Evolutionary history of mitochondrial ribosomal proteins of S18 family and Gly132 polymorphism in colon cancer

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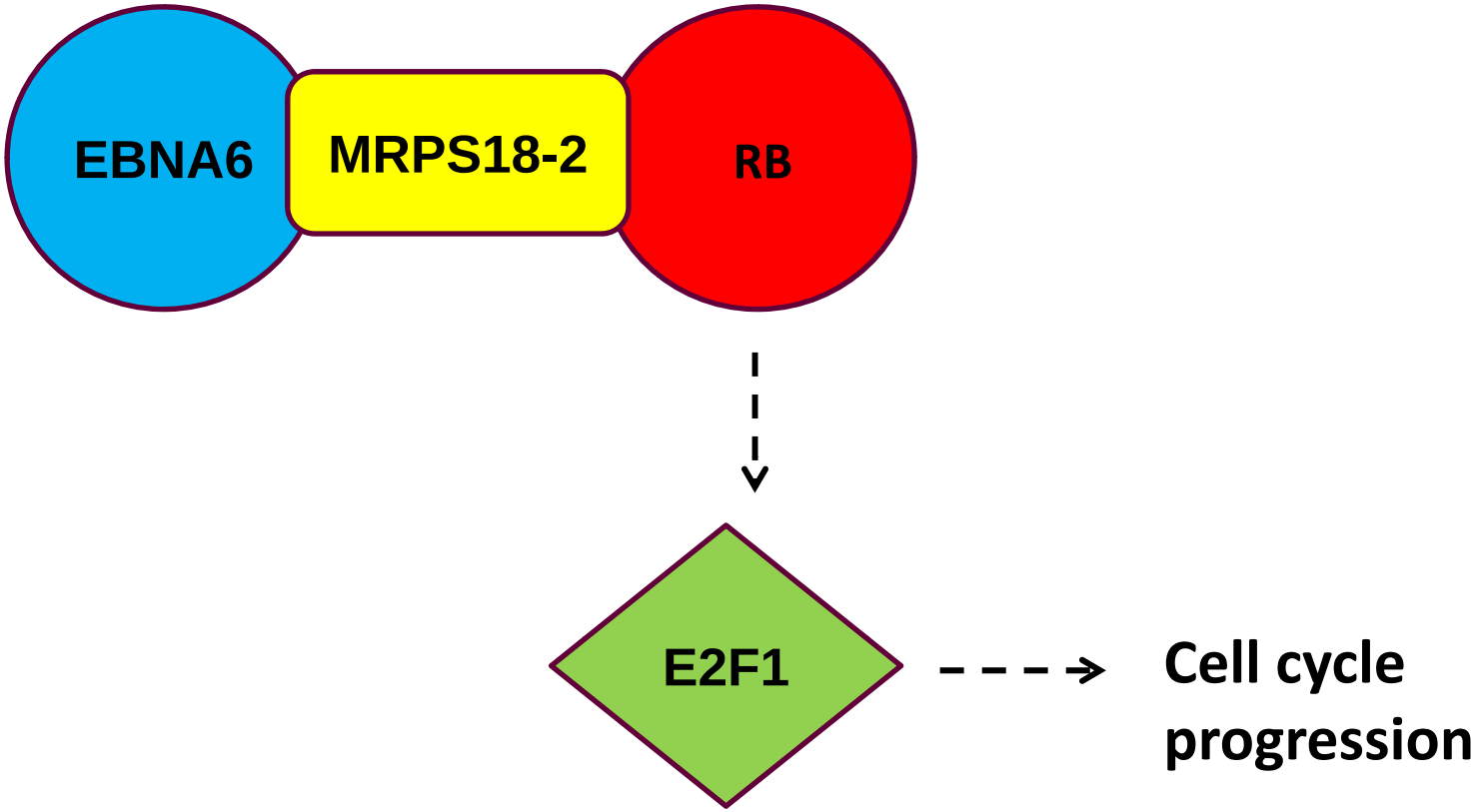
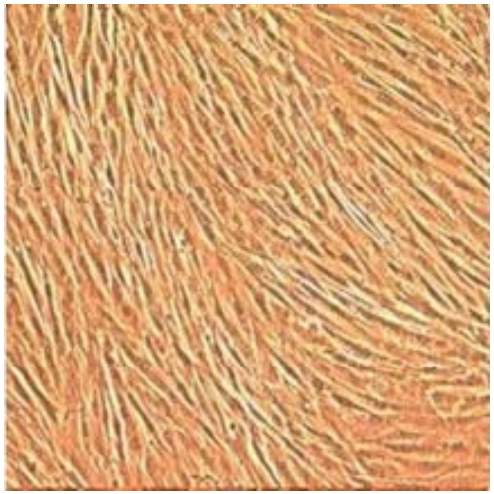
MRPS18-2 act as bridge between EBV encoded EBNA 6 and RB



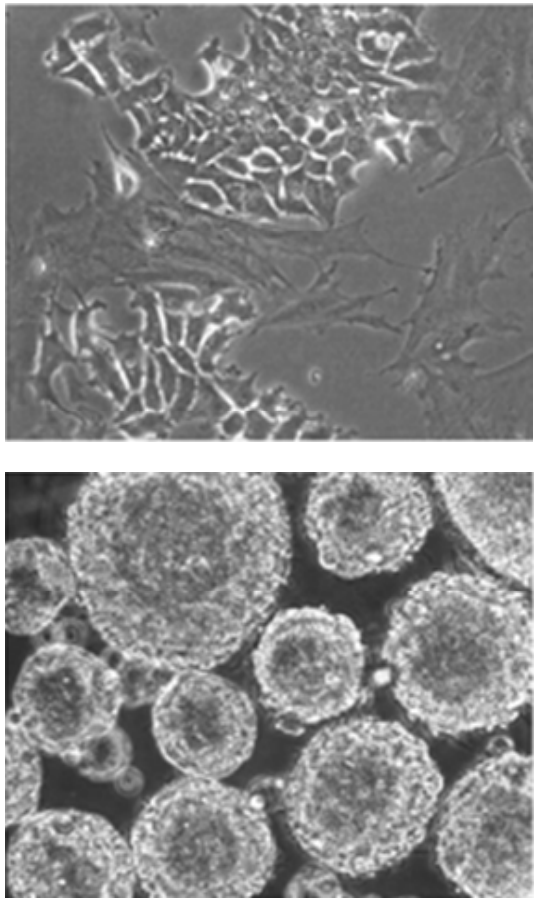
Overexpression of MRPS18-2 protein resulted in REF immortalization

Rat embryonic fibroblasts

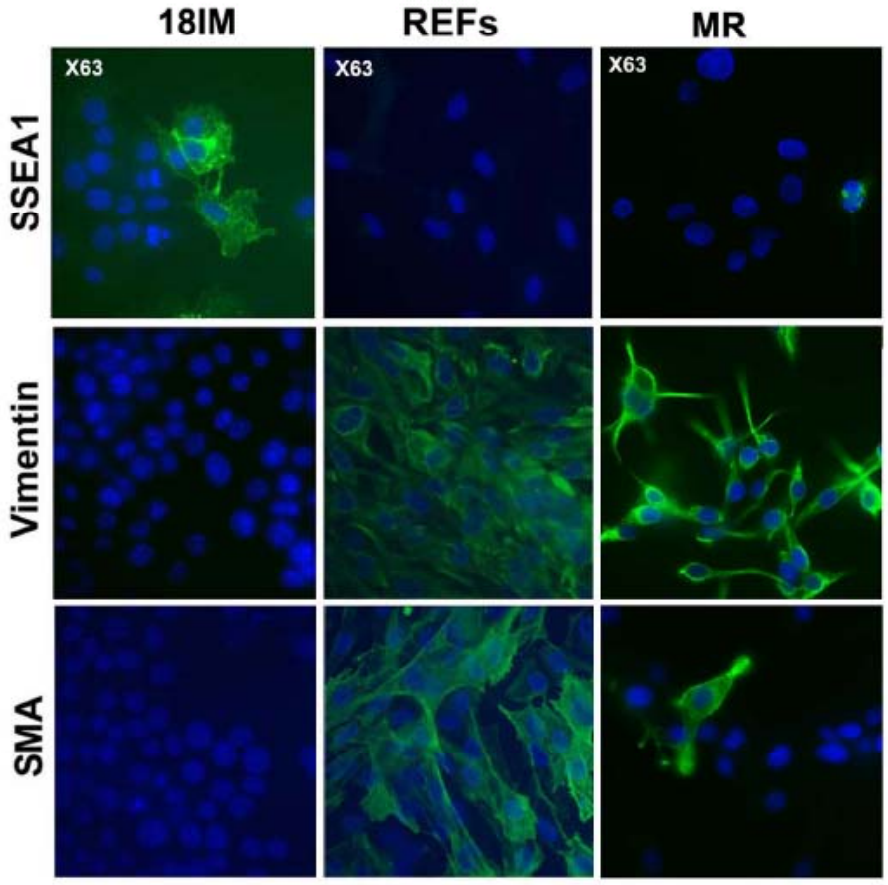


MRPS18-2

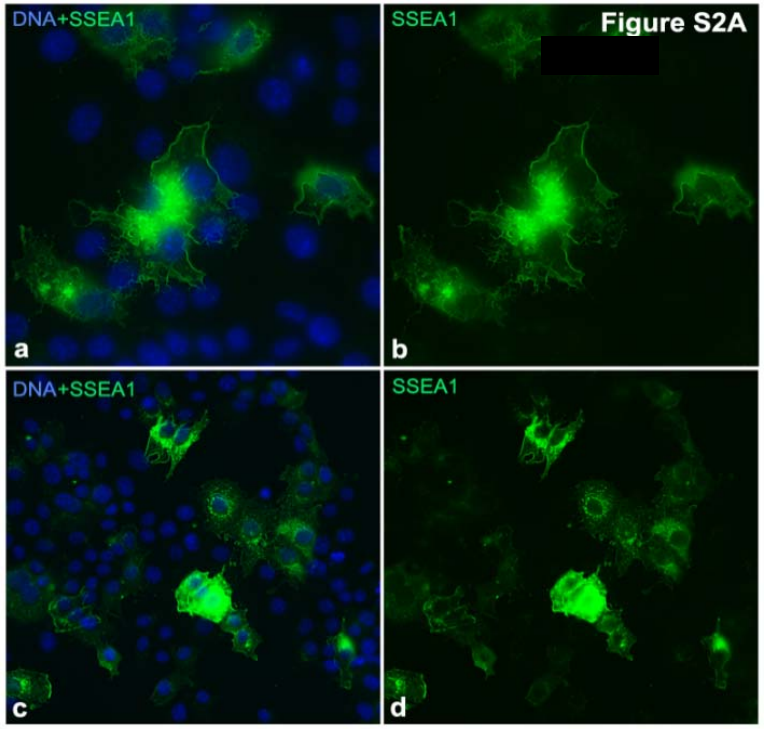
Immortalized cells (18IM)



Immortalized 18IM cells are de-differentiated



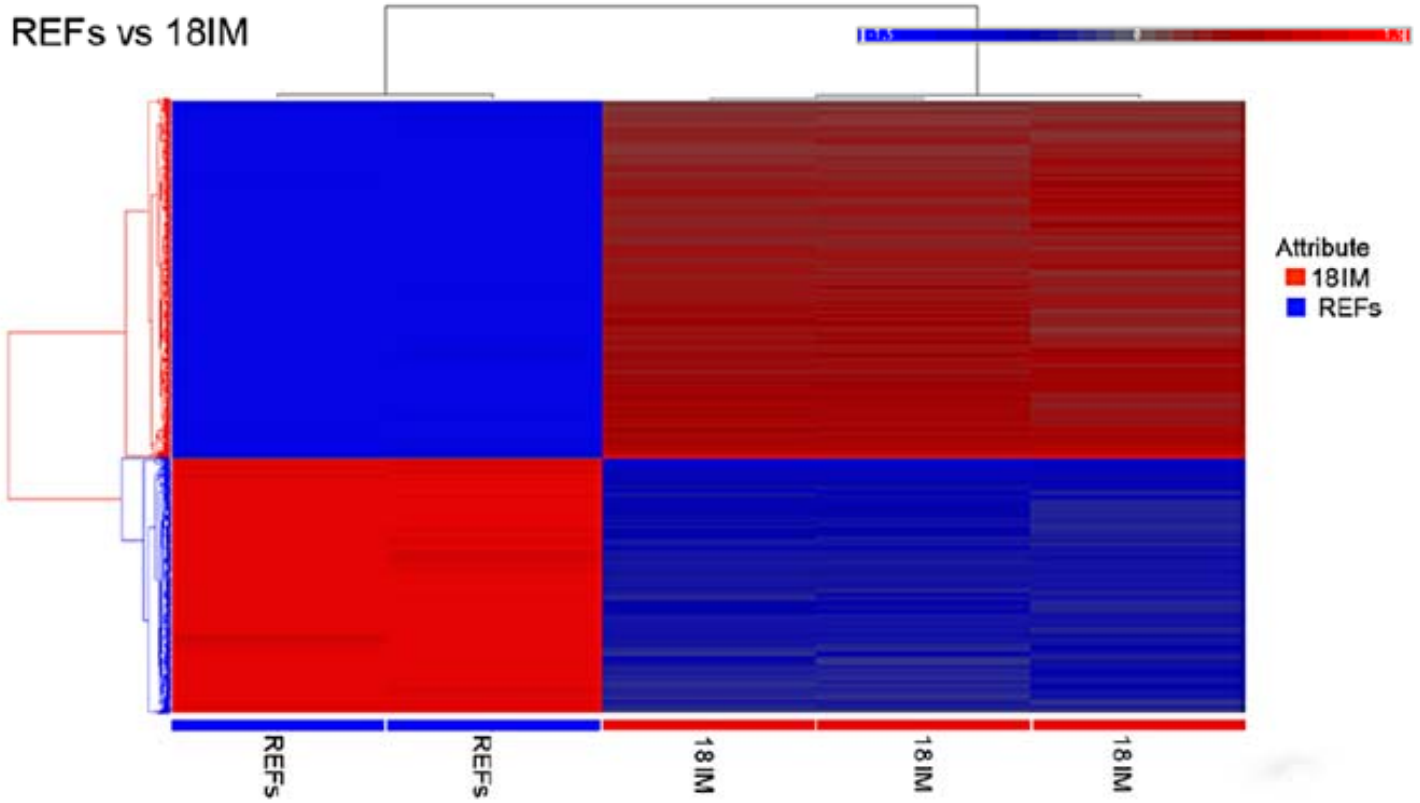
Growth for 2 years



Still 30 % of cells express SSEA-1

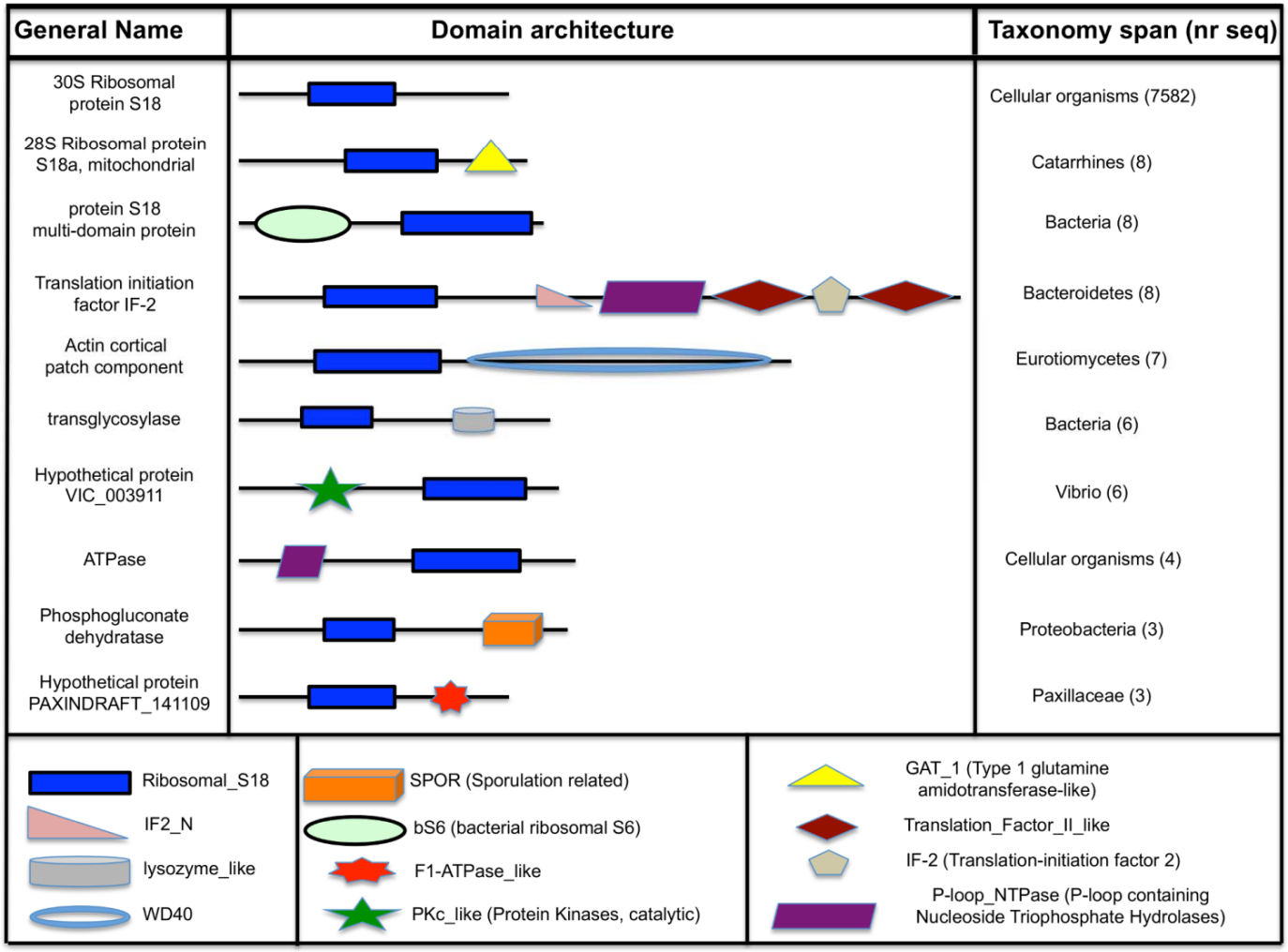
Microarray study confirmed stem cell phenotype of 18IM

Comparison of primary REFs and immortalized 18IM cells showed that 4209 genes and 19 pathways were changed

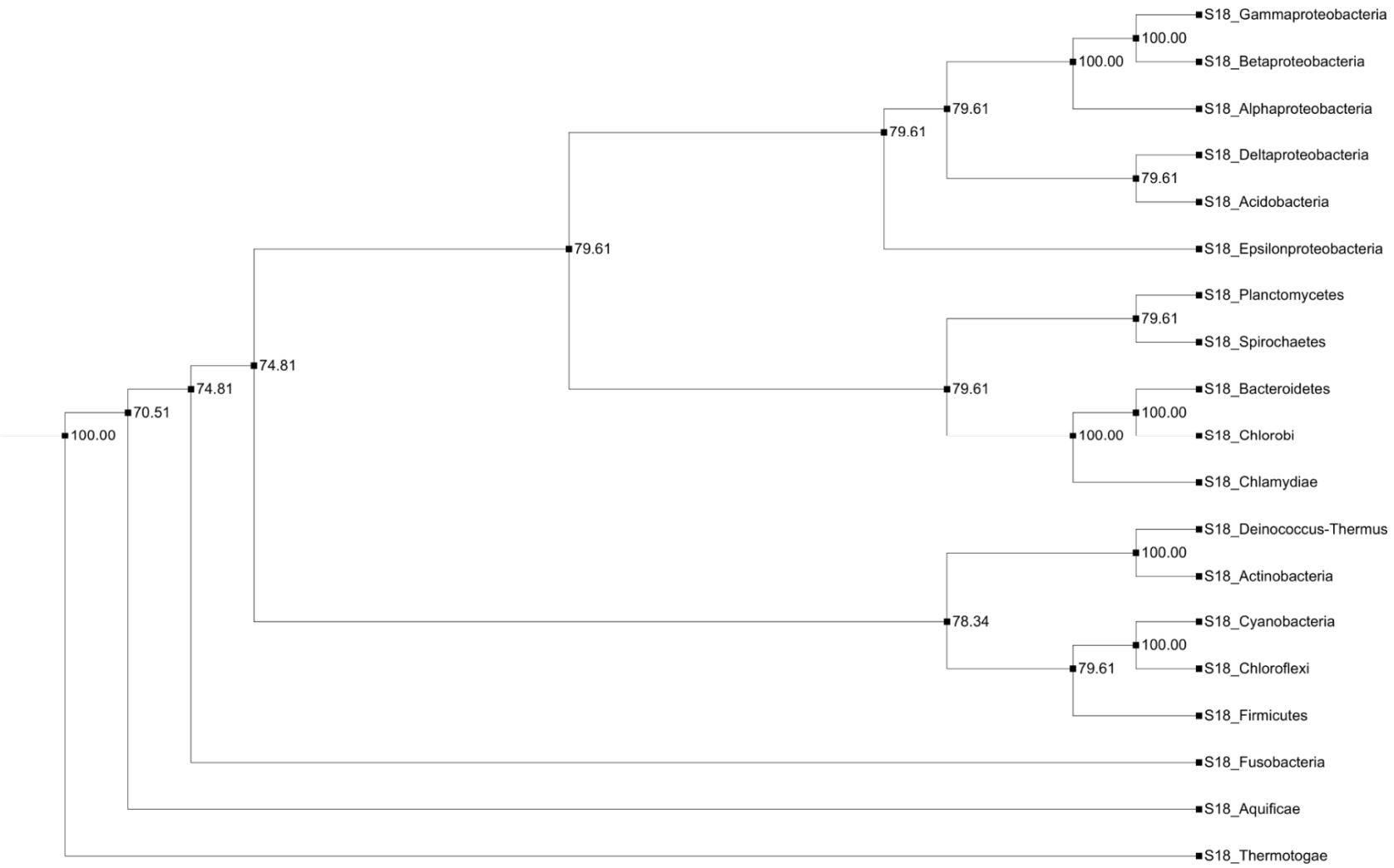


```
CLUSTAL W (1.82) multiple sequence alignment


S18_3          ----------------------------------------MAAVVAVCGGLGRKKLT 17
S18_1          ----------------------------------MAALKALVSGCGRLLRGLLAGPAAT 25
S18_2          MAASVLNTVLRRLPMLSLFRGSHRVQVPLQTLCTKAPSEEDSLSSVPISPYKDEPWKYLE 60

                                                          .
S18_3          HLVTAAVSLTHPGTHTVLWRRGCSQQVSSNEDLPISMENPYKEPLKKCILCGKHVDYKNV 77
S18_1          SWSRLPARGFREVVETQEGKTTIIEGRITATPKESPNPPNPSGQCPICRWNLKHKYNYDD 85
S18_2          SEEYQERYGSRPVWADYRRNHKGGVPPQRTRKTCIRRNKVVGNPCPICRDHKLHVDFRNV 120
                          :         .                                *    *    :

S18_3          QLLSQFVSPFTGCIYGRHITGLCGKKQKEITKAIKRAQIMGFMPVTYKDPAYLKDPKVCN 137
S18_1          VLLLSQFIRPHGGMLPRKITGLCQEEHRKIEECVKMAHRAGLLPNHRPRLPEGVVPKSKP 145
S18_2          KLLEQFVCAHTGIIFYAPYTGVCVKQHKRLTQAIQKARDHGLLIYHIPQVEPRDLDFSTS 180
                 **  .  .    * :     **:* ::::.: :.:: *:  *::

S18_3          IRYRE---------------------------------------------------- 142
S18_1          QLNRYLTRWAPGSVKPIYKKGPRWNRVRMPVGSPLLRDNVCYSRTPWKLYH--------- 196
S18_2          HGAVSATPPAPTLVSGDPWYPWYNWKQPPERELSRLRRLYQGHLQEESGPPPESMPKMPP 240


S18_3          ----------------
S18_1          ----------------
S18_2          RTPAEASSTGQTGPQSAL 258
```

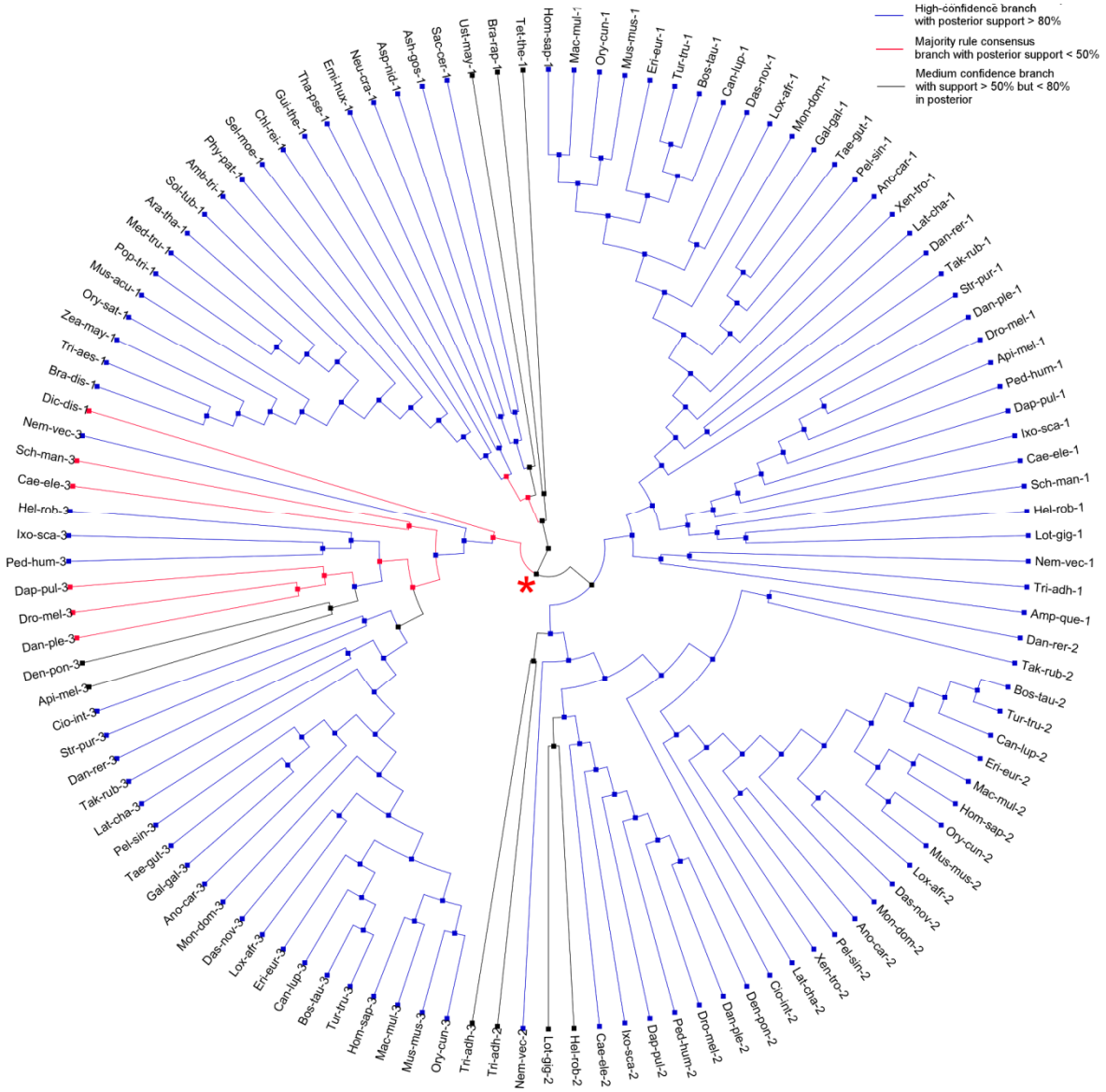
Domain architecture of S18 proteins



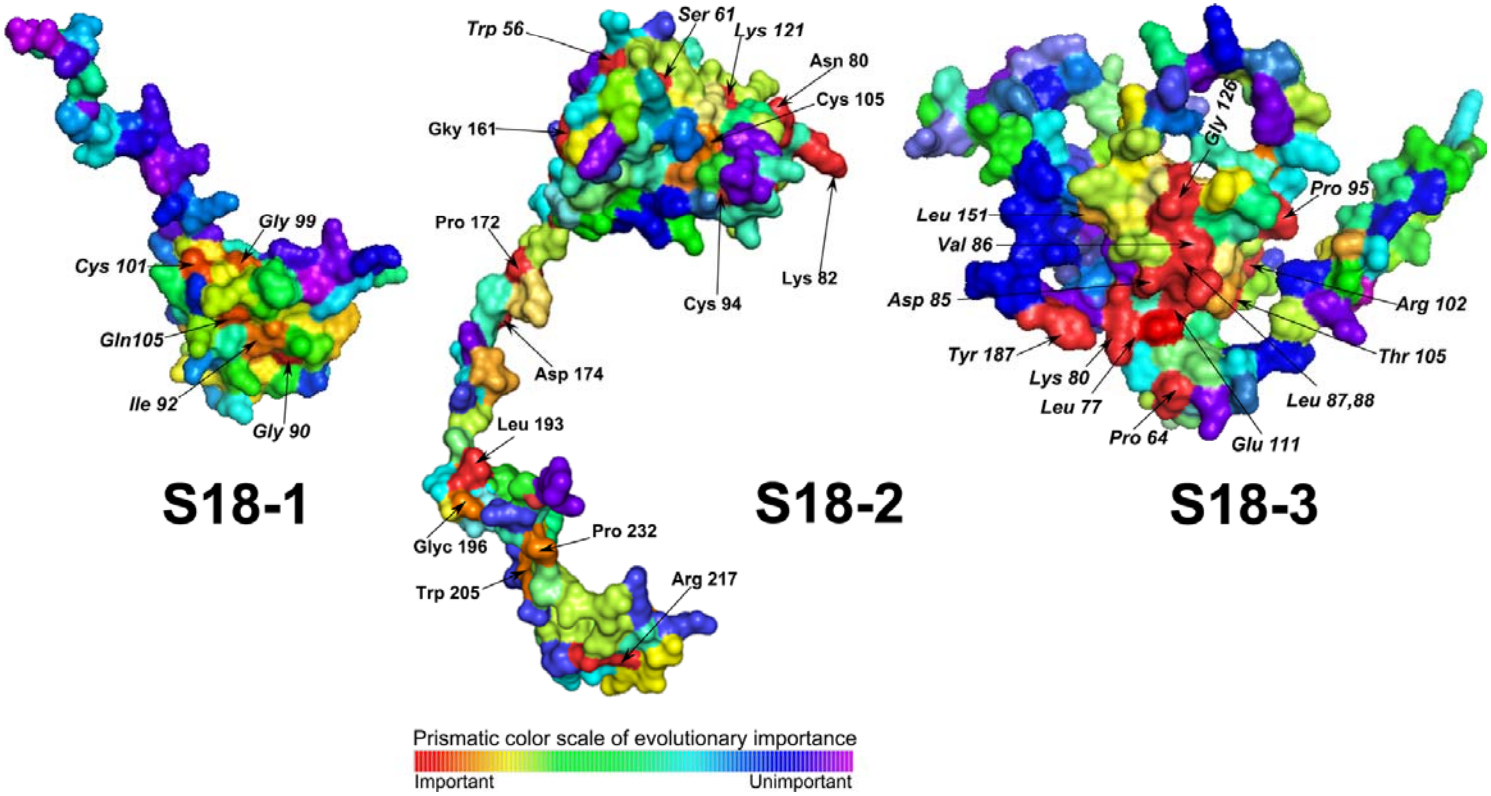
Consensus tree of bacterial S18 protein



Consensus tree of eukaryotic S18 proteins

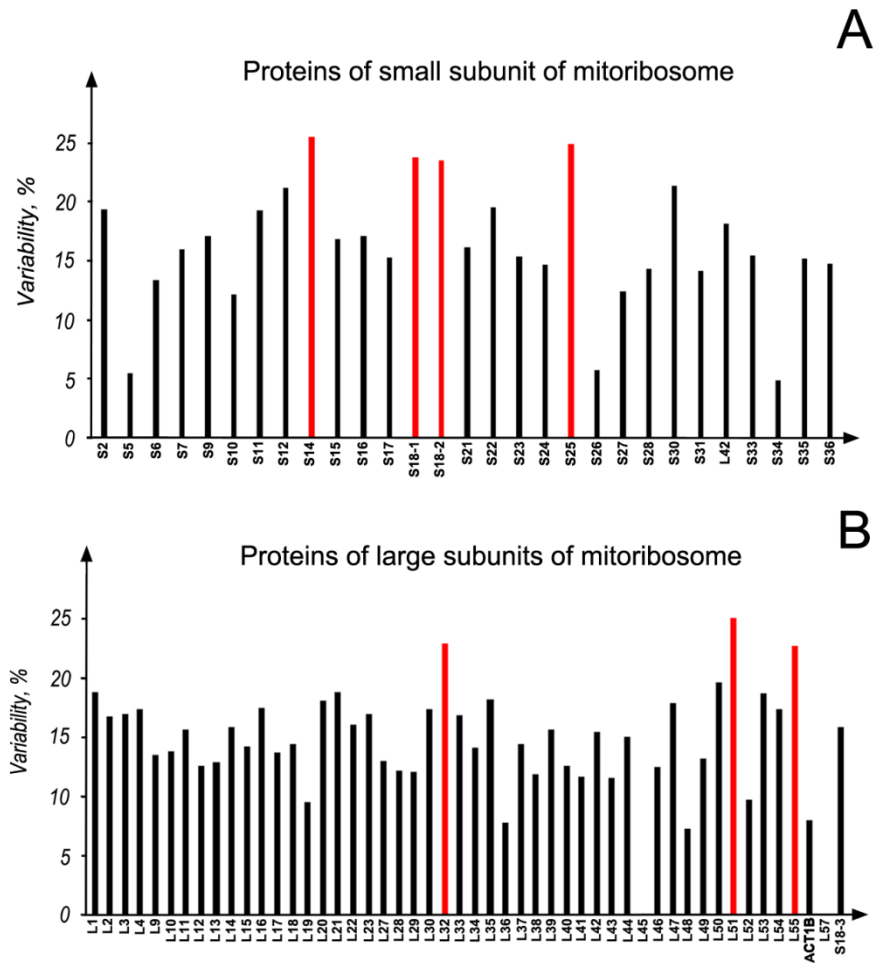


Evolutionary Trace Analysis (ETA)



Mutational analysis of all the mitoribosomal whole proteome in different cancers

Data derived from COSMIC database



Direct DNA sequencing of PCR products

Tumor DNA-Mutant Primer

```
Query  1      TTTGTCTGCGCCCACACGTGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  60
              |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  23095  TTTGTCTGCGCCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  23154
Query  61     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG  105
              |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG  23199
```

Tumor DNA-Wild Primer

```
Query  1      TTTGTCTGCGCCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23095  TTTGTCTGCGCCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  23154
Query  61     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG  105
              |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG  23199
```

Normal DNA-Mutant Primer

```
Query  3      TTTGTCTGCGCCCACACGTGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  62
              |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  23095  TTTGTCTGCGCCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  23154

Query  63     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTT  100
              |||||||||||||||||||||||||||||||||||||
Sbjct  23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTT  23192
```

Normal DNA-Wild Primer

```
Query  8      TTTGTCTGCGCCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  67
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23095  TTTGTCTGCGCCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC  23154

Query  68     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTT  109
              |||||||||||||||||||||||||||||||||||||||||
Sbjct  23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTT  23196
```

Conclusions

- S18 family of proteins are evolutionarily important proteins and the current study helps to know the evolutionaily history and pattern of duplications of S18 proteins during evolution.

- The Gly132 polymorphism might be an important biomarker for colon adenocarcinoma; therefore, it must be further investigated.

- The amino acids identified in ETA should be further investigated to identifty the physiological role of these proteins which is majorily unknown.

Acknowledgements

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- George Klein

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