Supporting Information

Arif et al. 10.1073/pnas.1011275108

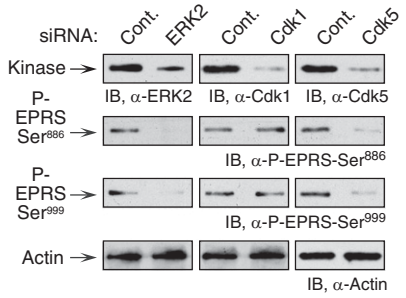

Fig. S1. ERK2 and Cdk5 are required for phosphorylation of endogenous EPRS. Human PBMs were transiently transfected with siRNAs targeting ERK2, Cdk1, and Cdk5. Scrambled siRNAs were used as controls (Cont.). After 24 h of recovery, cells were treated with IFN-γ for an additional 4 h. Knockdown efficiency was determined by immunoblot analysis. Phosphospecific EPRS antibodies were used to detect specific Ser^{886} and Ser^{999} phosphorylation. Immunoblot analysis with anti-actin antibody served as a loading control.

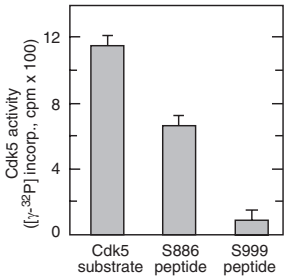

Fig. S2. Cdk5 specifically phosphorylates Cdk5 and EPRS Ser^{886} peptide substrates. Lysates from U937 cells treated with IFN-γ for 4 h were incubated with anti-Cdk5 antibody, and the precipitates were used for in vitro phosphorylation of Cdk5-specific peptide substrate and Ser^{886}- and Ser^{999}-containing EPRS peptide substrates. Incorporation of ^{32}P into peptides was determined by scintillation counting as described in Fig. 2*E* (mean ± SEM; *n* = 3 experiments).

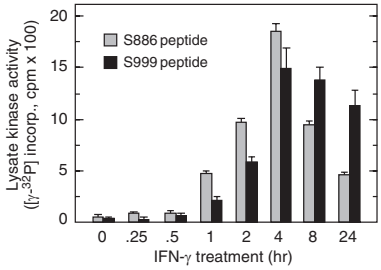

Fig. S3. Temporal induction of EPRS phosphorylation by IFN-γ. Lysates from U937 cells treated with IFN-γ for up to 24 h were used to directly phosphorylate Ser^{886}- and Ser^{999}-containing EPRS peptide substrates using [γ-^{32}P]ATP. Aliquots were analyzed for incorporation of ^{32}P into peptides as described in Fig. 2*E* (mean ± SEM; *n* = 3 experiments).

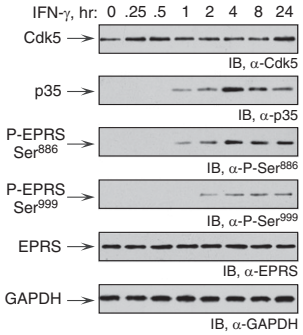

Fig. S4. IFN-γ induces p35 expression. Lysates from IFN-γ–treated PBMs were immunoblotted as shown.

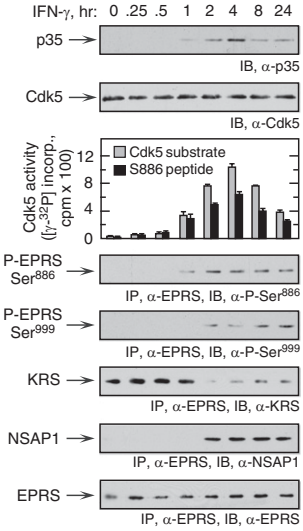

Fig. S5. Cdk5-mediated EPRS phosphorylation is required for the release of EPRS from the MSC. IFN-γ–induced Cdk5 activation and EPRS phosphorylation correlates with EPRS release from the MSC and binding to NSAP1. Lysates from U937 cells treated with IFN-γ for up to 24 h were analyzed for Cdk5 and p35 expression by immunoblot analysis and for Cdk5 activity after incubation with anti-Cdk5 antibody. The same lysates were immunoprecipitated with anti-EPRS antibody and immunoblotted with anti-phosphospecific EPRS, KRS, and NSAP1 antibodies. EPRS in the precipitate was detected by anti-EPRS antibody.