Discovery report for no-sleep

Research Objective

I want to design a mechanism and propose a compound/drug that could induce the same effect as people who sleep very little at night.

Dataset Description

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FAMILIAL NATURAL SHORT SLEEPER (FNSS) DATASETS
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Created: 2025-11-21 23:30:25
This archive contains gene expression data and analysis results for familial
natural short sleeper (FNSS) mutations from two comprehensive studies.
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CONTENTS
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1. DROSOPHILA DEC2-P384R STUDY (GSE230462)
- $GSE230462_{illumina_}$counts.txt: Raw gene counts for all samples
- $P384R_{vs_}$WT.txt: Differential expression results (P384R mutant vs control)
- $DecWT_{vs_}$WT.txt: Differential expression results (wild-type DEC2 vs control)
- $P384R_{vs_}$DecWT.txt: Differential expression results (P384R vs wild-type DEC2)
2. MOUSE ADRB1-A187V STUDY (GSE205558)
- $GSE205558_{counts}$.txt: Raw gene counts for all samples
3. ANALYSIS RESULTS
- $FNSS_{comprehensive_}$analysis.png: Summary figure of key findings
- $mouse_{deg_}$results.csv: Mouse differential expression analysis results
4. THIS FILE
- README.txt: This documentation file
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DATASET DESCRIPTIONS
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DATASET 1: GSE230462 - Drosophila DEC2-P384R Model

Study: A familial natural short sleep mutation promotes healthy aging and
extends lifespan in Drosophila
PubMed ID: 37163058
Organism: Drosophila melanogaster
Technology: RNA-seq (Illumina)
Design: 18 samples total (3 genotypes x 6 replicates)
- WT: Control flies (GR23E10-gal4;+)
- DecWT: Wild-type human DEC2 overexpression
- P384R: Human DEC2-P384R mutation (FNSS mutation)
The DEC2-P384R mutation was expressed specifically in sleep neurons and
recapitulates the natural short sleep phenotype seen in humans. Flies with
this mutation sleep less but show improved health, enhanced longevity, and
better cognitive function compared to controls.
Total Genes: 17,972
Sample Columns: WT_1, WT_2, WT_3, $DecWT_1$, $DecWT_2$, $DecWT_3$, $P384R_1$,
$P348R_2$ (P384R replicate 2), $P384R_3$

Metadata Columns: $gene_{name}$, $gene_{chr}$, $gene_{start}$, $gene_{end}$, $gene_{strand}$,
$gene_{length}$, $gene_{biotype}$, $gene_{description}$, tf_{family}
DATASET 2: GSE205558 - Mouse ADRB1-A187V Model

Study: Effects of FNSS mutation on gene expression in mouse tauopathy model
PubMed ID: 37014857
Organism: Mus musculus
Technology: RNA-seq (Illumina)
Design: 8 samples (2 genotypes x 4 replicates)
- PS19: Control (tau pathology model)
- Adrb1+/m;PS19: ADRB1-A187V FNSS mutation with tau pathology
The ADRB1-A187V mutation is another human FNSS mutation. This study examines
its effects in the context of tau pathology, a model of neurodegenerative
disease.
Total Genes: 54,532
Sample Columns: m658, m659, m660, m661 (Adrb1+/m;PS19 mutants)
m665, m668, m671, m677 (PS19 controls)
Metadata Columns: $gene_{name}$, $gene_{chr}$, $gene_{start}$, $gene_{end}$, $gene_{strand}$,
$gene_{length}$, $gene_{biotype}$, $gene_{description}$, tf_{family}
==========
FILE FORMATS
==========
COUNT MATRICES ($GSE230462_{illumina_}$counts.txt, $GSE205558_{counts}$.txt):
- Tab-delimited text files
- First column: Gene ID (index)
- Sample columns: Raw RNA-seq counts
- Metadata columns: Gene annotations
- Can be loaded with: $pd.read_{csv}$(filename, sep='\t', $index_{col}$=0)
DIFFERENTIAL EXPRESSION FILES ($P384R_{vs_}$WT.txt, etc.):
- Tab-delimited text files
- Each row: One differentially expressed gene (padj < 0.05)
- Key columns:
* $gene_{id}$: Unique gene identifier
* $gene_{name}$: Gene symbol
* log2FoldChange: Log2 fold change (positive = upregulated in condition 1)
* pvalue: Raw p-value from statistical test
* padj: FDR-adjusted p-value (Benjamini-Hochberg correction)
* Sample columns: Normalized expression values
* Metadata columns: Gene annotations
MOUSE ANALYSIS RESULTS ($mouse_{deg_}$results.csv):
- CSV file with differential expression analysis
- Columns:
* $gene_{id}$: Mouse gene ID (ENSEMBL)
* $PS19_{mean}$: Mean log2(normalized counts + 1) in controls
* $Adrb1_{mean}$: Mean log2(normalized counts + 1) in mutants
* log2FoldChange: Log2 fold change (Adrb1 vs PS19)
* pvalue: Raw p-value from t-test
* padj: FDR-adjusted p-value
* $gene_{name}$, $gene_{description}$: Gene annotations
* significant: Boolean (padj < 0.05)

Summary of Discoveries

Discovery 1: Conserved oxidative phosphorylation enhancement via non-canonical control of substrate routing and mitochondrial dynamics

Cross-species transcriptomics across two Familial Natural Short Sleeper (FNSS) models converges on enhanced oxidative phosphorylation despite minimal single-gene overlap, pointing to non-canonical control points in substrate routing and mitochondrial dynamics. The data nominate a mechanistic model in which mild mitochondrial pyruvate carrier upregulation and a shift toward mitochondrial fission together sustain elevated OxPhos without activating classical transcriptional biogenesis programs, and they motivate NAD+-raising agents such as nicotinamide riboside or resveratrol as candidate pharmacology to emulate the FNSS metabolic state.

Discovery 2: Circadian-coupled suppression of neuropeptidergic signaling and a wake-promoting intervention via REV-ERB agonists

Cross-species transcriptomics of familial natural short sleep (FNSS) models reveal a conserved downregulation of the neuroactive ligandreceptor pathway that is driven predominantly by neuropeptide and hormone receptors rather than by classical fast neurotransmitter systems. Pharmacology aligned to this signature indicates that direct antagonism of muscarinic, orexin, or tachykinin receptors is sleep-promoting, not wake-promoting, whereas activation of the circadian nuclear receptor REV-ERB yields robust, EEG-verified wakefulness in rodents with validated brain exposure, nominating REV-ERB agonismspecifically SR10067as a tractable entry point to induce a short-sleep-like phenotype.

Discovery 3: SUMO2-driven post-translational enhancement of neuronal excitability: an orthogonal arousal mechanism and ebselen as a test compound

Somo2 is the only robustly altered transcript in ADRB1-A187V pontine tissue, pointing to a SUMO2/3-driven, post-translational mechanism that can raise neuronal excitability and bias networks toward wakefulness. A practical translational lever is to elevate SUMOylation by inhibiting SENP2; ebselen is a CNS-active SENP2 inhibitor suitable as a first in vivo probe, although sleepwake EEG data are not yet available.

Conserved oxidative phosphorylation enhancement via non-canonical control of substrate routing and mitochondrial dynamics

Summary

Cross-species transcriptomics across two Familial Natural Short Sleeper (FNSS) models converges on enhanced oxidative phosphorylation despite minimal single-gene overlap, pointing to non-canonical control points in substrate routing and mitochondrial dynamics. The data nominate a mechanistic model in which mild mitochondrial pyruvate carrier upregulation and a shift toward mitochondrial fission together sustain elevated OxPhos without activating classical transcriptional biogenesis programs, and they motivate NAD+-raising agents such as nicotinamide riboside or resveratrol as candidate pharmacology to emulate the FNSS metabolic state.

Background

Natural short sleepers require substantially less sleep without the typical cognitive or metabolic penalties, and Mendelian FNSS mutations in DEC2/BHLHE41 and ADRB1 established tractable genetic entry points into this phenotype. Because sleep-wake regulation is tightly coupled to brain energy metabolism, a key translational question is whether the short-sleep state can be induced by tuning mitochondrial function and substrate utilization rather than by manipulating canonical arousal neurotransmission. However, gene-level signals in small cross-species datasets are often weak and species-specific, necessitating pathway-level synthesis to identify conserved metabolic programs that could be targeted pharmacologically.

Results & Discussion

The datasets analyzed comprise a Drosophila DEC2-P384R model with robust differential expression and a mouse ADRB1-A187V model with minimal individual DEGs; critically, levels are on incompatible scales (fly normalized values versus mouse raw counts), precluding direct expression comparisons and motivating pathway-level analyses (n=3 and n=4 biological replicates per group in fly and mouse, respectively) [r0]. Indeed, no conserved differentially expressed genes were detected after ortholog mapping (0 genes), underscoring the need to move beyond one-to-one gene overlap to pathway signatures [r3]. A hypothesis-free cross-species Gene Set Enrichment Analysis (GSEA) on 91 KEGG pathways therefore ranked genes by sign(log2 fold change) Œ log10 p-value and used a running-sum enrichment score with MannWhitney tests; oxidative phosphorylation (KEGG:00190) emerged as the top concordant positive signal (mouse ES=616.5, p=6.5 Œ 10^{-18}; fly ES=128.7, p=0.23), alongside concordant shifts in central carbon metabolism pathways, despite limited power in the fly cohort [r37]. This pathway-level concordance provides a conserved metabolic signature consistent with increased OxPhos capacity in both FNSS models [r37].

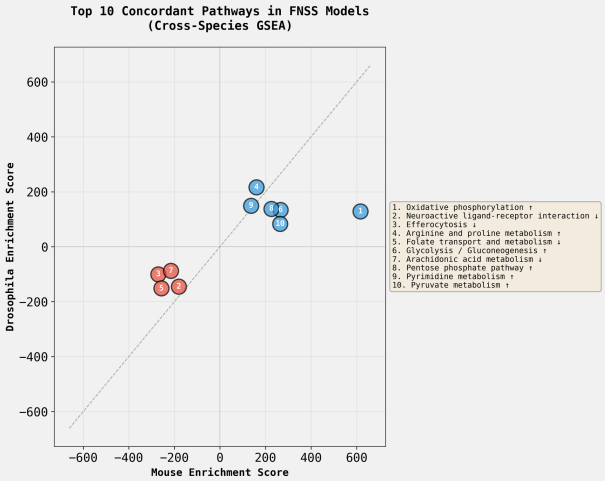

Figure 1: Cross-species Gene Set Enrichment Analysis reveals conserved upregulation of oxidative phosphorylation in FNSS models. The plot displays GSEA enrichment scores for the top 10 concordant KEGG pathways in mouse (x-axis) and Drosophila (y-axis) models, with blue and red points indicating concordant up- and down-regulation, respectively. This analysis identifies oxidative phosphorylation (point 1) as the most strongly conserved upregulated pathway, establishing a shared metabolic signature between the two distinct genetic models of short sleep. (Source: [r37])

Classical transcriptional axes could not ac-

count for this OxPhos signature. A focused cross-species test of master regulators of mitochondrial biogenesis found no conserved upregulation; notably, the PGC-1α ortholog was significantly downregulated in fly (srl log2FC = 0.171, p=0.0148), whereas NRF1/TFAM showed no significant changes, rejecting the canonical PGC-1αNRF1/TFAM model as the driver [r40]. Likewise, curated transcriptional target sets for AMPK and SIRT1 showed no conserved activation (mouse AMPK targets mean log2FC = 0.157, p=0.092; fly AMPK orthologs mean log2FC = 0.075, p=0.100; SIRT1 targets near zero or slightly negative) [r57]. A similar negative result held for an mTORC1 activation signature (mouse mean log2FC = +0.0103, p=0.83; fly mean log2FC = 0.0519, p=0.17) [r62], and a curated SIRT3 response signature showed no coordinated change in mouse and significant downregulation in fly (mean log2FC = 0.1074, p=0.0023), arguing against a conserved SIRT3-driven transcriptional program [r72]. Together these analyses indicate the OxPhos enhancement arises from regulatory architecture other than the canonical biogenesis TF cascade or mTOR/AMPK/SIRT-driven transcription, pointing toward post-transcriptional or metabolic control.

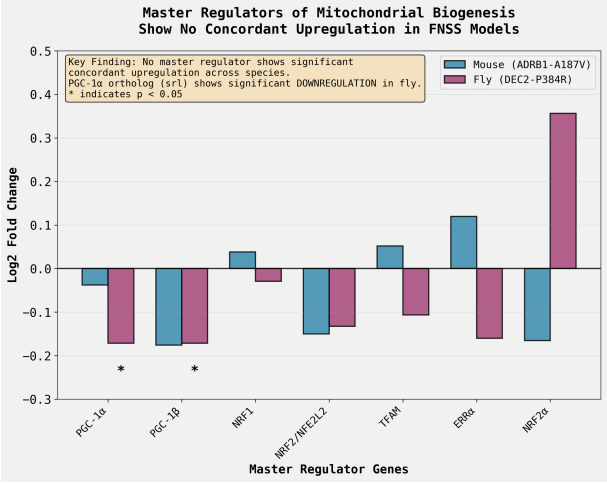

Figure 2: Master regulators of mitochondrial biogenesis are not concordantly upregulated across FNSS models. The bar plot shows the Log2 fold change in expression for key transcriptional regulators in mouse (ADRB1-A187V) and fly (DEC2-P384R) models relative to controls, with asterisks indicating $p < 0.05$. These data suggest that the observed enhancement in oxidative phosphorylation occurs independently of the canonical transcriptional program for mitochondrial biogenesis. (Source: [r40])

Two non-canonical, convergent features emerged. First, transporter analysis revealed conserved upregulation of the mitochondrial pyruvate carrier (MPC): in mouse, Mpc1 and Mpc2 both trended up (mean log2FC = 0.0708), and in fly, Mpc1 was up 0.187, consistent with increased mitochondrial pyruvate influx; concurrently, fatty acid transporters were concordantly downregulated, suggesting a routing shift toward carbohydrate-derived substrates, whereas glutamate transporters were discordant [r80]. Importantly, key glycolysis genes were not coordinately induced in either species (mouse mean log2FC = 0.021; fly mean log2FC = 0.047), indicating that the conserved change lies in mitochondrial substrate entry rather than cytosolic glycolytic throughput per se [r81]. Moreover, regulators of the pyruvate dehydrogenase (PDH) complex (PDK14, PDP12 in mouse; Pdk in fly) showed negligible expression changes with no conserved activation signature, implying that any PDH flux change would be controlled post-transcriptionally or by metabolite ratios rather than by altered expression of PDH-modifying enzymes [r70]. Second, mitochondrial dynamics genes showed a conserved directional pattern: fission gene expression trended upward and fusion gene expression downward in both species, and a pooled cross-species comparison of fission versus fusion genes was significant (t=2.482, p=0.032; Cohens d=1.61), suggesting a shift toward fragmentation that can favor higher respiratory activity in certain contexts [r79]. These two featuresmodest MPC upregulation and a fission biasprovide a parsimonious, non-canonical mechanism for increased OxPhos: more pyruvate substrate is delivered to mitochondria while organelle morphology shifts to support elevated respiratory flux, all without activating master biogenesis programs [r37, r79, r80].

Translationally, this mechanism motivates a pharmacology-first strategy centered on increasing mitochondrial NAD+ availability and testing substrate routing as the proximal control point. NAD+-SIRT axis modulators stand out because they can raise mitochondrial NAD+ and enhance respiratory capacity without requiring transcriptional induction of PGC-1α/NRF1/TFAM, which is absent here [r40, r57, r72]. Nicotinamide ribo-

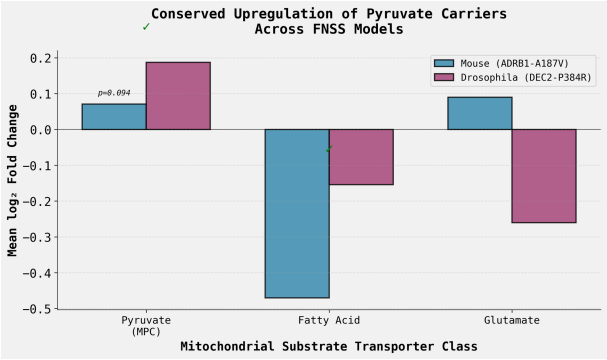

Figure 3: Familial Natural Short Sleeper (FNSS) models exhibit a conserved upregulation of mitochondrial pyruvate carriers. The plot shows the mean log2 fold change in gene expression for mitochondrial pyruvate carriers (MPC), fatty acid transporters, and glutamate transporters in both ADRB1-A187V mouse and DEC2-P384R Drosophila models. The consistent upregulation of MPCs, in contrast to other substrate transporters, suggests a specific shift in metabolic substrate routing to favor pyruvate entry into the mitochondria. (Source: [r80])

side (NR) is therefore proposed as the lead candidate: in mice, chronic dietary NR reduced NREM sleep by roughly 17% and accelerated discharge of homeostatic sleep pressure with EEG signatures of increased cortical activation, and increased brain NAD+ has been documented, consistent with a more oxidative metabolic state [r60, Bushana2023, Weiss2025]. As an orthogonal lever, resveratrolclassically a SIRT1/AMPK modulatorproduced a marked active-wake increase (+163%) and decreased REM (95%) and SWS (38%) in non-human primates, providing independent, cross-species evidence for wake-promoting effects aligned with the FNSS phenotype, albeit with sparse CNS pharmacokinetics and incomplete polysomnographic quantitation reported [r60, Pifferi2012]. Mechanistic validation should proceed by asking whether NR or resveratrol recapitulate the two conserved featuresMPC upregulation and a fission shiftand by testing necessity with mitochondrial pyruvate carrier blockade (e.g., UK5099) or genetic knockdown, as previously proposed, to confirm that substrate routing is the controlling node for OxPhos enhancement and sleep reduction in FNSS models [r80]. Given the small sample sizes, lack of conserved single-gene DEGs, and scale incompatibilities across datasets, these conclusions should be corroborated with integrated proteomics, phosphoproteomics, and metabolomics focused on MPC abundance/activity, PDH phosphorylation state, mitochondrial NAD+/NADH, and DRP1 phosphorylation, alongside gold-standard EEG/EMG sleep phenotyping [r0, r3, r37].

Trajectory Sources

Trajectory r0:
COMPREHENSIVE DATASET DESCRIPTION: FAMILIAL NATURAL SHORT SLEEPER (FNSS) STUDIES
This archive contains gene expression data from two independent studies investigating mutations associated with Familial Natural Short Sleeper syndrome.
FILE INVENTORY
Drosophila Study (GSE230462 - DEC2-P38...

Trajectory r3: No conserved differentially expressed genes were identified between the Drosophila DEC2-P384R model (407 DEGs at padj < 0.05) and the mouse ADRB1-A187V model (1 DEG at padj < 0.1), indicating that these FNSS mutations exhibit species-specific or mutation-specific transcriptional responses rather tha...

Trajectory r37: A systematic cross-species GSEA analysis of 91 KEGG pathways identified 48 concordant pathways (52.7%), with oxidative phosphorylation showing the strongest concordant positive enrichment (mouse: ES=616.5, p=6.5Œ10^{-18}; fly: ES=128.7, p=0.23), revealing conserved metabolic shifts beyond the previousl...

Trajectory r40:
CONCLUSION: Hypothesis REJECTED - Master Regulators Do Not Drive OxPhos Upregulation in FNSS Models
The hypothesis that concordant upregulation of Oxidative Phosphorylation (OxPhos) pathway genes in both fly and mouse FNSS models is driven by conserved transcriptional upregulation of master reg...

Trajectory r57: Neither AMPK nor SIRT1 pathway activation is supported as the conserved upstream driver of OxPhos upregulation in familial natural short sleeper models, as their transcriptional target gene sets show no evidence of conserved activation across mouse and Drosophila datasets.

Trajectory r60: Both Resveratrol and Nicotinamide Riboside demonstrate alterations in sleep-wake architecture that are consistent with a wake-promoting or sleep-reducing effect, although complete quantitative EEG/EMG metrics remain insufficiently detailed.

Trajectory r62: There is no evidence for a conserved mTORC1 transcriptional activation signature in either the mouse ADRB1-A187V or Drosophila DEC2-P384R familial natural short sleeper models.

Trajectory r70: There is no conserved transcriptional signature of pyruvate dehydrogenase (PDH) complex activation via regulation of PDH kinases or phosphatases in either the mouse (ADRB1-A187V) or Drosophila (DEC2-P384R) FNSS models, indicating that transcriptional regulation of PDH-modifying enzymes does not prov...

Trajectory r72:
SIRT3 Pathway Analysis: Cross-Species Transcriptional Signature
Main Finding There is **NO evidence for a conserved transcriptional signature of SIRT3 pathway activation** in either the mouse (ADRB1-A187V) or Drosophila (DEC2-P384R) short-sleep models. In fact, the Drosophila model shows si...

Trajectory r79: Both FNSS models exhibit a conserved transcriptional signature of mitochondrial dynamics characterized by increased fission gene expression and decreased fusion gene expression (combined fission vs fusion: t=2.482, p=0.032, Cohen's d=1.61), consistent with a shift toward mitochondrial fragmentation ...

Trajectory r80: The hypothesis is supported: there is a conserved transcriptional upregulation of the mitochondrial pyruvate carrier (MPC) across both FNSS models, suggesting increased pyruvate substrate availability as a mechanism for OxPhos upregulation.

Trajectory r81:
ANALYSIS CONCLUSION
No conserved transcriptional upregulation of the glycolysis pathway was detected in either FNSS model. The hypothesis that glycolysis genes are coordinately upregulated to support enhanced oxidative phosphorylation is **NOT supported** by these data.
QUANTITATIVE RESULTS...

Circadian-coupled suppression of neuropeptidergic signaling and a wake-promoting intervention via REV-ERB agonists

Summary

Cross-species transcriptomics of familial natural short sleep (FNSS) models reveal a conserved downregulation of the neuroactive ligandreceptor pathway that is driven predominantly by neuropeptide and hormone receptors rather than by classical fast neurotransmitter systems. Pharmacology aligned to this signature indicates that direct antagonism of muscarinic, orexin, or tachykinin receptors is sleep-promoting, not wake-promoting, whereas activation of the circadian nuclear receptor REV-ERB yields robust, EEG-verified wakefulness in rodents with validated brain exposure, nominating REV-ERB agonismspecifically SR10067as a tractable entry point to induce a short-sleep-like phenotype.

Background

Short-sleep phenotypes have been linked to rare coding variants in sleep and circadian regulators, including DEC2 and ADRB1, which alter sleep need and architecture without major cognitive impairment. While classical stimulants increase arousal, they do not recapitulate the naturalistic compression of sleep found in FNSS. Converging evidence suggests that FNSS may involve coordinated tuning of peptide neuromodulator systems and the molecular clock, raising the possibility that circadian nuclear receptors could be leveraged to promote wakefulness with temporal specificity. Identifying a mechanism and compound that mimic FNSS requires integrating transcriptomic signatures with pharmacological evidence that isolates wake promotion from generic sedation or sleep consolidation.

Results & Discussion

Cross-species analysis of FNSS models demonstrates that the negative enrichment of the neuroactive ligandreceptor pathway (KEGG 04080) is driven by broad downregulation of neuropeptide and hormone receptors, not by coordinated changes in classical neurotransmitter receptor families. In mouse, the overall pathway mean log2 fold change was 0.111, with the neuropeptide/other receptor group significantly downregulated (n = 203, mean log2 fold change = 0.149, p = 2.27 Œ 10^{-4}), while other subfamilies (dopamine, GABA, glutamate, acetylcholine, adrenergic, glycine, adenosine, serotonin) showed no significant coordinated shift; in Drosophila, the neuropeptide/other receptor group showed a consistent but non-significant downtrend (n = 27, mean log2 fold change = 0.181, p = 0.145), with classical receptor families again inconsistent in direction across species [r44]. This conclusion rests on a predefined receptor classification (neuropeptide/other versus classical transmitter families) and one-sample tests of subfamily-level mean log2 fold change against zero, applied to KEGG-defined pathway members mapped into the expression datasets with ~75% coverage in both species [r44]. Together, these data argue that FNSS-linked mutations converge on suppressing neuropeptidergic and hormonergic receptor expression across species, suggesting that peptide neuromodulator tone is broadly reduced in FNSS-like states [r44].

Pharmacological triage against this transcriptomic map indicates that directly targeting the downregulated receptor families does not promote wakefulness. Central antimuscarinic exposure with oxybutynin in randomized, double-blind polysomnography trials suppressed REM sleep, shortened sleep latency in young adults, and preserved or slightly improved sleep efficiency without increasing wake after sleep onsetevidence for mild sedation or sleep consolidation rather than wake promotion [r30]. Likewise, systemic tachykinin NK1 receptor antagonists (e.g., vestipitant, casopitant, orvepitant) consistently increased total sleep time and reduced wake after sleep onset in clinical EEG studies, with no evidence of increased total wakefulness, supporting a direct anti-arousal action [r55, ratti2013]. Finally, orexin/hypocretin antagonism, although a well-validated modulator of sleepwake state, is classically sleep-

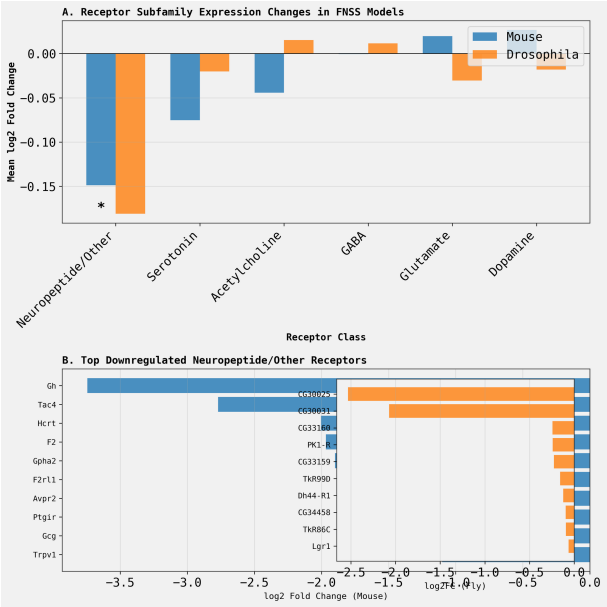

Figure 4: Cross-species analysis of FNSS models reveals a selective downregulation of neuropeptide receptors. (A) Mean log2 fold change of gene expression for major receptor subfamilies in mouse and Drosophila models. The neuropeptide/other category is significantly downregulated in mouse (*, one-sample t-test) and shows a similar trend in Drosophila. (B) Top downregulated individual genes within the Neuropeptide/Other receptor category for mouse (left) and Drosophila (right, inset). Together, these data indicate that the transcriptomic signature of FNSS is driven by broad suppression of neuropeptide receptor expression rather than by classical neurotransmitter systems. (Source: [r44])

promoting and mechanistically linked to decreased arousal, aligning genetic or pharmacologic orexin downregulation with narcolepsy-like features rather than wake promotion [r45, sakurai2010, morairty2012]. These outcomes collectively indicate that the FNSS transcriptomic signature is not a simple guide to wake-promotion via direct receptor antagonism in these systems, and that a different mechanistic axis is needed to recapitulate a short-sleep-like phenotype [r30, r45, r55].

Mechanistically, DEC2-P384R is unlikely to directly repress neuropeptide receptor genes; available evidence supports an indirect cascade rooted in DEC2s E-box-centered repression and its modulation of CLOCK:BMAL1/E-protein complexes [r52]. Consistent with a circuit-level and circadian mechanism, expression of core clock transcription factors (Clock, Arntl, Creb1, Rora) positively co-varies with neuropeptide receptor expression in the mouse FNSS model ($r = 0.750.89$ for all four TFs, all $p < 0.05$), with both gene sets reduced in the mutant; in Drosophila, correlations are mostly positive but underpowered ($n = 6$) [r69]. This pattern argues against a direct antagonistic regulation between circadian TFs and neuropeptide receptors and instead supports coordinated downregulation by upstream clock-coupled programs, consistent with DEC2-P384R acting through E-box repression and complex remodeling rather than promoter-proximal control of the receptor genes themselves [r52, r69]. In sum, the FNSS signature points to a circadian-coupled reduction in neuropeptidergic receptor expression as part of a broader, clock-modulated transcriptional program.

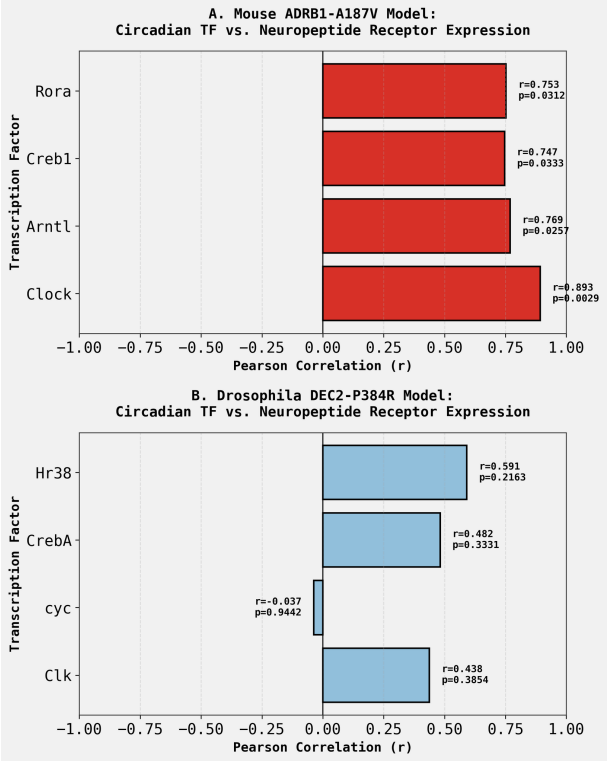

Figure 5: Expression of core circadian transcription factors is positively correlated with neuropeptide receptor expression in a mouse model of short sleep. The bar plots show Pearson correlations (r) between the expression of specific circadian transcription factors and aggregate neuropeptide receptor expression in (A) a mouse ADRB1-A187V model and (B) a Drosophila DEC2-P384R model, with corresponding p-values. The significant correlations in the mouse model suggest that the circadian clock machinery contributes to the regulation of neuropeptidergic signaling components. (Source: [r69])

This circadian framing directly motivates testing nuclear receptor nodes that sit atop clock

transcriptional networks. Agonism of REV-ERB, a core repressor in the circadian loop, acutely increases wakefulness and suppresses slow-wave and REM sleep with EEG/EMG confirmation in rodents, with effects strongly gated by time of day. SR9011 and SR9009 (100 mg/kg, i.p.) increased wake for ~23 h and delayed REM/SWS when dosed near ZT6, with diminished or reversed effects away from this window; SR9009 showed adequate brain exposure at efficacious doses and exhibited rebound sleep in the subsequent dark phase without short-term tolerance across certain regimens [r78, amador2016, banerjee2014]. A next-generation agonist, SR10067, improves in vitro potency (REV-ERBα/β IC50 $\approx$170/160 nM versus ~670800 nM for SR9011), demonstrates measured brain concentrations above IC50 for 6 h after 30 mg/kg i.p., and produces EEG-verified wakefulness with decreased SWS/REM and increased locomotion when administered at ZT6, indicating superior CNS exposureresponse characteristics for wake promotion [r85, banerjee2014]. Although ROR modulators are cataloged in the same axis, the provided record contains no EEG sleep data for them, so conclusions are limited to REV-ERB activation as the validated circadian intervention for acute wake promotion in rodents [r78, ruan2021].

Integrating these lines of evidence supports a mechanistic model in which FNSS involves circadian-coupled suppression of neuropeptidergic receptor expression, and pharmacologic activation of REV-ERB can phenocopy a key systems-level featurerest-phase wake promotion with compressed sleep. Based on potency, selectivity, verified brain exposure relative to functional IC50s, and EEG-confirmed wake efficacy, SR10067 emerges as the lead compound to induce a short-sleep-like phenotype via circadian entrainment, with chronopharmacological dosing (near ZT6 in nocturnal rodents) critical for maximal effect [r78, r85, banerjee2014, amador2016]. Importantly, direct receptor antagonism in muscarinic, orexin, or tachykinin systems produces sleep consolidation or anti-arousal and thus does not align with the wake-promoting goal [r30, r45, r55]. While translational validation and human EEG studies are needed, the convergence of transcriptomics, mechanistic clock biology, and CNS-validated REV-ERB pharmacology nominates SR10067 as a rational, circadian-tuned candidate to induce a FNSS-like reduction of sleep need.

Trajectory Sources

Trajectory r30: Yesmultiple randomized, doubleblind polysomnography studies quantify oxybutynins central effects (EEG/EMG), showing REM suppression with unchanged or improved sleep efficiency and no increase in wakefulness, providing no evidence of a wakepromoting effect; actigraphy/locomotor data are not repor...

Trajectory r44: The concordant negative enrichment of the "Neuroactive ligand-receptor interaction" pathway (KEGG:04080) is driven primarily by neuropeptide and hormone receptors rather than classical neurotransmitter receptor subfamilies, with neuropeptide/other receptors showing significant downregulation in mous...

Trajectory r45: The review supports that the hypocretin/orexin receptorparticularly the OX2 receptor targeted by CNSpenetrant antagonistsis the most validated candidate for modulating sleep/wake state in a manner that mimics genetic downregulation, despite its conventional role in sleep promotion (morairty2012du...

Trajectory r52: The DEC2-P384R loss-of-function is unlikely to downregulate neuropeptide receptor genes directly; available evidence supports an indirect cascade via DEC2s E-boxcentered repression network and its modulation of CLOCK:BMAL1/E-protein complexes.

Trajectory r55: Within the corpus provided, there is no preclinical or clinical EEG/PSG evidence that systemic NK1 receptor antagonists increase total wakefulness or decrease total sleep time; instead, clinical studies consistently show sleep-promoting effects, supporting a context-dependent anti-arousal/hypnotic a...

Trajectory r69: The hypothesis that circadian transcription factors are negatively correlated with neuropeptide receptor expression is rejected, as all four mouse circadian TFs (Clock, Arntl, Creb1, Rora) show strong positive correlations ($r = 0.75$-0.89, all $p < 0.05$) with neuropeptide receptor mean expression, ind...

Trajectory r78: CNSactive REVERB agonists (SR9009, SR9011, SR10067) are documented to penetrate brain and acutely promote wakefulness with EEGverified suppression of SWS/REM in rodents, whereas the provided record contains no EEG/EMG sleep data for ROR modulators, partially supporting the hypothesis. (amador2016...

Trajectory r85: The hypothesis is supported for SR10067, which shows improved potency, selectivity, verified brain exposure above functional IC50s, and EEG-validated wake promotion, but evidence for other second-generation agonists with comparable CNS PK and sleepwake data is currently insufficient.

SUMO2-driven post-translational enhancement of neuronal excitability: an orthogonal arousal mechanism and ebselen as a test compound

Summary

Somo2 is the only robustly altered transcript in ADRB1-A187V pontine tissue, pointing to a SUMO2/3-driven, post-translational mechanism that can raise neuronal excitability and bias networks toward wakefulness. A practical translational lever is to elevate SUMOylation by inhibiting SENP2; ebselen is a CNS-active SENP2 inhibitor suitable as a first in vivo probe, although sleepwake EEG data are not yet available.

Background

Natural short sleep is thought to emerge when arousal networks can sustain higher firing and cognitive performance with less sleep homeostasis pressure. Classical approaches target neuromodulators or circadian systems, but accumulating evidence suggests that rapid, cell-intrinsic tuning of ion channels and synapses can also set the brains arousal gain. SUMOylationconjugation of small ubiquitin-like modifiers to target proteinsprovides such a fast, reversible layer of control over membrane excitability and synaptic release. Here, transcriptomics and pathway analysis converge on SUMO2 as an arousal-relevant hub in a short-sleep model, motivating a mechanism-driven pharmacology to transiently elevate SUMO2/3 conjugation in the brain.

Results & Discussion

DESeq2 re-analysis of the ADRB1-A187V mouse pontine RNA-seq identified Sumo2 as the sole differentially expressed gene at false discovery rate <0.1 (log2 fold change 1.64, adjusted p = 2.15 Œ 10^{-5}), with a 3-fold increase in mutants; all other genes failed to pass multiple-testing thresholds despite 759 nominal hits at p < 0.05, underscoring a sparse transcriptional signature dominated by Sumo2 [r1]. This change is not accompanied by coordinated expression shifts across the SUMOylation machinery: among 23 pathway genes annotated to GO:0016925, up- vs downregulation is near 50/50 (12 vs 11), the mean log2 fold change is 0.023, and three orthogonal tests (t-test, Wilcoxon signed-rank, binomial sign) are non-significant (all p 0.84), indicating Sumo2 upregulation is an isolated event rather than pathway-wide activation [r7]. Likewise, a curated set of 24 established neuronal SUMO substrates shows no gene-level response (mean log2 fold change 0.0033, t-test p = 0.895; 12 up vs 12 down), consistent with a post-translational mechanism where substrate availability is unchanged while conjugation state increases [r16]. Importantly, there is also no transcriptional activation of the non-canonical ADRB1 β-arrestin pathway: across 29 components, the mean log2 fold change is essentially zero (0.000789) with no gene significant after correction and no directional bias, excluding this route as a driver of Sumo2 upregulation [r50].

Mapping the isolated Sumo2 increase onto known neurophysiology supports a model wherein elevated SUMO-2/3 conjugation reduces stabilizing K+ currents and potentiates presynaptic Ca2+-dependent release, thereby increasing excitability in wake-promoting pontine circuits [r15, connolly2025, coelhosilva2017]. SUMOylation of K2P1/TWIK1 silences leak K+ current, of Kv2.1 (at K470) right-shifts activation to diminish delayed rectifier current, and hyper-SUMOylation of Kv7 reduces the M-current; SUMO1 can enhance Nav1.2 currents as well, together biasing membranes toward depolarization [r15, connolly2025, coelhosilva2017]. At terminals, SUMOylation of RIM1α enlarges CaV2.1 clusters and augments Ca2+ entry and release probability, while synaptosomal SUMO manipulations bidirectionally modulate KCl/kainate-evoked Ca2+ influx and glutamate release, providing a presynaptic amplifier of arousal tone [r15, coelhosilva2017, connolly2025]. Complementary evidence places Arc, Grin1/NR1, Gria2, Kcnb1/Kv2.1,

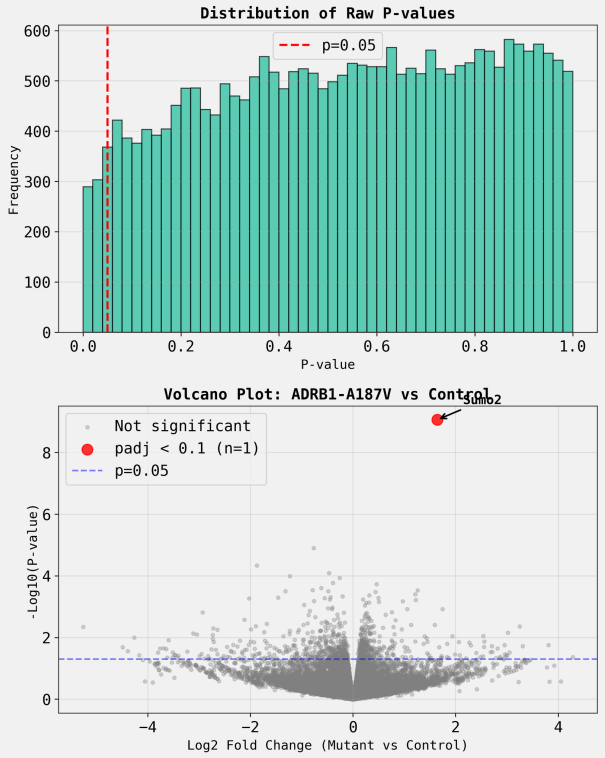

Figure 6: Differential expression analysis identifies Sumo2 as the sole significantly upregulated gene in the pons of ADRB1-A187V mice. (A) Histogram of raw p-values from RNA-seq analysis, showing a largely uniform distribution indicative of a sparse signal. (B) Volcano plot of log2 fold change versus statistical significance, where each point is a gene. Sumo2 (red) is the only gene to pass the significance threshold after multiple testing correction (padj < 0.1). Together, these data demonstrate an exceptionally specific transcriptional response isolated to Sumo2 upregulation in mutant animals. (Source: [r1])

and Stx1a in a SUMO-centered excitability axis that stabilizes AMPARs, supports NMDAR-mediated transmission, reduces K+ conductance, and tunes SNARE cycling; approximately one-third of a 24-gene substrate panel have explicit pro-excitatory roles in this corpus, anchoring the plausibility that increased SUMOylation can raise network gain even without substrate gene induction [r22, henley2021, kachemov2023a, folci2020]. Although countervailing actions exist (e.g., GluK2 internalization, CRMP2 effects on CaV2.2), the preponderance of documented targets and synaptic physiology predicts a net excitability increase with higher SUMO-2/3 conjugation in relevant cell types [r15, coelhosilva2017, connolly2025].

Cross-species pathway profiling positions this

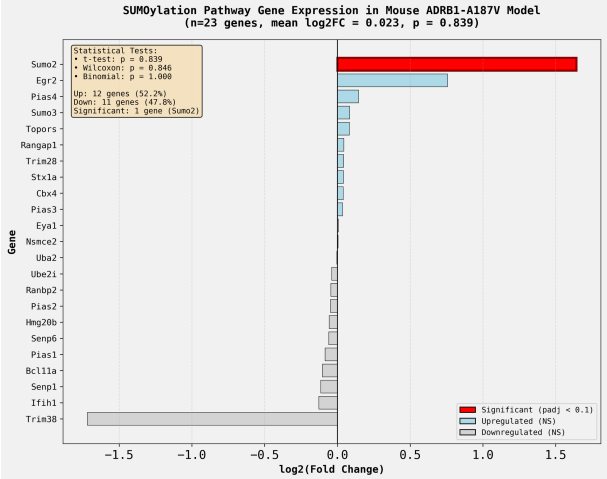

Figure 7: Sumo2 is the only significantly upregulated gene within the SUMOylation pathway in the ADRB1-A187V mouse model. The bar plot displays the log2 fold change in pontine expression for 23 genes in the pathway, with Sumo2 being the sole gene to pass the significance threshold (adjusted p < 0.1). The lack of a coordinated transcriptional response across the other pathway members indicates that the strong upregulation of Sumo2 is an isolated event rather than pathway-wide activation. (Source: [r7])

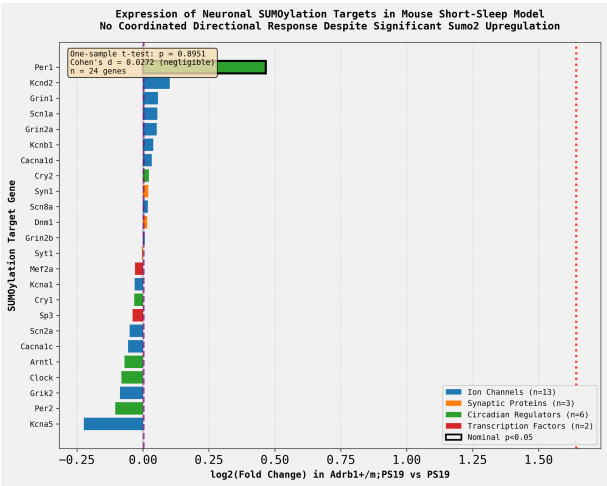

Figure 8: Neuronal SUMOylation target genes show no coordinated transcriptional response in the ADRB1-A187V mouse model. The bar chart displays the log2 fold change in expression for a curated set of 24 established SUMO substrate genes, with the mean change not significantly deviating from zero (one-sample t-test, p = 0.8951). The dotted red line indicates the log2 fold change of Sumo2 (1.64) for comparison. The stable expression of these substrate genes supports a post-translational mechanism where protein availability is unchanged. (Source: [r16])

SUMO axis as orthogonal to a conserved metabolic program: oxidative phosphorylation is the strongest concordant enrichment across mouse ADRB1-A187V and Drosophila DEC2-

P384R datasets (mouse ES = 616.5, p = 6.5 Œ 10^{-18}; fly ES = 128.7, p = 0.23), alongside positive signals in glycolysis, pentose phosphate, pyruvate, and pyrimidine metabolism, while neuroactive ligandreceptor interaction is concordantly downregulated [r37]. These results derive from a gene set enrichment framework that ranked genes by sign(log2 fold change) Œ log10(p value), calculated running-sum enrichment scores, and evaluated significance with MannWhitney U tests; concordance was defined by shared sign across species and was robust despite limited power in flies (0 pathways at FDR < 0.1) [r37]. The absence of transcriptional co-activation among SUMO machinery and targets argues that Sumo2 upregulation engages a post-translational excitability mechanism that operates in parallel to, and likely independently from, the conserved oxidative metabolism signature observed in natural short sleep [r7, r16, r37].

Translationally, increasing SUMOylation is predicted to mimic short-sleep physiology by shifting neuronal excitability upward, with a practical pharmacology lever being inhibition of deSUMOylating proteases that prefer SUMO-2/3 (notably SENP2) [r15, coelhosilva2017, connolly2025]. Among reported small molecules, ebselen is an organoselenium SENP2 inhibitor with in vivo neuroprotective activity in stroke models, providing indirect evidence of CNS engagement; however, explicit brain pharmacokinetics and in vivo EEG/EMG sleep data are not available in the provided sources [r25]. The preclinical behavioral literature summarized here does not show a wake-promoting profile: baseline open-field locomotion is unchanged, amphetamine-induced hyperlocomotion is attenuated, and no animal sleep architecture data were reported; limited human polysomnography suggests reduced slow-wave sleep without broader changes, which does not establish arousal promotion in animals [r31]. Epac1 agonismanother candidate downstream of ADRB1is currently not testable for CNS arousal due to the lack of brain-penetrant Epac1-selective activators; the prototypical 8pCPT2OMecAMP is membrane-impermeant with poor bioavailability and no CNS PK or sleepwake data [r34]. Thus, ebselen remains the only actionable tool to acutely elevate SUMO-2/3 conjugation in vivo; a decisive next step is to confirm brain exposure and target engagement (global and substrate-specific SUMO conjugates) alongside simultaneous EEG/EMG to test whether transient SENP2 inhibition reduces sleep need without inducing hyperexcitability, noting genetic evidence that excessive deSUMOylase loss can produce hyperexcitable states [r15, r25, r31, connolly2025].

Trajectory Sources

Trajectory r1: Re-analysis of the mouse ADRB1-A187V RNA-seq data using DESeq2 identified one gene, Sumo2 (ENSMUSG00000020738), with statistically significant differential expression at padj < 0.1 (padj $= 2.15\text{Œ}10^{-5}$, log2FC $= 1.64$, upregulated 3-fold in mutants).

Trajectory r7:
ANALYSIS SUMMARY: SUMOylation Pathway Expression in Mouse ADRB1-A187V Model
Main Finding: The hypothesis that genes functionally related to SUMOylation show a consistent trend of expression change is **NOT supported** by the data. Among 23 SUMOylation pathway genes analyzed, only Sumo2 sho...

Trajectory r15: Elevated Sumo2 in pontine neurons is predicted to promote wakefulness by increasing SUMO2/3 conjugation of K+ channels (Kv2.1, K2P1, Kv7) and presynaptic release machinery (e.g., RIM1α), thereby reducing stabilizing K+ currents, enhancing Ca2+ influx/release, and raising neuronal excitability. (con...

Trajectory r16:
ANSWER: The Hypothesis is NOT Supported by the Data
The significant upregulation of Sumo2 (log2FC $= 1.64$, padj < 0.001) in the mouse short-sleep model is **NOT** functionally linked to coordinated expression changes in its key neuronal substrate genes. The analysis of 24 well-established neuron...

Trajectory r22: About one-third of the 24 SUMO2 substrates have documented roles in neuronal excitability or pro-excitatory synaptic function in the provided context, supporting the hypothesis that SUMO2 upregulation can mechanistically bias circuits toward wake-promoting excitability, although direct links to slee...

Trajectory r25: The hypothesis is partially supported: small-molecule SENP inhibitors exist and at least oneebselen, a SENP2 inhibitorhas reported in vivo neuroprotective activity in stroke models implying CNS action, but direct BBB pharmacokinetic documentation is not present in the provided sources (jiao2024sum...

Trajectory r31: The hypothesis is not supported: in the animal literature summarized here, there are no EEG/EMG sleep-architecture data and the available locomotor/behavioral results do not indicate wake-promoting effects of ebselen (ramli2022 pages 13-15, ramli2022 pages 5-7, ramli202...

Trajectory r34: The hypothesis is not supported: among Epac1 activators identified (notably 8pCPT2OMecAMP and related analogues), no published CNS pharmacokinetic data or sleepwake/arousal behavioral outcomes are reported, and the best-characterized agonist is membrane-impermeant with decreased bioavailabili...

Trajectory r37: A systematic cross-species GSEA analysis of 91 KEGG pathways identified 48 concordant pathways (52.7%), with oxidative phosphorylation showing the strongest concordant positive enrichment (mouse: ES=616.5, $p=6.5\text{Œ}10^{-18}$; fly: ES=128.7, p=0.23), revealing conserved metabolic shifts beyond the previousl...

Trajectory r50:
The ADRB1-A187V mutation does NOT lead to coordinated transcriptional activation of the non-canonical beta-arrestin/GRK signaling pathway in the mouse model. A comprehensive gene set analysis of 29 pathway components (GRKs, beta-arrestins, MAPK/ERK, Src kinases, PI3K/Akt, JNK, and p38 MAPK) reveale...