

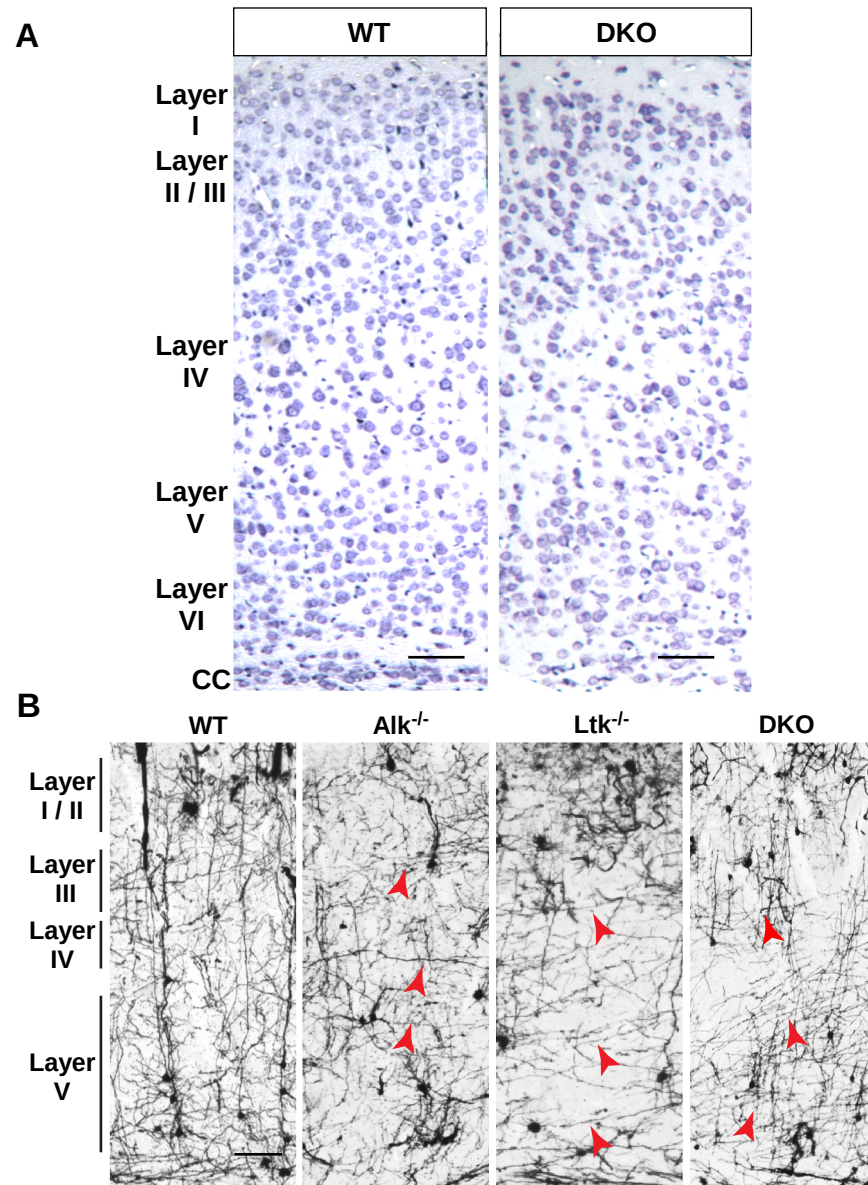
APPENDIX

LTK and ALK promote neuronal polarity and cortical migration by modulating IGF1R activity

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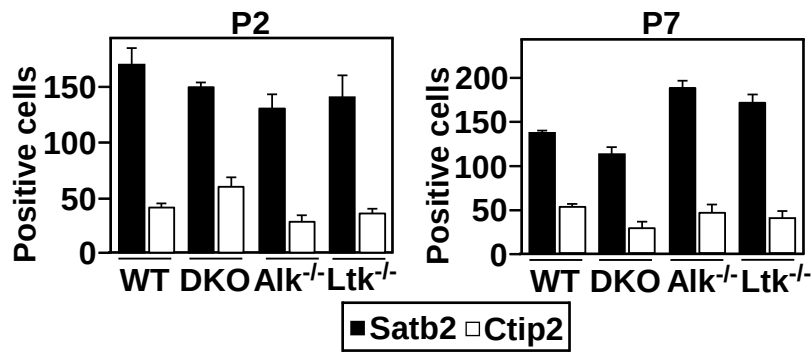
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Appendix Figure S1. Analysis of Coronal sections of WT and Ltk/Alk knockout mice by Nissl and Golgi staining.

- A** Analysis of cortical layering *in vivo* by Nissl staining of adult WT and DKO murine brains. Nissl-stained coronal sections reveal a similar pattern of cortical layering in wild type (WT) and DKO adults. Scale bars are 100 μ m.
- B** Golgi stains of adult cortices in wild-type (WT), single ($Ltk^{-/-}$ and $Alk^{-/-}$) and double (DKO) knockout mice show defects in neuronal projections. Representative coronal sections obtained from an independent biological experiment from those in Fig. 3E are shown. Examples of aberrant projections in pyramidal neurons in knockout mice are marked (red arrowheads). Scale bars are 100 μ m.

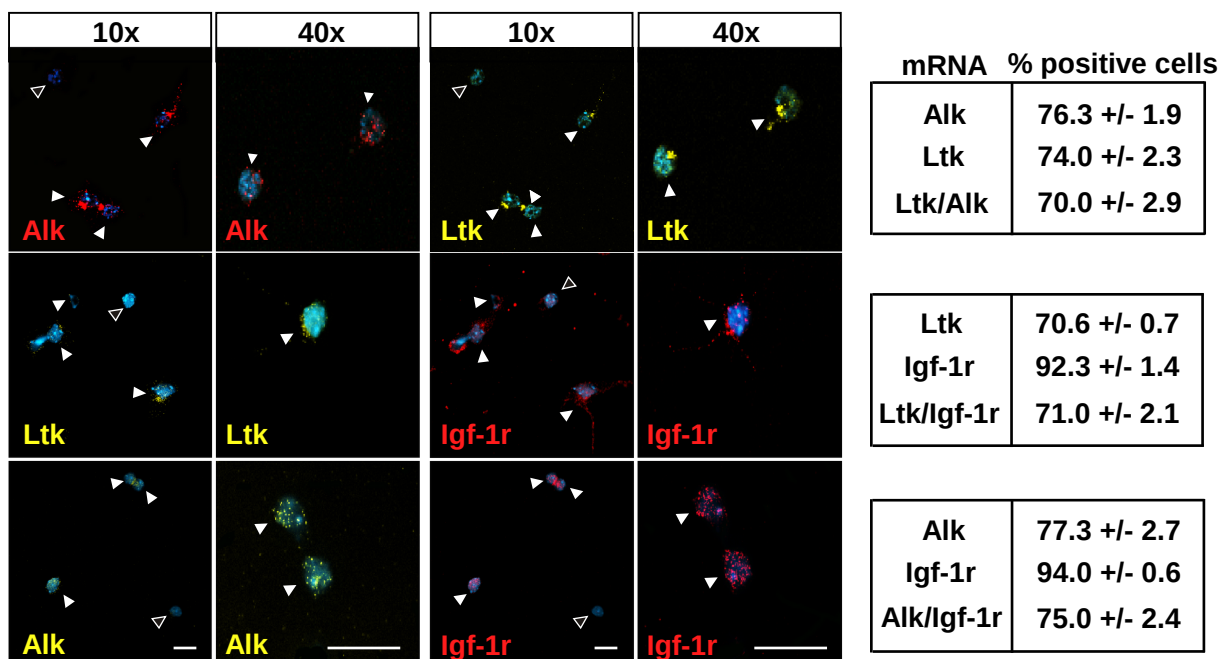


Appendix Figure S2. Ctip2 and Satb2 positive cells in WT, single and DKO mice at P2 and P7.

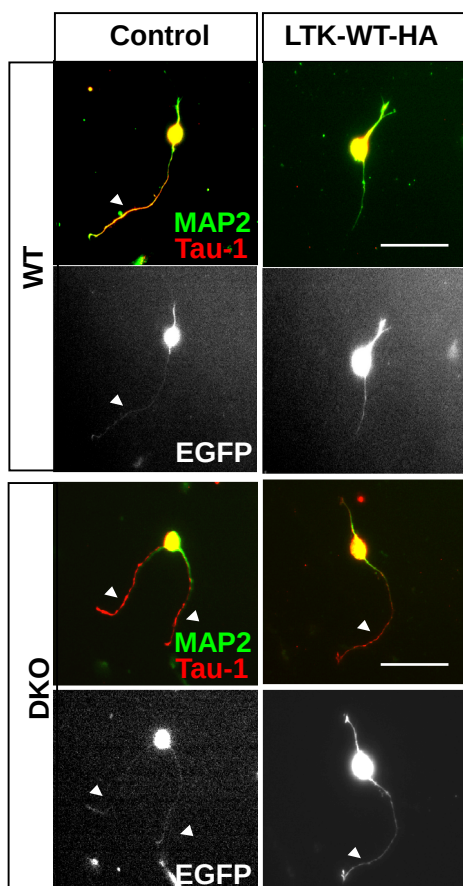
Quantitation of Ctip2 and Satb2 positive cells in all 8 bins within the cortex of WT, single and DKO mice at P2 and P7 in a total area corresponding to 100 μ M x 600 μ M.

Data information: Data are presented as the mean $\pm$ SEM of 3 independent experiments.

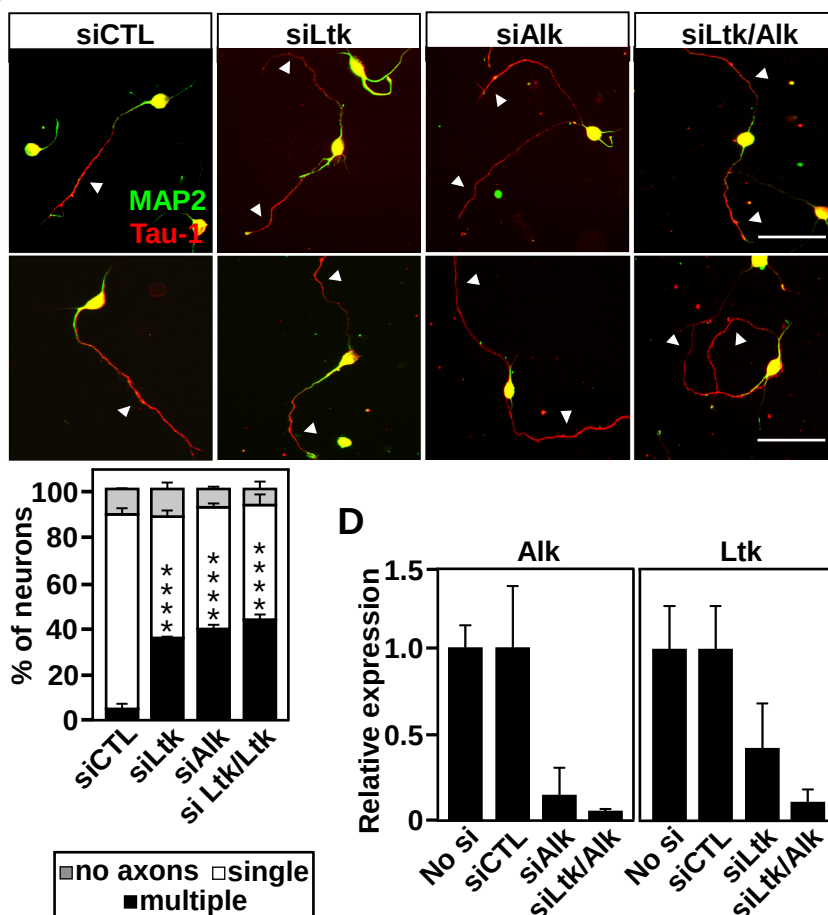
A



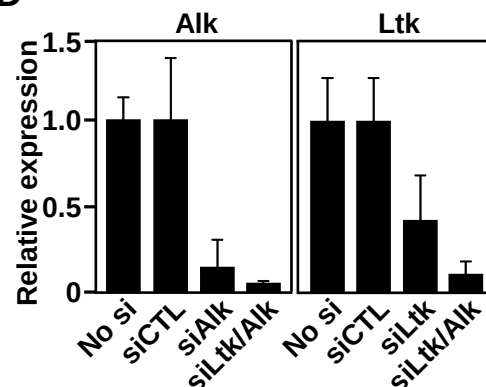
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C



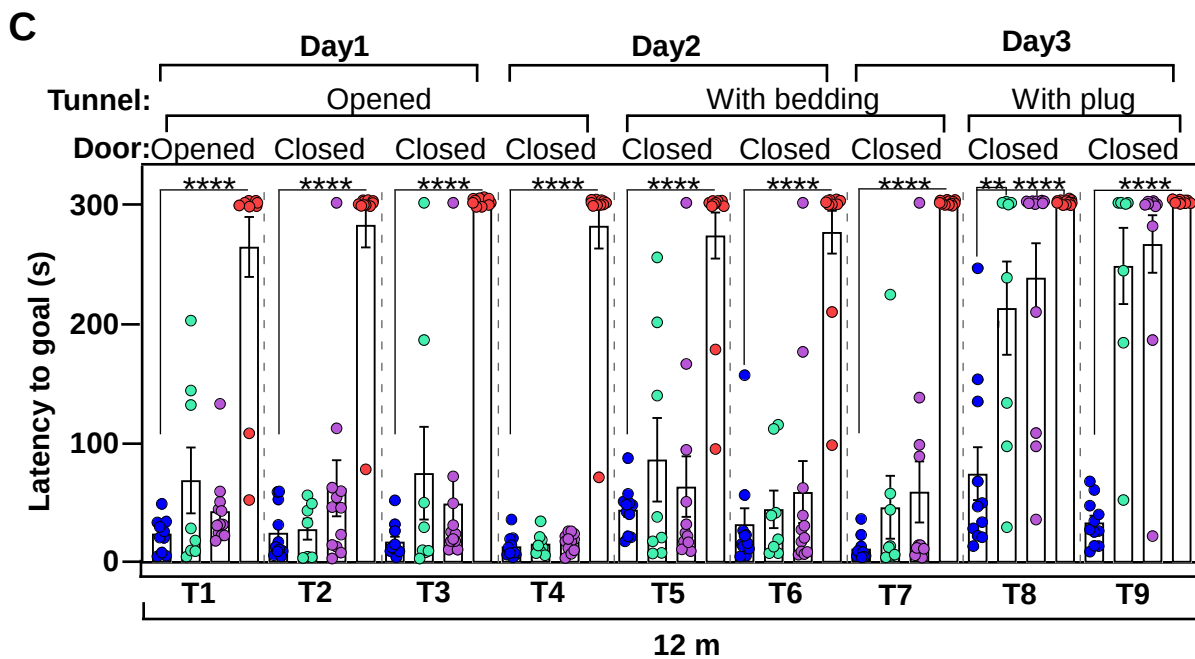
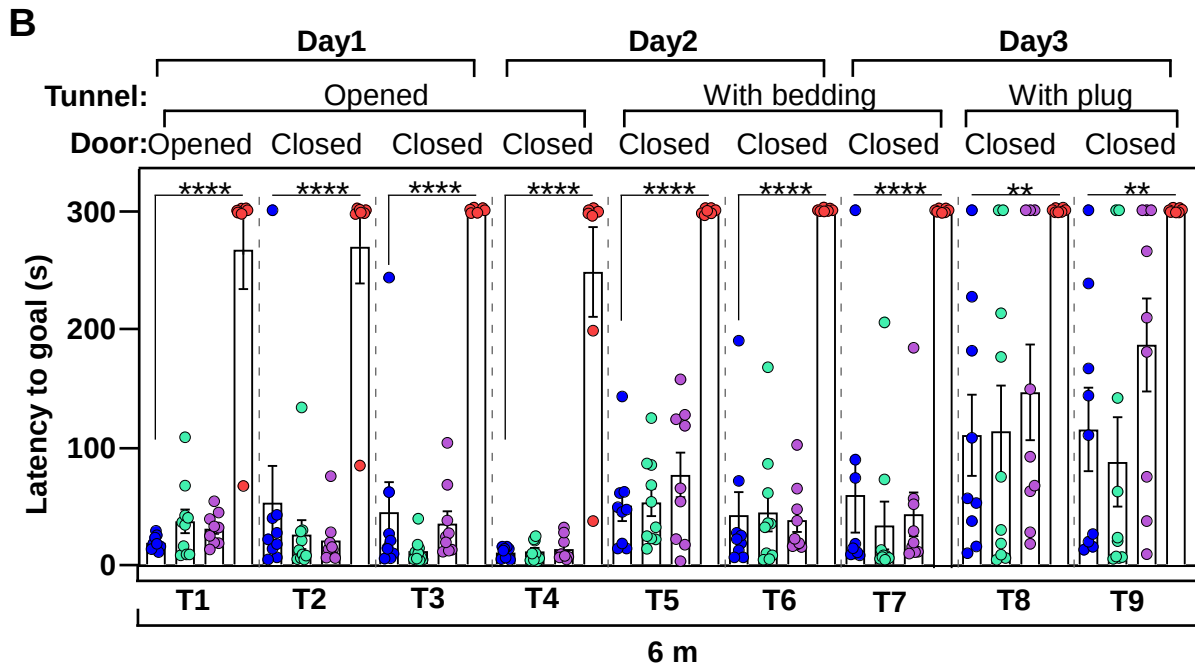
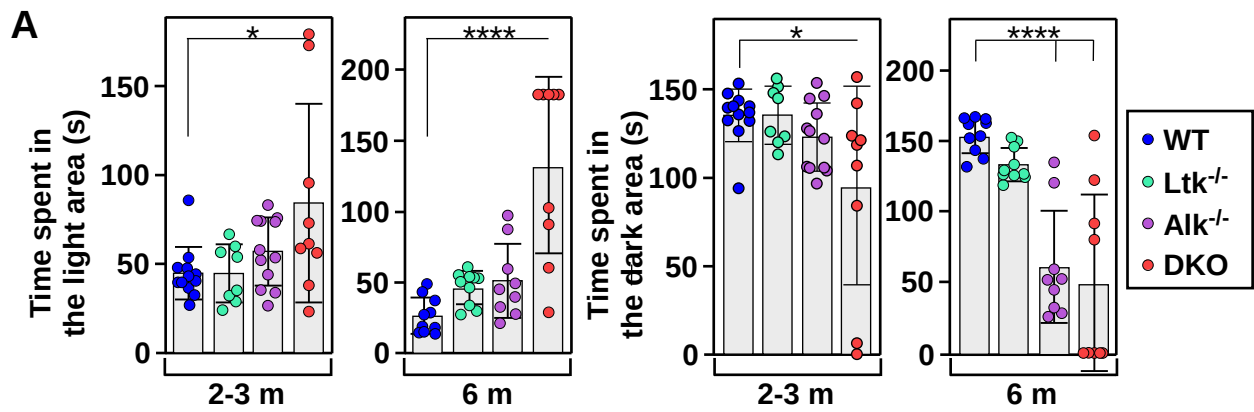
D



Appendix Figure S3. Change of expression or activity of Ltk and Alk results in defects in neuronal polarity.

- A Analysis of mRNA expression of Ltk, Alk and Igf-1r in isolated neurons. Expression of Alk, Ltk and Igf-1r in isolated primary cortical neurons from WT mice was determined by single molecule *in situ* analysis. Independent images taken at 10X and 40X magnification are shown. Filled arrowheads mark positive cells and open arrowheads mark negative cells. Scale bars are 200 μ m. Quantitation of the percent of positive cells was tabulated (right).
- B Additional representative images of dissociated WT and DKO cortical neurons, electroporated with plasmids encoding EGFP alone (control) or together with HA-tagged LTK-WT (as in Figure 3C) are shown. Cells were fixed 38 h after plating and then stained for Tau-1 (axons, red) and MAP2 (dendrites, green) with transfected cells identified by EGFP staining (white). Scale bars are 20 μ m.
- C Abrogation of the expression of Ltk, Alk or both in cortical neurons using siRNAs results in defects similar to that observed in neurons isolated from knockout mice. WT cortical neurons were transfected with siCtl, siLtk and/or siAlk. Representative images of cells fixed at 38 h and stained with Tau-1 (axons, red) and MAP2 (dendrites, green) are shown. Scale bars are 20 μ m.
- D Knockdown efficiency in neurons was evaluated by real-time PCR.

Data information: Quantitation of the percent of positive cells in (A) was plotted from 3 independent biological experiments and a minimum of 50 cells per condition. Quantitation of percent of neurons with multiple, single or no axons in (C) is plotted as the mean $\pm$ SEM of n=150 neurons from 3 independent biological experiments. Data in (D) are plotted as mean $\pm$ SD from 3 independent biological experiments. Statistical significance: ****p<0.0001 by one-way ANOVA using Dunnett's test.



Appendix Figure S4. Performance of mice in light-dark box and in puzzle box tests.

- A Light-dark box. Time spent in the light or dark areas of individual mice of 2-3 or 6 months of age of the indicated genotypes is plotted.
- B, C Puzzle-box test. Latency to reach the goal box during the 9 trials of the test is plotted for each individual 6 (B) or 12 (C) month old mouse of the indicated genotypes.

Data information: Results in all panels are plotted as mean $\pm$ SEM. Data were analyzed using one-way ANOVA and comparisons to WT were performed using Dunnett's test (**** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, ns, not significant, $n = 8-12$ mice/group, n represents biological replicates).

Appendix Table S1: Sequences of primers used for genotyping

Gene	Forward	Reverse
Alk WT	ACC CCC TCA CAG CGG ACA CCT ATC	TGG GGA CAG GGG CAG ATG ATT GAC
Alk neo	ATC TCC TGT CAT CTC ACC TTG CTC	GTA AAG CAC GAG GAA GCG GTC AGC
Ltk WT	GCC AAC CTA CCG CAA GCC CTC TGA T	AAT AGT AAG GGT TGG GTG CTG TCC
Ltk neo	ATC TCC TGT CAT CTC ACC TTG CTC	GTA AAG CAC GAG GAA GCG GTC AGC

Appendix Table S2: Antibody list

Antibody	Host	Application	Dilution	Suppliers	Catalog number
MAP2	Mouse	ICC	1:400	Millipore	MAB3418
NeuN	Rabbit	IHC	1:500	Cell Signaling	12943
Tau-1 (PC1C6)	Mouse	ICC	1:200	Millipore	MAB3420
SOX2	Mouse	IHC	1:50	Santa Cruz	sc-365823
TBR2	Rabbit	IHC	1:200	Abcam	ab23345
SATB2	Rabbit	IHC	1:500	Abcam	ab51502
PAX6	Rabbit	IHC	1:100	Covance	PRB-278P
TUJ-1	Mouse	ICC	1:500	BioLegend	MMS-435P
BrDU	Rat	IHC	1:1000	Abcam	ab142567
CTIP2	Rat	IHC	1:300	Abcam	ab18465
ALK (D5F3)	Rabbit	WB	1:1000	Cell Signaling	3633
ALK (D5F3)	Rabbit	IP	1:100	Cell Signaling	3633
pALK (Tyr 1586)	Rabbit	WB	1:1000	Cell Signaling	3348
pAKT (Ser 473)	Rabbit	WB	1:1000	Cell Signaling	9271
AKT	Rabbit	WB	1:1000	Cell Signaling	9272
GSK3 β (27C10)	Rabbit	WB	1:1000	Cell Signaling	9315
pGSK3 β (Ser 9)	Rabbit	WB	1:1000	Cell Signaling	9323
IGF-1R β (D23H3)	Rabbit	WB	1:1000	Cell Signaling	9750
IGF-1R β (D23H3)	Rabbit	IP	1:100	Cell Signaling	9750
pIGF-1R β (Tyr 1135/1136)	Rabbit	WB	1:1000	Cell Signaling	3024
IGF-1R α (24-60)	Mouse	NA	1.5 μ g/ μ l	ThermoFisher	MA5-13817
Flag M2	Mouse	WB	1:3000	Sigma	F3165
Flag M2	Mouse	IP	1:1000	Sigma	F3165
HA	Rat	WB	1:1000	Roche	11867423001
GST	Rabbit	WB	1:1000	Cell Signaling	2625
GFP	Rabbit	WB	1:1000	Cell Signaling	2555
GFP	Mouse	IP	1:100	Santa Cruz	sc9996
GAPDH	Rabbit	WB	1:10000	Sigma	G9545

Appendix Table S3: Sequences of primers used for Real-Time PCR

Gene	Forward	Reverse
Ltk	CTC ATT CTG CTG GAG CTG ATG TCT	TGC AAT AGG TCC TGC ATG GTC AGA
Alk	CCC GAG TAT AAG CTG AGC AAG	TCA GGT CAC TGA TGG AGG AA
Gapdh	CAC ACC GAC CTT CAC CAT TTT	GAG ACA GCC GCA TCT TCT TGT
Igf-1r	CAG CAC TCG TTG TTC TCG GT	AGC CCA TGT GTG AGA AGA CC
Hprt1	TCA GTC AAC GGG GGA CAT A	GGG GCT GTA CTG CTT AAC CAG
Alkal2	TGA AAC ATC TCA CAG GTC CTC T	GAT GGT GCA GT TCT CGT GT