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Supplementary Materials for

Augmented noncanonical BMP type II receptor signaling mediates the synaptic abnormality of fragile X syndrome

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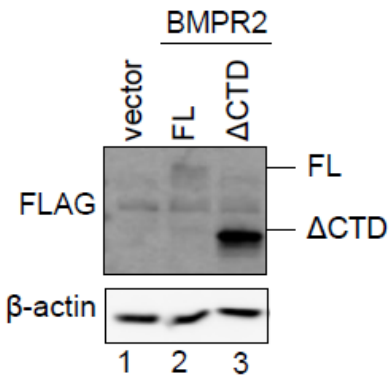

Figure S1. Translational regulation of BMPR2 through the mRNA sequence encoding the CTD (*CTDseq*). Cos7 cells were transfected with FLAG-tagged BMPR2 FL or ΔCTD expression vectors, and immunoblotting analysis was performed using FLAG monoclonal β-actin (loading control) antibodies. Blot is representative of 10 experiments.

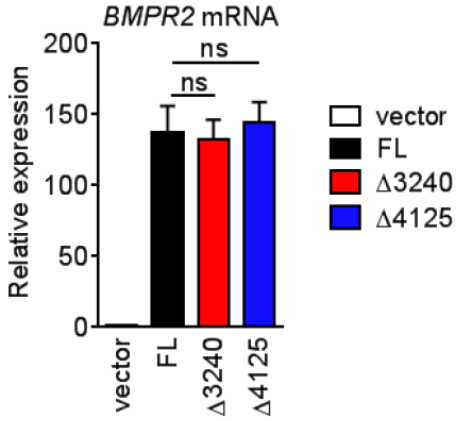

Figure S2. FMRP binds BMPR2-*CTD*$_{seq}$ and suppresses translation. HEK293 cells were transfected with BMPR2 FL, Δ3240 or Δ4125 and mRNA expression of *BMPR2 FL* and deletion mutants were examined by qRT-PCR and normalized to *GAPDH*. Data are means ± SD (N=3 experiments). ns, no significance by ANOVA with post hoc Tukey's test.

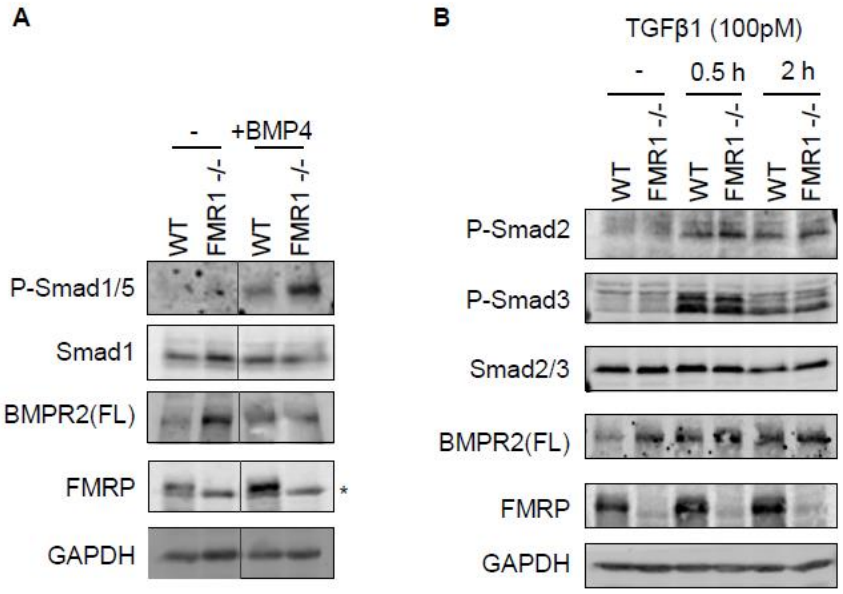

Figure S3. BMP4-SMAD1/5 signaling is increased, but TGFβ-SMAD2/3 signaling is not altered in *FMR1*-null cells. MEFs isolated from *FMR1* knock-out (FMR1 -/-) and wild-type (WT) mice were treated with 500 pM BMP4 (**A**) for 2 hours or 100 pM of TGFβ1 (**B**) for indicated periods. Phosphorylated SMAD (P-Smad), total SMAD, BMPR2(FL), FMRP, or GAPDH protein abundance was analyzed by immunoblotting. Asterisk indicates a non-specific band in the FMRP panel. Blots are representative of 3 experiments.

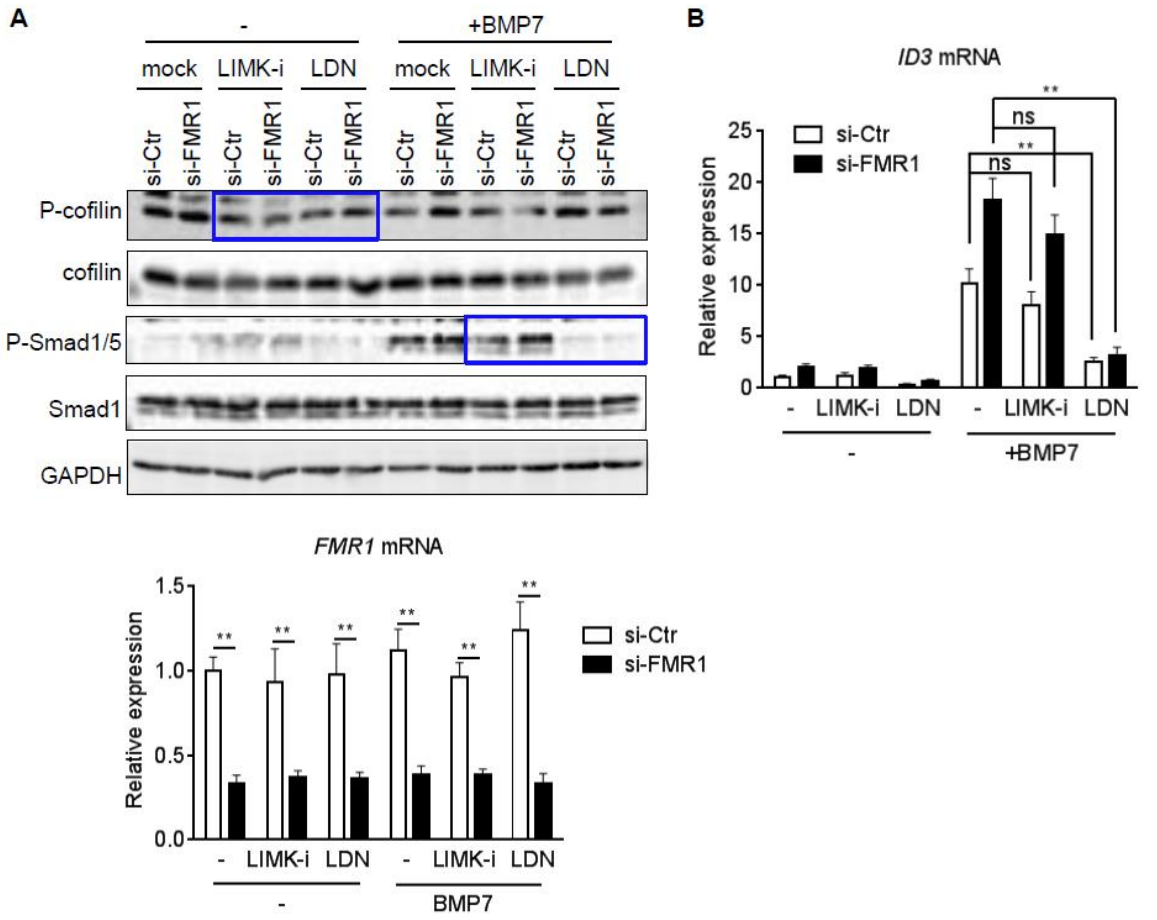

Figure S4. LIMK-i and LDN effectively inhibit LIMK1 and BMPR1 kinase activity in N1E cells. N1E cells transfected with si-Control (si-Ctr) or si-*FMR1* were treated with mock (DMSO), LDN-193189 (LDN) or LIMKi-3 (LIMK-i) with or without 10 ng/ml BMP7 for 30 min, followed by (**A**) Immunoblot analysis of P-cofilin, total cofilin, P-SMAD1/5, total SMAD1 and GAPDH (top). Knock down of FMR1 by si-*FMR1* was confirmed by qRT-PCR analysis (bottom). Data are means ± SD (N=3). ***P*<0.001 by Student's t-test. (**B**) qRT-PCR analysis of *Id3* mRNA, a transcriptional target of Smad1/5, indicates effective inhibition of the canonical Smad pathway by LDN, but not by LIMK-i. Data are means ± SD (N=3 experiments). ***P*<0.001 by ANOVA with post hoc Tukey's test.

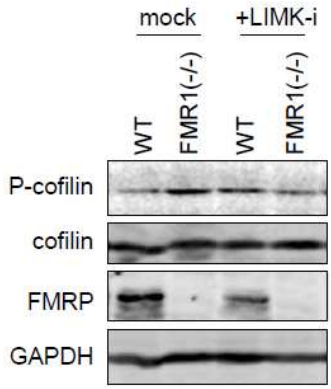

Figure S5. In vivo administration of LIMK-i inhibits phosphorylation of cofilin in mouse brain. *FMR1*$^{(-/-)}$ or littermate wild-type (WT) mice were treated with LIMK-i by intracerebroventicular injection at P1 and P4, and the brains were isolated at P7. Protein abundance was analyzed by immunoblotting using indicated antibodies. Blots are representative of 3 experiments.

Gene	species	forward primer	reverese primer
BMPR2	human	5'- GGGTAAGCTCTTGCCGTCTTGCT -3'	5'- CCCCTGGGCGCACCAGTCTAT -3'
BMPR2 FL	human	5'- GAAGACTGTTGGGACCAGGA -3'	5'- TGTGACAGGTTGCGTTCATT -3'
BMPR2 Δ CTD	human	5'- GAAGACTGTTGGGACCAGGA -3'	5'- TCCTGATTTGCATCTTGTG -3'
BMPR2 FL	mouse	5'- GAAGACTGCTGGGACCAGGAT -3'	5'- TGTGACAGGTTGCGTTCATT -3'
BMPR2 Δ CTD	mouse	5'- GAAGACTGCTGGGACCAGGAT -3'	5'- GCCATCTTGTGTTGACTCACCTA -3';
BMPR2	rat	5'- CAGACCCGTTGAGCAGTAC -3'	5'- GCCCTATGTGCCACTATG -3'
FMR1	human, monkey, mouse and rat	5'- GGCTCCAATGGCGCTTTCTAC -3'	5'- TAGCTAACCACCAACAGCAAGGCT -3'
LIMK1	mouse	5'- GGAGCCGGTGTCCCTCAAG -3'	5'- CTCGCTTCCTTCCTCTCCCATAC -3'
Smad7	mouse	5'- TCTCAAACCAACTGCAGGCT -3'	5'- TCTTGCTCCGCACTTTCTGT -3'
CTGF	mouse, rat	5'- GATGGTGCACCCTGTGTCTT -3'	5'- AGTCGGTAGGCAGCTAGGG -3'
Id1	rat	5'- GAACCGCAAAGTGAGCAAGG -3'	5'- AACACATGCCGCCTCGG -3'

Table S1: qRT-PCR primers. The sequences of the primers used in the qRT-PCR assays are provided.

position	forward primer	reverese primer
BMPR2 1820 – 1995	5'- GCGTCCAGTTGCTGTAAAAGTG -3'	5'- GATAGTACTCCATCACAAGCAAATATTCC -3'
BMPR2 2041 – 2250	5'- GGGTAAGCTCTTGCCGTCTTGCT -3'	5'- CCCCTGGGCGCACCAGTCTAT -3'
BMPR2 2513 – 2693	5'- GGAAAAACAGAGACCCAAGTTCCCA -3'	5'- GCTCACAGATTTGTTTCTTTCCCAAATCATCA -3'
BMPR2 2736 – 2928	5'- AACCTGTCACATAATAGGCGTGTGCCAA -3'	5'- CTTGCTGTCGTTCATAGTTAATTGAATTTCGGT -3'
BMPR2 3116 – 3278	5'- ACAGCTGACAGAAGAAGACTTGGAA -3'	5'- GAGTGGGTAAAGCAAGCTAGAACTA -3'
BMPR2 3240 – 3409	5'- CCACTGAGCAGTACTAGTTCTAGCT -3'	5'- CTCTTGGGAAGGTTCTGCTGCTT -3'
BMPR2 3630 -3780	5'- GGGACAGTACTATCTGGCCAAACAA -3'	5'- CAGCTTGTTGCTCTCGTCTCAGT -3'
BMPR2 4125 –FLAG	5'- ACTGAATCGCTGGACTGTGAAGTCA -3'	5'- CCTCTAGACATATGTTATTTGTCATCATCGTCCTTATA -3'
BMPR2 3468 – 3641	5'- GGCAGCAAGCACAAATCAAACTTG -3'	5'- TGTGGCAGCATGGGAGTTAACACT -3'

Table S2: RIP PCR primers. The sequences of the primers used in the RIP assays are provided.