Figure S1. In-vitro ubiquitination by Ubc5 requires intact RING domains of Slx5 and Slx8. Dimers of Slx5-Slx8 were expressed and purified as in Fig. 4A. These proteins were then assayed in ubiquitination assays using 10,000 cpm of ^{32}P-labeled Ub (Ub*) in which all lysine residues had been mutated to arginine. Assays contained Uba1 (13 nM), recombinant Ubc5 (30 nM), and the indicated Slx5-Slx8 preparation at either 10 nM (left) or 100 nM (right). Following a 15 min incubation at 30ºC, the reaction products were resolved by SDS-PAGE and autoradiography. Mono-ubiquitinated Slx5 and Slx8 are indicated.

Figure S2. Slx5-Slx8 interacts with Ubc5 in vivo. W303-1a cells were transformed with the indicated MORF expression plasmid[30] and either a bi-cistronic plasmid expressing *SLX5* and *SLX8* under the control of the *GAL1-10* promoter (pNJ6847), or an empty control vector (pRS425). Extracts were prepared as described[30] and 200 µl of extract was diluted with 800 µl wash buffer (buffer A containing 250 mM NaCl and 1% NP-40). 10 µl of IgG beads (GE Healthcare) were mixed with the extract for one hour after which the beads were washed two times with wash buffer and the bound proteins eluted in SDS-PAGE sample buffer. Proteins were then identified by immunoblotting with anti-serum against Slx5 (A) or Slx8 (B) as described.[16] Note that the Ubc-MORF proteins are immunoreactive due to the presence of a Protein A epitope-tags.