

Supplementary material for:

Protein synthesis levels are increased in a subset of individuals with Fragile X syndrome

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Contents

Supplementary Figures:

Figure S1. Global protein synthesis and FMRP levels in human fibroblasts, mouse primary neurons and embryonic fibroblasts (MEFs).

Figure S2. *De novo* protein synthesis in multiple culture passages.

Supplementary Tables:

Table S1. Cohort Demographic information.

Table S2. *FMRI* mutation category, *FMRI* mRNA and FMRP expression levels.

Table S3. Clinical assessments in the Lausanne and Sacramento (MIND) cohorts.

Supplementary Figures

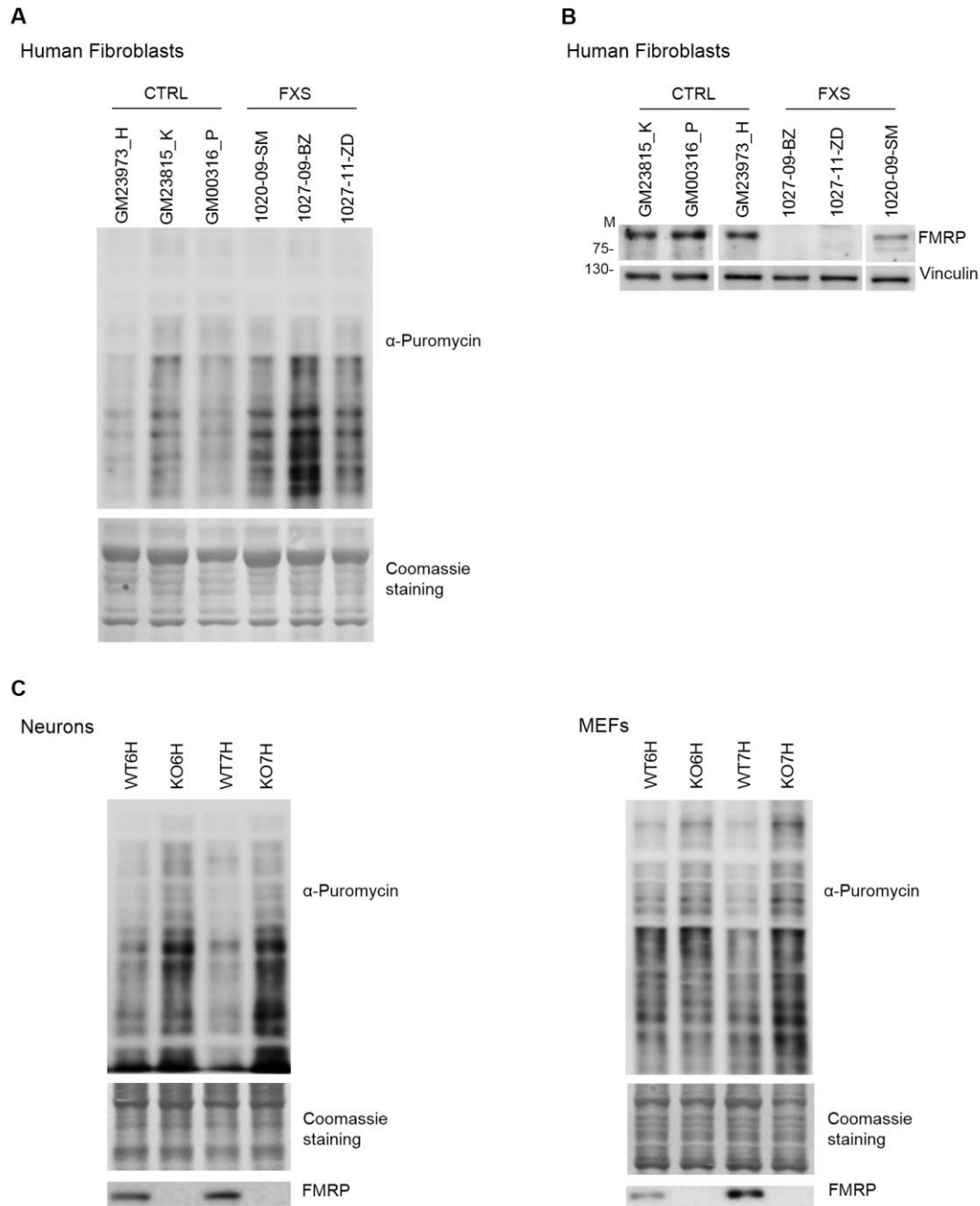


Figure S1. Global protein synthesis and FMRP levels in human fibroblasts, mouse primary neurons and embryonic fibroblasts (MEFs). **A)** Representative western blot showing puromycin incorporation and coomassie staining in control and FXS human fibroblasts. Puromycin incorporation in newly synthesized proteins was quantified by Western blot using an anti-Puromycin Antibody (SUnSET method, see Materials and Methods). **B)** FMRP levels in human fibroblasts. Representative Western blot showing FMRP and

Vinculin in control and FXS human fibroblasts. C) Representative Western blot showing puromycin incorporation, FMRP and coomassie staining in primary cortical neurons and in MEFs, WT and *Fmr1* KO derived from the same animal.

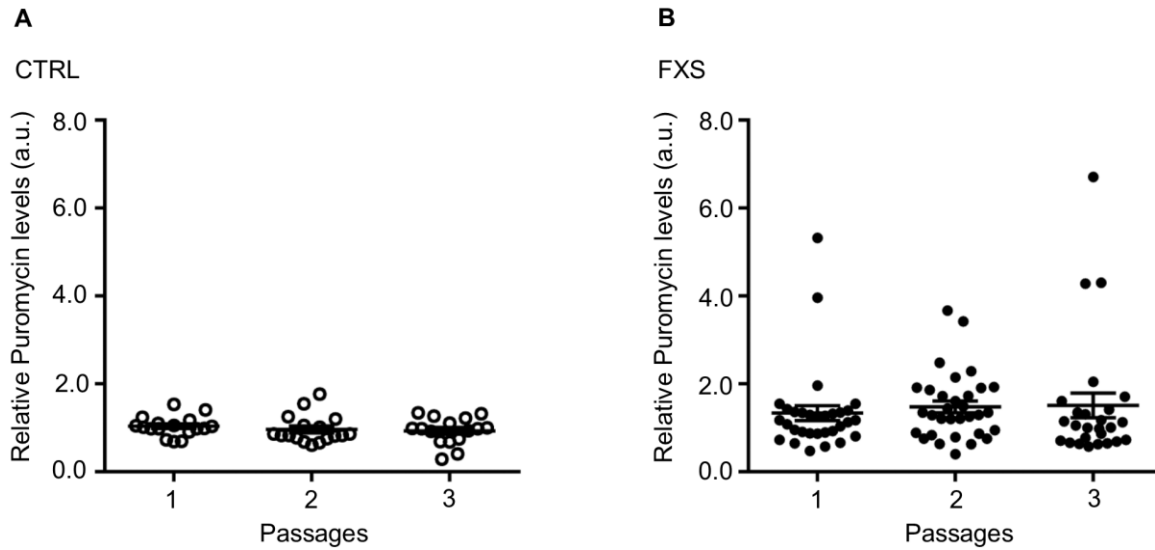


Figure S2. *De novo* protein synthesis in multiple culture passages.

Puromycin level measures for cell passage 1, 2 and 3 in controls **A**) and FXS **B**). Each dot represents the average of at least two technical replicates per individual. The bars represent the SEM, $P = 0.40$ non-parametric ANOVA (WT $n = 17$), $P = 0.31$ non-parametric ANOVA (FXS $n = 32$).

Supplementary Tables**Table S1: Cohort Demographic information.**

	Participants with Fragile X			Controls* (n = 17)
	CHUV Lausanne (n = 12) (FXS1)	Erasmus MC Rotterdam (n = 7) (FXS2)	MIND Institute Davis (n = 13) (FXS3)	
Age (year)				
Mean (SD)	26.0 (9.8)	21.0 (11.4)	28.2 (16.4)	23.7 (13.6)
Median	26.0	22.0	24.0	19.0
Rang	12 to 38	6 to 37	12 to 69	10 to 50
Sex, n (%)				
males / females	12/0	7/0	13/0	14/2/1**
FXS diagnosis				
Full mutation, non-mosaic, n (%)	8 (66.7)	5 (71.4)	4 (30.7)	0
Full mutation, mosaic, n (%)	4 (33.3)	2 (28.6)	9 (69.3)	0
Vineland§				
<i>Communication domain</i>				
Mean (SD)	79.2 (15.3)	NA	56.3 (27.2)	NA
Range	54 to 110	NA	21 to 93	NA
<i>Daily living skills domain</i>				
Mean (SD)	114 (17.9)	NA	55.3 (23.6)	NA
Range	75 to 136	NA	23 to 79	NA
<i>Socialization skills domain</i>				
Mean (SD)	84.58 (13.4)	NA	59.9 (22.6)	NA
Range	66 to 102	NA	20 to 88	NA
<i>Adaptive behavior composite</i>				
Mean (SD)	40.83 (25.9)	NA	54.8 (23.9)	NA
Range	20 to 117	NA	20 to 77	NA
<i>Motor skills domain</i>				
Mean (SD)	67.42 (5.7)	NA	NA	NA
Range	54 to 72	NA	NA	NA
IQ				
Full scale IQ (FSIQ)				
Mean (SD)	4.5 (1.1) ***	NA	63.8 (19.3)	NA
Range	2.5 to 6.7 ***	NA	40 to 117	NA
Verbal IQ (VIQ)				
Mean (SD)	NA	NA	65.6 (18.5)	NA
Range	NA	NA	42 to 113	NA

Non-verbal IQ (NVIQ)				
Mean (SD)	NA	NA	66.9 (19.5)	NA
Rang	NA	NA	43 to 121	NA

*Control fibroblasts came from the following companies or laboratories: CliniScience (<http://www.clinisciencences.com/>), Coriell (<https://catalog.coriell.org/>) and Lonza (<http://www.lonza.com/>).

** For one control the gender is unknown.

*** Developmental age was based on the scores of the Wechsler nonverbal scale; WPPSI, Wechsler preschool and primary scale of intelligence.

§ Vineland was available for 12/12 and 9/13 from the Lausanne and MIND Institute cohorts respectively.

Table S2: *FMRI* mutation category, *FMRI* mRNA and FMRP expression levels.

	<i>ID</i>	<i>FMRI</i> mutation status	<i>FMRI</i> CGG repeated size ¹	% Meth	<i>FMRI</i> mRNA ²	Std. Error	FMRP ³	Std. Error
Lausanne Hospital Cohort	NV00001	Full mutation	340, 720, 1020	100%	n.d	-	0,48	0,20
	NV00002	Mosaic	240, 300, 1150 (124)	>95%	0,208	0,012	0,48	0,24
	NV00005	Full mutation	310, 995	-	0,087	0,005	0,40	0,27
	NV00006	Full mutation	750	-	-	-	0,13	0,02
	NV00008	Mosaic	260, 510, 770,1030 (121)	>95%	0,138	0,059	0,29	0,05
	NV00009	Full mutation	370, 1060	-	n.d	-	0,23	0,07
	NV00010	Full mutation	345, 540, 700	100%	0,001	0,000	0,15	0,07
	NV00012	Mosaic	240, 300 (185)	>95%	0,075	0,005	0,12	0,01
	NV00013	Full mutation	450, 570	-	-	-	0,08	0,06
	NV00014	Full mutation	330, 575	-	0,068	0,025	0,22	0,04
	NV00017	Mosaic	350, 740, 1020 (133)	>95%	0,005	0,001	0,29	0,05
	NV00019	Full mutation	250, 640	100%	0,086	0,022	0,19	0,12
MIND Institute Cohort	1001-10-BZ	Mosaic	390, 490, 950, 1140 (160-230)	94%	0,216	0,072	0,39	0,09
	1017-09-MR	Mosaic	190, 220, 400, 500, 800 (65)	49%	0,537	0,003	0,80	0,11
	1022-10-ER	Mosaic	235, 350, 650 (smear)	82%	0,247	0,015	0,50	0,05
	1043-10-SS	Full mutation	290, 540, 790 (light smear)	100%	0,466	0,083	0,36	0,05
	1055-10-TG	Mosaic	255 (260-380)	12%	1,365	0,014	1,03	0,15
	TS114-12-KB	Mosaic	230, 330 (30-200)	18%	1,422	0,186	0,94	0,33
	1020-09-SM	Mosaic	180 (200-270)	16%	2,212	0,076	0,85	0,07
	1027-09-BZ	Full mutation	510, 590, 670, 810	100%	0,270	0,021	0,37	0,19
	1027-11-ZD	Full mutation	370	100%	0,005	0,001	0,34	0,10

	1032-10-SF	Mosaic	200 (250-760)	21%	2,130	0,194	0,40	0,06
	1035-09-LD	Mosaic	290 (200-250)	78%	0,353	0,001	0,97	0,24
	1060-10-GK	Mosaic	425, 580, 680 (210-280)	79%	0,358	0,001	0,42	0,06
	TS106-13-JS	Full mutation	500, 820, 960	100%	0,037	0,001	0,48	0,17
Rotterdam University Cohort	81E0152	Full mutation	490, 730	100%	0,034	0,036	0,27	0,07
	92E0198	Fragile X syndrome [#]	> 200	>95%	0,214	0,001	0,38	0,07
	84E0275	Full mutation	500	100%	0,001	0,001	0,46	0,12
	86E0633	Mosaic	240 (30-330)	<5%	2,888	0,006	1,09	0,25
	86E1377	Mosaic	410 (smear)	>95%	0,017	0,001	0,32	0,06
	94E0363	Fragile X Syndrome ^{&}	> 200	>95%	0,067	0,003	0,48	0,11
	86E0681	Fragile X Syndrome [§]	> 200	-	0,001	0,000	0,43	0,10
Control Cohort	449121 (FB1)	CTRL	-	-	0,953	0,046	0,94	0,09
	448766 (FB2)	CTRL	-	-	1,332	0,190	0,72	0,27
	321585 (FB7)	CTRL	-	-	1,090	0,129	0,95	0,21
	GM00316-P	CTRL	-	-	0,755	0,020	1,18	0,11
	GM00500_J	CTRL	-	-	1,265	0,090	1,44	0,16
	GM02037-A	CTRL	-	-	0,893	0,015	1,43	0,17
	GM02673_E	CTRL	-	-	0,914	0,054	1,18	0,10
	GM03349-D	CTRL	-	-	0,642	0,095	1,13	0,09
	GM07753-E	CTRL	-	-	1,220	0,179	0,70	0,12
	GM09503_C	CTRL	-	-	1,101	0,061	1,73	0,25

	GM23815_K	CTRL	-	-	0,886	0,009	2,07	0,16
	GM23971_D	CTRL	-	-	0,669	0,092	1,60	0,59
	GM23973_H	CTRL	-	-	1,075	0,088	1,53	0,23
	GM01864_G	CTRL	-	-	1,467	0,080	2,62	0,28
	GM23976_O	CTRL	-	-	0,645	0,025	1,32	0,18
	HDF	CTRL	-	-	1,063	0,248	0,90	0,19
	L	CTRL	-	-	-	-	1,01	0,09

¹Determined by Southern blot and PCR or/and standard karyotyping; numbers between parentheses indicate unmethylated alleles. ²Determined by RT-qPCR; ³Determined by Western blotting, where 0,4 has to be considered the baseline.

Abbreviations: CTRL, controls; (-), not available; n.d, not detected; % Meth, methylation percentage; *FMRI*, fragile X mental retardation 1; mRNA, messenger RNA; Std. Error, Standard Error.

Initially determined cytogenetically as Fragile X sample; during the revision process the sample was re-analyzed by Southern blotting and PCR confirming the presence of a full mutation allele in addition to a normal allele of 25 CGG repeats. Methylation status > 95%.

& Initially determined cytogenetically as Fragile X sample; during the revision process the sample was re-analyzed by Southern blotting and PCR confirming the presence of a full mutation allele in addition to a broad spectrum of unmethylated alleles present in less than 5% of the cells. Methylation status > 95%.

\$ Determined cytogenetically as Fragile X sample.

Table S3: Clinical assessments in the Lausanne and Sacramento (MIND) cohorts.

Cohort	ID	FMR1 mutation status	Sex	Age	ASD (Y/N)	IQ			Mental age	IQ test	Vineland				
						FSIQ	VIQ	NVIQ			Co	DL	S	AB	MS
Lausanne Cohort	NV00001	Full	M	38		-	-	-	3.6	S-B	75	113	80	30	71
	NV00002	Mosaic	M	35		-	-	-	4.9	S-B	91	124	102	39	70
	NV00005	Full	M	38		-	-	-	4.9	S-B	80	136	87	38	72
	NV00006	Full	M	22		-	-	-	3.6	S-B	58	88	68	20	54
	NV00008	Mosaic	M	23		-	-	-	6.8	S-B	81	125	97	36	70
	NV00009	Full	M	30		-	-	-	6.4	S-B	89	136	102	42	72
	NV00010	Full	M	37		-	-	-	4.1	S-B	69	107	71	24	70
	NV00012	Mosaic	M	17		-	-	-	4.5	S-B	110	119	86	40	61
	NV00013	Full	M	13		-	-	-	3.7	S-B	54	75	75	28	69
	NV00014	Full	M	18		-	-	-	6.4	S-B	91	119	80	117	66
	NV00017	Mosaic	M	12		-	-	-	4.7	S-B	78	118	101	54	72
NV00019	Full	M	29		-	-	-	4.2	S-B	74	108	66	22	62	
Sacramento Cohort	1055-10-TG	Mosaic	M	12	Y	63	73	56	-	S-B	-	-	-	-	-
	1001-10-BZ	Mosaic	M	13	Y	73	75	75	-	WPPSI-3	70	58	59	61	-
	TS114-12-KB	Mosaic	M	15	N	66	68	68	-	S-B	67	79	75	72	-
	1022-10-ER	Mosaic	M	22	Y	61	61	69	-	WAIS 3	66	65	71	65	-
	1017-09-MR	Mosaic	M	21	N	79	83	78	-	WAIS 3	69	69	80	71	-
	1043-10-SS	Full	M	23	Y	51	56	55	-	WAIS 3	23	25	46	29	-
	TS106-13-JS	Full	M	25	-	54	62	51	-	S-B	-	-	-	-	-
	1020-09-SM	Mosaic	M	24	N	117	113	121	-	WAIS 3	93	76	67	76	-
	1027-11-ZD	Full	M	27	Y	53	46	64	-	S-B	21	27	33	22	-
	1060-10-GK	Mosaic	M	29	Y	45	48	49	-	WAIS 3	21	23	20	20	-
	1027-09-BZ	Full	M	32	-	63	63	69	-	WAIS 3	77	76	88	77	-

Jacquemont *et al.*

	1032-10-SF	Mosaic	M	55	-	64	63	72	-	WAIS 3	-	-	-	-	-
	1035-09-LD	Mosaic	M	69	Y	40	42	43	-	S-B	-	-	-	-	-

Abbreviations: IQ, intelligence quotient; FSIQ, full scale IQ; VIQ, verbal IQ; NVIQ, Non-verbal IQ; Vineland (Co, communication domain; DL, daily living skills domain; S, socialization domain; AB, adaptive behavior; MS, motor skills domain; S-B, Stanford–Binet Intelligence Scales; WPPSI 3, Wechsler Preschool and Primary Scale of Intelligence; (-), not available; Y/N, yes/no