Melanoma cells implanted in the skin of aged mice result in lung metastases at greater rates than those implanted in younger mice. Whether this is due to increased dissemination from the primary site, or because the aged microenvironment at metastatic sites promotes outgrowth, remains unclear. To investigate this question, we intradermally implanted Yumm1.7 (mCherry) melanoma cells into young (8 weeks) and aged (more than 52 weeks) C57BL6 mice. The primary tumour grew faster in young mice, as shown previously (Fig. 1a).

We examined distal lung metastases at weeks 1, 3 and 5 using an immunohistochemistry (IHC) analysis of mCherry-positive cells in the lung. At week 1, we did not detect melanoma cells. At week 3, we found that melanoma cells seeded the lung in equal numbers in young and aged mice as single cells (Fig. 1b, c, Extended Data Fig. 1a). At week 5, larger metastatic colonies formed (more than 10 cells clustered together) in the aged lung, but single cells persisted in the young lungs (Fig. 1b, c). Single mCherry-positive melanoma cells in young mice stained negative for the proliferative marker Ki-67, whereas colonies in aged mice stained positive (Fig. 1d), suggesting that the aged lung promotes proliferation and melanoma outgrowth, compared to the young (which was growth-restrictive). Of note, although the number of cells seeded in young versus aged lungs was similar at three weeks, the metastatic efficiency (number of cells disseminating per mm³ of tumour volume) was far higher in aged mice. To determine whether the differences in lung outgrowth at week 5 were due to an overall increase in dissemination from aged primary tumours, we surgically removed the primary tumours from aged mice after three weeks to prevent further dissemination compared to mice containing primary tumours. This did not affect the rate of metastatic outgrowth and proliferation in aged mice lungs (Extended Data Fig. 1c–e). To further determine whether the aged lung was more growth-permissive for melanoma cells, we performed a tail vein colonization assay with our Yumm1.7 mCherry melanoma cells (Fig. 1e, f). We intravenously injected 1 × 10⁷ Yumm1.7 cells into young
and aged mice. Mice were euthanized once both groups showed clinical signs of metastasis, which occurred at day 35. We analysed the area of visible lung metastases relative to the total lung area and found that the aged mice had more metastases: 9 out of 10 aged mice had more metastases than the young lung is growth-restrictive and may promote a dormant melanoma phenotype.

It was previously shown that skin fibroblasts drive changes in the behaviour of melanoma cells in young versus aged primary tumours. We therefore investigated whether lung fibroblasts similarly affect melanoma cell growth at distal sites. We co-cultured melanoma cells with human skin or lung fibroblasts from young (35 years or younger) or aged (55 years or older) healthy donors in a three-dimensional (3D) collagen reconstruction. Consistent with our in vivo data, melanoma cells in an aged lung fibroblast microenvironment proliferated at a faster rate than those in a young lung fibroblast environment. Conversely, melanoma cells in an aged skin fibroblast microenvironment proliferated more slowly than those in a microenvironment with young skin fibroblasts. Notably, treating human melanoma cells (Extended Data Fig. 2a–h) with conditioned medium from young or aged lung or skin fibroblasts over a 10-day period in two-dimensional (2D) culture phenocopied these results, although the 2D growth curves eventually equalized, suggesting that while changes in secreted soluble factors are key in promoting these phenotypic differences, 3D interactions between the tumour and stroma are likely to be critical for maintaining dormancy.

We performed a proteomic analysis on the secretome of fibroblasts from the lungs of healthy young (35 years or younger) and aged (55 years or older) individuals (Fig. 1k), and found that sFRP1—a non-canonical WNT antagonist—was one of the most differentially secreted factors; this difference was confirmed in a western blot analysis of five different samples from the lungs of healthy young (35 years or younger) and aged (55 years or older) individuals, each in triplicate. A false discovery rate of less than 5% was considered significant (unpaired two-sided t-test).
but invasive melanoma phenotype\textsuperscript{19-21}. This was also interesting given that recent studies have shown that sFRP2 is secreted by type 1 alveolar cells to promote dormancy in breast cancer cells, in young mice\textsuperscript{22}. Previous observations in prostate cancer revealed that invasive pathways such as WNT5A (ref.\textsuperscript{23}) and AXL (ref.\textsuperscript{24}) independently promote tumour cell dormancy (and, potentially, survival) within metastatic bone microenvironments after dissemination\textsuperscript{25}. We therefore analysed other well-defined markers of tumour dormancy in prostate cancer and other cancer types, such as NR2F1, p-p38 and TGFB2 (refs.\textsuperscript{26,27}). All were increased in WNT5A\textsuperscript{high}AXL\textsuperscript{high} slower-growing aged primary tumours (Extended Data Fig. 1b), which implicates WNT5A and AXL in age-related signalling changes in melanoma dormancy. This finding also suggests that although sFRP2 promotes such a phenotype in melanoma and other cancers\textsuperscript{28}, sFRP1 within the lung microenvironment may, conversely, decrease WNT5A and other dormant signalling to promote reactivation from dormancy. To test this hypothesis, we treated human melanoma cells with conditioned medium from three young or aged healthy donors of lung fibroblasts. Relative to young donors, treatment with conditioned medium from aged lung fibroblasts consistently reduced non-canonical WNT5A signalling (Fig. 2a, Extended Data Fig. 3a, c), as well as downstream dormancy-associated pathways and non-canonical WNT pathways such as phosphorylated PKC, cleaved filamin A and ROR2. By contrast, proliferative pathways increased (Fig. 2a, Extended Data Fig. 3a, c), as did β-catenin and ROR1, both of which are antagonists of non-canonical WNT signalling. Treatment with conditioned medium from aged skin fibroblasts had the opposite effect on protein expression, promoting non-canonical WNT5A downstream dormancy pathways but inhibiting proliferative pathways (Fig. 2b, Extended Data Fig. 3b, d).

Next, we treated melanoma cells with recombinant (r) sFRP1. We observed decreased levels of WNT5A and other downstream dormancy markers such as AXL and p21, and increased levels of proliferative markers such as MITF and MER (Fig. 2c, Extended Data Fig. 3e), as well as increased proliferation (Fig. 2d, Extended Data Fig. 3f). Furthermore, knockdown of sFRP1 in aged lung fibroblasts (Fig. 2e) ablated the previous growth phenotype and decreased the proliferation of melanoma cells treated with conditioned medium relative to empty control (Fig. 2f, Extended Data Fig. 3g). We next treated young lung fibroblasts with conditioned medium from intrinsic WNT5A\textsuperscript{low} and WNT5A\textsuperscript{high} melanoma cells for 48 h to see whether soluble factors from either phenotype could alter the expression of sFRP1 in fibroblasts (Extended Data Fig. 3j).

We found that neither cell type increased the expression of sFRP1 in young fibroblasts relative to aged fibroblasts, suggesting an age-specific sFRP1 effect that was not altered by the presence of melanoma cells. To test the hypothesis that age-induced sFRP1 secretion from fibroblasts can reactivate melanoma cells from dormancy in vivo, we intradermally injected aged mice with Yumm1.7 mCherry cells to form primary tumours. Tumours were grown for three weeks in aged mice to allow single-cell seeding within the lung, as shown in Fig. 1c, and were then treated by intraperitoneal injection with a neutralizing antibody against sFRP1 until week 5. Depletion of sFRP1 markedly reduced the formation of metastatic colonies in the aged lungs compared with IgG-treated mice (Fig. 2g, h, Extended Data Fig. 4a, b). Colonies in the IgG control were Ki-67-positive, whereas single cells located in the lungs that were treated with the sFRP1 neutralizing antibody were Ki-67-negative (Extended Data Fig. 4a). Overall, these results show that aged lung fibroblasts promote the phenotypic reactivation of dormant melanoma cells in the lung through increased secretion of soluble factors such as sFRP1 (Fig. 2i).

Given the importance of WNT5A in prostate cancer dormancy\textsuperscript{18}, coupled with its role as a downstream antagonistic target of sFRP1, we chose to investigate it as a primary factor in metastatic melanoma dormancy in the lung. We first stratified samples from patients with melanoma (across both patients with primary melanoma and those with metastatic melanoma) from The Cancer Genome Atlas (TCGA) into the top and bottom 25th percentile of WNT5A-expressing primary and metastatic tumours. WNT5A\textsuperscript{high} patient samples expressed several well-defined dormancy related genes from other cancers\textsuperscript{18-20} (Fig. 3a). Many established proliferative melanoma markers correlated with WNT5A\textsuperscript{low} patient samples (Fig. 3b). Increasing the expression of WNT5A in low-expressing human melanoma cells through lentiviral transduction (Fig. 3c, Extended Data Fig. 4c) or treatment with WNT5A (Extended Data Fig. 4g) increased many of these dormancy targets, while reducing proliferative markers and melanoma cell growth in vitro (Extended Data Fig. 4d–f). We next performed an IHC analysis of WNT5A in single-cell colonies in our young mice versus the larger colonies in our aged mouse model (Fig. 3d). We found that in younger mice, which contained more single-cell colonies, WNT5A was highly expressed, but the cells were Ki-67-negative. When analysing the larger metastatic colonies in aged lungs, we found that WNT5A staining was weak and cells stained strongly for Ki-67. This highly suggests that downregulation of WNT5A is required to allow age-induced emergence from dormancy and efficient metastatic outgrowth.

WNT5A is correlated in many melanoma datasets with more aggressive and deadly disease, and studies from our laboratory and others implicate WNT5A in the establishment of an invasive phenotype\textsuperscript{21,22}, dissemination\textsuperscript{23-25} and therapeutic resistance\textsuperscript{26-28} within primary tumours. Notably, however, our current data suggest that simply analysing primary tumours for the expression of genes and correlating them with survival and progression may not be sufficient to fully show their role in the metastatic cascade. Our data suggest that to form competent, proliferative metastases at distal sites, WNT5A needs to be downregulated. This is supported by the fact that WNT5A expression is reduced in established distant metastatic tumour sites versus primary tumours in the TCGA (Extended Data Fig. 5a).

To investigate this point in vivo, we generated Yumm1.7 mCherry doxycycline (dox)-inducible cells that express short hairpin RNA against Wnt5a (shWnt5a). Treatment with dox (0.5 μg ml\textsuperscript{-1}) successfully reduced the expression of WNT5A, as well as reducing downstream dormancy markers and increasing proliferative markers and overall proliferation (Extended Data Fig. 5b, c). To investigate how WNT5A affects the dissemination and dormancy of disseminated cancer cells across different time points, we induced the formation of intradermal tumours in our young C57BL6 mouse model; these tumours disseminated to the lungs by week 3 but maintained dormant cell colonies in the lungs through week 5 (Fig. 1c). We investigated the temporal downregulation of WNT5A starting at two different time points: (1) beginning at day 3, to investigate the ablation of WNT5A throughout the entirety of tumour progression (growth and dissemination); and (2) beginning at day 21, to investigate the downregulation of WNT5A after cells had successfully disseminated and formed viable dormant, micrometastatic colonies within the lung. Analysis showed that inducible knockdown at either time point increased primary tumour growth (Extended Data Fig. 5d), providing further evidence of the role of WNT5A in inducing a slower-growing cell state within primary tumours. However, only mice treated with dox starting at day 21 formed metastatic colonies (more than 10 cells) after five weeks; non-treated mice predominantly contained single dormant disseminated cancer cells (Fig. 3e, f). These larger colonies were positive for Ki-67, whereas single cells were negative (Extended Data Fig. 5e). Of note, inducible knockdown beginning at day 3 resulted in significantly fewer single disseminated cancer cells seeded in the lung compared with mice that were not treated with dox (Extended Data Fig. 5f), further implicating WNT5A in initial dissemination and seeding.

We next tested whether maintaining high expression of WNT5A in aged mice (through dox-inducible overexpression) enforces dormancy in disseminated cancer cells and stops the formation of larger metastatic colonies (as shown in Fig. 1c). We found that only treatment with dox beginning at day 3 significantly slowed tumour growth (Extended Data Fig. 5g), probably owing to already-high levels of WNT5A and initial slower growth of aged primary tumours relative to young (Extended Data Fig. 1i); however, treatment with dox in vitro decreased melanoma cell growth (Extended Data Fig. 5h). Notably,
Fig. 2 | The aged lung fibroblast secretome promotes metastatic melanoma outgrowth through secretion of sFRP1. a, b, Protein lysate was collected from 1205Lu human melanoma cells treated with young (≤35 years) or aged (≥55 years) lung (a) or skin (b) fibroblast conditioned medium (CM) for five days compared to control (C). Western blot analysis was performed for dormant (WNT5A, AXL, p21, p-p38 and p-PKC) and proliferative (β-catenin, MER and MITF) melanoma markers. HSP90 was used as a loading control. c, Yumm1.7 (mouse) and 1205Lu (human) melanoma cells were treated with 500 ng of recombinant sFRP1 (rsFRP1) every 2 days; protein expression was assessed after 5 days (western blot). HSP90 was used as a loading control. d, 1205Lu (bottom) and Yumm1.7 (top) GFP melanoma cells were seeded at a density of 2 × 10^5. Cells were grown for 10 days, treated with 1 mg kg −1 neutralizing sFRP1 or IgG control antibody through intraperitoneal injection every 4 days; lung metastasis was assessed at week 5 and the number of mCherry+ colonies was quantified (more than 10 mCherry+ cells per lesion) per lung section (g). Data are mean ± s.e.m. (Student’s t-test; P < 0.0001). Representative images of Yumm1.7 mCherry staining in aged mouse lungs are displayed (h). Scale bars, 100 μm. I. Key differences in the metastatic cascade between young and aged patients. The diagram shows that a switch from sFRP2 secretion by aged skin fibroblasts to sFRP1 secretion by aged lung fibroblasts promotes efficient metastatic progression of melanoma cells in aged models (schematic made with Biorender.com).

within the lung, we found that mice that were not treated with dox exhibited significantly higher levels of colony formation compared to mice that were treated with dox beginning at days 3 or 21 (Fig. 3g, h), suggesting that maintaining WNT5A within an aged microenvironment is sufficient to maintain a more dormant melanoma phenotype. The larger colonies in dox-treated mice were Ki-67-positive, whereas single cells in dox-treated groups were Ki-67-negative (Extended Data Fig. 5i). We also found that mice that began dox treatment on day 3 had a significantly greater average number of infiltrating melanoma cells compared to mice that began treatment on day 21 (Extended Data Fig. 5i). This suggests that further increasing the expression of WNT5A at earlier time points increases dissemination into the lung. However, it is clear from both models that WNT5A expression needs to be downregulated once seeded within the lung to allow the outgrowth of larger metastatic colonies.
Fig. 3 | Temporal downregulation of WNT5A promotes metastatic melanoma outgrowth in previously dormant lung microenvironments.

**Analysis of the melanoma TCGA dataset (all samples) was performed on human samples stratified into the top (WNT5A top) and bottom (WNT5A bottom) 25th percentile of WNT5A expression and presented as a heat map of the average z-score. An unpaired two-sided t-test was performed to estimate the significance of the difference between conditions; P < 0.05 was considered significant (n = 120 per group). The panels represent common dormancy markers from other cancers (a) and common proliferative markers from melanoma (b). Protein expression analysis was performed on protein lysates from WNT5A human melanoma samples (FS13 and FS14) that underwent lentiviral-induced overexpression of WNT5A (OE). HSP90 was used as a loading control. (d) Lungs from young and aged mice containing Yumm1.7 mCherry tumour cells (Fig. 1) underwent IHC analysis for mCherry, WNT5A and Ki-67 to analyse colocalization. Scale bars, 100 μm. (e, f) Five aged mice per group were intradermally injected with 2.5 × 10^5 Yumm1.7 dox-inducible shWNT5A mCherry melanoma cells. Dox-treated mice (2 mg ml^{-1} in water) were treated beginning at day 3 and analysed until visible clinical signs of metastasis (weight loss, hunched posture), which took 35 days. Lungs were PFA-embedded, stained with H&E and sections were scanned at 40× using an Aperio Slide Scanner. Whole sections were analysed for total lung tissue area versus metastatic tissue area using Concentriq software (b). Data are mean ± s.e.m. (P = 0.0003; Student’s two-sided t-test). Representative images are displayed (j). Scale bars, 2.5 mm. (k, l) Lungs from three of the previous intravenously injected mice underwent IHC analysis for Ki-67. Images (l) were taken blind at 40× from five metastatic lesions from each lung section of appropriate size. The percentage of Ki-67 nuclei relative to total nuclei from each metastasis was assessed using ImageJ (k). Data are mean ± s.e.m. (P = 0.0186; Student’s two-sided t-test). (m) Melanoma metastatic progression and dormancy reactivation in young versus aged patients, highlighting the complex nature of WNT5A expression required at each metastatic time point (schematic made with Biorender.com).
Finally, to investigate the role of WNT5A in the dormancy of melanoma disseminated cancer cells in the absence of primary tumour—where WNT5A cannot alter dissemination ability—we performed tail vein intravenous injections of shWNT5A dox cells in young mice. We began treatment with dox three days after intravenous injection and euthanized mice when clinical signs of metastasis were present, which took 35 days. We found that knockdown of WNT5A markedly increased the total area of lung metastases compared to mice that were not treated with dox (Fig. 3i, j). Analysis of Ki-67 in lung metastases also showed that the percentage of Ki-67-positive nuclei relative to total nuclei increased in dox-treated mice (Fig. 3k, l). Overall, these data show that non-canonical WNT5A signalling is required for efficient dissemination from the primary tumour, but then maintains a dormant phenotype after seeding in the lung. Once these cells have successfully seeded as viable single dormant cells, temporal down-regulation of WNT5A promotes reactivation from dormancy, which allows metastatic outgrowth even in previously growth-restrictive young mouse lungs (Fig. 3m).

To further examine the mechanisms that underlie the complexity of dormancy versus outgrowth in ageing, we investigated the TYRO3, AXL and MER (TAM) family of tyrosine kinase receptors. AXL is strongly positively correlated with WNT5A in melanoma and is involved in driving dormancy in metastatic prostate cancer. In melanoma, studies have shown that MER and TYRO3 show a differential pattern of expression to that of AXL (in contrast to immune cells where expression correlates positively and often shows redundancy), and promote a proliferative phenotype. We hypothesized that differential expression of AXL versus either MER or TYRO3 within melanoma cells may constitute a dormancy–reactivation axis within metastatic tissues. To investigate this, we performed a western blot analysis of three previously characterized WNT5Ahigh melanoma cell lines (FS4, FS5 and 1205Lu) versus two characterized WNT5Alow cells (FS13 and FS14) (Fig. 4a). AXL expression showed a strong positive correlation with WNT5A expression, and these cells expressed the AXL ligand GAS6. Several established dormancy markers, including p21, p-p38 and NR2F1, were also increased in these WNT5AhighAXLhigh cells (Fig. 4a). Although there was no consistent difference in the expression of TYRO3 across the cell lines, MER showed an inverse pattern of expression to that of AXL and WNT5A. MERhigh cell lines also showed strong expression of the MER-specific ligand PROS1 and the proliferative markers MITF and MART1 (Fig. 4a).

To expand on this analysis in melanoma cells specifically, we analysed an RNA-sequencing (RNA-seq) dataset from the Cancer Cell Line Encyclopedia (CCLE) that contains over 60 human melanoma cell lines. We stratified these into AXLhigh and AXLlow subpopulations. We found that AXLhigh cells had significantly increased levels of WNT5A and dormancy markers (Extended Data Fig. 6a), reduced expression of melanoma proliferative markers (Extended Data Fig. 6b) and low expression of MER. Subsequent stratification and analysis of MERhigh and MERlow cell lines from within this dataset revealed the opposite trend, in that WNT5A, AXL and dormancy markers were reduced in MERhigh cells (Extended Data Fig. 6c), whereas proliferative markers were markedly increased (Extended Data Fig. 6d).

To assess this signalling axis in the context of human primary tumours and lung metastases, we performed an IHC analysis in matched patient primary melanoma tumours versus concurrent lung metastases (Fig. 4b, Extended Data Fig. 7a–d). A clear and consistent trend could be seen: MER expression was much higher in lung metastatic samples relative to the primary tumours, whereas the expression of WNT5A and AXL was much higher in primary tumours relative to lung metastases.

We next manipulated the expression of AXL and MER in human melanoma samples. Knockdown of AXL in WNT5Ahigh lines did not reduce the expression of WNT5A, which suggests that AXL is downstream of WNT5A within this dormancy axis; however, the levels of MER and p21 increased (Fig. 4c) alongside a modest increase in proliferation after 10 days (Extended Data Fig. 6f). Conversely, shRNA-mediated knockdown of MER increased the expression of WNT5A, AXL and p21, while reducing the expression of MITF (Fig. 4d, Extended Data Fig. 6g) and proliferation (Extended Data Fig. 6h–i). We next performed an IHC analysis of MER and AXL in metastatic lung tumours from our young and aged mouse models. We found high co-expression of both WNT5A and AXL in younger metastatic tumours, whereas the expression of MER and Ki-67 was very low (Fig. 4e). Conversely, in aged metastatic tumours, we observed very high levels of expression of MER correlating with strong expression of Ki-67, whereas the expression of WNT5A and AXL was markedly decreased (Fig. 4e). On the basis of these data, we hypothesized that MER promotes the reactivation of disseminated cancer cells from dormancy. We thus created a dox-inducible Yumm1.7 mCherry-tagged melanoma cell line. Inducible overexpression of MER decreased the expression of AXL and WNT5A, and increased MITF (Extended Data Fig. 6j) and overall proliferation in vitro (Extended Data Fig. 6k). Further validation of the role of MER in metastatic outgrowth comes from analysis of the TCGA. MER expression is significantly increased in metastatic samples compared to primary tumours (Extended Data Fig. 6e), and in patients with stage III or IV disease relative to stage I or II (Extended Data Fig. 6i), which strongly suggests that MER has an important role in driving metastatic growth and progression.

To investigate the expression of MER in metastatic reactivation in vivo, we used the dox-inducible MER-overexpressing Yumm1.7 mCherry cell line. These cells were implanted intradermally into our dormant young mouse model to form primary tumours. We again began treatment with dox at either day 3 or day 21. Dox treatment at both time points increased overall primary tumour growth (Extended Data Fig. 6m), which confirms that MER expression promotes a more proliferative melanoma phenotype in primary tumours. As in our WNT5A knockdown experiment, however, only mice that began treatment with dox on day 21 produced larger metastatic colonies (Fig. 4f, h), which were Ki-67-positive (Extended Data Fig. 8a). Mice that were treated with dox beginning at day 3, and mice that were not treated with dox, did not produce any larger colonies and were Ki-67-negative (Extended Data Fig. 8a). Notably, significantly more single cells were seeded in the lungs of non-treated mice versus mice that were treated with dox on day 3 (Fig. 4g, h). This again highlights the importance of the phenotypic switch in the full metastatic cascade, suggesting that low expression of MER is required for efficient dissemination from the primary tumour (an opposing role to WNT5A). After seeding within the lung, increased MER allows larger metastatic colony outgrowth. To investigate this further in the absence of a primary tumour, we performed intravenous injection of these MER-inducible cells in our young mouse model. We began treatment with dox on day 3 to allow time for cells to seed. We found that overexpression of MER through dox treatment markedly increased the total area of metastatic lung tissue compared to mice that were not treated with dox (Fig. 4i, j), and increased the percentage of Ki-67-positive cells relative to total nuclei in metastatic tumours (Fig. 4k, l).

We next hypothesized that AXL acts downstream of WNT5A to induce dormancy within this axis. To investigate this, we created a dox-inducible AXL mCherry Yumm1.7 melanoma cell line to determine whether AXL could inhibit reactivation through inhibition of this MER phenotypic switch within our metastatic aged mouse model. Inducible activation of AXL in these cells decreased the expression of MER and MITF (Extended Data Fig. 6n), as well as proliferation in vitro (Extended Data Fig. 6o); however, WNT5A remained unchanged during AXL overexpression, further implying that AXL is downstream of WNT5A in this dormancy axis (Extended Data Fig. 6n). This is notable given that the expression of AXL appeared to mimic that of WNT5A in human metastatic lung tumours (Fig. 4b, Extended Data Fig. 7a–d) and mouse lung tumours (Fig. 4e). To investigate the potential for AXL to induce dormancy in vivo, AXL-inducible cells were intradermally implanted in the aged metastatic mouse model to form primary tumours, with dox treatment beginning at either day 3 or day 21. Despite the already-high levels of AXL within these tumours (Fig. 1a, Extended Data Fig. 1i), induction of AXL produced a modest decrease in tumour growth, but only
were intradermally injected with $2.5 \times 10^5$ Yumm1.7 Dox-inducible melanoma cells. Scale bars, 100 μm.

**Fig. 4** An age-induced differential AXL–MER axis promotes a dormancy–reactivation axis during metastatic melanoma. **a.** Protein expression analysis of lysate from three WNTSAΔΔ (FS4, FS5 and 1205Lu) and two WNTSAΔΔ (FS13 and FS14) human melanoma samples. HSP90 was used as a loading control. **b.** IHC analysis was performed on matched human primary melanoma tumours and resulting lung metastases for MER, WNT5A and AXL. Scale bars, 100 μm. **c, d.** Western blot analysis of 1205Lu human melanoma cells that underwent lentiviral shRNA-mediated knockdown of AXL (c) or MER (d) using two independent shRNAs against a scrambled control. HSP90 was used as a loading control. **e–h.** Five young mice per group were intradermally injected with $2.5 \times 10^5$ Yumm1.7 Dox-inducible MER-overexpressing mCherry melanoma cells. Mice were treated with dox (2 mg ml$^{-1}$ in water) from day 3 (dox day 3) or day 21 onwards (dox day 21), with tumours measured for 5 weeks. Lungs were PFA-embedded and underwent IHC analysis for mCherry, WNT5A, MER, AXL and Ki-67 to analyse co-localization. Scale bars, 100 μm. **h.** Five young mice per group were intradermally injected with $2.5 \times 10^5$ Yumm1.7 Dox-inducible MER-overexpressing mCherry melanoma cells. Dox treated mice (2 mg ml$^{-1}$ in water) were treated beginning at day 3. Mice were analysed until visible clinical signs of metastasis (weight loss, hunched posture), at day 35. Lungs were PFA-embedded, stained with H&E and sections were scanned at 40× (Aperio Slide Scanner). Whole sections were analysed for total lung tissue area versus metastatic tissue area using Concentrix software (i). Data are mean ± s.e.m. ($P < 0.0041$; Student’s two-sided t-test). Representative images are displayed (j). Scale bars, 2.5 mm. **k, l.** Lungs from four of the previous intravenously injected mice underwent IHC analysis for Ki-67. Scale bars, 100 μm. Images (l) were taken blind at 40× from five metastatic lesions from each lung section of appropriate size. The percentage of Ki-67+ nuclei relative to total nuclei was assessed using ImageJ (k). Data are mean ± s.e.m. ($P = 0.001$; Student’s two-sided t-test). **m.** Five aged mice per group were intradermally injected with $2.5 \times 10^5$ Yumm1.7 dox-inducible AXL-overexpressing mCherry melanoma cells. Mice were treated with dox (2 mg ml$^{-1}$ in water) from day 3 (dox day 3) or day 21 onwards (dox day 21), with tumours measured for 5 weeks. Lungs were PFA-embedded and underwent IHC analysis of mCherry+ melanoma cells. Mice were treated with dox (2 mg ml$^{-1}$ in water) from day 3 (dox day 3) or day 21 onwards (dox day 21), with tumours measured for 5 weeks. Lungs were PFA-embedded and underwent IHC analysis of mCherry+ melanoma cells, after quantifying the number of mCherry+ colonies (more than 10 mCherry+ cells per section) per lung section (m). Data are mean ± s.e.m. ($P < 0.0001$ for comparisons between no dox compared to both dox day 3 and dox day 21; one-way ANOVA with Tukey’s post hoc test for multiple comparisons). Representative images are displayed for no dox and dox day 21 (n). Scale bars, 100 μm. **o, p.** Seven aged mice per group were injected intravenously via the tail vein with $1 \times 10^5$ Yumm1.7 dox-inducible AXL mCherry melanoma cells. Dox treated mice (2 mg ml$^{-1}$ in water) were treated beginning at day 3. Mice were analysed until visible clinical signs of metastasis (weight loss, hunched posture) in both groups. Lungs were PFA-embedded, stained with H&E and sections were scanned at 40× (Aperio Slide Scanner). Whole sections were analysed for the total lung tissue area versus the metastatic tissue area using Concentrix software (q). Data are mean ± s.e.m. ($P < 0.0001$; Student’s two-sided t-test).
for treatment starting at day 3 (Extended Data Fig. 6p). Induction of AXL at days 3 and 21 significantly reduced colony formation in the aged lung and largely resulted in single-cell colonies compared to the no-dox control (Fig. 4m, n). The larger colonies were Ki-67-positive, whereas the single cells were Ki-67-negative (Extended Data Fig. 8b). There was no difference in the average number of single-cell infiltrations between AXL induction at day 3 and day 21, which may suggest that AXL does not have as major a role as WNT5A in initial dissemination from the primary tumour (Extended Data Fig. 8c). Finally, we tested the ability of AXL to promote dormancy in the aged lung in the absence of a primary tumour by intravenously injecting dox-AXL-overexpressing cells into our aged mouse model, with dox treatment beginning at day 3. Overexpression of AXL significantly reduced the area of metastatic lung tissue relative to total tissue compared to mice that were not treated with dox (Fig. 4o–p), and decreased the percentage of Ki-67-positive cells (Fig. 4q–r). Overall, these data suggest that an AXL–MER differentially regulated signalling pathway has a key role in producing a dormancy–reactivation axis in metastatic tissues such as the lung.

We next sought to ascertain whether activation of this differential TAM axis by its respective primary ligands has a role in dormancy and reactivation. GAS6 is the prominent ligand involved in activating AXL, although it binds both MER and TYRO3 at much lower affinity\(^{30,31}\). PROS1 is the prominent ligand for MER, and is unable to bind the AXL receptor in most contexts\(^{32,33}\). IHC analysis of metastatic lung tumours in young and aged mice showed that the levels of GAS6 were higher in younger than in aged metastases (Fig. 5a). Conversely, PROS1 expression was higher in aged relative to young metastatic tumours (Fig. 5a). This is consistent with our data showing that AXL\(^{\text{high}}\) cells express higher levels of GAS6 in human melanoma samples and CCEL samples, whereas MER\(^{\text{high}}\) cells express higher levels of PROS1 (Fig. 4a, Extended Data Fig. 6a–d). Of note, we also found that WNT5A\(^{\text{high}}\)AXL\(^{\text{high}}\) human melanoma cells secrete higher amounts of GAS6 into conditioned medium in vitro (Fig. 5b), whereas MER\(^{\text{high}}\) cells secrete higher amounts of PROS1 (Fig. 5b), suggesting the potential for paracrine-related modulation of neighbouring melanoma cells in the microenvironment.

We first examined the effect of GAS6 on melanoma cell phenotype. Treatment of WNT5A\(^{\text{high}}\)AXL\(^{\text{high}}\) melanoma cells with rGAS6 consistently decreased the proliferation of melanoma cells across these cell lines (Fig. 5c, Extended Data Fig. 9a). However, WNT5A\(^{\text{high}}\)AXL\(^{\text{low}}\) melanoma cells treated with GAS6 showed increased proliferation (Fig. 5d, Extended Data Fig. 9b). We hypothesized, given the promiscuity of GAS6 among TAM family members, that it might actually be acting through the MER receptor in AXL\(^{\text{high}}\) cell lines. Consistently, shRNA-mediated knockdown of MER in AXL\(^{\text{high}}\) human melanoma cells—which increased the expression of AXL in previous experiments—indeed decreased the proliferation of melanoma cells in response to treatment with rGAS6 (Extended Data Fig. 9c). Conversely, shRNA-mediated knockdown of AXL in WNT5A\(^{\text{high}}\)AXL\(^{\text{high}}\) cells treated with GAS6 rescued the phenotype and reversed the decreased proliferative response (Extended Data Fig. 9d). Treating dox-inducible AXL melanoma cells with rGAS6 after AXL induction decreased the growth of melanoma cells when compared with untreated AXL-induced cells or with rGAS6 treatment in control cells (Fig. 5e). Analysis of protein expression of these cells confirmed that treating Yumm1.7 cells with rGAS6 produced only a modest decrease in MER and MITF and a slight increase in WNT5A (Fig. 5f). When expression of AXL was induced by dox treatment, administration of rGAS6 markedly increased the levels of WNT5A and produced a much more pronounced decrease in MER and MITF, suggesting that GAS6 regulation of melanoma cells relies heavily on whether they are in an AXL\(^{\text{high}}\) or a MER\(^{\text{high}}\) state. We previously showed that both overexpression (Extended Data Fig. 6n) and knockdown of AXL (Fig. 4c) do not alter the expression of WNT5A, which suggests that AXL is downstream of WNT5A. These data provide evidence that expression of AXL is not sufficient to regulate WNT5A, but that AXL activation through GAS6 promotes WNT5A expression. To investigate the effect of GAS6 in vivo, we formed primary tumours in our aged metastatic mouse models (aged lung metastases had lower GAS6 levels; Fig. 5a). We began treating these mice with rGAS6 (intraperitoneal injection) at three weeks to allow initial dissemination and seeding in the lung (Fig. 1c). We found that treatment with GAS6 decreased the formation of metastatic colonies after five weeks (Fig. 5g, h); however, this decrease was modest. Colonies were still consistently found in treated mice and were Ki-67 positive (Extended Data Fig. 9f). These data suggest that GAS6 alone cannot fully inhibit the phenotypic switch towards reactivation in the aged lung microenvironment. This may be a result of competition with other soluble factors in the aged lung, such as sFRP1, which promotes a MER\(^{\text{high}}\) cell state in melanoma cells and thus may limit the ability of GAS6 to decrease proliferation—as seen when we treated MER\(^{\text{high}}\) cells in vitro (Fig. 5d, Extended Data Fig. 9b).

Although GAS6 shows promiscuous regulation of cell growth on the basis of the adopted cell phenotype, PROS1 is thought to have very little receptor interaction with AXL. We showed previously that even in WNT5A\(^{\text{high}}\)AXL\(^{\text{high}}\) melanoma cell lines in vitro, expression of MER was still detectable (Fig. 4a). On the basis of this observation, we hypothesized that PROS1 could promote increased growth and reactivation regardless of cell phenotype. Indeed, treatment of either WNT5A\(^{\text{high}}\)AXL\(^{\text{high}}\) or MER\(^{\text{high}}\) human and mouse melanoma cells significantly increased melanoma growth (Extended Data Fig. 10a–e). Notably, shRNA-mediated knockdown of MER ablated this effect (Extended Data Fig. 10f). Protein expression analysis of Yumm1.7 cells treated with rPROS1 showed that PROS1 decreased the expression of WNT5A and AXL, but further increased that of MER and MITF (Extended Data Fig. 10g). To examine whether PROS1 could facilitate the reactivation of dormant melanoma cells, we implanted primary tumours in young mice using Yumm1.7 mcherry cells. Tumours were grown for three weeks to allow single-cell seeding in the lung (Fig. 1c). Mice were then treated with rPROS1 every two days through intraperitoneal injection. Treatment with rPROS1 significantly increased metastatic colonies in the previously dormant young lung microenvironment at five weeks compared with a phosphate-buffered saline (PBS) control, which showed only single melanoma cells (Fig. 5i, j). The colonies were Ki-67 positive, whereas the single cells were Ki-67 negative (Extended Data Fig. 10i).

Finally, given that we saw a prominent role for fibroblast secreted factors such as sFRP1 in promoting reactivation, along with melanoma secreted factors such as PROS1 in promoting a growth-permissive metastatic phenotype, we wanted to investigate whether both factors were required for efficient metastatic outgrowth. Using our young mouse model that promotes a dormant melanoma phenotype, we implanted primary tumours using Yumm1.7 mcherry cells. Tumours were grown for three weeks to allow single-cell seeding and mice were treated with rPROS1, sFRP1 or a combination of both recombinant proteins every two days through intraperitoneal injection versus a PBS control. The PBS control mice did not form any colonies (Fig. 5k–l). Both sFRP1 treatment and PROS1 treatment alone were able to induce larger colonies in the young mouse lung, but treatment with both factors produced significantly more colonies overall (Fig. 5k–l). These colonies were all Ki-67 positive (Extended Data Fig. 10j). Notably, treatment with both factors promoted larger, more macrometastatic colonies (more than 30 cells) in 3 out of 5 mice (represented in Fig. 5k), highlighting the importance of both fibroblast and melanoma secreted factors in efficient metastatic colonization of the lung.

Our study has some limitations. First, our in vitro data examining the proliferation rates of melanoma cells exposed to conditioned medium from young and aged fibroblasts, or when treated with recombinant proteins such as sFRP1 or GAS6, showed that despite initial differences in growth rate, cell growth eventually equalizes. However, this concern is offset by the fact that in vivo this was not the case and we continued to see small colonies of tumour cells persisting in young mice after six to eight weeks—as opposed to the case in aged mice, in which they grew rapidly. Given that nine days in the life of a mouse is roughly equal to one
human year\(^{31}\), these data, when extrapolated to human years, suggest that in a span of roughly five to seven human years (equal to six to eight weeks in the mouse), tumour cells remain in slow cycling colonies in the young mouse lung (as evidenced by colony size and Ki-67 staining). The discrepancies between the in vitro and the in vivo data point to the critical importance of the immune, stromal and tumour interactions in 3D in the tumour microenvironment. Another limitation is the use of a fluorescent reporter to detect single melanoma cells within our metastatic lung model. Recent studies have shown that fluorescent markers such as GFP are immunogenic across various models\(^{34}\); this
potentially affects our data as we used immunocompetent C57BL6 mice. It was previously established through tail-vein studies that using parental Yumm1.7 cells results in a marked increase in metastatic outgrowth in aged (52 weeks) C57BL6 mice, which matches our mCherry fluorophore studies. To confirm that the mCherry fluorophore did not alter our intradermal model, we injected parental Yumm1.7 cells and allowed tumour growth for five weeks in our young and aged models. We identified metastatic colonies and associated pathological features using haematoxylin and eosin (H&E) staining in the aged mouse lungs; colonies stained positive for MIF and Ki-67 (Extended Data Fig. 11a). We did not detect colonies in younger mice, which is consistent with our current data using mCherry-positive cells.

Overall, these data reveal a complexity in the role of WNT signalling and other downstream pathways in melanoma cell dormancy and metastasis initiation that is regulated by ageing (Fig. 5m). We show that WNT5A promotes initial dissemination of the tumour cells as previously defined, but that it then maintains cells in a dormant state to allow survival and adaptation in the lung microenvironment until age-related changes induce emergence from tumour dormancy. We also show that sFRP1 is secreted at higher levels by aged lung fibroblasts and decreases the expression of WNT5A to allow reactivation from dormancy in the aged mouse lung.

We further define an AXL–MER dormancy–reactivation axis downstream of WNT5A. MER expression is required to induce a phenotypic downregulation of the WNT5AAXLMEA1k dormant state to allow age-induced metastatic reactivation and outgrowth, whereas maintaining AXL expression inhibits reactivation. We find that whereas GAS6-mediated regulation of this axis is promiscuous, the MER-mediated ligand PROS1 can be secreted in a paracrine manner by MERhigh reactivated cells and is capable of driving reactivation and metastatic outgrowth in the previously dormant young lung microenvironment (Fig. 5m). These observations may explain the predisposition of older patients to aggressive metastatic melanoma, and the critical role of the ageing microenvironment in the emergence of tumour cells from dormancy.

It is important to note that this study specifically undertook analysis of the lung only, owing to the fact that the Yumm1.7 model is only capable of seeding the lung as a metastatic tissue site. However, analysis of sFRP1 expression (through The Human Protein Atlas) in human cell types across various tissues reveals that sFRP1 expression is highest in fibroblasts, located specifically in the lung, liver, pancreas and skeletal muscle (Extended Data Fig. 11b). The liver is a common metastatic site for melanoma. Of note, liver lysates from aged (more than 52 weeks) mice show increased expression of sFRP1 relative to young mouse lysates (Extended Data Fig. 11c). We were only able to source human liver fibroblasts from one healthy young (20) and one healthy aged (79) patient (Extended Data Fig. 11d). The aged human liver fibroblasts expressed much higher levels of sFRP1 relative to the young fibroblasts. Furthermore, we found that human melanoma cells grown in 3D sandwich reconstructions with aged liver fibroblasts grew much more efficiently than those grown with young fibroblasts (Extended Data Fig. 11e, f), and much faster in conditioned medium from aged liver fibroblasts (Extended Data Fig. 11g, h)—all of which matches our observations within the lung. Although these data are preliminary because of the lack of human liver fibroblast samples and the metastatic limitations of our model, they highlight the potential role of sFRP1—and other age-related soluble factors that are secreted by stromal cells—in promoting melanoma growth and reactivation from dormancy in metastatic tissue sites outside the lung. Together, these data strongly suggest that we need to consider age as a parameter in the design and delivery of cancer therapy, and as a critical modulator of tumour dormancy and progression.

Online content
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Antibodies were purchased from the following commercial vendors and probed with the corresponding HRP-conjugated secondary antibody incubated at 4°C overnight. The membranes were washed in TBST and TBST for 1 h. Primary antibodies were diluted in 5% milk–TBST and membrane using the iBlot system (Invitrogen) and blocked in 5% milk–Scientific) and run at 160 V. Proteins were then transferred onto a PVDF into NuPAGE 4–12% Bis-Tris Protein Gels (NP0321BOX, Thermo Fisher 50 μg of protein was prepared in sample buffer, heated and loaded into NuPAGE 4–12% Bis-Tris Protein Gels (NP0321BOX, Thermo Fisher 50 μg of protein was prepared in sample buffer, heated and loaded into NuPAGE 4–12% Bis-Tris Protein Gels (NP0321BOX, Thermo Fisher into an 3D reconstruction proliferation assay was performed by seeding young or aged fibroblasts into a top and bottom collagen layer (col). The 3D reconstruction proliferation assay was performed by seeding young or aged fibroblasts into a top and bottom collagen layer (col) and Run 10-day experiment using a two-way ANOVA with a post hoc Holm–performed by analysing the total number of cells at the end of the and the results are presented as mean ± s.e.m. Statistical analysis was performed by analysing the normal control DNA was routinely performed. However, each short tandem repeat profile is compared with our internal database of over 200 melanoma cell lines, as well as control lines, such as HeLa and 293T. Short tandem repeat profiles are available upon request. Cell culture supernatants were tested for mycoplasma using a Lonza MycoAlert assay at the University of Pennsylvania and Johns Hopkins University Cell Center Services, and used only if they tested negative for mycoplasma contamination.

Western blot
Cell lines were plated and lysed with RIPA buffer supplemented with protease and phosphatase inhibitors. Total protein lysate was quantified using a Pierce BCA assay kit (23225, Thermo Fisher Scientific) and 50 μg of protein was prepared in sample buffer, heated and loaded into NuPAGE 4–12% Bis-Tris Protein Gels (NP0321BOX, Thermo Fisher Scientific) and run at 160 V. Proteins were then transferred onto a PVDF membrane using the iBlot system (Invitrogen) and blocked in 5% milk–TBST for 1 h. Primary antibodies were diluted in 5% milk–TBST and incubated at 4°C overnight. The membranes were washed in TBST and probed with the corresponding HRP-conjugated secondary antibody at 0.2 μg ml⁻¹. Proteins were visualized using ECL Prime and detected using ImageQuant LAS 4000 (GE Healthcare Life Sciences).

Antibodies
Antibodies were purchased from the following commercial vendors and used at the indicated dilutions for western blot: GAPDH (1:10,000, Cell Signaling, 2118S), HSP90 (1:10,000, Cell Signaling, 4877S), human AXL (1:1,000, Cell Signaling, 8661S), human MER (1:1,000, Cell Signaling, 4319), p21 (1:1,000, Cell Signaling, 2947), p27 (1:1,000, Cell Signaling, 36865), Coup TF/NRZF1 (1:1,000, Cell Signaling, 63645), phospho-p38 (1:500, Cell Signaling, 9212S), H2AFZ (1:1,000, Cell Signaling, 2718S), MITF (1:1,000, Cell Signaling, 12590), human GAS6 (1:1,000, Cell Signaling, 67202S), human PROS1 (1:1,000, R&D Systems, AF-4036), WNT5A (1:50, R&D Systems, biotin-labelled, BA645), mouse AXL (1:1,000, R&D Systems, AF854), mouse MER (1:1,000, R&D Systems, MAB591-100), mouse p21 (1:1,000, Abcam, ab188224), MART1 (1:1,000, Thermo Fisher Scientific, MS176P0), β-catenin (1:1,000, Cell Signaling, 8541S), TGFβ2 (1:500, Abcam, ab36495), human phospho-PKCα/β II (Thr638/641) (1:1,000, Cell Signaling, 9375), ROR1 (D6TSC) (1:1,000, Cell Signaling, 16540), ROR2 (D3B6F) (1:500, Cell Signaling, 86639), filamin A (1:500, Cell Signaling, 4762), mouse GAS6 (1:1,000, R&D Systems, MAB9868) and mouse PROS1 (1:1,000, R&D Systems, 4976). The antibodies used for IHC were mCherry (1:200, Novus Biological NB2-25157 and ABCAM AB64753), mouse Ki-67 (1:200, Novus Biological, NB600-1252), mouse MER (1:200, Abcam, ab184086), mouse AXL (1:200, Thermo Fisher Scientific, 50-198-3691), human MER (Thermo Fisher Scientific, MAS-31991), human AXL (1:200, Cell Signaling, 8661S), WNT5A (1:200, Abcam, ab229200), GAS6 (1:200, Thermo Fisher Scientific, PA3-79300) and PROS1 (1:200, Thermo Fisher Scientific, BS-9512R). The neutralizing antibodies used in in vivo experiments were anti-sFRP1 (1 mg kg⁻¹ Sigma Aldrich, AB519539) and IgG control (1 mg kg⁻¹ R&D Systems, AB-105-C).

shRNA and overexpression, lentiviral production and infection
MERTK, AXL and sFRP1 shRNA was obtained from the TRC shRNA library available at the Wistar Institute (TRCN00000000865, CTGCATGACTT ACTATCTTTA; TRCN00000000862, GCTTCGGGCTGTAGTATT; TRCN0000000572, CTTTAGGCCTTGTGGCAT; TRCN0000000573, GCCGGTTCTGATGAGAATTT; TRCN00000026170, CCACCTCTAGTCC GTTGTTAA and TRCN00000026171, CGAGTTGGAATCTGAGGCCAT). The dox-inducible on system (Tet3g vector: pLV[Exp]-EGFP-T2A:Puro-EF1A>Tet3G VB190411-1425srq) was sourced from VectorBuilder; the plasmid used for the inducible MER component was a pLV-mCherry-TRE3G MER cloned vector, the AXL component was a PLV-mCherry-TRE3G AXL vector and the WNT5A-overexpressing component was a pLV-mCherry-TRE3GWNT5A cloned vector, all sourced from VectorBuilder. The mCherry (pLV[Exp]-Bsd-EF1A-mCherry, VB180821-1150kgq) and GFP (pLV[Exp]-Puro-EF1A-EmGFP, VB171103-1159dvu) were purchased from VectorBuilder. The dox-inducible shRNA WNT5A (clone ID: V2THS, 172016- TAGGCCATTTAGAATATGAC) TRIP2 system was purchased from Horizon Discovery. The WNT5A overexpression in cells was driven by the cloned vector pLU-EF1WNT5A-iCherry. Lentiviral production was performed according to the protocol suggested by the Broad Institute. In brief, 293T cells were at 70% confluency and cotransfected with shRNA or overexpressing plasmids and the lentiviral packaging plasmids (pCMV-dr8.74psPAX2, pMD2.G for second generation; pMDL/pRRE, pRSV/REV and PMD2.G for third generation). Appropriate empty vector and scrambled controls were created for overexpressing and shRNA constructs, respectively. Cells were transduced with lentivirus for 48 h, and then treated with an appropriate selection of antibiotics (puromycin, hygromycin and blasticidin) with previously established kill curves for each cell line, or were sorted for their selection marker (mCherry or GFP). For the dox-inducible models, cells were first transduced with a Tet3g plasmid mentioned above, followed by transduction of a second plasmid containing MER, AXL or WNT5A driven by a TRE3G promoter.

In vivo allograft assays
All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Wistar Institute (Protocol 112503X: Microenvironmental Regulation of Metastasis and Therapy Resistance) or the Johns Hopkins University (Protocol M019H421: Microenvironmental Regulation of Metastasis and Therapy Resistance) and were performed by analysing the total number of cells at the end of the 10-day experiment using a two-way ANOVA with a post hoc Holm–Sidak’s multiple comparisons test. P < 0.05 was considered significant. The 3D reconstruction proliferation assay was performed by seeding young or aged fibroblasts into a top and bottom collagen layer (collagen I, Gibco A1048301) and GFP melanoma cells in a middle layer at a density of 2 × 10⁴ in a 24-well plate. The cells were imaged using a Nikon TE automated microscope and the number of cells per field was quantified using NIS-Elements, with the data represented in GraphPad Prism as mean ± s.e.m.
performed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility. The mice were housed in a vivarium maintained at 20 ± 2 °C, 42% humidity, with a 12-h light–dark cycle with free access to food and water. The housing group was five at maximum for young mice and one mouse per cage for aged mice. The maximum tumour size allowed under this protocol was 2,000 mm³. No tumours in our experiments exceeded this size.

Young versus aged mouse experiments. YUMM1.7 mouse melanoma cells (2.5 × 10⁴) overexpressing mCherry were injected intradermally into 5 aged (more than 300 days old) and 5 young (6–8 weeks old) C57BL6 mice (Charles River). Tumours were allowed to grow for a period of 1, 3 and 5 weeks for appropriate experiments.

Neutralizing sFRP1 experiments. YUMM1.7 mouse melanoma cells (2.5 × 10⁴) expressing mCherry were injected intradermally into five aged mice per group. After 3 weeks of tumour growth, mice were intraperitoneally injected with anti-sFRP1 or an IgG control at a concentration of 1 mg kg⁻¹ every 4 days until 5 weeks of total tumour growth.

Dox-inducible shWnt5a, WNT5A, MER and AXL dox experiments. YUMM1.7 (mCherry) mouse melanoma cells (2.5 × 10⁴) transduced with a lentiviral TRIPZ dox-inducible shWnt5a vector or a MER, AXL, or WNT5A dox-inducible overexpression vector were injected intradermally into five young (eight-week-old) C57/BL6 mice (Charles River) per group. Expression of these vectors was controlled by administration of dox (2 mg ml⁻¹ in the water) and was given at day 3 or day 21 of tumour growth.

Recombinant protein treatments. YUMM1.7 (mCherry) mouse melanoma cells (2.5 × 10⁴) were injected intradermally into young (6–8-week-old) C57BL6 mice (Charles River) via the tail vein. Mice were checked every two days for clinical signs of metastasis (weight loss, altered posture, scruffed hair and inactivity) and were ultimately euthanized at day 35. These experiments were repeated using dox-inducible shWnt5a, dox overexpressing AXL and dox overexpressing MER YUMM1.7 mCherry melanoma cell lines. A total of 1 × 10⁴ cells were injected into nine young (MER and shWnt5a dox lines) or eight aged (AXL dox) mice. One mouse died from each group for the shWnt5a mice, and two mice died per group from the AXL dox mice. Dox treatment began at day 3 for one group (2 mg ml⁻¹ in the water), whereas the other group contained no dox. Mice were euthanized and the lungs were taken at day 35.

Tumour sizes for each appropriate experiment were measured every 2–4 days using digital calipers, and tumour volumes were calculated using the following formula: volume = 0.5 × (length × width)³. Mice were euthanized after 5 weeks or when a group reached 1,500 mm³, and tumour and lung tissue was preserved. Half of the tissue was embedded in paraffin and the other half was flash-frozen and processed for protein analysis. All reagents injected in live mice were tested for endotoxin levels at the University of Pennsylvania or Johns Hopkins Cell Center Services using The Associates of Cape Cod LAL test.

Immunohistochemistry

Mouse tumour and lung sections were paraffin-embedded and sectioned. Paraffin-embedded sections were rehydrated through a series of xylene and different concentrations of alcohol, which was followed with a rinse in water and washing in PBS. Slides were put with an antigen retrieval buffer (3300. Vector Labs) and steamed for 20 min. Slides were then blocked in a peroxide blocking buffer (TA060H202Q, Thermo Fisher Scientific) for 15 min, followed by protein block (TA-060-UB, Thermo Fisher Scientific) for 5 min, and were incubated with the primary antibody of interest, which was prepared in antibody diluent (S0809, Dako). Slides were put in a humidified chamber at 4 °C overnight. Samples were washed with PBS and incubated in biotinylated anti-rabbit (ab64256 Abcam), followed by streptavidin–HRP solution at room temperature for 20 min (TS-060-HR Thermo Fisher Scientific). Samples were then washed with PBS and incubated with AEC (3-amino-9-ethylcarbazole) chromogen for the appropriate amount of time after optimization (TA060SA, Thermo Fisher Scientific). Slides were then washed with water and incubated in Mayer’s haematoxylin (MHS1, Sigma) for 1 min, rinsed with water, and mounted in Aquamount (143905, Thermo Fisher Scientific). Lungs were assessed for localization of mCherry-positive melanoma cell colonies containing 10 or more cells in a cluster (not spaced individually) using a Nikon Eclipse 80i digital. The number of mCherry cells per high-power field (HPF) per lung section was also assessed in mice that did not display any colonies and only had single cells, and was quantified using automated NIS-Elements software and analysed using GraphPad Prism.

TCGA and CCLE analysis

RNA-seq data from the TCGA and the CCLE were downloaded from the TCGA database using cBioportal (http://www.cbioportal.org). Individual gene expression values for genes of interest were retrieved as normalized RNA-seq by expectation maximization (RSEM) read counts processed through the TCGA and CCLE cBioportal. The data were then transformed to represent a standardized z-score for each gene and further transformed such that genes of interest (WNT5A, AXL and MER) were separated into two groups on the basis of the top 25 percentile and bottom 25 percentile of gene expression within the samples from patients with melanoma from the TCGA dataset (n = 120) and the top and bottom 50th percentile for melanoma cells within the CCLE (n = 34). A Student’s two-sided t-test (unpaired) was then performed on each gene in the dataset to individually analyse the difference in average z-score in low- versus high-percentile melanoma samples. Data are presented as a heat map based on high (red, 0.5) and low (blue, −0.5) z-scores and associated P-values. Significance was taken at P < 0.05.

Proteomics

Fibroblasts were plated at an equal number and grown to 70% confluency in a 10-cm dish. They were washed once with PBS and five times with serum-free medium, and then grown for 16 h in 10 ml serum-free medium. Conditioned medium was collected, centrifuged at a low speed to remove cells and debris, and then centrifuged through a 0.22-μm filter. Protease inhibitors (PMSF, pepstatin A and leupeptin) were added and samples at this time were flash-frozen at −80 °C until ready. Fibroblasts from the 10-cm dish were disassociated, the number of single cells, and was quantified using automated NIS-Elements software and analysed using GraphPad Prism.

Heat and tumour volume were determined using digital calipers, and tumour volume was calculated using the following formula: volume = 0.5 × (length × width)³. Mice were euthanized after 5 weeks or when a group reached 1,500 mm³, and tumour and lung tissue was preserved. Half of the tissue was embedded in paraffin and the other half was flash-frozen and processed for protein analysis. All reagents injected in live mice were tested for endotoxin levels at the University of Pennsylvania or Johns Hopkins Cell Center Services using The Associates of Cape Cod LAL test.
was used to estimate the significance of the difference between conditions and the false discovery rate was estimated. Proteins that passed the criterion of a false discovery rate of less than 5% were considered significant and the top changed proteins with nominal \( P < 0.05 \) were also reported on a scatter plot.

**Statistical analysis and reproducibility**

For in vitro studies, a Student’s two-sided \( t \)-test or Mann–Whitney test was performed for two-group comparisons. An estimate of variance was performed and unequal variances for the \( t \)-test were adjusted accordingly using Welch’s correction. Multiple comparisons were performed using an ANOVA or Kruskal–Wallis test with post hoc Holm–Sidak’s adjusted \( P \)-values. The indicated sample size for each in vivo study was designed to have 80% power at a two-sided \( \alpha \) of 0.05 to detect a difference of large effect size of about 1.5 between two groups on a continuous measurement. GraphPad Prism 8 was used for plotting graphs and statistical analysis. Significance was designated as follows: * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \); **** \( P < 0.0001 \). All western blots represented have been repeated a minimum of two times. Representative IHC images are from a minimum of \( n = 3 \) from each lung section.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

Any data requested will be made available upon request. The mass spectrometry proteomics data have been deposited into the Massive (http://massive.ucsd.edu) and ProteomeXchange (http://www.proteomexchange.org) data repositories with accession numbers MSV000088977 and PXD032025, respectively.

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Extended Data Fig. 1 | Aged lung versus skin fibroblasts promote opposing melanoma phenotypes. **a**, **b**, 5 Young (8 week) and aged (>52 week) C57BL6 mice per group were subdermally injected with 2.5x10⁵ Yumm1.7 mCherry-labelled mouse melanoma cells. Lungs were collected and PFA embedded from 5 tumour bearing young and aged mice at week 3 and 5. IHC was performed using an mCherry antibody. Lung sections were analysed and quantified for the average number of single cell colonies per high powered frame (20 x) across entire lung sections and presented as mean ± SEM. A student’s two-way t-test was performed **P** = 0.3064 for 3 weeks and **P** = 0.2011 for 5 weeks.

**c**, **d**, **e** 2.5 x10⁵ Yumm1.7 melanoma cells were intradermally implanted into 3 C57BL6 aged mice (>52 weeks) per group. Tumours were left to grow for three weeks. Mice underwent survival surgery to allow excision of the primary tumour. After 5 total weeks, Lungs were taken, PFA embedded and underwent IHC analysis for the number of mCherry positive colonies (>10 mCherry positive cells per lesion) per section (**p** = 0.2879) along with the average number of single cell colonies per high powered frame (20 x) across entire lung sections (**p** = 0.081) presented as mean ± SEM. A student’s two-way t-test was performed with **P** = 0.05 designated as significant. Representative images were displayed across three independent mouse lung samples for mCherry positive cells and Ki-67 across subsequent sections with the scale bar representing 100um. **f**, **g**, **h** Collagen sandwich reconstructions were formed with healthy human lung or skin fibroblasts from young (>35) or aged (> 55) patients in the top and bottom layer. The middle layer contained FS4 GFP human melanoma cells seeded at the same density at day 0. GFP positive melanoma cells were imaged using an automated Nikon TI and an average count per field was quantified using imaging software NIS elements at day 4. Representative images are displayed for each condition (**N** = 3 independent wells in triplicate). A student’s two-way t-test was performed. **P** = 0.0432 for FS4 lung and **P** = 0.0088 for FS4 skin. **i**, **j** Western blot analysis was performed on primary tumour protein lysate from 5 young and 5 aged mice investigating dormancy and proliferative associated expression. HSP90 was used as a loading control.
Extended Data Fig. 2 | Aged lung conditioned medium promotes melanoma outgrowth in vitro. a, b FS13 c, d FS4 e, f SF14 g, h and 1205Lu GFP melanoma cells were seeded at a density of 2x10^4. Cells were then grown over a 10-day period in conditioned medium from young or aged lung or skin fibroblasts and underwent counting using a haemocytometer at days 3, 5 and 10 and were further assessed via automated counting using NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. ****= P < 0.0001. **= P = 0.0084 for Fig. 2d. Data were presented as the mean +/- the SEM for each time point.
Extended Data Fig. 3 | sFRP1 promotes melanoma outgrowth. a, b, Protein lysate was collected from 1205Lu human melanoma cells treated with young (under 35) or aged (over 55) lung or skin fibroblast conditioned medium over a 5-day period. Western blot analysis was performed examining NR2F1, H2AFZ, Filamin A, Cleaved Filamin A, ROR2 and ROR1. HSP90 was used as a loading control.

c, d Protein lysate was collected from FS4 human melanoma cells treated with young or aged lung or skin fibroblast conditioned medium over a 5-day period. Western blot analysis was performed examining dormant (WNT5A, AXL, p21, p27, p-P38, p-PKC, NR2f1, Filamin A, ROR2) and proliferative (β-catenin, H2AFZ, MER, MITF, ROR1) melanoma markers. HSP90 was used as a loading control.

e FS4 melanoma cells were treated with 500ng/ml of recombinant sFRP1, and protein expression was assessed after 5 days via western blot. HSP90 was used as a loading control.

f Proliferation of the above cells was assessed by seeding them at a density of 2x10^4. Cells were then grown over a 10-day period while being treated with 500 ng/ml of recombinant sFRP1 or a PBS control (Experiment was performed in biological triplicates with 3 technical replicates each) every 2 days and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/- the SEM for each time point.

g Aged lung human fibroblasts were transduced with lentiviral sh-sFRP1 or scrambled control vectors. Conditioned medium was taken from fibroblasts after 72 h and FS4 melanoma cells were then grown in this conditioned medium or a DMEM control medium, with proliferation assessed over 10 days. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. ****= P < 0.0001 for Aged conditioned medium compared to both Aged conditioned medium in SHsFRP1 and DMEM conditions. Data were presented as the mean +/- the SEM for each time point.

h Protein lysate was collected from the lungs of 5 young and 5 aged healthy mouse tissues. Western blot analysis was performed looking at sFRP1. HSP90 was used as a loading control.

i Protein lysate was taken from healthy human young vs aged lung fibroblasts and a 1205Lu human melanoma cell line. Western blot analysis was performed examining sFRP1 and sFRP2 expression and HSP90 was used as a loading control. j Protein lysate was taken from two independent young lung fibroblasts and a 1205Lu human melanoma cell line. Western blot analysis was performed examining sFRP1 and sFRP2 expression and HSP90 was used as a loading control.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | WNT5A regulates dormant pathways and slows proliferation. a, b, Five aged mice per group were intradermally injected with $2.5 \times 10^5$ Yumm1.7 mCherry melanoma cells. After three weeks of tumour growth, mice were treated with 1 mg/kg of a neutralizing sFRP1 or an IgG control antibody via IP injection every 4 days. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67. The number of single cell (>10 mCherry positive cells) was assessed at week 5 and quantified per section and presented as mean $\pm$ SEM. Scale bar represents 100 μm. A student’s two-way t-test was performed. P = 0.244.

c Protein expression analysis was performed on WNT5A low human melanoma samples (FS13, FS14) that underwent lentiviral induced overexpression of WNT5A. HSP90 was used as a loading control.

d, e FS13 and FS14 WNT5A overexpressing mCherry human melanoma cells were seeded at a density of $2 \times 10^4$. Cells were then grown over a 10-day period and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean $\pm$ SEM for each time point. P < 0.0001 for both conditions.

f FS13 GFP melanoma cells were seeded at a density $2 \times 10^4$. Cells were treated with 200ng of r-WNT5A every 2 days and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean $\pm$ SEM for each time point. P < 0.0001.

g Western blot analysis was performed on protein lysates from FS13 melanoma cells treated with 200ng of recombinant WNT5A for five days. HSP90 was used as a loading control.
Extended Data Fig. 5 | WNT5A promotes in vivo metastatic dormancy.

a, TCGA analysis of relative mRNA expression of WNT5A in patient tumour samples comparing primary vs metastatic (distant mets only) tissue sites and presented as a violin plot, with quartiles represented by black lines and the median by the broken line (N=104, 68). A student’s two-way t-test was performed. P = 0.003

b Western blot analysis was performed on protein lysate from Yumm1.7 doxycycline (Dox) inducible shWnt5a mCherry melanoma cells comparing control and Dox (0.5ug/ml) treated cells over 48 h. HSP90 was used as a loading control.

c Yumm1.7 Dox-inducible shWnt5a mCherry cells were seeded at a density of 2x10^4 and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/- the SEM for each time point. P<0.0001.

d Tumour volumes from young mice (N = 9 for Dox Day 21, N = 8 for Dox Day 3 and No Dox due to mouse death) subdermally injected with 2.5x10^5 Yumm1.7 Dox inducible shWnt5a mCherry melanoma cells. Mice were treated with Dox (2ug/ml in water) from day 3 or day 21 onwards, with tumours measured for 5 weeks. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 37. Data were presented as the mean +/- the SEM for each time point. **** P < 0.0001, ** P = 0.0038.

e, f 5 young mice per group were intradermally injected with 2.5x10^5 Yumm1.7 Dox inducible shWnt5a mCherry melanoma cells. Mice were treated with Dox (2mg/mL in water) from day 3 (Dox day 3) or day 21 onwards (Dox day 21), with tumours measured for 5 weeks. Lungs were taken, PFA embedded and underwent IHC analysis of mCherry positive melanoma cells. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67 with the scale bar representing 100um. Given that the no Dox and Dox day 3 groups failed to produce larger lesions, these groups were assessed for the average number of single cell colonies per high powered frame (20 x) across entire lung sections and presented as mean +/- SEM. A student’s two-way t-test was performed. P = 0.0224.

g Tumour volumes from 6 aged mice subdermally injected with 2.5x10^5 Yumm1.7 Dox inducible WNT5A overexpressing mCherry melanoma cells. Mice were treated with Dox (2ug/ml in water) from day 3 or day 21 onwards, with tumours measured for 5 weeks. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 37. Data were presented as the mean +/- the SEM for each time point. * P = 0.0235 comparing No Dox with Dox Day 3.

h Yumm1.7 Dox-inducible WNT5A mCherry cells were seeded at a density of 2x10^4 and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/- the SEM for each time point. P<0.0001.

i, j 5 Aged mice per group were subdermally injected with 2.5x10^5 Yumm1.7 Dox inducible WNT5A overexpressing mCherry melanoma cells. Mice were treated with Dox (2ug/ml in water) from day 3 (Dox day 3) and day 21 (Dox day 21) with tumours measured for 5 weeks. Lungs were taken, PFA embedded and underwent IHC analysis of mCherry positive melanoma cells. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67. Given that the Dox day 3 and Dox day 21 groups failed to produce larger lesions, these groups were further assessed for the average number of single cell colonies per high powered frame (20 x) across entire lung sections and presented as mean +/- SEM. A student’s two-way t-test was performed. P = 0.001.
Extended Data Fig. 6 | Role of MER and AXL in metastatic dormancy and reactivation. a, b, Analysis of melanoma cell lines from the Cancer Cell line Encyclopedia (CCLE) dataset were performed on human samples stratified into the top (AXL high) and bottom (AXL low) 50th percentile of AXL expression (N = 30) and presented as a heat map of the average Z-score. An unpaired two-way t-test was performed on each condition, investigating previously established dormancy and proliferative genes, with P < 0.05 considered significant. c, d, Analysis of MER high and low cells from the CCLE as described above. e, TCGA analysis of relative mRNA expression of MER in patient tumour samples stratified into stage I/II/III vs metastatic (distant metastases only) tissue sites and presented as a violin plot, with quartiles represented by black lines and the median by the broken line (N = 104, 68). A student's two-way t-test was performed. P = 0.0023. f, F2OSLu shAXL and control human mCherry melanoma cells were seeded at a density of 2x10⁴. Cells were then grown over a 10-day period and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/− the SEM for each time point. P < 0.0001. g, Western blot analysis was performed on protein lysates of Yumm1.7 Dox inducible AXL overexpressing mCherry mouse melanoma cells comparing control and Dox (0.5ug/ml) treated cells over 48 h. HSP90 was used as a loading control. h, Yumm1.7 Dox inducible AXL overexpressing mCherry mouse melanoma cells were seeded at a density of 2x10⁴. Cells were then grown over a 10-day period and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/− the SEM for each time point. P = 0.0025. i, TCGA analysis of relative mRNA expression of MER in patient samples stratified into stage I/II/III/IV and presented as a violin plot, with quartiles represented by black lines and the median by the broken line (N = 219, 197). A student’s two-way t-test was performed. P = 0.0025. j, Tumour volumes from young mice (N = 9 for Dox Day 21, N = 8 for Dox Day 3, N = 7 for No Dox due to mouse deaths) subdermally injected with 2.5x10⁵ Yumm1.7 Dox inducible MER mCherry melanoma cells. Mice were treated with Dox (2 μg/ml in water) from day 3 or day 21 onwards, with tumours measured for 5 weeks. A two-way ANOVA was performed on each condition, investigating previously described above. k, Yumm1.7 Dox inducible AXL mCherry melanoma cells. Mice were treated with Dox (2 μg/ml in water) from day 3 or day 21 onwards, with tumours measured for 5 weeks. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 31. Data were presented as the mean +/− the SEM for each time point. *** P < 0.0004 comparing No Dox with Dox Day 3. ** P = 0.0281 comparing No Dox with Dox Day 21. n, Western blot analysis was performed on protein lysates of Yumm1.7 Dox inducible AXL overexpressing mCherry mouse melanoma cells comparing control and Dox (0.5ug/ml) treated cells over 48 h. HSP90 was used as a loading control. o, Yumm1.7 Dox inducible AXL overexpressing mCherry mouse melanoma cells were seeded at a density of 2x10⁴. Cells were then grown over a 10-day period and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/− the SEM for each time point. P < 0.0001. p, Tumour volumes from aged mice (N = 8 for No Dox, N = 5 for Dox Day 3 and N = 7 for Dox Day 21 due to mouse deaths) per group were subdermally injected with 2.5x10⁵ Yumm1.7 Dox inducible AXL mCherry melanoma cells. Mice were treated with Dox (2 μg/ml in water) from day 3 or day 21 onwards, with tumours measured for 5 weeks. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 36. **** P < 0.0001 for comparisons between No Dox with both Dox Day 3 and Dox Day 21. Data were presented as the mean +/− the SEM for each time point.
Extended Data Fig. 7 | MER expression is inversely correlated with WNT5A and AXL and is higher in lung metastases. a–d, IHC analysis was performed on matched human primary melanoma tumours and lung metastases for MER, WNT5A and AXL. Scale bar represents 100um with representative images displayed for each patient sample.
Extended Data Fig. 8 | MER promotes emergence from metastatic dormancy whereas AXL induces dormancy. **a**, Five young mice per group were intradermally injected with 2.5x10^5 Yumm1.7 Dox inducible MER overexpressing mCherry melanoma cells. Mice were treated with Dox (2 mg/mL in water) from day 3 (Dox day 3) or day 21 onwards (Dox day 21), with tumors measured for 5 weeks. Lungs were taken, PFA embedded and underwent IHC analysis of mCherry positive melanoma cells. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67 with the scale bar representing 100 um. **b, c**, 5 aged mice per group were intradermally injected with 2.5x10^5 Yumm1.7 Dox inducible AXL overexpressing mCherry melanoma cells. Mice were treated with Dox (2 mg/mL in water) from day 3 (Dox day 3) or day 21 onwards (Dox day 21), with tumors measured for 5 weeks. Lungs were taken, PFA embedded and underwent IHC analysis of mCherry positive melanoma cells. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67 with the scale bar representing 100 um. Given that both Dox groups failed to produce larger lesions, these groups were assessed for the average number of single cell colonies per high powered frame (20 x) across entire lung sections and presented as mean +/− SEM. A student’s two-way t-test was performed. P = 0.5240.
Extended Data Fig. 9 | GAS6 regulates proliferation in a context-specific manner. a, b, FS4 and FS13 human GFP melanoma cells were seeded at a density of 2x10^4. Cells were then grown over a 10-day period, while being treated with 500ng of r-GAS6 every 2 days and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +− the SEM for each time point. P = 0.033 for 9A, P = 0.0205 for 9B c, d, FS14 control vs SH MER and 1205Lu control vs shAXL human melanoma cells were seeded at a density of 2x10^4. Cells were treated with 500ng of r-GAS6 vs a PBS control every two days and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. P-values were taken from day 10. ****P < 0.0001 shMER + GAS6 compared to control, **P = 0.0013 Control vs Control + GAS6, *P = 0.0238 shMER vs shMER + GAS6. ****P < 0.0001 Control + GAS6 vs Control, *P = 0.0161 Control + GAS6 vs shAXL + GAS6. e, f Aged mice were intradermally injected with 2.5x10^5 Yumm1.7 mCherry melanoma cells (5 mice for PBS, 4 mice for GAS6 treatment due to one early death). After three weeks of tumour growth, mice were subsequently treated with 500 ng of rGAS6 or PBS via IP injection every 2 days. After 5 total weeks, Lungs were taken, PFA embedded and underwent IHC analysis of mCherry positive melanoma cells. Both groups were further assessed for the average number of single cell colonies (<10 mCherry-positive cells) per high powered frame (20 x) across entire lung sections and presented as mean +/- SEM. A student’s two-way t-test was performed. P = 0441. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67 with the scale bar representing 100 um.
Extended Data Fig. 10 | PROS1 promotes melanoma growth. a–e, 1205Lu (P < 0.0001), FS4 (P < 0.0054), FS13 (P = 0.0015), FS14 (P < 0.0001) and Yumm1.7 (P < 0.0001). Fluorescent melanoma cells were treated every two days with 500 ng of r-PROS1 every 2 days vs a PBS control assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10 and P < 0.05 was designated significant. Data were presented as the mean +/− the SEM for each time point.

f, FS14 shMER knockdown cells and control cells were seeded at a density of 2x10⁴. Cells were then grown over a 10-day period, while being treated with 500 ng of r-PROS1 every two days and assessed for proliferation at days 3-5 and 10 using a haemocytometer and further assessed using automated counting via NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post-hoc Holm-Sidak’s multiple comparisons test for each time point. P-values were taken from day 10. Data were presented as the mean +/− the SEM for each time point. **** P < 0.0001 for control + PROS1 vs all other treatment groups.

g, Protein lysate from Yumm1.7 cells treated with 500 ng of r-PROS1 for 48 h underwent western blot analysis. HSP90 was used as a loading control.

h, i Young mice were intradermally injected with 2.5x10⁵ Yumm1.7 mCherry melanoma cells (5 mice per group). After three weeks of tumour growth, mice were subsequently treated with 500 ng of rPROS1 or PBS via IP injection every 2 days. After 5 total weeks, lungs were taken, PFA embedded and underwent IHC analysis of mCherry positive melanoma cells. Both groups were further assessed for the average number of single cell colonies (<10 mCherry positive cells) per high powered frame (20 x) across entire lung sections and presented as mean +/− SEM. A student’s two-way t-test was performed. Representative images are displayed across three independent mice for mCherry positive cells and Ki-67 with the scale bar representing 100 um. P = 0.8757.

j, 5 young mice per group were intradermally injected with 2.5x10⁵ Yumm1.7 mCherry melanoma cells. After three weeks of tumour growth, mice were subsequently treated with 500 ng of r-PROS1, r-SFRP1, a combination of both or PBS via IP injection every 2 days. After 5 total weeks, lungs were taken, PFA embedded and subjected to IHC analysis of mCherry positive melanoma cells. Representative images were displayed for mCherry and Ki-67 with the scale bar representing 100 um.
Extended Data Fig. 11 | Aged liver fibroblasts express sFRP1 and are growth permissive for melanoma cells. a, Five young (8 week) and five aged (>52 week) C57BL6 mice were intradermally injected with 2.5x10^5 Yumm1.7 parental mouse melanoma cells and tumours were measured over 36 days. Lungs were collected and PFA embedded from 5 tumour bearing young and aged mice at weeks 3 and 5 and IHC was performed using MITF, KI-67 and H&E. Representative images are displayed across three independent aged mice for MITF positive cells and Ki-67 with the scale bar representing 100 um. b, Data were generated using the Human Protein Atlas looking at RNA expression of sFRP1 in specific cell types across various tissue types. Green circles indicate high levels of expression, grey circles low expression and empty spaces no expression. c, Western blot was performed on protein lysate taken from the healthy liver of young (<8 weeks) and aged (>52 weeks) C57BL6 mice. GAPDH was used as a loading control. d, Protein lysate from one healthy human young (<35) and aged (>55) liver fibroblast. HSP90 was used as a loading control. e, Collagen sandwich reconstructions were formed with healthy human Liver fibroblasts from young (<35) or aged (>55) patients in the top and bottom layer. The middle layer contained FS4 or 1205Lu GFP human melanoma cells seeded at the same density at day 0. GFP positive melanoma cells were imaged using an automated Nikon TI and an average count per field was quantified using imaging software NIS elements at day 4. Representative images are displayed for each condition (N = 3 independent wells in triplicate). A student’s two-way t-test was performed. 1205Lu P = 0.0001, FS4 P = 0.0055. g, h 1205Lu and FS4 GFP melanoma cells were seeded at a density of 2x10^4. Cells were then grown over a 10-day period in conditioned medium from young or aged lung or skin fibroblasts and underwent counting using a haemocytometer at days 3, 5 and 10 and were further assessed via automated counting using NIS elements. Experiment was performed in biological triplicates with 3 technical replicates each. A two-way ANOVA was performed with a post hoc Holm-Sidak’s multiple comparisons test for each time point. ** P = 0.0018, **** P < 0.0001. Data were presented as the mean +/- the SEM for each time point.
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Reporting Summary

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Statistics

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- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Proteomics data were searched using MaxQuant v.1.6.0.16

Data analysis

Image analysis was performed with NIS elements Advanced Research ver 5.21.03

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Any data requested will be made available upon request. The mass spectrometry proteomics data have been deposited into the MassIVE (http://massive.ucsd.edu) and ProteomeXchange (http://www.proteomexchange.org) data repository with the accession number MSV000088977 and PXD032025, respectively.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- **Life sciences**
- **Behavioural & social sciences**
- **Ecological, evolutionary & environmental sciences**

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](http://nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

### Sample size
We wished to have 80% power at a two-sided alpha level of 0.05 to detect a minimum difference of an effect size of about 2.75 in vitro and 1.25 in vivo, in means in terms of standard deviation, between different groups using a two-sample t-test, requiring 3 samples (in vitro) and at least 5 mice per group (in vivo).

### Data exclusions
Data was only excluded from mouse groups if mice were sick or had died prior to experimental endpoint.

### Replication
For in vitro studies, a student’s two-sided t-test or Mann Whitney was performed for two group comparisons. Estimate of variance was performed and unequal variances for the t-test were addressed by using the F-test. Multiple comparisons were performed using ANOVA or Kruskal-Wallis test with post-hoc Bonferroni or Sidak adjusted p-values. The indicated sample size for each in vivo study was designed to have 80% power at a two-sided alpha of 0.05 to detect a difference of large effect size of about 1.5 between two groups in a continuous measurement. GraphPad Prism 8 was used for plotting graphs and statistical analysis. Significance was designated as follows: *p* < 0.05, **p** < 0.01, ***p*** < 0.001, ****p*** < 0.0001. All western blots represented have been repeated a minimum of 3 times. Representative blots are shown from a minimum of N=3 from each long section:

### Randomization
Allocation was random, except as it pertained to age, i.e., aged mice were randomly assigned into Cohorts, vs young mice that were randomly assigned into Cohorts.

### Blinding
Group allocation was not blind as Dox, recombinant and neutralizing treatments were unable to be done in this fashion. Assessment of proliferation was performed using automated software where possible. Assessment of metastatic area and ki67 area was performed using automated software. Smaller metastases were confirmed by a pathologist and counted blindly.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✓   | Antibodies            |
| ✓   | Eukaryotic cell lines |
| ✓   | Palaeontology and archaeology |
| ✓   | Animals and other organisms |
| ✓   | Human research participants |
| ✓   | Clinical data |
| ✓   | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✓   | ChIP-seq              |
| ✓   | Flow cytometry        |
| ✓   | MRI-based neuroimaging |

### Antibodies

Antibodies were purchased from the following commercial vendors and used at the indicated dilutions for western blot: GAPDH (1:100000, Cell Signaling #2118S), HSP90 (1:10000, Cell Signaling #4875S), Human AXL (1:10000, Cell Signaling #8493S), Human MER (1:10000, Cell Signaling #4119S), p21 (1:10000, Cell Signaling #7360S), p27 (1:10000, Cell Signaling #8248S), GAPDH (1:10000, Cell Signaling #67458), ERK1/2 (1:10000, Cell Signaling #9102S), AKT (1:1000, Cell Signaling #2273S), MAPK (1:1000, Cell Signaling #12590S), Human GAB1 (1:10000, Cell Signaling #67202), Human PRAS40 (1:10000, R & D Systems #84306), Wnt5A (1:50, R & D Systems #114864), mouse AXL (1:1000, R & D Systems #A8514), mouse MER (1:10000, R & D Systems #MB131-100), mouse p21 (1:1000, Abcam ab52242), MAP4K1 (1:1000, Mouse Specific Antibodies #64503), E-cadherin (1:1000, Cell Signaling #7368S), β-Catenin (1:1000, Cell Signaling #8432), TIMP-2 (1:1000, Cell Signaling #67504), TIMP-1 (1:1000, Cell Signaling #67503), TIMP-3 (1:1000, Cell Signaling #67505), TIMP-4 (1:1000, Cell Signaling #67506), Antibodies used for IHC were mAb22 (1:200, Novus Biological #BP2-2527), Mouse Ki-67 (1:200, Novus Biological #NB600-2251), Mouse MER (1:200, Abcam ab34086), Mouse AXL (1:200, Thermo Fisher #51-2883), Human MER (Thermo Fisher #MAS-31991), Human AXL (1:200, Cell Signaling #86615), Wnt5A (1:200, Abcam ab23023), GSK3β (1:200 Thermo Scientific #9200), PROS1 (1:200, Thermo Fisher #8B-5626). Neutralizing antibodies used in in vivo experiments were α-SRP1 (1mg/Kg, Sigma Aldrich #A185193) and IgG control (1mg/Kg, R & D Systems #AB-105-C).

### Eukaryotic cell lines

Policy information about [cell lines](http://celllines.org/). Cell lines are available from ATCC, Catalog #CRL-3362.

### Cell line source(s)

- Fs4, Fs5, Fs13, Fs14, from Franklin Square Hospital, 1205lu from the Wistar Institute, Fibroblast lines from Coriell Institute, Yumm1.7 Cell lines are available from ATCC, Catalog #CRL-3362.

### Authentication

Cell stocks were fingerprinted using an AmpFLSTR Identifier PCR Amplification Kit from Life Technologies at The Wistar Institute Genomics Facility. Although it is desirable to compare the profile with the tissue or patient of origin, our cell lines were established over the course of 40 years, long before acquisition of normal control DNA was routinely performed. However, each short tandem repeat profile is compared with our internal database of over 200 melanoma cell lines, as well as control lines, such as HeLa and 293T. Short tandem repeat profiles are available upon request.

### Mycoplasma contamination

Cell culture supernatants were tested for mycoplasma using a Lonza MycoAlert assay at the University of Pennsylvania and Johns Hopkins University Cell Center Services. Cells were only used if they tested negative for mycoplasma contamination.
Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals
All experiments were performed with the protocols approved by the Johns Hopkins University Animal Care and Use Committee. The mice were housed in the vivarium maintained at 20 ± 2°C, at 42% humidity, with 12-h light/dark cycle with free access to food and water. The housing group was 5 at maximum for young mice and 1 mouse per cage for aged mice.

Wild animals
No wild animals were used in this study.

Field-collected samples
No field-collected samples were used in this study.

Ethics oversight
All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Wistar Institute (Protocol # 112503X: Microenvironmental Regulation of Metastasis and Therapy Resistance) or the Johns Hopkins University (Protocol # M019H421: Microenvironmental Regulation of Metastasis and Therapy Resistance) and were performed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility. The mice were housed in the vivarium maintained at 20 ± 2°C with 12-h light/dark cycle with free access to food and water. The housing group was 5 at maximum for young mice and 1 mouse per cage for aged mice. Maximal tumor size allowed under this protocol was 2000 mm³. No tumors in our experiments exceeded this size.

Note that full information on the approval of the study protocol must also be provided in the manuscript.