Supplementary Methods

Intravital imaging

Mice were anesthetised with 100mg/kg ketamine/zylaxine and 5mg/kg xylazine solution i.p. and the tail vein was cannulated. Mice were injected with 20μg of anti-F4/80 mAb (UCSF Hybridoma Core Facility) via the cannula and the skin flap prepared. Each *xy* plane was 512 × 512 pixel at 0.83μm/pixel resolution and 50 optical sections 3μm apart were imaged at 30s intervals. N-BP (AF647-RIS or OsteoSense680) or calcein was injected via the cannula 4 cycles (2 minutes) after commencement of imaging. Images were processed and analysed with Imaris software (Bitplane, CT, USA) to correct for drift and reduce background noise. Colocalisation of N-BP and calcein was determined using the ColoC function and calcein levels were determined using the spot detection function in Imaris. Time-lapse images were exported, compiled and annotated using Adobe AfterEffects. Images were analysed using the Mean ROI function in ZEN software (Carl Zeiss, Germany) to determine dynamic changes in mean pixel intensities for the full Field of View.

Histology and immunostaining

To detect AF647-RIS *in situ*, whole 4T1 tumours were removed 24 hours after *s.c.* injection of AF647-RIS and then frozen in OCT medium. The frozen blocks, or 10μm sections, were then scanned on a LiCor Odyssey scanner using the 680nm laser.

For immunohistochemical analysis of $F4/80^+$ cells in 4T1 tumours, the tumour explants were fixed in 4% paraformaldehyde in PBS, pH8 for 24 hours at 4^0C then processed to paraffin wax. 3μm sections were cut and mounted on superfrost slides (Thermo-fisher) and left overnight at 37^0C. After dewaxing and hydrating, slides were washed in PBS containing 0.05% Tween 20 (PBST) for 5 minutes. Antigen retrieval was performed using trypsin (Millenium Sciences). Slides were then washed in PBST, blocked with 10% fetal calf serum/10% normal goat serum in PBS, then incubated in 5μg/ml rat anti-mouse F4/80 (AbD Serotec) for 1 hour at room temperature, and then washed twice in PBST. Endogenous peroxidase was blocked using 3% w/v hydrogen peroxide in PBS before slides were incubated in 2.5μg/ml goat anti-rat biotinylated secondary antibody (Vector Laboratories) diluted

in PBS for 30 mins at room temperature. Slides were washed twice in PBST and localisation of the antigen/antibody complex was visualised using Vector Elite ABC/ImmPACT DAB system (Vector Laboratories). Slides were counterstained using Gills haematoxylin (Thermo-fisher) dehydrated, cleared and mounted in Eukitt (Sigma).