Pericyte-Specific Secretome Profiling in Hypoxia Using TurboID in a Multicellular *In Vitro* Spheroid-Model

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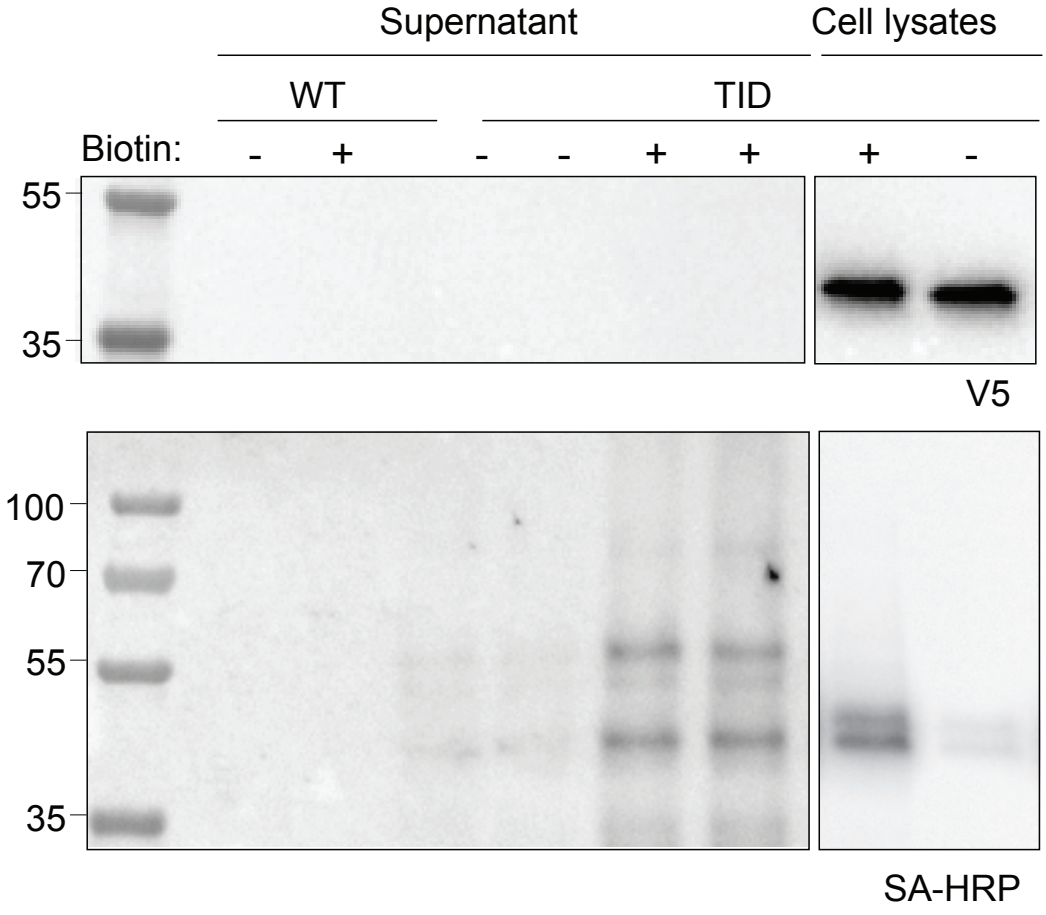
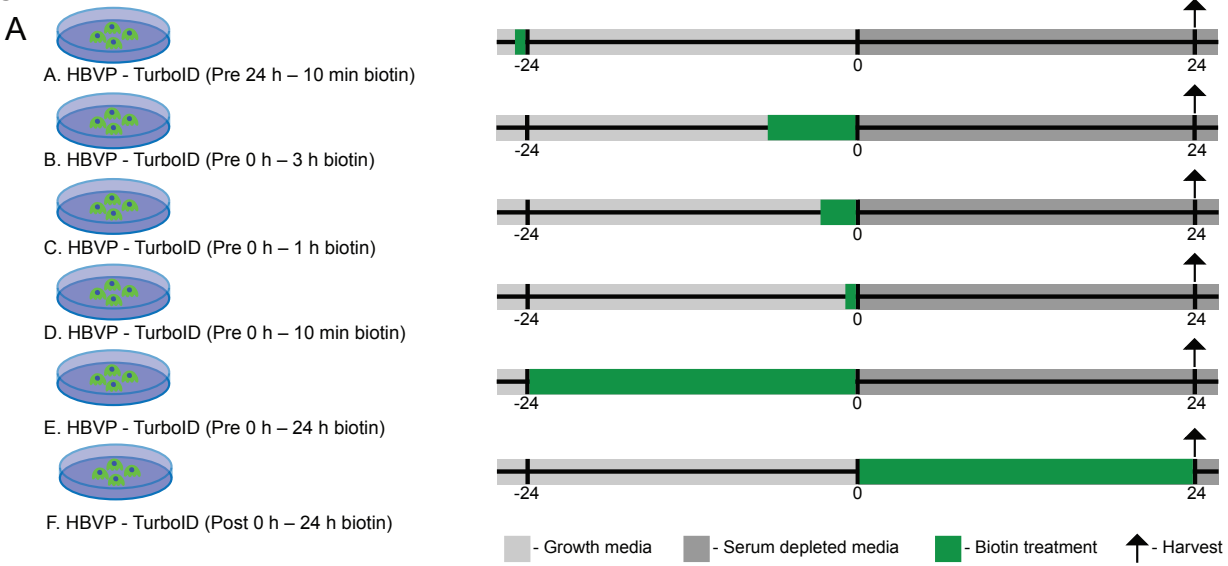
Contains Supplemental Figs. S1-S8.

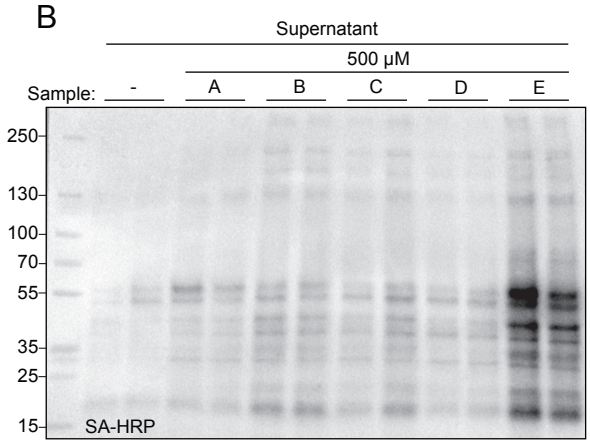
Fig. S1

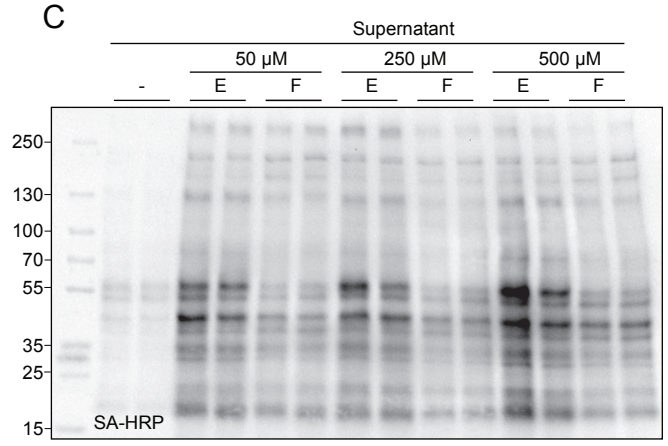


Supplemental Fig. S1. **Verification of pericyte-specific expression of TurboID.** Detection of biotinylated proteins or TurboID in the supernatant was evaluated by westerns blot with either SA-HRP or anti-V5 antibodies, respectively (left panel). Western blotting of intracellular proteins from cell lysates with either SA-HRP or anti-V5 antibodies, respectively.

Fig. S2

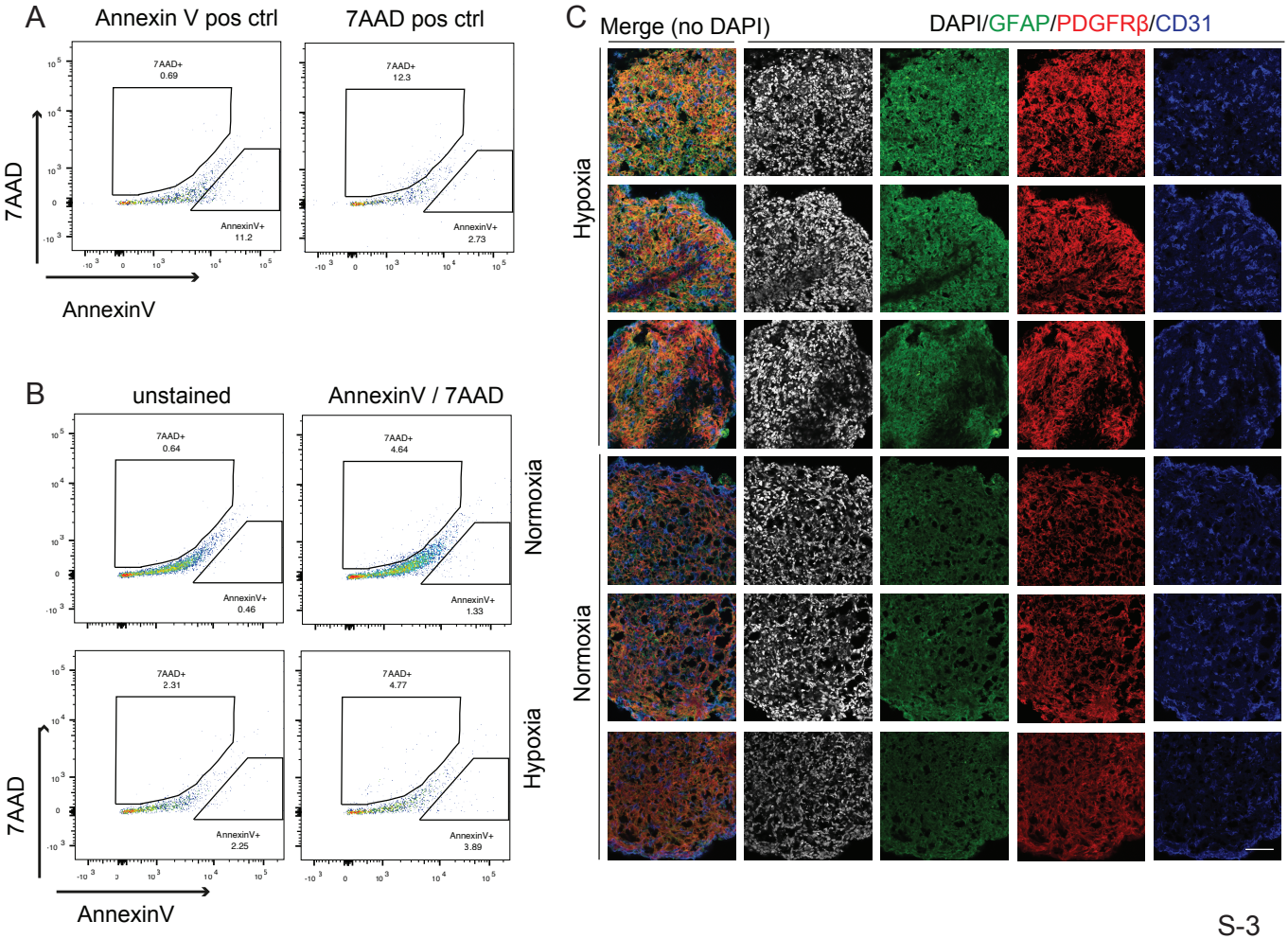






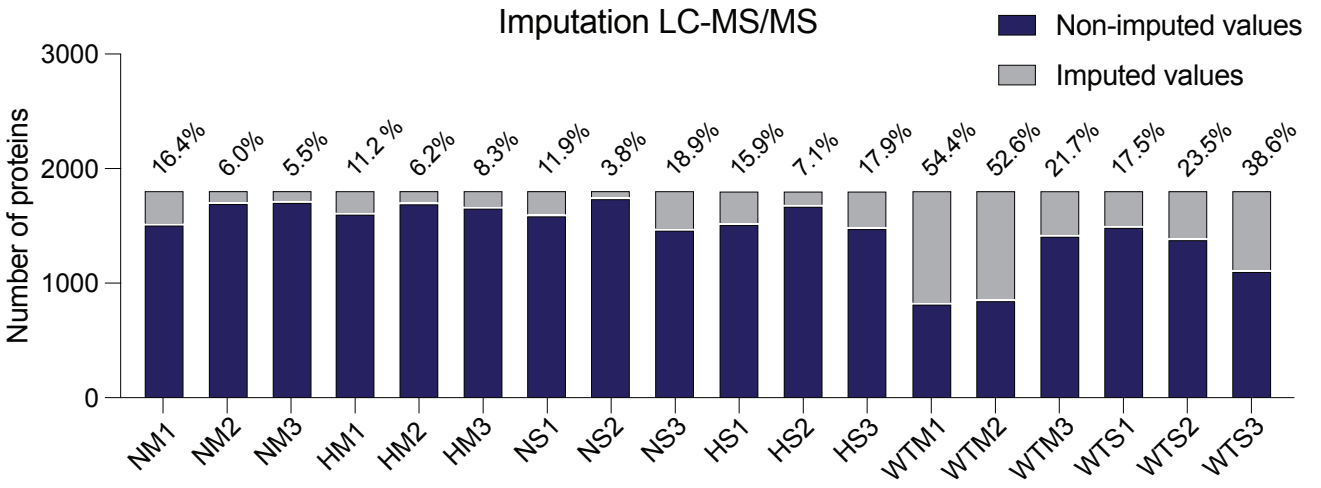
Supplemental Fig. S2. **The abundance of secreted biotinylated proteins from TurboID-pericytes is dependent on duration of biotin treatment.** A, Description of the different durations of biotin treatment. Green bar represents duration of biotin treatment, light grey bar represents growth media prior to experiment time-point 0, dark grey bar represents experimental condition of serum depleted media and arrow is time of supernatant extraction. B, Western blot with SA-HRP for the different durations of biotin treatment. C, Different concentrations of biotin (50 µM, 250 µM and 500 µM) were examined using western blot SA-HRP from supernatant of TurboID pericytes.

Fig. S3



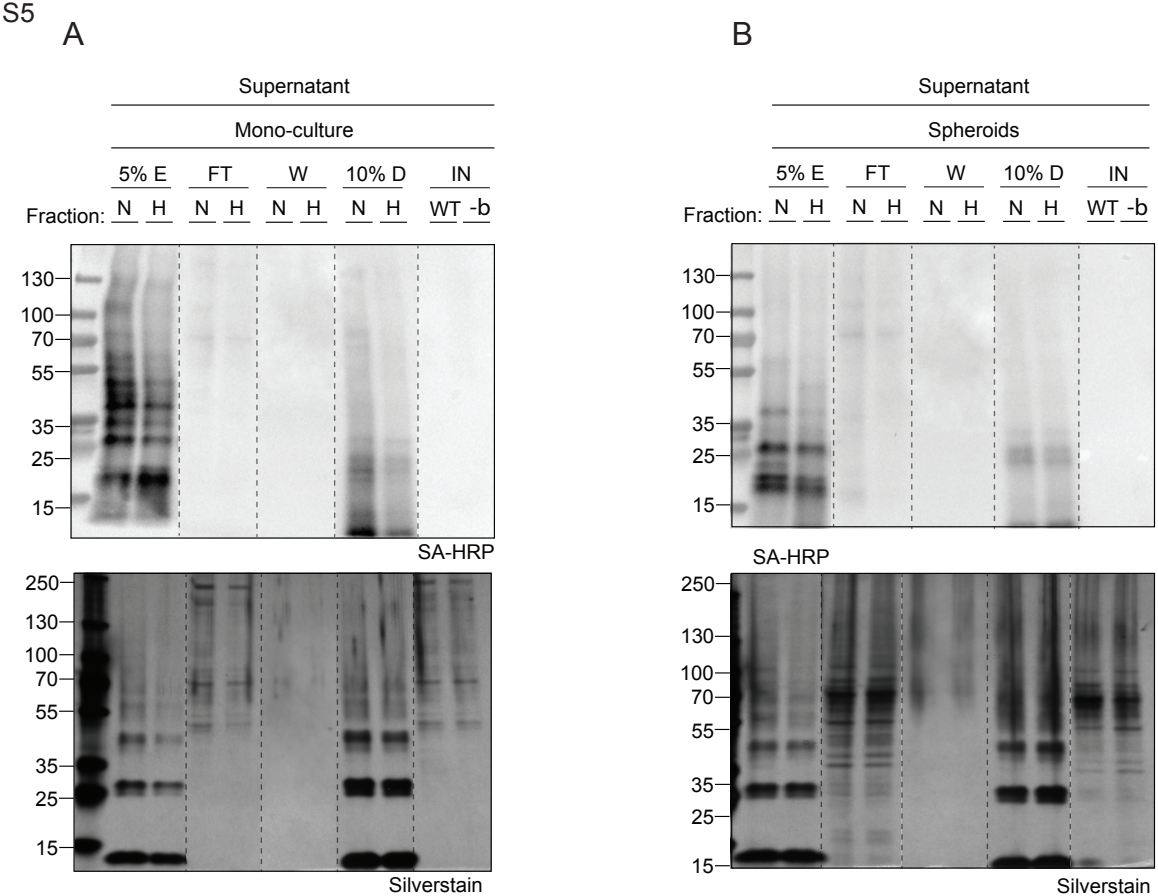
Supplemental Fig. S3. **Measurement of spheroid cell death and cell populations in normoxia and hypoxia.** A, Positive controls for the AnnexinV or 7AAD stainings of apoptotic or dead cells, respectively. B, Dissociated cells from 24h normoxic, or hypoxic spheroid cultures stained for apoptosis (AnnexinV; Normoxia=1.33%, Hypoxia=3.89%) and dead cells (7AAD; Normoxia=4.64%, Hyoxiap=4.77%), n=1. C, Confocal images with GFAP (astrocytes), PDGFRβ (pericytes), and CD31 (endothelial cells). Scale bar is 100 μm.

Fig. S4



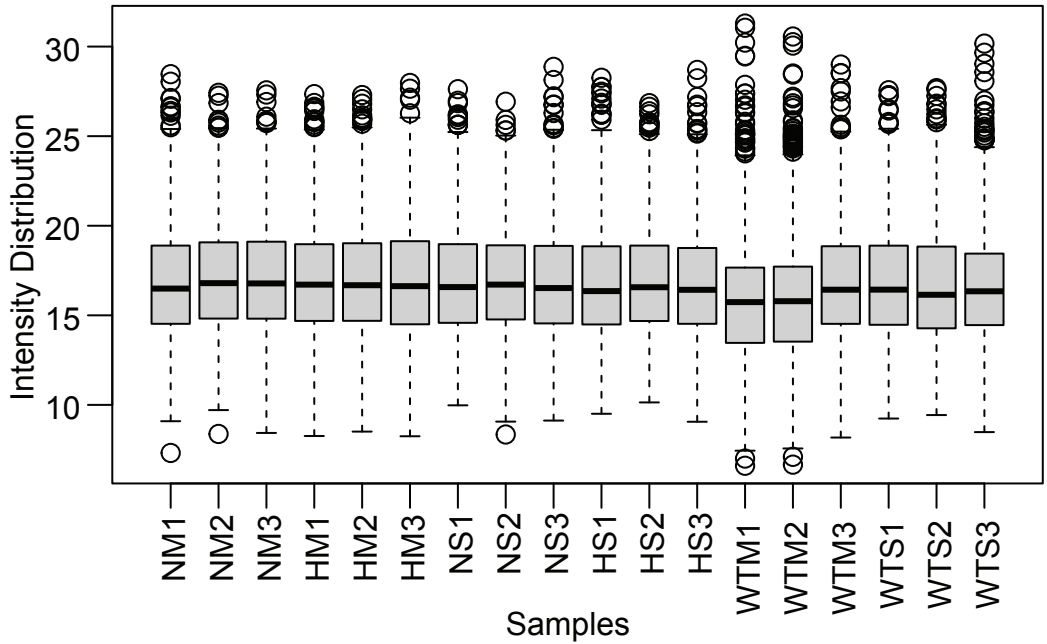
Supplemental Fig. S4. **Imputations for LC-MS/MS.** Bar plots showing the number and percentage of imputed values in grey and non-imputed values in blue. Abbreviations: NM, Normoxia–Monoculture; HM, Hypoxia–Monoculture; NS, Normoxia–Spheroids; HS, Hypoxia–Spheroids; WTM, wild-type monoculture; WTS, wild-type spheroids.

Fig. S5

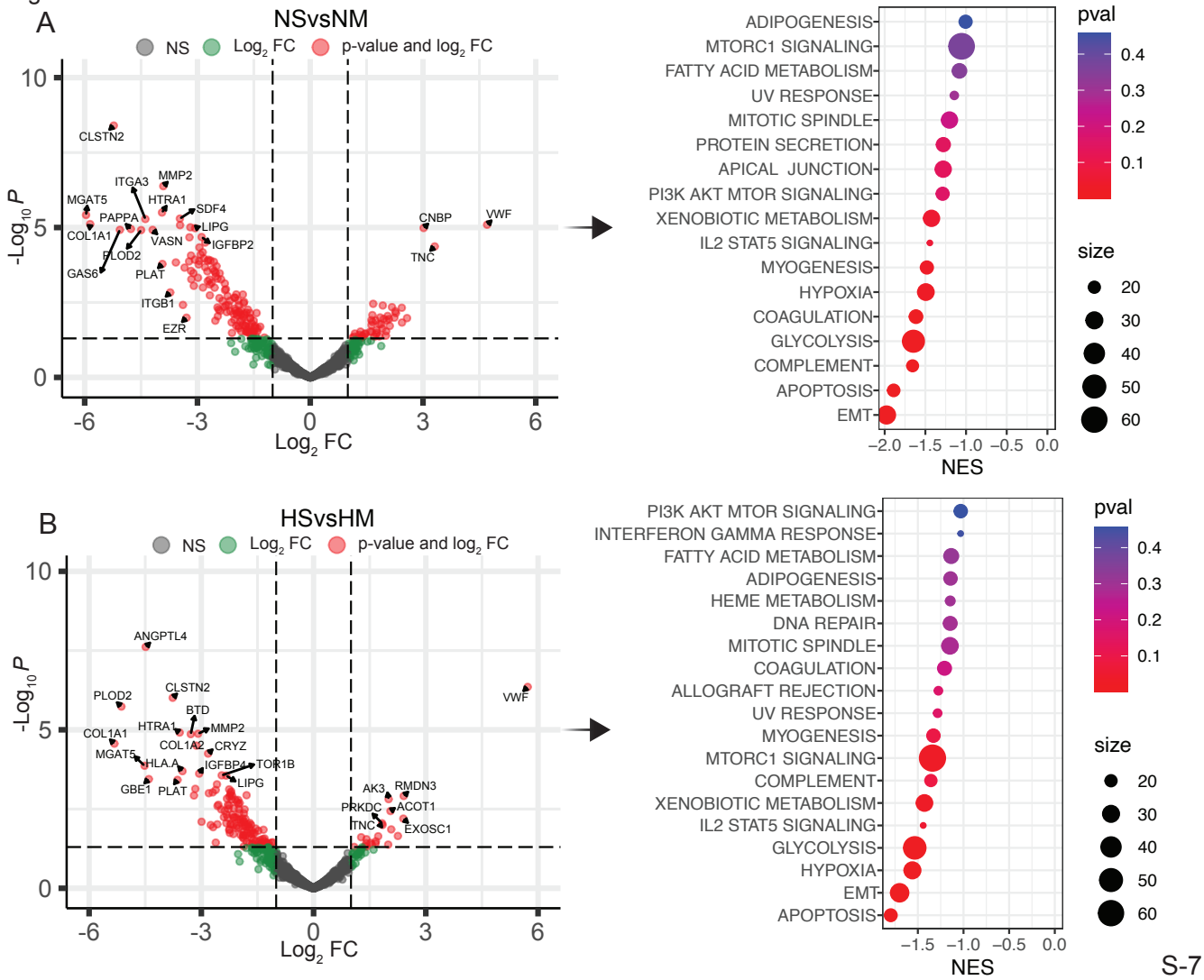


Supplemental Fig. S5. **Characterizing the protein-purification efficiency for LC-MS/MS.** A, Western blotting with SA-HRP or silver stain were used to evaluate the purification efficiency of supernatant samples used for LC-MS/MS analysis originating from monocultures of TurboID-pericytes and negative controls. B, Western blotting with SA-HRP or silver stain were used to evaluate the purification efficiency of supernatant samples used for LC-MS/MS analysis originating from spheroids of TurboID-pericytes and negative controls. The 5% E enriched fraction corresponds to 5% of the protein enriched PrS beads prior to on-bead digestion. The 10% D digestion fraction corresponds to the PrS beads post on-bead digestion. Abbreviations: FT, flow-through; W, wash; IN, input.

Fig. S6

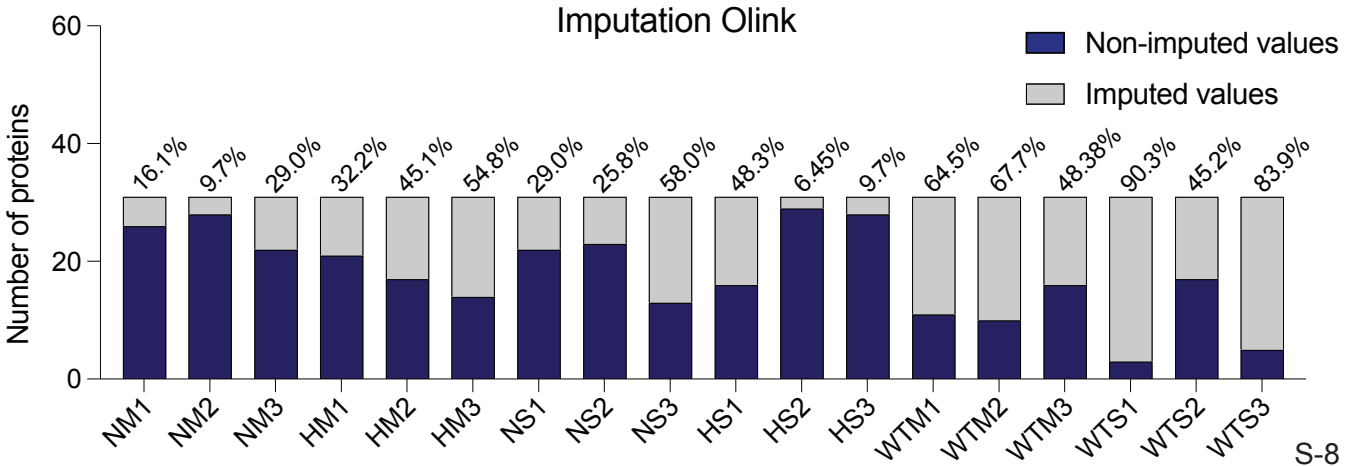


Supplemental Fig. S6. **Median-normalization of LC-MS/MS proteomic data.** The median protein signal intensity from each replicate (1-3) is illustrated in a box plot of imputed, normalised MS/MS data. The numbering indicate independent replicates, n=3. Abbreviations: NM, Normoxia–Monoculture; HM, Hypoxia–Monoculture; NS, Normoxia–Spheroids; HS, Hypoxia–Spheroids; WTM, wild-type monoculture; WTS, wild-type spheroids.



Supplemental Fig. S7. **The pericyte secretome changes depending on mono- or spheroid-culture conditions.** A, Volcano plot showing the differentially expressed proteins between NS compared to NM (left panel) and the significantly regulated signalling pathways from the reactome database (right panel). B, Volcano plot showing the differentially expressed proteins between HS compared to HM (left panel) and the significantly regulated signalling pathways from the reactome database (right panel), n=3. Abbreviations: NM, Normoxia–Monoculture; HM, Hypoxia–Monoculture; NS, Normoxia–Spheroids; HS, Hypoxia–Spheroids.

Fig. S8



Supplemental Fig. S8. **Imputations for Olink.** Bar plots showing the number and percentage of imputed values in grey and non-imputed values in blue. Abbreviations: NM, Normoxia–Monoculture; HM, Hypoxia–Monoculture; NS, Normoxia–Spheroids; HS, Hypoxia–Spheroids; WTM, wild-type monoculture; WTS, wild-type spheroids.