

Additional File 8

Alignment of characteristic domains of putative regulatory subunit proteins of *L. gigantea* (p47phox-, p67phox-, p22phox-like proteins), *N. vectensis* (p47phox-, p22phox-like proteins), and *M. brevicollis* (p47phox-, p22phox-like proteins). The following naming was used to describe species of the sequences: Lg (*L. gigantea*), Nv (*N. vectensis*), Mb (*M. brevicollis*). Human (Hs, *H. sapiens*) and sea urchin (Sp, *S. purpuratus*) sequences were used as references.

Alignment of PX, bis-SH3 domains, and predicted AIR-like region of p47phox-like sequences. *Gray boxes* indicate the predicted domains corresponding to the PX (residues 3-132) and bis-SH3 (residues 151-285) domains of human p47phox. *Yellow boxes* indicate the predicted motif corresponding to the crucial residues of the AIR (residues 299-305 of human p47phox) containing a putative phosphorylation site by PKC (*bold letter*) consistent with a PKC substrate consensus motif (R/K-x-S/T-x-R/K). Polybasic region of *N. vectensis* is indicated by *underline*.

	PX-domain	
Hs-p47phox	-----MGDTFIRHIALLGFEKRFVPSQHYVYMFLVKWQDLSEKV	39
Nv-p47phox	-----MTKRTIKDVKIVDVQKRKIPSKHYVYVSVTWSDGSVNV	39
Sp-p47phox	-----MGKRTVVNANVTDIEKRREPTKHVYVYI IHVTWSDGSVNV	39
Lg-p47phox	MFLIYLSIRDDTYITFSIQTYIKFLSVFDSHTITFYIRRLCYIFQVYVIFVTWSDSGTYY	60
Mb-p47phox	-----MAKFVKNVTVIDYQRRRAPSKHYVYVITVVWSDGSEVT	38
Hs-p47phox	VYRRFTEIYEFHKTLEKEMFPIEAGAINPENRIIPHLPAKWFDFG---QRAAENRQGTLTE	96
Nv-p47phox	VYRRYSKFFDLQTKLLEEFPEDEGGVKDPSARVLPFLPGKILFGRSHVRDVAVKRKEPIQE	99
Sp-p47phox	VYRRYSTFFDFQNKLLSKFPPEEAGANNPSSRCIPFLPGKILFGRSHIREVALKRLSPIDE	99
Lg-p47phox	VYRRYSRFFDFQSALLDKFPIEGGSIDPESRIIPFLPGKIIFGRSHIRDVAVKRLGPIND	120
Mb-p47phox	IYRRYSQLFEFHTSLLDRLFPEAAGATG--EERIIPFLPGKILFGRSHTHKVAQSRAPIDE	97
Hs-p47phox	YCGTLMSLPTKISRCPHLLDFFKVRPDDLKLPDNTQTKKPETYLMPKDGKSTATDITG--	154
Nv-p47phox	YCSKLVLPKISQSGLVLFKFFPKPEDIELPLEKK---KKKKKTADV--ISDPV-----	149
Sp-p47phox	YCTALVKLPGKISDSKEVINFFTPEDVSPSPDGGESTRGRADIGNISEPI-----	153
Lg-p47phox	YCKAVIALPPKISQCEEVLDFFEVETDDLDPKAEKKKKDEGAKKAENISDPK-----	174
Mb-p47phox	YLVKLVISLPAELSRCDLALFELFEATNTDIAPPSEQERERRPTGIFKKLSKSDSGKEERE	157

bis-SH3 domain	
Hs-p47 <i>phox</i>	-----PIILQTYRAIANYEKTSGSEMALSTGDDVVEVVEKSESGWWFCQMKAKRGWIPASF 209
Nv-p47 <i>phox</i>	-----SLEQYVAIADYQKQNRNEITMVAGDIVEIDKNENGGWVFNLDDEEQGWVPAAY 202
Sp-p47 <i>phox</i>	-----QAEQYIVVADYKQKQKNEVELTAGDLVEVFEKNDNGWVFTVHDQHGWAPGTF 206
Lg-p47 <i>phox</i>	-----TLTQYRVTCDYEKQDRGEIDLEAGMIVEVAEKSETGWWFVNSDDAQGWVPSTY 227
Mb-p47 <i>phox</i>	ISVGDVMVLDQYRAVADYTKQDRKELSFKTGDI FEVVEKNDNGWVFNDSAS----- 210
Hs-p47 <i>phox</i>	LEPLDSPDETEDP--EPNYAGEPYVAIKAYTAVEGDEVSLLEGEAVEVIHKLLDGGWVIR 267
Nv-p47 <i>phox</i>	LESVDGHSDDAAPIEGPTVEVGQYITTTSHKAELDDEITFETGVIVSVIQKNFDGWWLIR 262
Sp-p47 <i>phox</i>	LQNPDGQEEDEETLIPGNDE-SYITNNAYQGQAEDEISFETGVVTVIQKSLDGGWVKVS 265
Lg-p47 <i>phox</i>	LEPADGSTTDNVVLAQPGQEEKFICIEMFQSNNGNDEISLEKGA VVEVLEKNLSGWWLVR 287
Mb-p47 <i>phox</i>	-----QDEKYITTAAYAASSDDEIGYEKGVVVRVLEKLLDGGWVQVE 251
Hs-p47 <i>phox</i>	KDDVTGYFPSMYLQK----- 282
Nv-p47 <i>phox</i>	YQKKEGWAPAMYL RKPDP-PS---QLHAAQAVG-----GLPSKDRVG-AVTAPAAVRPG 310
Sp-p47 <i>phox</i>	YQGKQGWAPATFLQIYKGPSGVT PKHPTQSIGNVMLLKSGSDSKPKPGPGSGSRPQPP 325
Lg-p47 <i>phox</i>	HQKKEGFAPATYLNKTEDQFAV-SLAKRKS LDVEIITNLSDISNIMKGDSPRNSLASTET 346
Mb-p47 <i>phox</i>	YQKGVGWTGPTFLKRIENS NAG-----PTTLKSTPASST 284
predicted AIR-like region	
Hs-p47 <i>phox</i>	-----SGQDVSQAQRQIKRGA PPRRSSIR NVHSIHQRSRKRLSQDAYRRNSV----- 329
Nv-p47 <i>phox</i>	VNPVTDRCCKTINFKAETETVVT RKRKAH SVNVYGGKIEAMPALKSP----- 357
Sp-p47 <i>phox</i>	VQPRDEPGLYSNYDAE--VKPT PPRRATVK KSVRRGGVVRQTKPKLKK----- 373
Lg-p47 <i>phox</i>	PKPVTEMYSKVKSRSRLERGGNIK PPPRQSMR MVSLSF TSPSPSHK----- 390
Mb-p47 <i>phox</i>	AATNGGVVPPAKPPAPQLRNEKE PPPRRESIR RPVSIHSGPFAEIERAR----- 334

Alignment of TRP and AD-like regions of p67*phox*-like sequences

Gray and yellow boxes indicate predicted motifs corresponding to the tetratricopeptide repeat (TPR, residues 6-153) and activation domain (AD, 196-210 residues) of human p67*phox*. Red letters indicate the amino acid residues corresponding to the two residues (D67 and R102 of human p67*phox*) crucial for binding to Rac.

TPR	
Lg-p67 <i>phox</i>	MSLKQNIILWAEAVKLYEDGDLIECLEQLNKVN-VSAKIRYNGGIVNSRLGNSDKAIEYL 59
Hs-p67 <i>phox</i>	MSLVEAISLWNEGVLAAADKKDKWKGALDAFSAVQDPSRRCFNIGCMYTI LKNMTEAEKAF 60
Lg-p67 <i>phox</i>	SQALQLDSFMSVAYFQRGLLFFDLHRYNEAEKDLED SLINLRGSMVIDYKQLGLYTLHL 119
Hs-p67 <i>phox</i>	TRSINRDKHLAVAYFQRGMLYYQTEKYDLAIKDLKEALIQLRGNQLIDYKILGLQFKLFA 120
Lg-p67 <i>phox</i>	AQVLYNLAVIKKFKG---DVNGFELYSTKFNPNFNTL FSSVNTKLFNTVNV--LRPLY 174
Hs-p67 <i>phox</i>	CEVLYNIAFMYAKKEEWKKAEEQLALATSMKSEPRHSKIDKAMECVWKQLYEPVVIPVG 180

	predicted AD	
Lg-p67 <i>phox</i>	NIFCPPKGYVSNLLK KDFLGKSEVVS VEDND-----	206
Hs-p67 <i>phox</i>	KLFRPNERQVAQLAK KDYL GKATVVASVVDQD-----	212

Alignment of p22*phox*-like sequence

Gray boxes indicate the amino acid sequences corresponding to the polybasic region (basic, residues 55-61 of human p22*phox*), predicted 1st and 2nd transmembrane segments (TM1-TM2, residues 91-120 of human p22*phox*). Proline-rich region (PRR, residues 151-164 of human p22*phox*) are indicated by yellow boxes.

		basic
Lg-p22 <i>phox</i>	MGKIEWAMWANEQAIASSAVVLLGGIIAVAGQFPNWPFGAYGIVAGILVALLEYPRSKRQ	60
Nv-p22 <i>phox</i>	MGHTIEWAMWANEQGVITSFVLALGGTLGVAGMFKKYEFGAYSIALGVLVFLIEYPRGKRK	60
Hs-p22 <i>phox</i>	MGQIEWAMWANEQALASGLILITGGIVATAGRFTQWYFGAYSIVAGVFVCLLEYPRGKRK	60
Mb-p22 <i>phox</i>	-----GVLVLFVEWPRSARL	15
	TM1-TM2	
Lg-p22 <i>phox</i>	NGK-CVERK FQRPLSRVVQCCGLITRNYFVRFV FYLLLVTPCCFILPTLLGGMCFEFTSI	119
Nv-p22 <i>phox</i>	QGKKTQERSYQRCLTRLVSLGIVGRNYYIRFV FYLLS CIPCCFFLPTILGGFSLFFTSI	120
Hs-p22 <i>phox</i>	KGS-TMERWGQKYMTAVVKLFGPFTRNYYVRAVLHLLSV PAGFL LATILGTACLAIASG	119
Mb-p22 <i>phox</i>	KGS-TIPRLYQYSIANIVDKLGPVARNYFARA IFWLGAS IPCFFVFPALAGALTLAVGAL	74
	PRR	
Lg-p22 <i>phox</i>	IYFVA AIK GEWKPVLEDYKP-SQGPTVIEA PRHPPPRHPP THNPN-----	164
Nv-p22 <i>phox</i>	IYLCAALSGEKWQPCLDKADRTPGINPSHH PTVPPPRN PRSQSLDNLGV-----	170
Hs-p22 <i>phox</i>	IYLLAAVRGEQWTPIEPKRER PQIGGTIKQPPSNPP PRPPAEARKKPSEEEAAVAAGGP	179
Mb-p22 <i>phox</i>	VYFLAA AFK GEVWVKLE PQKER ---ARGKV YEAP TRAPPR-----	110
Lg-p22 <i>phox</i>	-RASEV-----	169
Nv-p22 <i>phox</i>	-SSSSVNRV-----	178
Hs-p22 <i>phox</i>	PGGPQVNP IPVT DEVV	195
Mb-p22 <i>phox</i>	-----	