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**4T1 METASTASES** 



Supplemental Figure 1. Additional staining controls for IL-33 immunohistochemistry (IHC). IHC to detect IL-33 in tumour-bearing and naïve BALB/c and C57BL/6 lung tissue was performed over two consecutive days. A positive control (papain) and negative control (IL-33 KO) from the second day of staining are shown above as well as an additional sample from a 4T1 metastases (20x, scale =  $50\mu$ m, inset 100x, scale =  $10\mu$ m), highlighting prominent nuclear and cytoplasmic IL-33 staining in the lungs of 4T1 tumour-bearing mice 3 weeks after orthotopic implant.

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**Supplemental Figure 2.** ST2 is highly co-expressed with CCR5 on Tregs in the lungs of mice bearing 4T1 and 4T07 tumours. Data show n = 4-6 mice per group from two independent experiments analyzed using Student's t-test \*\*\*  $p \le 0.001$ .

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**Supplemental Figure 3.** Representative images for pEGFR staining of lungs from additional 4T1 tumor-bearing mice treated with intranasal PBS or rmIL-33. Scale bars are 1000µm (left column) and 200µm (right column). Positive control lung tissue for pEGFR staining derived from CCSP-rtTA; TetO-EGFR<sup>L858R</sup> mice; scale bars =100µm and 25µm (inset)

pEGFR positive control

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**Supplemental Figure 4.** Flow cytometry analyses of ST2 and IL-18R expression showing no expression of either receptor by tumour cell lines *in vitro*. Rat IgG2ak isotype control is shown in grey for comparison to samples stained with ST2 and IL-18R (coloured lines).

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**Supplemental Figure 5.** Determination of  $IC_{50}$  for cell lines treated with Afatinib. The viability of 4T1 and 4T07 murine mammary tumour lines and of H23 and H1975 human lung adenocarcinoma cell lines is decreased in a dose-dependent manner with increasing concentrations of the EGFR inhibitor Afatinib.  $IC_{50}$  values were determined from the curve and used to guide dose selection for clonogenic assays shown in Figure 6.

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**Supplemental Figure 6.** Purity of Tregs isolated using CD25<sup>+</sup> EasySep magnetic bead separation. The viability of isolated cells was >90%, and the Foxp3 expression was >90% as validated by flow cytometry analysis.