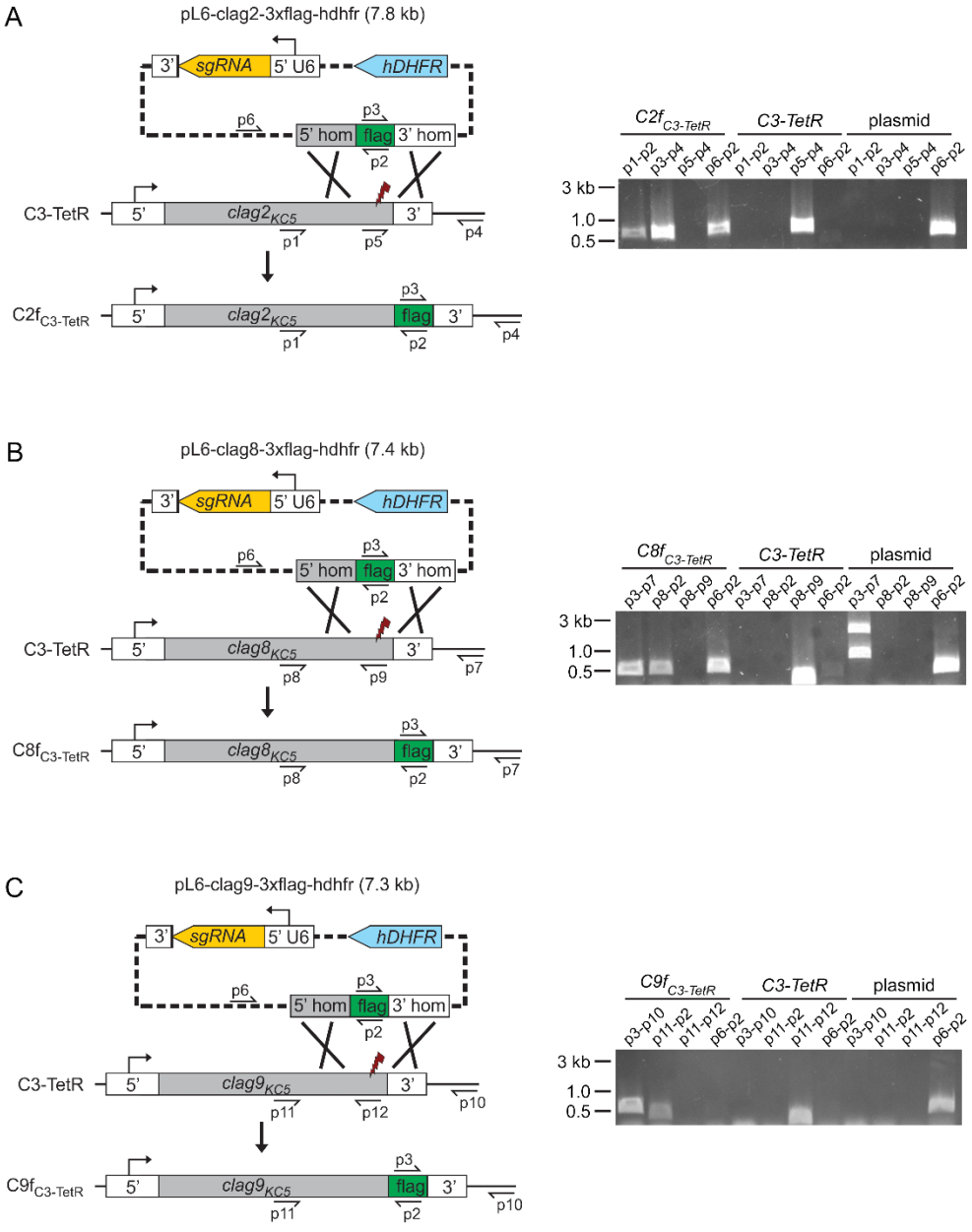

Figure S1. Strategy and production of double-tagged clones. **(A-C)** CRISPR-Cas9 transfection to produce *C2f*$_{C3\text{-}TetR}$, *C8f*$_{C3\text{-}TetR}$, and *C9f*$_{C3\text{-}TetR}$, respectively. In each panel, the schematic shows the transfection plasmid, homologous recombination at the genomic cleavage site, the resulting modification, and primer binding sites. Primers are listed in Table S1. Ethidium-stained gel at right shows PCR confirming integration (first two lanes, absence of residual wildtype sequence (third lane) and presence of the transfection episome (fourth lane). Control PCRs using the parental *C3-TetR* line and the transfection plasmid are also shown. Expected amplicon sizes (in bp) for panel A: p1-p2, 586; p3-p4, 582; p5-p4, 704; p6-p2, 603. For panel B: p3-p7, 483; p8-p2, 505; p8-p9, 357; p6-p2, 554. For panel C: p3-p10, 552; p11-p2, 440; p11-p12, 346; p6-p2, 500. The two bands seen with plasmid in the p3-p7 lane reflect spurious priming in the absence of cognate template.

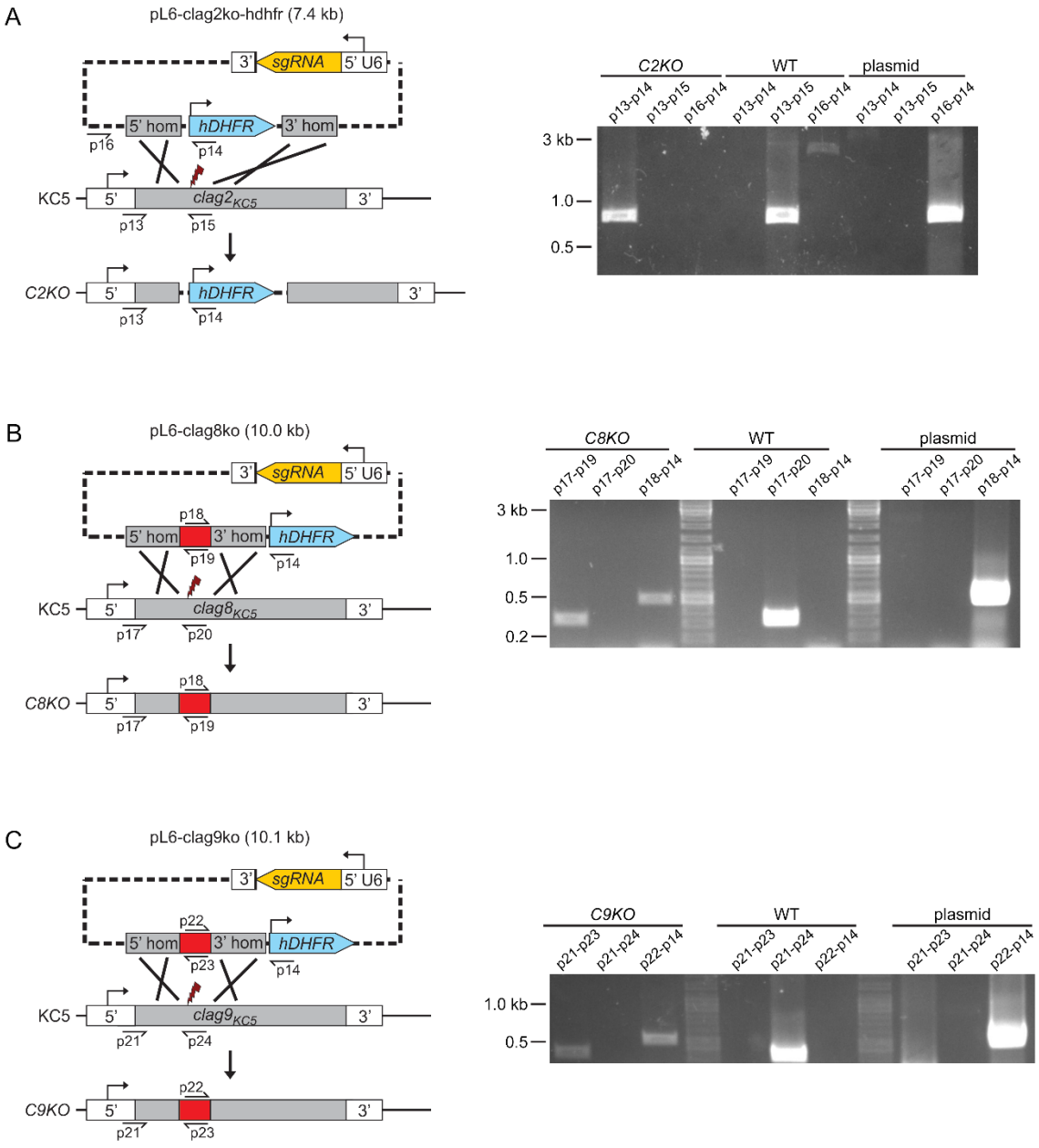

Figure S2. CRISPR knockout of CLAG paralogs. **(A-C)** Transfection to produce *C2KO, C8KO* and *C9KO* lines, respectively. Schematics show CRISPR-Cas9 mediated knockout of indicated *clag* paralogs and positions of primers (Table S1). Ethidium-stained gel in each panel shows PCR confirming integration to produce knockout (first lane), absence of residual wildtype sequence (second lane) and retention of the transfection episome (third lane). Control PCRs using the parental KC5 wild-type (WT) and the transfection plasmid are also shown. Expected amplicon sizes (in bp) for panel A: p13-p14, 750; p13-p15, 752; p16-p14, 750. For panel B: p17-p19, 301; p17-p20, 319; p18-p14, 483. For panel C: p21-p23, 392; p21-p24, 390; p22-p14, 530.

Table S1. Primers used in this study.

primer	primer alias	Sequence	primer usage
p1	clag2_LE-F	5'-AACGAAACTTAAAAAGTATACTACTGATGT-3'	clag2 PCR check
p2	3xFLAG-R	5'-TTTGTCATCGTCATCTTTGTAGTCGATATC-3'	epitope tag integration PCR check
p3	3xFLAG-F	5'-GATATCGACTACAAAGATGACGATGACAAA-3'	epitope tag integration PCR check
p4	clag2_3utr_wt-R	5'-ACATTTTCTTATAAGTAATAATTTTATATTGTTTA-3'	clag2 PCR check
p5	c2_tag_WT-F	ACATACTAGTAATATCCAAAAGGT	clag2 PCR check
p6	5Apt_Epis-F	CGTATGTTGTGTGGAATTGTGAGC	pL6 plasmid backbone PCR check
p7	clag8_3utr-R	TAGTTTAATTATATATAAAATCAAAATACAGAAAGAAAAAAA	clag8 PCR check
p8	clag8_LE-F	GATGAGGCAATGGAAGCTAGAATT	clag8 PCR check
p9	c8_tag_WT-R	ATTTTCTTCTTCCGATATTTCTGTTGAAAC	clag8 PCR check
p10	c9_3utr-R	GAAATATGATATTCTCCTTATATTTATTTATATATTCTTTTT	clag9 PCR check
p11	clag9_LE-F	TCAATAAATTGACAGTTGATAAATTACTCA	clag9 PCR check
p12	c9_tag_WT-R	ACGATAACGTTGCAAAGGAA	clag9 PCR check
p13	clag2_5utr-F	GGATTAACGAAATGTTATAATTTCTCATTT	clag2 knockout PCR check
p14	New_hDHFR-R	CAAGTATATATTTTGTTTCTATAAATTGATATCTT	pL6 plasmid hDHFR cassette primer
p15	clag2_WT-R	CTTTTTCATTTTCAGTTACATTTACATTTA	clag2 knockout PCR check
p16	c2ko_Epis-F	GCTATGACCATGATTACGCCAAGC	pL6 clag2 knockout plasmid backbone primer
p17	clag8_5utr-F	TGATACATTGGTATGAAATGTTCATGCAGG	clag8 knockout PCR check
p18	clag8ko_FLAG-F	TGATGACTATAAGGACGATGACGATAAGTG	pL6 plasmid FLAG tag primer
p19	clag8ko_Flag-R	CTTATCGTCATCGTCCTTATAGTC	pL6 plasmid FLAG tag primer
p20	clag8_WT-R	ATACTTGTCGGTTTCTTGTTTTACTAGAGG	clag8 knockout PCR check
p21	clag9_5utr-F	GTAATAGATTTTAATTCATGCATAAATTAG	clag9 knockout PCR check
p22	clag9_V5-F	TTACTTGGTCTTGATTCTACATGATGATGA	pL6 plasmid V5 tag primer
p23	clag9ko_V5-R	AGAATCAAGACCAAGTAAGGGATT	pL6 plasmid V5 tag primer
p24	clag9_WT-R	TATGTTCATATTTTACTATATCATTAGCAC	clag9 knockout PCR check

qRT-PCR primers			
	KC5NLE_RT-F	TCTTTTATGTGAATATCAGGCTGTGGCAAG	Forward primer - KC5 *clag3h*
	KC5NLE_RT-R	GCATGTGTCCAACTAATTCTCATTACTAGA	Reverse primer - KC5 *clag3h*
	7G8_c3.1-F	CATATTTTCTAGTAATGAGAATTAGTTGGA	Forward primer - 7G8 *clag3.1*
	7G8_c3.1_HVR-R	ATAATTACCACCATTACATTGAGAATTAGTACC	Reverse primer - 7G8 *clag3.1*
	Clag3.2_7G8-F	ACCCATAACTACATATTATCTAGTAATG	Forward primer - 7G8 *clag3.2*
	Clag3.2_7G8-R	TTTATTTATAGTACTTGAATTATCAGTATTAG	Reverse primer - 7G8 *clag3.2*
	PS_clag2-F	CTCTTACTACTTATTATCTATCTCTCA	Forward primer - *clag2*
	PS_clag2-R	CCAGGCGTAGGTCCTTTAC	Reverse primer - *clag2*
	Clag8-F_1	GATGATTGTGGTAAAAATGAGGAATTTCTAAATG	Forward primer - *clag8*
	Clag8-R_1	CTTGTGTTATAGCCTTACTATTTGACGATA	Reverse primer - *clag8*
	Clag9-F	TGTTTTATACACTTAAGGCAAGAACAG	Forward primer - *clag9*
	Clag9-R	ATATAATATCCAAAATATGGCCA	Reverse primer - *clag9*
	Rhoph2-F	GACATGATATCCAAAAAGGTAATATCA	Forward primer - *rhoph2*
	PS_Rhoph2-R	ACTAGAAAAATCATATACTGGTTTGTG	Reverse primer - *rhoph2*
	Rhoph3-F	GTAGATGAAGATGCTCACCATG	Forward primer - *rhoph3*
	PS_Rhoph3-R	GTATAATTCTATTTCTAAATCTTGATCCTT	Reverse primer - *rhoph3*
	PF07_0073_ctrl-F	AAGTAGCAGGTCATCGTGGTT	Forward primer - Pf07_0073 housekeeping control
	PS_PF07_0073-R	CATAAAAAATGGAGGATATACAGGTAT	Reverse primer - Pf07_0073 housekeeping control

Primers used for CRISPR-Cas9 transfections			
	c2_3xFLAG_843966gRNA-F	TAAGTATATAATATTACATACTAGTAATATCCAAAGTTTTAGAGCTAGAA	CLAG2 epitope tag sgRNA InFusion cloning (protospacer underlined)
	c2_3xFLAG_843966gRNA-R	TTCTAGCTCTAAAACTTTGGATATTACTAGTATGTAATATTATATACTTA	CLAG2 epitope tag sgRNA InFusion cloning (protospacer underlined)
	c8_3xFLAG_5223gRNA-F	TAAGTATATAATATTAGAAGTTTCAACAAAAATATGTTTTAGAGCTAGAA	CLAG8 epitope tag sgRNA InFusion cloning (protospacer underlined)
	c8_3xFLAG_5223gRNA-R	TTCTAGCTCTAAAACATATTTTTGTTGAAACTTCTAATATTATATACTTA	CLAG8 epitope tag sgRNA InFusion cloning (protospacer underlined)
	c9_3xFLAG_1419717gRNA-F	TAAGTATATAATATTTCATAACGATAACGTTGCAAGTTTTAGAGCTAGAA	CLAG9 epitope tag sgRNA InFusion cloning (protospacer underlined)
	c9_3xFLAG_1419717gRNA-R	TTCTAGCTCTAAAACTTGCAACGTTATCGTTATGAAATATTATATACTTA	CLAG9 epitope tag sgRNA InFusion cloning (protospacer underlined)
	C2_ko_328gRNA-F	TAAGTATATAATATTAAACTCCCCTTAATAAAAGAGTTTTAGAGCTAGAA	CLAG2 knockout sgRNA InFusion cloning (protospacer underlined)
	C2_ko_328gRNA-R	TTCTAGCTCTAAAACTCTTTTATTAAGGGGAGTTTAATATTATATACTTA	CLAG2 knockout sgRNA InFusion cloning (protospacer underlined)
	c8_ko_282gRNA-F	TAAGTATATAATATTAGATAAATCCAAATACTTGTGTTTTAGAGCTAGAA	CLAG8 knockout sgRNA InFusion cloning (protospacer underlined)
	c8_ko_282gRNA-R	TTCTAGCTCTAAAACACAAGTATTTGGATTTATCTAATATTATATACTTA	CLAG8 knockout sgRNA InFusion cloning (protospacer underlined)
	c9_ko_467gRNA-F	TAAGTATATAATATTGTATTTTTATAAGCACTCATGTTTTAGAGCTAGAA	CLAG9 knockout sgRNA InFusion cloning (protospacer underlined)
	c9_ko_467gRNA-R	TTCTAGCTCTAAAACATGAGTGCTTATAAAAATACAATATTATATACTTA	CLAG9 knockout sgRNA InFusion cloning (protospacer underlined)