Wehr et al., 'Monitoring Regulated Protein-Protein Interactions Using Split-TEV'

Supplementary Figure 8

Western blot of Split-TEV interaction pair dependent cleavage of GV-2ER.

Split-TEV interaction pair dependent cleavage of GV-2ER.

COS1 cells were transfected with equal amounts of the indicated expression constructs and lysed 40h after transfection. GV-2ER (*), unspecific band (open arrowhead), single cleaved GV-ER/ER-GV (arrowhead) and the ER domain (arrow) were detected with an α-ER antibody. Flag tagged GCNcc-N-TEV, GCNcc-C-TEV and GBR1acc-C-TEV were detected with an α-FLAG antibody. Equal loading was verified with an α-actin antibody. Boxed, contrast enhanced lower part of the α-ER probed western blot to better visualize the double-cleaved ER domain. Co-transfection of the interacting GCN4cc-TEV fragment pairs results in a more efficient GV-2ER cleavage when compared to the GCN4cc/GBR1acc control pair. Calculated protein sizes (in kDa): GV-2ER, 113.4; GV-ER, 77.1; ER-GV, 76.8; ER, 36.4; GCN4cc-N-TEV, 26.2; GCN4cc-C-TEV, 26.7; GBR1acc-C-TEV, 32.9. Due to the similar sizes, GV-ER/ER-GV and GCN4cc-N-TEV/GCN4cc-C-TEV appear as single bands.