Supplementary Table 1: Mutated β2 N-termini and artificial N-termini

	1	11	21	31	41
β2	FIWTSGRTS	SSYRHDEKRN	IYQKIRDHDL	LDKRKTVTAL	KAGEDRAILL
6Q	FIWTSGQTS	SSYQHDEQQN	IYQQIQDHDL	LDKRKTVTAL	KAGEDRAILL
10Q	FIWTSGQTS	SSYQHDEQQN	IYQQIQDHDL	LDQQQTVTAL	QAGEDRAILL
6QR#R					
6QR8R	FIWTSGRTS	SSYQHDEQQN	IYQQIQDHDL	LDKRKTVTAL	KAGEDRAILL
6QR14R	FIWTSGQTS	SSYRHDEQQN	IYQQIQDHDL	LDKRKTVTAL	KAGEDRAILL
6QK18K	FIWTSGQTS	SSYQHDEKQN	IYQQIQDHDL	LDKRKTVTAL	KAGEDRAILL
6QR19R	FIWTSGQTS	SSYQHDEQRN	IYQQIQDHDL	LDKRKTVTAL	KAGEDRAILL
6QK24K	FIWTSGQTS	SSYQHDEQQN	IYQKIQDHDL	LDKRKTVTAL	KAGEDRAILL
6QR26R	FIWTSGQTS	SSYQHDEQQN	IYQQIRDHDL	LDKRKTVTAL	KAGEDRAILL
10QR#R					
10QR8R	FIWTSGRTS	SSYQHDEQQN	IYQQIQDHDL	LDQQQTVTAL	QAGEDRAILL
10QR14R	FIWTSGQTS	SSYRHDEQQN	IYQQIQDHDL	LDQQQTVTAL	QAGEDRAILL
10QK18K	FIWTSGQTS	SSYQHDEKQN	IYQQIQDHDL	LDQQQTVTAL	QAGEDRAILL
10QR19R	FIWTSGQTS	SSYQHDEQRN	IYQQIQDHDL	LDQQQTVTAL	QAGEDRAILL
10QK24K	FIWTSGQTS	SSYQHDEQQN	IYQKIQDHDL	LDQQQTVTAL	QAGEDRAILL
10QR26R	FIWTSGQTS	SSYQHDEQQN	IYQQIRDHDL	LDQQQTVTAL	QAGEDRAILL
10QK33K	FIWTSGQTS	SSYQHDEQQN	IYQQIQDHDL	LDKQQTVTAL	QAGEDRAILL
10QR34R	FIWTSGQTS	SSYQHDEQQN	IYQQIQDHDL	LDQRQTVTAL	QAGEDRAILL
10QK35K	FIWTSGQTS	SSYQHDEQQN	IYQQIQDHDL	LDQQKTVTAL	QAGEDRAILL
10QK41K	FIWTSGQTS	SSYQHDEQQN	IYQQIQDHDL	LDQQQTVTAL	KAGEDRAILL
Artificial N-termini					
FIW30Q	FIW	QQQQQQQQQQ	QQQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW KK28Q	FIW	KKQQQQQQQQ	QQQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW 2QKK27Q	FIW	QQ-KKQQQQQ	QQQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW 4QKK24Q	FIW	QQQQKKQQQQ	QQQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW 6QKK22Q	FIW	QQQQQQKKQQ	QQQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW 8QKK20Q	FIW	QQQQQQQQKK	QQQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW 10QKK18Q	FIW	QQQQQQQQQQ	KKQQQQQQQQ	QQQQQQQQQQ	RAILL
FIW 11QKK15Q	FIW	QQQQQQQQQQ	Q--KKQQQQQ	QQQQQQQQQQ	RAILL
FIW 17QKK10Q	FIW	QQQQQQQQQQ	QQQQQQQ-KK	QQQQQQQQQQ	RAILL
FIW 20QKK8Q	FIW	QQQQQQQQQQ	QQQQQQQQQQ	KKQQQQQQQQ	RAILL
FIW 22QKK6Q	FIW	QQQQQQQQQQ	QQQQQQQQQQ	QQKKQQQQQQ	RAILL
FIW 24QKK4Q	FIW	QQQQQQQQQQ	QQQQQQQQQQ	QQQQKKQQQQ	RAILL
FIW 25QKK2Q	FIW	QQQQQQQQQQ	QQQQQQQQQQ	QQQQQ-KKQQ	RAILL
FIW 27QKK	FIW	QQQQQQQQQQ	QQQQQQQQQQ	QQQQQQQ-KK	RAILL

Zhang et al., Supplementary Figure 1.

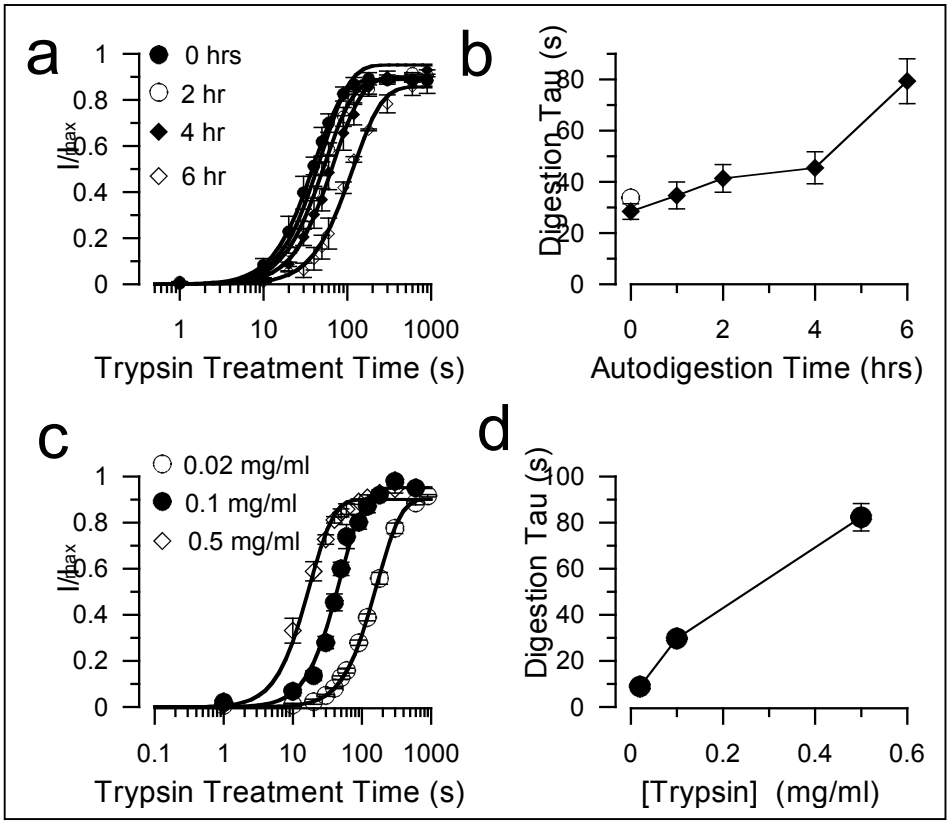

Figure S1. **Controls for autodigestion and linearity of the trypsin digestion rates. (a)** Autodigestion by trypsin at 0.1 mg/ml is minimal over a 6 hour period. Solutions of 0.1 mg/ml trypsin were incubated at room temperature for various times and then tested for ability to remove inactivation. Patches were exposed to trypsin within 15 minutes of the nominal time of trypsin incubation. Curves reflect fits of Eq. 1 to the recovery time course. **(b)**, Dependence of digestion time constant from **(a)** is plotted as a function of trypsin preincubation time. The points under each condition indicate the mean and SEM for a set of 3-4 patches. The open circle represents recovery time course determined at 0.1 mg/ml in a separate set of patches. **(c)** The dependence of removal of inactivation is plotted as a function of trypsin concentration. The removal of inactivation by trypsin was examined at three different trypsin concentrations (3 patches: 0.02 mg/ml; 7 patches: 0.1 mg/ml; 5 patches: 0.5 mg/ml). Lines represent fits of Eq. 1. **(d)**, Digestion time constants from (c) are plotted as a function of [trypsin].

Supplementary Figure 2.

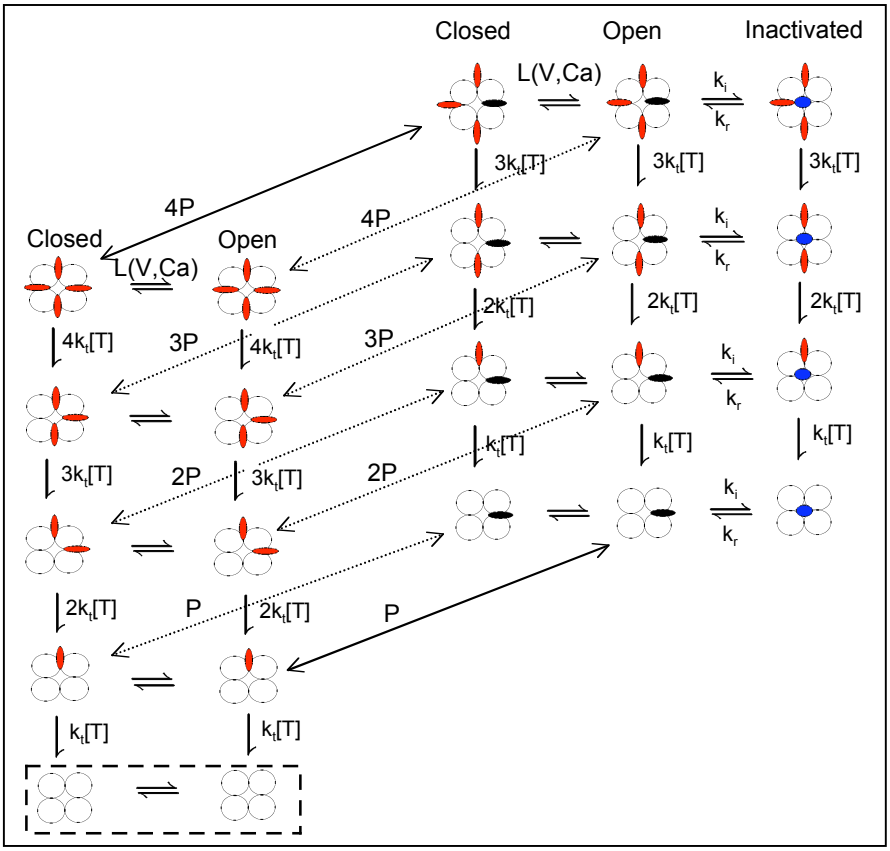

Figure S2. A complete model of protection against trypsin digestion of β2-subunit inactivation domains during resting conditions and during inactivation. The model incorporates both of the inactivation-dependent protection and resting potential models given in the main text. The entry of channels into the states within the dotted box describes the time course of removal of inactivation by trypsin digestion. Inactivation domains in red are considered accessible to trypsin, in black are protected with the antechamber, and, in blue, are inactivated (and protected). All terms are identical to those given for Fig. 4 and 6 in the main paper. Simulations based on this full model using the same parameters as those used in Fig. 4 and Fig. 6 to define resting and inactivated conditions yielded results identical to those in the main text paper.