Supplemental Methods:

Cell Culture

The 66cl4 mammary carcinoma cell lines were a generous gift from Fred Miller (1). Eya3 was knocked down in these cell lines using two different shRNAs (clone TRCN0000029858 antisense: TAATCATTGGATGAGCGAGGG and clone TRCN0000029855 TTTGTGAGGTATAATCATTGG Dharmacon, Lafayette, CO) and control scramble shRNA (CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG) (Addgene, plasmid 1864, Cambridge, MA). ShRNAs were lentivirally introduced into cells according to the pLK01 manufacturer's protocol (Addgene, Cambridge, MA), selected with puromycin (2.5ug/mL), and clonally isolated. These cells were tagged with luciferase using the MSCV Luciferase PGK-hygro vector (Addgene, plasmid #18782, Cambridge, MA) and transduced cells were selected as a pooled population with hygromycin (200μg/mL). Cells were determined to have equal levels of luciferase activity before injection.

The Met1 mammary carcinoma cell lines were a generous gift from Jeffrey Greg(2). Eya3 was knocked down using the same shRNA constructs as above and selected as a pooled population with puromycin (2.5μg/mL). The cells were luciferase tagged using the same constructs and methods as above.

To reintroduce Eya3 into Eya3 KD cells, mEya3 isoform 1 (CCDS18729.1) was subcloned into the pMSCVneo vector (Clonetech, Cat No. 631461, Mountain View, CA) altered with wobble mutations to inhibit targeting by Eya3 shRNAs. Primers used for these wobble mutations were: Fwd: CCCCCGATCGTCTAACGACTA and Rev: TAGTCGTTAGACGATCGGGGG. Threonine phosphatase dead, Tyrosine phosphatase dead, and Six1 binding mutations were

made in the Eya3 wobble constructs using two step cloning. Primers used for these mutations were: H79A Fwd: TATGCAGCCATCCTCTCAGTTCCTGTTTCGGAAACCAC and H79A Rev: GAGGATGGCTGCATAAGGTTTTGCAGAATACATTTGTG, D262N Fwd: CTCTGGAACTTGGACGAAACCATCATCATCTTTC and D262N Rev: CCAAGTTCCAGAG AAATACCCGTTCCAATTCAC, and A520R Fwd: CCAGCGTTTAGAGCTTGACTTCCTCTGA and A520R Rev: CTAAACGCTGG TGCAGGGACACCAGATCTCC. WT, Threonine phosphatase dead, Tyrosine phosphatase dead, and Six1-binding mutant versions of Eya3 were reintroduced into Eya3 KD cells and transduced cells were selected with neomycin (500μg/mL).

To restore PD-L1 expression in 66cl4 lines, mPD-L1 cDNA was kindly provided by Yuwen Zhu (clone identified in(3)) and was cloned into a pMSCV neomycin vector. Transduced cells were selected as a pooled population using neomycin (500μg/mL).

To transiently knockdown c-Myc in 66cl4 and Met1 cells, siRNA was transfected into cells as follows: cMyc siRNA pool (Santa Cruz, Dallas, TX cat sc-29227) and N-TARGETplus Non-targeting Control Pool (cat D-001810-10-20, Lafayette, CO) at a final siRNA concentration of 22.5nM using XtremeGene (Sigma Aldrich cat 4476093001, St. Louis, MO) for transfection. Cells were collected and RNA was isolated 48hrs post transfection.

The MDA-MB-231 cell line used was described previously(4) and STR profiling was performed in August of 2017 to verify the identity of the line used. Eya3 was knocked down using two different shRNAs (clone TRCN0000051606 antisense: AATGGGATAAGAATAATGTGC and clone TRCN0000051607 antisense: TTAATGCAGTTCCTAACCAGG Dharmacon Lafayette, CO) and an off target control scramble shRNA was used to transfect control cells (CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG) (Addgene plasmid 1864,

Cambridge, MA). ShRNAs were lentivirally introduced into cells according to the pLK01 manufacturing protocol (Addgene, Cambridge, MA), and transduced cells were selected as pooled populations using puromycin (1.5μg/mL).

All cells were routinely tested for mycoplasma infection, and were only utilized if negative.

In Vitro Phosphatase Assay

For in vitro Thr phosphatase assays, HEK293T cells were transfected with PEF-Flag-Eya3 Eya3 WT and H79A vectors provided by Shigekazu Nagata using the CaCl2 transfection method(5). Cells were lysed (in TBS buffer: 50 mM Tris HCl, pH 7.4 and 150 mM NaCl, including 1 mM EDTA, 1mM DTT and 0.5% TRITON X-100) and Eya3 WT and H79A proteins were purified using pull down with anti-Flag M2 affinity Gel (Sigma Aldrich cat A2220, St. Louis, MO). The resin was washed in TBS, 250mM NaCL, and 500mM NaCl. Eya3 was competitively eluted using a 142 ng/μl 3X Flag peptide (Sigma Aldrich. cat F3290, St. Louis, MO).

Phosphatase activity was measured using 250ng purified Eya3 protein and 300μM K-R-pT-I-R-R peptide (Millipore, cat 12-219, Billerica, MA) in buffers/colorimetric solution from the Millipore Ser/Thr Phosphatase Assay Kit (Millipore, cat 17-127, Billerica, MA). Absorbance was measured at OD 620 on the Modulus Microplate (Turner Biosystems, cat 998-9300, Sunnyvale, CA). Percentage of phosphatase activity was determined as level of absorbance OD 620 relative to that of Eya3 WT.

qRT-PCR

All RNA was extracted using the RNAeasy isolation kit (Qiagen, cat 74136, Germantown, MA) followed by cDNA synthesis using the iScript cDNA Synthesis Kit (Biorad, cat 1708891, Hercules CA) using 1μg of RNA. All qRT–PCR assays were performed using 1μg cDNA diluted 1:25 in ddH20 and ssoFast Evagreen supermix (BioRad, cat 1725200, Hercules, CA) on the Bio-Rad CFX-96 real time system machine (Biorad, cat 1855195, Hercules, CA). Gene expression was normalized to internal control using CFX Manager Software. Primer pairs used include: mEya3 Fwd: CCGACTCCTCTGCAAGCCCA, Rev: CTGGGAAGAGCTGGCATCAGCT; mPD-L1 Fwd: GACGCCTCACTTGCTCATTA, Rev: CAGAGCTAATGGGCTCCTTC; mc-Myc Fwd: ATGCCCCTCAACGTGAACTT, Rev: CCAGATATCCTCACTGGGCG; mGAPDH Fwd: CATCACCATCTTCCAGGAGC, Rev: ATGCCAGTGAGCTTCCCGTC; hEya3 Fwd: TGCTTACCCTGGACAGACTCA, Rev: CACAATGCACCAAAAGGAGGT; hPD-L1 Fwd: TGTCAGTGCTACACCAAGGC, Rev: ACAGCTGAATTGGTCATCCC; hGAPDH Fwd: CATCACCATCTTCCAGGAGC, Rev: ATGCCAGTGAGCTTCCCGTC; hPB1B Fwd: GGAGATGGCACAGGAGGAAA, Rev: CGTAGTGCTTCAGTTTGAAGT.

Western Blotting

Whole-cell extracts were isolated using RIPA buffer(6) with protease inhibitors (ThermoFisher Scientific, cat A32963, Waltham, MA). 30-50μg of protein was electrophoresed on 10% SDS-Page gels, and transferred to PVDF membranes. Membranes were blocked in 5% milk/TBST (137 mM NaCl, 2.7 mM KCl, 19mM Tris base, 0.1% Tween-20), exposed to primary

and secondary antibodies, and developed using chemoluminescence (ThermoFisher Scientific, cat 34080, Waltham, MA). Antibodies used can be found in Supplemental Table 1.

Cell Growth Assay

Cell growth was measured by plating 2000 66cl4 cells or 3000 Met1 cells in triplicate and their confluence was measured over time with the Incucyte Zoom (Essenbioscience, cat 4647, Ann Arbor, MI). Relative growth was measured by normalizing confluence from a time point back to starting confluence at time 0h.

Migration Assay

$1x10^4$ 66cl4 cells were plated in 400μL serum-free DMEM medium in triplicate into the top of 8μM 6.5cm transwell chambers (Sigma-Aldrich, cat CLS3422, St. Louis, MO). Chambers were placed in 12-well plates containing 1200μL serum containing DMEM medium and incubated for 4hrs. Transwells were then removed and the inner cell monolayer was removed with cotton tipped applicators. Migrated cells were fixed on the transwell with 95% ethanol and stained with Crystal Violet. 5 fields of view of each transwell were photographed and migrated cells counted and triplicates averaged.

Cell migration for the Met1-Eya3 SCR and KD system was measured using a modified scratch assay with a uniform 500μM gap (Ibidi, cat 80209, Madison, WI). $5\text{-}6x10^5$ cells/mL were plated in 70μL in each insert and incubated overnight. Inserts were removed and distance cells migrated in 5hrs was measured using DP2-BSW software.

Data mining

Correlation between EYA3 and PD-L1 in human cohorts was done using the Oncomine database (www.oncomine.org). Statistical analysis was performed using linear regression and Pearson correlations.

To measure the correlations between Eya3 and PD-L1 in tumors without lymphocytic and stromal cell gene expression pollution, expression data for EYA3 and PD-L1 (RNAseq median z-score) was downloaded for TCGA patients directly from cBioPortal using the cgdsr R package (7, 8). Tumor purity measures, consensus measurement of purity estimations (CPE), were taken from (9). Expression values, CPE measures, and receptor status of TCGA patients were compiled for a total of 123 patients that were reported to be triple negative. Patients were separated based on CPE (> 0.80 (C) or < 0.60 (D)). Correlation between EYA3 and PD-L1 was calculated for each patient group based on Spearman's rank correlation.

Cibersort analysis(10) was performed with gene expression data from the TCGA dataset (obtained from https://portal.gdc.cancer.gov) and stratified by EYA3 expression. "High EYA3" was defined as EYA3 expression above the 75th percentile and "Low EYA3" was defined as EYA3 expression below the 25th percentile. The CIBERSORT algorithm was then applied to the gene expression profiles of both groups (10). To estimate cell type abundance, the LM22 signature was used, which contains known gene expression signatures for 22 subsets of leukocytes. CIBERSORT estimated the relative proportions of cell populations within the TCGA samples, outputting an estimated proportion of each of the 22 leukocyte subsets for each breast cancer tumor. For each of the 22 leukocyte subsets in the LM22 signature, we compared the estimated

proportion of that cell type in the "EYA3 high" tumors and the "EYA3 low" tumors using a Student's T-test and multiple testing correction with the FDR procedure.

Animal Studies

In the orthotopic models of tumor growth either $5x10^5$ 66cl4-Eya3 SCR/KD cells or Met1-Eya3 SCR/KD cells in 50μL DMEM medium were injected into the fourth mammary fat pad of 6-8 week-old female BALB/c or FVB mice respectively (Jackson, stock 000651 and 001800, Bar Harbor, ME) and tumor growth was measured weekly using calipers. Mice were imaged with the IVIS Spectrum machine (PerkinElmer, cat 128201, Waltham, MA) weekly after receiving an intraperitoneal (i.p.) injection of 100μL of 100x d-luciferin (Gold Biotechnology cat LUCK-2G, Olivette, MO). 2 hours before euthanization mice were weighed and ip injected with 1mL/100g body weight BrdU Labeling Reagent (Invitrogen, cat 00-0103, Carlsbad, CA). Mice were then euthanized and their primary tumors were taken for analysis once tumors reached a 3-5 week time point or a volume of 1 or $2cm^3$ using the formula: volume = ($width^2$ x length)/2.

For CD8 depletion experiments, 6-8 week female BALB/c or FVB mice were given i.p. injections of rat IgG2a clone 20LC11.1 or anti-mouse CD8α clone 53.6-7 (BioXCell, cat BE0089 and BE0004-1, Lebanon, NH). The schedule of antibody administration was as follows: Day 1, 500μg antibody was given followed by 250μg on day3 and 250μg weekly for the remainder of the experiment. CD8α depletion was confirmed through flow cytometry analysis using an anti-CD8β PE antibody on blood collected by submandibular bleeds from treated animals. Blood samples were depleted of RBCs with ACK Lysing Buffer (Gibco, cat A1049201, Waltham, MA), followed by centrifugation and multiple washes with FACS buffer (2%BSA and 10mM Hepes in PBS), and antibody staining with CD8β antibody. Information on these antibodies can be found

in Supplemental Table 1. After CD8 depletion was confirmed on Day 4, mice were injected with 66cl4 or Met1 cell lines as described above on day 5.

Immunohistochemistry

Five micron thick paraffin sections were deparaffinized for immunodetection of CD8 (Bioss; Woburn, MA; #bs-0648R, 1:500) and were retrieved in 10 mM sodium citrate (pH 6.0 + 0.1% Tween 20) for 10 minutes at 110°C (NxGen Decloaker, Biocare Medical, Concord, CA). CD8 required standard manual detection with a 3% hydrogen peroxidase blocker for 10 minutes and 2.5% horse serum blocker for 20 minutes. Primary antibody was incubated at room temperature for 60 minutes in a humidity chamber followed by Rabbit ImmPress polymer detection system (Vector Laboratories, Burlingame, CA) for 30 minutes. Antibody complexes were visualized with ImmPACT DAB (Vector) for 5 minutes.

Additional sections were deparaffinized, antigen retrieval performed and immunostained for Ki-67 (Neomarkers/Thermo Scientific, Waltham, MA; clone SP6; 1:300) and BrdU (DAKO; Santa Clara, CA; #M-0744; 1:25). Ki-67 and BrdU antigens were revealed in pH 9.5 BORG solution (Biocare Medical, Concord, CA) and 10 mM sodium citrate (pH 6.0 + 0.1% Tween 20), respectively, for 10 minutes at 110°C. Immunodetection was performed on the Benchmark XT autostainer (Ventana Medical Systems/Roche, Indianapolis, IN) at 37°C with primary incubation for 32 minutes using UltraView DAB polymer detection (Ventana). All sections were counterstained in Harris hematoxylin for 2 minutes, blued in 1% ammonium hydroxide, dehydrated in graded alcohols, cleared in xylene and coverglass mounted using synthetic resin.

Negative controls to confirm the specificity of the immunostaining included omission of the primary antibody incubation step in the IHC protocol.

Flow Cytometry

Tumors from 66cl4 tumor bearing mice were isolated at $2cm^3$ size, weighed and 0.5g tumor removed. Tumors were diced with scalpels into small pieces and shaken at 37°C 2hr in 5mL serum free medium containing 0.1mg/mL Liberase DL (Sigma Aldrich, cat 5401160001, St. Louis MO). Large tumor clumps were then dissociated through an 18g needle and the whole slurry was filtered through a 100μM filter. The slurry was then centrifuged and washed several times with FACS buffer (2%BSA and 10mM Hepes in PBS) and then lysed for RBCs as described above. After lysis and wash, cells were counted on a hemocytometer. Cells were then stained for: DAPI, CD25 APCeFluor780, CD8α FITC, CD3ε PE-Cy7, CD4 PE, NK1.1 APC, and CD45.2 BV510 or DAPI, MHCII APCeFluor780, CD11c PEeFluour610, CD11b PerCP-Cy5.5, F480 FITC, Ly6G PE-Cy7, CD206 PE, CD80 APC, and CD45.2 BV510. Samples were filtered with 0.45 μM filter and run on the Galios 561 10-color and compensation/analysis performed using FlowJo software.

For an examination of markers associated with T cell exhaustion, flow cytometry on cells from isolated tumors was performed as described above 5 weeks post tumor cell injection. Cells were treated with 1x Brefeldin A (Biolegend cat 420601, San Diego, CA) with or without 1mg/mL Ionomycin and 1mg/mL PMA for 4hrs at 37°C. After the washes, cells were stained for: CD8α BUV395, IL2 BV421, CD4 BV711, TNFα PE, B220 PerCP-Cy5.5, MHCII PerCP-Cy5.5, CD3ε PE-Cy7, CD44 AF700 and GhostRed780; CD8α BUV395, Tbet BV42, CD4 BV711, CD3ε PE-Cy7, Blimp1 PE, B220 PerCP-Cy5.5, MHCII PerCP-Cy5.5, Tim3 PE-Cy7, Eomes APC, CD44 AF700 and

Ghostred780; or CD3ε PE/Dazzle594, IFNγ APC, CD4 BV510, CD45 BV421, B220 PE, MHCII PE, CD8α AF700 and GhostRed780; or B220 PE, MHCII PE, CD45 BV421, Annexin V, and PI. Cells were fixed and permeablized using the FoxP3/Transcription Factor Staining Buffer Set (eBioscience, cat 00-5523, Waltham, MA). Stained cells were then assessed using the BD LSR Fortessa X20 or the Gallios 561 10-color and compensation/analysis performed using FlowJo software.

For the PD-L1 addback flow cytometry experiments, tumors were isolated 6 weeks post tumor cell injection, weighed and treated as above. Cells were stained with: BD45.2 BV510, CD3ε PE-Cy7, CD8α FITC, PD-L1 PE, BrdU APC, anti-firefly luciferase, anti-rabbit AF594, and GhostRed780.

More information on all antibodies used can be found in Supplemental Table 1.

Statistics

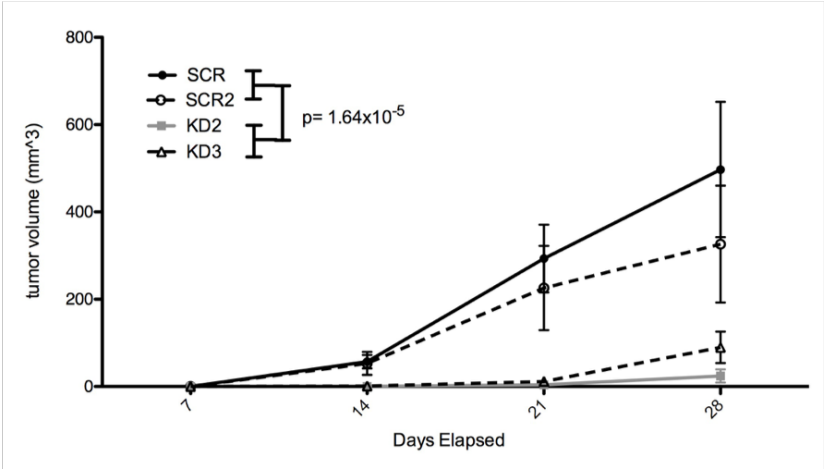
When conditions tested were over a time period, data were fit using a mixed effects model and examined for a statistically significant difference between the effect of SCR control vs average the effect of Eya3 KD2 and KD3, and reported as the corresponding p-value.

When exactly two conditions were compared, an unpaired two-tailed Student's T-test was used and corresponding p-values were reported. When correlations were tested, a linear regression test and correlation test were performed and corresponding p and r-values were reported. When comparisons were tested with more than two conditions, a one-way ANOVA was used followed by a Tukey's multiple comparison test. When two or more conditions were tested at a static time point we tested whether there is a significant difference between SCR vs Eya3 KD2 and KD3. This was done using an Analysis of Variation (ANOVA) by comparing the

effect of SCR against the average effect of KD2 and KD3 (done using sum contrasts in R) and the corresponding p-value was reported.

All graphs were created using GraphPad Prism software. If exact p-values were not stated, reported p-values are: *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$.

A



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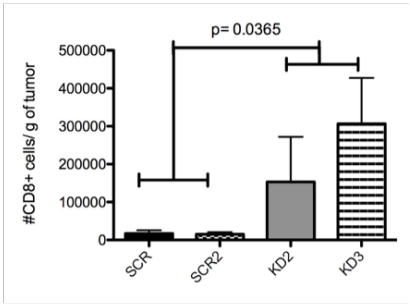
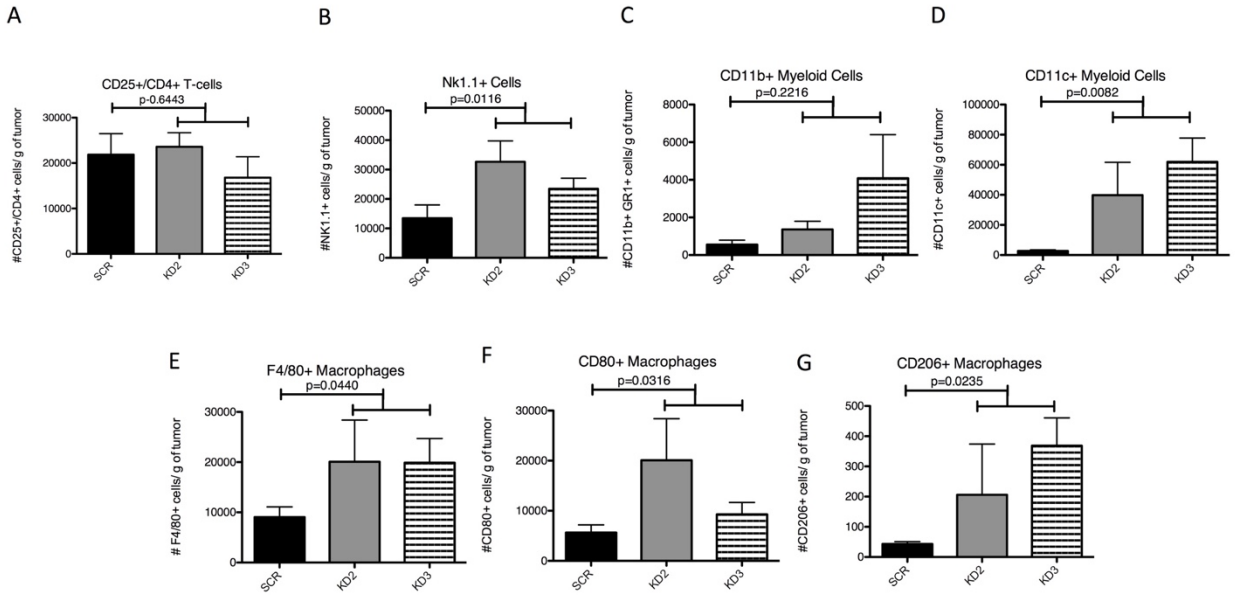
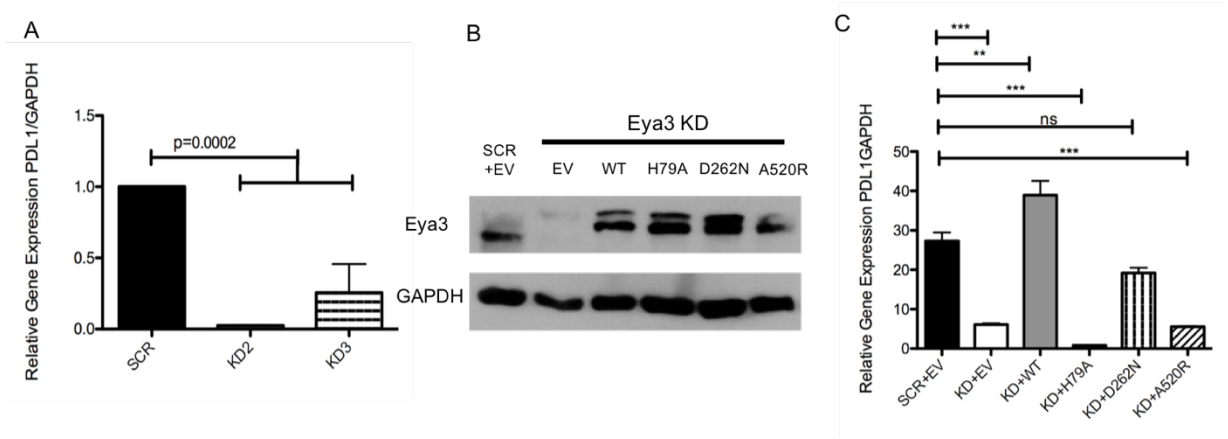


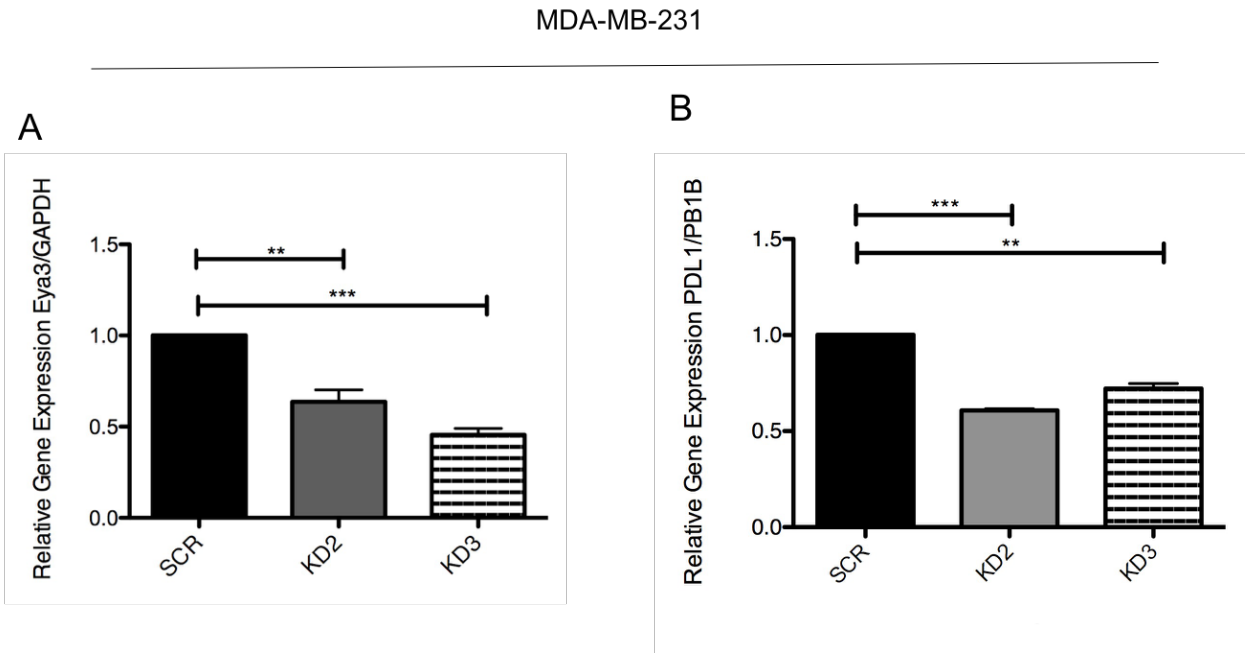
Supplemental Figure 1- Growth Differences Observed Between 66cl4 SCR and Eya3 KD tumors are not due to Differences in Clonal Antigenicity. (A) Tumor volume of 66cl4 SCR and Eya3 KD tumors in BALB/c mice as measured using calipers. Each point represents the mean tumor size ± SEM at that time point post injection and a mixed effects model was used to measure significance. n=10 mice per cell line. Solid black line represents SCR tumors, dotted black line with open circles represents SCR2 tumors, solid gray line represents KD2 tumors, dotted black line with open triangles represents KD3 tumors. (B) Calculated number of CD8+ T cells per gram of 66cl4 SCR and Eya3 KD tumors. Tumors were isolated (SCR n=5, SCR2 n=5, KD2 n=4, KD3 n=5), digested and analyzed by flow cytometry. CD8+ T cells were defined as: CD45+/CD3+/CD8+/CD4-. Data represent mean ± SEM. Significance was measured using ANOVA with sum contrasts in R. Representative experiment



Supplemental Figure 2- Eya3 Regulates Additional Immune Cell Types in 66cl4 Mammary Carcinomas. Calculated number of immune cells present per gram of 66cl4 SCR and Eya3 KD tumor. Tumors were isolated (SCR n=5, KD2 n=5, KD3 n=7), digested and analyzed by flow cytometry. Significance was measured using ANOVA with sum contrasts in R. Representative experiment (n=2). (A)CD24+/CD4+ T cells were defined as: CD45+/CD3+/CD8-/CD4+/CD24+. (B) Nk1.1+ cells were defined as: CD45+/CD3+/CD8-/CD4-/NK1.1+. (C) “MDSC like“ CD11b+/Gr1+ cells were defined as: CD45+/MHCII+/CD11b+/Gr1+. (D) “DC like“ CD11c+ cells were defined as: CD45+/MHCII+/CD11c+. (E) F4/80+ cells were defined as: CD45+/MHCII+/F480+. (F) “M1 like“ CD80+ macrophage cells were defined as: CD45+/MHCII+/F480+/CD80+. (G) “M1 like“ CD206+ macrophage cells were defined as: CD45+/MHCII+/F480+/CD206+.



Supplemental Figure 3- Eya3 Threonine Phosphatase Activity Regulates PD-L1 Expression in Met1 Mammary Carcinoma Cells. (A) qRT-PCR analysis on cDNA derived from RNA isolated from Met1 SCR and Eya3 KD cells. PD-L1 was normalized to GAPDH levels. Data represent mean ± SEM and significance was measured using ANOVA with sum contrasts in R for biological triplicates for three combined experiments. (B) Met1- Eya3 KD cells were stably rescued with empty vector (EV), Eya3 WT, Eya3 H79A, Eya3 D262N, or Eya3 A520R. Western blot analysis performed on membranes containing whole cell lysates from these cells, as well as the Scr+EV controls, using antibodies against Eya3 and GAPDH to probe the membranes. Representative image of experiments performed ≥3 times. (C) qRT-PCR analysis for PD-L1 using cDNA derived from cell lines shown in panel B. PD-L1 was normalized to GAPDH levels. Data represent mean ± SEM. Significance was measured using ANOVA. Representative experiment (n=3).



Supplemental Figure 4- Eya3 Regulates PD-L1 Expression in MDA-MB-231 Breast Cancer Cells. (A) qRT-PCR analysis on cDNA derived from RNA isolated from MDA-MB-231 SCR and Eya3 KD cells. Eya3 was normalized to GAPDH. Data represent mean ± SEM and significance was measured using ANOVA biological triplicates for three combined experiments. (B) qRT-PCR analysis on cDNA derived from RNA isolated from MDA-MB-231 SCR and Eya3 KD cells. PD-L1 was normalized to PB1B. Data represent mean ± SEM and significance was measured using ANOVA biological triplicates for three combined experiments.

Supplemental Table 1- Antibodies Used for Western Blot and Flow Cytometry

Antibody	Company	Catalog Number	Dilution/Concentration Used	Use
mEya3	Bethyl Montgomery,TX	A302-689A-M	1:500	Western Blot
c-Myc	AbCam Cambridge, MA	Ab32	1:500	Western Blot
pT58 c-Myc	ABM Richmond, BC, Canada	Y011034	1:1000	Western Blot
β-actin	Sigma-Aldrich St. Louis, MO	A5316	1:10000	Western Blot
GAPDH	Cell Signaling	D16H11	1:3000	Western Blot
DAPI	Sigma Aldrich St. Louis, MO	D9542	1:1000	Flow Cytometry
CD3ε PE-Cy7	eBioscience Waltham, MA	25-0031	1:200	Flow Cytometry
CD4 PE	eBioscience Waltham, MA	12-0041	1:200	Flow Cytometry
CD8α FITC	eBioscience Waltham, MA	11-0081	1:500	Flow Cytometry
CD8β PE	eBioscience Waltham, MA	12-0083	1:200	Flow Cytometry
CD25 APCeFluor780	eBioscience Waltham, MA	47-0251	1:200	Flow Cytometry
NK1.1 APC	eBioscience Waltham, MA	17-5941	1:200	Flow Cytometry
CD45.2 BV510	Biolegend San Diego, CA	103137	1:100	Flow Cytometry
MHCII APCeFluor780	eBioscience Waltham, MA	47-5321	1:200	Flow Cytometry
CD11c PEeFluour610	eBioscience Waltham, MA	61-0114	1:200	Flow Cytometry
CD11b PerCP-Cy5.5	eBioscience Waltham, MA	45-0112	1:200	Flow Cytometry
F480 FITC	eBioscience Waltham, MA	11-4801	1:200	Flow Cytometry
Ly6G PE-Cy7	eBioscience Waltham, MA	25-5931	1:200	Flow Cytometry
CD206 PE	Biolegend San Diego, CA	141705	1:200	Flow Cytometry
CD80 APC	eBioscience Waltham, MA	17-0801	1:200	Flow Cytometry

CD8α BUV395	BD Biosciences San Jose, CA	563786	1:200	Flow Cytometry
IL2 BV421	BioLegend San Diego, CA	503825	1:200	Flow Cytometry
CD4 BV711	BioLegend San Diego, CA	100447	1:200	Flow Cytometry
TNFα PE	BD Biosciences San Jose, CA	554419	1:200	Flow Cytometry

Supplemental Table 1- Antibodies Used for Western Blot and Flow Cytometry

Antibody	Company	Catalog Number	Dilution/Concentration Used	Use
B220 PerCP-Cy5.5	BioLegend San Diego, CA	103236	1:200	Flow Cytometry
MHCII PerCP-Cy5.5	BioLegend San Diego, CA	107626	1:200	Flow Cytometry
CD44 AF700	Tonbo BioSciences San Diego, CA	80-0441	1:200	Flow Cytometry
Tbet BV421	BioLegend San Diego, CA	644816	1:200	Flow Cytometry
Blimp1 PE	BioLegend San Diego, CA	150005	1:200	Flow Cytometry
Tim3 PE-Cy7	BioLegend San Diego, CA	119715	1:200	Flow Cytometry
Eomes APC	eBioscience Waltham, MA	12-4875	1:200	Flow Cytometry
PD-L1 PE	BioLegend San Diego, CO	124307	1:100	Flow Cytometry
BrdU APC	eBioscience Waltham, MA	17-5071	1:20	Flow Cytometry
Anti-Firefly Luciferase	Abcam Cambridge, MA	ab21176	1:100	Flow Cytometry
Anti-rabbit AF594	Invitrogen Carlsbad, CA	A11012	1:500	Flow Cytometry
GhostRed780	Tonbo BioSciences San Diego, CA	13-0865	1:1000	Flow Cytometry
PD1 FITC	Invitrogen Carlsbad, CA	11-9985-85	1:50	Flow Cytometry
CD3ε PE/Dazzle 594	BioLegend San Diego, CA	100347	1:100	Flow Cytometry

CD45.2 BV421	BioLegend San Diego, CA	109831	1:100	Flow Cytometry
CD8α AF700	BioLegend San Diego, CA	100729	1:100	Flow Cytometry
CD4 BV510	BioLegend San Diego, CA	100553	1:100	Flow Cytometry
MHCII PE	BioLegend San Diego, CA	107607	1:100	Flow Cytometry
B220 PE	BioLegend San Diego, CA	103207	1:100	Flow Cytometry
IFNγ APC	BioLegend San Diego, CA	505809	1:50	Flow Cytometry
Annexin V FITC and Propidium Iodide	Invitrogen Carlsbad, CA	88-8005-72	1:20	Flow Cytometry
24G2	BioLegend San Diego, CA	101302	1:200	Flow Cytometry

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