

# Dectin-2, a Novel Target for Tumor Macrophage Reprogramming in Cancer Immunotherapy

ABSTRACT 862

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### INTRODUCTION

Tumor-associated macrophages (TAMs) are an abundant immune cell population in most cancers that support tumor progression through their immunosuppressive effects. We discovered that TAMs express the pattern recognition receptor Dectin-2 (Clec4n/CLEC6A), an activating C-type lectin receptor (CLR) that binds to high-mannose glycans on fungi and other microbes and induces protective immune responses against infectious disease. Dectin-2 is selectively expressed by myeloid cells, and upon ligation, mediates enhanced phagocytosis, antigen processing and presentation, and pro-inflammatory cytokine production. Given these properties, we evaluated the therapeutic potential of targeting Dectin-2 using naturally derived ligands. We also generated human Dectin-2-targeted agonistic antibodies capable of robustly activating immunosuppressive "M2" or TAM-like macrophages.

### Dectin-2 agonism activates TAMs and elicits anti-tumor immune response

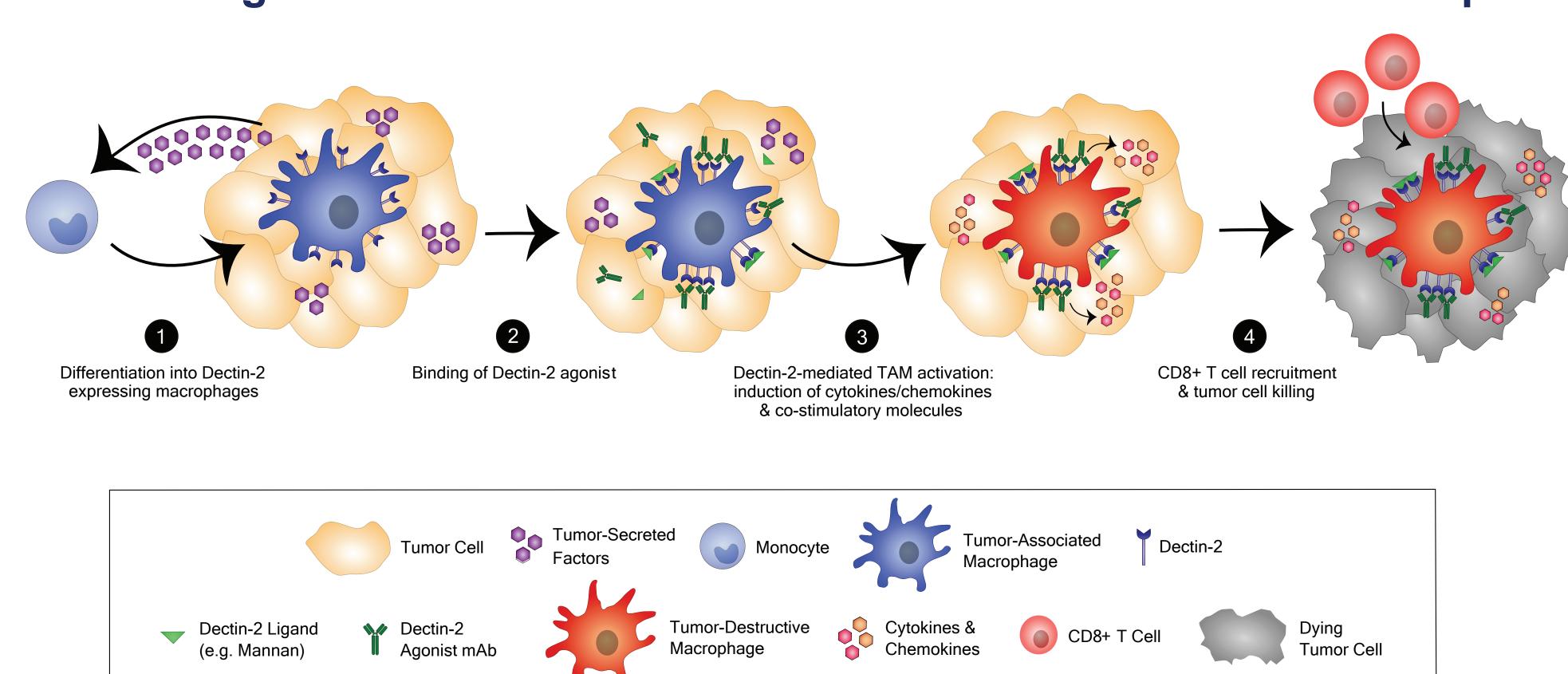


Figure 1: Schematic of proposed mechanism of action driving Dectin-2-mediated anti-tumor activity.

## RESULTS

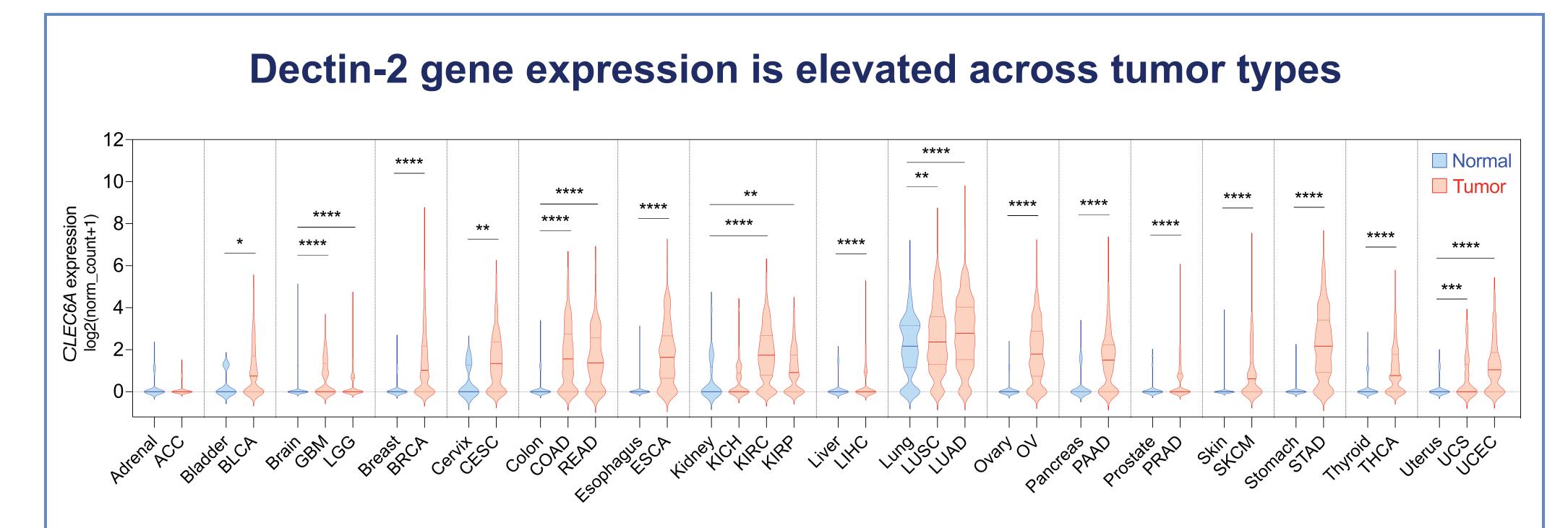


Figure 2: Dectin-2 gene expression is elevated in tumors but low in most normal tissues. Dectin-2/CLEC6A mRNA expression in human tissue samples from the TCGA (tumor) and GTEX (normal) datasets. TCGA study abbreviations are shown for the tumor subtypes. Data were processed using a uniform bioinformatic pipeline and obtained from UCSC Xena (xena.ucsc.edu). Data shown as median with interquartile range. Statistics were calculated by Mann-Whitney U test;  $*P \le 0.05$ ,  $**P \le 0.01$ ,  $***P \le 0.001$ ,  $****P \le 0.0001$ .

### Dectin-2 is selectively expressed by TAMs in a range of human tumors

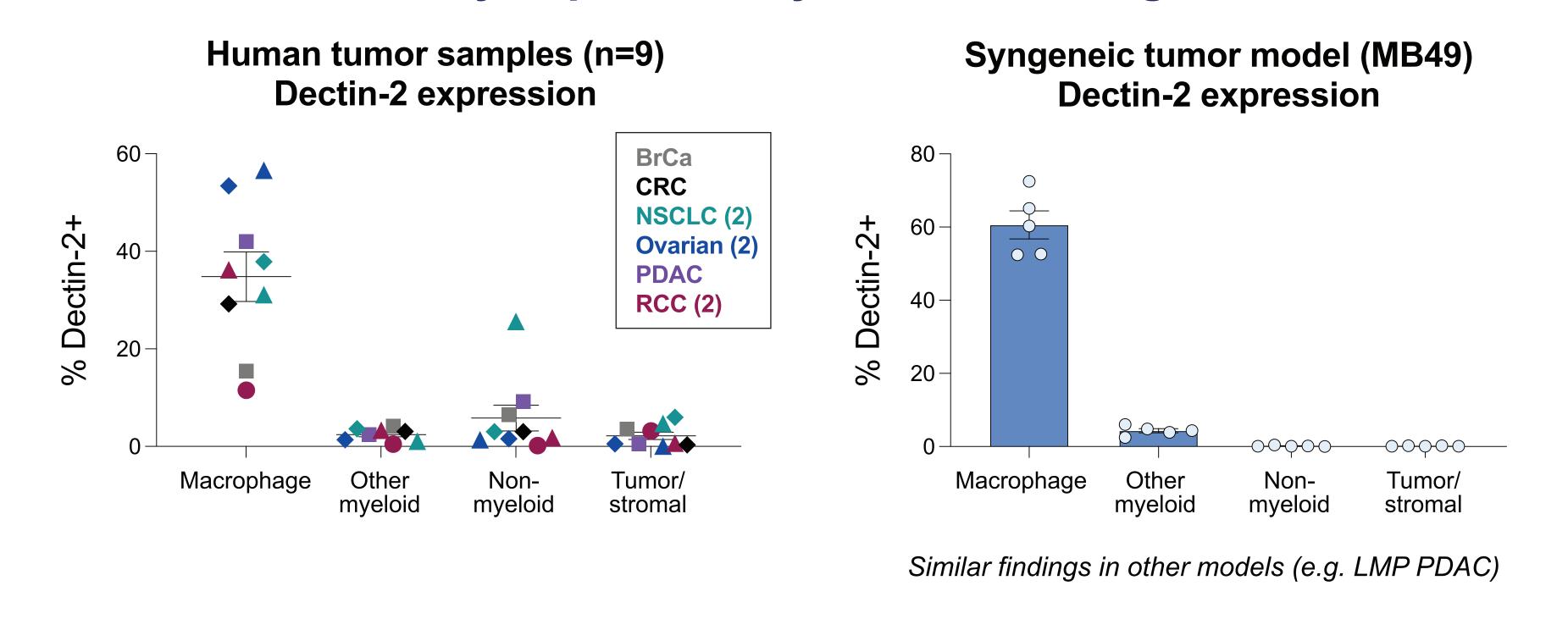
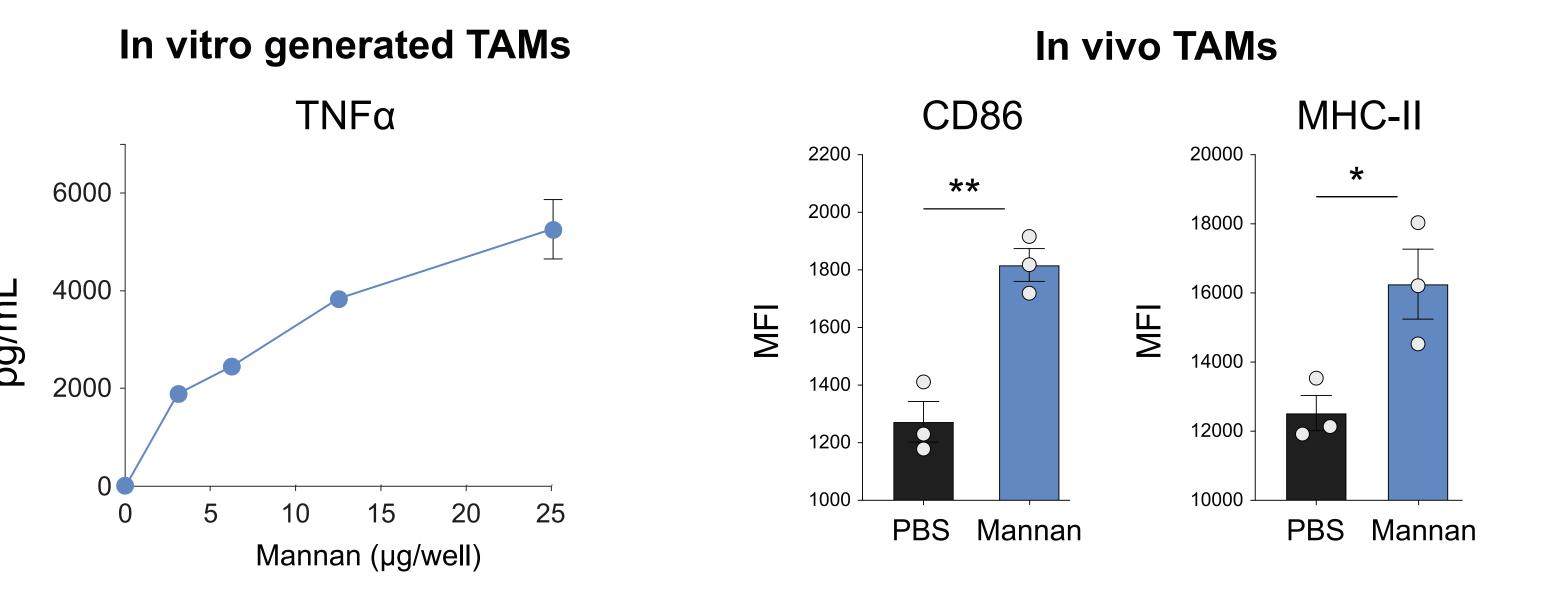


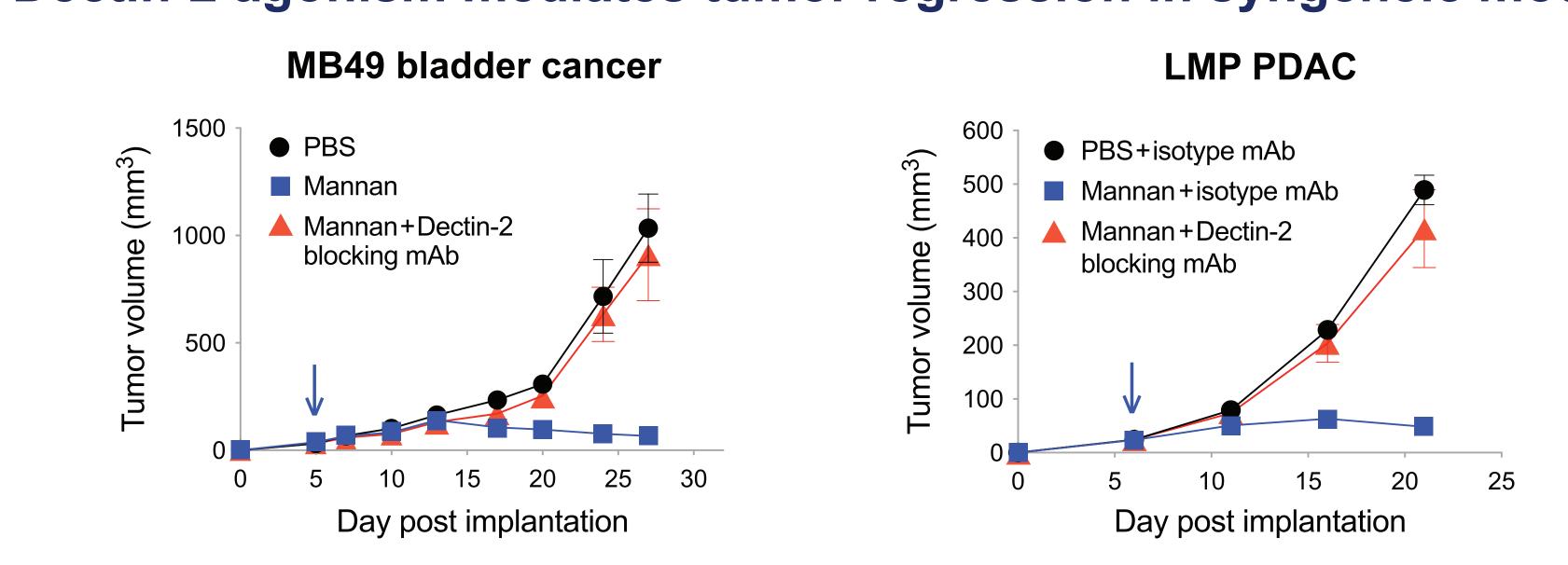
Figure 3: Dectin-2 is expressed by TAMs in primary human tumor samples. (Left) Human tumors were processed into single-cell suspensions and analyzed by flow cytometry. TAMs were defined as viable CD45+CD11b+CD14+HLA-DR+ cells. (Right) Tumors were harvested from MB49- or LMP-bearing mice and analyzed as single-cell suspensions by flow cytometry for Dectin-2 expression.

### Dectin-2 agonism activates murine TAMs in vitro and in vivo



**Figure 4: Mannan, a natural ligand for Dectin-2, activates TAMs.** (Left) TNFα secretion by in vitrogenerated murine TAMs stimulated overnight with plate-bound *S. cerevisiae* mannan. (Right) Murine TAMs from LMP PDAC tumors were analyzed by flow cytometry for activation markers 6 hours following treatment with systemically administered mannan or PBS control. Data are shown as mean with SEM (n=3 mice per group).

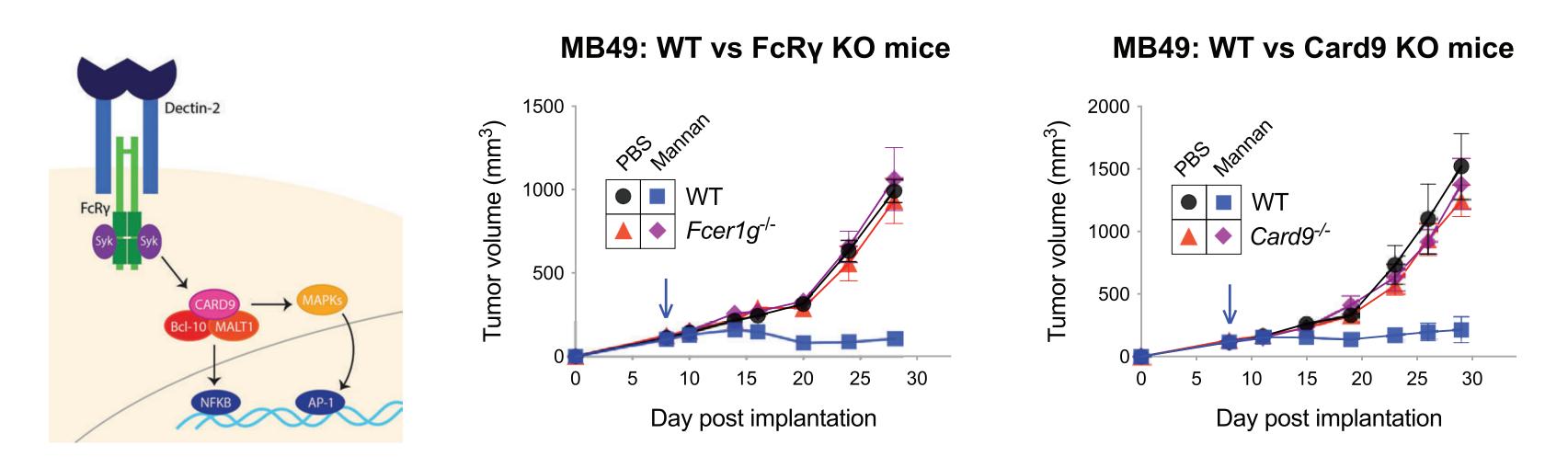
### Dectin-2 agonism mediates tumor regression in syngeneic models



**Figure 5: Mannan elicits tumor regression in a Dectin-2-dependent manner.** MB49 (Left) or LMP (Right) tumor-bearing mice were treated systemically every 2 days with *S. cerevisiae* mannan (12.5 mg/kg iv) with or without co-administration of Dectin-2 blocking antibody (5-10 mg/kg ip). Data are shown as mean with SEM and n=4-5 mice per group; day of treatment initiation is marked with a blue arrow.

# RESULTS

#### Anti-tumor efficacy requires Dectin-2 signaling components



**Figure 6: Mannan efficacy requires Dectin-2 signaling components.** MB49 tumor-bearing WT or KO (FcRγ or Card9) mice were treated systemically every 2 days with 10 mg/kg mannan. Data are shown as mean with SEM and n=5-6 mice per group; day of treatment initiation is marked with a blue arrow.

# Dectin-2 agonist activity is CD8 T cell dependent and elicits immunological memory

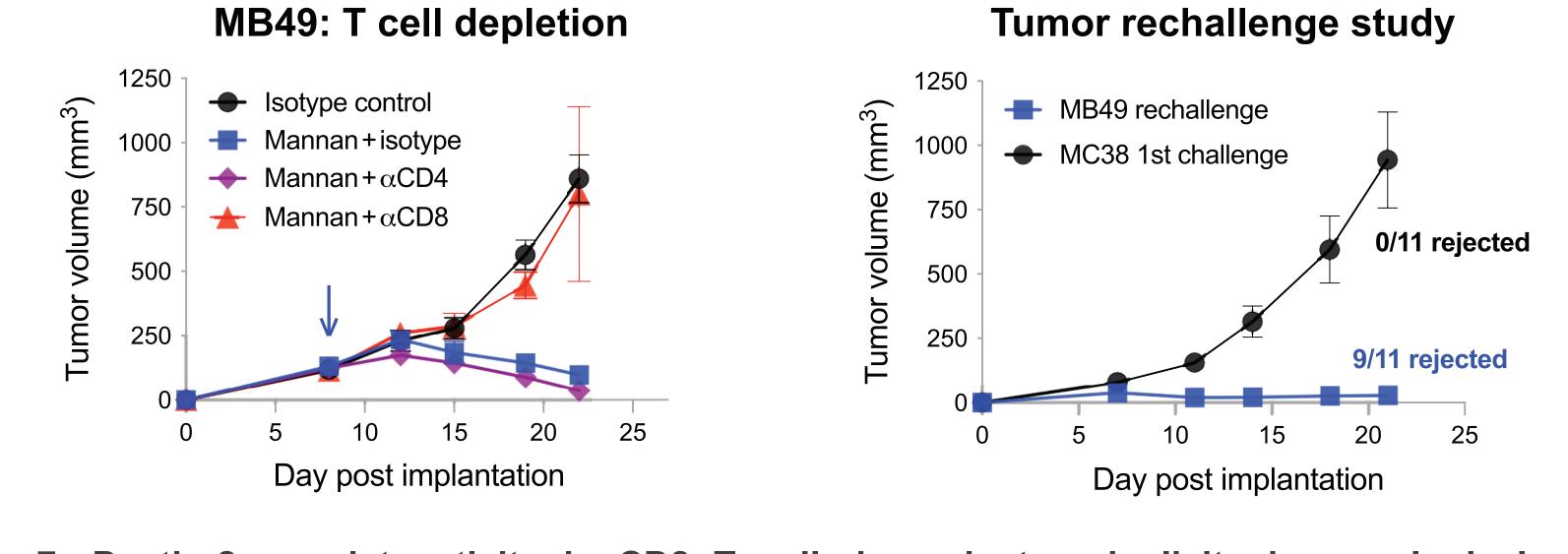


Figure 7: Dectin-2 agonist activity is CD8 T cell dependent and elicits immunological memory. (Left) MB49 tumor-bearing mice were treated systemically with mannan, and 250 ug of isotype IgG, anti-CD4, or anti-CD8 depleting antibody every 3 days (n=4-5 mice, day of treatment initiation marked with a blue arrow). (Right) Mice that experienced complete regression of MB49 tumors following mannan treatment were rechallenged with MB49 cells as well as an unrelated tumor cell line (MC38) (n=11 mice). Data are shown as mean with SEM.

### Agonist mAbs bind Dectin-2 and activate human macrophages

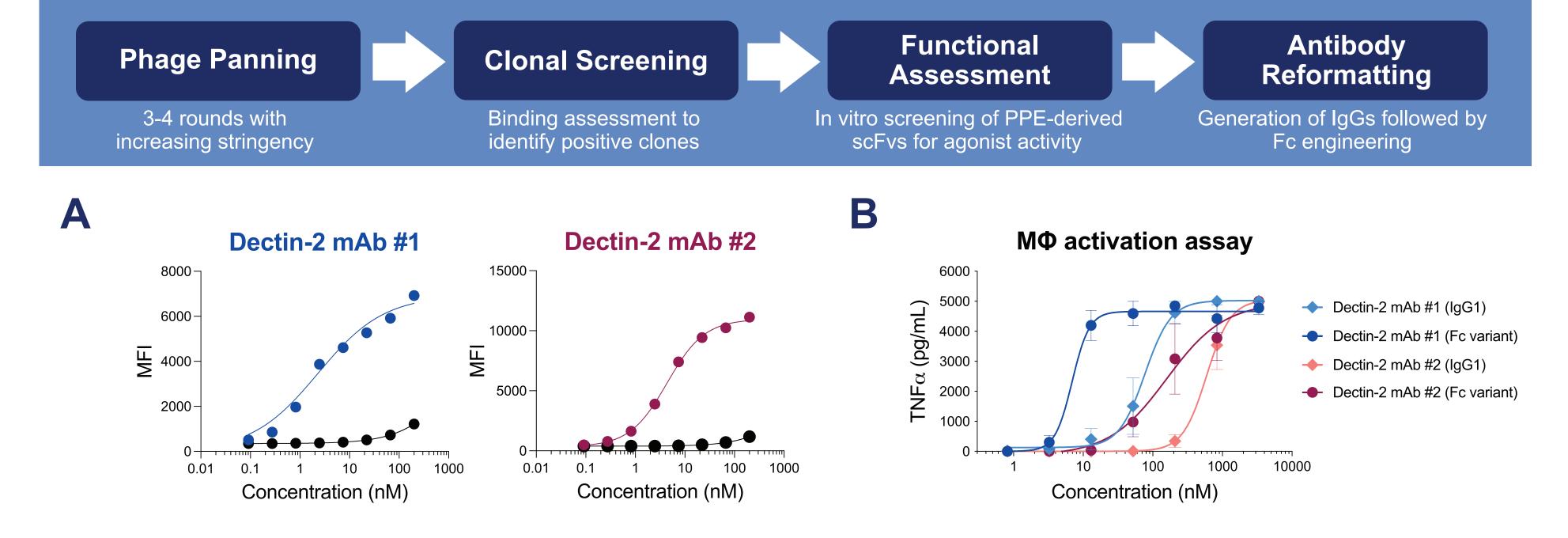


Figure 8: Agonist mAbs bind Dectin-2 and activate human macrophages. (A) Dectin-2 agonist mAbs bind to cells expressing Dectin-2 with single digit nM EC50s, while minimal binding is detected with HEK293T cells lacking Dectin-2 expression (black circles). (B) Human monocytes isolated from healthy human blood (n=5) were differentiated with M-CSF for 5 days and then stimulated overnight with indicated mAbs. Activation was measured as TNFα secretion by ELISA.

#### Fc-engineered agonist mAb potently activates human macrophages

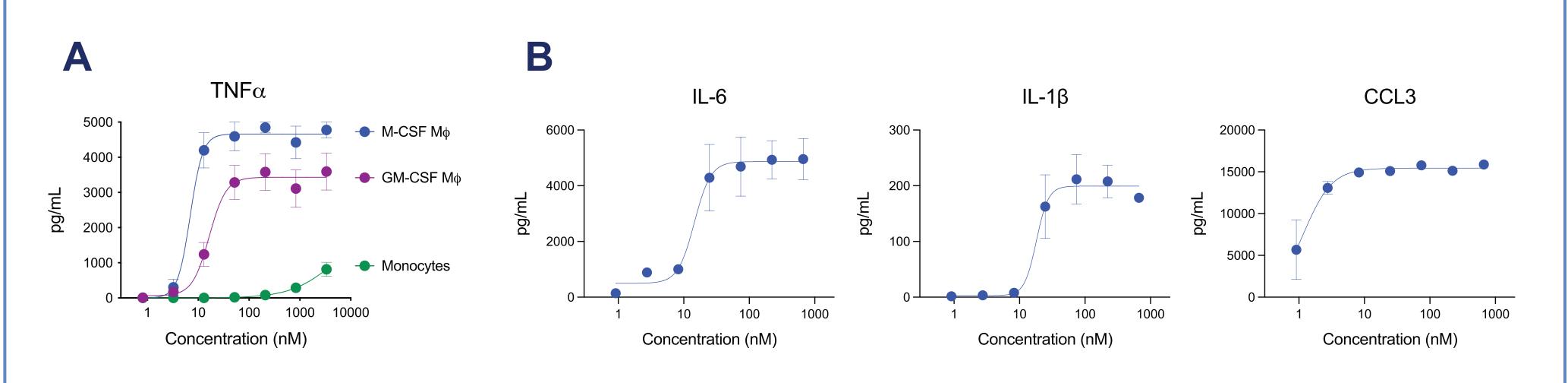


Figure 9: Dectin-2 agonist mAb 1 (Fc variant) elicits proinflammatory cytokine and chemokine production by human macrophages. (A) Fresh human monocytes (n=12 donors) or monocyte-derived macrophages generated with M-CSF (n=5) or GM-CSF (n=12) were stimulated overnight with the Dectin-2 agonist mAb, followed by cytokine analysis by ELISA. (B) Human M-CSF macrophages (n=3) were stimulated overnight with the Dectin-2 mAb, followed by cytokine and chemokine analysis using MSD kits.

### Dectin-2 agonist mAb activates primary human TAMs ex vivo

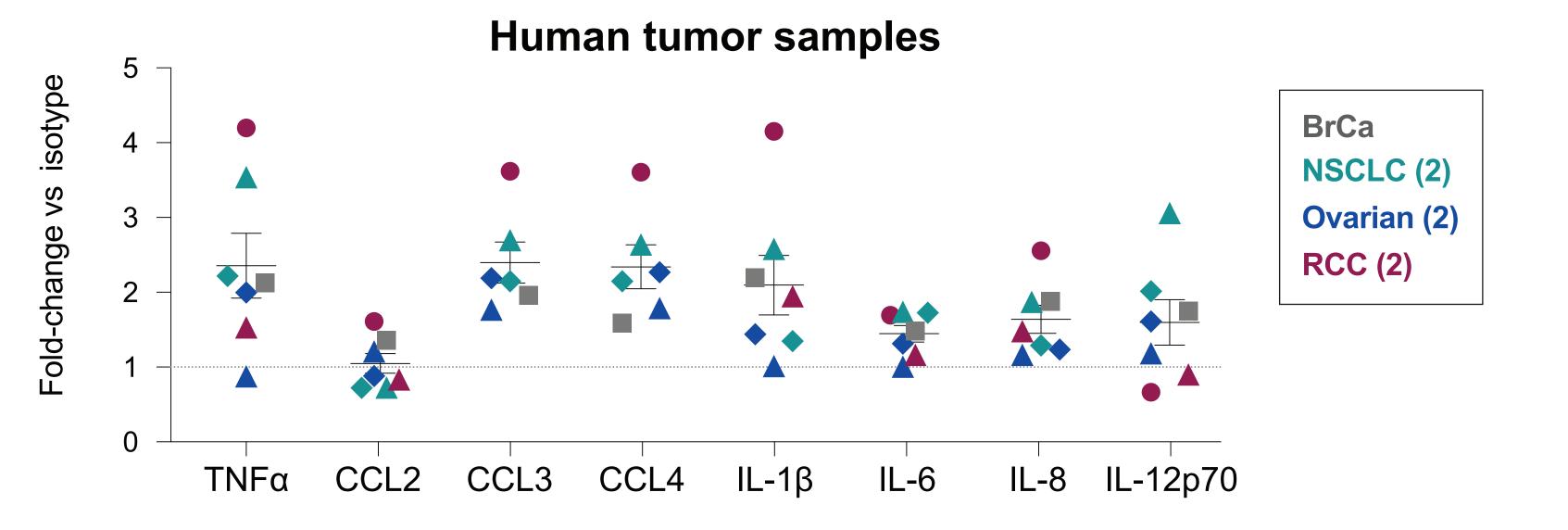


Figure 10: Dectin-2 agonist mAb 1 (Fc variant) activates primary human TAMs. Primary human tumor samples were processed into single-cell suspensions and cultured overnight with a Dectin-2 agonist antibody or non-binding isotype control antibody. Increased secretion of potent pro-inflammatory cytokines and chemokines was measured across all tumor samples tested. Data are shown as mean with SEM; Breast Cancer (BrCa, n=1), Non-Small Cell Lung Cancer (NSCLC, n=2), Ovarian (n=2), Renal Cell Carcinoma (RCC, n=2).

### CONCLUSIONS

- Dectin-2 is a novel target expressed by tumor-associated macrophages (TAMs)
- Agonism of Dectin-2 on TAMs elicits secretion of pro-inflammatory cytokines and chemokines to stimulate a productive anti-tumor immune response
- Dectin-2 agonism mediates anti-tumor efficacy in a CD8 T cell-dependent manner and elicits immunological memory
- Discovery and lead optimization identified a potent agonist antibody targeting Dectin-2 with an engineered Fc domain
- Bolt Biotherapeutics' agonist antibody has the potential to reprogram tumor-supportive macrophages into tumor-destructive macrophages as a novel anti-tumor immunotherapy