Supplemental Materials

Rare human nicotinic acetylcholine receptor alpha 4 subunit (*CHRNA4*) variants affect expression and function of high affinity nicotinic acetylcholine receptors

McClure-Begley, TD, Papke, RL, Stone, KL, Stokes, C, Levy, AD, Gelernter, J, Xie P, Lindstrom J, Picciotto, MR

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Supplemental Methods

cDNA Preparations:

Mutagenesis primers were:

α4R336C:	5'-CGCTCGCCATGCACGCACACC-3'
	5'-GGTGTGCGTGCATGGCGAGCG-3'
α4P451L:	5'-CCCTGCCGCCTGCCCCACGGC-3'
	5'-GCCGTGGGGCAGGCGGCAGGG-3'
α4R487Q:	5'-GCCGGTCTCAGAGCATCCAG-3'
	5'-CTGGATGCTCTGAGACCGGC-3'.

Top10 competent bacteria were transformed with 1 µl of the PCR product and streaked onto LB agar plates supplemented with 100u/ml ampicillin. Individual colonies were picked from the plate and used to inoculate 5 ml cultures of Luria broth (LB) supplemented with 100u/ml ampicillin and grown at 37^{o}C with agitation overnight. A 1 ml aliquot of each culture was used for DNA purification (Qiagen Miniprep). DNA was sequenced (WM Keck Foundation Genomics Services, Yale University School of Medicine) and confirmation of the mutagenesis primer sequence in the proper orientation was performed with 4Peaks (4peaks.en.softonic.com) and

BLASTn (blast.ncbi.nlm.nih.gov). For expression studies in HEK293 cells, the α4 cDNA was excised from psp64 by restriction digestion with BamH1 and HindIII (NEB) and ligated into identical restriction sites in pcDNA3.1 Hygro (Invitrogen) with T4 DNA ligase (Invitrogen). Human β2 cDNA for HEK 293 cell transfection was kindly provided by Dr. Jon Lindstrom (University of Pennsylvania) in a pRc/CMV expression vector (Invitrogen), cloned as described previously (Wang et al, 1998). Verified mutants were amplified by inoculating 400 ml of sterile LB supplemented with 100u/ml ampicillin with 1 ml of the verified culture. cDNA was purified from the large cultures with a Qiagen Endo-free Maxi-prep kit.

Cell Culture and Transfection:

Transient transfections were performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Briefly, cells were plated at an initial density of 50,000/cm^2 in DMEM+10% heat-inactivated fetal bovine serum (FBS), 100u/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B and maintained at 37^{o}C and 5% CO_2. Cells were allowed to reach ~90% confluence before transfection (typically ~96 hours). On the day of transfection, media was removed and cells rinsed once with warm DMEM+10% FBS, and covered with fresh DMEM+10% FBS. cDNA was mixed with Lipfectamine 2000 (7 μl/µg cDNA) in Optimem (200 µl/µg cDNA) and incubated at room temp for 30 min. The Optimem:Lipofectamine:cDNA mixture was gently layered onto the media and cells were returned to the incubator. Cells were harvested 24 hr following transfection.

[^{3}H]-Epibatidine Binding:

Cell membranes were collected by scraping the cells into 1 ml of 0.1X binding buffer (in mM: 14 NaCl, 0.15 KCl, 0.2 $CaCl_2$, 0.1 $MgSO_4{\cdot}7H2O$, 2.5 HEPES hemi-Na) and aspirating from the culture surface with a pipette. The lysed cell suspension was centrifuged at 10,000g for 10 min, and frozen under a fresh volume of 0.1X binding buffer at -80^{o}C until assay. On assay day, cell membranes were thawed and resuspended in 0.1X binding buffer. A 10 μl aliquot of membrane suspension was incubated with 10 μl of [^{3}H]-epibatidine in ddH2O and 10 μl 3X binding buffer to yield a final reaction volume of 30 μl. Nonspecific binding was determined in the presence of 200 μM (-)-cytisine. For most binding studies, the final concentration of [^{3}H]-epibatidine was 2 nM, and was varied linearly from 10 nM-4.9 pM for estimation of binding kinetic parameters. Binding took place either at 23^{o}C for 4 hr, or overnight at 4^{o}C. Binding was terminated by filtering the labeled cell membrane suspension onto a glass fiber filter sandwich (Pall type A/D and A/C) presoaked in 0.5% polyethylinimine. The filter was rinsed 3 times with ice-cold binding buffer, individual filters were picked and bound radioactivity was measured by scintillation counting in a Beckmann LS6000LL at 45% efficiency. Total protein content was determined by the method of Lowry (1954).

Cell surface biotinylation and biotinylated nAChR capture:

Briefly, HEK 293 cells were grown to ~80% confluence in 6-well culture plates and transfected as described above. Cells were rinsed twice with 2ml of ice-cold PBS(in mM: 136.89 NaCl, 2.68 KCl, 10.14 Na_2HPO_4, 1.76 KH_2PO_4; pH 7.4), and 1ml of PBS containing 0.25mg/ml sulfo-NHS-SS-biotin added to each well. Cell surface proteins were biotinylated by incubating with gentle agitation at 4^{o}C for 30 minutes. Biotinylation was quenched by the addition of 10μl/well of 1mM Tris-HCl (pH 7.4; 10mM final concentration) and further incubation for 5 minutes at 23^{o}C. Cells were collected from the wells by gentle suction and centrifuged at 800xG for 2 minutes. The supernatant was removed and the cells gently resuspended in 1ml of TBS (in mM: 50 Tris, 150 NaCl; pH 7.5). The cells were rinsed once more with PBS and then the resulting pellet resuspended in 135μl of PBS and frozed at -80^{o}C until further use. On assay day, cell samples were thawed at 23^{o}C and vigorously resuspended in 0.1x binding buffer. Aliquots of this suspension were taken for measurement of [^{3}H]-epibatidine binding sites and determination of total protein content. The remaining cell sample was mixed 1:1 with PBS supplemented with 4% Triton X-100, 2mM PMSF, and 20μg/ml each of aprotonin, leupeptin, and pepstatin A to effectively solubilize all proteins. Extraction in Triton X-100 took place with gentle agitation at 23°C for one hour, and samples were clarified by centrifugation at 4°C for two minutes at 10,000xG. Aliquots of clarified detergent extracts were taken to measure total protein content. Measurement of biotinylated nAChRs was achieved by incubating 10μl of detergent extract labeled with 2nM [^{3}H]-epibatidine with avidin-coated sepharose beads with gentle agitation for one hour at room temperature. Beads were recovered by filtering the suspension onto glass fiber filters and cpm captured was quantified by liquid scintillation counting.

Specific Immunocapture of β2* nAChRs for Proteomic Analysis:

M270-mAb295 beads were generated by reacting 0.5 μg mAb/mg of beads according to the manufacturer's instructions and stored in PBS+0.5% Triton/0.02% NaN_3 at 4°C until use. HEK 293 cells were grown and transfected as described above. For identification of the nAChR-associated proteome following IP, cells were grown in 10 cm culture plates at an initial density of 50,000 cells/cm^2, and protein:protein interactions were stabilized by crosslinking with dithiobis(succinimidyl)proprionate (DSP; Pierce). 24 hr following transfection, cells were dissociated from the culture surface by incubation with ice-cold PBS and gentle agitation. Cells were aspirated and placed in a 15 ml sterile conical tube and centrifuged at 800g for two minutes. The supernatant was removed and the cells resuspended in 5ml of PBS with 10% (v/v) DMSO and 1 mM DSP (final concentration). The cells were incubated with DSP on ice for 30 min, and crosslinking was terminated by the addition of 10 μl of 1M Tris-HCl, and incubation on ice for an additional 15 min. The crosslinked cells were centrifuged at 800g for 2 min, the supernatant was removed and the cells washed gently with 5 ml of ice-cold PBS. The cells were rinsed with PBS an additional two times before being resuspended in 500 μl of extraction buffer

(PBS supplemented with 2% Triton X-100, 1 mM phenylmethane sulfonyl fluoride, and 10 μg/ml each of aprotonin, pepstatin and leupeptin). Solubilization of nAChRs was achieved by 27 passages through a sterile 200 μl pipette tip. The solubilized protein was clarified by centrifugation at 20,000g for 20 min. The supernatants were collected and a 40 μl aliquot of extract was taken to quantify [^{3}H]-epibatidine binding sites. Two 500 μl extracts were pooled for immunocapture by M270-mAb295 Dynabeads. An aliquot of 400 μl of bead suspension (10 mg/ml) was placed in a clean 1.5 ml Eppendorf tube and the beads were separated by placing the tube in a magnetic rack. The storage buffer was aspirated and the beads rinsed with 1 ml PBS by gentle pipetting. The clarified cell extract was then transferred to the tube containing the rinsed beads and placed in a rotating tube rack at 4^{o}C overnight. The following morning, beads were separated by placing the tube in a magnetic rack, and the supernatant was collected. A 40 μl aliquot of supernatant was collected following incubation with M270-mAb295 Dynabeads to quantify the amount of remaining [^{3}H]-epibatidine binding and to quantify captured nAChRs. The beads were rinsed twice with 1.0ml PBS+0.1% Tween-20 and once with 1.0ml PBS. Proteins were eluted from the beads by the addition of 500 μl of elution buffer (500 mM NH_4OH, 500 μM EDTA, pH 11) and incubated at 23^{o}C with gentle rotation for 20 min. This step was repeated once to ensure complete removal of the receptor complex from the beads. The eluant was lyophilized under vacuum overnight in a DNA120 Speedvac and this dried protein was used for peptide/protein identification by LC-MS/MS.

Enzyamtic digestion, LC-MS/MS, and Phosphopeptide analysis:

The dried protein pellet was dissolved in 12 μl 8M urea, 0.4M ammonium bicarbonate (pH 8.0) prior to reduction using 1.2μl 45mM dithiothreitol (DTT- Thermo Scientific Pierce) and incubating at 37°C for 20 minutes. Alkylation was performed with 1.2μl of 100 mM iodoacetamide (IAN-Sigma-Aldrich) and incubating at ambient temperature for 20 minutes. The urea concentration was decreased to 2M with water and the samples were digested with 3µg lysyl endopeptidase (Wako Chemicals) for 4 hours at 37°C, and then with 3µg of Promega sequencing grade trypsin for 16 hours at 37°C . After digestion, the digests were desalted using UltraMicroSpin™ C18 columns (#SUM SS18V The Nest Group Inc.) and dried under vacuum. The peptide pellets were dissolved in 50µl 0.5% TFA, 50% acetonitrile for phosphopeptide enrichment using titanium dioxide TopTips (Glygen). Each TopTip was washed 3 times (at 2,000 rpm for 1 minute each spin) with 40µl 100% acetonitrile, followed with a 0.2M sodium phosphate pH 7.0, and 0.5% TFA, 50% acetonitrile washes. The digests were then loaded and spun at 1,000 rpm for 1 minute, and then 3,000 rpm for 2 minutes. The phosphopeptides were eluted using 3 washes of 30µl 28% ammonium hydroxide. Both the enriched (Phospho) and non-bound or flow through (FT) peptide fractions were desalted using a C18 ZipTip® (Millipore). The Phospho fraction was dissolved in 3µl 70% formic acid, plus 9µl 50mM sodium phosphate; the FT fraction was dissolved in 5µl 70% formic acid, plus 20µl 50mM sodium phosphate, and 5µl of each was analyzed by LC-MS/MS. This analysis was performed on a Thermo Scientific LTQ Orbitrap Elite equipped with a Waters nanoACQUITY UPLC® system. Trapping was done at 5µl/min, 99% Buffer A (100% water, 0.1% formic acid), 1% Buffer B(100% acetonitrile, 0.1% formic acid) for 3 minutes on a Waters Symmetry® C18 180µm x 20mm trap column. Peptide separation was performed on a 1.7 µm, 75 µm x 250 mm nanoACQUITY UPLC® column (35ºC) at 300 nl/min using a 91 minute linear gradient with 5% buffer B at initial conditions, 40% B at 90 minutes, and 85% B at 91 minutes. MS/MS was performed using higher-energy collisional dissociation (*HCD*) in order to get higher-quality MS/MS spectra. All MS/MS spectra were searched against the NCBInr human database using the Mascot algorithm (Matrix Science) and the Mascot Distiller program to generate Mascot compatible files. Search parameters included a peptide mass tolerance of 15 ppm with a fragment mass tolerance of 0.2Da and variable modifications of carbamidocysteine, methionine oxidation, and serine, threonine and tyrosine phosphorylation. Peptides used for protein identification had a confidence interval greater than

95%. For inclusion in subsequent analysis, a protein had to be present in at least one biological replicate and be identified by more than one unique peptide.

Electrophysiological Recording of Macroscopic Currents from α4β2 nAChRs Expressed in Xenopus Oocytes:

cRNA was prepared from cDNA clones of human α4 and β2 nAChR subunits in psp64 poly-A vectors. Subunit cRNAs (20 ng each) were injected into stage 5 oocytes and recordings were obtained within 7 days. Voltage-clamp recordings were made using OpusExpress 600A, with 300 μM acetylcholine (ACh) used as a control concentration. Raw data was processed with pClamp10 (Molecular Devices).

Figure S1

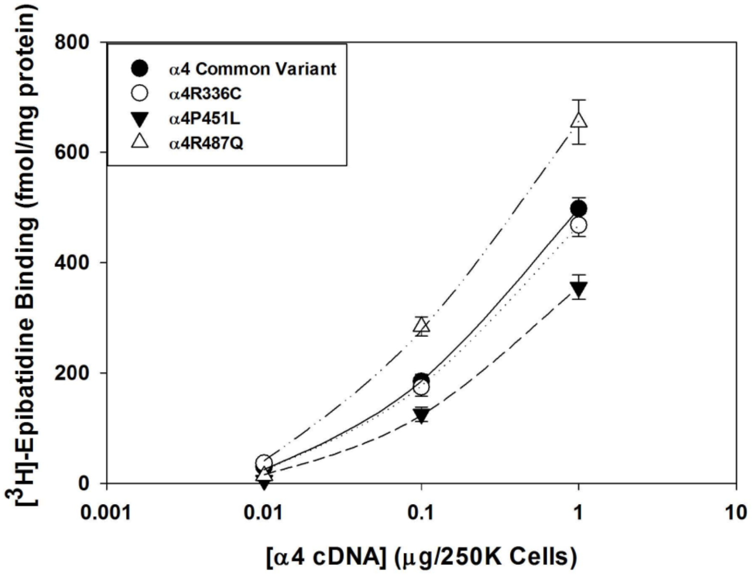

Figure S 1. Assembly of nicotinic binding sites is dependent on the concentration of α4 and β2 nAChR subunit-encoding cDNAs. [^{3}H]-Epibatidine binding increases with the concentration of α4 cDNA transfected along with 1 µg of the β2 cDNA. The amount of binding sites produced is saturable, with ½ maximal α4 cDNA concentration identical across α4 variants examined. Estimated maximal binding site production with respect to the common variant of α4 (black circles), is slightly elevated for α4R487Q (white triangles), decreased for α4P451L (black triangles) and identical for α4R336C (white circles).

Figure S2

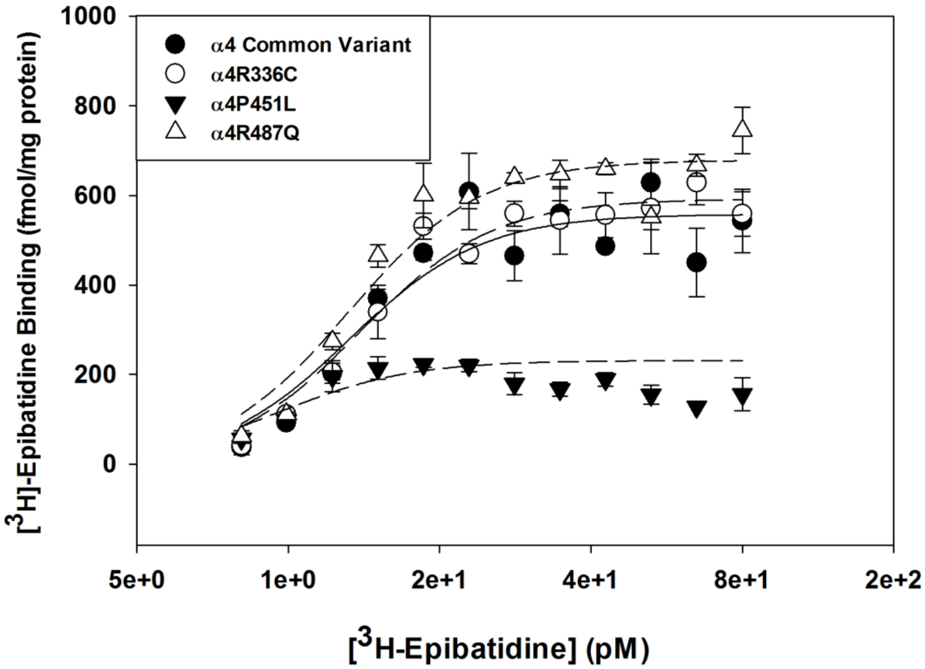

Figure S2. α4 rare variants do not affect nicotinic binding affinity of nAChRs. Saturation of [3H]-epibatidine binding illustrates that the calculated Kd for [3H]-epibatidine is not affected by any of the α4 polymorphisms examined. The Bmax (relative to common variant; black circles), is significantly lower for α4P451L (black triangles), and slightly higher for α4R487Q (white triangles). The Bmax of α4R336C variant (white circles) is identical to the common variant.

Figure S3

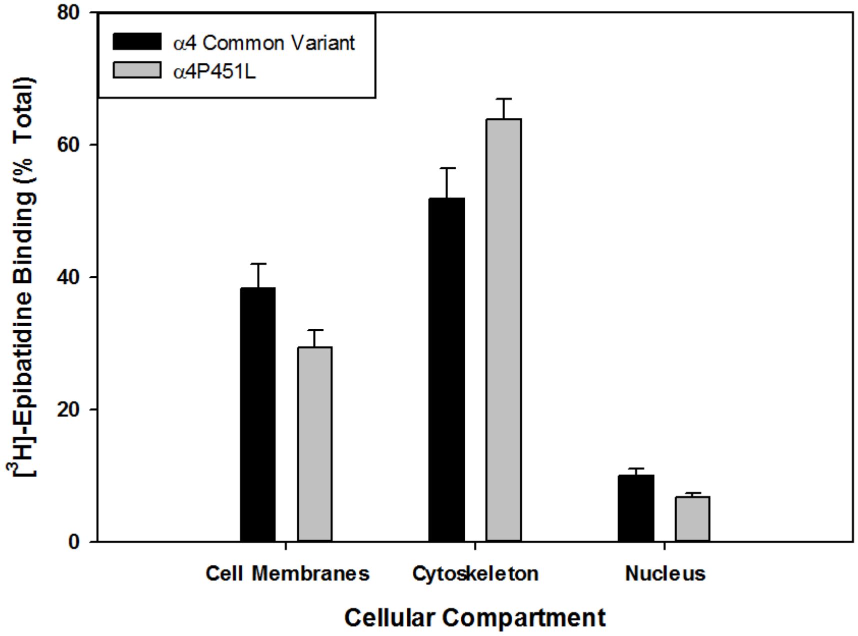

Figure S3. The α4P451L variant does appreciably affect the subcellular distribution of [^{3}H]-epibatidine binding sites expressed as percent of total compared to the common hα4 variant.

Figure S4

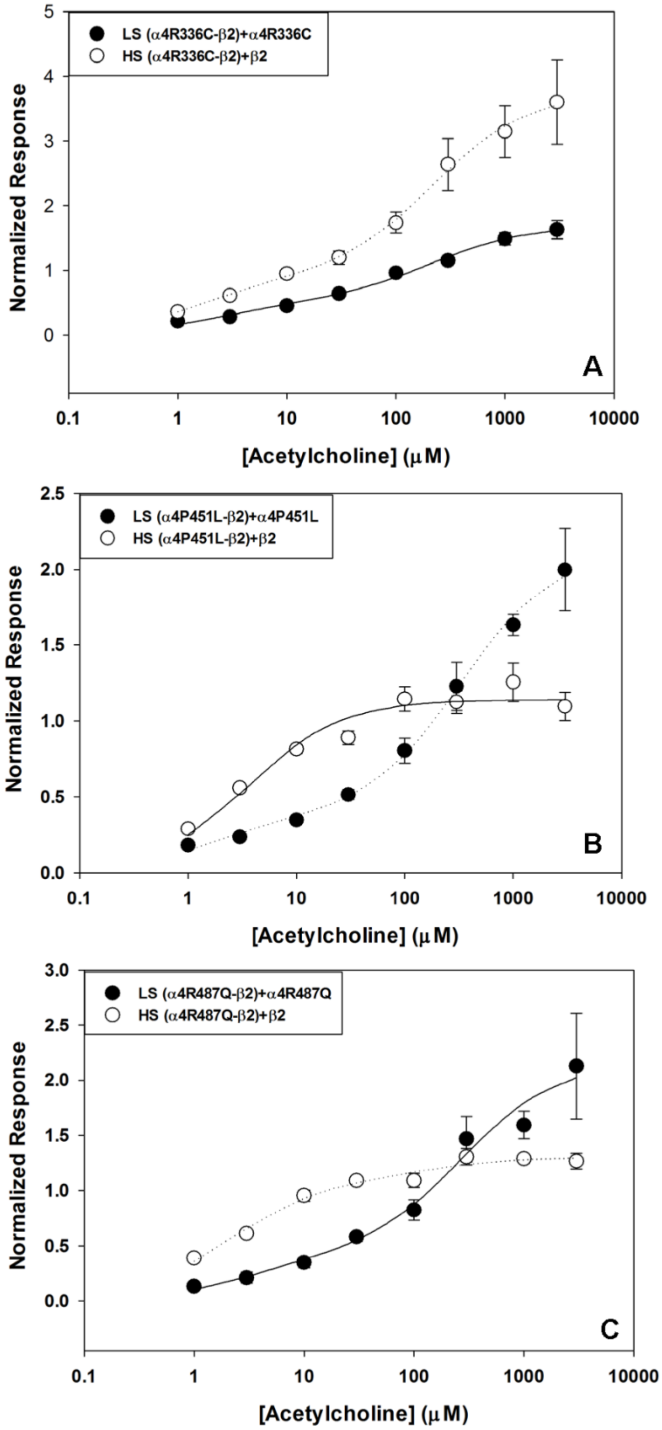

Figure S4. Electrophysiological recordings of concatenated α4 variants expressed in Xenopus oocytes activated by acetylcholine. A. The concentration-response curve for α4R336C-β2 + α4R336C to produce LS receptors (black circles) and α4R336C-β2 + β2 to produce HS receptors (white circles) both show the presence of two distinct activation components. B. The concentration-response curve for α4P451L-β2 + α4P451L to produce LS receptors (black circles) shows the presence of two distinct activation components, while the concentration-response curve for α4P451L-β2 + β2 is dominated by a single HS component (white circles). C. Acetylcholine evoked responses from α4R487Q-β2 + α4R487Q are monophasic and indicative of a single LS activation component (black circles), while α4R487Q-β2 + β2 evidences a single HS activation component (white circles).

Figure S5

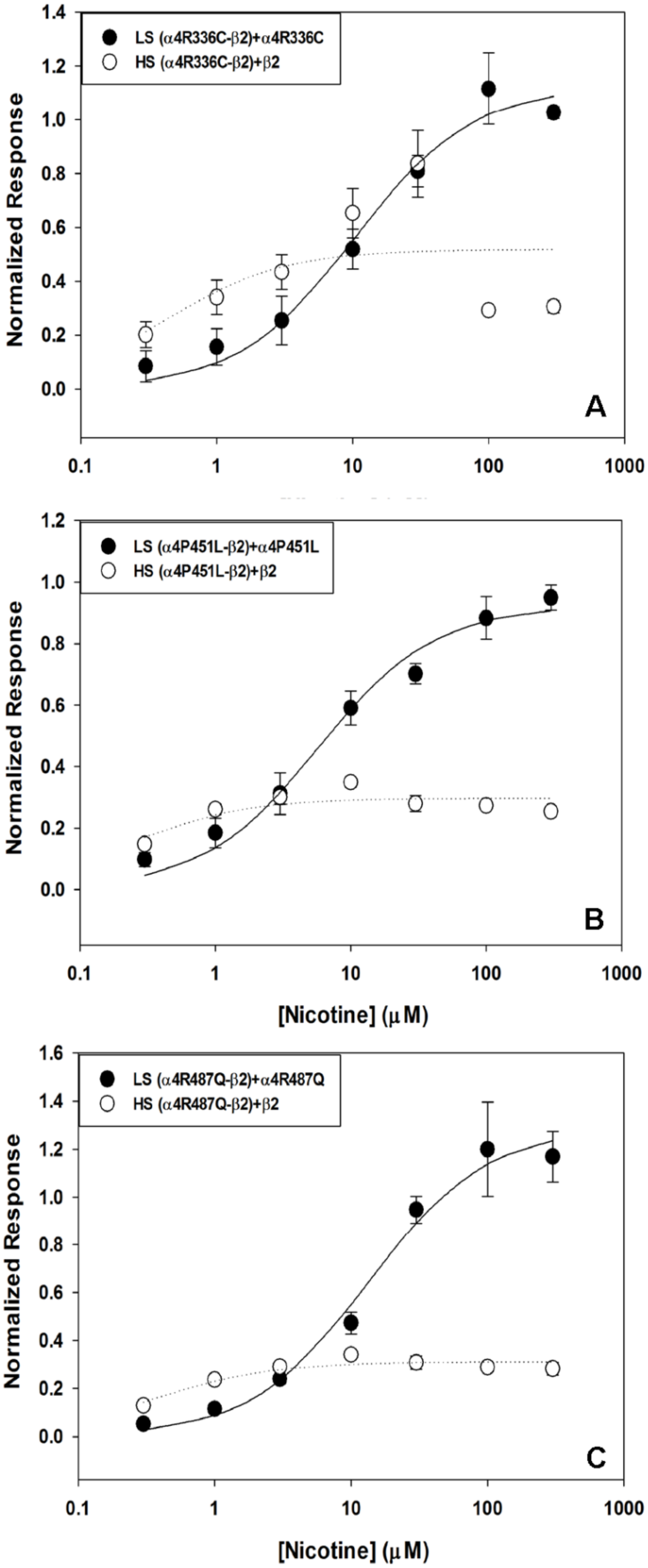

Figure S5. Electrophysiological recordings of concatenated α4 variants expressed in Xenopus oocytes activated by nicotine. A. Expression of α4R336C-β2 + α4R336C produces a monophasic concentration-response curve indicative of a single LS component (black circles), while expression of α4R336C-β2 + β2 produces a monophasic HS curve (white circles). B. The concentration-response profile of α4P451L-β2 + α4P451L activated by nicotine is monophasic and LS (black circles) while α4P451L-β2 + β2 is monophasic and HS (white circles). C. Activation of α4R487Q-β2 + α4R487Q by nicotine yields a monophasic LS concentration-response profile (black circles), and nicotine activation of α4R487Q-β2 + β2 is monophasic and HS in nature (white circles).

Functional Characterization of hα4β2 Rare Variants as Concatamers

Since the functional parameters of α4β2 nAChRs are strongly dependent on the pentameric arrangement in which they assemble, we tested the three rare α4 variants as components of nAChR concatamers. Concatenated subunit dimers (α4-β2) expressed in *Xenopus* oocytes either alone or with additional free α or β subunits reliably assemble into defined stoichiometries. We expected that constraining nAChR stoichiometry by expressing the rare α4 variants as part of a concatenated dimer with β2 would allow measures of functional parameters of HS and LS forms in isolation. Surprisingly, curve-fit calculations of the concentration-response relationships for ACh were not uniform, and under certain conditions the presence of two distinct activation components were observed. For (α4R336C-β2)+β2, the ACh concentration-response curve shows the presence of both HS and LS components, even though the mix of concatamer with free β2 typically produces a pure HS population of nAChRs (Table S3A). For (α4R336C-β2)+α4R336C, again we observed a concentration-response profile with both HS and LS components (Figure S4A). Instead, (α4P451L-β2)+β2, reliably produced a single HS activation profile, whereas (α4P451L-β2)+α4P451L, resulted in both HS and LS ACh responses (Figure S4B). Finally, either (α4R487Q)+α4R487Q or +β2, produces a uniform population of LS or HS activation components, respectively (Figure S4C). In contrast, there was only a single activation component in response to nicotine challenge for each α4 rare variant (Figure S5, Table S3B). Concatenated α4-β2 dimers with the addition of free α4 or β2 subunits normally produce highly constrained expression of LS or HS receptors (as it did in the case of α4R487Q). With activation of α4R336C and α4P451L concatamers by ACh, there was still a significant biphasic concentration-response profile. This effect was absent when nicotine was applied as the agonist, probably because of greater channel blockade by nicotine at concentrations >30μM. We hypothesize that assembly of α4P451L is so inefficient that a significant portion of the concatamers form dipentamers rather than incorporate free α4P451L subunit. When free β2 is added to the α4P451L-β2 concatamer, by contrast, HS receptors are assembled as expected.

Table S1

Protein ID	Protein Name	MW	Uniprot ID	Subcellular location	CV Score	R336C Score	P451L Score	R487Q Score
194386896	cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta	79145	B4DMA2	Cytoplasm	NP	NP	NP	89
194379304	ATP synthase subunit alpha	57542	B4DY56	Cell Membrane	NP	NP	NP	117
2102696	Importin-5	123512	O00410	Cytoplasm	NP	NP	91	NP
5453998	Importin-7)	119440	O95373	Cytoplasm	NP	NP	79	NP
4885165	Cystatin-A	11000	P01040	Cytoplasm	NP	NP	NP	71
122920512	Serum albumin	66412	P02768	Secreted	NP	115	NP	NP
110590597	Serotransferrin	74643	P02787	Secreted	245	NP	NP	NP
4506675	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	68527	P04843	Endoplasmic reticulum	NP	NP	NP	77
21361181	Sodium/potassium-transporting ATPase subunit alpha-1	112824	P05023	Cell membrane	93	104	NP	114
4506773	Protein S100-A9	13234	P06702	Secreted	NP	NP	76	90
32488	Heat shock protein HSP 90-alpha	84621	P07900	Cytoplasm	NP	NP	NP	135
386785	Heat shock 70 kDa protein 1A/1B	69825	P08107	Cytoplasm	NP	128	91	191
306891	Heat shock protein HSP 90-beta	83242	P08238	Cytoplasm	NP	87	NP	NP
30866	Signal recognition particle receptor subunit alpha	69880	P08240	Endoplasmic Reticulum	NP	71	NP	NP
5030431	Vimentin	41537	P08670	Cytoplasm	NP	106	69	83
109157809	Polyubiquitin-C	9016	P0CG48	Cytoplasm	NP	69	NP	87
6470150	78 kDa glucose-regulated protein	70888	P11021	Endoplasmic reticulum	243	343	196	391
5729877	Heat shock cognate 71 kDa protein	70854	P11142	Cytoplasm	102	133	194	235

307066	Inosine-5'-monophosphate dehydrogenase 2	55885	P12268	Cytoplasm	NP	69	93	75
4503483	Elongation factor 2	95277	P13639	Cytoplasm	NP	84	67	NP
4758304	Protein disulfide-isomerase A4	72887	P13667	Endoplasmic reticulum	83	75	NP	108
35505	Pyruvate kinase isozymes M1/M2	57841	P14618	Cytoplasm	NP	83	NP	89
4507677	Endoplasmin	92411	P14625	Endoplasmic reticulum	NP	NP	NP	95
15055539	40S ribosomal protein S2	31305	P15880	Cytoplasm	NP	NP	NP	85
3766451	Neuronal acetylcholine receptor subunit beta-2	57149	P17787	Cell Membrane	265	426	165	392
338490	E3 ubiquitin-protein ligase TRIM21	54109	P19474	Cytoplasm	92	122	85	245
32532	40S ribosomal protein S3	26703	P23396	Cytoplasm	72	79	NP	76
5803227	14-3-3 protein theta	27747	P27348	Cytoplasm	135	206	NP	151
4757900	Calreticulin	48112	P27797	Endoplasmic reticulum	78	109	NP	174
10716563	Calnexin	67526	P27824	Endoplasmic reticulum	171	338	155	338
860986	Protein disulfide-isomerase A3	56644	P30101	Endoplasmic reticulum	NP	83	NP	73
30268331	Myosin-9	154064	P35579	Cytoplasm	116	NP	234	159
641958	Myosin-10	228798	P35580	Cytoplasm	107	71	194	NP
4758032	Coatomer subunit beta	102422	P35606	Cytoplasm	NP	84	NP	98
337580	60S ribosomal protein L3	45440	P39023	Cytoplasm	69	NP	NP	NP
4432748	40S ribosomal protein S27	7686	P42677	Cytoplasm	NP	NP	NP	82
854159	Neuronal acetylcholine receptor subunit alpha-4	67363	P43681	Cell Membrane	1060	1400	318	1289

4826960	Glutamine--tRNA ligase	87743	P47897	Cytoplasm	NP	71	NP	NP
550343	B-cell receptor-associated protein 31	27989	P51572	Endoplasmic reticulum	NP	112	NP	103
1002369	Coatomer subunit alpha	138244	P53621	Cytoplasm	NP	127	NP	90
6005942	Transitional endoplasmic reticulum ATPase	89266	P55072	Cytoplasm	NP	71	NP	80
188590	Myosin light polypeptide 6	16920	P60660	Cytoplasm	72	NP	87	89
28336	Actin, cytoplasmic 1	41786	P60709	Cytoplasm	200	213	410	299
5803225	14-3-3 protein epsilon	29155	P62258	Cytoplasm	73	165	NP	135
4506701	40S ribosomal protein S23	15798	P62266	Cytoplasm	NP	69	90	74
6755368	40S ribosomal protein S18	17708	P62270	Cytoplasm	NP	NP	83	NP
4506681	40S ribosomal protein S11	18419	P62280	Cytoplasm	NP	NP	NP	88
337930	40S ribosomal protein S4, X isoform	27386	P62701	Cytoplasm	92	166	76	161
4507953	14-3-3 protein zeta/delta	27728	P63104	Cytoplasm	100	113	NP	87
181486	Nuclease-sensitive element-binding protein 1	39954	P67809	Cytoplasm	91	NP	69	NP
31092	Elongation factor 1-alpha 1	50095	P68104	Cytoplasm	NP	74	NP	NP
32015	Tubulin alpha-4A chain	49761	P68366	Cytoplasm	NP	84	NP	NP
4758012	Clathrin heavy chain 1	191493	Q00610	Cytoplasm	NP	NP	94	NP
36138	60S ribosomal protein L6	32841	Q02878	Cytoplasm	NP	108	NP	81
190406	Profilaggrin	133528	Q05331	Cytoplasm	NP	72	NP	NP
119618177	Cytoskeleton-associated protein 4	37383	Q07065	Endoplasmic reticulum	NP	106	NP	117
1060888	26S proteasome non-ATPase regulatory subunit 2	100122	Q13200	Proteasome	NP	NP	NP	96
7661948	Malectin	32214	Q14165	Endoplasmic	NP	NP	NP	96

7661948	Malectin	32214	Q14165	reticulum	NP	NP	NP	96
7705855	Estradiol 17-beta-dehydrogenase 12	34302	Q53GQ0	Endoplasmic reticulum	NP	113	NP	85
62896517	Ribosomal protein S4, X-linked X isoform variant	29560	Q53HV1	Cytoplasm	NP	NP	136	NP
46329880	SPTLC1 protein	57360	Q6NUL7	Endoplasmic Reticulum	NP	83	NP	NP
296164	Kinectin	155997	Q86UP2	Endoplasmic Reticulum	NP	71	NP	NP
19263767	Similar to cytoskeleton-associated protein 4	62023	Q8TB01	Cytoplasm	86	NP	NP	NP
9622124	Retinol dehydrogenase 11	35391	Q8TC12	Endoplasmic Reticulum	NP	77	NP	75
30578410	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B	93614	Q8TCJ2	Endoplasmic reticulum	NP	NP	NP	77
18204869	TUBA1B protein	37194	Q8WU19	Cytoplasm	NP	NP	NP	90
4506651	60S ribosomal protein L36a-like	12461	Q969Q0	Cytoplasm	NP	NP	69	NP
4240263	FAS-associated factor 2	52388	Q96CS3	Cytoplasm	NP	76	NP	121
4884343	Extended synaptotagmin-1	81391	Q9BSJ8	Cell Membrane	79	124	NP	131
9453734	Ras-related GTP-binding protein D	28593	Q9NQL2	Cytoplasm	NP	96	NP	NP
3329386	Very-long-chain enoyl-CoA reductase	36038	Q9NZ01	Endoplasmic reticulum	91	154	84	135
6851089	Protein sel-1 homolog 1	88667	Q9UBV2	Endoplasmic Reticulum	NP	94	NP	71

Table S2

Protein ID	Protein Name	MW	Uniprot ID	Subcellular location	CV Score	R336C Score	P451L Score	R487Q Score
4502209	ADP-ribosylation factor 5	20517	A4D0Z3	Cytoplasm	72	NP	NP	NP
7022616	SHINC3	27933	A6QKW0	Cell membrane	82	131	NP	96
158261809	cDNA FLJ78433, highly similar to Homo sapiens chaperonin containing TCP1, subunit 5	59692	A8K2X8	Cytoplasm	NP	114	NP	NP
189054116	cDNA, FLJ94551	26178	B2R9T9	Unknown	NP	79	NP	NP
193785552	cDNA FLJ10609 fis, clone NT2RP2005276, highly similar to Long-chain-fatty-acid--CoA ligase 3)	51273	B3KMA6	Cytoplasm	NP	93	NP	NP
193785682	cDNA FLJ12728 fis, clone NT2RP2000040, highly similar to Protein FAM62A	122829	B3KMV5	Cell membrane	92	NP	NP	258
193783680	cDNA FLJ40884 fis, clone UTERU2000607, highly similar to Alpha-actinin-1	54247	B3KUX9	Cytoplasm	NP	78	NP	127
193785970	cDNA FLJ16143 fis, clone BRAMY2038516, highly similar to Protein disulfide-isomerase A6	53256	B3KY95	Endoplasmic reticulum	155	214	NP	NP
194378936	cDNA FLJ56344, highly similar to Implantation-associated protein	41432	B4DH58	Cell membrane	NP	89	NP	NP
194386896	cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta	79145	B4DMA2	Cytoplasm	NP	515	506	548
194379448	unnamed protein product	53862	B4DYC8	Cytoplasm	NP	108	112	NP
194385368	unnamed protein product	99616	B4E299	Cytoplasm	NP	NP	119	NP
212293358	Actinin alpha 1 isoform 3	107074	B7TY16	Cytoplasm	NP	NP	NP	98
221040644	cDNA FLJ54432, highly similar to Alpha-actinin-1	67100	B7Z2W3	Cytoplasm	NP	104	NP	NP
221041874	cDNA FLJ58023, highly similar to Protein disulfide-isomerase A6	48670	B7Z4M8	Endoplasmic reticulum	184	NP	NP	NP
221042282	cDNA FLJ53638, highly similar to Annexin A6	61814	B7Z582	Secreted	NP	143	NP	NP

221043506	unnamed protein product	26690	B7Z6Z4	Cytoplasm	NP	NP	238	NP
221045918	cDNA, FLJ79164, highly similar to Tubulin beta-7 chain	46537	B7ZAF0	Cytoplasm	NP	471	NP	NP
31179	enolase	49446	E2DRY6	Cell membrane	NP	NP	82	NP
119590078	Transducin beta-like protein 2	46318	E9PF19	Unknown	NP	NP	NP	146
5729875	Membrane-associated progesterone receptor component 1	21658	O00264	Endoplasmic reticulum	NP	276	NP	222
24797086	importin-5	125464	O00410	Cytoplasm	NP	NP	316	NP
6755448	Vesicle-trafficking protein SEC22b	24725	O08547	Endoplasmic reticulum	178	217	176	172
4504733	Insulin receptor substrate 4	133685	O14654	Cell membrane	NP	111	86	71
15809016	myosin regulatory light chain 12B	19767	O14950	Cytoplasm	NP	NP	131	NP
5454084	Serine palmitoyltransferase 1	52710	O15269	Endoplasmic reticulum	105	112	86	107
2337920	Syntaxin-7	29827	O15400	Cell membrane	NP	103	NP	NP
4506133	phosphoribosyl pyrophosphate synthase-associated protein 2 isoform 1	40899	O60256	Cytoplasm	NP	136	95	NP
4507285	Syntaxin-10	28096	O60499	Golgi apparatus	74	NP	NP	NP
3037013	Heterogeneous nuclear ribonucleoprotein Q	69590	O60506	Cytoplasm	NP	NP	NP	87
4885637	Target of Myb protein 1	53785	O60784	Cytoplasm	NP	NP	NP	NP
62896519	Erlin-1	38929	O75477	Endoplasmic reticulum	NP	135	NP	127
6005721	Erlin-2	37815	O94905	Endoplasmic reticulum	320	362	196	376

4759302	Vesicle-associated membrane protein-associated protein B/C	27211	O95292	Endoplasmic reticulum	87	131	NP	72
5453998	importin-7	119440	O95373	Cytoplasm	NP	NP	556	NP
5031857	L-lactate dehydrogenase A chain isoform 1	36665	P00338	Cytoplasm	124	NP	136	NP
284448551	NADH-cytochrome b5 reductase 3	38202	P00387	Endoplasmic reticulum	NP	94	NP	NP
4885165	Cystatin-A	11000	P01040	Cytoplasm	72	NP	NP	NP
122920512	Serum albumin	66412	P02768	Secreted	75	114	NP	NP
31645	Glyceraldehyde-3-phosphate dehydrogenase	36031	P04406	Cytoplasm	146	242	NP	164
4506675	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	68527	P04843	Endoplasmic reticulum	467	503	348	455
209413738	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	67681	P04844	Endoplasmic reticulum	338	518	357	498
34740335	Tubulin alpha-1B chain	50120	P05213	Cytoplasm	378	599	564	413
4506671	60S acidic ribosomal protein P2	11658	P05387	Cytoplasm	91	NP	144	NP
693933	Alpha-enolase	47079	P06733	Cytoplasm	94	190	77	113
114155146	Tropomyosin alpha-3 chain	28938	P06753	Cytoplasm	150	76	240	109
4557032	L-lactate dehydrogenase B chain	36615	P07195	Cytoplasm	119	159	82	149
34364597	Annexin A2	40328	P07355	Secreted	102	141	87	103
338695	beta-tubulin	49727	P07437	Cytoplasm	302	664	361	NP
31958	Bifunctional glutamate/proline--tRNA ligase	162923	P07814	Cytoplasm	NP	70	NP	NP
61656603	Heat shock protein HSP 90-alpha	98052	P07900	Cytoplasm	318	450	476	548
4529892	Heat shock 70 kDa protein 1A/1B	69982	P08107	Cytoplasm	411	712	634	668
				Cell				

178685	4F2 cell-surface antigen heavy chain	57926	P08195	Cell membrane	NP	91	NP	NP
306891	Heat shock protein HSP 90-beta	83242	P08238	Cytoplasm	375	NP	NP	NP
23308697	Signal recognition particle receptor subunit alpha	69767	P08240	Endoplasmic reticulum	NP	94	NP	NP
62414289	Vimentin	53619	P08670	Cytoplasm	419	134	691	441
119624911	Polyubiquitin-B	16792	P0CG47	Cytoplasm	NP	373	254	NP
31615803	Polyubiquitin-C	8555	P0CG48	Cytoplasm	162	NP	NP	356
6470150	78 kDa glucose-regulated protein	70888	P11021	Endoplasmic reticulum	1157	1095	1012	1079
5729877	Heat shock cognate 71 kDa protein	70854	P11142	Cytoplasm	543	897	640	813
693937	polyadenylate binding protein II	58481	P11940	Cytoplasm	NP	NP	217	NP
66933016	inosine-5'-monophosphate dehydrogenase 2	55770	P12268	Cytoplasm	NP	NP	205	104
28334	Alpha-actinin-1	102910	P12814	Cytoplasm	NP	NP	169	92
4503483	Elongation factor 2	95277	P13639	Cytoplasm	148	259	202	242
4758304	Protein disulfide-isomerase A4	72887	P13667	Endoplasmic reticulum	86	127	NP	NP
182855	Glucosidase 2 subunit beta	59259	P14314	Endoplasmic reticulum.	121	112	NP	88
35505	Pyruvate kinase isozymes M1/M2	57841	P14618	Cytoplasm	NP	88	NP	NP
4507677	Endoplasmin	92411	P14625	Endoplasmic reticulum	238	244	164	273
15055539	40S ribosomal protein S2	31305	P15880	Cytoplasm	NP	NP	229	NP
4502285	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	109620	P16615	Endoplasmic reticulum	212	379	230	347
				Cell				

4502833	Neuronal acetylcholine receptor subunit beta-2	56982	P17787	Cell membrane	1371	1855	802	1625
1827881	Tyrosine-protein phosphatase non-receptor type 1	37273	P18031	Endoplasmic reticulum	NP	114	NP	73
35903	ribosomal protein L7	29164	P18124	Cytoplasm	NP	NP	87	NP
14994115	E3 ubiquitin-protein ligase TRIM21	54234	P19474	Cytoplasm	202	232	257	378
254540166	78 kDa glucose-regulated protein precursor	72377	P20029	Endoplasmic reticulum	NP	NP	850	NP
181250	Peptidyl-prolyl cis-trans isomerase B	22597	P23284	Endoplasmic reticulum	87	122	NP	NP
7765076	40S ribosomal protein S3	26699	P23396	Cytoplasm	181	242	326	277
119604798	Elongation factor 1-beta	31167	P24534	Cytoplasm	NP	86	71	NP
29383	60S ribosomal protein L13	24277	P26373	Cytoplasm	74	NP	139	97
4503481	Elongation factor 1-gamma	50087	P26641	Cytoplasm	165	260	236	213
5803227	14-3-3 protein theta	27747	P27348	Cytoplasm	401	492	132	382
184407	60S ribosomal protein L10	24561	P27635	Cytoplasm	NP	109	135	111
4757900	Calreticulin	48112	P27797	Endoplasmic reticulum	123	108	149	92
10716563	Calnexin	67526	P27824	Endoplasmic reticulum	462	635	442	541
558526	Proteasome subunit beta type-5	22882	P28074	Cytoplasm	76	96	NP	NP
35553	ATP-binding cassette sub-family D member 3	75539	P28288	Peroxisome	NP	NP	NP	118
38522	Elongation factor 1-delta	31202	P29692	Cytoplasm	NP	NP	NP	75
5803013	Endoplasmic reticulum resident protein 29	28975	P30040	Endoplasmic reticulum	NP	71	NP	NP

4506597	60S ribosomal protein L12	17808	P30050	Cytoplasm	NP	NP	95	NP
220702506	Protein disulfide-isomerase A3	54199	P30101	Endoplasmic reticulum	313	323	256	292
122921194	Chain A, Crystal Structure Of Protein Phosphatase 2a (Pp2a) Holoenzyme With The Catalytic Subunit Carboxyl Terminus Truncated	64375	P30153	Cytoplasm	NP	NP	95	NP
219588	DnaJ protein homolog	44848	P31689	Membrane	NP	NP	88	NP
4507949	14-3-3 protein beta/alpha	28065	P31946	Cytoplasm	374	369	NP	326
438069	Peroxiredoxin-2	21843	P32119	Cytoplasm	NP	84	NP	91
12667788	Myosin-9	226392	P35579	Cytoplasm	262	121	875	196
119610456	myosin, heavy polypeptide 10, non-muscle, isoform CRA_c	228886	P35580	Cytoplasm	NP	NP	318	NP
4758032	Coatomer subunit beta	102422	P35606	Cytoplasm	107	117	92	124
16579885	60S ribosomal protein L4	47667	P36578	Cytoplasm	NP	NP	156	NP
239938926	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	50769	P39656	Endoplasmic reticulum	NP	329	279	305
4506711	40S ribosomal protein S27	9455	P42677	Cytoplasm	NP	68	70	NP
854159	Neuronal acetylcholine receptor subunit alpha-4	67363	P43681	Cell membrane	2067	2787	627	2340
4506625	60S ribosomal protein L27a	16551	P46776	Cytoplasm	123	99	159	141
550019	60S ribosomal protein L28	15752	P46779	Cytoplasm	NP	NP	107	91
550023	ribosomal protein S9	22558	P46781	Cytoplasm	69	NP	103	NP
550021	40S ribosomal protein S5	22763	P46782	Cytoplasm	101	176	106	118
624704	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A	80419	P46977	Endoplasmic reticulum	NP	116	NP	101
793843	ribosomal protein L29	17656	P47914	Cytoplasm	NP	NP	78	NP

13491174	MARCKS-related protein	19517	P49006	Cytoplasm	NP	80	NP	NP
915392	Fatty acid synthase	272919	P49327	Cytoplasm	NP	NP	NP	81
28375485	Transmembrane emp24 domain-containing protein 10	25619	P49755	Golgi apparatus	76	117	NP	145
119617519	hCG2014475	23244	P50914	Cytoplasm	NP	NP	112	84
1136741	T-complex protein 1 subunit theta	58465	P50990	Cytoplasm	NP	NP	NP	118
1200184	stimulator of TAR RNA binding	57803	P50991	Cytoplasm	NP	NP	117	NP
34147513	Ras-related protein Rab-7a	23475	P51149	Cytoplasm	204	292	NP	252
4759012	Ras-related protein Rab-9A	22823	P51151	Cell membrane	NP	82	NP	NP
5454090	Translocon-associated protein subunit delta	18987	P51571	Endoplasmic reticulum	NP	118	131	114
15680023	B-cell receptor-associated protein 31	27975	P51572	Endoplasmic reticulum	112	98	86	80
5032137	Vesicle-associated membrane protein 7	24919	P51809	Cytoplasm	481	131	NP	71
4504897	importin subunit alpha-2	57826	P52292	Cytoplasm	NP	NP	141	NP
119573109	coatomer protein complex, subunit alpha, isoform CRA_b	136721	P53621	Cytoplasm	NP	NP	196	NP
6005942	Transitional endoplasmic reticulum ATPase	89266	P55072	Cytoplasm	NP	708	584	561
224589127	Transmembrane protein 33	27960	P57088	Cell membrane	NP	129	NP	NP
5803165	Protein transport protein Sec61 subunit beta	9968	P60468	Endoplasmic reticulum	NP	72	NP	NP
467828	Myosin light polypeptide 6	17601	P60660	Cytoplasm	99	NP	312	116
4501885	Actin, cytoplasmic 1	41710	P60709	Cytoplasm	471	NP	682	562
4503529	Eukaryotic initiation factor 4A-I	46125	P60842	Cytoplasm	118	134	167	178

4506697	40S ribosomal protein S20	13364	P60866	Cytoplasm	93	104	88	102
4506127	ribose-phosphate pyrophosphokinase 1 isoform 1	34812	P60891	Cytoplasm	NP	NP	192	NP
4506365	Ras-related protein Rab-2A	23531	P61019	Endoplasmic reticulum	NP	141	NP	NP
12052826	Ras-related protein Rab-10	22455	P61026	Cytoplasm	127	NP	NP	163
16758368	Ras-related protein Rab-14	23912	P61107	Golgi apparatus	NP	112	NP	NP
119612991	hCG1998718	24786	P61247	Cytoplasm	NP	NP	87	NP
12006350	60S ribosomal protein L15	24145	P61313	Cytoplasm	NP	NP	82	81
4503253	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1	12489	P61803	Endoplasmic reticulum	NP	88	NP	NP
460789	Heterogeneous nuclear ribonucleoprotein K	51040	P61978	Cytoplasm	104	NP	224	195
9507245	14-3-3 protein gamma	28285	P61983	Cytoplasm	262	256	105	256
4506743	40S ribosomal protein S8	24190	P62241	Cytoplasm	168	111	198	145
4506691	40S ribosomal protein S16	16435	P62249	Cytoplasm	105	126	110	114
5803225	14-3-3 protein epsilon	29155	P62258	Cytoplasm	707	895	161	552
5032051	40S ribosomal protein S14	16263	P62263	Cytoplasm	117	141	92	103
4506701	40S ribosomal protein S23	15798	P62266	Cytoplasm	75	NP	89	NP
6755368	40S ribosomal protein S18	17708	P62270	Cytoplasm	194	184	184	168
4506681	40S ribosomal protein S11	18419	P62280	Cytoplasm	82	134	152	113
1526426	26S protease regulatory subunit 10B	44133	P62333	Cytoplasm	NP	72	NP	68
4506661	60S ribosomal protein L7a	29977	P62424	Cytoplasm	NP	NP	109	NP
337930	40S ribosomal protein S4, X isoform	27386	P62701	Cytoplasm	134	188	267	163

306549	homology to rat ribosomal protein L23, partial	16730	P62750	Cytoplasm	NP	NP	106	
337514	40S ribosomal protein S6	28614	P62753	Cytoplasm	NP	77	142	120
4758988	Ras-related protein Rab-1A	22663	P62820	Golgi apparatus	187	217	127	235
4506605	60S ribosomal protein L23	14856	P62829	Cytoplasm	143	210	183	179
119579121	hCG27283	13014	P62854	Cytoplasm	NP	NP	83	NP
6680045	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	37353	P62874	Cell membrane	NP	105	NP	113
1655596	ribosomal protein L31	14084	P62899	Cytoplasm	NP	NP	67	NP
4506635	60S ribosomal protein L32	15850	P62910	Cytoplasm	NP	NP	91	NP
291463380	Peptidyl-prolyl cis-trans isomerase A	18537	P62937	Cytoplasm	86	192	115	127
4507953	14-3-3 protein zeta/delta	27728	P63104	Cytoplasm	515	564	169	449
5174447	Guanine nucleotide-binding protein subunit beta-2-like 1	35055	P63244	Cell membrane	92	106	220	111
181486	Nuclease-sensitive element-binding protein 1	39954	P67809	Cytoplasm	143	99	112	133
10441386	TPM4-ALK fusion oncoprotein type 2	27513	P67936	Cytoplasm	NP	NP	84	NP
31092	Elongation factor 1-alpha 1	50095	P68104	Cytoplasm	183	218	182	203
4501881	Actin, alpha skeletal muscle	42024	P68133	Cytoplasm	359	NP	NP	NP
5174735	Tubulin beta-4B chain	49799	P68371	Cytoplasm	89	573	185	552
5453603	T-complex protein 1 subunit beta isoform 1	57452	P78371	Cytoplasm.	NP	NP	NP	97
4506619	60S ribosomal protein L24	17768	P83731	Cytoplasm	NP	NP	91	NP
1065361	ADP-ribosylation factor 1	20553	P84077	Golgi apparatus	NP	68	71	NP
				Golgi				

4502209	ADP-ribosylation factor 5	20517	P84085	Golgi apparatus	90	97	78	82
119619805	hCG39240, isoform CRA_a	20120	P84098	Cytoplasm	NP	NP	91	NP
7106439	Tubulin beta-5 chain	49639	P99024	Cytoplasm	393	NP	615	611
4758012	Clathrin heavy chain 1	191493	Q00610	Cytoplasm	110	70	426	172
11415026	60S ribosomal protein L18a	20749	Q02543	Cytoplasm	NP	NP	79	NP
2624694	Single-stranded DNA-binding protein, mitochondrial	15186	Q04837	Cytoplasm	459	NP	NP	NP
437363	14-3-3 protein eta	28172	Q04917	Cytoplasm	196	160	NP	138
4505591	Peroxiredoxin-1	22096	Q06830	Cytoplasm	182	197	138	175
4506607	60S ribosomal protein L18	21621	Q07020	Cytoplasm	75	NP	203	86
63102283	Cytoskeleton-associated protein 4	58171	Q07065	Endoplasmic reticulum	NP	NP	NP	198
89574029	ATP synthase subunit beta	48083	Q0QEN7	Golgi apparatus	349	NP	NP	NP
5803023	Vesicular integral-membrane protein VIP36	40203	Q12907	Endoplasmic reticulum	NP	74	77	70
5453549	Peroxiredoxin-4	30521	Q13162	Cytoplasm	NP	103	85	93
1060888	26S proteasome non-ATPase regulatory subunit 2	100122	Q13200	Cytoplasm	NP	75	NP	NP
1805280	alpha II spectrin	284891	Q13813	Cytoplasm	NP	NP	87	NP
181914	Heterogeneous nuclear ribonucleoprotein D0	35801	Q14103	Endoplasmic reticulum	96	NP	NP	NP
119618619	Malectin	40066	Q14165	Endoplasmic reticulum	96	120	NP	122
19923142	importin subunit beta-1	97108	Q14974	Cytoplasm	NP	NP	656	NP
7661910	ER membrane protein complex subunit 2	34811	Q15006	Endoplasmic	135	NP	NP	NP

7661910	ER membrane protein complex subunit 2	34811	Q15006	reticulum	135	NP	NP	NP
1710248	protein disulfide isomerase-related protein 5	46170	Q15084	Endoplasmic reticulum	NP	NP	211	205
22477159	Protein transport protein Sec23A	86109	Q15436	Endoplasmic reticulum	NP	96	NP	NP
392890	drebrin E2	71381	Q16643	Cytoplasm	NP	NP	107	NP
84569966	Unknown (protein for MGC:131531)	21747	Q2NKY6	Cytoplasm	NP	NP	74	NP
126723336	Prohibitin 2	33276	Q3V235	Cytoplasm	176	NP	NP	NP
68533125	ACLY variant protein	124477	Q4LE36	Cytoplasm	NP	81	69	NP
62897773	Ribosomal protein S5 variant	22950	Q53G25	Cytoplasm	NP	NP	NP	130
62897625	Beta actin variant	41738	Q53G99	Cytoplasm	632	469	849	567
62897409	Beta actin variant	41696	Q53GK6	Cytoplasm	NP	554	106	NP
7705855	Estradiol 17-beta-dehydrogenase 12	34302	Q53GQ0	Endoplasmic reticulum	NP	82	NP	159
62896547	B-cell receptor-associated protein 31 variant	27944	Q53HT6	Cytoplasm	76	NP	NP	NP
62089222	Heat shock 70kDa protein 1A variant	77448	Q59EJ3	Endoplasmic reticulum	446	NP	NP	NP
62088648	Tumor rejection antigen	65912	Q59FC6	Endoplasmic reticulum	140	252	NP	145
62087780	ribosomal protein L21 variant	18879	Q59GK9	Cytoplasm	NP	NP	NP	NP
194382322	ER membrane protein complex subunit 4	15079	Q5J8M3	Cell membrane	NP	89	NP	NP
122891870	Melanoma inhibitory activity protein 3	213570	Q5JRA6	Endoplasmic reticulum	NP	94	NP	NP
54300702	Ubiquitin C splice variant	17142	Q5UGI3	Cytoplasm	NP	229	NP	NP

55665780	Tropomyosin 3	27158	Q5VU59	Cytoplasm	NP	82	NP	NP
5729875	PGRMC1 protein	21658	Q6IB11	Cell membrane	185	NP	112	NP
4200241	STX12 protein	30859	Q6LEU0	Cell membrane	NP	143	NP	98
45829841	SLC25A5 protein	35271	Q6NVC0	Cell membrane	210	286	228	NP
45501009	CKAP4 protein	67777	Q6NWZ1	Cytoplasm	163	197	187	NP
457262	Nuclease sensitive element binding protein-1	34650	Q7KZ24	Cytoplasm	95	NP	NP	NP
799177	100 kDa coactivator	99628	Q7KZF4	Cytoplasm	83	74	118	NP
20336290	putative ATP-dependent RNA helicase DHX30 isoform 2	129357	Q7L2E3	Cytoplasm	NP	74	87	NP
5729999	Ras-related GTP-binding protein A	36543	Q7L523	Cytoplasm	NP	NP	NP	NP
27477136	Zinc finger CCCH-type antiviral protein 1	101367	Q7Z2W4	Cytoplasm	NP	92	NP	76
119586864	Transmembrane protein 55B	22014	Q86T03	Cell membrane	NP	71	NP	NP
30582727	Dihydropyrimidinase-like 2	66975	Q86U75	Cytoplasm	NP	118	NP	NP
119631364	DnaJ homolog subfamily C member 10	94501	Q8IXB1	Endoplasmic reticulum	NP	75	NP	NP
21755136	Transmembrane protein 192	30469	Q8IY95	Cell membrane	NP	132	NP	NP
56605994	CDGSH iron-sulfur domain-containing protein 2	15268	Q8N5K1	Endoplasmic reticulum	NP	72	NP	NP
22095331	ER membrane protein complex subunit 1	111689	Q8N766	Endoplasmic reticulum	120	128	NP	101
19263767	Similar to cytoskeleton-associated protein 4	62023	Q8TB01	Cytoplasm	144	239	NP	165
30578410	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit	93614	Q8TCJ2	Endoplasmic	125	123	109	161

30578410	STT3B	93614	Q8TCJ2	reticulum	125	125	109	161
18676757	Probable glutathione peroxidase 8	23894	Q8TED1	Cell membrane	NP	99	NP	75
5138928	vesicle transport-related protein	72434	Q8WVM8	Cytoplasm	NP	NP	74	NP
1518259	GPI-anchor transamidase	45358	Q92643	Endoplasmic reticulum	NP	76	NP	NP
4680651	GPI transamidase component PIG-T	65353	Q969N2	Endoplasmic reticulum	NP	NP	NP	69
40254924	Leucine-rich repeat-containing protein 59	34909	Q96AG4	Endoplasmic reticulum	NP	79	NP	75
21361454	pyrroline-5-carboxylate reductase 2	33616	Q96C36	Cytoplasm	NP	NP	170	NP
4240263	FAS-associated factor 2	52388	Q96CS3	Cytoplasm	NP	72	88	106
13027602	DDRGK domain-containing protein 1	35589	Q96HY6	Endoplasmic reticulum	NP	NP	NP	82
7022978	Vacuolar protein sorting-associated protein 35	91551	Q96QK1	Cytoplasm	94	150	83	139
25777600	26S proteasome non-ATPase regulatory subunit 1 isoform 1	105769	Q99460	Proteasome	NP	NP	96	NP
5453607	T-complex protein 1 subunit eta isoform a	59329	Q99832	Cytoplasm	NP	NP	75	NP
13542987	Similar to ribosomal protein S8	9277	Q9BS10	Cytoplasm	NP	105	NP	NP
3882215	Extended synaptotagmin-1	119783	Q9BSJ8	Cell membrane	180	272	138	244
34392	RPS2 protein	24191	Q9BSW5	Cytoplasm	NP	NP	NP	128
12698013	KIAA1734 protein	143387	Q9C0C9	Cytoplasm	NP	NP	130	NP
6048243	Toll-interacting protein	30191	Q9H0E2	Cytoplasm	NP	78	NP	NP
22761454	Magnesium transporter protein 1	38011	Q9H0U3	Cell membrane	NP	NP	NP	71

22761456	Thioredoxin-related transmembrane protein 1	31743	Q9H3N1	Endoplasmic reticulum	83	135	91	114
10436857	cDNA: FLJ20897 fis, clone ADKA03573	71374	Q9H7G6	Cytoplasm	NP	77	75	NP
9802306	DNA-binding protein TAXREB107	32871	Q9HBB3	Cytoplasm	NP	NP	214	NP
11275389	Stromal cell-derived factor 2-like protein 1	23497	Q9HCN8	Endoplasmic reticulum	115	125	117	122
9858861	Probable cation-transporting ATPase 13A1	121032	Q9HD20	Cell membrane	NP	70	NP	NP
1581615	Oligosaccharyltransferase complex subunit OSTC	46216	Q9NRP0	Cell membrane	311	298	152	338
7019854	DnaJ homolog subfamily B member 12	41760	Q9NXW2	Cell membrane	NP	NP	NP	107
3329386	Very-long-chain enoyl-CoA reductase	36038	Q9NZ01	Endoplasmic reticulum	90	69	NP	76
6735452	Very-long-chain (3R)-3-hydroxyacyl-[acyl-carrier protein] dehydratase 3	43515	Q9P035	Endoplasmic reticulum	153	257	106	135
94721250	Vesicle-associated membrane protein-associated protein A	32593	Q9P0L0	Endoplasmic reticulum	NP	91	NP	NP
2935281	7-dehydrocholesterol reductase	54480	Q9UBM7	Endoplasmic reticulum	91	120	NP	97
7706495	DnaJ homolog subfamily B member 11	40489	Q9UBS4	Endoplasmic reticulum	180	120	102	140
6841440	Stomatin-like protein 2	37122	Q9UJZ1	Cell membrane	72	NP	74	67
3283893	Transmembrane and coiled-coil domain-containing protein 1	25934	Q9UM00	Endoplasmic reticulum	NP	87	NP	NP
4028622	chaperonin 10-related protein	10289	Q9UNM1	Cytoplasm	NP	NP	171	NP

7549793	transducin beta-like protein 2 precursor	49766	Q9Y4P3	Unknown	110	193	150	NP
38649415	Signal recognition particle receptor subunit beta	29698	Q9Y5M8	Endoplasmic reticulum	NP	181	NP	92
11559929	Coatomer subunit gamma-1	97655	Q9Y678	Cytoplasm	93	119	NP	118
5410278	Ancient ubiquitous protein 1	45768	Q9Y679	Endoplasmic reticulum	NP	70	NP	NP

Table S3

a

hα4 Concatamer Construct	HS EC_{50} (μM)	HS Max Response	LS EC_{50} (μM)	LS Max Response
α4R336C(α4β2)β2	1.70±0.69	0.92±0.12	220.0±154	2.80±0.12
α4R336C(α4β2)α4	2.5±1.30	0.53±0.10	210±703	1.16±0.10
α4P451L(α4β2)β2	3.55±0.76	1.14±0.04	--	--
α4P451L(α4β2)α4	1.63±0.96	0.38±0.06	331.4±59.88	1.76±0.07
α4R487Q(α4β2)β2	2.86±0.50	1.24±0.03	--	--
α4R487Q(α4β2)α4	--	--	108.3±35.4	2.0±0.15

b

hα4 Concatamer Construct	HS EC_{50} (μM)	HS Max Response	LS EC_{50} (μM)	LS Max Response
α4R336C(α4β2)β2	0.43±0.60	0.52±0.11	--	--
α4R336C(α4β2)α4	--	--	10.47±2.17	1.13±0.05
α4P451L(α4β2)β2	0.23±0.11	0.30±0.02	--	--
α4P451L(α4β2)α4	--	--	5.8±1.06	0.92±0.04
α4R487Q(α4β2)β2	0.35±0.10	0.31±0.01	--	--
α4R487Q(α4β2)α4	--	--	13.50±2.37	1.29±0.06

Section S1:

1. Main effect of [α4 cDNA]: One-way ANOVA, [α4 cDNA]: α4: $F_{(2,11)}$=251.2, p<0.0009; α4R336C: $F_{(2,11)}$=183.4, p<0.0009; α4P451L: $F_{(2,11)}$=20.5, p<0.0009; α4R487Q: $F_{(2,11)}$=161.1, p<0.0009
2. Main effect of hα4 Variant on ½ max [α4 cDNA]: One-way ANOVA,1/2max [cDNA]: $F_{(3,15)}$=1.71, p=0.22
3. Main effect of hα4 Variant on estimated max [^{3}H]-epibatidine binding: One-way ANOVA Max Expression: $F_{(3,15)}$=5.2, p=0.016
4. Main effect of hα4 Variant on [^{3}H]-epibatidine Bmax: One-way ANOVA Bmax: $F_{(3,11)}$=29.14, p<0.0009; Dunnett's post-hoc: Bmax: control vs P451L, p=0.001
5. Main Effect of hα4 Variant on [^{3}H]-epibatidine Kd: One-way ANOVA Kd: $F_{(3,11)}$=1.25, p=0.35
6. Main effect of 24hr nicotine treatment on [^{3}H]-epibatidine binding: one-way ANOVA, [Nicotine]: α4control:$F_{(5,47)}$=7.72, p<0.0009; α4R336C: $F_{(5,47)}$=19.42, p<0.0009; α4P451L: $F_{(5,47)}$=13.52, p<0.0009; α4R487Q: $F_{(5,47)}$=4.22, p=0.003
7. Main effect of hα4 Variant on estimated max upregulation: one-way ANOVA, MaxUpregulation: $F_{(3,29)}$=0.8, p=0.5
8. Main effect of hα4 Variant on estimated nicotine EC_{50} for upregulation: one-way ANOVA NicEC_{50}: $F_{(3,29)}$=1.03, p=0.4
9. Main effect of hα4 Variant on fold change following 24hr nicotine: one-way ANOVA, Max Fold Change: $F_{(3,29)}$=8.24, p=0.001; Dunnett's post-hoc: control vs P451L: p=0.002

Section S2

1. Main effect of hα4 Variant on total membrane [^{3}H]-epibatidine binding: one-way ANOVA, $F_{(3,23)}$=12.6, p<0.0009; Dunnett's post-hoc: control vs P451L: p<0.0009
2. Main effect of hα4 Variant on plasma membrane binding: one-way ANOVA, $F_{(3,23)}$=1.82, p=0.18
3. Main effect of hα4 Variant on percent plasma membrane [^{3}H]-epibatidine binding: one-way ANOVA, $F_{(3,23)}$= 2.81, p=0.07; Dunnett's post-hoc control vs P451L: p=0.04
4. Main Effect of nicotine on total and plasma membrane [^{3}H]-epibatidine binding: Total Binding, one-way ANOVA: α4control $F_{(1,11)}$=98.30, p<0.0009; α4R336C $F_{(1,11)}$=44.50, p<0.0009; α4P451L $F_{(1,11)}$=58.50, p<0.0009; α4R487Q $F_{(1,11)}$=56.10, p<0.0009; Surface Receptor Binding, one-way ANOVA: α4control $F_{(1,11)}$=22.40, p<0.0009; α4R336C $F_{(1,11)}$=7.90, p=0.02, α4P451L $F_{(1,11)}$=154.50, p<0.0009; α4R487Q $F_{(1,11)}$=60.70, p<0.0009Main effect of hα4 Variant on fold change in [^{3}H]-epibatidine binding following 24hr nicotine: Total Binding, one-way ANOVA: $F_{(3,23)}$=17.10, p<0.0009; Dunnett's post-hoc control vs P451L: p<0.0009; Surface Receptor Binding, one-way ANOVA: $F_{(3,23)}$=9.44, p<0.0009; Dunnett's post hoc WT vs P451L: p=0.007