The American Journal of Human Genetics, Volume *98*

Supplemental Data

Mutations in the Histone Modifier *PRDM6* Are Associated with Isolated Nonsyndromic Patent Ductus Arteriosus

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Supplemental Note: Case Report

The index case (III-2, fig.1A, arrow) was diagnosed with PDA at age 17 after she developed shortness of breath and peripheral edema during her first pregnancy. She delivered a healthy full-term child that was found to have a heart murmur and PDA. Further evaluation of the family at that time identified four additional family members with PDA. Individual II-2, who was known to have heart murmur, had fathered affected children by two different spouses, and 4 of his offspring were affected (fig. 1A). Two affected offspring also had children with PDA. In order to determine whether additional cases of PDA have occurred since the initial report, the kindred was reinvestigated and medical records of participating family members were reviewed. Individuals were classified as affected with PDA on the basis of cardiac catheterization or postmortem diagnosis of PDA. Eight individuals have undergone invasive procedures to close the patent ductus between the neonatal period and age 17. One neonate had died from congestive heart failure; with autopsy revealing a hypoplastic left ventricle, ventricular septum defect, mild pulmonary artery stenosis and a large and severely dilated PDA. There were no distinctive syndromic features noted in any of the affected subjects.

Supplemental Figures

Figure S1

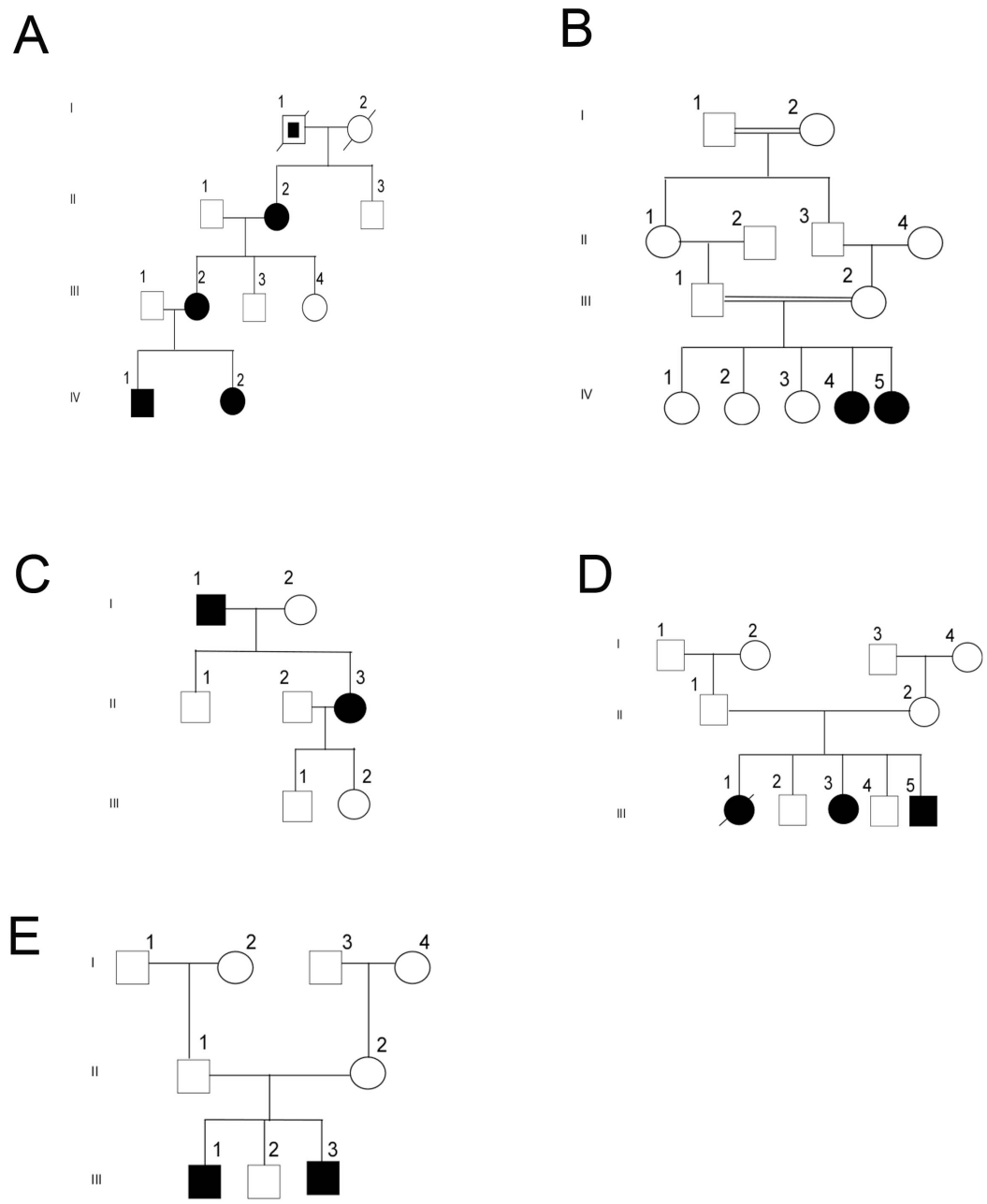
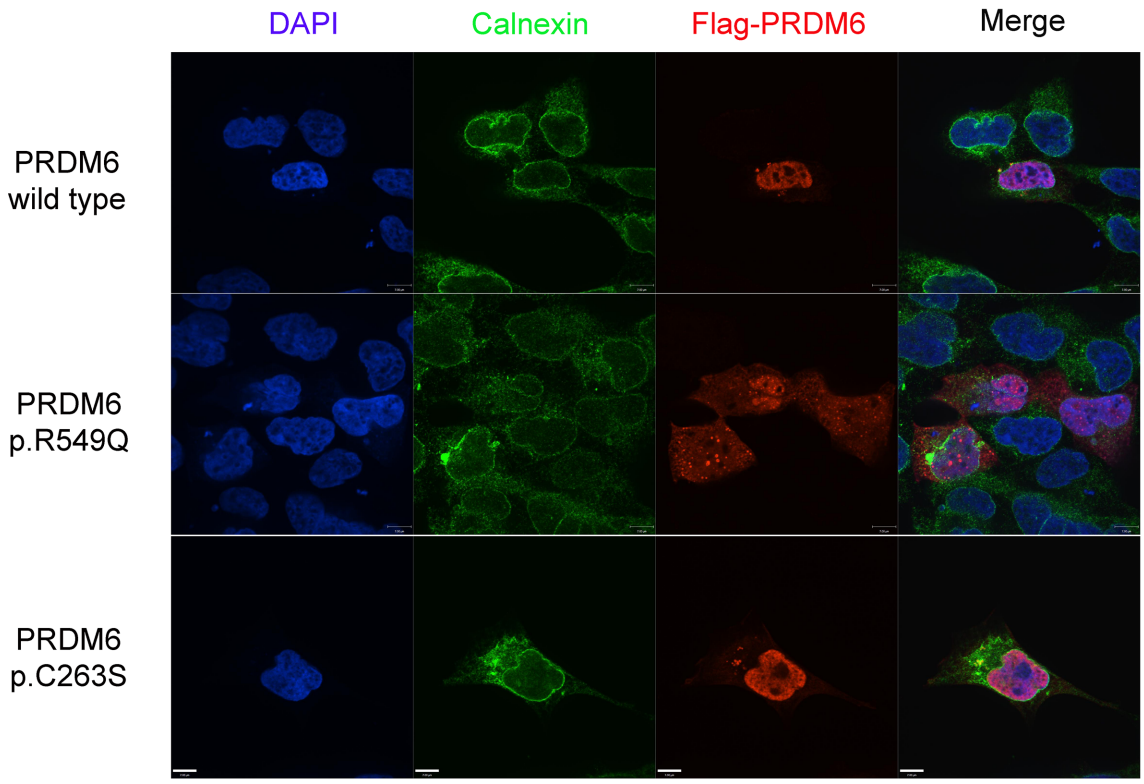


Figure S2

A)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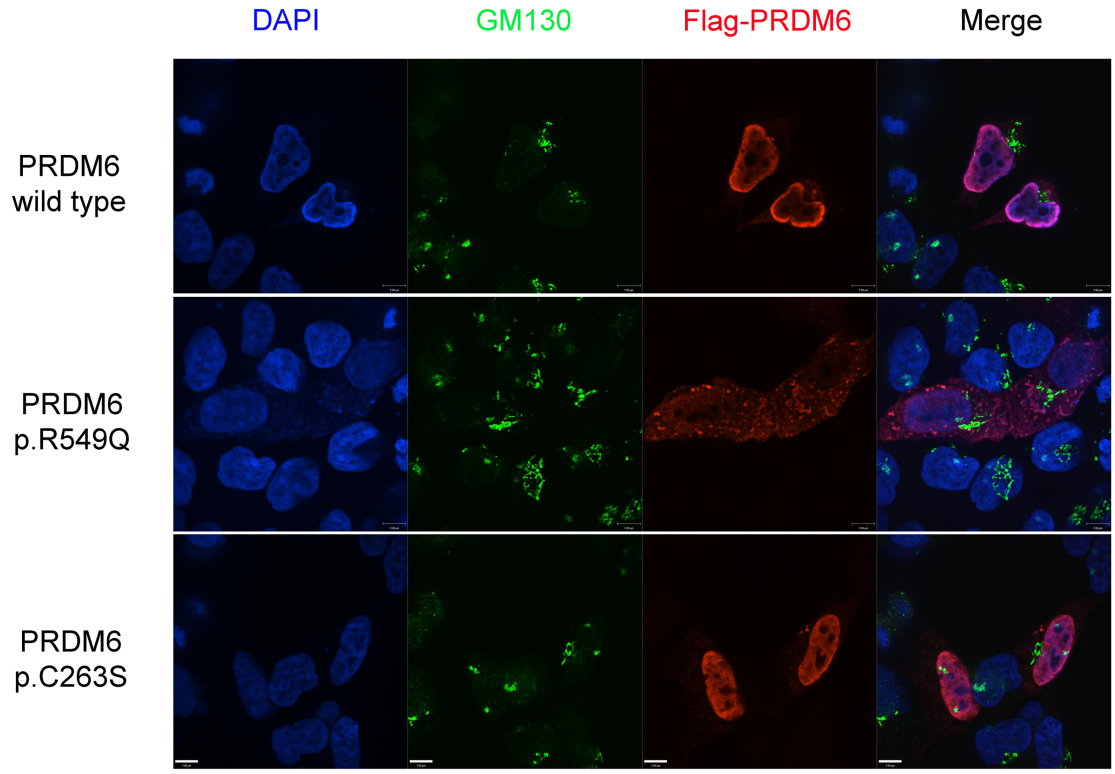
Calnexin
Flag-PRDM6
Merge
PRDM6
wild type
PRDM6
p.R549Q
PRDM6
p.C263S

B)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GM130
Flag-PRDM6
Merge
PRDM6 wild type
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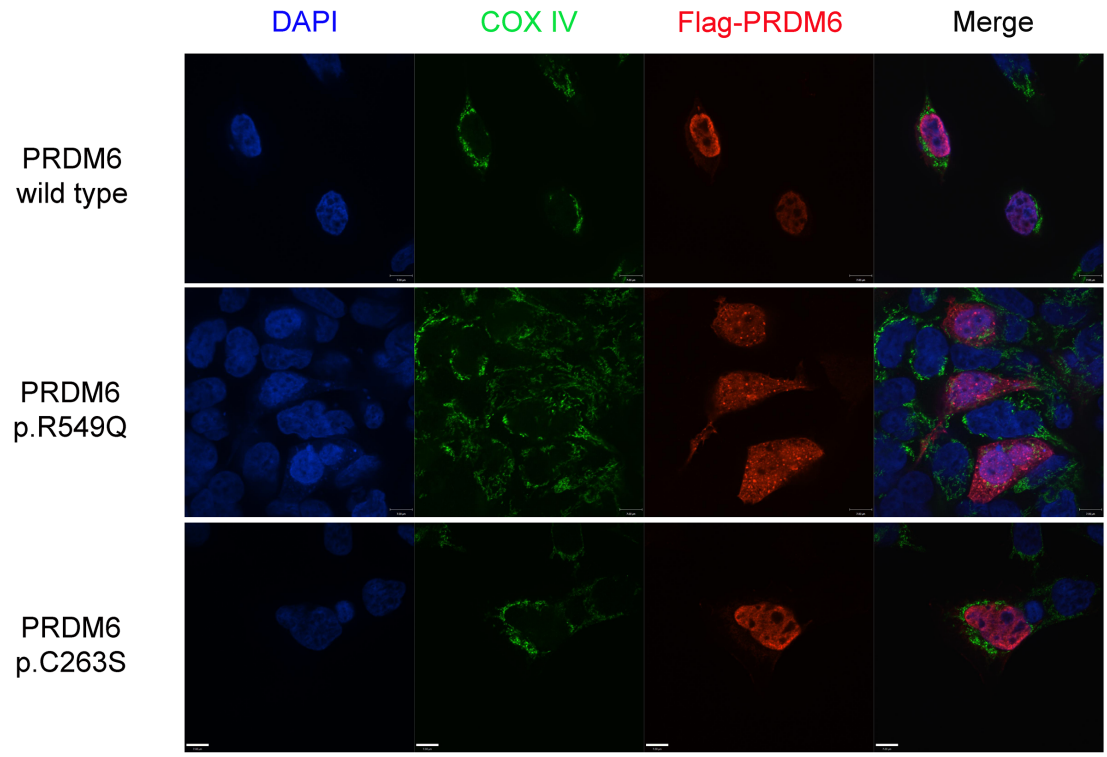
PRDM6 p.C263S

C)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Merge
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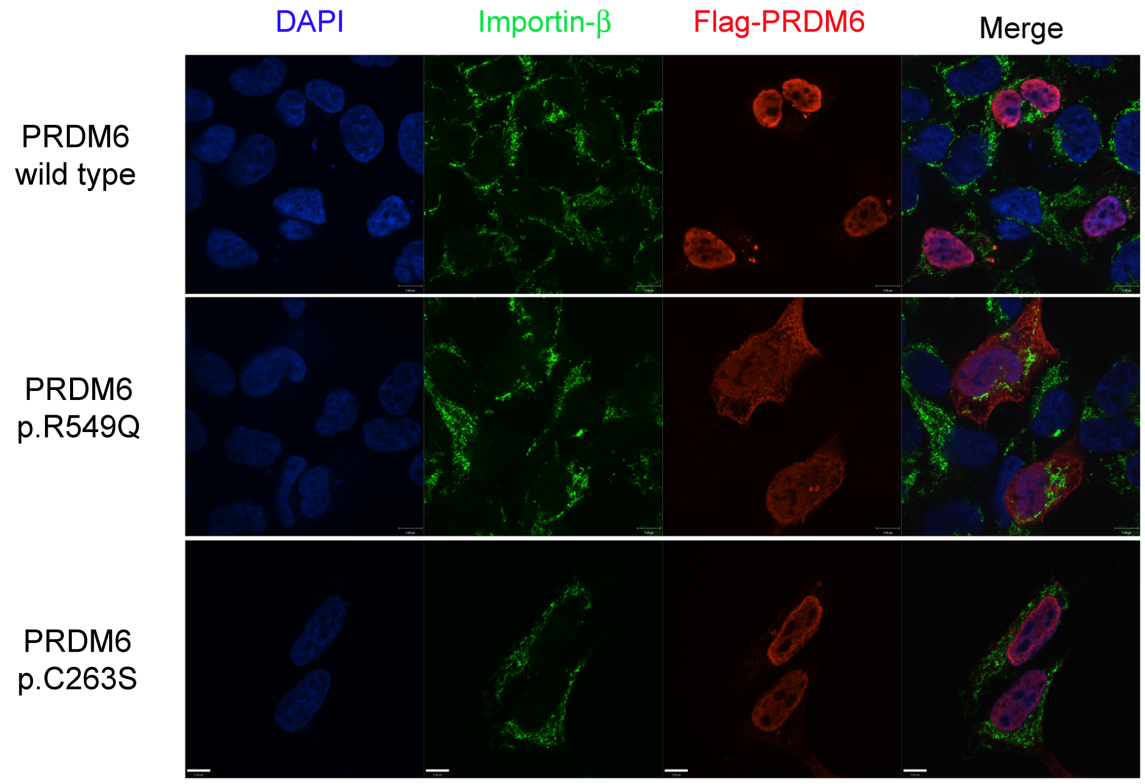
PRDM6 p.C263S

D)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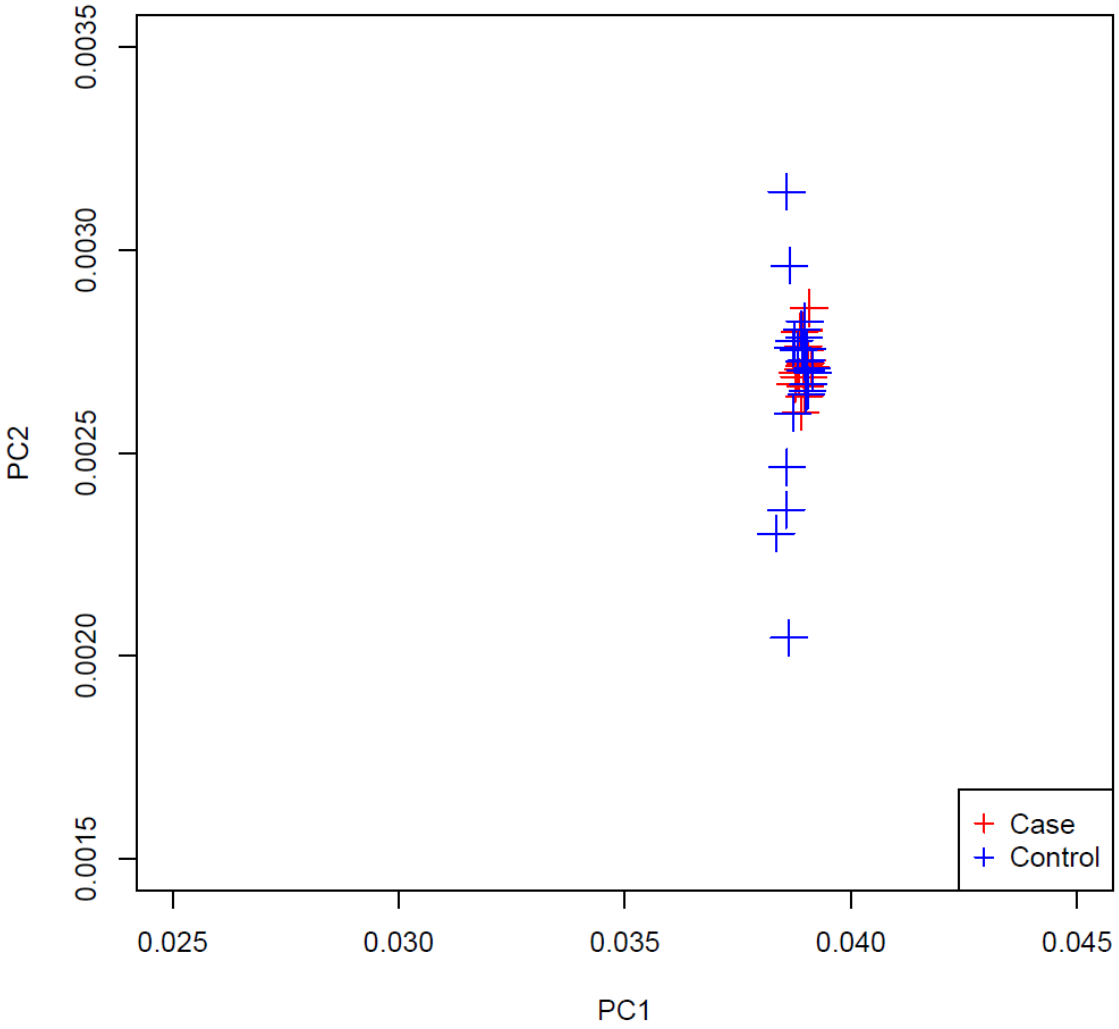
Flag-PRDM6
Merge
PRDM6
wild type
PRDM6
p.R549Q
PRDM6
p.C263S

Figure S3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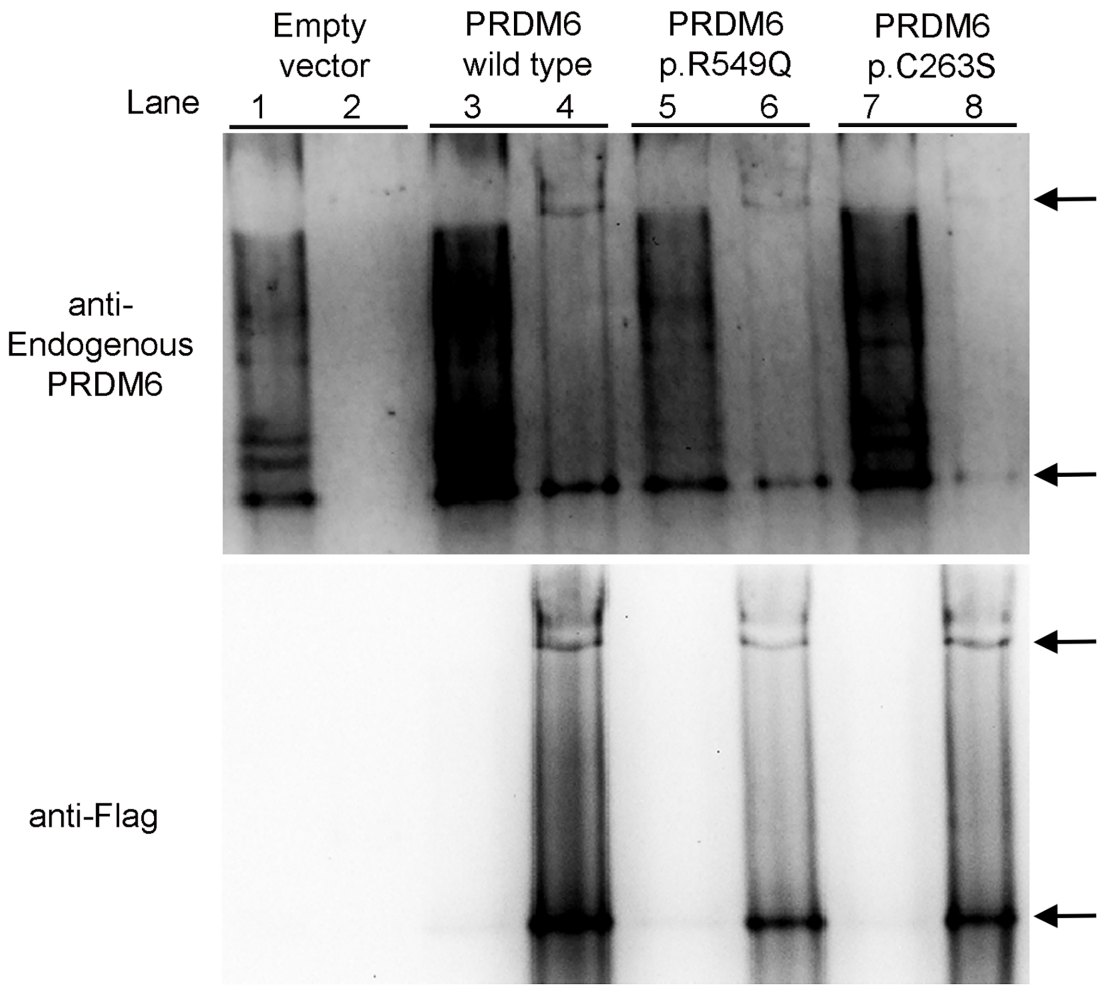
0.0030
0.0025
0.0020
0.0015
PC2
0.025
0.030
0.035
0.040
0.045
PC1
Case
Control

Figure S4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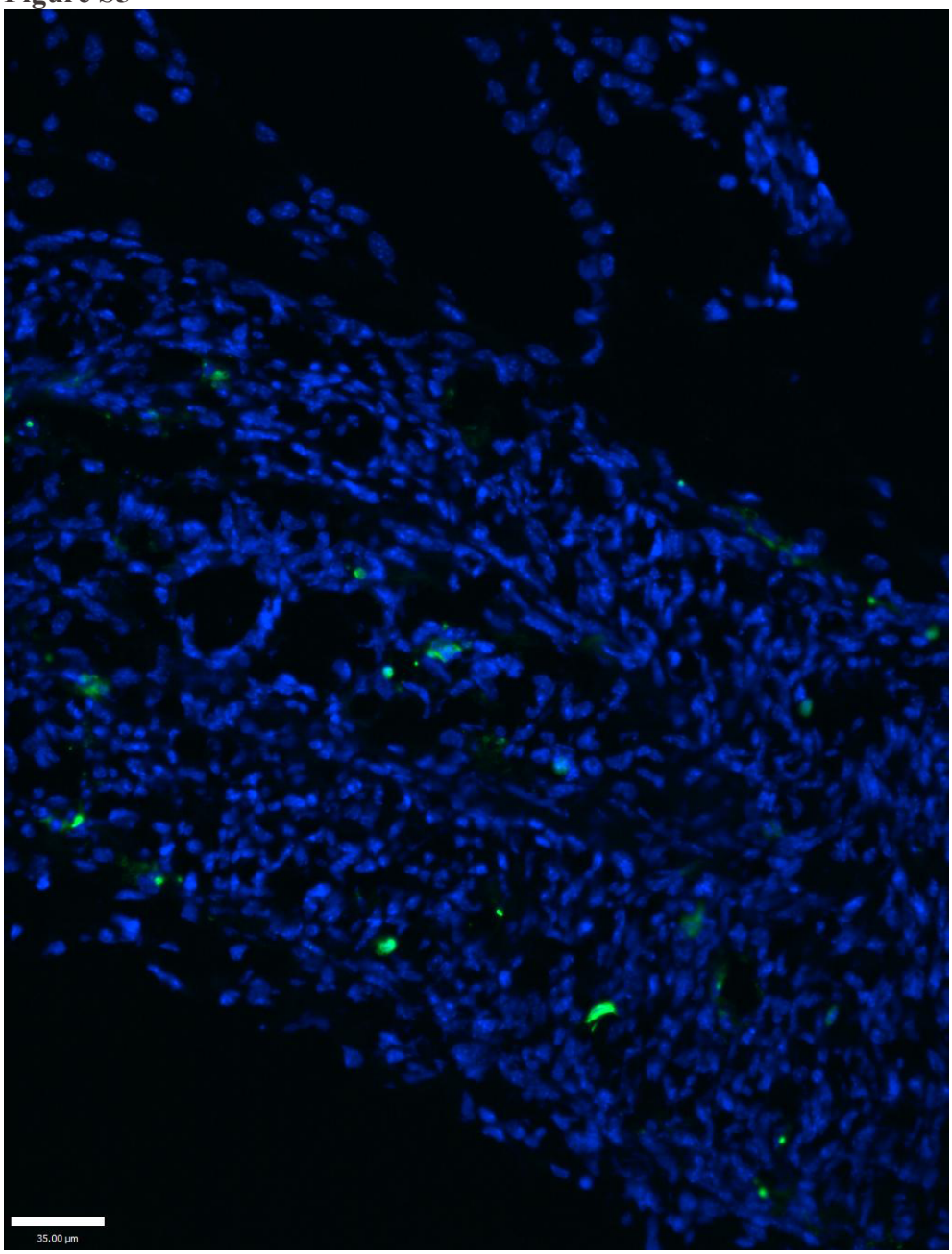
PRDM6 p.R549Q
PRDM6 p.C263S
Lane
1 2 3 4 5 6 7 8
anti-Endogenous PRDM6
anti-Flag

Figure S5



Supplemental Figure Legends:

Figure S1 – Representative pedigrees from other Caucasian families in our study. Individuals with PDA are indicated by black symbols; unaffected individuals are shown as unfilled symbols; individuals with unknown status have a dotted symbol. Circles represent females; squares represent males and symbols with a slash through them indicate deceased subjects.

Figure S2 - Immuofluorescent pictures localizing wild type and two mutants *PRDM6* variants $PRDM6_{Arg549Gln}$ (p.R549Q) and $PRDM6_{Cys263Ser}$ (p.C263S) in relationship to the ER (calnexin) (A), Golgi apparatus (GM130) (B), mitochondria (COX IV) (C) and Importin-β (D) in HEK293 cells. $PRDM6_{R549Q}$ retains largely in cytoplasm but not in Golgi or the ER.

Figure S3 – The visualization of the principle component analysis (PCA) using the EIGENSTRAT program to compare SNP genotypes with MAF >5% between probands and 2000 North European controls. SNPs without evidence for significant linkage disequilibrium were used. The analysis excluded population stratification. Changing control population to the population from 1,000 Genomes did not change the result of analysis. A separate analysis was carried out using 2000 Northern European unaffected subjects sequenced in the same facility using the same WES protocol as a second control group (data not shown). Population stratification was excluded using this analysis, supporting consistent comparison between burden of variants in cases and controls.

Figure S4 - PRDM6 homodimerization. Human aortic VSMC were transduced with adenovirus (empty adenoviruses vectors or vectors containing Flag-tagged mouse PRDM6 wild type, PRDM6p.R549Q, and PRDM6p.C263S constructs) and cultured for 48hr. Cells were washed twice with phosphate-buffered saline, scraped and lysed in 10mM Tris, pH7.4, 150mM NaCl, 1mM EDTA, 1% Triton X-100, and a cocktail of protease inhibitors. The supernatant was clarified by centrifugation at 20,000 g 4°C for 20min. Subsequently, lysates were immunoprecipitated with anti-Flag resin, washed three times in lysis

buffer at 4°C and bound protein was eluted with 0.1mg/ml 3X FLAG peptide (Sigma-Aldrich, St. Louis, MO) for 1hr at 4°C with gentle rotation before subjecting to native PAGE according to manufacture's instructions (Bio-rad). Monomeric endogenous PRDM6 and dimeric complexes were detected by immunoblotting with anti-human PRDM6. Monomeric exogenous PRDM6 and dimeric complexes were then detected by immunoblotting with anti-Flag antibody. Lanes 1, 3, 5, and 7 indicate the input controls. Lanes 2, 4, 6, and 8 indicates proteins after Flag-tag purification. The arrows indicate homodimers (the upper bands) and monomers (the lower bands).

Figure S5 - Immunofluoresence staining showing apoptotic cells (green color) in DA at stage P0.5. Blue color represents DAPI staining of the nucleus. Scale bar represents 35um. Tissue Cryosections were air dried for 1hr, and subjected to fixation in 1% PFA. Then the sections were processed using ApopTag Plus Fluorescein In Situ Apoptosis Detection Kit (EMD Millipore) according to manufacture's instructions. Briefly, cryosections were post-fixed in precooled ethanol: acetic acid (2:1) for 5 min at -20°C, and washed in PBS twice. Equilibration buffer was applied on the specimen and incubated for 10 seconds at room temperature. Then the cryosections were incubated with TdT enzyme for 1hr at 37°C and the reaction was stopped by incubating with stop/wash buffer, and incubated with anti-digoxigenin conjugate for 30 min at room temperature avoid exposure to light. Finally, the cryosections were washed in PBS four times and mounted with ProLong Gold antifade reagent with DAPI (Life technologies)

Supplemental Tables

Table S1: Variants within the recombination interval on 5q23

Position	Gene or rs#	Mutation Type	Frequency for Exonic Variants from EXAC
118500981	rs11306851	Intronic	
118639270	rs1394631	Intronic	
120191189	rs1422377	Intronic	
121488635	ZNF474	Exonic	0.09423
122091535	rs763497	Intronic	
122287705	rs717503	Intronic	
122915059	rs171546	Intronic	
122920306	rs246266	Intronic	
122515990	PRDM6	Exonic	0
123736339	rs51777	Intronic	
125246089	rs719829	Intronic	
125929031	ALDH7A1	Intronic	

Table S2: Pathways identified as significantly enriched for novel and deleterious genetic variants in 32 PDA samples

GO Term	Background Frequency	Sample Frequency	P-Value*
Biological Process	15968	226	9.17E-08
Cellular Process	13503	201	1.68E-06
Histone Modification	304	17	9.14E-04
Chromatin Modification	307	7	1.00E-03
Histone Lysine Methylation	57	8	3.10E-03
Multicellular Organismal Development	4256	81	7.35E-03
Neuron Development	793	24	1.30E-01
Axon Guidance	404	17	8.40E-01

* P-Values are corrected for multiple comparisons

Table S3: Histone Modification Genes Novel and Predicted to be deleterious by Polyphen and Sift (n=32)

Sample ID	Gene	Mutation	Het/Hom	Pathway	PolyPhen/SIFT score
0115	*GLI3*	p.A909G	Het	Histone acetyltransferase binding, Histone deacetylase binding	0.991/0
77-3	*HDAC11*	p.R281Q	Het	Histone deacetylase complex, Histone deacetylase activity, Histone deacetylation	1/?
100	*TET3*	p.K344R	Het	Histone H3-K4 trimethylation*	0.996/0.13
105	*RBM14*	p.K149R	Het	Histone deacetylation	0.99/0.03
115	*L3MBTL1*	p.C101F	Het	Nucleosomal histone binding, Methylated histone residue binding, Histone binding*	0.999/0.01
119	*ASH1L*	p.R1646K	Het	Histone-lysine N-methyltransferase activity*	0.974/0.09
120	*SETD1B*	p.R882H	Het	Histone methyltransferase complex, Histone methyltransferase activity H3-K4 specific, Histone H3-K4 methylation*	1/0
124	*HJURP*	p.N306K	Het	Histone binding	0.985/0
	L3MBTL2	p.S46C	Het	Methylated histone residue binding, Histone binding*	0.999/0.01
125	*BAP1*	p.C103Y	Het	Monoubiquinated histone H2A deubiquination	0.999/0.04
135	*KMT2A*	p.R4597C	Het	Histone-lysine N-methyltransferase activity*	1/0.08
138	*PPAGC1A*	p.R282C	Het	Positive regulation of histone acetylation	0.999/0
171	*NASP*	p.G407R	Het	Histone exchange	1/0
	PRDM2	p.K946R	Het	Histone-lysine N-methyltransferase activity*	1/0.01
256-3	*TUT1*	p.R623W	Het	Histone mRNA catabolic process	1/0.05
263	*SP1*	p.T265P	Het	Histone acetyltransferase binding, Histone deacetylase binding	0.992/0.05
327-3	*TRRAP*	p.N28301	Het	Histone acetylation, Histone deubiquination, NuA4 histone acetyltransferase complex, Histone H4 acetylation, Histone H2A acetylation	0.993/0