Coordination between extrinsic extracellular matrix cues and intrinsic responses to orient the centrosome in polarizing cerebellar granule neurons

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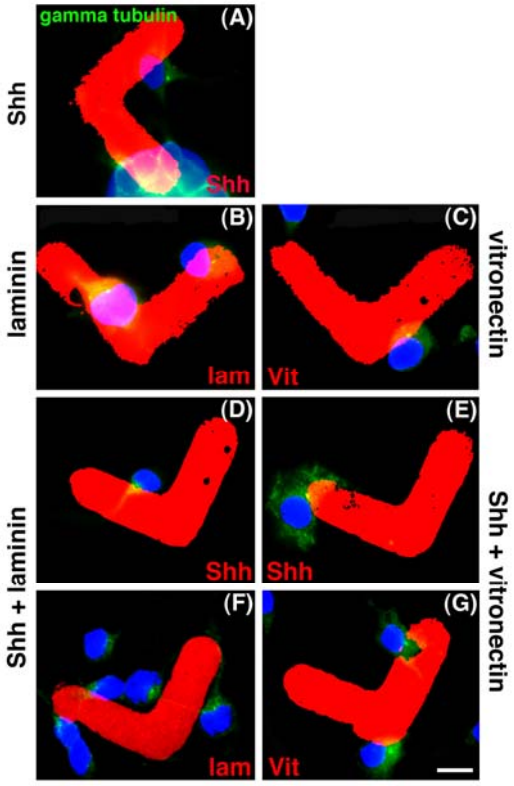
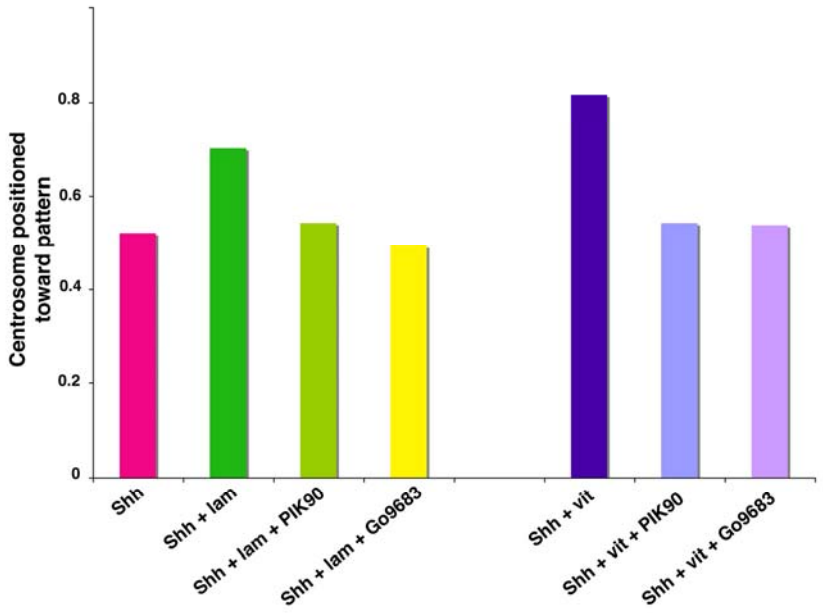


Supplementary Figure 1: Contact staining.

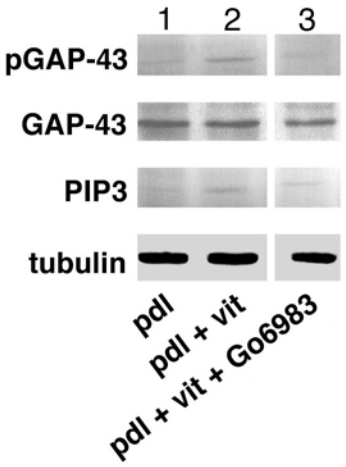
Immunoreactivity of printed protein patterns (red) and cells labeled with γ-tubulin (green). (A) Shh printed and stained (red), (B) Laminin printed and stained (red), (C) vitronectin printed and stained (red), (D and F) Shh+laminin is printed and stained for

(D) Shh and (F) laminin (red), (E and G) Shh+vitronectin is printed and stained for (E) Shh and (G) vitronectin (red) showing the retention of proteins on the coverslip after printing. Scale bar = 8μm.



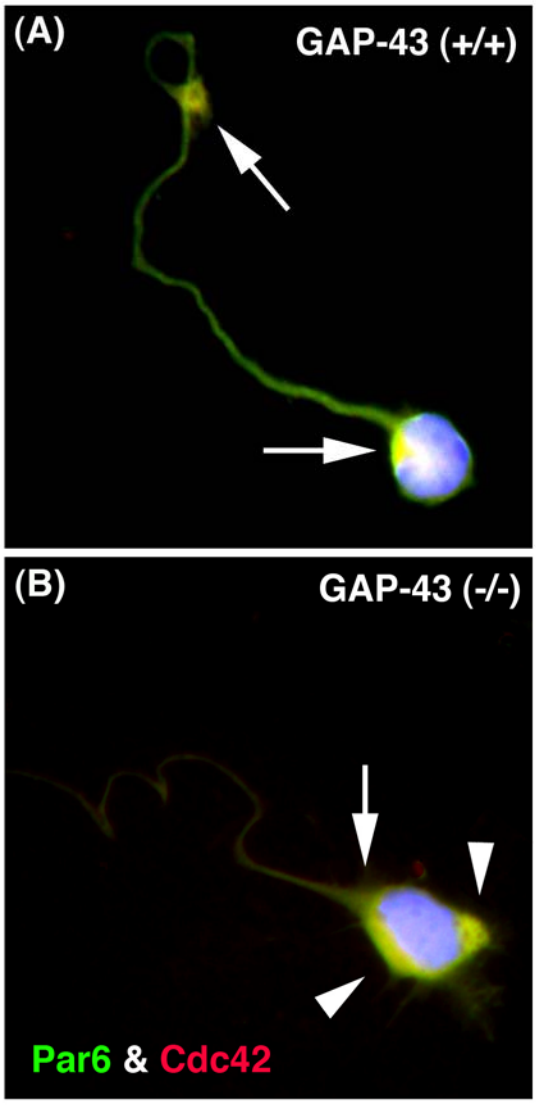
Supplementary Figure 2: Inhibitor contact quantitation.

Proportion of P8 granule cells with centrosomes positioned toward Shh (pink bar), Shh + laminin (dark green bar), Shh + laminin with 1μM PIK90 (light green bar), Shh + laminin with 1μM Go9683 (yellow bar), Shh + vitronectin (dark blue bar), Shh + vitronectin with 1μM PIK 90 (light blue bar), Shh + vitronectin with 1μM Gö 6983 (violet bar). Data is the mean from 2 independent experiments. (≥400 cells were counted in each experiment).



Supplementary Figure 3: PKC inhibition western.

Representative Western blot from P8 granule cells after 48 hours of culture probed with anti-pGAP-43, GAP-43, PIP3 and tubulin. Lane 1, poly-d-lysine (PDL); Lane 2, PDL + vitronectin; Lane 3, PDL + vitronectin in the presence of 1μM Go9683 (PKC inhibitor).



Supplementary Figure 4: Cdc42/Par6.

Immunoreactivity of Par6 (green) and Cdc42 (red) in P0 granule cells. (A) GAP-43 (+/+); (B) GAP-43 (-/-). Arrow in (A) indicates regions where Par6 and Cdc42 are colocalized at the base of the primary process. Arrow in (B) indicates the expression not being localized at the base of the axon. Arrowheads in (B) indicates region where only Par6 is localized and region where Par6 and Cdc42 are colocalized but not at the base of the

primary process. Representative images from n≥40 cells from two independent experiments. Scale bar = 8μm.

TABLE-1

PROTEIN PRINTED ⬇	ANIMALS USED ➡	P0 (WT)	P0 (KO)	P8 (WT)
Shh	Contact	136	165	142
	Non-contact	266	317	401
Laminin	Contact	101	110	166
	Non-contact	298	322	193
Shh+ Laminin	Contact	155	121	236
	Non-contact	394	315	409
Vitronectin	Contact	138	120	108
	Non-contact	398	374	262
Shh+Vitronectin	Contact	139	173	127
	Non-contact	294	255	274
Laminin+α6β1	Contact			095
	Non-contact			226
Vitronectin+α5β3	Contact			112
	Non-contact			254
Shh+Vitronectin +Cyclopamine	Contact	089		088
	Non-contact	239		201
Shh+Vitronectin +Shh antibody	Contact	064		071
	Non-contact	159		178
Shh+Vitronectin +α6β1	Contact			089
	Non-contact			172
Shh+Vitronectin +α5β3	Contact	093		087
	Non-contact	204		254
Shh+Laminin+ Wartmanin	Contact			086
	Non-contact			248
Shh+Vitronectin+ Wartmanin	Contact			074
	Non-contact			241
Shh+Laminin+ BIS.	Contact			067
	Non-contact			227
Shh+Vitronectin+ BIS.	Contact			063
	Non-contact			228

Supplementary Table 1: Total number of cells counted on protein patterns.

The number of contact and non contact cells included in the centrosome position analysis of each experimental condition.

Contact cell = cells touching the protein pattern.

Non contact cell = cells not touching the protein pattern.