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Peripheral positioning of lysosomes supports melanoma aggressiveness

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1 **Emerging evidences suggest that both function and position of** 37
2 **organelles are pivotal for tumor cell dissemination. Among** 38
3 **them, lysosomes stand out as they integrate metabolic sens-** 39
4 **ing with gene regulation and secretion of proteases. Yet, how** 40
5 **the function of lysosomes is linked to their position and how** 41
6 **this controls metastatic progression remains elusive. Here,** 42
7 **we analyzed lysosome subcellular distribution in micropat-** 43
8 **terned patient-derived melanoma cells and found that lysosome** 44
9 **spreading scales with their aggressiveness. Peripheral lyso-** 45
10 **somes promote invadopodia-based matrix degradation and in-** 46
11 **vasion of melanoma cells which is directly linked to their lyso-** 47
12 **somal and cell transcriptional programs. When controlling lyso-** 48
13 **somal positioning using chemo-genetical heterodimerization in** 49
14 **melanoma cells, we demonstrated that perinuclear clustering** 50
15 **impairs lysosomal secretion, matrix degradation and invasion.** 51
16 **Impairing lysosomal spreading in a zebrafish metastasis model** 52
17 **significantly reduces invasive outgrowth. Our study provides a** 53
18 **mechanistic demonstration that lysosomal positioning controls** 54
19 **cell invasion, illustrating the importance of organelle adaptation** 55
20 **in carcinogenesis.** 56

21 **Keywords:** lysosome, invasion, metastasis

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25 Main

26 Metastases are responsible for the majority of cancer-related 62
27 deaths (Dillekås et al., 2019). Melanoma shows strong 63
28 negative correlation between cancer stage and 5-year pa- 64
29 tient survival, making it an ideal model to study phenotypic 65
30 changes leading to cancer cell invasion, adaptation and sur- 66
31 vival. Melanoma progression consists of multiple sequen- 67
32 tial events. First, melanocytes are transformed and grow in 68
33 the epidermis during radial growth phase (RGP), forming a 69
34 premalignant lesion. Changes in their transcription program 70
35 lead to expression of matrix-degrading enzymes and to inva- 71
36 sion through the dermis during vertical growth phase (VGP) 72

followed by cancer dissemination through vascular and lym-
phatic routes, progressing into metastatic stages (Brauer et al., 2011). To colonize secondary organs during metastasis, melanoma cells sense their microenvironment and react by locally degrading and remodeling the extracellular matrix (ECM). Invading melanoma cells frequently form dynamic actin-rich protrusive structures with high proteolytic activity, called invadopodia, that mediate ECM degradation (Augoff et al., 2020). Invadopodia formation involves the local docking of endosomal compartments, such as multivesicular bodies, and their fusion with the plasma membrane (Hoshino et al., 2013). This suggests that melanoma metastasis requires specific invasion programs for an efficient and targeted delivery of ECM-degrading enzymes to highly-specialized structures. Yet how this is orchestrated in invasive cells remains unclear. Over the past decade, several reports indicated that lysosomes constitute novel regulators of invasion by allowing cells to sense their microenvironment and trigger adapted responses, notably through the exocytic release of their content (Ballabio and Bonifacino, 2020). For instance, lysosomal exocytosis drives the formation of invasive protrusions resulting in basement membrane breaching in *C. elegans* (Naegeli et al., 2017). In addition, secretion of lysosomal cathepsin B promotes cancer cell invasion and metastasis (Bian et al., 2016). Besides, lysosome secretion contributes to the repair of plasma membrane damages occurring during cell migration and results in better cell survival under mechanical stress (Corrotte and Castro-Gomes, 2019). Importantly, lysosomal activity is regulated by their subcellular location (Johnson et al., 2016; Korolchuk et al., 2011). Peripheral lysosomes are prone to exocytosis and drive growth factor signaling (Jia and Bonifacino, 2019), while perinuclear lysosomes have a decreased pH and higher proteolytic activity (Johnson et al., 2016). Lysosome distribution is in turn directly impacted by the cellular microenvironment (Steffan et al., 2009). Lysosomes are transported to the plasma membrane via kinesins

(anterograde transport) in response to growth factors and nutrients presence, conversely, during starvation and in alkaline environment, lysosomes are transported to the perinuclear region (retrograde transport) in a dynein-dependent manner (Ballabio and Bonifacino, 2020). While the molecular mechanisms driving lysosomal positioning have been partially elucidated, it remains unclear whether it can control invasion programs of melanoma cells. A parallel study by Marwaha and colleagues (Marwaha et al., 2023) demonstrates that peripheral lysosomes control the emergence of leader cells during epithelial collective migration events, suggesting that lysosomal positioning is likely a universal mechanism controlling cell invasion within tissues. Here, we investigated how lysosome dynamics contribute to cancer aggressiveness and metastatic progression. We reveal a phenotypic switch concerning lysosome positioning in aggressive melanoma that is supported by distinct transcriptional programs and controls migration and invasion. Our study illustrates the importance of organelle adaptation in carcinogenesis by providing mechanistic evidences that lysosomal positioning controls secretory pathways of malignant transformation.

Melanoma invasiveness scales with lysosome spreading

Cells progressing through the metastatic cascade display tremendous phenotypic plasticity in the benefit of increased invasion and ECM degradation potential. Transition of melanoma cells from RGP to VGP involves a series of invasion-promoting programs, particularly the concerted expression of matrix-degrading enzymes (Braeuer et al., 2011) and the maturation of invadopodia (Weidmann et al., 2016). In order to investigate which organelles control such invasion-promoting programs, we first characterized the invasive properties of a collection of patient-derived melanoma cells from different stages (RGP: WM1552c, WM1862, VGP: WM115, WM983A and metastatic: WM983B, A375 cells). Using collagen invasion assay (Fig 1a), we identified three patient-derived cell lines with low, medium and high invasion potential (Fig 1b,c), which correlated with their cancer progression state (RGP, VGP, metastatic). Using gelatin degradation assay (Fig 1d), we further showed that cells with high invasion index displayed significantly increased gelatin degradation frequency and area (Fig 1e,f) confirming previous observations (Mousson et al., 2021). Notably, gelatin degradation areas were mainly located at invadopodia identified by the invadopodia markers, actin and cortactin (Fig 1e). Because ECM degradation is mostly mediated by enzymes, such as metalloproteases (MMPs) or cathepsins, contained in lysosomes, and released by exocytosis at invadopodia (Jacob and Prekeris, 2015), we tested whether lysosomal marker LAMP1 co-localizes with active invadopodia. We found that LAMP1 colocalized with actin and cortactin in a subset of degradation areas (Fig S1 a-d) suggesting at least transient presence of lysosomes at podosomes as reported previously in v-*Src* transformed fibroblasts (Tu et al., 2008). We accessed transcriptional programs of the three representative patient-derived cell lines with graded invasion index (WM1862, WM983A, WM983B) using RNAseq and

Gene Ontology analysis. This showed an overrepresentation of metabolism, actin cytoskeleton and cell migration pathways associated with a concomitant reduction of transcripts linked to the lysosomal pathway (Fig.1g and Table 1). We thus hypothesized that increased invasion occurs through a phenotypic switch from a lysosomal signature characteristic of RGP cells (WM1862) to a migratory signature found in metastatic cells. We next investigated the sub-cellular localization of LAMP1, a marker of late endosomes and lysosomes (referred to as lysosomes hereafter) in these cell lines. Micropatterning allows for high-throughput study of cells with reproducible shapes (Fig 1h) and facilitates the comparison and quantitative positional analysis of lysosomes in 2D (Figure 1i) or in full cell volume (Figure 1j). While RGP cells had mostly perinuclear lysosomes, metastatic cells showed significant dispersion of LAMP1-compartments towards the cell periphery (Fig 1h-j) characterized by a significant increase in the mean inter-organelle distance and the mean distance to the barycenter (Figure 1k,l). Moreover, LAMP1-compartments were smaller and more numerous in metastatic cells (Fig S1e,f), revealing that the observed transcriptional changes in melanoma cells correlate with changes in LAMP1 distribution. Our results identify and characterize a remarkable cellular phenotype of scattered lysosomes associated with aggressive malignancy in melanoma. Since peripheral lysosome positioning has been reported earlier in other cancer types (breast cancer (Ping-Hsiu Wu et al., 2020) and bladder cancer (Mathur et al., 2023)), it is tempting to speculate that peripheral lysosome repositioning could represent a universal feature of cancer progression. Indeed, our transcriptomics data demonstrate that genes known to promote perinuclear localization of lysosomes, such as RILP (Pu et al., 2016) or RNF167 (Nair et al., 2020), show decreased expression, and conversely, genes linked to anterograde transport, such as KIF1B and KIF5B (Moamer et al., 2019), are overexpressed in metastatic cells. Interestingly, lysosomal genes previously associated with cancer progression such as LAMP1 (Machado et al., 2015; Wang et al., 2017), lysosomal Ca²⁺ channel MCOLN1 (Medina et al., 2011) and several MMPs (Hua et al., 2011) (MMP2, MMP15, MMP16, MMP17) are upregulated in metastatic cells, besides an overall loss of lysosomal signature in these cells. Yet, lysosomal dispersion could be uncoupled from lysosome biogenesis as some studies report its induction in highly metastatic breast cancer cells (Glunde et al., 2003) or during pancreatic ductal adenocarcinoma progression (Perera et al., 2015). Further work is therefore needed to identify what are the molecular programs controlling the positioning and the biogenesis of lysosomes in invasive melanomas cells and what triggers their activation.

Forcing lysosome perinuclear clustering in melanoma cells

We next engineered melanoma cells employing a chemo-genetic strategy based on the heretodimerization of the FKBP-FRB domains by Rapalog or derivatives (Kapitein et al., 2010) for lysosome positioning control. We chose WM983A and WM983B cell lines, derived from the primary tumor and metastatic site of the same patient, respectively.

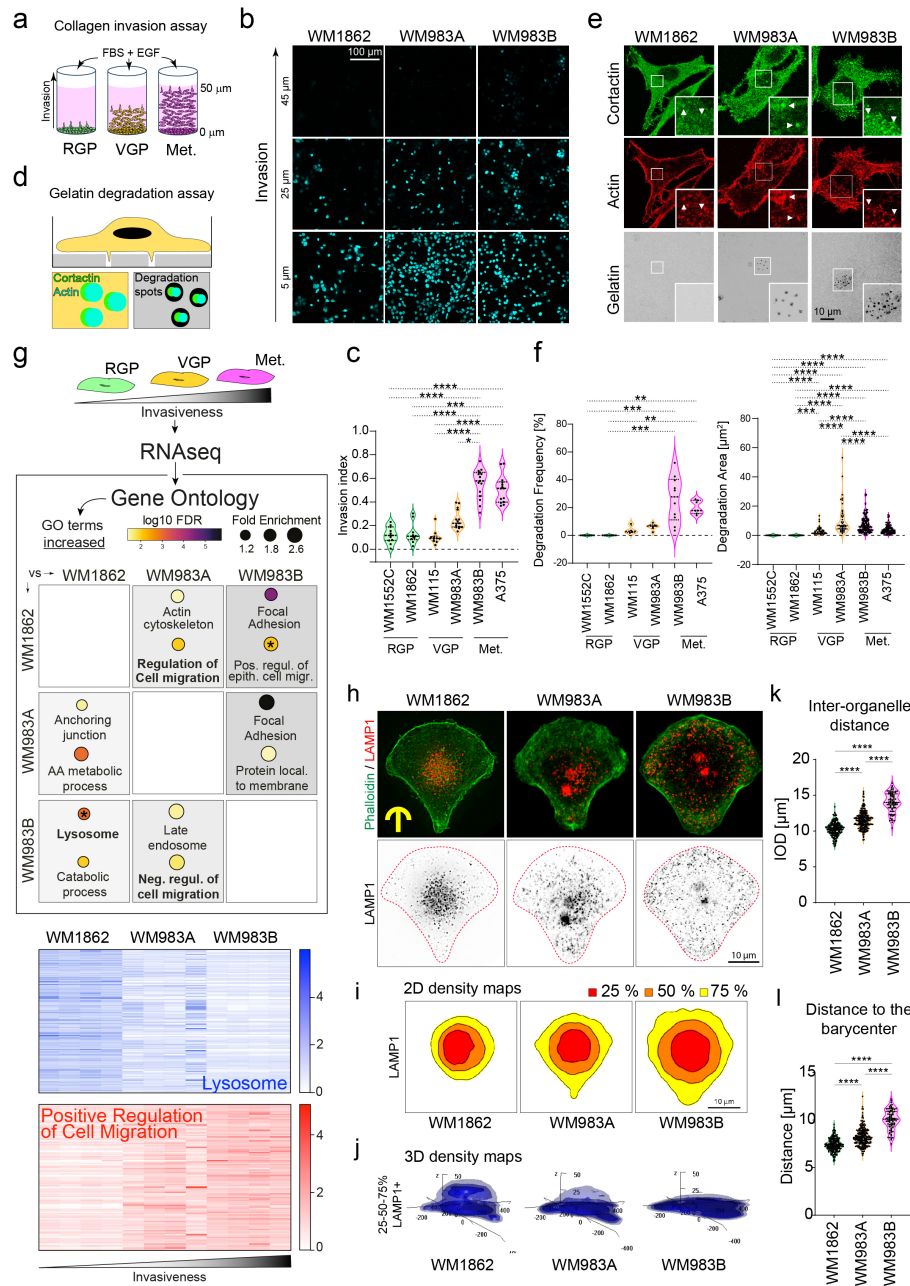


Fig. 1. Melanoma invasiveness correlates with lysosome spreading a-c) Invasive potential of human melanoma cells (WM1552c, WM1862, WM115, WM983A, WM983B, A375) was analyzed using collagen invasion assay. b) Cells were seeded into a collagen plug, cultured for 24 hours and their spreading at distance of the seeding position was analyzed by confocal imaging. c) For each cell line, the invasion index equals the number of nuclei above 10 μm distance divided by the total number of cells. Invasion index (Mean ± SD) = 0.1213 ± 0.070, 0.1334 ± 0.089, 0.2500 ± 0.080, 0.1036 ± 0.060, 0.5562 ± 0.125, 0.5070 ± 0.117, respectively, in triplicate, one dot represents 1 field of view. Kruskal-Wallis with Dunn's multiple comparison post-hoc test. d-f) Degradation capacity of the melanoma cell lines was assessed using gelatin degradation assay allowing to visualize actin, cortactin and degradation spots. e) Cells were seeded on FITC-gelatin for 24 hours, fixed and stained with Cortactin antibody and Phalloidin to label invadopodia and imaged using confocal microscopy. f) Degradation frequency (DF) was calculated as a percentage of cells displaying gelatin degradation activity. DF (Mean ± SD) = 0.00, 0.00, 6.19 ± 2.26, 4.00 ± 2.94, 26.08 ± 16.52, 20.08 ± 4.79, respectively, in triplicate one dot represents 1 field of view. For cells displaying degradation activity, the degradation area (DA, total area per cell) was quantified. DA (Mean ± SD) = 0.00, 0.00, 11.13 ± 11.00, 3.19 ± 2.76, 7.48 ± 5.59, 3.99 ± 2.97, respectively, in triplicate one dot represents 1 cell. Kruskal-Wallis with Dunn's multiple comparison post-hoc test. g) Transcriptomics analysis of WM1862, WM983A and WM983B cell lines was performed in quadruplicate, genes showing statistically significant differential expression (pAdj<0,01) were analyzed using gene ontology (GO). Selected GO terms of differentially regulated pathways are listed showing their fold enrichment and log10 FDR in paired comparisons (left panel) and heatmaps are shown for the two main pathways identified (right panel). h-j) WM1862, WM983A, WM983B cells were analyzed using micropatterning. Cells were seeded on 36μm crossbow micropatterns, let spread for 4 hours, fixed and stained with LAMP1 antibody and cell spreading was controlled by actin staining (Phalloidin). 2D (i) and 3D (j) density maps of LAMP1 staining were calculated using R software, displaying the smallest area that can be occupied by 25, 50 and 75per cent of all compartments. k) Inter-organelle distance (IOD) represents average of distances between all lysosomes. IOD (Mean ± SD) = 10.30 ± 0.94, 11.56 ± 1.15, 13.99 ± 1.40, respectively. One dot represents 1 cell. l) Distance to barycenter (DB) represents the distance from each lysosome to the center of mass. DB (Mean ± SD) = 7.40 ± 0.69, 8.21 ± 0.93, 10.03 ± 1.07, respectively. One dot represents 1 cell. Kruskal-Wallis with Dunn's multiple comparison post-hoc test. (i,j,k,l) WM1862, n= 182 cells, WM983A, n= 231 cells, WM983B, n= 82 cells, in triplicate. *p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001.

187 We stably expressed FKBP domain fused to LAMP1 and the 244
188 FRB domain fused to dynein adaptor BicD2. Rapalog treat- 245
189 ment forced binding of BicD2 and recruitment of Dynein to 246
190 LAMP1 for the movement towards the minus end of mi- 247
191 crotubules, and thus perinuclear clustering of the LAMP1 248
192 compartment around the microtubule organizing center (Fig. 249
193 3a, S2d). Clustering was fast, dose-dependent (Figure 2a,b) 250
194 and persistent in time (Figure 2b, c, Figure S2c), as previ- 251
195 ously described (Kapitein et al., 2010), allowing precise con- 252
196 trol of lysosomal positioning in vitro and in vivo investiga- 253
197 tions. Engineered control cells expressing only single domain 254
198 FKBP (FKBP only) did not display lysosome clustering upon 255
199 Rapalog treatment (Fig 2b, c, S2A). Correlative light and 256
200 electron microscopy (CLEM) of control- or Rapalog-treated 257
201 WM983B-LAMP1-mCherry cells showed colocalization be- 258
202 tween LAMP1-mCherry and vesicular compartments, which 259
203 clustered as expected in the perinuclear region upon Rapalog 260
204 treatment (Fig 2d). Notably, Rapalog had no effect on the 261
205 colocalization between LAMP1-mCherry and the BODIPY- 262
206 Pepstatin A (Fig S2e), which is delivered to lysosomes via 263
207 endocytic pathway (Chen et al., 2000) and binds to the ac- 264
208 tive site of cathepsin D under acid condition. Thus, clus- 265
209 tering by Rapalog did not disrupt cargo delivery and lyso- 266
210 somal catabolic activity in melanoma cells, indicating that 267
211 we could control lysosomal positioning in relevant patient- 268
212 derived cell lines, without interfering with basic lysosomal 269
213 functions. While our study exploited a chemo-genetic model 270
214 of forced lysosome clustering, the study by (Marwaha et al., 271
215 2023) built on the induction of lysosomal spreading demon- 272
216 strating that fine control or organelle positioning is now at 273
217 reach. Both approaches require cell engineering and would 274
218 benefit from the complementary use of small molecules that 275
219 regulate lysosome positioning, particular for clinical appli- 276
220 cations. We previously identified PI3K inhibitors as potent 277
221 lysosome clustering agents in bladder cancer (Mathur et al., 278
222 2023). Indeed, lysosome positioning, and more general or- 279
223 ganelle topology (Wang et al., 2023), were used as a readout 280
224 in a screen for novel therapeutic drugs and targets (Circu et 281
225 al., 2016). This opens an exciting area of research leading to 282
226 a wider drug discovery approach centered on organelle posi- 283
227 tioning. 284

228 **Peripheral lysosomes promote secretion and matrix** 286
229 **degradation** Lysosome exocytosis of different proteases and 287
230 subsequent extracellular matrix degradation promotes inva- 288
231 sion to adjacent tissues (Monteiro et al., 2013; Naegeli et 289
232 al., 2017). We thus first investigated how altering position- 290
233 ing of the LAMP1 compartment impacts the cell secretome. 291
234 We analyzed the concentrated cell supernatant of WM983B 292
235 cells in the presence and absence of Rapalog treatment (Fig 293
236 3a) by mass spectrometry and identified a significant de- 294
237 crease of several lysosome-associated proteins upon clus- 295
238 tering (Fig 3a), including Cathepsins. These enzymes con- 296
239 tribute to ECM degradation (Vidak et al., 2019) and have 297
240 been linked with metastatic progression, for instance in the 298
241 case of Cathepsin S or B in gastric and colorectal cancers 299
242 respectively (Bian et al., 2016; da Costa et al., 2020). To 300
243 confirm that clustering inhibits lysosome secretion, we im-

aged VAMP7-pHluorin, a v-SNARE involved in the fusion of lysosome with plasma membrane (Chaîneau et al., 2009) using TIRF microscopy (Fig 3b). We mixed cells expressing either FKBP only or FKBP-FRB and treated them with 25nM Rapalog (Fig 3c). Cells with clustered LAMP1 compartment showed significantly reduced numbers of VAMP7 secretory events (Fig 3d left, Fig 3e), with no impact on the duration of the secretion process (Fig 3d right). Together, these experiments demonstrated that perinuclear localization of lysosomes strongly decreased lysosome secretion in melanoma cells. These results are in line with a previous observation of peripheral lysosomes promoting their fusion with plasma membrane and thus exocytosis in a model of lysosomal storage disease (Medina et al., 2011). Next, we investigated whether lysosome clustering impairs invadopodia-based ECM degradation (Fig 3f). In both cell lines (WM983A, WM983B), we observed a significant decrease in the degradation area (Fig 3g top) upon LAMP1-clustering. While degradation frequency in VGP WM983A cells remained unaltered, metastatic WM983B cells showed a significant decrease (Fig 3g bottom). These results further confirmed that peripheral lysosome positioning promotes lysosome exocytosis and ECM degradation. Forcing perinuclear lysosome localization rescues this phenotype, suggesting that that lysosome position and subsequent secretion promotes ECM remodeling, feature often seen in aggressive cancers (Winkler et al., 2020). More globally, cancer cell secretion is likely to further shape pro-metastatic features of the tumor microenvironment, and favor the emergence of, for example, cancer associated fibroblasts, whose ECM remodeling expertise is pivotal during tumor progression (Kalluri, 2016; Sahai et al., 2020) Our results further suggest that the formation of functional invadopodia requires local lysosome exocytosis. Similarly, targeted secretion of CD63-positive multi-vesicular bodies promotes invadopodia formation. Indeed, other secretory organelles are relocalized to the cell periphery and protrusive structures, and favor, for example, the secretion of pro-tumorigenic extracellular vesicles (Ghoroghi et al., 2021) as it has been shown for CD63+ endo/lysosomes (Hoshino et al., 2013). Whether distinct types of late endosomes act in concert to favor invadopodia-mediated ECM degradation, or whether it involves hybrid late endo-lysosome compartments remains to be determined.

Forcing lysosomal clustering impairs invasion potential of melanoma cells Building on the stability of Rapalog-mediated lysosomal clustering in patient-derived cells and the demonstration that it impairs ECM degradation, we sought to monitor if lysosome position impacted the cancer cell dissemination in vivo. To do so, we intravenously injected melanoma cells with different clustering status in two days post-fertilization (dpf) zebrafish embryos (Follain et al., 2018) to probe lysosomal clustering while assessing the metastatic and invasive potential of melanoma cells over time (Fig 4a). Rapalog treatment (5nM) had no effect on cell proliferation or cell viability (Fig S3a, b) during the 3-days time course. Lysosome clustering was stable in vivo and visible in round circulating tumor cells that had just performed

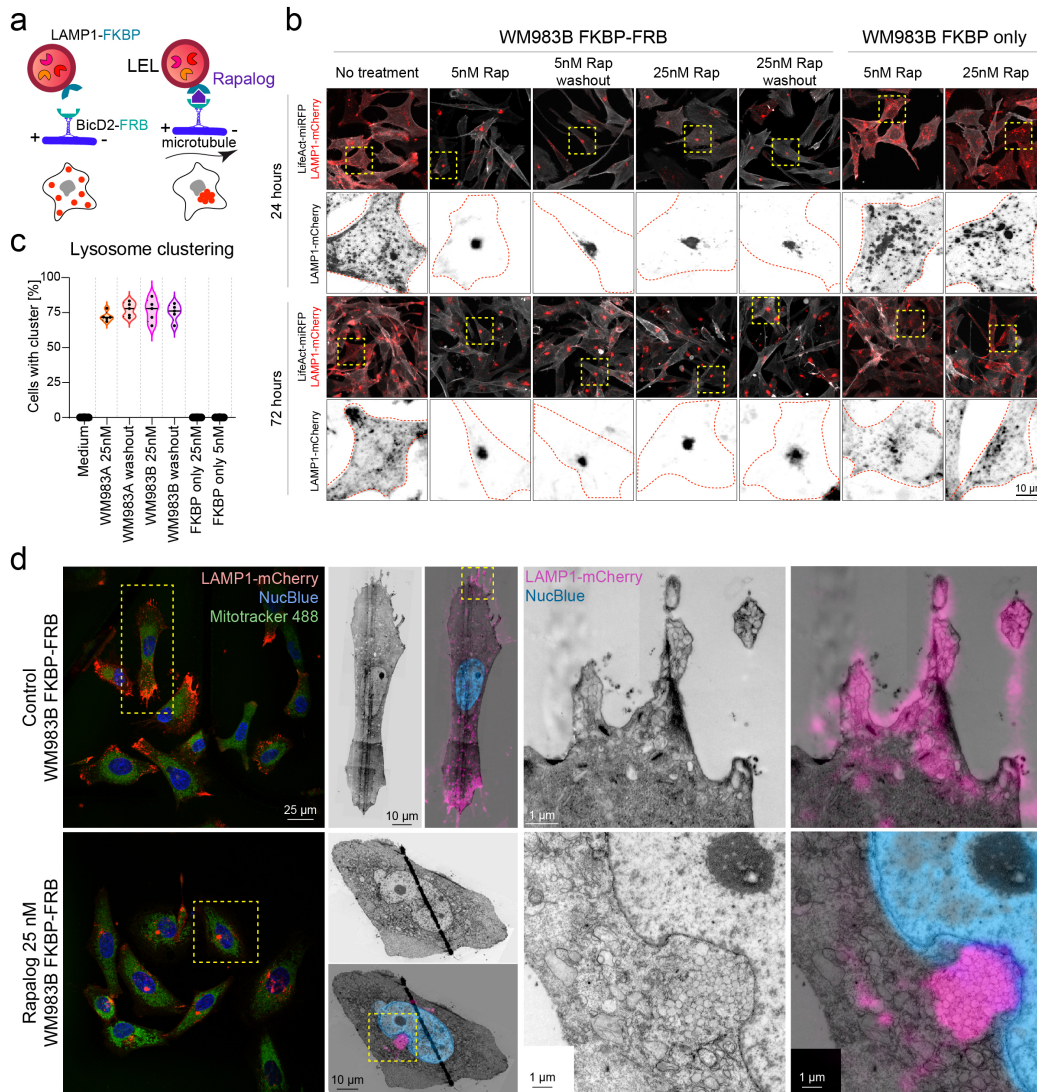


Fig. 2. Induced lysosome clustering in melanoma cells a) Lysosomes in WM983A or WM983B cells stably expressing LAMP1-mCherry-FKBP and BicD2-FRB can be clustered using the compound Rapalog (Rap) which induces FKBP-FRB rapid heterodimerization. b) Cells were seeded in glass-bottom dishes, treated with 5nM or 25nM Rapalog and imaged at 24 hours and 72 hours timepoints. Washout condition – cells were treated for 1 hour with Rapalog, washed 3x in PBS and cultured in normal growth medium. WM983B FKBP-FRB = cells expressing both heterodimerizing domains, lysosomes can cluster. WM983B FKBP only = cells expressing only one heterodimerizing domain, clustering is not achieved. Yellow square delineates the zoomed region shown below; red dotted line shows the cell contour. c) Percentage of cells displaying clustered lysosomes was quantified for each condition at 72 hours timepoint. Percentage = 0.0, 73.3 ± 3.6, 78.5 ± 5.1, 77.7 ± 8.3, 76.4 ± 6.2, 0.0, 0.0 respectively. Data come from five randomly selected fields of view per condition. One dot represents 1 field of view. Kruskal-Wallis with Dunn's multiple comparison post-hoc test, adjusted p-value: WM983A Rap vs. washout, $p > 0.9999$, WM983B Rap vs. washout, $p > 0.9999$. d) Correlative light and electron microscopy. WM983B cells expressing LAMP1-mCherry-FKBP and BicD2-FRB were cultured in control medium, or in medium supplemented with 25 nM Rapalog for one hour and imaged by spinning disc microscope. Samples were processed for electron microscopy and imaged by TEM. Representative images with high magnification of spread or clustered lysosomes and overlay of fluorescent and electron microscopy images (LAMP1 appears in pink, nucleus in blue) are shown for each condition.

301 arrest in the vasculature after injection (Fig 4b). Melanoma 313
 302 cells with spread lysosomes efficiently extravasated, and dis- 314
 303 played high metastatic potential in vivo (Fig 4c, left). On 315
 304 the contrary, when lysosomes were clustered in melanoma 316
 305 cells before injection, their ability to invade from the pri- 317
 306 mary tumor site was strongly impaired (Fig 4c, right) al- 318
 307 though they had similarly completed intravascular arrest and 319
 308 extravasation as quantified by their post-extravasation inva- 320
 309 sion potential (Figure 4d, Figure S4a, b). Collectively, these 321
 310 data show that lysosome positioning is an important driver 322
 311 of cell aggressiveness, contributing to the ECM remodeling 323
 312 and to cell invasiveness. As seen in our study, cells with 324
 peripheral lysosomes have higher invasion potential in vitro
 as well as in vivo, which can be rescued by promoting the
 perinuclear lysosome clustering and thus reducing their ma-
 lignancy, providing the first in vivo evidence for the role of
 lysosome positioning in metastatic progression. Organelles
 are dynamic, self-organized structures whose specific func-
 tion is inevitably linked to their position and morphology
 (Schauer et al. 2010), in space and time within cells (Bal-
 labio and Bonifacino, 2020; van Bergeijk et al., 2016). In
 this study, we provide the first mechanistic demonstration
 that the position of lysosomes within cells tightly controls the
 targeted secretion of matrix-degrading enzymes which subse-

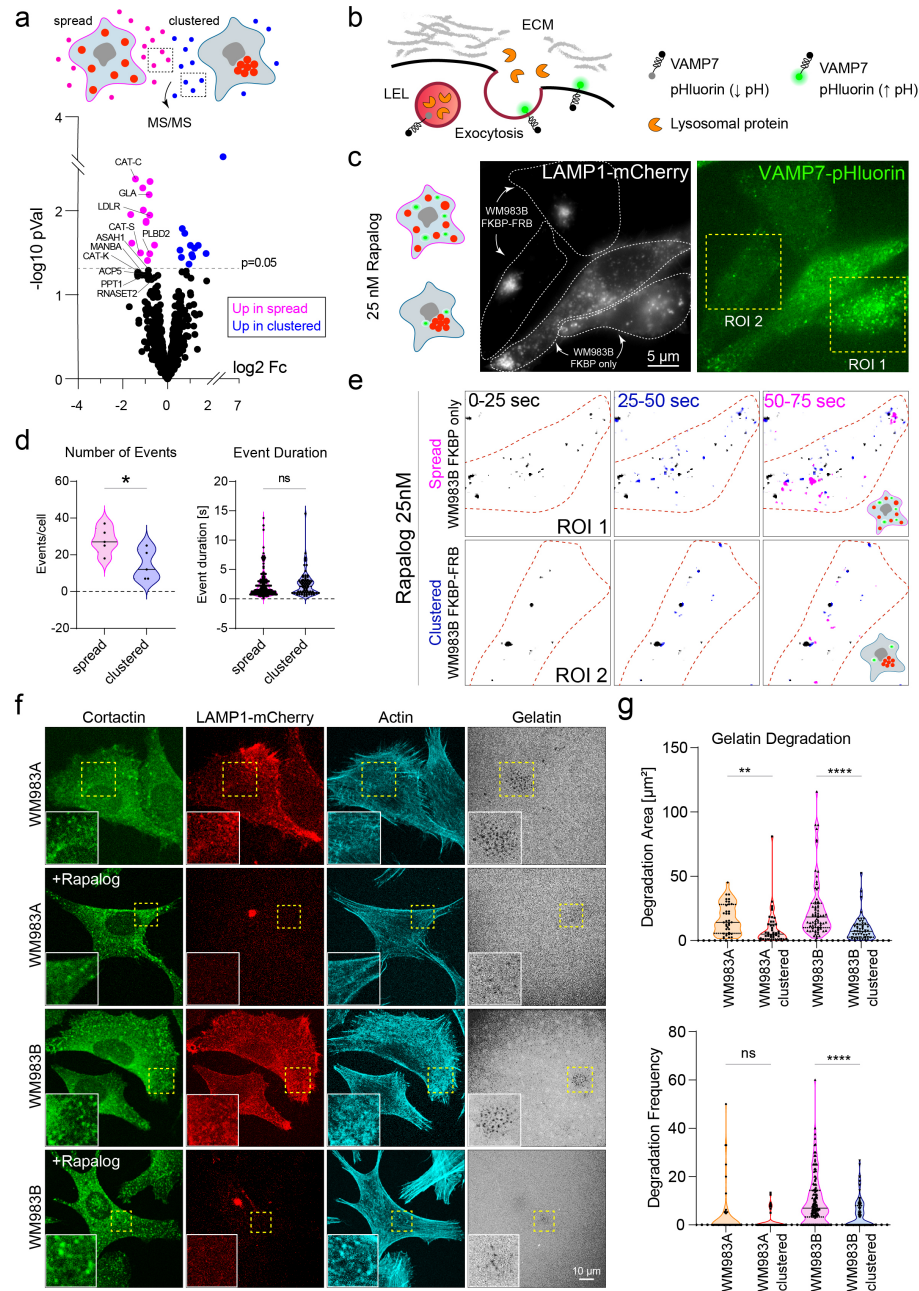


Fig. 3. Lysosome clustering inhibits lysosome secretion and matrix degradation a) Differential quantitative mass spectrometry analysis of proteins secreted in the cell medium by WM983B cells expressing LAMP1-mCherry-FKBP and BicD2-FRB. Cells were cultured in serum-free medium for 24 hours in the absence or presence of 5nM Rapalog. Each dot represents a protein. Proteins known to be lysosome-associated are labeled with their name. Magenta = proteins upregulated in cells with spread lysosomes, blue = proteins upregulated in cells with clustered lysosomes ($p < 0,05$). b-e) Lysosome secretion was assessed by TIRF microscopy using VAMP7-pHluorin probe, which is quenched in lysosome acidic environment and has bright fluorescence once exposed to the alkaline pH of the extracellular space. c) WM983B-LAMP1-mCherry cells expressing single heterodimerizing domain (FKBP only) and both the domains (FKBP-FRB) were co-cultured, treated with 25nM Rapalog for 1 hour and analyzed by TIRF microscopy. d) Number of secretion events (left graph, one dot represents one cell) and event duration (right graph, one dot represents one event) were manually counted in five different cells of each type. Events per cell, spread = 27.80 ± 7.19 , clustered = 14.40 ± 8.23 , p value = 0.0397, Mann-Whitney test. Event duration, spread = 2.46 ± 2.36 , clustered = 2.56 ± 2.20 seconds, p value = 0.3518, Mann-Whitney test. e) TIRF movie was divided into three time-segments (black: 0-25 seconds, blue: 25-50 seconds, magenta: 50-75 seconds) and displayed as maximum projection showing the number of events per each time-segment. Two representative examples from panel c are shown, ROI 1 = spread lysosomes, ROI 2 = clustered lysosomes. f-g) WM983A or WM983B cells expressing LAMP1-mCherry-FKBP and BicD2-FRB were cultured on FITC-gelatin in presence or absence of 25nM Rapalog for 24 hours, fixed and stained with cortactin antibody and Phalloidin and imaged by confocal microscopy. g) Degradation area (DA) per cell and degradation frequency (DF) were quantified from the immunofluorescence images. DA (Mean \pm SD) = 16.94 ± 11.71 n= 36 cells, 9.38 ± 13.47 n= 45 cells, 24.75 ± 23.29 n= 73 cells, 9.41 ± 9.94 n= 50 cells, respectively, in triplicate. DF (Mean \pm SD) = 3.62 ± 9.73 n= 57 cells, 1.49 ± 3.44 n= 58 cells, 10.14 ± 10.45 n= 138 cells, 4.43 ± 6.04 n= 102 cells, respectively, in triplicate, Kruskal-Wallis with Dunn's multiple comparison post-hoc test. One dot represents one cell. * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; **** $p < 0,0001$.

quently promotes melanoma cell invasion and metastatic progression. This echoes a parallel study showing that relocalization of lysosomes to the cell periphery promotes the emergence of leader cells in collective epithelial cell migration (Marwaha et al., 2023). While more work is required to understand the switches that relocalize lysosomes, both studies demonstrate that peripheral lysosome positioning can impact cell migration, through different mechanisms, either through lysosomal secretion (our study) or through a Rac1-dependent control of actin polymerization and lamellipodium formation (Marwaha et al., 2023). In addition, lysosome repositioning could potentially alter tumor progression by mediating nutrient sensing (Korolchuk et al., 2011) or chemoresistance through the secretion of chemotherapeutics stored in lysosomes (Machado et al., 2015). Probing of spatial distribution and morphology of lysosomes in tumors could constitute a novel indicator of tumor progression, as it is the case for cell shape or additional morphometric analysis (Sero et al., 2015; Pei-Hsun Wu et al., 2020). Several other organelles, such as mitochondria, are intimately linked to cancer progression and probing simultaneously all organelles, as it can be done using whole-cell segmentation of high-resolution images (Heinrich et al., 2021), could document precisely which organelles, and their contacts, are repositioned during melanoma progression. Interestingly, deep learning-based approaches recently demonstrated the power of correlating breast cancer status with organelle topology, which out-performed morphology-based features, further highlighting the need to consider organelles positioning, and their interactome, as a new cancer rheostat that could be exploited for better diagnosis (Wang et al., 2023).

Methods

Antibodies Anti-Cortactin (p80/85) clone 4F11 (ref. n°05-180-I) and Anti-LAMP1 (ref. n°L1418) are from Merck (Sigma-Aldrich). Alexa Fluor™ 488 Phalloidin (ref. n°A12379), Alexa Fluor™ 568 Phalloidin (ref. n°A12380), Alexa Fluor™ 647 Phalloidin (ref. n°A22287) and Alexa Fluor-conjugated secondary antibodies, are all from Thermo Fisher Scientific. Secondary antibodies include Alexa Fluor™ 405 conjugated Goat anti-Rabbit (ref. n°A-31556), Alexa Fluor™ 488 conjugated Goat anti-Rabbit (ref. n°A-11034), Alexa Fluor™ 555 conjugated Goat anti-Rabbit (ref. n°A21429) Alexa Fluor™ 647 conjugated Goat anti-Rabbit (ref. n°A-21244).

Cell culture Primary human melanoma cell lines were purchased from Rockland and cultured in MCDB153 (ref. n°M7403, Merck/Sigma-Aldrich) and Leibovitz's L-15 medium (ref. n°11415064, Thermo Fisher Scientific) in a 4 to 1 ratio, supplemented with 2 per cent foetal bovine serum, 1.68 mM CaCl₂ and 1 per cent penicillin/streptomycin.

Lentivirus transduction and plasmid transfection Transduction: pLSFFV-LAMP1-mCherry-FKBP (FK506 binding protein), pLSFFV-BicD-FRB (FKBP-rapamycin binding domain) and pLSFFV-LifeActin-miRFP lentivirus were produced in HEK293T cells using JetPRIME® transfection reagent (Polyplus). WM983B cells were infected by

lentivirus in the presence of 5µg/mL polybrene (ref. n°TR-1003, Merck/Sigma-Aldrich) followed by antibiotic selection (puromycin 1µg/mL, blasticidin 5µg/mL, hygromycin 200µg/ml). Transfection: Cenexin-GFP plasmid was transfected using JetOPTIMUS® transfection reagent (Polyplus) in a live-video dish, 46h prior experiment.

Invasion assay The 3D collagen invasion assay was adapted from (Sadok et al., 2015). Briefly, melanoma cell lines at 3x10⁵ cells/ml were labelled with Hoechst 33342 and suspended in 3mg/mL of serum free solution of neutralized Type I Bovine Collagen (PureCol® 5005-B, Advanced Biomatrix). Then, 600µL were distributed into black 24 well plates (ref. n°058062, Dutscher) coated with bovine serum albumin. The plates were centrifuged at 1500 rpm at 4°C for 5min and incubated at 37°C for 3 hours. Once collagen had polymerized, medium supplemented with 10 per cent foetal bovine serum, 100ng/mL EGF was added on top of the collagen. 24h after, cells were observed using a Leica TSC SPE confocal microscope (x20 HCX PI Apo 0.7 NA objective, Wetzlar, Germany) and z-stacks were acquired. Four fields per sample were imaged. Nuclear localization was quantified by IMARIS (interactive microscopy image analysis software) at each plane. The invasion index was calculated by reporting the number of cells above 10µM on the total number of cells by field.

Matrix degradation assay and immunofluorescence Glass coverslips were coated with fluorescent-labelled-FITC gelatine or Cy3 gelatine as described previously (Kolli-Bouhafs et al., 2014). Then, previously starved melanoma cells expressing or not LAMP1-mCherry-FKBP and BicD2-FRB were plated on fluorescent gelatine and incubated at 37°C for 6 to 24h in medium supplemented with 10 per cent foetal bovine serum with or without Rapalog. Cells were fixed using 4 per cent paraformaldehyde permeabilized using triton-X-100 at 0,1 per cent and incubated in 2 per cent of bovine serum albumin at room temperature. Cells were then labelled for 1h with Anti-Cortactin (1/250). After three washes with PBS, cells were incubated with either Alexa Fluor™ 405 conjugated Goat anti-Rabbit (1/1000), Alexa Fluor™ 488 conjugated Goat anti-Rabbit (1/1000) or Alexa Fluor™ 647 conjugated Goat anti-Rabbit (1/1000) and Alexa Fluor™ 568 Phalloidin (1/250) or Alexa Fluor™ 647 Phalloidin (1/250) for 1h, washed and mounted in ProLong™ Gold Antifade Mountant (ref. n°P10144, Thermo Fisher Scientific). Cells were imaged using Leica TSC SPE or SP8 confocal microscope (x63 HCX PI Apo 1.40 NA or x20 HCX PI Apo 0.7 NA objective, Wetzlar, Germany). Invadopodia were identified as actin and cortactin rich punctate structure. Areas of degradation were identified as "black holes" within the fluorescent gelatine. Invadopodia and areas of degradations were quantified using ImageJ software. Degradation areas measurements were based on cells displaying degradation activity, and the frequency of degradation was based on randomly selected cells. Maximum filter, background subtraction and gaussian blur filters were then applied to extract the gel degradation areas by thresholding. Then, M1 Manders coefficients between Lamp1 and inverted Gelatin inten-

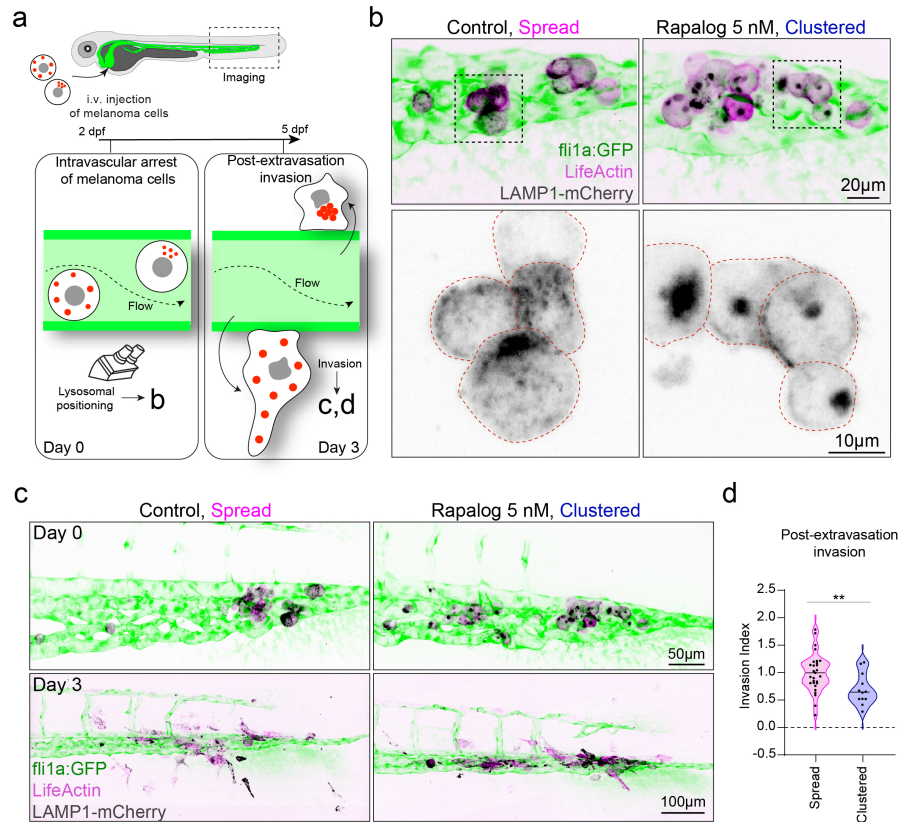


Fig. 4. Lysosome clustering impairs cancer cell invasion in vivo a) Fli1a:GFP (green endothelium) zebrafish embryos (48 hpf) were injected intra-vascularly with WM983B cells having either spread or clustered lysosomes and imaged by confocal microscopy at day 0 and day 3 post injection to assess the post-extravasation invasion potential of cancer cells with different lysosomal positioning. b) WM983B cells stably expressing LAMP1-mCherry-FKBP, BicD2-FRB and LifeActin-miRFP were cultured in medium or in medium supplemented with 5nM Rapalog for 1 hour before injection. Representative confocal images at 3 hours post-injection are shown as maximum z projection for each condition, control = spread lysosomes, 5nM Rapalog = clustered lysosomes. c) Zebrafish embryos were imaged at day 0 (3 hours after cell injection) and at day 3 (72 hours post injection) using a spinning disc microscope. Representative images are shown as a maximum z projection. d) Post-extravasation invasion (PEI) potential was calculated as a proportion of cells (area) that migrated outside of the vasculature region to the total area of cells. PEI (Mean \pm SD), control = 1.000 ± 0.3491 n = 29 embryos, clustered = 0.696 ± 0.285 n = 12 embryos, in triplicate, p value = 0.0090, Mann-Whitney test. One dot represents one fish, normalized to control.

438 sity pictures for these selections were calculated using the 459
439 colocalization finder plugin. 460

440 **Clustering dynamics, washout experiments proliferation** 462
441 **assay** Mechanism of lysosome clustering: clustering was per- 463
442 formed by heterodimerization between LAMP1-mCherry- 464
443 FKBP (lysosomes) and BicD-FRB (dynein adaptor) by the 465
444 use of Rapalog (A/C Heterodimerizer ref. n°635057, from
445 Takara Bio Inc.). Cells were cultured in glass bottom dishes 466
446 and imaged at different time points (24, 48 and 72 hours) 467
447 using Olympus Spinning Disk (60X objective, N.A. 1.2). 468
448 To establish time needed for lysosome clustering, WM983A 469
449 and WM983B cells expressing both heterodimerizing do- 470
450 mains (FKBP-FRB) were treated with 5nM or 25nM Ra- 471
451 palog and followed in time. Time to appearance of lyso- 472
452 some cluster was counted. Washout experiments: WM983B 473
453 cells expressing either one (FKBP only) or two (FKBP-FRB) 474
454 heterodimerizing domains were cultured in medium supple- 475
455 mented with 5nM or 25nM Rapalog. To assess the stability of 476
456 clustering, cells were treated for 1h with 5nM or 25nM Rapa- 477
457 log, washed 3x in PBS and cultured in normal medium for the 478
458 duration of the experiment. Cells cultured in normal growth 479

medium were used as a control. Proliferation assay: prolif-
eration rates of WM983B cells treated with increasing con-
centrations of Rapalog were analyzed using the Incucyte®
Live-Cell Analysis System. Confluences were automatically
calculated by the Incucyte® software based on bright field
images, all values are normalized to time zero. Acquisition
was performed for 96h.

Micropatterning Immunofluorescence Micropatterns were
prepared using photo-lithography methods as previously de-
scribed (Duong et al., 2012; Schauer et al., 2010). Briefly,
cover slides were cleaned in EtOH, dried, cleaned with UV
for 10 minutes, coated in PLL-PEG and exposed to UV 10
minutes through a Photomask with 36µm crossbow micropat-
terns. Coverslips were coated in Fibronectin (40µg/ml –
ref. n°F1141 Merck/Sigma-Aldrich). WM1862, WM983A,
WM983B cells were trypsinized, resuspended in 5ml culture
medium and seeded an micropatterned coverslips. Cells were
let to spread for 4 hours before fixing them in 4 per cent
PFA. For micropatterning, cells were fixed in 4 per cent
PFA, washed 3x in PBS, permeabilized in saponin 0.5 per cent /
2 per cent BSA in PBS for 20 minutes, blocked 30 minutes

in 2 per cent BSA, stained with Anti-LAMP1 (ref. n°L1418) primary antibody for 1 hour, washed 3x in PBS, stained with Alexa Fluor™ 555 secondary Goat anti-Rabbit (1:500) and Alexa Fluor™ 488 Phalloidin (1:200) 45 minutes. Mounted in Fluoromount-GTM mounting medium (ref. n°00-4958-02, Thermo Fisher Scientific with DAPI. Z stacks were acquired for the full cell volume (0.2µm between layers) using an Olympus Spinning Disk (60X objective, N.A. 1.2). Image analysis and processing were performed using the Fiji (2.0.0) (Schindelin et al., 2012), Cell Profiler (4.2.1) (Stirling et al., 2021), Metamorph and R software.

Live cell imaging For live cell imaging, Lamp1-mCherry and Lifeact-miRFP expressing cells were plated on fluorescent gelatin-coated coverslips and mounted in a Ludin Chamber (Life Imaging Services). The cells were then placed at 37°C, 5 per cent CO₂ on an iMIC microscope equipped with a multi-LED Lumencor Spectra X. Images were acquired with an Olympus 60x TIRFM (1.45 NA) objective every 2.5min during 4h and a Hamamatsu Flash 4 V2+ camera (Iwata) piloted by the Live Acquisition software (Till Photonics). Expressing cells were initially located via both the mCherry and Lifeact-miRFP signals, and were subsequently followed via dual phase contrast/fluorescent signal together with the FITC-coupled gelatin substrate. 10 to 20 different fields were sequentially recorded during each experiment using a Marzhauser Motorized Stage piloted by the iMIC software. JeasyTFM software were then used for automatic selection and repositioning of the best focused images in all channels and time-points. Mean fluorescence intensity of actin, Lamp1 and the underlying substrate at each time points were calculated at invadopodia identified using gelatin degradation.

TIRF microscopy WM983B cells expressing FKBP only (spread) and WM983B cells expressing both FKBP and FRB (clustered) were mixed in an 1:1 ratio, seeded in fibronectin-coated low glass bottom µ-Dish 35mm (ref. n°80137, Ibidi) 48h prior to imaging, treated with 25nM Rapalog for 1h. Imaging was performed in culture medium using an inverted Leica DMI8 microscope (objective 100X HC PL APO 1.47 oil). Recording was done with an Evolve® 512 camera (for TIRF-HILO), at 512X512 pixels resolution, at an acquisition rate of 250ms between frames, for a total duration of 90 seconds, with AFC (Adaptive Focus Control). Exocytosis events were identified based on VAMP7-pHluorin signal, marking lysosome exocytosis (Lachuer et al., 2023). Secretion events were detected and counted manually.

Experimental metastasis assay in zebrafish All zebrafish (ZF) procedures were performed in accordance with French and European Union animal welfare guidelines and supervised by local ethics committee (Animal facility A6748233; APAFIS 2018092515234191). Tg(fli1a:eGFP) (Lawson and Weinstein, 2002) embryos were maintained in Danieau 0.3X medium (17.4mM NaCl, 0.2mM KCl, 0.1mM MgSO₄, 0.2mM Ca(NO₃)₂) buffered with HEPES 0.15mM (pH=7.6), supplemented with 200µM of 1-phenyl-2-thiourea (PTU, ref. n°P7629, Merck/Sigma-Aldrich) to avoid pigmentation. Two days post-fertilization (2dpf) embryos were mounted in 0.8 per cent ultrapure low melting point agarose (Invitro-

gen) containing 0.17mg/ml tricaine (ethyl-3-aminobenzoate-methanesulfonate, ref. n°E10521, Merck/Sigma-Aldrich). WM983B cells of different lysosome clustering status (spread, clustered) were injected with a Nanoject II Auto-Nanoliter Injector (Drummond Scientific Company) and microforged glass capillaries (25 to 30µm inner diameter) filled with mineral oil (ref. n°M5904, Merck/Sigma-Aldrich). 13.8nL of cell suspension from confluent T25 flasks (50x10⁶ cells per ml approx.) were injected in the duct of Cuvier under a M205 FA Fluorescence stereomicroscope (Leica), as previously described (Stoletov et al., 2010). Embryos were injected with WM983B cells or with WM983B cells treated with 5nM rapalog 1h prior to injection, and then kept in Danieau with PTU. Caudal plexus was recorded at day 0 (injection day) and 3dpi using the inverted spinning-disk Olympus IXplore Spin, 30x / 1.05 NA (silicone) objective. Z-stacks of the caudal plexus were acquired for each embryo (3µm or 5µm between layers, at day 0 and 3dpi respectively) with the following settings: 488nm laser at 2 per cent for 100ms / 561nm laser at 15 per cent for 300ms / 640nm laser at 15 per cent for 300ms). Detailed lysosome status (Fig.4b) was imaged at 3 hpi using a 60x/1.2 NA (water) objective.

Correlative Light and Electron Microscopy (CLEM)

WM983B cells expressing LAMP1-mCherry-FKBP and BicD2-FRB were cultured in control medium, or in medium supplemented with 25nM Rapalog for one hour and imaged with an Olympus Spinning Disk (60X objective, N.A. 1.2). The samples were then chemically fixed right after the photonic acquisition with 0,05 per cent malachite green, 2.5 per cent glutaraldehyde in 0.1M sodium cacodylate buffer (NaCac), pH7.4 during 30min in an ice bath. Subsequently the samples were post-fixed in 1 per cent OsO₄ - 0.8 per cent K₃[Fe(CN)₆] - 0.1M NaCac buffer pH7.4 (under a fume hood) kept in an ice bath for 50 min, and then washed 2 times with ice-cold 0.1M NaCac. Then the samples were incubated in 1 per cent aqueous tannic acid solution for 25 min in an ice bath and finally washed 5 times with distilled water. Samples were then kept in 1 per cent uranyl acetate aqueous solution overnight at 4°C sheltered from the light. The samples were serially dehydrated with ethanol solutions (25 per cent, 50 per cent, 70 per cent 95 per cent and 100 per cent). Subsequently the samples were incubated in a serial resin-ethanol 100 per cent mix (1:3; 1:1; 3:1), ending with an incubation in 100 per cent Epon resin 3 times 1h at room temperature. The samples were allowed to polymerize in an oven at 60°C for 48h. The resin blocks were trimmed by ultramicrotomy, 90nm thin sections were collected and placed in copper/formvar slot grids. The transmission electron microscopy (TEM) data sets were acquired with a Hitachi 7500 TEM, with 80 kV beam voltage, and the 8-bit images were obtained with a Hamamatsu camera C4742-51-12NR. Correlative light and electron images were obtained/combined using Adobe Photoshop v.24.4.

Image analysis Organelles segmentation was performed using MetaMorph Microscopy Automation and Image Analysis Software (Molecular Devices) and the ImageJ Modular Image Analysis (MIA) plugin. Segmentation on LAMP1

594 images were performed to get coordinates for individual 651
595 LAMP1+ objects and their number. 3D Density maps and 652
596 inter-organelle distance and distance to barycenter were ob- 653
597 tained through the use of R software. Cell invasion in ze- 654
598 brafish was performed by ImageJ Cell Profiler (Molecular 655
599 Devices) software. Zebrafish images were divided into 6 re- 656
600 gions – plexus and 5 regions outside (bins). Area of cells was 657
601 quantified for each region using Cell Profiler and percentage 658
602 of cells (from total) was calculated per each region and plot- 659
603 ted in a graph. Invasion potential was calculated as area under 660
604 the curve for all cells that extravasated and invaded outside of 661
605 the vasculature region. 662

606 **Mass Spectrometry - Quantitative Proteomics** A 664
607 PierceTM 660nm protein assay quantification (ref. n°22660, 665
608 Thermo Fisher Scientific), 2µg of each protein extract 666
609 were digested using the automated Single Pot Solid Phase 667
610 enhanced Sample Preparation (SP3) protocol as described 668
611 in (Hughes et al., 2019) on the Bravo AssayMAP plat- 669
612 form (Agilent Technologies). Extracted peptides were 670
613 cleaned-up using automated C18 solid phase extraction on 671
614 the same platform and analysed by nanoLC-MS/MS on a 672
615 nanoUPLC system (nanoAcquityUPLC, Waters) coupled 673
616 to a quadrupole-Orbitrap mass spectrometer (Q-Exactive 674
617 HF-X, Thermo Scientific). Chromatographic separation was 675
618 conducted over a 60 minutes linear gradient from 2 to 40 per 676
619 cent of solvent B (0.1 per cent formic acid in acetonitrile) 677
620 at a flow rate of 350 nL/min. A Top 10 method was used 678
621 with automatic switching between MS and MS/MS modes 679
622 to acquire high resolution MS/MS spectra. To minimize 680
623 carry-over, a solvent blank injection was performed after 681
624 each sample. NanoLC-MS/MS data was interpreted to do 682
625 label-free extracted ion chromatogram-based differential 683
626 analysis. Searches were done using Mascot software (version 684
627 2.5.1, MatrixScience) against a composite database including 685
628 Homo Sapiens and Bos Taurus protein sequences, which 686
629 were downloaded from UniProtKB-SwissProt (28-07-2021; 687
630 26.031 sequences, Taxonomy ID: 9913 and 9606 respec- 688
631 tively) to which common contaminants and decoy sequences 689
632 were added. One trypsin missed cleavage was tolerated. 690
633 Carbamidomethylation of cysteine residues was set as a fixed 691
634 modification. Oxidation of methionine residues and acetyla- 692
635 tion of proteins n-termini were set as variable modifications. 693
636 Identification results were imported into the Proline software 694
637 (version 2.2.0) (Bouyssie et al., 2020) and validated. The 695
638 maximum false discovery rate was set at 1 per cent at peptide 696
639 and protein levels with the use of a decoy strategy. Peptides 697
640 abundances were extracted with cross assignment between 698
641 all samples. Protein abundances were computed using the 699
642 best ion of the unique peptide abundances normalized at the 700
643 peptide level using the median. To be considered, proteins 701
644 must be identified in at least three out of the four replicates in 702
645 at least one condition. The imputation of the missing values 703
646 and differential data analysis were performed using the 704
647 open-source ProStaR software (version 1.30.7) (Wieczorek 705
648 et al., 2017). Imputation of missing values was done using 706
649 the approximation of the lower limit of quantification by 707
650 the 2.5 per cent lower quantile of each replicate intensity 708

distribution (“det quantile”). A Limma moderated t-test was applied on the dataset to perform differential analysis. The adaptive Benjamini-Hochberg procedure was applied to adjust the p-values and False Discovery Rate. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (Perez-Riverol et al., 2022) with the dataset identifier PXD042007.

Transcriptomic analysis RNA integrity was assessed by Bioanalyzer (total RNA Pico Kit, 2100 Instrument, Agilent Technologies, Palo Alto, CA, USA). All samples had RNA integrity numbers above 9.5. Sequencing libraries were prepared using “NEBNext Ultra II Directional RNA Library Prep Kit for Illumina” combined with “NEB Ultra II polyA mRNA magnetic isolation” for mRNA enrichment (New England Biolabs, Ipswich, MA, USA). Libraries were pooled and sequenced (single-end, 100bp) on a NextSeq2000 according to the manufacturer’s instructions (Illumina Inc., San Diego, CA, USA). For each sample, quality control was carried out and assessed with the NGS Core Tool FastQC (Andrews S, 2010). Sequence reads (minimum 33 Million per sample) were mapped to Homo Sapiens genome version GRCh38 using STAR (Dobin et al., 2013) to obtain a BAM (Binary Alignment Map) file. An abundance matrix was generated based on read counts identified by Featurecounts (Liao et al., 2014) using default parameters. At last, differential expression analyses were performed using the DESeq2 (Love et al., 2014) package of the Bioconductor framework for RNASeq data (Gentleman et al., 2004). Up- and down-regulated genes were selected based on the adjusted p-value (< 0.05) and the fold-change (> 1.5). Functional enrichment analyses were performed using STRING v11 (Szklarczyk et al., 2019) and Gene Ontology (Carbon et al., 2021). Raw data (FASTQ files) are available at the EMBL-EBI ArrayExpress archive (Accession number E-MTAB-13165).

Statistical analyses Statistical analysis of the results was done using GraphPad Prism 9 (version 9.5.1 for Windows). Mann-Whitney (two groups) or Kruskal-Wallis (>2 groups) statistical tests were performed as specified in the figure legends, to the exception of quantitative proteomics and transcriptomic analysis which have dedicated statistical methodologies specified above. Illustrations of the statistical analyses are displayed in the figures as the mean +/- standard deviation (SD). p-Values smaller than 0.05 were considered as statistically significant. *, p<0.05, **, p<0.01, ***, p<0.001, ****, p<0.0001.

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733

Author Contributions KJR, VH, KS, PR and JGG con-
734 ceived project and designed the experiments. KJR performed
735 most of the experiments and analysis. AM, RK, HJ, LML,
736 PC, ES and PR performed collagen gel invasion assays and
737 invadopodia experiments. KJR, NA and MP performed ze-
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739 ments. AL and OL performed molecular biology. KJR per-
740 formed and analyzed (with help from HL) micropatterning
741 experiments. AP, TS, AM and RC performed and analyzed
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749 Bibliography

750 Andrews S, 2010. FastQC: a quality control tool for high
751 throughput sequence data. 807
752 Augoff, K., Hryniewicz-Jankowska, A., Tabola, R., 809
753 2020. Invadopodia: clearing the way for cancer 810
754 cell invasion. *Ann. Transl. Med.* 8, 902. 811
755 <https://doi.org/10.21037/atm.2020.02.157> 812
756 Ballabio, A., Bonifacino, J.S., 2020. Lysosomes as 813
757 dynamic regulators of cell and organismal homeosta- 814
758 sis. *Nat. Rev. Mol. Cell Biol.* 21, 101–118. 815
759 <https://doi.org/10.1038/s41580-019-0185-4> 816
760 Bian, B., Mongrain, S., Cagnol, S., Langlois, M.-J., 817
Boulanger, J., Bernatchez, G., Carrier, J.C., Boudreau, F.,
Rivard, N., 2016. Cathepsin B promotes colorectal tumori-
genesis, cell invasion, and metastasis. *Mol. Carcinog.* 55,
671–687. <https://doi.org/10.1002/mc.22312>
Bouyssié, D., Hesse, A.-M., Mouton-Barbosa, E., Rompais,
M., Macron, C., Carapito, C., Gonzalez de Peredo, A.,
Couté, Y., Dupierriis, V., Burel, A., Menetrey, J.-P.,
Kalaitzakis, A., Poisat, J., Romdhani, A., Burlet-Schiltz,
O., Cianférani, S., Garin, J., Bruley, C., 2020. Pro-
line: an efficient and user-friendly software suite for
large-scale proteomics. *Bioinformatics* 36, 3148–3155.
<https://doi.org/10.1093/bioinformatics/btaa118>
Braeuer, R.R., Zigler, M., Villares, G.J., Dobroff,
A.S., Bar-Eli, M., 2011. Transcriptional Control of
Melanoma Metastasis: The Importance of the Tumor Mi-
croenvironment. *Semin. Cancer Biol.* 21, 83–88.
<https://doi.org/10.1016/j.semcancer.2010.12.007>
Chaîneau, M., Danglot, L., Galli, T., 2009. Multiple
roles of the vesicular-SNARE TI-VAMP in post-Golgi and
endosomal trafficking. *FEBS Lett.* 583, 3817–3826.
<https://doi.org/10.1016/j.febslet.2009.10.026>
Chen, C.-S., Chen, W.-N.U., Zhou, M., Arttamangkul, S.,
Haugland, R.P., 2000. Probing the cathepsin D using a BOD-
IPY FL–pepstatin A: applications in fluorescence polariza-
tion and microscopy. *J. Biochem. Biophys. Methods* 42,
137–151. [https://doi.org/10.1016/S0165-022X\(00\)00048-8](https://doi.org/10.1016/S0165-022X(00)00048-8)
Circu, M.L., Dykes, S.S., Carroll, J., Kelly, K., Galiano, F.,
Greer, A., Cardelli, J., El-Osta, H., 2016. A Novel High Con-
tent Imaging-Based Screen Identifies the Anti-Helminthic
Niclosamide as an Inhibitor of Lysosome Anterograde Traf-
ficking and Prostate Cancer Cell Invasion. *PLOS ONE* 11,
e0146931. <https://doi.org/10.1371/journal.pone.0146931>
Corrotte, M., Castro-Gomes, T., 2019. Chapter One
- Lysosomes and plasma membrane repair, in: An-
drade, L.O. (Ed.), *Current Topics in Membranes, Plasma
Membrane Repair*. Academic Press, pp. 1–16.
<https://doi.org/10.1016/bs.ctm.2019.08.001>
da Costa, A.C., Santa-Cruz, F., Mattos, L.A.R., Rêgo
Aquino, M.A., Martins, C.R., Bandeira Ferraz, Á.A.,
Figueiredo, J.L., 2020. Cathepsin S as a target in
gastric cancer. *Mol. Clin. Oncol.* 12, 99–103.
<https://doi.org/10.3892/mco.2019.1958>
Dillekås, H., Rogers, M.S., Straume, O., 2019. Are 90 per
cent of deaths from cancer caused by metastases? *Cancer
Med.* 8, 5574–5576. <https://doi.org/10.1002/cam4.2474>
Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski,
C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013.
STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*
29, 15–21. <https://doi.org/10.1093/bioinformatics/bts635>
Duong, T., Goud, B., Schauer, K., 2012. Closed-form
density-based framework for automatic detection of cellu-
lar morphology changes. *Proc. Natl. Acad. Sci.* 109,
8382–8387. <https://doi.org/10.1073/pnas.1117796109>
Follain, G., Osmani, N., Azevedo, A.S., Allio, G., Mercier,
L., Karreman, M.A., Solecki, G., Garcia León, M.J., Lefeb-
vre, O., Fekonja, N., Hille, C., Chabannes, V., Dollé, G.,
Metivet, T., Hovsepian, F.D., Prudhomme, C., Pichot, A.,

- 818 Paul, N., Carapito, R., Bahram, S., Ruthensteiner, B., Kemm- 875
819 ling, A., Siemonsen, S., Schneider, T., Fiehler, J., Glatzel, M., 876
820 Winkler, F., Schwab, Y., Pantel, K., Harlepp, S., Goetz, J.G., 877
821 2018. Hemodynamic Forces Tune the Arrest, Adhesion, and 878
822 Extravasation of Circulating Tumor Cells. *Dev. Cell* 45, 33- 879
823 52.e12. <https://doi.org/10.1016/j.devcel.2018.02.015> 880
- 824 Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Det- 881
825 tling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, 882
826 J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, 883
827 R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Sawitzki, 884
828 G., Smith, C., Smyth, G., Tierney, L., Yang, J.Y., Zhang, J., 885
829 2004. Bioconductor: open software development for compu- 886
830 tational biology and bioinformatics. *Genome Biol.* 5, R80. 887
831 <https://doi.org/10.1186/gb-2004-5-10-r80> 888
- 832 Ghoroghi, S., Mary, B., Larnicol, A., Asokan, N., Klein, A., 889
833 Osmani, N., Busnelli, I., Delalande, F., Paul, N., Halary, 890
834 S., Gros, F., Fouillen, L., Haeberle, A.-M., Royer, C., 891
835 Spiegelhalter, C., André-Grégoire, G., Mittelheisser, V., De- 892
836 tappe, A., Murphy, K., Timpson, P., Carapito, R., Blot- 893
837 Chabaud, M., Gavard, J., Carapito, C., Vitale, N., Lefeb- 894
838 vre, O., Goetz, J.G., Hyenne, V., 2021. Ral GTPases 895
839 promote breast cancer metastasis by controlling biogene- 896
840 sis and organ targeting of exosomes. *eLife* 10, e61539. 897
841 <https://doi.org/10.7554/eLife.61539> 898
- 842 Glunde, K., Guggino, S.E., Solaiyappan, M., Pathak, A.P., 899
843 Ichikawa, Y., Bhujwalla, Z.M., 2003. Extracellular Acidifica- 900
844 tion Alters Lysosomal Trafficking in Human Breast Cancer 901
845 Cells. *Neoplasia* 5, 533–545. [https://doi.org/10.1016/S1476-](https://doi.org/10.1016/S1476-902)
846 [5586\(03\)80037-4](https://doi.org/10.1016/S1476-5586(03)80037-4) 903
- 847 Heinrich, L., Bennett, D., Ackerman, D., Park, W., Bogovic, 904
848 J., Eckstein, N., Petruncio, A., Clements, J., Pang, S., Xu, 905
849 C.S., Funke, J., Korff, W., Hess, H.F., Lippincott-Schwartz, 906
850 J., Saalfeld, S., Weigel, A.V., 2021. Whole-cell organelle 907
851 segmentation in volume electron microscopy. *Nature* 599, 908
852 141–146. <https://doi.org/10.1038/s41586-021-03977-3> 909
- 853 Hoshino, D., Kirkbride, K.C., Costello, K., Clark, 910
854 E.S., Sinha, S., Grega-Larson, N., Tyska, M.J., 911
855 Weaver, A.M., 2013. Exosome secretion is en- 912
856 hanced by invadopodia and drives invasive behav- 913
857 ior. *Cell Rep.* 5, 10.1016/j.celrep.2013.10.050. 914
858 <https://doi.org/10.1016/j.celrep.2013.10.050> 915
- 859 Hua, H., Li, M., Luo, T., Yin, Y., Jiang, Y., 2011. 916
860 Matrix metalloproteinases in tumorigenesis: an evolving 917
861 paradigm. *Cell. Mol. Life Sci. CMLS* 68, 3853–3868. 918
862 <https://doi.org/10.1007/s00018-011-0763-x> 919
- 863 Hughes, C.S., Moggridge, S., Müller, T., Sorensen, P.H., 920
864 Morin, G.B., Krijgsvelde, J., 2019. Single-pot, solid-phase- 921
865 enhanced sample preparation for proteomics experiments. 922
866 *Nat. Protoc.* 14, 68–85. [https://doi.org/10.1038/s41596-018-](https://doi.org/10.1038/s41596-018-923)
867 [0082-x](https://doi.org/10.1038/s41596-018-0082-x) 924
- 868 Jacob, A., Prekeris, R., 2015. The regulation of MMP target- 925
869 ing to invadopodia during cancer metastasis. *Front. Cell Dev.* 926
870 *Biol.* 3, 4. <https://doi.org/10.3389/fcell.2015.00004> 927
- 871 Jia, R., Bonifacino, J.S., 2019. Lysosome Positioning Influ- 928
872 ences mTORC2 and AKT Signaling. *Mol. Cell* 75, 26-38.e3. 929
873 <https://doi.org/10.1016/j.molcel.2019.05.009> 930
- 874 Johnson, D.E., Ostrowski, P., Jaumouillé, V., Grinstein, 931
S., 2016. The position of lysosomes within the cell de-
termines their luminal pH. *J. Cell Biol.* 212, 677–692.
<https://doi.org/10.1083/jcb.201507112>
- Kalluri, R., 2016. The biology and function of fibroblasts in cancer. *Nat. Rev. Cancer* 16, 582–598. <https://doi.org/10.1038/nrc.2016.73>
- Kapitein, L.C., Schlager, M.A., van der Zwan, W.A., Wulf, P.S., Keijzer, N., Hoogenraad, C.C., 2010. Probing Intracellular Motor Protein Activity Using an Inducible Cargo Trafficking Assay. *Biophys. J.* 99, 2143–2152. <https://doi.org/10.1016/j.bpj.2010.07.055>
- Kolli-Bouhafs, K., Sick, E., Noulet, F., Gies, J.-P., De Mey, J., Rondé, P., 2014. FAK competes for Src to promote migration against invasion in melanoma cells. *Cell Death Dis.* 5, e1379. <https://doi.org/10.1038/cddis.2014.329>
- Korolchuk, V.I., Saiki, S., Lichtenberg, M., Siddiqi, F.H., Roberts, E.A., Imarisio, S., Jahreiss, L., Sarkar, S., Futter, M., Menzies, F.M., O’Kane, C.J., Deretic, V., Rubinsztein, D.C., 2011. Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* 13, 453–460. <https://doi.org/10.1038/ncb2204>
- Lachuer, H., Le, L., Lévêque-Fort, S., Goud, B., Schauer, K., 2023. Spatial organization of lysosomal exocytosis relies on membrane tension gradients. *Proc. Natl. Acad. Sci.* 120, e2207425120. <https://doi.org/10.1073/pnas.2207425120>
- Lawson, N.D., Weinstein, B.M., 2002. In vivo imaging of embryonic vascular development using transgenic zebrafish. *Dev. Biol.* 248, 307–318. <https://doi.org/10.1006/dbio.2002.0711>
- Liao, Y., Smyth, G.K., Shi, W., 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930. <https://doi.org/10.1093/bioinformatics/btt656>
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>
- Machado, E., White-Gilbertson, S., van de Vlekkert, D., Janke, L., Moshiah, S., Campos, Y., Finkelstein, D., Gomero, E., Mosca, R., Qiu, X., Morton, C.L., Annunziata, I., d’Azzo, A., 2015. Regulated lysosomal exocytosis mediates cancer progression. *Sci. Adv.* 1, e1500603. <https://doi.org/10.1126/sciadv.1500603>
- Marwaha, R., Rawal, S., Khuntia, P., Banerjee, S., Manoj, D., Jaiswal, M., Das, T., 2023. Mechanosensitive dynamics of lysosomes along microtubules regulate leader cell emergence in collective cell migration. <https://doi.org/10.1101/2022.08.03.502740>
- Mathur, P., De Barros Santos, C., Lachuer, H., Patat, J., Latgé, B., Radvanyi, F., Goud, B., Schauer, K., 2023. Transcription factor EB regulates phosphatidylinositol-3-phosphate levels that control lysosome positioning in the bladder cancer model. *Commun. Biol.* 6, 1–14. <https://doi.org/10.1038/s42003-023-04501-1>
- Medina, D.L., Fraldi, A., Bouche, V., Annunziata, F., Mansueto, G., Spanpanato, C., Puri, C., Pignata, A., Martina, J.A., Sardiello, M., Palmieri, M., Polishchuk, R., Puertollano,

- R., Ballabio, A., 2011. Transcriptional Activation of Lysosomal Exocytosis Promotes Cellular Clearance. *Dev. Cell* 21, 421–430. <https://doi.org/10.1016/j.devcel.2011.07.016>
- Moamer, A., Hachim, I.Y., Binothman, N., Wang, N., Lebrun, J.-J., Ali, S., 2019. A role for kinesin-1 subunits KIF5B/KLC1 in regulating epithelial mesenchymal plasticity in breast tumorigenesis. *EBioMedicine* 45, 92–107. <https://doi.org/10.1016/j.ebiom.2019.06.009>
- Monteiro, P., Rossé, C., Castro-Castro, A., Irondelle, M., Lagoutte, E., Paul-Gilloteaux, P., Desnos, C., Formstecher, E., Darchen, F., Perrais, D., Gautreau, A., Hertzog, M., Chavrier, P., 2013. Endosomal WASH and exocyst complexes control exocytosis of MT1-MMP at invadopodia. *J. Cell Biol.* 203, 1063–1079. <https://doi.org/10.1083/jcb.201306162>
- Mousson, A., Legrand, M., Steffan, T., Vauchelles, R., Carl, P., Gies, J.-P., Lehmann, M., Zuber, G., De Mey, J., Dujardin, D., Sick, E., Rondé, P., 2021. Inhibiting FAK–Paxillin Interaction Reduces Migration and Invadopodia-Mediated Matrix Degradation in Metastatic Melanoma Cells. *Cancers* 13, 1871. <https://doi.org/10.3390/cancers13081871>
- Naegeli, K.M., Hastie, E., Garde, A., Wang, Z., Keeley, D.P., Gordon, K.L., Pani, A.M., Kelley, L.C., Morrissey, M.A., Chi, Q., Goldstein, B., Sherwood, D.R., 2017. Cell invasion in vivo via rapid exocytosis of a transient lysosome-derived membrane domain. *Dev. Cell* 43, 403–417. <https://doi.org/10.1016/j.devcel.2017.10.024>
- Nair, S.V., Narendradev, N.D., Nambiar, R.P., Kumar, R., Srinivasula, S.M., 2020. Naturally occurring and tumor-associated variants of RNF167 promote lysosomal exocytosis and plasma membrane resealing. *J. Cell Sci.* 133, jcs239335. <https://doi.org/10.1242/jcs.239335>
- Perera, R.M., Stoykova, S., Nicolay, B.N., Ross, K.N., Fita-mant, J., Boukhali, M., Lengrand, J., Deshpande, V., Selig, M.K., Ferrone, C.R., Settleman, J., Stephanopoulos, G., Dyson, N.J., Zoncu, R., Ramaswamy, S., Haas, W., Bardeesy, N., 2015. Transcriptional control of autophagy–lysosome function drives pancreatic cancer metabolism. *Nature* 524, 361–365. <https://doi.org/10.1038/nature14587>
- Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D.J., Prakash, A., Frericks-Zipper, A., Eisenacher, M., Walzer, M., Wang, S., Brazma, A., Vizcaíno, J.A., 2022. The PRIDE database resources in 2022: a hub for mass spectrometry based proteomics evidences. *Nucleic Acids Res.* 50, D543–D552. <https://doi.org/10.1093/nar/gkab1038>
- Pu, J., Guardia, C.M., Keren-Kaplan, T., Bonifacino, J.S., 2016. Mechanisms and functions of lysosome positioning. *J. Cell Sci.* 129, 4329–4339. <https://doi.org/10.1242/jcs.196287>
- Sadok, A., McCarthy, A., Caldwell, J., Collins, I., Garrett, M.D., Yeo, M., Hooper, S., Sahai, E., Kuemper, S., Mar-dakheh, F.K., Marshall, C.J., 2015. Rho Kinase Inhibitors Block Melanoma Cell Migration and Inhibit Metastasis. *Cancer Res.* 75, 2272–2284. <https://doi.org/10.1158/0008-5472.CAN-14-2156>
- Sahai, E., Astsaturon, I., Cukierman, E., DeNardo, D.G., Egeblad, M., Evans, R.M., Fearon, D., Gretchen, F.R., Hingorani, S.R., Hunter, T., Hynes, R.O., Jain, R.K., Janowitz, T., Jorgensen, C., Kimmelman, A.C., Kolonin, M.G., Maki, R.G., Powers, R.S., Puré, E., Ramirez, D.C., Scherz-Shouval, R., Sherman, M.H., Stewart, S., Tlsty, T.D., Tuveson, D.A., Watt, F.M., Weaver, V., Weeraratna, A.T., Werb, Z., 2020. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* 20, 174–186. <https://doi.org/10.1038/s41568-019-0238-1>
- Schauer, K., Duong, T., Bleakley, K., Bardin, S., Bornens, M., Goud, B., 2010. Probabilistic density maps to study global endomembrane organization. *Nat. Methods* 7, 560–566. <https://doi.org/10.1038/nmeth.1462>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682. <https://doi.org/10.1038/nmeth.2019>
- Sero, J.E., Sailem, H.Z., Ardy, R.C., Almuttaqi, H., Zhang, T., Bakal, C., 2015. Cell shape and the microenvironment regulate nuclear translocation of NF- κ B in breast epithelial and tumor cells. *Mol. Syst. Biol.* 11, 790. <https://doi.org/10.15252/msb.20145644>
- Steffan, J.J., Snider, J.L., Skalli, O., Welbourne, T., Cardelli, J.A., 2009. Na⁺/H⁺ exchangers and RhoA regulate acidic extracellular pH-induced lysosome trafficking in prostate cancer cells. *Traffic Cph. Den.* 10, 737–753. <https://doi.org/10.1111/j.1600-0854.2009.00904.x>
- Stirling, D.R., Swain-Bowden, M.J., Lucas, A.M., Carpenter, A.E., Cimini, B.A., Goodman, A., 2021. CellProfiler 4: improvements in speed, utility and usability. *BMC Bioinformatics* 22, 433. <https://doi.org/10.1186/s12859-021-04344-9>
- Stoletov, K., Kato, H., Zardoujian, E., Kelber, J., Yang, J., Shattil, S., Klemke, R., 2010. Visualizing extravasation dynamics of metastatic tumor cells. *J. Cell Sci.* 123, 2332–2341. <https://doi.org/10.1242/jcs.069443>
- Tu, C., Ortega-Cava, C.F., Chen, G., Fernandes, N.D., Cavallo-Medved, D., Sloane, B.F., Band, V., Band, H., 2008. Lysosomal cathepsin B participates in the podosome-mediated extracellular matrix degradation and invasion via secreted lysosomes in v-Src fibroblasts. *Cancer Res.* 68, 9147–9156. <https://doi.org/10.1158/0008-5472.CAN-07-5127>
- van Bergeijk, P., Hoogenraad, C.C., Kapitein, L.C., 2016. Right Time, Right Place: Probing the Functions of Organelle Positioning. *Trends Cell Biol.* 26, 121–134. <https://doi.org/10.1016/j.tcb.2015.10.001>
- Vidak, E., Javoršek, U., Vizovišek, M., Turk, B., 2019. Cysteine Cathepsins and Their Extracellular Roles: Shaping the Microenvironment. *Cells* 8, 264. <https://doi.org/10.3390/cells8030264>
- Wang, L., Goldwag, J., Bouyea, M., Barra, J., Matteson, K., Maharjan, N., Eladdadi, A., Embrechts, M.J., Intes, X., Kruger, U., Barroso, M., 2023. Spatial topology of organelle is a new breast cancer cell classifier. *iScience* 107229. <https://doi.org/10.1016/j.isci.2023.107229>

1046 Wang, Q., Yao, J., Jin, Q., Wang, X., Zhu, H., Huang, F.,
1047 Wang, W., Qiang, J., Ni, Q., 2017. LAMP1 expression is
1048 associated with poor prognosis in breast cancer. *Oncol. Lett.*
1049 14, 4729–4735. <https://doi.org/10.3892/ol.2017.6757>
1050 Weidmann, M.D., Surve, C.R., Eddy, R.J., Chen, X.,
1051 Gertler, F.B., Sharma, V.P., Condeelis, J.S., 2016.
1052 MenaINV dysregulates cortactin phosphorylation to pro-
1053 mote invadopodium maturation. *Sci. Rep.* 6, 36142.
1054 <https://doi.org/10.1038/srep36142>
1055 Wieczorek, S., Combes, F., Lazar, C., Gai Gianetto,
1056 Q., Gatto, L., Dorffer, A., Hesse, A.-M., Couté, Y.,
1057 Ferro, M., Bruley, C., Burger, T., 2017. DAPAR
1058 ProStaR: software to perform statistical analyses in quan-
1059 titative discovery proteomics. *Bioinformatics* 33, 135–136.
1060 <https://doi.org/10.1093/bioinformatics/btw580>
1061 Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K.J., Werb, Z.,
1062 2020. Concepts of extracellular matrix remodelling in tu-
1063 mour progression and metastasis. *Nat. Commun.* 11, 5120.
1064 <https://doi.org/10.1038/s41467-020-18794-x>
1065 Wu, Pei-Hsun, Gilkes, D.M., Phillip, J.M., Narkar,
1066 A., Cheng, T.W.-T., Marchand, J., Lee, M.-H., Li,
1067 R., Wirtz, D., 2020. Single-cell morphology en-
1068 codes metastatic potential. *Sci. Adv.* 6, eaaw6938.
1069 <https://doi.org/10.1126/sciadv.aaw6938>
1070 Wu, Ping-Hsiu, Onodera, Y., Giaccia, A.J., Le, Q.-T.,
1071 Shimizu, S., Shirato, H., Nam, J.-M., 2020. Lysosomal traf-
1072 ficking mediated by Arl8b and BORC promotes invasion of
1073 cancer cells that survive radiation. *Commun. Biol.* 3, 620.
1074 <https://doi.org/10.1038/s42003-020-01339-9>

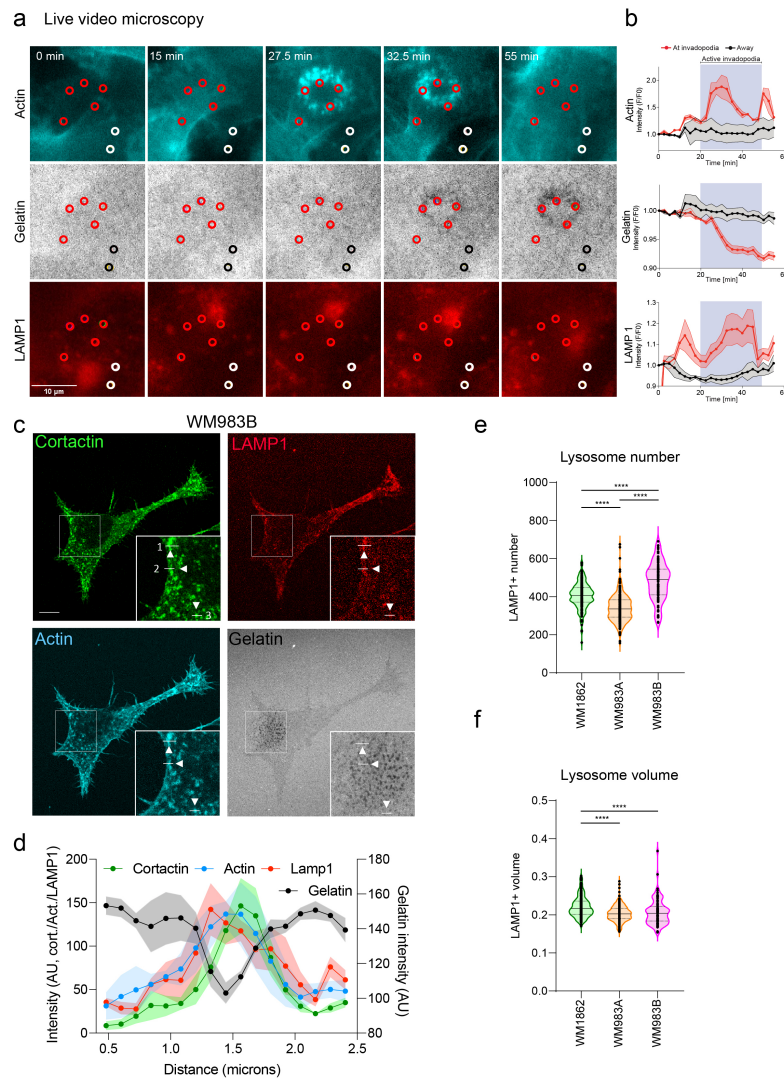


Fig. S1. Lysosomal marker LAMP1 colocalizes with active invadopodia a) WM983B cells stably expressing lysosome marker LAMP1-mCherry and LifeActin-miRFP were seeded on gelatin 24 hours prior to the experiment. Time-lapse movies were acquired during one hour and the signal intensity was quantified in five invadopodia-rich regions (red) and in two regions outside of active invadopodia (gray), blue rectangle marks the duration of active invadopodia. b) Intensity profiles over time are plotted for actin (invadopodia), gelatin (degradation) and LAMP1 (lysosomes). c) WM983B cells were seeded on gelatin 24 hours prior to the experiment, fixed and stained with cortactin, and LAMP1 antibody and with phalloidin (actin). d) The level of colocalization was quantified by line profiling in ImageJ. e-f) Cells were grown on crossbow micropatterns for 4 hours, fixed and stained with LAMP1 antibody. Images were segmented in Metamorph. R software was used to quantify total lysosome number per cell and the average volume of lysosome per cell. One dot represents 1 cell. Kruskal-Wallis with Dunn's multiple comparison post-hoc test. WM1862, n= 182 cells, WM983A, n= 231 cells, WM983B, n= 82 cells, in triplicate. e) Lysosome number (Mean \pm SD) = 405.9 \pm 68, 341.9 \pm 74, 479.7 \pm 99, respectively. f) Lysosome volume = 0.2211 \pm 0.023, 0.2035 \pm 0.021, 0.2071 \pm 0.034, respectively. *p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001.

1075

1076 **Supplementary material** Table 1: Lysosome genes differentially expressed (refers to Fig1g)

1077 Table 2: Positive Regulation of cell migration genes differentially expressed (refers to Fig1g)

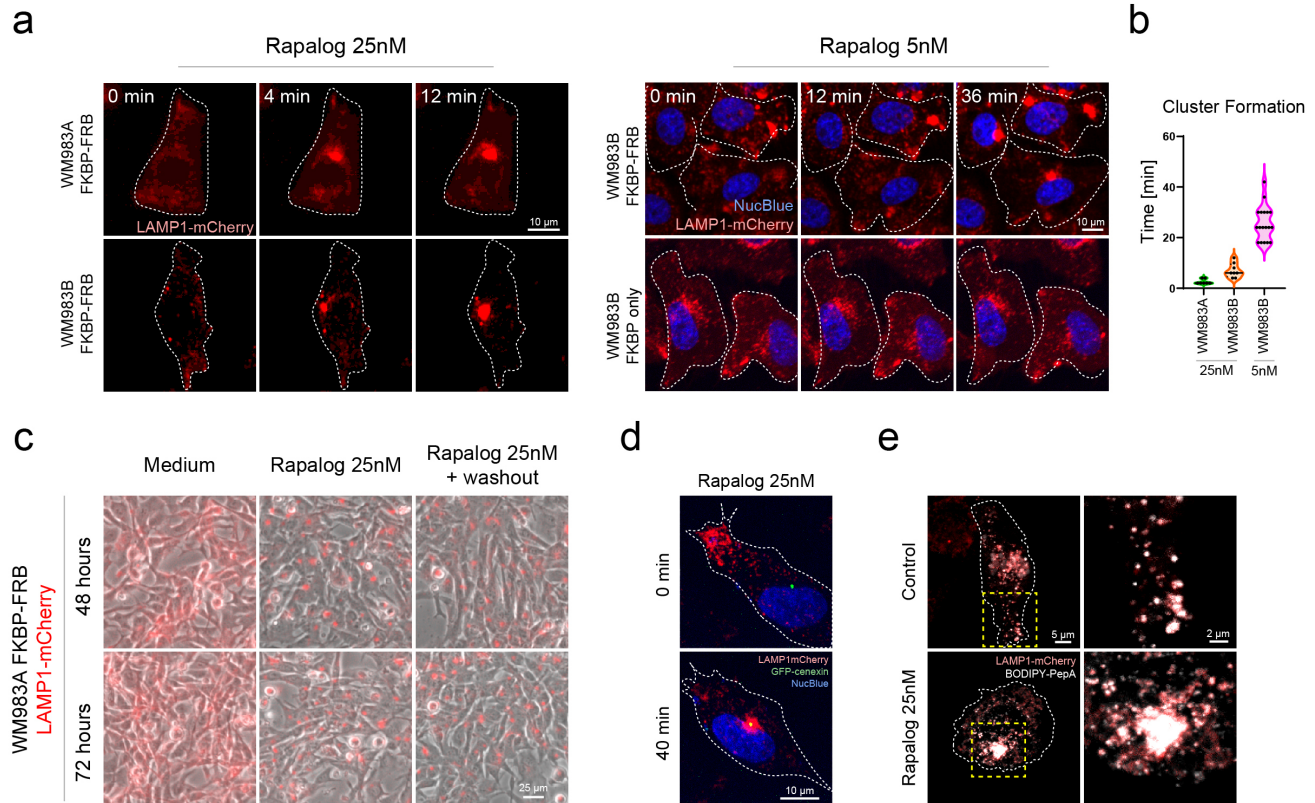


Fig. S2. Dynamics of Rapalog-induced lysosome clustering a) WM983A and WM983B cells expressing two heterodimerizing domains (FKBP-FRB) and WM983B cells expressing only one heterodimerizing domain (FKBP only) were seeded in glass bottom dishes and 1-hour time-lapse movies were acquired using spinning disc. After acquiring the first time-frame, cells were treated with 25 nM Rapalog (left) or with 5 nM Rapalog (right). b) Cluster formation was quantified as the time needed until a visible cluster appeared. Time [min] = 2.6 ± 1.0 , 7.0 ± 2.8 , 25.7 ± 6.8 , respectively. One dot represents 1 cell. c) WM983A (FKBP-FRB) cells were treated with 25 nM Rapalog and imaged at 48 hours and 72 hours timepoints. Washout condition – cells were treated for 1 hour with Rapalog, washed 3x in PBS and cultured in normal growth medium for the duration of the experiment. d) WM983B (FKBP-FRB) cells were seeded in glass bottom dishes and 45 minutes time-lapse movies were acquired using spinning disc. Rapalog induces lysosome clustering around the microtubule organizing center, labelled by cenexin-GFP. e) WM983B (FKBP-FRB) cells expressing LAMP1-mCherry (red) were cultured in medium or in medium with 25 nM Rapalog for 1 hour and then treated with PepstatinA-BODIPY (white) probe for 45 minutes. Cells were imaged live using spinning disc.

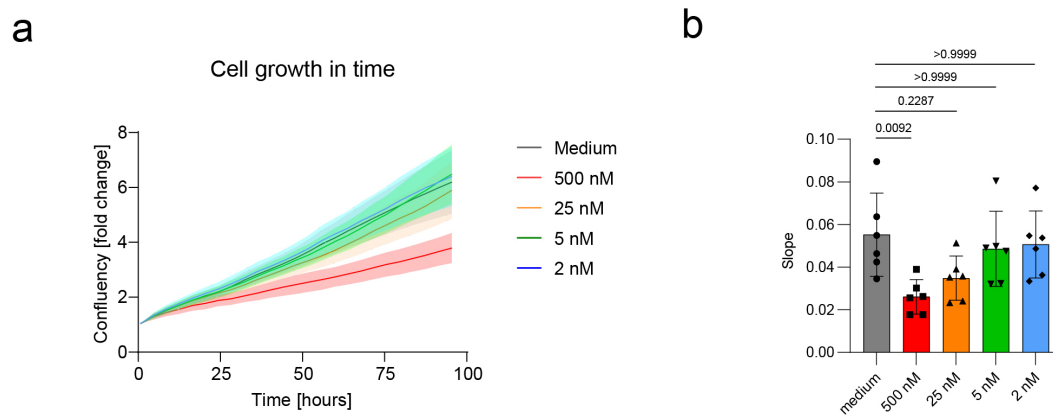


Fig. S3. Low dose of Rapalog treatment does not inhibit cell proliferation a-b) Proliferation rate of WM983B cells treated with increasing concentrations of Rapalog was analyzed using incuCyte. a) Cell confluency was quantified for each time-point, all values were normalized to time 0 and the growth curves were plotted. Colored lines depict different culture conditions: medium (black), 2 nM (blue), 5 nM (green), 25 nM (orange), 500 nM Rapalog (red) and are representing average value of three experiments \pm SD. b) Slopes were calculated for the different growth conditions. Slope (Mean \pm SD) = 0.0552 ± 0.02 , 0.0261 ± 0.01 , 0.0348 ± 0.01 , 0.0486 ± 0.02 , 0.0507 ± 0.02 , respectively. Kruskal-Wallis test with Dunn's multiple comparison post-hoc test, respective adjusted p-values are shown in the graph. One dot represents one experiment (performed in technical triplicate).

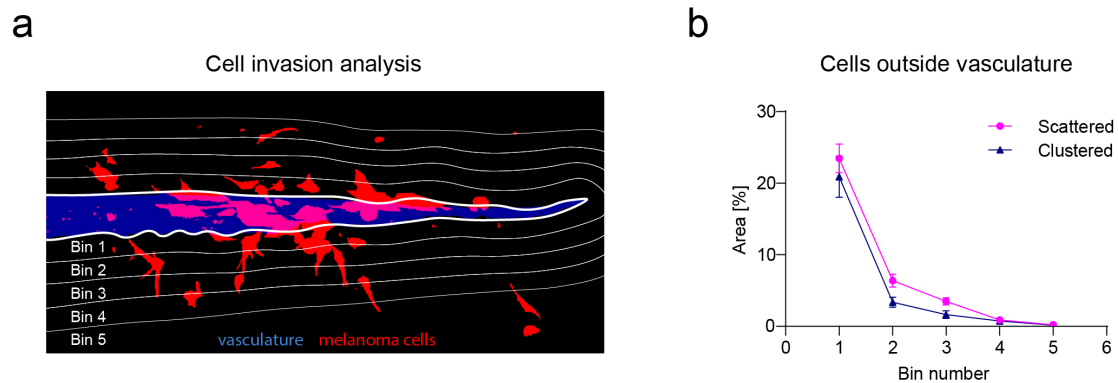


Fig. S4. Monitoring cell invasion in vivo a-b) Quantification of the post-extravasation invasion potential at 72 hours post injection. a) Representative example of the segmented image. Vasculature (blue) and WM983B melanoma cells (red) were segmented using ImageJ. Segmented images were further analyzed using CellProfiler: Image was divided into five regions (bins) with increasing distance from the vasculature region and the total cell area in each region was quantified. b) Percentage of cells (measured from their area) present in each bin was calculated and plotted for both conditions. Magenta = cells with spread lysosomes, blue = cells with clustered lysosomes. Each line represents mean value from three independent experiments ± SEM. Percentage (scattered) = 23.5 ± 2.0, 6.4 ± 0.9, 3.5 ± 0.5, 0.9 ± 0.2, 0.2 ± 0.1, respectively, n = 29 embryos. Percentage (clustered) = 20.9 ± 2.9, 3.4 ± 0.7, 1.6 ± 0.5, 0.74 ± 0.2, 0.2 ± 0.1, respectively, n = 12 embryos.