Online Supplemental Materials

Interaction of filamin C with actin is essential for cardiac development and function

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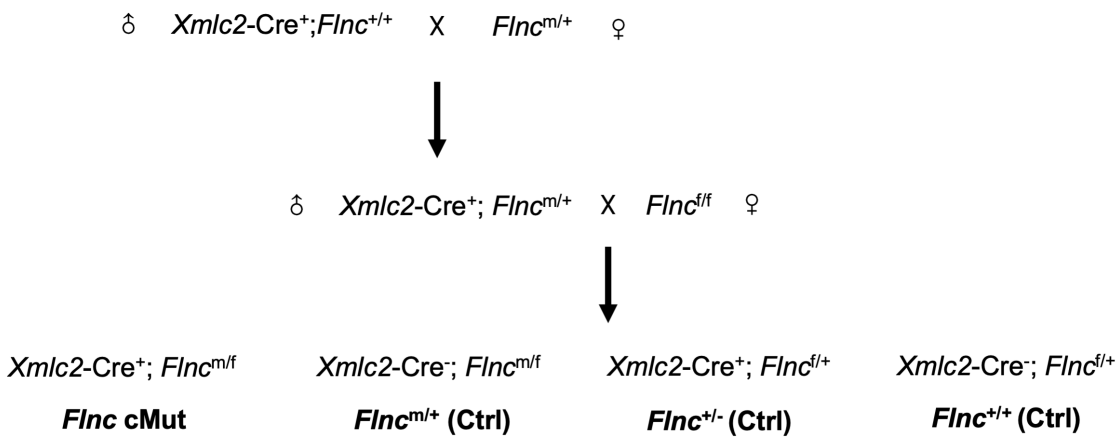
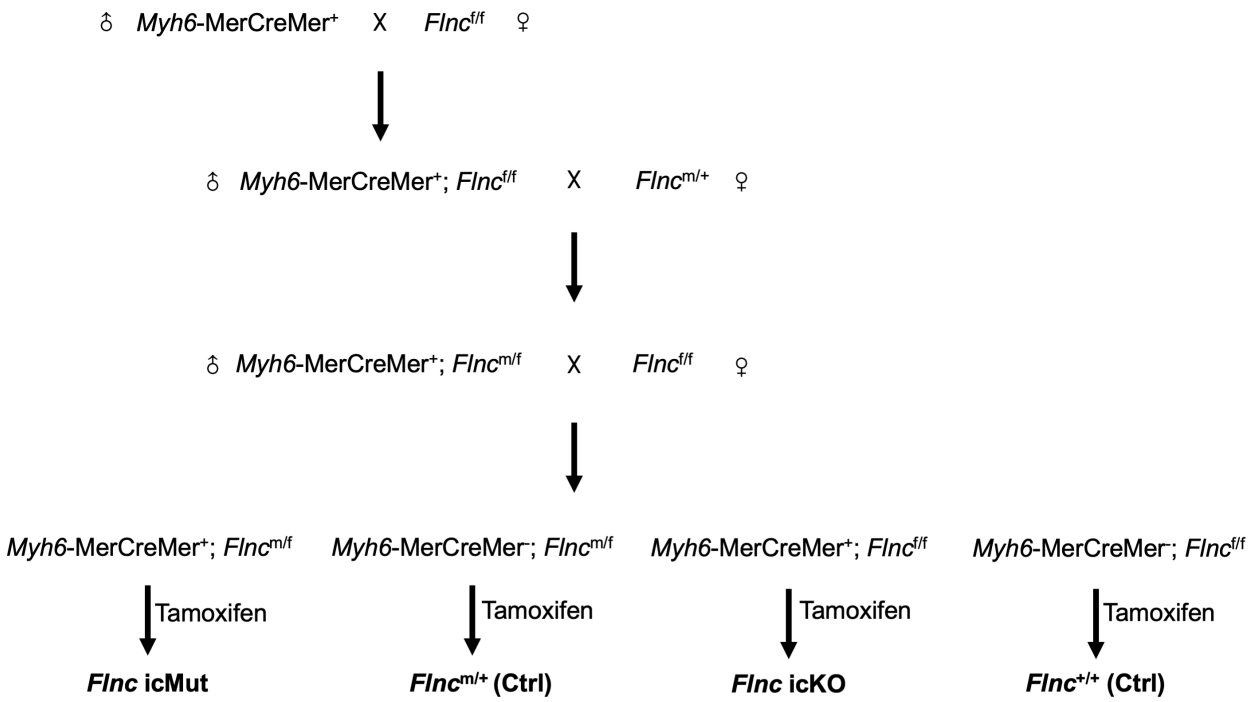
1) Supplemental Figures 1-7 and Figure Legends;

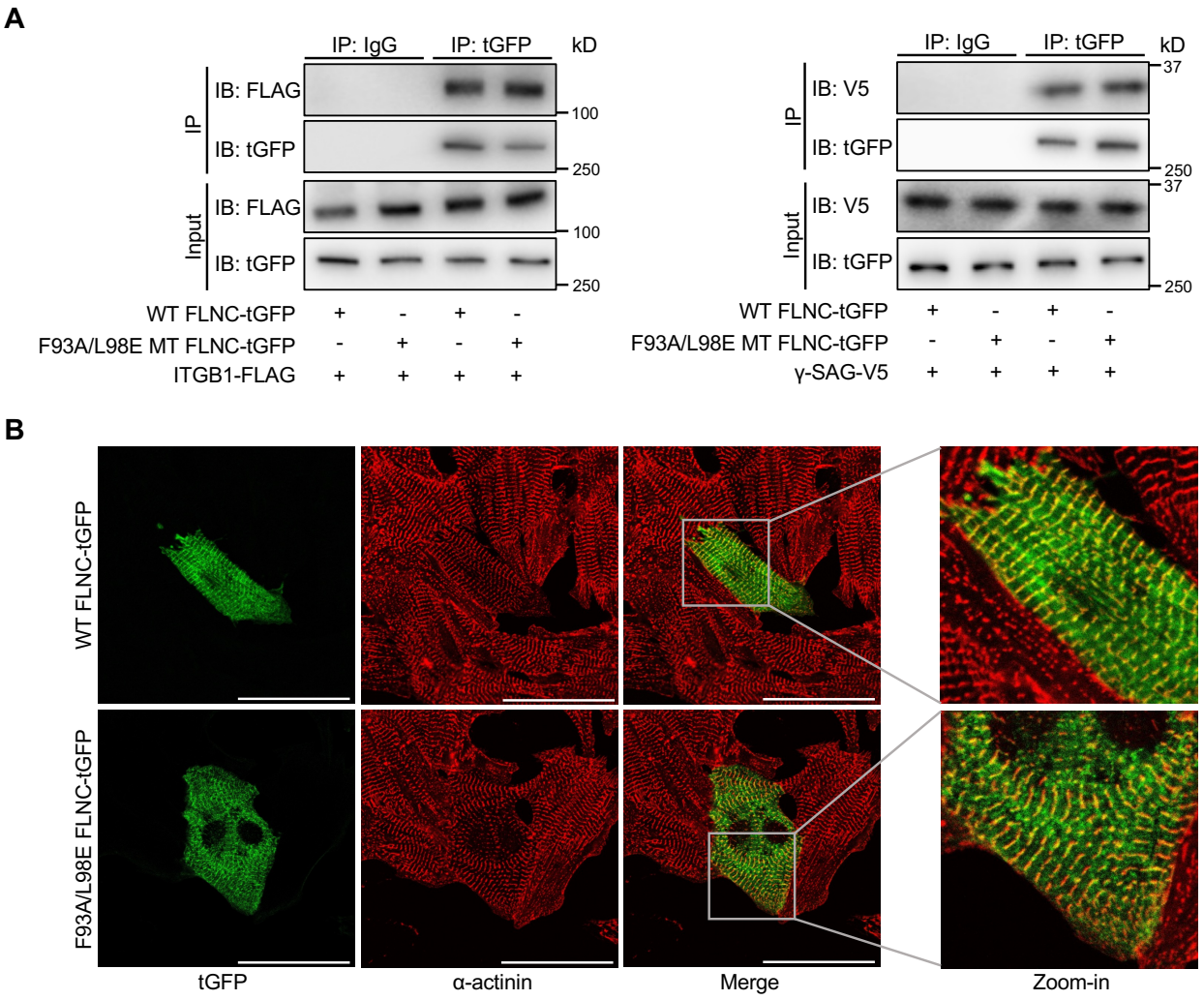
2) Supplemental Tables1-2



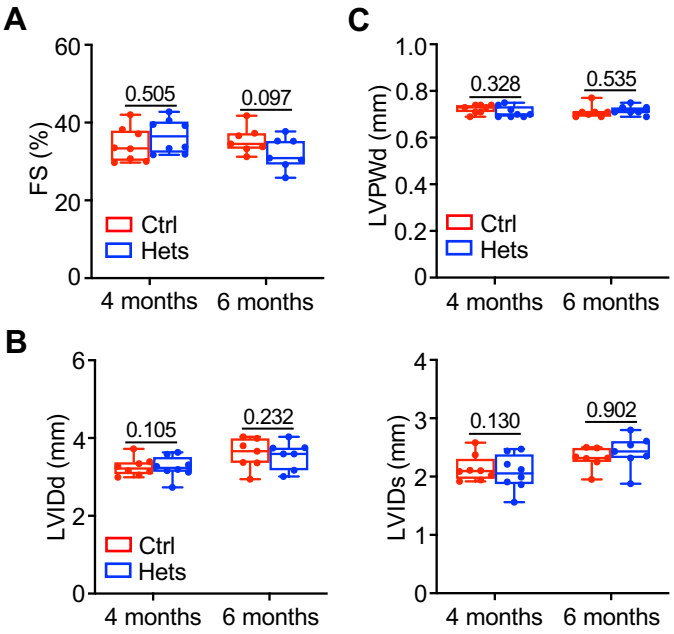
Supplemental Fig. 1 Breeding scheme for generating *Xmlc2*-Cre^{+};*Flnc*$^{mutant/flox}$ (cMut) and the corresponding control (Ctrl) mice. m: FLNC F93A/L98E mutant allele; f: *Flnc* floxed allele.



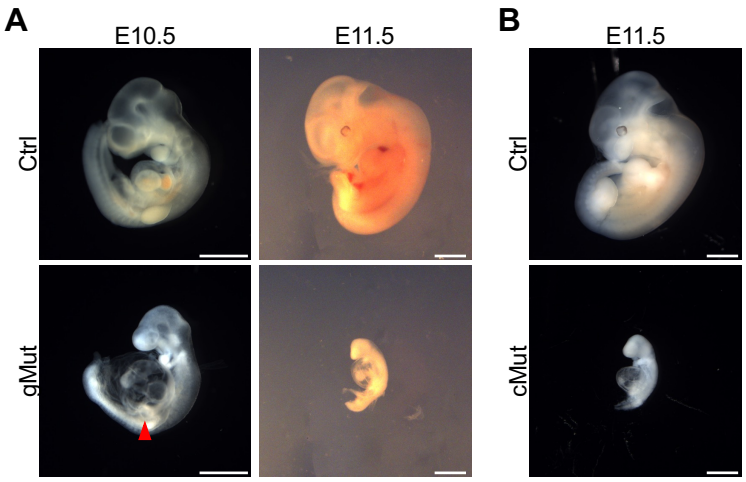
Supplemental Fig. 2 Breeding scheme for generating *Myh6*-MerCreMer^{+};*Flnc*$^{mutant/flox}$ (icMut) and the corresponding control (Ctrl) mice. m: FLNC F93A/L98E mutant allele; f: *Flnc* floxed allele.



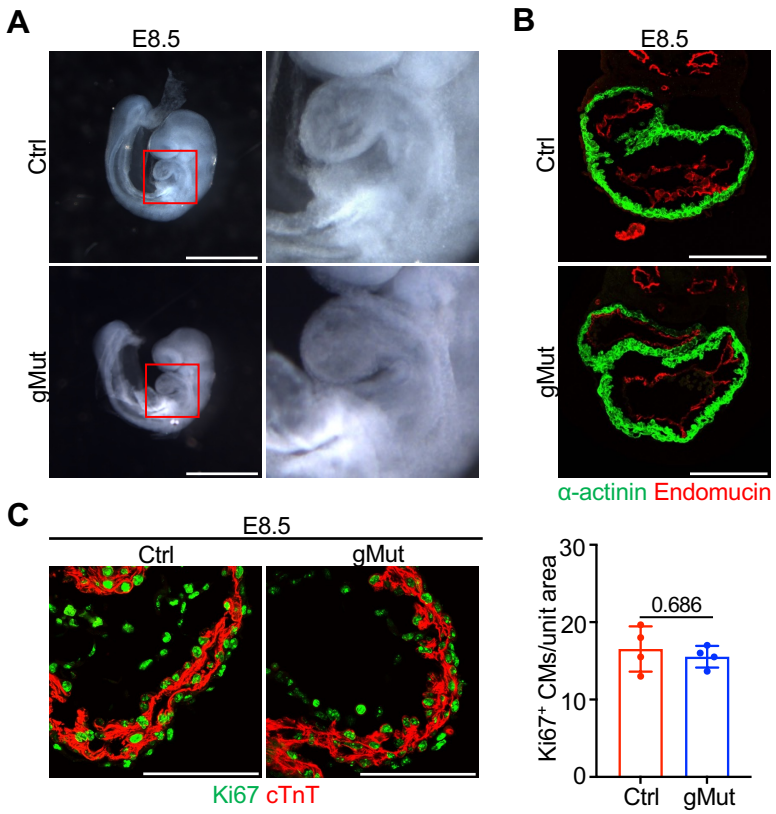
Supplemental Fig. 3 Loss of actin binding activity in FLNC does not affect its interaction with other binding partners or its subcellular localization. (A) Co-immunoprecipitation (Co-IP) of TurboGFP (tGFP) tagged wild type (WT) or F93A/L98E mutant filamin C (FLNC) with FLAG tagged β1 integrin (ITGB1) and V5 tagged γ-sarcoglycan (γ-SAG). The vectors containing cDNA sequences encoding all fusion proteins were co-transfected into HEK 293 cells for overexpression. n = 3 independently repeated experiments. (B) Representative immunofluorescence of tGFP and α-actinin on neonatal cardiomyocytes expressing tGFP tagged WT or F93A/L98E mutant FLNC. n ≥ 20 cells/group. Scale bar: 50 μm.



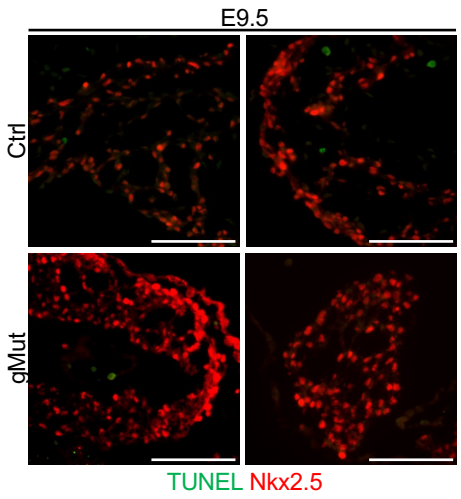
Supplemental Fig. 4 No cardiac defects in heterozygous FLNC F93/L98E mutant mice. Measurements of cardiac function index FS (A) , as well as the left ventricular dilatation indexes LVIDd, LVIDs (B) and LVPWD (C) from echocardiographic assays on heterozygous FLNC F93/L98E mutant (Hets) and wild-type control (Ctrl) and control mice at 4 months (n = 8 animals/group) and 6 months (n = 7 animals/group) of age. FS: fractional shortening; LVIDd: left ventricular internal diameter, end diastole; LVIDs: left ventricular internal diameter, end systole; LVPWD: left ventricle posterior wall thickness, end diastolic. All quantitative data shown in this figure are presented as mean values ± SD. Mann–Whitney U test was used to calculate the presented p-values.



Supplemental Fig. 5 Disruption of FLNC-actin binding leads to cardiac developmental defects and embryonic lethality. (A) Representative images of whole embryos of wild-type control (Ctrl) and global FLNC F93/L98E mutant (gMut) mice at E10.5 and E11.5. n ≥ 5 biologically independent samples/group. The red arrowheads indicate the pericardial effusion. (B) Representative images of whole embryos of *Xmlc2*-Cre^{+};*Flnc*$^{mutant/flox}$ (cMut) and control (Ctrl) mice at E11.5. n ≥ 5 biological independent samples/group. Scale bar: 1 mm.



Supplemental Fig. 6 No cardiac developmental defects in FLNC F93/L98E mutant mice at E8.5. (A) Representative images of whole embryos and hearts from wild-type control (Ctrl) and global FLNC F93/L98E mutant (gMut) mice at E8.5. n ≥ 5 biologically independent samples/group. scale bar: 1 mm. **(B)** Representative immunofluorescence of α-actinin and Endomucin on wild-type control (Ctrl) and gMut heart sections at E8.5. n = 4 biologically independent samples/group. Scale bar: 100 μm. **(C)** Representative immunofluorescence and corresponding quantitative analysis of Ki67 and cardiac troponin T (cTnT) on heart sections from wild-type control (Ctrl) and gMut embryos at E8.5. n = 4 biologically independent samples/group. Scale bar: 100 μm. All quantitative data shown in this figure are presented as mean values ± SD. Mann–Whitney U test was used to calculate the presented p-values in **C**.



Supplemental Fig. 7 Loss of FLNC-actin binding has no effect on the level of cardiac cell death: Representative TUNEL staining of wild-type control (Ctrl) and global FLNC F93/L98E mutant (gMut) hearts at E9.5. Immunostaining of cTnT was used to co-stain cardiomyocyte nuclei. n = 3 biologically independent samples/group. Scale bar: 100 μm.

Supplemental Table 1. Primer sequences for genotyping and qRT-PCR

Primers for genotyping		
	Forward	**Reverse**
Flnc wild type allele	GCCACCATGATGAACAACAG	TCCAGCTTCATTTGgCGGAA
Flnc mutant allele	GCCCGCCAAATGAAGGAG	CTCAGTACCCGCACCTATGT
Flnc sequencing	GGAAGAAGATCCAGCAGAACAC	TAGGGAGGCTGAAGCTAAGATG
Flnc floxed allele	GAATGGAGGTTGTAGGATCCCAG	TATATGTTAGTAGTCAGGGAGAGG C
Xml2-Cre	AGCCATCTTTGGTTCTCTGC	TCCCTGAACATGTCCATCAG
Myh6-MerCreMer	GCCATAGGCTACGGTGTAAAAG	GTTGGTCAATAAGCCCATCATT

Primers for qRT-PCR		
mRNA	**Forward**	**Reverse**
Anf	GATAGATGAAGGCAGGAAGCCGC	AGGATTGGAGCCCAGAGTGGACTAGG
Bnp	TGTTTCTGCTTTTCCTTTATCTGTC	CTCCGACTTTTCTCTTATCAGCTC
Myh6	GCTGGAAGATGAGTGCTCAGAG	CCAGCCATCTCCTCTGTTAGGT
Myh7	GCTGGAAGATGAGTGCTCAGAG	TCCAAACCAGCCATCTCCTCTG
Col1a1	ACTGCAACATGGAGACAGGTCAGA	ATCGGTCATGCTCTCTCCAAACCA
Col3a1	ACGTAGATGAATTGGGATGCAG	GGGTTGGGGCAGTCTAGTG
Polr2a	CGAGAAGGTCTCATTGACACGG	ACCACCTGGTTGATGGAGTTCC

Supplemental Table 2. Antibody list

Antibodies for immunofluorescence		
Antigen	**Producer**	**Cat. No**
TurboGFP	OriGene	MG222854
α-actinin	Abcam	ab68167
Ki67	Abcam	Ab15580
Cardiac troponin T	Thermo Fisher	MS-295-P1
Endomucin	Thermo Fisher	14-5851-82
Collagen I	Abcam	ab34710
9EG7	BD Biosciences	553715
Nkx2.5	R&D Systems	AF2444
Normal Rabbit IgG	Cell Signaling	2729
Normal Mouse IgG	Santa Cruz	sc-2025
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	711-545-152
Alexa Fluor® 594 AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch	715-585-150
Alexa Fluor® 488 AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch	715-545-150
Alexa Fluor® 594 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	711-585-152

Antibodies for western blot and immunoprecipitation		
Antigen	**Producer**	**Cat. No**
FLAG tag	Sigma-Aldrich	F7425
HA tag	Santa Cruz	sc-7392
TurboGFP	OriGene	MG222854

V5	Abcam	ab-27671
FLNC	Novus Biologicals	NBP1-89300
GAPDH	Santa Cruz	sc-32233
β1D integrin	Thermo Fisher	MA1-06906
Talin1	Bio-Rad	MCA4770
ILK	Cell Signaling	3862
γ-SAG	Vector Laboratories	VP-G803
Dystrophin	Abcam	ab-15277
Desmin	Santa Cruz	sc-7559
Xirp2	Proteintech	11896-1-AP
JUP	Sigma-Aldrich	P8087
Normal Rabbit IgG	Cell Signaling	2729
Normal Mouse IgG	Santa Cruz	sc-2025
Polyclonal Goat Anti-Rabbit Immunoglobulin/HRP	Dako	P0448
Polyclonal Goat Anti-Mouse Immunoglobulin/HRP	Dako	P0447