Online Supplement

Supplementary Figure S1. Exposure to GSTπ increased the signal for glutathionylation of the β1 Na^+/K^+-ATPase subunit

Na^+/K^+-ATPase-enriched membrane fragments from the outer medulla of pig kidneys obtained from the slaughter house were prepared by the SDS extraction procedure of Jorgensen (1). The protein concentration was ~4.5 mg/ml measured with the Peterson's modification of the Lowry method (2) and bovine serum albumin as a standard. The specific activity of the preparation was ~30 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ at 37 °C.

Susceptibility of the β1 subunit depends on the conformational poises of Na^+/K^+-ATPase (3) and the Na^+/K^+-ATPase was stabilized in the $E1Na_3$ conformation with solutions that included 100 mM NaCl, 4 mM $MgCl_2$, and 20 mM histidine at a pH of 7.35 with or without 2 mM GSH (Sigma) and 10 μg/ml GSTπ (Sigma). Na^+/K^+-ATPase was incubated with GSTπ for 30 min at 37 °C.

After the incubation the Na^+/K^+-ATPase-enriched membrane fragments were immunoprecipitated (IP) with a mosue antibody against β1 Na^+/K^+-ATPase subunit (Sigma) and then with protein A/G plus agarose beads (Santa Cruz Biotechnology). Immunoprecipitates in the beads were collected and immunoblotted with an anti-GSH antibody (Virogen) (Santa Cruz Biotechnology) as previously described (4) (Virogen). Non-immune IgG was used as negative control for the Co-Ip.

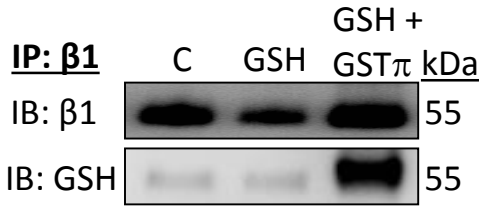


Fig. S1. Glutathionylation of β1 subunit in Na^+/K^+-ATPase-enriched membrane fragments exposed to GSH with or without exposure to GSTπ. C indicates exposure to GSH- and GSTπ-free solutions. IP indicates immunoprecipitate and IB indicates immunoblot. One of two similar experiments is shown.

Supplementary Figure S2. Ang II increased β_1 subunit/ GSTπ Co-immunoprecipitation

To examine if physiological oxidative stress increases the association between the β_1 Na^+/K^+-ATPase subunit and GSTπ we exposed freshly isolated rabbit cardiac myocytes to solutions containing 100 nM angiotensin II (Ang II) for 15 min to activate NADPH oxidase (5) or to Ang II-free solutions. Cells were lysed and Co-Ip of the β_1 Na^+/K^+-ATPase subunit and GSTπ determied.

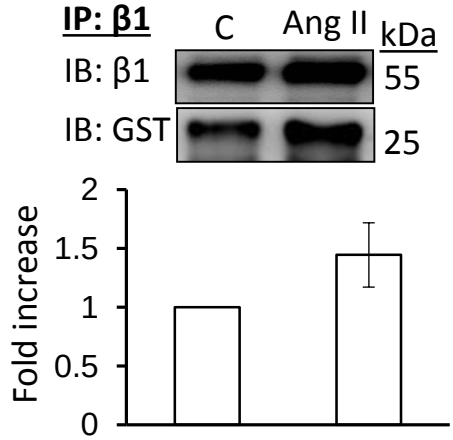


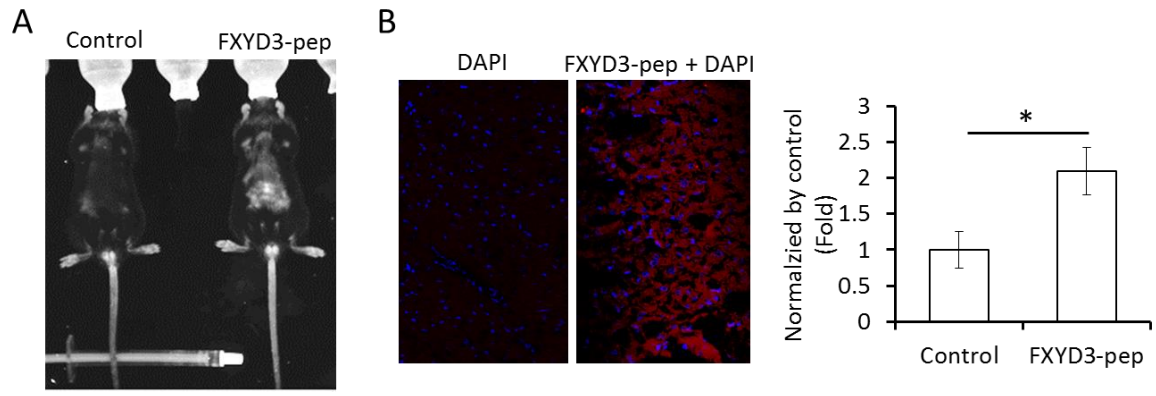
Fig. S2. Co-Ip of GSTπ with β1 subunit after exposure of myocytes exposed to angiotensin II (Ang II). N = 4.

Figure S3. In vivo imaging FXYD3-pep in C57BL/6 mice

Mice were given 3.3 mg/kg TRITC labeled FXYD3-pep by i.p. injection. The dose was calculated to give an average concentration of ~ 1 μM if the distribution was uniform. Two hours after injection, animals were anesthetized by isoflurane inhalation and then imaged using the Carestream Molecular Imaging System (Carestream Health Inc., USA) (6). Protocols were approved by institutional animal care and ethics committees. Fluorescence of mice 2 hours after the injection indicated uptake of the compound (**Fig. S3 A**)

We independently confirmed uptake of 3.3 mg/kg labeled FXYD3-pep with fluorescence microscopy of the myocardium of mice sacrificed 2 hours after the injection. Heart tissue samples were mounted in OCT embedding compound (ProSciTech, AUS) and frozen at -80 °C. Sections (6 μm thick) were then fixed in -20 °C acetone for 10 mins and stained with DAPI. Images of single confocal planes were obtained. Myocardial fluorescence indicated tissue uptake of the peptide.

There was no apparent acute toxic in mice allowed to recover from anesthesia after having been given the peptide, even when a dose of 10 mg/kg was given to 5 mice.



Supplementary Fig. S3 A, Fluorescence in a control mouse and a mouse given 3.3 mg/kg TRITC labeled FXYD3-pep, imaged 2 hours after i.p injection. **B**, Fluorescence (red) in myocardium of mouse sacrificed 2 hours after i.p. injection of peptide. DAPI was used to counter-stain the nucleus (blue). Bar graph shows mean fluorescence in 11 tissue slides from 4 mice. *, $p<0.05$.

References

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