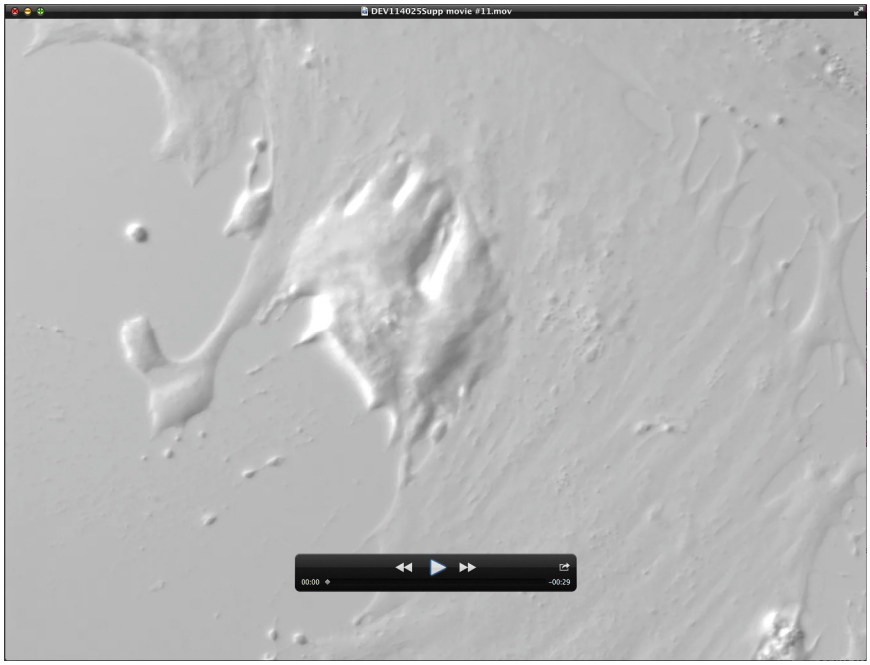
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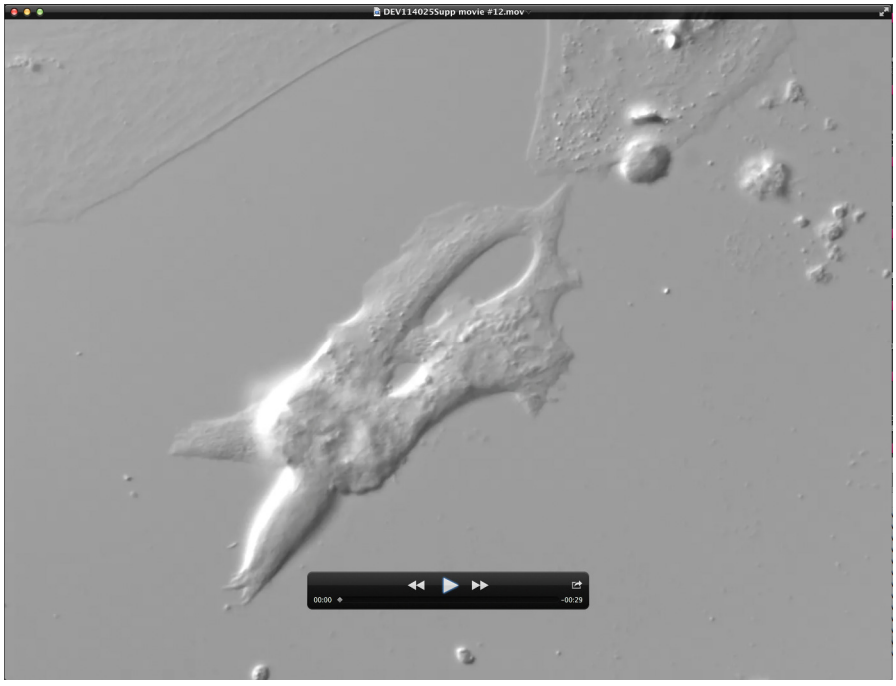
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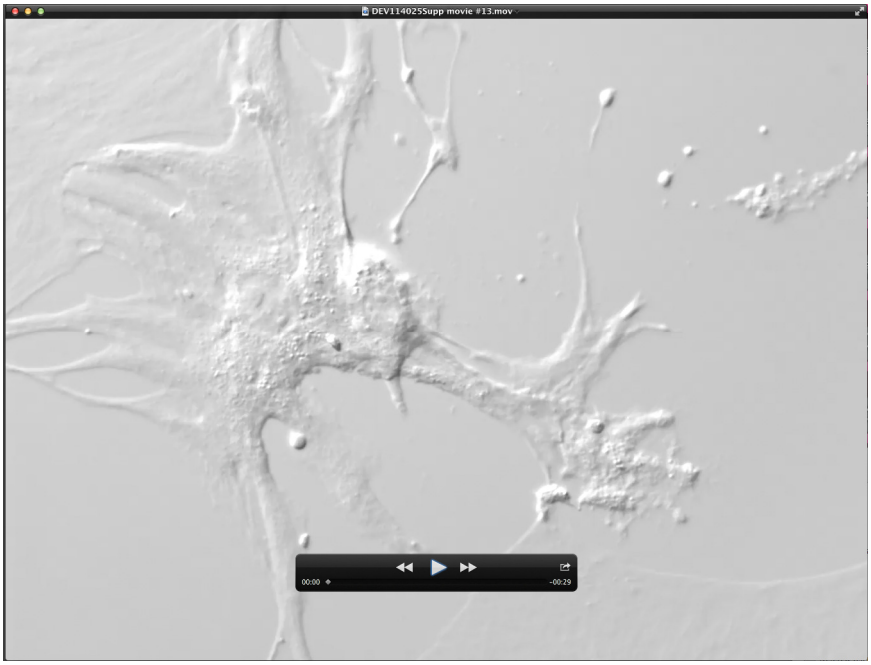

Movies 1-13. Spontaneously beating iCLMs 2-3 weeks post-infection of MEFs isolated from Hcn4-GFP reporter mice or WT mice with GHMT. The movies were recorded immediately prior to intracellular action potential recordings and/or immunostaining of indicated beating iCLMs.