ALS TYPE	LOCI	GENE
ALS1	21q22	SOD1
ALS2	2q33	Alsin2
ALS3	18q21	?
ALS4	9q34	SETX
ALS5	15q15-21	?
ALS6	16q12	FUS
ALS7	20p13	?
ALS8	20q13	VAPB
ALS9	14Q11.2	Angiogenin
ALS10	1p36.2	TARDBP
ALS11	6q21	FIG4
ALS12	10p15-p14	OPTN

TABLE 1: A list of Genetic Loci that are causative factors in ALS

TABLE 2: List of Suppressors of VAPB

ANNOTATION	GO BIOLOGICAL PROCESS	NAME	
CG3231	Unknown	something that sticks	
		like glue	
CG5014	neurotransmitter secretion neuromuscular junction	Vap-33-1	
	development		
CG5325	nervous system development	-	
CG17327	Translation	-	
CG30043	Proteolysis	-	
CG6048	Proteolysis	-	
CG3476	mitochondrial transport	-	
CG4486	Unknown	Cytochrome P450-9b2	
CG10207	phosphate transport	Na[+]-dependent	
		inorganic phosphate	
		cotransporter	
CG12822	Unknown	-	
CG5847	cell-matrix adhesion actin filament organization	Zye	
CG30060	Unknown	-	
CG32437	Unknown	-	
CG14032	hormone metabolic process ,oxidation -reduction	Cyp4ac1	
CG1539	cytoskeleton organization	Tropomodulin	
CG4913	transcription initiation from RNA polymerase II	ENL/AF9-related	
	promoter regulation of transcription, DNA-		
	dependent		
CG8465	Unknown	lethal (1) G0222	
CG9543	retrograde vesicle-mediated transport, Golgi to ER	epsilonCOP	
CG18110	sodium ion transport B66	-	
CG11560	Unknown	-	
CG6345	regulation of cyclin-dependent protein kinase	-	
	activity		
CG33090	bile acid metabolic process	-	
CG6950	Unknown	-	
CG7314	transmembrane transport	Bmcp	
CG5953	Unknown	-	
CG32685	Unknown	-	
CG32703	Proteinphosphorylation	-	

CG17760 G-protein coupled receptor protein signaling			
pathway signal transduction			
CG7217 cellredox homeostasis	Peroxiredoxin 5		
CG4646 Unknown	-		
CG17985 cell wall macromolecule catabolic process	-		
CG6342 regulation of translational initiation by iron	Iron regulatory protein 1B		
CG4627 Unknown	-		
CG7254 glycogen catabolic process	Glycogen phosphorylase		
CG17982 Unknown	-		
CG7899 Unknown	Acid phosphatase 1		
CG3975 DNA replication B108	-		
CG4068 Unknown	-		
CG4012 actin polymerization or depolymerization	genghis khan		
CG1409 Unknown	-		
CG5925 lipid metabolic process	desat2		
CG8465 Unknown	lethal (1) G0222		
CG6337 Proteolysis	-		
CG10327 neuromuscular junction development	ТВРН		
CG16935 fatty acid metabolic process	-		
CG3024 chaperone mediated protein folding requiring cofactor	torp4a		
CG7777 transmembrane transport	-		
CG7756 protein folding	Heat shock protein cognate 2		
CG31147 G-protein coupled receptor protein signaling pathway	methuselah-like 11		
CG3309 Unknown	-		
CG10908 ER-associated protein catabolic process	Derlin-1		

TABLE 3: List of Enhancers of VAP

ANNOTATION	GO BIOLOGICAL PROCESS	NAME
CG9172	mitochondrial electron transport, NADH to	-
	ubiquinone	
CG1059	protein import into nucleus	Karyopherin beta 3
CG5092	response to nutrien	Target of rapamycin
	positive regulation of cell size	
	autophagyendocytic recycling	
CG6238	actin cytoskeleton organization	Slingshot
	mushroom body development	
CG6341	translational elongation	Elongation factor 1
		beta
CG7843	response to arsenic	Ars2
	nuclear mRNA splicing, via spliceosome	
CG15160	Unknown	-
CG9638	axon target recognition	Ada2b
	chromatin remodeling	
CG5733	SMAD protein nuclear translocation	Nucleoporin 75
	NLSunknownbearing substrate import into nucleus	
CG6220	Unknown	-
CG7776	chromatin organization	Enhancer of
		Polycomb
CG12743	oogenesisPunknownbody organization	ovarian tumor
CG3884	Unknown	-
CG14222	metabolic process	-
CG8771	Unknown	-
CG5181	Unknown	-
CG6502	chromatin silencing, axon guidance	Enhancer of zeste
CG4715	Oogenesis	Iris
•		

ANNOTATION	GO BIOLOGICAL PROCESS	NAME
CG7306	chitin metabolic process	obstructor-F
CG14435	Unknown	*
CG8571	Unknown	smallminded
CG9107	Unknown	*
CG9391	Dephosphorylation	*
CG8863	protein folding	DnaJ-like-2
CG12359	SUMOylation	Ulp1
CG13387	protein export from nucleus ,centiole replication	embargoed

TABLE 4: List of Weak Enhancers of VAPB

GENE	FUNCTION	MODIFICATION OF NDs	ENHANCER/ SUPPRESOR	REFERENCE
Karyopherin beta-3	Nuclear export/import	Htt	Enhancer	Zhang et. al. (2010)
Target of Rapamycin	Phosphatase/kinase	Htt	Enhancer	Ravikumar et. al. (2004
Aa2b *	Chromatin remodelling	Htt	Enhancer	Zhang et. al. (2010)
Ars2	mRNA binding	Htt	Enhancer	Zhang et. al. (2010)
TDP-43/ TBPH	mRNA binding	ALS	Suppressor	Elden et. al. (2010)
CG6950	Kynurenine- OxologlutarateTransaminase	PD	Suppressor	Hartai et. al. (2005)
CG9172	NADH dehydrogenase I (ubiquinone) Fe-s	Htt	Enhancer	Kaltenbach et. al. (2007)
Ulp1	SUMOylation	Htt, ALS1	Weak Enhancer	Steffan et. al. (2004), Zhang et. al. (2010)

Table 5: Modifiers that were also identified in other Neurodegenerative diseases.

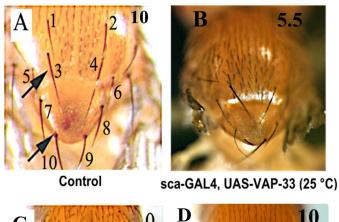
125

Figure1A: Analysis of VAPB primary structure; VAPB protein has three domain: N terminal cytosolic MSP domain(125 amino acid) having evolutionary conserved 16 amino acid known to interact with FFAT motif of cellular proteins and mutation in this domain at 56 position(P56S)is known to cause mis-folding of the protein and thus alters its ability to interact with other cellular proteins , variable coil coiled domain(187-231) known to interact with vesicle associated SNARE proteins; trans membrane domain(TMD)(249-269), having variability in proteins from other animals , the G*****G sequence in it is known for dimerization .

Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved

) 20	30	40	50
vpr_1_C_elegans MSEK HSLL	QVTPNRELVF	TGPFSDVVTS		P V C F K V K T T A
VAPB_H_sapiens MAKVEQVL	S L E P Q H E L <mark>K</mark> F	R G P F T D V V T T	N L K L G N P T D R	N V C F K V K T T A
VAPB_A_californ MASHEQAL	ILEPAGELRF		D L K <mark>L S N</mark> P T D R	
VAPB_D_melanoga <mark>M S K S L F D L P L</mark>	TIEPEHELRF	V G P F T R P V V T		
Scs2p_S_cerevis MSAV	EISP-DVLVY			TIAFKVKTTA
Consistency * 7 3 1 0 0 3 2 4 8	386*236*48	47*7854775	26594*4754	385**9****
61) 70	80	90	100
vpr_1_C_elegans PKQYCVRPNS	GLLKSGDSKO			KFMVOS CVAP
VAPB_H_sapiens P R R Y C V R P N S	GI I DAGASIN	VS VML OPFDY		KF MV OS MF A P
VAPB_A_californ PKRYCVRPNS	GI LEPKTSIA		DP NEKNKH	KFMVQSMYAP
VAPB_D_melanoga <mark>P K R Y C V R P N I</mark>	GKIIPFRSTQ	VEICLQPFVY	DQQEKNKH	KFMVQSVLAP
Scs2p_S_cerevis PKFYCVRPNA	AV VAPGETIQ	VQVI FLGLTE	E P A A D F K C R D	KFLVITLPSP
Consistency * 8 5 * * * * * 6	7 5 8 3 5 4 3 8 4 5	9495766634	5700557387	* * 8 * 6 8 5 3 8 *
	10 12	0 120	140	150
	10 12 VWKI I DP	AELTYSKLMV		
	VWKEAKP	EDLMDSKLRC		
	LWKDAPP			
VAPB_D_melanoga MDADLSDLNK				AENTSGGGAV
Scs2p_S_cerevis YDLNGKAVAD	VWSDLEAEFK	QQAISKKIKV	KYLISPDVHP	A Q N Q N I Q E N K
Consistency 2 6 0 3 4 2 5 3	7 * 7 5 5 4 7 0 0 0	657645*965	5844534334	5344433343
vpr_1_C_elegans N E D S F VAPB_H_sapiens S T T A S	A <u>A S</u>	SGQAQELG <mark>S</mark> S		VNS LRKS LKS SSL DDTEVKK
VAPB_H_sapiens STTAS VAPB_A_californ DAGAHFS		EDPTVASRKT		VGS AGED VKK
VAPB D melanoga G G G T G A A G G G				LLETSESLDL
Scs2p_S_cerevis E T V E P			SEPKE VPAVV	NEKEVPAEPE
	000000131	1101112164		3 2 4 3 2 4 5 5 5 4
		0		
vpr_1_C_elegans TVDEKEELQK	K <mark>V HGLEQE</mark> IE	VMLK <mark>K</mark> - NRKL	QQSHSD	<mark>G A L V</mark>
vpr_1_C_elegans TVDEKEELQK VAPB_H_sapiens VMEECKRLQG	K <mark>V HGL</mark> EQ <mark>E</mark> IE E <mark>V QRL</mark> REENK	VMLKK - NRKL QFKEE <mark>D</mark> GLRM	<mark>Q Q S H S D</mark> R K T V Q S <mark>N</mark> <mark>S</mark>	PISALAPTGK
vpr_1_C_elegans T V E E E L Q VAPB_H_sapiens V ME E C K R L Q VAPB_A_californ L Q H L K K A Q	K <mark>V</mark> HGLEQEIE EVQRLREENK EITSLKG <mark>E</mark> NS	VMLK <mark>K</mark> - NRKL QFKEEDGLRM QLKDE - GIRL	<mark>Q Q S H S D</mark> R K T V Q S N <mark>S R K V A M T D <mark>T V</mark> S</mark>	PISALAPTGK PTPLNPSPAP
vpr_1_C_elegans T V E E E L Q VAPB_H_sapiens V ME E C K R Q G VAPB_A_californ L Q H E L K A Q S VAPB_D_melanoga L S G E I K A L R	KVHGLEQEIE EVQRLREENK EITSLKGENS CNIELRENL	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF	Q Q S H S D R K T V Q S N S R K V A M T D T V S R S S P A V K Q	••••••••••••••••••••••••••••••••••••••
vpr_1_C_elegansTVDEKELQRVAPB_H_sapiensVMMECKRLQGVAPB_A_californLQHELKAQSVAPB_D_melanogaLSGEIKALREScs2p_S_cerevisTQPVQVKK	KVHGLEQEIE EVQRLREENK EITSLKGENS CNIELRENL	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	PISALAPTGK PTPLNPSPAP
vpr_1_C_elegansTVDEKELQRVAPB_H_sapiensVMMECKRLQGVAPB_A_californLQHELKAQSVAPB_D_melanogaLSGEIKALREScs2p_S_cerevisTQPVQVKK	KV HGLEQEIE EV QRLREENK EI TSLKGENS CNIELRRENL EV PPVVQKTV	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T
vpr_1_C_elegansTVDEKELQRVAPB_H_sapiensVMECKRLQGVAPB_A_californLQHELKAQSVAPB_D_melanogaLSGEIKALREScs2p_S_cerevisTQPVQVKKEConsistency5427373564	K V H G L E Q E I E E V Q R L R E N K E N K E I T S L K G N S C N I E L R R N L E V P P V Q K T V Q K T V 4 6 2 3 8 4 4 4 3 50	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN 3455702464	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T
vpr_1_C_elegansTVDEKELQRVAPB_H_sapiensVM ECKRLQGCKAQGVAPB_A_californLQHELKKAQSGEIKALRESSSGEIKALRESSSGIKALRESSSGALRCNNN <td< td=""><td>K V H G L E Q E I E E V Q R L R E N K E I T S L K G N S C N I E L R R N L E V P P V Q K T V 4 6 2 3 8 4 4 8 4 3 3 50. .</td><td>VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN 3455702464</td><td>Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q</td><td>G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T</td></td<>	K V H G L E Q E I E E V Q R L R E N K E I T S L K G N S C N I E L R R N L E V P P V Q K T V 4 6 2 3 8 4 4 8 4 3 3 50. .	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN 3455702464	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T
vpr_1_C_elegansTVDEKELQRVAPB_H_sapiensVM ECKRQQ<	K V H G L E Q E I E E V Q R L R E E N K E I T S L K G E N S C N I E L R R E N L E V P P V Q K T V 4 6 2 3 8 4 4 8 4 3 3 50. 1 A V A L L I G A L V V L F F I V G . . .	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN 3455702464 0	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T
vpr_1_C_elegansTVDEKELQRVAPB_H_sapiensVM ECKRLQGALQGALRCSCLRLSGEIKALRESSCSTQPVQVKKECSSCSTQPVQVKKECSSSGAYTLQQVKKECSSSGAYTLQQVKKECSSSGAYTLQIFVYYTLQIFYYYTLQIFYYYYHLLSTLLYYY<	K V H G L E Q E I E E V Q R L R E E N K E I T S L K G E N S C N I E L R R E N L E V P P V Q K T V 4 6 2 3 8 4 4 8 4 3 50. 1 A V A AL L I G A L V L F F I V G V Y V A AL I L G	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN 3455702464 0 LIVGRLF- VIIGKIAL LIIGKFLL	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T
vpr_1_C_elegansTVDEKELQQVAPB_H_sapiensVM ECKRLQGVM ECKRQQVKKLQBLSGEIKALRESSSCIKALRESCSTQPVQVKKECSSGALCNAAA	K V H G L E Q E I E E V Q R L R E E N K E I T S L K G E N S C N I E L R R E N L E V P P V Q K T V 4 6 2 3 8 4 4 8 4 3 50. 2 I A L V Q K T V 4 6 2 3 8 4 4 8 4 3 . . 50. 60. 7 L I A V A A L L I G A L V V L F F I V G Y G Y V A A I I L G A V A I A A A I V S . .	VMLKK - NRKL QFKEEDGLRM QLKDE - GIRL HLKDQ - ITRF PHENEKQTSN 3455702464 0	Q Q S H S D R K T V Q S N S R K V A MT D T V S R S S P A V K Q S T P A P Q	G A L V P I S A L A P T G K P T P L N P S P A P V N E P Y A P V L A - N Q I K E A A T
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Figure1B: Alignment of the Amino Acid Sequences of VAPBamong representative specie: VAPB expressed in all eukaryotic organisms ranging from yeast to mammals. The VAP consensus sequence (42-59 amino acids) and proline residue at 56th position is also highly conserved.





x UAS-VAP-33 RNAi (28°C)

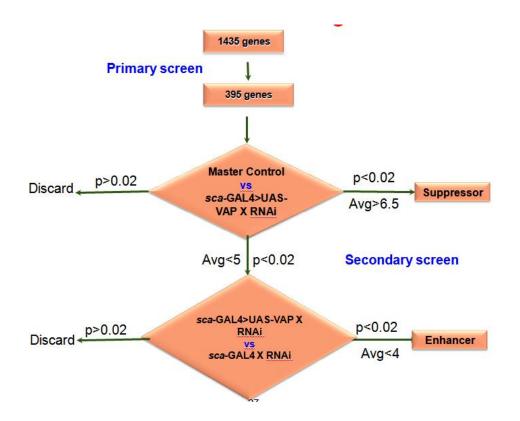
Figure 2: The basis of the Quantitative, secondary Genetic Screen at 25°C: The secondary genetic screen was carried out to validate the modifiers identified in the primary screen. As in the case of the primary screen, the starting line was a recombinant line expressing VAP in the Scabrous expression domain. This line had, on average, 5.5 bristles in females with VAP expression. In the secondary screen, the starting line was crossed with individual RNAi lines and bristles for 10 adult females were counted in the F1 generation.

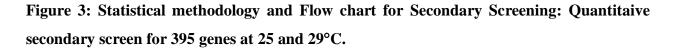
(A) Control, wild type line with 10 Macrochaetae.

(B) Starting line for the screen, where VAP is expressed in Macrochaetae utilizing the Scabrous-Gal4 driver. On average for 10 females, the line shows 5.5 bristles.

(C) When the experiment was done at 28 °C, the starting line had no macrochaetae.

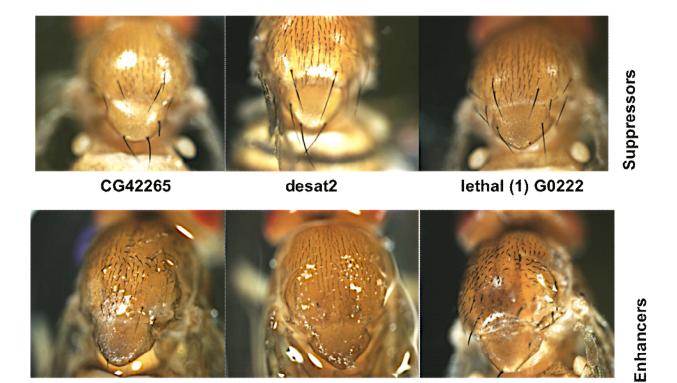
(D) The reduction of VAP transcript, using a VAP RNAi line leads to a rescue of the phenotype observed in C.





Suppressors: Experimental cross (Sca –Gal4 >UAS-VAP *UAS-RNAi) with average phenotype >6.5 was qualified as putative suppressor.Independent T test between individual of experiment cross Sca-Gal 4 >UAS-VAP × UAS- RNAi (experiment) and Sca-Gal 4 >UAS-VAP (master cross) was performed to analyze the statistical significance of the observed difference inavg phenotype.

Enhancers: For RNAi lines showing experiment cross average phenotype <4 macrochaetae, each individual fly's phenotype score was normalized against master control average phenotype(5.5 macrochaetae), similarly phenotype of individual flies of control cross'(sca-Gal4 x UAS-RNAi) was normalized against sca-Gal4 average phenotype(10 macrochaetae). Then an independent t-test was performed between these two normalized data score for each RNAi line to analyze the statistical significance in between them.



GstD6

hy

hyrax

CG3224

Figure 4: Primary screen modifiers: VAP function Suppressor was identified at 29 °C as number of macrochaetae greater than 6 while VAP function Enhancer was scored at 25 °C for number of macrochaetae less than 3.

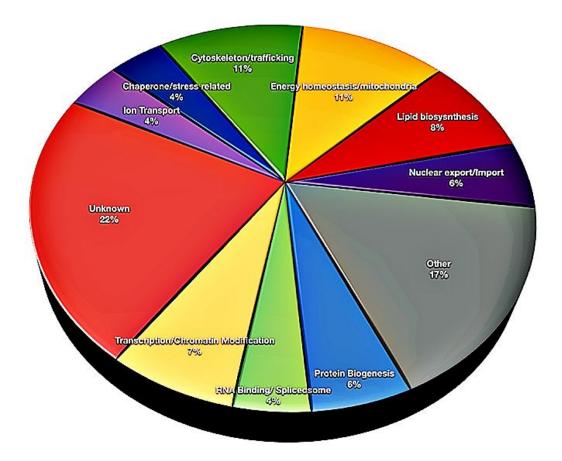
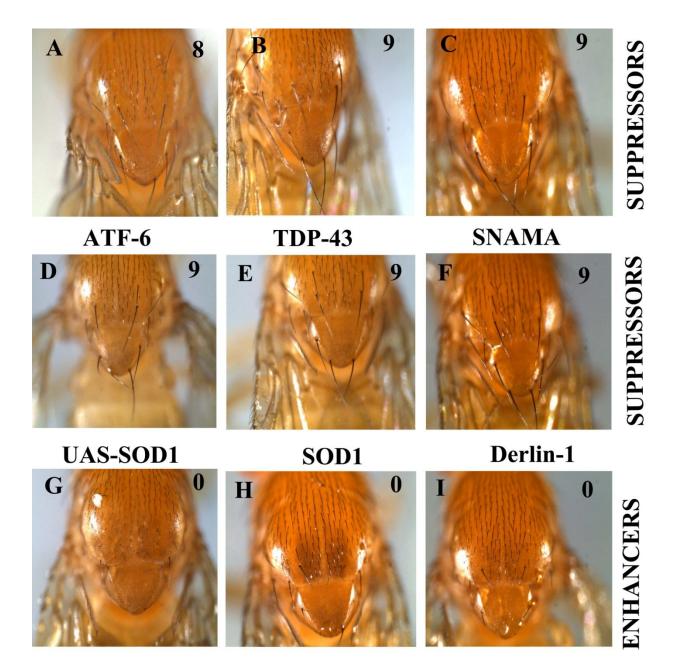


Figure 5: Gene Ontology (GO) distribution of Validated Modifiers: GO analysis indicates that 11% of identified modifier have their role in energy homeostasis, 6% in nuclear export/import, 22% have unknown function ,11% in cytoskeleton dynamics, 8% in lipid biosynthesis, 4% in ER stress response and 7% in chromatin modification.



- **UAS-TOR**
- CG 9172R-4 Karyopherin beta3

Figure 6: Macrochaetae phenotype of VAPB modifiers. Suppressors (A-F) and Enhancers (G-I).In (D) SOD1 has been over-expressed and in (G) TOR has been overexpressed and in others genes has been knockdown using RNAi.