Supplementary Material

Alpha7 Nicotinic Acetylcholine Receptor Mediates Chronic Nicotine Inhalation-Induced Cardiopulmonary Dysfunction

Anna K. Whitehead[1†], Nicholas D. Fried[1†], Zhen Li[2,3], Kandasamy Neelamegam[1], Charlotte S. Pearson[2], Kyle B. LaPenna[2,3], Thomas E. Sharp[3,4], David J. Lefer[2,3], Eric Lazartigues[2,3,5*], Jason D. Gardner[1*] and Xinping Yue[1*]

[1]Department of Physiology,[2]Department of Pharmacology & Experimental Therapeutics, [3]Cardiovascular Center of Excellence, and [4]Department of Medicine Section of Cardiology, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA; [5]Southeast Louisiana Veterans Health Care Systems, New Orleans, LA 70119, USA

[†]These authors contributed equally to this work.

Running title: Nicotine Impairs Cardiopulmonary Function via α7-nAChR

***Corresponding authors:**

Xinping Yue, MD, PhD; 504-568-2024; xyue@lsuhsc.edu; Department of Physiology

Jason D. Gardner, PhD; 504-568-7252; jgardn@lsuhsc.edu; Department of Physiology

and Eric Lazartigues, PhD; 504-568-3210; elazar@lsuhsc.edu; Department of Pharmacology

Louisiana State University Health Sciences Center, New Orleans, LA 70112

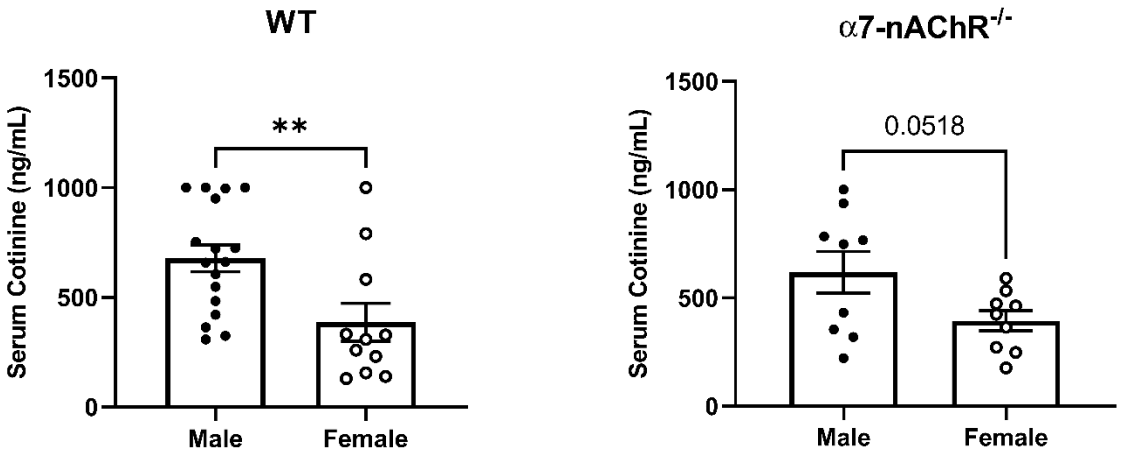


Figure S1. Serum cotinine levels in wildtype (WT) and α7-nAChR$^{-/-}$ male and female mice exposed to nicotine. $^{**}P < 0.01$.

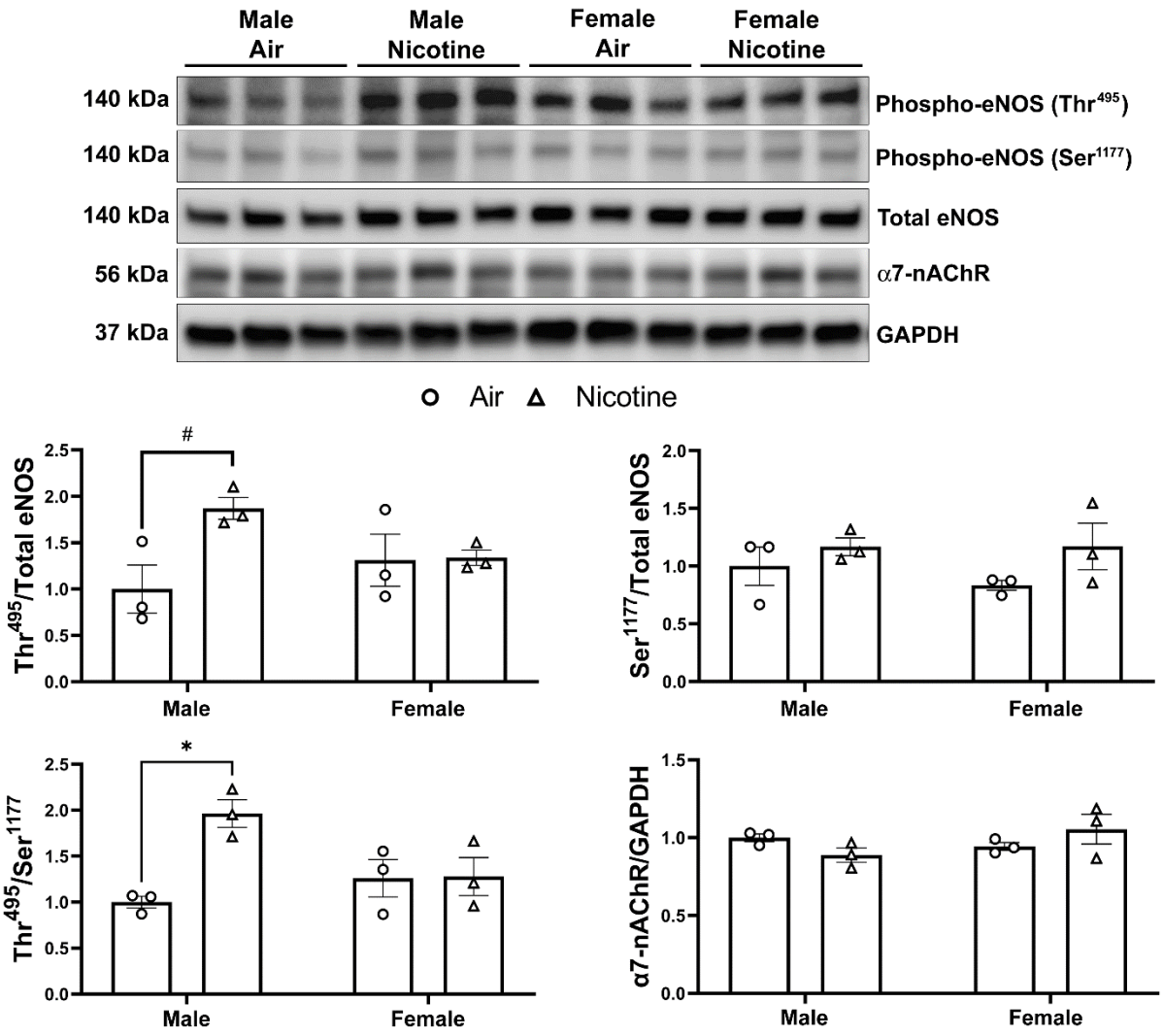


Figure S2. Comparison of the expression levels of total and phosphorylated eNOS and α7-nAChR between wildtype male and female mice. Western blot (top) analysis and quantification (bottom) of phospho-eNOS at Thr^{495}, phospho-eNOS at Ser^{1177}, total eNOS, and α7-nAChR in right ventricular samples collected from wildtype male and female mice exposed to air (control) or nicotine. $^{*}P < 0.05$, analyzed via two-way ANOVA followed by Tukey-Kramer post hoc test; $^{\#}P < 0.05$, analyzed by two-tail student t-test.

Table S1. List of genes differentially regulated by nicotine in wildtype male mice.

Gene Symbol	baseMean	log2FoldChange (Nicotine/Air)	lfcSE	stat	pvalue	padj
A530064D06Rik	5.76296126	6.004438512	1.732198	3.466369	0.000528	0.059887
RP24-492L15.6	9.80919374	4.274460383	1.153528	3.705554	0.000211	0.034104
Osm	9.9095008	3.821824764	1.067233	3.581059	0.000342	0.043745
Il6	12.5566953	3.546360484	1.042764	3.400923	0.000672	0.070456
Tnni2	9.56593576	3.45123752	0.945807	3.648989	0.000263	0.038883
Cxcl2	11.1563266	3.413310992	1.024859	3.330518	0.000867	0.08399
Nts	11.7619339	3.240390059	0.963486	3.363193	0.00077	0.076603
Slfn4	20.3558712	2.967855148	0.876093	3.387601	0.000705	0.071983
Egr2	317.523646	2.955708364	0.734759	4.022689	5.75E-05	0.015355
Ccl2	45.9930875	2.917982694	0.491438	5.937647	2.89E-09	6.28E-06
Apold1	116.25715	2.713269372	0.443162	6.122517	9.21E-10	2.34E-06
Fam167a	28.5980128	2.670813881	0.541548	4.931814	8.15E-07	0.000729
Il1b	40.6789558	2.381712448	0.685852	3.472635	0.000515	0.059393
Cyp2e1	1617.0202	2.35649886	0.6607	3.566668	0.000362	0.045832
Hdc	65.2569392	2.356310055	0.425854	5.533138	3.15E-08	4.63E-05
Atp2a1	23.8572923	2.305482378	0.678913	3.395844	0.000684	0.0708
Wnt2	24.4838303	2.284339239	0.558194	4.092376	4.27E-05	0.013598
Has2	19.0942902	2.265987376	0.693814	3.265988	0.001091	0.097407
Cish	64.9100246	2.245235242	0.548854	4.090769	4.30E-05	0.013598
Cxcr4	54.9127116	2.242303374	0.594188	3.773729	0.000161	0.029476
Cd83	84.7729648	2.205374247	0.482248	4.573108	4.81E-06	0.002809
Dnajb1	1192.66633	2.168507813	0.622074	3.485933	0.00049	0.057387
Agtr1a	21.4514253	2.163944126	0.591686	3.657251	0.000255	0.038397
Mfsd2a	30.4369303	2.154943034	0.547799	3.93382	8.36E-05	0.018703
Has1	61.4700354	2.104107112	0.339576	6.196285	5.78E-10	1.76E-06
Stil	20.2571145	2.084634977	0.575736	3.62082	0.000294	0.040246
Errfi1	2821.12901	2.053837504	0.259721	7.907851	2.62E-15	3.98E-11
Cxcl1	49.3168934	2.014029749	0.422219	4.770111	1.84E-06	0.001489
Sele	44.2154416	2.013736319	0.508109	3.963195	7.40E-05	0.01744
Adam8	47.381225	1.935010785	0.539808	3.584629	0.000338	0.043516
Prox1	49.4659636	1.832934833	0.504807	3.630965	0.000282	0.039771
Kcnk5	27.7555063	1.814825536	0.459653	3.94825	7.87E-05	0.017968
Ces1f	165.525113	1.807972495	0.485411	3.724623	0.000196	0.032343
Nfil3	333.033316	1.772005143	0.405265	4.372465	1.23E-05	0.005663
Anxa8	46.5609926	1.747417512	0.418284	4.17759	2.95E-05	0.010826
Muc16	42.8020191	1.743639759	0.471865	3.695207	0.00022	0.034104
Clca3a1	39.8907997	1.735929126	0.479271	3.62202	0.000292	0.040246
Phlda1	76.4316836	1.702338627	0.358564	4.747651	2.06E-06	0.001489

BB365896	83.2195771	1.698339056	0.451151	3.764457	0.000167	0.030227
Cd33	90.2795556	1.57400593	0.373185	4.21776	2.47E-05	0.009877
Ifi205	57.1065287	1.565576602	0.364488	4.295277	1.74E-05	0.007728
Slc27a1	888.804302	1.532311802	0.3363	4.556378	5.20E-06	0.002809
Tlr7	51.3305973	1.518790783	0.381094	3.985343	6.74E-05	0.017373
Ifi204	145.054865	1.483009491	0.330704	4.484403	7.31E-06	0.003588
Orm1	157.75427	1.471068178	0.452211	3.253058	0.001142	0.099538
Bcl3	82.4183086	1.45313997	0.356345	4.077897	4.54E-05	0.013667
Dusp4	211.872408	1.451048801	0.445418	3.257726	0.001123	0.099328
Chst1	104.663367	1.423975947	0.333866	4.265118	2.00E-05	0.008443
Tst	103.244989	1.422624316	0.373084	3.813149	0.000137	0.02688
Ms4a6b	72.8145655	1.418047656	0.371403	3.818083	0.000134	0.02688
Fam46a	1816.44078	1.408582969	0.2531	5.565315	2.62E-08	4.42E-05
Col6a5	34.1269983	1.391682554	0.428834	3.245273	0.001173	0.099718
Myh14	122.072687	1.380239868	0.355735	3.879967	0.000104	0.022694
Junb	1608.01598	1.340012175	0.36682	3.653056	0.000259	0.038647
Hmcn1	71.3860712	1.324116381	0.368511	3.593153	0.000327	0.043516
Ptgs2	663.801704	1.319221859	0.348554	3.784846	0.000154	0.028533
Ccl6	371.797185	1.311083986	0.317725	4.126472	3.68E-05	0.012679
Cilp	123.434481	1.290829656	0.337516	3.824498	0.000131	0.02688
Slc1a3	697.571205	1.28217463	0.28164	4.55253	5.30E-06	0.002809
Tnfaip6	100.265352	1.281860143	0.306602	4.180856	2.90E-05	0.010826
Rcan1	572.705447	1.281484115	0.200638	6.387034	1.69E-10	6.43E-07
Csrnp1	451.325688	1.279006562	0.320444	3.991358	6.57E-05	0.01723
Dnajb4	5305.51016	1.271424576	0.254789	4.990114	6.03E-07	0.000574
Islr2	67.7542813	1.270406951	0.358134	3.547291	0.000389	0.048136
Abra	246.760911	1.264229413	0.31415	4.024285	5.71E-05	0.015355
Serpine1	12059.8748	1.261370124	0.265634	4.748523	2.05E-06	0.001489
Sfrp4	1702.88766	1.242208158	0.242228	5.128268	2.92E-07	0.000318
Slfn2	113.831469	1.237949394	0.334802	3.697557	0.000218	0.034104
Csf2rb	81.6844797	1.237437857	0.35098	3.525664	0.000422	0.051407
Fosl2	3036.05577	1.231775769	0.167622	7.348542	2.00E-13	1.52E-09
Me1	2750.00905	1.230791182	0.328959	3.741475	0.000183	0.030922
Gpm6a	74.4527924	1.213030604	0.358836	3.380459	0.000724	0.073388
Atp1a3	97.4472947	1.210625875	0.368635	3.284074	0.001023	0.094331
AI607873	253.372834	1.210601402	0.266044	4.550386	5.35E-06	0.002809
Cebpb	813.410013	1.20331283	0.259114	4.643944	3.42E-06	0.002225
Gem	1043.85278	1.194893336	0.258077	4.629981	3.66E-06	0.002225
Plk3	111.331538	1.188637135	0.365893	3.248596	0.00116	0.099538
Nr4a3	1955.85709	1.176486329	0.313018	3.758524	0.000171	0.030588
Car5b	413.987584	1.158155803	0.339188	3.414494	0.000639	0.067976

Nrros	119.239154	1.152098787	0.354778	3.247383	0.001165	0.099538
Hmox1	161.046342	1.148083166	0.296157	3.876606	0.000106	0.022694
Siah2	140.152024	1.145709716	0.308544	3.713281	0.000205	0.033465
Mrc1	839.688537	1.13781156	0.206049	5.522037	3.35E-08	4.63E-05
Arid5a	939.923819	1.131669087	0.216409	5.229319	1.70E-07	0.000216
Slc5a6	152.523192	1.12880637	0.263059	4.291069	1.78E-05	0.007728
Fcgr3	150.032907	1.12050863	0.263259	4.256299	2.08E-05	0.008545
Bmp2	313.712387	1.119195278	0.29113	3.844318	0.000121	0.025541
St3gal6	201.643178	1.117394141	0.273439	4.086453	4.38E-05	0.013598
Ccl9	186.263536	1.113387822	0.289943	3.840029	0.000123	0.025635
Sik1	1445.45424	1.111258886	0.305726	3.634821	0.000278	0.039677
Irs2	2668.41392	1.108285173	0.26885	4.122317	3.75E-05	0.012679
Itgam	223.040416	1.089097351	0.294505	3.698061	0.000217	0.034104
Myc	150.818804	1.072474507	0.298575	3.591977	0.000328	0.043516
Mafb	440.514532	1.07039959	0.207647	5.154901	2.54E-07	0.000297
Aacs	1022.26773	1.069105538	0.268625	3.979916	6.89E-05	0.01744
Spry4	361.708468	1.062283503	0.222882	4.766116	1.88E-06	0.001489
Cadm3	175.318706	1.05929495	0.29245	3.622136	0.000292	0.040246
Lbp	218.154585	1.059247477	0.278429	3.804372	0.000142	0.027033
Klf16	147.225809	1.047564955	0.27473	3.813074	0.000137	0.02688
Bag3	2661.08016	0.995843566	0.273182	3.645345	0.000267	0.039059
Trib1	608.593366	0.995490776	0.245912	4.048155	5.16E-05	0.014817
Ccdc117	536.468254	0.994921455	0.246307	4.039363	5.36E-05	0.015098
Igfbp6	994.652238	0.987577372	0.151667	6.511466	7.44E-11	3.77E-07
Fam107b	248.565821	0.966160021	0.273993	3.526222	0.000422	0.051407
H2-Ab1	674.04376	0.959954208	0.261591	3.66968	0.000243	0.036943
Ssc5d	165.063833	0.954510341	0.235582	4.051715	5.08E-05	0.014817
Spry2	374.008774	0.951033964	0.240951	3.946999	7.91E-05	0.017968
Bhlhe40	1329.24748	0.930414932	0.165952	5.606532	2.06E-08	3.93E-05
Gpt2	252.160926	0.920878152	0.266983	3.449199	0.000562	0.062336
Gfpt2	1093.90096	0.916158229	0.219476	4.174292	2.99E-05	0.010826
C4b	1981.13885	0.914790444	0.263157	3.47621	0.000509	0.059054
Csf1r	700.87136	0.904507136	0.195309	4.63115	3.64E-06	0.002225
H2-Aa	475.417734	0.900281939	0.259551	3.468609	0.000523	0.059837
Thbs1	3753.03171	0.885902312	0.249058	3.557014	0.000375	0.047156
Ugdh	590.22487	0.88153662	0.175033	5.036412	4.74E-07	0.000481
Lyve1	428.925393	0.877108413	0.196525	4.463085	8.08E-06	0.00384
Jmjd6	304.402126	0.870374183	0.242763	3.585286	0.000337	0.043516
Klf4	1381.4757	0.854138104	0.205096	4.16457	3.12E-05	0.011035
Dcun1d3	567.460179	0.849291818	0.236448	3.591869	0.000328	0.043516
Wsb1	415.354397	0.797913741	0.245138	3.254958	0.001134	0.099538

C1qc	410.534969	0.785105819	0.2294	3.422435	0.000621	0.066958
Litaf	468.161669	0.783514951	0.191501	4.091449	4.29E-05	0.013598
Hes1	333.782893	0.779077555	0.208033	3.744976	0.00018	0.030922
F13a1	1096.48633	0.769722411	0.184024	4.182723	2.88E-05	0.010826
C1qb	317.651237	0.758872247	0.20634	3.677775	0.000235	0.036152
Lyz2	1584.0642	0.754983496	0.190579	3.961522	7.45E-05	0.01744
Tsc22d3	1100.55696	0.750742336	0.186274	4.030311	5.57E-05	0.015355
Cyth1	518.573724	0.740493865	0.22674	3.265826	0.001091	0.097407
Ctgf	34005.1436	0.739708579	0.190433	3.884348	0.000103	0.022621
Mxd1	415.521786	0.734036717	0.209378	3.505795	0.000455	0.053684
Dusp16	609.89697	0.727313596	0.216985	3.351902	0.000803	0.078767
Fndc1	1504.396	0.687140646	0.200582	3.42573	0.000613	0.066623
Arid5b	3488.2493	0.682799282	0.144096	4.738508	2.15E-06	0.001489
Pxdc1	515.944832	0.667049131	0.201046	3.317887	0.000907	0.085698
Akap12	2490.03709	0.641718374	0.186392	3.442852	0.000576	0.062995
Dusp8	758.263365	0.636571307	0.194362	3.275191	0.001056	0.096499
Rgs2	887.721363	0.635336032	0.178916	3.55104	0.000384	0.047845
Rnf2	364.699582	0.634035596	0.174319	3.637225	0.000276	0.039677
Cttnbp2nl	1540.79827	0.626496965	0.138692	4.517187	6.27E-06	0.003178
Jdp2	325.243326	0.624363267	0.187423	3.331308	0.000864	0.08399
Dlc1	4144.8743	0.622750778	0.165954	3.752549	0.000175	0.030922
Bach1	664.514551	0.607329064	0.159364	3.810952	0.000138	0.02688
Slfn5	896.680684	0.589909226	0.177491	3.323599	0.000889	0.085312
Efemp1	575.50149	0.582072466	0.168141	3.461819	0.000537	0.060013
Lima1	1427.58058	0.543149063	0.145325	3.737483	0.000186	0.031071
C3	9721.07529	0.540421026	0.159069	3.397408	0.00068	0.0708
Slc3a2	853.414207	0.526041378	0.159469	3.29871	0.000971	0.090647
Col14a1	6800.17094	0.509533008	0.156859	3.248358	0.001161	0.099538
Chd1	1751.54605	0.484613689	0.135066	3.587983	0.000333	0.043516
Zfp386	260.112351	-0.684238712	0.205932	-3.32264	0.000892	0.085312
A430105I19Rik	834.655224	-0.751476721	0.230097	-3.26591	0.001091	0.097407
Zfp626	193.46282	-0.774543099	0.204046	-3.79592	0.000147	0.027625
Jph2	4219.18174	-0.82358423	0.244457	-3.36904	0.000754	0.075989
Kcnq3	161.578406	-0.864021776	0.245791	-3.51528	0.000439	0.052286
Gm13111	306.888393	-0.890926779	0.238022	-3.74305	0.000182	0.030922
Pde4a	510.718963	-0.902443723	0.268203	-3.36478	0.000766	0.076603
Zfp810	153.251604	-0.910038086	0.246138	-3.69727	0.000218	0.034104
Ky	121.71229	-0.917030879	0.280072	-3.27426	0.001059	0.096499
Grip2	705.73336	-0.933506156	0.229026	-4.07599	4.58E-05	0.013667
9530077C05Rik	102.970663	-0.942186797	0.268057	-3.51488	0.00044	0.052286
Sel1l3	279.160274	-0.944641612	0.237767	-3.97298	7.10E-05	0.01744

Rasl10b	396.889177	-0.970467176	0.289206	-3.35562	0.000792	0.078219
Fam84a	233.108615	-0.979485968	0.282808	-3.46343	0.000533	0.060013
Zfp39	111.109767	-0.981729683	0.289681	-3.389	0.000701	0.071983
Cav3	105.028962	-0.981892852	0.300741	-3.26492	0.001095	0.097407
Mir143hg	660.703481	-0.98476378	0.258544	-3.80889	0.00014	0.02688
Fbxl22	659.882444	-0.987645267	0.275096	-3.59018	0.00033	0.043516
Bves	110.049191	-1.018373687	0.299236	-3.40324	0.000666	0.070346
Bmf	108.356416	-1.024591207	0.299841	-3.41712	0.000633	0.067798
Slc8a3	92.3263819	-1.139692886	0.32414	-3.51606	0.000438	0.052286
Fam212b	297.134851	-1.148406807	0.289763	-3.96326	7.39E-05	0.01744
Zfp41	57.4621181	-1.193084369	0.359877	-3.31526	0.000916	0.085975
4930522L14Rik	56.5169899	-1.215037279	0.334354	-3.63398	0.000279	0.039677
Zfp273	63.0959205	-1.314562621	0.350778	-3.74756	0.000179	0.030922
Smim5	127.090327	-1.535022113	0.445238	-3.44764	0.000566	0.062336
Nkx3-1	34.4951129	-1.659295212	0.418869	-3.96137	7.45E-05	0.01744
Rab17	20.5341665	-1.787771308	0.550073	-3.25006	0.001154	0.099538
Ifitm10	22.0248459	-1.807132025	0.549337	-3.28966	0.001003	0.093042
Gm10451	10.715006	-2.584308547	0.778404	-3.32001	0.0009	0.085582

Table S2. List of genes differentially regulated by nicotine in α7-nAChR knockout male mice.

Gene Symbol	baseMean	log2FoldChange (Nicotine/Air)	lfcSE	stat	pvalue	padj
Gpx3	4154.5886	1.37678613	0.309004	4.455558	8.37E-06	0.025371
Gmpr	248.99189	1.212432919	0.272859	4.443447	8.85E-06	0.025371
Zbtb16	1832.0117	0.815583834	0.155112	5.25804	1.46E-07	0.001252
Adamts1	3603.1786	0.62740568	0.143309	4.377984	1.20E-05	0.029424
Gm13889	638.55829	-0.751783997	0.165682	-4.5375	5.69E-06	0.025371
Adprhl1	29.913821	-2.570242834	0.572401	-4.49028	7.11E-06	0.025371
Jchain	53.829692	-2.616767358	0.385938	-6.78028	1.20E-11	2.06E-07

Table S3. RNA-seq data of up- and down-regulated pathways by nicotine in wildtype male mice.

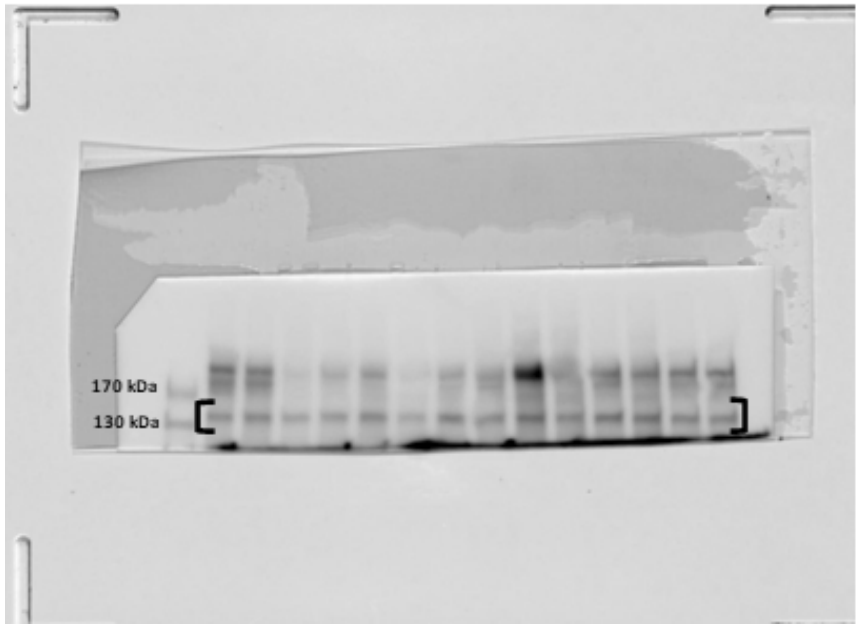
Upregulated Pathways by Nicotine	**Differentially Regulated Genes**	**p-value**	**Z-Score**
Acute phase response signaling	C3, C4b, Cebpb, Hmox1, Il6, Il1b, Lbp, Osm, Serpine1	4.91E-06	2.646
Complement system	C3, C1qb, C1qc, C4b, Itgam	5.71E-06	2.000
Role of pattern recognition receptors in recognition of bacteria and viruses	C3, C1qb, C1qc, Il6, Il1b, Osm, Tlr7	1.08E-04	2.449
TREM1 signaling	Cd83, Cxcl2, Il6, Il1b, Tlr7	1.83E-04	2.236
Neuroinflammation signaling pathway	Csf1r, H2-Aa, H2-Ab1, Hmox1, Il6, Il1b, Ptgs2, Slc1a3, Tlr7	2.65E-04	2.121
Cardiac hypertrophy signaling (enhanced)	Agtr1a, Atp2a1, Csf2rb, Il6, Il1b, Myc, Osm, Pde4a, Ptgs2, Rcan1, Wnt2	6.54E-04	2.714
Dendritic cell maturation	Cd83, Fcgr3, H2-Aa, H2-Ab1, Il6, Il1b	1.85E-03	2.449
IL6 signaling	Cebpb, Il6, Il1b, Lbp, Tnfaip6	1.88E-03	2.000
Coronavirus pathogenesis pathway	Agtr1a, Il6, Il1b, Ptgs2, Serpine1	4.12E-03	2.236
HMGB1 signaling	Il6, Il1b, Osm, Sele, Serpine1	6.14E-03	2.000
Th1 Pathway	H2-Aa, H2-Ab1, Il6, Nfil3	1.05E-02	2.000
ILK signaling	Bmp2, Irs2, Myc, Myh14, Ptgs2	1.10E-02	2.000
Systemic lupus erythematosus in B cell signaling pathway	Fcgr3, Il1b, Il6, Myc, Osm, Tlr7	1.29E-02	1.633
STAT3 pathway	Cish, Csf2rb, Il1b, Myc	1.51E-02	1.000
Hepatic fibrosis signaling pathway	Agtr1a, Cebpb, Il1b, Irs2, Myc, Serpine1, Wnt2	1.51E-02	2.646
Osteoarthritis pathway	Bmp2, Cebpb, Hes1, Il1b, Ptgs2	1.66E-02	2.236
Colorectal cancer metastasis signaling	Il6, Myc, Ptgs2, Tlr7, Wnt2	3.31E-02	2.236
Role of NFAT in regulation of the immune response	Fcgr3, H2-Aa, H2-Ab1, Rcan1	3.89E-02	1.000
Downregulated Pathways by Nicotine	**Differentially Regulated Genes**	**p-value**	**Z-Score**
LXR/RXR activation	C3, C4b, Il6, Il1b, Lbp, Lyz2, Ptgs2	2.32E-05	-1.134
cAMP-mediated signaling	Agtr1a, Akap12, Dusp4, Pde4a, Rgs2	2.23E-02	-1.000
Protein kinase A signaling	Akap12, Dusp4, Dusp8, Dusp16, Pde4a, Ptgs2, Tnni2	2.24E-02	-1.342

Supplementary Material Original Western Blots

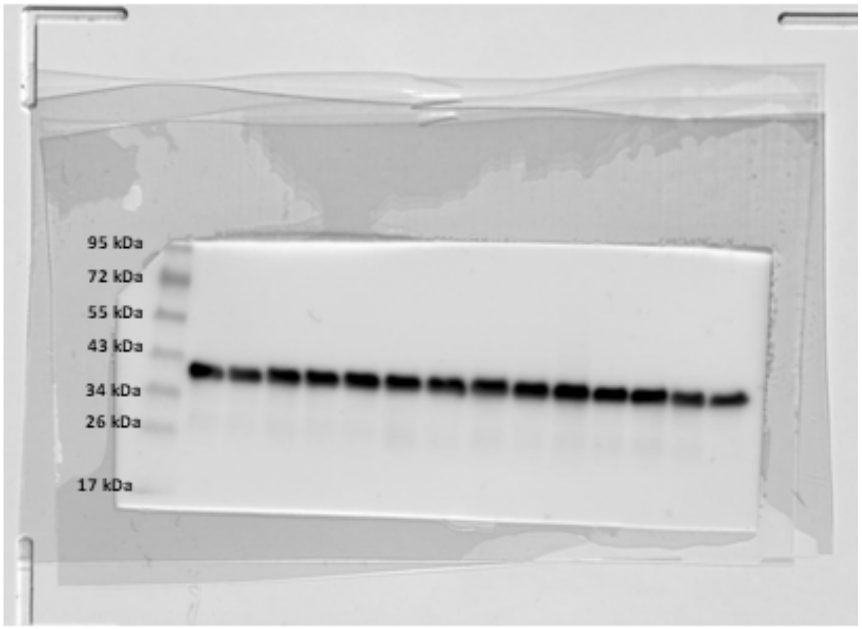
Methods: Equal amounts of proteins were run on 4-15% Tris-Glycine (TGX) gels (Bio-Rad # 4561086) with EZ-Run Pre-Stained Protein Ladder (Fisher BioReagents #BP3603-500), and positive bands were detected using ECL Prime Western Blotting Detection Reagent (Amersham #RPN2232). Some blots were cut into two pieces after transfer to probe for targets of different molecular weights.

[ ] Bands shown in Figure 5A (wildtype males: 1st 7 bands, air; 2nd 7 bands, nicotine)

Wildtype
Phospho-eNOS Ser1177

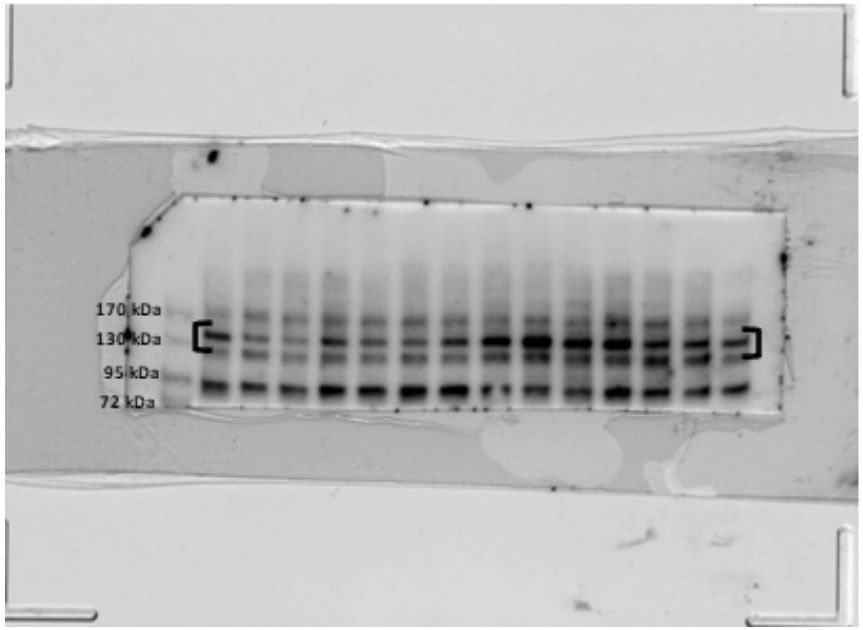


Wildtype GAPDH
For quantification and in figure

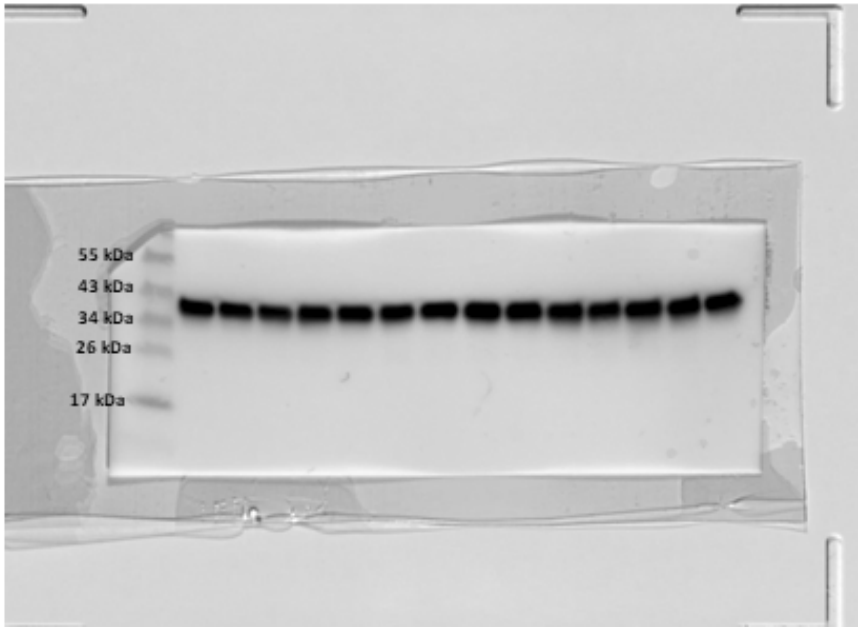


[ ] **Bands shown in Figure 5A (wildtype males: 1st 7 bands, air; 2nd 7 bands, nicotine)**

Wildtype
Phospho-eNOS Thr^{495}

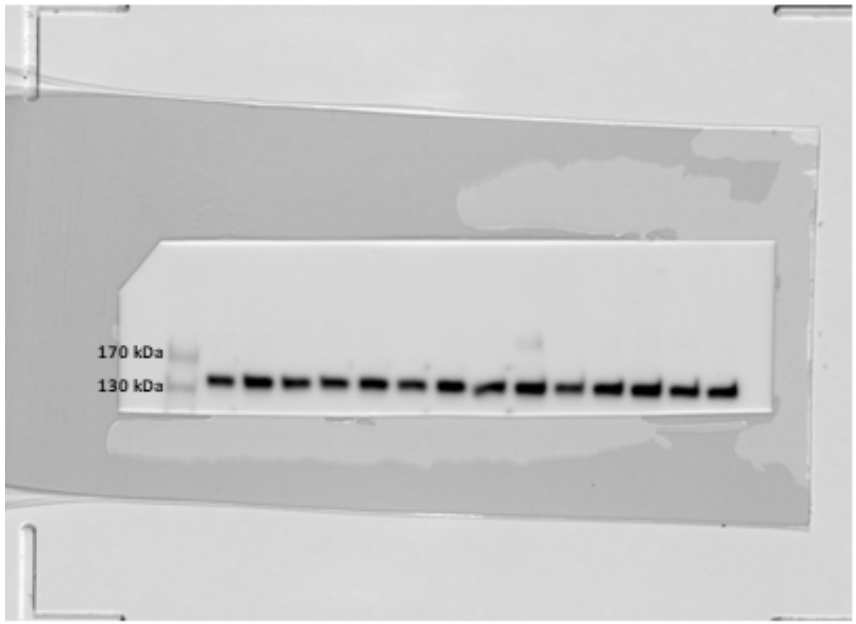


Wildtype GAPDH
Used for quantification

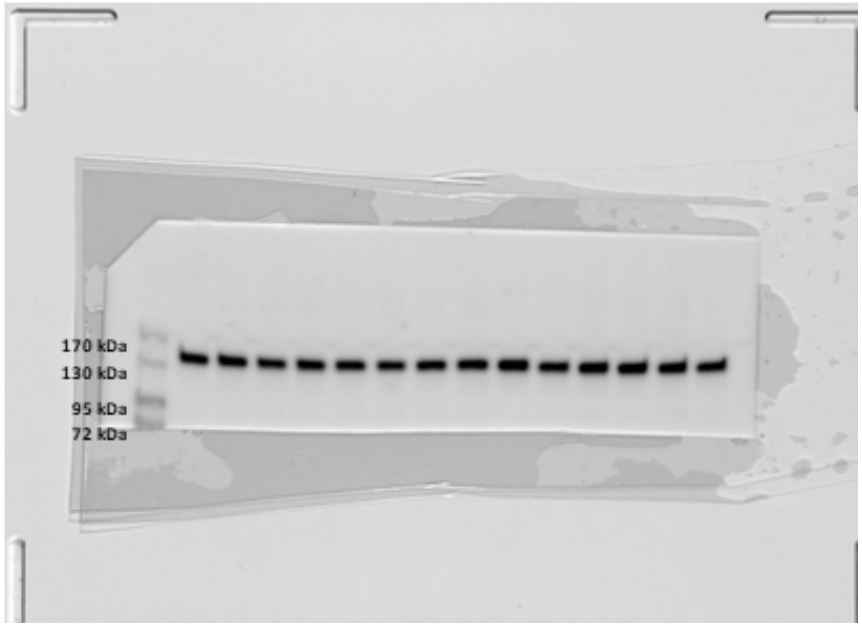


[ ] **Bands shown in Figure 5A (wildtype males: 1st 7 bands, air; 2nd 7 bands, nicotine)**

Wildtype
Total eNOS on stripped blot from
Phospho-eNOS Ser^{1177}
Used in quantification

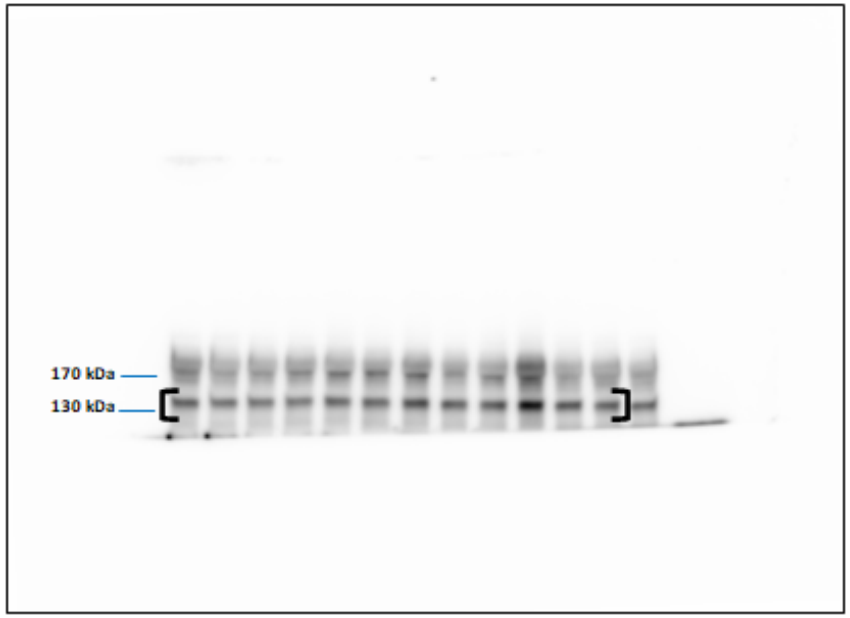


Wildtype
Total eNOS on stripped blot from
Phospho-eNOS Thr^{495}
Used in quantification and figure

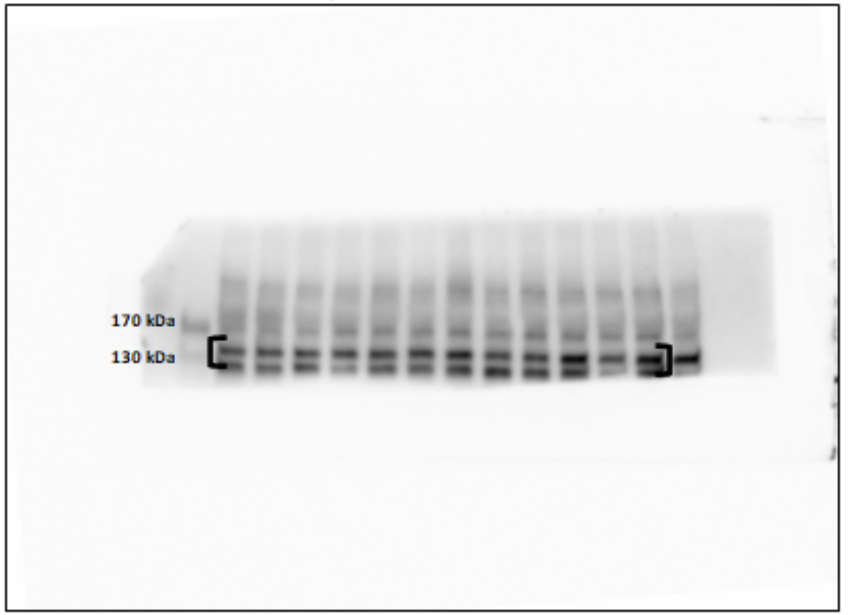


[ ] **Bands shown in Figure 5B (α7-nAChR$^{-/-}$ males: 1st 6 bands, air; 2nd 6 bands, nicotine)**

α7-nAChR$^{-/-}$
Phospho-eNOS Ser^{1177}



α7-nAChR$^{-/-}$
Phospho-eNOS Thr^{495}



[ ] **Bands shown in Figure 5B ((α7-nAChR$^{-/-}$ males: 1st 6 bands, air; 2nd 6 bands, nicotine)**

α7-nAChR$^{-/-}$ Total eNOS
Used for quantification of both Phospho-eNOS Thr^{495} and Ser^{1177} and in figure

α7-nAChR$^{-/-}$ GAPDH
Used for quantification of both Phospho-eNOS Thr^{495} and Ser^{1177} and used in figure