Supplementary information

To validate the BiFC assay using known interaction partners, we monitored active Ras by the BiFC method using the Ras-binding domain (RBD) of Raf-1, which is known to specifically bind the active, GTP-bound form of Ras (Fridman et al., 2000). YN was fused to the N-terminus of RBD (YN-RBD). YC was fused to the N-terminus of Ras (YC-H-RasG12V to allow post-translational modifications of the Ras protein's C-terminal domain. All BiFC constructs used in the study are presented schematically in Figure S1. HEK293 cells were co-transfected with YC-H-RasG12V, a constitutively active, predominantly GTP-bound Ras, and YN-RBD or with YC-H-Ras and YN-RBD. Non-fused YC and YN were used as controls. The cells were then monitored by fluorescence confocal microscopy. Results of a typical experiment clearly demonstrate strong fluorescence in cells co-transfected with YC-H-RasG12V and YN-RBD and only weak fluorescence in YC-H-Ras and YN-RBD co-transfectants (Figure S2). Fluorescence was recorded at the plasma membrane and at internal membranes. It was barely detectable, however, in control cells co-transfected with YC-H-RasG12V and YN (Figure S2) or with YN-RBD and YC (Figure S2). Thus, fluorescence complementation occurred only when YC and YN were each fused to an appropriate binding partner. These findings show that the BiFC method is a valid tool for studying the binding of active H-Ras to its interaction partners.

Supplementary figure legends

Figure S1. Scheme depicting the BiFC constructs used in this study. The name of each construct and its schematic representation is shown. YN corresponds to residues 1−154 and YC to residues 155−238 of the yellow fluorescent protein (YFP). RBD corresponds to Ras binding domain of Raf-1.

Figure S2. BiFC detects specific interaction between active H-Ras and the Ras-binding domain of Raf-1 (RBD) in live cells. HEK293 cells were co-transfected with YC-H-Ras and YN-RBD or YC-H-RasG12V and YN-RBD. Control transfections YC-H-RasG12V and YN or YN-RBD and YC are included. Cells were imaged using an LSM 510 confocal microscope fitted with a YFP filter 48 hours after co-transfection to detect BiFC. Typical images are shown. Similar images

were recorded for at least 30 different cells and the experiment was repeated four times. Note the clear fluorescence in cells co-transfected with YC-H-RasG12V and YN-RBD compared to the lack of fluorescence in the controls. BiFC of cells co-transfected with YC-H-RasG12V and YN-RBD is observed in the plasma membrane and in the perinuclear region. Only very weak BiFC is detected between YC-H-Ras and YN-RBD. Scale bar, 20 μm.

Fig. S1

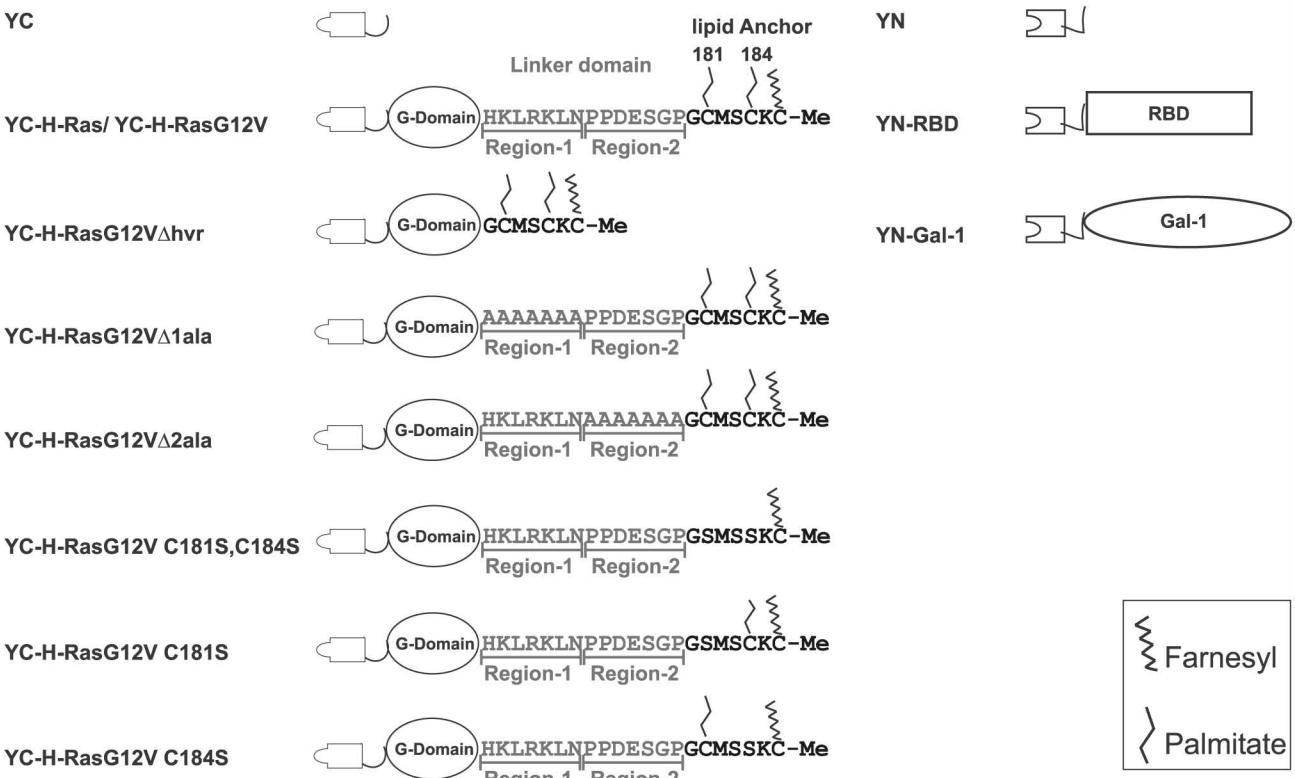
YC
YC-H-Ras/ YC-H-RasG12V
YC-H-RasG12VΔhvr
YC-H-RasG12VΔ1ala
YC-H-RasG12VΔ2ala
YC-H-RasG12V C181S,C184S
YC-H-RasG12V C181S
YC-H-RasG12V C184S
G-Domain
Linker domain
lipid Anchor
181
184
HKLRKLNPPDESGPGCMSCKC-Me
GCMSCKC-Me
AAAAAAAPPDESGPGCMSCKC-Me
HKLRKLNAAAAAAAGCMSCKC-Me
HKLRKLNPPDESGPGSMSSKC-Me
HKLRKLNPPDESGPGSMSCKC-Me
HKLRKLNPPDESGPGCMSSKC-Me
Region-1
Region-2
YN
YN-RBD
RBD
YN-Gal-1
Gal-1
Farnesyl
Palmitate

Fig. S2