Human DPP9 represses NLRP1 inflammasome and protects against auto-inflammatory diseases via both peptidase activity and FIIND domain binding

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Supporting information
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Supplemental Videos 1-3

Figure S1. Supporting evidence for DPP9 as a specific interacting partner of human NLRP1 and inhibitor of human NLRP1 inflammasome

A. The most enriched proteins found in the full length NLRP1-FLAG IP eluate measured by quantitative mass spectrometry. The CRAPPome contaminant cut-off was set at maximal spectral count of 5.
B. 293T cells were treated with pooled siRNAs against *DPP8* and *DPP9* for 3 days. 20 μg of whole cell lysate was used for Western blot.
C. 293T cells were transfected with FLAG tagged full length NLRP1, a.a. 1213-1474 and a.a. 1213-1373. Whole cell lysate from $1x10^8$ cells were used for anti-FLAG immunoprecipitation followed by mass spec. 1% of the eluates were analyzed by SDS-PAGE and Western blot.
D. Domain structure of DPP9.
E. 293T-NLRP1 cell were transfected with 3xFLAG tagged DPP9L, DPP9S, DPP9LΔhydrolase. 0.5 mg of whole cell lysate was used for anti-FLAG IP.
F. 293T-ASC-GFP cells were transfected with an empty vector, wild-type or NLRP1-M77T mutant. 24 hours after transfection, cell were treated with 3 μM Talabostat or 50 μM sitagliptin. Whole cell lysate was prepared in TBS with 1% NP40 and the insoluble material was crosslinked with 1% DSS in PBS before solubilization in 1x Laemmli's buffer.
G. Single cell clones of DPP8 knockout and DPP8/9 DKO cells were expanded. 20 μg of whole cell lysate was analyzed by SDS-PAGE and Western blot for endogenous DPP8 and DPP9.
H. Amino acid sequence of the NLRP1 FIIND auto-cleavage junction. The sequences of the tryptic peptides identified in the mass spec of NLRP1-FLAG IP eluate are shown below.
I. Thermoshift assay to test Talabostat binding to DPP9 and/or NLRP1. 293T-NLRP1-HA cells were treated with 3 μM Talabostat or DMSO for 24 hours. Whole cell lysates were heated in a temperature gradient from 25 ºC to 85 ºC in 20 μl aliquots at 1 μg/μl. Denatured proteins were removed by centrifugation at 20,000g for 20 minutes before SDS-PAGE.

Figure S2. Additional evidence demonstrating that NLRP1 inflammasome activation by DPP8/9 inhibitors involves ASC oligomerization and IL-1β cleavage in human keratinocytes.

A. Immortalized keratinocytes were treated with varying doses of Talabostat for 24 hours. Conditioned media were analyzed by IL-1β ELISA.
B. Conditioned media and cell lysate from A were used to measure the percentage of LDH activity released into the media. Statistical significance was calculated with One-way ANOVA with Bonferroni's Multiple Comparison Test to compare to all treatment groups. Bar graph represents data from biological triplicates.
C. Overlap between the cytokines induced by Talabostat (3 μM) and those enriched in MSPC patient-derived primary keratinocytes.
D. Quantification of the percentage of cells displaying ASC specks and PI influx. Error bars represent three independent imaging experiments.
E. Immortalized keratinocytes (N/TERT1) cells were treated with siRNAs against NLRP3, NLRP1, ASC/PYCARD or CASP1 for 48 hours and further treated with 3 μM Talabostat or 10 μM 1G244 for 24 hours. Cell culture media was harvested and analyzed by ELISA. NLRP3 siRNAs were used as a negative control as N/TERT1 cells do not express NLRP3. P-values were calculated by One-Way ANOVA within each drug treated group, as compared to control treated cells.
F. Immortalized keratinocytes (N/TERT1) cells were treated siRNAs and Talabostat as in Fig. 2F. The percentage of dead cells was calculated based on Trypan exclusion. We found this to be a much more sensitive measure of cell death than LDH release. P values were calculated with Student's T test from technical triplicates from one of two independent biological experiments.
G. Immortalized keratinocytes (N/TERT1) cells were treated with siRNAs against NLRP3, NLRP1 or CASP1 for 48 hours and Talabostat for another 24 hours. Culture media was concentrated 10 times before SDS-PAGE.

Figure S3. Supporting information on the role of the DPP9 NLRP1-binding and enzymatic function.

A. SyPRO-Ruby protein staining of the NLRP1-FLAG IP eluates in mock- and 3 μM Talabostat treated cells.
B. 293T ASC-GFP DPP8/9 DKO Clone 1 was co-transfected with NLRP1-HA and DPP9 rescue constructs. Whole cell lysate was harvested 48 hours after transfection and analyzed by SDS-PAGE.

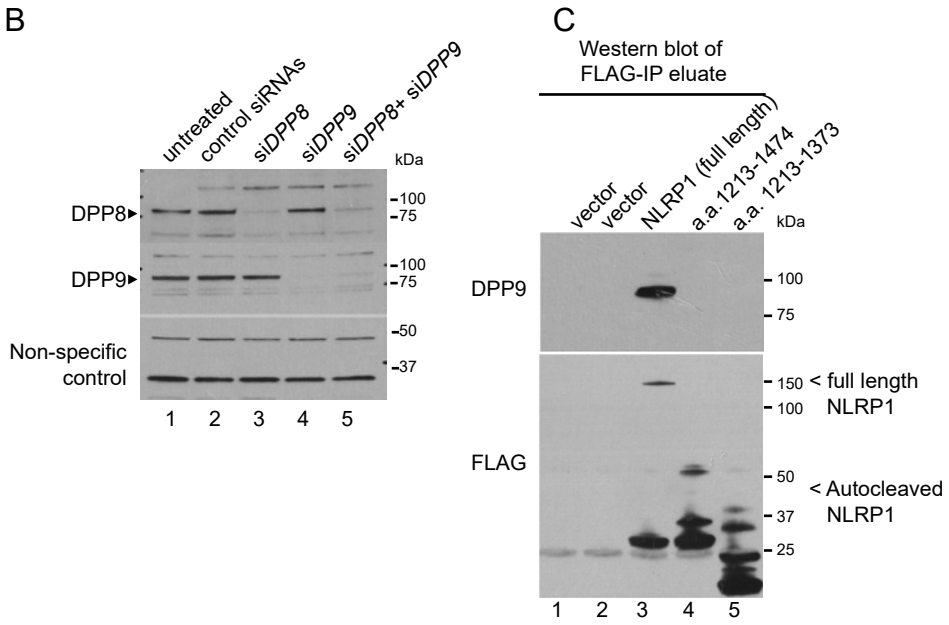
Figure S4. Additional evidence demonstrating that DPP9 interacts with CARD8 and the effect of DPP9 overexpression in keratinocytes.

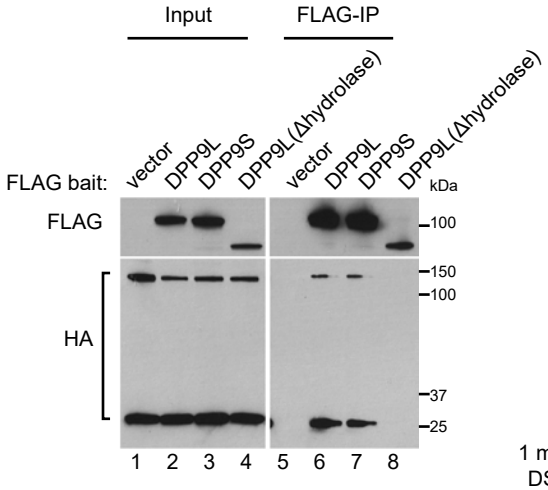
A. Coomassie staining of anti-FLAG-CARD8 IP eluates in 293T cells expressing FLAG-CARD8. Note that CARD8 undergoes auto-cleavage within the FIIND domain.
B. Mass spec identification of the DPP9 band in A. The timestamp (2011) demonstrates that these data were obtained independently and without prior knowledge of the data shown in other parts of this manuscript.
C. Cas9-expressing control, NLRP1 KO and PYCARD/ASC KO immortalized keratinocytes were transfected with vector, wild-type NLRP1 or the P1214R mutant. Cell culture media were harvested 24 hours after transfection and analyzed by IL-1β ELISA. Error bars were calculated by One-Way ANOVA comparing the indicated treatment groups. Bar graph represents one of two independent experiments.
D. Immortalized keratinocytes were transduced with lentiviral constructs encoding GFP, FLAG-tagged DPP9 and a DPP9 Δhydrolase mutant and selected with puromycin. Note that DPP9 Δhydrolase does not bind NLRP1 or display enzymatic activity.
E. Stably transduced immortalized keratinocytes in D were transfected with vector, wild-type NLRP1 or NLRP1 mutants M77T (MSPC) and R726W (AIADK). Cell culture media were harvested 24 hours after transfection and analyzed by IL-1β ELISA. Error bars were calculated by One-Way ANOVA comparing the indicated treatment groups. Bar graph represents one of two independent experiments.

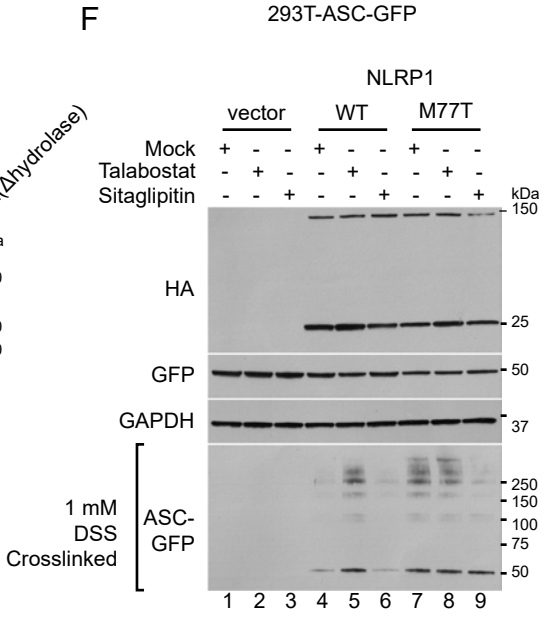
Figure S1

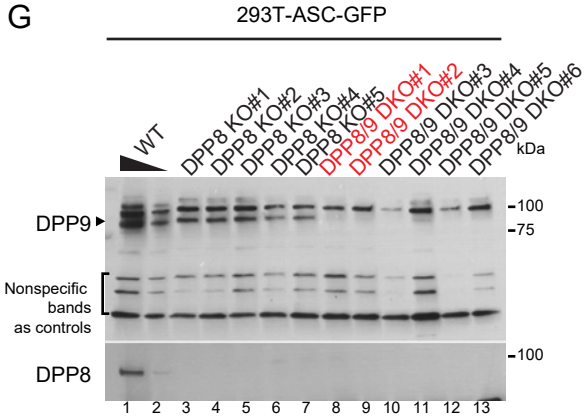
A

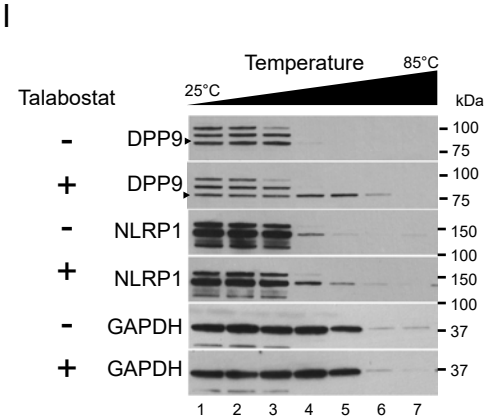
Protein	Mascot score	Fold change (NLRP1/ Vector)	CRAPome contaminant
	1078.0	72.2	Yes
ANP32B	337.1	18.4	No
UBTF	403.1	13.2	Yes
DPP9	865.7	12.7	No
NLRP1	**15007.3**	**10.6**	**No**
HADHB	619.8	10.2	Yes
TXN	370.9	10.1	Yes
HADHA	779.7	9.3	Yes
ANP32A	273.6	7.3	Yes
NCL	1538.7	6.5	Yes











H

Tryptic peptides identified in full length NLRP1-FLAG IP eluate

	Quality q value
VELHHIVLENPSFSPLGVLLK	0
SPLGVLLK	0.0217434

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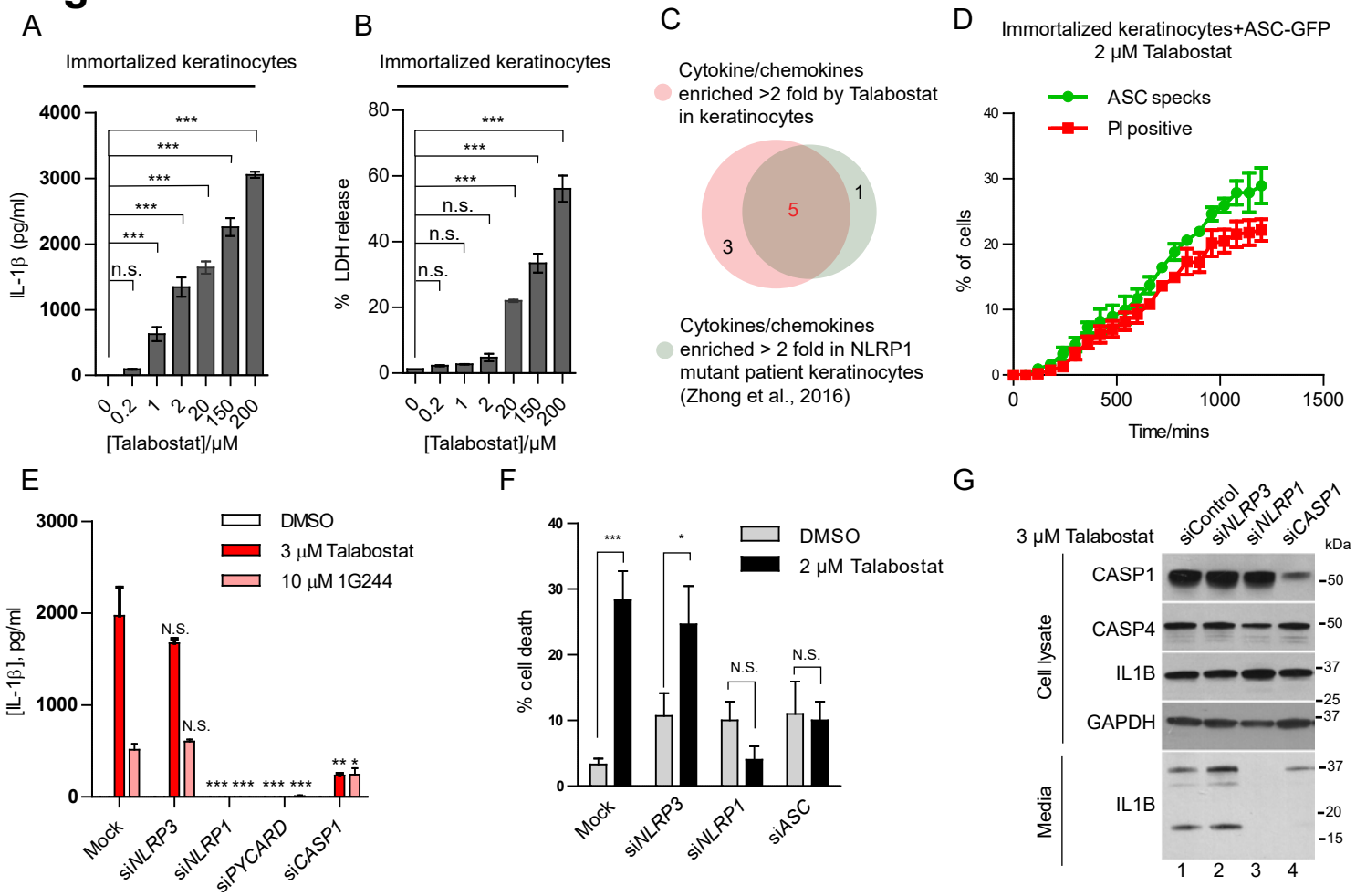
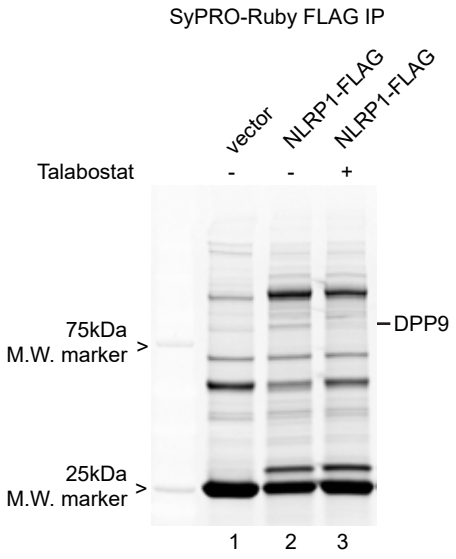
Figure S2



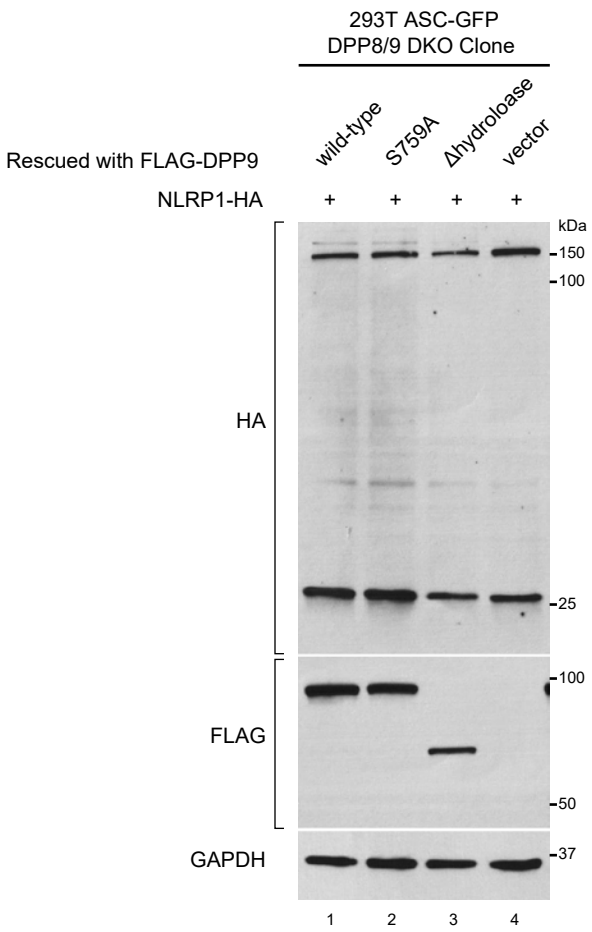
Zhong et al. 2018. Figure S2

Figure S3

A


SyPRO-Ruby FLAG IP
vector
NLRP1-FLAG
NLRP1-FLAG
Talabostat
- - +
75kDa
M.W. marker
25kDa
M.W. marker
DPP9
1 2 3

B


293T ASC-GFP
DPP8/9 DKO Clone
Rescued with FLAG-DPP9
wild-type
S759A
Δhydroloase
vector
NLRP1-HA
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kDa
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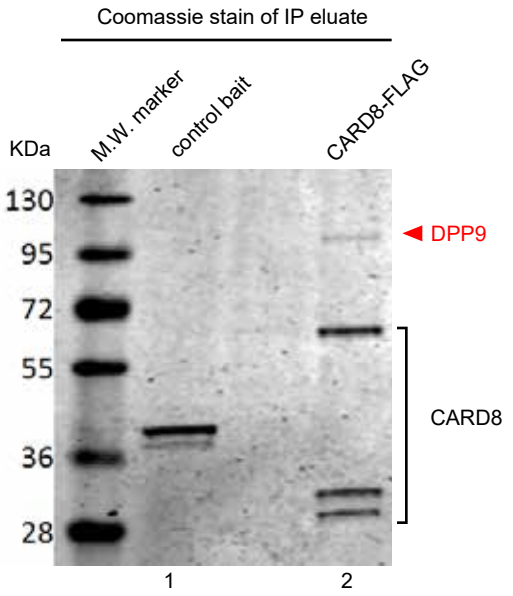
GAPDH
37
1 2 3 4

Figure S4

A



B

Protein: IPI00940180

>IPI00940180 IPI:IPI00940180.1 Tax_Id=9606 Gene_Symbol=DPP9 Isoform 2 of Dipeptidyl peptidase 9

LSRRVPCVRR GCRPPLPPLP GSQSRAWSRD REAPLDPGRP AQSGRRPTSR SVSHACSWNG GSLDPLEGTP
ALLRSAERLM RKVKKLRLDK ENTGSWRSFS LNSEGAERMA TTGTPTADRG DAAATDDPAA RFQVQKHSWD
GLRSIIHGSR KYSGLIVNKA PHDFQFVQKT DESGPHSHRL YYLGMPYGSR ENSLLYSEIP KKVRKEALLL
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GFLSLMGLIH KPQVFKVAIA GAPVTVWMAY DTGYTERYMD VPENNQHGYE AGSVALHVEK LPNEPNRLLI
LHGFLDENVH FFHTNFLVSQ LIRAGKPYQL QIYPNERHSI RCPESGEHYE VTLLHFLQEY L

MONO MW: **110175**, pI: **6.63**
Database = **/home/sorcerer/fasta/ipi.HUMAN.v3.73.fasta**
Links: Google Google Scholar NCBI ExPASy SGD

Observed Peptides

Position	Mass	Peptide
160-169	1215.60	APHDFQFVQK
180-190	1318.64	LYYLGMPYGSR
191-201	1291.67	ENSLLYSEIPK
824-831	969.49	YGFIDLSR

Coverage: AA 4.1% (40 / 971 residues) **Mass 4.3%** (4741 / 110175 Da)

COMET Sequence View by J.Eng (c) ISB 2001

1 of 2

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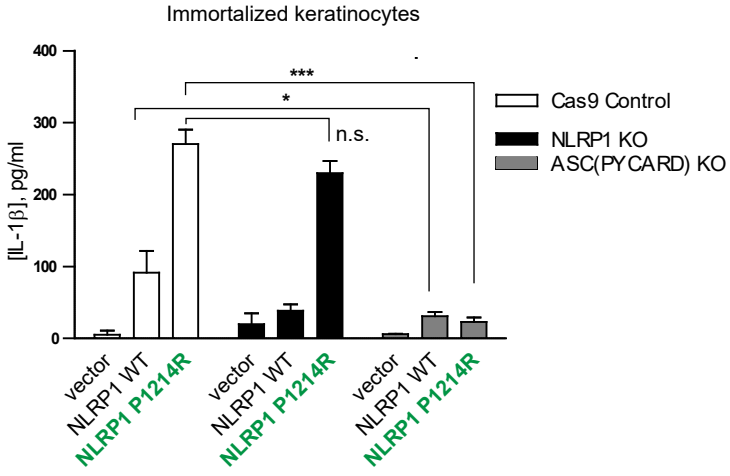

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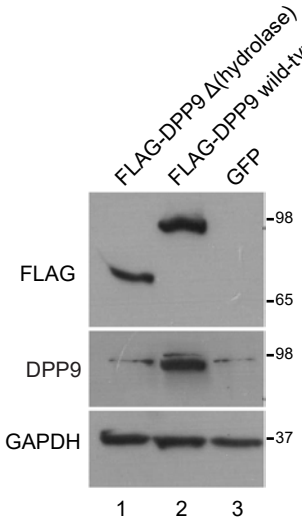

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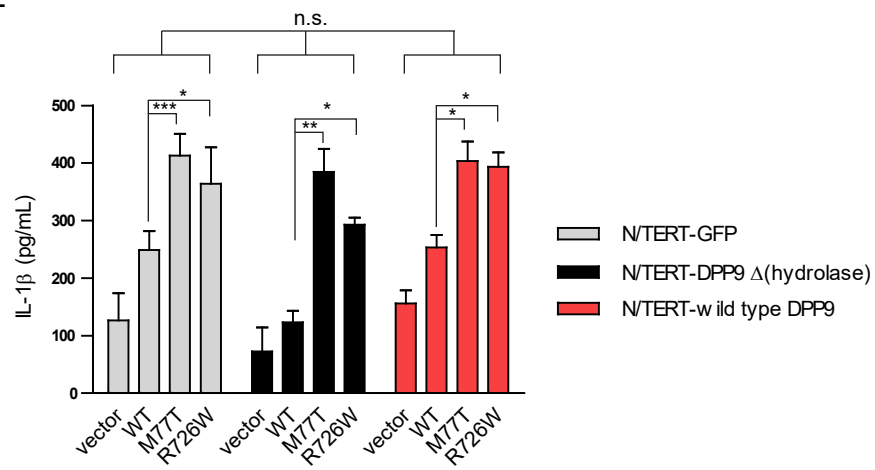

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