Supplementary information

Covariates

Child ethnicity was defined using the ethnicity categorization of 'Statistics Netherlands' (1). Handedness of the child was obtained using the Edinburgh Handedness Inventory (2). Maternal education was defined as highest education completed (3) and household income was defined by the total net monthly income of the household. Information on maternal alcohol use and smoking during pregnancy was obtained using questionnaires from each trimester of pregnancy. Child attention problems, which are known to be highly comorbid with autistic traits (4), were measured at the age of 6 years using the Attention Problems (AP) syndrome scale of the Child Behavior Checklist (CBCL) for ages 1.5-5. Non-verbal IQ at age 6 was estimated from the Mosaics and Categories subtest of the Snijders-Oomen Non-Verbal Intelligence Test –Revised (5). Total brain volume was calculated by adding up the bilateral supratentorial volumes and cerebellum.

In all regression analyses in SPSS, missing values of potential confounding (family) risk factors (7.5% for IQ, 0.1% for handedness, 2.6% for maternal education, 3.9% for household income, 9.1% for alcohol use during pregnancy and 3.1% for smoking during pregnancy) were imputed using the multiple imputation (Markov chain Monte Carlo) method in SPSS with 5 imputations and 10 iterations

The Social Responsiveness Scale

The Social Responsiveness Scale (SRS) is a 65-item questionnaire that represents the parent's observation of the child's social behavior during the past six months. Each item is scored from 0 ('never true') to 3 ('almost always true'). The SRS can be scored on a total scale and on social cognition, social communication and social mannerism subscales. Higher scores indicate more problems. The SRS covers various dimensions of interpersonal behavior, communication and repetitive/stereotypic behavior characteristics of autism spectrum disorders. When using a clinical cut-off score, the SRS was found to have high sensitivity (0.85) and moderate specificity (0.75) in a sample of 61 child psychiatric patients (6). Associations of SRS total scores with ADI-R algorithm scores for DSM-IV criterion sets were on the order or 0.7 in that same sample and in another sample of 119 children with special educational needs ADI-R total scores correlated 0.59 with SRS total scores (7).

The 18-items questionnaire in the current study contained items from the following subscales: social cognition, social communication and autistic mannerism. In the Generation R sample, the Cronbach's alpha indicated high inter-item reliability for the SRS (alpha=0.79). In a sample of 3857 children aged 4-18 years (as part of the Social Spectrum Study, a multicenter study social development in the children referred to a mental health care institution in the South-West of the Netherlands from 2010-2012) the correlation between total scores derived from the selected 18 items (SRS short-form) and the SRS scores derived from the complete test was r=0.95 ($p<0.001$) (unpublished data). The correlation between total scores derived by the SRS short-form and the SRS in the Missouri Twin Study was 0.93 in monozygotic male twins (n=98) and 0.94 in dizygotic male twins (n=134). In a sample of 2719 children from the Interactive Autism Network (unpublished data), the corresponding correlation was 0.99.

In this study, the Dutch version of the Social Responsiveness Scale was administered as part of a written questionnaire on the child's behavior and growth around age 6 (8). The questionnaires were mailed to the parents. In 92% of cases, the questionnaires were filled out by the biological mother. Scores of questionnaires filled out by the mother were not significantly different from those filled out by fathers (p=.478). For individual items contributing to the Social Responsiveness Scale scores, a maximum of 25% missing items were allowed. Total scores were weighted by the number of non-missing items. In all analyses, Social Responsiveness Scale scores were square root transformed to approach a normal distribution.

Magnetic Resonance Imaging

Structural MRI scans were obtained on a 3-Tesla scanner (Discovery MR750, GE Worldwide, Milwaukee, USA). Using an 8-channel head coil, a whole-brain high-resolution T_1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained. The scan parameters were: TR = 10.3 ms, TE = 4.2 ms, flip angle = 16º, 186 contiguous slices with a thickness of 0.9 mm, and in-plane resolution = 0.9 × 0.9 mm.

All T_1-weighted scans were rated on a 6-item scale for quality (unusable, poor, fairly good, good, very good, excellent). Scans rated as 'fairly good' or better were included. After processing by FreeSurfer, all images were again visually inspected to rate the segmentation quality. Processed data rated as unusable or poor was excluded from analyses, as well as the subjects for whom the required output could not be constructed.

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 5.1 (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures have been fully described in prior publications (9). Briefly, cortical thickness was calculated as the closest distance from the gray/white matter boundary of the cortex to the gray matter/cerebral spinal fluid boundary at the cortical vertex for each tessellated surface (10). Thickness maps were smoothed with a 10 mm full-width half-maximum (FWHM) Gaussian kernel prior to statistical analysis. Numerous studies using FreeSurfer in typical and atypical developing school-age children are available (11).

To assess the local gyrification index (LGI) we used the method of Schaer et al. (12), that is implemented in FreeSurfer. This approach provides an estimation of the local gyrification index, taking into account the three-dimensional cortical surface. Identification of the pial and white matter surfaces against an additional surface that tightly wraps the pial surface are used to estimate the degree of cortical folding at a 25 mm spherical vertex-based region. This method has been validated and used in several studies focusing on childhood and adolescent psychopathology (13, 14). The surface based LGI maps were smoothed prior to the analyses using a 5 mm full-width half-maximum (FWHM) Gaussian kernel, consistent with several comparable studies (13).

Supplementary References

1. Allochtonen in Nederland. Voorburg/Heerlen: Amsterdam University Press; 2004.
2. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 1971;9(1):97-113.
3. Netherlands S. Standaardonderwijsindeling. Voorburg/Heerlen2004.
4. Grzadzinski R, Di Martino A, Brady E, Mairena MA, O'Neale M, Petkova E, Lord C, Castellanos FX. Examining autistic traits in children with ADHD: does the autism spectrum extend to ADHD? J Autism Dev Disord. 2011;41(9):1178-91.
5. Tellegen PJ W-WB, Laros JA. Snijders-Oomen Niet-Verbale Intelligentietest: SON-R 2.5 - 7/. Amsterdam: Boom Testuitgevers; 2005.
6. Constantino JN. Social Responsiveness Scale (SRS), Manual. Los Angeles: Western Psychological services; 2002.
7. Charman T, Baird G, Simonoff E, Loucas T, Chandler S, Meldrum D, Pickles A. Efficacy of three screening instruments in the identification of autistic-spectrum disorders. Brit J Psychiat. 2007;191:554-9.
8. Roeyers H TM, Druart C, De Schryver, M, & Schittekate, M. SRS Screeningslijst voor autismespectrumstoornissen. Handleiding Amsterdam: Hogrefe; 2011.
9. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis - I. Segmentation and surface reconstruction. Neuroimage. 1999;9(2):179-94.
10. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. P Natl Acad Sci USA. 2000;97(20):11050-5.
11. Ghosh SS, Kakunoori S, Augustinack J, Nieto-Castanon A, Kovelman I, Gaab N, Christodoulou JA, Triantafyllou C, Gabrieli JD, Fischl B. Evaluating the validity of volume-based and surface-based brain image registration for developmental cognitive neuroscience studies in children 4 to 11 years of age. Neuroimage. 2010;53(1):85-93.
12. Schaer M, Ottet MC, Scariati E, Dukes D, Franchini M, Eliez S, Glaser B. Decreased frontal gyrification correlates with altered connectivity in children with autism. Frontiers in Human Neuroscience. 2013;7.
13. Wallace GL, Robustelli B, Dankner N, Kenworthy L, Giedd JN, Martin A. Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders. Brain. 2013;136:1956-67.
14. Kelly PA, Viding E, Wallace GL, Schaer M, De Brito SA, Robustelli B, McCrory EJ. Cortical Thickness, Surface Area, and Gyrification Abnormalities in Children Exposed to Maltreatment: Neural Markers of Vulnerability? Biol Psychiat. 2013;74(11):845-52.

Supplementary Table 1.

Non-response analysis

Child characteristics	**Filled out SRS questionnaire (n=5298)**					**Imaging first wave of datacollection (n=1070)**									
						Current study sample (n=717)					**Not in sample (n=353)**				
	observations					**observations**					**observations**				
Ethnicity (%)	5275					717					353				
Dutch		65.3					74.3					54.7			
Other Western		9.4					6.7					8.5			
Non-Western		25.3					19.0					36.8			
		mean	**SD**	**range**			**mean**	**SD**	**range**			**mean**	**SD**	**range**	
Social Responsiveness Scale	5043					717					139				
weighted score		0.23	0.25	0	3		0.27	0.29	0	3		0.35		0	3
Age at SRS (years)	5298	6.18	0.49	4.89	8.90	717	6.17	0.47	4.89	8.90	157	5.19	0.47	6.22	8.9
Child Behavior Checklist	5043					704					271				
attention problems score		1.46	1.69	0	10		1.98	2.05	0	9		2.48	2.48	0	9
IQ (non-verbal)	4444	102.69	14.69	50	150	663	102.86	14.42	50	142	319	99.31	14.52	56	139
Maternal characteristics															
Education level (%)	5054					698					273				
High		60.9					58.6					16.5			
Medium		28.5					30.2					38.1			
Low		10.6					11.2					16.5			
Monthly household income (%)	4784					680					257				
High		80.5					80.7					60.3			
Medium		14.1					14.3					25.7			

Low	5.4	4.7	14.0

Supplementary Table 2.
Total brain volume corrected analyses (n=717)

			B	SE B	*p*	β
Left Hemisphere						
	Temporal/precuneus	Model 1	-0.202	0.049	.000	-0.150
		Model 2 (adjusted)	-0.133	0.051	.009	-0.099
	Frontal	Model 1	-0.135	0.037	.000	-0.137
		Model 2 (adjusted)	-0.080	0.037	.032	-0.080
	Pre/postcentral	Model 1	-0.197	0.075	.009	-0.097
		Model 2 (adjusted)	-0.092	0.079	.243	-0.045
Right Hemisphere						
	Temporal/frontal	Model 1	-0.361	0.086	.000	-0.153
		Model 2 (adjusted)	-0.219	0.077	.004	-0.093
	Cingulate	Model 1	-0.304	0.078	.000	-0.143
		Model 2 (adjusted)	-0.204	0.087	.019	-0.096
	Frontal/cingulate	Model 1	-0.110	0.037	.003	-0.112
		Model 2 (adjusted)	-0.056	0.039	.148	-0.057

Note. Local gyrification indices were residualized for age at scanning. Model 1 adjusted for age when Social Responsiveness Scale was completed and gender. Model 2 additionally adjusted for child ethnicity, maternal education, maternal alcohol use, maternal smoking, Child Behavior Checklist attention problem scores, non-verbal IQ and total brain volume.

Supplementary Table 3.

Case control analyses of ADI-R/ADOS confirmed ASD cases (n=6, all male) vs. age and gender-matched controls (n=24)

		Mean ASD	Mean control	t	*p*
Left Hemisphere					
	Precuneus	0.01	0.07	1.01	.321
	Superior Frontal	0.07	0.04	-0.70	.492
	Precentral	0.01	0.04	0.29	.776
Right Hemisphere					
	Temporal	0.01	0.11	0.81	.427
	Posterior Cingulate	–0.01	0.16	0.98	.335
	Superior Frontal	0.04	0.05	0.14	.893

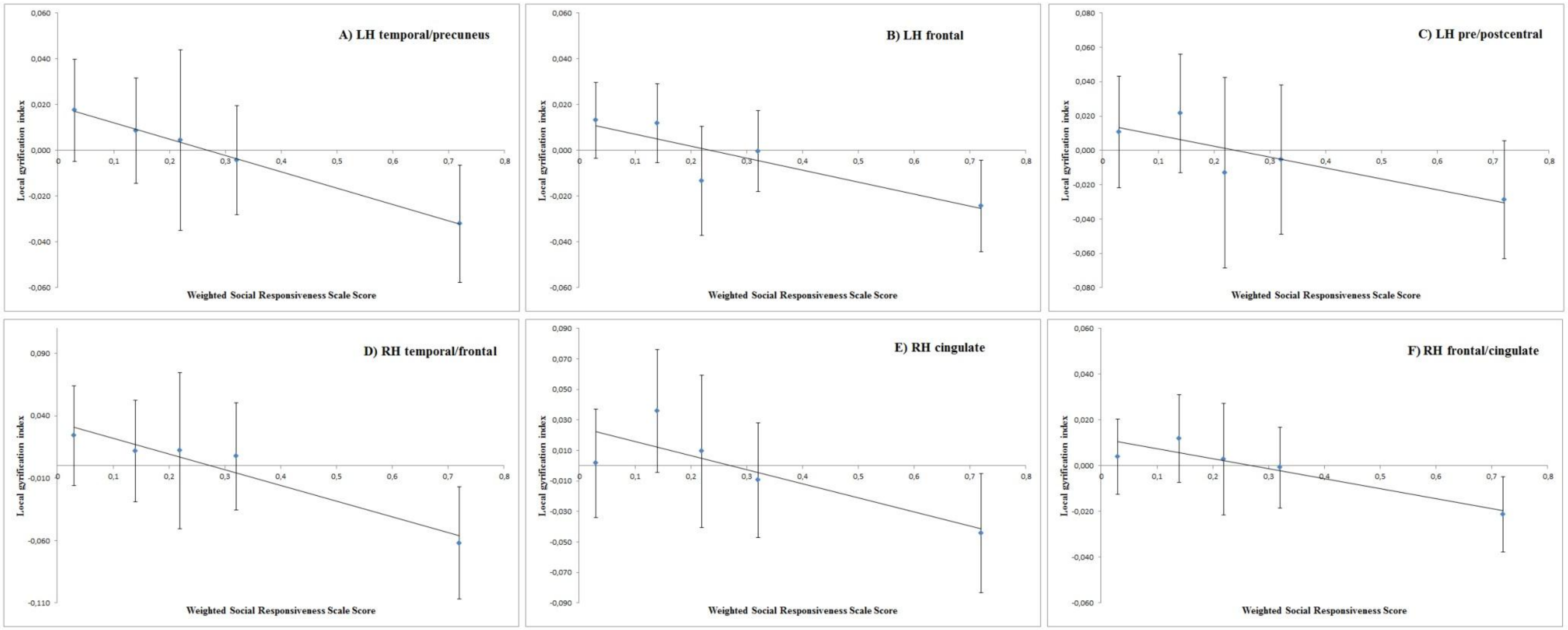
Note. Local gyrification indices were residualized for age at scanning in all analyses.

Supplementary Figure 1.
Flowchart


Imaging datalock August 2013
N=1070
SRS missing
N=214
SRS available
N=856
Insufficient quality 105
Incidental finding: 2
Gyrification output not
constructed: 15
Sufficient quality scans and
output
N=734
Siblings/twins N=17
Final sample
N=717

Supplementary Figure 2.

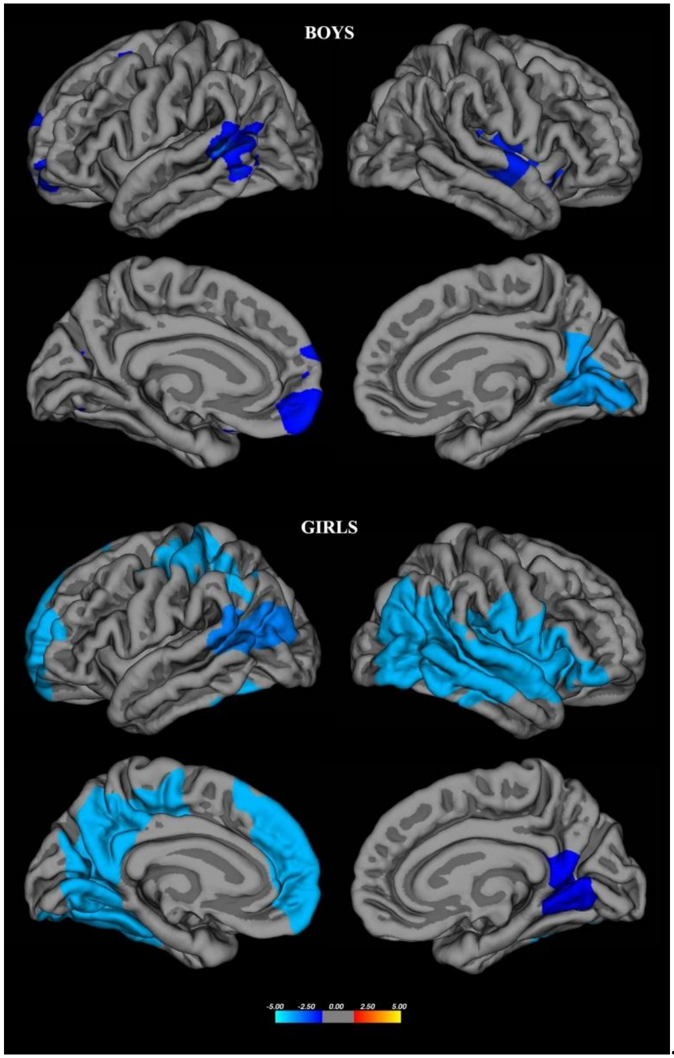
Plots of age-residualized local gyrification indices against quintiles of Social Responsiveness Score



Note. Means and 95% confidence intervals of Local Gyrification Indices are plotted against mean Social Responsiveness Scores per quintile. This is why distances are not equal.

Supplementary Figure 3.

Gyrification and autistic traits in the full sample, boys and girls shown separately



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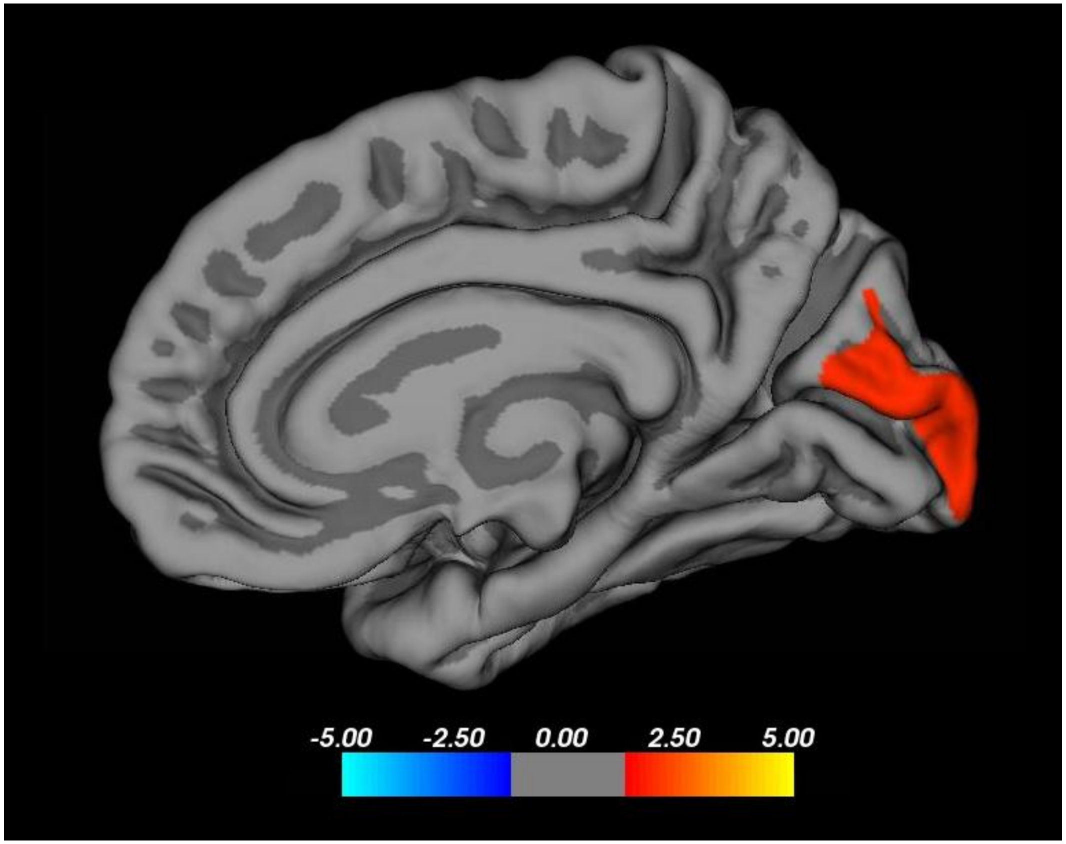
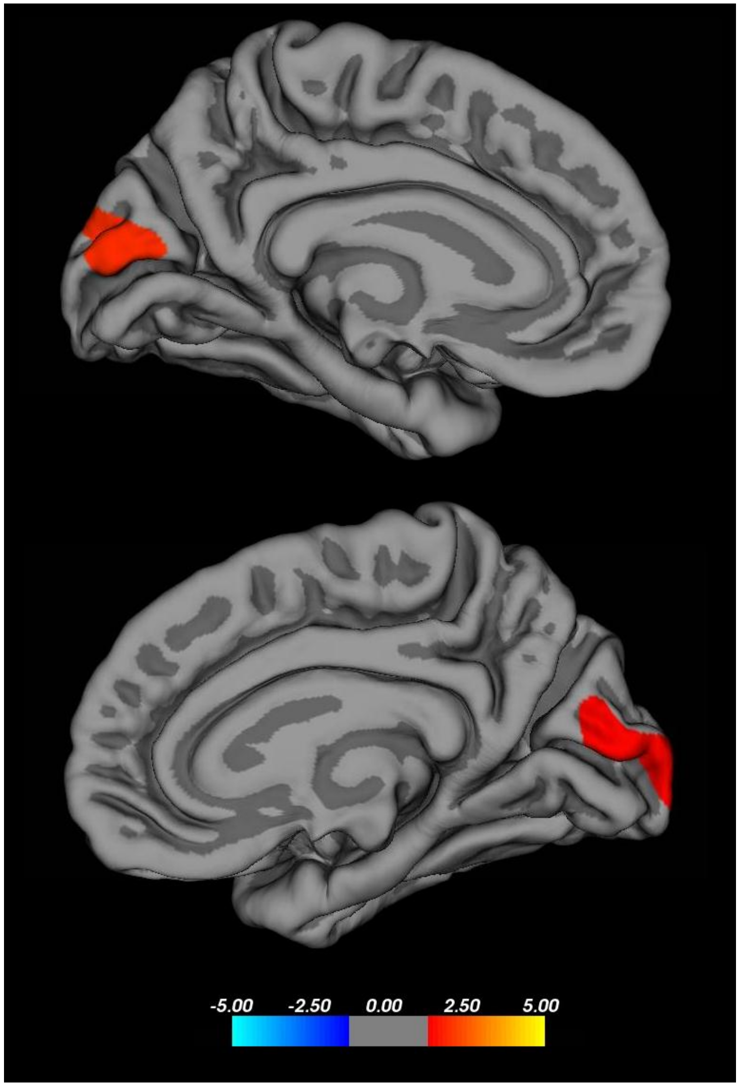
Supplementary Figure 4.
Cortical thickness and autistic traits in boys (right hemisphere)



Note. Analyses were adjusted for age. Colors represent the –log(p-value). Red clusters represent a positive correlation.

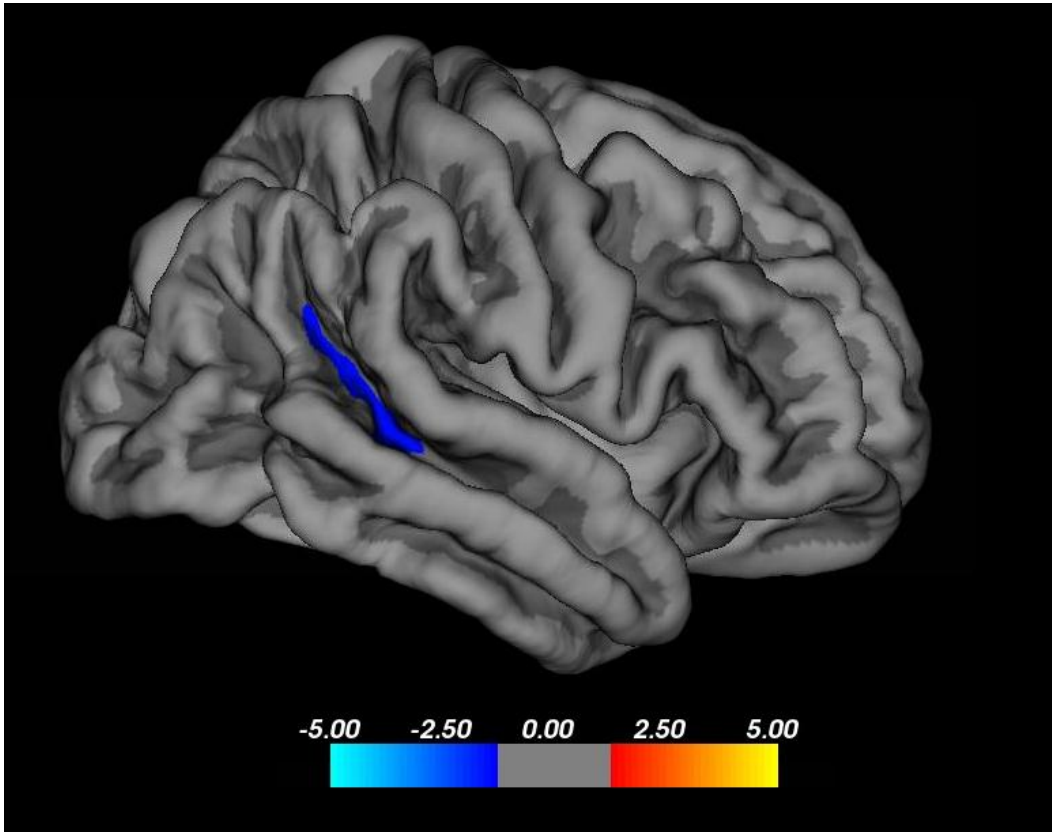
Supplementary Figure 5.
Cortical volume and autistic traits.



Note. Analyses were adjusted for age. Colors represent the –log(p-value). Red clusters represent a positive correlation.

Supplementary Figure 6.

Sulcal depth and autistic traits.



Note. Analyses were adjusted for age and in the full sample also for gender. Colors represent the -log10(p-value). Blue clusters represent a negative correlation with autistic traits.