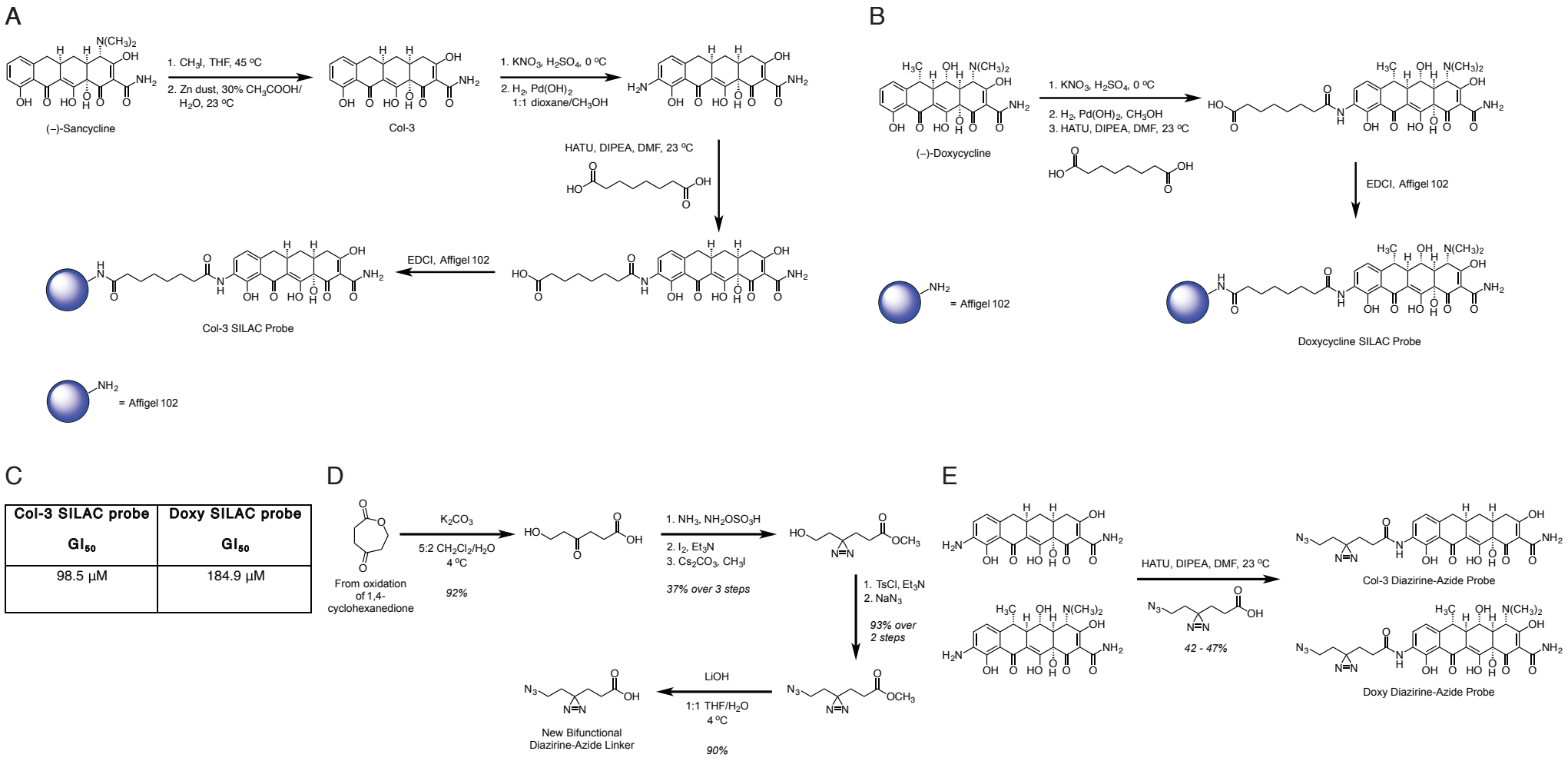

C

Col-3 SILAC probe GI_{50}	Doxy SILAC probe GI_{50}
98.5 µM	184.9 µM

Figure S1 (Related to Figure 1) – Scheme for synthesis of tetracycline affinity matrices and bifunctional diazirine-azide tetracycline probes. (**A**) Synthesis of Col-3 SILAC affinity matrix and (**B**) Synthesis of Doxycycline SILAC affinity matrix. (**C**) GI_{50} values for the tetracycline Col-3 and doxycycline SILAC probes (ethyl amide derivatives). (**D**) Synthesis of a novel bifunctional diazirine-azide linker. (**E**) Synthesis of bifunctional Col-3 and doxycycline crosslinking probes.

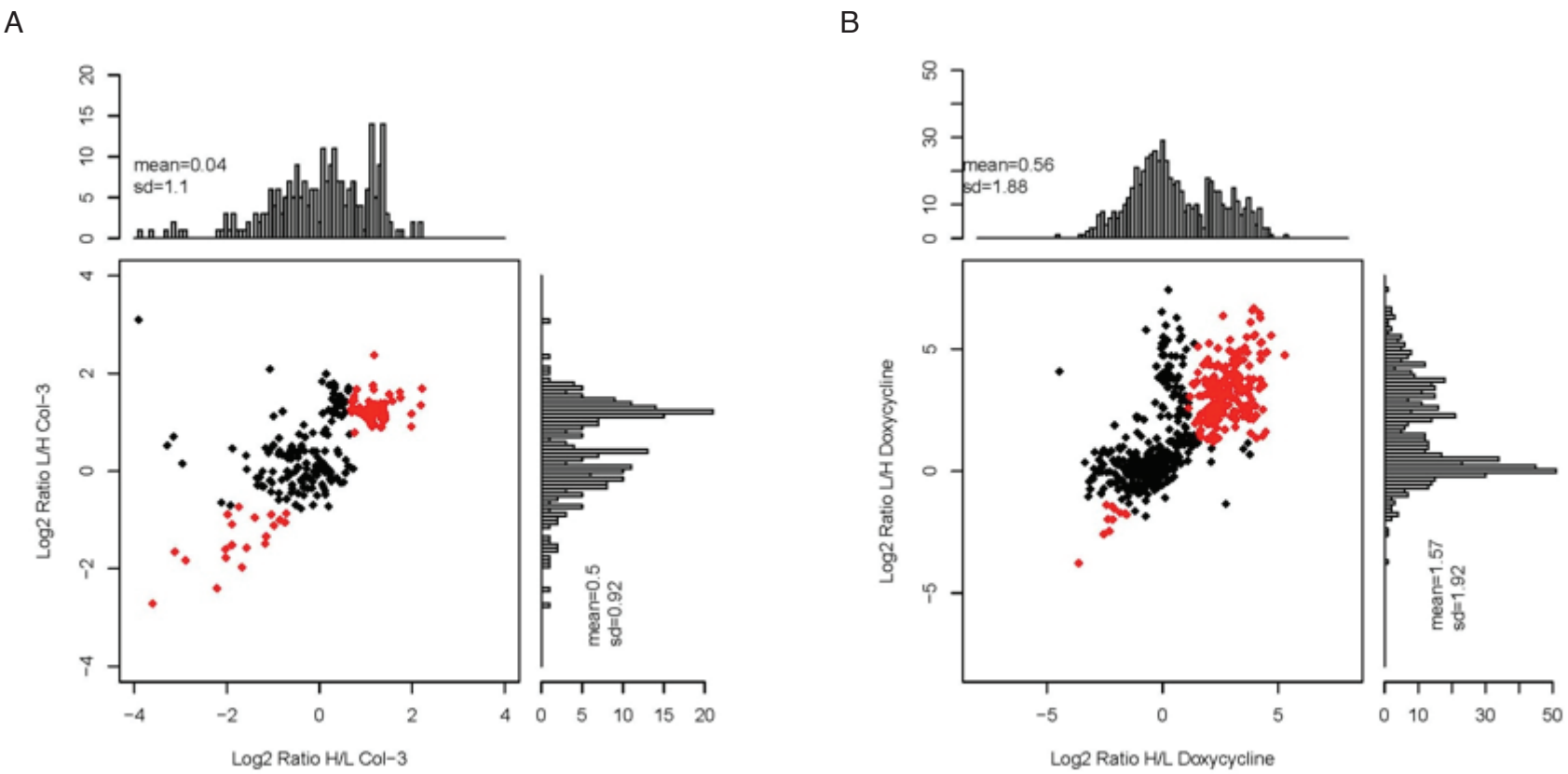

Figure S2 (Related to Figure 1) – Histograms and scatter plots for proteins identified from SILAC tetracycline affinity isolation experiements. (A) Plot showing proteins identified from Col-3 affinity probe pulldowns. **(B)** Plot showing proteins identified from doxycycline affinity probe pulldown experiments. Proteins with significantly different SILAC ratios (p value < 0.05) are shown in red. L = light SILAC lysate fraction and H = heavy SILAC lysate fraction

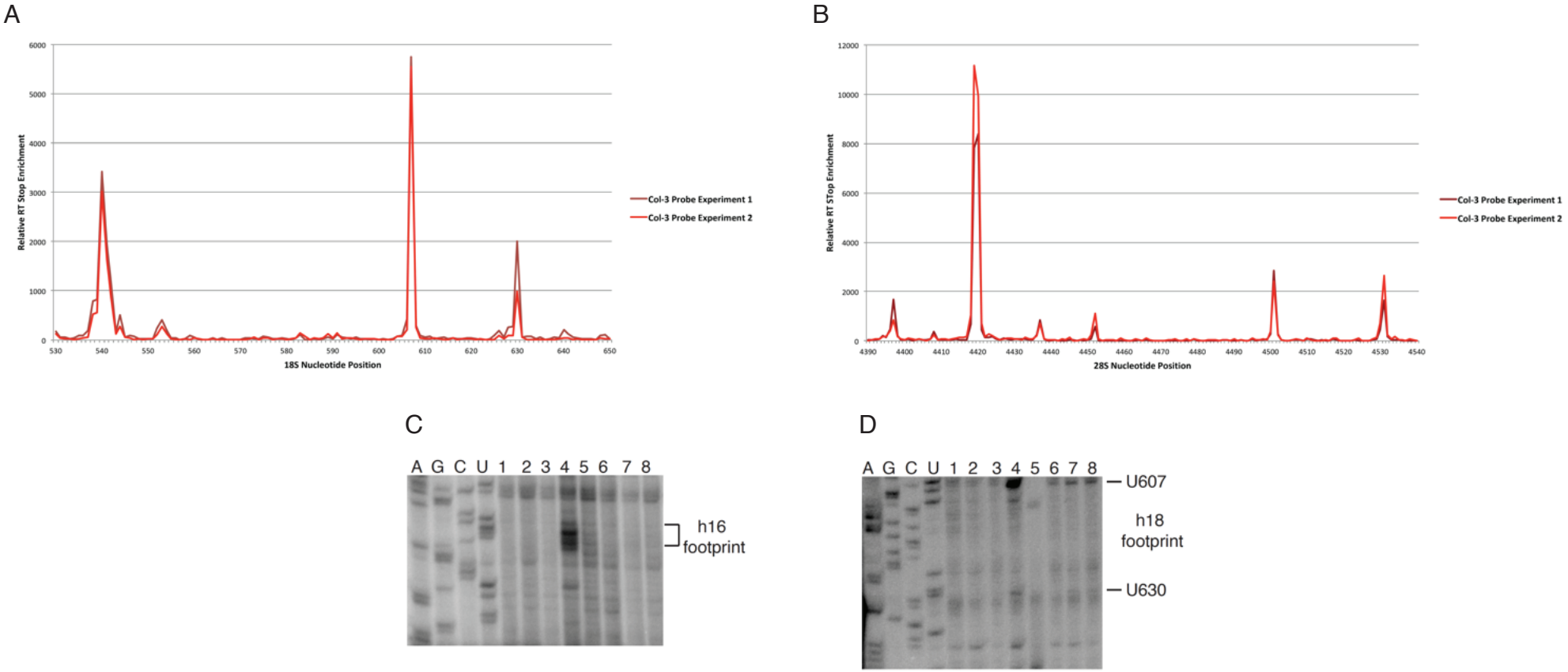

Figure S3 (Related to Figure 2) – icCL-Seq shows high reproducibility across independent experiments and is confirmed in radiolabeled footprinting experiments. (**A**) Comparison of reverse transcription stop enrichment from two independent in-cell crosslinking experiments showing highly reproducible Col-3 probe crosslinking at helices h16 and h18 on the 18S human ribosomal RNA. (**B**) Comparison of reverse transcription stop enrichment from two independent in-cell crosslinking experiments showing highly reproducible Col-3 probe crosslinking at helix H89 and the peptidyl exit tunnel on the 28S human ribosomal RNA. (**C**) ^{32}P radio-labeled primer extension for footprinting of tetracycline crosslinks on helix h16 (nucleotides 538-542) in A375 cells. (**D**) ^{32}P radio-labeled primer extension for footprinting of tetracycline crosslinks on helix h18 (U607 and U630) in A375 cells. For footprinting assays, lanes 1–8 correspond to RNA samples from A375 cells treated with DMSO, 20 μM aniline control probe, 20 μM inactive Col-3 control probe, 20 μM Col-3 diazirine-azide probe, 20 μM Col-3 diazirine-azide probe + 100 μM Col-3 soluble competitor, 20 μM inactive doxycycline control probe, 20 μM doxycycline diazirine-azide probe, 20 μM doxycycline diazirine-azide probe + 100 μM doxycycline soluble competitor

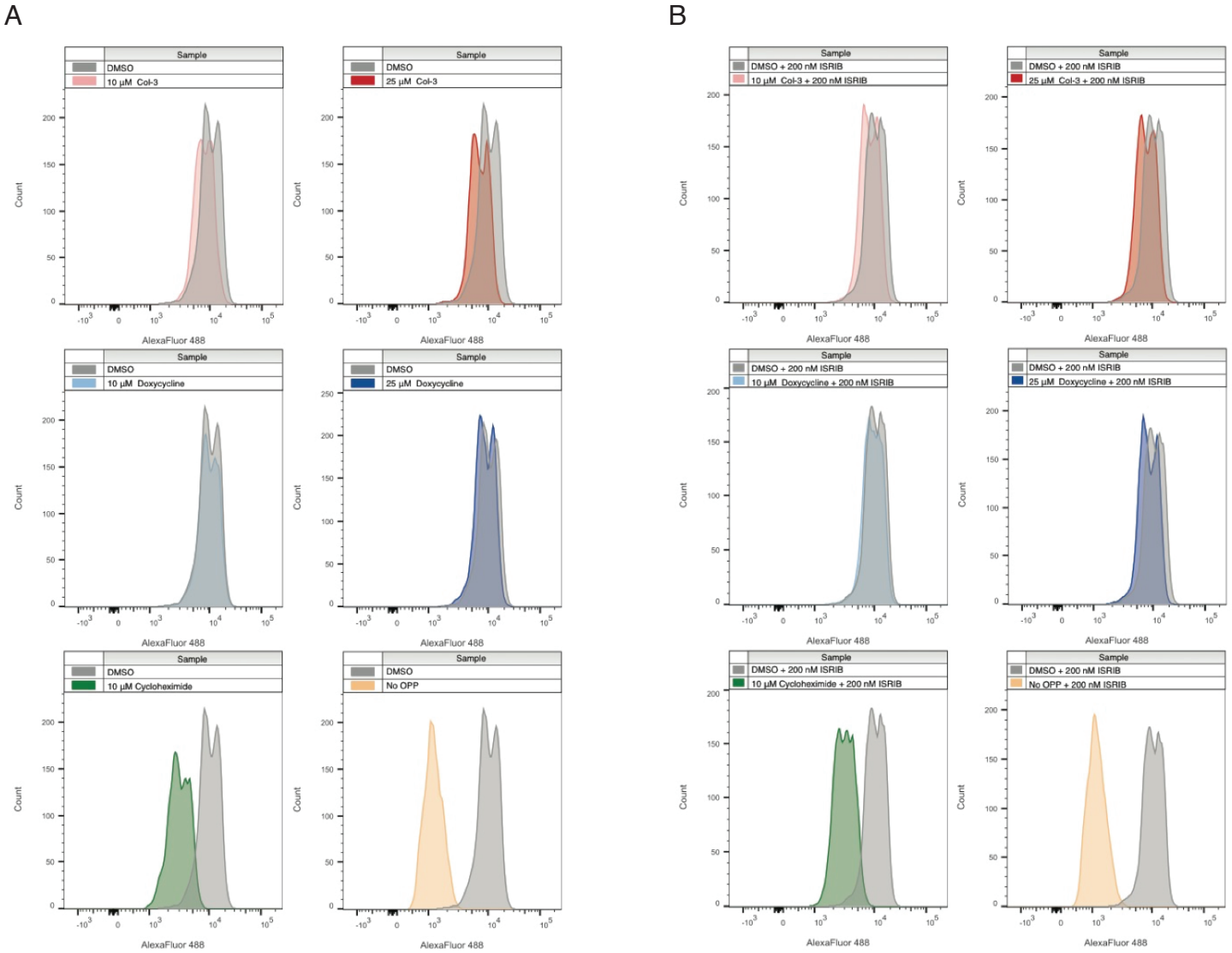

Figure S4 (Related to Figure 5) – Tetracyclines inhibit human translation independent of ISR activation. (**a**) Histogram plots for relative incorporation of OPP-Alexa Fluor 488 into nascent peptides in A375 cells dosed with tetracyclines at 3 h (**b**) Histogram plots for relative incorporation of OPP-Alexa Fluor 488 into nascent peptides in A375 cells co-dosed with tetracyclines plus 200 nM ISRIB at 3 h.

Col-3 Average GI_{50} – NCI 60 panel	Col-3 Average GI_{50} – adult human fibroblasts	Doxy Average GI_{50} – adult human fibroblasts
2.1 μM	16.0 ± 1.5 μM	>100 μM

Table S1 (Related to Figure 4) – Comparison of tetracycline-mediated cellular growth inhibition in the NCI 60 cancer cell lines versus normal human fibroblasts shows a potential therapeutic window for tetracyclines as anti-proliferative agents.

Col-3 GI_{50} data across the NCI 60 panel data is from NCI COMPARE database (https://dtp.cancer.gov/databases_tools/compare.htm). Col-3 NSC identifier: S683551.