## Supplementary Methods

### Exome sequencing

DNA was enriched for exonic regions with SureSelect 38Mbp All exon kit v. 2.0 (Agilent Technologies, Santa Clara, CA, USA), prepared according to manufacturer protocols and 100x2 bp paired-end sequenced on HiSeq2000 (Illumina Inc., San Diego, CA, USA) with a 50-80x medium coverage. Reads were aligned to reference genome NCBI build 37/UCSC hg19 with CASAVA software (Illumina Inc., San Diego, CA, USA) and variant calling (SNPs, Single Nucleotide Polymorphisms, and DIPs, Deletion Insertion Polymorphisms) was performed with CLC Bio Genomics Workbench software (CLC bio, Aarhus, Denmark). Subsequent variant annotation and filtering was performed by using ANNOVAR software (http://www.openbioinformatics.org/annovar/). An Excel file listing SNPs and DIPs was sent to us by the IGA Technology Service. All the subsequent bioinformatics and statistical analyses were done using functions available in the Microsoft Excel datasheet software.

### Lymphoblastoid cell line generation

Blood samples from II-7, III-26, IV-44, IV-43, IV-46, 156, 157, were collected in lithium heparin, diluted 1:2 with 1X PBS and stratified on Histopaque®-1077 (Sigma). After centrifugation the "buffy coat" was collected and placed in a flask with 2 ml of EBV-rich RPMI-1640 medium and 2 ml of RPMI-1640 (VWR), supplemented with 10% FBS (GIBCO), 1X Penicillin-Streptomycin (5000U/ml-5mg/ml, GIBCO) and 1X L-Glutamine (200 mM Biowest). B lymphocytes were positively selected adding cyclosporine A the day after the infection (2 µg/ml). Wild type controls were already available in our laboratory.

### RNA Extraction and qRT-PCR

To study the expression of *SPRY2* mRNA the total RNA was extracted from lymphoblastoid cell lines at three different time points or from a fresh blood sample using the TriReagent (Sigma), according to manufacturer instructions.

The total RNA was reverse transcribed in cDNA using RevertAid Premium First Strand cDNA Synthesis (Thermo Scientific) with random primers. cDNA from each sample was amplified with Taqman technology on a 7900HT Fast Real-time PCR System (Applied Biosystem). Primers and 5'-FAM labeled probes for *SPRY2* and *GAPDH* were purchased from IDT Technology, while the amplification reaction was performed using the 2X KAPA PROBE FAST ABI Prism qPCR kit. Per each qRT-PCR experiment each run was done in triplicates using 3 µl of cDNA. The semi quantitative expression of the *SPRY2* transcript was indirectly evaluated using the $2^{-\Delta\Delta Ct}$ analysis method [1].

**Western Blot and antibodies**

Whole cell lysates from lymphoblastoid cell lines were obtained spinning down the cell culture and washing it with 1X PBS. Cells were then resuspended and lysed in RIPA buffer, protease and phosphatase cocktail inhibitors (Sigma). Proteins were quantified using a Bio-Rad Protein Assay (Bio-Rad). Proteins were separated on an 8% SDS-PAGE gel and then transferred to a PVDF membrane. Membranes were blocked with 5% no-fat milk (Santa Cruz Biotechnology) in TBS-T buffer, followed by overnight incubation at 4°C with the appropriate primary antibody. After three washes, the membrane was incubated for 1 h at room temperature with the HPR-conjugated appropriate secondary antibody in 5% TBS-T no-fat milk. Anti-actin and anti tubulin antibodies (Sigma) were used as loading controls. All the images were acquired with VisionWorksLS Analysis software and then modified and quantified with Image J.

In this study the following primary antibodies were used: anti-human IgA (Novus Biological, 1:5000), anti-human SPRY2 (Novus Biological, 1:500; Santa Cruz Biotechnology, 1:250), anti-human ERK1/2 (Cell Signalling, 1:1000), anti-human Phospho ERK1/2 (Pierce, 1:1000; Cell Signalling, 1:2000), anti-BrdU (Sigma, 1:1000) and the following secondary antibodies anti-rabbit-HRP conjugated (Pierce, 1:2000), anti-mouse-HRP conjugated (Pierce, 1:2000)

**SPRY2 protein stability study**

The whole SPRY2 gene was subcloned in pJET1.2 (Thermo Scientific) following amplification with the primers: forward 5'-GGATCCgaccATGGAACAGAAACTGATCTCTGAAGAAGACCTGGAGGCCAGAGCTCAGAGTGG-3', reverse CTATGTTGGTTTTTCAAAGTTCCTAGG. The Forward primer has a BamHI site followed by the MYC tag and then the 5' sequence of SPRY2 gene downstream of the ATG. The reverse primer was located on SPRY2 stop codon. To carry out the PCR amplification the DNA of II7 was used. By screening different clones the wild type and the mutated sequence were identified and verified by Sanger sequencing. The mutated and wild type MYCSPRY2 were cut with BamHI-BglII from pJET1.2 and inserted in the BamHI of pIRES2EGP (Clontech), upon transfection the MYC tagged SPRY2 would be expressed along with the GFP protein as internal control. The pMYC-SPRY2IRES2-EGF plasmid was verified by sequencing and then used in subsequent transfection experiments.

HEK293 cells (300,000/30-mm dish) were transfected with the mutated and wild type plasmid, after 48 hours cells were treated with 200μM cycloheximide and protein extracted at the indicated time points. Cell lysates were tested with an anti-MYC (Bethyl, 1:10000) and anti-GFP antibody (Novus Biological, 1:10000).

The bands from the Western blot were analyzed using IMAGE J and the stability of MYC-SPRY2 was evaluated respect to GFP as described in this work2.

**BrdU analysis**

For the kinetic study BrdU (Sigma-Aldrich) was added to the cell culture at 100mM final concentration for 1 hour, cells were then washed in 1X PBS and $5x10^5$ cells in 100μl were spun on a glass slide, fixed for 20 minutes in methanol, blocked with 1% normal goat serum, 0.1% Triton X-100 in 1X PBS for 30 minutes, then the DNA double strand was denatured with 2N HCl for 20 minutes and after three washes, cells were incubated for 1 hour with the anti BrdU antibody, followed by hybridization with a 488 Alexa Fluor-conjugated secondary antibody. Images were

captured on a Zeiss epifluorescent microscope equipped with a CCD camera. Image manipulation and cell counting were performed with Image J software.

**Sorafenib treatment**

Two flasks with the LCL157 cell line were treated once a day, for 6 consecutive days with Sorafenib (a kind gift from Dr. G. Pani) at a final concentration of 20 μM, and with DMSO, as control. One hour after last treatment proteins and total RNA were extracted as indicated above and phosphoERK1/2 and total IgA assessed by western blot, while the *SPRY2* mRNA level was tested by qRT-PCR. This experiment was repeated twice.

**Flow cytometry**

Lymphoblasts cells ($5\times10^5$ cells per sample) were washed with washing buffer (phosphate-buffered saline, 0.1% sodium azide and 0.5% bovine serum albumin), stained with 10 μL of anti-IgGA PE-conjugated (Miltenyi) and incubated for 30 minutes at 4°C in the dark. Samples were washed, centrifuged (at 4 °C, 400g for 8 minutes) and immediately acquired on a FACSCanto II flow cytometer, running with FACSDiVa software (BD, USA); $10^4$ events were recorded for each sample. Data were analyzed using FACSDiva software, v6.1.3 (BD). Debris was excluded from the analysis by gating on morphological parameters. Mean fluorescence intensity (MFI) can differ between different experiments and therefore had to be standardized to allow comparisons between different samples. Individual values were standardized by calculating MFI ratio values by dividing the MFI of positive events by the MFI of negative events.

Peripheral blood cells (1 ml of fresh blood) were lysed to eliminate red blood cells; white blood cells were washed and then stained with anti-CD19 APC-conjugated (Miltenyi) and anti-IgA PE-conjugated. Samples were washed, centrifuged and immediately acquired as mentioned above. $2.5x10^6$ events were recorded for each sample.

**Supplementary Figure 1. SPRY2 transcript level. qRT-PCR showing the SPRY2 mRNA levels in LCLIII26, LCLIV46 and LCLWT.** LCLIII26 carries the p.(Arg119Trp) variant, while LCLIV46 and WT are wild type for SPRY2. Per each experiment 2 different RNA preparations, each one run in duplicates, were used. For the graphical representation it was used the data of just one wild type control.

**Supplementary Figure 2. Stability of mutated and wild type SPRY2**. HEK293 cells were transfected with a plasmid expressing mutated or wild-type SPRY2 (tagged with MYC) along with a GFP downstream of an IRES sequence. After 48 hours cells were treated with 200μM of cycloheximide (CHX) for the indicated times (4 and 8 hours). Cell lysates were then analysed by Western blot. An antibody against the tag (MYC) identified the mutated or the wild type SPRY2 protein while an antibody for the GFP was used to normalize the level of SPRY2 protein. The upper panel shows a representative western blot experiment. The graph below shows the increased stability of the mutated protein compared to the wild type one. The data are representative of two independent experiments. The density of the MYC bands at each time point was expressed as a ratio of the density of the GFP band and then normalized to percent of MYC at time 0.

**Supplementary Figure 3. Characterization of the lymphoblastoid cell lines by flow cytometry. A.** Flow cytometry analysis of the LCL156 and LCL157 and two wild-type controls, to identify whether the increased amount of IgA was related to an increased production of IgA per single cell. The fluorescence was indicated as Mean Fluorescence Intensity (MFI) between the P2 IgA stained population and the P3 unstained negative population. **B.** Flow cytometry analysis of LCLII7 showing one single IgA+ population, probably due to the aspecific binding of IgA present in the culture media.

**Supplementary Figure 4. Flow cytometry analysis of fresh blood samples from *SPRY2* mutation carriers.** White blood cells were stained with an anti-CD19 APC-conjugated and anti-IgA PE-conjugated antibodies and then analysed on a FACSCanto II flow cytometer. $2.5x10^6$ events were recorded for each sample. No significant differences were found among those cell populations.

**Supplementary Figure 5. Proliferative analysis of the different cell lines. A**. After a pulse of BrdU for one hour, cells were harvested and stained with an anti-BrdU antibody, per each sample 10 indipedent fields were captured and BrdU+ cells and the total number of cells were counted. There was no statistical difference among the different cell lines in two different experiments in the amount of BrdU+ cells. **B.** Representative picture of one of the fields analyzed. DAPI was used to counterstain nuclei.

Supplementary Table 1. Clinical synopsy of the IgAN family. NA not assessed, ESRD (end stage renal disease), “+” indicates presence of the specific finding, “-“ indicates lack of the specific finding.

| Individual | sex | age | SPRY2 mutation | Serum IgA (ref. 70-400 mg/dL) | Haematuria | Proteinuria | Hypertension | ESRD | biopsy | status |
|---|---|---|---|---|---|---|---|---|---|---|
| II-7 | M | 74 | + | NA | NA | NA | + | + | - | affected |
| III-21 | M | 46 | + | NA | NA | NA | NA | - | + | affected |
| III-23 | M | 43 | - | NA | - | - | - | - | - | unaffected |
| II-10 | M | 68 | + | NA | NA | NA | + | + | + | affected |
| II-11 | F | 67 | - | NA | - | - | - | - | - | unaffected |
| III-24 | F | 43 | + | NA | + | - | - | - | + | affected |
| III-26 | F | 41 | + | 158 | + | - | - | - | - | affected |
| III-28 | F | 35 | - | NA | - | - | + | - | - | unaffected |
| IV-41 | F | 21 | + | NA | + | - | - | - | - | affected |
| IV-42 | F | 17 | + | NA | - | - | - | - | - | unaffected |
| IV-43 | F | 23 | + | NA | - | - | - | - | - | unaffected |
| IV-44 | M | 20 | + | 183 | + | + | + | - | - | affected |
| IV-45 | F | 19 | + | NA | - | - | - | - | - | unaffected |
| IV-46 | M | 10 | - | 50 | - | - | - | - | - | unaffected |
| III-29 | M | 36 | - | NA | - | - | - | - | - | unaffected |
| IV-47 | M | 11 | - | NA | - | - | - | - | - | unaffected |
| IV-48 | F | 6 | - | NA | - | - | - | - | - | unaffected |
| II-18 | M | 57 | + | NA | + | - | - | - | - | affected |
| II-17 | F | 56 | - | NA | - | - | - | - | - | unaffected |

Supplementary Table 2. Summary of the bioinformatics analysis of the DIPs assuming III-23 unaffected.

| | **Individual II-7** | **Individual IV-44** | **Individual III-23** |
|---|---|---|---|
| **Total DIPs** | 7131 | 8398 | 9339 |
| **DIPs present in dbSNP132** | 4509 | 5170 | 5761 |
| **DIPs present in 1000g** | 666 | 769 | 837 |
| **New or very rare DIPs** | 1956 | 2459 | 2741 |
| **DIPs within coding regions** | 41 | 46 | 53 |
| **DIPs shared by II-7 and IV-44, absent in III-23** | 4 | | |

Supplementary Table 3. Summary of the bioinformatics analysis of the SNPs assuming III-23 unaffected.

| | **Individual II-7** | **Individual IV-44** | **Individual III-23** |
|---|---|---|---|
| **Total SNPs** | 53490 | 53455 | 64333 |
| **SNPs shared by II-7 and IV-44, absent in III-23** | 8891 | | |
| **SNPs present in dbSNP132 and 1000g** | 8836 | | |
| **SNP within coding regions** | 55 | | |
| **Non synonymous variants** | 11 | | |

Supplementary Table 4. Linkage blocks location, number of variants present in each region, size of the region and number of non synonymous variants in each region.

| | Number of variants | Size in megabases | Non synonymous variants |
|---|---|---|---|
| Locus chr 1 | 5 | 4 | 1 |
| Locus chr 13 | 7 | 31 | 1 |
| Locus chr 16 | 2 | 2 | 1 |
| Locus chr 2 | 6 | 18 | - |
| Locus chr 22 | 2 | 20 | 1 |
| Locus chr 3 | 9 | 13 | 3 |
| Locus chr 5 | 3 | 19 | 1 |
| Locus chr 5bis | 2 | 2 | - |
| Locus chr 8 | 3 | 4 | 1 |
| Locus chr 9 | 7 | 22 | 2 |

Supplementary Table 5. Bioinformatics analysis of the SNPs assuming III-23 affected.

| | **Individual II-7** | **Individual IV-44** | **Individual III-23** |
|---|---|---|---|
| **Total SNPs** | 53490 | 53455 | 64333 |
| **SNPs absent in dbSNP132 and 1000g** | 1081 | 1028 | 1322 |
| **SNPs shared by II-7 and IV-44** | 174 | | |
| **Non synonymous variants** | 35 | | 305 |
| **Shared by all 3 individuals** | 24 | | |

Supplementary Table 6. Bioinformatics analysis of the DIPs assuming III-23 affected.

| | **Individual II-7** | **Individual IV-44** | **Individual III-23** |
|---|---|---|---|
| **Total DIPs** | 7131 | 8398 | 9339 |
| **DIPs present in dbSNP132** | 4509 | 5170 | 5761 |
| **DIPs present in 1000g** | 666 | 769 | 837 |
| **New or very rare DIPs** | 1956 | 2459 | 2741 |
| **DIPs within coding regions** | 41 | 46 | 53 |
| **DIPs shared by II-7, IV-44 and III-23** | 14 | | |

**Supplementary references:**


1. **Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.** ***Methods (San Diego, Calif*** **2001; 25: 402-408.**

2. **Edwin F, Anderson K, Patel TB: HECT domain-containing E3 ubiquitin ligase Nedd4 interacts with and ubiquitinates Sprouty2. The Journal of biological chemistry 2010; 285: 255-264.**

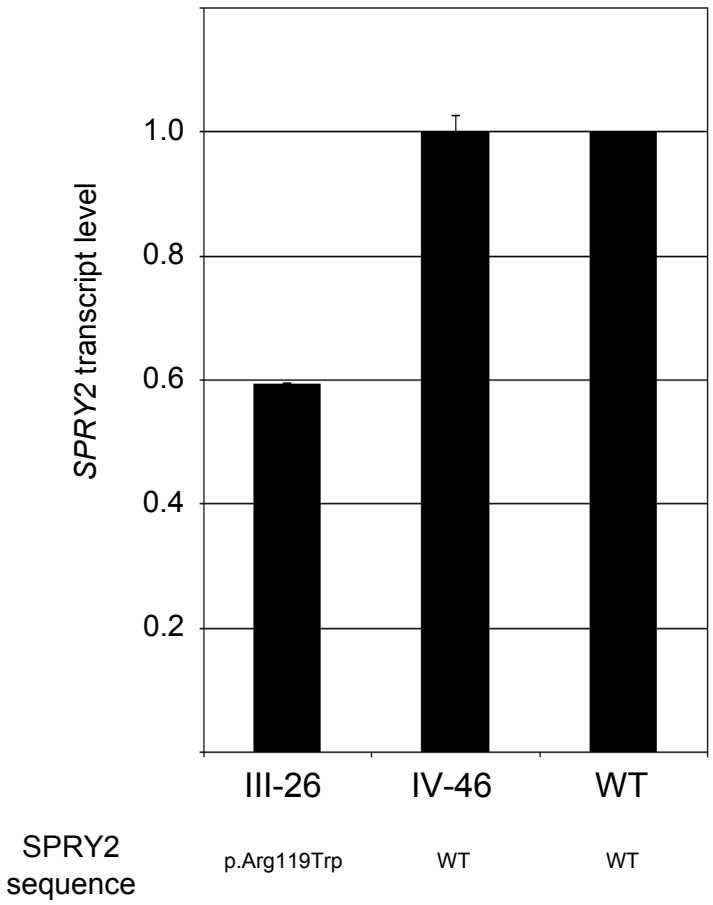

1.0
0.8
0.6
0.4
0.2
SPRY2 transcript level
III-26
IV-46
WT
SPRY2 sequence
p.Arg119Trp
WT
WT

Figure S1

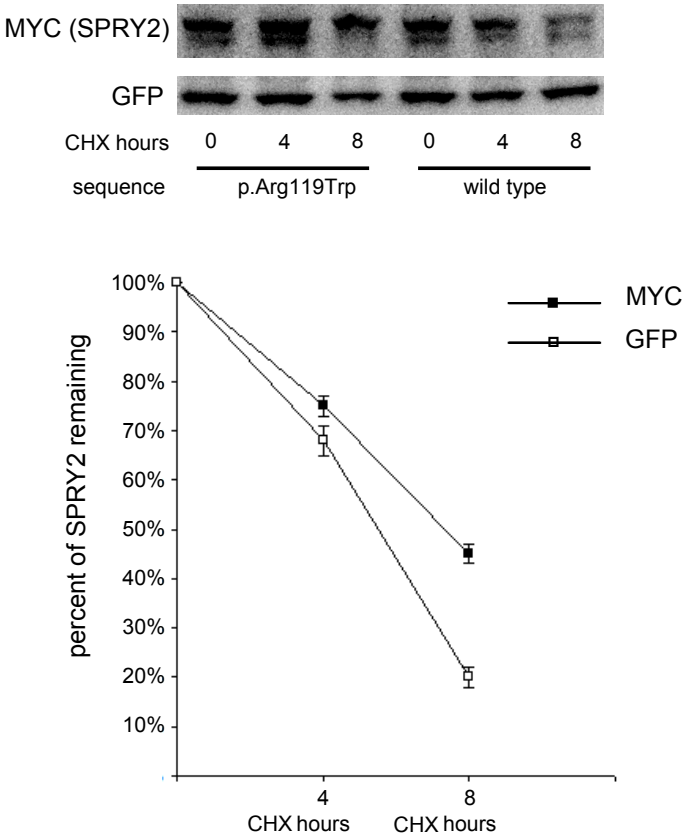
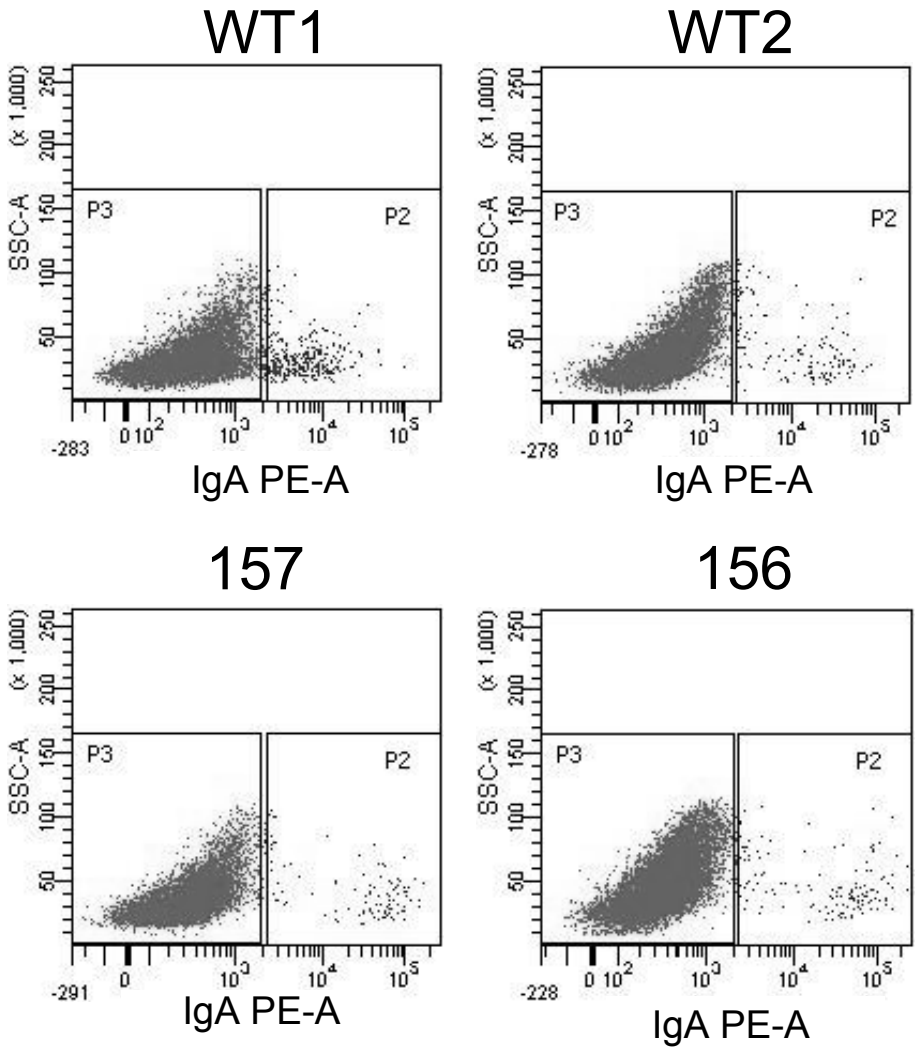
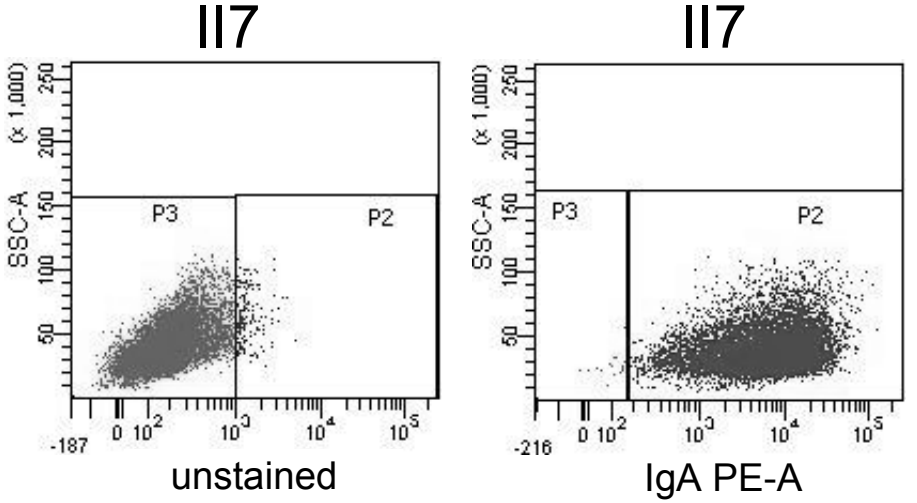

MYC (SPRY2)
GFP
CHX hours 0 4 8 0 4 8
sequence p.Arg119Trp wild type
MYC
GFP
percent of SPRY2 remaining
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
4
CHX hours
8
CHX hours

Figure S2

a

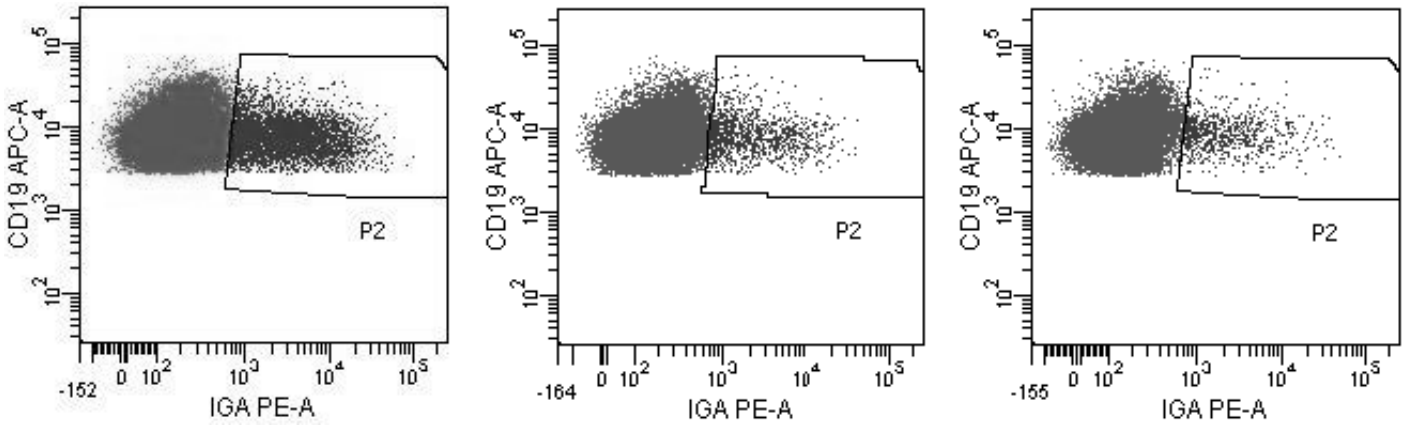



b

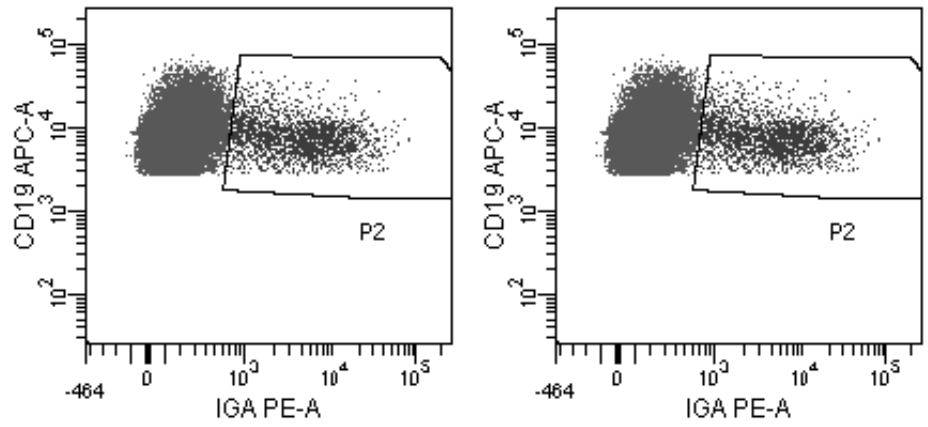



| | % of P2 | MFI |
|---|---|---|
| WT1 | 2.9 | 51.92 |
| WT2 | 1.4 | 115.80 |
| 156 | 1.1 | 247.40 |
| 157 | 1.0 | 319.26 |

Figure S3



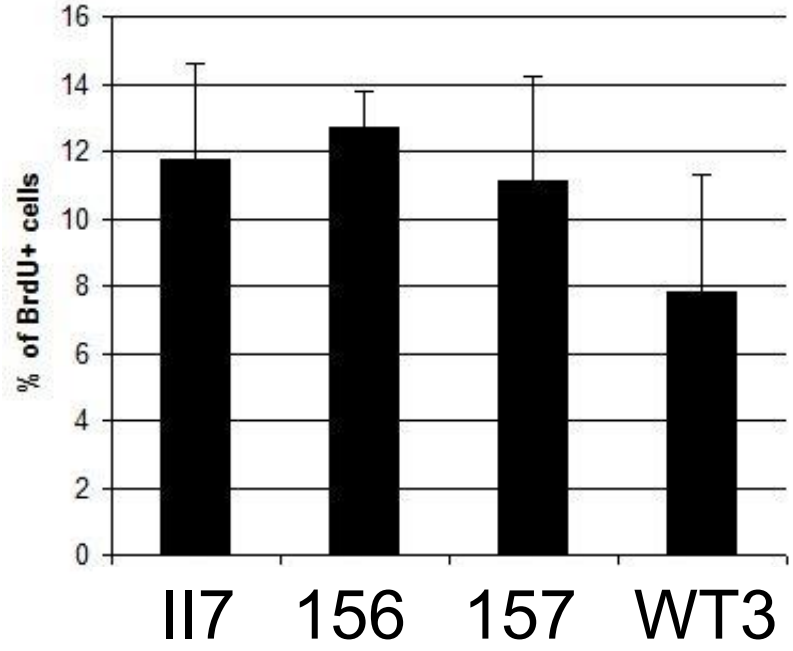

WT III-26 IV-44




IV-46 IV-45

Figure S4

a

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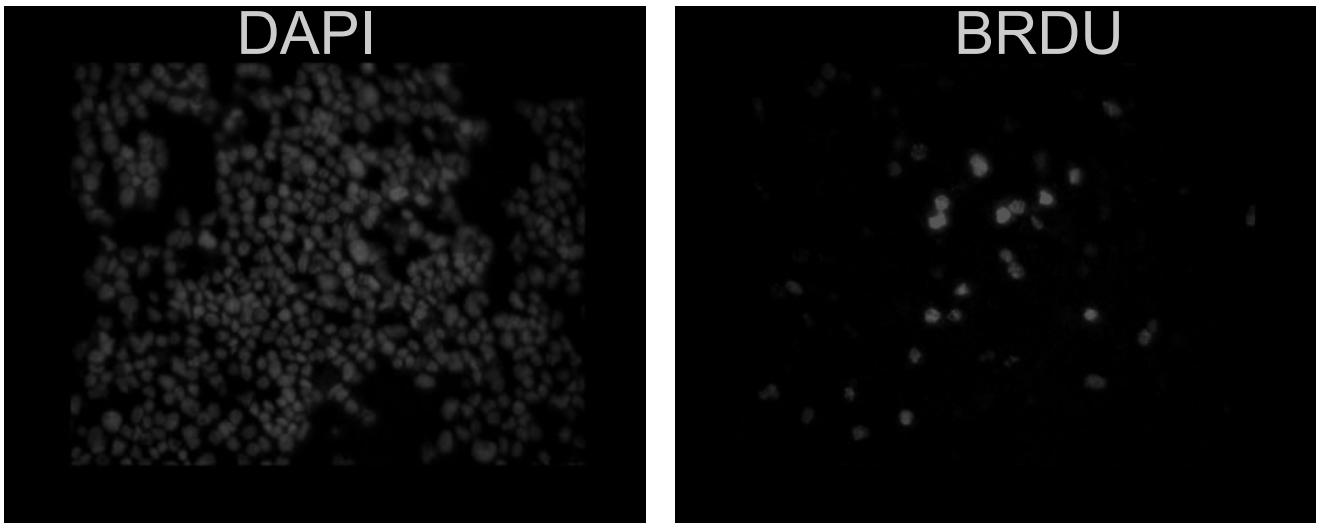


Figure S5