



**Figure S5. Sequence alignment of various LeuRS or IleRS enzymes from representative bacteria, archaea and eukaryotes.**

Both LeuRS (left panel) and IleRS (right panel) contain aligned sequences from five bacterial enzymes: *Escherichia coli*, *Bacillus subtilis*, *Helicobacter pylori*, *Staphylococcus aureus* and *Thermus thermophilus*; five archaeal enzymes: *Halobacterium salinarum*, *Methanocaldococcus vulcanius*, *Methanothermobacter thermautotrophicus*, *Pyrococcus horikoshii* and *Sulfolobus solfataricus*; and five eukaryotic cytosolic enzymes: *Giardia lamblia*, *Homo sapiens*, *Mus musculus*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Methionine at position 40 (*E. coli* numbering; the position is marked with an asterisk) is strictly conserved in bacterial LeuRSs, while most archeal enzymes have alanine instead of methionine, and eukaryotic cytosolic enzymes typically have proline (left panel). Homologous position in IleRS (right panel; the position is marked with an asterisk) has a strictly conserved glycine.