1. Co-expression of MOX-genes with *NvTwist*

MOX-genes (*NvMoxA, NvMoxB, NvMoxC* and *NvMoxD*) are members of the homeobox protein family. Homeobox proteins direct the formation of body structures and hence fulfil import functions during early development.

I looked into the co-expression of NvMox\_genes along with NvTwist among different animals.



log2FC = -**0.27**; p.adj = 1

Figure 1: log-normalized counts (y-axis) are plotted for NvTwist and MOX-genes respectively. Note that each black dots represents one log-normalized expression quantity observed in either animal (panel header). MOX-genes expression data on the x-axis include all four different proteins (NvMoxA – D).

Although log-normalized counts for *NvTwist* are persistently higher than those observed for Mox-genes, there is no significant difference detected when conducting DGE analysis. This result suggests that neither Mox- nor Twist are up-and downregulated and hence a co-expression pattern can be assumed.

1. Co-expression pattern of Paraxis, and tbx4/5

The transcription factor *NvParaxis* (NV2.10865) is not significantly up- or downregulated between TwiHead and WTHead (log2FC = **-0.37**, p.adj = 1). The overall baseMean (i.e., average expression quantity among all libraries) is low for *NvParaxin* (~118). Similar observation for T-box transcription factor *TBX5-A*. Note however, that TBX5-A expression quantities are abundantly enriched in early developmental stage (4 dpf.) in both mutant and wt-animals.

‘Bubble-forming’ animals show depletion (log2FC = -1.60) in *NvParaxin* when comparing to wt animals, however, don’t reach statistical significance as specified at p.adj < 0.01. Interestingly, tbx4 is highly enriched in ‘bubble’ (log2FC = 3.31) although, again, not reaching statistical significance (p.adj = 0.06).

When comparing ‘bubble’ with its genotypic analogue without the phenotype, we see that NvParaxin is again depleted in ‘bubble-forming’ animals (log2FC = -1.23), however, again, not reaching statistical significance (p.adj = 1). Similar for tbx4 (upregulation in Bubble; not significant).