

## Supporting Information

### **A Chicken Tapasin ortholog can chaperone empty HLA-B\*37:01 molecules independent of other peptide-loading components**

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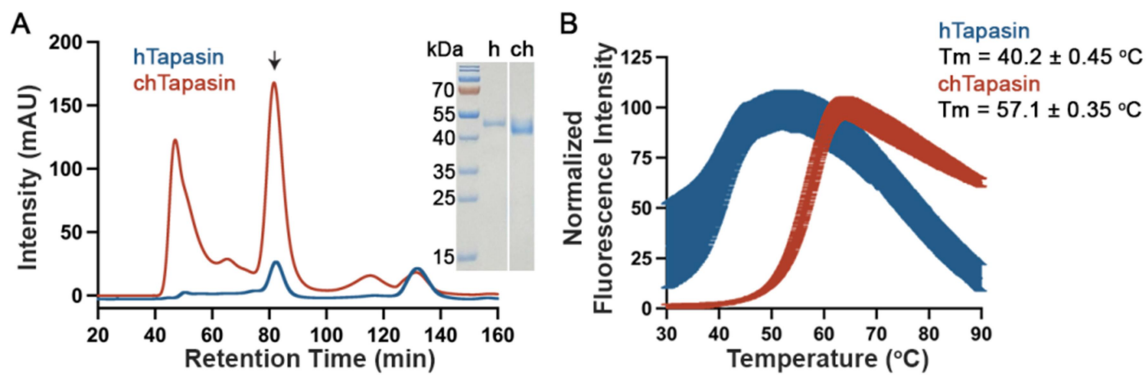
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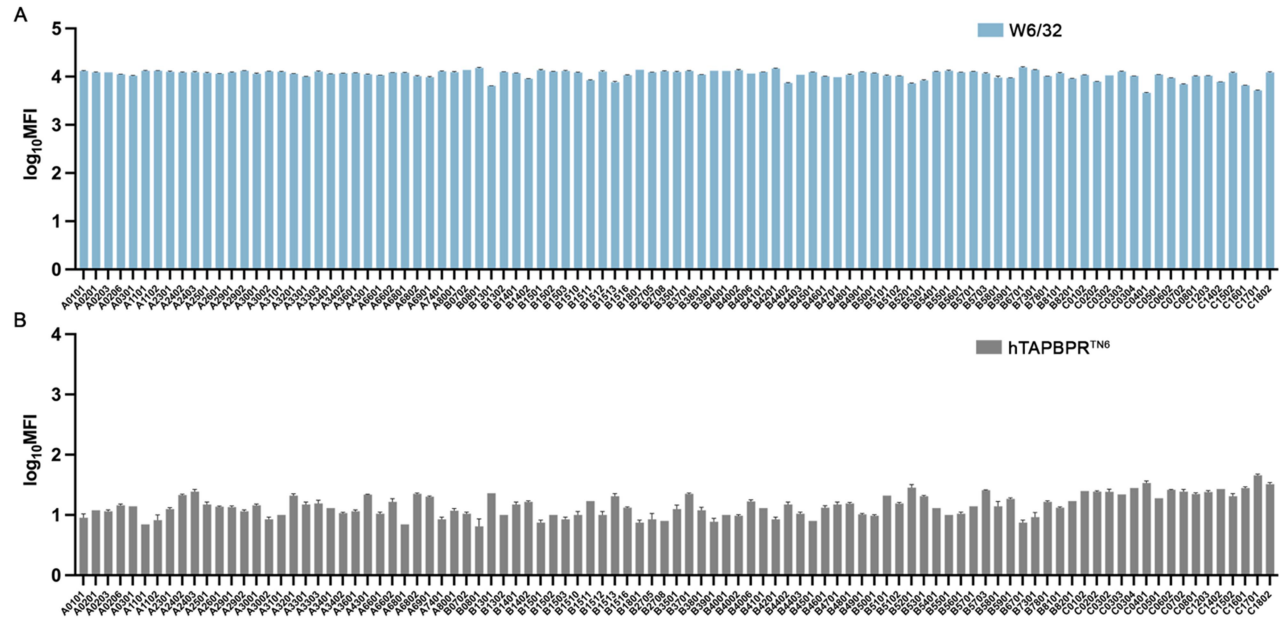
Figures S1 to S5

Table S1

## Figures



**Figure S1. Chicken Tapasin can be expressed in high yields with improved stability.** **A.** Size exclusion chromatography (SEC) traces of human (h) and chicken (ch) Tapasin proteins expressed in insect cells. The protein peaks are indicated by the arrow and were further validated by SDS-PAGE analysis. The additional peaks correspond to protein aggregates (40-70 min). **B.** Differential scanning fluorimetry (DSF) of human versus chicken Tapasin. Melting temperatures are in degrees Celsius ( $T_m$ ). Data are mean  $\pm$  SD obtained from  $n = 3$  independent experiments.



**Figure S2. Binding levels of human and chicken Tapasin on HLA single antigen beads. A.** Similar levels of peptide-loaded MHC-I molecules on the beads were observed upon staining with the primary anti-HLA class I antibody W6/32 conjugated with PE (Biolegend, 311406). **B.** Bar graph showing the binding levels of tetramerized hTAPBPR<sup>TN6</sup> (negative control) to 97 different HLA allotypes on the SABs expressed as logarithm of Mean Fluorescence Intensity (MFI). The plotted data are mean  $\pm$  SD of  $n = 3$  independent experiments.

7TUE  $\beta 1$   $\beta 2$

1 10 20 30

hTapasin . . . . . GP AVIEWFVE DASGKGL . . . . . AKRPGA LLLR QGPG . . . . .

chTapasin AS PPPP . . . . . P APVRCALLEGVGRGG LFGGGNAR PALTRFGGDAE . . . . .

hTAPBPR . . KHPAEGQWRAVD VVLD CFLVKDGAHR GALASSEDRAAS LVLK QVPVLDDGSLEDFTD

chTAPBPR VE GLTPVPELRRVD VVLGCSYVWEGGLSRA FGGSEH . . PATL VLR GLSVTDDGTLGDVTD

▲▲▲▲▲ ▲▲▲

7TUE  $\beta 3$   $\alpha 1$   $\beta 4$   $\eta 1$

40 50 60 70 80 90

hTapasin . . E P P P P R D L D P E L Y L S V H D P A G A L Q A A F . . R Y P R G A P A P H C F M S R F V P L P A S A K W A S G

chTapasin . . T . P P E P G P E P E V T F N V S D P W G T L . . . . . T P L G V P P R T P P S C E L N P T N P Q T G S D P W S R P

hTAPBPR F Q G G T L . A Q D D P I I F E A S V D L V Q I P Q A E A L L H A D C S G K E V T C E I S R Y F L Q M T E . . . . .

chTAPBPR Y E I P Q A D H S S S P I I F E A S E Q L V S I P Y A E A L L H V D C S G E E V S C E L S P Y S F Q Q E G . . . . .

▲▲▲▲▲ ▲▲▲

7TUE  $\eta 2$   $\beta 5$   $\beta 6$

90 100 110 120 130

hTapasin L T P A Q N C P R A L D G A W L M V S I S S P V L S L . . S L L R P . . . . . Q P E P Q Q E P V

chTapasin L H P D A R S P P T A G G Q W W V A A V G T P Q Y . . . . . G V T A L L Q G G M G T E

hTAPBPR . . . . . T T V K T A A W F M A N V Q V S G G G P S I S L V M K T P R . . . V A K N E V L W H P T L N L F L S P Q

chTAPBPR . . . . . N G L C S A S W F L A T I R L S S G I S . I V L L L R G P S C S S Q K E G H D V T L H P K L R I P M S K E

▲▲▲▲▲

7TUE  $\beta 7$   $\beta 8$   $\beta 9$   $\beta 10$   $\beta 11$

140 150 160 170 180 190

hTapasin L I T M A T V V L T V L T H T P A P R V R I G Q D A L L D L S F A Y M P P T S E A A S S L A P G P P P F G L E W R R O H

chTapasin G T I T A A V A L A V L T H T P T L R A R V G S P I H L H C A F A A P . . . . . P S S F . . V L E W R H Q N

hTAPBPR G T V R T A V E F Q V M T Q T Q S L S F L L G S S A S L D C G S M A . . . . . P G L D L I S V E W R L Q H

chTAPBPR G T L L T T V E F Q S S S N N T S L R T R L G S S I T L D C H E A L A . . . . . P S F L L S S L E W R R O H

▲▲▲▲▲

7TUE  $\beta 12$   $\beta 13$   $\beta 14$

200 210 220 230 240

hTapasin L G K G H L L L A A T P G L N G Q M P . . A A Q E G A . V A F A A W D D E P W G P W T G N G T F W L P T V Q P F Q E G

chTapasin R G A G R V L L A Y D S S T A R A P . . . R A T P G A E L L L G T R D G . . . . . D G V T A V T L R L A R P S P G D E G

hTAPBPR K G R G Q L V Y S W T A G Q G Q A V . R K G A T L E P A Q L G M A R D . . . . . A S L T L P G L T I Q D E G

chTAPBPR R G S G R S L F R Y R V G N A G L T A Q P K V H V D V E Q L L G N G D . . . . . A S L T L Q E A T V N D E G

▲▲▲▲▲

7TUE  $\beta 15$   $\beta 16$   $\beta 17$   $\beta 18$

250 260 270 280 290 300

hTapasin T Y L A T I H L P Y L Q G Q V T L E L A V Y K P P K V S L M P A T L A R A A P G E A P P E L L C L V S H F Y P S G L E

chTapasin T Y I C S V F L P H G H T Q T V L Q L H V F E P P K V T L S P K N L V A . . P G M S A E L R C H V S G F Y P L D . V T

hTAPBPR T Y I C Q I T T S L Y R A Q Q I I O L N I Q A S P K V R L S L A N E A . . . . . L L P T L I C D I A G Y P L D . V V

chTAPBPR T Y I C L V S T A Q H Q V Q H N I O L V S E P P R V R V F T E A S L K . . R D E T I T L T C N I A G Y P L D . I S

▲▲▲▲▲

7TUE  $\beta 19$   $\beta 20$   $\beta 21$   $\beta 22$

310 320 330 340 350 360

hTapasin V E W E L R G G P G G R S Q K A E . . . Q R W L S A L R H H S D G S V S L S G H L Q P P P V T T E Q H G A R Y A C R I

chTapasin V T W Q R R A G G S G T S R S P R D T V M D S W T S G H R Q A A D G T Y S R T A A R L I P A R P Q H H G D V Y S C V V

hTAPBPR V T W T R E E L G G S . P A Q V S G A . . . S F S S L R O S V A G T Y S I S S S L T A . E . . P G S A G A T Y T C Q V

chTAPBPR V S W I Q K T P E D E V E I S P S N T . . . . . R F S S H R O S Q D G T Y S I N S Y L S V . N L A T A Q A P A T Y T C H V

▲▲▲▲▲

7TUE  $\beta 23$

370 380

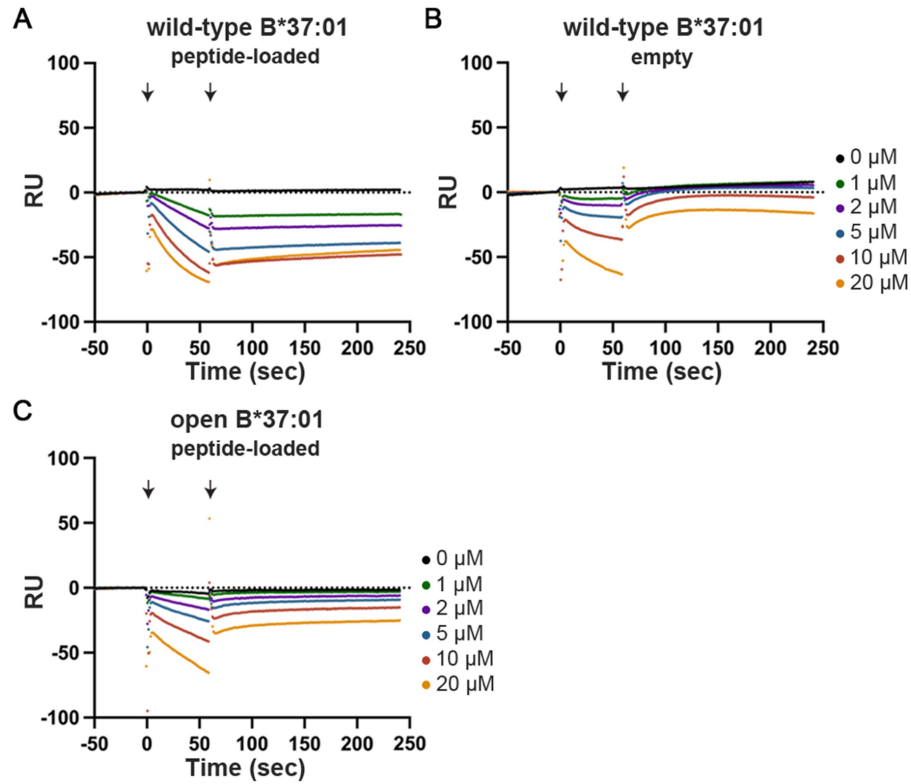
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chTapasin T H T A L A K P M R V S V R L L L A G T E G P H L E D

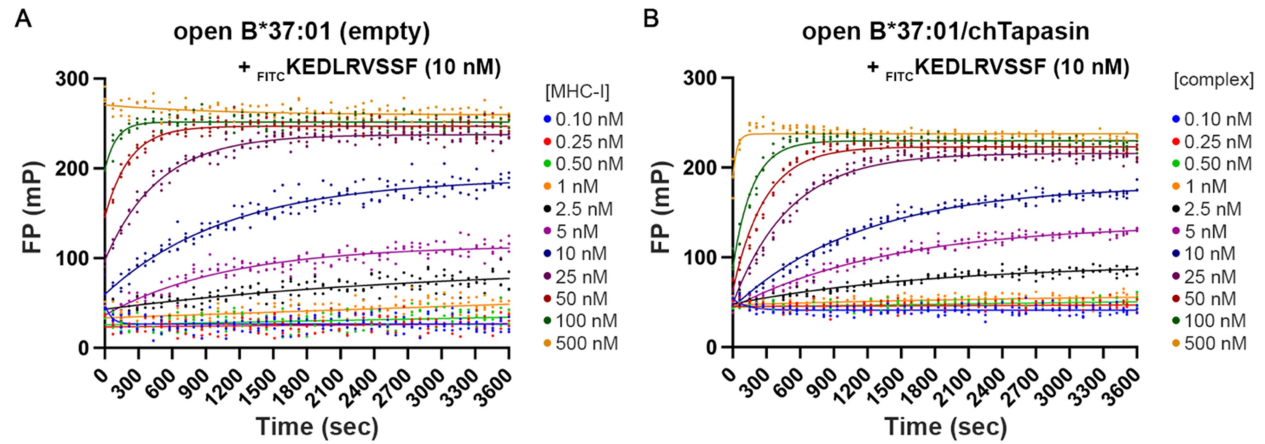
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chTAPBPR S H V A L E A P I S I S T H L K A P E H T . . E L E .

**Figure S3. Sequence alignment of human and chicken Tapasin and TAPBPR.** Alignment of the luminal domains of human Tapasin (hTapasin; UniProt: O15533), chicken Tapasin (chTapasin; UniProt: A4F5A9), human TAPBPR (hTAPBPR; UniProt: Q9BX59), and chicken TAPBPR (chTAPBPR; NP\_001382952.1). Conserved or polymorphic residues between human and chicken Tapasin on the interface with HLA-B\*37:01 are shown in black or red, respectively. The secondary structure of human Tapasin (PDB ID: 7TUE) is provided as reference (1). Conserved residues are marked in blue boxes. Alignment was performed in ClustalOmega (2) and processed in ESPript (3).



**Figure S4. Chicken Tapasin interactions are restricted to the open, empty B\*37:01.** SPR sensorgrams of varying concentrations of soluble wild-type **A.** peptide-loaded, and **B.** -deficient B\*37:01 or **C.** open, peptide-loaded B\*37:01 flown over a streptavidin chip coupled with biotinylated chTapasin. Injection and washing start points are indicated by arrows. RU, resonance units. The plotted data are mean  $\pm$  SD of  $n = 3$  independent experiments.



**Figure S5. The open B\*37:01/chTapasin complex is peptide receptive.** Association profile of the fluorophore-conjugated peptide  $_{FITC}$ KEDLRVSSF (10 nM) to a series of **A.** open, empty B\*37:01, and **B.** open B\*37:01/chTapasin complex concentrations. The data were fitted to a one-phase association model. Data from  $n = 3$  technical replicates are plotted. FP, fluorescence polarization.

**Table S1.** Summary of MHC-I contact residues with hTapasin (4) from all HLA allotypes on the SABs. We can distinguish 12 distinct groups (A-L) based on their polymorphic sites (P), which are highlighted in bold in the amino acid sequence.

Group	HLA allotypes	P	Amino Acid Position [111, 113, 127, 128, 131, 135, 136, 141, 142, 144, 145, 193, 195, 197, 200, 202, 212, 225, 226, 229, 231, 234, 244, 248]
<b>A</b>	<b>B*37:01</b> , B*14:01, B*14:02, B*15:02, B*15:13, B*27:05, B*27:08, B*44:02, B*44:03, B*45:01, B*47:01, B*49:01, B*50:01	-	RYNESAAQIQRPSHTRETQEVRRV
<b>B</b>	B*15:01, B*15:03, B*15:10, B*15:11, B*15:12, B*15:16, B*18:01, B*35:01, B*38:01, B*39:01, B*46:01, B*51:01, B*51:02, B*52:01, B*53:01, B*54:01, B*55:01, B*56:01, B*57:01, B*57:03, B*58:01, B*59:01, B*67:01, B*78:01, B*82:01	Y113H	<b>R</b> HNESAAQIQRPSHTRETQEVRRV
<b>C</b>	A*30:01, A*30:02, B*73:01, C*02:02, C*03:02, C*03:03, C*03:04, C*04:01, C*05:01, C*06:02, C*07:02, C*08:01, C*12:03, C*14:02, C*17:01, C*18:02	S131R	RYNERAAQIQRPSHTRETQEVRRV
<b>D</b>	B*07:02, B*08:01, B*40:01, B*40:02, B*40:06, B*41:01, B*42:01, B*48:01, B*81:01, C*15:02	Y113H, S131R	<b>R</b> HNERAAQIQRPSHTRETQEVRRV
<b>E</b>	A*01:01, A*03:01, A*11:01, A*11:02, A*36:01, A*80:01	S131R, Q144K	RYNERAAQ <b>I</b> KRPSHTRETQEVRRV
<b>F</b>	A*25:01, A*26:01, A*29:01, A*29:02, A*31:01, A*32:01, A*33:01, A*33:03, A*34:01, A*34:02, A*43:01, A*66:01, A*66:02, A*74:01	S131R, P193A	RYNERAAQIQR <b>A</b> SHTRETQEVRRV
<b>G</b>	C*16:01	S131R, P193L	RYNERAAQIQ <b>R</b> LSHTRETQEVRRV
<b>H</b>	B*13:01, B*13:02	Y113H, R145L	<b>R</b> HNESAAQIQ <b>L</b> PSHTRETQEVRRV
<b>I</b>	A*23:01	N127K, S131R	RY <b>K</b> ERAAQIQRPSHTRETQEVRRV
<b>J</b>	A*24:02, A*24:03	N127K, S131R, Q144K	RY <b>K</b> ERAAQ <b>I</b> KRPSHTRETQEVRRV
<b>K</b>	A*02:01, A*02:03, A*02:06,	N127K,	RY <b>K</b> ERAAQ <b>T</b> KHASHTRETQEVRRV



	A*68:01, A*68:02, A*69:01	S131R, I142T, Q144K, R145H, P193A	
<b>L</b>	C*01:02	V248M	<b>RYNERAAQIRPSHTRETQEV RWM</b>

## References

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