SUPPLEMENTAL MATERIAL

Supplementary Methods

Animals

The genotype of Ts1Rhr mice [1] (generated from timed matings between Ts1Rhr males and C57BL/6J females) was determined by PCR analysis using primers CCGTCAGGACATTGTTGGA and CCGTAACCTCTGCCGTTCA (Reeves, unpublished data).

In situ hybridisation

In order to generate species-specific probes for Sh3bgr, primers (5' GAAGAGGGAGGAGAGGGAGA 3', 5' TTGTTCTGAAAGCCAAGCCT 3' and 5' ATTCCTTCCTTGGTTTGGCT 3', 5' AGCATAAGGCGCATTTGAAG 3') were used to amplify part of the first exon of murine Sh3bgr and human Sh3bgr gene, respectively, using cDNA from a Tc1 positive mouse. PCR products (516 bp and 632 bp) were cloned into pCR 2.1-TOPO vector using TOPO TA cloning (Invitrogen). Correctly cloned vectors were confirmed by sequencing and the insert was amplified by PCR with M13 forward and reverse primers. The resulting PCR product was used for *in vitro* transcription with T7 polymerase and DIG labeling mix (Roche) to generate DIG-labelled probes for *in situ* hybridisation (ISH). Overnight matings between 129S8:C57BL/6J (F1) Tc1 females and C57BL/6J males were set up as previously described. Pregnant females were sacrificed by cervical dislocation and the embryos were explanted. The head and lower torso at the level of the liver of each embryo was removed and the remaining torso was placed in modified Carnoy's fix (60% EtOH, 30% 37%formaldehyde, 10% glacial acetic acid) overnight. Samples were placed in 70% ethanol then processed through graded alcohols and xylene in a Leica Tissue Processor (ASP300), before being embedded in paraffin to give a transverse orientation through the torso. Samples were sectioned at 7µm thickness, placed onto Superfrost Plus slides and stored at 4ºC until required. ISH was carried out as described previously [2] with slight modifications; proteinase K treatment and 4% PFA fixation were not applied, the post-hybridisation buffer comprised 50% formamide, 1X SSC, 0.1% Tween-20 and hybridization was carried out at 68ºC.

Supplementary Table 1

Heart Defect combinations	Wild type	Tc1
VS alone	7 (8.6)	13 (16.7)
Multiple VS	2 (2.5)	2 (2.6)
VS + OFT	1 (1.2)	7 (9.0)
AVC only	0 (0.0)	1 (1.3)
VS + AVC	0 (0.0)	5 (6.4)
VS + AVC + OFT	0 (0.0)	10 (12.8)

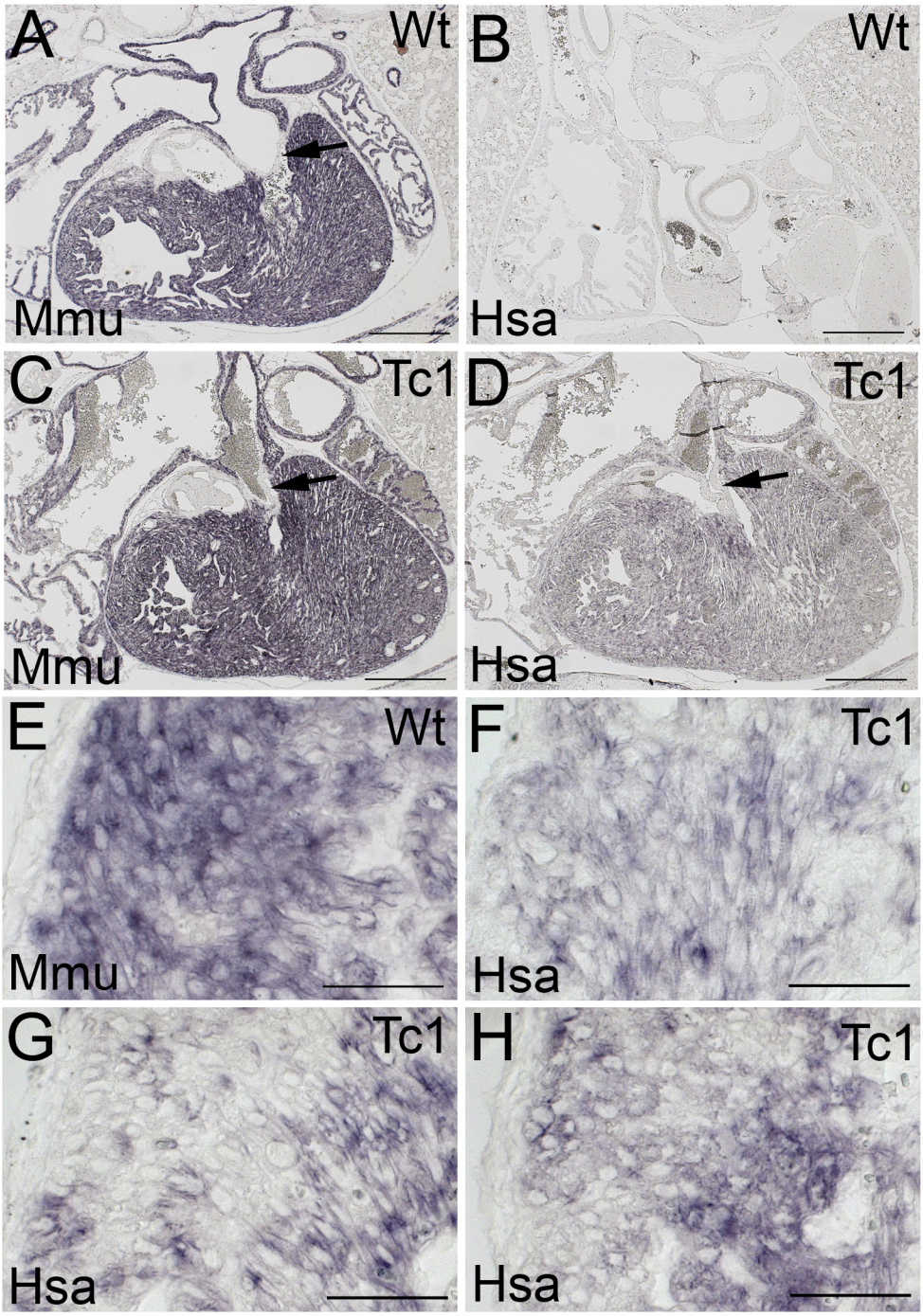
Malformations of more than one region of the embryonic heart are observed in E14.5 Tc1 embryos. Heart defects that affect the ventricular septum; VS, atrioventricular canal; AVC, and outflow tract; OFT are observed alone or in combination in E14.5 Tc1 embryonic hearts. Figures in parentheses show the frequency as the percentage of total samples analysed for each genotype, 81 wild type and 79 Tc1 hearts.

Supplementary Figure 1.

Expression of human-specific transcripts is mosaic in embryonic Tc1 hearts.

In the Tc1 mouse, tissue retention of Hsa21 is mosaic, with approximately 20-50% of cells in the hearts of adult Tc1 mice retaining the Hsa21transchromosome [3] but their distribution is unknown. In human mosaic DS cases, variation in phenotypes have been associated with the percentage of trisomic cells [4] and to examine if number or distribution of Hsa21-containing cells correlates with phenotype incidence in the Tc1 heart, we have used RNA *in situ* hybridisation with species-specific probes for the myocardially-expressed, SH3 domain-binding glutamic acid-rich protein [5] to identify Hsa21-containing cells in sections from Tc1 hearts.

Both human and mouse specific probes gave the expected expression pattern on transverse sections through E14.5 and E18.5 Tc1 embryo torsos, whilst only expression of the mouse transcript was detected on sections from wild type littermates. In all eight Tc1 samples analysed (three at E14.5 and five at E18.5), the human transcript was detected in all regions of the myocardium. Higher magnifications revealed some apparent variation in the proportion of cells expressing the human transcript (panels F-H), presumably reflecting mosaicism of Hsa21 distribution or retention. Three of the eight samples analysed in this way showed VSD, but these were not associated with any preferential localisation of Hsa21-expressing cells or degree of mosaicism as assessed by RNA *in situ* hybridisation. These results indicate that, at least in the myocardium, Hsa21-containing cells were not preferentially localised nor was their any evident link between their frequency and the presence of morphological abnormalities. Other tissues such as the endocardial cushions, the mesenchymal cap of primary atrial septum and dorsal mesenchyme protrusion (DMP or vestibular spine) are important for septation and AV junction formation, but for these no equivalent species-specific gene expression markers have so far been identified.



Supplementary Figure 1 legend
In situ hybridisation with probes for the mouse *Sh3bgr* and its human orthologue at E18.5 (**A**-**D**) and E14.5 (**E**-**H**) using paraffin sections from Tc1 (**C**, **D**, **F**-**H**) and wild type (**A**, **B** and **E**) littermates. Each species-specific probe gave expression restricted to the myocardium, with no signal in the valve tissue (black arrows, **A**, **C** and **D**). Some variation in the proportion of cells expressing SH3BGR can be seen between Tc1 samples (compare **F**-**H,** right ventricle).
(Scale bars:**A**-**D** 500µm; **E**-**H** 50µm).

Supplementary Movies Legends

Supplementary movie S1
E14.5 3D model eroded in the four-chamber plane to reveal a membranous VSD.
Supplementary movie S2
E14.5 3D model eroded in the short axis from the apex to reveal a muscular VSD.
Supplementary movie S3
E14.5 3D model eroded in the short axis from the atria to reveal DORV.
Supplementary movie S4
E14.5 3D model eroded in the short axis from the atria and in the four-chamber plane to reveal an overriding aorta.
Supplementary movie S5
E14.5 3D model eroded in the short axis from the atria to reveal AVSD with a balanced, common valvar orifice.
Supplementary movie S6
E14.5 3D model eroded in the short axis from the atria to reveal AVSD with separate left and right valvar orifices.
Supplementary movie S7
E18.5 3D model eroded in an oblique plane to reveal DORV with subpulmonary interventricular communication and an anterior aorta
Supplementary movie S8
E18.5 3D model eroded in the short axis to reveal AVSD with a balanced common AV junction, guarded by a common five-leaflet valve.

References

[1] Olson LE, Richtsmeier JT, Leszl J, Reeves RH. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. *Science* 2004;**306**:687-690.
[2] Etchevers HC, Vincent C, Le Douarin NM, Couly GF. The cephalic neural crest provides pericytes and smooth muscle cells to all blood vessels of the face and forebrain. *Development* 2001;**128**:1059-1068.
[3] O'Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, Cooke S*, et al.* An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. *Science* 2005;**309**:2033-2037.
[4] Papavassiliou P, York TP, Gursoy N, Hill G, Nicely LV, Sundaram U*, et al.* The phenotype of persons having mosaicism for trisomy 21/Down syndrome reflects the percentage of trisomic cells present in different tissues. *Am J Med Genet A* 2009;**149A**:573-583.
[5] Egeo A, Di Lisi R, Sandri C, Mazzocco M, Lapide M, Schiaffino S*, et al.* Developmental expression of the SH3BGR gene, mapping to the Down syndrome heart critical region. *Mech Dev* 2000;**90**:313-316.