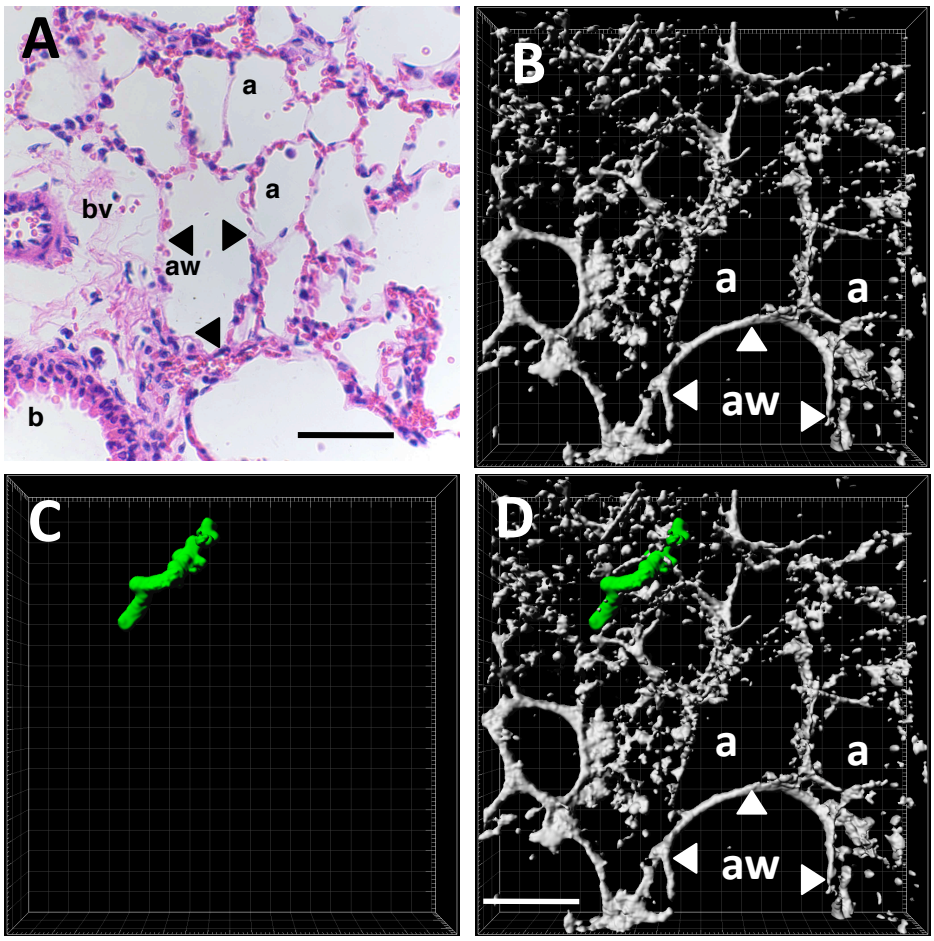
Supplemental Data

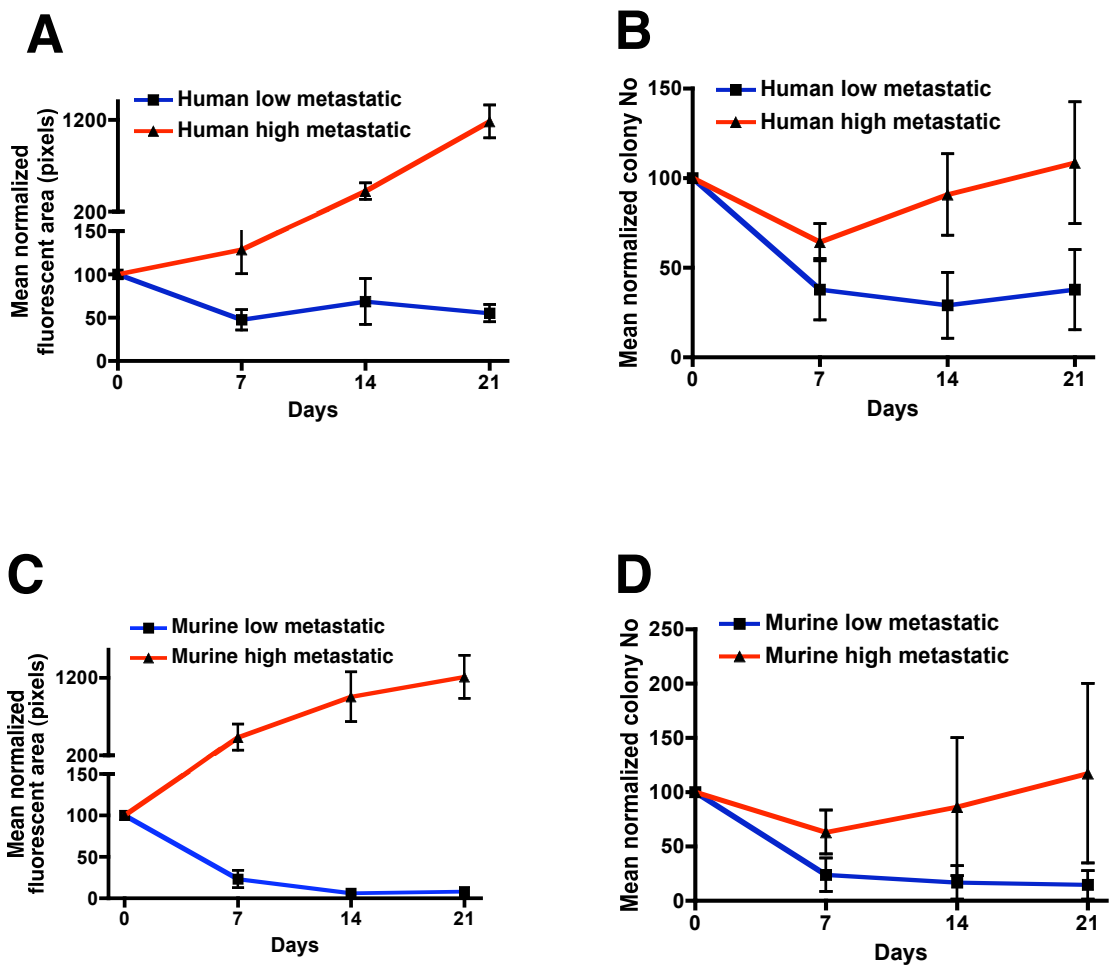
Methods

Confocal second harmonic generation (SHG) microscopy. Confocal assessment of SHG of PuMA lung sections was acquired using a plan-apochromat 40x/1.3 NA oil objective on a Zeiss LMS 510 NLO system (Zeiss). The IR laser was tuned to 780 nm for the SHG, while the 488 nm line of the argon laser was used for the GFP signal. With a multi-track configuration each signal was collected as a separated track, a 390-400 nm BP (band pass) filter was used for the SHG and a 500-550 nm band pass filter was used for the GFP fluorescence. Z-stacks were collected at 1 mm intervals at various locations in the lung organ sections. Imaris software (Bitplane) was used for surface rendering of the Z-stacks images.

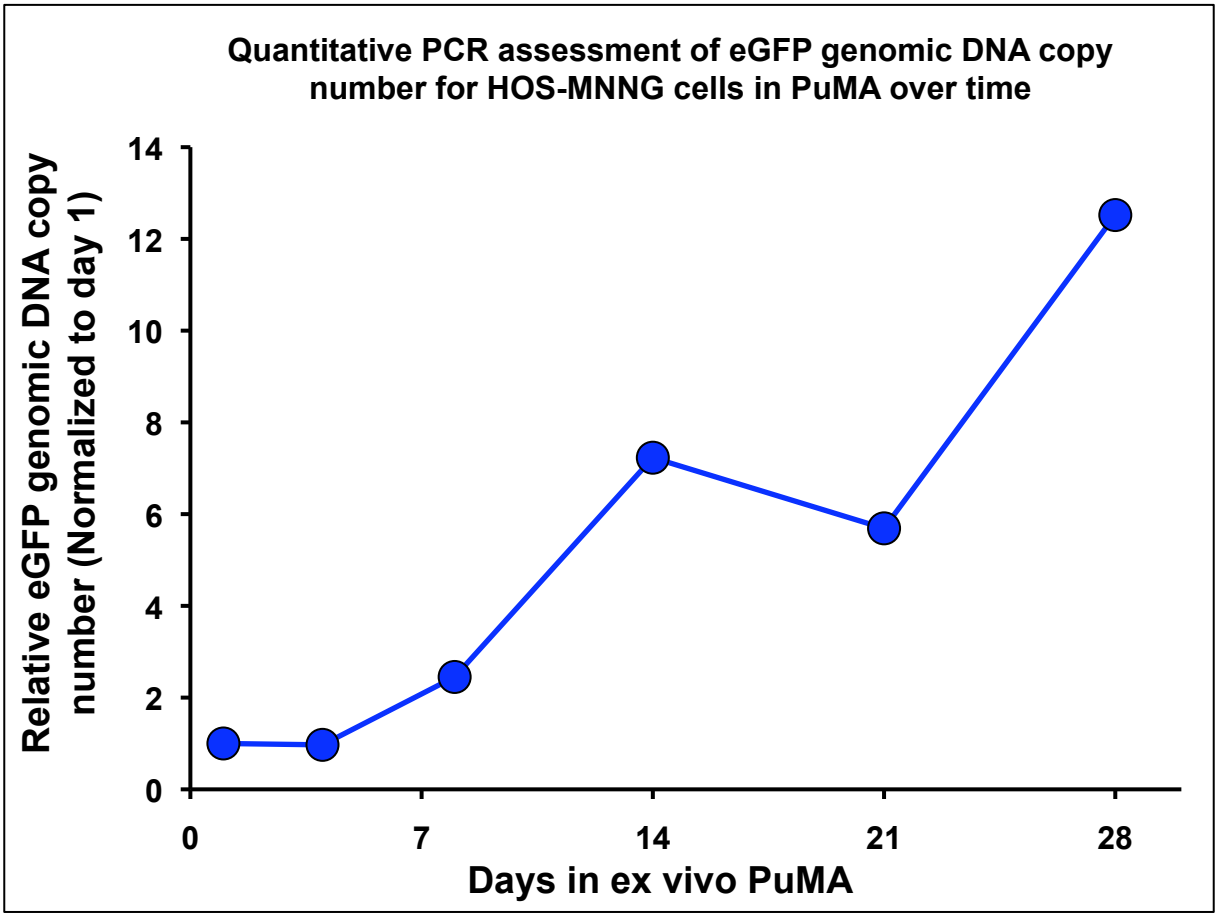
DNA extraction and quantitative real-time PCR analysis. Genomic DNA was isolated from the lung slices using the ZR Genomic DNA II Kit (Zymo Research Corp.) according to the manufacturers instructions. Samples were quantitated using a Nanodrop (Thermo) apparatus and DNA integrity verified by agarose gel electrophoresis and ethidium bromide staining. Purified, linearized plasmid DNA (pEGFP-C1, Clontech Corporation) was serially diluted and served as the template for generating the standard curve for absolute quantitation of eGFP copy number. PCR reactions were conducted using the Bio-Rad iQ Supermix for Taqman probes and assayed using an iQ5 real time thermocycler (Bio-Rad Laboratories, Inc.). The following primers were used at a final concentration of 300 nM each: eGFP primer 1, 5'-agtccgccctgagcaaaga-3' (Integrated DNA Technologies), eGFP primer 2, 5'-tcacgaactccagcaggacc-3' (Integrated DNA Technologies). The following fluorescent eGFP probe was used at a final concentration of 250 nM: 5'-FAM-cccaacgagaagcgcgatcaca-3'-BHQ (Integrated DNA Technologies). 50 ng of genomic DNA was used for each reaction and each sample was assayed in triplicate. Thermocycling parameters were as follows: cycle 1 = 95 ºC for 3 min, cycle 2 = 95 ºC for 15 seconds, 60 ºC for 1 min with cycle 2 repeated 43 times. Mean threshold cycle (cT) values for each sample were plotted against the standard curve, run on the same plate, in order to determine relative eGFP copy number for each condition.



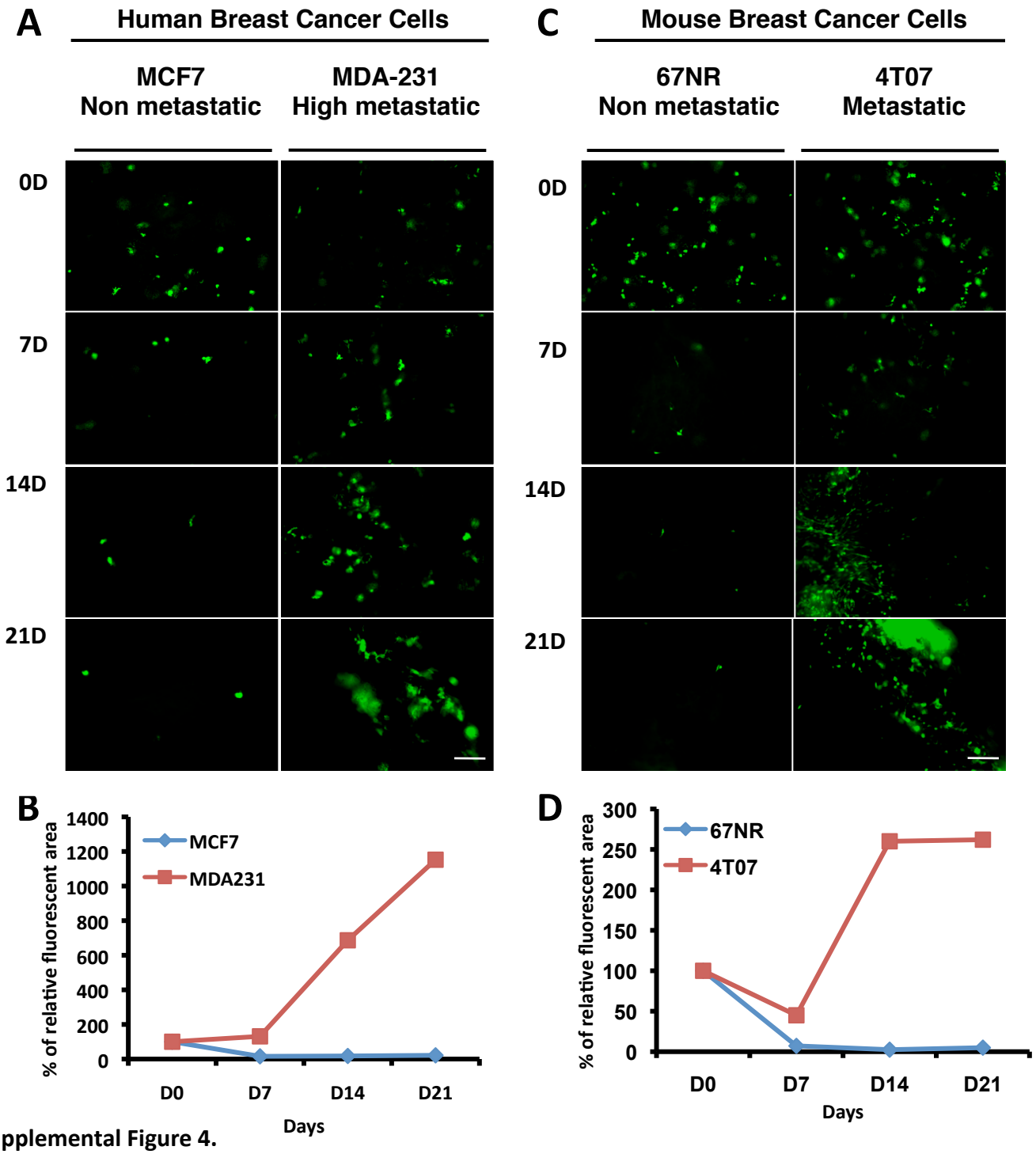
Supplemental Figure 1.
Three dimensional volume rendering images of PuMA and second generation confocal imaging demonstrate localization of green tumor cells within collagen lung architecture. **(A)** H&E image of PuMA microarchitecture (**B**) Three dimensional volume rendered flattened Second Harmonic Generation (SHG) images of PuMA. (**C)** Three dimensional volume rendered green tumor cells, imaged early after their arrival in the lung using PuMA. (**D**) Three dimensional volume rendered flattened combined SHG and green tumor cells images. Alveoli (a), alveolar walls (aw with arrow heads), blood vessels (bv), Bronchus (b). Scale bar: 50µm



Supplemental Figure 2.
Concordance of quantification of metastatic progression using metastatic area and metastatic colony count in the PuMA. Quantification of metastatic burden was undertaken in high and low metastatic pairs of human **(A and B)** and murine **(C and D**) osteosarcoma cancer cells (taken from Figure 4 A and B), using either mean normalized fluorescent area **(A** and **C)** or mean normalized colony number (**B** and **D**). Both methods of quantification revealed highly similar results. The use of colony number as a means to assess metastatic burden is complicated by coalescence of lesions often seen late during metastatic progression in PuMA. Similar difficulties with this method of quantification of metastatic burden have been described using conventional in vivo models of both experimental and spontaneous metastasis.

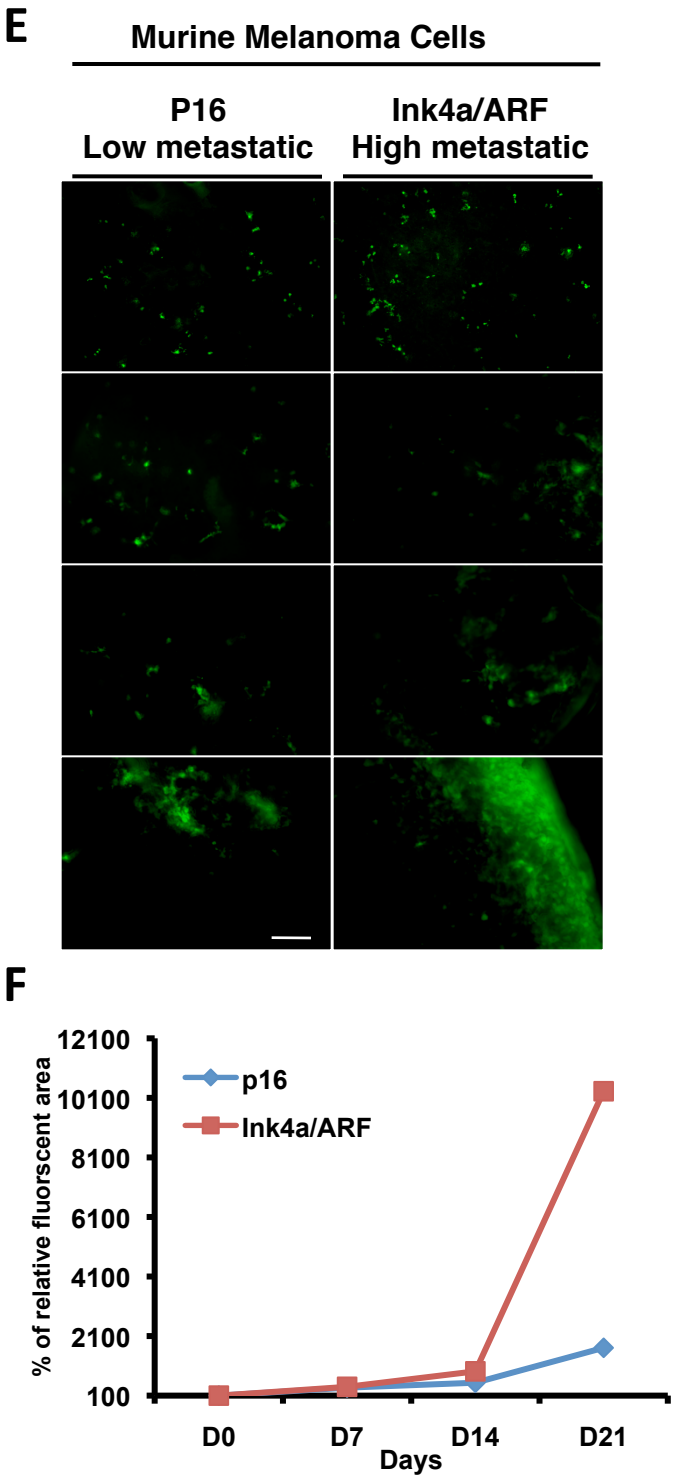


Supplemental Figure 3.
Quantitative PCR analysis of eGFP genomic DNA copy number confirms fluorescent microscopy quantification of metastatic progression in PuMA. Isolation of genomic DNA from PuMA lung sections during metastatic progression confirmed progression and expansion of metastatic (HOS-MNNG) burden in the PuMA. These data confirm assessment of metastatic progression provided by florescent imaging of metastatic events over time. Mean threshold cycle (cT) values for each sample were plotted against a standard curve in order to determine relative eGFP copy number in each sample for each time point.

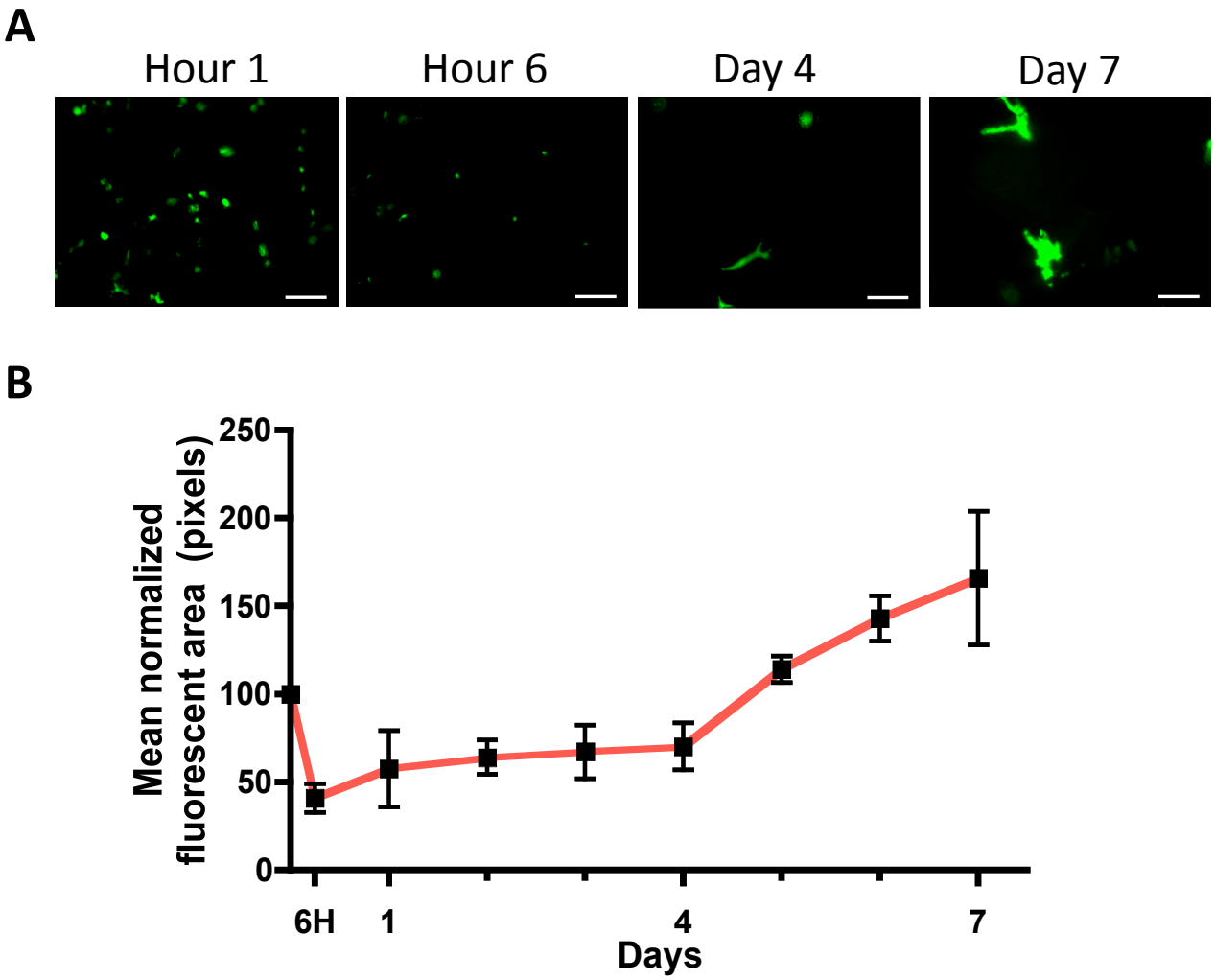


Supplemental Figure 4.

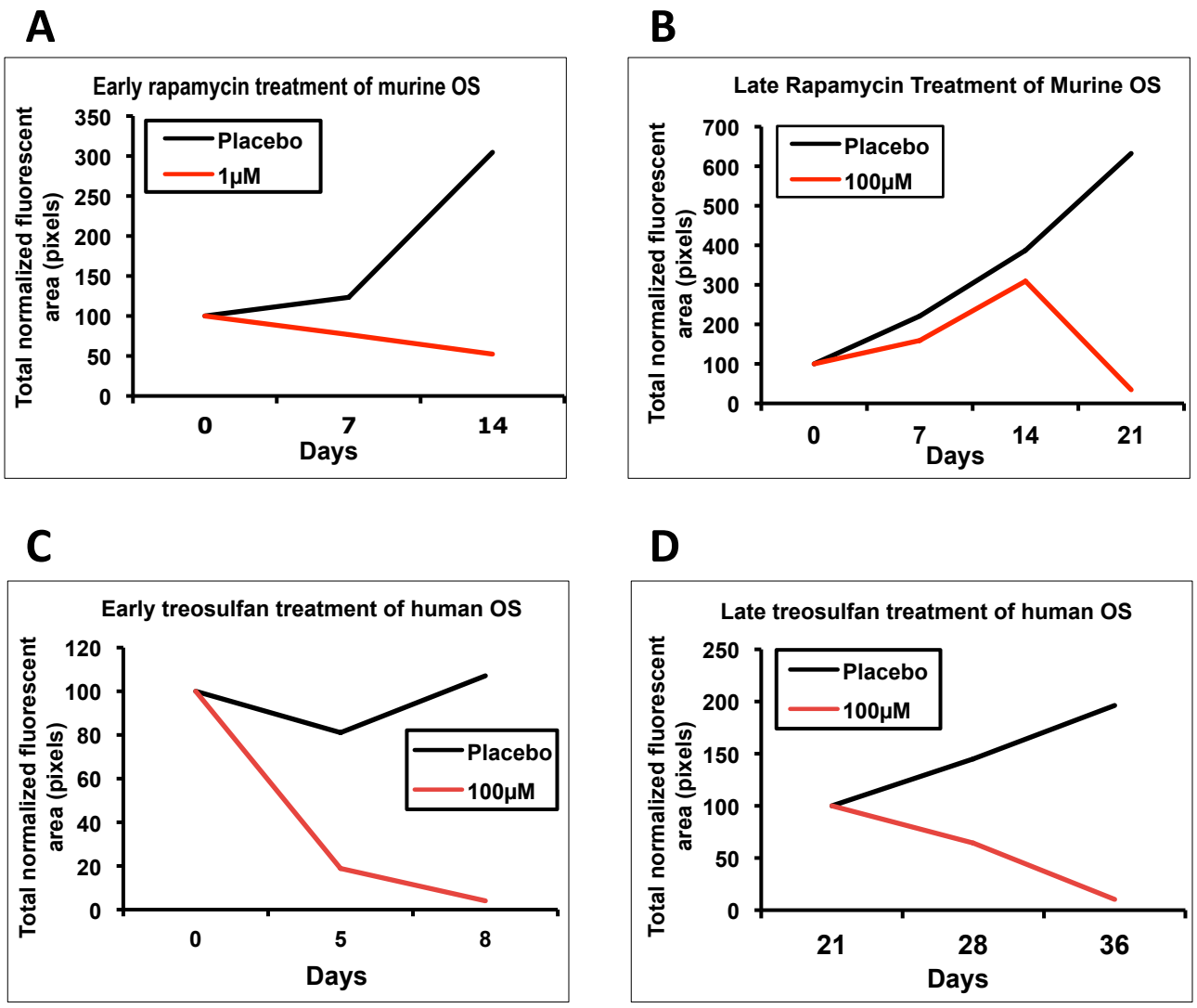
The PuMA distinguishes the phenotype of metastatic from non-metastatic cell lines. Summary in Table 1. (**A, C, E)** Serial imaging of fluorescently labeled metastatic and non-metastatic human (A; MDA-231 and MCF7) and murine (C; 4T07 and 67NR) breast cancer cells and murine melanoma cells (E; Ink4a/ ARF and P16). The 67NR murine mammary cancer cell line is consistently reported as non-metastatic; whereas the 4T07 cell line colonizes the lung but is variably reported as leading to gross metastasis. Similar numbers of cells are seen at day 0; however, a difference between metastatic and non-metastatic cells can be seen at day 7. Representative fields from lung are shown. Scale bar: 200 µm. (**B, D, F**) Quantification of metastatic burden (mean normalized fluorescent area) from **A, C and E,** respectively reveals significantly lower sum of GFP positive cells area in the lung sections following injection of low metastatic cells.



Supplemental Figure 4 (continued).



Supplemental Figure 5.
The phenomenon of metastatic inefficiency is seen in PuMA. **(A)** Serial imaging of fluorescently labeled human HOS-MNNG osteosarcoma cells following injection by tail vein. A substantial decrease in the density of metastatic cells is seen between hours 1 and 6, followed by a steady increase in size of metastatic colonies through days 4 and 7. Scale bar: 200µm. **(B)** Quantification of metastatic burden from A reveals a significant decrease in area of GFP positive tumor cells between hours 1 and 6, followed by slow growth in fluorescent area between days 1-4 and rapid growth between days 4-7.



Supplemental Figure 6.
PuMA provides an opportunity to evaluate the activity of novel cancer agents against metastatic progression and metastatic lesions. Highly metastatic tumors cells were injected by tail vein to mice. **(A)** PuMA cultures were treated continuously through early metastatic progression on day 0 through day 14 using either placebo or rapamycin at 1µM. **(B)** Metastatic progression in PuMA was allowed to advance without treatment for 7 days. Treatments of established metastatic lesions began thereafter on day 7 through 21 with placebo or rapamycin at 100µM. **(C)** PuMA cultures were treated continuously through early metastatic progression (day 0, 5 and 8) using either placebo or Treosulfan at 100µM. **(D)** Metastatic progression in PuMA was allowed to progress without treatment for 21 days. Treatments of established metastatic lesions began thereafter (day 21, 23, 25, 27) with placebo or Treosulfan at 100µM. Quantification of metastatic burden is presented as total normalized fluorescent area (pixels) captured in 5 sections of lung. A-C; Metastatic burden is normalized to day 0. D; Metastatic burden is normalized to day 21.