A

Lgals4

Exon 1

Exon 2

Fw:GAAGTCTCCACCACCAGAGAGACC
rv:CTGTCTCACCGTCTCATGTTCTC

Fw:CTCATCTCCCCCGCTTAAGG
rv:CCCAGTCATAGAGCCACCTC

B

	R26	Gal 4 Exon1				Gal 4 Exon 2				
Clone:	1	1	2	3	4	1	2	3	4	5

Vinculin — 150 kDa / — 100 kDa

Gal 4 — 37 kDa / — 25 kDa

C

Competition assay

- Gal 4 Exon 1
- Gal 4 Exon 2
- R26
- RPA3

D

R26 Control vs Gal 4-KO proliferation

- R26 (n=2)
- Gal 4 Exon 1 (n=4)
- Gal 4 Exon 2 (n=5)

ns. ns. ns. ns.

E

Reference genome	GCCCTACAAGAGACCCATCCCAGGTGGCCTCAGTGTCGGGATGTCCGTTTACATCCAAGGAATG
Clone 1, allele 1	GCCCTACAAGAGACCCATCCCAGGTGGCCTCA----GTTTACATCCAAGGAATG
Clone 1, allele 2	GCCCTACAAGAGACCCATCCCAGGTGGCCTCA---TGTCGGGATGTCCGTTTACATCCAAGGAATG

Reference genome	GCCCTACAAGAGACCCATCCCAGGTGGCCTCAG-TGTCGGGATGTCCGTTTACATCCAAGGAATG
Clone 4, allele 1	GCCCTACAAGAGACCCATCCCAGGTGGCCTCAGTGTCGGGATGTCCGTTTACATCCAAGGAATG
Clone 4, allele 2	GCCCTACAAGAGACCCATCCCAGGTGGCCTCAG---TCGGGATGTCCGTTTACATCCAAGGAATG

Reference genome	ACCTCTGAGCGCTGTTCACCTGTCGCCTTCTACAGACTCTGCC...GTGAGACAGTCCCCGCCGCACTTC
Clone 5, allele 1	ACCTCTGAGC-----------GCTGCTTCTACAGACTCTGCC---------GCACTTC
Clone 5, allele 2	ACCTCTGAGCGCAGGTGAAGAGCGCTCAGAGGTG-----------GCGCACTTC

F

Wildtype
Lgals4

GLECT   GLECT

Predicted Peptides

Clone 1 allele 1

Clone 1 allele 2

Clone 4 allele 1

Clone 4 allele 2

Clone 5 allele 1

Clone 5 allele 2

- Frameshift
- Wt Sequence
- Stop Codon

G

Tumor Weight

ns.

R26 (n=11)   Gal 4-KO (n=14)

H

CD8+ T-cell Gating Strategy

Live / Lymphocytes / CD45+ / Single Cells

I

Activated CTLs (CD69+)

ns.

R26 (n=4)   Gal 4-KO (n=6)

Degranulating CTLs (CD107a+)

ns.

R26 (n=4)   Gal 4-KO (n=6)

J

Tnf

ns.

R26 (n=4)   Gal 4-KO (n=4)

Nfkb1

*

R26 (n=4)   Gal 4-KO (n=4)

A

Scr 78 79 80 81 82

Vinculin — 150 kDa
Galectin 4 — 37 kDa

Galectin 4/Vinculin (scramble=1)

1.0

Scr 78 79 80 81 82

B

T3   T5   T8   T9
scr sh scr sh scr sh scr sh

Vinculin — 150kDa
Galectin 4 — 37kDa

C

Gal 4-KD vs Scr Proliferation comparison

Luminescence

100000
80000
60000
40000
20000
0

ns. ns.

● Ctrl (scramble)
■ Gal 4-KD (shGal4)

0   72   120
Time (h)

D

EMT Markers

Fold Change

40
35
30
15
10
5
0

● Gal 4-KD
● Scramble

*   *   *   *   *   *

Vim Fn1 Mmp10 Zeb1 Sox9 Axin2

E

CD4+ Cells

Cells/mm²

30
20
10
0

ns.

Scramble Ctrl   Gal 4-KD
(n=4)   (n=2)

CD8+ Cells

Cells/mm²

15
10
5
0

ns.

Scramble Ctrl   Gal 4-KD
(n=4)   (n=2)

F

Tumor Weight

Weight (g)

6
4
2
0

ns.

Scramble Ctrl   Gal 4-KD
(n=8)   (n=9)

G

Epithelial Cell Apoptosis

Cells/HPF

1.5
1.0
0.5
0.0

ns.

Scramble Ctrl   Gal 4-KD
(n=8)   (n=5)

H

Scramble   Gal 4-KD

Galectin 4

A

Matrigel Dome

Ex vivo Model system

B

Matrigel Dome

SSC

Epithelial Cells

Immune cells

CFSE

C

Monolayer

SSC (Height)

Single Cells
99.3

SSC (Area)

Size

CD3+ Cells
31.6

CD3

Size

CD8+ Cells
53.1

CD8

Size

7AAD+ Cells
43.1

7AAD

D

```
Human CD3D  KIPIEELEDRVFVNCNT---SITWVEG--TVGTLLSDITRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMCQSCVELD
Mouse CD3D  KIQVTEYEDKVFVTCNT--SVMHLDG--TVEGWFAKNKTLNLGKGVLDPRGIYLCNGTEQLAKVVSSVQVHYRMCQNCVELD
Sheep CD3D  ALEVLEAEDKVILKCNS--SITLLQG--TAGQEVSDNKTLNLGKRIEDPRGMYQCGEN----AKSFTLQVYYRMCQNCVELD
                            38              55          74

Human CD3G  KVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLTEDKKKWNLGSNAKDPRGMYQCKGS--QNKSKPLQVYYRMCQNCIELN
Mouse CD3G  QVDGSRGDGSVLLTCGLTDKTIKWLKDGSIISPLNATKNTWNLGNNAKDPRGTYQCQGA--KETSNPLQVYYRMCENCIELN
Sheep CD3G  KVDDNREDGSVILICVTDEKKITWLKDMKEISSGDTNKLTWDLGSSTKDPRGIYECKGS--SNESKSLQIYYRMCQNCIELN
                            52          66              92
```

E

SPR binding
hgal 4 - mCD3εδ Chain

Response Units (RU)

$K_d \sim 2\mu M$

Time (s)

F

Monolayer

SSC Area

FSC Area

SSC Area

CFSE

FSC Height

FSC Area

SSC Area

7AAD

SSC Area

Annexin V