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446 **Figure S22. Comparison of alternative methods for estimating reference signatures of**  
 447 **cell-types.**

448 Considered are 1) the Negative Binomial (NB) regression model, which accounts for batch  
 449 and technology effects, and 2) a hard-coded method that computes average count of each  
 450 gene in each reference cluster (see Suppl. Methods for details).

451 **A.** Consistency of cell abundance estimates when using cell2location with signatures  
 452 derived using the 2 considered methods (X-, Y-axis), pooled across all cell types and  
 453 locations. Left: mouse brain mapped using paired single nucleus RNA-seq reference  
 454 of 59 cell types. Right: human lymph nodes mapped using single cell RNA-seq  
 455 reference composed of multiple batches and 34 cell types (3 organs, 2 technologies).  
 456 2D histogram counts (colour) is shown and R2 denotes Pearson correlation.

457 **B.** Assessment of the accuracy of alternative variants of cell2location (Suppl. Methods).  
 458 Considered is the full cell2location model in conjunction with two alternative  
 459 approaches to estimate reference signatures (NB regression and hard-coded).

460 Additionally, we considered a simplified version of cell2location without specific  
461 features: prior factorisation of cell abundance  $w_{sf}$  and gene-specific technology  
462 scaling  $m_g$  (used in conjunction with the NB regression model, Suppl. Methods). The  
463 assessment was performed using the same procedure as shown in Fig 4F-I and Fig  
464 S21.

465 C. Spatial plots (X, Y-axis) display estimated relative cell abundance (colour intensity) of  
466 3 cell populations (columns) for 2 considered reference expression signature  
467 estimation methods (rows). T\_CD4+\_TfH\_GC is expected to be present in the GC  
468 zone whereas T\_CD4+\_TfH and T\_CD4+ are expected to be excluded from GC,  
469 especially dark zone GC (see Fig 4E).  
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