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

| 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 2: List of Suppressors of VAPB**

| ANNOTATION | GO BIOLOGICAL PROCESS | NAME |
|---|---|---|
| **CG3231** | Unknown | something that sticks like glue |
| **CG5014** | neurotransmitter secretion neuromuscular junction development | Vap-33-1 |
| **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 promoter regulation of transcription, DNA-dependent | ENL/AF9-related |
| **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 | TBPH |
| **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 positive regulation of cell size autophagyendocytic recycling | Target of rapamycin |
| **CG6238** | actin cytoskeleton organization mushroom body development | Slingshot |
| **CG6341** | translational elongation | Elongation factor 1 beta |
| **CG7843** | response to arsenic nuclear mRNA splicing, via spliceosome | Ars2 |
| **CG15160** | Unknown | - |
| **CG9638** | axon target recognition chromatin remodeling | Ada2b |
| **CG5733** | SMAD protein nuclear translocation NLSunknownbearing substrate import into nucleus | Nucleoporin 75 |
| **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 |

**TABLE 4: List of Weak Enhancers of VAPB**

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

| 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) |

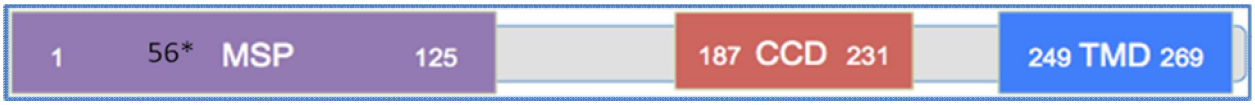


**Figure1A: Analysis of VAPB primary structure;** VAPB protein has three domain: N terminal cytosolic MSP domain(125 amino acid) having evolutionary conserved 16 amino acid known to interact with FFAT motif of cellular proteins and mutation in this domain at 56 position(P56S)is known to cause mis-folding 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 .

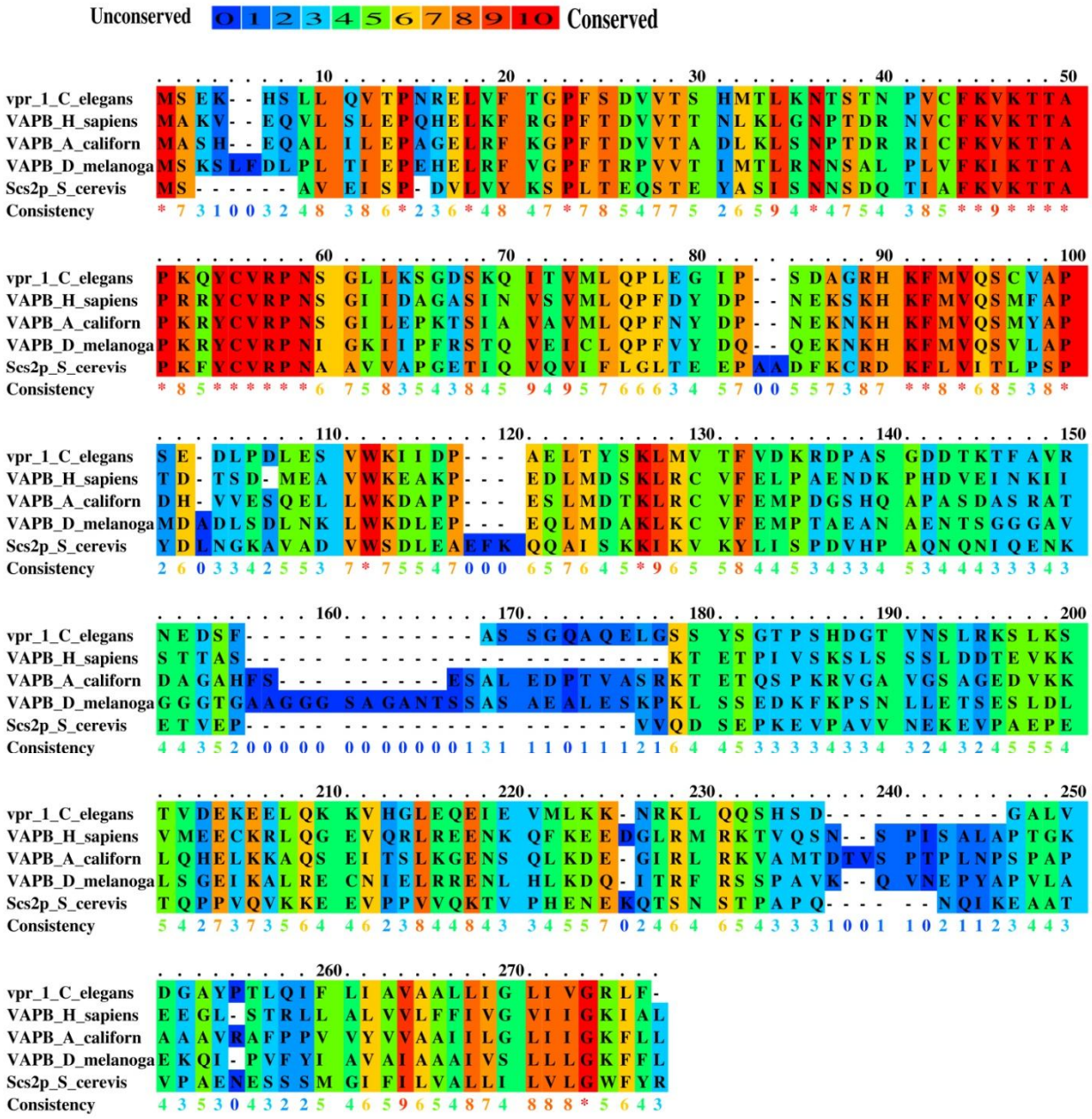


**Figure1B: Alignment of the Amino Acid Sequences of VAPBamong representative specie:** VAPB expressed in all eukaryotic organisms ranging from yeast to mammals.The VAP consensus sequence (42-59 amino acids) and proline residue at 56$^{th}$ position is also highly conserved.

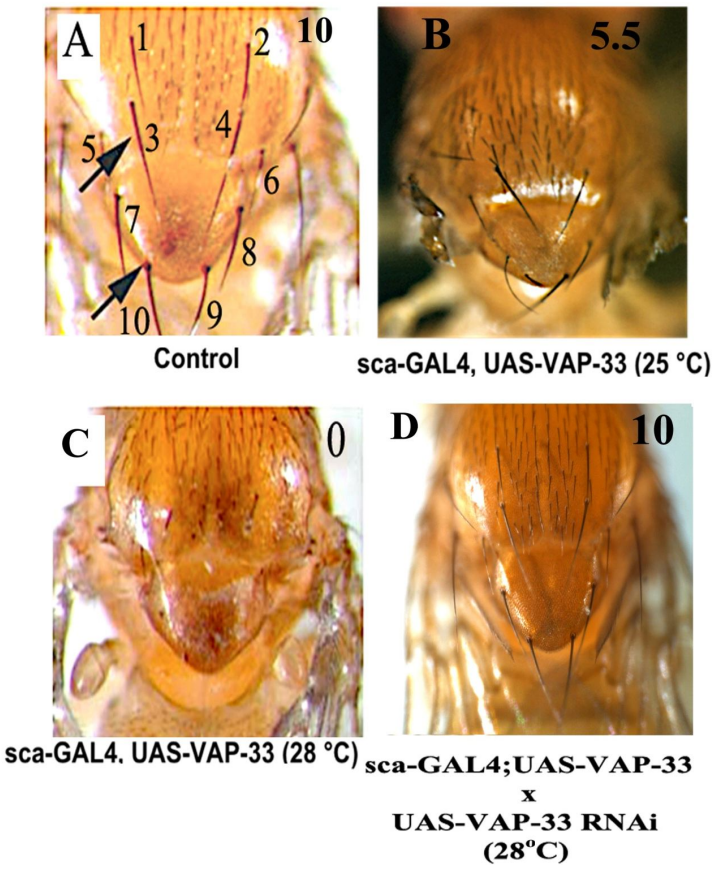


**Figure 2: The basis of the Quantitative, secondary Genetic Screen at 25**°C**:** The secondary genetic screen was carried out to validate the modifiers identified in the primary screen. As in the case of the primary screen, the starting line was a recombinant line expressing VAP in the Scabrous expression 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.

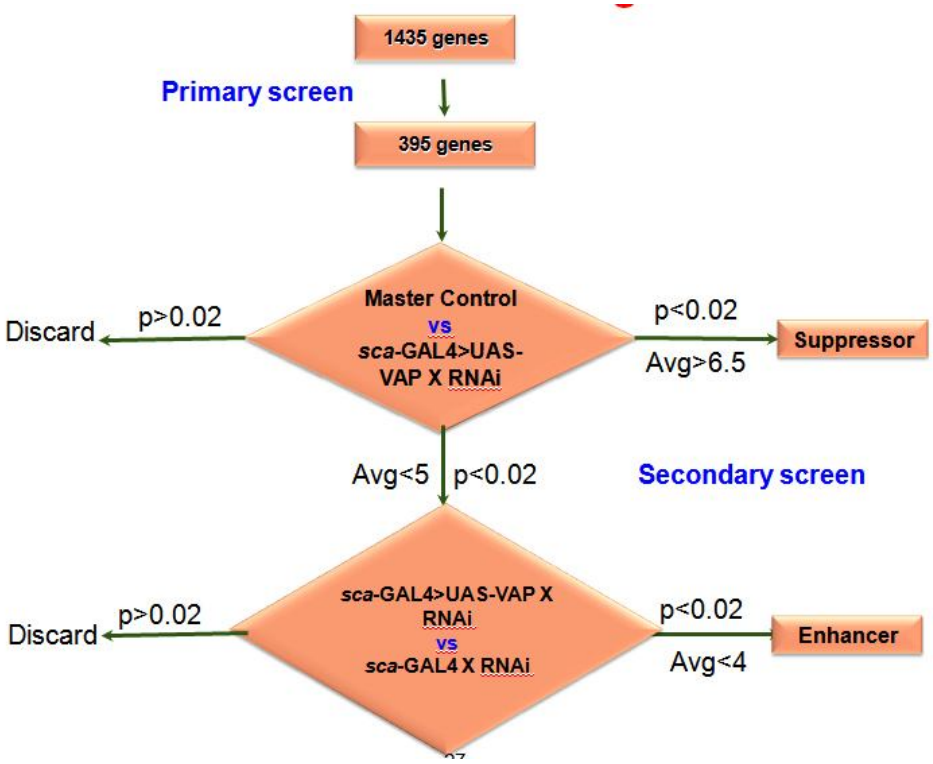


**Figure 3: Statistical methodology and Flow chart for Secondary Screening: Quantitaive secondary screen for 395 genes at 25 and 29°C.**

**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.

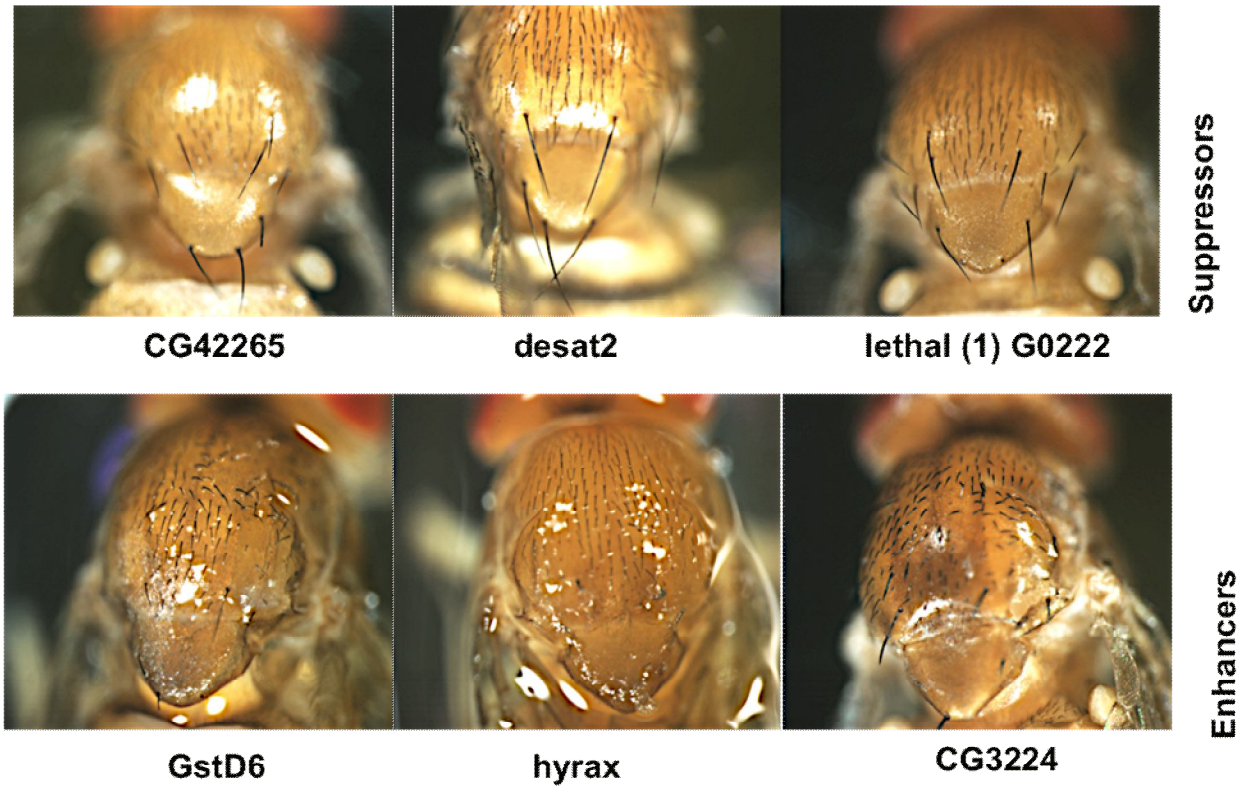


**Figure 4: Primary screen modifiers:** VAP function Suppressor was identified at 29 °C as number of macrochaetae greater than 6 while VAP function Enhancer was scored at 25 °C for number of macrochaetae less than 3.

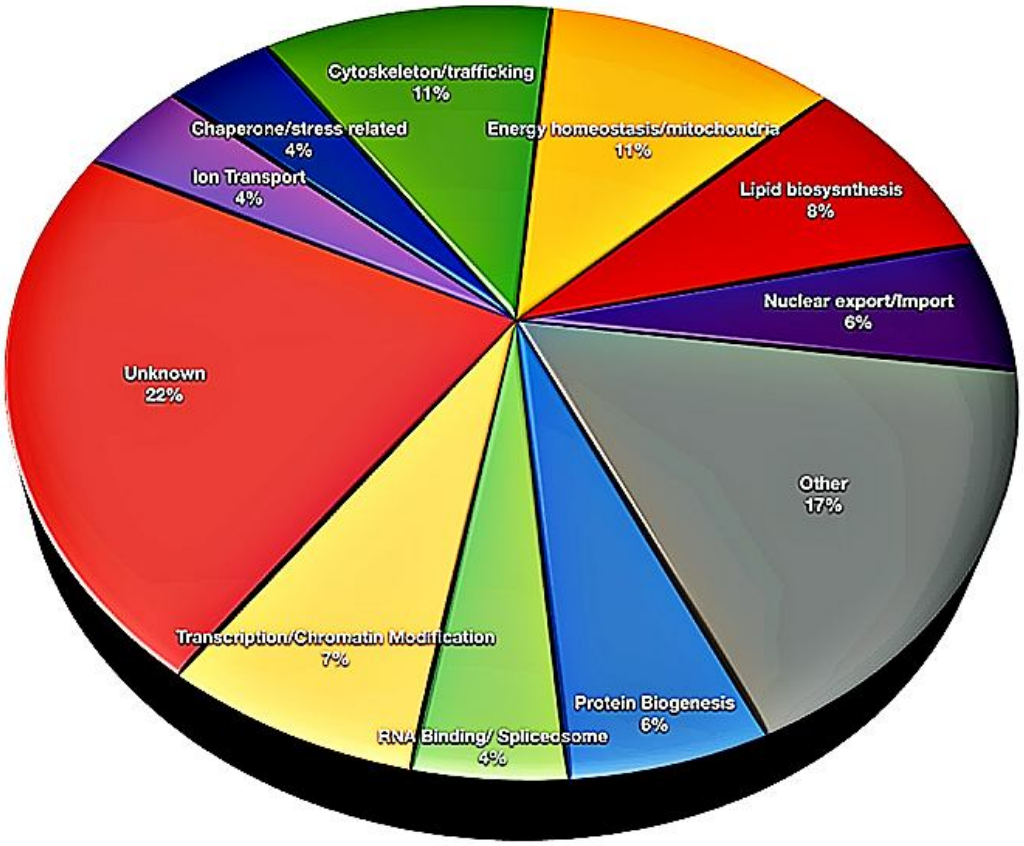


**Figure 5: Gene Ontology (GO) distribution of Validated Modifiers:** GO analysis indicates that 11% of identified modifier have their role in energy homeostasis, 6% in nuclear export/import, 22% have unknown function ,11% in cytoskeleton dynamics, 8%in lipid biosynthesis, 4% in ER stress response and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

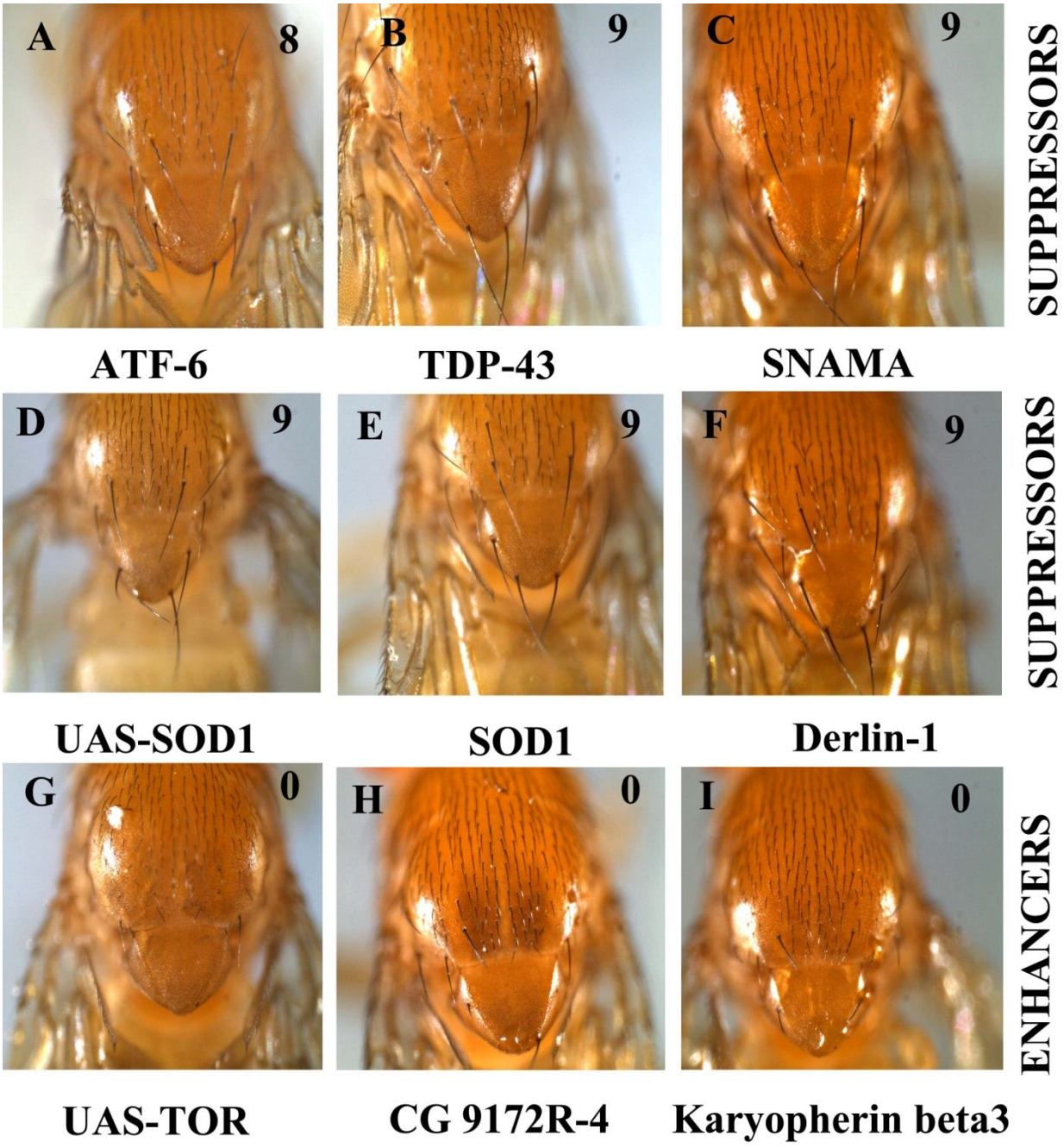


**Figure 6: Macrochaetae phenotype of VAPB modifiers**. Suppressors (A-F) and Enhancers (G-I).In (D) SOD1 has been over-expressed and in (G) TOR has been overexpressed and in others genes has been knockdown using RNAi.