Figure S1  **Alignment of the 20Rs of human and Drosophila APCs.** The 20Rs of APC contain an N-terminal Extended Region followed by a serine/leucine rich Phospho Region. It is thought that βcat initially binds to the core Extended Region, but clamps down to on the Phospho Region upon phosphorylation by CK1/GSK3. In addition, βcat binding 20Rs contain two acidic residues that contact critical lysine residues on βcat in the crystal structure (Ha et al., 2004; Xing et al., 2004). Notably, 20R2 from all species lack the upstream acidic residue. Interestingly, some additional 20Rs in human APC2 (APCL) also lack this upstream residue. It remains to be determined if these 20Rs also fail to bind βcat, although this would be consistent with the apparent weak activity of hAPC2 in βcat destruction given that hAPC2 also naturally lacks 15Rs(Schneikert et al., 2013).
Figure S2  APC2 transgenes are expressed at similar levels. Immunoblot on transgenic Drosophila embryos expressing the panel of GFP-tagged APC2 mutants. We previously demonstrated that the full-length APC2 transgene approximates endogenous APC2 protein levels (Roberts et al., 2011; Roberts et al., 2012b).
Figure S3  Flow cytometry analysis to measure βcat protein levels in transfected SW480 cells. Transfected cells were gated to quantify βcat protein in cells expressing GFP-tagged APC2 constructs. Q1 represents βcat levels in untransfected control cells, whereas Q2 represents transfected cells. Treatment with the MG132/ALLN cocktail blocked βcat destruction in APC2 transfected cells.