SUPPLEMENTAL DATA

Interaction of Hsp90β variants with WT p53 in vitro, measured by ultracenrifugation. To further confirm our finding, that ATP binding to Hsp90 destabilizes the Hsp90-p53 complex, we performed ultracentrifugation in 10-30% (w/v) glycerol gradient (Sup. 1). This method was applied previously to isolate steroid hormone receptor complex with Hsp90 (1). It is particularly useful in separation of large complexes of hydrophobic proteins, since the separation takes place in solution and improper interactions with the resin may be omitted. WT Hsp90β or E42A and D88N variants were preincubated with WT p53 protein at 37ºC and subsequently loaded on the top of 10-30% (w/v) glycerol gradient and separated by ultracentrifugation. We show that in the presence of ATP, only the Hsp90β D88N variant is still found in the same fractions as WT p53, while WT Hsp90 and E42A variant do not co-sediment with a significant amount of p53, upon treatment with ATP (Sup. 1). This suggests that similarly to ELISA experiments (Fig. 2, main text), the p53-Hsp90β complex is dissociated upon ATP binding to Hsp90β.

Interestingly, the amount of WT p53 protein detected after incubation with ATP was lower than in case of incubation without nucleotide. This observation indicates that ATP has a direct effect on p53 as was previously reported (2,3), here most likely represented by a change in a WT p53 aggregation level. However, the Hsp90β D88N variant, in the presence or absence of ATP, is able to shift the WT p53 sedimentation towards lighter fractions. This fact may aid to explain the limited, positive effect of Hsp90β D88N on WT p53 function and stability *in vitro* and in cells (Fig. 3-6, main text), most likely achieved by a passive WT p53 aggregation prevention.

Moreover, p53 incubated in 37ºC had propensity to form a high molecular mass aggregates, too large to separate in a used gradient and sedimenting at the bottom of the centrifuge tube. Hsp90β was mostly dimeric as judged by a size exclusion chromatography (data not shown) and in 10-30% glycerol gradient sedimented in lighter fractions (Sup.1, shown only for WT Hsp90β; E42A and D88N variants sediment in the same fractions – not shown). Native p53 tetramer at 4°C sedimented in 10-30% glycerol gradient in similar density fractions (Sup. 1, controls, 4°C). The Hsp90β-p53 complex sedimented in most dense fractions, that suggested stoichiometry of dimer Hsp90 to tetramer p53 or an even larger mass complex.

Hsp90β E42A variant is unable to reactivate the R249S p53 in cells. We have tested the Hsp90 variants solo and in combination with the Hsc70-Hsp40 system in attempt to restore the transcriptional activity to selected oncogenic p53 variants in H1299 cells. The cellular background was therefore the same as in experiments with WT p53 described in the main text (Fig. 4-6). The example of the activity of R249S p53 is shown in Sup. 2. In this experiment, E42A Hsp90β – the most efficient Hsp90β variant in the reactivation of R249S p53 in vitro (Fig. 3, main text) was transiently overproduced in cells together with R249S p53, also combined with the Hsc70-Hsp40 chaperone system. We have previously shown that Hsc/p70-Hdj1/2 setups cooperate with Hsp90β in the R249S reactivation *in vitro* (4). However, none of the used combinations was sufficient to restore the transcriptional activity of R249S significantly in H1299 cells, as tested by the luciferase reporter assay (Sup. 2). Such was also the case of other tested p53 oncogenic variants, namely V143A, R175H, G245S, R248Q, R273H and R282W (not shown). This suggests that while Hsp90 E42A is effective in increasing the WT p53 activity (Fig. 5, main text), it does not provide a straightforward solution for a reactivation of the most frequent p53 oncogenic variants.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Analytical Hsp90β – p53 complex ultracentrifugation. For analytical centrifugation 10 µmol Hsp90 and 0,2 µmol p53 were incubated for 1 hour at 37ºC in buffer 25mM Tris 7,5, 10 mM $MgCl_2$, 50 mM KCl. Samples were cooled and immediately loaded on the top of 13ml 10-30% w/v glycerol gradient in the same buffer prepared on Gradient Master ip 170 (BioComp) device. When indicated 5mM ATP was added during Hsp90 and p53 incubation. Sample were centrifuged at 140 000 g in SW41 rotor (Beckman) for 16h at 4ºC. 46 samples of volume 250 µl were taken starting from the bottom of the gradient tube (sample 1), and 1 µl of each was spotted on nitrocellulose. Dot- blot membranes were developed with anti p53 DO-1

(Moravian Biotechnology) or anti- Hsp90 SPA-846 (Sterssgen) antibodies. Blots were scanned on GS-800 scanner (Biorad) and the density of the dots quantified with program ImageQuant TL (Amersham Pharmacia).

Luciferase reporter assay. The activity measurement of WT and R249S p53 was carried out using luciferase reporter assay, as described in main text Experimental procedures. Vectors transiently transfected in R249S p53 reactivation attempts were as follows: pCMV-WTp53, pCMV-R249Sp53, pCDNA-FLAG-HA, pCDNA-FLAG-HA-Hsp90β WT/E42A, pCDNA-Hsp40 (Hdj1), pE-HA-Hsc70.

REFERENCES:

1. Inano, K., Haino, M., Iwasaki, M., Ono, N., Horigome, T., and Sugano, H. (1990) *FEBS Lett* **267**(1), 157-159
2. Warnock, L. J., and Raines, S. A. (2004) *Cancer Biol Ther* **3**(7), 634-637
3. Okorokov, A. L., and Milner, J. (1999) *Mol Cell Biol* **19**(11), 7501-7510
4. Walerych, D., Olszewski, M. B., Gutkowska, M., Helwak, A., Zylicz, M., and Zylicz, A. (2009) *Oncogene* **28**(48), 4284-4294