Resveratrol is reported to extend lifespan and provide cardio-neuro-protective, anti-diabetic, and anti-cancer effects by initiating a stress response that induces survival genes. Because human tyrosyl transfer-RNA (tRNA) synthetase (TyrRS) translocates to the nucleus under stress conditions, we considered the possibility that the tyrosine-like phenolic ring of resveratrol might fit into the active site pocket to effect a nuclear role. Here we present a 2.1 Å co-crystal structure of resveratrol bound to the active site of TyrRS. Resveratrol nullifies the catalytic activity and redirects TyrRS to a nuclear function, stimulating NAD+–dependent auto-poly-ADP-ribosylation of poly(ADP-ribose) polymerase 1 (PARP1). Downstream activation of key stress signalling pathways are causally connected to TyrRS–PARP1–NAD+ collaboration. This collaboration is also demonstrated in the mouse, and is specifically blocked in vivo by a resveratrol-displacing tyrosyl adenylate analogue. In contrast to functionally diverse tRNA synthetase catalytic nulls created by alternative splicings and RSV-promoted conformational change near the linker to the C-domain (Fig. 1b and Extended Data Table 1; Protein Data Bank (PDB) accession numbers 4Q93 and 4QBT).

While the phenolic ring of RSV and of the tyrosine have the same disposition in the respective co-crystals, accommodation of the cis-conformation of the dihydroxy ring of RSV forces a local structural change near the linker to the C-domain (Fig. 1b and Extended Data Fig. 2a). An RSV-promoted conformational change in TyrRS may drive the predominant trans RSV (in solution) into a cis conformation (Extended Data Fig. 2c, d).

Associated with a previous study, a distinct TyrRS–PARP1 interaction was observed. PARP1 is a major modulator of NAD+ metabolism and its related signalling. Because RSV acts through NAD+–dependent proteins, the TyrRS–PARP1 interaction was further studied. Given that RSV treatment elicits a stress response, serum starvation was used to mimic a general ‘stand-alone’ stress condition so that common signalling pathways, if any, between RSV treatment and a general stress condition, could be compared ex vivo. Either serum starvation or RSV treatment promoted nuclear translocation of endogenous TyrRS in HeLa cells (Fig. 1c). Translocation was observed under different stress conditions (heat shock and endoplasmic reticulum stress; Extended Data Fig. 3a), suggesting that TyrRS is a general stress transducer. Nuclear translocation of endogenous TyrRS was concomitant with strong auto-PARylation of PARP1 (PARP1-PAR), but not by Gly-SA (a control targeting GlyRS) (Extended Data Fig. 3b, c). Similar, but less pronounced, PARylation was seen with serum starvation. Enhanced PARylation correlated with increased amounts of TyrRS in the nucleus, which occurred upon serum starvation. Thus, both serum starvation and RSV promoted nuclear translocation of TyrRS and activation of PARP1.

Cell lysates treated with the PARG hydrolyase and its hydrolase-inactive mutant supported the idea that TyrRS preferentially bound to non-PARYlated PARP1 (Extended Data Fig. 3d, e). TyrRS interacted specifically with the C-domain of PARP1 (CT-PARP1) (Extended Data Fig. 3f). Neither mini-TyrRS nor the TyrRS C-domain interacted with PARP1; only full-length native TyrRS bound PARP1 (Extended Data Fig. 3g, h). In the absence of RSV, concentration-dependent activation of PARP1 by TyrRS was observed in vitro (Fig. 2a, top, and Extended Data Fig. 2b). In vivo, resveratrol strongly inhibited TyrRS with an inhibition constant (Kι) value of 22 μM (Extended Data Fig. 1a–c). Crystallization of Hs mini-TyrRS with RSV and, separately, with tyr-AMP analogue (Tyr-AMP) facilitated the nuclear translocation of TyrRS with a pronounced, PARylation was seen with serum starvation. Enhanced PARylation correlated with increased amounts of TyrRS in the nucleus, which occurred upon serum starvation. Thus, both serum starvation and RSV promoted nuclear translocation of TyrRS and activation of PARP1.

**Figure 1** | Resveratrol binds at the active site of TyrRS. a, Cartoon illustration of the domain organization of human TyrRS. Both domains are connected by a linker of approximately 20 amino acids. b, Left, Electron density of co-crystal X-ray structures (2.1 Å) of RSV bound to cis-resveratrol and to L-tyrosine. Right, resveratrol induced a local conformational change relative to bound tyrosine at the active site. c, Both serum starvation and resveratrol treatment (5 μM) facilitated the nuclear translocation of TyrRS with a concomitant increase in the PARylation of PARP1.

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Data Fig. 4a, b). RSV enhanced in vitro auto-PARYlation with the half-maximal effect at roughly 10 nM (Fig. 2a, middle), well below the $K_i$ (about 22 μM) in Extended Data Fig. 1a–c. Thus, PARP1 may alter the apparent affinity of RSV for TyrRS. Also, concentration-dependent quenching of PARYlation of PARP1 by Tyr-SA was evident (Fig. 2a, bottom). Lastly, while broken DNA normally activates PARP1 (ref. 13), Tyr-SA did not interfere with this DNA-dependent-pathway of PARP1 activation in vitro (Extended Data Fig. 4c). Therefore, TyrRS–RSV activation of PARP1 is distinct.

Ectopically expressed TyrRS in HeLa cells for 0–24 h caused progressive increase in cellular concentrations of the synthetase (Fig. 2b, top) and a correlated progressive increase in PARP1 PAR (Fig. 2b, middle). A TyrRS mutant (TyrRS-dNLS), with reduced nuclear localization, reduced activation of PARP1 (Extended Data Fig. 4d). Effects of RSV ex vivo at various concentrations (Fig. 2b, middle) and of Tyr-SA (Fig. 2b, bottom) mirrored those seen in vitro (Fig. 2a, middle and bottom).

RSV and l-tyrosine produce different bound conformations of TyrRS, seen locally in the high-resolution structures of the two co-crystals (Fig. 1b and Extended Data Fig. 2a). These differences could affect the disposition of the C-domain needed for the interaction of TyrRS with PARP1. This domain is tethered to the mini-TyrRS catalytic unit by a flexible linker (Fig. 2c, left). By breaking the Y341 OH–H bond to a backbone carbonyl oxygen, a Y341A mutation releases tight tethering of the C-domain to the catalytic domain and its reorientation (Fig. 2c, right).

In the absence of RSV, Y341A-TyrRS showed robust interaction with and activation of PARP1 in vitro (Fig. 2d). If Y341A is shifted more towards a conformation needed to bind to PARP1, then it would be more sensitive than wild-type TyrRS to low concentrations of RSV. This expectation was fulfilled (Extended Data Fig. 4e). TyrRS-Tyr may thus have a conformation that prevents interaction with PARP1, while TyrRS–RSV has a distinct conformation that binds PARP1. In the absence of either ligand, a dynamic equilibrium populates both forms (Fig. 2c).

A working model of downstream signalling markers was developed as detailed in Methods (Fig. 3a). Effects from serum starvation and RSV administration involve cascades through protein acetylation and phosphorylation effects of serum starvation and of RSV, which have not been linked to TyrRS nor activation of PARP1. Under conditions of serum starvation or RSV administration, the response in HeLa cells of PARylated and acetylated proteins, together with the aforementioned cell-signalling proteins, was monitored for up to 1 h. Either RSV or serum-starvation promoted production of AcK-Tip60 and AcK-ATM (Fig. 3b). In a temporal response from 0 to 8 h, either or both of serum starvation or 1 μM RSV promoted increased levels of whole-cell PARylated and acetylated proteins (Extended Data Fig. 5a, b). In a temporal response from 0 to 8 h, either or both of serum starvation or 1 μM RSV promoted increased levels of whole-cell PARylated and acetylated proteins (Extended Data Fig. 5a, b).
proteins, Hsp72, AcK382-p53, p-AMPK, pSer36-H2B, p21, FOXO3A, 14-3-3, HO-1, NAMPT, SIRT1, and SIRT6 (Fig. 3c, d). Also, RSV activated acetylation of Tip60 ex vivo in a concentration-dependent manner (Extended Data Fig. 5a). As expected from previous reports that acetylation of p53 is indispensable for its activation, a transient increase in acetylation of p53 (AcK382) was evident (Fig. 3c, d), possibly through a transient inhibition of SIRT1 by the nicotinamide being produced. Consistently, at 15 min, an RSV concentration-dependent reduction in total NAD+ with concomitant production of nicotinamide and ADP-ribose was observed (Extended Data Fig. 5b, c). Thus, serum starvation and RSV rapidly increased all monitored proteins in a way that is dependent on having active PARylation. This rapid increase is consistent with work showing rapid upregulation of PARP1 target genes, possibly because of a broader effect of PARP1 on transcription and associated stress signalling.

At longer times, and continuing at low concentrations (≤10 μM) of RSV, similarity between responses to RSV and serum starvation was ostensibly seen. By 1 h, low concentrations of RSV elevated the NAD+ levels similar to those of serum-starvation conditions (Extended Data Fig. 5d). Responses of key stress-signalling markers in a more extended period of 8–24 h at 5 μM RSV were mostly similar to those of serum starvation (Extended Data Fig. 5e, f). At 5 μM RSV, levels of PARP1PAR were sustained longer compared with what was observed at 1 μM RSV (compare 2 h and 8 h time points in Fig. 3d with Extended Data Fig. 5f) and we thus continued further studies at 5 μM RSV.

A short interfering RNA (siRNA) directed against PARP1 (siRNA PARP1) effectively abrogated the RSV-stimulated expression of Hsp72, p-AMPK, SIRT1, FOXO3A, SESN2, NAMPT, PUMA, and SIRT6 (Fig. 4a). Also, 5 μM RSV promoted induction of BRCA1 and p14ARF (whose genes are directly regulated by PARP1 (refs 22, 23)). Consistently, the RSV-stimulated inductions of BRCA1 and p14ARF were prevented by siRNA PARP1 (Fig. 4a), further showing involvement of PARP1 in RSV-mediated induction of these proteins.

In Fig. 4a, RSV treatment led to enhanced activation of p53. However, in the absence of RSV, knockdown of PARP1 resulted in a background increase in the products of these p53-regulated genes. Possibly p53 was activated by the knockdown of PARP1, because removal of PARP1 would enhance background levels of DNA damage. Indeed, activation of p53, seen as enhanced acetylation and phosphorylation of p53, was observed (Fig. 4a).

siRNA TyrRS knockdown at 5 μM RSV eliminated induction of, and dramatically reduced amounts of, PARylated proteins, and of Ack382-p53, Ack16-H4 (Tip60 activation), p-AMPK, and pSer36-H2B (AMPK activation) (Fig. 4b). (Separately, RSV (5 μM) addition did not affect the viability of HeLa cells expressing either siRNA TyrRS or siRNA PARP1 (Extended Data Fig. 6).) In contrast, siRNA SIRT1 knockdown did not affect RSV (5 μM)-mediated production of whole acetylated proteins or, among other downstream markers, activation of PARP1 and induction of SIRT6, FOXO3A, NAMPT, Ack16-H4, p-AMPK, and pSer36-H2B (Extended Data Fig. 7). Collectively, Fig. 4a, b shows that ex vivo TyrRS and PARP1 collaborate to activate RSV- and serum-starvation responses.

Although the RSV–TyrRS–PARP1 axis initially consumes NAD+, at low RSV (5 μM), NAD+ levels are transiently raised after 1 h owing to NAMPT activation (Extended Data Fig. 5d). At 1 h at low RSV, induction of NAD+ levels was abolished with either siRNA TyrRS or siRNA PARP1 (Extended Data Fig. 8). NAD+ depletion by NAMPT inhibition abolished RSV-mediated induction of BRCA1, FOXO3A, NAMPT, SESN2 and SIRT6 (Fig. 4c). These results are consistent with those using inhibitors against PLC-γ and the ryanodine receptor, which inhibit PARP1 activation and ADP-ribose-mediated calcium signalling, and which prevented RSV-mediated AMPK activation. Similarly, a PARP1 inhibitor prevented LKB1 activation by RSV.

Mice were injected intravenously the through tail vein with 100 μl of 10 μM RSV and, after 30 min, were euthanized. Compared with the PBS intravenously-injected control, PARylated and acetylated proteins, along with Ack16-H4 and pSer36-H2B, were significantly increased in skeletal muscle (Extended Data Fig. 9a, b). Results in cardiac tissue were similar (Extended Data Fig. 9c, d). In addition, PARP1 activation diminished back to normal by 24 h (Extended Data Fig. 9e). Consistent with ex vivo assays, tissue samples from RSV-injected mice showed higher levels of TyrRS–PARP1 interaction together with increased auto-PARYlation (Extended Data Fig. 9f).

Tyr-SA (5 μM) was added to the intravenous-injection with RSV. No interaction of TyrRS with PARP1 could be detected in skeletal or cardiac muscle and levels of PARylated and acetylated proteins did not increase. In contrast, co-injection of Gly-SA or cycloheximide (CHX, protein synthesis inhibitor) did not block RSV–TyrRS-mediated activation of PARP1 and its downstream signals (Fig. 4d (muscle) and Extended Data Fig. 9g, h (cardiac)). Consistent with ex vivo assays, immunoprecipitation of PARP1 (from harvested muscle tissue) pulled down TyrRS, and immunoprecipitation of p53 showed its increased...
acetylation. Pull-down of TyrRS and p53 acetylation were blocked in mice that had co-injections of Tyr-5A or the AGI4361 (SelleckChem) PARP1 inhibitor, but not of Gly-5A or cycloheximide (Fig. 4e). These responses in vivo parallel ex vivo assays, and support the idea that TyrRS is a major effector target for RSV that acts through PARP1 as described in Fig. 3a.

The mechanisms of action of RSV and of the stress response are both linked here to the activation of PARP1 through TyrRS and NAD⁺. The interaction of RSV with TyrRS could be viewed as an example of xenohormesis through interactions of a natural ligand with a protein target. In this instance, the natural ligand blocks the active site to create a RNA synthetase catalytic null with a new, orthogonal function. This kind of catalytic null is in contradistinction to those created by alternative splicing events that specifically remove the active site. The RSV–TyrRS activation of PARP1 is readily observable in a functional in vitro assay even at sub-micromolar concentrations (for example, 10 nM; Fig. 2a, middle). Thus, TyrRS–RSV–induced PARP1 activation appears at significantly lower RSV concentrations than seen with RSV functional binding to other targets. As a consequence, the direct effects of RSV binding to these targets of RSV are layered over a pre-existing foundation that comes from the TyrRS–RSV–PARP1–NAD⁺ connection.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions M.S. and P.S. conceived the idea, designed the research, analysed the data, and wrote the manuscript. M.S. performed the experiments.

Author Information X-ray structure coordinates of resveratrol-bound TyrRS and l-Tyr bound TyrRS have been deposited in Protein Data Bank under accession numbers 4Q93 and 4QBT, respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper, Correspondence and requests for materials should be addressed to P.S. (schimmel@scripps.edu).
using Lipofectamine RNAiMAX (Invitrogen). NAMPT, the rate-limiting enzyme for NAD synthesis, is the major regulator of NAD levels in the cell. NAD serves as a substrate for AMPK activation by resveratrol and the stress response is dependent on NAD at the Ca2+ channel opener27,41, a substrate for AMP production42, and hence an indispensable component for AMPK activation by resveratrol and the stress response by phosphorylating H2B25. AMPK is known to upregulate NAMPT expression43, the major regulator of NAD levels in the cell. NAD is also a major regulator of NRF2 through p21 induction33 and inducible expression level of NAMPT.

Preparation of cell fractions. HeLa cells were cultured in a humidified incubator with 5% CO2 in DMEM medium (Invitrogen) supplemented with 10% FBS (Invitrogen) and 1 × penicillin/streptomycin. The cells were transfected with pcDNA6-TyrRS-V5 (wild type), or pcDNA3.1-ZZ-PARP1 using Lipofectamine LTX (Invitrogen), Resveratrol (Santa Cruz Biotechnologies), at a series of concentrations from 0 to 50 μM, was used to treat HeLa cells 0–24 h in experiments described. As the effects of RSV are strongly influenced by the background level of PARP1 activity, the experiments were done only after checking to verify that background PARP1 activation was low. Our criterion was to see PARP1 activation upon serum starvation (DMEM medium without FBS), along with Hsp72 induction12. When serum starvation induced PARP1 activation with Hsp72 induction within 30–45 min (from the same batch of a split of HeLa cells), we consistently saw PARP1 activation at lower concentrations of RSV (1 μM). Cell culture medium was supplemented with Tyr-SA or Glx-SA (RNA-Tec) where mentioned. Silencer Select Pre-Designed siRNA against PARP1 (siRNA#1099, 5'-GGACCUUGUAACCAAGGATT-3'), SIRT1 (siRNA#23770, 5'-GGCCUGU UGGGUAACUGAAtt-3') and TyrRS (siRNA#443, 5'-GGACUUGUUGCGCU GAGGUn-3') were purchased from Invitrogen and transfected into HeLa cells using Lipofectamine RNAiMAX (Invitrogen). NAMPT, the rate-limiting enzyme in the NAD salvage pathway, was used in inhibiting STEF-118804 according to the manufacturer’s protocol (SelleckChem). The effect of a concomitant increase in nicotinamide production with a reduction of NAD metabolism, leading to SIRT1 inhibition was monitored by immunoblotting with α-ACK382-p53, an acetylation site known to be specifically targeted by SIRT1 (ref. 1). Monitoring of α-ps3er 15-53 p53 indicated that enhanced acetylation preceded the phosphorylation event. Total p53 was blotted with α-p53. Transactivation of p53 was determined by monitoring its known targets, such as p21, PUMA, I4-3-3, FOXO3A, SESN2, and SIRT6. Activation of NRF2 was further confirmed by following HO-1 expression, using the cognate antibodies. Activation of AMPK (Thr 172 phosphorylation) mediated through AMP and Ca2+ influx was monitored using α-Thr172-AMPK. Activation of AMPK on its targets was further determined by both α-sPr36 H2B and by the expression level of NAMPT.

Cell viability assay. HeLa (1 × 105) cells were reverse-transfected with siRNAs and viability was monitored using an RTCA ICCellSensor System (ACEA Biosciences). HeLa cells were cultured in a humidified incubator with 5% CO2 in DMEM medium (Invitrogen) supplemented with 10% FBS (Invitrogen) and 1 × penicillin/streptomycin.

Preparation of cell fractions. Protocol for cell fractionation was followed as described previously12. Briefly, for whole-cell lysis preparation, HeLa cells were dissolved in 1× SDS–polyacrylamide gel electrophoresis (SDS–PAGE) loading buffer containing 300 mM NaCl. For cytoplasmic fraction preparation, HeLa cells were suspended in 0.1 ml of swelling buffer for 6 min on ice, then incubated with 0.1 ml of plasma membrane lysis buffer for 5 min on ice. The cells were immediately passed through a 21-gauge needle ten times and centrifuged for 10 min at 3220g, 4°C. The supernatant was harvested, while the pellets were used for the preparation of nuclear fractions. For this purpose, the pellet (nuclei) was incubated for 30 min at 4°C with 0.2 ml nuclear extraction buffer. Whenever required, PARP1 (AG14361) and PARP inhibitors (ADP-HPD, Millipore) were added to the cell lysis buffer to ensure unwanted PARylation and removal of PAR chains with PARP.

Immunoprecipitation and Ni-NTA pull-down assays. Protocol for immunoprecipitation was followed as described previously12. Briefly, the supernatants were pre-cleared by incubation with protein G beads. The pre-cleared cell lysates were incubated at 4°C for 1h with either α-PARP1, α-ATM, α-DNA-PKcs, α-TyrRS, α-Tip60, or non-immune immunoglobulin-G (IgG), at a concentration of 5 μg ml−1 followed by incubation with 30 μl Protein G beads (pretreated with 10 μg ml−1 BSA) at 4°C for 1h with rotation. PARP1 (AG14361) and PARP inhibitors (ADP-HPD, Millipore) were added to the cell lysis buffer to ensure unwanted PARylation and removal of PAR chains with PARP. Immunoprecipitates were washed three times, subjected to SDS–PAGE and immunoblotted with specific antibodies. Whenever mentioned, the ZZ domain allowed immunoprecipitation of ectopically expressed ZZ-PARP1, using anti-IgG. Whenever Ni-NTA pull-down was performed, proteins with a 6×-His tag were overexpressed in Escherichia coli. Cells were lysed and the supernatant fractions containing the soluble proteins were mixed with HeLa cell lysates. For Ni-NTA pull-downs, normal procedures for immunoprecipitation were followed with 15–20 μg mL−1 in the washing buffer.

Animal experiments. All experiments were approved by The Scripps Research Institute Institutional Animal Care and Use Committee (protocol number 13-0002) and conducted by the mice facility at The Scripps Research Institute. BALB/cByl mice were originally purchased from Jackson Laboratories. Six-week-old male mice were kept with a 12 h light–dark cycle with free access to food and water for 3 days before conducting the experiment. For all studies, mice were dosed once and collected sample after either at 30 min or at 24 h. Briefly, activation of PARP1 in mouse tissues treated with resveratrol was performed by the intravenous injection of a 100 μl sample of resveratrol (10 μM) in PBS into eight mouse tails (0.012 mg kg−1). A 100 μl PBS injection was used as a control in six mice. Tissue samples were collected from four resveratrol-treated mice and three control mice after 30 min treatment. Tissue samples from the remaining mice were collected after 24 h. In a different experiment, blocking of resveratrol-mediated activation of PARP1 by Tyr-SA was analysed with five groups of two mice each, a 100 μl sample of resveratrol (10 μM) in PBS alone and, separately, with Tyr-SA (5 μM), Gly-SA (5 μM), cycloheximide (5 μM), or the AG14361 PARP1 inhibitor (10 μM) was injected intravenously. A 100 μl PBS injection with Tyr-SA was used in a control group (two mice) as indicated. Tissue samples were collected after 30 min treatment. Muscle or heart tissues samples were homogenized and analyzed by immunoblotting for the status of poly-ADP-ribosylation and associated signalling events.

Recombiant protein purification. Human TyrRS, PARP1 and their variants were purified as previously described12,13. Briefly, DNA encoding either full-length TyrRS (amino acids 1–528), mini-TyrRS (amino acids 1–341), DN-TyrRS (amino acids 237–528), or CT-TyrRS (amino acids 324–528) was cloned into NdeI–HindIII sites of pET-20b vector (Novagen). The expressed proteins included a 6-His tag from the vector sequence. The full-length PARP1 and its variants (amino (N)- and C-terminal domains) were cloned into pET 20b vector. All proteins having a C-terminal His-tag were expressed in E. coli strain BL21 (DE3) by induction for 4 h with 1 mM isopropyl β-D-thiogalactopyranoside. Proteins were purified from the supernatants of lysed cells using Ni-NTA agarose (Qiagen) column chromatography according to the manufacturer’s instructions. The additional high-salt two-column wash (1–1.5 M NaCl), to remove the endogenous DNA/RNA associated with the purified protein. The NaCl concentration was increased to 1.5 M in increments and returned to final elution buffer having 250 mM NaCl in decrease. All the proteins were subjected to a gel-filtration (5–200) chromatography and the protein peak corresponding to homogenous protein was collected. As endotoxin associated with purified protein interfered with the inhibitor/activating effect of RSV on TyrRS/PARP1, the complete protein purification included an additional endotoxin removal step. The purified protein was further passed through a column containing Detoxi-GelTM (Pierce-Thermo Scientific) and followed the manufacturer’s instructions. Additional high-salt two-column wash (1–1.5 M NaCl) was measured using an Endosafe®-PTS kit (Charles River Laboratories). The quality of each protein purification was validated by SDS–PAGE analysis.

Protein crystallization and data collection. All the steps were followed as mentioned in the previous report for mini-TyrRS crystallization12. Briefly, mini-TyrRS was mixed with either 1 mM RSV or 2 mM tyrosine and incubated at 4°C for 16 h. Before setting up the crystallization trials, the protein samples were subjected to high-speed centrifugation (15,871 relative centrifugal force for 15 min) to remove all the precipitants and the clear soluble fraction transferred to a new tube. The crystallization trials were done using 2.1 M (NH4)2SO4, 0.1 M NaH2PO4/K2HPO4 (pH 7.5), and 0.5 mM 1,8-dioxo-2,7-naphthalimide, mini-TyrRS crystals with t-tyrosine were grown in 3 days (data collected at Stanford Synchrotron Radiation Lightsource), crystals with RSV were grown only after 3–4 months at around pH 7. X-ray data were collected at 2.1 A using the in-house X-ray diffraction facility (The Scripps Research Institute). Data were integrated and scaled using
ATP–pyrophosphate exchange assay. Tyrosine adenylate synthesis was measured by using the tyrosine-dependent ATP–pyrophosphate (PPI) exchange assay. A mixture containing 100 mM HEPES (pH 7.5), 20 mM KCl, 2 mM ATP, 1 mM NaPPi, 2 mM DTT, 1 mM 3-mercaptothiopropanol, 10 mM MgCl_2, and about 0.01 mM [32P]NAD was incubated for 30 min at 30°C. [32P]NAD was added to a final concentration of 0.01 mCi ml\(^{-1}\). The reaction was stopped by the addition of 20 μl of 5 M HClO_4 and heated at 95°C for 1 min. The [32P]-adenylylated TyrRS was separated from free [32P]NAD by using Spin-X Centrifuge Filters (Corning) containing 40 mM NaPPi, 1.4% HClO_4, 0.4% HCl, and 8% (w/v) of activated charcoal.

In vitro PARylation assay. Protocol was as previously described\(^{32}\). Briefly, 5 μl recombinant PARP1 (2 μg) was mixed with 5 μl recombinant TyrRS (0–50 μM) and, depending on the experiment, either RSV, TyrSA, DNA, or AG14361 were also added (5 μl) and adjusted the volume to 25 μl. This mixture was incubated at 4°C for 15 min, then incubated with 25 μl 2× PAR assay cocktail (50 mM HEPES, pH 7.5, 100 mM KCl, 10 mM MgCl_2, 0.2 mM EGTA, 0.1 mM EDTA, 40 nM NAD, and 1 μCi [32P]NAD (Perkin-Elmer)) at 37°C for 30 min. The RSV concentration used was 0.01 mCi ml\(^{-1}\), endotoxin free, 0.5 EU ml\(^{-1}\), and adjusted the volume to 25 μl. After thoroughly mixing, the charcoal was filtered and washed with a solution of 7% HClO_4 and 200 mM NaPPi using Spin-X Centrifuge Filters (Corning) containing 0.45-μm pore-size cellulose acetate filters. After drying, the charcoal was punched into scintillation vials and the radioactivity of the ATP bound to the charcoal mixture was measured by scintillation counting.

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Extended Data Figure 1 | Resveratrol inhibits TyrRS activation. a, The ATP–PPi exchange assay as described in Methods demonstrated the inhibitory effect of resveratrol on TyrRS and b resveratrol shifts the Michaelis constant ($K_m$) for tyrosine. c, Resveratrol binds TyrRS better than tyrosine. The apparent $K_i$ for resveratrol was deduced by varying the concentration of RSV and plotting the slope of $(1/v$ versus $1/[Tyr])$ versus $[RSV]$ as indicated.
Extended Data Figure 2 | Resveratrol induces a distinct conformational change upon binding to active site of TyrRS.  

a, Comparison of the overall conformational change induced by resveratrol at the active site of TyrRS by structure-based superposition (yellow, tyrosine-bound structure; magenta, resveratrol-bound structure). Note the conformational change near the helix region (P331–P342) that connects the linker region with the C-domain.

b, Illustration of the extensive interactions of resveratrol with the active site.

c, The trans-resveratrol (dark blue) docks (manual) into TyrRS active site without significant structural disturbances. 
d, Generation of a new pocket through a RSV-induced conformational change in TyrRS accommodates the dihydroxy phenolic ring of RSV (otherwise exposed to the destabilizing aqueous environment in the trans form) and hence facilitates the trans (dark blue) to cis (light blue) conversion of RSV.
Extended Data Figure 3 | Resveratrol facilitates the TyrRS–PARP1 interaction in an active-site-dependent manner. a, Both heat shock (42 °C for 30 min) and tunicamycin-treatment (10 μg ml⁻¹, endoplasmic reticulum stress) facilitated the nuclear translocation of TyrRS and activation of PARP1. b, Resveratrol or serum starvation facilitate TyrRS interaction with PARP1, and Tyr-SA prevents this interaction. ZZ-PARP1 was immunoprecipitated with IgG from HeLa cells treated with RSV or serum starvation alone or in combination with Tyr-SA. c, Resveratrol- or serum-starvation-mediated PARP1 activation is blocked only by Tyr-SA and not by Gly-SA. d, TyrRS interacts directly with PARP1. HeLa cell lysate after RSV treatment (5 μM, 30 min) was divided into three parts and treated with PARG and catalytically inactive PARG-MT. PARP1 was immunoprecipitated and analysed for TyrRS interaction. e, Model illustrating the mechanism of RSV-mediated TyrRS interaction with PARP1 and subsequent release after auto-PARylation. f, Ni-NTA pull-down of N- and C-terminal fragments of PARP1 overexpressed in E. coli demonstrates that TyrRS interacts with the C-terminal region of PARP1. g, Only the full-length TyrRS (1–528), but none of the various fragments of TyrRS (mini-TyrRS (1–364), ΔN-TyrRS (228–528), or the C-domain (328–528)), interacts with PARP1. h, Coomassie blue staining of a gel showing the total protein input in for the experiment of Extended Data Fig. 3g.
Extended Data Figure 4 | Tyrosyl-AMP analogue (Tyr-SA) does not affect DNA-dependent auto-PARylation of PARP1. 

a, Silver-stained SDS–PAGE gel showing the purity and input of PARP1 and TyrRS in the in vitro PARylation study of Fig. 2. 
b, Quantitation (Image J software) of the band intensity of PARylated PARP1 in Fig. 2a, top. 
c, Tyrosyl-AMP analogue (Tyr-SA) does not affect DNA-dependent auto-PARylation of PARP1. 
d, Overexpression of nuclear-translocation-weakened mutant of TyrRS is less effective in activating PARP1. 
e, Y314A-TyrRS is more sensitive to RSV than is TyrRS in facilitating PARP1 activation.
Extended Data Figure 5 | Resveratrol enhances the acetylation of Tip60 and modulates NAD⁺ concentration in a dose- and time-dependent manner.

a, Treatment of HeLa cells (1 h) with increasing concentration of resveratrol enhances the acetylation level of Tip60. Activation of Tip60 was monitored by histone acetylation status. b, Total NAD⁺ contents of serum-starved cells or RSV-treated samples were compared with untreated samples at 15 min using a commercially available BioVision NAD⁺/NADH quantitation colorimetric kit. c, Total nicotinamide or ADP-ribose produced was deduced from the difference in the amount of NAD⁺ in each sample with respect to the untreated sample (consumption of one mole of NAD⁺ would give rise one mole of nicotinamide and one mole of ADP-ribose). d, Total NAD⁺ content of the serum-starved cells or RSV-treated samples were compared with untreated samples at 1 h. (Although the experiments were done in biological triplicates (all samples showing similar results), the error bars in the figure represent the deviations from the mean of the technical triplicates from one representative biological sample.) e, f, Time course study of poly-ADP-ribosylation status and associated signalling events after (e) serum starvation (extended time course data of the same image shown in Fig. 3c) and (f) treatment with 5 μM RSV. Using the respective antibodies, Activation of p53 was monitored by the induction of p21 and SIRT6. Activation of NRF2 was monitored by HO-1 induction.
Extended Data Figure 6 | siRNA (siRNA$^{\text{TyrRS}}$ or siRNA$^{\text{PARP1}}$), with and without low RSV (5 μM), does not affect cell viability. HeLa cells ($1 \times 10^6$) were reverse-transfected with siRNA targeted to TyrRS or PARP1. An siRNA$^{\text{Con}}$ (a scrambled sequence of siRNA$^{\text{PARP1}}$) was used as a control. Viability was monitored using the RTCA iCELLigence System (ACEA Biosciences). Samples were treated with RSV (5 μM) at 60 h and monitoring was continued for another 2 h for siRNA$^{\text{TyrRS}}$ (a total of 62 h of monitoring) and for another 16 h for siRNA$^{\text{Con}}$ and siRNA$^{\text{PARP1}}$ (total 76 h monitoring).
Extended Data Figure 7 | siRNA<sup>SIRT1</sup> did not affect downstream signalling events at low RSV (5 μM). HeLa cells were treated with siRNA<sup>SIRT1</sup> for 60 h to knockdown SIRT1. HeLa cells were treated with RSV (5 μM) for another 4 h and samples were collected intervals as indicated. Samples were analysed for downstream signalling markers using appropriate antibodies.
Extended Data Figure 8 | siRNA- (siRNA\textsubscript{TyrRS} or siRNA\textsubscript{PARP1}) treated cells did not upregulate the levels of NAD\textsuperscript{+} in response to RSV (5 \textmu M) after 1 h. HeLa cells (1 \times 10^6) were reverse transfected, separately, with siRNA targeted to PARP1 or TyrRS. A scrambled sequence of target siRNA was used as a control. The total NAD\textsuperscript{+} content of RSV (5 \textmu M)-treated samples was compared with untreated samples at 1 h, using a commercially available BioVision NAD\textsuperscript{+}/NADH quantitation colorimetric kit. (Although the experiments were done in biological triplicates (all samples showing similar results), the error bars in the figure represent the deviations from the mean of the technical triplicates from one representative biological sample.) The comparator (shown as a dashed bar) is taken from Extended Data Fig. 5d.
Extended Data Figure 9 | Resveratrol treatment activates PARP1 and associated signalling events in the mouse tissues. a, Activation of PARP1 in mouse muscle tissue treated with resveratrol monitored by increased PARylation and by increased acetylation status. Activations of Tip60 and AMPK were monitored by using α-AcK16-H4 and α-pSer36-H2B, respectively. c, Activation of PARP1 in mouse heart tissue treated with resveratrol monitored by increased PARylation and d by increased acetylation status. e, Resveratrol treatment causes only a transient activation on PARP1. Immunoblotting of mouse muscle tissue samples after 24 h of RSV treatment showed no significant difference in the level of PARP1PAR compared with control. f, RSV treatment enhances TyrRS interaction with and activation of PARP1 in the muscle tissue. g and h, Resveratrol-mediated activation of PARP1 (g, monitored by PARylation status; h, monitored by acetylation status) is blocked by Tyr-SA in mouse heart tissues.
## Extended Data Table 1 | Data collection and refinement statistics

|                  | Resveratrol-TyrRS | L-Tyrosine-TyrRS |
|------------------|-------------------|------------------|
| **Data collection** |                   |                  |
| Space group      | P21212            | P21212           |
| Cell dimensions  |                   |                  |
| $a, b, c$ (Å)    | 73.97, 162.55, 35.0 | 73.97, 162.55, 35.0 |
| $\alpha, \beta, \gamma$ (°) | 90, 90, 90   | 90, 90, 90     |
| Resolution (Å)   | 37 - 2.1(2.15-2.09)* | 34.63 - 2.1(2.15-2.09)* |
| $R_{syn}$ or $R_{merge}$ | 27.1       | 44.7            |
| I/σI             | 4.1               | 4.6             |
| Completeness (%) | 98.6              | 98              |
| Redundancy       | 3.2               | 5.2             |
| **Refinement**   |                   |                  |
| Resolution (Å)   | 2.1               | 2.1             |
| No. reflections  | 25438             | 24513           |
| $R_{work}$, $R_{free}$ | 0.19/0.24   | 0.22/0.27       |
| No. atoms        | 2955              | 2858            |
| Protein          | 2653              | 2636            |
| Ligand/ion       | 16                | 12              |
| Water            | 284               | 238             |
| B-factors        |                   |                  |
| Protein          | 37.664            | 38.199          |
| Ligand/ion       | 43.2              | 42.3            |
| Water            | 47.3              | 48.4            |
| R.m.s deviations |                   |                  |
| Bond lengths (Å) | 0.018             | 0.017           |
| Bond angles (°)  | 2.017             | 2.008           |

*Highest resolution shell is shown in parenthesis.

Numerical parameters for the RSV-TyrRS and L-Tyrosine-TyrRS co-crystals are listed and compared side-by-side in separate columns.