a. iTAC mouse
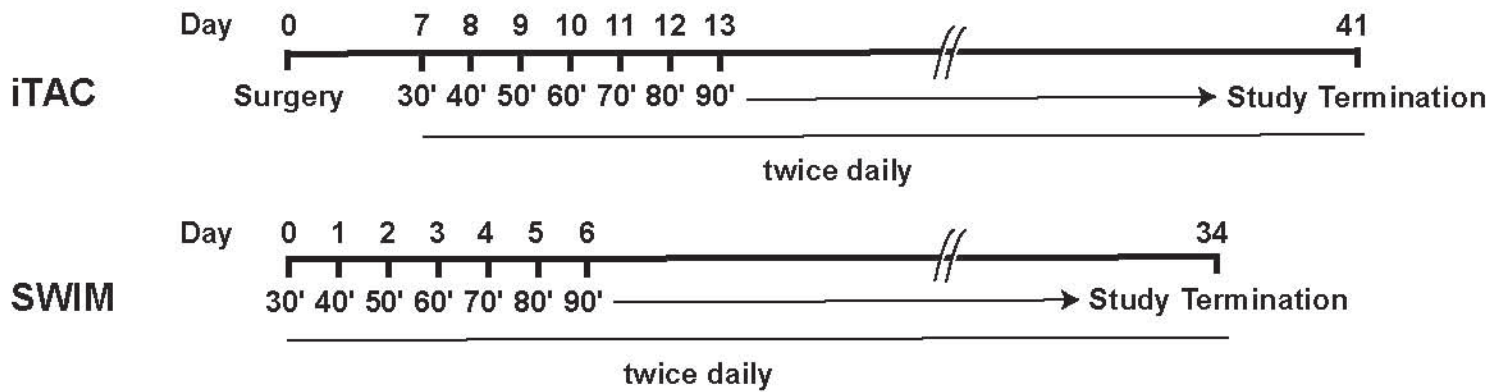

b. Experimental Design I (4 WEEKS)

iTAC

Day 0   7   8   9   10   11   12   13   41

Surgery   30' 40' 50' 60' 70' 80' 90' ⟶ Study Termination

twice daily

SWIM

Day 0   1   2   3   4   5   6   34

30' 40' 50' 60' 70' 80' 90' ⟶ Study Termination

twice daily

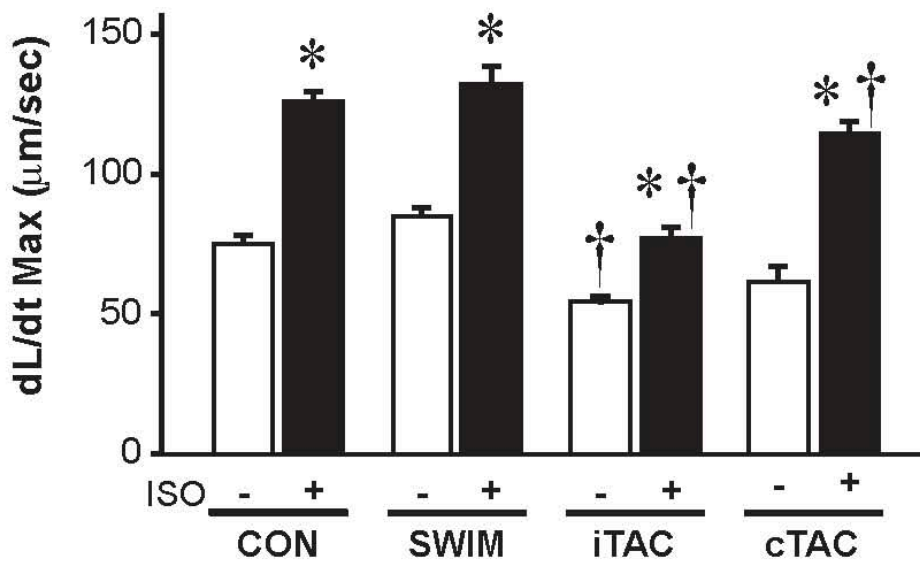# c. Experimental Design II (1 WEEK)

iTAC

Day 0   7   8   9   16

Surgery   30' 60' 90' ⟶ Study Termination

twice daily

SWIM

Day 0   1   2   9

30' 60' 90' ⟶ Study Termination

twice daily
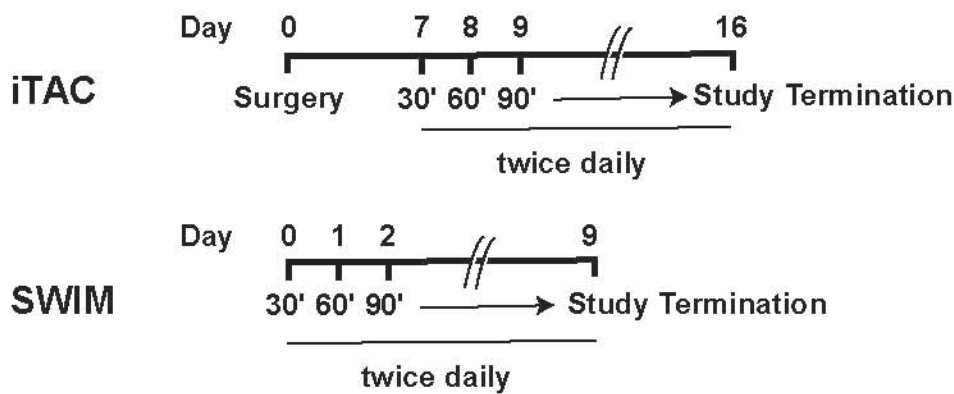
d. Experimental Design III (1 WEEK + metoprolol)

iTAC$_{meto}$

Day 0   7   14 15 16   22

Surgery   30' 60' 90' ⟶ Study Termination

twice daily

Metoprolol treatment
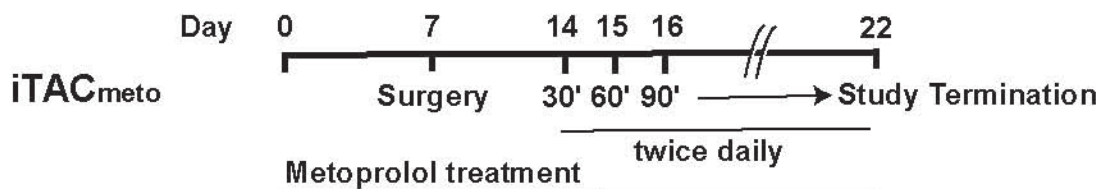
a

b

Abnormal relaxation in cells isolated from hearts exposed to intermittent pressure overload (iTAC) for 4 weeks

(a) dL/dt max in freshly isolated cardiomyocytes from CON, SWIM, iTAC and cTAC hearts under basal conditions (white bars) and following stimulation with isoproterenol (ISO) 1μM. In cells from iTAC hearts, dL/dt max was significantly reduced vs. CON under basal conditions (72.5 ± 2.4 % of basal CON) and following ISO stimulation (iTAC: 1.1 ± 0.07 fold over basal; CON: 1.7 ± 0.07, *p< 0.01 vs. respective basal; †p< 0.01 vs. correspondent CON or SWIM).
**(b)** Immunoblotting of cardiac cytosolic lysates for SERCA2a ATPase, total phospholamban (PLB) and phospho-phospholamban (pPLB-Ser 16).
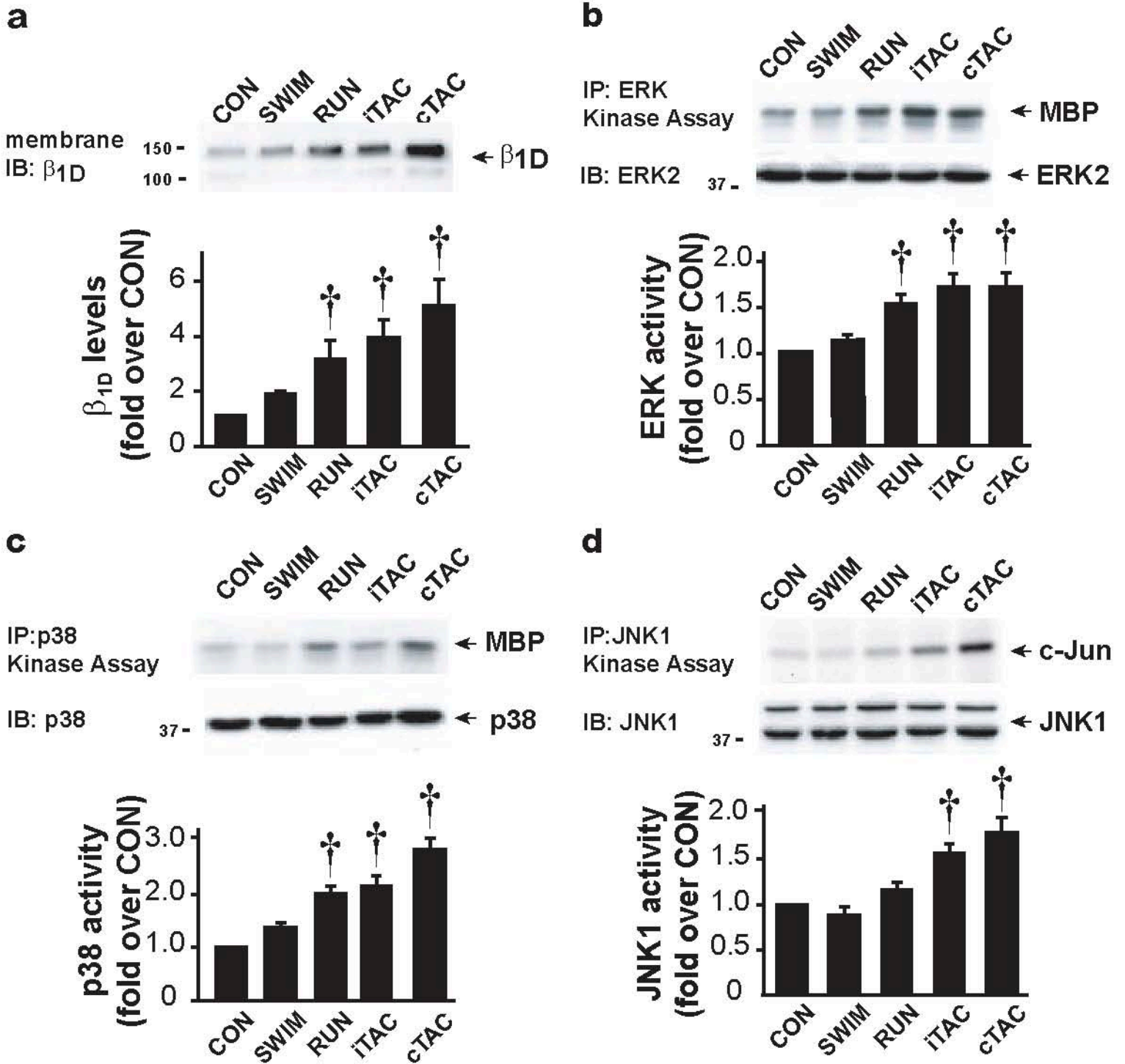
Physiological and pathological mechanical loads are differently sensed by the heart

(a) Representative immunoblotting showing integrin β_{1D} levels in membranes from mouse hearts of the different groups; densitometric analysis of at least five different experiments is shown in the lower panel ($\dagger$p<0.01 vs. CON or SWIM). (b-d top panels) MAPKs activation was determined for ERK and p38 by the ability to in vitro phosphorylate myelin binding protein (MBP); for JNK1, recombinant GST-cJun (cJun) was used as substrate. Immunoblotting was also carried out to evaluate total protein levels of each kinase (b-d, middle panels). Densitometric evaluation of the kinase assays in at least five experiments is shown for each kinase (b-d, lower panels, $\dagger$p<0.01 vs. CON or SWIM).
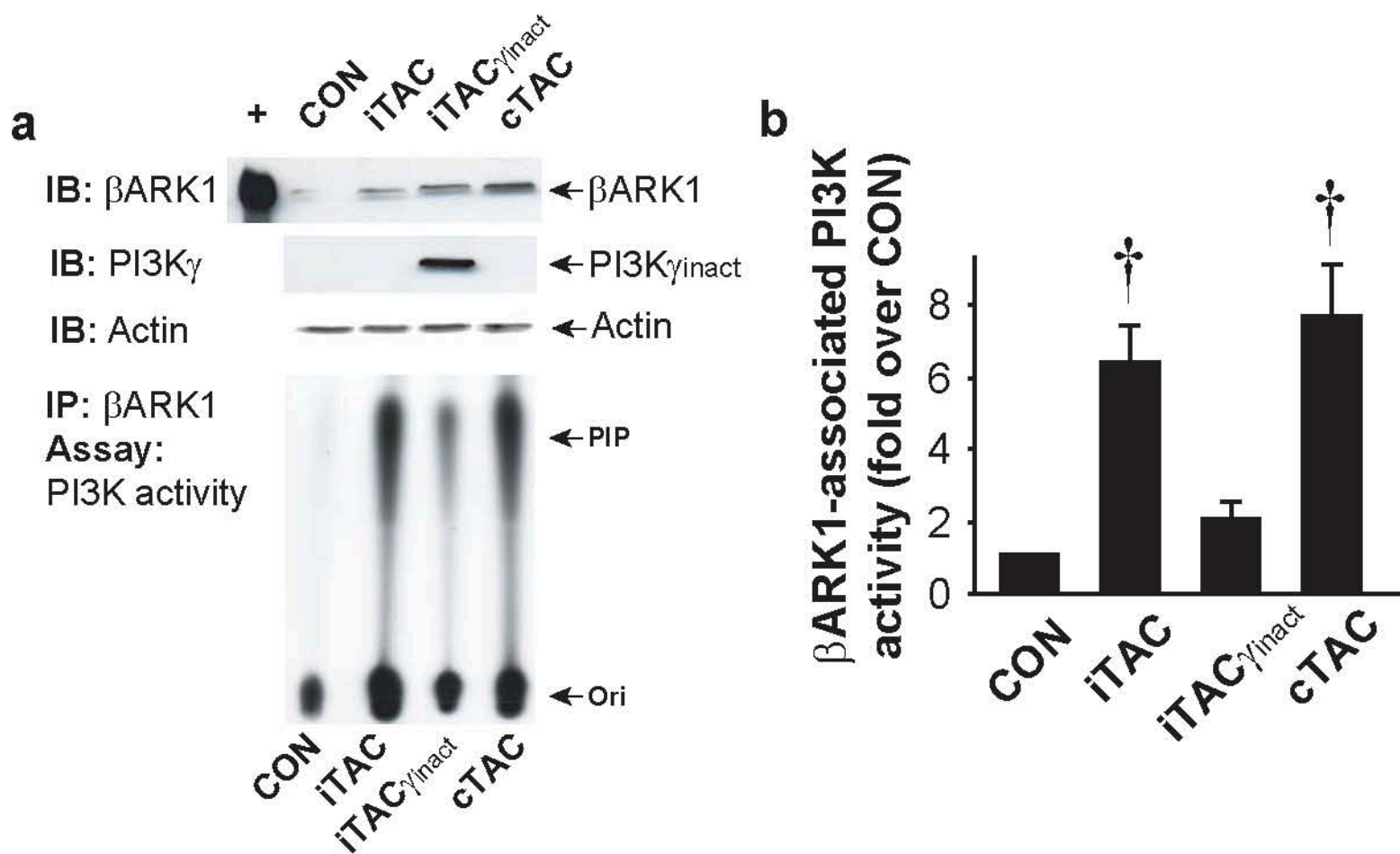
(a) Representative pictures showing βARK1 (upper panel), PI3Kγinact (middle panel), actin levels (lower panel) and resultant βARK1-associated PI3K activity in the membrane fractions of mouse hearts.
(b) Summary data of 8 different experiments and relative statistical analysis of βARK1-associated PI3K activity in the cardiac membrane from the different groups.