Nature Communications | 谱系示踪技术探索心肌致密化不全的发病机制

2017年7月20日，《Nature Communications 》发表了中国科学院生物化学与细胞生物学研究所周斌研究组题为“ Identification of a hybrid myocardial zone in the mammalian heart after birth ”的最新研究，研究中采用谱系示踪技术发现了哺乳动物心脏发育过程中心肌致密化的新机制。

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左心室致密化不全（LVNC）是继扩张型心肌病和肥大性心肌病之后第三种最常见的心肌病，主要起因是心肌过度小梁化和致密心肌层变薄。为了明确心肌小梁致密化的机制，文中采用遗传谱系示踪技术追踪了胚胎期心肌小梁和致密心肌层在出生后心脏中的命运。

通过基因敲入构建Nppa-GFP、Nppa-rtTa、Nppa-CreER、Hey2-CreER、Hey2-2A-CreER、Npr3-CreER和Sema3a-CreER工具小鼠模型，与Rosa26-LSL-Reporter 小鼠结合使用。特异性表达的Cre酶会切除loxP位点之间的转录终止序列，从而激活报告基因的持续表达。由于该修饰在DNA水平上进行，可以遗传到子代细胞，因而可以永久标记特定类型的细胞以研究其子代的命运。

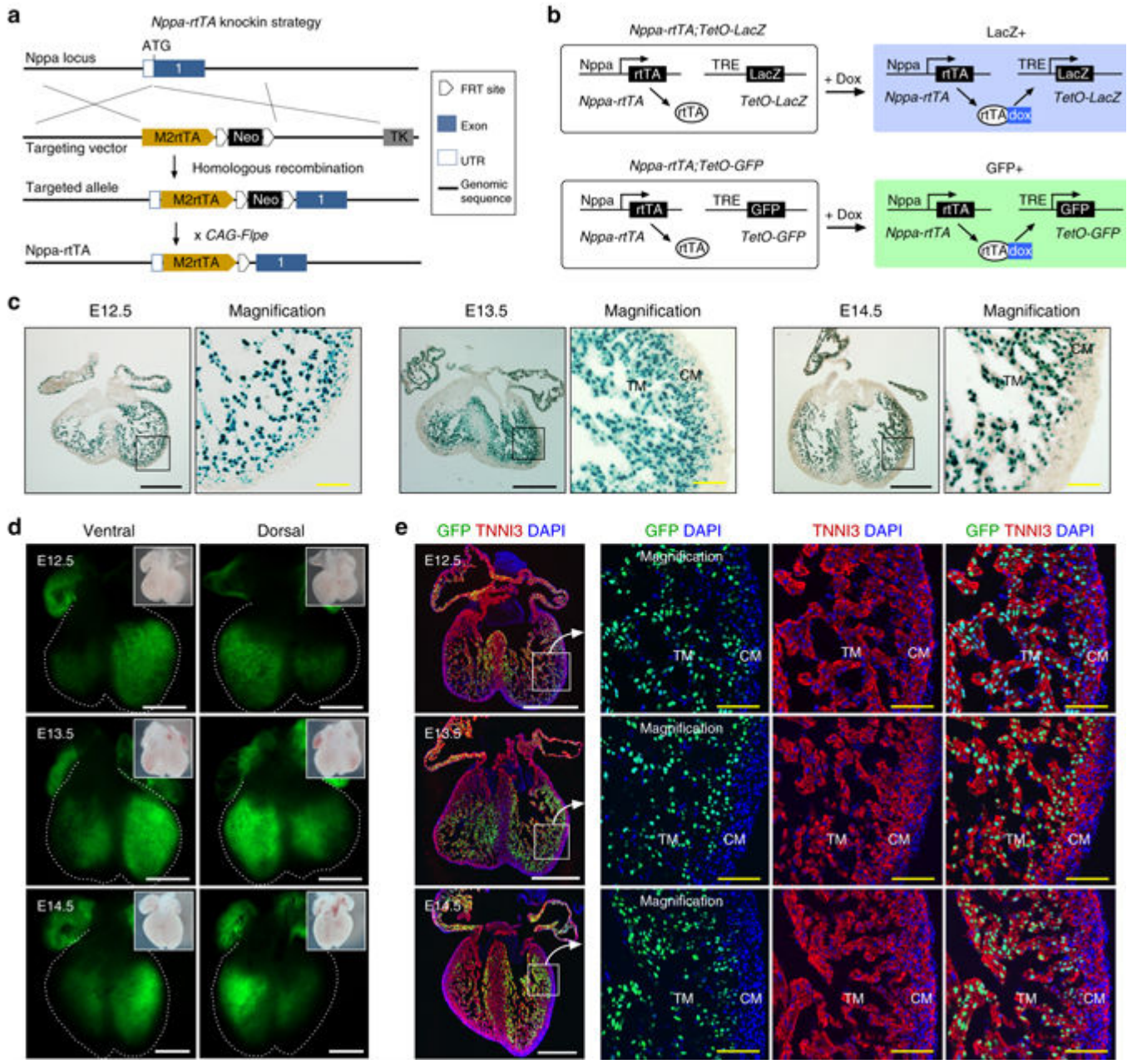

Fig1. **Generation and characterization of Nppa-rtTA mouse line.** a) Schematic showing knock-in strategy of Nppa-rtTA mouse line by homologous recombination. b)Schematic showing characterization of Nppa-rtTA by rtTA responding reporter mice TetO-LacZ or TetO-GFP. LacZ or GFP is expressed after doxycycline treatment (+Dox). c) X-gal staining of E12.5 to E14.5 Nppa-rtTA;TetO-LacZ heart sections. d Whole-mount fluorescence view of Nppa-rtTA;TetO-GFP mouse hearts. Inserts are bright-field images of same hearts. Dotted lines indicate epicardium. e Immunostaining for GFP and TNNI3 on Nppa-rtTA;TetO-GFP heart sections showed GFP is highly enriched in trabecular myocardium (TM) but sparse in compact myocardium (CM). Each image is a representative of four individual samples. Scale bars, 500 μm (white or black); 100 μm (yellow)

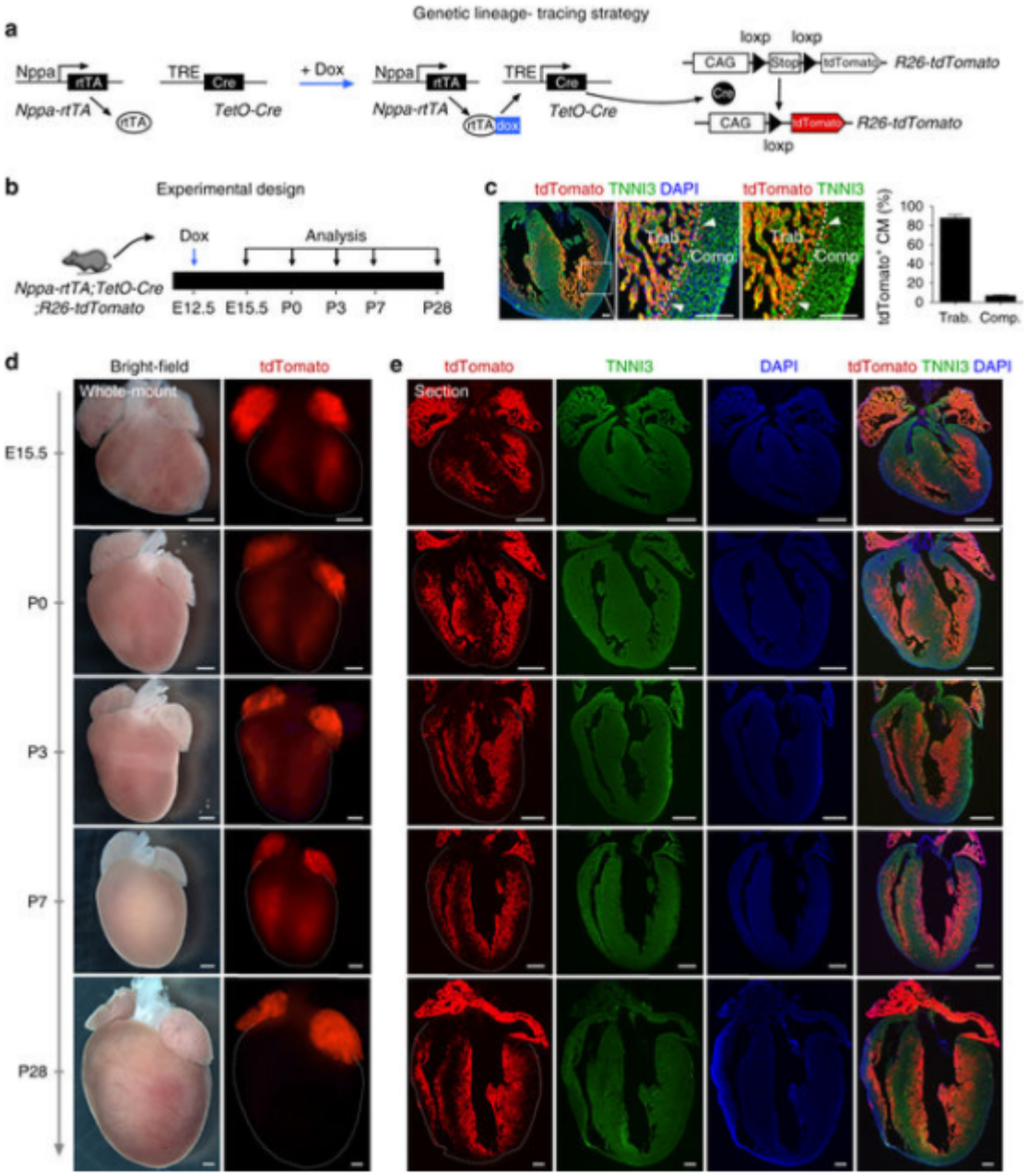

Fig2. **Trabecular myocardium develops into the inner myocardial wall.** a) Schematic diagram showing the genetic labeling strategy of Nppa+ cardiomyocytes by tet-on system. After doxycycline (Dox) treatment, rtTA binds to tetO promoter and drives Cre expression. Cre-loxp recombination leads to tdTomato expression. b) Schematic diagram showing the time point for doxycycline induction and tissue analysis. c) Immunostaining for tdTomato and TNNI3 on E15.5 heart sections. Arrowheads indicate a small number of Nppa+ cells (tdTomato+) in compact (Comp.) myocardium. Dotted line indicates border between trabecular (Trab.) and comp. myocardium. Quantification of the percentage of tdTomato+ cardiomyocytes (CM) in Trab. or Comp. layer is shown on the right panel. Data are mean ± s.e.m.; n = 4. d) Whole-mount bright-field and fluorescence views of hearts collected from Nppa-rtTA;tetO-Cre;R26-tdTomato mice. Dotted lines indicate the outline of hearts. e) Immunostaining for tdTomato and TNNI3 on heart sections. Dotted lines indicate epicardium. Nuclei were stained with DAPI. Each image is a representative of four individual samples. Scale bars, 100 μm in c; 500 μm in d, e

研究中的主要发现包括：

- 胚胎期的心肌小梁较少增殖，融合成心室壁的心内膜下心肌层。
- 致密心肌层在胚胎发育晚期和新生期显著增殖形成增厚的心外膜下心肌层。
- 位于这两层心肌之间的混合区是由来源于心肌小梁和致密心肌层的心肌细胞共同组成的，致密心肌层心肌细胞向心腔内增殖，使心肌小梁体积增大，压缩小梁间隙，促进心肌小梁融合成致密心肌。在心肌小梁融合的过程中，嵌入小梁之间的心内膜细胞参与了冠状动脉内皮的形成。

研究人员还通过特异性敲除小鼠胚胎期致密心肌层心肌细胞中的 Yap1 基因（Hey2-CreER；$Yap1^{flox/flox}$），使致密心肌层心肌细胞增殖减少，抑制其参与心室壁中间混合区的形成，导致小鼠出生后心肌过度小梁化和致密心肌层变薄。表明心肌致密化不全不能仅归因于心肌小梁本身过度化的缺陷，胚胎期致密心肌层的扩增及其参与心肌混合区的形成，对发育形成正常的心室壁起着至关重要的作用。

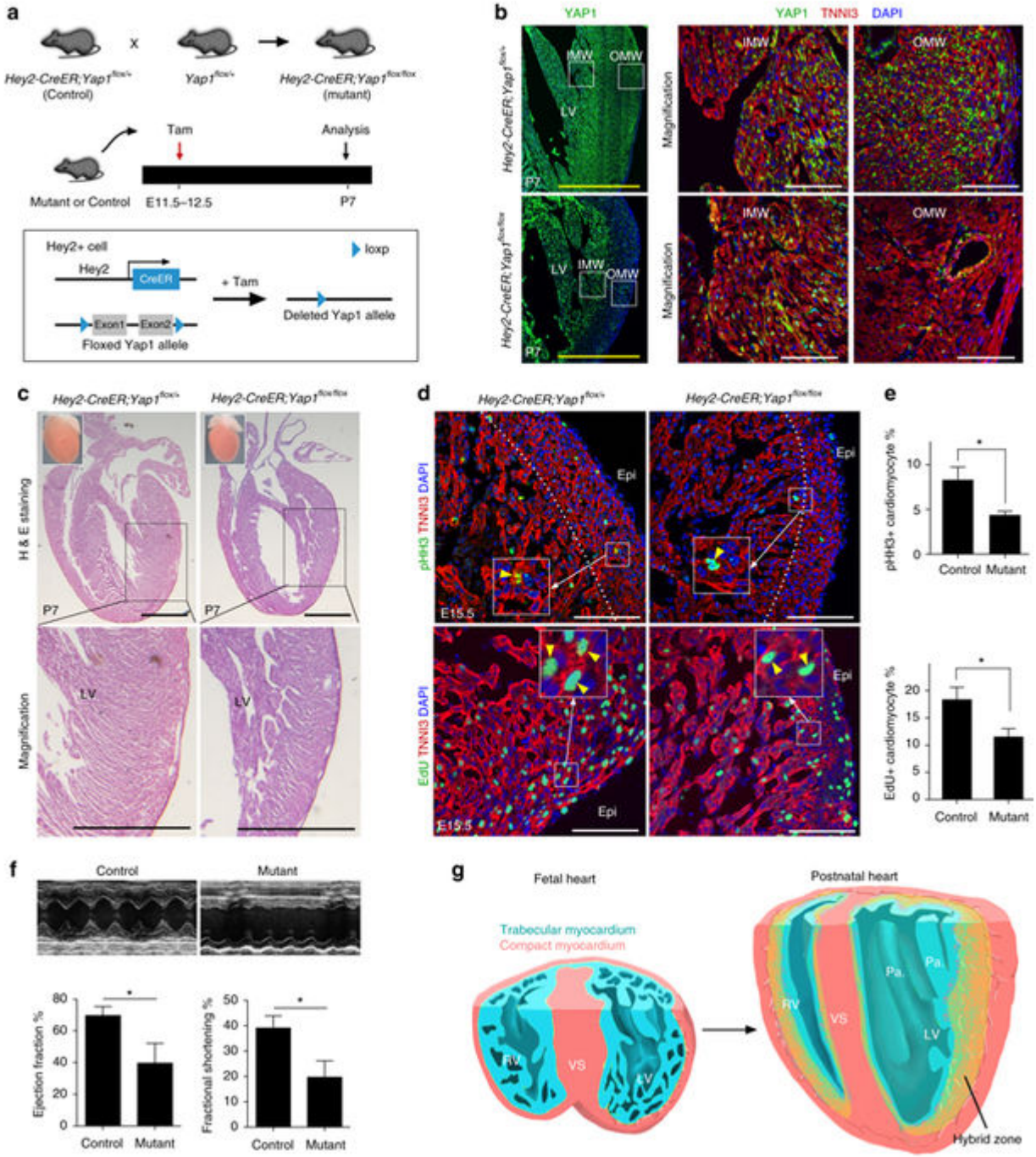

Fig3. **Inhibition of fetal compact myocardial expansion results in prominent trabeculae and thin compacted layer of postnatal heart.** a) Schematic figure showing generation of Yap1 gene deletion in Hey2+ cells by tamoxifen injection at embryonic stage. b) Immunostaining for YAP1 and TNNI3 on postnatal day 7 (P7) control and mutant heart sections. Although YAP1 is detected in the inner myocardial wall (IMW) of both control and mutant hearts, YAP1 is reduced in the outer myocardial wall (OMW) of mutant heart compared with that in the control heart. c) Hematoxylin and eosin (H&E) staining of heart sections from P7 control (left) and mutant (right) mice. Inserts indicate whole-mount images of hearts. d) Immunostaining for TNNI3 and pHH3 or EdU on control and mutant heart sections. Yellow arrowheads indicate proliferating cardiomyocytes in the magnified inserts. e) Quantification on the percentage of pHH3+ or EdU+ cardiomyocytes is shown on the right panel. f) Echocardiographic analysis of heart function showed a significant reduction of ejection fraction and fractional shortening in the mutant,

compared with the control. *P□<□0.05; n□=□4 for mutant and n□=□5 for control. g) Cartoon image showing hybrid myocardial zone in the postnatal heart derived from trabecular layer and compact myocardium. The hybrid zone (yellow) is interposed between the inner myocardial wall (blue) produced mainly by trabecular coalescence and the outer myocardial wall (red) produced by compact myocardial expansion. RV right ventricle, LV left ventricle, VS ventricular septum; Pa. papillary muscle. Scale bars, 1□mm (black or yellow); 100□μm (white)

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