Supporting Information

Lerner-Marmarosh *et al*. 10.1073/pnas.0800750105

SI Text

This study reveals a previously unknown mechanism for the activation of the stress- and growth-factor-responsive genes in the MAPK/ERK pathway that involves transcriptional activity of hBVR and its phosphorylation by ERK. An outcome of hBVR activation by ERK is induction of stress-responsive gene expression. hBVR is a transcription factor for those nuclear factors that bind AP-1 sites; the transcriptional activity is dependent on its phosphorylation (1).

1. Miralem T, Hu Z, Torno MD, Lelli KM, Maines MD (2005) Small interference RNA-mediated gene silencing of hBVR, but not that of HO-1, attenuates arsenite-mediated induction of the oxygenase and increases apoptosis in 293A kidney cells. *J Biol Chem* 280:17084–17092.

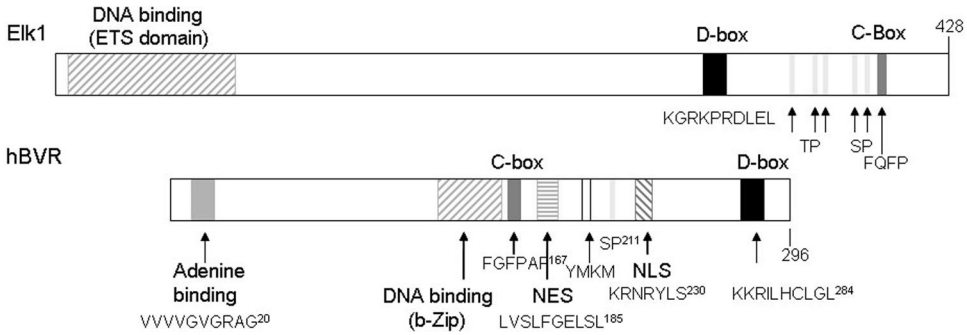

Fig. S1. Comparison of structural features of human Elk1 and hBVR. The positions of the motifs in Elk1 (GenBank accession NP_005220) and hBVR (GenBank accession NP_000703) are indicated by shading (2, 3); maps for each sequence (428 and 296 aa, respectively) are drawn to scale.

2. Jacobs D, Glossip D, Xing H, Muslin AJ, Kornfeld K (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev* 13:163–175.
3. Maines MD, Polevoda BV, Huang TJ, McCoubrey WK, Jr (1996) Human biliverdin IXα reductase is a zinc-metalloprotein. Characterization of purified and Escherichia coli expressed enzymes. *Eur J Biochem* 235:372–381.

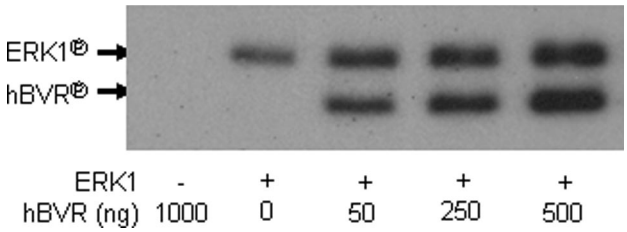

Fig. S2. Concentration-dependent activation of ERK1 by hBVR substrate. Recombinant active ERK1 was obtained from EMD. 5 ng active ERK1 was incubated in an assay system optimized for ERK kinase activity together with the indicated amounts of GST-hBVR. The reaction was initiated by the addition of 100 μM [γ-^{32}P]ATP, and terminated by the addition of Laemmli buffer followed by SDS/PAGE, transfer to PVDF membrane and autoradiography. Experimental details are provided in ref. 4.

4. Lerner-Marmarosh N, *et al*. (2005) Human biliverdin reductase: A member of the insulin receptor substrate family with serine/threonine/tyrosine kinase activity. *Proc Natl Acad Sci USA* 102:7109–7114.

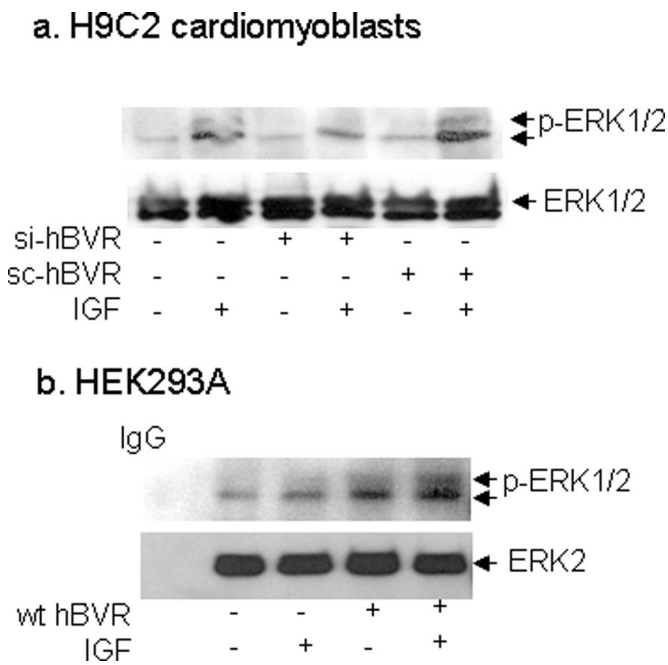

Fig. S3. Activation of ERK by hBVR is not cell line-specific. (*a*) hBVR enhances ERK2 phosphorylation in response to IGF-1 in cardiomyoblasts. The cardiomyoblast cell line, H9C2 (a generous gift from W. Mao, Department of Medicine, University of Rochester, NY), was maintained in DMEM containing penicillin and streptomycin and supplemented with 10% FBS and 5% horse serum (HS). Cells were treated with rat si-BVR or sc-BVR, as described in the text. Cells were starved in medium with 0.1% FBS and 0.05% HS, treated with 20 ng/ml IGF1 or vehicle for 15 min, then harvested. Cell lysates were prepared in RIPA buffer. Equal amounts of protein were immunoprecipitated with antibody to rat ERK2, and the immunoprecipitated proteins were separated by SDS/PAGE, blotted onto PVDF and the membrane was probed sequentially with anti-phospho-ERK1/2 and anti-rat ERK2 antibodies. The antibody to rat ERK2 shows some cross-reactivity to ERK1. (*b*) Endogenous ERK1/2 phosphorylation is enhanced by hBVR in HEK293A cells. HEK293A cells were transfected with wt hBVR or empty vector, starved in medium with 0.1% FBS and treated with 20 ng/ml IGF1 or vehicle for 15 min. Total cell lysates were immunoprecipitated with anti-ERK2 antibodies, and analyzed as in *a*.

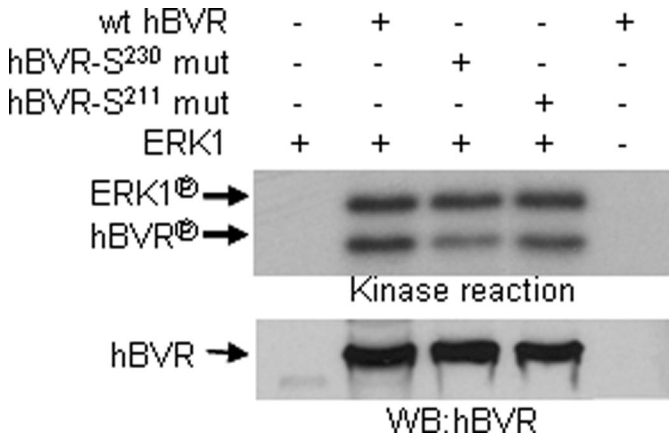

Fig. S4. hBVR serine target sites of ERK phosphorylation—testing site-specific serine mutants as substrates for ERK1. Binding of ERK requires the FXFP motif in the substrate (2, 3); S/T P motifs in substrates are phosphorylation target(s) of ERK, as is the case for Elk1. These sites are shown in Fig. S1. In Elk1, the FXPF motif lies C-terminal to the S/TP motifs. There is one SP sequence in hBVR, at Ser^{211}, and the $S^{211}\rightarrow A$ and $S^{230}\rightarrow A$ mutants were examined as kinase substrates. ERK kinase reactions *in vitro* were performed as described in the legend to Fig. S2 using wt hBVR and mutant forms of GST-hBVR as substrates. After allowing the radioactivity to decay, the membrane was probed with anti-hBVR antibody, to confirm that an equal amount of GST-hBVR was present in each assay. As shown in the figure, Ser^{211} is not a target of ERK kinase activity; rather, Ser^{230}, in the SH2 binding motif Y^{228}LSF, is an ERK target. It should be noted that this site is well conserved in mammals (5).

2. Jacobs D, Glossip D, Xing H, Muslin AJ, Kornfeld K (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev* 13:163–175.
3. Maines MD, Polevoda BV, Huang TJ, McCoubrey WK, Jr (1996) Human biliverdin IXα reductase is a zinc-metalloprotein. Characterization of purified and Escherichia coli expressed enzymes. *Eur J Biochem* 235:372–381.
5. Maines MD (2005) New insights into biliverdin reductase functions: Linking heme metabolism to cell signaling. *Physiology* 20:382–389.

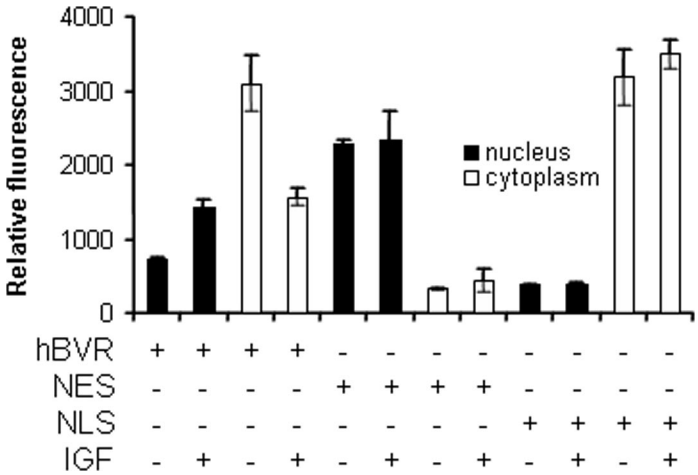

Fig. S5. Quantification of hBVR in nuclei and cytoplasm. HEK293 cells were transfected with wt pEGFP-hBVR or its NES and NLS mutants, starved in 0.1% FBS and treated with 20 ng/ml IGF1 or vehicle for 15 min. After washing in cold PBS, the cells were harvested by scraping and followed by centrifugation at 500 × *g* for 5 min. Intact cell pellets were resuspended in buffer containing 0.28 M sucrose, 50 mM Tris·HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 μg/ml each of leupeptin, pepstatin, and aprotinin and 1 mM PMSF, and homogenized by passing five times through a 25-gauge needle. The homogenates were centrifuged at 120 × *g* for 25 min at 4°C to separate the nuclei. The supernatants were collected and centrifuged at 13,000 × *g* for 15 min at 4°C, to remove mitochondria, then at 25,000 × *g*, for 60 min 4°C, to separate the plasma membrane (pellet) from cytoplasm. The nucleus and cytoplasm fractions were solubilized in RIPA buffer (Triton X-100 was added to the nuclei to give a final concentration of 1%). Equal amounts of nuclear and cytoplasmic proteins were used for quantitative determination of GFP using a Turner Biosystems fluorometer. The relative fluorescence, in arbitrary units, of each sample is shown; data are the mean ± SD of three samples.