Supplement: Evidence of traumatic brain injury in muskoxen (Ovibos moschatus). Code for heatmaps and linegraphs

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Packages library(ggplot2) library(readxl) library(plotly) library(ggpubr) library(viridis) Heatmaps Muskox 1 / MO1 / Generic 0191 Read in data NTCs <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=3) neuropils <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=4) neurons <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=5) Heat Map 1 Muskox1\_heatmap <- ggplot(neuropils, aes(x=x, y=y))+ stat\_density\_2d(aes(fill=..level..), geom=“polygon”)+ scale\_fill\_viridis\_c(option = “viridis”, alpha=0.7)+ geom\_point(data=NTCs, color=“black”, size=2, alpha=0.5)+ geom\_point(data=neurons, shape=23, colour=“pink”, fill=“pink”, size=2, alpha=0.8)+ labs(title=“A”)+ theme\_void()

Muskox1\_heatmap

Muskox 2 / MO2 / Generic 0194 Read in data NTCs <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=6) neuropils <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=7) neurons <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=8) Heat Map 2 Muskox2\_heatmap <- ggplot(neuropils, aes(x=x, y=y))+ stat\_density\_2d(aes(fill=..level..), geom=“polygon”, bins=20)+ scale\_fill\_viridis\_c(option = “viridis”, alpha=0.7)+ geom\_point(data=NTCs, color=“black”, size=1, alpha=0.5)+ geom\_point(data=neurons, shape=23, colour=“pink”, fill=“pink”, size=2, alpha=0.8)+ labs(title=“B”)+ theme\_void()

Muskox2\_heatmap

Muskox 3 / MO3 / Generic 0193 Read in data NTCs <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=9) neuropils <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=10) neurons <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=11) Heat map 3 Muskox3\_heatmap <- ggplot(neuropils, aes(x=x, y=y))+ stat\_density\_2d(aes(fill=..level..), geom=“polygon”)+ scale\_fill\_viridis\_c(option = “viridis”, alpha=0.7)+ geom\_point(data=NTCs, color=“black”, size=2, alpha=0.5)+ geom\_point(data=neurons, shape=23, colour=“pink”, fill=“pink”, size=2, alpha=0.8)+ labs(title=“C”)+ theme\_void() Muskox3\_heatmap # all three together AllHeatmaps<-ggarrange(Muskox1\_heatmap, Muskox2\_heatmap, Muskox3\_heatmap, nrow=3, common.legend = TRUE, legend=“right”)

AllHeatmaps

Linegraphs Read in Data AllData <- read\_excel(“NA\_BHP\_Supp\_211022.xlsx”, sheet=2) make characters non numerical AllDataIndividual) AllDatalayer) Subset NTCsub <- subset(AllData, marker==“NTC” & layer==“all”) Neuropilsub <- subset(AllData, marker==“neuropil” & layer==“all”) Neuronsub <- subset(AllData, marker==“neuron” & layer==“all”) Marker count per layer per Individual sub\_NTC <- subset(AllData, marker==“NTC”) sub\_Neuropil <- subset(AllData, marker==“neuropil”) sub\_Neuron <- subset(AllData, marker==“neuron”) Make line graphs 3 Individuals NTC p1 <- ggplot(data=sub\_NTC, aes(x=layer, y=count, group=Individual, colour=Individual)) + geom\_line()+ geom\_point()+ scale\_color\_manual(values=viridis(3))+ labs(title=“B”,x=element\_blank(), y=element\_blank())+ theme( plot.title = element\_text(size=11, hjust=0.5), panel.border = element\_blank(),  
panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_blank(), axis.text=element\_text(size=7), legend.position=“none”) + geom\_path(size=1)+ scale\_x\_discrete(limits=c(“I”,“II”,“III”,“IV-VI”,“WM”))

p1 ## Warning: Removed 3 row(s) containing missing values (geom\_path). ## Warning: Removed 3 rows containing missing values (geom\_point). ## Warning: Removed 3 row(s) containing missing values (geom\_path).

3 Individuals Neuropil p2 <- ggplot(data=sub\_Neuropil, aes(x=layer, y=count, group=Individual, colour=Individual)) + geom\_line()+ geom\_point()+ scale\_color\_manual(values=viridis(3))+ labs(title=“A”,x=element\_blank(), y=element\_blank())+ theme( plot.title = element\_text(size=11, hjust=0.5), panel.border = element\_blank(),  
panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_blank(), axis.text=element\_text(size=7), legend.position=“none”) + geom\_path(size=1)+ scale\_x\_discrete(limits=c(“I”,“II”,“III”,“IV-VI”,“WM”))

p2 ## Warning: Removed 3 row(s) containing missing values (geom\_path). ## Warning: Removed 3 rows containing missing values (geom\_point). ## Warning: Removed 3 row(s) containing missing values (geom\_path).

3 Individuals Neuron p3 <- ggplot(data=sub\_Neuron, aes(x=layer, y=count, group=Individual, colour=Individual)) + geom\_line() + geom\_point()+ scale\_color\_manual(values=viridis(3))+ labs(title=“C”,x=“Brain layers”, y=element\_blank())+ theme( plot.title = element\_text(size=11, hjust=0.5), panel.border = element\_blank(),  
panel.grid.major = element\_blank(), panel.grid.minor = element\_blank(), panel.background = element\_blank(), axis.line = element\_blank(), axis.title.x = element\_text(size=8), axis.text=element\_text(size=7)) + geom\_path(size=1)+ scale\_x\_discrete(limits=c(“I”,“II”,“III”,“IV-VI”,“WM”))

p3 ## Warning: Removed 3 row(s) containing missing values (geom\_path). ## Warning: Removed 3 rows containing missing values (geom\_point). ## Warning: Removed 3 row(s) containing missing values (geom\_path).

##arrange on page all3<-ggarrange(p2, p1, p3, nrow=3, common.legend = TRUE, legend=“right”) all3<-annotate\_figure(all3, left=text\_grob(“Estimated population / volume”~(mm^3),rot = 90))

all3