

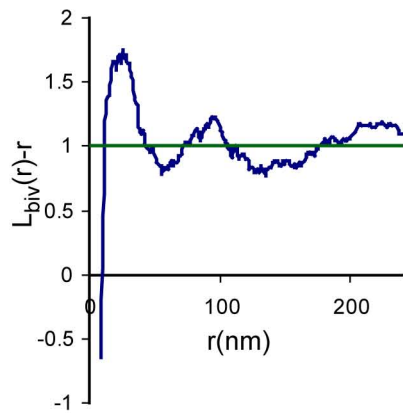
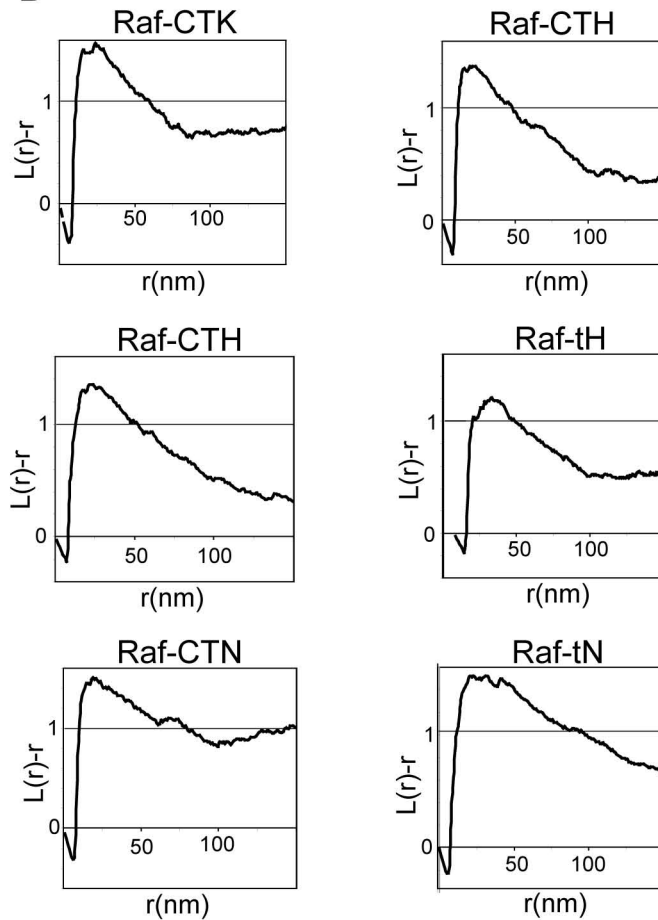
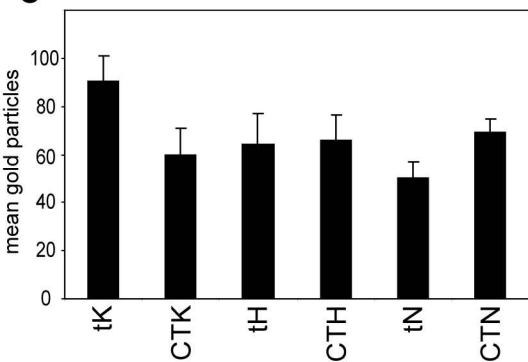
Supplementary Figure Legends

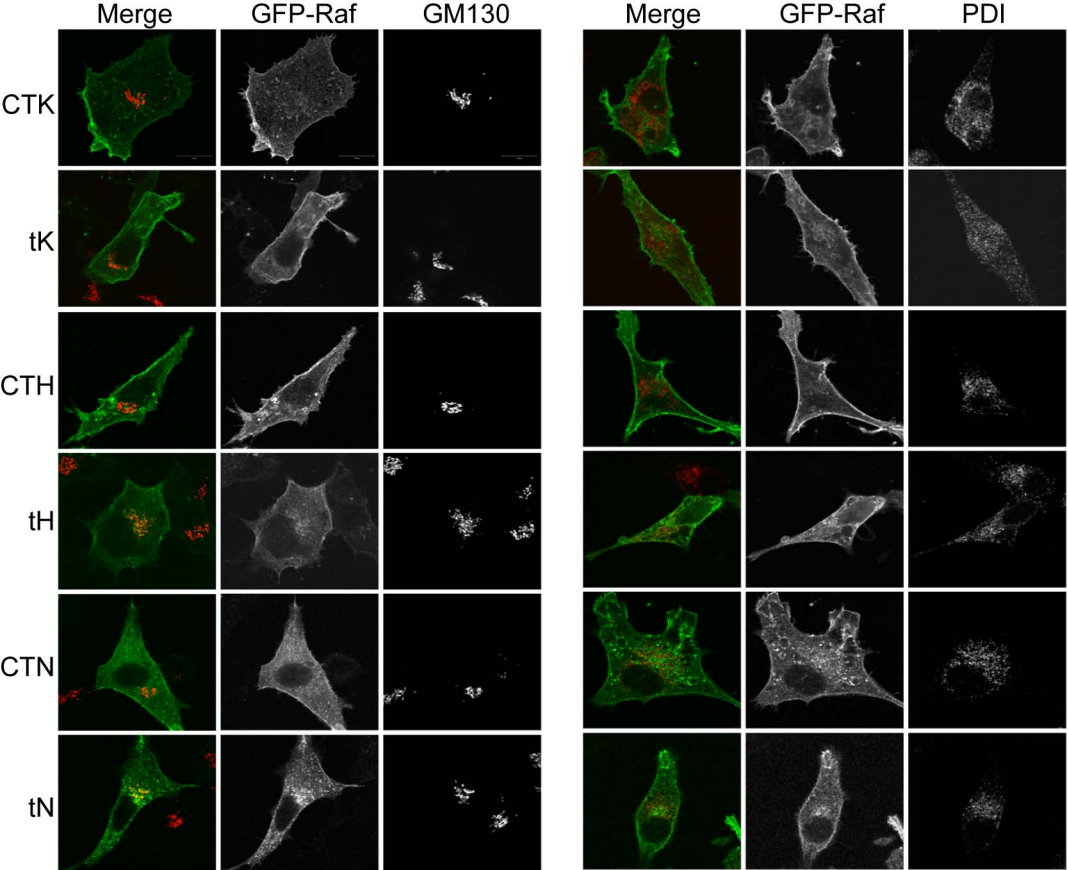
Figure S1. Raf-1 directed to different Ras plasma membrane nanodomains. (A) CTH co-localizes with active H-Ras. Apical plasma membrane sheets from BHK cells transiently co-expressing GFP-CTH and RFP-H-RasG12V were co-labeled with anti-GFP tagged 6nm and anti-RFP tagged 2nm gold particles. The immunogold point patterns were analysed using Ripley's bivariate K-function. The graphs show weighted mean K-functions ($n \geq 8$ sheets) standardized on the 99% confidence interval (CI) for complete spatial randomness. The weighted mean bivariate K function shows that GFP-CTH and RFP-H-RasG12V significantly co-cluster. (B) Apical plasma membrane sheets from BHK cells transiently expressing Raf-CTK, Raf-tK, Raf-CTH, Raf-CTN and Raf-tH, Raf-tN were labelled with anti-GFP antibodies coupled to 5 nm gold. The immunogold point patterns were analysed using Ripley's K-function. The graphs show weighted mean K-functions ($n \geq 8$ sheets) standardized on the 99% confidence interval (CI) for complete spatial randomness. The graphs show that all of the Raf constructs exhibit nanoclustering on the plasma membrane. (C) The graph shows the mean number of gold particles/ μm^2 of plasma membrane sheet (mean \pm SEM, $n = 8$) for each of the plasma membrane targeted Raf constructs.

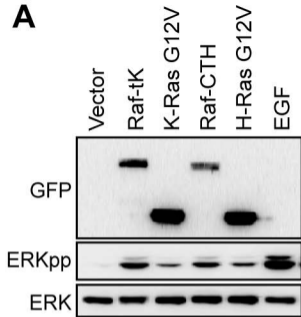
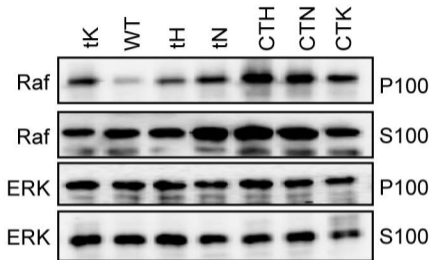
Figure S2. Raf-1 directed to the plasma membrane. (A) BHK cells transfected with the plasma membrane targeted Raf-1 constructs Raf-CTK, Raf-tK, Raf-CTH, Raf-tN, Raf-CTN, or Raf-tH were co-localized with organelle markers, GM130 (Golgi) and PDI (ER), and imaged by confocal microscopy.

Figure S3 (A) BHK cells expressing vector only, GFP-Raf-tK, GFP-K-ras G12V, GFP-Raf-CTH, GFP-H-Ras G12V, and EGF stimulated cells were serum starved for 3h. Activation of ERK was assayed in whole cell lysates with a phospho-specific antibody to ERKpp, total ERK levels were evaluated with anti-ERK2 and expression of the constructs was evaluated anti-GFP. (B) BHK cells expressing Raf-1 constructs Raf-CTK, Raf-tK, Raf-CTH, Raf-tN, Raf-CTN, Raf-tH or wild type Raf (WT) were separated into a membrane fraction (P100) and soluble fraction (S100) by hypotonic lysis and ultracentrifugation. The western blots were probed with antibody against Raf-1 and ERK

to show equal loading. (C) Raf-CTK, Raf-tK, Raf-CTH, Raf-tN, Raf-CTN, and Raf-tH were transiently expressed in BHK cells and cell lysates prepared after 3h of serum starvation. Empty vector and wild type Raf-1 (WT) were used as controls. The Raf-1 western blot shows the Raf constructs expressing at levels similar to endogenous Raf. Activation ERK was assayed with phospho-specific antibody to ERKpp.

A**B****C**



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