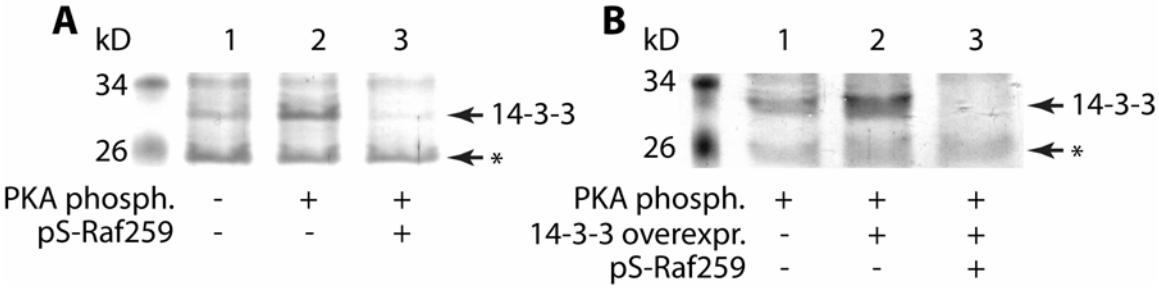
Supplementary information

Table 1. Oligonucleotide sequences for PCR and *in vitro* site-directed mutagenesis. Start codons are in italic. Restriction enzyme sites for cloning or those introduced by silent mutations for identifying mutant clones are underlined. Mutations are marked with lowercase letters.

Primer	Sequence (5' → 3')	Restriction enzyme
Xen-s	AATTCGAACCTAAGGAAGCTTCGCGATCCGGACCTTGGCATGCAGATC	not shown
Xen-a	TCGAGATCTGCATGCCAAGGTCCGGATCGCGAAGCTTCCTTAGGTTCG	not shown
pQ-s	CTTGAGCATCAGCAGCAACAGCAACAGCAGCAGCAA	-
pQ-a	CAAGTTGCTGCTGCTGTTGCTGTTGCTGCTGATGCT	-
mTRESK-s	ATCGAATTCCAAGAGG*ATG*GAGGCTGAGG	EcoRI
mTRESK-a	CCTGGTCCGGACCCCAAGGTAGCGAAACTTCCCTTTG	Kpn2I
h14-3-3η-s	GGGGGGGCATGCAG*ATG*GGGGACCGGGAGCAGCTG	PaeI
h14-3-3η-a	GTGCTCGAGTCAGTTGCCTTCTCCTGCTTCTTCATC	XhoI
m14-3-3β-s	GCGGAATTCCTCCGCGAAA*ATG*ACCATG	EcoRI
m14-3-3β-a	GCGCTCGAGTTAGTTCTCTCCCTCTCCAGCATC	XhoI
m14-3-3γ-s	GCGGAATTCAGCCCTGTGAAG*ATG*GTGG	EcoRI
m14-3-3γ-a	GCGCTCGAGCACCTGGGGCCTTAGTTGTTGC	XhoI
m14-3-3ε-s	GCGGAATTCGCTGCCGCA*ATG*GATGATC	EcoRI
m14-3-3ε-a	GCGCTCGAGTCACTGATTCTCATCTTCCACATCC	XhoI
m14-3-3ζ-s	GCGGAATTCCCACTCCGGACACAGAATATCAG	EcoRI
m14-3-3ζ-a	ATACCGTCGACGGCCGGTTAATTTTCCCCTCC	SalI
m14-3-3σ-s	GCGGAATTCCTCGCAGTC*ATG*GAGAGAG	EcoRI
m14-3-3σ-a	GCGCTCGAGTCAGCTCTGGGGCTCCTCCG	XhoI
m14-3-3τ-s	GCGGAATTCGCTCGCC*ATG*GAGAAGACC	EcoRI
m14-3-3τ-a	ATACCGTCGACTTAGTTTTCGGCCCCCTCTGCTG	SalI
β-R58,62A-s	GTGGTAGGTGCCCGggcCTCTTCCTGGgcTGTCATCTCCAGCATC	SmaI
γ-R57,61A-s	GGTGGGGGCTCGCgctagCTCCTGGgcGGTCATCAGCAGCATC	NheI
ε-R57,61A-s	TGATTGGAGCCAGAgcgGCgTCCTGGgcAATAATCAGCAGCATTG	BglI
ζ-R56,60A-s	GTTGTAGGAGCCCGggcGTCATCGTGGgcGGTCGTCTCAAGTATTG	SmaI
σ-R56,60As	GGTGGGCGGCCAGgcgGCcGCCTGGgcGGTCCTGTCCAGCATC	NotI
τ-R56,60A-s	GTAGGGGGCCGCgctagCGCCTGGgcGGTCATCTCGAGCATTG	NheI
mTRloop-s	CAGGACC*ATG*GGCCACGATATCTTCAAATGGCGC	NcoI
mTRloop-H8-a1	ATGATGATGATGGTCCAGACGCTCCACCTGCTGGCCCAC	-
mTRloop-H8-a2	GCGCTCGAGTCAATGATGATGATGATGATGATGGTCCAGACG	XhoI
mTR-S192A-s	CTTCAAATGGCGagCtCTCCCGCTCTGC	SacI
mTR-S202A-s	GGAAGCAGCCTGACgcCAAACCGGTGGAGGAAG	Hin1I, BstXI
mTR-S227,232,234A-s	AACCCGCAGCCggcCAAGGACCCCCCCgCTCCGgcaTGCAATGTGGAGCTG	PdiI, PaeI
mTR-T256A-s	GAACAAGCTACAACCgCCggCGCGTCCCGTGGAG	PdiI
mTR-T256A,S262A-s	CTACAACCACCCgCGCGTCCCGTGGAGAGGgcCAACTCCTGTCCCGAG	(Δ MluI)
mTR-S262,264A-s	CGCGTCCCGTGGAGcGcgcgAAtgCCTGTCCCGAGCTGG	BssHII, BsmI
mTR-S274,(279)A-s	CTGGTGCTGGGGCGgCTagCCTGTTCTATTCTCGC	NheI
mTR-S279A-s	CCTGTTCTATTCTCgcgAATCTGGATGAAGTGG	Bsp68I
mTR-S274,276,279A-s	CTGGGGCGACTGgCaTGcgCTATTCTCgcgAATCTGGATGAAGTGG	PaeI, Bsp68I

Supplementary Figure S1.



We examined whether the interaction of endogenous *Xenopus* 14-3-3 with TRESK could be biochemically verified, whether the 14-3-3 level remained high for 3 days after the microinjection of 14-3-3 cRNA, and whether pS-Raf259 competed with TRESK for the binding to (endogenous and overexpressed) 14-3-3 present in the *Xenopus* oocyte cytosol.

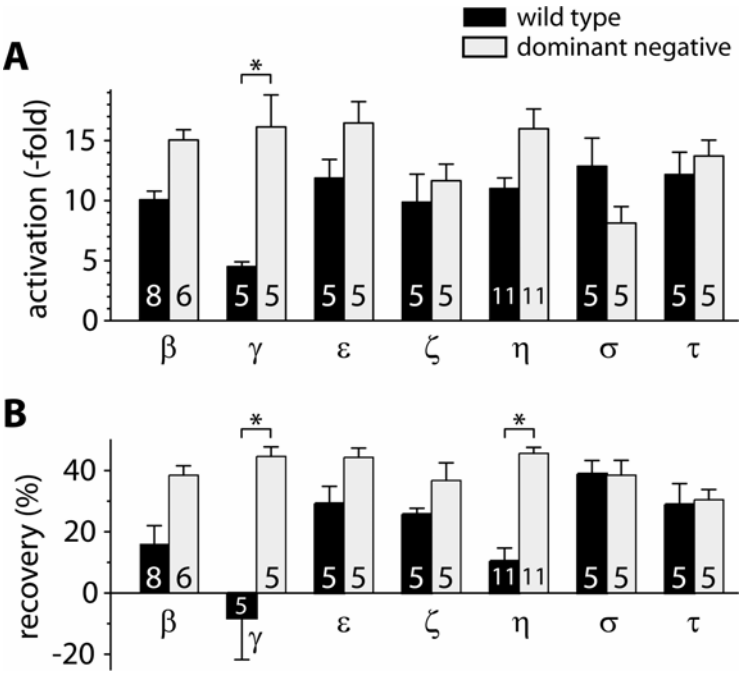
His-tag pulldown experiments were performed (similarly as in Fig. 5. D-F) with cytosol extracts prepared from *Xenopus laevis* ovarian lobes (Panel A) or oocytes (Panel B, 100 oocytes/lane). The cytosols were incubated with 'wild type' TRESKloop-H_8 immobilized on Ni-NTA resin (see the *Experimental procedures*).

A. The endogenous *Xenopus* 14-3-3 protein (molecular weight between 26 and 34 kD) was pulled down with PKA-phosphorylated TRESKloop-H_8 protein from the cytosol extract of *Xenopus* ovarian lobes (*lane 2*). The interaction was phosphorylation-dependent, since the pulldown of 14-3-3 was much smaller with non-phosphorylated TRESKloop-H_8 (*lane 1*). In the presence of pS-Raf259 (600 μM), the pulldown of endogenous 14-3-3 was prevented (*lane 3*). (Several contaminating protein bands, binding non-specifically to the Ni-NTA resin, are also visible; one being especially prominent at 26 kD, marked with an *arrow* and an *asterisk*.)

B. The endogenous *Xenopus* 14-3-3 protein was also pulled down with PKA-phosphorylated TRESKloop-H_8 protein from the cytosol extract of 100 non-injected oocytes (*lane 1*). If the oocytes were microinjected with 14-3-3η cRNA (identical quantity as used in the electrophysiological experiments) 3 days before the preparation of the cytosol, then more 14-3-3 was pulled down than from the non-injected oocytes (*lane 2*). The addition of pS-Raf259 (600 μM) eliminated the binding of both the endogenous and overexpressed 14-3-3 (*lane 3*).

The endogenous *Xenopus* 14-3-3 bound phosphorylation-dependently to the intracellular loop of mouse TRESK (similarly to the recombinant 14-3-3 fusion proteins expressed in *E.coli*.) The 14-3-3 level in the cRNA-injected cells exceeded that of the non-injected cells 3 days after the microinjection. The pS-Raf259 phosphopeptide prevented the binding of the (endogenous and overexpressed) 14-3-3 to TRESK.

Supplementary Figure S2.



The effects of the different 14-3-3 isoforms on the calcium-dependent regulation of TRESK.
A. Activation of the K^+ current in response to ionomycin (measured as detailed in Fig. 1.A) in oocytes coexpressing different wild type (*black* columns) or dominant negative (*gray* columns) 14-3-3 isoforms (as indicated by *Greek letters* below the panel) with mouse TRESK. **B.** Recovery of the K^+ current after a washout period of 5 minutes in the same cells as in A. (The negative recovery (γ isofrom) indicates that the current was even larger at the end of the washout period than immediately after the ionomycin stimulation. The data for the human η isoform were pooled and repeated from Fig. 1.B-E.)