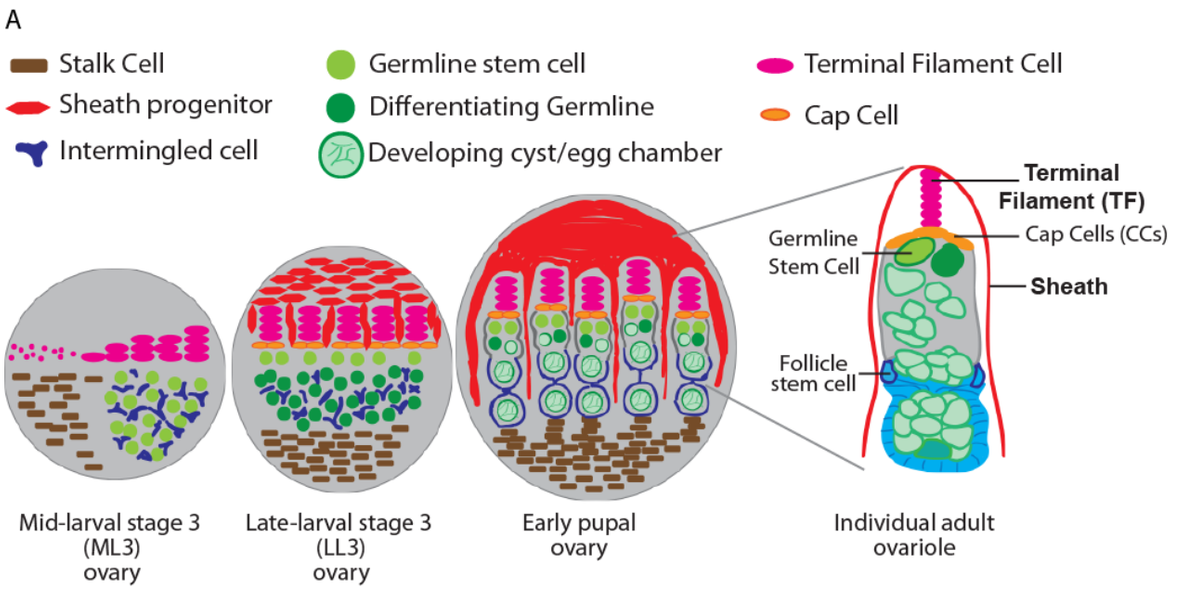
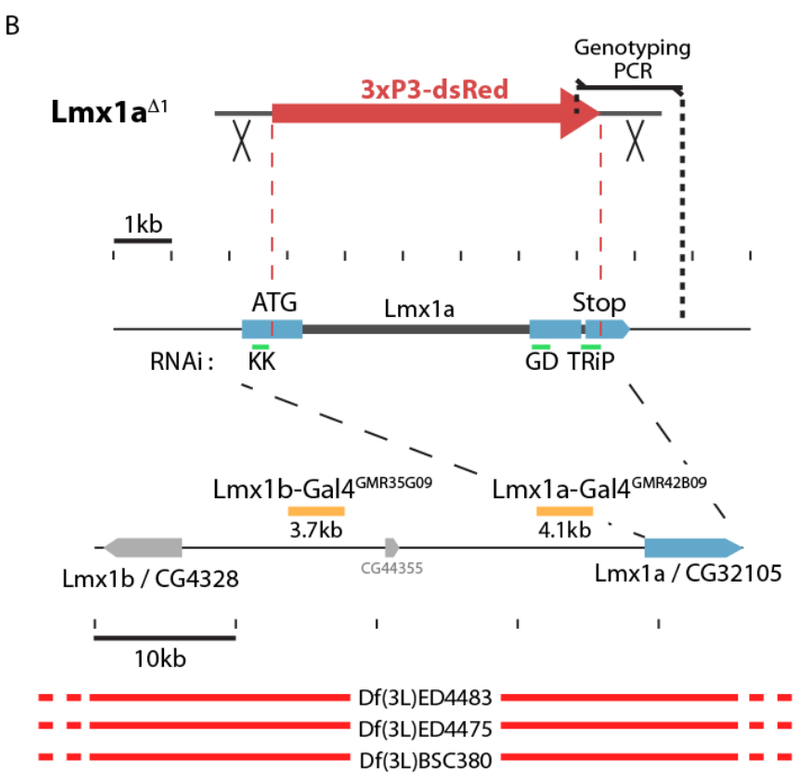
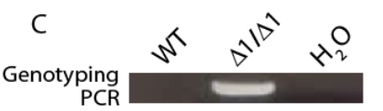
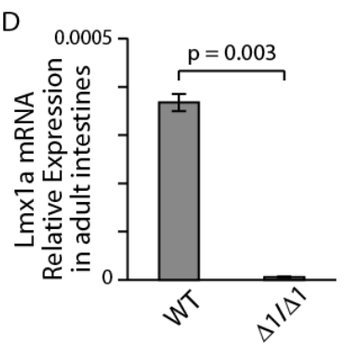
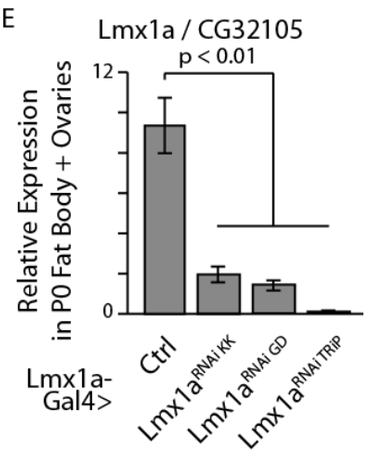
Supplementary Information (7 figures and 3 tables)

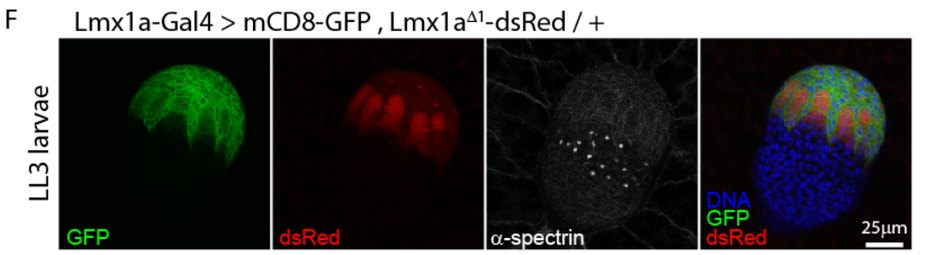




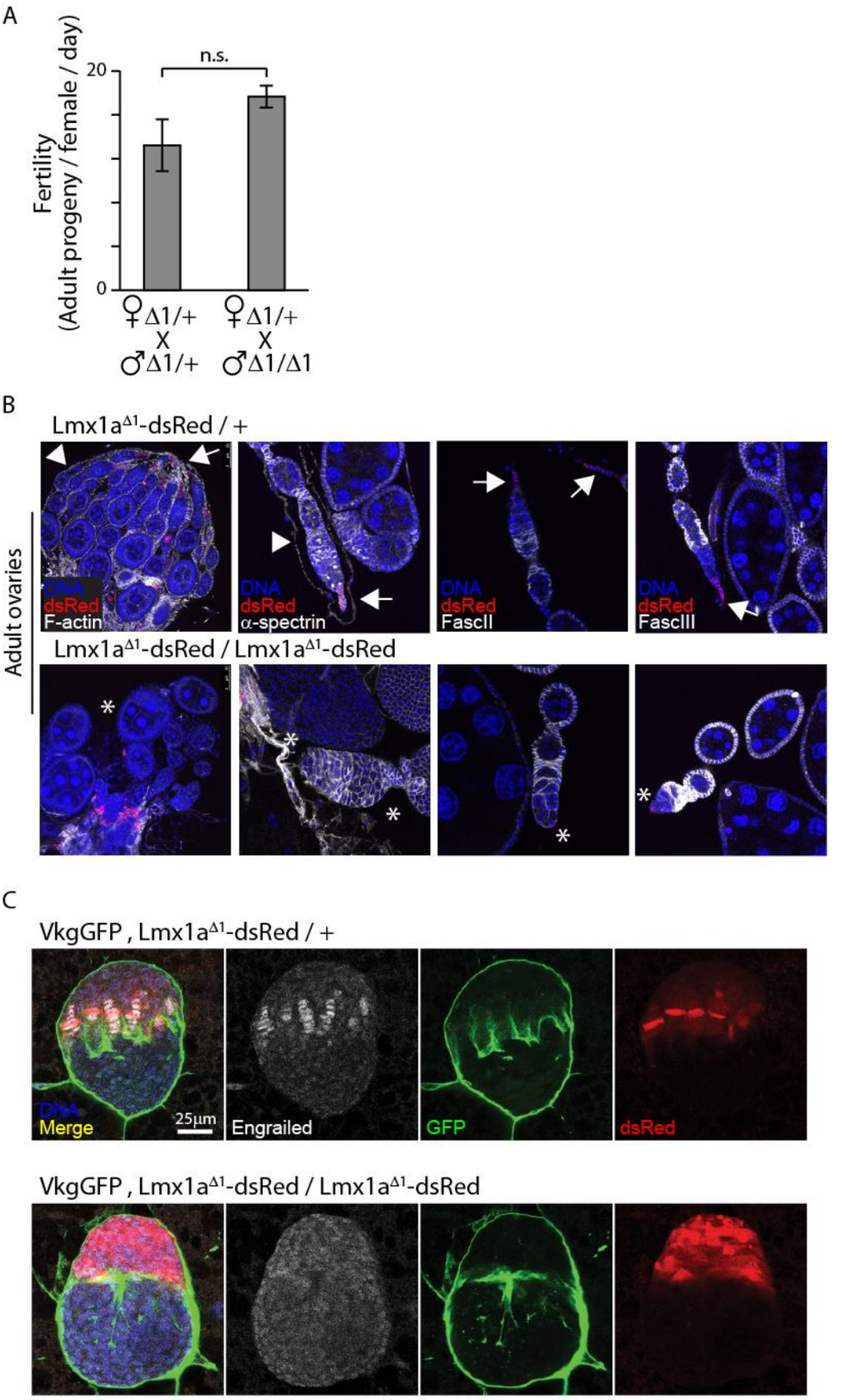




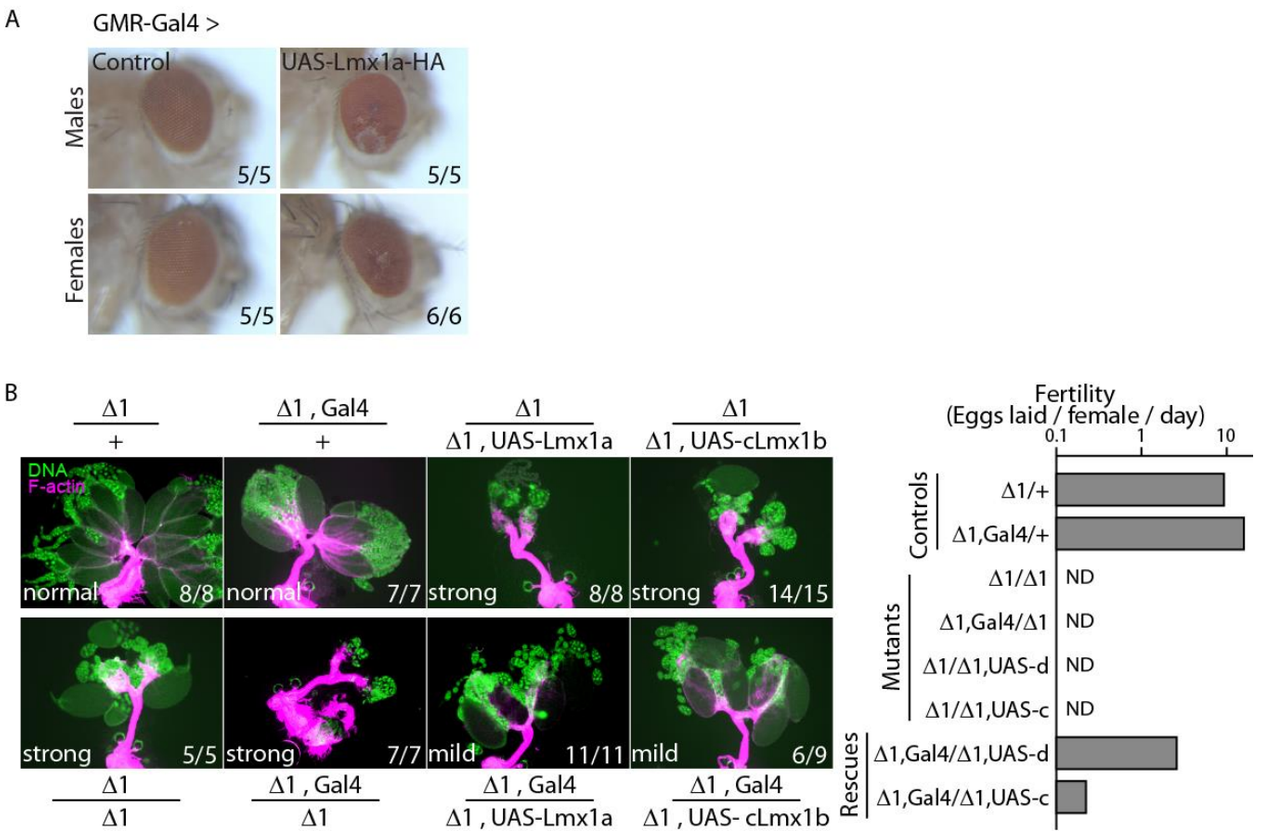




Supplementary Figure 1. Drosophila ovary development, the Lmx1a locus, Lmx1a knock out allele and confirmed Lmx1a knock-down efficiency. (**A**) Schematic representation of Drosophila ovary development and the main cell types involved, from larval stages to adulthood. The terminal filament and cap cells form the stem cell niche in each ovariole. (**B**) Schematic of the Lmx1a locus and tools used to manipulate its expression. Shown at top is the strategy to generate Lmx1a$^{\Delta 1}$-dsRed via Cas9-mediated double strand break and Homology Directed Repair (HDR), replacing the Lmx1a open reading frame with 3xP3-dsRed. The designed gRNA sequence directed Cas9 to the second exon. The 1kb homology arms of the HDR construct are indicated. Indicated in green are the regions targeted by the three RNAi constructs used to knock-down Lmx1a expression in this study. Below is indicated where the Lmx1a locus lies in relation to Lmx1b/CG4328 as well as the locations of the enhancer regions represented by the Lmx1a-Gal4 and Lmx1b/a-Gal4 lines used. The three red lines below indicate that the three deficiency lines used to validate the Lmx1a$^{\Delta 1}$-dsRed phenotype span the full locus of Lmx1a and dLmx1b. (**C**) Validation of the Lmx1a$^{\Delta 1}$-dsRed insertion using PCR amplification of a 3' region of dsRed and the locus downstream of the 3' homology arm, as represented in (B). (**D**) Further validation of Lmx1a$^{\Delta 1}$-dsRed by qPCR, showing full loss of detectable Lmx1a transcript in adult midgut mRNAs. Lmx1a expression is normalized to Actin5c. (**E**) Lmx1a transcript knock down efficiency of Lmx1a-Gal4-driven Lmx1a dsRNA expression as detected by qPCR relative to Vasa transcript in LL3/P0 fat bodies containing ovaries. (**F**) dsRed (from the Lmx1a$^{\Delta 1}$ locus) and Lmx1a-Gal4-driven GFP co-localize in terminal filaments, cap cells and apical cells within the anterior domain of the LL3 ovary, adjacent to germline marked by brightly α-spectrin positive fusomes. α-spectrin also stains plasma membranes.



Supplementary Figure 2. Lmx1a$^{\Delta 1}$ male fertility, Lmx1a$^{\Delta 1}$ ovarian defects in rare genetic backgrounds where residual adult ovariole material is present, and loss of niche architecture as revealed by GFP-labeled collagen IV. (**A**) Fertility assay showing that Lmx1a$^{\Delta 1}$-dsRed homozygous males have no defects in fertility compared to heterozygous siblings. Five cohorts of four females were analyzed per genotype. Values are presented as mean +/- s.e.m., P value is calculated using two-tailed t-test. (**B**) In rare genetic backgrounds, including a non-backcross Lmx1a$^{\Delta 1}$-dsRed/TM3 balanced stock shown here, residual ovarian tissue can be recovered from newly eclosed virgin homozygous mutant females. Left panel shows phalloidin staining and dsRed expression, revealing loss of both terminal filaments (arrows) and sheath (arrowheads) in Lmx1a$^{\Delta 1}$-dsRed homozygotes compared to heterozygotes. Anterior is top right, posterior is bottom left. The asterisk marks absence of sheath in mutant ovaries. The three panels to the right show zoomed-in images of mature ovaries of the same stock, again revealing the presence of terminal filaments (arrows) and sheath (arrowheads) in heterozygotes and the loss of these structures in rare ovarioles found in homozygotes (asterisks). (**C**) GFP-labeled collagen IV subunit (Vkg-GFP) demarcates forming TF-cap structures in control ovaries. This pattern is lost in Lmx1a$^{\Delta 1}$ homozygotes. Loss of En/Inv immunostaining and dsRed pattern also reveal defective terminal filament development as also shown in Fig. 2 E.



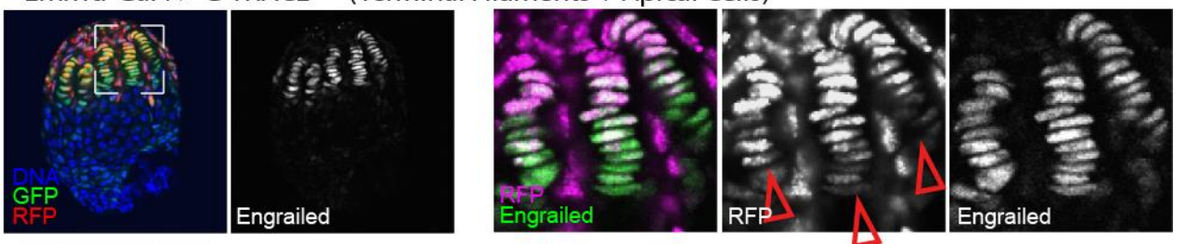
Supplementary Figure 3. Genetic rescue by re-expression of Lmx1a and cLmx1b in developing TF-cap structures. (**A**) Wang et al. reported in 2015 that ectopic expression of Lmx1a in the eye imaginal disc induces eye defects. We show that expression of the HA-tagged Lmx1a construct that we used to rescue Lmx1a function in developing TFs also causes eye-defects when expressed using the GMR-Gal4 driver. This further confirms the Lmx1a fusion protein is functional. (**B**) Expression of UAS-Lmx1a and UAS-cLmx1b using the Lmx1a-Gal4 driver rescues the Lmx1a$^{\Delta 1}$ homozygous phenotype when cultured at 25ºC, as shown by both ovary morphology and fertility. Rescue of Lmx1a$^{\Delta 1}$-dsRed homozygous fertility by cLmx1b appears less pronounced at 25ºC, as compared to 18ºC shown in figure 3A. For each condition, the severity of the phenotype and the proportion of ovary pairs observed are indicated. ND, No laid eggs Detected. Three to four cohorts of four females were analyzed per genotype. Values are presented as mean +/- s.e.m. and P values are calculated using two-tailed t-test.

RFP expression = Driver activity in LL3 / P0 stages
GFP expression = Lineage tracing

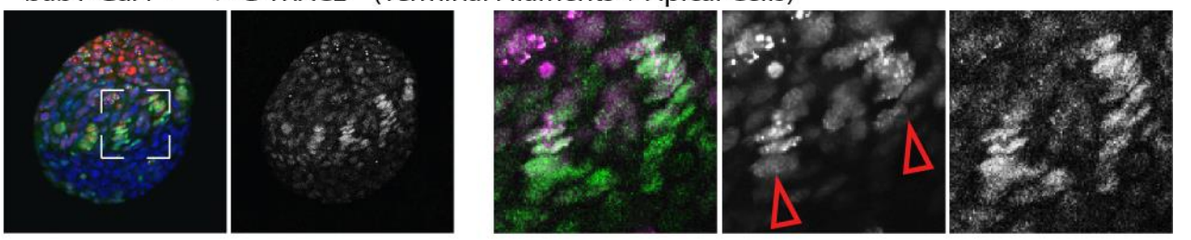
Terminal Filament in which the driver is active during the LL3/P0 transition

Terminal Filament without driver activity during the LL3/ P0 transition

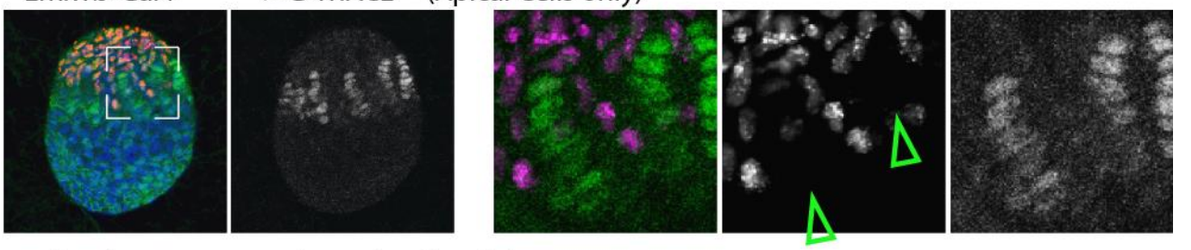
Lmx1a-Gal4 > G-TRACE (Terminal Filaments + Apical Cells)



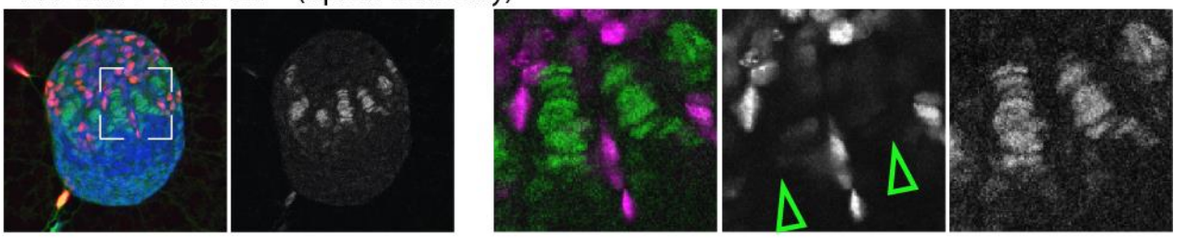
bab1-Gal4$^{Agal4\text{-}5}$ > G-TRACE (Terminal Filaments + Apical Cells)



Lmx1b-Gal4^{GMR35G09} > G-TRACE (Apical Cells only)

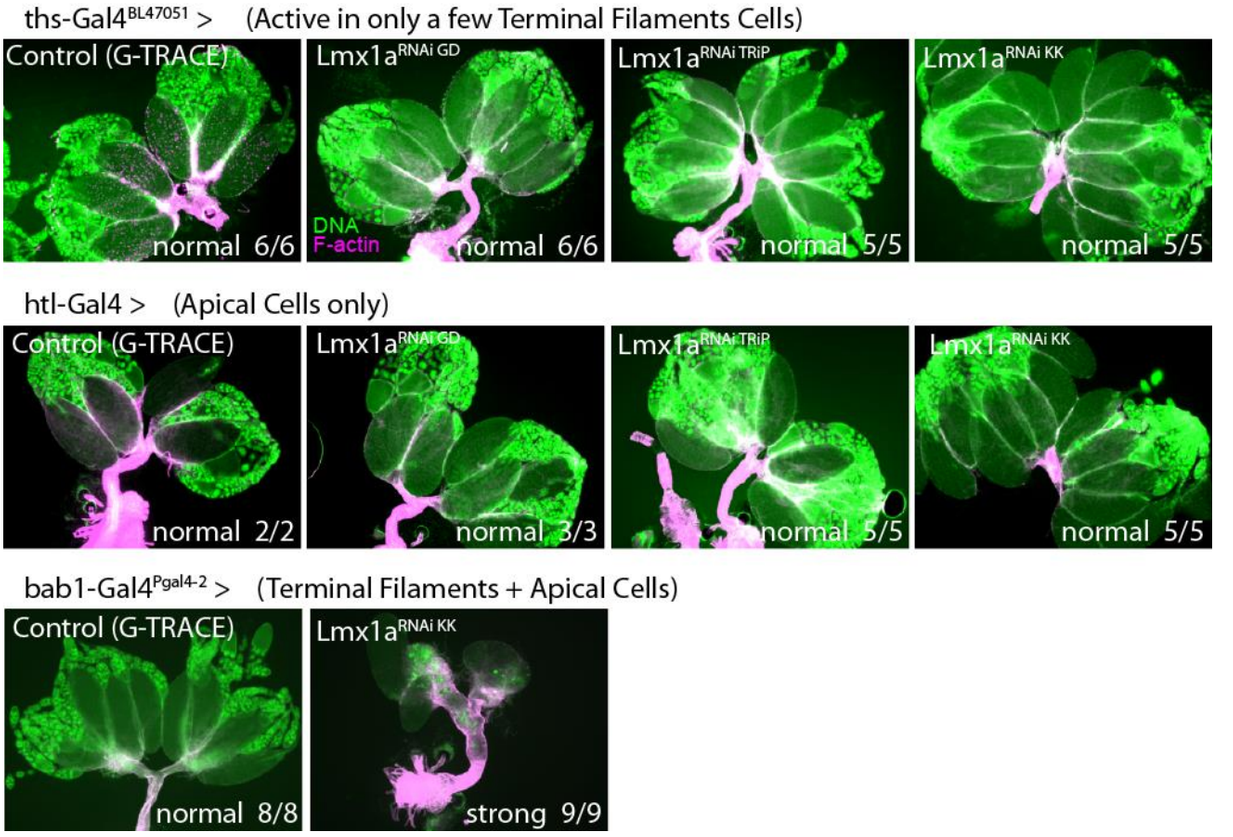


Htl-Gal4 > G-TRACE (Apical Cells only)

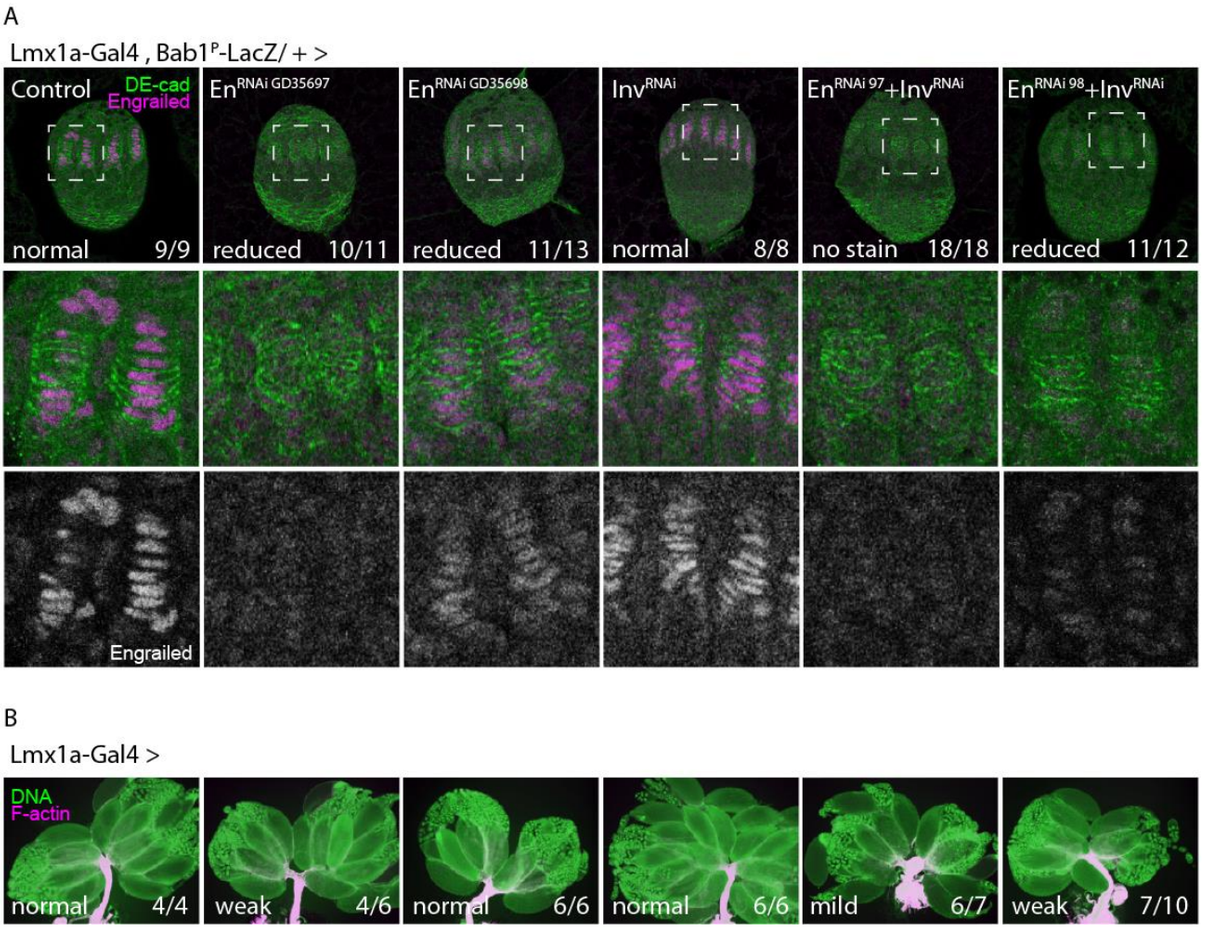


ths-Gal4^{BL47051} > G-TRACE (Active in only a few Terminal Filaments Cells)

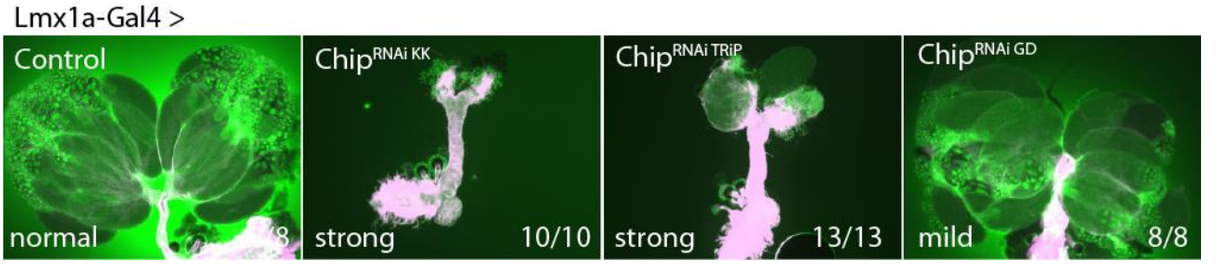
Supplementary Figure 4. P0 expression patterns of Gal4 lines used to demonstrate the requirement of Lmx1a specifically in forming TF-cap structures. All Gal4 driver lines were crossed with the G-TRCAE reporter to both permanently label each driver's full lineage (GFP) and mark current Gal4 activity (RFP) at the time of fixation (P0). Lmx1a-Gal4 and bab1-Gal4$^{Agal4\text{-}5}$ demonstrate activity in both TFs and apical cells at P0 (red arrowheads). dLmx1b-Gal4^{GMR35G09}, which represents control of Gal4 by an enhancer region upstream of the dLmx1b locus, demonstrates activity in only apical cells at P0, not in TFs (green arrowheads). Htl-Gal4, as previously reported by Irizarry et al. 2015, also demonstrates activity in only apical cells at P0. Also reported by Irizarry et al. 2015, a Gal4 driver under the influence of the thisbe (ths) locus, which encodes an FGF ligand secreted by terminal filaments, drives expression specifically in terminal filaments. However, GTRACE RFP analysis of this Gal4 line reveals expression only in a small subset of TF-cap cells (asterisk) by the time of larval-pupal transition. This suggests that this driver expresses Gal4 in TF-cap structures after the time at which they form.



Supplementary Figure 5. Additional tissue-specific Lmx1a knock-down further demonstrates the specificity of Lmx1a requirement to TF-cap structures. ths-Gal4^{BL47051}, which drives expression in only a small subset of terminal filament cells by the time of larval-pupal transition, does not induce adult ovary defects based on ovariole (Hoechst) and sheath (F-actin) morphology. htl-Gal4, an additional Gal4 line active only in apical cells at P0, also does not induce adult ovary defects. bab1-Gal4$^{Pgal4-2}$, an additional Gal4 line active in both apical cells and terminal filaments at P0, does induce adult ovary defects based on loss of ovariole and sheath morphology. For each condition, the severity of the phenotype and the proportion of ovary pairs observed for that phenotype are indicated. See Fig. S3 for the activity of the drivers in P0 ovaries.



Supplementary Figure 6. Efficacy of Engrailed and Invected knock down in developing ovaries and corresponding phenotypes. Engrailed/Invected immunostaining of P0 ovaries demonstrating the efficacy of UAS- En and Inv dsRNA constructs in knocking down En/Inv expression when driven by Lmx1a-Gal4 (**A**). Corresponding adult ovary phenotypes are displayed below (**B**). The $En^{RNAi\ GD35697}$ recombined with Inv^{RNAi} reveals the most effective knock down and induces a mild phenotype. En/Inv knock down was further assessed using this recombinant, as displayed in figure 7.



Supplementary Figure 7. Chip is required in the Lmx1a-Gal4 lineage of the ovary. Three UAS-RNAi lines targeting two separate regions of the Chip transcript induce ovary defects when driven by the Lmx1a-Gal4 driver, as shown by loss of overall ovary material and ovariole morphology (nuclei, Hoechst, green) and sheath (F-actin stain, Phalloidin, magenta).

Supplementary Table 1. RNA sequencing analysis: Genes enriched in ovary-containing samples whose expression is affected in Lmx1a homozygous mutants.

		Enrichment in Ovaries+FatBody samples versus FatBody in Δ1/+		Δ1/Δ1 versus Δ1/+ in Ovaries+FatBody samples	
	Gene Name	**log2FoldChange**	**Padj**	**log2FoldChange**	**Padj**
Genes down-regulated in Δ1/Δ1 ovaries	CG9518	2.01	2.63E-04	-3.71	2.53E-08
	HGTX	3.85	3.41E-10	-3.25	4.36E-07
	wb	0.26	1.27E-02	-3.03	2.65E-20
	otp	3.79	1.20E-50	-2.98	7.31E-05
	rt	4.47	1.32E-24	-2.43	1.22E-13
	CG9492	3.87	5.53E-26	-2.41	4.48E-06
	en	0.17	3.79E-03	-2.28	4.63E-05
	CG15822	1.55	8.42E-09	-1.99	3.88E-04
	ea	0.37	8.69E-03	-1.88	5.76E-03
	CG34347	0.78	4.48E-05	-1.86	2.06E-04
	CG18586	1.00	7.16E-03	-1.85	4.41E-02
	rtet	0.27	1.37E-02	-1.83	2.41E-17
	inv	3.54	4.88E-11	-1.81	2.28E-03
	hh	0.44	4.61E-05	-1.73	1.12E-08
	Nrt	2.84	3.23E-55	-1.69	1.51E-03
	Sox100B	1.34	1.50E-04	-1.58	7.64E-06
	CG10738	1.37	1.76E-05	-1.57	2.59E-06
	hbs	2.86	1.13E-06	-1.56	4.23E-03
	CG42260	1.57	4.59E-09	-1.54	6.22E-03
	Mur89F	3.53	3.33E-15	-1.51	7.01E-04
	CG12592	0.21	1.70E-02	-1.51	2.82E-02
	Timp	2.75	3.19E-05	-1.38	2.98E-02
	CG42346	0.29	2.26E-02	-1.29	6.40E-03
	Ser	2.53	4.88E-10	-1.29	2.28E-03
	RhoGAP100F	0.32	2.85E-03	-1.27	9.78E-06
	form3	2.03	3.76E-04	-1.18	3.27E-02
	CG32165	1.43	3.61E-03	-1.15	1.96E-03
	dysc	3.44	1.99E-12	-1.11	3.07E-02
	CG4415	2.10	2.71E-03	-1.09	3.76E-02
	CG13928	4.64	1.02E-51	-1.06	3.20E-02
	ko	4.13	8.30E-23	-0.96	4.19E-03
	stumps	1.96	1.23E-06	-0.92	2.70E-03
	rec	2.69	6.35E-08	-0.91	4.43E-03
	wake	3.20	1.62E-36	-0.84	2.34E-03
	CG6812	2.17	1.58E-05	-0.73	1.11E-03
	ptc	1.60	5.59E-04	-0.66	2.06E-03
	Ime4	3.25	4.29E-13	-0.50	5.42E-03
	Uch-L5	1.01	1.05E-02	-0.48	6.13E-06
	janA	1.05	6.08E-04	-0.42	2.68E-03
	PIG-S	0.55	1.06E-02	-0.39	2.20E-02
	Cpsf73	4.58	2.29E-25	-0.39	1.07E-03
	trsn	0.75	5.61E-07	-0.37	4.24E-03
	dalao	0.29	4.13E-03	-0.33	1.58E-02
	CG5641	4.60	5.92E-28	-0.30	2.78E-02
	Hpr1	0.35	1.40E-03	-0.27	4.19E-02
	Ge-1	5.83	7.36E-44	-0.27	1.43E-05
	Ddx1	1.68	5.31E-04	-0.23	2.87E-02
	w	3.78	2.20E-27	-0.21	8.91E-01
Genes up-regulated in Δ1/Δ1 ovaries	rdgC	3.40	4.07E-11	1.75	1.99E-08
	Fas3	3.57	4.68E-18	1.54	2.11E-06
	CG6293	2.32	1.72E-12	1.50	6.94E-05
	CG4096	1.17	3.40E-02	1.43	1.59E-07
	CG13032	3.87	1.48E-17	1.40	1.24E-02
	Nlg1	0.35	9.35E-03	1.30	3.88E-04
	Tollo	2.84	2.89E-34	1.28	6.71E-12
	Shaw	1.49	6.16E-13	1.25	2.39E-07
	CG9411	2.93	2.40E-09	1.18	5.28E-04
	ab	0.36	2.96E-03	0.66	3.27E-02
	Spc105R	0.54	1.93E-13	0.66	1.32E-02
	CTCF	3.22	1.72E-20	0.52	3.14E-05
	CG3967	1.62	3.88E-06	0.40	7.66E-03
	Atu	2.83	8.61E-17	0.28	4.31E-02
	Dref	0.37	4.43E-04	0.20	4.42E-02

Supplementary Table 2. Detailed genotypes of animals described in each figure.

Figure	Genotype
1A	Lmx1a$^{\Delta 1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
1B	Lmx1a-Gal4/+
	Lmx1a$^{RNAi\ KK}$ / + ; Lmx1a-Gal4/+
	Lmx1a-Gal4/Lmx1a$^{RNAi\ TRiP}$
	Lmx1a-Gal4/Lmx1a$^{RNAi\ GD}$
1C	Lmx1a-Gal4 / UAS-mCD8-GFP
1D	Lmx1a-Gal4, UAS-mCD8-GFP, Lmx1a$^{\Delta 1}$-dsRed / +
1E	Gtrace / + ; Lmx1a-Gal4
1F	Gtrace / + ; Lmx1a-Gal4
2A	Lmx1a$^{\Delta 1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
2B	Lmx1a$^{\Delta 1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
	Lmx1a$^{\Delta 1}$-dsRed /Df(3L)ED4475
	Lmx1a$^{\Delta 1}$-dsRed /Df(3L)ED4483
	Lmx1a$^{\Delta 1}$-dsRed /Df(3L)BSC380
2C	Lmx1a$^{\Delta 1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
2D	Lmx1a$^{\Delta 1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
2E	Lmx1a$^{\Delta 1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
2F	Lmx1a$^{\Delta 1}$-dsRed, Bab1^{P}-LacZ /TM3-GFP
	Lmx1a$^{\Delta 1}$-dsRed, Bab1^{P}-LacZ / Lmx1a$^{\Delta 1}$-dsRed
3A	Lmx1a$^{\Delta 1}$-dsRed /+
	Lmx1a$^{\Delta 1}$-dsRed, Lmx1a-Gal4/+
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed
	Lmx1a$^{\Delta 1}$-dsRed, Lmx1a-Gal4/ Lmx1a$^{\Delta 1}$-dsRed
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed, UAS-Lmx1a
	Lmx1a$^{\Delta 1}$-dsRed / Lmx1a$^{\Delta 1}$-dsRed, UAS-cLmx1b
	Lmx1a$^{\Delta 1}$-dsRed, Lmx1a-Gal4/ Lmx1a$^{\Delta 1}$-dsRed, UAS-Lmx1a
	Lmx1a$^{\Delta 1}$-dsRed, Lmx1a-Gal4/ Lmx1a$^{\Delta 1}$-dsRed, UAS-cLmx1b
3B,C	Gtrace/+ ; Lmx1a-Gal4/+
	Lmx1a$^{RNAi\ KK}$ / + ; Lmx1a-Gal4/+
	Lmx1a-Gal4/Lmx1a$^{RNAi\ TRiP}$
	Lmx1a-Gal4/Lmx1a$^{RNAi\ GD}$
	Gtrace/+ ; Bab-Gal4$^{Agal4-5}$/+
	Lmx1a$^{RNAi\ KK}$ / + ; Bab-GalAgal4-5/+

	Bab-Gal4$^{Agal4-5}$/Lmx1a$^{RNAi\ TRiP}$
	Bab-Gal4$^{Agal4-5}$/ Lmx1a$^{RNAi\ GD}$
	Gtrace/+ ; dLmx1b^{GMR35G09}-Gal4/+
	Lmx1a$^{RNAi\ KK}$ / + ; dLmx1b-Gal4 ^{GMR35G09}/+
	dLmx1b-Gal4 ^{GMR35G09}/Lmx1a$^{RNAi\ TRiP}$
3D	Lmx1a-Gal4/+
	Lmx1a-Gal4/Lmx1a$^{RNAi\ TRiP}$
	Gtrace/+ ; Bab-Gal4$^{Agal4-5}$/+
	Bab-Gal4$^{Agal4-5}$/Lmx1a$^{RNAi\ TRiP}$
4A	+ / tub-Gal80^{ts} ; Lmx1a-Gal4 / +
	Lmx1a$^{RNAi\ KK}$ / tub-Gal80^{ts} ; Lmx1a-Gal4 / +
4B	+ / tub-Gal80^{ts} ; Lmx1a-Gal4, Lmx1a$^{\Delta1}$-dsRed / +
	+ / tub-Gal80^{ts} ; Lmx1a-Gal4, Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed
	+ / tub-Gal80^{ts} ; Lmx1a-Gal4, Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed, UAS-Lmx1a
5A-D	Lmx1a$^{\Delta1}$-dsRed /TM3-GFP
	Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed
6A	Lmx1a-Gal4, Bab1^{P}-LacZ / +
	Lmx1a-Gal4, Bab1^{P}-LacZ / Lmx1a$^{RNAi\ KK}$
	Lmx1a-Gal4, Bab1^{P}-LacZ / Lmx1a$^{RNAi\ TRiP}$
6B	Lmx1a-Gal4 / +
	Lmx1a-Gal4 / Bab1$^{RNAi\ 57410}$
	Lmx1a-Gal4 / Bab1$^{RNAi\ 49042}$
6C	Bab1^{P}-LacZ / +
	Bab1^{P}-LacZ / Bab1^{P}-LacZ
7A	Lmx1a-Gal4 / +
	Lmx1a-Gal4 / Hh $^{RNAi\ TRiP\ 25794}$
	Sox100B $^{RNAi\ TRiP\ 57417}$ / + ; Lmx1a-Gal4 / +
	Lmx1a-Gal4 / Invected$^{RNAi\ TRiP\ 41675}$, Engrailed$^{RNAi\ GD\ 35697}$
7B	Lmx1a-Gal4, Bab1^{P}-LacZ / +
	Lmx1a-Gal4, Bab1^{P}-LacZ / Hh $^{RNAi\ TRiP\ 25794}$
	Sox100B $^{RNAi\ TRiP\ 57417}$ / + ; Lmx1a-Gal4, Bab1$^{P\text{-}LacZ}$ / +
	Lmx1a-Gal4, Bab1^{P}-LacZ / Invected$^{RNAi\ TRiP\ 41675}$, Engrailed$^{RNAi\ GD\ 35697}$
S1C	W^{1118}
	Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed
S1D	W^{1118}
	Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed
S1E	Gtrace/+ ; Lmx1a-Gal4/+
	Lmx1a$^{RNAi\ KK}$ / + ; Lmx1a-Gal4/+
	Lmx1a-Gal4/Lmx1a$^{RNAi\ GD}$
	Lmx1a-Gal4/Lmx1a$^{RNAi\ TRiP}$
S1F	Lmx1a-Gal4, UAS-mCD8-GFP, Lmx1a$^{\Delta1}$-dsRed / +
S2A	Lmx1a$^{\Delta1}$-dsRed /TM3-GFP

	Lmx1a$^{\Delta1}$-dsRed / Lmx1aΔ1-dsRed
S2B	Lmx1a$^{\Delta1}$-dsRed (non-isogenic) / TM3
	Lmx1a$^{\Delta1}$-dsRed (non-isogenic) / Lmx1aΔ1-dsRed (non-isogenic)
S2C	Vkg-GFP, Lmx1a$^{\Delta1}$-dsRed / TM3-GFP
	Vkg-GFP, Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed
S3A	w ; GMR-Gal4 , UAS-GFP / +
	w ; GMR-Gal4 , UAS-GFP / + ; UAS-Lmx1a-HA
S3B	Lmx1a$^{\Delta1}$-dsRed /+
	Lmx1a$^{\Delta1}$-dsRed, Lmx1a-Gal4/+
	Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed
	Lmx1a$^{\Delta1}$-dsRed, Lmx1a-Gal4/ Lmx1a$^{\Delta1}$-dsRed
	Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed, UAS-Lmx1a
	Lmx1a$^{\Delta1}$-dsRed / Lmx1a$^{\Delta1}$-dsRed, UAS-cLmx1b
	Lmx1a$^{\Delta1}$-dsRed, Lmx1a-Gal4/ Lmx1a$^{\Delta1}$-dsRed, UAS-Lmx1a
	Lmx1a$^{\Delta1}$-dsRed, Lmx1a-Gal4/ Lmx1a$^{\Delta1}$-dsRed, UAS-cLmx1b
S4	Gtrace ; Lmx1a-Gal4/+
	Gtrace ; Bab1-Gal4$^{Agal4-5}$
	Gtrace ; dLmx1b-Gal4^{GMR35G09}
	Gtrace ; htl-Gal4^{BL40669}
	Gtrace ; ths-Gal4^{BL47051}
S5	Gtrace ; ths-Gal4^{BL47051}
	ths-Gal4^{BL47051}/ Lmx1a $^{RNAi\ GD}$
	ths-Gal4^{BL47051}/ Lmx1a $^{RNAi\ TRiP}$
	Lmx1a $^{RNAi\ KK}$ / + ; ths-Gal4^{BL47051}/ +
	Gtrace ; htl-Gal4^{BL40669}
	htl-Gal4^{BL40669}/ Lmx1a $^{RNAi\ GD}$
	htl-Gal4^{BL40669}/ Lmx1a $^{RNAi\ TRiP}$
	Lmx1a $^{RNAi\ KK}$ / + ; htl-Gal4^{BL40669}/ +
	Gtrace ; Bab1-Gal4$^{Agal4-2}$
	Lmx1a $^{RNAi\ KK}$ / + ; bab1-Gal4$^{Pgal4-2}$/ +
S6	Lmx1a-Gal4, Bab1^{P}-LacZ / +
	Lmx1a-Gal4 Bab1^{P}-LacZ / + ; Engrailed $^{RNAi\ GD\ 35697}$ / +
	Lmx1a-Gal4 Bab1^{P}-LacZ / + ; Engrailed $^{RNAi\ GD\ 35698}$ / +
	Lmx1a-Gal4 Bab1^{P}-LacZ / + ; Invected$^{RNAi\ TRiP\ 41675}$ / +
	Lmx1a-Gal4 Bab1^{P}-LacZ / + ; Invected$^{RNAi\ TRiP\ 41675}$, Engrailed$^{RNAi\ GD\ 35697}$ / +
	Lmx1a-Gal4 Bab1^{P}-LacZ / + ; Invected$^{RNAi\ TRiP}$ 41675, Engrailed$^{RNAi\ GD\ 35698}$ / +
	Lmx1a-Gal4 / +
	Lmx1a-Gal4 / + ; Engrailed $^{RNAi\ GD\ 35697}$ / +
	Lmx1a-Gal4 / + ; Engrailed $^{RNAi\ GD\ 35698}$ / +
	Lmx1a-Gal4 / + ; Invected$^{RNAi\ TRiP\ 41675}$ / +
	Lmx1a-Gal4 / + ; Invected$^{RNAi\ TRiP\ 41675}$, Engrailed$^{RNAi\ GD\ 35697}$ / +

Lmx1a-Gal4 / + ; Invected$^{RNAi\ TRiP}$ 41675, Engrailed$^{RNAi\ GD\ 35698}$ / +

S7

Lmx1a-Gal4 / +
Chip$^{RNAi\ KK}$ / + ; Lmx1a-Gal4 / +
Lmx1a-Gal4 / Chip$^{RNAi\ TRiP}$
Chip$^{RNAi\ GD}$ / + ; Lmx1a-Gal4 / +

Supplementary Table 3. Oligonucleotide primers used in this study.

	Forward	**Reverse**
qPCR primers		
Actin5c	5'-CTCGCCACTTGCGTTTACAGT-3'	5'-TCCATATCGTCCCACTTGGTC-3'
Lmx1a/CG32105	5'-CAGTAGCCACCTCGCAATTA-3'	5'-CGAAGTTCTTCTCGCACTTGA-3'
Vasa	5'-CGGTCGGCTGTACGAAA-3'	5'-CCCTCTTTCACCACGTTCA-3'
Fbp2	5'-TCGACAAGGATGTGGAGACTA-3'	5'-CAGAGGACATGTTAACCACCAT-3'
bab1	5'-CGAGATGATCCGAGAGGAAG-3'	5'-GGTTGGTGTTCCAGCACTTT-3'
bab2	5'-GGAGATCAAGCCAGAAATCG-3'	5'-TCTGCTGATTGGTGTTCCAG-3'
Hh	5'-CCACATCTACTGCTCCGTCA -3'	5'-GTTTTGCATCTGCTCGAGGT-3'
Ser	5'-TGCCTGCAACTTAATTGCTTT-3'	5'-CTATCGTCTTGGTGGCCCTAAG-3'
Lmx1aΔ1-dsRed mutant		
Lmx1a$^{\Delta 1}$-dsRed genotyping	5'-CTCCCACAACGAGGACTACA-3'	5'-GCTGAGCTGCCATCTGTTAAT-3'
Lmx1a gRNA	5'-CTTCGATTTGCTGAGGCCACGGGA-3' (sense)	5'-AAACTCCCGTGGCCTCAGCAAATC-3' (antisense)
HDR 5' Homology Arm	5'-AAGAATTCCTGCCTCTACTGCTGCCACTGC-3'	5'-AAGCGGCCGCTCGTATACGAGTTGCCACA-3'
HDR 3' Homology Arm	5'-AAACTAGTGATGGCCATTGGCTGGAATG-3'	5'-AACTCGAGTTGGTCGAGTTTCATAAAAGC-3'