The molecular control of calcitonin receptor (CTR) signaling

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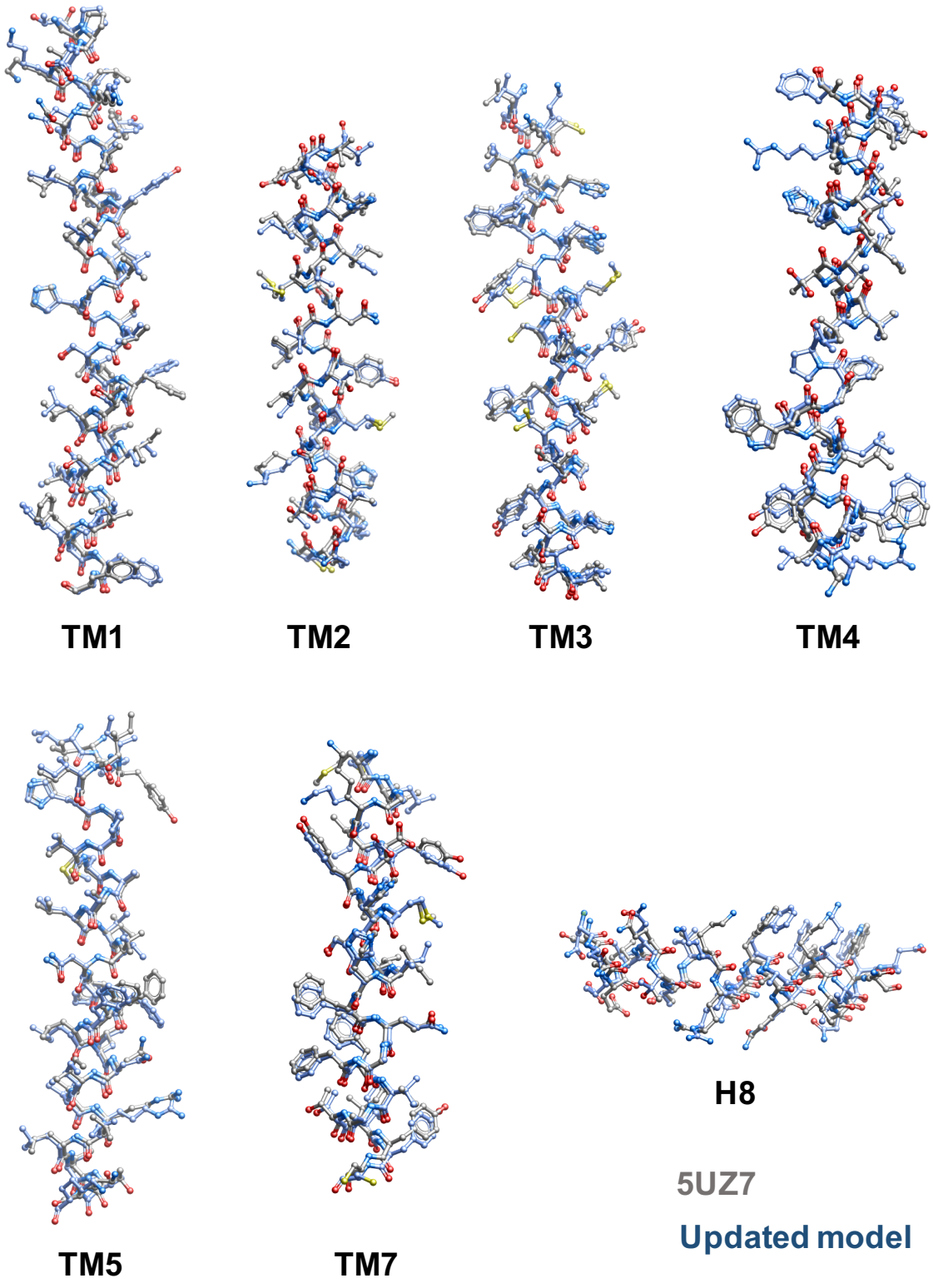
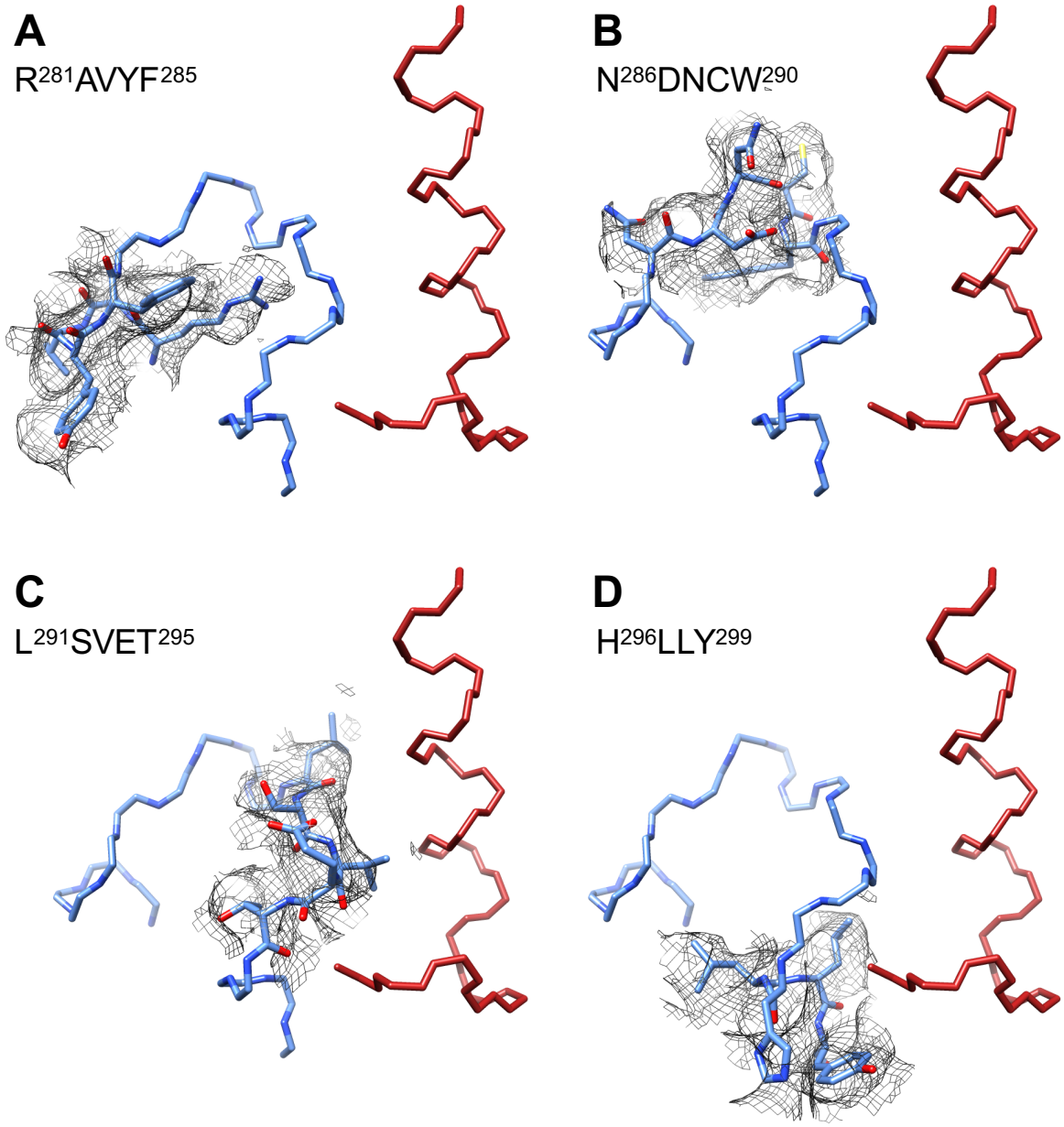
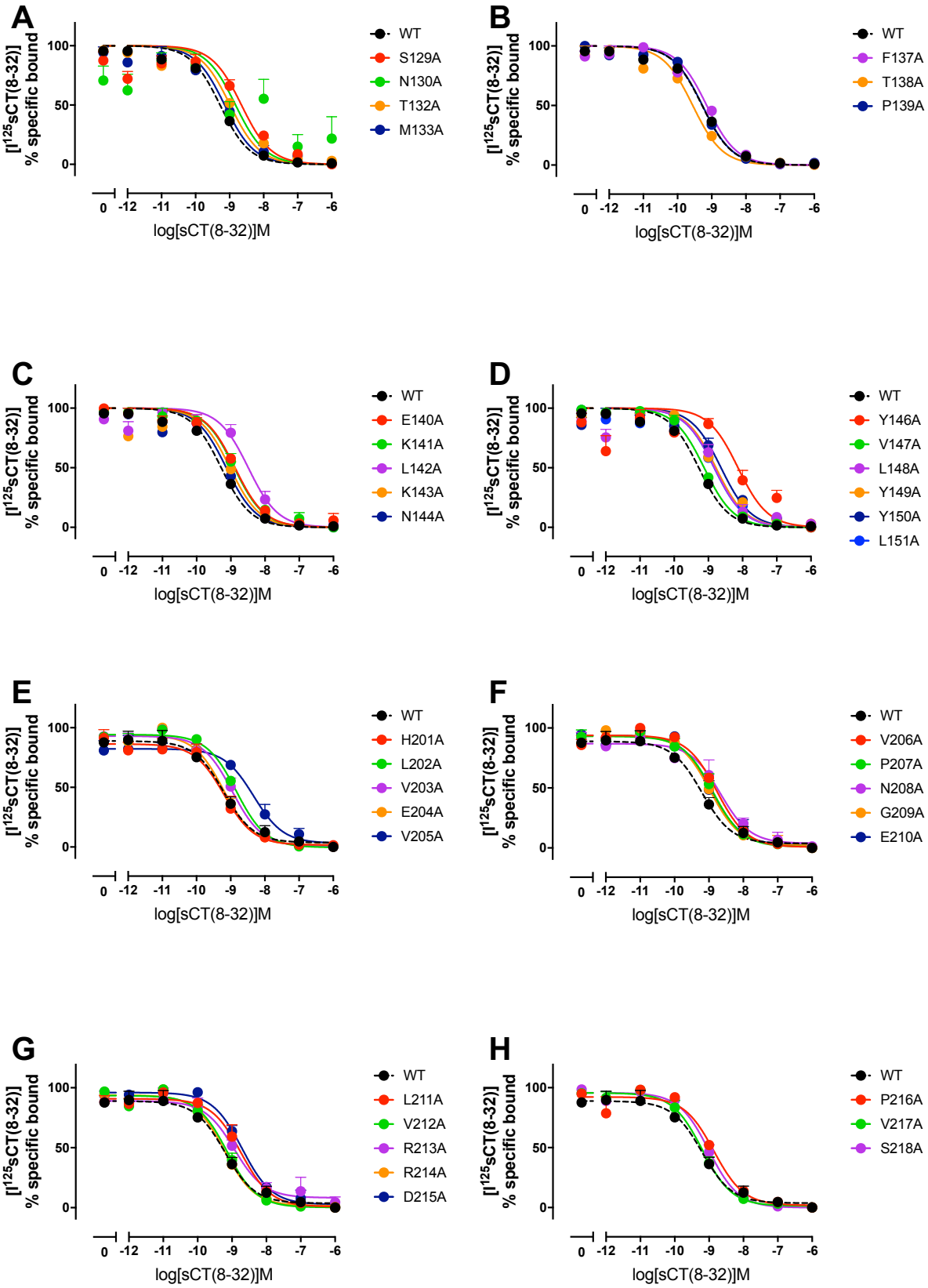
Supplementary Data.



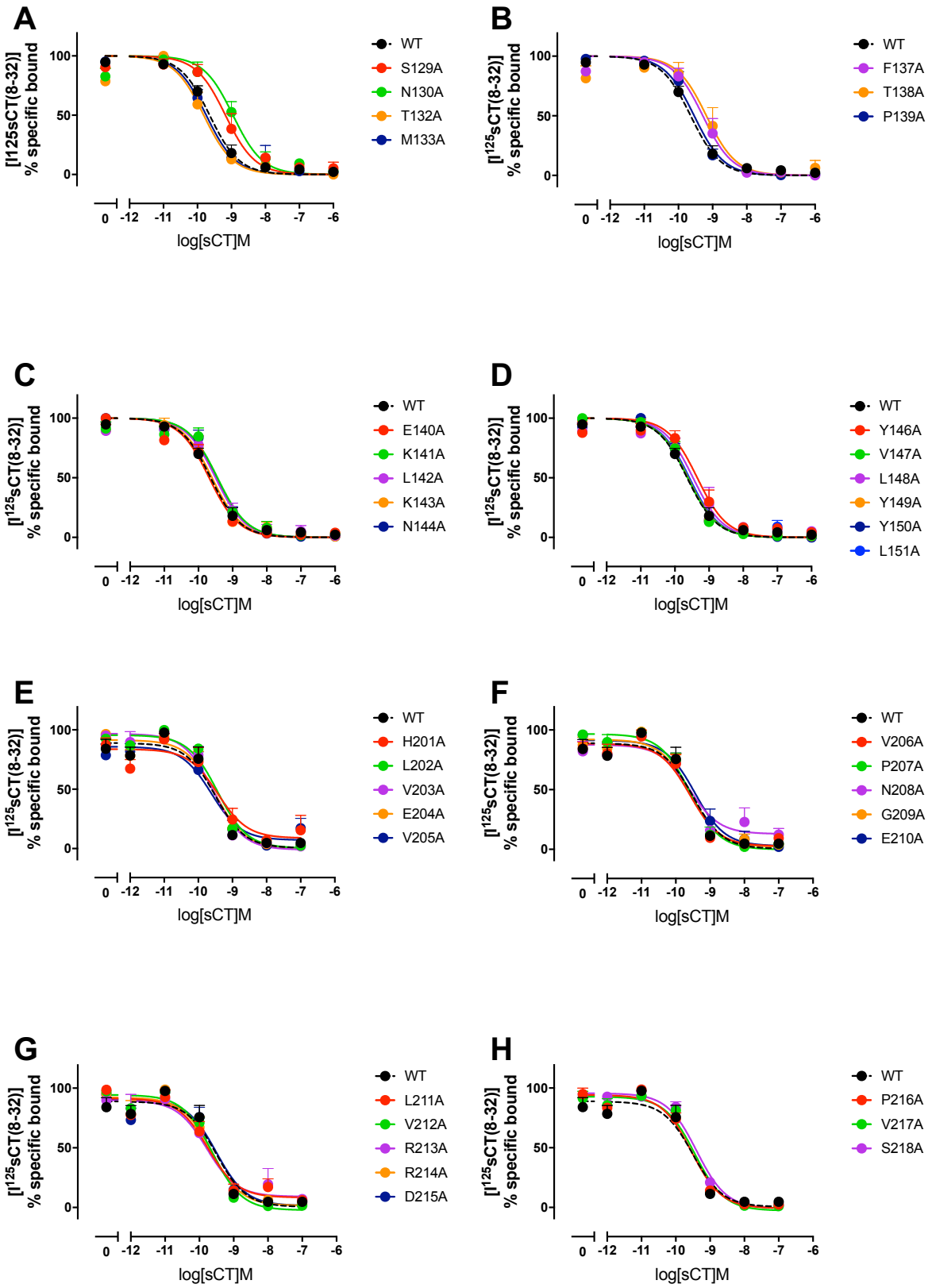
Supplementary Figure 1. Alignment of transmembrane helices (TM1-TM5 and TM7) and helix 8 of models of the sCT:CTR:Gs:Nb35 complex derived from the original 4.1 A EM map (PDB: 5UZ7, grey) and the current 3.3 A EM map (blue). Helices are depicted as x-stick, coloured by atom type. There was an excellent correlation between models for these segments of the receptor structure, consistent with the relative quality of the original EM map for the TM domain of the CTR.



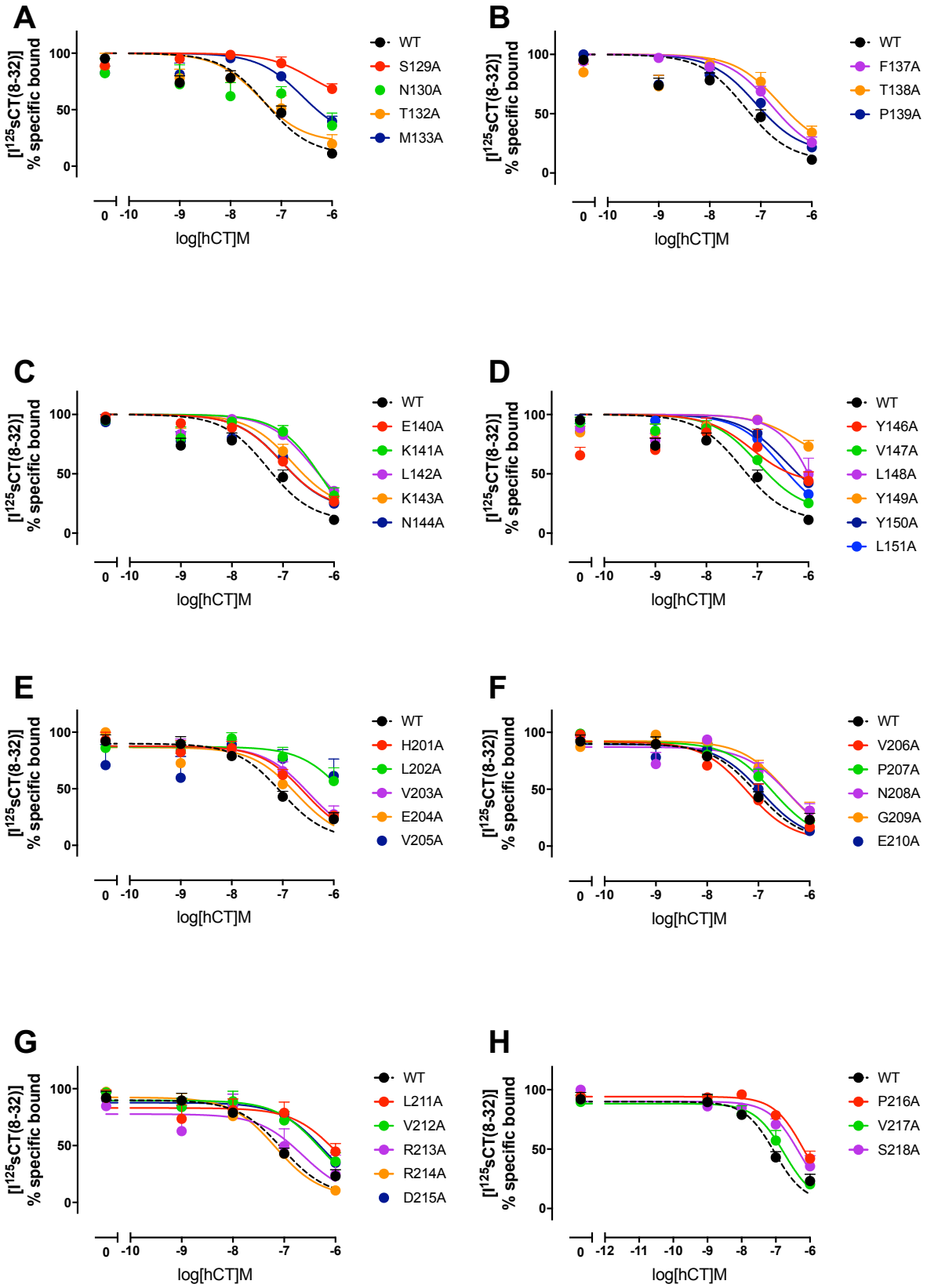
Supplementary Figure 2. Atomic-resolution model of the CTR ECL2 in the cryo-EM density map of the active complex. Cryo-EM density map and side-chain modelling are shown for in step wise increments along ECL2. **A,** R281-F285. **B,** N286-W290. **C,** L291-T295. **D,** H296-Y299. The EM map was zoned at 2.5 Å around the protein segments. The ECL2 backbone is shown in blue, and the sCT peptide backbone in dark red for each panel to assist reader orientation.



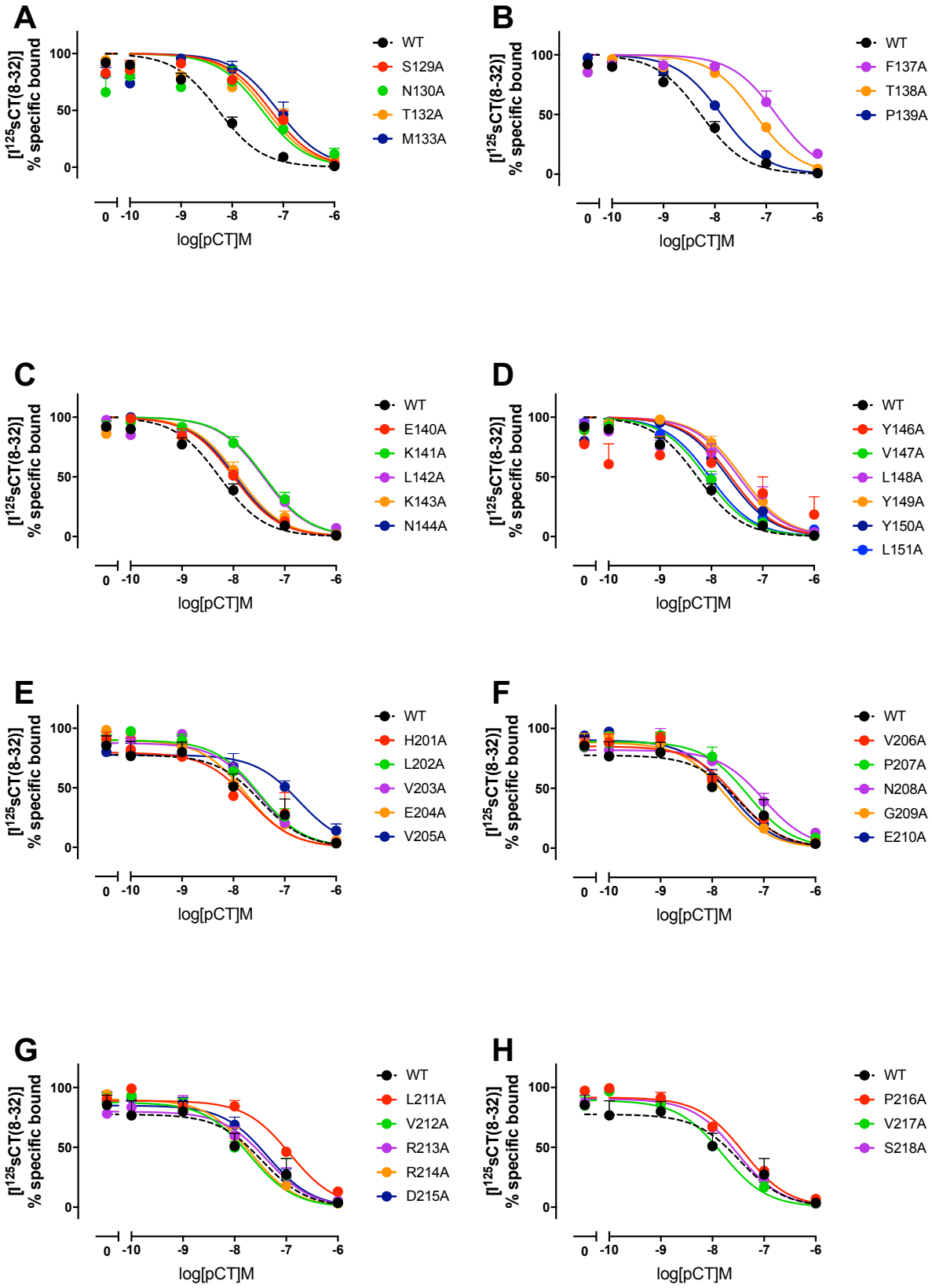
Supplementary Figure 3. Competition binding isotherms for sCT(8-32) in competition for 125I-sCT(8-32) at the WT and mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the total binding for each receptor mutant (or wild-type) (100%) and non-specific binding, defined by 1 μM sCT(8-32), has been subtracted (giving % specific binding). Data have been fit with a 3-parameter logistic equation. Data are mean + SEM from 4-9 independent experiments (specific "n" numbers are shown in Table 1).



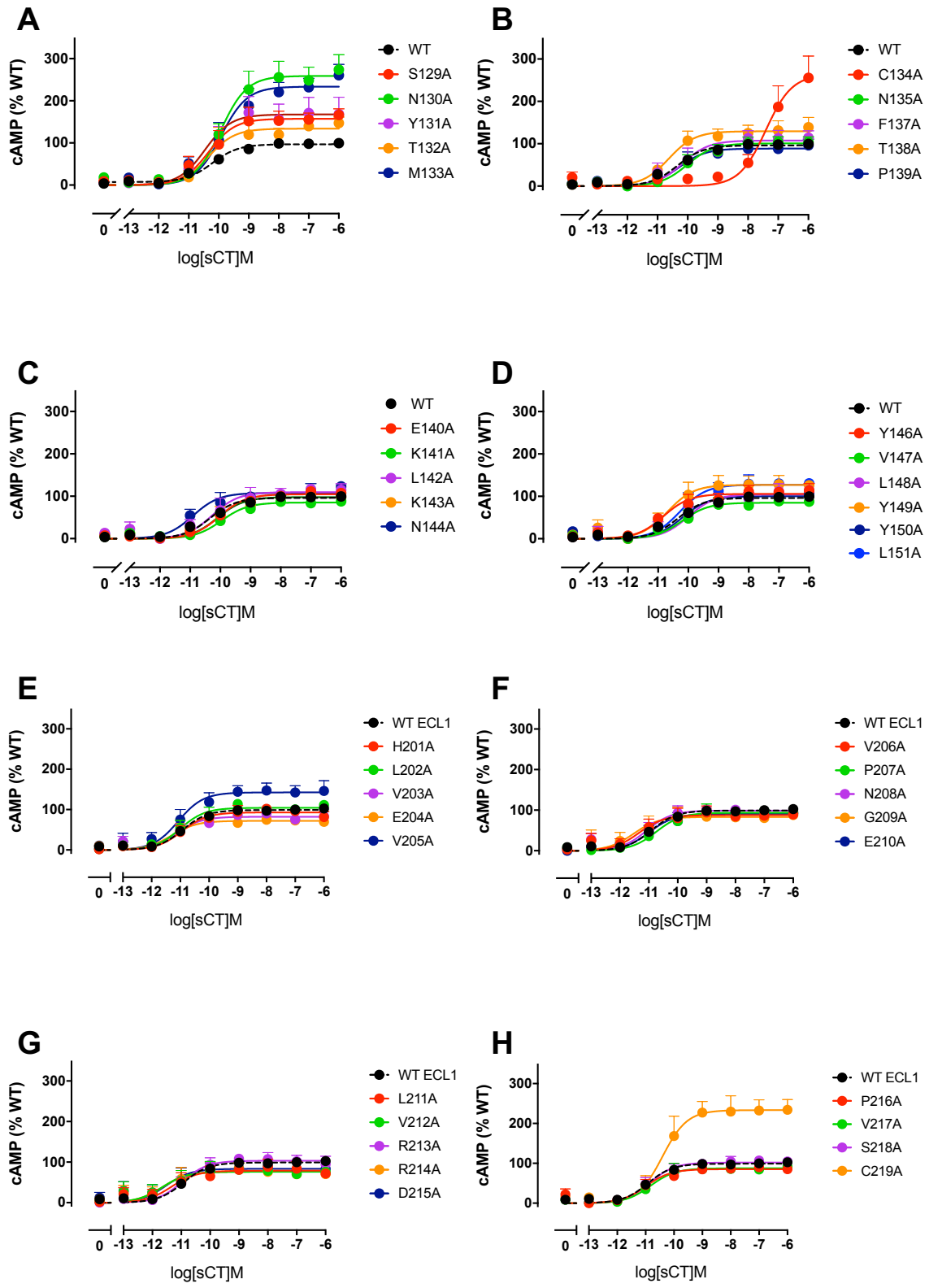
Supplementary Figure 4. Competition binding isotherms for sCT in competition for 125I-sCT(8-32) at the WT and mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the total binding for each receptor mutant (or wild-type) (100%) and non-specific binding, defined by 1 μM sCT(8-32), has been subtracted (giving % specific binding). Data have been fit with a 3-parameter logistic equation. Data are mean + SEM from 4-7 independent experiments (specific "n" numbers are shown in Table 1).



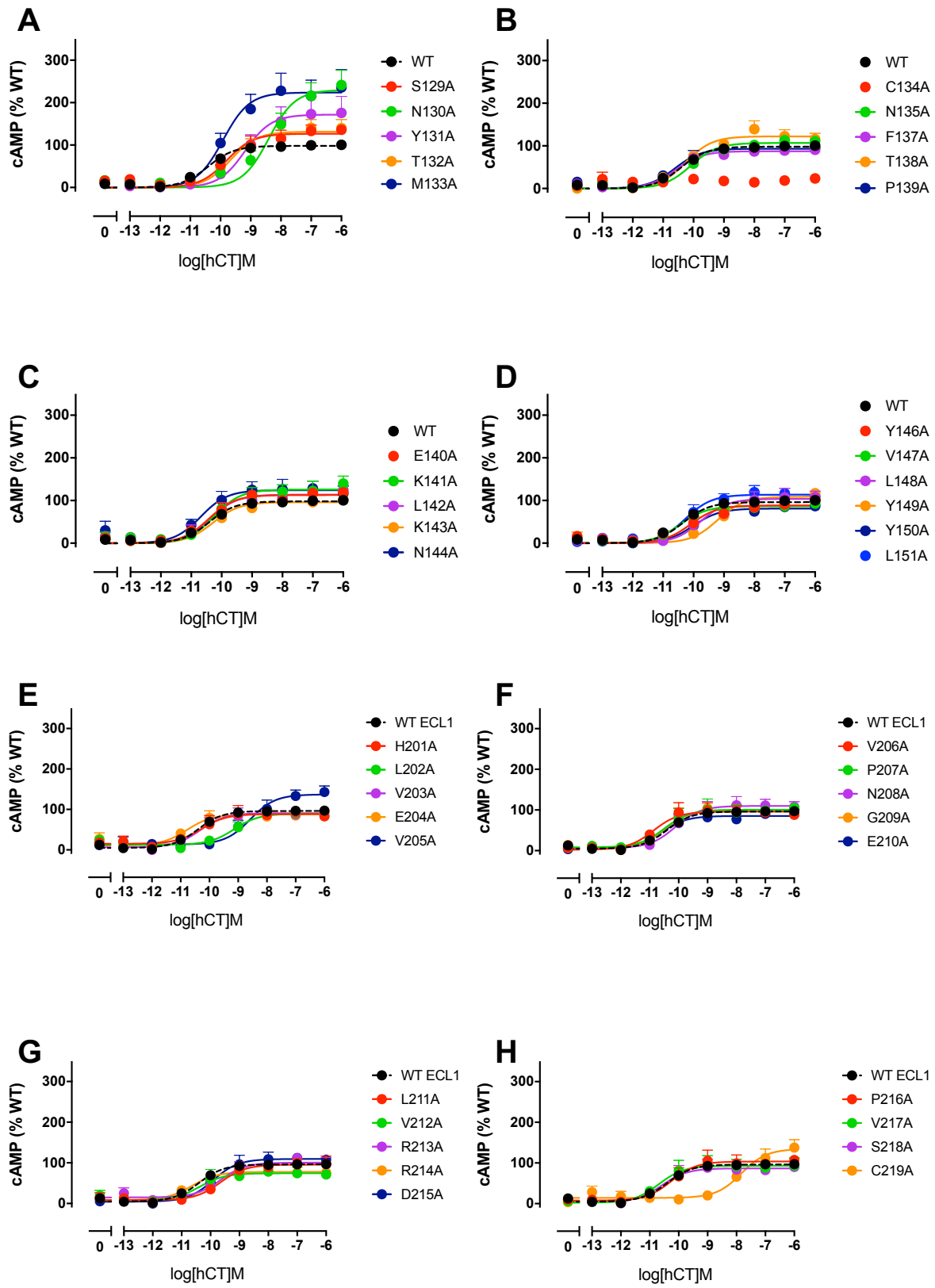
Supplementary Figure 5. Competition binding isotherms for hCT in competition for 125I-sCT(8-32) at the WT and mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the total binding for each receptor mutant (or wild-type) (100%) and non-specific binding, defined by 1 μM sCT(8-32), has been subtracted (giving % specific binding). Data have been fit with a 3-parameter logistic equation. Data are mean + SEM from 3-7 independent experiments (specific "n" numbers are shown in Table 1).



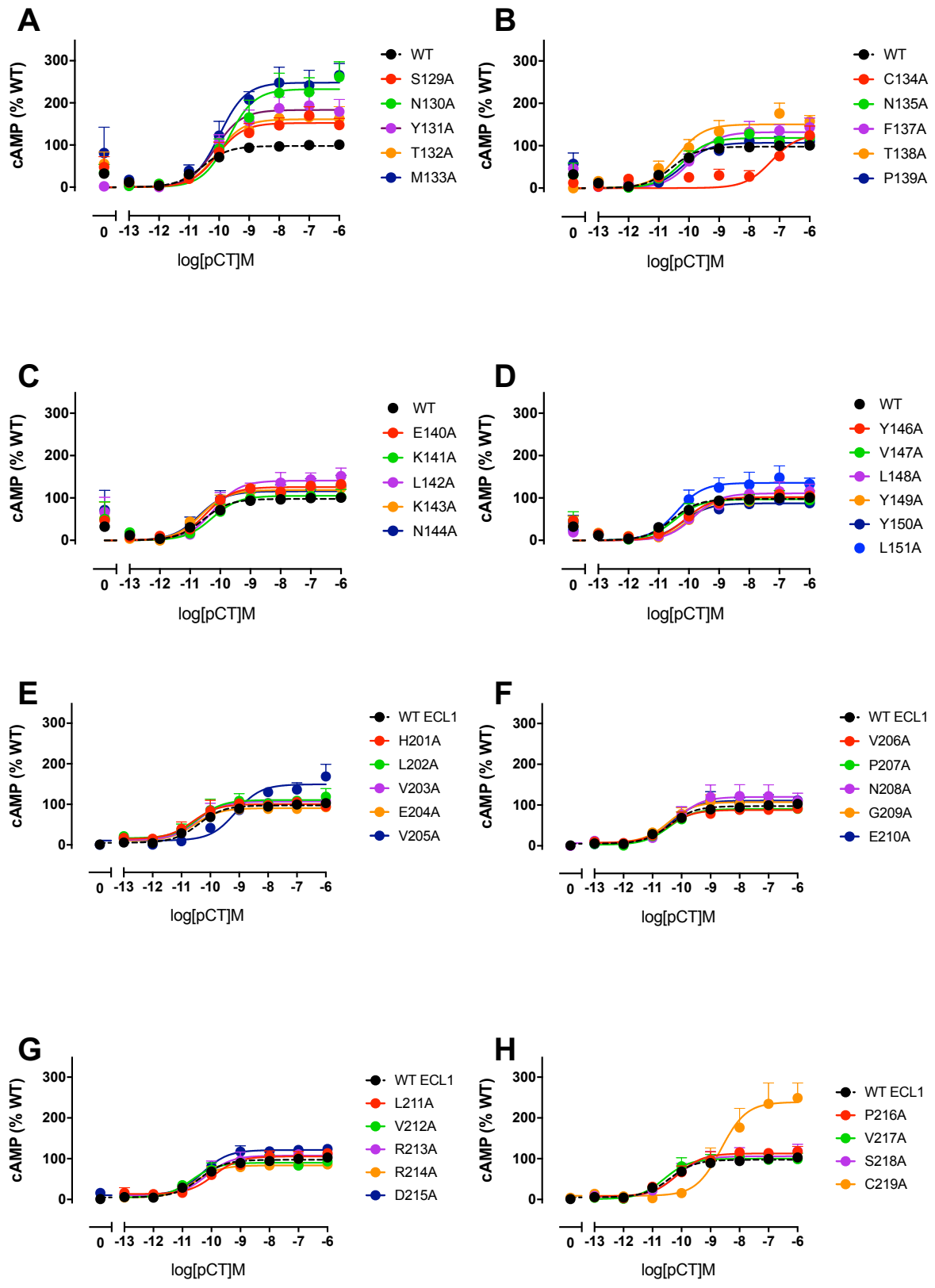
Supplementary Figure 6. Competition binding isotherms for pCT in competition for 125I-sCT(8-32) at the WT and mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the total binding for each receptor mutant (or wild-type) (100%) and non-specific binding, defined by 1 μM sCT(8-32), has been subtracted (giving % specific binding). Data have been fit with a 3-parameter logistic equation. Data are mean + SEM from 3-8 independent experiments (specific "n" numbers are shown in Table 1).



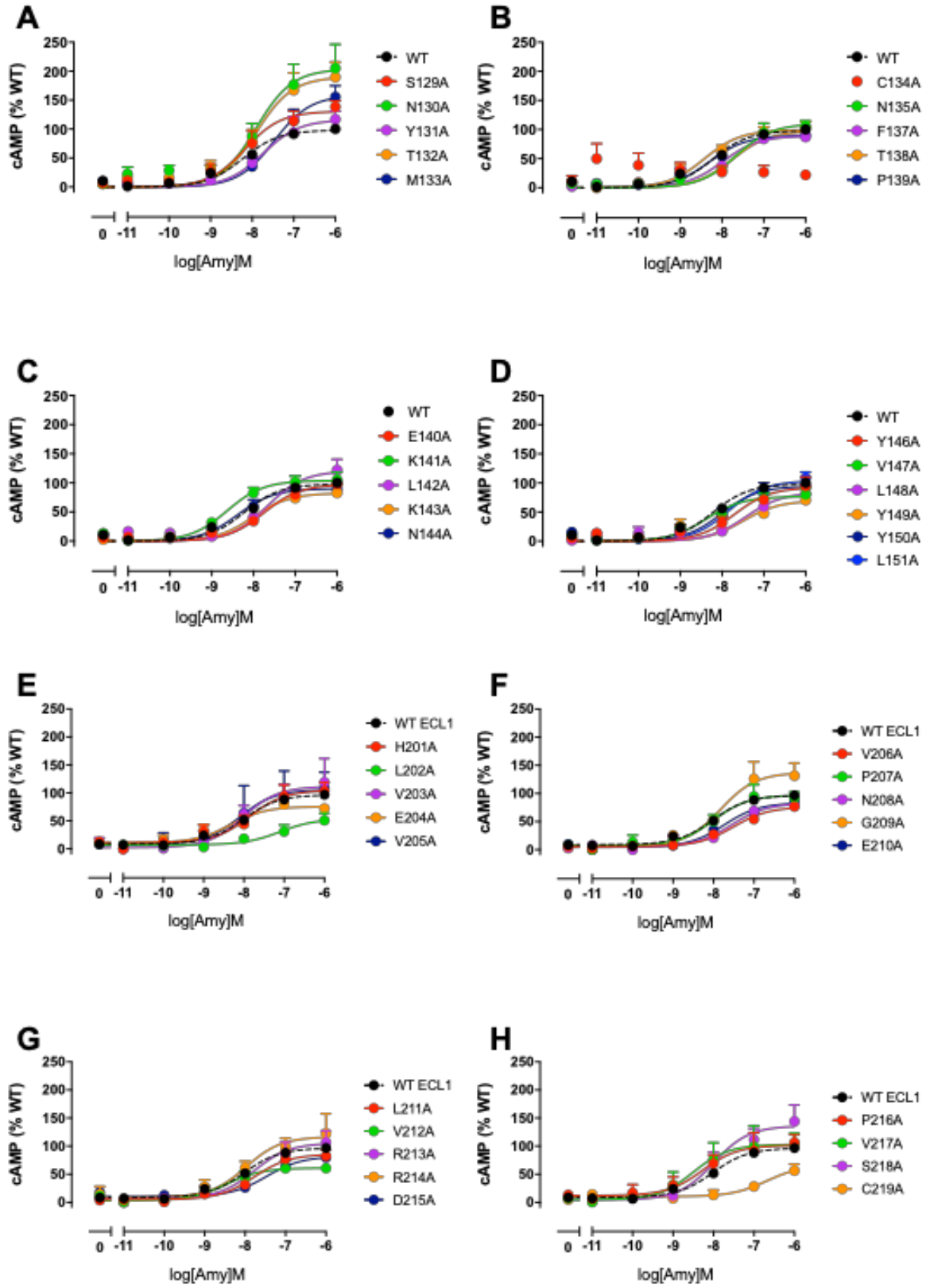
Supplementary Figure 7. Concentration-response curves for sCT in cAMP accumulation assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 4-39 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 2).



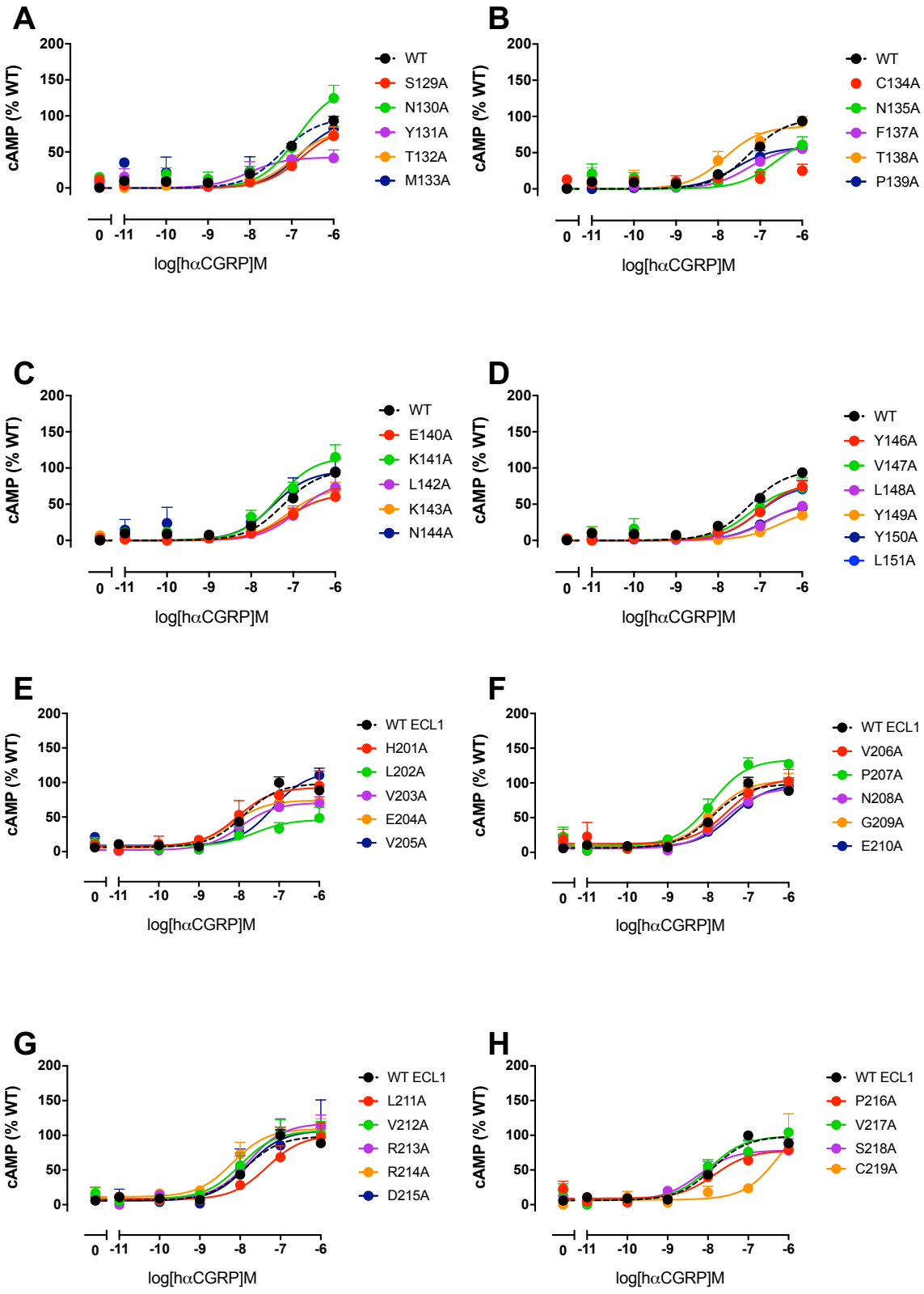
Supplementary Figure 8. Concentration-response curves for hCT in cAMP accumulation assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 4-38 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 2).



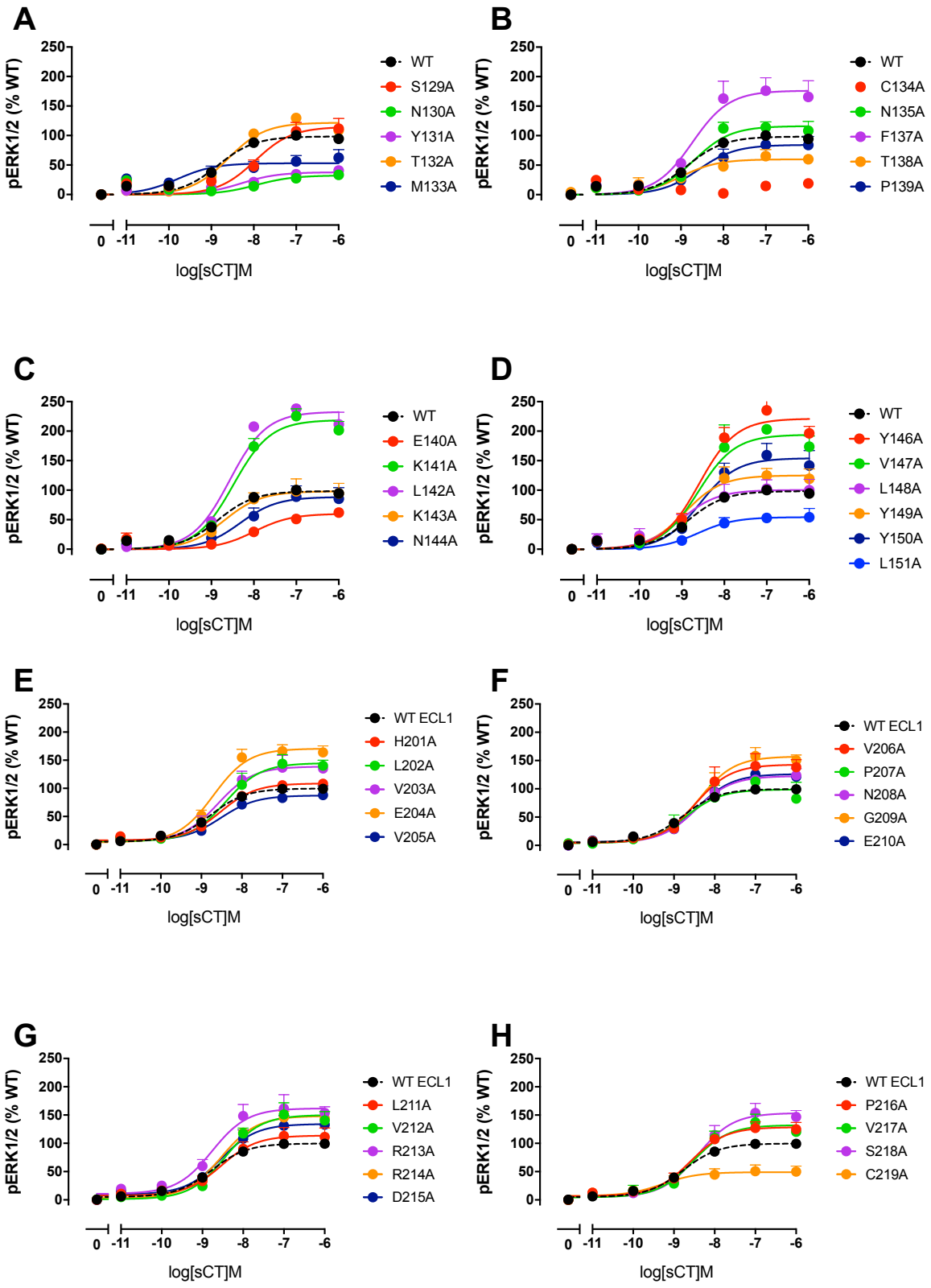
Supplementary Figure 9. Concentration-response curves for pCT in cAMP accumulation assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 3-39 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 2).



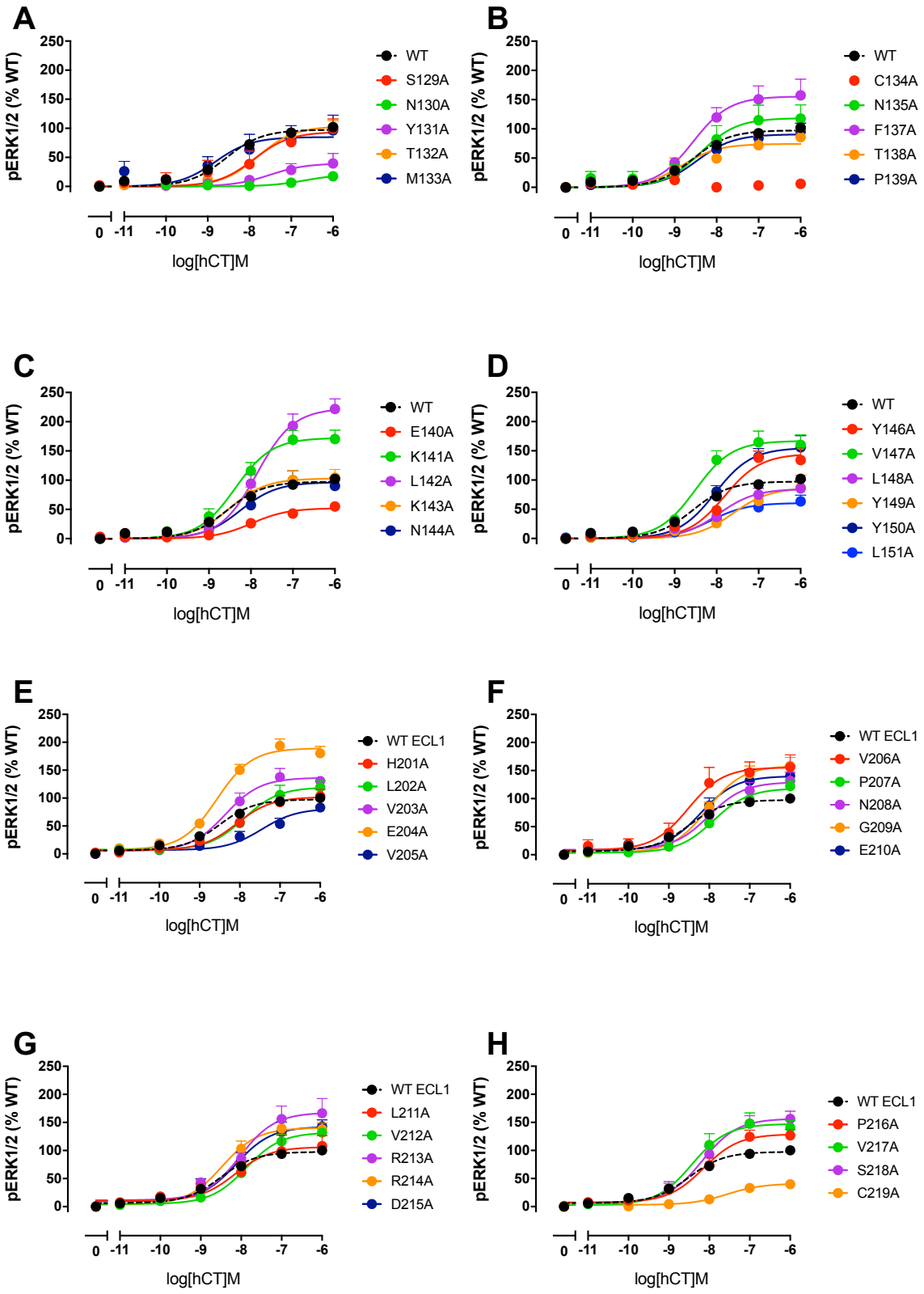
Supplementary Figure 10. Concentration-response curves for rat amylin in cAMP accumulation assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 4-23 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 2).



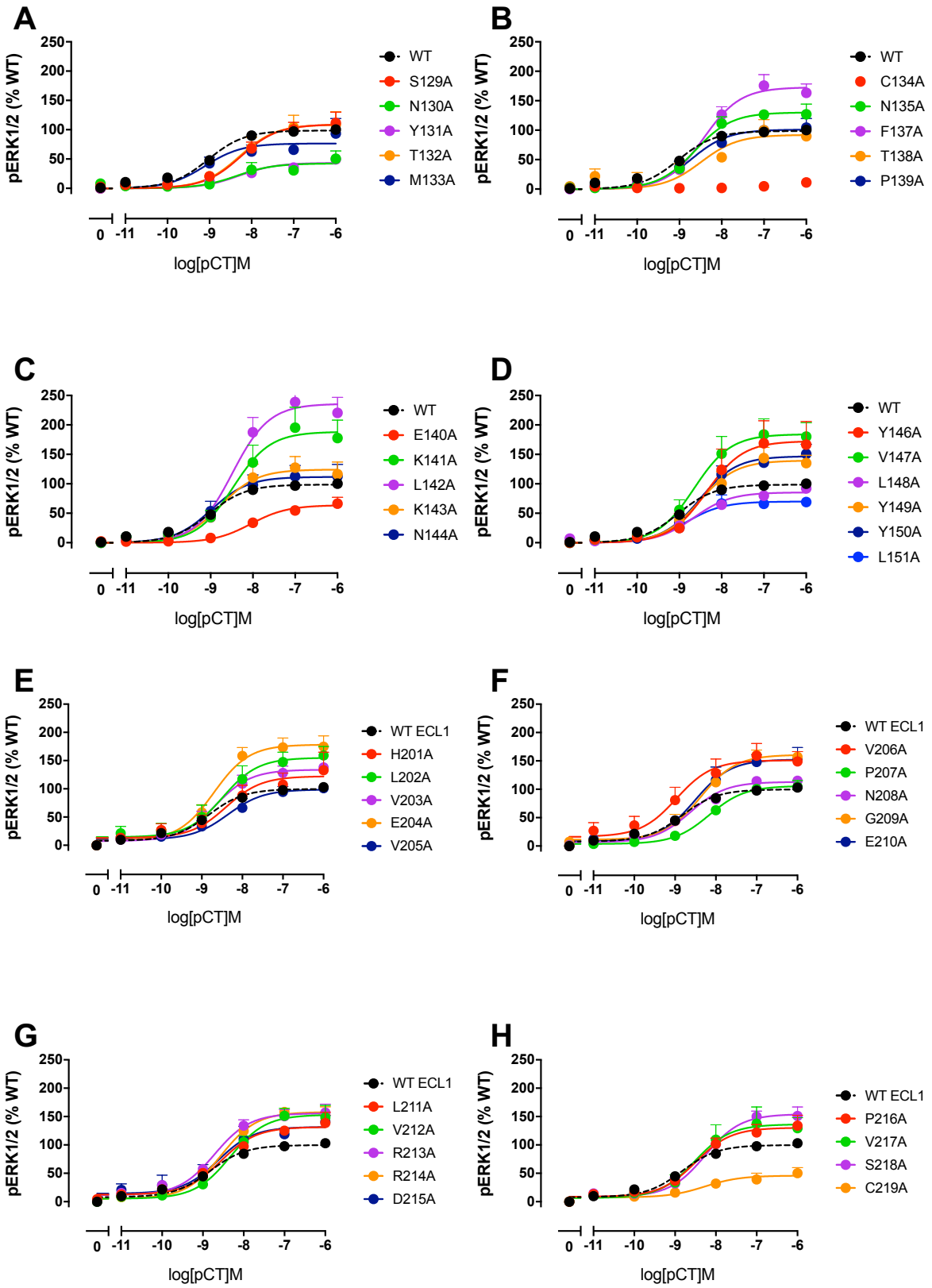
Supplementary Figure 11. Concentration-response curves for CGRP in cAMP accumulation assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 3-16 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 2).



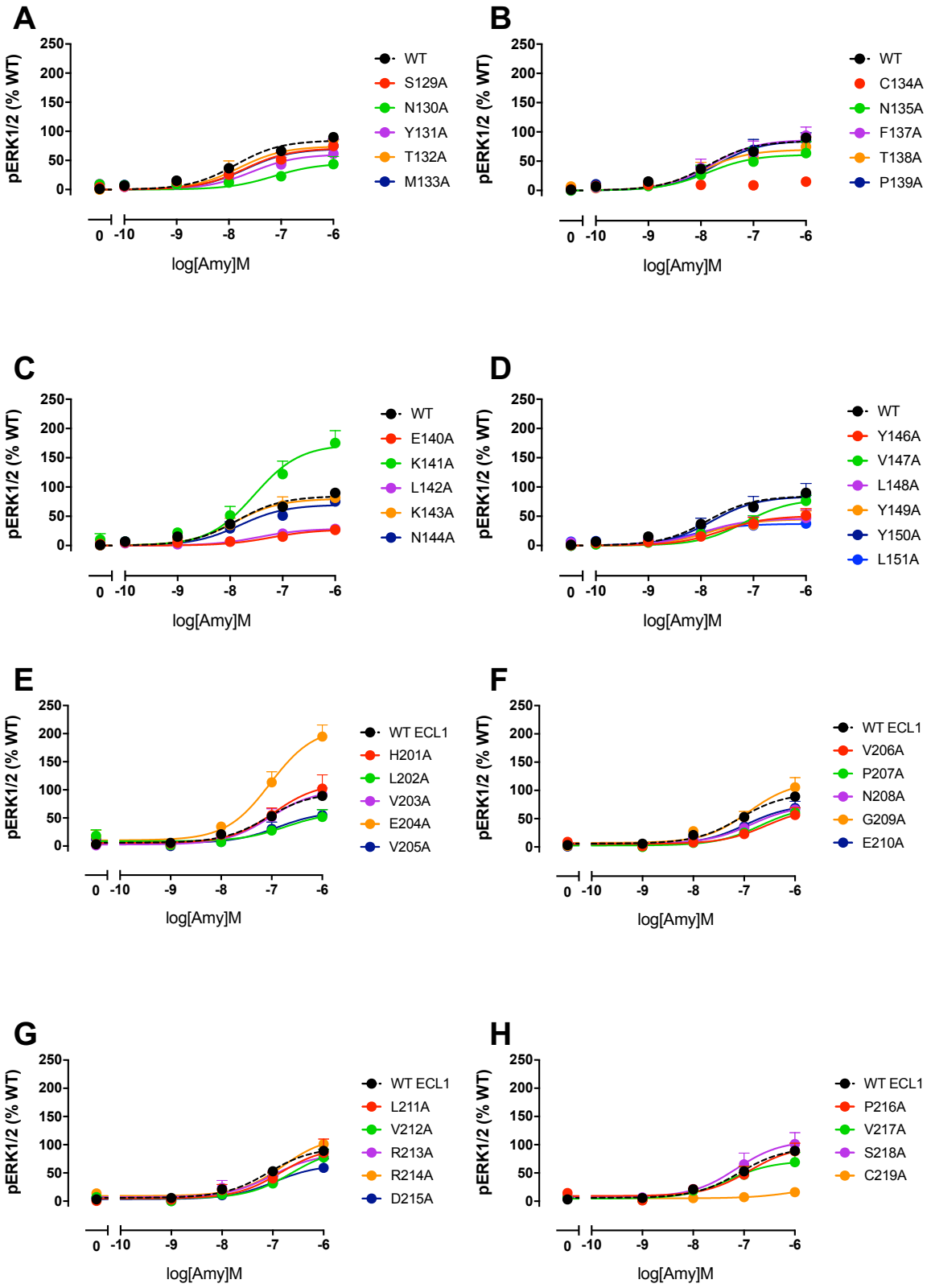
Supplementary Figure 12. Concentration-response curves for sCT in pERK assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 3-23 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 3).



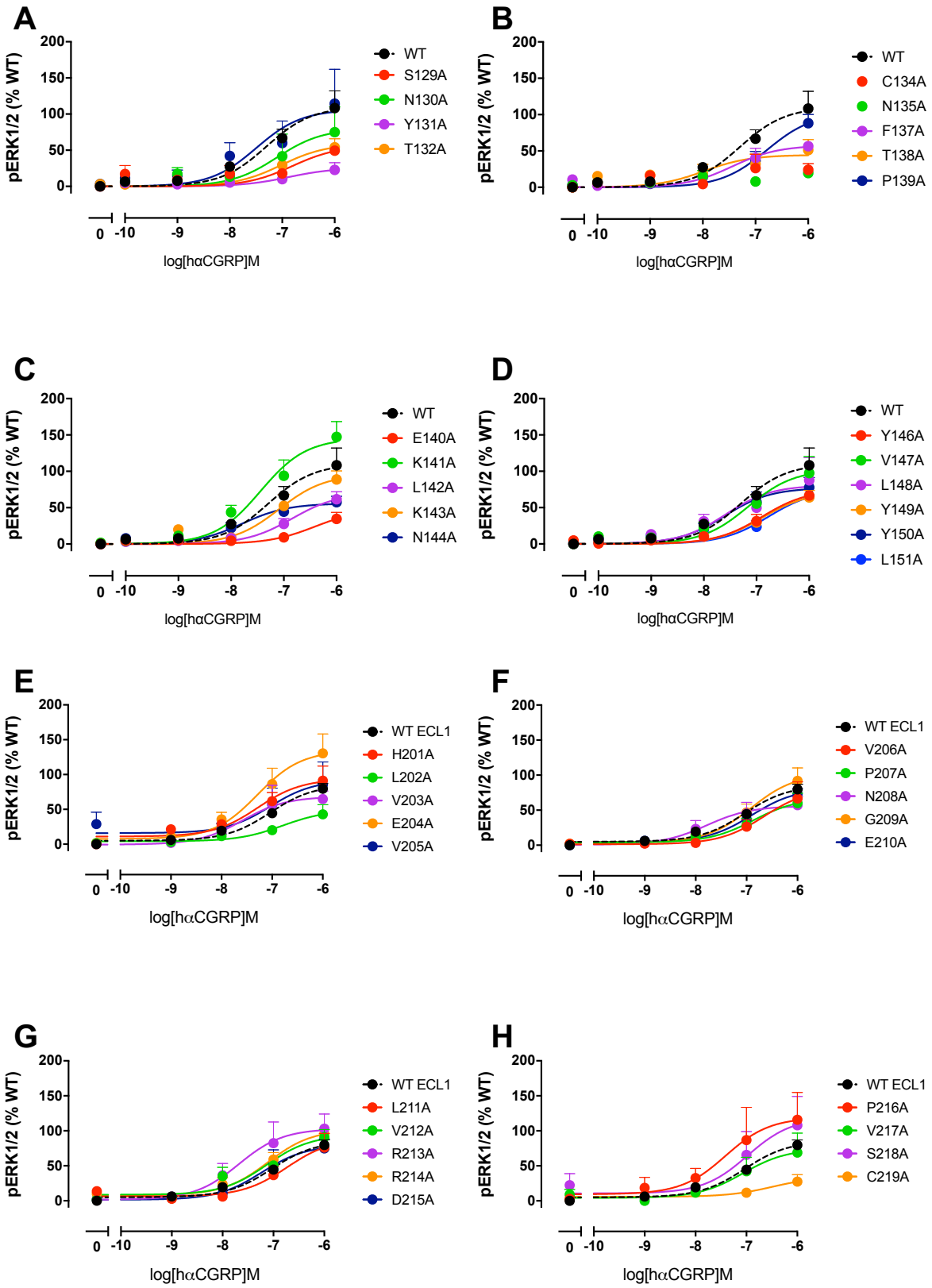
Supplementary Figure 13. Concentration-response curves for hCT in pERK assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 3-23 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 3).



Supplementary Figure 14. Concentration-response curves for pCT in pERK assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 3-21 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 3).



Supplementary Figure 15. Concentration-response curves for rat amylin in pERK assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 3-25 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 3).



Supplementary Figure 16. Concentration-response curves for CGRP in pERK assays in cells expressing the wild-type or mutant CTR. **A-D.** Mutations of the TM1 stalk. **E-H.** Mutations of ECL1. Data have been normalized to the maximal response of the wild-type receptor (100%). Data have been fit with a 3-parameter logistic equation. The response at the wild-type receptor is shown as a dashed line. Data are mean + SEM from 4-25 independent experiments (specific "n" numbers for functional cAMP experiments are shown in Table 3).