Supplemental Table 1. Publications utilizing *αMyHC-Cre* mice and controls included. Results of literature search for primary research papers citing [1] in which *αMyHC-Cre* mice were used to conditionally delete or overexpress a floxed gene in cardiac myocytes. Control group genotypes, if specified, are noted. Approximately 20% of papers cite *αMyHC-Cre* mice as controls while nearly 50% cite floxed littermates as controls. WT: Wild type, fl/fl: Gene of interest with flanking *loxP* sites.

Supplemental Table 2. Primer sequences.

Supplemental Table 3. Morphometric and functional M-mode echocardiography. Summary of echocardiography measurements made in 3 and 6 month old male and female mice. N/group is displayed in column heading **P* < 0.05, ***P* < 0.01 vs. WT, age-matched control.

Supplemental Figure 1. *αMyHC-Cre* ventricular mRNA and Protein expression. *Cre* mRNA expression by qRT-PCR **(A)** and Cre protein expression by immunoblot **(B)**. Error bars: SEM, N=4-6/group ****P* < 0.001 vs. WT control.

Supplemental Figure 2. Cre expression correlates with cardiac function at 3 months. (A). %EF in 3 month-old mice. **(B)** *Cre* expression by qRT-PCR. *Cre* expression is grouped into "high" (solid line) and "low" (dashed line). **(C)** %EF separated into *Cre* "high" and *Cre* "low" demonstrates dose-dependence of *Cre* effect on %EF. N=4-6/group **P* < 0.05.

Supplemental Figure 3. Myocardial analysis of inflammatory cells. Histochemical assessment of activated macrophages (F4/80^{+} cells) in ventricular myocardium. Arrows indicate F4/80^{+} cells. Scale bar: 100μm.

Supplemental Figure 4. Ventricular gene expression changes in 6 month-old *αMyHC-Cre*$^{+/-}$ males. Many of the gene expression changes observed in *αMyHC-Cre*$^{+/-}$ females were confirmed in *αMyHC-Cre*$^{+/-}$ males. Error bars: SEM, N=4-5/group, **P* < 0.05, ***P* < 0.01 vs. age-matched and sex-matched WT controls.

Supplemental Figure 5. Gene expression is disrupted in a subset of genes harboring degenerate *loxP* sites. (A) qRT-PCR mRNA expression in 6 month old *αMyHC-Cre*$^{+/-}$ females of a subset of genes harboring degenerate *loxP* sites. 27/55 (49%) genes identified were tested. Expression changes were observed for 7/27 (26%) of genes tested. **(B)** Protein expression (immunoblot) for ME3 normalized to Gapdh. N=3-4/group **P* < 0.05 & *P* < 0.1 vs. WT control.

Supplemental Figure 6. Genomic insertion site of *αMyHC-Cre* transgene does not likely account for cardiotoxicity. (A) PCR-based strategy for mapping transgene insertion site utilizes sequential, forward gene-specific primers (GSP1-3) in 3' end of *αMyHC-Cre* transgene and degenerate, reverse primers in flanking genomic DNA. **(B)** Screenshot of UCSC Genome Browser window displaying Chromosome 6 genomic locus of transgene to which PCR products were mapped. The region is intergenic and non-transcriptionally active based on displayed Genome Browser tracks. **(C)** Genomic locus was verified using PCR of additional animals. Products of expected length based on primer design were only amplified in *αMyHC-Cre*$^{+/-}$ mice. Lane 1: 1kb+ DNA ladder (Invitrogen), Lane 2: LML (low mass ladder, Invitrogen), Lane 3: *Cre* PCR product, positive control, Lanes 4-17: *αMyHC-Cre* genotype (+ or -) or no template control (NTC).

Supplemental Figure 7. *αMyHC-Cre* transgene copy number in *αMyHC-Cre*$^{+/-}$ mice. Copy number was estimated based on standard curves generated with known copy numbers of either *Cre* **(A)** or a control gene, *Myh15* (2 copies/genome) (**B**). **(C)** An average of 6 copies of the *αMyHC-Cre* transgene were detected per genome, when normalized to *Myh15* copy number, N=8.

Supplemental Figure 8. Lower levels of myocardial Cre expression do not result in cardiotoxicity. Cre expression and cardiotoxicity were assessed in male WT, “conventional” *αMyHC-Cre*$^{+/-}$ mice [1] and “alternate” *αMyHC-Cre*$^{+/-}$ mice [2]. Although both transgenes drive *Cre* expression under the *αMyHC* promoter, the transgene structures differ in their 3’UTRs and promoter sequences [1,2]. Differences in transgene structure, copy number and insertion site may all contribute to expression differences in Cre. **(A)** Cre protein expression (immunoblot). **(B)** Cardiac function (%EF). **(C)** Gene expression (qRT-PCR). Error bars: SEM, N=4-5/group, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 vs. age-matched and sex-matched WT controls.

References

[1] Agah R, Frenkel PA, French BA, Michael LH, Overbeek PA, Schneider MD. Gene recombination in postmitotic cells. Targeted expression of Cre recombinase provokes cardiac-restricted, site-specific rearrangement in adult ventricular muscle in vivo. 1997;100:169–79.

[2] Abel ED, Kaulbach HC, Tian R, Hopkins JCA, Duffy J, Doetschman T, et al. Cardiac hypertrophy with preserved contractile function after selective deletion of GLUT4 from the heart 1999;104:1703–14.

S Table 3.

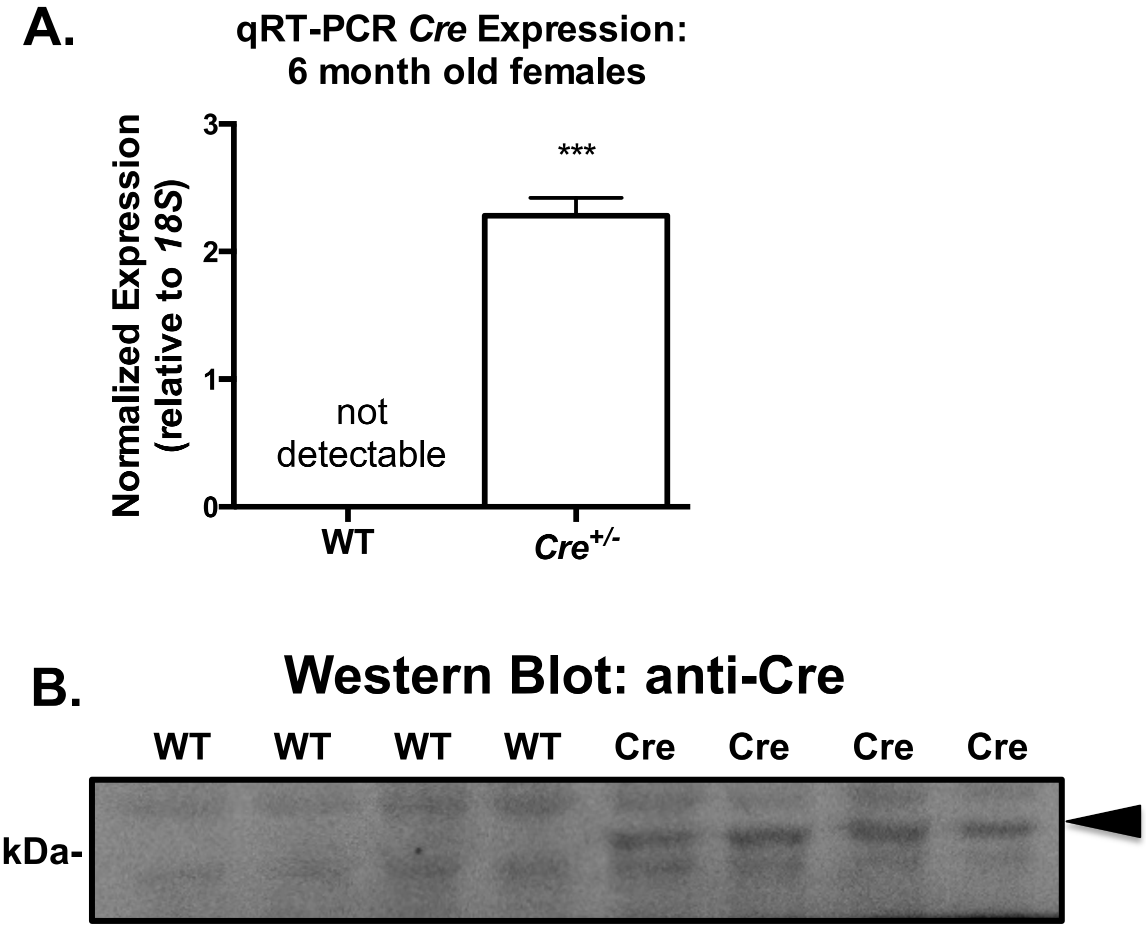
3 months

(N)	WT Female (6)	*Cre*$^{+/-}$ Female (14)	WT Male (6)	*Cre*$^{+/-}$ Male (10)
%EF	59.57 ± 2.13	62.92 ± 1.41	54.55 ± 1.90	**64.24 ± 1.72
LVID;s (cm)	0.270 ± 0.0057	0.258 ± 0.00283	0.294 ± 0.00835	*0.259 ± 0.00618
LVID;d (cm)	0.388 ± 0.00471	0.384 ± 0.00355	0.408 ± 0.00991	0.408 ± 0.00844
LVAW;s (cm)	0.12 ± 0.0032	0.12 ± 0.0023	0.12 ± 0.0043	0.13 ± 0.0033
LVAW;d (cm)	0.062 ± 0.0018	0.058 ± 0.0010	0.066 ± 0.0015	0.066 ± 0.0012
LVPW;s (cm)	0.0983 ± 0.00320	0.102 ± 0.00156	0.102 ± 0.00198	*0.109 ± 0.0014
LVPW;d (cm)	0.062 ± 0.0014	0.061 ± 0.00074	0.067 ± 0.0010	0.067 ± 0.00090
Heart Rate (bpm)	434 ± 10.3	443 ± 12.7	439 ± 13.8	**503 ± 9.3
LV Vol;s	26.36 ± 1.40	23.56 ± 0.77	33.65 ± 2.26	26.78 ± 2.46
LV Vol;d	65.32 ± 1.87	63.92 ± 1.42	74.09 ± 4.29	74.08 ± 3.73
LV mass	64.22 ± 1.21	*58.57 ± 1.45	77.26 ± 4.44	76.39 ± 2.09

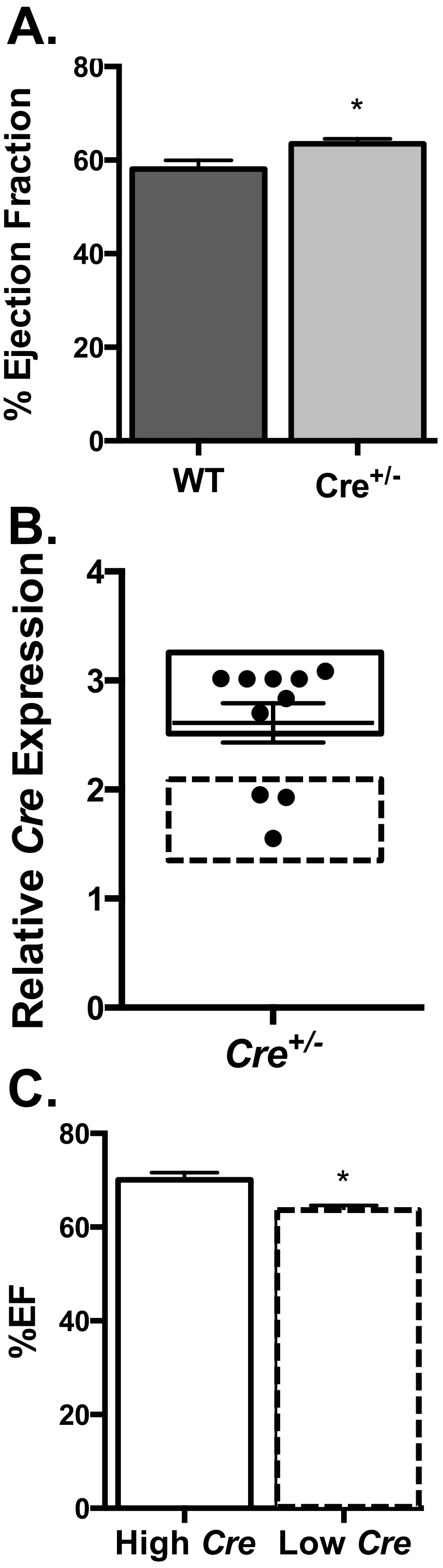
6 months

(N)	WT Female (4)	*Cre*$^{+/-}$ Female (6)	WT Male (4)	*Cre*$^{+/-}$ Male (8)
%EF	68.41 ± 2.70	**57.80 ± 1.67	65.07 ± 1.60	*57.24 ± 1.72
LVID;s (cm)	0.232 ± 0.0117	**0.275 ± 0.00693	0.256 ± 0.0100	0.276 ± 0.0118
LVID;d (cm)	0.360 ± 0.00466	**0.397 ± 0.0029	0.395 ± 0.0123	0.397 ± 0.0110
LVAW;s (cm)	0.12 ± 0.0033	0.12 ± 0.0028	0.13 ± 0.0026	0.12 ± 0.0022
LVAW;d (cm)	0.059 ± 0.0	***0.067 ± 0.0010	0.068 ± 0.0	0.068 ± 0.00037
LVPW;s (cm)	0.106 ± 0.0052	0.104 ± 0.0038	0.109 ± 0.0016	0.107 ± 0.0032
LVPW;d (cm)	0.059 ± 0.0	*0.067 ± 0.0016	0.068 ± 0.0016	0.067 ± 0.00037
Heart Rate (bpm)	531 ± 8.8	506 ± 9.6	466 ± 30.7	492 ± 18.9
LV Vol;s	18.82 ± 2.41	**28.54 ± 1.67	23.97 ± 2.31	29.27 ± 2.88
LV Vol;d	59.45 ± 4.98	67.39 ± 1.76	68.41 ± 4.88	69.56 ± 4.49
LV mass	57.36 ± 3.00	**72.40 ± 2.53	74.78 ± 2.91	75.29 ± 3.48

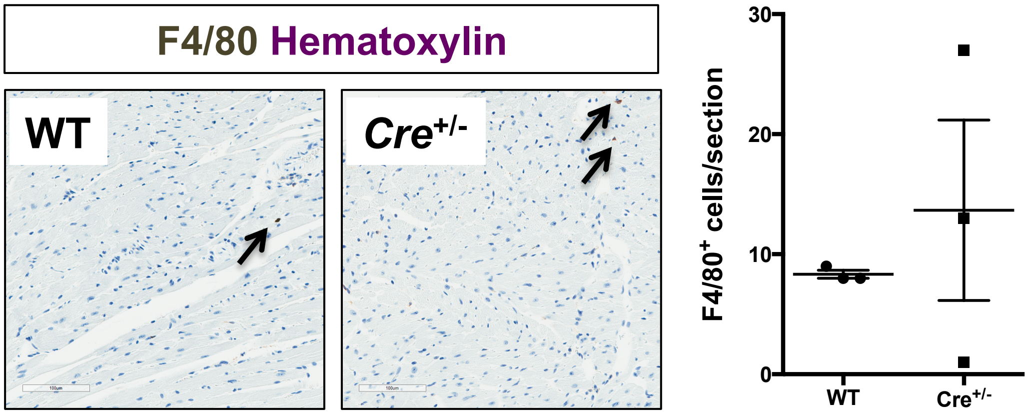
S1.


A.
qRT-PCR Cre Expression:
6 month old females
Normalized Expression
(relative to 18S)
3
2
1
0
***
not
detectable
WT
Cre+/-
B.
Western Blot: anti-Cre
WT
WT
WT
WT
Cre
Cre
Cre
Cre
37 kDa-

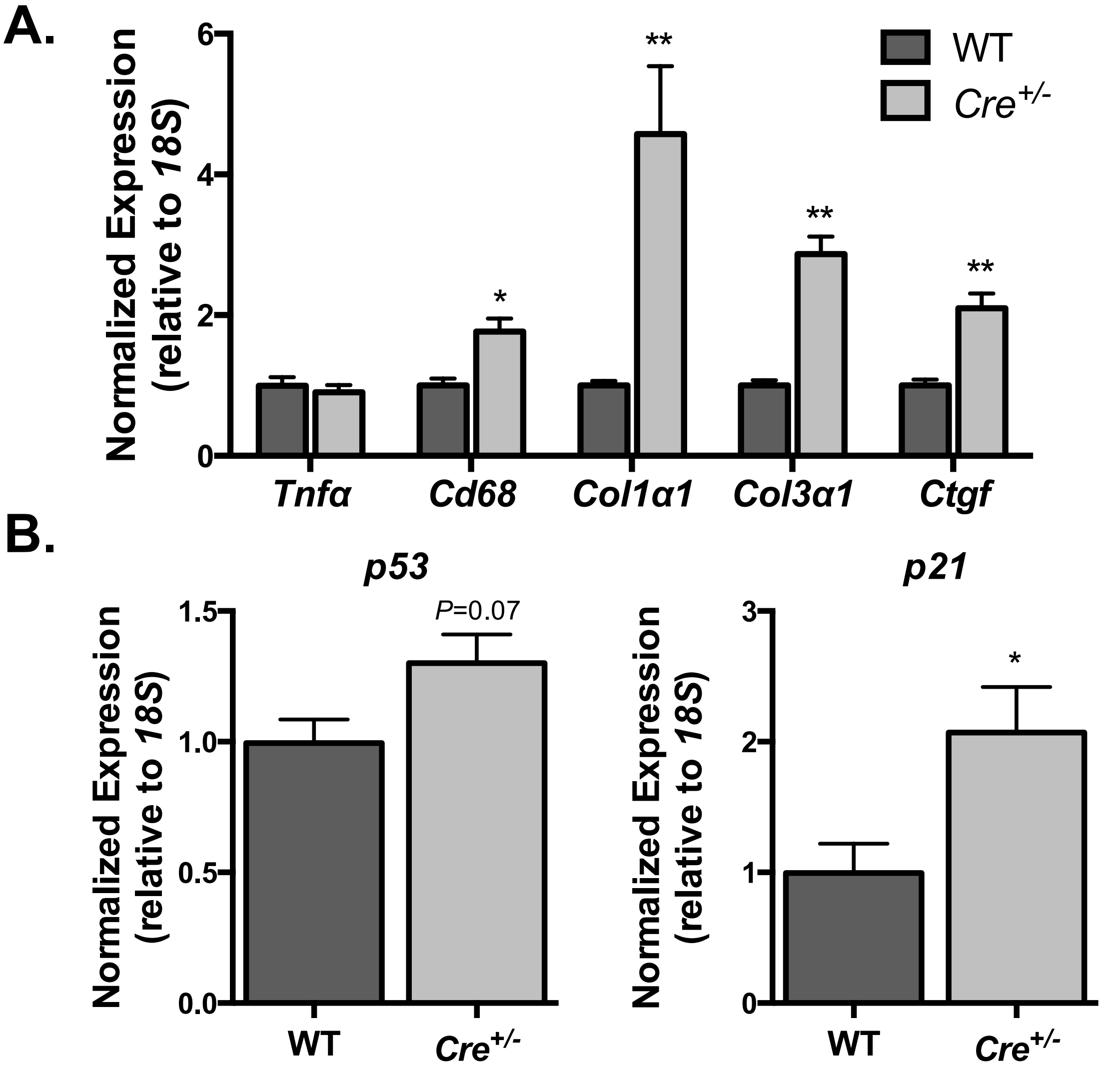
S2.


A.
% Ejection Fraction
80
60
40
20
0
*
WT
Cre+/-
B.
Relative Cre Expression
4
3
2
1
0
Cre+/-
C.
%EF
80
60
40
20
0
*
High Cre
Low Cre

S3.

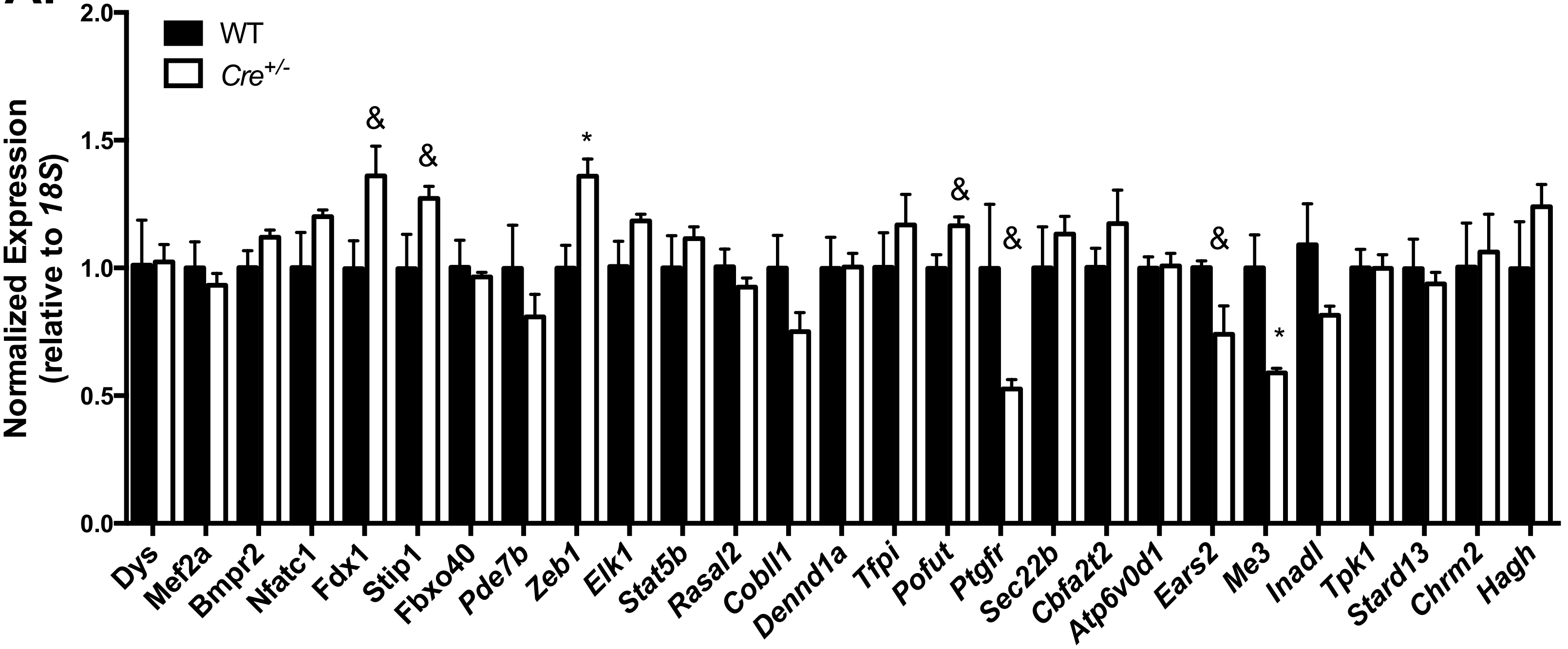


S4.

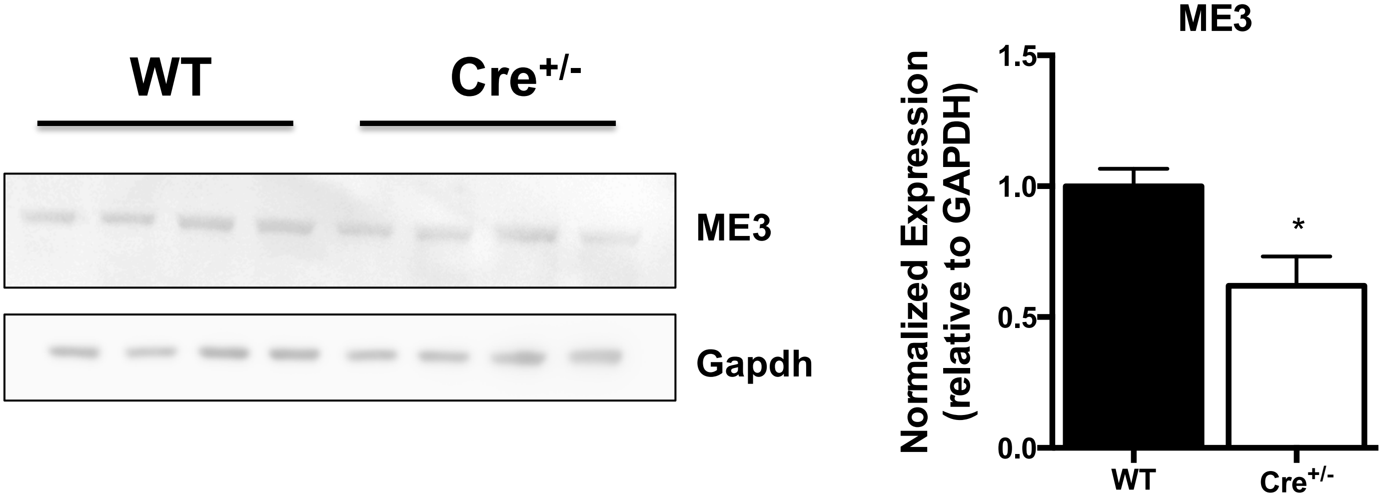

A.
Normalized Expression (relative to 18S)
0
2
4
6
WT
Cre+/-
*
**
**
**
Tnfα
Cd68
Col1α1
Col3α1
Ctgf
B.
p53
Normalized Expression (relative to 18S)
0.0
0.5
1.0
1.5
P=0.07
WT
Cre+/-
p21
Normalized Expression (relative to 18S)
0
1
2
3
*
WT
Cre+/-

S5.

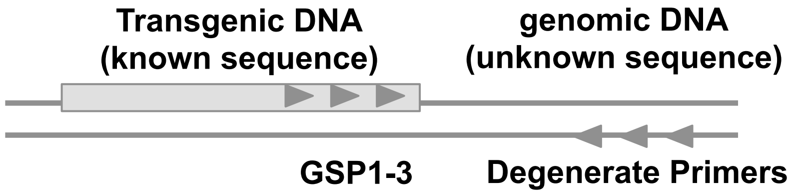
A.


WT
Cre+/-
Normalized Expression (relative to 18S)
2.0
1.5
1.0
0.5
0.0
&
&
*
&
&
&
*
Dys
Mef2a
Bmpr2
Nfatc1
Fdx1
Stip1
Fbxo40
Pde7b
Zeb1
Elk1
Stat5b
Rasal2
Cobll1
Dennd1a
Tfpi
Pofut
Ptgfr
Sec22b
Cbfa2t2
Atp6v0d1
Ears2
Me3
Inadl
Tpk1
Stard13
Chrm2
Hagh

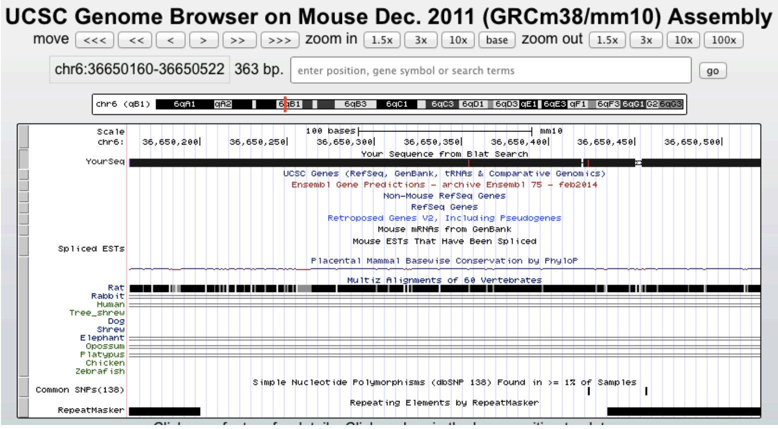
B.


WT
Cre+/-
ME3
Gapdh
ME3
Normalized Expression (relative to GAPDH)
1.5
1.0
0.5
0.0
*
WT
Cre+/-

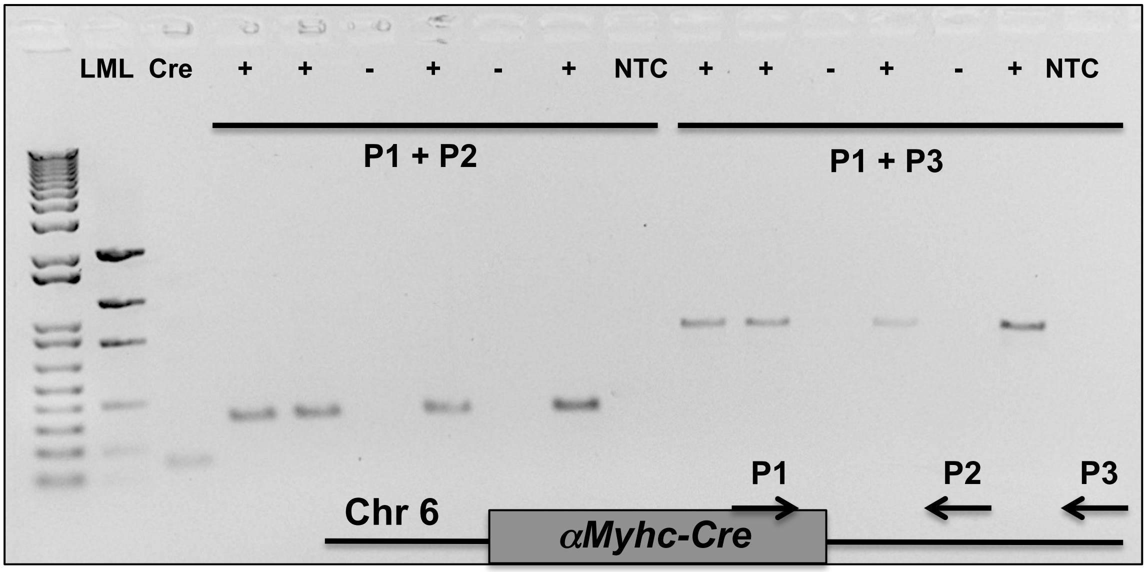
S6. A. TAIL-PCR Strategy



B. Genomic locus of transgene

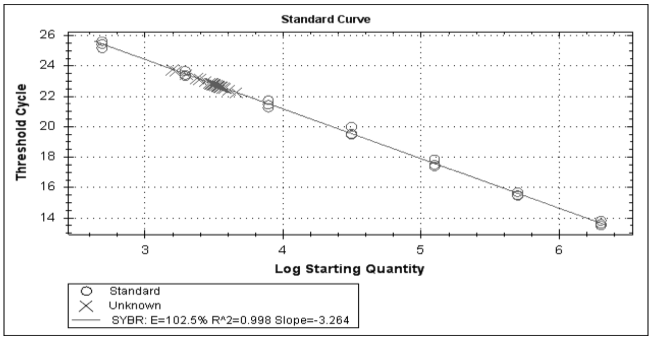


C. PCR verification of locus



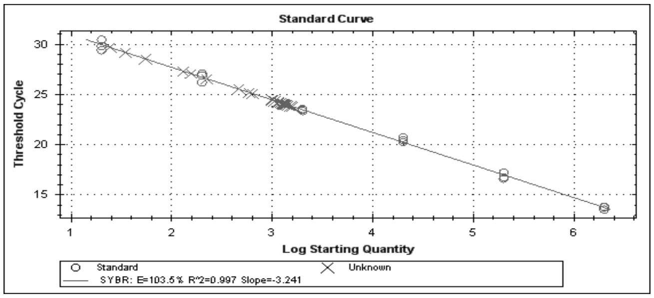
S7.

A. ***Cre* standard**

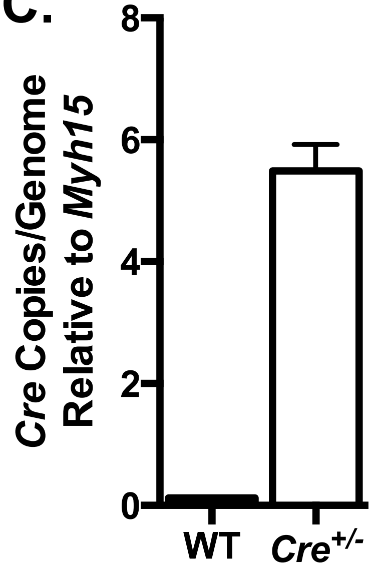


***Myh15* standard**

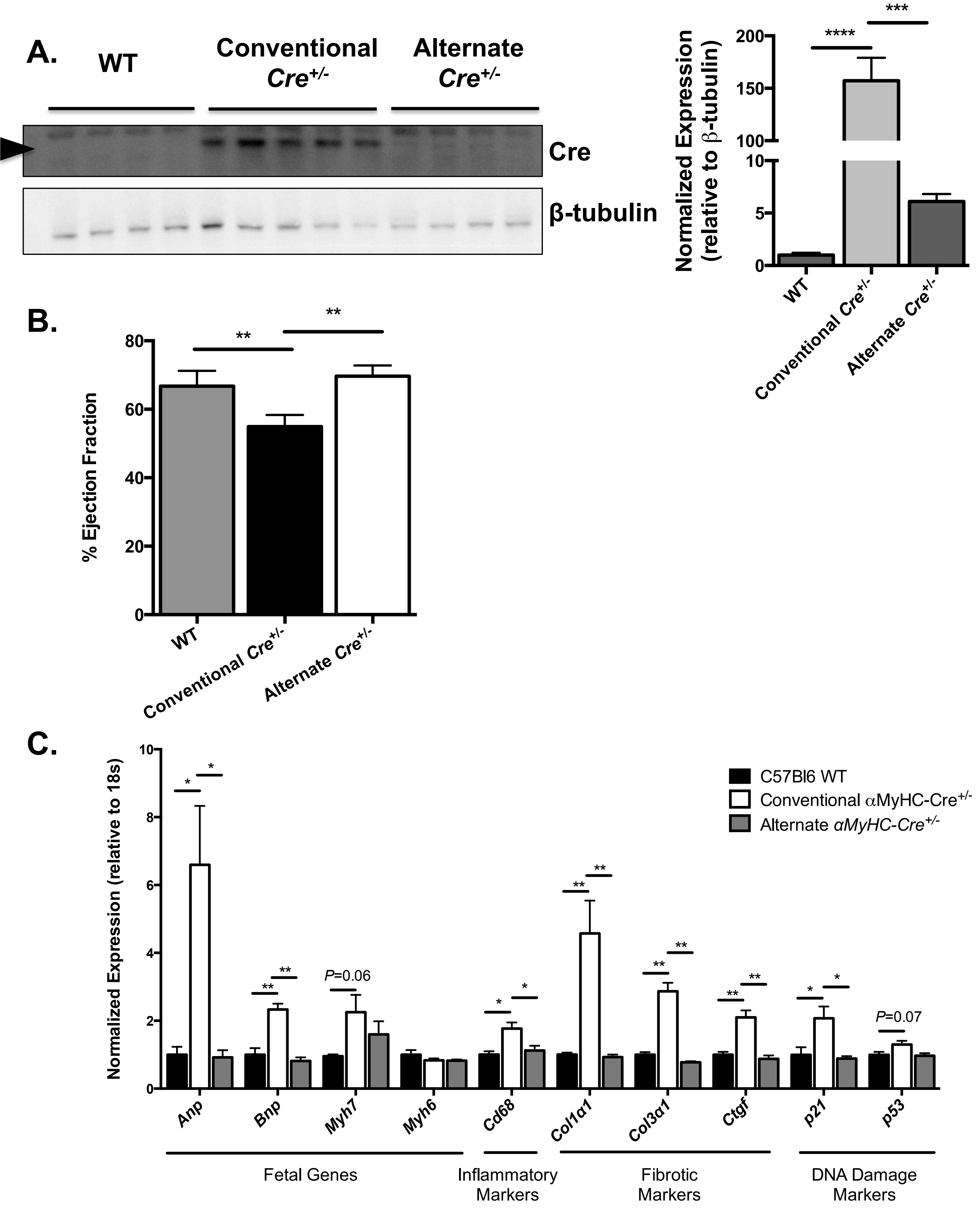
B.



C.



S8.



A.
WT
Conventional Cre+/-
Alternate Cre+/-
Cre
β-tubulin
Normalized Expression (relative to β-tubulin)
200
150
100
10
5
0
****
***
WT
Conventional Cre+/-
Alternate Cre+/-
B.
% Ejection Fraction
80
60
40
20
0
**
**
WT
Conventional Cre+/-
Alternate Cre+/-
C.
Normalized Expression (relative to 18s)
10
8
6
4
2
0
C57Bl6 WT
Conventional αMyHC-Cre+/-
Alternate αMyHC-Cre+/-
*
*
**
**
P=0.06
*
*
**
**
**
**
**
**
*
*
P=0.07
Anp
Bnp
Myh7
Myh6
Cd68
Col1α1
Col3α1
Ctgf
p21
p53
Fetal Genes
Inflammatory Markers
Fibrotic Markers
DNA Damage Markers

Column Numbers

Figure 1: 2

Figure 2: 2

Figure 3: 1.5

Figure 4: 2

Supplemental Figure 1: 1.5

Supplemental Figure 2: 1

Supplemental Figure 3: 2

Supplemental Figure 4: 1.5

Supplemental Figure 5: 1.5

Supplemental Figure 6: 1.5

Supplemental Figure 7: 1.5

Supplemental Figure 8: 2

Supplemental Table 1: 2

Supplemental Table 2: 1

Supplemental Table 3: 1.5

Supplemental Table 4: 1.5