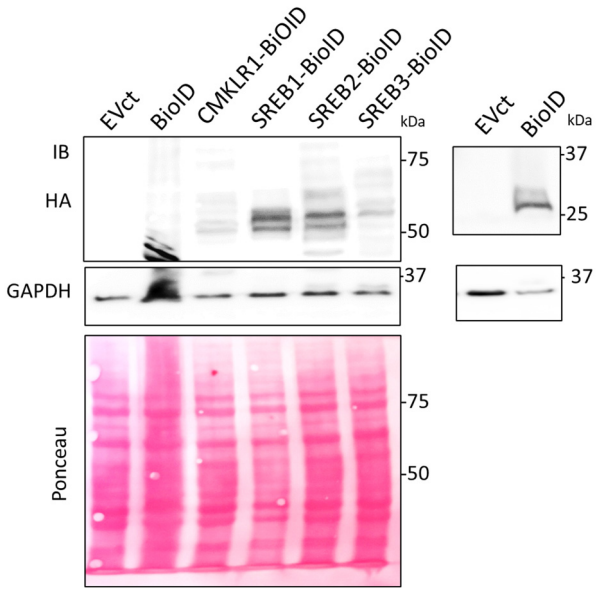
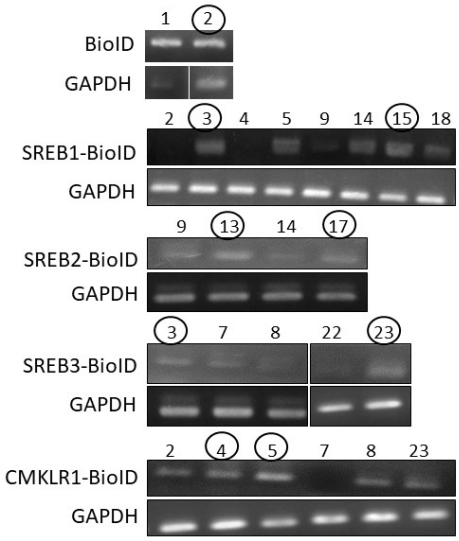
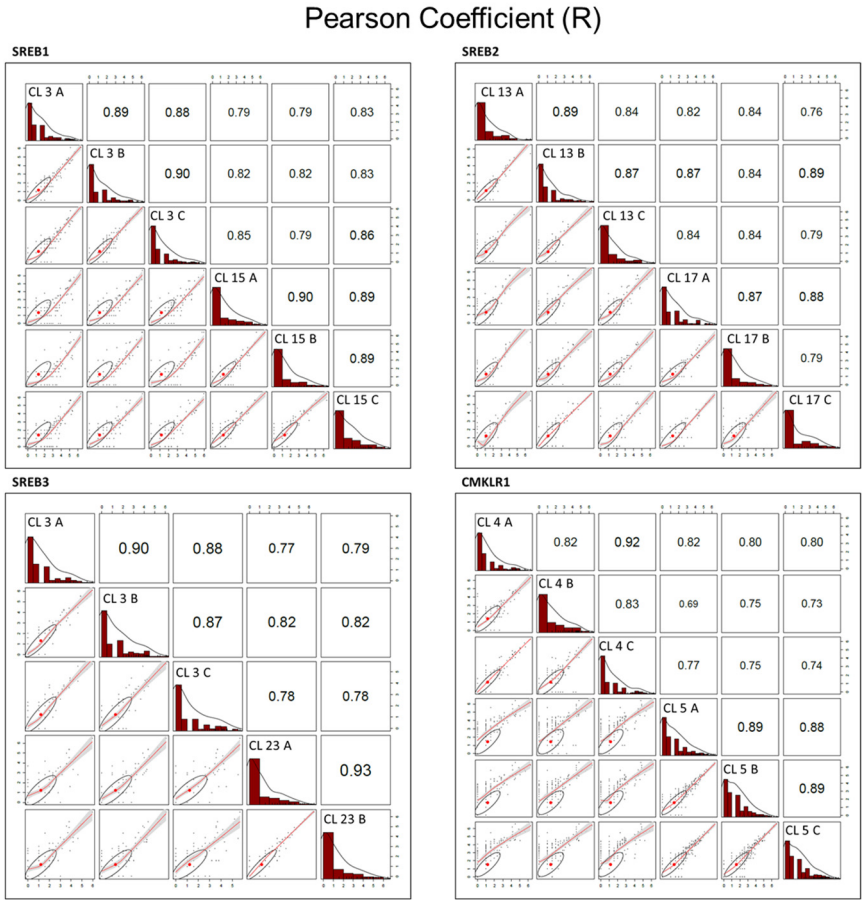
**Supplementary Materials:**



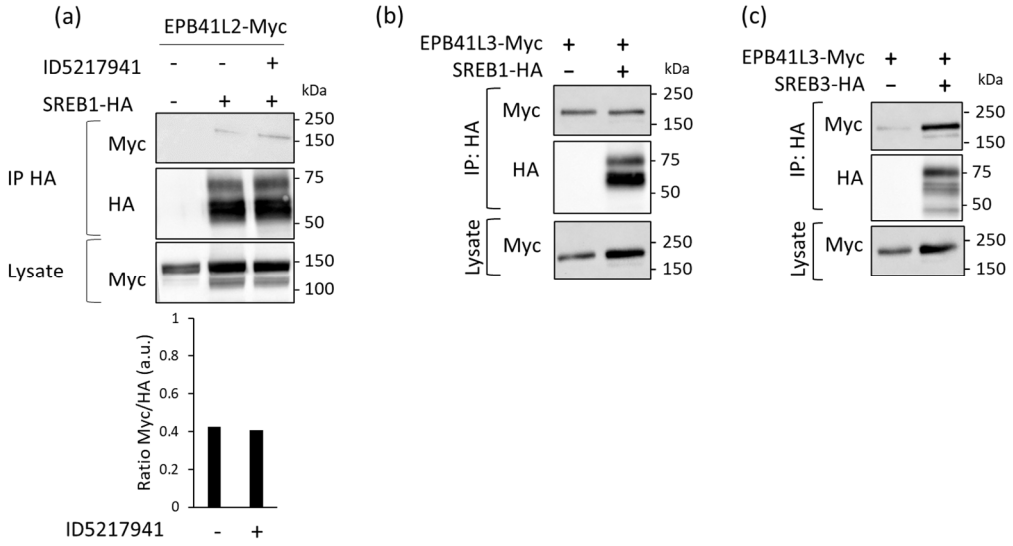

**Supplementary Figure S1**: BioID-SREBs are expressed in HEK293 cells. Total cell lysates of transfected HEK293T cells were analyzed by Western blotting (following 8% polyacrylamide gels or 12% gels for BioID-alone) and the fusion proteins were detected using anti-HA antibodies (or anti-GAPDH as reference). The protein load was monitored by Ponceau staining. Western blots are representative of three independent experiments.




**Supplementary Figure S2** : Selection of HEK293 clones stably expressing BioID fusion proteins based on RT-PCR. Clones resulting from G418 selection were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) for expression of the receptor-BioID fusions. Representative agarose gels show expression of BioID, SREB1-BioID, SREB2-BioID, SREB3-BioID, and CMKRL1-BioID in different HEK293 clones. The reference gene used was glyceraldehyde phosphate dehydrogenase (GAPDH). Black circles indicate the clones selected for further experiments.

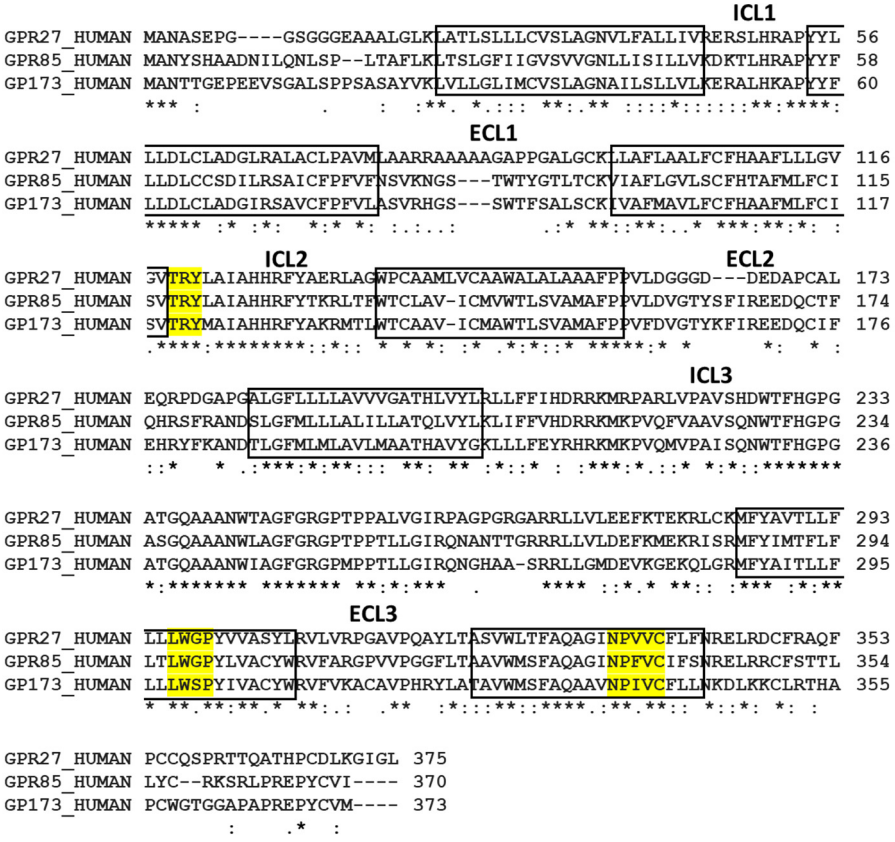



**Supplementary Figure S3** : Pair panel matrices of spectral counts pairwise combinations of all replicates in BioID assay. Scatter plots of the number of spectral counts of all combinations of the two biological clones (CL) and three technical replicates (A, B, and C) of each GPCR and its relative Pearson coefficient (R)




**Supplementary Figure S4** : Physical interactions of SREB1 and SREB3 with EPB41L proteins. (a) The interaction of SREB1 and EPB41L2 was not affected by the presence of the ID5217941 agonist. SREB1-BioID2-HA and Myc-tagged EPB41L2 were co-transfected in HEK293T cells and treated with 15 μM of ID5217941. Expression of EPB41L2 was monitored by immunoblotting of the total cell

lysate. Anti-HA immunoprecipitates were analyzed by Western blotting with the indicated antibodies. The EPB41L2-Myc/SREB1-BioID2-HA ratio (quantification using Image J) showed no change in the interaction between SREB1 and EPB41L2 as a result of ID5217941 treatment. HEK293T cells were transfected with (b) SREB1-BioID2-HA or (c) SREB3 -BioID2-HA, together with the proximal interactor EPB41L3 identified in the BioID assay. Expression of the myc-tagged EPB41L3 was confirmed by immunoblotting (Lysate). Anti-HA immunoprecipitates analyzed by Western blotting (b) did not show any specific pull-down of EPB41L3 with SREB1 however (c) EPB41L3 was precipitated with SREB3.




**Supplementary Figure S5:** Alignment of amino acid sequences of human SREB members. Putative transmembrane domains are boxed. Relatively conserved GPCR motifs are highlighted in yellow (TRY motif at the junction of TM III and ICL2, NPxxC motif in TM VII, LWxP motif in TM VI). A particularly high amino acid conservation is observed in ICLs of human *GPR27* (SREB1), *GPR85* (SREB2), and *GPR173* (SREB3). Asterisks (*) indicate fully conserved residues, colons (:) strong functional conservation, and dots (.) weak conservation. TM: transmembrane domain, ECL: extracellular loop, ICL: intracellular loop.

**Supplementary Table S1**: Internal primer sequences used to validate the expression of the genes of interest and the housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH) (as a reference) in BioID constructs.

| Gene | Sequence | Amplicon | Position |
|---|---|---|---|
| SREB1-BioID Forward | TCCTCTTCAACAGGGAGCTG | 239 bp | 1015-1254 bp |
| SREB1-BioID Reverse | GTAGCTCACGTTCCACTCCT | | |
| SREB2-BioID Forward | CAGCACAACCCTTCTTTAC | 188 bp | 1050-1234 bp |
| SREB2-BioID Reverse | TCACGTTCCACTCCTTCAGTC | | |
| SREB3-BioID Forward | AATTGTCTGCTTCCTGCTCAAC | 233 bp | 1010-1234 bp |
| SREB3-BioID Reverse | TCACGTTCCACTCCTTCAGTC | | |
| CMKLR1-BioID Forward | ACTCTTCCTACCCCAGCCATAG | 213 bp | 1024-1237 bp |
| CMKLR1-BioID Reverse | TCACGTTCCACTCCTTCAGTC | | |
| BioID-only Forward | CGGGTCTGGAGGCGGGGGTAG | 318 bp | 12-329 bp |
| BioID-only Reverse | CACTTCAGGCTGAAGGGGATC | | |
| GAPDH Forward | GGATTTGGTCGTATTGGGCG | 240 bp | 28-267 bp |
| GAPDH Reverse | ATCGCCCCACTTGATTTTGG | | |