Supplementary Figures for "Digital expression profiling of the compartmentalized translatome of Purkinje neurons"

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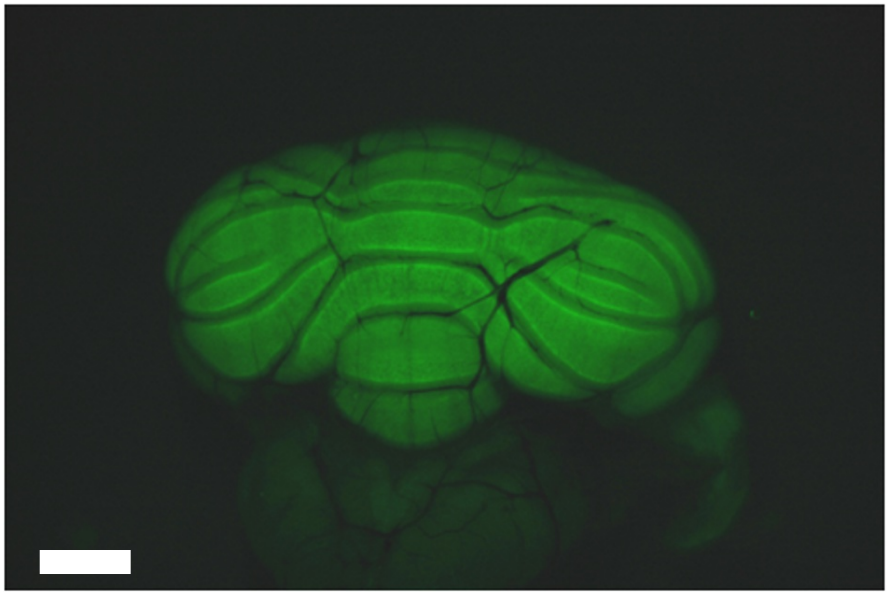


Figure S1: Transduction of EYFP-RPL10A fusion protein into Purkinje cells, by a AAV2-2/8 hybrid virus, 28 days after injection in the cerebellum at PN4. Convection-Enhanced Diffusion resulted in widespread viral transduction in the vermis along the rostro-caudal axis and often in lateral hemispheres. Note that all slices used in this study were taken from the vermis. Size bar 10 mm.

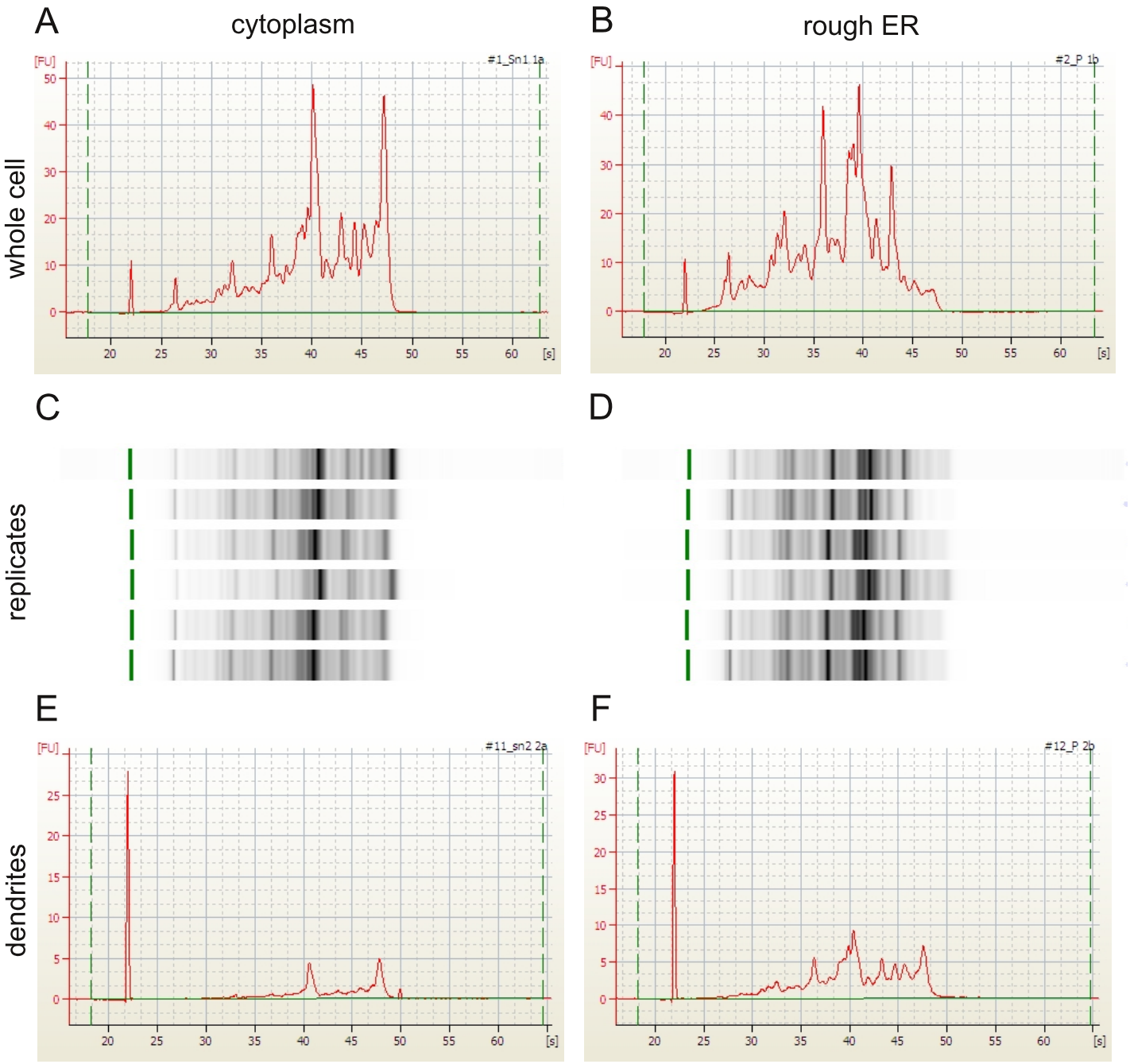


Figure S2: Size profiles for (A) one replicate of whole cell, cytoplasm, (B) one replicate of whole cell, rER fraction, (C) all six replicates of whole cell, cytoplasm (D) all six replicates of whole cell, rER fraction, (E) one replicate of dendrites, cytoplasmic fraction, (F) one replicate of dendrites, rER fraction.

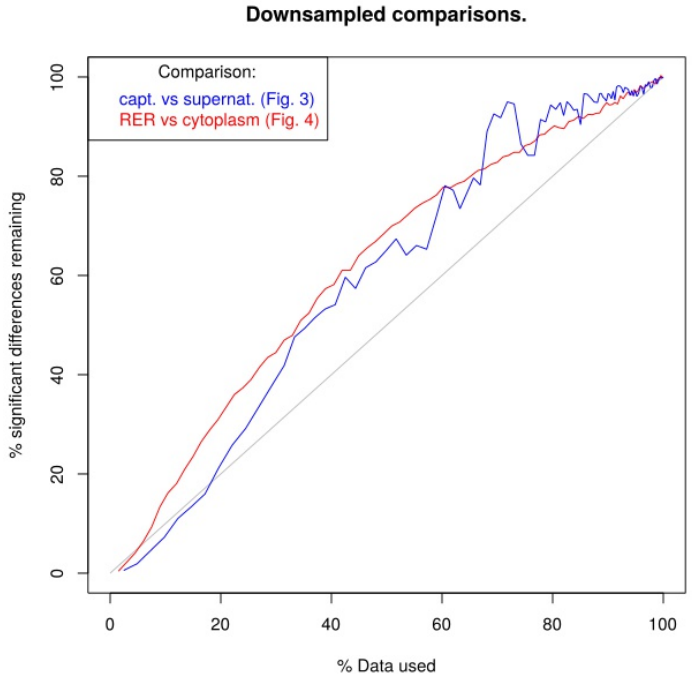


Figure S3: Effect of the downsampling of the libraries on the number of significant differences observed in the statistical tests performed for Figure 3 (ribosome capture vs. supernatant) and Figure 4 (rough ER vs. cytoplasm). The figure shows diminishing returns for adding extra data. For instance, with only 50 % of the data, more than 60% of the differences are observed. The trend of the curves suggests that doubling the depth (and cost) of the libraries would be far from doubling the the number of significant differences.

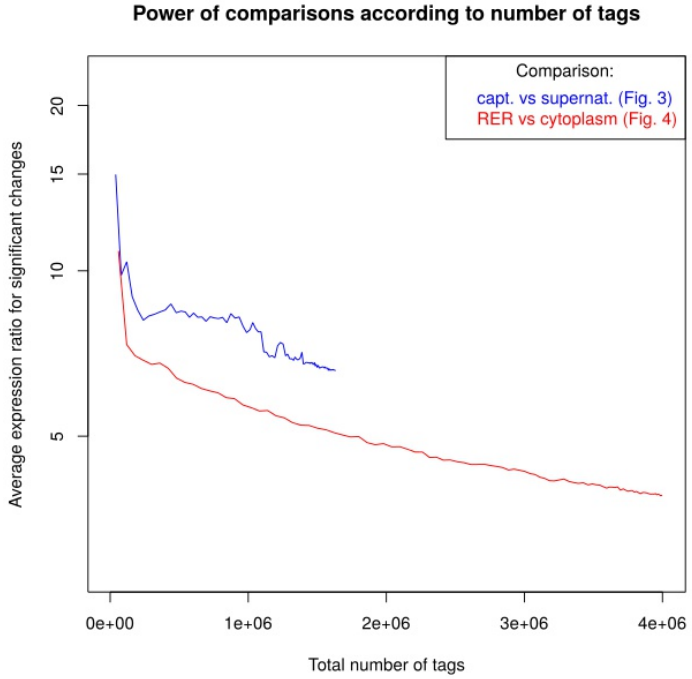
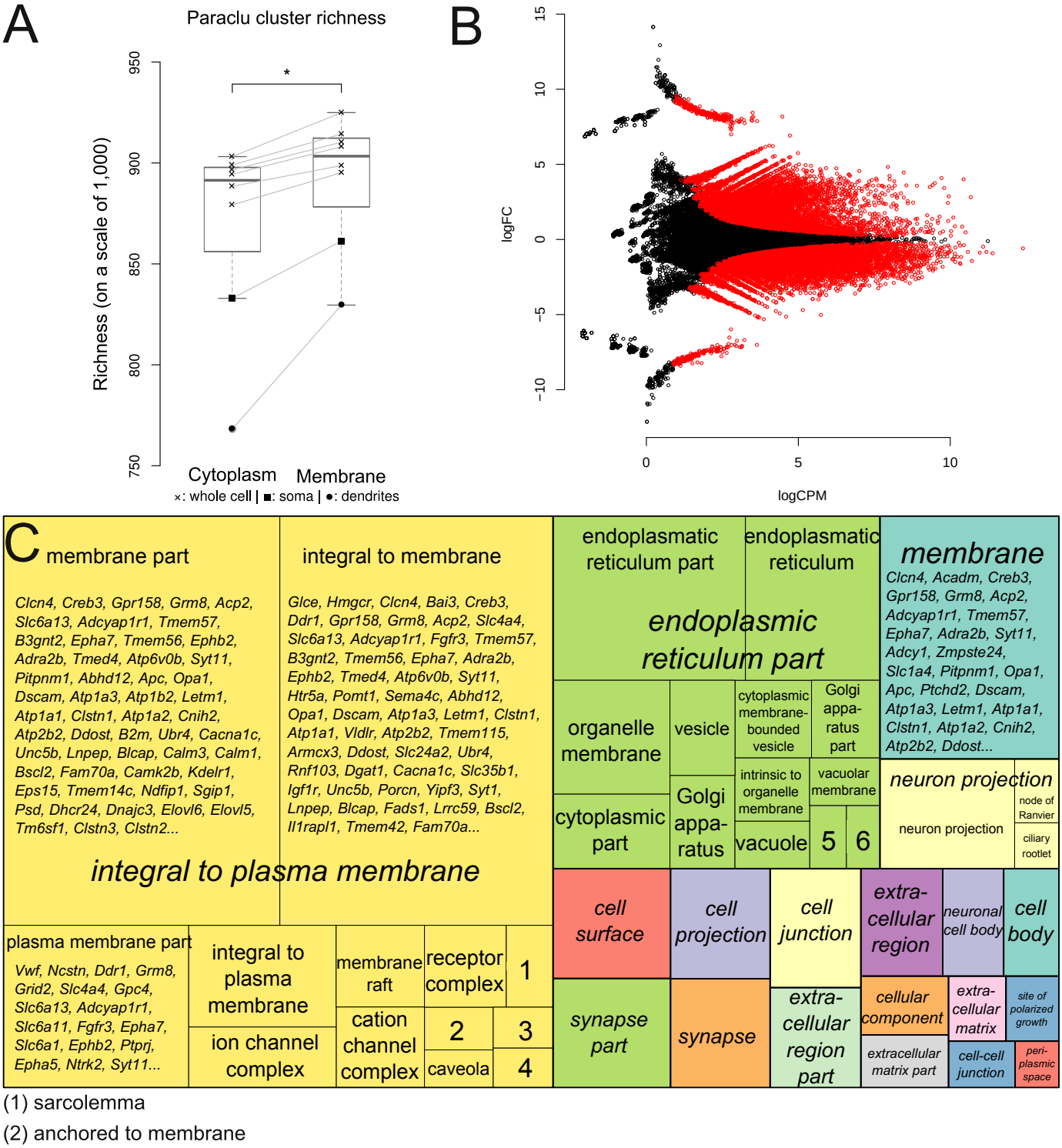
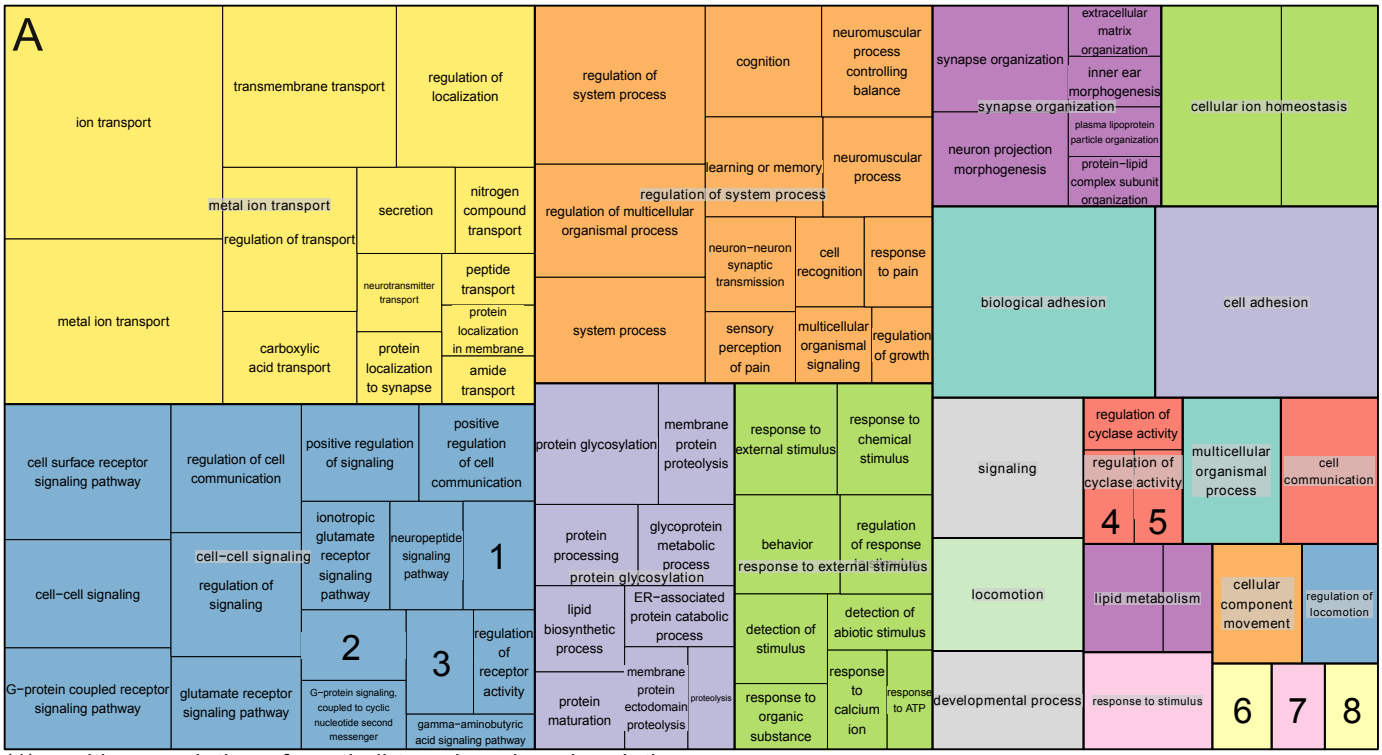


Figure S4: Effect of the downsampling of the libraries on the power to detect smaller significant differences in the statistical tests performed for Figure 3 and 4. In average, the absolute fold change of the differentially represented clusters in ribosome capture vs. supernatant (blue curve) is 6.6 times, and would still be in that order of magnitude with half of the data removed. Since this comparison is aimed at discovering markers of Purkinje cells, that are strong changes, the current data provides enough power. In the case of the comparison between cell compartments (red curve), the power was higher because of the larger number of replicates (n=6) and the deeper data (more than twice the number of tags in total). The trend of the curve suggests that sequencing deeper will not dramatically change the power of the comparison.



(1) sarcolemma
(2) anchored to membrane
(3) T-tubule
(4) external encapsulating structure part
(5) endoplasmic reticulum - Golgi intermediate compartment
(6) endoplasmic reticulum - Golgi intermediate compartment membrane

Figure S5: (A) Richness in cytoplasmic and rER (membrane) fractions. Crosses: whole slices; dots: dendritic libraries; squares somatic libraries. Thin lines join pairs of libraries from the same pool of lobules. (B) Differential gene expression between the rough endoplasmic reticulum (up) and cytoplasm (down). Clusters with statistically significant (FDR < 0.1) expression are red, otherwise green. (C) Gene Ontology analysis of rER-specific, Purkinje-enriched genes visualized as a treemap. Representative terms (shown in italics) have been chosen for each cluster of similar terms based on their enrichment p-values. A few genes comprising some of the representative Gene Ontology terms are shown.



(1) positive regulation of peptiydl-tyrosine phosphorylation
(2) regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate selective glutamate receptor activity
(3) second-messenger-mediated signaling
(4) regulation of cAMP metabolic process
(5) regulation of lyase activity
(6) biological process
(7) developmental maturation
(8) negative regulation of epithelial cell proliferation

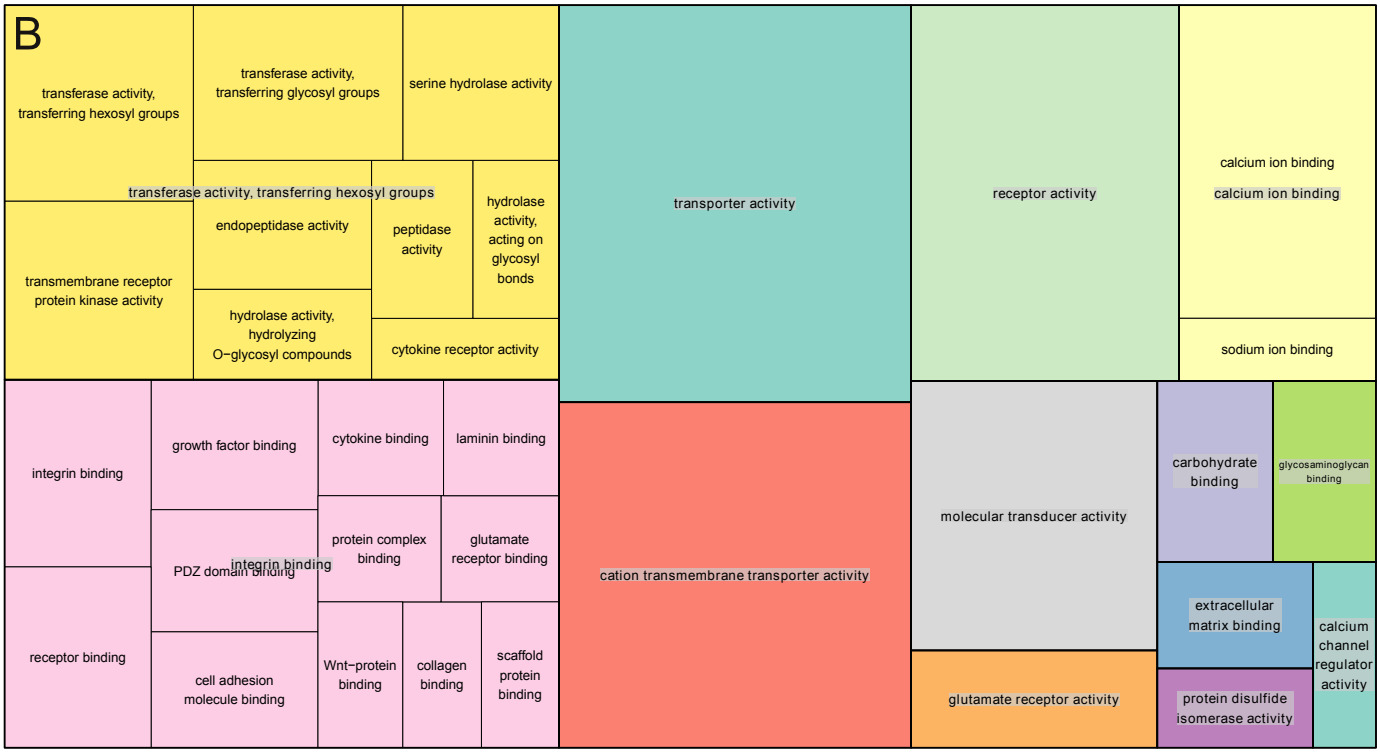


Figure S6: Result of the same Gene Ontology test as in Fig. 5, but for the categories (A) Biological Process and (B) Molecular Function, visualized as a treemap. The enriched Gene Ontology terms can be found in Table S5.

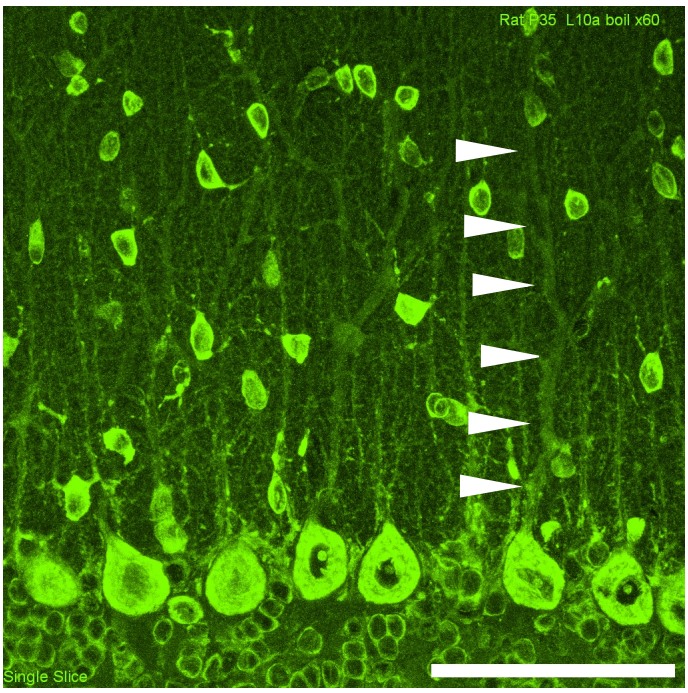
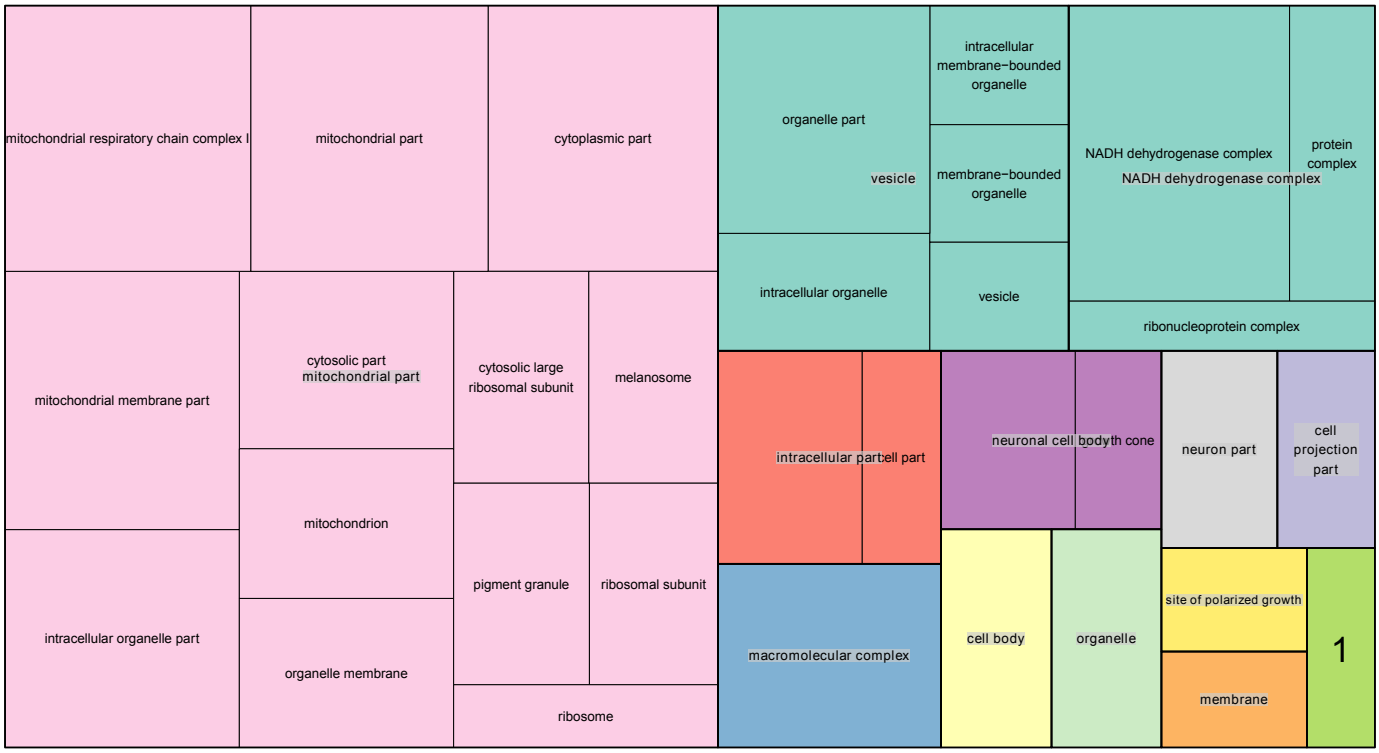


Figure S7: Micrograph of endogenous RPL10A in PCs, detected by immunofluorescence. Its expression is predominantly somatic but is also present at a much lower level in the dendrites of the PC (arrowheads). Size bar = 100 μm.



(1) membrane-enclosed lumen

Figure S8: Treemap of Gene Ontology terms significantly enriched for the dendritic clusters, cellular component category. The enriched Gene Ontology terms can be found in Table S7.

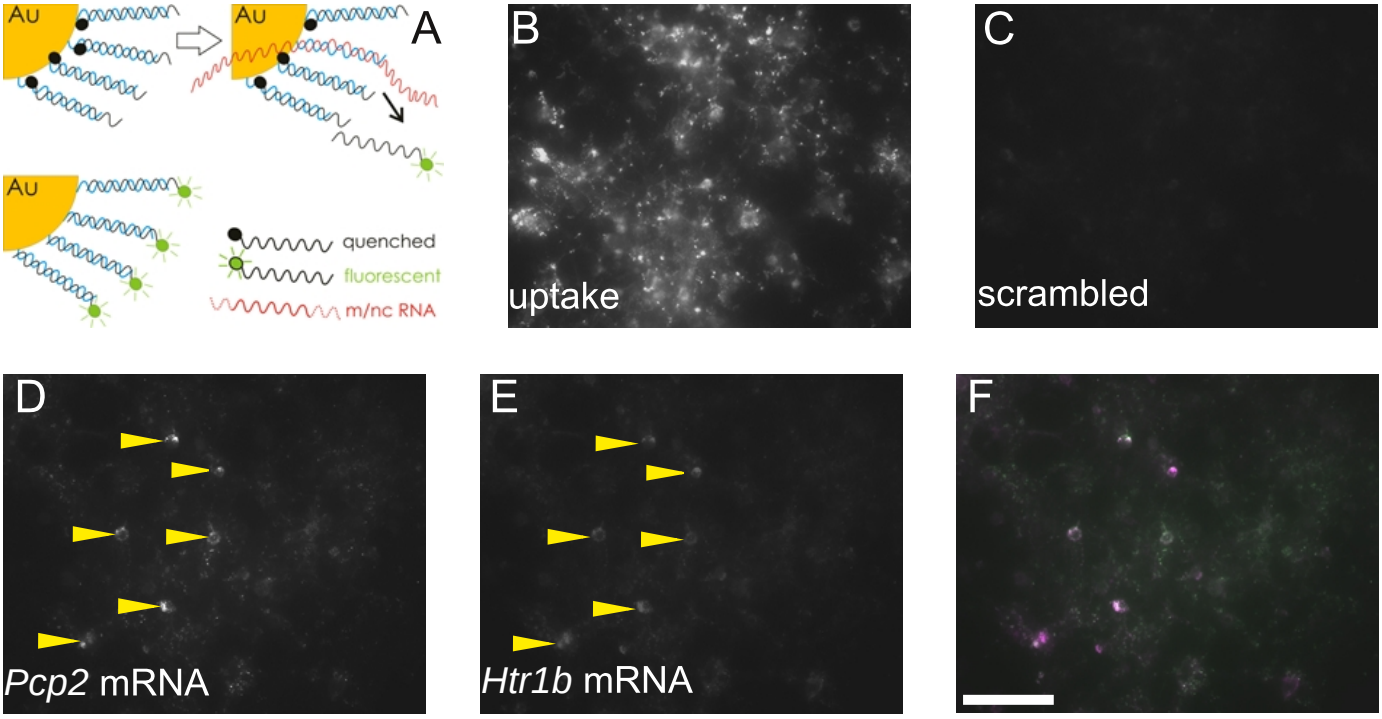


Figure S9: Live cell in situ hybridization with SmartFlare. (A) a: fluorophore-conjugated DNA probe (black) is quenched when bound to gold nanocluster-conjugated complementary DNA (blue). b: hybridization with endogenous RNA displaces the probes, unquenching the fluorophore. c: uptake control. (B) Ubiquitous endocytosis of the uptake control in cerebellar culture. (C) A scrambled probe with no endogenous target. (D) Detection of *Pcp2* transcripts, arrows indicate putative Purkinje cells. (E) Detection of *Htr1b* mRNA. (F) colocalization of *Pcp2* (green) and *Htr1b* (magenta). All images background-substracted, scalebar: 100µm.

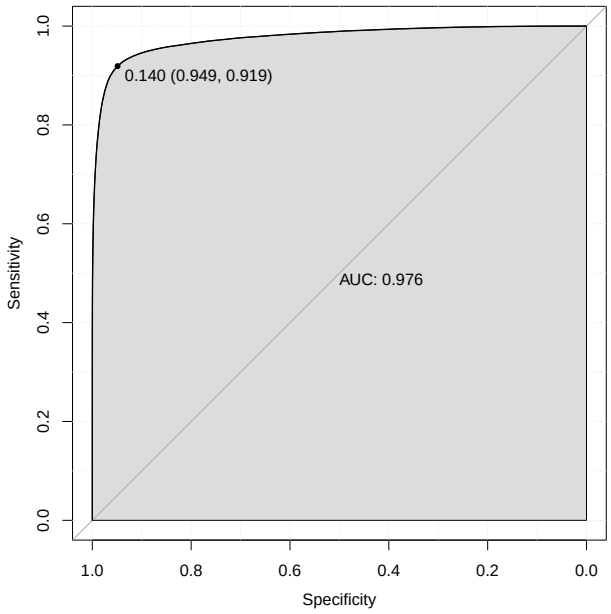


Figure S10: AUC (Area-Under-Curve) of the trained classifier. The classification score of 0.14 at a specificity of 0.949 and sensitivity of 0.919 yields the best separation of clusters into low and high confidence.