

Supplementary Figures

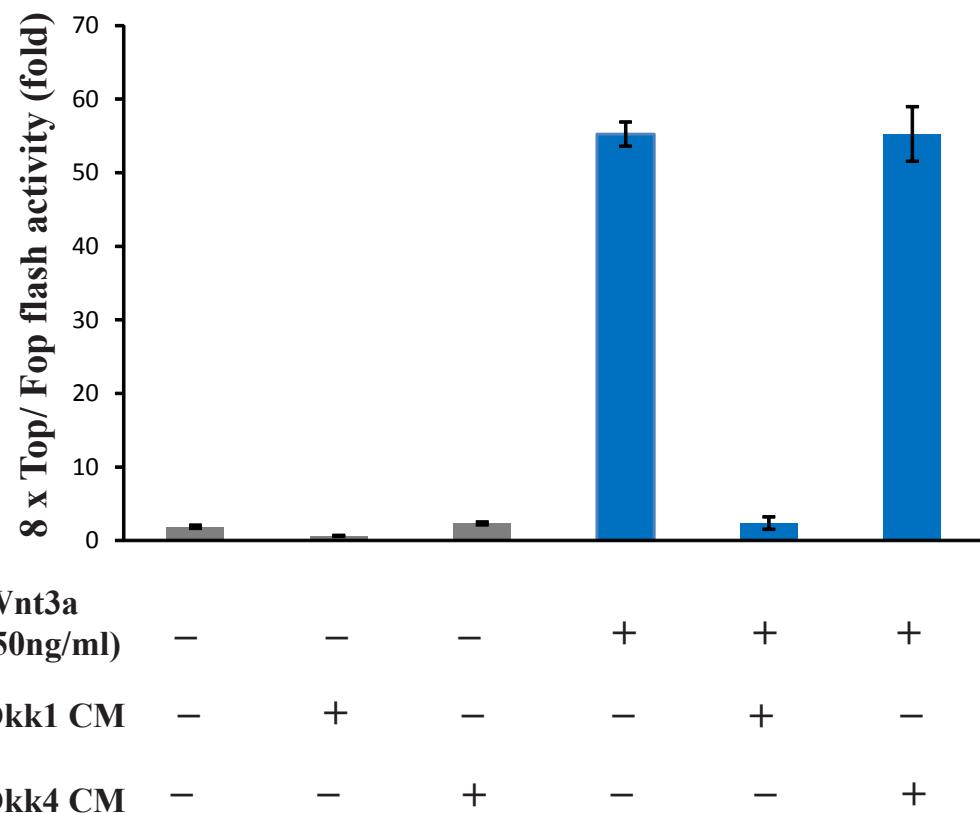


Fig. S1: Dkk1 but not Dkk4 blocks Wnt3a-induced Topflash luciferase activity.

Kera308 cells were transfected with 8×Topflash or Fopflash vector and cultured for 24 h, then incubated with control CM, Dkk1CM or Dkk4CM for another 2 h, followed by application with or without recombinant Wnt3a (50ng/ml) for an additional 24 h culture. Luciferase activity was measured and the ratio of Top/Fop was shown in histogram. Transfection and luciferase assays were performed in triplicate. Error bars, mean ± SEM.

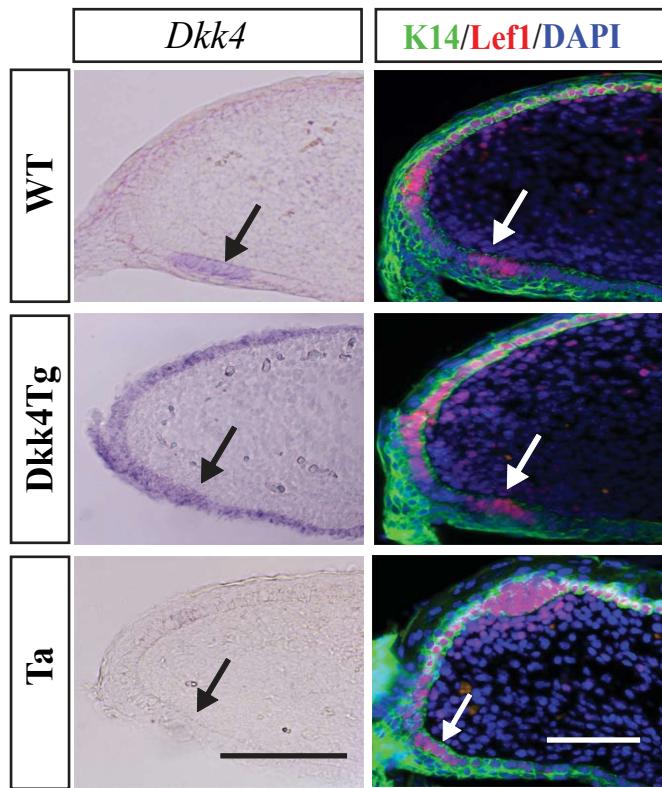


Fig. S2: MG initial induction is not affected in Dkk4Tg and Tabby mice.

Dkk4 mRNA was expressed in Dkk4Tg, as expected, throughout epidermis of eyelids; but was sharply reduced in Tabby mice in contrast to WT (left panel, black arrows). Right panel shows the expression of Lef1, unaffected in Dkk4Tg or Tabby at E15.5 (white arrows). Scale bar, 50 μ m.

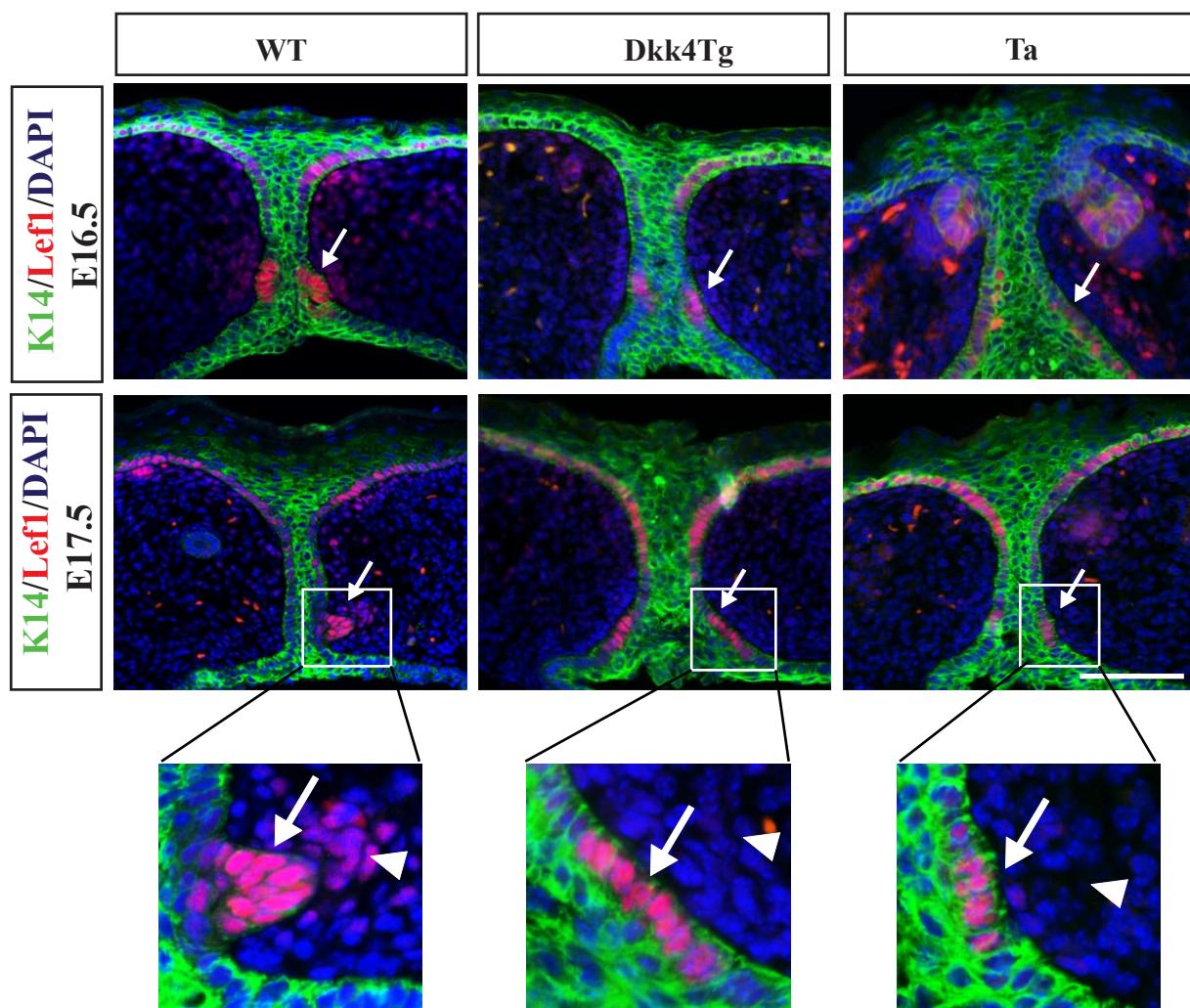


Fig. S3: Growth of MG germs is arrested at E16.5 / E17.5 in both Dkk4Tg and Tabby mice.

IHC staining showed the expression of K14 (green) and Lef1 (red) in eyelids with MG germs (white arrows) in WT, Dkk4Tg and Tabby at E16.5 (upper panels), or at E17.5 (lower panels). Two or more layers of Lef1 positive cells were observed in WT at both E16.5 and E17.5, but Lef1 positive cells were maintained as single layer in Dkk4Tg and Tabby. Scale bar, 50 μ m.

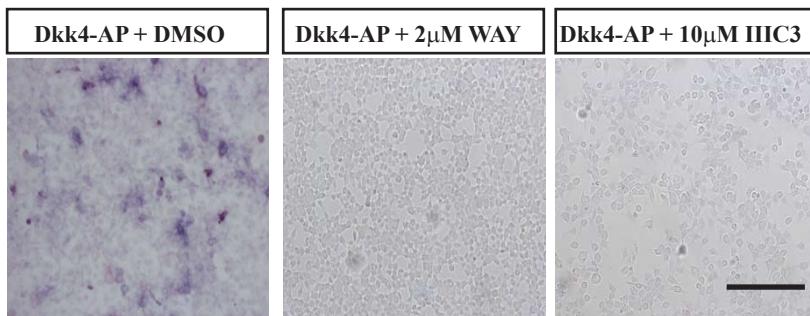
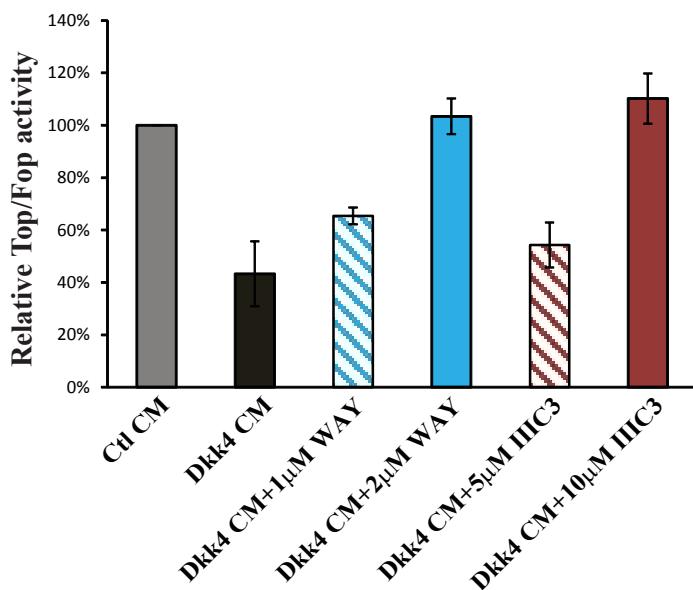
A**B**

Fig. S4: Dkk inhibitors block Dkk4 function by disrupting Dkk4-Lrp6 complex.

(A) 2 μ M WAY-262611 or 10 μ M IIIC3 totally blocked Dkk4-Lrp6 complex formation on the cell surface. HEK293 cells transfected with Lrp6 expressing vector for 24 h and then incubated with 2 μ M DMSO, 2 μ M WAY-262611 or 10 μ M IIIC3 for 2 h, then with Dkk4-AP CM added for 2 h. Images show AP staining (blue). Scale bar, 50 μ m. (B) Effect of Dkk inhibitors on Wnt10b-induced Topflash activity. Kera308 cells expressing Top or Fop flash reporter constructs were pre-incubated with DMSO, WAY-262611 or IIIC3 for 2 h, followed by incubation with 293 control CM or Dkk4 CM for another 2 h. Then recombinant Wnt10b (50ng/ml) was added to each culture for 24 h. Top- or Fopflash luciferase

activity was measured and results in histogram represent the average calculated Top/Fop ratio in each culture. Top/Fop ratio of control CM-treated cells was normalized to 100%. Experiments were done three times. Error bars, mean \pm SEM of triplicates.

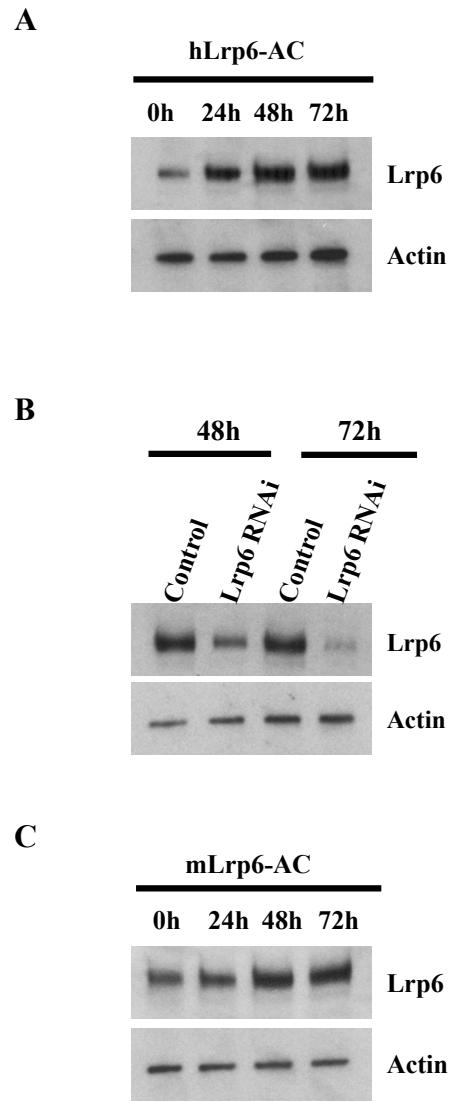


Fig. S5: Efficacy of Lrp6 shRNA and Lrp6-AC

(A) Efficiency of human Lrp6 (hLrp6) CRISPR activation plasmid (hLrp6-AC). hLrp6-AC plasmids were transfected into 293 cells and cell lysate at each time points were analyzed by Lrp6 immunoblotting. (B, C) Efficacy of mouse Lrp6 (mLrp6) shRNA and mLrp6-AC lentiviral particles. Control (LentiCon), mLrp6 shRNA and mLrp6-AC lentiviral particles were applied into Kera308 cell culture for up to 72 h culture. Lrp6 and Actin immunoblots were showed.

Supplementary Table 1: Full list of gene expression profile of Wnt signaling pathway at early phases of MG development.

Red color: reduced gene expression in Tabby mic (at least at one stage with Ta/WT ≤ 0.8); blue color: genes with low expression level (average log intensity < 2.0)

Gene	E14.5 Ta/WT (fold)	E15.5 Ta/WT (fold)
Lrp6	0.56	0.68
Dkk4	0.58	0.81
Wnt10b	0.78	0.56
Fzd10	0.81	0.76
Dkk1	1.03	0.95
Dkk2	0.93	0.98
Dkk3	1.00	1.03
Wnt1	0.95	0.96
Wnt2	0.94	1.20
Wnt2b	1.03	0.98
Wnt3	1.03	0.93
Wnt3a	0.99	0.91
Wnt4	1.01	0.92
Wnt5a	0.99	0.99
Wnt5b	1.01	1.13
Wnt6	1.01	0.97
Wnt7a	0.94	0.95
Wnt7b	1.26	1.20
Wnt8a	0.84	0.89
Wnt8b	1.37	0.74
Wnt9a	0.99	1.05
Wnt9b	1.09	0.91
Wnt10a	1.02	0.87
Wnt11	0.99	0.99
Wnt16	1.07	1.10
Fzd1	1.01	0.97
Fzd2	0.97	0.97
Fzd3	1.01	0.97
Fzd4	1.17	1.20
Fzd5	0.83	1.01

Fzd6	0.91	1.05
Fzd7	0.99	0.93
Fzd8	1.19	1.26
Fzd9	0.95	1.25
Lrp5	1.13	0.93
Kremen1	1.01	1.08
Kremen2	0.96	0.89
Musk	0.95	1.10
Ptk7	1.05	0.94
Ror1	0.98	1.03
Ror2	1.06	1.01
Ryk	1.00	1.01
Sdc1	1.05	0.93
Sdc2	1.02	0.91
Sdc3	0.96	1.08
Sdc4	0.96	1.01
Gpc1	0.95	1.01
Gpc2	1.12	1.13
Gpc3	0.99	1.00
Gpc4	1.04	1.13
Gpc6	0.93	1.04
Sfrp1	1.02	0.96
Sfrp2	1.00	1.11
Sfrp4	1.03	1.14
Sost	0.97	1.08
Cer1	1.22	1.90
Igfbp4	1.00	1.04
Wif1	1.01	0.96
Aes	1.05	1.08
APC	1.01	0.94
Dvl1	1.03	0.97
Dvl2	0.99	1.02
Axin1	1.00	1.09
Axin2	1.08	1.03
Ctnnb1	1.03	1.00
Gsk3a	0.99	1.04
Gsk3b	0.99	0.99
Lef1	1.00	1.00
Tcf7	0.84	0.96

Supplementary Table S2: Antibodies used in this study.

Antibody	Vendor
Anti-K14 (1:2000)	Progen biotechnik (# GP-CK14)
Anti-Lef1(1:200)	Cell signaling Tech (#2230)
Anti-Lrp6 (1:1000)	Cell signaling Tech (#3395)
Anti-Flag (1: 1000)	Sigma Aldrich (#F1804)
Anti-Dkk4 (1:500)	R&D systems (#AF3105)
Anti-Actin (1:1000)	Bethyl Lab (#A300-491A)
Anti-β-catenin (1:1000)	Cell signaling Tech (#9587)
Anti-active β-catenin (Non-phospho) (1:1000)	Cell signaling Tech (#8814S)
Anti-Digoxigenin (1:3000)	Roche (#1093274)
Anti-NF-κB1(1:500)	Cell signaling Tech (#12540)
Anti-rabbit IgG (1:5000)	Cell signaling Tech (#5127)
Anti-GFP (1:1000)	Invitrogen (#A11122)

Supplementary Table S3: PCR Primers used in this study.

Primer name	DNA sequence (5'-3')
<i>Eda</i> RT forward	GGAAAATCAGCCAGCTGTGG
<i>Eda</i> RT reverse	CAGCCAGGAGAGAAGAACGCC
<i>Shh</i> RT forward	GGCAGATATGAAGGGAAGAT
<i>Shh</i> RT reverse	ACTGCTCGACCCTCATAGTG
<i>Dkk1</i> RT forward	TCCCAGAACCAACACTGACTTC
<i>Dkk1</i> RT reverse	TCTTGGACCAGAACGTCTTGCAC
<i>Dkk4</i> RT forward	CTGTGTGAATGATGTTGCAC
<i>Dkk4</i> RT reverse	GTCAGAGGTTCTAAGACAGC
<i>Lrp5</i> RT forward	CTTCCACACACCATGCGAGG
<i>Lrp5</i> RT reverse	GTCAGACAGGTCTATGTACTC
<i>Lrp6</i> RT forward	CTGGATTATTGTCCCCGGAT
<i>Lrp6</i> RT reverse	GTGTGTATTCCAGTCAGTCC
<i>Fzd10</i> RT forward	ATCGGCACCCCTCATCCTGTC
<i>Fzd10</i> RT reverse	TCTTCCAGTAGTCCATGTTGAG
<i>Wnt10a</i> RT forward	GCTGGAGCCGGCCCCGCGGGAA

<i>Wnt10a</i> RT reverse	AGACCCAGAGGAGCTTGGGT
<i>Wnt10b</i> RT forward	GAATGCTGCTCCGCCGAGG
<i>Wnt10b</i> RT reverse	CCTCCAGCATGTCGAAGCC
<i>Lrp6</i> Luc forward	AGCTGGTACCGTGGCCTGGATAA GTCACC
<i>Lrp6</i> Luc reverse	ATTCCCTCGAGCATTCAGCCTCTTCC TCTCG
<i>Lrp6_1</i> Mu Luc forward	TTTGGGCTATTACTTCAAATGACC ACTGCACATGTAG
<i>Lrp6_2</i> Mu Luc forward	ATTTCCCTACGTCCCATTAGCCCCGGGGT CCGCA
<i>Lrp6_1</i> CHIP forward	TGCAGAAAGGGTGTGTGATG
<i>Lrp6_1</i> CHIP reverse	CCCCAAAACAACCTCTACCA
<i>Lrp6_2</i> CHIP forward	GGCCCATCTGATAAATTGCT
<i>Lrp6_2</i> CHIP reverse	CTACATGTGCAGTGGGCATT
<i>Lrp6_c</i> CHIP forward	ACCACACCCTGCAGCACTGA
<i>Lrp6_c</i> CHIP reverse	CTCTCCGTGTATGGGAAGG
<i>Dkk4</i> CHIP forward	ACATCCTCTTGCTGGGAG
<i>Dkk4</i> CHIP reverse	TCAAATCTTGATCAATAG
WT-Dkk4-Flag forward	AGCTAAGCTTGCACCATGGTACT GGTACCTTGCTTGGAG
WT-Dkk4-Flag reverse	GCGTGGATCCTATTCTTGGCATAAC TCTTAGCC
CL-Dkk4-Flag forward	ATCTAAGCTTGATGTTGCACTGCA GTGGAAG
CL-Dkk4-Flag reverse	AGCTGGATCCTATTCTTGGCATAAC TCTTAGCC
PM-Dkk4-Flag forward	GGAGCTGCAGTTGCTAACGCTTGCT GTTGCACTGCAGT