Supplementary Material

Allosteric modulation of the GTPase activity of a bacterial LRRK2 homologue by conformation-specific Nanobodies

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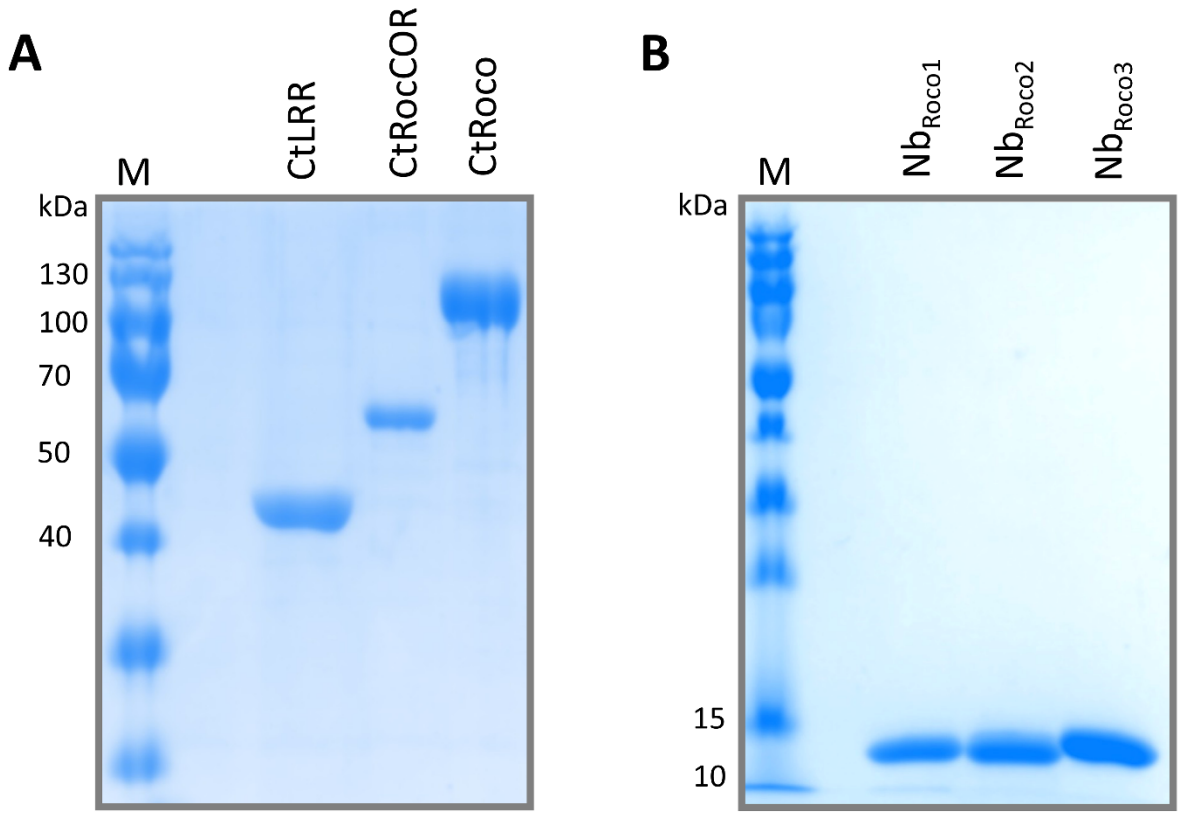
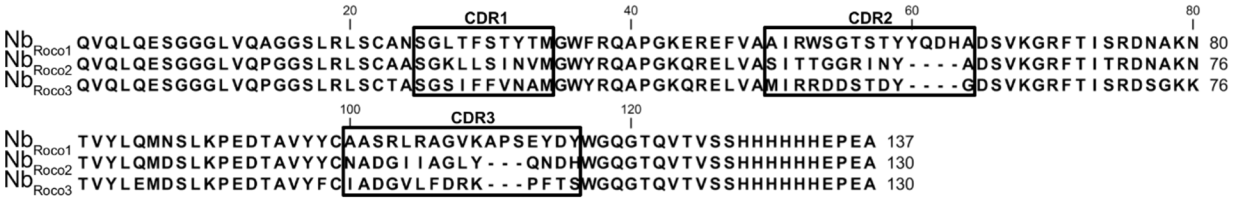
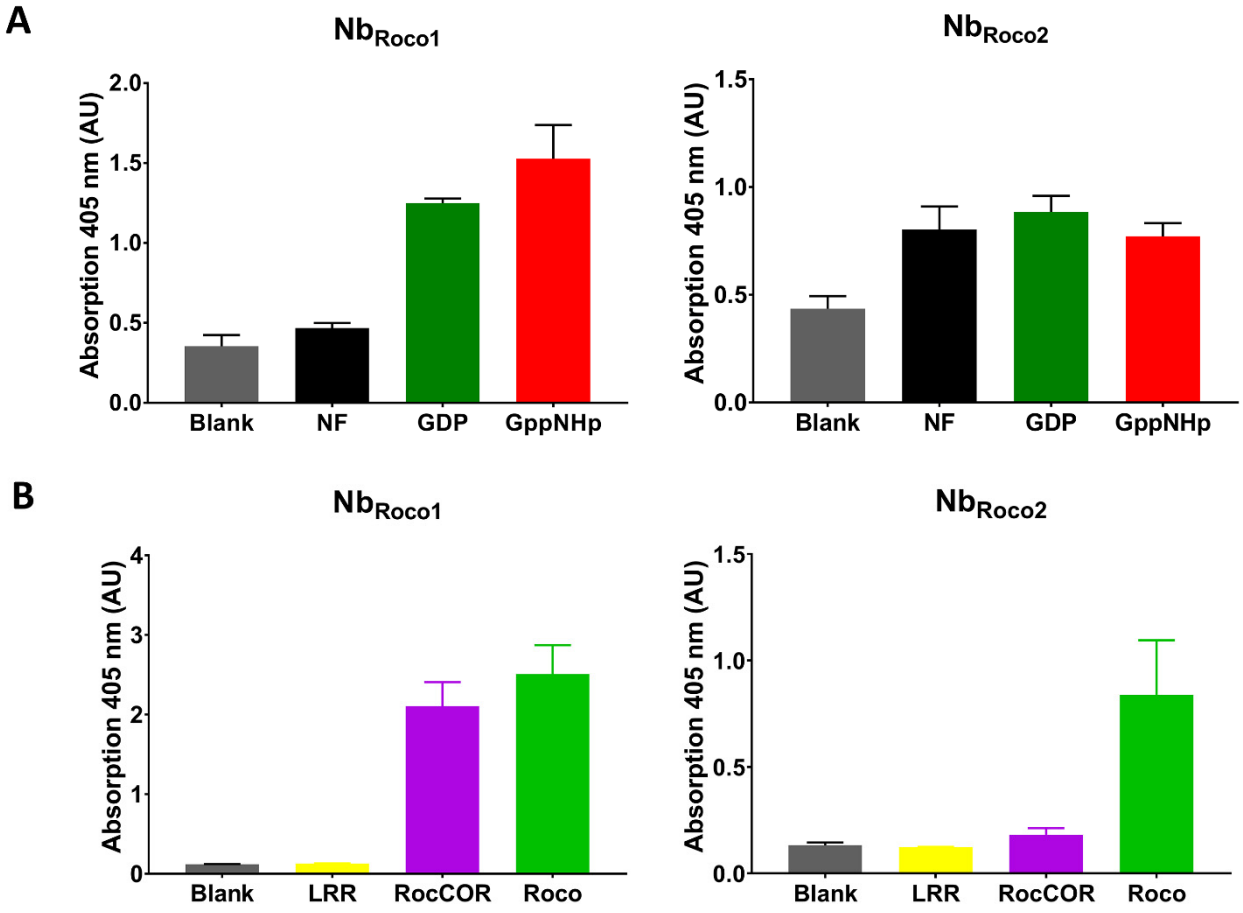


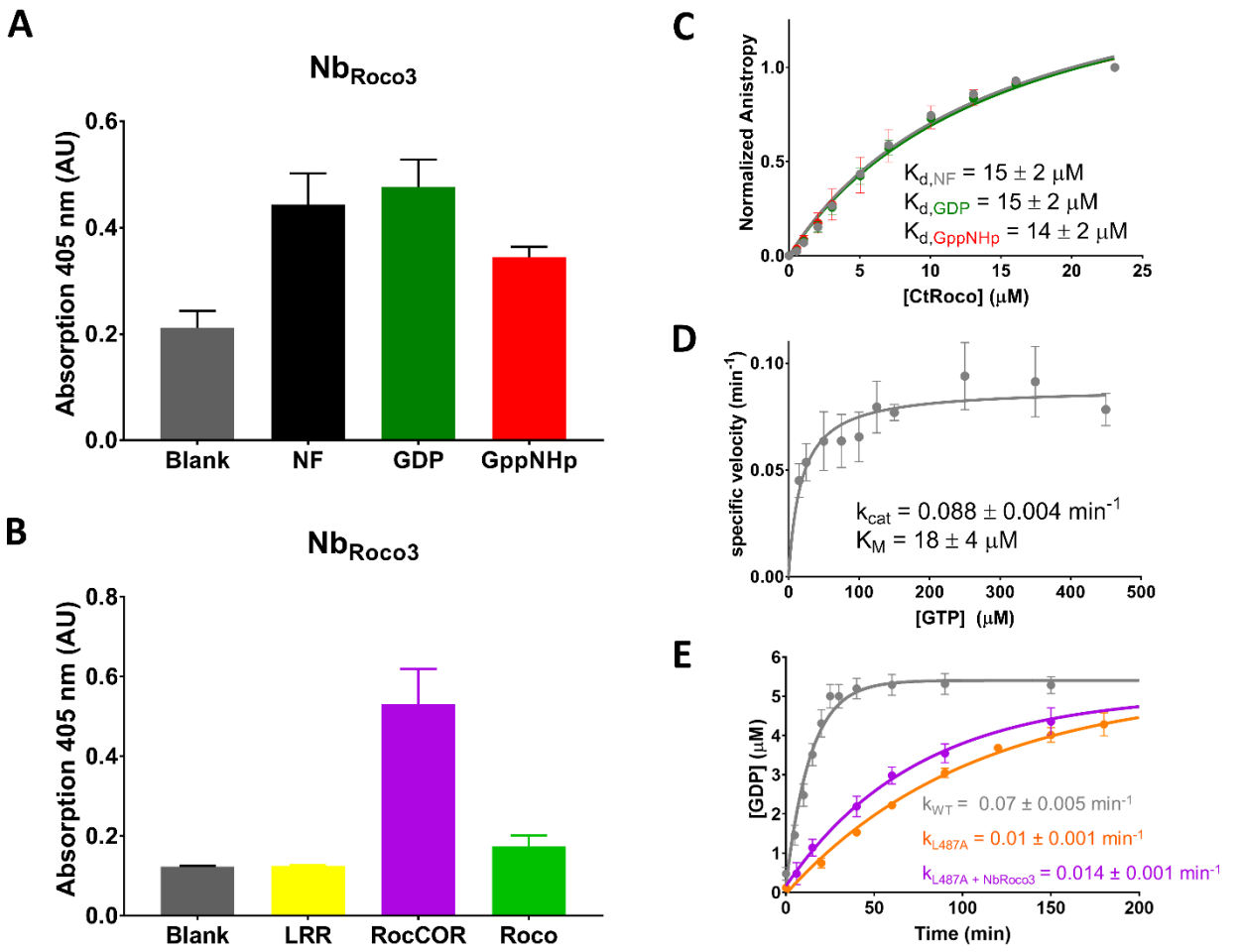
Supplementary Figure S1. Purity of proteins used in this study. (A) SDS-PAGE after purification of CtRoco and its RocCOR (CtRocCOR) and LRR (CtLRR) domain constructs. 15 µg of each protein has been loaded on gel. **(B)** SDS-PAGE after purification of Nb_{Roco1} (Nb6946), Nb_{Roco2} (Nb8175) and Nb_{Roco3} (Nb9221). 15 µg of each protein has been loaded on gel. M: PageRuler Prestained Protein Ladder.



Supplementary Figure S2. Sequence alignment of Nb_{Roco1} (Nb6946), Nb_{Roco2} (Nb8175) and Nb_{Roco3} (Nb9221). The CDR regions (CDR1-3) are indicated.

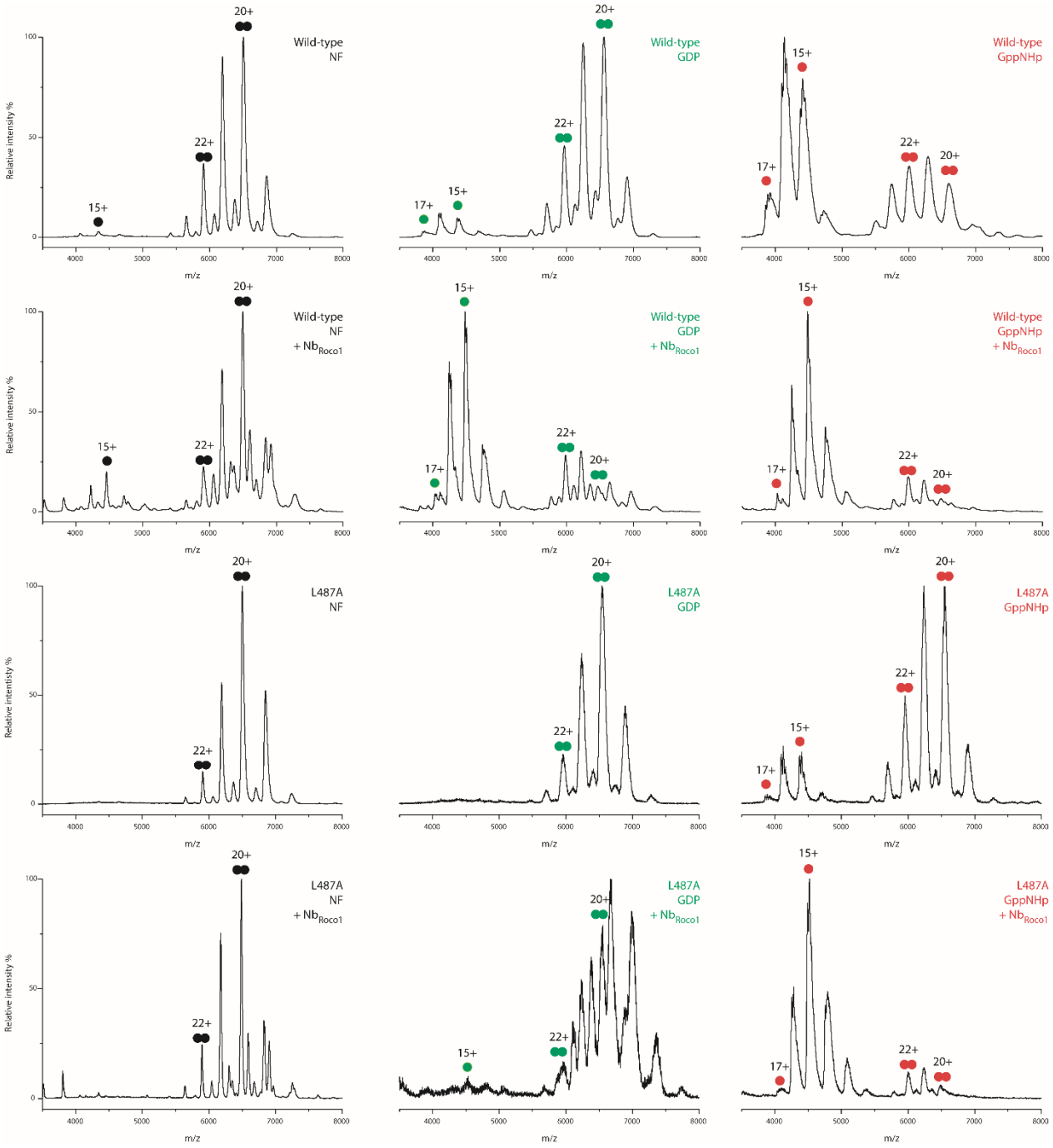


Supplementary Figure S3. ELISAs to determine the conformational and domain specificity of Nb_{Roco1} and Nb_{Roco2}. (A) ELISAs to determine the conformational specificity of Nb_{Roco1} and Nb_{Roco2} toward CtRoco. The following conditions were used to coat the ELISA plates: Blank: no protein, NF: nucleotide free CtRoco, GDP: GDP-loaded CtRoco, GppNHp: GppNHp-loaded CtRoco. **(B)** ELISAs to determine the domain specificity of Nb_{Roco1} and Nb_{Roco2}. The ELISA plates were coated with either CtLRR, CtRocCOR or CtRoco proteins, or no protein as blank. All measurements were done in quintuplicate of which the mean and standard deviations are depicted.



Supplementary Figure S4: Characterization of Nb$_{Roco3}$. (A) ELISAs to determine the conformational specificity of Nb$_{Roco3}$ toward CtRoco. The following conditions were used to coat the ELISA plates: Blank: no protein, NF: nucleotide free CtRoco, GDP: GDP-loaded CtRoco, GppNHp: GppNHp-loaded CtRoco. All measurements were done in quintuplicate of which the mean and standard deviations are depicted. **(B)** ELISAs to determine the domain specificity of Nb$_{Roco3}$. The ELISA plates were coated with either CtLRR, CtRocCOR or CtRoco proteins, or no protein as blank. All measurements were done in quintuplicate of which the mean and standard deviations are depicted. **(C)** Influence of the nucleotide bound state of CtRoco on the affinity for Nb$_{Roco3}$ assessed by fluorescence anisotropy titrations. The fluorescence anisotropy signal of the FITC-labelled Nb is monitored upon titration with increasing concentrations of CtRoco in the nucleotide-free state (grey), or bound to GDP (green) or GppNHp (red). The corresponding equilibrium dissociation constants (K_d ± standard error) obtained by fitting on a quadratic binding equation are given (each data point is the average of three independent measurements with the error bars representing the standard deviation). **(D)** Steady state Michaelis-Menten kinetics of CtRoco in the presence of an excess of Nb$_{Roco3}$. The rate of GDP production in function of time was analyzed via reversed-phase HPLC, and specific velocities (initial rate divided by enzyme concentration) were plotted against the GTP

concentration. k_{cat} and K_M values (± standard error) resulting from fitting with the Michaelis-Menten equation are shown (each data point is the average of three independent measurements with the error bars representing the standard deviation). **(E)** Effect of Nb_{Roco3} on the single turnover GTP hydrolysis kinetics of the CtRoco L487A mutant. 5 µM of CtRoco (grey) or the CtRoco L487A mutant either in absence (orange) or presence (purple) of an excess of Nb_{Roco3} was mixed with 5 µM of GTP, and production of GDP was followed in time. The observed rate constants (k ± standard error) obtained by fitting on a single exponential equation are shown. Each data point is the average of three independent measurements with the error bars representing the standard deviation.



Supplementary Figure S5: Native mass spectra of CtRocCOR and the CtRocCOR L487A mutant either in the absence or in the presence of a molar excess of Nb_{Roco1} as indicated in each mass spectrum, and for three different nucleotide states: nucleotide free (NF, black), GDP-bound (green) and GppNHp-bound (red). Peaks corresponding to dimeric and monomeric species are labeled with two circles and one circle, respectively.