Rapid conjugation of antibodies to toxins to select candidates for the development of anti-cancer Antibody-Drug Conjugates (ADCs)

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Supplementary Materials: Fig. S1: Streptavidin-Biotin conjugation of Saporin to trastuzumab can be performed at different molar ratios resulting in decreased cell binding cell viability of ADCs conjugated at higher ratios. Fig. S2: Streptavidin-Biotin conjugation of Saporin to trastuzumab does not potentiate off-target toxicity compared to clinically available T-DM1, Fig. S3: Streptavidin-Biotin conjugation of DM1 to trastuzumab does not potentiate off-target toxicity compared to clinically available T-DM1.

a

Heavy chain trastuzumab

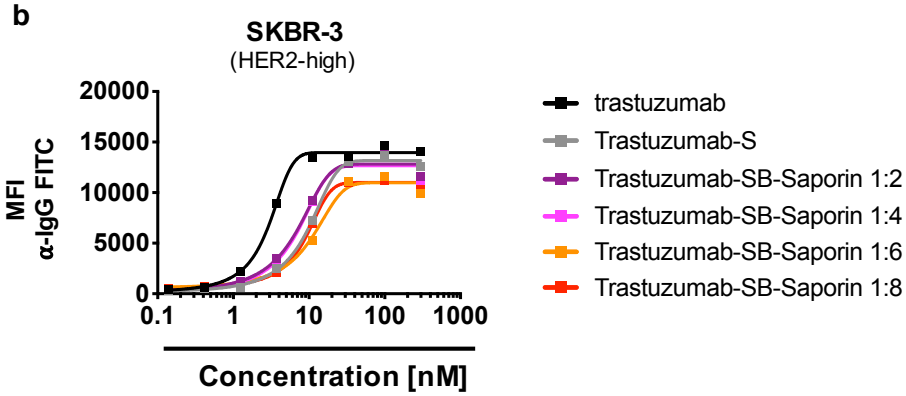
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNT
AYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light chain trastuzumab

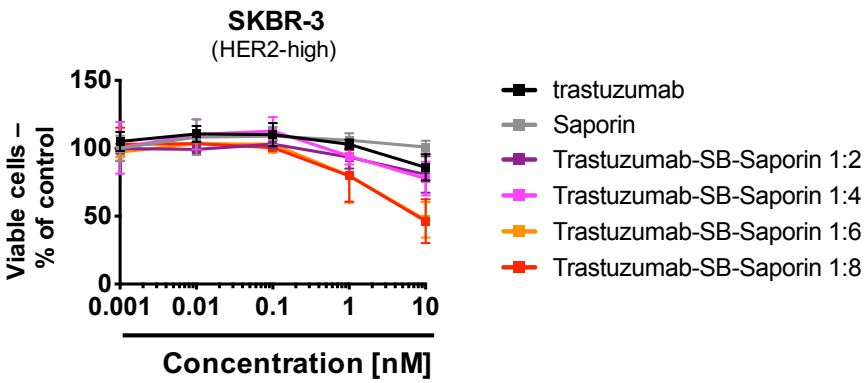
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSL
QPEDFATYYCQQHYTTPPTFGQGTKVEIRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

V_H and V_L: Framework regions (FRs), Complementarity-determining regions (CDRs)
C_H and C_L: Constant region
mAb: **Lysines (K)**

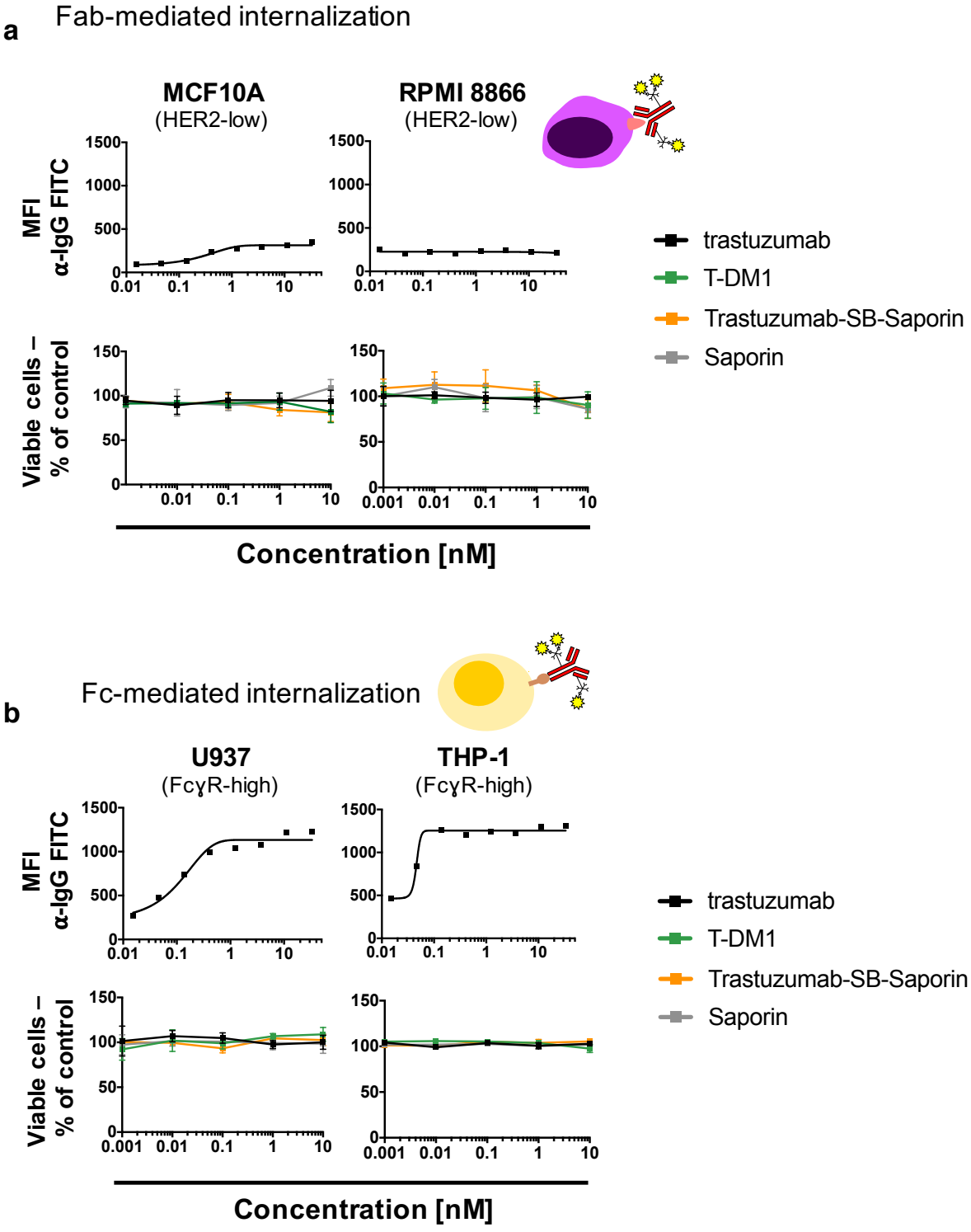
b



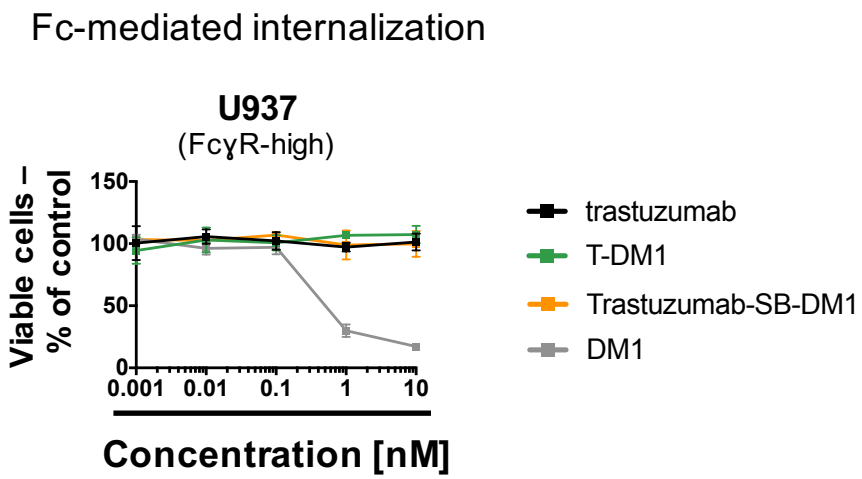
c



Supplementary Fig. 1. Streptavidin-Biotin conjugation of Saporin to trastuzumab can be performed at different molar ratios resulting in decreased cell binding and cell viability of ADCs conjugated at higher ratios. (**a**) Sequence of heavy and light chain of trastuzumab highlighting lysine (K) residues available for conjugation to Streptavidin in framework regions, complementarity-determining regions and constant regions. Streptavidin labelling by using the LightningLink Streptavidin kit results in the conjugation of an average of 2 molecules of Streptavidin per antibody at 41 possible lysine residues. (**b**) Investigation of cell binding of breast cell line SKBR-3 with high Her2 expression of Trastuzumab-SB-Saporin ADCs conjugated with 4 different ratios from 1:2 to 1:8. Streptavidin-linked trastuzumab shows reduced binding affinity to naked trastuzumab. (**c**) Investigation of cell viability of breast cell line SKBR-3 with high Her2 expression of Trastuzumab-SB-Saporin ADCs conjugated with 4 different ratios from 1:2 to 1:8 showing reduction in cell viability for ADCs with more Saporin conjugated per antibody. N=1 for all binding assays and N=3 independent experiments for all MTS studies, each condition performed in triplicate, error bars represent Standard Deviation (SD).



Supplementary Fig. 2. Streptavidin-Biotin conjugation of Saporin to trastuzumab does not potentiate off-target toxicity compared to clinically available T-DM. (**a**) Investigation of cell viability of breast cell line MCF10A and myeloid cell line RPMI 8866 with low Her2 expression. (**b**) Investigation of cell viability of monocytic cell lines U937 and THP-1 with high Fcγ-receptor expression. Cells were treated with the naked antibody trastuzumab, control ADC T-DM1, Trastuzumab-SB-Saporin or Saporin toxin alone. N=1 for all binding assays and N=3 independent experiments for all MTS studies, each condition performed in triplicate, error bars represent Standard Deviation (SD).



Supplementary Fig. 3. Streptavidin-Biotin conjugation of DM1 to trastuzumab does not potentiate off-target toxicity compared to clinically available T-DM1. Investigation of cell viability of monocytic cell line U937 with high Fcγ-receptor expression. Cells were treated with the naked antibody trastuzumab, control ADC T-DM1, Trastuzumab-SB-DM1 or DM1 toxin alone. N=3 independent experiments, each condition performed in triplicate, error bars represent Standard Deviation (SD).