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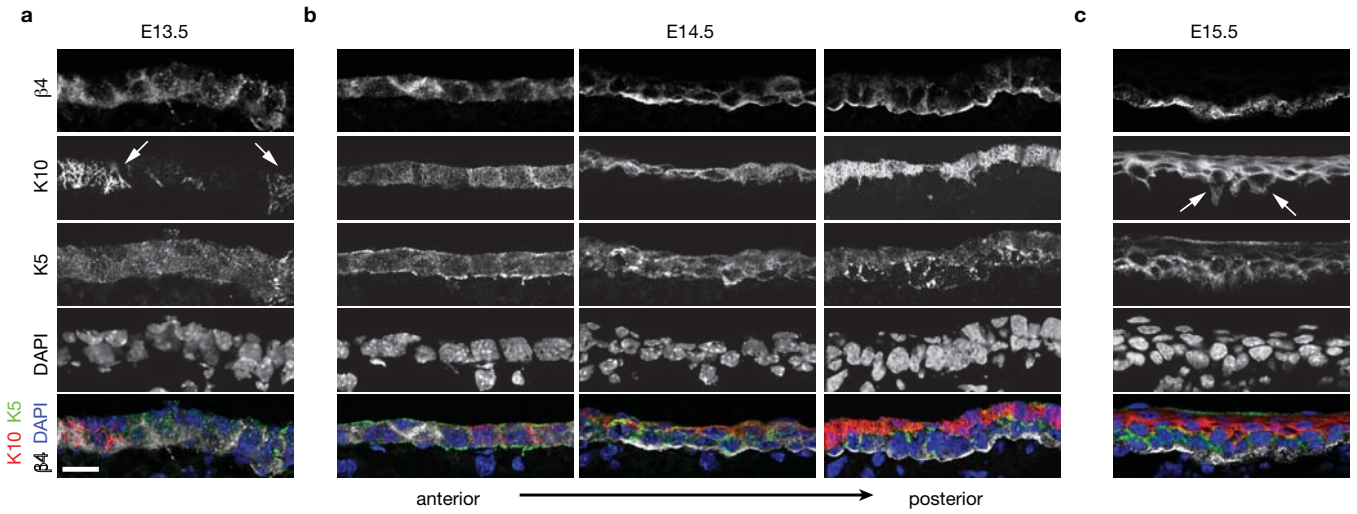
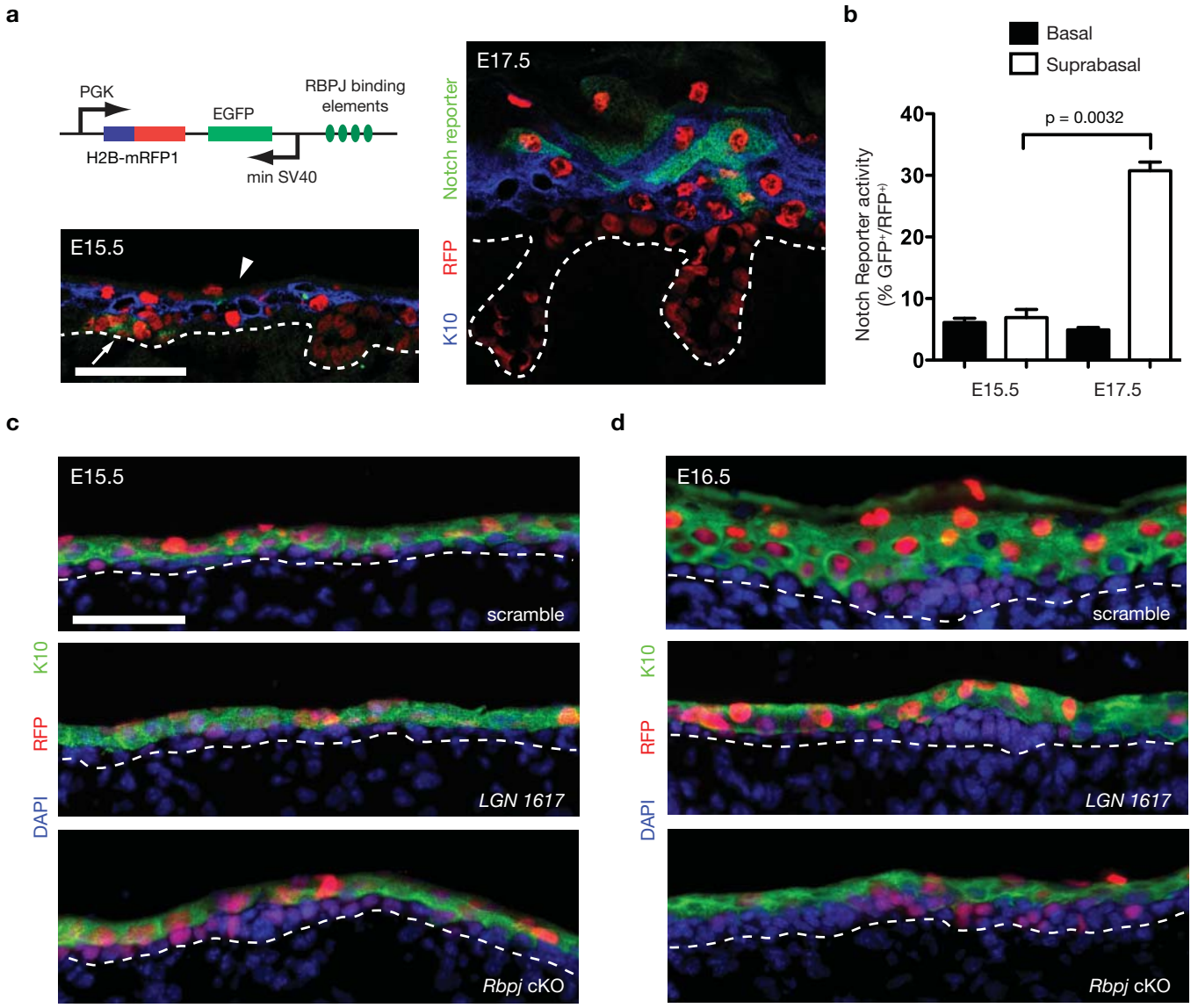
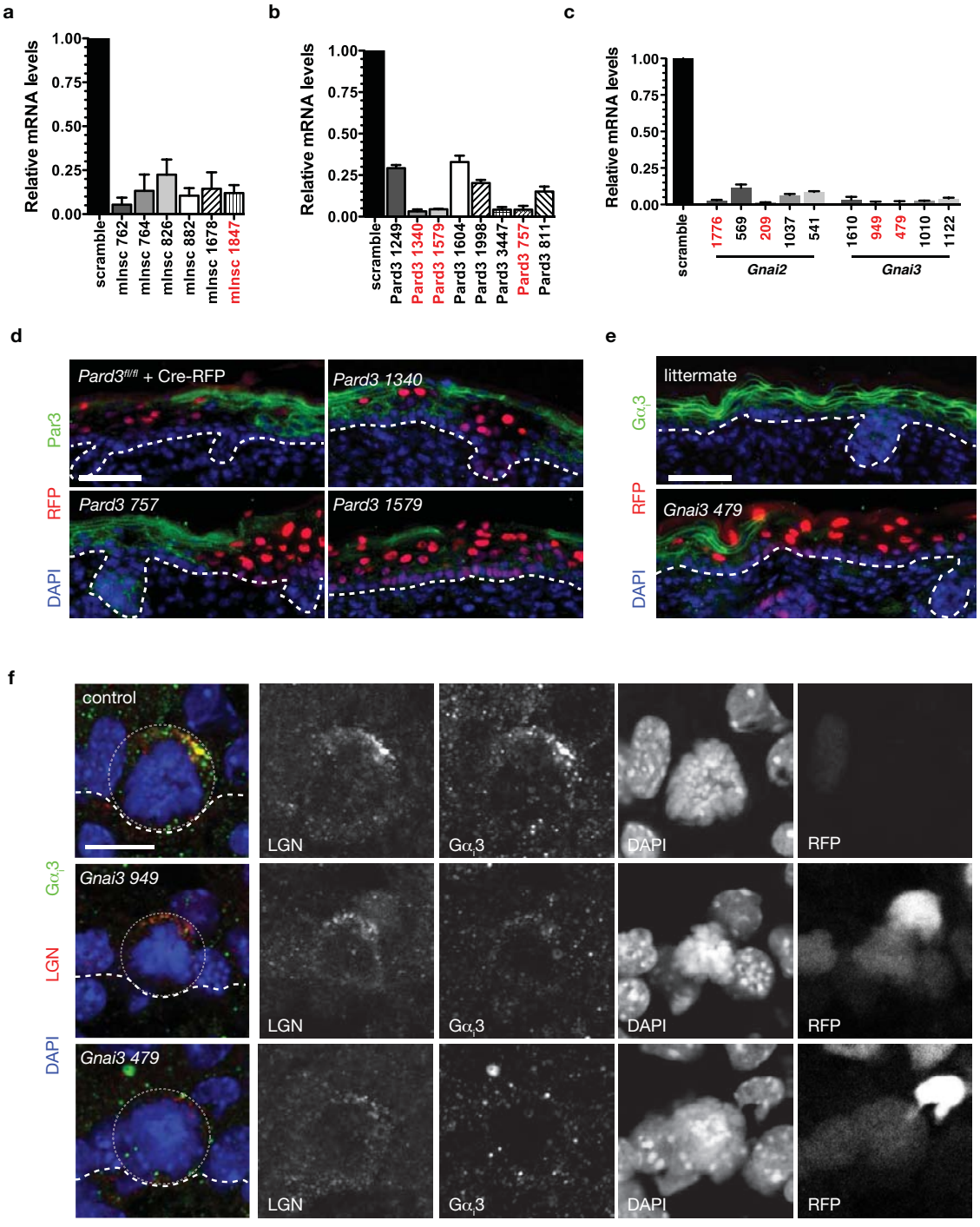


Supplementary Figure 1 Maturation of differentiation markers in developing epidermis (**a**) Beginning around E13.5, at the stage when suprabasal cells (except the periderm) are absent, sporadic basal cells coexpress the spinous keratin, K10, along with basal keratins K5 and K14 (not shown). Note also that at this age, the basement membrane marker and hemidesmosome constituent β4-integrin is broadly and diffusely expressed throughout basal cells at E13.5, while it progressively becomes more basally restricted at later ages. (**b**) Sections of wild-type E14.5 back skin from anterior (less differentiated) to posterior (more differentiated). In anterior regions of single-layered epidermis, K10 and K5 are broadly coexpressed, while β4-integrin remains diffuse. In areas where β4-integrin begins to show some apical enrichment and suprabasal cells are present, K10 becomes more restricted to suprabasal cells, though the basal keratin K5 is diffusely coexpressed there. In posterior regions, the segregation of K10 and K5 becomes more apparent. (**c**) At E15.5, β4-integrin becomes more restricted to the epidermal-dermal boundary, while K10 and K5 are expressed in opposing domains. An exception is the appearance of sporadic cells positioned in the basal layer which coexpress K10 and K4 (arrows). We suggest that these are cells undergoing differentiation by delamination rather than asymmetric cell division.



Supplementary Figure 2 Both Notch and LGN are dispensable for early stage stratification (**a**) (Top left) Schematic of lentiviral Notch reporter construct. Nuclear H2B-RFP is expressed under a constitutive reporter to mark cells transduced with the reporter construct, while cytosolic GFP reveals cells in which Notch signaling has been activated. At E17.5 (right), robust Notch/GFP+ cells are observed in suprabasal (K10^{+}, blue) layers. In contrast, at E15.5 (bottom left), few cells show detectable Notch activity, and those that do are weakly positive, and appear in both basal (arrow) and suprabasal (arrowhead) layers. (**b**) Quantification of the percentage of cells expressing the Notch reporter construct (RFP+) which show detectable Notch activity (GFP+). At E17.5, there is a strong bias toward suprabasal Notch activity, while at E15.5 Notch activity is lower overall, and present equally in basal and suprasal cells. (**c,d**) Spinous differentiation as indicated by K10 (green) at E15.5 (**c**) and E16.5 (**d**) in shScramble control (top), *LGN* knockdown (middle) and *Rbpj* knockout (bottom) sections of back skin. Note that the formation of the initial spinous layer is not impacted by impairing spindle orientation or Notch. Unlike controls, however, differentiation fails to progress in these mutants. RFP (red) indicates H2B-mRFP1 in top and middle panels, and Cre-mRFP1 in bottom panels. *Rbpj* loss at this age was confirmed by absence of the target gene Hes1 in suprabasal layers (not shown, [17]). Scale bars: 50 μm.



Supplementary Figure 3 Characterization of shRNA knockdown efficiency *in vitro* and *in vivo*. (**a**) Quantification of *mInsc* knockdown efficiency in keratinocytes stably-transduced with retroviral mInsc, necessary due to low endogenous levels of mInsc in low calcium conditions. (**b**) Quantification of *Pard3* knockdown in keratinocytes. Red letters indicate those with the highest levels of knockdown (94-97%), subsequently used for *in vivo* studies. (**c**) Quantification of mRNA knockdown efficiency in keratinocytes for *Gnai2* (left) and *Gnai3* (right) shRNA clones. Those with the strongest knockdown (>97%) are shown in red letters, and were subsequently used *in vivo*. Bars are the mean ± SD. (**d**) Back skin sections from E17.5 epidermis showing specific loss of Par3 expression in mosaic *Pard3* knockout (top left) or knockdown tissue. (**e**) Confirmation of knockdown efficiency by immunofluorescence in E17.5 back skin. In addition to its polarized localization in mitotic basal cells, Ga_i3 is also localized to cell membranes suprabasally, like Par3 (top panel). This localization is specifically lost in RFP^+ regions of mosaic knockdown tissue (bottom panel). (**f**) Ga_i3 is normally enriched apically in mitotic basal cells (left, see also Fig. 5b), but following *Gnai3* knockdown, LGN cortical expression is frequently reduced (middle row, weaker hairpin) or delocalized (bottom row, stronger hairpin).

Supplementary Table 1 Chi-square analyses of division angle distributions.

Condition 1	Condition 2	Figure	Chi square p-value	Significance
E12.5	E13.5	1f	0.0176	*
E13.5	E14.5	1f	0.6431	n.s.
E14.5	E15.5	1f	0.1413	n.s.
E15.5	E16.5	1f	< 0.0001	***
E16.5	E17.5	1f	0.0938	n.s.
E15.5 scramble EYFP-mInsc	E15.5 YFP- internal controls§	3d	0.8169	n.s.
E15.5 scramble EYFP-mInsc	E15.5 littermates§	3d	0.8096	n.s.
E15.5 scramble EYFP-mInsc	E15.5 scramble H2B-RFP	3d	0.0589	n.s.
E15.5 scramble H2B-RFP	E15.5 LGN 1617 H2B-RFP	3d	0.5394	n.s.
E17.5 scramble EYFP-mInsc	E17.5 YFP- internal controls§	3e	0.0316	*
E17.5 scramble EYFP-mInsc	E15.5 scramble EYFP-mInsc	3d, 3e	< 0.0001	***
E17.5 scramble H2B-RFP	E17.5 LGN 1617 H2B-RFP	3e	< 0.0001	***
mInsc cKO (Cre-RFP)	mInsc$^{fl/fl}$ littermates	4f	< 0.0001	***
mInsc cKO (K14-Cre)	mInsc$^{fl/fl}$ littermates	4f	< 0.0001	***
mInsc cKO (Cre-RFP)	mInsc cKO (K14-Cre)	4f	0.7446	n.s.
mInsc 1847 H2B-YFP	YFP- internal controls§	4f	< 0.0001	***
mInsc 1847 H2B-YFP	mInsc cKO (Cre-RFP)	4f	0.0254	*
mInsc 1847 H2B-YFP	mInsc cKO (K14-Cre)	4f	0.0632	n.s.
Pard3 1340 H2B-RFP	Pard3 1340/1579/757 littermates†	5d	< 0.0001	***
Pard3 1340 H2B-RFP	Pard3 1340 littermates‡,§	5d	< 0.0001	***
Pard3 1579 H2B-RFP	Pard3 1340/1579/757 littermates†	5d	< 0.0001	***
Pard3 1579 H2B-RFP	Pard3 1579 littermates‡,§	5d	< 0.0001	***
Pard3 757 H2B-RFP	Pard3 1340/1579/757 littermates†	5d	< 0.0001	***
Pard3 757 H2B-RFP	Pard3 757 littermates‡,§	5d	< 0.0001	***
Pard3 1340 H2B-RFP	Pard3 1579 H2B-RFP	5d	0.0855	n.s.
Pard3 1340 H2B-RFP	Pard3 757 H2B-RFP	5d	0.1065	n.s.
Pard3 757 H2B-RFP	Pard3 1579 H2B-RFP	5d	0.9071	n.s.
Pard3 cKO (Cre-RFP)	Pard3$^{fl/fl}$ littermates§	5d	0.0024	**
Pard3 cKO (Cre-RFP)	Pard3 1340 H2B-RFP	5d	0.0028	**
Pard3 cKO (Cre-RFP)	Pard3 1579 H2B-RFP	5d	0.2557	n.s.
Pard3 cKO (Cre-RFP)	Pard3 757 H2B-RFP	5d	0.3046	n.s.
Pard3b 2571 H2B-YFP	Pard3b 2571 littermates§	5e	0.5244	n.s.
Pard3$^{fl/fl}$ + Pard3b 2571 Cre-RFP	Pard3$^{fl/fl}$ +Cre-RFP (cKO)	5e	0.8017	n.s.
Pard3 1340 EYFP-mInsc	YFP- internal controls	5h	< 0.0001	***
Pard3 1340 EYFP-mInsc	Pard3 1340 H2B-RFP	5d, 5h	0.7474	n.s.
scramble EYFP-mInsc	Pard3 1340 EYFP-mInsc	3e, 5h	< 0.0001	***
Gnai2 1776 H2B-YFP	Gnai2 209/1776 littermates†	6c	0.9709	n.s.
Gnai2 209 H2B-YFP	Gnai2 209/1776 littermates†	6c	0.3643	n.s.
Gnai2 1776 H2B-YFP	scramble	6c, 3e	0.8641	n.s.
Gnai2 209 H2B-YFP	scramble	6c, 3e	0.6512	n.s.
Gnai3 479 H2B-RFP	RFP- internal controls	6d	< 0.0001	***
Gnai3 479 H2B-RFP	Gnai3 479/949 littermates†	6d	< 0.0001	***
Gnai3 479 H2B-RFP	scramble	6d, 3e	< 0.0001	***
Gnai3 479 H2B-RFP	Gnai3 949 H2B-RFP	6d	0.5653	n.s.
Gnai3 949 H2B-RFP	Gnai3 479/949 littermates†	6d	< 0.0001	***
Gnai3 949 H2B-RFP	scramble	6d, 3e	< 0.0001	***
LGN 1617 RFP-LGNΔC	LGN 1617 H2B-RFP	6e, 3e	0.1554	n.s.
LGN 1617 RFP-LGNΔC	Gnai3 479 H2B-RFP	6e, 6d	0.9901	n.s.
mInsc cKO (Cre-RFP)	mInsc$^{fl/fl}$ littermates	7e	< 0.0001	***
Gnai3 479 H2B-RFP	Gnai3 479 littermates	7e	< 0.0001	***
Gnai3 479 H2B-RFP	mInsc cKO (Cre-RFP)	7e	0.1394	n.s.
Gnai3 479 H2B-RFP	LGN 1617 H2B-RFP	7e	< 0.0001	***
mInsc cKO (Cre-RFP)	LGN 1617 H2B-RFP	7e	< 0.0001	***
mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP	Gnai3 479 H2B-RFP	7e	0.0003	***
mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP	mInsc cKO (Cre-RFP)	7e	< 0.0001	***
mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP	LGN 1617 H2B-RFP	7e	0.1179	n.s.
mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP+	mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP-	7e	< 0.0001	***
mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP	mInsc$^{fl/fl}$ littermates	7e	0.828	n.s.
mInsc$^{fl/fl}$ + Gnai3 479 Cre-RFP	mInsc 1847 + Gnai3 479 (RFP+ YFP+)	7e, 7f	0.2147	n.s.
wild-type (YFP- RFP-)	Gnai3 479 (RFP+)	7f	0.0008	***
wild-type (YFP- RFP-)	mInsc 1847 (YFP+)	7f	0.0008	***
wild-type (YFP- RFP-)	mInsc 1847 + Gnai3 479 (RFP+ YFP+)	7f	0.0017	**
mInsc 1847 (YFP+)	Gnai3 479 (RFP+)	7f	0.4903	n.s.
mInsc 1847 (YFP+)	mInsc 1847 + Gnai3 479 (RFP+ YFP+)	7f	0.1319	n.s.
Gnai3 479 (RFP+)	mInsc 1847 + Gnai3 479 (RFP+ YFP+)	7f	0.0081	**

Chi-square test values were calculated using two degrees of freedom.

Data were categorized as follows: perpendicular (70-90°); planar (0-20°); oblique (20°-70°).

As opposed to equal 30° bins, these weighted bins were chosen based on the observation that in a wild-type "bimodal" division distribution, 80-90% of all divisions fell into the perpendicular and parallel categories. Thus a 20°-70° "oblique" bin better represents a deviation from normal than a 30°-60° bin would. All data were categorized in the same way.

† Indicates that littermates were pooled from multiple hairpins for the same gene (e.g. *Pard3 1340* littermates and *Pard3 1579* littermates), which is the actual data shown in the main text figure. This was done to avoid reproducing multiple control graphs in the main text. No statistical differences were ever found between age-matched littermates.

‡ Denotes a specific cohort of littermates associated with a single hairpin, in cases where the "littermates" data shown is pooled as above.

§ Indicates a data set which is not shown graphically in the main text figure panel.

Supplementary Table 2 | Clone distribution of lentiviral lineage tracing

	E15.5		E16.5		
	n	%	n	%	p-value
Mitotic clones	43	36.1%	32	57.1%	p = 0.0137
Delamination clones	76	63.9%	24	42.9%	
ACD clones	18	41.9%	20	62.5%	p = 0.1032
SCD clones	25	58.1%	12	37.5%	
3 cell ACD/SCD clones	6	5.0%	4	7.1%	

p-values determined by two-tailed Chi-square test

Supplementary Table 3 | Effect of Par3 loss on mInsc apical localization

	YFP$^-$ internal controls (n=50)		scramble EYFP-mInsc (n=44)			*Pard3 1340* EYFP-mInsc (n=36)		
	n	%	n	%	p-value†	n	%	p-value‡
Polarized LGN	38	76.0%	41	93.2%	0.0266	23	63.9%	0.0016
Polarized EYFP-mInsc			40	90.1%		15	41.7%	< 0.0001
Colocalized LGN/mInsc			39	88.6%		10	27.8%	< 0.0001

p-values determined by Chi-square: †relative to YFP$^-$ control; ‡relative to scramble EYFP-mInsc

n = number of mitotic pHH3+ mitotic basal cells examined per condition from ≤ 4 biological replicates