SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Species specificity of the anti $\alpha 1^h$ and $\alpha 1^r$ subunit antibodies. We transfected HEK293T cells with empty vector (negative control, shaded histograms), or 0.250 μg of β2 and γ2 subunit cDNA and hemizygous expression of either 0.125 μg of $\alpha 1^h$ (dotted line) or 0.125 μg of $\alpha 1^r$ (dashed line). We stained surface (A-B) or total (C) receptors with the anti $\alpha 1^h$ antibody (A, C) or anti $\alpha 1^r$ antibody (B) and quantified antibody staining by flow cytometry. These data demonstrated that these antibodies were species specific; staining of the $\alpha 1^h$ subunit by the anti $\alpha 1^h$ antibody or staining of the $\alpha 1^r$ subunit by the anti $\alpha 1^h$ antibody accounted for $0 \pm 0.4\%$ that of specific staining (N ≥ 3).

Supplemental Figure 2: Effect of the transfection of the pmaxGFP plasmid on $\alpha 1\beta 2\gamma 2$ and $\alpha 3\beta 2\gamma 2$ receptor expression. We transfected HEK293T cells with hemizygous $\alpha 1^h \beta 2\gamma 2$ (A, B) or wild type $\alpha 3\beta 2\gamma 2$ (C) receptors and 2 μg of either empty plasmid (control), or the pmaxGFP, or $\alpha 1(AD)^r$ plasmids (N \geq 3). Surface (A, C) and total (B) $\alpha 1^h$ (A-B) or $\alpha 3$ subunit expression was determined by flow cytometry using anti $\alpha 1^h$ -647 or anti $\alpha 3$ subunit antibodies. The bar graphs depict the mean specific $\alpha 1^h$ -647 or $\alpha 3$ subunit fluorescence normalized to that of control samples. While the $\alpha 1(AD)^r$ subunit caused significant reductions in $\alpha 1^h$ and $\alpha 3$ subunit expression (P < 0.004), transfection of the pmaxGFP plasmid did not alter the expression of either of these wild type receptors (P > 0.513).

Supplemental Figure 3: Effect of the $\alpha 1(AD)$ subunit on surface expression of the TGF^{HA} protein. We transfected HEK293T cells with wild type $\alpha 1\beta 2\gamma 2$ receptors in addition to 0.250 µg of cDNA encoding TGF^{HA} and with or without 2 µg of the cDNA encoding the $\alpha 1(AD)$ subunit. We performed flow cytometry using an anti-HA-647 antibody. A sample histogram is shown in panel A with negative control cells depicted as a shaded histogram, samples transfected in the absence of $\alpha 1(AD)$ shown with the solid line and samples transfected with $\alpha 1(AD)$ depicted in a dashed line. Quantification (B) demonstrated that the $\alpha 1(AD)$ subunit did not reduce expression of the TGF^{HA} protein (116 ± 10%; P = 0.169; N = 5).

