

Supplemental Material for:
Rheostats and toggle switches for modulating protein function
File 4: Parallel amino acid substitutions

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Figures S13-S24. These plots compare the outcomes of parallel amino acid substitutions among the LacI/GalR homologs. Results are organized to show all variants obtained at a position on one page; five amino acids are shown per panel, as indicated in the legends at the right.

In the left-hand panels, data for each variant were normalized relative to the starting protein, which had different repression values (Table 1 in the main document). If an amino acid substitution caused no change, this corresponds to a value of 1 on the plots (dashed black line). As discussed in Methods, data within 2-fold are considered equivalent to each other, which is indicated by the straight dotted lines on the plots; this range is usually larger than the error bars of a repression measurement. Substitutions that enhanced repression have increased fold-change (>2). Substitutions that diminished repression have decreased fold-change (<2). The jagged dotted line labeled MIN shows the no repressor DEL control relative to the starting protein and represents the lowest possible value for each homolog. The colored connecting lines are to aid visual inspection of the data.

The right-hand panels show amino acid frequency in the naturally occurring proteins at the relevant positions, as calculated from the YPAL-MSA. The following definitions were used to assign the results in Table 5:

- A = amino acid absent from the YPAL-MSA but substitution allowed repression near the parent value or better in two or more chimeras.
- P1 = amino acid present in the YPAL-MSA but substitution diminished repression below the biologically determined threshold of 13 Miller units (not shown) for at least one chimera.
- P2 = amino acid present in the YPAL-MSA but substitution diminished repression to MIN; these designations were only used for parent chimeras with repression better than 13 Miller units.
- D = substitution caused widely different outcomes among several chimeras.
- L = absent in the YPAL- MSA but allowed strong repression in LacI-11; LacI data are commonly used as a single representative of the family for benchmarking MSA analyses.

Position 46

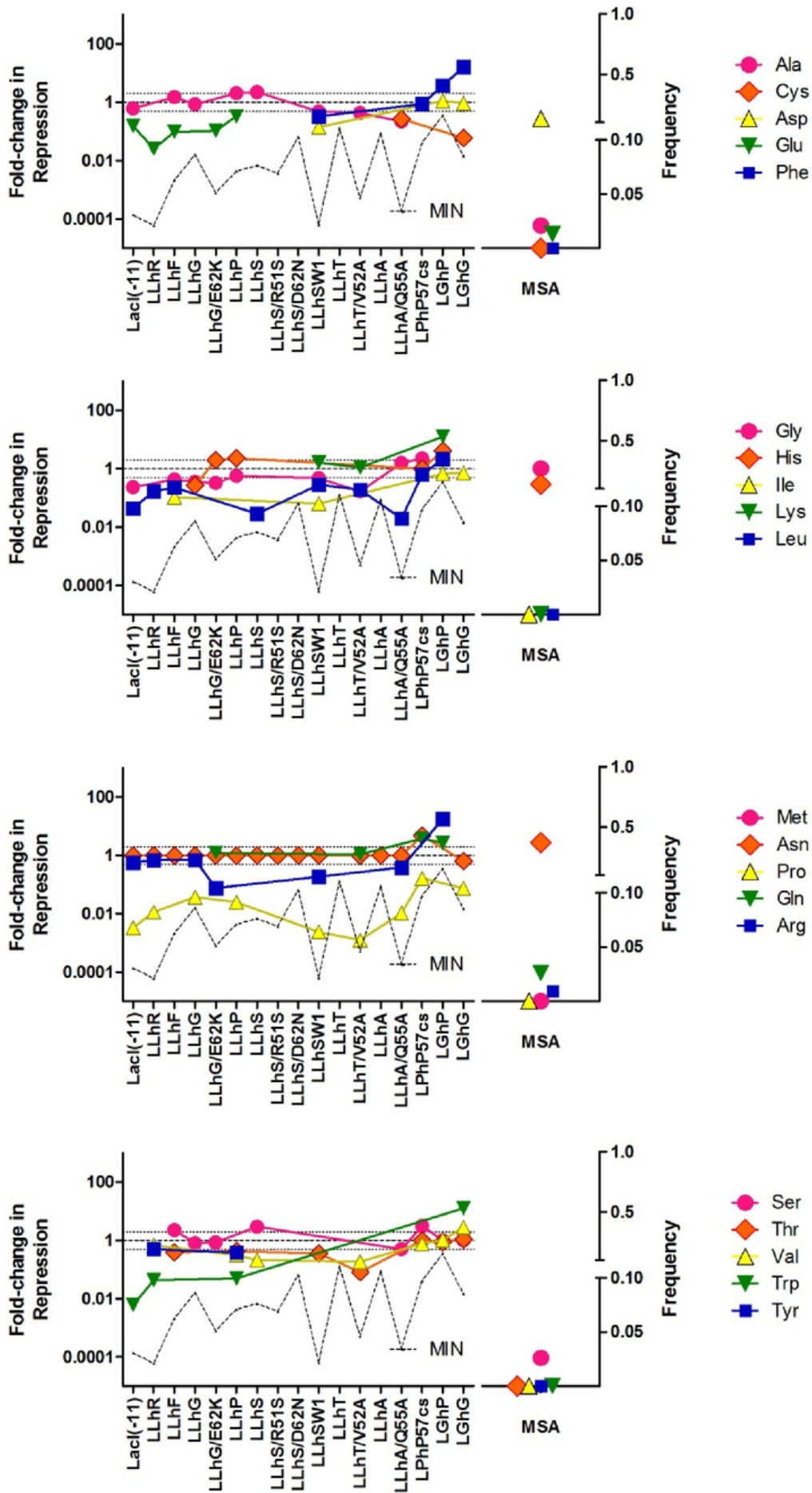


Figure S13: Parallel amino acid substitutions: Position 46

Position 48

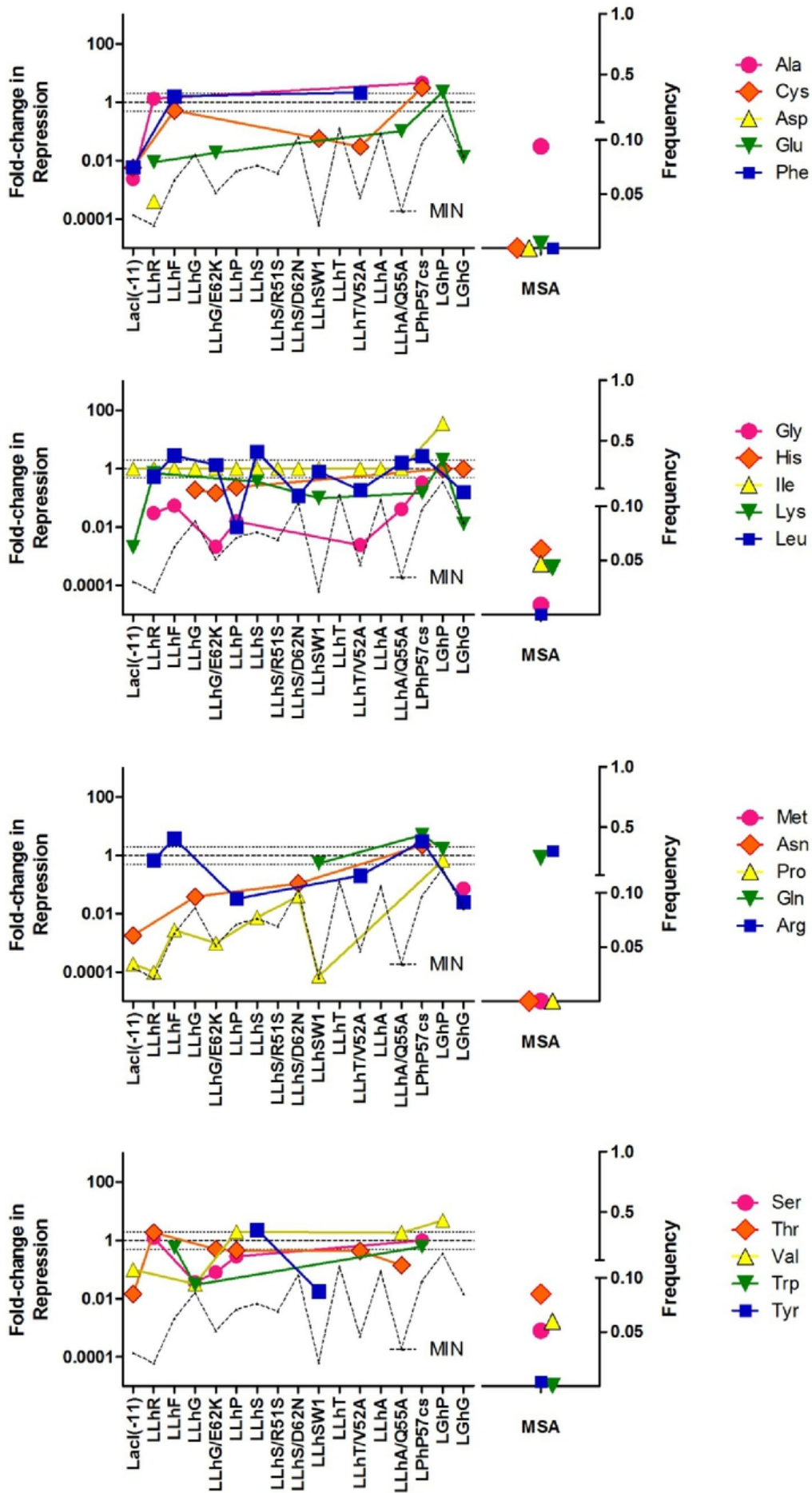


Figure S14: Parallel amino acid substitutions: Position 48

Position 50

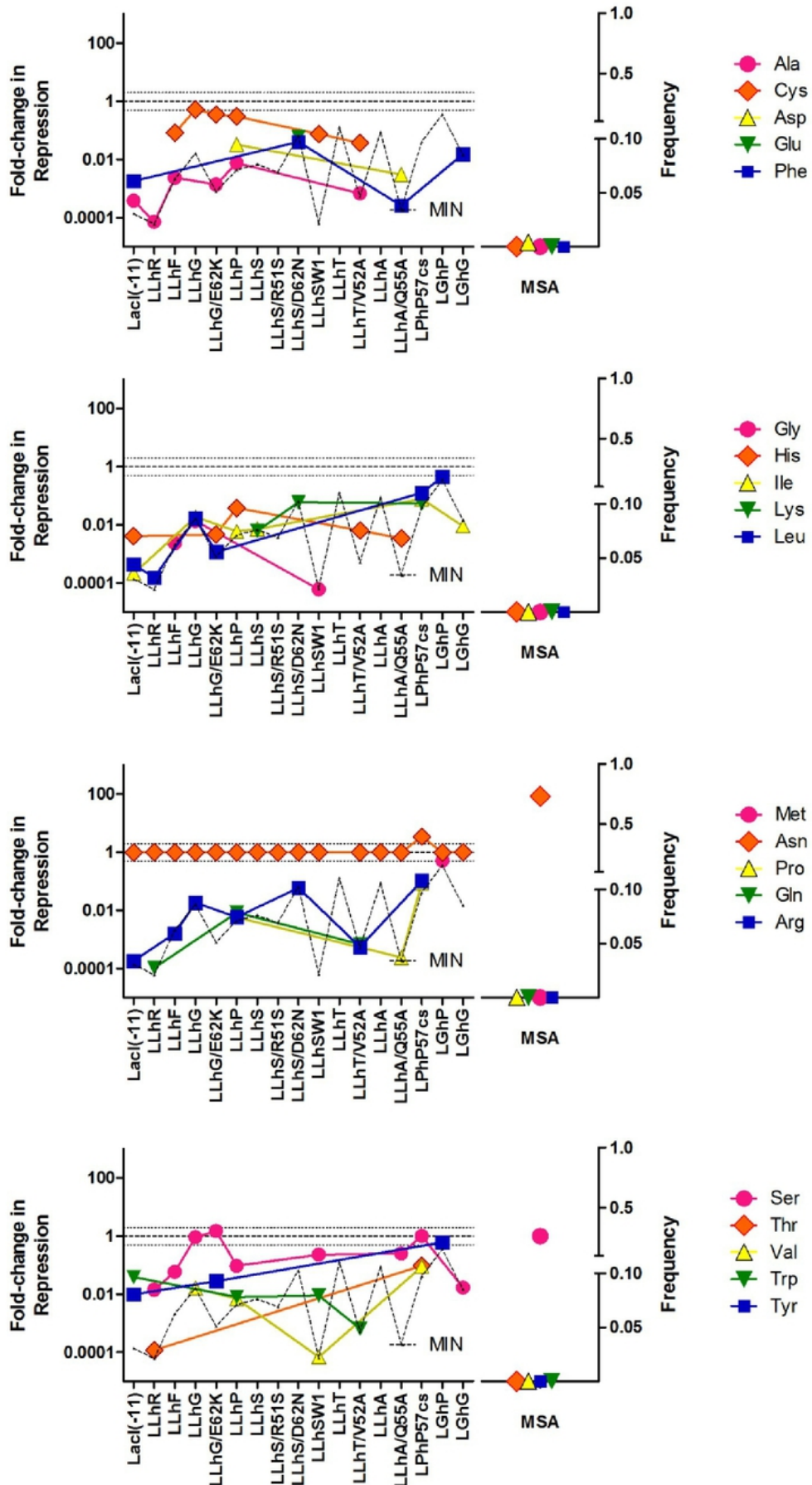


Figure S15: Parallel amino acid substitutions: Position 50

Position 51

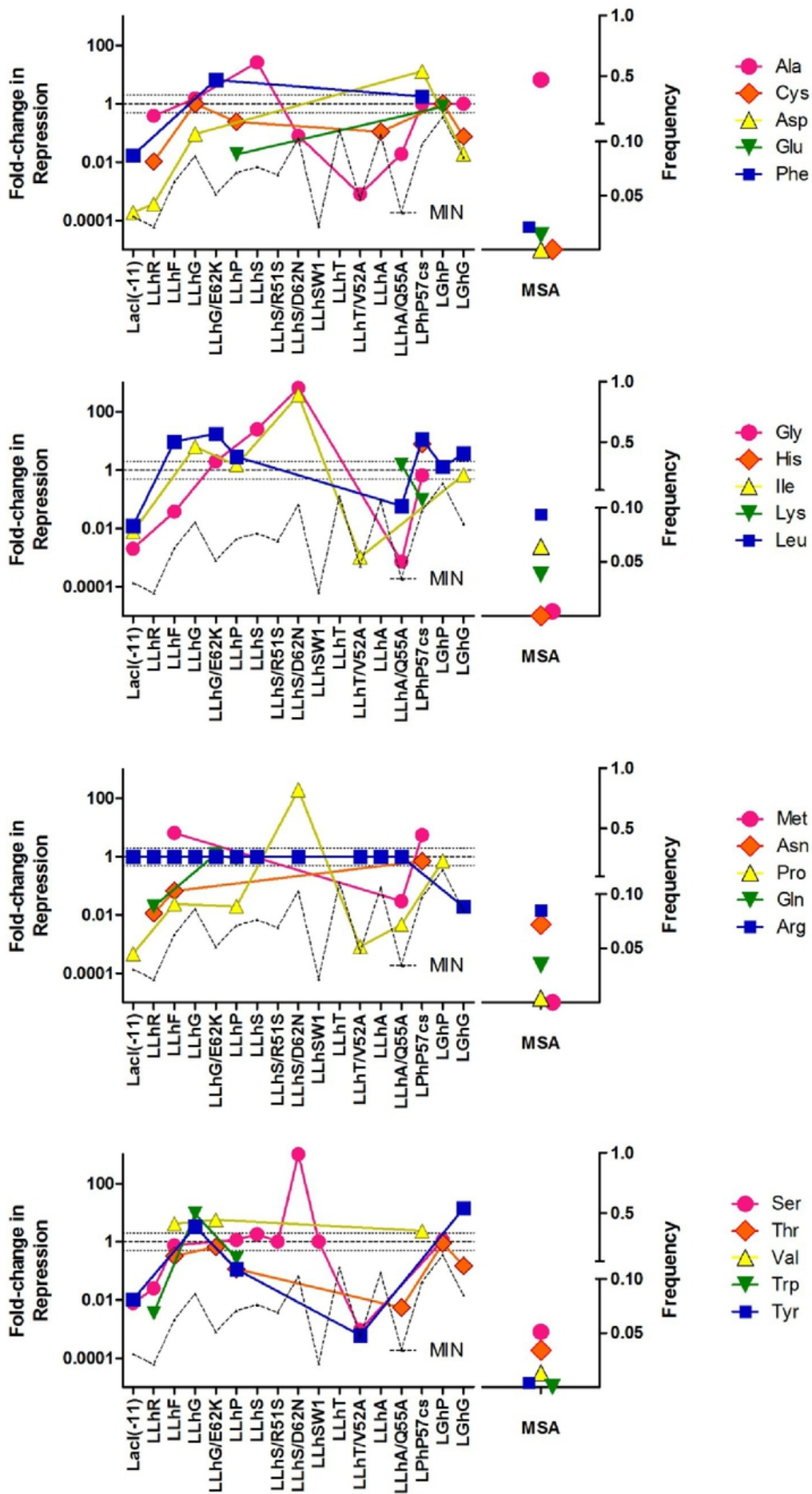


Figure S16: Parallel amino acid substitutions: Position 51

Position 52

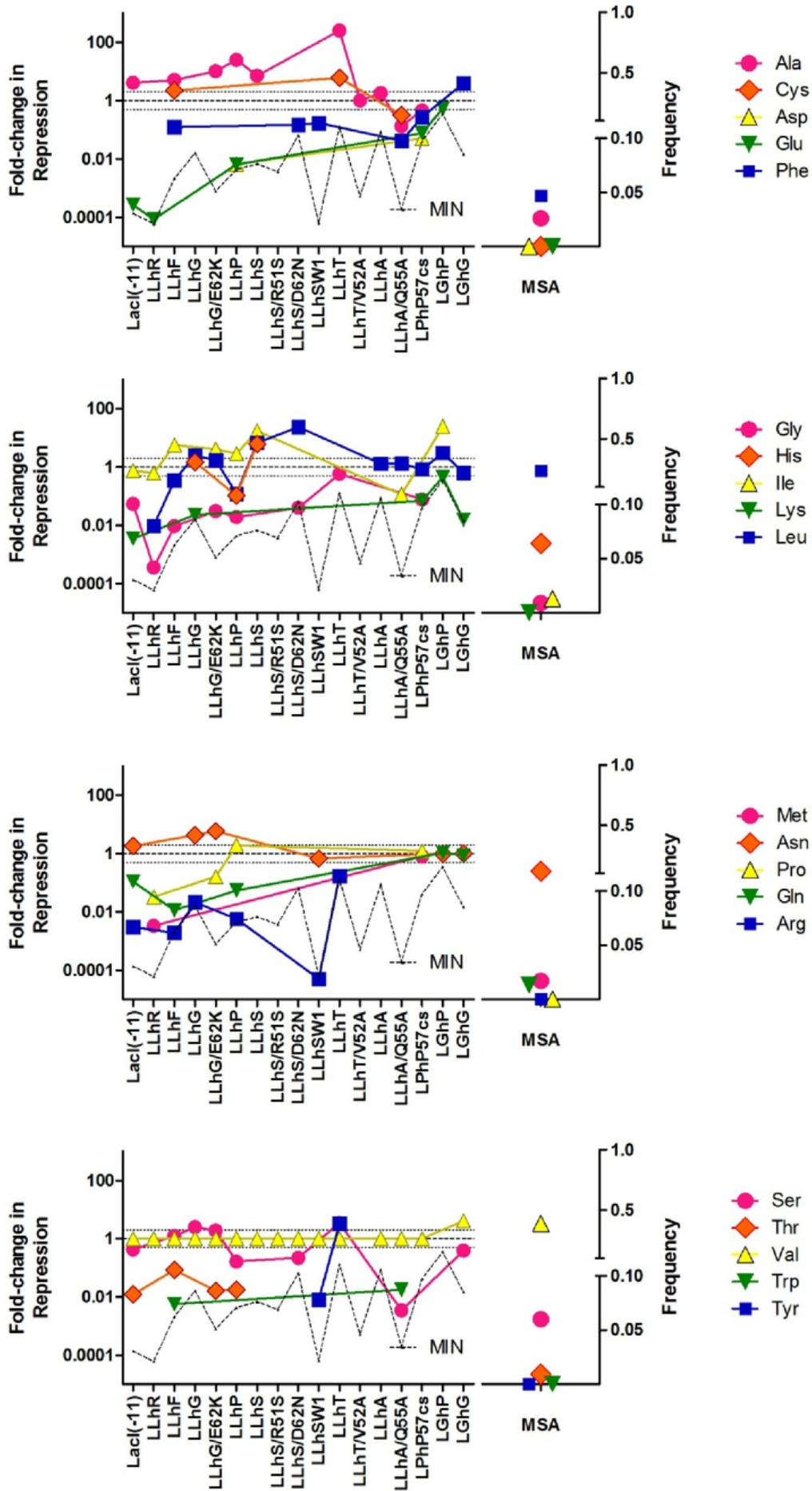


Figure S17: Parallel amino acid substitutions: Position 52

Position 54

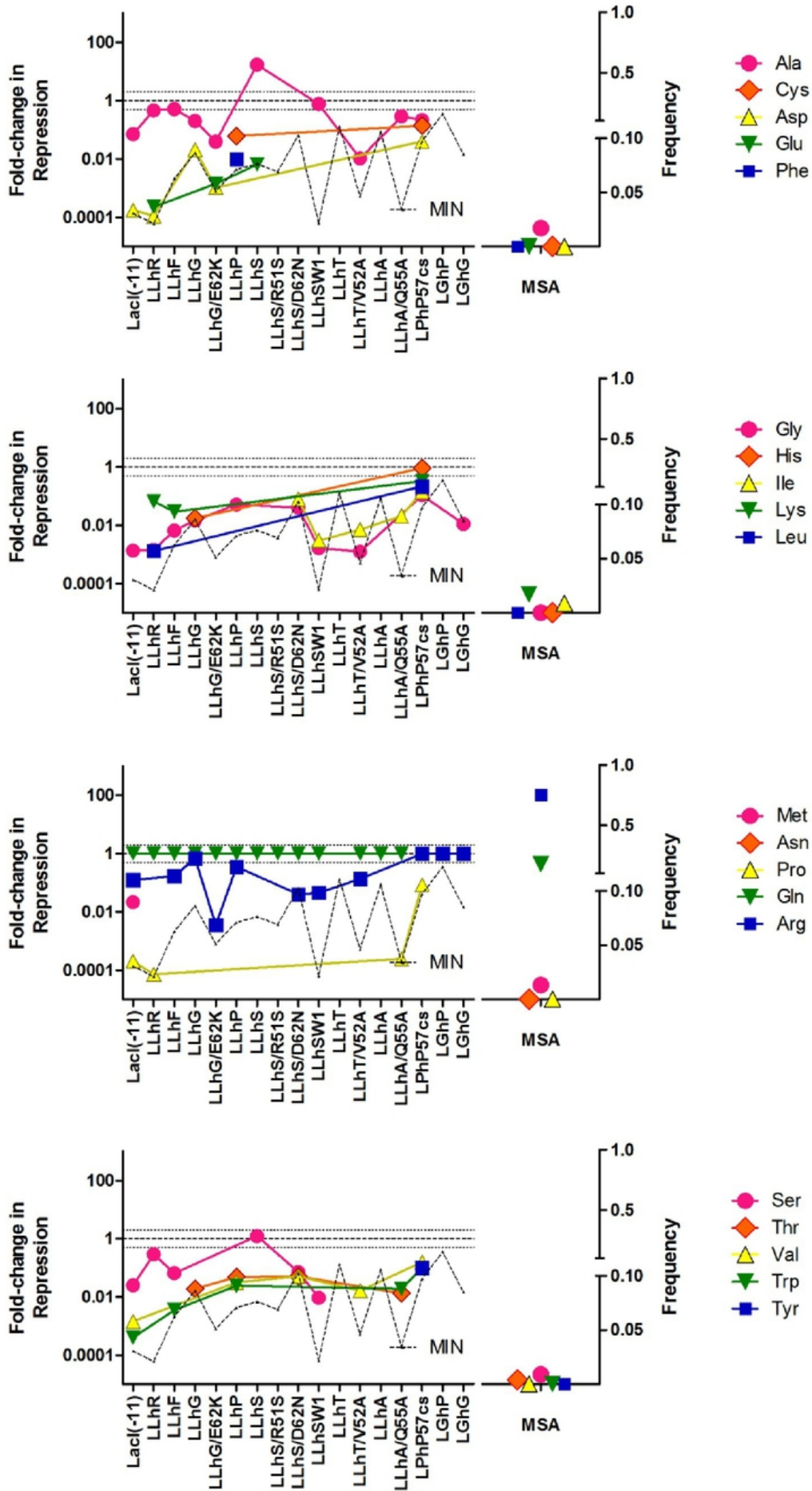


Figure S18: Parallel amino acid substitutions: Position 54

Position 55

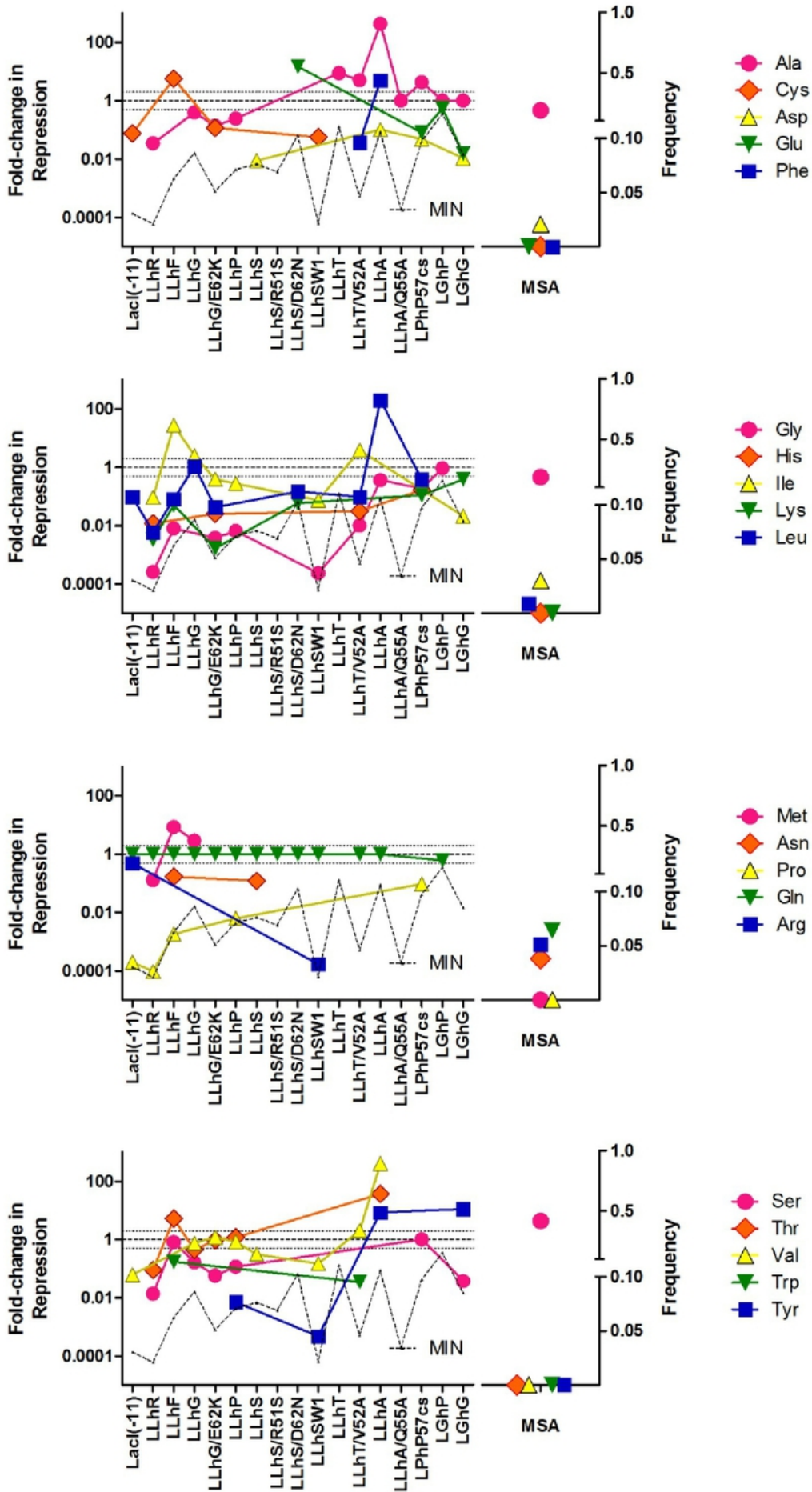


Figure S19: Parallel amino acid substitutions: Position 55

Position 58

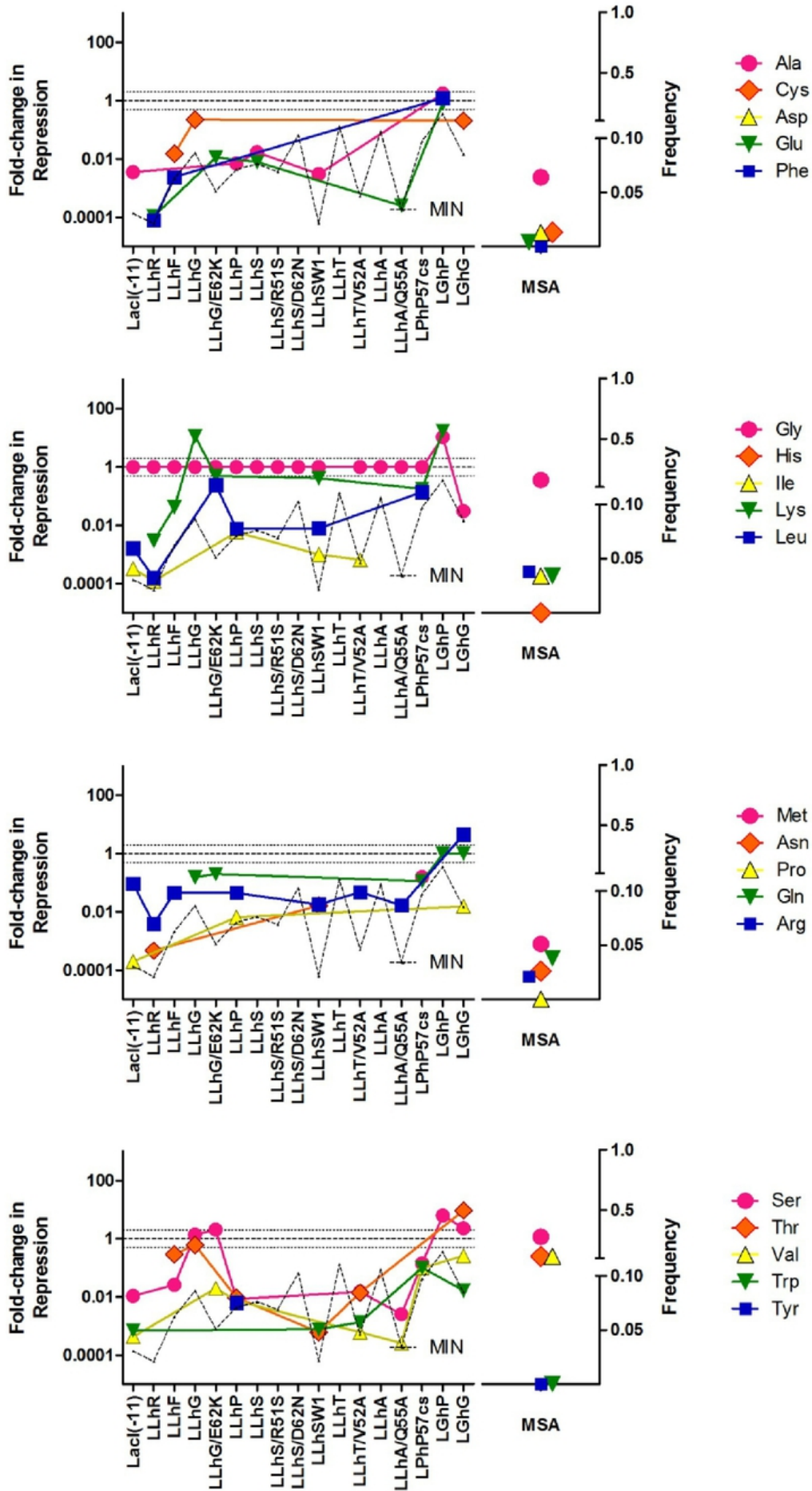


Figure S20: Parallel amino acid substitutions: Position 58

Position 59

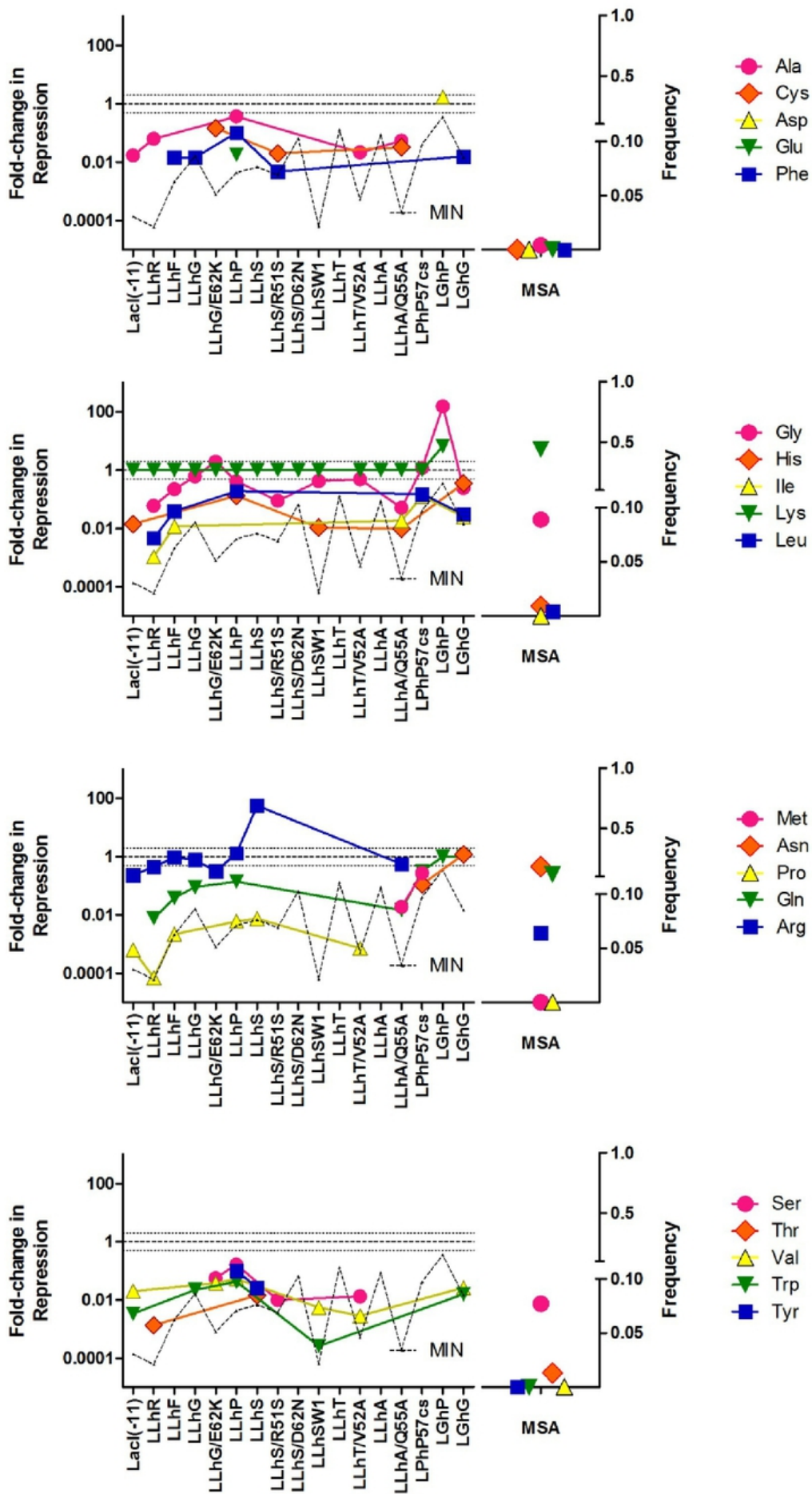


Figure S21: Parallel amino acid substitutions: Position 59

Position 60

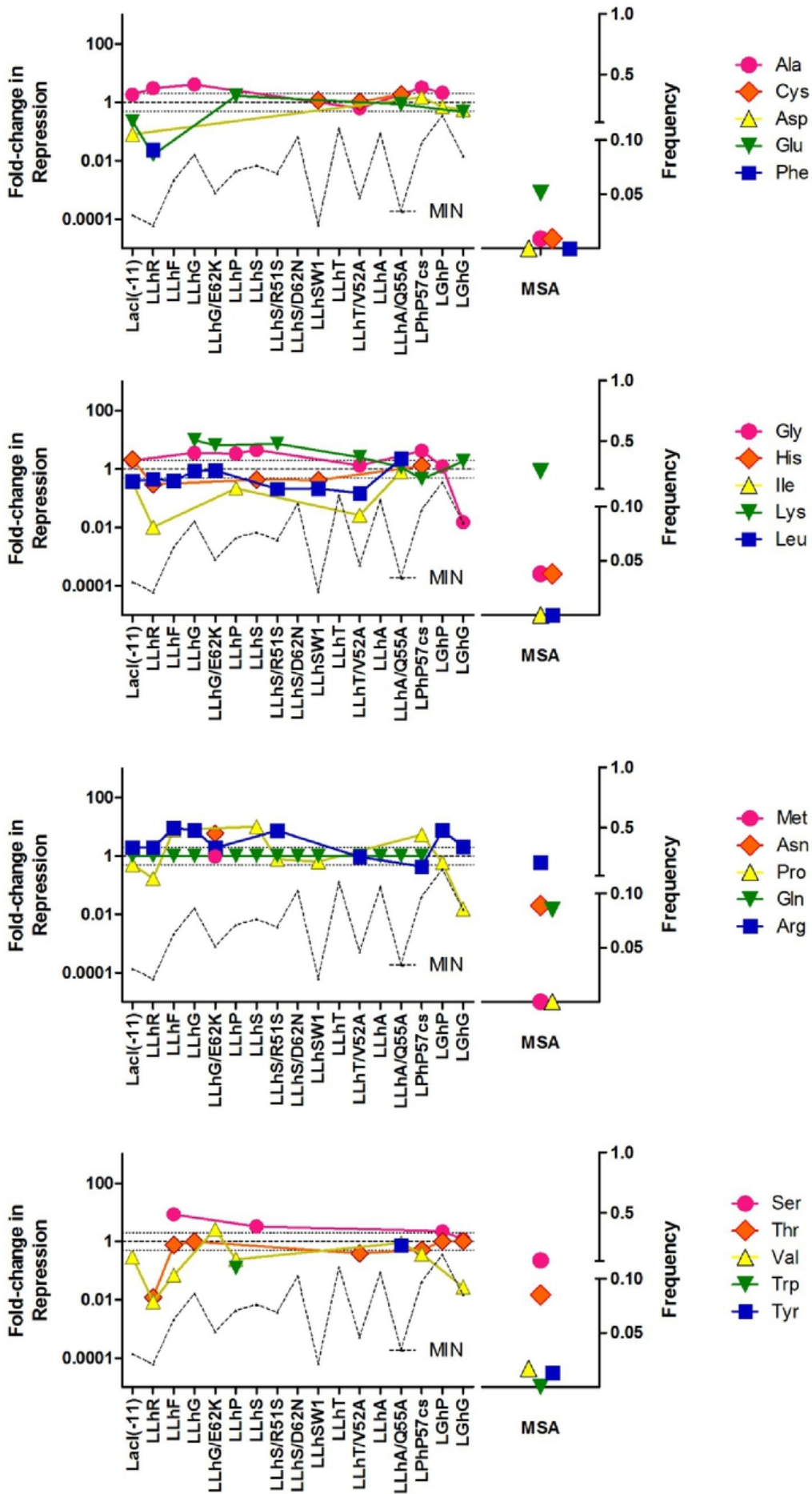


Figure S22: Parallel amino acid substitutions: Position 60

Position 61

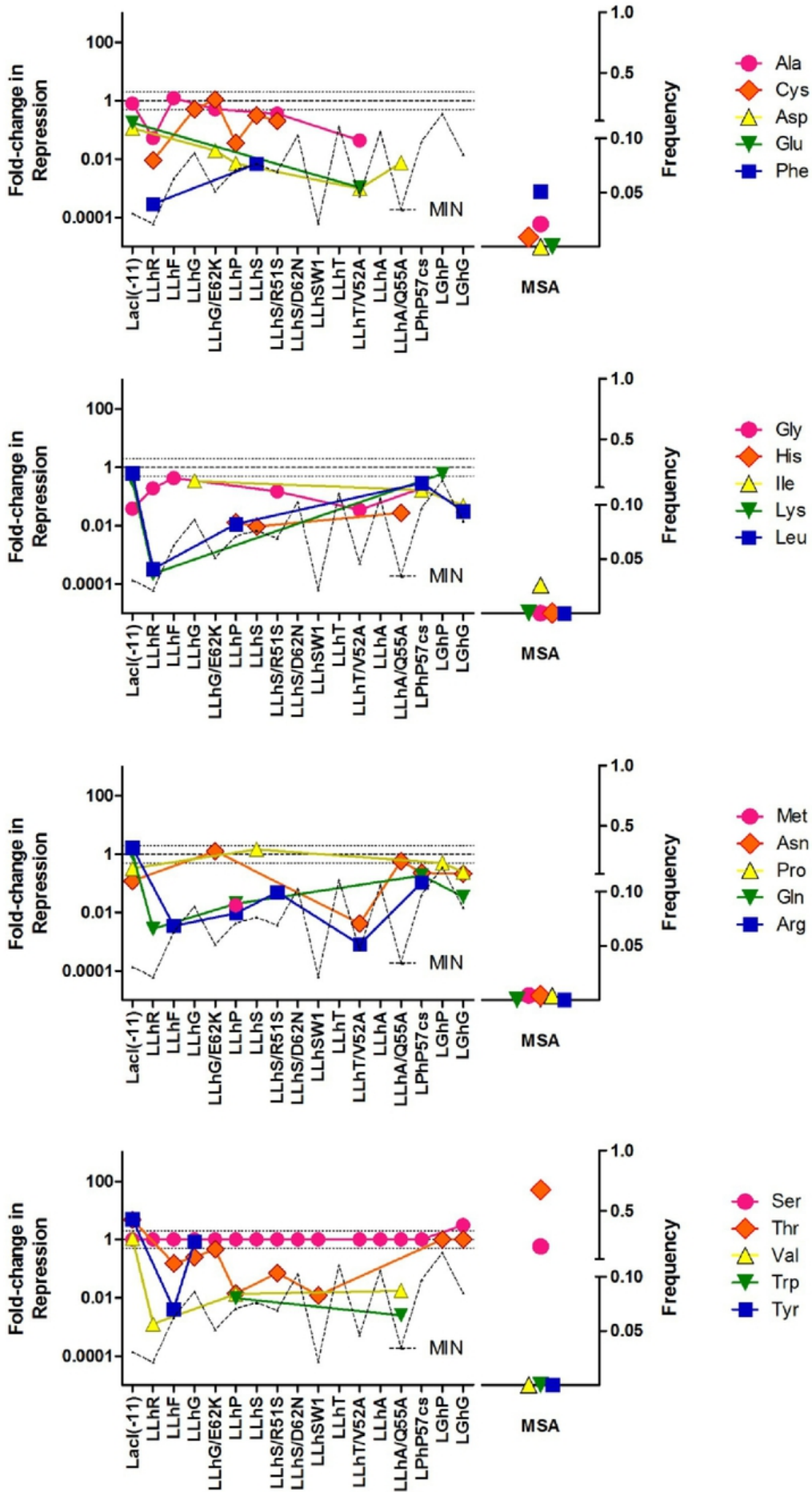


Figure S23: Parallel amino acid substitutions: Position 61

Position 62

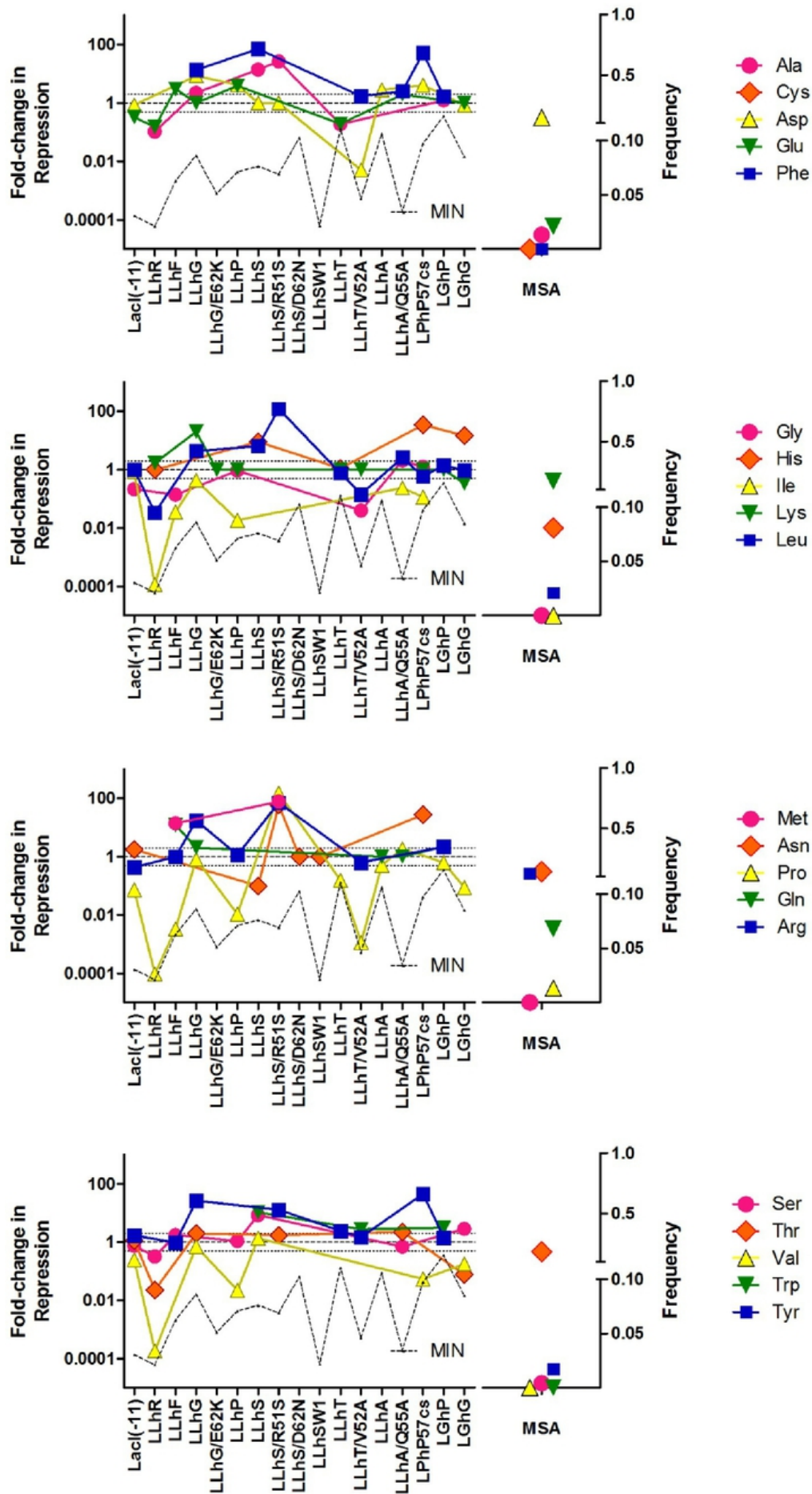


Figure S24: Parallel amino acid substitutions: Position 62