Supplemental Figure Legends

Figure S1. Schematic of MYBPC3 transgenic models developed.

(**A**) Mice with a genetically modified knockout first allele of the murine Mybpc3 gene (Mybpc3^{tm1a}) were crossed with the Actb:Flp deleter line to remove the lacZ/Neo transcriptional disruption cassette. The resultant line, Mybpc3^{tm1c}, was then crossed with a Cmv:Cre line to create a germline MYBPC3 null. Alternatively, the Mybpc3^{tm1c} line was crossed with the Tg(Myh6-Cre/Esr1) line to create mice that allowed the conditional disruption of MYBPC3 protein expression after tamoxifen administration. (**B**) Mice with a genetically modified knockout first allele of the murine Mybpc3 gene (Mybpc3^{tm1a}) were crossed with a mouse line that expresses a tamoxifen inducible Flp protein (R26^{FlpoER}). The resulting mouse line allows for conditional reactivation of Mybpc3 protein expression after administration of tamoxifen by Flp recombinase mediated removal of the lacZ/Neo transcriptional disruption cassette.

Figure S2. Comparison of Mybpc3^{tm1a} and Mybpc3^{tm1d} mouse lines.

(**A**) Measurement of Nppb gene expression at postnatal day 25 (P25) from Ctl (n=3) and Null (n=3) hearts and postnatal day 90 (P90) control (Ctl) (n=4) and conditional MYBPC3 knockout (Null C) (n=4) hearts. BNP expression was normalized to Rpl32 expression. Fold changes are shown relative to respective controls. (**B**) Western blot of MYBPC3 protein levels in heart lysate from mice with a germline deletion of exon 3-5 of the Mybpc3 gene (Mybpc3^{tm1d}, tm1d) (*top*) and from mice with a knockout first allele of the Mybpc3 gene (Mybpc3^{tm1a}, tm1a) (*bottom*). (**C and D**) Post-natal day 2 (P2), 7 (P7), and 25 (P25) heart weights (HW) (**C**) and heart weight to body weight ratios (HW/BW) (**D**) of wild-type (+/+), Mybpc3 tm1a Null (tm1a/tm1a) and Mybpc3 tm1d Null (tm1d/tm1d) mouse lines. An average of 6 mice per age was used for each group. (**E**) Beta-galactosidase (β-gal) staining of heart section from P25 Null reactivation mice receiving different amounts of tamoxifen. Presence of β-gal identifies cardiomyoctyes lacking MYBPC3. (**F**) Quantification of β-gal negative cardiomyocytes. (**G**) Western blot of MYBPC3 demonstrating reactivation of MYBPC3 after 60 days following injection with tamoxifen at post-natal day 30 (Null R-P30). All results are shown as mean±s.e.m. Statistical analysis performed using an unpaired, 2-tailed Student's t test.

Figure S3. Cardiomyocyte nuclei labeling of control and MYBPC3 null myocardial tissue

(**A**) Immunofluorescence staining of phospho histone H3 (pH H3) (red) in control (Ctl) and MYBPC3 null (Null) hearts at postnatal day 2 (P2), 7 (P7), and 25 (P25). Cardiomyocytes were identified with sarcomeric α-actinin (green) with nuclei labeled with DAPI (blue). Scale bars, 100 μm. (**B**) Ki67 (red) staining in P7 hearts identifying Ki67-positive cardiomyocyte nuclei (arrow head) and Ki67-positive non-cardiomyocyte nuclei (arrow). Cardiomyocytes were identified with sarcomeric α-actinin (green) with nuclei labeled with DAPI (blue). Scale bars,

100 μm. (**C**) pH H3 (red) staining in P7 hearts identifying pH H3-positive cardiomyocyte nuclei (arrow head) and pH H3-positive non-cardiomyocyte nuclei (arrow). Cardiomyocytes were identified with sarcomeric α-actinin (green) with nuclei labeled with DAPI (blue). Scale bars, 100 μm. (**D**) Cardiomyocyte-specific pericentriolar material 1 protein (PCM-1) (red) staining in P25 hearts identifying cardiomyocyte PCM-1-positive nuclei (arrow head) and non-cardiomyocyte nuclei (arrow). Cardiomyocytes were identified with sarcomeric α-actinin (green) with nuclei labeled with DAPI (blue). Scale bars, 100 μm. (**E**) Wheat-germ agglutinin (green) and PCM-1 (red) staining in P25 hearts identifying cardiomyocyte PCM-1-positive nuclei (arrow head) and non-cardiomyocyte nuclei (arrow). Nuclei are labeled with DAPI (blue). Scale bars, 100 μm. (**F**) Isolectin B4 (red) and PCM-1 (green) staining in P25 hearts identifying cardiomyocyte PCM-1-positive nuclei (arrow head) and endothelial cells (arrow). Nuclei are labeled with DAPI (blue). Scale bars, 100 μm. (**G**) Ki67 (red) and PCM-1 (green) staining in P7 hearts identifying cardiomyocyte nuclei positive for Ki67 (arrow head). Nuclei are labeled with DAPI (blue). Scale bars, 100 μm. (**H**) Quantification of PCM-1 and Ki67 positive nuclei in Ctl (n=3) and Null (n=3) hearts. A minimum of 500 PCM-1 positive nuclei per heart were counted. (**I**) Aurora B (red) and PCM-1 (green) staining in P7 hearts identifying Aurora B in the cleavage furrow of cardiomyocytes. Nuclei are labeled with DAPI (blue). Scale bars, 100 μm. (**J**) Quantification of PCM-1 and Aurora B cleavage furrow positive nuclei in Ctl (n=3) and Null (n=3) hearts. A minimum of 500 PCM-1 positive nuclei per sample were counted. All results are shown as mean±s.e.m. Statistical analysis performed using an unpaired, 2-tailed Student's t test.

Figure S4. Measurement of cardiomyocyte cell cycle activity in adult mice after condition deletion of MYBPC3.

(**A**) Immunofluorescence staining of Ki67 (red) in postnatal day 90 (P90) Ctl mice and P90 mice that had conditional disruption of MYBPC3 protein expression at post-natal day 30 (Null C). Cardiomyocytes were identified with sarcomeric α-actinin (green) with nuclei labeled with DAPI (blue). Scale bars, 100 μm. (**B**) Quantification of Ki67 positive cardiomyocytes (CM) in Ctl (n=3) and Null C (n=4) hearts (minimum of 4,000 cells per sample). (**C**) Schematic illustration demonstrating how the total number of cardiomyocytes per left ventricular (LV) wall section was calculated. Cardiomyocyte borders were identified by wheat-germ agglutinin staining. Non-cardiomyocytes were excluded based on their smaller size. All results are shown as mean±s.e.m. Statistical analysis performed using an unpaired, 2-tailed Student's t test.

Figure S5. Evaluation of cardiomyocyte apoptosis and cardiomyocyte number after CDK4/6 inhibition from postnatal day 2 to 7.

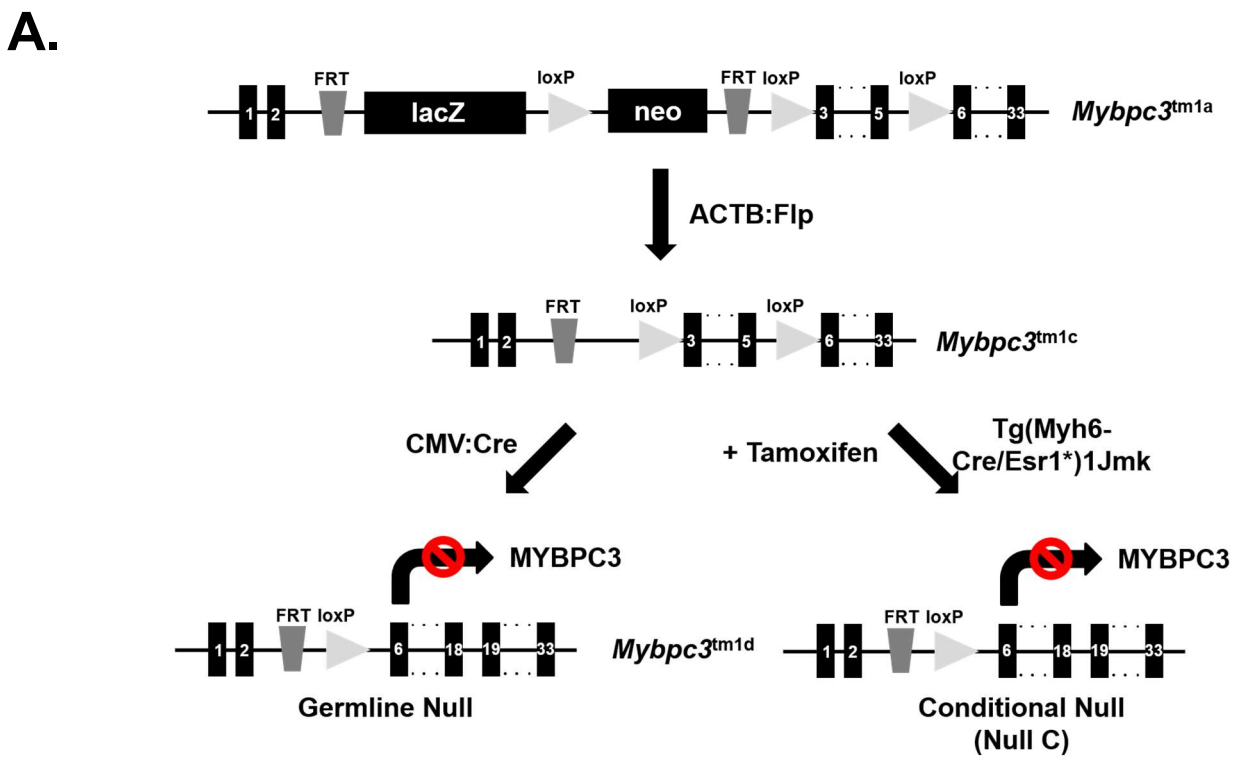
(**A**) Cardiomyocyte apoptosis was assessed using TUNEL staining of postnatal day 7 (P7) control (Ctl) and MYBPC3 null (Null) hearts from mice exposed to vehicle or 150 mg/kg PD-0332991 from P2 until P7. TUNEL positive cardiomyocytes are identified (red, white

arrowhead) with nuclei labeled with DAPI (blue). Scale bars, 100 μm. (**B**) Quantification of TUNEL positive cardiomyocytes (CM) in Ctl (n=3), Ctl+150 mg/kg PD-0332991 (n=3), Null (n=3), and Null+150 mg/kg PD-0332991 (n=3) hearts sections. (**C**) Hearts from mice treated with 0 or 150 mg/kg PD-0332991 were stained for wheat-germ agglutinin to identify cardiomyocyte boarders. Scale bars, 100 μm. (**D**) Cardiomyocytes per field from 0 mg/kg (n=3) and 150 mg/kg (n=3) PD-0332991 treated MYBPC3 null mice at P7. (**E**) Heart weights of Ctl (n=10), Ctl+75 mg/kg PD-0332991 (n=10), Ctl+150 mg/kg PD-0332991 (n=7), Null (n=7), Null+75mg/kg PD-0332991 (n=5), and Null+150 mg/kg PD-0332991 (n=5) mice. (**F**) Body weights of Ctl (n=10), Ctl+75 m/kg PD-0332991 (n=10), Ctl+150 mg/kg PD-0332991 (n=7), Null (n=7), Null+75mg/kg PD-0332991 (n=5), and Null+150 mg/kg PD-0332991 (n=5) mice. All results are shown as mean±s.e.m. Statistical analysis performed using an unpaired, 2-tailed Student's t test.

Figure S6. Measurement of cardiac structure in MYBPC3 Null mice exposed to CDK4/6 inhibitor.

(**A**) M-mode echocardiography images of postnatal day 7 (P7) Ctl and Null mice exposed to vehicle or 150 mg/kg PD-0332991 from P2 to P7. (**B-D**) (**B**) interventricular septal thickness at end diastole (IVSd), (**C**) left ventricular posterior wall thickness at end diastole (LVPWd), and (**D**) left ventricular internal diameter at end diastole (LVIDd) of Ctl (n=7), Ctl+150 mg/kg PD-0332991 (n=4), Null (n=8), and Null+150 mg/kg PD-0332991 (n=6) mice. All results are shown as mean±s.e.m. Statistical analysis performed using an unpaired, 2-tailed Student's t test.

Figure S1


A.
FRT
loxP
lacZ
neo
FRT loxP
loxP
1
2
3
5
6
33
Mybpc3tm1a
ACTB:Flp
FRT
loxP
loxP
Mybpc3tm1c
CMV:Cre
+ Tamoxifen
Tg(Myh6-Cre/Esr1*)1Jmk
MYBPC3
FRT loxP
18
19
Mybpc3tm1d
Germline Null
Conditional Null
(Null C)

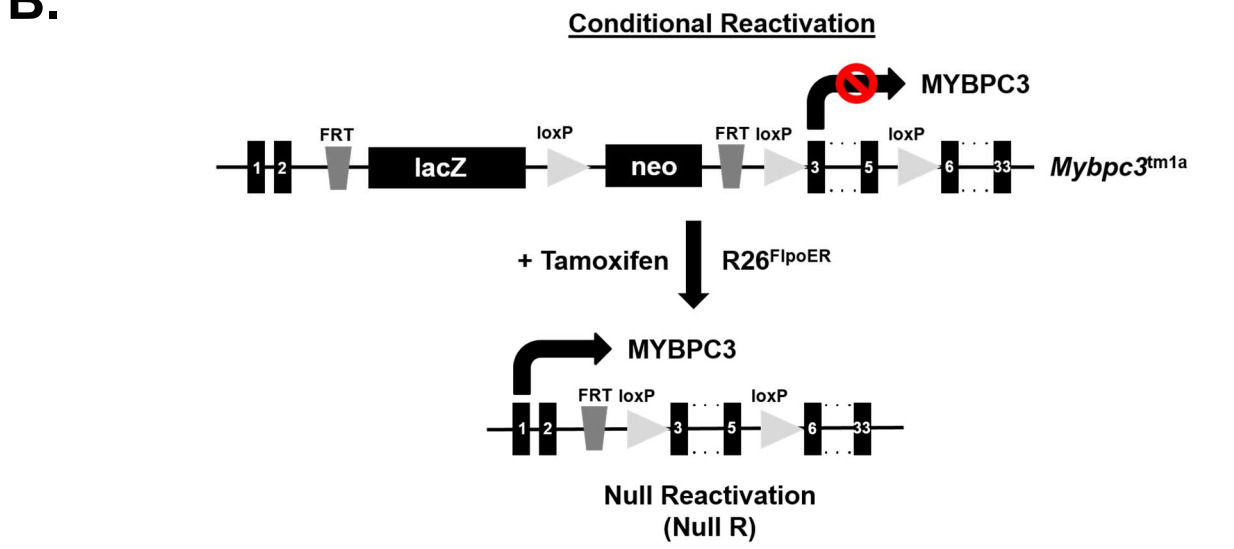

B.
Conditional Reactivation
MYBPC3
FRT
loxP
lacZ
neo
FRT loxP
loxP
1
2
3
5
6
33
Mybpc3tm1a
+ Tamoxifen
R26FlpoER
MYBPC3
FRT loxP
loxP
Null Reactivation
(Null R)

Figure S2

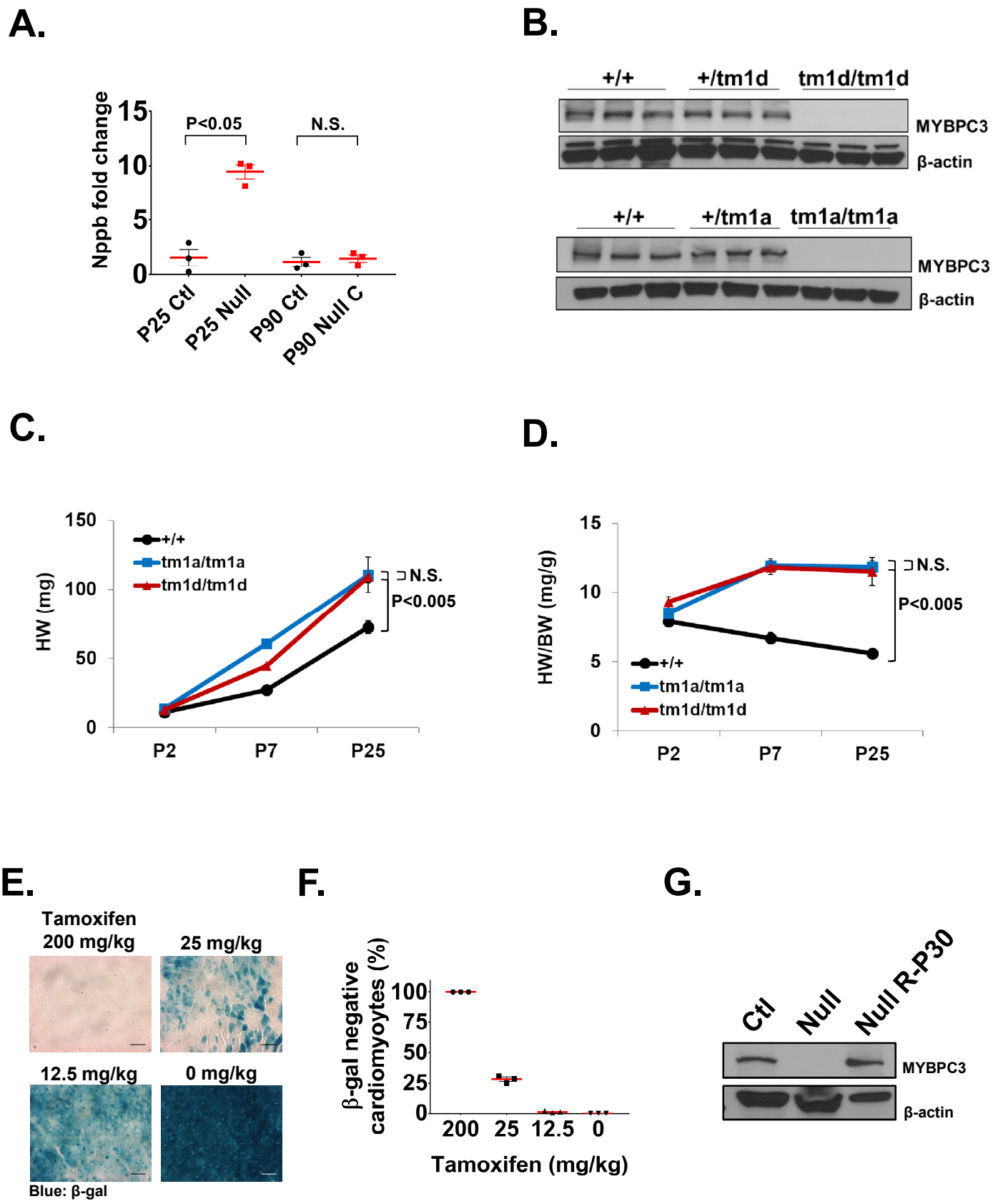

A.
Nppb fold change
15
10
5
0
P<0.05
N.S.
P25 Ctl
P25 Null
P90 Ctl
P90 Null C
B.
+/+
+/tm1d
tm1d/tm1d
MYBPC3
β-actin
+/+
+/tm1a
tm1a/tm1a
MYBPC3
β-actin
C.
HW (mg)
150
100
50
0
+/+
tm1a/tm1a
tm1d/tm1d
N.S.
P<0.005
P2
P7
P25
D.
HW/BW (mg/g)
15
10
5
0
N.S.
P<0.005
+/+
tm1a/tm1a
tm1d/tm1d
P2
P7
P25
E.
Tamoxifen
200 mg/kg
25 mg/kg
12.5 mg/kg
0 mg/kg
Blue: β-gal
F.
β-gal negative cardiomyoytes (%)
100
75
50
25
0
200
25
12.5
0
Tamoxifen (mg/kg)
G.
Ctl
Null
Null R-P30
MYBPC3
β-actin

Figure S3

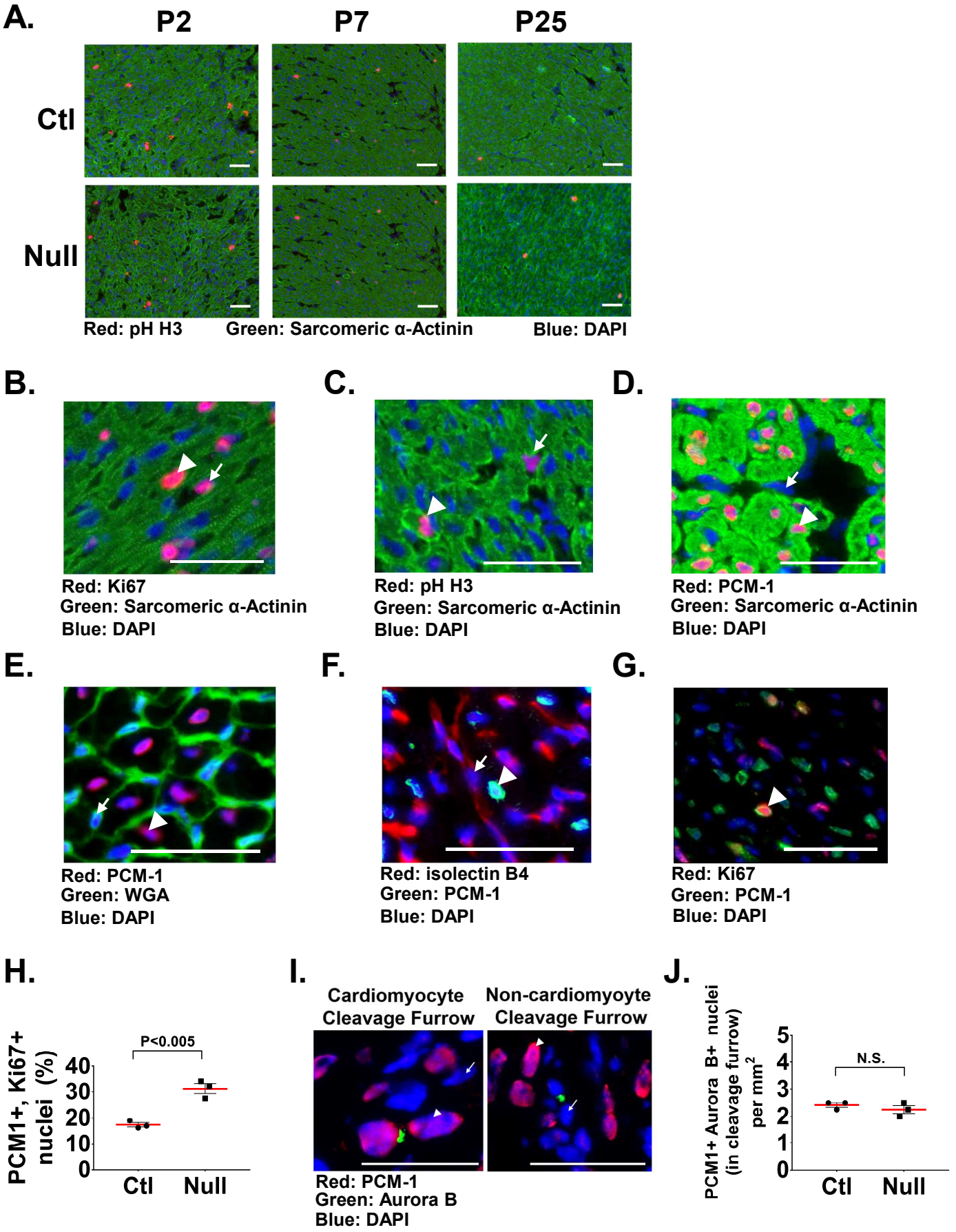
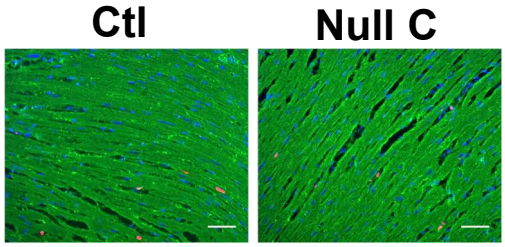

A.
P2
P7
P25
Ctl
Null
Red: pH H3
Green: Sarcomeric α-Actinin
Blue: DAPI
B.
Red: Ki67
Green: Sarcomeric α-Actinin
Blue: DAPI
C.
Red: pH H3
Green: Sarcomeric α-Actinin
Blue: DAPI
D.
Red: PCM-1
Green: Sarcomeric α-Actinin
Blue: DAPI
E.
Red: PCM-1
Green: WGA
Blue: DAPI
F.
Red: isolectin B4
Green: PCM-1
Blue: DAPI
G.
Red: Ki67
Green: PCM-1
Blue: DAPI
H.
PCM1+, Ki67+ nuclei (%)
P<0.005
40
30
20
10
0
Ctl
Null
I.
Cardiomyocyte Cleavage Furrow
Non-cardiomyoyte Cleavage Furrow
Red: PCM-1
Green: Aurora B
Blue: DAPI
J.
PCM1+ Aurora B+ nuclei (in cleavage furrow) per mm2
N.S.
5
4
3
2
1
0
Ctl
Null

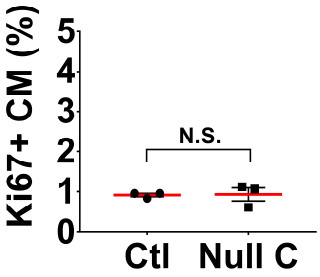
Figure S4

A.

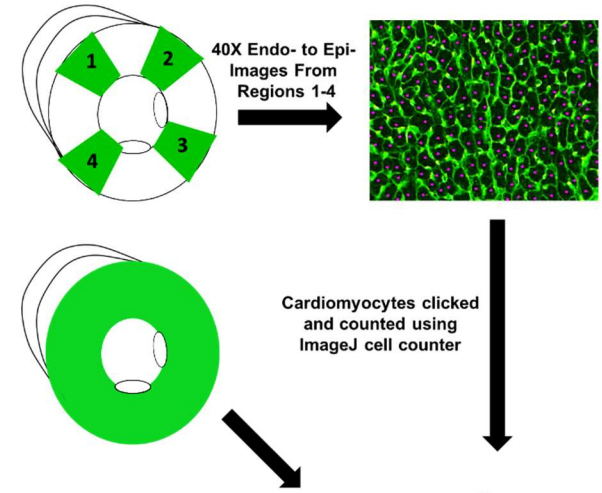


Red: Ki67
Green: Sarcomeric α-Actinin
Blue: DAPI

B.



C.



$$Total\ cardiomyocytes = total\ LV\ area\ (mm^2) * \frac{x\ cardiomyocytes}{image\ area\ (mm^2)}$$

Figure S5

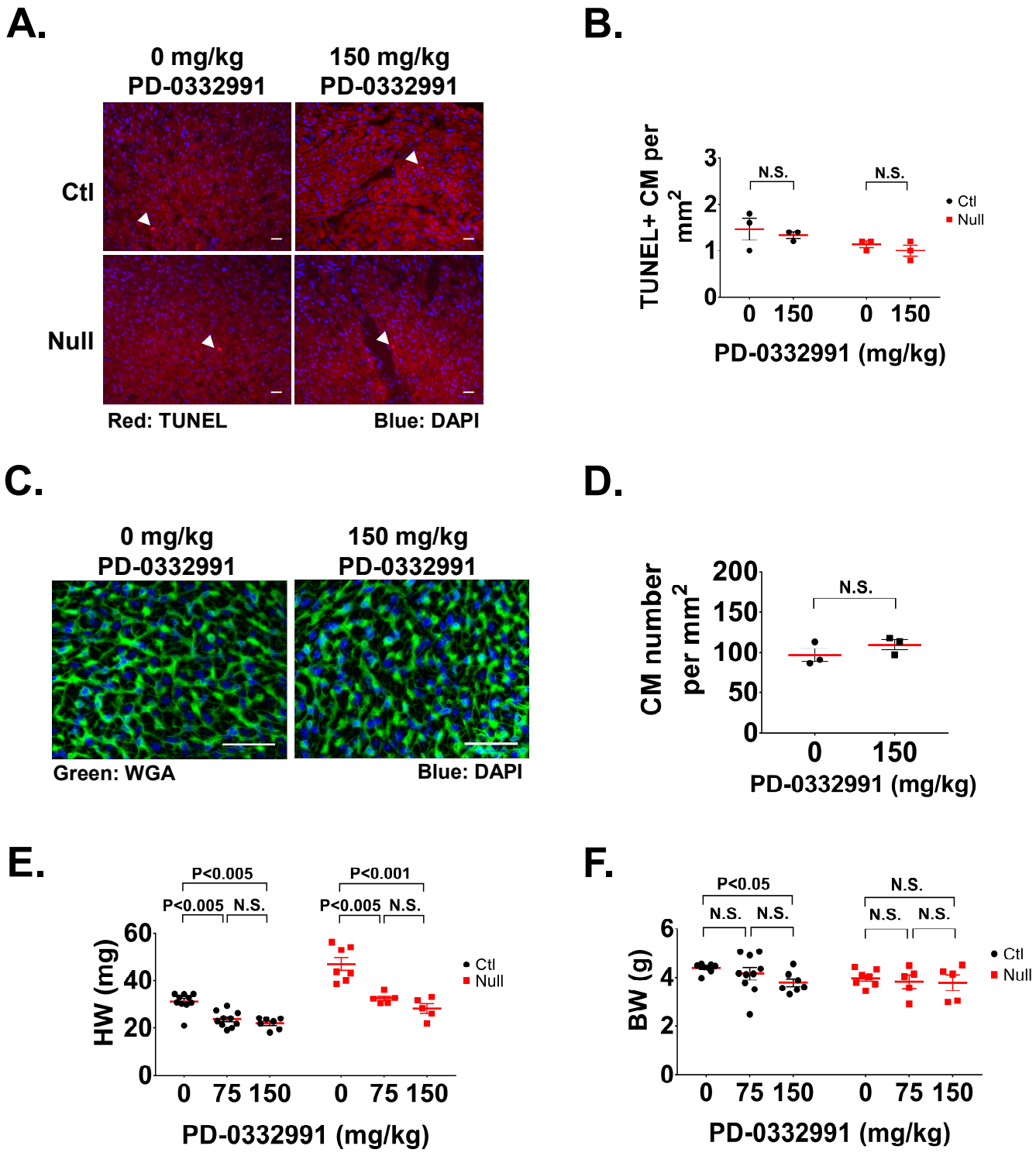
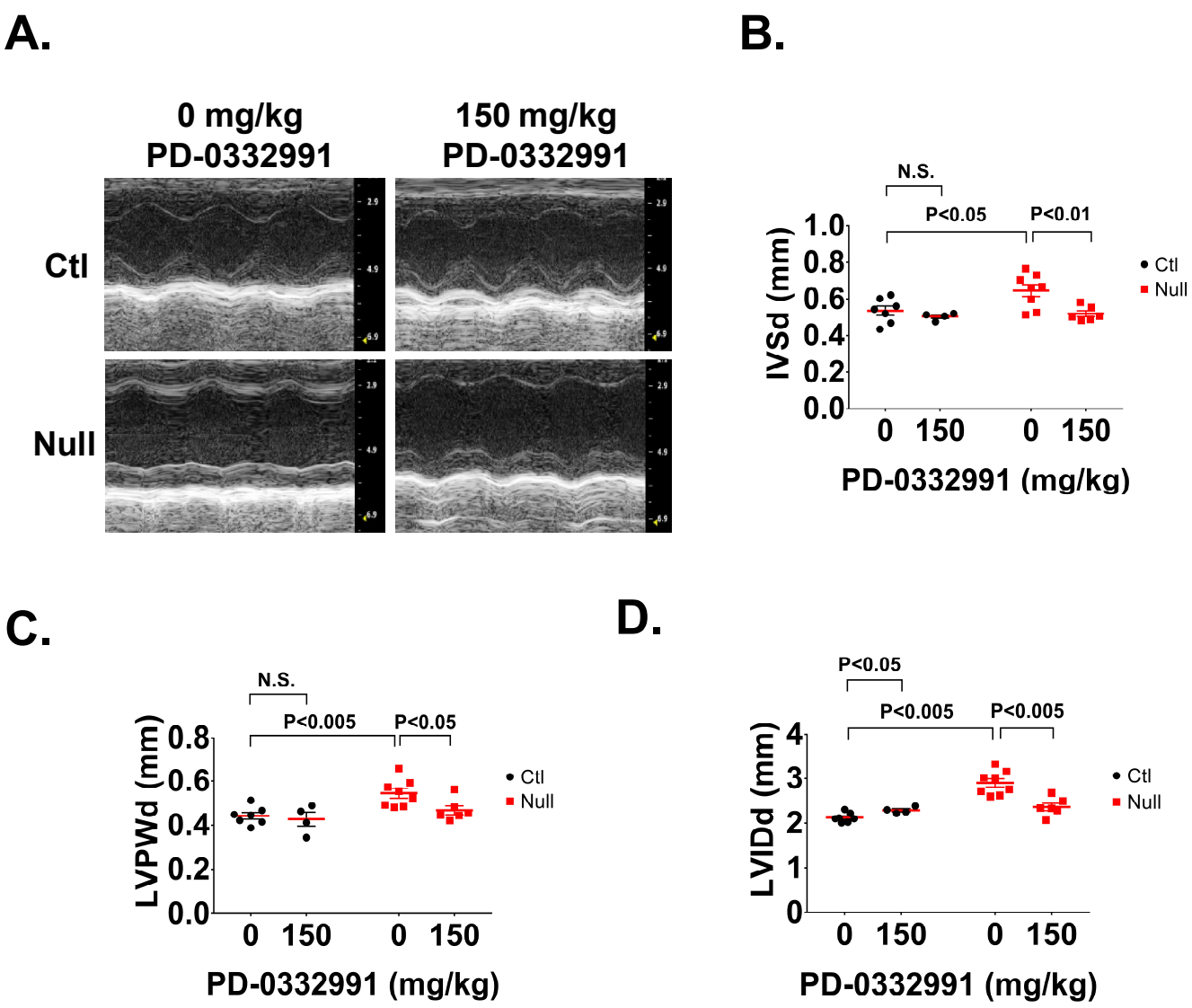

A.
0 mg/kg PD-0332991
150 mg/kg PD-0332991
Ctl
Null
Red: TUNEL
Blue: DAPI
B.
TUNEL+ CM per mm2
N.S.
N.S.
Ctl
Null
0 150 0 150
PD-0332991 (mg/kg)
C.
0 mg/kg PD-0332991
150 mg/kg PD-0332991
Green: WGA
Blue: DAPI
D.
CM number per mm2
N.S.
0 150
PD-0332991 (mg/kg)
E.
P<0.005
P<0.005 N.S.
P<0.001
P<0.005 N.S.
HW (mg)
Ctl
Null
0 75 150 0 75 150
PD-0332991 (mg/kg)
F.
P<0.05
N.S. N.S.
N.S.
N.S. N.S.
BW (g)
Ctl
Null
0 75 150 0 75 150
PD-0332991 (mg/kg)

Figure S6


A.
0 mg/kg
PD-0332991
150 mg/kg
PD-0332991
Ctl
Null
B.
N.S.
P<0.05
P<0.01
IVSd (mm)
1.0
0.8
0.6
0.4
0.2
0.0
0
150
0
150
PD-0332991 (mg/kg)
Ctl
Null
C.
N.S.
P<0.005
P<0.05
LVPWd (mm)
0.8
0.6
0.4
0.2
0.0
0
150
0
150
PD-0332991 (mg/kg)
Ctl
Null
D.
P<0.05
P<0.005
P<0.005
LVIDd (mm)
4
3
2
1
0
0
150
0
150
PD-0332991 (mg/kg)
Ctl
Null

Supplemental Table 1. List of oligonucleotide primer sequences used for qRT-PCR.

Murine Gene	Forward Primer	Reverse Primer
Ccnd1	ACCTGGATGCTGGAGGTCT	CAGGCGGCTCTTCTTCAA
Ccnd2	CCACCTGGATGCTAGAGGTC	CCAAGAAACGGTCCAGGTAA
Ccne1	CGTCTTGAATTGGGGCAATA	AGCCAATCCAGAAGAACTGC
Ccne2	TTTAAGCTGGGCATGTTCAC	CCTCATCTGTGGTTCCAGGT
Ccna1	TGCTGGATTTCAACACAGTTTC	CCGTTACGTTAATCACATCTGAAC
Ccna2	CCTTAGGGAAATGGAGGTTAAA	TCTTCTCCCACCTCAACCAG
Ccnb1	GGCTGCTTCAGGAGACCAT	TGCAATAAACATGGCCGTTA
Ccnb2	CTCAAAAGCCGGAGAGGTG	CCTGAGAAGGATGGTAGTGC
Ccnb3	GTGCAAAAGAAACTGAGATAACCAT	AGACGTCTTTGGCATATATAGTGTT
Cdkn1a	GCAGACCAGCCTGACAGATT	CTGACCCACAGCAGAAGAGG
Nppb	GGTCCAGCAGAGACCTCAAA	AACAACTTCAGTGCGTTACAGC
Rpl32	CACCAGTCAGACCGATATGTGAAAA	TGTTGTCAATGCCTCTGGGTTT