

Supplement to "Isolation and Characterization of Mutant *Sinorhizobium meliloti* NodD1

Proteins With Altered Responses to Luteolin" by Melicent C. Peck^a, Robert F. Fisher^{b,#},

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Sm NodD1	M - R F R G L D L N L L V A L D A L M T E R - K L T A A A R R I N I L S Q P A M S A A I A R L R T Y F G D E L F S M Q G R E L I P T	63
Rlv NodD	M - R F K G L D L N L L V A L D A L M T E R - K L T A A A R S I N L S Q P A M S A I S R L R D Y F R D D L F I M Q Q R R L V P T	63
Rlt NodD	M - R F K G L D L N L L V A L D A L M T E R - K L T A A A R S I N L S Q P A M S A I S R L R D Y F R D D L F I M Q Q R R L V P T	63
Bsp DntR	M - D L R D I D L N L L V V F N Q L L L D R - S V S T A G E K L G L T Q P A V S N S L K R L R T A L N D D L F L R T S K G M E P T	63
Pp NahR	M - E L R D D L N L L V V F N Q L L L D R - R V S I T A E N L G L T Q P A V S N A L K H L R T S L O D P L F V R T H Q G M E P T	63
Ec XopR	M E R V V Y R T D L K L L R Y F L A V A E E L - H F G R A A A R L N M S Q P F L S I H I K E L L E N Q L G T Q L F I R H S R S V V L T	64
Ct Tsrf	M - - - - - K L Q T L Q A L I C I E E V G - S L R A A A A Q L L H L S Q P A M S A A I Q Q L E D E L K A P L L V T K R G V S L T	58
At OccR	M - - - - - N L R Q V E A F R A V M L T G - Q M T A A A A Q L M L V T Q P A I S R L I K D F E Q A T K L Q L F E E R G N H I I P T	58
Cf AmpR	M T R S Y - I P L N S E R A F E A A A R H - L S F T R A A I E L N V T H S A I S Q H V K S L E Q Q L N C Q L E V R G S R G L M L T	63
Ec OxyR	M - - - - - N I N D E L E Y L V A L A E H R - H F R R A A D S C H V S Q P F M I S G Q I R K L E D E L G V M M L L E T S R K V L F T	58
Rs CbbR	M V R L D A I T L Q L R A L V A V A G S A - S L T G G A T R L G L T P P A I H N L E E A F G V P L L H R P P E T G S F T	64
Ab BenM	M E - - - - - L R H R L R Y F V A V V E E Q - S F T K A A D K L C I A Q P P L S R Q I Q N L E E E L G I Q Q L L E R G S R P V K T T	58
Ec Cbl	M - - - - - N F Q Q L K I I R E A A R Q D Y N L T E V A N M L F T S Q S G V S R H I R E L E D E L G I E I F V R R G K R L L G M	59
St CysB	M - - - - - K L Q Q L R Y I V E V V N H N L N V S S T A E G L Y T S Q P G I S K Q V R M L E D E L G I Q F A R S G K H L T Q V	59
Sm NodD1	P [I] - - A E A L A P A R D A L L H I Q L S V I A W D P L N P A Q S D R R F R I [L] S D F M L V F F A R I V E R V A R E A P G V	126
Rlv NodD	P R - - A E A L A P A V R E A L L H I Q L S V I A W D P L N P A E S D R R F R I [L] S D F M L V F F D K I I L R L A R E A P G V	126
Rlt NodD	P R - - A E A L A P A V R E A L L H I Q L S V I A W D P L N P A E S D R R F R I [L] S D F M L V F F D K I I L R L A R E A P G V	126
Bsp DntR	P Y - - A L H L A E P V I Y A L N T I Q T A L T T R D S F D P F A S T R T M A I D O G I E Y P M P R L M D V L A H Q A P N [C]	126
Pp NahR	P Y - - A A H L A E P V T S A M H A L R N A L Q H H E S F D P L T S E R T T R L A M T D I G E Y P M P R L M D V L A H Q A P N [C]	126
Ec XopR	H A - - G K I L M E E S P R L L V N V A N N V L A R I E Q I G R G E A G R - J E G V V V G T A M N G R M R P V M R R F L R E N P N V	126
Ct Tsrf	S F - - G Q A F M K H A R L I T V E S R A Q A E E Q I G Q R R W E G H - J T F A A S P A I A A A L P I A L A S F A R E F P D V	128
At OccR	Q E - - A K T L W K E V D R A F V Q L N H I G N L A A D I G R Q A A G T - L R I A A M P A L A N G L L P R F L A Q F I R D R P N [D]	128
Cf AmpR	T E - - G E S L L P V L N D S V L N D S F D R M A G M L D R F A - T K Q T K L - K I G V V G I F A I G C L P F L L S D F K R S Y P H I	123
Ec OxyR	Q A - - G M L L V D Q A R T V L R E V K Y V K E M A S S Q Q E T M S G P - L H I Q L P W P H I H M L H Q T F P K	120
Rs CbbR	P T L A G I A V L E E A A Q R I E V I L S Q C S Y Q V M A V S E G R A Q Q - V T L G V V S T - G R Y F A P R L V K M L S L A C P E I	127
Ab BenM	P E - - G H F F Y Q Y A K I L L S N V D Q M Y S M T K R I A S V E K T - - I R I G F V G S L S D F G L P R I H Y R Q A H P N L	119
Ec Cbl	T E - P G K A L L V I A E R I L N E A S N V R R L A D L F T N D T S G V - L T I A T T H T D A Y E L P E V I K A F R E L F P E V	122
St CysB	T P - A G Q E I I R I A K E V L S K V D A I K S V A G E H T W P D K G S - L Y I A T H H A R Y A L P G V I K G F I E R Y P R Y	122
Sm NodD1	S F E L L P L D D D P - H E L L R R G D V D F L I - - F P D V F M S S A H P K A K L F D E A L V C V G C P T N K K L L G N I S F E	188
Rlv NodD	S F E L L P L D D D P - E E L L R G D V D F L I - - L P D L F M S G A H P K A R L F E E R I L V C V G C P T N E Q L Q Q G Q L S L E	188
Rlt NodD	S F E L L H V N D D P - D E R L R S G B L D F L I - - L P D Q F Q M S A T H P S A K L F E D K L V C V G C P S N Q Q L R G K L S L K	188
Bsp DntR	Q I S T L R P N A G N L K E D M E S G A V D O L A G L L P E - - L T Q T G F F Q R E R F R Y V C M F R K D H P S A K S P M S L	189
Pp NahR	Q I S T V A D D S S M S L M Q A L Q N G T V D L A V G L P D N - - L Q T G F F Q R R L L Q N H Y V L C R K D H P V T R E P L T L E	189
Ec XopR	D V L F R E K M P A M Q M A L L E R E L D A G I W R M A T E P - P T G F T S R L H E S A F L V A M P P E E H H L S S F S T - - V	188
Ct Tsrf	T V N V R D G H Y P M P A V S P Q L R D G T L D F A L T A A H K H D I D T D L E A Q P L V W S D V Y V G Q R H P M A N - - - - A T	181
At OccR	Q V S L M G L P S M V M E A V A S G R A D I G Y A D Q P Q E R Q G F Q L I E T R S L - - P A V V A P M G H R L A G L D R - - V	188
Cf AmpR	D L H I - S T H N N R V D P A E G L D Y T I R Y G G A H W D T D A Q Y L C S A L - - - - - - - - M S P L C S P T L	173
Ec OxyR	E Q M Y V H E A Q T H Q L L A Q L D S G K L D C V I L L - V K E - S E A F I E V P L F D E P M L L A I Y E D H P W A N - - R E C V	181
Rs CbbR	R I A I R V G N R E Q L I D D I L A R H M V D L A V M G H P P R Q P E - - V A S V A L G P H P H G I V A P P D H P L A G L A E - - V	188
Ab BenM	R I E K Y V M G K R A Q T E A L K E G R I D A G F G R I K N S D - P A I K R T L - L K N E R L M V A V A H S H P L N Q M K D K G Y	182
Ec Cbl	R E L I O G T P Q E A T A L L Q N G E A D I G T A S E R L S N D P Q - L V A F W P E R W H S S L V P H D H P L T Q I S P - - L	184
St CysB	S L H M H D G S P T Q I A E A V S K G N A D F A I A E A L H L Y D D - L V M L P C Y H W R N S I V V T P D H P L A A T S S - - V	184
Sm NodD1	T Y M S K I G H V A A Q - F G R E M K P S V E Q W - - - L L E H G F N R R I E L V V P G F T L - I P R L L S G T W R I A T L P	246
Rlv NodD	Q Y M S M G H V A A K - F G R G K P S V E Q W - - - L L M Q H G L K R R I E L V V P G F N L - I P P L L S G T W R I A T L P	246
Rlt NodD	R F M S M G H V A A M - F G R T L K P S I E Q W - - - L L E H G F K R R V E I V V P G F N S - I P M L L Q G T H R I A T L P	246
Bsp DntR	Q F T E L E H V G V V - A L N T G Q G I E V D G - - - L L E H G F K R R V E I V V P G F N S - I P M L L Q G T H R I A T L P	246
Pp NahR	R F C S Y G H V R V I - A A Q I G H G E V D T - - - Y M T R V G I R R D I R L E V V H F A A - V G H I L Q R U D D L A H V P	246
Ec XopR	P L E A L D R E - - - Y V T M P D V Y T D W D F L Q R V C Q V Q G F S P V V I R E V N E P Q T V L - A M V S M G I G I T L I A	248
Ct Tsrf	R L A E L Q E C R W A F S A P I R G P G A T I R N A F A R Y - - - G L P E P M L L A I Y E D H P W A N - - R E C V	242
At OccR	T P Q D L A G E R I I K - - - Q E T G T L F A M K R E V E V A I - - - G G I Q R R P S I E V S L S H T A L S - - L V R E G A G I A I I	238
Cf AmpR	A S Q I Q T P A D I L K F P P L L R S Y R D E W A L W M Q A G A E A P P S P T H N V M V F D S S V T V M L E A A Q G G M G V A I A P	238
Ec OxyR	P M A D L A G E K L L M - - - L E D G H C R Q D A Q M G Q P G A E G A D E T H E R A T S C T L R - N M V A A G S G I T L I W	241
Rs CbbR	P V P D L L S Q T F L - - - A R A R G S G T R V L M S R Y L D R L G E Q Q V V V D L I E M S N E T I Q K S V I A G L G A F L S	249
Ab BenM	H K N D I D E K I L L Y P S S P K H N F S I - - - H V M N H I F S D H G M E P T K I N E V K H D O A L - - G I V A A G E G I S L V P	244
Ec Cbl	T L E S I A K W P L I T Y - - - R Q I G R S R I D D A F A R K G L L A D I V L S A Q S D S W - - I K I Y V A L G G I G I G L V A	244
St CysB	T I E A L A Q Y P L V T Y - - - T F G F T G R S E L D T A F N R A G L T P R I V F T A D A V Y - - I K T Y V R L G L G V G V I A	244
Sm NodD1	L R L V K Y F E Q T I P L R I V T - - S P L P P L F F T E A I Q W P A D H N T D P G N I - - W L R E I L L Q E A S R - - I D P Q	304
Rlv NodD	L L V K Y H Y E R I I P L R I I E - - H P L P L V S I F L A V Q W P A N H N I D P G N I - - W M R E I M Q I Q I D L K - - M E S E	304
Rlt NodD	L L V R H F E P T I P L Q I V D - - - H P L P L S F T E A L Q W P L Q H N S P G N I - - W M R N I I L E E A S R - - I E T S	304
Bsp DntR	Q I F A V R C E V P F G L T T S P - - H P A K L P D I A I N L F W H A K Y H N R D P G N M - - W L R Q L F V E - - - - L F T D	300
Pp NahR	I R I A D Q C V E P F G L S A L P - - H P V V L P E I A I N M F W H A K Y H K D L A N I - - W L Q L M F D - - - - L F T D	300
Ec XopR	D - - S Y A Q M N W P G V I F R P L K Q R I P - - A D L Y I V Y E T Q Q V T P A M V K - - L L A I L T Q	294
Ct Tsrf	R T L Y E R N A F K D Q L C S I P L Q D A P N P T H V Y V L R L R H D L P V T A A G L I R V I Q H H A - - - - -	294
At OccR	P A A A - - - - - I E F T D R I V L R P F S I F I D A G F L E V R S A I G A P S T I V D R F T T E F W R - - - - -	285
Cf AmpR	V R M F T H L L S S E R I V Q - P F L T Q I D L G S W I T R L Q S R P E T P A M R E F S R W L T - - - - -	286
Ec OxyR	A L A V P P E R K R D G V V Y L P C I K P E P R R T I G L V Y R P G S P L R S R Y E Q L A E A I R A M D G H F - - - - -	297
Rs CbbR	L H V V M D E L R F G Q L V Q L A A P G L P I E R H W F L V H P V D R P L N P A A L - - - - - - R V Q G E I V K - - - - -	299
Ab BenM	A - - S T Q S I O I F N L S Y V P L L D P D A I T P I Y I A V R N M E E S T Y I Y S - L Y E T I R Q I Y A - - Y - - - - -	295
Ec Cbl	E W S S - G E Q E E N L I R L D T R H L F D A N T V W L G L K R G Q L Q R N Y V W R F L E L C N A G L S V E D I K R Q V M E S S	308
St CysB	S M A V - D P L A D P D L V R I D A H D I F S H S T K I G F R R S T F L R S Y M D F I Q R F A P H L T R D V V D T A V A L R S	308
Sm NodD1	S D T C	308
Rlv NodD	M E S C T S	310
Rlt NodD	S E R C S Q E P R A T Q S W	318
Bsp DntR	A	301
Pp NahR		300
Ec XopR		294
Ct Tsrf	- - - - - L Q T G	
At OccR	- - - - - F H D D L M K Q N G L M E	
Cf AmpR	- - - - - G V L H K	
Ec OxyR	- - - - - D K V L K Q A V	
Rs CbbR	- - - - - L K G A Y L P G A P - - A	
Ab BenM	- - - - - E G F T E P P N W	
Ec Cbl	- E E E I D - - Y Q I	
St CysB	N E E I E A M F Q D I K L P E K	

LTTR mutants

- █ Type I: Activation-deficient
- █ Type II, IV: Constitutive
- █ Type III: Enhanced-sensitivity

LTTR crystal structure

- █ Oligomerization
- █ Ligand-binding cavity

Figure S1. Locations of mutant residues based on structural and functional studies in LTTRs: *S. meliloti* NodD1 (1), *R. l. bv viciae* NodD (2, 3) (Peck and Long, unpublished data), *R. l. bv trifolii* NodD (4) (Peck and Long, unpublished data), *Burkholderia* sp. DntR (5), *P. putida* NahR (6) (7), *E.coli* XapR (8), *C. testosteroni* T-2 TsaR (9, 10), *A. tumefaciens* OccR (11) (12), *C. freundii* AmpR (13, 14), *E. coli* OxyR (15) (16, 17), *R. sphaeroides* CbbR (18), *A. baylyi* BenM (19) (20), *E.coli* Cbl (21) (22), and *S. typhimurium* CysB (23). Numbers to the right indicate amino acid residue. Residues shaded yellow are identical to *S. meliloti* NodD1. Outlined residues indicate the following mutant phenotypes based on mutations in LTTRs: green: activation-deficient except in Cbl in which mutants are unable to repress target transcription; red: constitutively-active; purple: enhanced inducer sensitivity. Mutant phenotypes based on LTTR crystal structures: orange: oligomerization; grey: ligand binding (i.e., lining the inducer binding pocket or contacting the ligand). Boxed areas indicate mutational hotspots at sites of mutant residues discussed in this study.

TABLE S1. Strains and Plasmids

Strain or Plasmid	Relevant Characteristics	Source/Reference
<i>Strains</i>		
DH5 α	<i>Escherichia coli supE44 ΔlacU169 (Φ80lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	(24)
XL1-Red	$\Delta[mcrA]183, \Delta[mcrCB-hsdSMR-mrr]173, endA1, supE44, thi-1, gyrA96, relA1, mutS, mutT, mutD5, lac Tn10 (Tet^R)$	Stratagene
XL1-Red Tc ^S	$\Delta[mcrA]183, \Delta[mcrCB-hsdSMR-mrr]173, endA1, supE44, thi-1, gyrA96, relA1, mutS, mutT, mutD5, lac Tc^S$	This study
Rm 1021	<i>Sinorhizobium meliloti SU47 str-21 Sm^R</i>	(25)
A2105	<i>Rm1021 nodD1::Tn5(9B7) nodD2::tm nodD3::sp/g-1 nodC'-lacZ</i>	(26)
BTH101	<i>F', cya-99, araD139, galE15, galK16, rpsL1(Str^R), hsdR2, mcrA1, mcrB1</i>	(27)
<i>Plasmids</i>		
pASK75B	Vector encoding 10 amino-acid Strep-tag, Tc-inducible promoter	(28)
pUC119	Cloning vector, Ap ^R	(29)
pBluescript SK	Cloning vector, Ap ^R	Stratagene
pCRII-TOPO	TOPO Cloning vector, Ap ^R	Invitrogen
pGroESL	<i>E. coli groESL</i> in pTG10	(30)
pKT25	<i>B. pertussis cya</i> T25-expression plasmid, Kn ^R	(27)
pKT25-zip	<i>B. pertussis cya</i> T25-leucine zipper fusion, Kn ^R	(27)
pUT18	<i>B. pertussis cya</i> T18-expression plasmid, Ap ^R	(27)

pUT18c-zip	<i>B. pertussis cya</i> T18-leucine zipper fusion, Ap ^R	(27)
pNodD1-ST	C-terminal fusion of NodD1 to Strep-tag in pASK75B	(31)
pMP40	C-terminal fusion of NodD1 D284N to Strep-tag in pASK75B	This study
pMP41	C-terminal fusion of NodD1 K205N to Strep-tag in pASK75B	This study
pMP42	C-terminal fusion of NodD1 L280F to Strep-tag in pASK75B	This study
pMP43	C-terminal fusion of NodD1 L69F to Strep-tag in pASK75B	This study
pMP44	C-terminal fusion of NodD1 S104L to Strep-tag in pASK75B	This study
pMP45	C-terminal fusion of NodD1 D134N to Strep-tag in pASK75B	This study
pMP46	C-terminal fusion of NodD1 M193I to Strep-tag in pASK75B	This study
pMP50	<i>S. meliloti nodD1</i> in pCRII	This study
pMP60	<i>S. meliloti nodD1</i> D284N in pCRII	This study
pMP61	<i>S. meliloti nodD1</i> K205N in pCRII	This study
pMP62	<i>S. meliloti nodD1</i> L280F in pCRII	This study
pMP63	<i>S. meliloti nodD1</i> D284A in pCRII	This study
pMP64	<i>S. meliloti nodD1</i> L280A in pCRII	This study
pMP66	<i>S. meliloti nodD1</i> P285A in pCRII	This study
pMP67	<i>S. meliloti nodD1</i> P285Q in pCRII	This study
pMP68	<i>S. meliloti nodD1</i> G286A in pCRII	This study
pMP69	<i>S. meliloti nodD1</i> P285Q G286A in pCRII	This study
pMP70	<i>S. meliloti nodD1</i> K205A in pCRII	This study
pMP86	<i>S. meliloti nodD1</i> M193I in pCRII	This study
pAB 100	<i>S. meliloti nodD1</i> L69F in pCRII	This study
pAB 101	<i>S. meliloti nodD1</i> L103F in pCRII	This study
pAB 102	<i>S. meliloti nodD1</i> S104L in pCRII	This study
pAB 103	<i>S. meliloti nodD1</i> D134N in pCRII	This study
pAB 104	<i>S. meliloti nodD1</i> D135N in pCRII	This study
pRF796	<i>S. meliloti nodF nod</i> box in pCRII	This study
pRF1049	NodD1-T18 fusion in pUT18, Ap ^R	This study

pRF1050	T18-NodD1 fusion in pUT18c, Ap ^R	This study
pRF1051	T25-NodD1 fusion in pKT25, Kn ^R	This study
pRF1099	NodD1 L69F -T18 fusion in pUT18, Ap ^R	This study
pRF1101	NodD1 S104L -T18 fusion in pUT18, Ap ^R	This study
pRF1104	NodD1 M193I -T18 fusion in pUT18, Ap ^R	This study
pRF1105	NodD1 K205N -T18 fusion in pUT18, Ap ^R	This study
pRF1107	NodD1 D284N -T18 fusion in pUT18, Ap ^R	This study
pRF1108	T25-NodD1 S104L fusion in pKT25, Kn ^R	This study
pRF1113	T25-NodD1 L280F fusion in pKT25, Kn ^R	This study
pRF1114	T25-NodD1 D284N fusion in pKT25, Kn ^R	This study
pRF1115	NodD1 L103F -T18 fusion in pUT18, Ap ^R	This study
pRF1117	NodD1 L280F -T18 fusion in pUT18, Ap ^R	This study
pRF1119	T25-NodD1 L69F fusion in pKT25, Kn ^R	This study
pRF1120	T25-NodD1 K205N fusion in pKT25, Kn ^R	This study
pRF1134	T25-NodD1 M193I fusion in pKT25, Kn ^R	This study
pRF1138	T25-NodD1 L103F fusion in pKT25, Kn ^R	This study
pRF1145	NodD1 D134N/D135N -T18 fusion in pUT18, Ap ^R	This study
pRF1151	NodD1 L103F/S104L -T18 fusion in pUT18, Ap ^R	This study
pRF1153	T25-NodD1 L103F/S104L fusion in pKT25, Kn ^R	This study
pRF1155	T25-NodD1 D134N/D135N fusion in pKT25, Kn ^R	This study
pRF1151	NodD1 L103F/S104L -T18 fusion in pUT18, Ap ^R	This study
pRF1161	NodD1 (BglII/BamHIΔ)-T18 fusion in pUT18, Ap ^R	This study
pRF1163	T25-NodD1 (BglII/BamHIΔ) fusion in pKT25, Kn ^R	This study

Broad host-range plasmids

pRK2013	RK2 derivative helper plasmid, Kn ^R	(32)
pTE3	IncP cloning vector, Tc ^R , <i>S. typhimurium trp</i> promoter	(1)
pRF771	pTE3 with modified polylinker	(33)
pRmE43	<i>nodD1</i> in pTE3, Tc ^R	(34) (35)

pMP53	<i>S. meliloti nodD1</i> in pRF771	This study
pMP150	<i>S. meliloti nodD1</i> D284N in pRF771	This study
pMP151	<i>S. meliloti nodD1</i> K205N in pRF771	This study
pMP152	<i>S. meliloti nodD1</i> L280F in pRF771	This study
pMP153	<i>S. meliloti nodD1</i> D284A in pRF771	This study
pMP154	<i>S. meliloti nodD1</i> L280A in pRF771	This study
pMP156	<i>S. meliloti nodD1</i> P285A in pRF771	This study
pMP157	<i>S. meliloti nodD1</i> P285Q in pRF771	This study
pMP158	<i>S. meliloti nodD1</i> G286A in pRF771	This study
pMP159	<i>S. meliloti nodD1</i> P285Q/G286A in pRF771	This study
pMP160	<i>S. meliloti nodD1</i> K205A pRF771	This study
pMP173	<i>S. meliloti nodD1</i> M193I pRF771	This study
pAB 110	<i>S. meliloti nodD1</i> L69F pRF771	This study
pAB 111	<i>S. meliloti nodD1</i> L103F pRF771	This study
pAB 112	<i>S. meliloti nodD1</i> S104L pRF771	This study
pAB 113	<i>S. meliloti nodD1</i> D134N pRF771	This study
pAB 114	<i>S. meliloti nodD1</i> D135N pRF771	This study

Table S2. Dimerization of NodD1 mutants

	T18 fusion	T25 fusion	β -gal ^a	SD
1		D1(L69F)-T18	T25-zip	26.2 9.2
2		D1(L69F)-T18	T25-D1	540.3 59.5
3		D1(L69F)-T18	T25-D1(L69F)	657.7 118.6
4		D1-T18	T25-D1(L69F)	356.1 125.2
5		T18-zip	T25-D1(L69F)	27.6 9.5
6		T18 vector	T25-D1(L69F)	27.5 18.9
7		D1(L103F/S104L)-T18	T25-zip	29.1 7.7
8		D1(L103F/S104L)-T18	T25-D1	413.8 122.9
9		D1(L103F/S104L)-T18	T25-D1(L103F/S104L)	728.7 90.8
10		D1-T18	T25-D1(L103F/S104L)	302.6 105.3
11		T18-zip	T25-D1(L103F/S104L)	27.6 4.3
12		T18 vector	T25-D1(L103F/S104L)	29.5 4.8
13		D1(D134N/D135N)-T18	T25-zip	27.0 8.6
14		D1(D134N/D135N)-T18	T25-D1	712.9 103.3
15		D1(D134N/D135N)-T18	T25-D1(D134N/D135N)	818.0 104.2
16		D1-T18	T25-D1(D134N/D135N)	396.9 40.0
17		T18-zip	T25-D1(D134N/D135N)	34.1 8.8
18		T18 vector	T25-D1(D134N/D135N)	23.1 9.5
19		D1(M193I)-T18	T25-zip	25.1 10.2
20		D1(M193I)-T18	T25-D1	570.7 47.9
21		D1(M193I)-T18	T25-D1(M193I)	534.0 40.7
22		D1-T18	T25-D1(M193I)	387.3 56.6
23		T18-zip	T25-D1(M193I)	51.4 28.5
24		T18 vector	T25-D1(M193I)	14.1 7.5
25		D1(K205N)-T18	T25-zip	26.0 32.5
26		D1(K205N)-T18	T25-D1	562.4 180.8
27		D1(K205N)-T18	T25-D1(K205N)	709.1 138.5
28		D1-T18	T25-D1(K205N)	459.2 227.7
29		T18-zip	T25-D1(K205N)	108.5 59.0
30		T18 vector	T25-D1(K205N)	12.1 11.6
31		D1(L280F)-T18	T25-zip	16.6 22.5
32		D1(L280F)-T18	T25-D1	627.0 154.5
33		D1(L280F)-T18	T25-D1(L280F)	774.9 143.0
34		D1-T18	T25-D1(L280F)	263.7 116.0
35		T18-zip	T25-D1(L280F)	30.4 21.1
36		T18 vector	T25-D1(L280F)	20.1 8.5
37		D1(D284N)-T18	T25-zip	26.3 16.4
38		D1(D284N)-T18	T25-D1	579.2 110.2
39		D1(D284N)-T18	T25-D1(D284N)	764.4 164.3
40		D1-T18	T25-D1(D284N)	361.4 163.4
41		T18-zip	T25-D1(D284N)	44.0 21.8
42		T18 vector	T25-D1(D284N)	17.1 5.5
43		D1(L103F)-T18	T25-zip	15.7 7.9
44		D1(L103F)-T18	T25-D1	460.8 266.6
45		D1(L103F)-T18	T25-D1(L103F)	557.7 105.8
46		D1-T18	T25-D1(L103F)	659.4 128.5
47		T18-zip	T25-D1(L103F)	51.5 36.6
48		T18 vector	T25-D1(L103F)	52.7 12.7
49		D1(S104L)-T18	T25-zip	13.1 14.8
50		D1(S104L)-T18	T25-D1	553.9 99.9
51		D1(S104L)-T18	T25-D1(S104L)	767.4 177.6
52		D1-T18	T25-D1(S104L)	369.5 49.0
53		T18-zip	T25-D1(S104L)	20.8 24.3
54		T18 vector	T25-D1(S104L)	14.1 13.4

^a β -galactosidase activity (Miller units) of wild-type lacZ in cyA E.coli

Table S2. Dimerization of NodD1 mutants. *cya E. coli* BTH101 was co-transformed with plasmids expressing the indicated T18- and T25-NodD1 fusions. Six colonies from each co-transformation were patched onto minimal maltose medium (left) and assayed for cAMP-dependent β-galactosidase activity (right). D1 is wild-type NodD1; mutations in NodD1 give rise to the residues in parentheses. Data is presented as mean +/- SD from a representative assay.

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