**File S4**

**Assessing pool complexity**

Restriction digests were used diagnostically at each step of the mutagenesis and processing of the mutagenized pool. This enabled us to monitor if donor vectors or unmutagenized expression plasmids were errantly maintained in the pool. Also, double digests with enzymes that cut in the backbone and in the epitope tag allowed us to qualitatively assess pool complexity. Digesting a mutagenized plasmid pool in this way should result in smeared bands after gel electrophoresis, where the range of fragment sizes should reflect epitope tags inserted throughout the target ORF (data not shown).