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Supplementary Information for

Calcineurin Controls Proximodistal Blastema Polarity in Zebrafish Fin Regeneration

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Other supplementary materials for this manuscript include the following:

- Movies S1

Supplementary Figures and Figure legends:

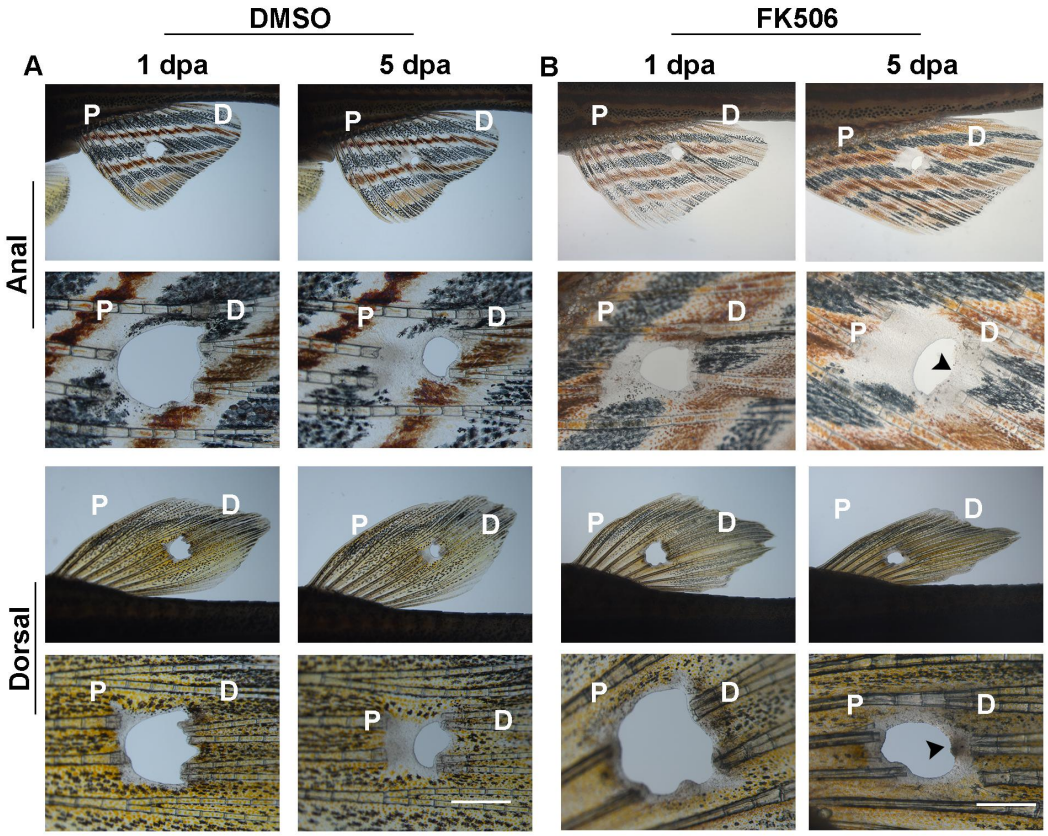

Fig. S1. PCE blastemas of anal and dorsal fins were induced by the FK506 treatment. (A) Anal and dorsal PCE fins were amputated and failed to form blastemas (n = 6/6). (B) PCE regeneration of anal and dorsal fins was induced by the FK506 treatment (n = 5/10). Scale bars: 500 μM.

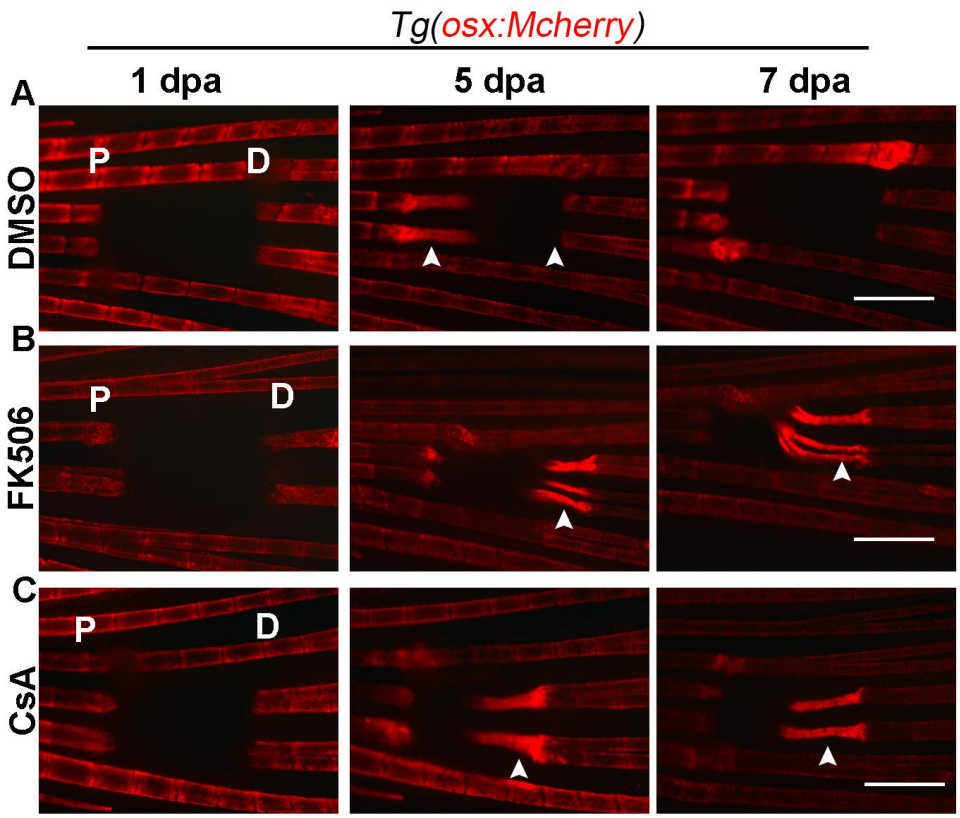

Fig. S2. Osteoblasts of the PCE were induced by calcineurin inhibition. (A-C) Transgenic *Tg(osx:mCherry)* fins were amputated and osteoblasts of PCE (labeled by mCherry, arrowheads) were induced by FK506 (n= 4/10) and CsA (n= 5/10) compared with DMSO (n= 8/10) . Scale bars: 500 μM.

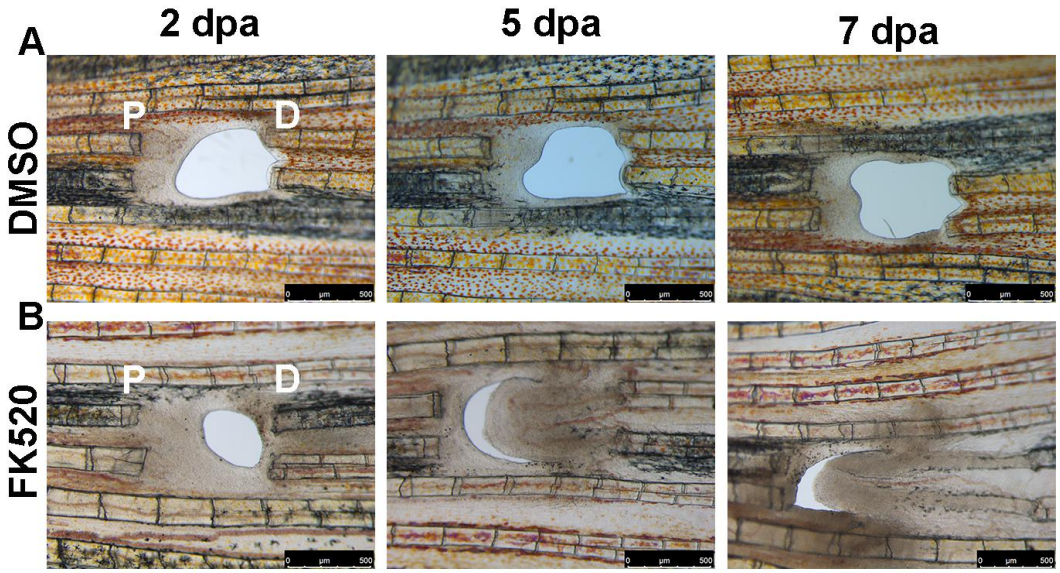

Fig. S3. PCE regeneration was induced by the FK520 treatment. (A, B) FK520 (2 μm), a FK506 derivative, induced the formation of PCE regeneration (5/7) compared with DMSO (8/10). Scale bars: 500 μM.

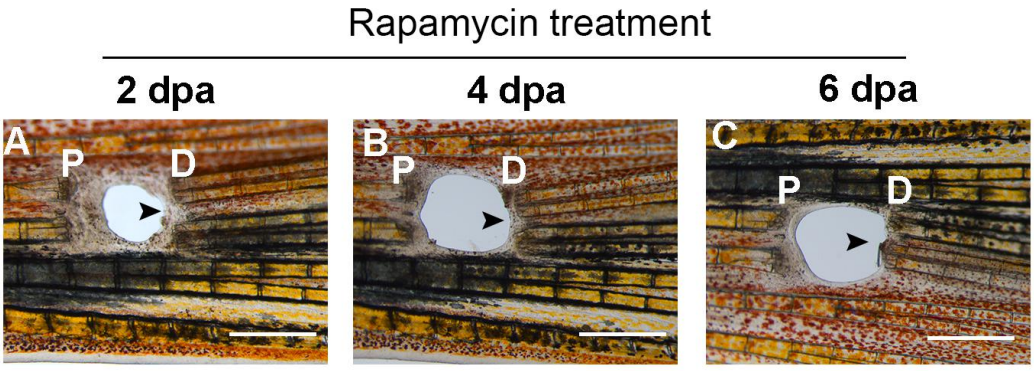

Fig. S4. FK506-induced PCE regeneration was not due to immunosuppression. (A-C) PCE blastemas failed to form at 5 dpa after rapamycin treatment (n = 7/7). Scale bars: 500 μM.

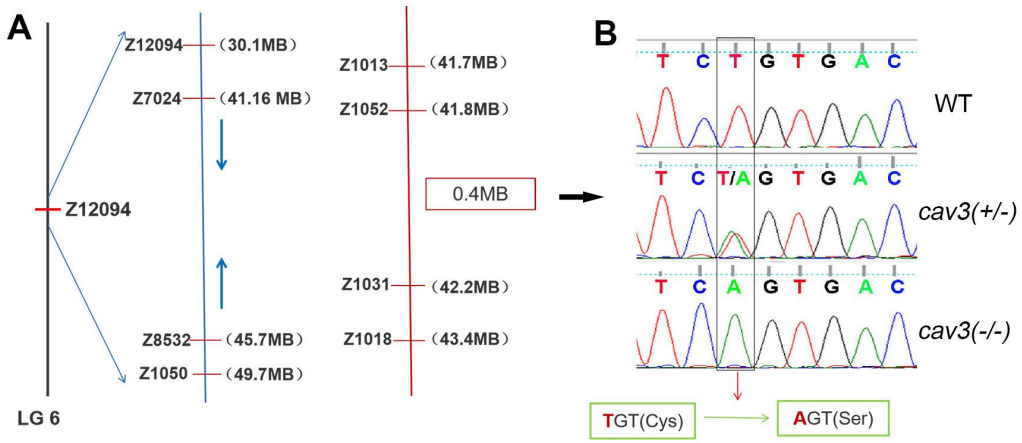

Fig. S5. Identification of the *cav3*^{cq105} mutant.

(A) Gene mapping with the SSLP marker. (B) Single base with the nonsense mutation in the *cav3*^{cq105} mutant.

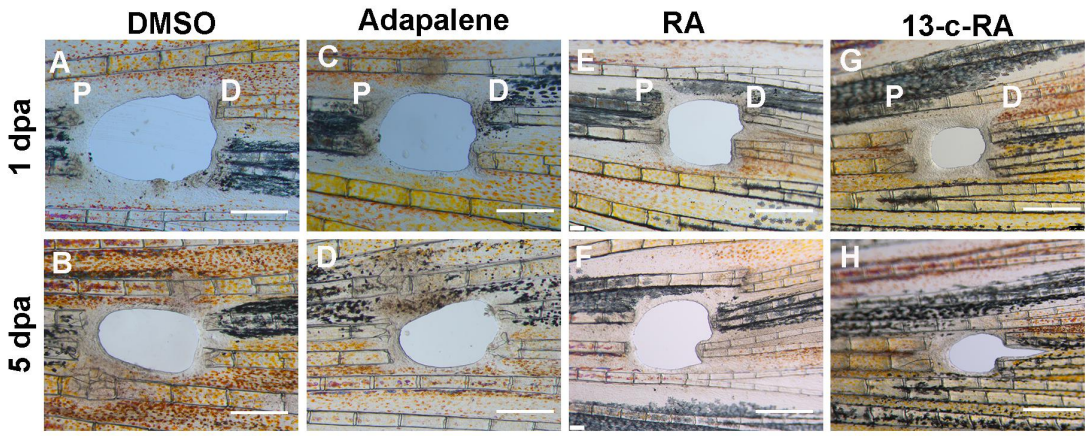

Fig. S6. Wnt, Fgf and notch signaling were unable to induce regeneration of the PCE. (A, B) Controls with DMSO treatment (n =6/7). (C-H) XAV939 (Wnt inhibitor) (n =7/7), BML284 (Wnt accelerator) (n=7/7), and FK506 after XAV939 treatment (n =6/6). (I-N) Overexpression of *fgf20a* (n =11/12) and *dnfgfr* (n =12/14) by heat-shock of the transgenic fish *Tg(hsp70l:fgf20a-mCherry)* and *Tg(hsp70l:dnfgfr-GFP)* for separately activating and suppressing Fgf signaling and BGJ398 (Fgf inhibitor)

treatment (n=7/8). (O, P) NICD (a notch accelerator) treatment from 1 to 5 dpa (n=7/7). Scale bars: 500 μM.

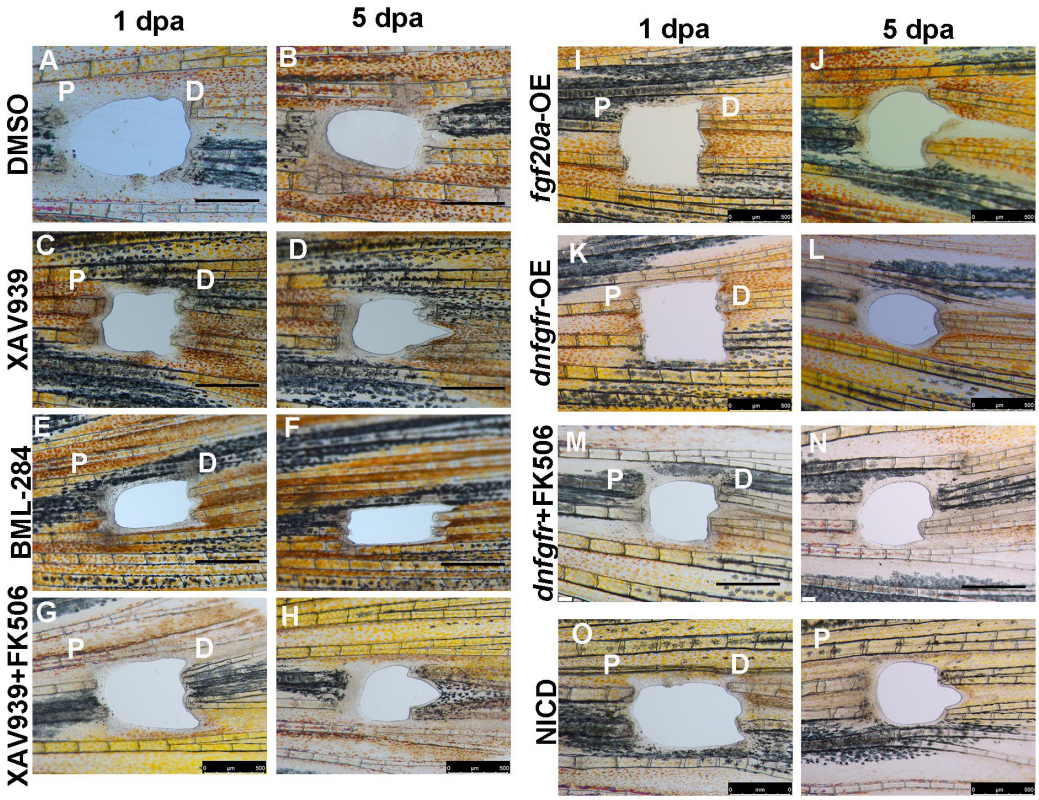

Fig. S7. RA signaling was unable to induce PCE regeneration.

(A–H) Adapalene, RA, and 13-c-RA (RA inhibitor) treatment failed to induce PCE regeneration (n = 8/8). Scale bars: 500 μM.

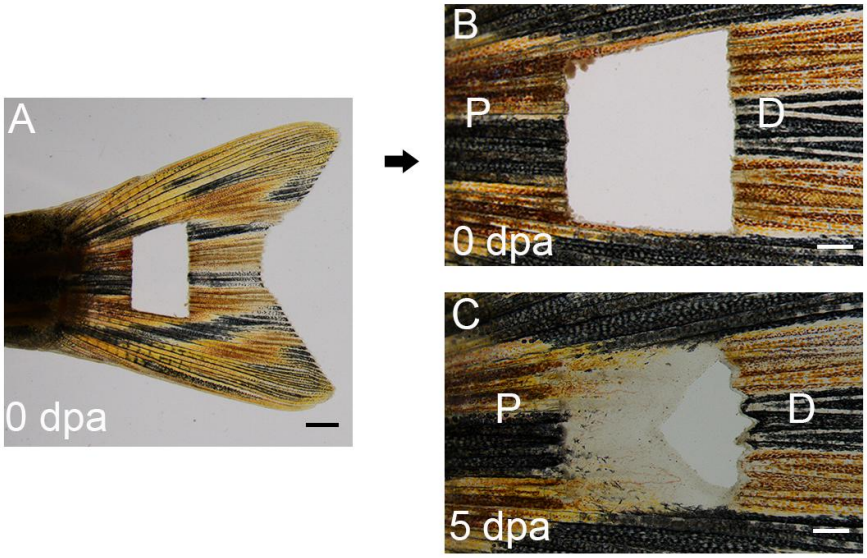

Fig. S8. The regenerative direction was from the ACE to the PCE in the largest holes of zebrafish caudal fins.

(A-C) The largest holes (2.2 x 2 mm) in zebrafish caudal fins were regenerated and the regenerative direction was still from the ACE to the PCE (n = 6/7). Scale bars: 1 mm (A), 500 μM (B and C).

Tabel S1. FPKM values of ACE and PCE transcriptome sequencing

Gene	control	ACE_24h	PCE_24h	ACE_48h	PCE_48h	ACE_72h	PCE_72h
fkbp9	20.19	36.27	21.05	112.07	20.26	182.46	40.63
fkbp10b	10.64	16.99	6.20	27.74	6.29	54.74	18.87
fkbp7	29.56	38.99	8.55	80.88	30.58	172.47	26.82
fkbp14	5.86	8.43	4.91	27.51	6.23	40.25	7.44

Table S2. List of primers used for qRT-PCR and probes.

Primers for qRT-PCR	
fgf20a	FP: AAAAGCTGTCAGCCGAGTGT
	RP: TGGACGTCCCATCTTTGTTG
lef1	FP: AATGATCCCGTTCAAAGACG
	RP: CGCTAAGTCTCCCTCCTCCT
msxb	FP: ACACTTTGTCGAGCGTTTCGG
	RP: TCTTGTGCTTGCGTAAGGTGC
osn	FP: GTGGAGGATGTTATTGCTGAG
	RP: GGGGCAGGTCAAAGGGTC
raldh2	FP: AACCACTGAACACGGACCTC
	RP: CTCCAGTTTGGCTCCTTCAG
wnt10a	FP: CATGAGTGCCAGCATCAGTT
	RP: CTCTGAAACCCCTGCTGAAG
wnt5b	FP: TAGGATGGGGAACATCAAGG
	RP: AGCAAGGTGGAGTGTGTGTG
cyp26a1	FP: GATGGGAGCTGATAATGTG
	RP: CCTGAACCTCCTCTCTGACC
ppp3ca	FP: GGGCTTCTATCCTACGCCAG
	RP: TTCAGCGACCACAGGTACAG
Primers for probes	
lef1	FP: CAGTCACGACGCAGCTAGAC

	RP: CTCTGGCCTGTACCTGAAGC
runx2b	FP: ATGCGCATTCCCGTAGATCC
	RP: TCAATACGGCCTCCAAACGCC
msxb	FP: GAGAATGGGACATGGTCAGG
	RP: GCGGTTCCTCAGAATAATAAC
raldh2	FP: GGCTGATCTGGTGGAGAGAG
	RP: TGAATCCTCCGAAAGGACAC
and1	FP:ATGGCTCATTTGAGAGGATCTTCC
	RP: TTATTTCTTTCTGTAGTCTCC
and2	FP: ATGGCCAGACTCATTAAGATC
	RP: TCATTTCTTGTAGCCACCCAT

Movie S1. Blood flow (erythrocytes labeled by *Tg(gata1:DesRed)*) in the lepidotrichia between two holes at 3 dpa.