Novel Mechanisms of Lymphomagenesis from Precision Proteogenomics

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April 23, 2026

Disclosure statement

Genomenon Inc: Co-Founder, Adviser

ThermoFisher Inc: Research support, Advisory Panel

Outline

- Background
- Hypothesis
- Experimental Approach
- Conclusions
- Future Directions

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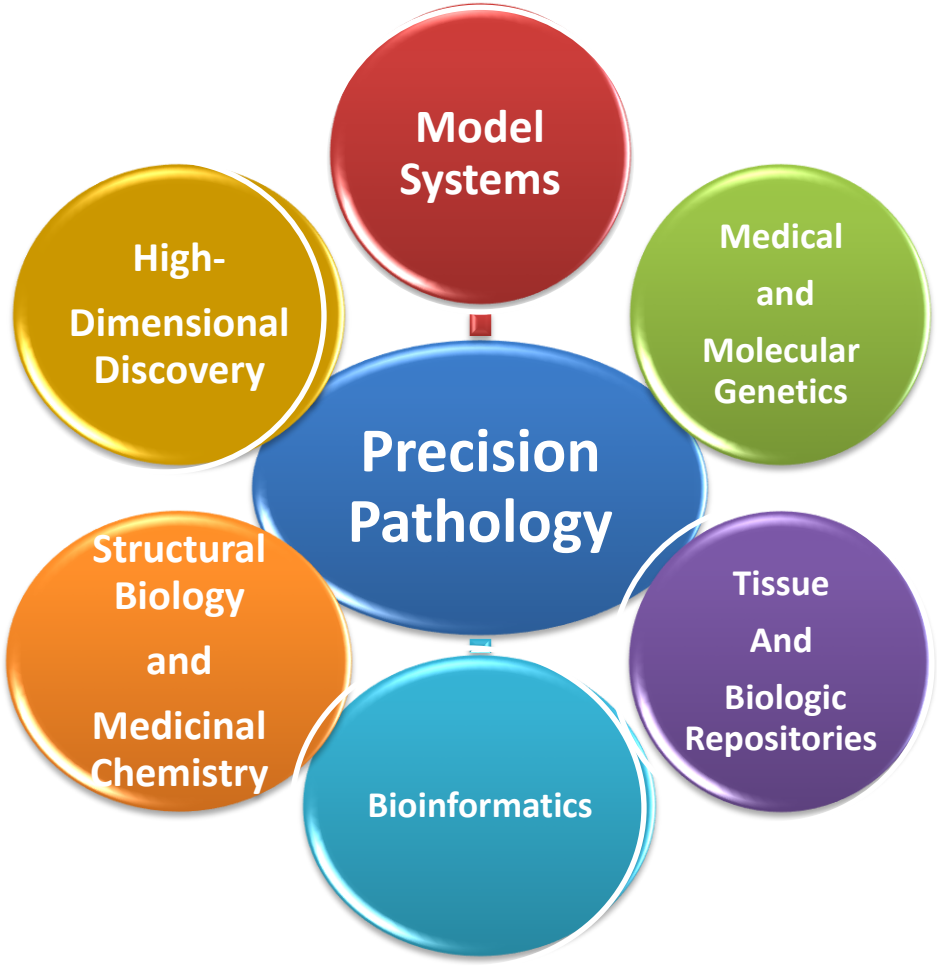
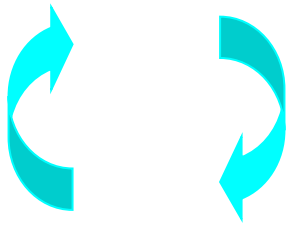
Integrated Systems As Drivers of Precision Medicine



Basic science



Translational medicine

New insights into pathogenesis

BIOMARKER DISCOVERY/INTELLECTUAL PROPERTY

Diagnostics **Early Detection** **Prognostics** **Therapeutics**

Elenitoba-Johnson/Lim Lab Research Strategy

Primary Patient Specimens

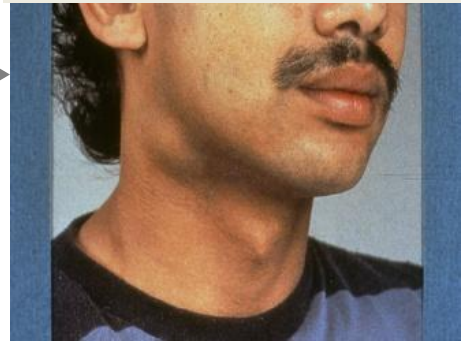
Outcomes

Novel biology

Biomarkers:
- Diagnostic
- Predictive

Novel therapies

Lymphoma



Animal Model

DNA

RNA

Protein

PTMs

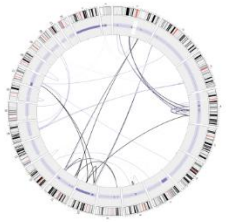
Function

Synthesis

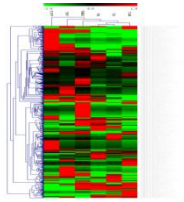
Amino Acids

Proteolysis

Genomic Sequencing

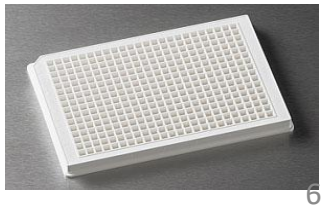


Gene Expression



Proteomic Profiling

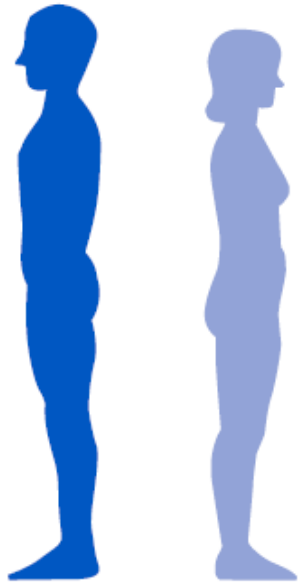
Functional Screens



Estimated US Cancer Deaths

Top 10

Male		
Lung & bronchus	67,160	21%
Prostate	34,700	11%
Colon & rectum	28,470	9%
Pancreas	26,620	8%
Liver & intrahepatic bile duct	19,000	6%
Leukemia	13,900	4%
Esophagus	12,920	4%
Urinary bladder	12,160	4%
Non-Hodgkin lymphoma	11,780	4%
Brain & other nervous system	11,020	3%
All sites	**322,080**	

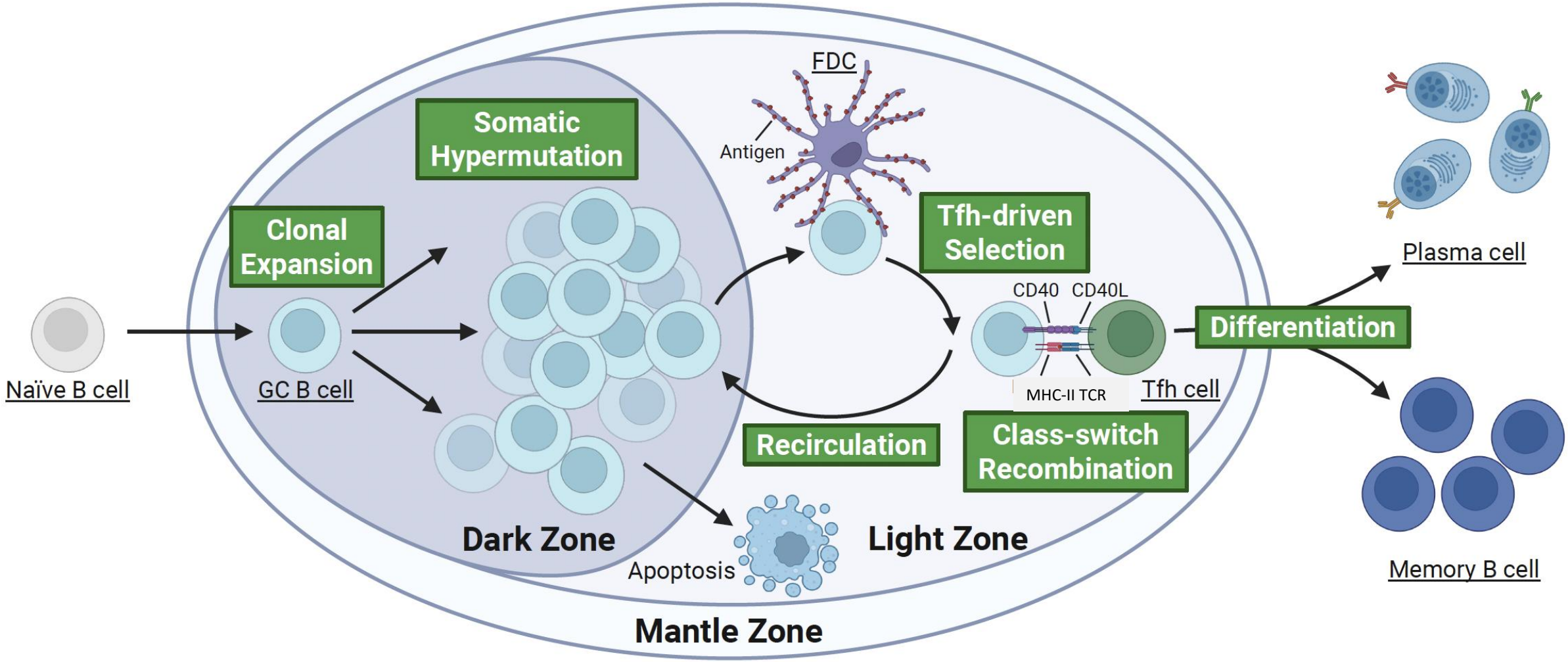


Female		
Lung & bronchus	59,910	21%
Breast	43,170	15%
Colon & rectum	24,080	8%
Pancreas	23,930	8%
Ovary	13,270	5%
Uterine corpus	13,030	5%
Liver & intrahepatic bile duct	10,380	4%
Leukemia	9,810	3%
Non-Hodgkin lymphoma	8,400	3%
Brain & other nervous system	7,970	3%
All sites	**287,740**	

American Cancer Society

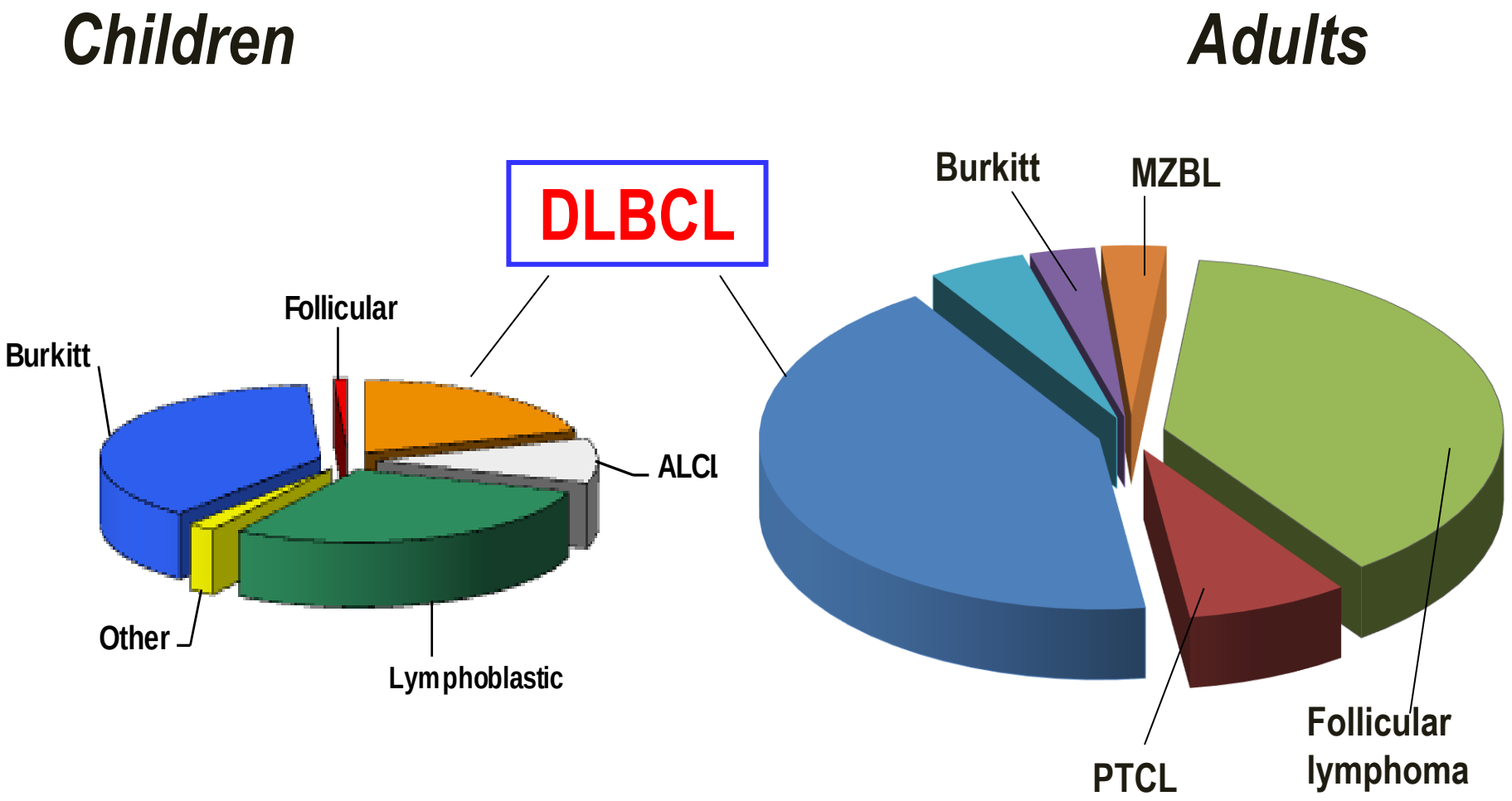
Cancer Facts and Figures 2023

B cell differentiation



Germinal Center Reaction

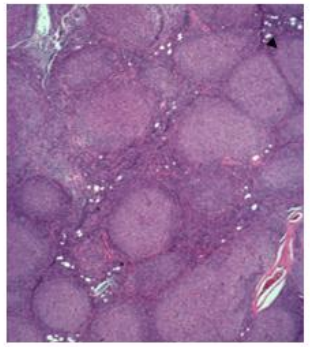
Distribution of NHL



Adapted from: Swerdlow S et al., 2008

Transformation is the leading cause of FL mortality

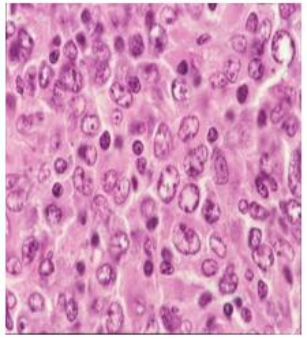
Follicular lymphoma



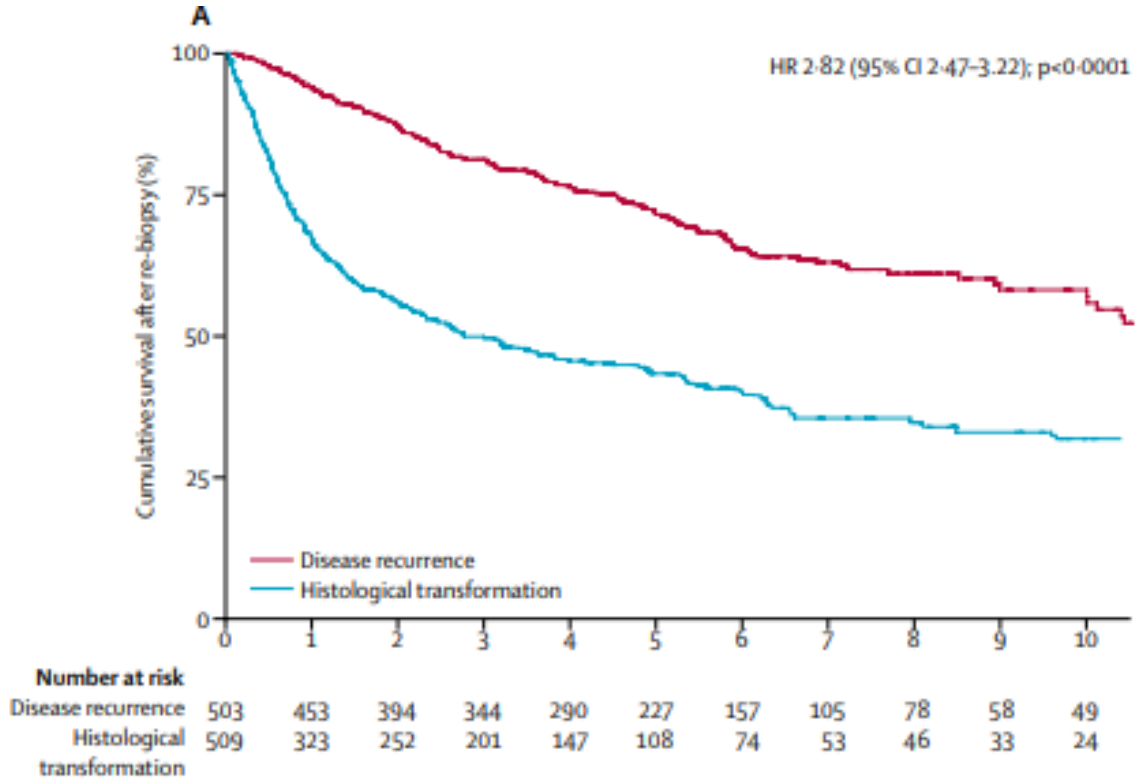
Indolent

Progression

Transformed FL



Aggressive



Federico et al. Lancet Haematol 2018;

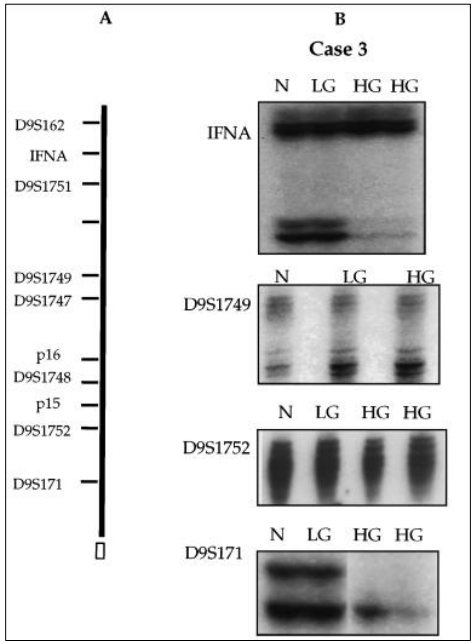
Genomic Events in Follicular Lymphoma Transformation

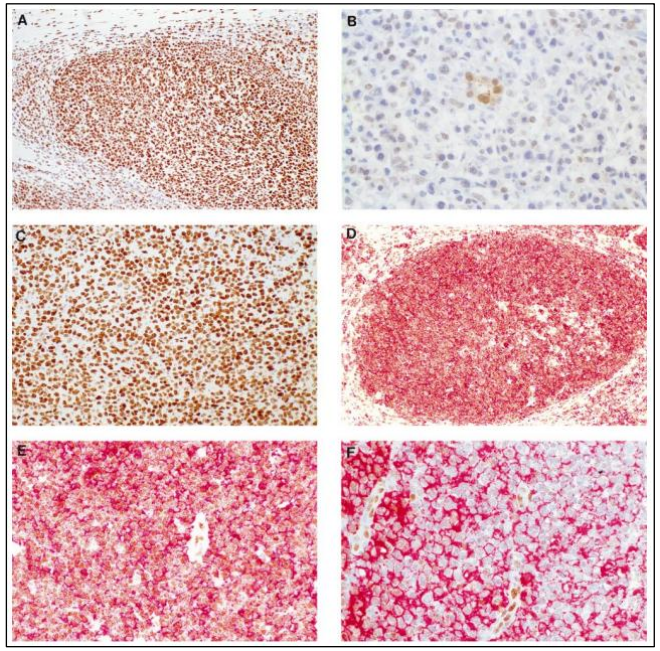
blood
Volume 91, Issue 12, 15 June 1998, Pages 4677-4685

Vol 91, Issue 12, 15 June 1998, Pages 4677-4685

Homozygous Deletions at Chromosome 9p21 Involving p16 and p15 Are Associated With Histologic Progression in Follicle Center Lymphoma

By Kojo S.J. Elenitoba-Johnson, Randy D. Gascoyne, Megan S. Lim, Mukesh Chhanabai, Elaine S. Jaffe, and Mark Raffeld





blood
Volume 91, Issue 12, 15 June 1998, Pages 4677-4685

p16^{INK4a} Gene Inactivation by Deletions, Mutations, and Hypermethylation Is Associated With Transformed and Aggressive Variants of Non-Hodgkin's Lymphomas

By Magda Pinyol, Francesc Cobo, Silvia Bea, Pedro Jares, Iracema Nayach, Pedro L. Fernandez, Emilio Montserrat, Antonio Cardesa, and Elias Campo

Blood, Vol 91, No 8 (April 15), 1998: pp 2977-2984

Regular Article

LYMPHOID NEOPLASIA

BLOOD, 13 MARCH 2014 x VOLUME 123, NUMBER 11,

Genome-wide copy-number analyses reveal genomic abnormalities involved in transformation of follicular lymphoma

Alyssa Bouska,[1] Timothy W. McKeithan,[1] Karen E. Deffenbacher,[1] Cynthia Lachel,[1] George W. Wright,[2] Javeed Iqbal,[1] Lynette M. Smith,[3] Weiwei Zhang,[1] Can Kucuk,[1] Andrea Rinaldi,[4] Francesco Bertoni,[4,5] Jude Fitzgibbon,[6] Kai Fu,[1] Dennis D. Weisenburger,[7] Timothy C. Greiner,[1] Bhavana J. Dave,[8] Randy D. Gascoyne,[9] Andreas Rosenwald,[10] German Ott,[11] Elias Campo,[12] Lisa M. Rimsza,[13] Jan Delabie,[14] Elaine S. Jaffe,[15] Rita M. Braziel,[16] Joseph M. Connors,[17] Louis M. Staudt,[18] and Wing-Chung Chan[1]

blood
Volume 91, Issue 12, 15 June 1998, Pages 4677-4685

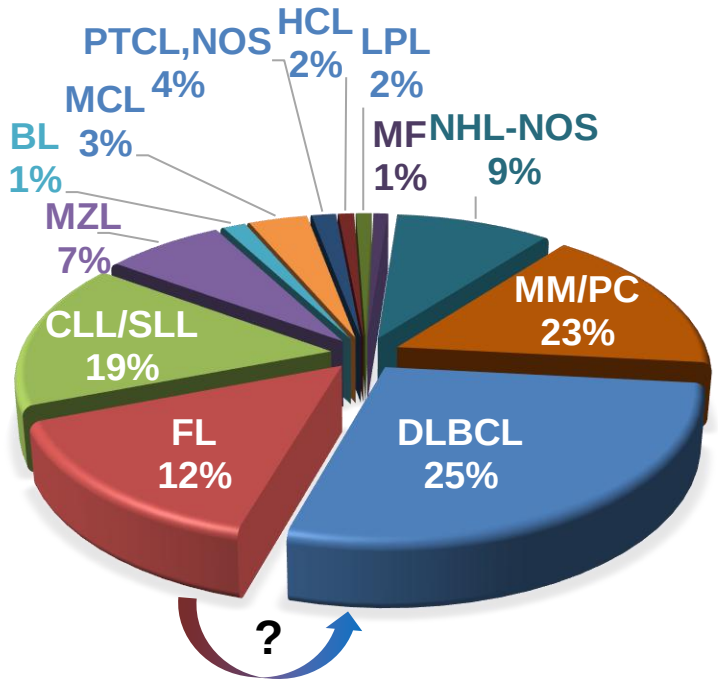
Blood, Vol82, No 7 (October 1). 1993: pp 1994-2004

RAPID COMMUNICATION

p53 Mutation Is Associated With Progression in Follicular Lymphomas

By Christian A. Sander, Takahiro Yano, Helen M. Clark, Cynthia Harris, Dan L. Longo, Elaine S. Jaffe, and Mark Raffeld

Molecular events in FL transformation

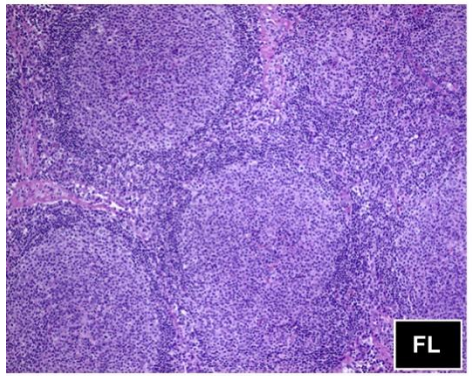


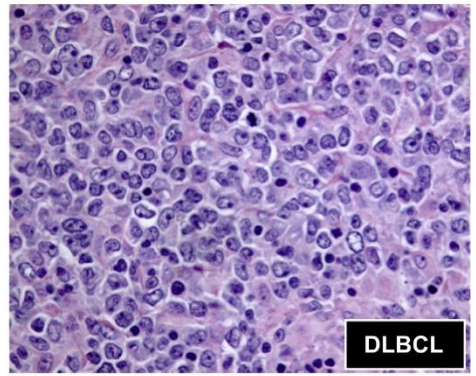
10-60%

Teras L.R. et.al., CA Cancer J Clin. 2016

Genetic lesions in the tumor cells

- *p53* loss
- *p16/CDKN2A* loss
- *MYC* deregulation
- +12/12q13-14 gain
- *BCL6* translocations
- Mutations in *BCL2/BCL6*
- Deletion/UPD 1p36 (*TNFRSF14*)
- Deletion 6q
- Trisomy 7
- *FAS* mutations
- UPD 16p
- CD9 loss





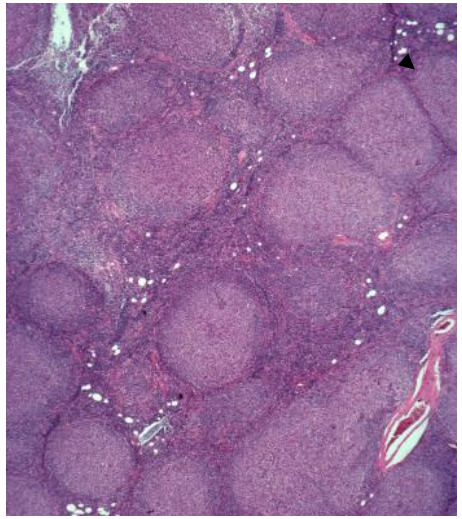
Changes in the micro environment (non-neoplastic cells)

- FDC loss or immaturity
- Increased intrafollicular $CD4^+$ T cells
- Pattern and number of regulatory T cells
- Decreased $PD\text{-}1^+$ T cells
- Increased MVD

I.S. Lossos, R.D. Gascoyne
Best Practice & Research Clinical Haematology 24 (2011) 147–163

Transformation is the leading cause of FL mortality

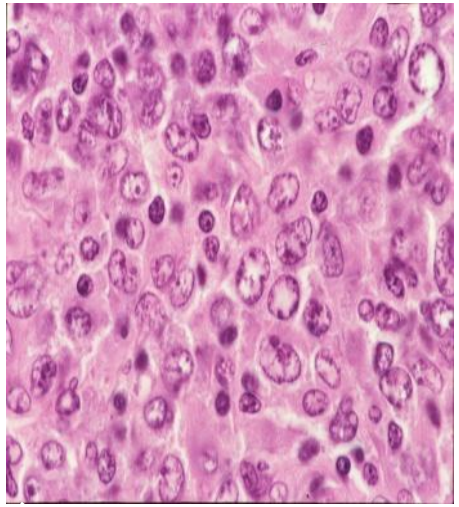
Follicular lymphoma



Indolent

Progression

Transformed FL

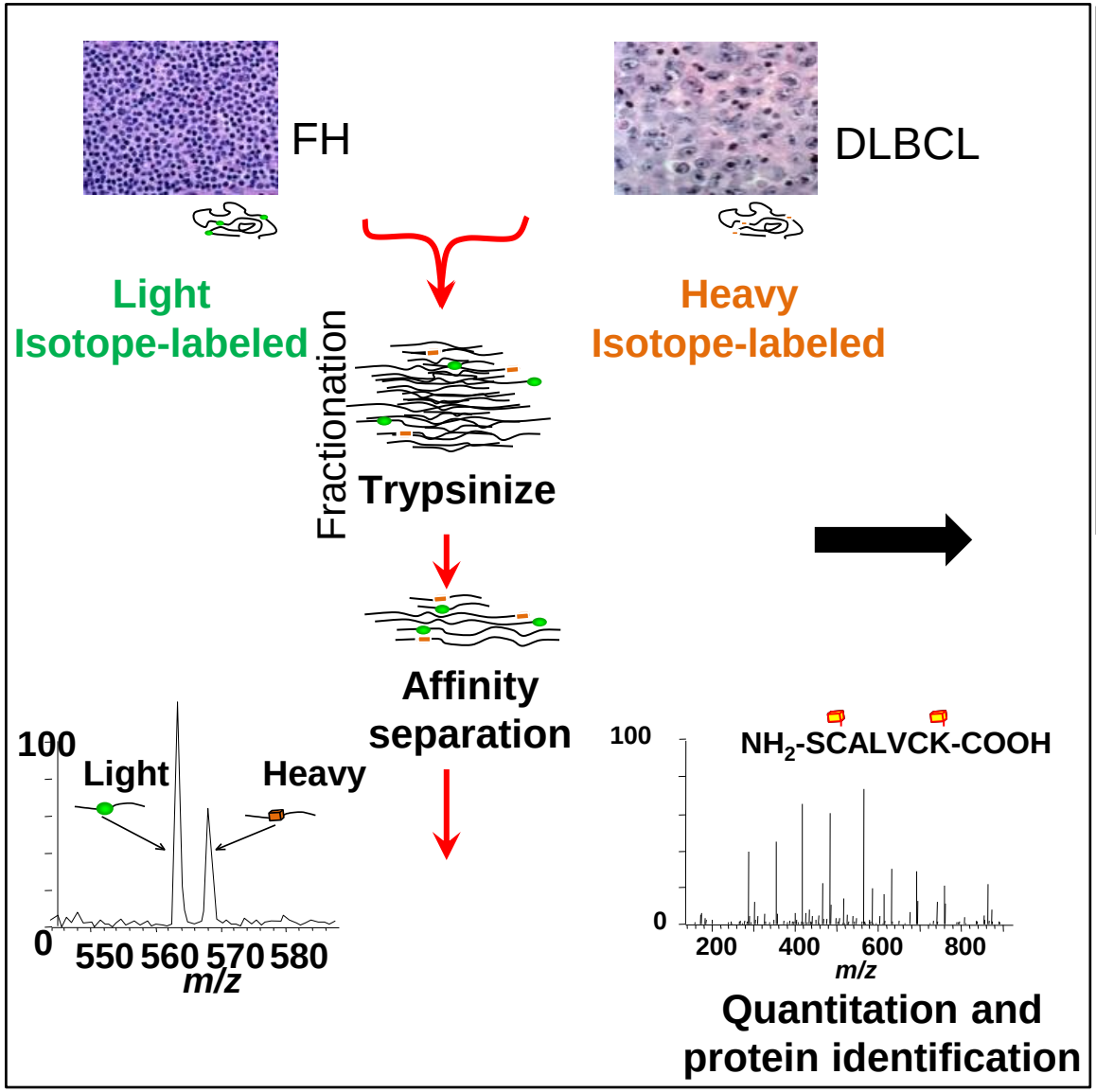


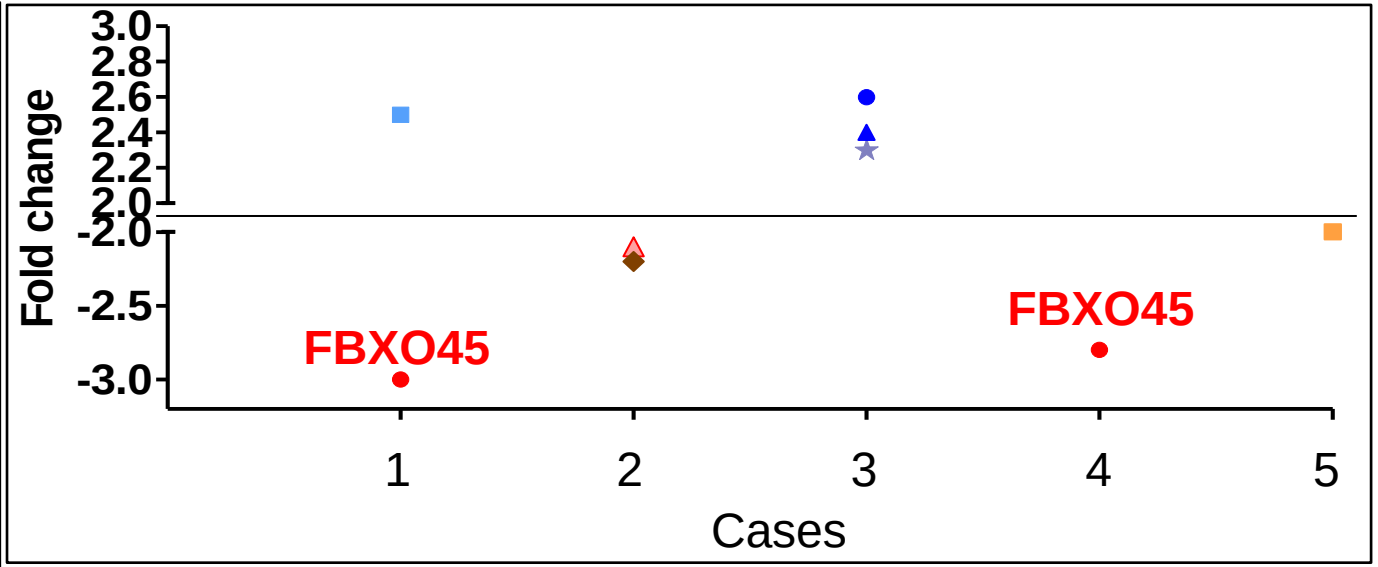
Aggressive

Proteomic analysis of FL transformation

Post-translational events

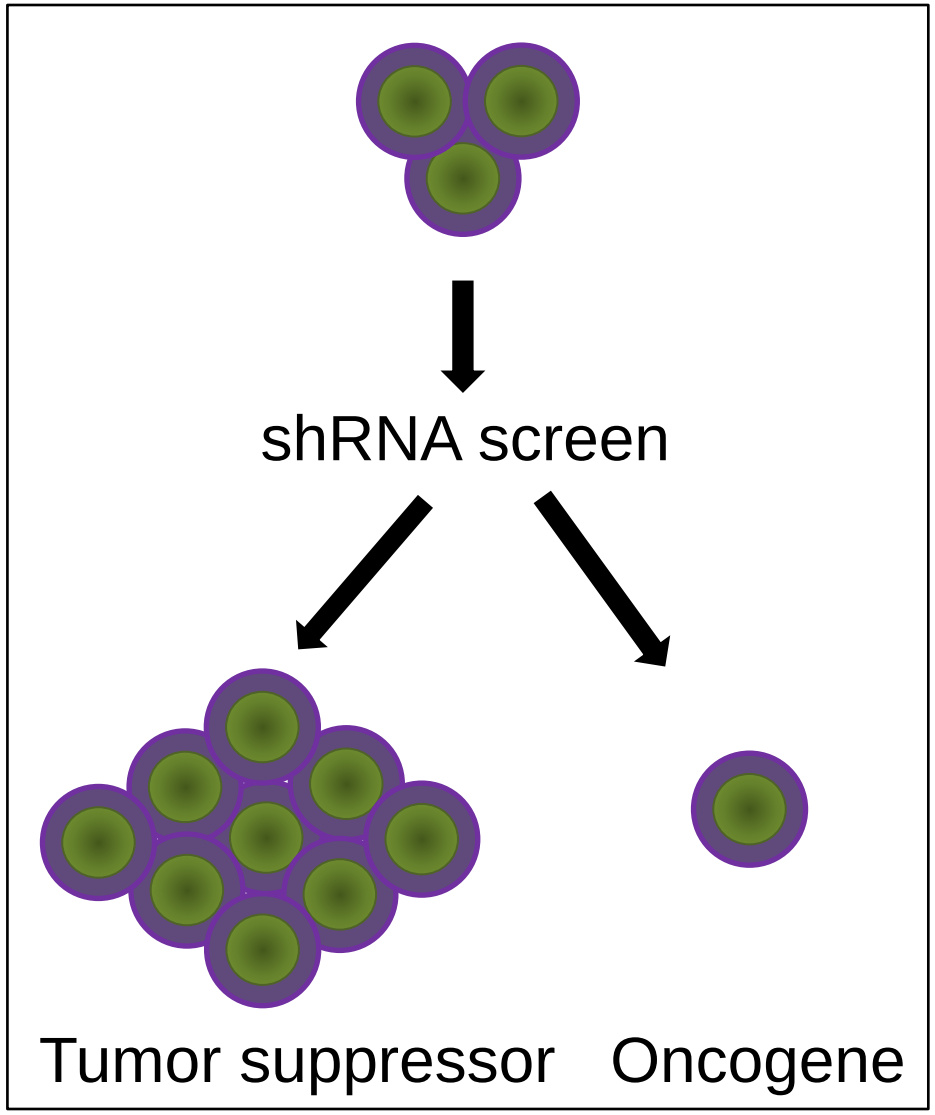
Quantitative mass spectrometry-based proteomics identifies recurrent downregulation of FBXO45 in transformed DLBCL

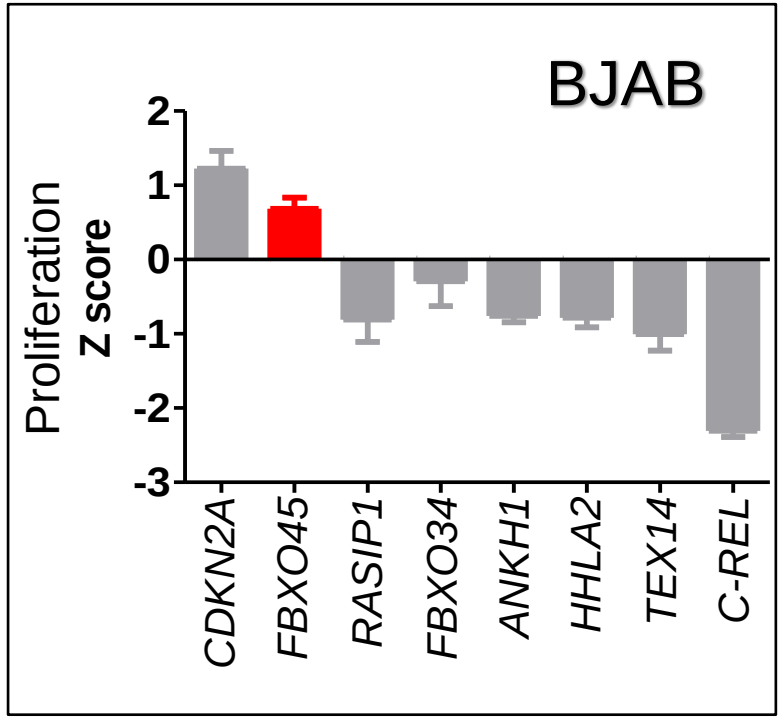


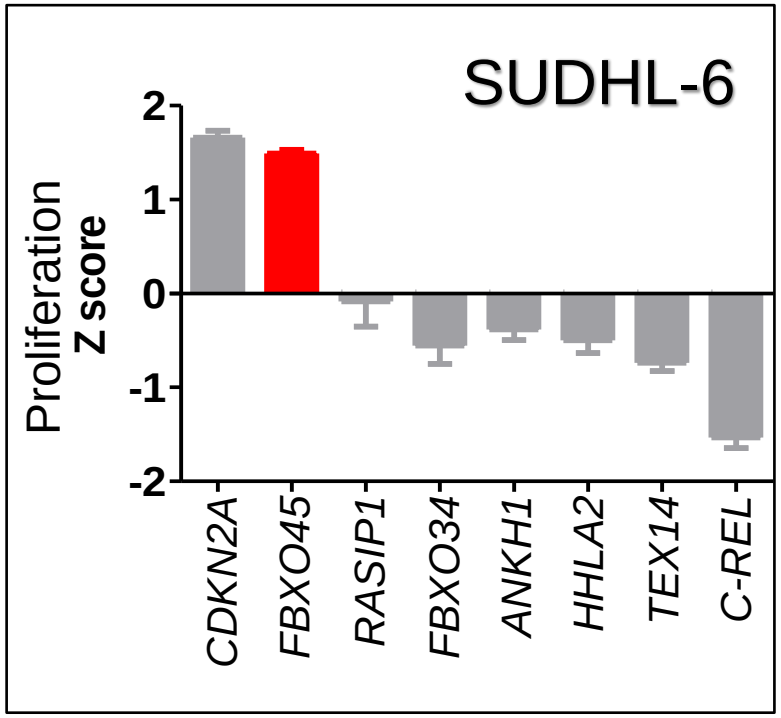


- TEX14
- ANKH1
- RASIP1
- C-REL
- FBXO34
- HHLA2
- **FBXO45**
- **FBXO45**
- CDKN2A

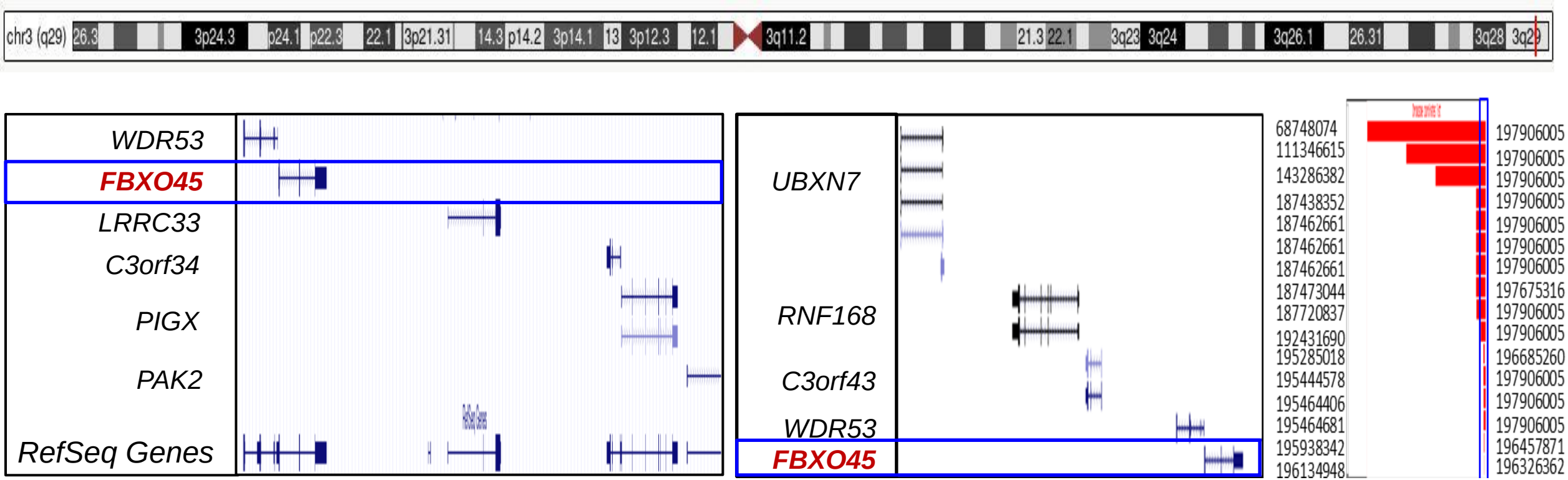
Functional genomic *RNAi* screen reveals *FBXO45* depletion promotes lymphoma growth







Genomic losses targeting 3q29 are recurrent in DLBCL



Deffenbacher KE, et. al. Blood 2012

Schmitz,R. et. al. NEJM 2018

- **The functionally-relevant genes that are targeted at this locus are unknown**
- **The minimal deletional region recurrently targets *FBXO45* in independent studies**

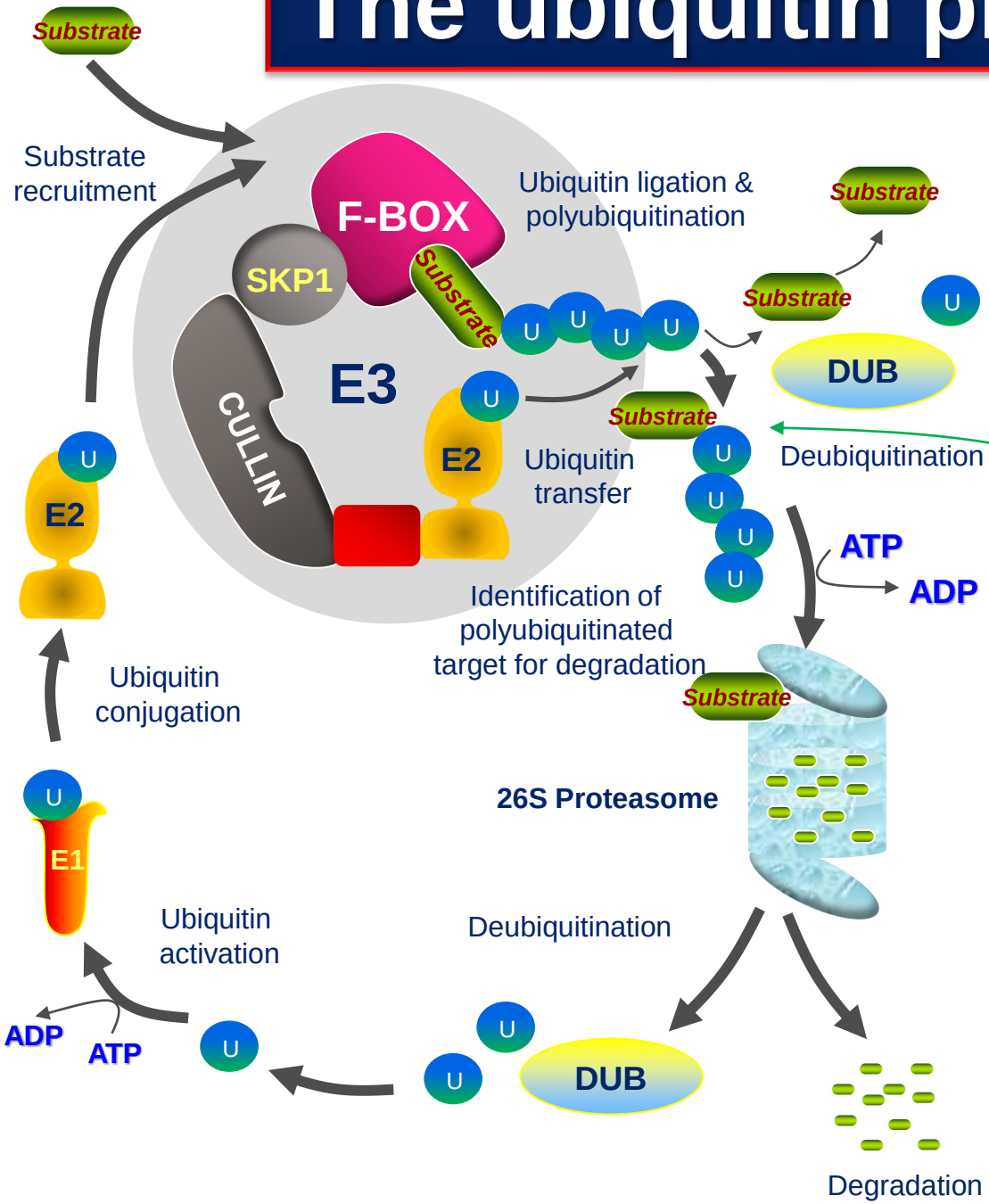
Hypothesis

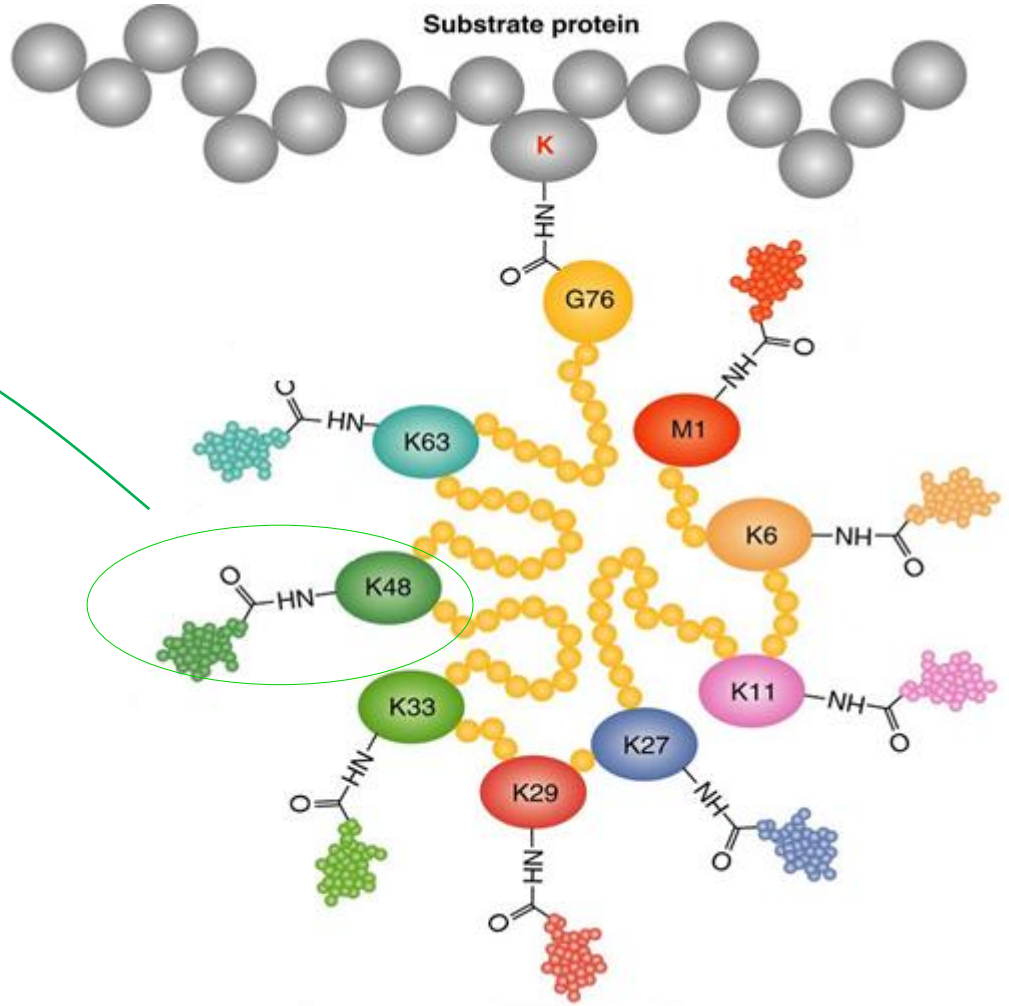
FBXO45 is a novel tumor suppressor in B-cell lymphoma

Memorial Sloan Kettering Cancer Center

- FBXO45 protein is **downregulated** in transformed follicular lymphoma
- FBXO45 depletion by **RNAi promotes cell proliferation** and **colony formation**
- Recurrent deletions target FBXO45 in pediatric DLBCL
- ***Ectopic overexpression of FBXO45 inhibits B cell growth***

The ubiquitin proteasome system



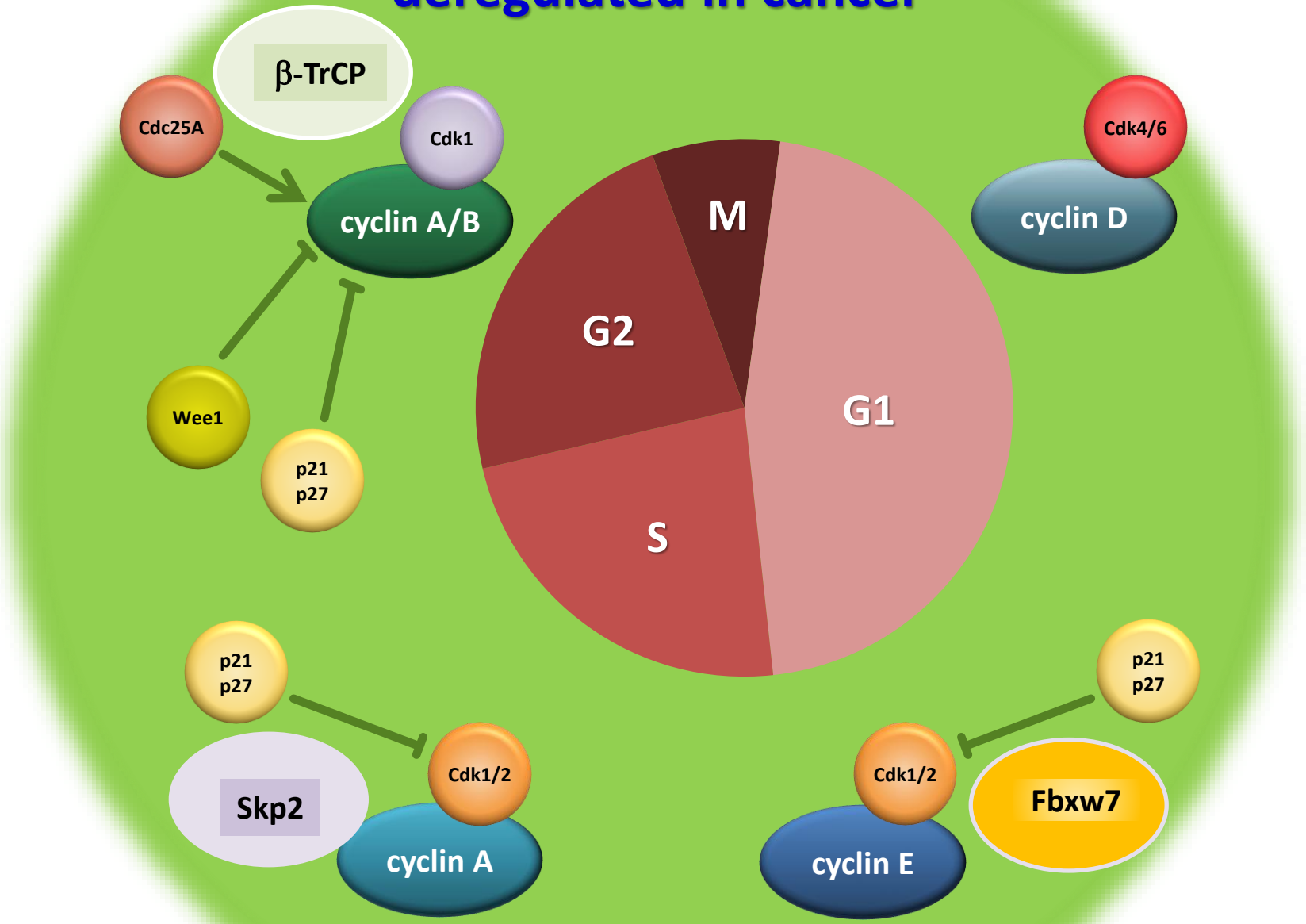


E3 Ubiquitin Ligases Regulate Protein Degradation

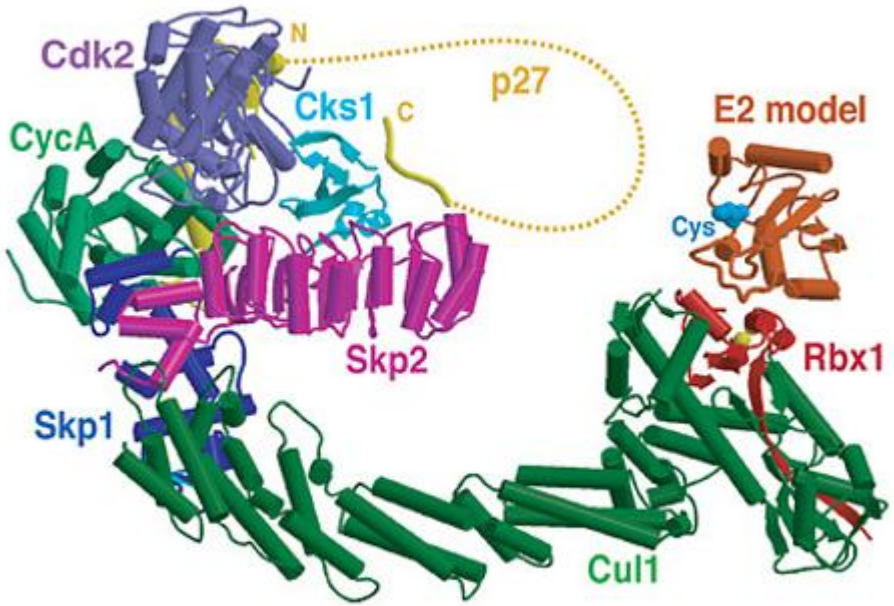
Advantages of regulated proteolysis

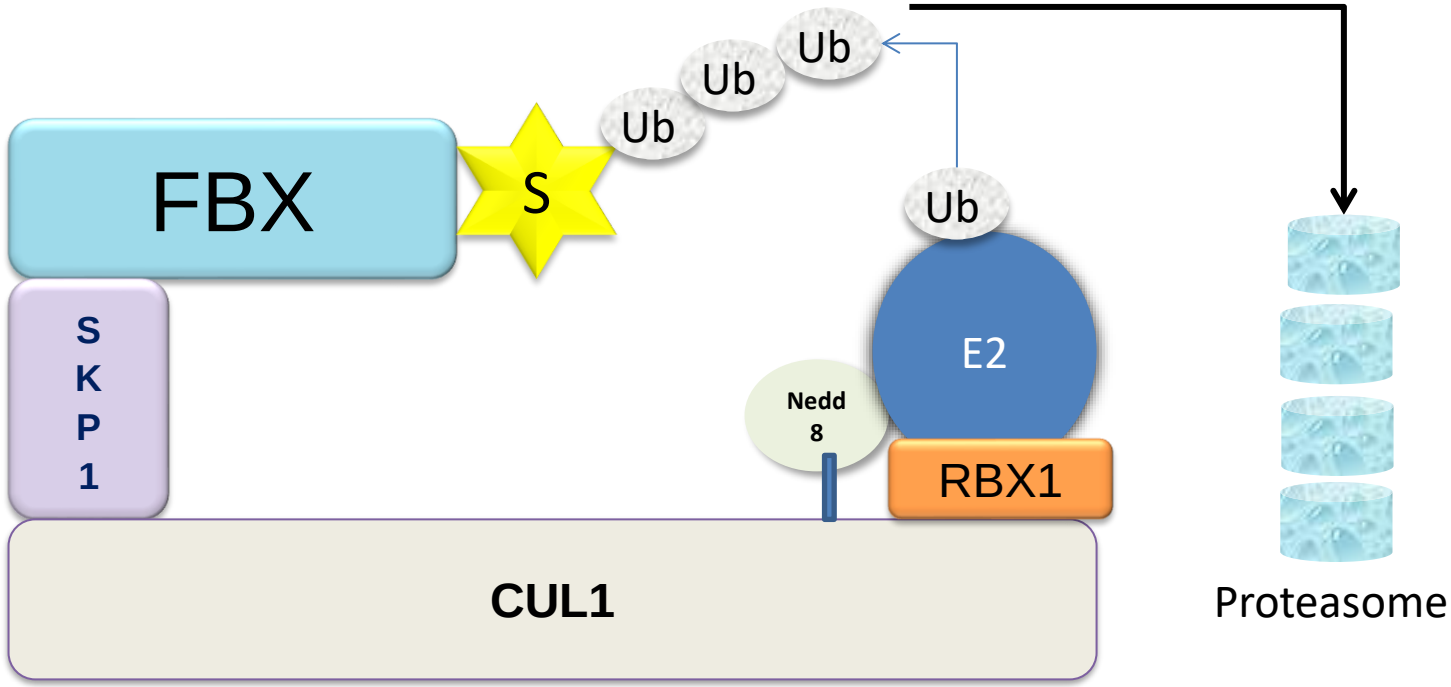
- Unidirectional
- Rapid
- Fine adjustment of levels of regulatory proteins
- Localized: in specific subcellular compartments
- Highly specific

Ubiquitin ligases and their substrates are frequently deregulated in cancer



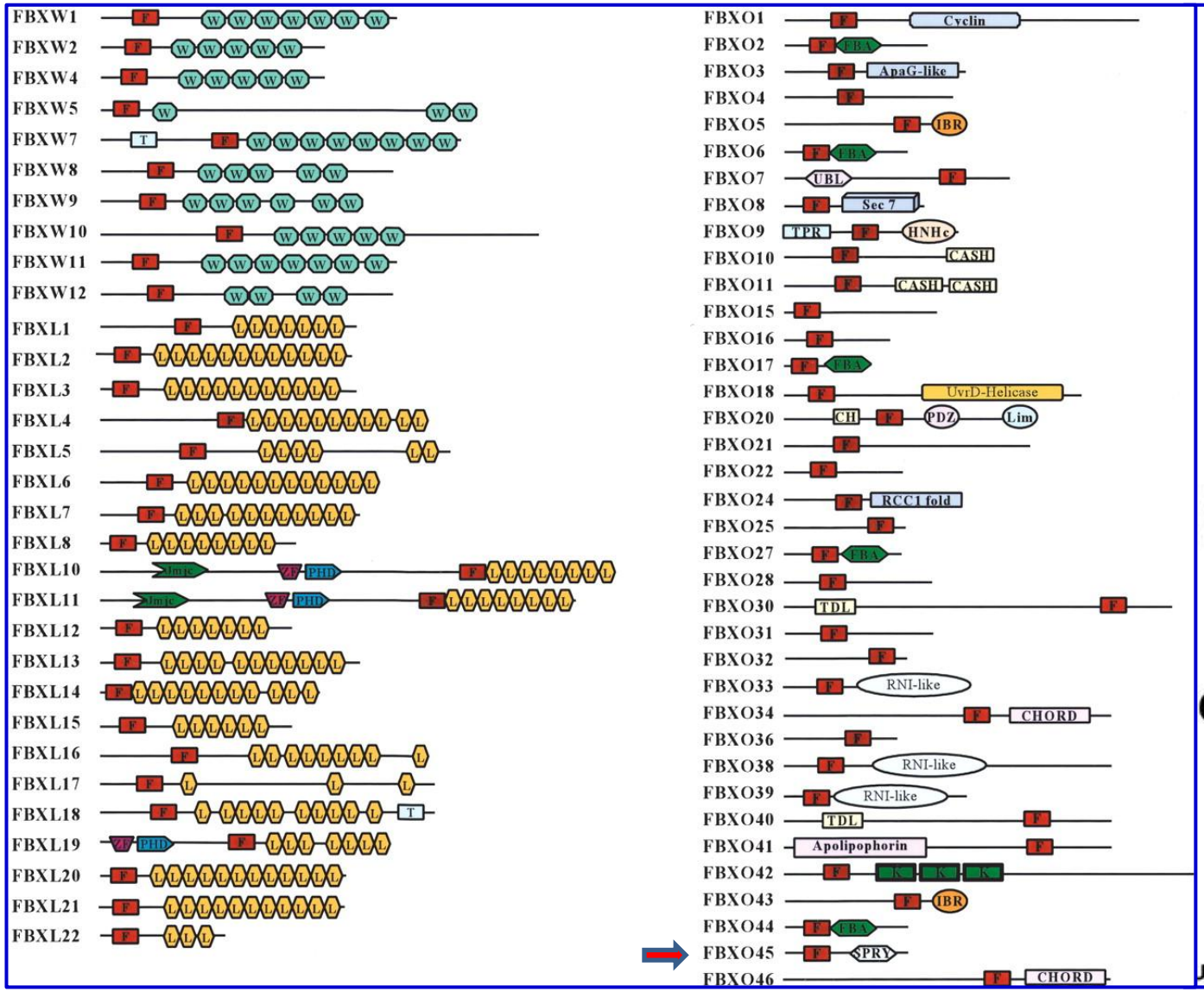
Prototypical SCF E3 Ligase



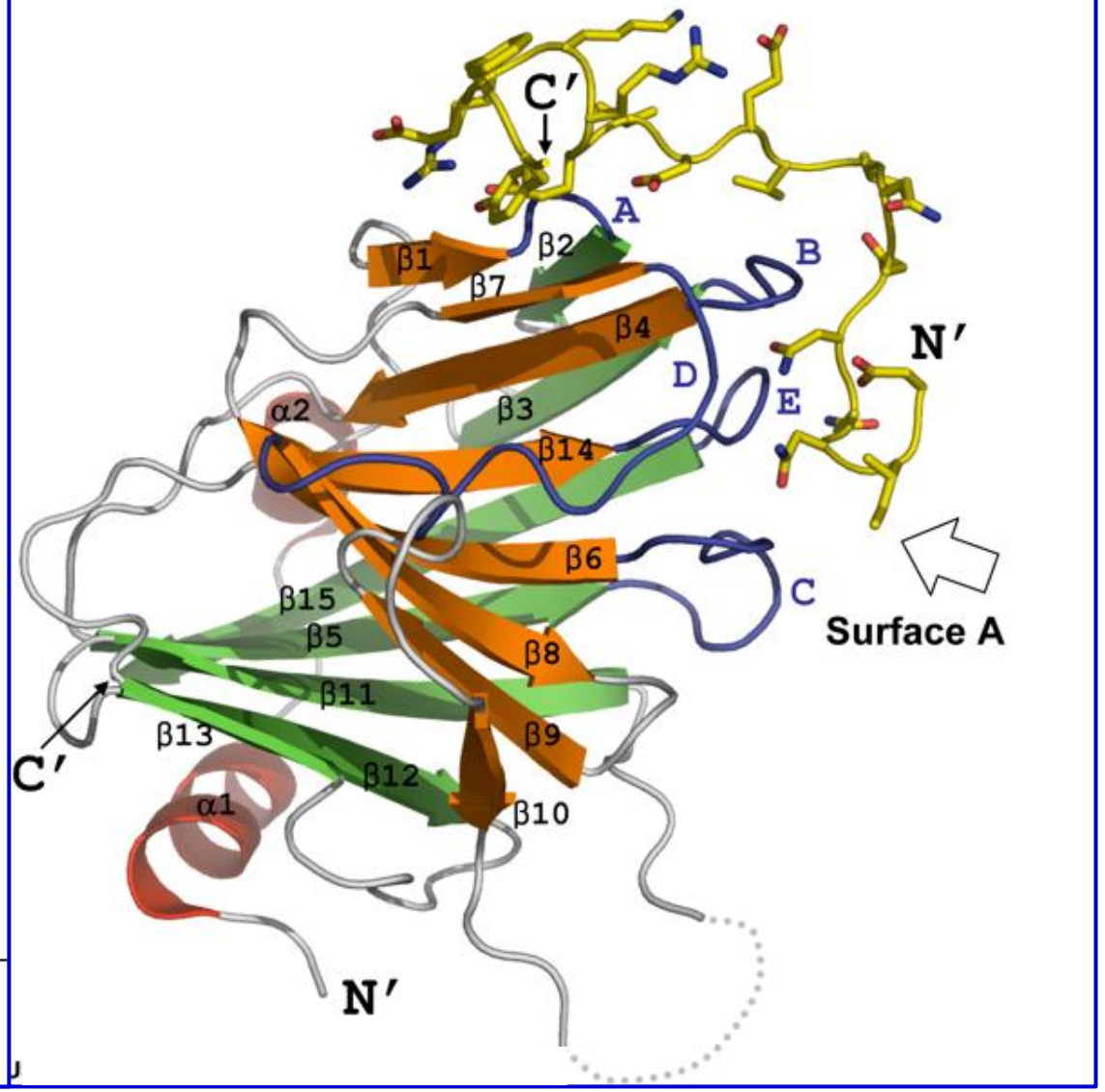


Zhang N et.al., 2002

FBXO45: Only F-box protein known to contain a SPRY domain

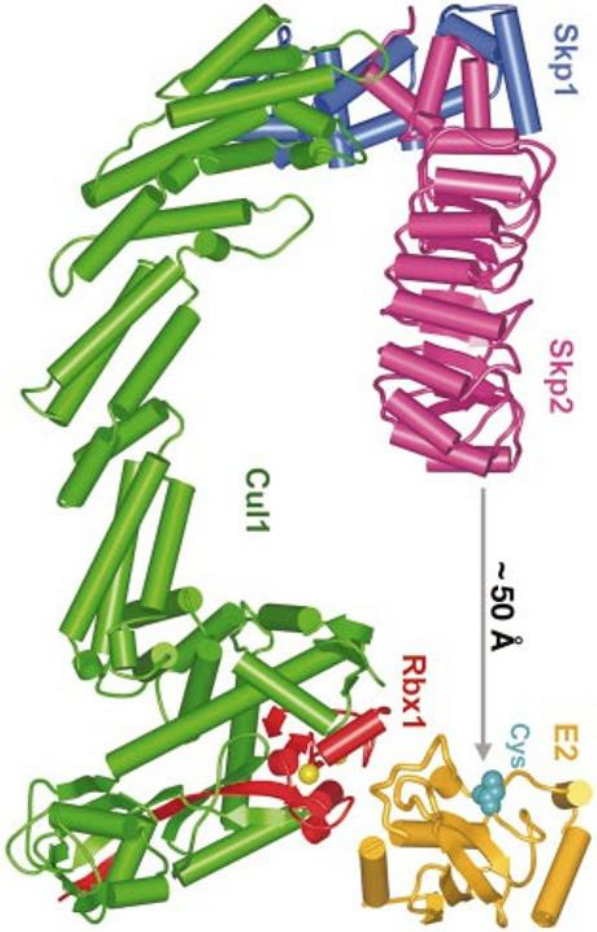


Jin J et al. Genes Dev. 2004;18:2573-2580



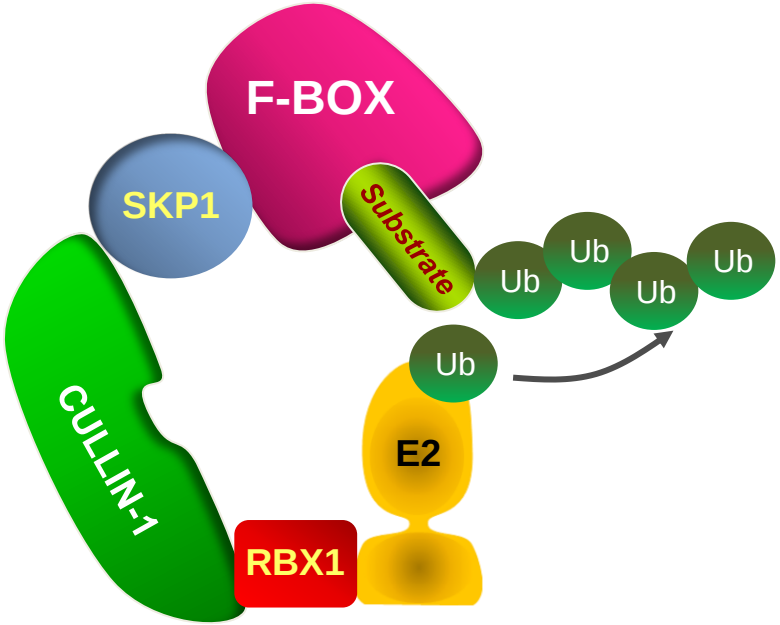
Woo J –S et al. Mol Cell 2006

FBXO45 is an atypical SCF E3 ligase

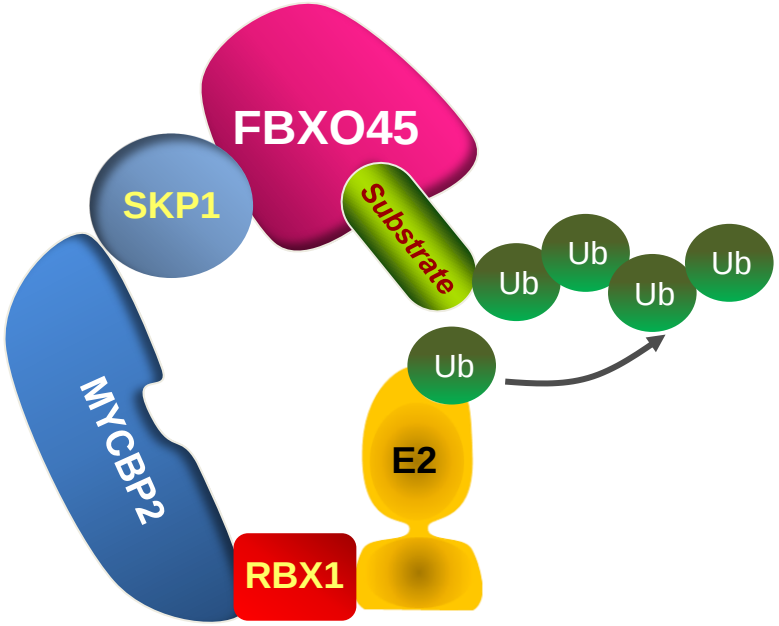


Hao B, Zheng N, Schulman BA, Wu G, Miller JJ, Pagano M, Pavletich NP .Mol Cell. 2005

Typical

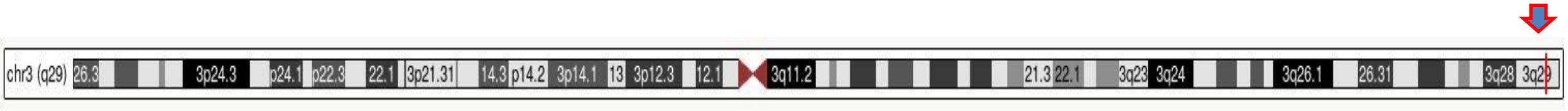


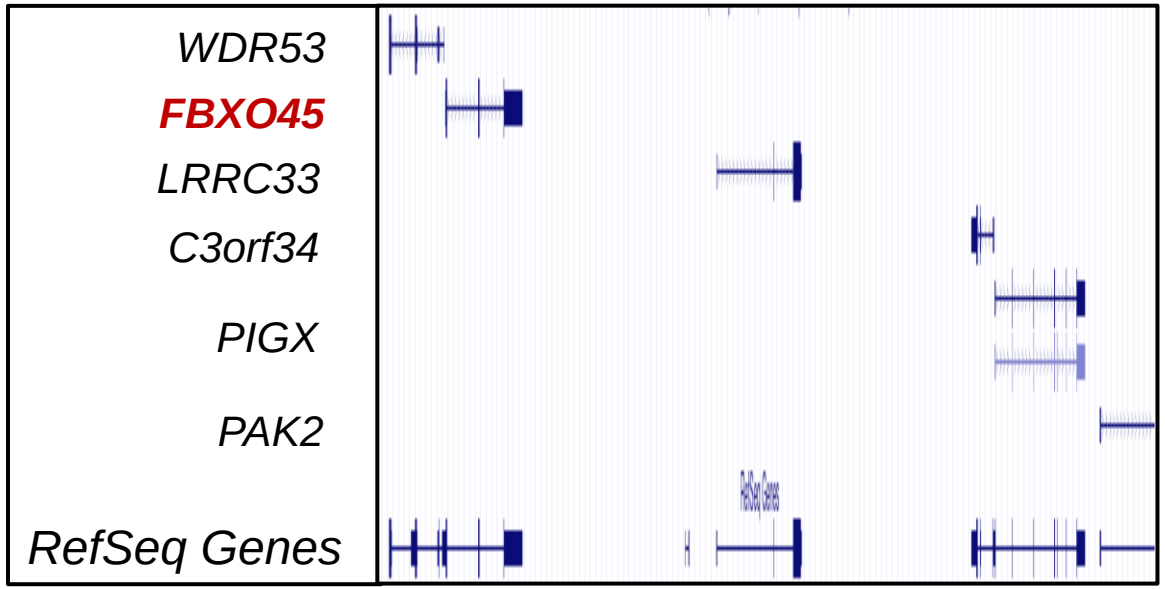
Atypical



FBXO45 is a substrate recruitment unit that interacts with MYCBP2 and SKP1 to form a non-canonical SCF E3 ligase complex.

Human FBXO45 is on 3q29

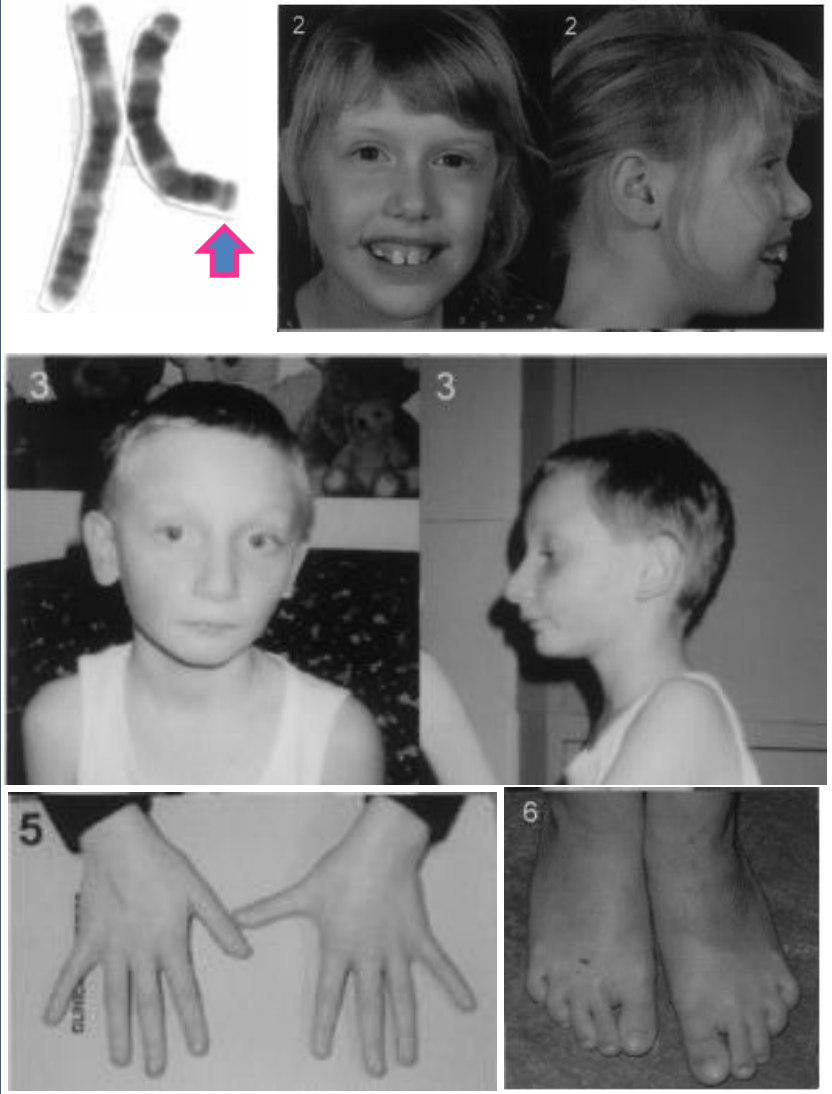




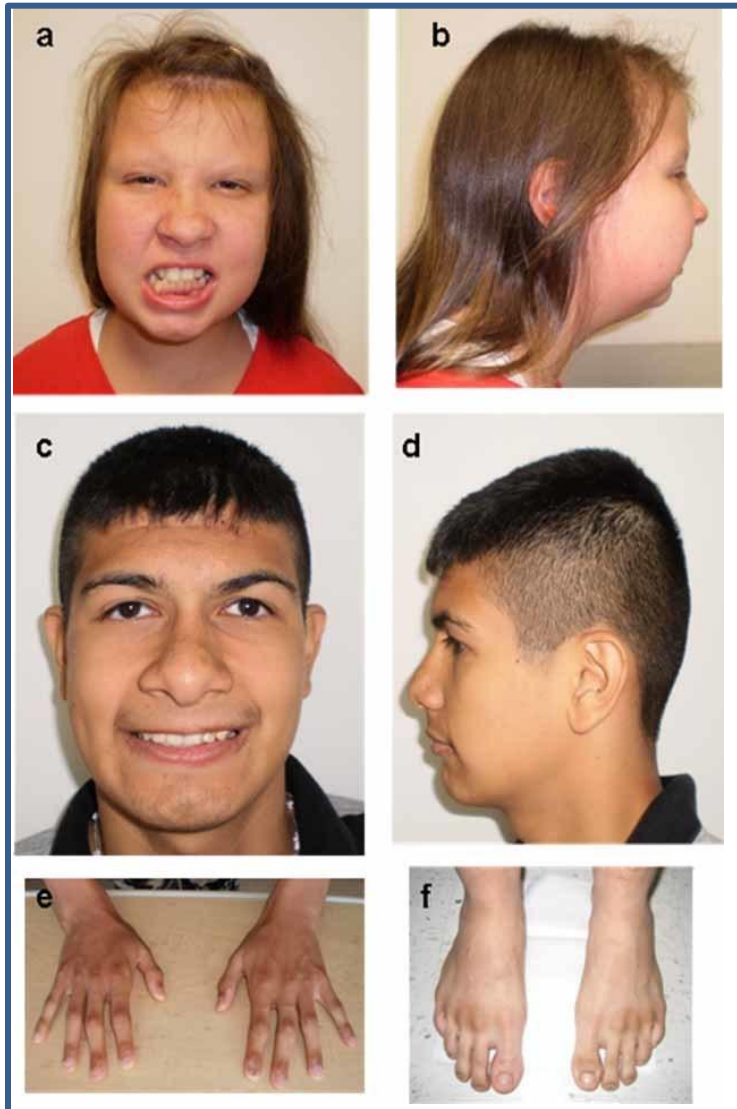
- **In review**

• 2005

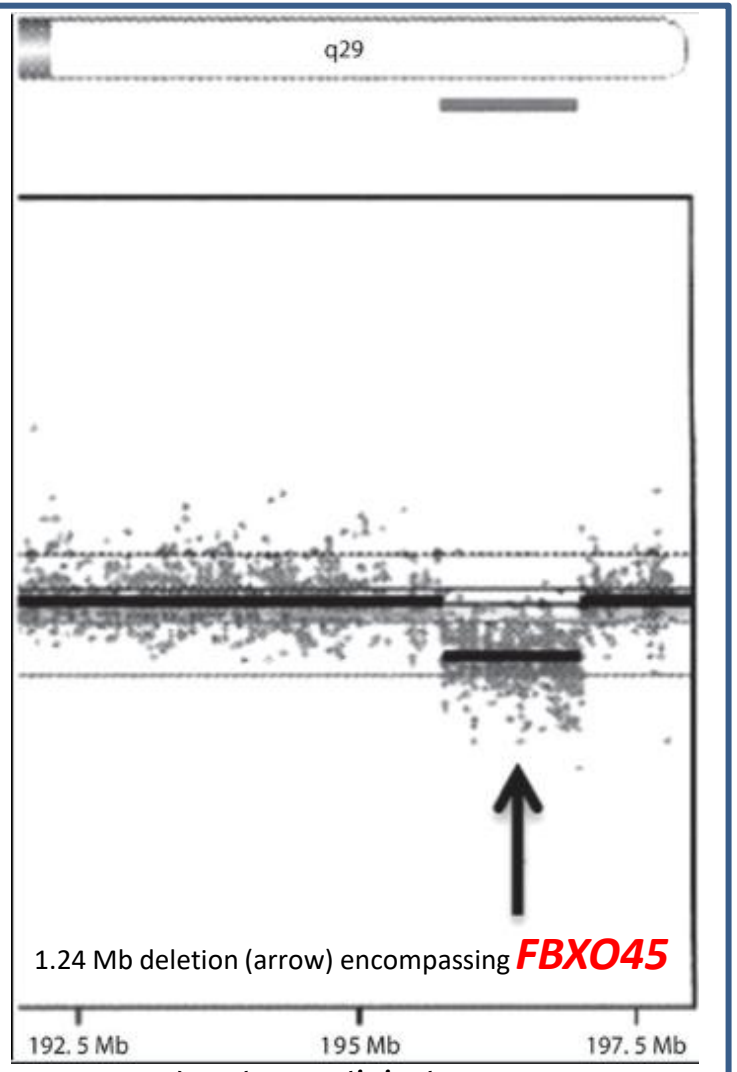
3q29 microdeletion syndrome



Willatt L.et al, Am J. Hum Genet 2005

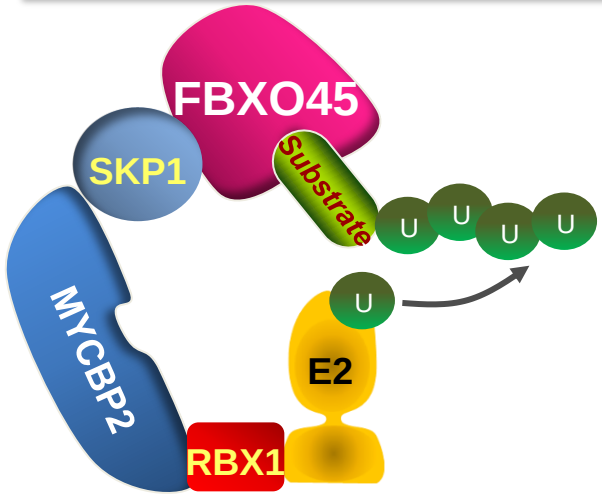


Quintero-Rivera, F. et al. Am J Med Genet 2010

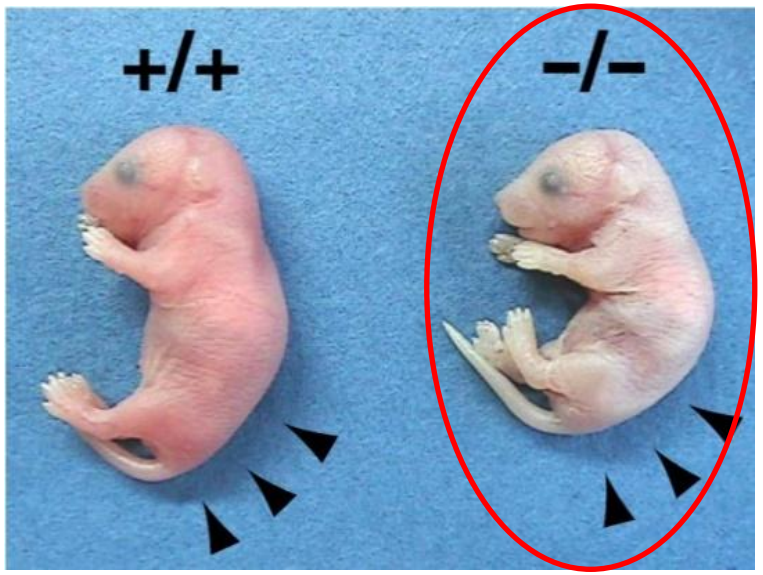


Cox D. and Butler M. Clinical Dysmorphology24(3):89-94, July 2015.

FBXO45 E3 ligase



- One of only six F-box proteins that are conserved in metazoans.
- Homologues in *Drosophila and C. elegans* play critical roles in synaptic development.



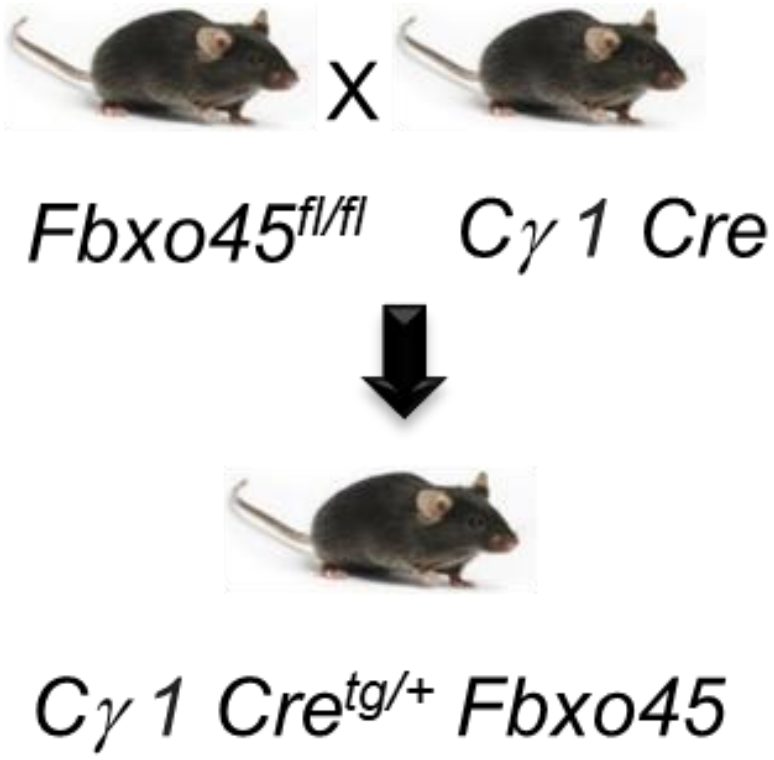
- Constitutional *Fbxo45*$^{-/-}$ knockout results in perinatal lethality.
- The functional role of FBXO45 in the hematopoietic system and cancer is not established.

Saiga, T. et.al., MCB 2009;13:3529-43

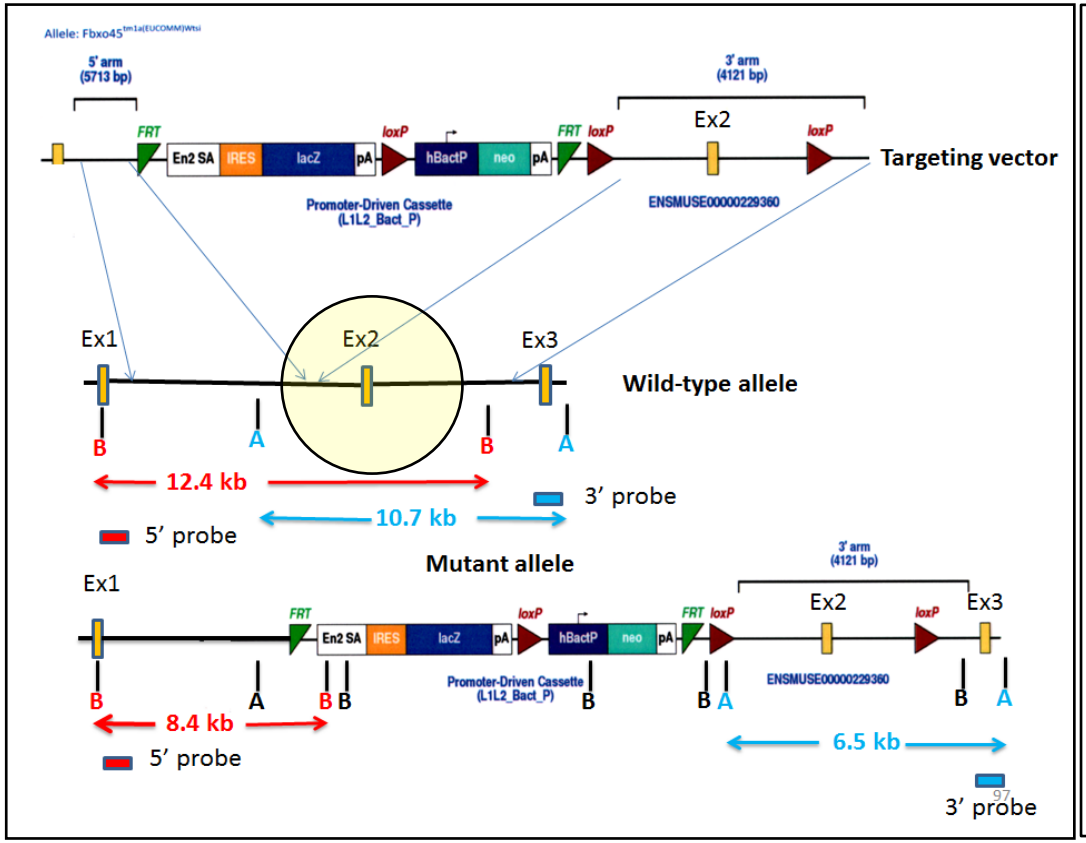
Outline

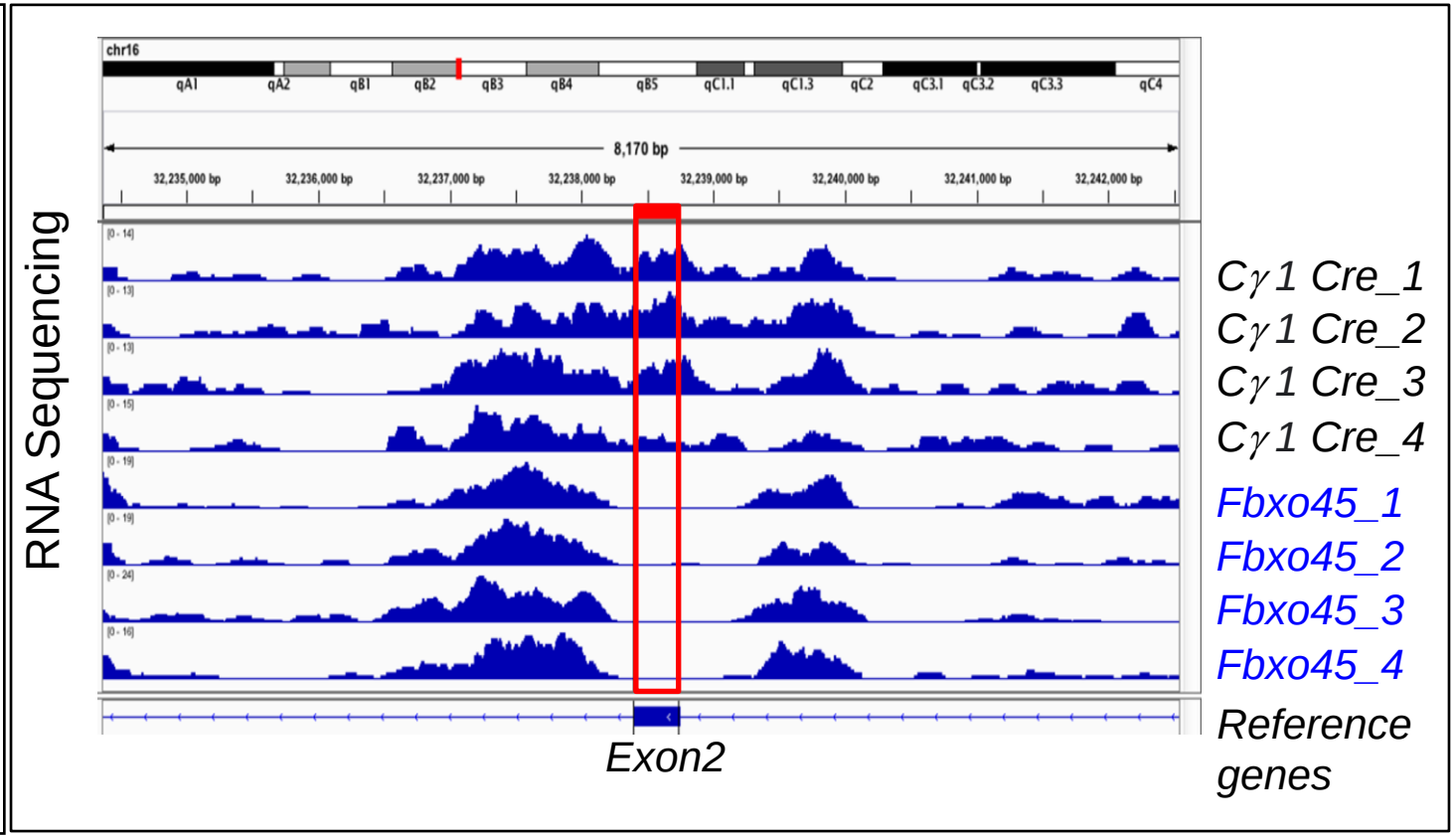
- Background
- Hypothesis
- **Experimental Approach**
- Conclusions
- Future Directions

Conditional *Fbxo45* knockout in germinal center B cells



Generation of conditional *Fbxo45* KO mice



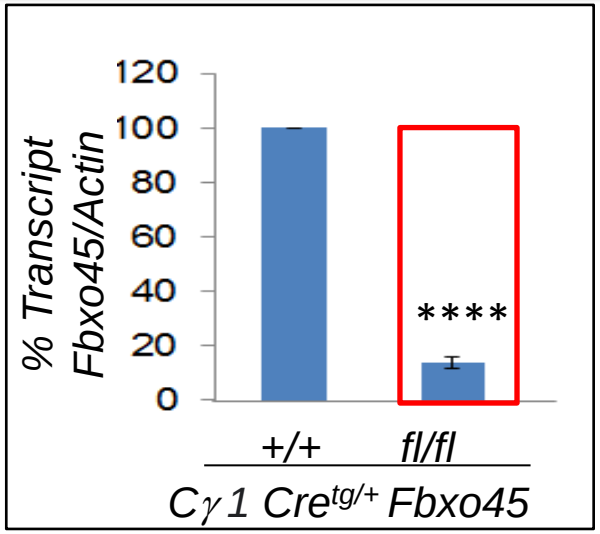


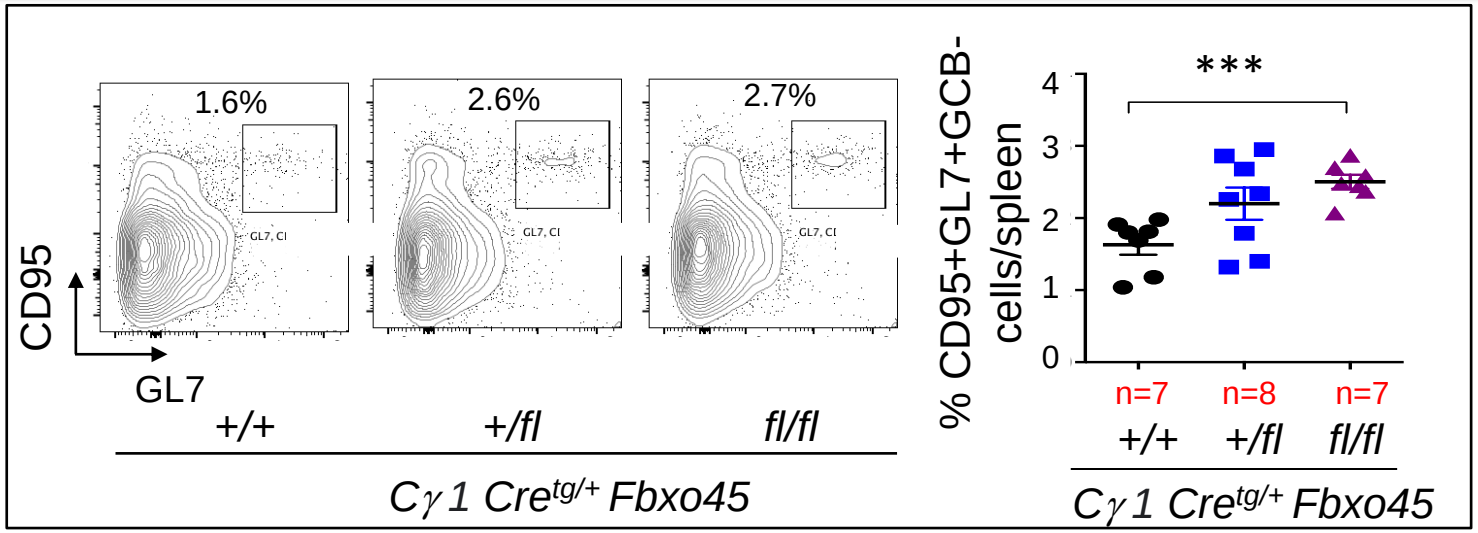
1. **Understand the physiological function of FBXO45 in germinal center development**
2. **Investigate the role of FBXO45 in B-cell lymphoma**

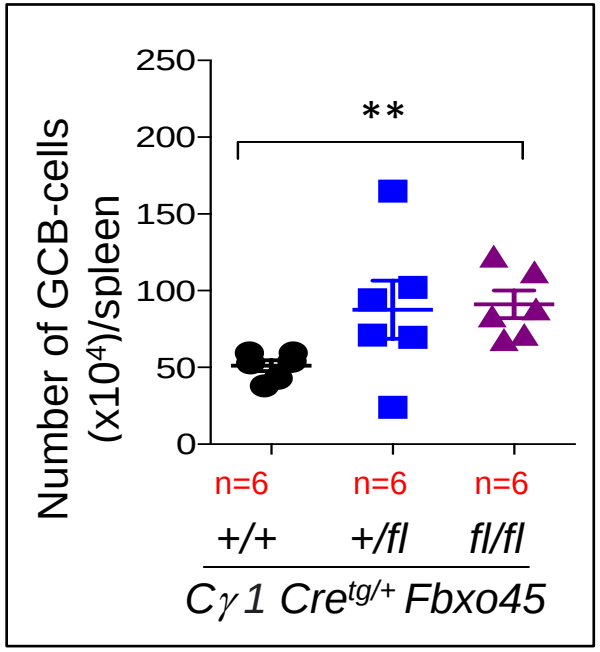
Fbxo45 deletion results in abnormal germinal center formation

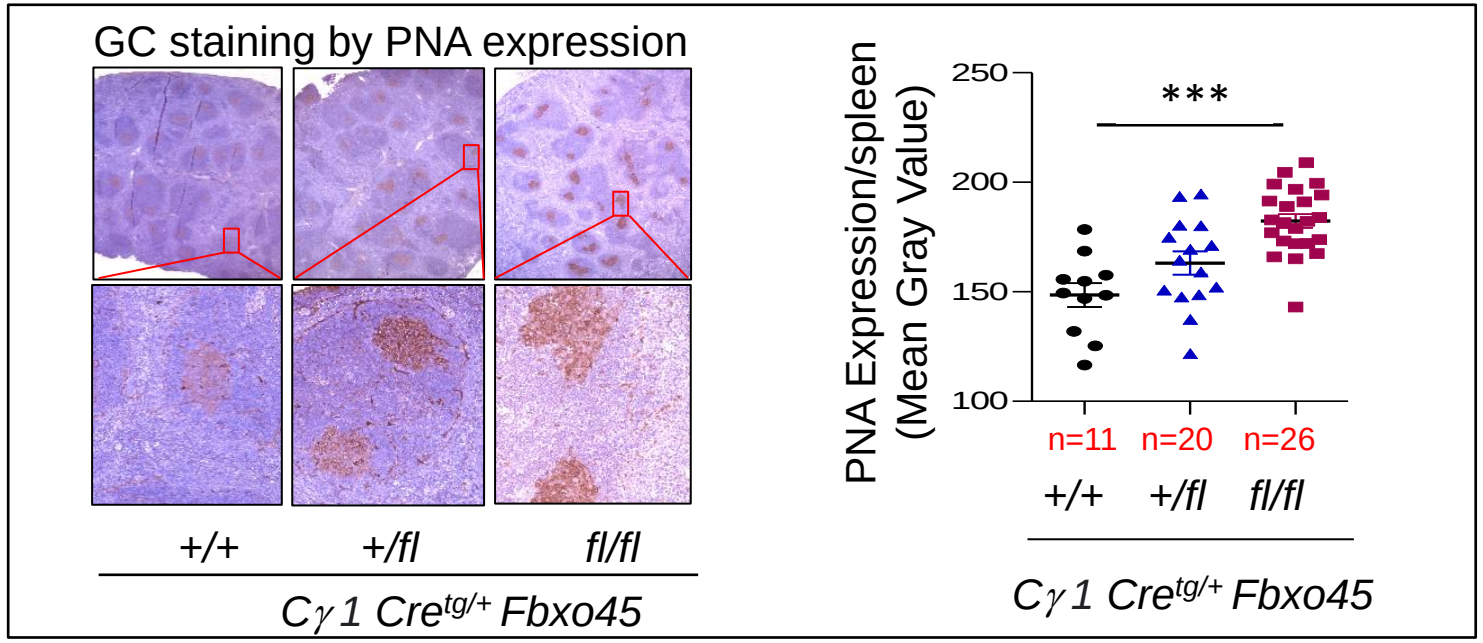
$Fbxo45^{fl/fl}$ X $C\gamma1\ Cre$

$C\gamma1\ Cre^{tg/+}\ Fbxo45$



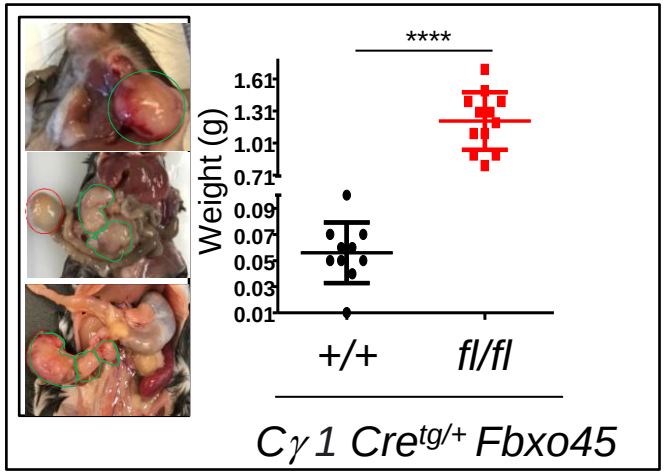


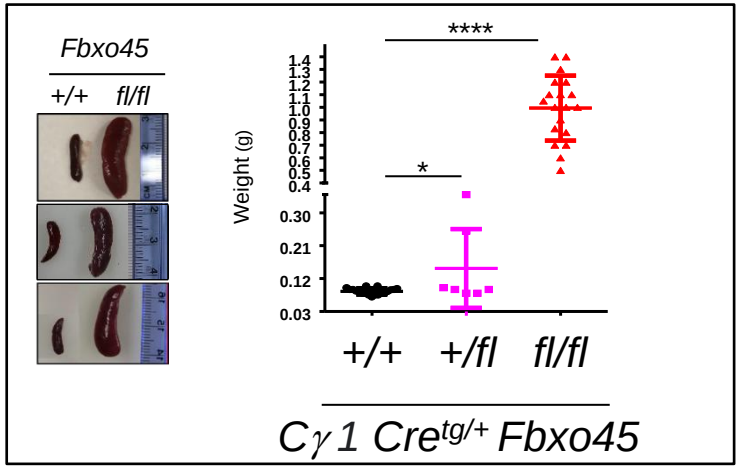


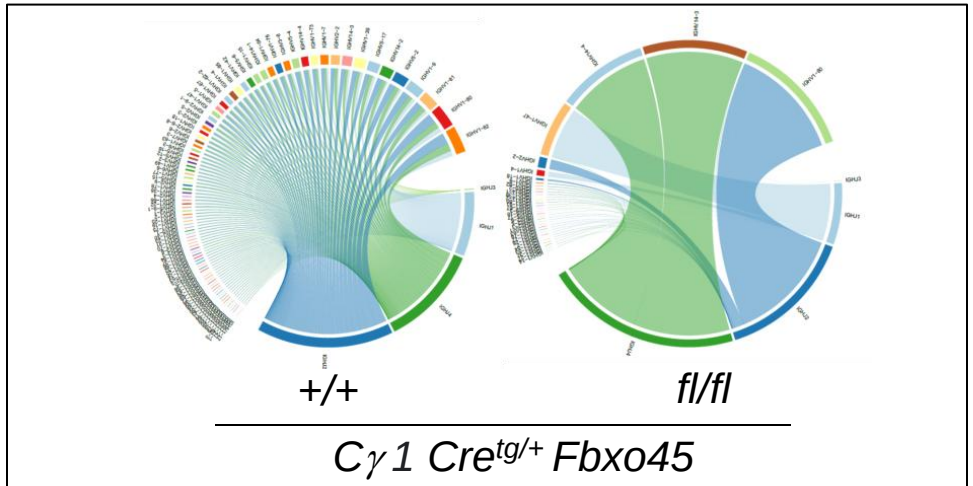


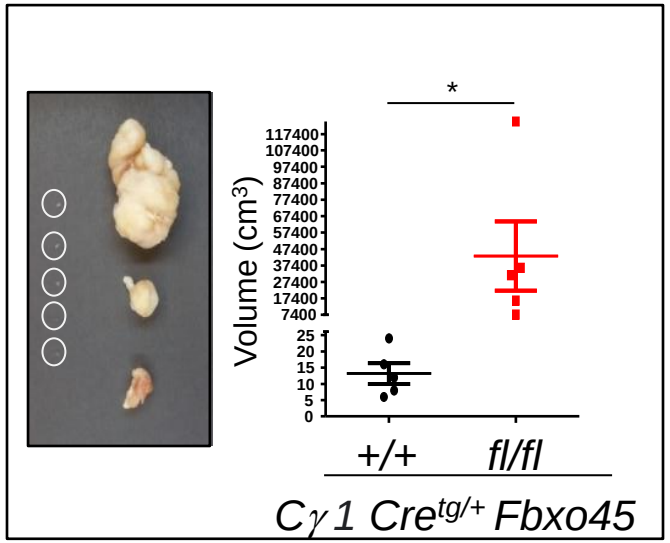
Targeted genetic ablation of *Fbxo45* results in increased germinal center number and size

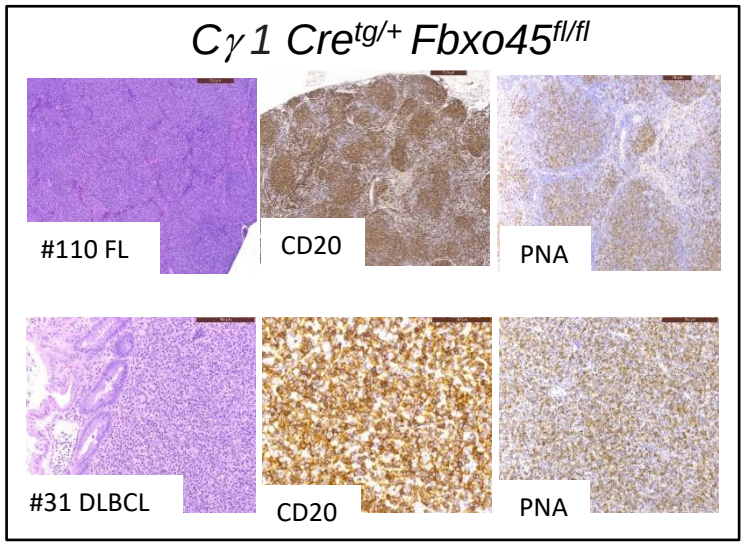
In vivo conditional targeting of *Fbxo45* in GCB-cells results in spontaneous B-cell lymphomagenesis (FL and DLBCL)

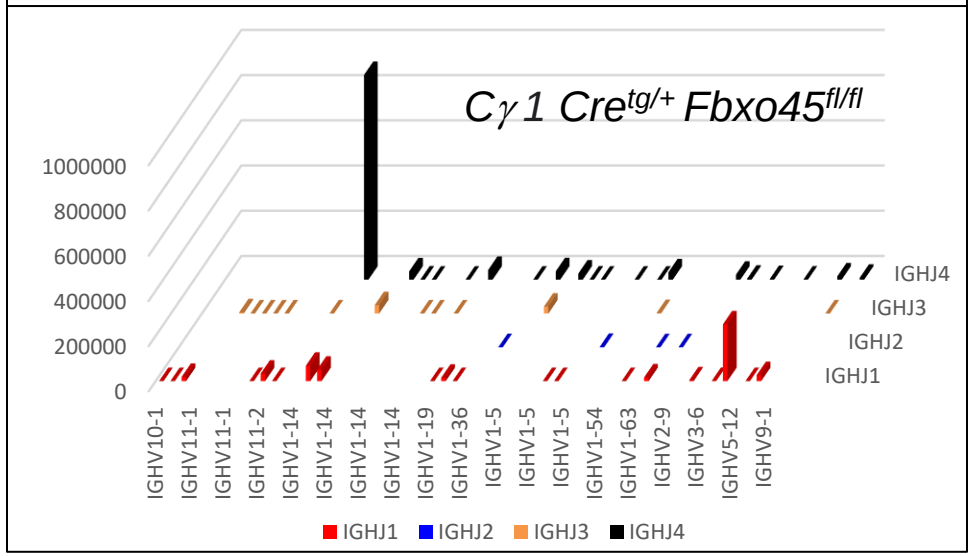




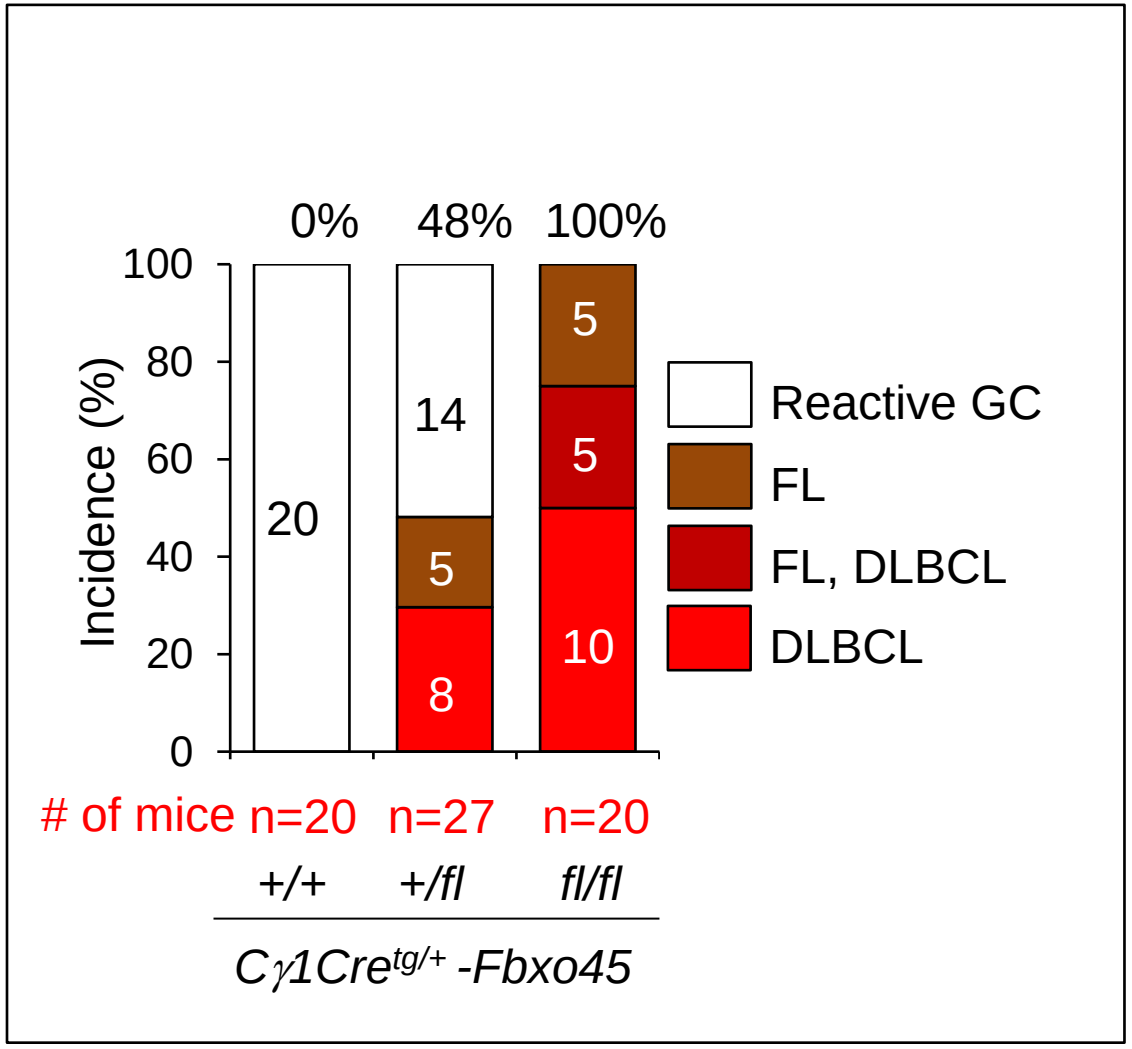


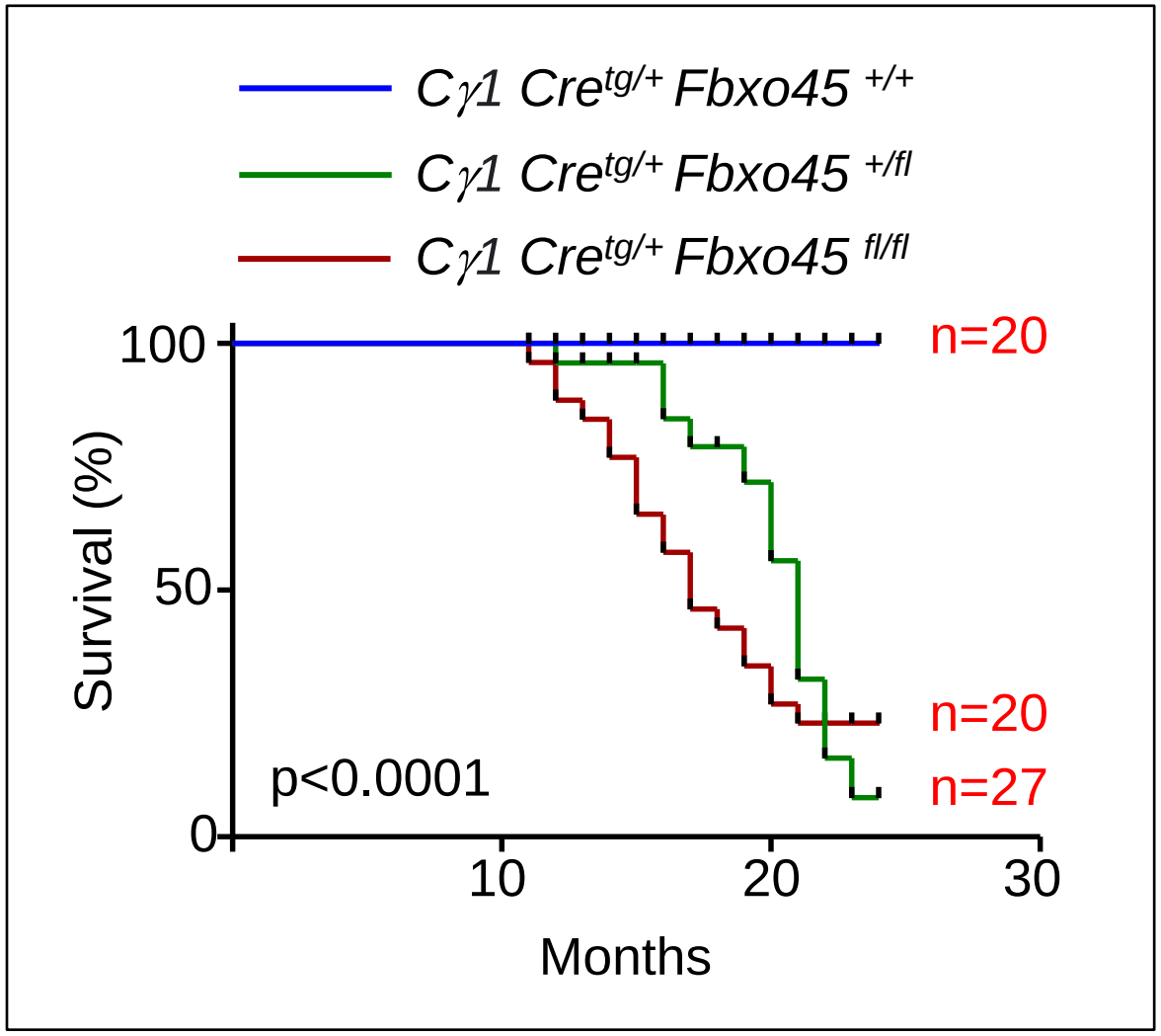






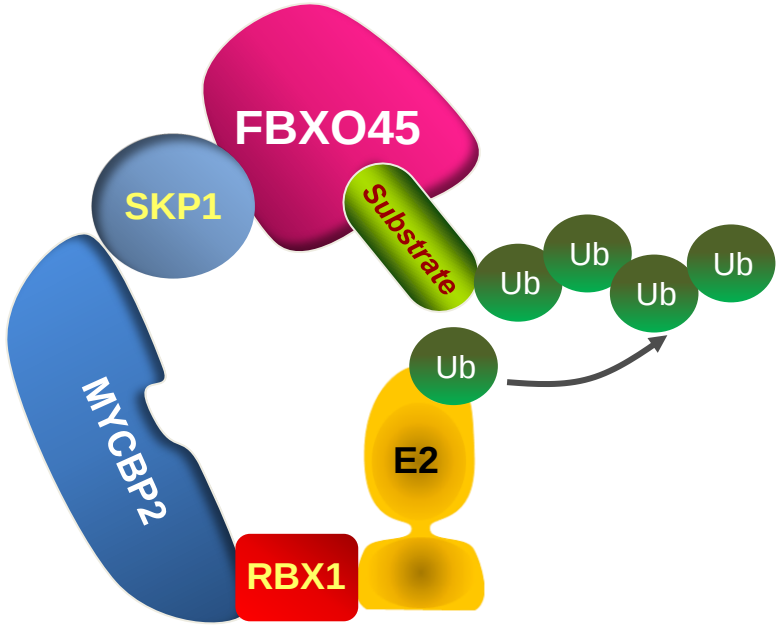
In vivo conditional targeting of *Fbxo45* in GCB cells results in spontaneous B cell lymphomagenesis





Identification of FBXO45 substrate

Integrated functional proteomic strategy

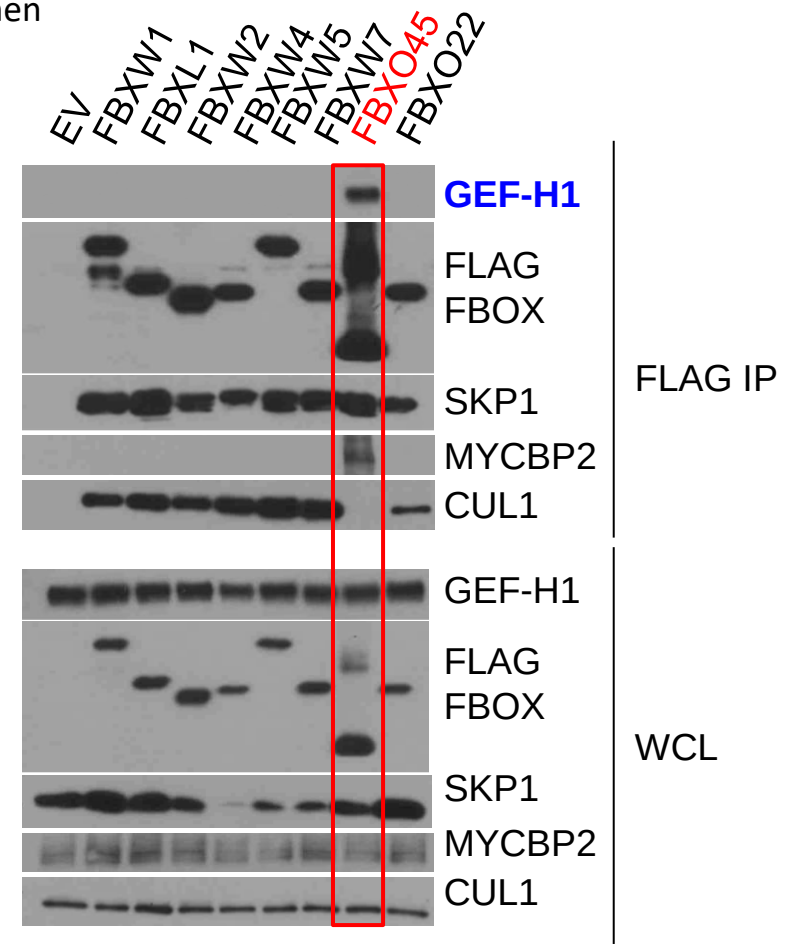


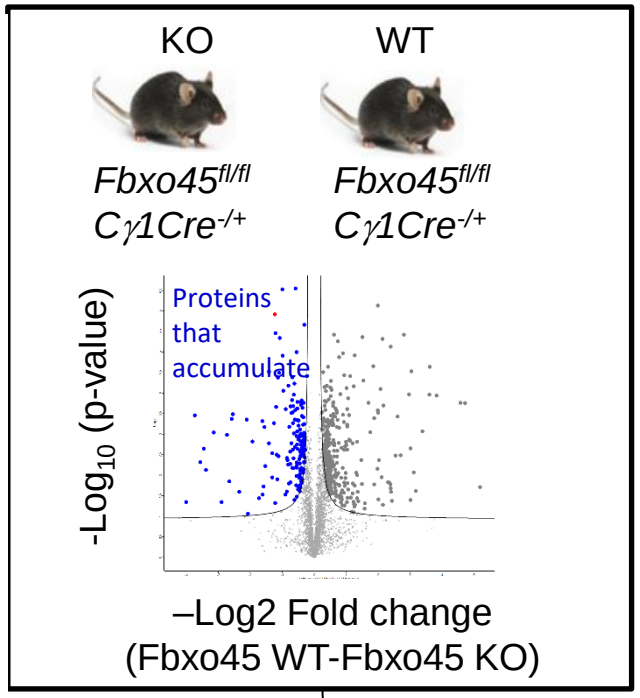
Differential proteomic analysis

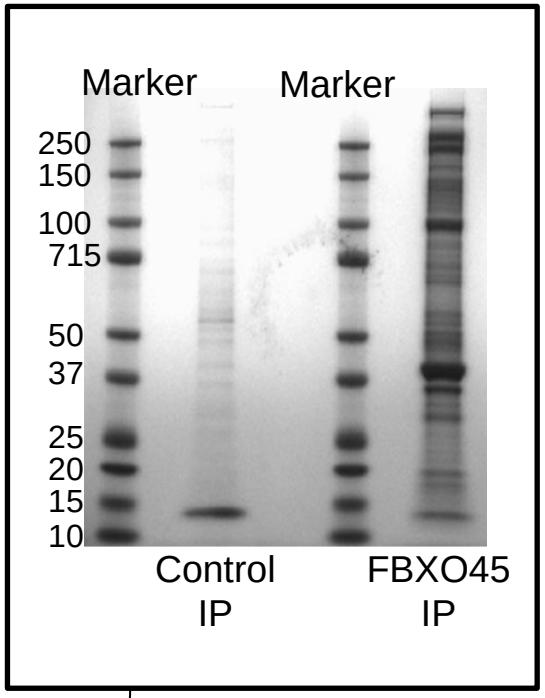
FBXO45 IP-MS

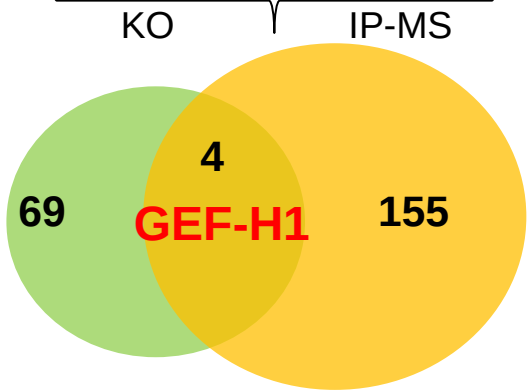

Xiaofei Chen

Identification of candidate substrate of FBXO45





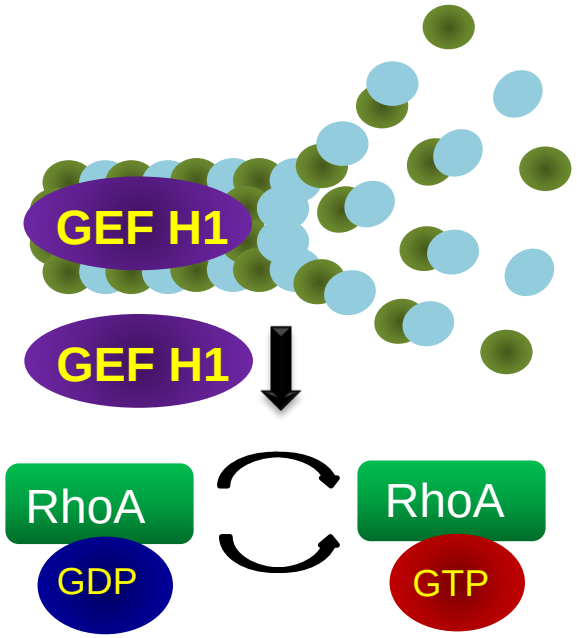




GEF-H1 is an exclusive interactor of FBXO45

Identification of GEF-H1 as the candidate substrate of FBXO45

GEF-H1
(RhoA specific guanine nucleotide exchange factor)



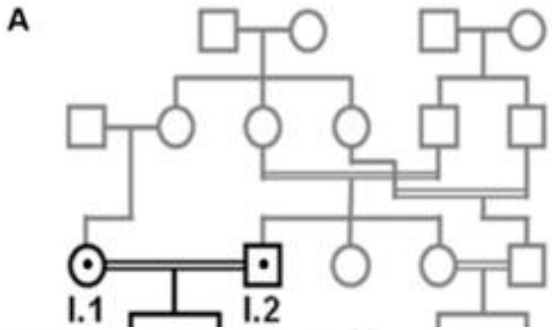
- GEF-H1 is a Dbl family RhoA regulatory guanine nucleotide exchange factor that **activates Rho** guanosine triphosphatases (GTPases).
- GEF-H1 is sequestered on microtubules and regulated by the polymerization state of microtubule networks.
- GEF-H1 is implicated in numerous cellular processes including cell motility and polarization, cell cycle regulation, activation of **MAPK signaling** and **cancer**.

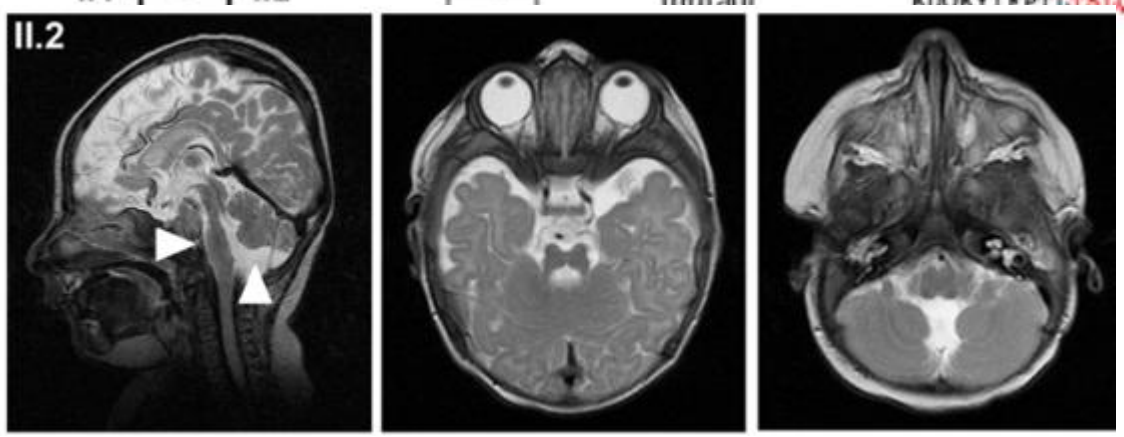
PLOS GENETICS

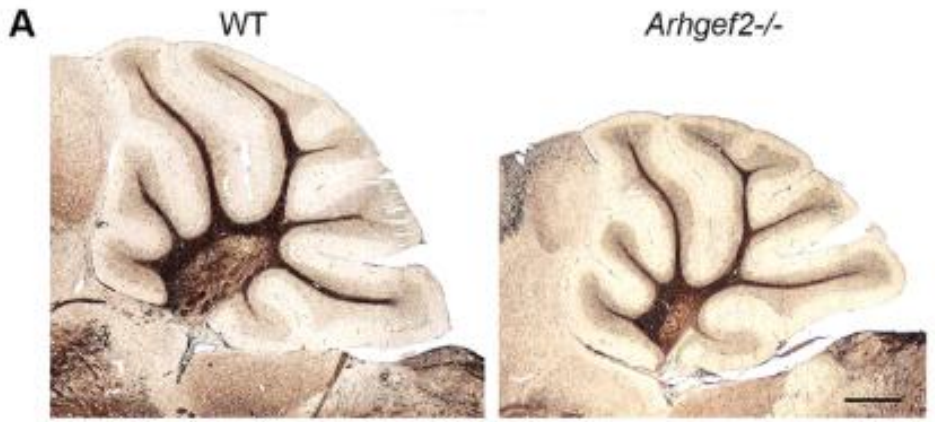
RESEARCH ARTICLE

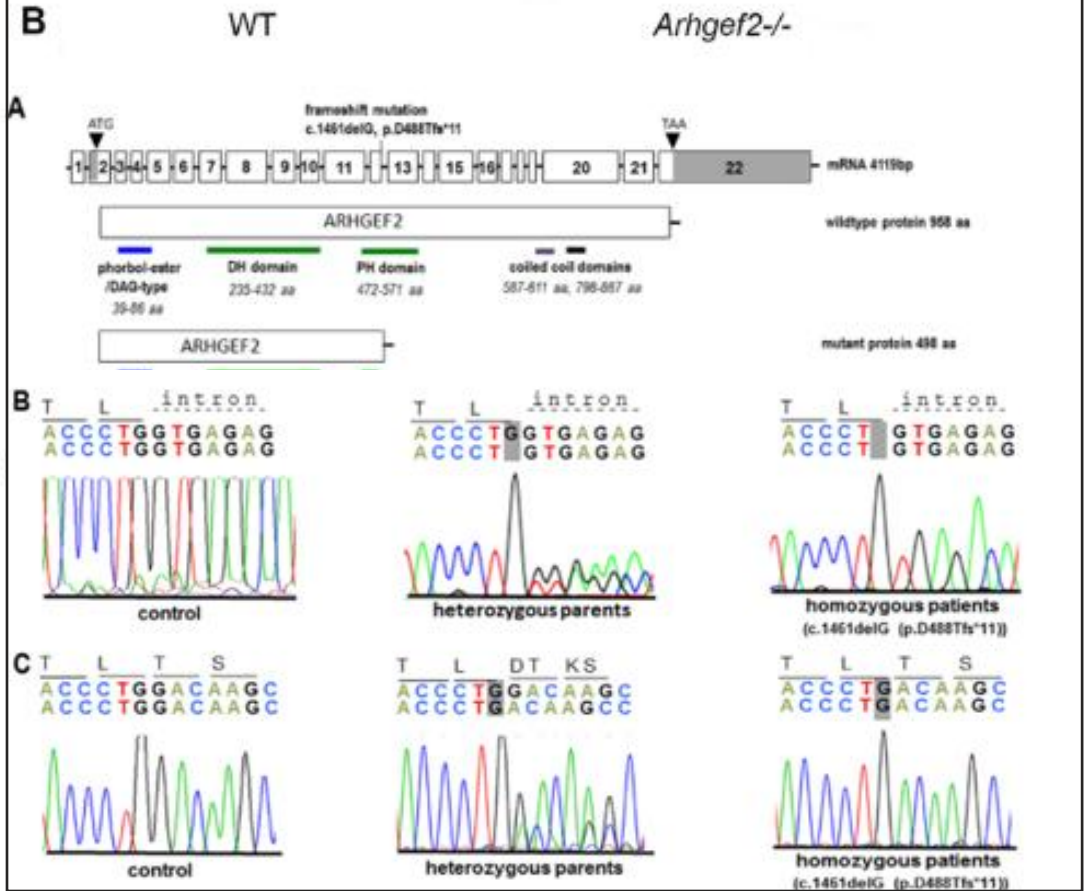
Homozygous *ARHGEF2* mutation causes intellectual disability and midbrain-hindbrain malformation

Ethiraj Ravindran[1,2,3], Hao Hu[4,5], Scott A. Yuzwa[6,7], Luis R. Hernandez-Miranda[8], Nadine Kraemer[1,2,3], Olaf Ninnemann[1], Luciana Musante[4], Eugen Boltshauser[9], Detlev Schindler[10], Angela Hübner[11], Hans-Christian Reinecker[12], Hans-Hilger Ropers[4], Carmen Birchmeier[8], Freda D. Miller[6,7], Thomas F. Wienker[4], Christoph Hübner[2], Angela M. Kaindl[1,2,3]*

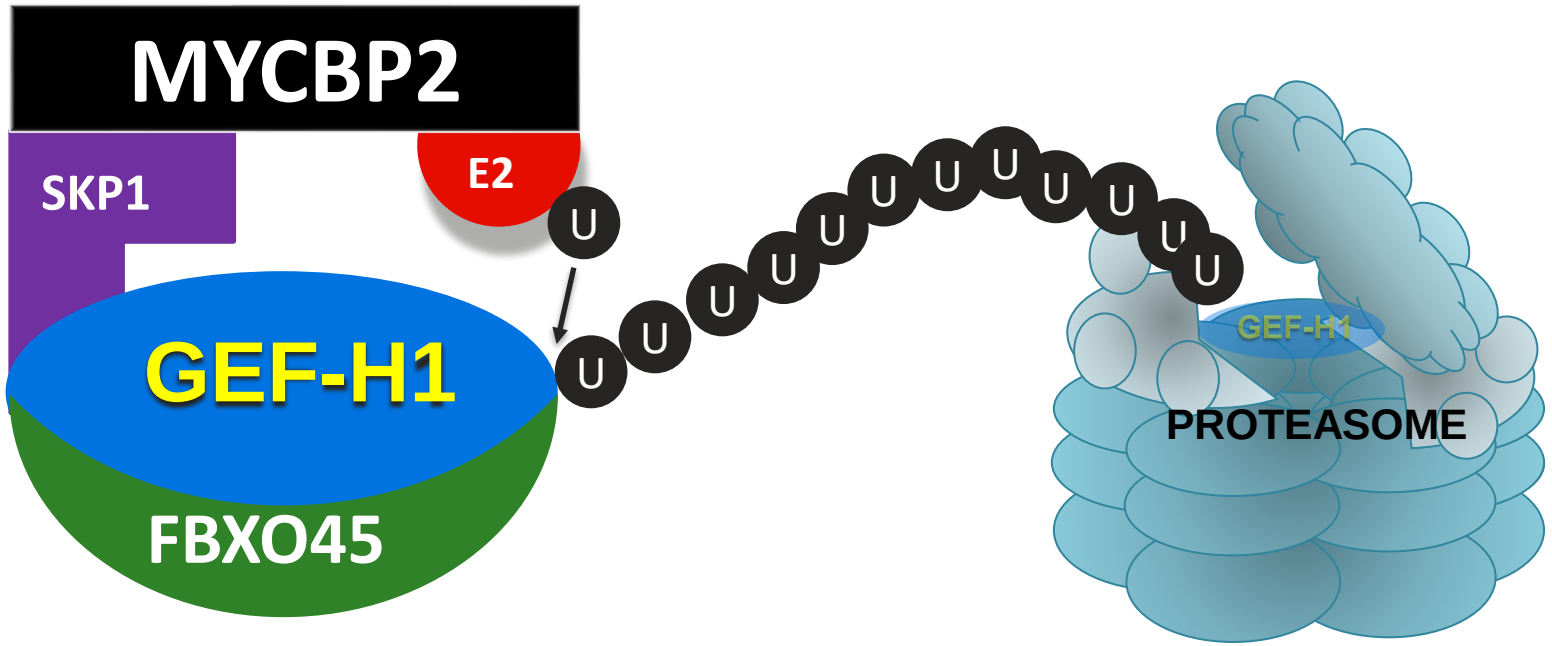






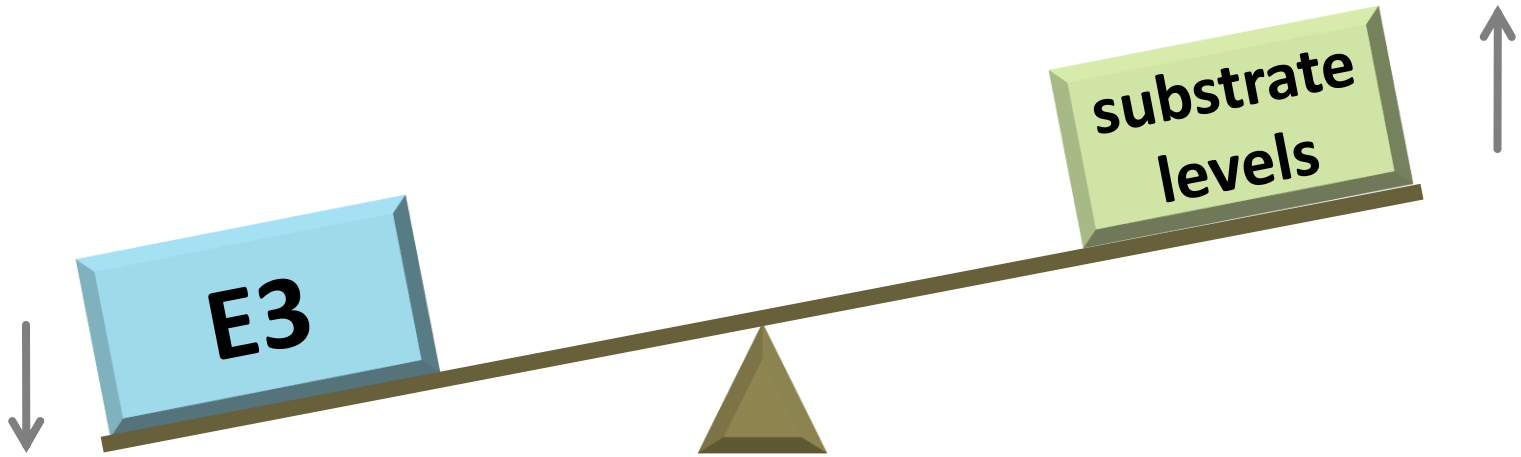


Hypothesis

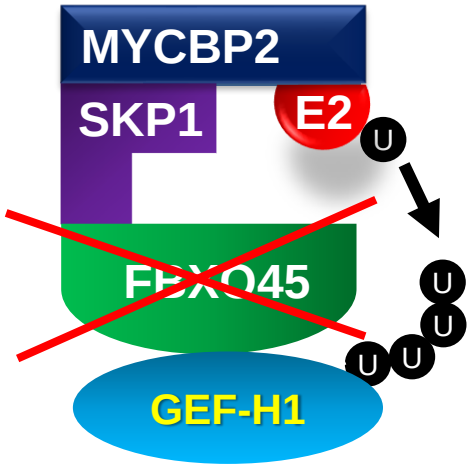


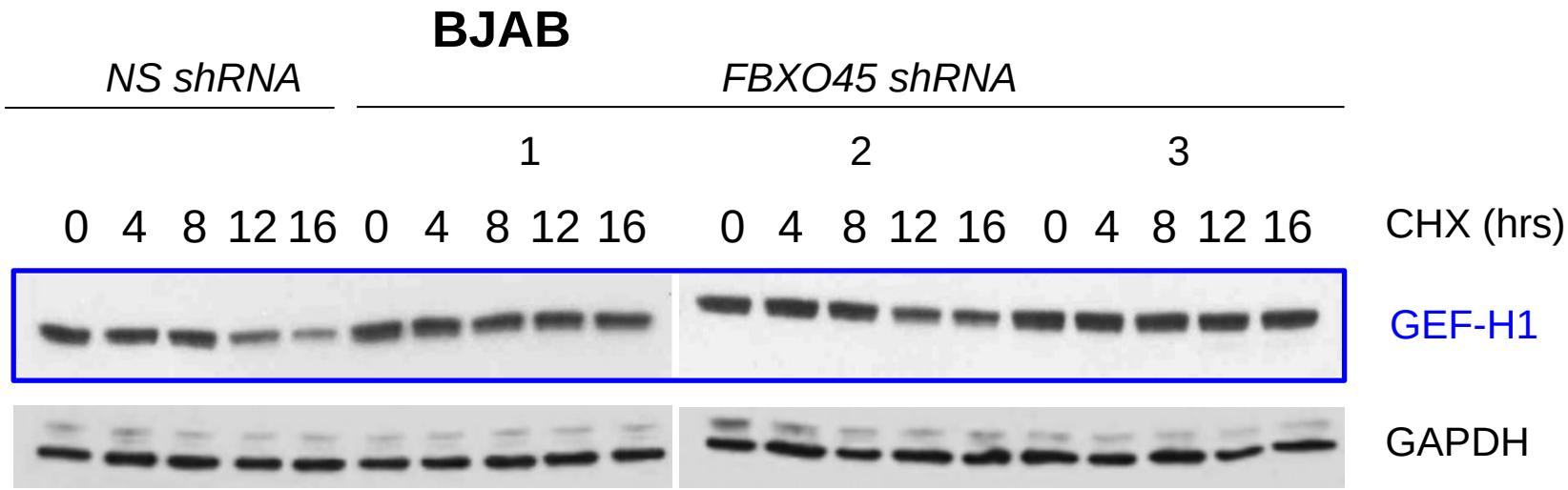
GEFH1 is a substrate of FBXO45 E3 ligase

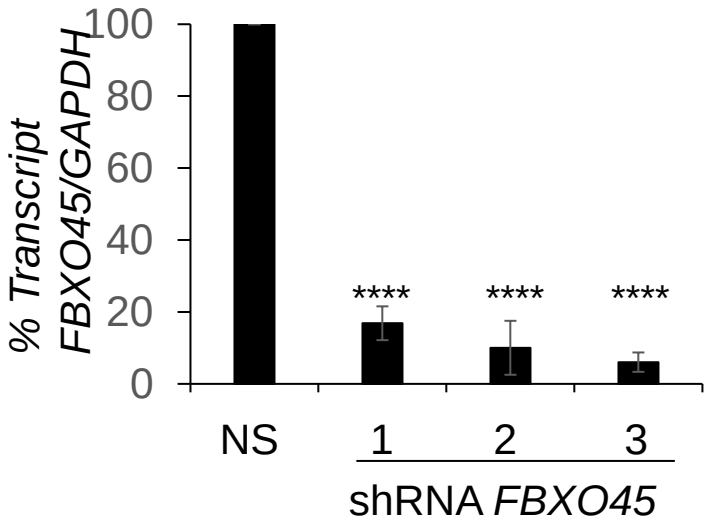
How does FBXO45 perturbation affect GEF-H1 stability?

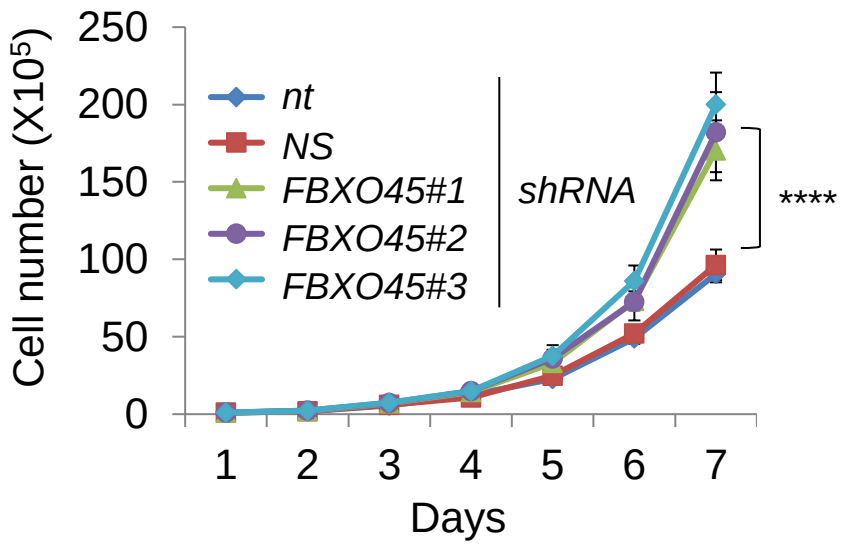


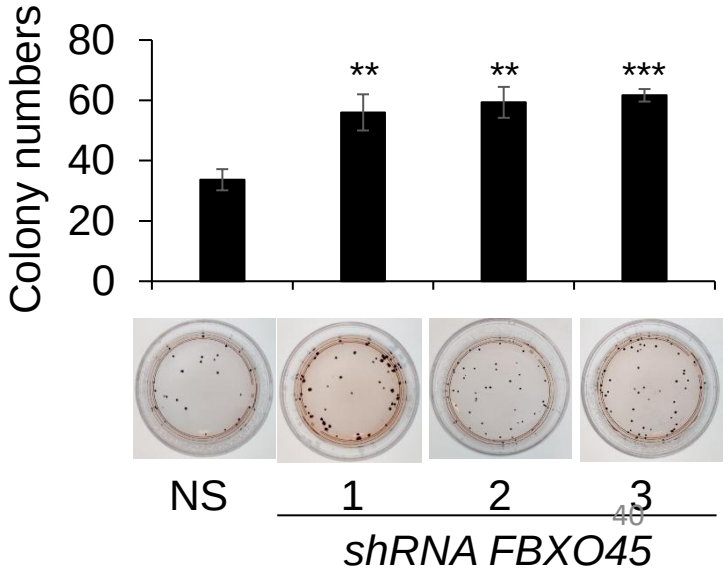
FBXO45 silencing stabilizes GEF-H1 and promotes cell proliferation and colony formation



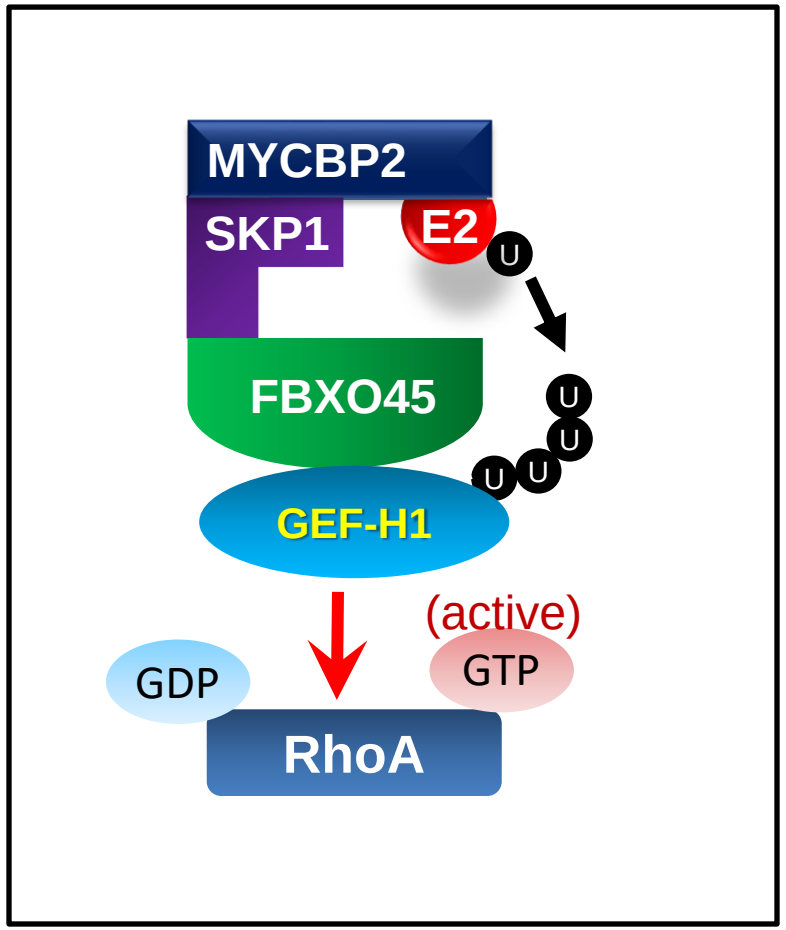


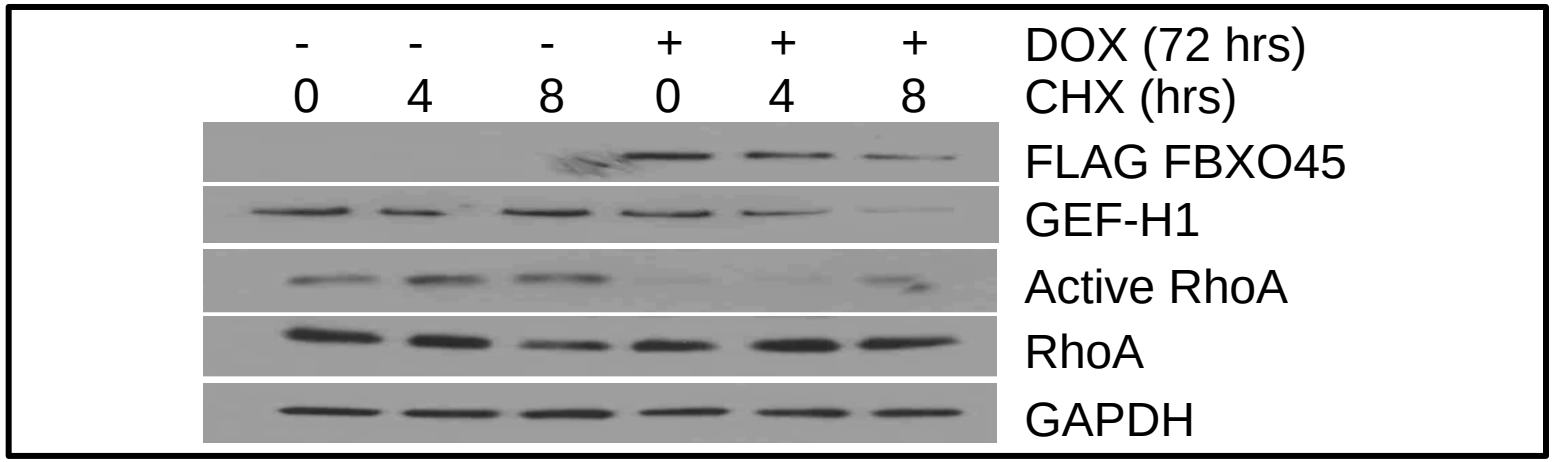






FBXO45 regulates GEF-H1 associated RhoA activation

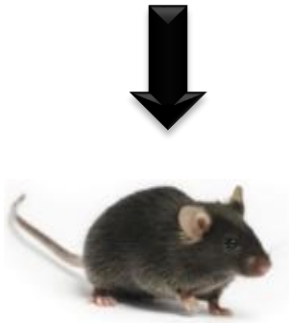




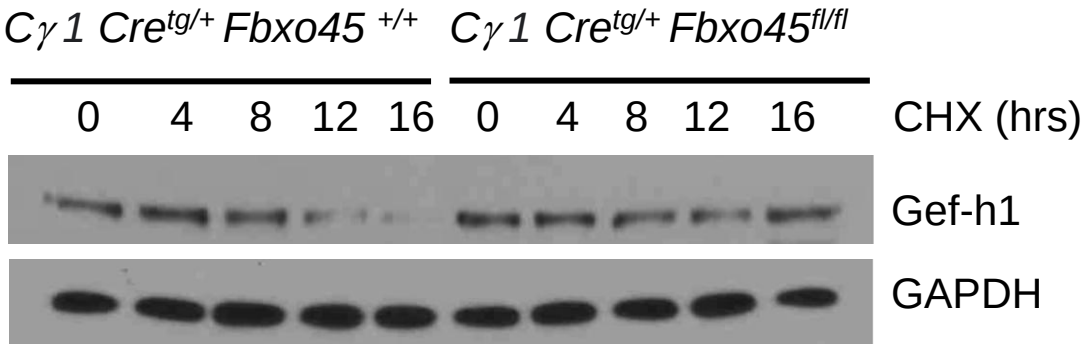
- **Overexpression of *FBXO45* accelerates GEF-H1 turnover and downregulates RhoA activity**

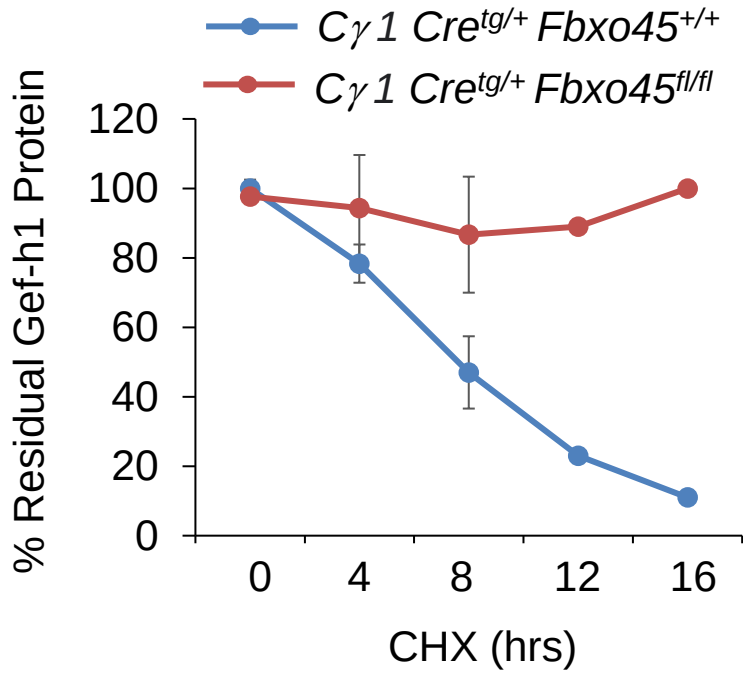
Increased stability of Gef-h1 protein in splenic GCB-cells of conditional Cγ1Cre$^{tg/+}$*Fbxo45*$^{fl/fl}$ transgenic mice

Fbxo45$^{fl/fl}$ X *Cγ1 Cre*



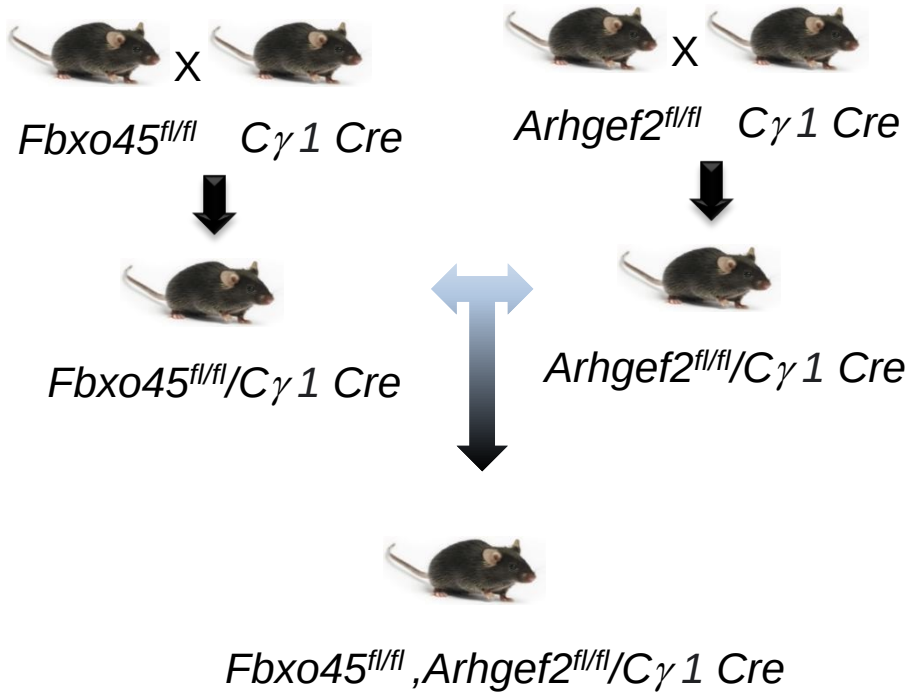
Cγ1 Cre$^{tg/+}$ *Fbxo45*$^{fl/fl}$

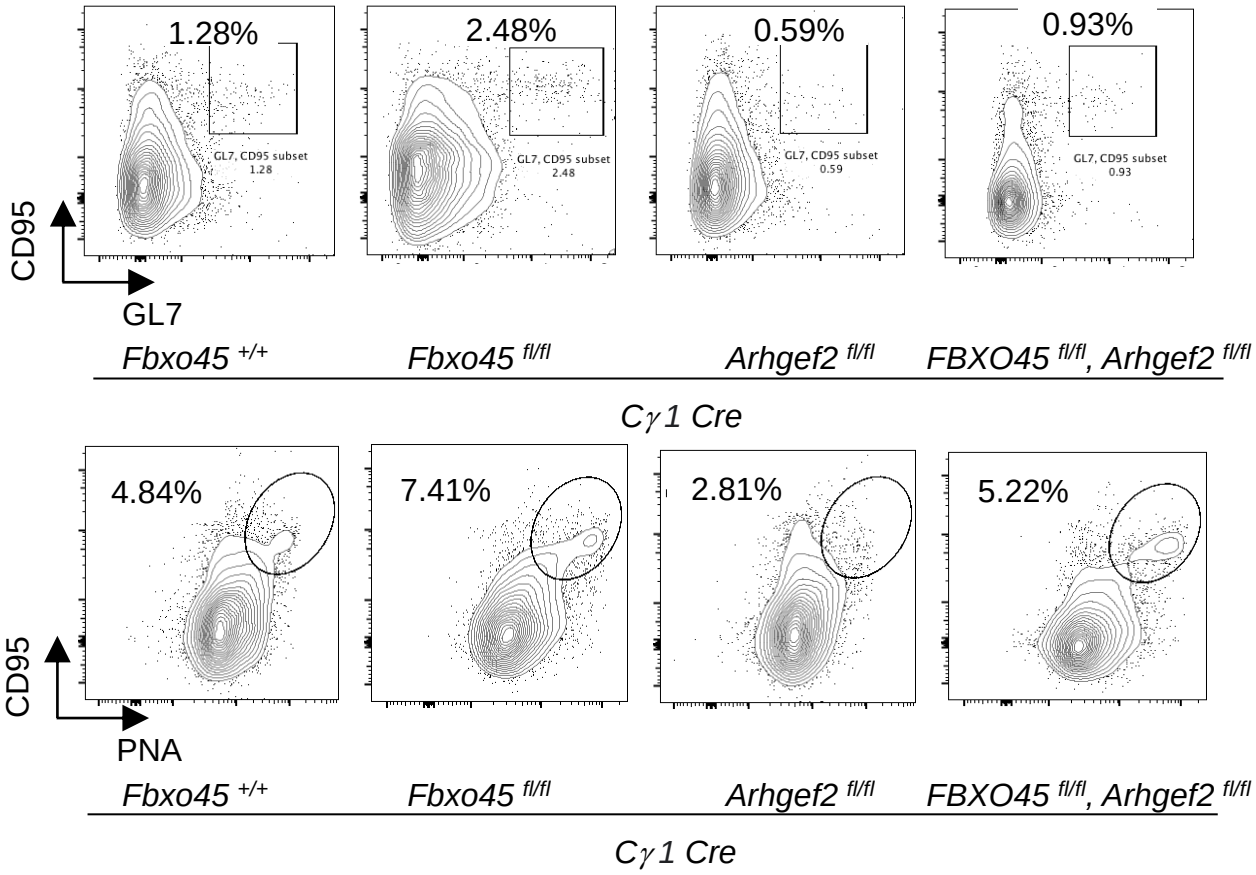




Kaiyu Ma

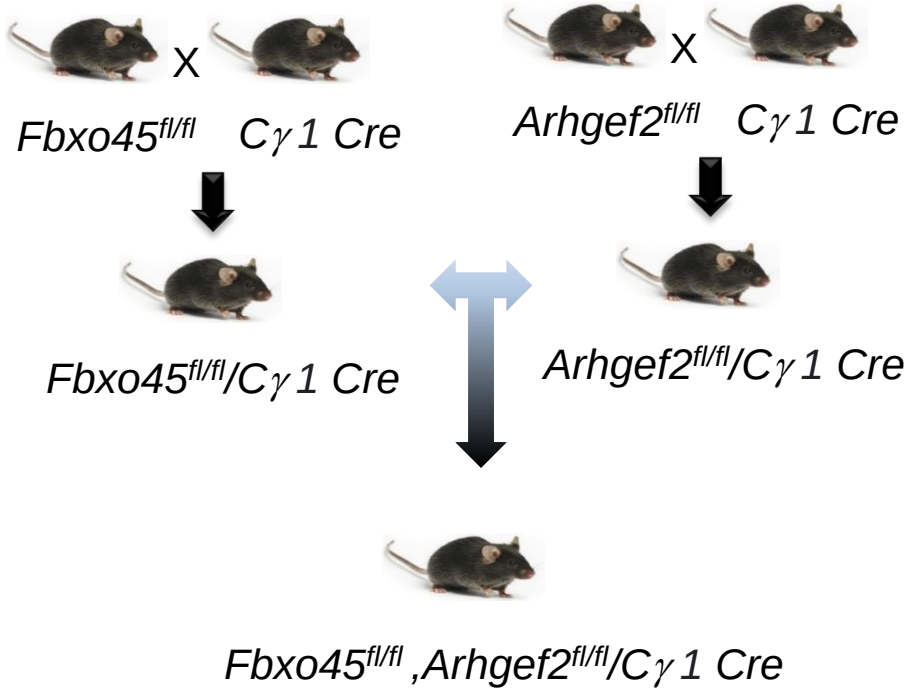
Arhgef 2 deletion in B-cells suppresses *Fbxo45*-mediated GCB-cell proliferation

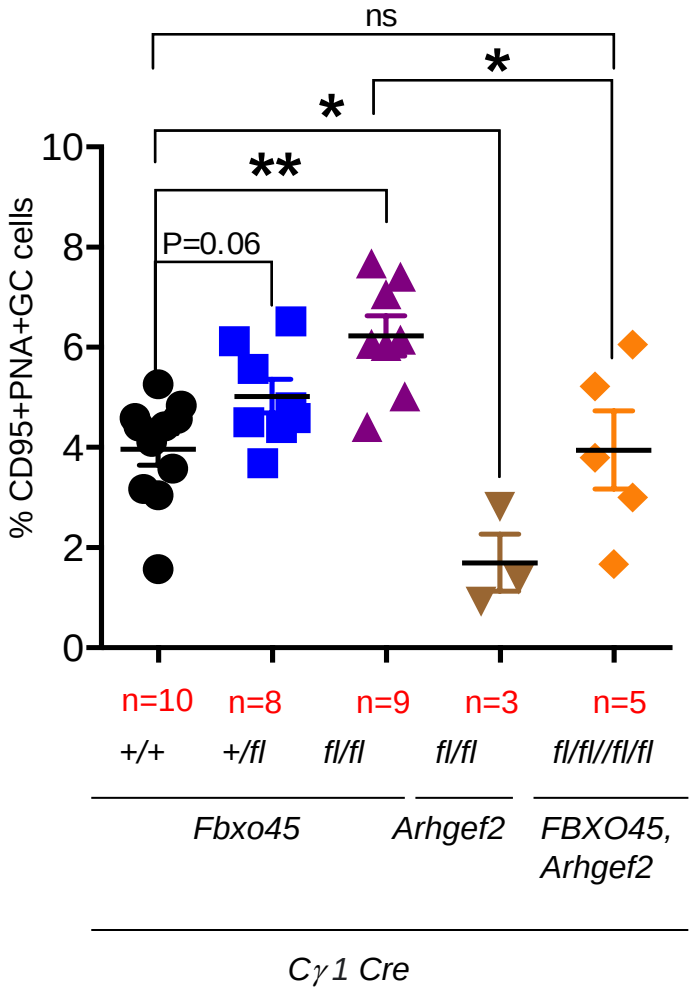




GCB-cell expansion caused by loss of *Fbxo45* is rescued by double knockout of *Fbxo45* and *Arhgef2*

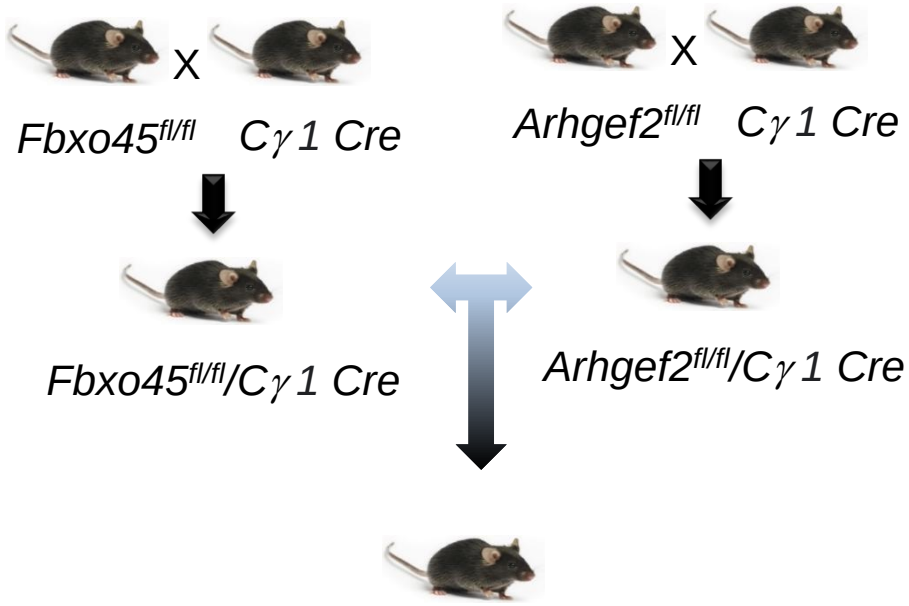
Arhgef 2 deletion in B-cells suppresses *Fbxo45*-mediated GCB-cell proliferation

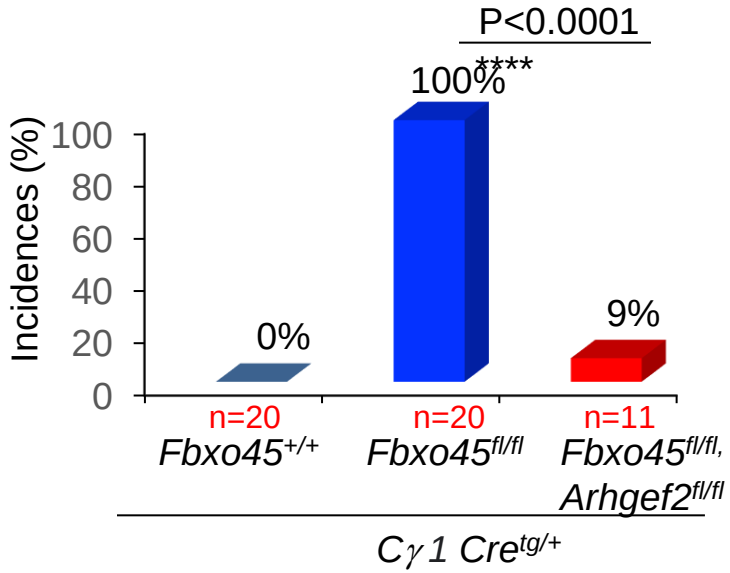


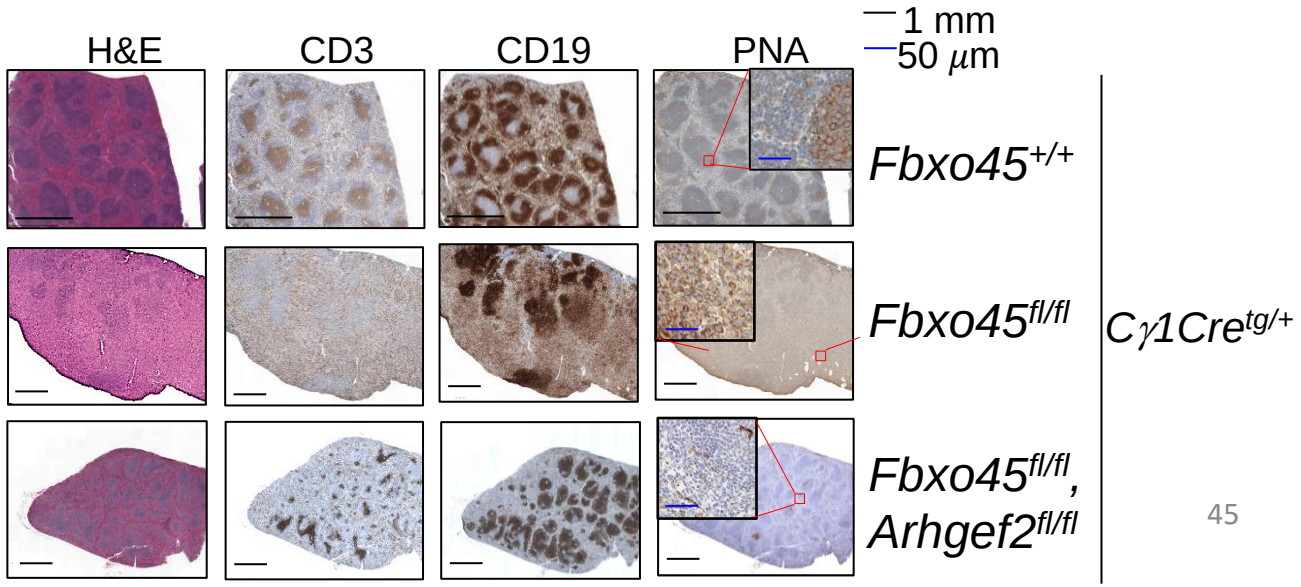


GCB-cell expansion caused by loss of *Fbxo45* rescued by double knockout of *Fbxo45* and *Arhgef2*

Arhgef2 deletion in B-cells reverts *Fbxo45* mediated lymphomagenesis

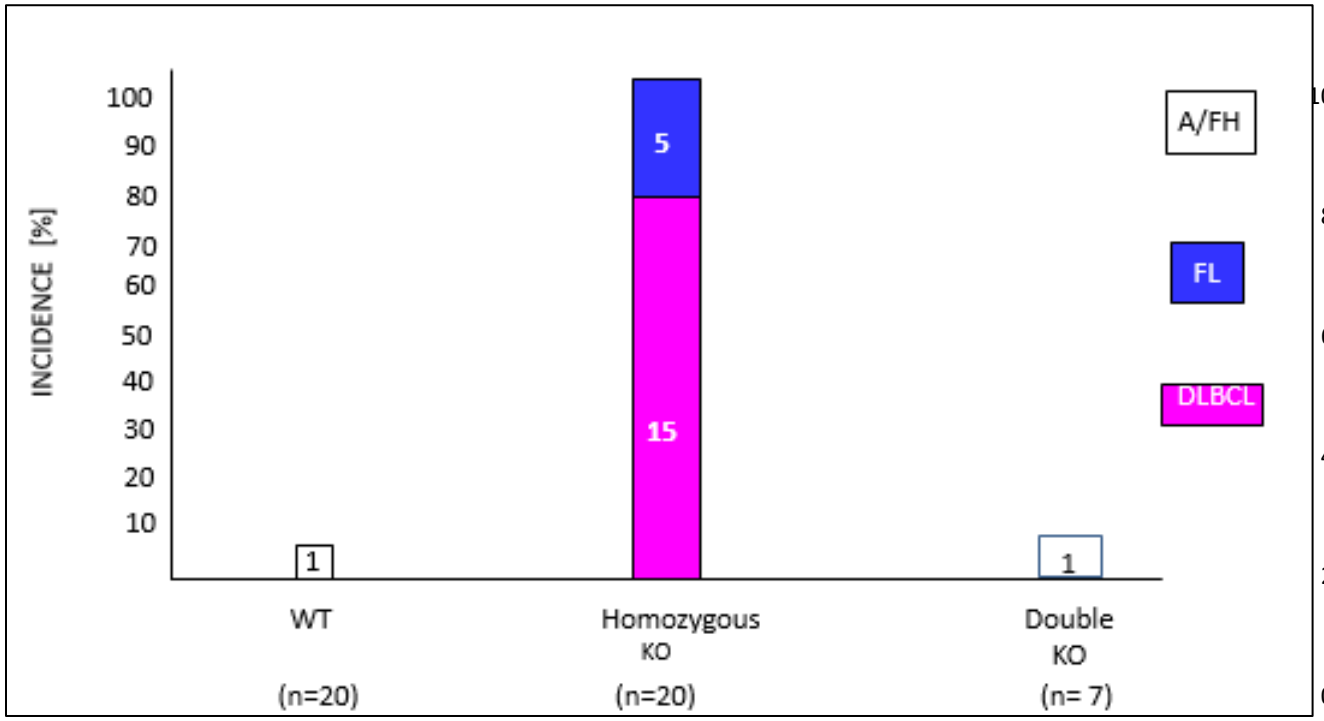


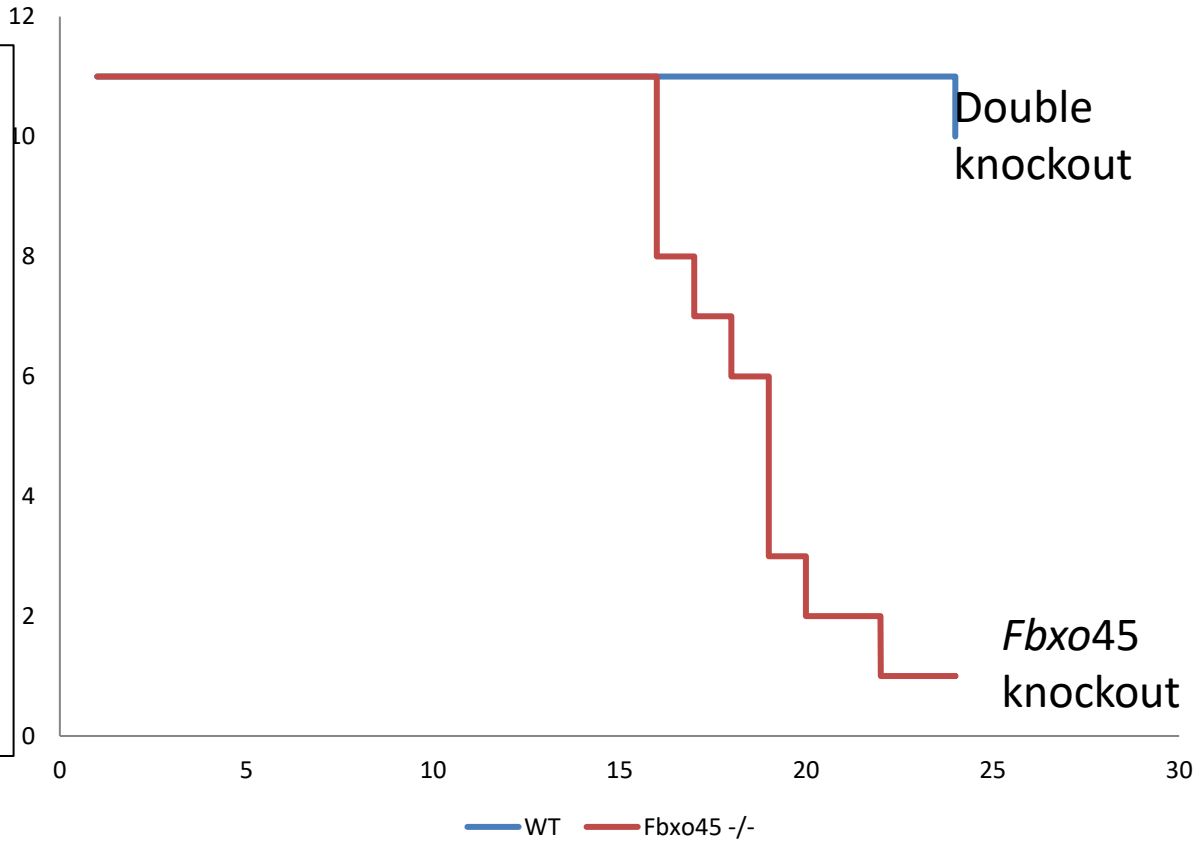




B-cell lymphomagenesis caused by loss of *Fbxo45* is reverted by co-deletion of *Fbxo45* and *Arhgef2*

Fbxo45-Cγ1Cre$^{fl/fl}$-Arhgef2$^{Δ/Δ}$ abrogrates lymphoma





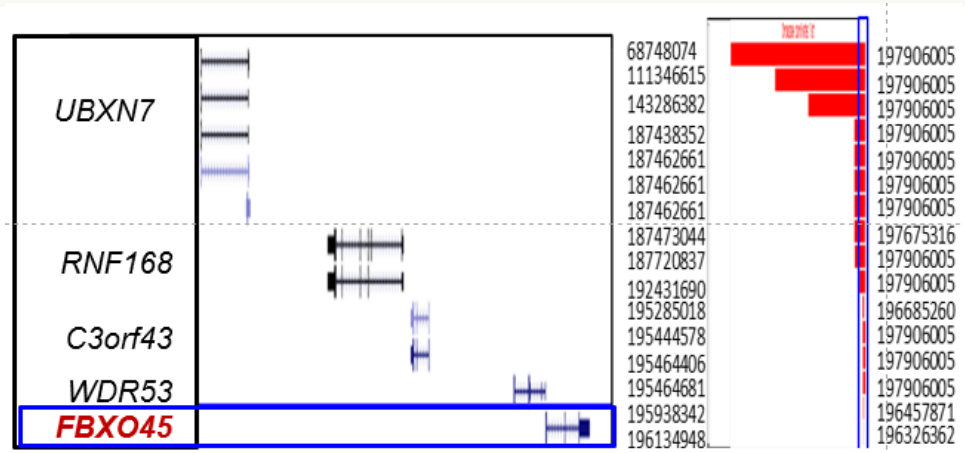
Summary

Conditional knockout of *Fbxo45* in germinal center B-cells:

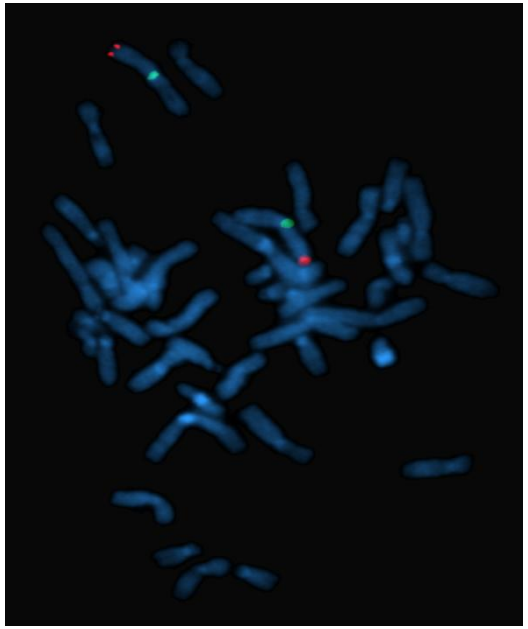
- Results in expansion of percentage and absolute number of GC B-cells, increase in mean GC size, surface area and total numbers of GCs/spleen.
- Develops lymphoma at various anatomic locations and exhibited significant splenomegaly and lymphadenopathy.
- Results in GCB-derived lymphoma at 100% penetrance in homozygous null mice and reduces survival of mice.

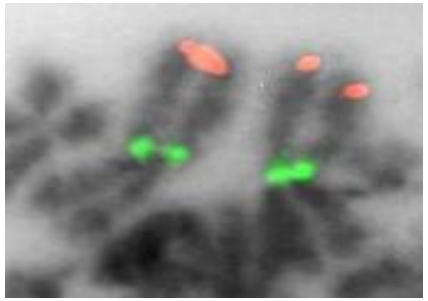
Genomic structural alterations targeting *FBXO45* and *ARHGEF2* in primary human lymphoma

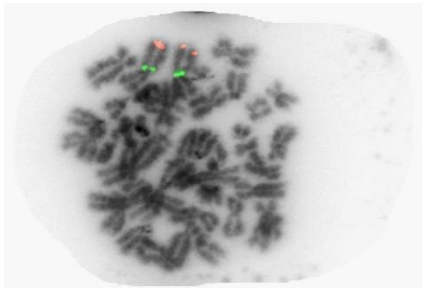
Genomic losses targeting 3q29 are recurrent in DLBCL



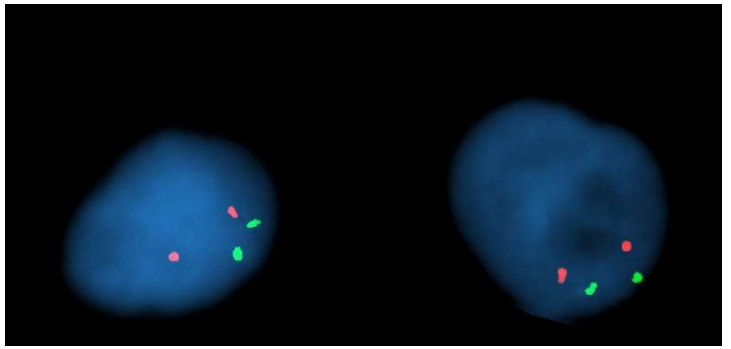
FISH 3cen (green)/3q29 (red)



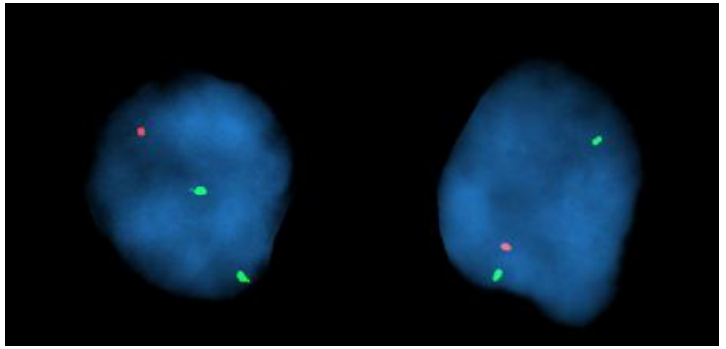




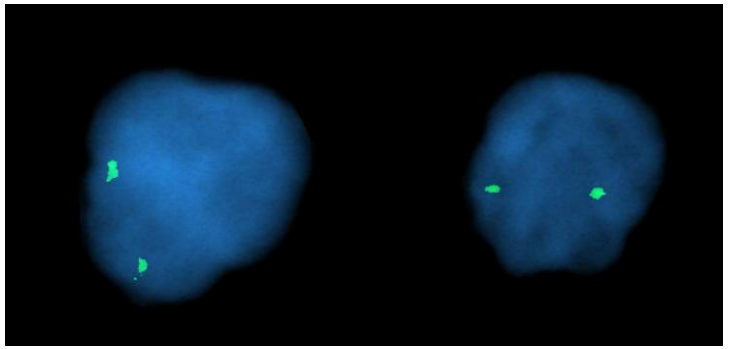
Normal control



Normal Pattern
FISH 3cen (green)/3q29 (red)
Negative cells

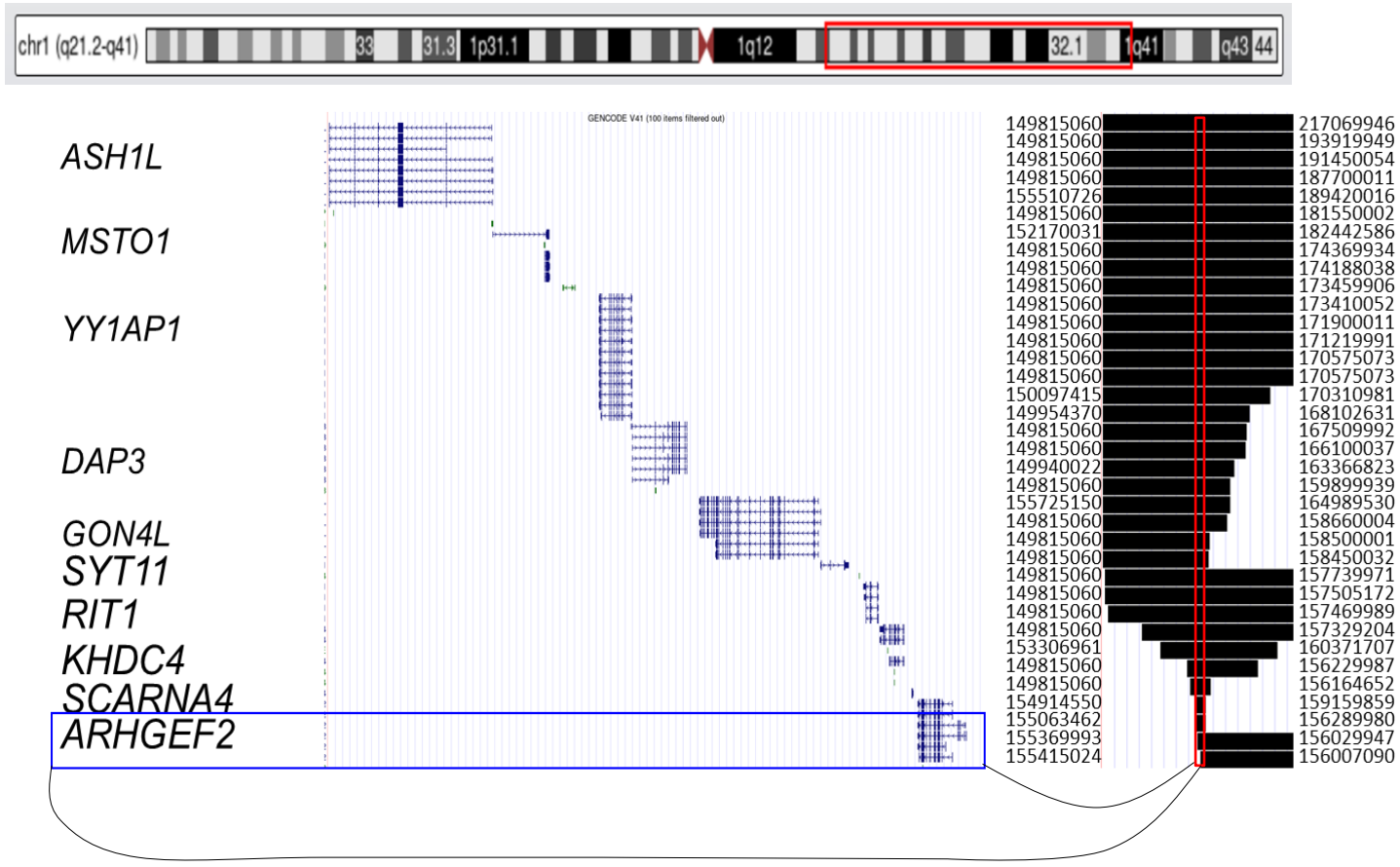


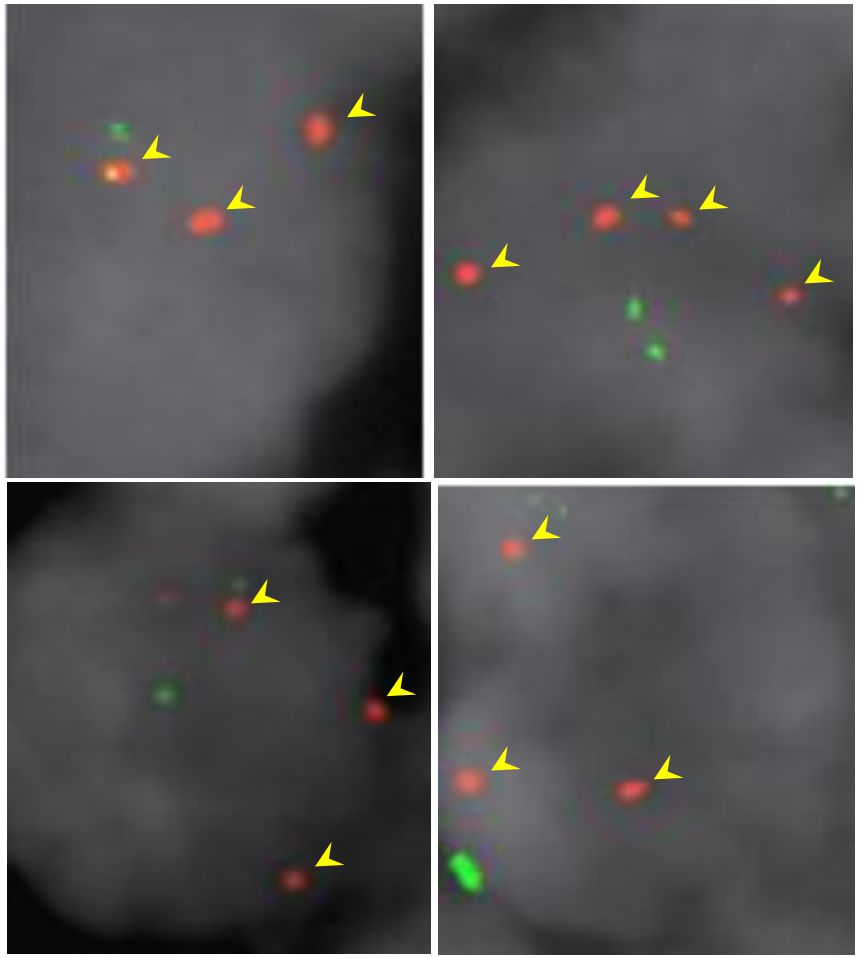
Hemizygous deletion
FISH 3cen (green)/3q29 (red)
1R 2G



Homozygous deletion
FISH 3cen (green)/3q29 (red)
0R 2G

Copy number gain of *ARHGEF2* in transformed DLBCL





WGS identifies copy number loss of *FBXO45* and gains of *ARHGEF2* in primary human B-cell lymphoma

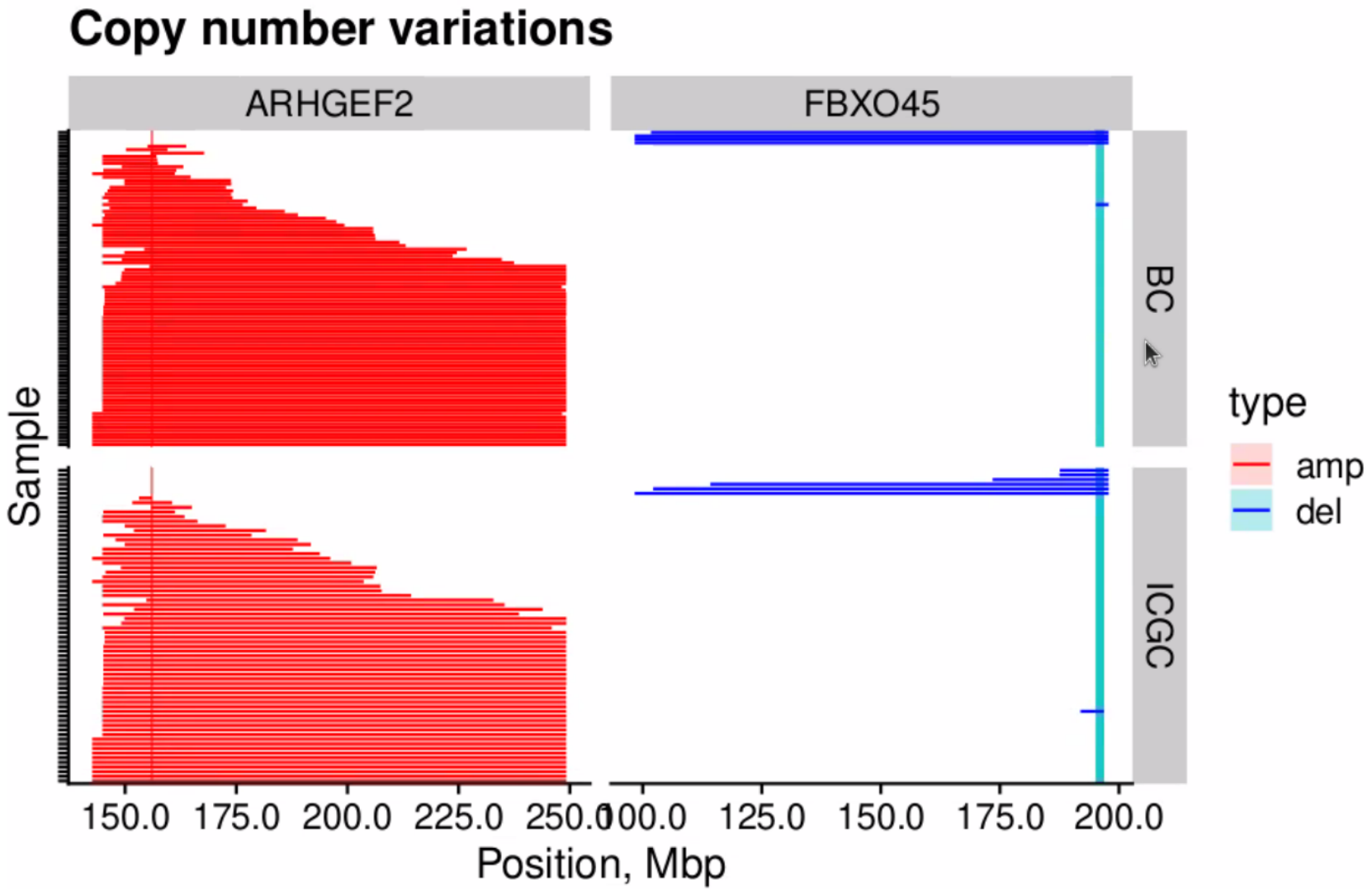
Cases with DEL of *FBXO45*	Number	Percent	Total cohort
Total	11	6.2	178
DLBCL	5	6.6	76
FL	2	2.3	86
FL-DLBCL	4	**25.0**	16

Cases with GAINS of *ARHGEF2*	Number	Percent	Total cohort
Total	36	20.2	178
DLBCL	20	26.3	76
FL	9	10.5	86
FL-DLBCL	7	**43.8**	16

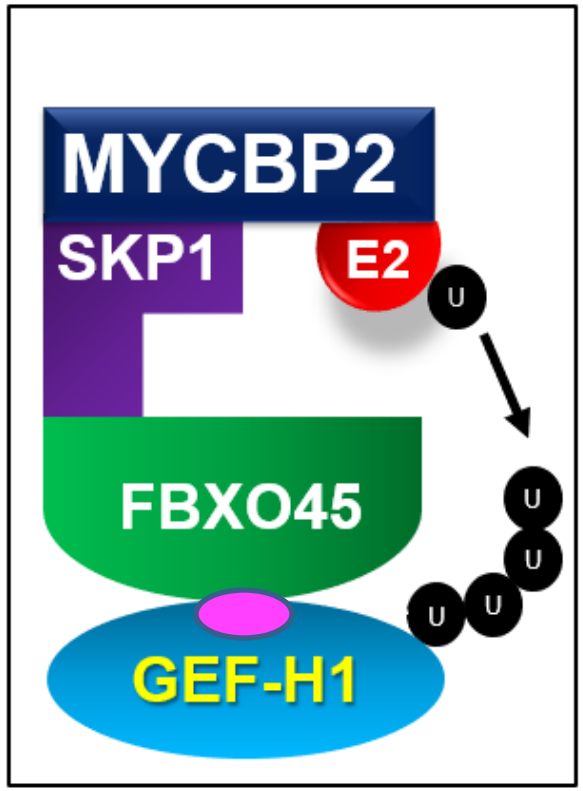
FBXO45 loss is frequent in transformed DLBCL

ARHGEF2 gains are frequent in transformed DLBCL

FBXO45 loss and *ARHGEF2* gains are mutually exclusive in DLBCL

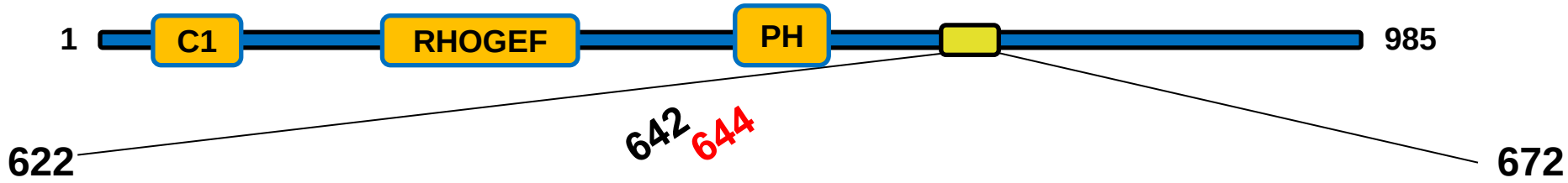


FBXO45 targets GEF-H1 for degradation

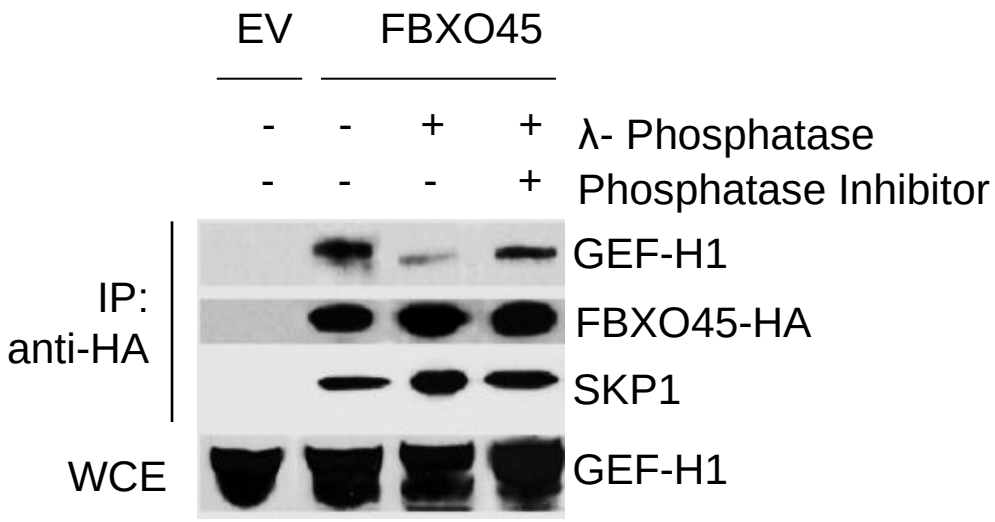


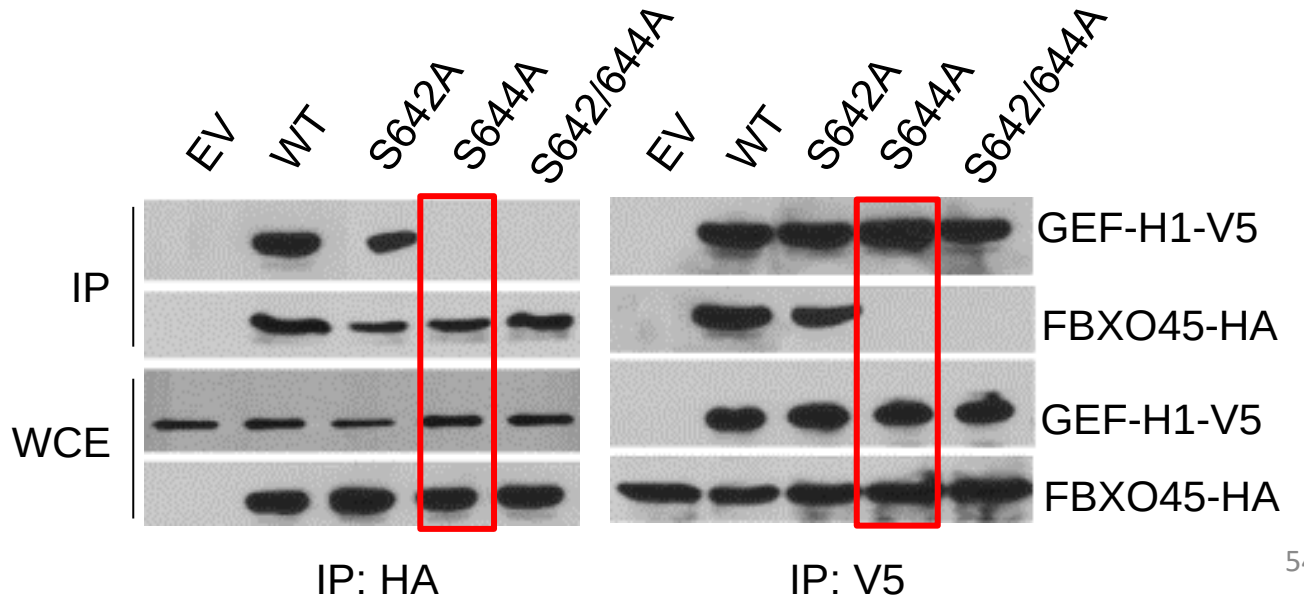
Identification of the degron

GEF-H1 S644 is critical for its interaction with FBXO45



Homo sapiens	AEEDGGSGMALPTLPRGLFRSE**S**LESPRGERLLQDAIREVEGLKDLLVGPG
Canis lupus familiaris	VEEDSG-GVALPALPRGLFRSE**S**LECPRGERLLQDAIREVEGLKDLLVGPG
Bos Taurus	VEEDGSSGVPLPTLPRGLFRSE**S**LESPRGERLLQDAIREVEGLKDLLVGPG
Mus musculus	ALKAGFVGMPPPALPRGLFRLE**S**FESLRGERLLKDALREVEGLKDLLLGPC
Rattus norvegicus	ALKAGFIGMPPPTLPRGLFRLE**S**FESLRGERLLKDALREVEGLKDLLLGPC
Danio rerio	AEVMGGYEVMLPACSRNLFRAE**S**PQAPRGEQLLTQAITEVDRLTELLLGSG
Xenopus laevis	QIQEGENSSLTGVSTRCLFRTD**S**MDSYRGEKLISEAIKEVEALKDVIVGSA

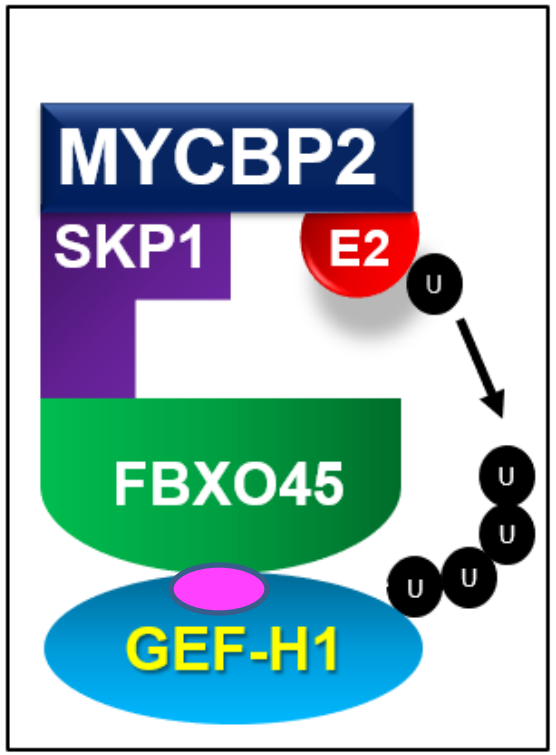


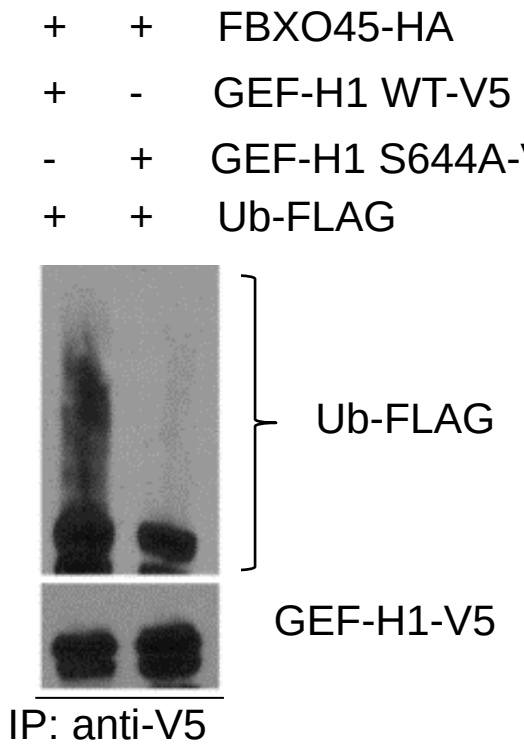


FBXO45 polyubiquitinates GEF-H1

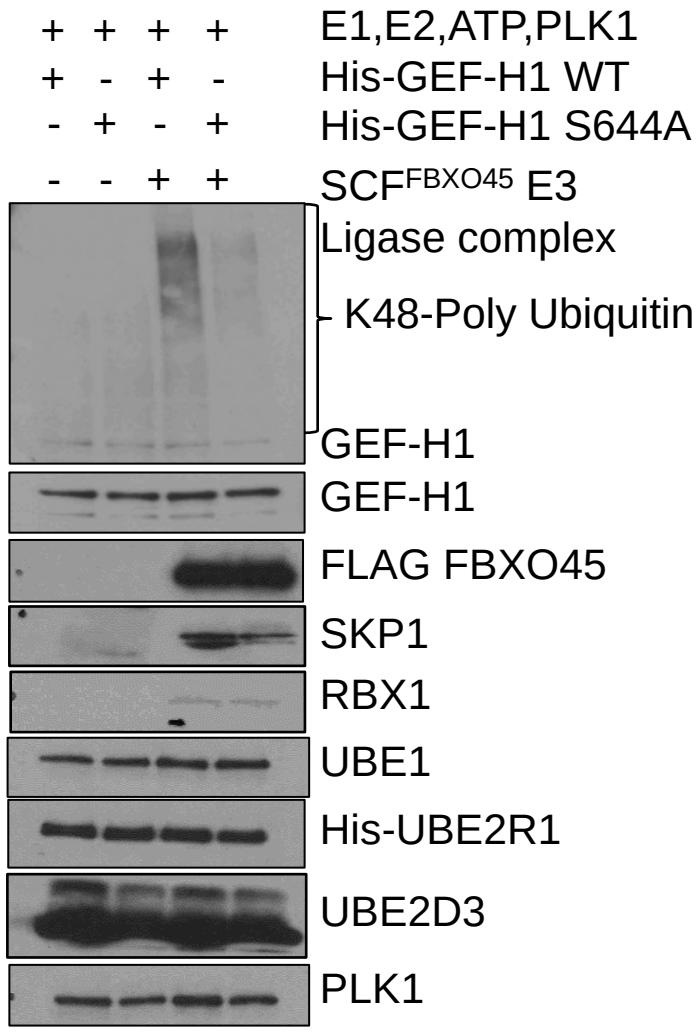
Xiaofei Chen

Anagh Sahasrabuddhe



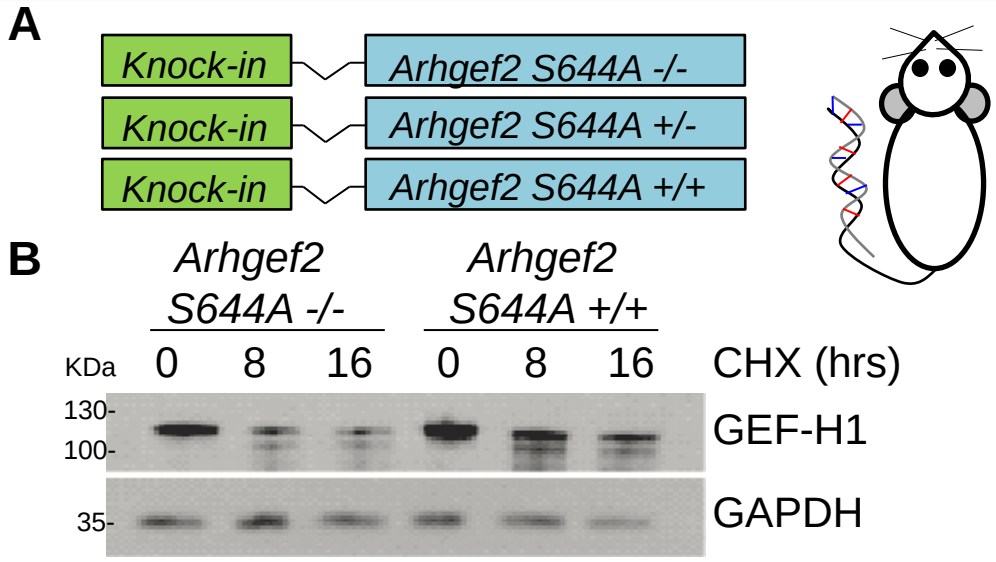


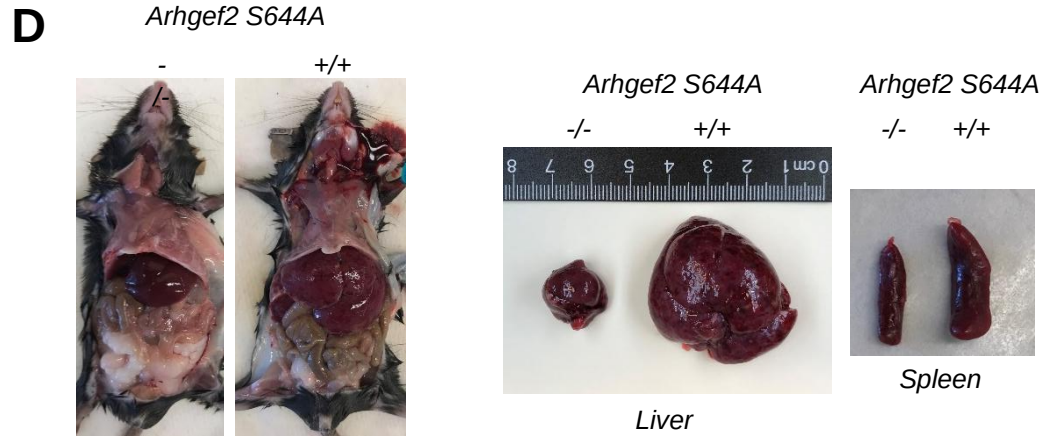
In-cell polyubiquitination assay

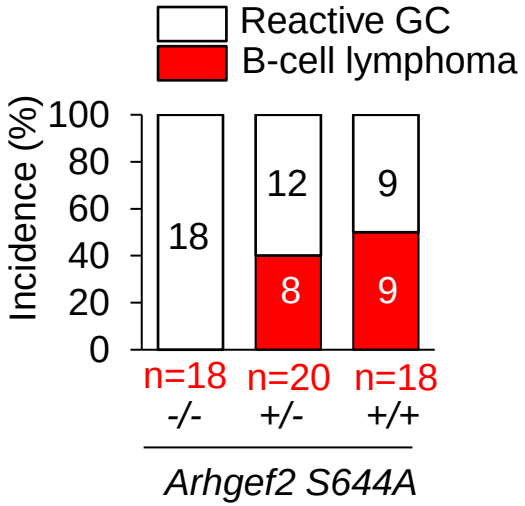


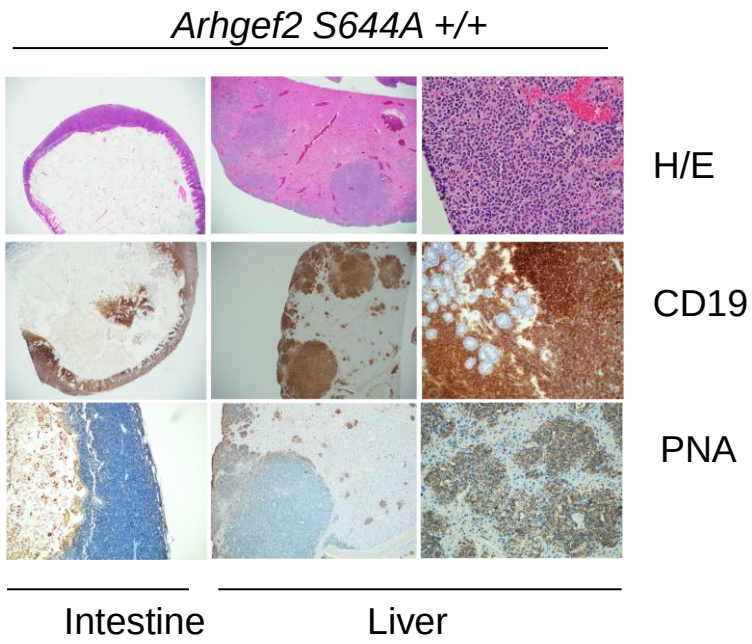
In-vitro polyubiquitination assay

GEF-H1 S644A *knock in* stabilizes GEF-H1 protein and leads to B-cell lymphomagenesis

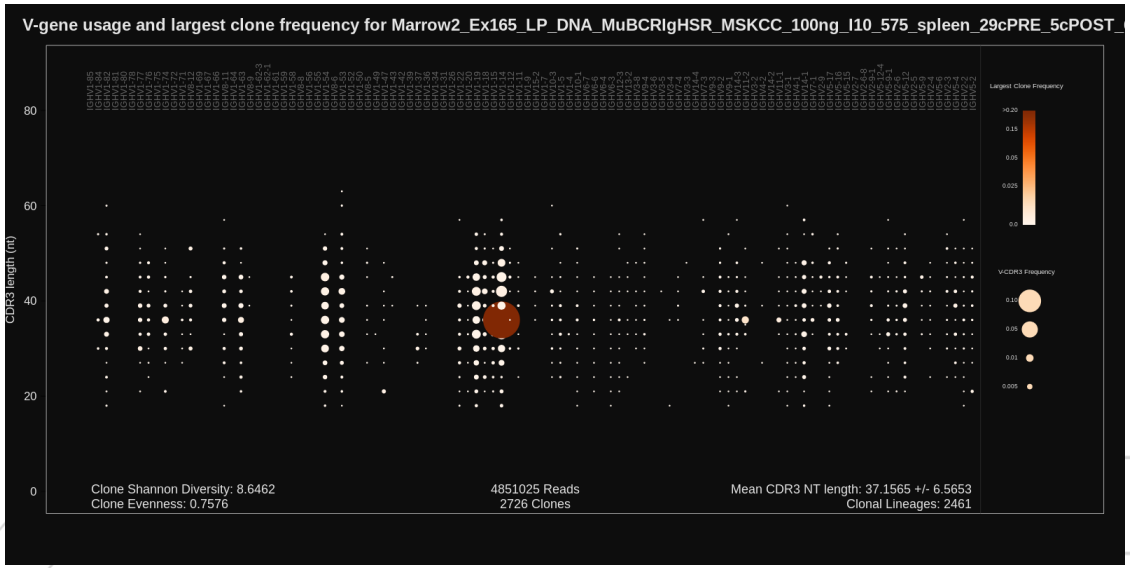


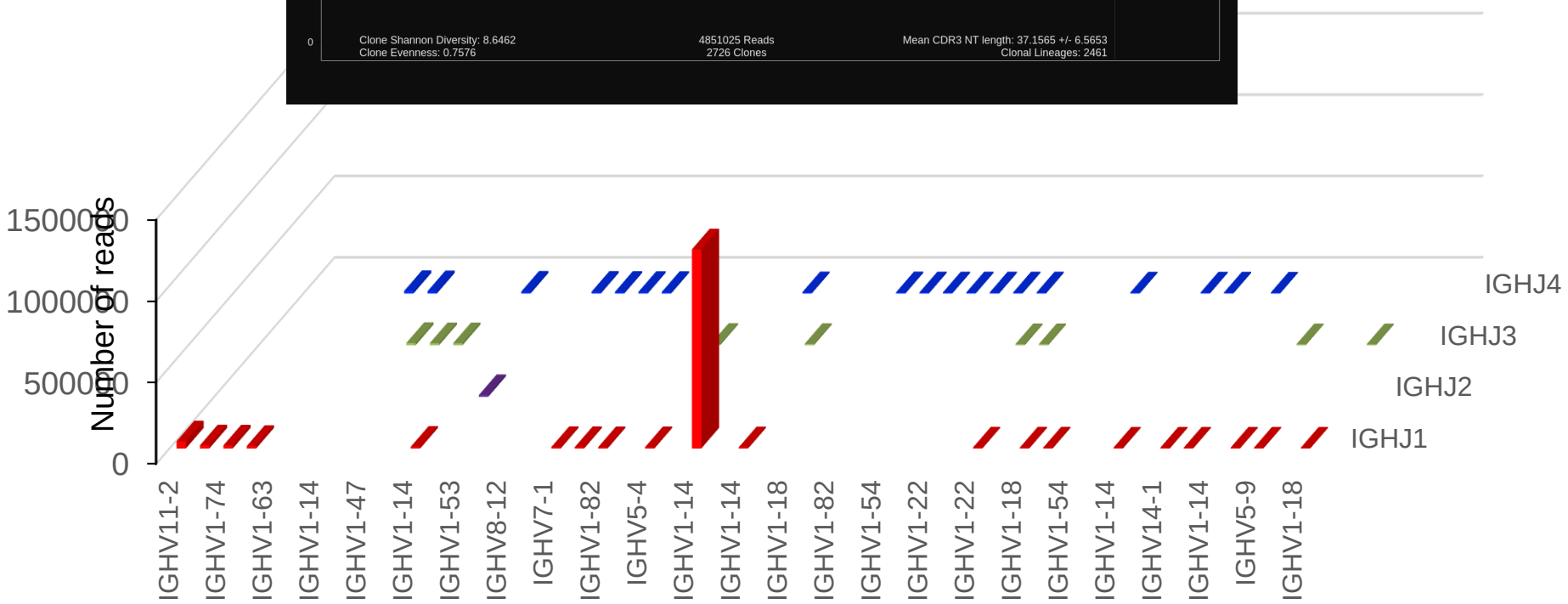




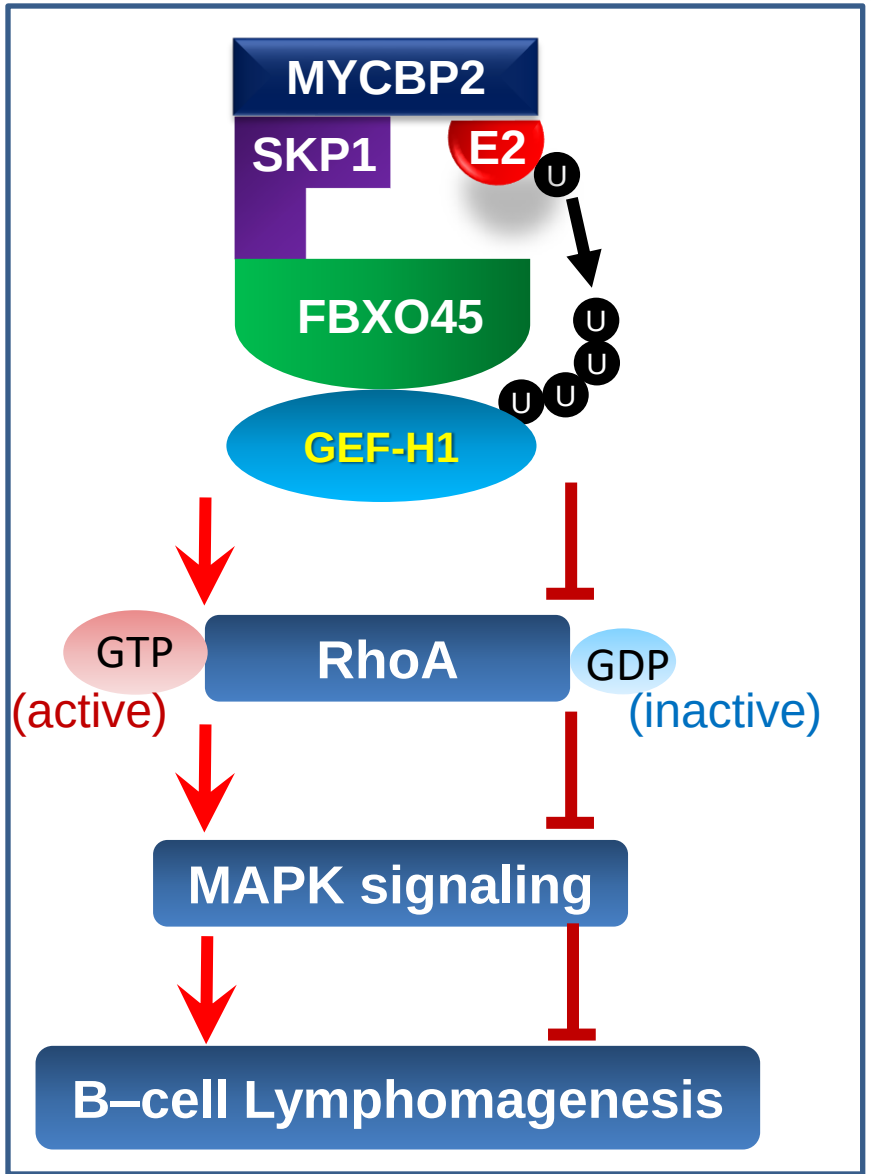


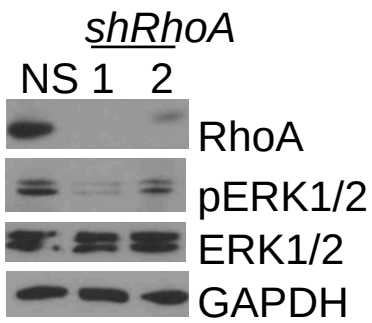
Lymphoid proliferations in GEF-H1 S644A transgenic mice are monoclonal



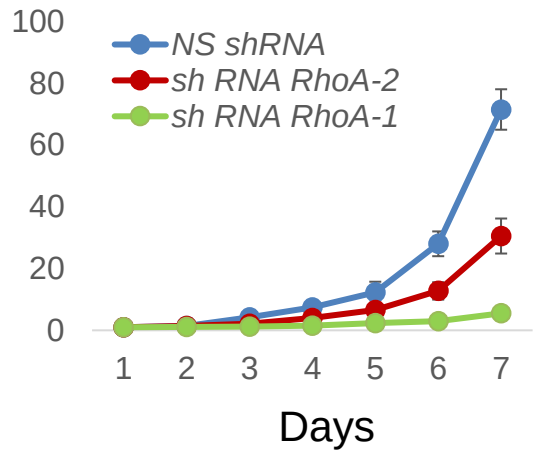


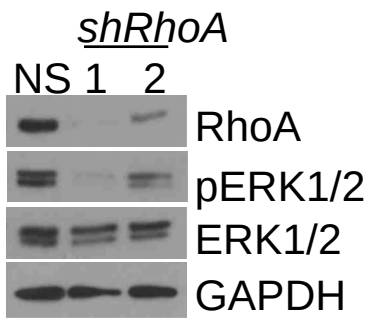
Silencing of *RHOA* downregulates pERK signaling and concomitantly reduces cell proliferation





BJAB





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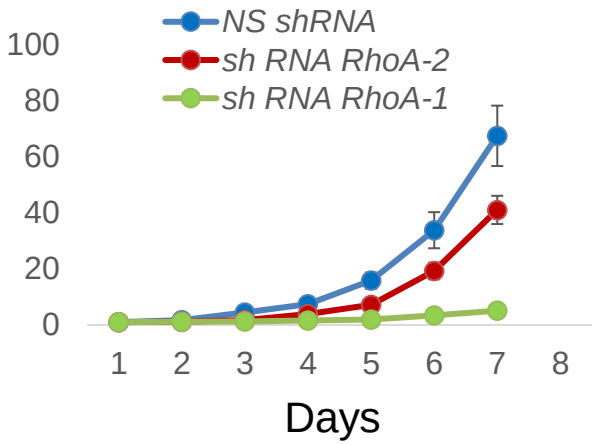
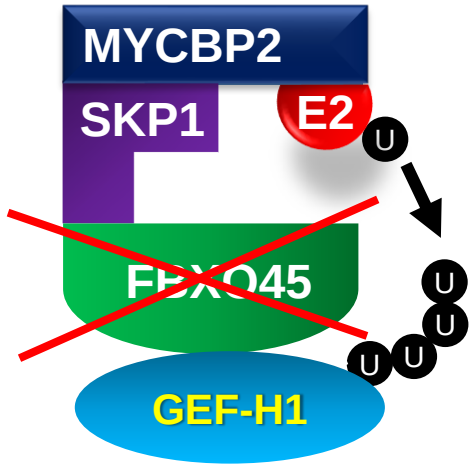
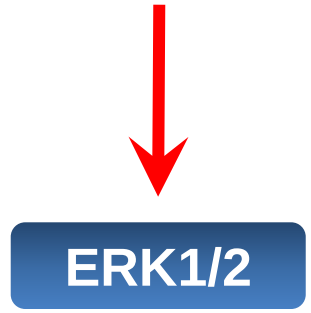


FBXO45 silencing stabilizes GEF-H1 and promotes hyperactivation of MAP Kinase pathway





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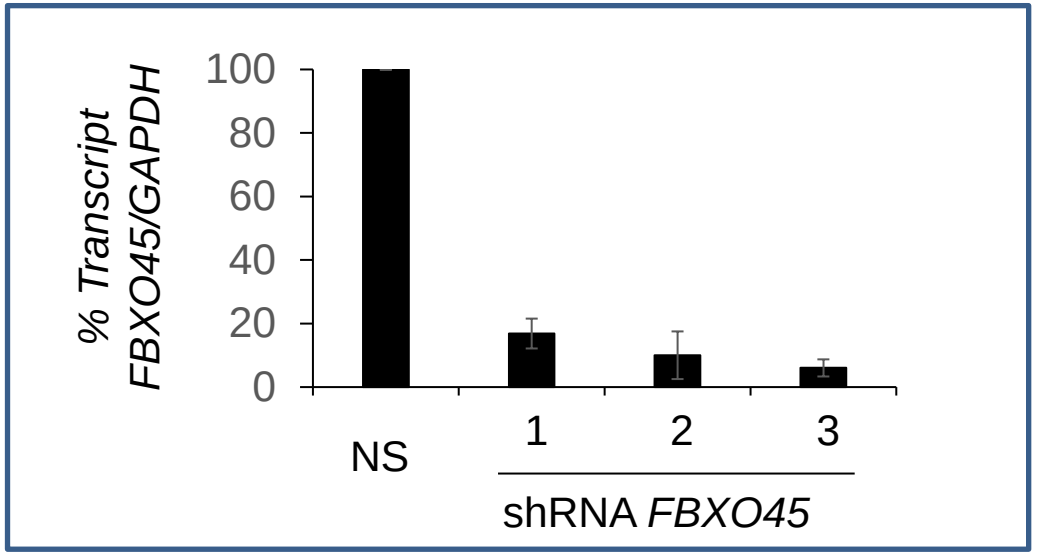
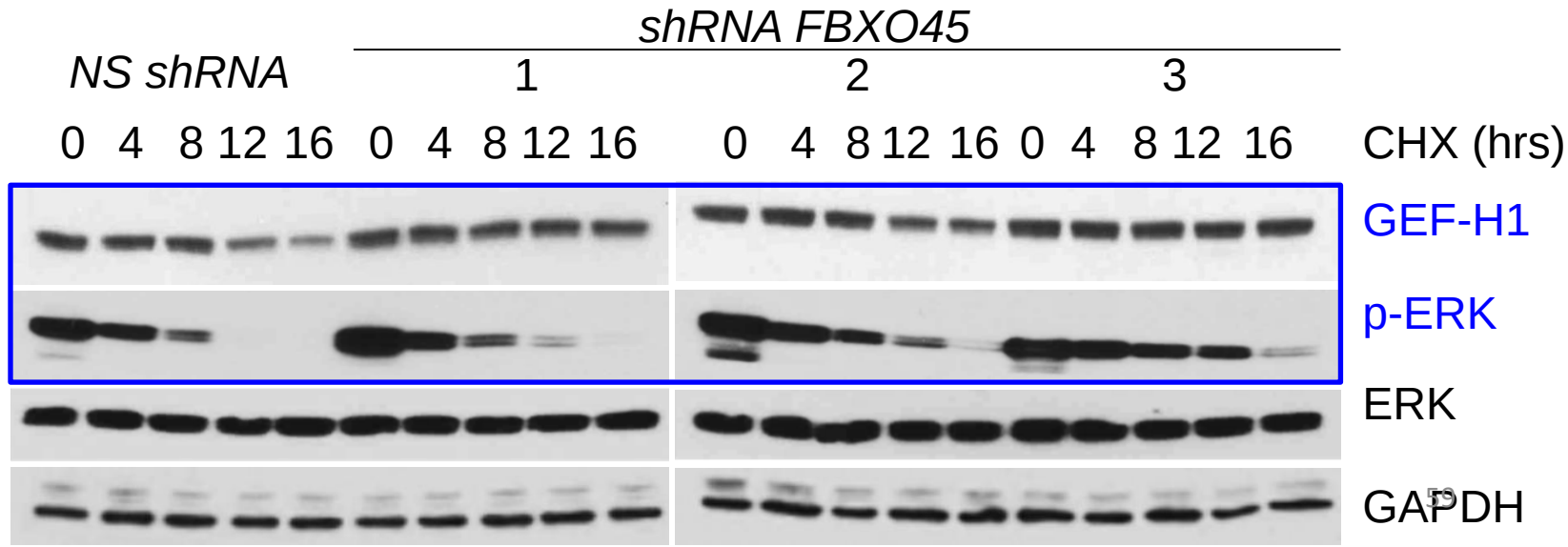
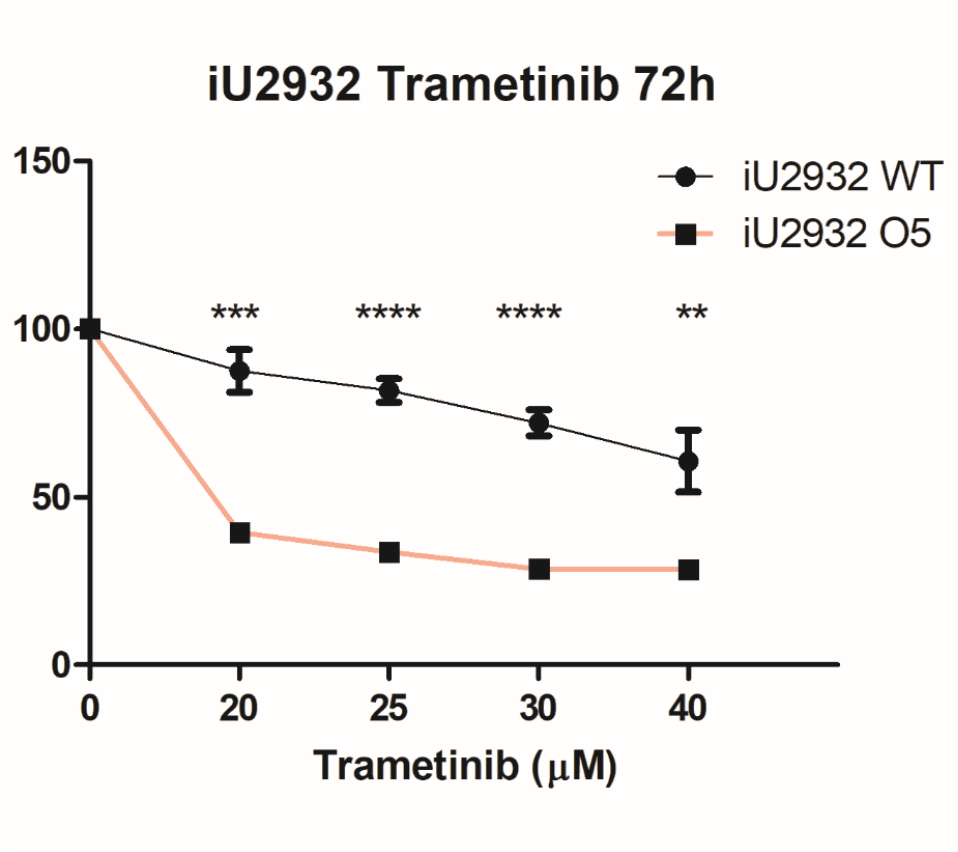


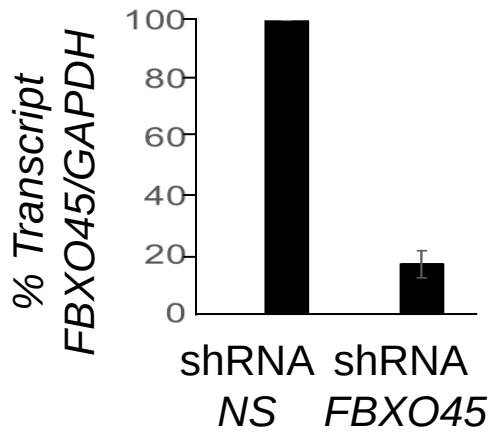


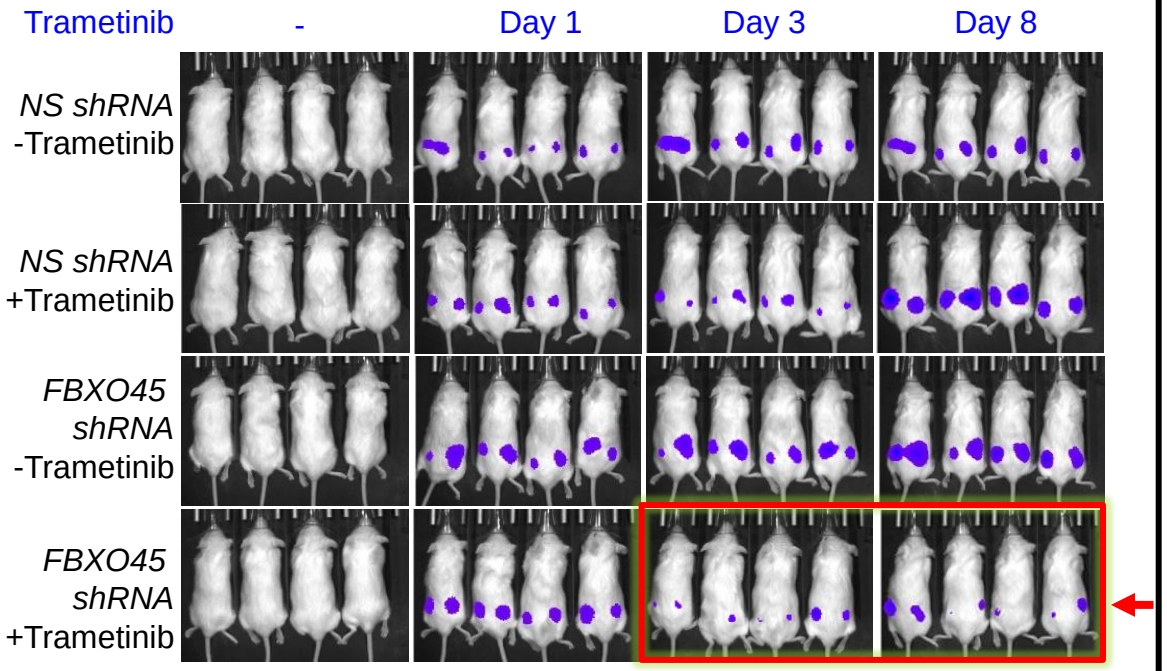
Fbxo45 KO in B cell lymphoma cells enhances sensitivity to MEK1/2 inhibitor

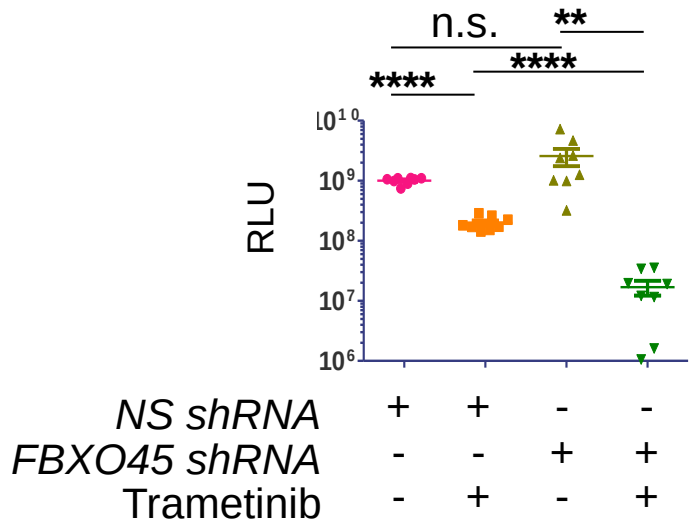


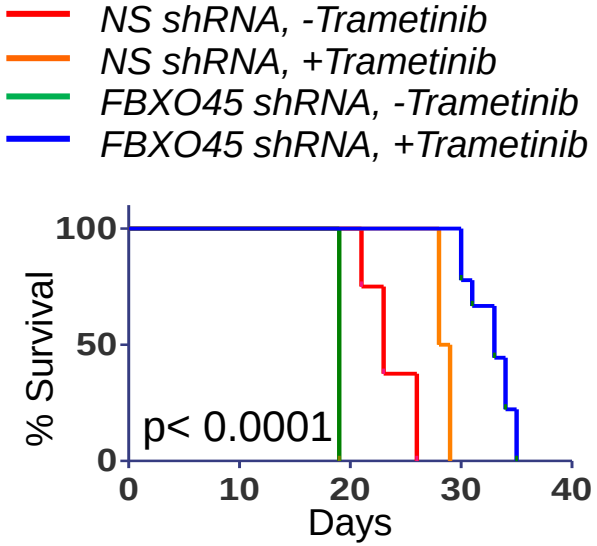
FBXO45 silencing promotes synthetic lethality to MAP kinase pathway inhibition in DLBCL

FL518









Outline

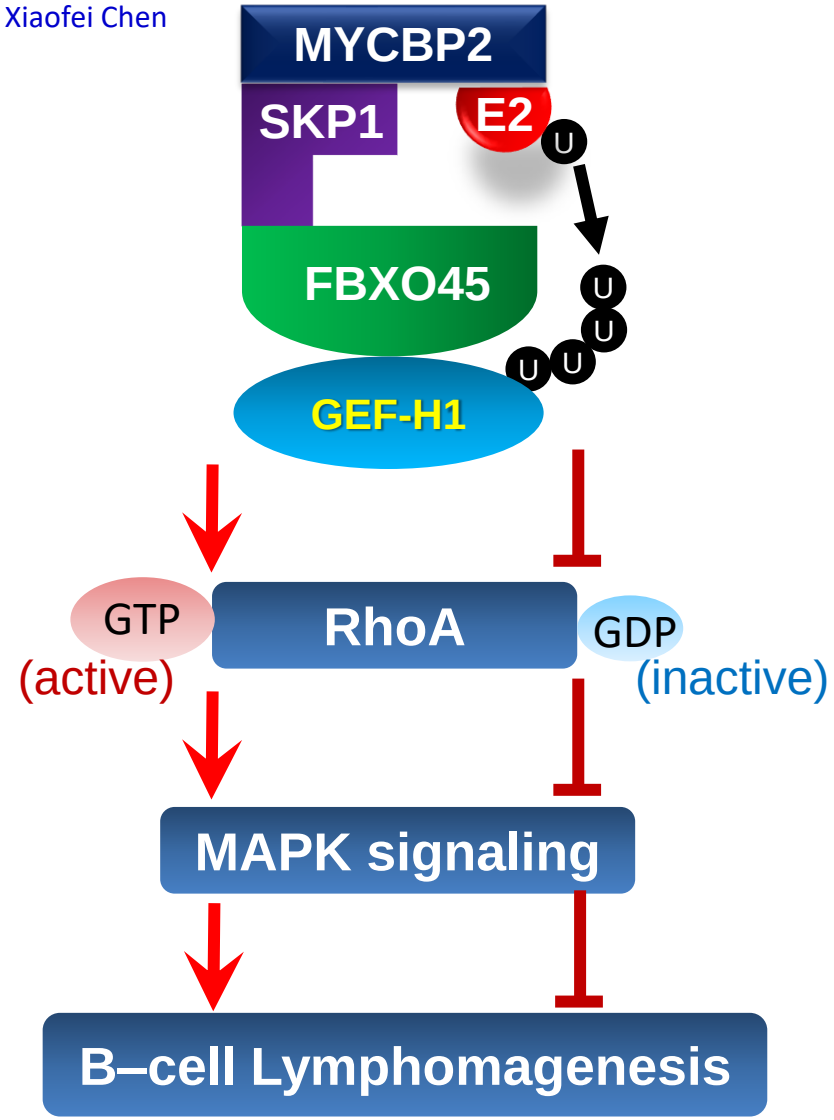
- Background
- Hypothesis
- Experimental Approach
- **Conclusions**
- Future Directions

Conclusions

Xiaofei Chen

A novel FBXO45-GEF-H1 axis controls oncogenic signaling in B-cell lymphoma

Anagh Sahasrabuddhe



- **FBXO45** is a **novel haploinsufficient tumor suppressor** in B-cell lymphoma
- **FBXO45** regulates proper **germinal center B cell development**
- **GEF-H1** is required for proper **germinal center B cell** formation
- **GEF-H1** is a novel B cell **oncogene** in humans

Sahasrabuddhe AA, Chen X *et al., The FBXO45-GEF-H1 axis controls germinal center formation and B-cell lymphomagenesis. Cancer Discov. 2025*

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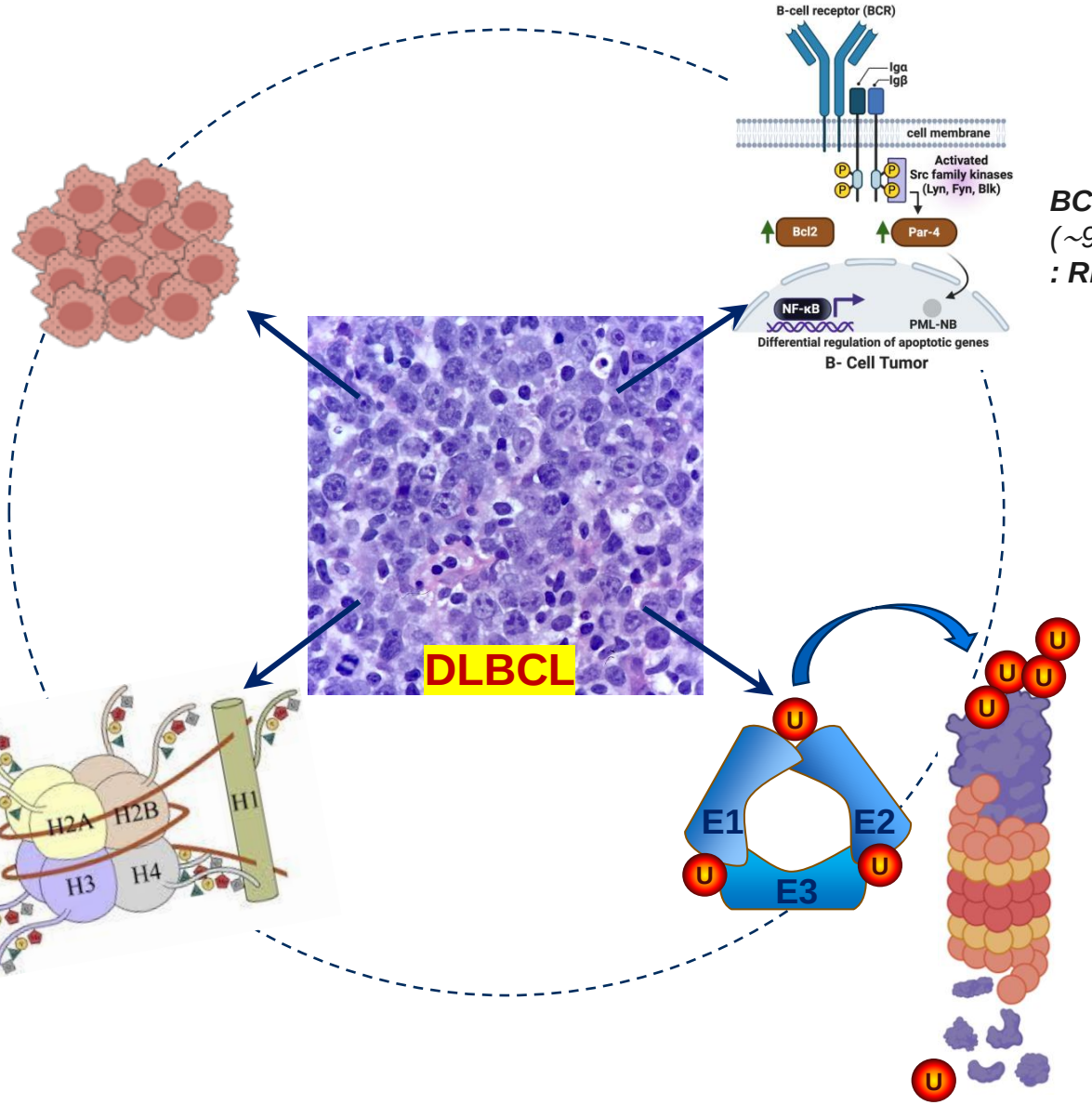
Pathogenetic mechanisms in DLBCL

Activation of anti-apoptotic signaling

BCL2 (~30%), MYC (~10%), BCL6 (20%)

Cell signaling activation

BCR : CD79B *(~20%),* ***NFKB : CARD11*** *(~9%),* ***PI3K-AKT : FOXO1*** *(~10%),* ***GTPases : RHOA*** *(~9%)*



Inactivation of histone/chromatin modifiers

KMT2D *(~30%),* ***CREBBP*** *(~25%),* ***EP300*** *(~5%)*

Inactivation of regulated proteolysis

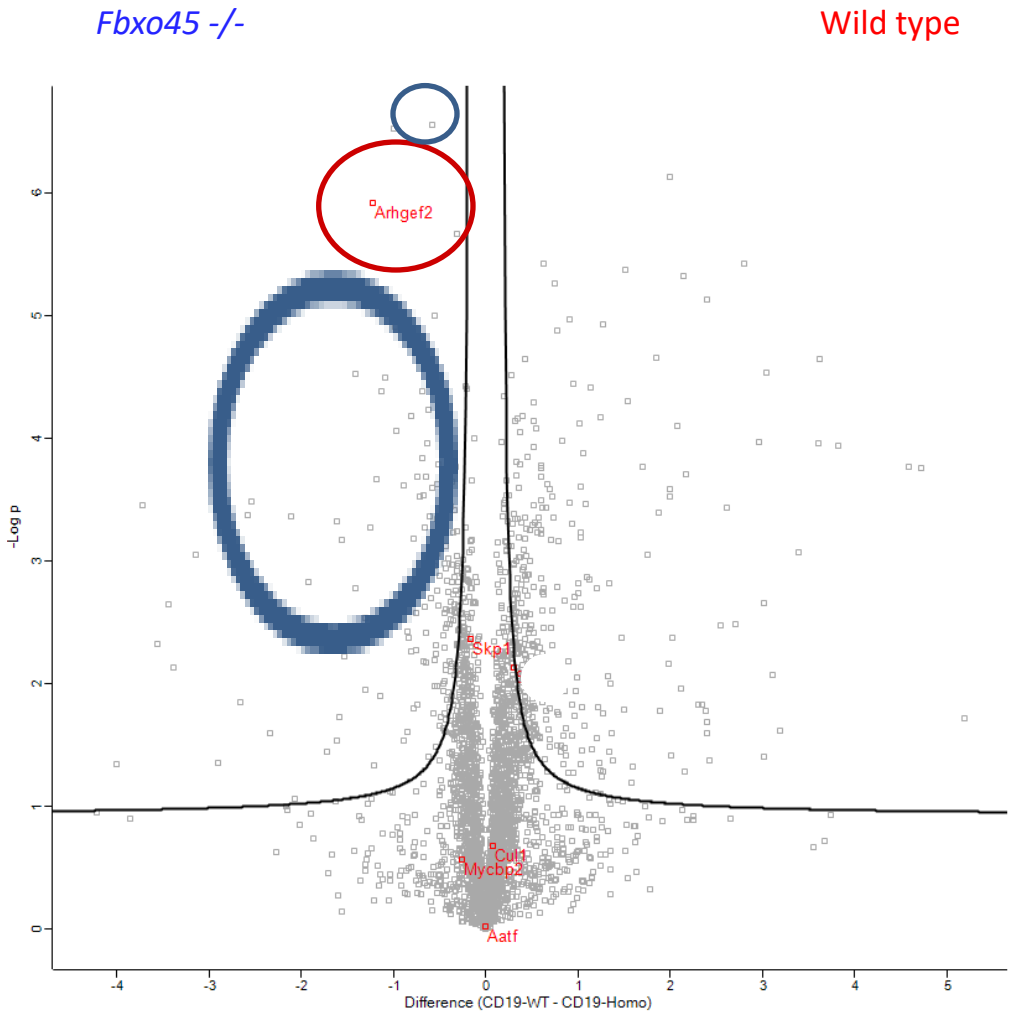
FBXO10 *(mutational inactivation),* ***FBXO11*** *(~6%),* ***KLHL6*** *(~15%)* ***FBXO45*** *(~3%)*

Pasqualucci L. et. al., Blood 2018
Schmitz R. et. al., NEJM 2018
Chiorazzi M. et. al., PNAS 2013
Duan S. et. al., Nature 2012
Choi et al., Nature Cell Biology
Sahasrabuddhe A.A. et. al., Cancer Discovery 2025

Increased steady state levels of GEF-H1 protein in splenic GCB-cells of conditional *Fbxo45*$^{-/-}$ transgenic mice

Significantly Differentially Expressed Proteins

- Significance determined by combination of t-test and bootstrapping (250 randomizations)
- FDR <0.05



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