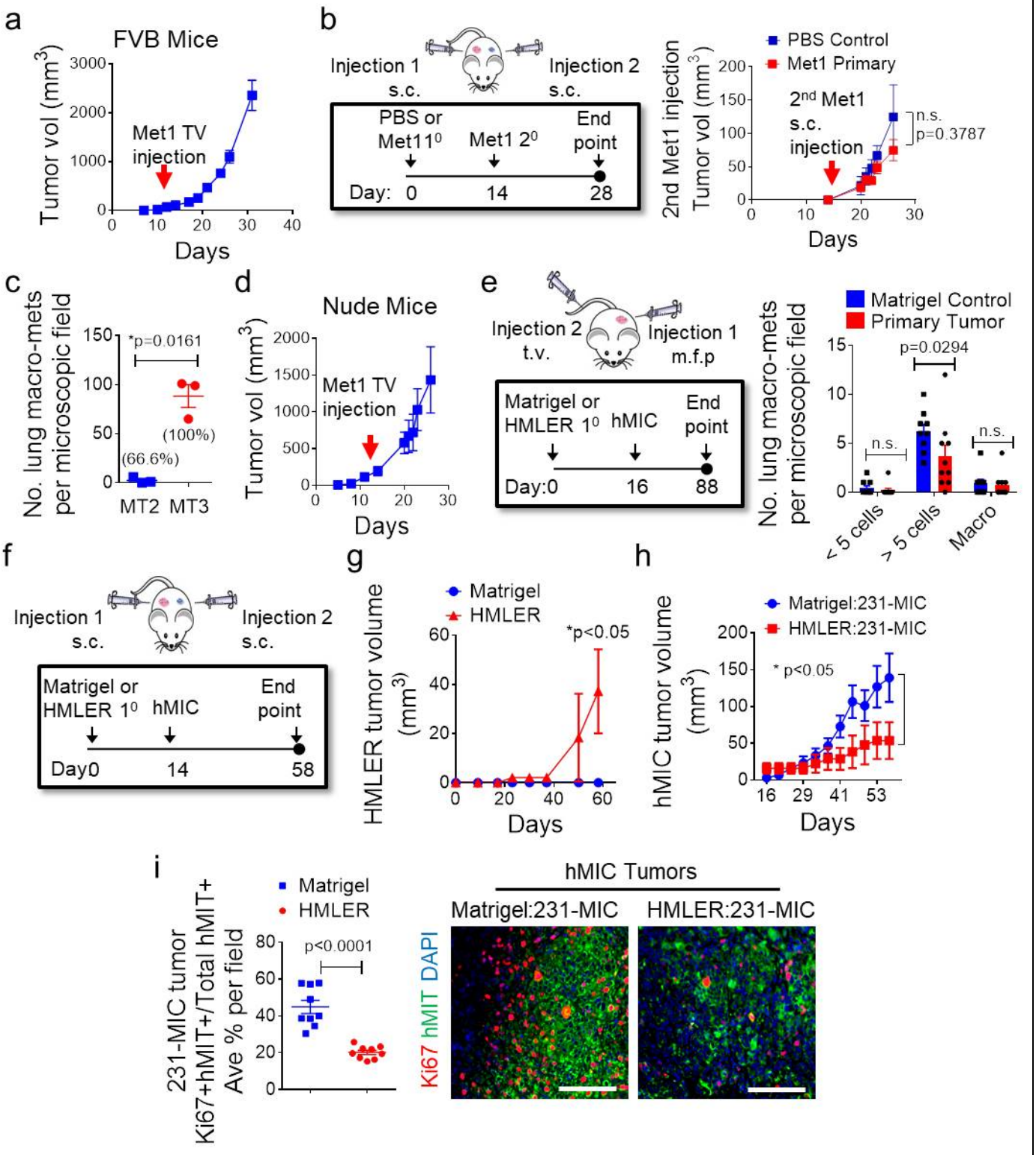
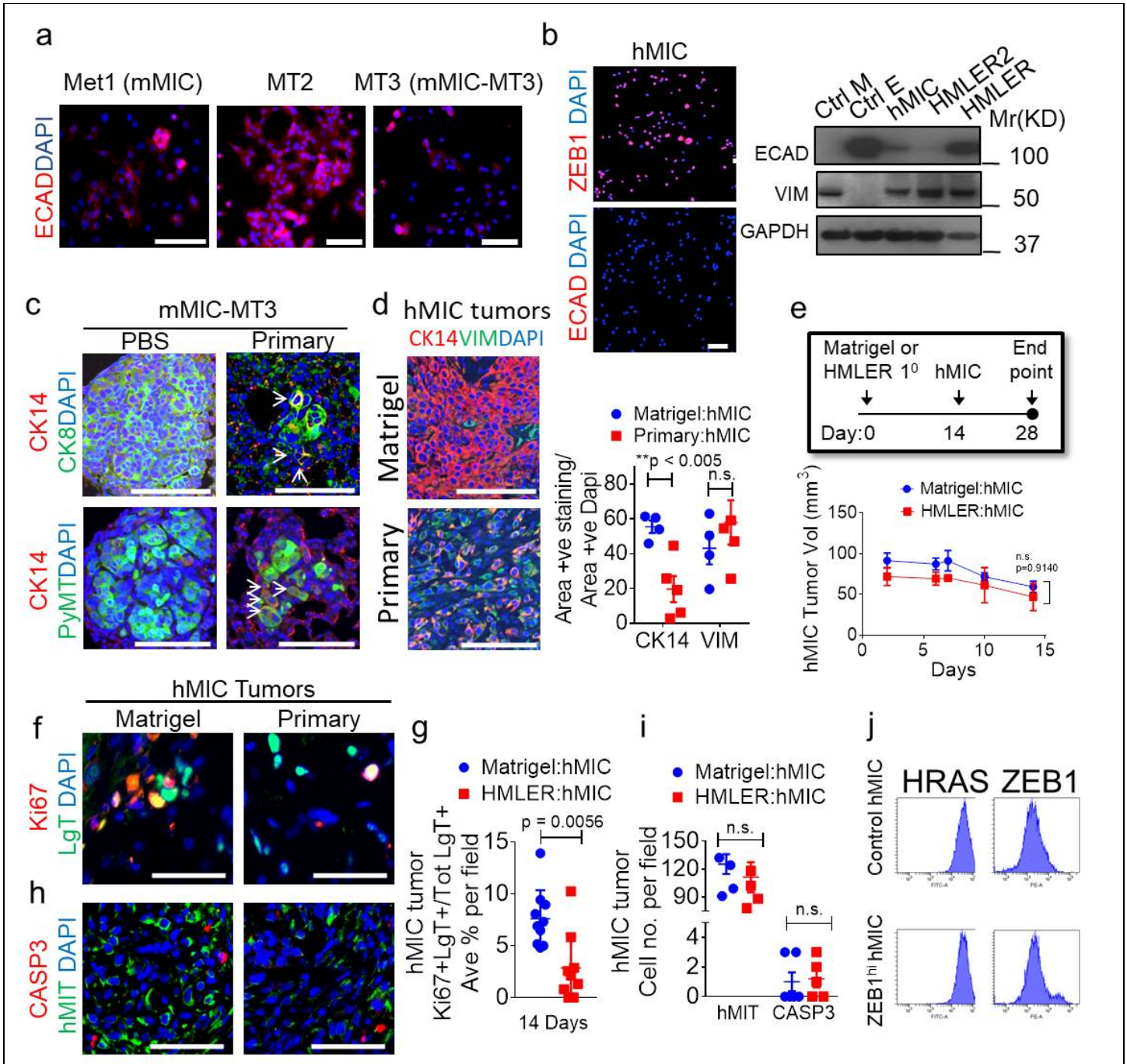


Supplementary Figure 1

Primary tumors inhibit outgrowth of distant metastasis-initiating cells

a, Met1 orthotopic primary tumor growth kinetics, FVB mice, represented in Fig. 1b (n=10). **b,** Schematic of sequential subcutaneous experimental model (left) and growth kinetics (right) of Met1 secondary tumors (2.5 x 10^5/mouse; implanted at day 14) in mice with PBS control or Met1 primary tumor (2.5 x 10^5/mouse; n=5 animals/cohort). **c,** Numbers of pulmonary macrometastases following tail vein injection of MT2 or MT3 cells (7.5x10^5cells/mouse; n=3). Macrometastases >100 microns were quantified on microscope tissue sections from 4 lung lobes per mouse. **d,** Met1 orthotopic tumor growth kinetics, Nude mice, represented in Fig. 1f (n=5). **e,** Schematic of experimental model (left), and number of pulmonary macrometastases following tail vein injection of 2.5x10^5 hMICs into Nude mice with either Matrigel (n = 10) or HMLER primary tumors (n=9; original injection of 5.0x10^5 cells/mouse) (right). Macrometastases (>100 microns) or micrometastases (>5 cells or <5 cells) were quantified from microscopic whole lung tissue sections. **f**, Schematic of experimental model (applies to g and h). **g,** Growth kinetics of HMLER primary tumors, Nude mice, described in Figure 1h (n=10). **h,** MIC-231 tumor growth kinetics, Nude mice, opposite Matrigel control (n=12) or HMLER primary tumors (n=5). Representative of 2 experiments. **i,** Images: representative immunofluorescent images of 231-MIC tumors grown opposite Matrigel control or an HMLER primary tumor (represented in Supplementary Fig. 1h) stained with Ki67 (red), hMIT to identify human mitochondria (green), DAPI (nuclei, blue); Scale bars=100 μm. Graph: Quantification of Ki67+hMit+ cells as a percentage of the total number of hMit+ tumor cells/microscopic field (n=9 images; 3 tumors/cohort). Source data for **a, b, c, d, e, g, h, i** in Supplementary Table 1. 2-way ANOVA, followed by Sidak's multiple comparison test **(b, g, h)**; 1-sided Welch's t test (**e**); 2-sided Welch's t test (**c, i**).

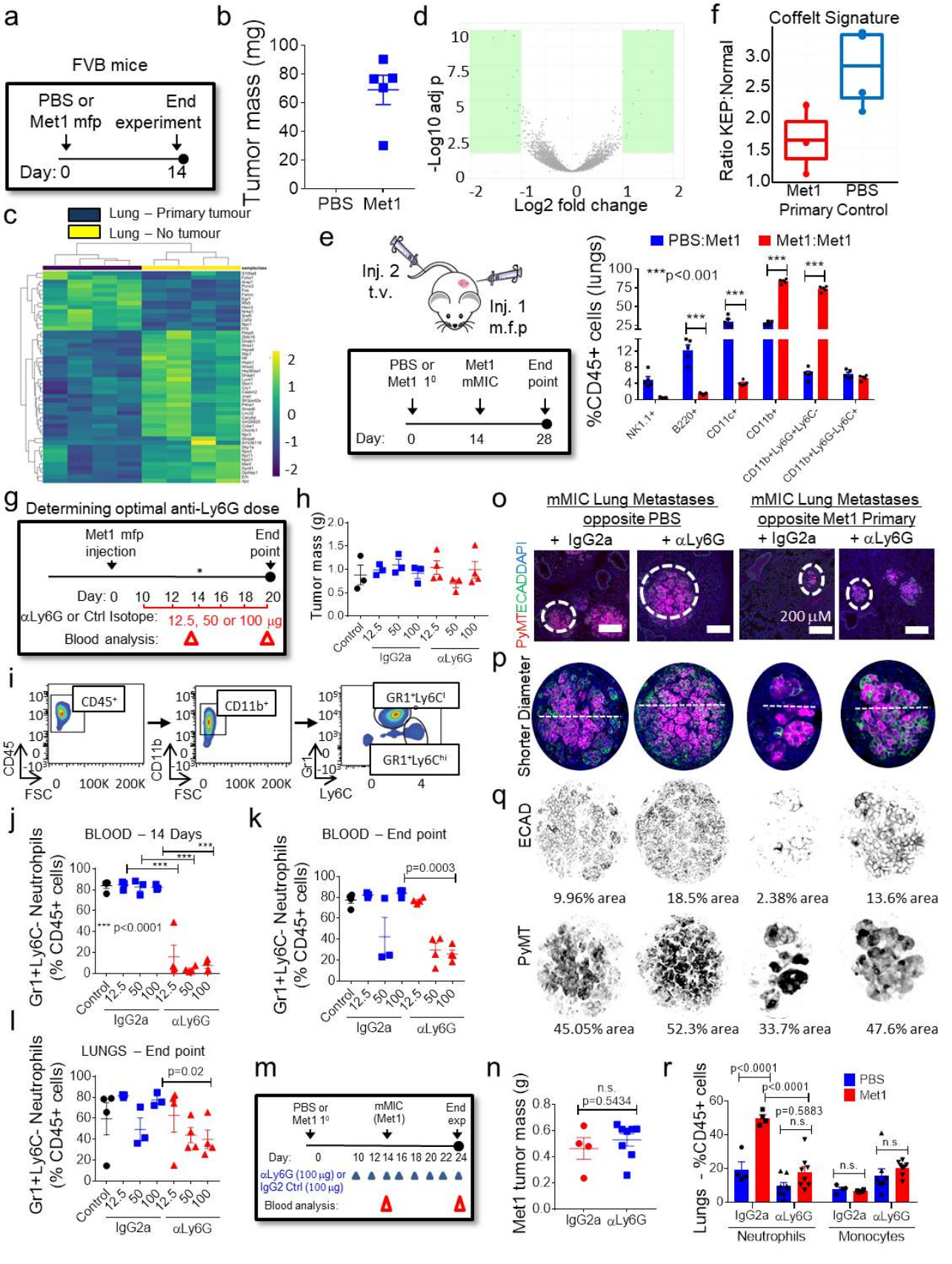


Supplementary Figure 2

MIC Differentiation is Perturbed by the Presence of a Primary Tumor

a, In vitro immunocytochemical flourescence showing E-cadherin (ECAD, red) and DAPI (nuclei, blue) in Met1 parental cell line (mMIC) and Met1-derived clones, MT2 and MT3 (mMIC-MT3). **b,** Images: Immunofluorescence showing ZEB1 and ECAD expression in cultured hMICs prior to xenotransplantation. Western blot: mesenchymal marker Vimentin (VIM) and epithelial marker ECAD protein in polyclonal HMLER cells and derivative hMIC and HMLER2 cells. GADPH shown as internal control. Positive controls: Ctrl E (epithelial-MCF7Ras); Ctrl M (mesenchymal CD44^{hi} HMLER cells). **c,** Merged immunofluorescent images of mMIC-MT3 tumors (described in Fig. 1d) stained for basal cytokeratin 14 (CK14, red), luminal CK8 (green) or PyMT antigen (expressed by tumor cells only-green). Arrows - CK14+ tumor cells. **d**, Images: hMIC tumors (from Fig 1i) stained with CK14 (red), VIM (green) and DAPI (blue); Graph: quantification of indicated

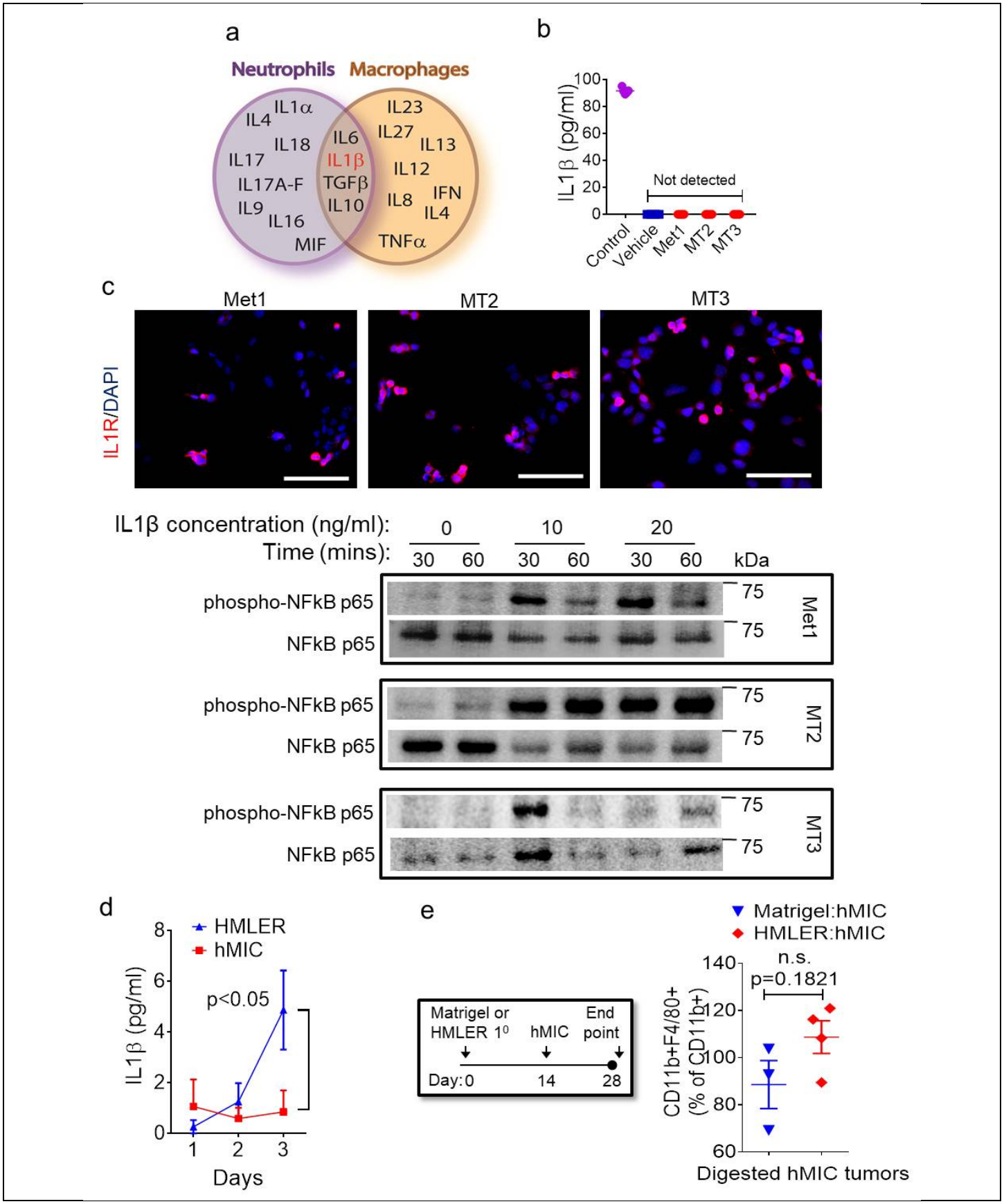
stains on hMIC tumors grown opposite Matrigel (n=4) or primary tumor (n=5). **e,** Schematic: modeling early stages of hMIC colonization. Graph: hMIC tumor growth kinetics opposite Matrigel control or HMLER primary tumor (n=4/group); differences not statistically significant. **f, g,** Immunofluorescent images (**f**) and quantification (**g**) of hMIC tumors stained for ki67 (red), LgT antigen (tumor cells, green), and DAPI (nuclei, blue) as a percentage of total LgT+ cells. Control, n=10 images, 4 tumors; HMLER cohort, n=9 images, 4 tumors. **h, i,** Immunofluorescent images (**h**) and quantification (**i**) of staining hMIC tumors for cleaved caspase3 (CASP3, red), human-specific mitochondria (hMIT, green), and DAPI (nuclei, blue) grown in mice with Matrigel control (n=6 images, 4 tumors) or HMLER primary tumors (n=5 images, 4 tumors). **j,** Expression of ZEB1 (ZEB1-GFP construct) or HRAS (HRAS-tomato construct) analyzed by FACS ($1.0x10^5$ cells) in Control hMIC or $ZEB1^{hi}$ hMIC (from Fig. 2n-p). All scale bars=100 μm. Source data for **d, e, g, i** in Supplementary Table 1 and d on Supplementary Figure 9. 2-way ANOVA (**e**); 2-sided Welch's t test (**d, i**); 2-sided Mann-Whitney test (**g**).


a
FVB mice
PBS or Met1 mfp
End experiment
Day: 0
14
b
Tumor mass (mg)
PBS
Met1
d
-Log10 adj p
Log2 fold change
f
Coffelt Signature
Ratio KEP:Normal
Met1
PBS
Primary Control
c
Lung – Primary tumour
Lung – No tumour
e
Inj. 2 t.v.
Inj. 1 m.f.p
PBS or Met1 1⁰
Met1 mMIC
End point
Day: 0 14 28
PBS:Met1
Met1:Met1
%CD45+ cells (lungs)
***p<0.001
NK1.1+
B220+
CD11c+
CD11b+
CD11b+Ly6G+Ly6C-
CD11b+Ly6G-Ly6C+
g
Determining optimal anti-Ly6G dose
Met1 mfp injection
End point
Day: 0 10 12 14 16 18 20
αLy6G or Ctrl Isotope:
12.5, 50 or 100 μg
Blood analysis:
h
Tumor mass (g)
Control
IgG2a
αLy6G
o
mMIC Lung Metastases opposite PBS
mMIC Lung Metastases opposite Met1 Primary
+ IgG2a
+ αLy6G
PyMT ECAD DAPI
200 μM
i
CD45+
CD11b+
GR1+Ly6Cl
GR1+Ly6Chi
CD45
CD11b
Gr1
Ly6C
FSC
p
Shorter Diameter
q
ECAD
PyMT
9.96% area
18.5% area
2.38% area
13.6% area
45.05% area
52.3% area
33.7% area
47.6% area
j
BLOOD – 14 Days
Gr1+Ly6C- Neutrophils (% CD45+ cells)
*** p<0.0001
k
BLOOD – End point
p=0.0003
l
LUNGS – End point
p=0.02
m
PBS or Met1 1⁰
mMIC (Met1)
End exp
Day: 0 10 12 14 16 18 20 22 24
αLy6G (100 μg) or IgG2 Ctrl (100 μg)
Blood analysis:
n
Met1 tumor mass (g)
n.s.
p=0.5434
r
Lungs - %CD45+ cells
p<0.0001
p<0.0001
p=0.5883
n.s.
n.s.
n.s.
n.s.
PBS
Met1
Neutrophils
Monocytes

Supplementary Figure 3

Innate Inflammatory Cells are Necessary for MIC Colonization

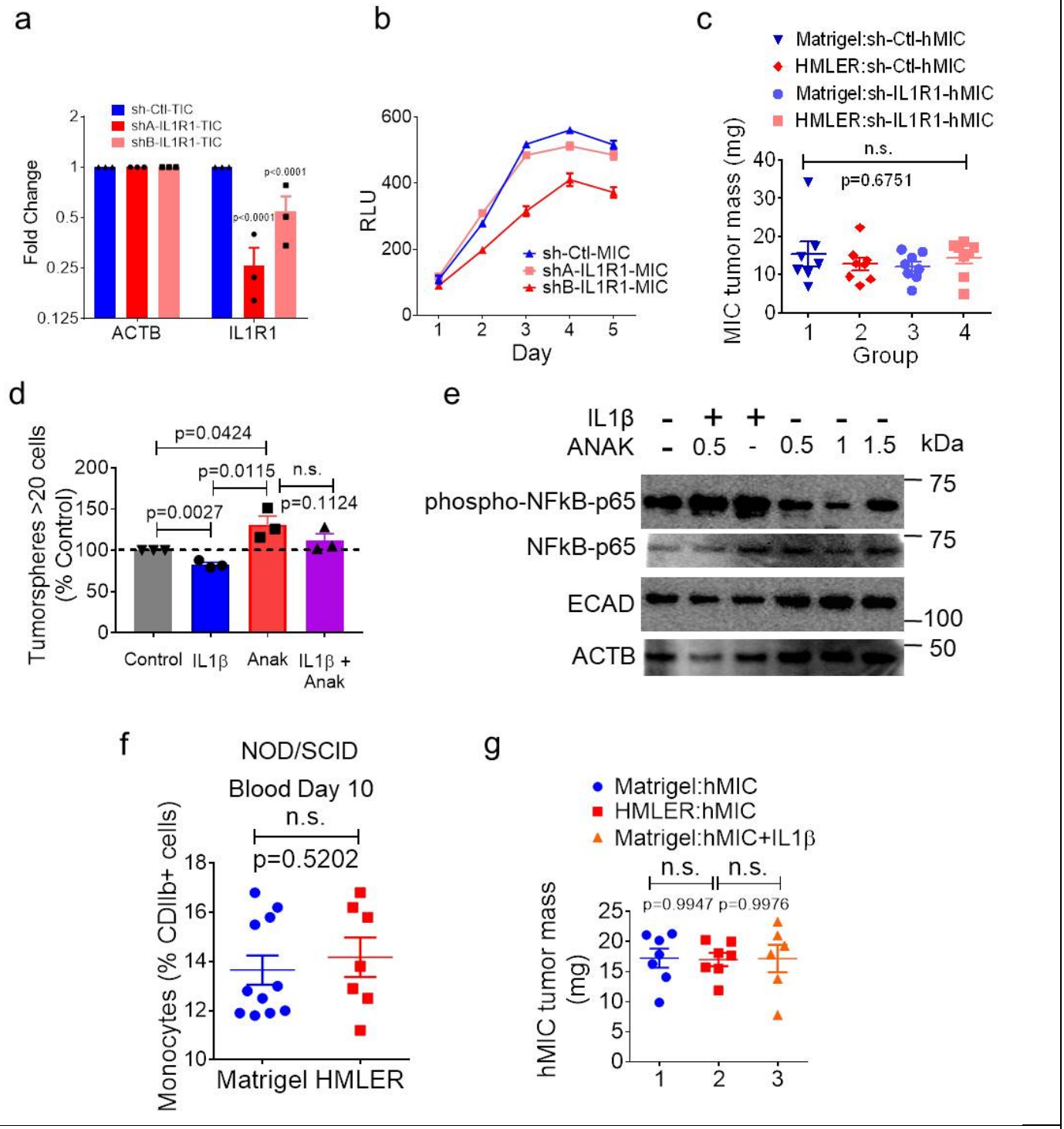
a, Experimental schematic for tissues used for RNA-seq analysis (Fig. 3a-c and Supplementary Fig. 3b-e). **b,** Met1 primary tumor mass in FVB mice (n=5). **c, d,** RNA-seq analysis on lungs from mice with PBS control (n=4) or a Met1 primary tumor (n=4). Heatmap (**c**): top 50 differentially expressed genes (by adjusted p-value, DESeq2). Blue=low, green=mean, and yellow=high relative expression levels. PBS control lungs (yellow), Met1 primary tumor-bearing lungs (purple). Volcano plot (**d**): DESeq2 comparison Single gene with Padj<0.05 and absolute log2(FoldChange)>1 (green). **e**, Experimental schematic and flow cytometric quantification of immune cell populations in lungs of indicated FVB mice at 28-day end point (see Fig. 1a). **f,** Ratio of genes expressed by pro-metastatic immunosuppressive neutrophils from *K14cre;Cdh1F/F;Trp53F/F* (KEP) mice to control neutrophils from wild type littermates (KEP:Normal)[13] extrapolated onto our signatures from control (blue) primary tumor-bearing lungs (red). Higher ratios indicate higher pro-metastatic KEP signature. Box plot is median, 25th and 75th percentiles; whiskers extend to minimum and maximum values. **g,** Experimental design to identify optimal anti-Ly6G dose for neutrophil depletion. **h**, Primary tumor mass in Control anti-IgG2a (n=3 mice/cohort) and anti-Ly6G (n=4 mice/cohort). **i,** Flow cytometric gating strategy for neutrophils (CD45+CD11b+Gr1+Ly6Clo) and monocytes (CD45+CD11b+Gr1+Ly6Chi). **j-l,** Flow cytometric analysis of blood at 14d (**j**), 20d (**k**), and lungs 20d (**l**) in experiment described in (**g**). Control anti-IgG2a n=3 mice/cohort; anti-Ly6G n=4 mice/cohort. **m**, Schematic modeling neutrophil depletion experiment (**n-r)**. **n,** Primary tumor mass represented in Fig. 3i (control IgG2a n=4 mice/cohort; anti-Ly6G n=8 mice/cohort). **o,** Immunofluorescent images of Met1 lung metastasis. Scale bars= 200 µm. **p,** Metastases at higher magnification (indicated in circles in (**o**) analyzed by ImageJ to measure shortest-length diameter)**. q,** Single channels of ECAD and PyMT staining measured by ImageJ analysis (Fig. 3k, l). **r,** End point flow cytometric analysis of neutrophils and monocytes in lungs of mice treated with control IgG2a (n=4 mice/cohort) or anti-Ly6G (n=8 mice/cohort). Source data for **b, e, h, j, k, l, n, r** in Supplementary Table 1. DESeq2's Wald test two-sided (**c)**, 2-sided Welch's t-test (**f**); 2-sided Welch's t test (**n**); 2-way ANOVA followed by Sidak's multiple comparison test (**f, h, j, k, l, o**).


a
Neutrophils
Macrophages
IL1α
IL4
IL18
IL17
IL17A-F
IL9
IL16
MIF
IL6
IL1β
TGFβ
IL10
IL23
IL27
IL13
IL12
IL8
IFN
IL4
TNFα
b
IL1β (pg/ml)
100
80
60
40
20
0
Not detected
Control
Vehicle
Met1
MT2
MT3
c
Met1
MT2
MT3
IL1R/DAPI
IL1β concentration (ng/ml):
0
10
20
Time (mins):
30
60
30
60
30
60
kDa
phospho-NFkB p65
NFkB p65
75
75
Met1
MT2
MT3
d
HMLER
hMIC
p<0.05
IL1β (pg/ml)
8
6
4
2
0
1
2
3
Days
e
Matrigel or HMLER 10
hMIC
End point
Day: 0
14
28
Matrigel:hMIC
HMLER:hMIC
n.s.
p=0.1821
CD11b+F4/80+ (% of CD11b+)
140
120
100
80
60
Digested hMIC tumors

Supplementary Figure 4

Innate Inflammatory Response is sufficient for MIC colonization

a, Some of the predominant cytokines expressed by monocytes, neutrophils or by both. **b,** ELISA to detect cumulative IL-1β in the conditioned media of Met1 cell line and its derived clones after 3 days *in vitro* (n=5/group). **c**, Met1 parental cells and the MT2 and MT3 subclones stained as indicated (upper panel). Scale bars=100 μm. Western blot detecting phospho-NFkB p65 (activated form of NF-kB p65) or total NF-kB p65 (NFkB p65) in Met1, MT2 and MT3 treated *in vitro* with indicated doses of IL-1β at indicated time points (lower panel). **d,** ELISA to detect cumulative IL-1β in the conditioned media of HMLER and hMIC cell lines over 3 consecutive days of *in vitro* culture (n=4/group). **e,** Experimental schematic and flow cytometric quantification of immune cell populations in digested hMIC tumors from indicated cohorts in Nude mice at the 28-day experimental end point (see Fig. 2i) (n=3 in Matrigel control and 4 in HMLER cohort). Source data for **b, d, e** in Supplementary Table 1. 2-sided Welch's t test (**b, e**); Two-way ANOVA, Sidak's multiple comparisons test (**d**).

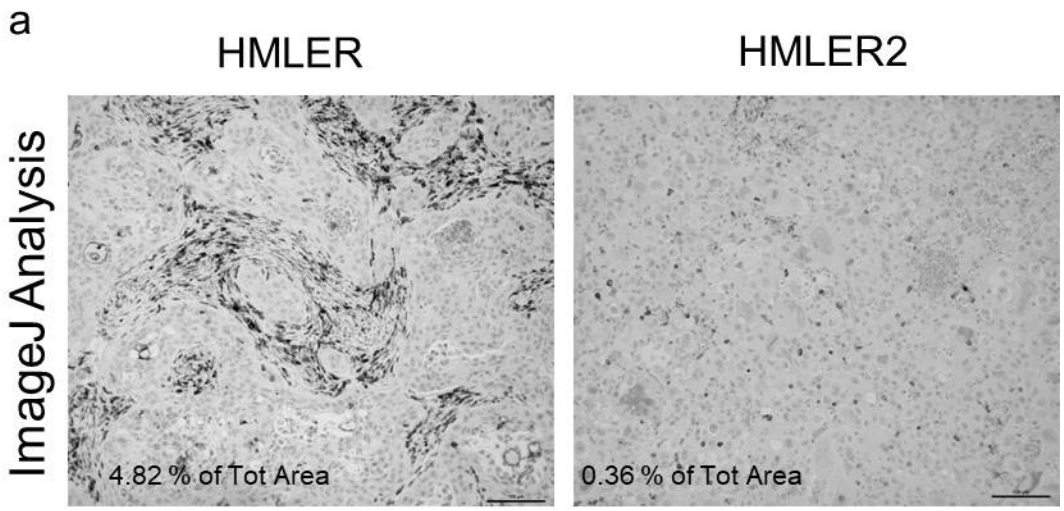


Supplementary Figure 5

IL-1R1 Pathway is sufficient to block MICs colonization

a, qRT-PCR to determine *IL-1R1* mRNA levels in hMIC expressing shRNA against IL-1R1 (shA-IL-1R1-MIC and shB-IL-1R1-MIC) compared to hMIC expressing a scrambled shRNA (sh-Ctl-MIC). B-actin (ACTB) served as the internal control (n=3, average technical triplicates). **b,** Proliferation analysis (CyQuant assay) comparing growth of MIC-control cells to those expressing shRNA against IL-1R1 (shA-IL-1R1-MIC and shB-IL-1R1-MIC; n=3 in triplicate representative of 5 independent experiments). **c,** hMIC tumor mass (mg) at the

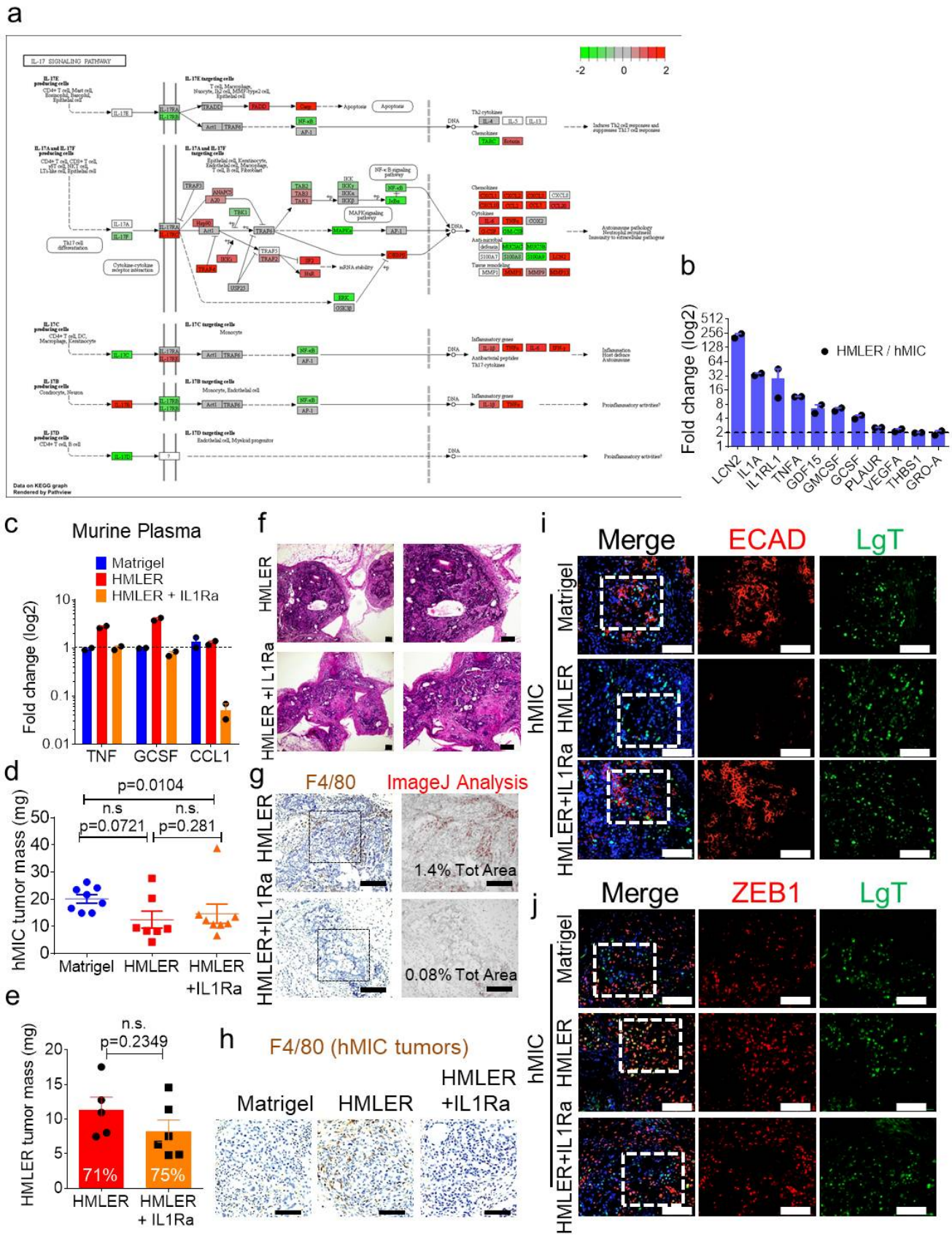
4-week experimental end point (n=8/cohort, except sh-Ctl-MIC n=7). **d**, Met1 tumorspheres generated in 3 independent experiments, treated with IL-1β (10 ng/mL), Anakinra (500 ng/ml) or IL-1β + Anakinra combination (n=3 replicates, 28 technical replicates). **e,** Western blot detecting phospho-NFkB p65, total NFkB p65, or ECAD in Met1 cells treated with 10ng/ml of IL-1β and increasing doses of ANAKINRA (IL-1Ra) for 48h. B-actin (ACTB) was used as a loading control. **f,** Flow cytometric analysis of monocytes ($CD11b^{+}Ly6C^{+}Ly6G^{lo}$) presented as % CD11b+ cells in blood at 10 days, prior to the injection of contralateral tumors (Matrigel n=11 (combined from Groups 1 and 3); HMLER n=7). **g,** Final mass (mg) of hMIC tumors from indicated cohorts (differences not statistically significant, Control n=7, HMLER n=6 mice/group). Representative of 1 experiment. Source data from **a, b, c, d, f, g** in Supplementary Table 1. 2-way ANOVA and Sidak's multiple comparisons test (**a, b**); 1-way ANOVA (**c, g**); 2-sided Student's t tests (**d**); 2-sided Mann-Whitney test (**f**).



Supplementary Figure 6

Primary tumors induce an inflammatory response

a, Representative images of F4/80 staining and ImageJ analysis used to quantify area covered by positive F4/80 staining in Figure 6b. Scale bar=100 μm.

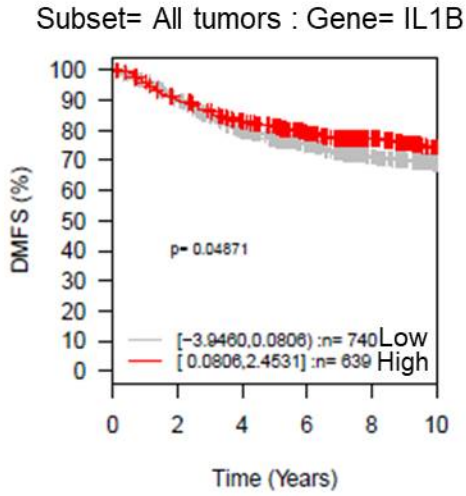

a
IL-17 SIGNALING PATHWAY
-2
0
2
Data on KEGG graph
Rendered by Pathview
b
Fold change (log2)
512
256
128
64
32
16
8
4
2
1
HMLER / hMIC
LCN2
IL1A
IL1RL1
TNFA
GDF15
GMCSF
GCSF
PLAUR
VEGFA
THBS1
GRO-A
c
Murine Plasma
Matrigel
HMLER
HMLER + IL1Ra
Fold change (log2)
10
1
0.1
0.01
TNF
GCSF
CCL1
d
p=0.0104
n.s
p=0.0721
n.s.
p=0.281
hMIC tumor mass (mg)
50
40
30
20
10
0
Matrigel
HMLER
HMLER +IL1Ra
e
n.s.
p=0.2349
HMLER tumor mass (mg)
20
15
10
5
0
71%
75%
HMLER
HMLER + IL1Ra
f
HMLER
HMLER +I L1Ra
hMIC
g
F4/80
ImageJ Analysis
HMLER
HMLER+IL1Ra
1.4% Tot Area
0.08% Tot Area
h
F4/80 (hMIC tumors)
Matrigel
HMLER
HMLER +IL1Ra
i
Merge
ECAD
LgT
Matrigel
HMLER
HMLER+IL1Ra
hMIC
j
Merge
ZEB1
LgT
Matrigel
HMLER
HMLER+IL1Ra
hMIC

Supplementary Figure 7

Inhibition of IL-1R1 at Primary Tumor Site Enables MIC Colonization

a, KEGG IL-17 signaling pathway (mmu04657). The log2 fold change values comparing primary tumor to control lungs. Red, higher expression in primary tumors; green, higher expression in normal lungs (n=3 for Met1 primary tumors, and n=4 control lungs). **b,** Inflammatory cytokine array analysis of conditioned media from HMLER and hMIC cells after 3 days in culture (n=2 biological replicates, 3 technical replicates). Results are fold change (Log2) HMLER/MIC. **c**, Mouse inflammatory cytokine array results from plasma of mice bearing Matrigel control, HMLER, or HMLER+IL-1Ra tumors after 2 weeks in vivo (n=2, plasma pooled from 3 individual animals per sample). **d**, hMIC tumor mass (4 weeks) grown opposite Matrigel control (n=8), HMLER primary tumor (n=7), or HMLER+IL-1Ra antagonist (IL-1Ra) (n=8). Representative of 1 experiment. **e**, Graph: HMLER primary tumor mass embedded in control Matrigel (n=5) or Matrigel with 100ng/ml IL-1Ra (n=6). Incidence (%) marked on graphs. **f**, Hematoxylin and eosin (H&E) stains from HMLER and HMLER+IL1Ra tumors (Figure 7c-g), low and high magnification. **g**, F4/80 staining and ImageJ analysis to quantify area covered by F4/80+ staining in Figure 7e. Boxes indicate area shown in Figure 7e. **h**, Immunohistochemistry showing macrophage infiltration (F4/80 stain) in hMIC tumors grown opposite Matrigel control, HMLER, or HMLER+IL-1Ra primary tumors. **i**, **j**, Photomicrographs of hMIC tumors grown opposite Matrigel control, HMLER primary tumor, or HMLER + IL-1Ra primary tumors. ECAD (red), LgT (green – to detect human tumor cells), DAPI (blue). (**h**) ZEB1 (red), LgT (green), DAPI (blue). Boxes indicate area shown in Figure **7f-g**. Scale bars=100 μm. Source data for **b, c, d, e** in Supplementary Table 1. DESeq2's Wald test two-sided (**a)**; 2-sided Mann-Whitney test (**d);** 2-sided Welch's t test (**e**).

a

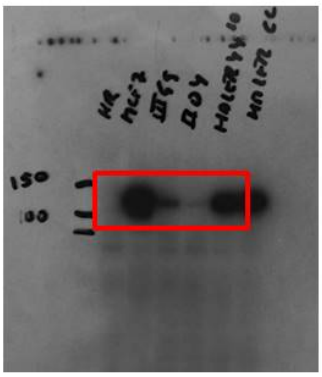


Supplementary Figure 8

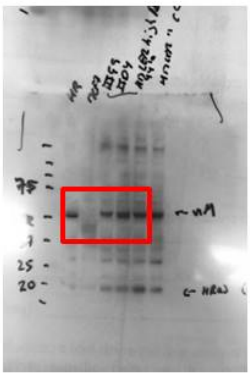
Analysis of IL1 pathway components as predictors of disease outcome in clinical breast cancer specimens

a, Kaplan-Meier analysis using distant metastasis-free survival (DMFS) as end point with 10-year censoring based on stratification of *IL1B* gene expression (log2; low, grey line, n=740; high, red line, n=639) in tumor tissue from 1,379 breast cancer patients. Logrank p value is shown. Analysis performed using the GOBO database (http://co.bmc.lu.se/gobo/gsa.pl).

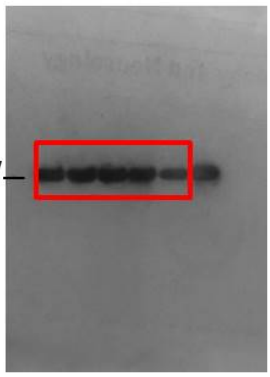
Suppl. Fig. 2b, CDH1



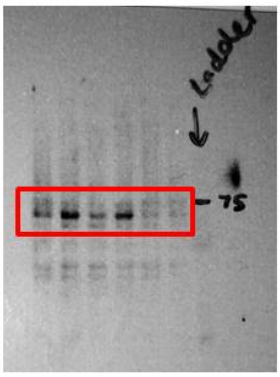
Suppl. Fig. 2b, VIM



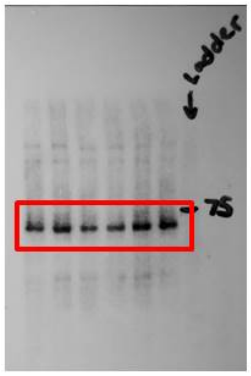
Suppl. Fig. 2b, GAPDH



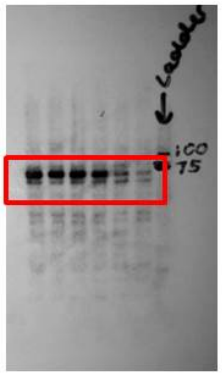
Suppl. Fig. 4c, Met1 P-P65



Suppl. Fig. 4c, Met1 Total-P65



Suppl. Fig. 4c, MT2 P-P65



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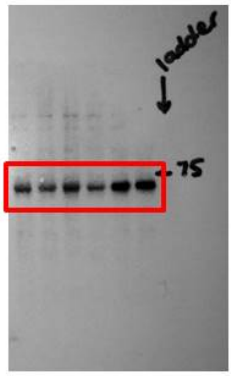

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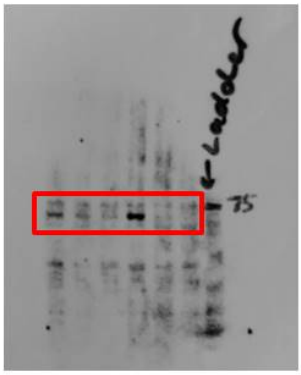
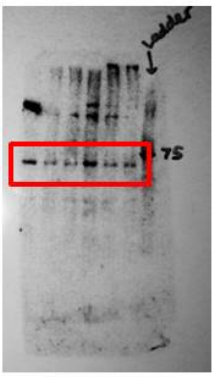
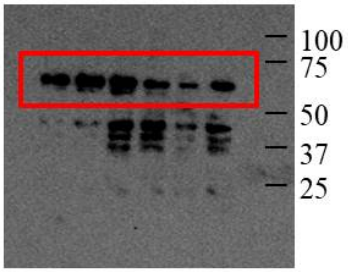


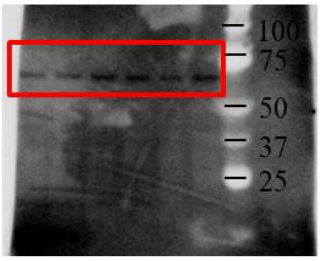
Suppl. Fig. 4c, MT3 Total-P65



Suppl. Fig. 5e, P-P65



Suppl. Fig. 5e, Total-P65



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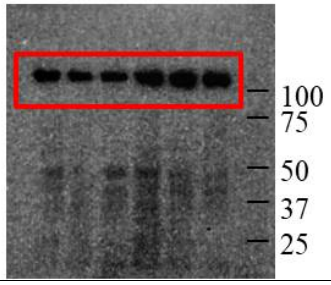
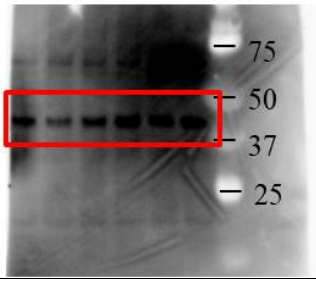


Suppl. Fig. 5e, ACTB



Supplementary Figure 9
Western blot scanned films
Boxes highlight lanes used in figures.