Supporting Information

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SI Materials and Methods

Preparation of Membranes and Biochemical Analysis. REV-transformed cells were cultured in RPMI 1640 (supplemented with glutamine, kanamycin, and 10% FCS) at 37 °C and at 5% CO_2. Cells were spun down and resuspended in 1 mL cold freeze–thaw buffer (1 mM $MgCl_2$, 10 mM TrisCl, pH 8), frozen and thawed twice, and centrifuged at 13,300 rpm at 4 °C for 1 h in a Haereus Fresco 17 microfuge (Thermo Scientific). The pellet was solubilized on ice for 30 min in digitonin lysis buffer [150 mM NaCl, 1 mM $MgCl_2$, 10 mM TrisCl, pH 8, with 1% digitonin (Calbiochem) and the serine protease inhibitor AEBSF (Pefabloc; Roche)] to give 10^8 cell equivalents per milliliter, and centrifuged at 13,300 rpm at 4 °C for 10 min in a Haereus Fresco 17 microfuge, and the supernatant was used immediately or frozen in aliquots.

For Fig. S1, aliquots corresponding to 5×10^5 REV-transformed IS19 cells were prepared as cell pellets, detergent lysates of a membrane pellet, and the supernatant containing the soluble material not spinning down as membranes. The three samples were heated to 95 °C for 5 min with SDS sample buffer (2% SDS, 50 mM TrisCl, pH 8, 5% glycerol, 0.1% bromophenol blue) and then SDS gel electrophoresis followed by staining with Coomassie Brilliant Blue in 10% methanol, 7.5% acetic acid, and by Western blot with the mAb 2G11 to chicken class II β-chain were performed as described in *Materials and Methods*.

For Fig. S2, aliquots of digitonin lysate from membranes were incubated with ATP-agarose (Sigma) in cold digitonin IP wash buffer (1 vol digitonin lysis buffer: 9 vol 150 mM NaCl, 50 mM TrisCl, pH 8) supplemented with 5 mM $MgCl_2$ overnight on ice (with or without 25 mM ATP), then washed three times with the same buffer. The affinity-purified molecules were heated to 95 °C for 5 min with SDS sample buffer, and then SDS gel electrophoresis followed by Western blot with mAbs were performed as described in *Materials and Methods*.

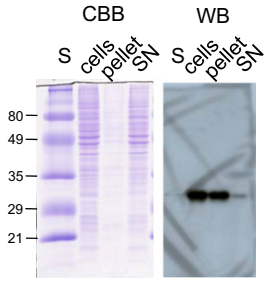

Fig. S1. A simple protocol separates membrane proteins from most other proteins. Chicken TAP proteins from REV-transformed cells were not detected until sufficient cell equivalents were used in the Western blot. This experiment shows that most proteins as detected by Coomassie Brilliant Blue (CBB) staining were found in the supernatant (SN) of a membrane preparation, whereas a typical membrane protein as detected by Western blot (WB) was found in the membrane pellet. Two samples of cells were centrifuged. One sample was resuspended in freeze–thaw buffer, frozen and thawed twice, and separated into a membrane pellet and supernatant by using a microcentrifuge (*Materials and Methods*). The cells and the membrane pellet were solubilized with detergent and subcellular material was removed by centrifugation (*Materials and Methods*). Samples representing equivalent numbers of cells were analyzed by SDS gel electrophoresis, with the gels either stained with CBB in 10% methanol, 7.5% acetic acid (*Left*), or Western blotted (*Materials and Methods*) with 2G11, an mAb to chicken class II β-chains (*Right*). S, standards (apparent molecule mass indicated in kDa).

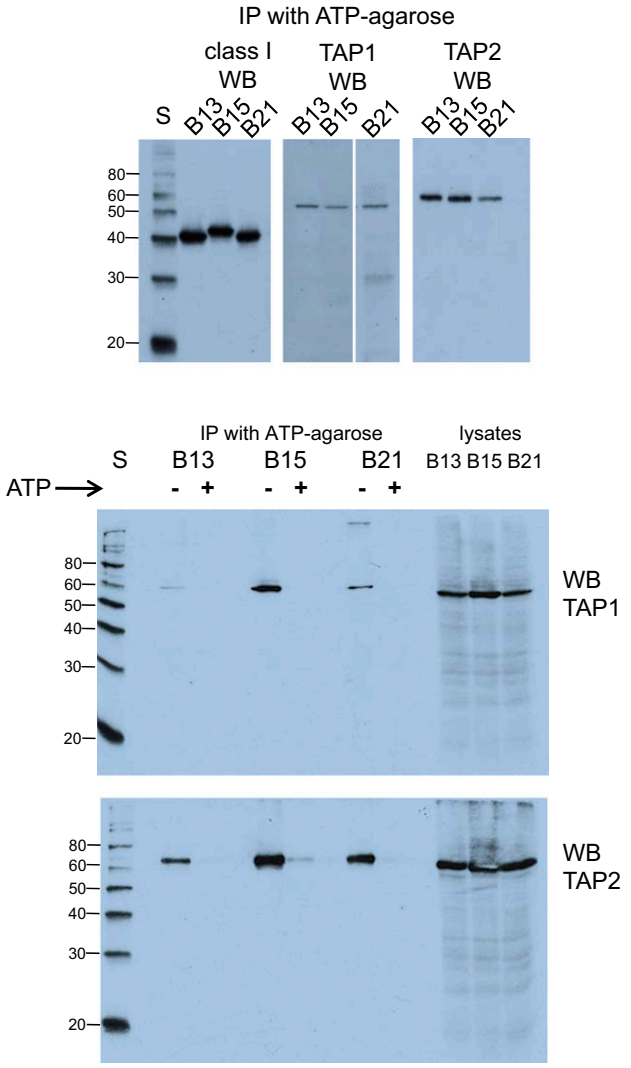

Fig. S2. Chicken class I, TAP1, and TAP2 molecules are all isolated by specific affinity chromatography with ATP-agarose. *Upper*: Digitonin lysates of membranes from UG5 (B13 haplotype, encoding molecules with identical sequence to B4), TG15 (B15), and TG21 (B21) cells were analyzed by Western blot with mAb to chicken class I heavy chain (F21-2), TAP1 (F1-11), or TAP2 (F1-3) after affinity purification (IP) with ATP-agarose. *Lower*: Aliquots of digitonin lysates (as above) were affinity purified (IP) by ATP-agarose in the presence or absence of ATP, followed by Western blot with mAb to chicken TAP1 (F1-3) or TAP2 (F1-2); equal aliquots of lysate were also analyzed directly. S, standards (apparent molecular mass indicated in kDa).

Table S1. Polymorphic residues in chicken TAP1 membrane-spanning domain

	3	54	92	116	131	162	179	289	327
B2	T	E	R	M	E	V	R	R	A
B4	T	K	R	M	K	A	R	R	A
B12	K	E	R	M	E	A	R	E	A
B14	T	E	H	M	E	A	R	E	A
B15	T	E	R	T	E	A	Q	E	A
B19	K	E	R	M	E	A	R	E	A
B21	T	E	R	M	E	A	R	R	T

In Tables S1–S5, MHC haplotypes are from the following lines at the Basel Institute for Immunology and the Institute for Animal Health (Compton, UK): B2 haplotype, lines H.B2, 6_1 and 7_2; B4, CC and C-B4; B12, CB and C-B12; B14, H.B14 and WL; B15, H.B15 and 15I; B19, H.B19 and P2a; B21, H.B21, N and 0. Basel and Compton lines were sequenced at the genomic level, and Compton lines at cDNA level as well.

Table S2. Polymorphic residues in chicken TAP1 nucleotide-binding domain

	437	446	455	487	537	C-terminus
B2	R	R	R	R	Q	SGGEG
B4	R	R	R	R	Q	SGGEG
B12	R	Q	R	R	E	IAGVMDGEGRGW
B14	R	R	L	Q	Q	SGGEG
B15	R	R	R	R	Q	SGGEG
B19	R	Q	R	R	E	IAGVMDGEGRGW
B21	W	R	R	R	Q	SGGEG

Table S3. Polymorphic residues in chicken TAP2 tapasin-binding domain

	9	37	64
B2	R	W	H
B4	R	W	R
B12	R	W	R
B14	H	W	H

Table S4. Polymorphic residues in chicken TAP2 membrane-spanning domain

	216	220	248	263	325	351	378	398	406	416	436	446	450
B2	R	I	A	A	D	H	R	R	S	K	A	N	A
B4	R	V	A	A	D	H	Q	R	S	K	V	D	P
B12	R	I	A	A	D	H	Q	H	G	K	A	N	A
B14	G	V	A	T	D	H	Q	R	S	N	A	D	A
B15	G	V	S	T	D	H	Q	R	S	N	A	D	A
B19	R	I	A	A	D	H	Q	H	G	K	A	N	A
B21	R	V	A	A	N	Y	Q	R	S	K	V	D	A

Table S5. Polymorphic residues in chicken TAP2 nucleotide-binding domain

	458	466	478	494	570	579	622	671
B2	V	V	R	G	E	R	H	I
B4	V	I	R	S	E	R	R	V
B12	V	V	R	G	E	R	H	I
B14	M	I	R	G	K	R	H	I
B15	M	I	R	G	E	R	R	I
B19	V	V	C	G	K	R	R	V
B21	V	V	R	G	E	K	R	V