

## Expanded View Figures

A

>sp|P22629|SAV\_STRAV Streptavidin OS=Streptomyces avidinii OX=1895 PE=1 SV=1

MRKIVVAAIAVSLTTVSITASASADPSKDSKAQVSAEAGITGTWYNQLGSTFIVTAGAD  
 GALTGTYESAVGNAESRYVLTGRYDSAPATDGSALTGWTVAWKNNYRNAHSATTWSGQY  
 VGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAACKAGVNNGNPLDA  
 VQQ

B

STLVGHDTFTK  $m/z = 603.3$

NAHSATTWSGQYVGGAEAR  $m/z = 655.3$

INTQWLLTSGTTEANAWK  $m/z = 1018.0$

C

		Intensity			
		Streptavidin	All proteins	Streptavidin (%)	
BioID Low amount	Ctr_wtS	R1	4.0E+09	6.3E+09	64.09
		R2	4.0E+08	6.2E+08	63.67
		R3	4.85+09	7.9E+09	61.49
	Ctr_prS	R1	6.5E+08	4.5E+10	1.46
		R2	7.5E+08	4.5E+10	1.67
		R3	1.0E+09	5.9E+10	1.73
	BioID_wtS	R1	3.4E+09	5.4E+09	63.85
		R2	3.3E+09	5.0E+09	67.00
		R3	4.4E+09	6.8E+09	64.72
	BioID_prS	R1	6.0E+08	5.7E+10	1.06
		R2	7.1E+08	5.6E+10	1.27
		R3	6.7E+08	6.1E+10	1.11

		Intensity						
		Streptavidin		All proteins		Streptavidin (%)		
		R1	R2	R1	R2	R1	R2	
ChIP-SiCAP	wtS_HpH fractions	fr1	3.2E+07	2.5E+07	2.6E+08	2.9E+08	12.51	8.78
		fr2	5.8E+06	1.6E+07	9.0E+07	1.1E+08	6.44	14.14
		fr3	8.9E+05	8.5E+05	2.0E+08	2.3E+08	0.45	0.36
		fr4	2.1E+08	2.3E+08	5.0E+08	5.7E+08	42.87	40.53
		fr5	8.2E+07	8.9E+08	1.2E+08	1.2E+09	68.70	76.30
		fr6	1.5E+08	2.6E+08	7.5E+08	1.1E+09	19.97	24.40
		fr7	5.6E+07	6.0E+07	4.5E+08	4.6E+08	12.35	12.93
		fr8	3.9E+07	6.3E+07	1.6E+08	2.0E+08	24.56	30.83
		fr9	1.8E+07	2.4E+07	2.2E+08	3.8E+08	8.11	6.24
		fr10	1.2E+07	2.4E+07	4.5E+08	6.0E+08	2.60	4.01
	Single injection	wtS	4.1E+09	3.3E+09	8.2E+09	6.3E+09	50.24	51.84
	prS	2.6E+06	0	1.7E+10	2.2E+10	0.01	0.00	

		Intensity			
		Streptavidin	All proteins	Streptavidin (%)	
BioID High amount	Ctr_wtS	R1	1.2E+11	2.1E+11	60.34
		R2	1.3E+11	2.0E+11	62.34
		R3	7.3E+10	1.5E+11	49.36
	Ctr_prS	R1	1.0E+10	4.6E+11	2.25
		R2	7.7E+09	4.1E+11	1.87
		R3	9.2E+09	5.0E+11	1.85
	BioID_wtS	R1	1.5E+11	1.9E+11	77.39
		R2	1.4E+11	1.9E+11	72.01
		R3	1.2E+11	1.8E+11	66.96
	BioID_prS	R1	8.2E+09	5.1E+11	1.60
		R2	7.4E+09	5.0E+11	1.48
		R3	6.1E+09	4.6E+11	1.33

		Intensity		
		Streptavidin	All proteins	Streptavidin (%)
Lipids	R1	1.9E+11	1.7E+12	9.76
	R2	1.4E+11	1.1E+12	11.44
	R3	1.4E+11	1.7E+12	7.19

		Intensity		
		Streptavidin	All proteins	Streptavidin (%)
Membrane proteins	wtS_10	1.1E+11	1.6E+11	70.58
	prS_10	9.4E+06	1.4E+10	0.07
	prS_90	1.6E+08	9.6E+10	0.18

Figure EV1. Tryptic digestion of streptavidin.

- A Amino acid sequence of streptavidin protein. Residues cleaved by trypsin are shown in red.  
 B Sequence and  $m/z$  of the top-3 streptavidin peptides, used to determine the extracted ion chromatogram (XIC) shown in main Fig 1E and F.  
 C Summary of the total iBAQ (All proteins) and streptavidin iBAQ values in all the experiments shown in main Figs 2 and 3.

**Figure EV2. Suz12 chromatin interactors identified via prS bead ChIP-SICAP experiment and single injection or wtS bead enrichment followed by HpH fractionation.**

- A Experimental procedure.
- B Intensity of all PRC2 complex components and streptavidin in ChIP-SICAP with wtS beads and subjected to HpH fractionation or prS bead and single injection. The error bars show standard deviation of two replicates
- C Digesting mock beads prepared according to Barshop *et al* (blue) and prS (red) protocols. Intensity of streptavidin peptides (left) and XICs of the top-two streptavidin peptides (right).
- D Comparing beads prepared according to Barshop *et al* with prS beads using a Suz12 ChIP-SICAP assay. Intensity of streptavidin (left). Number of identified proteins (right).
- E Comparative Suz12 ChIP-SICAP between 2i and serum. Full MS spectra between 612–618 *m/z* at retention time: 64–65 min are shown. Specific precursors (*m/z*: 617.2866 and 612.2821) from Aebp2 isoform 3/4 are detected in the forward experiment (top) and the reverse experiment (bottom).
- F MS/MS spectra indicating the N-terminal peptide of Aebp2 isoform 3/4 : mDIDSTISSGR.
- G Western blot of whole-cell lysate of mES cells grown in 2i and serum media. Top: anti-Aebp2; bottom: anti-GAPDH. Arrows A and B show two bands detected by Aebp2 antibody. The intensities of band A and B were compared between 2i and serum normalized to GAPDH as the loading control.

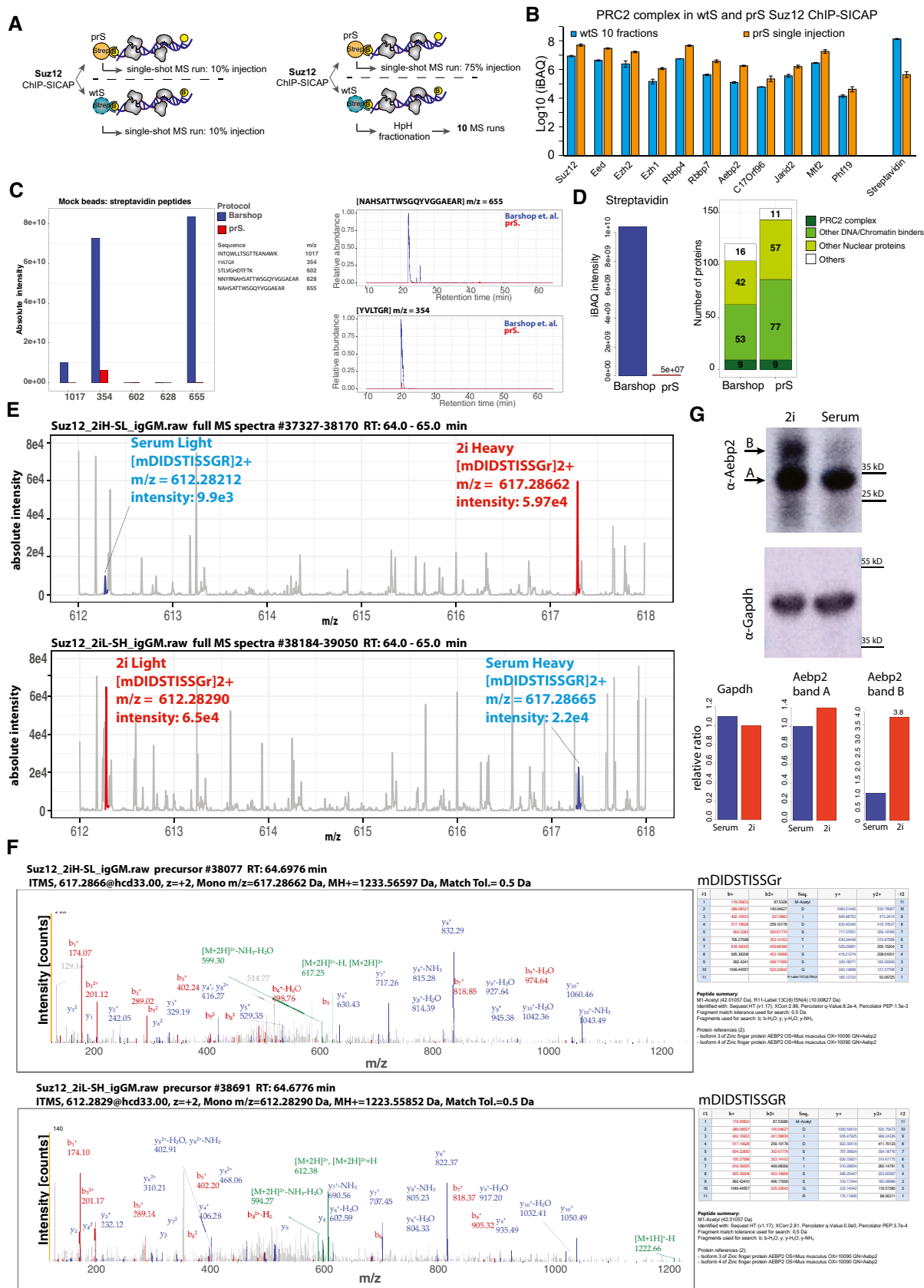


Figure EV2.

**Figure EV3. Comparing performance of prS and wtS beads in biotin-based enrichment experiments. BioID.**

- A Experimental procedure.
- B, C Ranking of proteins identified in BioID experiments performed with either prS or wtS beads in target (SEC61B) or control cells and injection of 100 ng (low amount) (B) or 1  $\mu$ g (high amount) (C).
- D The boxplots indicate the number of MS events, the percentage of MS/MS identified, and the peptide-spectrum matches (PSMs) in the different experiments. In the boxplots, the boxes show interquartile ranges (IQR = Q3-Q1). The whiskers extend to data points that are  $< 1.5 \times$  IQR away from Q1 or Q3 (Turkey whiskers), and the central lines show the medians. *T*-test *P*-values were calculated where \**P* < 0.05, \*\* = 0.001, \*\*\* < 0.001.
- E, F Integral membrane proteins. (E) Experimental procedure. (F) Intensity and iBAQ values of streptavidin peptides after injecting 10% of the wtS sample, 10% of the prS sample or 90% of the prS sample.
- G, H Lipid-interacting proteins. (G) Experimental procedure. (H) Histograms of proteins that were enrichment over control upon biotinylated sphingolipid pull-down with prS beads and on-bead digestion.

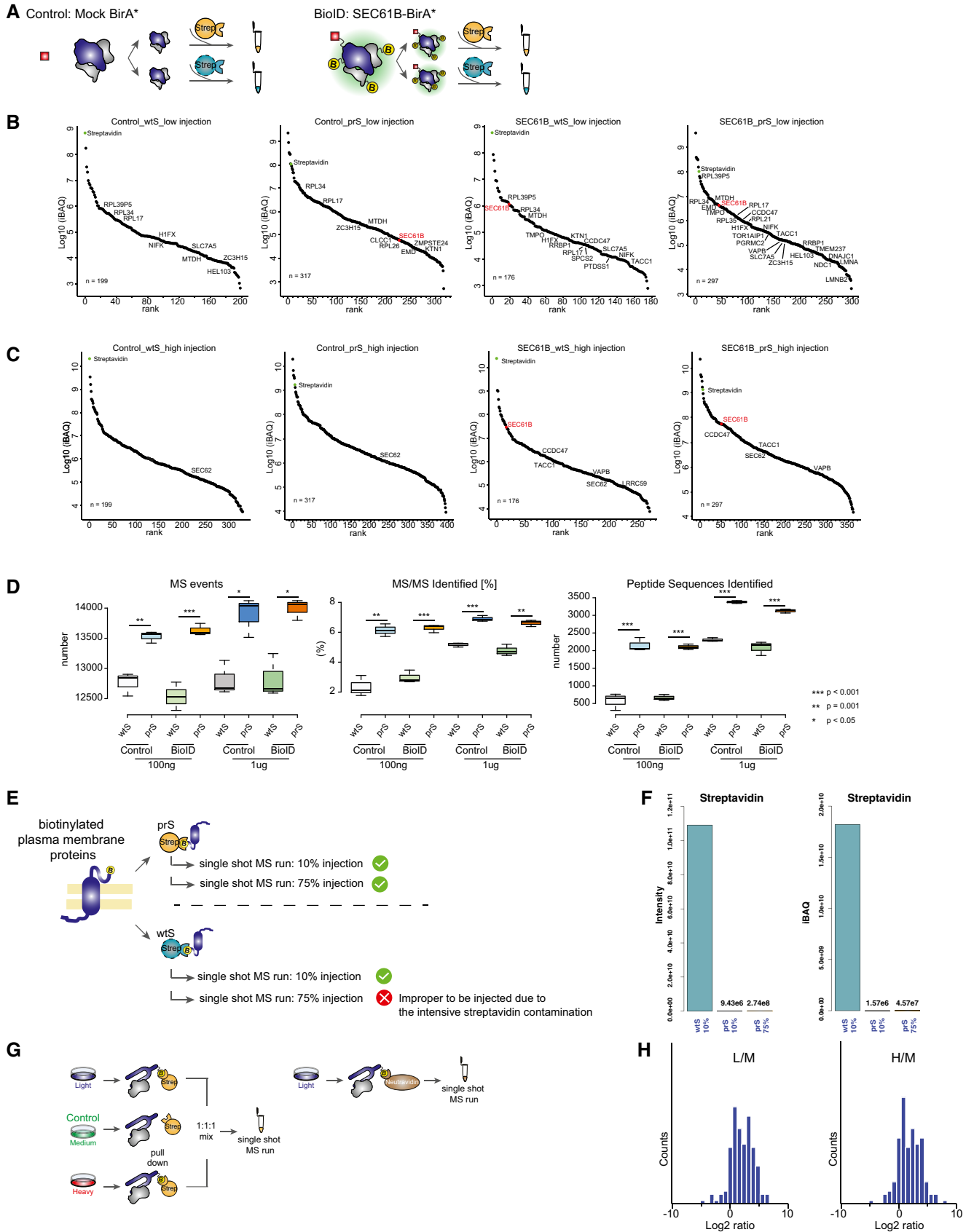


Figure EV3.