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Chen, L. *et al.* (2013) Endoplasmic reticulum aminopeptidase 1 (ERAP1) plays a critical role in determining the length and sequence of peptides bound and presented by HLA-B27.

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Supplementary materials

Table S1. Protein ID and accession numbers of extended peptides eluted from HLA-B27.

Figure S1. Stable and specific silencing of endogenous ERAP1 expression in HeLa.B27 and C1R.B27 cells transduced with lentiviral ERAP1-shRNA. Western blot showing stably silenced ERAP1 in (A) HeLa.B27 cells (B) C1R.B27 cells. Approximately 85% and 80% of ERAP1 expression was suppressed in HeLa.B27 and C1R.B27 cells respectively (quantified with ImageJ). (C) HeLa.B27 do not express ERAP2, however ERAP1 silencing shRNA does not have any effect on LNPEP or NPEPPS expression. (D) Western blot showing no change in ERAP2, LNPEP or NPEPPS expression in the presence of ERAP1-silencing shRNA.

Figure S2. ME1 stains only HeLa.B27 and C1R.B27 cells, W6/32 stains HeLa and C1R but to a lesser extent compared to cells expressing B27. ME1 and W6/32 were used to stain HeLa.B27/C1R.B27 and HeLa/C1R cells. Results shown are representative of n=3 experiments.

Figure S3. Extended peptides lacking Arginine at position 2 bind poorly to HLA-B27. T2.B27 cells were incubated with 100 μ M of various synthetic peptides for 3 hours before harvested for ME1 staining of cell surface HLA-B27 expression. The identified LRNQSVFNF, SR-LRNQSVFNF, GRLAAIVAK and GRLAAIVAK-QV were synthesized and tested for ability to stabilize HLA-B27 expression. An 11mer LL-GRLAAIVAK corresponding to the theoretical N-terminally extended version (not identified) of eluted GRLAAIVAK, and two artificial mutated control peptides (SA-LRNQSVFNF and LR-GRLAAIVAK) were also synthesized and tested. Mean and standard deviation MFI values are shown; $P < 0.001$ indicated using two-way ANOVA. Representative experiment of 3 shown.

Figure S4. Quantities of extended peptides are increased following ERAP1-silencing. Extracted ion chromatograms showing relative abundance of three representative peptide pairs (canonical 9mer and extended peptides) are shown comparing between control-shRNA and ERAP1-shRNA transduced cells. The 'core' sequences of the extended peptides are underlined. GRLPSSWALFSP, TRVMAPRTLIL and SRLRNQSVFNF are derived from ITFG1 (Q8TB96), HLA-C (P30504) and FDFT1 (P37268) shown in Table S1 respectively.

Figure S5. ERAP1 silencing effect does not substantially alter expression levels of cell surface HLA-B27 by HeLa.B27 and ERAAP-/- mFib B27 cells. ME1 antibody staining is shown. (A) ERAP1-shRNA or ctr-shRNA HeLa.B27 cells. (B) ERAAP-/- mFib B27 transduced with WT-ERAP1 or ctr-plasmid.

Figure S6. ERAP1 silencing reduces presentation of the KK10 B27 epitope to CTLs following transfection with minigenes expressing N-terminally extended peptides. HeLa.B27 transduced with ctr-shRNA or ERAP1-shRNA were transfected with minigenes to express an N-terminally extended KK10 15mer either in the cytosol (X5-KK10) or directed to the ER (ES-X5-KK10). 2 days after transfection, HeLa.B27 cells were used to activate KK10-CTLs (1×10^4 CTLs per well, target: effector = 20, 2 or 0.2). The amount of IFN-gamma secreted into medium was measured by ELISA. Mean and standard deviation were shown,

two-way ANOVA was used.

Figure S7. The Ankylosing Spondylitis protective variant K528R-ERAP1 decreases presentation of KK10 to CTLs. ERAP1^{-/-} mFib.B27 were first co-transfected with minigenes and ERAP1 variants (WT-ERAP1, K528R-ERAP1, R725Q-ERAP1, K528R/Q730E-ERAP1 and P127R/K528R/Q730E-ERAP1), then used to activate KK10 CTLs. (A) Mean and standard deviation of IFN-gamma ELISPOT are shown, two-way ANOVA was used. Experiment was repeated 3 times. (B) Western blot showing that ERAP1 variants have been expressed at similar level. (C) Cell surface HLA-B27 expression of ERAP1^{-/-} mFib.B27 transfected with ERAP1 variants. ME1 antibody was used to stain cell surface HLA-B27 of ERAP1^{-/-} mFib.B27 transfected to express ERAP1 variants (WT-ERAP1, K528R-ERAP1, R725Q-ERAP1, K528R/Q730E-ERAP1 and P127R/K528R/Q730E-ERAP1).

Table S1. Protein ID and accession number of extended peptides eluted from HLA-B27.

Peptide sequence	Reference	Protein	Accession Number
<i>HeLa.B27 ME1 - Ctr-shRNA (upper) vs ERAP1-shRNA (lower)</i>			
Extended peptides identified in ERAP1-silenced cells			
K R L E G L S K	This study	Ceramide synthase 5 (CERS5)	Q8N5B7
*K K R L E G L S K	This study		
G R I D K P I L K	(28, 32, 33)	60S ribosomal protein L8 (RPL8)	P62917
G R I D K P I L K A	(32)		
F R V P F I Y G H	(32)	Synaptic glycoprotein SC2 (TECR)	Q9NZ01
F R V P F I Y G H K Y	This study		
G R L P S S W A L	This study	T-cell immunomodulatory protein (ITFG1)	Q8TB96
G R L P S S W A L F S P	This study		
A R L D H K F D L	(32)	Tubulin alpha-1A chain (TUBA1A)	Q9BQE3
A R L D H K F D L M	(32)		
L R F P G Q L N A	(32)	Tubulin beta chain (TUBB)	P07437
L R F P G Q L N A D L	This study		
**A R F S P D G Q Y L	(28,32)	WD40 repeat-containing protein SMU1	Q2TAY7
**A R F S P D G Q Y L V	(32)		
**G R I L S G V V T K	(28,32,34)	40S ribosomal protein S11 (RPS11)	P62280
**G R I L S G V V T K M	(32)		
A R L D H K F D L M	(32)	Tubulin alpha-1A chain (TUBA1A)	Q9BQE3
A R L D H K F D L M Y	(32)		
A R L D H K F D L M Y A	This study		
G R W G P T F P S S Y	(32)	Coiled-coil domain-containing protein 34 (CCDC34)	Q96HJ3
G R W G P T F P S S A G F	This study		
Extended peptides identified in non-silenced cells			
S R V N I P K V L R	(32)	Clathrin heavy chain 1	Q00610
S R V N I P K V L	(28,32)		
S R F D A S F F G V H P K	(32)	Fatty acid synthase	P49327
S R F D A S F F G V	(32)		
<i>C1R.B27 ME1 - Ctr-shRNA vs ERAP1-shRNA</i>			
Extended peptides identified in ERAP1-silenced cells			
**L R V T P F I L K	(32)	Mitochondrial import receptor subunit TOM5 homolog	Q8N4H5
**L R V T P F I L K K	This study		
**G R L A A I V A K	(28,32)	60S ribosomal protein L13a (RPL13A)	P40429
**G R L A A I V A K Q V	This study		
**V R L L L P G E L A K	(28,32)	Histone H2B type 2-F (HIST2H2BF)	Q5QNW6
**V R L L L P G E L A K H	(32)		
Extended peptides identified in non-silenced cells			
F R Y N G L I H R K	(28, 32, 33)	60S ribosomal protein L28(RPL28)	P46779
F R Y N G L I H R	This study		

C1R.B27 W632 - Ctr-shRNA vs ERAP1-shRNA

Extended peptides identified in ERAP1-silenced cells

A H E V G W K Y *G R L A H E V G W K Y	This study (32)	60S ribosomal protein L13a (RPL13A)	Q8N4H5
Q I E D F E A R *R R L Q I E D F E A R	This study (32)	NADH dehydrogenase 1 alpha subcomplex subunit 13	Q9P0J0
L R N Q S V F N F *S R L R N Q S V F N F	(28,32) This study	Farnesyl-diphosphate farnesyltransferase 1 (FDFT1)	P37268
V M A P R T L I L *M R V M A P R T L I L	This study This study	HLA class I histocompatibility antigen (HLA-C)	P30504
L R V T P F I L K L R V T P F I L K K	(32) This study	Mitochondrial import receptor subunit TOM5 homolog	Q8N4H5
G R L A A I V A K G R L A A I V A K Q V	(28,32) This study	60S ribosomal protein L13a (RPL13A)	P40429
G R W P G S S L Y G R W P G S S L Y Y	(28) (28,34)	Lamin-B receptor (LBR)	Q14739
L R Y P M A V G L L R Y P M A V G L N K	(28,32) This study	60S ribosomal protein L36 (RPL36)	Q9Y3U8
S R I H P V S T M S R I H P V S T M V K	(28,32) (32)	L-lactate dehydrogenase B chain (LDHB)	P07195
A R A T L S S I R H A R A T L S S I R H M I R	This study This study	60S ribosomal protein L28 (RPL28)	P46779
A R F S P D G Q Y L A R F S P D G Q Y L V	(28,32) (32)	WD40 repeat-containing protein SMU1(SMU1)	Q2TAY7
G R I L S G V V T K G R I L S G V V T K M	(28,32,34) (32)	40S ribosomal protein S11 (RPS11)	P62280
G R W P K K S A E F G R W P K K S A E F L L	This study This study	60S ribosomal protein L17 (RPL17)	P18621
A R L H P R A V N C A R L H P R A V N C R	This study This study	Ubiquitin-60S ribosomal protein L40 (UBA52)	P62986
V R L L L P G E L A K V R L L L P G E L A K H	(28,32) (32)	Histone H2B (HIST1H2B)	Q5QNW6

Extended peptides identified in non-silenced cells

None

Data from 3 experiments (HeLa.B27-ME1, C1R.B27-ME1 and C1R.B27-W6/32 immuno-precipitation) were shown. Datasets of peptides identified from control (upper of each pair) and ERAP1-shRNA silenced cells (lower in bold) were cross searched for extended forms. 24 extended peptides were identified from ERAP1-silenced cells, of which only 5 were N-terminally extended (marked *). Only 3 from control-shRNA-transduced cells were identified. 5 pairs of peptides were identified in more than one experiments (marked **).

Fig. S1.

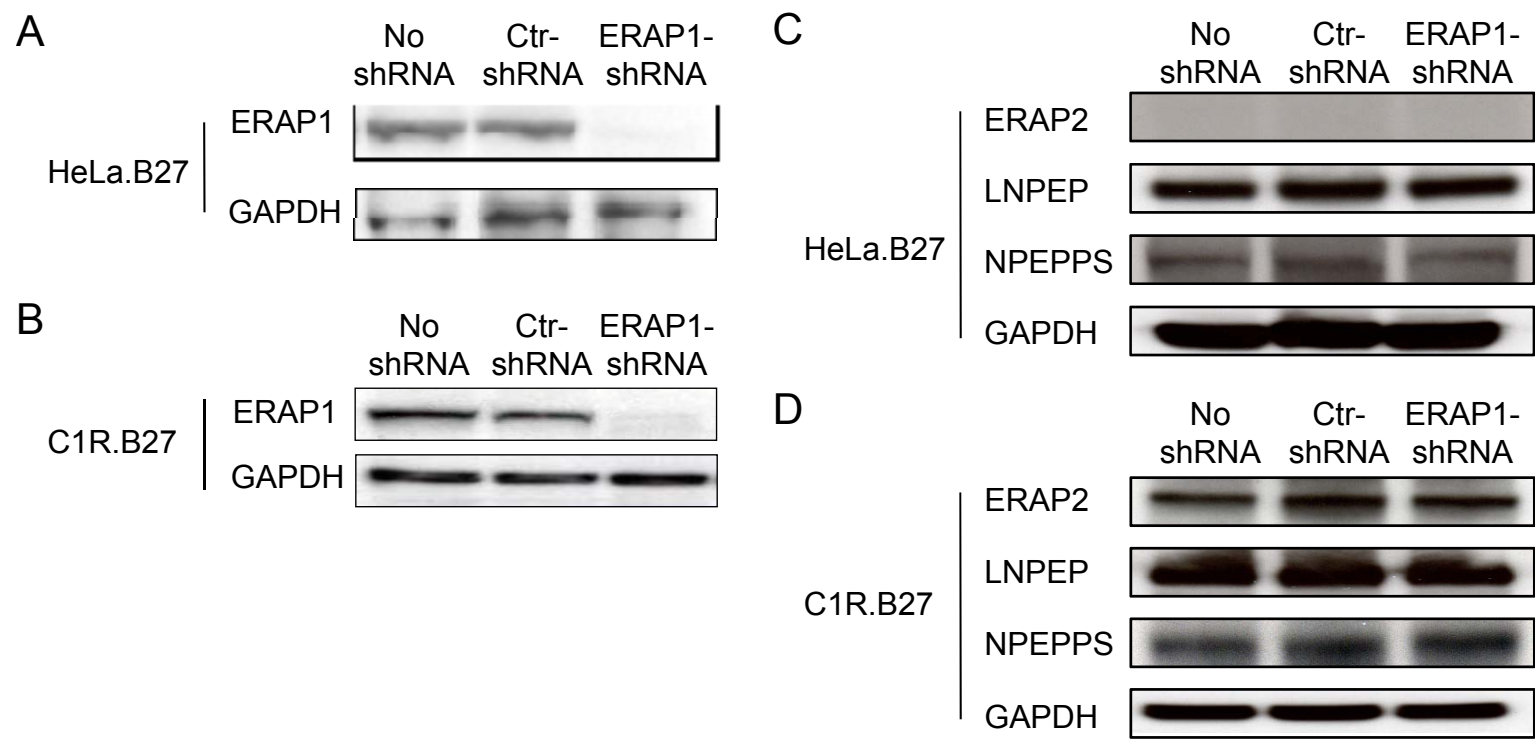


Fig. S2.

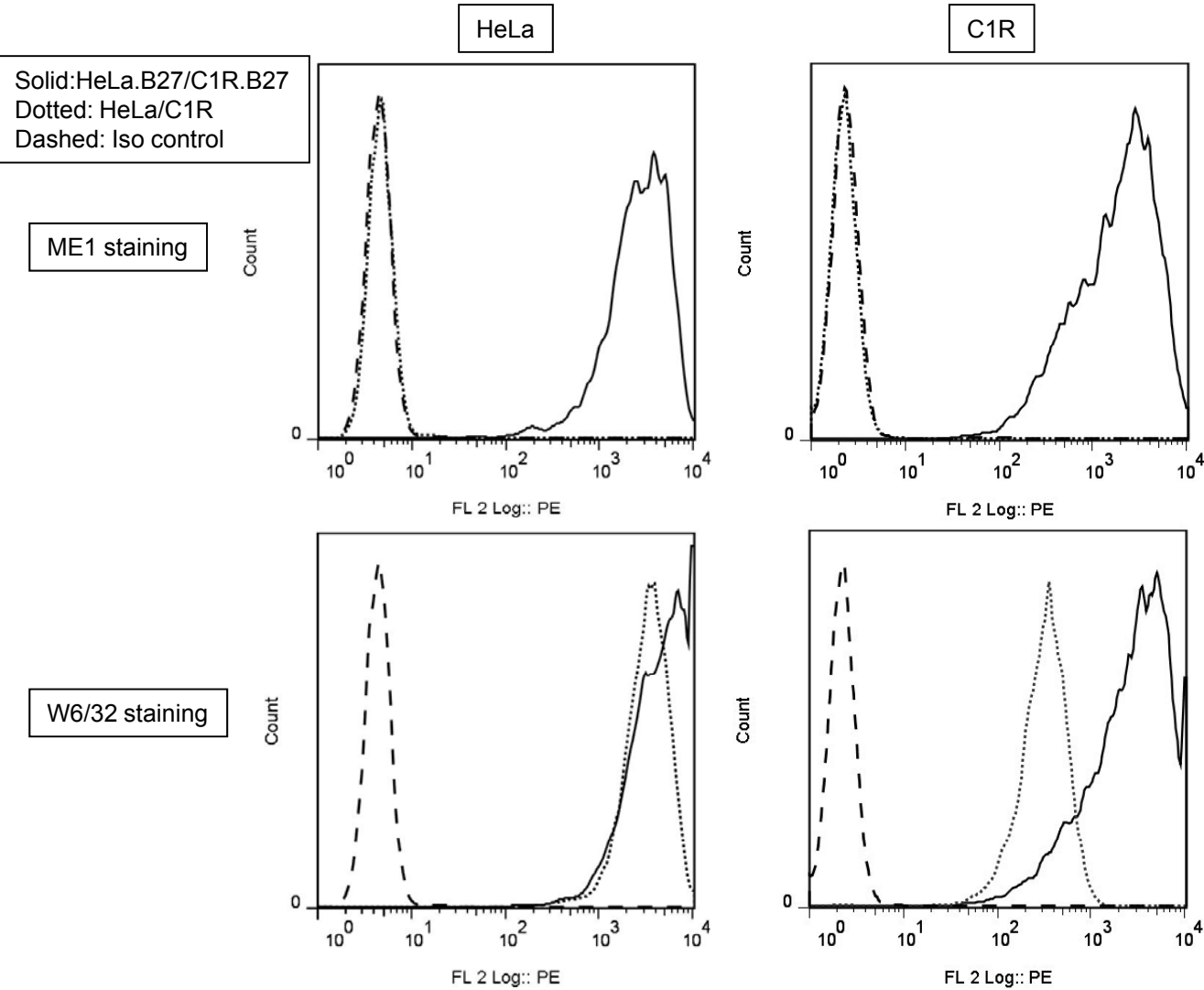


Fig. S3.

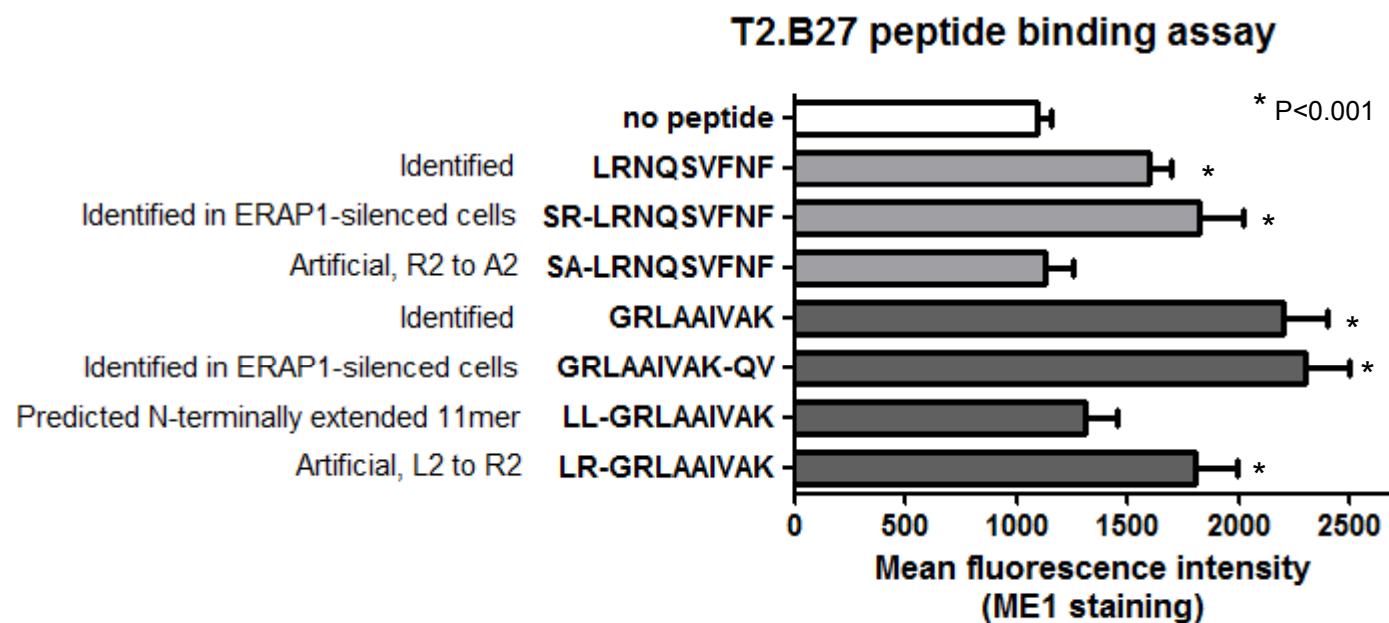


Fig. S4.

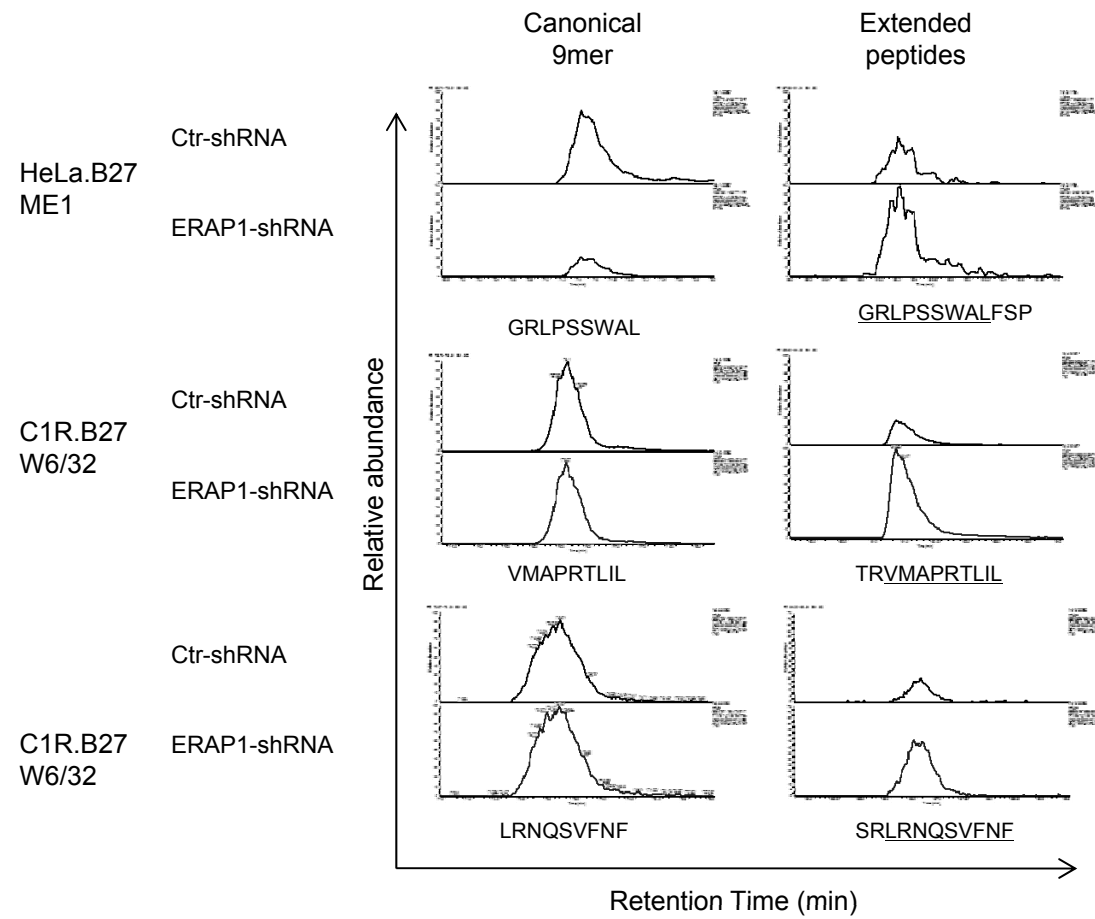


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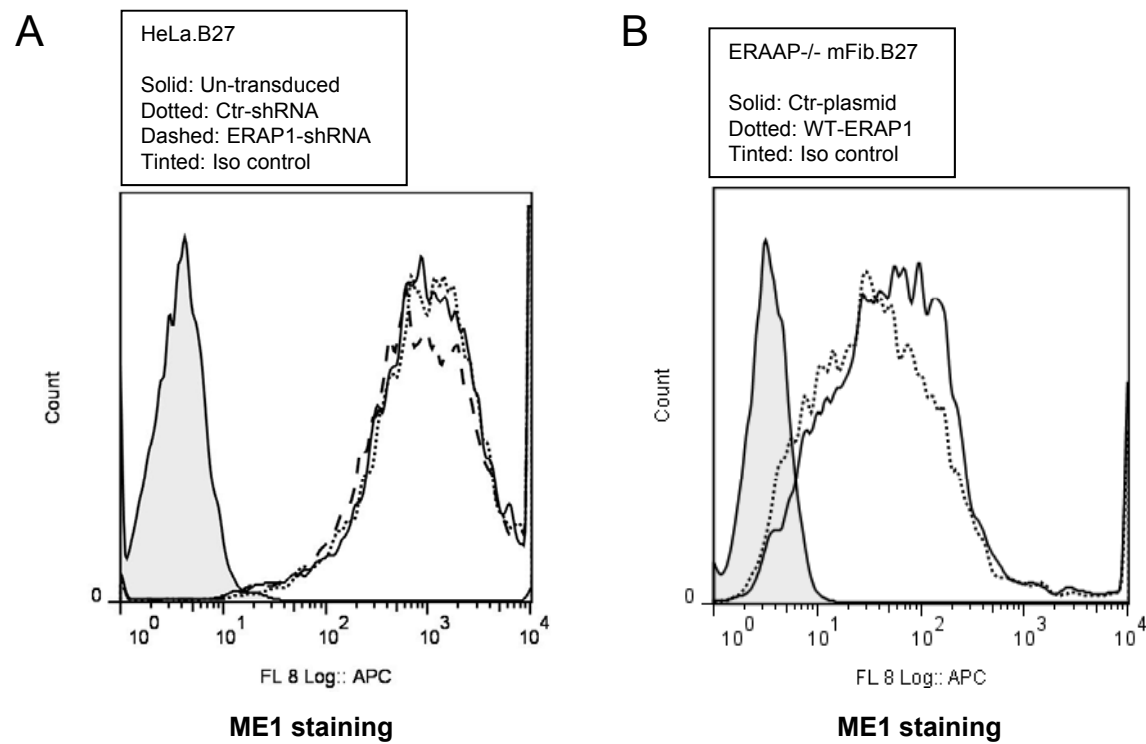


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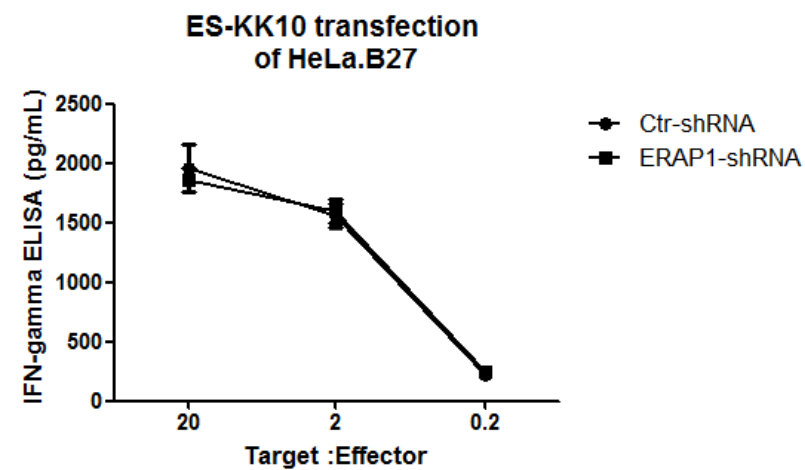
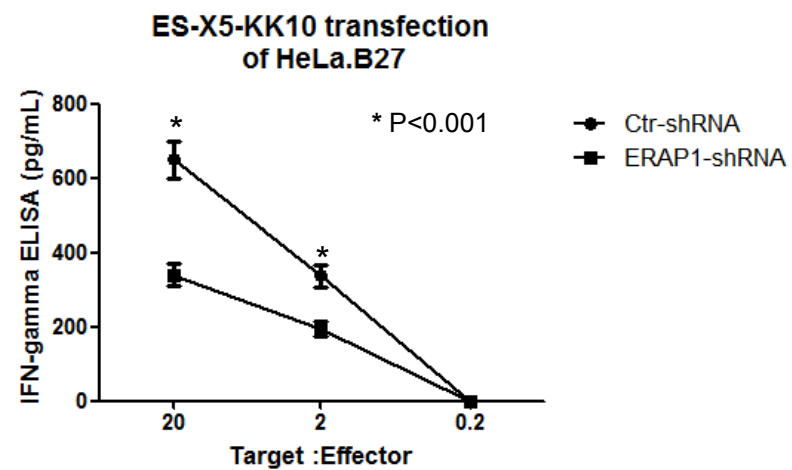
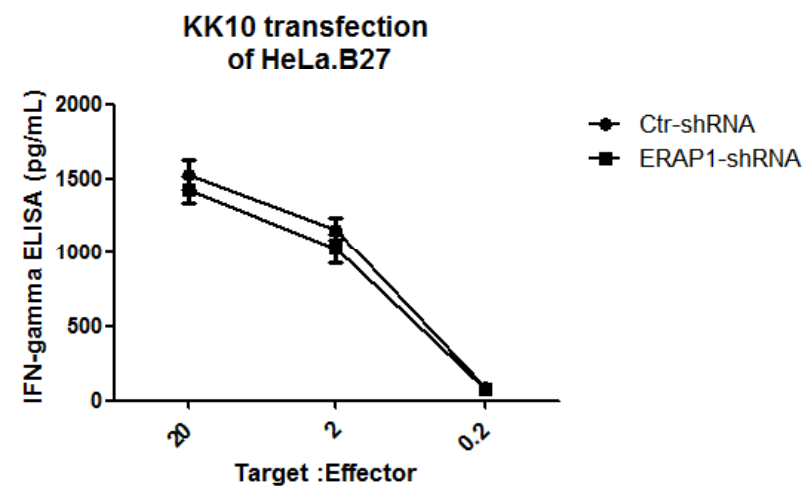
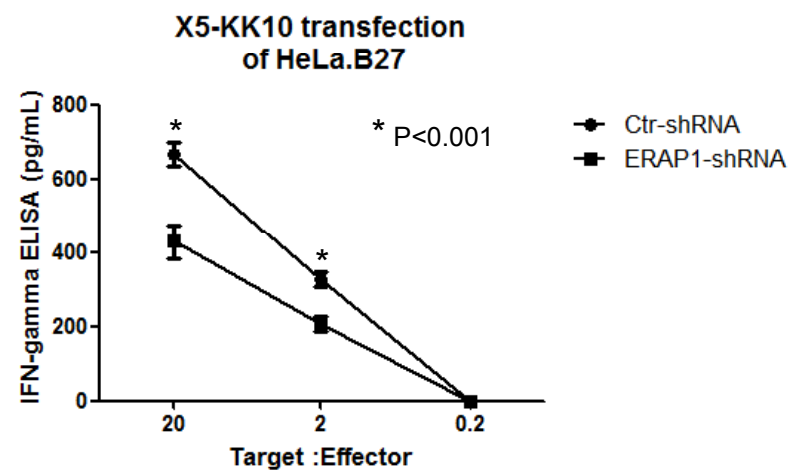


Fig. S7.

