

Figure S1. Effects of HuR Knockdown on  $\beta_2$ -AR expression in A431 cells.

**A**, Immunoblot analysis of cellular extracts from A431 cells expressing control shRNA (lane 1) and HuR specific shRNA (lane 2) using anti-HuR monoclonal antibody and  $\beta$ -tubulin (control) polyclonal antibody. **B**, RNase protection assay (RPA) for quantitative measurement of  $\beta_2$ -AR mRNA and GAPDH mRNAs in control (lane 1) and HuR knockdown A431 cells (lane 2). **C**, Radioligand binding assay using <sup>125</sup>I-CYP in control and HuR knockdown A431 cells. Ligand binding assays were performed as described in Figure 1C. **D**, Confocal microscopy images of A431 cells showing appearance of  $\beta_2$ -AR around the nucleus in HuR knockdown A431 cells.

Figure S2 & S3. Role of 3'-UTR on  $\beta_2$ -AR localization.



**S2**, Immunofluorescence staining and confocal images of DDT<sub>1</sub>-MF2 cell expressing full-length hamster  $\beta_2$ -AR cDNA shows over-expressed receptors are present on the plasma membrane. Magnified View of a single cell over-expressing  $\beta_2$ -AR. Scale bars 10  $\mu$ m

**S3**, Immunofluorescence staining and confocal images of DDT<sub>1</sub>-MF2 cell expressing 3'-UTR deletion constructs of hamster  $\beta_2$ -AR cDNA shows over-expressed receptors failed to traffic to plasma membrane and appeared around the nucleus. Magnified View of a single cell. Scale bars 10  $\mu$ m

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Figure S4 . Shows localization of enhanced GFP<sup>NLS/myr</sup> with and without  $\beta_2$ -AR 3'-UTR.

**S4.** DDT<sub>1</sub>-MF2 cells were transfected with GFP<sup>NLS/myr</sup> with (S4 A & B) or without (S4, C)  $\beta_2$ -AR 3'-UTR. GFP localization was examined in live cells by Confocal microscopy. To avoid cumulative effect of GFP expression, we used a destabilized version of GFP fused to a myristoylation consensus sequence that limits protein diffusion to its site of synthesis (25). Receptor 3'-UTR sequences limited the distribution of GFP to the cell periphery as shown in figure S4 A&B. S4, B shows the magnified view of the inset in Fig. S4, A. In the absence of  $\beta_2$ -AR 3'-UTR, GFP was distributed around the nucleus (S4, C) and failed to traffic to the cell periphery. Scale bars as shown in the figure.