

Supplemental Data to Brandmayr et al.

Suppl Table 1**Amino acids modified by protein kinases in $Ca_v\beta_2$ and $Ca_v1.2$.**

The publication reporting phosphorylation of a specific amino acid (a.a.) is indicated by a reference in brackets. A.a.'s without a reference are the corresponding a.a. in the other shown protein sequences taken from the gene bank.

Species	Prot.Seq-No	Subunit	Kinases			
			PKA	CaMKII	Akt/PKB	PKG
rabbit	P15381	$\alpha 1$	S1928 (1)	S1517, S1576		
	CAA39289.1	$\alpha 1$		S1512, S1570 (2)		
	X64297	$\beta 2$	S479, S480	T500	S576	S496
rat	M80545	$\beta 2$	S478, S479 (3,4)	T498 (5)	S574 (6)	S494 (7)
mouse	Q01815	$\alpha 1$	S1898	S1487, S1545		
	Q8CC27	$\beta 2$	S529, S530	T549	S625	S545

Suppl Table 2**Nomenclature and genotype of mouse lines**

Wt	$Ca_v\beta_2^{+/+}$
β Stop	$Ca_v\beta_2^{P501Stop}/Ca_v\beta_2^{P501Stop}$
Ctr β Stop	$Ca_v\beta_2^{P501Stop/+}$
SA β Stop	$Ca_v1.2^{S1928A}/Ca_v1.2^{S1928A}, Ca_v\beta_2^{P501Stop}/Ca_v\beta_2^{P501Stop}$
Ctr SA β Stop	$Ca_v1.2^{S1928A/+}, Ca_v\beta_2^{P501Stop/+}$
SF β Stop	$Ca_v1.2^{S1512/1570A}/Ca_v1.2^{S1512/1570A}, Ca_v\beta_2^{P501Stop}/Ca_v\beta_2^{P501Stop}$
Ctr SF β Stop	$Ca_v1.2^{S1512/1570A/+}, Ca_v\beta_2^{P501Stop/+}$
$Ca_v1.2^{stop}$	$Ca_v1.2^{N1904 stop}/Ca_v1.2^{N1904 stop}$

Suppl Tab 3**Inactivation time constants**

I_{Ca} inactivation time constants in ms for various CMs from mice expressing the mutations heterozygous (Ctr) and homozygous (β stop/ β stop). N, the number of CMs for Ctr; β stop/ β stop. The fast and the slow inactivation time constant \pm isoproterenol (0.1 μ M) did not differ significantly between groups as tested by ANOVA followed by Bonferroni's posttest.

	Ctr				β stop/ β stop				N
	-ISO		+ISO		-ISO		+ISO		
$Ca_v\beta_2$ Stop	17.5 \pm 1.6	73.6 \pm 4.5	24.8 \pm 2.9	84.8 \pm 5.1	22.6 \pm 3.7	80.4 \pm 6.1	31.4 \pm 1.7	96.4 \pm 6.4	6;5
$Ca_v\beta_2^{stop}$ x $Ca_v1.2^{SA}$	17.4 \pm 1.2	78.4 \pm 4.2	23.8 \pm 2.2	91.2 \pm 5.8	21.2 \pm 3.3	120 \pm 41	28.3 \pm 3.2	108.8 \pm 22.7	8;5
$Ca_v\beta_2^{stop}$ x $Ca_v1.2^{SF}$	20.6 \pm 1.7	133.8 \pm 29	28.2 \pm 3.5	147 \pm 40	27.6 \pm 3.2	138.3 \pm 19	27.1 \pm 4.2	162.7 \pm 29	10;7

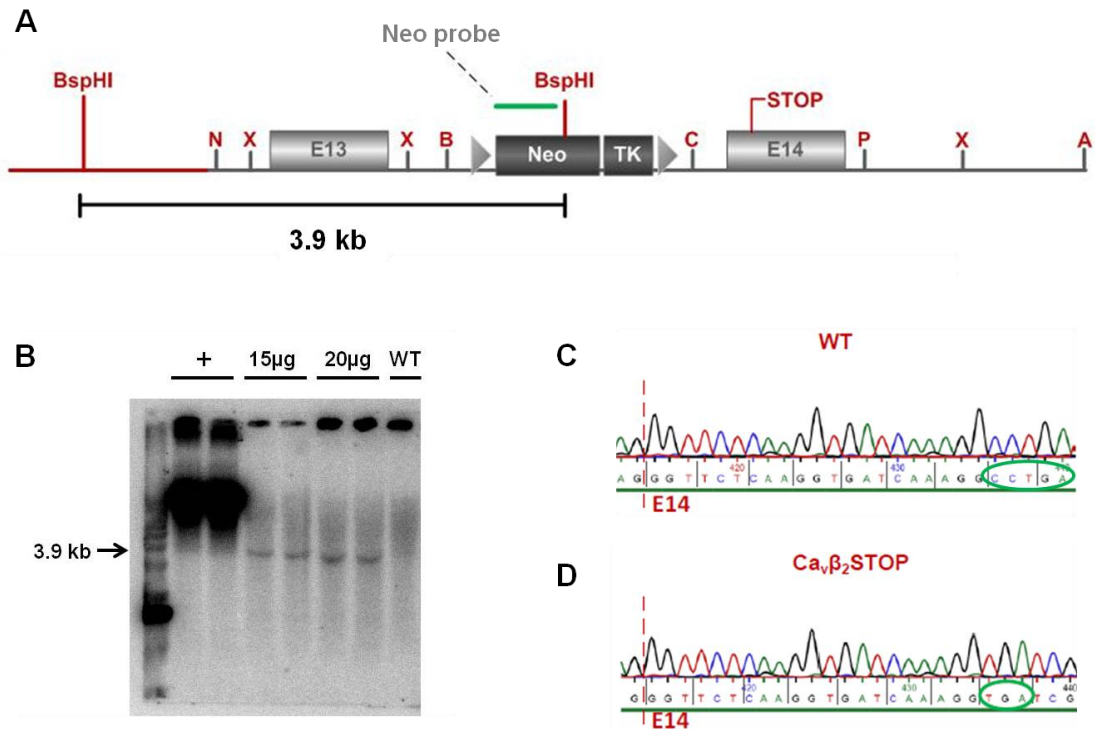
Suppl Figure 1**Southern Blot and sequence of the mutated $\beta 2$ gene.**

A: Southern blot strategy. After restriction digestion with BspHI the Neo probe detects a 3.9 kb fragment. The red sequence is outside of the targeting vector.

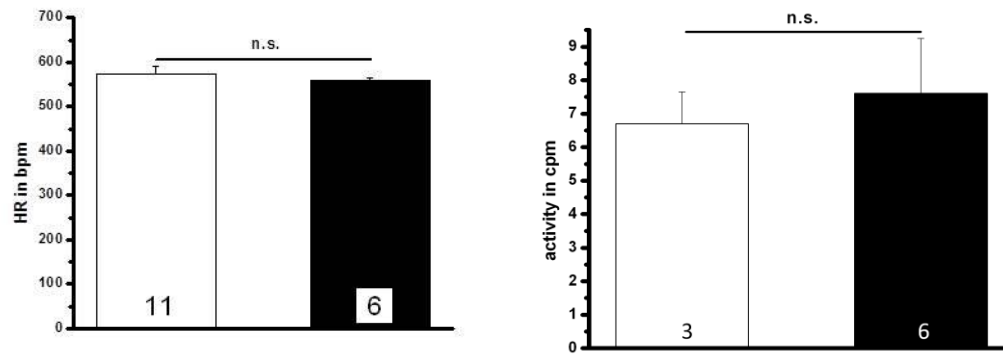
B: Southern blot with Neo probe. +: positive control (5' extended screening vector that was used to test primers and the Neo probe). 15 μ g/20 μ g: ES cell DNA of the $Ca_v\beta$ Stop clone after restriction digestion with BspHI. This clone was injected into the blastocytes and generated the $Ca_v\beta$ stop mouse line. WT: WT DNA after restriction digestion with BspHI.

C: sequence excerpt of exon 14 from a $Ca_v\beta_2$ WT mouse heart

D: sequence excerpt of exon 14 from a $Ca_v\beta_2$ Stop mouse heart; by deletion of the 2 cytosines the TGA stop-codon comes in frame

**Suppl Figure 2** **β stop mice have normal heart rate (HR, left panel) and activity (right panel) under control condition.**

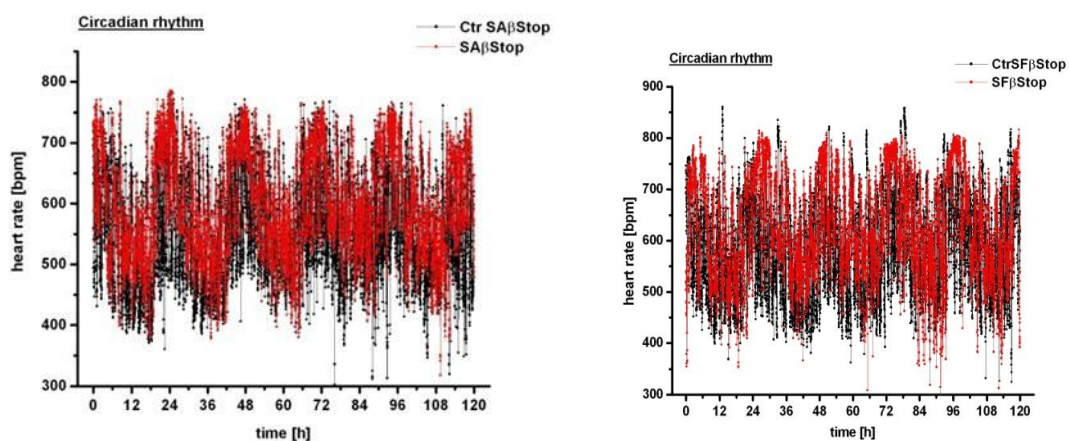
Heart rate is measured as beats per min (bpm) and activity as counts per min (cpm). Both values are obtained by ECG recording (DSI ETA-F20 transmitter) in awake, freely moving animals. The number of animal observed is given in the columns. Heterozygous (Ctr β stop) mice open columns; homozygous β stop mice black columns



Suppl Figure 3

Circadian cardiac rhythm in heterozygous Ctr SA β Stop and homozygous SA β Stop mice (left panel) and in heterozygous Ctr SF β Stop and homozygous SF β Stop mice (right panel).

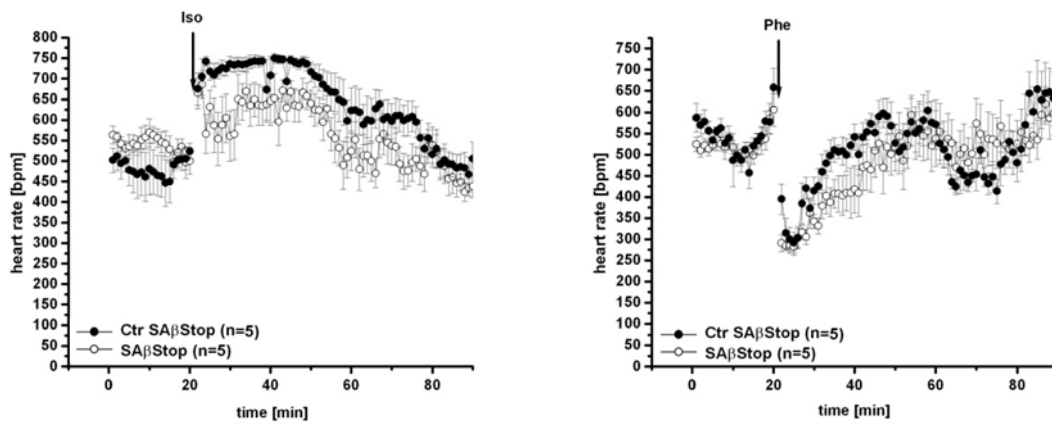
Heterozygous (Ctr SA β Stop/Ctr SF β Stop) mice are black; homozygous mice (SA β Stop/SF β Stop) are red.



Suppl Fig 4

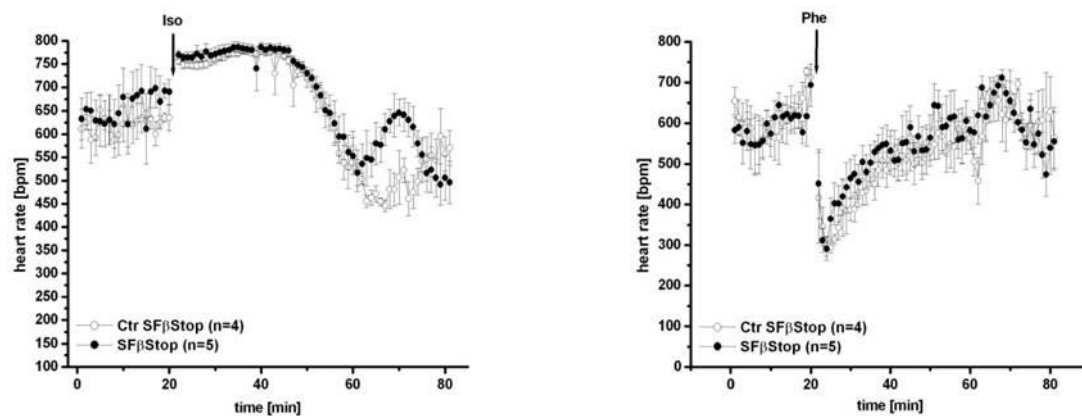
In vivo stimulation of heart rate by i.p. injection of isoproterenol (left panel) or phenylephrine (right panel) in Ctr SA β Stop and SA β Stop mice.

For experimental details see Experimental Procedures.

**Suppl Fig 5**

In vivo stimulation of heart rate by i.p. injection of isoproterenol (left panel) or phenylephrine (right panel) in Ctr SF β Stop and SF β Stop mice.

For experimental details see Experimental Procedures.

**References**

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