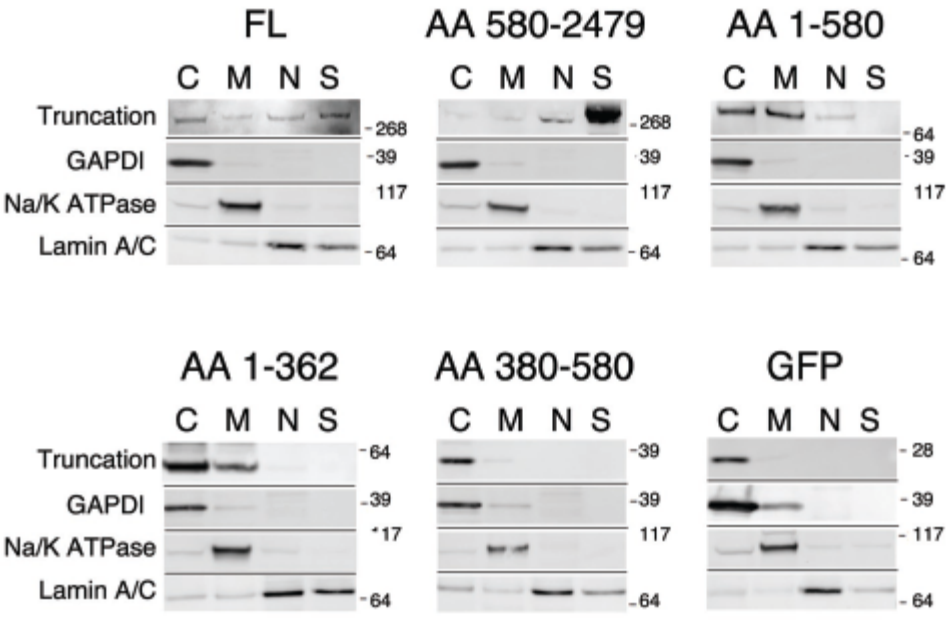
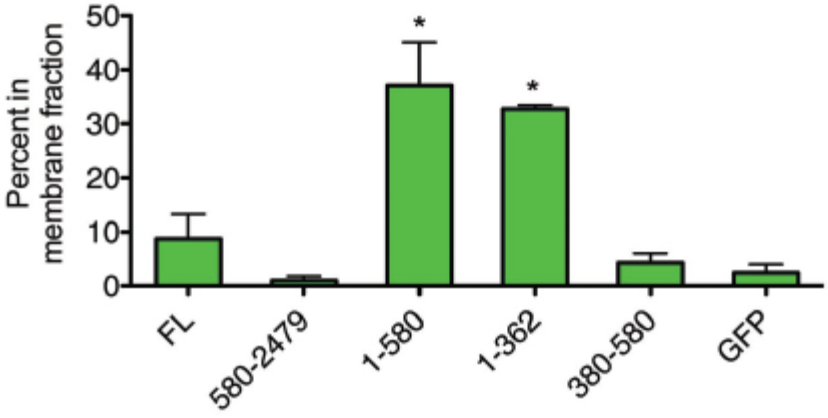
Supplemental Material

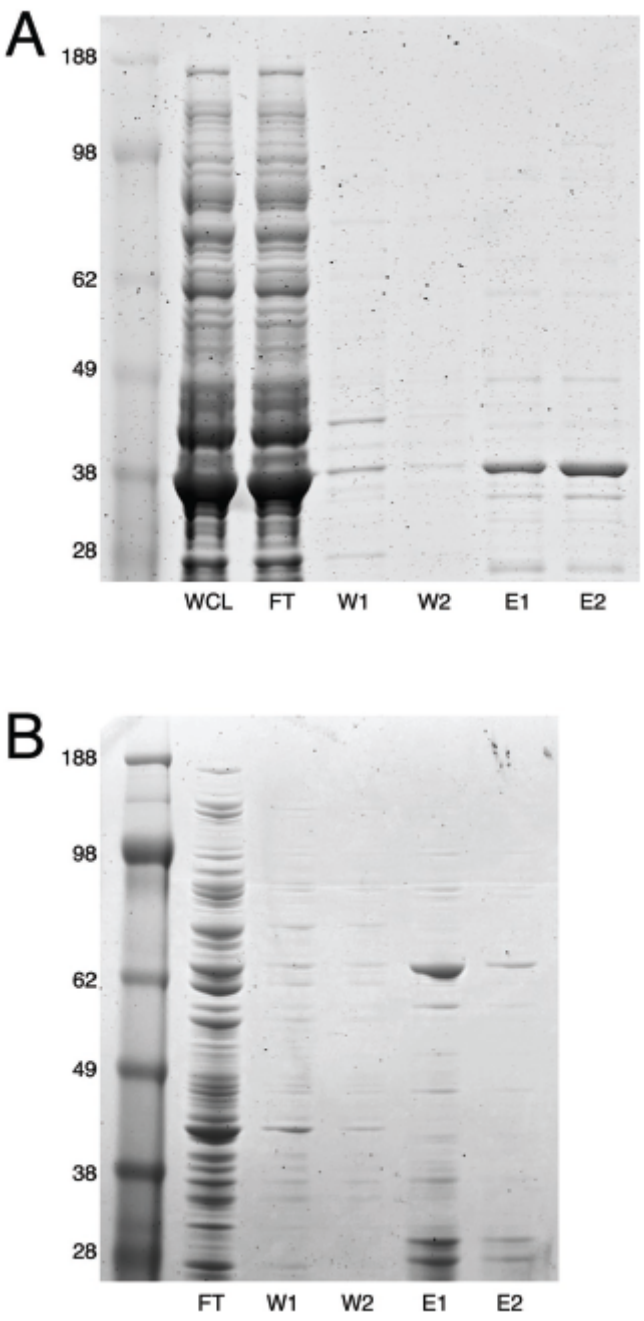




Supplemental Figure 1. Truncation of CEP290's C-terminus increases CEP290 membrane association

(A) Subcellular fractionation experiments performed on 293T cells expressing CEP290 constructs. Cells were fractionated into cytoplasmic (C), membrane (M), nuclear (N), and cytoskeletal (S) fractions and analyzed by western blotting. Relevant molecular weight markers are shown in kDa.

(B) Percent of each truncation present in the membrane fraction. Data are presented as mean ± SD, n=3. Asterisks indicate statistical significance over GFP-alone.



Supplemental Figure 2. Expression and purification of CEP290 truncation mutants in bacterial cells

CEP290 regions M (A) and aa 1-580 (B) were expressed from pDest-527 in E.coli BL21(DE3)pLysS by IPTG induction and purified by nickel affinity chromatography. WCL is the whole bacterial cell lysate. FT is the fraction of lysate that did not bind to the Ni-NTA resin. W1 and W2 are the material that washed off the resin during the first and second washes, respectively. E1 is the first elution fraction. E2 is the second elution fraction. Relavent molecular weights are indicated in kDa

Supplemental Table 1. Primers used to generate truncation mutants

Truncation	Primer	Sequence
aa 1-2479	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCCCCCAAACATCAATTGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATAGATCGGGAAGTTAACAGG
aa 580-2479	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGACGGAGAACATAAGCCAAGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATAGATCGGGAAGTTAACAGG
aa 1-580	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCCCCCAAACATCAATTGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAAGATTCAGATCCTCGGTAG
aa 1-362	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCCCCCAAACATCAATTGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATCCCTTTCTTGGATTCCCTGC
aa 380-580	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGAAAAACACTTGCATCATTGAGGAC
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAAGATTCAGATCCTCGGTAG
aa 1-1695	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCCCCCAAACATCAATTGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATCCAGCAGATACTTCAAATCC
aa 580-1695	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGACGGAGAACATAAGCCAAGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATCCAGCAGATACTTCAAATCC
aa 1-1966	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCCCCCAAACATCAATTGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATGTTTTCAGCTTTCTCTGCAG
aa 580-2479	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGACGGAGAACATAAGCCAAGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATAGATCGGGAAGTTAACAGG
aa 580-1966	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGACGGAGAACATAAGCCAAGG
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATGTTTTCAGCTTTCTCTGCAG
aa 1695-1966	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCAGTCCCAGAAGGAGTCAC
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATGTTTTCAGCTTTCTCTGCAG
aa 1966-2479	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGACAGGCATGACCGTGGAC
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATAGATCGGGAAGTTAACAGG
aa 1695-1903	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGCAGTCCCAGAAGGAGTCAC
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATTTCTCTTTCATGGGCTTAAGGTC
aa 1903-2479	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGACAGGCATGACCGTGGACC
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATAGATCGGGAAGTTAACAGG