Supporting Information

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SI Text

Effect of TAT-BH4 on IP3 Receptor Channel Opening *in Vitro*. Planar lipid bilayer studies were performed to determine whether the TAT-BH4 peptide regulates IP3 receptor function *in vitro,* as shown in Fig. S1. Detailed methods of planar lipid bilayer analysis were published previously (1). Briefly, single channel recordings of IP3 receptor type 1 activities, using cesium as the permeant ion, were performed by vesicle fusion of native rat cerebellar IP3 receptor type 1 microsomes in planar lipid bilayers. IP3 receptor channel opening was activated by adding 2 μM IP3 to the *cis* compartment (cytoplasmic side of channel) at 250 nM Ca^{2+}. Statistical analysis, data processing, and figure presentation were performed using Origin software (Microcal Software Inc. , Northampton, MA). The single channel activity of type 1 IP3 receptor, reconstituted into planar lipid bilayers, was visualized as a series of discrete positive current fluctuations in the presence of 2 μM IP3 and 250 nM Ca^{2+} in the *cis* compartment (cytoplasmic side of channel) (Fig. S1). The IP3 receptor open probability decreased significantly from 0.23 to 0.02 after adding 2 μM TAT-BH4 but was not affected by TAT-ctrl, a fusion peptide composed of TAT and a scrambled sequence of the BH4 domain. These results demonstrate that the BH4 domain is sufficient to inhibit IP3 receptor channel opening *in vitro.*

Effect of TAT-Pep2 on the Interactions of Bcl-2 with IP3 Receptor, Calcineurin, and VDAC. The BH4 domain of Bcl-2 is known to interact with calcineurin (2) and VDAC (3, 4). We reported previously that the IP3 receptor–derived peptide, referred to as Peptide 2, displaces Bcl-2 from the IP3 receptor (1). To determine whether Peptide 2 also interferes with the interactions of Bcl-2 with calcineurin and VDAC, Jurkat cell lysates were preincubated for 1 h in the presence or absence of 0.2 mM μM TAT-ctrl or TAT-Pep2. Bcl-2 was then immunoprecipitated from the cell lysates as described previously (1), followed by immunoblotting analysis of immunoprecipitates to detect IP3 receptors, calcineurin A, and/or VDAC. The results (Fig. S2) indicate that TAT-Pep2 interferes with the interaction of Bcl-2 with IP3 receptors but not with the interaction f Bcl-2 with either calcineurin or VDAC. Thus, TAT-Pep2 appears to be a relatively specific inhibitor of Bcl-2-IP3R interaction.

1. Rong Y, et al. (2008) Targeting Bcl-2-IP3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. *Mol Cell* 31:255–265.
2. Shibasaki F, Kondo E, Akagi T, McKeon F (1997) Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2. *Nature* 386:728–731.
3. Shimizu S, Narita M, Tsujimoto Y (1999) Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature* 399:483–412.
4. Shimizu S, Konishi A, Kodama T, Tsujimoto Y (2000) BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death. *Proc Natl Acad Sci USA* 97:3100–3105.

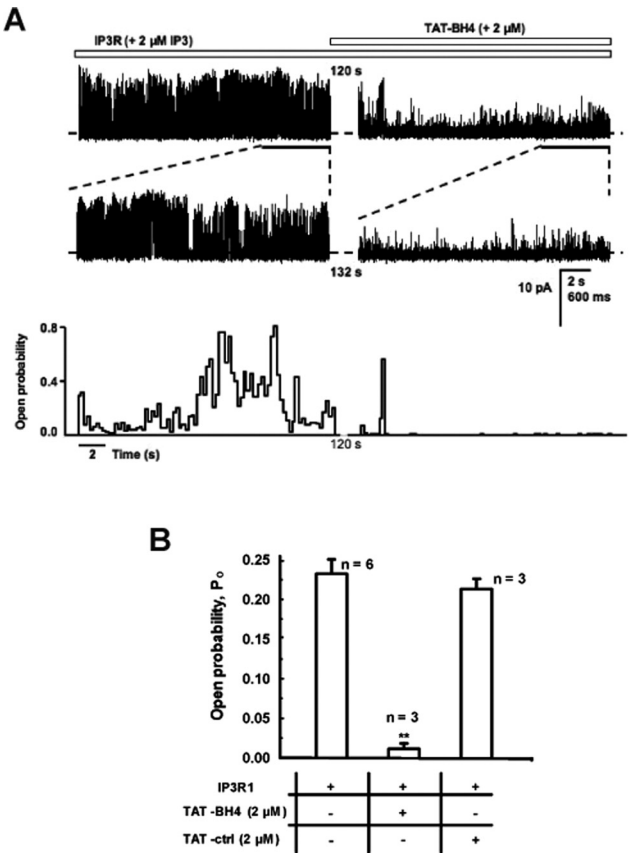

Fig. S1. TAT-BH4 peptide inhibits IP3-dependent channel opening *in vitro.* (*A*) IP3 receptor type 1 single channel recordings at 0 mV in planar lipid bilayers with 0.2 mM Ca^{2+} and 2 μM IP3 in the *cis* (cytosolic) compartment (zero-current level marked). Current traces at the expanded time scale are shown in the *bottom panel.* TAT-BH4 (2 μM), added to the *cis* compartment, blocked channel activity. (*B*) Bar graph summarizes multiple experiments (mean $\pm$ SEM; n = number of individual channels examined, *, $P < 0.05$).

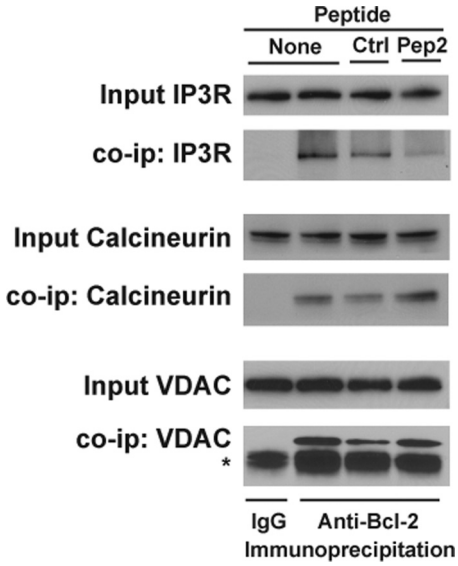

Fig. S2. Effect of TAT-Pep2 on the interaction of Bcl-2 with IP3 receptor, calcineurin, and VDAC. Jurkat cell lysates were preincubated in the presence or absence of 0.2 mM TAT-Pep2 or TAT-ctrl peptide for 1 h before immunoprecipitating Bcl-2. Co-immunoprecipitated proteins were detected by immunoblotting with antibodies recognizing type 1 IP3 receptor, calcineurin, and VDAC. The amounts of these proteins used as input into the immunoprecipitation are shown by immunoblotting in parallel panels. *, IgG band.