Supplementary Materials & Methods:

MILAN Multiplex staining and image analysis

Panel of primary antibodies used for staining consists of anti-CD3 (Abcam, cat# ab16669), anti-CD4 (Thermo-eBioscience, cat# 14-9766-82), anti-CD8 (Thermo-eBioscience, cat# 14-0808-82), anti-Foxp3 (Thermo-eBioscience, cat# 14-5773-82), anti-CD11c (Cell Signaling, cat# 97585S), anti-F4/80 (Bio-Rad, Serotec, cat# MCA497), anti-CD206 (R&D system, cat# AF2535), anti-CD11b (Abcam, cat# ab133357), anti-CD11C (Cell Signaling Technology, cat#97585). All the antibodies were validated with MILAN protocol on control tissue before performing actual staining. Immunofluorescence images were captured with Axio scan.Z1 (Zeiss, Germany) using 10X objective at a resolution of 0.65 µm/pixel. All samples were stained simultaneously. Image acquisition order was distributed spatially and independently of tumor replicates. The stainings were visually evaluated for quality by digital image experts and experienced pathologists (MDA, GM, FB, triple-blind). Multiple approaches were taken to ensure data quality. At the image level, focus, presence of external artefacts and tissue integrity were reviewed. Regions that contained severely deformed tissues and artefacts were identified and excluded from downstream analysis. Antibodies that gave low confidence staining patterns by visual evaluation were excluded from the analysis. Image analysis was performed following a custom pipeline. Briefly, flat field correction was performed using a custom implementation of a previously described algorithm [1]. Then, adjacent tiles were stitched by minimizing the Frobenius distance of the overlapping regions. Next, images from consecutive rounds were registered following an algorithm previously described [2]. During this process, the first round was always used as a fixed image, whereas all consecutive rounds were sequentially used as moving images. Transformation matrices were calculated using the DAPI channel and then applied to the rest of the channels. Registration results were visually inspected by domain experts (MDA, GM, FB). Next, tissue autofluorescence was subtracted using a baseline image with only secondary antibody. Finally, cell segmentation was applied to the DAPI channel using StarDist [3]. For every cell, topological features (X/Y coordinates), morphological features (nuclear size),

and molecular features (Mean Fluorescence Intensity (MFI) of each measured marker) were extracted.

For the cell Identification MFI values were normalized within each sample to Z-scores, as recommended in Caicedo et al. [4] . Z-scores were trimmed in the [0, 5] range to avoid a strong influence of possible outliers in downstream analyses. Single cells were mapped to known cell phenotypes using three different clustering methods: PhenoGraph [5], FlowSom [6], and KMeans as implemented in the Rphenograph, FlowSOM, and stats R packages. While FlowSom and KMeans require the number of clusters as input, PhenoGraph can be executed by defining exclusively the number of nearest neighbours to calculate the Jaccard coefficient. The number of clusters identified by PhenoGraph was then passed as an argument for FlowSom and KMeans. Clustering was performed exclusively in a subset of the identified cells (50,000) selected by stratified proportional random sampling. For each clustering method, clusters were mapped to known cell phenotypes following manual annotation from domain experts (FMB, MDA, double blind). If two or more clustering methods agreed on the assigned phenotype, the cell was annotated as such. If all three clustering methods disagreed on the assigned phenotype, the cell was annotated as “not otherwise specified” (NOS). Annotated cells were used to construct a template that was in turn used to extrapolate the cell labels to the rest of the dataset. To that end, a tSNE was built by sampling 500 cells for each identified cell type in the consensus clustering. The complete dataset was projected into the tSNE using the base predict R function. For each cell, the label of the closest 100 neighbors was evaluated in the tSNE space and the label of the most frequent cell type was assigned.

Flow cytometry panels

Panel 1: Total immune cell population was based on CD45 and endothelial was based on CD31 in the living cells on bulk tumor. Fibroblasts cells (FAP^+) and tumor cells (FAP^-) population were gated based in the tumor and stroma living cells.

Panel 2: Macrophages population was based on F4/80 and $CD11b^+$ living cells in bulk tumor. Dendritic cells were based on marker CD11c. Myeloid cells were based on the expression of CD11b marker and negative for F4/80 and CD11c.

Panel 3: the lymphoid panel is used to discriminate among T, B and NK cells. T cells phenotype was based on the expression of CD4 and CD8 in the living $CD3^+$ population. T regulatory cells (Treg) were gated on living $CD3^+$ $CD4^+$ $CD25^+$ cells. B cells were identified by the expression of CD19 and NK cells by the expression of NK1.1.

FM_0 controls were included in all experiments. Measurements were based on triplicates or quadruplicates of each condition. In the experiments using the transgenic mouse model BPA, CD45 fraction was enriched using MACS sort CD45 magnetic beads for panels 2 and 3.

The following fluorochrome-conjugated Abs, purchased from Biolegend (or otherwise indicated) as anti-mouse antibodies, were used for flow cytometry surface staining.

Panel 1: PE/Cy7-CD45(30-F11), FAP-biotinylated antibody (R&D systems), APC-Streptavidin, FITC-CD31 (390).

Panel 2: APC-F4/80 (BM8), FITC-CD11c (N418), BV650-CD11b or PE/Cy7-CD11b (M1/70).

Panel 3: PerCP-cy5.5-CD3 or FITC-CD3 (17A2), APC-CD8 (53-6.7), FITC-CD4 or Brilliant Violet 421-CD4 (GK1.5), BV421-CD25 (PC61) or PerCP-cy5.5-CD25 (3C7), BV605-CD19 (1D3) or PE/Cy5-CD19 (6D5), PE/Cy5-NK1.1 or APC/Cy7-NK1.1 (PK136). Live/dead cells were discriminated based on the Zombie Aqua Fixable Viability kit (BioLegend). To prevent unspecific binding, un-labeled anti-CD16/32 antibody was included in all staining setups.

T-Cell migration

The migration of T cells towards conditioned medium from BPA melanoma cells was measured using overnight-rested primary murine T cell isolated as described in the main text. At day 2, a total of 300.000 T cells in 200 µl of T cell medium supplemented with 3 µg/ml ConA (Sigma-Aldrich) was added to 6.5 mm transwell inserts with 5.0 µm pore size polycarbonate membranes (Corning). The T cells were placed above 500 ul of conditioned medium (with or w/o 10 ng/ml Il1α) supplemented with 3 µg/ml ConA (Sigma-Aldrich) in 24-well plates. Prior to use, conditioned medium was centrifuged at 300×g for 5 min and ConA (Sigma-Aldrich) was added to a final concentration of 3 µg/ml. As a control for maximal migration, 300.000 T cells were added directly to the wells of a 24-

well plate. The T-cells were allowed to migrate towards the conditioned medium for 26–28 h at 37˚C, 5% CO_2. Subsequently at day 3, all migrated cells were collected from the lower wells and stained with PerCP-Cy5.5-CD3, FITC-CD4, APC-CD8, BV421-CD25, and Zombie Aqua Fixable Viability Dye (BioLegend). To determine the number of migrated cells, all samples were resuspended in 100 µl of FACS Buffer and acquired on Cytek Aurora (Cytek Biosciences Inc) for 1 min on flow rate "high." Specific migration was determined by normalizing the data to the maximum control. Data were analyzed using FlowJo software. T-cells were gated on singlets, living cells, and $CD3^+$ cells. T-cell phenotype was based on the expression of CD4 and CD8 in the living $CD3^+$ population. Treg were gated on living $CD3^+$ $CD4^+$ $CD25^+$ cells. FM_0 controls were included in all experiments.

Antibodies for protein expression analysis

Antibodies used were: anti-Actin (Novus Biologicals, CO, USA cat# NB600-501), anti-Ambra1 (Merck-Millipore, MA, USA cat# ABC131), anti-AMBRA1 (Santa Cruz Biotechnology, TX, USA cat# sc-398204), anti-c-myc (Santa Cruz Biotechnology, TX, USA cat# sc-40), anti-Cyclin D1 (Abcam, UK cat# ab16663), anti-FAK1 (Cell Signaling, MA, USA cat# 13009S), anti-Lamin A/C (Santa Cruz Biotechnology, TX, USA cat# sc-6215), anti-LC3 (Cell Signaling, MA, USA cat# 3868S; Novus Biologicals, CO, USA cat# NB100-2220SS), anti-LDH (Santa Cruz Biotechnology, TX, USA cat# sc-33781), anti-N-Cadherin (Cell Signaling, MA, USA cat# 13116S), anti-pFak-Y397 (Cell Signaling, MA, USA cat# 8556S), anti-pSRC-Y416 (Cell Signaling, MA, USA cat# 6943S), anti-SRC (Cell Signaling, MA, USA cat# 2123S), anti-Tubulin (Cell Signaling, MA, USA cat# 2128S). Images were taken using a ChemiDoc™ MP System (Bio-Rad Laboratories, CA, USA; cat# 1708-280) provided with the Image Lab 6.0.1 Software (Bio-Rad Laboratories, CA, USA). Densitometry analyses were performed using ImageJ version 1.52.n (University of Wisconsin, WI, USA), as described in the main text.

Supplementary references

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5. Levine JH*, et al.* Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. *Cell* **162**, 184-197 (2015).

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