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The endocardial expression of ADGRG6 (Gpr126) is necessary for survival in mouse and sufficient to drive trabeculation in zebrafish

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Trabeculation is a crucial process in the developing vertebrate heart, by which clusters of ventricular cardiomyocytes extrude and expand into the lumen of ventricular chambers to form sheet like projections. Alterations in normal development of trabeculation lead to various cardiomyopathies, most widespread one being, left ventricular non compaction syndrome (LVNC). The underlying molecular mechanisms regulating trabeculation are still poorly understood. Our previous studies have demonstrated that adhesion-GPCR ADGR6 (Gpr126), expressed in the endocardium of the embryonic murine heart, plays an important role in the development of trabeculation. Lack of Gpr126 causes ventricular hypotrabeculation and mitochondrial dysfunction in mice and zebrafish, with inefficient conduction as secondary defects.

In our studies we utilize a knockout first allele mouse generated utilizing the EUCOMM construct Gpr126tm1a(EUCOMM)Hmgu. This model allows the expression of the LacZ gene under the control of GPR126 promoter, providing insight on the endogenous expression of Gpr126. The correct insertion of the cassette was verified by southern blot and genotyping. Furthermore mating of heterozygous Gpr126tm1a(EUCOMM)Hmgu mice yields no homozygous offspring at birth confirming that insertion of the cassette results in knockout mice. Utilizing this LacZ reporter mouse we confirm the expression of Gpr126 is restricted to the endocardium lining the ventricular wall but not only, at embryonic day E11.5. In addition Gpr126 expression is observed in the endocardium of the atrial chambers at E11.5. The localized expression of Gpr126 is supported by immunostaining of mouse embryo cryosections with anti-GPR126 antibody (Monk et al. 2015) which specifically recognizes the C-terminal part of GPR126. In order to generate a conditional knockout mouse with possibility of cell type specific deletion of Gpr126, we crossed Gpr126tm1a(EUCOMM)Hmgu mouse with Git(ROSA)26Sortm1(FLP1)Dym/J. This mating resulted in the deletion of the LacZ cassette. The new strain, Gpr126tm1b(EUCOMM)Hmgu presents 2 loxP sites flanking exon 7 and 8 of Gpr126. To verify that endocardial Gpr126 is essential for trabeculation, we crossed the Gpr126tm1b(EUCOMM)Hmgu with Tg(Tek-cre)1Ywa/J resulting in endocardial deletion of Gpr126. The endocardial deletion of Gpr126 resulted in embryonic lethality, confirmed by Prof. Kelly Monk laboratory in Washington (USA). In addition we performed overexpression experiments of full length Gpr126 in zebrafish by mRNA injection in single celled embryos expressing myocardial specific GFP. At 80 hours post fertilization, when trabeculae begin to appear, we observed a thinner ventricular wall and a higher number of trabeculae compared to the wildtype embryos. These data taken together indicate that the endocardial expression of Gpr126 is essential and sufficient for the initiation and development of heart trabeculation.