Supporting Information

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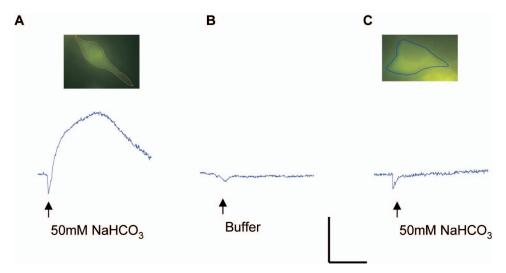


Fig. 51. FRET imaging showing that bicarbonate activates guanylyl cyclase-D (GC-D) activity. (A) Strong FRET signal after stimulation of a cell (inset) with 50 mM bicarbonate (arrow). This HEK-293T cell was transiently cotransfected with constructs encoding GC-D and a cGMP FRET signal probe cGES-DE5. (B) For the same cell as shown in A, no FRET signal was observed when same amount of buffer solution with no bicarbonate was applied. (C) No FRET signals were observed after bicarbonate application on a HEK-293T cell transfected only with FRET probe cGES-DE5. (Scale Bars, 20% ΔΥΕΡ/CFP and 200 s.)

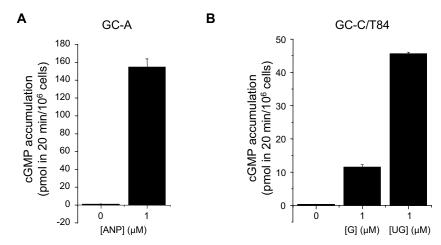


Fig. S2. GC-A and GC-C were activated by their cognate peptide ligands. (A) Application of atrial natriuretic peptide (ANP, 1 μ M) stimulated the accumulation of cGMP in HEK-293T cells transfected with ANP receptor GC-A. (B) Application of guanylin (G, 1 μ M) and uroguanylin (UG, 1 μ M) stimulated cGMP production in T-84 cells, which are frequently used for GC-C activity assay because of their high endogenous expression of GC-C.

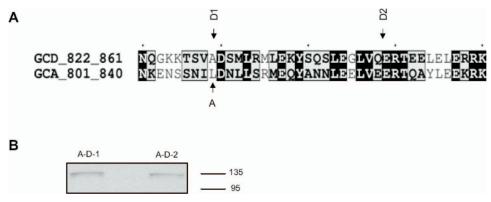


Fig. 53. Strategy for designing chimeric proteins. (A) Amino acid alignment between GC-D and GC-A. Identical amino acids are shaded black and conserved amino acids are shaded gray. Numbers indicate residue positions for each protein sequence. Arrows above indicate the swapping points for A-D-1 and A-D-2. A-D-1 contains GC-A residues 1–809 linked with GC-D residues 830–1,110; A-D-2, GC-A residues 1–829 with GCD residues 850–1,110. Arrow below indicates the swapping point for D-A. (B) Bottom panel shows that 2 chimeric proteins (A-D-1 and A-D-2) were expressed in the membrane of the transfected HEK-293T cells.