HIRamide
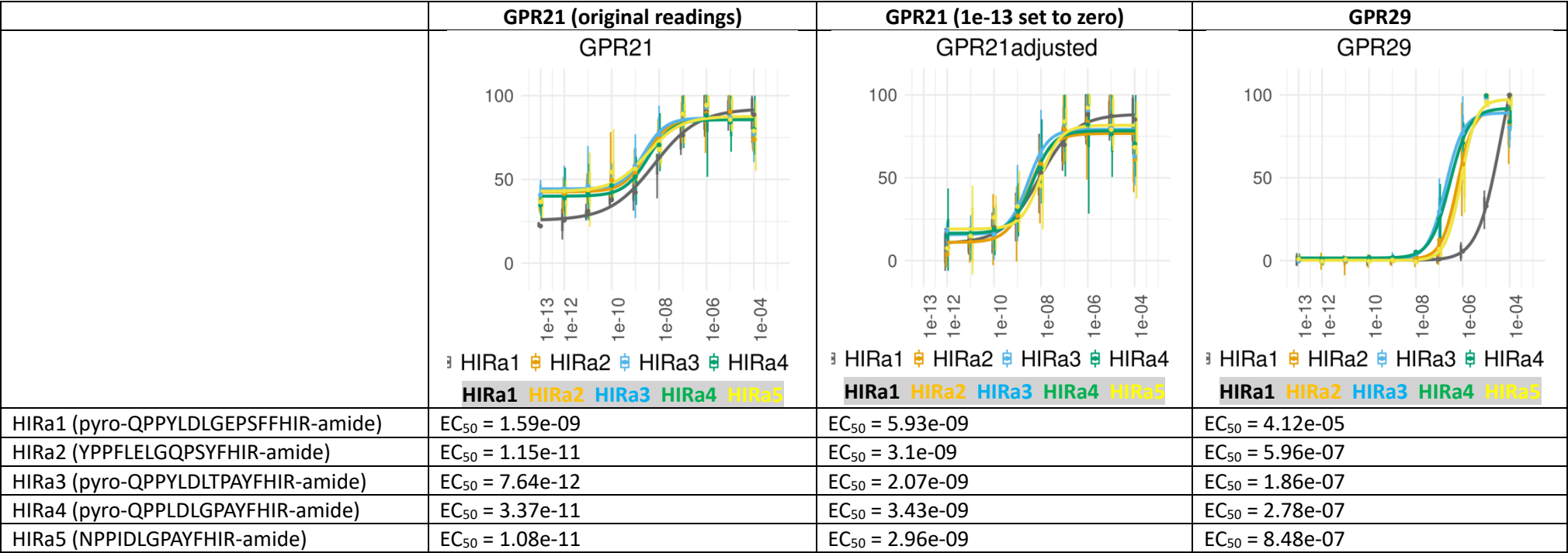
MRLYLFVPVFALVLAVEGASDEKRDSKQPPIDLSPAAYFHIRGKRTYNAPPLDLSGPAYFHIRGKRTAK**QPPYLDLGEPSFFHIR**GKRTEGPPYIDLTEPSFFHIRGKRSSE**QPPLDLGPAYFHIR**GK
RTK**NPPIDLGPAYFHIR**GKRLSGE**QPPYLDLTPAYFHIR**GKRTQQPPMIDLSEPAFFHIRGRRAVEQPPYLDLTPSYFHIRGKRTE**YPPFLELGQPSYFHIR**GRRAEKTTKD

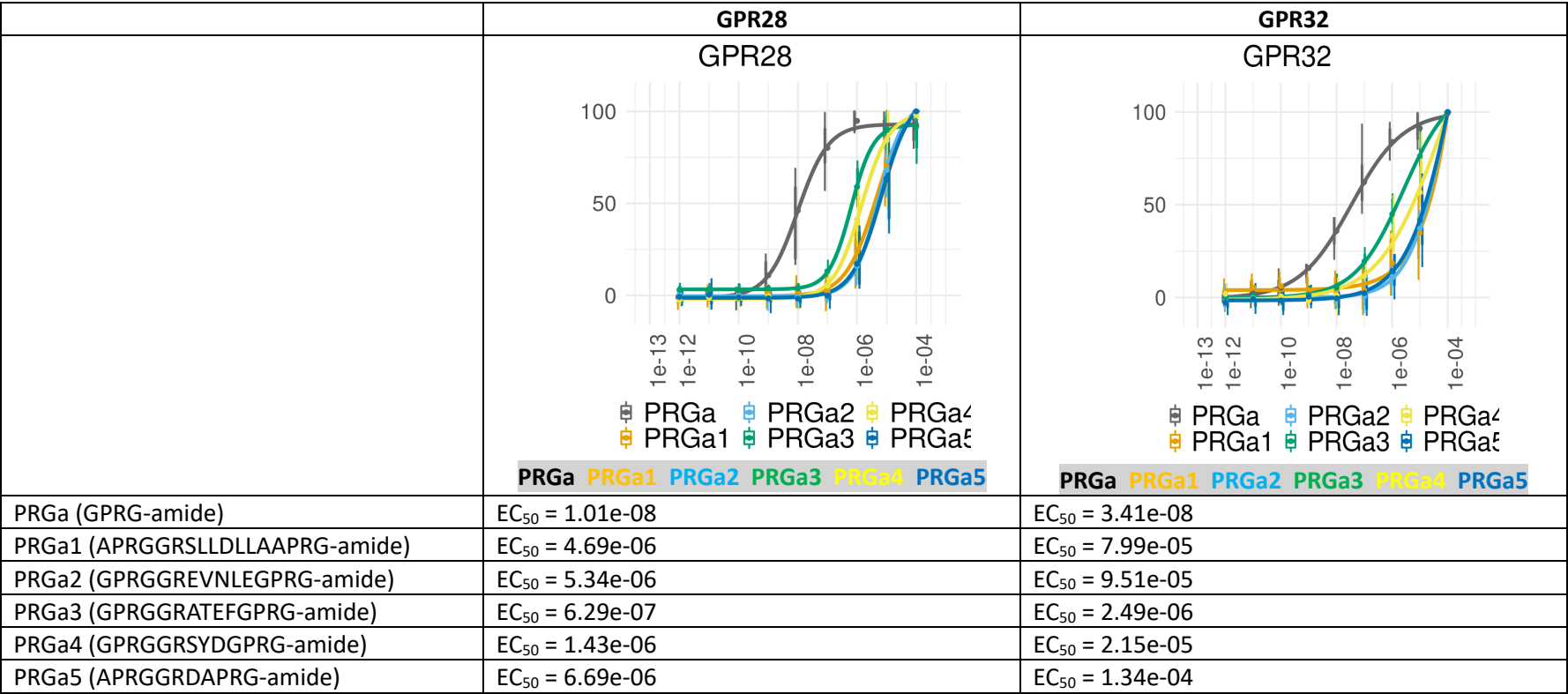|	GPR21 (original readings)	GPR21 (1e-13 set to zero)	GPR29
	GPR21	GPR21adjusted	GPR29
	HIRa1 HIRa2 HIRa3 HIRa4 / HIRa1 HIRa2 HIRa3 HIRa4 HIRa5	HIRa1 HIRa2 HIRa3 HIRa4 / HIRa1 HIRa2 HIRa3 HIRa4 HIRa5	HIRa1 HIRa2 HIRa3 HIRa4 / HIRa1 HIRa2 HIRa3 HIRa4 HIRa5
HIRa1 (pyro-QPPYLDLGEPSFFHIR-amide)	EC_{50} = 1.59e-09	EC_{50} = 5.93e-09	EC_{50} = 4.12e-05
HIRa2 (YPPFLELGQPSYFHIR-amide)	EC_{50} = 1.15e-11	EC_{50} = 3.1e-09	EC_{50} = 5.96e-07
HIRa3 (pyro-QPPYLDLTPAYFHIR-amide)	EC_{50} = 7.64e-12	EC_{50} = 2.07e-09	EC_{50} = 1.86e-07
HIRa4 (pyro-QPPLDLGPAYFHIR-amide)	EC_{50} = 3.37e-11	EC_{50} = 3.43e-09	EC_{50} = 2.78e-07
HIRa5 (NPPIDLGPAYFHIR-amide)	EC_{50} = 1.08e-11	EC_{50} = 2.96e-09	EC_{50} = 8.48e-07

Precursor sequence: N-terminal signal peptide is not highlighted, cleavage + amidation sites are highlighted in a darker gray. Tested peptides are underlined and coloured as shown underneath the activation curves.

Activation curves: Activation of receptors GPR21 and GPR29 with different HIRamide peptides from the same precursors. Sequences and EC_{50} values for different HIRamides are shown at the bottom. The graph in the main figure of the manuscript shows HIRamide3 as the peptide with the lowest EC_{50} values. GPR21 showed already at low concentrations a high base activation compared to the negative control, but did not show a clear increase in intensity at concentrations between 1e-13 and 1e-11. We therefore set the minimum to the value meassured at a concentration of 1e-13 instead of actual negativ control and show a comparison of the corresponding graphs and EC_{50} values here. The curve might look different and EC_{50} values might be even lower if more data points at lower concentrations were tested.

PRGamide

MAYFKWTLCAFVLAVLCLSTNYVAGEKNEEKSEKSTLLQELSKKDFDKELAADEAIVLELLKEKADDLDEESLLDLL**APRGGRDAPRG**GRSLLD**APRGGRSLLDLLAAPRG**GRDAPRGGRSLTELLNA
PRRGRSLADLLDAPRGGRSLAELLDAPRGGRSIEAPRGGRSLAELLEAPRGGRSLIELLEAPRGGRSVEAPRGGRSILELLTAPRGGRSAPRGGRSAEKSDAIHKEKKAPRGGRKRRSLPEEEMD**GPR
GGR**SAVSGRSSED**GPRG**GRAVY**GPRG**GRSFDGSRGGRSYD**GPRG**GRSMEN**GPRGGRATEFGPRG**GRSYEGSRGGREID**GPRG**GRSFKE**GPRG**GRAVE**GPRG**GRDLYEE**GPRG**GREVYLE**GPRG**GRDLV
LE**GPRG**GRDLYEE**GPRGGREVNLEGPRG**GRDLYEE**GPRGGRSYDGPRG**GRSAVY**GPRG**GRDAND**GPRG**GRDAND**GPRG**GRDAND**GPRG**GRDANE**GPRG**GRDANE**GPRG**GRDANK**GPRG**GRNTNTVGAQ
QADARQGQRGGRSTGMG**PRG**GRSVSSEKDNRH

	GPR28	GPR32
PRGa (GPRG-amide)	EC$_{50}$ = 1.01e-08	EC$_{50}$ = 3.41e-08
PRGa1 (APRGGRSLLDLLAAPRG-amide)	EC$_{50}$ = 4.69e-06	EC$_{50}$ = 7.99e-05
PRGa2 (GPRGGREVNLEGPRG-amide)	EC$_{50}$ = 5.34e-06	EC$_{50}$ = 9.51e-05
PRGa3 (GPRGGRATEFGPRG-amide)	EC$_{50}$ = 6.29e-07	EC$_{50}$ = 2.49e-06
PRGa4 (GPRGGRSYDGPRG-amide)	EC$_{50}$ = 1.43e-06	EC$_{50}$ = 2.15e-05
PRGa5 (APRGGRDAPRG-amide)	EC$_{50}$ = 6.69e-06	EC$_{50}$ = 1.34e-04

Precursor sequence: N-terminal signal peptide is not highlighted, cleavage + amidation sites are highlighted in a darker gray. Tested peptides are coloured as shown underneath the activation curves. The GPRGamide sequence is underlined as part of several of the longer peptides that were previously detected by mass spectrometry. These longer versions likely represent peptides that are not fully processed.

Activation curves: Activation of receptors GPR28 and GPR232 with different PRGamide peptide version from the same precursors. Sequences and EC$_{50}$ values for different PRGamides are shown at the bottom. The graph in the main figure of the manuscript shows the short PRGamide, which is the peptide with the lowest EC$_{50}$ values.