

ORIGINAL ARTICLE

Characterization of tumor-associated macrophages in prostate cancer transgenic mouse models

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Abstract

Background: Tumor-associated macrophages (TAMs) are critical components of the tumor microenvironment (TME) in prostate cancer. Commonly used orthotopic models do not accurately reflect the complete TME of a human patient or the natural initiation and progression of a tumor. Therefore, genetically engineered mouse models are essential for studying the TME as well as advancing TAM-targeted therapies. Two common transgenic (TG) models of prostate cancer are Hi-Myc and transgenic adenocarcinoma of the mouse prostate (TRAMP), but the TME and TAM characteristics of these models have not been well characterized.

Methods: To advance the Hi-Myc and TRAMP models as tools for TAM studies, macrophage infiltration and characteristics were assessed using histopathologic, flow cytometric, and expression analyses in these models at various timepoints during tumor development and progression.

Results: In both Hi-Myc and TRAMP models, macrophages adopt a more pro-tumor phenotype in higher histological grade tumors and in older prostate tissue. However, the Hi-Myc and TRAMP prostates differ in their macrophage density, with Hi-Myc tumors exhibiting increased macrophage density and TRAMP tumors exhibiting decreased macrophage density compared to age-matched wild type mice.

Conclusions: The macrophage density and the adenocarcinoma cancer subtype of Hi-Myc appear to better mirror patient tumors, suggesting that the Hi-Myc model is the more appropriate in vivo TG model for studying TAMs and TME-targeted therapies.

KEYWORDS

Hi-Myc, pro-tumor, TAM, TRAMP, tumor microenvironment

1 | INTRODUCTION

Prostate cancer tumor growth and disease progression are highly influenced by noncancerous host cells within the tumor micro-environment (TME). One particularly important TME cell type in prostate cancer is the tumor-associated macrophage (TAM) which can comprise up to 50% of prostate cancer bone metastases.¹ Examination of prostate cancer patient tissues revealed that the extent of infiltration of tumor tissue by TAMs was increased in aggressive and advanced disease.² In addition, TAMs in prostate cancer as well as other cancers are known to contribute to tumorigenesis.³

Macrophages are a plastic cell type and adopt different functions in response to signaling molecules in their environment. There are an array of subtypes that a macrophage can adopt which are categorized by the polarization stimuli, gene expression, and functional readouts (i.e., cytokine secretion, phagocytosis, and T cell activation).^{4–6} Given the plasticity of macrophages and their ability to repolarize, macrophages often do not fit neatly into these subtypes making classification of TAMs based on canonical subtypes complicated and imperfect.^{4,5,7–10}

To date, the field has relied on an M1–M2 dichotomy spectrum to associate macrophage characteristics with either antitumor (M1) or pro-tumor (M2) functions.^{8,11,12} While this has proved useful in providing a common language with which to describe TAMs, the field has advanced in its understanding of the nuances of TAM gene expression and behavior. It is becoming increasingly clear that the M1–M2 model no longer suffices in encapsulating the complexity and key characteristics of TAMs. In light of this, we consider the terms “M2-like” and “pro-tumor” to be appropriate means for referring to such TAMs that share tissue remodeling and immune-suppressing characteristics with M2s but differ in expression of specific characteristic genes.

The majority of TAMs associated with prostate cancer lesions are pro-tumor and M2-like. Similar to M2s, these TAMs promote tissue remodeling, cell growth and proliferation, and suppress a CD8⁺ cytotoxic T cell response which altogether support tumors.^{7,13} Given their pro-tumor functions and their prevalence in prostate cancer, targeting pro-tumor M2-like TAMs provides a pressing and promising adjunct therapeutic strategy.

To study effective ways to target M2-like TAMs in patients, it is important to have accurate in vivo prostate cancer models. Of the available prostate cancer mouse models, transgenic (TG) mice are among the most accurate in vivo models of tumor initiation and the evolving TME. Other models such as xenograft or syngeneic injected cancer cell lines and allograft tumor transplantation are insufficient given their methods of tumor induction. Such injected tumor models are poor models for tumor initiation as they involve implantation of bulk tumors that lack any semblance of the original tissue architecture seen in early-stage tumors and native TME components and heterogeneity.¹⁴ Additionally, because of the way these tumors are initiated, these models rely heavily on infiltrating host cells to reconstitute the TME which may not accurately reflect that of a patient's tumor.¹⁵ However, in TG mouse models, tumors are initiated

using the host's cells which shapes the tissue in a manner that is more histologically consistent with early-stage patient tumors. This provides a more accurate model for studying both tumor initiation and the TME.

Two common prostate cancer TG mouse models are the Hi-Myc and transgenic adenocarcinoma of the mouse prostate (TRAMP) models. The Hi-Myc model is genetically engineered to express the human c-myc oncogene under the prostate-specific probasin promoter and two androgen-regulated regions.¹⁶ Hi-Myc mice develop prostatic intraepithelial neoplasia (PIN) as early as 2 weeks and adenocarcinoma tumors are observed in all mice by 6 months. The TRAMP model contains a rat probasin promoter-driven simian virus 40 large tumor T antigen (SV40 T-Ag) gene which when expressed acts as an oncoprotein by inhibiting Rb and p53 tumor suppressors.¹⁷ While the line was initially thought to model adenocarcinoma, the large invasive tumors that develop in TRAMP mice were later found to be best classified as neuroendocrine prostate cancer.¹⁸ Carcinogenic tissue from these mice is observed in all mice by 10–12 weeks.^{18,19} These models offer a means for studying the TME and tumor initiation that more closely resembles patient tumor development.

These two TG models have been widely used and are advantageous for prostate cancer studies. The histology and characteristics of the carcinogenic cells in these models are well characterized and cell lines developed from these models have proven to be useful tools.^{20,21} However, with increased interest in immunotherapeutic treatments for prostate cancer, it has become increasingly clear how important it is to understand the immune components of these models to inform development of immune-related prostate cancer therapeutics. Our work endeavors to describe the macrophage characteristics and infiltration in Hi-Myc and TRAMP prostates over time and with tumor growth in these models.

2 | MATERIALS AND METHODS

2.1 | Mouse models

The Johns Hopkins Institutional Animal Care and Use Committee approved all experiments involving mice (protocol # MO19M41). FVB/N Hi-Myc and FVB/N TRAMP mice were a gift from Brian Simons (Baylor University). Mice were bred in monogamous pairs with a transgene heterozygote female and a nontransgene bearing male. Mice containing the transgene were identified by tail snip DNA extraction with Hot Shot Lysis Buffer (25 mM NaOH, 0.2 mM ethylenediaminetetraacetic acid [EDTA]) and PCR with *Taq* DNA polymerase (100021276; NEB) using primers forward 5'-AAACATGATGACTACCAAGCTTGGC-3' and reverse 5'-ATGATAGCATCTTGTTCTTAGTCTTTTCTTAATAGGG-3' for Hi-Myc and forward 5'-GCGCTGCTGACTTTCTAAACATAAG-3' and reverse 5'-GAGCTCACGTTAAGTTTGTATGTGT-3' for TRAMP. Mice were euthanized using CO₂ asphyxiation. Prostates were micro-dissected using protocols described previously.²²

TABLE 1 Upregulated differentially expressed genes (DEGs) in Hi-Myc 2-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Rnase2a^a</i>	NM_053113.2	9.05	32.42	293.26
<i>Adam8^a</i>	NM_007403.2	3.06	89.44	273.26
<i>Tgm2^a</i>	NM_009373.3	2.95	220.23	648.72
<i>Clec7a^a</i>	NM_020008.2	2.51	320.85	805.34
<i>Fyn^a</i>	NM_008054.2	2.13	77.14	164.40
<i>Retnla^a</i>	NM_020509.3	1.98	2343.21	4642.10
<i>Isg15^{a,b}</i>	NM_015783.3	1.78	270.54	480.98
<i>C4a^a</i>	NM_011413.2	1.72	139.74	241.05
<i>Trem2^a</i>	NM_031254.2	1.67	181.11	302.14
<i>Fcgr1^a</i>	NM_010186.5	1.63	183.34	298.81
<i>Ctsd^{a,b}</i>	NM_009983.2	1.60	2082.73	3329.11
<i>Fcgr2b^{a,b}</i>	NM_001077189.1	1.59	657.35	1043.06
<i>Osm^a</i>	NM_001013365.2	1.54	690.89	1065.27
<i>Psmb8^a</i>	NM_010724.2	1.53	226.94	347.69
<i>Apoe^{a,b}</i>	NM_001305844.1	1.52	7775.29	11849.07
<i>Csf2rb^a</i>	NM_007780.4	1.51	503.07	759.80
<i>Il1rn^a</i>	NM_031167.5	1.50	2134.15	3191.37
<i>Grn^{a,b}</i>	NM_008175.4	1.42	453.88	643.16

Abbreviation: TRAMP, transgenic adenocarcinoma of the mouse prostate.

^aCommonly upregulated across cohorts in TG compared to strain-matched, age-matched WT.

^bCommonly upregulated across cohorts in Hi-Myc 2 months, Hi-Myc 6 months, TRAMP 2 months, and TRAMP 5 months.

2.2 | Tissue fixation and immunohistochemistry

For prostates that were fixed, tissue was incubated in 10% neutral buffered saline for 48 h and stored in 70% ethanol. Tissue was paraffin-embedded, sectioned, and stained with hematoxylin and eosin by the Johns Hopkins Oncology Tissue Services Core. Immunolabeling for F4/80 was performed by the Johns Hopkins Oncology Tissue Services Core on formalin-fixed, paraffin embedded sections. Briefly, following dewaxing and rehydration, slides were immersed in 1% Tween-20, then heat-induced antigen retrieval was performed in a steamer using Target Retrieval Solution (S170084-2; Dako) for 45 min. Slides were rinsed in PBST and endogenous peroxidase and phosphatase was blocked (S2003; Dako) and sections were then incubated with primary antibody; anti-F4/80 (1:2000 dilution; MCA497R, lot 1365, Serotec; Bio-Rad) for 45 min at room temperature, followed by incubation with rabbit anti-rat antibody (1:500 dilution, AI-4001, lot ZC0603; Vectorlab). The linking antibodies were detected by 30-min incubation with HRP-labeled anti-rabbit secondary antibody (PV6119; Leica Microsystems) followed

TABLE 2 Downregulated differentially expressed genes (DEGs) in Hi-Myc 2-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Id1</i>	NM_010495.2	-3.07	133.04	43.32
<i>Hpgd^a</i>	NM_008278.2	-2.99	397.99	133.30
<i>Mmp13</i>	NM_008607.1	-2.79	741.20	265.48
<i>Il12b^a</i>	NM_001303244.1	-2.65	10624.92	4003.38
<i>Hpgds</i>	NM_019455.4	-2.59	618.22	238.83
<i>Ceacam1</i>	NM_001039185.1	-2.57	279.49	108.86
<i>Cxcl13^a</i>	NM_018866.2	-2.39	169.93	71.09
<i>Cyr61</i>	NM_010516.1	-2.17	181.11	83.31
<i>Birc2</i>	NM_007465.2	-2.12	276.13	129.97
<i>Cdh1</i>	NM_009864.2	-2.07	206.82	99.97
<i>Tlr1</i>	NM_030682.1	-1.99	404.69	203.28
<i>Mmp12^a</i>	NM_008605.3	-1.98	4029.06	2038.35
<i>Stat5a</i>	NM_011488.2	-1.96	211.29	107.75
<i>Mmp9^a</i>	NM_013599.2	-1.89	305.20	161.07
<i>Tuba4a^a</i>	NM_009447.3	-1.87	242.59	129.97
<i>Hivep1</i>	NM_007772.2	-1.83	302.96	165.51
<i>Cd163^a</i>	NM_053094.2	-1.75	382.34	218.83
<i>Ripk2</i>	NM_138952.3	-1.75	338.74	193.28
<i>Cd180</i>	NM_008533.2	-1.69	238.12	141.07
<i>Fosb</i>	NM_008036.2	-1.68	493.01	293.26
<i>Rin2</i>	NM_028724.4	-1.67	251.54	151.07
<i>Rgs1</i>	NM_015811.1	-1.67	2526.55	1508.49
<i>Cxcl1</i>	NM_008176.1	-1.67	11723.85	7040.35
<i>Igf1r</i>	NM_010513.2	-1.65	256.01	155.51
<i>Cd86</i>	NM_019388.3	-1.61	2294.02	1427.40
<i>Adamts1^a</i>	NM_009621.4	-1.59	323.09	203.28
<i>Klf4</i>	NM_010637.3	-1.59	402.46	253.27
<i>Il1b</i>	NM_008361.3	-1.58	7821.12	4949.79
<i>Malt1</i>	NM_172833.2	-1.56	906.65	582.07
<i>Ccl22</i>	NM_009137.2	-1.55	1119.06	720.92
<i>Cybb</i>	NM_007807.2	-1.55	1771.94	1144.14
<i>Il1a</i>	NM_010554.4	-1.54	880.94	572.07
<i>Cxcl2</i>	NM_009140.2	-1.53	12730.00	8321.12
<i>Nfkbiz^a</i>	NM_030612.1	-1.50	2095.02	1394.07
<i>Clic4</i>	NM_013885.2	-1.48	745.67	505.42
<i>Icosl^a</i>	NM_015790.3	-1.47	1548.35	1051.94
<i>Ccr7</i>	NM_007719.2	-1.46	960.31	656.49

(Continues)

TABLE 2 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Icam1</i>	NM_010493.2	-1.45	700.95	482.09
<i>Msr1</i>	NM_001113326.1	-1.44	1482.39	1028.61
<i>Dusp2</i>	NM_010090.2	-1.44	765.79	530.97
<i>Gem^a</i>	NM_010276.3	-1.43	851.87	594.29
<i>Nfkb1</i>	NM_008689.2	-1.43	1632.19	1144.14
<i>Nr4a1^a</i>	NM_010444.1	-1.42	822.81	578.73
<i>Irf1^a</i>	NM_008390.1	-1.42	1181.66	834.22
<i>Ptpcr</i>	NM_011210.3	-1.42	673.00	474.32
<i>Skil</i>	NM_011386.2	-1.40	1942.98	1384.08
<i>Ptgs2</i>	NM_011198.3	-1.39	6118.50	4402.16
<i>Birc3</i>	NM_007464.3	-1.39	746.79	538.75
<i>Nlrp3</i>	NM_145827.3	-1.38	1617.66	1171.91
<i>Tgfb²</i>	NM_029575.3	-1.37	1953.04	1428.51
<i>Ccl3^a</i>	NM_011337.1	-1.36	18022.34	13210.92
<i>Jun^a</i>	NM_010591.2	-1.36	2035.77	1496.27
<i>Cd83</i>	NM_009856.2	-1.26	10641.69	8422.20

^aCommonly downregulated across cohorts in TG compared to strain-matched, age-matched WT.

by detection with 3,3'-diaminobenzidine (D4293; Sigma-Aldrich), counterstaining with Mayer's hematoxylin, dehydration, and mounting.

2.3 | Immunohistochemistry (IHC) quantification

F4/80 IHC sections were scanned at ×20 resolution. Regions of PIN, cribriform PIN/carcinoma in situ (CribPIN/CIS), invasive adenocarcinoma, or higher-grade carcinoma were identified by a pathologist.²³ QuPath version 0.1.2 was used to define analysis regions and quantify DAB staining as a function of number of brown DAB pixels over total number of stained (DAB or hematoxylin) pixels in the defined region. One 6-month Hi-Myc wild type (WT) mouse, one 6-month Hi-Myc TG mouse, one 2-month TRAMP TG mouse, and one 5-month TRAMP WT mouse were identified as statistical outliers and removed from all IHC analyses.

2.4 | Flow-cytometric macrophage analysis

Prostate tissue was subjected to single cell dissociation using the MACS Mouse Tumor Dissociation Kit protocol and gentleMACS Dissociator (Miltenyi). Suspended cells were blocked with rat serum (012-000-120;

TABLE 3 Upregulated differentially expressed genes (DEGs) in Hi-Myc 6-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Arg1^b</i>	NM_007482.3	At least 31.39	Below thres-hold	627.79
<i>Rnase2a^a</i>	NM_053113.2	At least 10.14	Below thres-hold	202.72
<i>Emp1^b</i>	NM_010128.4	9.12	36.21	330.38
<i>Adam8^{a,b}</i>	NM_007403.2	7.93	32.11	254.62
<i>Tgm2^{a,b}</i>	NM_009373.3	7.24	142.80	1033.93
<i>Il1rn^a</i>	NM_031167.5	6.27	366.22	2294.43
<i>Vegfa^b</i>	NM_001025250.3	6.23	122.30	761.77
<i>Cd274</i>	NM_021893.2	5.52	120.25	664.27
<i>Cxcl3</i>	NM_203320.2	4.83	57.39	277.07
<i>Ccl8^b</i>	NM_021443.2	4.45	178.33	793.33
<i>Csf2rb^{a,b}</i>	NM_007780.4	3.69	203.61	751.95
<i>Irf7^b</i>	NM_016850.2	3.67	38.26	140.29
<i>Clec7a^a</i>	NM_020008.2	3.57	493.98	1762.73
<i>Arg2</i>	NM_009705.2	3.56	34.85	124.16
<i>Nfil3</i>	NM_017373.3	3.33	198.14	659.36
<i>Ccl7</i>	NM_013654.3	3.28	364.17	1195.26
<i>Fn1^b</i>	NM_010233.1	3.23	126.40	408.24
<i>Ccr1^b</i>	NM_009912.4	3.16	142.11	448.92
<i>Ctsd^{a,b,c}</i>	NM_009983.2	3.14	2308.67	7239.61
<i>Cd84^b</i>	NM_001252472.1	3.11	168.08	523.28
<i>Fcgr2b^{a,b,c}</i>	NM_001077189.1	3.10	566.41	1757.82
<i>Cytip</i>	NM_139200.4	2.97	179.69	533.10
<i>Osm^a</i>	NM_001013365.2	2.93	539.08	1577.55
<i>Trem1</i>	NM_021406.5	At least 2.91	Below thres-hold	58.22
<i>Furin</i>	NM_011046.2	2.64	380.57	1003.07
<i>Stat1</i>	NM_009283.3	2.55	73.79	187.99
<i>Cxcl9</i>	NM_008599.2	2.53	38.26	96.80
<i>Hif1a</i>	NM_010431.2	2.49	331.37	824.20
<i>Anxa1^b</i>	NM_010730.2	2.40	59.44	142.39
<i>Cd80</i>	NM_009855.2	2.35	64.22	150.81
<i>Fcgr4^b</i>	NM_144559.1	2.33	127.77	298.11
<i>Nampt</i>	NM_021524.1	2.33	392.18	911.88
<i>Isg15^{a,b,c}</i>	NM_015783.3	2.28	164.66	375.27

TABLE 3 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Crem</i>	NM_001110853.1	2.18	53.98	117.84
<i>Ccr2^b</i>	NM_009915.2	2.17	76.52	166.24
<i>Il10</i>	NM_010548.1	2.12	64.91	137.48
<i>Fyn^{a,b}</i>	NM_008054.2	2.11	57.39	121.35
<i>Grn^{a,b,c}</i>	NM_008175.4	2.04	426.34	867.69
<i>Cxcr4</i>	NM_009911.3	2.03	251.43	509.95
<i>Ccl2</i>	NM_011333.3	2.03	1663.01	3380.96
<i>Siglec1</i>	NM_011426.3	2.00	56.71	113.63
<i>Ccl6^b</i>	NM_009139.2	1.99	116.15	230.78
<i>C4a^{a,b}</i>	NM_011413.2	1.97	174.23	343.71
<i>Tlr8^b</i>	NM_133212.2	1.94	168.76	326.87
<i>S100a11</i>	NM_016740.3	1.92	144.85	277.77
<i>Tnfrsf1b</i>	NM_011610.3	1.89	190.62	360.54
<i>H2-D1</i>	NM_010380.3	1.86	2771.23	5157.72
<i>Pdgfa</i>	NM_008808.3	1.86	237.08	440.51
<i>Txn1^b</i>	NM_011660.3	1.86	506.97	942.04
<i>Anxa4</i>	NM_013471.2	1.82	215.22	391.41
<i>Alcam</i>	NM_009655.1	1.81	94.29	170.45
<i>Retnla^a</i>	NM_020509.3	1.80	1408.16	2532.21
<i>Ccl9^a</i>	NM_011338.2	1.80	871.82	1569.83
<i>Fem1c</i>	NM_173423.4	1.80	325.22	584.30
<i>Apoe^{a,b,c}</i>	NM_001305844.1	1.79	13407.25	24039.21
<i>Ptgs2</i>	NM_011198.3	1.76	1920.59	3371.14
<i>Ccr5</i>	NM_009917.5	1.72	349.82	601.14
<i>Il2rg</i>	NM_013563.3	1.71	79.94	136.78
<i>Msr1</i>	NM_001113326.1	1.69	537.71	906.27
<i>Ccl5</i>	NM_013653.1	1.67	182.43	305.13
<i>Trem2^{a,b}</i>	NM_031254.2	1.62	342.30	553.44
<i>Psmb8^a</i>	NM_010724.2	1.61	284.91	458.74
<i>Cebpb</i>	NM_009883.3	1.61	1694.44	2727.22
<i>Ctss</i>	NM_021281.2	1.61	4061.87	6543.07
<i>Cd47^b</i>	NM_010581.3	1.57	217.95	342.30
<i>Fcgr1^{a,b}</i>	NM_010186.5	1.56	377.83	588.51
<i>Serpine1</i>	NM_008871.2	1.56	233.67	364.75
<i>Smad7</i>	NM_001042660.1	1.56	183.79	286.89
<i>Plaur</i>	NM_011113.3	1.55	262.36	406.14
<i>Il17ra</i>	NM_008359.1	1.55	181.06	280.58
<i>Ccl12</i>	NM_011331.2	1.52	1422.51	2163.26

(Continues)

TABLE 3 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Mif^b</i>	NM_010798.2	1.51	148.26	223.76
<i>H2-Q1</i>	NM_010390.3	1.50	663.43	994.65
<i>Ctnnb1^b</i>	NM_007614.2	1.47	525.41	771.59
<i>C1qb^b</i>	NM_009777.2	1.46	3249.50	4741.06
<i>Malt1</i>	NM_172833.2	1.43	283.55	406.84
<i>Syk</i>	NM_001198977.1	1.43	315.66	451.73
<i>Cstb</i>	NM_007793.3	1.43	1421.83	2036.99
<i>Ier3</i>	NM_133662.2	1.42	924.43	1313.81
<i>Rhog</i>	NM_019566.3	1.42	610.13	866.99
<i>Lipa</i>	NM_021460.3	1.40	513.80	716.88
<i>Itgb1</i>	NM_010578.1	1.39	680.51	946.25
<i>Il1a</i>	NM_010554.4	1.38	474.85	654.45
<i>Cdkn1a</i>	NM_007669.4	1.38	512.43	707.76
<i>Psme2</i>	NM_001029855.1	1.37	392.18	537.31
<i>Cd14</i>	NM_009841.3	1.32	2825.20	3740.10
<i>Id2</i>	NM_010496.3	1.29	4426.04	5713.27

Abbreviation: TRAMP, transgenic adenocarcinoma of the mouse prostate.

^aCommonly upregulated across cohorts in TG compared to strain-matched, age-matched WT.^bCommonly upregulated across cohorts in Hi-Myc 6 months and TRAMP 5 months.^cCommonly upregulated across cohorts in Hi-Myc 2 months, Hi-Myc 6 months, TRAMP 2 months, and TRAMP 5 months.

Jackson ImmunoResearch), stained with FVS570 viability dye (1 µl/ml, 564995; BD Biosciences) in the dark for 15 min at room temperature. Samples were washed with phosphate-buffered saline (PBS) and incubated with Myeloid extracellular antibody panel (Table S1) or corresponding isotype panels diluted in Brilliant Stain Buffer (566349; BD Biosciences) in the dark for 30 min at 4°C. Cells were washed with FACS buffer (1X PBS, 1% bovine serum albumin, 2 mM EDTA), fixed with 1X Fixation Buffer (420801; BioLegend) in the dark for 20 min at room temperature, and stored overnight in FACS buffer at 4°C. Samples were incubated in 1X FoxP3 Fix/Perm Solution (421401; BioLegend) in the dark for 20 min at room temperature and washed with 1X FoxP3 Perm Buffer (421402; BioLegend). Cells were resuspended with Myeloid intracellular antibody panel (Table S1) or corresponding isotype panels diluted in FACS buffer in the dark for 30 min at room temperature under gentle agitation. Cell suspensions were washed with FACS buffer and analyzed with a Gallios flow cytometer (Beckman Coulter Life Sciences). Macrophages were defined as FVS570⁻CD45⁺CD11b⁺F4/80⁺CD68⁺ cells.

TABLE 4 Downregulated differentially expressed genes (DEGs) in Hi-Myc 6-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Adamts1</i> ^{a,b}	NM_009621.4	-6.18	663.43	107.32
<i>Fscn1</i> ^b	NM_007984.2	-4.38	116.83	26.65
<i>Mmp9</i> ^{a,b}	NM_013599.2	-4.21	333.42	79.26
<i>Hpgd</i> ^{a,b}	NM_008278.2	-3.62	556.16	153.62
<i>Cd163</i> ^{a,b}	NM_053094.2	-3.50	334.11	95.40
<i>Cxcl13</i> ^{a,b}	NM_018866.2	-2.69	215.22	79.96
<i>Btg2</i> ^b	NM_007570.2	-2.42	1991.65	823.50
<i>Pf4</i>	NM_019932.4	-2.26	481.69	213.24
<i>Alox5</i>	NM_009662.2	-2.24	217.95	97.50
<i>Jun</i> ^{a,b}	NM_010591.2	-2.19	4083.73	1863.74
<i>Mmp12</i> ^{a,b}	NM_008605.3	-2.15	3037.69	1410.60
<i>Tuba4a</i> ^{a,b}	NM_009447.3	-2.11	182.43	86.28
<i>H2-Ob</i> ^b	NM_010389.3	-2.01	191.99	95.40
<i>Icosl</i> ^{a,b}	NM_015790.3	-1.94	950.39	491.01
<i>Gem</i> ^{a,b}	NM_010276.3	-1.88	465.29	247.61
<i>Nfkbiz</i> ^{a,b}	NM_030612.1	-1.74	1987.55	1145.46
<i>H2-Eb1</i> ^b	NM_010382.2	-1.73	204.97	118.54
<i>Tnf</i> ^b	NM_013693.2	-1.72	1598.10	929.41
<i>Smad1</i>	NM_008539.3	-1.69	136.65	80.67
<i>Gas6</i>	NM_019521.2	-1.65	760.45	461.55
<i>Irf1</i> ^{a,b}	NM_008390.1	-1.64	698.96	427.18
<i>Klf10</i> ^b	NM_013692.2	-1.63	230.25	141.69
<i>Il12b</i> ^{a,b}	NM_001303244.1	-1.62	1506.55	928.71
<i>Stab1</i>	NM_138672.2	-1.60	758.40	472.77
<i>Col14a1</i> ^b	NM_181277.3	-1.56	642.93	411.05
<i>Myc</i> ^b	NM_010849.4	-1.55	194.04	125.56
<i>F11r</i> ^b	NM_172647.2	-1.48	243.23	164.14
<i>Insig1</i> ^b	NM_153526.5	-1.47	502.18	340.90
<i>Marcks1</i> ^b	NM_010807.4	-1.45	611.50	422.27
<i>Ccl3</i> ^{a,b}	NM_011337.1	-1.44	14366.52	10008.21
<i>Nr4a1</i> ^{a,b}	NM_010444.1	-1.43	854.73	598.33
<i>Ccl4</i> ^b	NM_013652.1	-1.43	3440.12	2404.55
<i>H2-Ea</i> ^{ps} ^b	NM_010381.2	-1.35	4549.02	3365.53
<i>Plau</i> ^b	NM_008873.2	-1.35	2649.61	1958.43
<i>Atf3</i> ^b	NM_007498.3	-1.33	6386.94	4802.09

Abbreviation: TRAMP, transgenic adenocarcinoma of the mouse prostate.

^aCommonly downregulated across cohorts in TG compared to strain-matched, age-matched WT.

^bCommonly downregulated across cohorts in Hi-Myc 6 months and TRAMP 5 months.

2.5 | Three-dimensional (3D) cell density analysis

Formalin-fixed paraffin-embedded prostate of a representative 14-month-old TG Hi-Myc mouse was serially sectioned into 150 4- μ m layers and stained in the following pattern: hematoxylin and eosin (H&E), F4/80 IHC, skip, H&E, F4/80, skip, and so on. H&E and F4/80 stains were scanned at $\times 20$ resolution. H&E stains were used to calculate total cell density of nucleated cells. The individual tissue images were aligned into a digital tissue volume using a nonlinear image registration program executed in MATLAB 2020a. Overall cell counts were determined using the hematoxylin channel of the images, and macrophage cell counts were determined using the antibody channel of the F4/80 stained IHC sections. Correction factors were used to estimate the true 3D cell count from the serial 2D images. 3D cell density as a function of distance from regions of interest were calculated for a number of locations in the tissue. Regions of carcinogenic tissue were identified by a pathologist.

2.6 | Macrophage gene expression

Prostate tissue was subjected to single cell dissociation using enzymatic digestion. Tissue was incubated in a collagenase and hyaluronidase mixture (07912; STEMCELL Technologies) in DMEM/F12K media supplemented with 5% heat-inactivated fetal bovine serum (FBS) for 3 h at 37°C under agitation. Red blood cell lysis was performed with a 1:4 mixture of Hank's Balanced Salt Solution Modified supplemented with 2% heat inactivated FBS and 1% wt/vol ammonium chloride in HBSS. Tissue was further digested using a 5:1 mixture of 5 U/ml Dispase (07913; STEMCELL Technologies) and 1 mg/ml DNase I (07469; STEMCELL Technologies) with continuous mild agitation for 1 min before filtering through a 40- μ m cell strainer. Due to the low cell and macrophage numbers, equivalent cell numbers from each of 10 mice within a cohort were pooled following single cell dissociation. Suspended cells were washed (PBS, 0.5% bovine serum albumin, 2 mM EDTA), blocked with mouse Fc block (Rat anti-mouse CD16/CD32, clone 2.4G2, 553141; BD Biosciences), and incubated with APC-conjugated anti-mouse CD11b (101212; Biolegend) and PE-conjugated anti-mouse F4/80 (123110; Biolegend) in the dark for 45 min at 4°C. Cells were washed and incubated with 1 μ g/million cells 7-aminoactinomycin D (7AAD, A1310; Thermo Fisher Scientific) for 10 min before sorting up to 20 million CD11b⁺F4/80⁺7AAD⁻ cells into Qiazol lysis buffer (Qiagen) using a FACSaria II cell sorter (BD Biosciences). RNA was purified using the miRNeasy Micro Kit (Qiagen). Expression levels of 770 immune-related genes were assessed by mouse nCounter Myeloid Innate Immunity Panel and custom Panel Plus (NanoString Technologies) containing the following RNA transcripts listed in Table S2. Hybridization for each sample was performed using 20 ng of RNA measured by Bioanalyzer

TABLE 5 Upregulated differentially expressed genes (DEGs) in TRAMP 2-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Cd84^a</i>	NM_001252472.1	4.83	53.8	260.11
<i>Tspan8</i>	NM_001168680.1	4.83	89.1	430.58
<i>Lpl</i>	NM_008509.2	4.21	53.8	226.31
<i>Il1a</i>	NM_010554.4	3.43	312.7	1071.3
<i>Clec7a</i>	NM_020008.2	3.22	154.67	498.18
<i>Retnla</i>	NM_020509.3	3.00	1220.54	3666.53
<i>Ptprc</i>	NM_011210.3	2.90	248.82	721.55
<i>Arg2</i>	NM_009705.2	2.82	67.25	189.57
<i>Crem</i>	NM_001110853.1	2.81	79.02	221.9
<i>Il13ra1</i>	NM_133990.4	2.44	164.76	401.19
<i>Gpr65</i>	NM_008152.2	2.33	147.94	345.34
<i>Msr1</i>	NM_001113326.1	2.32	593.46	1374.03
<i>Itgav</i>	NM_008402.2	2.29	206.79	473.2
<i>Ctss</i>	NM_021281.2	2.25	2311.63	5197.81
<i>Rgs1</i>	NM_015811.1	2.21	995.26	2201.39
<i>Cd36</i>	NM_007643.3	2.21	479.14	1061.02
<i>Nampt</i>	NM_021524.1	2.19	433.75	947.86
<i>Isg15^{a,b}</i>	NM_015783.3	2.15	257.22	554.02
<i>Hpgds</i>	NM_019455.4	2.14	210.15	449.68
<i>Ell2</i>	NM_138953.2	2.13	122.73	261.58
<i>Skil</i>	NM_011386.2	2.12	1055.79	2239.6
<i>Tnfrsf8</i>	NM_134131.2	2.10	215.19	451.15
<i>Malt1</i>	NM_172833.2	1.99	450.56	897.9
<i>Anxa4</i>	NM_013471.2	1.98	257.22	509.93
<i>Tgfb1</i>	NM_009370.2	1.94	1289.47	2498.24
<i>Hif1a</i>	NM_010431.2	1.93	376.59	727.43
<i>Fcgr2b^{a,b}</i>	NM_001077189.1	1.91	401.8	767.11
<i>H2-D1</i>	NM_010380.3	1.89	2686.54	5090.53
<i>Txn1^a</i>	NM_011660.3	1.86	536.3	996.36
<i>Ccl2</i>	NM_011333.3	1.86	3927.25	7286.04
<i>Grn^{a,b}</i>	NM_008175.4	1.84	430.38	793.56
<i>Ptgs2</i>	NM_011198.3	1.84	4171.02	7688.7
<i>Itgb1</i>	NM_010578.1	1.84	633.81	1163.89
<i>Mrc1</i>	NM_008625.1	1.83	911.2	1669.41
<i>Fem1c</i>	NM_173423.4	1.82	482.5	880.26
<i>Ctsd^{a,b}</i>	NM_009983.2	1.81	3263.18	5895.85
<i>S100a11</i>	NM_016740.3	1.79	198.38	355.63

(Continues)

TABLE 5 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Cxcl10</i>	NM_021274.1	1.77	2123.34	3763.52
<i>Ccr1^a</i>	NM_009912.4	1.76	161.39	283.62
<i>Ceacam1</i>	NM_001039185.1	1.74	193.34	336.53
<i>Apoe^{a,b}</i>	NM_001305844.1	1.73	10460.34	18115.18
<i>Cd86</i>	NM_019388.3	1.73	1284.43	2219.02
<i>Ccl12</i>	NM_011331.2	1.72	1202.05	2064.72
<i>Ifnb1</i>	NM_010510.1	1.71	769.98	1316.72
<i>Birc3</i>	NM_007464.3	1.69	440.47	743.59
<i>Ccl3</i>	NM_011337.1	1.64	12191.97	19981.51
<i>Gnai3</i>	NM_010306.2	1.62	258.9	418.82
<i>Cd274</i>	NM_021893.2	1.60	482.5	772.98
<i>Vegfa^a</i>	NM_001025250.3	1.55	285.8	442.34
<i>Ccl7</i>	NM_013654.3	1.55	1269.3	1972.14
<i>Cybb</i>	NM_007807.2	1.51	1101.18	1666.47
<i>Peli1</i>	NM_023324.2	1.48	521.17	770.05
<i>Il17ra</i>	NM_008359.1	1.48	479.14	709.79
<i>Mafb</i>	NM_010658.2	1.47	909.52	1337.29
<i>Osm</i>	NM_001013365.2	1.42	793.52	1127.15
<i>Cxcr4</i>	NM_009911.3	1.42	585.05	830.3
<i>Cdc42</i>	NM_009861.1	1.38	3024.46	4185.29
<i>Il1rn</i>	NM_031167.5	1.34	2928.63	3938.4

Abbreviation: TRAMP, transgenic adenocarcinoma of the mouse prostate.

^aCommonly upregulated across cohorts in TG compared to strain-matched, age-matched WT.^bCommonly upregulated across cohorts in Hi-Myc 2 months, Hi-Myc 6 months, TRAMP 2 months, and TRAMP 5 months.**TABLE 6** Downregulated differentially expressed genes (DEGs) in TRAMP 2-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Gsn^a</i>	NM_146120.3	-1.72	692.65	402.66
<i>Pf4</i>	NM_019932.4	-1.70	630.45	370.33
<i>Cxcl3^a</i>	NM_203320.2	-1.69	659.03	389.43
<i>Marcks1^a</i>	NM_010807.4	-1.56	1696.32	1086
<i>Hist2h2aa1</i>	NM_013549.2	-1.46	801.93	548.14
<i>Vaspa^a</i>	NM_009499.2	-1.44	1299.56	905.24
<i>Sqstm1^a</i>	NM_011018.2	-1.36	7950.33	5859.11

^aCommonly downregulated across cohorts in TG compared to strain-matched, age-matched WT.

TABLE 7 Upregulated differentially expressed genes (DEGs) in TRAMP 5-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Arg1</i> ^b	NM_007482.3	At least 7.80	Below threshold	156.07
<i>Fn1</i> ^b	NM_010233.1	6.82	48.96	334.07
<i>Adam8</i> ^b	NM_007403.2	6.26	43.81	274.09
<i>Chil3</i>	NM_009892.2	At least 6.06	Below threshold	121.25
<i>Cd38</i>	NM_007646.4	5.94	38.66	229.59
<i>Mmp19</i>	NM_021412.2	5.33	24.91	132.86
<i>Tuba1a</i>	NM_011653.2	3.69	85.04	313.44
<i>Ccl6</i> ^b	NM_009139.2	3.45	121.12	417.91
<i>Ccl8</i> ^b	NM_021443.2	3.36	404.60	1358.87
<i>Irf7</i> ^b	NM_016850.2	3.32	36.08	119.96
<i>Ctsd</i> ^{a,b,c}	NM_009983.2	3.31	2294.45	7601.78
<i>Lag3</i>	NM_008479.1	3.22	30.07	96.74
<i>Chil4</i>	NM_145126.2	At least 3.10	Below threshold	61.91
<i>Ear3</i>	NM_017388.1	At least 3.10	Below threshold	61.91
<i>Amica1</i>	NM_001005421.4	2.95	35.22	103.83
<i>Fyn</i> ^b	NM_008054.2	2.83	58.41	165.10
<i>Fcgr2b</i> ^{a,b,c}	NM_001077189.1	2.77	675.19	1867.72
<i>Emp1</i> ^b	NM_010128.4	2.57	94.49	242.49
<i>Ccl9</i> ^b	NM_011338.2	2.54	897.68	2279.83
<i>Cd84</i> ^{a,b}	NM_001252472.1	2.44	185.55	453.39
<i>Tgm2</i> ^b	NM_009373.3	2.44	295.50	721.68
<i>Hist1h1c</i>	NM_015786.3	2.40	230.22	552.06
<i>Top2a</i>	NM_011623.2	2.32	71.30	165.75
<i>Fcgr4</i> ^b	NM_144559.1	2.29	181.25	414.69
<i>C4a</i> ^b	NM_011413.2	2.24	256.85	575.92
<i>Vegfa</i> ^{a,b}	NM_001025250.3	2.21	182.97	403.73
<i>ApoE</i> ^{a,b,c}	NM_001305844.1	2.16	10583.17	22829.21
<i>Tlr8</i> ^b	NM_133212.2	2.12	201.87	427.59
<i>Gri</i> ^{a,b,c}	NM_008175.4	2.02	550.63	1110.57
<i>Mertk</i>	NM_008587.1	1.96	62.71	123.18
<i>Vcam1</i>	NM_011693.2	1.93	317.84	613.33
<i>Ccr2</i> ^b	NM_009915.2	1.89	79.89	150.91

TABLE 7 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Anxa1</i> ^b	NM_010730.2	1.86	66.14	123.18
<i>Ccr1</i> ^{a,b}	NM_009912.4	1.82	193.28	350.84
<i>Fcgr1</i> ^b	NM_010186.5	1.81	354.78	641.70
<i>Serpina6a</i>	NM_001164117.1	1.78	88.48	157.36
<i>Itgam</i>	NM_001082960.1	1.77	302.38	534.00
<i>Txn1</i> ^{a,b}	NM_011660.3	1.70	621.93	1059.62
<i>Adgre5</i>	NM_011925.1	1.68	128.85	216.70
<i>Mif</i> ^b	NM_010798.2	1.68	158.06	265.71
<i>Isg15</i> z ^{a,b,c}	NM_015783.3	1.65	181.25	298.60
<i>Tlr13</i>	NM_205820.1	1.64	217.33	355.36
<i>Acly</i>	NM_134037.2	1.61	154.62	248.94
<i>Csf2rb</i> ^b	NM_007780.4	1.57	284.34	446.29
<i>S100a4</i>	NM_011311.2	1.56	160.64	250.23
<i>Trem2</i> ^b	NM_031254.2	1.53	305.81	466.93
<i>Fcgr3</i>	NM_010188.5	1.48	801.47	1187.96
<i>Cd47</i> ^b	NM_010581.3	1.47	300.66	441.13
<i>Hist2h2aa1</i>	NM_013549.2	1.44	673.47	972.55
<i>Ctnnb1</i> ^b	NM_007614.2	1.43	547.20	781.01
<i>C1qb</i> ^b	NM_009777.2	1.38	3853.58	5321.96

Abbreviation: TRAMP, transgenic adenocarcinoma of the mouse prostate.

^aCommonly upregulated across cohorts in TG compared to strain-matched, age-matched WT.

^bCommonly upregulated across cohorts in Hi-Myc 6 months and TRAMP 5 months.

^cCommonly upregulated across cohorts in Hi-Myc 2 months, Hi-Myc 6 months, TRAMP 2 months, and TRAMP 5 months.

(Agilent). Gene expression was analyzed with nSolver software 4.0 (NanoString Technologies). The expression levels of each gene were normalized to those of control genes. Heat maps and unsupervised hierarchical clustering were generated in nSolver with agglomerative cluster analysis using average Euclidean distance. All genes with significant differential expression of $p < .01$ between strain-matched, age-matched TG and WT cohorts are listed in Tables 1–8. Thresholds for all genes were set to 20 counts.

2.7 | Statistical analysis

Differentially expressed gene analyses were performed in nSolver software 4.0 (NanoString Technologies) using the Differential Expression Call Error Model. Statistical analysis for IHC quantification analyses were performed using GraphPad Prism version 8. Outliers

TABLE 8 Downregulated differentially expressed genes (DEGs) in TRAMP 5-month transgenic (TG) compared to wild type (WT) prostate macrophages

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Adamts1^b</i>	NM_009621.4	-14.25	578.98	40.63
<i>Il12b^b</i>	NM_001303244.1	-12.78	4072.63	318.60
<i>Mmp12^b</i>	NM_008605.3	-9.39	4453.18	474.02
<i>Mmp9^b</i>	NM_013599.2	-6.50	427.79	65.78
<i>Tnf^b</i>	NM_013693.2	-6.45	1822.85	282.48
<i>Cxcl1</i>	NM_008176.1	-5.96	6806.04	1142.17
<i>Ccr7</i>	NM_007719.2	-5.76	367.66	63.85
<i>Plau^b</i>	NM_008873.2	-5.60	3150.90	562.38
<i>Gem^b</i>	NM_010276.3	-4.86	548.92	112.86
<i>Ccl22</i>	NM_009137.2	-4.86	397.73	81.91
<i>Ccl3^b</i>	NM_011337.1	-4.72	16382.43	3471.65
<i>Cyr61</i>	NM_010516.1	At least -4.64	92.77	Below threshold
<i>Il1a</i>	NM_010554.4	-4.54	626.23	138.01
<i>Ripk2</i>	NM_138952.3	-4.50	214.76	47.72
<i>Areg</i>	NM_009704.3	At least -4.34	86.76	Below threshold
<i>Maff</i>	NM_010755.3	-4.29	365.08	85.13
<i>Ifnb1</i>	NM_010510.1	-4.19	624.51	148.98
<i>Irf1^b</i>	NM_008390.1	-4.17	940.63	225.73
<i>H2-Eb1^b</i>	NM_010382.2	-4.05	373.68	92.22
<i>Icam1</i>	NM_010493.2	-4.03	468.17	116.09
<i>H2-Ea-ps^b</i>	NM_010381.2	-4.00	5752.02	1438.84
<i>Malt1</i>	NM_172833.2	-3.94	505.96	128.34
<i>Cd40</i>	NM_011611.2	-3.90	251.69	64.49
<i>Icosl^b</i>	NM_015790.3	-3.75	1401.93	373.41
<i>Nlrp3</i>	NM_145827.3	-3.74	1260.19	336.65
<i>Ccl4^b</i>	NM_013652.1	-3.72	3965.25	1066.71
<i>Cxcl2</i>	NM_009140.2	-3.72	7675.37	2061.84
<i>Tnfrsf12a</i>	NM_001161746.1	-3.67	747.35	203.80
<i>Hpgd^b</i>	NM_008278.2	-3.65	548.06	150.27
<i>Hbegf</i>	NM_010415.1	-3.62	133.15	36.76
<i>Btg2^b</i>	NM_007570.2	-3.41	1407.94	413.40
<i>Nfkbiz^b</i>	NM_030612.1	-3.40	1962.01	576.57
<i>Sqstm1^a</i>	NM_011018.2	-3.39	3775.41	1113.79
<i>Kitl</i>	NM_013598.1	-3.38	141.74	41.92

(Continues)

TABLE 8 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Tuba4a^b</i>	NM_009447.3	-3.36	164.93	49.01
<i>Dusp2</i>	NM_010090.2	-3.34	400.30	119.96
<i>Traf1</i>	NM_009421.3	-3.26	107.38	32.89
<i>Skil</i>	NM_011386.2	-3.25	2134.67	655.89
<i>Cd83</i>	NM_009856.2	-3.24	9731.88	3004.73
<i>Cd69</i>	NM_001033122.3	-3.23	137.44	42.57
<i>Tnfaip3</i>	NM_009397.2	-3.08	3514.26	1141.52
<i>Jun^b</i>	NM_010591.2	-3.06	3102.79	1012.54
<i>Nfkb1</i>	NM_008689.2	-2.97	926.89	312.15
<i>Cd86</i>	NM_019388.3	-2.89	1693.99	586.24
<i>Batf</i>	NM_016767.2	-2.89	329.86	114.15
<i>H2-Ob^b</i>	NM_010389.3	-2.80	232.80	83.20
<i>Ptgs2</i>	NM_011198.3	-2.79	3070.15	1098.96
<i>Il10</i>	NM_010548.1	-2.77	155.48	56.11
<i>Mmp13</i>	NM_008607.1	-2.77	369.38	133.50
<i>Cd36</i>	NM_007643.3	-2.76	1201.77	435.33
<i>Il1b</i>	NM_008361.3	-2.75	5181.63	1885.13
<i>Tlr2</i>	NM_011905.2	-2.73	2097.74	769.40
<i>Sema4a</i>	NM_013658.3	-2.73	207.88	76.10
<i>Gadd45b</i>	NM_008655.1	-2.70	1660.49	614.62
<i>Rab20</i>	NM_011227.1	-2.61	1502.43	574.63
<i>Nfkbia</i>	NM_010907.2	-2.55	974.13	382.44
<i>Insig1^b</i>	NM_153526.5	-2.53	485.35	191.54
<i>Clic4</i>	NM_013885.2	-2.47	552.35	223.79
<i>Ier3</i>	NM_133662.2	-2.46	1203.49	488.86
<i>Retnla</i>	NM_020509.3	-2.45	2962.77	1209.89
<i>Atf3^b</i>	NM_007498.3	-2.45	6289.77	2570.04
<i>Pdgfb</i>	NM_011057.3	-2.41	321.27	133.50
<i>Birc2</i>	NM_007465.2	-2.38	249.98	105.12
<i>Klf10^b</i>	NM_013692.2	-2.36	217.33	92.22
<i>Ccrl2</i>	NM_017466.4	-2.34	6117.96	2619.70
<i>Myc^b</i>	NM_010849.4	-2.30	240.53	104.48
<i>Birc3</i>	NM_007464.3	-2.29	632.24	276.67
<i>Nr4a1^b</i>	NM_010444.1	-2.29	864.18	377.28
<i>Cxcl10</i>	NM_021274.1	-2.26	1708.60	757.15
<i>Map2k3</i>	NM_008928.4	-2.26	284.34	125.76
<i>Cxcl16</i>	NM_023158.6	-2.24	5679.86	2538.44
<i>C3</i>	XM_011246258.1	-2.24	396.87	177.36

(Continues)

TABLE 8 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Pdgfra</i>	NM_008808.3	-2.21	396.01	179.29
<i>Tlr9</i>	NM_031178.2	-2.20	302.38	137.37
<i>Rgs1</i>	NM_015811.1	-2.19	3369.95	1539.45
<i>Gsn</i> ^a	NM_146120.3	-2.16	484.49	223.79
<i>Il1r2</i>	NM_010555.4	-2.16	116.83	54.17
<i>Hpgds</i>	NM_019455.4	-2.13	538.61	252.81
<i>Marcksl1</i> ^{a,b}	NM_010807.4	-2.13	761.95	357.94
<i>Mob3c</i>	NM_175308.4	-2.09	99.65	47.72
<i>Hivep1</i>	NM_007772.2	-2.07	224.21	108.35
<i>F11r</i> ^b	NM_172647.2	-2.06	248.26	120.60
<i>Vasp</i> ^a	NM_009499.2	-2.05	721.58	352.13
<i>Nfkbie</i>	NM_008690.3	-2.05	330.72	161.23
<i>Col14a1</i> ^b	NM_181277.3	-2.04	646.84	317.31
<i>Tlr1</i>	NM_030682.1	-2.02	329.01	162.52
<i>Cd14</i>	NM_009841.3	-2.02	4630.14	2289.50
<i>Peli1</i>	NM_023324.2	-2.01	578.12	287.64
<i>Ptafr</i>	NM_001081211.1	-2.00	1607.23	802.29
<i>Id2</i>	NM_010496.3	-1.98	5803.56	2929.27
<i>Vav1</i>	NM_011691.4	-1.98	839.27	423.07
<i>H2-Aa</i>	NM_010378.2	-1.96	49289.90	25211.58
<i>Cd74</i>	NM_001042605.1	-1.92	52908.97	27566.86
<i>Cxcl3</i> ^a	NM_203320.2	-1.91	203.59	106.41
<i>Gpr183</i>	NM_183031.2	-1.90	325.57	170.91
<i>Igf1r</i>	NM_010513.2	-1.88	182.11	96.74
<i>Ccl2</i>	NM_011333.3	-1.87	3364.79	1800.64
<i>Il21r</i>	NM_021887.1	-1.86	444.97	239.27
<i>Il10ra</i>	NM_008348.2	-1.82	586.71	323.11
<i>H2-Ab1</i>	NM_207105.2	-1.81	13811.38	7618.55
<i>Axl</i>	NM_009465.3	-1.79	2632.05	1474.31
<i>Socs3</i>	NM_007707.2	-1.78	715.57	401.15
<i>Rgl1</i>	NM_016846.3	-1.77	174.38	98.67
<i>Cxcl9</i>	NM_008599.2	-1.76	163.21	92.87
<i>Irf8</i>	NM_008320.3	-1.76	323.85	184.45
<i>Cd163</i> ^b	NM_053094.2	-1.75	346.19	197.99
<i>Tgfbr1</i>	NM_009370.2	-1.73	2756.61	1596.20
<i>Ets1</i>	NM_001038642.1	-1.72	175.24	101.90
<i>Il10rb</i>	NM_008349.5	-1.72	1634.72	948.05
<i>Stat6</i>	NM_009284.2	-1.70	512.84	302.47

TABLE 8 (Continued)

Gene	Accession #	Fold change	Normalized counts	
			WT	TG
<i>Traf2</i>	NM_009422.2	-1.70	147.75	87.07
<i>Fscn1</i> ^b	NM_007984.2	-1.70	144.32	85.13
<i>Ifnar1</i>	NM_010508.1	-1.68	691.51	410.82
<i>Arhgef6</i>	NM_152801.2	-1.67	363.37	217.99
<i>Clec5a</i>	NM_001038604.1	-1.66	201.87	121.25
<i>Il6ra</i>	NM_010559.2	-1.65	256.85	156.07
<i>Tgfbr2</i>	NM_029575.3	-1.62	1986.92	1229.88
<i>Irf5</i>	NM_001252382.1	-1.60	700.10	437.91
<i>Stat3</i>	NM_213659.2	-1.60	477.62	297.96
<i>Il13ra1</i>	NM_133990.4	-1.59	483.63	304.41
<i>Cxcl13</i> ^b	NM_018866.2	-1.58	293.79	185.74
<i>Cd180</i>	NM_008533.2	-1.58	256.85	162.52
<i>Ccl5</i>	NM_013653.1	-1.58	299.80	190.25
<i>H2-DMA</i>	NM_010386.3	-1.57	819.51	521.10
<i>Fem1c</i>	NM_173423.4	-1.56	595.30	381.80
<i>Cybb</i>	NM_007807.2	-1.56	1993.79	1281.47
<i>Dusp1</i>	NM_013642.3	-1.54	2810.72	1829.02
<i>Klf4</i>	NM_010637.3	-1.53	370.24	241.20
<i>Smad7</i>	NM_001042660.1	-1.53	222.49	145.75
<i>Csf1r</i>	NM_001037859.1	-1.52	3047.81	2007.67
<i>Nampt</i>	NM_021524.1	-1.51	666.60	440.49
<i>Lat2</i>	NM_020044.2	-1.51	329.01	217.99
<i>H2-K1</i>	NM_001001892.2	-1.48	6023.47	4082.40
<i>Serpine1</i>	NM_008871.2	-1.43	444.12	309.57
<i>Vwa5a</i>	NM_172767.3	-1.43	344.47	241.20
<i>C3ar1</i>	NM_009779.2	-1.43	2108.04	1474.95
<i>Il1rn</i>	NM_031167.5	-1.42	856.45	604.30
<i>Cxcl14</i>	NM_019568.2	-1.40	383.12	272.81
<i>Cdkn1a</i>	NM_007669.4	-1.40	603.89	430.17
<i>Adgre1</i>	NM_010130.1	-1.38	1803.09	1307.27
<i>Furin</i>	NM_011046.2	-1.38	657.15	476.60

Abbreviation: TRAMP, transgenic adenocarcinoma of the mouse prostate.

^aCommonly downregulated across cohorts in TG compared to strain-matched, age-matched WT.

^bCommonly downregulated across cohorts in Hi-Myc 6 months and TRAMP 5-months.

were identified by Grubbs' test with a false discovery rate (q) = 0.05. All results are expressed as means $\pm$ SD. Data were analyzed using one- or two-way analysis of variance as specified. Differences were considered significant at $p < .05$. Figures denote statistical significance of $p < .05$ as *, $p < .01$ as **, $p < .001$ as ***, and $p < .0001$ as ****.

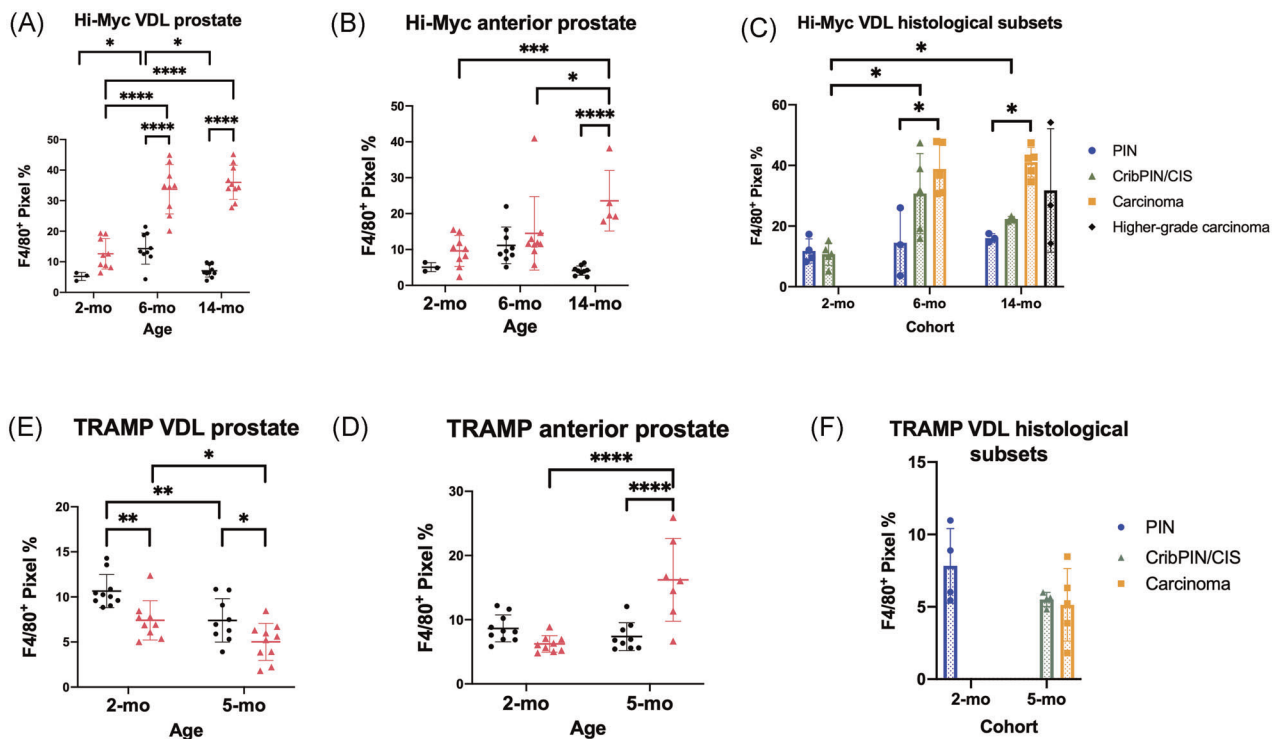


FIGURE 1 Macrophage infiltration into Hi-Myc and TRAMP prostate tissue. Macrophage density was measured by quantification IHC staining of F4/80⁺ DAB stain pixels normalized to the sum of hematoxylin pixels and F4/80⁺ DAB stain pixels. Each data point represents one region in one mouse. Macrophage density was measured for different subsets: (A) Hi-Myc ventral and dorsolateral (VDL) lobes, (B) Hi-Myc anterior lobes, (D) TRAMP VDL lobes, and (E) anterior lobes. Regions of (C) Hi-Myc and (F) TRAMP prostate H&E tissue were classified histologically as prostatic intraepithelial neoplasia (PIN), cribriform PIN/carcinoma in situ (CribPIN/CIS), carcinoma, or higher-grade carcinoma. Significance was determined by two-way ANOVA with * $p < .05$, ** $p < .01$, *** $p < .001$, and **** $p < .0001$. Black circles = WT. Pink triangles = TG. ANOVA, analysis of variance; H&E, hematoxylin and eosin; TG, transgenic; TRAMP, transgenic adenocarcinoma of the mouse prostate; WT, wild type [Color figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS AND DISCUSSION

3.1 | Macrophage infiltration increases in Hi-Myc prostates with age, tumor presence, and histological grade

In an analysis of macrophage densities using IHC quantification of the pan-macrophage marker F4/80, Hi-Myc prostates exhibited an overall increase in macrophage density in TG prostates compared to WT prostates at each age group (2, 6, and 14 months) (Figure 1A). Increased macrophage density was also observed in Hi-Myc prostate tissue as it progressed in either age or histological grade (Figure 1A-C).

The changes in macrophage infiltration were mainly observed in the ventral and dorsolateral (VDL) lobes which are the sites of most precancer and invasive carcinoma development in Hi-Myc TG mice. Interestingly, however, increased macrophage density with presence of TG compared to WT and with increasing age was also observed in the adjacent anterior lobe tissue at 14 months (Figure 1B). While 10% of anterior lobes from 6 months old and 80% of anterior lobes from 14-month-old Hi-Myc mice contained regions of cribriform PIN/carcinoma in situ (CribPIN/CIS) or invasive adenocarcinoma.

The increase in macrophage infiltration in Hi-Myc TG VDL lobes was corroborated by flow cytometry analysis of macrophage populations. Macrophage levels were significantly increased in prostates from 6- and 14-month old Hi-Myc TG mice compared to all other cohorts (Figure 2A). Overall, these data suggest that in Hi-Myc mice development of adenocarcinoma tissue induces higher levels of macrophages in the prostate and is similar to the increases in macrophage density observed in prostate cancer patients.²

3.2 | Macrophage density by 3D spatial analysis varies widely throughout Hi-Myc prostate tumor tissue

To better understand macrophage spatial density throughout the tissue, 3D cell density analysis was performed on a representative 14-month Hi-Myc TG prostate. Macrophage densities varied across different regions of the tissue (Figure 3A-C). Total cell densities within a tumor (ROI1) were higher than tissue adjacent to tumor (ROI2). Macrophage densities proximal to ROI1 were lower than those proximal to ROI2 (Figure 3D,E). In this comparison, macrophage infiltration was higher in the tumor-adjacent tissue than in the

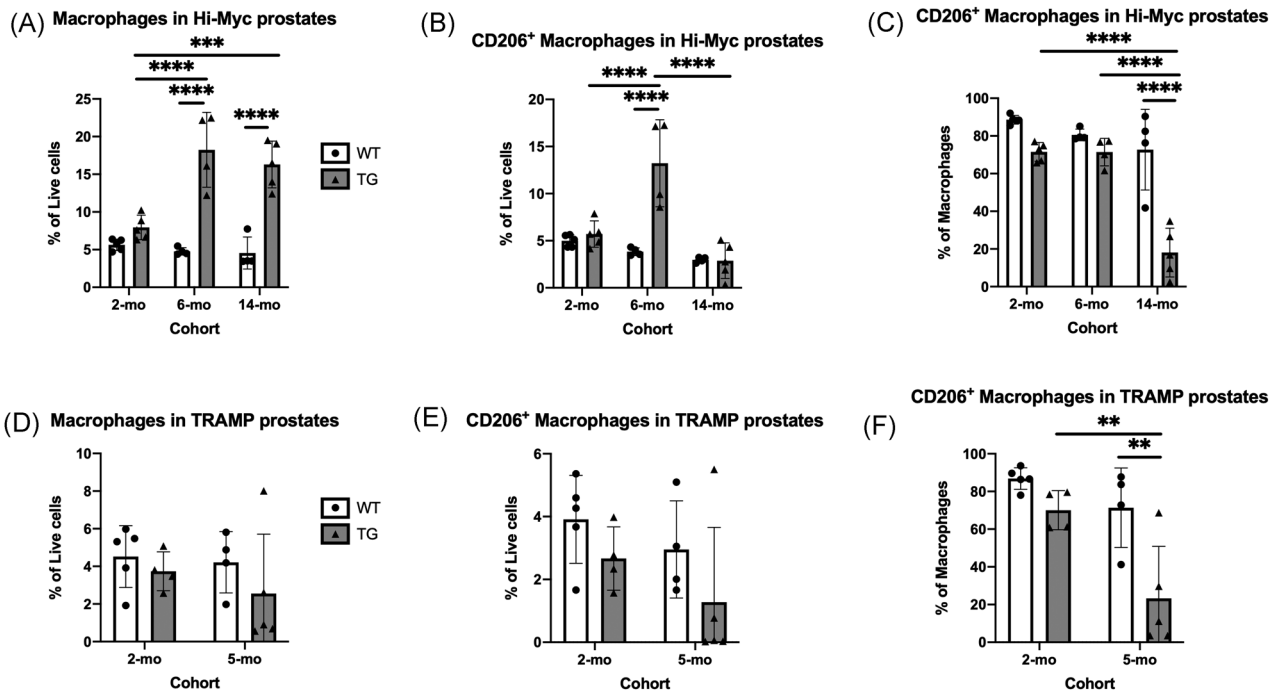


FIGURE 2 Macrophage populations in Hi-Myc and TRAMP prostate tissue. CD45⁺CD11b⁺F4/80⁺CD68⁺ macrophage populations were determined by flow cytometry. (A) Macrophages as a percentage of live cells in Hi-Myc prostates. (B) CD206⁺ macrophages as a percentage of live cells in Hi-Myc prostates. (C) CD206⁺ macrophages as a percentage of all macrophages in Hi-Myc prostates. (D) Macrophages as a percentage of live cells in TRAMP prostates. (E) CD206⁺ macrophages as a percentage of live cells in TRAMP prostates. (F) CD206⁺ macrophages as a percentage of all macrophages in TRAMP prostates. Significance was determined by two-way ANOVA with ***p* < .01, ****p* < .001, and *****p* < .0001. WT = wild type (white bars, circles); TG = transgenic (grey bars, triangles); mo = month. ANOVA, analysis of variance; TRAMP, transgenic adenocarcinoma of the mouse prostate

middle of a large region of tumor tissue. This is generally the trend throughout other points within this prostate however there are regions of tumor tissue that have higher macrophage infiltration. The variation in macrophage density throughout the dimensions of the tissue speak to the complexity of macrophage biology and the importance of spatial heterogeneity when considering how macrophages function within tumors. The 3D analysis technique provides a useful tool and further opportunity for investigating where TAMs and other cell types are acting within tumors. This information could be pertinent to determining how to target TAMs or other TME components.

3.3 | CD206⁺ macrophage populations decrease in late-stage Hi-Myc tumors

The pro-tumor macrophage marker mannose receptor CD206 expression was analyzed by flow cytometry to begin to differentiate the broad phenotypic characteristics of macrophages in the different models. CD206⁺ macrophage populations increased proportional to all live cells in 6-month-old TG mice compared to all other cohorts (Figure 2B). However, when analyzed as a proportion of macrophages, the percentage of CD206⁺ macrophages did not change between cohorts except in 14-month-old TG mice in which CD206⁺

macrophage proportions decreased (Figure 2C). This suggests that as prostate macrophage populations increase with tumor growth from 2 to 6 months, the proportion of macrophages that are CD206⁺ remains around 70%. As the tumor continues to grow out to 14 months, the number of CD206[−] macrophages overtake the number of CD206⁺ macrophages. Because CD206 is a pro-tumor macrophage marker, it can be concluded that 60%–80% of macrophages in WT prostates and 2- to 6-month-old TG prostates have some pro-tumor characteristics. While only about 20% of 14-month-old prostate macrophages express the pro-tumor marker CD206, the pro- and anti-tumor characteristics of these macrophages was then further explored.

3.4 | Macrophages in Hi-Myc TG prostates exhibit increased pro-tumor gene expression profiles

To further delineate macrophage phenotype in the Hi-Myc mice, FACS-separated CD11b⁺F4/80⁺ Hi-Myc prostate macrophages were analyzed for their myeloid gene expression by NanoString messenger RNA profiling. Overall, gene expression patterns of prostate macrophages from younger (2 months old) mice more closely resembled one another regardless of genotype, while older (6 and 14 months old) TG mice more closely resemble one another regardless of age

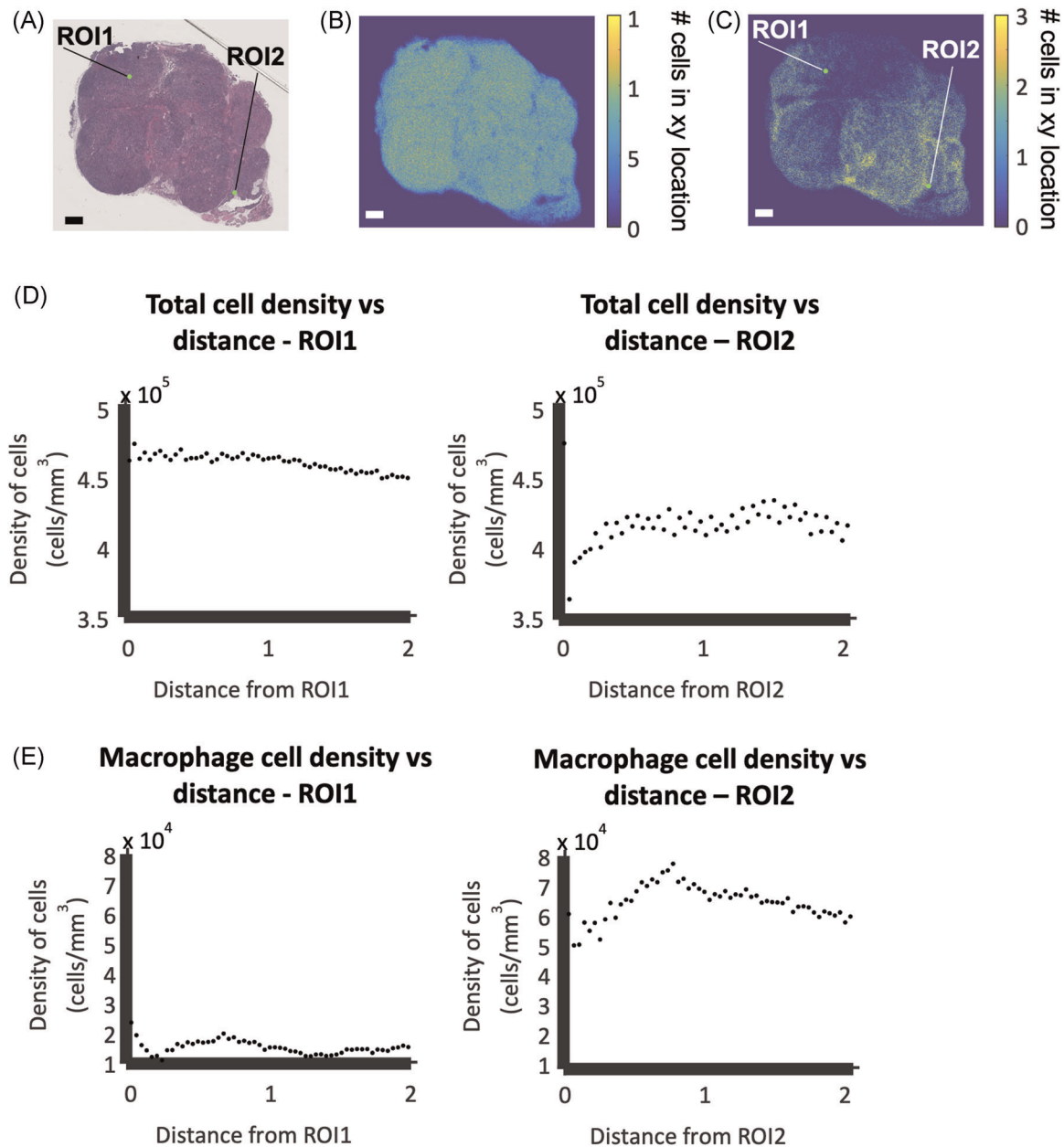


FIGURE 3 Macrophage 3D spatial analysis of Hi-Myc late-stage tumor. (A) H&E stain of a representative 4-μm section of a representative Hi-Myc 14-month-old TG prostate with regions of interest ROI1 and ROI2. (B) Compressed Z projection of all cells in the representative section. (C) Compressed Z projection of F4/80⁺ macrophages with ROI1 and ROI2. (D) Density of total cells measured by 3D density analysis compared to distance from ROI1 and ROI2. (E) Density of F4/80⁺ macrophages measured 3D density analysis compared to distance from ROI1 and ROI2. Scale bar = 2 mm. 3D, three dimensional; H&E, hematoxylin and eosin [Color figure can be viewed at wileyonlinelibrary.com]

(Figure 4A). The Hi-Myc 14-month WT sample was omitted as the sample input was below threshold. When limiting the analysis to key pro-tumor and anti-tumor macrophage genes, a similar trend was observed with the exception of 6-month WT macrophages clustering with 2-month WT and TG macrophages (Figure 4B). This suggests that tumor presence and growth is more influential on macrophage characteristics than age. Overall, prostate macrophages from Hi-Myc TG mice exhibited higher pro-tumor (*Cd206*, *Arg1*, *Il10*, *Vegfa*, and *Pd11*) and lower antitumor (*Tnf*, *Il1b*, *Il12b*, *Cd80*, and *Cd86*) macrophage gene expression compared to age-matched WT mice (Figure 4C–F). While not all genes (i.e., *Il1b* and *Cd80*) followed this

pattern, taken cumulatively the tumor-infiltrating prostate macrophages exhibited more pro-tumor characteristics which was further supported by the expression of various inflammatory genes including *Adam8*, *Adamst1*, *Ccl3*, *Cxcl13*, *Il1rn*, *Mmp9*, and *Mmp12* (Tables 1–4).

Notably, detection of CD206 differed between NanoString RNA expression analysis and flow cytometry surface protein expression. This is likely due to differences in RNA and protein expression and detection with flow cytometry detection of surface protein likely being the more biologically relevant assessment of CD206 expression. Apart from this discrepancy, prostate macrophages tended to demonstrate an overall pro-tumor RNA

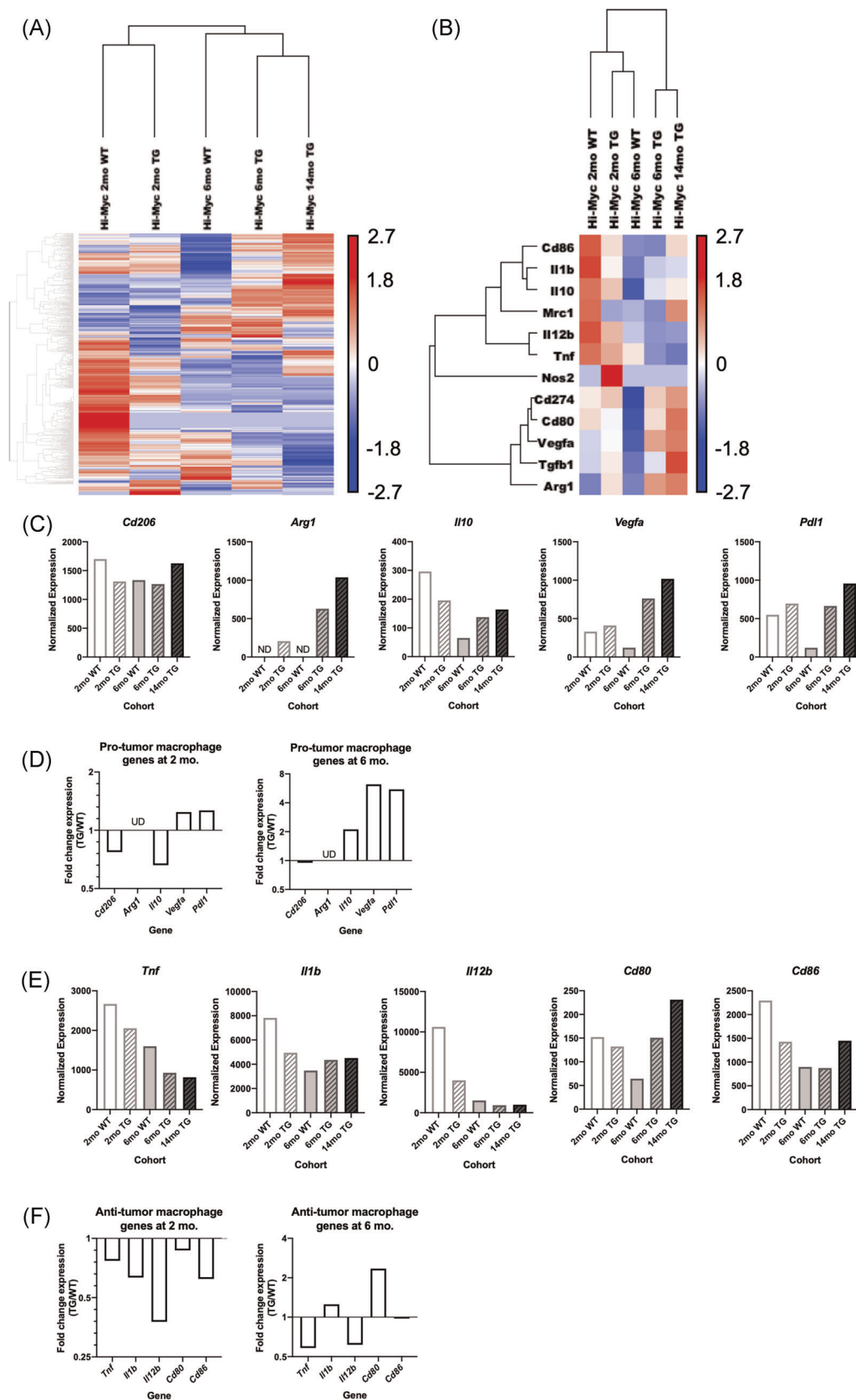


FIGURE 4 (See caption on next page)

expression and decrease in overall antitumor RNA expression in tumor-bearing mice.

Taken together, these data suggest that Hi-Myc adenocarcinoma tumor growth increases both macrophage density and the pro-tumor characteristics of infiltrating macrophages.

3.5 | Macrophage infiltration decreases in TRAMP prostates with tumor presence and age

When similar analyses were applied to TRAMP tissue, an opposite pattern of macrophage infiltration from the Hi-Myc mice was observed with decreased macrophage density in TRAMP TG tissue compared to WT at both 2 and 5 months (Figure 1D). Additionally, when comparing different ages within the same genotype, macrophage density decreased with age. However, no significant difference in macrophage density was observed between different histological lesion types within TRAMP TG VDL tissue (Figure 1F).

Counter to TRAMP VDL tissue but similar to Hi-Myc anterior tissue, macrophage density increased in older (5 months old) TRAMP TG anterior prostates (Figure 1E). At this age, 14% of anterior lobe samples had invasive carcinoma or CribPIN/CIS regions. However, given that disease stage did not correlate with higher macrophage density in TRAMP TG VDL, this increase in TG anterior lobe tissue is likely due to other factors such as increased stress and inflammation in the surrounding tumor-adjacent tissue.

Similar to the IHC results, flow cytometry analysis of macrophage populations in VDL tissue revealed that macrophage levels decreased in prostates from 5-month-old TG mice compared to all other cohorts (Figure 2D). Altogether, these data suggest that macrophage populations decrease with TRAMP tumor growth.

3.6 | CD206⁺ macrophage populations decrease in late-stage TRAMP tumors

Flow cytometry analysis showed a decrease in CD206⁺ macrophage populations in TRAMP TG mice compared to WT with the largest decrease at 5 months (Figure 2E). Additionally, the same trend was

observed when analyzing CD206⁺ macrophages as a proportion of all macrophages (Figure 2F). Similar to Hi-Myc mice, 60%–80% of macrophages in WT prostates and 2-month-old TRAMP TG prostates express the pro-tumor marker CD206. Also similar to Hi-Myc mice, only about 20% of macrophages from late stage tumor bearing mice express CD206. This suggests that as tumors grow and macrophage populations decrease, the proportion of macrophages that express CD206 also decrease. The following subsection further explores the pro- and anti-tumor characteristics of these macrophages with a larger array of genes.

3.7 | Macrophages in TRAMP TG prostates exhibit increased pro- and antitumor gene expression profiles at tumor initiation but decreased antitumor gene expression in late-stage tumors

FACS-separated macrophages from TRAMP prostates reveal that 5-month TG prostate macrophages have different expression profiles compared to 2-month WT, 2-month TG, and 5-month WT that all exhibit similar myeloid gene expression trends (Figure 5A). When limiting the analysis to key pro- and antitumor macrophage genes, a similar trend was observed with 2-month WT and TG macrophages closest in gene expression and 5-month WT macrophages more closely resembling 2-month WT and TG than 5-month TG macrophages (Figure 5B). Interestingly, the expression profiles of macrophages from 2-month TG and 5-month TG displayed opposite expression profiles suggesting macrophage expression changes drastically from early- to late-stage tumors (Figure 5C–F). This pattern of expression is also observed when limited to pro-tumor (*Cd206*, *Arg1*, *Il10*, *Vegfa*, and *Pdl1*) and antitumor (*Nos2*, *Tnf*, *Il1b*, *Il12b*, *Cd80*, and *Cd86*) macrophage gene expression with 2-month TG macrophages expressing high levels of all pro- and antitumor-associated genes but low levels of *Arg1* while 5-month TG macrophages expressed low levels of most pro- and antitumor-associated genes but high levels of *Arg1*. Unlike with Hi-Myc prostates, the decrease in CD206 expression in TRAMP prostates was consistent between RNA NanoString and flow cytometry analyses. Looking at select representative pro- and antitumorigenic macrophage RNA transcripts, prostate macrophages from 2-month-old TRAMP TG mice exhibit increase expression of both subsets suggesting these macrophages are generally more inflammatory compared to age-matched WT prostate macrophages. The reason for this is unknown,

FIGURE 4 Hi-Myc transgenic prostate macrophages express higher levels of pro-tumor genes. Hi-Myc wild type (WT) and transgenic (TG) prostate macrophages from 2-, 6-, and 14-month-old mice were analyzed by NanoString Myeloid Panel gene expression analysis. (A) Dendrograms and heat map of gene expression across all Myeloid Panel genes that were detected above background in at least one sample compared between each cohort. (B) Dendrograms and heat map of gene expression across select pro- and antitumor macrophage genes that were detected above background in at least one sample compared between each cohort. (C) Select pro-tumor macrophage genes normalized to control genes. (D) Fold change expression of pro-tumor macrophage genes in age-matched TG tissue relative to WT. (E) Select antitumor macrophage genes normalized to control genes. (F) Fold change expression of antitumor macrophage genes in age-matched TG tissue relative to WT. Heat maps were generated using unsupervised hierarchical clustering with average Euclidean distance. ND = not detected; UD = undefined due to below threshold expression of WT control [Color figure can be viewed at wileyonlinelibrary.com]

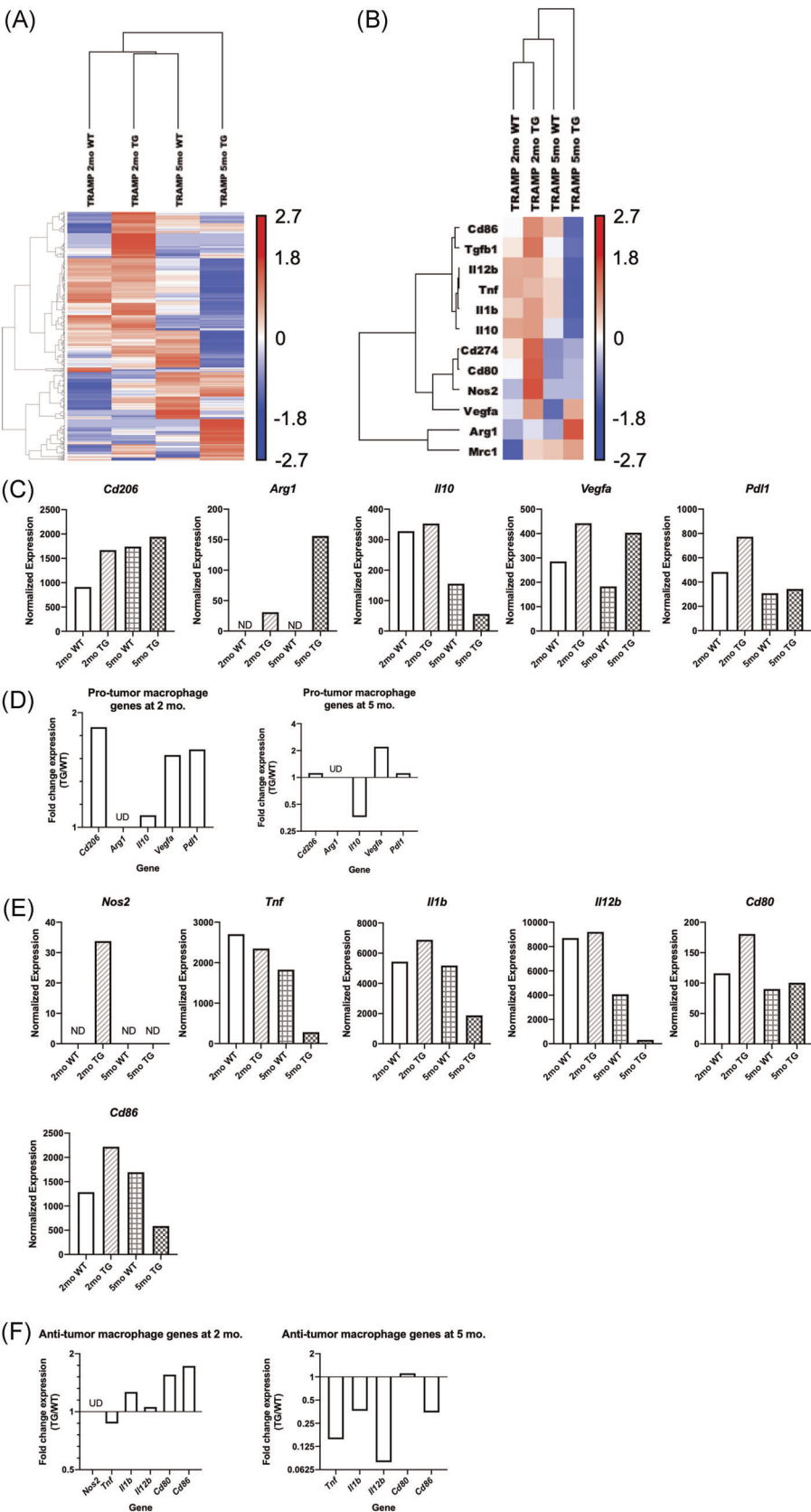


FIGURE 5 (See caption on next page)

though it is likely a response to the initial stages of tumor development and tissue reconstruction observed at this age in TRAMP mice.²⁴ Five-month-old TRAMP TG prostate macrophages exhibited more protumorigenic characteristics than those in the age-matched WT tissue, expressing higher levels of some protumorigenic genes and decreased levels of many antitumorigenic genes. As with the Hi-Myc mice, not all genes followed this pattern, but the cumulative gene expression changes point towards a more pro-tumor macrophage characteristic which was corroborated by various inflammatory genes such as *Ccr1*, *Cd84*, and *Cxcl3* in the Differential Expression Call analyses (Tables 5–8). Altogether these data suggest that while TRAMP neuroendocrine tumor growth decreases macrophage density, it increases the pro-tumor characteristics of infiltrating macrophages.

3.8 | Hi-Myc and TRAMP TAMs share common differentially regulated genes

Though Hi-Myc and TRAMP models exhibited unique macrophage characteristics, some similarities were also observed. Increases in *Apoe* (apolipoprotein E), *Ctsd* (cathepsin D), *Fcgr2b* (Fc receptor, immunoglobulin G, low affinity IIb), *Grn* (granulin), and *Isg15* (interferon-stimulated gene 15 ubiquitin-like modifier) were observed in all TG cohorts compared to age-matched WT mice. *Apoe* is a low-density lipoprotein ligand and promotes cholesterol uptake.²⁵ *Ctsd* promotes lysosomal activity and autophagy.²⁵ *Fcgr2b* is involved in antibody-mediated phagocytosis.²⁶ *Grn* promotes inflammation, is associated with proliferation, and promotes lysosomal function.^{27,28} *Isg15* has a variety of functions that include resolving viral infections, promoting exosome secretion, promoting cholesterol efflux, and promoting several inflammatory responses.^{29,30} Altogether these upregulated genes may play various roles in lipid metabolism, lysosomal activity, and the general inflammatory response of TAMs in these models. Targeting these proteins or their related pathways may provide a powerful method for disrupting TAM tumor promotion.

Interestingly, *Ccr2* expression is upregulated in TG prostates compared to WT in both Hi-Myc and TRAMP models. While Further investigation is needed to fully understand the origin of these TAMs,

this suggests that macrophages in these tumors may be infiltrating from the circulation rather than arising from local proliferation.

It should be noted that in both 6-month-old Hi-Myc and 5-month-old TRAMP TG mice *Cd163* expression unexpectedly decreased compared to age-matched WT mice. *Cd163* is often used as a pro-tumor macrophage marker, but in these instances was found to be decreased in pro-tumor macrophages from late-stage tumors. Thus, *Cd163* is not an effective marker for assessing pro- or anti-tumor characteristics of TAMs in Hi-Myc and TRAMP models.

3.9 | Hi-Myc is a more representative model than TRAMP for prostate cancer TAM studies

These macrophage studies reveal that the two prostate cancer TG models exhibit key similarities and differences in their TME. While macrophage infiltration increased with age and histological grade in Hi-Myc mice, the opposite was observed in TRAMP mice. However, the two models exhibited similar trends of increased pro-tumor gene expression in prostate macrophages with increasing age and presence of tumor in TG mice. This is consistent with current knowledge of TAM pro-tumor functions. The differences in TAM infiltration may be due to the differences in cancer type and biology as Hi-Myc tumors are more adenocarcinoma-like and TRAMP tumors are more neuroendocrine-like. Since adenocarcinoma is more commonly seen in patients, the Hi-Myc model is likely more representative of TAMs present in most patient tumors.² The difference in macrophage density trends between the two models suggests that TAMs play different roles in supporting these two cancer types. The increase in macrophage density in tumors from patients and Hi-Myc TG mice may suggest that macrophage-targeted therapies would be more effective against prostate adenocarcinomas. Due to its similarity to patient TAM trends, the Hi-Myc model is a better model for prostate cancer TAM-related studies. Ongoing work uses the Hi-Myc model to investigate prostate cancer TAM biology and macrophage-targeted therapeutic approaches. With this novel information on TAM characteristic in this model, prostate cancer research is better equipped to advance TAM-focused therapies.

FIGURE 5 TRAMP transgenic prostate macrophages express higher levels of pro-tumor genes. TRAMP wild type (WT) and transgenic (TG) prostate macrophages from 2- and 5-month-old mice were analyzed by NanoString Myeloid Panel gene expression analysis. (A) Dendrograms and heat map of gene expression across all Myeloid Panel genes that were detected above threshold (20 counts) in at least one sample compared between each cohort. (B) Dendrograms and heat map of gene expression across select pro- and antitumor macrophage genes that were detected above threshold (20 counts) in at least one sample compared between each cohort. (C) Select pro-tumor macrophage genes normalized to control genes. (D) Expression of pro-tumor macrophage genes in age-matched TG tissue relative to WT. (E) Select antitumor macrophage genes normalized to control genes. (F) Expression of antitumor macrophage genes in age-matched TG tissue relative to WT. Heat maps were generated using unsupervised hierarchical clustering with average Euclidean distance. ND = not detected; UD = undefined due to below threshold expression of WT control. TRAMP, transgenic adenocarcinoma of the mouse prostate [Color figure can be viewed at wileyonlinelibrary.com]

4 | CONCLUSION

Prostate cancer treatments currently neglect the role of the TME and TAMs in supporting cancer. Many in vivo models used for studying cancer do not properly recapitulate the complex TME. Studying TAMs in TG prostate cancer models which reflect more accurate TMEs may lead to more impactful studies for TAM-targeted therapies. TAMs from Hi-Myc adenocarcinoma and TRAMP neuroendocrine TG models on the FVB/N background both exhibit pro-tumor characteristics. However, there are key differences in macrophage infiltration levels with Hi-Myc tumors containing higher and TRAMP tumors containing lower macrophage densities than age-matched WT prostates. Because patient tumors are more often adenocarcinomas and also exhibit higher macrophage densities than normal prostate tissue, the Hi-Myc model should function as a more representative model for investigating prostate cancer TAM biology and pursuing TAM-targeted therapies.

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CONFLICT OF INTERESTS

Denis Wirtz is a cofounder and owns stock in AbMeta Therapeutics, Inc. Angelo M. De Marzo receives research support from Janssen R&D and Myriad and is a consultant for Cepheid. Kenneth J. Pienta is a consultant for CUE Biopharma, Inc., is a founder and holds equity interest in Keystone Biopharma, Inc., and receives research support from Progenics, Inc.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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