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Supplementary information

CRISPR screens in *Drosophila* cells identify Vsg as a Tc toxin receptor

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Supplementary Information

CRISPR screens in Drosophila cells identify Vsg as a Tc toxin receptor

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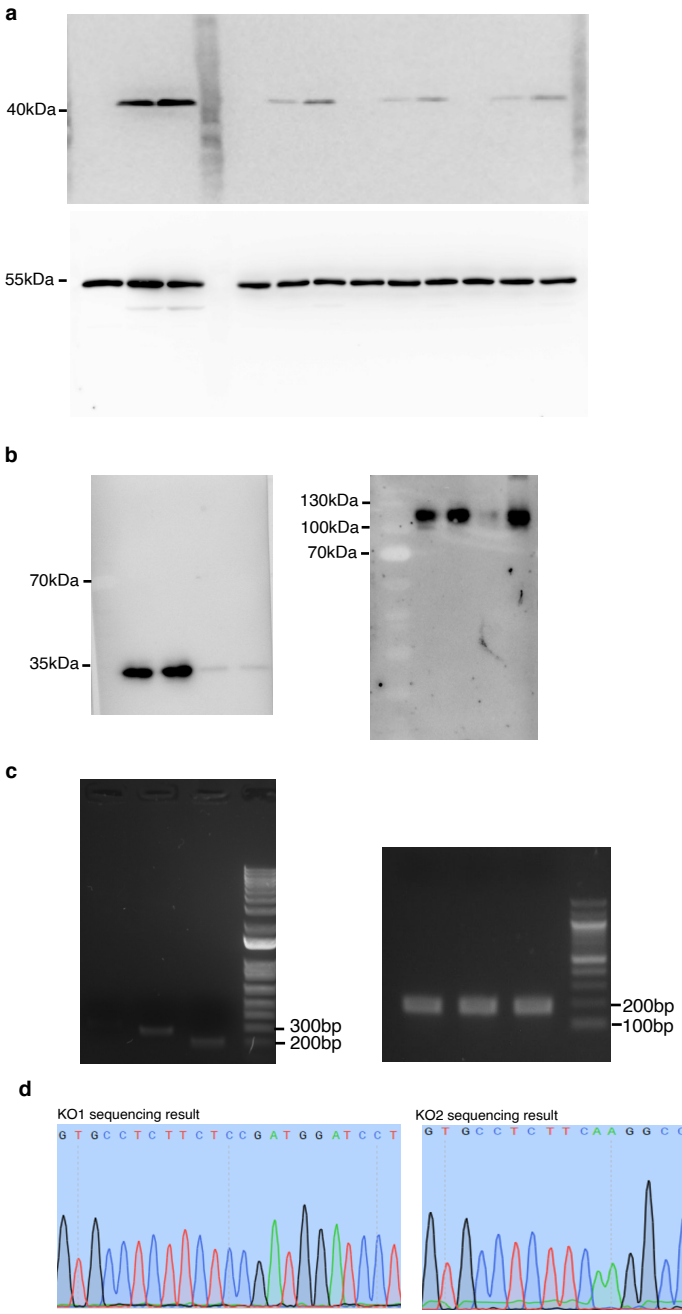


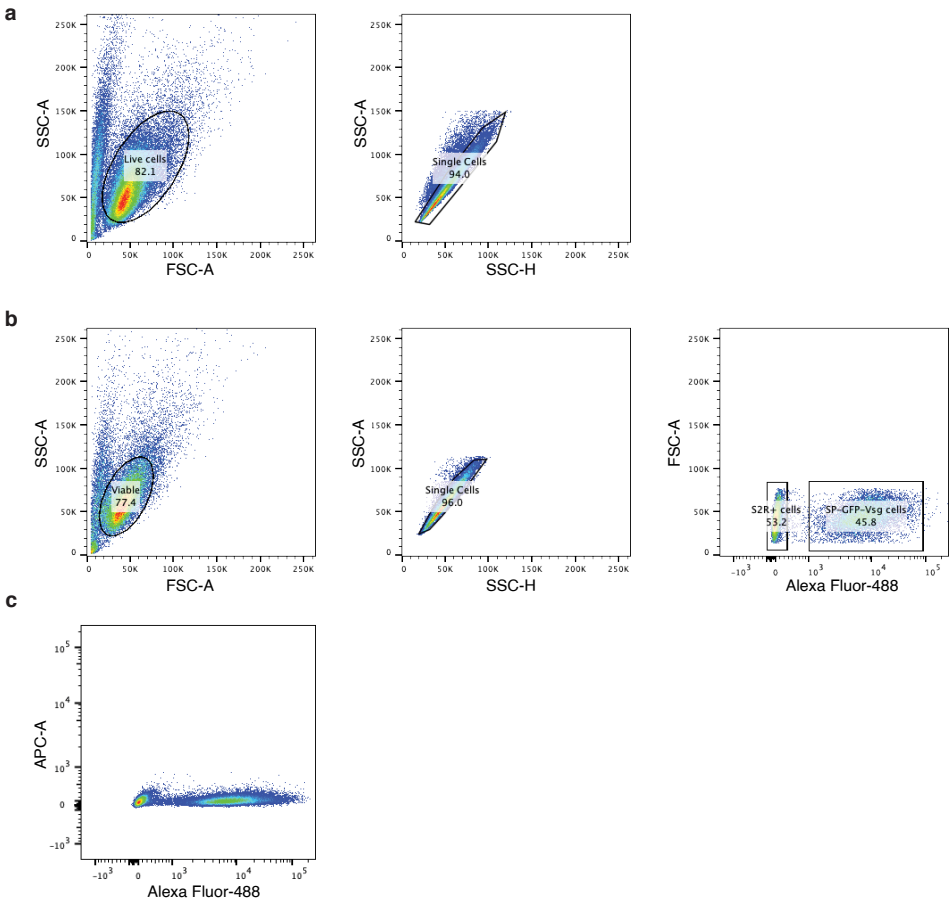
Supplementary Figure 1. Raw blot and gel source images.

a. Raw western blots for Fig. 2d.

b. Raw western blots for Extended Data Fig. 5c.

c. Raw DNA gel for Fig. 4a.

d. DNA sequencing results near the truncation regions for Fig. 4a.



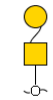
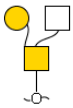
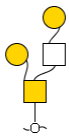
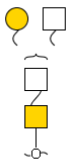
Supplementary Figure 2. Gating for flow cytometry data.

a. Gating strategy relevant to Fig. 2i. The FSC-A/SSC-A profile used to select viable cells (~80%), and then the SSC-A/SSC-H profile used to select singlets (~95%).

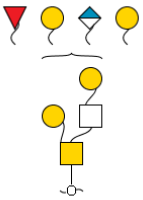
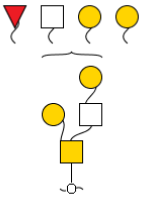
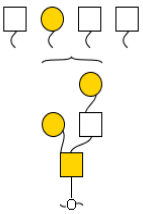
b. Gating strategy relevant to Extended Data Fig. 5a. The FSC-A/SSC-A profile used to select viable cells (~77%). Next, the SSC-A/SSC-H profile used to seletct singlets (~95%). Finally, the FSC-A/Alexa Fluor-488 profile was used to seperate cell populations into S2R+ cells (~55%) and SP-GFP-Vsg cells (~45%)

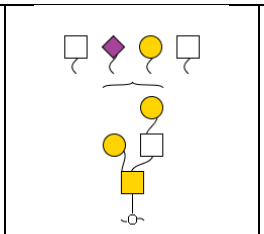
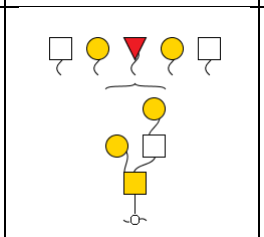
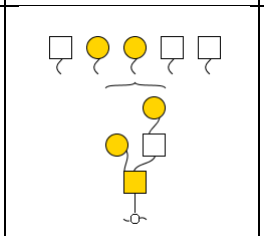
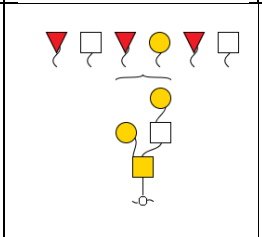
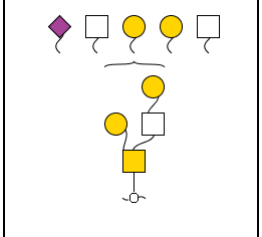
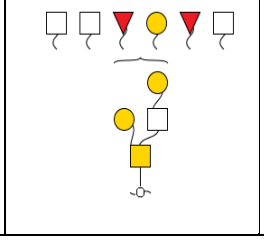
c. The APC-A/Alexa Fluor-488 profile of a mixture of S2R+ cells and SP-GFP-Vsg cells prior to tretment with TcdA1-647 is shown, verifying that there is no crosstalk between SP-GFP-Vsg and APC-A channel in Extended Data Fig. 5a..

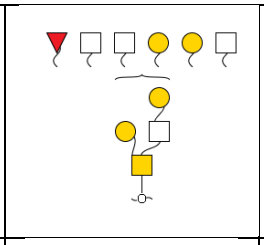
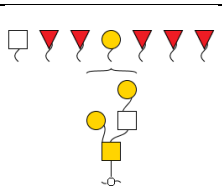
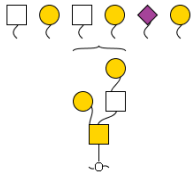
Supplementary Data 1. Mass spectrometry analysis of glycan moieties in Vsg-ECD and sVsg-ECD.

Mass (*m/z*)		Composition	Graphic	Relative intensity	
Theoretical	Observed			VSG	SVSG
534.3	534.3	Hex1HexNAc1		27.61%	30.77%
779.4	779.5	Hex1HexNAc2		0.04%	0.03%
942.5	942.6	Hex3HexNAc1		0.34%	2.05%
953.5	953.6	Hex1HexNAc2Fuc1		0.02%	0.06%
983.6	983.6	Hex2HexNAc2		0.11%	1.43%
1024.6	1024.6	Hex1HexNAc3		0.01%	0.01%
1157.7	1157.7	Hex2HexNAc2Fuc1		7.01%	12.43%

1187.7	1187.7	Hex3HexNAc2		3.23%	9.41%
1228.7	1228.7	Hex2HexNAc3		0.09%	0.00%
1331.8	1331.7	Hex2HexNAc2Fuc2		0.54%	1.07%
1361.8	1361.8	Hex3HexNAc2Fuc1		44.10%	35.29%
1375.7	1375.8	Hex2HexNAc2Fuc1HexA1		2.98%	2.76%
1432.8	1432.8	Hex3HexNAc3		0.20%	0.28%
1579.8	1579.9	Hex3HexNAc2Fuc1HexA1		11.28%	3.09%

1606.9	1606.9	Hex3HexNAc3Fuc1		0.46%	0.42%
1719.0	1718.9	Hex2HexNAc5		0.26%	0.38%
1783.9	1784.0	Hex4HexNAc2Fuc1HexA1		1.20%	0.24%
1811.0	1811.0	Hex4HexNAc3Fuc1		0.15%	0.08%
1910.1	1910.0	Hex3HexNAc2NeuAc2		0.13%	0.00%
1923.1	1923.0	Hex3HexNAc5		0.10%	0.09%

2039.1	2039.1	Hex3HexNAc4NeuAc1		0.03%	0.01%
2056.2	2056.1	Hex4HexNAc4Fuc1		0.04%	0.02%
2127.2	2127.2	Hex4HexNAc5		0.03%	0.02%
2200.2	2200.3	Hex3HexNAc4Fuc3		0.01%	0.00%
2243.3	2243.2	Hex4HexNAc4NeuAc1		0.01%	0.00%
2271.3	2271.3	Hex3HexNAc5Fuc2		0.00%	0.01%

2301.3	2301.3	Hex4HexNAc5Fuc1		0.01%	0.01%
2303.3	2303.3	Hex3HexNAc3Fuc5		0.03%	0.02%
2447.4	2447.4	Hex5HexNAc4NeuAc1		0.00%	0.01%

Monosaccharide Key:

: Galactose
: Fucose
: *N*-acetyl glucosamine (GlcNAc)
: *N*-acetyl galactosamine (GalNAc)
: Glucuronic acid (GlcA)
: *N*-acetyl neuraminic acid (NeuAc)
: *N*-acetyl hexosamine (HexNAc, undefined stereochemistry)
: Hexose (Hex, undefined stereochemistry)