## Supplementary Figure S4

Α	Protein Name	Position	Modified Sequence	PEP	Charge	m/z
	MEKK2	K360	_LG <mark>K</mark> (gl)LLGQGAFGR_	0,005473	3	444,2579
	MEKK2	K450	_DQL <mark>K</mark> (gl)AYGALTENVTR_	1,25E-19	2	896,963
	MEKK2	K485	_D <mark>IK</mark> (gl)GANILR_	0,014834	2	557,3224
	MEKK2	K602	_IFVEA <mark>K</mark> (gl)LRPSADELLR_	0,050382	4	493,531
	MEKK3	K143	_I <mark>K(</mark> gl)ASQSAGDINTIYQPPEPR_	0,000485	3	767,0608
	MEKK3	K456	_DQL <mark>K</mark> (gl)AYGALTESVTR_	0,05263	3	589,3075
	MEKK3	K504	_DSAGNV <mark>K(</mark> gl)LGDFGASK_	0,000206	2	790,3892





## **Supplementary Figure S4**

## Characterization of the potential ubiquitination sites on MEKK2 targeted by XIAP.

(A) Enlisted are the lysines found to be ubiquitinated in MEKK2 and MEKK3 by XIAP and cIAP1. MEKK2 and MEKK3 were ubiquitinated *in vitro* using either cIAP1 or XIAP and run on an SDS-PAGE gel. The gel was stained with Coomassie and the observed MEKK2/3 ubiquitination bands were spliced and subjected to in-gel trypsin digestion. The peptides were then analyzed for ubiquitinated lysine residues by mass spectroscopy. (**B**) Representation of the ubiquitination sites on MEKK2 and MEKK3 by XIAP and cIAP1. (**C**) Mutation of K450 on MEKK2 enhances the activation of ERK5. HeLa cells were transiently transfected with either MEKK2 wt-V5, MEKK2 K360R-V5 or MEKK2 K450R-V5 constructs. Total lysates were analyzed by western blotting.