Establishment of Proximity-dependent Biotinylation Approaches in Different Plant Model Systems.

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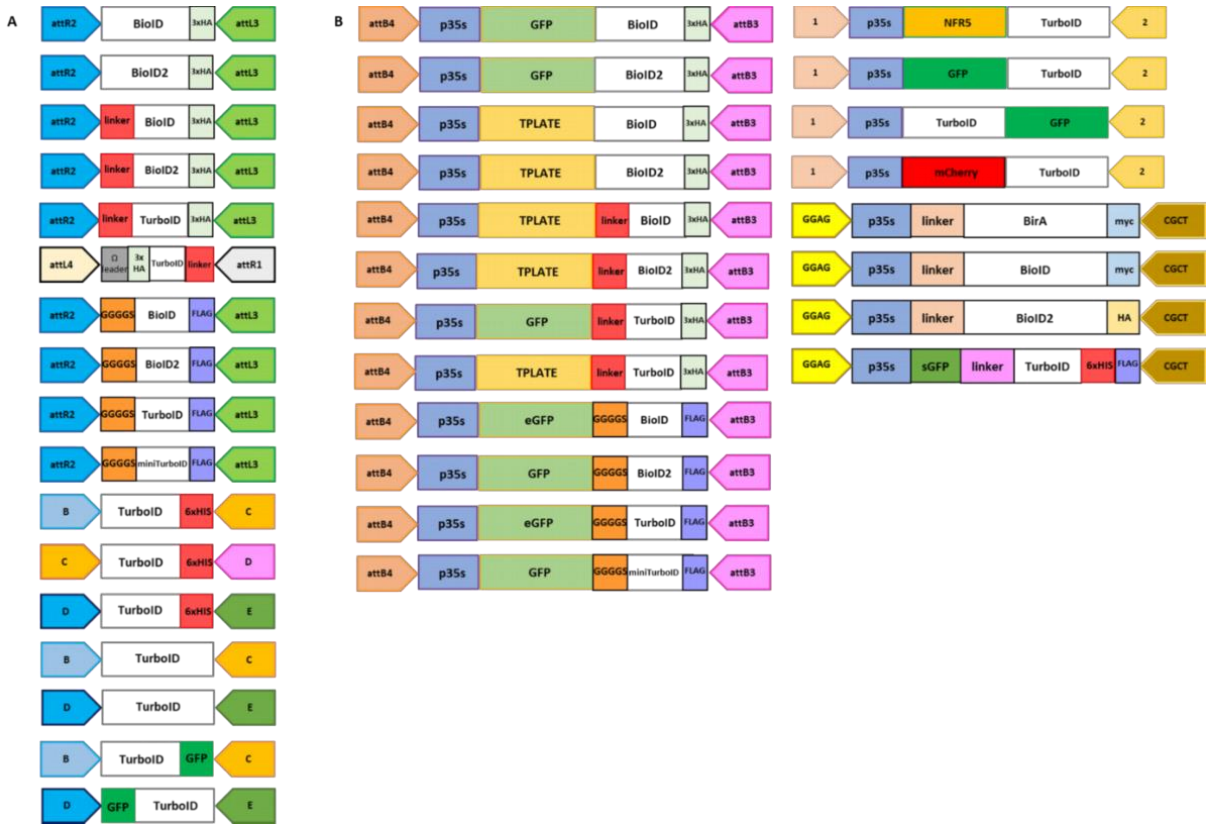
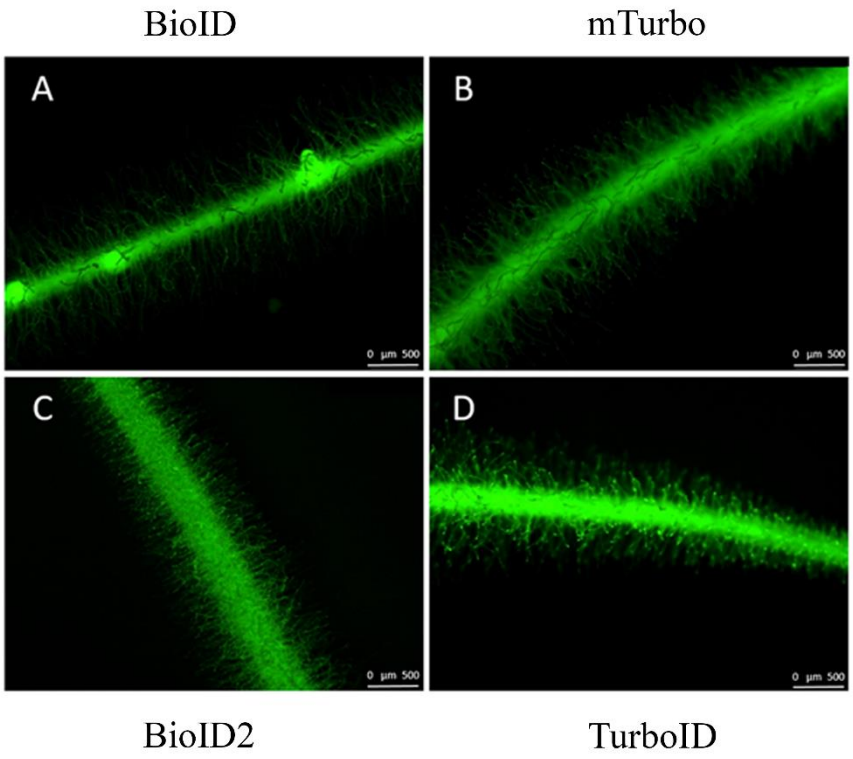
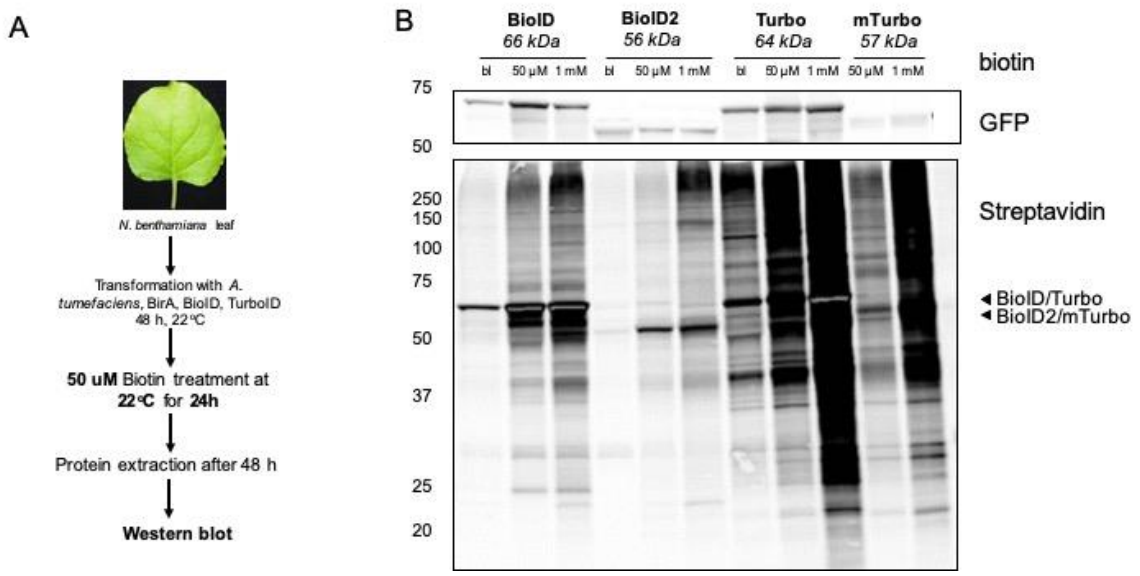
Supplemental material



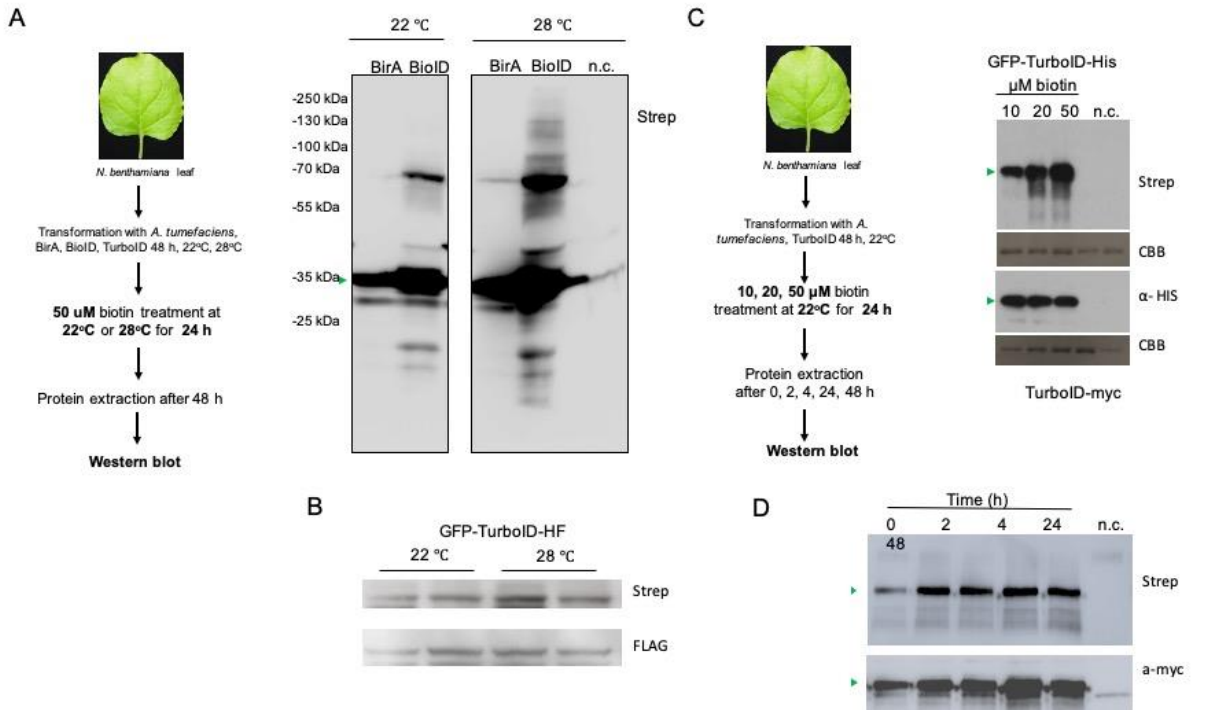
Supplemental Figure 1. Overview of available constructs for proximity biotinylation in plants.
Diagrammatic representation of plasmids used in the study and/or available for the community. (A) various entry clones or building blocks used to make the destination vectors in panel B. (B) Destination vectors used in this study and/or available to the community. Gateway att sites are according to Karimi et al., 2007. Borders of golden gateway B-E and 1,2 are according to GoldenGate from Binder et al 2014, whereas the 4 nucleotide borders of the golden gateway system are according to GoldenGate from Patron et al., 2015. The linker (in red) is a 65 aa long $(GGGGS)_{13}$ repeat. The linker (in light orange) is a 213bp GS linker while the one in grey is 30 bp GS linker. BioID, BioID2, TurboID and miniTurbo all represent promiscuous versions of the respective biotin ligases. BirA is the non-promiscuous negative control version of the biotin ligase. Myc, HA, 6xHis and 1x or 3xFLAG are added tags for Western Blot detection. All corresponding sequences can be found in supplemental sequences.



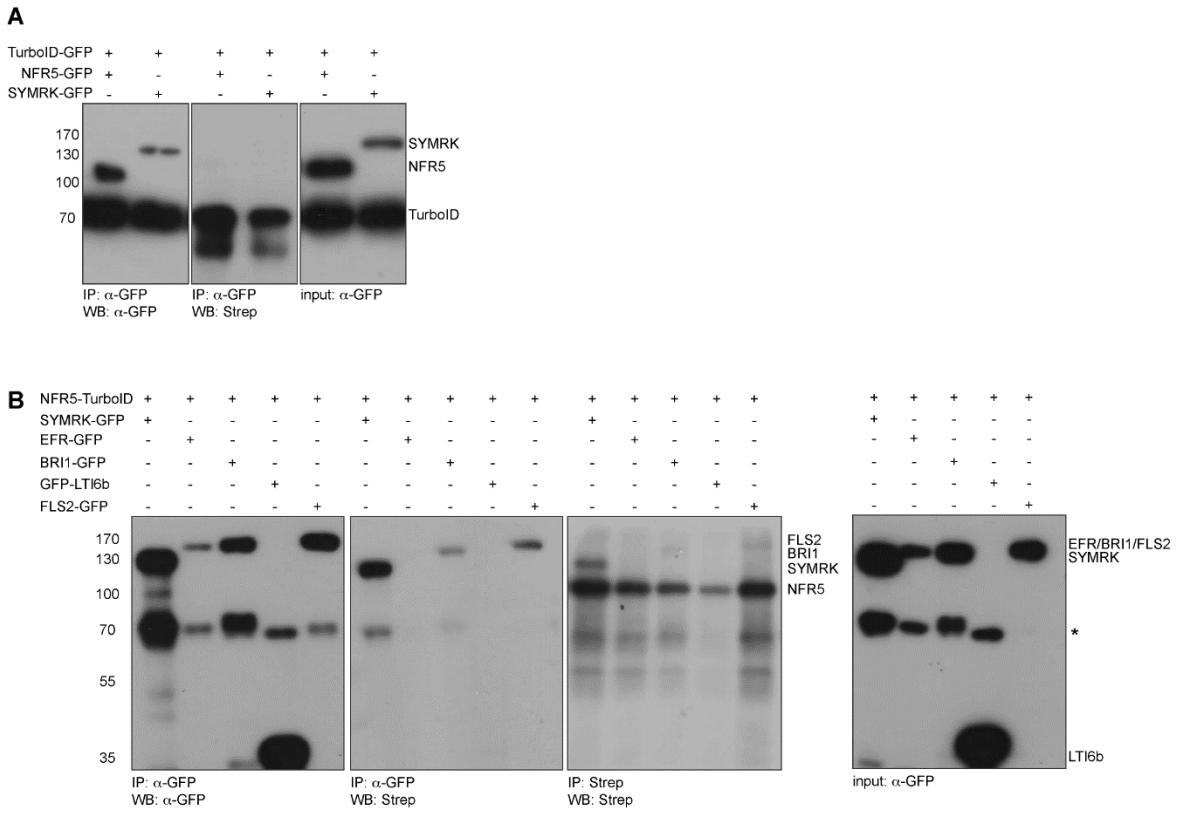
Supplemental Figure 2. GFP expression in tomato hairy root cultures transformed with rhizogenic *Agrobacterium*. Fluorescence micrograph images of eGFP expression were obtained from primary hairy roots (~14 days) transformed by rhizogenic *Agrobacterium* with the following expression constructs; Pro35S::eGFP-BioID, Pro35S::eGFP-BioID2, Pro35S::eGFP-TurboID, and Pro35S::eGFP-mTurbo. The scale bars are 500 µm. Images are representative for the four to ten independent roots selected for sub cultivation and showing expression of the marker per construct. This figure supports Figure 1.



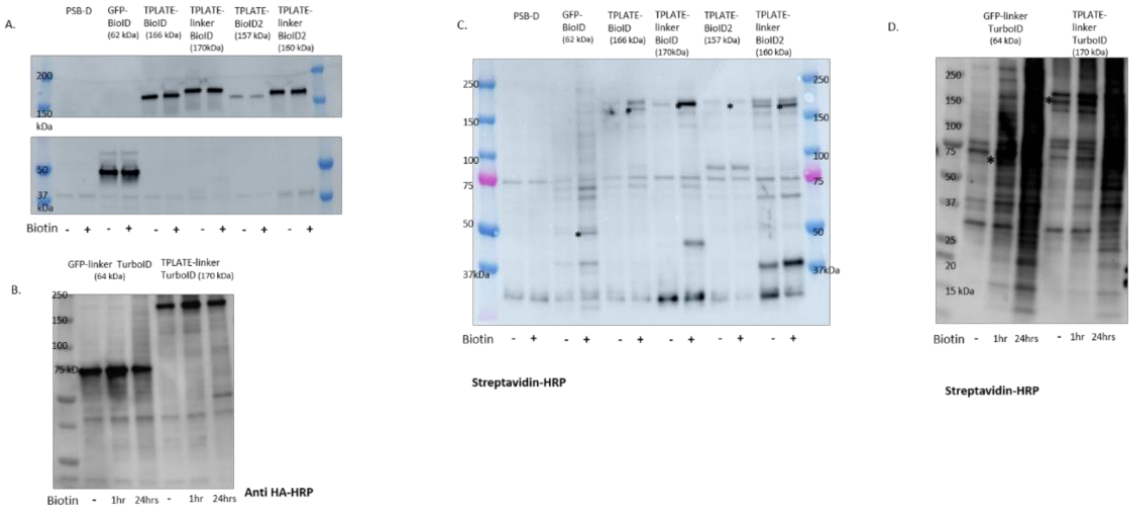
Supplemental Figure 3. Characterization of PBL-catalysed proximity labelling in *N. benthamiana*. (A) Experimental setup. (B) Comparison of biotinylation activity in *N. benthamiana* expressing eGFP-BioID (~66 kDa), eGPF-BioID2 (~56 kDa), eGFP-Turbo (~64 kDa) and eGPF-mTurbo (~57 kDa). Overlapping signal as indicated with a black arrow denote enzyme-catalysed *cis*-biotinylation. Gray bands in intense black areas represent saturation of the streptavidin-s680 signal and is most prominent in case of auto-biotinylation activity. Blank lanes (bl) depict endogenously biotinylated proteins. Two infiltrated tobacco leaf segments/leaves were analyzed per setup and the experiment was repeated twice with similar results. This figure supports Figure 1.



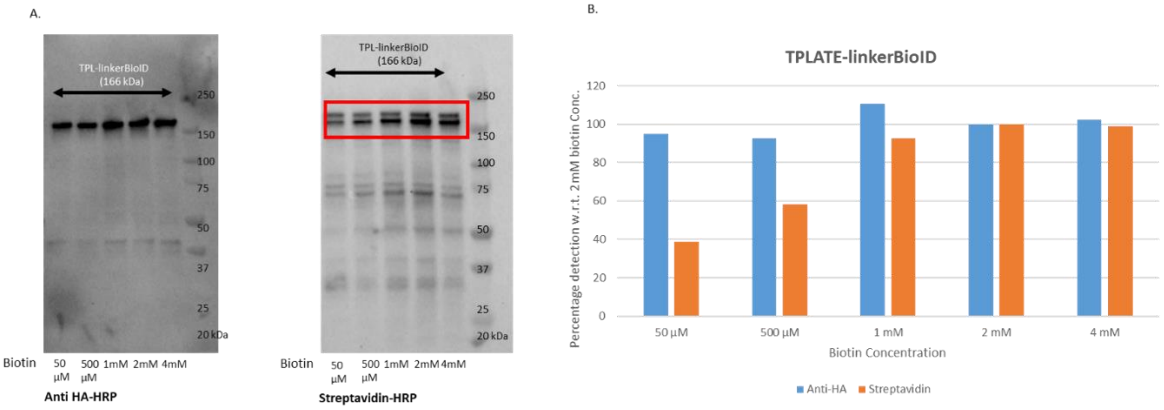
Supplemental Figure 4. Biotinylation of BioID increases at elevated growth temperature and biotin concentration in *Nicotiana benthamiana*. (A) The left panel shows the experimental setup. Western blot: The wild-type version of BioID (BirA) shows residual cis-biotinylation activity at both 22°C and 28°C. (B) BioID shows promiscuous biotinylation which increases with temperature. The samples were run on the same blot along with other ones. The blot was cropped to allow a direct comparison between the samples shown. GFP-TurboID-HF activity was ~2 fold increased at higher temperature (i.e. when going from 22°C to 28°C). Two biological replicates are depicted. (C) The left panel shows the experimental setup. Increasing biotin levels elevated biotinylation efficiency. (D) Efficient cis-biotinylation was observed already 2 h after biotin administration. For (C) and (D), green arrowheads show the corresponding BL. N.c. negative control (empty vector). This figure supports Figure 2.



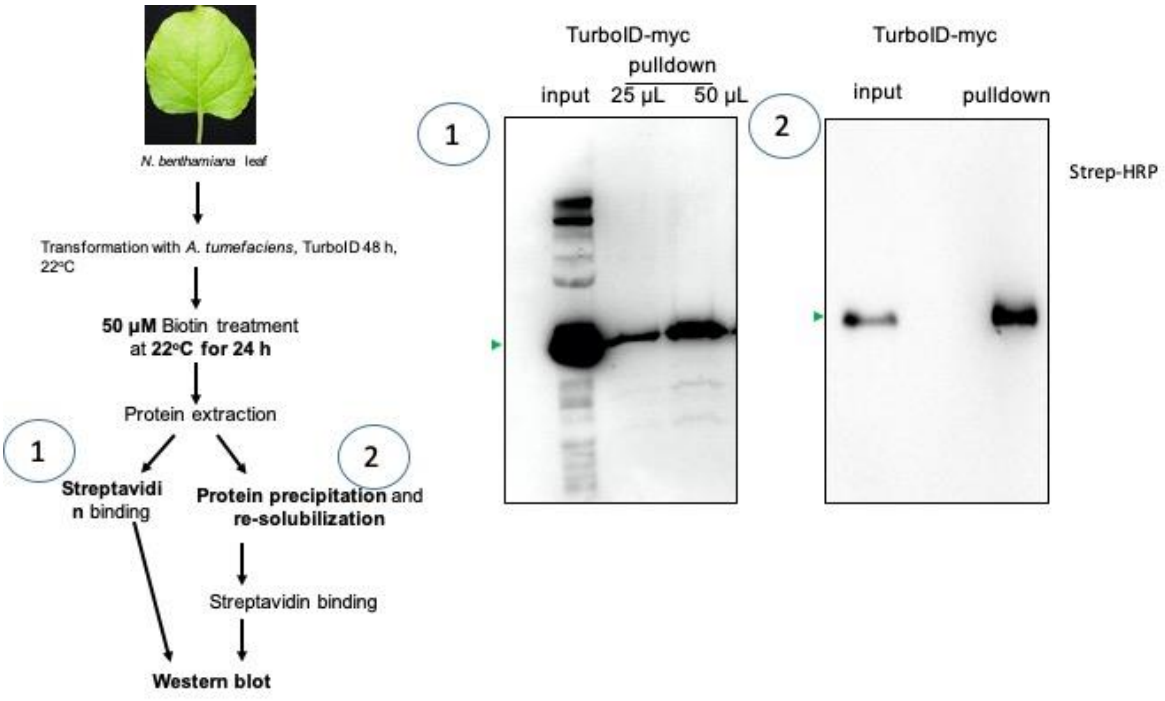
Supplemental Figure 5. *Trans*-biotinylation within membrane-resident receptor complexes. (A) TurboID-GFP was co-expressed with symbiotic RLKs to test for unspecific *trans*-biotinylation. While all proteins were detected before (right panel) and after immunoprecipitation (left panel), no *trans*-biotinylation of the receptors was observed under these conditions (middle panel). The activity of TurboID is indicated by *cis*-biotinylation of TurboID-GFP (70 kDa). (B) Fusing TurboID to NFR5 (120 kDa) resulted in strong *trans*-biotinylation of the known interaction partner SYMRK (150 kDa), weak signals were detected in case of the PM-resident receptors BRI1 and FLS2, while no *trans*-biotinylation of EFR and the PM-marker LTI6b were detected. Temporally limiting the reaction results in weak but specifically detectable bands in case of NFR5-TurboID and SYMRK-GFP. Biotin was applied for 2 hours. IP= immunoprecipitation; WB= Western Blot. *= unclassified band. Strep= Streptavidin. This figure supports Figure 2.



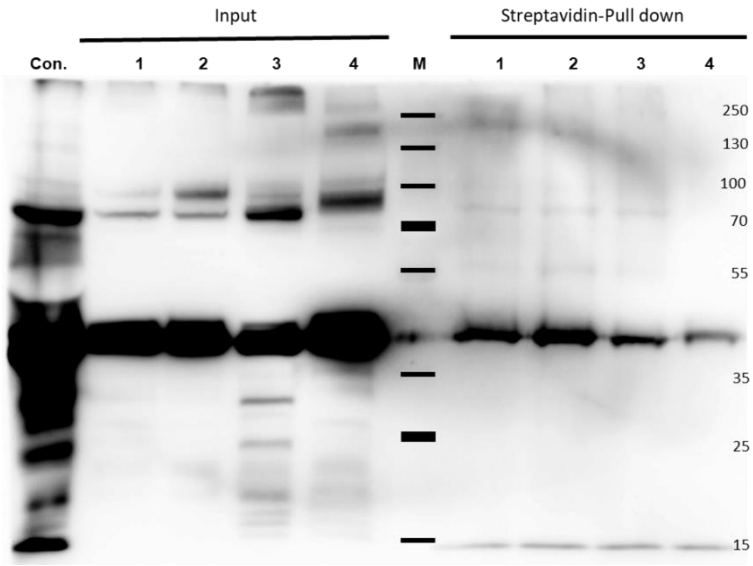
Supplemental Figure 6. Different PBL cause different *cis*- and *trans*-biotinylation. *Arabidopsis* cell cultures expressing different TPLATE-PBLs were incubated with 50µM biotin at 28°C for 24 h or for both 24 h and 1 h (linkerTurboID cultures) before harvesting. (A and B) Anti-HA HRP Western blotting was performed to visualize expression levels of the different cultures. (C and D) Streptavidin-HRP Western blotting of different TPLATE-PBLs, GFP-BioID and control cell cultures (PSB-D). *Cis*-biotinylation of the bait and *trans*-biotinylation can clearly be observed. * indicates *cis*-biotinylation of the bait. This figure supports Figure 4.



Supplemental Figure 7. *Cis*-biotinylation of TPLATE-linkerBioID increases at higher concentration of exogenous biotin. Cell cultures expressing TPLATE-linkerBioID were incubated with biotin (50µM to 4mM) at 28°C for 24 h. (A) Anti-HA HRP Western immunostaining was performed to check protein expression while streptavidin-HRP Western immunostaining was used to assess the biotinylation levels of TPLATE-BioID. (B) Quantification of the percentage of biotinylation (orange) as well as the expression (blue) for each biotin concentration normalized to the maximum biotinylation efficiency (2mM) using ImageJ. This figure supports Figure 4.

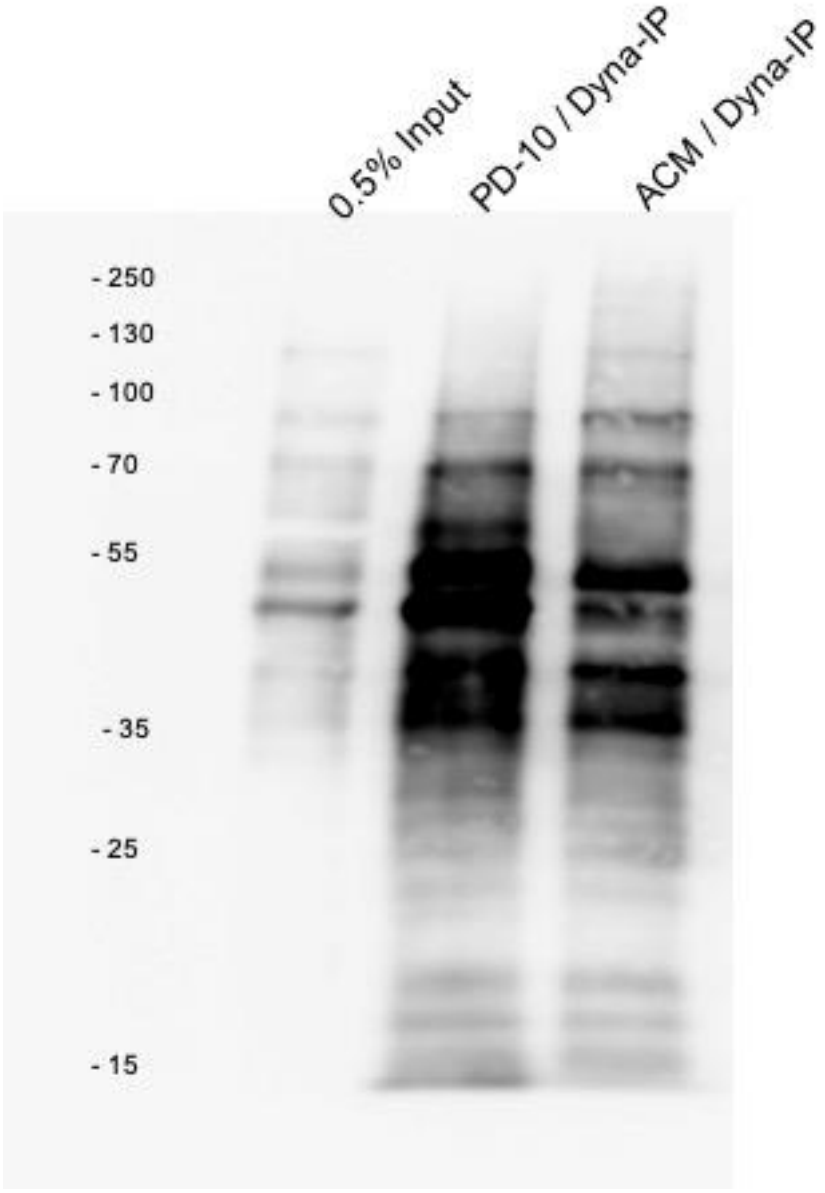


Supplemental Figure 8. Exogenous application of biotin can exceed the binding capacity of streptavidin beads. Blot on the left: input and IP with streptavidin using 25 or 50 µl of beads. Note that 2x more beads increased the recovery of the input signal, suggesting that the beads are saturated. Blot on the right: IP with 25 µl of streptavidin beads but in this case the supernatant was precipitated using ammonium acetate to remove excess biotin (in this case, the pulldown is much more than the input). Green arrowheads mark the position of the BL. This figure supports Figure 4.



Supplemental Figure 9. The biotin-streptavidin interaction is retained under harsh conditions. Different extraction buffers were used for testing the binding affinity of biotin-labelled proteins with streptavidin from equal amounts of plant protein material: 1. 50mM HEPES, 150 mM NaCl, 0.5% NP40,

10% Glycerol, 1mM PMSF; 2. 50mM Tris/HCl, 150 mM NaCl, 0.5% NP40, 10% Glycerol, 1mM PMSF; 3. 1x PBST, 1mM PMSF; 4. 2x Laemmli sample buffer (65 mM Tris-HCl, pH 6.8, 20% (w/v) glycerol, 2% SDS, 0.01% bromophenol blue, 10mM DTT). Con: control. M: marker. Note that the lack of enrichment in the pulldown (right panel) is due to the presence of biotin (see also Supplemental Figure 8). This figure supports Figure 4, Figure 5 and Figure 6.



Supplemental Figure 10. Comparing PD10 versus ammonium acetate for biotin removal. To test the best way for removing free biotin following sample extraction, the same amount of supernatant (2.5 mL) was either de-salted using PD-10 columns or precipitated by ammonium acetate method (ACM). Next, the PD10-cleared lysate and the ammonium acetate re-dissolved precipitate were incubated with Dynabeads (Dyna-IP) to capture biotinylated proteins. Western blot analysis using anti-streptavidin showed that majority of biotinylated proteins (ca. 15 to 70 kDa) were better enriched in the supernatant by using PD-10 columns. Larger proteins (ca. >100 kDa) were better enriched using ACM.

Supplemental Table1. List of expression vectors used in this study.

Name	Vector	Promoter	Gene	PBL used	Material used
GFP-BioID	pK7m34Gw	p35s	GFP	BioID	*Arabidopsis* cell culture
GFP-BioID2	pK7m34Gw	p35s	GFP	BioID2	*Arabidopsis* cell culture
TPLATE-BioID	pK7m34Gw	p35s	TPLATE	BioID	*Arabidopsis* cell culture
TPLATE-BioID2	pH7m34Gw-R	p35s	TPLATE	BioID2	*Arabidopsis* cell culture
TPLATE-linkerBioID	pH7m34Gw-R	p35s	TPLATE	$(GGGGS)_{13}$ BioID	*Arabidopsis* cell culture
TPLATE-linkerBioID2	pH7m34Gw-R	p35s	TPLATE	$(GGGGS)_{13}$ BioID	*Arabidopsis* cell culture
GFP linkerTurboID	pK7m34Gw	p35s	GFP	$(GGGGS)_{13}$ TurboID	*Arabidopsis* cell culture
TPLATE-linkerTurboID	pK7m34Gw	p35s	TPLATE	$(GGGGS)_{13}$ TurboID	*Arabidopsis* cell culture
BirA-myc	pICSL86900	p35s	BirA-myc	BirA-myc	*N.benthamiana*
BioID-myc	pICSL86900	p35s	BioID-myc	BioID-myc	*N.benthamiana*
HF-BioID2-HA	pICSL86900	p35s	BioID2-HA	BioID2-HA	*N.benthamiana*
GFP-TurboID-HF	pICSL86922	p35s	TurboID-HF	TurboID	*N.benthamiana*
GFP-TurboID-His	Xpre2-S (pCAMBIA)	p35s	GFP	TurboID	*N.benthamiana*
NFR5-TurboID	Xpre2-S (pCAMBIA)	p35s	NFR5	TurboID	*N.benthamiana*
GFP-LTI6b	Xpre2-S (pCAMBIA)	p35s	LTI6b		N.benthamiana
BiFC_CC_TPLATE_BIN2	BiFC_CC	p35s	TPLATE/BIN2	NA	*N.benthamiana*
BiFC_CC_TPLATE_TOL9	BiFC_CC	p35s	TPLATE/TOL9	NA	*N.benthamiana*
BiFC_CN_TPLATE_TOL9	BiFC_CN	p35s	TPLATE/TOL9	NA	*N.benthamiana*
BiFC_CC_TPLATE_SCAMP5	BiFC_CC	p35s	TPLATE/SCAMP5	NA	*N.benthamiana*
BiFC_CN_TPLATE_SCAMP5	BiFC_CN	p35s	TPLATE/SCAMP5	NA	*N.benthamiana*
GFP-GGGS-BioID-FLAG	pK7m34Gw	p35s	GFP	BioID	*Solanum lycopersicum* stable hairy root lines & *N. Benthamiana*
GFP-GGGS-BioID2-FLAG	pK7m34Gw	p35s	GFP	BioID2	*Solanum lycopersicum* stable hairy root lines & *N. Benthamiana*
GFP-GGGS-TurboID-FLAG	pK7m34Gw	p35s	GFP	TurboID	*Solanum lycopersicum* stable hairy root lines & *N. Benthamiana*
GFP-GGGGS-mTurbo-FLAG	pK7m34Gw	p35s	GFP	mTurbo	*Solanum lycopersicum* stable hairy root lines & *N. Benthamiana*

Supplemental Table 2: list of primers used.

Name	Primer
BirA	GTGGTCTC A T TCG GGA AAC GCGGCT ATT AGA TCA AAG GAT AAC ACC GTG CCA CTT A
BirA	GTGGTCTC A AAGC CTA CAG ATC CTC TTC TGA GAT GAG TTT TTG TTC TTT TTC TGC ACT ACG AAG G
BirA	GTGGTCTC A CGTAGAGGTCGTAAATGGTTTTC
BirA	GTGGTCTC C TACGTCCACGACCAGCCTGCT
HF-Module-Fw	GTGGTCTC ACCATGGGTTCCGGAAGAGGATCGCA
HF-Module-Rv	GTGGTCTC ACATTCCCTTGTCATCGTCATCCTTG
BioID2 with long-linker-Module-Fw	agGAAGACaaTTCGGGATCTGGAGGTGGCGGAAG
BioID2 without linker-Module-Fw	agGAAGACaaTTCGTTTAAGAACTTGATATGGCTGAAAG
ScFv-superfolder-GFP-Module-Fw	GTGGTCTC A ATGG GCCCCGACATCGT
ScFv-superfolder-GFP-Module-Fw	GTGGTCTC A CGAA CCACCTTTGTAGAGCTC
NotImcherryu	GCGGCCGCATGGTGAGCAAGGGCGAGGAG
NotImcherryd	GCGGCCGCTTACTTGTACAGCTCGTCC ATG
attB1_SCAMP5_FWD	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAATCGCCACCACGATCCCAATCC
attB4_SCAMP5_REV	GGGGACAACTTTGTATAGAAAAGTTGGGTGCTTGTTTCCCCTAAAGTAGAGGTAAATTTTCTG
attB3_TPLATE_FWD	GGGGACAACTTTGTATAATAAAGTTGTAATGGACATTCTTTTTGCTCAGATCCAGGC
attB2-TPLATE_REV	GGGGACCACTTTGTACAAGAAAGCTGGGTTGTTAACTTTGGTATATTTTCTATCTTTGCACAAGATG
attB1_TOL9_FWD	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTGAACGCTATGG
attB4_TOL9_REV	GGGGACAACTTTGTATAGAAAAGTTGGGTGTCACATGGTACCAGCTC

Supplemental Table 3: Cell cultures expressing different TPLATE-PBLs identifies TPC subunits with different amount of non-biotinylated peptides. The table shows the amount of non-biotinylated peptides identified for TPC subunits in GFP and TPLATE-BioIDs PBL cell cultures. Preparation of samples for LC-MS/MS involved the use of a buffer containing 8M Urea and 2% SDS. Cell cultures were incubated with either 50µM or 2mM biotin for 24 hours before harvesting. This table shows higher amount of peptides detected for all TPC subunits in case of linkerTurboID constructs than other BioIDs PBL. Also, it shows higher amounts of peptides detected in TurboID control experiments (GFP-linkerTurboID vs. GFP-BioID and GFP-BioID2) suggesting increased promiscuity of TurboID. This table supports Figure 4.

Total number of unique non-biotinylated peptides identified for each TPC subunit							
	AT1G20760	AT1G21630	AT2G07360	AT3G01780	AT3G50590	AT5G24710	AT5G57460
	AtEH1	AtEH2	TASH3	TPLATE	TWD40-1	TWD40-2	TML
TPLATE linkerTurboID	73	155	205	384	228	374	116
GFP linkerTurboID	40	98	122	27	102	271	21
TPLATE BioID	20	32	2	428	68	95	37
TPLATE linkerBioID	19	37	15	540	35	34	16
GFP BioID	-	-	-	-	-	26	-
TPLATE BioID2	9	30	-	157	42	38	51
TPLATE linkerBioID2	24	55	59	453	107	89	38
GFP BioID2	-	-	6	-	-	42	-
TPLATE linkerBioID 2mM	-	-	-	10	-	-	-

Supplemental Table 4. Full list of significantly enriched identifications with TPLATE-BioID versus GFP-BioID at different incubation temperatures for 24 hours with 50 µM biotin.
The table shows the amount of non-biotinylated peptides identified for TPC subunits in GFP and TPLATE-BioID PBLs at different temperatures (25°, 28°, 30° and 35°). Cell cultures were incubated with 50µM biotin for 24 hours. This table supports figure 3.

Supplemental Table 5. Full list of significantly enriched identifications with TPLATE as bait using BioID, BioID2, linkerBioID, linkerBioID2, linkerTurboID E1, linkerTurboID E2 and linkerTurboID E1+E2, versus the respective GFP PBLs. This table shows the amount of non-biotinylated peptides identified for TPC subunits in GFP and various TPLATE-PBLs. Cell cultures were treated with either 50 µM or 2mM biotin at 28°C for 24 hours. This table supports Figure 4.

Supplemental Table 6. Full list of significantly enriched hits with either TPLATE PBLs or GSrhino PD, including average iBAQ values normalized versus TPLATE. The table shows the normalized MaxQuant iBAQ values of all proteins that were significantly enriched in TPLATE-GSRhino (PD), TPLATE-linkerBioID (LBioID), TPLATE-linkerBioID2 (LBioID2) or TPLATE-linkerTurboID (LTurboID) compared to the respective control samples. Colored cells (red for PD, orange for LBioID, green for

LBioID2 and blue for LTurboID) are proteins that are significantly enriched for those experiments. This table supports Figure 5.

Supplemental Table 7. Full list of significantly enriched hits with TPLATE-linkerTurboID, including average iBAQ values normalized versus TPLATE, at different incubation times at 25 degrees and 24-hour incubation time at 28 degrees, all with 50 µM biotin. The table shows the normalized MaxQuant iBAQ values of all proteins that were significantly enriched in TPLATE-linkerTurboID (LTurboID) at different temperatures (25°C or 28°C) and biotin treatments (10 mins, 1 hour, 6 hours and 24 hours) compared to the respective control samples. Colored cells (red for 25°C,10 mins, orange for 25°C,1 hour, green for 25°C, 6 hours and blue triangle for 25°C, 24 hours and dark blue circle for 28°C, 24 hours) are proteins that are significantly enriched for those experiments. This table supports Figure 6.

Supplemental Table 8. MaxQuant TPC subunits peptides in TPLATE-LTurboID. The table shows the amount of biotinylated and non-biotinylated peptides identified for TPC subunits in the TPLATE-linkerTurboID culture. The cell culture was treated with 50 µM biotin at 28°C for 24 hours. This table supports Figure 7.

Supplemental sequences. List of all used PBL sequences.

AttR2_**BioID-**3xHA_attL3

AttR2_GACAAGGACAACACCGTACCCCTGAAGCTCATCGCACTACTTGCTAATGGTGAATTTCACTCCGGGGAA
CAGCTAGGAGAAACACTCGGCATGTCAAGAGCAGCAATAAACAAGCATATACAAACACTCCGTGACTGGGGCGTG
GATGTATTTACCGTCCCTGGTAAGGGATACTCCCTCCCGGAACCAATCCAACTTCTCAATGCCAAGCAGATTCTA
GGTCAACTCGATGGTGGCTCAGTGGCGGTTTTACCAGTAATAGACTCTACGAACCAGTATCTGCTCGACCGAATA
GGGGAGCTTAAATCCGGAGATGCTTGCATTGCGGAATATCAACAAGCCGGACGTGGTGGCAGGGGAAGGAAGTGG
TTCAGTCCCTTCGGCGCGAATCTCTATCTGAGCATGTTCTGGCGACTTGAACAGGGTCCCGCGGCCGCTATCGGC
CTATCCTTAGTTATAGGTATCGTCATGGCTGAGGTCCTCAGAAAGCTAGGGGCTGACAAGGTGAGGGTAAAATGG
CCTAATGACCTCTATCTTCAAGATAGAAAGTTGGCCGGTATCTTAGTGGAATTAACGGGAAAGACCGGCGATGCT
GCACAGATCGTGATCGGTGCGGGAATTAACATGGCCATGCGACGAGTGGAAGAAAGCGTTGTTAATCAGGGATGG
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GCGTTGGAACTTTTTGAACAGGAGGGTTTGGCACCTTACCTTAGCCGATGGGAAAAACTCGACAACTTCATTAAC
CGTCCCGTCAAATTGATTATTGGCGATAAGGAAATTTTCGGAATAAGTAGAGGGATCGATAAGCAGGGAGCGTTA
CTACTTGAGCAGGATGGTATCATAAAGCCGTGGATGGGAGGCGAAATTTCACTCCGTAGCGCCGAAAAGGCCTAC
CCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTAC
GCTTGA_attL3.

AttR2_**BioID2-**3xHA_attL3

AttR2_GACTTCAAAAACTTAATTTGGTTAAAAGAAGTGGATTCAACACAGGAGCGACTTAAAGAATGGAATGTA
AGTTACGGCACGGCATTAGTTGCCGATCGTCAAACGAAGGGAAGAGGAGGGTTGGGGCGTAAGTGGCTAAGTCAG
GAAGGTGGGCTATACTTCAGTTTTCTTCTGAATCCGAAGGAGTTCGAGAACCTGCTACAATTACCGCTCGTACTG
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AAAGAATTCAAAGGCAAGATTGAAAGCAAGATGCTATACCTCGGGGAAGAGGTTAAGTTGCTTGGGGAAGGAAAA
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TTGAGCGGAGAGTTCTCCTTGCGTCGAAGCGCCTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTT
CCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTGA_attL3.

AttR2_linker**BioID-**3xHA_attL3

AttR2_GGTGGCGGAGGTTCTGGCGGTGGAGGATCCGGAGGTGGCGGGTCCGGTGGTGGCGGATCAGGTGGAGGA
GGTTCGGGAGGCGGAGGTAGTGGAGGCGGTGGGAGCGGCGGAGGTGGGAGCGGTGGCGGAGGGAGTGGAGGTGGA
GGCTCGGGCGGAGGTGGCTCCGGCGGAGGTGGGTCTGGAGGCGGTGGCTCGGACAAGGACAACACCGTACCCCTG
AAGCTCATCGCACTACTTGCTAATGGTGAATTTCACTCCGGGGAACAGCTAGGAGAAACACTCGGCATGTCAAGA
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AGCATGTTCTGGCGACTTGAACAGGGTCCCGCGGCCGCTATCGGCCTATCCTTAGTTATAGGTATCGTCATGGCT
GAGGTCCTCAGAAAGCTAGGGGCTGACAAGGTGAGGGTAAAATGGCCTAATGACCTCTATCTTCAAGATAGAAAG
TTGGCCGGTATCTTAGTGGAATTAACGGGAAAGACCGGCGATGCTGCACAGATCGTGATCGGTGCGGGAATTAAC
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TGGATGGGAGGCGAAATTTCACTCCGTAGCGCCGAAAAGGCCTACCCATACGATGTTCCAGATTACGCTTACCCA
TACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTGA_attL3.

AttR2_linker**BioID2-**3xHA_attL3

AttR2_GGTGGCGGAGGTTCTGGCGGTGGAGGATCCGGAGGTGGCGGGTCCGGTGGTGGCGGATCAGGTGGAGGA
GGTTCGGGAGGCGGAGGTAGTGGAGGCGGTGGGAGCGGCGGAGGTGGGAGCGGTGGCGGAGGGAGTGGAGGTGGA
GGCTCGGGCGGAGGTGGCTCCGGCGGAGGTGGGTCTGGAGGCGGTGGCTCGGACTTCAAAAACTTAATTTGGTTA
AAAGAAGTGGATTCAACACAGGAGCGACTTAAAGAATGGAATGTAAGTTACGGCACGGCATTAGTTGCCGATCGT
CAAACGAAGGGAAGAGGAGGGTTGGGGCGTAAGTGGCTAAGTCAGGAAGGTGGGCTATACTTCAGTTTTCTTCTG
AATCCGAAGGAGTTCGAGAACCTGCTACAATTACCGCTCGTACTGGGCCTGAGTGTGAGCGAGGCTTTAGAAGAA
ATTACAGAAATCCCCTTCAGTTTGAAGTGGCCAAATGATGTCTATTTTCAGGAAAAGAAAGTCTCTGGCGTACTG
TGCGAACTATCAAAGGATAAGCTGATCGTAGGAATCGGTATAAATGTTAATCAACGAGAGATCCCGGAGGAAATC
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GGAG_ p35S_Linker_**BioID**-Myc_CaMV poly(A)_CGCT

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GGAG_ p35S_Linker_**BioID2**-HA_CaMV poly(A)_CGCT

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GGAG_ p35S_sGFP_Linker_**TurboID**_6xHIS-3xFLAG_CaMV poly(A)_CGCT

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