Fig. A: Model for KRAB-TRIM28 mediated transcriptional repression

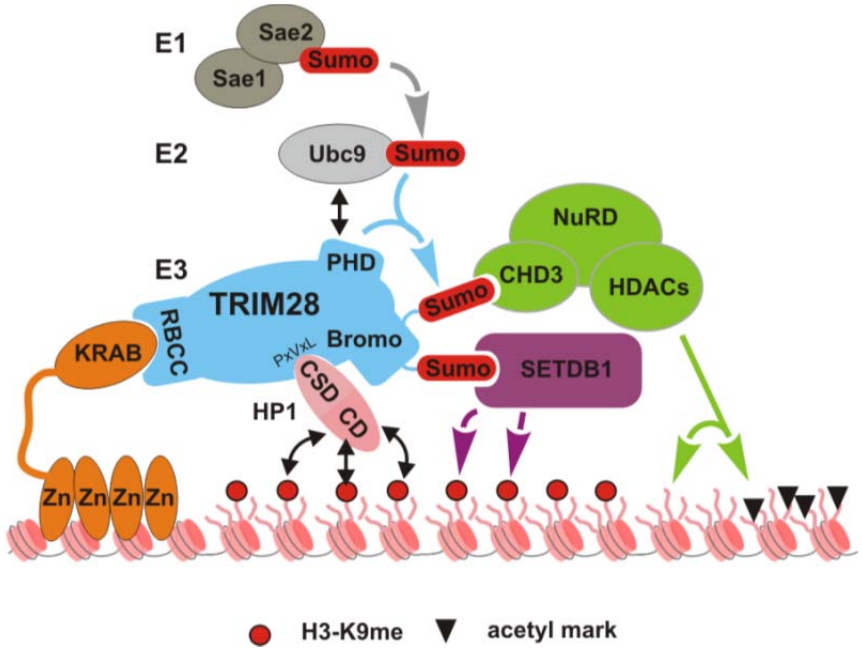


Fig. A: Model for KRAB-TRIM28 mediated transcriptional repression.

A KRAB-ZNF protein binds through its zinc finger (Zn) motifs specifically to a chromatin locus. KRAB interacts with the Ring finger, B-box, coiled-coil domain (RBCC) of the nuclear multimodular hub protein TRIM28. TRIM28 in turn recruits chromatin modifying complexes through its different modules. Its plant homeo domain (PHD) contacts the Sumo E2 transfer protein Ubc9 and serves as Sumo E3 ligase to sumoylate its own Bromo domain. The sumoylation sites are docking interfaces for CHD3, a component of NuRD complexes with its associated activities like histone deacetylases (HDACs), and the histone methyltransferase SETDB1, which is activated by this interaction. The HDACs remove histone acetyl marks (black triangles) while SETB1 methylates histone H3-K9 residues (red circles). The latter are recognized by the chromo domain (CD) of heterochromatin proteins (HP1) that are also part of the TRIM28 complex (interaction between the chromoshadow domain of HP1 and the HP1 box around the amino acids P-x-V-x-L of TRIM28). Ultimately, the KRAB-TRIM28 binding leads to chromatin re-organization towards a heterochromatin-like configuration non-permissive for transcription. Coloured arrows with large heads indicate enzymatic activities while small black double arrows depict interaction; adapted from Ivanov 2007 with modifications.

Fig. B: Epitope Mapping of KRAB ZNF Antibodies

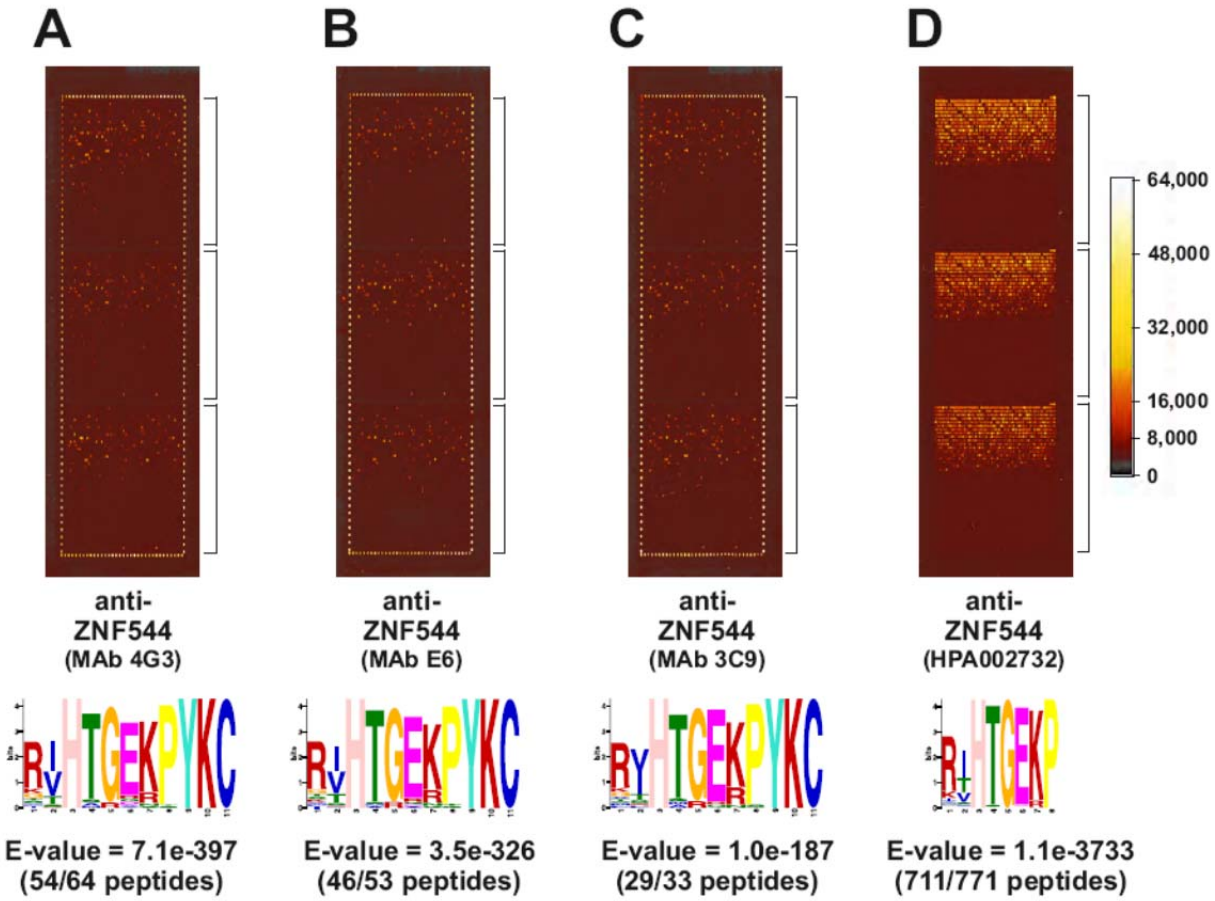
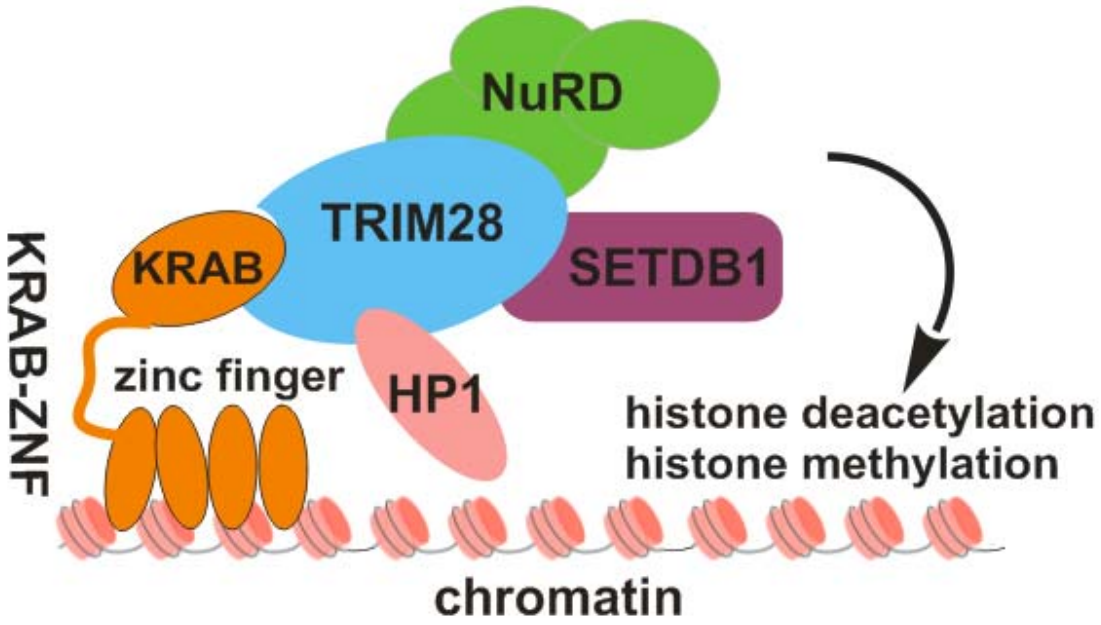


Fig. B: Epitope Mapping of KRAB ZNF Antibodies

Images of peptide microarrays stained with monoclonal antibodies against ZNF544 (A, B, C as indicated) at 2.5 µg/ml or the anti-ZNF544 rabbit polyclonal antibodies (D; same image as Fig. 1 in main manuscript). The strong spot signals around the border in A-C correspond to control stainings against HA tag peptides (sequence YPYDVPDYAG) using the monoclonal anti-HA antibody 12CA5 conjugated to Cy5.

Fig. C: Genes downregulated in HAP1 knockout cell lines



Knockout cell lines

Gene symbol	TRIM28	POGK	SETDB1	CBX1	CBX3	CBX5	ZNF267	ZNF764	Min_FC	Max_FC	ko Min	ko Max
POF1B	-812.43	-201.00	-758.67	-295.73	-35.62	-156.15	-270.44	-431.96	-812.43	-35.62	TRIM28	CBX3
ITM2A	-3.09	-1.99	-583.04	-2.02	-1.25	-1.25	1.78	2.02	-583.04	2.02	SETDB1	ZNF764
SLC7A3	3.86	19.86	14.39	15.35	-126.49	16.01	-135.87	-137.10	-137.10	19.86	ZNF764	POGK
HRASLS5	-14.73	-52.56	-16.81	-11.09	-83.23	-101.74	-83.24	-80.01	-101.74	-11.09	CBX5	CBX1
ATAD1	-1.10	-1.04	-1.35	1.03	-43.78	-1.10	-63.66	-48.49	-63.66	1.03	ZNF267	CBX1
RP11-297P16.4	-9.55	-42.71	-5.40	-53.91	-9.26	-50.19	-32.11	-35.97	-53.91	-5.40	CBX1	SETDB1
SERPINB9	-1.62	-1.19	1.31	-1.22	-49.35	-1.12	1.43	1.38	-49.35	1.43	CBX3	ZNF267
FASTKD2	-13.73	-14.52	-12.66	-12.55	-12.55	-13.47	-13.07	-12.55	-14.52	-12.55	POGK	CBX1/ZNF764

Fig. C: Genes downregulated in HAP1 knockout cell lines

HAP1 cells are currently used to knock out KRAB ZNF and KRAB ZNF associated genes in order to determine and validate protein protein interactions and their functionalities.

Fig. 1

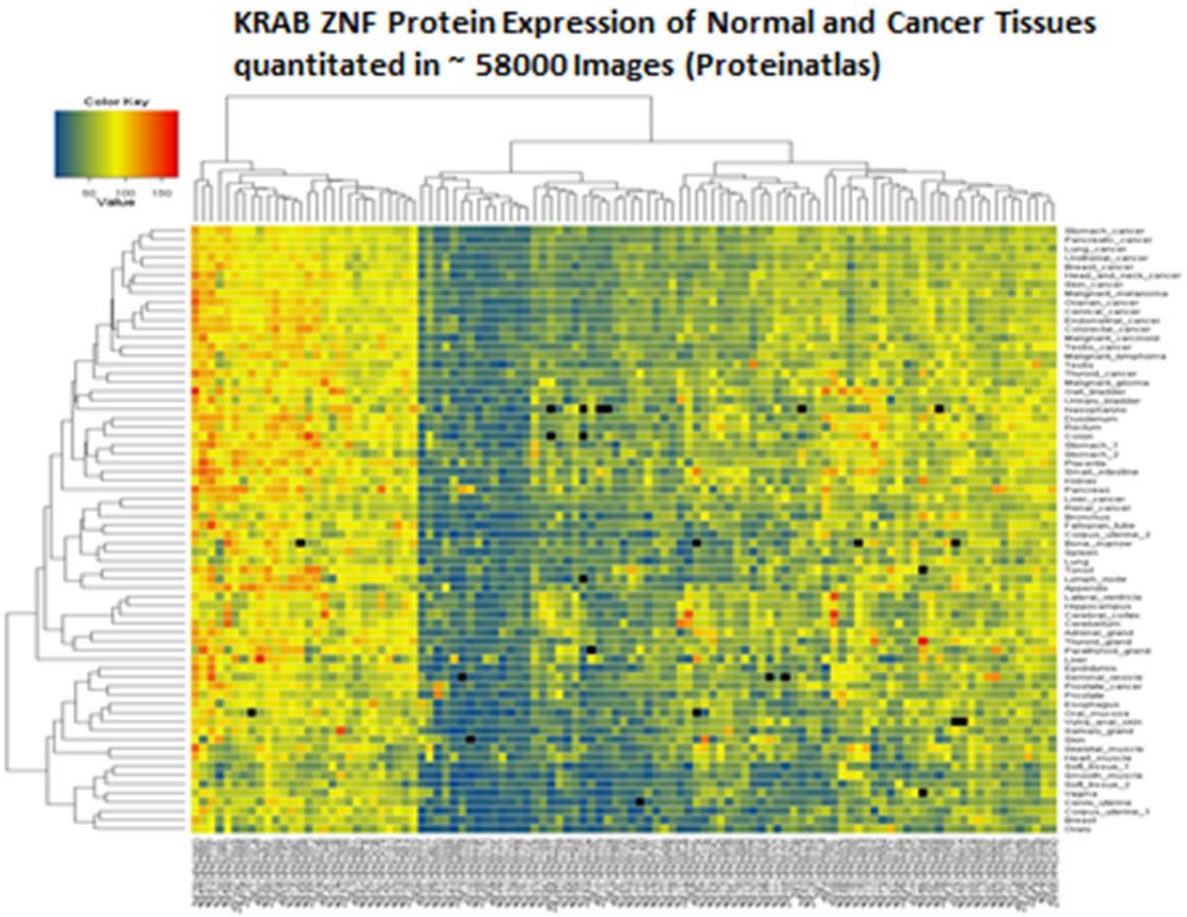


Fig. 1: 58 000 immunohistochemical images (IHCs) downloaded from the Proteinatlas-database have been quantitated and comparatively analysed.