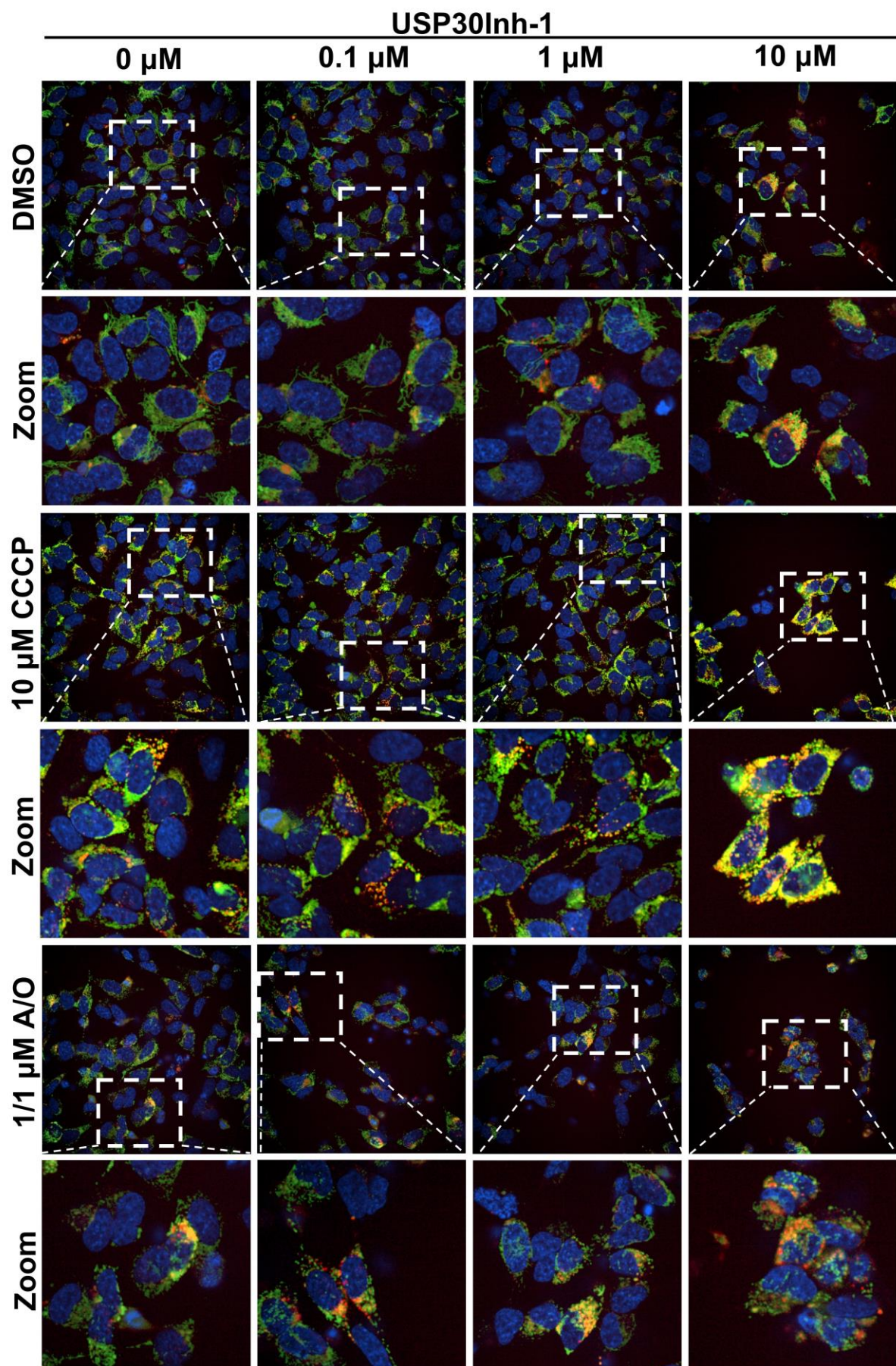


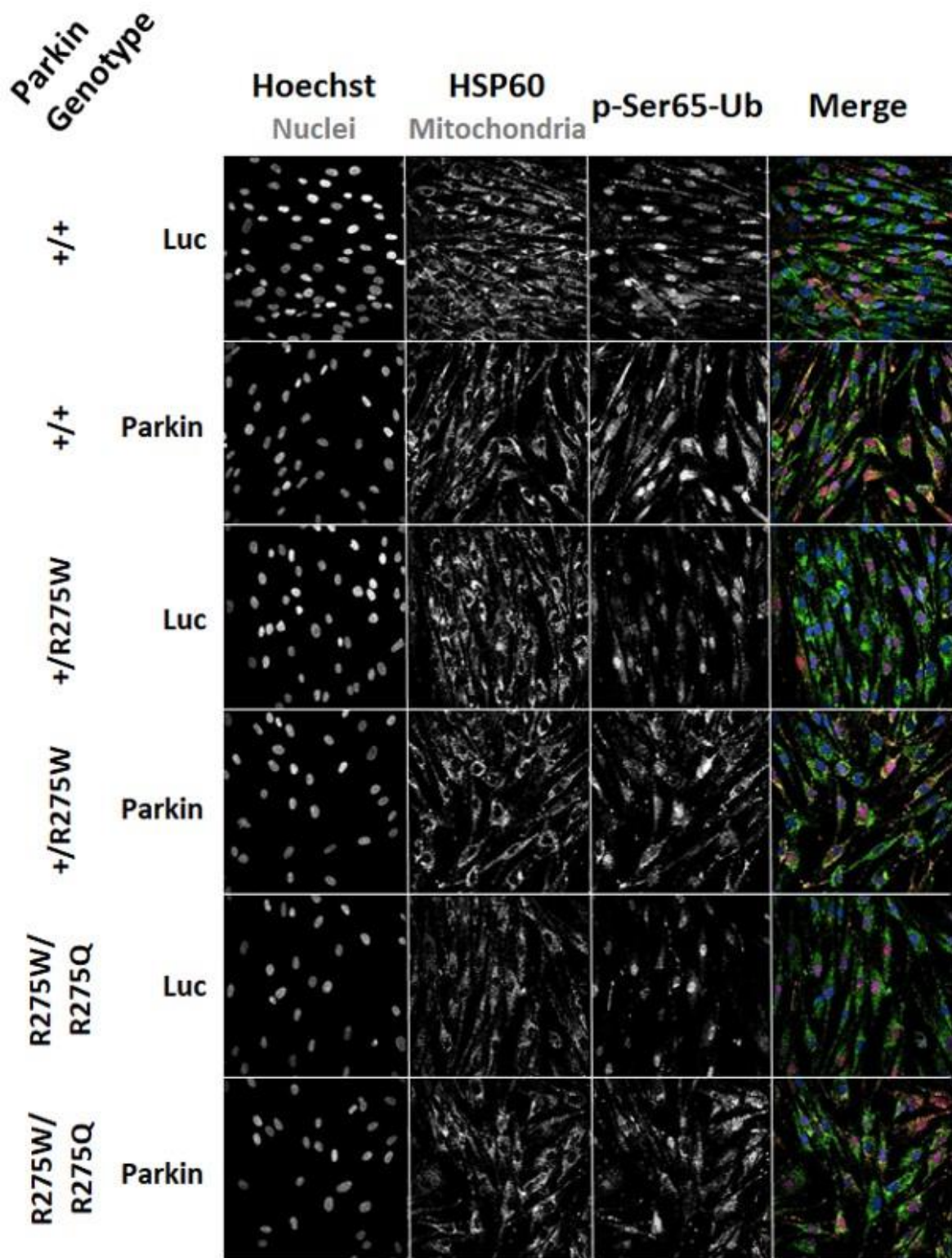
**Supplementary Figure S1. Changes of the mitoKeima signal after siRNA-mediated knockdown of USP30.**

Representative images of USP30 knockdown in SHSY5Y mitoKeima cells for 7 days before inducing mitophagy with 10  $\mu$ M CCCP or 1/1  $\mu$ M A/O or 0.1  $\mu$ M Val for 7 h.



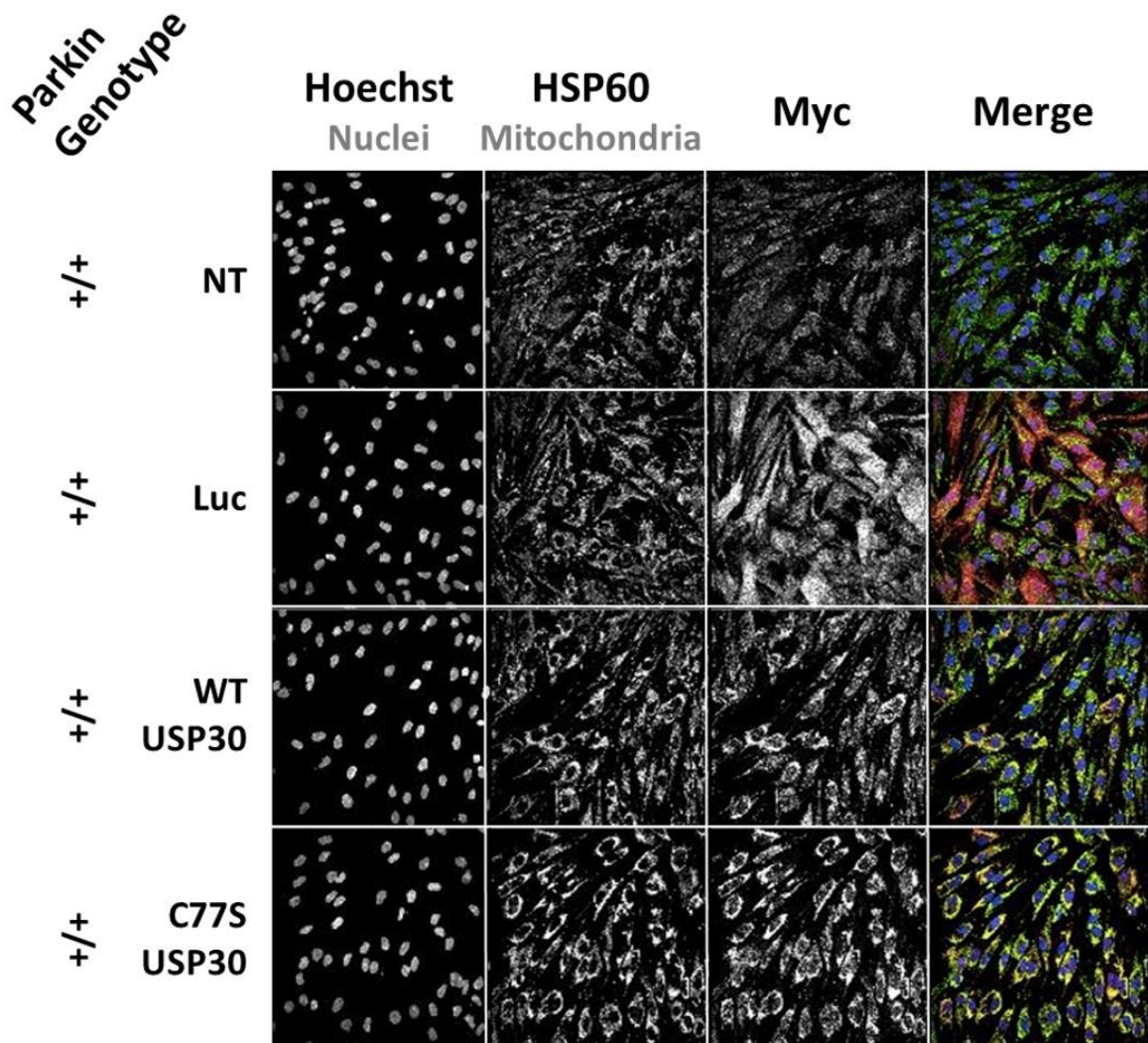
**Supplementary Figure S2. Changes of the mitoKeima signal after treatment with USP30Inh-1 for 3 days.**

Representative images of SHSY5Y mitoKeima cells treated with the indicated USP30Inh-1 concentration for 3 days. Mitophagy was induced with 1/1  $\mu\text{M}$  A/O or 10  $\mu\text{M}$  CCCP for 10 h.



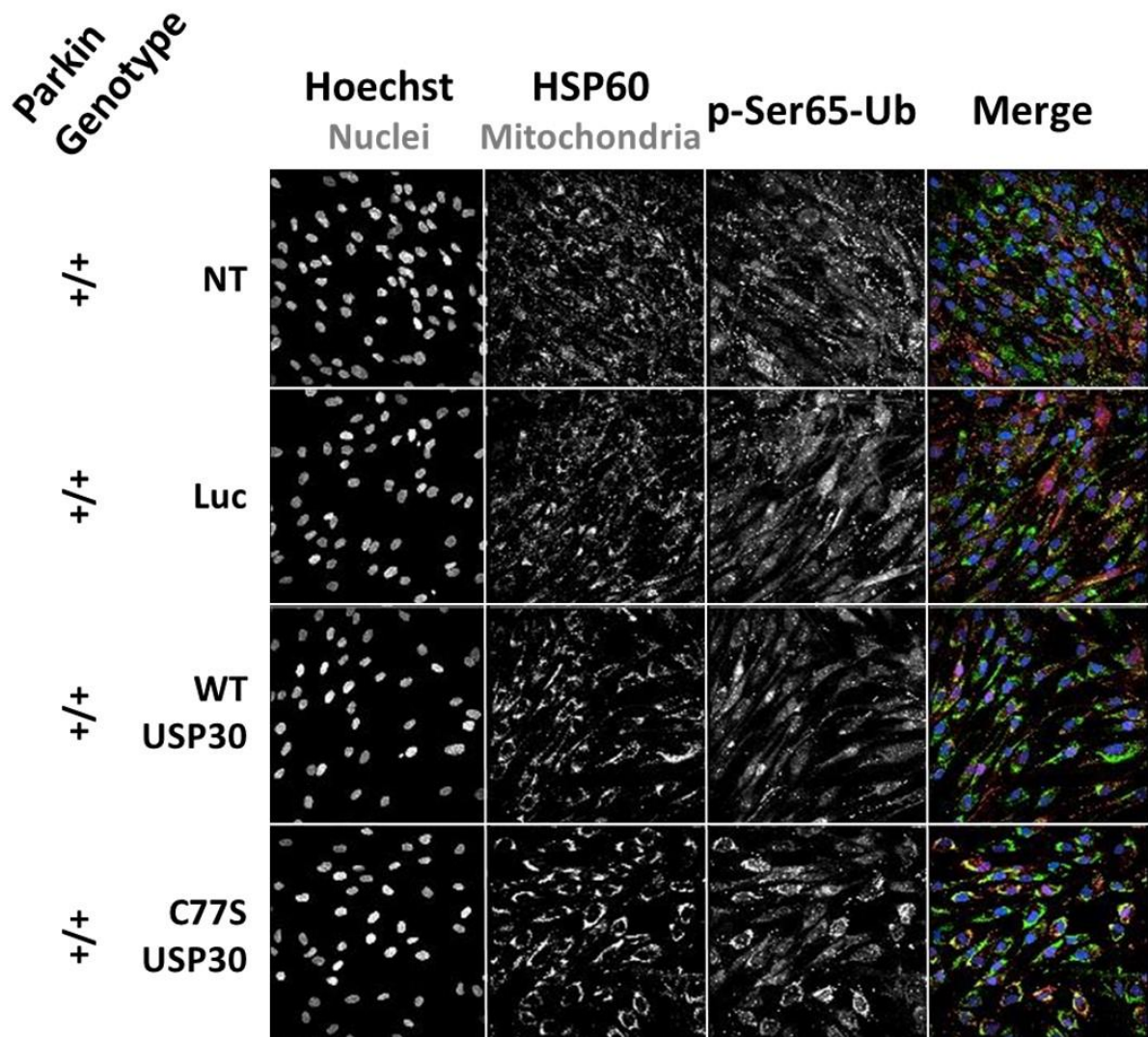
**Supplementary Figure S3. Over-expression of WT Parkin in patient-derived Parkin mutated fibroblasts.**

Parkin<sup>+/+</sup>, Parkin<sup>+/R275W</sup> and Parkin<sup>R275W/R275Q</sup> fibroblasts were transfected with a Parkin WT mRNA or Luciferase (Luc) mRNA for 20 h before inducing mitophagy for 2 h with 10  $\mu$ M FCCP. Representative images of the immuno-staining for p-Ser65-Ub and HSP60 are presented.



**Supplementary Figure S4. Over-expression of USP30-C77S in Parkin<sup>+/+</sup> fibroblasts.**

Representative images of Parkin<sup>+/+</sup> fibroblasts transfected with a non-targeted (NT), Luciferase (Luc), USP30-WT or USP30-C77S mRNA for 20 h. Transfection efficiency was assessed via immuno-staining for Myc (epitope tag for USP30) and HSP60 as mitochondria marker.



**Supplementary Figure S5. Over-expression of USP30-C77S affects the p-Ser65-Ub levels in Parkin<sup>+/+</sup> fibroblasts.**

The Parkin<sup>+/+</sup> fibroblasts were transfected with non-targeted (NT), Luciferase (Luc), USP30-WT or USP30-C77S mRNA for 20 h before inducing mitophagy for 2 h with 10  $\mu$ M FCCP. Representative images of the immuno-staining for p-Ser65-Ub and HSP60 are presented.