Fig. S1

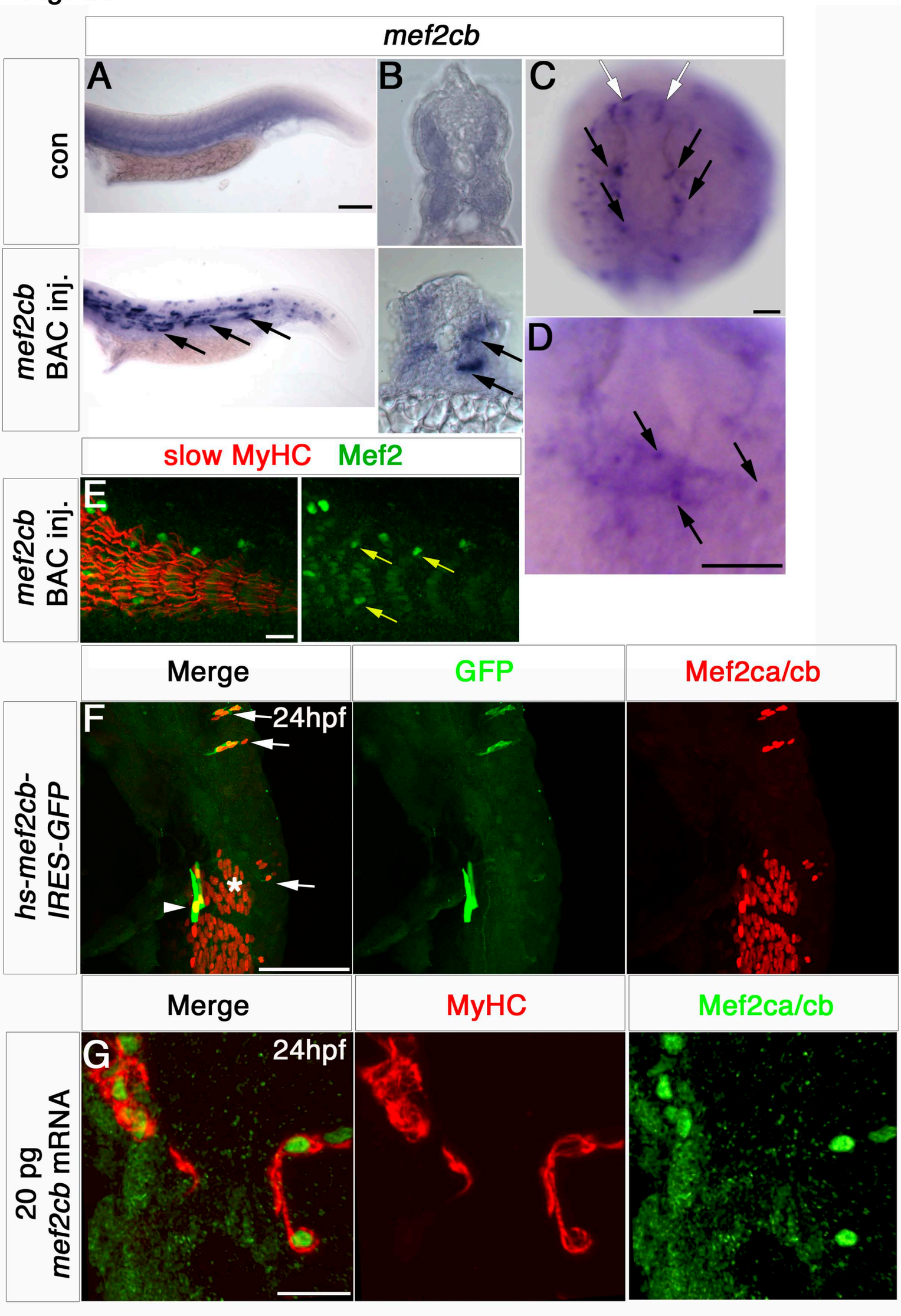

mef2cb
con
mef2cb BAC inj.
A
B
C
D
slow MyHC
Mef2
mef2cb BAC inj.
E
Merge
GFP
Mef2ca/cb
hs-mef2cb-IRES-GFP
F
24hpf
Merge
MyHC
Mef2ca/cb
20 pg mef2cb mRNA
G
24hpf

Fig. S2

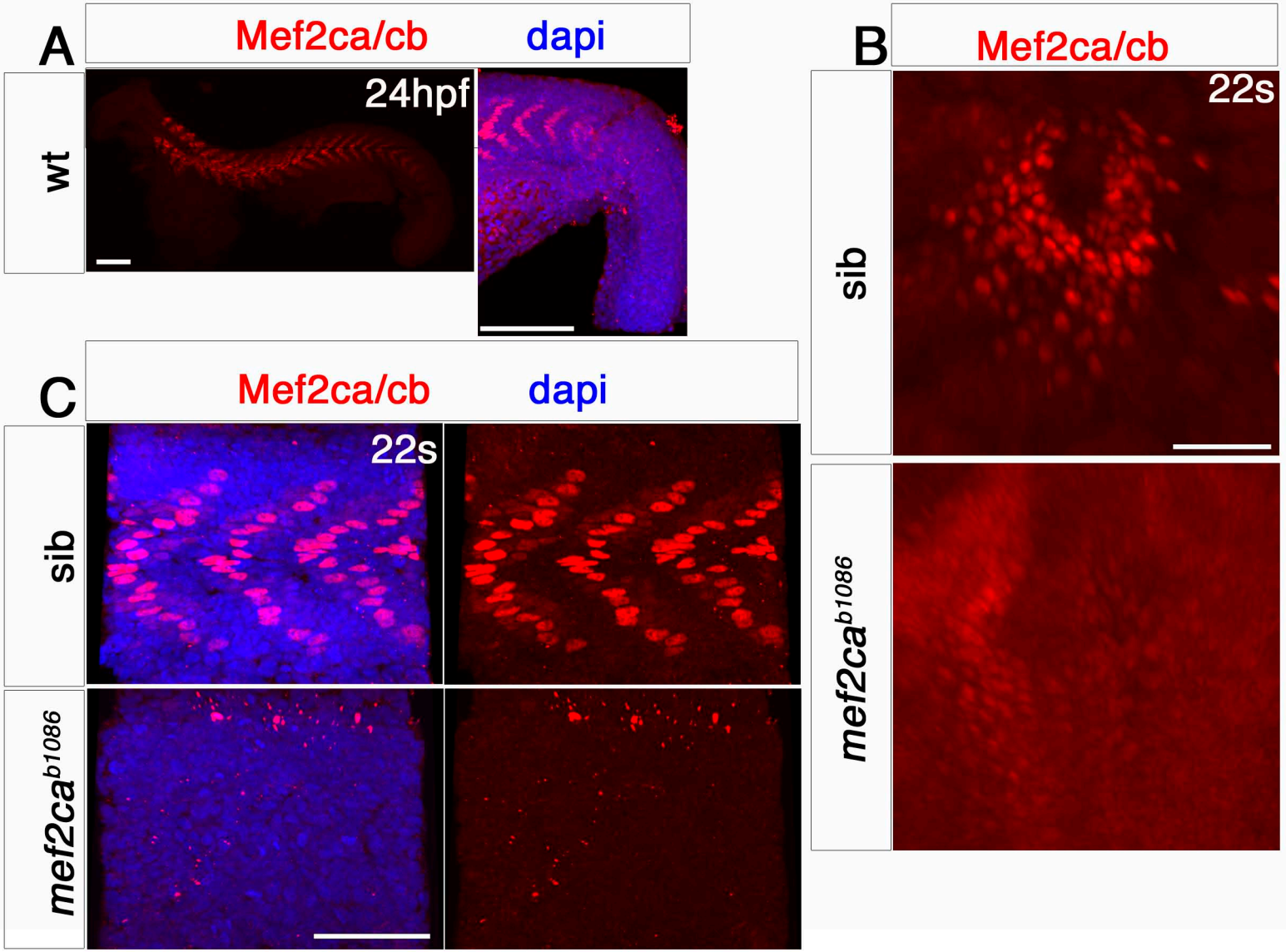
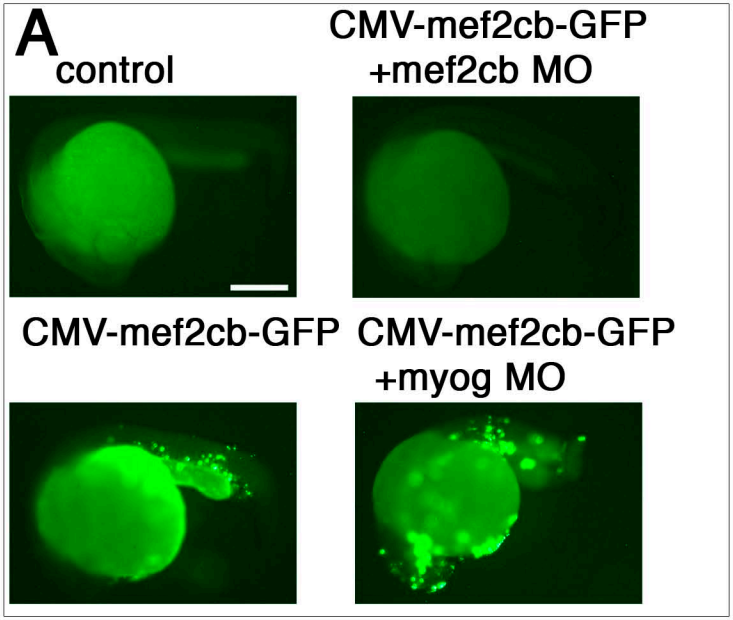
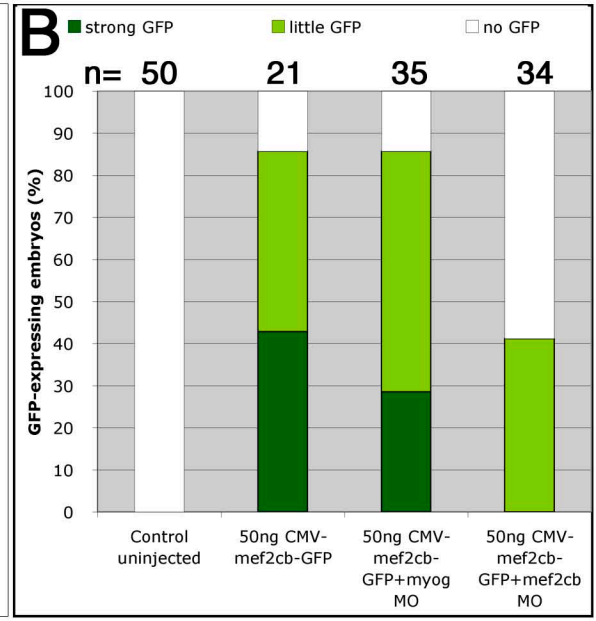
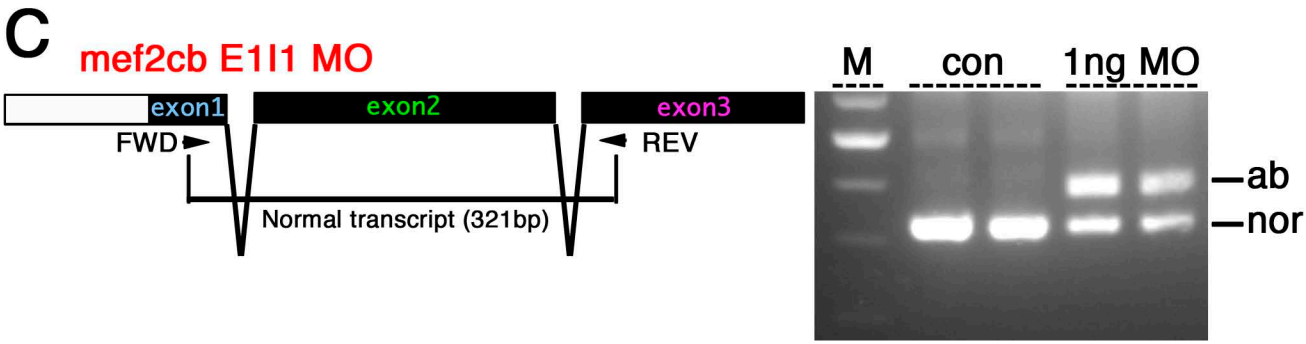

A
Mef2ca/cb
dapi
wt
24hpf
B
Mef2ca/cb
sib
22s
mef2ca^b1086
C
Mef2ca/cb
dapi
sib
22s
mef2ca^b1086

Fig. S3







aberrant transcript (403bp):

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--T--R--I--M--D--E--R--N--R--Q--V--S--H--A--D--R--K--E--P--K--N--T--S--F--T--Q--

cttgatgggttgttttagacagaaatagatcaaGTGACATTTACAAAGAGAAAGTTTGGCCTGATGAAGAAAGCCTACGA

A--*-

GCTGAGCGTGTTGTGTGACTGTGAGATTGCCCTGATCATCTTCAACAGCACCAACAAGCTGTTCCAGTACGCCAGCACAG

ATATGGACAAAGTGCTGCTCAAATACACAGAGTACAACGAGCCACACGAGAGCAGGACCAACTCGGACATAGTGGAGACC

CTGAGAAAGAAGGGTCTAAATGGCTGTGACAGCCCGGACCCCGACGCCGACGACTCGGTGGGCCACAGCCCAGAGTCAAA

GGA

Fig. S4

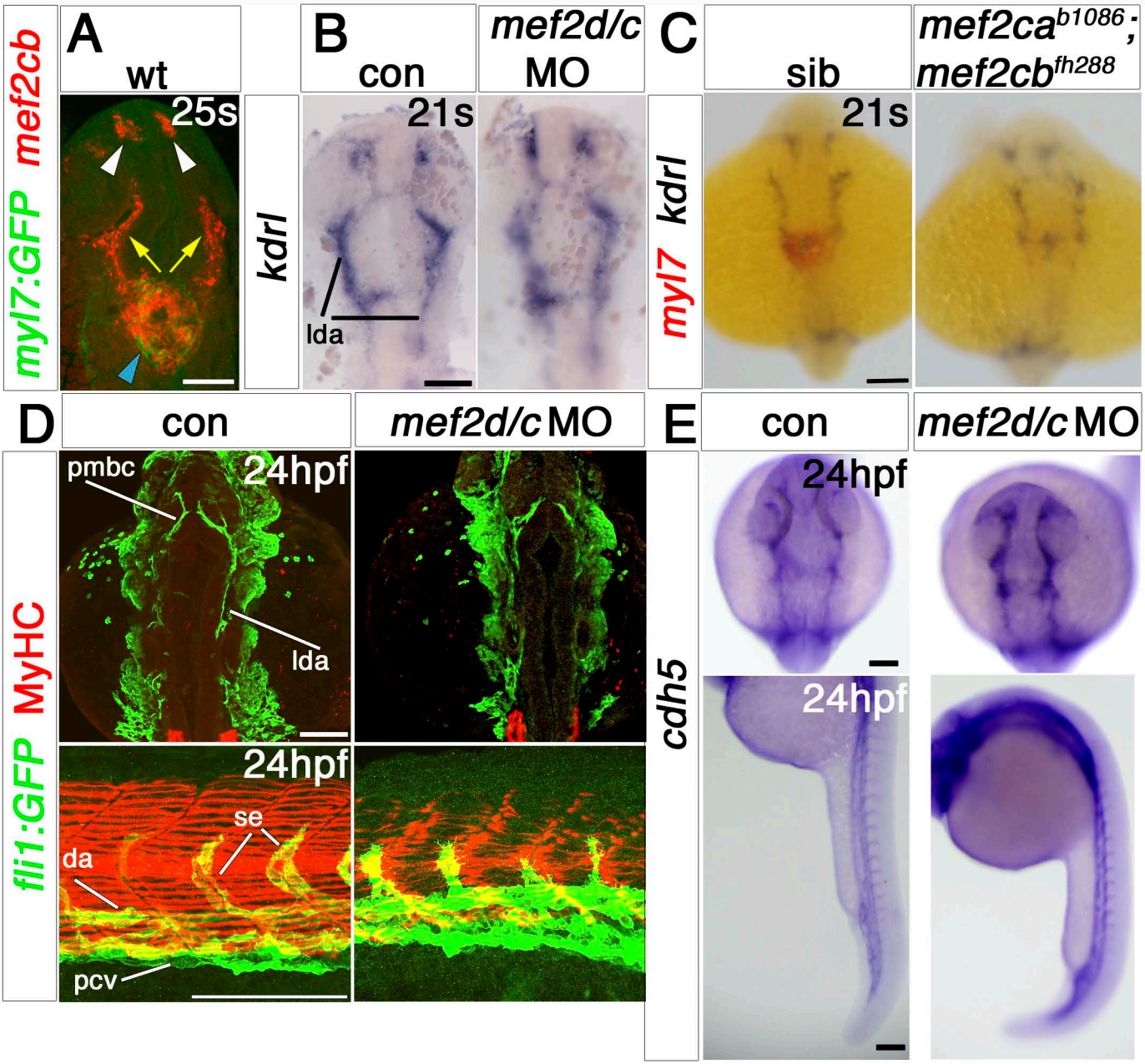
A
wt
B
con
mef2d/c MO
C
sib
mef2ca^b1086; mef2cb^fh288
mef2cb
myl7:GFP
25s
kdrl
21s
lda
myl7
kdrl
21s
D
con
mef2d/c MO
E
con
mef2d/c MO
MyHC
fli1:GFP
pmbc
24hpf
lda
24hpf
se
da
pcv
cdh5
24hpf
24hpf

Fig. S5

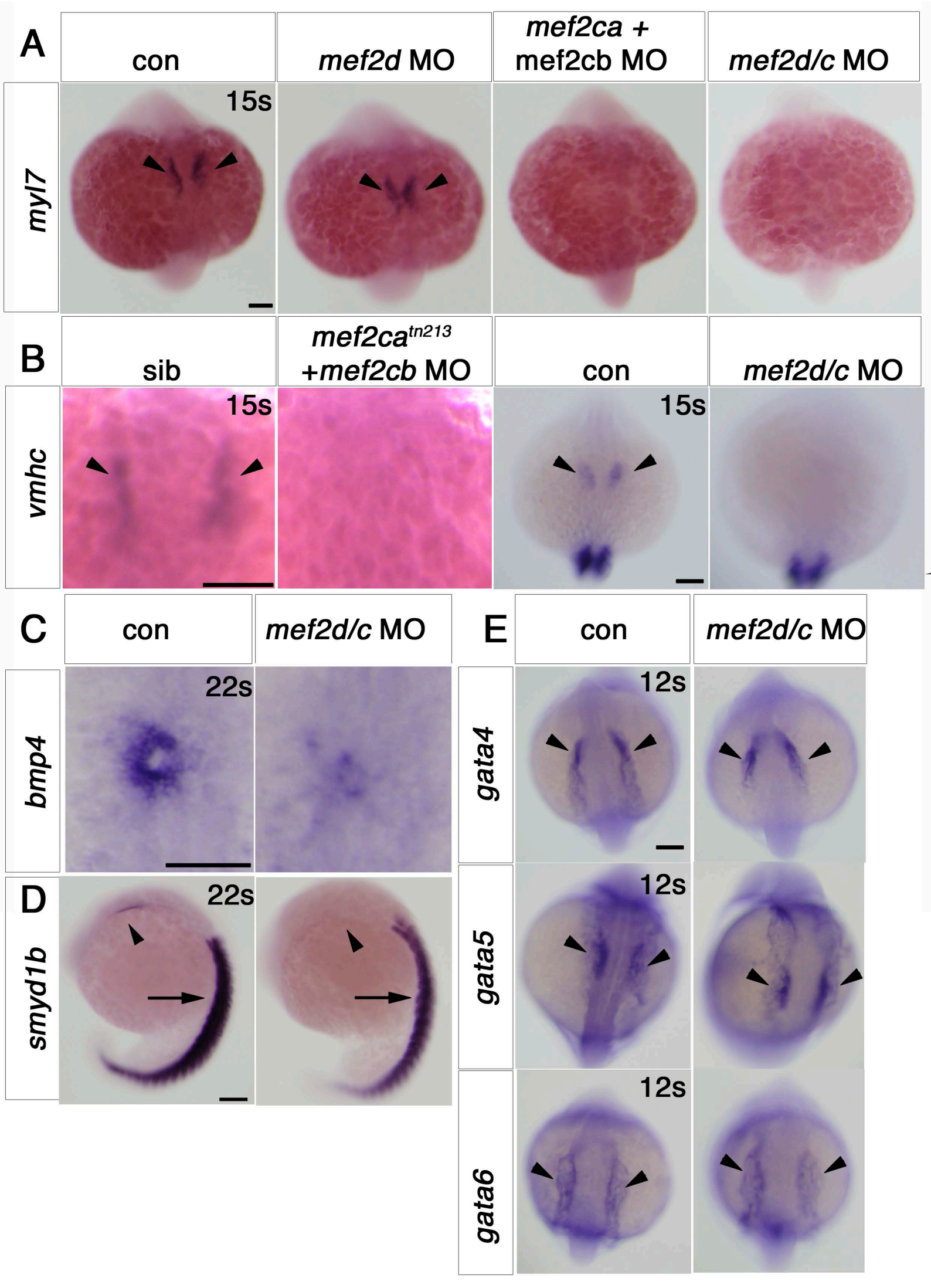
A
con
mef2d MO
mef2ca +
mef2cb MO
mef2d/c MO
myl7
15s
B
sib
mef2ca^tn213
+mef2cb MO
con
mef2d/c MO
vmhc
15s
15s
C
con
mef2d/c MO
bmp4
22s
D
smyd1b
22s
E
con
mef2d/c MO
gata4
12s
gata5
12s
gata6
12s

Fig. S6

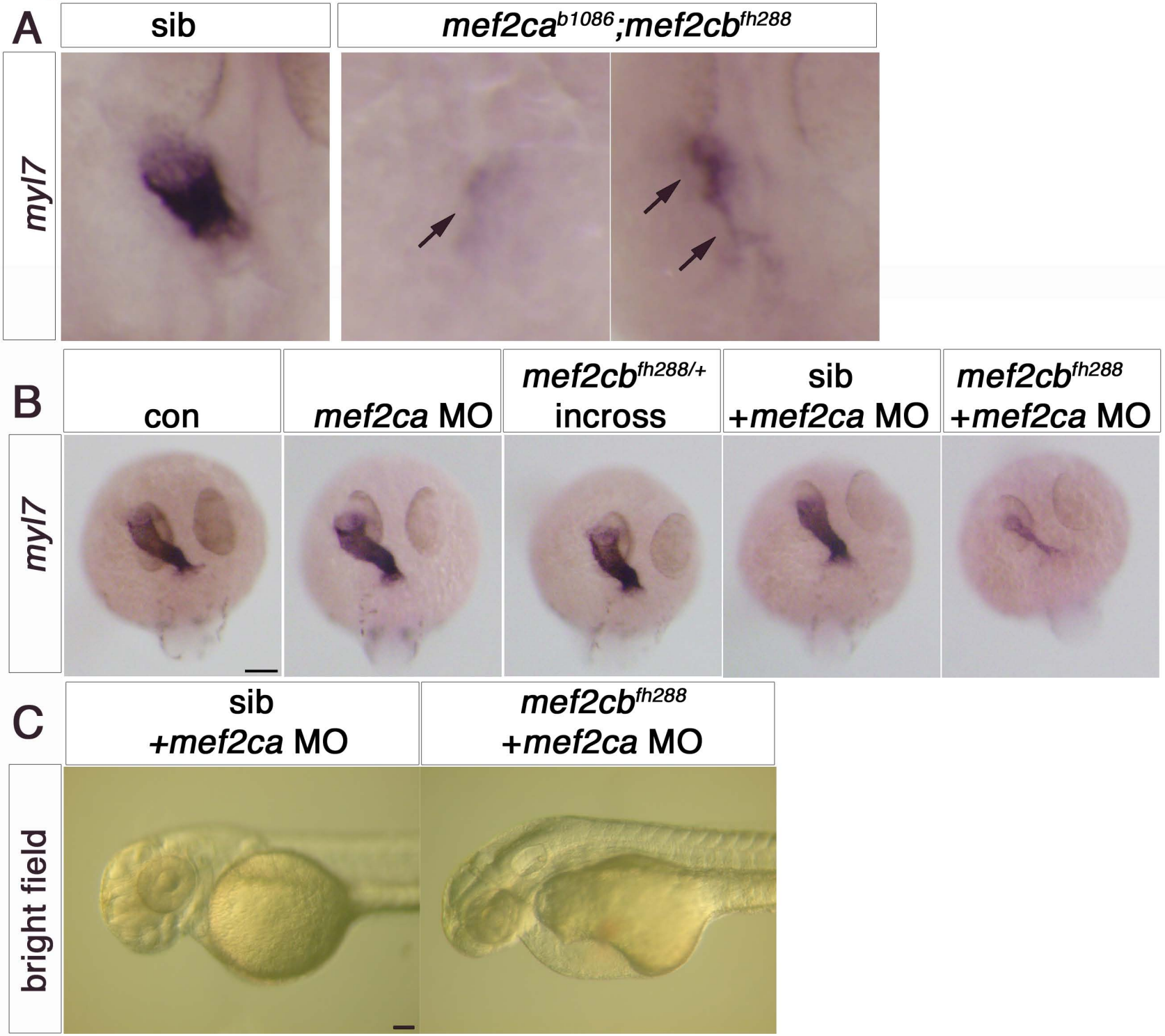
A
sib
mef2ca^b1086;mef2cb^fh288
myl7
B
con
mef2ca MO
mef2cb^fh288/+ incross
sib +mef2ca MO
mef2cb^fh288 +mef2ca MO
myl7
C
sib +mef2ca MO
mef2cb^fh288 +mef2ca MO
bright field

Fig. S7

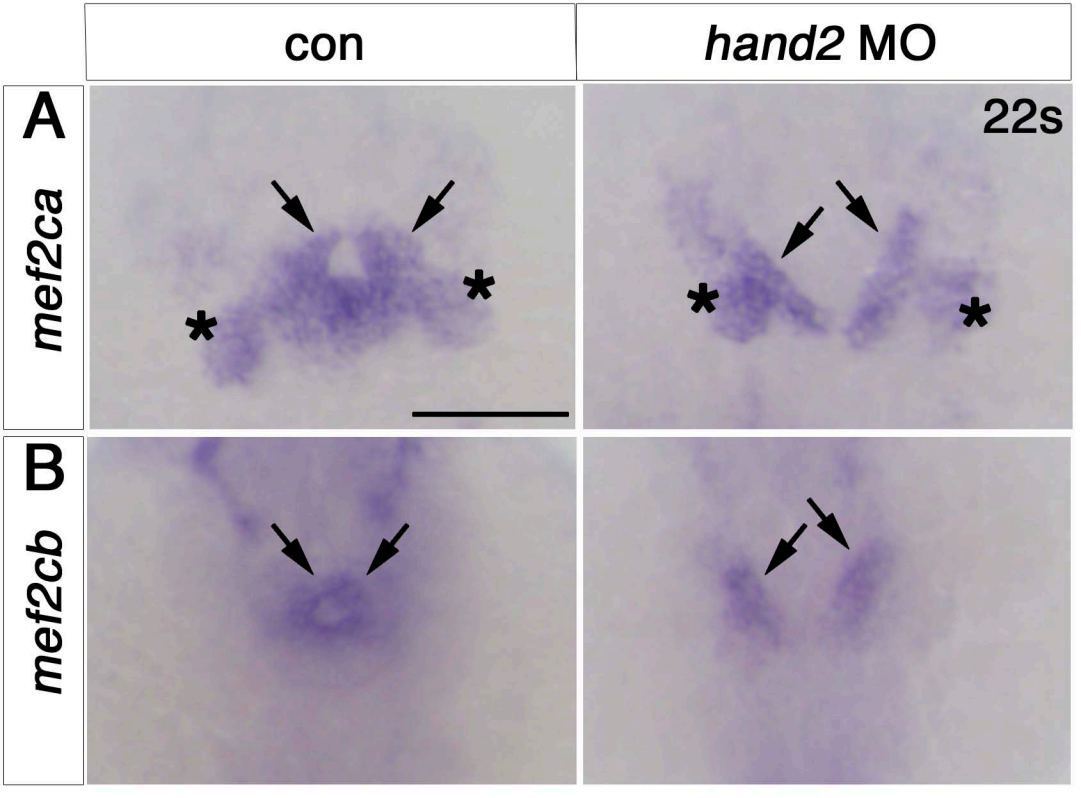

Fig. S8

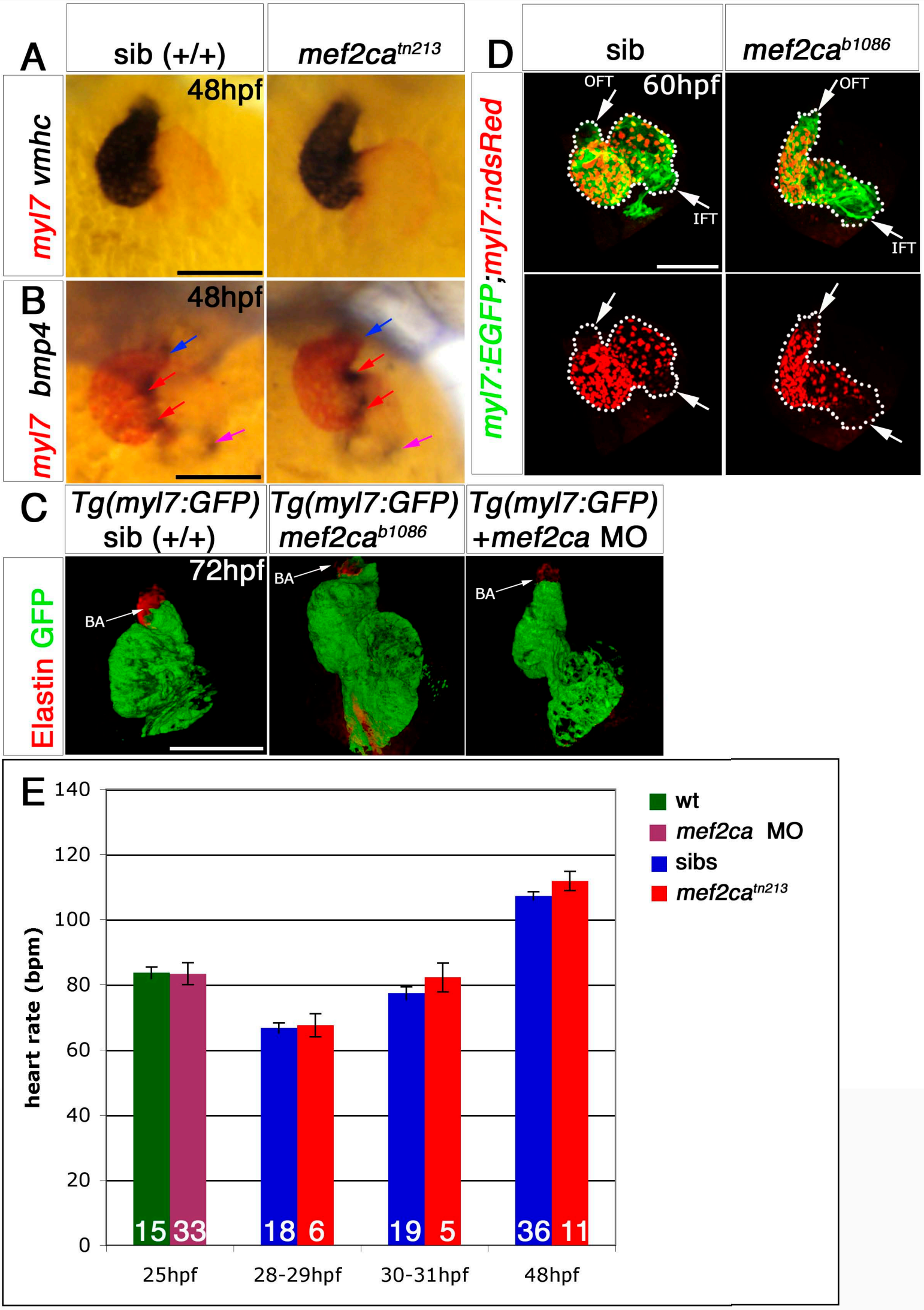
A
sib (+/+)
mef2ca tn213
myl7 vmhc
48hpf
B
myl7 bmp4
48hpf
C
Tg(myl7:GFP)
sib (+/+)
Tg(myl7:GFP)
mef2ca b1086
Tg(myl7:GFP)
+mef2ca MO
Elastin GFP
72hpf
BA
BA
BA
D
sib
mef2ca b1086
myl7:EGFP;myl7:ndsRed
60hpf
OFT
IFT
OFT
IFT
E
heart rate (bpm)
0
20
40
60
80
100
120
140
15
33
18
6
19
5
36
11
25hpf
28-29hpf
30-31hpf
48hpf
wt
mef2ca MO
sibs
mef2ca tn213

Fig. S9

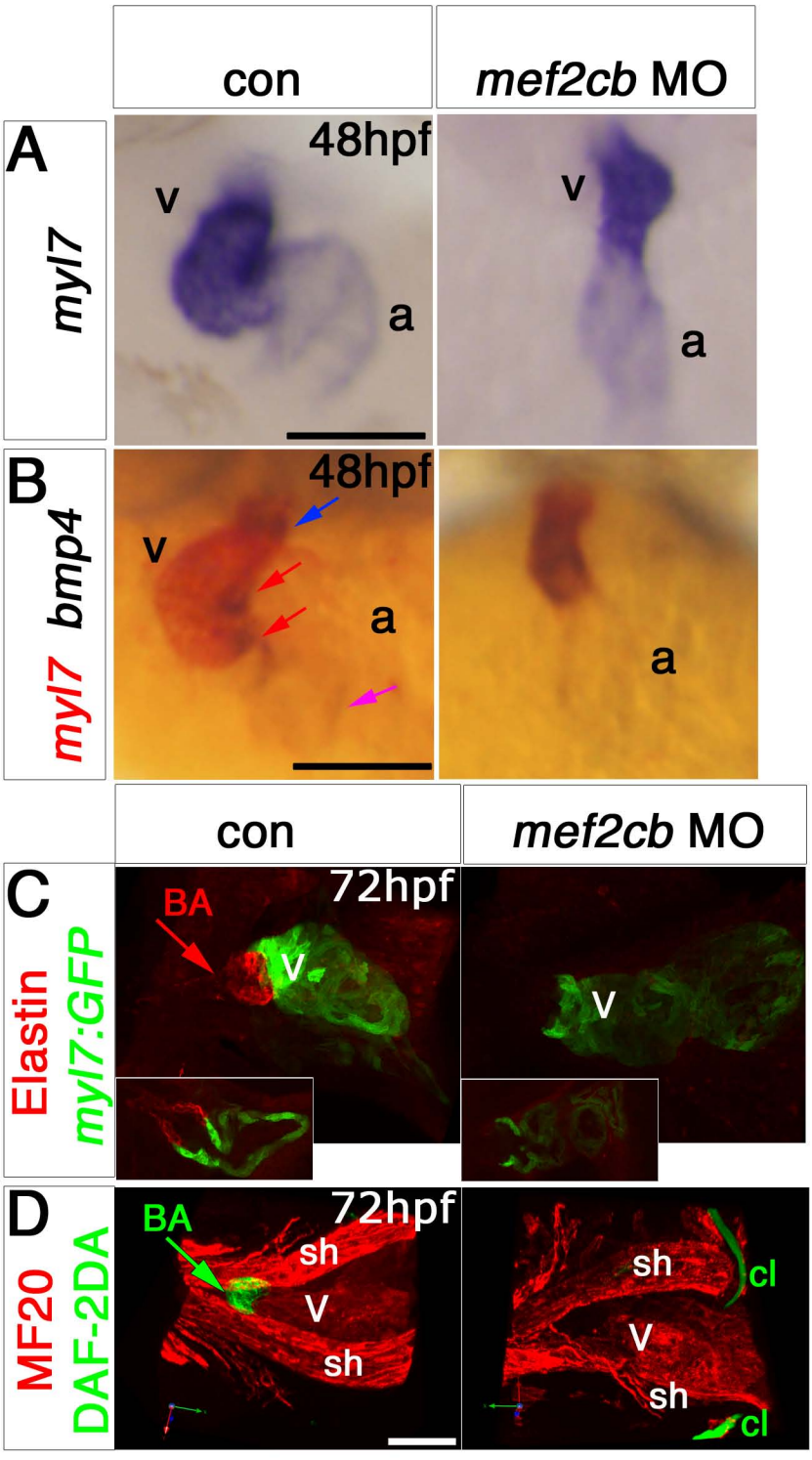
con
mef2cb MO
A
myl7
48hpf
v
a
v
a
B
myl7 bmp4
48hpf
v
a
a
con
mef2cb MO
C
Elastin
myl7:GFP
72hpf
BA
v
v
D
MF20
DAF-2DA
72hpf
BA
sh
v
sh
sh
v
sh
cl
cl

Table S1 Quantification of effects of loss- and gain-of-function.

Fig. No.		con	*mef2ca* MO	*mef2cb* MO	*mef2ca*+ *mef2cb* MO	*mef2ca*$^{+/fh1086}$ incross	*mef2ca*$^{+/fh1086}$ incross+ *mef2cb* MO	*mef2c*$^{+/tn213}$ incross	*mef2ca*$^{+/tn213}$ incross+ *mef2cb* MO	*mef2d/c* MO	*mef2d* MO	*mef2cb*$^{+/fh288}$ incross	*mef2cb*$^{+/fh288}$ incross +*mef2ca* MO	*mef2ca*$^{+/fh1086}$; *mef2cb*$^{+/fh288}$ incross
2A-F, S5A	***myl7*** **15s**	87/87 (100%) n	11/41 (27%) n, 30/41 (73%) r	39/39 (100%) n	7/17 (41%) n, 10/17 (59%) a	30/37 (81%) n, 7/37 (29%) r	44/57 (77%) n, 13/57(23%) a	30/45 (67%) n, 15/45 (33%) r,		5/27 (18%) sr, 22/27 (82%) a	21/21 (100%) n	19/19 (100%) n		58/62 (94%) n, 4/62(6%) a
2A-F, S5B	***vmhc*** **15s**	135/135 (100%) n		42/42 (100%) n		26/26 (100%) n	22/31 (71%) n, 9/31 (29%) a	22/33 (66%) n, 11/33 (34%) r,	8/46 (17%) n, 22/46 (48%) r, 16/46 (35%) a	4/65 (6%) n, 1/65 (2%) r, 60/65 (92%) a		20/20 (100%) n		79/86 (92%) n, 7/86 (8%) a
2A-D	***smyd1b*** **13s**	98/98 (100%) n		65/65 (100%) n		12/46 (26%) n, 23/46 (50%) r, 11/46 (24%) a	49/64 (77%) n, 15/64 (23%) a	7/31 (22%) n, 15/31 (48%) r, 9/31 (30%) a		3/67 (4%) n, 2/67 (3%) r, 62/67 (93%) a (in heart)				
2A-D	***smyd1b*** **17s**	28/28 (100%) n		25/25 (100%) n		21/22 (95%) n, 1/22 (5%) r	17/21 (81%) n, 4/21 (19%) a			13/55 (24%) n, 16/55 (29%) r, 26/55 (47%) a (in heart)				
2G	**MyHC** **24s**													94/107 (87%) n, 6/107 (6%) r, 7/107 (7%) a
3A-F	**MyHC** **24 hpf**	25/25 (100%) n		8/26 (31%) n, 18/26 (69%) sm				+/+*: 10/12 (83%) n, 2/12(17%) r *+/tn213**: 28/28 (100%) n *tn213/tn213**: 15/19 (79%) n, 4/19 (21%) r	+/+*: 5/19 (26%) n, 14/19(84%) r *+/tn213**: 9/35 (26%) n, 25/35 (71%) r, 1/35 (3%) a *tn213/tn213**: 1/17 (6%) n, 16/17 (94%) a			31/31 (100%) n		74/80 (93%) n, 6/80 (7%) res
3A-F	***bmp4*** **24 hpf**	23/23 (100%) n		1/22 (4%) n, 21/22 (96%) sm		27/27 (100%)	16/23 (70%) sm, 7/23 (30%) a					53/53 (100%) n		83/88 (94%) n, 5/88 (6%) res
3A-F, S6A,B	***myl7*** ***+vmhc*** **24 hpf** **or: *myl7* only**	122/128 (95%) n, 6/128 (5%) r *myl7* only: 48/48 (100%) n	*myl7* only: 56/56 (100%) n	16/33 (48%) n, 17/33 (52%) sm		49/49 (100%) n	3/59 (5%) n, 41/59 (69%) r, 15/59 (26%) a			3/36 (8%) n, 33/36 (92%) a		36/36 (100%) n *myl7* only: 78/78 (100%) n	*myl7* only: 58/80 (72%) n, 22/80 (28%) sr	83/86 (96%) n, 3/86 (4%) res *myl7* only: 71/79 (90%) n, 8/79 (10%) res
3G	***acta1b*** **24 hpf**	22/22 (100%) n								4/28 (14%) n, 10/28 (36%) r, 14/28 (50%) a				
3H	***tnnc2*** **24 hpf**	25/25 (100%) n	23/23 (100%) n							3/25 (12%) n, 22/25 (30%) a	24/24 (100%) n			
3I	***mybpc1*** **24 hpf**	38/38 (100%) n								6/41 (14%) n, 35/41 (86%)				
3J	***nppa*** **24 hpf**	16/16 (100%) n								5/21 (23%) n, 16/21 (77%) a				
4A, S6C	**Bright field** **48-72 hpf**											158/158 (100%) n	106/146 (73%) n, 40/146 (27%) sr + edema,	642/694 (93%) n, 52/694 (7%) a or res + edema,
4B	***myl7*** ***+vmhc*** **48 hpf**													69/74 (93%) n, 5/74 (7%) res
4C	**MyHC** **+Mef2, 50 hpf**													80/83 (96%) n, 3/83 (4%) res
4D	**MyHC** **(A4.1025); S46, 48 hpf**	15/15 (100%) n								1/26 (4%) n, 6/26 (23%) sr, 19/26 (73%) a				
4E	***nppa*** **48 hpf**													52/55 (95%) n, 3/55 (5%) res
4F	***myl7*** ***+bmp4*** **48 hpf**													46/50 (92%) n, 4/50 (8%) res
4G														73/78 (94%) n, 5/78 (6%) res

Fig. No.		con	*mef2ca* MO	*mef2cb* MO	*mef2ca+ mef2cb* MO	*mef2ca*$^{+/b1086}$ incross	*mef2ca*$^{+/b1086}$ incross+ *mef2cb* MO	*mef2c*$^{+/tn213}$ incross	*mef2ca*$^{+/tn213}$ incross+ *mef2cb* MO	*mef2d/c* MO	*mef2d* MO	*mef2cb*$^{+/fh288}$ incross	*mef2cb*$^{+/fh288}$ incross +*mef2ca* MO	*mef2ca*$^{+/b1086}$; *mef2cb*$^{+/fh288}$ incross
5A	***nkx2.5*** **12s**	27/27 (100%) n			22/22 (100%) n									60/60 (100%) n
5B	***hand2*** **12s**	16/16 (100%) n			24/24 (100%) n									68/68 (100%) n
5C	***gata4*** **12s**	20/20 (100%) n			27/27 (100%) n									70/70 (100%) n
5D	***tbx20*** **22s**	13/13 (100%) n								22/22 (100%) n (but loss of ring shape)				
5E	***gata6 (±myl7)*** **22s**	22/22 (100%) n								3/26 (11%) n, 23/26 (89%) n (but loss of ring shape)				57/61 (93%) n, 4/61 (7%) a *myl7*; loss of ring shape *gata6*
5F	***nkx2.5 (±myl7)*** **22s**	46/46 (100%) n,	46/46 (100%) n	15/40 (37%) n, 16/40 (40%) r, 9/40 (13%) sr						8/57 (14%) n, 49/57 (86%) sr,				55/58 (95%) n, 3/58 (5%) a *myl7*; res *nkx2.5*
5G	***hand2*** **20-25s**	15/15 (100%) n						34/34 (100%) n	13/49 (27%) n, 36/49 (73%) up	16/16 (100%) up				58/62 (94%) n, 4/62 (6%) up
5H	***cdh5;myl7*** **22s**	68/68 (100%) n								21/65 (32%) n, 44/65 (68%) sr or a *myl7*; up and expanded *cdh5*				79/85 (93%) n, 6/85 (7%) a *myl7*; up and expanded *cdh5*
5I	***cdh5;myl7*** **48 hpf**													87/92 (95%) n, 5/92 (5%) res *myl7*; up and expanded *cdh5*

		con	*mef2cb* BAC DNA	*mef2cb* mRNA	*mef2cb*$^{+/fh288}$ incross
6A, S1G	**MyHC; Mef2ca/cb 24 hpf**	20/20 (100%) n		5/22 (22%) n, 17/22 (78%) up+ectopic	
6B	***smyd1b*** **24 hpf**	25/25 (100%) n		5/23 (22%) n, 18/23 (78%) up+ectopic	
6C	***bmp4*** **24 hpf**	19/19 (100%) n		13/37 (35%) n, 24/37 (65%) up (in cardiac region)	
6D	***myod;myl7*** **14s**	9/9 (100%) n		5/22 (23%) n, 17/22 (77%) ectopic *myod*, no *myl7*	
6E	***myod;myl7*** **24s**	11/11 (100%) n		5/16 (31%) n, 10/16 (78%) ectopic *myod*, reduced *myl7*	
6F	***myl7;cdh5*** **24s**	14/14 (100%) n		5/24 (21%) n, 19/24 (79%) reduced *myl7* reduced *cdh5*	
6G	***myl7*** **24 hpf**	24/24 (100%) n	24/41 (58%) n, 17/41 (42%) ect		
7A	***myl7+vmhc,*** **50 hpf**				51/51 (100%) n; 12 genotyped
7B	***bmp4*** **48 hpf**				42/42 (100%) n; 12 genotyped
7C	**Zn-5+Elastin, 72 hpf**				38/38 (100%) n; 12 genotyped
S1A-D	***mef2cb*** **24 hpf**		25/34 (73%) up in chimeric distribution		

		con	*mef2cb* MO	*mef2ca*^{b1086} incross	*mef2d/c MO*	*mef2ca*^{tn213} incross	*hand2* MO	*mef2ca*$^{+/b1086}$; *mef2cb*$^{+/fh288}$ incross
S2B,C	**Mef2ca/cb**			29/38 (76%) n 9/38 (24%) a (somite), sr (heart)				
S4B	***kdrl (± myl7)*** **21s**	16/16 (100%) n			29/29 (100%) n			41/44 (93%) n, 3/44 (7%) a *myl7*; n *kdrl*
S4D	***fli1:GFP*** ***24 hpf***	23/23 (100%) n			18/18 (100%) n (heart region)			
S4E	***cdh5*** ***24 hpf***	26/26 (100%) n			6/21 (27%) n, 15/21 (73%) up			
S5A	***myl7*** **15s**	**See entry for 2A-F**						

S5B	***vmhc*** **15s**	**See entry for 2A-F**						
		con	***mef2cb* MO**	***mef2ca*^{b1086} incross**	***mef2d/c MO***	***mef2ca*^{tn213} incross**	***hand2* MO**	***mef2ca*$^{+/b1086}$; *mef2cb*$^{+/fh288}$ incross**
S5C	***bmp4*** **22s**	35/35 (100%) n			15/79 (19%) n, 19/79 (24%) r, 45/79 (57%) sr			
S5D	***smyd1b*** **22s**	41/41 (100%) n			36/36 (100%) a			
S5E	***gata4*** **12s**	21/21 (100%) n			35/35 (100%) n			
S5E	***gata5*** **12s**	26/26 (100%) n			26/26 (100%) n			
S5E	***gata6*** **12s**	24/24 (100%) n			24/24 (100%) n			
S6A,B	***myl7*** **24 hpf**	**See entry for 3A-F**						
S6C	**Bright field phenotype**	**See entry for 4A**						
S7A	***mef2ca*** **22s**	23/23 (100%) n					18/19 (94%) cb (nl) 1/19 (6%) n	
S7B	***mef2cb*** **22s**	31/31 (100%) n					23/24 (96%) cb (nl) 1/24 (4%) n	
S8A	***myl7+vmhc*** **48 hpf**					29/29 (100%) n**		
S8B	***myl7+bmp4*** **48 hpf**					36/36 (100%) n**		
S8A	***smyd1b*** **24 hpf**	53/53 (100%) n	5/32 (15%) n, 27/32 (85%) sm, up					
S9A	***myl7*** **48 hpf**	25/25 (100%) n	7/77 (9%) 70/77 (91%) small, unlooped heart					
S9B	***bmp4*** **48 hpf**	24/24 (100%) n	7/48 (15%) n, 41/48 (85%) up, unlooped heart					
S9D	**MF20; DAF-2DA 72 hpf**	12/12 (100%) n	2/14 (14%) n, 12/14 (86%) a					

		mef2ca*^{b1086}; *Tg(myl7:GFP; myl7;ndsRed)	***Tg (myl7:GFP)***	***Tg (myl7:GFP) + mef2cb MO***	***Tg (myl7:GFP) + mef2ca MO***	***mef2ca*^{b1086}; *Tg(myl7:GFP)***
S8C, S9C	***myl7:GFP;*** **Elastin,72 hpf**		12/12 (100%) n	3/15 (20%) n, 2/15 (43%) r, 10/15 (67%) a	8/10 (80%) n 2/10 (20%) r	12/12 (100%) n**
S8D	***myl7:GFP; myl7:ndsRed*, 60 hpf**	21/21 (100%) n**				

Fraction of embryos showing normal, or any deviated effect of mRNA expression, protein immunodetection or marker at the indicated stages.
Abbreviations: n, normal, ab, abnormal, r, reduced, sr, strongly reduced, res, absent except for a few residual cells, up, upregulated, a, absent, sm, small heart, cb, cardia bifida, nl, normal levels
* genotype verified by Mef2C immunostaining.
** Results verified by sequence genotyping.