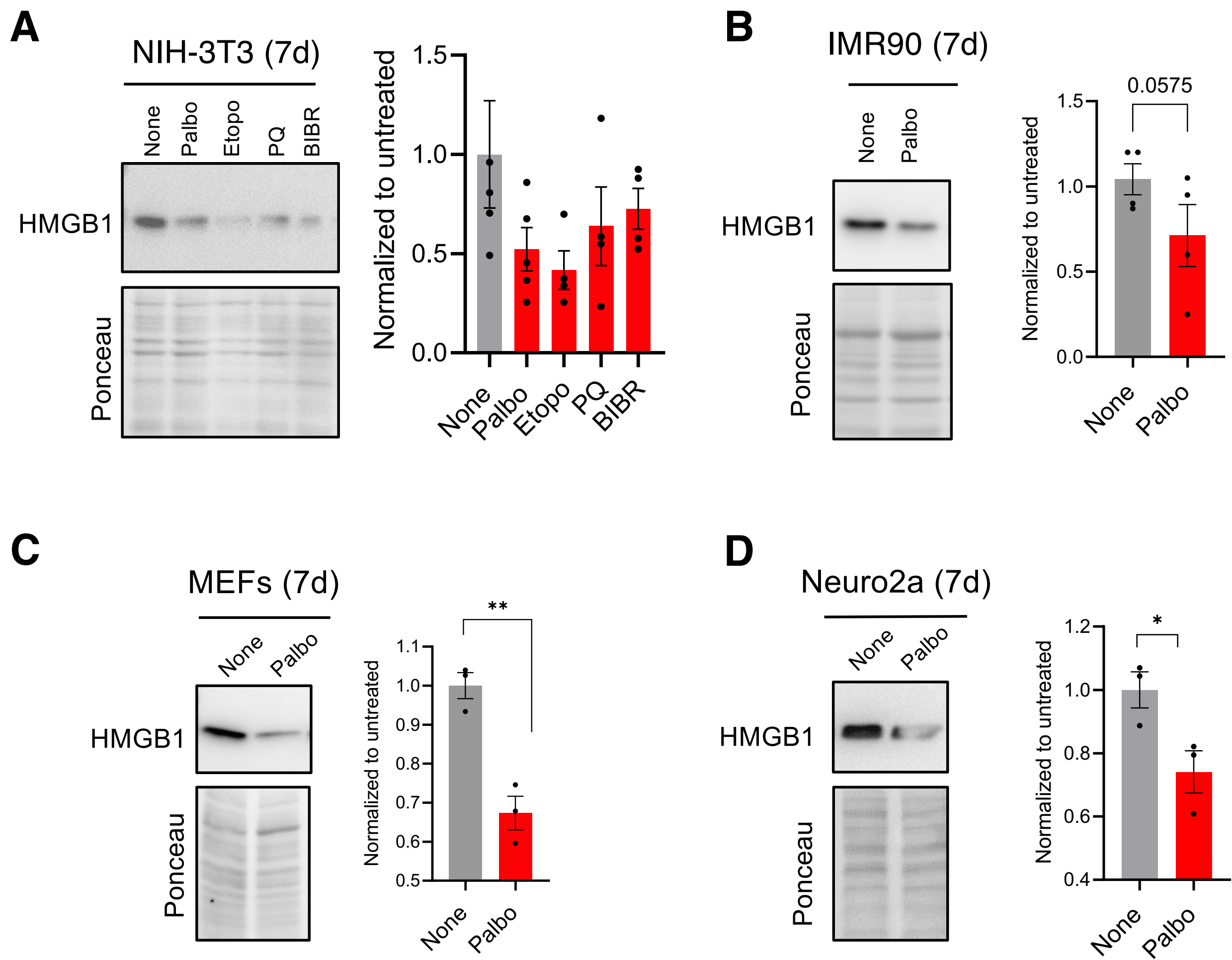


Supplementary Figure 1

Supplementary Figure 1.

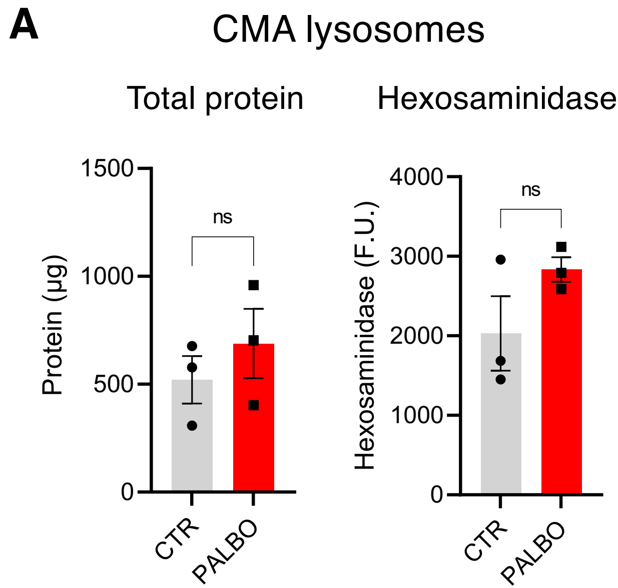
A, *Left:* Representative immunoblot for HMGB1 of NIH3T3 cells untreated (None) or 7 days after exposure to the indicated agents. Ponceau staining is shown as loading control. *Right:* Quantification of HMGB1 levels from n = 4 independent experiments. Values are expressed relative to untreated cells and are individual values and mean ± SEM.

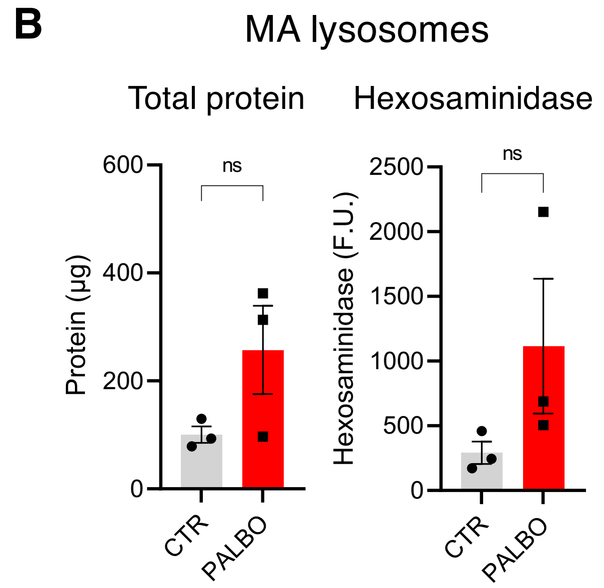
B, *Left:* Representative immunoblot for HMGB1 of human fibroblasts (IMR90 population doubling level (PDL) 20 untreated (None) or 7 days after exposure to palbociclib. Ponceau staining is shown as loading control. *Right:* Quantification of HMGB1 levels from n = 4 independent experiments. Values are expressed relative to untreated cells and are individual values and mean ± SEM of three independent experiments.

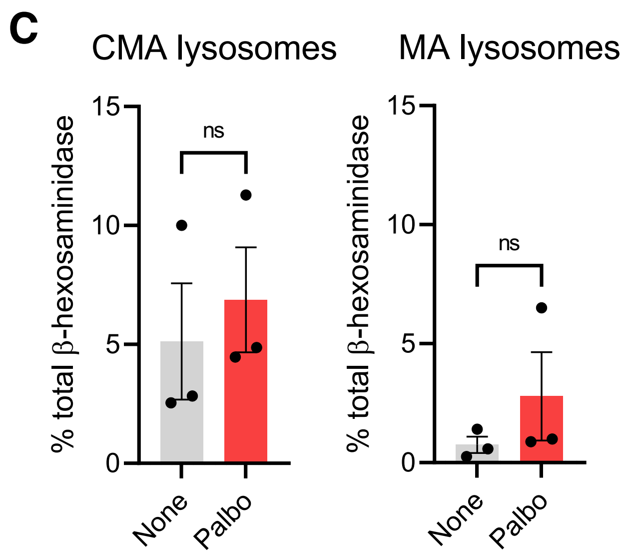
C, Similar to B using primary mouse embryonic fibroblasts (MEFs).

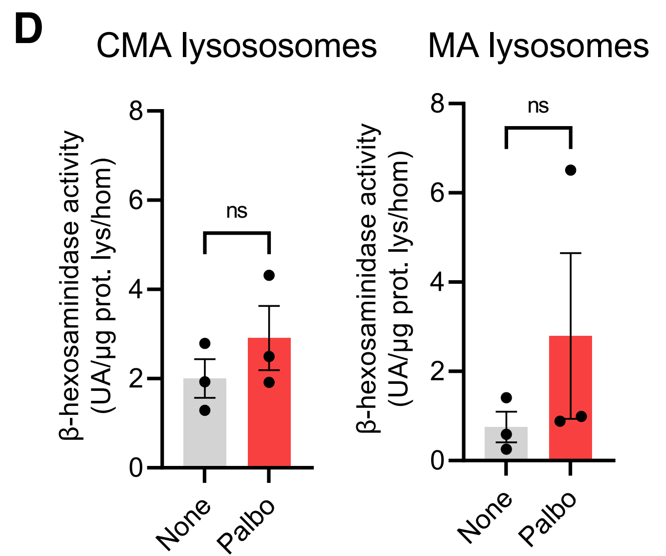
D, Similar to B using Neuro2a cells (N2a).

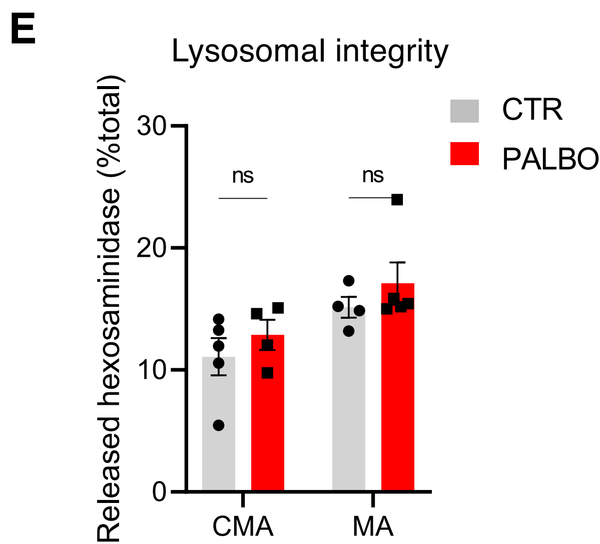
Statistical significance was estimated by 1-way ANOVA test (in A) and unpaired t-test in B-D, ** $p<0.01$, * $p<0.05$.

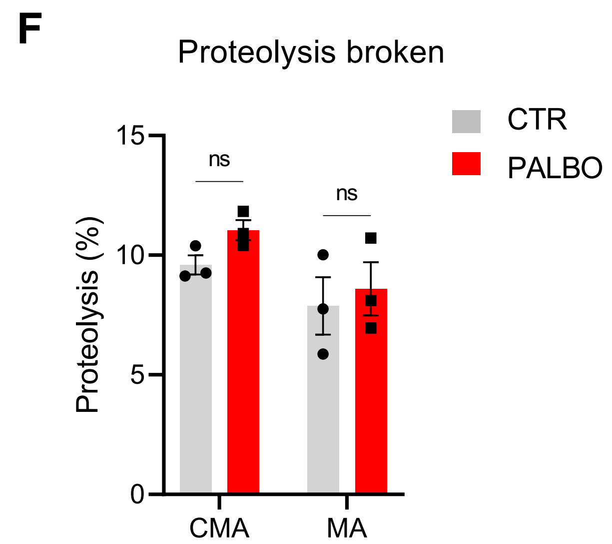












Supplementary Figure 2

Supplementary Figure 2.

A, Total protein (left) and hexosaminidase activity (right) in CMA lysosomes isolated from control or 7 days after palbociclib treatment of SK-MEL-103 cells. Values are individual values and mean ± SEM of three different isolations. FU: fluorescence units

B, Total protein (left) and hexosaminidase activity (right) in MA lysosomes isolated from control or 7 days palbociclib-treated SK-MEL-103 cells. Values are individual values and mean ± SEM of three different isolations. FU: fluorescence units

C, Percentage of total cellular hexosaminidase activity recovered in CMA (left) and MA (right) lysosomes isolated as in A and B. Values are individual values and mean ± SEM of three different isolations.

D, Enrichment for hexosaminidase activity in CMA (left) and MA (right) lysosomes isolated as in A and B calculated as units of activity (UA) per µg of protein in the fractions relative to activity in the homogenate. Values are individual values and mean ± SEM of three different isolations.

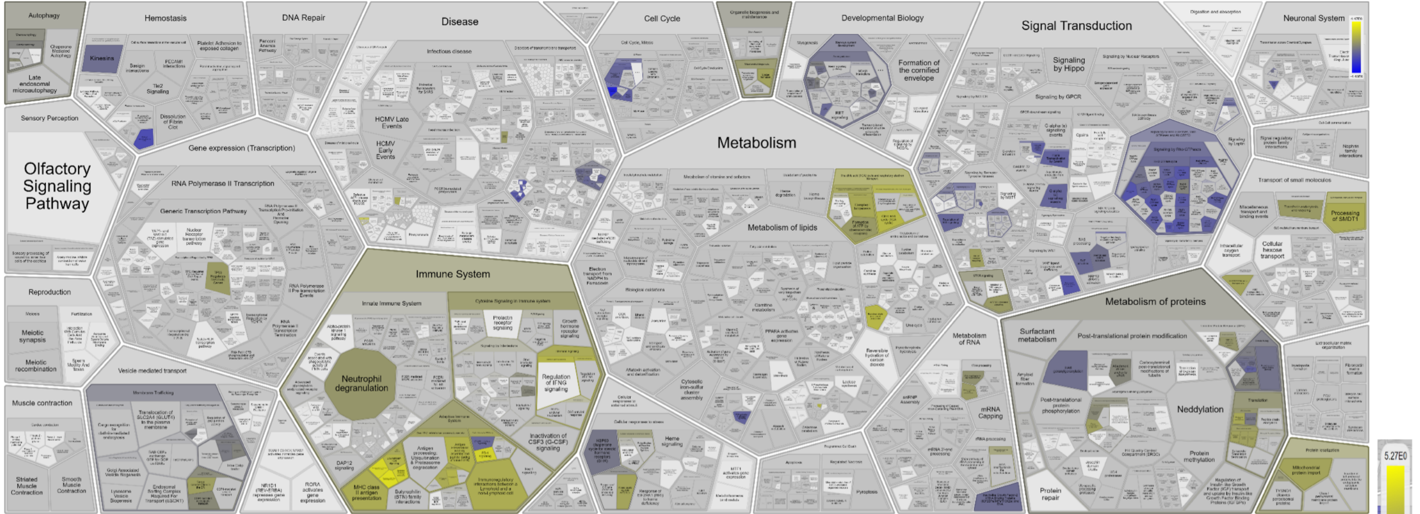
E, Lysosomal membrane stability measured as the percentage of lysosomal β-hexosaminidase activity detectable in the media relative to total β-hexosaminidase in the lysosomal preparations. Values are individual values and mean ± SD, n=4.

F, Proteolytic capacity of luminal content of CMA and MA lysosomes isolated from control or 7 days palbociclib-treated SK-MEL-103 cells. Values are individual values and mean ± s.e.m, n=3.

Statistical significance was estimated by unpaired t-test (A-D) and two-way ANOVA and Sidak's multiple comparisons test (E, F). n.s.: non-significant.

A

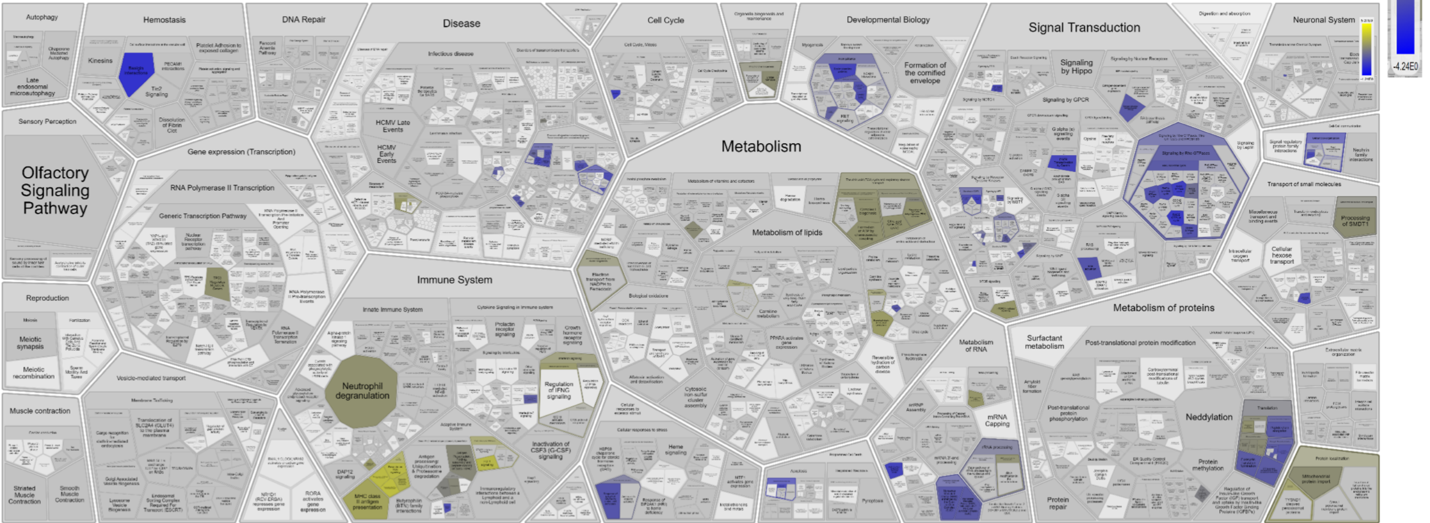
CMA LYSOSOMES



5.27E0

-4.24E0

MA LYSOSOMES



B

CHANGES IN CMA LYSOSOMES ONLY	CHANGES IN MA LYSOSOMES ONLY	CHANGES IN CMA AND MA LYSOSOMES
↑ Iron uptake/transport	↑ Cell junction organization	↑ mTOR signaling
↑ Cargo concentration in ER	↓ Strogen-stimulated signals	↑ TCR donswstream
↑ COPI-mediated transport	↓ Cell junction adherines	↑ MHC class II antigen presentation
↑ Transferrin endocytosis	↓ Apoptotic proteins	↑ PD-1 signaling
↓ Rab-generylation	↓ Reeling and Robo	↓ RhoGTPases
↓ Kinesins		↓ CTL4 inhibitory signaling
↓ Intra Golgi trafficking		

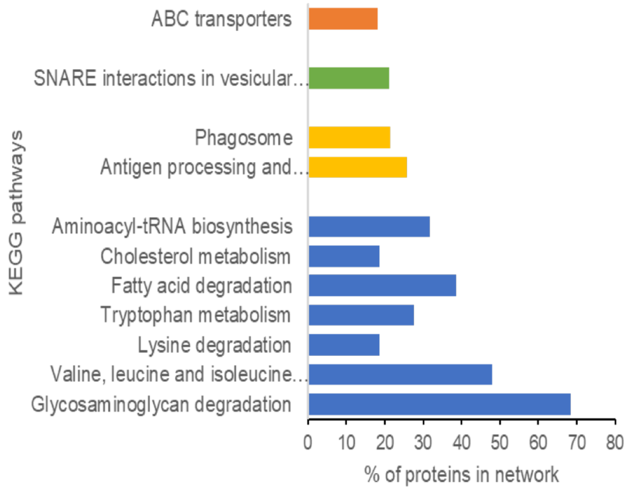
Supplementary Figure 3

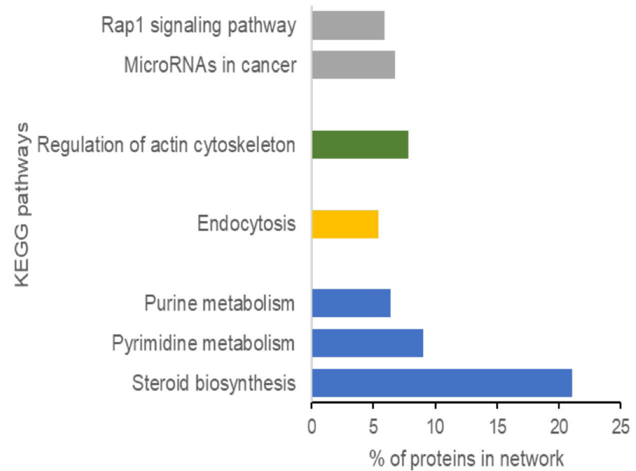
Supplementary Figure 3.

A, Mosaic gradient representation of the Reactome analysis of proteins with increased (yellow) or reduced (blue) abundance in CMA active lysosomes (top) and MA lysosomes (bottom) in 7 days palbociclib-treated cells SK-MEL-103 cells compared to control cells.

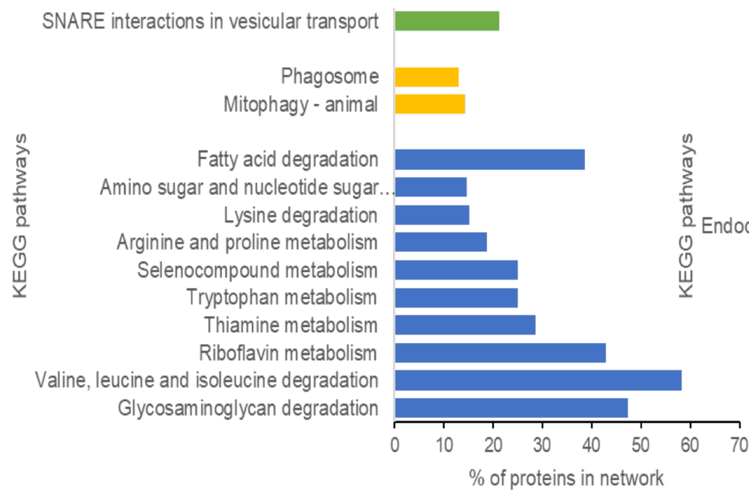
B, Cellular pathways of proteins that change in senescent SK-MEL-103 cells compared with control only in CMA lysosomes, only in MA lysosomes or that change in both. Arrow indicates decreased (blue) or increased (orange) levels in senescent cells.

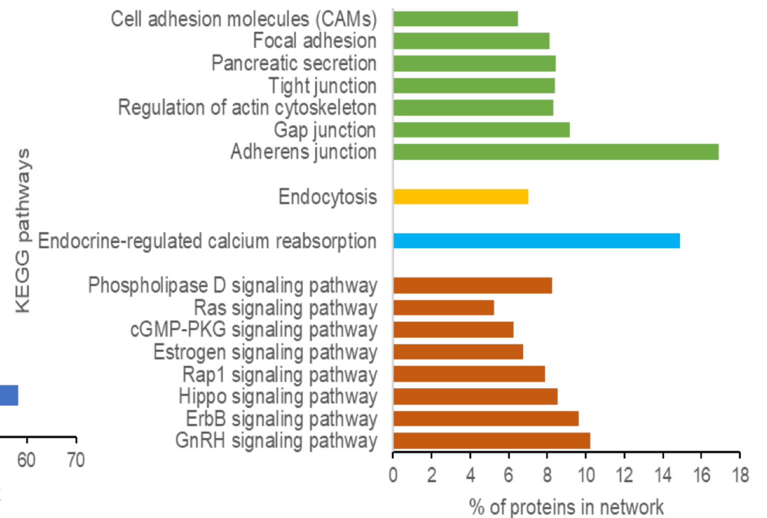
A





B





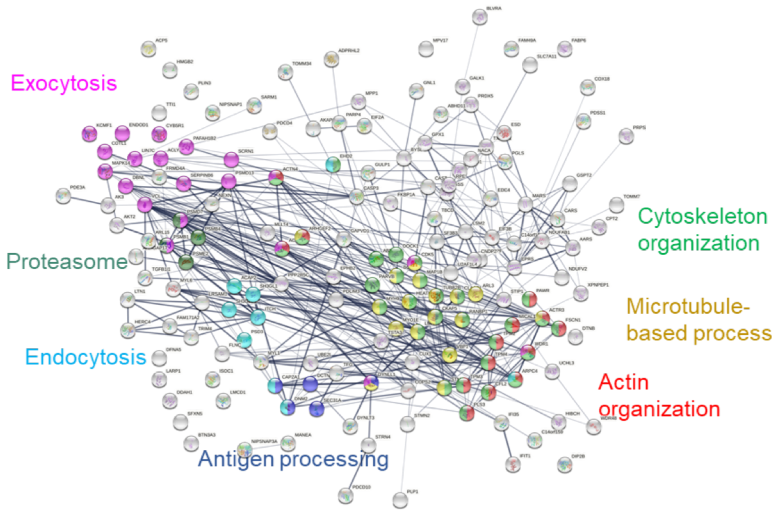
Supplementary Figure 4

Supplementary Figure 4.

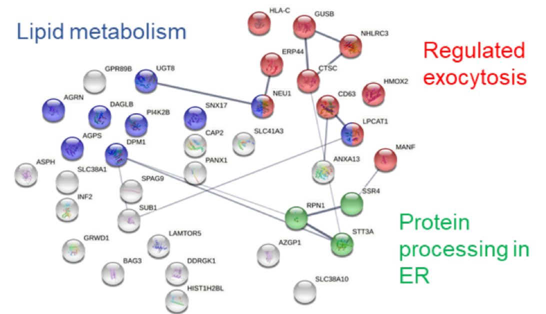
A, GO terms gene sets of proteins defined as resident lysosomal proteins that show higher (left) or lower (right) levels in CMA lysosomes from SK-MEL-103 senescent cells. Constitutive resident proteins were defined as those that do not change significantly $p > 0.05$ in N/L *vs* vehicle or that have a negative log2 fold change N/L *vs* vehicle.

B, GO terms gene sets of proteins defined as resident lysosomal proteins that show higher (left) or lower (right) levels in MA lysosomes from SK-MEL-103 senescent cells. Constitutive resident proteins were defined as those that do not change significantly $p > 0.05$ in N/L *vs* vehicle or that have a negative log2 fold change N/L *vs* vehicle.

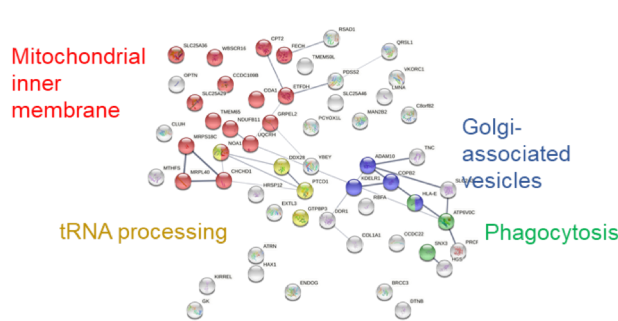
A Only degraded by MA in senescence (165)



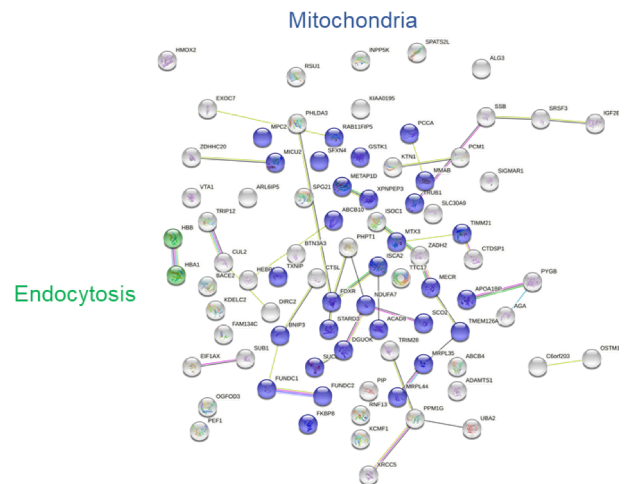
B No longer degraded by MA (37)



C Only degraded by CMA in senescence (47)



D No longer degraded by CMA (83)

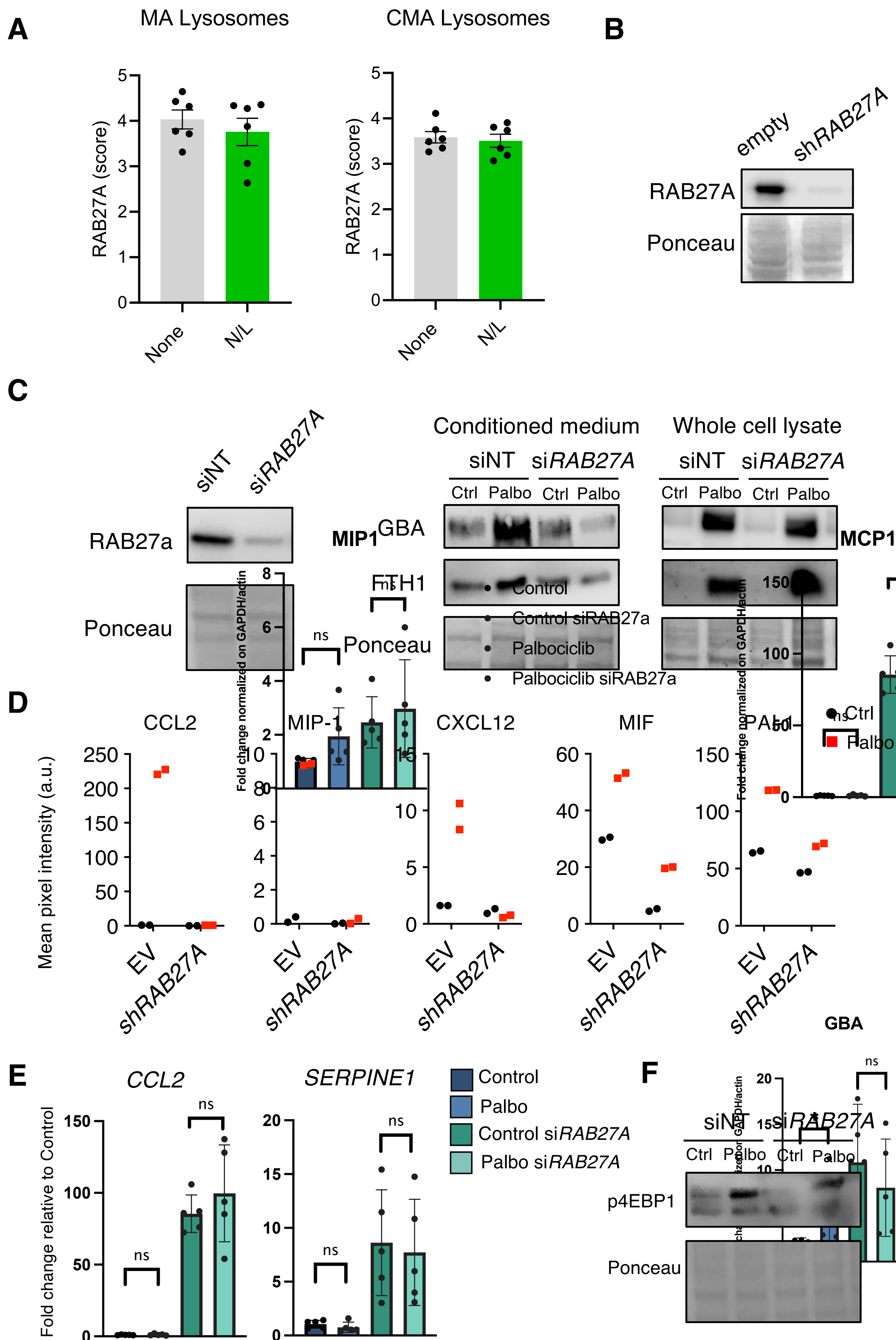


Supplementary Figure 5

Supplementary Figure 5.

A, B, STRING analysis of proteins identified in the proteomic analysis of lysosomes isolated from control or 7 days palbociclib-treated SK-MEL-103 cells that are only degraded (**A**) or no longer degraded (**B**) by MA in senescent cells. Substrate proteins were defined as those that accumulate significantly upon N/L treatment, *i.e.,* log2 fold change N/L *vs* vehicle >0.21 (fold >1.1) and p value <0.05.

C, D, STRING analysis of proteins identified in the proteomic analysis of lysosomes isolated from control or 7 days palbociclib-treated SK-MEL-103 cells that are only degraded (**C**) or no longer degraded (**D**) by CMA in senescent cells. Substrate proteins were defined as those that accumulate significantly upon N/L treatment, *i.e.,* log2 fold change N/L *vs* vehicle >0.21 (fold >1.1) and p value <0.05.



Supplementary Figure 6

Supplementary Figure 6.

A, Levels of RAB27A in MA (left) and CMA (right) lysosomes isolated from SK-MEL-103 cells cultured without additions (none) or in the presence of 10 mM ammonium chloride and 100 μM leupeptin for 16h before isolation. Individual values and mean ± SEM from three different isolations in duplicate are shown. Values are expressed as Z score were extracted from the MS proteomic analysis.

B, Confirmation of the knock-down of RAB27A using lentiviral shRNA or empty vector in SK-MEL-103 cells.

C, Confirmation of the knock-down of RAB27A using siRNAs in SK-MEL-103 cells (left). Analysis of the levels of GBA and FTH1 in SK-MEL-103 cells, control or senescent, treated or not with siRNAs targeting RAB27A (right). Protein levels were analyzed in the conditioned medium and in whole-cell extracts

D, Profiles of mean spot pixel density for the cytokines and chemokines indicated in Fig. 7E. EV, empty vector; sh, lentiviral sh*RAB27A*.

E, Levels of mRNA for the indicated genes in SK-MEL-103 cells, control or senescent (palbociclib), treated with non-targeting siRNAs (siNT) or with siRNAs targeting *RAB27A* (si*RAB27A*).

F, Levels of phospho-4EBP1 in the same cells as in panel E.

Statistical significance was estimated by t-test, n.s.: non-significant.