Supplementary Materials and Methods

Plasmid construction. All plasmids were constructed using standard molecular biology techniques, and the nucleotide sequences of DNA fragments generated by PCR were verified by sequencing. The sequences of primers used in plasmid construction are available upon request. All GAr-containing fusion proteins were cloned into the p414ADH yeast expression vector (Mumberg, Muller et al. 1995)using flanking SpeI and XhoI restriction sites introduced by PCR during their construction. The construction of the ODC::GAr_{30} expression vector, p414ADH-ODC::GAr_{30}, was described previously (Zhang and Coffino 2004). The GAr_{30} module contains a 30-mer Gly-Ala repeat and flanking 12 residue N-terminal (GSTRDYKDDDDK) and 13 residue C-terminal (YPYDVPDYAHMID) linker sequences including FLAG and HA epitope sequences (underlined), respectively. The DHFR-GAr_{30}-C37 ORF was constructed by splice overlap extension PCR (SOE-PCR) following PCR amplification of the human DHFR ORF from pKT7HDR (Schweitzer, Srimatkandada et al. 1989) and the GAr_{30} module and C-terminal 37 amino acid residues of mouse ODC from p414ADH-ODC::GAr_{30}. Amino acid substitutions were introduced into ODC::GAr_{30} and DHFR-GAr_{30}-C37 plasmids by SOE-PCR using primers containing the desired mutations and common end primers. N-terminal truncations of ODC::GAr_{30} were generated by PCR with a forward primer in which the initiator Met was followed immediately by His12 (Δ11ODC::GAr_{30}), Phe18 (Δ17ODC::GAr_{30}), or Asp35 (Δ34ODC::GAr_{30}) of mouse ODC, and a common reverse primer. ODC::GAr_{30} plasmids containing a 25 residue spacer in the GAr cassette (see the ODC[25]GAr_{30} fusion protein in Figure 5A, and Figure 6A) were constructed by successive rounds of SOE-PCR to introduce the linker sequence, using p414ADH-ODC::GAr_{30} as the template, and introducing a FLAG epitope on the N-terminus of the ODC moiety. The linker sequence (DDYKGVARYMASPKDYDTPDHDIYD) was designed by combining the 12 residue N-terminal and 13 residue C-terminal linker sequences of the GAr_{30} module and randomizing the amino acid sequence. ODC[n]GAr_{30} plasmids containing shorter spacer sequences were subsequently constructed from the ODC[25]GAr_{30} template by SOE-PCR. The linker sequence of ODC[n]GAr_{30} consists of the last n residues of the randomized sequence.

Mumberg, D., R. Muller, et al. (1995). "Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds." Gene **156**(1): 119-22.

Schweitzer, B. I., S. Srimatkandada, et al. (1989). "Probing the role of two hydrophobic active site residues in the human dihydrofolate reductase by site-directed mutagenesis." J Biol Chem **264**(34): 20786-95.

Zhang, M. and P. Coffino (2004). "Repeat Sequence of Epstein-Barr Virus EBNA1 Protein Interrupts Proteasome Substrate Processing." J. Biol. Chem. **279**: 8635-8641.