Supplemental Material

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***MATR3* Disruption in Human and Mouse Associated with Bicuspid Aortic Valve, Aortic Coarctation and Patent Ductus Arteriosus**

DGAP105 clinical history

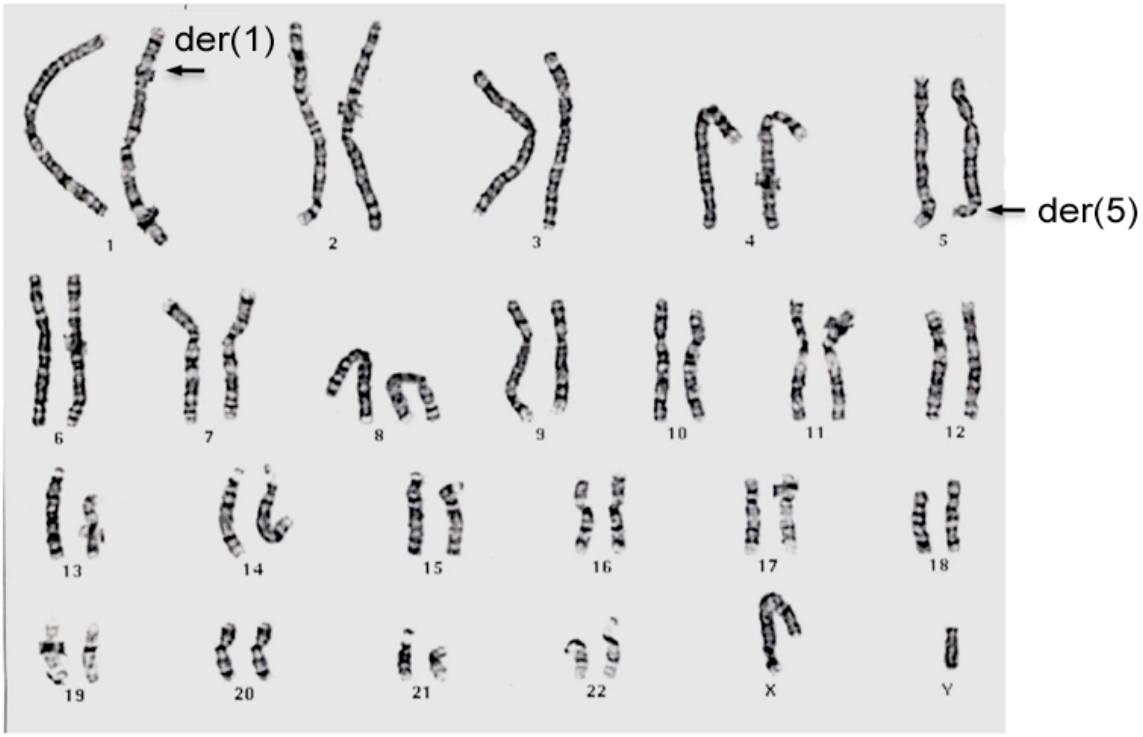
DGAP105 was born following a normal pregnancy with weight 3 kg, length 46 cm and occipitofrontal circumference (OFC) 33.5 cm (all between 3rd and 10th percentiles). **At birth,** he had no spontaneous respiratory effect, Apgars of 1 and 2, and was hypoxic. His cardiac phenotype is summarized in detail under Results. Family history was negative for Noonan Syndrome and for congenital heart disease. **At the age of 18 months** he first walked, and was diagnosed with intermittent exotropia, and hyperopia. **At age of two years,** he required glasses. He was diagnosed with dyspnea, respiratory disease with wheezing and asthma (requiring multiple hospitalizations), sleep disturbance, and had a tonsillectomy, adenoidectomy, and two inguinal hernia repairs for bilateral cryptorchidism and hypospadias. There was history of a single non-febrile seizure at age two, but none thereafter.

At four years plus eight months of age, he was developmentally delayed, with cognitive function comparable to that of a two year-old child; speech was markedly delayed and he could say seven words and knew 16 signs. Dysmorphic features at this time included down-slanting palpebral fissures, bilateral epicanthal folds, hypertelorism, strabismus, a broad nose, smooth philtrum, and thin vermilion border (Fig. 1A). Ears were simple, low set, and posteriorly rotated with thickened helices. The neck was short and the nipples widely spaced, but there was no pectus deformity. There were three posterior and one anterior hair whorls.

At five years of age, examination revealed weight was 18.2 kg (75th percentile), height 102 cm (between 25th and 50th percentiles), and OFC 51.5 cm (between 50th and 75th percentiles). At nine years of age his BMI was at the 90th percentile; height 147 cm (75th percentile), weight 47.3 kg (90th percentile).

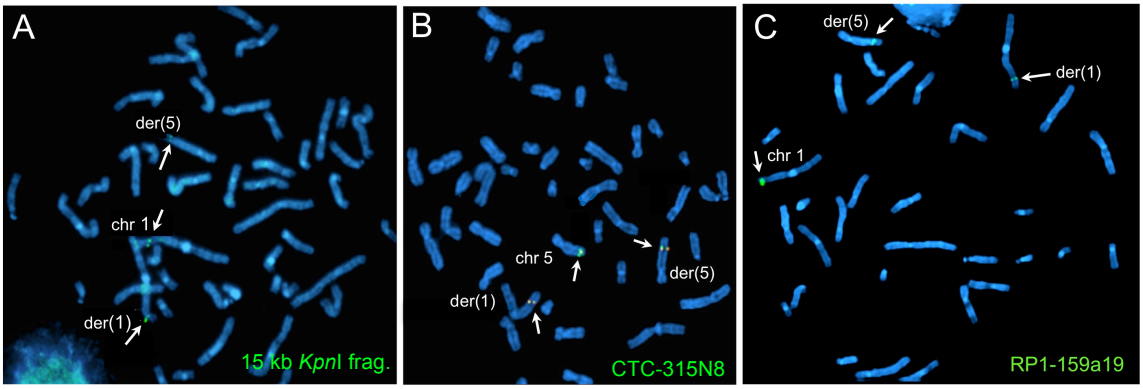
At ten years of age, he could use 50 words and 50 signs to communicate, and continued to have difficulty with fine motor skills. Notably, by this age he had developed significant issues with anxiety, emotional outbursts, and aggressive behavior, with non-compliance and autistic tendencies. He developed a large appetite. He was diagnosed with Pervasive Developmental Disorder, Not Otherwise Specified (NOS; *i.e.,* Autism Spectrum Disorder), with features of ADHD, and was followed in a behavioral clinic. His medications at that time included methylphenidate 36 mg every morning and melatonin 2 mg at bedtime. Additional testing within normal parameters included a renal ultrasound, chest X-ray, head CT scan, EEG, FISH for 22q11.2 deletion, and chromosome microarray analysis. NS due to mutation in *PTPN11*, *KRAS* or *SOS1* was suspected, but subsequently excluded by molecular testing (See Methods). Two younger siblings are in good health.

Supplemental Figure 1



Supp. Figure 1. Karyotype analysis of DGAP105. The GTG-banded karyotype is 46,XY, t(1;5)(p36.11;q31.2)dn (revised from t(1;5)(p35.3;q31.3)) indicated in ref. (36)). Derivative chromosomes are labeled and approximate positions of translocation breakpoints are indicated.

Supplemental Figure 2

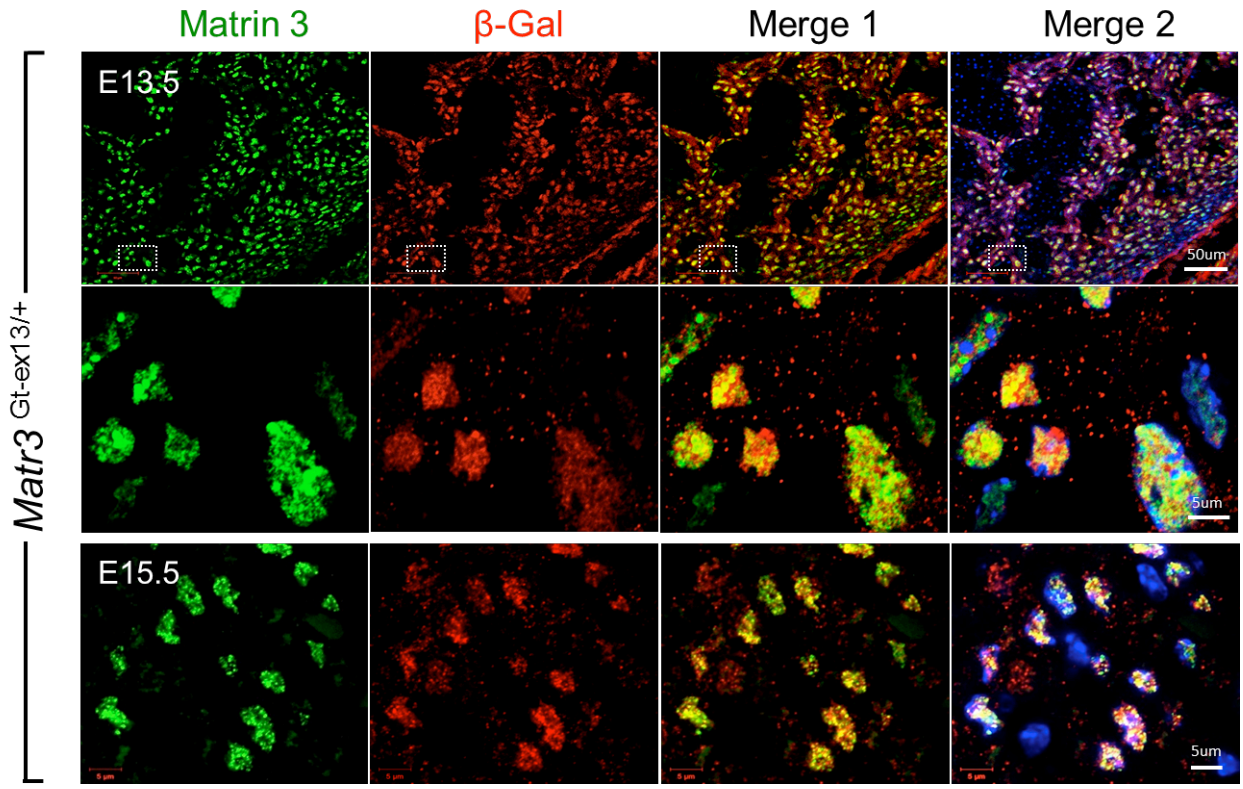


Supp. Figure 2. Fluorescence *in situ* hybridization mapping of the DGAP105 breakpoints. **(A)** Fine resolution mapping of the 1p36.11 breakpoint in DGAP105 using a 15 kb *Kpn*1 restriction fragment probe (Supp. Fig. 3A). Hybridization to the normal chromosome 1 and to both der(1) and der(5) chromosomes is observed. **(B)** FISH confirmation of 5q31.2 breakpoint in DGAP105 using BAC CTC-315N8 (green signals) shows hybridization present on the normal chromosome 5 and both der(1) and der(5) chromosomes. **(C)** The 1p breakpoint was reassigned to 1p36.11 based on FISH mapping with the split signal shown for PAC RP1-159A19.

Supplemental Figure 3

Supp. Fig. 3. Summary of the 1p36.11 and 5q31.2 breakpoints in DGAP105. DNA sequences of the der(1) and der(5) junction fragments near the breakpoint site show a 13-bp deletion of chromosome 1 sequence (green) at the der(1), but a 3-bp insertion (red) at the der(5) junction. MATR3 and AHDC1 sequence on derivative chromosomes shown in purple and blue in der (1) and der (5) respectively. Note that on the der(1) the underlined bases (TC) at the junction could theoretically derive from either chromosome. The bold A (black) represents a difference from the human reference sequence and exists on both normal and der(5).

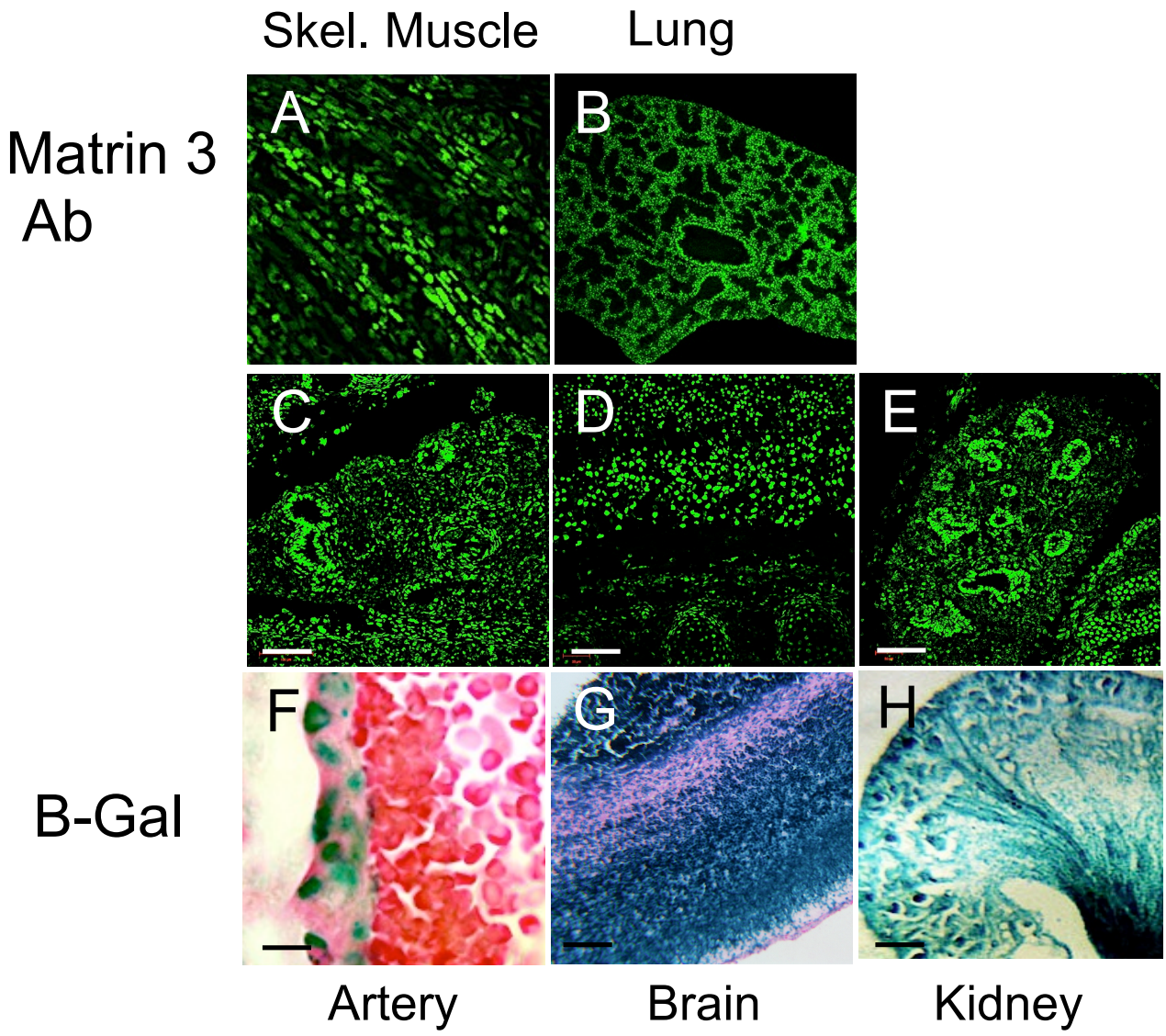
Supplemental Figure 4



Supplemental Figure 4. Matrin 3 fusion protein expression in embryonic *Matr3*$^{GT\text{-}ex13/+}$ heterozygous cardiomyocytes. Upper panels show immunofluorescent staining of *Matr*$^{Gt\text{-}ex13/+}$ heterozygous cardiomyocytes at E13.5. Middle panels show higher magnification of boxed regions in the upper panels. Lower panels show immunofluorescent staining at E15.5.

Co-localization of β-Gal and Matrin 3 in embryonic heart tissue at E13.5 and E15.5 is shown by yellow in cardiac muscle cell nuclei.

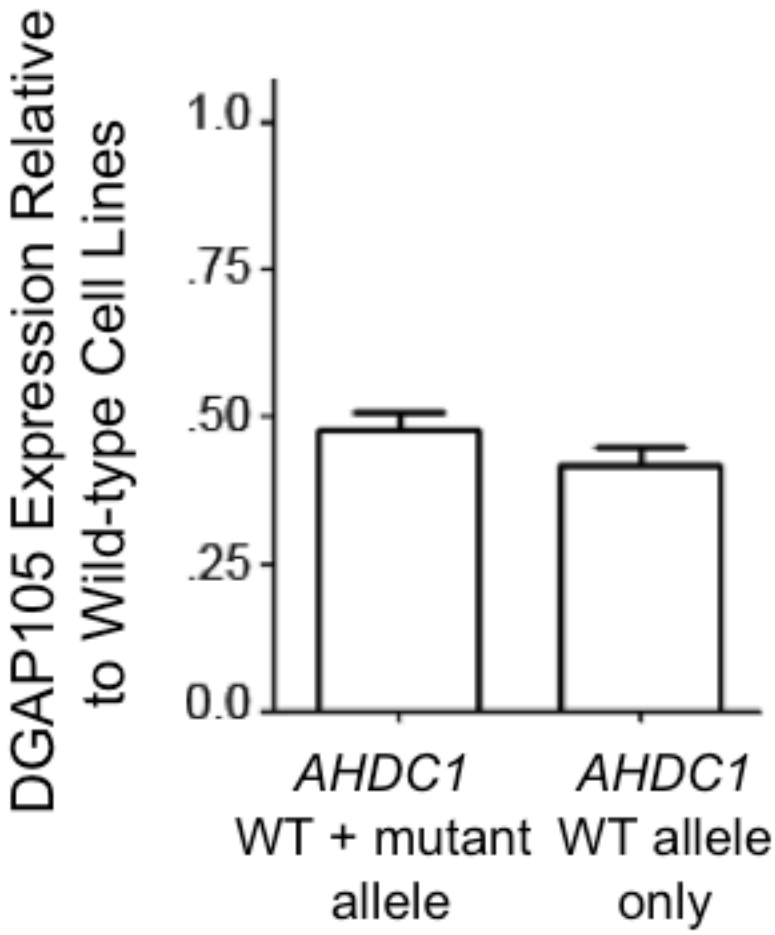
Supplemental Figure 5



Supplemental Figure 5. Matrin 3 expression in developing non-cardiac tissues. Matrin 3 expression is detected with a anti-Matrin 3 antibody by immunofluoresce in newborn wildtype (A-B) and E13.5 *Matr3*$^{GT\text{-}ex13}$ heterozygotes (C-E): (A) distal leg skeletal muscle (100×), and (B)

lung (pulmonary vasculature and pulmonary epithelial cells) (40×). (C-E) Immunofluorescent staining show Matrin 3 expression in the lung, brain and kidney tissues of E13.5 *Matr3*$^{GT\text{-}ex13}$ heterozygotes. Scale bar 50 microns. (F-H) Matrin 3 expression is detected via staining for β galactosidase activity of the β-geo cassette in *Matr3*$^{GT\text{-}ex13}$ heterozygotes in the artery smooth muscle (F), the brain (G) and the kidney (H) as follows: (F) Circumferential pulmonary arterial smooth muscle and endothelial expression; (G) Cerebral cortex expression with LacZ showing positive nuclei in both subventricular and ventricular layers, and outer cortical layers; (H) E13.5 kidney with LacZ expression in both tubular and glomerular components. Scale bar 50 microns (G, H); 10 microns in (F).

Supplement Figure 6



Supp. Figure 6. Expression analyses of human *MATR3*. qRT-PCR of DGAP105 lymphoblastoid cells for *AHDC1* transcripts. The ratio of expression in DGAP105 compared to the mean of five control male lymphoblast lines (defined as 1.0) was calculated by the $2^{-\Delta\Delta CT}$ method (46).