ASCC2 as a novel regulator of cardiac development

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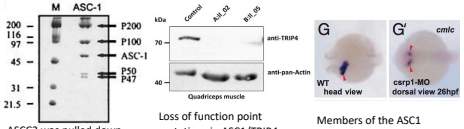
1 ASCC2 was identified as a member of the ASC1 transcription coactivator complex



ASCC2 was pulled down with ASC-1 along with two other members, ASCC1 and ASCC3, which form the ASC1 transcription coactivator complex in HeLa cells and has been shown to interact with transcription factors.

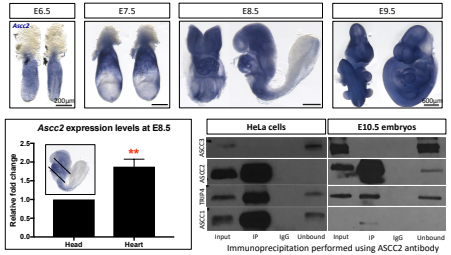
No *in vivo* models for Asc1 complex members!

Loss of function point mutations in ASC1/TRIP4 or ASCC1 in human patients led to defects in the musculo-skeletal, nervous, and cardiovascular systems resulting in post natal lethality.

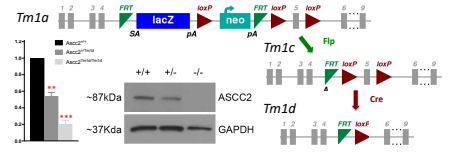
Members of the ASC1 complex interact with LIM domain superfamily protein CSRP1 in humans. Loss of *csrp1* in zebrafish leads to cardiac progenitor migration defects resulting in cardia bifida.

Knierim et al., The American Journal of Human Genetics, 2016.
Miyasaka, K. Y., et al., *PNAS*. 2007.
Jung et al., Molecular and Cellular Biology, 2002.

2 *Ascc2* is expressed broadly and interacts with other ASC1 complex members in early mouse embryos

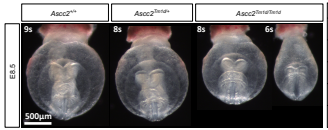


3 *Ascc2* alleles generated through the Knock Out Mouse Project result in loss of function mutants



Conditional ready *Ascc2*^{Tm1c} and knockout *Ascc2*^{Tm1d} alleles were generated from the *Ascc2*^{Tm1a} allele by recombination crosses using *Flp* and *Ella-cre* mice. *Ascc2* transcript levels were significantly reduced in *Ascc2*^{Tm1d}homozygous embryos at E8.5 and no protein was detected.

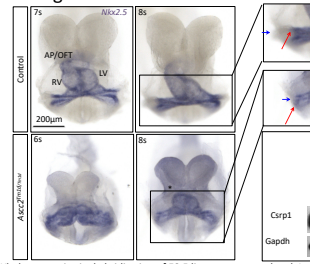
4 *Ascc2* mutants exhibit distinct embryonic phenotypes and early post implantation lethality



	WT	Het	Mutant
E3.5 (n=26)	23%	50%	27%
E7.5 (n=35)	26%	46%	28%
E8.5 (n=119)	27%	49%	24%
E9.5 (n=24)	21%	49%	30%
E12.5 (n=24)	29%	71%	0%

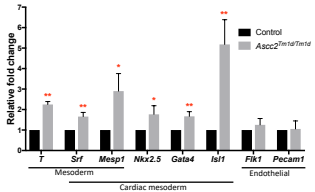
Bright field images of E8.5 embryos (Left): *Ascc2*$^{Tm1d/Tm1d}$ mutants are smaller in size and show underdeveloped hearts with no apparent looping. Table showing window of lethality (Right).

5 *Ascc2* mutants lack clear heart structures and possible defects in early cardiac mesoderm cell migration

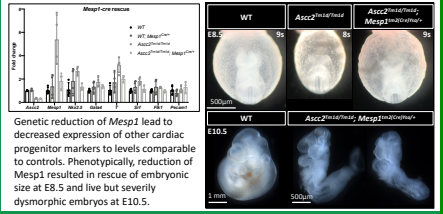


Whole mount *in situ* hybridization of E8.5 littermate control and *Ascc2* mutant embryos for *Nkx2.5* and western for Csrp1. AP/OFT arterial pole/out flow tract; RV right ventricle; LV left ventricle; asterisk indicates possible missing AP/OFT; red arrows: boundary of cardiac region; blue arrows: outer boundary of embryonic body. Western blot for CSRP1 shows higher expression in the cardiac region compared to the rest of the embryo.

6 Cardiac mesoderm markers are up regulated in *Ascc2* mutant embryos at E8.5



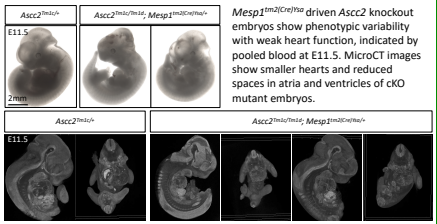
7 Reduction of *Mesp1* in *Ascc2* mutants results in partial molecular and phenotypic rescue



Genetic reduction of *Mesp1* lead to decreased expression of other cardiac progenitor markers to levels comparable to controls. Phenotypically, reduction of Mesp1 resulted in rescue of embryonic size at E8.5 and live but severily dysmorphic embryos at E10.5.

8 Loss of *Ascc2* in *Mesp1* expressing cells results in cardiac defects and embryonic lethality by E12.5

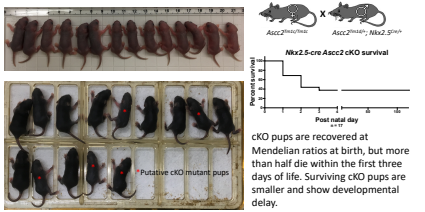
	Ascc2$^{Tm1c/+}$	Ascc2$^{Tm1c/Tm1d}$	Ascc2$^{Tm1c/+}$; Mesp1$^{Tm2(Cre)Yba/+}$	Ascc2$^{Tm1c/Tm1d}$; Mesp1$^{Tm2(Cre)Yba/+}$
E11.5 (n=10)	20%	10%	40%	30%
E13.5 (n=18)	17%	17%	27%	39%
P0 (n=26)	27%	35%	38%	0%



Mesp1$^{Tm2(Cre)Yba}$ driven *Ascc2* knockout embryos show phenotypic variability with weak heart function, indicated by pooled blood at E11.5. MicroCT images show smaller hearts and reduced spaces in atria and ventricles of cKO mutant embryos.

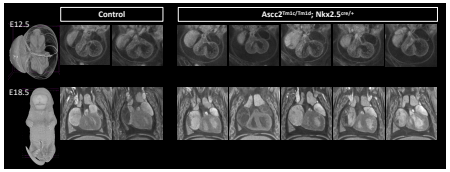
9 *Nkx2.5-cre* driven conditional knockout mutants are sub-viable during post natal stages

	Ascc2$^{Tm1c/+}$	Ascc2$^{Tm1c/Tm1d}$	Ascc2$^{Tm1c/+}$; Nkx2.5$^{cre/+}$	Ascc2$^{Tm1c/Tm1d}$; Nkx2.5$^{cre/+}$
P0 (n=60)	17%	22%	33%	28%
P21 (n=51)	20%	25%	39%	16%



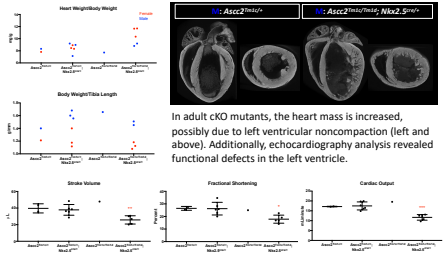
cKO pups are recovered at Mendelian ratios at birth, but more than half die within the first three days of life. Surviving cKO pups are smaller and show developmental delay.

10 *Nkx2.5 Ascc2* cKO mutants show varying degrees of cardiac abnormality during late gestation



MicroCT imaging of whole mount E12.5 and E18.5 control and Nkx2.5-cre cKO embryos. Isolated cardiac regions show slightly dysmorphic hearts in cKO mutants compared to controls at E12.5. By E18.5 a subset of cKO mutants hearts appear largely normal while the majority show ventricular defects similar to trabecular noncompaction.

11 *Nkx2.5 Ascc2* cKO adult mutants exhibit physiological and functional cardiac defects



In adult cKO mutants, the heart mass is increased, possibly due to left ventricular noncompaction (left and above). Additionally, echocardiography analysis revealed functional defects in the left ventricle.

Conclusions:

1) Loss of *Ascc2* globally results in early post-implantation lethality.
2) *Ascc2*$^{Tm1d/Tm1d}$ mutants show defects in heart tube formation possibly due to defects in cardiac progenitor migration as well as up regulation of cardiac mesoderm markers.
3) Genetic reduction of Mesp1 expression can rescue expression of other cardiac progenitor markers as well as partial phenotypic rescue
4) Conditional knockout experiments show the requirement of ASCC2 in cardiac mesoderm for normal development.

Future directions:

1) Determine whether ASCC2 directly modulates transcriptional activity of cardiac mesoderm genes by targeted ChIP as well as ChIP-seq experiments.
2) Identify potential downstream targets for ASCC2 during development by RNA-seq experiments on control and *Ascc2* mutant embryos.
3) Functional analysis of cardiomyocytes (*in vivo*: regeneration by apical resection; *ex vivo*: proliferation/beating in culture).

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