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Supplemental Material to:

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CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells

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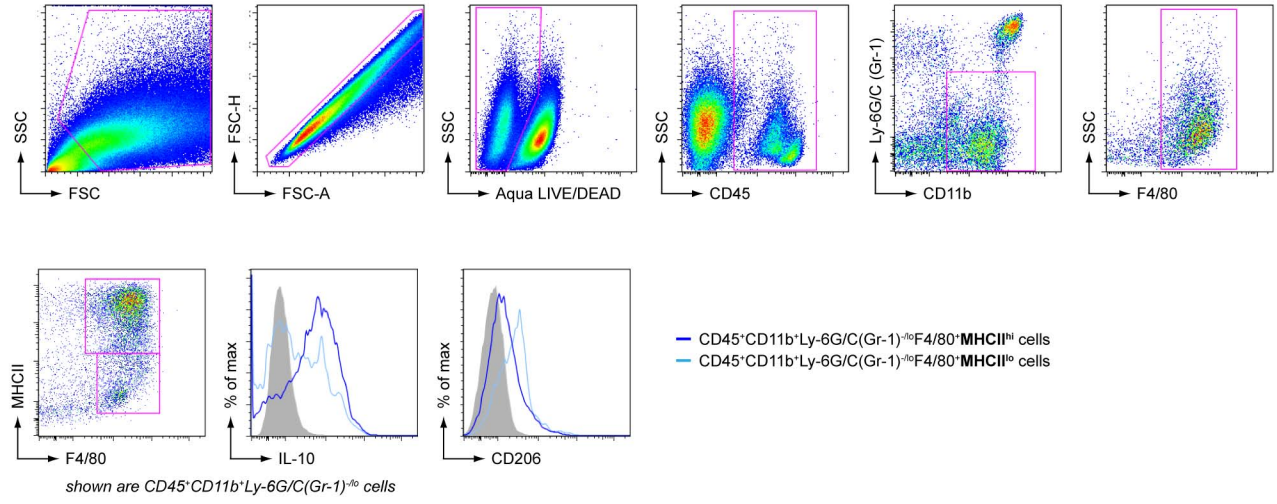
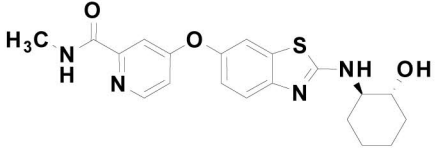
Supplemental Figure 1. Characterization of tumor-associated macrophages in the PyMT allograft model. Spontaneous tumors from MMTV-PyMT mice were pooled and digested to form a single-cell suspension. One million cells were implanted into a single mammary fat pad in syngeneic mice. Tumor allografts were digested and analyzed by flow cytometry. Gating strategy to identify macrophages in tumors. Gating was performed in sequence from left to right. Gates are outlined in pink. Cell surface expression of F4/80 and MHCII in $CD45^{+}CD11b^{+}Ly\text{-}6G/C(Gr\text{-}1)^{-/lo}$ cells to identify two distinct ($MHCII^{hi}$ and $MHCII^{lo}$) TAM subpopulations. Histogram overlays show intracellular staining of IL-10 and cell surface expression of CD206 in $MHII^{hi}$ (dark blue) and $MHCII^{lo}$ (light blue) TAM subpopulations. Isotype control-stained cells are shown as a solid histogram.

Supplemental Figure 2. Structure and activity of BLZ945, a CSF1R inhibitor. Chemical structure and biochemical profiling of BLZ945, a highly selective and potent CSF1R inhibitor.

Supplemental Figure 3. The CSF1R pathway regulates at least two regulatory TAM populations in mammary tumors. A, Pseudocolor dot plot showing cell surface-staining of F4/80 and MHCII in $CD45^{+}CD11b^{+}Ly\text{-}6G/C(Gr\text{-}1)^{-/lo}$ cells from spontaneous tumors in MMTV-PyMT transgenic mice. Two $F4/80^{+}$ populations ($MHCII^{hi}$ and $MHCII^{lo}$ TAMs) are boxed in magenta. Histogram overlay showing intracellular staining of IL-10 and cell-surface staining of CD206 in $MHCII^{hi}$ (dark blue) and $MHCII^{lo}$ (light blue) TAM subpopulations. Isotype control-stained cells are represented by the solid histogram. B, MMTV-PyMT transgenic mice were dosed with BLZ945 or vehicle (n = 4 per group) for 6 days. Spontaneous tumors were analyzed as described in Fig. 1A, using MHCII staining to separate out $IL\text{-}10^{+}$ TAM subpopulations. Data is quantified in C. D-E, Repopulation of $MHCII^{hi}$ and $MHCII^{lo}$ TAM subpopulations in mammary tumors over a period of 6 days following a 6-day treatment with BLZ945 (n = 4 per group). All graphs

show mean values, error bars represent SEM. *$p < 0.05$ versus vehicle, unpaired t-test, two-tailed.


SSC
FSC
FSC-H
FSC-A
SSC
Aqua LIVE/DEAD
SSC
CD45
Ly-6G/C (Gr-1)
CD11b
SSC
F4/80
MHCII
F4/80
% of max
IL-10
% of max
CD206
CD45+CD11b+Ly-6G/C(Gr-1)-/loF4/80+MHCIIhi cells
CD45+CD11b+Ly-6G/C(Gr-1)-/loF4/80+MHCIIlo cells
shown are CD45+CD11b+Ly-6G/C(Gr-1)-/lo cells



Biochemical Activity

CSF-1R	0.001 mM
PDGFRb	5.86 mM
PDGFRa	>10 mM
cKit	3.88 mM
Flt3	>10 mM

Invitrogen Kinase Panel

>10 mM	220 kinases

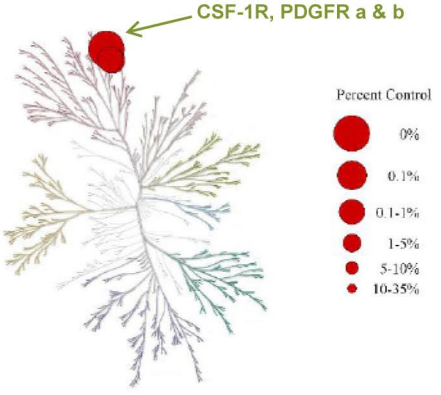
Cellular Target Modulation

p-CSF-1R (MNFS-60)	0.05
p-MEK$^{217/221}$ (MNFS-60)	0.06
p-CSF-1R (monocytes)	0.11
p-PDGFRb (HVSMC)	1.2
p-cKit (Mo7E)	>8

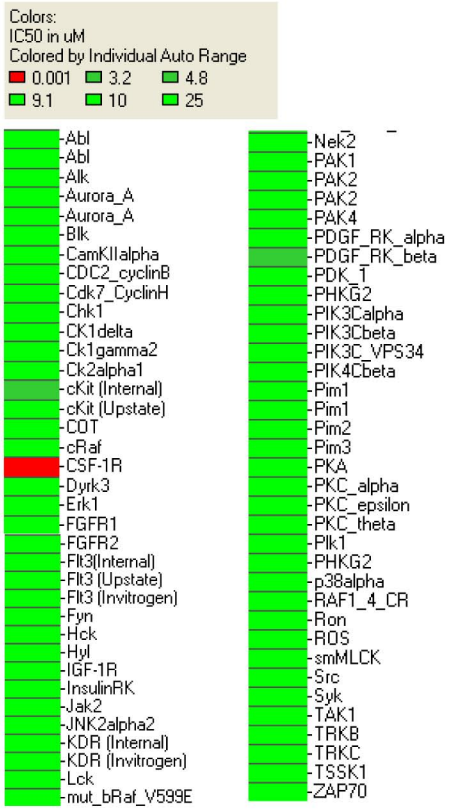
Cell Proliferation

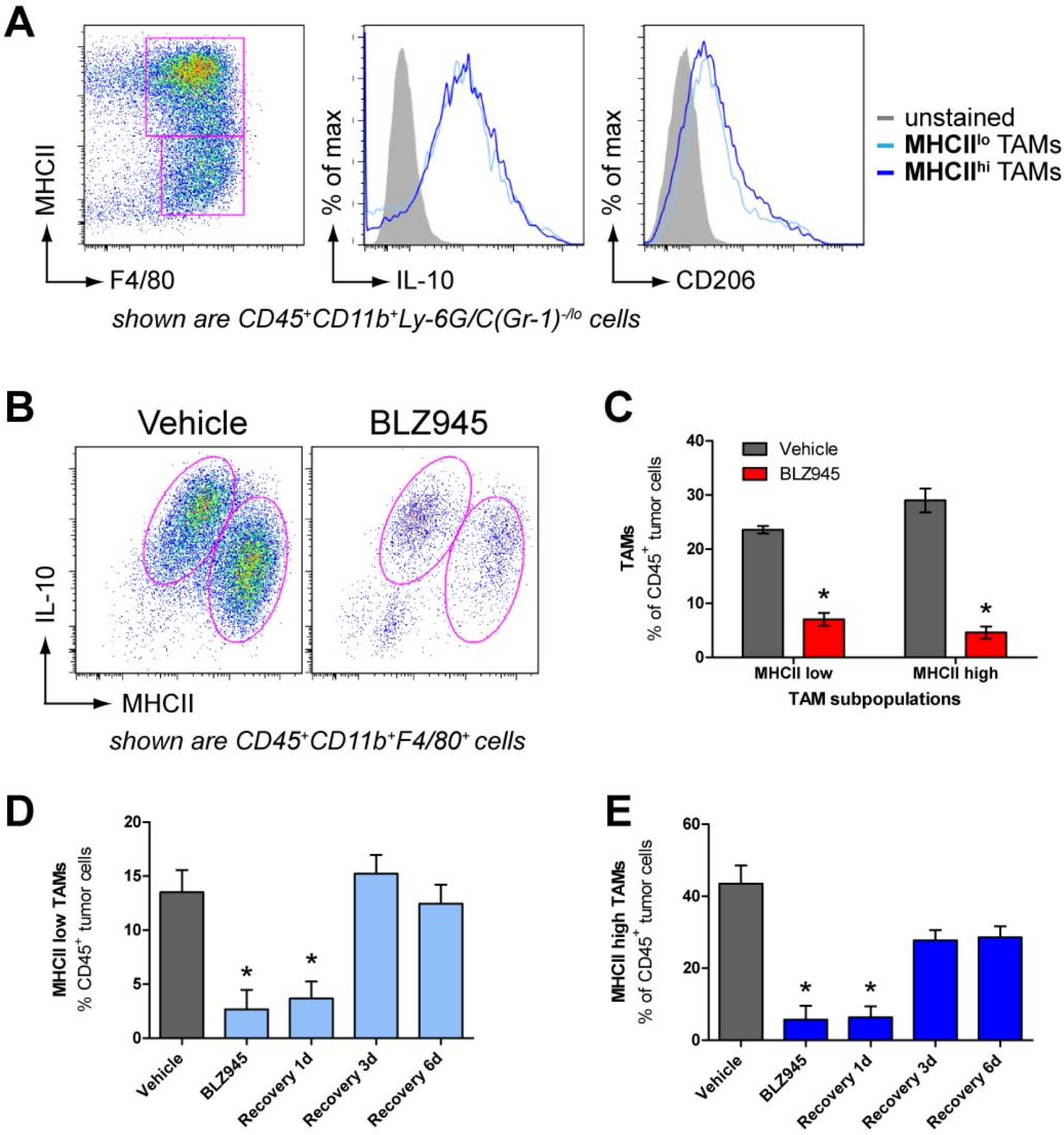
MNFS-60 (M-CSF)	0.067 mM
HVSMC (PDGF)	8.7 mM
Mo7e (SCF)	>10 mM

NVP-BLZ945-AA-3 @ 1000nM



Biochemical Selectivity Profiling




A
MHCII
F4/80
% of max
IL-10
% of max
CD206
unstained
MHCIIlo TAMs
MHCIIhi TAMs
shown are CD45+CD11b+Ly-6G/C(Gr-1)-/lo cells
B
Vehicle
BLZ945
IL-10
MHCII
shown are CD45+CD11b+F4/80+ cells
C
TAMs
% of CD45+ tumor cells
Vehicle
BLZ945
MHCII low
MHCII high
TAM subpopulations
D
MHCII low TAMs
% CD45+ tumor cells
Vehicle
BLZ945
Recovery 1d
Recovery 3d
Recovery 6d
E
MHCII high TAMs
% of CD45+ tumor cells
Vehicle
BLZ945
Recovery 1d
Recovery 3d
Recovery 6d