

SUPPLEMENTARY FIGURE AND MOVIE LEGENDS

Supplementary Figure 1. Deletion of *Ets2* in the TAMs does not affect primary tumor progression while diminishing lung metastases in the spontaneous *PyMT* breast cancer model

A- Schematic illustration of the breeding strategy to obtain *Ets2*-deficient macrophages *in vivo*. Wild-type *Ets2* contains conserved *Pointed* (gray box) and *DNA-binding* (black box) domains. Two different alleles of *Ets2* are used: a conditional *Ets2^{LoxP}* allele and a conventional *Ets2* DNA-binding-domain knock-out (*Ets2^{db}*) allele. Solid triangles represent the *LoxP* sites. **B-** PCR analysis of *Ets2* deletion in DNA extracted from purified mammary gland-derived macrophages. **C-** Analysis of tumor progression in *PyMT; Lys-Cre; Ets2^{LoxP/db}* (*E2-*, shown in triangles, *N*=27) and *PyMT; Ets2^{LoxP/db}* (*E2+*, shown in squares, *N*=24) mice. Each dot represents total tumor volume in an individual mouse. Average tumor volume in each genotype at each time point is indicated by the horizontal line. Statistical significance (*p* value evaluated by repeated measures ANOVA) is shown. **D-** Quantification of tumor burden (*top panel*) and tumor volume (*bottom panel*), of *E2-* and *E2+* mice at late-carcinoma stage. Data is presented as the average \pm SD tumor weight relative to whole body weight (*top panel*) or tumor volume (*bottom panel*). **E-** Wholemout images of hematoxylin-stained lungs obtained from *E2-* (*top*) and *E2+* (*bottom*) mice at late carcinoma stage. One representative lobe each for four different mice is shown.

Supplementary Figure 2. *Ets2* deletion in macrophages does not decrease the number of macrophages present in the primary and metastatic tumor sites

A- Quantification of number of metastatic tumors in mice with the indicated genotype. Average number of the metastases is indicated by the horizontal line. N indicates the number of mice per genetic group. Statistical significance (*p value* evaluated by non-parametric Kruskal-Wallis test) is shown. **B&C-** Micrographs of α -F4/80 immunostained primary tumor and metastatic lung tumor respectively, in *PyMT; Lys-Cre; Ets2^{LoxP/db}* (*E2-*) and *PyMT; Ets2^{LoxP/db}* (*E2+*) is shown. Quantification of F4/80 positive area in the primary tumor at the indicated stage (**B**) and lung metastases at late carcinoma stage (**C**) in *E2-* and *E2+* mice is presented as the average \pm SD percentage of cells that are positive in tumor area. **D-** Micrographs of lung sections from mice injected with the Met-1 cell line in the indicated genotypes, immunostained with α -active caspase-3. Five different lung areas from 5 different mice in each group were analyzed. Scale Bar = 100 μ m.

Supplementary Figure 3. Characterization of *c-fms-YFP* transgenic mouse as a tool to study the molecular function of *Ets2* in TAMs *in vivo*

A- Fluorescent images of an adenoma/early carcinoma (*top panel*) and metastatic lung tumor (*bottom panel*) in *PyMT; c-fms-YFP* female demonstrating YFP expression in tumor macrophages. Right panel indicates higher magnification image of the area demarcated in the box shown in the *left panel*. **B-** FACS histograms of YFP-positive cell population extracted from normal mammary gland (*left*), primary mammary tumor 4 weeks post tumor initiation (*middle*) and bronchiolar lavage (*right*) from a tumor bearing animal are shown. Data are presented as the percentage of YFP-positive cells in the total

population. **C**- Quantification of YFP and PE-conjugated α -F4/80 double positive cells. Data is presented as a dot plot of YFP and α -F4/80-PE populations. Percentage of double positive cells in the total population is indicated in red. **D**- Confirmation of genes identified by the microarray analysis using real-time qPCR. RNA was extracted from independently isolated sets of TAMs derived from mammary glands of the indicated genotype and stage of tumor development. Data is represented as average fold induction in samples analyzed in duplicates.

Supplementary Figure 4. ETS2 represses the expression of a set of anti-angiogenic genes in TAMs *in vivo*

A- Promoter scanning of the indicated genes reveals highly conserved *Ets* site (shown in box) among different species. Schematic diagram on the *top* illustrates the position of the *Ets* binding sites relative to the ATG start site. Arrows indicate the position of the primers used in qPCR of ChIP material to analyze the *Ets* binding sites. **B**- ChIP analysis of the distal *Ets*-binding sites in *Thbs1* promoter from YFP+ cells extracted from the primary tumor. ChIP was performed with α -ETS2 and α -HDAC1 antibodies and rabbit IgG control, as indicated. Subsequently, qPCR was performed on the immunoprecipitated chromatin. Data is represented as relative enrichment of the amplified chromatin.

Supplementary Figure 5. *Ets2* deficiency in TAMs de-represses the expression of Thrombospondin-1 and SPARC in metastatic lung tumors

A&B- Micrographs of lung sections from mice injected with the Met-1 cell line in the indicated genotypes, immunostained with α -Thrombospondin-1 and α -SPARC respectively. **Bottom panels in A and B** represent high magnification image of areas demarcated by the box in the respective top panels. Quantification of antibody staining is

presented as the average percentage of cells that are positive in the metastatic tumors (graph at right panel). Scale Bar = 100 μ m. Five different lung areas from 5 different mice in each group were analyzed. Statistical significance (*p-value* evaluated by unpaired Student's t-test) is shown.

Supplementary Figure 6. *Ets2* deficiency in TAMs de-represses the expression of Thrombospondin-1 and SPARC in macrophages associated at primary mammary tumors

A&B- Micrographs of mammary tumor sections from mice injected with the MVT-1 cell line in the indicated genotypes (harvested 1 week post-injection), double-immunostained with α -F4/80 (red, *left panel*) and α -Thrombospondin-1 (green, *middle panel*) and α -SPARC (green, *middle panel*) respectively. Co-distribution of F4/80-THBS1 (**A**) and F4/80-SPARC (**B**) -positive regions is indicated in the right panels. Yellow regions indicate co-localization of F4/80 and THBS2 immunostaining. Quantification of antibody staining is presented as the average percentage of F4/80 positive cells that are also positive for THBS1 (A) or SPARC (B) in the mammary tumors (graphs at right panels in A and B). Scale Bar = 20 μ m. Five different areas from 4 different mammary tumors in each group were analyzed. Statistical significance (*p-value* evaluated by unpaired Student's t-test) is shown.

Supplementary Figure 7. The *Ets2*-TAM signature predicts survival in lymph-node negative human breast cancer patients

A- Venn diagram indicating the overlap of genes between the *Ets2* mouse TAM microarray dataset and the Rosetta 98 patient dataset (distinguished by their lymphocyte/leukocyte infiltration status). 142 probesets had a significant *p-value* ($p <$

0.05). *P value* was calculated using the Fisher's Exact Test. **B**- Biological annotation based on cellular localization (*pie-chart on left*) and biological process (*pie-chart on right*) of the 133 genes differentially regulated lymphocyte/leukocyte infiltration -positive versus -negative breast cancer samples.

Supplementary Movie 1. *Ets2* deficiency in TAMs de-represses the expression of Thrombospondin-2 in TAMs at the mammary tumors

Confocal microscope movie depicting sequential set of 2 μ m optical slices of a 15 μ m mammary tumor section obtained from *E2*- mice injected with MVT-1 cells (harvested 1 week post injection). Tissue was immunolabeled with α -F4/80 (PE-conjugated) and α -THBS2 (FITC-conjugated). Yellow regions indicate co-localization of α -F4/80 and α -THBS2 immunostaining. DRAQ5 stained nuclei are seen in blue.

Supplementary Movie 2. *Ets2* deficiency in TAMs de-represses the expression of Thrombospondin-1 in TAMs at the mammary tumors

Confocal microscope movie depicting sequential set of 2 μ m optical slices of a 15 μ m mammary tumor section obtained from *E2*- mice injected with MVT-1 cells (harvested 1 week post injection). Tissue was immunolabeled with α -F4/80 (PE-conjugated) and α -THBS1 (FITC-conjugated). Yellow regions indicate co-localization of α -F4/80 and α -THBS1 immunostaining. DRAQ5 stained nuclei are seen in blue.

Supplementary Movie 3. *Ets2* deficiency in TAMs de-represses the expression of SPARC in TAMs at the mammary tumors

Confocal microscope movie depicting sequential set of 2 μ m optical slices of a 15 μ m mammary tumor section obtained from *E2*- mice injected with MVT-1 cells (harvested 1

week post injection). Tissue was immunolabeled with α -F4/80 (PE-conjugated) and α -SPARC (FITC-conjugated). Yellow regions indicate co-localization of α -F4/80 and α -SPARC immunostaining. DRAQ5 stained nuclei are seen in blue.