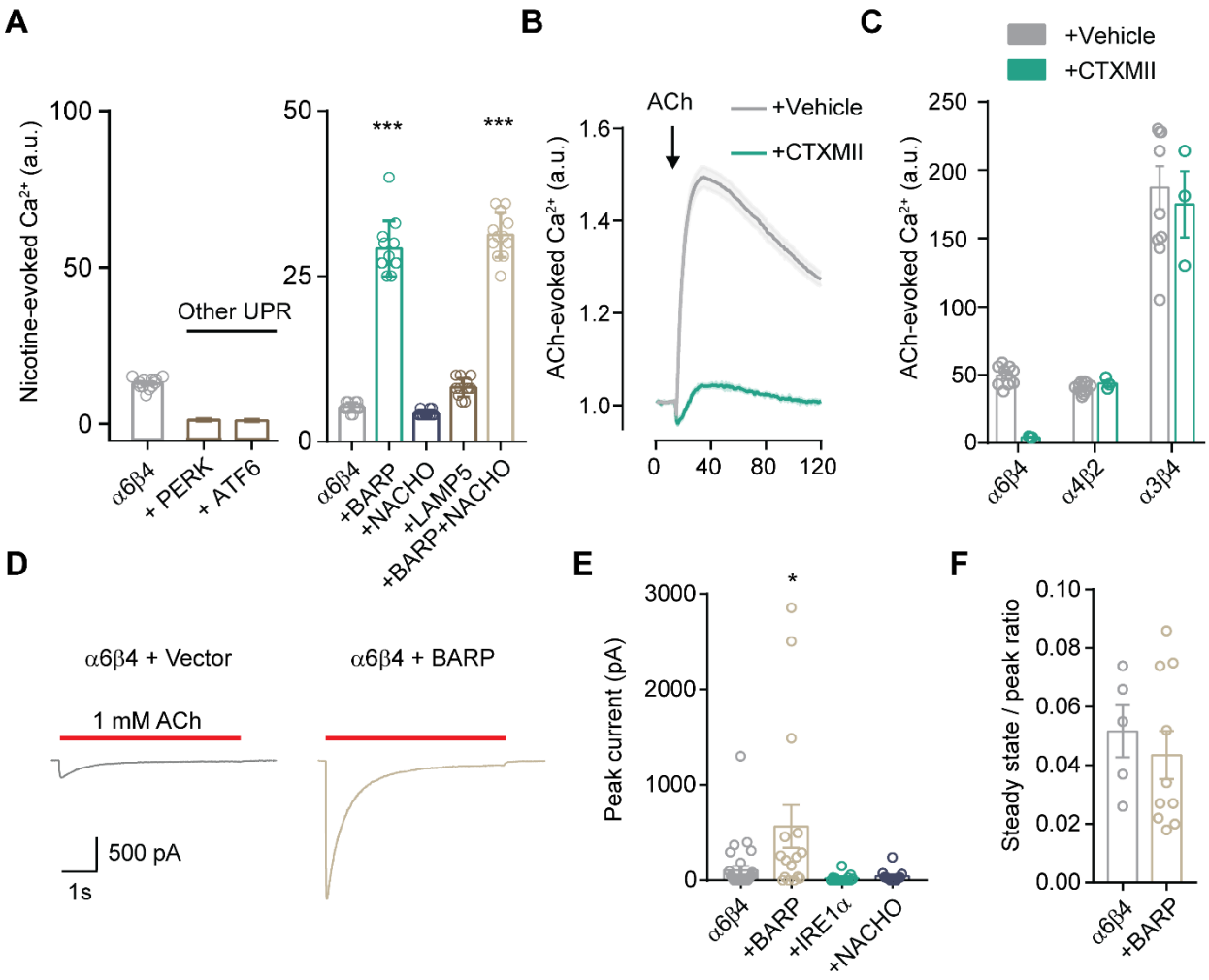
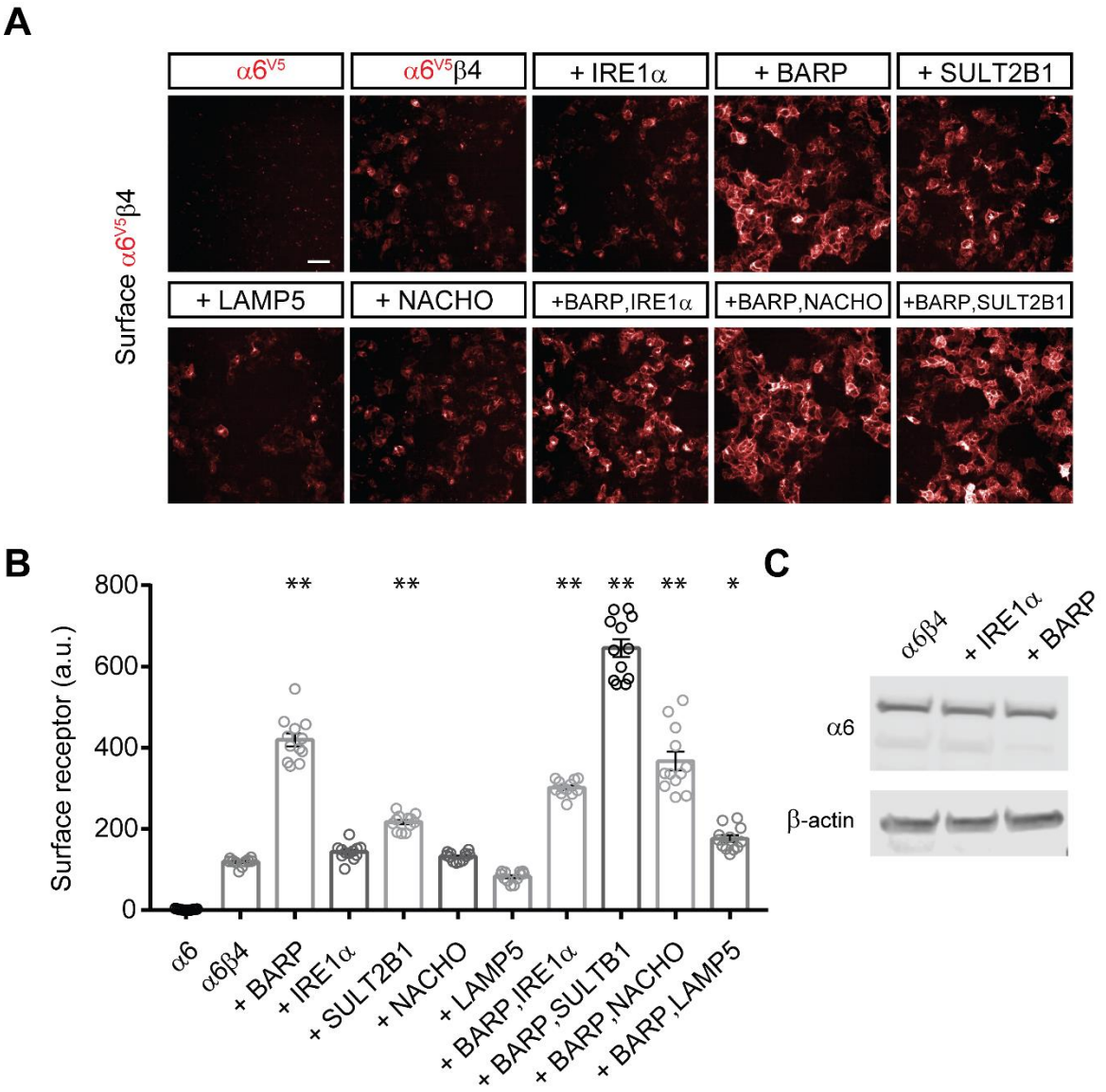


Supplemental Figure 1. Validation of α6β4 genomic screening hits BARP and IRE1α

A. Quantification of nicotine- (50 μM) evoked FLIPR responses in HEK cells transfected with α6β4 and indicated cDNAs. Other UPR members (left) and NACHO and LAMP5 (right) had no effect. **B-C** Traces (B) and quantification (C) of ACh- (25 μM) evoked FLIPR responses from HEK cells transfected with α6β4, BARP and IRE1α and pre-treated with α-CTXMII (500 nM) as indicated. CTXMII blocked responses from α6β4 but not from α4β2 or α3β4. n = 10, 4 for vehicle, CTXMII groups respectively. **D.** Traces of whole cell α6β4 currents elicited by 1 mM ACh in HEK cells co-transfected with either vector (left) or BARP (right). **E.** Quantification of whole-cell patch clamp currents in HEK cells transfected as indicated and stimulated with 1 mM ACh. n = 31, 12, 15, 16, cells for α6β4, +NACHO, +IRE1α, +BARP, respectively. **F.** Quantification of steady-state / peak ratio showed no significant changes. n=5, 10 cells α6β4 without and with BARP, respectively.

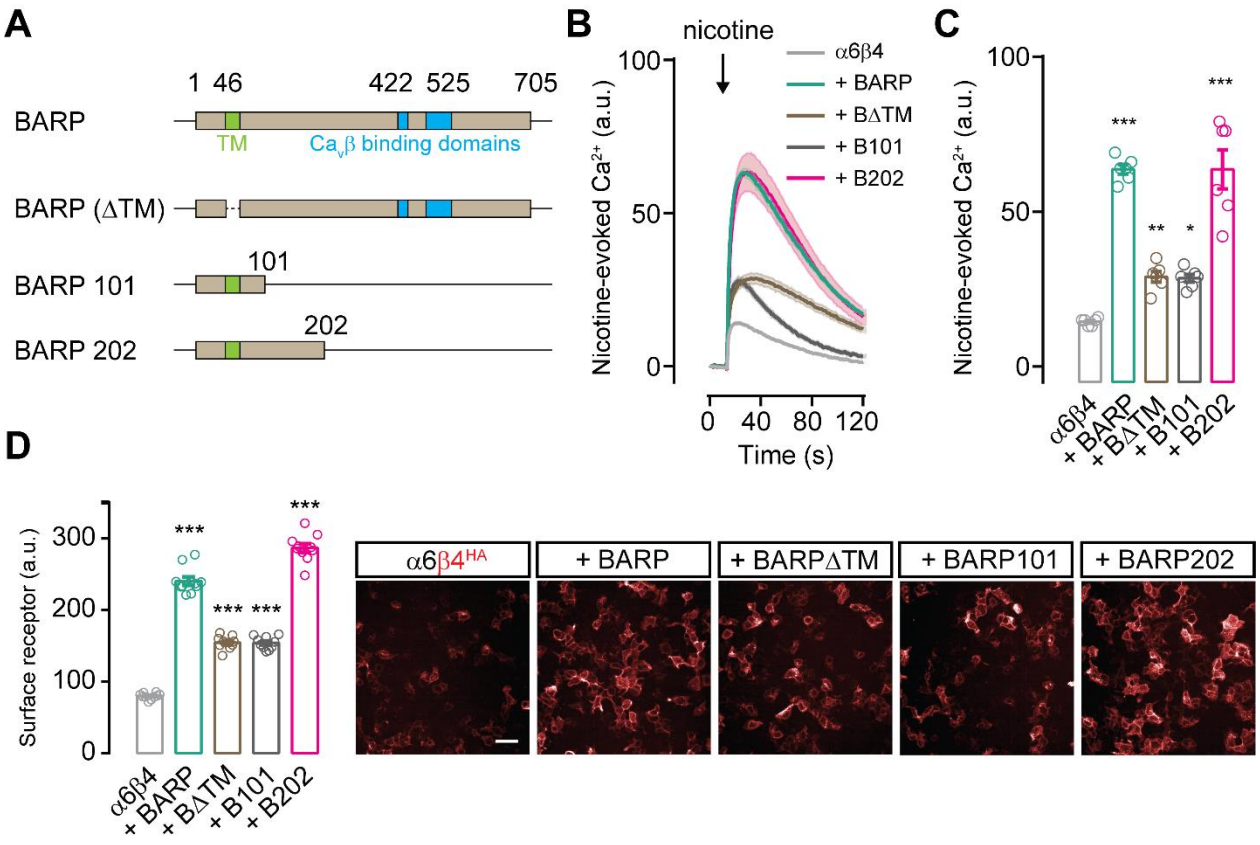
Graphs are average ± SEM. * p < .05, ** p < .01, *** p < .001. For A (right): One-way ANOVA with Tukey's post-hoc test for multiple comparisons. $F_{4,55}$ = 4.964. For presentation purposes, asterisks only shown comparing to α6β4 + vector. + BARP vs. + BARP + NACHO not significant. +BARP vs. +NACHO p < .001. E: Kruskall- Wallis with Dunnett's post-hoc test. For A: $F_{6,77}$ = 491.1. For f: unpaired t-test.



Supplemental Figure 2. BARP and SULT2BI promote surface expression of α6^{V5}β4 receptors

A. Confocal images of live surface staining of HEK cells transfected as indicated. The N-terminus of α6 was tagged with V5 to enable surface labeling with anti-V5-550 antibody. Scale = 50 μm. **B.** Quantification of surface α6-V5 intensity. BARP and SULT2B1, but not other cDNAs enhance α6β4 surface expression. n=10 each group. **C.** Immunoblotting shows that neither IRE1α nor BARP affect total α6 protein levels.

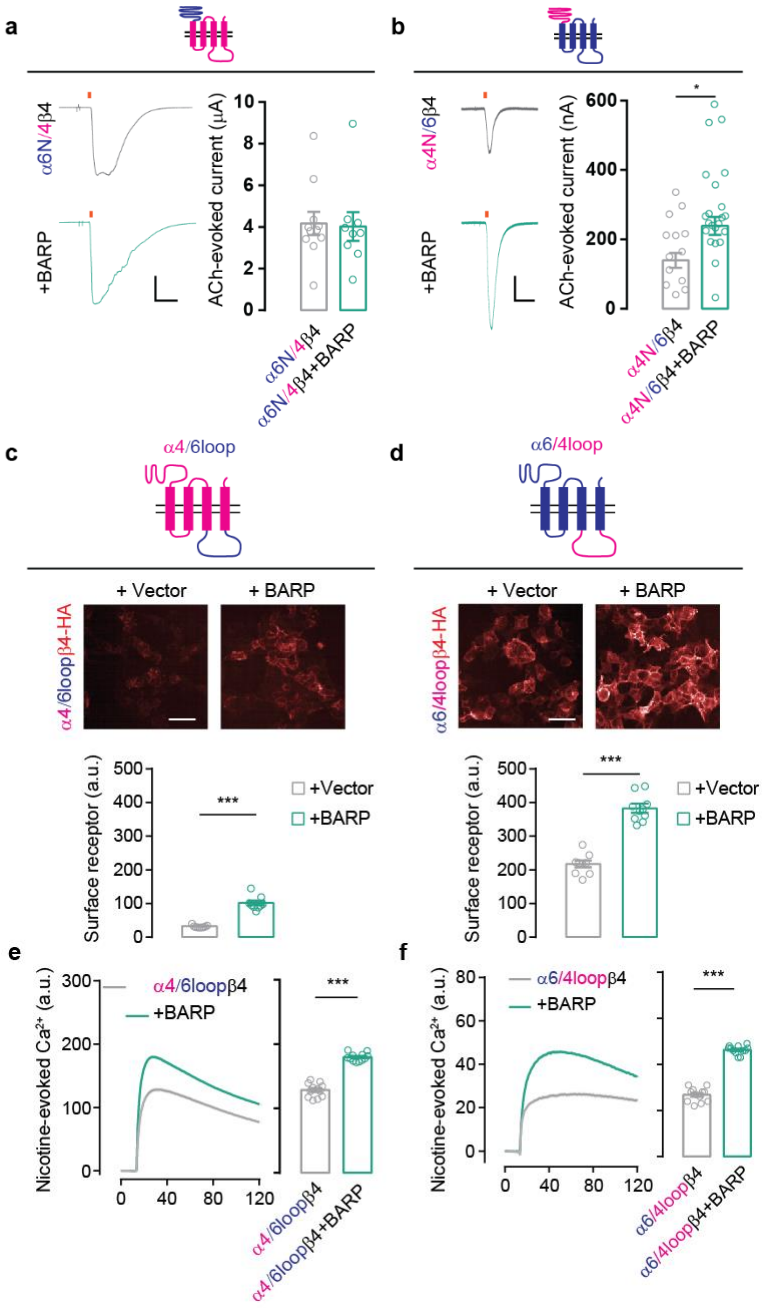
Graphs are average ± SEM. One-way ANOVA with Tukey's post hoc correction for multiple comparisons to vector, $F_{9,110} = 215$. * $p < .01$, ** $p < .001$. For presentation purposes, asterisks only shown comparing to α6β4 + vector. + BARP vs. + BARP + NACHO not significant. +BARP vs. +NACHO $p < .001$. +BARP vs. +BARP + SULT2B1 $p < .001$.



Supplemental Figure 3. BARP effects on α6β4 do not require the Ca_vβ binding domain

A. Schematic of BARP mutants. ΔTM lacks the transmembrane domain; BARP101 and BARP202 are truncated forms with stop codons after the 101st or 202nd amino acid, respectively. **B-C.** Traces (B) and quantification (C) of nicotine- (50 μM) evoked FLIPR responses in HEK cells transfected with α6β4 and BARP mutants. N = 6 each condition. **D.** Quantification (left) and confocal images (right) of α6β4 live surface staining of HEK cells co-transfected with indicated BARP constructs. Scale = 50 μm. n = 10 each condition.

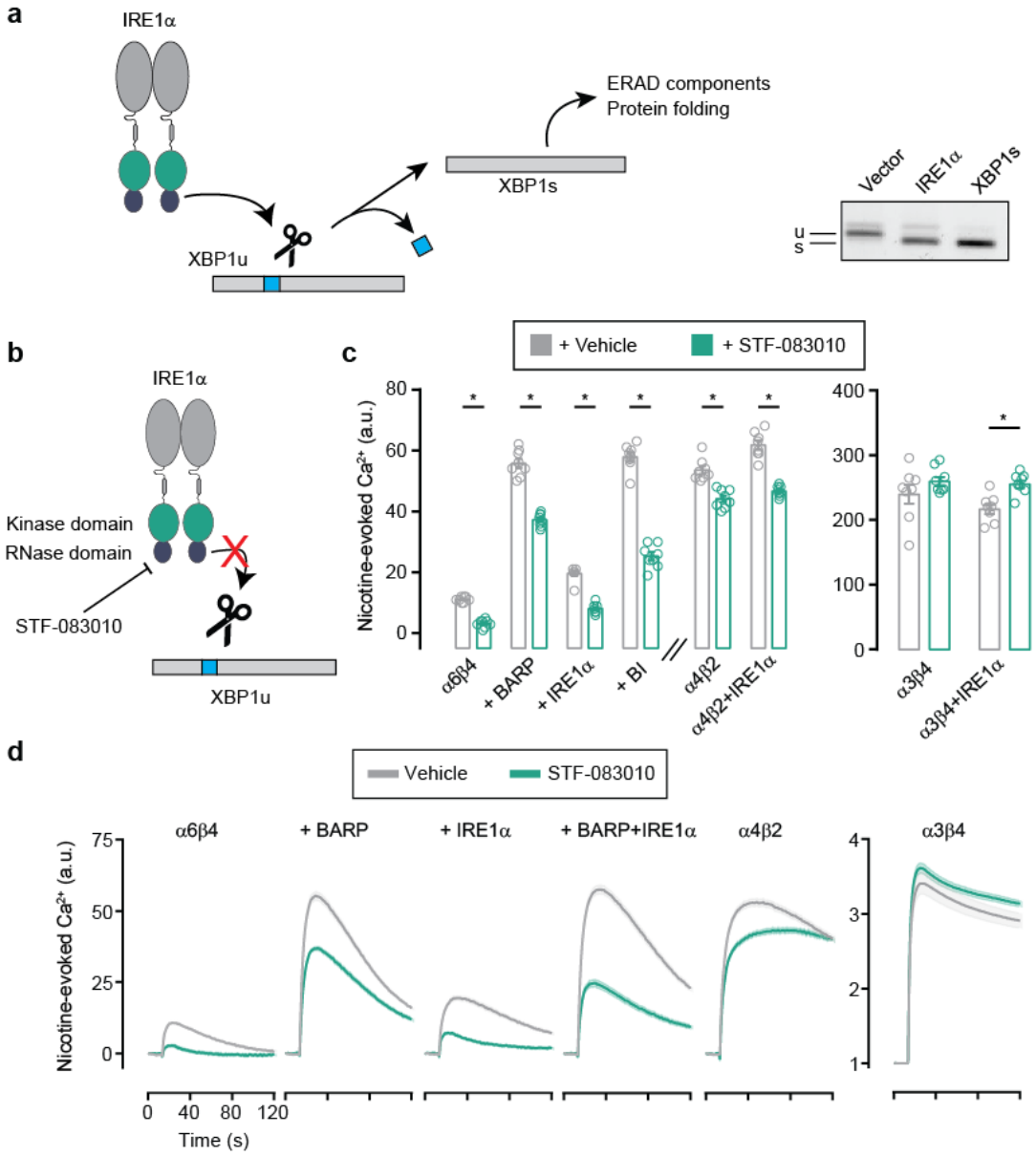
One-way ANOVA with Dunnett's post-hoc correction for multiple comparisons to vector. For C: $F_{4,25}$ = 53.68; for D: $F_{4,45}$ = 367.8. Graphs are average ± SEM. * p < .05, ** p < .01, *** p < .001.



Supplemental Figure 4. The α6 2nd intracellular loop is sufficient, but not necessary for BARP effects

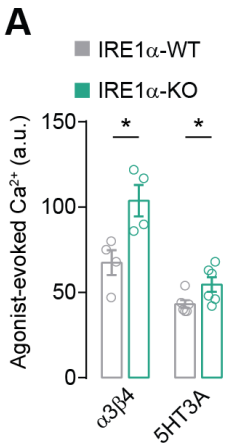
A-B. Chimeric α6 / α4 cRNAs were co-injected into oocytes with β4 +/- BARP. Shown are 250 μM ACh responses (left) and amplitude quantifications (right). For (A), n = 11, 9 oocytes for α6N/4, α6N/4 + BARP, respectively, scale = 1 μA, 10 seconds; for (B) n = 13, 22 oocytes for α4N/6, α4N/6 + BARP, respectively, scale: 100 nA, 10 seconds. **C-D.** (Top) Schematic of α6 and α4 loop chimeras. α4/6loop = α4 receptor with α6 loop; α6/4loop = α6 receptor with α4 loop. (Bottom) Confocal images and quantifications of surface staining live HEK cells transfected as indicated. Cells stained with anti-HA-650 antibody. BARP enhanced surface expression of both chimeras. N = 10 each condition. Scales = 50 μm. **E-F.** Traces (left) and quantification (right) of nicotine-evoked (50 μM) FLIPR responses from HEK cells transfected with α4/6loop chimera (E) and α6/4loop chimera (F) +/- BARP. N = 12 for each condition.

All experiments were replicated with similar results. Graphs are average ± SEM. A, B: Mann-Whitney test. C-F: Unpaired two-tailed t-tests. For B: U = 70; C: t = 10.15; D: t = 9.546; E: t = 14.19; F: t = 19.18. * p < .05, ** p < .01, *** p < .001.



Supplemental Figure 5. IRE1α effects on α6β4 require its RNase activity

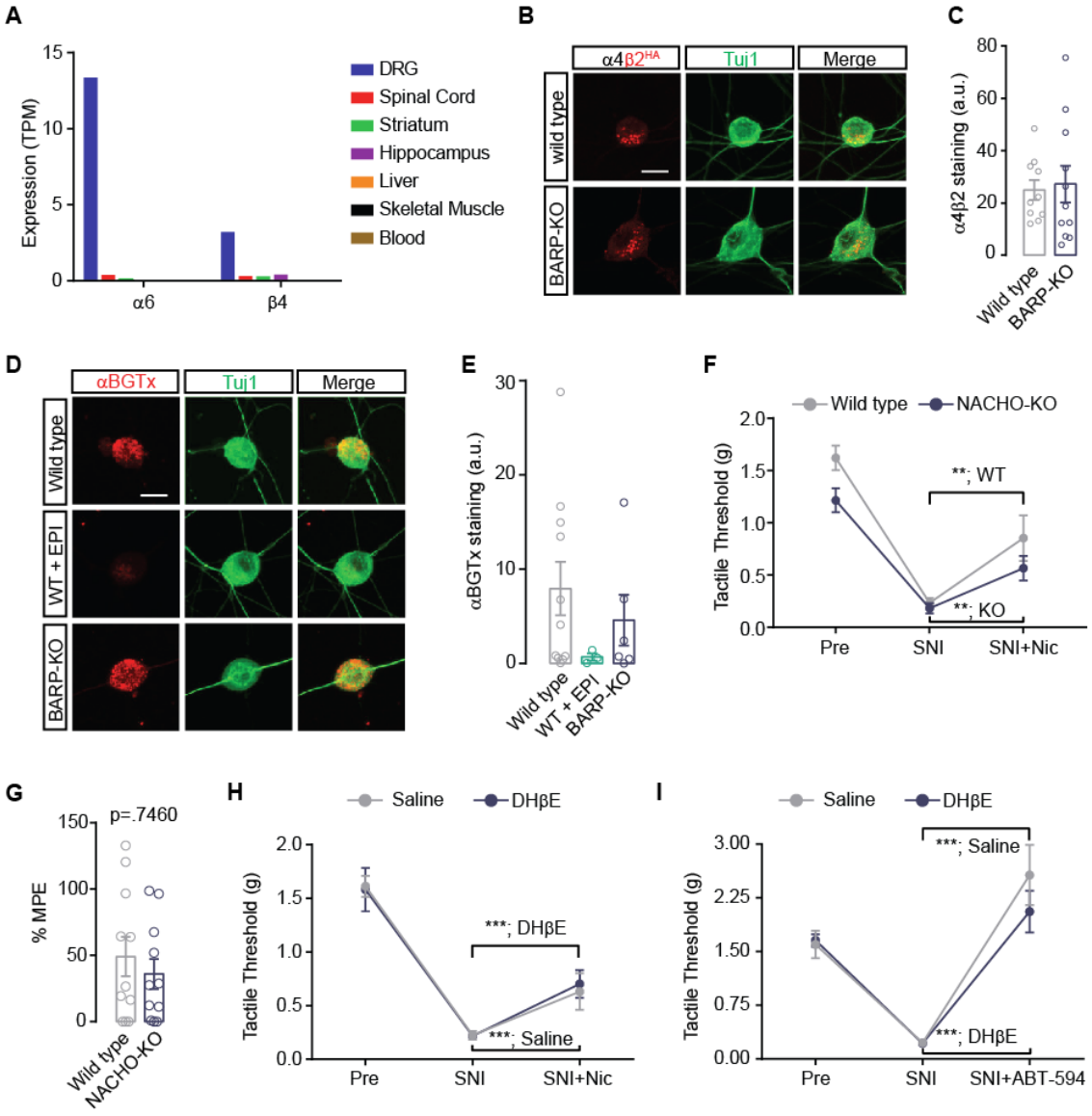
A. Schematic of IRE1α-induced splicing of full-length XBP1 (XBP1u) to spliced XBP1 (XBP1s). RT-PCR of XBP1s in HEK cells transfected with vector, IRE1α, or XBP1s. u = unspliced XBP1, s = XBP1s. **B.** STF-083010 (STF) blocks IRE1α RNase activity and splicing of XBP1. **C.** Quantification of nicotine- (50 μM) evoked Ca^{2+} responses by FLIPR. HEK cells were transfected as indicated and treated with 60 μM STF for 36-48 hours prior to FLIPR experiments. STF compound inhibits function of α6β4 and α4β2 in the presence or absence of transfected IRE1α but has no effect or α3β4. **D.** Nicotine- (50 μM) evoked FLIPR traces for indicated transfections with and without STF (60 μM) incubation for 36 hours. Unpaired t-test for each combination. C: α6β4 t = 14.56; +BARP t = 10.91; +IRE1α t = 11.64; +BI t = 16.23; α4β2 t = 5.367; α4β2+IRE1α t = 9.323; α3β4+IRE1α t = 4.172. * $p < .001$. Graphs are average ± SEM.



Supplemental Figure 6. α3β4 and 5-HT3A receptor responses are not reduced in *IRE1α-KO* cells

A. FLIPR responses in *IRE1α-WT* and *IRE1α-KO* cells transfected with α3β4 or 5-HT3A. Responses stimulated with 100 μM nicotine and 6 μM serotonin, respectively. n=4, 6 for α3β4 and 5HT3A, respectively.

Unpaired t-test. Graphs are averages $\pm$ SEM. * $p < .05$, ** $p < .01$, *** $p < .001$. Graphs depict one experiment that was replicated with similar results.



Supplemental Figure 7. Anti-allodynia is likely mediated through α6β4 nAChRs

A. RNAseq expression data of CHRNA6 (α6) and CHRNB4 (b4) in different tissues. CHRNA6 is greatly enriched in DRG. CHRNB4 also has highest expression in DRG with very low expression in rest of the brain. (raw data from UT Dallas RNAseq database (26)). **B-C**. Surface staining from dissociated dorsal root ganglia (DRG) neurons transduced with α4 and $\beta2^{HA}$ lentiviral particles. Imaging (B) and quantification (C) from wild type and BARP-KO animals (n = 10, 11 neurons, respectively). Scale = 10 mm. **D-E**. Surface staining from dissociated DRG neurons and stained with Alexa 550 conjugated α-Bungarotoxin (αBGTx). Imaging (D) and quantification (E) from wild type, wild type neurons co-treated with epibatidine (10 mM), and BARP-KO neurons (n = 11, 3, 6, respectively). Scale = 10 mm. Co-application of epibatidine displaces αBGTx and reveals non-specific binding. αBGTx binds endogenous α7 nAChRs. **F**. Mechanical allodynia was assessed before (pre), 4 weeks after SNI surgery (SNI), and after intraperitoneal nicotine (2 mg/kg) injection in wild type and NACHO-KO mice (n = 12, 11, respectively). Nicotine induced robust anti-allodynia in both wild type and NACHO-KO mice. **G**. Tactile threshold values comparing single timepoint nicotine-induced allodynia (from H) between wild type and NACHO-KO mice. No significant difference in nicotine-induced anti-allodynia observed between

genotypes. MPE = max percent effect of allodynia, normalized to allodynia after surgery compared to baseline. Data points represent individual mice. **H-I**. Same as in (F), except wild type animals were pre-treated with either saline or 1 mg/kg subcutaneous DHβE 20 minutes before nicotine (H; 1 mg/kg) or ABT-594 (I; 0.3 mg/kg) i.p. administration. For H: n = 9 each group. For I: n = 11 each group.

For F, H, I: linear mixed effects model for repeated measures comparing SNI-Base to SNI-treatment timepoint. Grubbs test (alpha = 0.05) was used to identify outliers. 1 outlier identified in WT group and removed from displayed data in (F), however this did not affect significance.

Supplemental Table 1

Maximum agonist efficacies on recombinant α6β4 receptors. Shown are agonist-evoked Ca^{2+}-responses (relative light units) and 95% confidence intervals from HEK cells. For α6β4, receptors were co-transfected with BARP, IRE1α, and SULT2B1. Related to Figure 6D.

	Nicotine	**Epibatidine**	**ABT-594**	**ABT-894**
α6β4	46.8 (44.6 – 49.2)	72.3 (68.4 – 76.3)	80.1 (75.0-86.1)	21.5 (19.9 – 23.2)
α4β2	50.0 (47.3 – 53.2)	79.0 (75.7 – 82.5)	81.7 (77.4 – 86.4)	46.8 (43.7 – 51.0)

Supplemental Table 2

Agonist EC_{50} values on recombinant α6β4 receptors co-transfected with BARP, IRE1α, and SULT2B1. Also shown in parentheses are 95% confidence intervals. Related to Figure 6D.

	Nicotine	**Epibatidine**	**ABT-594**	**ABT-894**
α6β4	9.5 µM (8.363 µM – 10.86 µM)	12.81 nM (9.62 nM – 16.63 nM)	239.1 nM (185 nM – 317 nM)	2.689 µM (2.131 µM – 3.314 µM)
α4β2	3.321 µM (2.693 µM – 4.12 µM)	25.55 nM (20.36 nM – 31.92 nM)	56.88 nM (43.18 nM – 74.8 nM)	1.139 µM (880.8 nM – 1.5 µM)