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Supplemental Data

The Roles of FMRP-Regulated Genes in Autism Spectrum Disorder: Single- and Multiple-Hit Genetic Etiologies

Julia Steinberg and Caleb Webber

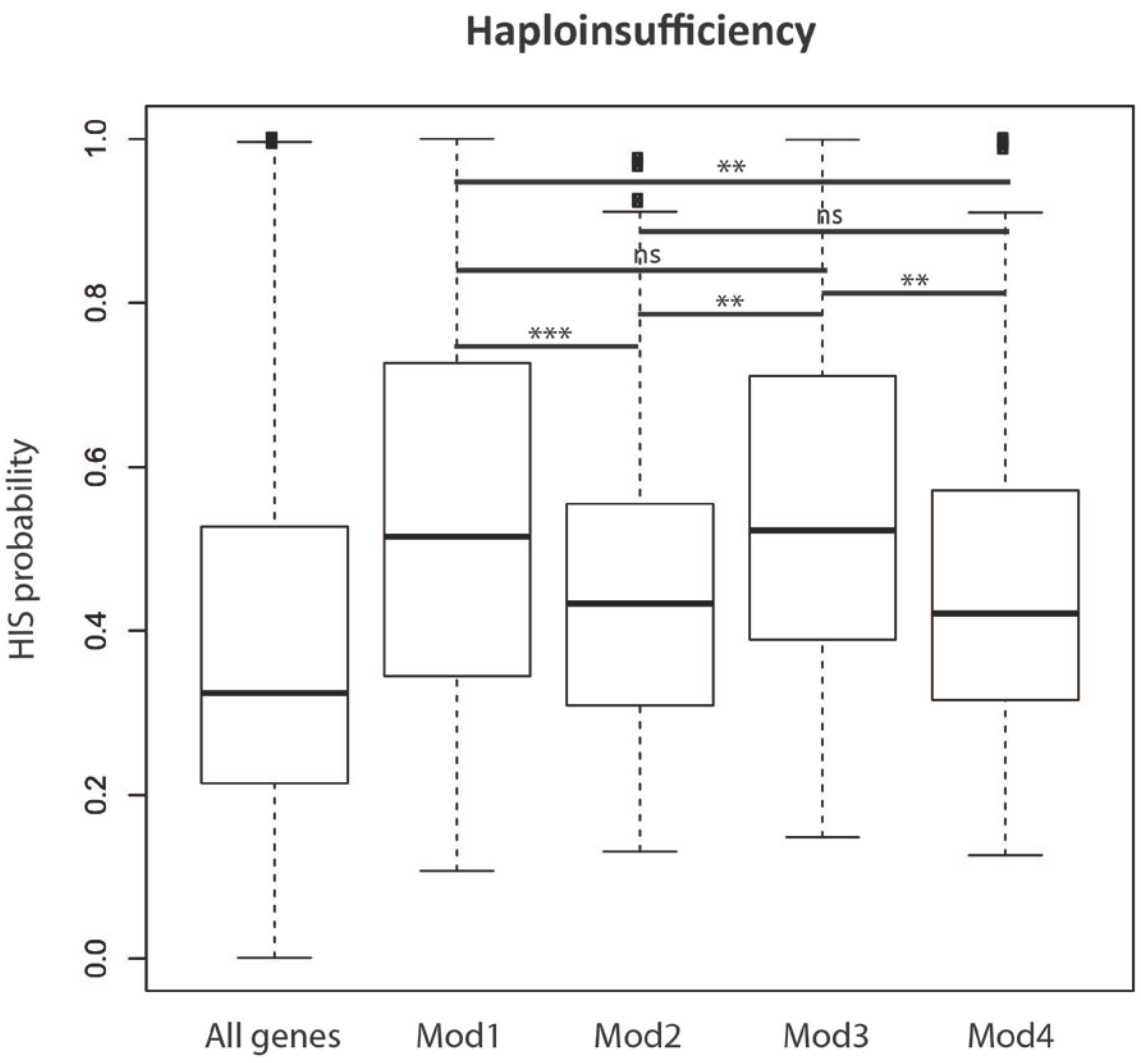

Figure S1. The distribution of predicted probabilities of haploinsufficiency varies between all genes and FMRP Modules. The difference in distributions was tested with the Mann-Whitney U-test and is significant between all genes and each of the FMRP Modules.
ns = not significant; ** = $p<0.01$; *** = $p<\times10^{-8}$; error bars: most extreme point with distance ≤1.5-fold interquartile range from the box.

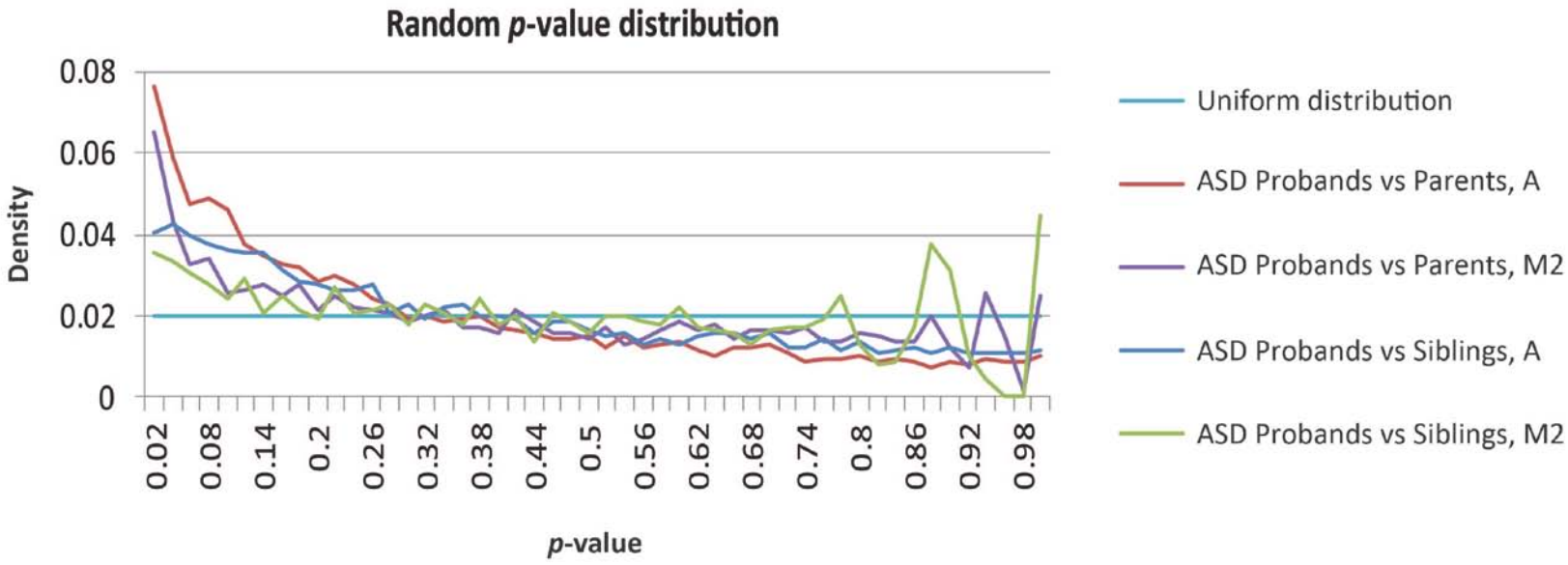

Figure S2.Trend test *p*-values for random gene sets matched to all FMRP targets (A) and FMRP Module 2 (M2), using the Sanders datasets (see Methods).

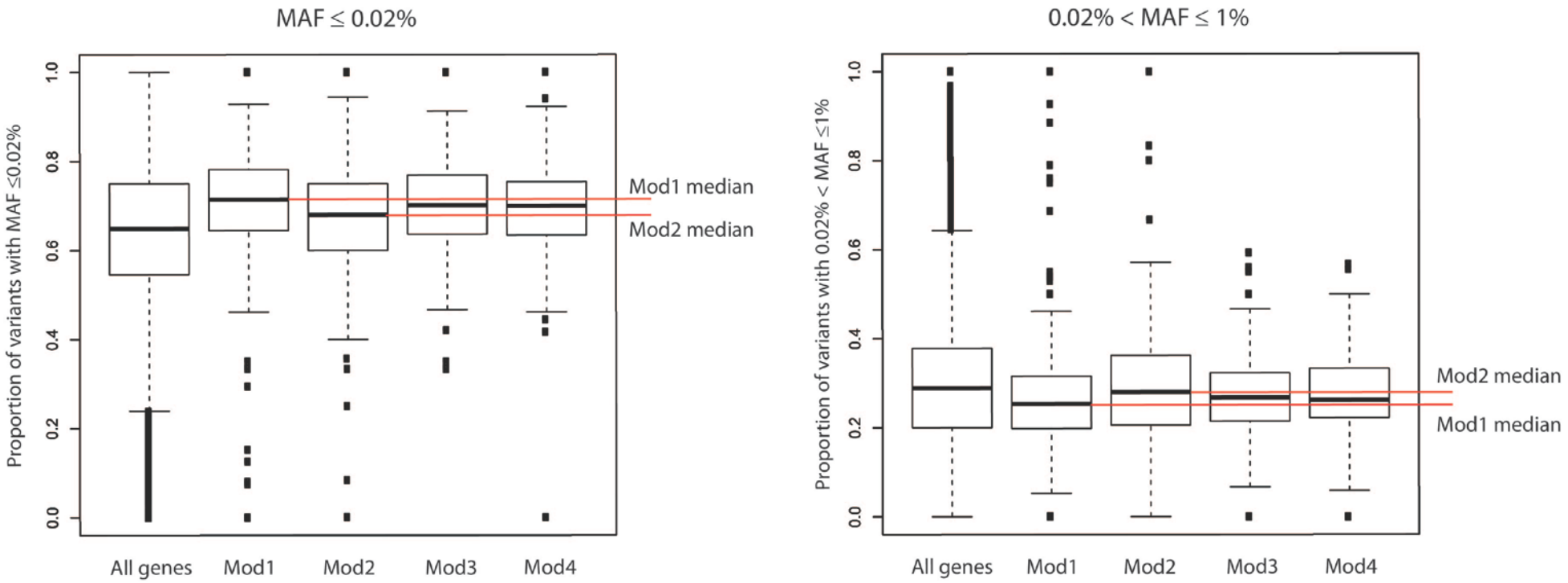

Figure S3. Differences in proportion of ultrarare (MAF<0.02%) and rare (0.02%≤MAF≤1%) nonsynonymous variants between all genes and FMRP Modules. Error bars: most extreme point with distance ≤1.5-fold interquartile range from the box.

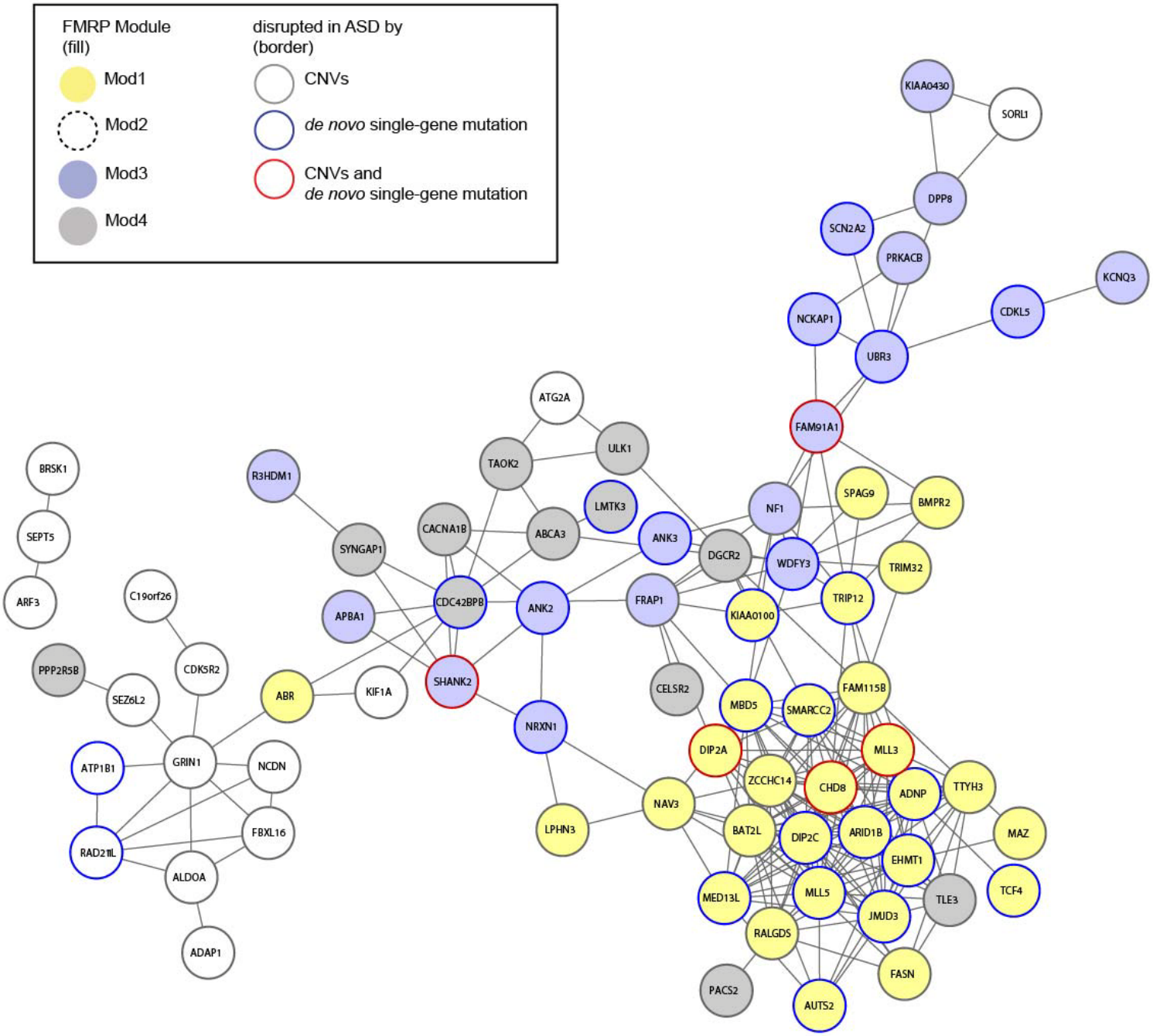

Figure S4. Brain co-expression network of FMRP targets disrupted in ASD probands by *de novo* single-gene mutations and rare deletion CNVs. The network confirms the modular pattern with the genes from Modules 3 and 4 linking the more distinct clusters of disrupted genes from Module 1 and, separately, Module 2 (see Appendices). Brain expression was taken from BrainSpan, with links between genes representing a correlation of ≥0.8. The disrupted hit reflect the functional enrichments in the modules: The 34 FMRP targets disrupted by damaging *de novo* single-gene mutations are significantly enriched in genes annotated with the GO terms "chromatin modification" (14.1-fold, p=4.5×10^{-7}), "regulation of transcription, DNA dependent" (3.2-fold, p=6.7×10^{-4}) when compared to both all genes and to all genes disrupted by damaging *de novo* single-gene mutations (**Table S15**). By contrast, the 76 FMRP targets disrupted by rare deletion CNVs are significantly enriched in genes annotated the GO terms "transmission of nerve impulse" (7.2-fold, p=1.1×10^{-6}) and "synaptic transmission" (7.6-fold, p=2.6×10^{-6}) when compared to the genomic background and to all genes disrupted by rare deletion CNVs in the cohorts from our study (**Table S16**).

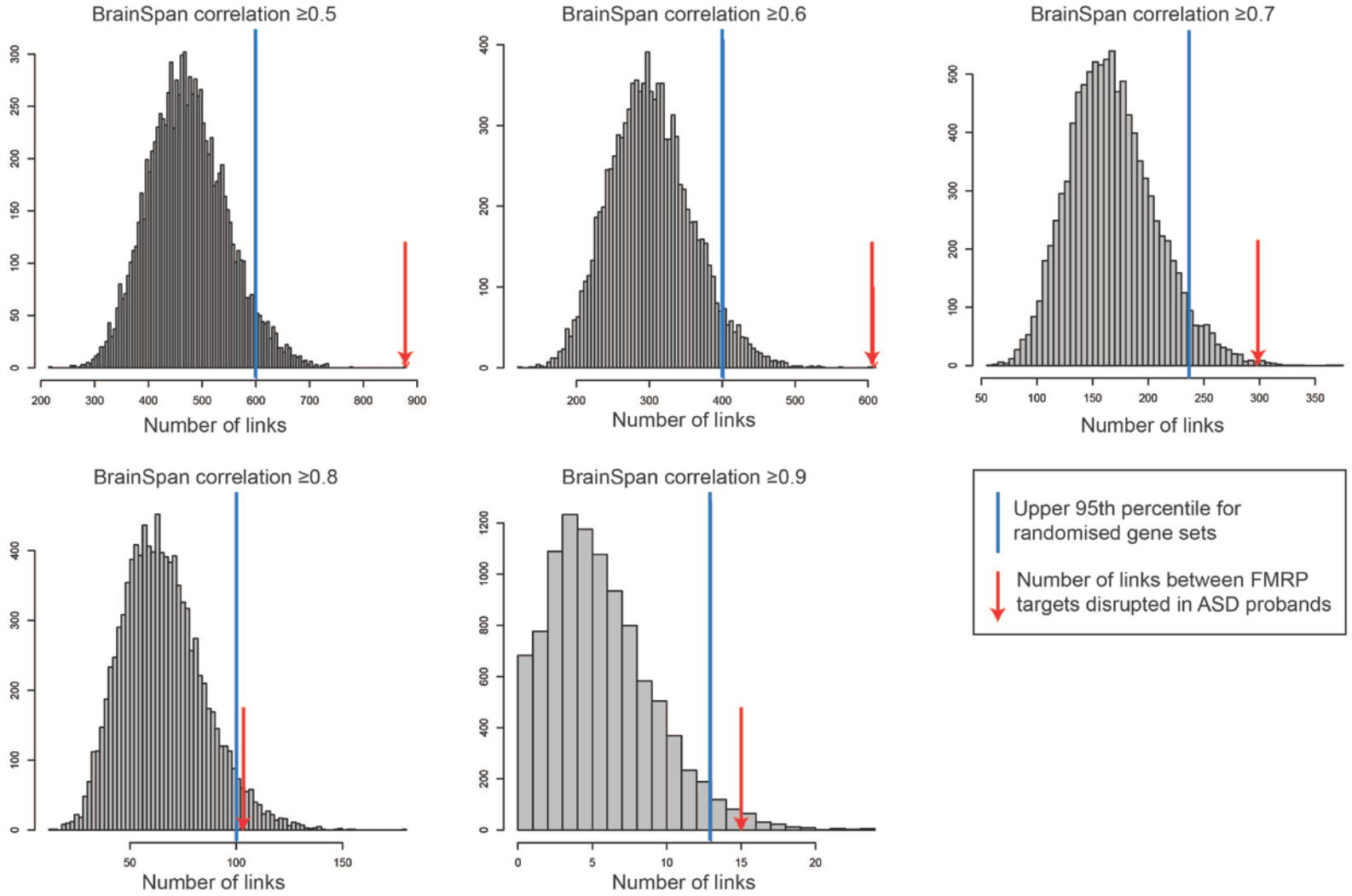

Figure S5. FMRP targets disrupted in ASD probands by *de novo* single-gene mutations and rare deletion CNVs are significantly co-expressed in the human brain. A gene co-expression network was constructed from BrainSpan, with genes linked if they were correlated above a threshold of 0.5. Randomised gene sets matched for the number of genes were constructed as described in the **Appendices**. The experiment was repeated with four more stringent correlation thresholds.

Variation type	Dataset	Reference	Description	Analysis
***De novo* nonsense, splice-site, frameshift mutations**	I-exomes	Iossifov *et al.*[10]	59 disrupted genes in ASD probands	Enrichment
	SON-exomes	Sanders *et al.*[13], O'Roak *et al.*[11], Neale *et al.*[12]	65 disrupted genes in ASD probands	Enrichment
Breakpoints of balanced chromosomal abnormalities	T-BCAs	Talkowski *et al.*[24]	32 disrupted genes in ASD probands	Enrichment
Rare deletion CNVs	Sanders probands/siblings	Sanders *et al.*[26]	Rare deletion CNVs of 872 ASD probands and matched unaffected siblings	Trend test
	Sanders probands/parents	Sanders *et al.*[26]	Rare deletion CNVs of 1124 ASD probands and their parents	Trend test
	AGP strict ASD/controls	Pinto *et al.*[27]	Rare deletion CNVs of 561 probands with "strict ASD" and 1146 unaffected controls	Trend test
SNPs	AGRE	AGRE; Wang *et al.*[30]	Autosomal SNPs of 1334 ASD cases and 1764 controls (family-based)	Transmission disequilibrium test and gene set analysis

Table S1◊ ASD datasets used in the analysis.

SNP	Minor/major allele	p-value (AGRE) Wang *et al*	tdt *p*-value
rs4307059	C/T	$1.13*10^{-5}$	$3.44*10^{-5}$
rs7704909	C/T	$1.63*10^{-5}$	$6.08*10^{-5}$
rs12518194	G/A	$1.33*10^{-5}$	$2.51*10^{-5}$
rs4327572	T/C	$2.23*10^{-5}$	$4.19*10^{-5}$
rs1896731	C/T	$1.73*10^{-3}$	0.01374
rs10038113	C/T	$1.43*10^{-3}$	0.0181

Table S2◊ Results from the transmission disequilibrium (tdt) test for AGRE data compared to analysis of highly overlapping cohort by Wang *et al*.

Foetal tissues	Adult tissues
Brain	Atrioventricular node
Liver	Adipocyte
Lung	Adrenal cortex
Thyroid	Adrenal gland
	Appendix
	Bronchial epithelial cells
	Cardiac myocytes
	Heart
	Kidney
	Liver
	Lung
	Ovary
	Pancreas
	Pancreatic Islets
	Pituitary gland
	Placenta
	Prostate
	Salivary gland
	Skeletal muscle
	Skin
	Smooth muscle
	Testis
	Testis Germ cell
	Thymus
	Thyroid
	Tougue
	Trachea
	Uterus
	Uterus corpus
	Whole blood
	Whole brain

Table S3◊ Foetal and adult tissues used to determine tissue-specificity of genes.

Gene list	Adult			
	Intercept	**BrainSpec$_{adult}$**	**CDS**	**FMRP**
I-exomes	-6.09 ($<2\times10^{-16}$)	-7.24×10^{-3} (0.80)	7.09×10^{-5} (0.001)	1.84 (7.85×10^{-9})
SON-exomes	-5.79 ($<2\times10^{-16}$)	-0.16 (0.14)	6.16×10^{-5} (0.001)	1.80 (8.23×10^{-8})
T-BCAs	-6.56 ($<2\times10^{-16}$)	-0.12 (0.35)	5.02×10^{-5} (0.019)	2.06 (2.58×10^{-6})
All combined	-5.20 ($<2\times10^{-16}$)	-0.038 (0.31)	1.22×10^{-4} (7×10^{-7})	1.62 (9.61×10^{-13})
Gene list	**Foetal**			
	Intercept	**BrainSpec$_{foetal}$**	**CDS**	**FMRP**
I-exomes	-6.10 ($<2\times10^{-16}$)	2.95×10^{-3} (0.67)	7.14×10^{-5} (0.001)	1.81 (4.97×10^{-9})
SON-exomes	-5.90 ($<2\times10^{-16}$)	-0.05.5 (0.47)	6.41×10^{-5} (0.001)	1.66 (6.31×10^{-7})
T-BCAs	-6.71 ($<2\times10^{-16}$)	5.23×10^{-3} (0.1)	5.23×10^{-5} (0.012)	1.86 (7.69×10^{-6})
All combined	-5.26 ($<2\times10^{-16}$)	3.91×10^{-3} (0.25)	1.27×10^{-4} (2×10^{-7})	1.53 (1.79×10^{-12})

Table S4◊ Logistic regression shows that after accounting for relative brain specificity and length of coding sequence (CDS; in base pairs), being targeted by FMRP significantly increases the probability of a gene to be found disrupted by a single-gene *de novo* mutation in ASD based on three such lists and their combination. Table shows the coefficients from logistic regression, with *p*-values in parentheses.

Gene Ontology gene set	Enrichment *p*-value	Fold enrichment	Module 1 genes in gene set	FMRP targets in gene set
transcription (BP,0006350)	8.84×10^{-12}	1.85	71	114
regulation of transcription (BP,0045449)	3.88×10^{-9}	1.65	77	139
chromatin modification (BP,0016568)	7.73×10^{-9}	2.26	32	42
chromatin organization (BP,0006325)	2.11×10^{-8}	2.21	32	43
chromosome organization (BP,0051276)	2.29×10^{-8}	2.18	33	45
nucleoplasm part (CC,0044451)	3.01×10^{-7}	2.07	32	46
regulation of transcription, DNA dependent (BP,0006355)	5.59×10^{-7}	1.66	57	102
regulation of RNA metabolic process (BP,0051252)	5.59×10^{-7}	1.66	57	102

Table S5◊ 8 GO biological processes (BP) and cellular component (CC) gene sets most significantly enriched among FMRP Module 1, with all FMRP targets as background. All shown enrichments are significant at 5% FDR (Benjamini-Hochberg).

MGI phenotype	Enrichment *p*-value	Fold enrichment	Module 2 genes annotated with phenotype	FMRP targets annotated with phenotype
abnormal synaptic transmission	2.5×10^{-6}	1.51	57	121
abnormal CNS synaptic transmission	9.90×10^{-6}	1.53	51	107
abnormal synaptic depression	6.26×10^{-5}	2.07	20	31
abnormal nervous system physiology	0.0002	1.23	80	209
convulsive seizures	0.0005	1.81	22	39

Table S6◊ MGI nervous system and behaviour/neurological subphenotypes significantly enriched among FMRP Module 2 genes at 5% FDR (Benjamini-Hochberg), with all FMRP targets as background.

Gene Ontology gene set	Enrichment *p*-value	Fold enrichment	Module 2 genes in gene set	FMRP targets in gene set
synapse part (CC,0044456)	4.90×10^{-6}	2.01	30	51
synapse (CC,0045202)	6.41×10^{-6}	1.77	41	79
plasma membrane (CC,0005886)	1.08×10^{-5}	1.33	107	276
cation transport (BP,0006812)	1.93×10^{-5}	1.85	33	61
metal ion transport (BP,0030001)	2.44×10^{-5}	1.90	30	54
ion transport (BP,0006811)	5.21×10^{-5}	1.73	36	71
cell junction (CC,0030054)	0.0002	1.64	37	77
membrane fraction (CC,0005624)	0.0003	1.59	39	84

Table S7◊ 8 GO biological processes and cellular components gene sets most significantly enriched among FMRP Module 2, with all FMRP targets as background. All shown enrichments are significant at 5% FDR (Benjamini-Hochberg).

Variants	MAF	Mann-Whitney U-test *p*-value		
		FMRP Module 1 vs all genes	FMRP Module 2 vs all genes	FMRP Module 1 vs Module 2
All variants	<0.02%	4.69×10^{-10}	0.3408	7.83×10^{-6}
	0.02%-1%	0.001	0.2617	9.40×10^{-5}
	>1%	0.00016	0.02515	0.2478
Missense, splice, introducing/removing stop codon	<0.02%	4.75×10^{-14}	0.0004104	0.003439
	0.02%-1%	1.99×10^{-6}	0.5304	0.003101
	>1%	0.0005735	5.63×10^{-5}	0.4649
Polyphen "possibly damaging" and "probably damaging"	<0.02%	1.00×10^{-06}	0.005737	0.164
	0.02%-1%	0.001202	0.15	0.2124
	>1%	0.1177	0.03083	0.4569

Table S8⇳ Differences in proportion of ultra-rare (MAF<0.002%), rare (0.002%≤MAF≤1%) and common (MAF>1%) variants between all genes, FMRP Modules 1 and 2. Yellow: significant at 5% FDR (correction for the number of tests in each variant classification).

FMRP gene set	I-exomes (59 genes)			SON-exomes (65 genes)		
	Number of genes in overlap	Enrichment *p*-value	Fold enrichment	Number of genes in overlap	Enrichment *p*-value	Fold enrichment
All FMRP targets[a]	15	6.07×10^{-7} [b]	4.53	13	5.49×10^{-5} [b]	3.56
Module 3	6	1.16×10^{-7} [b]	11.73	3	0.0188 [b]	5.33
Module 1[a]	6	0.0009 [b]	5.32	7	0.0002 [b]	5.63
Module 2	2	0.2420	2.13	0	1	-1.00
Module 4	1	0.3780	2.12	1	0.4074	1.92
FMRP gene set	**T-BCAs (32 genes)**			**T-BCAs without overlap with SON-exomes (29 genes)**		
	Number of genes in overlap	**Enrichment *p*-value**	**Fold enrichment**	**Number of genes in overlap**	**Enrichment *p*-value**	**Fold enrichment**
All FMRP targets[a]	8	0.0003 [b]	4.45	6	0.0048 [b]	3.68
Module 3	2	0.0312	7.21	2	0.0260	7.96
Module 1[a]	6	2.78×10^{-5} [b]	9.80	4	0.0021 [b]	7.21
Module 2	0	1	-1.00	0	1	-1.00
Module 4	0	1	-1.00	0	1	-1.00

Table S9◊ ASD *de novo* single-gene disruptions are significantly enriched in FMRP targets, particularly in Module 1 genes.

[a] = gene set significantly enriched in I-exomes, SON-exomes and T-BCAs; [b] = enrichment significant at 5% FDR (Benjamini-Hochberg)

Dataset	Intercept	CDS	Mod1	Mod2	Mod3	Mod4
I-exomes	-6.10 ($<2\times10^{-16}$)	6.77×10^{-5} (8×10^{-4})	1.94 (1.29×10^{-5})	1.14 (0.12)	2.72 (2.72×10^{-9})	1.04 (0.31)
SON-exomes	-5.93 ($<2\times10^{-16}$)	6.35×10^{-5} (0.001)	1.96 (2.17×10^{-6})	-1.28 (0.98)	1.86 (0.002)	0.90 (0.38)
T-BCAs	-6.70 ($<2\times10^{-16}$)	4.95×10^{-5} (0.03)	2.64 (1.66×10^{-8})	-1.3 (0.99)	2.30 (0.002)	-1.31 (0.99)
All combined	-5.22 ($<2\times10^{-16}$)	1.18×10^{-4} (2.41×10^{-6})	1.88 (3.34×10^{-10})	0.092 (0.90)	2.20 (3.97×10^{-10})	0.62 (0.39)

Table S10◊ Logistic regression shows that after accounting for relative brain specificity and length of coding sequence (CDS; in base pairs), being targeted by FMRP significantly increases the probability of a gene to be found disrupted by a single-gene *de novo* mutation in ASD based on three such lists and their combination. Table shows the coefficients from logistic regression, with *p*-values in parentheses.

a) 800 cases, 800 controls						
Fisher's test	**OR**					
Controls with 1+ hits	1.5	2.0	2.5	3.0	3.5	4.0
10	4.21×10^{-1}	9.56×10^{-2}	1.55×10^{-2}	1.96×10^{-3}	2.03×10^{-4}	1.79×10^{-5}
20	1.95×10^{-1}	1.18×10^{-2}	3.28×10^{-4}	5.08×10^{-6}	**4.99×10^{-8}**	**3.39×10^{-10}**
30	9.72×10^{-2}	1.52×10^{-3}	6.67×10^{-6}	**1.12×10^{-8}**	**8.82×10^{-12}**	**3.70×10^{-15}**
40	4.92×10^{-2}	1.91×10^{-4}	1.20×10^{-7}	**1.91×10^{-11}**	**9.90×10^{-16}**	**2.00×10^{-20}**
50	2.50×10^{-2}	2.28×10^{-5}	**1.86×10^{-9}**	**2.37×10^{-14}**	**6.53×10^{-20}**	**4.80×10^{-26}**
60	1.27×10^{-2}	2.54×10^{-6}	**2.40×10^{-11}**	**2.06×10^{-17}**	**2.37×10^{-24}**	**4.55×10^{-32}**
70	6.33×10^{-3}	2.62×10^{-7}	**2.53×10^{-13}**	**1.21×10^{-20}**	**4.36×10^{-29}**	**1.50×10^{-38}**
80	3.12×10^{-3}	2.49×10^{-8}	**2.14×10^{-15}**	**4.56×10^{-24}**	**3.77×10^{-34}**	**1.48×10^{-45}**
Trend test	**OR**					
Controls with 1+ hits	1.5	2.0	2.5	3.0	3.5	4.0
10	**3.13×10^{-1}**	**6.53×10^{-2}**	**1.04×10^{-2}**	**1.36×10^{-3}**	**1.57×10^{-4}**	**1.63×10^{-5}**
20	**1.51×10^{-1}**	**8.49×10^{-3}**	**2.46×10^{-4}**	**4.47×10^{-6}**	5.79×10^{-8}	5.76×10^{-10}
30	**7.60×10^{-2}**	**1.13×10^{-3}**	**5.54×10^{-6}**	1.23×10^{-8}	1.52×10^{-11}	1.17×10^{-14}
40	**3.89×10^{-2}**	**1.47×10^{-4}**	**1.11×10^{-7}**	2.62×10^{-11}	2.54×10^{-15}	1.18×10^{-19}
50	**1.99×10^{-2}**	**1.80×10^{-5}**	1.88×10^{-9}	4.05×10^{-14}	2.49×10^{-19}	5.27×10^{-25}
60	**1.01×10^{-2}**	**2.06×10^{-6}**	2.68×10^{-11}	4.40×10^{-17}	1.34×10^{-23}	9.40×10^{-31}
70	**5.06×10^{-3}**	**2.19×10^{-7}**	3.11×10^{-13}	3.22×10^{-20}	3.72×10^{-28}	5.94×10^{-37}
80	**2.50×10^{-3}**	**2.13×10^{-8}**	2.89×10^{-15}	1.52×10^{-23}	4.88×10^{-33}	1.16×10^{-43}
b) 600 cases, 600 controls						
Fisher's test	**OR**					
Controls with 1+ hits	1.5	2.0	2.5	3.0	3.5	4.0
10	4.19×10^{-1}	9.45×10^{-2}	1.51×10^{-2}	1.87×10^{-3}	1.89×10^{-4}	1.62×10^{-5}
20	1.93×10^{-1}	1.13×10^{-2}	2.95×10^{-4}	4.21×10^{-6}	**3.71×10^{-8}**	**2.20×10^{-10}**
30	9.44×10^{-2}	1.36×10^{-3}	5.21×10^{-6}	**7.16×10-9**	**4.30×10^{-12}**	**1.28×10^{-15}**
40	4.67×10^{-2}	1.56×10^{-4}	7.56×10-8	**8.12×10^{-12}**	**2.49×10^{-16}**	**2.56×10^{-21}**
50	2.30×10^{-2}	1.63×10^{-5}	8.61×10^{-10}	**5.67×10^{-15}**	**6.32×10^{-21}**	**1.41×10^{-27}**
60	1.12×10^{-2}	1.54×10^{-6}	**7.38×10^{-12}**	**2.26×10^{-18}**	**6.10×10^{-26}**	**1.69×10^{-34}**
Trend test	**OR**					
Controls with 1+ hits	1.5	2.0	2.5	3.0	3.5	4.0
10	**3.12×10^{-1}**	**6.45×10^{-2}**	**1.01×10^{-2}**	**1.30×10^{-3}**	**1.45×10^{-4}**	**1.46×10^{-5}**
20	**1.49×10^{-1}**	**8.07×10^{-3}**	**2.20×10^{-4}**	**3.67×10^{-6}**	4.25×10^{-8}	3.69×10^{-10}
30	**7.36×10^{-2}**	**1.01×10^{-3}**	**4.28×10^{-06}**	7.76×10^{-9}	7.29×10^{-12}	3.97×10^{-15}
40	**3.67×10^{-2}**	**1.19×10^{-4}**	**6.84×10^{-8}**	1.09×10^{-11}	6.24×10^{-16}	1.47×10^{-20}
50	**1.82×10^{-2}**	**1.27×10^{-5}**	**8.55×10^{-10}**	9.49×10^{-15}	2.35×10^{-20}	1.53×10^{-26}
60	**8.83×10^{-3}**	**1.23×10^{-6}**	8.05×10^{-12}	4.71×10^{-18}	3.41×10^{-25}	3.55×10^{-33}

Table S11. Trend and Fisher's tests p-values for case/control datasets of two sizes: a) 800 cases, 800 controls and b) 600 cases, 600 controls. In all scenarios, each individual has 0 or 1 hits; the smaller p-value from Fisher's/Trend comparison is in bold. $\mathbf{OR} = \frac{\textbf{Number of cases with 1 hit in pathway}}{\textbf{Number of controls with 1 hit in pathway}}$.

Gene set	a) ASD Matched Probands vs Siblings (Sanders)			
	Trend test *p*-value	**Cases with Hit**	**Controls with Hit**	**Emp. *p*-value**
Module 2[a]	0.0011	23	6	0.0016[b]
All FMRP targets[a]	0.0012	54	29	0.0017[b]
Module 4	0.0045	15	3	0.0111[b]
Module 1	0.0066	23	8	0.0080[b]
Module 3	1	9	9	N/A
Gene set	**b) ASD Probands vs Parents (Sanders)**			
	Trend test *p*-value	**Cases with Hit**	**Controls with Hit**	**Emp. *p*-value**
Module 2[a]	0.0008	26	21	0.0042[b]
All FMRP targets[a]	0.0039	68	89	0.0193[b]
Module 4	0.0650	20	23	N/A
Module 1	0.0514	29	36	N/A
Module 3	N/A			
Gene set	**c) ASD strict cases vs controls (AGP)**			
	Trend test *p*-value	**Cases with Hit**	**Controls with Hit**	**Emp. *p*-value**
Module 2[a]	0.0014	8	2	0.0134[b]
All FMRP targets[a]	2.76×10^{-5}	20	9	0.0009[b]
Module 4	N/A			
Module 1				
Module 3				

Table S12. The Trend test shows that disruptions of FMRP targets and Module 2 in particularly by rare deletion CNVs are significantly associated with ASD. "Hits" are defined as a rare deletion CNV overlapping a pathway gene such that at least one exon from every transcript is affected. Empirical *p*-values were obtained from 10,000 random gene sets matched for gene number and length (see **Methods**). Gene sets with significant results in a) were validated in b); likewise, results validated in b) were tested for replication in c).

[a] = empirical *p*-value for gene set is significant for a)-c) at 5% FDR (Benjamini-Hochberg);

[b] = significant at 5% FDR (Benjamini-Hochberg).

Module	Independent SNPs (r2=0.5)	Mean χ2 statistic	Empirical *p*-value
Mod1	4505	1.205	0.2879
Mod2	3211	1.289	0.0062 [a]
Mod3	3187	1.252	0.1345
Mod4	812	1.191	0.4509

Table S13. FMRP Mod2 is associated with ASD diagnosis based on a family-based GWAS (AGRE data), using a set-based transmission-disequilibrium test in Plink. [a] = significant at 5% FDR (Benjamini-Hochberg)

Gene Symbol	Ensembl ID	chr	FMRP mod	Disrupted in I- or SON-exomes, T-BCAs, Sanders or AGP CNVs	ASD cand. gene[a]	GO BP or CC[b]	MGI[c]
ABCA3	ENSG00000167972	16	4	San			BN,MA
ABR	ENSG00000159842	17	1	AGP		SC	BN,MA,NS
ADAP1	ENSG00000105963	7	2	San			
ADNP	ENSG00000101126	20	1	SON		TR	MA,NS,E
AKT3	ENSG00000117020	1	1	San			BN,MA,NS
ALDOA	ENSG00000149925	16	2	AGP, San			
ANK2	ENSG00000145362	4	3	I			BN,MA,NS
ANK3	ENSG00000151150	10	3	T	S	S	BN,MA,NS
APBA1	ENSG00000107282	9	3	San		S	BN,MA,NS
ARF3	ENSG00000134287	12	2	San		SC	
ARID1B	ENSG00000049618	6	1	SON	S	CM,TR	
ARVCF	ENSG00000099889	22	1	AGP, San			
ATG2A	ENSG00000110046	11	2	San			
ATP1B1	ENSG00000143153	1	2	I			MA
AUTS2	ENSG00000158321	7	1	T	A, S		
BMPR2	ENSG00000204217	2	1	San			MA,E
BRSK1	ENSG00000160469	19	2	AGP		SC	BN,MA,NS
C19orf26	ENSG00000099625	19	2	San			
CACNA1B	ENSG00000148408	9	4	San	S		BN,MA,NS
CDC42BPB	ENSG00000198752	14	4	SON		SC	
CDK5R2	ENSG00000171450	2	2	San			BN,MA,NS,E
CDKL5	ENSG00000008086	X	3	T	A, B, S		
CELSR2	ENSG00000143126	1	4	San		TR	NS
CHD8	ENSG00000100888	14	1	SON, T, San	S	CM,TR	MA,E
CTNND2	ENSG00000169862	5	1	San	A		BN,NS
DGCR2	ENSG00000070413	22	4	AGP, San			
DIP2A	ENSG00000160305	21	1	I, San			
DIP2C	ENSG00000151240	10	1	I			
DOCK4	ENSG00000128512	7	1	San			
DPP8	ENSG00000074603	15	3	San			
DST	ENSG00000151914	6	3	I			BN,MA,NS
EHMT1	ENSG00000181090	9	1	T	B (,S)	CM,TR	BN,MA,NS,E
FAM115B	ENSG00000198420	7	1	AGP			
FAM91A1	ENSG00000176853	8	3	I, AGP			
FASN	ENSG00000169710	17	1	San			BN,MA,NS
FBXL16	ENSG00000127585	16	2	San			
GRIN1	ENSG00000176884	9	2	San		TR,S	BN,MA,NS
GRIN2B	ENSG00000150086	12	1	T	S	S	BN,MA,NS
ITPR1	ENSG00000150995	3	2	San		S	BN,MA,NS
JMJD3	ENSG00000132510	17	1	I		CM,TR	MA
KCND2	ENSG00000184408	7	0	San		S	BN,NS
KCNQ3	ENSG00000184156	8	3	San			BN,MA,NS
KCNT1	ENSG00000107147	9	2	AGP			
KIAA0100	ENSG00000007202	17	1	SON			
KIAA0430	ENSG00000166783	16	3	AGP, San			
KIF1A	ENSG00000130294	2	2	San			BN,MA,NS

KLHL22	ENSG00000185214	22		AGP, San			
LMTK3	ENSG00000142235	19	4	I			
LPHN3	ENSG00000150471	4	1	San			
MAN2A2	ENSG00000196547	15	2	San			MA
MAZ	ENSG00000103495	16	1	AGP, San			
MBD5	ENSG00000204406	2	1	SON, T	B		
MED13L	ENSG00000123066	12	1	I		TR	
MLL3	ENSG00000055609	7	1	SON, San		CM,TR,SC	MA
MLL5	ENSG00000005483	7	1	I		CM,TR,SC	MA
MTOR	ENSG00000198793	1	3	San		SC	BN,MA,NS,E
MTSS1L	ENSG00000132613	16	0	San			
NAV3	ENSG00000067798	12	1	San			
NCDN	ENSG00000020129	1	2	San			BN,MA,NS,E
NCKAP1	ENSG00000061676	2	3	I	(S?)		MA,NS,E
NF1	ENSG00000196712	17	3	San	A, B, S	SC	BN,MA,NS,E
NLGN3	ENSG00000196338	X	4	San	A, B, S	S	BN,MA,NS
NOMO1	ENSG00000103512	16	2	AGP, San			
NRXN1	ENSG00000179915	2	3	I	A, B, S	S	MA,NS
PACS2	ENSG00000179364	14	4	San			
PGM2L1	ENSG00000165434	11	3	San			
PI4KA	ENSG00000133511	22		AGP, San		SC	
PIGQ	ENSG00000007541	16	4	San			
PLEC	ENSG00000178209	8	2	AGP			BN,MA,NS
PLXNB1	ENSG00000164050	3	0	SON		SC	NS
PPP2R5B	ENSG00000068971	11	4	San			
PREX1	ENSG00000124126	20	0	San		SC	BN,NS
PRKACB	ENSG00000142875	1	3	San		SC	BN,MA,NS,E
PRRC2B	ENSG00000130723	9	1	San			
PTPRT	ENSG00000196090	20	3	San			
R3HDM1	ENSG00000048991	2	3	AGP			
RAD21L1	ENSG00000101298	20	2	I		S	BN,NS
RALGDS	ENSG00000160271	9	1	AGP		SC	
RELN	ENSG00000189056	7	0	SON	S		BN,MA,NS
RTN4R	ENSG00000040608	22	2	AGP, San			BN,NS
SCN2A	ENSG00000136531	2	3	SON	S		
SEPT5	ENSG00000184702	22	2	AGP, San		S	BN,NS
SEZ6L2	ENSG00000174938	16	2	AGP, San	S		BN,NS
SHANK2	ENSG00000162105	11	3	SON, AGP	A, B, S	S,SC	
SLC8A2	ENSG00000118160	19	2	AGP			BN,NS
SMARCA2	ENSG00000080503	9	1	AGP		CM,TR	MA
SMARCC2	ENSG00000139613	12	1	SON		CM,TR	
SORL1	ENSG00000137642	11	2	San			NS
SPAG9	ENSG00000008294	17	1	San		SC	
SYNGAP1	ENSG00000197283	6	4	AGP	B, S		BN,MA,NS
TAOK2	ENSG00000149930	16	4	AGP, San		SC	
TCF25	ENSG00000141002	16	4	AGP		TR	
TCF4	ENSG00000196628	18	1	T		TR	MA,NS
TLE3	ENSG00000140332	15	4	San		TR	MA
TNRC6B	ENSG00000100354	22	1	San			
TNS3	ENSG00000136205	7	0	San		SC	MA
TRIM32	ENSG00000119401	9	1	AGP			BN,NS
TRIP12	ENSG00000153827	2	1	I			

TRPM3	ENSG00000083067	9	0	AGP			
TTYH3	ENSG00000136295	7	1	San			
UBR3	ENSG00000144357	2	3	SON			BN,MA
ULK1	ENSG00000177169	12	4	San		SC	MA
UNC13C	ENSG00000137766	15	2	San		S,SC	BN,NS
WDFY3	ENSG00000163625	4	3	I			
ZCCHC14	ENSG00000140948	16	1	San			

Table S14. FMRP targets disrupted in ASD by de novo single-gene mutations and rare deletion CNVs.

[a] = ASD candidate genes from AGP (A) [12], C. Betancur [36] and SFARI gene (S) [https://gene.sfari.org; 07/01/2013].

[b] = GO BP or CC annotations: CM (chromatin modification, BP,0016568), TR (regulation of transcription, DNA dependent, BP,0006355), S (synapse, CC,0045202), SC (intracellular signalling cascade, BP,0007242).

[c] = MGI annotations: NS (nervous system phenotype), BN (behaviour/neurological phenotype), MA (mortality/aging phenotype).

Gene Ontology gene set	Enrichment *p*-value	Fold enrichment	Disrupted FMRP targets in gene set	Total genes in gene set
chromatin modification (BP,0016568) [a]	4.48×10^{-7}	14.12	7	280
chromatin organization (BP,0006325) [a]	7.18×10^{-6}	9.30	7	425
chromosome organization (BP,0051276) [a]	3.22×10^{-5}	7.38	7	536
plasma membrane part (CC,0044459) [a]	5.95×10^{-5}	3.19	13	2299
cell junction (CC,0030054) [a]	0.000275	6.42	6	528
cell morphogenesis (BP,0000902) [a]	0.000399	7.78	5	363
synapse (CC,0045202) [a]	0.000399	7.78	5	363
cellular component morphogenesis (BP,0032989) [a]	0.000642	7.01	5	403
regulation of transcription DNA dependent (BP,0006355) [a]	0.000671	3.15	10	1790
regulation of RNA metabolic process (BP,0051252) [a]	0.000821	3.08	10	1836
cell part morphogenesis (BP,0032990) [a]	0.001033	8.79	4	257
neuron projection development (BP,0031175) [a]	0.001079	8.69	4	260
positive regulation of transcription DNA dependent (BP,0045893) [a]	0.001512	5.79	5	488
positive regulation of RNA metabolic process (BP,0051254) [a]	0.001568	5.74	5	492
regulation of transcription from RNA polymerase II promoter (BP,0006357)	0.001623	4.58	6	740
neuron projection (CC,0043005) [a]	0.002876	6.64	4	340
neuron development (BP,0048666) [a]	0.002938	6.60	4	342
positive regulation of transcription (BP,0045941) [a]	0.003118	4.90	5	576
positive regulation of gene expression (BP,0010628) [a]	0.003533	4.76	5	593
negative regulation of transcription DNA dependent (BP,0045892)	0.003638	6.22	4	363

Table S15. GO biological process (BP) and cellular component (CC) terms significantly enriched among FMRP targets disrupted by *de novo* single-gene mutations from I-exomes, SON-exomes and T-BCAs against all genes in the genome at 5% FDR (Benjamini-Hochberg). [a] = enrichments replicated against a background of all genes disrupted by *de novo* single-gene mutations from I-exomes, SON-exomes and T-BCAs at 5% FDR.

Gene Ontology gene set	Enrichment *p*-value	Fold enrichment	Disrupted FMRP targets in gene set	Total genes in gene set
transmission of nerve impulse (BP,0019226)	1.13×10^{-6}	7.18	10	351
synaptic transmission (BP,0007268)	2.61×10^{-6}	7.58	9	299
membrane bounded vesicle (CC,0031988)	1.58×10^{-5}	4.78	11	580
intracellular signaling cascade (BP,0007242)	3.70×10^{-5}	3.09	16	1305
cytoplasmic membrane bounded vesicle (CC,0016023)	6.84×10^{-5}	4.48	10	562
vesicle (CC,0031982)	7.02×10^{-5}	4.06	11	683
regulation of small GTPase mediated signal transduction (BP,0051056)	8.39×10^{-5}	6.66	7	265
synapse (CC,0045202)	9.04×10^{-5}	5.55	8	363
cytoplasmic vesicle (CC,0031410)	0.000237	3.85	10	654
synapse part (CC,0044456)	0.000494	5.98	6	253
metal ion transport (BP,0030001)	0.0005	4.32	8	467
cell cell signaling (BP,0007267)	0.000684	3.69	9	615
cation transport (BP,0006812)	0.001615	3.60	8	560
neuron projection (CC,0043005)	0.00226	4.45	6	340
regulation of phosphorylation (BP,0042325)	0.002662	3.72	7	474
regulation of protein kinase activity (BP,0045859)	0.002724	4.28	6	353
regulation of kinase activity (BP,0043549)	0.003213	4.14	6	365
regulation of phosphate metabolic process (BP,0019220)	0.00331	3.58	7	493
regulation of phosphorus metabolic process (BP,0051174)	0.00331	3.58	7	493
ion transport (BP,0006811)	0.003399	2.92	9	776
regulation of transferase activity (BP,0051338)	0.003964	3.97	6	381

Table S16. GO biological process (BP) and cellular component (CC) terms significantly enriched among FMRP targets disrupted by rare deletion CNVs in ASD probands from AGP and Sanders datasets against all genes in the genome at 5% FDR (Benjamini-Hochberg). All significant enrichments were replicated against a background of all genes disrupted by rare deletion CNVs in ASD probands from AGP and Sanders datasets at 5% FDR.