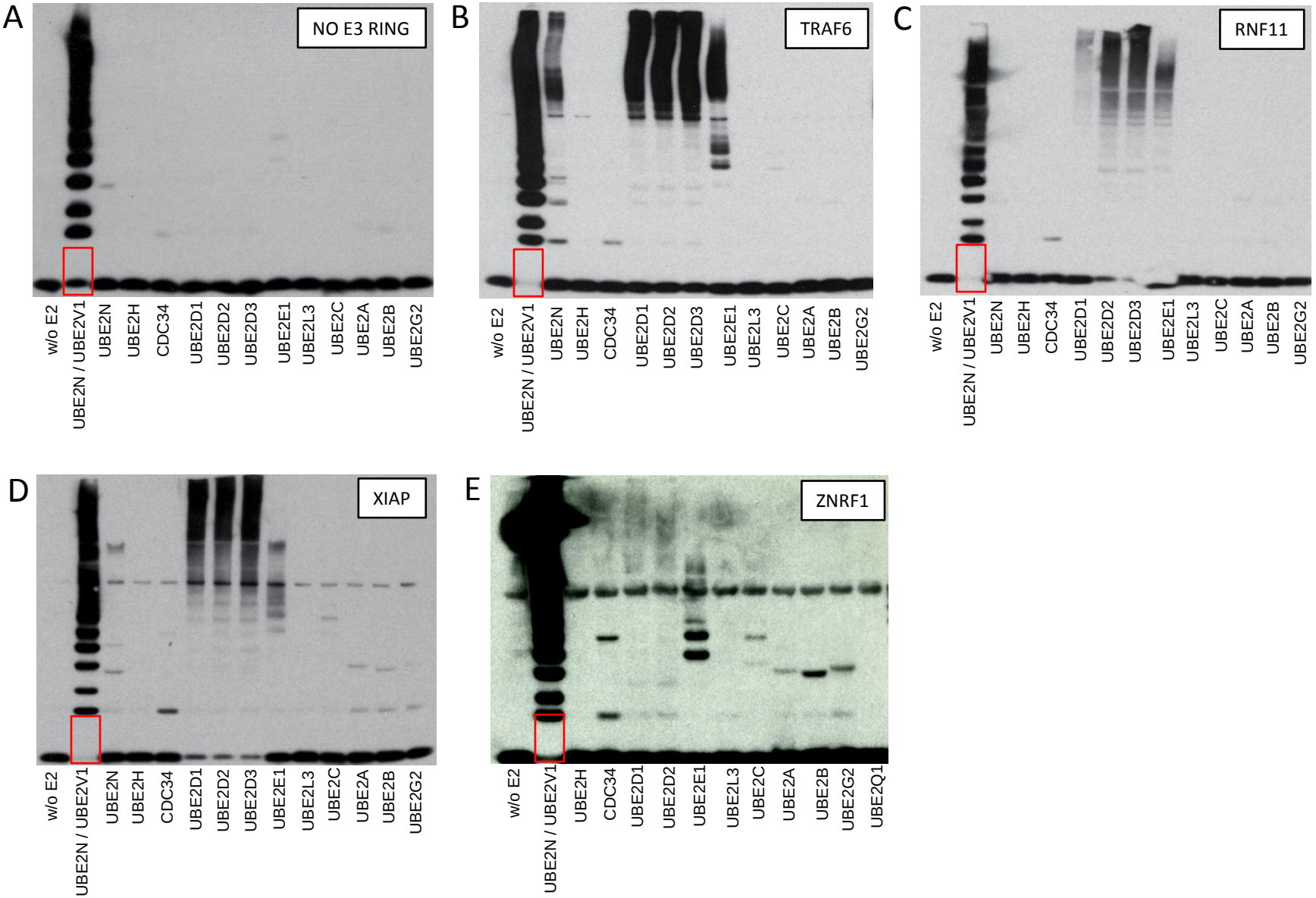
Supplementary File 6: Functional analysis of potential E2/E3-RING complexes



Comparison of functional profiles and yeast two-hybrid data

	No E3 RING			TRAF6			RNF11			XIAP			ZNRF1		
	ACTIVITY	Y2H/ -ADE	Y2H/ -HIS	ACTIVITY	Y2H/ -ADE	Y2H/-HIS	ACTIVITY	Y2H/ -ADE	Y2H/-HIS	ACTIVITY	Y2H/ -ADE	Y2H/-HIS	ACTIVITY	Y2H/ -ADE	Y2H/-HIS
wo/E2															
UBE2V1				NT			NT			NT			NT		
UBE2N/UBE2V1					NT	NT		NT	NT		NT	NT		NT	NT
UBE2N													NT		
UBE2H															
CDC34															
UBE2D1															
UBE2D2															
UBE2D3													NT		
UBE2E1															
UBE2L3															
UBE2C															
UBE2A															
UBE2B															
UBE2G2						AA		AA	AA		AA	AA		AA	AA
UBE2Q				NT			NT			NT					

To assess the ability of yeast two-hybrid studies to predict functional E2/E3-RING complexes 51 different E2/E3-RING combinations were systematically tested for autoubiquitination activity *in vitro.* Activity profiles are shown in Panels A-E. Corresponding yeast two-hybrid data is shown in the chart above. RED squares indicate strong positive results in functional assays or yeast two-hybrid studies. YELLOW squares indicate a weaker positive result in either assay. Reproducible positive yeast two-hybrid interactions detected on high stringency (-ADE) or lower stringency (-HIS) plates are shown for each potential E2/E3-RING complex. White squares indicate no functional activity ,or no detected interaction in yeast two-hybrid studies. UBE2G2 auto-activates (AA) in our yeast two-hybrid system and therefore does not represent a true positive interaction. UBE2N/UBE2V1 were used in combination in some activity assays, however, as these proteins were tested independently in yeast two-hybrid studies data for all potential complexes is shown. The UBE2N/UBE2V1 complex exhibits some independent auto-ubiquitination activity in the absence of E3-RING proteins (Panel A). However, this activity increases when an interacting E3-RING protein is included. This increase in activity can be monitored by observing the decrease in residual un-conjugated ubiquitin (see areas highlighted by red boxes), or an increased abundance of high molecular weight ubiquitination products (Panel E). The activity of UBE2V1 alone could not be tested (NT) as this is a catalytically inactive E2 protein., equally, interactions with the UBE2N/UBE2V1 complex were not tested (NT) in our yeast two-hybrid assays.