Supplemental Figure 1

IP: p110-α

myr-Akt  NTG

IP: p110-γ

myr-Akt  NTG

IP: p110-β

myr-Akt - + - + - + - +

PIP

Origin

PIP

Origin
Supplemental Figure 2

PI3K activity (fold over NTG control)

- NTG
- myr-Akt

Cont  I-20  I/R5  I/R40

*  **
Supplemental Figure 3

(a) Male and Female dn-Akt genotypes:
- **dn-Akt**
  - Male: 
    - -
    - +/-
    - +/+  
  - Female: 
    - -
    - +/-
    - +/+  
- Total Akt

(b) Baseline and Insulin treatment:
- **dn-Akt**
  - Baseline: 
    - -
    - +/-
    - +/+  
  - Insulin: 
    - -
    - +/-
    - +/+  
- Akt-kinase assay

(c) Baseline:
- **dn-Akt**
  - Baseline: 
    - -
    - +/-
    - +/+  
- p-GSK3 (Ser 9)
- Total GSK3
Supplemental Figure 5

Control | Wortmannin | Rapamycin

IRS-1

Insulin (20h)
Wortmannin

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<th>Wortmannin</th>
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<th>High</th>
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<td>Total GSK3</td>
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Supplemental Figure 6
Supplemental Figure Legends:

Supplemental Figure 1. Activity of PI3K isoforms in myr-Akt hearts. Cardiac lysates were immunoprecipitated with indicated antibodies, and immune-complexes were assayed by TLC. PI3K activity is comparable at baseline for all isoforms in myr-Akt-TG and NTG mice.

Supplemental Figure 2. The time course of PI3K activation during IRI. Quantitation of mean spot density from cumulative data in Figure 2a are shown. Each quantitation was normalized to the control-perfused NTG. *p<0.001 versus control-perfused NTG. **p<0.01 versus NTG after ischemia-reperfusion.

Supplemental Figure 3. Overexpression of dn-Akt in the heart. (a) Total Akt expression from cardiac tissue lysates of NTG (-), hemizygous (+/-) and homozygous (+/+) dn-Akt-TG mice is shown. (b) Total cardiac tissue lysates with or without insulin stimulation were analyzed for Akt kinase activity with GSK3α/β as substrate. (c) Representative immunoblot for phospho- and total GSK3 from whole heart tissue lysates.

Supplemental Figure 4. GSK3β inhibition does not restore function to myr-Akt-TG hearts during IRI. LVDP profiles during 40 min reperfusion after 20 min ischemia in NTG (closed squares; n=11), NTG with lithium (closed rhombuses; n=4), myr-Akt (open circles; n=11), myr-Akt with lithium (open rhombuses; n=5) and myr-Akt with SB216763 (open triangles; n=4). *p<0.001, **p<0.03 versus NTG alone.

Supplemental Figure 5. Wortmannin but not rapamycin block insulin-induced reduction of IRS-1 in NRCM. Cardiomyocyte lysates with or without prolonged (20h)
insulin stimulation were immunoblotted for IRS-1. Representative immunoblot from one of three independent experiments.

**Supplemental Figure 6. The effects of wortmannin on PI3K dependent pathways.**

After ischemia-reperfusion in the absence or presence of either low dose (100 nM, for 10 min before ischemia) or high dose (200 nM, for 10 min before ischemia and during entire 40-min reperfusion period) of wortmannin, tissue lysates were immunoblotted with the indicated antibodies.