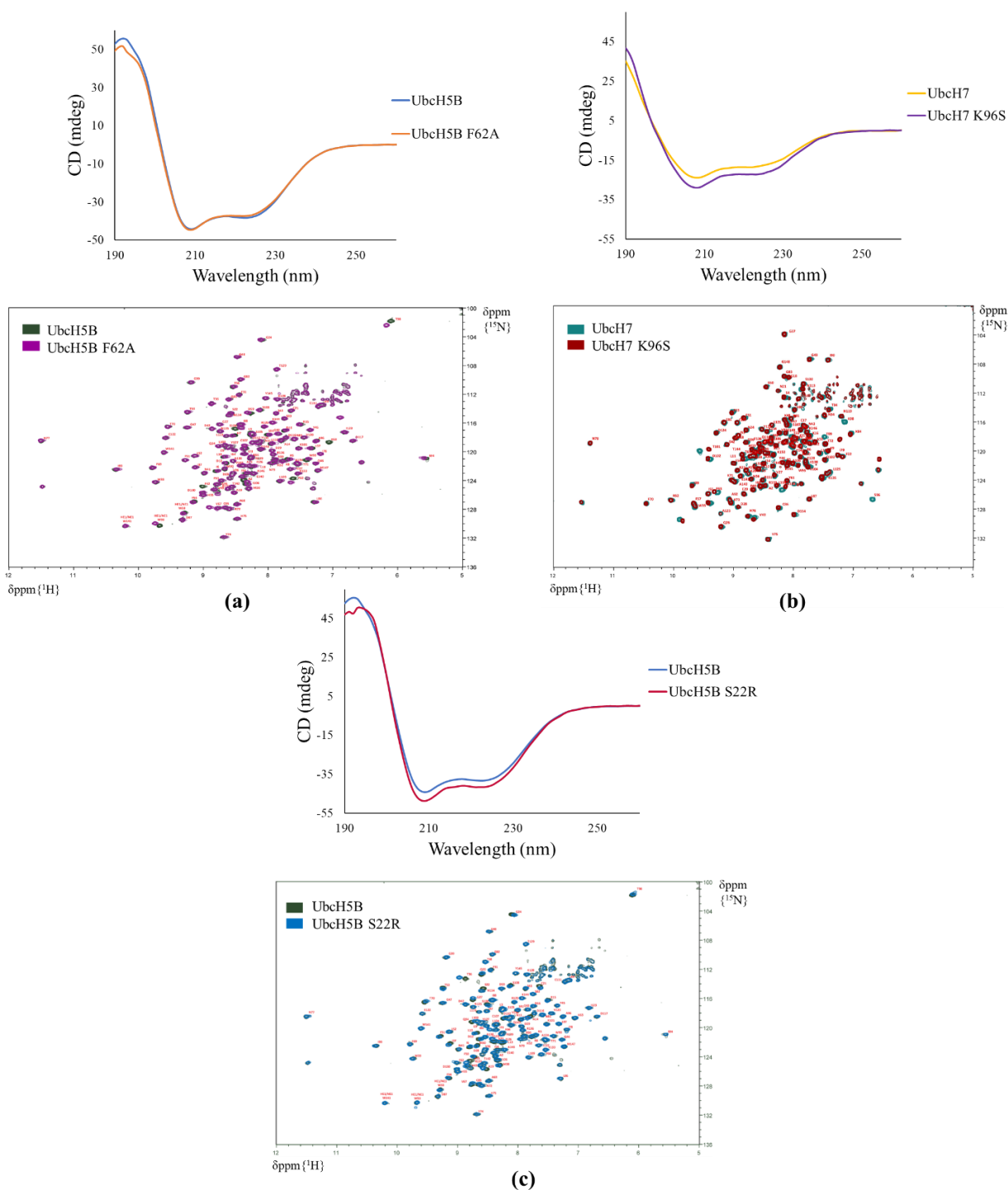


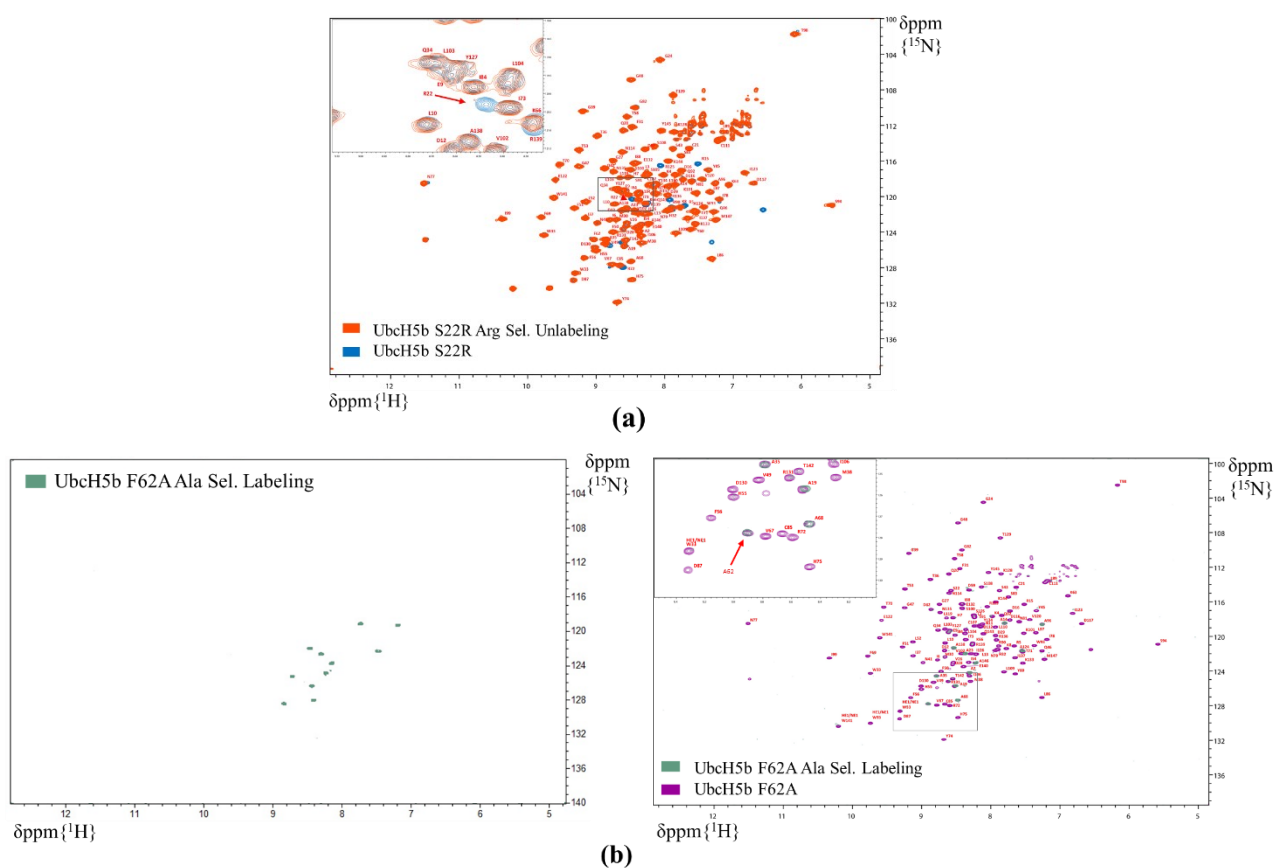
Ark RING 68aa  
927  
N-KQDGEEGTEEDTEEKCTICLSILEEGEDVRRLP~~CMHLFHQV~~CVDQWLITNKKCPICRV~~DIEAQLPSES~~-C994

Ark LONG 119aa  
876  
N-FRGNFEELIHLEERLGNVNRGASQGTIERCTYPHYKKVTTDFWSQRKLHCKQDGEEGTEEDTEEKCTICLSILEEGEDVRRLP~~CMHLFHQV~~CVDQWLITNKKCPICRV~~DIEAQLPSES~~-C994

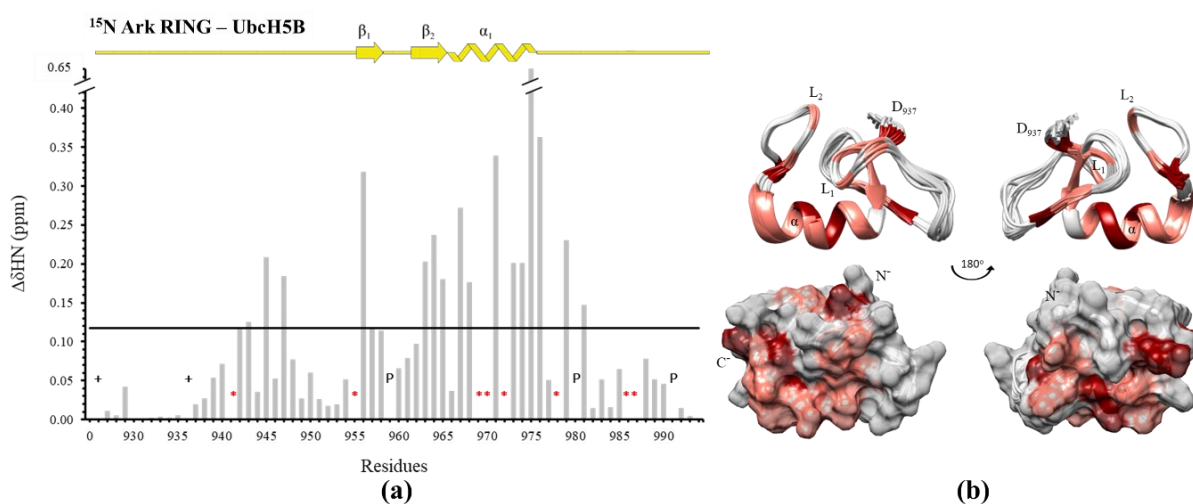
**Figure S1.** Ark RING and Ark LONG C-terminal constructs of Arkadia E3 ligase. Zinc binding amino acids are colored red.



**Figure S2.** (a) (top) CD spectra of wt UbcH5B and UbcH5B F62A. (bottom) Overlay  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of wt UbcH5B and UbcH5B F62A. (b) (top) CD spectra of wt UbcH7 and UbcH7 K96S. (bottom) Overlay  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of wt UbcH7 and UbcH7 K96S. (c) (top) CD spectra of wt UbcH5B and UbcH5B S22R. (bottom) Overlay  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of wt UbcH5B and UbcH5B S22R.



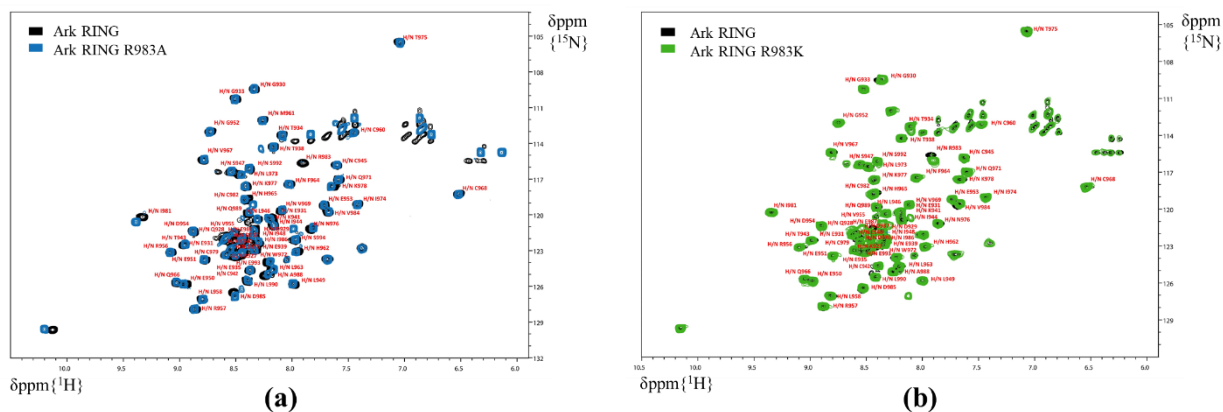
**Figure S3. (a)** Reverse labeling of  $^{15}\text{N}$  UbCH5B S22R. **(b)** Selective  $^{15}\text{N}$  labeling of UbCH5B F62A with  $^{15}\text{N}$ -Ala.



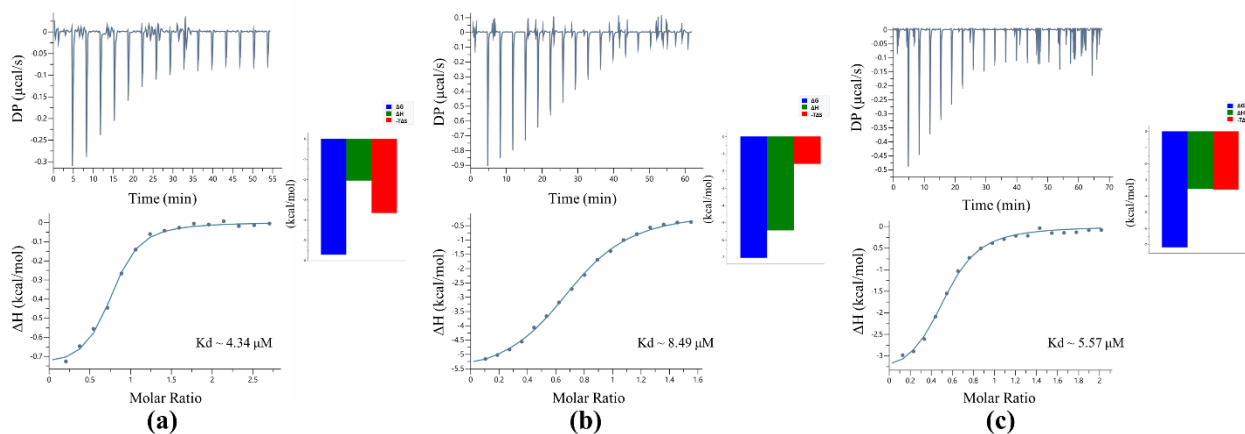
**Figure S4. (a)** Diagram of the total CSPs measured at 1:2 molar ratio (saturation point) for  $^{15}\text{N}$  Ark RING/ $^{14}\text{N}$  UbCH5B; \*: represents disappeared residues, +: represents residues with no information. **(b)** Ark RING mapping (PDBid: 2KIZ) after addition of UbCH5B (residues that disappeared during the interaction are colored red, while residues that exhibited fast exchange interaction are colored coral).

	945	955	965	975	983
<i>RNF111</i>	CTICLSILEEGEDVRRLP	CMHLLFHQV	CVDQWLITN	-KK--	CPICR
<i>RNF165</i>	CTICLSMLEEDGEDVRRLP	CMHLLFHQL	CVDQWLAMS	-KK--	CPICR
<i>RNF53</i>	CPICLELIEKPVSTK	--CDHIFCKF	CMLKLLNQK	-KGFSQ	CPLCK
<i>RNF38</i>	CVVCMCDFESRQLLRVLP	CNHEFHAK	CVDKWLKAN	-RT--	CPICR
<i>RNF55</i>	CKICA--	ENDKDKVKEIEPCGHL	LMCTS	CLTSWQESEGQG	--CPFCR

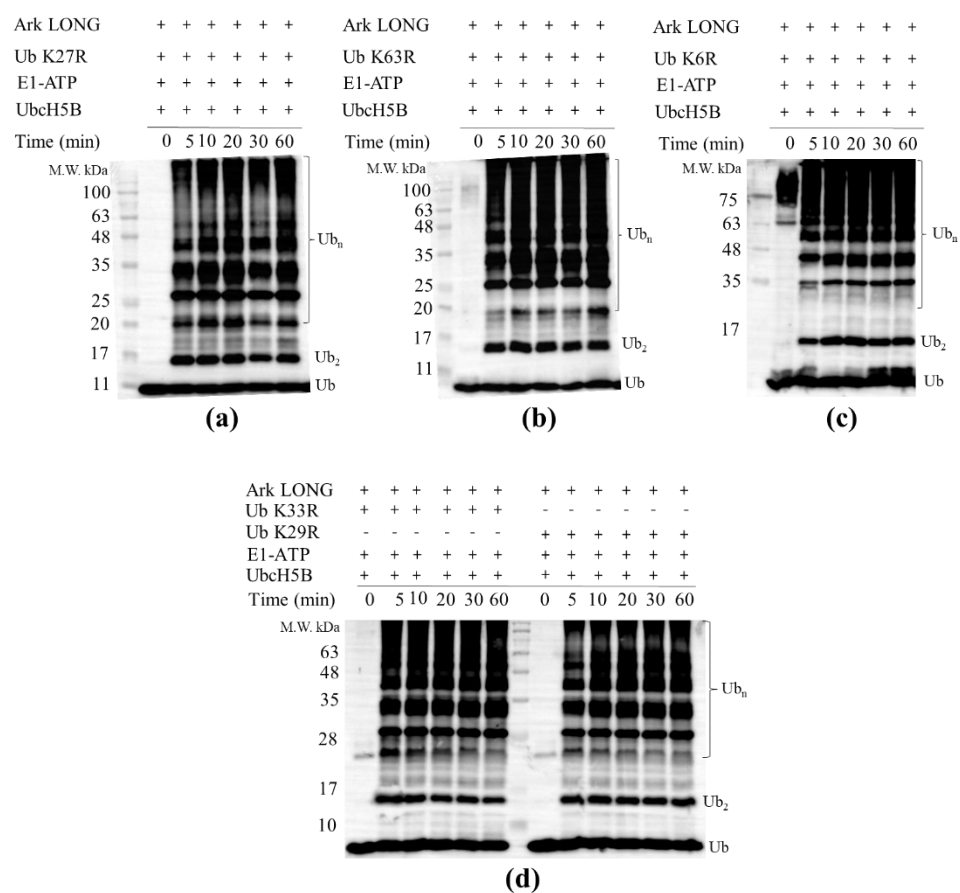
**Figure S5.** Sequence alignment of the RING domains of different E3 ubiquitin ligases numbered according to Arkadia/*RNF111*. The conserved arginine is highlighted with red box.



**Figure S6.** (a) Overlay  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of Ark RING R983A and Ark RING. (b) Overlay  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of Ark RING R983K and Ark RING.



**Figure S7.** (a) ITC data for the titration of wt UbcH7 into Ark LONG. (b) ITC data for the titration of UbcH5B into Ark LONG R983A. (c) ITC data for the titration of UbcH5B into Ark LONG R983K.



**Figure S8. (a)** In vitro ubiquitination assays using UbcH5B in the presence of Ub K27R, **(b)** Ub K63R, **(c)** Ub K6R, **(d)** Ub K33R and Ub K29R.