Cell Research | 点突变小鼠模拟人类心脏综合症模型，发现PRKAG2基因作为治疗遗传性心脏病潜在靶点

利用PRKAG2基因点突变小鼠，模拟了人类心脏综合症，并通过CRISPR/Cas9基因组编辑成功校正小鼠的PRKAG2突变。 复旦大学附属中山医院的主任医师颜彦课题组和武汉大学生命科学学院的宋保亮教授课题组的这项研究于2016年8月发表在《Cell Research》上，题为“Genome editing with CRISPR/Cas9 in postnatal mice corrects PRKAG2 cardiac syndrome”。

RKAG2心脏综合征是由PRKAG2基因突变造成的常染色体显性遗传疾病，包括家族性心室预激、传导系统病变及心肌肥厚，患者往往会出现室性心动过速和进程性心力衰竭。PRKAG2心脏综合征很难治疗，需要进行心脏移植。

研究人员在家族性预激综合征（WPW）中鉴定了PRKAG2的H530R突变，并构建了携带PRKAG2基因H530R点突变的小鼠模型。该模型能够反映包括心肌肥厚和糖原贮积在内的人类症状，说明H530R与PRKAG2心脏综合症的确有因果关系。

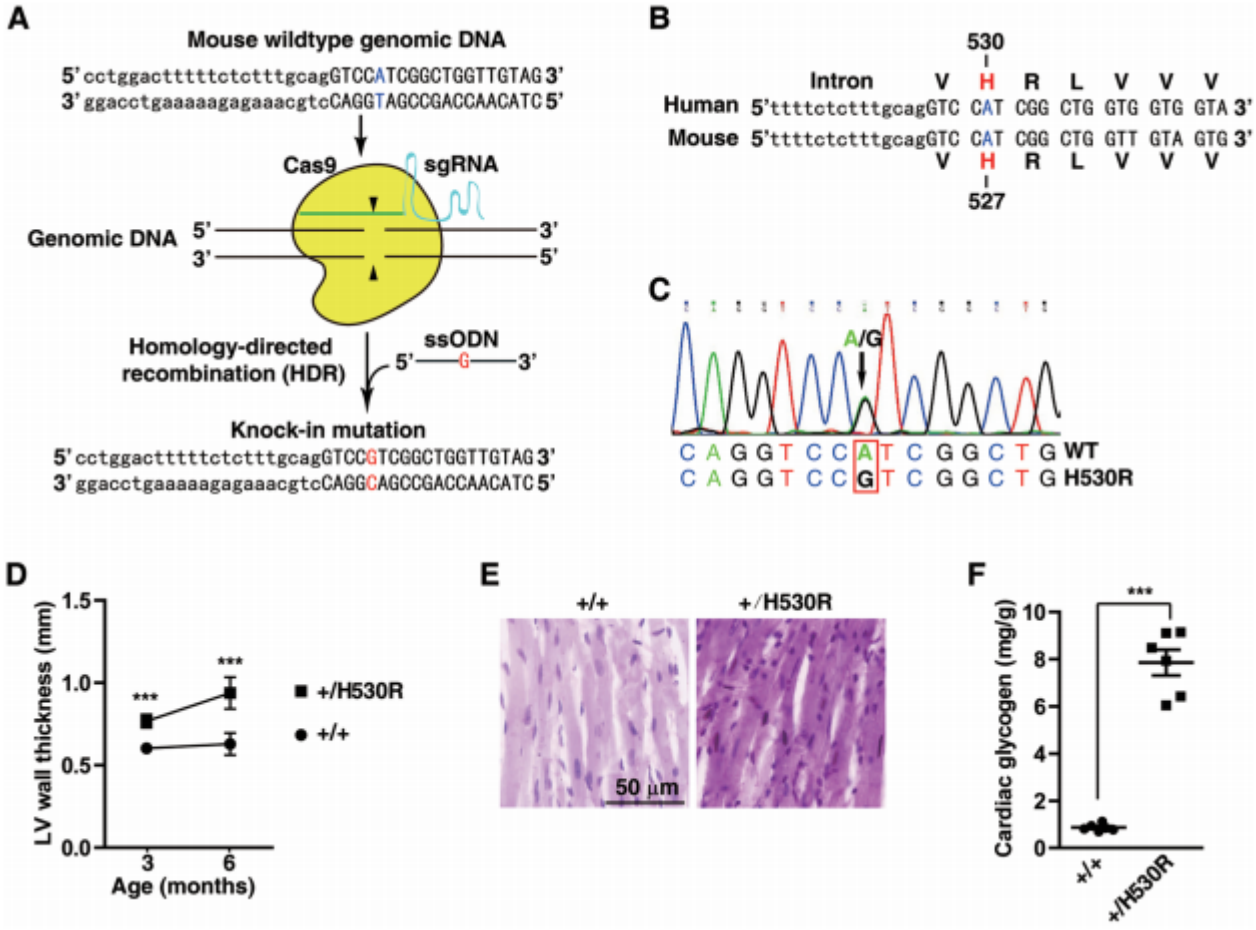

Fig1. **H530R knock-in mice recapitulate the PRAKG2 cardiac syndrome.** (A) Schematic showing the generation of H530R-PRKAG2 knock-in mouse model using the CRISPR/Cas9 system. Lowercase characters denote the intronic region and uppercase characters denote the exonic region. (B) Alignment of genomic DNA and protein sequences of human and mouse PRKAG2 near the H530 locus. The H530 of human PRKAG2 is equivalent to H527 of mouse PRKAG2. The obtained knock-in mouse model is referred to as the H530R-PRKAG2 knock-in model. (C) Validation of heterozygous H530R knock-in mice by sequencing. (D) LV wall thickness calculated by continuous echocardiography. Data represent mean ± SD. Two-way ANOVA was performed for overall differences (n = 6). Unpaired two-tailed Student's t-test was performed for single comparison (***P < 0.001). (E) PAS staining of paraffin-embedded sections. (F) Measurement of glycogen content in hearts from 3-month-old WT (+/+) and heterozygous H530R knock-in (+/H530R) mice. Data represent mean ± SD. One-way ANOVA was performed for overall differences (n = 6). Unpaired two-tailed Student's t-test was performed for single comparison (***P < 0.001).

研究人员利用AAV9载体结合CRISPR/Cas9系统，对出生后的小鼠进行基因编辑。该系统能够破坏带有H530R突变的PRKAG2等位基因，同时不影响野生型等位基因。这项研究表明，在小鼠出生第4天或42天一次性注射AAV9-Cas9/sgRNA，可以显著恢复小鼠心脏的形态和功能。研究人员指出，活体CRISPR/Cas9基因组编辑是一个有效的工具，能通过选择性破坏致病突变治疗PRKAG2心脏综合征和其他显性遗传的心脏病。

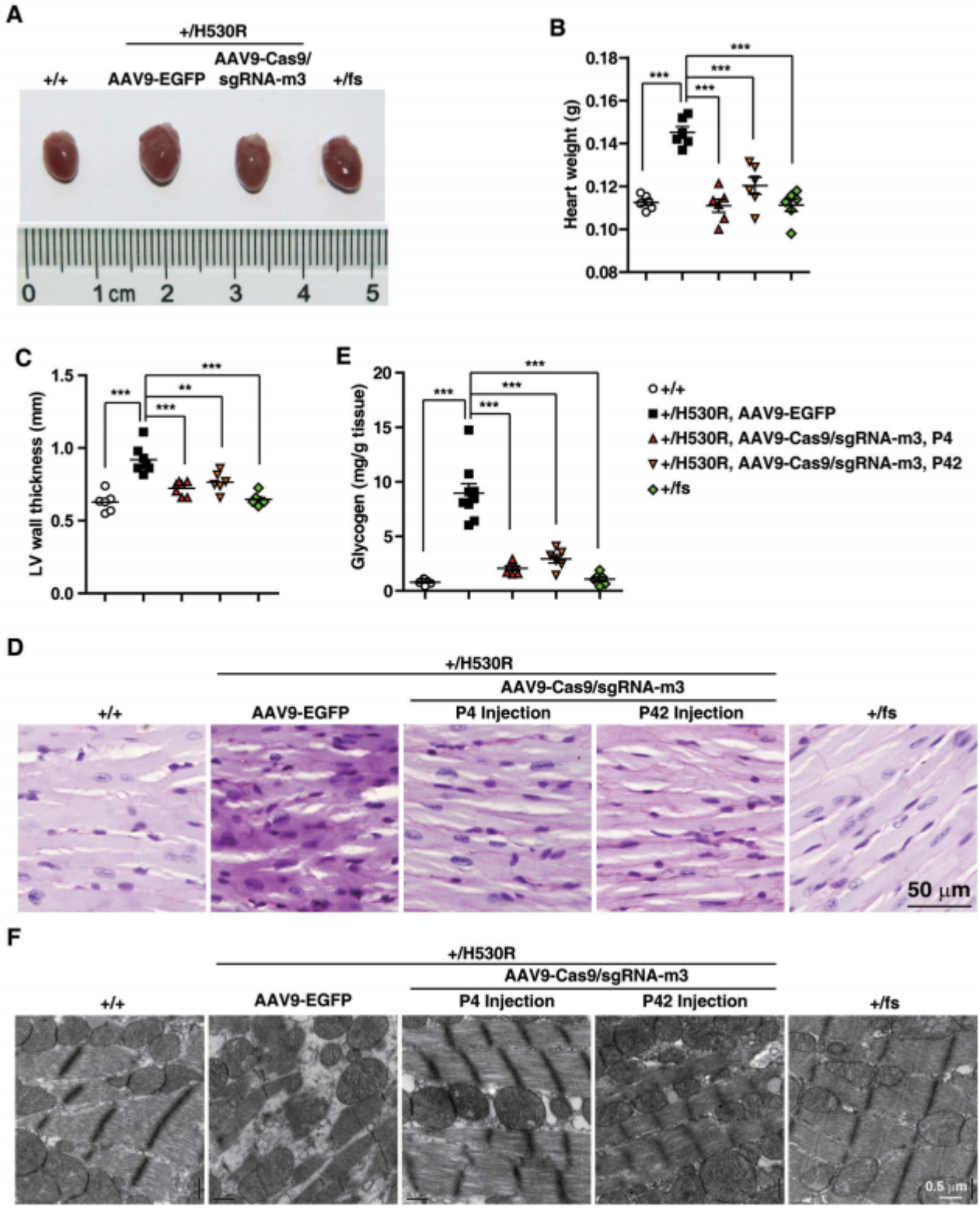

Fig2. **CRISPR/Cas9 system-mediated genome editing corrects PRKAG2 cardiac syndrome in the knock-in mouse model.** (A) Heart images. (B) Heart weight statistics. (C) LV wall thickness measured by echocardiography. (D) PAS staining of paraffin-embedded sections. (E) Glycogen measurement. In B, C and E, one-way ANOVA was performed for overall differences between all groups (n = 6). Unpaired two-tailed Student's t-test was performed for single comparison (**P < 0.01, ***P < 0.001). (F) Transmission electron microscopy of ultrathin sections of hearts from 6-month-old mice as indicated.

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