SUPPLEMENTAL DATA FIGURE LEGENDS

FIGURE S1. Manipulation of Neu 1 Expression in Airway ECs. (A) A549 cells were infected with increasing MOI's of Ad-Neu 1- FLAG. At 48 h, cells were lysed and the lysates processed for immunoblotting with anti-FLAG antibody. (B) A549 cells were transiently infected with FLAG-tagged Ad-Neu 1 at an MOI = 100. After 48 h, the cells were transfected with Neu 1-targeting or control siRNAs, cultured for 24, 48, or 72 h, lysed, and the lysates processed for immunoblotting with anti-FLAG antibody. (C) A549 cells were transiently infected with HA-tagged Ad-Neu 3 at an MOI = 100. After 48 h, the cells were transfected with either Neu 1-targeting, Neu 3-targeting, or control siRNAs, cultured for 48 h, lysed, and the lysates processed for immunoblotting with anti-HA antibody. (D) SAECs were infected with increasing MOI's of FLAG-tagged Ad-Neu 1. At 48 h, the cells were lysed and the lysates processed for immunoblotting with anti-FLAG antibody. (E) A549 cells were infected with increasing MOI's of HA-tagged Ad-Neu 3. At 48h, cells were lysed and the lysates processed for HA immunoblotting. In (A) and (D), to control for endogenous Neu 1 expression, the blots were stripped and reprobed with anti-Neu 1 antibody. In (A)-(E), to control for protein loading and transfer, blots were stripped and reprobed for β-tubulin. IB = immunoblot; IB* = immunoblot after stripping. MW in kDa is indicated on left. Each blot is representative of $\geq$3 independent experiments.

FIGURE S2. EGF Diminishes EGFR Expression in Neu 1-Manipulated Airway ECs. A549 cells (A, B) or SAECs (C) were transfected with Neu 1-targeting or control siRNAs, or were infected with Ad-Neu 1 or Ad-Null at an MOI = 100. The cells were incubated for 24 h, starved for 2 h in serum-free medium (A549) or basal medium (SAECs), and treated for 10 min with 100 ng/ml EGF or medium alone. Equal protein aliquots (70 μg) of cell lysates were resolved by SDS-PAGE, transferred to PVDF, and the blots probed with anti-EGFR antibody. To control for protein loading and transfer, the blots were stripped and reprobed for β-tubulin. IB = immunoblot; IB* = immunoblot after stripping. MW in kDa is indicated on left. Each blot is representative of 2 independent experiments.

FIGURE S3. NEU1 Associates with MUC1 in Airway ECs. (A) A549 cells were infected with increasing MOIs of Ad-NEU1, and after 48h, were lysed and the lysates were immunoprecipitated with anti-MUC1 antibody. The MUC1 immunoprecipitates were processed for FLAG immunoblotting. The blots are representative of 3 independent experiments. (B) Densitometric analyses of blots in (A) (n=3). (C) A549 cells uninfected (–) or were infected (+) with Ad-NEU1 (MOI=100). After 48h, cells were untransfected (–) or were transfected with NEU1-targeting or control siRNAs, after which they were lysed and the lysates were immunoprecipitated with anti-MUC1 antibody. The MUC1 immunoprecipitates were processed for FLAG immunoblotting. (D) Densitometric analyses of blot in (C). In (A) and (C), to control for loading and transfer of immunoprecipitates, blots were stripped and reprobed with anti-MUC1 antibody. IP = immunoprecipitation; IB = immunoblot; IB* = immunoblot after stripping. MW in kDa indicated on left. In (B) and (D), vertical bars represent mean ± SEM FLAG signal normalized to MUC1 signal in the same lane on the same blot.

FIGURE S4. Sialic Acid and Galactose are Undetectable in TLR5. (A) A549 cells were transfected with Neu 1-targeting or control siRNAs, or were infected with Ad-Neu 1 or Ad-Null at an MOI = 100. After 24 h, the cells were lysed and 1.0 mg aliquots of cell lysates were immunoprecipitated with anti-TLR5 antibody. The TLR5 immunoprecipitates were resolved by SDS-PAGE, transferred to PVDF, and the blots probed with MAL (A) or PNA (C). As a control for lectin specificity, parallel blots of fetuin and asialofetuin were simultaneously probed (lanes 5, 6). To control for protein loading and transfer, blots were stripped and reprobed with anti-TLR5 antibody. IP = immunoprecipitation; IB = immunoblot; IB* = immunoblot after stripping. MW in kDa is indicated on left. Each blot is representative of 2 independent experiments.

FIGURE S5. Manipulation of Neu 1 Does Not Alter siRNA-Induced MUC1 Silencing. A549 cells were transfected with Neu 1-targeting or control siRNAs (A), or were infected with Ad-Neu 1 or Ad-Null at an MOI = 100 (B). After 24 h, the cells were transfected with control or MUC1-targeting siRNAs. After an additional 24 h, the cells were lysed and 50 µg aliquots of cell lysates were resolved by SDS-PAGE, transferred to PVDF, and the blots probed with anti-MUC1 antibody. To control for protein loading and transfer, blots were stripped and reprobed with anti-β-tubulin antibody. IB = immunoblot; IB* = immunoblot after stripping. MW in kDa is indicated on left. Each blot is representative of 2 independent experiments.

Figure S1

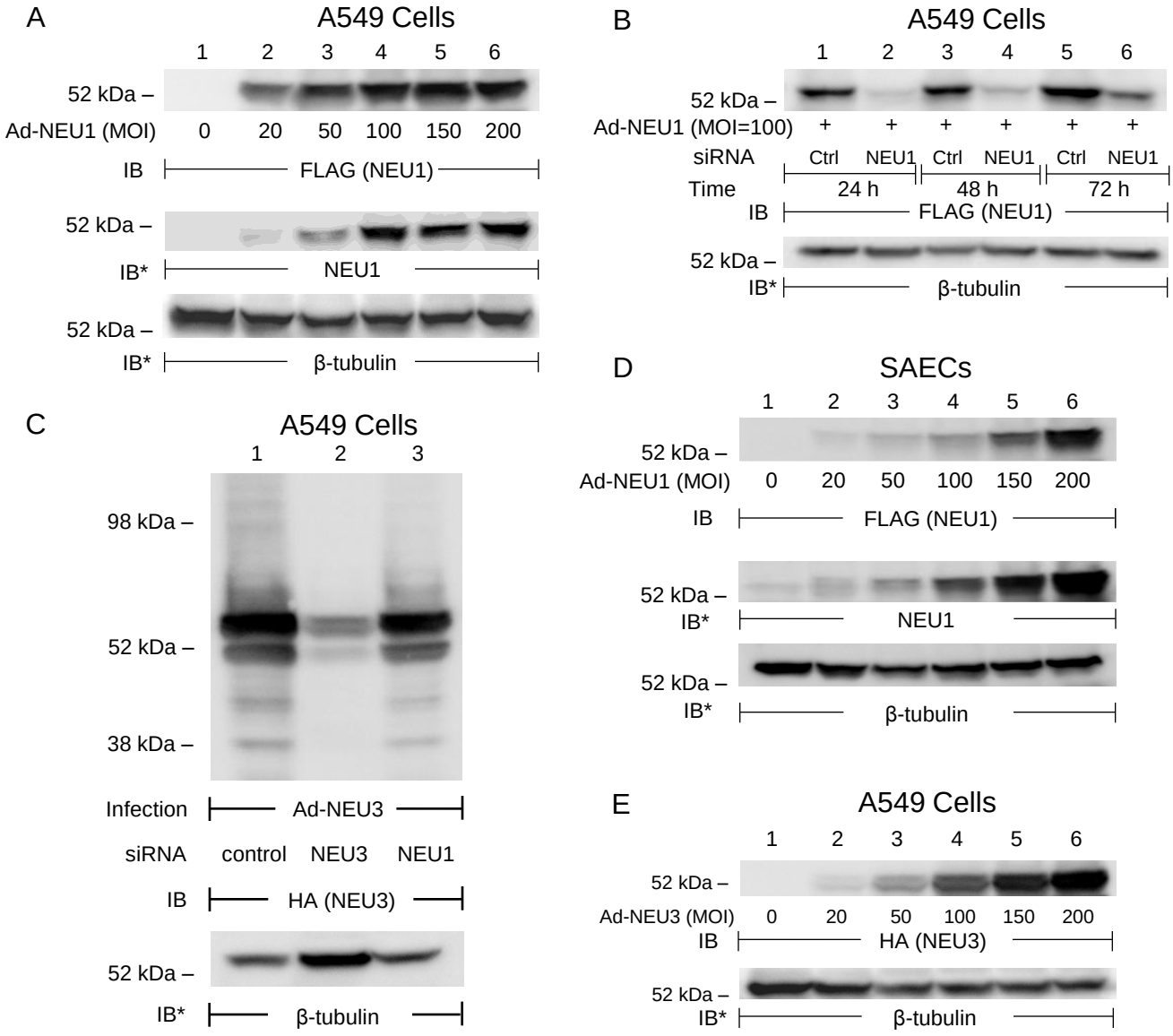

A
A549 Cells
1 2 3 4 5 6
52 kDa –
Ad-NEU1 (MOI) 0 20 50 100 150 200
IB FLAG (NEU1)
52 kDa –
IB* NEU1
52 kDa –
IB* β-tubulin
B
A549 Cells
1 2 3 4 5 6
52 kDa –
Ad-NEU1 (MOI=100) + + + + + +
siRNA Ctrl NEU1 Ctrl NEU1 Ctrl NEU1
Time 24 h 48 h 72 h
IB FLAG (NEU1)
52 kDa –
IB* β-tubulin
C
A549 Cells
1 2 3
98 kDa –
52 kDa –
38 kDa –
Infection Ad-NEU3
siRNA control NEU3 NEU1
IB HA (NEU3)
52 kDa –
IB* β-tubulin
D
SAECs
1 2 3 4 5 6
52 kDa –
Ad-NEU1 (MOI) 0 20 50 100 150 200
IB FLAG (NEU1)
52 kDa –
IB* NEU1
52 kDa –
IB* β-tubulin
E
A549 Cells
1 2 3 4 5 6
52 kDa –
Ad-NEU3 (MOI) 0 20 50 100 150 200
IB HA (NEU3)
52 kDa –
IB* β-tubulin

Figure S2

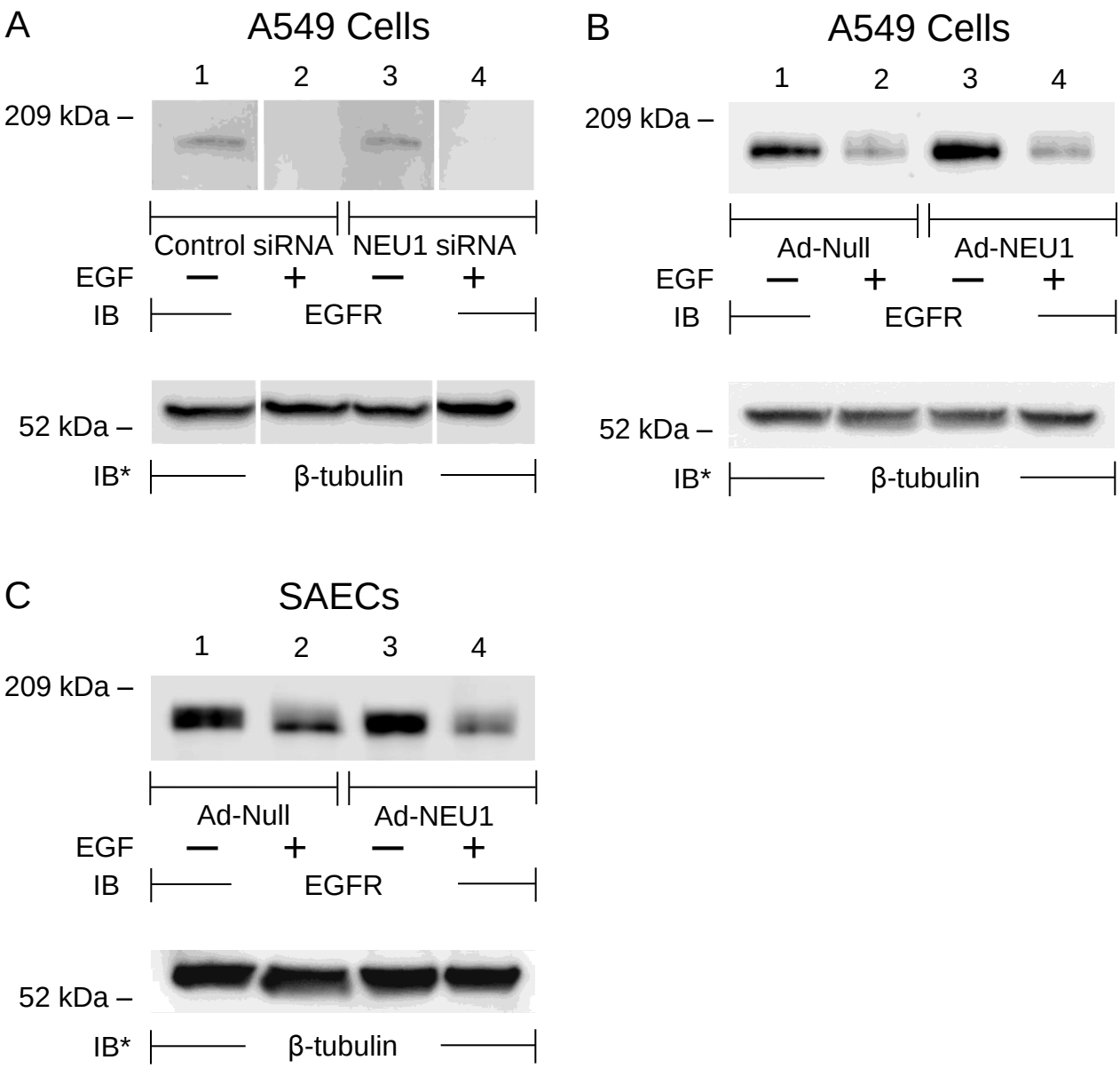

A
A549 Cells
1
2
3
4
209 kDa –
Control siRNA
NEU1 siRNA
EGF
–
+
–
+
IB
EGFR
52 kDa –
IB*
β-tubulin
B
A549 Cells
1
2
3
4
209 kDa –
Ad-Null
Ad-NEU1
EGF
–
+
–
+
IB
EGFR
52 kDa –
IB*
β-tubulin
C
SAECs
1
2
3
4
209 kDa –
Ad-Null
Ad-NEU1
EGF
–
+
–
+
IB
EGFR
52 kDa –
IB*
β-tubulin

Figure S3

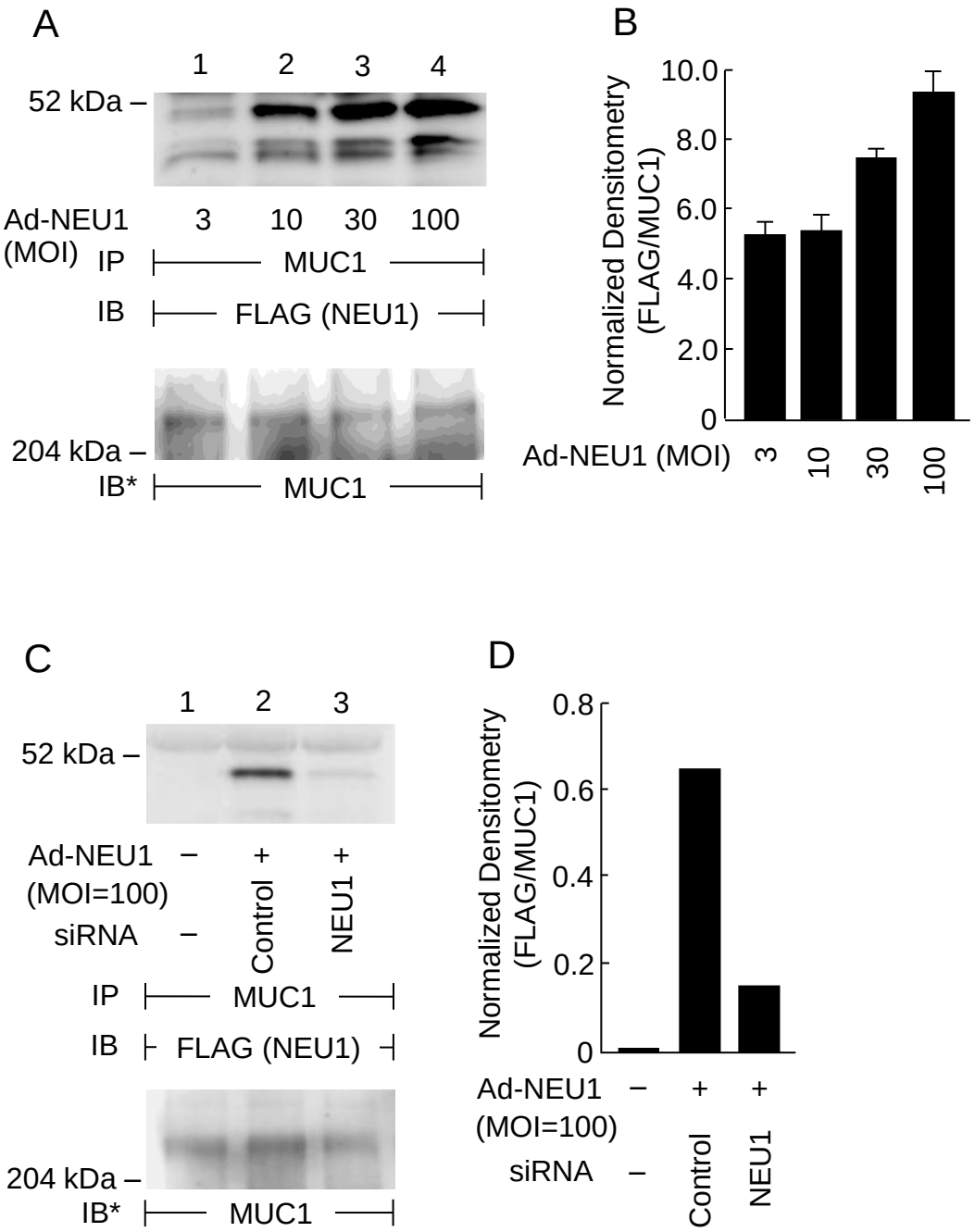

A
1
2
3
4
52 kDa –
Ad-NEU1 (MOI)
3
10
30
100
IP
MUC1
IB
FLAG (NEU1)
204 kDa –
IB*
MUC1
B
Normalized Densitometry (FLAG/MUC1)
10.0
8.0
6.0
4.0
2.0
0
Ad-NEU1 (MOI)
3
10
30
100
C
1
2
3
52 kDa –
Ad-NEU1 (MOI=100)
–
+
+
siRNA
–
Control
NEU1
IP
MUC1
IB
FLAG (NEU1)
204 kDa –
IB*
MUC1
D
Normalized Densitometry (FLAG/MUC1)
0.8
0.6
0.4
0.2
0
Ad-NEU1 (MOI=100)
–
+
+
siRNA
–
Control
NEU1

Figure S4

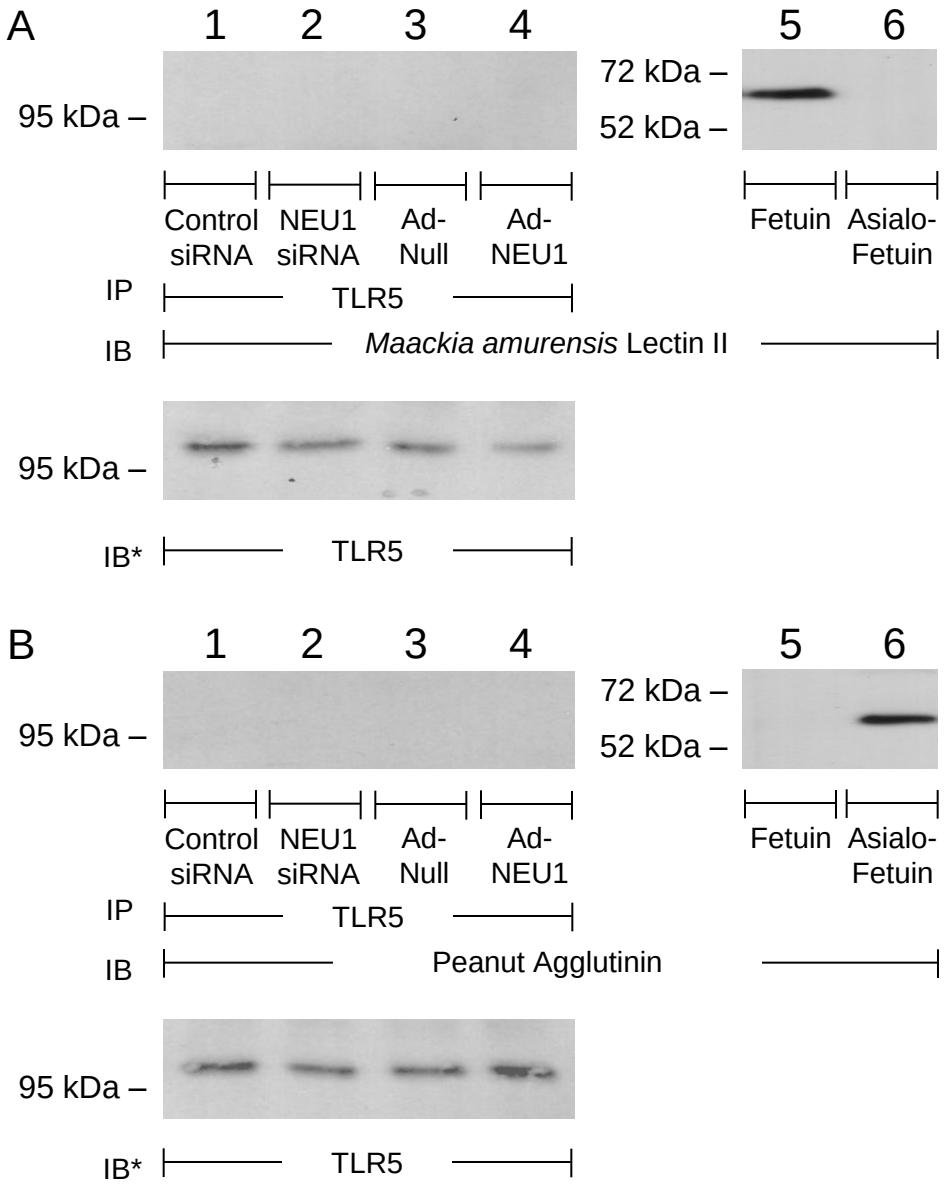
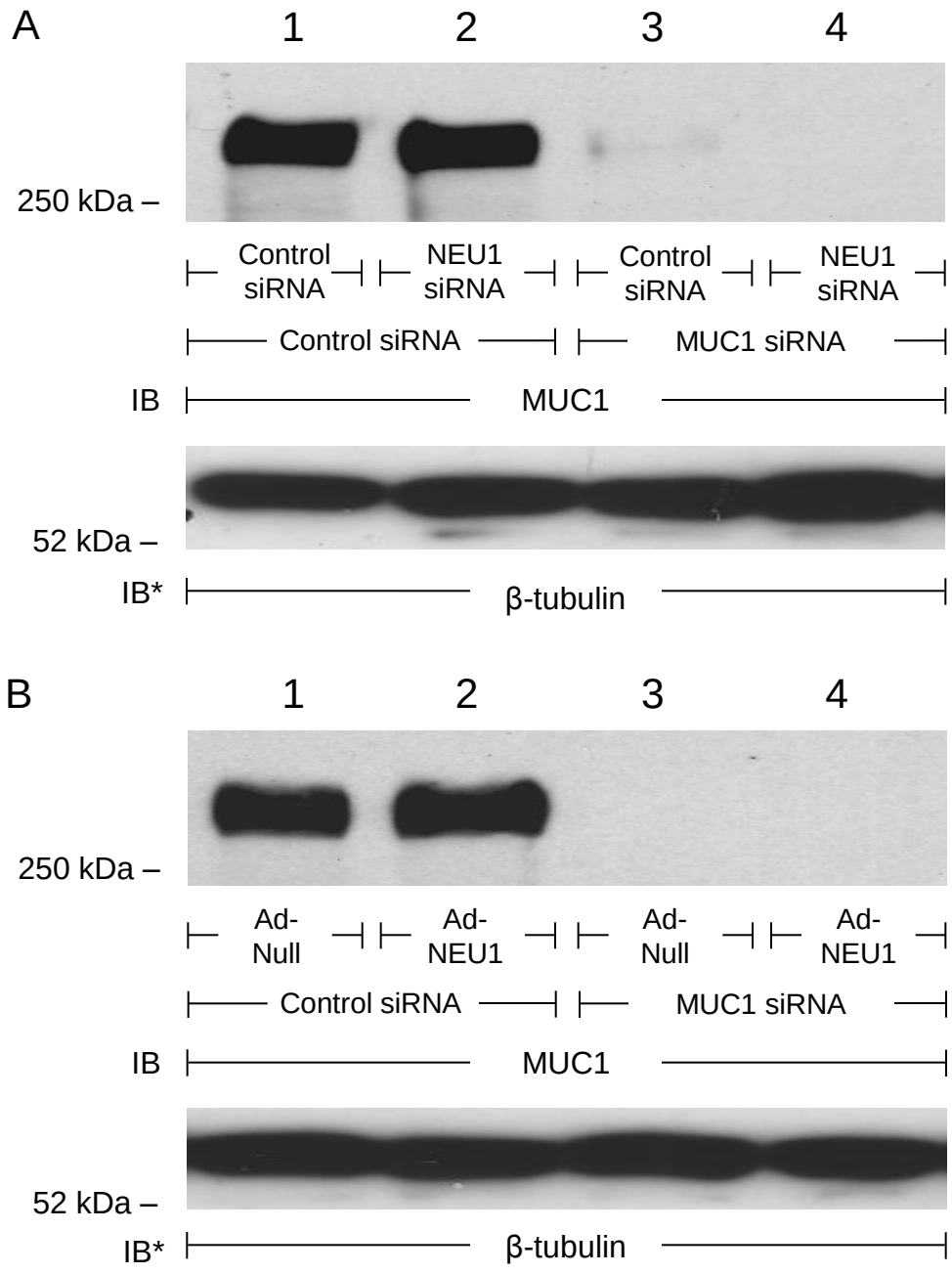

A
1
2
3
4
5
6
95 kDa –
72 kDa –
52 kDa –
Control siRNA
NEU1 siRNA
Ad-Null
Ad-NEU1
Fetuin
Asialo-Fetuin
IP
TLR5
IB
Maackia amurensis Lectin II
95 kDa –
IB*
TLR5
B
1
2
3
4
5
6
95 kDa –
72 kDa –
52 kDa –
Control siRNA
NEU1 siRNA
Ad-Null
Ad-NEU1
Fetuin
Asialo-Fetuin
IP
TLR5
IB
Peanut Agglutinin
95 kDa –
IB*
TLR5

Figure S5


A
1
2
3
4
250 kDa –
Control siRNA
NEU1 siRNA
Control siRNA
NEU1 siRNA
Control siRNA
MUC1 siRNA
IB
MUC1
52 kDa –
IB*
β-tubulin
B
1
2
3
4
250 kDa –
Ad-Null
Ad-NEU1
Ad-Null
Ad-NEU1
Control siRNA
MUC1 siRNA
IB
MUC1
52 kDa –
IB*
β-tubulin