

## A4

### **The sorting protein GASP-1 regulates the constitutive signaling capacity of the virally encoded chemokine receptor US28**

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#### **Background**

Human cytomegalovirus (HCMV) is a widespread pathogen that has been shown to be present in various malignancies and it is also thought to be linked to vascular diseases. HCMV encodes the seven transmembrane (7TM)/G protein-coupled receptor (GPCR) US28, which constitutively activates the  $G_{\alpha_q}$ /phospholipase C (PLC) pathway and downstream transcription factors such as the nuclear factor- $\kappa$ B (NF- $\kappa$ B) or the cyclic AMP responsive element binding protein (CREB). In this study we set out to elucidate the role of the GPCR-associated sorting protein-1 (GASP-1) in the regulation of the constitutive signaling capacity of US28.

#### **Methods**

To elucidate the role of GASP-1 in the regulation of the constitutive signaling capacity of US28 we disrupted the US28/GASP-1 interaction by either overexpression of dominant negative cGASP-1 or shRNA knock-down of endogenous GASP-1. To monitor the US28-mediated signaling we conducted inositol phosphate (IP) accumulation assays as well as luciferase reporter gene assays to check the activation of the transcription factors NF- $\kappa$ B and CREB.

#### **Results**

We find that GASP-1 is indeed able to modulate the signaling activity of US28. Disruption of the GASP-1/US28 interaction by either i) overexpression of dominant negative cGASP-1 or by ii) shRNA knock-down of endogenous GASP-1 alters the US28-mediated  $G_{\alpha_q}$ /PLC/IP accumulation as well as the activation of the transcription factors NF- $\kappa$ B and CREB.

#### **Conclusions**

By identifying the sorting protein GASP-1 as a key regulator of the constitutive signaling activity of US28, we may be one step closer to gaining a better understanding of this viral receptor and its significance in the pathogenesis implicated by HCMV.