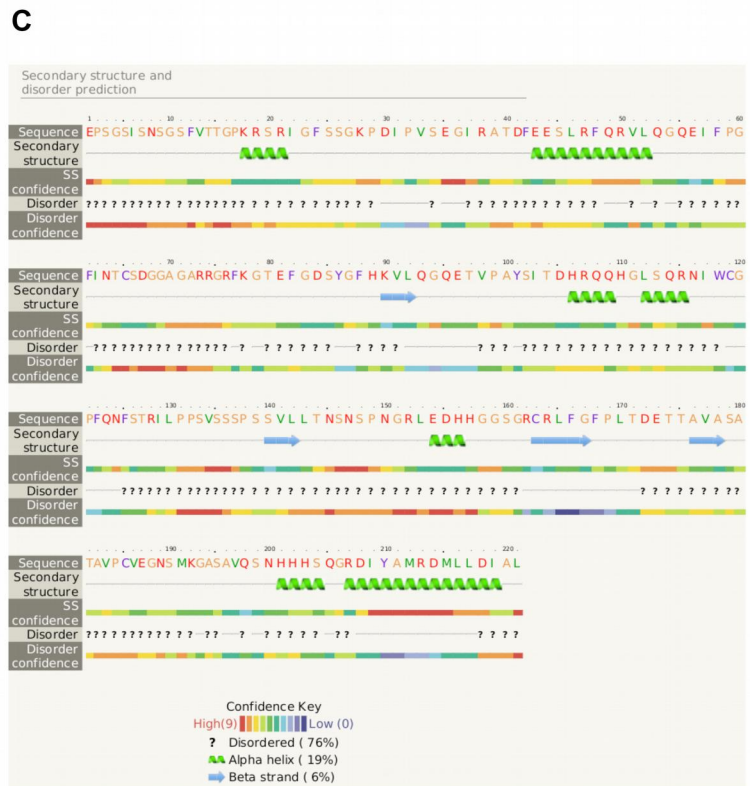
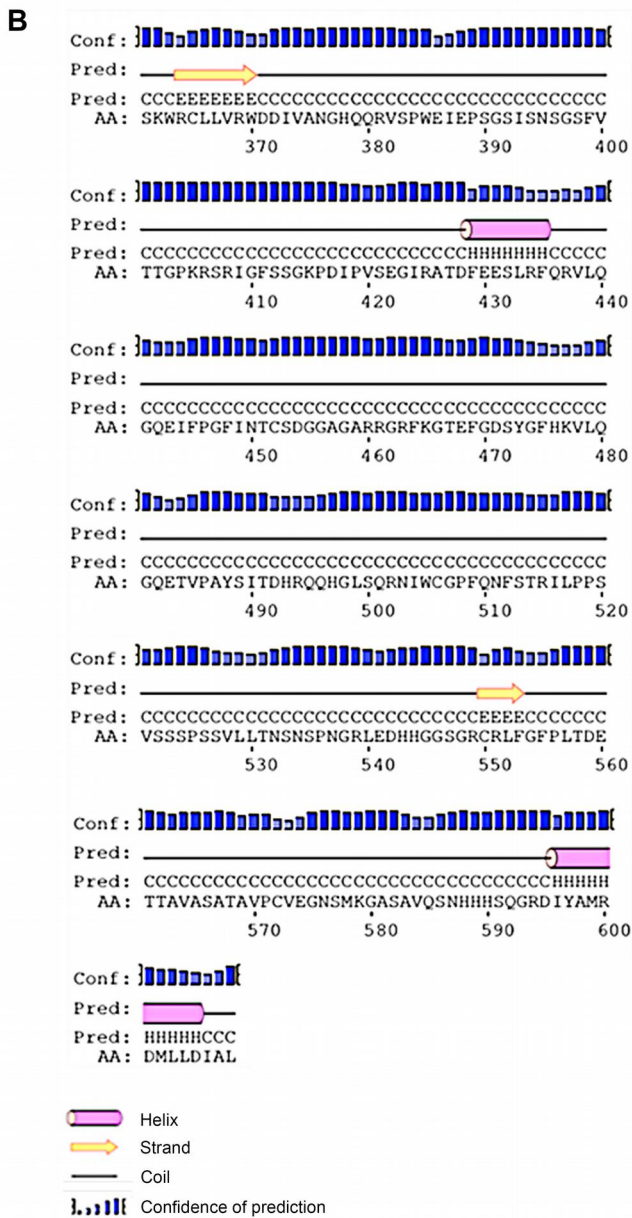
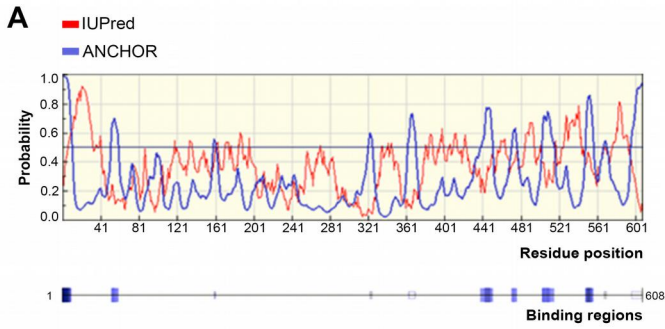


## **Supplementary Information**

# **Auxin sensing is a property of an unstructured domain in the Auxin Response Factor ETTIN of *Arabidopsis thaliana***

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### Supplementary Figure 1.

Structure prediction by (A) IUPred indicating predicted disorder in red and potential interaction sites in blue; (B) PsiPred indicating secondary structure; and (C) Phyre2 indicating predicted disorder and secondary structure.

## NLS

```

ARF10 LSP-FS--PRKKIRI-PQPPEF-----PFGHTKFFIFSP
ARF16 LTFSP--PRKKRL---PQHPDYNL-----INSIPVPSFNSPLIR
ARF17 TPF-PP---AKRLKY-PQGGGFLSGDD-----GEILYQSGLSAAA
ARF3 SFV-TT--C[KRSRL]-GFSSGKPIPVLS-----EGIRATDFEESLRFQVLQ
ARF4 IQS-SP--RFRKRWAGLLDTPPGNPIT-----KRGGLDFEESVRPSKVLQ
ARF13 QSS-L---KKKHMLQLNEIGAT-----LSNLWT
ARF14 RSS-FL--KNKRSRE-VNEIGSSSSHLL-----PPTLTQ
ARF23 -----
ARF15 RSS-FL--KNKRLRE-VNEFGSSSSHLL-----PPTLTQ
ARF20 RSS-LL--KNKRLRE-VNEF-----
ARF21 RSS-LL--KNKRLRE-VNEFGSSSSHLL-----PPTLTQ
ARF12 RSF-LL--KNKRLRE-VNEIGSSSSHLL-----PPTLTQ
ARF22 RPS-LL--KNKRLRE-VNEIGSSSSHLL-----PPTLTQ
ARF9 KSV-ML--KNKRPRQ-VSEVSALDVGIT-----ASNLWSSVLTO
ARF11 TPT-QQQSKKRSRPISEITGSPVASSF-----LSSF50
ARF18 TPAQQQSKKRSRPISEITGSPVASSF-----LYSLPQ
ARF1 SQP-QPPQRNKRPR-PGLPSPATPGSG-----PVTPDGWVKSADT
ARF2 PVP-MP--RPRKRPR-NIAPSPDSSML-----TREGTTKANMDPLPASGLSRVLQ
ARF5 SLT-SG--LKRQLHP-SYPAGETEWGSL-----IKRPLIRVPDSANGIMPYAS
ARF7 PPP-FF--RPRFSGQGMPPDDETDMEASALKRAMPWLDNSLEMKDPSTTFPGLSLVQWMM
ARF19 PPP-FF--RPKYRQPGMPDDELDMENAFKRAMPWGEDFGMKDAQSSMFPGLSLVQWMS
ARF6 SPF-PL--RLKRPWP-PGLSPFHGLKED-DMGMSMSPLMWRDLQSLNFGQMGVNPWMQ
ARF8 SLF-PL--RLKRPWHAGTSSLPDGRGDLGSLTLWRGGGGEQQGLLPLNYPVGLFPWMQ

```

## Motif 1A/1B

```

ARF10 -----PFGHTKFFIFSP-GFANNGGESMCYLSNDNN-----
ARF16 -----INSIPVPSFNSPLIR-SSPLSSVLDNVVGLQGAR-----
ARF17 -----GEILYQSGLSAAA-PDPSMFSYST-----
ARF3 -----EGIRATDFEESLRFQ[V]LQ-G[Q]EIPPGFINTCS-----
ARF4 -----KRGGLDFEESVRPSKVLQ-GQENIGSASPSQGFVMMNRILDFAMQ
ARF13 -----LSNLWT-CQEIQRSMNSPISVP-----
ARF14 -----PPTLTQ-GQEIQQSMATPMNI-----
ARF23 -----
ARF15 -----PPILTO-GQEIQLSVASPMNI-----
ARF20 -----GQEIQLSVASPMNT-----
ARF21 -----PPILTO-GQEIQLSVASPMNI-----
ARF12 -----PPILTO-GQENEQLSVASPMNI-----
ARF22 -----PPILTO-GQEIQLSVASPMNI-----
ARF9 -----ASNLWSSVLTO-PHEFAQSCITSQWSSP-----
ARF11 -----LSSF50-SHESNPSV-----
ARF18 -----LYSLPQ-SQDSINAS-----
ARF1 -----PVTPDGWVKSADT-PSSVPLFSPPAK-----
ARF2 -----TREGTTKANMDPLPASGLSRVLQ-GQEYSTRKHTESVECDAPENSVWQ
ARF5 -----IKRPLIRVPDSANGIMPYAS-FPSMASEQLMKMMRPHNNQNVPSFMGE
ARF7 LKRAMPWLDNSLEMKDPSTTFPGLSLVQWMM-MOQONQLPSAAQPGFPPSMLSPTAA
ARF19 FKRAMPWGEDFGMKDAQSSMFPGLSLVQWMS-MOQNNPLSGSATPQLPSALSSFNL---
ARF6 -DMGMSMSPLMWRDLQSLNFGQMGVNPWMQ-PRLDTSGLLGMQNDV-----YQAMAA
ARF8 GSGLTLWRGGGGEQQGLLPLNYPVGLFPWMQ QRLDLSQMGTDNNQ-----YQAMLA

```

```

ARF10 -----QLFGSPSPSLLSDNLSSYTGNNKL-
ARF16 -----LSSSDLHHYYLNRPPPPPP
ARF17 -----
ARF3 -----TEFGDSYGFH[V]LQGGQETVP
ARF4 -----GVNPAQSGVMDLDRFPVLOGQEQICS
ARF13 -----EDSKFLSGLLNHSLLAIPN
ARF14 -----EDAMTSPRLLMSYVQPM-
ARF23 -----
ARF15 -----EDAMNPSRLLMSYVQPM-
ARF20 -----EDAMNPSRLLMSYVQPM-
ARF21 -----EAMNPSRLLMSYVQPM-
ARF12 -----EDAMNPSRLLMSYVQPM-
ARF22 -----EDVMNPSRLLMSYVQPM-
ARF9 -----EDAKKSDWLNNSYSVSNVAK
ARF11 -----
ARF18 -----LER-----
ARF1 -----NKSFGVSIQSAFWPTNADS-
ARF2 -----WMSSARHEPTYDLDLSSGFTNIDPSHGQRIP
ARF5 -----MMNQKSEMVPQNKLTVPNSASNTSGQEQL
ARF7 FHKONQQAQPMSQLPQPPTTLQQQQQLQQLLHSLNHHQQQSSQSQQQQQQLQ
ARF19 -----LSSANSQFNKPNVNHISQMQAQ
ARF6 -----SPGSMQSPSLVQPMQLQQQLSQQQQL
ARF8 -----HOYLQQSASHNSDLMLQQQQQQQASR

```

## Ser Patch

```

ARF10 -VLF-----GQPILTEQQVMMNRKRL-----EE
ARF16 -VLF-----GKLILPEEQLEKGSTDTANIEK-----TC
ARF17 -QLF-----TNNFLSPPLDLGKVS-----TE
ARF3 -QNF-----STRIL[PPSVSSPSV]LLTNSN-----SF
ARF4 -INF-----GEETRKFDAQNEGGLPNNVTADLP-----FF
ARF13 -LLF-----GVDL-----
ARF14 -RLF-----GVSLATPSVIK-----DF
ARF23 -----
ARF15 -RLF-----GVSLATPPVIK-----DF
ARF20 -RLF-----GVSLAIPLVIK-----DF
ARF21 -RLF-----GVTLDTPPMIK-----DF
ARF12 -RLF-----GVTLDTPPMIK-----DF
ARF22 -RLF-----GVSLVTPSVIK-----DF
ARF9 -RLF-----GIDLMSSSLAVPEEKTPMRPINISKPTM-----DE
ARF11 -RLF-----GFDLTSKPASATIPHD-----KC
ARF18 -RLF-----GFDLTSNSPAP-----IF
ARF1 -RLF-----GFELVENNVNDECFSAASVSGAVAVD-----QE
ARF2 -RLF-----GIPLTNNMNGTDSMTSQRNNDLAAGLTQIAS-----PF
ARF5 -PLFHDVNDTQLNGLKFDQFSLMQQDLYASQNICMSNTSTSNILDPLSNTVLDQCAIF
ARF7 :SGFLNRSQ---SGPAILLPDAIDMSGNLVQDLYSKDMRLKQELVQOQ-----KSKAE
ARF19 *NFLSRNQQQQAASVSDSDFERASNPVQELYTKTESRI-SQGMNMMKSAGEHFRFKSF
ARF6 .QL-----GQSTNSNVPPNAVSLPFPGGRECSI-----EQ
ARF8 -NLL-----NFSITGQSVLPEQLTEGWSPKASNTFPELSLQA-----YI

```

## Motif 2

```

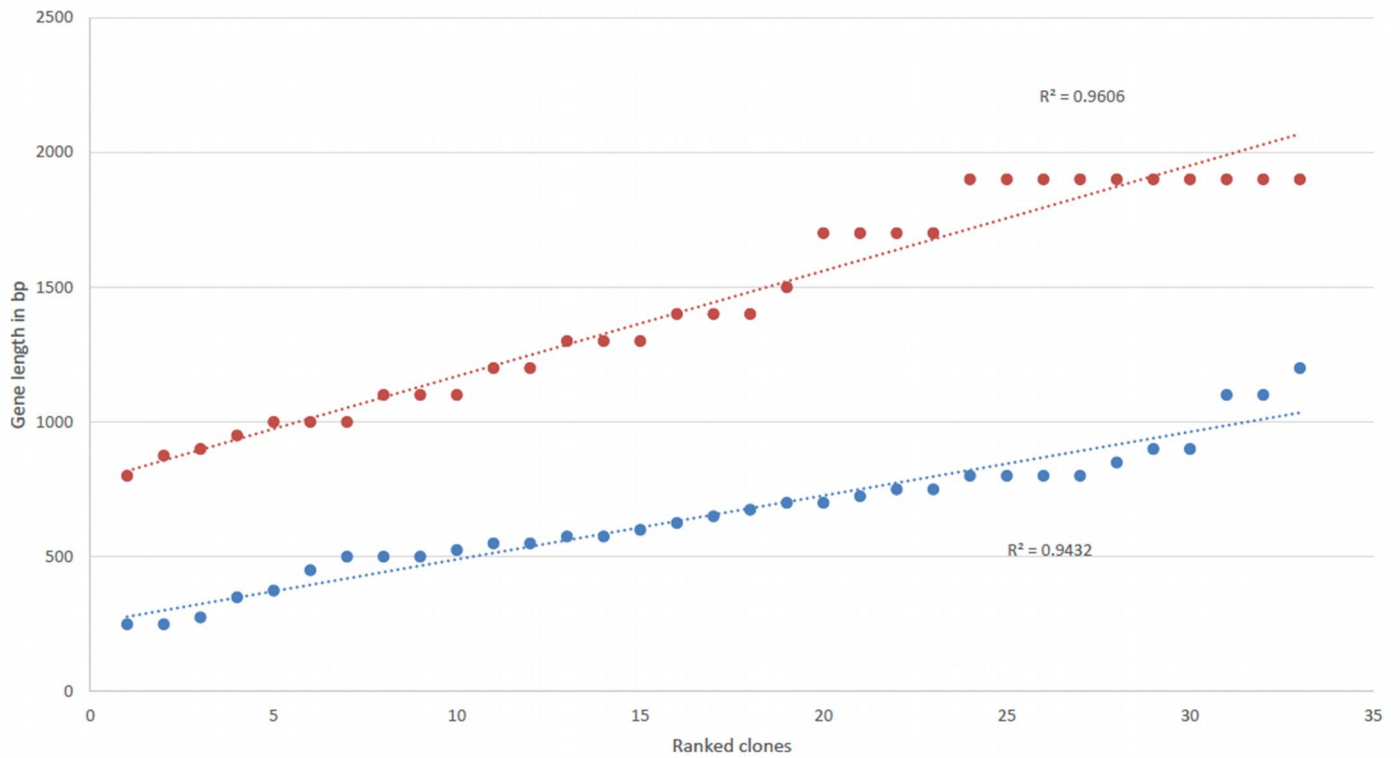
ARF10 -VARGLTWNY-----S
ARF16 --VAGREFSS-----SDEGSPCKKVVHD
ARF17 -NLSFNSNTT-----NLSGNDLVG
ARF3 -SG[R]CLFGF-----PLTDETTA
ARF4 ASSGCKLFGF-----SLPVETPASK
ARF13 -----
ARF14 -LTQEKKFGQ-----SILRSPTETQS
ARF23 -----
ARF15 -LTEGKKFGQ-----SOTLRSPTKIQS
ARF20 -LTEGKKFGQ-----SOTLRSPTETQS
ARF21 -LTERKKFGQ-----SOTLRSPTETQS
ARF12 -LTEGKKFGL-----SOTLRSPTETQN
ARF22 -LTEGKKFGQ-----SOTLRSPTETQS
ARF9 -VSEKKQEP-----AEGSPKEVQS
ARF11 -TTRKQDPNS-----SNSPKEQ
ARF18 -AAKQCEPIT-----PTSMKEQ
ARF1 -QSEPLNINQ-----SDIPSGSGDPEKSSLSRPOE--
ARF2 STNDHREOGR-----RFQTNPHPKDAQT
ARF5 QITSASFADSOAFS-----RQDFPDNSGGTSSSNVDFDCCSLRQNSKGSWQK
ARF7 QNFLAPTGLDGDNRNLLGGANVDNGFVPTDLLSRGYDSQKDLQNMLSNYGGVTNDIGT
ARF19 QTFFPLSPFG-DGDCQSHHPRNLLAFPGNLEAVTSDPLYQKDFQNLVFNYPNTPRIET
ARF6 GMSNLRISGI-----EGGDSTLFPFSSNFNDFSGNLAMTTPSSC
ARF8 -PQNPSLFGVDDPDSGLFELPTVRFASSSGDAEASPMSLTDSGFQNSLYSCMQDTTHELL

```

## Supplementary Figure 2.

Amino acid alignment of the 23 ARFs of *A. thaliana*

### ETT 5' medium and large sublibraries random colony PCR

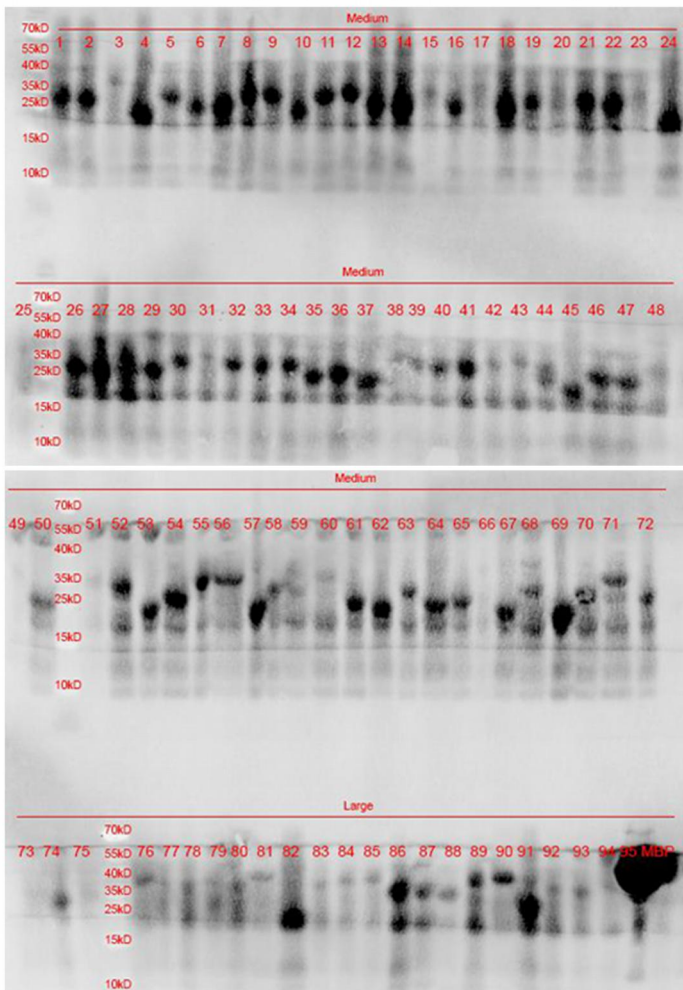


### Supplementary Figure 3.

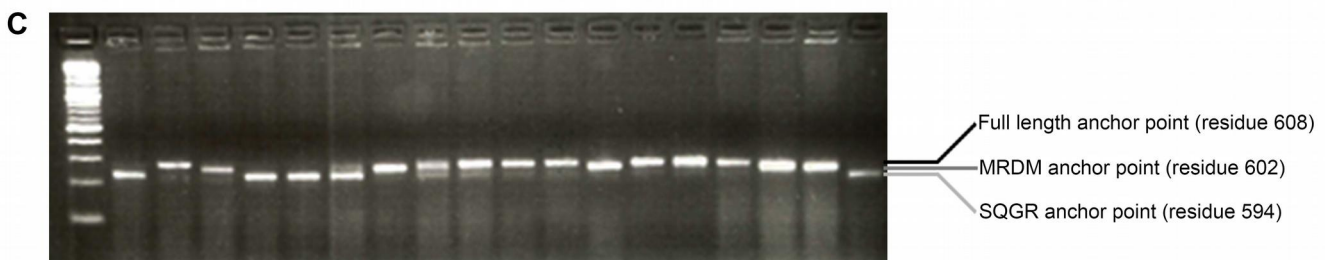
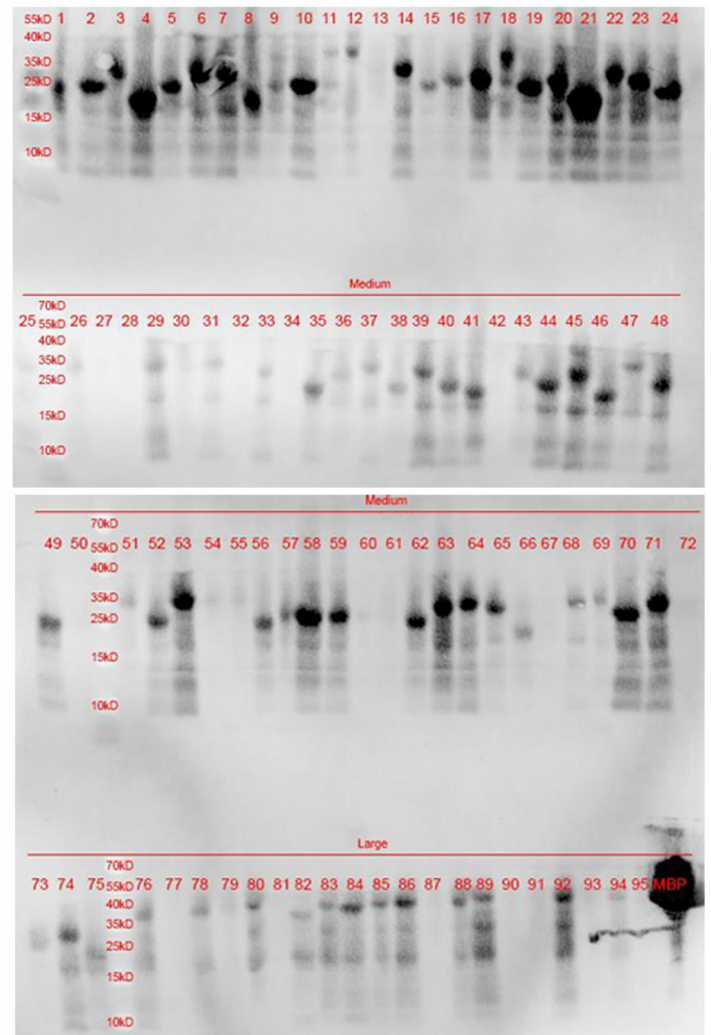
Ranked plot of *ETT* gene insert lengths (bp) by colony PCR of randomly selected clones from medium and large insert sublibraries showing even distribution of construct sizes in both. Correlation coefficients ( $R^2$ ) are shown for both sublibraries.



**A** WB BAP-Tag detection (-IAA condition)



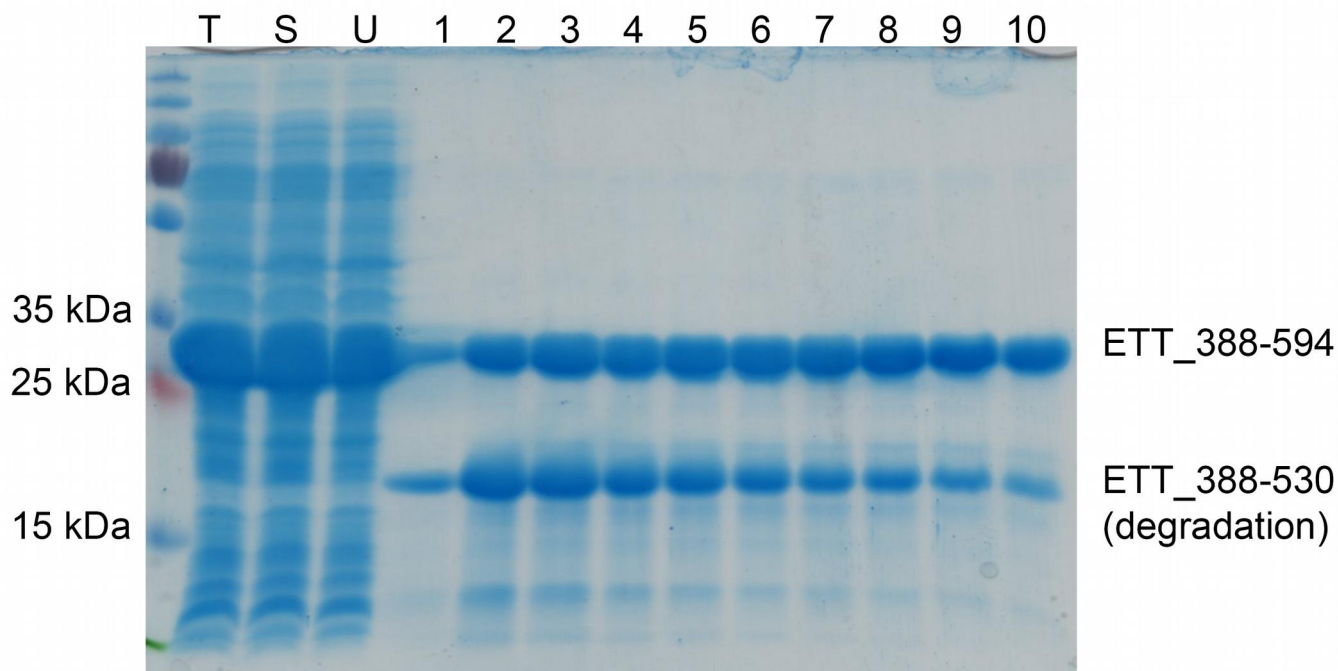
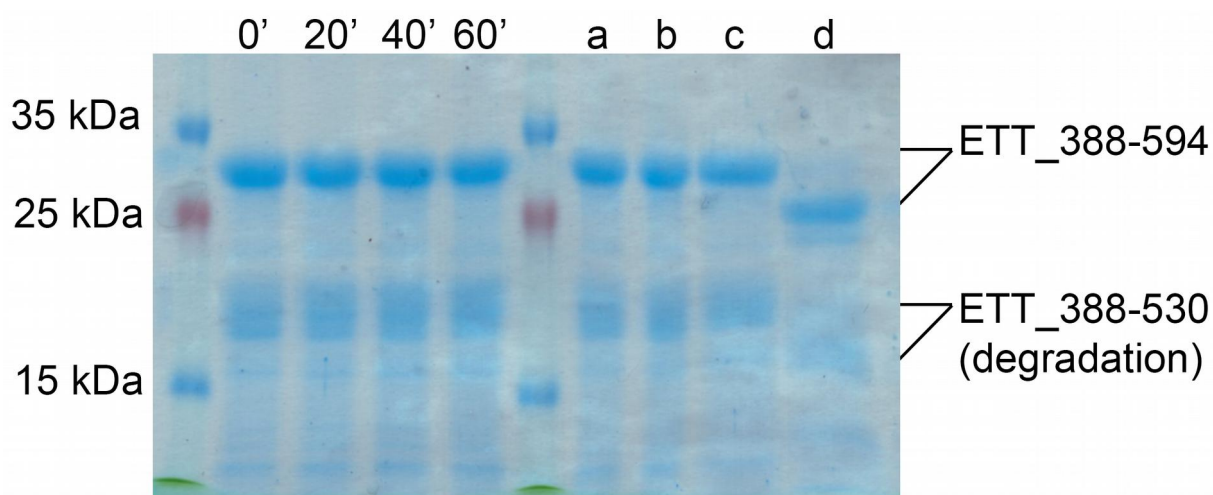
**B** WB BAP-Tag detection (+IAA condition)



**Supplementary Figure 4.**

(A-B) Fluorescent streptavidin blot against C-terminal biotin acceptor peptide following  $\text{Ni}^{2+}$ -NTA purifications from 4 mL small-scale cultures of the 95 selected clones from (A) Minus (-)IAA and (B) Plus (+)IAA conditions plus 6xHis-MBP-BAP control (lane 96). The first three gels show clones from the medium sub-library (DNA fragments from 250 to 1200 bp); the last gel from the large sub-library (DNA fragments from 800 to 1900 bp). Lanes correspond to constructs shown in Fig. 3 as follows: 442-594, gel +IAA, lane 4; 439-594, gel -IAA, lane 4; 416-594, gel -IAA, lane 54; 415-594, gel -IAA, lane 18; 413-594, gel -IAA, lane 27; 397-594, gel -IAA, lane 29; 396-594, gel -IAA, lane 26; 391-594, gel -IAA, lane 19; 388-594, gel -IAA, lane 8; 380-594, gel -IAA, lane 33; 373-594, gel -IAA, lane 9; 340-594, gel -IAA, lane 86.

(C) Agarose gel showing insert sizes by colony PCR on randomly selected clones from the library prior to screening confirming that all the three fixed 3' ends were present.

**A****B****Supplementary Figure 5.**

(A) SDS-PAGE analysis of Ni<sup>2+</sup>-NTA purified ETT\_388-594 with hexahistidine and biotin acceptor peptide tags from 2 L of bacterial culture. Elution fractions comprised a strong major species and a minor component of lower molecular weight. T; Total fraction, S: soluble fraction; U: unbound fraction during resin binding step; 1-10: 1 mL elutions. Fraction 10 was used for CD analysis and thermal stability assays.

(B) Left side of gel: Thermal stability assay of purified tagged ETT\_388-594 (fraction 10). Protein was heated at 95 °C for 0, 20, 40 and 60 minutes and the soluble fraction analysed after centrifugation. No change in solubility was observed. Right side of gel: lane a: ETT\_388-594 after 1 week at RT; lane b: sample from lane a after centrifugation (no precipitation observed); lane c: overnight mock digestion without TEV protease; d: overnight digestion with TEV protease.

GGAAGAGGGTGATGTTGATACAGATGG	ETT ES S523T rev
CCATCTGTATCATCAACACCCTCTTCCG	ETT ES S524T fw
CGGAAGAGGGTGTTGATGATACAGATGG	ETT ES S524T rev
ATCATCACCCACTTCCGTCTTGC	ETT ES S526T fw
GCAAGACGGAAGTGGGTGATGAT	ETT ES S526T rev
CACCCTCTACCGTCTTGCTTACCA	ETT ES S527T fw
TGGTAAGCAAGACGGTAGAGGGTG	ETT ES S527T rev
TCTAGAGGCCGAGGCGGCCGACATGCTAGAGAGCAATGTCTAGCAAC	REV on ETT for pGADT7 recombination
AAGCAGTGGTATCAACGCAGAGTGGCCATTATGGCCATGATCTCCAATTCAGGCAGCTTCG	FW ETT-ES domain for error-prone analyses pGADT7 rec
GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAACCATCTGGTTCCATCTCCAATTC	ETT ESPRIT clone fw gw
GGGGACCACTTTGTACAAGAAAGCTGGGTGCTACCTTCCTTGCGAATGATGATGATTGC	ETT ESPRIT clone rev gw (ends in SQGR)

**Supplementary Figure 6.**

Table of primers used in this study. Sequences are shown 5' to 3'.