S1 Table. Primer pairs used for reverse-transcribed RT-qPCR.

JUNV-SF	GGGGCAGTTCATTAGCTTCATGC
JUNV-SR	CAAAGGTAGGTCATGTGGATTGTTGG
LCMV-SF	AGA ATC CAG GTG GTT ATT GCC
LCMV-SR	GTT GTA GTC AAT TAG TCG CAG C
VSV-NP-F	TGA ATG TGC CTC GTT CAG ATA
VSV-NP-R	CCA AAG TCG ATC AAA TAA GGC
GAPDH-F	CCCCTTCATTGAC CTCAACTACA
GAPDH-R	CGCTCCTGGAGGATGGTGAT
TCRV-GPF	TCG GTC ACA GAT GGG ACC AGG
TCRV-GPR	CAG GGT TCT TCA CGT CCT CTG
A1S-F	GAC AGC AGA GGA GGA ACT GG
A1S-R	AGC AAA TTG GAG GGG TCT TT
A2D2-F	TGC CAA TCA GCA AAC TGA AG
A2D2-R	CCC GTG TCC AGA ATC AAG TT
B3-F	CAC CGG AGT CAT TTG ATG TG
B3-R	ATC AGA GGG CAT CAA GCT GT
DAG1-F	CAG TGA ACC CTC AGA GGC TGT
DAG1-R	GTT GGA ATG CTC ACT CGA AAT GA
hTfR1-F	GTA TCC CTC TAG CCA TCC AGT
hTfR1-R	CCT ATT GAA ACT TGC CCA GAT G

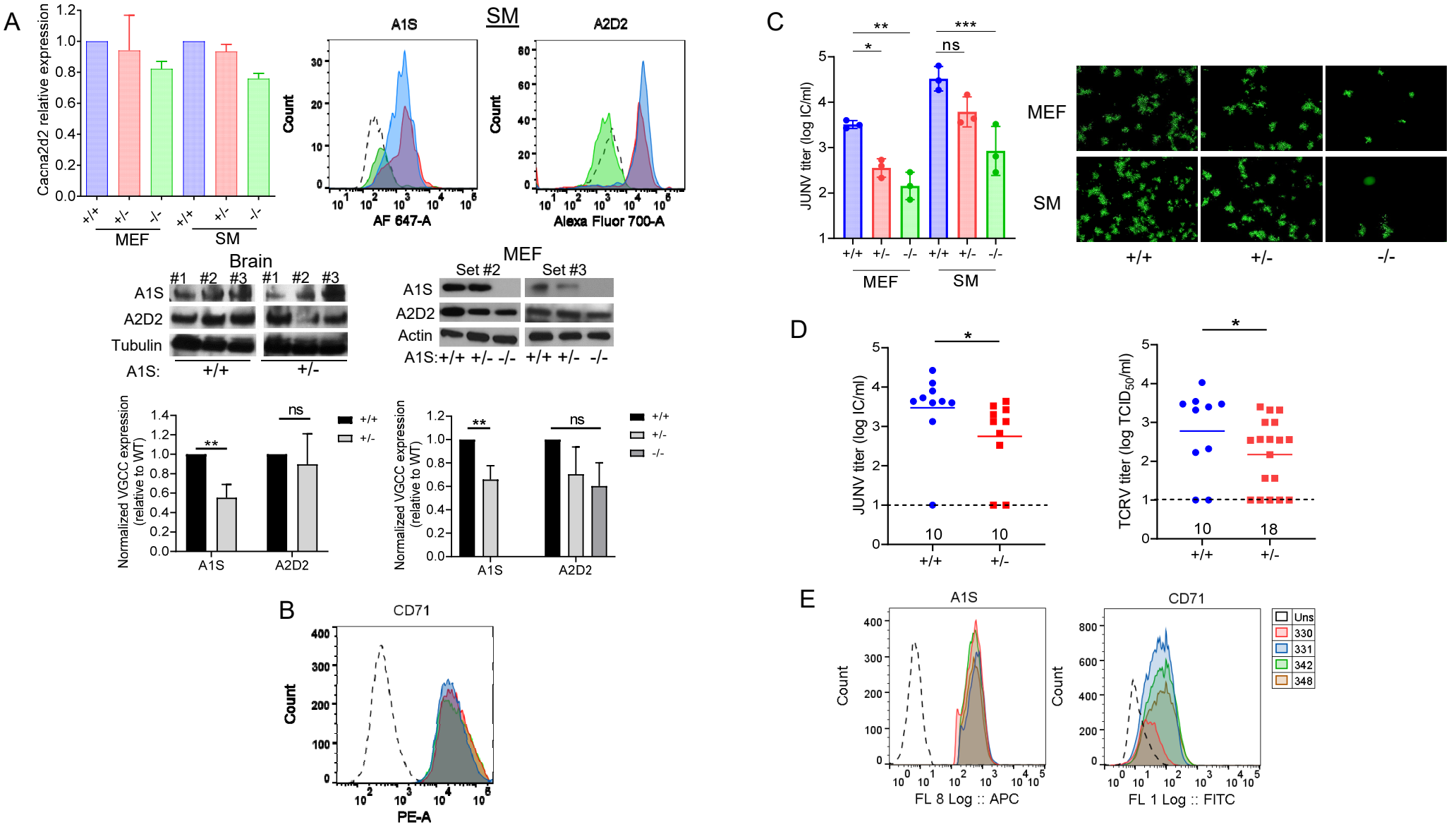
A
Cacna2d2 relative expression
+/+
+/-
-/-
MEF
SM
A1S
A2D2
Count
AF 647-A
Alexa Fluor 700-A
Brain
#1 #2 #3 #1 #2 #3
A1S
A2D2
Tubulin
A1S: +/+ +/-
MEF
Set #2 Set #3
Actin
A1S:+/+ +/- -/- +/+ +/- -/-
Normalized VGCC expression (relative to WT)
ns
B
CD71
PE-A
C
JUNV titer (log IC/ml)
MEF
SM
D
TCRV titer (log TCID50/ml)
10
18
E
FL 8 Log :: APC
FL 1 Log :: FITC
Uns
330
331
342
348

Fig. S1. A) A2D2 mRNA levels in primary cells (left), A1S and A2D2 surface expression on SM (right), A1S and A2D2 protein expression in whole brain (middle, left) and in MEFs (middle, right). Shown below the western blots is the quantification of A1S and A2D2 protein from 3 mice, using ImageJ software (imagej.nih.gov). Significance was determined by two-tailed Student T test (left) or ANOVA (right). $^{**}P \leq 0.005$. B) TfR1 (CD71) surface expression on MEFs. C) JUNV-C1 production in primary cells. MEFs and SMs were infected with JUNV-C1 (MOI = 1) and culture supernatant was collected at 5 days post-infection. Virus titers were determined by infectious center assay (top). A representative field from MEFs and SMs infection of each genotype is shown (bottom). One-way ANOVA Tukey's multiple comparisons test was used to determine significance. $^{*}P \leq 0.03$; $^{**}P \leq 0.0025$; $^{***}P \leq 0.0006$. D) A1S WT and +/- mice were either intracranially infected with JUNV-C1 for 5 days (left) or intraperitoneally with TCRV for 7 days (right). Virus titers were determined from brains and spleens, respectively. P values were determined by unpaired t tests. $^{*}P \leq 0.015$. Number of mice in each group is shown above the x-axis. E) A1S and TfR1 (CD71) surface expression on human LCLs.

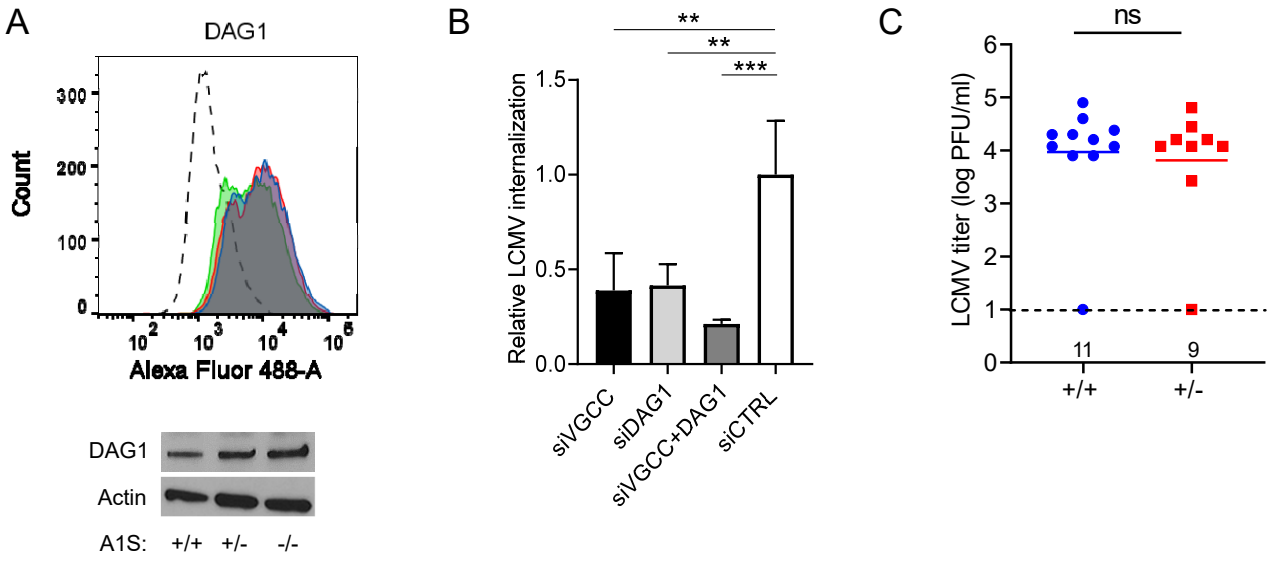

Fig. S2. A) αDG protein expression on MEFs, surface (top) and total (bottom). B) U2OS cells were transduced with siVGCC (A1S, A2D2, B3), siDAG1, siVGCC and DAG1 or siCtrl, infected with LCMV on ice for 1 hr, shifted to 37˚C for 45 min, virus was stripped off and RNA was isolated and analyzed by RT-qPCR. The data shown are the average and SD of 4 independent experiments. One-way ANOVA Tukey's multiple comparisons test was used to determine significance. ***P* ≤0.002; ****P* ≤0.0002. C) A1S WT and +/- mice were either intracranially infected with JUNV-C1 for 5 days (left) or intraperitoneally with TCRV for 7 days (right). Virus titers were determined from brains and spleens, respectively. P values were determined by unpaired t tests. **P* ≤0.015. Number of mice in each group is shown above the x-axis. ns=not significant.

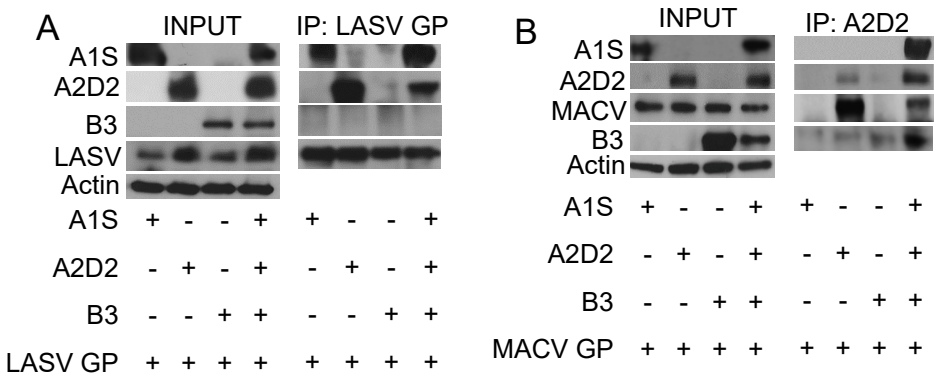

Fig. S3. IP of arenavirus GPs with VGCC subunits. A) Co-IP of LASV-GP with VGCC subunits. 293T cells were co-transfected with VGCC subunits (A1S, A2D2, B3) constructs, singly or combined, along with a tagged LASV GP construct. Cell extracts were immunoprecipitated with anti-FLAG and western blots were subjected to probing with anti-A1S, anti-A2D2, and anti-B3 antibodies; anti-Actin antibody served as a control. B) Reciprocal Co-IP of HA-tagged A2D2. 293T cells were transfected with A1S, A2D2-HA and B3 constructs, singly or together, with the MACV GP-FLAG construct. Cell extracts were immunoprecipitated with an anti-HA antibody and western blot were probing with anti-A1S, anti-A2D2, anti-B3 and anti-FLAG antibodies; anti-Actin antibody served as a control. Shown is a representative blot from 2-3 independent experiments.

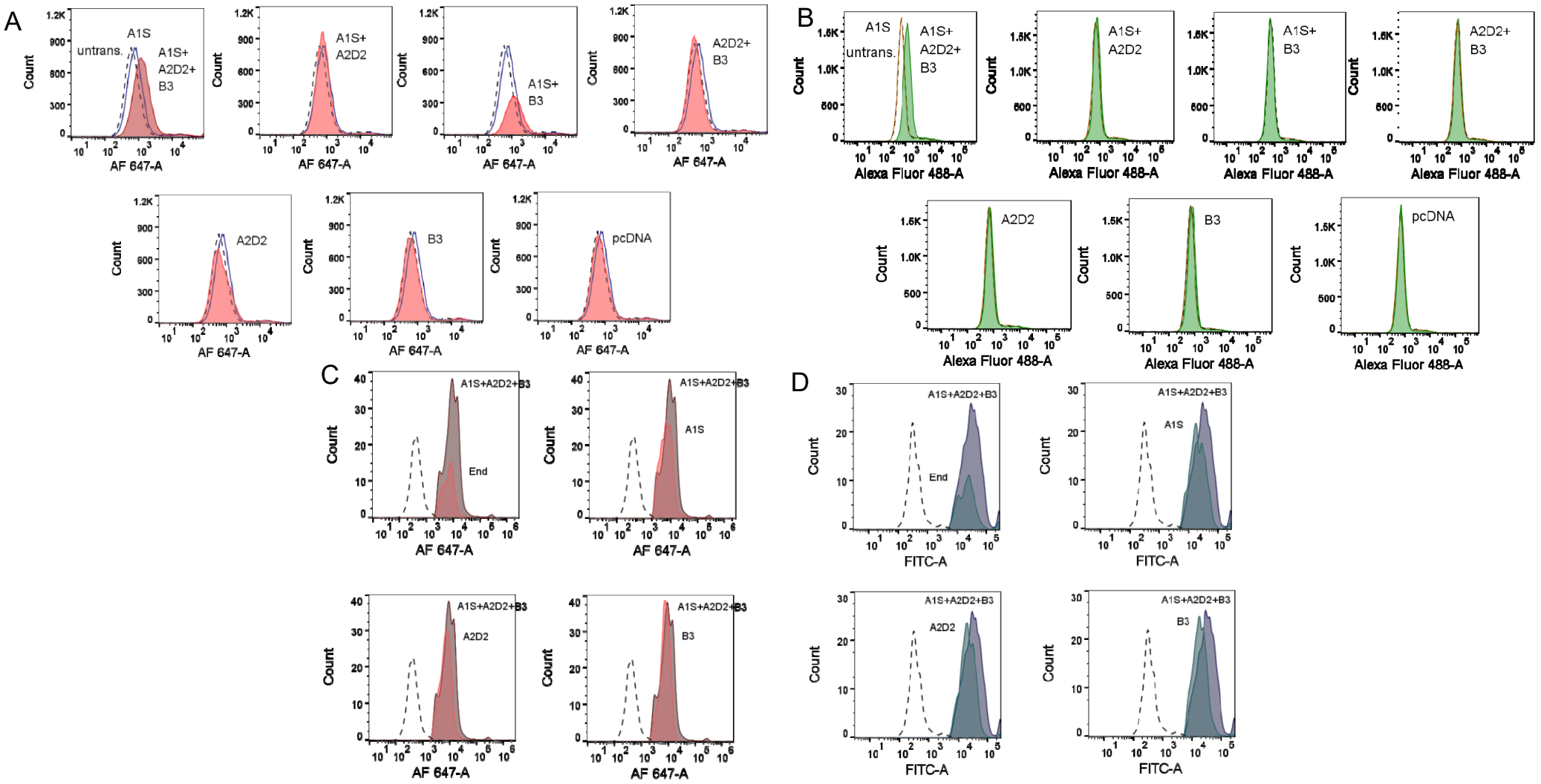

Fig. S4. Validation of VGCC subunit overexpression by FACS analysis (related to Fig. 4). A) – B) 293T cells were co-transfected with A1S, A2D2, B3 constructs singly or in combination and A1S (A) and A2D2 (B) surface expression was determined by FACS.C) – D) U2OS cells were equally transfected with the 3 subunit constructs and A1S (C) and A2D2 (D) surface expression was assayed.

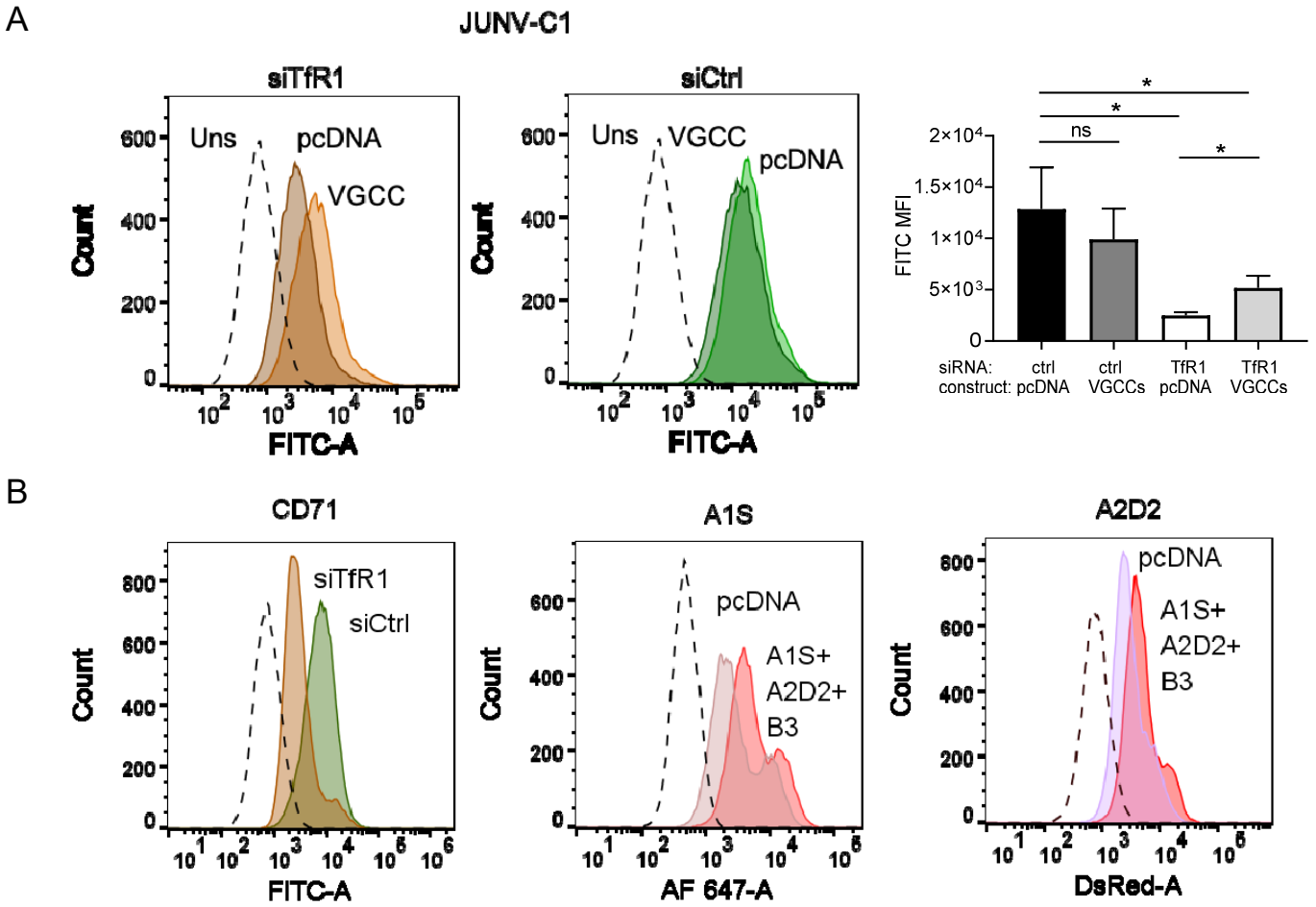

Fig. S5. VGCC overexpression alone does not alter Candid 1 binding to human cells. A) U20S cells were transduced with siTfR1 (left) or siControl (right) and A1S, A2D2 and B3 expression vectors and bound with FITC-labeled Candid 1. Shown is a representative FACS plot for each condition (left) and the virus median fluorescence for 3 independent experiments (right). Significance was determined by unpaired T test. **P* ≤0.03. B) Surface expression of CD71 (TfR1), A1S and A2D2 under siTfR1 knockdown and VGCCs over-expression. Shown above the panel is the protein detected.

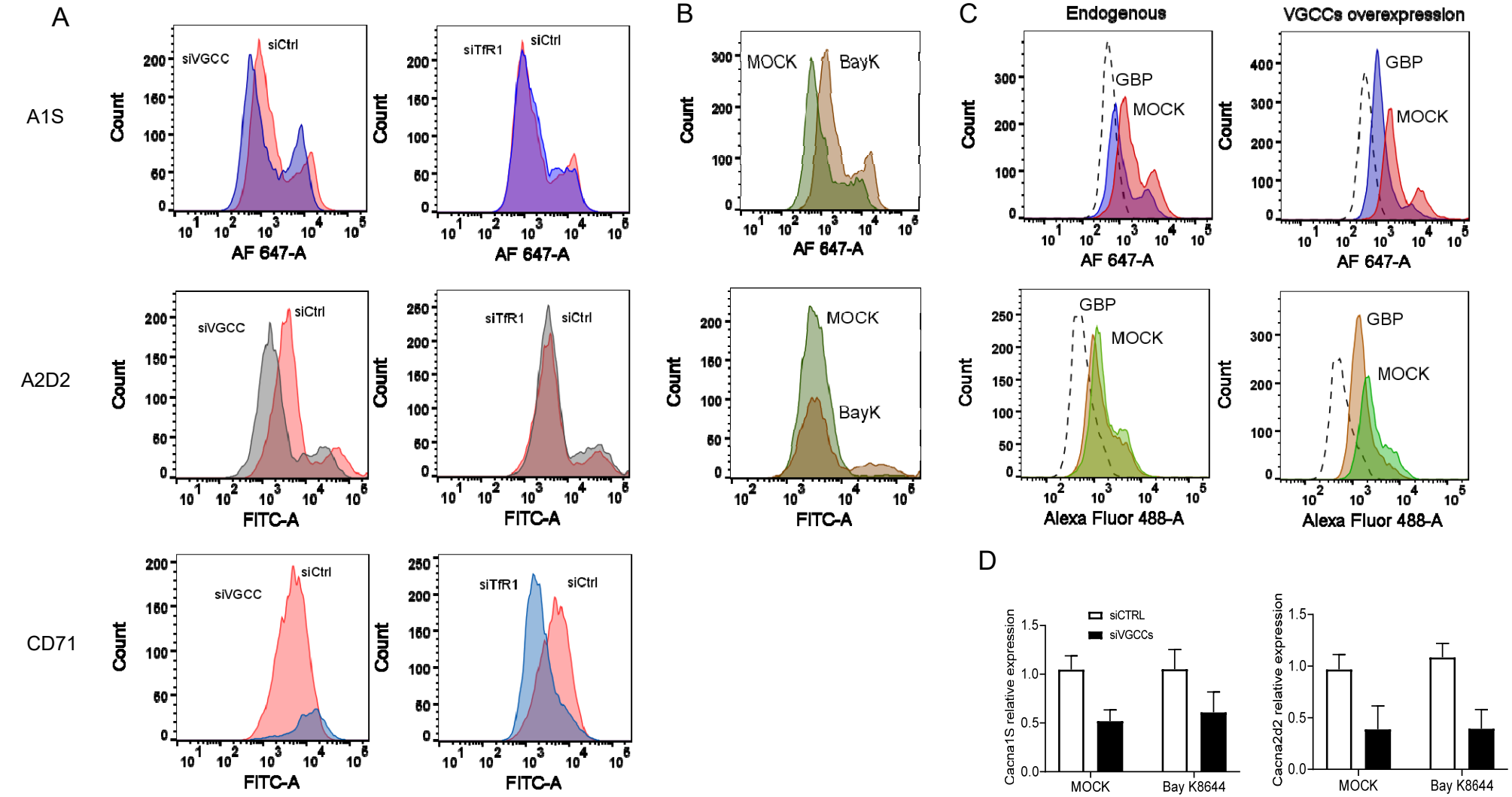
A
A1S
A2D2
CD71
siVGCC
siCtrl
siTfR1
Count
AF 647-A
FITC-A
B
MOCK
BayK
C
Endogenous
VGCCs overexpression
GBP
MOCK
Alexa Fluor 488-A
D
siCTRL
siVGCCs
Cacna1S relative expression
Cacna2d2 relative expression
MOCK
Bay K8644

Fig. S6. Validation of VGCC subunit expression by FACS analysis, related to Fig. 5. A) U2OS cells were transduced with siVGCC (A1S, A2D2, B3), siTfR1 or siCtrl (A) or treated with the VGCC agonist Bay K8644 (B) and A1S, A2D2 and TfR1 surface expression was determined. C) A1S and A2D2 surface expression on U2OS cells after GBP treatment; endogenous levels (left), VGCC overexpression (A1S, A2D2, B3) (right). Shown is a representative FACS plot from three independent experiments. D) Relative levels of A1S and A2D2 RNA upon siRNA knockdown in U2OS cells treated with BayK8644.

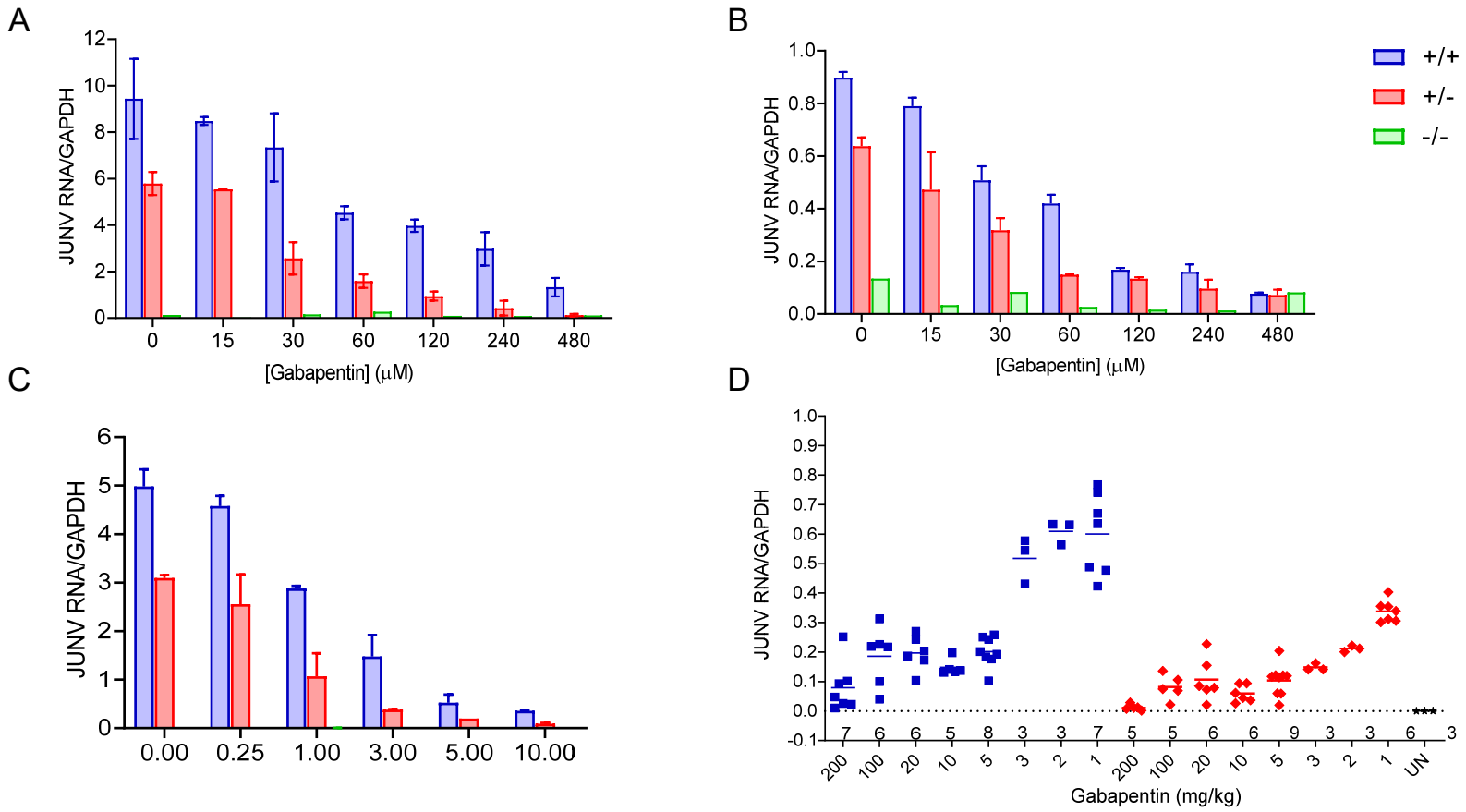

Fig. S7. A1S +/- mice are more resistant to JUNV-C1 infection and more susceptible to VGCC-targeting drugs, related to Fig. 6. A) MEFs and B) SMs were pre-treated with increasing doses of GBP for 5 hr, infected with JUNV-C1 for 1 hr at 37º with GBP-supplemented media, and incubated for another 40 hr where JUNV-C1 RNA levels were analyzed. C) MEFs were pre-treated with increasing dosages of verapamil for 1 hr and infected with JUNV-C1 as described for GBP treatment. D) Eight- to twelve-week old A1S WT and +/- mice were treated with GBP by intraperitoneal injection and 1 hr afterwards, intracranially infected with JUNV-C1. GBP was administrated daily and on day 5 post-infection brains were harvested and viral RNA was analyzed by RT-qPCR. Number of mice (N) in each group is above below the x-axis.