**Supplementary Material**

Methods:

Sample Characterization: Analyses were conducted using the SPSS 22 software package. Independent t-tests and χ2-square tests (for continuous and categorical variables, respectively) tested the similarity of each group within age for demographic characteristics (Table 1). Linear regression models tested the relationship between gross motor scores (dependent variable) and functional connectivity (fc, the independent variable) when controlling for site, based on values for the ten region of interest (ROI) pairs with the highest fc-behavior correlations, given that the enrichment analysis identifies networks based on ROI pairs with strong fc-behavior relationships.

Results:

*Details of Participant Characteristics*

There were no significant differences at 12 versus 24 months in proportions of participants by sex (χ2(1) = 2.19, p = .14), site (χ2(3) = 7.11, p = .068), outcome group based on autism spectrum disorder (ASD) diagnosis (χ2(2) = 3.30, p =.19), or overall cognitive development, as indexed by the Mullen Early Learning Composite (t(226) = .214, p = .83). In this subsample, all children with ASD diagnoses were at elevated familial risk for ASD, with 18.9% of high-risk infants receiving an ASD diagnosis, consistent with prior infant sibling studies (Ozonoff et al. 2011). The proportion of high-risk to low-risk infants did not differ between those with longitudinal (n = 42) versus cross-sectional data (n = 145) (χ2(1) = .041, p = .84).

Walking and gross motor scores for this subsample did not differ from the larger Infant Brain Imaging Study sample without fcMRI data [Walking: 12 months: t(597) = .086, p = .93; 24 months: t(512) = -.512, p = .61; Gross Motor: 12 months: t(604) = -.489, p = .63; 24 months: t(518) = -1.11, p = .267)]. Gross motor scores for analyzed subjects did not significantly differ by sex (12 months: t(128) = .86, p = .93; 24 months: t(97) = 1.72, .089) or site at 12 months (F(3,126) = 1.57, p = .20). At 24 months, a gross motor score difference related to site (F(3,95) = 4.52, p = .005) was attributable to the site with the least participants (Philadelphia), which displayed lower scores than the other sites. The relationship between fc and gross motor behavior (based on the 10 ROI pairs with the strongest fc-behavior correlations) remained highly significant when adjusting for site (12 months: all ten regressions with F(4,125), p < .0001; 24 months: all ten regressions with F(4,94), p ≤ .004).

*Fc-walking Analyses: Examination of tDMN ROIs Implicated in Multiple Network Blocks*

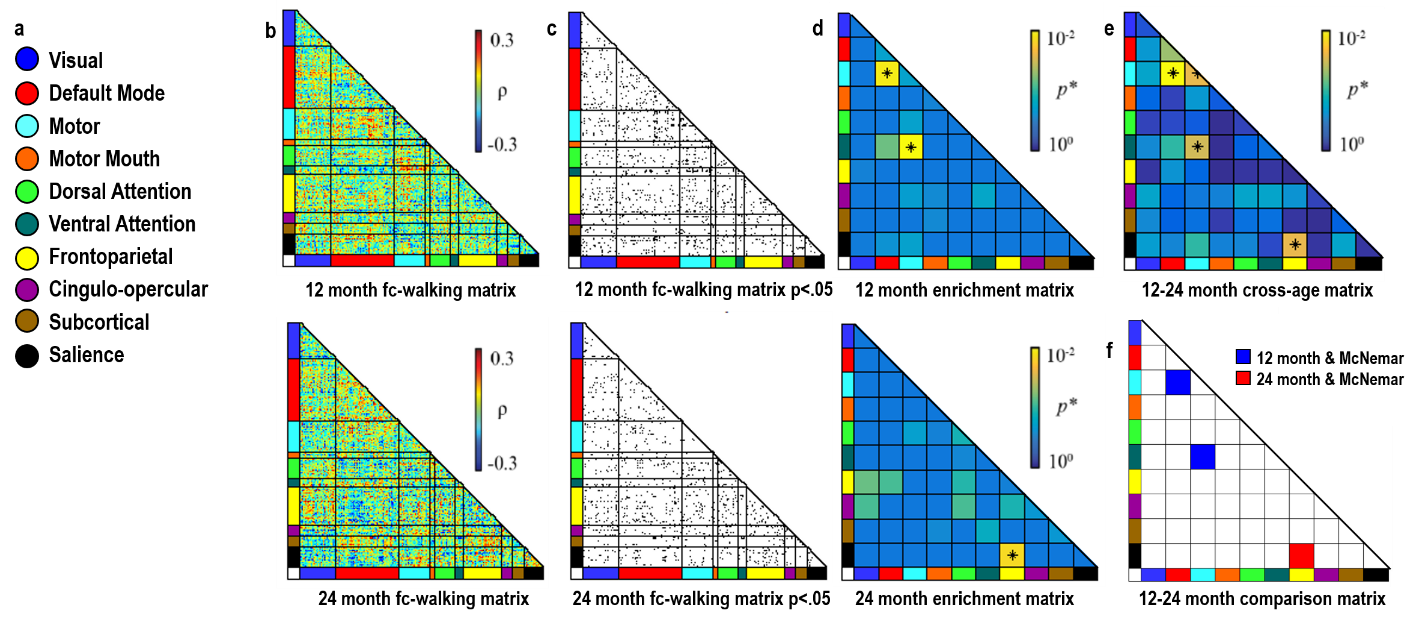
As described in the main text, results of fc-walking analyses showed that at 12 months, the temporal default mode network (tDMN) was implicated in multiple enriched network blocks. ROI pairs contributing to enrichment showed uniformly positive brain-behavior relationships (red lines) in tDMN-SMN (temporal default mode-somatomotor and temporal default mode-somatomotor 2 networks), but negative brain behavior relationships in tDMN-tDMN (blue lines) (Figure 4). To examine whether this inverse pattern (i.e., positive between-network versus negative within-network fc-walking relationships) was accounted for by specific tDMN ROIs, we compared tDMN ROIs contributing to enrichment between networks to those contributing to enrichment within the tDMN. Of the sixteen tDMN ROIs comprising ROI pairs in tDMN-SMN, fifteen also contributed to enrichment within the tDMN and accounted for 92% of all ROI pairs in tDMN-SMN. All of these connections preserved the pattern of opposite sign noted above for fc-walking relationships. Similarly, fifteen of the seventeen tDMN ROIs in ROI pairs for tDMN-SMN2 also contributed to enrichment within the tDMN. This accounted for 93% of all ROI pairs in tDMN-SMN2, with 90% of these ROI pairs preserving an inverse pattern of sign for fc-walking relationships.

*Sign of Functional Connectivity in Enriched Network Blocks for fc-Walking Analyses*

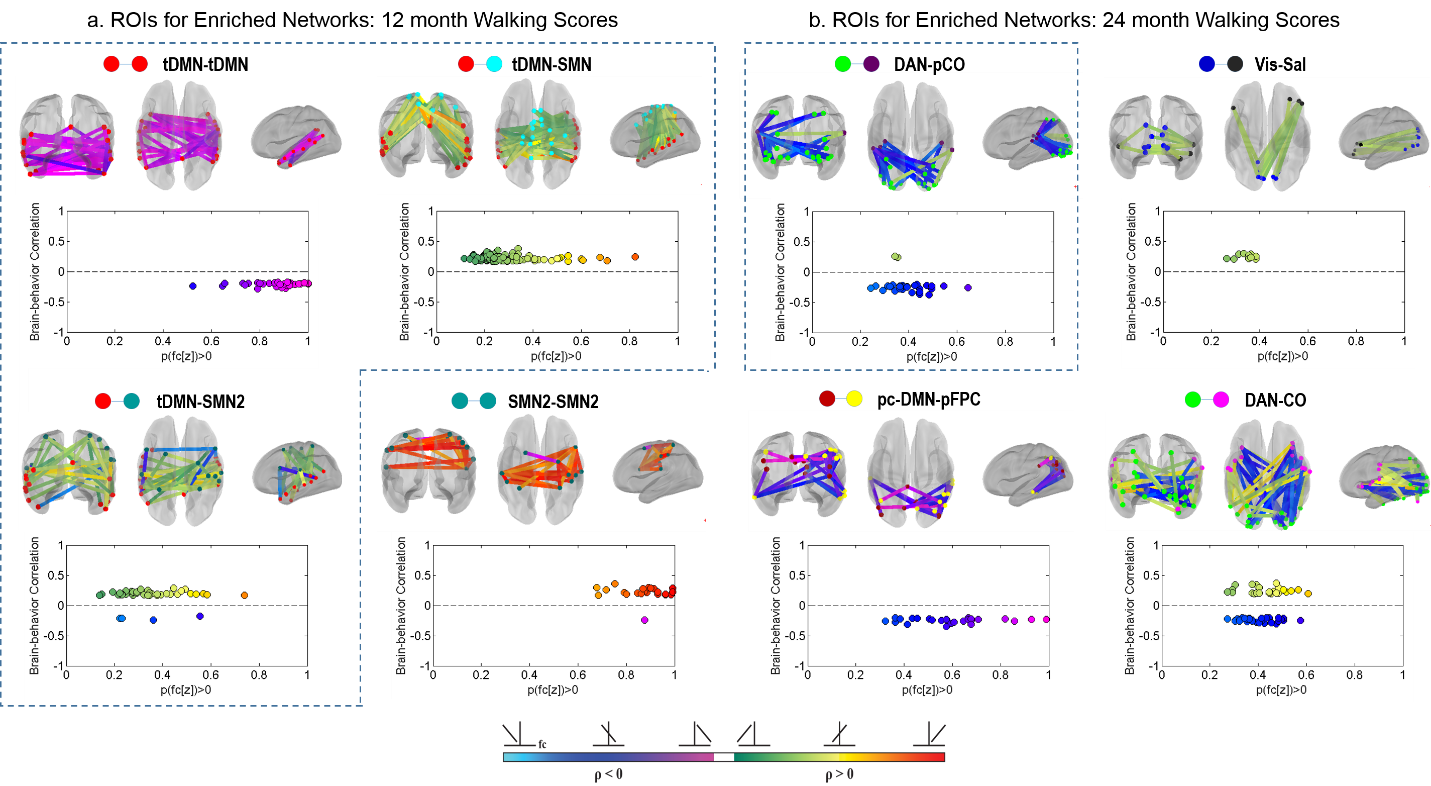
Fc values were quantified in terms of the proportion of positive fc values for a given ROI pair across all subjects, where proportions above 0.5 indicate primarily positive fc values, and proportions below 0.5 indicate primarily negative fc values. In the case of the tDMN-tDMN at 12 months, negative fc-walking relationships involved ROI pairs with largely positive fc values, whereby more positive fc between tDMN ROIs was associated with lower walking scores. For tDMN-SMN and tDMN-SMN2, positive fc-walking relationships involved ROI pairs with mostly negative fc values, whereby increased fc between ROI pairs was associated with higher walking scores. At 24 months, the negative fc-walking relationship for DAN-pCO (dorsal attention-posterior cingulo-opercular networks) involved ROI pairs with primarily negative fc values, with fc increases associated with lower walking scores. Fc values among individual subjects for these ROI pairs revealed a broad spread of positive and negative fc values, consistent with sufficient variation being represented in fc-walking correlations (Figure S3).

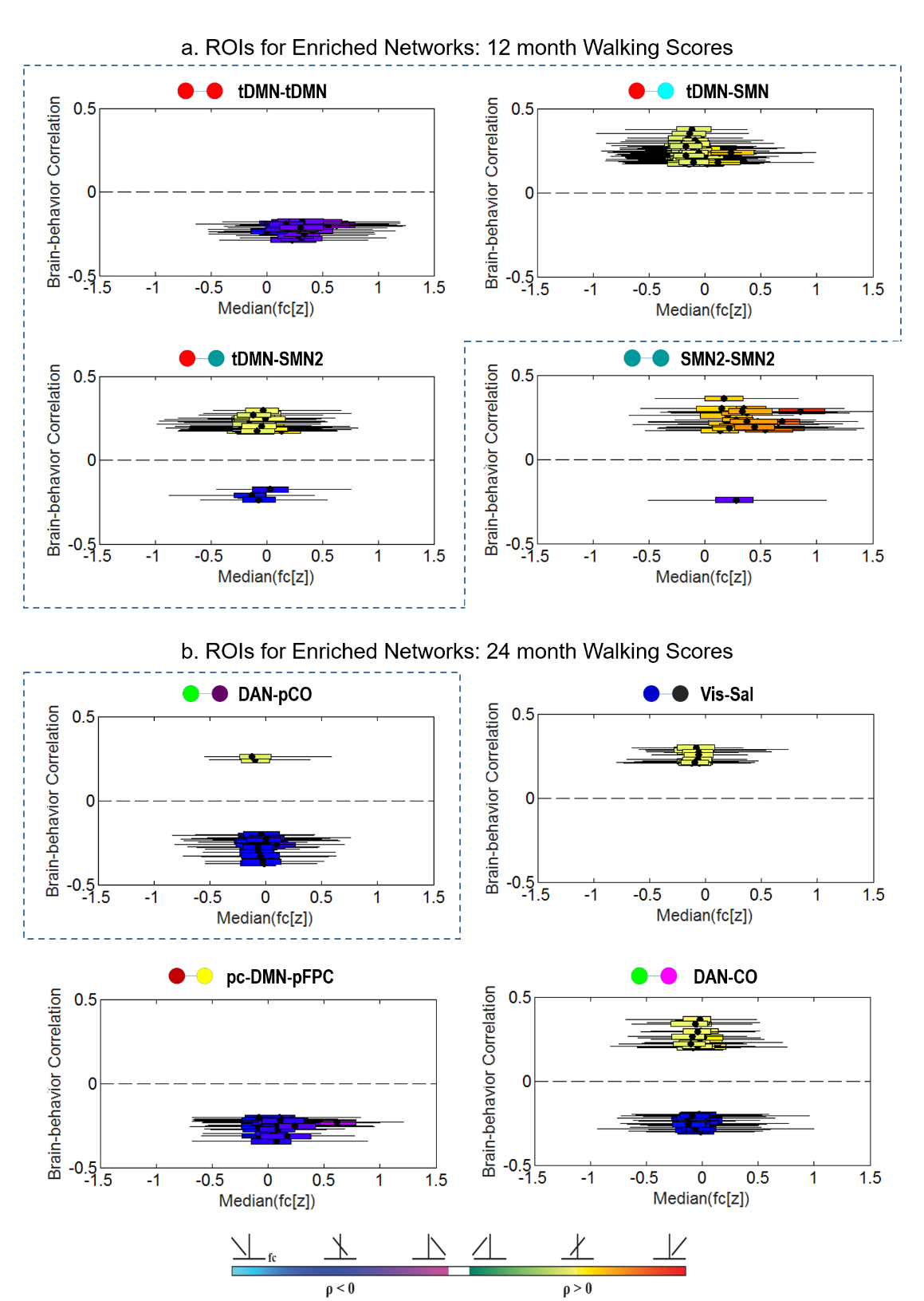
References:

Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, Zwaigenbaum L, Bryson S, Carver LJ, Constantino JN, Dobkins K, Hutman T, Iverson JM, Landa R, Rogers SJ, Sigman M, Stone WL. 2011. Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. Pediatrics. 128:e488-95.

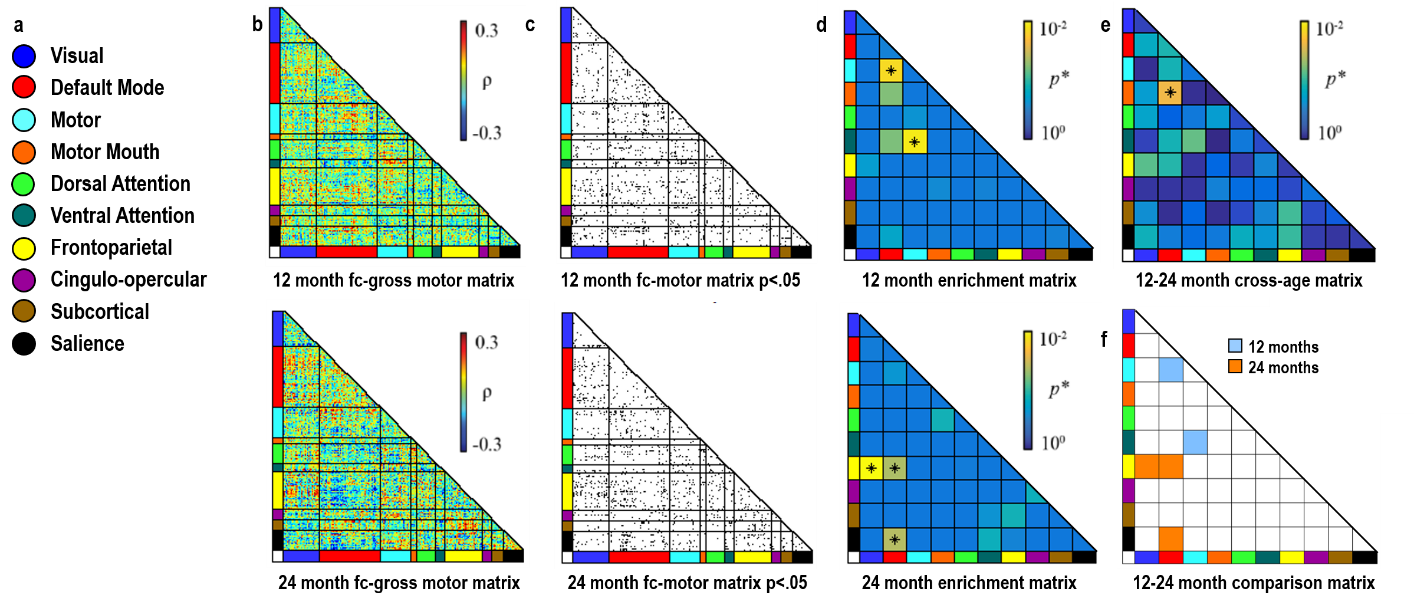
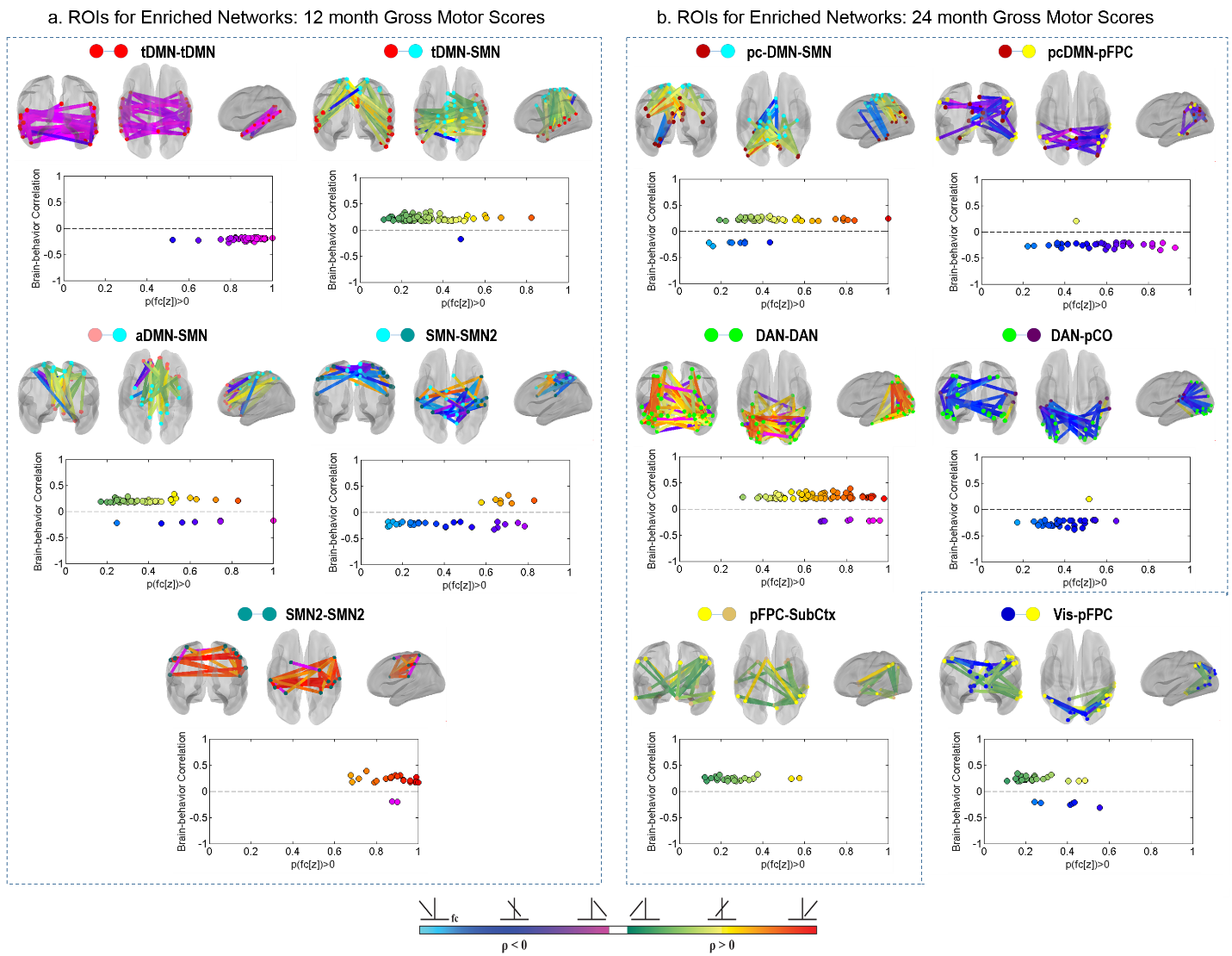
Supplementary Figures

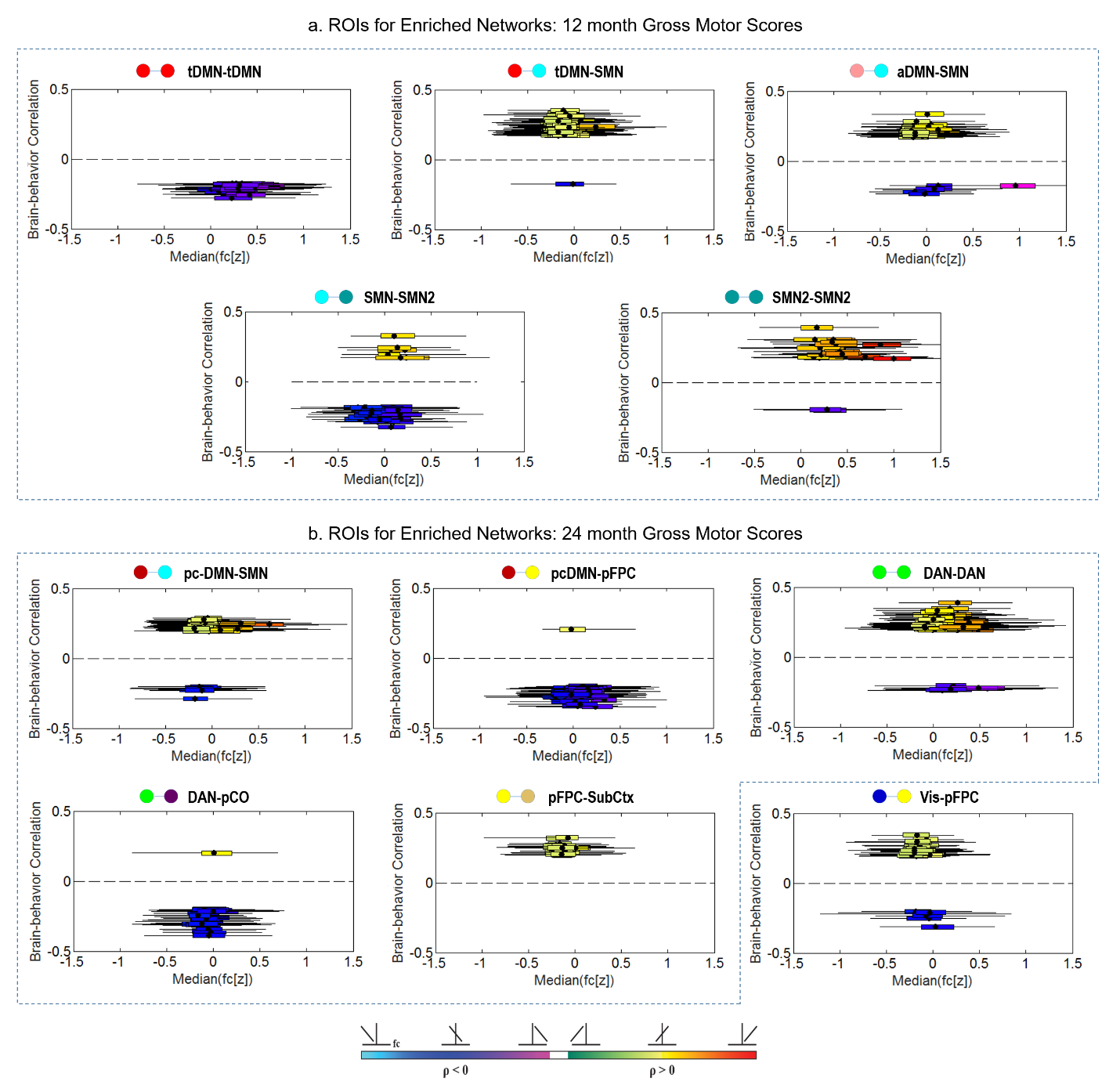
**Supplemental Figure 1. Enrichment Analyses Using Adult Networks Shows Specific Brain-Behavior Relationships of Functional Connectivity and Walking Scores at 12 and 24 months** (a) A color-coded key of 10 putative adult networks is shown. (b) Matrices describe relationships between functional connectivity (fc) and walking score. Data at 12 and 24 months are shown in the top and bottom rows, respectively (b-d). The 230 ROIs comprising the networks are sorted by assigned network along the X and Y axes. Hot colors indicate strong positive relationships of fc to walking score; whereas cool colors indicate strong negative fc-walking relationships. (c) The fc-walking matrices for 12 and 24 months are thresholded to show those ROI pairs with fc-walking correlations significant at an uncorrected threshold of *p* ≤ .05. (d) Matrices are colored by *p*-values for enrichment. Enriched blocks are labeled with an asterisk. (e) This matrix is colored by *p*-values for the McNemar test, which evaluated differences in the level of enrichment for network blocks at 12 vs. 24 months. Asterisks indicate significant differences. (f) Blocks are colored based on whether they are enriched at a particular time point and whether their level of enrichment also differs between time points. Here, significant network blocks (dark blue and red squares) are enriched at either 12 or 24 months and significantly different between 12 and 24 months. There were no additional discovery results.

**Supplemental Figure 2. ROI Pairs Contributing to Enriched Networks for Walking Analyses Include Positive and Negative fc Values** Functional connections between ROI pairs are colored according to 1) the sign of the relationship between functional connectivity (fc) value and walking score (i.e., a positive brain-behavior relationship or a negative brain-behavior relationship) and 2) the proportion of positive fc values across participants for a given ROI pair. As indicated by the color bar, blue to purple colors indicate negative fc-walking correlations, and green to red colors indicate positive fc-walking correlations. Color gradients reflect the proportion of fc values greater than 0 for each ROI pair, which was obtained by pooling fc values across participants. Values greater than 0.5 indicate primarily positive fc and values less than 0.5 indicate mostly negative fc for an ROI pair. Each dot represents an ROI pair contributing to enrichment; spatial locations of these ROI pairs are provided in the ball and stick diagrams. In several network blocks, such as tDMN-tDMN, most ROI pairs show a higher proportion (greater than 0.5) of positive fc values. Other network blocks (e.g., tDMN-SMN, tDMN-SMN2, and DAN-pCO) show more ROI pairs with lower proportions (values less than 0.5), indicating primarily negative fc for ROI pairs contributing to enrichment. Hatch marks enclose enriched network blocks found to meet criteria for significance.

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**Supplemental Figure 3. ROI Pairs in Enriched Network Blocks for Walking Behavior Show a Range of fc Values** Plots display the relationship of fc (in Z scores) on the x axis for each ROI pair contributing to enriched network blocks vs. fc-walking correlations on the y axis. Lines comprise the range of fc values observed across subjects for a given ROI pair. Bars represent the 25th and 75th percentiles and dots in the middle of the bars represent the median. Bar colors indicate the sign of both fc values and the relationship of fc to walking score, as shown in color bar. (a) 12-month results comprise a range encompassing both positive and negative fc values. (b) Similar findings are observed at 24 months.

**Supplementary Figure 4. Enrichment Analyses Using Adult Networks Show Specific Brain-Behavior Relationships for Functional Connectivity and Gross Motor Scores at 12 and 24 months** (a) A color-coded key of 10 putative adult networks is provided for reference. (b) Matrices describe relationships between functional connectivity (fc) and gross motor score. Data at 12 and 24 months are shown in the top and bottom rows, respectively (b-d). The 230 ROIs comprising the networks are sorted by assigned network along the X and Y axes. Hot colors indicate strong positive relationships of fc to gross motor score; whereas cool colors indicate strong negative fc-gross motor relationships. (c) The fc-gross motor matrices for 12 and 24 months are thresholded to show those ROI pairs with fc-gross motor correlations significant at an uncorrected threshold of *p* ≤ .05. (d) Matrices are colored by *p*-values for enrichment. Enriched blocks are labeled with an asterisk. (e) This matrix is colored by *p*-values for the McNemar test, which evaluated differences in the level of enrichment for network blocks at 12 vs. 24 months. Asterisks indicate significant differences. (f) Blocks are colored based on whether they are enriched at a particular time point and whether their level of enrichment also differs between time points. In this analysis, enriched network blocks qualify as discovery results but not primary results, because these blocks, although enriched at one time point, do not show significant differences in enrichment across time or enrichment at both times. This finding illustrates the importance of conducting infant-toddler brain-behavior analyses in reference to networks derived from infant-toddler fcMRI data.**Supplemental Figure 5. ROI Pairs Contributing to Enriched Networks for Gross Motor Analyses Include Positive and Negative fc Values** Functional connections between ROI pairs are colored according to 1) the sign of the relationship between fc value and gross motor score and 2) the proportion of positive or negative fc values between ROI pairs for a given connection. The color bar details these relationships: blue to purple colors indicate negative fc-gross motor correlations and green to red colors indicate positive fc-gross motor correlations. Color gradients reflect the proportion of fc values greater than 0 for each ROI pair, which was obtained by pooling fc values across participants. Values greater than 0.5 indicate primarily positive fc, and values less than 0.5 indicate mostly negative fc for an ROI pair. Each dot represents an ROI pair contributing to enrichment; spatial locations of these ROI pairs are provided in the ball and stick diagrams. In several network blocks, e.g. tDMN-tDMN and DAN-DAN, most ROI pairs show a higher proportion (greater than 0.5) of positive fc values, while ROI pairs in others (e.g., DAN-pCO and pFPC-SubCtx) show lower proportions (values less than 0.5), indicating primarily negative fc. In several network blocks (e.g., SMN-SMN2, pcDMN-SMN, and pcDMN-pFPC) the proportions for ROI pairs contributing to enrichment involve a range of positive or negative fc values around 0.5. Hatch marks enclose enriched network blocks found to meet criteria for significance.



**Supplemental Figure 6. ROI Pairs in Enriched Network Blocks for Gross Motor Behavior Show a Range of fc Values** Plots display the relationship of fc (in Z scores) on the x axis for each ROI pair contributing to enriched network blocks vs. fc-gross motor correlations on the y axis. Lines comprise the range of fc values observed across subjects for a given ROI pair. Bars represent the 25th and 75th percentiles and dots in the middle of the bars represent the median. Bar colors indicate the sign of both fc values and the relationship of fc to gross motor score, as shown in color bar. (a) 12-month results comprise a range encompassing both positive and negative fc values. (b) Similar findings are observed at 24 months.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Network Block | Age  (mos.) | Percent of ROI Pairs with p≤.05 | χ2 Statistic | χ2 asymptotic p value | χ2 FPR p value | HG asymptotic p value | HG FPR p value | McNemar χ2 statistic | McNemar asymptotic p value | McNemar FPR p value |
| **Walking Scores: Infant/Toddler Network** | | | | | | | | | | |
| *tDMN-tDMN* | 12 | 14.6% | 36.80 | 1.31e-09 | .0139 | 1.17e-07 | .0121 | 23.68 | 5.68e-07 | .0106 |
| *tDMN-SMN* | 12 | 15.3% | 149.75 | 1.96e-34 | .0025 | 2.73e-25 | .0002 | 65.33 | 3.16e-16 | 1.78e-04 |
| *tDMN-SMN2* | 12 | 19.6% | 32.31 | 1.32e-08 | .0169 | 2.35e-07 | .0134 | 20.48 | 3.01e-06 | .0161 |
| SMN2-SMN2 | 12 | 12.7% | 51.57 | 6.92e-13 | .0084 | 1.02e-09 | .0064 | 10.70 | 5.35e-04 | .0649 |
| *DAN-pCO* | 24 | 21.0% | 15.85 | 6.85e-05 | .0454 | .000121 | .0352 | 13.44 | 1.23e-04 | .0421 |
| DAN-CO | 24 | 9.1% | 19.97 | 7.87e-06 | .0339 | 6.75e-05 | .0275 | 1.06 | .1512 | .4944 |
| pcDMN-pFPC | 24 | 10.7% | 18.77 | 1.51e-05 | .0365 | .000227 | .0348 | 5.45 | .0098 | .1679 |
| Vis-Sal | 24 | 11.6% | 21.25 | 4.03e-06 | .0316 | 1.71e-05 | .0392 | 3.76 | .0262 | .2400 |
| **Walking Scores: Adult Network** | | | | | | | | | | |
| *DMN-SMN* | 12 | 10.2% | 36.02 | 1.96e-09 | .0117 | 9.96e-09 | .0049 | 36.92 | 6.15e-10 | .0025 |
| *SMN-VAN* | 12 | 17.1% | 40.40 | 2.065e-10 | .0098 | 2.16e-09 | .0062 | 15.36 | 4.43e-05 | .0318 |
| *FP-Sal* | 24 | 10.1% | 34.94 | 3.39e-09 | .0141 | 3.81e-08 | .0090 | 16.62 | 2.29e-05 | .0268 |
| **Gross Motor Scores: Infant/Toddler Network** | | | | | | | | | | |
| *tDMN-tDMN* | 12 | 15.8% | 59.42 | 1.27e-14 | 0.0067 | 7.95e-11 | 0.0047 | 20.45 | 3.05e-06 | 0.0160 |
| *tDMN-SMN* | 12 | 17.2% | 123.05 | 1.36e-28 | 0.0028 | 4.37e-21 | 0.0005 | 38.44 | 2.82e-10 | 0.0020 |
| *aDMN-SMN* | 12 | 9.6% | 14.63 | 1.31e-04 | 0.0489 | 1.14e-04 | 0.0378 | 18.06 | 1.07e-05 | 0.0217 |
| *SMN-SMN2* | 12 | 10.6% | 15.06 | 1.04e-04 | 0.0469 | 1.97e-04 | 0.0421 | 28.44 | 4.82e-08 | 0.0060 |
| *SMN2-SMN2* | 12 | 22.9% | 76.40 | 2.32e-18 | 0.0047 | 2.67e-11 | 0.0041 | 22.04 | 1.33e-06 | 0.0131 |
| *DAN-pCO* | 24 | 12.9% | 22.61 | 1.99e-06 | 0.0289 | 4.78e-06 | 0.0220 | 13.30 | 1.33e-04 | 0.0437 |
| *DAN-DAN* | 24 | 9.9% | 28.30 | 1.04e-07 | 0.0211 | 3.70e-07 | 0.0152 | 30.75 | 1.47e-08 | 0.0045 |
| *pcDMN-SMN* | 24 | 12.1% | 26.61 | 2.49e-07 | 0.0236 | 6.77e-07 | 0.0165 | 14.75 | 6.12e-05 | 0.0350 |
| *pcDMN-pFPC* | 24 | 17.6% | 51.16 | 8.53e-13 | 0.0090 | 4.52e-10 | 0.0065 | 14.53 | 6.88e-05 | 0.0354 |
| *pFPC-SubCtx* | 24 | 14.1% | 19.93 | 8.04e-06 | 0.0340 | 5.03e-05 | 0.0332 | 17.39 | 1.52e-05 | 0.0238 |
| Vis-pFPC | 24 | 14.2% | 25.59 | 4.23e-07 | 0.0247 | 4.12e-06 | 0.0217 | 7.53 | .0133 | 0.1146 |
| **Gross Motor Scores: Adult Network** | | | | | | | | | | |
| DMN-SMN | 12 | 9.4% | 32.14 | 1.44e-08 | .0141 | 1.26e-08 | .0065 | 6.55 | 0.0052 | 0.132 |
| SMN-VAN | 12 | 16.7% | 36.10 | 1.88e-09 | .0117 | 4.45e-08 | .0078 | 9.8 | 8.73e-04 | 0.0741 |
| Vis-FP | 24 | 10.8% | 43.91 | 3.45e-11 | .0101 | 1.77e-10 | .0044 | 9.52 | 0.0010 | 0.0775 |
| DMN-FP | 24 | 8.1% | 15.26 | 9.39e-05 | .0435 | 3.91e-05 | .0283 | 7.67 | 0.0028 | 0.1079 |
| DMN-Sal | 24 | 8.6% | 16.27 | 5.51e-05 | .0405 | 3.24e-05 | .0273 | 2.11 | 0.0732 | 0.356 |

**Supplemental Table 1. Enrichment Statistics**

Values for enrichment statistics are provided for network blocks which were enriched at 12 months or 24 months. Of these results, a subset of network blocks, indicated in italics, qualified as significantly enriched. These blocks were enriched either at 12 or 24 months and also displayed significant differences in the level of enrichment at 12 and 24 months. See Figure 1 for key for network abbreviations. Mos. refers to months. ROI stands for “region of interest”. HG refers to hypergeometric, as in the hypergeometric test. FPR stands for false positive rate.