Supplementary Materials for

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

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The PDF file includes:

- Fig. S1. Translational regulation of BMPR2 through the mRNA sequence encoding the CTD.
- Fig. S2. FMRP binds BMPR2-CTDseq and suppresses translation.
- Fig. S3. BMP4-SMAD1/5 signaling is increased, but TGFβ-SMAD2/3 signaling is not altered in FMR1-null cells.
- Fig. S4. LIMK-i and LDN effectively inhibit LIMK1 and BMPR1 kinase activity in N1E cells.
- Fig. S5. In vivo administration of LIMK-i inhibits phosphorylation of cofilin in mouse brain.
- Table S1. qRT-PCR primers.
- Table S2. RIP PCR primers.
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<sub>seq</sub> 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 intracerebroventricular 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.

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

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