



Supplementary Information

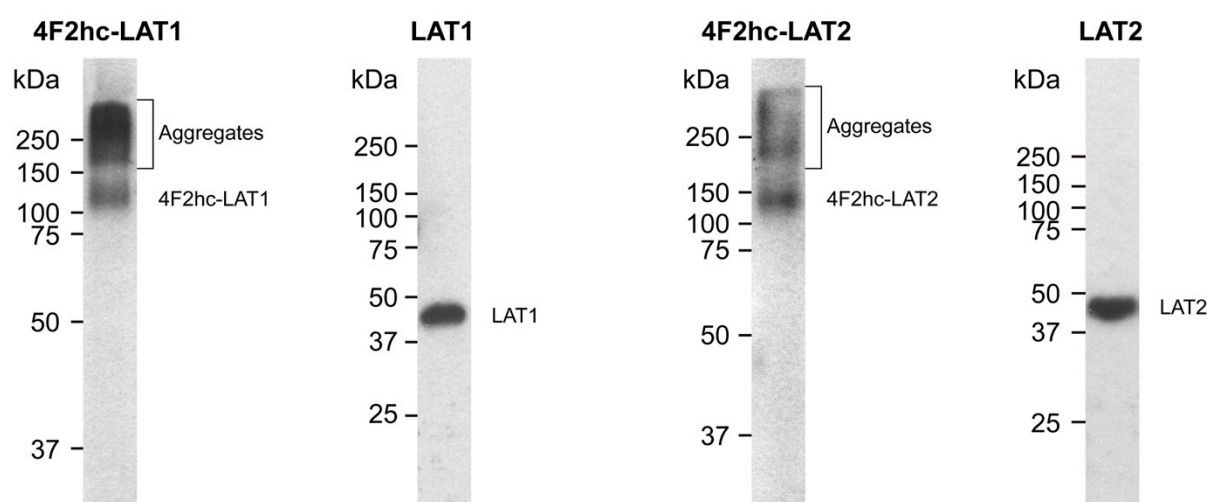
# The Heavy Chain 4F2hc Modulates the Substrate Affinity and Specificity of the Light Chains LAT1 and LAT2

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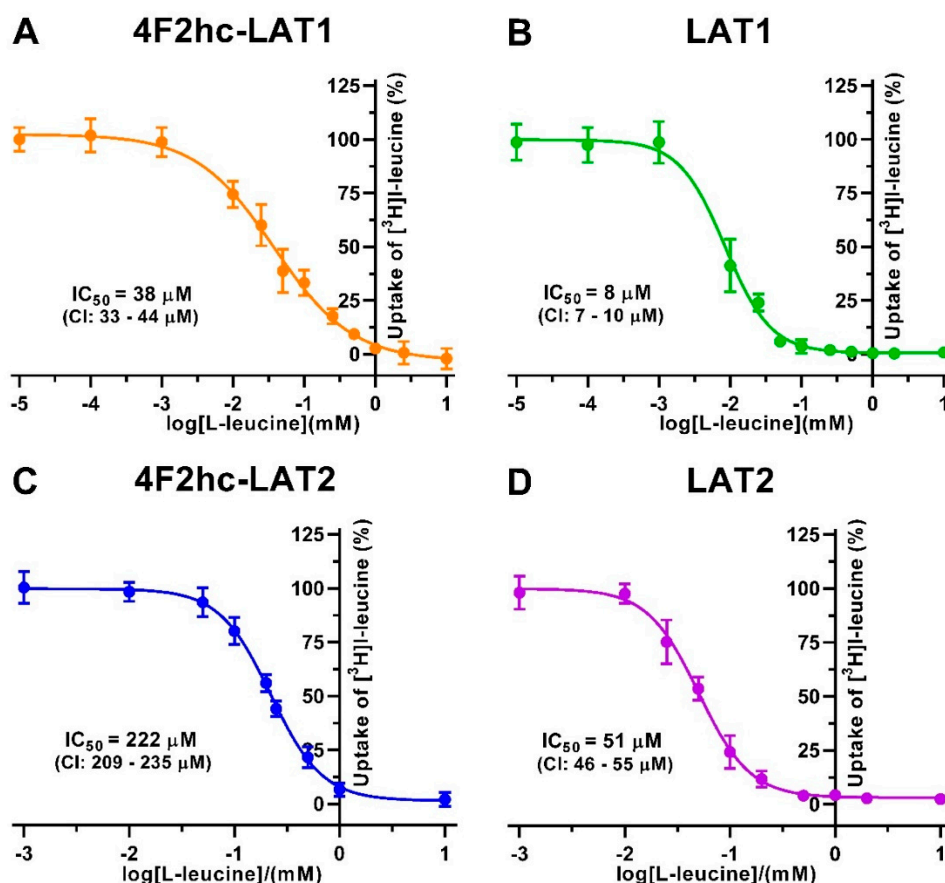
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**Figure S1.** Western blot analysis of SDS-solubilized *Pichia pastoris* cells overexpressing human 4F2hc-LAT1, LAT1, 4F2hc-LAT2 or LAT2. For 4F2hc-LAT1 and 4F2hc-LAT2, a prominent band at ~120 kDa was observed. This molecular weight is in line with the expected mass of the heteromeric amino acid transporters (HATs) [1]. As observed previously with human 4F2hc-LAT2, HATs had to some extent the tendency to aggregate in SDS-PAGE [1]. LAT1 and LAT2 Western blots displayed a single band near the 50 kDa marker, in agreement with previously described migration properties [1]. See Materials and Methods for a detailed description of immunoblotting experiments and Figure S3 for full original images of Western blots.



**Figure S2.**  $IC_{50}$  determination of L-leucine for human 4F2hc-LAT1 (A; orange), LAT1 (B; green), 4F2hc-LAT2 (C; blue) and LAT2 (D; violet). Determined  $IC_{50}$  values and 95% confidence intervals (CIs) are indicated. For data analysis, the signal of the untransformed *P. pastoris* KM71H was subtracted from all transporter signals. Cpm values of each experiment were normalized with respect to the determined upper plateau value, i.e., the fitted upper plateau value corresponds to 100%. A sigmoidal model curve was fitted to the net transport signals to obtain the  $IC_{50}$ . Data points represent means with SD from normalized data of three independent experiments, each at least in triplicate are shown. If not visible, error bars are smaller than symbols.

## References

- Costa, M.; Rosell, A.; Alvarez-Marimon, E.; Zorzano, A.; Fotiadis, D.; Palacin, M. Expression of human heteromeric amino acid transporters in the yeast *Pichia pastoris*. *Protein Expr. Purif.* **2013**, *87*, 35-40.

