

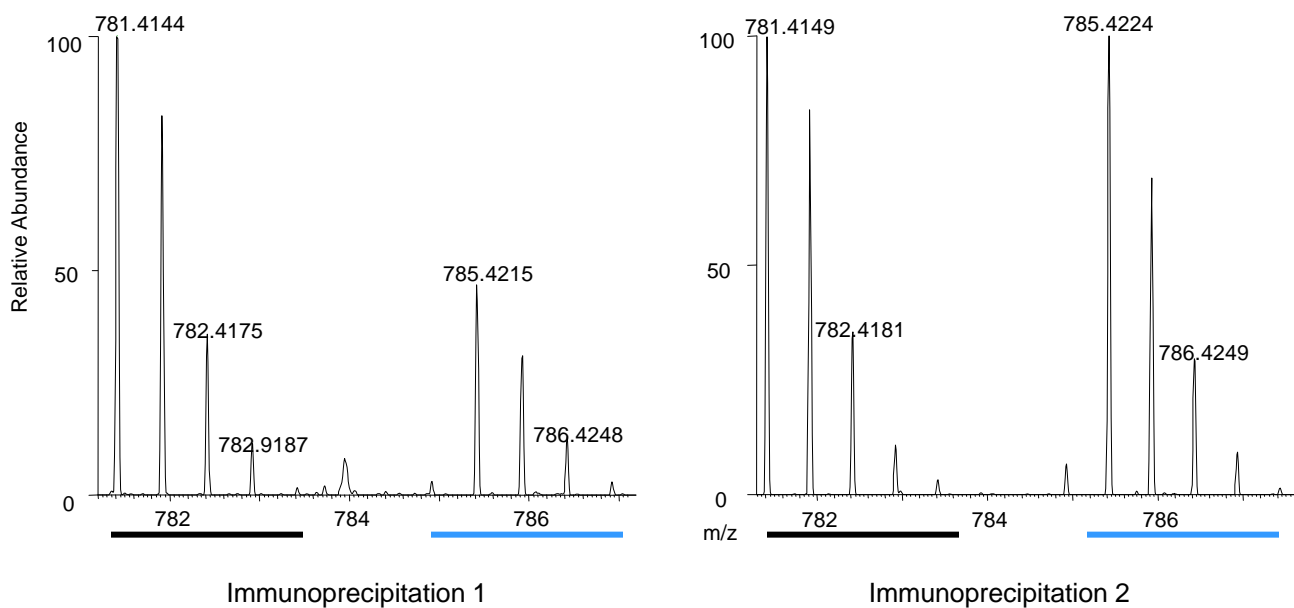
Supplementary Figure 1

a CDC27 Input

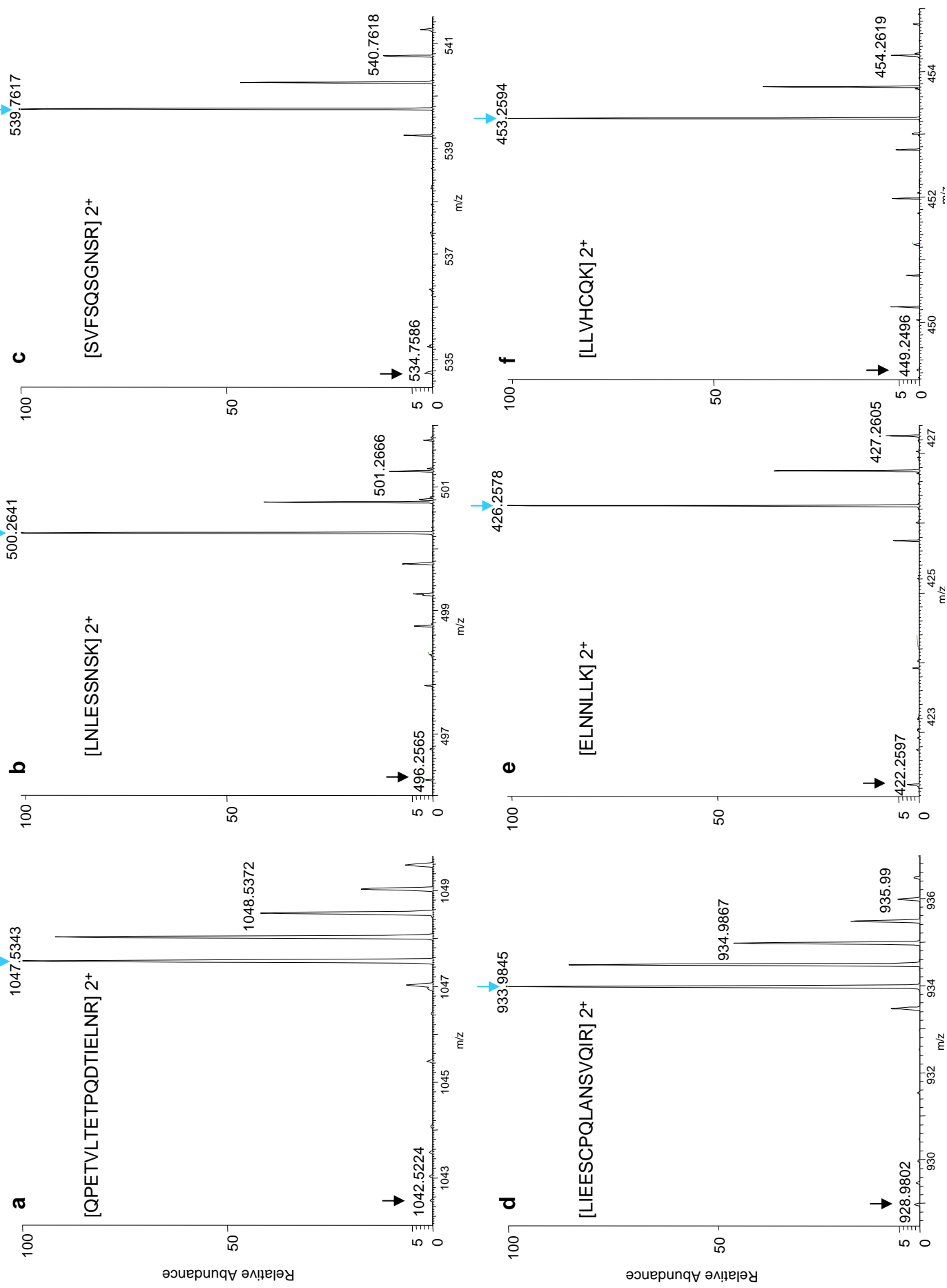
	HeLa cell lysate				Wheat germ extract			
Input concentration	10 mg/ml				2 mg/mL			
Volume analyzed per well	10 uL				10 uL			
Final Dilution	[1/16]	[1/32]	[1/68]	[1/136]	[1/16]	[1/32]	[1/68]	[1/136]



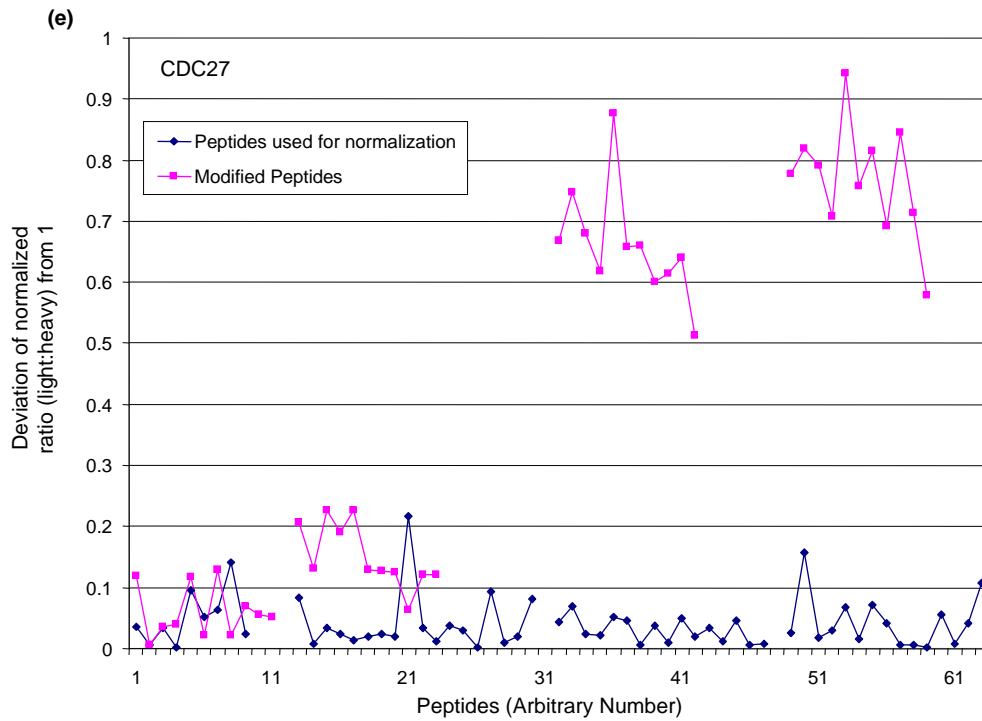
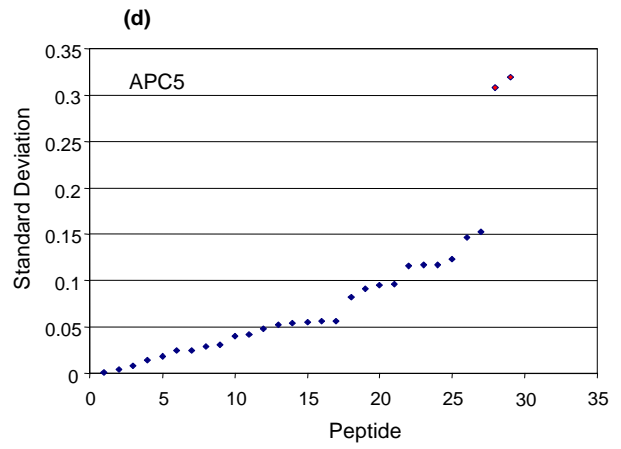
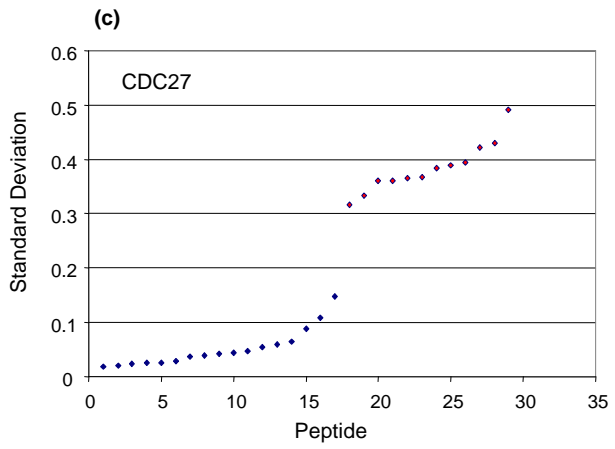
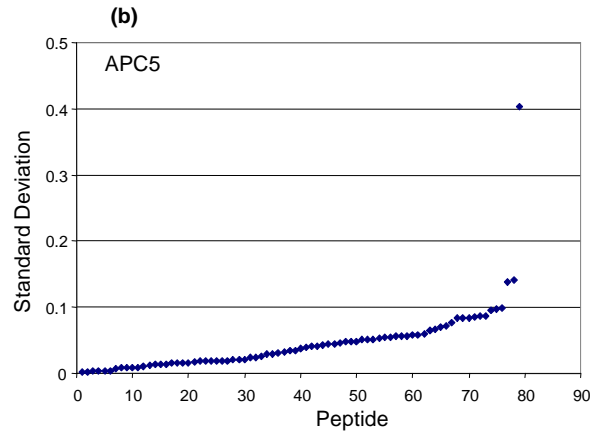
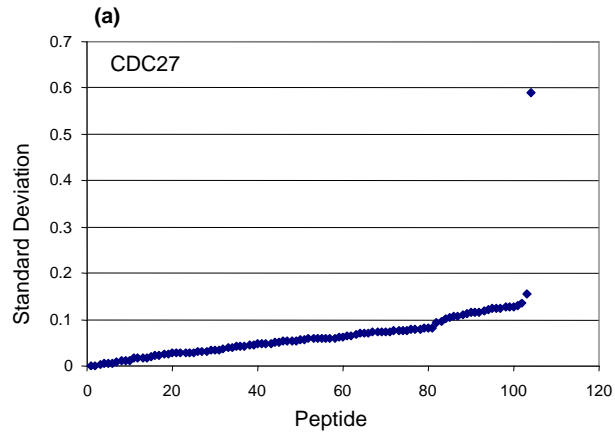
b CDC27 Output



Supplementary Figure 2



Supplementary Figure 3



Vector	Tag	Source
pEU-E01-His-TEV-N1	MGHHHHHHYDIPTTENLYFQGDISR	Cell Free Sciences
pEU-E01-His-TEV-C1	LEENLYFQGDYDIPPTTHHHHHH-	Cell Free Sciences
pEU-E01-His-N1-FLEX	MGHHHHHHK TENLYFQGDISR	This study
pEU-E01-His-C1-FLEX	LEK TENLYFQGDISR PTTHHHHHH-	This study

Site-directed mutagenesis required to generate the FLEX-tag peptide

Mutation/Vector	Primers (forward listed)	Template
N1 T14K/pEU-E01-His-N1-FLEX	catcaagattacgacatcccaaaaaccgaaaaccgtattttcagggc	pEU-E01-His-TEV-N1
C1insertKT	ccaagatatacactagttctcgagagagaccgaaaaccgtattttcaggg	pEU-E01-His-TEV-C1
C1 DI>SR	cctgtatttcagggagattactcgaggccaacgaccatcatcatc	C1 insertKT
C1 Y>I/pEU-E01-His-C1-FLEX	cctgtatttcagggagatatatcaggccaacgaccatcatcatc	C1 DI>SR

APC constructs

APC subunit clone	Primers	Backbone/restriction sites
cdc27	gcgcccaggatgacggctgcgaggaaccgctc gcgcggtacccttaaaatcattcatttcagctgca	pEU-E01-His-TEV-N1-AQUA Xho1, Kpn1
apc5	gcctcgaggatggccagcgtccacgagagcctctac gcactagctagagatgggtttatcaagggtaacccc	pEU-E01-His-TEV-N1-AQUA Xho1, SpeI

Supplementary Table 1. Vectors used for *in vitro* expression of the APC subunits. The residues surrounding the TEV site (italicized) were substituted to generate the FLEX-tag peptide (bolded). Restriction sites used to subclone the APC subunits are underlined.