**Van der Horst et al., Figure S1**

**Figure S1.** Localisation of FOXO4-K199,211R is similar to localisation of wild-type FOXO4. A14 cells expressing wild-type HA-FOXO4 or HA-FOXO4-K199,211R were treated with 200 μM hydrogen peroxide for 30 minutes and stained using α-HA antibody. (top) Staining was analysed using an immunofluorescence microscope. Green, α-mouse-Alexa488; blue, DAPI counterstaining. (bottom) Twenty-five cells per experiment were scored for FOXO4 localisation (c=cytosol, n=nucleus). Representative examples for scoring are shown in Supplementary Information, Fig. S5. Data presented are the mean ± s.d. of two independent experiments. Bar, 10 μm.
Van der Horst et al., Figure S2

**Figure S2.** Mutant USP7 and USP14 do not deubiquitinate FOXO4. (a) To show dependence on enzymatic activity and specificity of USP7, respectively myc-USP7-CS and HA-USP14 were used, as in Fig. 3c. (b) Co-immunoprecipitation of FOXO4 and USP7 or USP14 in HEK293T cells treated with hydrogen peroxide (as in Fig. 3b).
Figure S3. Knockdown of USP7 increases FOXO4 monoubiquitination and FOXO4 activity. HEK293T cells were transfected with HA-FOXO4, his-ubiquitin and either a non-targeting siRNA oligo or a combination of two siRNAs against USP7. Cells were treated with 50 μM hydrogen peroxide for 30 minutes. Experiments were performed as described in Figure 1a. In addition, Western blot analysis for p27kip1, p130, USP7 and tubulin expression was performed.
Van der Horst et al., Figure S4

Figure S4. Half-life analysis of FOXO in HEK293T cells overexpressing HA-FOXO4 and myc-USP7. (a) Puromycin-selected cells were treated with 50 μM hydrogen peroxide for 24 hours and with cycloheximide (CHX) for the indicated times. Protein levels were equalized and samples were analysed using the indicated antibodies. (b) Using the Odyssey Infra-red imaging system relative expression levels were calculated and displayed in a graph.
Van der Horst et al., Figure S4

Figure S4. Half-life analysis of FOXO in HEK293T cells overexpressing HA-FOXO4 and myc-USP7. (a) Puromycin-selected cells were treated with 50 μM hydrogen peroxide for 24 hours and with cycloheximide (CHX) for the indicated times. Protein levels were equalized and samples were analysed using the indicated antibodies. (b) Using the Odyssey Infra-red imaging system relative expression levels were calculated and displayed in a graph.

Van der Horst et al., Figure S6

Figure S6. Representative examples of FOXO4 localisation for scoring the experiments shown in Figs. 2b, 5e and S2. Bar, 10 μm.