SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE AND TABLE LEGENDS

Supplemental Figure I: Generation of a *Brd4* conditional allele (*Brd4^{flox}*).

(A) Targeting strategy to flank *Brd4* exon 3 (containing the canonical translation start site) with loxP sites. **(B-C)** Southern blotting using both 5′ (B; wild type band 17.6Kb, knock-in band 6.4Kb) and 3′ (C; wild type band 12.4Kb, knock-in band 7.2Kb) probes confirms appropriate targeting of mouse embryonic stem cells (images from two representative clones displayed). **(D)** Representative image of PCR genotyping assay used for *Brd4^{flox}* allele that resolves an 82 bp difference (representing the residual FRT scar and 5′ loxP site) between the wild type and knock-in allele.

Supplemental Figure II: Immunohistochemical analysis of BRD4 in *Brd4*-KO and control hearts.

(A-F) Sections stained with DAPI (blue), TNNT2 (green), and BRD4 (red) in vehicle (VEH; A-C) or tamoxifen treated (TAM; D-F) *Myh6-MCM; Brd4$^{flox/flox}$* mice at 10-weeks of age. **(G)** Quantification of percentage of TNNT2+ cells with BRD4+ nuclei. Mean ± 1 SD shown, n=4 sections quantified. **** represents $p<0.0001$. Scale bars = 20 μm.

Supplemental Figure III: Cleaved caspase 3 staining in *Brd4*-KO and control hearts.

(A,B) Sections from 10-week old *Myh6-MCM; Brd4$^{flox/flox}$* mice treated with vehicle (VEH; A) or tamoxifen (TAM; B) stained with cleaved caspase 3 (CC3; green) and TNNT2 (orange). Mean ± 1 SD shown, n=3 sections quantified. Scale bars = 100 μm. (**C**) Quantification of CC3 positive nuclei in TNNT2 positive cells in sections from 10-week old *Myh6-MCM; Brd4$^{flox/flox}$* mice treated with vehicle (VEH; A) or tamoxifen (TAM; B). n=3. Student's t-test was performed to assess significance.

Supplemental Figure IV: A 5-day regimen of 50 μg/g/day tamoxifen is not sufficient to effectively delete BRD4 in *Myh6-MCM; Brd4$^{flox/flox}$* cardiomyocytes.

(A) Immunoblot of isolated *Myh6-MCM* and *Myh6-MCM; Brd4$^{flox/flox}$* cardiomyocyte lysates from mice treated with 50 μg/g/day tamoxifen (TAM) for 5 days using BRD4 or vinculin

(loading control) antibodies. (**B**) Ejection fraction and **(C)** left ventricular end systolic volume (LVESV) of indicated mice treated with 50 µg/g/day tamoxifen (TAM) or vehicle (VEH) at indicated days after injection**.** Individual points and mean ± SD shown. Two-way ANOVA analysis was used to assess significance.

Supplemental Figure V: Gene expression changes following tamoxifen-mediated Cre nuclear localization in Cre-control adult cardiac myocytes.

(A) Table indicating naming convention and genotypes of various samples used in this study. **(B)** PCA plot of individual replicates of each genotype used in RNA-seq analyses. **(C-D)** Volcano plots of differential gene expression in Cre-control vs. Control at day 2 (C) and day 5 (D) with associated enriched terms from gene ontology analysis. Genes are assigned with specific colors following DE analysis: grey (not significant), green (Log2 FC<-1 or >+1), blue (adj. p<0.05) or red (Log2 FC<-1 or >+1 and adj. p<0.05).

Supplemental Figure VI: Electron micrographs of mitochondria in *Brd4*-KO and control hearts.

(A, B) Electron micrographs of cardiac tissue from 10-week old *Myh6-MCM; Brd4$^{flox/flox}$* mice treated with vehicle (VEH; A) or tamoxifen (TAM; B) highlighting the loss of normal mitochondrial morphology. Scale bars = 100 nm.

Supplemental Figure VII: Immunohistochemical analysis of BRD4 in *Tnnt2-Cre; Brd4$^{flox/flox}$* and control embryonic hearts.

(**A-F**) Immunohistochemical analysis of cross sections from *Brd4$^{flox/flox}$*, *Tnnt2-Cre; Brd4$^{flox/+}$*, and *Tnnt2-Cre; Brd4$^{flox/flox}$* embryonic hearts stained with DAPI (blue), TNNT2 (green), and BRD4 (red).

Supplemental Figure VIII: Assessment of H3K27Ac/BRD4/GATA4 enriched regions in proximity to dysregulated genes following BRD4 CM-deletion.

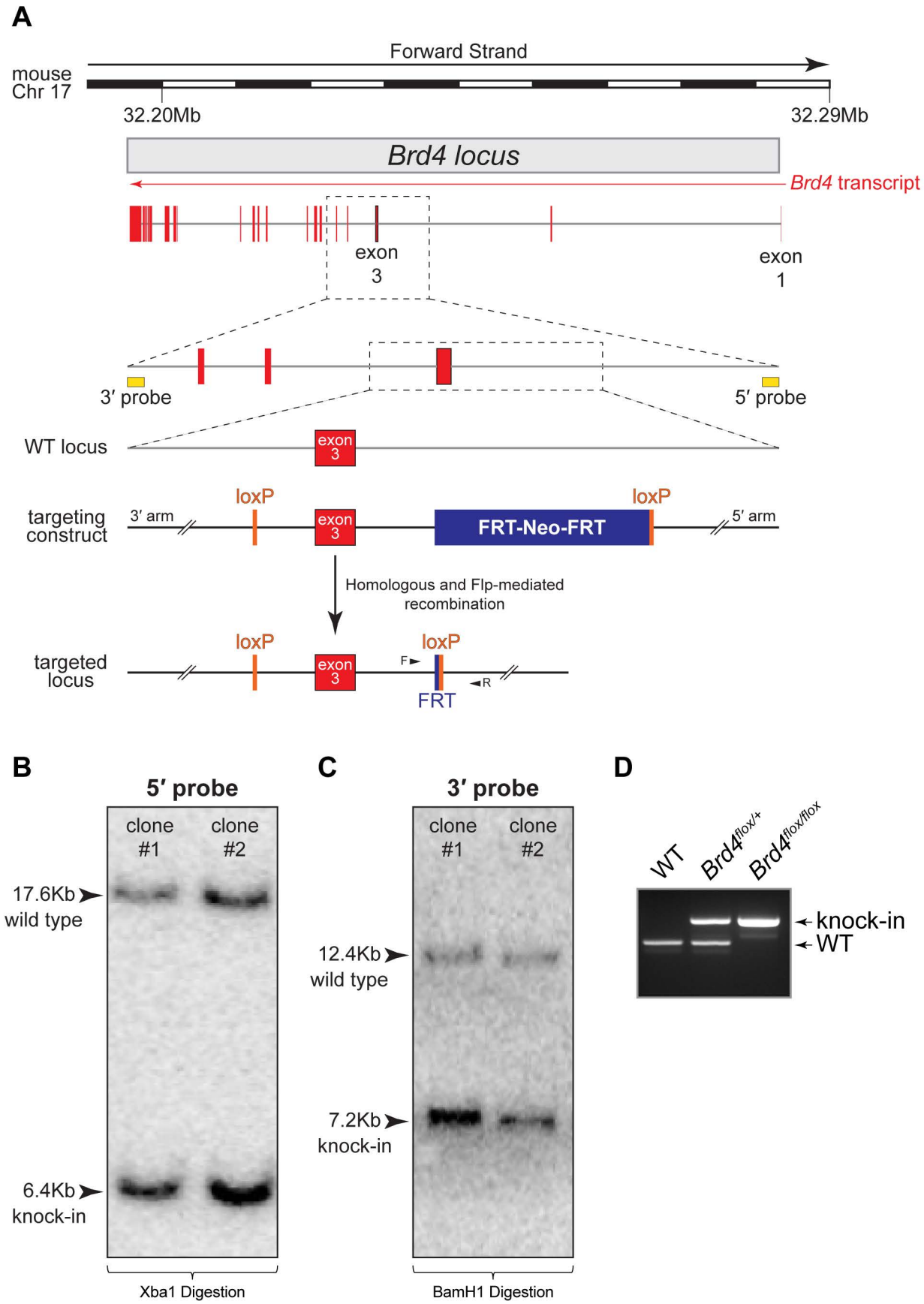
(**A-C**) Heatmaps showing enrichment of H3K27Ac, BRD4, and GATA4 occupancy from adult mouse hearts under basal homeostatic conditions. Unbiased K-means clustering identified H3K27Ac positive regions with highest density of BRD4 (n=18,348; B) and

GATA4 (n=24,608; C) occupancy. (**D**) Venn diagram depicting the regions that are enriched with: H3K27Ac-BRD4 (left), H3K27Ac-BRD4-GATA4 (center) and H3K27Ac-GATA4 (right). (**E**) Percentage of DE transcripts at Day 2 and Day 5 that have a H3K27Ac-BRD4-GATA4 enriched region within 1 kb to 25 kb from their TSS.

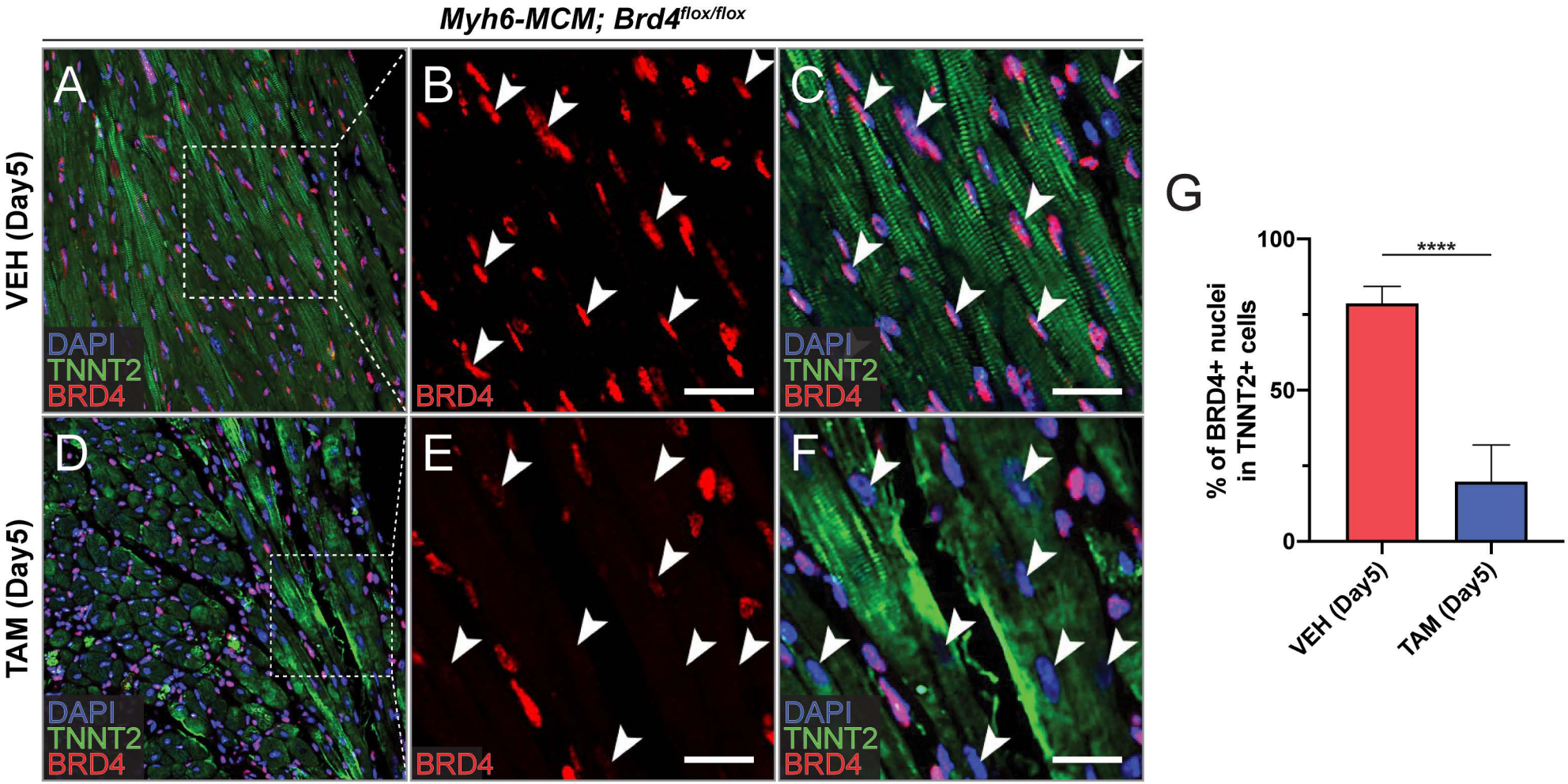
Supplemental Excel Tables I-V:

RNA-seq data at Day 2 or Day 5 in *Myh6-MCM; Brd4*$^{flox/flox}$ animals treated with tamoxifen (*Brd4*-KO) or vehicle (control) and RNA-seq data at Day 2 or Day 5 of Cre-control (*Myh6-MCM*) versus Control. RNA-seq data at E14.0 in *Tnnt2-Cre; Brd4*$^{flox/flox}$ animals versus *Tnnt2-Cre; Brd4*$^{flox/+}$.

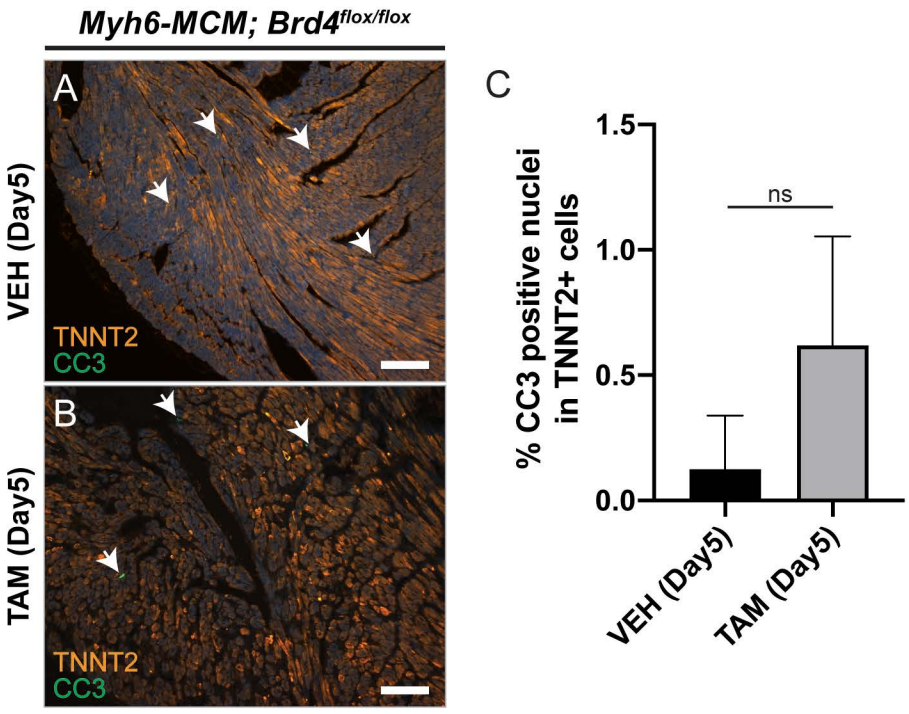
Supplemental Figure I



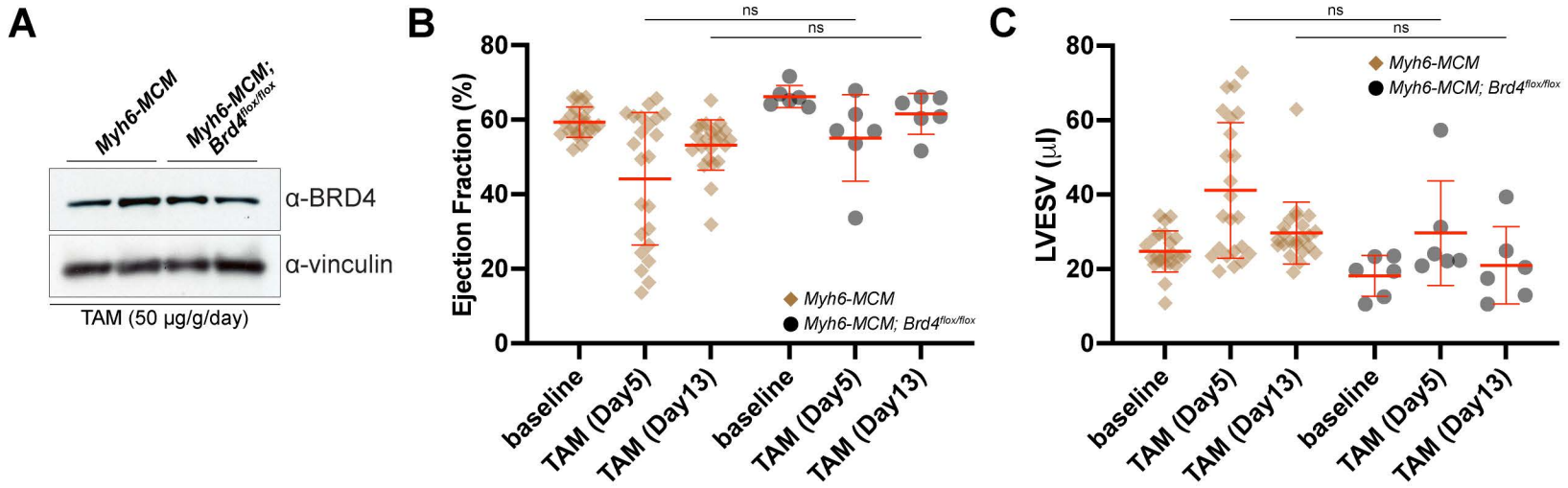
Supplemental Figure II



Supplemental Figure III



Supplemental Figure IV



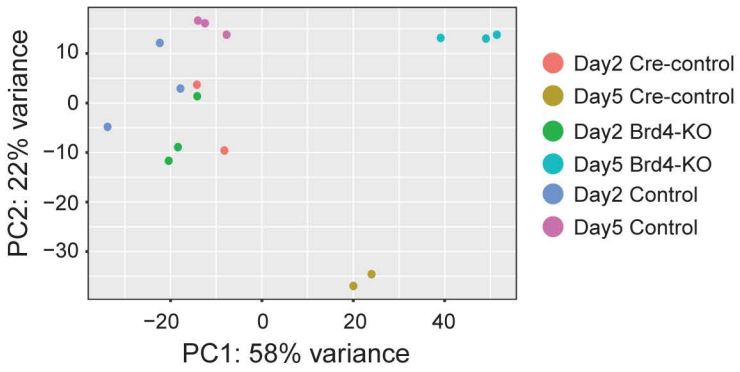
Supplemental Figure V

A

Mouse/Sample nomenclature for RNA/ATAC sequencing

Sample name	Genotype	Treatment
Cre-control	*Myh6-MCM*	Tamoxifen
Control	*Myh6-MCM; Brd4$^{flox/flox}$*	Vehicle
Brd4-KO	*Myh6-MCM; Brd4$^{flox/flox}$*	Tamoxifen

B



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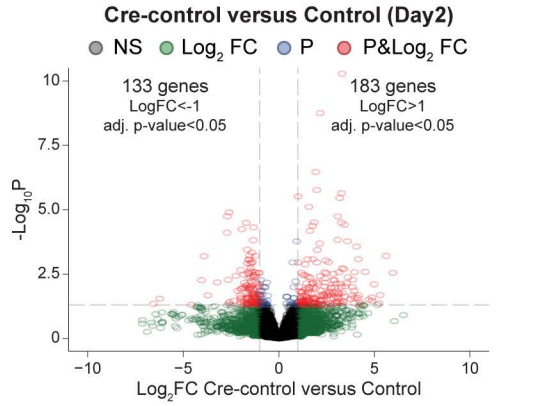


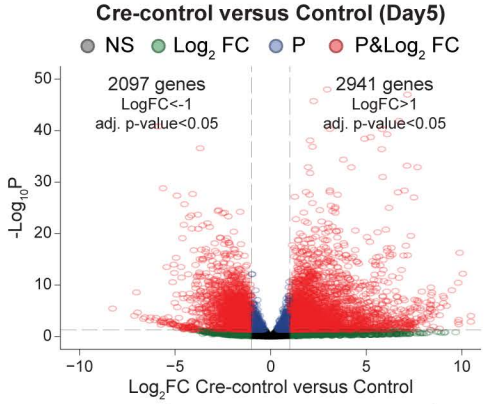
Down-regulated Cre-control

Biological process	p-val
Cardiac muscle cell development	$4.4e^{-3}$
Reg. of cardiac muscle cell contraction	$4.2e^{-2}$
Reg. of cardiac muscle cell action potential	$4.6e^{-2}$

Up-regulated Cre-control

Biological process	p-val
Pos. reg. of cellular metabolic process	$8.5e^{-4}$
Negative regulation of neuron death	*NS*
Inorganic cation transmem. transport	*NS*

D



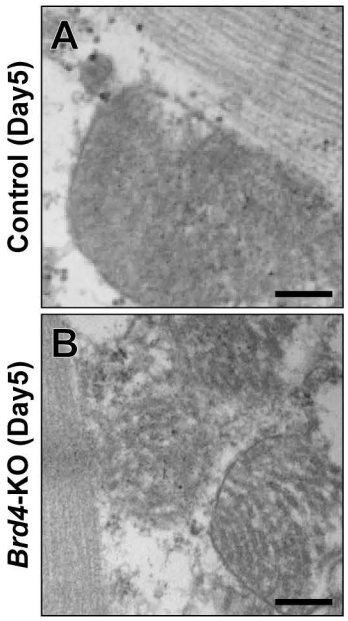
Down-regulated Cre-control

Biological process	p-val
Cellular Respiration	$3.1e^{-3}$
Reg. of heart contraction	$1.5e^{-2}$
Cardiac muscle cell membrane repol.	$1.6e^{-2}$

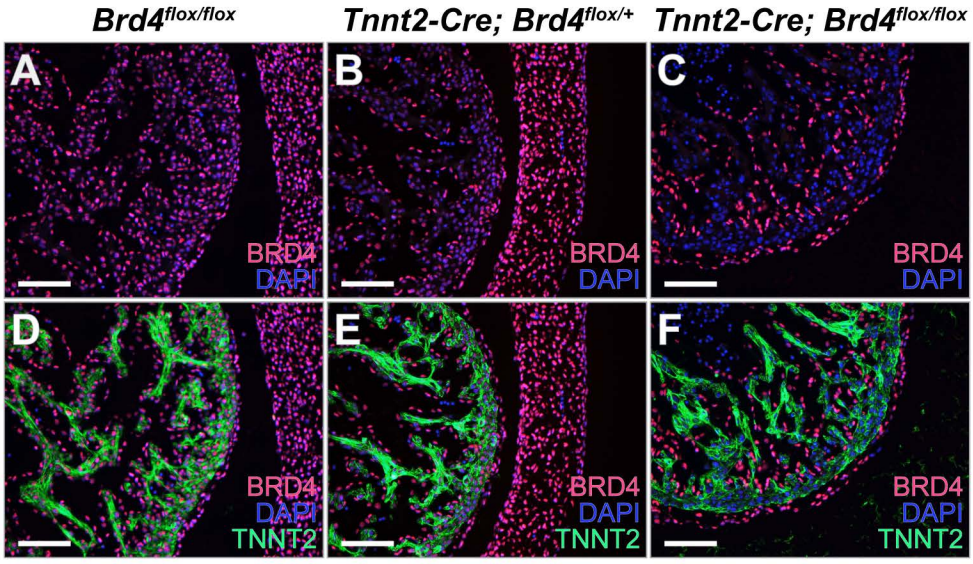
Up-regulated Cre-control

Biological process	p-val
rRNA processing	$3.3e^{-18}$
Ribosome biogenesis	$4.5e^{-17}$
Protein targeting to ER	$4.5e^{-16}$

Supplemental Figure VI



Supplemental Figure VII



Supplemental Figure VIII