Supplemental Material:

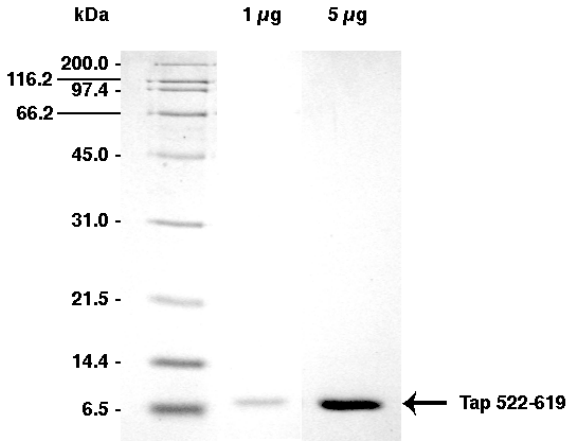
Production of polyclonal Antibody (pAB) against Tap. Tap residues 522-619 were digested from FLAG-Tap WT vector using NheI and EcoRI and inserted into the NheI and EcoRI site of the bacterial expression vector pET23b. Digestion of the pET23b vector with Nhe1 and EcoRI also removed the T7 tag. Tap 522-619 was expressed in *E. coli* and induced with IPTG. The bacterial pellet was solubilized in TNE (50 mM Tris pH 8, 50 mM NaCl, 1 mM EDTA) and Tap 522-619 enriched through sequential ammonium sulfate precipitation steps. Significant enrichment of the Tap protein was recovered from 15%-35% ammonium sulfate pellet (Figure S1) and used to immunize two New-Zealand White rabbits (Cocalico Biologicals, Inc., Reamstown, PA).

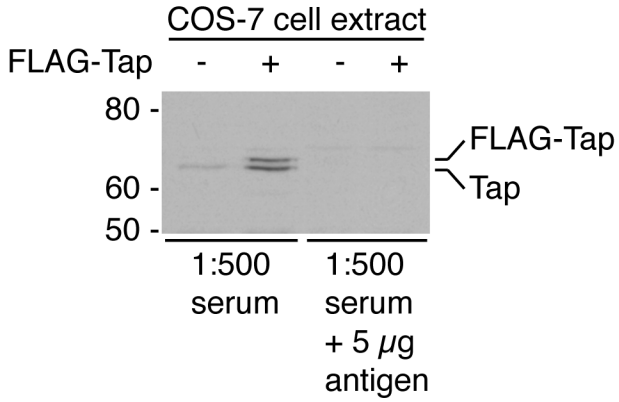
Specificity of Tap pAb. Extract from COS-7 cells untransfected or transfected with FLAG-Tap WT construct were separated by SDS-PAGE and transferred to Immobilon-P membrane. Blots were blocked in 5% milk and incubated with immune serum overnight. Some of the serum was preadsorbed with recombinant Tap 522-619 protein (5 ug/μL of serum) for 3 hours prior to incubation with the blot (Figure S2). The serum detected endogenous Tap in both transfected (lane 2) and untransfected cells (lane 1). Expression of FLAG-Tap was also observed in transfected cell extract. Preadsorption of the serum with antigen prior to WB eliminated the reactivity (Figure S2, lanes 3 and 4).

Supplemental Figure 1: Recombinant Tap 522-619 protein used for pAb production. Recombinant Tap 522-619 was enriched from bacterial extract by sequential ammonium sulfate precipitation steps. Aliquots of the enriched Tap 522-619 fraction containing 1 μg or 5 μg of proteins were resolved on SDS-PAGE and visualized by Coomassie R250.

Supplemental Figure 2: Characterization of the Tap pAb by WB. Extract from untransfected COS-7or COS-7 cells expressing FLAG-Tap were resolved by SDS-PAGE and transferred to Immobilon-P membrane. Membrane was incubated with either serum

derived from a rabbit immunized with Tap 522-619 protein or the same serum pre-incubated with antigen. Proteins were visualized by standard WB protocol.


kDa
1 µg
5 µg
200.0
116.2
97.4
66.2
45.0
31.0
21.5
14.4
6.5
Tap 522-619


COS-7 cell extract
FLAG-Tap
-
+
-
+
80 -
60 -
50 -
FLAG-Tap
Tap
1:500 serum
1:500 serum + 5 μg antigen