**Supplementary File 2:** Summarizing the results and functions of the validated target genes (not part of sex-determination pathway) at which splicing is regulated by CLAMP

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| **Gene name** | **Function** | **Effect on splicing** |
| Fus (Fusilli) | Fus regulates alternative splicing of specific genes and plays a role in embryonic dorsoventral patterning (Wakabayashi-Ito 2001)1. Its human ortholog ESRP1 (Epithelial splicing regulatory protein) regulates splicing during the epithelial to mesenchymal transition and is implicated in autosomal recessive non-syndromic deafness 109. Alternative splicing of both Fus and ESRP1 has been shown to confer distinct subcellular localization (Yang and Carstens 2017)2. | CLAMP regulates splicing of retained intron in exon 97 of *fus* in males (**Figure 2-figure supplement 3A, D, G**). |
| Wnd (Wallenda) | encodes for a MAP Kinase with roles in axonal injury signaling and in regulation of presynaptic bouton structure (Russo et al 2019)3 | one of the isoforms isoA significantly downregulated only in males (**Figure 2-figure supplement 3B, E, H**) |
| PEP (Protein on ecdysone puffs) | PEP is part of the catalytic step 2 spliceosome (Herold et al 2009)4 and physically interacts with MLE (Cugusi et al 2015)5, Squid (Amero et al 1993)26, Ubx and Abd-A (Bischof et al 2018)6. | Splicing of intron between exon 6-5 in *pep* is regulated by CLAMP in males (**Figure 2-figure supplement 3E, J, O**). |
| spen | encodes an RRM (RNA recognition motif) domain protein that interacts with the *Hox* pathway (Willette et al 1999)7. It is orthologous to human SPEN (spen family transcriptional repressors) which recruits histone deacetylases. *de novo* truncating variants in *SPEN* have been linked to a neurodevelopmental disorder associated with obesity and increased BMI in females who also have a distinctive X chromosome epi-signature (Radio et al 2021)8. | *spen* exon5 skipped transcript is significantly upregulated in females (**Figure 2-figure supplement 3J, N**) and not in males |
| Ama (Amalgam) | regulates receptor ligand activity during cell-cell adhesion and positively regulates glial cell proliferation (Seeger et al. 1988, Fremion et al. 2000)9,10. Human ortholog LSAMP is implicated in ovarian and prostate cancer (Spears et al 2006, Petrovics et al 2015)11,12 | Isoform B show significant down-regulation in males after CLAMP RNAi (**Figure 2-figure supplement 3K, O**) compared to females |
| iab4 | non-coding RNA regulating *abd-A,* located within the essential *Hox* cluster that controls body plan patterning. CLAMP directly binds and regulates chromatin accessibility at this gene (Duan et al 2021)13. | retained intron isoform is significantly down-regulated in males in absence of CLAMP (**Figure 2-figure supplement 3L, P).** |
| sc35 (SR family splicing factor) | Sc35 regulates mRNA alternative splicing, the processing of mRNA 3’ends, and transcription start site selection. The human ortholog, SRSF2, is linked to acute myeloid leukemia and myelodysplastic syndrome in which females show a significant survival advantage over their male counterparts (Hossain and Xie 2015, Wang et al 2019)14,15. Affected men have overall more mutations in genes involved in RNA splicing and epigenetic regulation with a higher risk of disease progression and overall poor outcome (Karantanos et al 2021)16. | Splicing of a *sc35* isoform with exon7 is significantly affected in males and not females (**Figure 2-figure supplement 3M, Q**). |
| Bacc (Bacchus) | encodes for tyramine dependent nuclear regulators involved in ethanol sensitivity (Chen et al 2013)17. | CLAMP-dependent splicing in both males and females (**Figure 2-figure supplement 3M, R**), However, isoform B with exon3 is significantly down-regulated in males compared to females in absence of CLAMP. |

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