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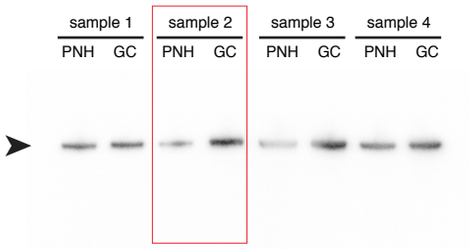
Supplementary information

Neuronal subtype-specific growth cone and soma purification from mammalian CNS via fractionation and fluorescent sorting for subcellular analyses and spatial mapping of local transcriptomes and proteomes

In the format provided by the authors and unedited

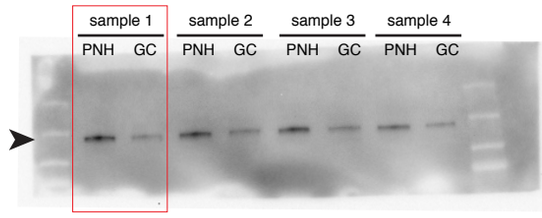
GAP43

mouse-anti-GAP43
(MAB347, Chemicon, RRID:AB_94881, 1:2,000)



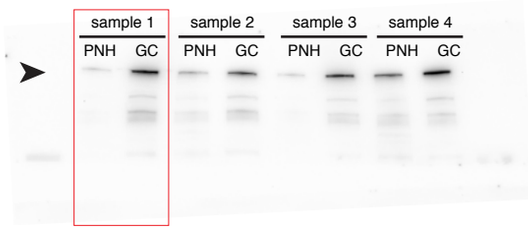
Gm130

mouse-anti-Gm130
(610823, BD Biosciences, RRID:AB_398142, 1:3,000)



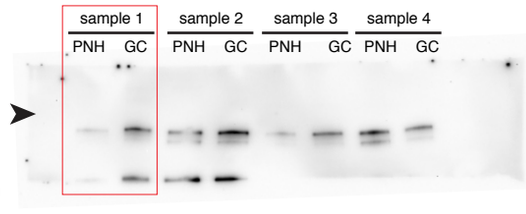
mTOR

rabbit-anti-mTOR
(affinity purified, A300-504A, Bethyl Labs, RRID:AB_2105648, 1:500)



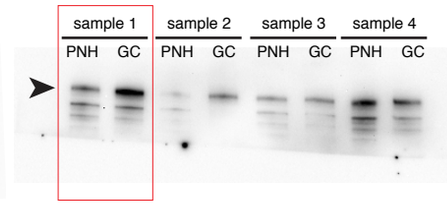
Larp1

rabbit-anti-LARP1
(PA5-62398, ThermoFisher, RRID:AB_2643281, 1:1,000)



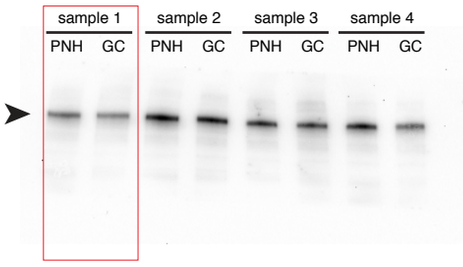
Raptor

rabbit-anti-raptor
(42-4000, ThermoFisher, RRID:AB_2533523, 1:1,000)



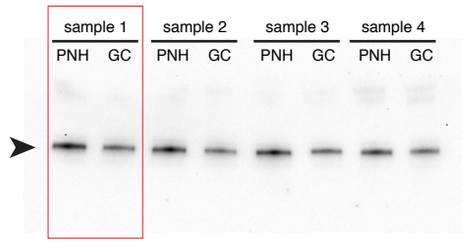
Rictor

rabbit-anti-RICTOR
(2140, Cell Signaling Technology, RRID:AB_2179961, 1:1,000)



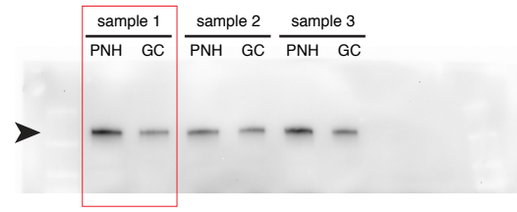
TSC1

rabbit-anti-TSC1
(PA5-20131, ThermoFisher, RRID:AB_11154814, 1:1,000)



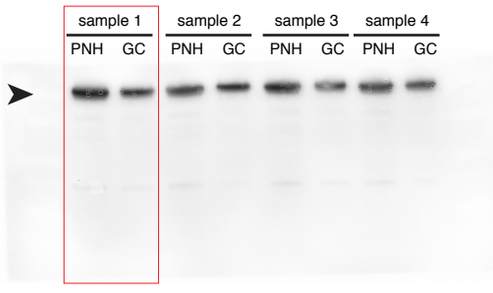
Lamp1

mouse-anti-LAMP1
(1D4B, Developmental Studies Hybridoma Bank, RRID:AB_2134500, 1:500)



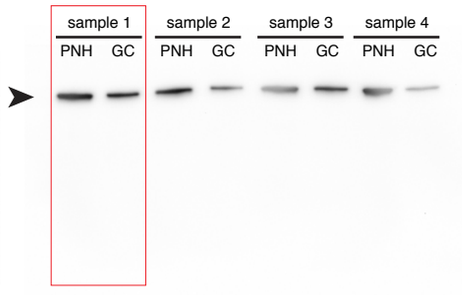
Tubb3

mouse-anti-tubulin
(MMS-435P, Covance, RRID:AB_2313773, 1:2,000)



Actb

mouse-anti- -actin
(A5441, Sigma, RRID:AB_476744, 1:2,000)



Supplementary Figure 1: GCF samples show robust enrichment/depletion for compartment-specific markers, mTOR machinery, and cytoskeletal components. Replicates of 3-4 independent biological samples for PNH and GCF western blotted for various compartment-specific marker proteins (GAP43, Gm130), mTOR and associated proteins (mTOR, Larp1, Raptor, Rictor, TSC1, Lamp1) and cytoskeletal components (Tubb3, Actb). This supplemental figure shows the full blots of the condensed data presented in Figure 3C to visualize specificity of antibody detection, and robustness across independent biological replicates. Representative samples depicted in Figure 3C are highlighted by red rectangles, and, in instances when antibodies detected several bands, the band of interest is highlighted with an arrow head. (mouse-anti-β-actin (A5441, Sigma, RRID:AB_476744, 1:2,000); mouse-anti-GAP43 (MAB347, Chemicon, RRID:AB_94881, 1:2,000); mouse-anti-Gm130 (610823, BD Biosciences, RRID:AB_398142, 1:3,000); mouse-anti-LAMP1 (1D4B, Developmental Studies Hybridoma Bank, RRID:AB_2134500, 1:500); rabbit-anti-LARP1 (PA5-62398, ThermoFisher, RRID:AB_2643281, 1:1,000); mouse-anti-MAP2 (M1406, Sigma, RRID:AB_477171, 1:1,000); rabbit-anti-mTOR (affinity purified, A300-504A, Bethyl Labs, RRID:AB_2105648, 1:500); rabbit-anti-raptor (42-4000, ThermoFisher, RRID:AB_2533523, 1:1,000); rabbit-anti-RICTOR (2140, Cell Signaling Technology, RRID:AB_2179961, 1:1,000); rabbit-anti-TSC1 (PA5-20131, ThermoFisher, RRID:AB_11154814, 1:1,000); mouse-anti-tubulin (MMS-435P, Covance, RRID:AB_2313773, 1:2,000); isotope-specific secondary antibodies were HRP-conjugated and cross-absorbed (Life technologies)). For detailed methods, please see Poulopoulos*, Murphy* et al., Nature, 201913.