

SUPPLEMENTARY INFORMATION

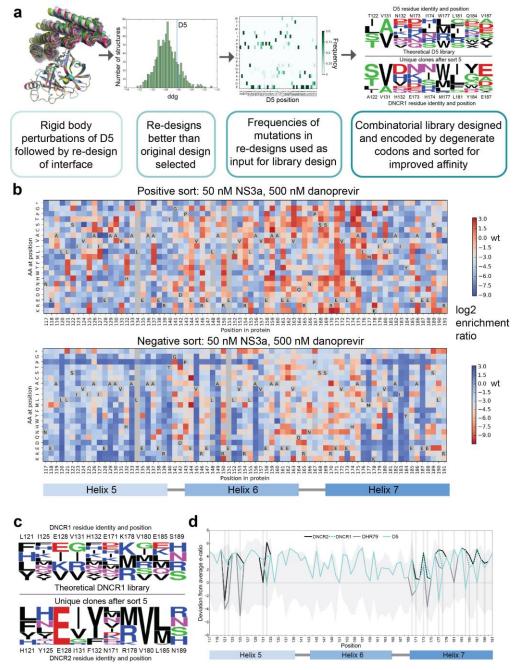
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Multi-input chemical control of protein dimerization for programming graded cellular responses

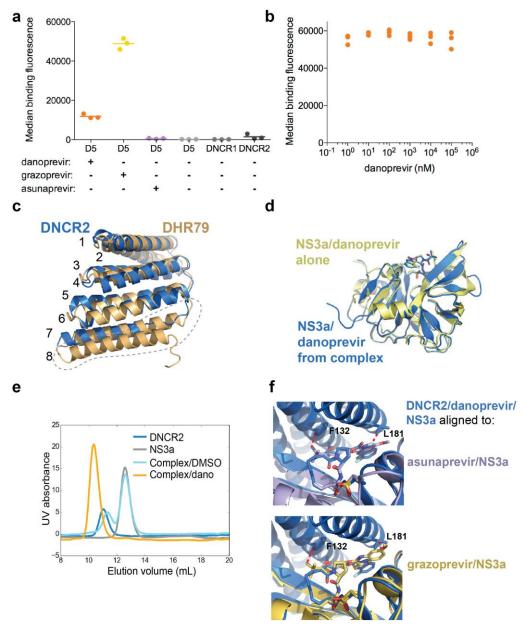
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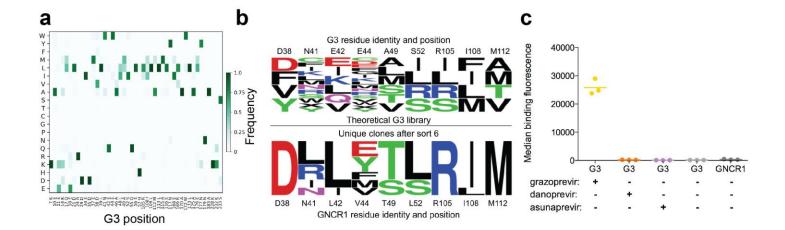
Design and characterization of danoprevir:NS3a complex reader libraries.

a, Process of Rosetta re-design-informed design of a combinatorial D5 interface library. **b**, Enrichment ratios of the DNCR1 site saturation mutagenesis (SSM) library sorted for (positive sort, top) or against (negative sort, bottom) binding to 50 nM NS3a in the presence of 500 nM danoprevir. The color scale has been flipped for the negative sort such that for both sorts, blue corresponds to predicted weaker binders, and red corresponds to predicted tighter binders. Gray boxes with letters are the wild-type residue and other gray boxes are positions with <15 counts in the naïve library sequencing results. **c**, Sequence logos of the theoretical library for the second combinatorial library varying the DNCR1 interface (top), and the mutations found in the final enriched clones (bottom). Residue identities at the varied positions are indicated for the starting DNCR1 and final DNCR2. **d**, Progression of binding improvement from DHR79 to D5 to DNCR1 to DNCR2 as measured by the deviation from average enrichment ratio of the DNCR1 SSM values at each position. Gray shaded region indicates the range of enrichment ratios of all amino acids at each position, and vertical gray bars indicate positions at the interface.



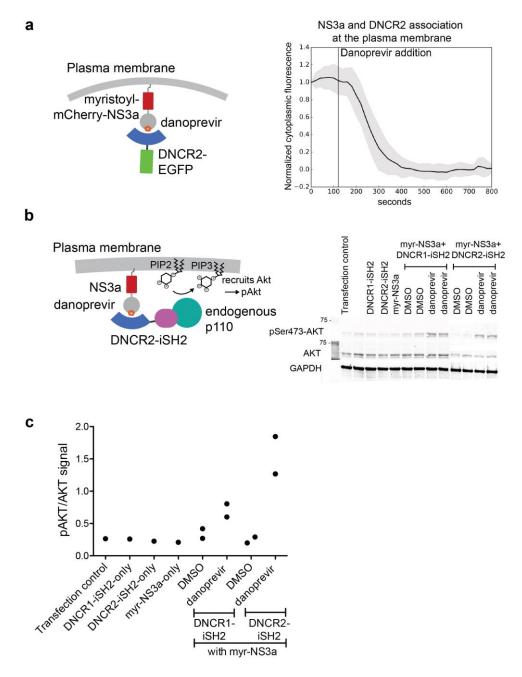
Analysis of the DNCR2:danoprevir:NS3a complex crystal structure and the specificities of drug/NS3a complex reader proteins.

a, 1 μM NS3a with avidity binding to yeast-displayed D5, DNCR1, or DNCR2. Technical triplicates and their mean are shown. **b**, Binding of 1 nM NS3a to DNCR2 displayed on the surface of yeast in the presence of increasing concentrations of danoprevir. Technical triplicates are shown. **c**, An overlay of DNCR2 (blue) from the DNCR2:danoprevir:NS3a complex with the original DHR79 scaffold (orange) crystal structure (PDBID: 5CWP) (Nature 528, 580-584, 2015). Regions where there are modest changes in the backbone conformation are circled with a dotted line, including missing density for helix 8 and an unraveled helix 7 N-terminus. **d**, NS3a:danoprevir (blue) from the DNCR2:danoprevir:NS3a complex aligns closely to a crystal structure of NS3a:danoprevir (yellow) alone (PDBID: 3M5L) (PNAS 107, 20986-20991, 2010). **e**, Size exclusion chromatograms of DNCR2, NS3a, or DNCR2:NS3a complexes in the presence or absence of danoprevir. Representative of three technical replicates. **f**, Crystal structure of DNCR2:danoprevir:NS3a (blue) aligned to structures of asunaprevir:NS3a (lavender, PDBID: 4WF8) or grazoprevir:NS3a (yellow, PDBID: 3SUD) with clashes (red) between residues of DNCR2 and asunaprevir and grazoprevir highlighted (PLoS Pathog 8, e1002832, 2012; ACS Chem Biol 9, 2485-2490, 2014).



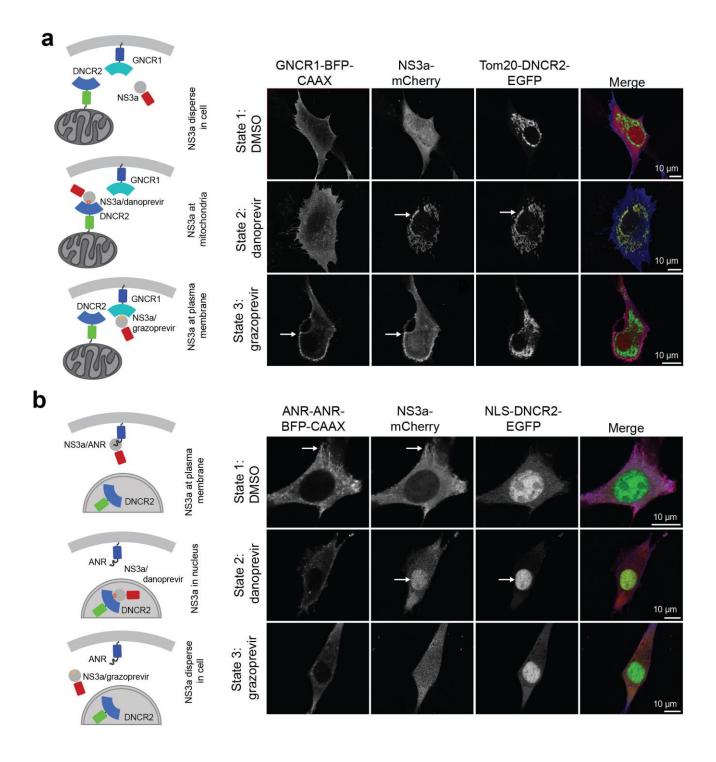
Grazoprevir:NS3a complex reader binding and improvement.

a, Predicted mutational preferences of the G3 interface for binding to NS3a:grazoprevir, as defined by the frequencies of mutations found in Rosetta re-designs of the interface. **b**, Sequence logos of the theoretical library for the combinatorial library varying the G3 interface (top), and the mutations found in the final enriched library (bottom). Residue identities at the varied positions are indicated for the first-generation reader G3 and optimized reader GNCR1. **c**, Binding of 1 µM NS3a with avidity to yeast-displayed G3 or GNCR1 in the presence of grazoprevir, danoprevir, asunaprevir, or DMSO. Technical triplicates and their means are shown.



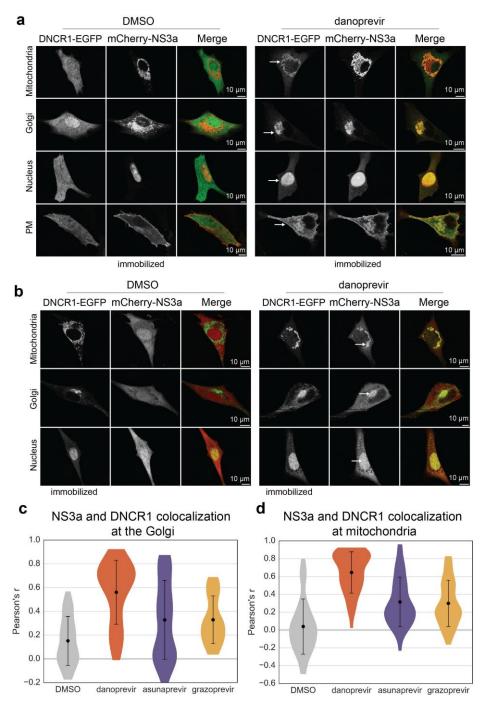
Characterization of the kinetics and affinity of the DNCR2:danoprevir:NS3a complex in mammalian cells.

a, Kinetics of DNCR2-EGFP association with myristoylated NS3a-mCherry after adding 5 μM danoprevir (time of drug addition is denoted by the dark gray vertical line). Mean and standard deviation of the cytoplasmic EGFP fluorescence (normalized to first and last frame) of 18 NIH3T3 cells collected from 4 separate experiments. **b**, Schematic of danoprevir-mediated Pl3K-Akt pathway activation through recruitment of an inter-Src homology 2 domain (iSH2) of the regulatory Pl3K subunit p85/DNCR2 fusion (DNCR2-iSH2) to myristoylated NS3a-mCherry (left panel). Western blots of phospho-AKT (pSer473) and AKT performed in COS-7 cells transfected with control plasmid (GFP), DNCR1-iSH2-only, DNCR2-iSH2-only, myristoyl-NS3a-only, DNCR1-iSH2 or DNCR2-iSH2 co-transfected with myristoyl-NS3a treated with DMSO or 10 μM danoprevir from one experiment. See Supplementary Figure 15 for the full Western blots. **c**, Quantification of Western blot in (b) (singlicate for controls; two well replicates for DMSO and danoprevir conditions).



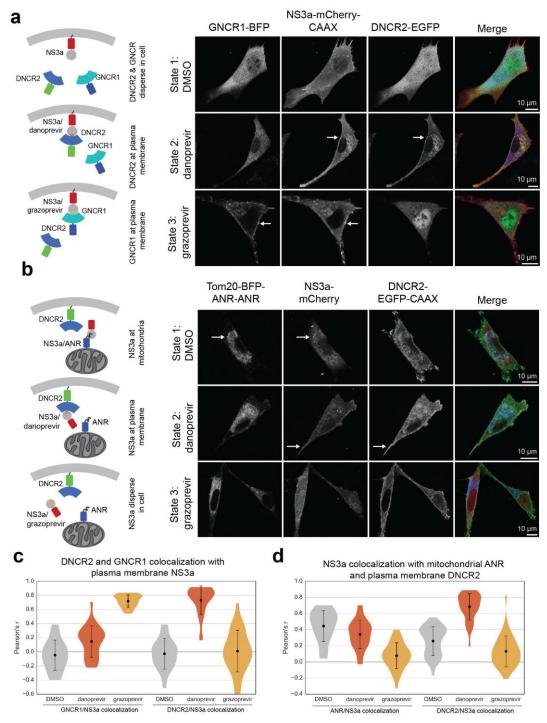
Combination of reader pairs for inducible two-location and colocalization control with NS3a.

a, Colocalization of NS3a-mCherry with GNCR1-BFP-CAAX or Tom20-DNCR2-EGFP after treatment with danoprevir (5 μ M), grazoprevir (5 μ M), or DMSO. **b**, Colocalization of NS3a-mCherry with ANR-BFP-CAAX or NLS-DNCR2-EGFP after treatment with danoprevir (5 μ M), grazoprevir (5 μ M), or DMSO. See Fig. 2c,d for quantification of multiple cells and associated entry in Supplementary Table 3 for the number of cells and replicates of which these images are representative.



Drug-regulated control of subcellular protein localization with intermediate-affinity danoprevir:NS3a reader DNCR1.

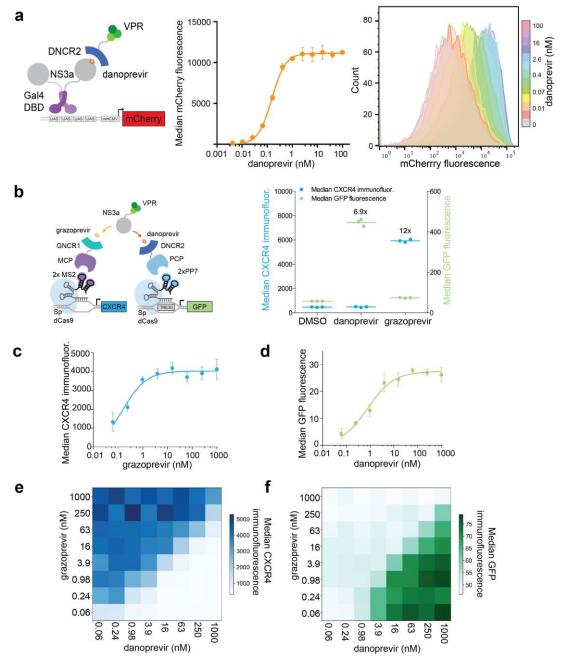
a, Colocalization of DNCR1-EGFP with mitochondria-, Golgi-, nuclear-, or plasma membrane-localized NS3a-mCherry under DMSO (left panel) or 10 µM danoprevir (right panel) treatment. **b**, Colocalization of mCherry-NS3a with mitochondria-, Golgi-, or nuclear-localized DNCR1-EGFP under DMSO (left panel) or 10 µM danoprevir (right panel) treatment. Each panel in (a,b) is representative of the majority population of n≥18 NIH3T3 cells from one well. Quantification of colocalization of mCherry-NS3a with **c**, Golgi- or **d**, mitochondrially-localized DNCR1-EGFP after treatment with grazoprevir (10 µM), danoprevir (10 µM), asunaprevir (10 µM), or DMSO. The mean and standard deviation of the Pearson's r of red/green pixel intensities is given for the number of cells stated in Supplementary Table 3 for each condition, along with the distributions for multiple NIH3T3 cells. See Supplementary Table 3 for sample sizes and P values.



Supplementary Figure 7

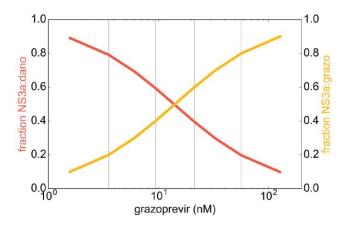
Additional PROCISiR combinations for two-location control of NS3a.

a, Colocalization of GNCR1-BFP or DNCR2-EGFP with NS3a-mCherry-CAAX after treatment with danoprevir (5 μ M), grazoprevir (5 μ M), or DMSO. **b**, Colocalization of NS3a-mCherry with Tom20-BFP-ANR or DNCR2-EGFP-CAAX after treatment with danoprevir (5 μ M), grazoprevir (5 μ M), or DMSO. Images are representative of two separate wells and the number of cells given in Supplementary Table 3. **c,d**, The mean (marked by dot) and standard deviation (error bars) of the Pearson's r of red/blue or red/green pixel intensities for the number of cells stated in Supplementary Table 3 is given for each condition in (a,b), along with the distributions of Pearson's r.



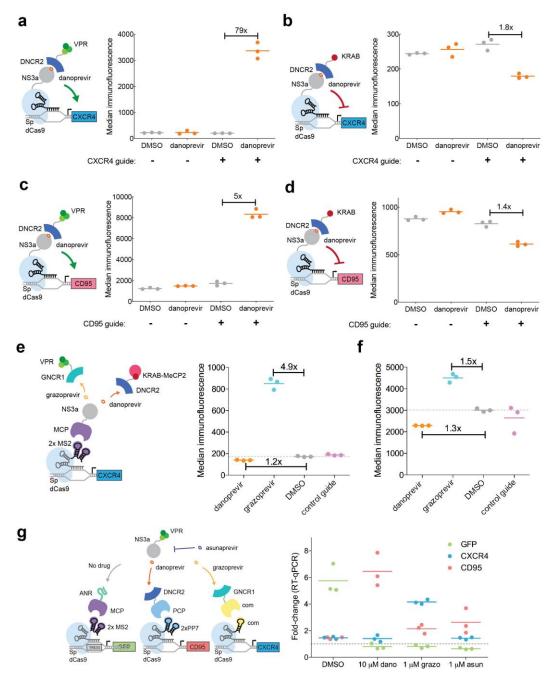
Titration of gene expression with Gal4/UAS and a two-gene dCas9 system.

a, Titration of mCherry expression from a UAS-minCMV promoter using Gal4-NS3a and DNCR2-VPR (left). Median mCherry values are shown in the middle panel, with the histograms for one replicate shown on the right to illustrate that the full population shifts to intermediate levels of gene expression. b, Expression of CXCR4 and GFP in cells expressing an MS2 scRNA targeting CXCR4, a PP7 scRNA targeting a GFP reporter, GNCR1-MCP, DNCR2-PCP, and NS3a-VPR after treatment with DMSO, danoprevir, or grazoprevir. Fold changes relative to DMSO are given for each 10 μM drug response for three biological replicates from one experiment. See Supplementary Table 3 for P values. c, CXCR4 immunofluorescence from titration of grazoprevir alone in the same system as (b). d, GFP fluorescence from titration of danoprevir alone in the same system as (b). (a,c,d) are fit to a one-site, specific binding Hill equation, and each point shows the mean and standard deviation of 3 biological replicates from one experiment, with background fluorescence levels from a DMSO-only condition subtracted. e,f, Raw median fluorescence values for experiment shown in Fig. 3e and 3f (mean of two replicates, not DMSO-subtracted).



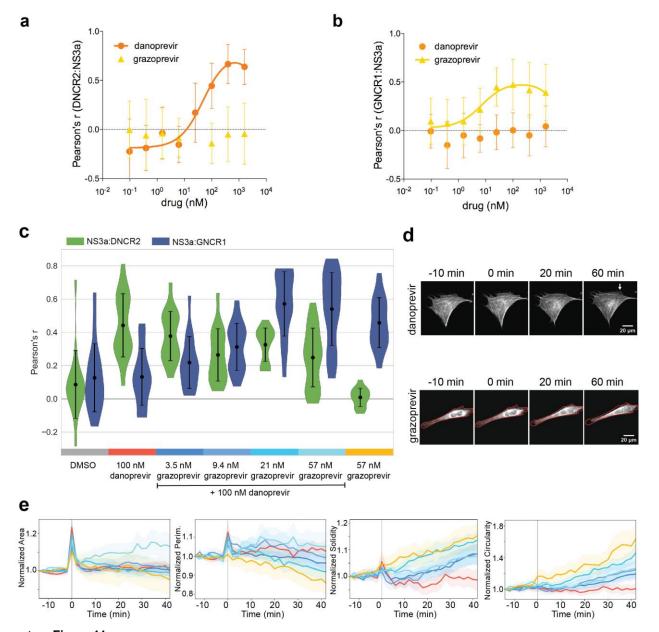
Modeling of NS3a:danoprevir and NS3a:grazoprevir occupancies.

The fraction of NS3a bound to danoprevir (orange, left axis) and the fraction of NS3a bound to grazoprevir (yellow, right axis) was calculated for a constant concentration of 100 nM danoprevir, with increasing concentrations of grazoprevir. The vertical gray lines mark the grazoprevir concentrations used for the experiments in Fig. 4, Supplementary Fig. 11, and Supplementary Fig. 12.



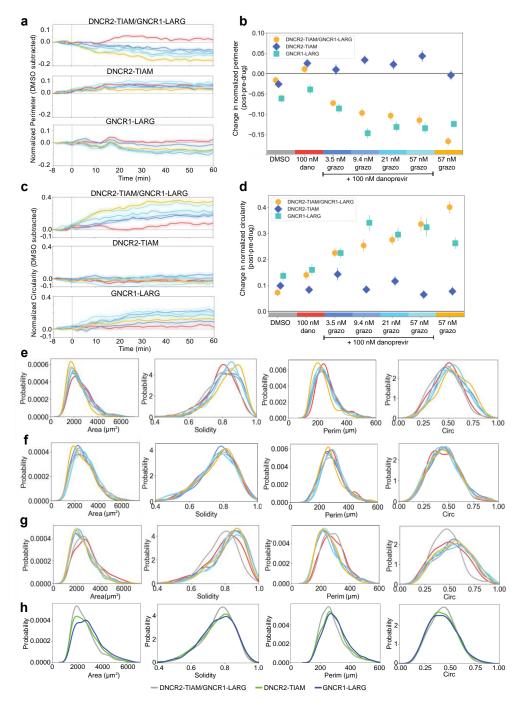
Switchable repression, overexpression, and three-gene control.

Median immunofluorescence of CXCR4 (a,b) or CD95 (c,d) expression controlled by danoprevir-promoted recruitment of (a,c) DNCR2-VPR or (b,d) DNCR2-KRAB to NS3a-dCas9 in the absence or presence of guides targeting the CXCR4 (a,b) or CD95 (c,d) promoter region. Fold change (a,c) or inverse fold change (b,d) are given above each DMSO/danoprevir condition pair. e, Switching between repression and overexpression is achieved from endogenous promoters for CXCR4 and CD95 (f) using dCas9 with MCP-NS3a, GNCR1-VPR, and DNCR2-KRAB-MeCP2. Fold change or inverse fold change is shown for treatment with 100 nM grazoprevir or danoprevir, respectively. (a-f) The mean of the median immunofluorescence intensities are given in arbitrary units for data from 3 independent wells from one experiment. g, Expression of GFP, CD95, and CXCR4 using a MS2 scRNA targeting a GFP reporter, a PP7 scRNA targeting CD95, and a com scRNA targeting CXCR4 with MCP-ANR, PP7-DNCR2, and com-GNCR1, respectively. The mean of 3 independent wells from one experiment is given for each gene relative to untransfected cells. See Supplementary Table 3 for all P values.



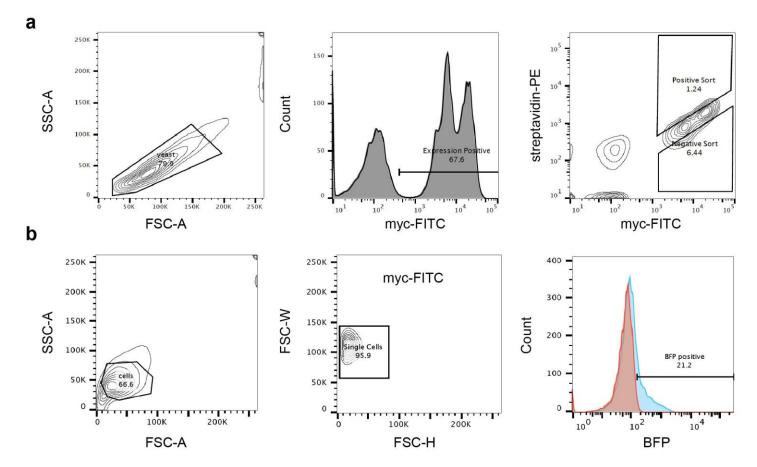
Membrane localization control in Hela and NIH3T3 cells.

a,b, Confocal image quantification of EGFP-DNCR2 (a) or BFP-GNCR1 (b) colocalization with mCherry-NS3a-CAAX for single-drug titrations in HeLa cells. Mean and standard deviation of the number of cells per condition given in Supplementary Table 3 from one well. Curves are fit to a one-site, total binding equation. **c**, Confocal image quantification of EGFP-DNCR2 (green) and BFP-GNCR1 (blue) colocalization with mCherry-NS3a-CAAX for drug combinations shown in NIH3T3 cells. The mean (marked by dot) and standard deviation (error bars) of the Pearson's r of red/blue or red/green pixel intensities for the number of cells stated in Supplementary Table 3 is given for each condition along with the distributions of Pearson's r. **d**, Representative images from two experiments of NIH3T3 cells transiently co-expressing EGFP-DNCR2-TIAM, BFP-GNCR1-LARG, and NS3a-CAAX treated with danoprevir (top) or grazoprevir (bottom) for the times indicated (drug was added at t=0). Lifeact-mCherry was also co-expressed to allow visualization of F-actin. Danoprevir treatment led to membrane ruffling (arrow) and grazoprevir treatment led to cell contraction (a red outline illustrating the cell boundary at -10 min is overlaid to illustrate cell contraction). **e**, Cell morphology parameters (area, perimeter, solidity, and circularity) of NIH3T3 cells transiently co-expressing EGFP-DNCR2-TIAM, BFP-GNCR1-LARG, NS3a-CAAX, and Lifeact-mCherry that were treated with the drug combinations shown in (C) for the times indicated (drug was added at t=0). Mean and s.e.m. for the number of per condition given in Supplementary Table 3 normalized to first frame, from one well.



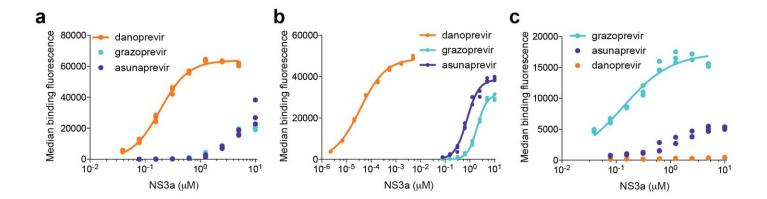
Proportional control of RhoA and Rac1 activation in HeLa cells.

a,c, Change in normalized perimeter (a) or circularity (c) (DMSO baseline subtracted) over time (drug addition at 0 min) in HeLa cells expressing NS3a-CAAX with either DNCR2-TIAM and GNCR1-LARG, DNCR2-TIAM alone, or GNCR1-LARG alone. Lines are colored according to the drug conditions shown on the x-axis of the plot in (b). **b,d**, Change in normalized perimeter (b) or circularity (d) (average last 10 min-first 10 min). (a-d) Mean and s.e.m. of the number cells per condition listed in Supplementary Table 3 from four independent wells. **e-g**, Kernel density estimates of distributions of morphology statistics at 60 min post-drug addition in Hela cells expressing NS3a-CAAX with either (e) DNCR2-TIAM and GNCR1-LARG, (f) DNCR2-TIAM alone, or (g) GNCR1-LARG alone. **h**, Kernel density estimates of the distributions of morphology statistics at the first frame (before drug addition) for the three HeLa cell lines.



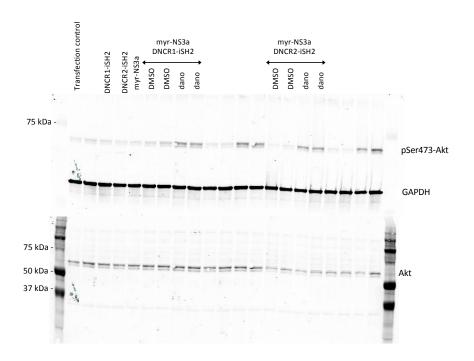
Yeast and human cell FACS gating strategies.

a, Yeast gating for all events (left), expression positive events used to report median binding fluorescence values (middle), and example sorting gates (right). **b**, HEK293T gating for all events (left), followed by single cell gating (middle), and gating for transfection-positive cells (BFP positive, right). Untransfected cells (red), NS3aH1-BFP-dCas9-transfected cells (blue).



Yeast-displayed design variants binding to NS3a:drug complexes.

Binding of NS3a:drug complexes (danoprevir, grazoprevir, asunaprevir) to yeast-displayed $\bf a$, DNCR1, $\bf b$, DNCR2, $\bf c$, GNCR1. Shown are 3 technical replicates fit with the Hill equation for complete curves.



Full western blots for Supplementary Figure 4b.

Full Western blots (top, anti-rabbit secondary with anti-pSer473-Akt and anti-GAPDH primaries; bottom, anti-mouse secondary with anti-Akt primary). Molecular weight markers are indicated on both blots.

Supplementary Notes

Protein engineering details and biochemical characterization of DNCR and GNCR

The danoprevir:NS3a complex reader design process started with docking a set of highly stable, de novo designed proteins on a danoprevir:NS3a structure using PatchDock: leucine-rich repeat proteins, designed helical repeat proteins (DHRs), ferredoxins, and helical bundles. The scaffolds were docked over the danoprevir:NS3a binding surface by directing their docking in PatchDock to the danoprevir molecule. Rosetta design was used to optimize the residues on the scaffold that came in contact with danoprevir and NS3a, largely resulting in designs with a hydrophobic interface with danoprevir, as most danoprevir hydrophilic groups are inaccessible when it is bound to NS3a. One design, D5, based on a DHR, showed danoprevir-dependent binding to NS3a when assayed via yeast surface display.

To improve D5's affinity for the danoprevir:NS3a complex, we used two sequential yeast surface display libraries (Supplementary Fig. 1). First, a combinatorial library was designed based on the frequencies of mutations present in re-designs of the D5 interface (Supplementary Fig. 1a). A.5 These Rosetta re-designs were obtained after small rigid-body perturbations of D5 relative to the danoprevir:NS3a complex. Sorting this library with increasingly stringent conditions led to a variant, danoprevir:NS3a complex reader 1 (DNCR1), that specifically bound the danoprevir:NS3a complex with high nanomolar affinity (Supplementary Table1). Next, we characterized a single-site saturation mutagenesis (SSM) library of DNCR1's two designed primary interface helices (5 and 7) and the non-interface helix 6. Enrichment ratios, calculated after sorting for both danoprevir:NS3a complex binders and non-binders, supported the overall designed binding mode (Supplementary Fig. 1b). Interestingly, the negative sort, which enriched for non-binders, gave us further structural insight into the binding mode of DNCR1. The surface

residues of helix 6, which faces away from the interface, were very permissive of substitution. Likewise, a region spanning the C-terminus of helix 6 to the N-terminus of helix 7 was permissive of mutation to nearly any residue, including proline. The helices in this region were found to be unfolded in the DNCR2:danoprevir:NS3a structure, and the shift of DNCR2 results in this region of the DHR not participating in the interface (Supplementary Fig. 2c). The trends seen in the negative sort SSM library enrichment ratios support the hypothesis that DNCR1 likely binds similarly to DNCR2. A second combinatorial library was designed based on the positive sort enrichment ratios, and enrichment of this library for danoprevir:NS3a binding resulted in multiple high affinity clones, of which one, DNCR2 (Supplementary Fig. 1c), was chosen for further characterization, based on its superior expression on the surface of yeast. The progression of improved binding from the original scaffold DHR79, to the design D5, and through two libraries resulting in DNCR1, and finally DNCR2, are illustrated by the DNCR1 SSM enrichment ratios in Supplementary Fig. 1d.

We performed a detailed biochemical analysis of the DNCR2:danoprevir:NS3a complex to confirm that it had the expected properties of a chemically-induced heterodimer. DNCR2 does not appear to bind substantially to danoprevir alone based on the inability of a high concentration (100 µM) of the free drug to disrupt the DNCR2:danoprevir:NS3a complex on yeast (Supplementary Fig. 2b). Size exclusion chromatography demonstrated that DNCR2 and NS3a behave as expected, forming a 1:1 complex only in the presence of danoprevir (Supplementary Fig. 2e). This behavior, along with the drug specificity described in the main text (Supplementary Fig. 2a,f), indicated that we had successfully designed and engineered a CID that was only inducible by danoprevir.

For our second drug:NS3a complex reader, we targeted the grazoprevir:NS3a complex. Grazoprevir is an FDA-approved drug with picomolar affinity to NS3a (K_i of 140 pM).⁶ For this round of design, we exclusively used DHR scaffolds, as our first-generation design had indicated that they were more suitable scaffolds for our design goal. We assembled a DHR scaffold set of many curvatures and sizes from published DHR crystal structures, as well as an in-house set of models (available upon request). We used both PatchDock and a new rotamer interaction field docking protocol (RIFDock) to center the DHR scaffolds over grazoprevir, followed by the same design approach that was used for the danoprevir CID design.⁷ We ordered and tested 29 designs by yeast surface display. Five designs based on DHR models showed very weak, but grazoprevir-dependent binding (data not shown). One design, G3, based on the crystal structure of DHR18, showed modest binding, similar to the first-generation danoprevir reader design, D5 (Supplementary Fig. 3c).

We computationally characterized the mutational preferences of the G3 interface via a similar Rosetta-based approach used to predict the mutational preferences of D5. The predicted mutational preferences at the G3 interface are shown in Supplementary Fig. 3a. These mutational frequencies were used to design a combinatorial library varying 9 positions of G3, which was sorted for sequences with increased affinity to grazoprevir:NS3a (Supplementary Fig. 3b). Both G3 and the final high-affinity clone, grazoprevir:NS3a complex reader 1 (GNCR1), showed high specificity for binding grazoprevir:NS3a over complexes of NS3a with danoprevir or asunaprevir, or apo NS3a (Supplementary Fig. 3c, Supplementary Table 1). GNCR1 had a similar affinity for the grazoprevir:NS3a complex as DNCR1 had for the danoprevir:NS3a complex (<200 nM). Because this affinity was found to be adequate for functioning as a chemically-inducible dimerizer in mammalian cells, we did not engineer GNCR1 further.

Further subcellular localization control with PROCISiR

As an assay for colocalization of NS3a and DNCR1, we used confocal fluorescence microscopy of NIH3T3 cells transiently transfected with pairs of NS3a-mCherry and DNCR1-EGFP constructs. NS3a was localized to different subcellular compartments via an N-terminal Tom20 tag (mitochondria), a nuclear localization signal (NLS, nucleus), an N-terminal myristoyl group (plasma membrane), or a C-terminal Giantin tag (Golgi). DNCR1-EGFP was diffuse throughout the cell under DMSO treatment (Supplementary Fig. 6a, left), and colocalized with NS3a-mCherry after treatment with 10 μM danoprevir (Supplementary Fig. 6a, right). The intermediate affinity reader also exhibited colocalization when the orientation was switched and DNCR1 was fused to the localization tags (Supplementary Fig. 6b), demonstrating that these components are interchangeable and modular. DNCR1 also demonstrated partial binding specificity for the danoprevir:NS3a complex over other drug:NS3a complexes, as quantification of EGFP/mCherry signal correlation for multiple cells showed lower (but non-zero) correlation in cells treated with 10 μM asunaprevir or grazoprevir (Supplementary Fig. 6c,d).

In addition to the GNCR1/DNCR2 and DNCR2/ANR combinations used for the control of NS3a's subcellular localization shown in Fig. 2c,d and Supplementary Fig. 5, we also tested two other PROCISiR combinations for localization control. Colocalization of untagged DNCR2-EGFP and GNCR1-BFP with NS3a-mCherry-CAAX clearly exhibited 3 states: no colocalization with no drug, DNCR2/NS3a colocalization with danoprevir, and GNCR1/NS3a colocalization with grazoprevir (Supplementary Fig. 7a,c). Likewise, NS3a-mCherry could be pre-localized to mitochondria with Tom20-BFP-ANR, and recruited to membrane-targeted DNCR2-EGFP-CAAX after treatment with danoprevir (Supplementary Fig. 7b,d). Thus, PROCISiR's readers

can be combined to specifically respond to different drug treatments and provide multiple localization state outputs.

Modeling of NS3a:drug complex binding

To predict drug concentration regimes that would yield intermediate levels of NS3a:DNCR2 and NS3a:GNCR1 complexes, we modeled the fraction of NS3a bound to danoprevir and grazoprevir. For this, we simply used NS3a:drug K_i values and the Cheng-Prussoff approximation for equilibrium drug:receptor binding in the presence of a competitive inhibitor:

$$f_{N_d} = \frac{D}{\left(1 + \frac{C}{K_{i,c}}\right)K_{i,d} + D}$$

$$f_{N_c} = \frac{C}{\left(1 + \frac{D}{K_{i,d}}\right)K_{i,c} + C}$$

where f_{Nd} is the fraction of NS3a bound to the target drug, and f_{Nc} is the fraction of NS3a bound to the competitor drug, D is the free concentration of target drug, C is the free concentration of competitor drug, $K_{i,d}$ is the NS3a K_i for the target drug, and $K_{i,c}$ is the NS3a K_i for the competitor drug. The following NS3a:drug K_i values used are from published enzyme inhibition studies: danoprevir:NS3a = 1.0 nM, and grazoprevir:NS3a = 0.14 nM. There are several assumptions made in applying these equations that are unlikely to be valid in all cellular conditions, including the direct inverse relationship between f_{Nd} and f_{Nc} , which is unlikely to be true when intracellular NS3a concentrations are high. Additionally, in applying these equations to model the fractions of NS3a:drug:reader complexes, we make the further approximation that all NS3a:drug complexes will be stoichiometrically bound to their corresponding reader. Despite these assumptions, we

see very good correspondence between our model and the experimental results in Fig. 3e. The predicted fraction of NS3a bound to danoprevir or grazoprevir at different drug concentrations closely matches transcriptional outputs resulting from NS3a:danoprevir:DNCR2 or NS3a:grazoprevir:GNCR1 complex formation. We also used the same equations to model the amount of DNCR2 and GNCR1 recruited to membrane-bound NS3a using different drug treatment regimes (Supplementary Fig. 9, Fig. 4b, Supplementary Fig. 12c). In both transcriptional and signaling output applications, we see that the relative EC50 values between DNCR2 and GNCR1 outputs match the ratio of their drug:NS3a K_i values. See discussion below.

Additional transcriptional control modes

In Supplementary Fig. 10a-d, we used a direct fusion of NS3a to dCas9 to recruit the transcriptional activator DNCR2-VPR or the transcriptional repressor DNCR2-KRAB to specific genomic loci. 9,10 We used this system to control the expression of two endogenous genes in HEK293 cells, CXCR4 and CD95. Detection of expression by immunofluorescence and FACS revealed expression induction of 79-fold (CXCR4) or 5-fold (CD95) over a DMSO-treated control for the DNCR2-VPR constructs, and repression induction of 1.8-fold (CXCR4) or 1.4-fold (CD95) for the DNCR2-KRAB constructs. Danoprevir had no effect on gene expression in the absence of the guide RNA. The gene induction for CXCR4 and CD95 from DNCR2-VPR is similar to that obtained on these same genes for a direct dCas9-VPR fusion and two other inducible dCas9/VPR systems employing the gibberellin and abscisic acid CIDs. 11 To the best of our knowledge, inducible repression using dCas9 on endogenous promoters has not previously been demonstrated.

To enable temporal switching or graded control of gene expression from repression to overexpression, we utilized a scaffold RNA/RNA-binding protein (RBP) system with NS3a fused to the RBP MS2, GNCR1 fused to VPR, and DNCR2 fused to KRAB-MeCP2, a repressor with enhanced activity over KRAB. While the transcriptional activation and repression observed with the scaffold RNA/RBP system was more modest than with the direct dCas9-NS3a fusion, this switchable system also demonstrated statistically significant overexpression (from grazoprevir treatment) or repression (from danoprevir treatment) of CXCR4 and CD95 (Supplementary Fig. 10e,f). Notably, this was using guides that were previously published as optimal for inducing overexpression of these genes and that anneal 5' to the transcription start site for each gene. Optimization of guide positions, or utilization of multiple guides that tile before and after the transcription start site could be explored in the future to improve the dynamic range of this switchable VPR/KRAB-MeCP2 system.

Finally, in a demonstration of the multi-state transcriptional outputs that can be achieved with PROCISiR, we combined GNCR1, DNCR2, and ANR with three orthogonal scRNA/RBP pairs (com/com, PP7/PCP, and MS2/MCP) to control the expression of CXCR4, CD95, and GFP, respectively, in a GFP-report HEK293 cell line (Supplementary Fig. 10g). This system exhibited four distinct transcriptional output states under four input states: DMSO (GFP expression under control of ANR), $10~\mu M$ danoprevir (CD95 expression under control of DNCR2), $1~\mu M$ grazoprevir (CXCR4 expression under control of GNCR1), and $1~\mu M$ asunaprevir (no gene expression, as asunaprevir disrupts ANR's interaction with NS3a-VPR but does not promote complex formation with DNCR2 or GNCR1). This demonstrates that all 3 readers can be used orthogonally to control multiple output states.

Membrane colocalization in HeLa and NIH3T3 cells

We performed single-drug titrations of membrane colocalization in HeLa cells coexpressing EGFP-DNCR2, BFP-GNCR1, and mCherry-NS3a-CAAX. The dose-responses, quantified by Pearson's r of EGFP/mCherry or BFP/mCherry correlation, are shown in Supplementary Fig. 11a,b. Neither DNCR2 nor GNCR1 colocalizes with NS3a in response to the non-target inducer, grazoprevir or danoprevir, respectively. One-site, total binding curves fit to the DNCR2:danoprevir and GNCR1:grazoprevir response gave EC₅₀ values ~50-fold higher than the K_i values for those drugs and NS3a, which is in contrast to the close agreement between drug:NS3a K_i values and EC₅₀ values for transcriptional activation we observed (Supplementary Fig. 8c,d). This disparity may be due to what is likely the high expression levels of NS3a-CAAX in HeLa cells. The EC₅₀ for DNCR2:danoprevir:NS3a colocalization was 51 ± 16 nM and the EC₅₀ for GNCR1:grazoprevir:NS3a colocalization was 7.3 ± 3.6 nM (mean, standard deviation), which accurately reflects the seven-fold difference in affinity for NS3a of the two drugs. Therefore, in order to identify drug concentrations that would achieve a range of intermediate levels of DNCR2:NS3a and GNCR1:NS3a colocalization, we used the drug:NS3a K_i values (1.0 and 0.14 nM for danoprevir and grazoprevir, respectively) to model the predicted fraction of NS3a:drug complexes formed in response to different combinations of danoprevir and grazoprevir (Supplementary Notes, "Modeling of NS3a drug complex binding"). We quantified colocalization from these drug concentrations in HeLa cells (Fig. 4b), and NIH3T3 cells (Supplementary Fig. 11c) co-expressing EGFP-DNCR2, BFP-GNCR1, and membrane-localized NS3a with confocal microscopy. NIH3T3 cells showed the predicted graded and proportional membrane colocalization of DNCR2:NS3a and GNCR1:NS3a (Supplementary Fig. 9 and Supplementary Fig. 11c). HeLa cells showed the expected graded membrane colocalization of

GNCR1:NS3a, with more modest gradation of DNCR2:NS3a membrane colocalization at higher grazoprevir concentrations (Fig. 4b). Future models could be further parameterized to improve accuracy, but our simple steady-state modeling based on drug:NS3a K_i values was sufficient to predict a useful regime for achieving intermediate response levels.

Cell morphology changes in single-effector versus dual-effector HeLa cells

The distributions of morphology statistics for the three HeLa cell lines (DNCR2-TIAM/GNCR1-LARG co-expressing and single-effector versions thereof: DNCR2-TIAM (in the presence of GNCR1 that is not fused to an effector) and GNCR1-LARG (in the presence of DNCR2 that is not fused to an effector)) after 60 minutes of drug treatment are shown in Supplementary Fig. 12e-g. Interestingly, the area and perimeter changes appear graded on the single-cell level, while solidity changes appear more binary. This trend of cells partitioning between two extremes of solidity defined by the single-drug conditions is more obvious in the dual-effector cells, which have a larger range of solidity changes. In the dual-effector cells, the intermediate drug combinations with low grazoprevir exhibit a bimodal or broadened solidity distribution when compared to the single-drug conditions, indicative of some cells withdrawing protrusions and achieving the high solidity state, while others retain their original morphology (Supplementary Fig. 11e). This binary cell solidity change was previously noted when modulating Rac/Rho activity indirectly with Pak inhibitors. 13 These results are suggestive of some events leading to changes in cell morphology being more abrupt (withdrawal of protrusions following Rho activation), while others (changes to cell size) exhibit more gradual behavior. The three HeLa cell lines showed no systematic differences in starting morphology (Supplementary

Fig. 11h), validating that the observed morphological changes are due to TIAM and LARG recruitment in response to danoprevir and grazoprevir, respectively.

Graded and proportional control of GTPase-driven signaling pathways in NIH3T3 cells

Plasma membrane colocalization of DNCR2-TIAM and GNCR1-LARG with NS3a-CAAX was also investigated in NIH3T3 cells using the danoprevir/grazoprevir combinations suggested from modeling. Danoprevir treatment appeared to increase membrane ruffling, which is a known result of Rac1 activation, while grazoprevir treatment caused stress fiber formation and cell contraction from RhoA activity (Supplementary Fig. 11d). The drug combinations from Supplementary Fig. 11c were applied to NIH3T3 cells transiently co-expressing DNCR2-TIAM, GNCR1-LARG, and NS3a-CAAX, and the area, perimeter, solidity, and circularity were tracked with wide-field microscopy over time (Supplementary Fig. 11e). Although danoprevir treatment and subsequent Rac1 activation led to the formation of observable lamellipodia, it did not induce measurable changes in morphology. This suggests that Rac1 activity may have different effects in NIH3T3 cells than in HeLa cells. NIH3T3 cells are fibroblasts, and therefore start with a highly adherent, flat, and elongated morphology, while HeLa cells are an epithelial line and start with a more rounded morphology. Visually observed membrane ruffling from Rac1 activity in NIH3T3 cells may not be captured by the gross cell size and shape parameters quantified, and as NIH3T3 cells are already fairly flat and spread out, there is little further spreading from Rac1 activation. In HeLa cells expressing DNCR2-TIAM, danoprevir treatment caused significant cell spreading, as indicated by increases in area and perimeter (Fig. 4, Supplementary Fig. 12). In NIH3T3 cells, grazoprevir treatment (LARG/RhoA activation) caused significant contraction of cell length and protrusions as indicated by increases in circularity and solidity, respectively,

which was consistent between the cell lines (Supplementary Fig. 11e, Fig. 4). Gradations in RhoA effect on cell morphology were observable at the intermediate danoprevir/grazoprevir concentrations. The NIH3T3 data is from approximately an order of magnitude fewer cells ($n \ge 22$) than the HeLa datasets ($n \ge 201$) and is therefore considerably noisier than the HeLa data, including a spike at t=0 min due to focusing and segmentation issues when drug was added. Nevertheless, these data illustrate that graded and proportional control of signaling pathways can be performed with PROCISiR in cells of different types and uncover differences in cell response to the same stimuli.

Supplementary Table 1 \mid Apparent dissociation constants for yeast-displayed design variants to NS3a:drug complexes

Clone	K_D (nM) \pm standard Drug deviation or relative binding		Fold drug specificity
	danoprevir	++	<u>-</u>
D5	grazoprevir	+++	none
	asunaprevir	+	modest
	danoprevir	190 ± 10 nM	-
DNCR1	grazoprevir	2900 ± 100 nM	15
	asunaprevir	6300 ± 2600 nM	33
	danoprevir	$0.036 \pm 0.0002 \text{ nM}$	-
DNCR2	grazoprevir	2000 ± 100 nM	56,000
	asunaprevir	770 ± 50 nM	21,000
	grazoprevir	++	-
G3	danoprevir	no binding	high
	asunaprevir	+	modest
	grazoprevir	140 ± 20 nM	-
GNCR1	danoprevir	>10,000 nM	>71
	asunaprevir	>10,000 nM	>71

Data are from three technical replicates. Relative binding of different NS3a:drug complexes is given for conditions where binding was too weak to achieve full titration curves on yeast, indicating binding in the low-to-mid micromolar-range, or weaker. Curves for DNCR1, DNCR2, and GNCR1 are shown in Supplementary Fig. 14. See Supplementary Fig. 2A, 3C for single concentration point data for D5 and G3.

Supplementary Table 2 | Crystallography data collection and refinement statistics

	DMCD2 MC2 - 1
	DNCR2:NS3a:danoprevir
Data collection	
Space group	$P2_1$
Cell dimensions	
a,b,c (Å)	70.84, 69.26, 99.34
α, β, γ (°)	90, 108.59, 90
Resolution (Å)	50.0 - 2.30 (2.37 - 2.30) *
$R_{ m sym}$	0.102 (0.340)
$I/\sigma I$	14.4 (2.6)
Completeness (%)	99.3 (93.6)
Redundancy	4.1 (3.0)
•	
Refinement	
Resolution (Å)	50.0 - 2.30
No. reflections	38559
$R_{ m work}$ / $R_{ m free}$	0.203 / 0.240
No. atoms	
Protein	5818
Ligand/ion	134
Water	209
B-factors	
Protein	29.9
Ligand/ion	33.4
Water	37.4
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.545
This differential data and an	

This diffraction data set was collected from a single crystal. *Values in parentheses are for highest-resolution shell.

Supplementary Table 3 | Statistics and reproducibility
Values are from one-way ANOVA with significance for each comparison (P < 0.05) determined by Tukey's multiple comparisons test, except where noted.

						ANOVA	results
Figure	Condition	Condition 1 Condition 2		Number of cells (1,2)	Number of wells (1,2)	Mean difference	Signif- cant
Fig. 2B			One-	way ANOV	A P value	<0.00	001
		DMSO	10 µM danoprevir	26, 26	3, 3	-0.6277	Yes
		DMSO	10 µM asunaprevir	26, 32	3, 4	0.004479	No
		DMSO	10 µM grazoprevir	26, 34	3, 4	0.08568	No
		10 µM danoprevir	10 µM asunaprevir	26, 32	3, 4	0.6321	Yes
		10 µM danoprevir	10 μM grazoprevir	26, 34	3, 4	0.7133	Yes
Fig.	GNCR1/NS3a			way ANOV	A P value	<0.00	01
2C		DMSO	5 μM danoprevir	37, 30	3, 3	0.04179	No
		DMSO	5 μM grazoprevir	37, 35	3, 3	-0.6140	Yes
		5 μM danoprevir	5 μM grazoprevir	30, 35	3, 3	-0.6558	Yes
	DNCR2/NS3a		One-way ANOVA P va				001
		DMSO	5 μM danoprevir	37, 30	3, 3	-0.6409	Yes
		DMSO	5 μM grazoprevir	37, 35	3, 3	0.2358	Yes
		5 μM danoprevir	5 μM grazoprevir	30, 35	3, 3	0.8767	Yes
Fig.	ANR/NS3a			way ANOV	A P value	<0.0001	
2D		DMSO	5 µM danoprevir	29, 25	3, 3	0.4612	Yes
		DMSO	5 μM grazoprevir	29, 26	3, 3	0.3235	Yes
		5 μM danoprevir	5 μM grazoprevir	25, 26	3, 3	-0.1377	Yes
	DNCR2/NS3a			way ANOVA P value		<0.0001	
		DMSO	5 μM danoprevir	29, 25	3, 3	-0.6039	Yes
		DMSO	5 μM grazoprevir	29, 26	3, 3	-0.4252	Yes
		5 μM danoprevir	5 μM grazoprevir	25, 26	3, 3	0.1786	Yes
Fig. 4b		DMSO	1 O	18	1		
		100 nM danoprevir		18	1		
		100 nM danoprevir, 3.5 nM grazoprevir			1		

		100 nM danop	rovir Q 4 pM	1			
		•	ilevii, 9.4 mivi	14	1		
		grazoprevir 100 nM danop	rovin 21 nM	14			
		grazoprevir	revir, Z i flivi	20	1		
		20					
		100 nM danop	revir, 57 nivi	16	1		
		grazoprevir					
		57 nM grazopi	evir DMSO	20	1		
Fig.	DNCR2-		385	4			
4d-g	TIAM/GNCR1-	1	00 nM danoprevir	340	4		
and	LARG	100 nM d	328	4			
Supp.			grazoprevir				
Fig 12		100 nM d	anoprevir, 9.4 nM	201	4		
			grazoprevir	201	4		
		100 nM c	lanoprevir, 21 nM	248	4		
			grazoprevir	240	4		
		100 nM c	lanoprevir, 57 nM	200	4		
			grazoprevir	260	4		
		5	7 nM grazoprevir	266	4		
	DNCR2-TIAM		DMSO	374	4		
		1	00 nM danoprevir	273	4		
			anoprevir, 3.5 nM				
			grazoprevir	292	4		
		100 nM d	anoprevir, 9.4 nM		_		
			grazoprevir	387	4		
		100 nM c	lanoprevir, 21 nM	309	4		
			grazoprevir				
		100 nM c	lanoprevir, 57 nM	274			
		10011111	grazoprevir		4		
		F	57 nM grazoprevir		4		
	GNCR1-	DMSO		323 307	4		
	LARG	100 nM danoprevir		313	4		
	2, (()	100 nM danoprevir, 3.5 nM			7		
		grazoprevir		328	4		
		100 nM d	anoprevir, 9.4 nM				
		100 11111 01	grazoprevir	237	4		
		100 nM c	lanoprevir, 21 nM				
		100 11101 0	grazoprevir	286	4		
		100 nM c		247			
		100 11101 0	100 nM danoprevir, 57 nM grazoprevir		4		
		-	7 nM grazoprevir	298	4		
Supp.				way ANOV		<0.0	001
Fig.		DMSO	10 µM	25, 42	3, 5		
6C		DIVIOU	danoprevir	20, 72	0, 0	-0.4154	Yes
		DMSO	10 µM	25, 27	3, 3		
		DIVIOU	asunaprevir	20, 21	0, 0	-0.1721	No
		DMSO	10 µM	25, 27	3, 3		
		DIVIOU	grazoprevir	20, 21	5, 5	-0.1751	No
		10 µM	10 µM	42, 27	5, 3		
		danoprevir	asunaprevir	72, 21	0, 0	0.2433	Yes
	danoprevir 10 μM		10 µM	42, 27	5, 3		
		danoprevir	grazoprevir	72, 21	5, 5	0.2402	Yes
						2 -	004
Fig.6D						<0.0	UU1
1 19.00		DMSO	10 μM	33, 45	3, 5	-0.6159	Yes
			danoprevir			-0.0108	162
			•				

ANR/NS3a ANR/NS3a			DMSO	10 μΜ	33, 37	3, 3	0.0054	Vaa
Supp. Pig. ANR/NS3a Supp. Pig. ANR/NS3a Supp. Pig. DMSO Supp. Pig. Pig. Pig. Pig. Pig. Pig. Pig. Pig		1		asunaprevir	·		-0.2954	res
10 μM 10 μM 10 μM 45, 37 5, 3 0.3205 Yes			DMSO	•	33, 32	3, 3	-0.2641	Yes
To pM danoprevir				10 μM	45, 37	5, 3	0.3205	Yes
Supp. Fig. 7C Supp. Fig. 8B Supp. Pig. 8 Pig. 8 Supp. Pig. 8 Pig. 9 Pig. 8 Pig. 9			10 μM	10 μΜ	45, 32	5, 3	0.3519	Yes
Pig.		011004/1100	danoprevir		41101			
DMSO		GNCR1/NS3a	DIAGO				<0.0	001
DNCR2/NS3a DMSO	Fig. 7C			danoprevir	·		-0.2066	Yes
10 μM danoprevir 10 μM grazoprevir 27, 29 2, 2 -0.5855 Yes			DMSO	•	26, 29	2, 2	-0.7921	Yes
DNCR2/NS3a DNSO 10 μM 26, 29 2, 2 -0.0729 No				10 μM	27, 29	2, 2	-0.5855	Yes
DMSO		DNCR2/NS3a	uanoprevii			/A P value	<0.0	001
DMSO		DINGINZ/INOJA	DMSO					
DMSO			DIVISO		20, 21	۷, ۷	-0.7846	Yes
Supp. Fig. 7D ANR/NS3a DMSO S μM danoprevir DMSO S μM danoprevir DMSO S μM danoprevir DMSO S μM danoprevir DMSO S μM danoprevir DMSO S μM danoprevir NA 3, 3 -11.67 No DMSO danoprevir NA 3, 3 -5447 Yes danoprevir GFP DMSO danoprevir NA 3, 3 -5447 Yes DMSO danoprevir NA 3, 3 -5447 Yes DMSO danoprevir NA 3, 3 -16.70 No danoprevir Grazoprevir NA 3, 3 -16.70 No danoprevir Grazoprevir NA 3, 3 -16.70 No danoprevir Grazoprevir NA 3, 3 -16.70 No danoprevir NA 3, 3 -16.70 No danopre			DMSO	10 μM	26, 29	2, 2	-0.0729	No
Supp. Fig. 7D				10 μM	27, 29	2, 2	0.7117	Yes
Pig. 7D	Supp	ANR/NS3a	danopievii		·way ANO\	/A P value	<0.0001	
DMSO		71111111000	DMSO					
DMSO				•	00,00	0, 0	0.1010	Yes
DNCR2/NS3a			DMSO	5 µM	39, 41	3, 4	0.3764	Yes
DNCR2/NS3a DMSO 5 μM 39, 33 3, 5 -0.4278 Yes				5 μM	33, 41	5, 4	0.2754	Yes
DMSO		DNCR2/NS3a					<0.0	001
DMSO			DMSO	5 µM			-0.4278	Yes
Supp. Fig. 8B CXCR4 CXCR4 DMSO danoprevir NA 3, 3 -11.67 No			DMSO	5 µM	39, 41	3, 4	0.1308	Yes
Supp. CXCR4 One-way ANOVA P value < 0.0001 Fig. 8B DMSO danoprevir NA 3, 3 -11.67 No DMSO grazoprevir NA 3, 3 -5458 Yes danoprevir grazoprevir NA 3, 3 -5447 Yes One-way ANOVA P value <0.0001				5 μΜ	33, 41	5, 4	0.5587	Yes
DMSO	Supp.	CXCR4	I		way ANO\	/A P value	<0.0	001
DMSO grazoprevir NA 3, 3 -5458 Yes	Fig. 8B		DMSO					
One-way ANOVA P value			DMSO	grazoprevir	NA	3, 3	-5458	Yes
DMSO danoprevir NA 3, 3 -388.2 Yes DMSO grazoprevir NA 3, 3 -16.70 No danoprevir grazoprevir NA 3, 3 371.5 Yes Supp. No CXCR4 guide DMSO danoprevir NA 3, 3 0.8382a Fig. guide DMSO danoprevir NA 3, 3 <0.0001a Supp. No CXCR4 guide DMSO danoprevir NA 3, 3 0.3309a Fig. guide CXCR4 guide DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a Supp. Su			danoprevir					
DMSO grazoprevir NA 3, 3 -16.70 No		GFP						
Supp. No CXCR4 guide DMSO danoprevir NA 3, 3 371.5 Yes Supp. No CXCR4 guide DMSO danoprevir NA 3, 3 0.8382a Fig. guide DMSO danoprevir NA 3, 3 <0.0001a								
Supp. No CXCR4 guide DMSO danoprevir danoprevir NA 3, 3 0.8382a Fig. 10A CXCR4 guide DMSO danoprevir NA 3, 3 <0.0001a				<u> </u>				
Fig. guide CXCR4 guide DMSO danoprevir NA 3, 3 <0.0001a Supp. No CXCR4 guide DMSO danoprevir NA 3, 3 0.3309a Fig. guide DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0008a								
Supp. No CXCR4 guide DMSO danoprevir NA N	Fig.	guide		danoprevir				
Fig. guide 0.5309 10B CXCR4 guide DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0040a	10A	CXCR4 guide	DMSO	danoprevir	NA	3, 3	<0.00	001ª
10B CXCR4 guide DMSO danoprevir NA 3, 3 0.0008a Supp. No CD95 DMSO danoprevir NA 3, 3 0.0040a			DMSO	danoprevir	NA	3, 3	0.33	09ª
Supp. No CD95 DMSO danoprevir NA 3, 3			DMSO	danoprevir	NA	3, 3	0.00	08 ^a
- 1 N		Supp. No CD95 DMSO danoprevir NA 3, 3						
10C CD95 guide DMSO danoprevir NA 3, 3 <0.0001a			DMSO	danoprevir	NA	3, 3	<0.00	001 ^a

Supp. Fig.	No CD95 guide	DMSO	NA	3, 3	0.0	1 ^a	
10D	CD95 guide	DMSO	NA	3, 3	0.0006 ^a		
Supp.					VA P value	<0.0	
Fig.		DMSO	danoprevir	NA	3, 3	33.67	No
10E		DMSO	grazoprevir	NA	3, 3	-677.7	Yes
		DMSO	Control guide	NA	3, 3	-15.00	No
Supp.			One-		VA P value	0.00	
Fig.		DMSO	danoprevir	NA	3, 3	718.0	No
10F		DMSO	grazoprevir	NA	3, 3	-1499	Yes
		DMSO	Control guide	NA	3, 3	358.3	No
Supp.	GFP		One-	way ANO'	VA P value	<0.0	001
Fig. 10G		DMSO	10 µM danoprevir	NA	3, 3	4.949	Yes
		DMSO	1 µM grazoprevir	NA	3, 3	4.937	Yes
		DMSO	1 μM asunaprevir	NA	3, 3	5.105	Yes
		10 μM danoprevir	1 µM grazoprevir	NA	3, 3	-0.0118	No
		10 µM danoprevir	1 μM asunaprevir	NA	3, 3	0.1562	No
		1 μM grazoprevir	1 μM asunaprevir	NA	3, 3	0.1680	No
	CXCR4	One-way ANOVA P value				<0.0001	
		DMSO	10 µM danoprevir	NA	3, 3	0.06206	No
		DMSO	1 μM grazoprevir	NA	3, 3	-2.691	Yes
		DMSO	1 μM asunaprevir	NA	3, 3	0.03393	No
		10 µM danoprevir	1 μM grazoprevir	NA	3, 3	-2.753	Yes
		10 µM danoprevir	1 μM asunaprevir	NA	3, 3	-0.0281	No
		1 μM grazoprevir	1 μM asunaprevir	NA	3, 3	2.725	Yes
	CD95			way ANO'	VA P value	0.0003	
		DMSO	10 µM danoprevir	NA	3, 3	-5.006	Yes
		DMSO	1 µM grazoprevir	NA	3, 3	-0.6903	No
		DMSO	1 µM asunaprevir	NA	3, 3	-1.180	No
		10 µM danoprevir	1 μM grazoprevir	NA	3, 3	4.316	Yes
		10 µM danoprevir	1 µM asunaprevir	NA	3, 3	3.826	Yes
		1 μM grazoprevir	1 μM asunaprevir	NA	3, 3	-0.4897	No
Supp.		1600.0 nM		25	1		
Fig		400.0 nM		28	1		
11a		100.0 nM			1		
		25.0 nM		18 20	1		

1.6 nM		6.3 nM	21	1	
O.4 nM					
Supp. Fig					
Supp. 1600.0 nM 25 1					
Fig 11b	Supp				
11b					
25.0 nM					
Supp. DMSO 15 1	116				
1.6 nM 22 1					
0.4 nM					
Dusco Dusc					
DMSO					
Tight 100 nM danoprevir 15					
11c					
Grazoprevir 15			15		
100 nM danoprevir, 9.4 nM 1 1 1 1 1 1 1 1 1	11c			1	
grazoprevir 15			15		
100 nM danoprevir, 21 nM grazoprevir 100 nM danoprevir, 57 nM 1 1 grazoprevir 18 57 nM grazoprevir 14 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		100 nM danoprevir, 9.4 nM		1	
grazoprevir			15		
100 nM danoprevir, 57 nM		100 nM danoprevir, 21 nM		1	
grazoprevir		grazoprevir	14		
Supp. 100 nM danoprevir 22 1		100 nM danoprevir, 57 nM		1	
Supp. 100 nM danoprevir 22 1 Fig 100 nM danoprevir, 3.5 nM 1 11e grazoprevir 30 100 nM danoprevir, 9.4 nM 1 grazoprevir 39 100 nM danoprevir, 21 nM 1 grazoprevir 57 100 nM danoprevir, 57 nM 1		grazoprevir			
Fig 100 nM danoprevir, 3.5 nM 30 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		57 nM grazoprevir	14	1	
11e grazoprevir 30 1 1 1 1 1 1 1 1 1	Supp.		22	1	
100 nM danoprevir, 9.4 nM		100 nM danoprevir, 3.5 nM		1	
grazoprevir 39 100 nM danoprevir, 21 nM 1 grazoprevir 57 100 nM danoprevir, 57 nM 1	11e	grazoprevir	30		
100 nM danoprevir, 21 nM 1 1 grazoprevir 57 100 nM danoprevir, 57 nM 1		100 nM danoprevir, 9.4 nM		1	
grazoprevir 57 100 nM danoprevir, 57 nM 1		grazoprevir	39		
100 nM danoprevir, 57 nM 1		100 nM danoprevir, 21 nM		1	
100 nM danoprevir, 57 nM 1			57		
				1	
		grazoprevir	33		
57 nM grazoprevir 30 1			30	1	

^aP values from unpaired, two-sided t-test.

Supplementary Table 4 | Sequences of constructs and primers and usage in figures

Sequence ID	Plasmid ID	Description	Figures	Sequence (Reader/NS3a component in bold, fusions/tags/linkers in regular
NS3a_1	GWF020	Tagless NS3a/4a- solubility optimized- S139A (His- SMT3 removed by ULP1 cleavage) (E. coli)	Fig. 1D,E; Supp. Fig. 2C-F	font) MGHHHHHHHHHHHGSLQDSEVNQEA KPEVKPEVKPETHINLKVSDGSSEIFF KIKKTTPLRRLMEAFAKRQGKEMDSL RFLYDGIRIQADQAPEDLDMEDNDIIE AHREQIGGMKKKGSVVIVGRINLSG DTAYAQQTRGEEGCQETSQTGRDK NQVEGEVQIVSTATQTFLATSINGVL WTVYHGAGTRTIASPKGPVTQMYTN VDKDLVGWQAPQGSRSLTPCTCGS SDLYLVTRHADVIPVRRRGDSRGSL LSPRPISYLKGSAGGPLLCPAGHAV GIFRAAVSTRGVAKAVDFIPVESLET TMRSP
DNCR2_1	GWF017	Tagless DNCR2 (His- SMT3 removed by ULP1 cleavage) (E. coli)	Fig. 1D,E; Supp. Fig. 2C-F	MGHHHHHHHHHHGSLQDSEVNQEA KPEVKPEVKPETHINLKVSDGSSEIFF KIKKTTPLRRLMEAFAKRQGKEMDSL RFLYDGIRIQADQAPEDLDMEDNDIIE AHREQIGGSSDEEEARELIERAKEA AERAQEAAERTGDPRVRELARELK RLAQEAAEEVKRDPSSSDVNEALKL IVEAIEAAVDALEAAERTGDPEVREL ARELVRLAVEAAEEVQRNPSSSDVN EALHSIVYAIEAAIFALEAAERTGDPE VRELARELVRLAVEAAEEVQRNPSS RNVEHALMRIVLAIYLAEENLREAEE SGDPEKREKARERVREAVERAEEV QRDPSGWLNH
NS3a_2	GWF016	Avi-His ₆ - NS3a/4a- solubility optimized S139A (<i>E. coli</i>)	Library sorting;	MAGGLNDIFEAQKIEWHEDTGGSSH HHHHHGSGSGSMKKKGSVVIVGRIN LSGDTAYAQQTRGEEGCQETSQTG RDKNQVEGEVQIVSTATQTFLATSIN GVLWTVYHGAGTRTIASPKGPVTQM YTNVDKDLVGWQAPQGSRSLTPCT CGSSDLYLVTRHADVIPVRRRGDSR GSLLSPRPISYLKGSAGGPLLCPAG HAVGIFRAAVSTRGVAKAVDFIPVES LETTMRSP
NS3a_3	GWF005	Avi-His6- NS3a/4a- solubility optimized catalytically active (E. coli)	Fig. 1C; Fig. 2A; Supp. Fig. 2A,B; Supp. Fig. 3C; Supp. Table 1	MAGGLNDIFEAQKIEWHEDTGGSSH HHHHHGSGSGSMKKKGSVVIVGRIN LSGDTAYAQQTRGEEGCQETSQTG RDKNQVEGEVQIVSTATQTFLATSIN GVLWTVYHGAGTRTIASPKGPVTQM YTNVDKDLVGWQAPQGSRSLTPCT CGSSDLYLVTRHADVIPVRRRGDSR GSLLSPRPISYLKGSSGGPLLCPAG HAVGIFRAAVSTRGVAKAVDFIPVES LETTMRSP
G3	GWF041	Yeast surface display G3, C-terminal	Fig. 1A; Supp. Fig.	KDNSSTIEGRYPYDVPDYALQASG GGGSGGGGSGGGSASHMDIEKLC KKAEEAKEAQEKADELRQRHPDS

		fusion to Aga2	3C; Supp. Table 1	QAAEDAEDLANEAEAAVLAACSLA QEHPNADIAKLCIKAASEAAEAASK AAELAQRHPDSQAARDAIKLASQAA RAVILAIMLAAENPNADIAKLCIKAAS EAAEAASKAAELAQRHPDSQAARD AIKLASQAAEAVERAIWLAAENPNA DIAKKCIKAASEAAEEASKAAEEAQ RHPDSQKARDEIKEASQKAEEVKER CKSLEGGGSEQKLISEEDL
GNCR1	GWF351	Yeast surface display GNCR1, C- terminal fusion to Aga2	Supp. Fig. 3C; Supp. Table 1	KDNSSTIEGRYPYDVPDYALQASG GGSGGGGSGGGSASHMDIEKLC KKAEEEAKEAQEKADELRQRHPDS QAAEDAEDLANLAVAAVLTACLLAQ EHPNADIAKLCIKAASEAAEAASKA AELAQRHPDSQAARDAIKLASQAAR AVILAIMLAAENPNADIAKLCIKAASE AAEAASKAAELAQRHPDSQAARDAI KLASQAAEAVERAIWLAAENPNADI AKKCIKAASEAAEEASKAAEEAQRH PDSQKARDEIKEASQKAEEVKERCK SLEGGGSEQKLISEEDL
D5	GWF036	Yeast surface display D5, C-terminal fusion to Aga2	Fig. 1C; Supp. Fig. 2A	KDNSSTIEGRYPYDVPDYALQASG GGSGGGGSGGGSASHMSSDEE EARELIERAKEAAERAQEAAERTGD PRVRELARELKRLAQEAAEEVKRDP SSSDVNEALKLIVEAIEAAVDALEAA ERTGDPEVRELARELVRLAVEAAEE VQRNPSSSDVNEALLTIVIAIEAAVNA LEAAERTGDPEVRELARELVRLAVE AAEEVQRNPSSREVNIALWKIVLAIQ EAVESLREAEESGDPEKREKARERV REAVERAEEVQRDPSGWLNHLEGG GSEQKLISEEDL
DNCR1	GWF040	Yeast surface display DNCR1, C- terminal fusion to Aga2	Supp. Fig. 2A	KDNSSTIEGRYPYDVPDYALQASG GGGSGGGSGGGGSASHMSSDEE EARELIERAKEAAERAQEAAERTGD PRVRELARELKRLAQEAAEEVKRDP SSSDVNEALKLIVEAIEAAVDALEAA ERTGDPEVRELARELVRLAVEAAEE VQRNPSSSDVNEALLSIVIAIEAAVH ALEAAERTGDPEVRELARELVRLAV EAAEEVQRNPSSREVEHALMKIVLAI YEAEESLREAEESGDPEKREKARER VREAVERAEEVQRDPSGWLNHLEG GGSEQKLISEEDL
DNCR2	GWF352	Yeast surface display DNCR2, C- terminal fusion to Aga2	Supp. Fig. 2A,B	KDNSSTIEGRYPYDVPDYALQASG GGSGGGGSGGGSASHMSSDEE EARELIERAKEAAERAQEAAERTGD PRVRELARELKRLAQEAAEEVKRDP SSSDVNEALKLIVEAIEAAVDALEAA ERTGDPEVRELARELVRLAVEAAEE VQRNPSSSDVNEALHSIVYAIEAAIF ALEAAERTGDPEVRELARELVRLAV EAAEEVQRNPSSRNVEHALMRIVLAI YLAEENLREAEESGDPEKREKARER

				VREAVERAEEVQRDPSGWLNHLEG GGSEQKLISEEDL
DNCR2- EGFP	GWF122	(in pcDNA5/FRT /TO)	Fig. 2B; Supp. Fig. 4A; Supp. Fig. 7A,C	MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLGSGTGSGTMVSK GEELFTGVVPILVELDGDVNGHKFSV SGEGEGDATYGKLTLKFICTTGKLPV PWPTLVTTLTYGVQCFSRYPDHMKQ HDFFKSAMPEGYVQERTIFFKDDGN YKTRAEVKFEGDTLVNRIELKGIDFKE DGNILGHKLEYNYNSHNVYIMADKQK NGIKVNFKIRHNIEDGSVQLADHYQQ NTPIGDGPVLLPDNHYLSTQSALSKD PNEKRDHMVLLEFVTAAGITLGMDEL YK
Tom20- DNCR1- EGFP	GWF115	(in pcDNA5/FRT /TO)	Supp. Fig. 6B,D	MVGRNSAIAAGVCGALFIGYCIYFDR KRRSDPNFSSDEEEARELIERAKEA AERAQEAAERTGDPRVRELARELK RLAQEAAEEVKRDPSSSDVNEALKL IVEAIEAAVDALEAAERTGDPEVREL ARELVRLAVEAAEEVQRNPSSSDVN EALLSIVIAIEAAVHALEAAERTGDPE VRELARELVRLAVEAAEEVQRNPSS REVEHALMKIVLAIYEAEESLREAEE SGDPEKREKARERVREAVERAEEV QRDPSGWLNHEQKLISEEDLGSGTG SGTMVSKGEELFTGVVPILVELDGDV NGHKFSVSGEGEGDATYGKLTLKFIC TTGKLPVPWPTLVTTLTYGVQCFSRY PDHMKQHDFFKSAMPEGYVQERTIF FKDDGNYKTRAEVKFEGDTLVNRIEL KGIDFKEDGNILGHKLEYNYNSHNVYI MADKQKNGIKVNFKIRHNIEDGSVQL ADHYQQNTPIGDGPVLLPDNHYLST QSALSKDPNEKRDHMVLLEFVTAAGI TLGMDELYK
DNCR1- EGFP- Giantin	GWF114	(in pcDNA5/FRT /TO)	Supp. Fig. 6B,C	MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALLSIVIAI EAAVHALEAAERTGDPEVRELAREL VRLAVEAAEEVQRNPSSREVEHALM KIVLAIYEAEESLREAEESGDPEKRE KARERVREAVERAEEVQRDPSGWL NHEQKLISEEDLGSGTGSGTMVSKG EELFTGVVPILVELDGDVNGHKFSVS GEGEGDATYGKLTLKFICTTGKLPVP WPTLVTTLTYGVQCFSRYPDHMKQH

				DFFKSAMPEGYVQERTIFFKDDGNY KTRAEVKFEGDTLVNRIELKGIDFKED GNILGHKLEYNYNSHNVYIMADKQKN GIKVNFKIRHNIEDGSVQLADHYQQN TPIGDGPVLLPDNHYLSTQSALSKDP NEKRDHMVLLEFVTAAGITLGMDELY KGSGTGSGSGEPQQSFSEAQQQLC NTRQEVNELRKLLEEERDQRVAAEN ALSVAEEQIRRLEHSEWDSSRTPIIGS CGTQEQALLIDLTSNSCRRTRSGVG WKRVLRSLCHSRTRVPLLAAIYFLMI HVLLILCFTGHL
3xNLS- DNCR1- EGFP	GWF111	(in pcDNA5/FRT /TO)	Supp. Fig. 6B	MDPKKKRKVDPKKKRKVDPKKKRKV SSDEEEARELIERAKEAAERAQEAA ERTGDPRVRELARELKRLAQEAAEE VKRDPSSSDVNEALKLIVEAIEAAVD ALEAAERTGDPEVRELARELVRLAV EAAEEVQRNPSSSDVNEALLSIVIAIE AAVHALEAAERTGDPEVRELARELV RLAVEAAEEVQRNPSSREVEHALM KIVLAIYEAEESLREAEESGDPEKRE KARERVREAVERAEEVQRDPSGWL NHEQKLISEEDLGSGTGSGTMVSKG EELFTGVVPILVELDGDVNGHKFSVS GEGEGDATYGKLTLKFICTTGKLPVP WPTLVTTLTYGVQCFSRYPDHMKQH DFFKSAMPEGYVQERTIFFKDDGNY KTRAEVKFEGDTLVNRIELKGIDFKED GNILGHKLEYNYNSHNVYIMADKQKN GIKVNFKIRHNIEDGSVQLADHYQQN TPIGDGPVLLPDNHYLSTQSALSKDP NEKRDHMVLLEFVTAAGITLGMDELY K
DNCR1- EGFP	GWF112	(in pcDNA5/FRT /TO)	Supp. Fig. 6A	MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALLSIVIAI EAAVHALEAAERTGDPEVRELAREL VRLAVEAAEEVQRNPSSREVEHALM KIVLAIYEAEESLREAEESGDPEKRE KARERVREAVERAEEVQRDPSGWL NHEQKLISEEDLGSGTGSGTMVSKG EELFTGVVPILVELDGDVNGHKFSVS GEGEGDATYGKLTLKFICTTGKLPVP WPTLVTTLTYGVQCFSRYPDHMKQH DFFKSAMPEGYVQERTIFFKDDGNY KTRAEVKFEGDTLVNRIELKGIDFKED GNILGHKLEYNYNSHNVYIMADKQKN GIKVNFKIRHNIEDGSVQLADHYQQN TPIGDGPVLLPDNHYLSTQSALSKDP NEKRDHMVLLEFVTAAGITLGMDELY K
mCherry- NS3a	GWF104	NS3a solubility	Supp. Fig. 6B,C,D	MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK

	•	110		
		optimized,		AYVKHPADIPDYLKLSFPEGFKWERV
		S139A		MNFEDGGVVTVTQDSSLQDGEFIYK
		(in		VKLRGTNFPSDGPVMQKKTMGWEA
		pcDNA5/FRT		SSERMYPEDGALKGEIKQRLKLKDG
		/TO)		GHYDAEVKTTYKAKKPVQLPGAYNV
				NIKLDITSHNEDYTIVEQYERAEGRHS
				TGGMDELYKGSGTGDYKDDDDK KK
				KGSVVIVGRINLSGDTAYAQQTRGE
				EGCQETSQTGRDKNQVEGEVQIVST
				ATQTFLATSINGVLWTVYHGAGTRTI
				ASPKGPVTQMYTNVDKDLVGWQAP
				QGSRSLTPCTCGSSDLYLVTRHADV
				IPVRRRGDSRGSLLSPRPISYLKGSA
				GGPLLCPAGHAVGIFRAAVSTRGVA
				KAVDFIPVESLETTMRSP
Tom20-	GWF105	NS3a	Fig. 2B;	MVGRNSAIAAGVCGALFIGYCIYFDR
mCherry-	0111100	solubility	Supp. Fig. 6A	KRRSDPNFGSGMVSKGEEDNMAIIK
NS3a		optimized,	Cupp. 1 ig. 6/ (EFMRFKVHMEGSVNGHEFEIEGEGE
11000		S139A		GRPYEGTQTAKLKVTKGGPLPFAWD
		(in		ILSPQFMYGSKAYVKHPADIPDYLKL
		pcDNA5/FRT		SFPEGFKWERVMNFEDGGVVTVTQ
		/TO)		DSSLQDGEFIYKVKLRGTNFPSDGPV
		/10)		MQKKTMGWEASSERMYPEDGALKG
				EIKQRLKLKDGGHYDAEVKTTYKAKK
				PVQLPGAYNVNIKLDITSHNEDYTIVE
				QYERAEGRHSTGGMDELYKGSGTG
				DYKDDDDK KKKGSVVIVGRINLSGD
				TAYAQQTRGEEGCQETSQTGRDKN
				QVEGEVQIVSTATQTFLATSINGVLW
				TVYHGAGTRTIASPKGPVTQMYTNV
				DKDLVGWQAPQGSRSLTPCTCGSS
				DLYLVTRHADVIPVRRRGDSRGSLL
				SPRPISYLKGSAGGPLLCPAGHAVGI
				FRAAVSTRGVAKAVDFIPVESLETTM
				RSP
mCherry-	GWF107	NS3a	Supp. Fig. 6A	MVSKGEEDNMAIIKEFMRFKVHMEG
NS3a-Giantin		solubility		SVNGHEFEIEGEGEGRPYEGTQTAK
		optimized,		LKVTKGGPLPFAWDILSPQFMYGSK
		S139A		AYVKHPADIPDYLKLSFPEGFKWERV
		(in		MNFEDGGVVTVTQDSSLQDGEFIYK
		pcDNA5/FRT		VKLRGTNFPSDGPVMQKKTMGWEA
		/TO)		SSERMYPEDGALKGEIKQRLKLKDG
				GHYDAEVKTTYKAKKPVQLPGAYNV
				NIKLDITSHNEDYTIVEQYERAEGRHS
				TGGMDELYKGSGTGDYKDDDDK KK
				KGSVVIVGRINLSGDTAYAQQTRGE
				EGCQETSQTGRDKNQVEGEVQIVST
				ATQTFLATSINGVLWTVYHGAGTRTI
				ASPKGPVTQMYTNVDKDLVGWQAP
				QGSRSLTPCTCGSSDLYLVTRHADV
				IPVRRRGDSRGSLLSPRPISYLKGSA
				GGPLLCPAGHAVGIFRAAVSTRGVA
				KAVDFIPVESLETTMRSPGSGTGSG
				SGEPQQSFSEAQQQLCNTRQEVNEL
				RKLLEEERDQRVAAENALSVAEEQIR
1	I		1	RLEHSEWDSSRTPIIGSCGTQEQALL

				IDLTSNSCRRTRSGVGWKRVLRSLC HSRTRVPLLAAIYFLMIHVLLILCFTGH L
3xNLS- mCherry- NS3a	GWF106	NS3a solubility optimized, S139A (in pcDNA5/FRT /TO)	Supp. Fig. 6A	MDPKKKRKVDPKKKRKVDPKKKRKV GSGMVSKGEEDNMAIIKEFMRFKVH MEGSVNGHEFEIEGEGEGEGRPYEGTQ TAKLKVTKGGPLPFAWDILSPQFMYG SKAYVKHPADIPDYLKLSFPEGFKWE RVMNFEDGGVVTVTQDSSLQDGEFI YKVKLRGTNFPSDGPVMQKKTMGW EASSERMYPEDGALKGEIKQRLKLKD GGHYDAEVKTTYKAKKPVQLPGAYN VNIKLDITSHNEDYTIVEQYERAEGRH STGGMDELYKGSGTGDYKDDDDKK KKGSVVIVGRINLSGDTAYAQQTRG EEGCQETSQTGRDKNQVEGEVQIVS TATQTFLATSINGVLWTVYHGAGTR TIASPKGPVTQMYTNVDKDLVGWQA PQGSRSLTPCTCGSSDLYLVTRHAD VIPVRRRGDSRGSLLSPRPISYLKGS AGGPLLCPAGHAVGIFRAAVSTRGV AKAVDFIPVESLETTMRSP
Myristoyl-tag- mCherry- NS3a	GWF100	NS3a solubility optimized, S139A (in pcDNA5/FRT /TO)	Supp. Fig. 4A; Supp. Fig. 6A; Supp. Fig. 4B	MGCGCSSHPEDDGSGTGSGMVSKG EEDNMAIIKEFMRFKVHMEGSVNGH EFEIEGEGEGRPYEGTQTAKLKVTKG GPLPFAWDILSPQFMYGSKAYVKHP ADIPDYLKLSFPEGFKWERVMNFED GGVVTVTQDSSLQDGEFIYKVKLRGT NFPSDGPVMQKKTMGWEASSERMY PEDGALKGEIKQRLKLKDGGHYDAE VKTTYKAKKPVQLPGAYNVNIKLDITS HNEDYTIVEQYERAEGRHSTGGMDE LYKGSGTGDYKDDDDKKKKGSVVIV GRINLSGDTAYAQQTRGEEGCQETS QTGRDKNQVEGEVQIVSTATQTFLA TSINGVLWTVYHGAGTRTIASPKGPV TQMYTNVDKDLVGWQAPQGSRSLT PCTCGSSDLYLVTRHADVIPVRRRG DSRGSLLSPRPISYLKGSAGGPLLC PAGHAVGIFRAAVSTRGVAKAVDFIP VESLETTMRSP
NS3aH1- mCherry	GWF094	ANR-binding- competent NS3a, catalytically active (in pcDNA5/FRT /TO)	Fig. 2C,D; Supp. Fig. 5	MKKKGSVVIVGRINLSGDTAYSQQT RGLEGCQETSQTGRDKNQVEGEVQ VVSTATQSFLATSINGVLWTVYHGA GTRTIASPKGPVTQMYTNVDKDLVG WQAPQGSRSLTPCTCGSSDLYLVT RHADVIPVRRRGDSRGSLLSPRPISY LKGSSGGPLLCPAGHAVGIFRAAVS TRGVAKAVDFIPVESLETTMRSPGS GTGSGMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEG TQTAKLKVTKGGPLPFAWDILSPQFM YGSKAYVKHPADIPDYLKLSFPEGFK WERVMNFEDGGVVTVTQDSSLQDG EFIYKVKLRGTNFPSDGPVMQKKTM GWEASSERMYPEDGALKGEIKQRLK

	T	1	1	
				LKDGGHYDAEVKTTYKAKKPVQLPG
				AYNVNIKLDITSHNEDYTIVEQYERAE
				GRHSTGGMDELYKGSGTGDYKDDD
				DK
3xNLS-	GWF124	(in	Fig. 2D;	MDPKKKRKVDPKKKRKVDPKKKRKV
DNCR2-		pcDNA5/FRT	Supp. Fig. 5B	GSG SSDEEEARELIERAKEAAERAQ
EGFP		/TO)		EAAERTGDPRVRELARELKRLAQEA
				AEEVKRDPSSSDVNEALKLIVEAIEA
				AVDALEAAERTGDPEVRELARELVR
				LAVEAAEEVQRNPSSSDVNEALHSI
				VYAIEAAIFALEAAERTGDPEVRELA
				RELVRLAVEAAEEVQRNPSSRNVEH
				ALMRIVLAIYLAEENLREAEESGDPE
				KREKARERVREAVERAEEVQRDPS
				GWLNH EQKLISEEDLGSGTGSGTMV
				SKGEELFTGVVPILVELDGDVNGHKF
				SVSGEGEGDATYGKLTLKFICTTGKL
				PVPWPTLVTTLTYGVQCFSRYPDHM
				KQHDFFKSAMPEGYVQERTIFFKDD
				GNYKTRAEVKFEGDTLVNRIELKGID
				FKEDGNILGHKLEYNYNSHNVYIMAD
				KQKNGIKVNFKIRHNIEDGSVQLADH
				YQQNTPIGDGPVLLPDNHYLSTQSAL
				SKDPNEKRDHMVLLEFVTAAGITLGM
				DELYK
ANR-ANR-	GWF135	/in	Fig. 2D;	MGELDELVYLLDGPGYDPIHSDGSG
BFP-CAAX	GWF135	(in pcDNA5/FRT		TGSGTGSGTGTTSGTGTGGSTG GE
BFF-CAAX			Supp. Fig. 5B	
		/TO)		LDELVYLLDGPGYDPIHSDGSGTGS
				GTGSGTGTTSGTGTGGSTGEQKLIS
				EEDLGSGSSELIKENMHMKLYMEGT
				VDNHHFKCTSEGEGKPYEGTQTMRI
				KVVEGGPLPFAFDILATSFLYGSKTFI
				NHTQGIPDFFKQSFPEGFTWERVTT
				YEDGGVLTATQDTSLQDGCLIYNVKI
				RGVNFTSNGPVMQKKTLGWEAFTET
				LYPADGGLEGRNDMALKLVGGSHLI
				ANIKTTYRSKKPAKNLKMPGVYYVDY
				RLERIKEANNETYVEQHEVAVARYCD
				LPSKLGHKLNRKHKEKMSKDGKKKK
				KKSKTKCVIM
Tom20-BFP-	GWF143	(in	Supp. Fig.	MVGRNSAIAAGVCGALFIGYCIYFDR
ANR-ANR-		pcDNA5/FRT	7B,D	KRRSDPNFMSELIKENMHMKLYMEG
P2a-DNCR2-		/TO)		TVDNHHFKCTSEGEGKPYEGTQTMR
EGFP-CAAX				IKVVEGGPLPFAFDILATSFLYGSKTFI
				NHTQGIPDFFKQSFPEGFTWERVTT
				YEDGGVLTATQDTSLQDGCLIYNVKI
				RGVNFTSNGPVMQKKTLGWEAFTET
				LYPADGGLEGRNDMALKLVGGSHLI
				ANIKTTYRSKKPAKNLKMPGVYYVDY
				RLERIKEANNETYVEQHEVAVARYCD
				LPSKLGHKLNSGSGEQKLISEEDLGS
				GTGSGTGSGTGTTSGTGTGGSTG G
				ELDELVYLLDGPGYDPIHSD GSGTG
				SGTGSGTGTTSGTGTGGSTGGELD
				ELVYLLDGPGYDPIHSD GSGATNFSL
				LKQAGDVEENPGPM SSDEEEARELI
	1	1		LINGAGD VEENT GPIVIOODEEEARELI

Tom20- DNCR2- EGFP GWF125 GNCR1- EGFP GNCR1- GNC				1	
MEALKLIVAIEAAVDALEAAERTGOD PEWRELARELURLAVEAAEEVQRNP SSDVNEALHSIVYAIEAAIFALEAAE RTGDPEWRELARELURLAVEAAEEV QRNPSSRNVEHALMRIVLAYILAEEN LREAEESGOPEKREKARERVREAVE RAEEVQRDPSGWINHEGKILSEEDL GSGTGSGTMVSKGEELFTGVVPILVE LDGDVNGHKFSVSGEGGGDATYGKL TLKFICTTGKLPVPPWPTLYTTLTYGV QCFSRYPDHMKQHDFFKSAMPEGY VQERTIFFKDDGNYKTRAEVKFEGDT LVMRIELKGIDFKEDGNILGHKLEYNY NSHNVYIMADKGKNGIKVNFKIRNNIE DGSVQLADHYQQNTPIGGGPVLLPD NHYLSTGSALSKOPNEKRDHMVLLE FVTAAGITLGMDELYKRKHKEKMSKG GKKKKKSKTKCVIM WGRNSAIAAGVCGALFIGYCIYFDR KRRSDPNFSSDEEARELIERAKEA GKKKKKKSTKCVIM WGRNSAIAAGVCGALFIGYCIYFDR KRRSDPNFSSDEEARELIERAKEA KRLQEAAEEVKRDPSSSDVNEALKL IVEAIEAAVDALEAAERTGOPEVREL ARELVRLAVEAAEEVQRNPSS RNVEHALMRIVLAIPLAEENLERAEE SGDPEKREKARERVREAVERAEEV QRDPSGMLNHEQKLISEEDLGSGTG SGTMVSKGEELFTGVVPILVELDGDV NGHKFSVSGEGGBDATYGKLTLKFIG TTGKLPVPPTLYTTLTYGVGCFSRY PDHMKQHOFFKSAMPEGYVGERTIF FKDDGNYKTRAEVKFEGDTLVNRIEL KGIDFKEDGNILGHKLEYNYNSHNVY MADKQKNGIKVNFKIRRNIEDGSVQL ADHYQQNTPIGGPVLLPDNYLST QSALSKOPNEKRDHMVLLEFVTAAGI TLGMDELYK MADKQKNGIKVNFKIRRNIEDGSVQL ADHYQQNTPIGGPVLLPDNYLST QSALSKOPNEKRDHMVLLEFVTAAGI TLGMDELYK LTGMDELYK LTGMCTATAGI TLGMCTATAGI TCGCTATAGI TLGMCTATAGI TLGMCTAGI					ERAKEAAERAQEAAERTGDPRVRE
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PDHMKQHDFFKSAMPEGYVQERTIF FKDDGNYKTRAEVKFEGDTLVNRIEL KGIDFKEDGNILGHKLEYNYNSHNVYI MADKQKNGIKVNFKIRHNIEDGSVQL ADHYQQNTPIGDGPVLLPDNHYLST QSALSKDPNEKRDHMVLLEFVTAAGI TLGMDELYK GNCR1- BFP-CAAX (in pcDNA5/FRT //TO) Fig. 2C; Supp. Fig. 5A MDIEKLCKKAEEEAKEAQEKADELR QRHPDSQAAEDAEDLANLAVAAVL TACLLAQEHPNADIAKLCIKAASEAA EAASKAAELAQRHPDSQAARDAIKL ASQAARAVILAIMLAAENPNADIAKL CIKAASEAAEASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
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MADKQKNGIKVNFKIRHNIEDGSVQL ADHYQQNTPIGDGPVLLPDNHYLST QSALSKDPNEKRDHMVLLEFVTAAGI TLGMDELYK GNCR1- BFP-CAAX (in pcDNA5/FRT /TO) Fig. 2C; Supp. Fig. 5A MDIEKLCKKAEEEAKEAQEKADELR QRHPDSQAAEDAEDLANLAVAAVL TACLLAQEHPNADIAKLCIKAASEAA EAASKAAELAQRHPDSQAARDAIKL ASQAARAVILAIMLAAENPNADIAKL CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
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BFP-CAAX pcDNA5/FRT /TO) Supp. Fig. 5A QRHPDSQAAEDAEDLANLAVAAVL TACLLAQEHPNADIAKLCIKAASEAA EAASKAAELAQRHPDSQAARDAIKL ASQAARAVILAIMLAAENPNADIAKL CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
TACLLAQEHPNADIAKLCIKAASEAA EAASKAAELAQRHPDSQAARDAIKL ASQAARAVILAIMLAAENPNADIAKL CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ		GWF148			•
EAASKAAELAQRHPDSQAARDAIKL ASQAARAVILAIMLAAENPNADIAKL CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ	BFP-CAAX			Supp. Fig. 5A	
ASQAARAVILAIMLAAENPNADIAKL CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ			/TO)		TACLLAQEHPNADIAKLCIKAASEAA
CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					EAASKAAELAQRHPDSQAARDAIKL
CIKAASEAAEAASKAAELAQRHPDS QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					ASQAARAVILAIMLAAENPNADIAKL
QAARDAIKLASQAAEAVERAIWLAA ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
ENPNADIAKKCIKAASEAAEEASKA AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
AEEAQRHPDSQKARDEIKEASQKAE EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
EVKERCKSEQKLISEEDLGSGSSELI KENMHMKLYMEGTVDNHHFKCTSE GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					
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GEGKPYEGTQTMRIKVVEGGPLPFA FDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQ					· ·
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QSFPEGFTWERVTTYEDGGVLTATQ					•
DTSLQDGCLIYNVKIRGVNFTSNGPV					
		<u></u>			DTSLQDGCLIYNVKIRGVNFTSNGPV

	T		ı	
				MQKKTLGWEAFTETLYPADGGLEGR
				NDMALKLVGGSHLIANIKTTYRSKKP
				AKNLKMPGVYYVDYRLERIKEANNET
				YVEQHEVAVARYCDLPSKLGHKLNR
				KHKEKMSKDGKKKKKKSKTKCVIM
NS3aH1-	GWF096	ANR-binding-	Supp. Fig.	MKKKGSVVIVGRINLSGDTAYSQQT
mCherry-		competent	7A,C	RGLEGCQETSQTGRDKNQVEGEVQ
CAAX		NS3a,	174,0	VVSTATQSFLATSINGVLWTVYHGA
0,000		catalytically		GTRTIASPKGPVTQMYTNVDKDLVG
		active		WQAPQGSRSLTPCTCGSSDLYLVT
				RHADVIPVRRRGDSRGSLLSPRPISY
		(in		
		pcDNA5/FRT		LKGSSGGPLLCPAGHAVGIFRAAVS
		/TO)		TRGVAKAVDFIPVESLETTMRSPGS
				GTGSGMVSKGEEDNMAIIKEFMRFK
				VHMEGSVNGHEFEIEGEGEGRPYEG
				TQTAKLKVTKGGPLPFAWDILSPQFM
				YGSKAYVKHPADIPDYLKLSFPEGFK
				WERVMNFEDGGVVTVTQDSSLQDG
				EFIYKVKLRGTNFPSDGPVMQKKTM
				GWEASSERMYPEDGALKGEIKQRLK
				LKDGGHYDAEVKTTYKAKKPVQLPG
				AYNVNIKLDITSHNEDYTIVEQYERAE
				GRHSTGGMDELYKGSGTGDYKDDD
				DKQHKLRKLNPPDESGPGCMSCKC
				VLS
GNCR1-BFP	GWF146	(in	Supp. Fig.	MDIEKLCKKAEEEAKEAQEKADELR
		pcDNA5/FRT	7A,C	QRHPDSQAAEDAEDLANLAVAAVL
		/TO)		TACLLAQEHPNADIAKLCIKAASEAA
		,		EAASKAAELAQRHPDSQAARDAIKL
				ASQAARAVILAIMLAAENPNADIAKL
				CIKAASEAAEAASKAAELAQRHPDS
				QAARDAIKLASQAAEAVERAIWLAA
				ENPNADIAKKCIKAASEAAEEASKA
				AEEAQRHPDSQKARDEIKEASQKAE
				EVKERCKS EQKLISEEDLGSGSSELI
				KENMHMKLYMEGTVDNHHFKCTSE
				GEGKPYEGTQTMRIKVVEGGPLPFA
				FDILATSFLYGSKTFINHTQGIPDFFK
				QSFPEGFTWERVTTYEDGGVLTATQ
				DTSLQDGCLIYNVKIRGVNFTSNGPV
				MQKKTLGWEAFTETLYPADGGLEGR
				NDMALKLVGGSHLIANIKTTYRSKKP
				AKNLKMPGVYYVDYRLERIKEANNET
				YVEQHEVAVARYCDLPSKLGHKLN
DNCR2-iSH2	GWF129	Inter-SH2	Supp. Fig. 4B	MSSDEEARELIERAKEAAERAQEA
DINCKZ-ISHZ	GVVF 129		Supp. Fig. 4B	
		domain of		AERTGDPRVRELARELKRLAQEAAE
		human		EVKRDPSSSDVNEALKLIVEAIEAAV
		PIP3K fused		DALEAAERTGDPEVRELARELVRLA
		to C-term of		VEAAEEVQRNPSSSDVNEALHSIVY
		DNCR2		AIEAAIFALEAAERTGDPEVRELARE
		(in		LVRLAVEAAEEVQRNPSSRNVEHAL
		pcDNA5/FRT		MRIVLAIYLAEENLREAEESGDPEKR
		/TO)		EKARERVREAVERAEEVQRDPSGW
		,		LNHEQKLISEEDLGSGTGSGTRLLYP
				VSKYQQDQIVKEDSVEAVGAQLKVY HQQYQDKSREYDQLYEEYTRTSQEL

				QMKRTAIEAFNETIKIFEEQGQTQEK CSKEYLERFRREGNEKEMQRILLNSE
				RLKSRIAEIHESRTKLEQQLRAQASD NREIDKRMNSLKPDLMQLRKIRDQYL VWLTQKGARQKKINEWLGIKNETED QYALMEDEDDLP
DNCR2-VPR	GWF180	In pB-CAG- DNCR2- VPR-IRES- Puro-WPRE- SV40PA- PGK- NS3aH1- tagBFP- SpdCas9	Fig. 2A,B,C; Supp. Fig. 10A,C	MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLEFSSAAGTSDALD DFDLDMLGSDALDDFDLDMLGSDAL DDFDLDMLGSDALDDFDLDMLGSPK KKRKVGSQYLPDTDDRHRIEEKRKR TYETFKSIMKKSPFSGPTDPRPPRR IAVPSRSSASVPKPAPQPYPFTSSLS TINYDEFPTMVFPSGQISQASALAPA PPQVLPQAPAPAPAPAMVSALAQAP APVPVLAPGPPQAVAPPAPKPTQAG EGTLSEALLQLQFDDEDLGALLGNST DPAVFTDLASVDNSEFQQLLNQGIPV APHTTEPMLMEYPEAITRLVTGAQRP PDPAPAPLGAPGLPNGLLSGDEDFS SIADMDFSALLSQISSGSGSGSRDSR EGMFLPKPEAGSAISDVFEGREVCQ PKRIRPFHPPGSPWANRPLPASLAPT PTGPVHEPVGSLTPAPVPQPLDPAP AVTPEASHLLEDPDEETSQAVKALRE MADTVIPQKEEAAICGQMDLSHPPPR GHLDELTTTLESMTEDLNLDSPLTPE LNEILDTFLNDECLLHAMHISTGLSIFD TSLF
DNCR2- KRAB	GWF181	In pB-CAG- DNCR2- KRAB-IRES- Puro-WPRE- SV40PA- PGK- NS3aH1- tagBFP- SpdCas9	Supp. Fig. 10B,D	MSSDEEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLEFSSAAGTSGG GGMDAKSLTAWSRTLVTFKDVFVDF TREEWKLLDTAQQIVYRNVMLENYK NLVSLGYQLTKPDVILRLEKGEEP
NS3aH1- tagBFP- SpdCas9	GWF180,181	ANR-binding- competent NS3a, catalytically active,	Fig. 2A,B,C; Supp. Fig. 10A-D	MKKKGSVVIVGRINLSGDTAYSQQT RGLEGCQETSQTGRDKNQVEGEVQ VVSTATQSFLATSINGVLWTVYHGA GTRTIASPKGPVTQMYTNVDKDLVG WQAPQGSRSLTPCTCGSSDLYLVT RHADVIPVRRRGDSRGSLLSPRPISY

In pB-CAG-DNCR2-VPR/KRAB-IRES-Puro-WPRE-SV40PA-PGK-NS3aH1tagBFP-SpdCas9

LKGSSGGPLLCPAGHAVGIFRAAVS TRGVAKAVDFIPVESLETTMRSPHM SSAAGATMSELIKENMHMKLYMEGT VDNHHFKCTSEGEGKPYEGTQTMRI KVVEGGPLPFAFDILATSFLYGSKTFI NHTQGIPDFFKQSFPEGFTWERVTT YEDGGVLTATQDTSLQDGCLIYNVKI RGVNFTSNGPVMQKKTLGWEAFTET LYPADGGLEGRNDMALKLVGGSHLI ANIKTTYRSKKPAKNLKMPGVYYVDY RLERIKEANNETYVEQHEVAVARYCD LPSKLGHKLNSSAAGATMDKKYSIGL AIGTNSVGWAVITDEYKVPSKKFKVL **GNTDRHSIKKNLIGALLFDSGETAEAT** RLKRTARRRYTRRKNRICYLQEIFSN **EMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRK** KLVDSTDKADLRLIYLALAHMIKFRGH FLIEGDLNPDNSDVDKLFIQLVQTYN QLFEENPINASGVDAKAILSARLSKSR RLENLIAQLPGEKKNGLFGNLIALSLG LTPNFKSNFDLAEDAKLQLSKDTYDD DLDNLLAQIGDQYADLFLAAKNLSDAI LLSDILRVNTEITKAPLSASMIKRYDE **HHQDLTLLKALVRQQLPEKYKEIFFD** QSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTF DNGSIPHQIHLGELHAILRRQEDFYPF LKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPK **HSLLYEYFTVYNELTKVKYVTEGMRK** PAFLSGEQKKAIVDLLFKTNRKVTVK QLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDI LEDIVLTLTLFEDREMIEERLKTYAHL FDDKVMKQLKRRRYTGWGRLSRKLI NGIRDKQSGKTILDFLKSDGFANRNF **MQLIHDDSLTFKEDIQKAQVSGQGDS** LHEHIANLAGSPAIKKGILQTVKVVDE LVKVMGRHKPENIVIEMARENQTTQK **GQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMY** VDQELDINRLSDYDVDAIVPQSFLKD DSIDNKVLTRSDKNRGKSDNVPSEE VVKKMKNYWRQLLNAKLITQRKFDN LTKAERGGLSELDKAGFIKRQLVETR QITKHVAQILDSRMNTKYDENDKLIRE VKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLE SEFVYGDYKVYDVRKMIAKSEQEIGK ATAKYFFYSNIMNFFKTEITLANGEIR KRPLIETNGETGEIVWDKGRDFATVR KVLSMPQVNIVKKTEVQTGGFSKESI LPKRNSDKLIARKKDWDPKKYGGFD SPTVAYSVLVVAKVEKGKSKKLKSVK

				ELLGITIMERSSFEKNPIDFLEAKGYK EVKKDLIIKLPKYSLFELENGRKRMLA SAGELQKGNELALPSKYVNFLYLASH YEKLKGSPEDNEQKQLFVEQHKHYL DEIIEQISEFSKRVILADANLDKVLSAY NKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLI HQSITGLYETRIDLSQLGGDAYPYDV PDYASLGSGSPKKKRKVEDPKKKRK VDGIGSGSNG
CXCR4-C1	GWF271	C1 guide in gRNA_Clonin g Vector	Fig. 2A,B; Supp. Fig. 10A,B	GCGGGTGGTCGGTAGTGAGTC
CXCR4-C2	GWF272	C2 guide in gRNA_Clonin g Vector	Fig. 2A,B; Supp. Fig. 10A,B	GCAGACGCGAGGAAGGAGGCGC
CXCR4-C3	GWF273	C3 guide in gRNA_Clonin g Vector	Fig. 2A,B; Supp. Fig. 10A,B	GCCTCTGGGAGGTCCTGTCCGGCT C
CD95-1	GWF279	CD95-1 guide in gRNA_Clonin g Vector	Fig. 2C; Supp. Fig. 10C,D	GTACAGCAGAAGCCTTTAGAA
CD95-2	GWF280	CD95-2 guide in gRNA_Clonin g Vector	Fig. 2C; Supp. Fig. 10C,D	GTGGCATGCTCACTTCAGGTG
CD95-3	GWF281	CD95-3 guide in gRNA_Clonin g Vector	Fig. 2C; Supp. Fig. 10C,D	GAAGCCTCGCTGGGGAACGCC
CXCR4-C1- 2xMS2	GWF310	scRNA, wt + f6 MS2 expressed with NLS- MCP- GNCR1-P2a- BFP	Fig. 2D,E,F; Supp. Fig. 8B,C,D	GCGGGTGGTCGGTAGTGAGTCGTT TAAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAAGTGGCACCGA GTCGGTGCGGGAGCACATGAGGAT CACCCATGTGCGACTCCCACAGTC ACTGGGGAGTCTTCCCTTTTTTTGT TTTTTATGTCT
CXCR4-C2- 2xMS2	GWF311	scRNA, 2x wt MS2 expressed with NLS- MCP- GNCR1-P2a- BFP	Fig. 2D,E,F; Supp. Fig. 8B,C,D	GCAGACGCGAGGAAGGAGGCGC GTTTAAGAGCTATGCTGGAAACAGC ATAGCAAGTTTAAATAAGGCTAGTC CGTTATCAACTTGAAAAAGTGGCAC CGAGTCGGTGCGGGAGCACATGAG GATCACCCATGTGCCACGAGCGAC ATGAGGATCACCCATGTCGCTCGT GTTCCCTTTTTTTTTT
CXCR4-C3- 2xMS2	GWF312	scRNA, wt + f6 expressed with NLS- MCP-	Fig. 2D,E,F; Supp. Fig. 8B,C,D	GCCTCTGGGAGGTCCTGTCCGGCT CGTTTAAGAGCTATGCTGGAAACAG CATAGCAAGTTTAAATAAGGCTAGT CCGTTATCAACTTGAAAAAGTGGCA CCGAGTCGGTGCGGGAGCACATGA

	1	GNCR1-P2a-	<u> </u>	COATCACCCATCTCCCACTCCCAC
				GGATCACCCATGTGCGACTCCCAC
		BFP		AGTCACTGGGGAGTCTTCCCTTTTT
				TTGTTTTTATGTCT
NLS-MCP-	GWF310-2	Expressed	Fig. 2D,E,F;	MPKKKRKVGSMASNFTQFVLVDNG
GNCR1-P2a-		with CXCR4-	Supp. Fig.	GTGDVTVAPSNFANGIAEWISSNSRS
BFP		2xMS2	8B,C,D	QAYKVTCSVRQSSAQNRKYTIKVEV
		scRNAs		PKGAWRSYLNMELTIPIFATNSDCELI
				VKAMQGLLKDGNPIPSAIAANSGIYG
				SGGSG DIEKLCKKAEEEAKEAQEKA
				DELRQRHPDSQAAEDAEDLANLAV
				AAVLTACLLAQEHPNADIAKLCIKAA
				SEAAEAASKAAELAQRHPDSQAAR
				DAIKLASQAARAVILAIMLAAENPNA
				DIAKLCIKAASEAAEAASKAAELAQ
				RHPDSQAARDAIKLASQAAEAVERA
				IWLAAENPNADIAKKCIKAASEAAEE
				ASKAAEEAQRHPDSQKARDEIKEAS
				QKAEEVKERCKSEQKLISEEDLGSG
				ATNFSLLKQAGDVEENPGPSELIKEN
				MHMKLYMEGTVDNHHFKCTSEGEG
				KPYEGTQTMRIKVVEGGPLPFAFDIL
				ATSFLYGSKTFINHTQGIPDFFKQSFP
				EGFTWERVTTYEDGGVLTATQDTSL
				QDGCLIYNVKIRGVNFTSNGPVMQK
				KTLGWEAFTETLYPADGGLEGRNDM
				ALKLVGGSHLIANIKTTYRSKKPAKNL
				KMPGVYYVDYRLERIKEANNETYVE
				QHEVAVARYCDLPSKLGHKLN
CXCR4-C1-	GWF313	scRNA	Supp. Fig.	GCGGGTGGTCGGTAGTGAGTC GTT
com		expressed	10G	TAAGAGCTATGCTGGAAACAGCATA
33		with NLS-		GCAAGTTTAAATAAGGCTAGTCCGT
		com-GNCR1-		TATCAACTTGAAAAAGTGGCACCGA
		P2a-BFP		GTCGGTGCCTGAATGCCTGCGAGC
		1 24-511		ATCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
CXCR4-C2-	GWF314	scRNA	Cupp Fig	GCAGACGCGAGGAAGGAGGCGC
	GWF314		Supp. Fig. 10G	GTTTAAGAGCTATGCTGGAAACAGC
com		expressed	100	
		with NLS-		ATAGCAAGTTTAAATAAGGCTAGTC
		com-GNCR1-		CGTTATCAACTTGAAAAAGTGGCAC
		P2a-BFP		CGAGTCGGTGCCTGAATGCCTGCG
			_	AGCATCTTTTTTTGTTTTTATGTCT
CXCR4-C3-	GWF315	scRNA	Supp. Fig.	GCCTCTGGGAGGTCCTGTCCGGCT
com		expressed	10G	C GTTTAAGAGCTATGCTGGAAACAG
		with NLS-		CATAGCAAGTTTAAATAAGGCTAGT
		com-GNCR1-		CCGTTATCAACTTGAAAAAGTGGCA
		P2a-BFP		CCGAGTCGGTGCCTGAATGCCTGC
				GAGCATCTTTTTTTGTTTTTTATGTC
				T
NLS-com-	GWF313-5	Expressed	Supp. Fig.	MPKKKRKVGSMKSIRCKNCNKLLFK
GNCR1-P2a-		with CXCR4-	10G	ADSFDHIEIRCPRCKRHIIMLNACEHP
BFP		com scRNAs		TEKHCGKREKITHSDETVRYGSGSG
		JOHN JOHN NA		SGDIEKLCKKAEEEAKEAQEKADEL
				•
				RQRHPDSQAAEDAEDLANLAVAAV
1				LTACLLAQEHPNADIAKLCIKAASEA
				AEAASKAAELAQRHPDSQAARDAIK
				AEAASKAAELAQRHPDSQAARDAIK LASQAARAVILAIMLAAENPNADIAK LCIKAASEAAEAASKAAELAQRHPD

				SQAARDAIKLASQAAEAVERAIWLA AENPNADIAKKCIKAASEAAEEASK AAEEAQRHPDSQKARDEIKEASQK AEEVKERCKSEQKLISEEDLGSGAT NFSLLKQAGDVEENPGPSELIKENMH MKLYMEGTVDNHHFKCTSEGEGKPY EGTQTMRIKVVEGGPLPFAFDILATS FLYGSKTFINHTQGIPDFFKQSFPEG FTWERVTTYEDGGVLTATQDTSLQD GCLIYNVKIRGVNFTSNGPVMQKKTL GWEAFTETLYPADGGLEGRNDMALK LVGGSHLIANIKTTYRSKKPAKNLKM PGVYYVDYRLERIKEANNETYVEQHE VAVARYCDLPSKLGHKLN
CD95-1- 2xPP7	GWF303	scRNA expressed with NLS- PCP- DNCR2-P2a- BFP	Supp. Fig. 10G	GTACAGCAGAAGCCTTTAGAAGTT TAAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAAGTGGCACCGA GTCGGTGCGGGAGCTAAGGAGTTT ATATGGAAACCCTTAGCCTGCTGC GTAAGGAGTTTATATGGAAACCCTT ACGCAGCAGTTCCCTTTTTTTTTT
CD95-2- 2xPP7	GWF304	scRNA expressed with NLS- PCP- DNCR2-P2a- BFP	Supp. Fig. 10G	GTGGCATGCTCACTTCAGGTGGTT TAAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAAGTGGCACCGA GTCGGTGCGGGAGCTAAGGAGTTT ATATGGAAACCCTTAGCCTGCTGC GTAAGGAGTTTATATGGAAACCCTT ACGCAGCAGTTCCCTTTTTTTTTT
CD95-3- 2xPP7	GWF305	scRNA expressed with NLS- PCP- DNCR2-P2a- BFP	Supp. Fig. 10G	GAAGCCTCGCTGGGAACGCCGT TTAAGAGCTATGCTGGAAACAGCAT AGCAAGTTTAAATAAGGCTAGTCCG TTATCAACTTGAAAAAGTGGCACCG AGTCGGTGCGGGAGCTAAGGAGTT TATATGGAAACCCTTAGCCTGCC GTAAGGAGTTTATATGGAAACCCTT ACGCAGCAGTTCCCTTTTTTTTTT
TRE3G- 2xPP7	GWF306	scRNA expressed with NLS- PCP- DNCR2-P2a- BFP	Fig. 2D,E,F; Supp. Fig. 8B,C,D	GTACGTTCTCTATCACTGATAGTTT AAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAAGTGGCACCGA GTCGGTGCGGGAGCTAAGGAGTTT ATATGGAAACCCTTAGCCTGCTGC GTAAGGAGTTTATATGGAAACCCTT ACGCAGCAGTTCCCTTTTTTTTTT
NLS-PCP- DNCR2-P2a- BFP	GWF303-6	Expressed with TRE3G- 2xPP7 or CD95-2xPP7 scRNAs	Fig. 2D,E,F; Supp. Fig. 8B,C,D; Supp. Fig. 10G	MPKKKRKVGSMSKTIVLSVGEATRTL TEIQSTADRQIFEEKVGPLVGRLRLTA SLRQNGAKTAYRVNLKLDQADVVDS GLPKVRYTQVWSHDVTIVANSTEAS RKSLYDLTKSLVATSQVEDLVVNLVP LGRGSGSGSSDEEEARELIERAKEA

				AERAQEAAERTGDPRVRELARELK RLAQEAAEEVKRDPSSSDVNEALKL IVEAIEAAVDALEAAERTGDPEVREL ARELVRLAVEAAEEVQRNPSSSDVN EALHSIVYAIEAAIFALEAAERTGDPE VRELARELVRLAVEAAEEVQRNPSS RNVEHALMRIVLAIYLAEENLREAEE SGDPEKREKARERVREAVERAEEV QRDPSGWLNHEQKLISEEDLGSGAT NFSLLKQAGDVEENPGPSELIKENMH MKLYMEGTVDNHHFKCTSEGEGKPY EGTQTMRIKVVEGGPLPFAFDILATS FLYGSKTFINHTQGIPDFFKQSFPEG FTWERVTTYEDGGVLTATQDTSLQD GCLIYNVKIRGVNFTSNGPVMQKKTL GWEAFTETLYPADGGLEGRNDMALK LVGGSHLIANIKTTYRSKKPAKNLKM PGVYYVDYRLERIKEANNETYVEQHE VAVARYCDLPSKLGHKLN
TRE3G- 2xMS2	GWF297	scRNA, wt+f6 MS2 expressed with NLS- MCP-ANR- ANR-P2a- BFP	Supp. Fig. 10G	GTACGTTCTCTATCACTGATAGTTT AAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAAGTGGCACCGA GTCGGTGCGGGAGCACATGAGGAT CACCCATGTGCGACTCCCACAGTC ACTGGGGAGTCTTCCCTTTTTTTGT TTTTTATGTCT
NLS-MCP- ANR-ANR- P2a-BFP	GWF297	Expressed with TRE3G- 2xMS2	Supp. Fig. 10G	MPKKKRKVGSMASNFTQFVLVDNG GTGDVTVAPSNFANGIAEWISSNSRS QAYKVTCSVRQSSAQNRKYTIKVEV PKGAWRSYLNMELTIPIFATNSDCELI VKAMQGLLKDGNPIPSAIAANSGIYG SGGSGTGSGTGSGTGTTSGTGTGG STGGELDELVYLLDGPGYDPIHSDG SGTGSGTGSGTGTTSGTGTGGSTG GELDELVYLLDGPGYDPIHSDGSGA TNFSLLKQAGDVEENPGPSELIKENM HMKLYMEGTVDNHHFKCTSEGEGK PYEGTQTMRIKVVEGGPLPFAFDILA TSFLYGSKTFINHTQGIPDFFKQSFPE GFTWERVTTYEDGGVLTATQDTSLQ DGCLIYNVKIRGVNFTSNGPVMQKKT LGWEAFTETLYPADGGLEGRNDMAL KLVGGSHLIANIKTTYRSKKPAKNLK MPGVYYVDYRLERIKEANNETYVEQ HEVAVARYCDLPSKLGHKLN
NS3aH1- VPR	GWF196	ANR-binding- competent NS3a, catalytically active, In pcDNA5/FRT /TO	Fig. 2D,E,F; Supp. Fig. 8B,C,D; Supp. Fig. 10G	MKKKGSVVIVGRINLSGDTAYSQQT RGLEGCQETSQTGRDKNQVEGEVQ VVSTATQSFLATSINGVLWTVYHGA GTRTIASPKGPVTQMYTNVDKDLVG WQAPQGSRSLTPCTCGSSDLYLVT RHADVIPVRRRGDSRGSLLSPRPISY LKGSSGGPLLCPAGHAVGIFRAAVS TRGVAKAVDFIPVESLETTMRSPGS GTGSGEQKLISEEDLEFSSAAGTSDA LDDFDLDMLGSDALDDFDLDMLGSD

dCas9	GWF198	N-terminal	Fig. 2D,E,F;	ALDDFDLDMLGSDALDDFDLDMLGS PKKKRKVGSQYLPDTDDRHRIEEKR KRTYETFKSIMKKSPFSGPTDPRPPP RRIAVPSRSSASVPKPAPQPYPFTSS LSTINYDEFPTMVFPSGQISQASALA PAPPQVLPQAPAPAPAMVSALAQ APAPVPVLAPGPPQAVAPPAPKPTQ AGEGTLSEALLQLQFDDEDLGALLGN STDPAVFTDLASVDNSEFQQLLNQGI PVAPHTTEPMLMEYPEAITRLVTGAQ RPPDPAPAPLGAPGLPNGLLSGDED FSSIADMDFSALLSQISSGSGSRD SREGMFLPKPEAGSAISDVFEGREV CQPKRIRPFHPPGSPWANRPLPASL APTPTGPVHEPVGSLTPAPVPQPLD PAPAVTPEASHLLEDPDEETSQAVKA LREMADTVIPQKEEAAICGQMDLSHP PPRGHLDELTTTLESMTEDLNLDSPL TPELNEILDTFLNDECLLHAMHISTGL SIFDTSLF MDYKDDDDKDKKYSIGLAIGTNSVG
		FLAG-Sp- dCas9-SV40- NLS in pCDNA5/FR T/TO	Supp. Fig. 8B,C,D	WAVITDEYKVPSKKFKVLGNTDRHSI KKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDD SFFHRLEESFLVEEDKKHERHPIFGNI VDEVAYHEKYPTIYHLRKKLVDSTDK ADLRLIYLALAHMIKFRGHFLIEGDLN PDNSDVDKLFIQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRRLENLIAQ LPGEKKNGLFGNLIALSLGLTPNFKS NFDLAEDAKLQLSKDTYDDDLDNLLA QIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYA GYIDGGASQEEFYKFIKPILEKMDGT EELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWM TRKSEETITPWNFEEVVDKGASAQSF IERMTNFDKNLPNEKVLPKHSLLYEY FTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYF KKIECFDSVEISGVEDRFNASLGTYH DLLKIIKDKDFLDNEENEDILEDIVLTL TLFEDREMIEERLKTYAHLFDDKVMK QLKRRRYTGWGRLSRKLINGIRDKQ SGKTILDFLKSDGFANRNFMQLIHDD SLTFKEDIQKAQVSGQGDSLHEHIAN LAGSPAIKKGILQTVKVVDELVKVMG RHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENT QLQNEKLYLYYLQNGRDMYVDQELD INRLSDYDVDAIVPQSFLKDDSIDNKV LTRSDKNRGKSDNVPSEEVVKKMKN YWRQLLNAKLITQRKFDNLTKAERG GLSELDKAGFIKRQLVETRQITKHVA

	Ī		I	QILDSRMNTKYDENDKLIREVKVITLK
				SKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYG DYKVYDVRKMIAKSEQEIGKATAKYF FYSNIMNFFKTEITLANGEIRKRPLIET NGETGEIVWDKGRDFATVRKVLSMP QVNIVKKTEVQTGGFSKESILPKRNS DKLIARKKDWDPKKYGGFDSPTVAY SVLVVAKVEKGKSKKLKSVKELLGITI MERSSFEKNPIDFLEAKGYKEVKKDL IIKLPKYSLFELENGRKRMLASAGELQ KGNELALPSKYVNFLYLASHYEKLKG SPEDNEQKQLFVEQHKHYLDEIIEQIS EFSKRVILADANLDKVLSAYNKHRDK PIREQAENIIHLFTLTNLGAPAAFKYF DTTIDRKRYTSTKEVLDATLIHQSITG LYETRIDLSQLGGDSRADPKKKRKV
CXCR4-C1-	GWF324	pU6-guide-	Supp. Fig.	GCGGGTGGTCGGTAGTGAGTC GTT
2xMS2		2xMS2(wt+f6)/CMV-BFP	10E	TAAGAGCTATGCTGGAAACAGCATA
)/CIVIV-BFP		GCAAGTTTAAATAAGGCTAGTCCGT
				TATCAACTTGAAAAAGTGGCACCGA
				GTCGGTGCGGGAGCACATGAGGAT
				CACCCATGTGCGACTCCCACAGTC
				ACTGGGGAGTCTTCCCTTTTTTGT
				TTTTTATGTCT
CXCR4-C2- 2xMS2	GWF325	pU6-guide- 2xMS2(wt+f6)/CMV-BFP	Supp. Fig. 10E	GCAGACGCGAGGAAGGAGGCGC GTTTAAGAGCTATGCTGGAAACAGC ATAGCAAGTTTAAATAAGGCTAGTC CGTTATCAACTTGAAAAAAGTGGCAC CGAGTCGGTGCGGGAGCACATGAG GATCACCCATGTGCGACTCCCACA GTCACTGGGGAGTCTCCCTTTTTT TGTTTTTTATGTCT
CXCR4-C3- 2xMS2	GWF326	pU6-guide- 2xMS2(wt+f6)/CMV-BFP	Supp. Fig. 10E	GCCTCTGGGAGGTCCTGTCCGGCT CGTTTAAGAGCTATGCTGGAAACAG CATAGCAAGTTTAAATAAGGCTAGT CCGTTATCAACTTGAAAAAGTGGCA CCGAGTCGGTGCGGGAGCACATGA GGATCACCCATGTGCGACTCCCAC AGTCACTGGGGAGTCTTCCCTTTTT TTGTTTTTTATGTCT
CD95-1- 2xMS2	GWF321	pU6-guide- 2xMS2(wt+f6)/CMV-BFP	Supp. Fig. 10F	GTACAGCAGAAGCCTTTAGAAGTT TAAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAAGTGGCACCGA GTCGGTGCGGGAGCACATGAGGAT CACCCATGTGCGACTCCACAGTC ACTGGGGAGTCTTCCCTTTTTTTGT TTTTTATGTCT
CD95-2- 2xMS2	GWF322	pU6-guide- 2xMS2(wt+f6)/CMV-BFP	Supp. Fig. 10F	GTGGCATGCTCACTTCAGGTGGTT TAAGAGCTATGCTGGAAACAGCATA GCAAGTTTAAATAAGGCTAGTCCGT

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				TATCAACTTGAAAAAGTGGCACCGA GTCGGTGCGGGAGCACATGAGGAT CACCCATGTGCGACTCCACAGTC ACTGGGGAGTCTTCCCTTTTTTTGT
				TTTTTATGTCT
CD95-3-	GWF323	pU6-guide-	Supp. Fig.	GAAGCCTCGCTGGGGAACGCCGT
2xMS2		2xMS2(wt+f6)/CMV-BFP	10F	TTAAGAGCTATGCTGGAAACAGCAT AGCAAGTTTAAATAAGGCTAGTCCG TTATCAACTTGAAAAAGTGGCACCG AGTCGGTGCGGGAGCACATGAGGA TCACCCATGTGCGACTCCCACAGT CACTGGGGAGTCTTCCCTTTTTTTG TTTTTTATGTCT
MCP-NS3a- P2a-DNCR2- KRAB- MeCP2-P2a- GNCR1- VPR-(IRES- BFP)	GWF169	NS3a solubility optimized S139A, DNCR2, and GNCR1 all in bold (in pCDNA5/FR T/TO)	Supp. Fig. 10E,F	MPKKKRKVGSMASNFTQFVLVDNG GTGDVTVAPSNFANGIAEWISSNSRS QAYKVTCSVRQSSAQNRKYTIKVEV PKGAWRSYLNMELTIPIFATNSDCELI VKAMQGLLKDGNPIPSAIAANSGIYG SGGSGTGEQKLISEEDLGGKKKGSV VIVGRINLSGDTAYAQQTRGEEGCQ ETSQTGRDKNQVEGEVQIVSTATQT FLATSINGVLWTVYHGAGTRTIASPK GPVTQMYTNVDKDLVGWQAPQGSR SLTPCTCGSSDLYLVTRHADVIPVRR RGDSRGSLLSPRPISYLKGSAGGPL LCPAGHAVGIFRAAVSTRGVAKAVD FIPVESLETTMRSPGSGATNFSLLKQ AGDVEENPGPMSSDEEEARELIERA KEAAERAQEAAERTGDPRVRELAR ELKRLAQEAAEEVKRDPSSSDVNEA LKLIVEAIEAAVDALEAAERTGDPEV RELARELVRLAVEAAEEVQRNPSSS DVNEALHSIVYAIEAAIFALEAAERTG DPEVRELARELVRLAVEAAEEVQRN PSSRNVEHALMRIVLAIYLAEENLRE AEESGDPEKREKARERVREAVERA EEVQRDPSGWLNHEQKLISEEDLSG GGSGGSGSMDAKSLTAWSRTLVTFK DVFVDFTREEWKLLDTAQQIVYRNV MLENYKNLVSLGYQLTKPDVILRLEK GEEPWLVSGGGSGGSGSSPKKKRK VEASVQVKRVLEKSPGKLLVKMPFQ ASPGGKGEGGGATTSAQVMVIKRPG RKRKAEADPQAIPKKRGRKPGSVVA AAAAEAKKKAVKESSIRSVQETVLPIK KRKTRETVSIEVKEVVKPLLVSTLGEK SGKGLKTCKSPGRKSKESSPKGRSS SASSPPKKEHHHHHHHHAESPKAPMP LLPPPPPPEPQSSEDPISPPEPQDLS SSICKEEKMPRAGSLESDGCPKEPA KTQPMVAAAATTTTTTTTTTVAEKYKH RGEGERKDIVSSSMPRPNREEPVDS RTPVTERVSGSGATNFSLLKQAGDV EENPGPDIEKLCKKAEEEAKEAQEK
				ADELRQRHPDSQAAEDAEDLANLA VAAVLTACLLAQEHPNADIAKLCIKA

		1		T
				ASEAAEAASKAAELAQRHPDSQAA
				RDAIKLASQAARAVILAIMLAAENPN
				ADIAKLCIKAASEAAEAASKAAELA
				QRHPDSQAARDAIKLASQAAEAVER
				AIWLAAENPNADIAKKCIKAASEAAE
				EASKAAEEAQRHPDSQKARDEIKEA
				SQKAEEVKERCKS EQKLISEEDLEFS
				SAAGTSDALDDFDLDMLGSDALDDF
				DLDMLGSDALDDFDLDMLGSDALDD
				FDLDMLGSPKKKRKVGSQYLPDTDD
				RHRIEEKRKRTYETFKSIMKKSPFSG
				PTDPRPPPRRIAVPSRSSASVPKPAP
				QPYPFTSSLSTINYDEFPTMVFPSGQ
				ISQASALAPAPPQVLPQAPAPAPA
				MVSALAQAPAPVPVLAPGPPQAVAP
				PAPKPTQAGEGTLSEALLQLQFDDE
				DLGALLGNSTDPAVFTDLASVDNSEF
				QQLLNQGIPVAPHTTEPMLMEYPEAI
				TRLVTGAQRPPDPAPAPLGAPGLPN
				GLLSGDEDFSSIADMDFSALLSQISS
				GSGSGSRDSREGMFLPKPEAGSAIS
				DVFEGREVCQPKRIRPFHPPGSPWA
				NRPLPASLAPTPTGPVHEPVGSLTPA
				PVPQPLDPAPAVTPEASHLLEDPDEE
				TSQAVKALREMADTVIPQKEEAAICG
				QMDLSHPPPRGHLDELTTTLESMTE
				DLNLDSPLTPELNEILDTFLNDECLLH
				AMHISTGLSIFDTSLF
LifeAct-	GWF214	(in	Fig. 4C-G,	MGVADLIKKFESISKEEGDPPVATMV
mCherry	OWI Z 14	pCDNA5/FR	Supp. Fig.	SKGEEDNMAIIKEFMRFKVHMEGSV
Incherry		T/TO)	11, Supp.	NGHEFEIEGEGEGRPYEGTQTAKLK
		1/10)	Fig. 12C-E	VTKGGPLPFAWDILSPQFMYGSKAY
			Fig. 120-E	VKGGFLFFAWDILSFQFMTGSKAT
				I VKOPALJPLJI KLOPPCIPKVVPKVIVL I
				NFEDGGVVTVTQDSSLQDGEFIYKV
				NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS
				NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG
				NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI
				NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST
				NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK
mCherry-	GWF223	NS3a	Fig. 4B;	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG
NS3a-CAAX-	GWF223	solubility	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK
NS3a-CAAX- (IRES)-	GWF223			NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG
NS3a-CAAX-	GWF223	solubility	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK
NS3a-CAAX- (IRES)-	GWF223	solubility optimized	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK
NS3a-CAAX- (IRES)- EGFP-	GWF223	solubility optimized S139A.	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown.	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a,	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG GKKKGSVVIVGRINLSGDTAYAQQT
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in bold (in	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG GKKKGSVVIVGRINLSGDTAYAQQT RGEEGCQETSQTGRDKNQVEGEVQ
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG GKKKGSVVIVGRINLSGDTAYAQQT RGEEGCQETSQTGRDKNQVEGEVQ IVSTATQTFLATSINGVLWTVYHGAG
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in bold (in	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG GKKKGSVVIVGRINLSGDTAYAQQT RGEEGCQETSQTGRDKNQVEGEVQ IVSTATQTFLATSINGVLWTVYHGAG TRTIASPKGPVTQMYTNVDKDLVGW
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in bold (in	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG GKKKGSVVIVGRINLSGDTAYAQQT RGEEGCQETSQTGRDKNQVEGEVQ IVSTATQTFLATSINGVLWTVYHGAG TRTIASPKGPVTQMYTNVDKDLVGW QAPQGSRSLTPCTCGSSDLYLVTRH
NS3a-CAAX- (IRES)- EGFP- DNCR2-P2a-	GWF223	solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in bold (in	Supp. Fig.	NFEDGGVVTVTQDSSLQDGEFIYKV KLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGG HYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHST GGMDELYK MVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAK LKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERV MNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKGSGTGEQKLISEEDLG GKKKGSVVIVGRINLSGDTAYAQQT RGEEGCQETSQTGRDKNQVEGEVQ IVSTATQTFLATSINGVLWTVYHGAG TRTIASPKGPVTQMYTNVDKDLVGW

				GVAKAVDFIPVESLETTMRSPSAGG SAGGEKMSKDGKKKKKKSKTKCVIM – (IRES) – MVSKGEELFTGVVPILVELDGDVNGH KFSVSGEGEGDATYGKLTLKFICTTG KLPVPWPTLVTTLTYGVQCFSRYPD HMKQHDFFKSAMPEGYVQERTIFFK DDGNYKTRAEVKFEGDTLVNRIELKG IDFKEDGNILGHKLEYNYNSHNVYIM
				ADKQKNGIKVNFKIRHNIEDGSVQLA DHYQQNTPIGDGPVLLPDNHYLSTQ SALSKDPNEKRDHMVLLEFVTAAGIT LGMDELYKSGSGEQKLISEEDLGSG SSDEEEARELIERAKEAAERAQEAA ERTGDPRVRELARELKRLAQEAAEE VKRDPSSSDVNEALKLIVEAIEAAVD ALEAAERTGDPEVRELARELVRLAV EAAEEVQRNPSSSDVNEALHSIVYAI EAAIFALEAAERTGDPEVRELARELV
				RLAVEAAEEVQRNPSSRNVEHALM RIVLAIYLAEENLREAEESGDPEKRE KARERVREAVERAEEVQRDPSGWL NHSAGGSAGGSAGGSGASGS GATNFSLLKQAGDVEENPGPSELIKE NMHMKLYMEGTVDNHHFKCTSEGE GKPYEGTQTMRIKVVEGGPLPFAFDI LATSFLYGSKTFINHTQGIPDFFKQSF
				PEGFTWERVTTYEDGGVLTATQDTS LQDGCLIYNVKIRGVNFTSNGPVMQK KTLGWEAFTETLYPADGGLEGRNDM ALKLVGGSHLIANIKTTYRSKKPAKNL KMPGVYYVDYRLERIKEANNETYVE QHEVAVARYCDLPSKLGHKLNSGSG EQKLISEEDLGSGTGSGTGSTT SGTGTGGSTGMDIEKLCKKAEEEAK EAQEKADELRQRHPDSQAAEDAED
				LANLAVAAVLTACLLAQEHPNADIA KLCIKAASEAAEAASKAAELAQRHP DSQAARDAIKLASQAARAVILAIMLA AENPNADIAKLCIKAASEAAEAASK AAELAQRHPDSQAARDAIKLASQAA EAVERAIWLAAENPNADIAKKCIKAA SEAAEEASKAAEEAQRHPDSQKAR DEIKEASQKAEEVKERCKSSAGGSA GGSAGGSAGGSAG
NS3a-CAAX- (IRES)- EGFP- DNCR2- TIAM-P2a- BFP- GNCR1- LARG	GWF243, GWF228	NS3a solubility optimized S139A. CAAX from KRAS4b. IRES not shown. NS3a, DNCR1, and GNCR1 in	Fig. 4C-G; Supp. Fig. 11A-E,H; Supp. Fig. 12D,E	MEQKLISEEDLGGKKKGSVVIVGRIN LSGDTAYAQQTRGEEGCQETSQTG RDKNQVEGEVQIVSTATQTFLATSIN GVLWTVYHGAGTRTIASPKGPVTQM YTNVDKDLVGWQAPQGSRSLTPCT CGSSDLYLVTRHADVIPVRRRGDSR GSLLSPRPISYLKGSAGGPLLCPAG HAVGIFRAAVSTRGVAKAVDFIPVES LETTMRSPSAGGSAGGEKMSKDGK KKKKKSKTKCVIM – (IRES) – MVSKGEELFTGVVPILVELDGDVNGH

bold. TIAM	KFSVSGEGEGDATYGKLTLKFICTTG
and LARG	KLPVPWPTLVTTLTYGVQCFSRYPD
underlined	
PB501B or	
pEF5-FRT)	
	ADKQKNGIKVNFKIRHNIEDGSVQLA
	DHYQQNTPIGDGPVLLPDNHYLSTQ
	SALSKDPNEKRDHMVLLEFVTAAGIT
	LGMDELYKSGSGEQKLISEEDLGSG
	SSDEEEARELIERAKEAAERAQEAA
	ERTGDPRVRELARELKRLAQEAAEE
	VKRDPSSSDVNEALKLIVEAIEAAVD
	ALEAAERTGDPEVRELARELVRLAV
	EAAEEVQRNPSSSDVNEALHSIVYAI
	EAAIFALEAAERTGDPEVRELARELV
	RLAVEAAEEVQRNPSSRNVEHALM
	RIVLAIYLAEENLREAEESGDPEKRE
	KARERVREAVERAEEVQRDPSGWL
	NH SAGGSAGGSAGGSGAS <u>RQ</u>
	<u>LSDADKLRKVICELLETERTYVKDLN</u>
	<u>CLMERYLKPLQKETFLTQDELDVLFG</u>
	<u>NLTEMVEFQVEFLKTLEDGVRLVPDL</u>
	<u>EKLEKVDQFKKVLFSLGGSFLYYADR</u>
	<u>FKLYSAFCASHTKVPKVLVKAKTDTA</u>
	FKAFLDAQNPKQQHSSTLESYLIKPIQ
	RILKYPLLLRELFALTDAESEEHYHLD
	VAIKTMNKVASHINEMQKIHEEGSGA
	TNFSLLKQAGDVEENPGPSELIKENM
	HMKLYMEGTVDNHHFKCTSEGEGK
	PYEGTQTMRIKVVEGGPLPFAFDILA
	TSFLYGSKTFINHTQGIPDFFKQSFPE
	GFTWERVTTYEDGGVLTATQDTSLQ
	DGCLIYNVKIRGVNFTSNGPVMQKKT
	LGWEAFTETLYPADGGLEGRNDMAL
	KLVGGSHLIANIKTTYRSKKPAKNLK
	MPGVYYVDYRLERIKEANNETYVEQ
	HEVAVARYCDLPSKLGHKLNSGSGE
	QKLISEEDLGSGTGSGTGTTS
	GTGTGGSTGMDIEKLCKKAEEEAKE
	AQEKADELRQRHPDSQAAEDAEDL
	ANLAVAAVLTACLLAQEHPNADIAK
	LCIKAASEAAEAASKAAELAQRHPD
	SQAARDAIKLASQAARAVILAIMLAA
	ENPNADIAKLCIKAASEAAEAASKA
	AELAQRHPDSQAARDAIKLASQAAE
	AVERAIWLAAENPNADIAKKCIKAAS
	EAAEEASKAAEEAQRHPDSQKARD
	EIKEASQKAEEVKERCKSSAGGSAG
	GSAGGSAGGSAG <u>TPPNWQQLVSRE</u>
	VLLGLKPCEIKRQEVINELFYTERAHV
	RTLKVLDQVFYQRVSREGILSPSELR
	KIFSNLEDILQLHIGLNEQMKAVRKRN
	<u>ETSVIDQIGEDLLTWFSGPGEEKLKH</u>
	AAATFCSNQPFALEMIKSRQKKDSRF
	QTFVQDAESNPLCRRLQLKDIIPTQM
	QRLTKYPLLLDNIAKYTEWPTEREKV
1	<u> </u>

NS3a-CAAX- GWF246 NS3a Fig. 4D-G; Supp. Fig. L Optimized S139A.	KKAADHCRQILNYVNQAVKEAENKQ R MEQKLISEEDLGGKKKGSVVIVGRIN
(IRES)- solubility Supp. Fig. Loptimized DNCR2- S139A.	
IRES not shown. NS3a, DNCR1, and GNCR1 in bold. TIAM underlined (in PB501B) IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	LSGDTAYAQQTRGEEGCQETSQTG RDKNQVEGEVQIVSTATQTFLATSIN GVLWTVYHGAGTRTIASPKGPVTQM YTNVDKDLVGWQAPQGSRSLTPCT CGSSDLYLVTRHADVIPVRRGDSR GSLLSPRPISYLKGSAGGPLLCPAG HAVGIFRAAVSTRGVAKAVDFIPVES LETTMRSPSAGGSAGGEKMSKDGK KKKKSKTKCVIM – (IRES) – MVSKGEELFTGVVPILVELDGDVNGH KFSVSGEGEGDATYGKLTLKFICTTG KLPVPWPTLVTTLTYGVQCFSRYPD HMKQHDFFKSAMPEGYVQERTIFFK DDGNYKTRAEVKFEGDTLVNRIELKG IDFKEDGNILGHKLEYNYNSHNVYIM ADKQKNGIKVNFKIRHNIEDGSVQLA DHYQQNTPIGDGPVLLPDNHYLSTQ SALSKDPNEKRDHMVLLEFVTAAGIT LGMDELYKSGSGEGKLISEEDLGSG SSDEEEARELIERAKEAAERAQEAA ERTGDPRVRELARELKRLAQEAAEE VKRDPSSSDVNEALKLIVEAIEAAVD ALEAAERTGDPEVRELARELVRLAV EAAEEVQRNPSSSDVNEALHSIVYAI EAAIFALEAAERTGDPEVRELARELV RLAVEAAEEVQRNPSSRNVEHALM RIVLAIYLAEENLREAEESGDPEKRE KARERVREAVERAEEVQRDPSGWL NHSAGGSAGGSAGGSGASRQ LSDADKLRKVICELLETERTYVKDLN CLMERYLKPLQKETFLTQDELDVLFG NLTEMVEFQVEFLKTLEDGVRLVPDL EKLEKVDQFKKVLFSLGGSFLYYADR FKLYSAFCASHTKVPKVLVKAKTDTA FKAFLDAQNPKQQHSSTLESYLIKPIQ RILKYPLLLRELFALTDAESEEHYHLD VAIKTMNKVASHINEMQKIHEEGSGA TNFSLLKQAGDVEENPGPSELIKENM HMKLYMEGTVDNHHFKCTSEGEGK PYEGTQTMRIKVVEGGPLPFAFDILA TSFLYGSKTFINHTQGIPDFFKQSFPE GFTWERVTTYEDGGVLTATQDTSLQ DGCLIYNVKIRGVNFTSNGPVMQKKT LGWEAFTETLYPADGGLEGRNDMAL KLVGGSHLIANIKTTYRSKKPAKNIK MPGVYYVDYRLERIKEANNETYVEQ HEVAVARYCDLPSKLGHKLNSGSGE QKLISEEDLGSGTGSGTGSTTS GTGTGGSTGMDIEKLCKKAEEEAKE AQEKADELRQRHPDSQAAEDAEDL ANLAVAAVLTACLLAQEHPNADIAK

•	1	1	T	
				ENPNADIAKLCIKAASEAAEAASKA
				AELAQRHPDSQAARDAIKLASQAAE
				AVERAIWLAAENPNADIAKKCIKAAS
				EAAEEASKAAEEAQRHPDSQKARD
				EIKEASQKAEEVKERCKS SAGGSAG
				GSAGGSAGGSAG
NS3a-CAAX-	GWF248	NS3a	Fig. 4D-G;	MEQKLISEEDLGG KKKGSVVIVGRIN
(IRES)-		solubility	Supp. Fig.	LSGDTAYAQQTRGEEGCQETSQTG
EGFP-		optimized	12A-D,G,H	RDKNQVEGEVQIVSTATQTFLATSIN
DNCR2-P2a-		S139A.		GVLWTVYHGAGTRTIASPKGPVTQM
BFP-		CAAX from		YTNVDKDLVGWQAPQGSRSLTPCT
GNCR1-		KRAS4b.		CGSSDLYLVTRHADVIPVRRRGDSR
LARG		IRES not		GSLLSPRPISYLKGSAGGPLLCPAG
		shown.		HAVGIFRAAVSTRGVAKAVDFIPVES
		NS3a,		LETTMRSP SAGGSAGGEKMSKDGK
		DNCR1, and		KKKKKSKTKCVIM – (IRES) –
		GNCR1 in		MVSKGEELFTGVVPILVELDGDVNGH
		bold. LARG		KFSVSGEGEGDATYGKLTLKFICTTG
		underlined (in		KLPVPWPTLVTTLTYGVQCFSRYPD
		PB501B)		HMKQHDFFKSAMPEGYVQERTIFFK
				DDGNYKTRAEVKFEGDTLVNRIELKG
				IDFKEDGNILGHKLEYNYNSHNVYIM
				ADKQKNGIKVNFKIRHNIEDGSVQLA
				DHYQQNTPIGDGPVLLPDNHYLSTQ
				SALSKDPNEKRDHMVLLEFVTAAGIT
				LGMDELYKSGSGEQKLISEEDLGSG
				SSDEEEARELIERAKEAAERAQEAA
				ERTGDPRVRELARELKRLAQEAAEE
				VKRDPSSSDVNEALKLIVEAIEAAVD
				ALEAAERTGDPEVRELARELVRLAV
				EAAEEVQRNPSSSDVNEALHSIVYAI
				EAAIFALEAAERTGDPEVRELARELV
				RLAVEAAEEVQRNPSSRNVEHALM
				RIVLAIYLAEENLREAEESGDPEKRE
				KARERVREAVERAEEVQRDPSGWL
				NH SAGGSAGGSAGGSGASGS
				GATNFSLLKQAGDVEENPGPSELIKE
				NMHMKLYMEGTVDNHHFKCTSEGE
				GKPYEGTQTMRIKVVEGGPLPFAFDI
				LATSFLYGSKTFINHTQGIPDFFKQSF
				PEGFTWERVTTYEDGGVLTATQDTS
				LQDGCLIYNVKIRGVNFTSNGPVMQK
				KTLGWEAFTETLYPADGGLEGRNDM
				ALKLVGGSHLIANIKTTYRSKKPAKNL
				KMPGVYYVDYRLERIKEANNETYVE
				QHEVAVARYCDLPSKLGHKLNSGSG
				EQKLISEEDLGSGTGSGTGTT
				SGTGTGGSTGMDIEKLCKKAEEEAK
				EAQEKADELRQRHPDSQAAEDAED
				LANLAVAAVLTACLLAQEHPNADIA
				KLCIKAASEAAEAASKAAELAQRHP
				DSQAARDAIKLASQAARAVILAIMLA
				AENPNADIAKLCIKAASEAAEAASK
				AAELAQRHPDSQAARDAIKLASQAA
				EAVERAIWLAAENPNADIAKKCIKAA
				SEAAEEASKAAEEAQRHPDSQKAR

Gal4DBD- S3a-P2a- DNCR2-VPR Gal4DBD- NS3a-P2a- DNCR2-VPR MALTEVELQUEVRYSPERTERSPLTRA HITEVESRLERLEQLFLLIFFREDLDM ILKMDSLQDIKALIGIBT VEGEVOLVER With NS3a and DNCR2 in bold Gal4DBD- NS3a-P2a- DNCR2-VPR With NS3a and DNCR2 in bold Gal4DBD- NS3a-P2a- DNCR2-VPR With NS3a AND NCR2- DNCR2-VPR With NS3a AND NCR2 IN DOLG2 IN DOLG					
Gal4DBD- NS3a-P2a- DNCR2-VPR GWF058 From pLenti- UAS- minCMV- mCherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR With NS3a and DNCR2 in boid GSAGSGGKKKGSVYRGPRLEMIKS-GDT- NS3a-P2a- DNCR2-VPR With NS3a and DNCR2 in boid GSAGSGGKKGSVYRGPLT- WITH SAGSGATIN-SLLKQAGDYEBNEPG- MSSGATIN-SLLKQAGDYEBNEPG- MSSGATIN-SLLKQAGDYEBNEPG- MSSGATIN-SLLKQAGDYEBNEPG- MSSGATIN-SLLKQAGDYEBNEPG- MSSGATIN-SLLKQAGDYEBNEPG- MSSDEEGARELIERAKE-AAERAGEA AERTGDPEVRELARELKRLAGEAAE EVKRDPSSDVNEALKLIVEAIEAA DALEAAERTGDPEVRELARELL- LVRLAVEAAEEVQRNPSSRVHEALAE LVRLAVEAAEEVQRNPSSRNVEALKLIVEAIEAA MRIVLAIYLAENLERAEERGDPLK MRIVLAIYLAENLERAEERGDPLK KSPSGPTDPRPPPRRIAVPSRSSAS VPKPAPOPYPPTSSLSTINYDEFPTM VFPSGQISQASALAPAPOQULPQAPA PAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAPAMWSALAQAPAPYPULAPOAP PAPAPAPAMWSALAQAPAPYPULAPOAP PAPAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAPAWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPYPULAPOAP PAPAPAMWSALAQAPAPAPAPAMPAPAPAPAMBALAQAPAPAPAP					DEIKEASQKAEEVKERCKS SAGGSA
Gal4DBD- NS3a-P2a- DNCR2-VPR GWF058 GWGMCHKKLSCKEKPK MKLLSIGACDICKKLKCSKEKPK GWF058 GWGCMCHKRIKGNECH GWF058 GWGMCHALGHDIA GWF058 GWGMCHALGHDIA GWF058 GWF058 GWGMCHATHOR GWF058 GWF058 GWGMCHALGHDIA GWF058 GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGCMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHALGH GWGMC					GGSAGGSAGGSAGTPPNWQQLVSR
Gal4DBD- NS3a-P2a- DNCR2-VPR GWF058 GWGMCHKKLSCKEKPK MKLLSIGACDICKKLKCSKEKPK GWF058 GWGCMCHKRIKGNECH GWF058 GWGMCHALGHDIA GWF058 GWGMCHALGHDIA GWF058 GWF058 GWGMCHATHOR GWF058 GWF058 GWGMCHALGHDIA GWF058 GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHANACH GWF058 GWGCMCHANACH GWF058 GWGMCHANACH GWF058 GWGMCHALGH GWGMC					
Gal4DBD- NS3a-P2a- DNCR2-VPR Gil4DBD- NS3a-P2a- DNCR2-VPR Gil4DBC- Gil4DBC- Gil4DBC- Gil4DBC- Gil4DBC- Gil4DBC- Gil4DBC- Gil					
Gal4DBD- NS3a-P2a- DNCR2-VPR Gwross From plenti- UAS- mincMV- mCherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR Gwross About Nore in bold From plenti- UAS- mincMV- mCherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR With NS3a and DNCR2 in bold About Nore in bold Gwross About Nore in bold From plenti- UAS- mincMV- mCherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR With NS3a and DNCR2 in bold From plenti- UAS- mincMV- WCHAPT- With NS3a And DNCR2 In bold From plenti- UAS- mincMV- WCHAPT- With NS3a And DNCR2 In bold From plenti- UAS- MKLLSSIEQACDICRLKKLKCSKEKPK MKLLSSIEQACDICRLKKLKCSKEKPK WKSADDHCRYSFKTKRSPLTRA HLTEVESRLERLEQLFLLIFPREDLOM ILKMDSLQDIKALIGTPAAASTLEGG GSAGSGKKKGSWIVGRINLSGDT AYAQQTRGEEGCQETSQTGRDKNQ VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTNVNV VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTMVNV VYHGAGTRTIASPKGPVTQWTMVNV VYHGAGTRTIASPKGPVTQWTMVNV VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTMVNV VYHGAGTRTIASPKGPVTQWTMVNV VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTMVNV VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTMVNV VYHGAGTRTIASPKGPVTQWTMVNV VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTMVNT VYHGAGTRTIASPKGPVTQWTMVNV VEGEVQIWSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTQWTMVNT VYHGAGTRTIASPKGPVTQWTMVNT VYHGAGTRTIASPKGPVTQWTMVNT VYHGAGTRTIASPKGPVTQWTMVT VYHGAGTRTIASPKGPVTQW					
Gal4DBD- NS3a-P2a DNCR2-VPR GWF058 From plenti- UAS- mincMV- mCherry(M V-Gal4DBD- NS3a-P2a DNCR2-VPR, with NS3a and DNCR2 in bold From bold From plenti- UAS- DNCR2-VPR, with NS3a And DNCR2 in bold From plenti- RAMSTRIAPKERD- R					
Gal4DBD- NS3a-P2a- DNCR2-VPR GWF058 GWF058 GWF058 GWF058 From plenti- UAS- mincMV- mcherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, With NS3a And DNCR2 In bold GSAGSGCKKKGSVWGRINLSGDT AYAQQTRGEGCQETGTGRDKNQ VFGEVQIVSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPVTGWTNVD KDLVGWAQPQGSRSLTPCTCGSSD LYLVTRHADVIPVRRRGDSRGSLLS PRPISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPCP MSSDEEEARELIERAKEAAERAQEA AERTGDPRVRELARELKPLAQGDAE EVKRDPSSDVNEALKLIVEAIEAA DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSDNVBALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSRDNVEALH MRIVLAIYLAEENLEAAERTGDPEKRE LVRLAVEAAEEVQRNPSSRDNVEALH MRIVLAIYLAEENLEAGRTGDPEKRE LVRLAVEAAEEVQRNPSSRNVEALL MRIVLAIYLAEENLEAGRTGDPEKRE LVRLAVEAAEEVGRPSSSDNVBALHSIVY AIEAAIFALLEAAERTGDPEVRELARE LVRLAVEAAEEVGRPSSSDNVBALHSIVY AIEAAIFALLAEARTGDPEVRELARE LVRLAVEAAEEVGRPSSDDVBALHSIVY AIEAAIFALLAEARTGDPEVRELARE LVRLAVEAAEEVGRPSSSDVBALHSIVY AIEAAIFALLAEARTGDPUR AIEAAIFALAEARTGDPUR AIEAAIFALAEARTGD					
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Gal4DBD- NS3a-P2a- DNCR2-VPR GWF058 MICHAUSA- MINCMV- MCherry/CM MCLICK SASGGKKKGSVVIVGRINLSGDT AYAQQTRGEEGCQETSQTGRDKNQ VEGEQUISTATQTFLATSINGULWT VYHGAGTRTIASPKGPVTOMYTNVD KDLVGWQAPQGSRSLTFCGSSD LYLVTRHADVIPVRRRGDSRGSLLS PRPISYLKGSAGGPLLCPAGHAVGIF RAAVSTROVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAARAGEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSDVMEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVMEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA WEAVEAAEEVQRNPSSSNVEAHAL MRIVLAIVLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRPSGW LNHEQKLISEEDLDALDDFDLDMLG SDALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSDALDDFDLDMLGDFDLDMLGDFDLDMLGDBALDDFDL					
GalADBD- NS3a-P2a- DNCR2-VPR GWF058					<u>KVKKAADHCRQILNYVNQAVKEAEN</u>
NS3a-P2a- DNCR2-VPR Micherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, With NS3a and DNCR2 in bold NS4-P2a- DNCR2-VPR, With NS3a AND SDEEARELIERAGUFLUTHATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WEGEVQIVSTATQTFLATSINGVLWT RSPCSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAAERAQEA AERTGDPPVRELARELWRLAQEAAE EVKRDPSSSDVNEALHSIVY AIEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAERTGDPEVRELARELVRLA WEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLG SDALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSPKKKRKVGSQY LPDTDDRHRIEEKRKRTYETFKSIMK KSPFSGFTDRRPPRRRIAVPSRSSAS VPKPAPDQPYPFTSSITNYDEFPTM VFPSGQISQASALAPAPPQVLAPGP PQAVAPPAPKFTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPAPTTDAPAP PAPAMVSALAQAPAPVVLAPGP PQAVAPPAPKFTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAGRPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSGSTSTADFOFFSHEPVG SPWANRPLPASLAPTPTGSPVHEPVG STUARPPVQPCLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRENLILDTFLINDE					KQR
NS3a-P2a- DNCR2-VPR Micherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, With NS3a and DNCR2 in bold NS4-P2a- DNCR2-VPR, With NS3a AND SDEEARELIERAGUFLUTHATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMYTNVD WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WEGEVQIVSTATQTFLATSINGVLWT WHGAGTRITIASPRGPYTQMSTSINGVLWT WEGEVQIVSTATQTFLATSINGVLWT RSPCSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAAERAQEA AERTGDPPVRELARELWRLAQEAAE EVKRDPSSSDVNEALHSIVY AIEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAERTGDPEVRELARELVRLA WEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLG SDALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSPKKKRKVGSQY LPDTDDRHRIEEKRKRTYETFKSIMK KSPFSGFTDRRPPRRRIAVPSRSSAS VPKPAPDQPYPFTSSITNYDEFPTM VFPSGQISQASALAPAPPQVLAPGP PQAVAPPAPKFTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPAPTTDAPAP PAPAMVSALAQAPAPVVLAPGP PQAVAPPAPKFTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAGRPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSGSTSTADFOFFSHEPVG SPWANRPLPASLAPTPTGSPVHEPVG STUARPPVQPCLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRENLILDTFLINDE	Gal4DBD-	GWF058	From pLenti-	Supp. Fig. 8A	MKLLSSIEQACDICRLKKLKCSKEKPK
DNCR2-VPR mincMv- mcherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold mchery/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold mchery/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold mchery/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold mchery/CM V-GartalsprkGpvtQmytnvD KDLVGWQAPQGSRSLTPCTCGSSD LYLVTRHADVIPVRRRGDSRGSLLS PRPISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVESNPGP MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELAREL LVRLAVEAAEEVQRNPSSSNVEALLAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRPPSGW LNHEGKLISEEDLDALDDFDLDMLGS DALDDFDLDMLGSPKKKRKVGSQY LPDTDDRHRIEEKRKRTYETFKSIMK KSPFSGPTDPRPPPRISLSTINYDEFPTM VFPSGQISQASALAPAPPQVLPQAPA PAPAPMVSALAOAPAPVVLPQAPA PAPAPMVSALAOAPAPPVVLPQAPA PAPAPMVSALAOAPAPPVVLPQAPA PAPAPMSALAOAPAPPVVLPQAPA PAPAPMSALAOAPAPPVVLPQAPA PAPAPMSALAOAPAPPVTDLASV DNSEFGQLLNGGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRFFHPPG SPWANRPLASLAPTTTGVHIEPVG SLTPAPVPQELDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTTFLNDE	NS3a-P2a-		·	'' 5	CAKCLKNNWECRYSPKTKRSPLTRA
mCherry/CM V-Gal4DBD- NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold MSDEER ART SUPPRESSIDE ARE SUPPRESSIDATE. SUPPRESSIDE ARE SUPPRESSIDE ARE SUPPRESSIDE ARE SUPPRESSIDE					
V-Gald/BBD- NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold WEGEVQIVSTATQTFLATSINGVLWT WHQAGTRIASPKGPVTQMYTNVD KDLVGWQAPQGSRSLTPCTCGSSD LYLVTRHADVIPVRRRGDSRGSLLS PRISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALLKIVA DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSSRVVEHAL MRIVLAIYLAEBILREAEESGDPEKR EKARERVREAVERAEEVGRDPSGW LNHECKLISEEDLDALLDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSDALDFPLDMLG SDALDDFDLDMLGSDALDFPLDMLG SDALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSDALDFPLDMLG SDALDDFDLDMLGSDALDFPLDASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPDPAPAPLGA PGPAMVSALAQAPAPVVLAPGP PQAVAPPAPKPTOAGEGTLSEALLOL QFDDEDLGALLGNSTDPAPATDFDASVL SQISSGSGSGSRSRSREGMELPKPEA GSALSDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTTTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE	DITORE VITE				·
NS3a-P2a- DNCR2-VPR, with NS3a and DNCR2 in bold NS4-P25- in bold NS5-P25- In bold NS5-P25-P25- In bold NS5-P25-P25-P25-P25-P25-P25-P25-P25-P25-P2			•		•
DNCR2-VPR, with NS3a and DNCR2 in bold WEGEVQIVSTATQTFLATSINGVLWT VYHGAGTRTIASPKGPYTQMYTINVD KDLVGWQAPQGSRSLTPCTCGSSD LYLVTRHADVIPVRRRGDSRGSLLS PRPISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRPSSSDVMEALKLIVEAIEAAV DALEAAERTGDPEVRELARELKRLA VEAAEEVQRNPSSSDVMEALHSIVY AIEAAIFALEAAERSGDPEVRELAREL LVRLA VEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLGS DALDDFDLDMLGS DALDDFDLDMLGS DALDDFDLDMLGS DALDDFDLDMLGS SOALDDFDLDMLGS					
with NS3a and DNCR2 in bold WHGAGTRTIASPKGPVTQMYTNVD KDLVGWQAPQGSRSI, LPCTCGSSD LYLVTRHADVIPVRRRGDSRGSLLS PRPISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTIM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAAERAQEA AERTGDPEVRELARELKRLAQEAAE EVKRDPSSSDVNBEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLG SDALDDFDLDMLG					
and DNCR2 in bold KDLVGWQAPQGSRSLTPCTCGSSD LYLVTRHADVIPVERRGDSRGSLLS PRRISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEVQRNPSSSDVNEALKSLIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSSDVNEALKSLIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSROWHALL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLGS DALDDFDLDMLGS DALDDFDLDMLGS DALDDFDLDMLGS DALDDFDLDMLGS SDALDDFDLDMLGSPKKKRKYGSQY LPDTDDRHRIEEKRKRTYETFKSIMK KSPFSGPTDPRPPPRRIAVPSRSSAS VPKPAPQPYPFTSSLSTINYDEFPTM VFPSGQISQASALAPAPPQVLPQAPA PAPAPAMWSALAQAPAPVPVLAPGP PQAVAPPAPKPTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQOLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPRGHLDELTTTLE			,		
in bold LYLVTRHADVIPVRRRGDSRGSLLS PRPISYLKGSAGGFLLCPAGHAVGIF RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAAERAQEA AERTGDPRVRELARELKLLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAAETGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSPKKKRKVGSQY LPDTDDRHRIEKRKTYETFKSIMK KSPFSGPTDPRPPRRIAVPSRSSAS VPKPAPQPYPFTSSLSTINYDEFPTM VFPSGQISQASALAPAPPQVLAPAP PAPAPMVSALAQAPAPVVLAPGP PQAVAPPAFKPTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE			with NS3a		I
PRPISYLKGSAGGPLLCPAGHAVGIF RAAVSTRGVAKAVOFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEEARELIERAKEAAERAQEA AERTGDPRVRELARELKRLAQEAAE EVKRDPSSSDVNEALKILVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAEAETGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVLAIYLAEENLREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLG SDALDDFDLDMLGSPKKKRKVGSQY LPDTDDRHRIEEKRKRTYETFKSIMK KSPFSGPTDPRPPPRRIAVPSRSSAS VPKPAPQPYPFTSSLSTINYDEFPTM VFPSGQISQASALAPAPPQVLPQAPA PAPAAMVSALAQAPAPVPVLAPGP PQAVAPPAPKPTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGRILDELTTTLE			and DNCR2		KDLVGWQAPQGSRSLTPCTCGSSD
RAAVSTRGVAKAVDFIPVESLETTM RSPGSGATNFSLLKQAGDVEENPGP MSSDEEARELIERAKEAAERAGEA AERTGDPVRELARELKRLAQEAAE EVKRDPSSSDVNEALKLIVEAIEAAV DALEAAERTGDPEVRELARELVRLA VEAAEEVQRNPSSSDVNEALHSIVY AIEAAIFALEAAERTGDPEVRELARE LVRLAVEAAEEVQRNPSSRNVEHAL MRIVALAIEALREAEESGDPEKR EKARERVREAVERAEEVQRDPSGW LNHEQKLISEEDLDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLGS DALDDFDLDMLGSDALDDFDLDMLGS SDALDDFDLDMLGSPKKKRKVGSQY LPDTTDDRHRIEEKRKRTYETFKSIMK KSPFSGPTDPRPPPRRIAVPSRSSAS VPKPAPQPYPFTSSLSTINYDEFPTM VFPSGQISQASALAPAPPQVLPQAPA PAPAPAMVSALAQAPAVVPVLAPGP PQAVAPPAPKPTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSGRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGMDLSHPPPRGHLDELTTTLE			in bold		LYLVTRHADVIPVRRRGDSRGSLLS
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PQAVAPPAPKPTQAGEGTLSEALLQL QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					
QFDDEDLGALLGNSTDPAVFTDLASV DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					
DNSEFQQLLNQGIPVAPHTTEPMLM EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					
EYPEAITRLVTGAQRPPDPAPAPLGA PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					· ·
PGLPNGLLSGDEDFSSIADMDFSALL SQISSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					DNSEFQQLLNQGIPVAPHTTEPMLM
SQISSGSGSRDSREGMFLPKPEA GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					EYPEAITRLVTGAQRPPDPAPAPLGA
GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					PGLPNGLLSGDEDFSSIADMDFSALL
GSAISDVFEGREVCQPKRIRPFHPPG SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					SQISSGSGSGSRDSREGMFLPKPEA
SPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					,
SLTPAPVPQPLDPAPAVTPEASHLLE DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					
DPDEETSQAVKALREMADTVIPQKEE AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					
AAICGQMDLSHPPPRGHLDELTTTLE SMTEDLNLDSPLTPELNEILDTFLNDE					
SMTEDLNLDSPLTPELNEILDTFLNDE					
					CLLHAMHISTGLSIFDTSLF

CXCR4_fwd	qPCR primer	Fig. 4A; Supp. Fig. 10G	GAAGCTGTTGGCTGAAAAGG
CXCR4_rev	qPCR primer	Fig. 4A; Supp. Fig. 10G	CTCACTGACGTTGGCAAAGA
GAPDH_fwd	qPCR primer	Supp. Fig. 10G	ACAGTCAGCCGCATCTTCTT
GAPDH_rev	qPCR primer	Supp. Fig. 10G	ACGACCAAATCCGTTGACTC
GFP_fwd	qPCR primer	Supp. Fig. 10G	AGCAGAAGAACGGCATCAAG
GFP_rev	qPCR primer	Supp. Fig. 10G	GGGGTGTTCTGCTGGTAGTG
CD95_fwd	qPCR primer	Supp. Fig. 10G	ATGGTGTCAATGAAGCCAAA
CD95_rev	qPCR primer	Supp. Fig. 10G	TGATGCCAATTACGAAGCAG

Supplementary Table 5 | Drug concentrations used for Figure 3b,c

	grazoprevir (nM)				
	low proportion	medium proportion	high proportion		
	grazoprevir	grazoprevir	grazoprevir		
danoprevir	2-fold	1.5-fold	1.25-fold		
(nM)	serial	serial	serial		
	dilutions	dilutions	dilutions		
1000.00	10.00	10.00	10.00		
400.00	5.00	6.67	8.00		
160.00	2.50	4.44	6.40		
64.00	1.25	2.96	5.12		
25.60	0.63	1.98	4.10		
10.24	0.31	1.32	3.28		
4.10	0.16	0.88	2.62		
1.64	0.08	0.59	2.10		
0.66	0.04	0.39	1.68		
0.26	0.02	0.26	1.34		
0.10	0.01	0.17	1.07		
0.04	0.005	0.12	0.86		

Design scripts

```
ROSETTA Script cid design.xml:
<dock design>
 <SCOREFXNS>
  <sfxn hard weights=talaris2014/>
  <sfxn soft weights=soft rep/>
  <sfxn_hard_cst weights=talaris2014>
   <Reweight scoretype=coordinate constraint weight=1 />
  </sfxn hard cst>
  ### score function that contains the full atom attractive forces only
  <VDW weights="empty">
          <Reweight scoretype="fa atr" weight=1.0/>
  </VDW>
 </SCOREFXNS>
 <RESIDUE SELECTORS>
  In the setup below, chain A is target (NS3a) and chain B is scaffold, X is ligand.
  In the input pdb, chain B should be 1st, followed by chain A and chain X.
  <Chain name=chA chains=A,X/>
  <Chain name=chX chains=X/>
  <Chain name=chB chains=B/>
  <InterfaceByVector name=intf cb dist cut=10 nearby atom cut=5.5 vector angle cut=75.0 vector dist cut=9.0</p>
grp1 selector=chA grp2 selector=chB/>
  <InterfaceByVector name=ligand intf cb dist cut=10 nearby atom cut=5.5 vector angle cut=75.0</p>
vector dist cut=9.0 grp1 selector=chX grp2 selector=chB/>
  <ResidueName name=hp residue name3="VAL,PHE,TYR,ILE,LEU,TRP,MET" />
  <And name=hp in A selectors=intf,hp,chA/>
                                                 hydrophobic residues on target
  <Or name=hp in A and X selectors=hp in A,chX/> union: hydrophobic residues on target + Ligand +
contacting residues on scaffold
  <Neighborhood name=hp neighbor selector=hp in A and X distance=10.0/>
  <And name=hp contact in B selectors=chB,hp neighbor/>contacting residues on scaffold
  <Or name=hpA contactB selectors=hp in A,hp contact in B/> union: hydrophobic residues on target +
contacting residues on scaffold
  <Not name=not hpA contactB selector=hpA contactB/> negation of hpA contactB for restriction
  <Not name=not ligand intf selector=ligand intf/> negation of hpA contactB for restriction
  # hp revert
  <Neighborhood name=hp revert selector=chX distance=10.0/>
  <And name=hp rvt selectors=hp revert/>
 </RESIDUE_SELECTORS>
 <TASKOPERATIONS>
  <ProteinLigandInterfaceUpweighter name=up interface weight=1.5/>
  <DesignAround name=da design_shell=10 resnums=1X repack_shell=10.0 allow_design=1</p>
resnums allow design=0/>
  <IncludeCurrent name=inclcur/>
  <PreventChainFromRepacking chain=2 name=pctr2/>
  <PreventChainFromRepacking chain=3 name=pctr3/>
  <ExtraRotamersGeneric name=exrot ex1=1 ex2=1 extrachi cutoff=1/>
```

```
<InitializeFromCommandline name=init/>
  <LimitAromaChi2 name=arochi2 chi2max=110 chi2min=70 />
  <RestrictAbsentCanonicalAAS name=nohis keep aas="ACDEFGIKLMNPQRSTVWY"/>
  <RestrictAbsentCanonicalAAS name=nocys keep_aas="ADEFIKLMNPQRSTVWYH"/>
  #noCvs & noGlv
  # this allows PRO, GLY, CYS in the interface it seems that CYS is overfavoured so restrict this might be
necessary
  <ProteinInterfaceDesign name=dzn inter design chain1=1 design chain2=0 repack chain1=1 repack chain2=0</p>
jump=1 interface distance cutoff=11.0 allow all aas=1/>
  Design ONLY near the hydrophobic residues on target
  <OperateOnResidueSubset name=hp task selector=not hpA contactB >
   <Pre><PreventRepackingRLT/>
  </OperateOnResidueSubset>
  <OperateOnResidueSubset name=ligand interface selector=not ligand intf>
   <Pre><PreventRepackingRLT/>
  </OperateOnResidueSubset>
  <DisallowIfNonnative name=dis_charge resnum=0 disallow_aas=RQKDEG/>
  <DesignAround name=da10 design_shell=12 resnums=1X repack_shell=14.0 allow_design=1</p>
resnums allow design=0/>
  # Selector hp revert contains all residues within the ligand
  # these are set to prevent repacking
  <OperateOnResidueSubset name=nodesignaroundligand</p>
    selector=hp revert >
   <Pre><PreventRepackingRLT/>
  </OperateOnResidueSubset>
  ### this task operation defines an extended generous interface for minimization
  <ProteinInterfaceDesign name="pack_long" design_chain1="0" design_chain2="0" jump="1"</p>
interface distance cutoff="15"/>//
 </TASKOPERATIONS>
 <MOVERS>
  <MinMover name=min rb chi=1 bb=0 jump=1>
   <MoveMap>
    <Chain number=1 chi=1 bb=0/>
                                        only chi for LRR
    <Chain number=2 chi=1 bb=0/>
                                        only chi for target
    <Chain number=3 chi=0 bb=0/>
                                        not chi for Ligand fixed as in crystal structure
   </MoveMap>
  </MinMover>
  <MinMover name=min init chi=1 bb=0 jump=0>
   <MoveMap>
    <Chain number=1 chi=1 bb=0/>
                                        only chi for LRR
    <Chain number=2 chi=0 bb=0/>
                                        only chi for target
    <Chain number=3 chi=0 bb=0/>
                                        only chi for Ligand
   </MoveMap>
  </MinMover>
```

```
<TaskAwareMinMover name=min chi bb=0 chi=1 jump=0 scorefxn=sfxn hard
task operations=init.arochi2.inclcur.exrot.dzn inter/>
  <AtomTree name=simple ft docking ft=0 simple ft=1/>
  # Design based on ligand task operation
  <PackRotamersMover name=dzn packrot layer soft ligand scorefxn=sfxn soft
task operations=init,exrot,inclcur,arochi2,nohis,pctr2,da,up,nocys,dis charge/>
   <PackRotamersMover name=dzn packrot layer hard ligand scorefxn=sfxn hard
task operations=init,exrot,inclcur,arochi2,nohis,pctr2,da,up,nocys,dis charge/>
  # Normal design
  <PackRotamersMover name=dzn packrot soft scorefxn=sfxn soft</pre>
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,pctr2,pctr3,up,nocys/>
  <PackRotamersMover name=dzn packrot hard scorefxn=sfxn hard
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,pctr2,pctr3,nocys/>
  <DumpPdb name=d1 fname=dump1.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d2 fname=dump2.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d3 fname=dump3.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d4 fname=dump4.pdb scorefxn=sfxn hard cst />
  <DumpPdb name=d5 fname=dump5.pdb scorefxn=sfxn hard cst />
  # Normal design
  <PackRotamersMover name=dzn packrot soft last scorefxn=sfxn soft
task operations=init,exrot,inclcur,arochi2.nohis,dzn inter,nocys,pctr2,pctr3,up.nodesignaroundligand/>
  <PackRotamersMover name=dzn packrot hard last scorefxn=sfxn hard
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,nocys,pctr2,pctr3,nodesignaroundligand/>
  ### minimization of the extended PPI
  <TaskAwareMinMover name=min scorefxn=sfxn hard bb=0 chi=1 task operations=pack long/>
 </MOVERS>
<FILTERS>
  <Sasa name=sasa jump1 threshold=1100 confidence=1 jump=1/> # scaffold. Confidence=1 used for grazoprevir
CID design, but not danoprevir CID design
  <Sasa name=sasa jump2 confidence=0 jump=2/> # ligand
  <Sasa name="interface hydrophobic sasa" confidence=0 hydrophobic=True/>
  <Sasa name="interface polar sasa" confidence=0 polar=True/>
  <Ddg name=ddg jump1 threshold=-10 scorefxn=sfxn hard jump=1 repack=1 relax mover=min chi repeats=1
confidence=0/> # ligand
  <Ddg name=ddg_jump2 threshold=-10 scorefxn=sfxn_hard jump=2 repack=1 relax_mover=min_chi repeats=1</p>
confidence=0/> # ligand
  <Ddg name="ddg fa atr" threshold=-10 jump=1 repeats=5 repack=1 relax mover=min confidence=0
scorefxn=VDW />
  <Ddg name="ddg fa atr norepack" threshold=-10 jump=1 repeats=1 repack=0 confidence=0 scorefxn=VDW/>
  <ScoreType name=total score scorefxn=sfxn hard score type=total score threshold=0 confidence=0/>
  <AverageDegree name=deg threshold=8.6 distance threshold=8 task operations=dzn inter confidence=0/>
  <CalculatorFilter name="ddg fa atr per 1000sasa" equation="1000 * ddg / (sasa+0.01)" threshold="1"
confidence="0">
    <VAR name="ddg" filter="ddg fa atr"/>
    <VAR name="sasa" filter=sasa jump1/>
  </CalculatorFilter>
```

```
<CalculatorFilter name="ddg fa atr norepack per 1000sasa" equation="1000 * ddg / (sasa+0.01)"
threshold="1" confidence="0">
    <VAR name="ddg" filter="ddg fa atr norepack"/>
        <VAR name="sasa" filter=sasa jump1/>
  </CalculatorFilter>
  <ShapeComplementarity name=sc jump1 jump=1 verbose=1 min sc=0.6 confidence=0/>
                                                                                         #scaffold
  <ShapeComplementarity name=sc_jump2 jump=2 verbose=1 min_sc=0.6 confidence=0/>
                                                                                         #ligand
  <LigInterfaceEnergy name=interfE scorefxn=sfxn hard jump number=2 energy cutoff=-5 confidence=0/>
  <DesignableResidues name=dr task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,pctr3 designable=1</p>
packable=1/>
  <InterfaceHoles name="interface holes" confidence=0/>
  <Sasa name="hydrophobic sasa" confidence=0 jump=1 hydrophobic=True/>
  <BuriedUnsatHbonds2 name="interface unsat hbond" confidence=0/>
  <Rmsd name=rmsdB chains="B" threshold=2.0 superimpose=0 confidence=0/>
 </FILTERS>
 <APPLY_TO_POSE>
 </APPLY_TO_POSE>
 <PROTOCOLS>
  # Setting up atomtree with simple foldtree
  <Add mover=simple ft/>
  # Movemaps to minimize scaffold
  <Add mover=min init/>
  # PackRotamer with soft score function
  <Add mover=dzn packrot soft/>
  <Add mover=min chi/>
  <Add mover=dzn packrot hard/>
  <Add mover=min chi/>
  <Add mover=dzn packrot soft/>
  <Add mover=min chi/>
  <Add mover=dzn_packrot_hard/>
  <Add mover=min chi/>
  #Design around the ligand
  <Add mover=dzn packrot layer soft ligand/>
  <Add mover=min chi/>
  <Add mover=dzn_packrot_layer_hard_ligand/>
  <Add mover=min chi/>
  <Add mover=min rb/>
  <Add mover=dzn packrot layer soft ligand/>
  <Add mover=min chi/>
  <Add mover=dzn packrot layer hard ligand/>
  <Add mover=min chi/>
  <Add mover=dzn_packrot_soft_last/>
  <Add mover=dzn packrot hard last/>
  <Add filter=sasa jump1/>
```

```
<Add filter=sasa jump2/>
  <Add filter=deg/>
  <Add filter=total score/>
  <Add filter=sc jump1/>
  <Add filter=sc jump2/>
  <Add filter=ddg jump1/>
  <Add filter=ddg jump2/>
  <Add filter=interfE/>
  <Add filter=dr/>
  <Add filter="interface holes"/>
  <Add filter="hydrophobic sasa"/>
  <Add filter="rmsdB"/>
  <Add filter="interface unsat hbond"/>
  <Add filter="interface hydrophobic sasa"/>
  <Add filter="interface polar sasa"/>
  <Add filter="ddg fa atr"/>
  <Add filter="ddg fa atr norepack"/>
  <Add filter="ddg fa atr per 1000sasa"/>
  <Add filter="ddg_fa_atr_norepack_per_1000sasa"/>
 </PROTOCOLS>
</dock design>
```

ROSETTA Script cid_roll_design.xml:

```
<dock roll design>
 <SCOREFXNS>
  <sfxn hard weights=talaris2014/>
  <sfxn soft weights=soft rep/>
  <sfxn hard cst weights=talaris2014>
   <Reweight scoretype=coordinate constraint weight=1 />
  </sfxn hard cst>
  ### score function that contains the full atom attractive forces only
  <VDW weights="empty">
        <Reweight scoretype="fa atr" weight=1.0/>
  </VDW>
 </SCOREFXNS>
 <RESIDUE SELECTORS>
  In the setup below, chain A is target and chain B is scaffold, X is ligand.
  In the input pdb, chain B should be 1st, followed by chain A and chain X.
  <Chain name=chA chains=A,X/>
  <Chain name=chX chains=X/>
  <Chain name=chB chains=B/>
  <InterfaceByVector name=intf cb dist cut=10 nearby atom cut=5.5 vector angle cut=75.0 vector dist cut=9.0</p>
grp1 selector=chA grp2 selector=chB/>
```

```
<InterfaceByVector name=ligand intf cb dist cut=10 nearby atom cut=5.5 vector angle cut=75.0</p>
vector dist cut=9.0 grp1 selector=chX grp2 selector=chB/>
  <ResidueName name=hp residue name3="VAL,PHE,TYR,ILE,LEU,TRP,MET" />
  <And name=hp in A selectors=intf,hp,chA/>
                                                hydrophobic residues on target
  <Or name=hp in A and X selectors=hp in A,chX/> union: hydrophobic residues on target + Ligand +
contacting residues on scaffold
  <Neighborhood name=hp neighbor selector=hp in A and X distance=10.0/>
  <And name=hp_contact_in_B selectors=chB,hp_neighbor/>contacting residues on scaffold
  <Or name=hpA contactB selectors=hp in A,hp contact in B/> union: hydrophobic residues on target +
contacting residues on scaffold
  <Not name=not hpA contactB selector=hpA contactB/> negation of hpA contactB for restriction
  <Not name=not ligand intf selector=ligand intf/> negation of hpA contactB for restriction
  # hp revert
  <Neighborhood name=hp revert selector=chX distance=10.0/>
  <And name=hp rvt selectors=hp revert/>
 </RESIDUE_SELECTORS>
 <TASKOPERATIONS>
  <ProteinLigandInterfaceUpweighter name=up interface weight=1.5/>
  <DesignAround name=da design_shell=10 resnums=1X repack_shell=10.0 allow_design=1</p>
resnums allow design=0/>
  <IncludeCurrent name=inclcur/>
  <PreventChainFromRepacking chain=2 name=pctr2/>
  <PreventChainFromRepacking chain=3 name=pctr3/>
  <ExtraRotamersGeneric name=exrot ex1=1 ex2=1 extrachi cutoff=1/>
  <InitializeFromCommandline name=init/>
  <LimitAromaChi2 name=arochi2 chi2max=110 chi2min=70 />
  <RestrictAbsentCanonicalAAS name=nohis keep_aas="ACDEFGIKLMNPQRSTVWY"/>
  <RestrictAbsentCanonicalAAS name=nocys keep_aas="ADEFIKLMNPQRSTVWYH"/>
  #noCys & noGly
  # this allows PRO, GLY, CYS in the interface it seems that CYS is overfavoured so restrict this might be
necessary
  <ProteinInterfaceDesign name=dzn inter design chain1=1 design chain2=0 repack chain1=1 repack chain2=0</p>
jump=1 interface distance cutoff=11.0 allow all aas=1/>
  Design ONLY near the hydrophobic residues on target
  <OperateOnResidueSubset name=hp task selector=not hpA contactB >
   <Pre><PreventRepackingRLT/>
  </OperateOnResidueSubset>
  <OperateOnResidueSubset name=ligand interface selector=not ligand intf>
   <Pre><PreventRepackingRLT/>
  </OperateOnResidueSubset>
  <DisallowIfNonnative name=dis charge resnum=0 disallow aas=RQKHDEG/>
  <DesignAround name=da10 design_shell=12 resnums=1X repack_shell=14.0 allow_design=1</p>
resnums allow design=0/>
  # Selector hp revert contains all residues within the ligand
  # these are set to prevent repacking
  <OperateOnResidueSubset name=nodesignaroundligand</p>
    selector=hp revert >
   <Pre><PreventRepackingRLT/>
```

```
</OperateOnResidueSubset>
  ### this task operation defines an extended generous interface for minimization
  <ProteinInterfaceDesign name="pack long" design chain1="0" design chain2="0" jump="1"</p>
interface_distance_cutoff="15"/>//
 </TASKOPERATIONS>
 <MOVERS>
  <RollMover name="randomroll" chain=1 random roll=1 random roll angle mag=4.0
random roll trans mag=3.0/>
  <MinMover name=min rb chi=1 bb=0 jump=1>
   <MoveMap>
    <Chain number=1 chi=1 bb=0/>
                                         only chi for scaffold
    <Chain number=2 chi=1 bb=0/>
                                         only chi for target
    <Chain number=3 chi=0 bb=0/>
                                         not chi for Ligand fixed as in crystal structure
   </MoveMap>
  </MinMover>
  <MinMover name=min init chi=1 bb=0 jump=0>
   <MoveMap>
    <Chain number=1 chi=1 bb=0/>
                                         only chi for scaffold
    <Chain number=2 chi=0 bb=0/>
    <Chain number=3 chi=0 bb=0/>
   </MoveMap>
  </MinMover>
  <TaskAwareMinMover name=min chi bb=0 chi=1 jump=0 scorefxn=sfxn hard
task operations=init,arochi2,inclcur,exrot,dzn inter/>
  <a href="#">AtomTree name=simple_ft docking_ft=0 simple_ft=1/></a>
  # Design based on ligand task operation
  <PackRotamersMover name=dzn packrot layer soft ligand scorefxn=sfxn soft
task operations=init,exrot,inclcur,arochi2,nohis,pctr2,da,up,nocys,dis charge/>
  <PackRotamersMover name=dzn_packrot_layer_hard_ligand scorefxn=sfxn_hard</pre>
task operations=init,exrot,inclcur,arochi2,nohis,pctr2,da,up,nocys,dis charge/>
  # Normal design
  <PackRotamersMover name=dzn packrot soft scorefxn=sfxn soft</pre>
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,pctr2,pctr3,up,nocys/>
   <PackRotamersMover name=dzn_packrot_hard scorefxn=sfxn_hard</pre>
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,pctr2,pctr3,nocys/>
  <DumpPdb name=d1 fname=dump1.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d2 fname=dump2.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d3 fname=dump3.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d4 fname=dump4.pdb scorefxn=sfxn hard cst/>
  <DumpPdb name=d5 fname=dump5.pdb scorefxn=sfxn hard cst/>
  # Normal design
  <PackRotamersMover name=dzn packrot soft last scorefxn=sfxn soft
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,nocys,pctr2,pctr3,up,nodesignaroundligand/>
  <PackRotamersMover name=dzn packrot hard last scorefxn=sfxn hard
task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,nocys,pctr2,pctr3,nodesignaroundligand/>
```

```
### minimization of the extended PPI
  <TaskAwareMinMover name=min scorefxn=sfxn hard bb=0 chi=1 task operations=pack long/>
 </MOVERS>
<FILTERS>
  <Sasa name=sasa jump1 confidence=0 jump=1/> # scaffold
  <Sasa name=sasa jump2 confidence=0 jump=2/> # ligand
  <Ddg name=ddg jump1 threshold=-10 scorefxn=sfxn hard jump=1 repack=1 relax mover=min chi repeats=1
confidence=0/> # ligand
  <Ddg name=ddg jump2 threshold=-10 scorefxn=sfxn hard jump=2 repack=1 relax mover=min chi repeats=1
confidence=0/> # ligand
  <ScoreType name=total score scorefxn=sfxn hard score type=total score threshold=0 confidence=0/>
  <AverageDegree name=deg threshold=8.6 distance threshold=8 task operations=dzn inter confidence=0/>
  <ShapeComplementarity name=sc jump1 jump=1 verbose=1 min sc=0.6 confidence=0/>
                                                                                         #scaffold
  <ShapeComplementarity name=sc_jump2 jump=2 verbose=1 min_sc=0.6 confidence=0/>
                                                                                         #ligand
  <LigInterfaceEnergy name=interfE scorefxn=sfxn_hard jump_number=2 energy_cutoff=-5 confidence=0/>
  <DesignableResidues name=dr task operations=init,exrot,inclcur,arochi2,nohis,dzn inter,pctr3 designable=1</p>
packable=1/>
  <InterfaceHoles name="interface holes" confidence=0/>
  <Sasa name="hydrophobic sasa" confidence=0 jump=1 hydrophobic=True/>
  <BuriedUnsatHbonds2 name="interface unsat hbond" confidence=0/>
  <Rmsd name=rmsdB chains="B" threshold=2.0 superimpose=0 confidence=0/>
  <Sasa name="interface hydrophobic sasa" confidence=0 hydrophobic=True/>
  <Sasa name="interface polar sasa" confidence=0 polar=True/>
  <Ddg name="ddg fa atr" threshold=-10 jump=1 repeats=5 repack=1 relax mover=min confidence=0
scorefxn=VDW />
  <Ddg name="ddg fa atr norepack" threshold=-10 jump=1 repeats=1 repack=0 confidence=0 scorefxn=VDW/>
  <CalculatorFilter name="ddg fa atr per 1000sasa" equation="1000 * ddg / (sasa+0.01)" threshold="1"
confidence="0">
        <VAR name="ddg" filter="ddg fa atr"/>
        <VAR name="sasa" filter=sasa jump1/>
  </CalculatorFilter>
  <CalculatorFilter name="ddg fa atr norepack per 1000sasa" equation="1000 * ddg / (sasa+0.01)"
threshold="1" confidence="0">
        <VAR name="ddg" filter="ddg fa atr norepack"/>
        <VAR name="sasa" filter=sasa jump1/>
  </CalculatorFilter>
 </FILTERS>
 <APPLY TO POSE>
 </APPLY_TO_POSE>
 <PROTOCOLS>
```

```
# Setting up atomtree with simple foldtree
  <Add mover=simple ft/>
  #RollMover to perturb scaffold
  <Add mover name="randomroll"/>
  # PackRotamer with soft score function
  <Add mover=dzn packrot soft/>
  <Add mover=min_chi/>
  <Add mover=dzn_packrot_hard/>
  <Add mover=min_chi/>
  <Add mover=dzn packrot soft/>
  <Add mover=min chi/>
  <Add mover=dzn packrot hard/>
  <Add mover=min chi/>
  #Design around the ligand
  <Add mover=dzn packrot layer soft ligand/>
  <Add mover=min chi/>
  <Add mover=dzn packrot layer hard ligand/>
  <Add mover=min chi/>
  <Add mover=min rb/>
  <Add mover=dzn_packrot_layer_soft_ligand/>
  <Add mover=min chi/>
  <Add mover=dzn packrot layer hard ligand/>
  <Add mover=min chi/>
  <Add mover=dzn packrot soft last/>
  <Add mover=dzn_packrot_hard_last/>
  <Add filter=sasa jump1/>
  <Add filter=sasa jump2/>
  <Add filter=deg/>
  <Add filter=total score/>
  <Add filter=sc jump1/>
  <Add filter=sc jump2/>
  <Add filter=ddg jump1/>
  <Add filter=ddg jump2/>
  <Add filter=interfE/>
  <Add filter=dr/>
  <Add filter="interface holes"/>
  <Add filter="hydrophobic sasa"/>
  <Add filter="rmsdB"/>
  <Add filter="interface unsat hbond"/>
  <Add filter="interface hydrophobic sasa"/>
  <Add filter="interface polar sasa"/>
  <Add filter="ddg fa atr"/>
  <Add filter="ddg fa atr norepack"/>
  <Add filter="ddg fa atr per 1000sasa"/>
  <Add filter="ddg fa atr norepack per 1000sasa"/>
 </PROTOCOLS>
</dock roll design>
```

RIF generation flags, rifgen grazo.flags:

```
-rifgen::rif type RotScore64
-rifgen:extra rotamers false
-rifgen:extra rif rotamers true
-rif accum scratch size M 24000
                  input files/3SUD SUE.prelax.pdb
-rifgen:target
-rifgen:target res
                    input files/3SUD SUE target.txt
-extra_res_fa input_files/SUE.fa.params
-renumber pdb
-hash cart resl
                      0.7
-hash angle resl
                      14.0
-rifgen::rosetta field resl 0.25
-rifgen::search resolutions 3.0 1.5 0.75
-rifgen:score threshold -0.5
-rifgen:hbond weight 1.0
                              # max score per-hbond
-rifgen:upweight multi hbond 1.0 # extra score factor for bidentate hbonds
-rifgen:data cache dir ~directorypath/data/scheme data exrots2
-rifgen:outdir
                   output rifgen
-rifgen:outfile
                   3SUD SUE.rif.gz
-rifgen:score cut adjust 0.7
-rifgen:apores VAL ILE LEU MET PHE TRP
-rifgen:donres SER THR TYR GLN ASN HIS HIS D TRP
-rifgen:accres SER THR TYR GLU GLN ASP ASN HIS HIS_D
-hbond cart sample hack range 0.33
-hbond cart sample hack resl 0.33
-rifgen:tip tol deg
                      60.0 # for now, do either 60 or 36
-rifgen:rot samp resl
                        6.0
-rifgen:rif hbond dump fraction 0.000001
-rifgen:rif apo dump fraction 0.000001
-add orbitals
-rifgen:beam_size_M 10000.0
-rifgen:hash preallocate mult 0.125
-rifgen:max rf bounding ratio 4.0
-rifgen:hash cart resls 16.0 8.0 4.0 2.0 1.0
```

-rifgen:hash cart bounds 512 512 512 512 512

```
-rifgen:lever_bounds 16.0 8.0 4.0 2.0 1.0

-rifgen:hash_ang_resls 38.8 24.4 17.2 13.6 11.8 # yes worky worky

-rifgen:lever_radii 23.6 18.785501 13.324600 8.425850 4.855575

-database ~directorypath/rosetta/librosetta_update/database
```

RIF docking flags (rifdock grazo.flags):

```
#### the block below comes from the bottom of the log file from rif generation
output rifgen/3SUD SUE.rif.gz target.pdb.gz
-rif dock:target pdb
-in:file:extra res fa
                    input files/SUE.fa.params
-rif dock:target res
                    input files/3SUD SUE target.txt
-rif dock:target rf resl
                     0.25
-rif dock:target rf cache
output rifgen/ RF 3SUD SUE.prelax.pdb CEN trhash29405780 resl0.25 osamp2 replonlybdry
-rif dock:target bounding xmaps output rifgen/3SUD SUE.rif.gz BOUNDING RIF 16.xmap.gz
-rif dock:target bounding xmaps output rifgen/3SUD SUE.rif.gz BOUNDING RIF 08.xmap.gz
-rif dock:target bounding xmaps output rifgen/3SUD SUE.rif.gz BOUNDING RIF 04.xmap.gz
-rif dock:target bounding xmaps output rifgen/3SUD SUE.rif.gz BOUNDING RIF 02.xmap.gz
-rif dock:target bounding xmaps output rifgen/3SUD SUE.rif.gz BOUNDING RIF 01.xmap.gz
                    output rifgen/3SUD SUE.rif.gz
-rif dock:target rif
-rif dock:extra rotamers
-rif dock:extra rif rotamers 1
#### this is where the output will go, and how much
-rif dock:outdir rifdock grazo 30junc
-rif dock:dokfile all.dok
-rif dock:n pdb out 20 # max number of output pdbs
-rif dock:redundancy filter mag 1.0
-rif dock:align output to scaffold false
#-rif dock:target tag conf01 # optional tag to add to all outputs
-beam size M 5
-hsearch scale factor 1.2
#### to use -beta scoring function (betaJuly16)
```

```
-beta
-score:weights beta soft
-add orbitals false
#### if you DO NOT supply scaffold res files, this will attempt to pick which residues on the scaffold
#### can be mutated based on sasa, internal energy, and bb-sc hbonds
-scaffold res use best guess true
#### if scaffold res is NOT used, this option will cause loop residues to be ignored
#### scaffold res overrides this
-rif dock::dont use scaffold loops True
#### these cause the non-designable scaffold residues to still contribute sterically
#### and to the 1 body rotamer energies. use these flags if you have a fully-designed scaffold
-rif dock:scaffold to ala false
-rif dock:scaffold to ala selonly true
-rif dock:replace all with ala 1bre false
#### if you don't have a fully designed scaffold, treat non-designable positions as alanine
#-rif dock:scaffold to ala true
#-rif dock:scaffold to ala selonly false
#-rif dock:replace all with ala 1bre true
-rif dock:hbond weight 1.0
-rif dock:upweight multi hbond 1.0 # value of 1.0 could up to double hbscore if bidentate, triple if tridentate... best
in conjunction with low-ish starting hbweight
#### weight interactions with the target more than intra-scaffold interactions
#### kinda like the rosetta_score_*_weight flags, but applies to the rif calculations
-rif dock:upweight iface 2.0
############ rif packing options, probably don't change
-hack pack true
-rif dock:pack n iters 2
-rif dock:pack iter mult 2.0
-rif dock:hack pack frac 0.1
-rif dock:packing use rif rotamers
-rif dock:extra rotamers
                                false
-rif dock:always available rotamers level 0
-rif dock::rf resl 0.5
-rif dock::rf oversample 2
-rif dock:use scaffold bounding grids 0
#### negative interactions are always full-weight, this is positive only
-rif dock:rosetta score rifres rifres weight 0.6
-rif dock:rosetta score rifres scaffold weight 0.4
#### score cut for the rosetta "score," which is kinda a ddg, but with hbond weighs highern
-rif dock:rosetta score cut -15.0
-rif dock:rosetta score fraction 0.006
-rif dock:rosetta min fraction 0.07
-rif dock:rosetta min scaffoldbb false
```

```
-rif dock:rosetta min targetbb false
-rif dock:rosetta hard min false
#-rif dock:rosetta score then min below thresh -20.0 # this is in "rif docking score units"
#-rif dock:rosetta score at least 3000
#-rif dock:rosetta score at most 30000
#### details for how twobody rotamer energies are computed and stored, don't change
-rif dock:rotrf resl 0.25
-rif_dock:rotrf_spread 0.0
-rif dock:rotrf scale atr 1.0
-rif dock:rotrf cache dir ~directorypath/data/scheme data exrots2 by structure/rot rf tables 025 0 atr1
-rif dock:data cache dir ./rifdock v4 scaffdata 025 0 atr1
-rif dock:cache scaffold data true
#### options to favor existing scaffold residues
-add native scaffold rots when packing 0 # 1
-bonus to native scaffold res
                                    0 # -0.5
-rif dock:pdb info pikaa false # this is default I think
-rif dock:global score cut -0.0
# disulfides seem to cause problems... ignoring them isn't really an issue unless
# you do bbmin where there should be disulfides
-detect disulf 0
-database ~directorypath/rosetta/librosetta update/database
-rif_dock:target_rf_oversample 2
-mute core.scoring.ScoreFunctionFactory
-mute core.io.pose from sfr.PoseFromSFRBuilder
```

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