RESEARCH ARTICLE SUMMARY

PROTEIN HOMEOSTASIS

Dimerization quality control ensures neuronal development and survival

Elijah L. Mena, Rachel A. S. Kjolby, Robert A. Saxton, Achim Werner, Brandon G. Lew, John M. Boyle, Richard Harland, Michael Rape*

INTRODUCTION: Protein complex formation is at the heart of all metazoan signal transduction networks. Facilitating cellular information flow, modular BTB domains, leucine zippers, or coiled coils have been reused in many proteins, where they often mediate crucial homodimerization events. While mutation of a single allele encoding homodimeric proteins might poison signaling complexes, aberrant heterodimerization between related modules can also inhibit or alter the output of signal transduction cascades. Whether cells detect and eliminate protein complexes of aberrant composition has remained unknown.

The globular BTB domain is found in ~220 human proteins that function as substrate adapters of CUL3 E3 ligases, transcription factors, or membrane channels. Proteins containing homodimeric BTB domains, such as KEAP1, KLHL3, KBTBD8, or BCL6, are essential for metazoan development, and their mutation or aberrant expression causes hypertension, cancer, or neurodegeneration. As it is not understood how organisms control the expression or activity of homodimeric BTB proteins, the BTB domain provides a physiologically important model to dissect the regulation of recurrent interaction modules.

RATIONALE: To identify regulatory mechanisms that impinge on modular interaction domains, we searched for shared binding partners of BTB proteins. Having found an E3 ligase that targets multiple BTB proteins for proteasomal degradation, we used biochemical reconstitution and protein complex engineering to dissect the underlying molecular control mechanism. Finally, we relied on *Xenopus laevis* embryos to study the organismal consequences of aberrant regulation of recurrent protein interaction modules.

RESULTS: Affinity purification and mass spectrometry experiments revealed that many BTB proteins heterodimerize, but also interacted with FBXL17, the substrate adapter of the SCF^{FBXL17} E3 ligase. SCF^{FBXL17} catalyzed the polyubiquitylation of BTB proteins to trigger their proteasomal degradation. SCF^{FBXL17} is therefore a rare example of an E3 ligase that targets a domain shared by many proteins, rather than a specific substrate.

As shown by biochemical reconstitution and affinity purification from cells and animals, SCF^{FBXL17} is a quality control enzyme that detects and ubiquitylates inactive BTB heterodimers, yet ignores active homodimers of the same domains. Accordingly, the loss of FBXL17 increased heterodimerization of BTB proteins, yet at the same time reduced the ability of BTB proteins to engage their downstream targets. SCF^{FBXL17} therefore ensures that only functional BTB dimers are present in cells, an activity that we refer to as dimerization quality control (DQC).

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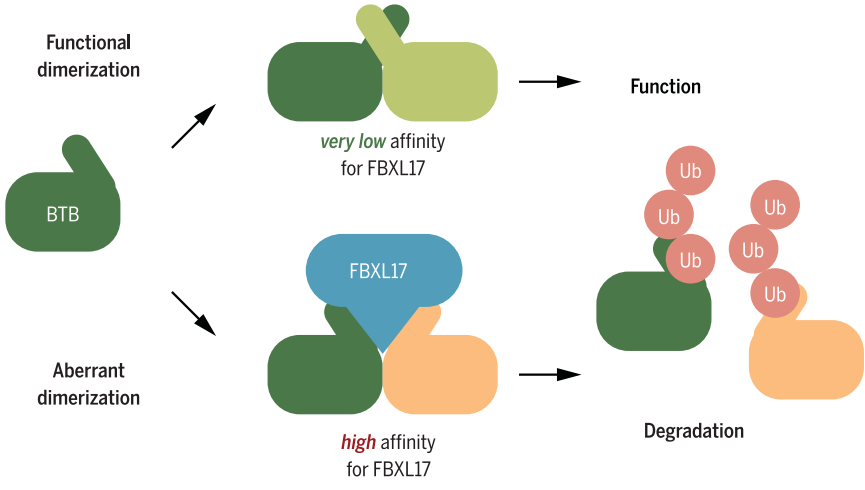
Depletion of FBXL17 in differentiating human embryonic stem cells showed that DQC prevented heterodimerization of KBTBD8, a BTB protein that is an essential regulator of neural crest specification. In line with this observation, the loss of DQC from *Xenopus laevis* embryos interfered with the differentiation, function, and survival of cells of the central and peripheral nervous system, including the neural crest. By contrast, somitogenesis or general body plan formation were initially unaffected. Similar to other quality control networks, the loss of DQC thus caused specific neuronal phenotypes. However, in addition to the known consequence of muted quality control, i.e. premature neuronal death, the effects of aberrant DQC were already observed early during differentiation.

CONCLUSION: We discovered DQC as a surveillance pathway that detects protein complexes of aberrant composition, rather than protein misfolding. We speculate that other recurrent interaction modules, such as leucine zippers or coiled coils, are monitored by similar DQC networks that rely on distinct E3 ligases. The neuronal phenotypes caused by DQC inactivation point to an active role of quality control in fate decisions in the nervous system. During evolution, DQC appeared at the same time as BTB domains multiplied in the vertebrate genome, suggesting that the ability to eliminate inactive heterodimers formed by related BTB domains contributed to the widespread use of this domain as a dimerization module. ■



Dimerization quality control eliminates inactive heterodimers of a recurrent interaction module but leaves functional homodimers intact. SCF^{FBXL17} selectively ubiquitylates inactive BTB dimers, such as BTB heterodimers or dimers containing mutant BTB domains, which triggers their proteasomal degradation. Functional BTB homodimers escape detection by SCF^{FBXL17}.

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RESEARCH ARTICLE

PROTEIN HOMEOSTASIS

Dimerization quality control ensures neuronal development and survival

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Aberrant complex formation by recurrent interaction modules, such as BTB domains, leucine zippers, or coiled coils, can disrupt signal transduction, yet whether cells detect and eliminate complexes of irregular composition is unknown. By searching for regulators of the BTB family, we discovered a quality control pathway that ensures functional dimerization [dimerization quality control (DQC)]. Key to this network is the E3 ligase SCF^{FBXL17}, which selectively binds and ubiquitylates BTB dimers of aberrant composition to trigger their clearance by proteasomal degradation. Underscoring the physiological importance of DQC, SCF^{FBXL17} is required for the differentiation, function, and survival of neural crest and neuronal cells. We conclude that metazoan organisms actively monitor BTB dimerization, and we predict that distinct E3 ligases similarly control complex formation by other recurrent domains.

As revealed by large-scale affinity-purification and mass spectrometry, human proteins associate on average with five partners (*1*), which frequently controls signal transduction by establishing the specificity of kinases, transcription factors, or E3 ligases (*2*, *3*). Modular domains, such as leucine zippers, coiled coils, or BTB domains, are found in functionally diverse proteins and often mediate dimerization. If homodimerization is required for function, mutation of a single allele can poison the complex and disrupt signaling, as seen with the tumor suppressor FBXW7 (*4*). Moreover, recurrent interaction modules may retain affinity for homologous domains in other proteins (*1*, *5*), which can result in heterooligomers with altered signaling output. Whether and how cells recognize aberrant dimers and prevent their formation is not known.

Providing a model to address this question, the BTB domain is a globular interaction fold that often mediates dimerization (*6*, *7*). The ~200 human BTB domain–containing proteins function as CUL3 E3 ligase subunits, transcription factors, or membrane channels (*8*–*12*). Members of this family, including the transcriptional regulators BCL6 and BACH1 or the E3 ligase subunits KLHL3 and KEAP1, have important roles in metazoan development (*13*–*18*), and their aberrant activity results in a wide range of diseases including hypertension, cancer, and neurodegeneration (*18*–*22*). Proteomic studies suggested that overexpressed BTB proteins heterodimerize with related family members (*1*, *5*), yet endogenous BTB proteins typically act as homodimers (*7*, *23*–*27*). These observations implied that cells prevent aberrant dimerization of BTB proteins, but the nature of this protective mechanism remains unknown.

SCF^{FBXL17} targets multiple BTB proteins

To identify regulators of the BTB family, we subjected 20 BTB proteins to heterologous expression, affinity purification, and mass spectrometry. As seen before (*1*, *5*, *25*), we found that several BTB proteins formed complexes with other members of this family (Fig. 1A), including KLHL12, KEAP1, and KBTBD8, which require homodimerization for function (*23*, *25*, *27*). By subjecting these immunoprecipitations to CompPASS analysis (*1*, *28*), we noticed that many BTB proteins bound the SCF E3 ligase adaptor F-box/LRR-repeat protein 17 (FBXL17) and the SCF core components SKP1 and CUL1, in line with recent high-throughput studies that independently pointed to potential interactions between FBXL17 and BTB proteins (*1*, *5*, *29*, *30*). Reciprocal immunoprecipitation of FBXL17 confirmed its association with BTB proteins, CUL1, and SKP1 (Fig. 1A). Notably, unsupervised clustering of these data revealed that FBXL17 preferentially recognized BTB proteins that engaged in heterodimerization (Fig. 1A).

To validate these interactions, we used CRISPR-Cas9 genome editing to append FLAG epitopes to all *FBXL17* alleles in 293T cells and analyzed FBXL17^{3xFLAG} immunoprecipitates by mass spectrometry or Western blotting. These experiments confirmed that endogenous FBXL17 interacted with many BTB proteins, as well as with CUL1 and SKP1 (Fig. 1B and fig. S1A). Expression of a dominant negative mutant of CUL1, which prevents ubiquitylation of SCF targets, stabilized the interaction between FBXL17 and BTB proteins (Fig. 1C and fig. S1B). By contrast, mutation of a conserved Cys residue of FBXL17 (FBXL17^{C627R}), a variant that was present in the COSMIC database of somatic breast cancer mutations, severely impaired the ability of FBXL17 to bind BTB proteins, but not SKP1 (Fig. 1C and fig. S1B). We conclude that SCF^{FBXL17} specifically binds and thus potentially regulates many BTB domain–containing proteins.

Overexpression of FBXL17 reduced the levels of many BTB proteins, which was rescued by dominant negative CUL1, proteasome inhibition, or mutation of Cys^{627} in FBXL17 (Fig. 1, C and E, and fig. S1C). Increased expression of FBXL17 also reduced the abundance of BTB proteins in complexes with endogenous CUL3 (fig. S1D), which together implied that SCF^{FBXL17} can ubiquitylate BTB proteins to trigger their proteasome-dependent degradation. Consistent with this, overexpressed FBXL17 ubiquitylated the BTB protein KLHL12, as shown by denaturing His-Ub (ubiquitin) pull-down experiments (fig. S1E), while depletion of FBXL17 or expression of dominant negative CUL1 reduced KLHL12 modification (Fig. 1D and fig. S1E). Moreover, deletion of *FBXL17* increased the stability of BTB proteins in cycloheximide chase experiments (Fig. 1F and fig. S2A), without strongly affecting mRNA levels (fig. S2B), and these effects were rescued by introduction of wild-type FBXL17, but not FBXL17^{C627R} (Fig. 1E and fig. S2C). However, overexpression of FBXL17 induced degradation of endogenous BTB proteins with a marked delay (fig. S2D), and deletion of *FBXL17* did not strongly affect the abundance of most endogenous BTB proteins (Fig. 1F). Thus, although SCF^{FBXL17} can induce the degradation of BTB proteins when overexpressed, it likely targets a specific subset of BTB proteins at the endogenous level.

SCF^{FBXL17} is a quality control enzyme for dimeric BTB complexes

To understand how SCF^{FBXL17} selects its substrates, we reconstituted the ubiquitylation of the BTB protein KLHL12 in vitro. After experiments using recombinant KLHL12 failed to produce modification by SCF^{FBXL17}, even if lysate was provided to modify substrate or enzyme, we asked whether SCF^{FBXL17} functions as a quality control enzyme to eliminate defective BTB proteins. As many quality control enzymes detect protein conformations that are transiently populated during folding (*31*), we tested whether SCF^{FBXL17} could recognize nascent BTB proteins that still expose surfaces buried in the mature state. Notably, if SCF^{FBXL17} was present as KLHL12 was being translated, it readily associated with KLHL12 and triggered the ubiquitylation and degradation of this substrate (Fig. 2, A to C). SCF^{FBXL17} also targeted nascent KLHL12 still bound to tRNA, owing to deletion of the stop codon (fig. S3, A to C). By contrast, if FBXL17 was added after synthesis of KLHL12 had been completed, substrate recognition was strongly impaired (Fig. 2, A to C), and even a large excess of purified BTB proteins failed to compete with recognition of nascent KLHL12 by SCF^{FBXL17} (fig. S3D). Time-of-addition experiments showed that wild-type KLHL12 was accessible

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to FBXL17 only during a very brief time window during its synthesis, and KLHL12 ubiquitylation was prevented almost immediately after it became competent to bind CUL3 (fig. S3, E and F). SCF^{FBXL17} recognized the same BTB proteins in vitro that were substrates in cells (fig. S3G) and showed the strong preference for K48-linked ubiquitin chains that is expected of SCF ligases triggering proteasomal degradation (fig. S3, H and I). Thus, SCF^{FBXL17} recognizes wild-type BTB proteins only if these are incompletely folded, suggesting that it is a quality control enzyme specializing on BTB-domain containing proteins.

To identify which aspect of BTB structure or function is monitored, we searched for the substrate motif, or degron, recognized by SCF^{FBXL17}. After domain swap experiments showed that the BTB domain was required and sufficient to impose SCF^{FBXL17}-dependent degradation (fig. S4, A and B), we mutated ~50 residues in the BTB domain of KLHL12 and asked whether the resulting variants were protected from FBXL17 in cells. We found that His^{15}, Asp^{34}, and Ala^{60}, whose mutation in KLHL3 causes hypertension (*22*), were required for degradation of KLHL12 by FBXL17 (Fig. 2D and fig. S4C). The residue corresponding to Ala^{60}, Ser^{59}, was important for the SCF^{FBXL17}-dependent degradation of a BTB domain transcription factor, BCL6 (fig. S4D), while reintroduction of Ala^{60} converted the nontargeted

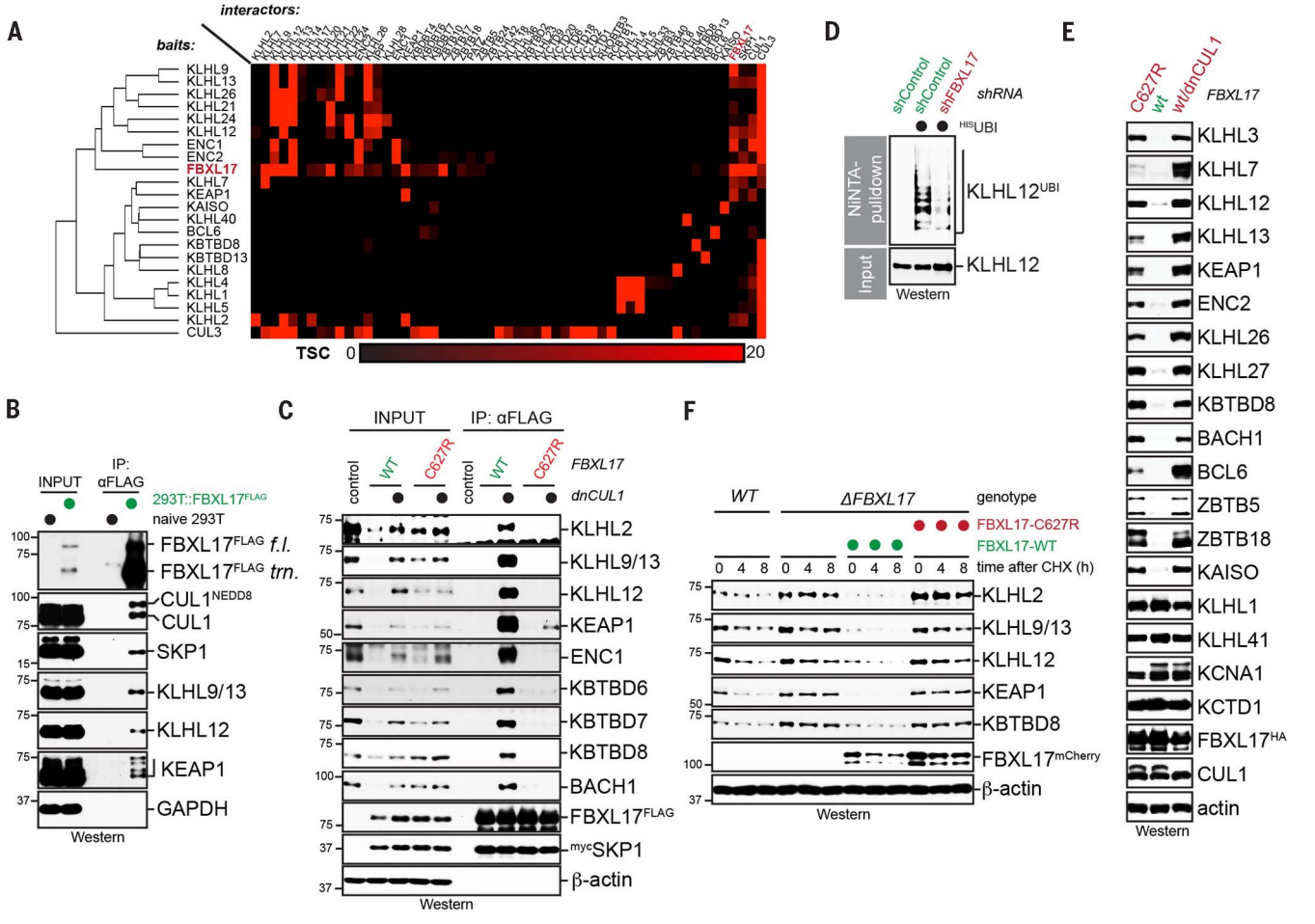

Fig. 1. SCF^{FBXL17} controls the stability and abundance of BTB proteins. (**A**) Proteomic analysis of binding partners of 20 BTB domain–containing proteins reveals FBXL17 as a common interactor. Twenty FLAG-tagged BTB proteins were affinity-purified from 293T cells and analyzed for binding partners by mass spectrometry. BTB proteins, CUL3, and components of SCF^{FBXL17} are shown. Shading corresponds to the abundance (total spectral counts, TSC) of interacting proteins. The reciprocal immunoprecipitation of $FBXL17^{FLAG}$ was from cells also expressing dominant-negative CUL1 to prevent substrate degradation. Bait proteins were ordered by unsupervised clustering. (**B**) Endogenous FBXL17 interacts with many BTB proteins. *FBXL17* loci were tagged in 293T cells with a 3xFLAG epitope by CRISPR-Cas9–dependent genome editing, and endogenous $FBXL17^{3xFLAG}$ was affinity-purified and analyzed by Western blotting for endogenous BTB proteins or components of the SCF machinery. (**C**) SCF^{FBXL17} specifically binds and regulates endogenous BTB proteins. Cells were transfected with $FBXL17^{FLAG}$, $FBXL17^{C627R/FLAG}$, and ^{6myc}SKP1. When shown, dominant negative CUL1 (dnCUL1) was coexpressed. FBXL17 variants were affinity-purified and analyzed for binding to endogenous BTB proteins by Western blotting. (**D**) FBXL17 promotes ubiquitylation of the BTB protein KLHL12 in cells. Cells stably transduced with control short hairpin RNAs (shRNAs) or shRNAs against FBXL17 were transfected with ^{HIS}ubiquitin and treated with proteasome inhibitor MG132. Ubiquitylated endogenous proteins were purified under denaturing conditions and analyzed for modified KLHL12 by Western blotting. (**E**) SCF^{FBXL17} induces the degradation of many, but not all, BTB proteins. Cells were transfected with distinct FLAG-tagged BTB proteins, $FBXL17^{HA}$ or $FBXL17^{C627R/HA}$, and ^{6myc}SKP1. As indicated, dominant negative CUL1 was coexpressed. The abundance of BTB proteins was determined by αFLAG Western blotting. Representative FBXL17, CUL1, and β-actin blots are shown. (**F**) SCF^{FBXL17} controls the stability of BTB proteins. 293T cells lacking functional FBXL17 were engineered by CRISPR-Cas9–dependent genome editing, and cell lines were verified after clonal selection by PCR. As indicated, cells were produced to stably express doxycycline-inducible FBXL17 or $FBXL17^{C627R}$. After 12 hours of induction, cycloheximide was added to measure the stability of endogenous proteins, using Western blotting and specific antibodies.

KLHL41 into an SCF^{FBXL17} substrate (Fig. 2E). In line with these results, mutation of Ala^{60} in KLHL12 or Ser^{59} in BCL6 interfered with the ubiquitylation or degradation of these SCF^{FBXL17} substrates in vitro (Fig. 2, A to C, and fig. S4E). The degron residues were conserved in most of 24 BTB proteins that were turned over by SCF^{FBXL17}, and at least one mutation was present in 20 out of 21 BTB proteins that were resistant to SCF^{FBXL17} (Fig. 2F).

When mapped onto the structure of a BTB domain, all SCF^{FBXL17} degron residues were in close proximity to the dimer interface, without involving residues required for dimerization (Fig. 2G, upper panel). Whereas Ala60 is surface exposed, His^{15} and Asp^{34} are buried in mature BTB dimers resistant to FBXL17 (Fig. 2G, lower panel). Thus, FBXL17 either targets monomeric BTB proteins, or it binds immature dimers that still present His^{15} and Asp^{34} on their surface.

To distinguish between these possibilities, we mutated KLHL12 to prevent BTB dimerization (*25*) and found that monomeric KLHL12 was severely impaired in binding to and receiving ubiquitin chains by SCF^{FBXL17} (fig. S5, A and B). An excess of FBXL17 did not destabilize BTB dimers in vitro, which implied that FBXL17 does not interact with BTB monomers (fig. S5, C and D), and FBXL17 strongly bound KLHL12 dimers in cells (fig. S5E). Together, these findings indicated that SCF^{FBXL17} targets dimeric BTB proteins that have not yet matured into their final conformation.

SCF^{FBXL17} specifically targets inactive BTB dimers

To assess whether endogenous SCF^{FBXL17} recognizes nascent BTB dimers in vivo, we exposed cells to the mRNA translation inhibitor cycloheximide and determined binding partners of endogenous FBXL17 by mass spectrometry. FBXL17 interacted with the same BTB proteins if mRNA translation had been prevented (fig. S6A), showing that without overexpression, SCF^{FBXL17} engages most substrates posttranslationally. Other quality control enzymes, such as HSP70, similarly bind nascent wild-type clients cotranslationally, but posttranslationally detect proteins destabilized by mutations or stress (*32*). To test whether this was the case for SCF^{FBXL17}, we assessed whether unfolding or mutation of the BTB domain induced recognition by FBXL17. Whereas unfolding by heat, a condition that eliminates any structural information, did not enable BTB recognition (fig. S6B), specific mutations in the BTB domains of KLHL12 or KBTBD8 allowed for posttranslational binding and ubiquitylation by SCF^{FBXL17} (Fig. 3, A and B, and fig. S6, C and D). Time-of-addition experiments confirmed that these mutants remained accessible to SCF^{FBXL17}

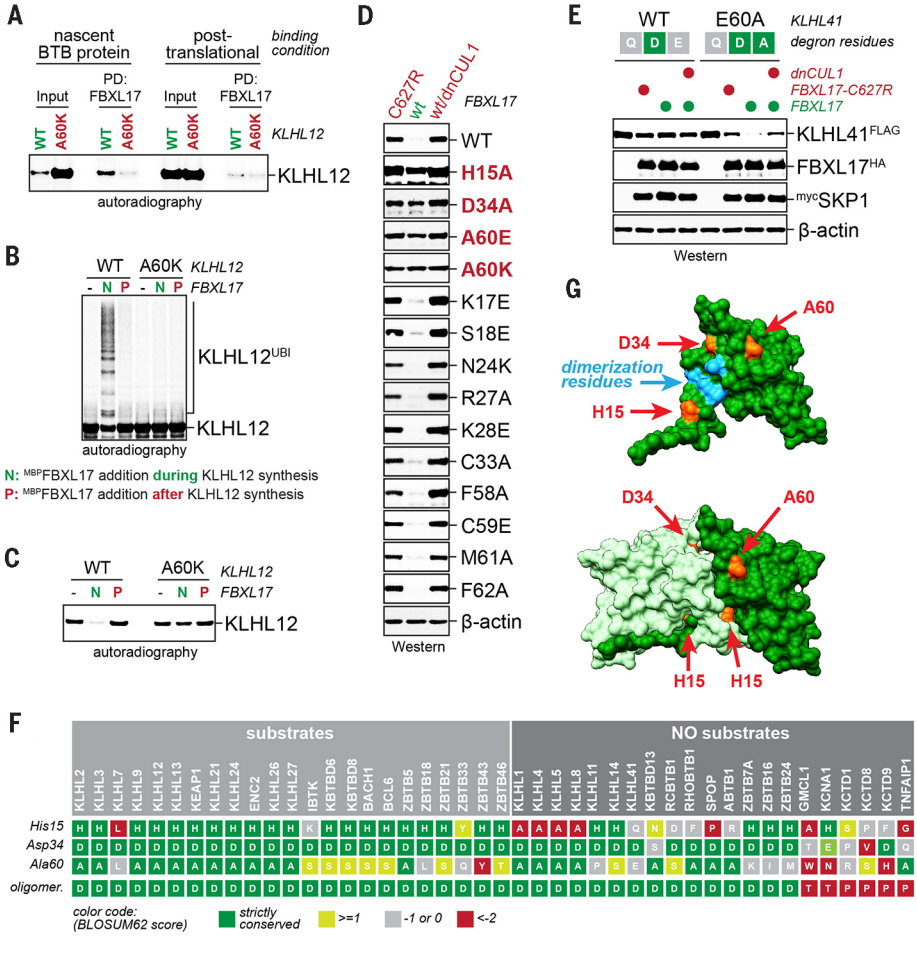

Fig. 2. SCF^{FBXL17} recognizes a conserved degron at the BTB dimer interface. (**A**) FBXL17 recognizes nascent wild-type BTB proteins. Recombinant ^{MBP}FBXL17 was added either during ("N"; nascent BTB protein binding) or after ("P"; posttranslational interaction) the in vitro synthesis of ^{35}S-labeled KLHL12 or KLHL12^{A60K}, immobilized, and analyzed for bound KLHL12 proteins by autoradiography. The input levels of KLHL12 or KLHL12^{A60K} are shown on the left. (**B**) SCF^{FBXL17} ubiquitylates KLHL12, but not KLHL12^{A60K}, cotranslationally in vitro. Recombinant FBXL17-SKP1 was added either during ("N") or after ("P") in vitro synthesis of KLHL12 or KLHL12^{A60K}. Ubiquitylated higher molecular weight KLHL12 species were detected after SDS gel electrophoresis by autoradiography. (**C**) FBXL17 induces cotranslational degradation of wild-type BTB proteins in vitro. ^{35}S-labeled KLHL12 or KLHL12^{A60K} were incubated either during ("N") or after ("P") in vitro synthesis with recombinant FBXL17 in rabbit reticulocyte lysate, which contains proteasomes. KLHL12 levels were analyzed by autoradiography. (**D**) Identification of an SCF^{FBXL17} degron in cells. The indicated KLHL12^{FLAG} variants were expressed in 293T cells together with either FBXL17^{HA} or FBXL17$^{C627R/HA}$. Dominant negative CUL1 (dnCUL1) was coexpressed as indicated. The levels of KLHL12 variants were analyzed by αFLAG-Western. (**E**) Key degron residues are required and sufficient for SCF^{FBXL17}-dependent degradation. KLHL41 or KLHL41^{E60A} were expressed in 293T cells with FBXL17, FBXL17^{C627R}, or FBXL17 and dnCUL1, as indicated. KLHL41 levels were analyzed by Western blotting. (**F**) Key degron residues are conserved in SCF^{FBXL17} targets, but not in BTB proteins that are resistant to FBXL17. Moreover, all FBXL17 targets are dimeric ("D") BTB proteins, whereas tetra- ("T") or pentameric ("P") family members are not recognized. The indicated BTB proteins were expressed in cells together with FBXL17, FBXL17^{C627R}, or FBXL17 and dnCUL1 and analyzed for FBXL17-dependent degradation by Western blotting. Substrates are marked with a green box, whereas BTB proteins resistant to FBXL17 degradation are marked with a red box. The conservation of key degron residues is shown, with the coloring of mutations according to BLOSUM62 matrix scores. (**G**) The SCF^{FBXL17} degron residues are in direct proximity to the dimerization interface and become buried upon completion of BTB dimer formation. Upper panel: Residues whose mutation caused significant stabilization of KLHL12 (red) were mapped onto the crystal structure of a KEAP1 BTB monomer (green; derived from Protein Data Bank: 4CXI). Residues required for dimerization (*25*) are shown in blue. Lower panel: Degron residues H15 and D34 (red) are buried in the folded KEAP1 BTB dimer (light and dark green). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; F, Phe; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

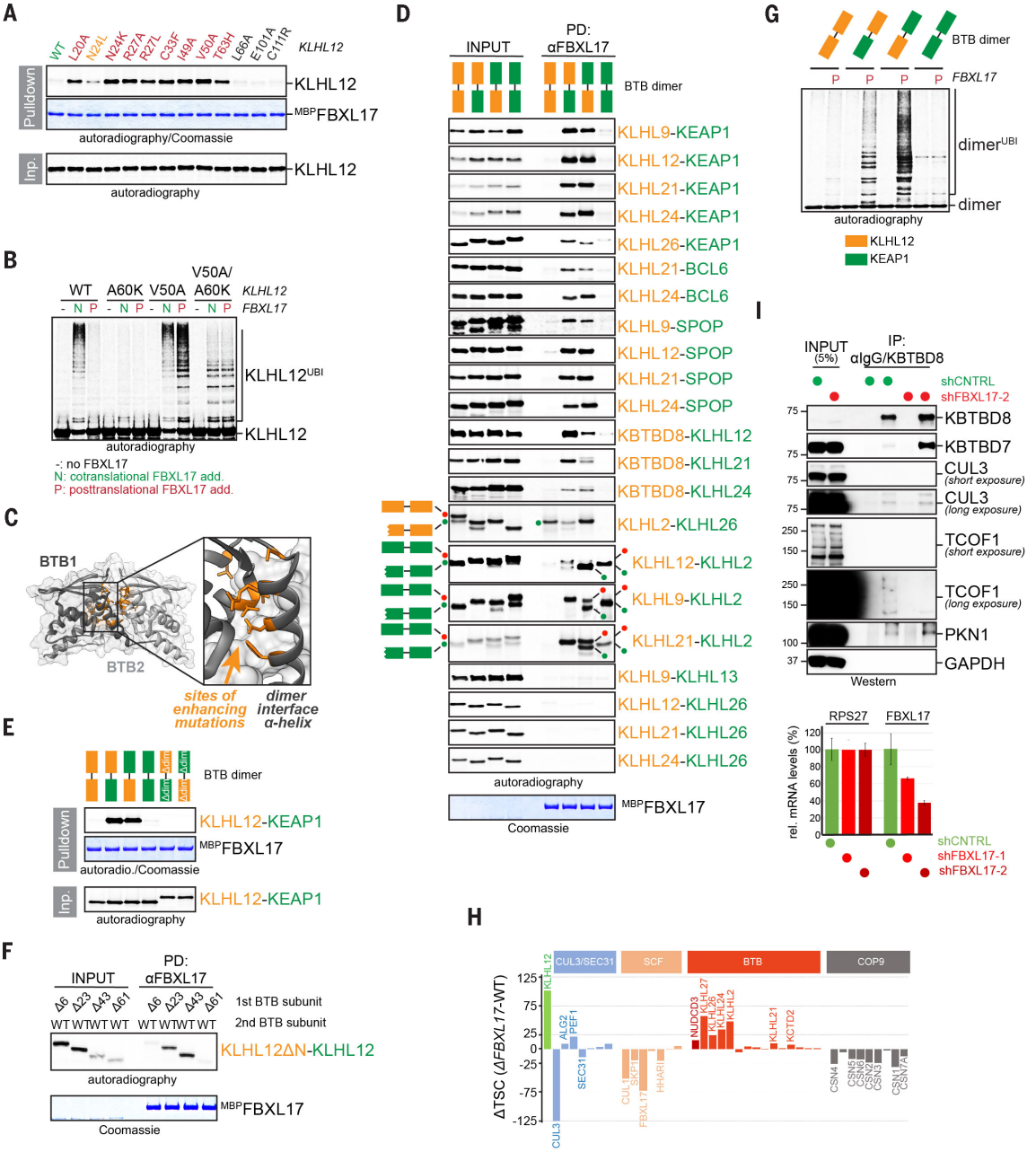

Fig. 3. SCF^{FBXL17} preferentially recognizes aberrant BTB dimers. (**A**) BTB mutations near the dimerization interface enhance substrate recognition by SCF^{FBXL17}. Variants of ^{35}S-labeled KLHL12 were tested for posttranslational binding to immobilized ^{MBP}FBXL17/SKP1 complexes. Binding was detected by autoradiography. (**B**) BTB mutations allow for posttranslational ubiquitylation by SCF^{FBXL17}. Recombinant FBXL17/SKP1 complexes were added either during ("N") or after ("P") synthesis of the indicated KLHL12 variants, and ubiquitylation was detected by SDS gel electrophoresis and autoradiography. (**C**) Structural representation of BTB mutations that allow for posttranslational substrate recognition by SCF^{FBXL17}. Sites of enhancing mutations (orange) were mapped onto the KEAP1-BTB homodimer (light and dark gray). The inset demonstrates that all enhancing mutations map to a central α helix at the interface of two BTB domains; note that an orange residue in the background binds the same α helix of the opposing BTB subunit. (**D**) FBXL17 preferentially binds BTB heterodimers. BTB domain fusions indicated on the right were produced as ^{35}S-labeled proteins and tested for posttranslational binding to ^{MBP}FBXL17-SKP1. Binding was monitored by SDS gel electrophoresis and autoradiography. The green dots mark heterodimers containing a truncated BTB domain of KLHL2 that lacks an amino-terminal region making stabilizing contacts with the opposing BTB subunit; the red dots mark the heterodimers with only full-length KLHL2 BTB domains. (**E**) Formation of a dimer interface between BTB domains is required for substrate binding by SCF^{FBXL17}. Mutations in residues required for BTB dimerization were introduced into heterodimers composed of KEAP1 and KLHL12 BTB domains, as indicated (Δdim). ^{35}S-labeled fusions were then tested for posttranslational binding to ^{MBP}FBXL17/SKP1, as described above. (**F**) Amino-terminal truncations that remove residues required for stabilization of the BTB dimer interface induce posttranslational recognition by SCF^{FBXL17}. Amino-terminal truncations were introduced into the first BTB domain of KLHL12-KLHL12 homodimers, as indicated. Posttranslational binding to ^{MBP}FBXL17-SKP1 was then analyzed as described above. (**G**) SCF^{FBXL17} preferentially ubiquitylates BTB heterodimers. Different combinations of the BTB domains of KLHL12 and KEAP1 were used to produce the forced dimers indicated above. BTB dimers were synthesized as ^{35}S-labeled proteins in vitro and incubated with recombinant FBXL17-SKP1 in reticulocyte lysate. Ubiquitylation was monitored by SDS gel electrophoresis and autoradiography. (**H**) Deletion of *FBXL17* stabilizes BTB heterodimers but reduces binding to substrate. All *KLHL12* alleles in 293T wild-type (WT) or Δ*FBXL17* cells were fused to FLAG-epitopes using CRISPR-Cas9-dependent genome editing. Endogenous KLHL12^{FLAG} was affinity-purified from both cell types and analyzed for binding partners by CompPASS mass spectrometry. The difference in total spectral counts (TSC) between Δ*FBXL17* and WT cells is shown. (**I**) Depletion of FBXL17 from differentiating hESCs induces heterodimer formation of KBTBD8. hESCs were stably transfected with control shRNAs or shRNAs depleting FBXL17. Cells were induced to undergo neural conversion, and at the time of neural crest specification (NC1), endogenous KBTBD8 was affinity-purified. Bound proteins were analyzed by Western blotting using specific antibodies. The efficiency of FBXL17 depletion was analyzed by quantitative reverse transcription (qRT)–PCR (below); RPS27 levels were tested as specificity control.

long after their synthesis had been completed (fig. S3, E and F). Although conservative mutations enhanced recognition by SCF^{FBXL17}, drastic substitutions that likely interfere with BTB folding, or unfolding by heat, obliterated this effect (fig. S6, B to D). Recognition and ubiquitylation of mutant BTB proteins was specific and relied on the SCF^{FBXL17} degron identified above (Fig. 3B and fig. S6E), and as shown for $KLHL12^{V50A}$, also resulted in more efficient detection by FBXL17 in cells (fig. S6F).

All mutations that enabled posttranslational recognition by SCF^{FBXL17} clustered around an α helix at the BTB dimer interface (Fig. 3C). As shown for $KLHL12^{V50A}$, these mutants formed stable dimers that did not noticeably dissociate within 24 hours (fig. S5, C and D), yet were strongly impaired in binding to substrate, i.e., SEC31 (fig. S6, G and H). Thus, SCF^{FBXL17} binds preferentially to BTB proteins that form inactive heterodimers (Fig. 1A), and conversely, mutations that inactivate BTB dimers promote posttranslational recognition by SCF^{FBXL17} (fig. S6, G and H). Together, these observations suggested that SCF^{FBXL17} might detect and eliminate aberrant BTB dimers, such as heterodimers, yet spare functional homodimers.

To test this hypothesis, we developed a system to control the composition of BTB dimers. Guided by structural analyses (*7*), we used a short linker to fuse the carboxy terminus of one BTB domain to the amino terminus of a second so that the high local BTB concentration competes off BTB proteins in trans and thus establishes defined BTB dimers. A fused homodimer of KLHL12 BTB domains was co-translationally recognized by SCF^{FBXL17} with the same efficiency as BTB domains that dimerize in trans (fig. S7A). Mutation of the degron residues Asp^{34} or Ala^{60} in one position of the fused dimer reduced binding by SCF^{FBXL17}, whereas loss of both degrons abolished its recognition (fig. S7, A and B). Notably, mutations that prevented dimerization of unfused BTBs also disrupted the recognition of fused BTB domains (fig. S5A), showing that even in the context of covalently linked BTB domains, formation of a dimer interface is required for substrate binding by SCF^{FBXL17}.

Having established this platform, we generated 55 distinct homo- or heterodimers of BTB domains and tested for posttranslational recognition by FBXL17. Notably, although FBXL17 readily bound many heterodimers composed of distinct BTB domains, it did not readily detect any homodimer (Fig. 3D). As revealed by mutation of dimerization residues in KEAP1-KLHL12 BTB heterodimers, formation of a dimer

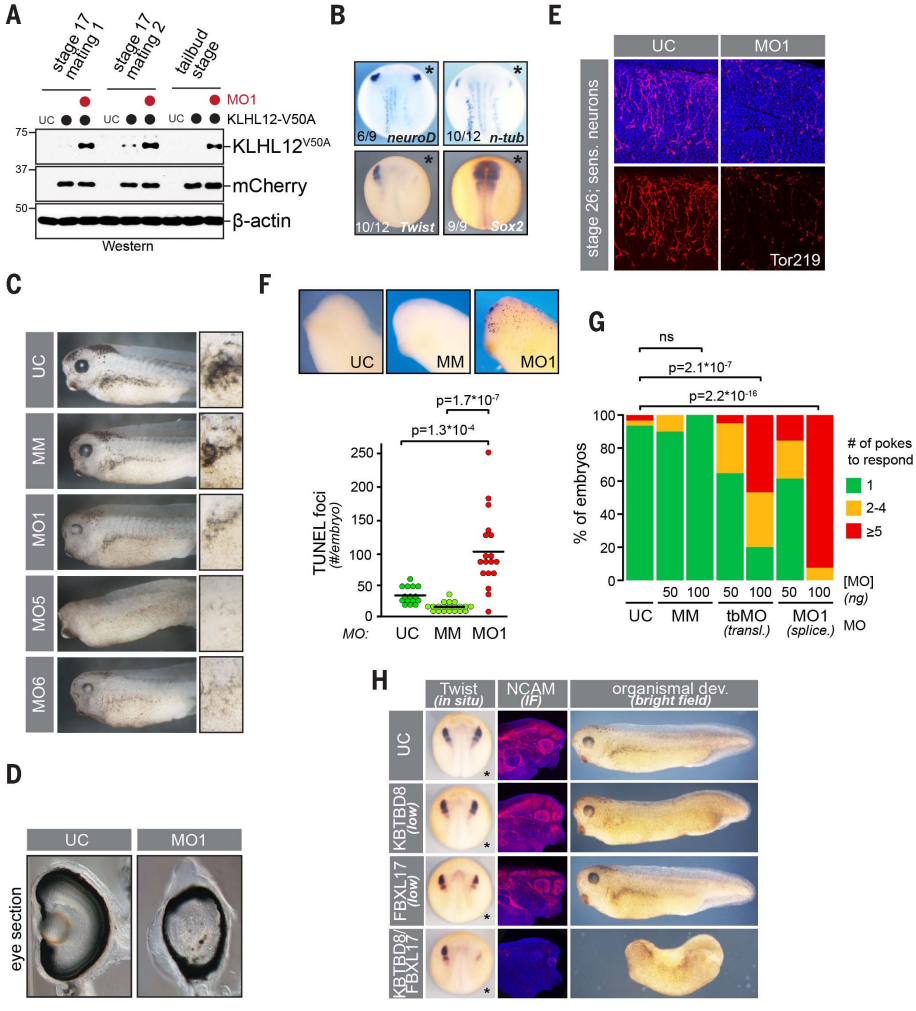

Fig. 4. FBXL17 is required for neuronal differentiation, survival, and function. (**A**) SCF^{FBXL17} functions in intact *X. laevis* embryos. Embryos derived from independent matings were injected with mRNA encoding $KLHL12^{V50A/3xFLAG}$, mCherry, and as indicated, morpholino (MO) 1 against *FBXL17*. Embryos were allowed to develop to stage 17 or the tailbud stage, lysed as pools of five embryos, and analyzed for levels of $KLHL12^{V50A}$ by Western blotting. UC: uninjected control. (**B**) Two-cell-stage *X. laevis* embryos were injected with morpholinos targeting FBXL17 (MO1: *n-tub; neuroD; Sox2*; MO5: *Twist*) on the side marked with an asterisk and allowed to develop to stage 18. In situ hybridization was used to monitor expression of developmental markers. (**C**) Depletion of FBXL17 impairs melanocyte differentiation. *X. laevis* embryos were injected with a mismatch control (MM) or three different morpholinos targeting FBXL17 and allowed to develop to the tailbud stage. Insets show the same embryonic area that is rich in melanocytes in uninjected (UC) or MM-injected embryos, but reduced in FBXL17-depleted embryos. (**D**) FBXL17 depletion impairs eye development. *X. laevis* embryos were uninjected (UC) or injected with MO1 targeting FBXL17, and eyes were sectioned and imaged. (**E**) FBXL17 is essential for neuronal development. Uninjected (UC) *X. laevis* embryos or those injected with MO1 against FBXL17 were stained for mechanosensory neurons with Tor219 antibodies [blue: DAPI (4′,6-diamidino-2-phenylindole); red: Tor219]. (**F**) Depletion of FBXL17 triggers apoptosis in heads of embryos. Apoptotic cells were detected by TUNEL staining in embryos left uninjected (UC) or those injected with either a mismatch (MM) control morpholino or an FBXL17-targeting morpholino (MO1). (**G**) FBXL17 is required for the escape response in *X. laevis* embryos. Embryos were left uninjected (UC) or were injected with a mismatch control morpholino (MM) or with two different morpholinos that either prevent translation (tbMO) or splicing (MO1) of FBXL17. Embryos were poked with a thin pipette tip to elicit a movement described as the escape response. Shown is the number of pokes required to elicit a response. Typically, 15 to 30 embryos were injected and measured per condition. (**H**) FBXL17 maintains KBTBD8 activity in developing *X. laevis* embryos. Morpholinos against FBXL17 and KBTBD8 were titrated so that only minor effects on neural crest development were observed, before both morpholinos were combined to test for synergistic effects of FBXL17 and KBTBD8 co-depletion. As seen by in situ hybridization against Twist, immunofluorescence analysis against NCAM, and bright-field microscopy of whole embryos, co-depletion of FBXL17 and KBTBD8 reveals their strong genetic interaction during neural crest development.

interface was essential for posttranslational heterodimer recognition by SCF^{FBXL17} (Fig. 3E). A single SCF^{FBXL17} degron was sufficient to mediate heterodimer recognition: Even though the BTB domain of SPOP lacks a key degron residue and is not recognized by itself (Fig. 2F), it induces binding by SCF^{FBXL17} when paired with BTB domains of KLHL9, KLHL12, KLHL21, or KLHL24 (Fig. 3D). SCF^{FBXL17} also detected heterodimers formed by distinct variants of the same BTB domain: In fusions of KLHL2 BTB domains, FBXL17 bound a truncated $KLHL2^{\Delta N}$-KLHL2 heterodimer, but not the more abundant full-length homodimer present in the same reaction (Fig. 3D); this specificity could be recapitulated by heterodimers between truncated and full-length BTB domains of a different protein, KLHL12 (Fig. 3F). Although SCF^{FBXL17} efficiently detects inactive BTB complexes, we found that it ignores functional heterodimers: Consistent with KLHL9 and KLHL13 binding each other to control mitosis (*33*), heterodimers composed of the KLHL9 and KLHL13 BTB domains escaped detection by SCF^{FBXL17} (Fig. 3D). We also noticed that KLHL26, which is present in many BTB affinity purifications (Fig. 1A), frequently formed heterodimers that were not recognized by FBXL17 (Fig. 3D), and we speculate that KLHL26 might establish BTB heterodimers with cellular activity. Consistent with these binding studies, SCF^{FBXL17} ubiquitylated BTB heterodimers, but not the respective homodimers (Fig. 3G), which required the SCF^{FBXL17} degron in at least one subunit (fig. S7, C and D). Together, these findings revealed that SCF^{FBXL17} indeed detects and ubiquitylates aberrant BTB dimers but ignores their functional counterparts.

To test whether SCF^{FBXL17} functions similarly in vivo, we used mass spectrometry to probe the dimerization status of FLAG-tagged endogenous KLHL12, an SCF^{FBXL17} substrate that needs to homodimerize to bind its substrate (*25*). Notably, *FBXL17* deletion increased the abundance of KLHL12 in heterodimers with KLHL27, KLHL26, KLHL24, and KLHL2 (Fig. 3H). At the same time, loss of FBXL17 reduced binding of KLHL12 to its substrate SEC31, suggesting that lack of SCF^{FBXL17} activity decreased overall KLHL12 activity. We made similar observations by affinity purification of endogenous KBTBD8, which formed heterodimers with KBTBD7 and ZBTB32 if *FBXL17* had been deleted, or KEAP1, which in the absence of FBXL17 formed heterodimers with KLHL2 (fig. S7, E and F). Similar to these experiments in 293T cells, SCF^{FBXL17} also counteracted heterodimerization in physiologically relevant settings: Depletion of FBXL17 strongly increased the heterodimerization of KBTBD8 with KBTBD7 in human embryonic stem cells instructed to differentiate into a neural crest fate (Fig. 3I), which is dependent upon KBTBD8 function (*15*). Depletion of FBXL17 also reduced the association of KBTBD8 with its key substrate, TCOF1, an interaction that requires KBTBD8 dimerization (*27*), whereas the dimerization-independent binding of KBTBD8 to CUL3 or PKN1 was unaffected. Thus, SCF^{FBXL17} eliminates BTB dimers of aberrant composition in cells. We propose to refer to the ability of SCF^{FBXL17} to detect and eliminate faulty dimers as dimerization quality control, or DQC.

SCF^{FBXL17} ensures nervous system development and function

We next wished to determine whether DQC, similar to other quality control networks, plays a role in organismal development or homeostasis and thus depleted FBXL17 from *Xenopus laevis* embryos (fig. S8A). We injected morpholino oligonucleotides that target either the start codon or four distinct exon-intron splice boundaries of FBXL17 into one cell of two-cell *X. laevis* embryos, thereby depleting FBXL17 by preventing mRNA translation or splicing. We confirmed the efficiency of splice site–directed morpholinos by quantitative polymerase chain reaction (qPCR) (fig. S8B) and analyzed uninjected embryos, untreated sides of injected embryos, or embryos injected with a mismatch morpholino as controls. All phenotypes described below were observed with multiple morpholinos depleting FBXL17, but not with ineffective morpholinos or any control approach.

Establishing that SCF^{FBXL17} prevents accumulation of aberrant BTB proteins in *X. laevis* embryos, down-regulation of FBXL17 resulted in a marked increase in the levels of coinjected $KLHL12^{V50A}$ (Fig. 4A), a prototypic SCF^{FBXL17} substrate in our cellular studies. Accumulation of $KLHL12^{V50A}$ was observed in early stage 17 embryos and the later tailbud stage. As in 293T cells or differentiating human embryonic stem cells (hESCs), depletion of FBXL17 also stabilized KBTBD7-KBTBD8 heterodimers in *X. laevis* embryos (fig. S8C). The inactivation of SCF^{FBXL17} had consequences for neuronal differentiation, as markers for neuronal identity (*n-Tubulin; Neuro D*) were strongly reduced, whereas those of early neural progenitors (*Sox2*) were unaffected (Fig. 4B and fig. S8D). The epithelial mesenchymal transition marker Twist was reduced; melanocyte specification and migration were diminished, indicative of impaired neural crest differentiation; and eye development was also impaired (Fig. 4, B to D). Analysis of Neurofilament, NCAM, or the Tor219 marker of sensory neurons revealed inefficient production of Rohon-Beard neurons and deficits at later stages of neuronal development (Fig. 4E and fig. S8, E and F), and TUNEL (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling) staining documented increased cell death specifically in the heads of embryos (Fig. 4F). In accordance with aberrant nervous system development, depletion of FBXL17 resulted in a strong and dose-dependent loss of the tadpole response to gentle touch (Fig. 4G and Movies 1 and 2). Loss of SCF^{FBXL17}, however, did not affect somitogenesis (fig. S8G), and FBXL17-depleted embryos initially did not show gross morphological changes (fig. S8H). Consistent with FBXL17 being expressed in the neuro-ectoderm of *X. laevis* embryos (fig. S8I), these observations thus revealed that DQC is particularly important for the differentiation, function, and survival of cells in the peripheral and central nervous system.

To assess whether phenotypes of aberrant DQC were caused by misregulation of specific BTB proteins, we tested for a genetic interaction between FBXL17 and KBTBD8 in neural crest specification. KBTBD8 is a prototypic DQC substrate: Although SCF^{FBXL17} posttranslationally bound heterodimers containing the KBTBD8 BTB domain, it did not detect KBTBD8 homodimers (Fig. 3D); KBTBD8 showed increased heterodimerization in 293T cells, differentiating hESCs, or *X. laevis* embryos lacking FBXL17 (Fig. 3I and fig. S7, E and F); and the heterodimerization in hESCs lacking FBXL17 was accompanied by a decrease of KBTBD8-substrate binding (Fig. 3I). We first titrated morpholinos against KBTBD8 or FBXL17 to cause only very mild neural crest phenotypes and then combined both morpholinos: Additive effects of co-depletion would indicate independent functions of KBTBD8 and FBXL17; rescue of KBTBD8 phenotypes by FBXL17 co-depletion would suggest that FBXL17 degrades and inactivates KBTBD8; yet, synthetic lethality

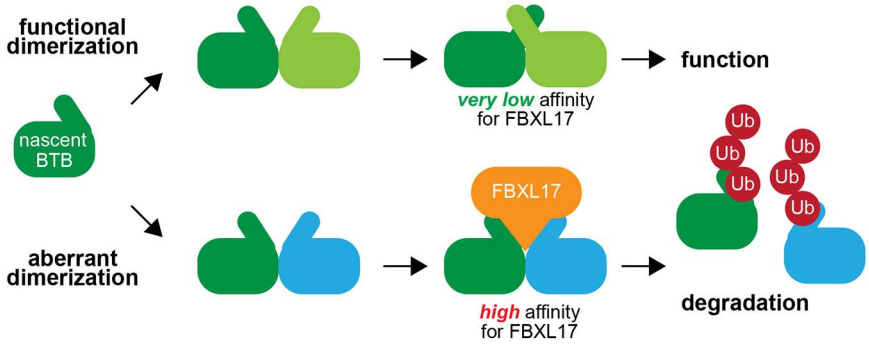

Fig. 5. Model of dimerization quality control (DQC). BTB domain–containing proteins rapidly dimerize upon synthesis. Wild-type BTB homodimers or functional BTB heterodimers mature quickly to bury essential SCF^{FBXL17}-degron residues. Mature and active dimers therefore escape recognition by SCF^{FBXL17}. By contrast, the maturation of mutant BTB homodimers or aberrant heterodimers into a closed conformation is delayed, and such inactive dimers therefore remain accessible to SCF^{FBXL17}, leading to their ubiquitylation and proteasomal degradation. Thus, DQC detects and eliminates complexes of aberrant composition, rather than targeting misfolded and aggregation-prone proteins.

Movie 1. *Xenopus laevis* embryos respond to touch. Embryos were poked with a pipette tip to evoke a movement referred to as escape response.

Movie 2. *Xenopus laevis* embryos lacking FBXL17 fail to respond to touch. Embryos treated with morpholino oligonucleotides targeting FBXL17 were analyzed for their escape response, as described above.

would be anticipated if quality control by SCF^{FBXL17} ensured KBTBD8 activity. Notably, in situ hybridization against Twist, immunofluorescence against NCAM, and bright-field microscopy all revealed strong synthetic lethality of KBTBD8 and FBXL17 depletion (Fig. 4H). This demonstrates that FBXL17 sustains neural crest differentiation by maintaining the cellular pool of active KBTBD8, which highlights the physiological importance of DQC for specific BTB proteins in embryos.

Discussion

Dimerization quality control monitors a recurrent interaction module

We have identified a quality control system, DQC, that ensures dimerization and function of a widespread protein interaction module, the BTB domain. By monitoring complex composition, rather than folding, DQC complements quality control networks that provide defense against unfolded and aggregation-prone proteins (*31*, *32*, *34*). DQC relies on SCF^{FBXL17}, which selectively ubiquitylates aberrant BTB dimers that arise as a consequence of heterodimerization or BTB mutation (Fig. 5). Our results suggest that SCF^{FBXL17} accesses residues at the interface of BTB subunits that are transiently exposed in nascent BTB dimers or persistently presented by mutant homo- or aberrant heterodimers, yet buried upon successful dimerization, as shown in structures of mature KEAP1 and KLHL3 (*35*, *36*). The mechanism of substrate detection by SCF^{FBXL17} allows for the possibility that cellular conditions, such as oxidative stress (*30*), induce BTB recognition—for example, by post-translational modifications that expose degron residues. Conversely, localized mRNA translation or cotranslational BTB homodimerization might limit heterodimerization and reduce the burden on DQC. Our model of DQC is consistent with the finding that overexpressed FBXL17 degrades wild-type BTB proteins (Fig. 1C), as overexpressed FBXL17 could capture nascent BTB dimers before these have reached their mature conformation.

Specific roles of dimerization quality control in the nervous system

The ability of SCF^{FBXL17} to eliminate compromised BTB dimers ensures that only functional BTB complexes are present in cells, and genetic interaction studies demonstrated the importance of DQC for maintaining active KBTBD8 for neural crest specification. The compromised neuronal differentiation and survival that are caused by aberrant DQC may result from a combinatorial effect of dysregulating multiple BTB proteins at a time. We also do not wish to exclude the possibility that SCF^{FBXL17} has targets in addition to inactive BTB dimers, whose misregulation contributes to the aberrant neuronal function observed in the absence of this E3 ligase. Notably, phenotypes similar to those of FBXL17 depletion have been observed in neurodegenerative diseases, and mutation of the F-box of *FBXL17* has been reported in Parkinson's disease (*37*). However, whether and how *FBXL17* mutations affect dopaminergic neurons remains to be determined.

Implications for the evolution of protein interaction modules

Basal eukaryotes lack a recognizable FBXL17 and accordingly contain few BTB proteins that could heterodimerize; *Drosophila melanogaster* also does not possess a FBXL17 ortholog but uses alternative splicing to link a single BTB domain to multiple proteins for homodimerization (*38*). Conversely, FBXL17 is present in basal metazoans and conserved in most metazoans, which correlates with the rapid expansion of dimeric BTB proteins early in the metazoan lineage (*39*). We speculate that FBXL17-dependent DQC allowed expansion of BTB proteins by ensuring homodimerization despite a steep increase in the number of proteins that are prone to aberrant heterodimerization. In addition to BTB domains, recurrent dimerization modules include leucine zippers, coiled coils, or zinc fingers. We predict that the interaction status of these domains is surveilled by additional branches of the DQC pathway that depend on E3 ligases distinct from SCF^{FBXL17}. Analyzing the coevolution of E3 ligases with dimerization modules could allow us to identify such DQC E3 ligases that we expect to be similarly important for metazoan development as SCF^{FBXL17}.

Materials and methods

Detailed materials and methods can be found in the supplementary materials. In vitro binding and ubiquitylation assays were performed with in vitro–transcribed and –translated substrates, as described (*40*). Cellular experiments used 293T cells, as well as differentiating hESCs subjected to neural conversion (*15*). Animal experiments were performed with embryos of *X. laevis*.

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SUPPLEMENTARY MATERIALS

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Science

Dimerization quality control ensures neuronal development and survival

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A way to prevent deadly interaction

Many metazoan proteins form oligomers, which is often mediated by modular domains such as BTB domains. Mena *et al.* now describe a quality control pathway they term dimerization quality control (DQC) (see the Perspective by Herhaus and Dikic). DQC monitors and prevents aberrant dimerization of BTB domain–containing proteins. The system relies on FBXL17, an adaptor protein that recruits an E3 ligase that specifically ubiquitylates nonfunctional BTB heterodimers, triggering their degradation. FBXL17 accesses a degradation signal at the BTB dimer interface in nonphysiological, nonfunctional complexes. The loss of DQC from *Xenopus laevis* embryos leads to lethal neurodevelopmental defects.

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