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Supplementary Materials for

RACK7 recognizes H3.3G34R mutation to suppress expression of MHC class II complex components and their delivery pathway in pediatric glioblastoma

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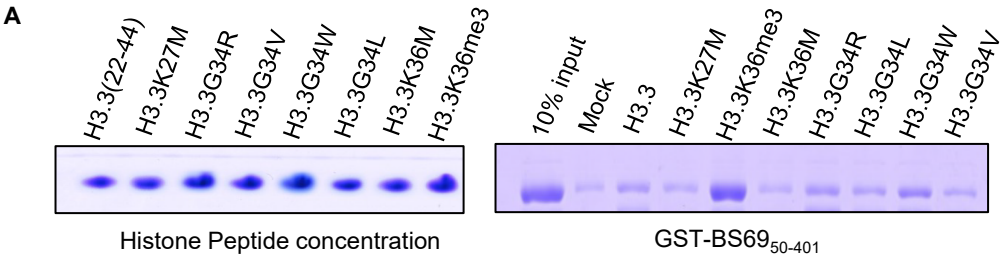
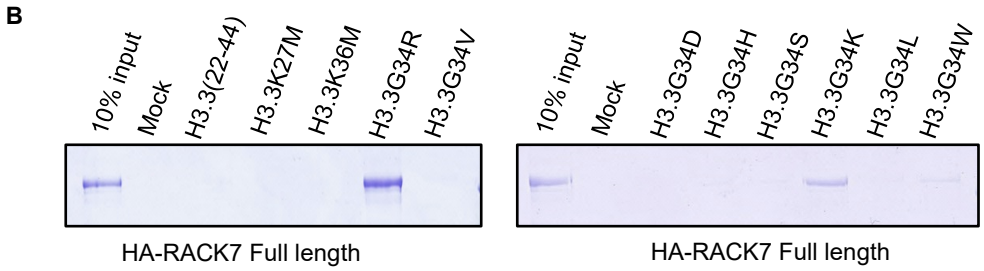
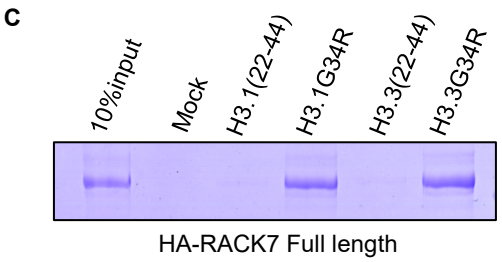
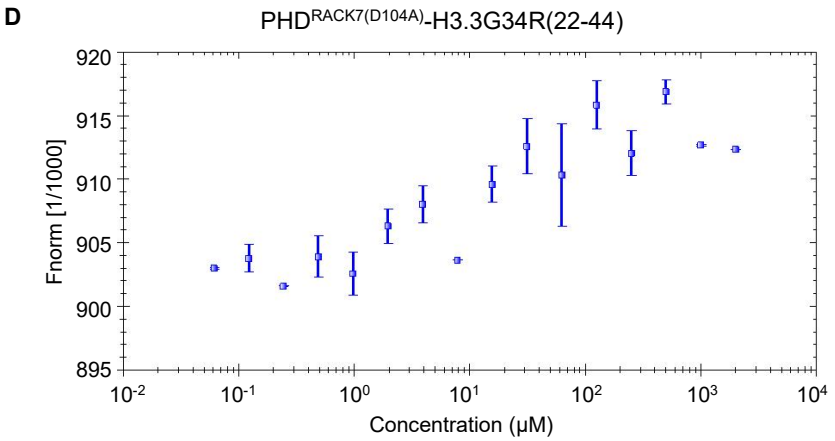
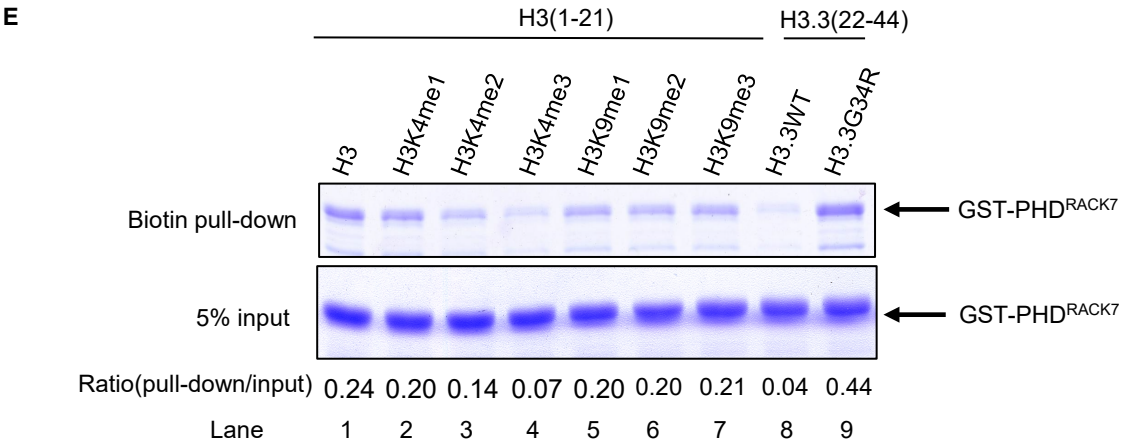
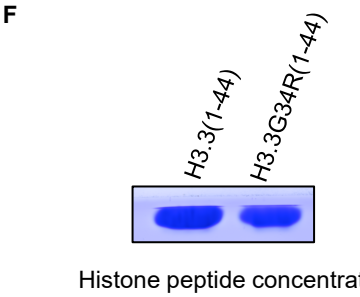

A
H3.3(22-44)
H3.3K27M
H3.3G34R
H3.3G34V
H3.3G34W
H3.3G34L
H3.3K36M
H3.3K36me3
Histone Peptide concentration
10% input
Mock
H3.3
H3.3K27M
H3.3K36me3
H3.3K36M
H3.3G34R
H3.3G34L
H3.3G34W
H3.3G34V
GST-BS69$_{50-401}$

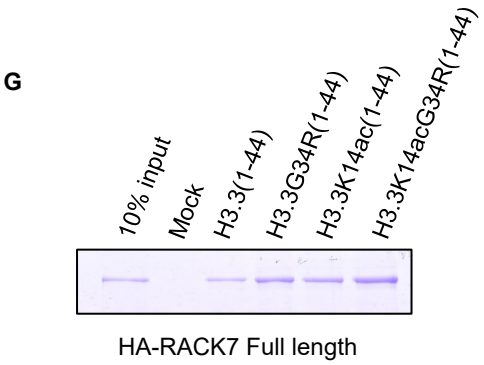

B
10% input
Mock
H3.3(22-44)
H3.3K27M
H3.3K36M
H3.3G34R
H3.3G34V
HA-RACK7 Full length
10% input
Mock
H3.3G34D
H3.3G34H
H3.3G34S
H3.3G34K
H3.3G34L
H3.3G34W
HA-RACK7 Full length


C
10%input
Mock
H3.1(22-44)
H3.1G34R
H3.3(22-44)
H3.3G34R
HA-RACK7 Full length


D
PHD$^{RACK7(D104A)}$-H3.3G34R(22-44)
Fnorm [1/1000]
920
915
910
905
900
895
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
Concentration (μM)


E
H3(1-21)
H3.3(22-44)
H3
H3K4me1
H3K4me2
H3K4me3
H3K9me1
H3K9me2
H3K9me3
H3.3WT
H3.3G34R
Biotin pull-down
GST-PHD^RACK7
5% input
GST-PHD^RACK7
Ratio(pull-down/input) 0.24 0.20 0.14 0.07 0.20 0.20 0.21 0.04 0.44
Lane 1 2 3 4 5 6 7 8 9


F
H3.3(1-44)
H3.3G34R(1-44)
Histone peptide concentration


G
10% input
Mock
H3.3(1-44)
H3.3G34R(1-44)
H3.3K14ac(1-44)
H3.3K14acG34R(1-44)
HA-RACK7 Full length

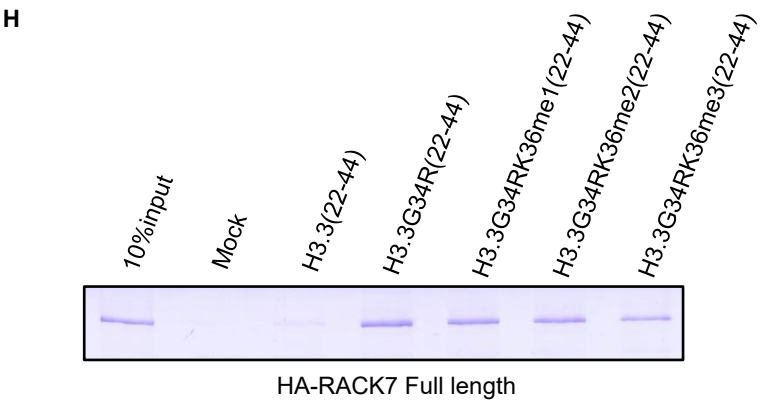
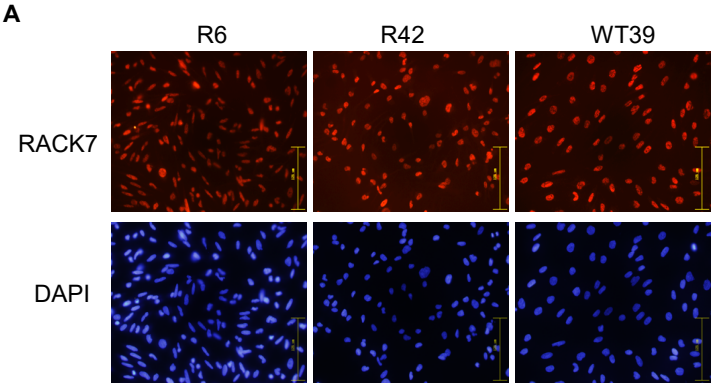

H
10%input
Mock
H3.3(22-44)
H3.3G34R(22-44)
H3.3G34RK36me1(22-44)
H3.3G34RK36me2(22-44)
H3.3G34RK36me3(22-44)
HA-RACK7 Full length

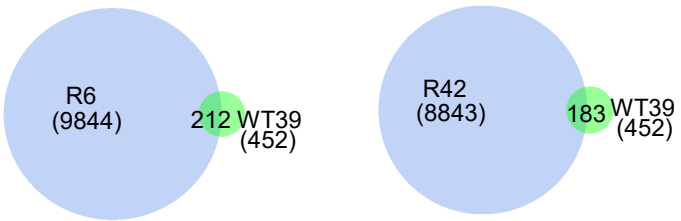
Fig. S1.

RACK7 recognizes H3.3G34R mutation. (**A**) *In vitro* peptide pull-down assays using various H3.3 peptides and GST-tagged $BS69_{50-401}$ purified from *E. coli.* Left panel: the concentration of the peptides used in the peptide pull-down assays. Right panel: the biotinylated peptides were immobilized on streptavidin beads and the bound proteins were subject to SDS-PAGE and visualized by Coomassie blue staining. (**B**) Peptide pull-down assays using indicated biotinylated peptides and the HA-tagged full length RACK7. Glycine at position 34 was replaced with various amino acids as indicated. (**C**) Peptide pull-down assays using the indicated biotinylated peptides and the HA-tagged full length RACK7. (**D**) MicroScale Thermophoresis analysis of the interaction between GST-tagged $PHD^{RACK7(D104A)}$ protein purified from *E.coli* with H3.3G34R(22-44) peptide. Data are represented as mean ±SD from 3 biological replicates. (**E**) Peptide pull-down assays using the indicated biotinylated peptides and the GST-tagged PHD^{RACK7} (**F**) Concentration of the peptides used in Fig. 1H and Fig. 1I. (**G**) Peptide pull-down assays using the indicated biotinylated peptides and the HA-tagged full length RACK7. (**H**) Peptide pull-down assays using the indicated biotinylated peptides and the HA-tagged full length RACK7. All experiments were repeated three times.

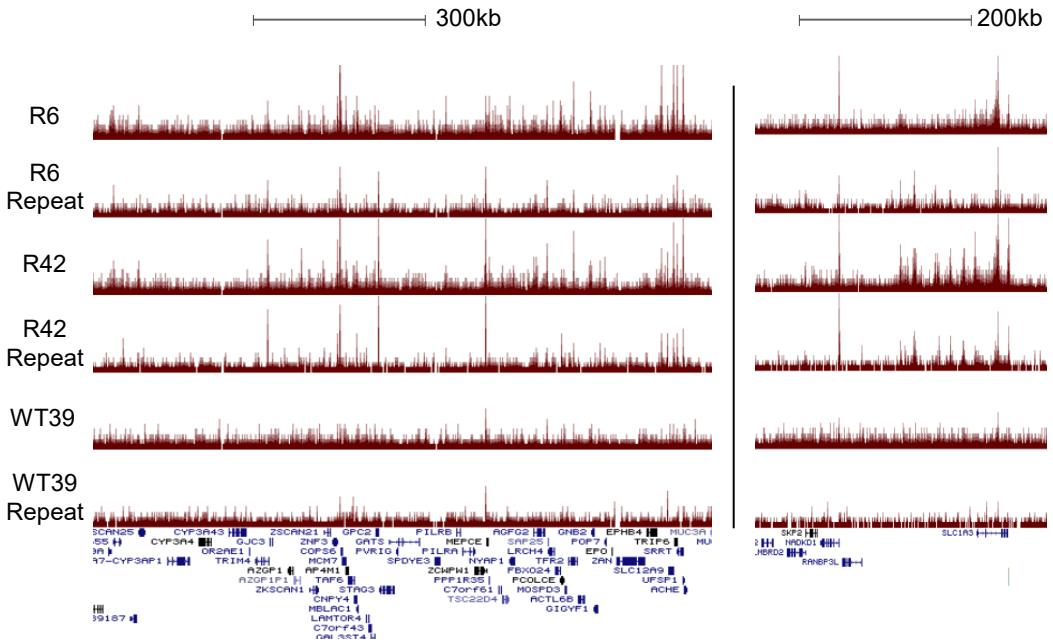
A



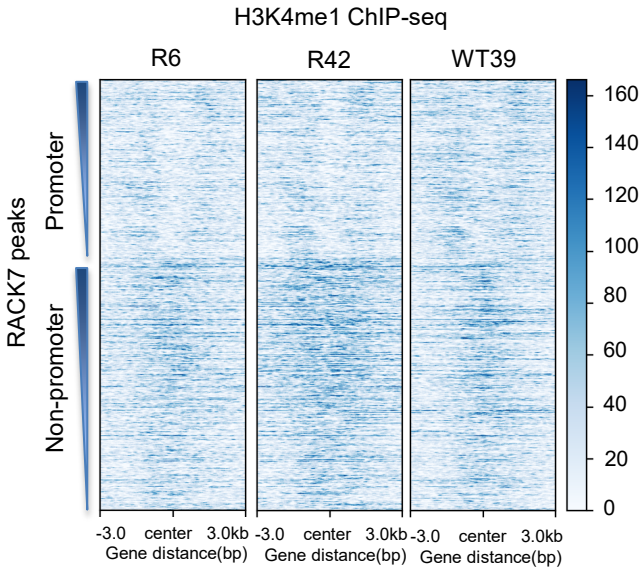
B RACK7 ChIP-seq peaks



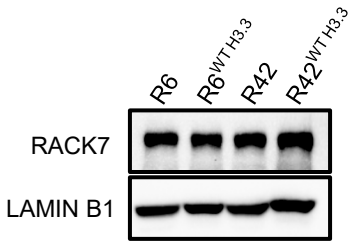
C RACK7 ChIP-seq



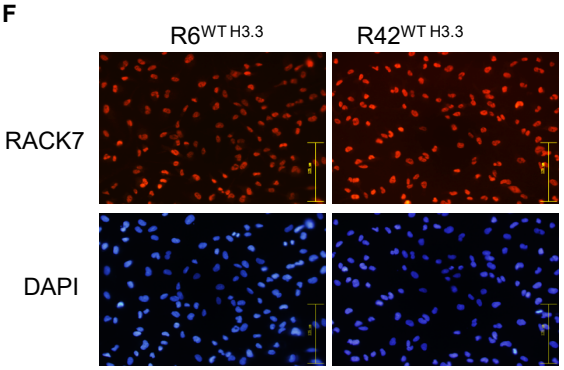
D


H3K4me1 ChIP-seq
R6
R42
WT39
RACK7 peaks
Promoter
Non-promoter
160
140
120
100
80
60
40
20
0
-3.0 center 3.0kb
Gene distance(bp)

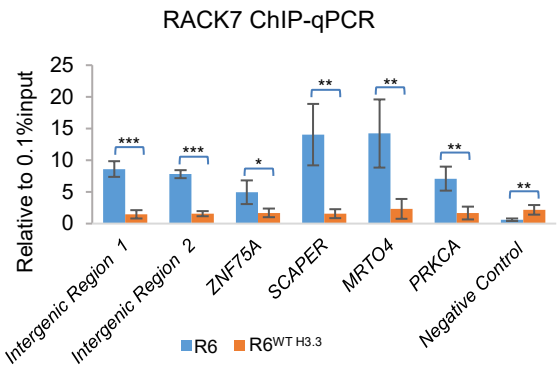
E


R6
R6WT H3.3
R42
R42WT H3.3
RACK7
LAMIN B1

F


R6WT H3.3
R42WT H3.3
RACK7
DAPI

G


RACK7 ChIP-qPCR
Relative to 0.1%input
25
20
15
10
5
0
Intergenic Region 1
Intergenic Region 2
ZNF75A
SCAPER
MRTO4
PRKCA
Negative Control
R6
R6WT H3.3

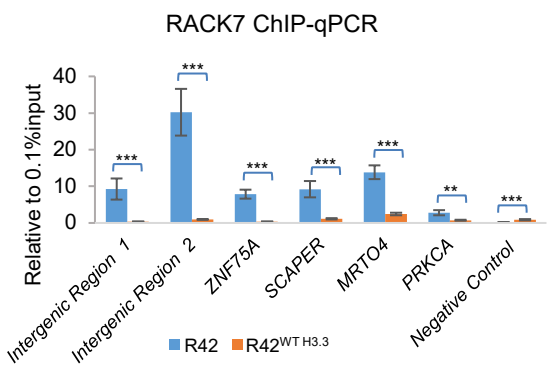
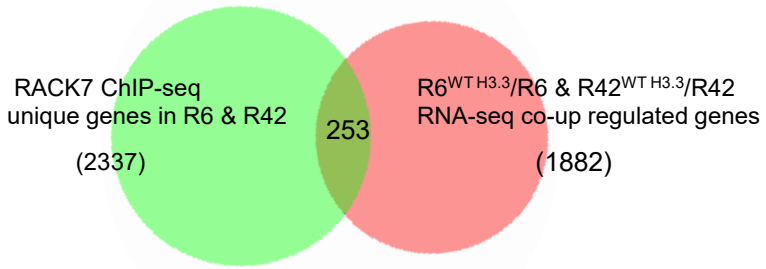

RACK7 ChIP-qPCR
Relative to 0.1%input
40
30
20
10
0
Intergenic Region 1
Intergenic Region 2
ZNF75A
SCAPER
MRTO4
PRKCA
Negative Control
R42
R42WT H3.3

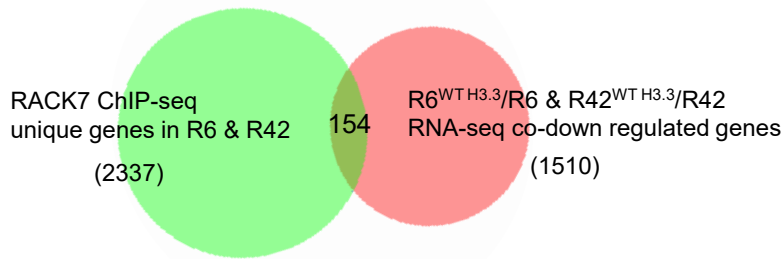
Fig. S2.

RACK7 binds H3.3G34R chromatin in cells. (**A**) Immunofluorescence assay of RACK7 in R6(left), R42(middle) and WT39(right) cells. (**B**) Venn diagram analysis of RACK7 ChIP-seq peaks in R6, R42 and WT39 cells. (**C**) Genome browser snapshots of selected RACK7 ChIP-seq signals in R6, R42 and WT39 cells from two biological repeats. (**D**) Heatmap analysis of H3K4me1 distribution, H3K4me1 ChIP-seq peaks were sorted by RACK7 enrichment. (**E**) Western blot of RACK7 level in R6, $R6^{WT\ H3.3}$, R42, $R42^{WT\ H3.3}$ cells. (**F**) Immunofluorescence assay of RACK7 in $R6^{WT\ H3.3}$(left) and $R42^{WT\ H3.3}$ (right) cells. (**G**) ChIP-qPCR validation of selected RACK7-bound peaks in R6, $R6^{WT\ H3.3}$ (left) and R42, $R42^{WT\ H3.3}$(right) cells. Data are represented as mean ± SD from 3 biological replicates, **P*<0.05, ***P*<0.01, ****P*<0.001, two-tailed Student's *t*-test.

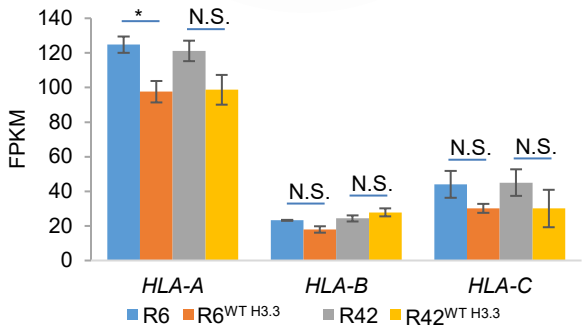
A


RACK7 ChIP-seq
unique genes in R6 & R42
(2337)
253
R6WT H3.3/R6 & R42WT H3.3/R42
RNA-seq co-up regulated genes
(1882)

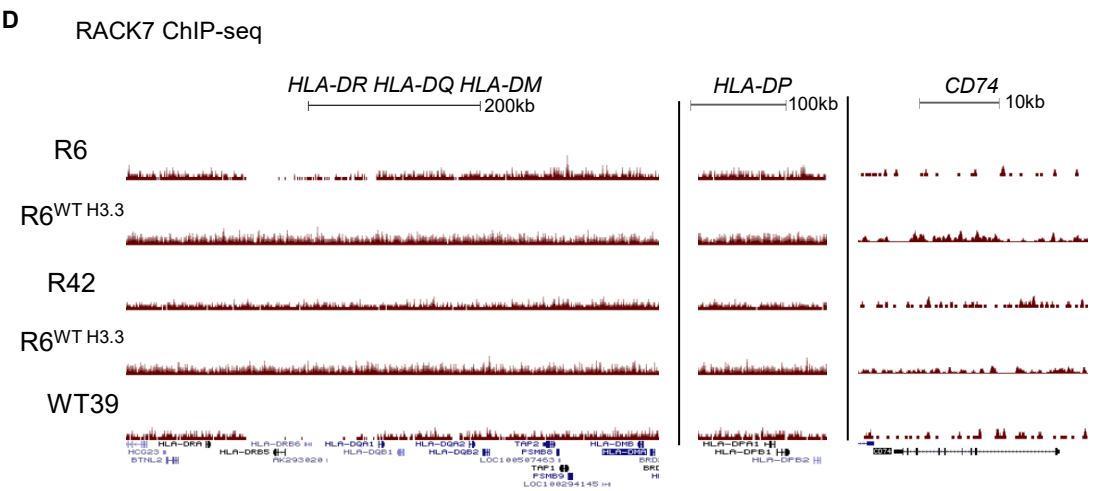
B


RACK7 ChIP-seq
unique genes in R6 & R42
(2337)
154
R6WT H3.3/R6 & R42WT H3.3/R42
RNA-seq co-down regulated genes
(1510)

C


FPKM
140
120
100
80
60
40
20
0
*
N.S.
N.S.
N.S.
N.S.
N.S.
N.S.
HLA-A
HLA-B
HLA-C
R6
R6WT H3.3
R42
R42WT H3.3

D


RACK7 ChIP-seq
HLA-DR HLA-DQ HLA-DM
200kb
HLA-DP
100kb
CD74
10kb
R6
R6WT H3.3
R42
R6WT H3.3
WT39

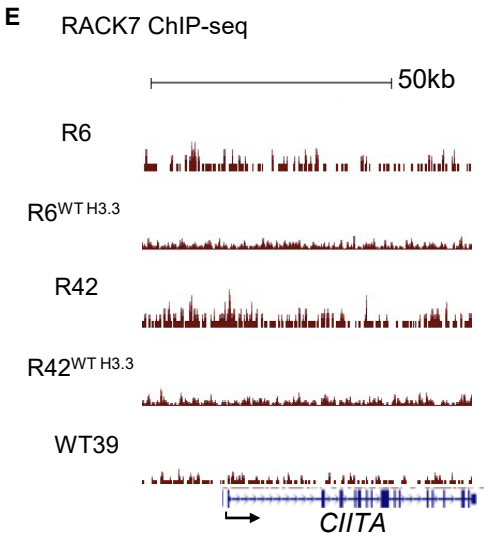
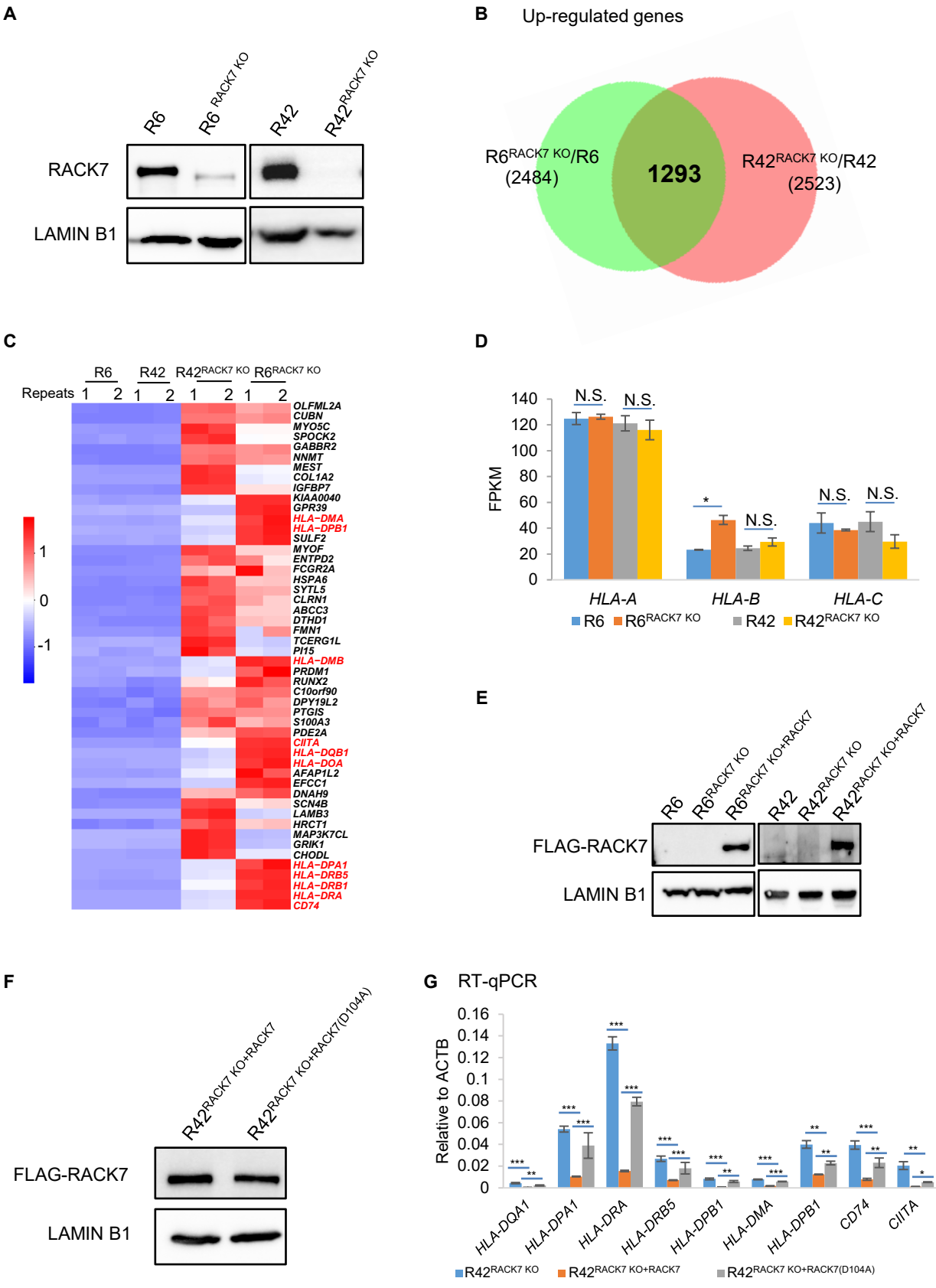
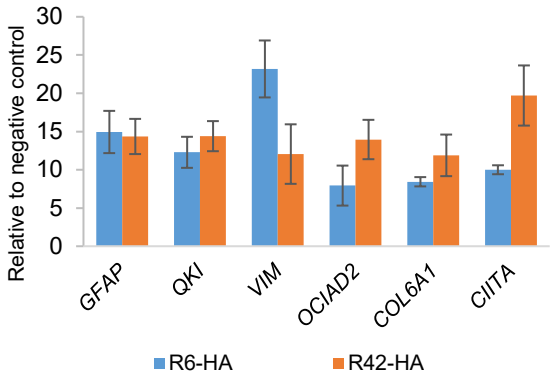


Fig. S3.

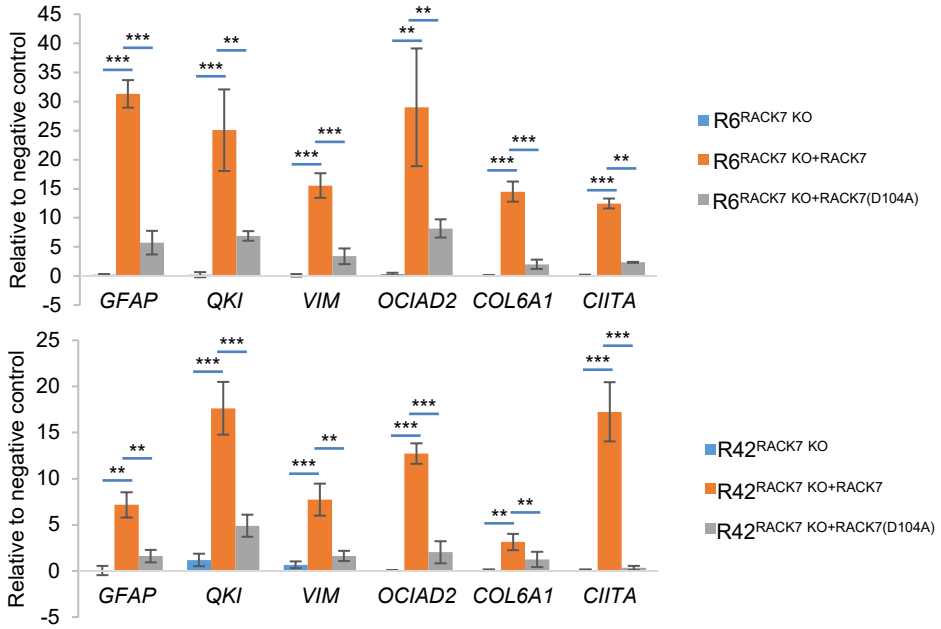
RACK7 represses gene transcription through its binding to H3.3G34R mutation. (**A**) Venn diagram analysis of RACK7 ChIP-seq unique genes in R6 and R42 relative to their knock-in cells ($R6^{WT\ H3.3}$ and $R42^{WT\ H3.3}$) and RNA-seq data of co-up regulated genes (1.5-fold (P<0.05)) in $R6^{WT\ H3.3}$, $R42^{WT\ H3.3}$ relative to their parental cells (R6 and R42), respectively. (**B)** Venn diagram analysis of RACK7 ChIP-seq unique genes in R6 and R42 relative to their knock-in cells ($R6^{WT\ H3.3}$ and $R42^{WT\ H3.3}$) and RNA-seq data of co-down regulated genes (1.5-fold (P<0.05)) in $R6^{WT\ H3.3}$, and $R42^{WT\ H3.3}$ relative to their parental cells (R6 and R42), respectively. (**C**) RNA expression of MHC class I molecules from RNA-seq in R6, $R6^{WT\ H3.3}$, R42 and $R42^{WT\ H3.3}$ cells. Data are represented as mean ± SD from 2 biological replicates, *P<0.05, two-tailed Student's t-test. (**D**) Genome browser snapshots of RACK7 ChIP-seq signals of MHC class II molecules and *CD74* in R6, $R6^{WT\ H3.3}$, R42, $R42^{WT\ H3.3}$ and WT39 cells. (**E**) Genome browser snapshot of *CIITA* RACK7 ChIP-seq peaks in R6, $R6^{WT\ H3.3}$, R42, $R42^{WT\ H3.3}$ and WT39 cells.


A
R6
R6 RACK7 KO
R42
R42RACK7 KO
RACK7
LAMIN B1
B
Up-regulated genes
R6RACK7 KO/R6
(2484)
1293
R42RACK7 KO/R42
(2523)
C
R6
R42
R42RACK7 KO
R6RACK7 KO
Repeats 1 2 1 2 1 2 1 2
OLFML2A
CUBN
MYO5C
SPOCK2
GABBR2
NNMT
MEST
COL1A2
IGFBP7
KIAA0040
GPR39
HLA-DMA
HLA-DPB1
SULF2
MYOF
ENTPD2
FCGR2A
HSPA6
SYTL5
CLRN1
ABCC3
DTHD1
FMN1
TCERG1L
PI15
HLA-DMB
PRDM1
RUNX2
C10orf90
DPY19L2
PTGIS
S100A3
PDE2A
CIITA
HLA-DQB1
HLA-DOA
AFAP1L2
EFCC1
DNAH9
SCN4B
LAMB3
HRCT1
MAP3K7CL
GRIK1
CHODL
HLA-DPA1
HLA-DRB5
HLA-DRB1
HLA-DRA
CD74
1
0
-1
D
FPKM
140
120
100
80
60
40
20
0
N.S.
N.S.
*
N.S.
N.S.
N.S.
HLA-A
HLA-B
HLA-C
R6
R6RACK7 KO
R42
R42RACK7 KO
E
R6
R6RACK7 KO
R6RACK7 KO+RACK7
R42
R42RACK7 KO
R42RACK7 KO+RACK7
FLAG-RACK7
LAMIN B1
F
R42RACK7 KO+RACK7
R42RACK7 KO+RACK7(D104A)
FLAG-RACK7
LAMIN B1
G
RT-qPCR
Relative to ACTB
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
HLA-DQA1
HLA-DPA1
HLA-DRA
HLA-DRB5
HLA-DPB1
HLA-DMA
HLA-DPB1
CD74
CIITA
R42RACK7 KO
R42RACK7 KO+RACK7
R42RACK7 KO+RACK7(D104A)

H H3.3G34R-HA ChIP-qPCR



I RACK7 ChIP-qPCR



J

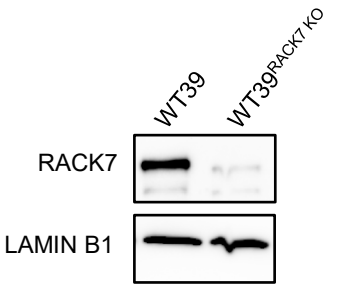


Fig. S4.

RACK7 knockout has similar effects on gene expression as does H3.3 knock-in which corrects the G34R mutation. (**A**) Western blot of RACK7 in indicated cells. (**B**) Venn diagram analysis of up-regulated genes (1.5-fold (P<0.05)) in RACK7 KO cells compared to their parental cells, respectively. (**C**) Heatmap analysis of top-50 up-regulated genes in RACK7 KO cells compared with their parental cells. Sample order is based on the sum of $\log_2$(Fold Change). Scale indicated Z-score, defined as Δ (FPKM- mean FPKM)/SD. (**D**) FPKM value of MHC class I molecules based from RNA-seq in indicated cells, two biological repeats for each cell lines were used, *P<0.05. (**E** and **F**) Western blot of FLAG-RACK7 in RACK7 KO cells rescued with wildtype (E) or D104A mutated (F) RACK7 transgene. (**G**) RT-qPCR analysis of mRNA expression in wildtype or D104A mutated RACK7 rescued cells. (**H**) ChIP-qPCR of selected H3.3G34R-bound peaks in R6 and R42 cells overexpressed with HA-tagged histone H3.3G34R. (**I**) ChIP-qPCR of selected RACK7-bound peaks in wildtype or D104A mutated RACK7 rescued cells. (**J**) Western blot of RACK7 in indicated cells. Data in G to I, are represented as mean ± SD from 3 biological replicates, *P<0.05, **P<0.01, ***P<0.001, two-tailed Student's t-test.

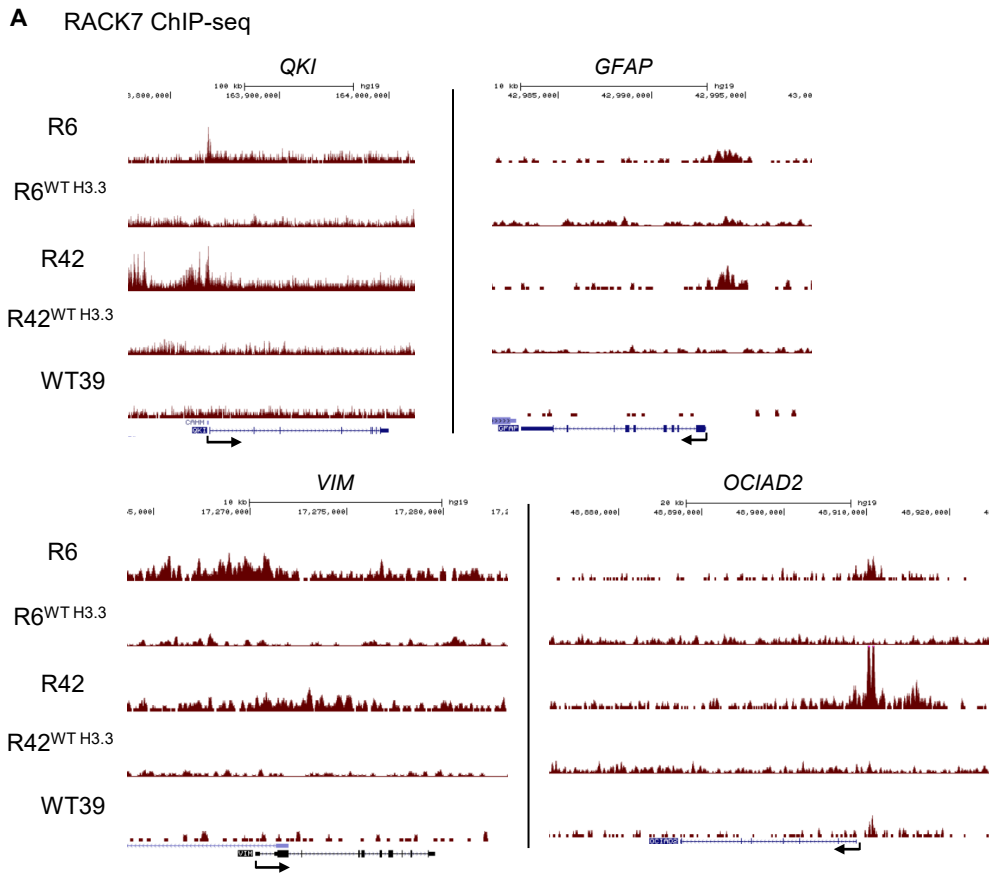


Fig. S5.

RACK7 directly regulates *CIITA* and genes involved in vesicular transportation through its chromatin binding. (**A**) Genome browser snapshots of selected RACK7 ChIP-seq signals in R42, R42$^{WT\ H3.3}$, R6, R6$^{WT\ H3.3}$and WT39 cells.

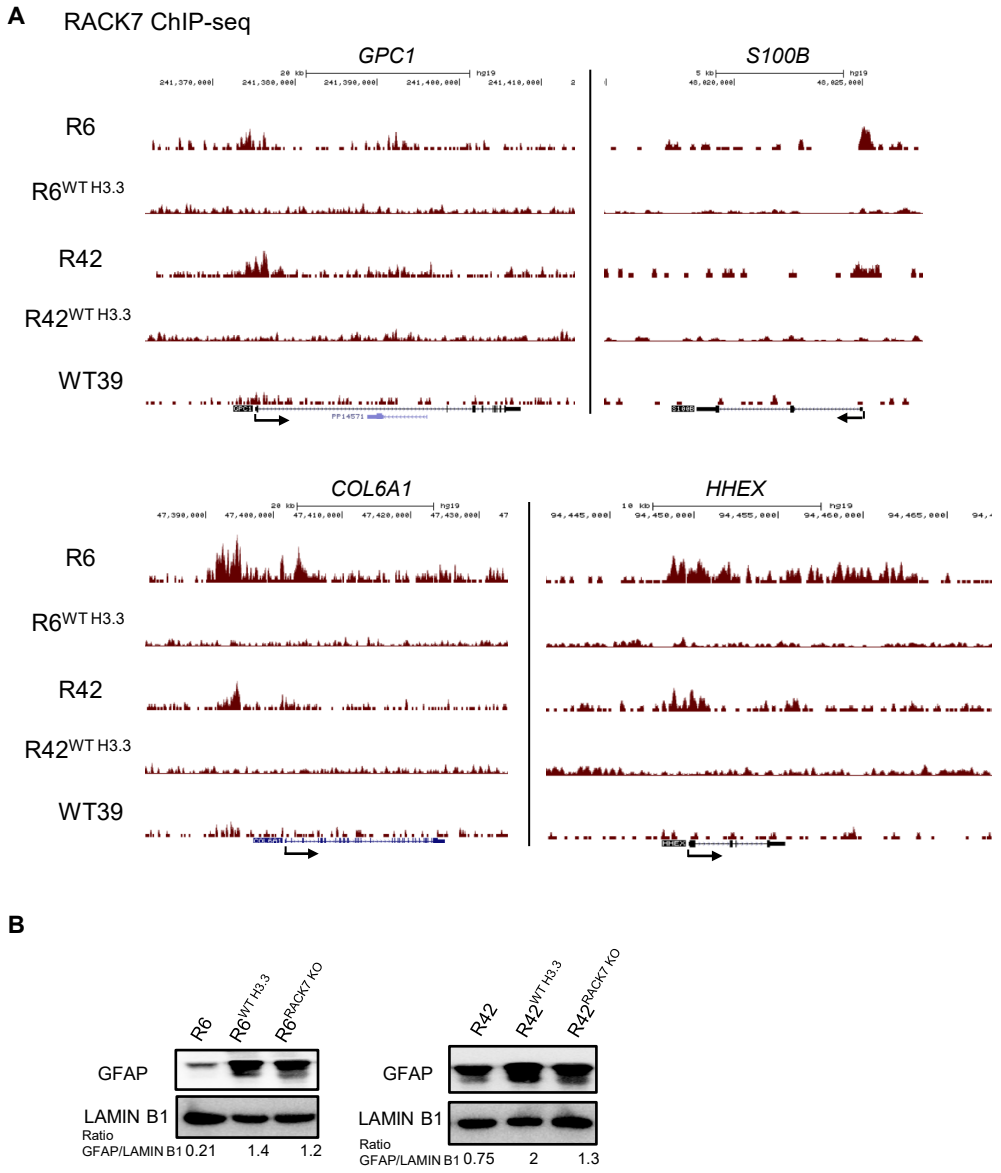


Fig. S6.

RACK7 KO or H3.3 knock-in upregulates cell differentiation genes and inhibits cell migration and invasion. (**A**) Genome browser snapshots of selected RACK7 ChIP-seq signals in R42, R42$^{WT\ H3.3}$, R6, R6$^{WT\ H3.3}$ and WT39 cells. (**B**) Western blot analysis of GFAP in R6, R6$^{WT\ H3.3}$, R6$^{RACK7\ KO}$ (left) and R42, R42$^{WT\ H3.3}$, R42$^{RACK7\ KO}$ (right) cells.

Table S1.

Effects of treatments on MHC class II level in tumor cells[37-40].

Treatment	Type of tumor cells	Effects
DNA-alkylating agents	lymphoma cell line	Activates CIITA and induces MHC-II
JAK1/2 inhibitor ruxolitinib (RUX)	myeloid leukemia (CML) cells	Activates CIITA and induces MHC-II
BRAF inhibitors	melanoma cells	Enhances IFN-inductibility of MHC-II
HDAC inhibitors	various cancer cells	Upregulates MHC-II
Demethylating agents-5-azacytidine	various cancer cells	Upregulates MHC-II
IFN-γ	various cancer cells	Upregulates MHC-II
Bacillus Calmette-Gue´rin	bladder cancer	Induces expression of MHC-II
TLR7 agonists-Imiquimod	cutaneous cancer cells	Enhances expression of MHC-II
TLR7/8 agonists-Resiquimod (R-848)	AML blasts	Upregulates MHC-II
CpG oligodeoxynucleotide	tumor B cells	Upregulates MHC-II
CD40 agonists	malignant B cells	Enhances expression of MHC-II

Table S2.

List of antibodies used in the study.

Antibodies for immunoblotting				
Primary Abs	Clone	Source	Catalog number	Dilution
Rabbit anti-RACK7 Antibody	Polyclonal	Bethyl	A302-089A	1:6000
Mouse anti-LAMIN B1 Antibody	3C10G12	Proteintech	66095-1-Ig	1:3000
Mouse anti-FLAG-tag Antibody	M2	Sigma	F1804	1:3000
Rabbit anti-GFAP Antibody	Polyclonal	Proteintech	16825-1-AP	1:1000
Rabbit anti-HLA-DRA Antibody	Polyclonal	Proteintech	17221-1-AP	1:2000
Rabbit anti-HLA-DRB5 Antibody	Polyclonal	Proteintech	21702-1-AP	1:500
Antibodies for immunofluorescent (IF)				
Primary Abs	Clone	Source	Catalog number	Dilution
Rabbit anti-RACK7 Antibody	Polyclonal	Bethyl	A302-089A	1:500
Rabbit anti-GFAP Antibody	Polyclonal	Proteintech	16825-1-AP	1:250
Rabbit anti-CD74 Antibody	D5N3I	Cell signaling technology	77274	1:200
Antibody for Chromatin immunoprecipitation (ChIP) and ChIP-Seq				
Antibody	Clone	Source	Catalog number	Amount
Rabbit anti-RACK7 Antibody	Polyclonal	Bethyl	A302-089A	2μg
Rabbit anti-histone H3K4me1 Antibody (pAb)	Polyclonal	Active motif	39297	5μl
Antibody for Flow Cytometry				
Antibody	Clone	Source	Catalog number	Amount
APC Mouse Anti-Human HLA-DR	G46-6	BD bioscience	560896	20μl

Table S3.

List of DNA oligonucleotides used in ChIP-qPCR assay.

DNA oligonucleotides for ChIP-qPCR	
Intergenic region1 F	TGTGTCTGTCCATCCCCTCTG
Intergenic region1 R	AGAGAAACGGGCCTCCCTTAG
Intergenic region2 F	GGAAGTCATGGGCATGAGGAT
Intergenic region2 R	GTAGGTCTCTTCTCACGGCAG
ZNF75A F	GCTTCAGCGGGTCTGGATAC
ZNF75A R	AGTGGAAGTGACGCTTTCCG
SCAPER F	GGGAGGTGTGAGGGTTGATT
SCAPER R	ACGAGAAAGACCTGTGTGGC
MRTO4 F	CACGGGAAGTTGCCCTACAG
MRTO4 R	GTGCACCCCGTTAGGACC
PRKCA F	GCACAGTCCTCACCTGGTTTC
PRKCA R	GCATTTCTGAGCATCCCTTCTG
CIITA F	TTGGCATTAGGTCGCACTGT
CIITA R	CAGTACCCAAGCACTCAGCA
HLA-DRA F	GCCTTTCATCCTTCTCCAGTGT
HLA-DRA R	TTGGCAGTACTGAGGACATAGC
HLA-DQA1 F	GCTACCACCAGCAGAGATCC
HLA-DQA1 R	GCCCTTTCTCTGGCTTCTGT
CD74 F	CAGGCAAATGTCCACTCCCT
CD74 R	TCCTCCTGCGTGTTTGAGAC
Negative control F	CCATTCAACCCAAGGATGAGC
Negative control R	TCTGCAATCATGGTCCTCACT
GFAP F	TCGAGTTCCCACACATCAGC

GFAP R	CCAGGTCTCAGGCTCCTAGT
QKI F	CATTTCGAGCACGACTGACG
QKI R	GATCTTGTTGCACCTTCCCG
VIM F	AAGTCGATGGACAGAGGCG
VIM R	AGATCTGAAGTCGCGGAGAA
OCIAD2 F	CTGCCAGGACTGACTGAGTG
OCIAD2 R	GGTACAGGGACCAATTCGCA
COL6A1 F	CACGCTGGTTTTCAGACGTT
COL6A1 R	TGCAGTTCAGTTCCCGTGTC

Table S4.

List of DNA oligonucleotides used in RT-qPCR assay.

DNA oligonucleotides for RT-qPCR	
CIITA F	TGAGGCTGTGTGCTTCTGAG
CIITA R	ACACTGTGAGCTGCCTTGG
HLA-DRB5 F	CCTGGAGGTTCCTACATGGC
HLA-DRB5 R	AAGAAACGTGGTCGGGTGTC
HLA-DQA1 F	GTGGCAAAACACAACTTGAACA
HLA-DQA1 R	TGACCTCAGGAACCTCATTGG
HLA-DPA1 F	ATGCGCCCTGAAGACAGAAT
HLA-DPA1 R	GACACATGGTCCGCCTTGAT
HLA-DRA F	GGGTCTGGTGGGCATCATTA
HLA-DRA R	CCATCACCTCCATGTGCCTT
HLA-DPB1 F	GCAGGGCCACTCCAGAGAATTA
HLA-DPB1 R	CCCCACGTCGCTGTCGAA
HLA-DMA F	CTATTGGGTACCCCGGAACG
HLA-DMA R	ATCAGTCACCTGAGCAAGGC
HLA-DRB1 F	GGTGGACAACTACTGCAGACA
HLA-DRB1 R	CACCTTAGGATGGACTCGCC
CD74 F	GAGGTCCCCAACACCAGAAG
CD74 R	CTCTCACATGGGGACTGGGC
QKI F	GGTACCTGCAGCAGAAGGAG
QKI R	GCAAGAGAAAAGGCAAGGGC
GFAP F	GAGCCTCAAGGACGAGATGG
GFAP R	TCCAGGCTGGTTTCTCGAAT

VIM F	CGGGAGAAATTGCAGGAGGA
VIM R	AAGGTCAAGACGTGCCAGAG
OCIAD2 F	ACCAAGCAAGCAGAGCCTGT
OCIAD2 R	CCAAGGCCAAATCCCAAGAGA
GPC1 F	GAGGCTGGTGGCTGCTATG
GPC1 R	GCAGGTGCTCACCCGAGAT
S100B F	GGTGAGACAAGGAAGAGGATG
S100B R	TGTCTCCCTCCCTTCCAGAAT
COL6A1 F	CCCGTGGACCTGTTCTTTGT
COL6A1 R	CACAGCGGTAGTACCTGTCC
HHEX F	CCCTGGGCAAACCTCTACTC
HHEX R	GGTTTTGACCTGTCTCTCGC
ACTB F	CATCCGCAAAGACCTGTACG
ACTB R	CCTGCTTGCTGATCCACATC