Supplementary Information

Conditions for qPCR

RT-qPCR were performed using the following primers:
human *TDO2* (Forward: CATGGCTGGAAAGAACTC, Reverse: CTGAAGTGCTCTGTATGAC, Probe: TTTAGAGCCACATGGATTTAACTTCTGGG); human *GAPDH* (Forward: TCAACGACCACTTTGTCAAGC, Reverse: CCAGGGGTCTTACTCCTTGG, Probe: CCTGGTATGACAACGAATTTGGCTACAGC); mouse *Tdo2* (Forward: GTATCTATGGAGGACAATGAAG, Reverse: GATGAATAGGTGCTCGTCATG, Probe: CCTCCTTTGCTGGCTCTGTTTACACC); mouse *β-Actin* (Forward: CTCTGGCTCCTAGCACCATGAAG, Reverse: GCTGGAAGGTGGACAGTGAG, Probe: ATCGGTGGCTCCATCCTGGC). The probes were labeled with 5' FAM and 3'TAMRA. Standard curves were added for m*Tdo2* and m*β-Actin.* Cycling conditions were usually: denaturation at 95°C for 15 sec, and hybridation and elongation at 60°C for 1min, except for m*Tdo2* (3 min at 95°C, then 40 cycles of 10 sec at 95°C and 1 min at 60°C), and for m*β-Actin* (3 min at 95°C, then 40 cycles of 3 sec at 95°C and 30 sec at 60°C).

Construction of plasmids producing deleted and mutated TDO proteins

Deletions were introduced into the ORF of *TDO2* by using PCR with two divergent oligonucleotides corresponding to either border of the deleted sequence. The oligonucleotides (100pmoles of each) were first phosphorylated with 5U of T4 polynucleotide kinase (NEB) in 10µl of T4 DNA ligation buffer (NEB) for 30 min at 37°C followed by 20 min at 65°C to inactivate the enzyme. These primers (0.5µM each) were then used to amplify the recombinant vector (10ng) in 50µl of Phusion GC buffer (Finnzyme) with 1U of Phusion DNA polymerase (Finnzyme). PCR conditions were 1 cycle at 98°C for 30 sec, 30 cycles at 98°C for 10 sec, at a temperature corresponding to the lower Tm + 2°C for 30 sec and at 72°C for 4 min and finally 1 cycle at 72°C for 10 min. 5µl of the PCR reaction were separated in an agarose-ethidium bromide gel where we usually observed a bright PCR product of 7.1kb. The PCR products were purified on size-exclusion chromatography columns. One µl (about 40ng) of PCR product was then incubated with 5U of T4 DNA ligase in 50µl of T4 DNA ligation buffer (NEB) for 1h30 at 37°C to circularize the vector. One twentieth (2.5µl) of the ligation reaction was used to transform XL1-Blue competent bacteria (Agilent Technologies). Plasmid DNA was purified from 3-5 colonies and sequenced. Deletions were found in most of the

sequenced plasmids. Oligonucleotides used to introduce the deletions: human TDO del200-213 (Forward: GGTTTAGAGCCACATGGATTTAAC, Reverse: CTTTTCCTGCTCAGATTTAAGTAGC); human TDO del98-105 (Forward: AGGAACATGCTTAAGGTTGTTTCTCG, Reverse: AAAGATCTCTCGAACAGAATCCAAC); human TDO del169-187 (Forward: GAAAATGAACTGCTACTTAAATCTGAG, Reverse: AAGAACACCTATCTTGTTTTCTAATAGTC). Point mutations were introduced into the ORF of *TDO2* with the QuikChange II site-directed mutagenesis kit (Agilent Technologies) following the protocol of the manufacturer. Oligonucleotides used to introduce the mutations: human TDO mut208-211 (Forward: GTGGAGGCAGCGGCGGCAGCAACTCCAGGTTTAGAGCCACATGG, Reverse: CCATGTGGCTCTAAACCTGGAGTTGCTGCCGCCGCTGCCTCCAC); human TDO mut204-206 (Forward: CACTTCTGGAAGCAGCGGCGGCATGGCTGGAAAGAACTCCAGG, Reverse: CCTGGAGTTCTTTCCAGCCATGCCGCCGCTGCTTCCAGAAGTG).

Supplementary Figures and Tables

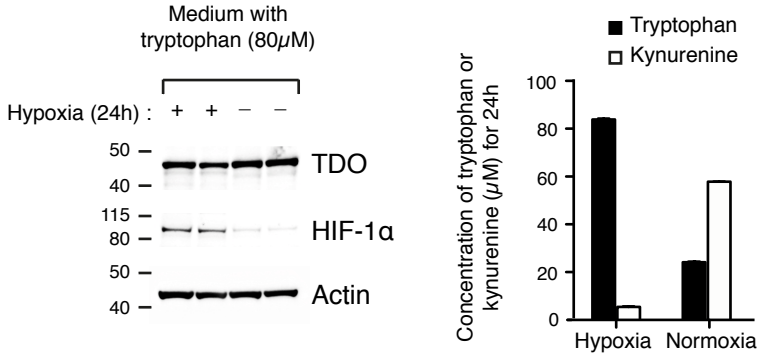

Figure S1. Hypoxia does not decrease TDO protein levels, while it reduces TDO catalytic activity.
A172 cells cultured in tryptophan-containing medium (80μM) were exposed to normoxia (20% oxygen) or hypoxia (1% oxygen) for 24h. TDO and HIF1α protein levels were analyzed by western blot. Stabilization of HIF1α confirmed effective hypoxic conditions. One representative experiment out of two independent is shown. The right panel illustrates the expected reduction of TDO catalytic activity in hypoxic conditions, with the measurement of tryptophan and kynurenine in the 24h supernatant of A172 cells.

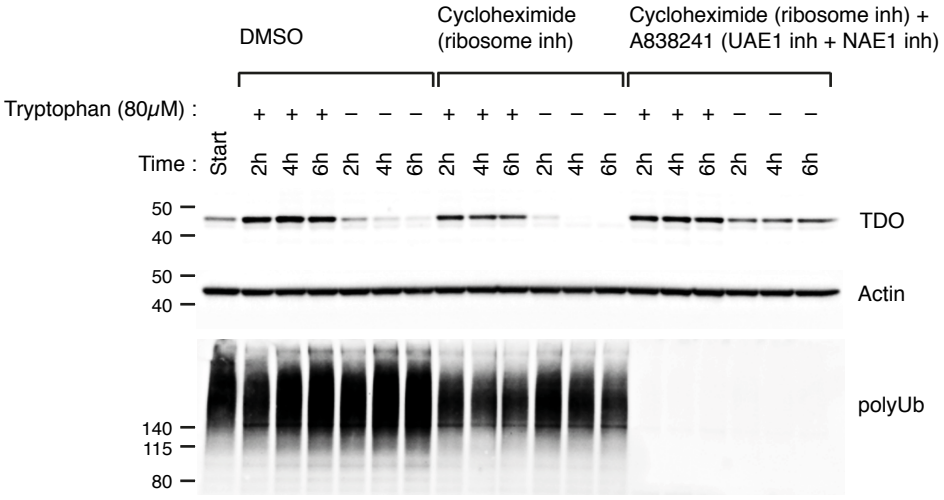

Figure S2 (related to Fig. 3D). Blocking polyubiquitination prevents TDO degradation in the absence of tryptophan.
A172 cells initially grown in tryptophan-containing medium (start) were then incubated in medium with or without 80μM tryptophan, in the presence of 50μg/ml cycloheximide and 10μM A838241, an inhibitor of E1 ubiquitin ligases and NEDD8 activating enzymes*. Western blots were performed to analyze the level of TDO protein and of polyubiquitinated proteins, as a control for E1 ubiquitin ligases inhibition. One representative experiment out of three independent is shown.

* Lukkarila, J. L. et al. Identification of NAE Inhibitors Exhibiting Potent Activity in Leukemia Cells: Exploring the Structural Determinants of NAE Specificity. ACS medicinal chemistry letters 2, 577-582, doi:10.1021/ml2000615 (2011).

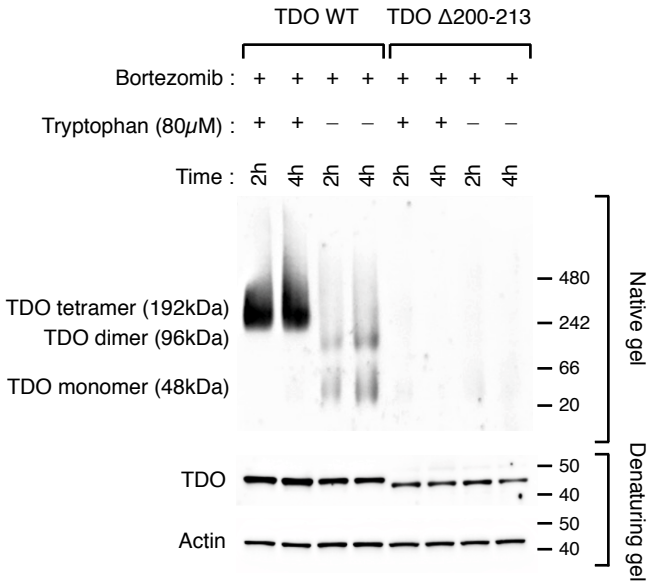

Figure S3 (related to Fig. 6). Deleting residues 200-213 in the exosite does not stabilize the tetrameric conformation of TDO.
HEK293 cells stably transfected with expression vectors encoding TDO WT or TDO Δ200-213 were incubated in medium with or without 80μM tryptophan in the presence of bortezomib (1μM). TDO molecular mass was evaluated by performing a western blot analysis in native (top) or denaturing conditions (bottom). One representative experiment out of three independent is shown.

Table S1 (related to Fig. 3 and 4).
Inhibition of the ubiquitin-proteasome system increases TDO half-life in the absence of tryptophan

Inhibitors	**Cycloheximide**	**Cycloheximide Bortezomib**	**Cycloheximide MLN7243**	**Cycloheximide Pevonedistat/MLN4924**
Targets	**Ribosomes**	**Ribosomes Proteasomes**	**Ribosomes Ubiquitin activating E1**	**Ribosomes NEDD8 activating enzymes**
Medium with tryptophan Half-life (± SD)	> 8h	> 8h	> 6h	> 6h
Medium without tryptophan Half-life (± SD)	1h22 (±13) min	2h04 (±18) min	> 6h	2h02 (±33) min
Related figure	Fig 3A	Fig 3B	Fig 3D	Fig 4A
Number of experiments quantified	2	2	3	3

The table shows the half-life of TDO in A172 cells incubated in medium with or without tryptophan and treated or not with proteasome and ubiquitin ligase inhibitors from Fig 3 and 4. Signals of TDO/Actin were quantified from kinetic degradations and related to the value at the time 0h. Data were plotted by experiments and half-life values represent the mean of half-lives from independent experiments, as indicated.

Table S2 (related to Fig. 4B-D).
Transfection of the dominant-negative CUL1 increases TDO half-life in the absence of tryptophan

Vector (dominant negative)	**Empty vector**	**CUL1DN**	**CUL3DN**	**CUL4ADN CUL4BDN**	**CUL5DN**
Medium with tryptophan Half-life (± SD)	> 4h	> 4h	> 4h	> 4h	> 4h
Medium without tryptophan Half-life (± SD)	47 (±7) min	1h29 (±23) min	46 (±9) min	39 min	49 min
Related figure	Fig 4B	Fig 4B	Fig 4B	Fig 4C	Fig 4D
Number of experiments quantified	2	2	2	1	1

The table shows the half-life of TDO in HEK293 cells stably transfected with *TDO2* (HEK293 hTDO cl19) transiently transfected with dominant negative cullins incubated in medium with or without tryptophan (in presence of cycloheximide). Signals of TDO/Actin were quantified from kinetic degradations and related to the value at the time 0h. Data were plotted by experiments and half-life values represent the mean of half-lives from independent experiments or the value of a single experiment, as indicated. For Fig 4C and 4D, the experiment was repeated twice, but only one experiment could be quantified.