TARGETING GALECTIN-3 WITH A HIGH-AFFINITY ANTIBODY FOR INHIBITION OF HIGH GRADE SEROUS OVARIAN CANCER AND OTHER MUC16/CA-125–EXPRESSING MALIGNANCIES

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1S. Expression of LGALS3 and CA125 (MUC16) in human ovarian cancer cell lines. OVCAR3 and SKOV3^{c344} cell lines were stained for extracellular MUC16 and intracellular LGAL3 and evaluated via flow cytometry.

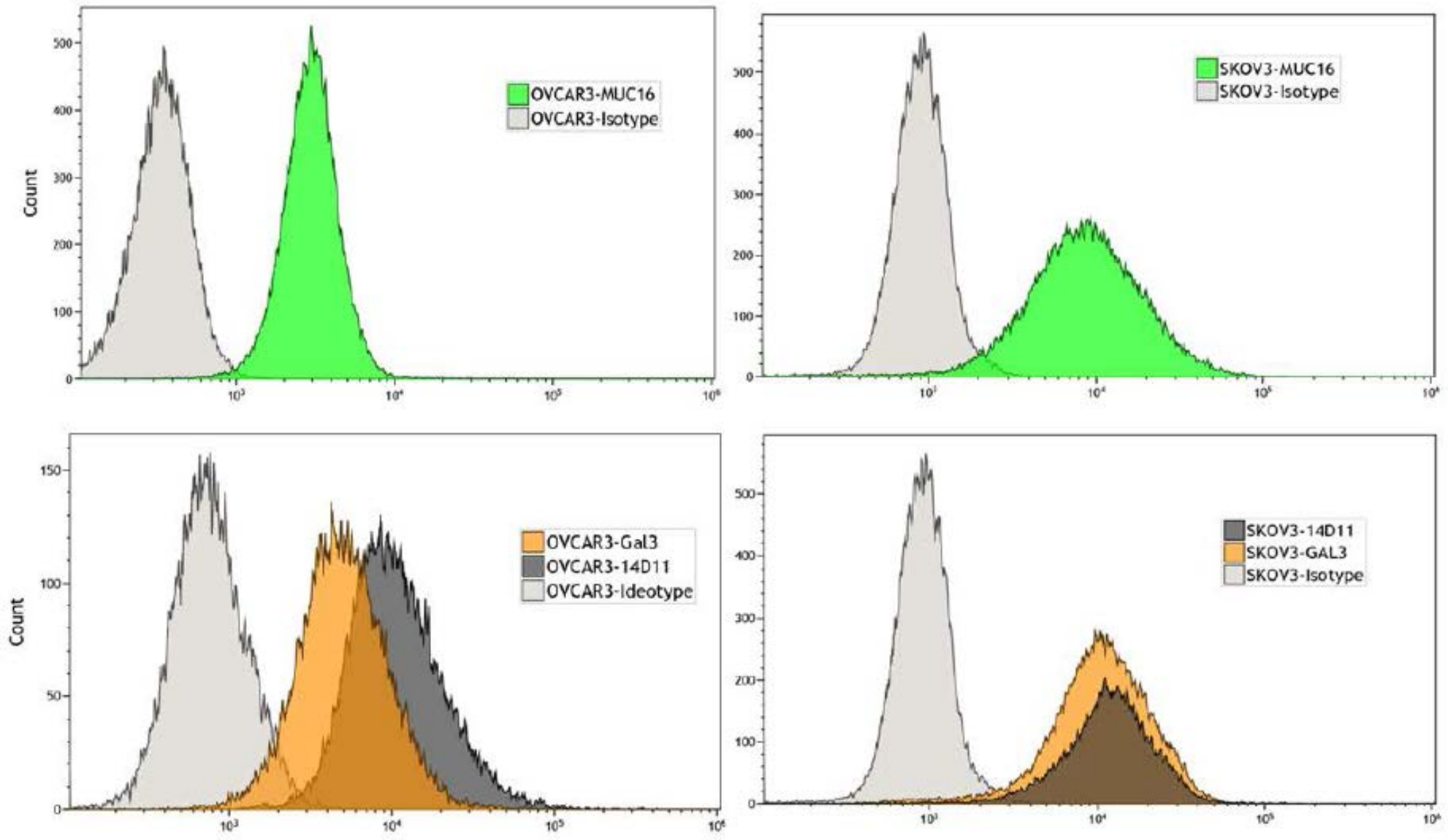
Supplemental Figure S2. Anti–Gal3 monoclonal antibody, 14D11, demonstrates binding to Gal3. **A.** Binding of antibody to human galectin-3 (R&D Systems; Minneapolis, MN) by surface plasmon resonance (SPR; GE Biacore; Pittsburgh, PA) according methods described by Mehta-D'souza et al (33). The antibodies to be tested were bound to a gold chip activated with mouse Fc receptor. Galectin-3 (R and D Systems) was used as ligand and delivered at a constant flow rate. 1D1 was used as a isotype control antibody without desired characteristics. **B.** A series of increasing concentrations Gal3 and analysis to fit the binding curves. The dissociation constant for the 14D11 antibody was estimated as 14.6 nM.

Supplemental Figure S3: Uncropped Western Blots. A. Cropped Western Blot (from Figure 2A) confirming silencing of *LGALS3* in the *LGALS3* knockdown cell line, sh*LGALS3*-MDA-MB-231 and confirming silencing of *LGALS1* in the *LGALS1* knockdown cell line, sh*LGALS1-MDA-MB-231*. β-Actin normalized densitometry quantification values are shown below each Western blot band. **B.** Uncropped Western Blots

Supplemental Figure 4S: Cropped Western Blot (Figure 4B) showing the effect of 14D11 on MUC16-mediated activation of ERK/AKT signaling in MUC16-expressing A2780^{c114}, A2780^{c344}, SKOV3^{c114} and SKOV3^{c344} cells after 48-h exposure to antibody. MUC16-negative A2780 and SKOV3 cells were used as controls. β-Actin normalized densitometry quantification values are shown below each western blot band. **B.** Uncropped Western Blots

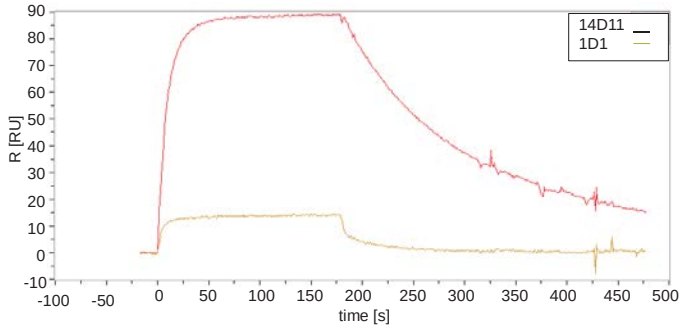
Supplemental Table 1: Immunization schedules used for the three separate immunization campaigns to generate anti-galectin 3 monoclonal antibodies.

Supplemental Figure 1: Expression of MUC16 and Galectin-3

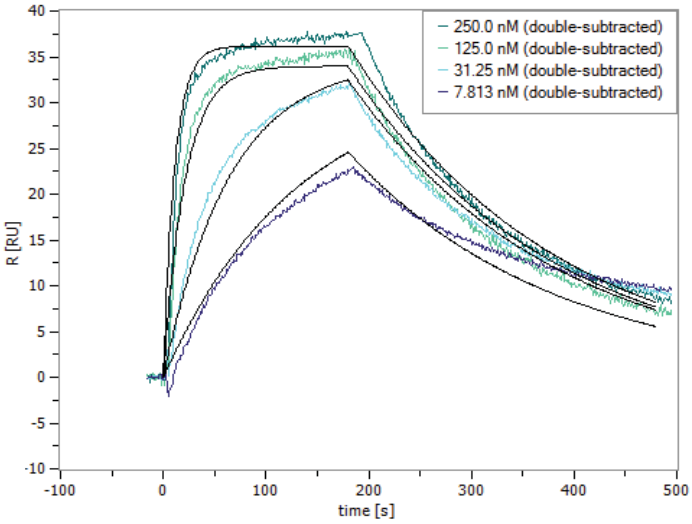

Count
OVCAR3-MUC16
OVCAR3-Isotype
SKOV3-MUC16
SKOV3-Isotype
Count
OVCAR3-Gal3
OVCAR3-14D11
OVCAR3-Ideotype
SKOV3-14D11
SKOV3-GAL3
SKOV3-Isotype

SUPPLEMENTAL FIGURE S2

A.

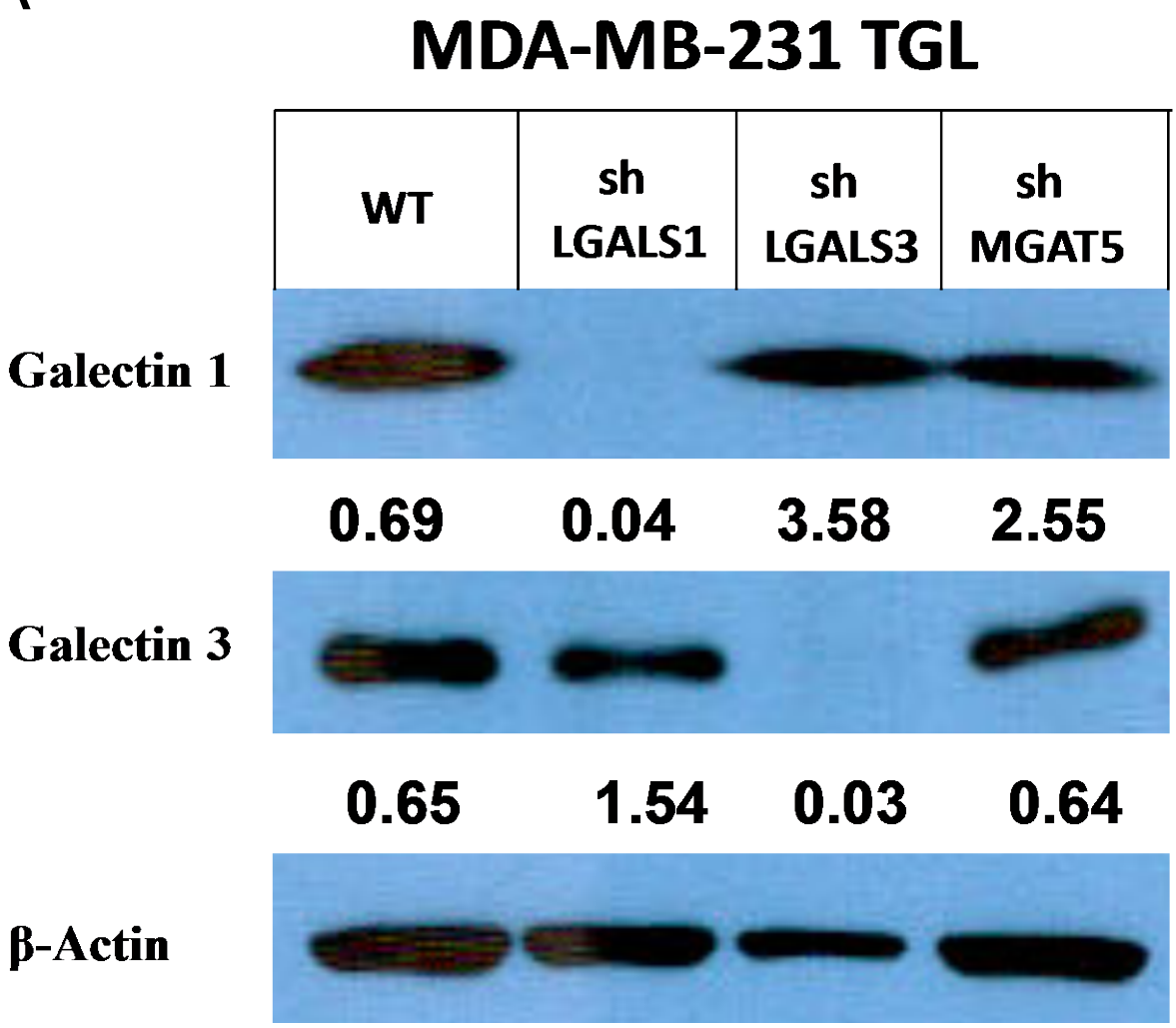

14D11
1D1
R [RU]
time [s]

B.

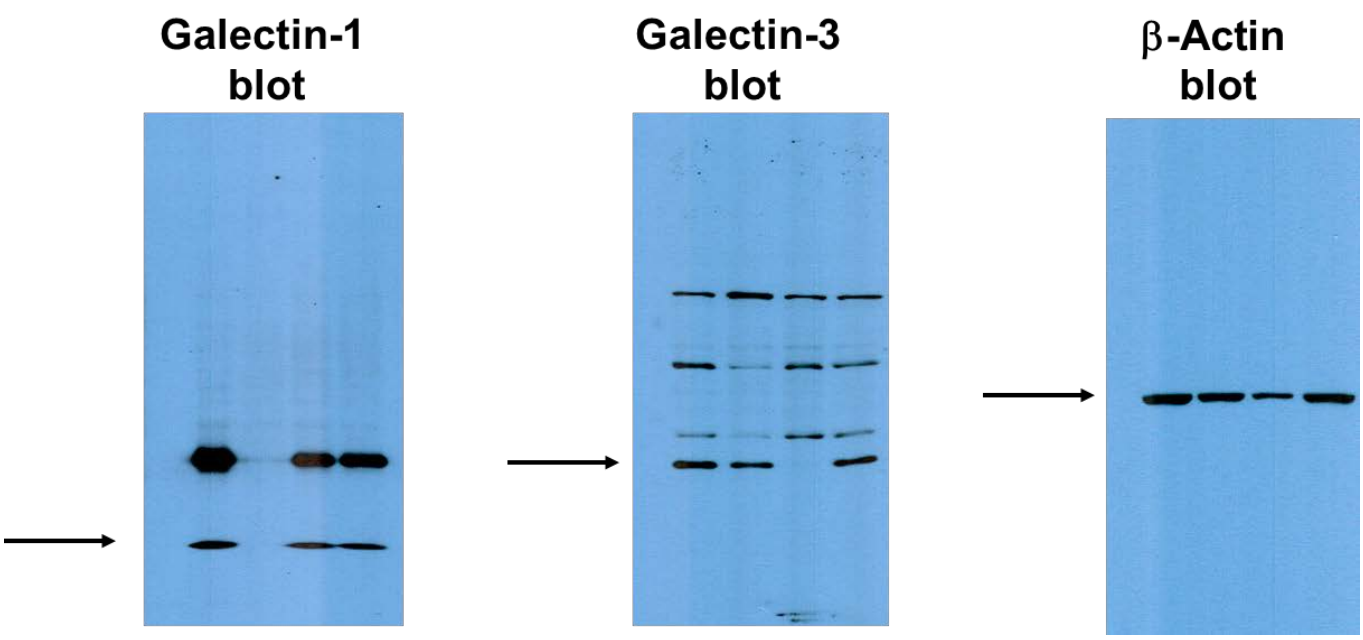

250.0 nM (double-subtracted)
125.0 nM (double-subtracted)
31.25 nM (double-subtracted)
7.813 nM (double-subtracted)
R [RU]
time [s]

SUPPLEMENTAL FIGURE S3

A

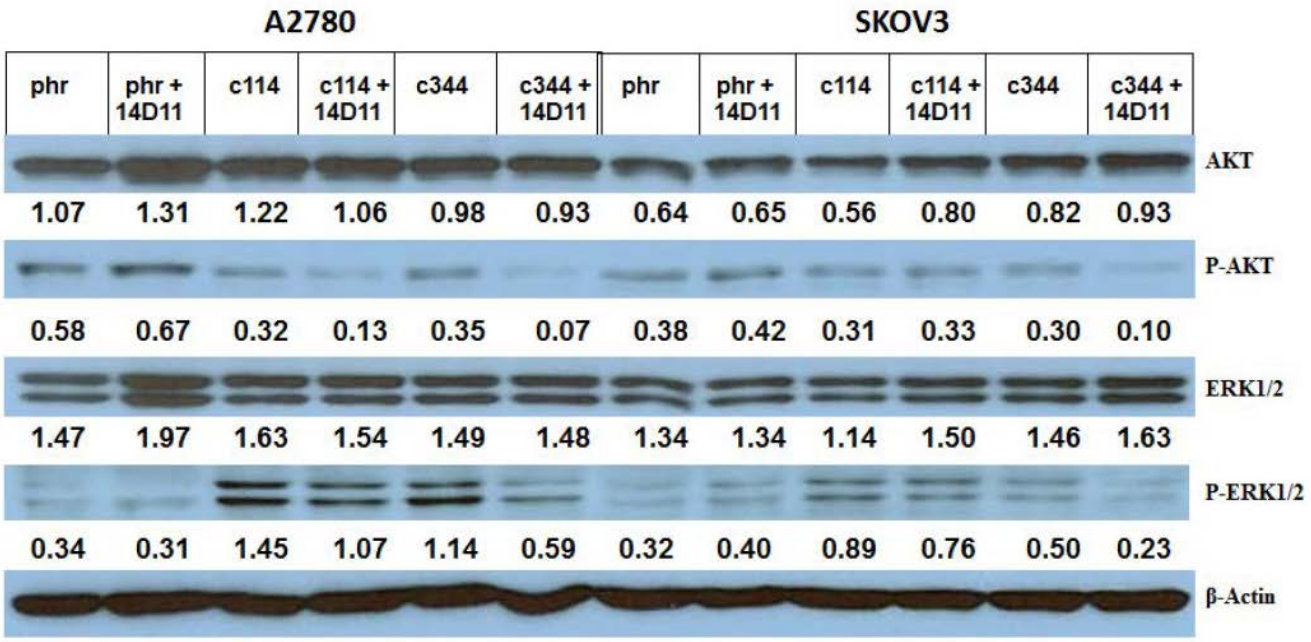

MDA-MB-231 TGL
WT
sh LGALS1
sh LGALS3
sh MGAT5
Galectin 1
0.69
0.04
3.58
2.55
Galectin 3
0.65
1.54
0.03
0.64
β-Actin

B

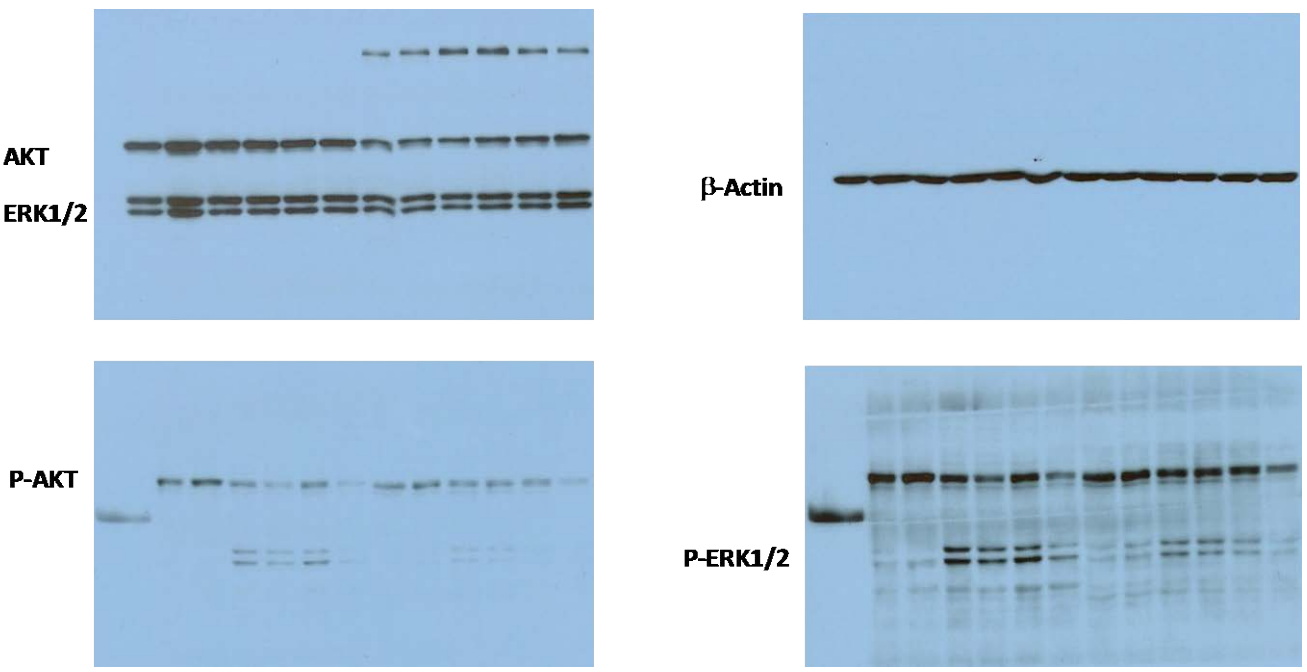

Galectin-1 blot
Galectin-3 blot
β-Actin blot

SUPPLEMENTAL FIGURE S4

A


A2780
SKOV3
phr
phr + 14D11
c114
c114 + 14D11
c344
c344 + 14D11
phr
phr + 14D11
c114
c114 + 14D11
c344
c344 + 14D11
AKT
1.07 1.31 1.22 1.06 0.98 0.93 0.64 0.65 0.56 0.80 0.82 0.93
P-AKT
0.58 0.67 0.32 0.13 0.35 0.07 0.38 0.42 0.31 0.33 0.30 0.10
ERK1/2
1.47 1.97 1.63 1.54 1.49 1.48 1.34 1.34 1.14 1.50 1.46 1.63
P-ERK1/2
0.34 0.31 1.45 1.07 1.14 0.59 0.32 0.40 0.89 0.76 0.50 0.23
β-Actin

B


AKT
ERK1/2
β-Actin
P-AKT
P-ERK1/2

SUPPLEMENTAL TABLE S1

Date	Immunogen	Amount ug	Route Of Injection	Sera Id	Bleed Date
Campaign 1					
7/22/2016	LGals3 - Fc Fusion	50ug	SC (CpG+adjuvant)	Pre immune	7/22/2016
8/12/2016	LGals3 - Fc Fusion	50ug	SC (with adjuvant)	TB1	8/19/2016
9/2/2016	LGals3 - Fc Fusion	50ug	SC (with adjuvant)	TB2	9/9/2016
9/19/2016	LGals3 - Fc Fusion	50ug	SC (with adjuvant)	TB3	9/26/2016
10/10/2016	LGals3 - Fc Fusion	50ug (M2 M3)	SC (with adjuvant)	TB4	10/17/2016
10/18/2016	LGALS3 Protein	50 ug (M1, M4, M5)	SC (with adjuvant)	TB4	10/25/2016
10/31/2016	LGALS3 Protein	5ug	SC (with adjuvant)	TB5	11/7/2016
Campaign 2					
12/2/2016	Peptide - LGALS3 binding domain	50 ug	SC (with adjuvant)	TB6	12/9/2016
12/23/2016	Peptide - LGALS3 binding domain	50ug	SC (with adjuvant)	TB7	12/30/2016
1/13/2017	Peptide - LGALS3 binding domain	50 ug	SC (with adjuvant)	TB8	1/20/2017
4/7/2017	LGALS3 N-Terminus Peptide	50 ug	SC (with adjuvant)	TB9	4/14/2017
5/2/2017	LGALS3 N-Terminus Peptide	50 ug	SC (with adjuvant)	TB10	5/9/2017
5/23/2017	LGALS3 N-Terminus Peptide	50 ug	SC (with adjuvant)	TB11	5/30/2017
6/26/2017	LGALS3 N-Terminus Peptide	5 ug (M1)	IV - tail vein	Terminal	6/29/2017
6/26/2017	Peptide - LGALS3 binding domain	5 ug (M1)	IV - tail vein	Terminal	6/30/2017
Campaign 3					
12/16/2016	LGALS3 conserved region Peptide 1-KLH	20 ug	SC (with adjuvant)	Pre-immune	12/15/2016
12/30/2016	LGALS3 conserved region Peptide 1-KLH	50ug	SC (with adjuvant)	TB1	1/6/2017
1/20/2017	LGALS3 conserved region Peptide 1-KLH	50 ug	SC (with adjuvant)	TB2	1/27/2017
2/21/2017	LGALS3 conserved region Peptide 1-KLH	10 ug	IV - tail vein	Terminal	2/24/2017
4/7/2017	LGALS3 N-Terminus Peptide 9-KLH	50 ug	SC (with adjuvant)	TB3	4/14/2017
5/2/2017	LGALS3 N-Terminus Peptide 9-KLH	50 ug	SC (with adjuvant)	TB4	5/9/2017
5/23/2017	LGALS3 N-Terminus Peptide 9-KLH	50 ug	SC (with adjuvant)	TB5	5/30/2017
6/26/2017	LGALS3 N-Terminus Peptide 9-KLH	5 ug (M2)	IV - tail vein	Terminal	6/30/2017