Supplementary Fig. 1. *Galectin-3 is present within tumors.* (A) mRNA expression levels of *Lgals3* (galectin-3) and *Lgals8* (galectin-8) in the four classes of cell lines as determined by gene expression microarray analysis. (B) Western blot analysis of galectin-3 and -8 expression in lysates from representative cell lines of the T\textsubscript{nonMet} (802T4), T\textsubscript{Met} (393T5), and M (393M1) classes. (C) Immunofluorescence imaging of transplanted M tumors excised from mice. Tumors were stained for macrophages (F4/80, green), galectin-3 (pink), isotype controls (IgG), and nuclei (blue). Dashed boxes in the top row highlight the area shown in the middle row. Scale bars in the top and bottom rows are 100µm. Scale bars in the middle row are 25µm.
Supplementary Fig. 2. Analysis of peripheral blood from tumor-bearing mice by flow cytometry. (A-C) Gating strategy for isolating living single cells. (D) Gating of galectin-3 positive cells. (E) Gating of CD11b positive cells. (F) FSC vs. SSC plot depicting all cells (red) and those that are galectin-3+ (blue). Galectin-3+ cells are SSC mid to SSC hi. (G) Gating of Gr-1+ cells. (H) Gating of CD11b+Ly-6Chi and CD11b+Ly-6Clo cells. (I) CD115 (Csf1r) staining in Gr-1- population.
Supplementary Fig. 3. Galectin-3 expression on CD11b⁺ cells is not dependent on the presence of tumors. Peripheral blood from naïve and tumor-bearing mice were analyzed for galectin-3 levels on various CD11b⁺ populations. (A) Galectin-3 geometric mean of all CD11b⁺ cells. (B) Galectin-3 geometric mean of all Gr-1⁺ cells. (C) Percentage of CD11b⁺Gr-1⁻ cells that are galectin-3 positive.
Supplementary Fig. 4. Galectin-3 presentation on CD11b+ leukocytes is not carbohydrate dependent
Peripheral blood from mice bearing M flank tumors was harvested, and CD11b+ leukocytes were isolated
by MACS separation. Cells were incubated with β-lactose (hashed bars) or control (sucrose, solid bars)
and analyzed by flow cytometry for galectin-3 expression on the various CD11b+ subsets to determine
whether galectin-3 presentation is carbohydrate-mediated. ‘n.s.’ not significant. Significance was
determined by One-way ANOVA with Tukey’s Multiple Comparison Test. Error bars are s.e.m.
Supplementary Fig. 5. Tumor conditioned medium induces galectin-3-independent mobilization of CD11b\(^+\) galectin-3\(^+\) leukocytes. Analysis of myeloid cell mobilization to peripheral blood following injections of control medium (white), M line conditioned medium (red), or recombinant murine galectin-3 supplemented control medium (gray): (A) percentage of all cells that are CD11b\(^+\); (B) percentage of all cells that are galectin-3\(^+\); (C) ratio of CD11b\(^+\)Ly-6C\(^{hi}\) to CD11b\(^+\)Ly-6C\(^{lo}\) cells; (D) percentage of all cells that are CD11b\(^+\)Gr-1\(^+\); (E) galectin-3 geometric mean of the various CD11b\(^+\) subsets. P-values were calculated by One-way ANOVA with Tukey’s Multiple Comparison Test. *** P < 0.001. Error bars are s.e.m.
Supplementary Fig. 6. Galectin-3 is not necessary for mobilization of galectin-3⁺CD11b⁺ leukocytes or metastatic seeding. (A) Concentrations of galectin-3 in media taken from M cells expressing short hairpins against a control gene (shLuc, black) or galectin-3 (shGal3-3 and shGal3-5, red) as determined by ELISA. (B-F) Analysis of myeloid cell mobilization to peripheral blood following injections of control hairpin medium (shLuc, black) or shGalectin-3 medium (red): (B) percentage of all cells that are CD11b⁺; (C) percentage of all cells that are galectin-3⁺; (D) percentage of all cells that are CD11b⁺Gr-1⁻; (E) ratio of CD11b⁺Ly-6C⁺ to CD11b⁺Ly-6C⁻ cells; (F) galectin-3 geometric mean of the various CD11b⁺ subsets. Error bars are s.e.m. (G) Livers were harvested from mice bearing flank tumors expressing the hairpins and examined by IHC for the presence of F4/80⁺galactin-3⁺ macrophages. (H) Wild-type mice were administered M cell lines containing the control (shLuc) and galectin-3 hairpins by intrasplenic inoculation followed by splenectomy. Two weeks following injections, livers were harvested and imaged to visualize the presence of GFP⁺ tumor nodules. These images are representative livers from each of the conditions. No decrease in tumor formation was visible in the shGalectin-3 lines. Significance was analyzed by One-way ANOVA with Tukey’s Multiple Comparison Test. ‘n.s.’ not significant.
Supplementary Fig. 7. *IL-6 induces mobilization of galectin-3⁺CD11b⁺ leukocytes.* Analysis of myeloid cell mobilization to peripheral blood following injections with recombinant murine IL-6: (A) percentage of all cells that are CD11b⁺; (B) percentage of all cells that are galectin-3⁺; (C) percentage of all cells that are CD11b⁺Gr-1⁺; (D) ratio of CD11b⁺Ly-6C⁹⁰ to CD11b⁺Ly-6C⁹⁰ cells. *P*-values were determined by Student’s t-test. * P < 0.05; ** P < 0.01; *** P < 0.001.
Supplementary Fig. 8. **IL-6 induces the mobilization of galectin-3^+ CD11b^+ granulocytes.** Analysis of peripheral blood harvested from wild-type and Csf3r knockout mice following injections of recombinant IL-6 (gray) or PBS (white). (A) Percentage of all cells that are CD11b^+Gr-1^- (B) ratio of CD11b^+Ly-6C^hi to CD11b^+Ly-6C^lo cells; (C) percentage of all cells that are CD11b^+galectin-3^-; galectin-3 geometric mean of the various CD11b^+ subsets. *P*-values were calculated by One-way ANOVA with Tukey’s Multiple Comparison Test. *** *P* < 0.001. ‘n.s.’ not significant. Error bars are s.e.m.
Supplementary Fig. 9. Galectin-3 expression by leukocytes or stromal cells is not necessary for IL-6-dependent mobilization of myeloid cells. Analysis of peripheral blood harvested from wild-type and Lgals3 knockout mice following injections of recombinant IL-6 (pink) or PBS (white). (A) Percentage of all cells that are galectin-3⁺; (B) percentage of all cell that are CD11b⁺galectin-3⁺; (C) Percentage of all cells that are CD11b⁺Gr-1⁺; (D) ratio of CD11b⁺Ly-6Chi to CD11b⁺Ly-6Clo cells; galectin-3 geometric mean of the various CD11b⁺ subsets. P-values were calculated by One-way ANOVA with Tukey’s Multiple Comparison Test. * P < 0.05; *** P < 0.001. ‘n.s.’ not significant. Error bars are s.e.m.
Supplementary Fig. 10. *The Thomsen-Friedenreich Antigen is elevated in metastatic cells and human lung cancer tissue.* (A) PNA staining (green) of the clonally-related T<sub>Met</sub> and M pair of cell lines (393T5 and 393M1, respectively). Nuclei are stained with Hoechst (blue). (B) Quantification of human NSCLC tissue microarray (TMA) PNA staining in Fig. 4 by cancer subtype: ‘LN’: lymph node; ‘Adj Lung’ lung tissue adjacent to (but not containing) cancerous tissue; ‘Adeno’: adenocarcinoma; ‘LCC’: large cell carcinoma; ‘SCC’: squamous cell carcinoma.
Supplementary Fig. 11. Gene expression microarray analysis of glycosyltransferases shows no evidence of differential regulation of transferases that generate galectin-3 ligands. (A) Gene expression microarray analysis of glycosyltransferase expression for 216 glucosyltransferases for cell lines from each of the four classes (T_nonMet: blue; T_Met: green; N: orange; M: red). Yellow bar: primary tumor-derived lines; red bar: metastasis-derived lines. (B) Gene expression microarray analysis of glycosyltransferase expression for transferases that generate the glycan motifs known to bind galectin-3 and -8. The predicted glycan linkages are shown to the left of the heatmap, and the heatmap depicts the expression of the transferases that generate those linkages for cell lines from each of the four classes (T_nonMet: blue; T_Met: green; N: orange; M: red). Yellow bar: primary tumor-derived lines; red bar: metastasis-derived lines.
Supplementary Fig. 12. Differential regulation of select glycosyltransferase activity prevents glycan elongation and preserves T-Antigen presentation. Gene expression microarray analysis of differentially-regulated glycosyltransferases from each of the four classes (T_{nonMet}, T_{Met}, and N: n=3; M: n=2). Bottom: Glycan symbol depiction of the structures resulting from the respective transferase activity. * $P < 0.05$; ** $P < 0.01$. Error bars are s.e.m.
Supplementary Fig. 13. O-linked core 1 disaccharide synthesis is unaltered during metastatic progression, but branching and elongation affect T-Antigen surface presentation. (A) PNA lectin blot for whole cell lysates from the clonally-related T_Met and M cell lines. (B) Gene expression microarray analysis of the human NSCLC lines shown in Fig. 4F. Cosmc (C1GALT1C1) and T-synthase (C1GALT1) promote the formation of the core 1 disaccharide. GCNT3 and GCNT1 induce branching and formation of the core 2 structure and ST6GALNAC4, ST3GAL1, and ST3GAL2 result in sialylation of the T-Antigen.
Supplementary Fig. 14. Transfection of Gcnt3 and knockdown of St6galnac4. (A) qRT-PCR for Gcnt3 in 393M1 cells following transfection with Gcnt3. (B) qRT-PCR for St6galnac4 following transduction with short hairpins targeting St6galnac4 or control (targeting firefly luciferase). (C) PNA staining of non-sialylated T-Antigen on 393M1 cells following transduction with short hairpins. (D) qRT-PCR for St6galnac4 following transduction with a second set of short hairpins targeting St6galnac4 or control (targeting firefly luciferase). (E) Galectin-3 binding by flow cytometry of 393M1 cells following transduction with short hairpins. Error bars are s.e.m.
Supplementary Fig. 15. Transfection of Gcnt3 reduces T-Antigen expression and adhesion to galectin-3. (A) Analysis of PNA labeling of the M cell line 393M1 transfected with Gcnt3 by flow cytometry for T-Antigen expression. (B) Analysis of galectin-3 binding to the M cell line 393M1 transfected with Gcnt3 by flow cytometry. P-values were determined by Student’s t-test. ** P < 0.01; *** P < 0.001.
Supplementary Fig. 16. Knockdown of St6GalNAcIV reduces the formation of metastases in vivo. Hematoxylin and eosin staining of representative liver sections from mice two weeks following intrasplenic transplantation of M cells expressing short hairpins against St6galnac4 or control (Luc, firefly luciferase). Boxed areas in the top row highlight the regions shown in the bottom row. White arrowheads denote the locations of tumor nodules in the shSt6galnac4 livers. Scale bars on the top row are 2mm. Scale bars on the bottom row are 500µm.
Supplementary Fig. 17. Knockdown of St6galnac4 does not affect proliferation. (A) Cell counts of the M line harboring hairpins against the control gene (firefly luciferase, shLuc, black) or St6galnac4 (red) were made at 24 hour intervals in cell culture.
Supplementary Fig. 18. *Galectin-3 is elevated in mice bearing tumors.* Serum galectin-3 concentrations in control mice or mice bearing bilateral $T_{\text{nonMet}}$ and $T_{\text{Met}}$ tumors as quantified by ELISA. *P*-value in (B) was determined by Student’s t-test. **** $P < 0.0001$. 