

**CIS 2016 School in Primary
Immunodeficiency Diseases**

CIS Clinical
Immunology
Society

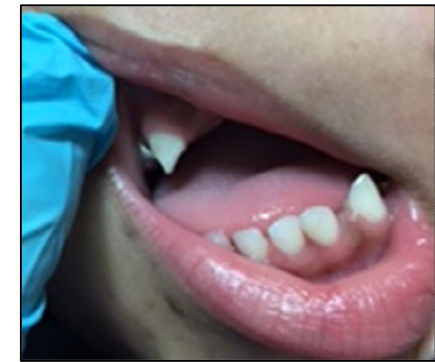
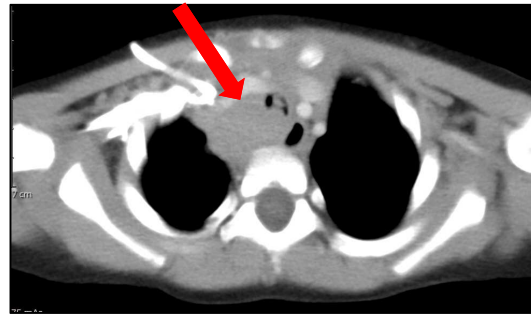
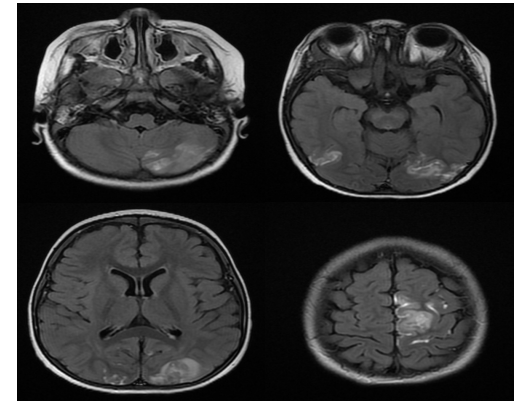
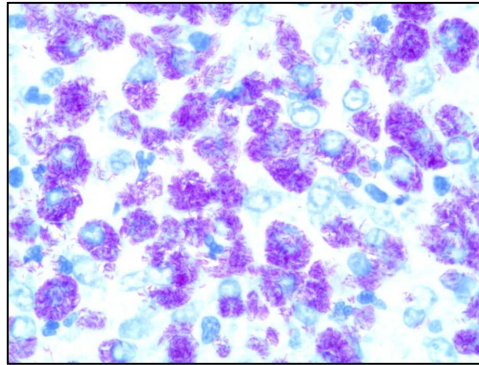
October 6-9, 2016
The Confidante
Miami Beach, Florida

**FUNCTIONAL EVALUATION OF A
[REDACTED] IN A
PATIENT WITH INCREASED SUSCEPTIBILITY
TO VIRAL AND MYCOBACTERIAL INFECTIONS,
AND DELAYED ABNORMAL DENTITION**

Cecilia Korol
National Institutes of Health
Clinical Center
Department of Laboratory Medicine

Patient

- Male
- African American
- Born to non-consanguineous parents

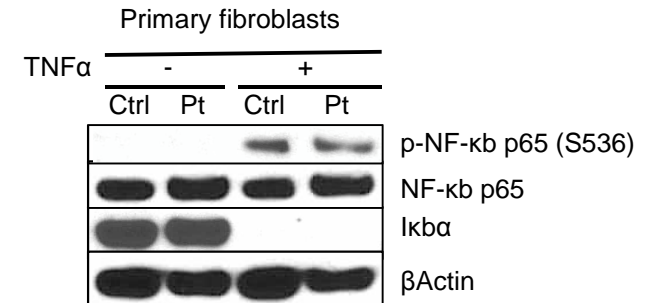


➤ Infections:

- Disseminated varicella post-VZV vaccination (17m).
 - Anterior mediastinal mass eroding the trachea. Positive for *Mycobacterium avium complex* (MAC).
 - CMV, HHV-6, adenovirus and enterovirus viremia.
 - Acute onset hyponatremia, seizures, and left hemiparesis. EBV DNA was found on CSF.
- Normal routine laboratories tests. Including lymphocyte phenotype/proliferation (PHA), Igs levels, protective titers to tetanus and diphtheria toxoids, 9/13 *S. pneumoniae* serotypes, DHR and NK cytotoxicity.
- Un-related matched cord blood transplantation (RIC 5/6) with complete engraftment (at 2y, now 1y3+ post-HSCT).
- Post-transplantation has shown irregular dentition (e.g. conical teeth) with delayed eruption.

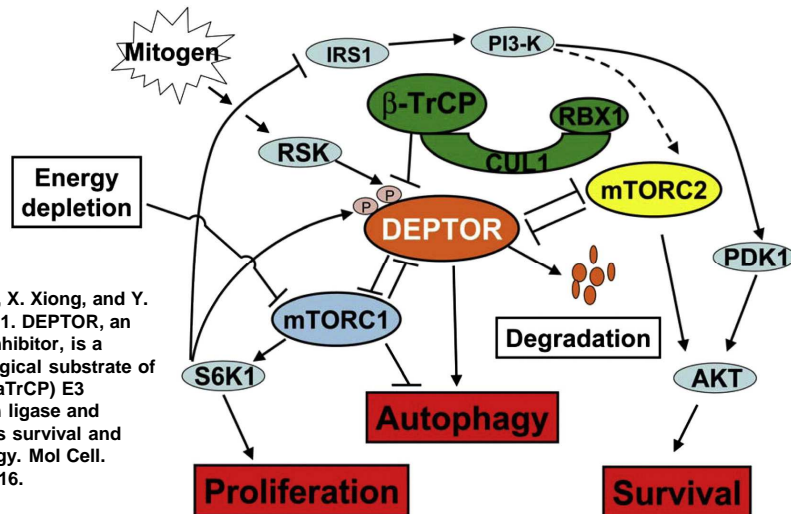
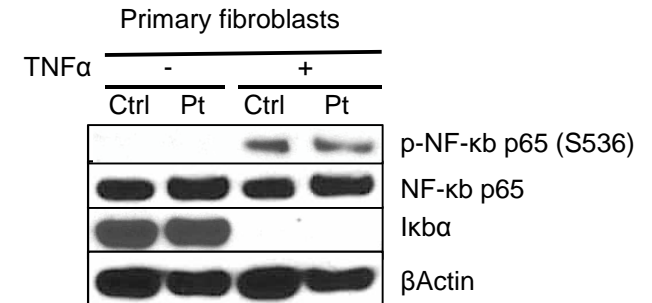
Congrats on the ones suspecting the NEMO pathway!!!!

- But you were **wrong**: Sequencing for all known primary IDPs was normal and functional test of the pathway on patient fibroblasts gave a comparable to normal response.
- So we did whole exome sequencing searching for a possible new gene causing disease.



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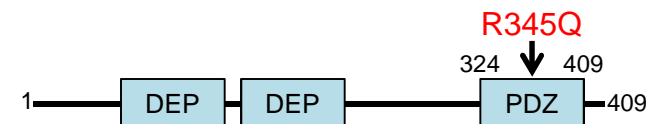


Zhao, Y., X. Xiong, and Y. Sun. 2011. DEPTOR, an mTOR inhibitor, is a physiological substrate of SCF(betaTrCP) E3 ubiquitin ligase and regulates survival and autophagy. Mol Cell. 44:304-316.

- **DEP** domain containing **mTOR**-interacting protein

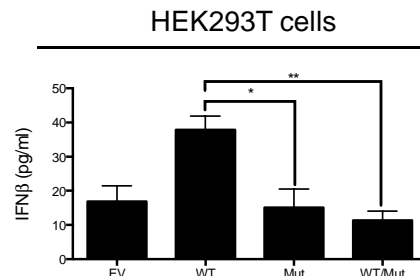
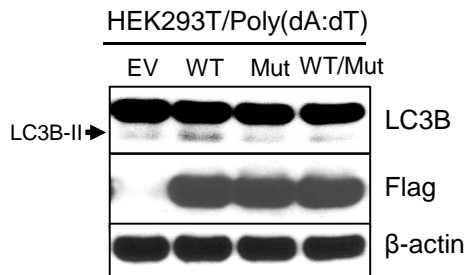
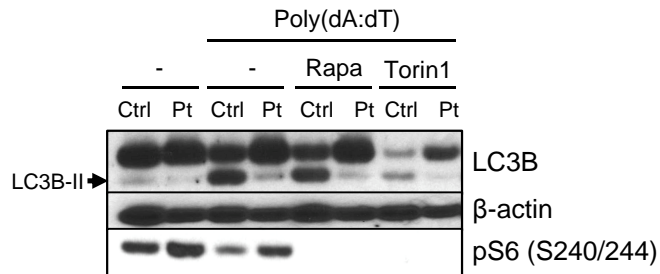
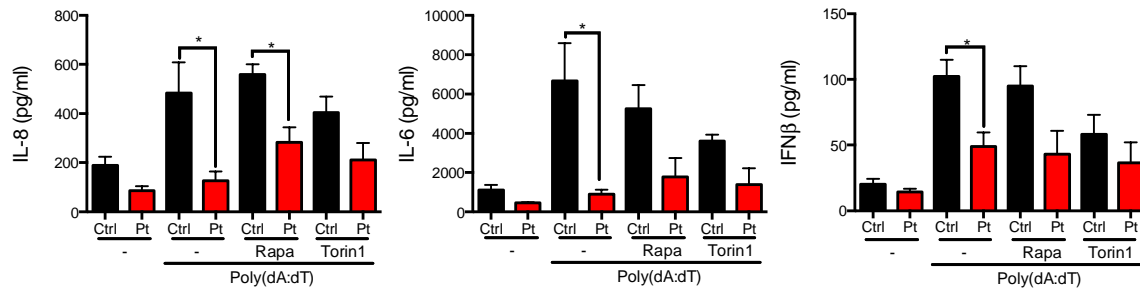
Predicted to be **damaging**; CADD C-score = 36 (Rockefeller Mutation Significance Cutoff MSC>11)

- Missense mutation in the PDZ domain of DEPTOR that may affect interaction with mTOR FRAP-ATM-TRAP (FAT) domain
 - Arg (positively charged, basic) > Gln (polar uncharged)

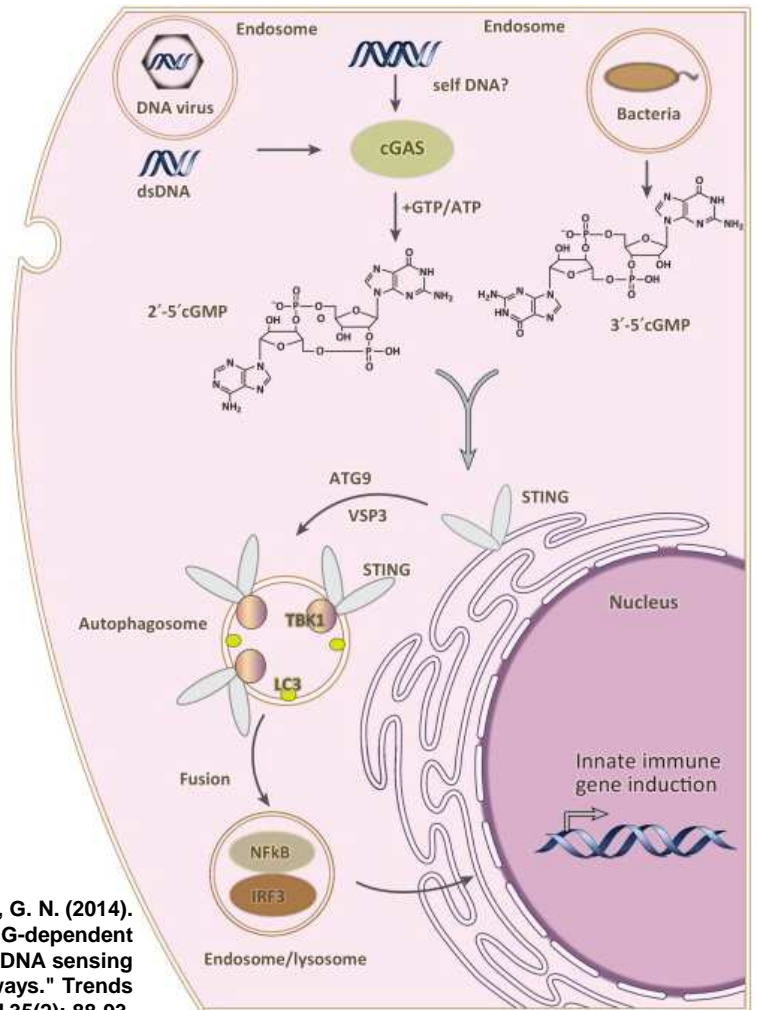


- Very high nucleotide position conservation scores.

Impaired response to cytosolic DNA sensing in patient cells. Independent of mTOR signaling.



Ctrl=Normal; Pt=Patient; EV=Empty vector; WT=Wild type DEPTOR; Mut=Mutant DEPTOR; Rapa=Rapamycin
*p<0.05 unpaired T-student Test.



Barber, G. N. (2014). "STING-dependent cytosolic DNA sensing pathways." Trends Immunol 35(2): 88-93.

Summary

- Patient with complicated infections to multiple viruses and *M. avium*. Post BMT he developed irregular dentition.
- WES revealed a heterozygous mutation in DEPTOR predicted to be damaging in the MTOR binding domain.
- Both his PBMCs and primary fibroblasts revealed hyperactive MTOR, with also enhanced NOTCH2 signaling resembling NOTCH2 g-o-f patients. On transfected HEK293T cells, lower binding of the mutant DEPTOR allele to MTORC1/2 was observed.
- Patient fibroblasts were not able to mount a response comparable to controls with cytosolic DNA stimulation either by autophagy or cytokine production. This impaired response was MTOR independent. This was recapitulated on HEK293T cells transfected with mutant or WT/Mutant Deptor producing less IFN β or LC3B-II than the cells transfected with the WT allele.
- We conclude that although further experiments are required given the nature of single patient studies, the susceptibility to infections and the abnormal dentition of the patient can be explained by a dominant negative effect on DEPTOR function by his mutation.

Acknowledgments

✧ NIH/Clinical Center:

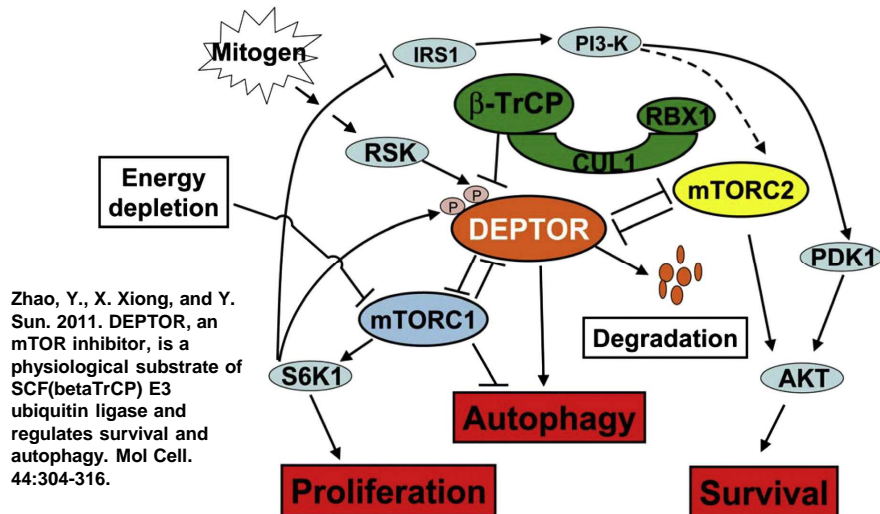
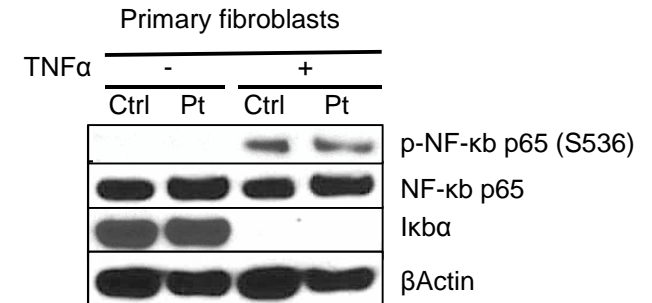
- Dr. Hyesun Khuen
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- Manuel Rovira-Gonzalez
- Dr. Uzel Gulbu
- Dr. Qingxue Li
- Dr. Jeffrey Cohen
- Dr. Adrian Zelazny
- Dr. Fleisher Thomas
- Dr. Sergio Rosenzweig

✧ Other centers:

- Hana Niebur-University of Nebraska/Department of Pediatrics Division of Allergy and Immunology
- Aleksandra Petrovic-All Children's Hospital Blood and Marrow Disorders
- Jennifer W. Leiding-University of South Florida/Department of Pediatrics/Division of Allergy and Immunology

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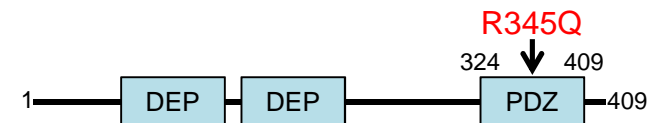
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- **DEP** domain containing **mTOR**-interacting protein

NM_022783 c.1034G>A, p.R345Q heterozygous rare SNP
 MAF A=0.000615 (6/10000)
 Predicted to be **damaging** by SIFT, PP2, LRT, Mutation Taster

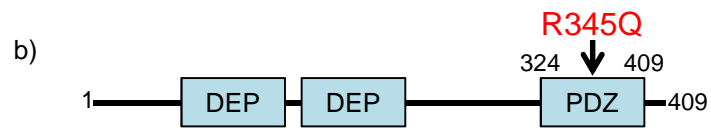
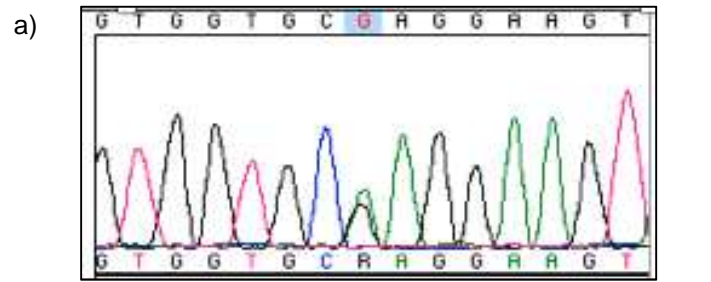
- Missense mutation in the PDZ domain of DEPTOR that may affect interaction with mTOR FRAP-ATM-TRAP (FAT) domain
 - Biochemically non-conservative aminoacid substitution
 - Arg (positively charged, basic) > Gln (polar uncharged)



- CADD C-score = 36 (Rockefeller Mutation Significance Cutoff MSC>11)

- Very high nucleotide position conservation scores (GERP=5.83, PhyloP=2.77, SiPhy=20.1338)

Figure 2



c)

```

shank1_ligand_seq      -----EAQTRL-----                6
mTOR_FAT_domain        TLVNDETQAKMARMAAAAAANGLGQWDSMEEYTCMIPRDTHDGAFYRAVLALHQDLFSLAQ    180
                        *:::
    
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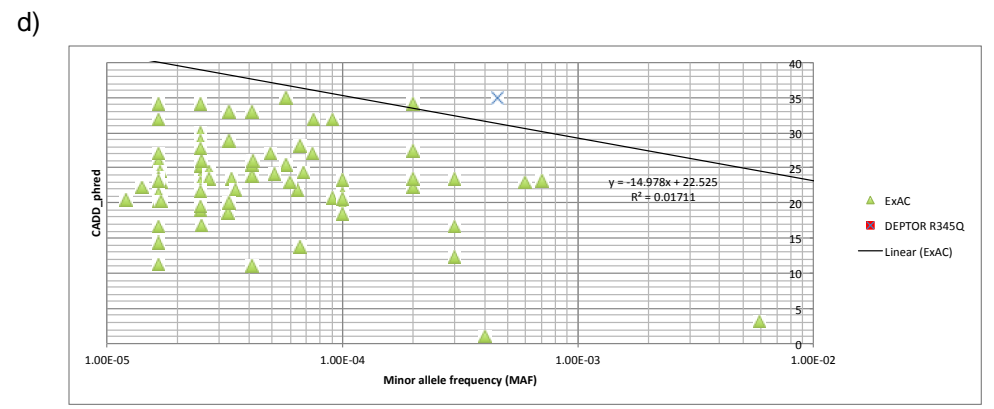
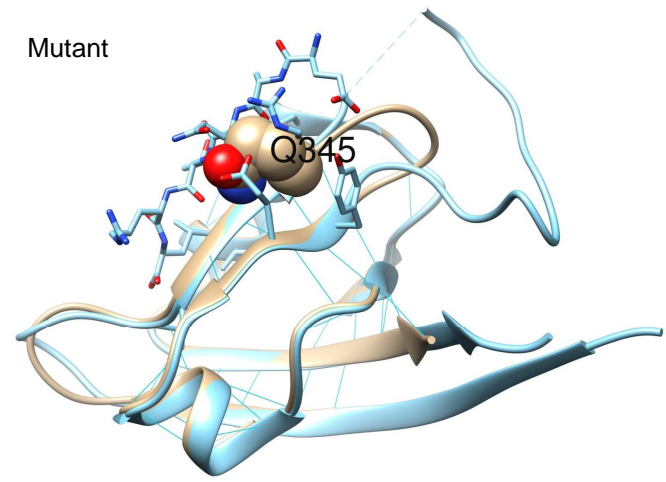
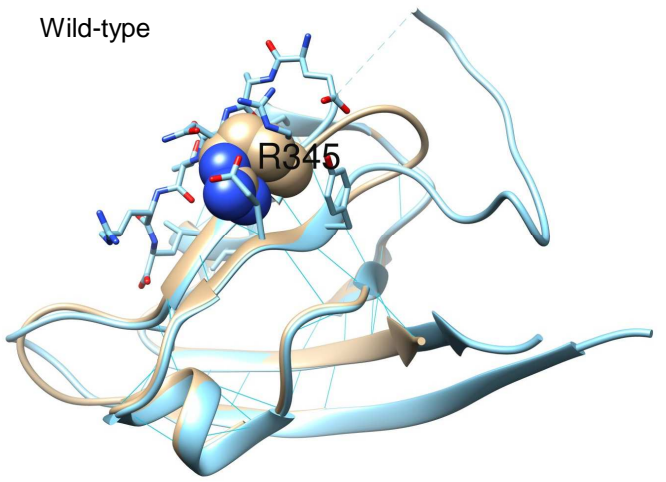


Figure 3

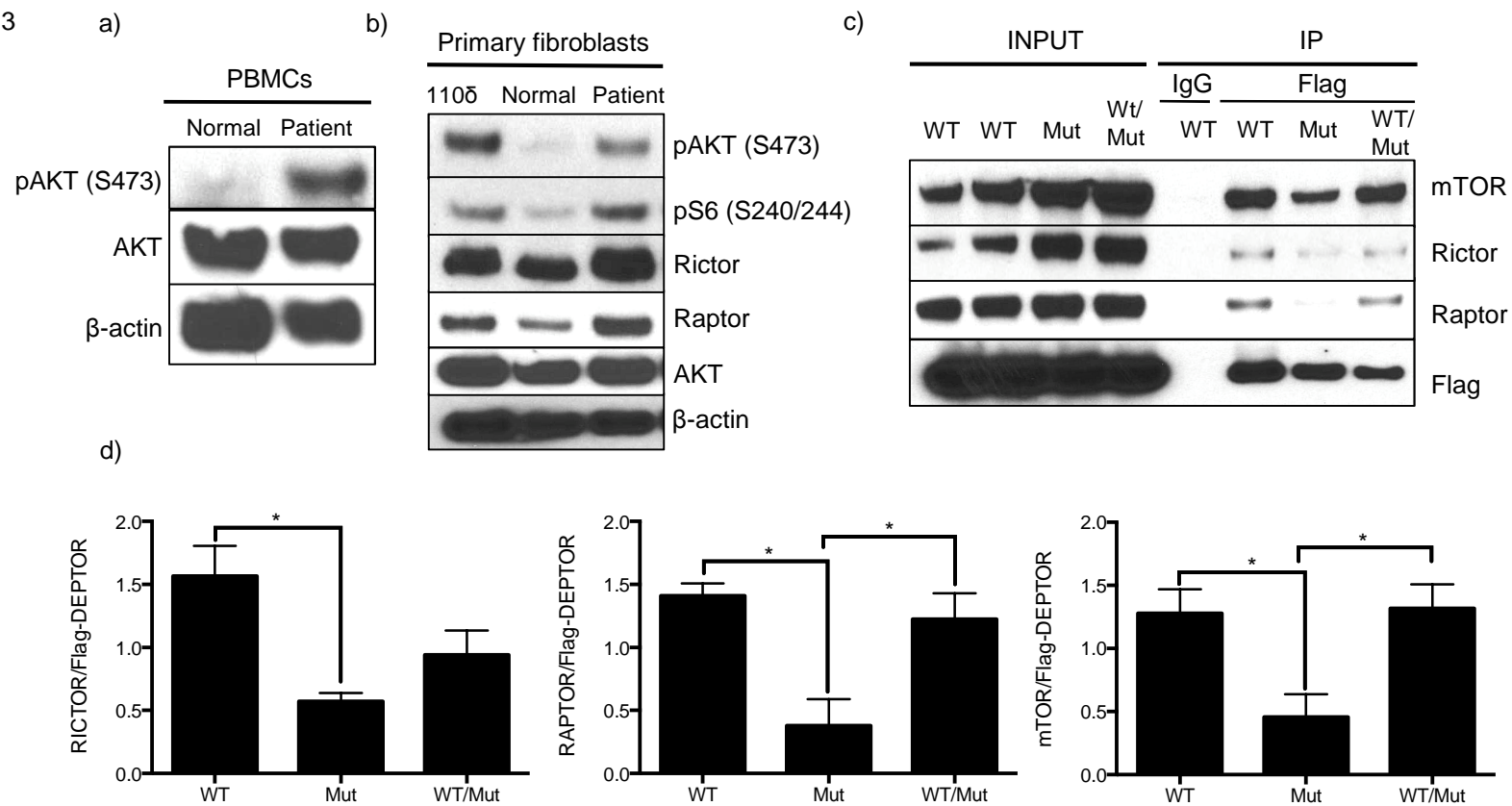


Figure 4

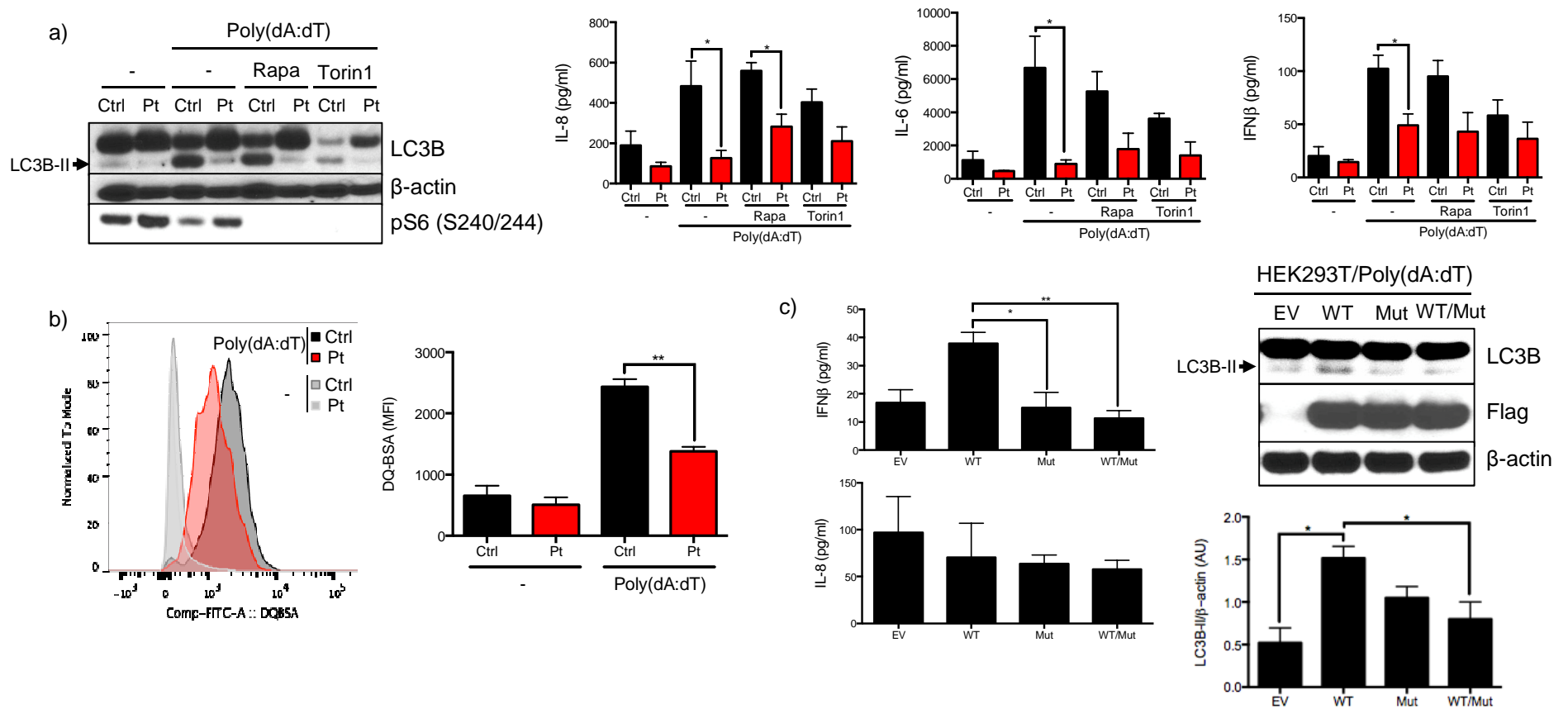
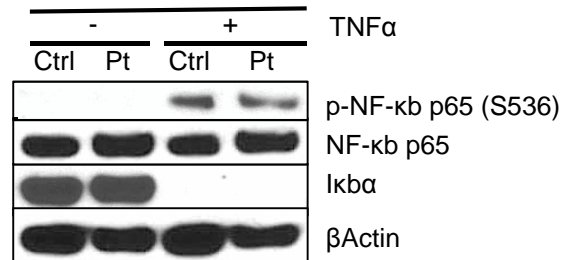


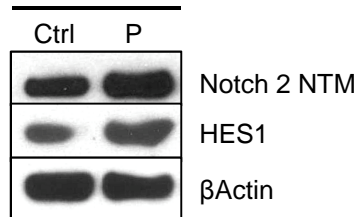
Figure 5

a) Primary fibroblasts



b)

Primary fibroblasts



Supplemental figure

