Calreticulin retrieves suboptimally loaded MHC class I molecules from the Golgi apparatus

Christopher Howe*, Malgorzata Garstka*, Mohammed Al-Balushi, Esther Ghanem, Antony N. Antoniou, Susanne Fritzsche, Gytis Jankevicius, Nasia Kontouli, Clemens Schneeweiss, Anthony Williams, Tim Elliott#, and Sebastian Springer#

Supplementary Information
Legends for Supplementary Figures

Figure S1: Transfected H-2K\(^b\)-GFP molecules are peptide-receptive in a post-ER compartment of COS cells. COS cells were transfected with H-2K\(^b\)-GFP and stained with the K\(^b\)-specific peptide, SIINFEKL, labeled with the fluorescent dye, TAMRA. The inserts show overlays of the two channels. The bottom row shows a field of cells that do not express H-2K\(^b\)-GFP (insert: phase contrast image). Scale bars, 20 µm.

Figure S2: Calreticulin colocalizes with peptide-receptive class I molecules in T2 cells. T2 cells were stained for endogenous HLA-B*5101 (with MAb HC10) and for calreticulin (PA3-900). Accumulations of calreticulin (arrows) colocalize with accumulations of HLA-B. Scale bar, 20 µm.

Figure S3: The calreticulin mutant HA\(\Delta\)KDEL accumulates in the cis-Golgi during a 20 ºC transport block. K42 CRT-HA\(\Delta\)KDEL cells were kept at 37 ºC or 20 ºC and stained for calreticulin and organelle markers.

Figure S4: Calreticulin accumulates in COS cells that express a single chain fusion of H-2D\(^b\)(T134K)-GFP. COS cells were transfected with single chain (sc) H-2D\(^b\)(T134K)-GFP and stained for calreticulin and the cis-Golgi marker, giantin. Scale bar, 10 µm.

Figure S5: Wild type calreticulin and HA-tagged transfected calreticulin (CRT-HAKDEL) have the same distribution in fibroblasts. K41 cells and K42 CRT-HAKDEL cells were kept at 37 ºC or 20 ºC and stained with PA3-900 antiserum or with anti-HA antibody and ER or Golgi markers.

Figure S6: Model of calreticulin-mediated class I recycling. MHC class I heavy chains (HC, red) assemble with beta-2 microglobulin (β\(_2\)m, pink) and the PLC in the ER. Optimally (blue pentagon) and suboptimally loaded (green triangle) class I/β\(_2\)m dimers (the latter in complex with calreticulin, CRT) exit the ER into COPII vesicles, carrying it to the ERGIC and the cis-Golgi. In the cis-Golgi, suboptimally loaded
class I accumulate prior to their retrieval, which is mediated by the KDEL tail of calreticulin. Optimally loaded class I molecules continue to the cell surface.
Howe et al.
Figure S1

H-2K^b-GFP | SIINFEKL-TAMRA
Howe et al.
Figure S2
Howe et al.

Figure S3

<table>
<thead>
<tr>
<th></th>
<th>K42 CRT-HA(\Delta)KDEL cells, 37 ºC</th>
<th>K42 CRT-HA(\Delta)KDEL cells, 20 ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Organelle</td>
<td>Calreticulin</td>
</tr>
<tr>
<td>ER</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>cis-Golgi</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td>Early Endosomes</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
</tr>
<tr>
<td>Lysosomes</td>
<td><img src="image19" alt="Image" /></td>
<td><img src="image20" alt="Image" /></td>
</tr>
</tbody>
</table>
β2m-H-2D^b(T134K)-GFP Calreticulin cis-Golgi Overlay
Howe et al.
Figure S5
Howe et al.
Figure S6