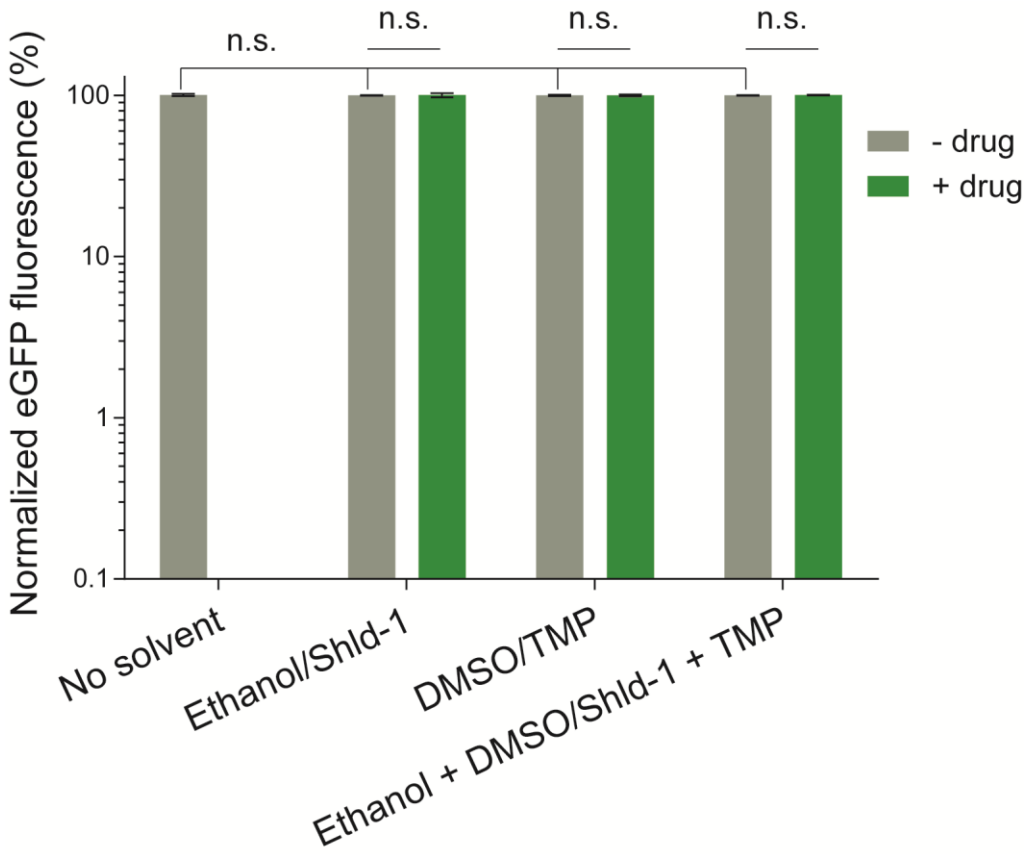
Supporting information for "Drug-Inducible Control of Lethality Genes: A Low Background Destabilizing Domain Architecture Applied to the Gal4-UAS System in *Drosophila*"

Manjunatha Kogenaru* **and Mark Isalan**

Department of Life Sciences, Imperial College London, London, SW7 2AZ, United Kingdom.

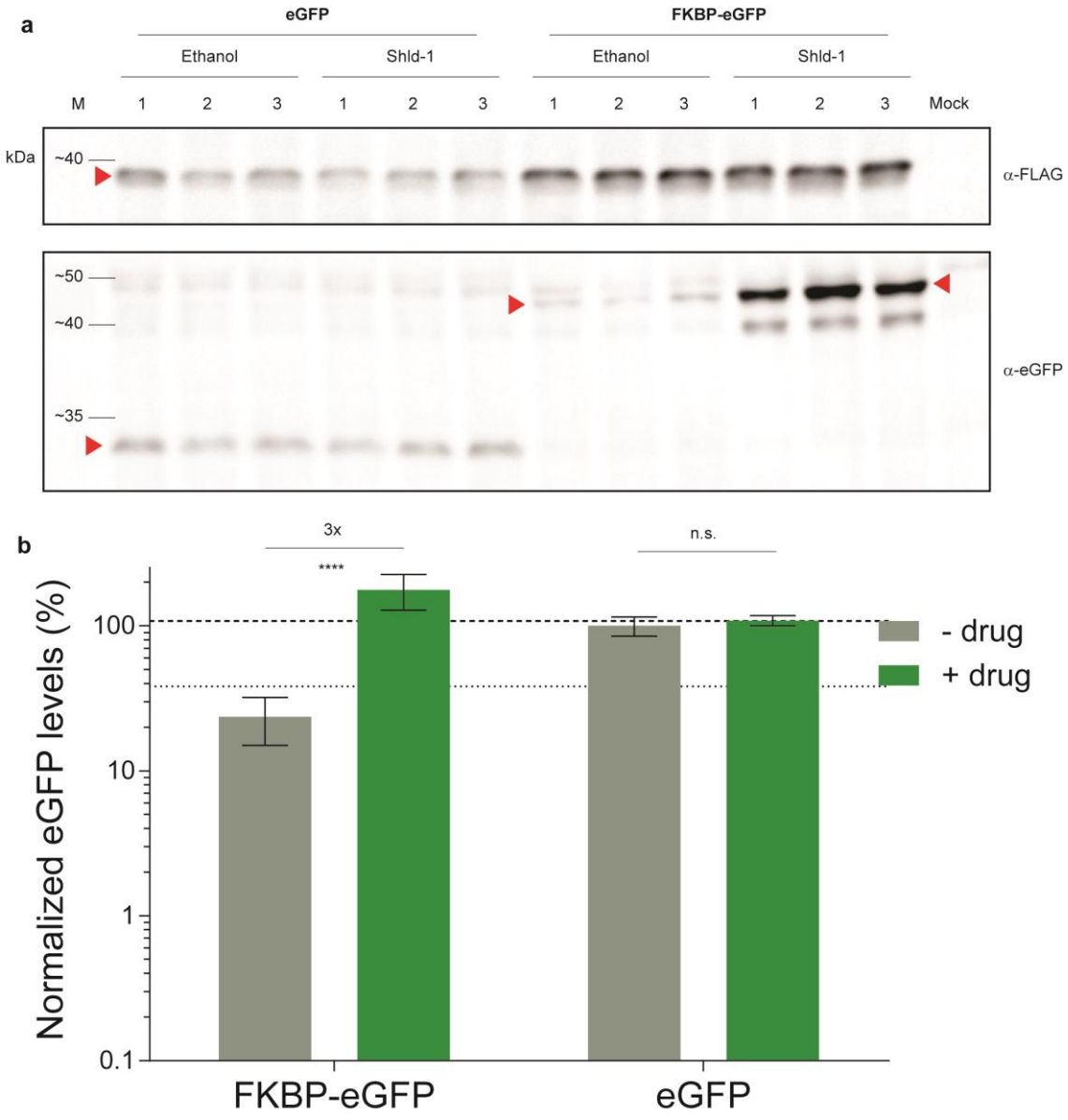
Imperial College Centre for Synthetic Biology, Imperial College London, London, SW7 2AZ, United Kingdom.

*Correspondence: m.kogenaru@imperial.ac.uk



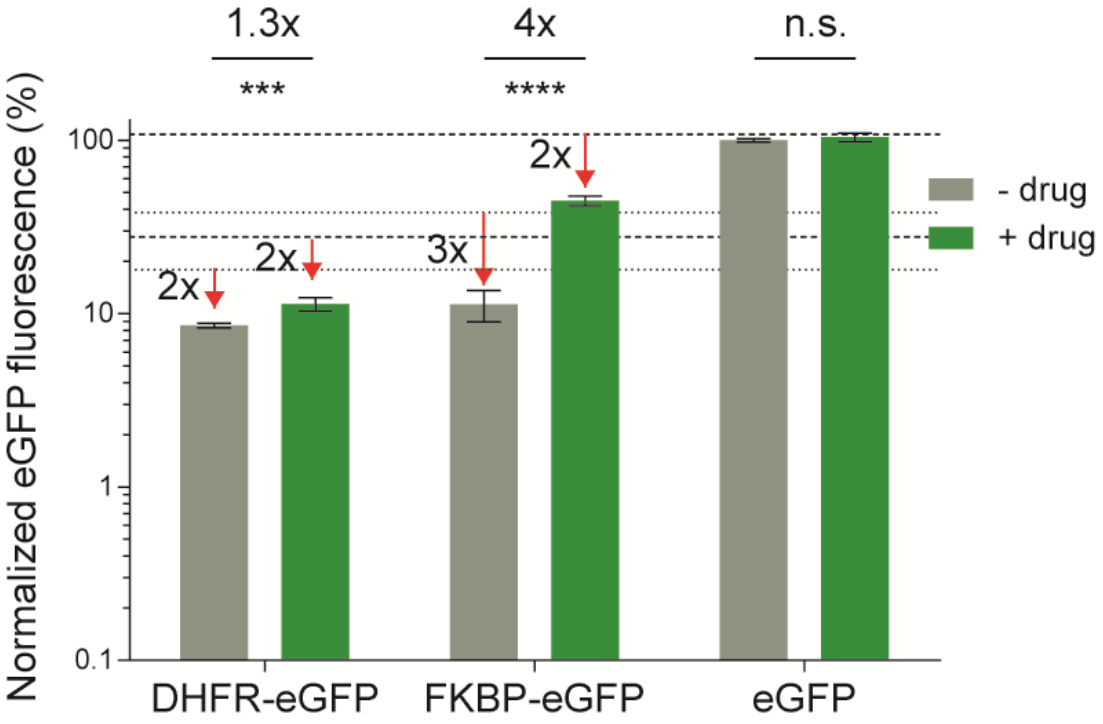
Supplementary Figure S1. The solvents and the drugs have no effect on the control eGFP fluorescence intensity. The histogram shows the normalized mean eGFP fluorescence for the eGFP control in the conditions of no solvent, absence (-) and presence (+) of the drugs (TMP, Shld-1). For the conditions without drug, corresponding volumes of the appropriate solvents were used. A *t*-test was performed on the indicated data set. All tests returned no significant results, *P*-value >0.05. The

error bars represent the standard deviation over the mean across the 5 biological replicates.

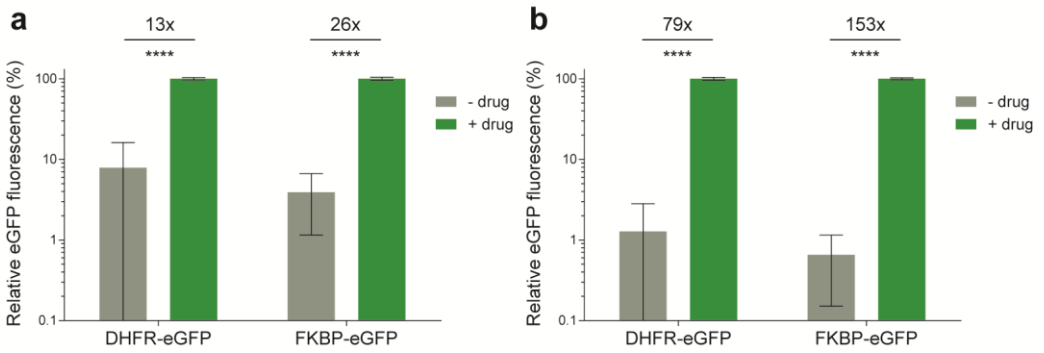


Supplementary Figure S2. Quantitative assessment of the FKBP parent DD in *Drosophila* S2R+ cells by a direct measurement of mCherry and eGFP protein levels, using Western blot. (a) A Western blot analysis was performed using *Drosophila* S2R+ cells transiently transfected with either control eGFP, or FKBP-eGFP fusion construct, or only mock-transfected (Mock). The FLAG-tagged mCherry and eGFP were detected by the anti-FLAG (α-FLAG) and eGFP (α-eGFP) antibodies, respectively (red arrowheads). The protein band intensities are reflective of the transfection rates (data not shown) and stabilization effect of the drug. Each construct

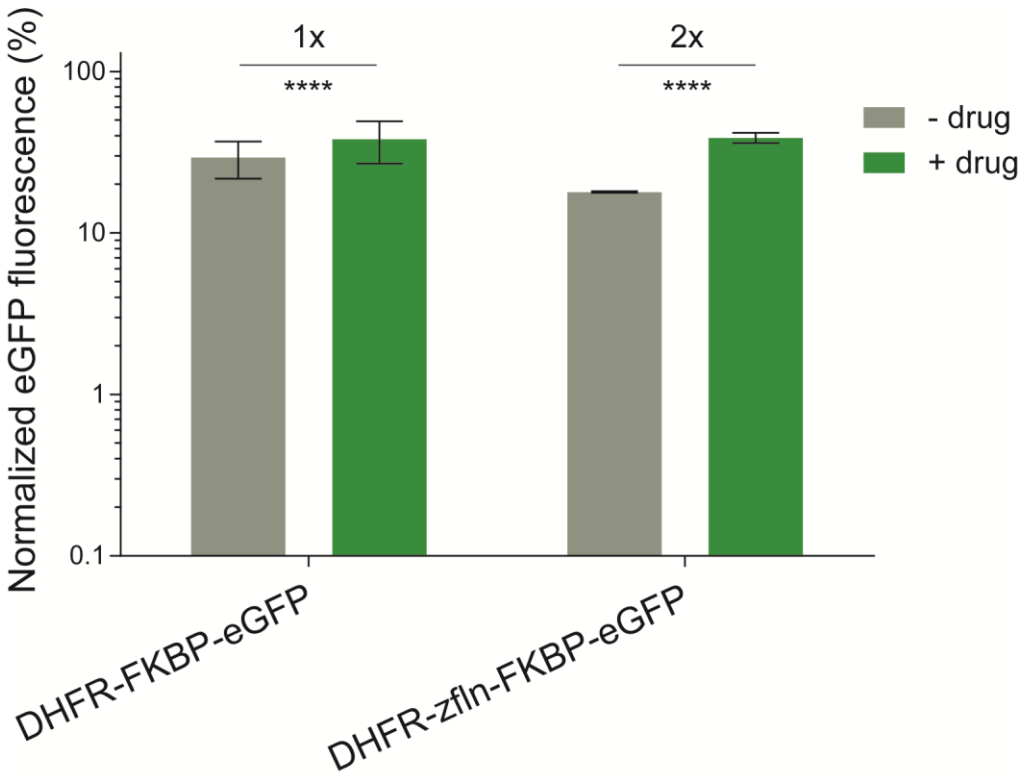
was performed in 3 replicates in the absence of drug (+Ethanol) or in the presence of drug (5 µM Shld-1). The molecular weights in kDa are indicated to the left (M). (b) The histogram shows the normalized mean eGFP protein levels for FKBP-eGFP and control eGFP in the absence (-) and presence (+) of the drug (5 µM Shld-1) conditions, derived from the Western blot presented in Supplementary Figure S2. The horizontal lines indicate the mean eGFP fluorescence of the parent FKBP DD determined from the fluorescence-based quantification method (indicated in absence (dot) and presence (hyphen) of drug). The statistical significance of results from *t*-tests are summarized with asterisk marks representing the level of significance (**** = *P*-value ≤ 0.0001 and n.s. = *P*-value >0.05). The error bars represent the standard deviation of the mean over 3 biological replicates.



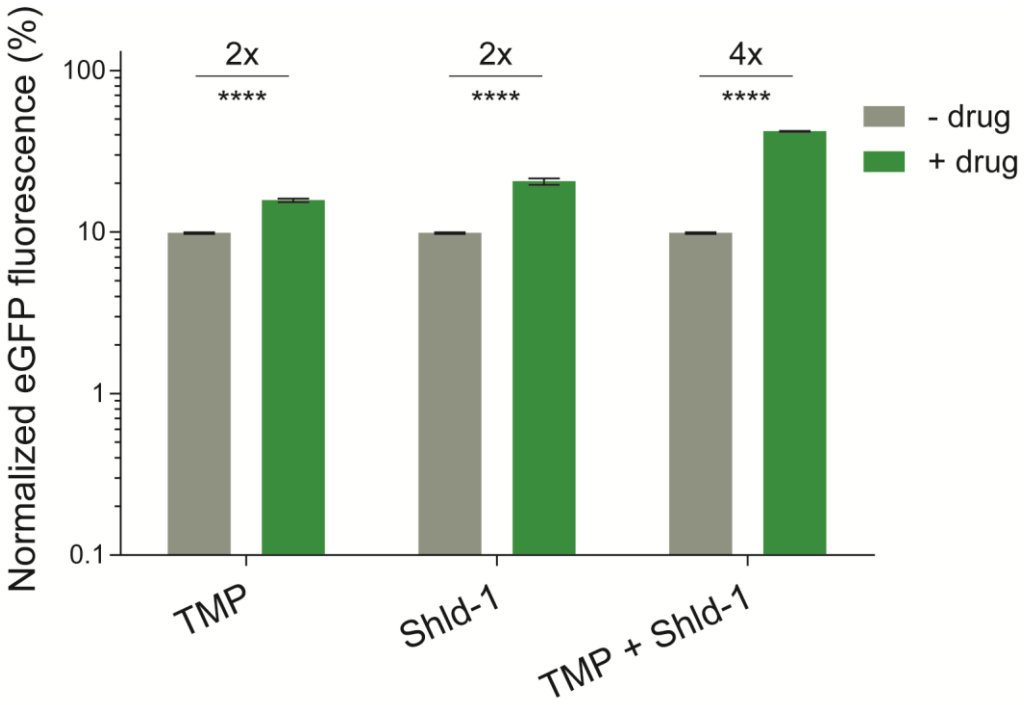
Supplementary Figure S3. Quantitative assessment of the parent DHFR and FKBP DD domains in stable *Drosophila* S2R+ cell lines. The histogram shows the normalized mean eGFP fluorescence for DHFR-eGFP, FKBP-eGFP and eGFP control in the absence (-) and presence (+) of the drugs (10 µM TMP, 5 µM Shld-1). Control eGFP without DD: DMSO and 10 µM TMP. The horizontal lines indicate the mean eGFP fluorescence of the parent DHFR and FKBP DDs determined from a transient transfection quantification; these are indicated in absence (dots) and presence (hyphens) of drug. The statistical significance of results from *t*-tests are summarized with asterisk marks representing the levels of significance (*** = *P*-value ≤ 0. 001, **** = *P*-value ≤ 0.0001 and n.s. = *P*-value >0.05). The error bars represent the standard deviation of the mean over 5 biological replicates.



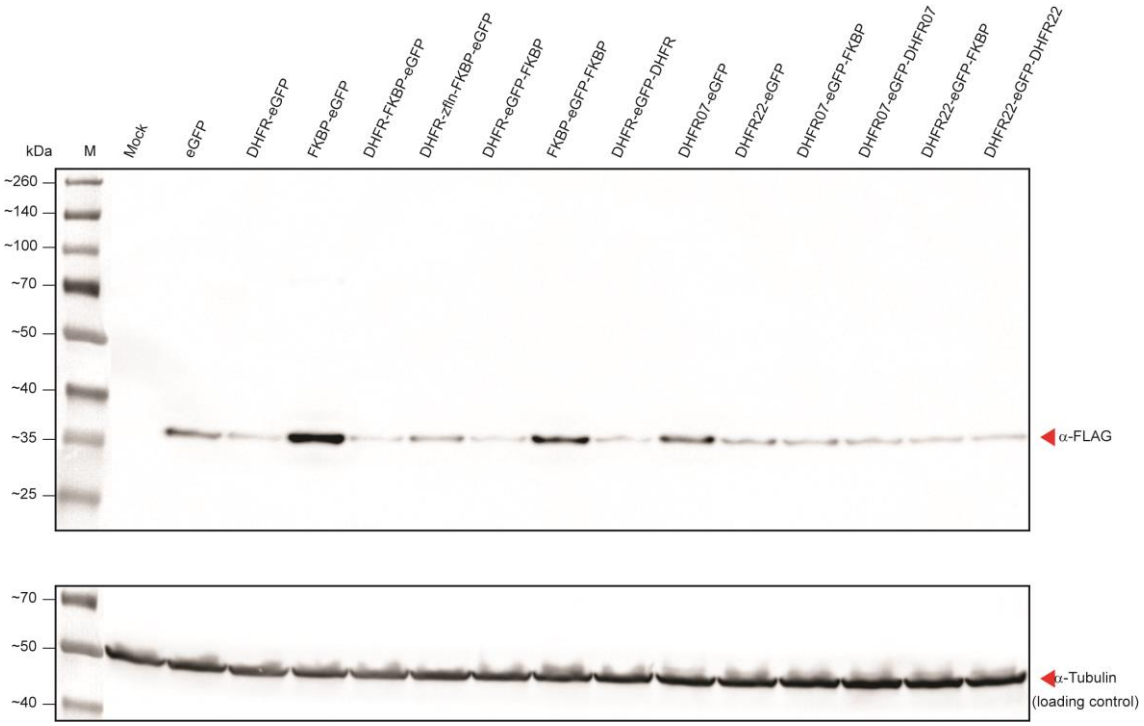
Supplementary Figure S4. Relative normalization of DHFR and FKBP DDs fused to the N-terminus of eGFP protein. This normalization process omits the mCherry fluorescence by considering only the eGFP fluorescence. The lowest mean eGFP fluorescence from the uninduced condition was used to baseline the signal and normalize the resulting data to the respective induced conditions (10 µM TMP for DHFR-eGFP or 10 µM Shld-1 for FKBP-eGFP). (a) Relative normalization of the data corresponding to the main Figure 1b for Drosophila S2R+ cells. (b) Relative normalization of the data corresponding to the main Figure 1d for HEK293T cells. The statistical significance results from *t*-tests are summarized with asterisk marks representing the level of significance (**** = *P*-value ≤ 0.0001). The error bars represent the standard deviation over the mean from 5 biological replicates.



Supplementary Figure S5. Quantitative assessment of the chimeric DD architecture at the N-terminus of an eGFP fusion protein. The normalized mean eGFP fluorescence is shown in the absence (-) and in the presence (+) of 10 µM TMP and 5 µM Shld-1 drug molecules. The first DD architecture results from a direct concatenation of the DHFR and FKBP DDs to the N-terminus of eGFP (DHFR-FKBP-eGFP). The second architecture uses a 33-amino acid residues long zinc finger domain as a structured linker (zfln) between the DHFR- and FKBP-DD (DHFR-zfln-FKBP-eGFP). The statistical significance resulting from a *t*-test are summarized with asterisk marks representing the level of significance (**** = *P*-value ≤ 0.0001). The error bars represent the standard deviation over the mean from 4 biological replicates.



Supplementary Figure S6. Orthogonality of the drug molecules assessed using the chimeric DD architecture DHFR-eGFP-FKBP. The normalized mean eGFP fluorescence is shown in the absence (-) and in the presence (+) of 5 µM TMP and/or 5 µM Shld-1 drug molecules. The statistical significance resulting from a *t*-test are summarized with multiple asterisk marks representing the level of significance (**** = *P*-value ≤ 0.0001). The error bars represent the standard deviation over the mean from 4 biological replicates.



Supplementary Figure S7. The T2A peptide sequences are processed correctly in both single and double DD architectures. A Western blot analysis was performed using *Drosophila* S2R+ cells transiently transfected with either control eGFP, DD-eGFP, and DD-eGFP-DD fusion constructs, or only mock-transfected (Mock). The FLAG-tagged mCherry and an endogenous α-Tubulin loading control were detected by the anti-FLAG (α-FLAG) and α-Tubulin (α-Tubulin) antibodies, respectively (red arrowheads). The protein band intensities are reflective of the transfection efficiency and/or the expression levels of the constructs (data not shown). The molecular weights in kDa are indicated to the left (M).

Supplementary DNA sequences

Following are the coding DNA sequences of the low background DD architecture applied to the Gal4VP16 transcriptional activator.

>DHFR22-Gal4VP16
ATGATCAGTCTGATTGCGGCGTTAGCGGTAGATCACGTTATCGGCATGGAAAACGCCATGCCGTGGAGCC
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>DHFR22-Gal4VP16-DHFR22
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