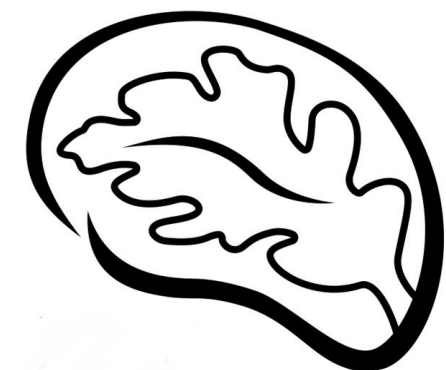




Cell-type-resolved *APOE* ε4 analysis reveals neuronal activation, *XIST* inversion, and pathology-linked regulatory programs in Alzheimer’s disease

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Background

APOE ε4 is the strongest genetic risk factor for late-onset Alzheimer’s disease (AD), yet its cell-type-specific mechanisms remain incompletely defined. We leveraged a 2.6-million-nucleus human dorsolateral prefrontal cortex single-nucleus RNA-seq (RNAseq) dataset to dissect *APOE* ε4 effects on cell composition, gene expression, gene–endophenotype coupling across neuropathology, cognition, and clinical diagnosis, and transcription factor regulon activity.

Table 1. Cohort Characteristics

	All	Female	Male	P value	<i>APOE</i> ε4 Carrier	<i>APOE</i> ε4 Non-carrier	P value
N	613	385	228		156	457	
— Demographics —							
Age at death (years)	88.50 ± 6.61	89.55 ± 6.53	86.72 ± 6.37	<0.001	87.87 ± 5.99	88.71 ± 6.80	0.15
Education (years)	17.60 ± 3.52	16.10 ± 3.28	17.43 ± 3.75	<0.001	16.88 ± 3.37	16.50 ± 3.56	0.24
PMI (hours)	7.64 ± 4.93	7.71 ± 4.97	7.53 ± 4.89	0.66	7.17 ± 4.37	7.81 ± 5.11	0.13
— Sex & Genetics —							
Female, % (n)	62.8% (385)	100.0% (385)	0.0% (0)	—	62.2% (97)	63.0% (288)	0.93
<i>APOE</i> ε4 carrier, % (n)	25.4% (156)	25.2% (97)	25.9% (59)	0.93	100.0% (156)	0.0% (0)	—
— Neuropathology —							
Amyloid burden (sqrt)	1.04 ± 0.79	1.13 ± 0.79	0.89 ± 0.78	<0.001	1.46 ± 0.68	0.90 ± 0.78	<0.001
Tangle burden (sqrt)	1.26 ± 0.89	1.38 ± 0.93	1.06 ± 0.78	<0.001	1.67 ± 1.09	1.12 ± 0.76	<0.001
Pathconf_AD, % (n)	76.5% (189)	83.5% (132)	64.0% (57)	<0.001	90.0% (81)	68.8% (108)	<0.001
Pathconf_control, % (n)	23.5% (58)	16.5% (26)	36.0% (32)	<0.001	10.0% (9)	31.2% (49)	<0.001
— Cognition —							
Cognitive slope	-0.13 ± 0.10	-0.13 ± 0.11	-0.12 ± 0.10	0.28	-0.17 ± 0.12	-0.11 ± 0.10	<0.001
Last cognitive score	-0.92 ± 1.20	-0.98 ± 1.22	-0.80 ± 1.15	0.07	-1.39 ± 1.34	-0.76 ± 1.10	<0.001
— Clinical diagnosis —							
No Impairment, % (n)	34.9% (214)	33.2% (128)	37.7% (86)	0.49	23.1% (36)	38.9% (178)	<0.001
MCI, % (n)	24.3% (149)	24.4% (94)	24.1% (55)	0.49	17.3% (27)	26.7% (122)	<0.001
AD/Other Dementia, % (n)	40.8% (250)	42.3% (163)	38.2% (87)	0.49	59.6% (93)	34.4% (157)	<0.001

*Values denoted as mean ± standard deviation or percentage

Methods

- 613 ROS/MAP donors (**Table 1**) including sporadic AD and control from the SEA-AD IAC dataset (syn64410371) were analyzed, spanning 24 transcriptomic subclasses.
- Cell composition was assessed using scCODA Bayesian compositional analysis at subclass resolution, with and without amyloid and tangle pathology covariates.
- Gene-level analyses were performed using Nebula mixed models to test *APOE* ε4 differential expression and sex- and *APOE* ε4-dependent gene–endophenotype associations across pathology, cognition, and AD diagnosis.
- Regulon activity per subclass was quantified with AUCell following pySCENIC analysis. Donor-aware linear mixed models and mediation analyses were performed to evaluate *APOE* ε4-associated regulon activity and pathology dependence.

Results

- Composition: *APOE* ε4 carriers showed broadly lower relative subclass abundance estimates across neuronal subclasses, while glial effects were more pathology-sensitive (**Figure 1**).
- Gene expression: Nebula models identified recurrent *APOE* ε4-associated neuronal activity/immediate early gene upregulation and sex-dependent gene–endophenotype coupling, including *XIST* inversion and female-biased non-*XIST* effects (**Figures 2–3**).
- Regulatory programs: *APOE* ε4-associated regulons were concentrated in Lamp5, L6 IT, Pvalb, and Microglia_PVM subclasses and were attenuated after amyloid/tangle adjustment, supporting substantial pathology-linked regulation (**Figure 4**).

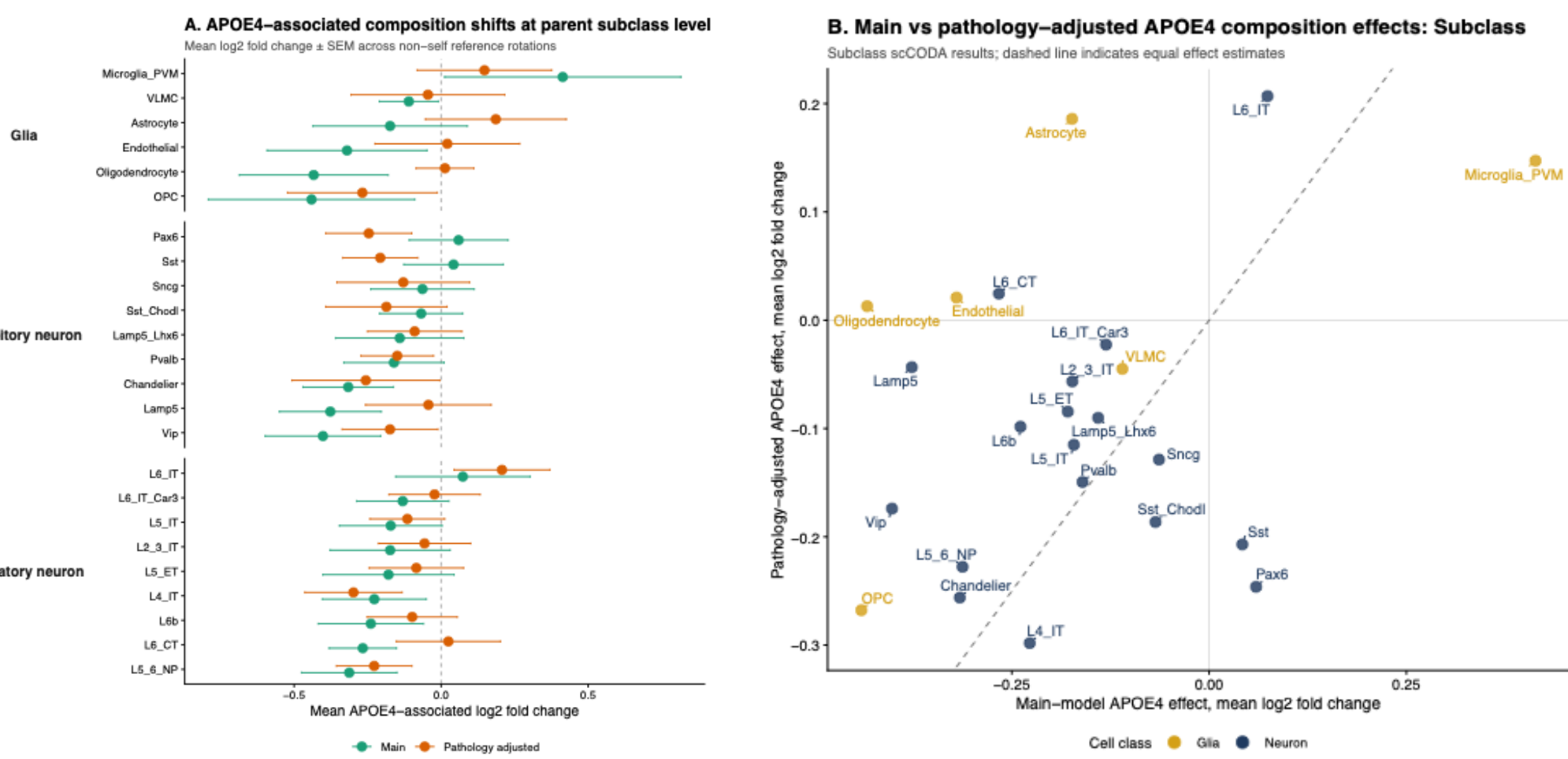


Figure 1. *APOE* ε4-associated composition shifts are modest, with broad neuronal decreases and pathology-sensitive glial effects

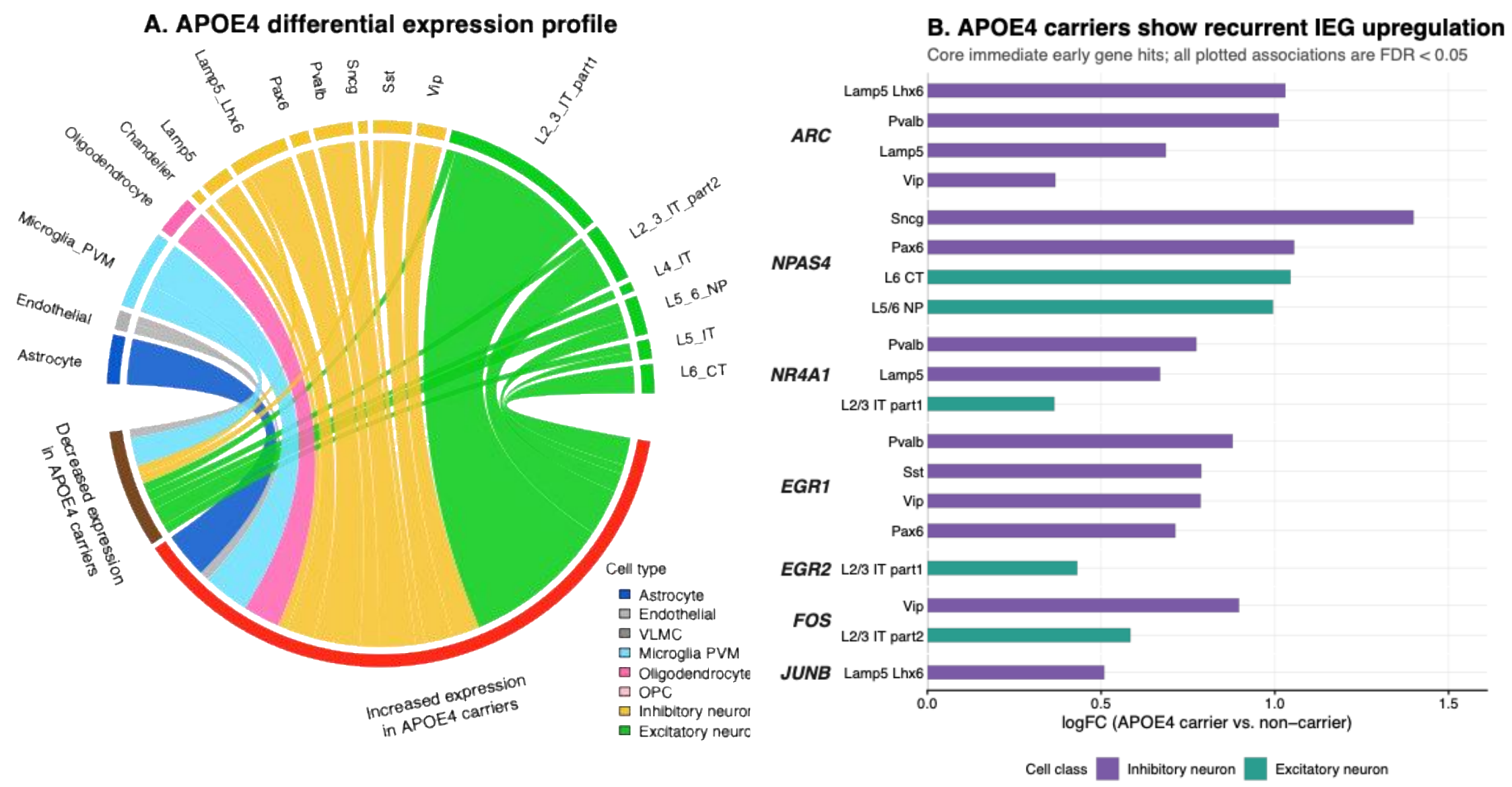


Figure 2. *APOE* ε4 differential expression is dominated by neuronal immediate early gene upregulation

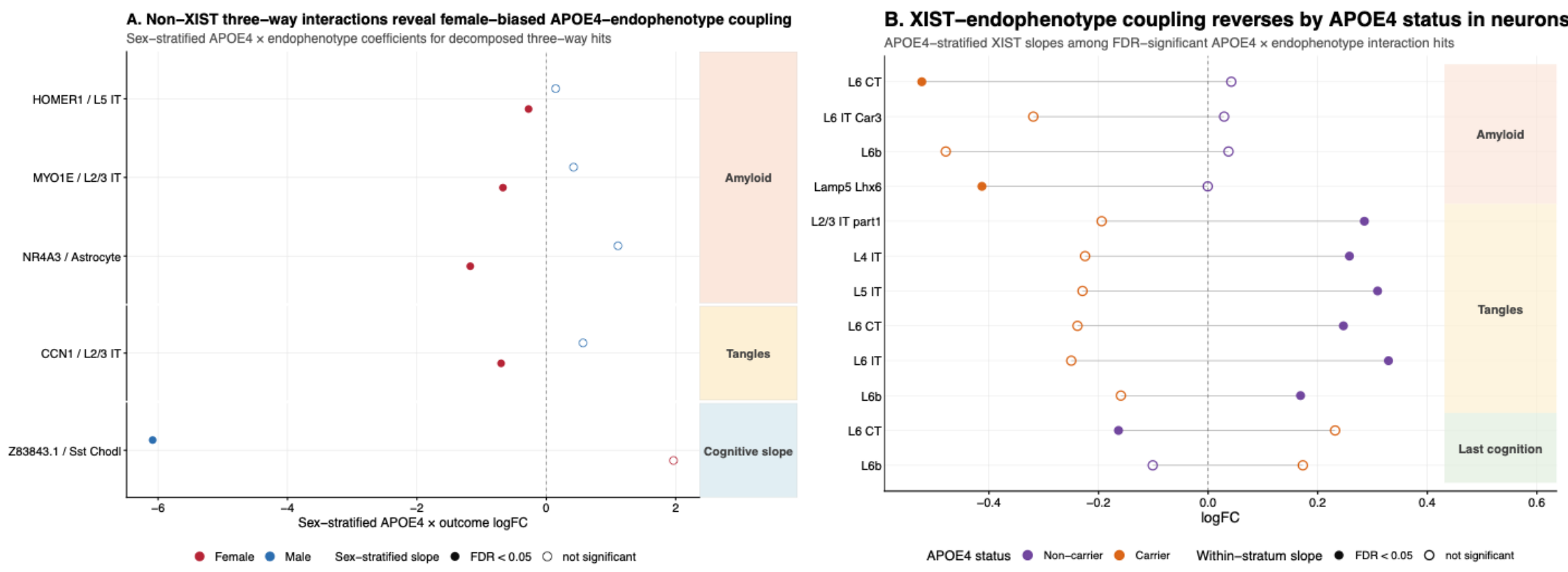


Figure 3. *APOE* ε4 reshaped AD endophenotype-associated transcriptional coupling, including *XIST* inversion and female-biased non-*XIST* effects

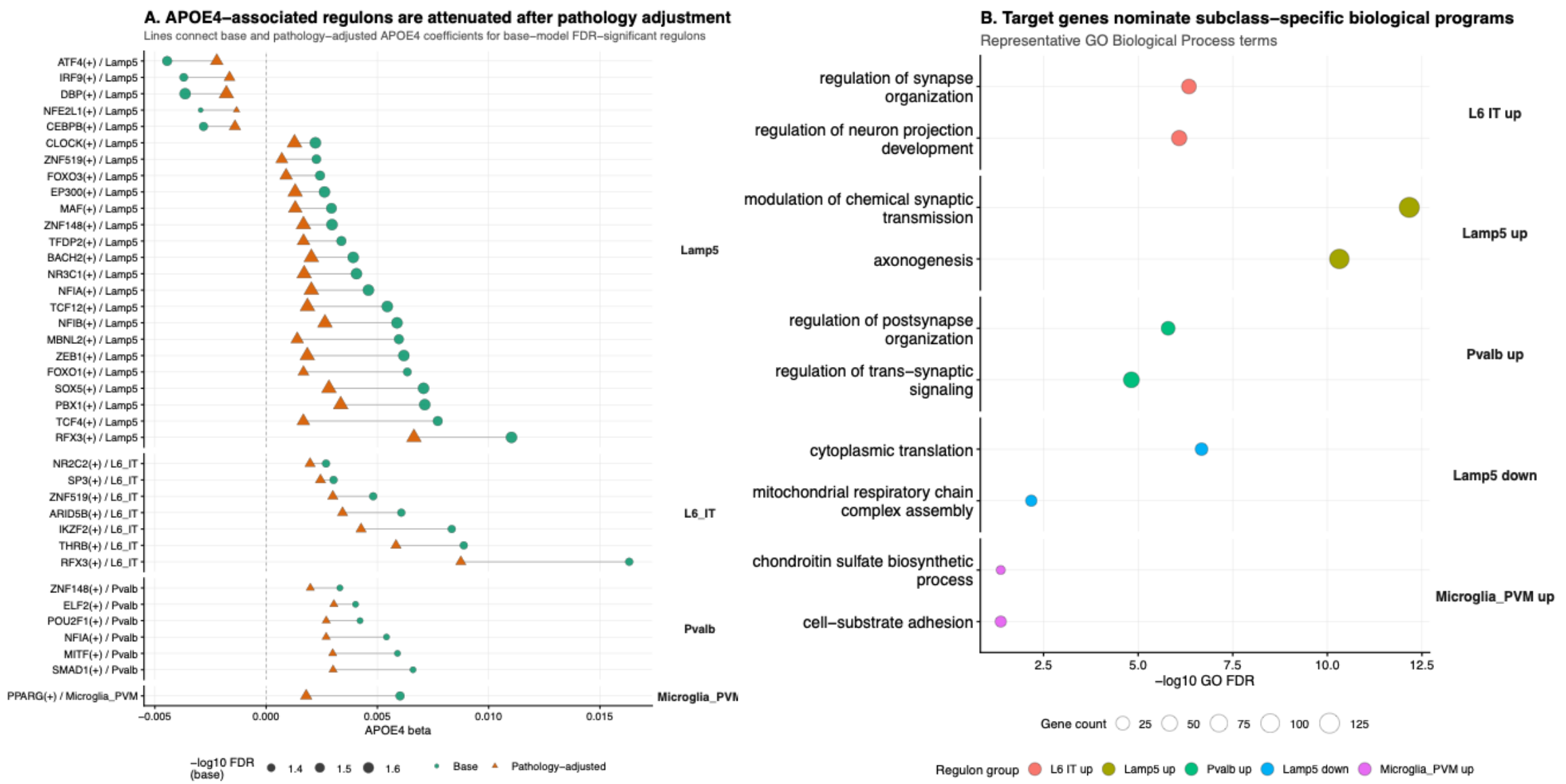


Figure 4. *APOE* ε4-associated regulon activity identifies pathology-linked regulatory programs

Conclusions

- APOE* ε4 is associated with broad, cell-type-specific compositional and transcriptional alterations in the aging cortex.
- Regulatory-program effects were most sensitive to amyloid/tangle adjustment, supporting pathology-linked *APOE* ε4 biology.
- Sex-dependent signatures, including *XIST*-associated and female-biased effects, indicate that sex modifies *APOE* ε4-related cellular vulnerability.

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