

Tubb5 knockdown exacerbates protein abundance changes in a mouse model of alcohol dependence

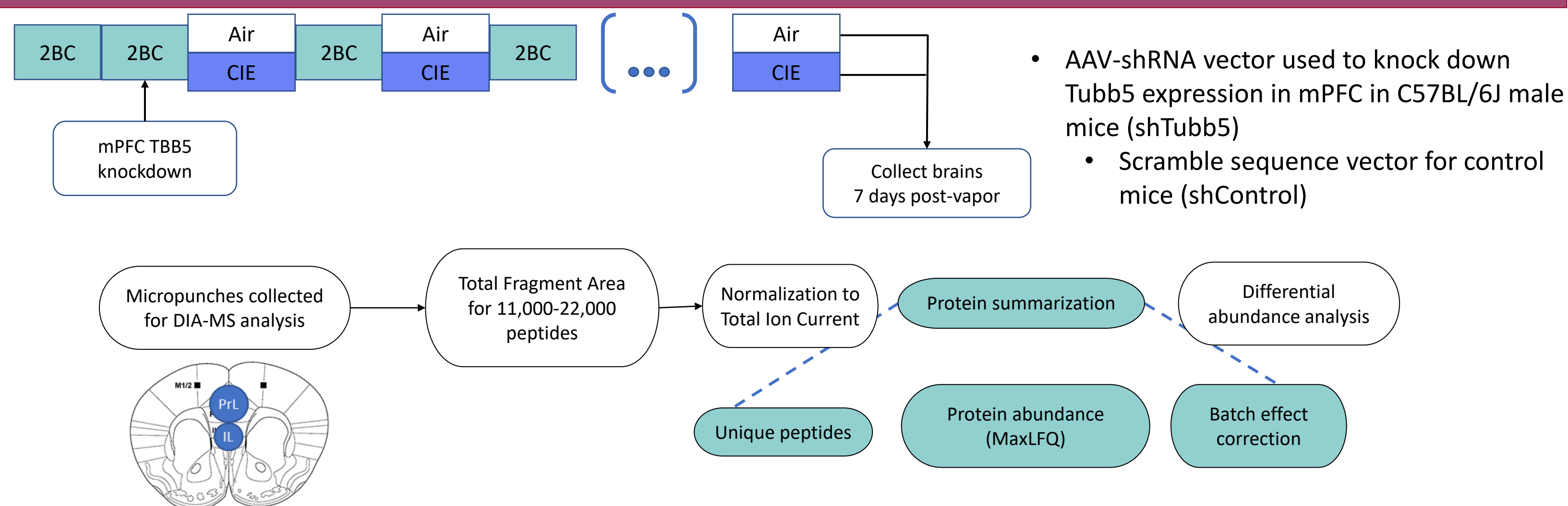
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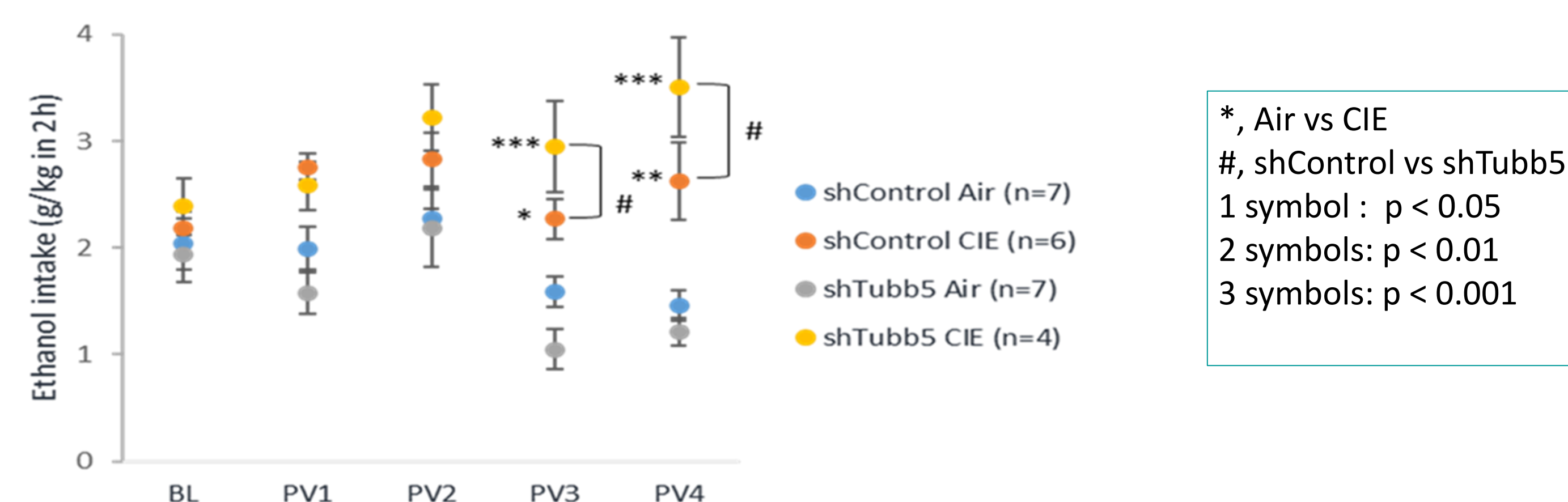
Rationale

- Microtubule cytoskeleton dysfunction associated with negative affect in psychiatric diseases and mood disease
- Tubulin protein abundance altered in the prefrontal cortex of individuals with AUD
- $\beta 5$ tubulin is significantly downregulated in the prefrontal cortex, but not the nucleus accumbens or amygdala, in 2 mouse models of excessive drinking (CIE-2BC and intermittent access). This provides evidence that excessive drinking remodels the microtubule cytoskeleton

Study design

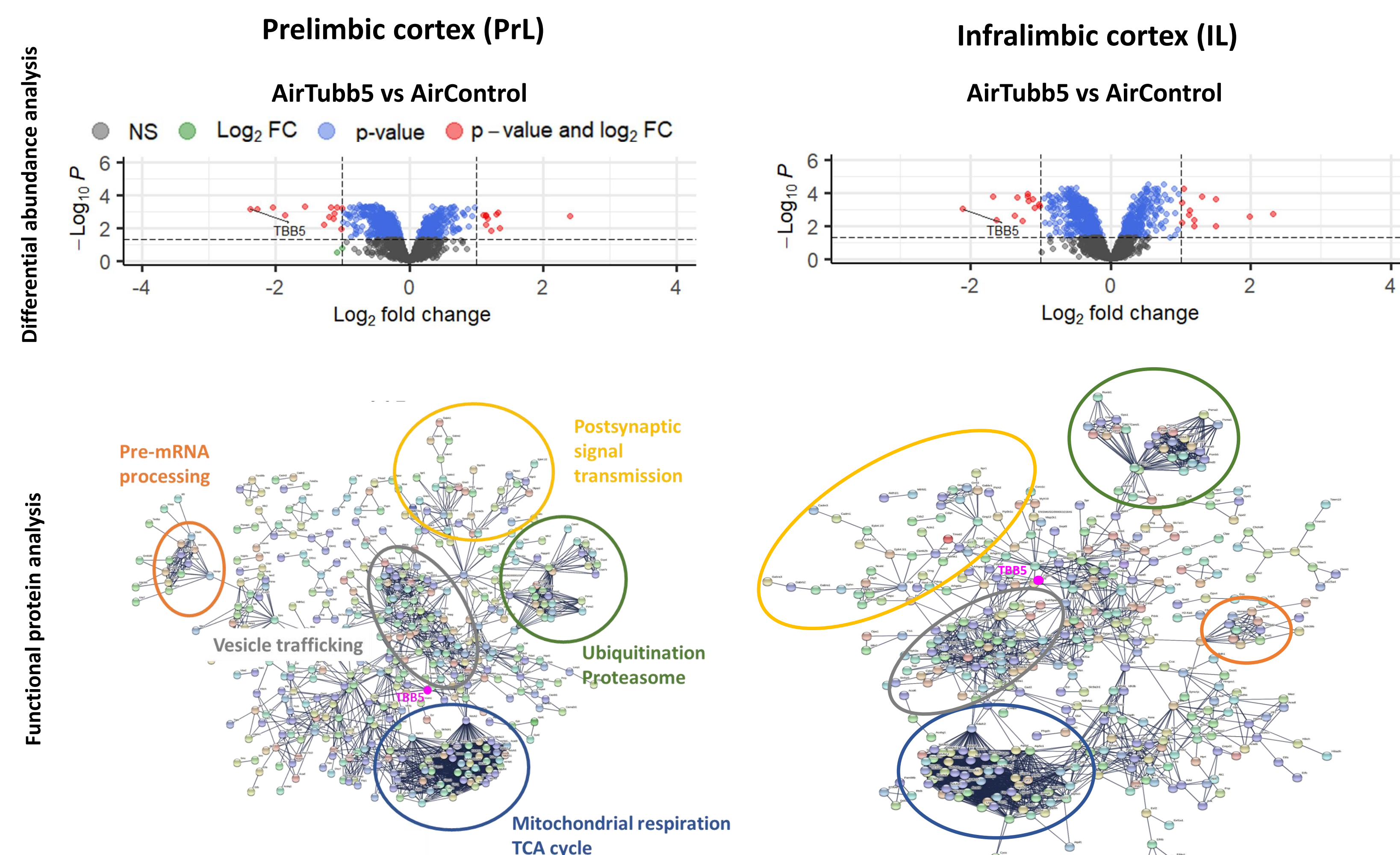


Tubb5 knockdown exacerbates ethanol intake escalation



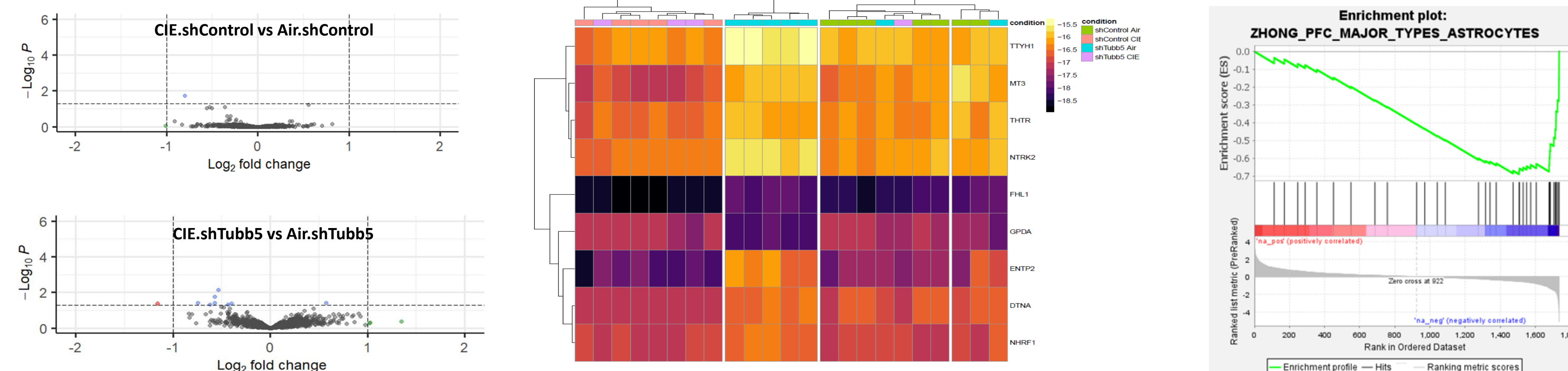
Two-way ANOVA detected a significant interaction between vapor and vector at PV3 [$F(1, 20)=7.5$, $p=0.013$] and PV4 [$F(1,20)=4.4$., $p=0.048$]

Tubb5 knockdown induces remodeling of proteome

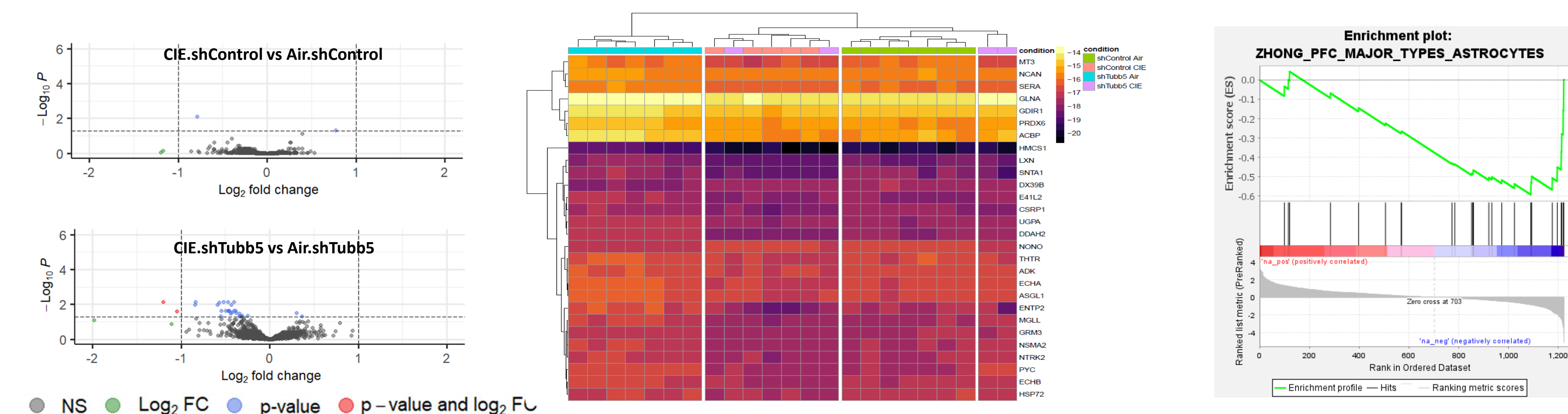


Abstinence from 2BC-CIE downregulates proteins enriched in astrocytes

PrL



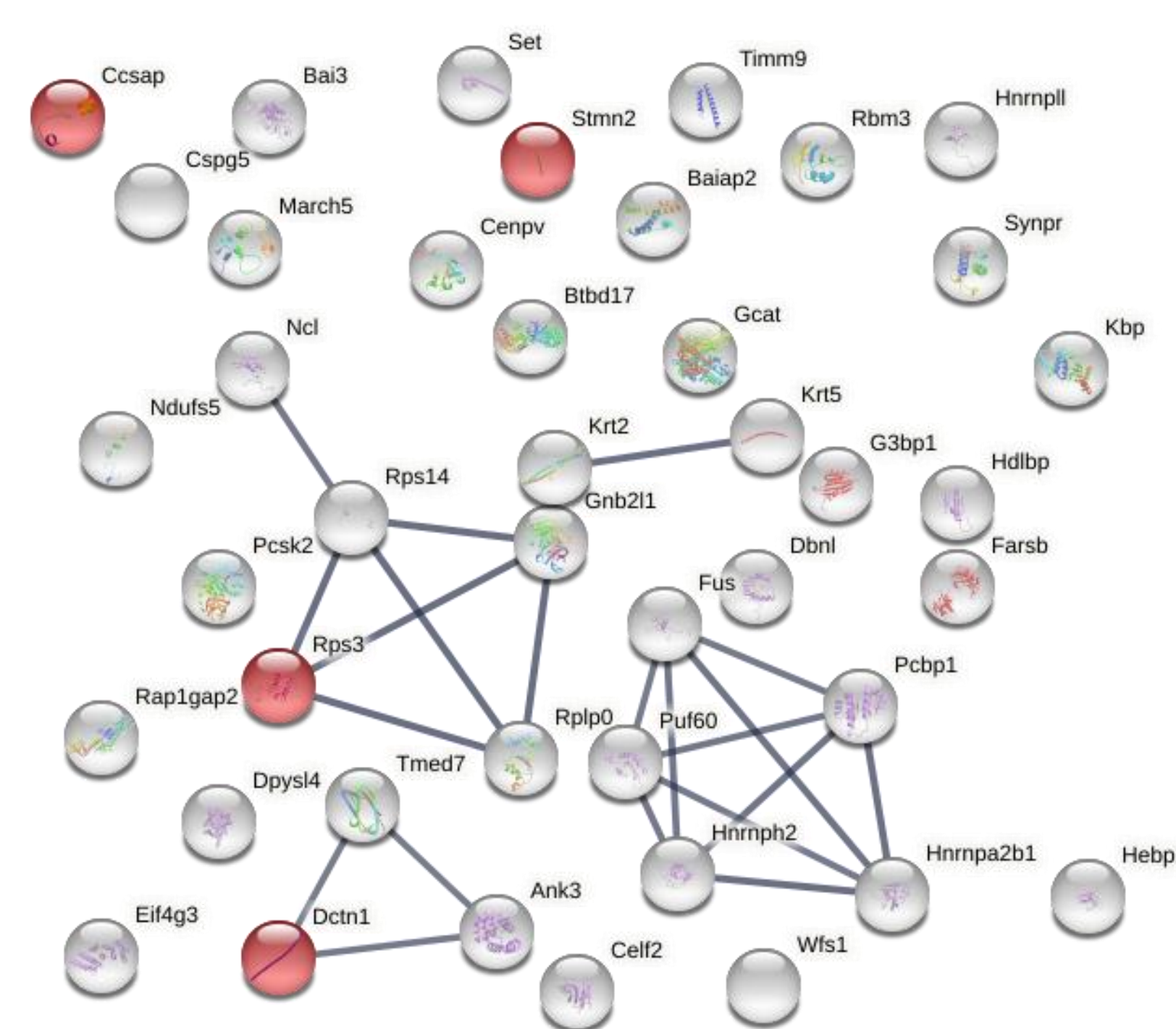
IL



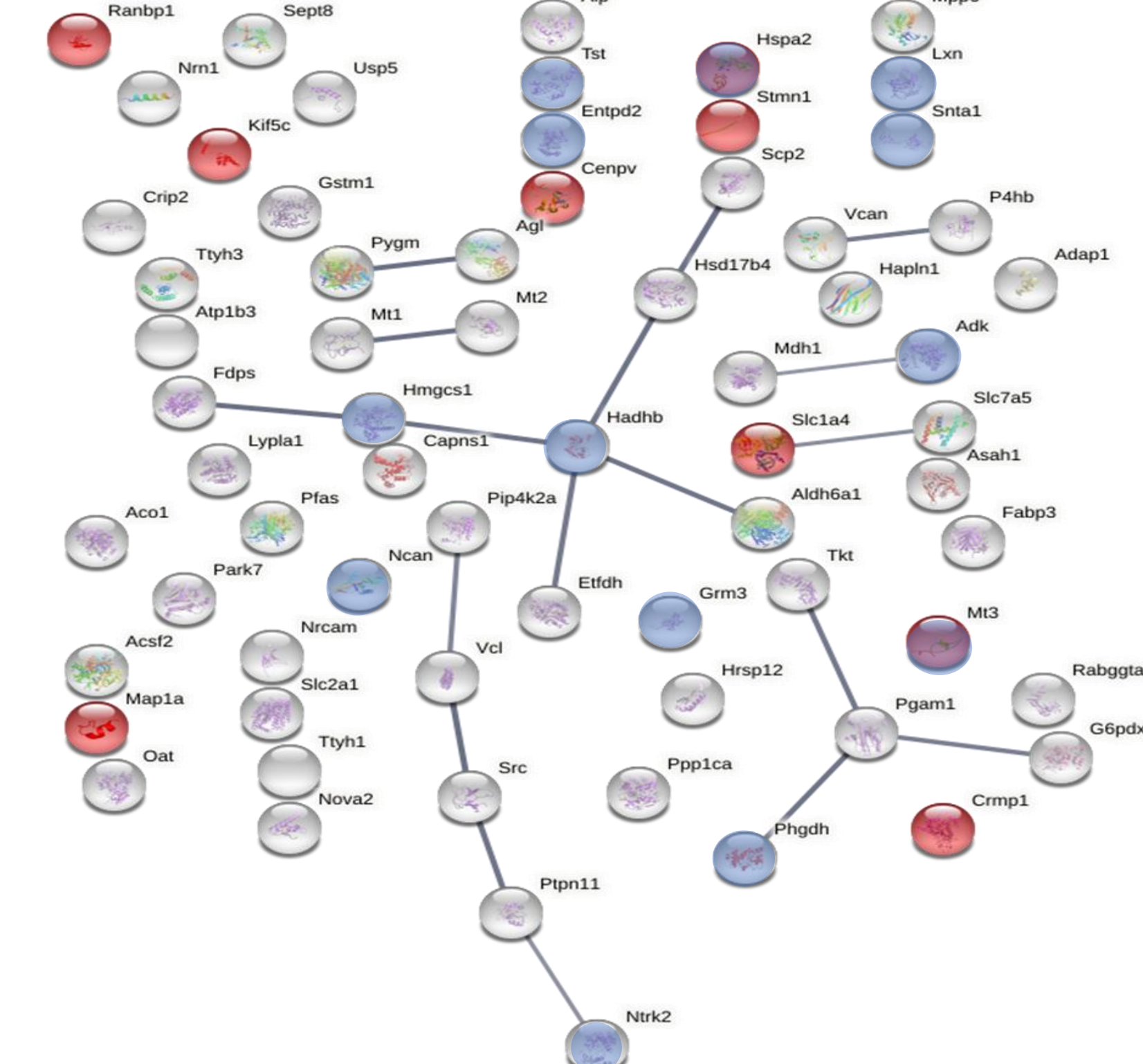
- Heatmaps show proteins identified as significantly differentially abundant in CIE.shTubb5 vs Air.shTubb5 comparison
- Gene set enrichment analysis (GSEA) was conducted using cell type gene sets from the Molecular Signature Database (MsigDB)
 - Astrocyte cell type gene set significant for both prelimbic and infralimbic comparisons (FDR <0.25)

Network analysis reveals EtOH intake correlates

PrL



IL



- Weighted co-expression network analysis revealed 42 and 69 proteins directly co-expressing with EtOH intake in the PrL and IL respectively ($p < 0.05$)
- Functional protein analysis with STRING revealed interactions with proteins associated with microtubule processes (highlighted in red)
- Several proteins identified in differential abundance analysis of infralimbic cortex were identified as co-expressors with EtOH intake (highlighted in blue)

Conclusions

- Knockdown of *Tubb5* in prefrontal cortex remodels proteome of prelimbic and infralimbic cortices
 - CIE-shTubb5 mice had significantly increased ethanol intake compared to CIE-shControl mice
 - shTubb5 mice had more significant changes at the protein level compared to controls
- Astrocytic proteins and proteins involved in microtubule processes co-express with ethanol intake

Acknowledgements

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