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Supplemental Information

Transcriptional Signature Derived from Murine Tumor-Associated Macrophages Correlates with Poor Outcome in Breast Cancer Patients

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Fig. S1 - Related to Fig. 1

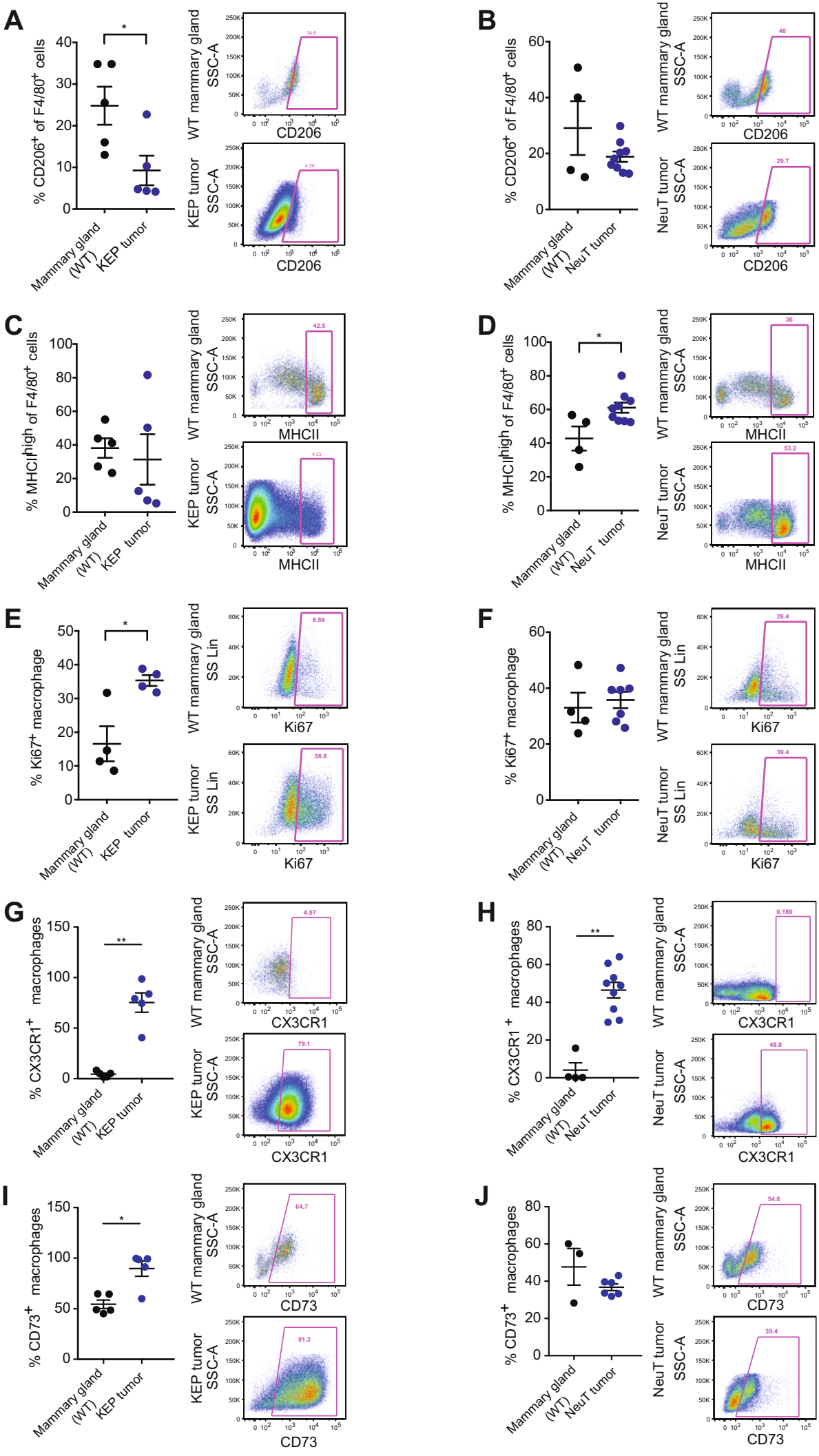


Fig. S1 - Related to Fig. 1. TAMs in *K14cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mammary tumors express low levels of CD206 and have a proliferative phenotype compared to TAMs from the *MMTV-NeuT* model. (**A-B**) Percentage of CD206^{+} macrophages in KEP tumors and WT FVB mammary glands (**A**) or NeuT tumors and WT Balb/c mammary glands (**B**) as determined by flow cytometry. The gating was based on fluorescence-minus-one sample for CD206. (**C-D**) Percentage of MHC-II^{+} macrophages in KEP tumors and WT FVB mammary glands (**C**) or NeuT tumors and WT Balb/c mammary glands (**D**) as determined by flow cytometry. (**E-F**) Percentage of Ki67^{+} macrophages in KEP tumors and WT FVB mammary glands (**E**) or in NeuT mammary tumor and WT Balb/c mammary glands (**F**) as determined by flow cytometry. Dot plots were gated on CD11b^{+}F4/80^{+} macrophages. (**G-H**) Percentage of CX$_3$CR1^{+} macrophages in KEP tumors and WT FVB mammary glands (**G**) or in NeuT mammary tumor and WT Balb/c mammary glands (**H**) as determined by flow cytometry. Dot plots were gated on CD11b^{+}F4/80^{+} macrophages. (**I-J**) Percentage of CD73^{+} macrophages in KEP tumors and WT FVB mammary glands (**I**) or in NeuT mammary tumor and WT Balb/c mammary glands (**J**) as determined by flow cytometry. Dot plots were gated on CD11b^{+}F4/80^{+} macrophages. Representative dot plots are shown in all panels. Data are mean values ± SEM from n=3 animals per group and were analyzed with Mann-Whitney test, *$p < 0.05$.

Fig. S2 – Related to Fig. 1

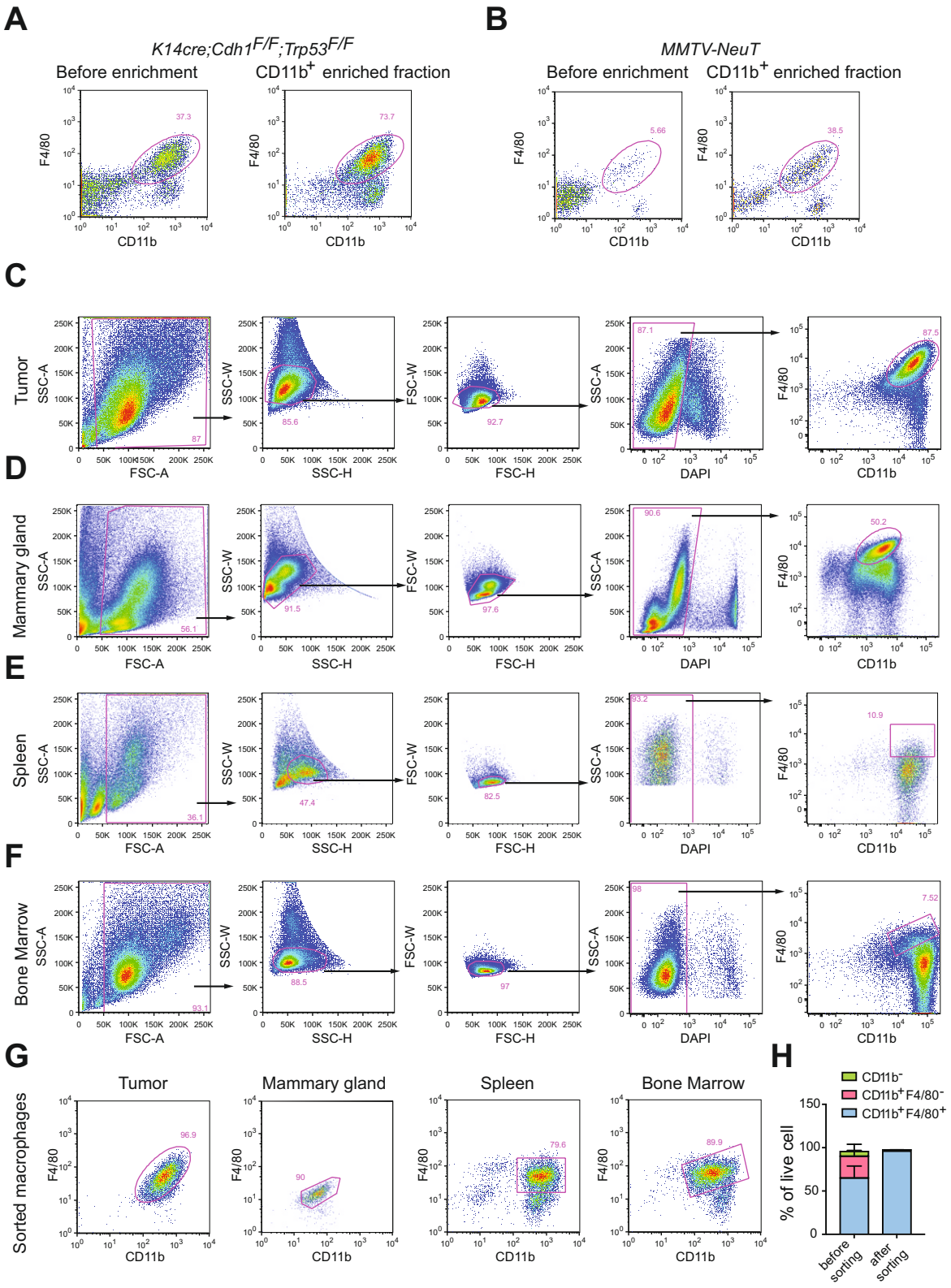


Fig. S2 - Related to Fig. 1. Isolation procedure of $CD11b^+F4/80^+$ macrophages from mammary tumor, mammary gland, spleen and bone marrow by magnetic and fluorescence-activated cell sorting. (**A-B**) Representative dot plots of KEP (**A**) and NeuT (**B**) mammary tumors showing the $CD11b^+F4/80^+$ population before and after pre-enrichment by magnetic-activated cell sorting for $CD11b^+$ cells. (**C-F**) Representative dot plots illustrating the gating strategy for the isolation of $CD11b^+F4/80^+$ macrophages from KEP tumors (**C**), mammary gland (**D**), spleen (**E**) and bone marrow (**F**) by fluorescence-activated cell sorting after enrichment of $CD11b^+$ cells. (**G**) Dot plots showing the purity of the sorted macrophages in tumor, mammary gland, spleen and bone marrow of KEP mice. (**H**) Stacked bar plots showing the composition of KEP tumors in $CD11b^-$, $CD11b^+F4/80^-$ and $CD11b^+F4/80^+$ cells before and after macrophage sorting. Data are mean values ± SEM from 3 preps.

Fig. S3 – Related to Fig. 2

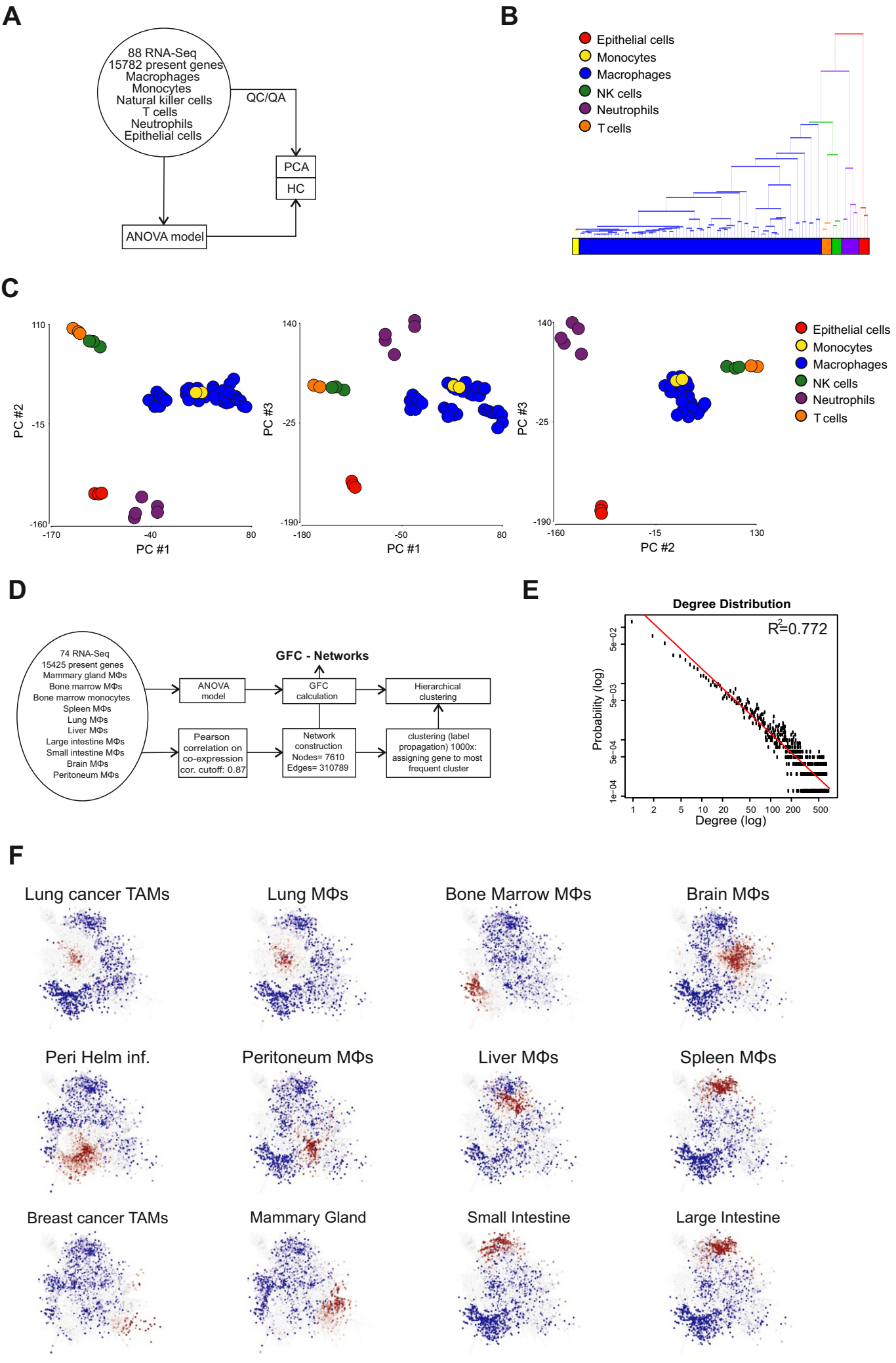
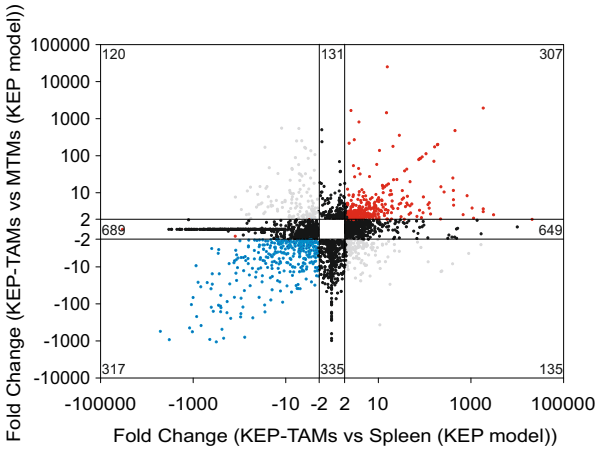


Fig. S3 - Related to Fig. 2. Murine tissue macrophages are distinguished from other immune cell populations and epithelial cells by their transcriptome. (**A**) Schematic representation outlining bioinformatics workflow. (**B**) HC based on the1,000 genes with the highest variance within the dataset. Macrophage transcriptomes derived from different organs and disease states were collectively named macrophages. (**C**) PCA using all present genes plotted in two-dimensional graphs. (**D**) Schematic representation of the bioinformatics workflow for Co-expression network analysis (CNA) of all present genes by CoCena2. (**E**) CoCena2 logged network degree distribution with linear fitting. (**F**) Networks were colored according to GFCs for each condition, respectively.

Fig. S4 – Related to Fig. 3

A



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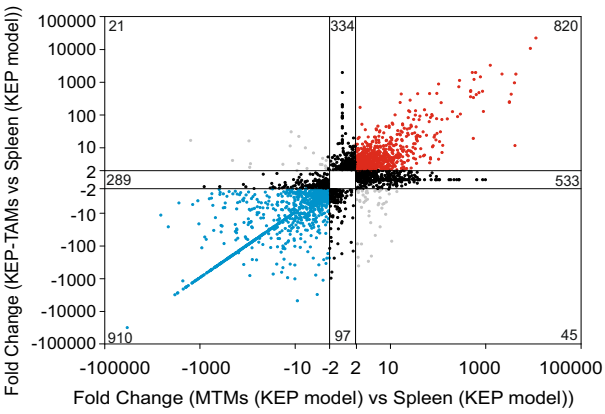

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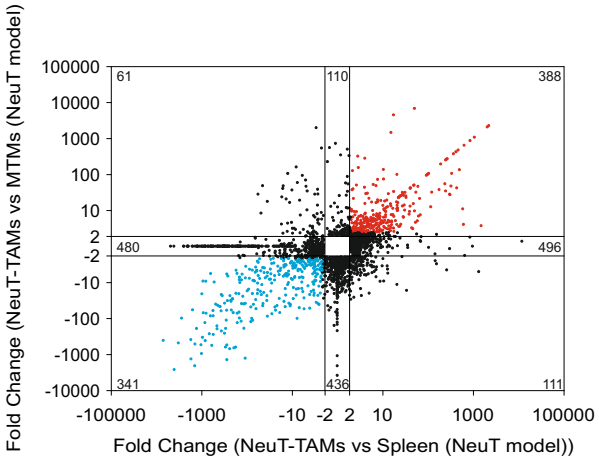

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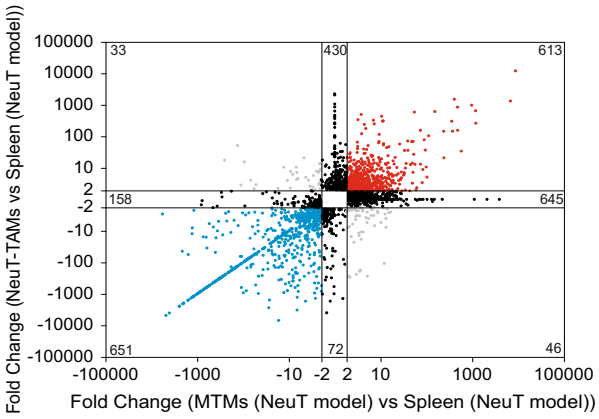

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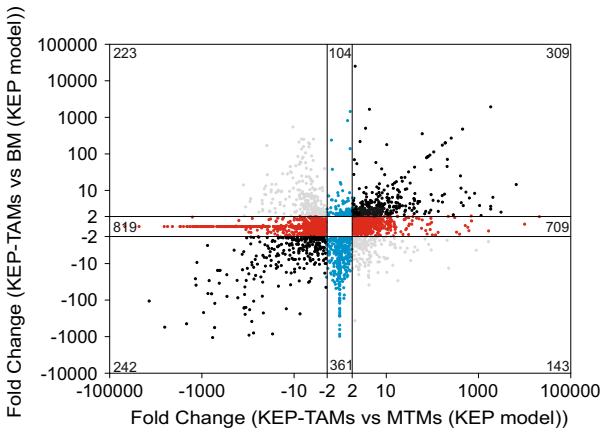
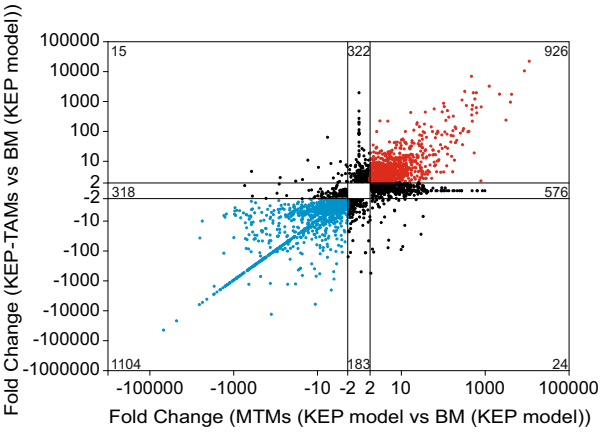
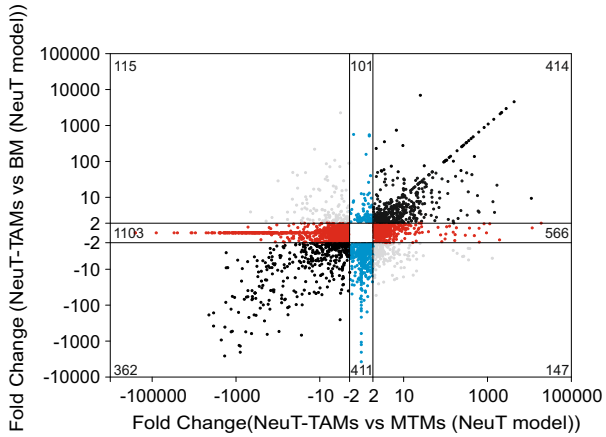


F



G



H

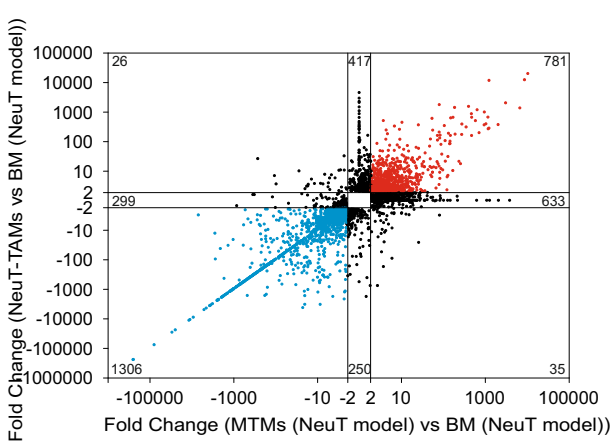


Fig. S4 - Related to Fig. 3. Inter-tissue comparison leads to false interpretation of changes in TAMs. FC/FC plot of the union of DE genes showing the fold change in expression of genes in (**A**) KEP-TAMs compared to MTMs (KEP model) (y-axis) against KEP-TAMs compared to splenic macrophages (KEP model) (x-axis), (**B**) KEP-TAMs compared to splenic macrophages (KEP model) (y-axis) against MTMs (KEP model) compared to splenic macrophages (KEP model) (x-axis), (**C**) NeuT-TAMs compared to MTMs (NeuT model) (y-axis) against NeuT-TAMs compared to splenic macrophages (NeuT model) (x-axis), (**D**) NeuT-TAMs compared to splenic macrophages (NeuT model) (y-axis) against MTMs (NeuT model) compared to splenic macrophages (NeuT model) (x-axis), (**E**) KEP-TAMs compared to bone marrow macrophages (KEP model) (y-axis) against KEP-TAMs compared to MTMs (KEP model) (x-axis), (**F**) KEP-TAMs compared to bone marrow macrophages (KEP model) (y-axis) against MTMs (KEP model) compared to bone marrow macrophages (KEP model) (x-axis), (**G**) NeuT-TAMs compared to bone marrow macrophages (NeuT model) (y-axis) against NeuT-TAMs compared to MTMs (NeuT model) (x-axis) and (**H**) NeuT-TAMs compared to bone marrow macrophages (NeuT model) (y-axis) against MTMs (NeuT model) compared to bone marrow macrophages (NeuT model) (x-axis). Each dot represents one gene where red and blue dots indicate positive or negative fold change differences in both comparisons and grey dots correspond to opposite fold change differences across the axes.

Fig. S5 – Related to Fig. 4 and Fig. 5

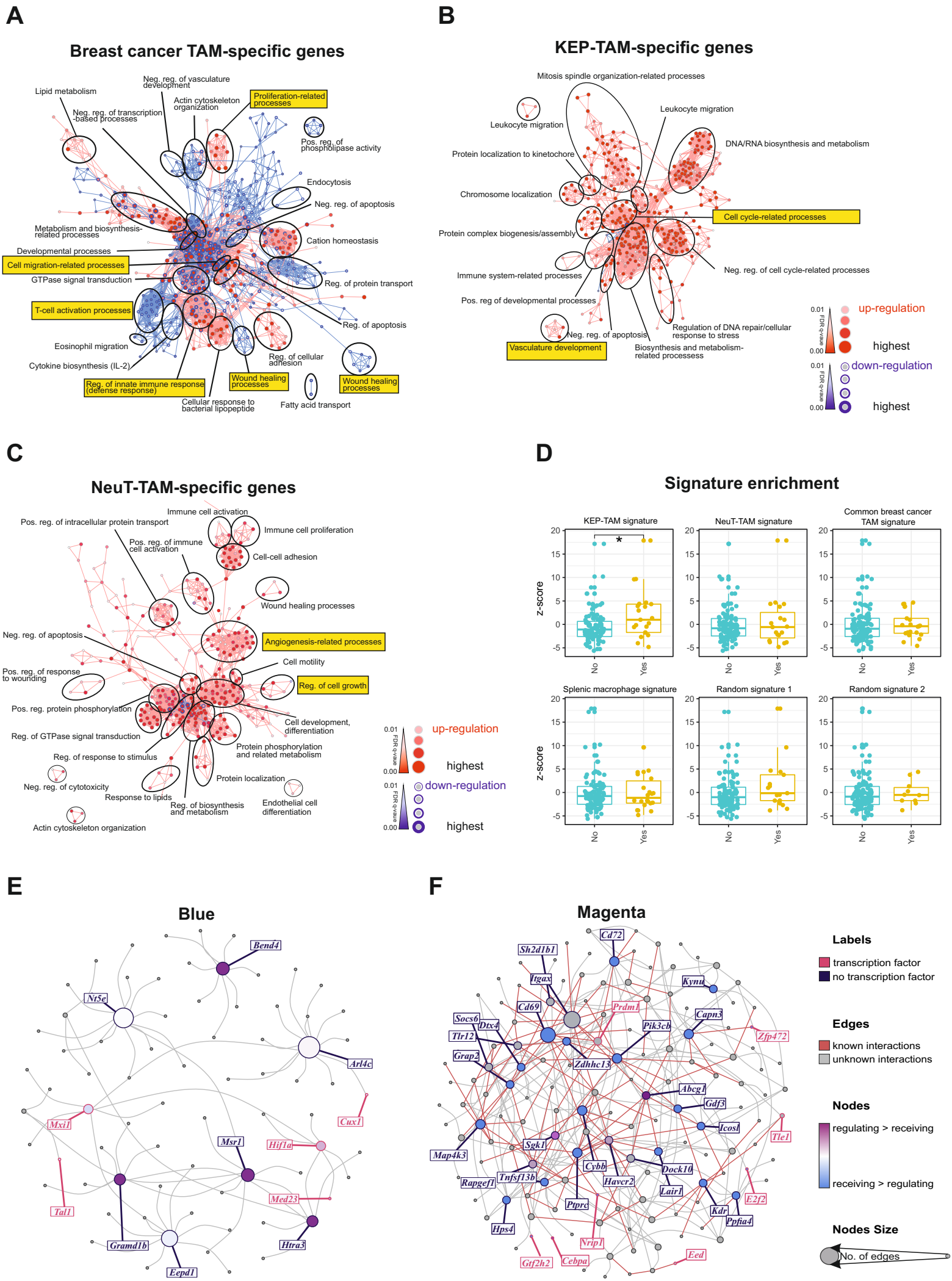
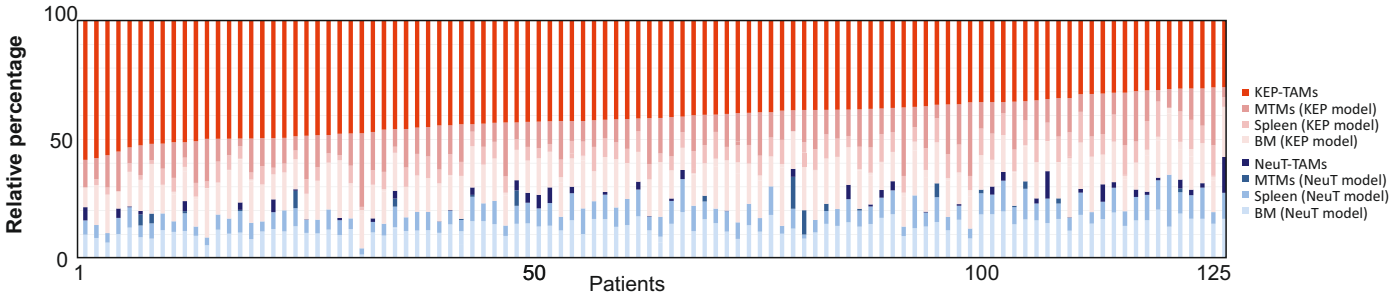


Fig. S5 - Related to Fig. 4 and Fig.5. *In silico* gene ontology enrichment and regulatory circuit analysis of TAM-specific gene modules. (**A-C**) Network visualization of GOEA using BiNGO and EnrichmentMap based on model-specific DE genes derived from (**A**) common breast cancer TAM-, (**B**) KEP-TAM- and (**C**) NeuT-TAM-specific genes. Node size and color (positively enriched GO terms) and node border width (negatively enriched GO terms) represent corresponding FDR-adjusted enrichment p-values (p-value: ≤0.05). Enriched gene ontology terms mentioned in the text are highlighted in yellow boxes. (**D**) ILC patients from the TCGA database enriched in the KEP-TAM gene signature exhibit higher enrichment in human breast cancer cell proliferation signatures. ILC specimens having a significant enrichment for the macrophage signatures are visualized in gold boxplots, whereas non-enriched specimens are marked in turquoise boxplots. Data were analyzed with t-test, *p < 0.05. (**E-F**) I-GIN construction for KEP-TAM-specific (blue cluster) (**E**) and NeuT-specific (magenta cluster) (**F**) genes. Node coloring represents regulatory or receiving status obtained from a Bayesian approach and points out highly co-expressed genes and their known or proposed links to strongest correlated neighbors. Candidate genes mentioned in the text are highlighted in yellow boxes.

Fig. S6 – Related to Fig. 6

A



		TCGA overall		METABRIC overall		METABRIC DSS	
		ILC	TNBC	ILC	TNBC	ILC	TNBC
B	KEP	*Fig. 6E*	p-value KM = 0.25	p-value KM = 0.048	p-value KM = 0.37	p-value KM = 0.066	p-value KM = 0.53
C	NeuT	*Fig. 6F*	p-value KM = 0.76	p-value KM = 0.34	p-value KM = 0.23	p-value KM = 0.34	p-value KM = 0.28
D	vsSpleen	p-value KM = 0.96	p-value KM = 0.24	p-value KM = 0.8	p-value KM = 0.43	p-value KM = 0.73	p-value KM = 0.68
E	Random1	*Fig. 6G*	p-value KM = 0.53	p-value KM = 0.14	p-value KM = 0.35	p-value KM = 0.54	p-value KM = 0.38
F	Random2	p-value KM = 0.4	p-value KM = 0.27	p-value KM = 0.49	p-value KM = 0.55	p-value KM = 0.42	p-value KM = 0.29
G	Abundance	*Fig. 6H*		p-value KM = 0.11		p-value KM = 0.16	

Fig. S6 - Related to Fig. 6. Validation of Kaplan-Meier survival analysis of breast cancer model-specific signatures. (**A**) KEP-TAM, NeuT-TAM, splenic and bone marrow macrophage and MTM signatures from both models were computed using CIBERSORT and their relative abundance in the ILC specimens was calculated. (**B-G**) Kaplan-Meier survival curves of overall TCGA (left, n=125), overall METABRIC (n=146) and disease-specific METABRIC (right, n=146) ILC (left subpanel) or TNBC (right subpanel) patients. Specimens having a significant enrichment for (**B**) KEP-TAM, (**C**) NeuT-TAM, (**D**) splenic macrophage, (**E-F**) random or (**G**) macrophage abundance signatures are visualized in red lines and numbers. The rest patients are visualized in green lines and numbers. Z score above 2 (p-value: ≤0.05) marks significant enrichment of gene signatures.