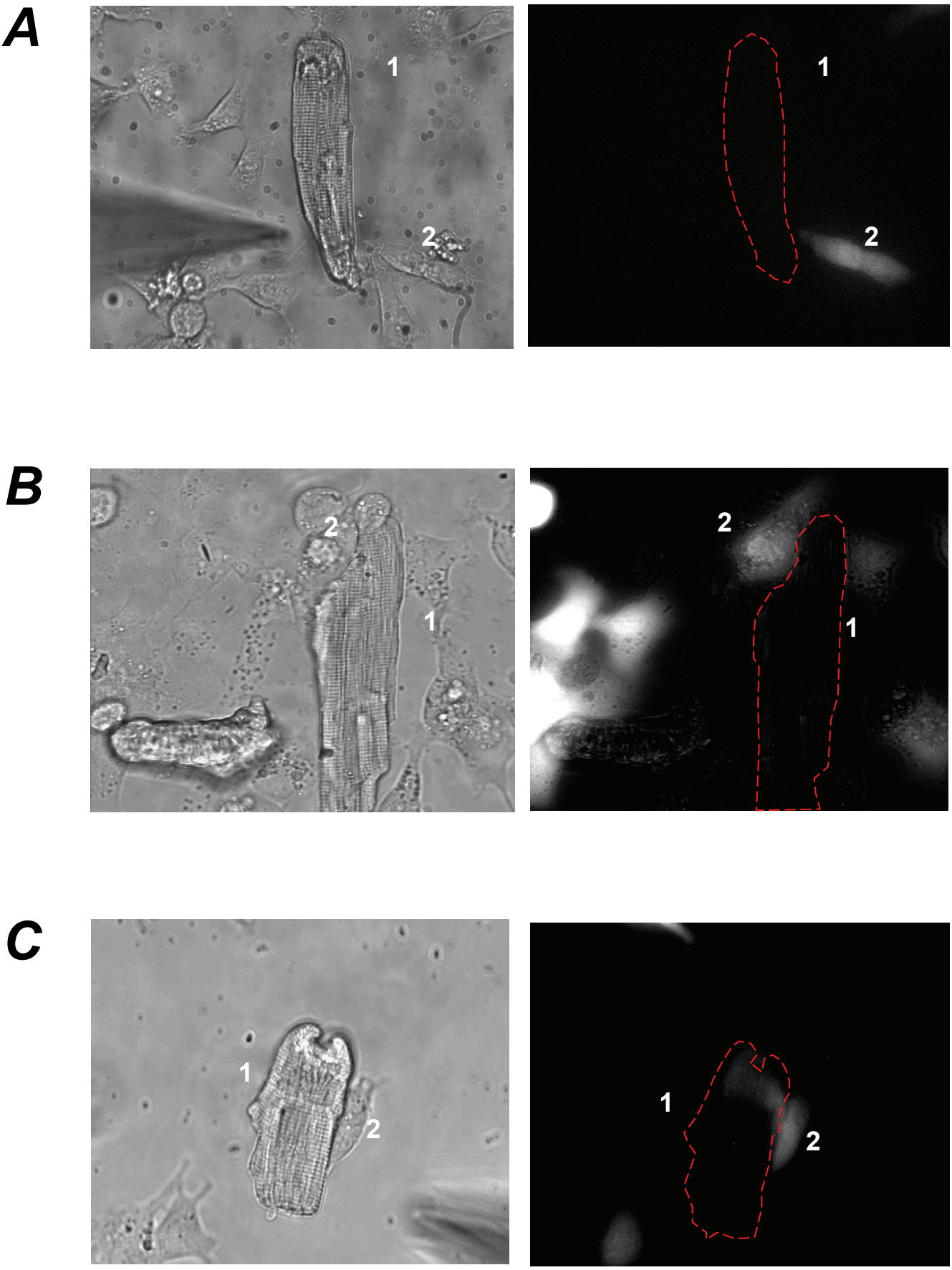
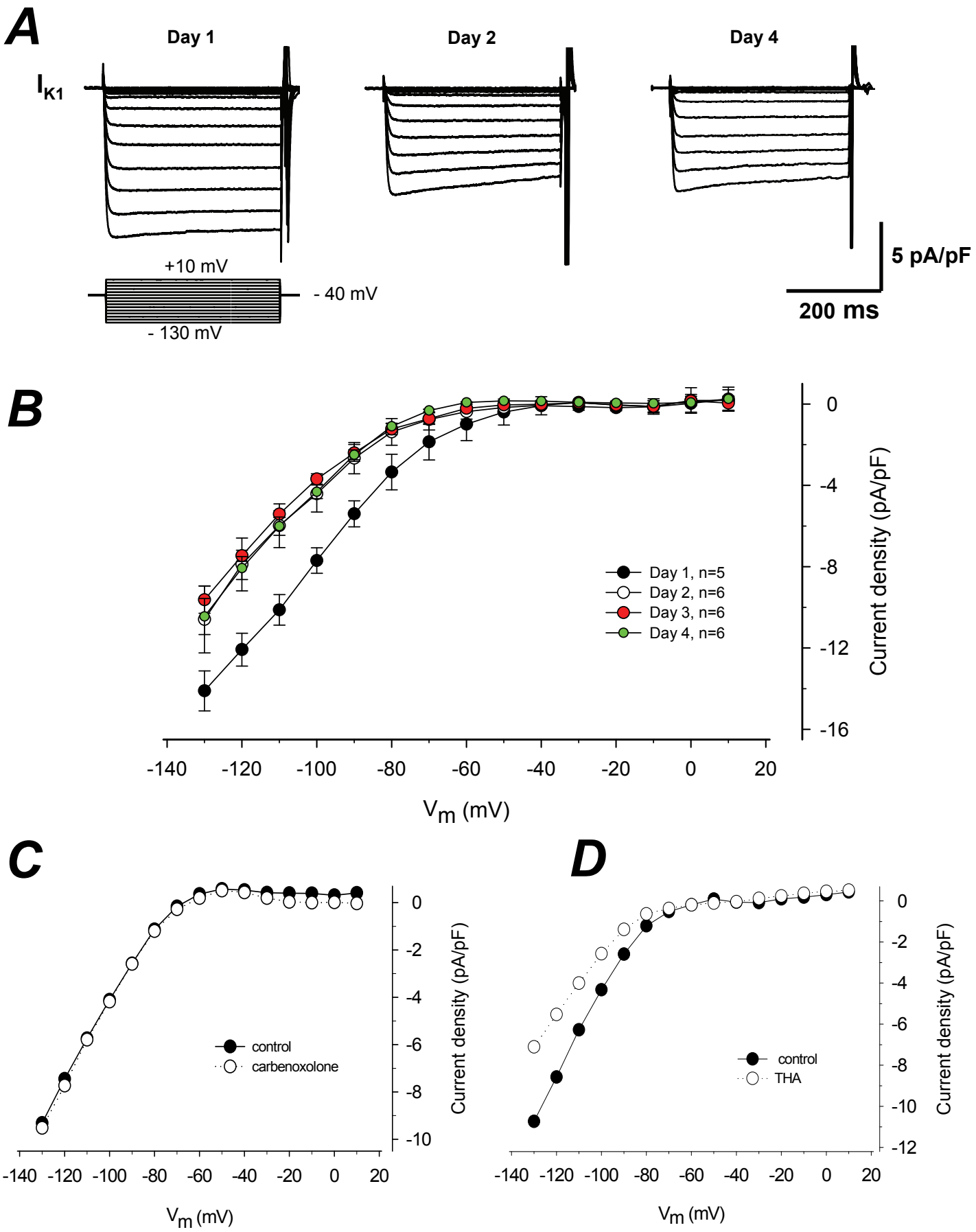
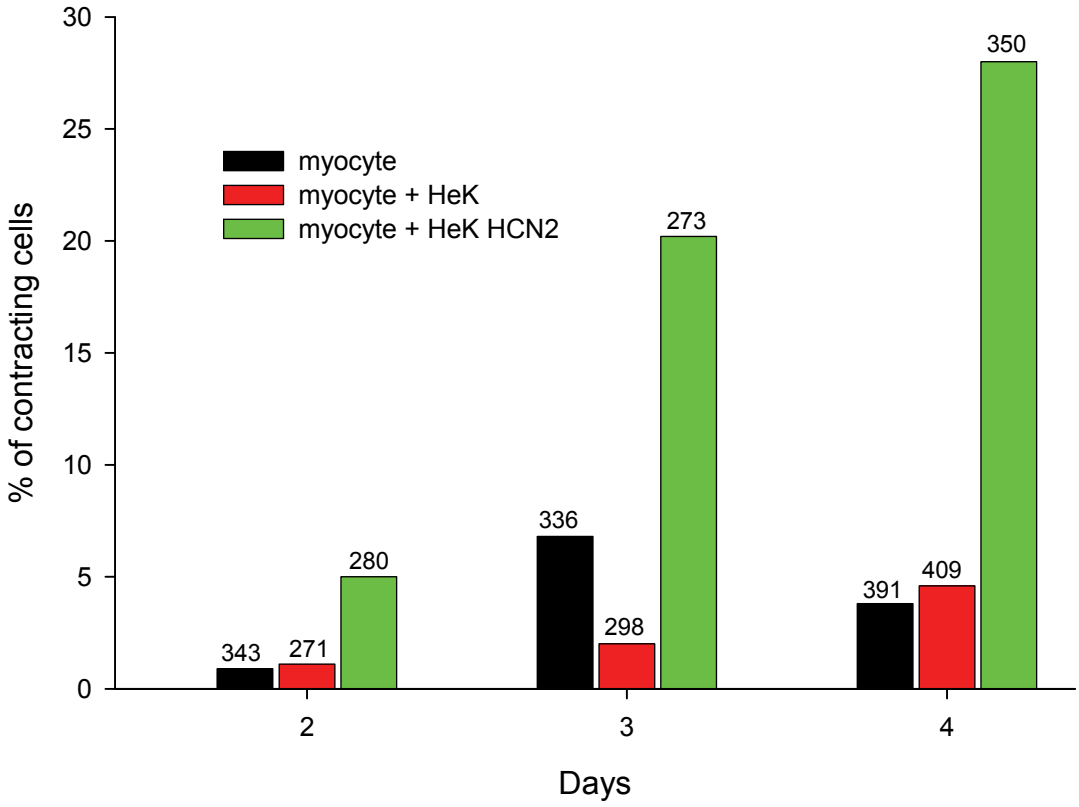


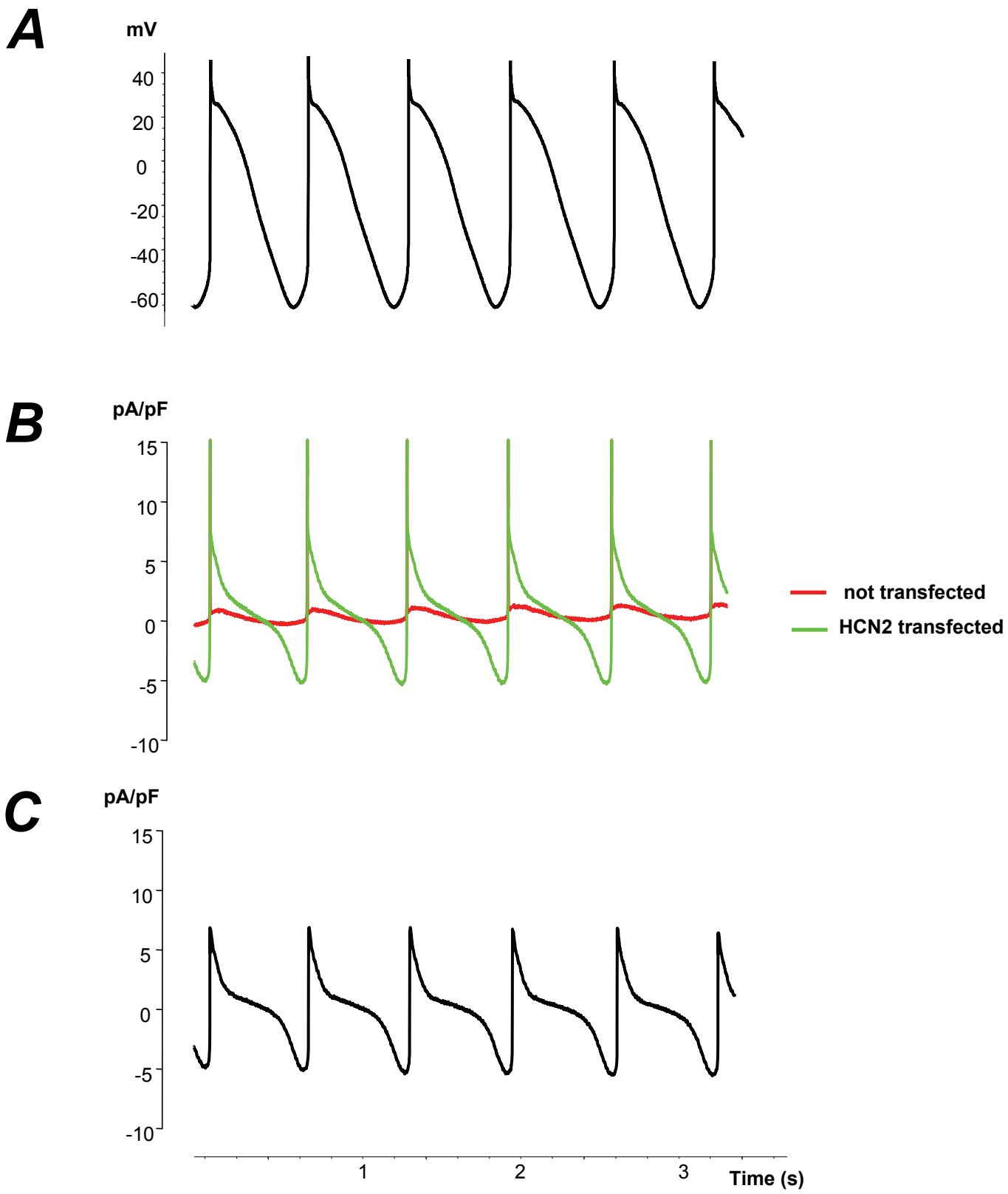
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Supplementary Figure legends

Figure 1

A Co-culture of canine ventricular myocytes (1) and HeLaCx43 cells (2) transfected with mHCN2 gene. The myocyte contracted spontaneously. When the HCN2 transfected HeLaCx43 cell was detached from the myocyte the contraction disappeared (see Movie 1).

B Cell1– canine ventricle myocyte; cell 2- HEK293 transfected with mHCN2. Application of 200 μm of carbenoxolone abolished spontaneous activity of this pair and myocyte contraction was restored during washout (see Movie 2).

C Cell1– canine ventricle myocyte; cell 2- HEK293 transfected with mHCN2. Application of 100 μM 9-amino-1,2,3,4-tetrahydroacridine (THA) abolished spontaneous activity which was recovered during washout (see Movie 3). Fluorescence indicates expression of eGFP which is expressed with mHCN2 in these cells (see Methods).

Figure 2. I_{K1} in canine ventricular myocytes

A Ba $^{2+}$-subtracted membrane currents elicited by 500 ms voltage steps ranging from -130 mV to +10 mV (by10 mV) from a holding potential of -40 mV.

B Peak I_{K1} densities from myocytes cultured for 1 day (isolation day) were significantly decreased ($P < 0.01$) after culturing for 24 hours (Day2) but stayed unchanged after 3 and 4 days (48 and 72 hours) in culture.

C Application of 200 μM carbenoxolone did not affect $I_{K1.}$

D Application of 100 μM THA reduced I_{K1} for membrane potentials between -130 mV and – 80 mV.

Figure 3. Pacing induced by HCN2 expressing cells.

Histogram showing the percent of contracting cells for myocytes alone(black bars), myocytes co-cultured with HEK cells (red bars) and myocytes co-cultured with HEK cells expressing HCN2 (green bars) at 2,3 and 4 day intervals. The number of contracting myocytes increased in co-culture with HEK cells expressing mHCN2. Only rode shaped and healthy looking myocyte cells were counted excluding unhealthy looking, blebbing and leaky myocytes.

The numbers on the bars represent the total number of myocytes alone (black) or in contact with HEK cells (red or green) counted.

Figure 4. HCN2 current activation by AP wave form stimulation.

A AP waveform command potential generated from AP shown in Figure 5B.

B Average currents from 4 HEK non-transfected cells (red trace) and 7 HEK HCN2 transfected cells (green trace) evoked by command potential shown in (**A).** cultured for 1 day (isolation day) were significantly decreased ($P < 0.01$) after culturing for 24 hours (Day2) but stayed unchanged after 3 and 4 days (48 and 72 hours) in culture.

C The current difference from transfected and non-transfected cells.