



Figure S1. Sequence alignment of Tim23 protein of different species. Orthologous Tim23 sequences from different species including *S. cerevisiae*, *N. crassa*, *S. pombe*, *A. thaliana* and *H. sapiens* are aligned using ClustalW program. The position of transmembrane helices TM1-TM4 has been indicated. The sequence corresponding to the GXXXG motif is shaded in gray and the glycine residue is highlighted by asterix. The black arrowhead indicates the position of various mutations isolated in the library.