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SUPPLEMENTARY ONLINE DATA

Cleavage of Notch1 by granzyme B disables its transcriptional activity

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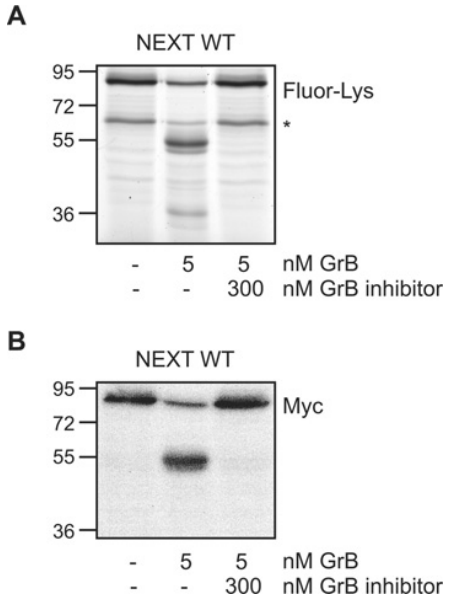

Figure S1 Specification of GrB activity

(**A**) *In vitro* translated NEXT fragments are cleaved in the presence of 5 nm GrB. This activity could be fully blocked by 300 nM of the GrB inhibitor Ac-IETD-CHO as assessed by fluorimetric detection of lysine residues (**A**) as well as Myc immunoblotting (**B**). Molecular mass markers are indicated in kDa.

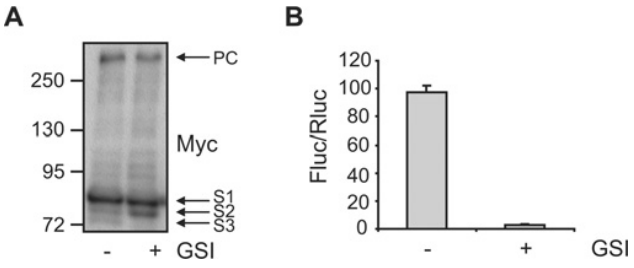

Figure S2 HeLa FRT N1 L1594P cell line characteristics

(**A**) Myc immunoblot of N1 L1594P HeLa cell lysates showing Notch is expressed and cleaved. PC, precursor; S1, S2 and S3 indicate cleavage products of Notch1. Molecular mass markers are indicated in kDa. (**B**) 12× CSL transcriptional activation assay showing Notch is active in N1 L1594P HeLa cells, which can be attenuated by the γ-secretase inhibitor (GSI) DBZ.

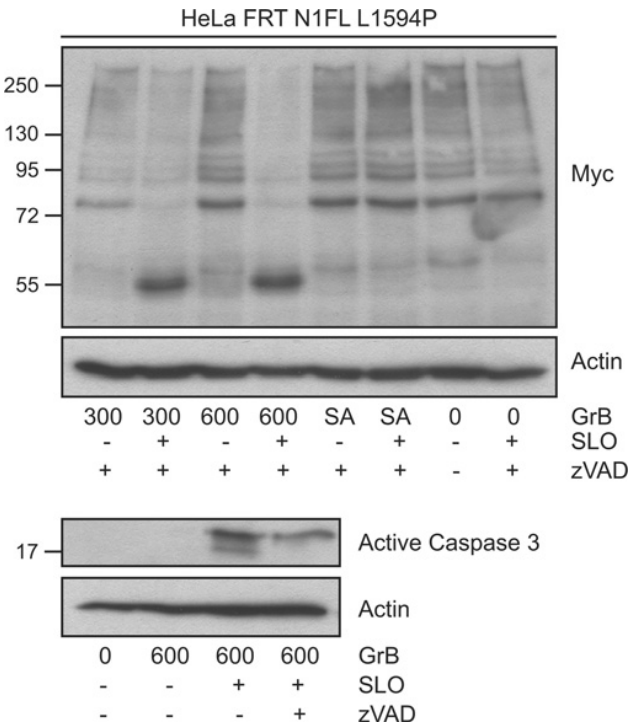

Figure S3 GrB action requires cell entry

Upper two panels, immunoblot showing N1 L1594P HeLa cells incubated with GrB in the absence or presence of Perorin. Only upon co-incubation with perforin was GrB able to cleave Notch in the intracellular domain. Lower two panels, Caspase immunoblot demonstrating caspases are only activated upon co-incubation of perforin with GrB. The pan-caspase inhibitor Z-VAD-FMK (zVAD) inhibits the activating autoproteolysis of caspase 3. Actin staining of the same immunoblots serves as a loading control. Molecular mass markers are indicated in kDa.

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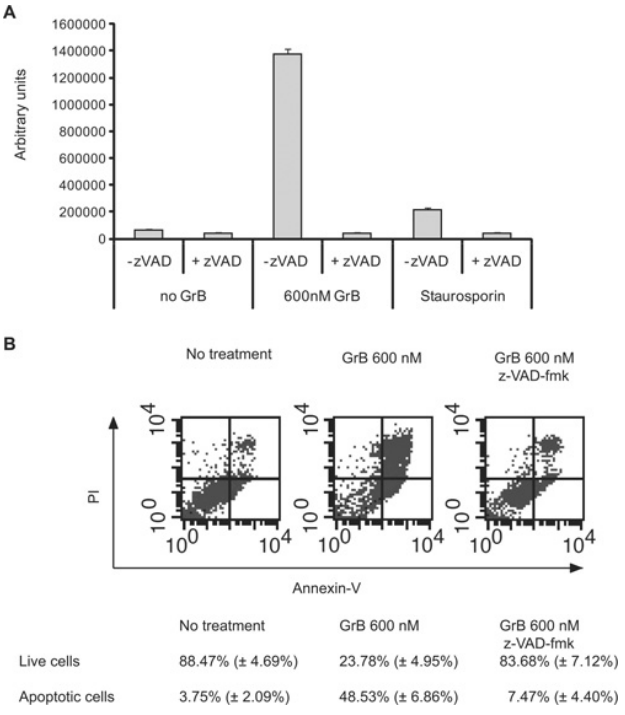

Figure S4 Z-VAD-FMK blocks caspase-dependent cell death

(**A**) Caspase GLO assay of N1 L1594P HeLa cells shows that upon GrB treatment both caspase 3 and 7 are activated. Both caspase-3 and caspase-7 activity is inhibited in Z-VAD-FMK (zVAD)-treated cells. Staurosporine is used as a positive control for caspase activation. (**B**) Annexin V and propidium iodide FACS plots demonstrating incubation of 600 nM GrB induces cell death. Upon treatment with Z-VAD-FMK, cell death can be prevented. Percentages ± S.D. are given in the table below.

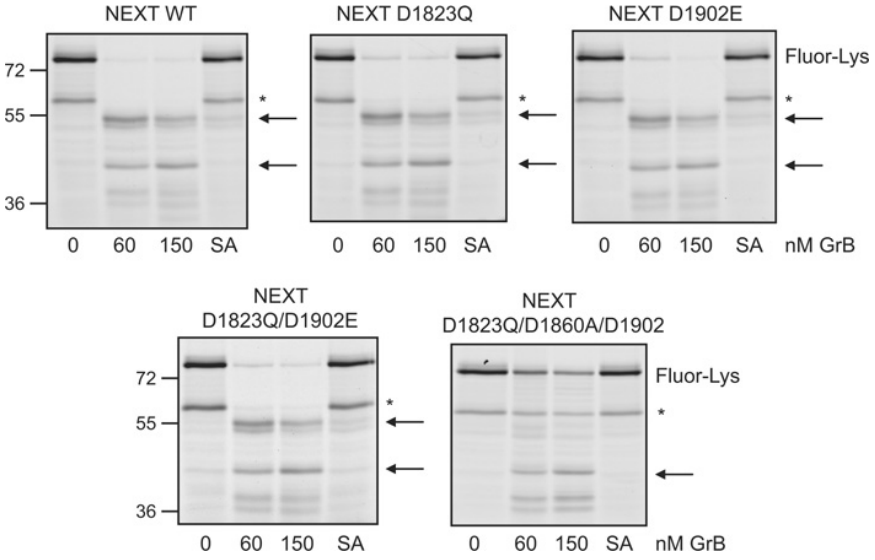

Figure S5 Possible NEXT-GrB site single mutants

The single site mutations at D1823Q and D1902E do not interfere with GrB processing of Notch1. Double D1823Q/D1902E Notch mutants are still processed normally by GrB. Only upon additionally mutating Asp^{1860} is cleavage affected.

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