Supporting Information

Allosteric Inhibition of Parkinson's-linked LRRK2 by Constrained Peptides

Leah G. Helton [1,†], Ahmed Soliman[2,†], Felix von Zweydorf[3], Michalis Kentros[4], Jascha T. Manschwetus[5], Scotty Hall[1], Bernd Gilsbach[3], Franz Y. Ho[2], Panagiotis S. Athanasopoulos[2], Ranjan K. Singh[6,7], Timothy J. LeClair[1], Wim Versées[6,7], Francesco Raimondi[8], Friedrich W. Herberg[5], Christian Johannes Gloeckner[3,9], Hardy Rideout[4], Arjan Kortholt[2,10]* and Eileen J. Kennedy[1]*

[1]Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, Georgia 30602, United States.

[2]Department of Cell Biochemistry, University of Groningen, 9747 Groningen, The Netherlands.

[3]DZNE German Center for Neurodegenerative Diseases, 72076 Tübingen, Germany.

[4]Center for Clinical, Experimental Surgery, and Translational Research, Biomedical Research Foundation of the Academy of Athens, 115 27 Athens, Greece.

[5]Department of Biochemistry, Institute for Biology, University of Kassel, 34132, Kassel, Germany.

[6]VIB-VUB Center for Structural Biology, Pleinlaan 2, 1050 Brussels, Belgium.

[7]Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium.

[8]Laboratorio di Biologia Bio@SNS, Scuola Normale Superiore, 56126, Pisa, Italy.

[9]Core Facility for Medical Bioanalytics, Center for Ophthalmology, Institute for Ophthalmic Research, University of Tübingen, 72076 Tübingen, Germany.

[10]Department of Pharmacology, Innovative Technologies Application and Research Center, Suleyman Demirel University, 32260 Isparta, Turkey.

† co-first authors

* Corresponding authors: Eileen Kennedy (ekennedy@uga.edu), Arjan Kortholt (a.kortholt@rug.nl)

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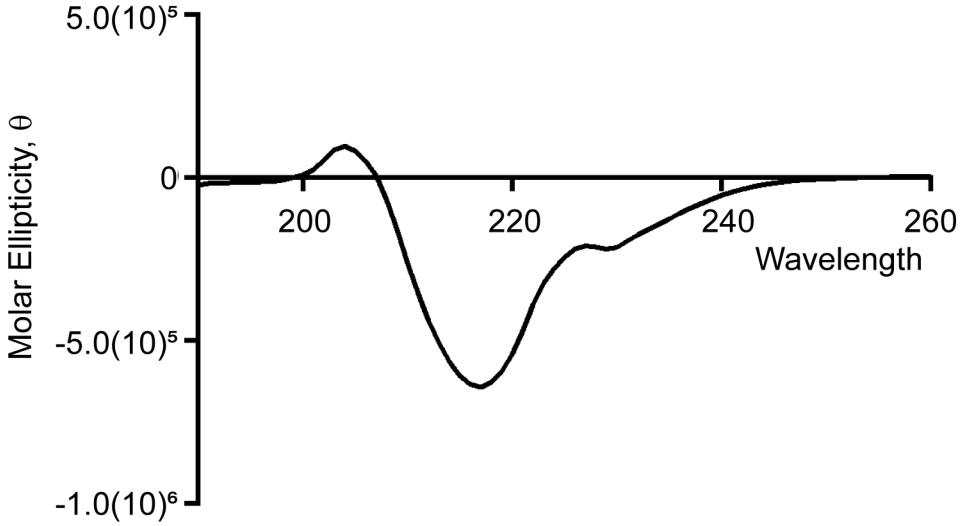
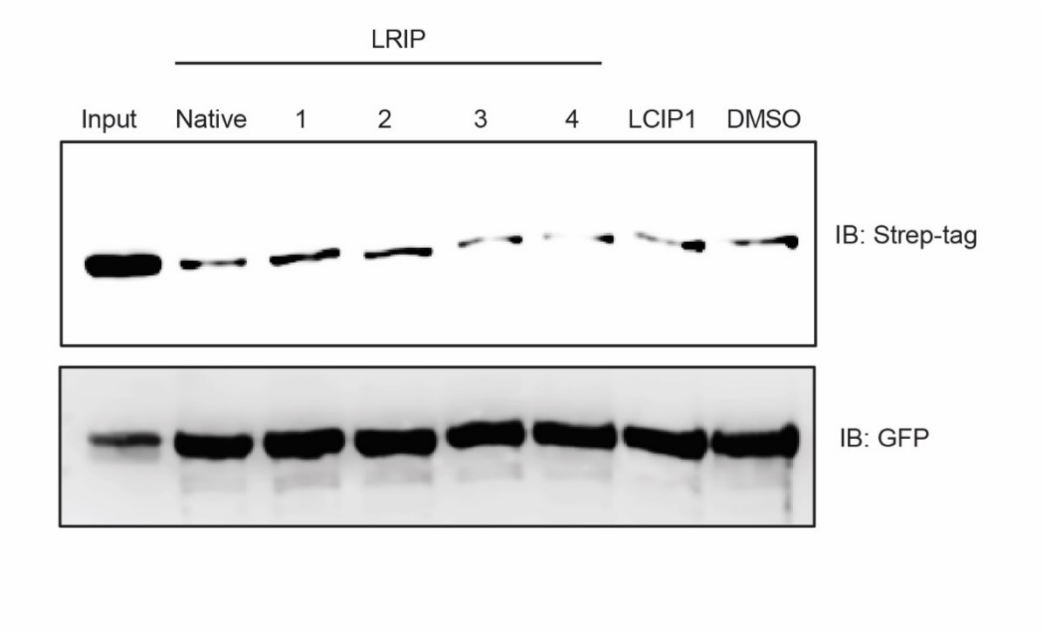
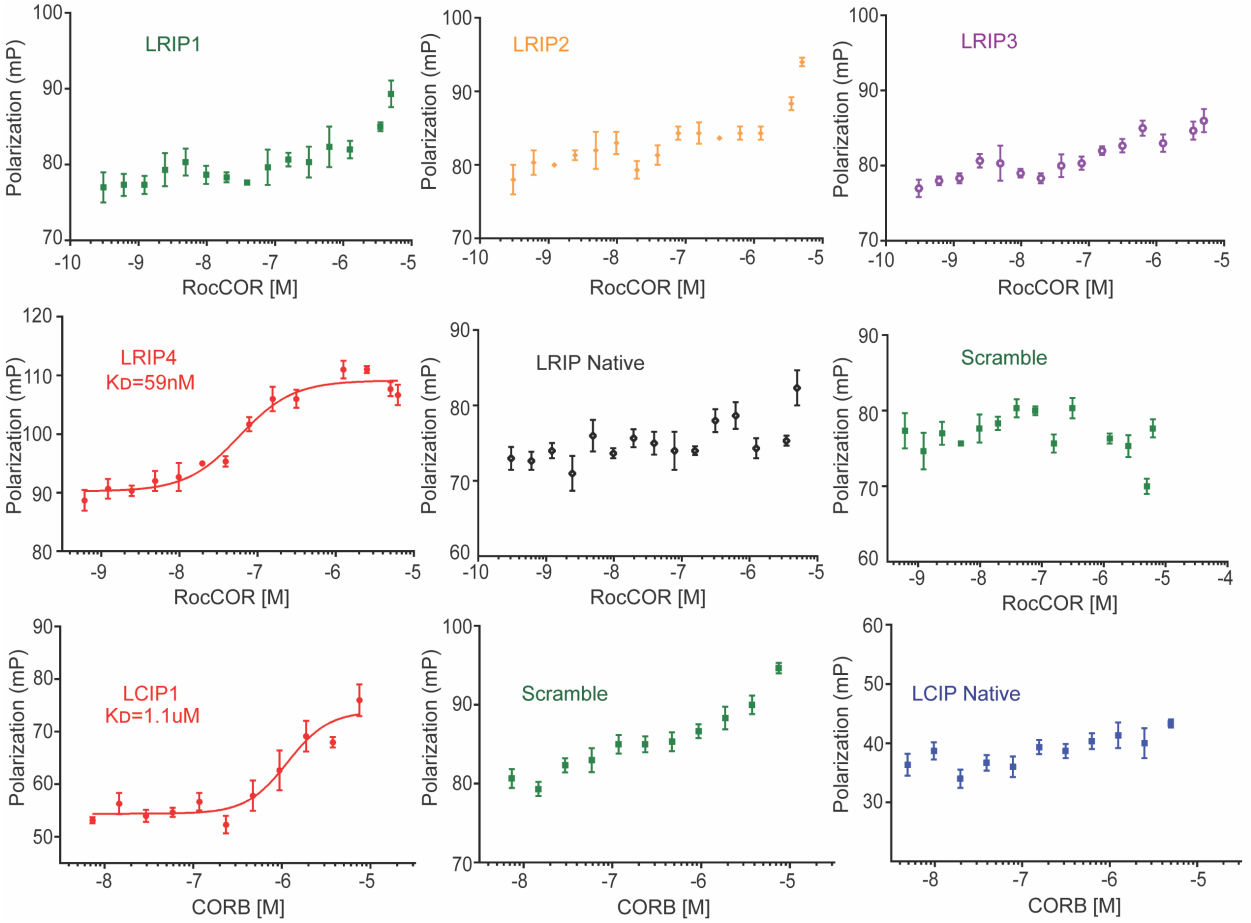


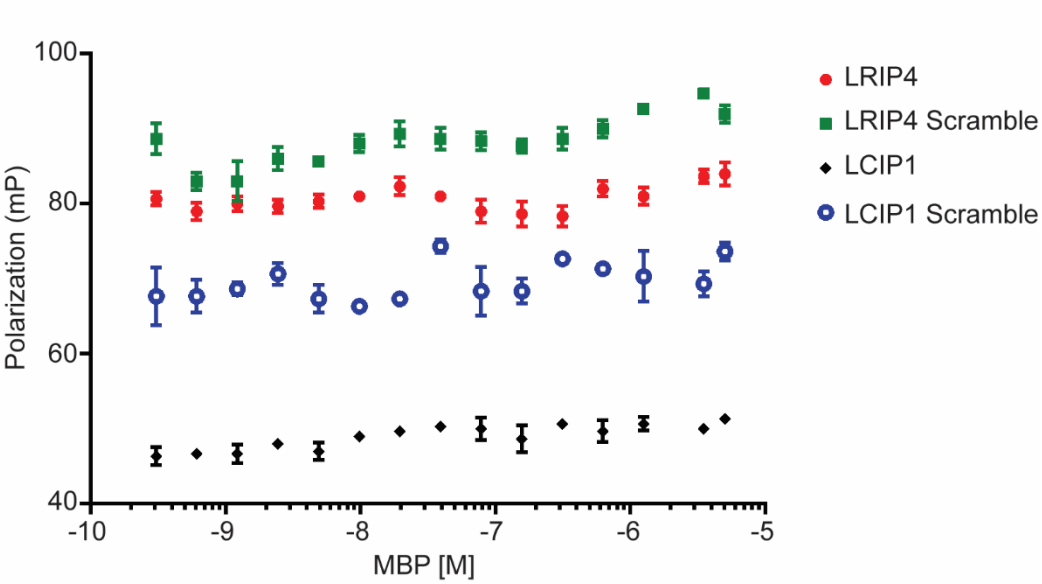
Figure S1. LCIP1 adopts a mixed secondary structure. Circular Dichroism was performed on FAM-labeled LCIP1. Minima were identified at 215 nm and 229 nm and maxima was identified at 204 nm, consistent with a mixed secondary structure.



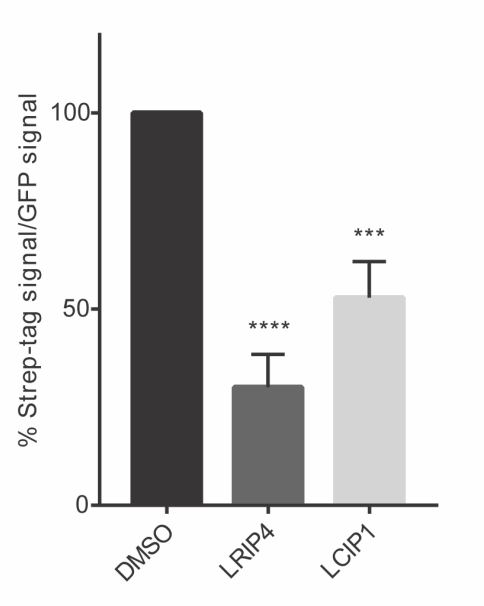
Supplementary Figure 2. LRIP4 and LCIP1 disrupt LRRK2 dimerization. HEK293 cells were co-transfected with GFP tagged LRRK2 and Strep-tagged LRRK2 before peptide treatment. Co-immunoprecipitation revealed that LRIP4 and LCIP1 had an inhibitory effect on LRRK2. Blot is representative of n=3.



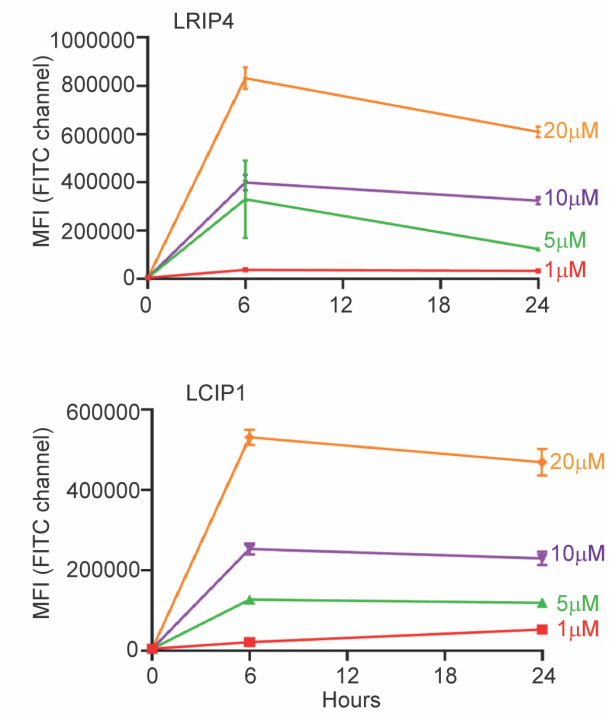
Supplementary Figure 3. LRIP4 and LCIP1 exhibit binding to LRRK2 Constructs. Fluorescence polarization assays indicated that while LRIP4 bound its RocCOR target with a K_D of approximately 60 nM, LCIP1 bound its CORB target with considerably less affinity with a K_D of 1 μM. Both scrambled peptide controls exhibited no binding (green). Additionally, the non-constrained native control peptides exhibited no binding as expected due to the anticipated loss of secondary structure in the absence of chemical constraints. Of the initial LRIP library, LRIP4 is the only peptide that exhibits binding. Each data point is representative of n =3.



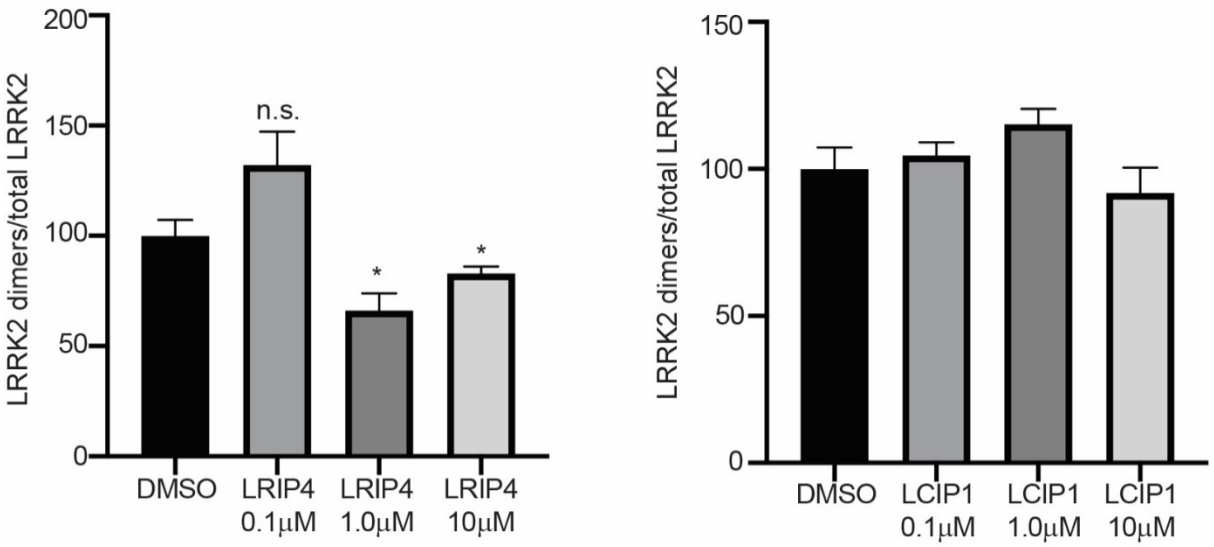
Supplementary Figure 4. Peptides exhibit no apparent binding to MBP alone. Fluorescence polarization assays indicated no detectable binding between MBP and LRIP4, LRIP4 scramble, LCIP1 or LCIP1 scramble, demonstrating that the peptides appear to bind their targeted LRRK2 domains.



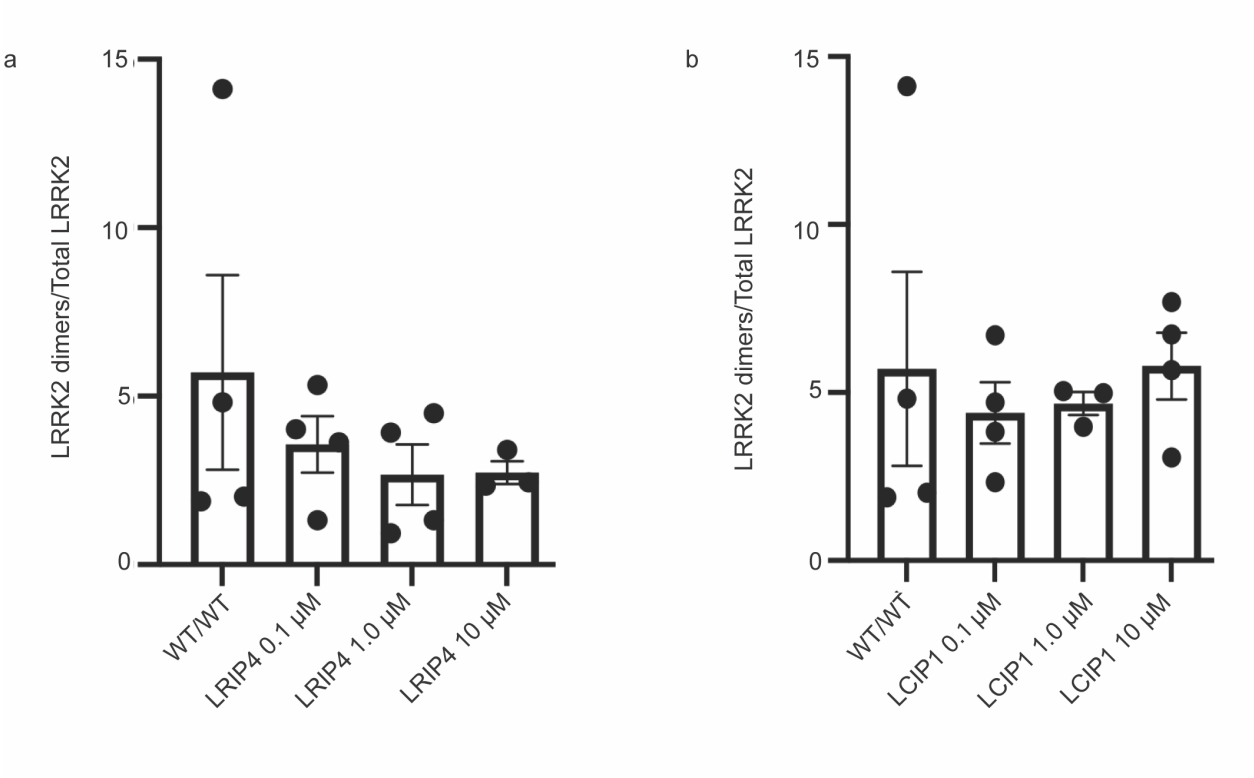
Supplementary Figure 5. Quantification of LRRK2 dimerization. Quantification of LRRK2 dimers in HEK293 cells that were transiently transfected with Strep-tagged LRRK2 and GFP or GFP-tagged LRRK2. Quantification was performed using one-way ANOVA with DMSO as a reference control from three independent experiments. *** $p<0.001$; **** $p<0.0001$.



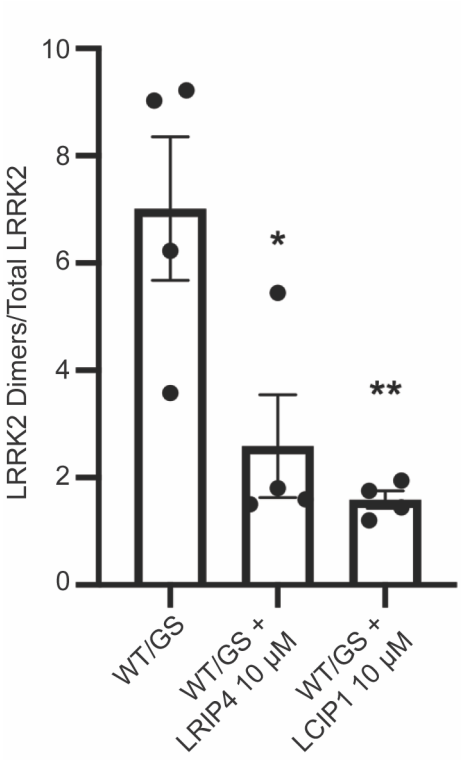
Supplementary Figure 6. Dose-dependent uptake of LCIP1 and LRIP4. HEK293 cells were treated with 1-20 μM FAM-labeled LRIP4 or LCIP1 over a 24-hour time course. Flow cytometry experiments demonstrate that both peptides yielded an increased shift in fluorescence maximally at 6 hours. Experiment was performed in triplicate.



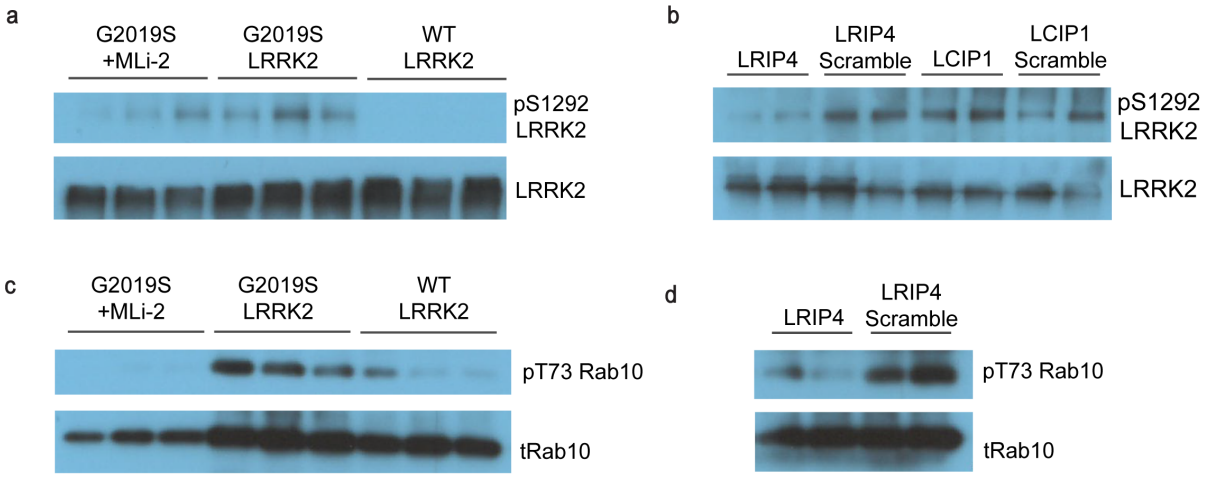
Supplementary Figure 7. LRIP4 effectively disrupts LRRK2 dimerization in cells. Using a LRRK2 proximity biotinylation assay, it was determined that LRRK2 could disrupt dimerization at concentrations ranging from 1 μM to 10 μM, whereas LCIP1 had no statistically significant effect on dimerization. *$p < 0.05$. n=3.



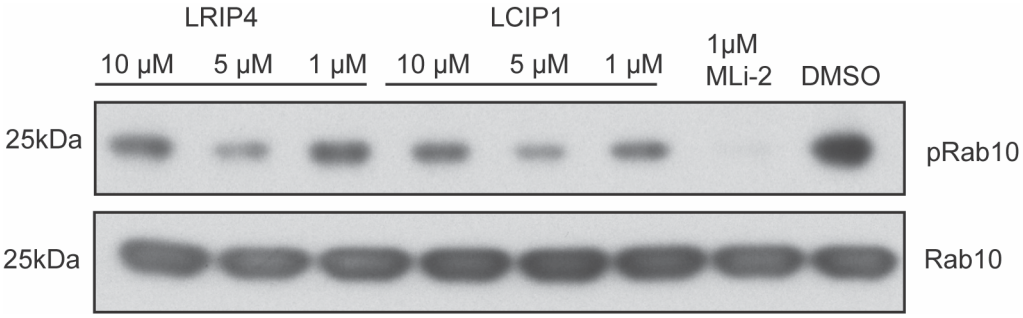
Supplementary Figure 8. LRRK2 dimerization was measured in cells using a proximity biotinylation ELISA-based assay. Dimeric LRRK2 was biotinylated *in situ* and purified on streptavidin-coated ELISA plates. At all concentrations, of LRIP4, WT/WT dimers were reduced compared to un-treated cells; however, the higher variability in the un-treated control group resulted in no statistical differences. In cells treated with increasing concentrations of LCIP1, as we have seen in other assays, the effects on LRRK2 are variable owing to the inconsistent uptake of these compounds. No statistically significant differences were found for dimer formation in this assay following treatment with LCIP1.



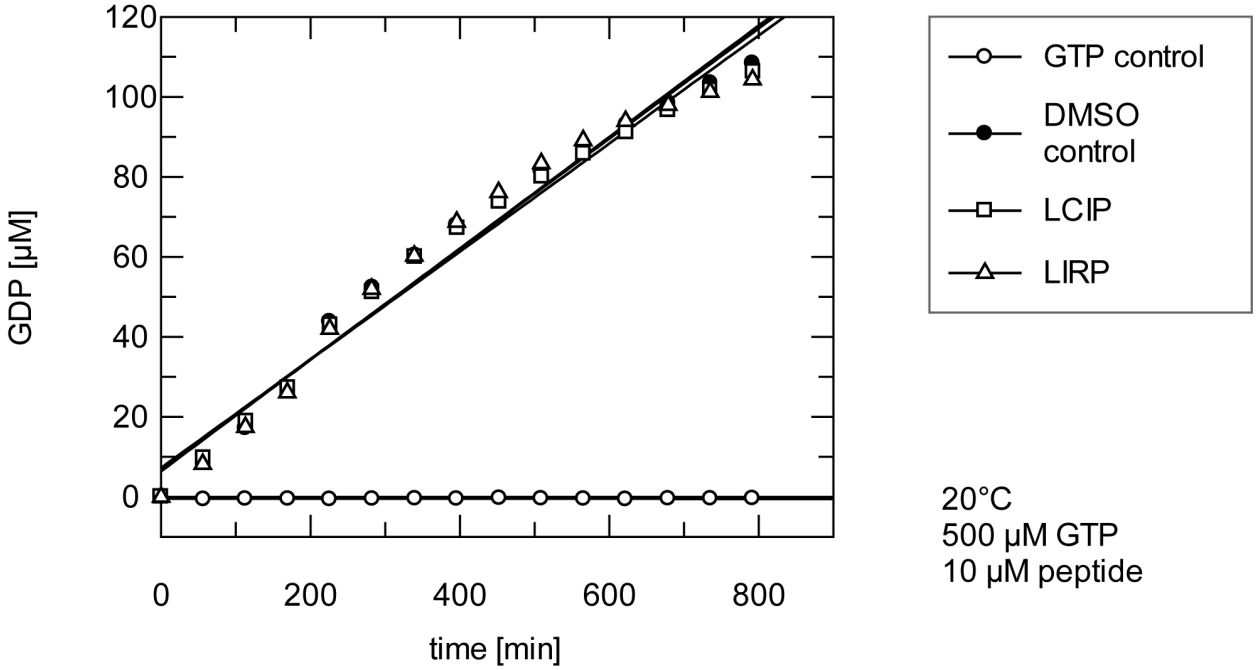
Supplementary Figure 9. LRIP4 and LCIP1 significantly decrease the formation of heterodimers. We assessed the effect of LRIP4 and LCIP1 to alter dimer formation of WT/G2019S heterodimers, as we show for WT/WT dimers. Dimeric LRRK2 was biotinylated *in situ* and purified on streptavidin-coated ELISA plates. In both cases, LRIP4 and LCIP1 at 10 μM, significantly reduced WT/G2019S dimers compared to in-treated cells. n=4 biological replicates; * $p < 0.05$, ** $p<0.01$.



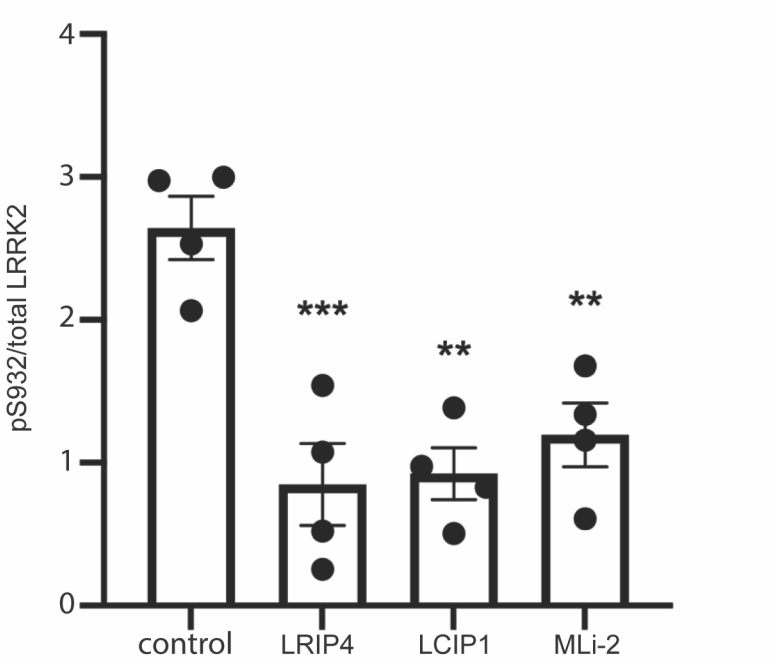
Supplementary Figure 10. LRIP4 decreases LRRK2 kinase activity in HEK293 cells expressing LRRK2 G2019S. a) Autophosphorylation of LRRK2 S1292 is enhanced in G2019S expressing HEK293 cells and can be inhibited with MLi-2. b) LRIP4 effectively downregulations S1292 phosphorylation, however LCIP1 does not appear to have any effect. c) Substrate phosphorylation of Rab 10 is enhanced in LRRK2 G2019S expressing HEK293 cells. d) LRIP4 decreased substrate phosphorylation as compared to its scrambled control.



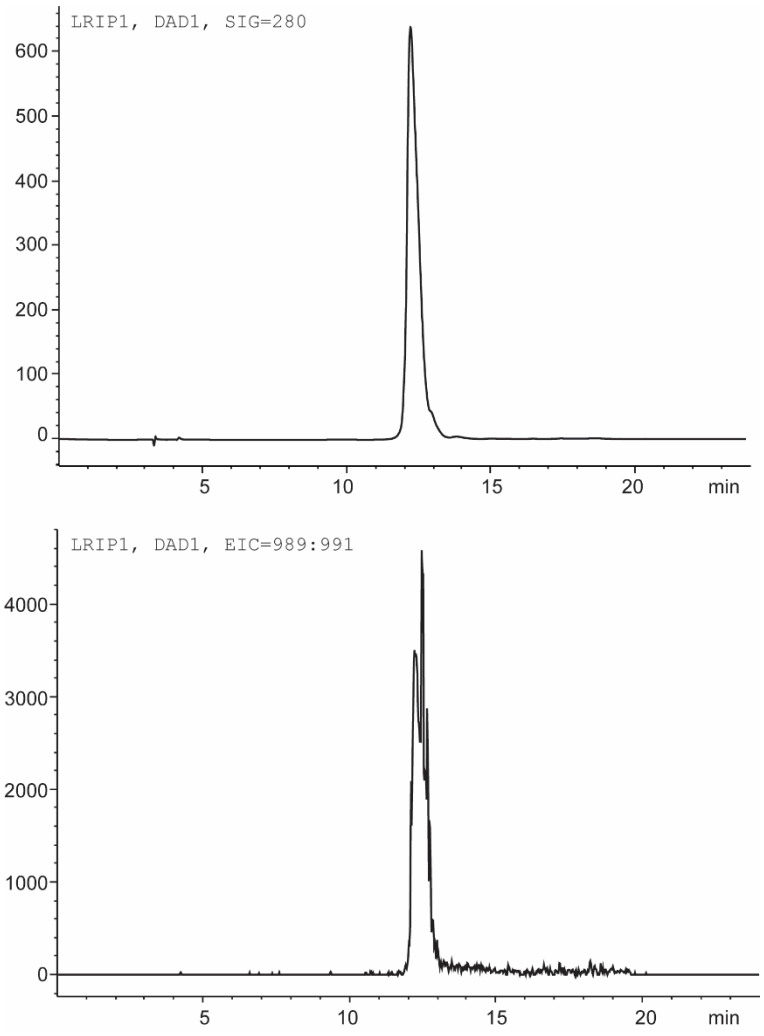
Supplementary Figure 11. Rab10 phosphorylation with different concentrations of peptide treatments. A549 cells with native expression of LRRK2 and Rab10 were used to investigate the inhibitory effect of LRIP4 and LCIP1 (1, 5, and 10 μM) on endogenous LRRK2 kinase activity as measured by Rab10 phosphorylation. LRIP4 demonstrated inhibition of Rab10 phosphorylation with variability in its inhibitory effects between 5-10 μM (n = 3).



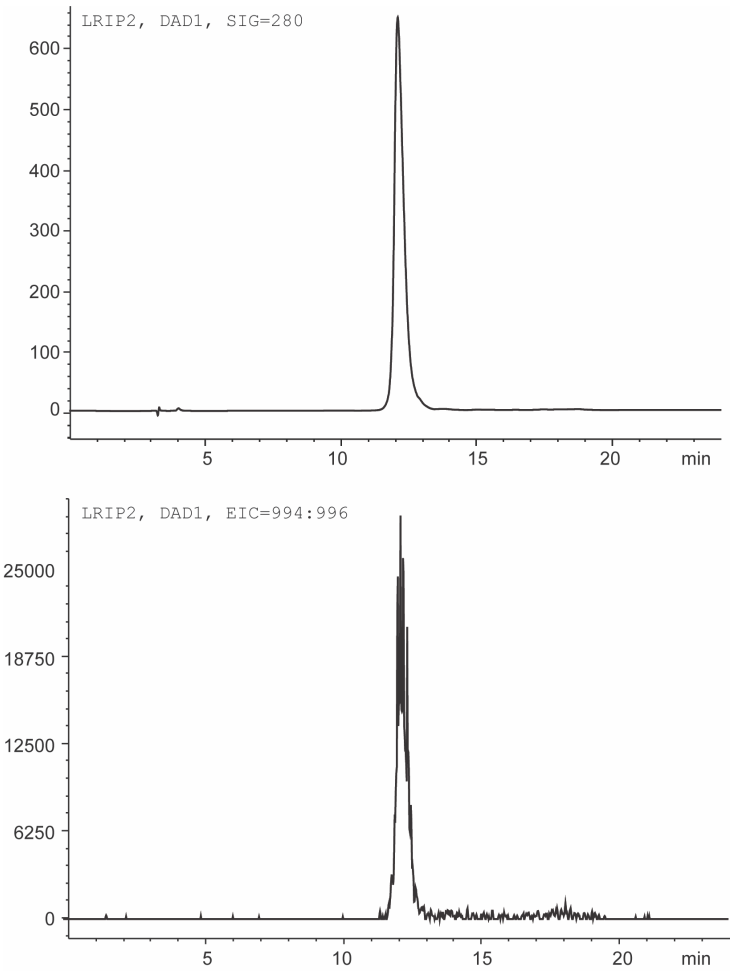
Supplemental Figure 12. GTPase activity is not affected by peptides. Full length WT LRRK2 was incubated with 10μM of peptide for 30 minutes at 20°C. 500uM GTP was added, and the production of GDP was monitored at 20°C. The amount of GDP and GTP was determined via RP-HPLC. As expected, the intrinsic hydrolysis of the protein was not affected by the peptides.



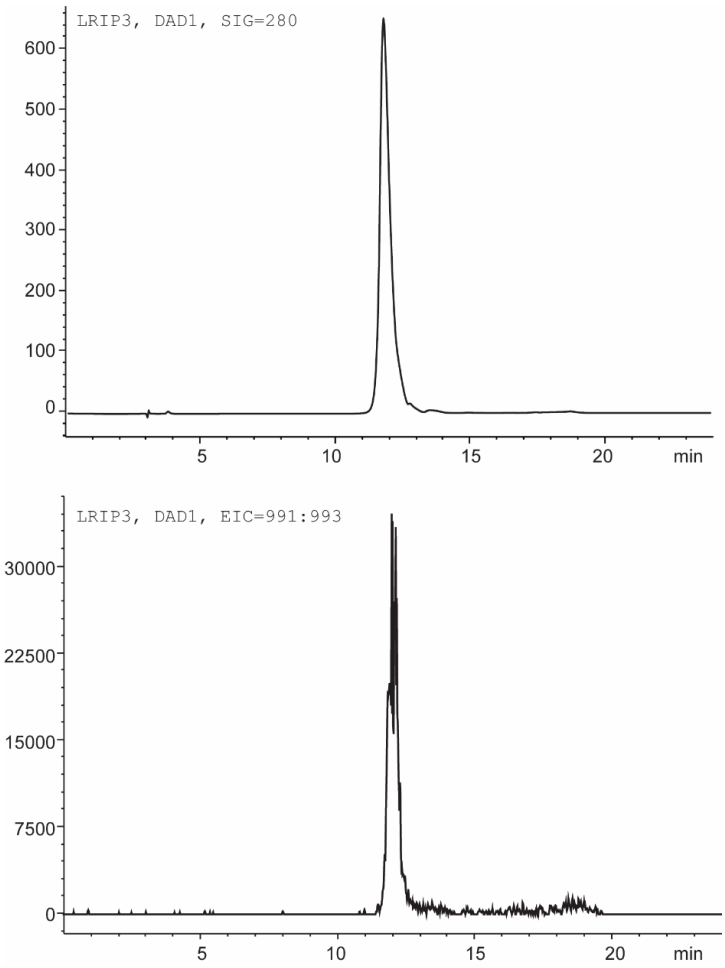
Supplemental Figure 13. pS935-LRRK2 levels are reduced in cortical neurons treated with peptides. As a pharmacodynamics readout of LRRK2 kinase inhibition, endogenous levels of pS935-LRRK2 in cortical neurons by ELISA. Neurons were treated with 10 μM of each peptide, or 200nM MLi-2, and lysates were subjected to parallel ELISA's for total and pS935-LRRK2. The chemiluminescence obtained for pS935-LRRK2 was normalized to total LRRK2. ** $p<0.01$; *** $p<0.001$.



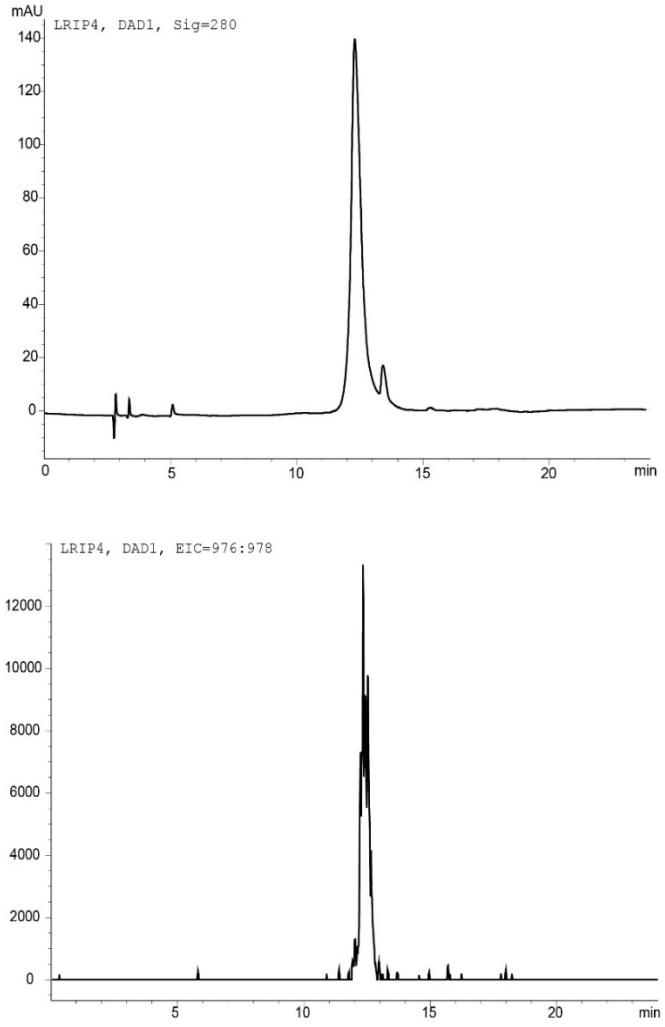
Supplemental Figure 14. ESI-Mass Spectrometry Analysis of LRIP1. Molecular Weight of the purified product of LRIP1 5(6)-FAM- PEG_3-DEK*RKA*(Nle)SKITKELLNKR, actual mass 2967.0 (expected mass: 2967.5). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



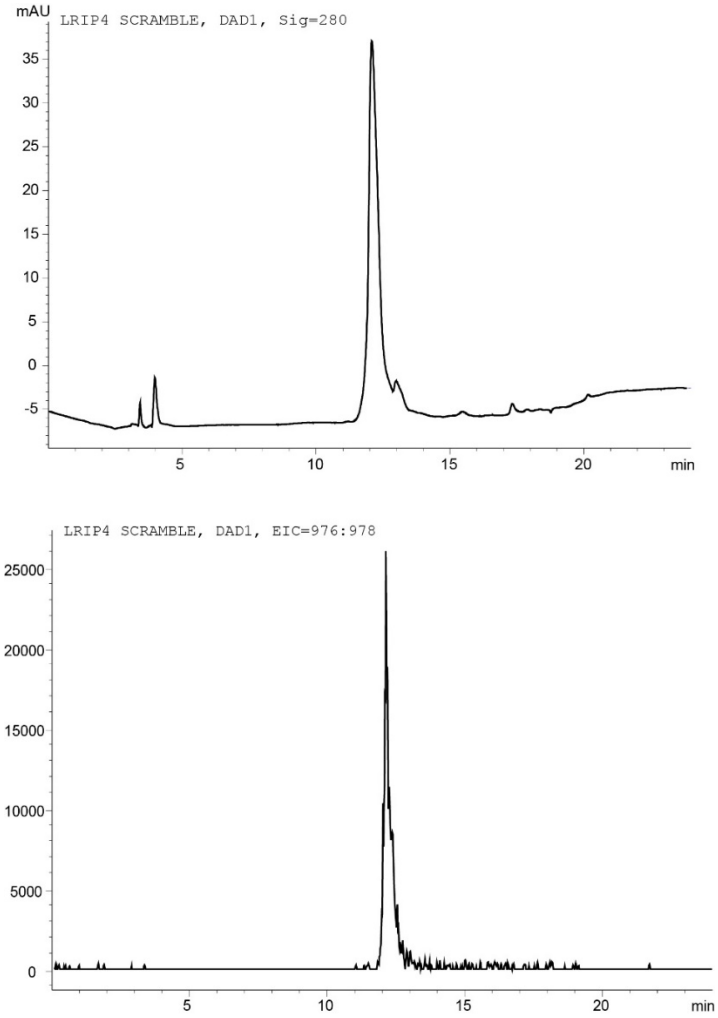
Supplementary Figure 15. ESI-Mass Spectrometry of LRIP2. Molecular Weight of the purified product of LRIP2 5(6)FAM- PEG_3-DEKQRKA*(Nle)SK*TKELLNKR actual mass 2981.4 (expected mass: 2982.4). * represents S)-N-Fmoc-2-(4-pentenyl) alanine. * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



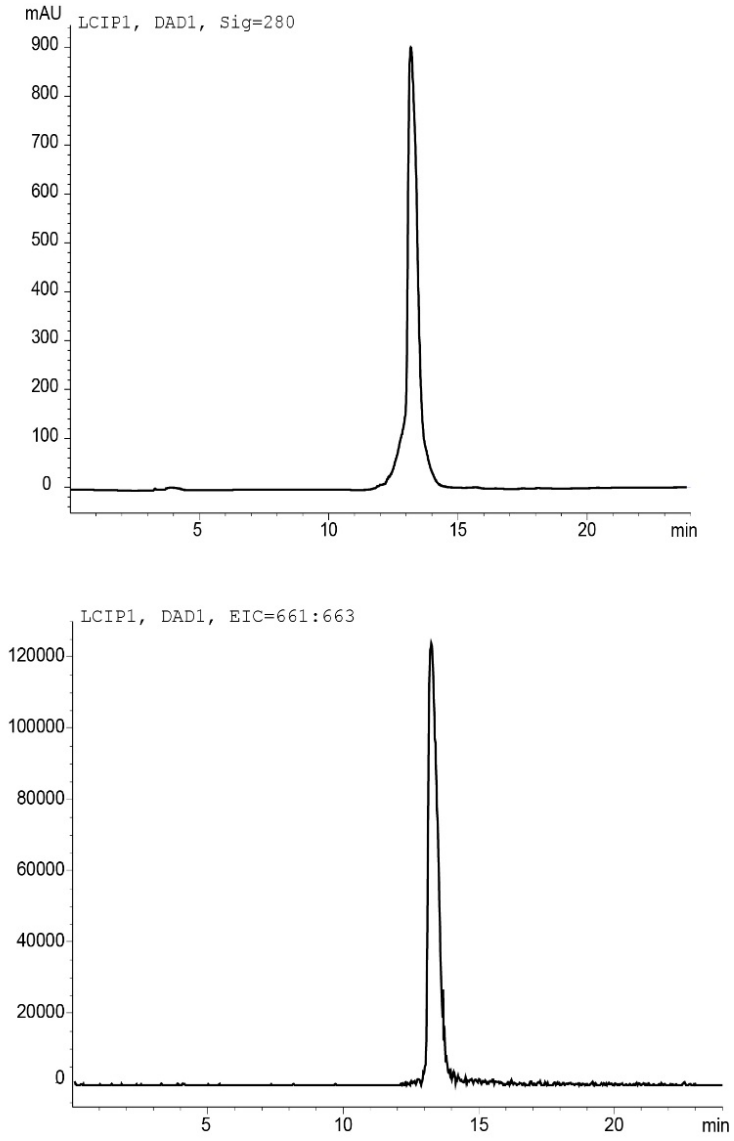
Supplementary Figure 16. ESI-Mass Spectrometry of LRIP3. Molecular Weight of the purified product of LRIP3 5(6)-FAM-PEG_3-DEKQRKAC(Nle)SK*TKE*LNKR actual mass 2972.0 (expected mass: 2972.4). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



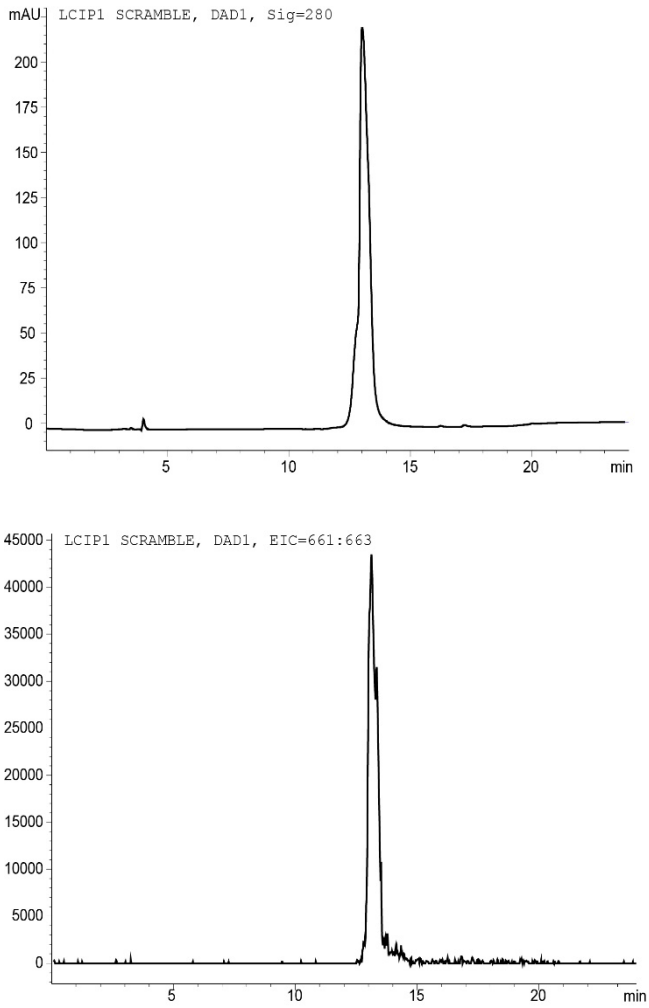
Supplementary Figure 17. ESI-Mass Spectrometry of LRIP4. Molecular weight of the purified product of LRIP4: 5(6)FAM-PEG_3-DEKQRKAC(Nle)SKITE*LNK*, actual mass 2928.0 (expected mass: 2929.4). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



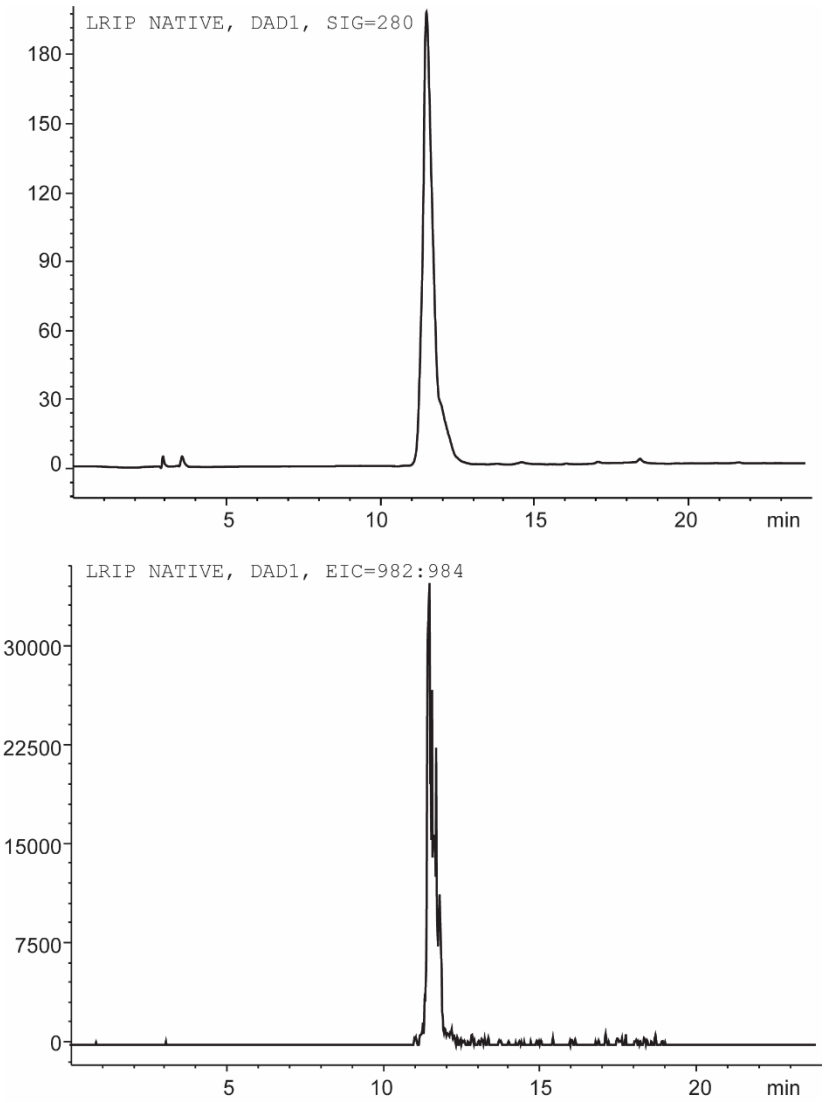
Supplementary Figure 18. ESI-Mass Spectrometry of LRIP4 scramble. Molecular weight of the purified product of LRIP4 Scramble: 5(6)FAM-PEG_3-Q(Nle)DKAESKNKERKLC(S5)TIK(S5), actual mass 2928.9 (expected: 2929.4). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



Supplementary Figure 19. ESI- Mass Spectrometry of LCIP1. Molecular weight of the purified product of LCIP1: 5(6)FAM-PEG_3-KGEGE*LLK*WK, actual mass 1983.6 (expected mass: 1984.3). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



Supplementary Figure 20. ESI-Mass Spectrometry of LCIP1 scramble. Molecular weight of the purified product of LCIP1 Scramble: 5(6)FAM-PEG_3- GKWEK*GEL*KL , actual mass 1983.6 (expected mass: 1984.3). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.



Supplementary Figure 21. ESI-Mass Spectrometry of LRIP Native. Molecular Weight of the purified product of LRIP Native: 5(6)-FAM-DEKQRKAC(Nle)SKITKELLNKR actual mass 2946.9 (expected mass: 2948.4). * represents (S)-N-Fmoc-2-(4-pentenyl) alanine.