Photoactivatable Hsp47: A tool to regulate collagen secretion & assembly

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ABSTRACT: Collagen is the most abundant structural protein in mammals and is crucial for the mechanical integrity of tissues. Hsp47, an endoplasmic reticulum resident collagen-specific chaperone, is involved in collagen biosynthesis and plays a fundamental role in the folding, stability and intracellular transport of procollagen triple helices. This article reports on a photoactivatable derivative of Hsp47 that allows regulation of collagen biosynthesis within mammalian cells using light. Photoactivatable Hsp47 contains a non-natural light-responsive tyrosine (o-nitro benzyl tyrosine – ONBY) at Tyr383 position of the protein sequence. This mutation renders Hsp47 inactive towards collagen binding. The inactive, photoactivatable protein was easily uptaken by cells within a few minutes of incubation, and accumulated at the endoplasmic reticulum (ER) via retrograde KDEL receptor-mediated uptake. Upon light exposure, the photoactivatable Hsp47 turned into functional Hsp47 *in situ*. The increased intracellular concentration of Hsp47 resulted in stimulated secretion of Collagen. The ability to promote collagen synthesis on-demand, with spatiotemporal resolution, and in disease state cells is demonstrated *in vitro*. We envision that photoactivatable Hsp47 will allow unprecedented fundamental studies of collagen biosynthesis, matrix biology, and inspire new therapeutic concepts in biomedicine and tissue regeneration.

■ INTRODUCTION

Collagen is a major component of the extracellular matrix, and the most abundant structural protein for the mechanical integrity of tissues[1]. A collagen molecule consists of a characteristic triple helix formed by three α-chains. *In vivo*, collagen molecules self-assemble to form collagen fibrils, beaded filaments or network structures depending on the collagen type. This assembly is the basis for achieving tissue-specific mechanical properties: rigid bone, compliant skin, or gradient mechanics in cartilage tissue[2]. The dynamics of collagen synthesis and deposition plays a crucial role in biological processes such as embryogenesis[3] and tissue regeneration[4], while it gets disregulated in pathologies like brittle-bone disease[5], fibrosis or tumor formation [6,7]. As the main underlying supportive structure for cells, the deposition of collagen typically precedes and guides the development of tissue architectures. For instance, in lungs, salivary and mammary glands, the spatial distribution and density of pre-deposited collagen directs the formation of branched tissue configurations[8]. In spinal cord[9] and arterial injuries,[10] it has been shown that initial collagen deposition by the underlying fibroblasts is vital for the remodeling of these tissues, resulting in their healing. While extensive efforts have been made to understand and replicate these phenomena, the existing molecular strategies to probe collagen deposition require the use of chemicals such as ascorbate[11], growth factors like TGF-β and FGF, and genetic modifications.[12-14] However, these molecules interfere with many cellular processes and do not allow localized manipulation at single cell level[15]. The possibility to regulate collagen deposition specifically, on demand and eventually in a spatially confined manner would allow precise studies in collagen-related cellular processes for fundamental and therapeutic purposes.

In this study, we have taken the first steps in this direction by developing and testing a molecular tool that enables spatiotemporal manipulation of collagen synthesis and deposition using light irradiation as remote trigger. We targeted a molecular chaperone, Hsp47 (Heat shock protein 47) that is known to be vital for collagen biosynthesis within cells[15-19]. It is a highly conserved, 47-kDa endoplasmic reticulum (ER)-resident protein known to bind collagen types I to V (and possibly others)[20], and plays a fundamental role in the folding, stability and intracellular transport of procollagen triple helices[18]. Procollagen is inherently unstable at physiological temperature and prone to intracellular degradation. Hsp47 protects it by stabilizing the triple helix, thereby providing a quality control mechanism for correct helical folding and assembly while simultaneously preventing premature aggregation of the helices[21]. Constitutive expression levels of

Hsp47 strongly correlate with the amounts of collagen being synthesized in the corresponding cells[19,22]. For example, expression of Hsp47 is up-regulated during the progression of various fibrotic lesions[23,24]. Studies have shown that suppression of Hsp47 expression can reduce accumulation of collagen and can delay the progression of fibrotic diseases in experimental animal models[25]. Alternatively, knocking out its expression in mouse embryos has been found to result in a lethal phenotype[26]. Collagen biosynthesis therefore strongly depends on the correct expression and function of Hsp47, and hence can be altered by interfering with Hsp47 activity. We hypothesized that a light-activatable version of Hsp47 would allow us to remotely control the biosynthesis and, subsequently, the deposition of collagen.

Controlling intracellular protein function by light is possible by introducing photoresponsive non-natural amino acids at the active site in the protein structure[27-30]. Hsp47 binds collagen at its typical Gly-X-Yaa amino acid repeats. Reported literature[16] has shown that when Arg occupies the Yaa position, the Asp385 rest in Hsp47 binds to the collagen triple helix strand by forming a salt bridge. Further stabilization of this complex is provided through Tyr383 and Leu381 through hydrophobic interactions. In its triple helical form, collagen forms a complex with Hsp47 with a total solvent-accessible surface area of 1000 ± 150 Å^2 buried between them, indicating tight interactions within the binding site[16]. Interestingly, while mutations in Hsp47's Asp385 and Leu381 seem to reduce its affinity with collagen, mutations in Tyr383 were shown to completely inhibit the interaction, indicating that this residue plays a major role in the binding [16,23]. Therefore, this position was identified as suitable site for the introduction of a photoactivatable rest to control Hsp47 binding to collagen with light. Luckily, synthetic tools to incorporate the non-natural light-responsive tyrosine (o-nitro benzyl tyrosine – ONBY) aminoacid on recombinant proteins have been previously developed.[31] It has been shown that replacement of Tyr by ONBY in Tyr-stabilized protein-protein or protein-DNA complexes lead to destabilization of the complex via steric hindrance and disruption of hydrophobic interactions due to the polar nitro group[31]. We hypothesized that a similar effect would occur when replacing Tyr383 by ONBY in Hsp47 structure to obtain the photoactivatable variant $H_{47Y<ONBY}$.

The delivery of recombinant proteins from the medium into the ER of cells was considered a challenge for our objective. Initially, microinjection of the protein into the ER was planned[32-34]. However, during the course of this study a KDEL receptor-mediated endocytosis mechanism capable of transporting proteins containing an ER retention motif (KDEL) to the ER was observed. By including KDEL motif in the recombinantly synthesized $H_{47<ONBY}$, the possibility to deliver it to the ER by simple incubation was attempted and successfully achieved. Using this strategy, collagen production and deposition in disease-state Hsp47-knocked out fibroblast cells was realized. This article demonstrates on demand deposition of collagen by activation of a new molecular tool with spatiotemporal control.

■ RESULTS AND DISCUSSION

Synthesis and Purification

A previously developed Hsp47 gene coding for residues 36-418 was used[16]. In order to improve Hsp47's solubility for heterologous expression in *E. coli*, an enhanced green fluorescent protein (EGFP) was genetically fused to its N-terminal[35]. This approach led to a significant increase in the synthesis yield (Table S1). A StrepII-tag was introduced at the C-terminal for affinity purification. This derivative of Hsp47 was successfully obtained in 207.8 µg yield from 200mL culture, as confirmed by absorbance with UV-Vis spectrophotometer, and was named H_{47N} (N refers to native). The o-nitrobenzyl tyrosine (ONBY) rest was incorporated at 383 position via a previously established bacteria engineering strategy[36,37] (see details in Fig S1 at the Supporting Information). An amber codon mutation TAG incorporated ONBY specifically at the 383rd position. This Hsp47 derivative was named $H_{47Y<ONBY}$.

In order to confirm the incorporation of ONBY, synthesis in the presence or absence of ONBY in the medium before induction was performed. In the presence of ONBY, the full protein would be expressed, whereas in the absence of ONBY, translation should terminate at the 383rd position and the StrepII-tag would not be included. Agarose beads with affinity towards the StrepII-tag were incubated with small amount of cleared lysates and purified Hsp47 control variants. The agarose beads became fluorescent within 5 mins when incubated with $H_{47Y<ONBY}$, indicating that the StrepII-tag had been incorporated at the C-terminus (ONBY present) whereas no fluorescence was observed in truncated version having no StrepII-tag (Fig 1B). The clear lysates were purified using StrepII-tag affinity purification and screened to affirm the presence of StrepII-tag. Western Blot was performed labeling StrepII-tag using Alexa488-Streptavidin after affinity purification which showed a clear fluorescent band when ONBY was present during synthesis, and no fluorescence was observed in the truncated version (Fig 1C). This result confirmed the incorporation of ONBY to $H_{47Y<ONBY}$. The yield of the synthesis was 43.56ng from 200ml culture. Two additional mutants of H_{47N} were developed as control proteins for further experiments: (i) $H_{47Y<R,}$ with Tyrosine mutated to Arginine at 383 position as an inactive version of H_{47N}[16]. (ii) H_{47Kdel} where the KDEL sequence at C terminus of H_{47N} was deleted, thereby preventing retrograde delivery of the protein into the ER. The yields of all the variants are included in Table S1.

Photoactivatable Hsp47 specifically binds to collagen upon activation

In order to verify the affinity of the different Hsp47 variants (H_{47N}, $H_{47Y<ONBY}$, $H_{47Y<R}$, H_{47Kdel}) for collagen, the obtained proteins were incubated with micropatterns of Collagen I on a substrate (see experimental section for details)[38] and imaged by fluorescence microscopy. H_{47N} and H_{47Kdel} showed fluorescence bands (green fluorescence due to EGFP tagged constructs) indicating

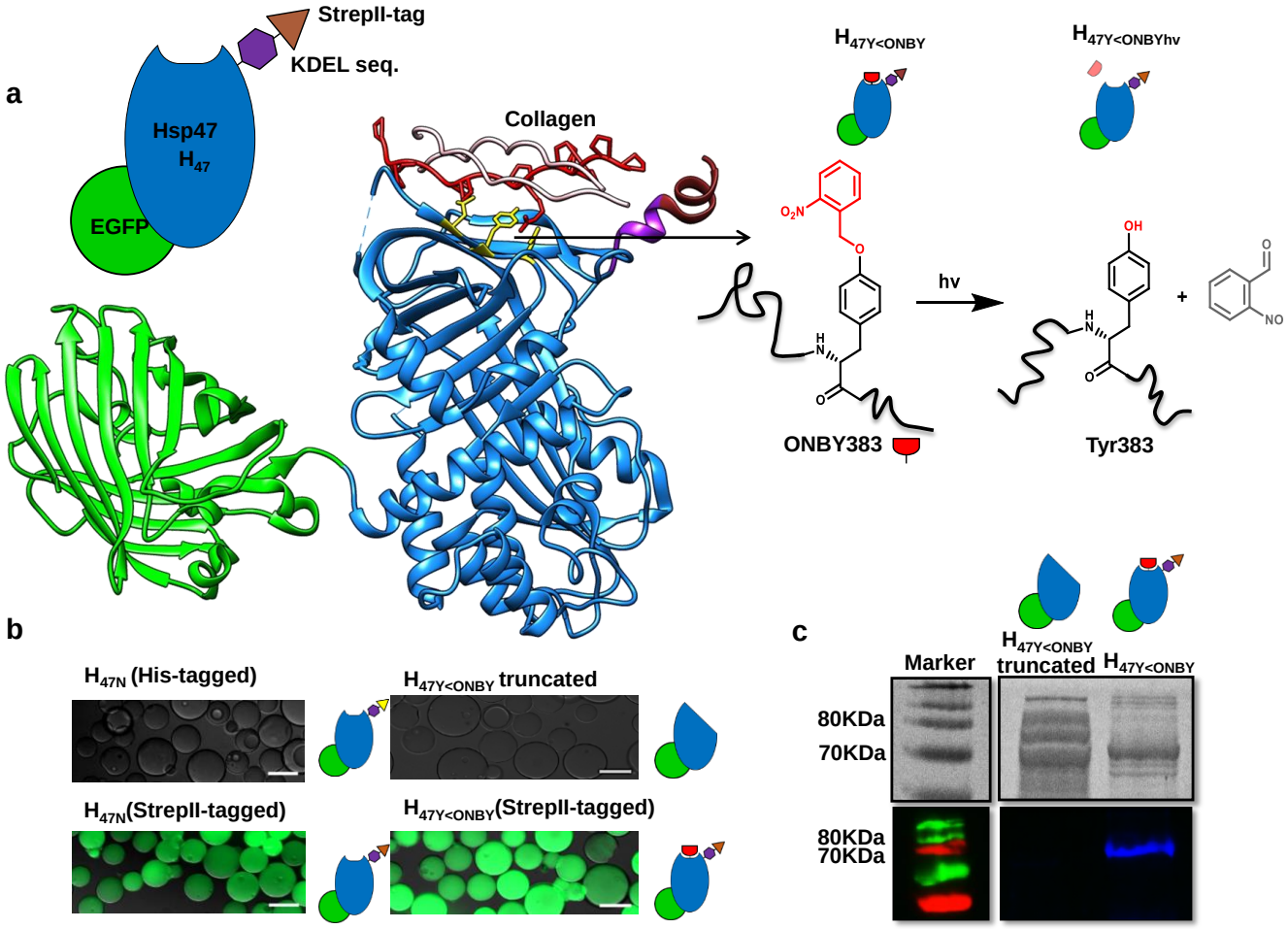

Figure 1. Structure and characterization of Photoactivatable Hsp47 ($H47_{Y<ONBY}$) **a**. Scheme showing the structure of Hsp47: Collagen peptide interaction [19] and the photocleavage reaction of ONBY. **b.** Microscopy images (merged phase contrast and epifluorescence green channels) of Strep-II-Tagged agarose beads incubated with supernatant of different Hsp47 variants showing the presence or absence of StrepII-tag in the protein structure (Scale bar 100µm). **c**.12% SDS Page gel and Western Blot of purified H47Y<ONBY (truncated and full) stained with Alexa 488 conjugated Streptavidin (blue).

interaction to collagen, whereas $H_{47Y<ONBY}$, the negative control $H_{47Y<R}$, and the EGFP did not bind to collagen (Fig 2A).Light irradiation of the $H_{47Y<ONBY}$ solution during incubation (*in situ* activation upon 10 sec exposure at 365 nm) lead to appearance of fluorescence bands. This result demonstrates that photochemical activation of $H_{47Y<ONBY}$ renders functional Hsp47 ($H_{47Y<ONBYhv}$), able to bind to collagen. Native PAGE-western blot analysis confirmed these results (Fig 2b). Co-localization of fluorescent antibody labeled bands of collagen and H_{47N} or light-activated $H_{47Y<ONBYhv}$ was observed. Conversely, $H_{47Y<ONBY}$, $H_{47Y<R}$ and EGFP did not co localize with the collagen band, demonstrating no collagen binding.

Hsp47 has been shown to prevent collagen fibrillogenesis *in vitro*. Previous reports have demonstrated that addition of Hsp47 in 2-fold molar excess to a 0.6 µM collagen solution in PBS delays collagen fibrillation at 34°C[17,39]. Similar fibrillation assays performed with H_{47N} solutions using turbidity measurements reproduced these results (see Fig S3). When using minimum essential medium (MEM) for the experiments, improved gelation was observed, and confirmed by rheology measurements (see Fig S4). MEM is considered more representative of physiological conditions for collagen association[40] and was used for further fibrillation assays with Hsp47 variants at concentrations between 0.1 µM and 1.2µM. $H_{47Y<ONBY}$, $H_{47Y<R}$ and EGFP did not show any effect on fibrillation kinetics of collagen solutions at any tested concentration (Fig 2C). Light-activated $H_{47Y<ONBYhv}$ reduced the rate of collagen fibrillation to a similar extent to H_{47N} at comparable concentrations. These results indicate that $H_{47Y<ONBYhv}$ enables light-triggered interference with lateral association of collagen triple helices and delay of fibril formation.

Hsp47 variants can be delivered into ER via KDEL receptor-mediated Endocytosis

Hsp47 is an ER resident protein with a C-terminal KDEL retention motif. This motif is recognized by KDEL receptor after Hsp47 release in the Golgi and is responsible for its retention in the ER[41].In fact, deletion of

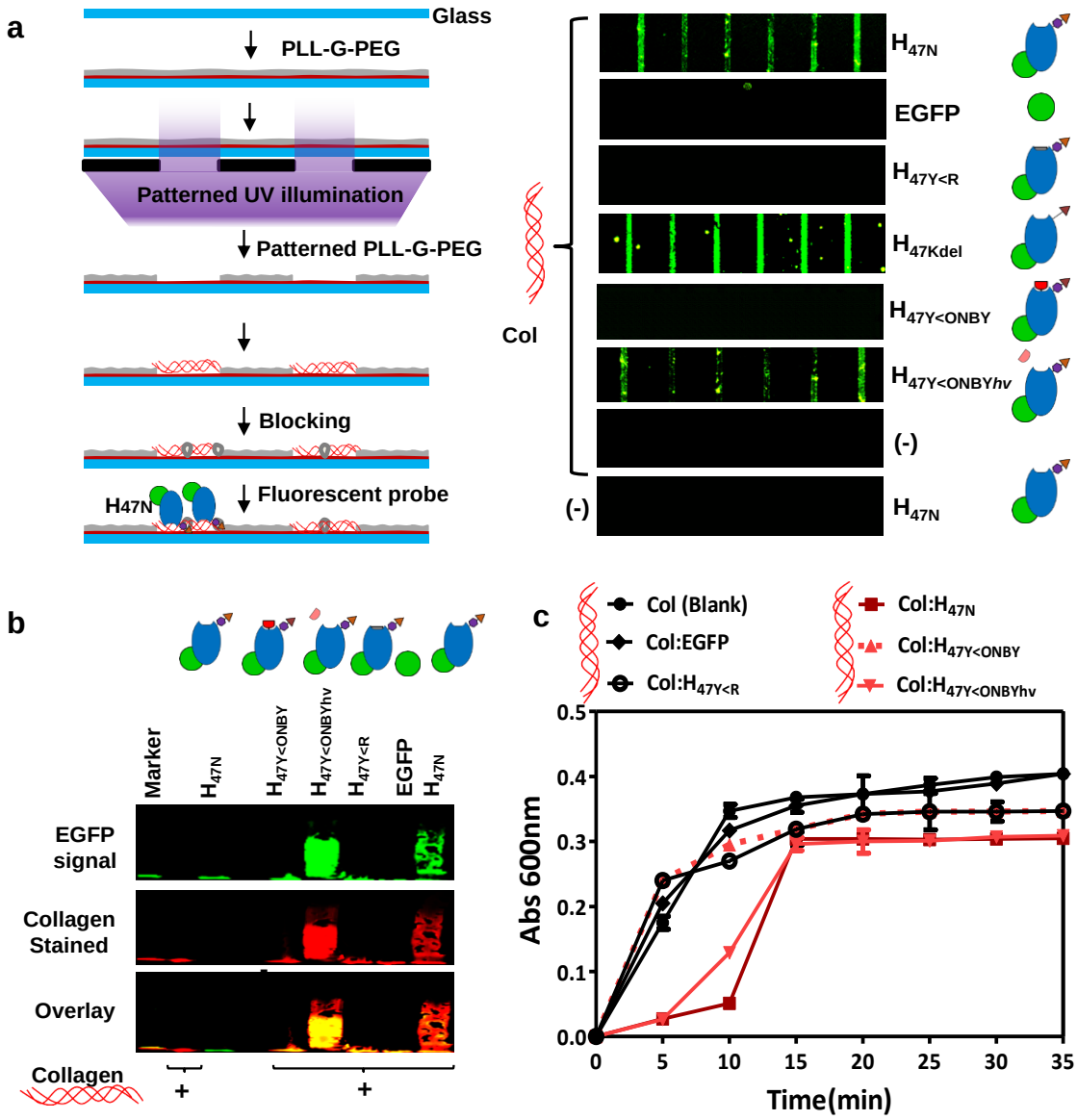

Figure 2. Photoactivatable Hsp47 affinity and function assay **a.** Schematic of affinity test using a protein micropattern designed to detect affinity of Hsp47 and its different variants to collagen. The corresponding fluorescence image of the binding assay shows the obtained results. **b.** Native PAGE Western Blot of rat tail Collagen Type 1 (200ug/mL, 0.6uM) mixed with $H47_N$, $H47_{Y<R}$, $H47_{Y<ONBYhv}$ or $H47_{Kdel}$ (1.2μM), demonstrating co-localization of Hsp47 variants with collagen in binding assays. **c.** Fibrillogenesis assay by turbidimetry measurements of collagen (200ug/mL, 0.6uM) mixed with $H47_N$, $H47_{Y<R}$, $H47_{Y<ONBYhv}$ or $H47_{Kdel}$ (1.2μM) at molar ratio 2:1(Hsp47: Collagen) at OD600 values (n=3 data point representing mean value of technical triplicates of each experiment with error bars representing standard deviation).

KDEL sequence has been shown to block the retention of Hsp47 into the ER[42]. The KDEL receptor is also present in the plasma membrane of cells, and has been shown to assist internalization of KDEL containing molecules from the extracellular space [43-45]. We tested if our Hsp47 variants could be delivered to ER using KDEL receptor-mediated endocytosis (Fig.3). For this purpose, L929 and MEFs fibroblasts were incubated with Hsp47 variants for 3 h and imaged. Interestingly, cells incubated with H_{47N}, $H_{47Y<R}$ and $H_{47Y<ONBYhv}$ showed co-localization of the EGFP signal (green in Fig.3) with the ER tracker dye (red), indicating successful uptake of the Hsp47 variant (see 10X-magnified images in Fig S7). Neither uptake of the H_{47Kdel} variant with deleted KDEL, nor uptake of EGFP was observed after 3 h incubation. In order to optimize the Hsp47 concentration for efficient delivery to fibroblast cells, experiments with concentrations of H_{47N} in the incubation medium between 0.01μM and 1μM were performed. 0.2-0.3μM concentration proved to be the best condition for the delivery of the recombinant constructs

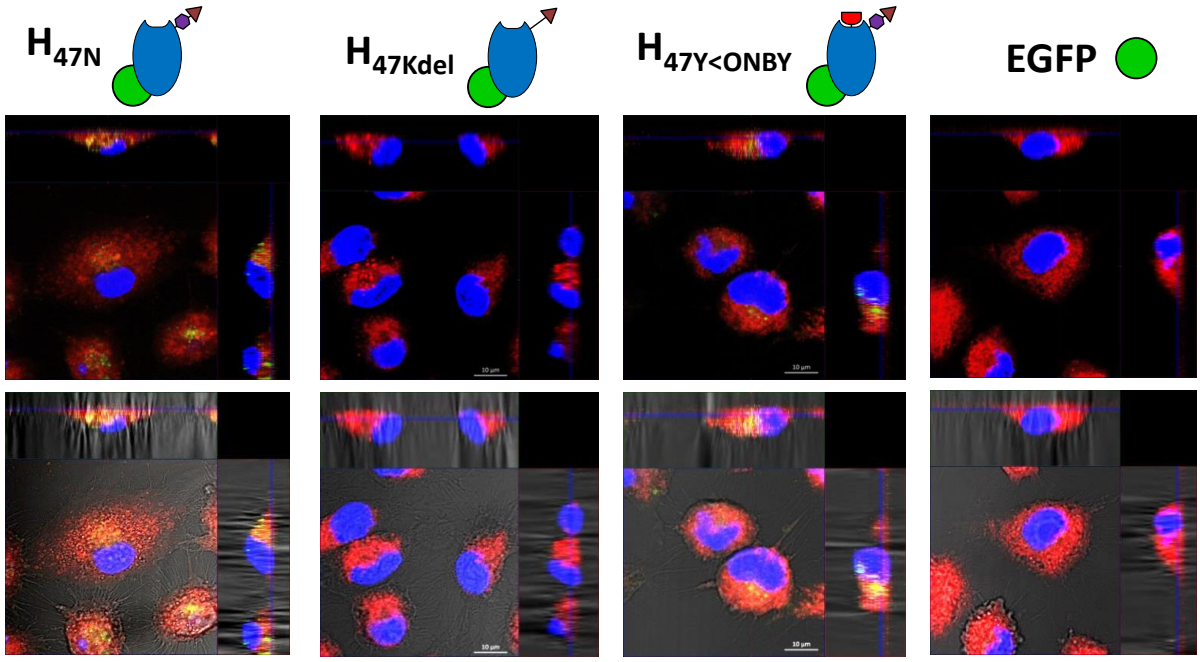

Figure 3. Photoactivatable Hsp47 delivery to ER via KDEL receptor-mediated endocytosis. Confocal Z stack images of L929 cells after incubation with Hsp47 variants showing co-localization of $H47_N$ or $H47_{Y<ONBY}$ signal and ER staining. No fluorescence was observed after incubation with EGFP or H47Kdel constructs (Blue: DAPI (Nucleus), Green: EGFP (Hsp47 variants) and Red: ER tracker Dye) Scale bar: 10 µm.

(see Fig S6). Protein concentrations above 0.3µM resulted in the formation of protein aggregates on the cell culture substrate. These results demonstrate that the photoactivatable $H_{47Y<ONBYhv}$ can be simply introduced into the ER of cells by short incubation, allowing easy experimental implementation of this tool for the study of Hsp47-specific roles in cellular pathways and collagen assembly.

Increase in collagen production in Hsp47 -/- cell cultures in response to photo activation of $H47_{Y<ONBY}$

Collagen biosynthesis is dependent on the expression level of functional Hsp47. To determine the bioactivity of up taken Hsp47 variants, we investigated collagen biosynthesis of Hsp47 -/- cells after incubation with the recombinant proteins. The deposited collagen was quantified using the Sirus Red assay, whose dye binds specifically to the [Gly-X-Y] n helical structure on fibrillar collagen types I to V [23]. Hsp47 –/– cells incubated with H_{47N} showed a significant increase in collagen production (approximately 15-20%). Hsp47 –/– cells incubated with $H_{47Y<ONBY}$ did not show increase in collagen production, in agreement with the lack of biofunctionality of $H_{47Y<ONBY}$ observed in previous experiments. Exposure of $H_{47Y<ONBY}$ treated cells with 405nm light *in situ* lead to an increase in collagen production. These results demonstrate biofunctionality of $H_{47Y<ONBY}$ intracellularly in response to light activation (Fig.4.b). Ascorbate (Vitamin C) is a widely used chemical inducer for collagen production at translational level by triple helix stabilization [46-48]. We compared collagen deposition in Hsp47 -/- cells treated with H_{47N}, with light exposed $H_{47Y<ONBY}$ and with ascorbate. Interestingly, we found higher levels of collagen production in H_{47N} and light exposed $H_{47Y<ONBY}$ incubated cells compared to ascorbate treated cells (Fig.4.b). Addition of ascorbate to cells containing H_{47N} or light exposed $H_{47Y<ONBYhv}$ had a synergistic effect for collagen production (see Fig 4.c). When Hsp47 +/+ cells were treated with H_{47N} and *in situ* activated $H_{47Y<ONBYhv}$ only a slight increase in collagen production was observed (see Fig 4.c).This might be due to saturation levels of Hsp47 in the ER. Interestingly, proliferation levels in Hsp47 –/– cultures treated with H_{47N} or *in situ* activated $H_{47Y<ONBY}$ slightly increased vs. non treated cultures (see Fig S5). Together, these results demonstrate the efficiency of exogenous $H_{47<ONBY}$ to up-regulate collagen biosynthesis upon light exposure (Fig.4).

Controlled light exposure allows spatial regulation of collagen production

Finally, we investigated the potential of $H_{47Y<ONBY}$ for spatiotemporal control of collagen production after *in situ* photoactivation of cell cultures. For this purpose an assay for imaging collagen deposition on the culture substrate was established. Hsp47 –/– cells were incubated with the different Hsp47 variants for 3 h. Then the medium was exchanged and cells were cultured for further 24 h, fixed and stained with Col1 Antibody for imaging deposited collagen type I on the culture substrates. Hsp47 –/– cells incubated with $H_{47Y<R}$, H_{47Kdel} or EGFP did not show any fluorescence signal, whereas cells incubated with H_{47N}

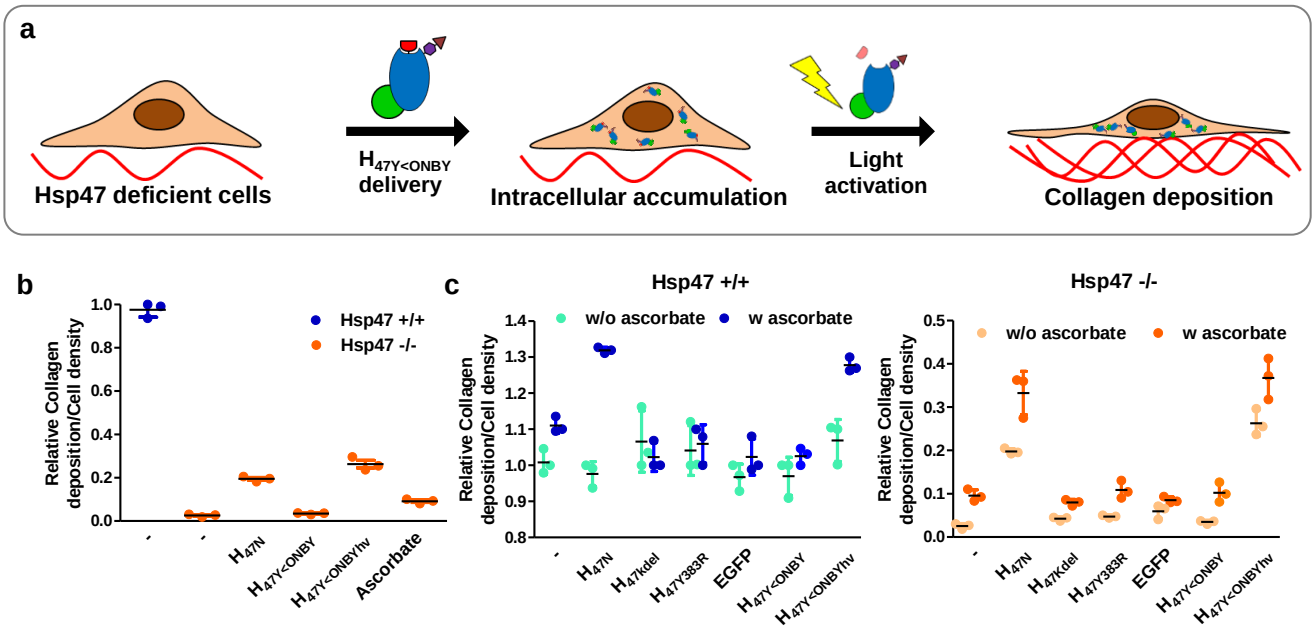

Figure 4. Stimulated collagen deposition using Photoactivatable Hsp47**. a.** Scheme showing photoactivation of H47Y<ONBY and stimulated collagen deposition in Hsp47 deficient cells. b. Relative collagen deposition in Hsp47 +/+ and Hsp47 -/- cultures treated with Hsp47 variants and ascorbate. c. Relative collagen deposition in Hsp47 +/+ and Hsp47 -/- cultures treated with Hsp47 variants with or without ascorbate. Collagen deposition was calculated using quantified data of Sirus Red Assay at 570 nm (n=3 data point representing mean value of technical triplicates of each experiment with whisker plots representing standard deviation).

showed a clear fluorescence corresponding to collagen production (Fig 5.B). H_{47N} and light exposed $H_{47Y<ONBY}$ showed EGFP fluorescence located at the ER, indicating that the recombinant protein delivered was still present intracellularly after 24 h(Fig 5). This is in agreement with the reported >24 h half-life of Hsp47[49]. $H_{47Y<R}$ did not show EGFP fluorescence after 24 h, which may be due to degradation because of lack of functional activity over time (Fig.5.B). Altogether these results demonstrate that $H_{47Y<ONBY}$ can be used in cell cultures to increase collagen production at a selected time point. In order to test the spatial resolution of the light-induced collagen deposition,1.8x1.2mm² areas of the Hsp47 -/- cell culture previously incubated with $H_{47Y<ONBY}$ were irradiated for 30 seconds at 405 nm an hour after medium exchange. Higher dose results in cell damage[50]. Cells at the exposed areas showed significant higher collagen deposition (Fig 5.A). Hsp47-/- and Hsp47 +/+ cells lacking exogenous $H_{47Y<ONBY}$ did not show any increase in collagen staining upon exposure, indicating that UV irradiation by itself had no effect on collagen production (Fig.5). These results demonstrate the possibility to use $H_{47Y<ONBY}$ to photoregulate collagen biosynthesis in cell cultures and build spatially defined collagen networks.

■ CONCLUSION

A photoactivatable variant of the collagen-specific protein Hsp47 has been developed by incorporating a photoactivatable Tyr rest at the 383rd amino acid of Hsp47, which is relevant for collagen binding. This recombinant protein could be effectively delivered to the ER of fibroblasts via KDEL receptor-mediated endocytosis. *In situ* light exposure allowed light-mediated increase of functional Hsp47 concentration inside the cells, and consequently localized upregulation of collagen production and deposition in cellular cultures. We envision that this tool will allow unprecedented studies of collagen synthesis and early assembly. Photoactivation at submicron level will allow precise control and simultaneous monitoring of collagen-related intracellular events. Moreover, photoactivatable Hsp47 might inspire new therapeutic concepts for treating collagen-related defects like Osteogenesis imperfecta, Ehlers-Danlos Syndrome and Epidermolysis Bullosa by promoting correct folding of collagen. Finally, the possibility of external upregulation of collagen synthesis and deposition might be advantageous for tissue regeneration and rebuilding of the extracellular scaffold.

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Notes

The authors declare no competing financial interest.

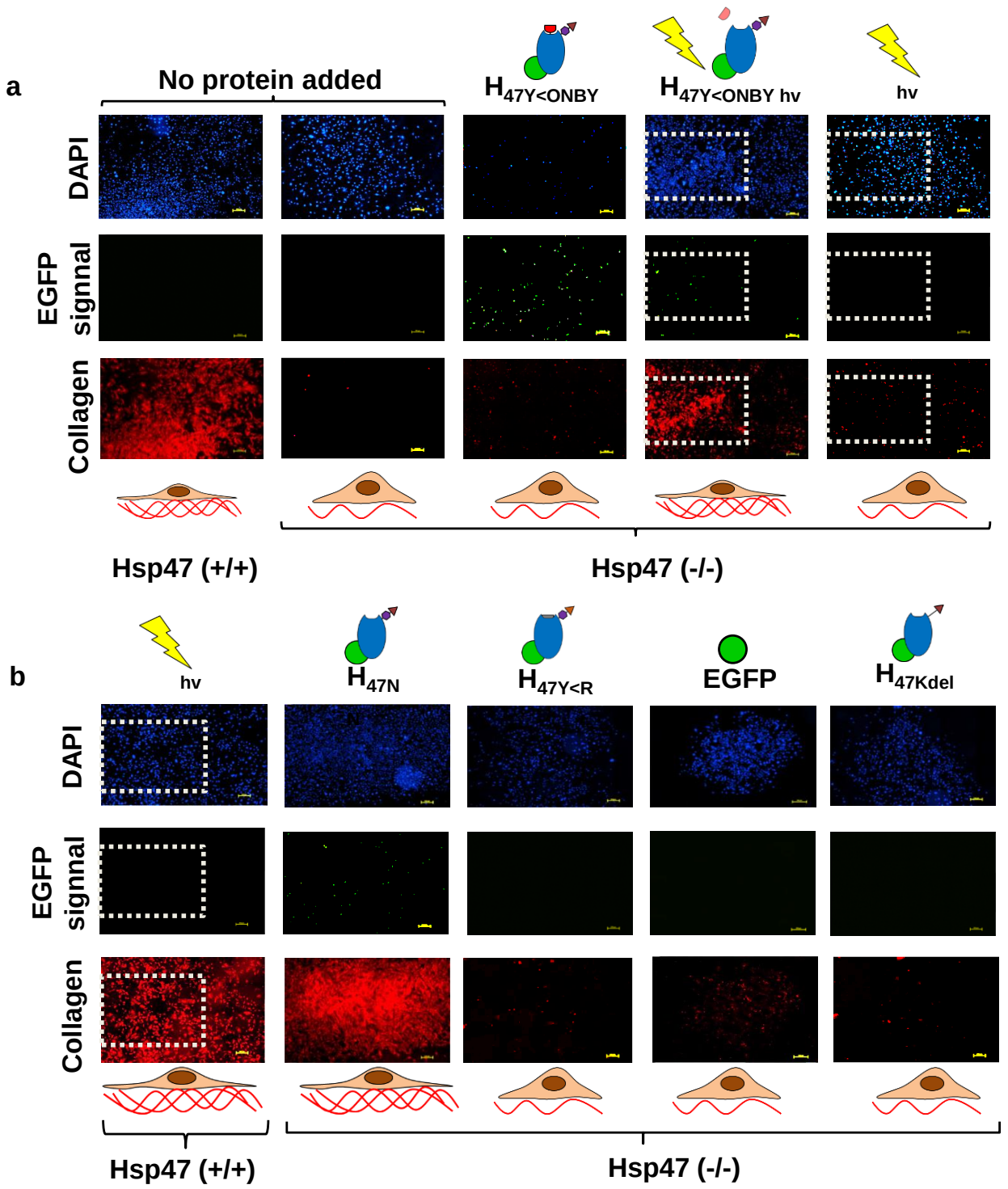

Figure5.Localized induction of collagen deposition by photoactivation of Photoactivatable Hsp47. Immunostaining of MEF Hsp47 +/+ and –/– cultures 24 h after treatment with different Hsp47 variants and controls Col1 Antibody staining in red, Hsp47 variants in green (EGFP), and nuclei in blue (DAPI). **a.** Hsp47 +/+ and –/– having no protein delivery were used as controls. The light exposed areas (1.8x1.2 mm^2) are highlighted with dotted square. Irradiation wavelength was 405 nm. **b.** Hsp47 +/+ cells incubated with other inactive mutants did not show enhanced collagen production, whereas cells incubated with H_{47N} showed higher collagen levels. All the experiment were done in triplicates. Scale bar: 250um

Author Contributions

AdC conceived the outline of the project. SK and JP designed the synthetic strategy. AdC and SK supervised the experimental and analytical work. EK performed the experiments with assistance from CM and inputs from SK for the biochemical and cellular assays. EK and MH contributed to the analysis and data representation of the cellular experiments. EK drafted the manuscript with further proof reading and modifications from AdC, SK, JP and MH.

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■ REFERENCES

(1) Brinckmann J. (2005) Collagens at a Glance. In: Brinckmann J., Notbohm H., Müller P.K. (eds) Collagen. Topics in Current Chemistry, vol 247. Springer, Berlin, Heidelberg.

(2) Boerboom, R. A.; Krahn, K. N.; Megens, R. T. A.; van Zandvoort, M. A. M. J.; Merkx, M.; Bouten, C. V. C., *J. Struct. Biol.* **2007,** *159*, 392.

(3) Varner, V. D.; Nelson, C. M., *Development* **2014,** *141*, 2750.

(4) Glowacki, J.; Mizuno, S., *Biopolymers* **2008,** *89*, 338.

(5) Byers, P. H.; Pyott, S. M. In *Annual Review of Genetics* 2012; Vol. 46, p 475.

(6) Wynn, T. A.; Ramalingam, T. R., *Nat. Med.* **2012,** *18*, 1028.

(7) Schedin, P.; Keely, P. J., *Cold Spring Harbor Perspect. Biol.* **2011,** *3*, a003228.

(8) Brownfield, D. G.; Venugopalan, G.; Lo, A.; Mori, H.; Tanner, K.; Fletcher, D. A.; Bissell, M. J., *Curr. Biol.* **2013,** *23*, 703.

(9) Wehner, D.; Tsarouchas, T. M.; Michael, A.; Haase, C.; Weidinger, G.; Reimer, M. M.; Becker, T.; Becker, C. G., *Nat. Commun.* **2017,** *8*, 126.

(10) Sluijter, J., *Cardiovasc. Res.* **2004,** *61*, 186.

(11) S. Murad, D. G., K. A. Lindberg, G. Reynolds, A. Sivarajah, S. R. Pinnell, *Proc. Natl. Acad. Sci. U S A* **1981,** *78*, 2879.

(12) Yeung, C.-Y. C.; Garva, R.; Pickard, A.; Chang, J.; Holmes, D. F.; Lu, Y.; Mallikarjun, V.; Swift, J.; Adamson, A.; Calverley, B.; Meng, Q. J.; Kadler, K. E., *bioRxiv 304014* **2018**.

(13) Pickard, A.; Chang, J.; Alachkar, N.; Calverley, B.; Garva, R.; Arvan, P.; Meng, Q. J.; Kadler, K. E., *bioRxiv 348078* **2018**.

(14) Pickard, A.; Adamson, A.; Lu, Y.; Chang, J.; Garva, R.; Hodson, N.; Kadler, K., *bioRxiv 331496* **2018**.

(15) Ohnishi, S.; Sumiyoshi, H.; Kitamura, S.; Nagaya, N., *FEBS Lett.* **2007,** *581*, 3961.

(16) Widmer, C.; Gebauer, J. M.; Brunstein, E.; Rosenbaum, S.; Zaucke, F.; Drogemuller, C.; Leeb, T.; Baumann, U., *Proc. Natl. Acad. Sci. U. S. A.* **2012,** *109*, 13243.

(17) Thomson, C. A.; Ananthanarayanan, V. S., *J. Biol. Chem.*

(18) Ishida, Y.; Nagata, K. In *Methods in Enzymology* 2011; Vol. 499, p 167.

(19) Ishida, Y.; Kubota, H.; Yamamoto, A.; Kitamura, A.; Bachinger, H. P.; Nagata, K., *Mol. Biol. Cell* **2006,** *17*, 2346.

(20) Natsume, T.; Koide, T.; Yokota, S.; Hirayoshi, K.; Nagata, K., *J. Biol. Chem.* **1994,** *269*, 31224.

(21) Koide, T.; Asada, S.; Takahara, Y.; Nishikawa, Y.; Nagata, K.; Kitagawa, K., *J. Biol. Chem.* **2006,** *281*, 3432.

(22) Kuroda, K.; Tsukifuji, R.; Shinkai, H., *J. Invest. Dermatol.* **1998,** *111*, 1023.

(23) Shinya Ito, K. O., Koh Takeuchi, Motoki Takagi, Masahito Yoshida, Takatsugu Hirokawa, Shoshiro Hirayama, Kazuo Shin-ya, Ichio Shimada, Takayuki Doi, Naoki Goshima, Tohru Natsume; Nagata, a. K., *J. Biol. Chem.* **2017,** *292*, 20076.

(24) Wynn, T. A., *J. Pathol.* **2008,** *214*, 199.

(25) Hagiwara, S.; Nakamura, K.; Hamada, H.; Sasaki, K.; Ito, Y.; Kuribayashi, K.; Sato, T.; Sato, Y.; Takahashi, M.; Kogawa, K.; Kato, J.; Terui, T.; Takayama, T.; Matsunaga, T.; Taira, K.; Niitsu, Y., *J. Gene Med.* **2003,** *5*, 784.

(26) Nagai, N.; Hosokawa, M.; Itohara, S.; Adachi, E.; Matsushita, T.; Hosokawa, N.; Nagata, K., *J Cell Biol.* **2000,** *150*, 1499.

(27) Nguyen, D. P.; Mahesh, M.; Elsasser, S. J.; Hancock, S. M.; Uttamapinant, C.; Chin, J. W., *J. Am. Chem. Soc.* **2014,** *136*, 2240.

(28) Hemphill, J.; Chou, C.; Chin, J. W.; Deiters, A., *J. Am. Chem. Soc.* **2013,** *135*, 13433.

(29) Xuan, W.; Yao, A.; Schultz, P. G., *J. Am. Chem. Soc.* **2017,** *139*, 12350.

(30) Ge, Y.; Fan, X.; Chen, P. R., *Chem. Sci.* **2016,** *7*, 7055.

(31) Chou, C.; Deiters, A., *Angew. Chem., Int. Ed. Engl.* **2011,** *50*, 6839.

(32) FitzHarris, G.; Marangos, P.; Carroll, J., *Dev. Biol.* **2007,** *305*, 133.

(33) Lippincott-Schwartz, J., *J Cell Biol.* **1995,** *128*, 293.

(34) Miles, S.; McManus, H.; Forsten, K. E.; Storrie, B., *J. Cell. Biol.* **2001,** *155*, 543.

(35) Snapp, E., *Curr. Protoc. Cell Biol.* **2005,** *Chapter 21*, 21 4 1.

(36) Arbely, E.; Torres-Kolbus, J.; Deiters, A.; Chin, J. W., *J. Am. Chem. Soc.* **2012,** *134*, 11912.

(37) Deiters, A.; Groff, D.; Ryu, Y.; Xie, J.; Schultz, P. G., *Angew. Chem., Int. Ed. Engl.* **2006,** *45*, 2728.

(38) Strale, P. O.; Azioune, A.; Bugnicourt, G.; Lecomte, Y.; Chahid, M.; Studer, V., *Adv. Mater.* **2016,** *28*, 2024.

(39) Abdul-Wahab, M. F.; Homma, T.; Wright, M.; Olerenshaw, D.; Dafforn, T. R.; Nagata, K.; Miller, A. D., *J. Biol. Chem.* **2013,** *288*, 4452.

(40) Piechocka, I. K.; van Oosten, A. S.; Breuls, R. G.; Koenderink, G. H., *Biomacromolecules* **2011,** *12*, 2797.

(41) Mirco Capitani; Sallese, M., *FEBS Lett.* **2009**, 3863.

(42) Satoh, M., *J Cell Biol.* **1996,** *133*, 469.

(43) Björn Becker, M. R. S., Domenik Rammo, Tobias Bubel, Ludger Santen & Manfred J. Schmitt, *Sci. Rep.* **2016**.

(44) Alice Chen, T. H., Carole Mikoryak, Rockford K. Draper *Biochim. Biophys. Acta* **2002**, 124.

(45) Björn Becker, A. B., Esther Gießelmann, Julia Dausend, Domenik Rammo, Nina C. Müller, Emilia Tschacksch, Miriam Steimer, Jenny Spindler, Ute Becherer, Jens Rettig, Frank Breinig & Manfred J. Schmitt, *Sci. Rep.* **2016**, 1.
(46) Gilkes, D. M.; Chaturvedi, P.; Bajpai, S.; Wong, C. C.; Wei, H.; Pitcairn, S.; Hubbi, M. E.; Wirtz, D.; Semenza, G. L., *Cancer Res.* **2013,** *73*, 3285.
(47) Makareeva, E.; Leikin, S., *PLoS One* **2007,** *2*, e1029.
(48) Schwarz, R. I., *Biochem.Biophys.Rep.* **2015,** *3*, 38.
(49) Nakai, A., *J Cell Biol.* **1992,** *117*, 903.
(50) Ramakrishnan, P.; Maclean, M.; MacGregor, S. J.; Anderson, J. G.; Grant, M. H., *Toxicol. In Vitro* **2016,** *33*, 54.

Supporting Information

Photoactivatable Hsp47: A tool to regulate collagen secretion & assembly.

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Experimental Procedures

Cloning and purification

The psfGFP150TAGPylT-His6pBad plasmid encoding for MbPylT and C-terminal hexahistidine-tagged sfGFP, with an amber stop codon at position 150 (pBad), and pBK-ONBYRS expressing MbPylS mutant was a gift from Prof. Dr. Jason Chin, Medical Research Council Laboratory of Molecular Biology, University of Cambridge, UK[1]. A construct encoding amino acids 36–418 of the canine Hsp47 (canine SERPINH1 mRNA, NCBI accession NM_001165888), cloned into the pJExpress vector with a C-terminal Strep II tag was gift from Prof. Dr. Ulrich Baumann, University of Cologne,Germany[2].

The canine-derived synthetic Hsp47 gene from pJExpress plasmid was cloned in pET28a plasmid at the C terminus of EGFP gene using EcoRI and XhoI sites and transformed in NEB5α cells using manufacturer's protocol (NEB C29871). Colonies were picked up and insert screening was performed using colony PCR and confirmed with sequencing following which positively cloned constructs were selected and were further cloned into psfGFP150TAGPylT-His6pBad plasmid at NcoI and XhoI site to develop pEGFPHsp47StrepIITagpBad construct. This construct was used to optimize protein expression conditions and was used as a backbone for developing the final construct pEGFPHsp47TAG 383 StrepIITagpBad construct (described later). Colonies were picked up and insert screening was performed using colony PCR approach and sequencing following which positively cloned constructs were selected and transformed into One shot top10 cells for protein expression using strepTag2 purification. Transformed cells were grown to OD600 of 0.8 in LB medium containing kanamycin (Kan) (25µg/ml) and tetracycline (Tet) (12.5µg/ml). These cells were pelleted and the medium was exchanged in sterile environment with pre-warmed Terrific broth containing kanamycin (25µg/ml) and tetracycline (12.5µg/ml) and protein expression was induced after half an hour with 0.2 % arabinose at 37°C / 250 rpm. After induction, the cultures were incubated for 1 hour at 37°C / 250 rpm following which it was kept at 25-30°C / 180

rpm cells overnight. The cultured cells were harvested and pellets stored at -80 °C. Cells were resuspended in lysis buffer (50 mM Tris ·HCl (pH 7.5), 150 mM NaCl, 100 µ M PMSF, 4 mM DTT) and lysed by sonication. Cleared lysates were loaded onto 1 ml Streptactin Superflow high capacity binding column (IBA) and eluted with 2.5 mM (D)-desthiobiotin in lysis buffer excluding PMSF.

For the expression of photoactivatable Hsp47 ($H_{47Y<ONBY}$), *E. coli* TOP10 cells co-transformed with pBK-ONBYRS and pEGFPHsp47TAG 383 StrepTagIIpBad (encoding for N terminal EGFP tagged cHsp47 and C-terminal strepIItag with an amber stop codon (TAG) at position 383). The TAG mutation at 383rd position was incorporated with NEB Q5 site-directed mutagenesis kit using manufacturer's protocol (NEB E0554S). Transformed cells were incubated overnight with shaking at 37°C in LB supplemented with Kan (25µg/ml) and Tet (12.5µg/ml). The overnight culture was diluted 1:100 in two separate volumes of terrific broth (TB) supplemented with the same concentration of antibiotics and incubated with shaking at 37°C. At OD600= 0.8 bacteria were isolated by centrifugation and re-suspended in equal volumes of warm TB supplemented with the same concentration of antibiotics, in the presence or absence of 0.4 mM of O-[(2-Nitrophenyl)methyl]-L-tyrosine hydrochloride (Santa Cruz Biotech). After 30 min, protein expression was induced with the addition of arabinose at a final concentration of 0.2% (w/v). Induction studies were performed with different temperature conditions after induction incubation for 1 hour at 37°C / 250 rpm following with 25-30°C / 180 rpm cells overnight was chosen as optimized expression condition. The cultured cells were harvested and pellets stored at -80 °C. Cells were resuspended in lysis buffer (50 mM Tris ·HCl (pH 7.5), 150 mM NaCl, 100 µ M PMSF) and lysed by sonication. 20µL of the clear lysates were taken incubated with 5 µL of Strep II tagged agarose beads (IBA Life sciences) for 5 min. Previously purified H_{47N} (His-taggged) and H_{47N} (strepIItaggged) were used as controls. The beads were then spun down at 1000 rcf for 30 s and washed 2 times with PBS. For purification, cleared lysates were loaded onto 1 ml Strepactin Superflow high capacity binding column (IBA Life sciences) and eluted with 2.5 The bead solutions were placed in 96 well-plate wells and imaged using the Nikon Ti-Eclipse microscope. mM (D)-desthiobiotin in lysis buffer excluding PMSF. The

soluble fraction for both H_{47N} and $H_{47Y<ONBY}$ was concentrated and purified further by gel filtration (Superdex S75; GE Healthcare) in buffer A [20 mM Hepes (pH 7.5), 300 mM NaCl, 4 mM DTT]. The strepIItag at C terminus was chosen as a selection marker for ONBY incorporation which was confirmed by Alexa 488 labeled Streptavidin against the strepIItag at the C terminus of $H_{47Y<ONBY}$. The bacteria which were not fed with ONBY in the medium produced truncated or incomplete $H_{47Y<ONBY}$ because of the TAG mutation. A Western Blot was performed by running protein samples on SDS page. The 12% SDS PAGE gel was transferred using blotting chamber to PVDF membrane. The Blotted PVDF Membrane was blocked with Blocking buffer (0.5% milk powder in PBST (0.1 w/v)) for 20 mins. The excess blocking buffer was washed of three times using PBST (0.1 w/v) and then stained using labeled Fluorescent Alexa 488 streptavidin with a dilution of 1:500 for 20 mins. The excess streptavidin was washed of three times using PBST (0.5 w/v) and image RGB Blot in Gel doc.

In order to have controls for protein and cell based assays the N-terminal E GFP tagged cHsp47 pet28a plasmid were modified to develop variants. The variants were **a.** EGFP-Hsp47 383 Y<R pet28a (encoding $H_{47Y<R}$ with Tyrosine mutated to Arginine at 383rd position).**b.** KDEL del EGFP-Hsp47 pet28a (encoding H_{47Kdel} without KDEL sequence at the C terminus). All the mutants were developed using NEB site-directed mutagenesis kit (NEB E0554S) and transformed into NEB 5 α cells for plasmid extraction and sequencing analysis. Positive clones were transformed in BL21 (DE3) Clear coli cells for His-tagged Ni-NTA chromatography. Expression was induced in BL21 (DE3) cells grown to an OD600 of 0.6–0.7 by adding 0.5 mM isopropyl-β-d-thio-galactoside and shaking overnight at 25 °C. Cells were resuspended in lysis buffer (300 mM NaCl, 10 mM imidazole, 20 mM Tris, pH 8) and lysed by sonication. Cleared lysate was purified by Ni-NTA affinity chromatography (Ni-NTA superflow; Qiagen). The soluble fraction was concentrated and purified further by gel filtration (Superdex S75; GE Healthcare) in buffer A [20 mM Hepes (pH 7.5), 300 mM NaCl, and 4 mM DTT]. EGFP-pet28a was purified using same approach.

Collagen micropatterned affinity binding assay with Hsp47 variants

Collagen micropatterns were made by adopting a reported protocol[3,4]. Glass cover-slips were cleaned by 5 min air plasma treatment (Harrick Plasma, Ithaca, NY, USA). In a 20×20x0.25 μm^3 Poly(dimethylsiloxane) (PDMS) membrane having square, nine 3x3 mm^2 wells. The PDMS membrane was placed on the plasma treated cover-slip. In each 3x3 mm^2 well, 5 µL of a 0.1 mg/mL PLL-g-PEG (PLL (20)-g [3.5]-PEG (5), SuSoS AG, Dübendorf, Switzerland) solution in phosphate buffered saline (PBS) was incubated for 1 h. Next, the wells were incubated with 5uL of photo initiator (4-benzoylbenzyltrimethyl ammonium chloride) solution (custom synthesis by Sigma-Aldrich outsourced to SinoChem, China) for 1 minute. A wide field mass less UV projection was employed for making 10 µm patterns for 10 sec having 50%intensity at 365 nm wavelength.The optical projection system is based on a standard epi-fluorescence inverted microscope (Nikon Ti-Eclipse microscope, Nikon Instrument, France) coupled with a Digital Light Processing device (Texas-Instrument DLP Discovery 4100 UV) including a DMD (PRIMO unit) to generate spatially modulated excitation patterns[3,4]. The UV exposure cleaves the PEG chains at the exposed sites on the substrate. After extensive rinsing with PBS, the micro wells were filled with 10 µL of 200ug/mL rat tail collagen I (Fisher Scientific) in H_2O (Diluted from Rat tail collagen stock of 3mg/mL). Collagen adsorbs on the UV exposed areas. The wells with the collagen patterns were incubated for 10 mins with 1µM solutions of H_{47N}, $H_{47Y<R,}$ $H_{47Kdel,}$ $H_{47Y<ONBY}$ or EGFP in PBS. For *in situ* photo activation of $H_{47Y<ONBY}$, the well filled with $H_{47Y<ONBY}$ was irradiated 10 s with a PRIMO unit at 365 nm and 50% intensity. Fluorescence imaging was done using a Nikon Ti-Eclipse microscope. All the experiments were done in triplicate.

Native PAGE Western Blot

3mg/mL rat tail collagen Type 1 (Fischer Scientific) was diluted in sterile D/W to a concentration of 200ug/mL. H_{47N}, $H_{47Y<R}$, $H_{47Y<ONBY}$, $H_{47Y<ONBYhv}$, H_{47Kdel} and EGFP solutions at 1.2μM concentration were mixed with collagen solutions in 2:1 ratio. The samples were incubated overnight at 4°C. 20ul

were run in Native PAGE 4-16% Bis-Tris Protein Gel (Invitrogen).The proteins were blotted on PVDF membrane for WB. Anti-Collagen Type I (RABBIT) Antibody - 600-401-103-0.1 (Rockland antibodies and assays), Secondary; Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647(Invitrogen) in 1:500 dilution were used for WB. All the experiments were done in triplicate.

Fibrillation Assay

Collagen Type I solution (3mg/mL, Fisher Scientific) was diluted to 0.4 mg/ml with minimal essential medium (MEM) Buffer (1x) and pH was adjusted to 7.5 with 1 M sodium hydroxide (Sigma-Aldrich). This process has to be done within 4 min to prevent premature polymerization.[5] The collagen solution was mixed with 1.2 mM solutions of H_{47N}, EGFP, $H_{47Y<R}$, $H_{47Y<ONBY}$ or $H_{47Y<ONBYhv}$ in MEM at molar ratio 1:2. The final concentration of collagen was 0.2 μg/ml(0.6 μM). A 0.2 μg/ml collagen solution was also used as a positive control. 100μl of the Col/Hsp47 variant mixture was transferred to a pre-cooled 96 well plate and absorbance at 600 nm was recorded with a Spectra Max UV plate reader during 40 min. The optical density of the Collagen solution at 600 nm was measured under different conditions. This wavelength does not interfere with the UV spectrum of ONBY. The $H_{47Y<ONBY}$ solution was irradiated at 365 nm for 5 mins for activation. All the experiments were done in triplicate.

KDEL mediated delivery of Hsp47 variants to fibroblasts

L929 fibroblasts (ATCC CRL-6364) and Mouse Embryonic Fibroblast (MEF) were seeded on 12 well μ-Slide Angiogenesis (Ibidi) with DMEM GlutaMax and RPMI GlutaMax medium containing 10% fetal bovine serum (FBS; Gibco), ascorbic acid phosphate, and antibiotics (20K cells per well). After 30

mins cells were incubated with 0.3 µM solution of Hsp47 variants in DMEM medium. After 3 hours incubation the medium was removed and cells were washed once with sterile Assay buffer (1X). Dual staining was prepared by mixing 1 µl of ER tracker (ER Staining Kit - Red Fluorescence - Cytopainter (ab139482)) with 1 µl of DAPI in 1 ml of ER Assay buffer (1X) provided in the kit. The cells were incubated with 60ul of Dual staining solution per well at 37°C for ½ hr. Cells were washed with Assay buffer (1X) once. Cells were fixed with 4% PFA for 10 mins. Washing was performed 3 times with Assay buffer (1X). All the experiments were done in triplicate.

Sirus Red Assay for quantification of Collagen deposition and Immunostanining:

Hsp47+/+ and Hsp47-/- MEFs derived from Lethal Mouse Embryos were gifted by Prof. Dr. Kazuhiro Nagata, Kyoto Sangyo University, Japan[6-11]. Cells were cultured in 24 well plate with high glucose DMEM Glutamax(Gibco) containing 10% FBS (Gibco), w/o or w/ ascorbic acid phosphate (0.1mM) (Ascorbate), and antibiotics for 24 hours (25K cells per well). Cells were incubated with 0.3µM solutions of Hsp47 variants or EGFP in medium for 3 h, followed by medium exchange w/ or w/o ascorbate, and cultured for additional 24 h. After PBS washing, cells were fixed using Bouin solution (75% picric acid, 10% formalin, and 5% acetic acid) (Sigma HT10132). Collagen deposited in the wells was stained by incubation with 0.1% Sirius red in picric acid (ab150681) for 1 hr and washing with 0.01 N HCl and 0.1 N NaOH[10]. Collagen deposition was quantified at 570 nm using a Biolumin960k spectrophotometer. Results were normalized by taking Hsp47 (+/+) as 1. Cell Counting was performed using Image J with references to DAPI stained cells. All the analysis was performed in triplicate.

For investigating the potential of $H_{47Y<ONBY}$ for spatiotemporal controllHsp47+/+ and Hsp47-/- cells (25K cells per well) were cultured in 24 well plates with high glucose DMEM Glutamax (Gibco) containing 10% FBS (Gibco), 0.1 mM ascorbate, and antibiotics. After 24 h, cells were incubated with 0.3 µM solution of H_{47N}, $H_{47Y<ONBY}$, H_{47Kdel} or EGFP in DMEM medium for 3 h. Afterwards the solution was exchanged by DMEM Glutamax with ascorbate and cells were cultured for further 24 h. The wells containing $H_{47Y<ONBY}$ were irradiated at 405 nm using Ti-Nikon Microscope with laser power of 50%

intensity for 30 seconds (~37mW exposure over the toal exposed area i.e1.7W/cm^2 (measured using ILX Lightwave OMM-68108 Optical Multimeter) which is equivalent to energy of 51.2 J/cm^2) with 10X objective exposed in an area of about 1.8x1.2mm^2, an hour after medium exchange. After 24 hours cells were fixed and immuno stained against collagen with Primary; Rabbit polyclonal anti-type I collagen, 600-401-103-0.1 (Rockland; dilution 1:200), Secondary; Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647, A-21245 (dilution 1:200) and stained Nucleus with DAPI (Thermofisher). Microscopic images were taken with Nikon Ti-Eclipse microscope. All the experiments were done in triplicates.

Statistics and Reproducibility

In Fibrillation Assay each data point represents the technical mean of (n=3) experiments including standard deviation represented by error bars. The data were plotted with the absorbance values. For Sirus Red assay each data point represents technical triplicates. We performed (n=4) number of experiment and plotted the graphs including whisker plots representing standard deviation by normalizing all the conditions with respect to Hsp47 (+/+) as 1. The samples sizes for all biochemical assays, imaging and immunoblotting reported have a sample sizes (n=3). All independent quantitative experiments were started from cell cultures. The quality of these quantitative datasets is assessed by the standard deviation value of all the measured and are provided in each main and supplementary dataset.

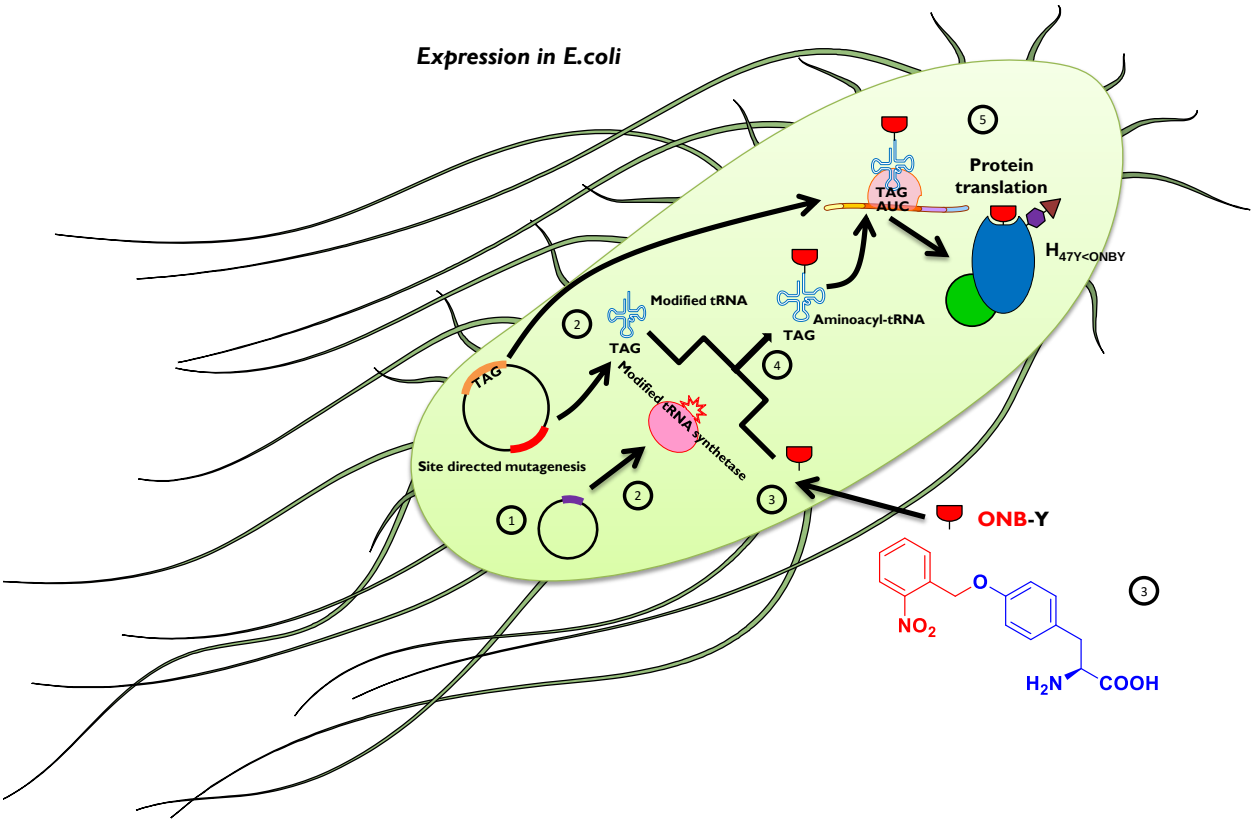

Figure S1. Schematic of synthesis of $H_{47Y<ONBY}$ 1. Co transformation of Hsp47383TAGpBAD and PBKONBYRS, 2. synthesis of orthogonal modified tRNA and tRNA synthetase, 3. uptake of ONBY by the *E.coli*, 4. Modified tRNA attaching to ONBY and 5. Protein translation.

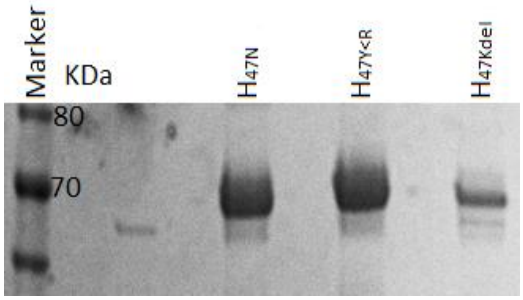

Figure S2. 12 % SDS PAGE gel of Hsp47 variants.

Sr.No	Title	Detail Plasmid & Genetic Construct	Yield (200 mL culture)
1	H_{47}	cHsp47strepPJexp411	183.26 ng (with impurities)
2	H_{47N}	EGFPHsp47strepTagpeT28a EGFPHsp47strepTagpBAD	207.8 µg 65.98 ng
3	$H_{47Y<ONBYhv}$	EGFPHsp47strepTag383TAGMutpBAD	43.56 ng
4	H_{47Kdel}	EGFPHsp47strepTag C terminal KDEL Deletion peT28a	65.98ng
5	$H_{47Y<R}$	EGFPHsp47strepTag MutY383R peT28a	45.85 ng
6	EGFP	EGFPpeT28a	359.95 ng

Table S1. Yield of the synthesis of Hsp47 mutants calculated from absorbance measurement using Nano drop UV spectrophotometer.

Sr.No	Title	Detail Plasmid & Genetic Construct	Primers
1	H_{47N}	EGFPHsp47strepTagpeT28a	ttaGAATTCATGCTGAGCCCGAAAGCC
			tttCTCGAGTTATTTCTCAAATTGCGGGTGG
		EGFPHsp47strepTagpBAD	tttCCATGGTGAGCAAGGGCGAG
			tttCTCGAGTTATTTCTCAAATTGCGGGTGG
2	$H_{47Y<ONBYhv}$	EGFPHsp47strepTag383TAGMutpBAD	AGCTGTTTTagGCGGATCATCCG
			TCGGGGAACGCAGCTCTT
3	H_{47Kdel}	EGFPHsp47strepTag C terminal KDEL Deleted peT28a	CTGGAATCCGCTTGGAGC
			GTCGCCCTTTGGACGGAC
4	$H_{47Y<R}$	EGFPHsp47strepTag MutY383R peT28a	GAAGCTGTTTcgCGCGGATCATCC
			GGGGAACGCAGCTCTTCA
5	EGFP	EGFPpeT28a	CTGGAATCCGCTTGGAGC
			GTCGCCCTTTGGACGGAC

Table S2.Primers used for developing $H_{47YN,}$ $H_{47Y<ONBY}$ and other mutants.

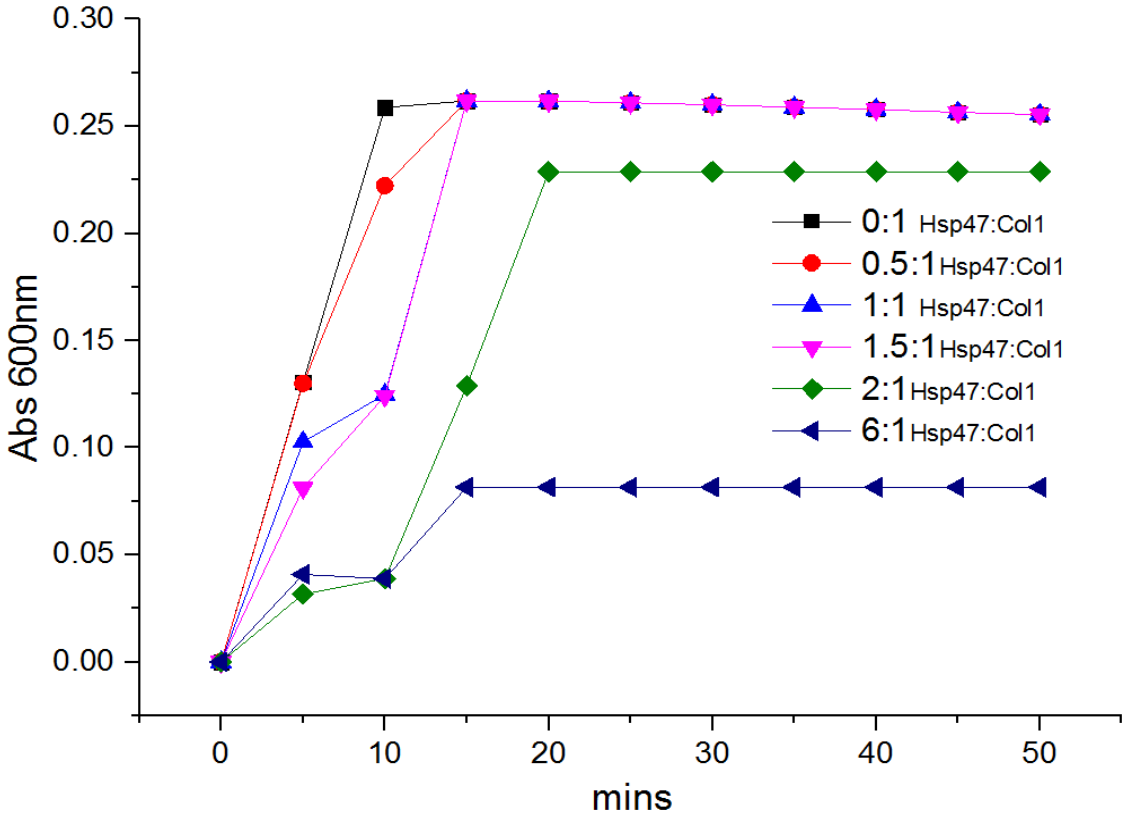

Figure S3. Turbidity measurements of Collagen/Hsp47 mixtures of different ratios. The increase in absorbance at 600nm reflects the formation of fibrils. Rat tail collagen (3mg/mL) was diluted to 0.4 mg/ml by addition of MEM Buffer (1x) and was adjusted to pH to 7.5 with sodium hydroxide within 4 min to prevent premature polymerization[5].The 0.4 mg/ml of collagen solution was mixed with H_{47N} at molar ratios of 0.5:1 (H_{47N} (0.3uM): Collagen (0.6uM)), 1:1 (H_{47N} (0.6uM): Collagen (0.6uM)), 2:1 (H_{47N} (1.2uM): Collagen (0.6uM)) and 6:1(H_{47N} (3.6uM): Collagen (0.6uM)) to a final concentration of 0.2 µg/ml. A 0.2 µg/ml collagen solution was used as a positive control. 75µl of each sample were transferred to a plate reader and absorbance was measured at 600 nm for 50 min at 34 ºC. The experiments were performed in triplicate. (n = 3 data point representing mean value of technical triplicates of each experiment).

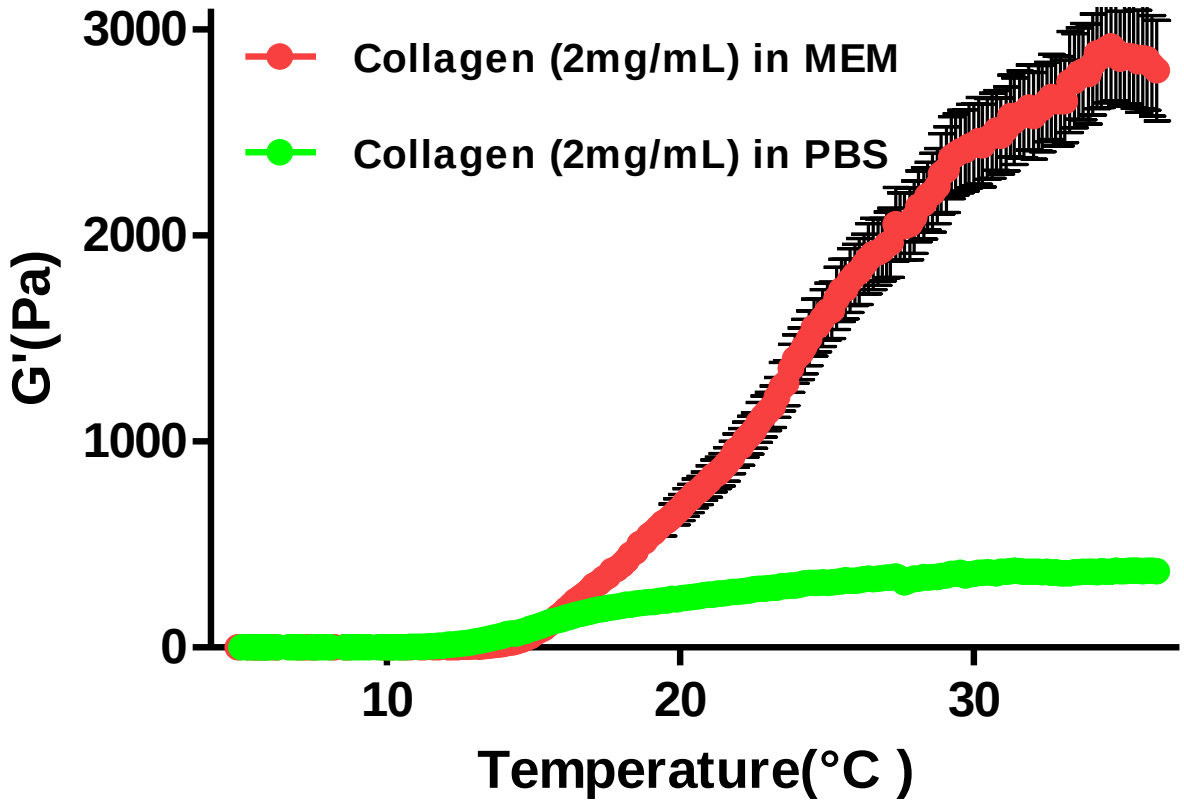

Figure S4. Rheology measurements of collagen solutions during fibrillation in MEM and PBS. Rat tail collagen (3mg/mL) was diluted to 2 mg/mL concentration with MEM or PBS and was adjusted to pH to 7.5 with sodium hydroxide within 4 min. A DHRIII Rheometer (TA Instruments) was used for the measurements. 50 µl of the collagen solution was placed between two parallel plates of 8 mm diameter cooled at 4°C. The shear moduli (G') were measured at frequency ω - 30-0.03 rad/s while temperature was increased to 40°C at 0.1°C/min. (n=3 data point representing mean value of technical triplicates of each experiment with standard deviation)

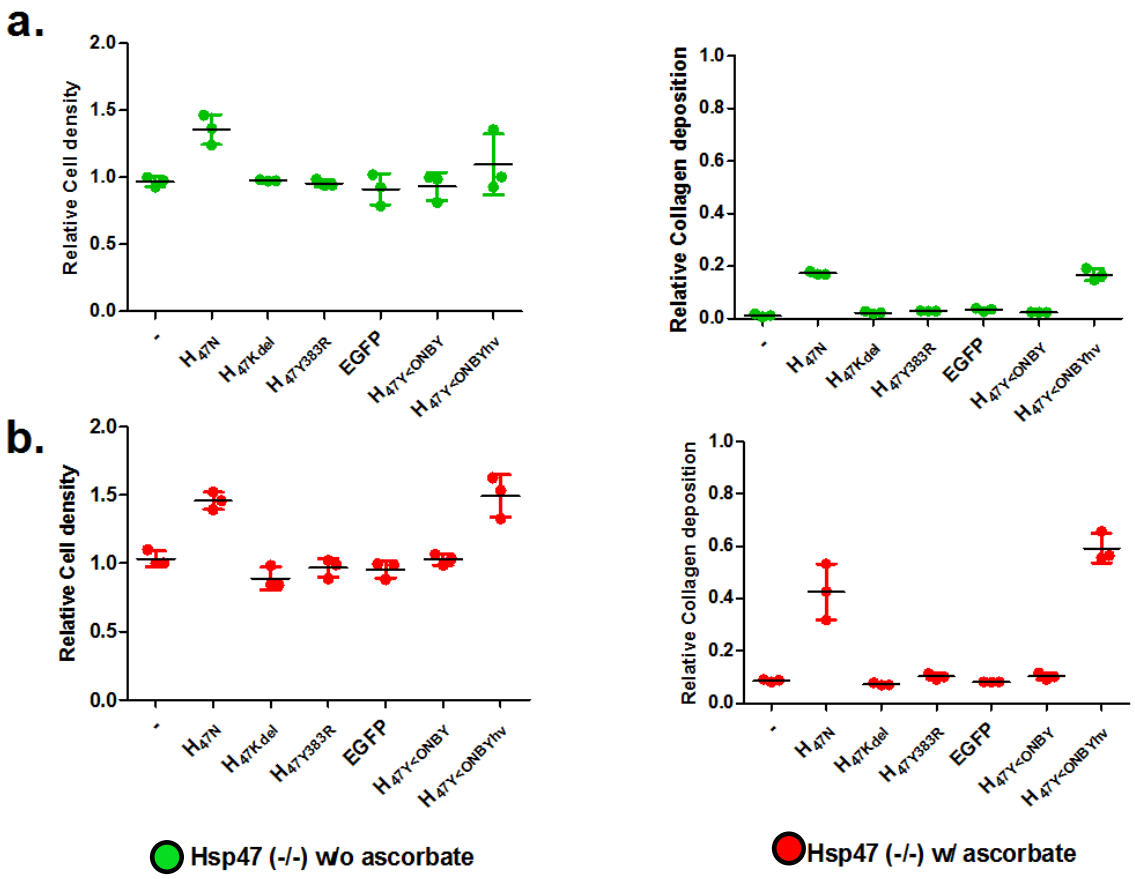

Figure S5. Relative collagen deposition and Relative Cell Density of MEF Hsp47 -/- in absence (a) and presence of ascorbate (b) with Hsp47 variants. Photo activation of $H_{47Y<ONBY}$ was done *in situ* by irradiating UV light for 30 sec using Nikon Ti-Eclipse microscope at 405 nm wavelength (n=3 data point representing mean value of technical triplicates of each experiment with whisker plots representing standard deviation standard deviation).

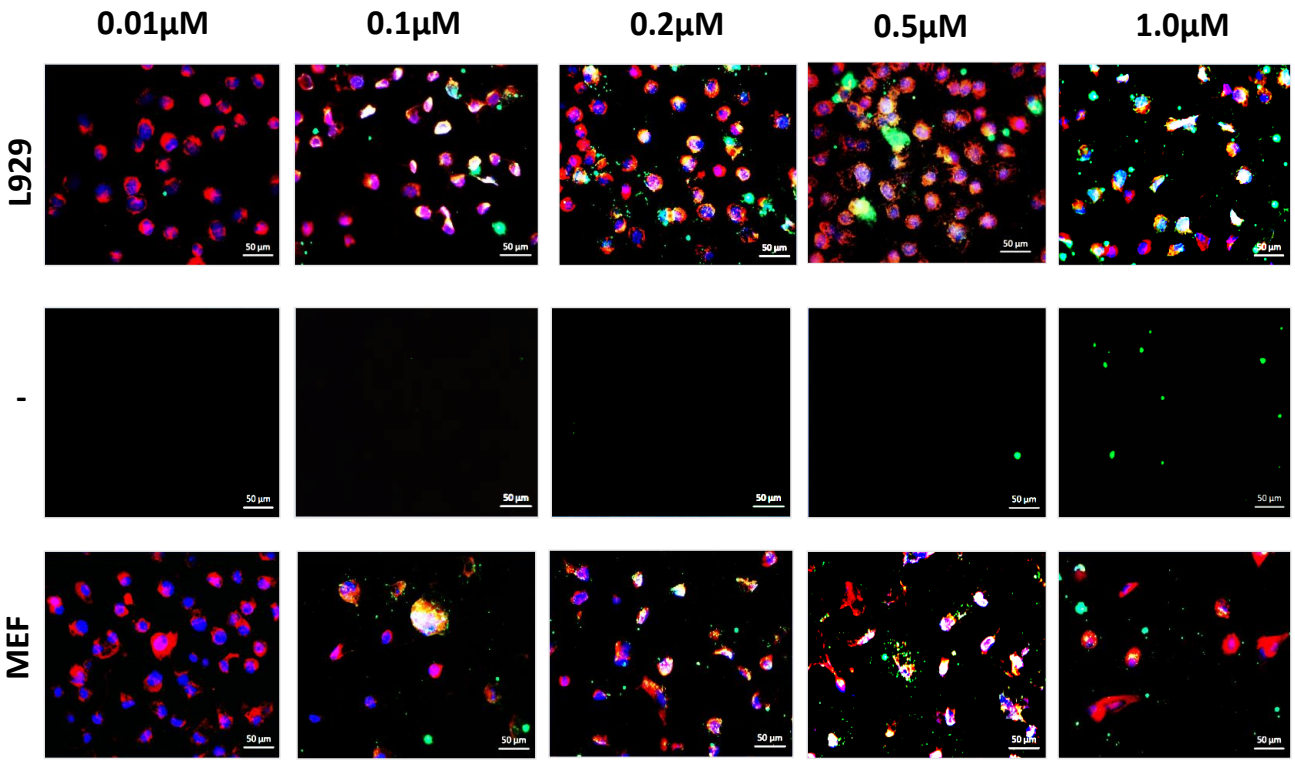

Figure S6. Fluorescence microscopy images of L929 and MEF cells after incubation with different concentration of H_{47} from 0.01 to 1 µm during 3 hours, washing and fixation. (Blue: DAPI (Nucleus), Green: EGFP Hsp47, Red: Rhoda mine labeled ER). The images were taken using a Zen Observer Video Microscope (Zeiss).

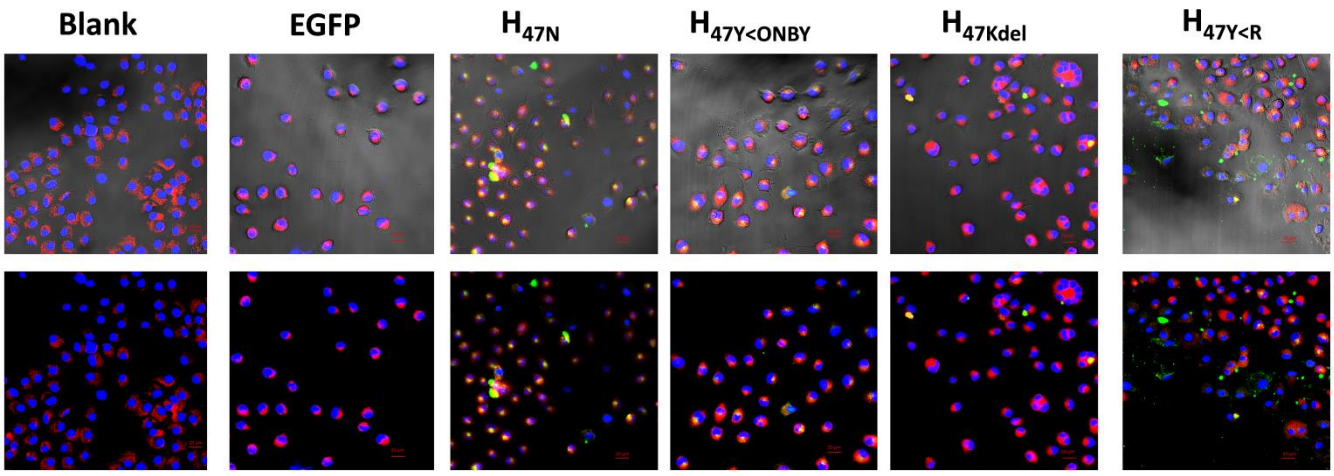

Figure S7. Confocal microscopy images of L929 cells after incubated with EGFP, H_{47}, $H_{47Y<ONBY}$, $H_{47Y<R}$ and H_{47Kdel}.Fixed and stained with Blue: DAPI (Nucleus), Green: EGFP Hsp47, Red: Rhoda mine labeled ER. Scale: 20 μm.

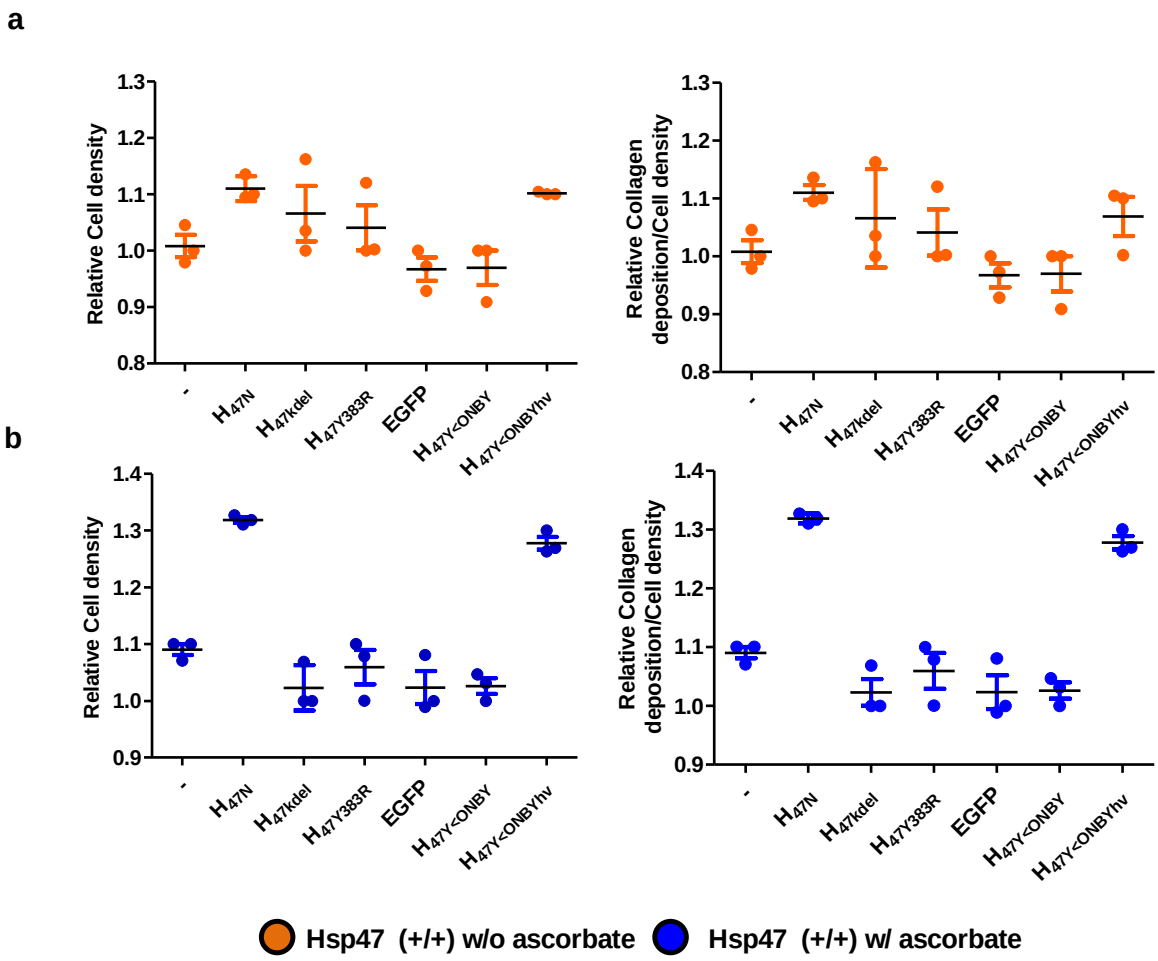

Figure S8. Relative collagen deposition, Relative Cell Density of Hsp47 +/+ in absence (a) and presence of ascorbate(b) with different Hsp47 mutants. Collagen deposition was quantified using Sirus Red Assay. Cell density was counted using DAPI staining of the nucleus and Image J software. Photo activation of $H_{47Y<ONBY}$ was done *in situ* by 30 sec exposure at 405 nm using PRIMO Unit in Nikon Ti-Eclipse microscope at 50% illumination intensity(n=3 data point representing mean value of technical triplicates of each experiment with whisker plots representing standard deviation standard deviation)

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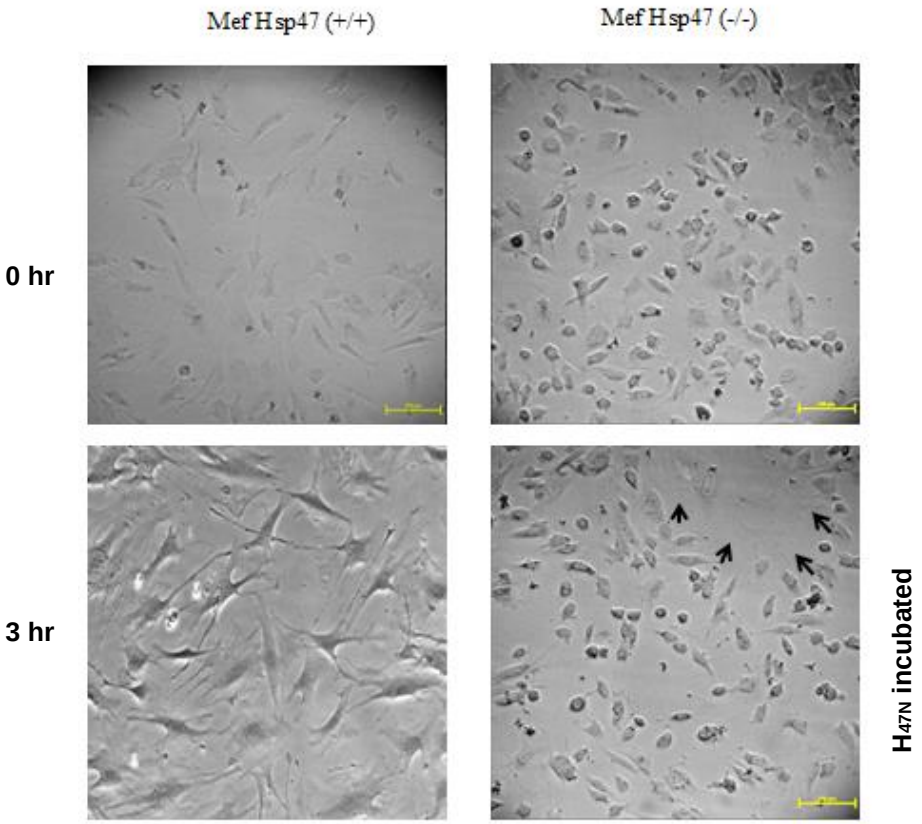

Figure S9. 20X Bright field images of Hsp47 (+/+) and Hsp47 (-/-) cells showing improvement shown with the small arrows in morphology after delivery of H_{47} in 3 hours (Scale:100 μm).

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Homo    MRSLLLLSAF CLLEAALAAE VKKPAAAAAP GTAEKLSPKA ATLAERSAGL
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Mus     .MRSLLLGTL CLLAVALAAE VKKPLEAAAP GTAEKLSSKA TTLAERSTGL
Cani    MRFLLLLNTC CLLAVVLAAE VKKPAAAAAP GSAEKLSPKA ATLAERSAGL
Gall    .......... ...MQIFLVL ALCGLAAAVP SEDRKLSDKA TTLADRSTTL
Zeb     .......... ...MWVSSLI ALCLLAVAVS GEDKKLSTHA TSMADTSANL
Gol     .......... ...MLVSSVV LLCLLATVSG ..DKALSSHA SILADNSANF
Mel     .......... .......... ...MEALKIT YKLERQFLVK FLFVLGATAL
Chan    .......... ...MWVKFLV GLCLLASVGA ..DKKLSSHA TILADNSANL
Amaz    .......... ...MWIILVL ALCGLAAAVP SEDRKLSDKA TTLADRSTTL
Bran    MRSLLLTSAF CLLAMALAAE VKKPAAPAAP GTAEKLSPKA TTLAERSAGL
Gree    .......... ...MWVTNLL ALCALVAAVP SEDKKLSDKA AALADRSATL
Roc     .......... ....MWIILV LALCGLAAAV PSEDRKLSDK ATTLADRSTT
Sque    MRSLLLLSTF CLLALAG... .......... .VLAAELSPK AATLAERSAG
Yak     MRALLLISTI CLLARALAAE VKKPAAAAAP .GTAEKLSPK AATLAERSAG
Blac    MRSLLLTSTF CLLAITLAAE VKKPAVAAAA PGTGEKLSPK AATLAERSAG
Nake    MRCLLLLGTF SLLAVALAAE VKKPAAAAAP G.TAEKLSSK AATLAERSAG
Chin    .MRSLLLASF CLLAVALAAE VKKPVEAAAP G.TAEKLSSK ATTLAERSTG
Dama    MRCLLLLGTF SLLAVALAAE VKKPAAVAAP G.TAEKLSSK AATLAERSAG
Medi    .......... .......... ....MMAYTY RHKLQIYFIA QILCTVWLTA
Aust    .......... .....MGMKL ILLSLLICVV KSEPVLLKEQ GPILGDSTVN

Homo    AFSLYQAMAK DQAVENILVS PVVVASSLGL VSLGGKATTA SQAKAVLSAE
Rat     AFSLYQAMAK DQAVENILLS PLVVASSLGL VSLGGKATTA SQAKAVLSAE
Mus     AFSLYQAMAK DQAVENILLS PLVVASSLGL VSLGGKATTA SQAKAVLSAE
Cani    AFSLYQAMAK DQAVENILLS PVVVASSLGL VSLGGKATTA SQAKAVLSAE
Gal     AFNLYHAMAK DKNMENILLS PVVVASSLGL VSLGGKATTA SQAKAVLSAD
Zeb     AFNLYHNVAK EKGLENILIS PVVVASSLGM VAMGSKSSTA SQVKSILKAD
Gol     AFNLYHNLAK EKDIENIVIS PVVVASSLGL VALGGKSNTA SQVKTVLSAT
Mel     AYGQDDGFAQ D...DNVYSW YILDVTRILQ NAESNIVVSP SNIRALLKTP
Chan    AFDLYHNMAK EKDMENILIS PVVVASSLGL VALGGKASTA SQVKTVLSGN
Amaz    AFNLYXAMAK DKNMENILLS PVVVASSLGL VSLGGKATTA SQAKAVLSAD
Bran    AFSLYQAMAK DQAVENILLS PVVVASSLGL VSLGGKATTA SQAKAVLSAE
Gree    AFNLYHTMAK DKNMENILVS PVVVASSLGL VSLGGKATTA SQAKAVLSAD
Roc     LAFNLYHAMA KDK..NMENI LLSPVVVASS LGLVSLGGKA ATASQAKAVL
Sque    LAFSLYQAMA KDQ..AVENI LLSPVVVASS LGLVSLGGKA ATASQAKAVL
Yak     LAFSLYQAMA KDQ..AVENI LLSPVVVASS LGLVSLGGKA ATASQAKAVL
Blac    LAFSLYQAMA KDQ..AVENI LLSPVVVASS LGLVSLGGKA TTASQAKAVL
Nake    LAFSLYQAMA KDQ..AVENI LLSPVVVASS LGLVSLGGKA ATASQAKAVL
Chin    LAFSLYQAMA KDQ..AVENI LLSPLVVASS LGLVSLGGKA TTASQAKAVL
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Dama    LAFSLYQAMA KDQ..AVENI LLSPVVVASS LGLVSLGGKA ATASQAKAVL
Medi    TVCGESDKYS EDEVVNENNV LAWYILDVSQ ILQNSKVNTI LSP...MNLL
Aust    LGLSLYQTMI KDQKLRSQNL LFSPVVVASS LGVMSMGAKD KTAKQVKSLL

Homo    QLRDEEVHAG LGELLRSLSN STARNVTWKL GSRLYGPSSV SFADDFVRSS
Rat     KLRDEEVHTG LGELLRSLSN STARNVTWKL GSRLYGPSSV SFADDFVRSS
Mus     KLRDEEVHTG LGELLRSLSN STARNVTWKL GSRLYGPSSV SFADDFVRSS
Cani    QLRDEEVHAG LGELLRSLSN STARNVTWKL GSRLYGPSSV SFAEDFVRSS
Gal     KLNDDYVHSG LSELLNEVSN STARNVTWKI GNRLYGPASI NFADDFVKNS
Zeb     ALKDEHLHTG LSELLTEVSD PQTRNVTWKI SNRLYGPSSV SFAEDFVKNS
Gol     TVKDEQLHSG LSELLTEVSN STARNVTWKI SNRLYGPSSV SFVDNFLKSS
Mel     PSMN..LRFG FDEKTMLG.. ...QNVIFAE SNMILNNP.. .......DTL
Chan    KVKDENLHSG LAELLSEVSN PKERNVTWKI TNRLYGPSSV SFSEDFVKNS
Amaz    KLNDDYVHSG LSELLNEVSN STARNVTWKI GNRLYGPASI NFADDFVKNS
Bran    QLRDEEVHAG LGELLRSLSN STARNVTWKL GSRLYGPSSV SFAEDFVRSS
Gree    KLNDDYIHGG LSELLNEVSN STARNVTWKL GNRLYGPSSI SFAEDFVKSS
Roc     SADKLNDDYL HSGLSELLNE VSNSTARNVT WKIGNRLYGP ASINFADDFV
Sque    SAEQLRDEEV HAGLGELLRS LSNSTARNVT WKLGSRLYGP SSVSFAEDFV
Yak     SAEQLRDDEV HAGLGELLRS LSNSTARNVT WKLGSRLYGP SSVSFAEDFV
Blac    SAEQLRDEEV HAGLGELLRS LSNNTARNVT WKLGSRLYGP SSVSFADDFV
Nake    SAEQLRDEEV HAGLGELLRS LSNSTARNVT WKLGSRLYGP SSVSFADDFV
Chin    SAEKLRDEEV HTGLGELLRS LSNSTARNVT WKLGSRLYGP SSVNFAEDFV
Dama    SAEQLRDEEV HTGLGELLRS LSNSTARNVT WKLGSRLYGP SSVSFADDFV
Medi    KTPAIVDLRF GADN.EMEGI NMILAESRRS LSRP...... ..........
Aust    NIN.LNDDTL HPAFSELLNE VSNETARNTT WKIGNCLYAP TSVNVRDDFV

Homo    KQHYNCEHSK INFRDKRSAL QSINEWAAQT TDGKLPEVTK DVERTDGALL
Rat     KQHYNCEHSK INFRDKRSAL QSINEWASQT TDGKLPEVTK DVERTDGALL
Mus     KQHYNCEHSK INFRDKRSAL QSINEWASQT TDGKLPEVTK DVERTDGALL
Cani    KQHYNCEHSK INFRDKRSAL QSINEWAAQT TDGKLPEVTK DVERTDGALL
Gal     KKHYNYEHSK INFRDKRSAL KSINEWAAQT TDGKLPEVTK DVEKTDGALI
Zeb     KKHYNYEHSK INFRDKRSAI NSINEWAAKT TDGKLPEITK DVKNTDGAMI
Gol     KKHYNCEHSK INFRDKRSAV KAINDWASKS TDGKLPEVTK DVEKTDGAMI
Mel     EWFYDCKIQE TSFTDKKKLI TSINDWSENI ADQTILKSSE MILKEENLQV
Chan    KKHYKYEHAK INFRDKKSAV NAINEWASKS TDGKLPEVTK DVEKTDGAMI
Amaz    KKHYNYEHSK INFRDKRSAL KSINEWAAQT TDGKLPEVTK DVEKTDGALI
Bran    KLHYNCEHSK INFRDKRSAL QSINEWASQT TDGKLPEVTK EVERTDGALL
Gree    KKHYNYEHSK INFRDKRSAL KSINEWASQT TNGKLPEVTT NVEKTDGALI
Roc     KNSKKHYNYE HSKINFRDKR SALKSINEWA AQTTDGKLPE VTKDVEKTDG
Sque    RSSKQHYNCE HSKINFRDKR SALQSINEWA AQTTGGKLPE VTSDVERTDG
Yak     RSSKQHYNCE HSKINFRDKR SALQSINEWA AQTTDGKLPE VTKDVERTDG
Blac    RSSKQHYNCE HSKINFRDKG SALQSINEWA AQTTDGKLPE VTKEVERTDG
Nake    RSSKQHYNCE HSKINFRDKR TALQSINEWA AQTTDGKLPE VTKDVERTDG
Chin    HSSKQHYNCE HSKINFRDKR SALQSINEWA SQTTDGKLPE VTKDVERTDG
Dama    RSSKQHYNCE HSKINFRDKR SALQSINEWA AQTTDGKLPE VTKDVERTDG
Medi    ENVEMFYNTK IQEISFADTA NHLVMINDWG KRVTNEEFPK LIESNSSLKN
Aust    QKTKTHYKYD HSQINFKDQR SALRSINQWA SQATEGKLSE ITAALSSTDG


Homo    VNAMFFKPHW DEKFHHKMVD NRGFMVTRSY TVGVMMMHRT GLYNYYDDEK
Rat     VNAMFFKPHW DEKFHHKMVD NRGFMVTRSY TVGVTMMHRT GLYNYYDDEK
Mus     VNAMFFKPHW DEKFHHKMVD NRGFMVTRSY TVGVTMMHRT GLYNYYDDEK
Cani    VNAMFFKPHW DEKFHHKMVD NRGFMVTRSY TVGVTMMHRT GLYNYYDDEK
Gal     VNAMFFKPHW DEKFHHKMVD NRGFMVTRSY TVGVPMMHRT GLYNYYDDEA
Zeb     VNAMFFKPHW DEKSHHKMVD NRGFLVTRSH TVSVPMMHRT GIYGFYEDTE
Gol     INAMFYKPHW DEQFHHKMVD NRGFLVHRSY TVSVPMMHRT GIYGLFDDTT
Mel     LILNMLNFKE TLQINFKYTL NATFHERPDS TIVLPAVETT EYLKYLDSQI
Chan    INAIFYKPHW DEQFHHQMVD NRAFLVHRSY TVSVPMMHRT GIYGFYDDTA
Amaz    VNAMFFKPHW DEKFHHKMVD NRGFMVSRSY TVGVPMMHRT GLYNYYDDET
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Bran    VNAMFFKPHW DERFHHKMVD NRGFMVTRSY TVGVTMMHRT GLYNYYDDEK
Gree    VNAMFFKPHW EERFHHKMVD NRGFMVTRSY TVGVPMMHRT GLYNYFDDEA
Roc     ALIVNAMFFK PHWDEKFHHK MVDNRGFMVT RSYTVGVPMM HRTGLYNYYD
Sque    ALLVNAMFFK PHWDERFHHK MVDNRGFMVT RSYTVGVTMM HRTGLYNYYD
Yak     ALLVNAMFFK PHWDERFHHK MVDNRGFMVT RSYTVGVTMM HRTGLYNYYD
Blac    ALLVNAMFFK PHWDERFHHK MVDNRGFMVT RSYTVGVTMM HRTGLYNYYD
Nake    ALLVNAMFFK PHWDEKFHHK MVDNRGFMVT RSYTVGVTMM HRTGLYNYYD
Chin    ALLVNAMFFK PHWDEKFHHK MVDNRGFMVT RSYTVGVTMM HRTGLYNYYD
Dama    ALLVNAMFFK PHWDEKFHHK MVDNRGFMVT RSYTVGVTMM HRTGLYNYYD
Medi    LQVLILNMFH FVETLEINFK YTANLLFYIT PKQRTKVPAV ETTEYLKYLD
Aust    AFIINANYFK PHWDESFQQT MVDKRGFIIT RTHTVSIPMM HQIRLCNYYE

Homo      EKLQIVEMPL AHKLSSLIIL MPHHVEPLER LEKLLTKEQL KIWMGKMQKK
Rat       EKLQLVEMPL AHKLSSLIIL MPHHVEPLER LEKLLTKEQL KTWMGKMQKK
Mus       EKLQMVEMPL AHKLSSLIIL MPHHVEPLER LEKLLTKEQL KAWMGKMQKK
Cani      EKLQIVEMPL AHKLSSLIIL MPHHVEPLER LEKLLTKEQL KIWMGKMQKK
Gal       EKLQVVEMPL AHKLSSMIFI MPNHVEPLER VEKLLNREQL KTWASKMKKR
Zeb       NRFLIVSMPL AHKKSSMIFI MPYHVEPLDR LENLLTRQQL DTWISKLEER
Gol       NNLLVLDMAL AHKMSSIVFI MPYHVESLER VEKLLTRQQL NTWISKMEQR
Mel       LDAKILELPY SNG.YFMYII LPHTKQGVIE TINNLGYEQL TRIEWMMKER
Chan      NSFFVLEMPL AHKKSSVIFI MPYHVESLER LEKMLTRKQL DIWQSKMEQK
Amaz      EKLQVVEMPL AHKLSSMIFI MPNHVEPLER VEKLLNREQL KTWAGKMKKR
Bran      EKLQIVEMPL AHKLSSLIIL MPHHVEPLER LEKMLTKEQL KTWMGKMQKR
Gree      EKLQIVEMPL AHKLSSMIFI MPNHVEPLER VEKLLTREQL KTWIGKLKKR
Roc       DETEKLQVVE MPLAHKLSSM IFIMPNHVEP LERVEKLLNR EQLKTWAGKM
Sque      DEKEKLQIVE MPLAHKLSSL IIIMPHHVEP LERLEKLLTK EQLKTWMGKM
Yak       DEKEKLQMVE MPLAHKLSSL IIIMPHHVEP LERLEKLLTK EQLKVWMGKM
Blac      DEKEKMQIVE MPLAHKLSSL IILMPHHVEP LERLEKMLTK EQLKIWMGKM
Nake      DEKEKVQILE MPLAHKLSSL IILMPHHVEP LERLEKLLTK EQLKAWTGKL
Chin      DEKEKLQILE MPLAHKLSSL IILMPHHVEP LERLEKLLTK EQLKAWMGKM
Dama      DEKEKVQILE MPLAHKLSSL IILMPHHVEP LERLEKLLTK EQLKVWMGKM
Medi      SQMLDAKILQ LPYSNG.FSM YILLPHTKAG LNELLSILGF EQLKRLQWMM
Aust      DNANSLQVLE LPLSHKHSSM IFIMTKHIEP LARLEKLLTK EQLNTWIGKL

Homo      AVAISLPKGV VEVTHDLQKH LAGLGLTEAI DKNKADLSRM SGKKDLYLAS
Rat       AVAISLPKGV VEVTHDLQKH LAGLGLTEAI DKNKADLSRM SGKKDLYLAS
Mus       AVAISLPKGV VEVTHDLQKH LAGLGLTEAI DKNKADLSRM SGKKDLYLAS
Cani      AVAISLPKGV VEVTHDLQKH LAGLGLTEAI DKNKADLSRM SGKKDLYLAS
Gal       SVAISLPKVV LEVSHDLQKH LADLGLTEAI DKTKADLSKI SGKKDLYLSN
Zeb       AVAISLPKVS MEVSHDLQKH LGELGLTEAV DKPKADLSNI SGKKDLYLSN
Gol       AVAVSLPKVS VEVSHDLQKH LTELGLTEAV DKAKADLSNI SGKKDLYLSN
Mel       RVNVVMPTFK YHFITNMREH IQKN....SA HRFDVDFEPA FG.VETKKIN
Chan      AVAVSLPKIS MEVSHNLQKY LGELGVTEAV DKTKADLSNI SGKKDLYLAN
Amaz      SVAISLPKVV LEVSHDLQKH LADLGLTEAI DKTKADLSKI SGKKDLYLSN
Bran      AVAISLPKGV VEVTHDLQKH LAGLGLTEAI DKNKADLSRM SGKKDLYLAS
Gree      AVAISLPKVS LEVSHDLQKH LADLGLTEAM DKNKADLSKI SGKKDLYLSN
Roc       KKRSVAISLP KVVLEVSHDL QKHLADLGLT EAIDKTKADL SKISGKKDLY
Sque      QTKAVAISLP KGVVEVTHDL QKHLATLGLT EAIDKNKADL SRMSGKKDLY
Yak       QKKAVAISLP KGVVEVTHDL QKHLAGLGLT EAIDKNKADL SRMSGKKDLY
Blac      QKKAVAISLP KGVVEVTHDL QKHLAGLGLT EAIDKNKADL SRMSGKKDLY
Nake      QKKAVAISLP KGVVEVTHDL QKHLAGLGLT EAIDKNKADL SRMSGKKDLY
Chin      QKKAVAISLP KGVVEVTHDL QKHLAGLGLT EAIDKNKADL SRMSGKKDLY
Dama      QKKAVAISLP KGVVEVTHDL QKHLAGLGLT EAIDKTKADL SRMSGKKDLY
Medi      EVRRVNVLMP TFKYSFITNL KEDILDK... .SDHRFDSDF ENSFSKEKDF
Aust      ERHTVSVSLP KVNLEVSHDL QKYLQELGLT EAVDKNKADF SGITGKKNLH

Homo      VFHATAFELD TDGNPFDQDI YGREELRSPK LFYADHPFIF LVRDTQSGSL
Rat       VFHATAFEWD TEGNPFDQDI YGREELRSPK LFYADHPFIF LVRDNQSGSL
Mus       VFHATAFEWD TEGNPFDQDI YGREELRSPK LFYADHPFIF LVRDNQSGSL
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Cani      VFHATAFEWD TEGNPFDQDI YGREELRSPK LFYADHPFIF LVRDTQSGSL
Gal       VFHAAALEWD TDGNPYDADI YGREEMRNPK LFYADHPFIF MIKDSKTNSI
Zeb       VFHASSLEWD TEGNPFDPSI FGSEKMRNPK LFYADHPFIF LVKDNKTNSI
Gol       VFHASAMEWD TEGNPPDTSI YGTDKLKTPK LFYADHPFIF LVKDKKTNSI
Mel       IFQTTLVQFD GSG.RARVED YEKIRTTKYE RFHVDRPFAF YIKEKSTGRI
Chan      VFHASAFEWD IAGNPADTSI FGTDKVKNPK LFYVDHPFIF LVKDKSTGSI
Amaz      VFHAAALEWD TEGNPYDADI YGREEMRNPK LFYADHPFIF MIKDSKTNSI
Bran      VFHATAFEWD TEGNPFDQDI YGREELRSPK LFYADHPFIF LVRDSQTGSL
Gree      VFHAAALEWD TEGNPFDADI YGREEMRNPK LFYADHPFVF VIKDNKTNSI
Roc       LSNVFHATAL EWDTEGNPYD ADIYGREEMR NPKLFYADHP FIFMIKDNKT
Sque      LASVFHATAF ELDTEGNPFD QDIYGREELR SPKLFYADHP FIFLVRDTQS
Yak       LASVFHATAF EWDTDGNPFD QDIYGREELR SPKLFYADHP FIFLVRDTQS
Blac      LASVFHATAF EWDTEGNPFD QDIYGREELR SPKLFYADHP FIFLVRDSQS
Nake      LASVFHATAF EWDTEGNPFD QDIYGREELR SPKLFYADHP FIFLVRDTQS
Chin      LASVFHATAF EWDTDGNPFD QDIYGREELR SPKLFYADHP FIFLVRDNQS
Dama      LASVFHATAF EWDTEGNPFD QDIYGREELR SPKLFYADHP FIFLVRDTQS
Medi      LN.IFKVAAI SFNGTGKPRV EDYQNIR.SA KYEKFHVDRP FVYYIEN.KY
Aust      LSGMLHATAI DWDTEGNQFD QDWNSPEITK SAKVFYADHA YIFLIRDNKT

Homo                  LFIGRLVRPK GDKMRDEL
Rat                   LFIGRLVRPK GDKMRDEL
Mus                   LFIGRLVRPK GDKMRDEL
Cani                  LFIGRLVRPK GDKMRDEL
Gal                   LFIGRLVRPK GDKMRDEL
Zeb                   LFIGRLVRPK GDKMRDEL
Gol                   LFMGRLVQPK GDKMRDEL
Mel                   VCIGKVLNPV Q.......
Chan                  LFIGRLVRPK GEKMRDEL
Amaz                  LFIGRLVRPK GDKMRDEL
Bran                  LFIGRLVRPK GDKMRDEL
Gree                  LFIGRLVKPK GDKMRDEL
Roc                   NSILFIGRLV RPKGDKMRDE L
Sque                  GSLLFIGRLV RPKGDKMRDE L
Yak                   GSLLFIGRLV RPKGDKMRDE L
Blac                  GSLLFIGRLV RPKGDKMRDE L
Nake                  GSLLFIGRLV RPKGDKMRDE L
Chin                  GSLLFIGRLV RPKGDKMRDE L
Dama                  GSLLFIGRLV RPKGDKMRDE L
Medi                  GEIVCIGKVE NPEQ...... .
Aust                  NSILLIGRLV KPKSNDHDEL .
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Figure S10. Multiple sequence alignment of Hsp47 showing conserved residues in different species. The KDEL sequence is highlighted in Green, active site 383 Y is highlighted in Blue, Histidine involved in protonation are highlighted in Red. RCL loop in Grey and Aspartic acid and Histidine are highlighted in Yellow and Pink.

References:

(1) Arbely, E.; Torres-Kolbus, J.; Deiters, A.; Chin, J. W., *Journal of the American Chemical Society* **2012,** *134*, 11912.

(2) Oecal, S.; Socher, E.; Uthoff, M.; Ernst, C.; Zaucke, F.; Sticht, H.; Baumann, U.; Gebauer, J. M., *J Biol Chem* **2016,** *291*, 12612.

(3) Strale, P. O.; Azioune, A.; Bugnicourt, G.; Lecomte, Y.; Chahid, M.; Studer, V., *Adv. Mater.* **2016,** *28*, 2024.

(4) Douglass, M. R.; Lee, B. L.; Strale, P.-O.; Azioune, A.; Bugnicourt, G.; Lecomte, Y.; Chahid, M.; Studer, V. In *Emerging Digital Micromirror Device Based Systems and Applications IX* 2017.

(5) Piechocka, I. K.; van Oosten, A. S.; Breuls, R. G.; Koenderink, G. H., *Biomacromolecules* **2011,** *12*, 2797.

(6) Greenspan, D. S. In *Collagen* 2005, p 149.

(7) He, W.; Dai, C., *Curr Pathobiol Rep* **2015,** *3*, 183.

(8) Ishida, Y.; Kubota, H.; Yamamoto, A.; Kitamura, A.; Bachinger, H. P.; Nagata, K., *Mol. Biol. Cell* **2006,** *17*, 2346.

(9) Ito, S.; Nagata, K., *Semin Cell Dev Biol* **2017,** *62*, 142.

(10) Ito, S.; Ogawa, K.; Takeuchi, K.; Takagi, M.; Yoshida, M.; Hirokawa, T.; Hirayama, S.; Shin-Ya, K.; Shimada, I.; Doi, T.; Goshima, N.; Natsume, T.; Nagata, K., *J Biol Chem* **2017,** *292*, 20076.

(11) Makareeva, E.; Leikin, S., *PLoS One* **2007,** *2*, e1029.