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Supplementary Information for

Galectin-1 fosters an immunosuppressive microenvironment in colorectal cancer by reprogramming $CD8^+$ regulatory T cells

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This PDF file includes:

Fig. S1 to S6

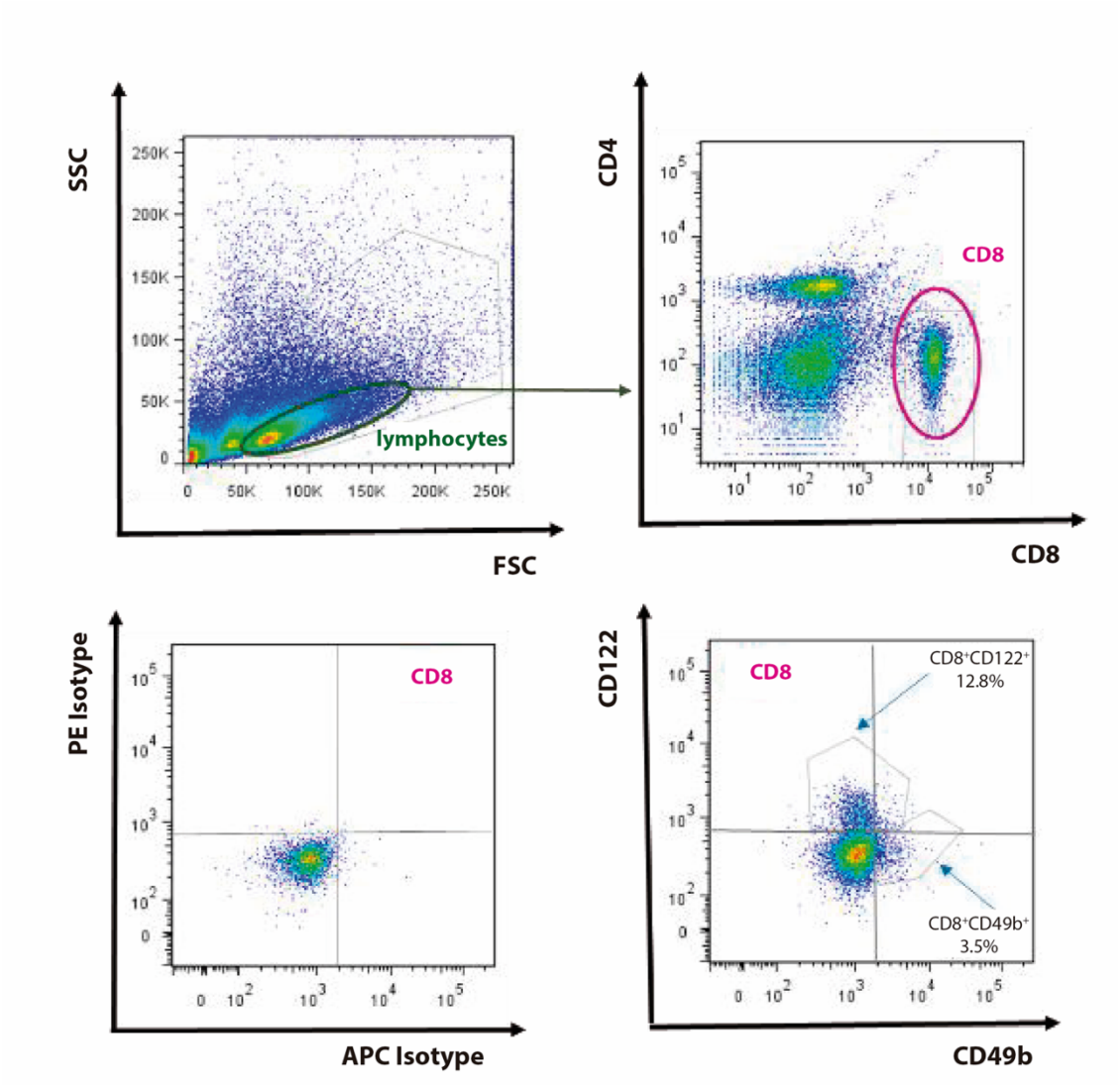

Fig. S1. Flow cytometry analysis of $CD8^+$ Tregs in draining lymph nodes (DLN) of healthy WT and *Lgals1*$^{-/-}$ mice. $CD8^+$ T cells were labeled for CD122 and the NKT cell marker CD49b.

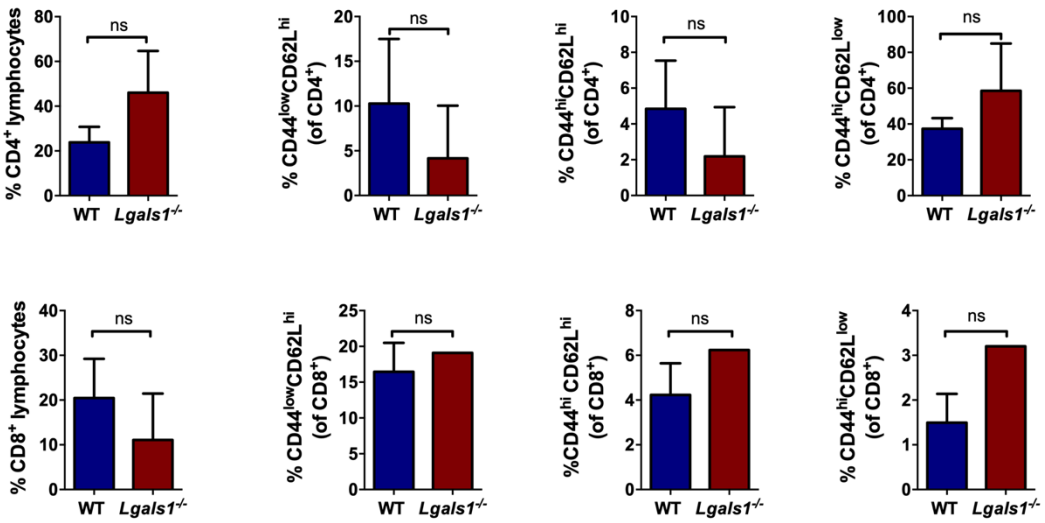

Fig. S2. Frequency of $CD4^+$, $CD4^+CD44^{low}CD62L^{hi}$, $CD4^+CD44^{hi}CD62L^{hi}$, $CD4^+CD44^{hi}CD62L^{low}$, $CD8^+$, $CD8^+CD44^{low}CD62L^{hi}$, $CD8^+CD44^{hi}CD62L^{hi}$ and $CD8^+CD44^{hi}CD62L^{low}$ T cells in mesenteric lymph nodes from WT and Gal-1 deficient (*Lgals1*$^{-/-}$) mice subjected to the azoxymethane (AOM)-dextran sodium sulfate (DSS) colitis-associated colorectal cancer (CRC) model. T_{EM} (effector memory cells) are $CD44^{hi}CD62L^{low}$, T_{CM} (central memory cells) are $CD44^{hi}CD62L^{hi}$, and naïve cells are $CD44^{low}CD62L^{low}$. Data presented are mean ± SEM from a representative of three independent experiments. N = 5-6 mice per group. Unpaired Student's *t*-test, ns = not significant.

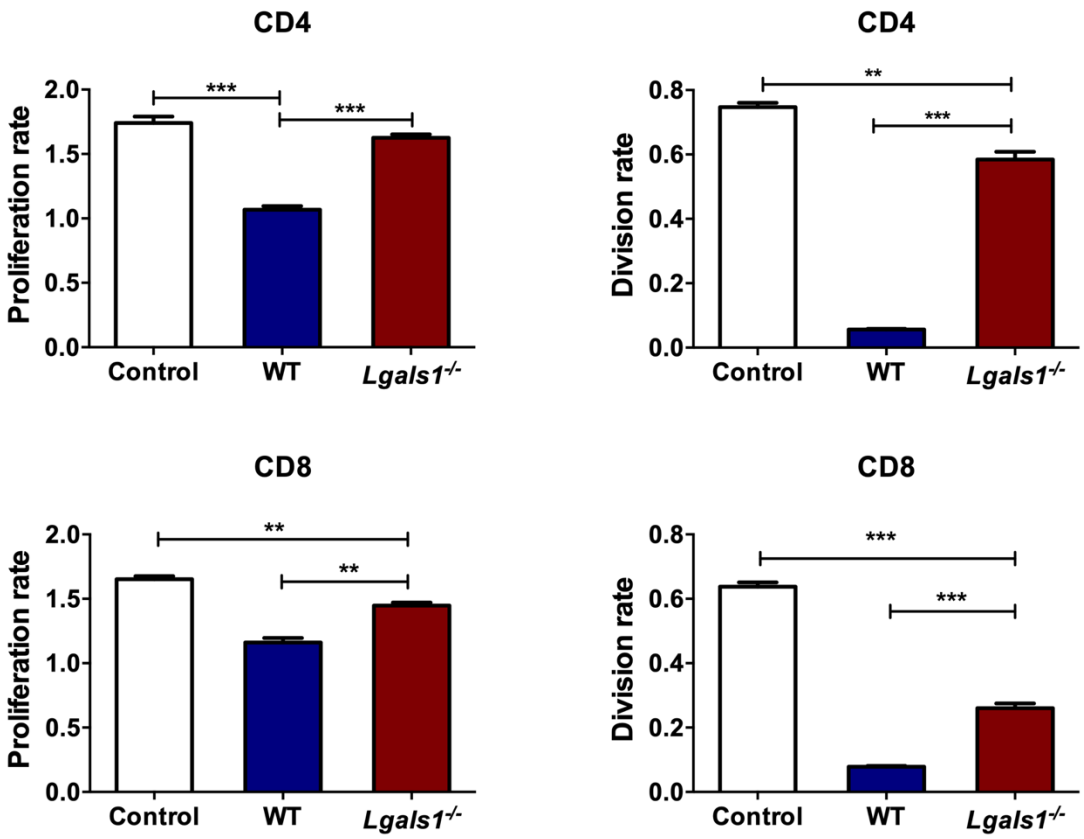

Fig. S3. Suppression of $CD4^+$ and $CD8^+$ T cell proliferation by $CD8^+CD122^+PD\text{-}1^+$ Tregs isolated from WT and *Lgals1*$^{-/-}$ BALB/c mice. Proliferation and division rate of $CD4^+$ and $CD8^+$ responder T cells incubated with control WT splenocytes, $CD8^+CD122^+PD\text{-}1^+$ WT Tregs or *Lgals1*$^{-/-}$ $CD8^+CD122^+PD\text{-}1^+$ Tregs co-cultured in a 1:4 ratio. Data presented are mean ± SEM from a representative of three independent experiments. ANOVA, Bonferroni multiple comparison test, *p<0.05, **p<0.01 and ***p<0.001.

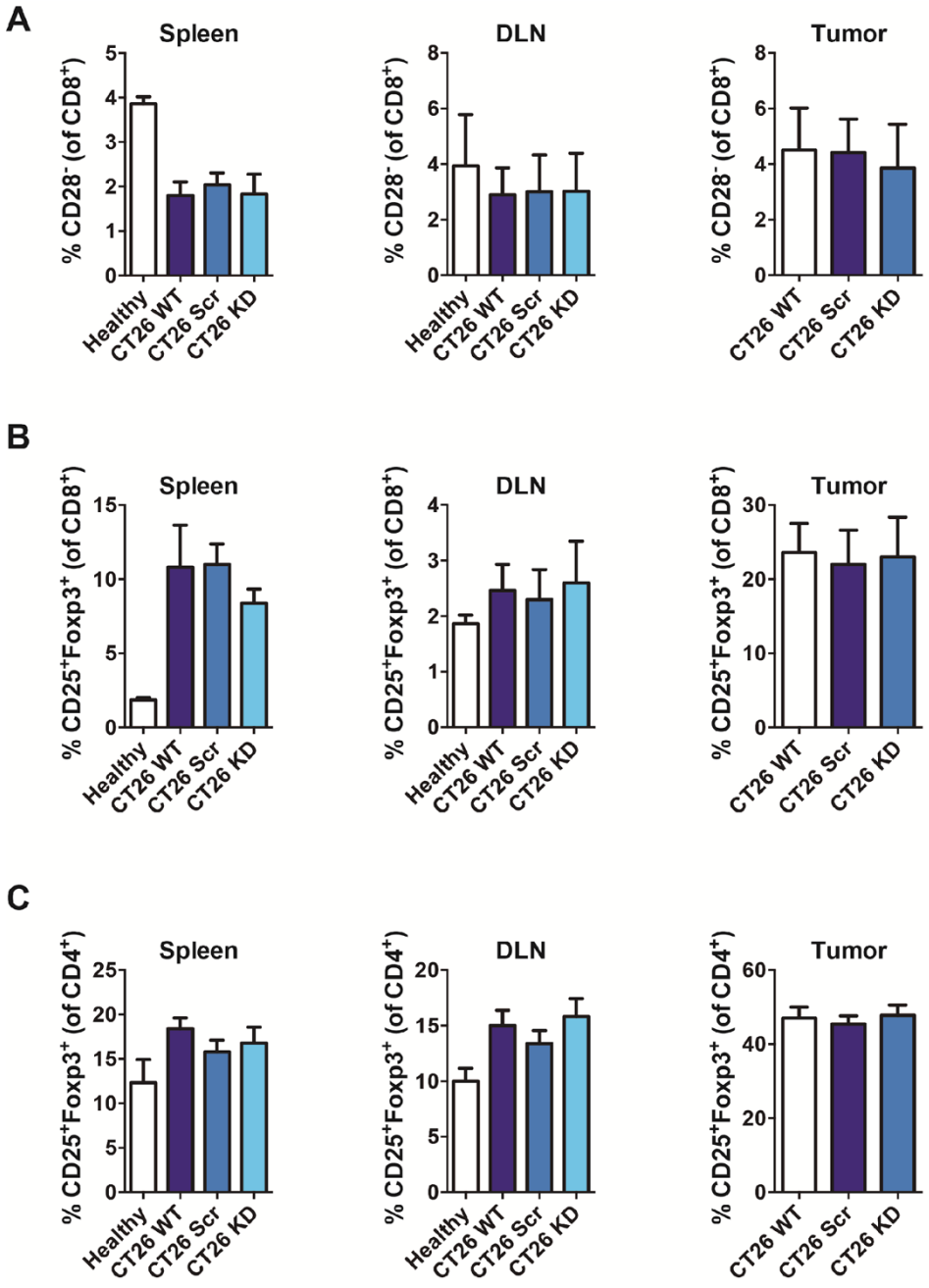

Fig. S4. Analysis of $CD8^+CD28^-$, $CD8^+CD25^+Foxp3^+$ and $CD4^+CD25^+Foxp3^+$ Tregs in the (*A*) spleen, (*B*) draining lymph nodes (DLN) and (*C*) tumors from mice inoculated with CT26 WT, scrambled (Scr) or Gal-1 knockdown (KD) cells. Data presented are mean ± SEM from a representative of three independent experiments. N = 5-6 mice per group. ANOVA, Bonferroni multiple comparison test, *p<0.05, **p<0.01 and ***p<0.001.

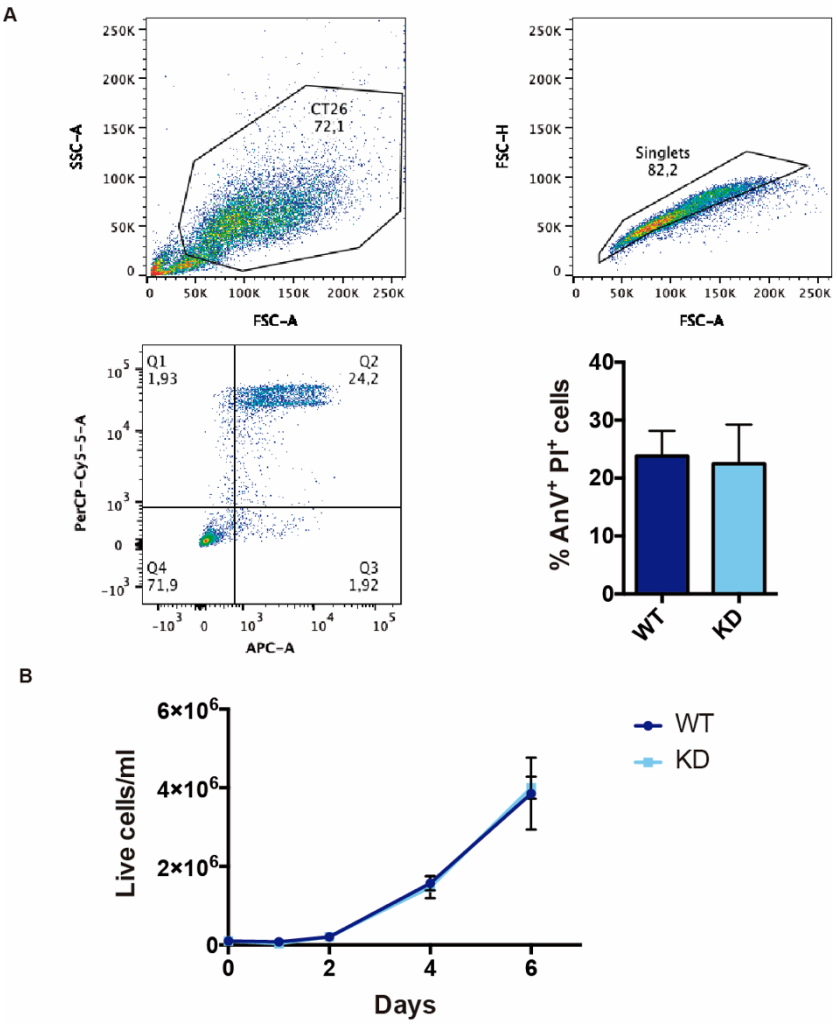

Fig. S5. Lack of differences in proliferation and survival of WT and Gal-1 KD CT26 cell lines. (A) Flow cytometry dot plot analysis showing gates for the selected CT26 apoptotic cells, identified by staining with APC–conjugated annexin V (AnV) and propidium iodide (PI). Analysis of $AnV^{+}PI^{+}$ cells on CT26 WT and Gal-1 KD cell lines. (B) Kinetics of cell proliferation in WT and Gal-1 KD CT26 cells. Data presented are mean ± SEM from a representative of three independent experiments. Unpaired Student's *t*-test.

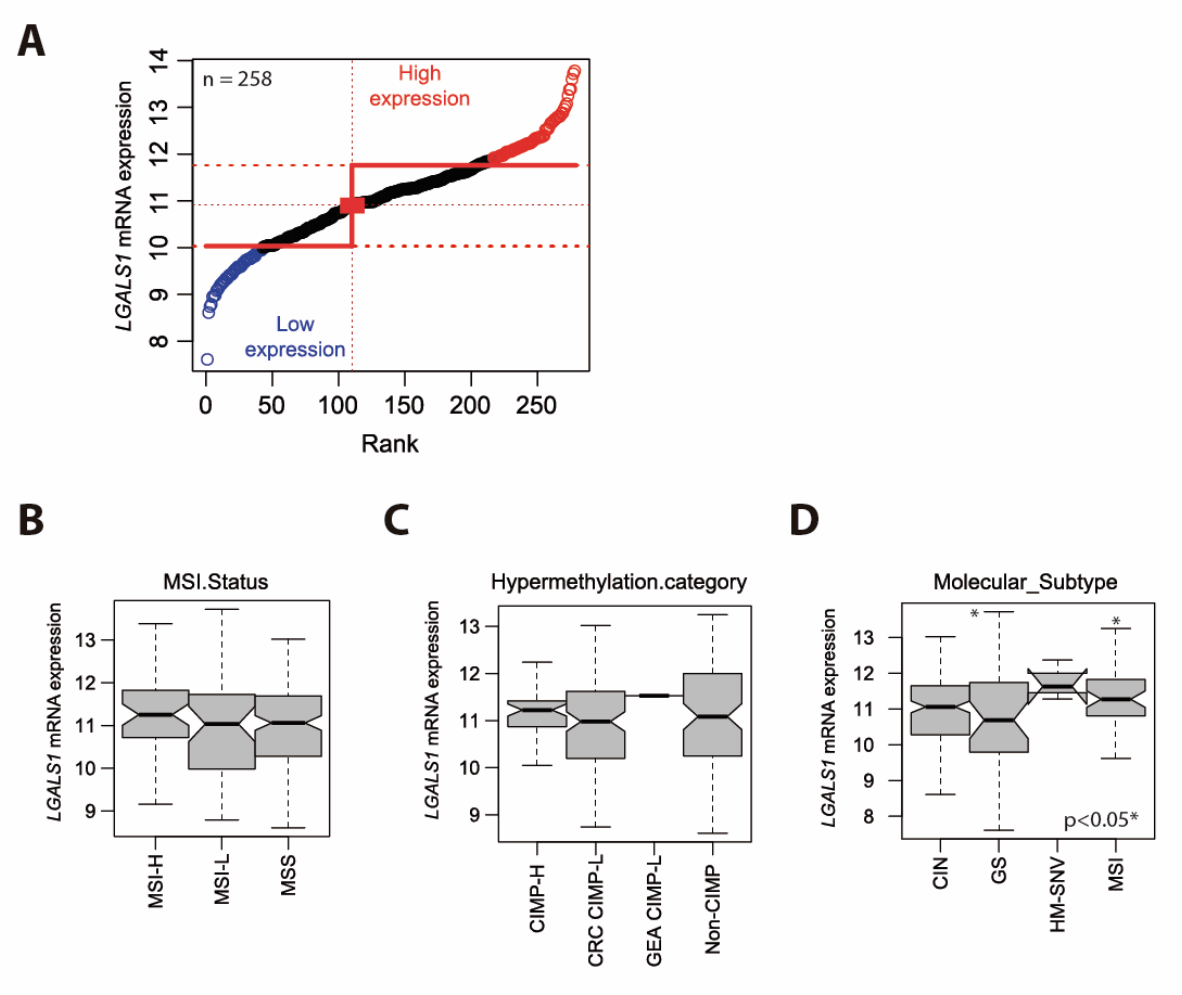

Fig. S6. (*A*) *LGALS1* expression dichotomization in tumors analyzed according to the Stepminer one-step algorithm. (*B-D*) *LGALS1* expression in colon adenocarcinoma samples categorized according to: (*B*) MSI status, (*C*) hypermethylation category and (*D*) molecular subtype. CIMP-H: CpG island methylation phenotype (CIMP) high; CRC CIMP-L: colorectal CIMP low; GEA CIMP-L: gastroesophageal CIMP-low; MSI: microsatellite instability; MSS: microsatellite stable; CIN: chromosomal instability; GS: genomically stable; HM-SNV: hypermethylated single-nucleotide variants. ANOVA, Bonferroni multiple comparison test; **P* < 0.05.