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Supplemental information

POLR1A variants underlie phenotypic heterogeneity in craniofacial, neural, and cardiac anomalies

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Supplemental note: Case Reports

All growth parameter standard deviations for height and weight were calculated using Centers for Disease Control (CDC) growth charts for individuals over age 2, and World Health Organization (WHO) growth charts for those under age 2 unless otherwise noted. Standard deviation for head circumference (HC) was calculated using Rollins data[1]. Individual genotypes and phenotypes are summarized in Table S5.

Individual 1 is the older brother of individual 2. He had bilateral cleft lip and palate, metopic craniosynostosis, and bilateral hydroceles. Echocardiogram obtained to evaluate a murmur was normal. He had a clinical single nucleotide polymorphism (SNP) microarray which identified a likely benign 220 kb deletion at 7p12.3. He died unexpectedly at age 3 months after undergoing cleft lip repair. Post-mortem testing confirmed he shares the *POLR1A* c.176A>T; p.(Asp59Val) variant identified in his brother (Individual 2).

Individual 2, brother of Individual 1, presented as an infant with left-sided cleft lip and palate and metopic craniosynostosis. He had a normal echocardiogram and brain magnetic resonance imaging (MRI), both obtained due to his brother's sudden death. As an infant, he had a polysomnography study which identified mixed obstructive and central apnea, treated with supplemental oxygen. At 4 months of age, his weight gain plateaued, and he was diagnosed with frank aspiration, requiring gastrostomy tube placement. Now age 5, he has some speech difficulties (articulation and resonance) but overall development is on track. He had clinical trio exome sequencing (GeneDx, Gaithersburg, MD) which identified a paternally inherited missense variant in *POLR1A* c. 176A>T; p.(Asp59Val), which was also confirmed to be present in his deceased brother. The father of individuals 1 and 2 (not included in this series) was noted to have bitemporal narrowing and a prominent metopic ridge.

Individual 3 was the first child to non-consanguineous parents, delivered at term following an uncomplicated pregnancy. Birth parameters were weight 3.145kg (40th centile), length 30.7cm (40th centile), head circumference 33.5cm (40th centile). She was noted to have congenital moderate-to-severe sensorineural hearing loss in the newborn period and was admitted to intensive care at 6 weeks of age due to poor feeding, lethargy and respiratory distress. She was found to have hypertrophic cardiomyopathy and elevated lactate. Physical exam was notable for microcephaly, congenital nevus on the scalp, hypotonia and micrognathia. Head circumference was 34.7 cm (<3rd centile) and weight was 3.26 kg (<3rd centile). She had a normal brain MRI. Clinical trio genome sequencing was performed as part of the Acute Care Genomics study (Victorian Clinical Genetics Services, Australia) and identified a *de novo* frameshift variant in *POLR1A* c.190delT; p.(Cys64Alafs*42). RNA sequencing was uninformative. She died at 8 weeks of age after a 2-week hospitalization for management of respiratory distress and heart failure.

Individual 4 is an 18-month-old male who presented to genetics for evaluation of bilateral vocal cord paralysis, feeding difficulties, and hypotonia. He was born at term with birth weight 2.7 kg, length 49.5 cm, and HC 35 cm. Vocal cord paralysis and a glottic web were diagnosed during an evaluation for inspiratory stridor. He required gastrostomy tube placement due to aspiration, and ultimately required tracheostomy for airway management. His physical exam was notable for long tapered fingers with bilateral fifth finger clinodactyly and single palmar crease on the right, mild toenail hypoplasia, and nevus flammeus on his forehead. He had tall and wide palpebral fissures, a short nose with upturned tip, and small slightly low-set ears. Echocardiogram demonstrated anomalous origin of the right coronary artery from the left cusp. Clinical trio exome sequencing (GeneDx, Gaithersburg, MD) identified a maternally inherited variant in *POLR1A* c.1178G>A; p.(Arg393His) and a maternally inherited variant in *PHEX* c.160T>C; p.(Cys54Arg) (NM_000444.6, GRCH 38), both variants of uncertain significance. Clinical SNP microarray was normal. Pathogenic variants in *PHEX* are associated with X-linked dominant

hypophosphatemic rickets and the referring clinician for Individual 4 and his mother (Individual 5) felt that this did not fit their phenotype.

Individual 5 is the mother of individual 4. She had congenital unilateral vocal cord paralysis, short stature (147.5 cm), small HC (< 3rd) ptosis, subaortic ventricular septal defect, myopia, and hypotonia. Prior to the exome which identified the *POLR1A* variant in herself and her son (individual 4), she had a normal karyotype and subtelomeric fluorescent in situ hybridization.

Individual 6 is a 10-year-old female with multiple congenital anomalies: choanal atresia requiring surgical repair, hydrocephalus with C0/C1 stenosis treated with ventriculocisternostomy, and cleft palate. She was noted to have hypertelorism, low-set ears, and micrognathia. She had a normal echocardiogram. At age 10.3 years, her head circumference is 55.5 cm (+2 SD), height is 134 cm (-0.6 SD), and weight is 31 kg (-0.31 SD). Clinical exome sequencing identified a maternally inherited missense variant in *POLR1A* c.1442G>A; p.(Arg481Lys).

Individual 7 is the mother of individual 6. She also has a cleft palate and similar facial features to Individual 6, as well as normal echocardiogram. At age 44 years, her head circumference is 57 cm (+2 SD), height is 160 cm, and weight is 110 kg.

Individual 8 is a 2-year-old boy (now deceased) who was born at 37 weeks gestation to his parents. Prenatally, polyhydramnios was present, as well as concern for micrognathia and craniosynostosis. At birth, he was noted to have craniofacial anomalies including partial acalvaria, marked hypertelorism and telecanthus, ablepharon with bilateral upper eyelid colobomas, low-set anteriorly placed ears with thickened helices and left ear pit, and retrognathia. He had a broad nasal bridge, short columella and large, anteverted nares. He had sparse eyelashes and arched eyebrows with short, neutral palpebral fissures. His second toe overlapped third toe on the right, and his left fourth toe was displaced inferiorly with third and fifth toes overlapping it. Additionally, he had bilateral cryptorchidism, an atrial septal

defect, and right-sided hydronephrosis. Brain MRI at six days of life demonstrated right parietal ischemia and posterior fossa subdural hemorrhage. Head CT at 3 days of life revealed almost completely absent ossified calvarium above the posterior fossa (Figure S1-B). At four weeks of age, he began having seizures, requiring multiple antiepileptic drugs. He also had significant neuro-irritability. Clinical trio exome sequencing (Children's Mercy Hospital, MO) identified a *de novo* missense variant in *POLR1A* c.1488G>T; p.(Met496Ile). At age 2 years, his development globally delayed. He was able to make sounds, laugh, roll over, sit with support, enjoy tastes by mouth, and bring items to midline and pass between his hands. Growth parameters at this time were a head circumference of 55.5cm (-2.31 SD), length of 82 cm (-2.21 SD), and weight of 9.6kg (-3.15 SD). Subsequent head CT scans showed mild progression of skull ossification (Figure S1-C). He passed away at 33 months of age in his sleep.

Individual 9 is an almost 4-year-old male who developed epileptic encephalopathy at age 2.5 years. Epilepsy is a combination of myoclonia, atonic seizures, and multifocal epileptic activity on EEG. He has experienced developmental regression since seizures began but was previously developmentally normal. His epilepsy has been refractive to multiple medications and he is currently on 5 antiepileptic medications (clobazam, topiramate, phenobarbital, brivaracetam,and rufinimide) having previously tried vigabatrin (ineffective), levetiracetam (behavioral problems), and lacosmide (ineffective). His head circumference is -1.3 SD and length is +2 SD. Trio exome sequencing identified a paternally inherited truncating variant in *POLR1A* c.2583_2586del; p.(Asp862*). His father has no history of developmental abnormalities and does not have epilepsy. Testing of paternal grandparents identified the variant in paternal grandfather as well.

Individual 10, previously reported in a cohort of congenital limb deficiency (da Rocha et al., 2021)[2] is a 7-year-old female born at 39 weeks gestation to healthy, non-consanguineous parents. Birth parameters were within normal range (weight 3.38 kg, 50th centile; length 47 cm, 10th centile; HC 35 cm, 75th centile, WHO growth chart). Fetal upper limb defects were diagnosed during the pregnancy and prenatal

karyotype was normal female. At birth she was noted to have a posterior cleft palate, micrognathia, radial dysplasia, and club feet. Physical examination at 9 months of age disclosed a weight of 7.5 kg (10^{th}-25^{th} centiles), length of 68 cm (25^{th} centile), HC of 45 cm (50^{th} centile), facial dysmorphisms (epicanthus, left palpebral ptosis, short nose, hypoplastic alae nasi, long philtrum, retro-micrognathia, posterior cleft palate); short and bowed forearms, with right preaxial polydactyly, adducted left thumb and 5^{th} finger clinodactyly; mild clubbing of the feet. X-rays demonstrate duplication of the right thumb with bifid distal phalanx, short medial phalanx of the 5^{th} fingers, radial hemimelia, as well as triphalangeal halluces. She had a normal abdominal ultrasound, echocardiogram, and cranial computed tomography (CT) scan. Surgical procedures were performed to correct the cleft palate, as well as strabismus. She evolved with normal growth and development but presenting language impairment. Genetic tests disclosed normal chromosomal microarray and normal number of repeats in 5´UTR of *EIF4A3* (for Richieri-Costa-Pereira syndrome). Exome sequencing disclosed a heterozygous, frameshift variant in *POLR1A*, c.3649delC; p.(Gln1217Argfs*10), previously reported (Weaver et al., 2015)[3], and classified as pathogenic. Segregation analysis of this variant in the parents by Sanger sequencing showed its presence in her apparently unaffected father. Review of confirmatory Sanger chromatogram demonstrated no evidence for paternal mosaicism, with equal-sized peaks in father and proband.

Individual 11 is an 11-year-old male with mild hypertelorism, gross motor delay, and spastic dystonia of the legs requiring tendon surgery. Otherwise, he had no intellectual problems and attends normal school. He had a normal brain MRI and normal EEG. Trio exome sequencing was performed as part of a research project identifying genes responsible for movement-disorder phenotypes (Helmholtz Center Munich and Technical University of Munich, Munich, Germany), and identified a *de novo* missense variant in *POLR1A* c.3721G>A; p.(Val1241Ile).

Individual 12 is a 4.25-year-old male with multiple anomalies including left hemifacial microsomia, unilateral radial aplasia (left), several small ventricular septal defects, and pulmonary artery stenosis.

Additionally, he had left diaphragmatic eventration, left enophthalmia with ptosis, left partial hearing loss, difficulty gaining weight but normal psychomotor development and learning. At 4 years 3 months weight is 12kg (-3.2 SD), height is 97 cm (-1.6 SD) and HC 48 cm (-2 SD). Prenatal history was significant for intrauterine growth retardation. Exome sequencing identified a maternally inherited variant in *POLR1A*, c. 3850C>T; p.(Gln1284*). Individual 12's mother is healthy and without congenital anomalies. Review of confirmatory Sanger chromatogram demonstrated no evidence for maternal mosaicism, with equal-sized peaks in mother and proband.

Individual 13 is a 24-year-old male with intellectual disability (non-verbal), autism, global developmental delay, hypotonia, ataxic gait, and generalized epilepsy (multi-drug resistant). Epilepsy began at age 6. Height is 162.6 cm (-1.98 SD). He struggles to maintain his weight, which is 49 kg (-2.69 SD) (body mass index 18.5). Physical exam was notable for ptosis, low-set ears, small feet with high arches and hammertoes, asymmetric thumb nailbeds with left wider than right, hypotonia, and poor coordination. Brain MRI was reportedly normal. Trio exome sequencing (GeneDx, Gaithersburg, MD) identified a *de novo* variant in *POLR1A* c.3988_3990delGAG; p. (Glu1330del) as well as a *de novo* variant in *ATP1A1* c.2769C>A; p.(Phe923Leu) (NM_000701.8, GRCH38). Both are classified as likely pathogenic. Heterozygous variants in *ATP1A1* have recently been associated with a small number of cases of variable neurologic phenotypes ranging from Charcot-Marie-Tooth (peripheral neuropathy) to seizures and intellectual disability[4-6]. Thus, it is possible that both de novo variants contribute to this individual's phenotype (*ATP1A1* and *POLR1A* to neurodevelopmental abnormalities, *ATP1A1* to hammer toes and high arches, *POLR1A* to craniofacial features and wide left thumb nailbed). Of note, an individual with a missense variant in *ATP1A1* at the same codon c.2768T>A, encoding p.(Phe923Tyr) and reported by Dohrn et al (2022), did not have epilepsy.

Individual 14 is a 6-month-old male with craniofacial anomalies, hypotonia, failure to thrive requiring gastrostomy tube placement, unilateral (left) cryptorchidism, right sided inguinal hernia, and

developmental delay. On exam he had hypertelorism and telecanthus with a broad and flat nasal bridge with an upturned nasal tip and anteverted nares, bilateral epicanthal folds, upslanting palpebral fissures, micrognathia, and low-set ears. He had trigonocephaly due to metopic craniosynostosis that was confirmed on a magnetic resonance imaging (MRI) scan of the brain and for which he underwent surgical repair with a strip craniectomy. MRI brain also showed a cavum septum pellucidum. Echocardiogram was normal. At 6 months, his head circumference was 42 cm (15th centile), length 60 cm (<3rd centile), and weight 6.68 kg (3-15th centile). He developed focal epilepsy at 2 months of age which is treated with Keppra. Trio exome sequencing (UCSF Genomic Medical Laboratory) identified a *de novo* variant in *POLR1A*, c.3988_3990delGAG; p.(Glu1330del).

Individual 15 is a 5-year-old male who presented at birth with neurologic and craniofacial anomalies. He was born at term to a 33-year-old gravida 1 mother. Birth weight was 3.03 kg, length 52 cm (85th), HC 33 cm (15-50th). High palate and poor suck were noted during newborn hospitalization. Echocardiogram demonstrated patent ductus arteriosus and patent foramen ovale. He was referred to genetics at 2 months of age for evaluation of hypotonia and craniofacial anomalies. On exam he had hypertelorism and telecanthus, anteverted nares, midline pseudocleft of his upper lip, and high narrow palate. He had low tone and low muscle bulk, and held his feet dorsiflexed and wrists hyperextended. Failure to thrive and poor feeding with aspiration led to gastrostomy tube placement at 6 months of age. He developed infantile spasms at 6 months of age which progressed to multi-drug resistant epilepsy. Trio exome sequencing (GeneDx, Gaithersburg, MD) identified a *de novo* variant in *POLR1A*, c.4685G>T; p.(Cys1562Phe). At age 5, he smiles and has partial head control. He is unable to roll and cannot sit independently. He is learning to communicate using eye gaze and non-verbal communication.

Individual 16 is a 3-year-old male with hypertelorism, hypotonia, and feeding difficulties requiring gastrostomy tube. Infantile spasms began at 3 months of age and progressed to multi-drug resistant epilepsy (absence, myoclonic, and generalized tonic-clonic seizures). Trio exome sequencing identified a

de novo variant in *POLR1A*, c.4685G>T; p.(Cys1562Phe). At age 3, he is non-verbal and is unable to sit independently. His height is 86.7 cm (-2.28 SD) and his head circumference is 46.1 cm (<-2 SD). He had an MRI which demonstrated peritrigonal high signal.

Individual 17 is a 7-year-old female with craniofacial anomalies, aqueductal stenosis (requiring a shunt), multiple vertebral anomalies, and developmental delay. Craniofacial anomalies include cleft palate, bilateral preauricular tags, cleft tragus, cleft eyelids, epibulbar dermoids, right ear canal atresia, and cup-shaped ears. She also had hearing loss, primarily in the left ear with conductive loss on right due to canal atresia. She has generalized epilepsy well controlled on lamotrigine. She required a gastrostomy tube for poor feeding. Exome sequencing (GeneDx, Gaithersburg, MD) of individual 14 identified a *POLR1A* variant c.4891G>A; p.(Val1631Met) which was not maternally inherited. Paternal testing was not performed.

Individual 18 is a 16-year-old female with mandibulofacial dysostosis (micrognathia, malar hypoplasia), congenital ptosis, congenital heart defects (atrial and ventricular septal defects, cleft mitral valve, bicuspid aortic valve, doming pulmonary valve, aortic and pulmonary artery dilation) and limb anomalies (small hands, single palmar creases, interlocked toes requiring surgery, reverse clubbed foot). She also has congenital hypotonia, requiring physical and occupational therapies. She had developmental delays and processing difficulty. Micrognathia was treated with mandibular distraction at age 7, and her photograph in Figure 1 was taken after the distraction surgery. Ascending aortic aneurysm required aortic replacement at age 13. Septal defects and mitral valve also required surgical repair. She had bilateral hip replacement at age 14 due to severe pain related to osteoarthritis and acetabular protrusion. Clinical exome sequencing (GeneDx, Gaithersburg, MD) of individual 18 identified a *POLR1A* variant c.4913C>T; p.(Pro1638Leu) which was not present in her mother. Paternal testing was not performed.

Supplemental Figures and Legends

Figure S1

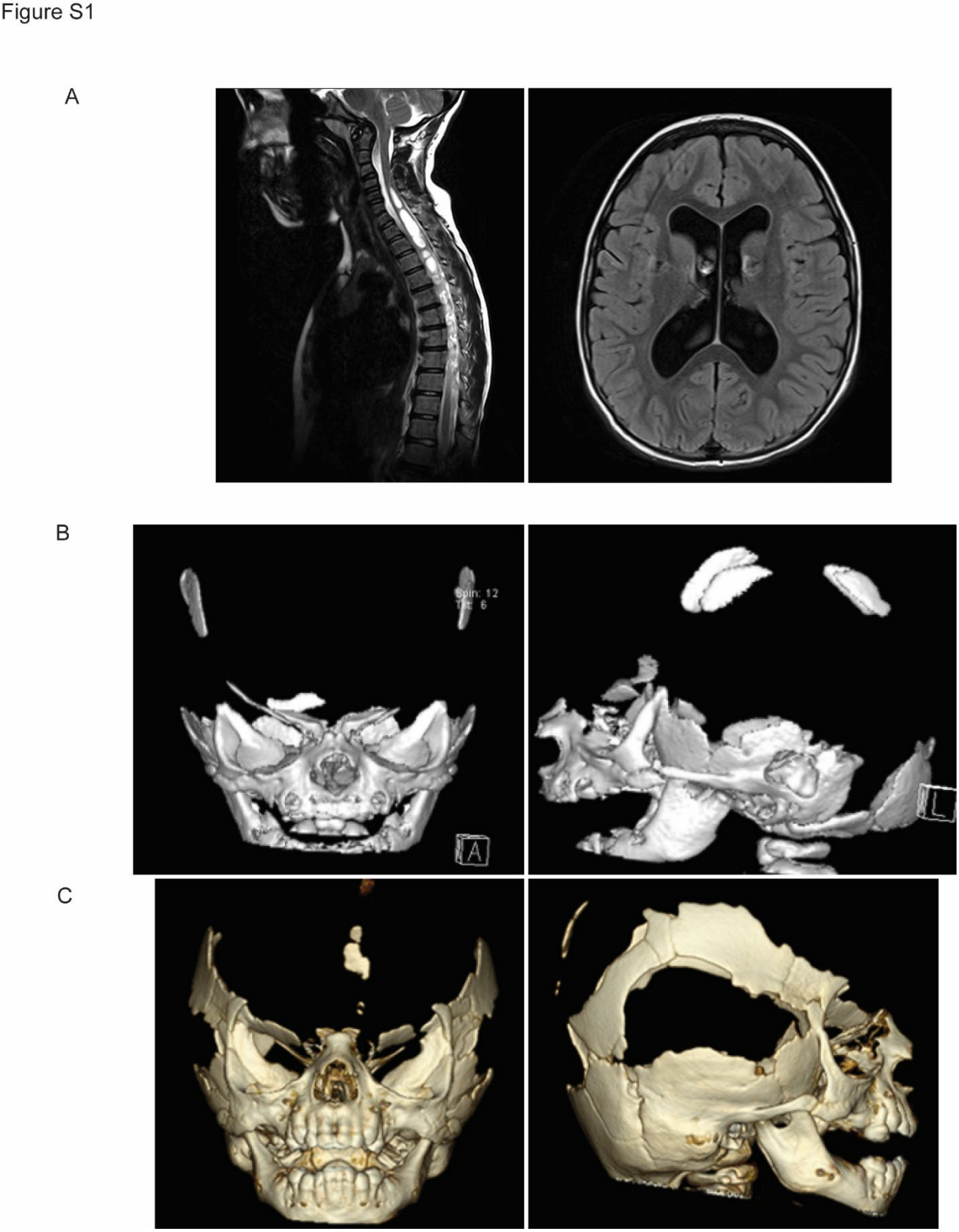
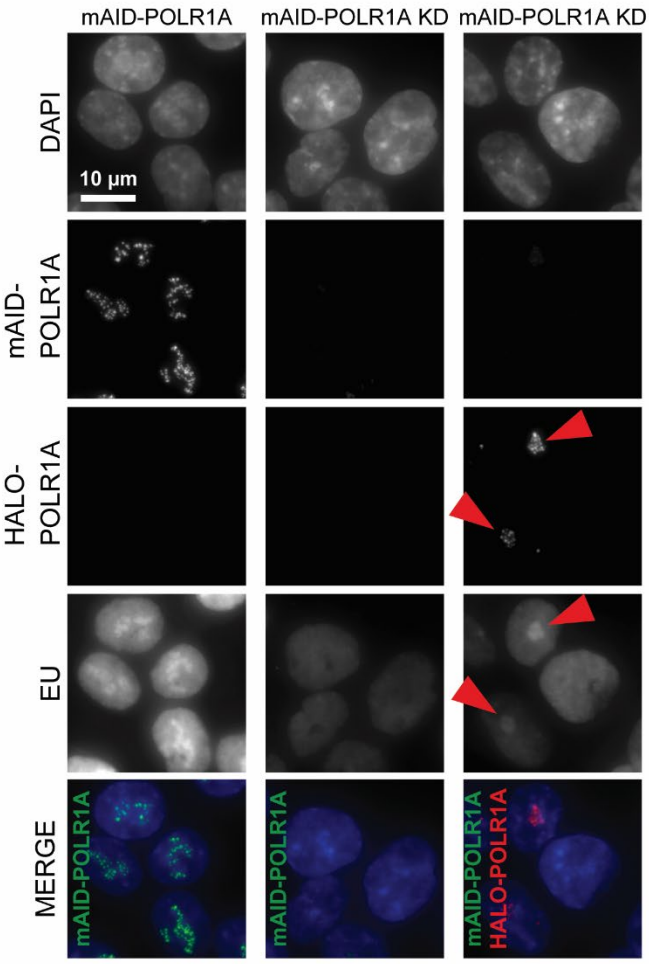


Figure S1: Imaging findings for Individuals 6 and 8. (A) MRI of individual 6 demonstrates extensive syringomyelia (Left) and ventriculomegaly (Right). Head CT with 3D reconstruction of Individual 8 demonstrates (B) almost complete acalvaria at 3 days of age and (C) progressive post-natal ossification evident at 26 months of age.

Figure S2

A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mAID-POLR1A KD
mAID-POLR1A KD
DAPI
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mAID-POLR1A
HALO-POLR1A
EU
MERGE
mAID-POLR1A
HALO-POLR1A

B

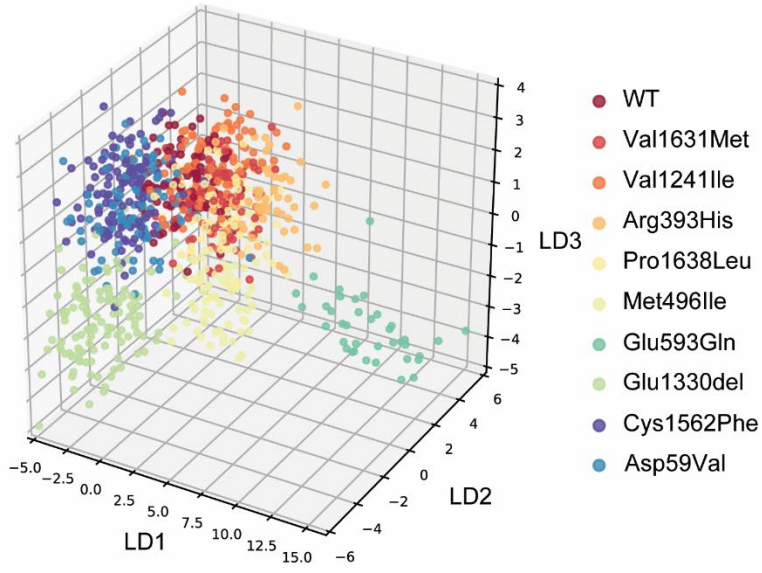
LD1
LD2
LD3
WT
Val1631Met
Val1241Ile
Arg393His
Pro1638Leu
Met496Ile
Glu593Gln
Glu1330del
Cys1562Phe
Asp59Val

Figure S2: (A) Fluorescence intensity of EU in nucleoli in cells without auxin (first column), or with auxin treatment for rapid degradation of endogenous POLR1A (second column) or with the auxin treatment after transfecting the plasmid encoding wild-type POLR1A (third column). First row, DAPI staining; second row, mAID-POLR1A; third row, HaloTag-POLR1A fluorescently labeled with HaloTag ligand TMR; fourth row, EU conjugated with Alexa Fluor 594. Fifth row merged image of DAPI, mAID-POLR1A and HaloTag-POLR1A. Note that EU signals colocalized with HaloTag-POLR1A foci were quantified for evaluation of the transcription activity of HaloTag-POLR1A (arrowheads in third and fourth row). Scale bar, 10 um. (B) Fisher's Linear discriminant analysis (FLDA) of HaloTag-POLR1A image sets of wild type and 9 POLR1A variants (LD1: 30.5%, LD2: 23.1%, and LD3: 9.6%): 3D scatter plotting showing the discrimination of wild type and 9 POLR1A variants. Dots labeled in different colors represent the images of different groups.

Figure S3

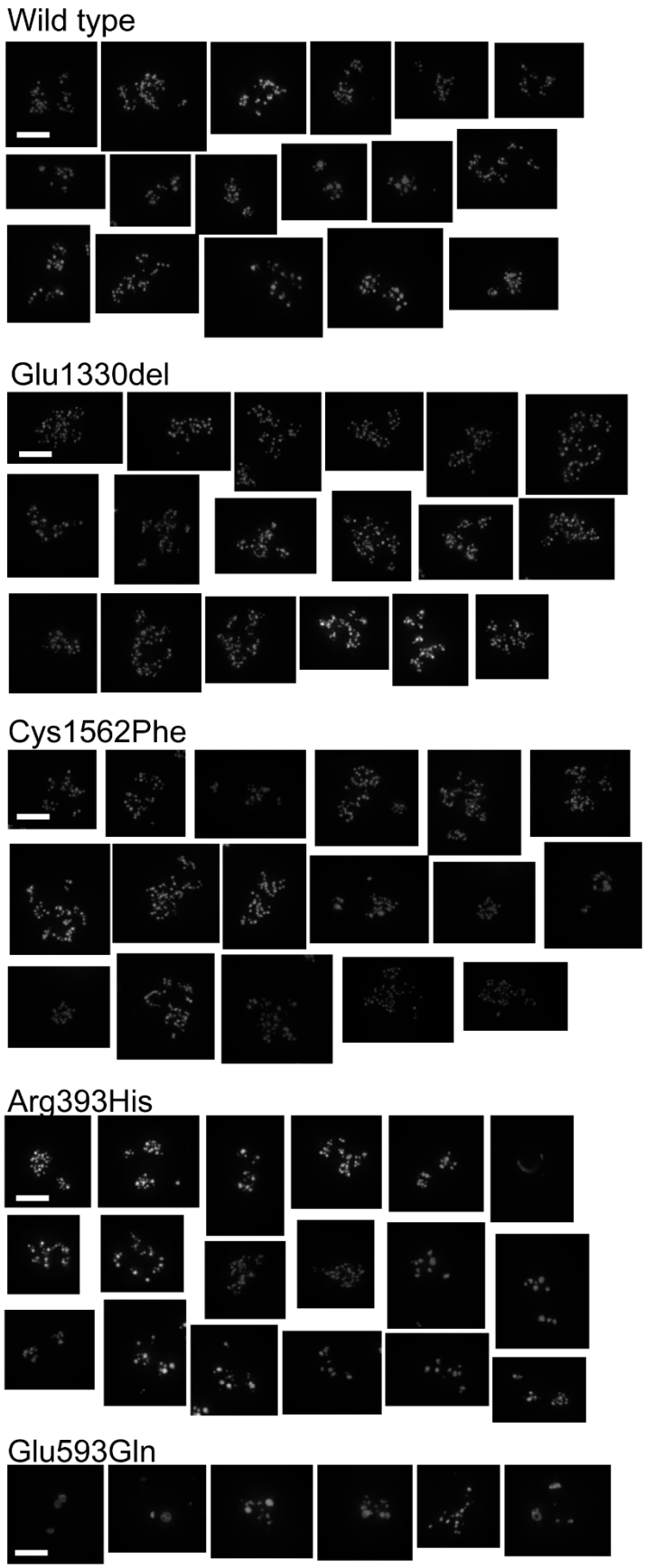

Figure S3: POLR1A variant localization images used for machine learning analysis. Image galleries (wild type, p.(Cys1562Phe), p.(Glu1330del), p.(Arg393His) and p.(Glu593Gln) used for wndchrm analyses described in Figure 2C. HaloTag-POLR1A was fluorescently labeled by TMR ligands. Scale bar, 5 µm.

Figure S4

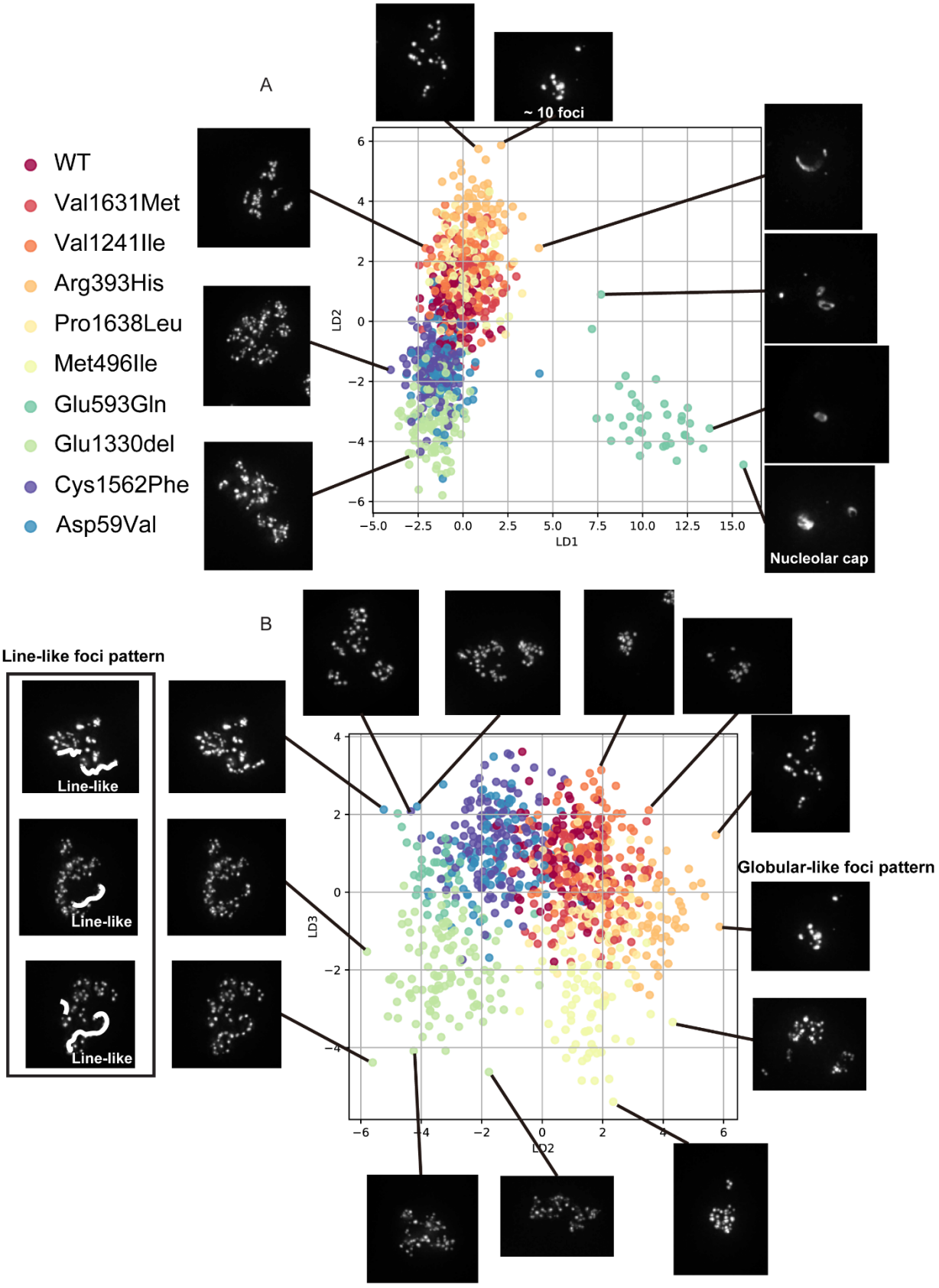
A
WT
Val1631Met
Val1241Ile
Arg393His
Pro1638Leu
Met496Ile
Glu593Gln
Glu1330del
Cys1562Phe
Asp59Val
~ 10 foci
Nucleolar cap
LD1
LD2
B
Line-like foci pattern
Line-like
Line-like
Line-like
Globular-like foci pattern
LD2
LD3

Figure S4: 2D scatter plotting of Fisher's Linear discriminant analysis of HaloTag-POLR1A. (A) X axis is LD1 and Y axis is LD2 of the 3D scatter plotting in Figure S2B. p.(Glu593Gln) foci are biggest and but fewest in number. p.(Arg393His) foci are smaller and more numerous than p.(Glu593Gln) foci. The foci number gradually increase in the order of p.(Arg393His), wild type, p.(Cys1562Phe) (or p.(Asp59Val) or p. (Glu1330del)), which are from top to bottom along the y-axis. (B) X axis is LD2 and Y axis is LD3. The foci clusters of p. (Glu1330del), p.(Asp59Vaol), and p.(Cys1562Phe) on the left side (with low LD2 value) are extensively stretched out and sometimes localized along a line (indicated as line-like foci pattern), while those in the right-side image (with high LD2 value) tend to gather and show globular distribution.

Figure S5

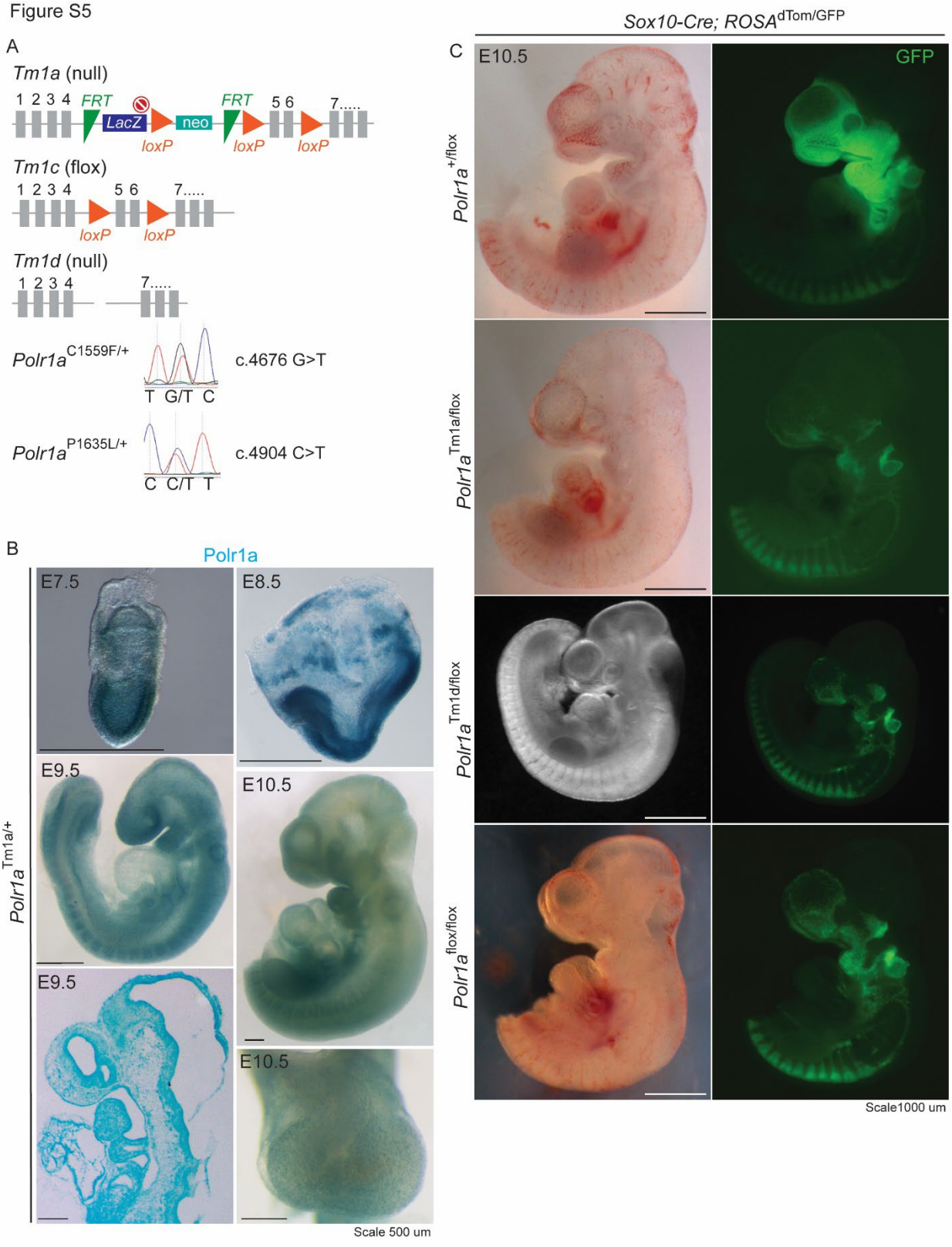
A
Tm1a (null)
1 2 3 4
FRT
LacZ
neo
FRT
5 6
7.....
loxP
loxP
loxP
Tm1c (flox)
1 2 3 4
5 6
7.....
loxP
loxP
Tm1d (null)
1 2 3 4
7.....
Polr1a C1559F/+
T G/T C
c.4676 G>T
Polr1a P1635L/+
C C/T T
c.4904 C>T
B
Polr1a
Polr1a Tm1a/+
E7.5
E8.5
E9.5
E10.5
E9.5
E10.5
Scale 500 um
C
Sox10-Cre; ROSA dTom/GFP
E10.5
GFP
Polr1a +/flox
Polr1a Tm1a/flox
Polr1a Tm1d/flox
Polr1a flox/flox
Scale1000 um

Figure S5: Overview of *Polr1a* alleles and *Polr1a* expression. (A) *Tm1a* and *Tm1d* are both null alleles. *Tm1a* contains a *LacZ* cassette. *Tm1c* is a flox (conditional) allele. *Polr1a*^{C1559F} and *Polr1a*^{P1635L} were created with CRISPR/Cas9 gene editing. (B) Expression of *Polr1a* is ubiquitous but enhanced in branchial arches, frontonasal prominence, and central nervous system at E9. At E10 differential expression in branchial arches is more pronounced. *Polr1a* is expressed in the heart at E10. Scale bar, 500 um. (C) *Polr1a*$^{Tm1a/flox}$, *Polr1a*$^{Tm1d/flox}$, and *Polr1a*$^{flox/flox}$ alleles produce the same phenotype when combined with Cre as exemplified here with *Sox10-Cre*. Sox10-expressing cells are labeled with GFP. Scale bar, 1000um.

Figure S6

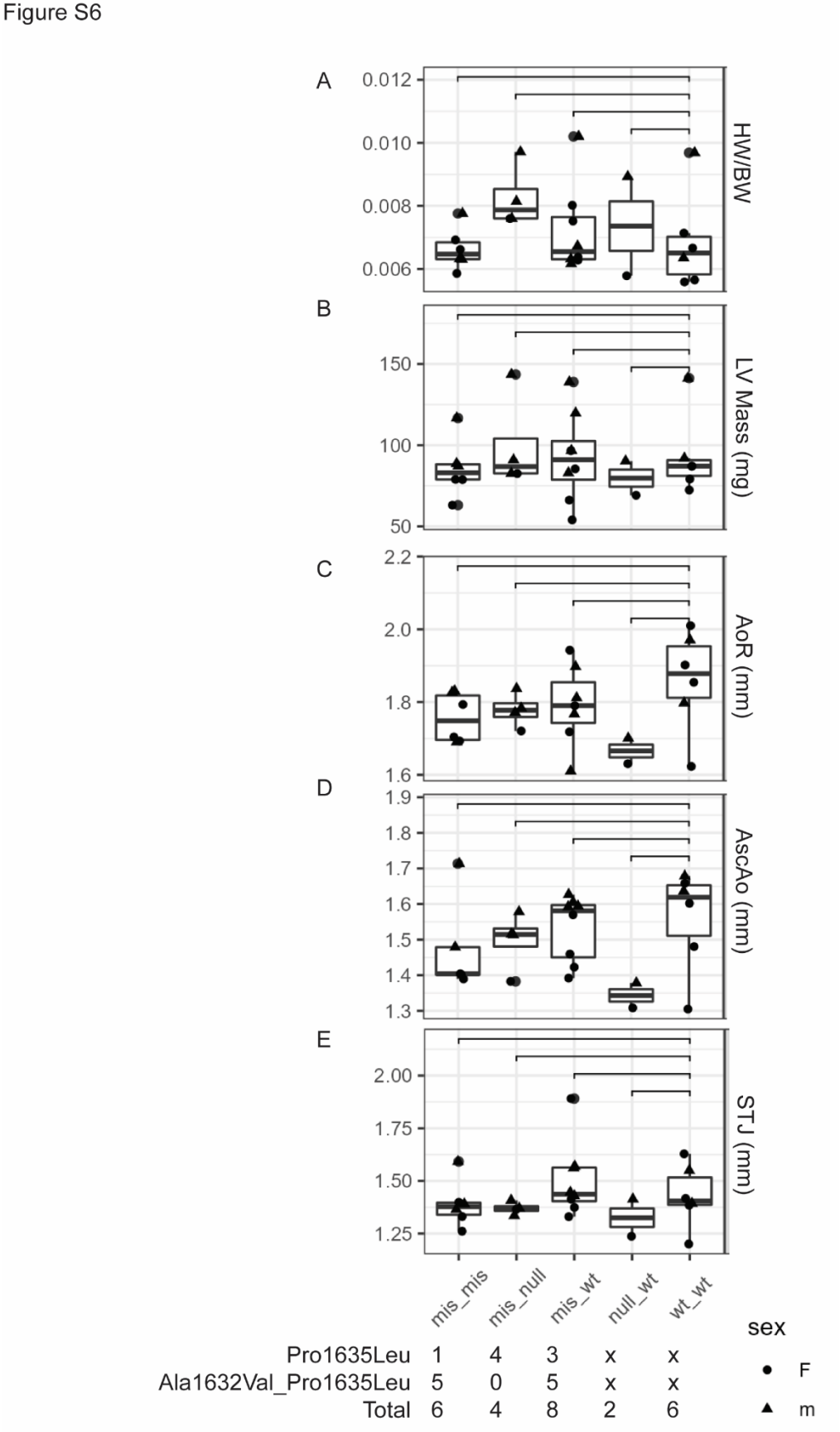
A
0.012
0.010
0.008
0.006
HW/BW
B
150
100
50
LV Mass (mg)
C
2.2
2.0
1.8
1.6
AoR (mm)
D
1.9
1.8
1.7
1.6
1.5
1.4
1.3
AscAo (mm)
E
2.00
1.75
1.50
1.25
STJ (mm)
mis_mis
mis_null
mis_wt
null_wt
wt_wt
sex
F
m
Pro1635Leu 1 4 3 x x
Ala1632Val_Pro1635Leu 5 0 5 x x
Total 6 4 8 2 6

Figure S6: *Polr1a*^{P1635L} and *Polr1a*$^{A1632V\_P1635L}$ homozygotes have normal echocardiograms. (A) Heart weight/Body weight (HW/BW) ratios, (B) Left ventricular (LV) mass, (C) Aortic root (AoR) diameter, (D) Ascending aorta (AscAo) diameter, and (E) Sinotubular junction (STJ) diameter of mutants are not significantly different from controls. Bars indicate comparisons made using Student's t-test. P value>0.05 for all comparisons.

Figure S7

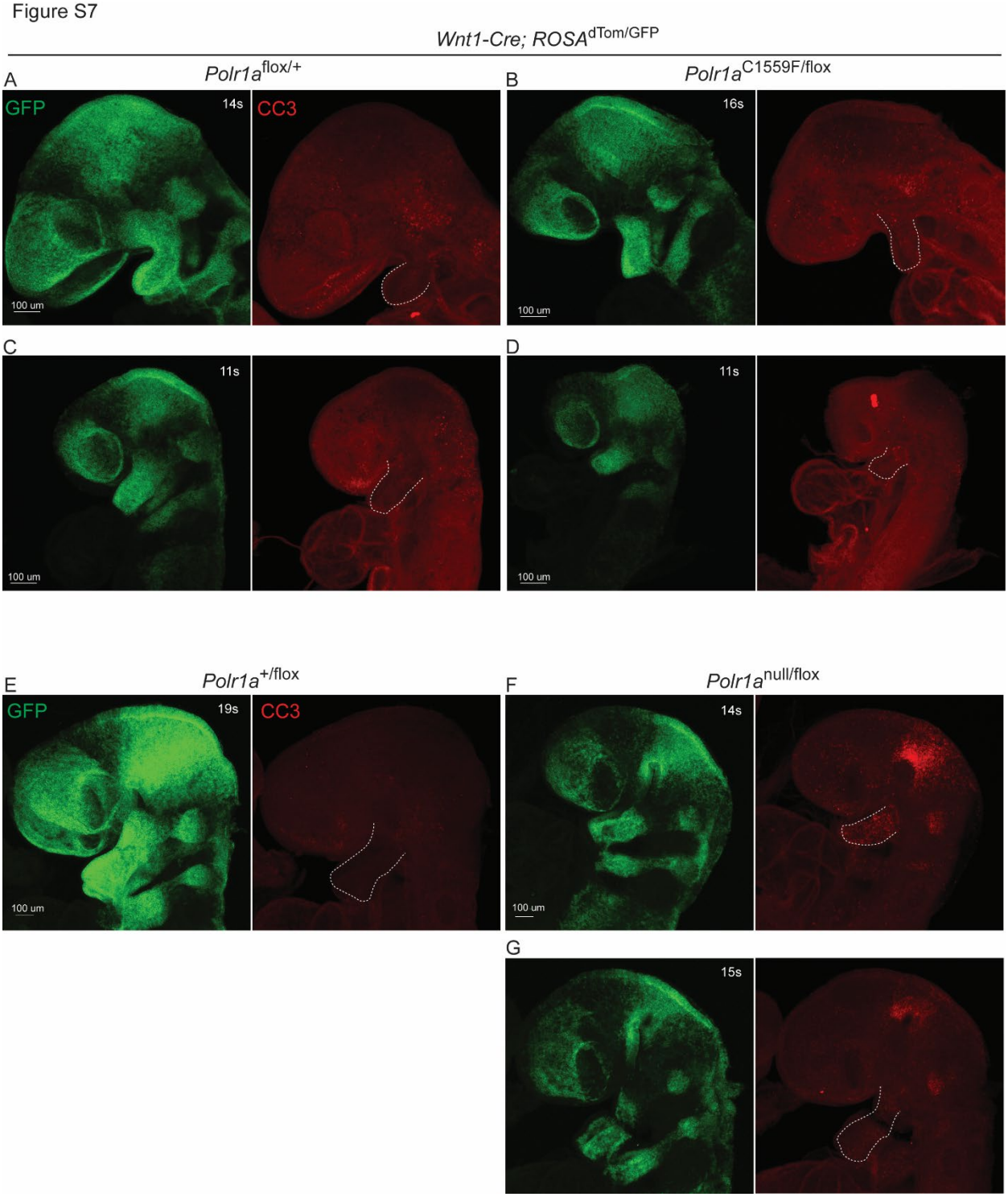
Wnt1-Cre; ROSA dTom/GFP
A
Polr1a flox/+
GFP
14s
CC3
100 um
B
Polr1a C1559F/flox
16s
100 um
C
11s
100 um
D
11s
100 um
E
Polr1a +/flox
GFP
19s
CC3
100 um
F
Polr1a null/flox
14s
100 um
G
15s

Figure S7: CC3 localization in early *Polr1a*$^{C1559F/flox}$ *and Polr1a*$^{null/flox}$*; Wnt1-Cre* mutants. (A-D) At 11-16 somite stages, *Polr1a*$^{C1559F/flox}$; *Wnt1-Cre* mutants do not yet have much CC3 localization apparent in their first arches (dashed white lines) and GFP-labeled NCCs are similar between controls and mutants. A and C each depict a control embryo while B and D depict matched mutant embryos. (E-G) At 14-19 somite stages, *Polr1a*$^{null/flox}$; *Wnt1-Cre* mutants demonstrate robust CC3 localization in their first arches and have reduced GFP-labeled NCCs present (1 mutant each in panels F and G). Scale bar, 100um.

Figure S8

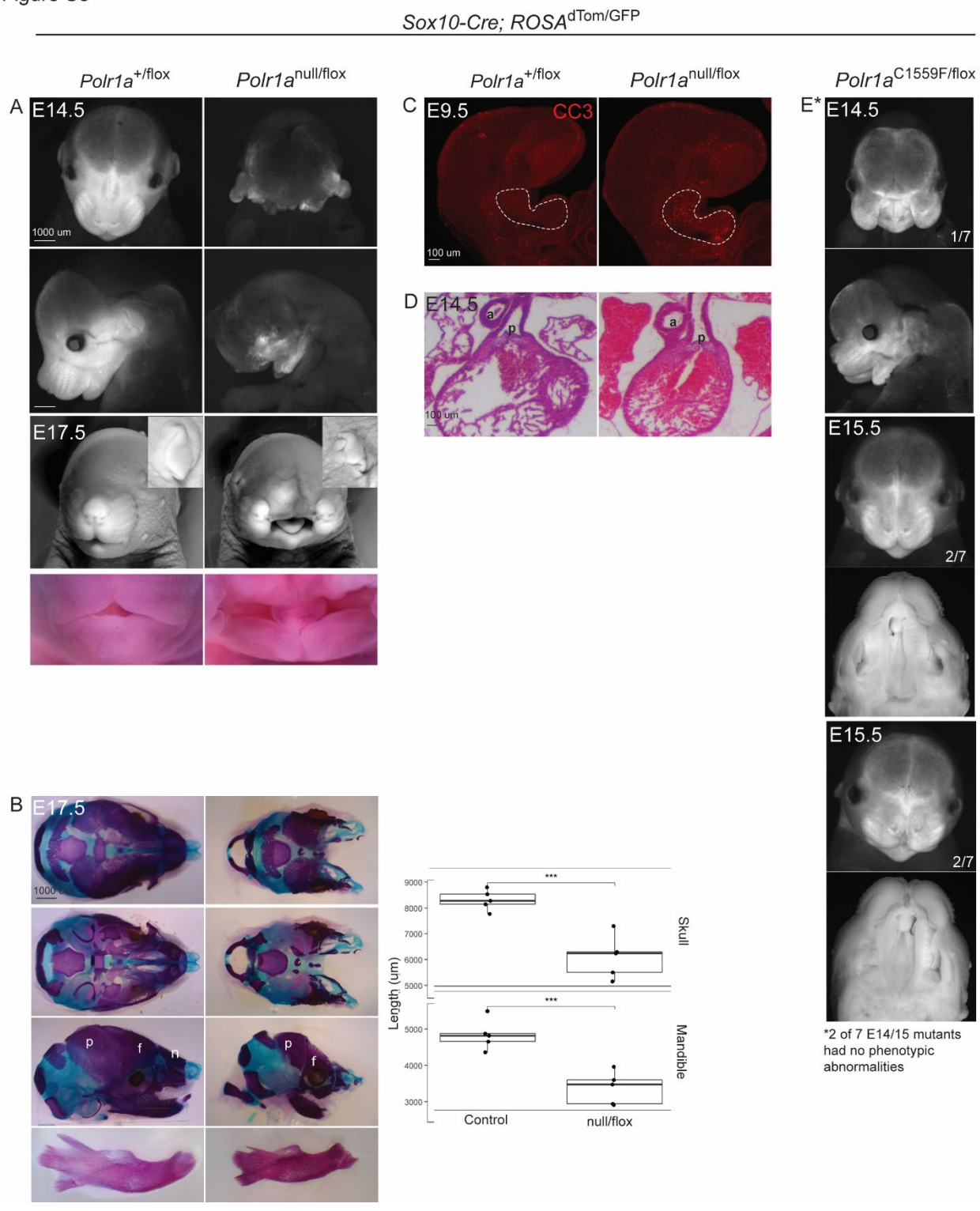
Sox10-Cre; ROSA dTom/GFP
Polr1a +/flox
Polr1a null/flox
A
E14.5
1000 um
E17.5
C
E9.5
CC3
100 um
D
E14.5
a
p
100 um
Polr1a +/flox
Polr1a null/flox
E*
Polr1a C1559F/flox
E14.5
1/7
E15.5
2/7
E15.5
2/7
*2 of 7 E14/15 mutants had no phenotypic abnormalities
B
E17.5
1000 um
p
f
n
p
f
Length (um)
9000
8000
7000
6000
5000
5000
4000
3000
***
***
Skull
Mandible
Control
null/flox

Figure S8: Phenotype of *Sox10-Cre* mutants. (A) Cleft face in *Polr1a*$^{null/flox}$; *Sox10-Cre* mutants at E14 and E17 with microtia and median mandibular cleft at E17. Scale bar, 1000um. (B) Skeletal preparations of *Polr1a*$^{null/flox}$*; Sox10-Cre* embryo skulls at E17 demonstrate hypoplastic skull bones, reduced skull and mandibular lengths, and wide cleft palate. "f" frontal, "p" parietal, and "n" nasal bones. Skull and mandible lengths were compared using Student's t test. Each dot represents a separate animal (n=5 for each group). *** p value < 0.001, * p value <0.05, "ns" not significant. Scale bar, 100um. (C) At E9.5, cell death (CC3 localization) is prominent in the first arch (dashed white line) of *Polr1a*$^{null/flox}$; *Sox10-Cre* mutants. Scale bar, 100um. (D) The outflow tract of *Polr1a*$^{null/flox}$; *Sox10-Cre* mutants is normally separated at E14. Scale bar, 100um. (E) Variable phenotype of *Polr1a*$^{C1559F/flox}$; *Sox10-Cre* mutants at E14-E15 (n=7 total mutants analyzed).

Supplemental References

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