Supplemental Material for "Atypical Protein Kinase C Dependent Polarized Cell Division is Required for Myocardial Trabeculation"

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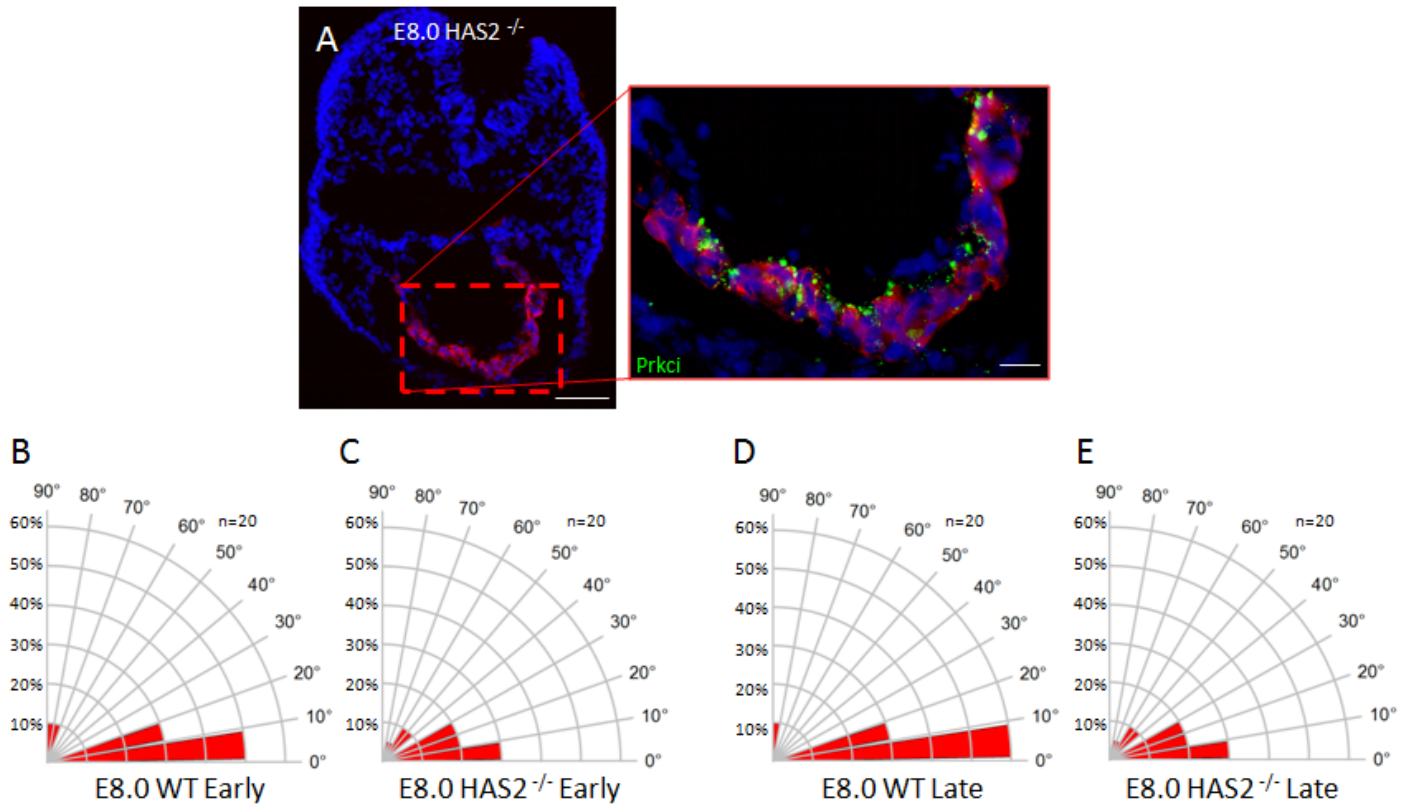
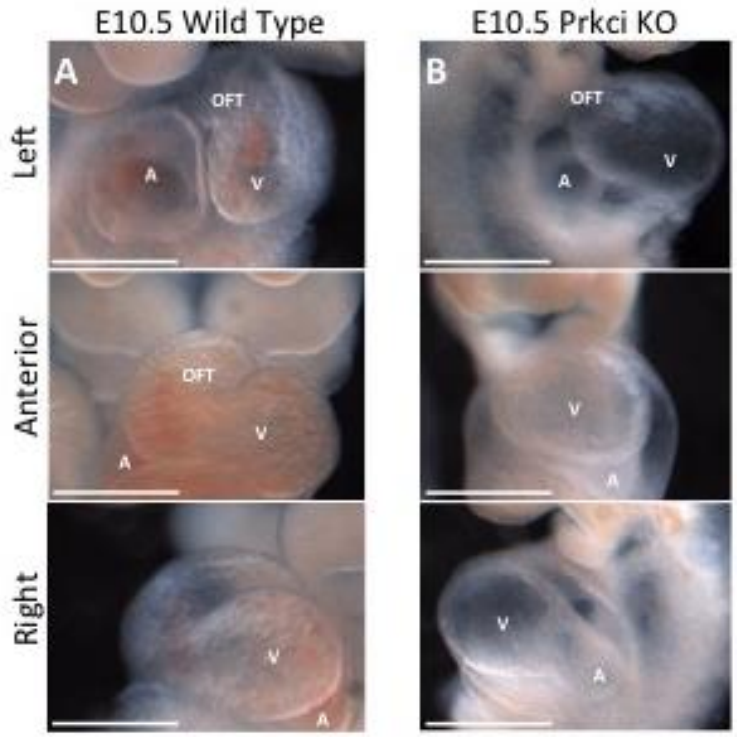


Figure S1 (related to figure 3): HA in the cardiac jelly is required for polarized cell division in E8.0 mouse hearts. A. E8.0 HAS2$^{-/-}$ embryo co-stained for TnT (purple), Prkci (green), and DAPI (blue). **B-C**. Radial histogram depicting the orientation of cardiomyocyte divisions in early-stage mitotic cells from E8.0 HAS2$^{-/-}$ hearts. **D-E**. Radial histogram depicting the orientation of cardiomyocyte divisions in late-stage mitotic cells from E8.0 HAS2$^{-/-}$ hearts. Scale bars: 100μm zoom out, 10μm zoom in. P values ($P \leq 0.001$ for early and late mitosis) determined by a chi-square test in B&C and D&E for determining significance of the difference between HAS2 null and wildtype cells. Chi-square P values related to B&C and D&E are in Table S1. n values in B&C and D&E represent the number of cells analysed from 3 to 5 independent animals.

Genotype	Observed (Expected) n=47
Nkx 2.5 $^{+/cre}$ Prkci$^{fl/fl}$	0 (12)
Nkx 2.5 $^{+/+}$ Prkci$^{fl/fl}$	15 (12)
Nkx 2.5 $^{+/cre}$ Prkci$^{+/fl}$	21 (12)
Nkx 2.5 $^{+/+}$ Prkci$^{+/fl}$	11 (12)

Figure S2 (related to figure 4): Genotypes of pups born from an Nkx 2.5$^{+/cre}$/Prkci$^{+/fl}$ male crossed with a Prkci$^{fl/fl}$ female. Graph showing observed and expected genotypes of pups born from an Nkx 2.5$^{+/cre}$/Prkci$^{+/fl}$ male crossed with a Prkci$^{fl/fl}$ female.



Online Figure S3 (related to figure 4): Gross morphological analysis of an E10.5 Prkci cardiac null mouse heart. External structures of the heart from an E10.5 wild-type (**A**) and an E10.5 Prkci cardiac null embryo (**B**). Scale bars: 1mm.

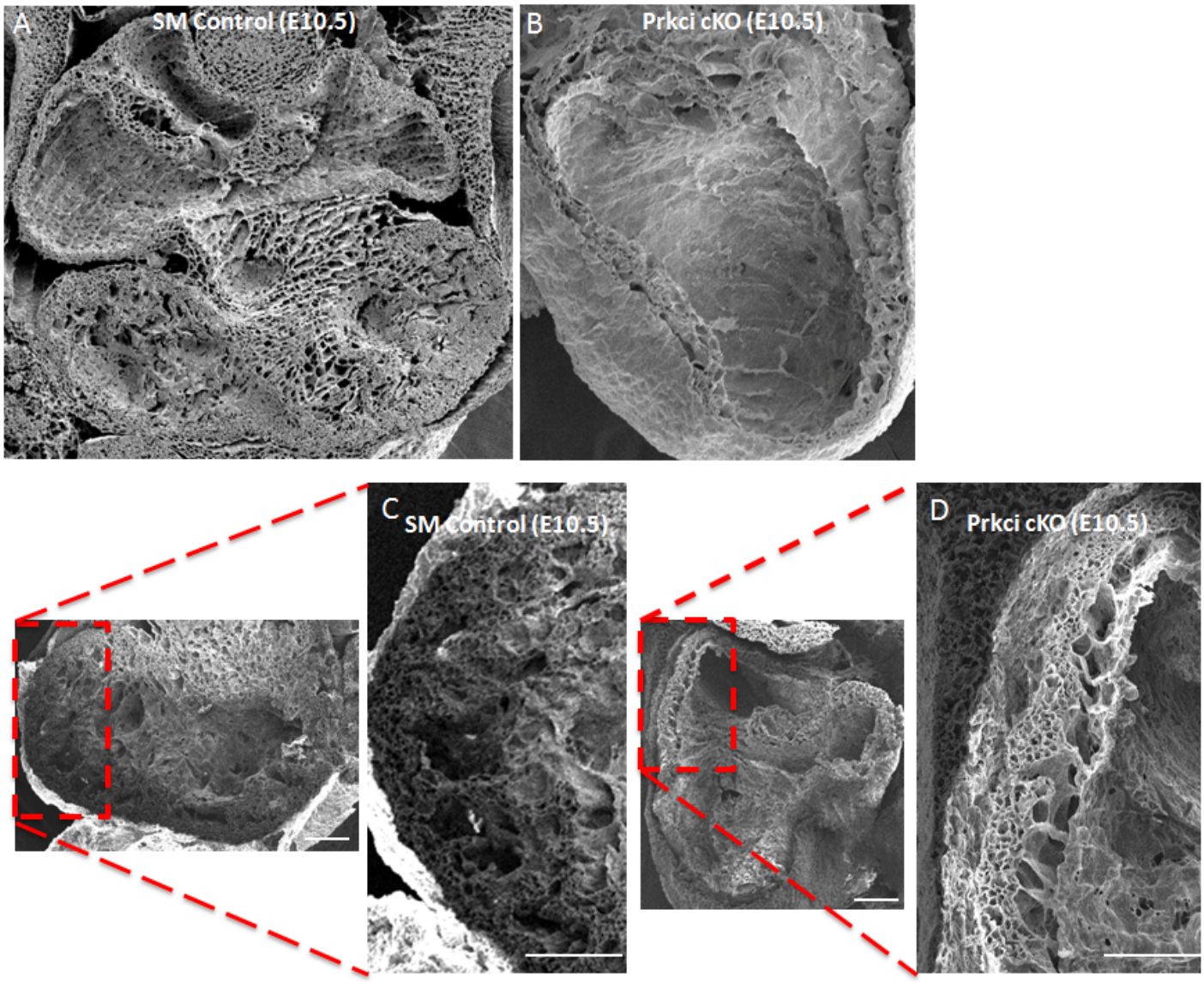


Figure S4 (related to figure 4): Scanning EM of an E10.5 Prkci cardiac null mouse heart. Scanning EM images of a cryosectioned E10.5 littermate heart (**A**) or E10.5 Prkci cardiac null heart (**B**). **C-D.** Zoomed out and zoomed in scanning EM images of a cryosectioned E10.5 littermate heart (**C**) or E10.5 Prkci cardiac null heart (**D**) showing the lack of trabeculations in the mutant heart. Scale bars: 100μm zoom out and 10μm zoom in.

Genotype	Observed (Expected) n=40
Nkx 2.5 $^{+/cre}$ NuMA$^{fl/fl}$	0 (10)
Nkx 2.5 $^{+/+}$ NuMA$^{fl/fl}$	11 (10)
Nkx 2.5 $^{+/cre}$ NuMA$^{+/fl}$	18 (10)
Nkx 2.5 $^{+/+}$ NuMA$^{+/fl}$	11 (10)

Figure S5 (related to figure 6): Genotypes of pups born from an Nkx 2.5$^{+/cre}$/NuMA$^{+/fl}$ male crossed with a NuMA$^{fl/fl}$ female. Graph showing observed and expected genotypes of pups born from an Nkx 2.5$^{+/cre}$/NuMA$^{+/fl}$ male crossed with a NuMA$^{fl/fl}$ female.

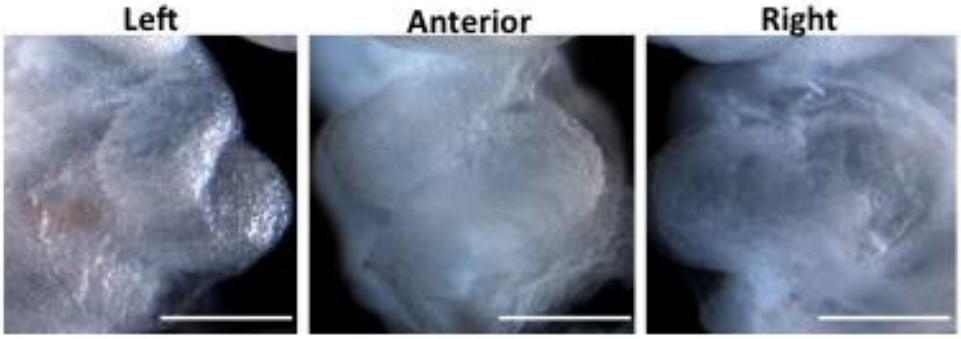


Figure S6 (related to figure 6): Gross morphological analysis of an E10.5 NuMA cardiac null mouse heart. External structures of the heart from an E10.5 NuMA cardiac null embryo. Scale bars: 1mm.

Condition 1	Condition 2	Figures	Chi² p value	Significance
E8.5 WT (Early)	E8.5 HAS2 null (Early)	2B & 3G	<0.001	***
E8.5 WT (Late)	E8.5 HAS2 null (Late)	2D & 3H	<0.001	***
E8.0 WT (Early)	E8.0 HAS2 null (Early)	S1B & S1C	<0.001	***
E8.0 WT (Late)	E8.0 HAS2 null (Late)	S1D & S1E	<0.001	***
E8.5 WT (Early)	E8.5 CD44 null (Early)	2B & 3O	0.1	NS
E8.5 WT (Late)	E8.5 CD44 null (Late)	2D & 3P	0.1	NS
E8.5 WT (Early)	E8.5 Prkci cKO (Early)	2B & 5B	<0.001	***
E8.5 WT (Late)	E8.5 Prkci cKO (Late)	2D & 5D	0.002	***
E8.5 WT (Early)	E8.5 NuMA cKO (Early)	2B & 7B	<0.001	***
E8.5 WT (Late)	E8.5 NuMA cKO (Late)	2D & 7D	0.01	**
E8.5 WT (Early)	E8.5 WT (Late)	2B & 2D	0.5	NS
E8.5 HAS2 null (early)	E8.5 HAS2 null (Late)	3G & 3H	.1	NS
E8.0 HAS2 null (early)	E8.0 HAS2 null (Late)	S1C & S1E	.1	NS
E8.5 CD44 null (Early)	E8.5 CD44 null (Late)	3O & 3P	.1	NS
E8.5 Prkci cKO (Early)	E8.5 Prkci cKO (Late)	5B & 5D	0.2	NS
E8.5 NuMA cKO (Early)	E8.5 NuMA cKO (Late)	7B & 7D	0.1	NS

Table S1: Chi-square test values for spindle orientation and cell division plane. Cells were categorized as perpendicular (70-90°), planar (0-20°), or oblique (20°-70°). No statistical differences were ever found between age-matched littermates.