Molecular mechanisms of B lymphomagenesis induced by TRAF3 inactivation

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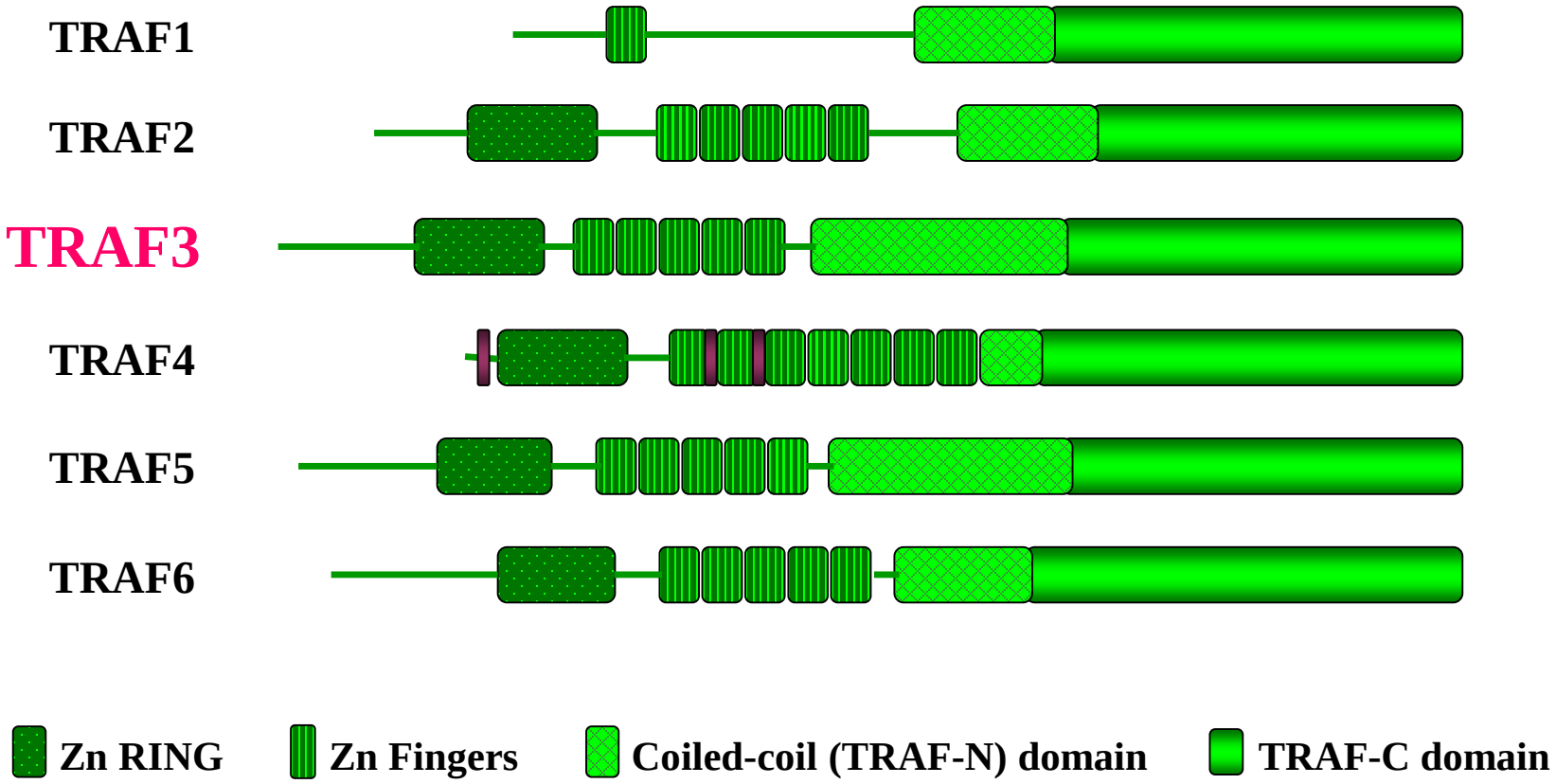
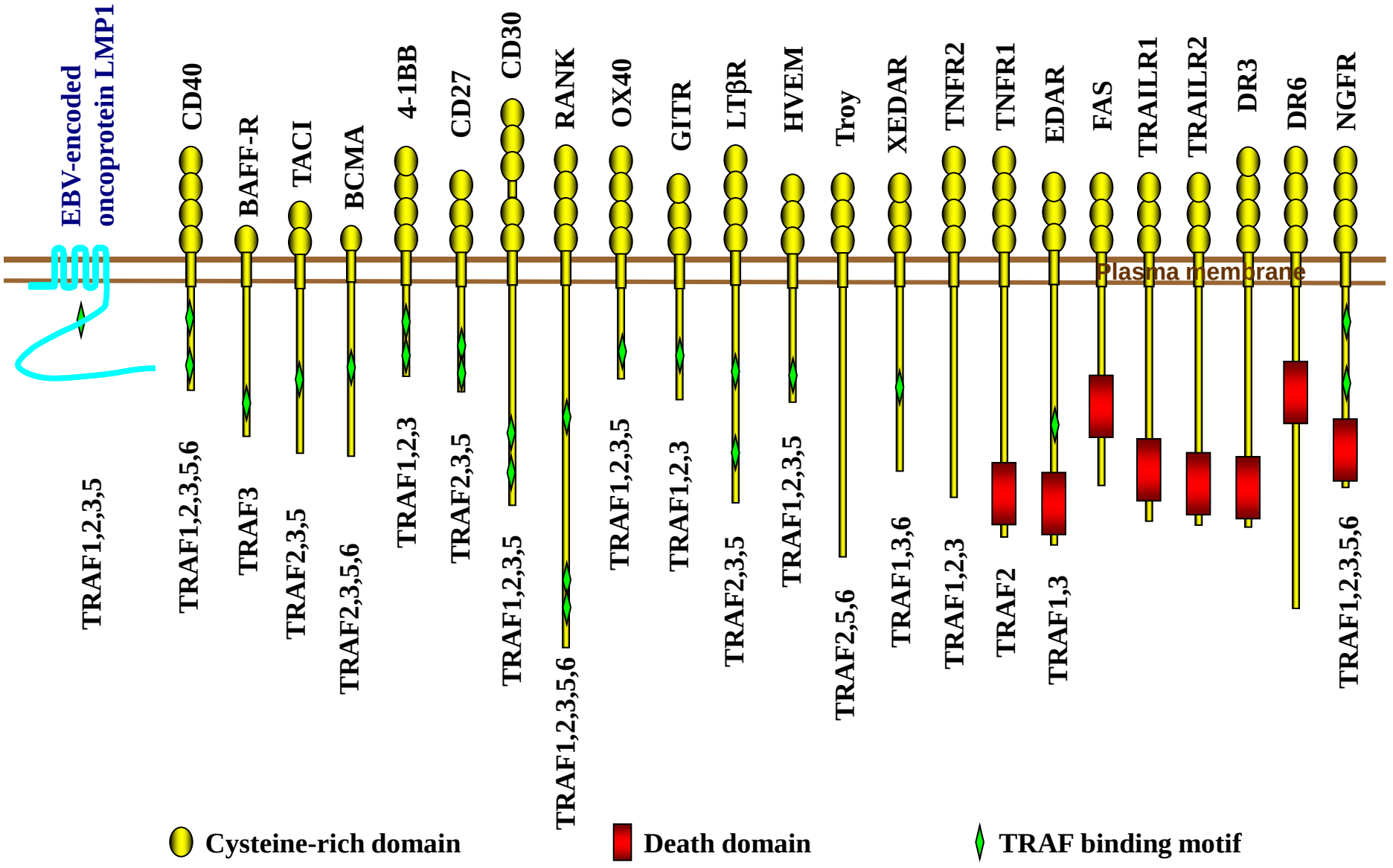
The TNFR-associated factor (TRAF) family of adaptor proteins



Shared use of TRAF3 by the TNF-R superfamily



Mice genetically deficient in TRAF3 show early lethality

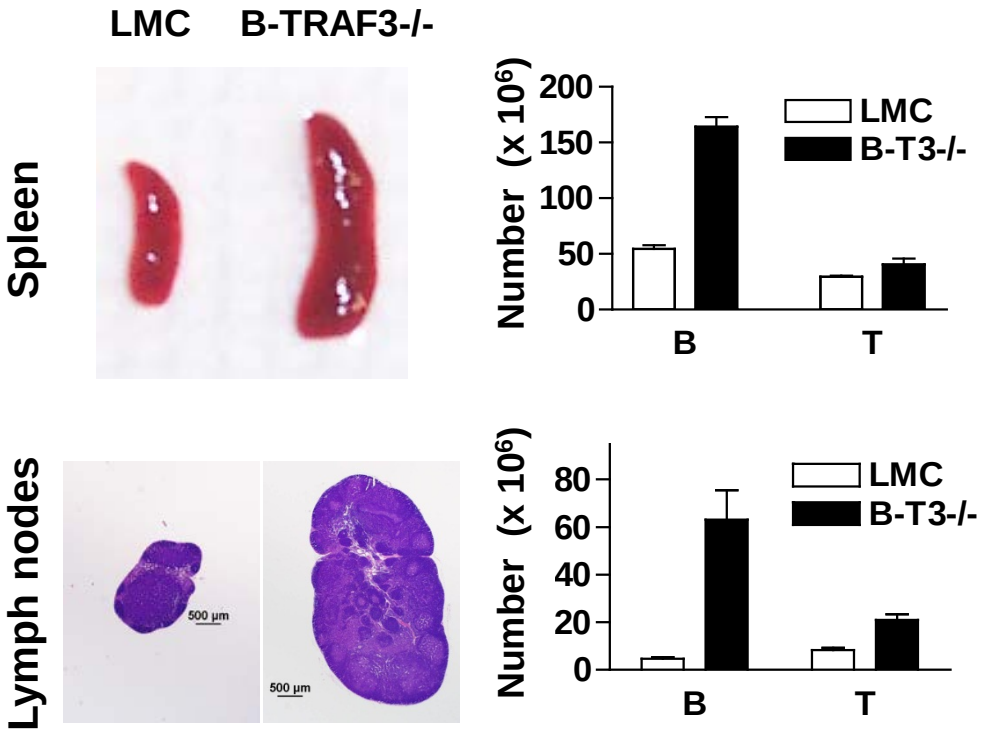
TRAF3$^{-/-}$ mice (Xu et al., 1996, *Immunity* 5: 407-415):

- **die by 10 days of age.**
- **have smaller lymphoid organs, and exhibit a progressive depletion in all lineages of white blood cells in the periphery.**

We generated conditional TRAF3 knockout mice (TRAF3$^{flox/flox}$) as a model system to investigate the *in vivo* function of TRAF3.

TRAF3$^{flox/flox}$CD19$^{+/Cre}$: B cell-specific TRAF3$^{-/-}$ (B-TRAF3$^{-/-}$) mice

B-TRAF3$^{-/-}$ mice exhibit enlarged spleen and lymph nodes



- **Prolonged survival of mature B cells**
- **Constitutive NF-κB2 activation**

➢ **Autoimmune manifestations**

Xie et al., *Immunity,* 27: 253-267, 2007

TRAF3 mutations in human patients with B cell malignancies

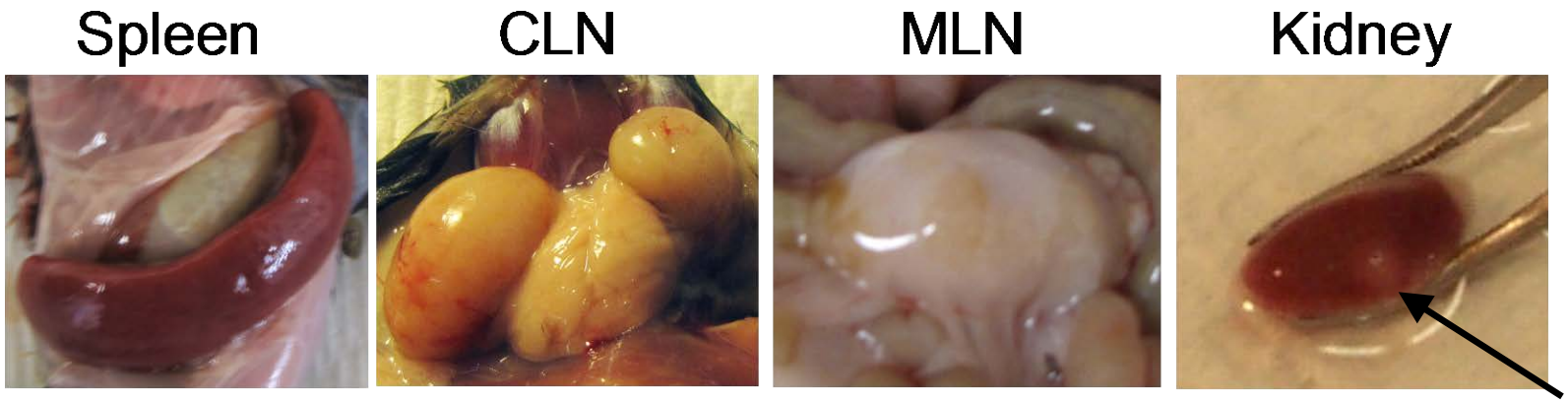
- Homozygous deletions and inactivating mutations of the TRAF3 gene
  - multiple myeloma (MM)
  - splenic marginal zone lymphoma (SMZL)
  - B cell chronic lymphocytic leukemia (B-CLL)
  - mantle cell lymphoma (MCL)
  - Waldenström's macroglobulinemia (WM)

Carissa Moore

TRAF3: a tumor suppressor gene in B cells?

Hypothesis: B-TRAF3$^{-/-}$ mice may spontaneously develop B lymphoma as they age.

B-TRAF3$^{-/-}$ mice spontaneously develop B lymphomas

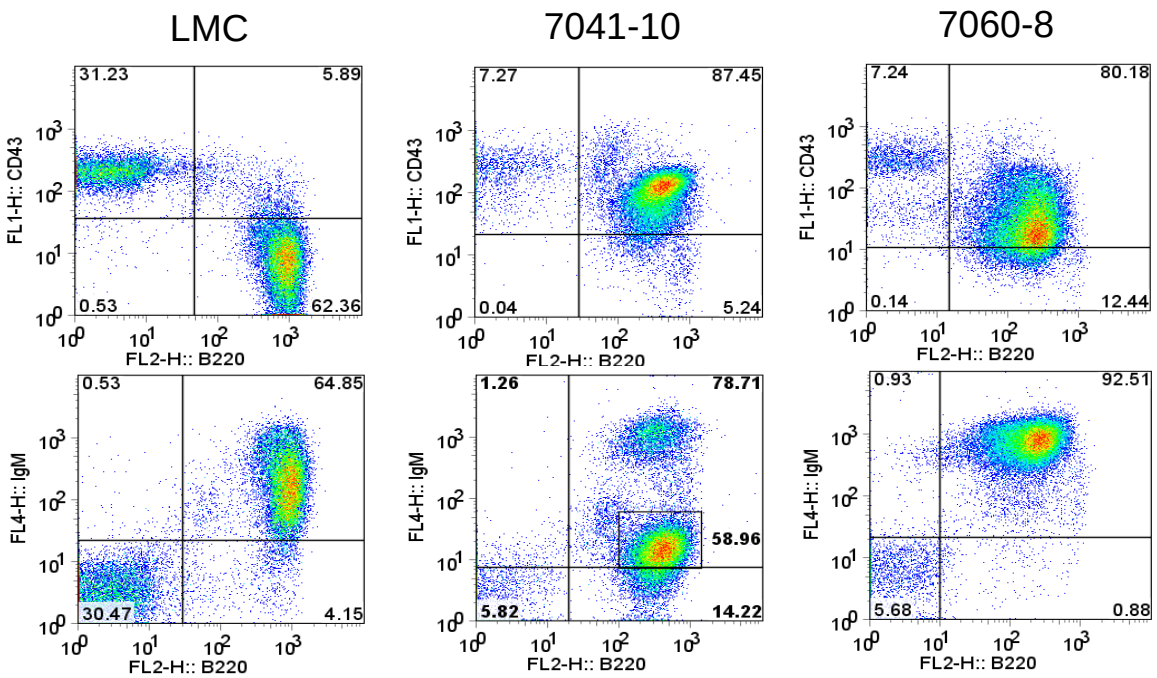


Mice examined	Number of mice with B lymphomas							
(total number)	Spleen	Ascites	BM	CLNs	MLNs	Kidney	Lung	Liver
Mice without overt external symptoms **n=32**	20	6	7	5	5	4	3	0
Moribund mice **n=18**	18	9	9	3	0	5*	6	3

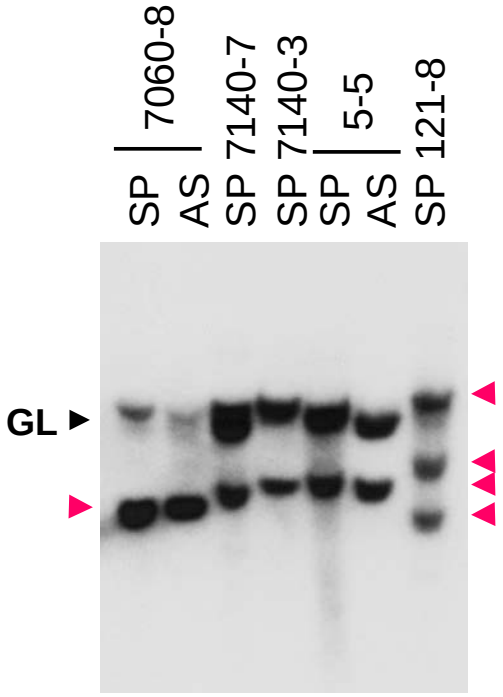
Moore et al., *Leukemia*, 26: 1122-1127, 2012

TRAF3$^{-/-}$ B lymphomas were distinguished from normal B lymphocytes

Flow cytometric analysis of splenic B cells



Southern blot of IgH



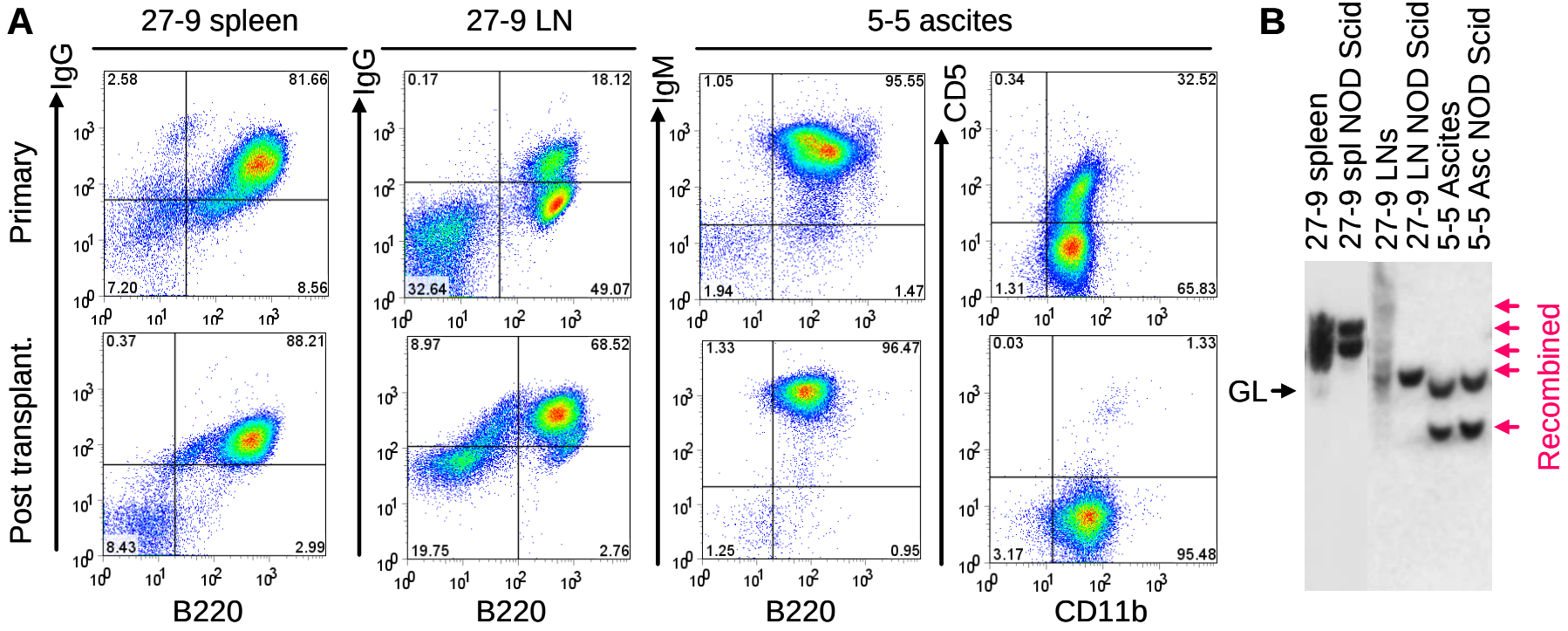
Moore et al., *Leukemia*, 26: 1122-1127, 2012

TRAF3$^{-/-}$ B lymphomas do not contain somatic hypermutation in the IgH gene V(D)J region

Mouse ID	Organ	IgH V gene	Frequency	Somatic hypermutation
7041-10	Spleen	VH36-60.a2.90	18/20	No
		VH7183.a19.31	2/20	No
7060-8	Spleen	VH7183.a25.43 (or VH283)	8/19	No
		VH36-60.a2.90	11/19	No
	Ascites	VH7183.a25.43 (or VH283)	11/18	No
		VH36-60.a2.90	6/18	No
		VH7183.a47.76	1/18	No
5-5	Spleen	VH98-3G (VH7183.a21.35)	15/21	No
		VS107.a3.106	3/21	No
		VH7183.a2.3	1/21	No
		VH36-60.a2.90	1/21	No
		VH7183.a7.10	1/21	No
7079-8	Ascites	VH7183.a2.3 (7183.2.3)	18/21	No
		V98-3G	2/21	K16?
		VH7183.a28.48	1/21	Yes

B-TRAF3$^{-/-}$ mice spontaneously develop marginal zone lymphoma (MZL) or B1 lymphomas.

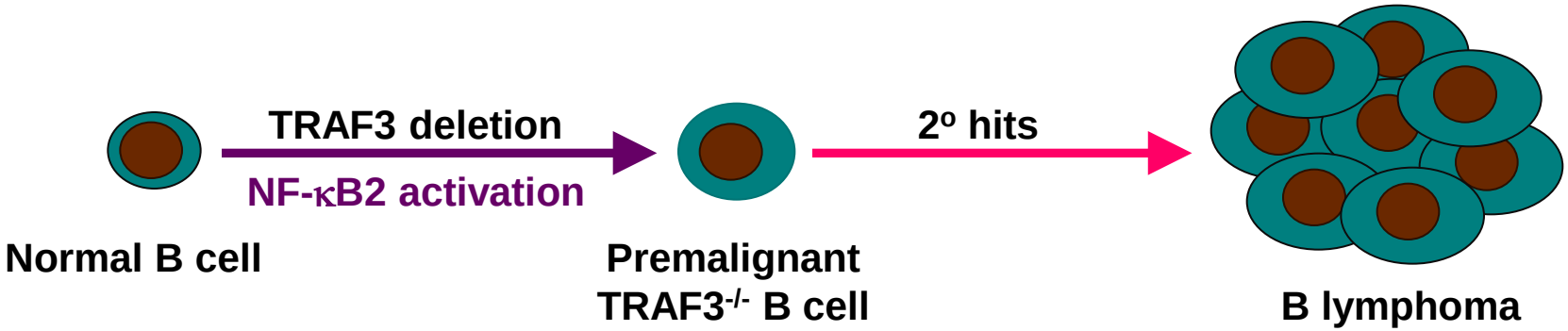
TRAF3$^{-/-}$ B lymphoma cells are transplantable in immunodeficient NOD SCID recipient mice



- **Immortalized cell lines**
  - » 105-8
  - » 27-9
  - » 115-6

Identification of secondary oncogenic hits involved in TRAF3 inactivation-induced B lymphagenesis

Microarray analyses: Dr. Ronald Hart



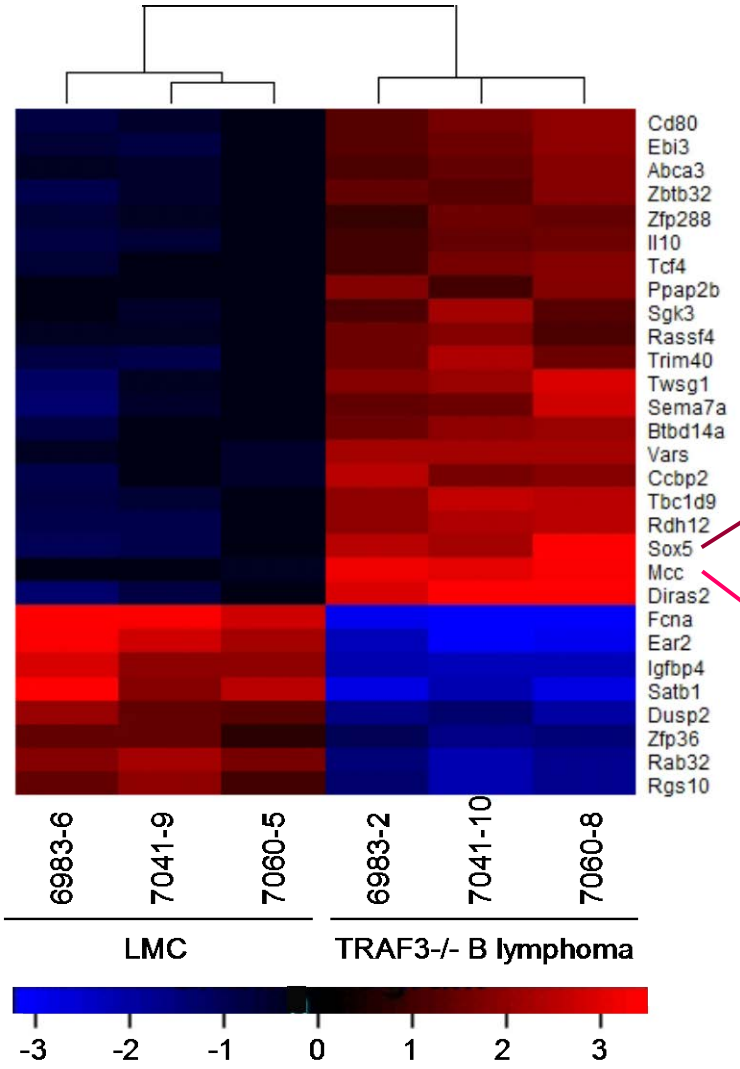
- **160 up-regulated genes**
- **244 down-regulated genes**

Up-regulation verified by Taqman qPCR:

MCC, *Sox5*, *Diras2*, *Tbc1d9*, *Ccbp2*, *Btbd14a*, *Sema7a*, *Twsg1*, *Ppap2b*, *TCF4*, *Tnfrsf19*, *Zcwpw1*, and *Abca3*, etc.

Sox5 and MCC are aberrantly up-regulated in TRAF3-/- mouse B lymphomas

Shanique Edwards



A novel isoform of Sox5

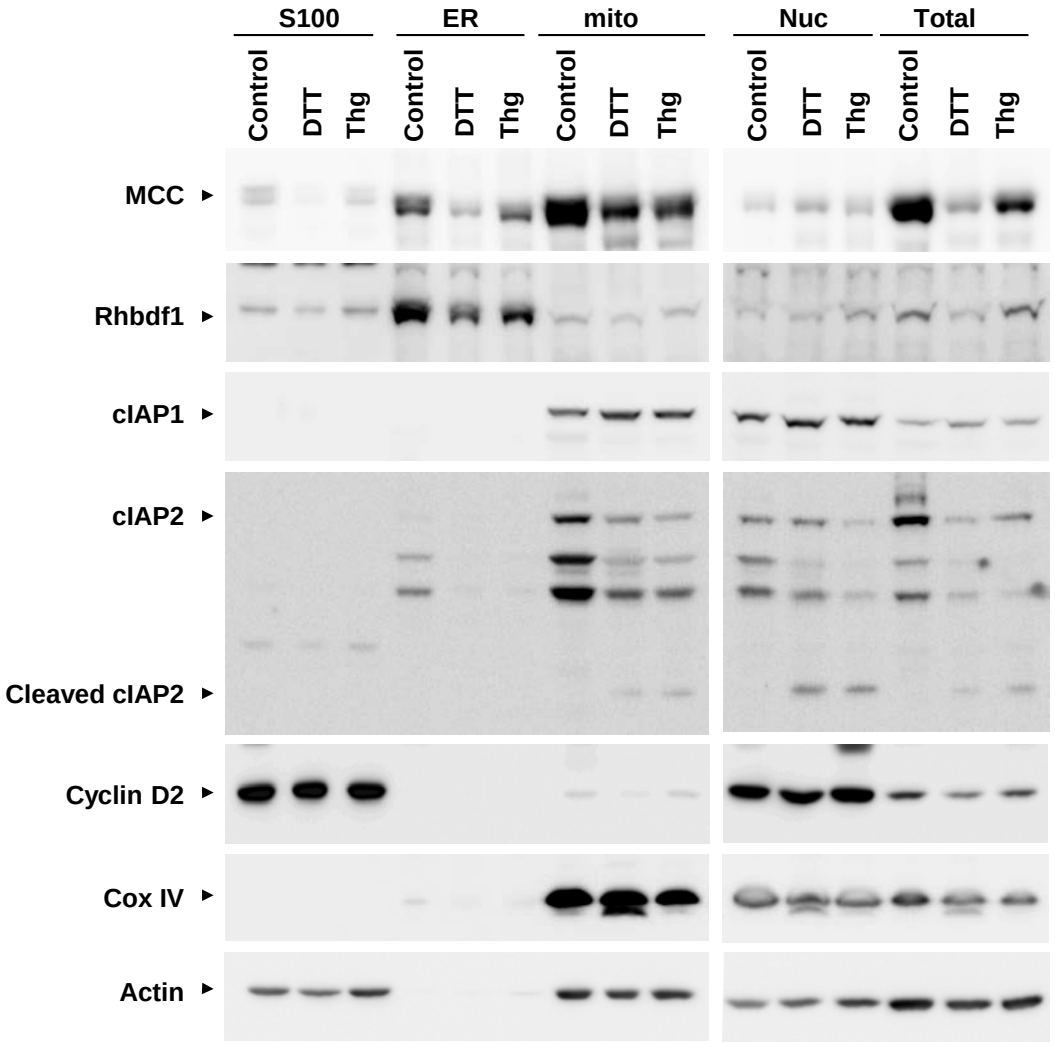
Edwards et al., *Leukemia Res.*, 38: 393-401, 2014

MCC:
Mutated in colorectal cancer

Evidence led us to further study MCC

- **MCC was identified as a tumor suppressive gene in colorectal cancer. However, the function of MCC in B cells has not been studied.**
- **MCC is aberrantly up-regulated in in TRAF3-deficient mouse B lymphomas and human patient-derived MM cell lines.**
- **Aberrant MCC up-regulation is frequently detected in a variety of primary human B cell neoplasms.**
  - **PEL, CBL, DLBCL, BL, and MM**
- **MCC expression was not detected in normal or premalignant TRAF3$^{-/-}$ B cells even after treatment with B cell stimuli.**
- **Lentiviral shRNA vector-mediated knockdown of MCC induced apoptosis and inhibited proliferation in human MM cells.**

MCC is mainly localized at mitochondria in human MM cells



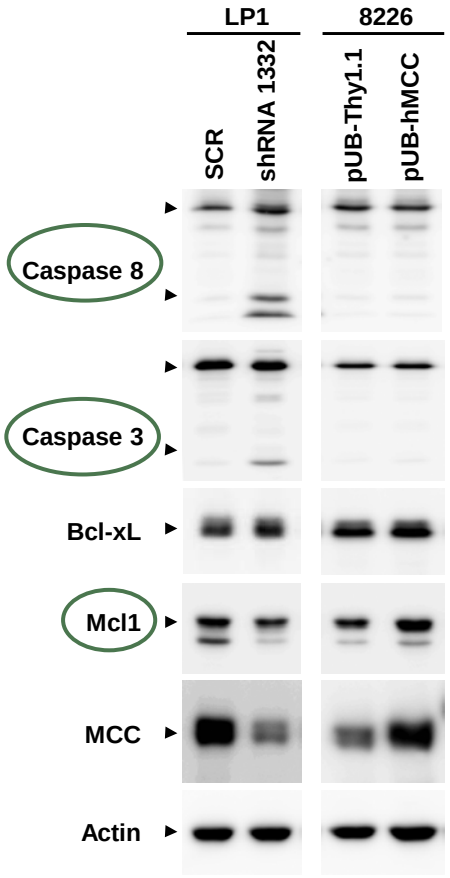
ER stress inducers

- DTT
- Thapsigargin (Thg)

- **The ER stress inducers DTT and thapsigargin induced apoptosis and decreased MCC protein levels in human MM cells.**

MCC regulates different signaling pathways in human MM cells versus other cancers

- **Known MCC targets in colorectal cancer and hepatocellular carinoma**
  - Phospho-β-catenin, β-catenin, IκBα, IκBβ, and RelA ✗



- **MCC downstream targets in human MM cells**
  - Mcl-1,caspase 8, and caspase 3
  - p27, cyclin B1, Phospho-ERK, c-Myc

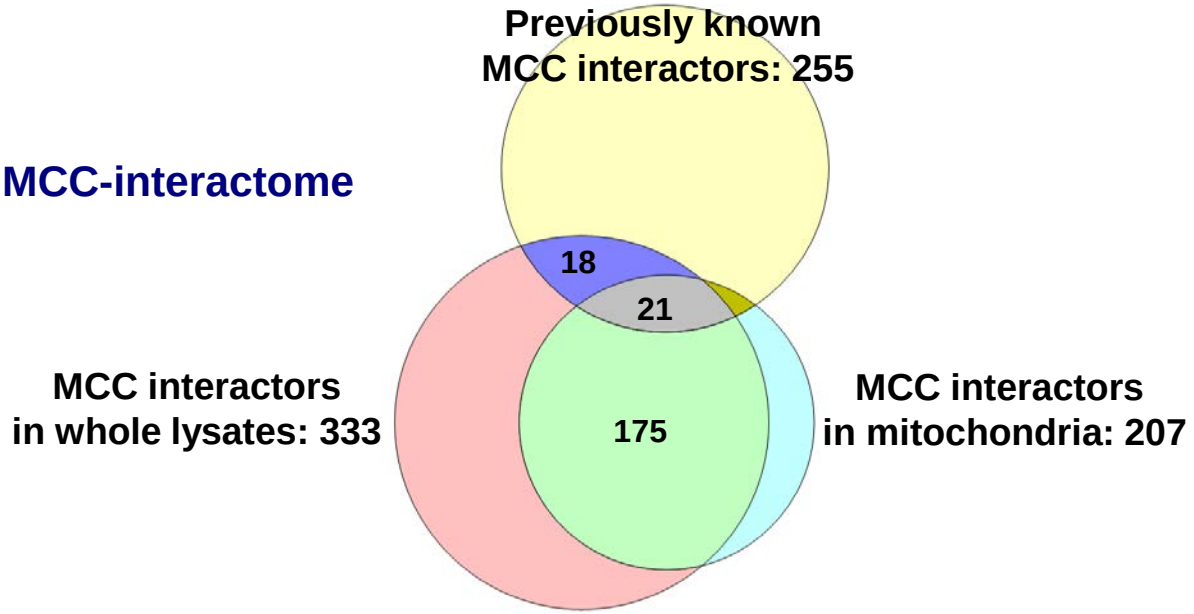
- **Additional regulators <u>not</u> changed by MCC knockdown or overexpression:**
  - Bcl2, Bim, Bad, Bid, Bik
  - cyclin D1, cyclin D2, p21, E2F1, p53
  - P-p38, P-JNK, P-Akt

MCC interacting proteins in human MM cells

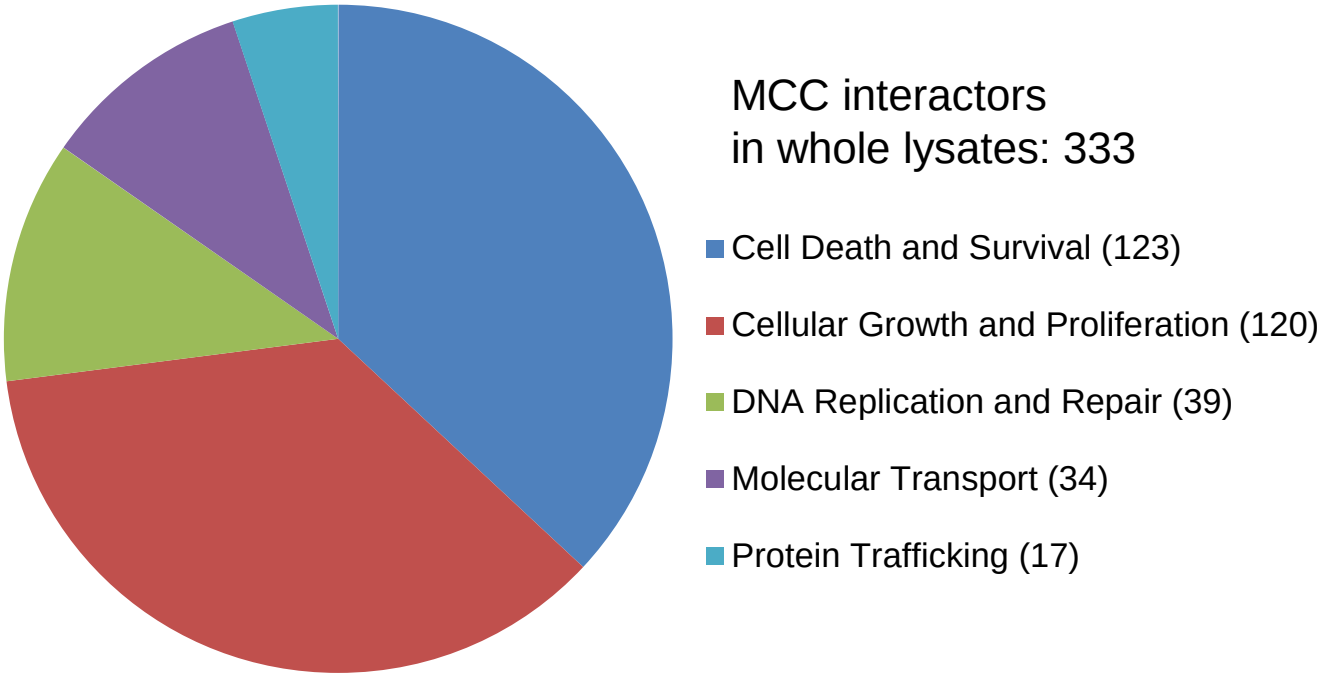
- **Lentiviral expression vectors of MCC for immunoprecipitation**
  - pUB-FLAG-hMCC
  - pUB-hMCC-SBP-6xHis
- **Known MCC interacting partners in colorectal cancer and 293T cells**
  - β-catenin, Mst3, VCP, PP2A, DFFA, VHL, VDAC, scribble, myosin IIb, etc.

LC-MS/MS: Dr. David Perlman, Princeton University

➢ **365 proteins of the MCC-interactome**

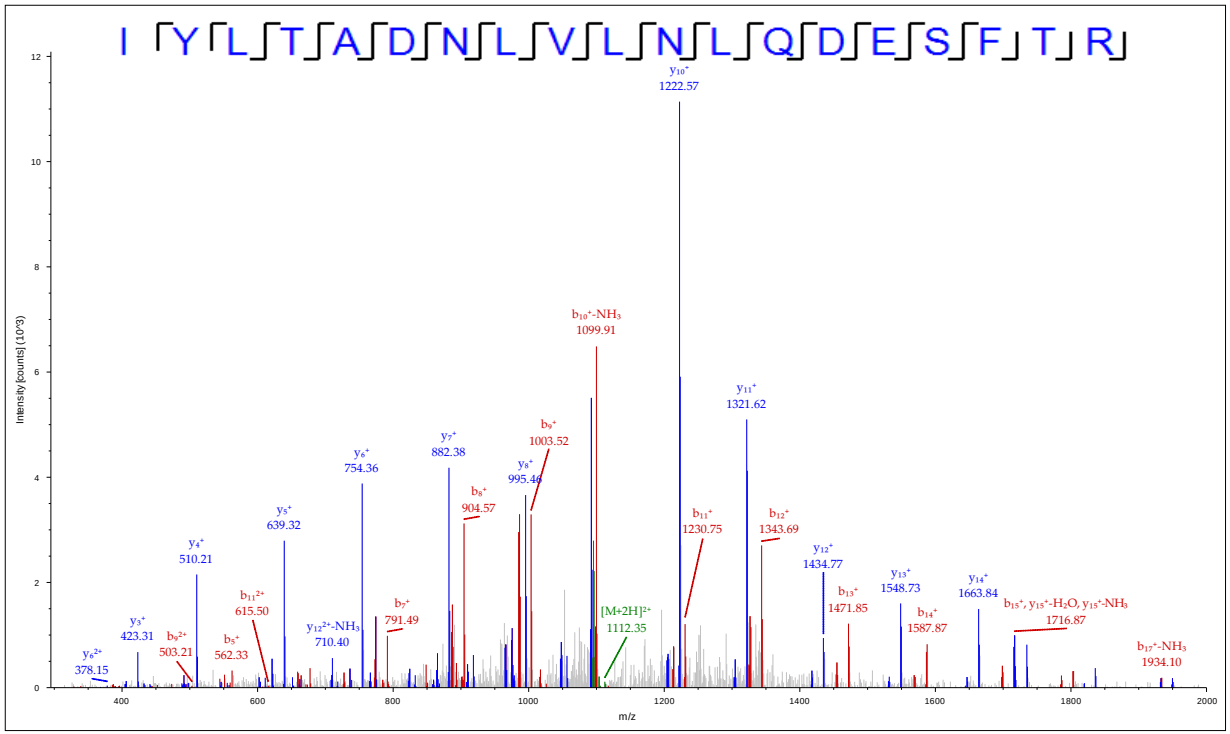


Functional clustering of the MCC-interactome Identified from human MM cells

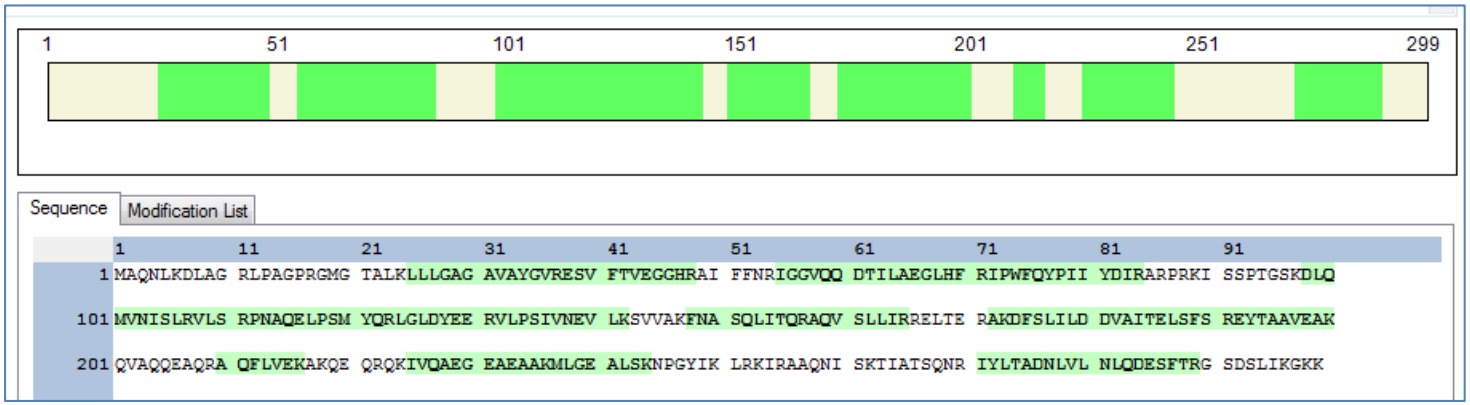


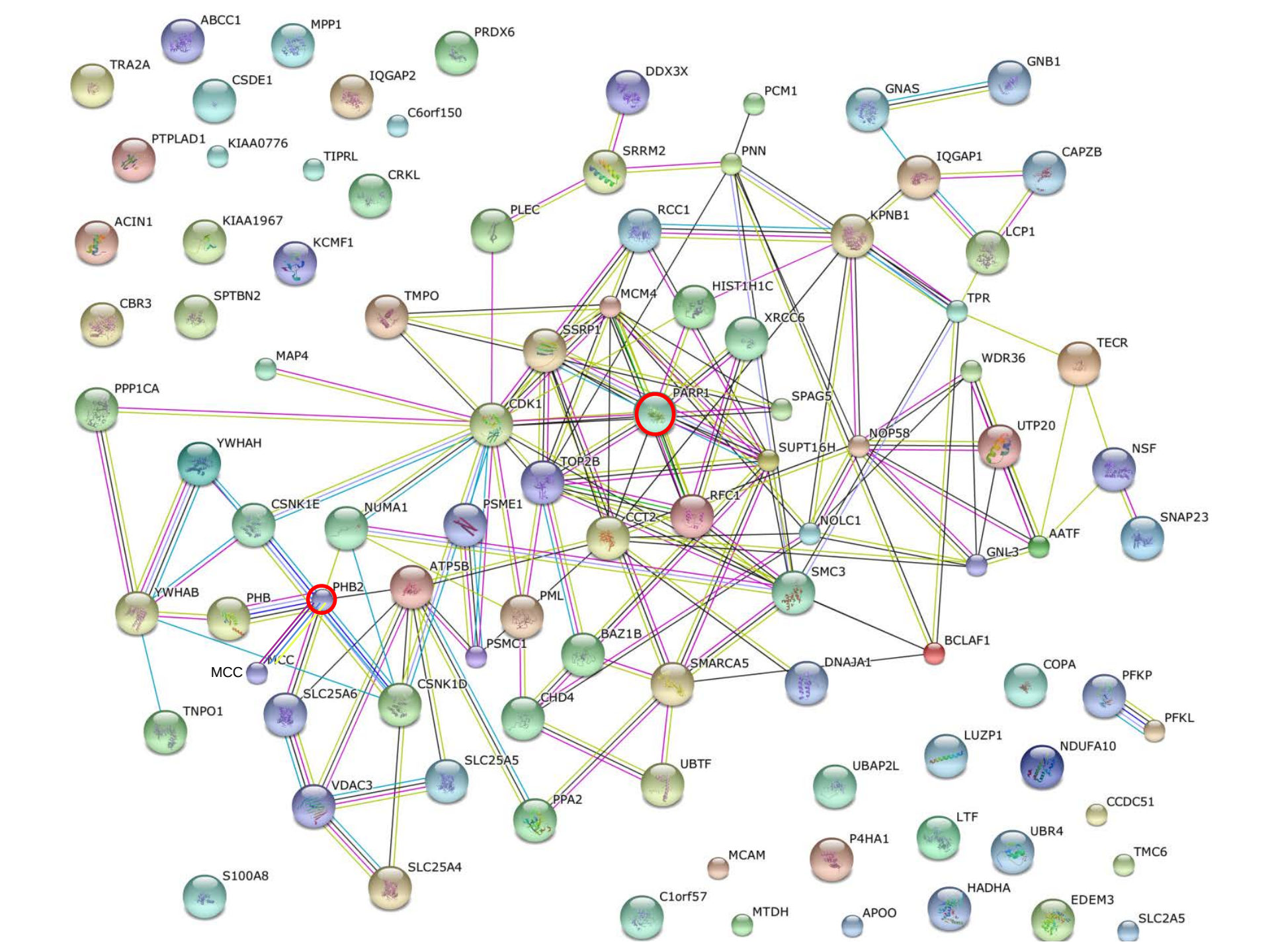
- **Disease association analysis by Ingenuity: cancer**
  - **195 of the 333 (58.5%) MCC interactors in whole lysates**
  - **91 of the 207 (43.9%) MCC interactors in mitochondria**

PHB2, a mitochondrial protein critical for survival, was identified as an MCC-interacting protein in human MM cells



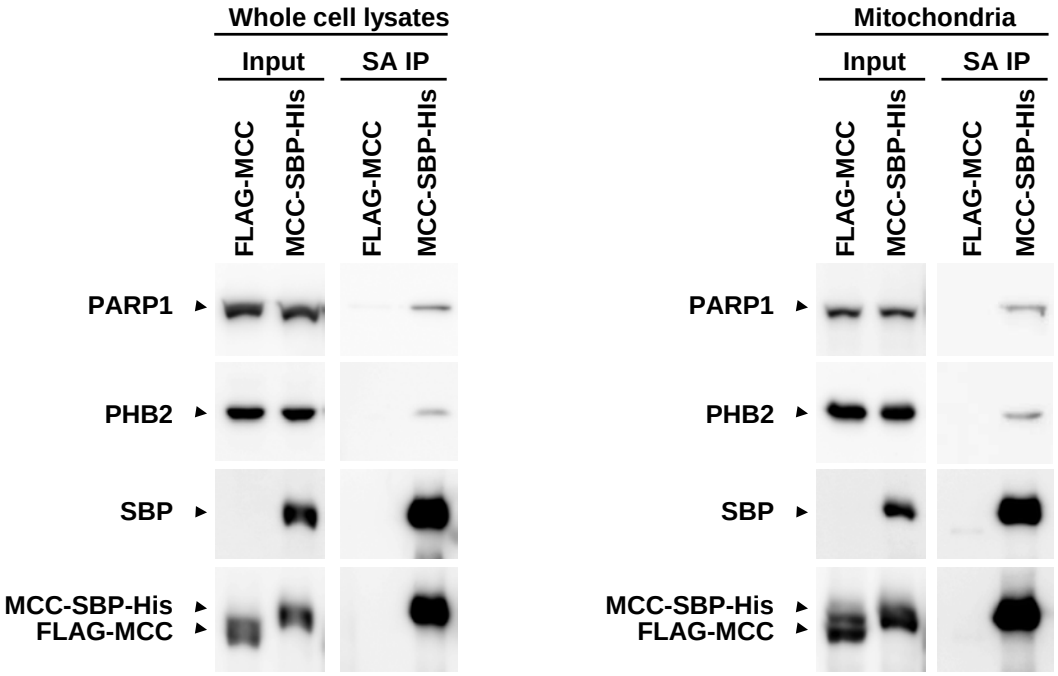
PHB2_Human: 64.21% coverage




ABCC1
MPP1
PRDX6
TRA2A
CSDE1
IQGAP2
C6orf150
DDX3X
PCM1
GNAS
GNB1
PTPLAD1
KIAA0776
TIPRL
CRKL
SRRM2
PNN
IQGAP1
CAPZB
ACIN1
KIAA1967
KCMF1
PLEC
RCC1
KPNB1
LCP1
CBR3
SPTBN2
TMPO
MCM4
HIST1H1C
TPR
XRCC6
SSRP1
MAP4
TECR
WDR36
PPP1CA
CDK1
PARP1
SPAG5
UTP20
YWHAH
NOP58
SUPT16H
NSF
TOP2B
CSNK1E
NUMA1
PSME1
RFC1
CCT2
NOLC1
SNAP23
AATF
GNL3
ATP5B
SMC3
YWHAB
PHB
PHB2
PML
BAZ1B
BCLAF1
PSMC1
MCC
SMARCA5
DNAJA1
COPA
PFKP
SLC25A6
CSNK1D
CHD4
PFKL
TNPO1
LUZP1
NDUFA10
SLC25A5
UBTF
UBAP2L
CCDC51
VDAC3
PPA2
LTF
UBR4
P4HA1
TMC6
MCAM
S100A8
SLC25A4
HADHA
C1orf57
MTDH
APOO
EDEM3
SLC2A5

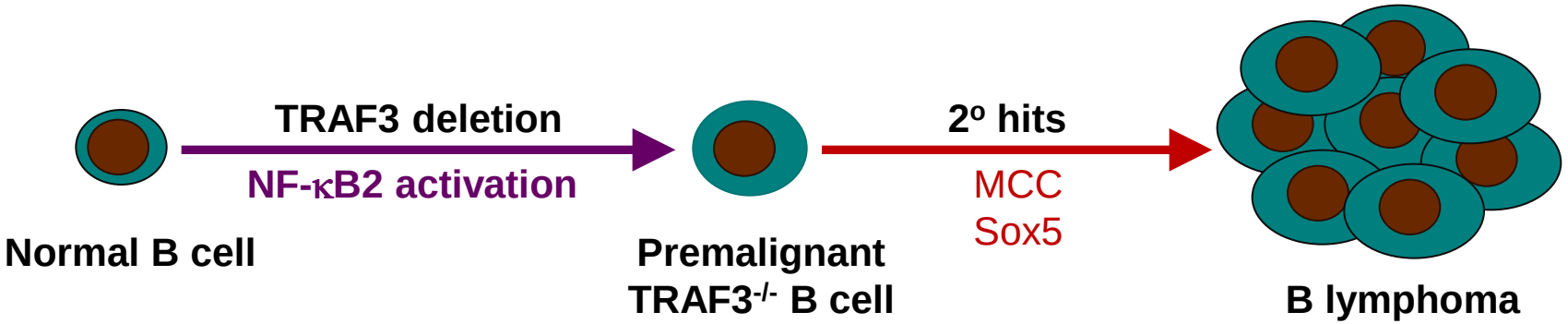
PARP1 and PHB2 were co-immunoprecipitated with MCC in human MM cells

- **PARP1: the top novel MCC-interacting protein**
- **PHB2: the top previously known MCC-interacting protein**
- **Both are known regulators of cell survival and proliferation.**
- **Both regulate the MCC targets identified by knockdown and overexpression**
  - Phospho-ERK, cyclin B1, p27, c-Myc
  - Mcl-1,caspase 8, and caspase 3



Edwards et al., *J. Hematol. & Oncol.*, 7:56, p1-24, 2014.

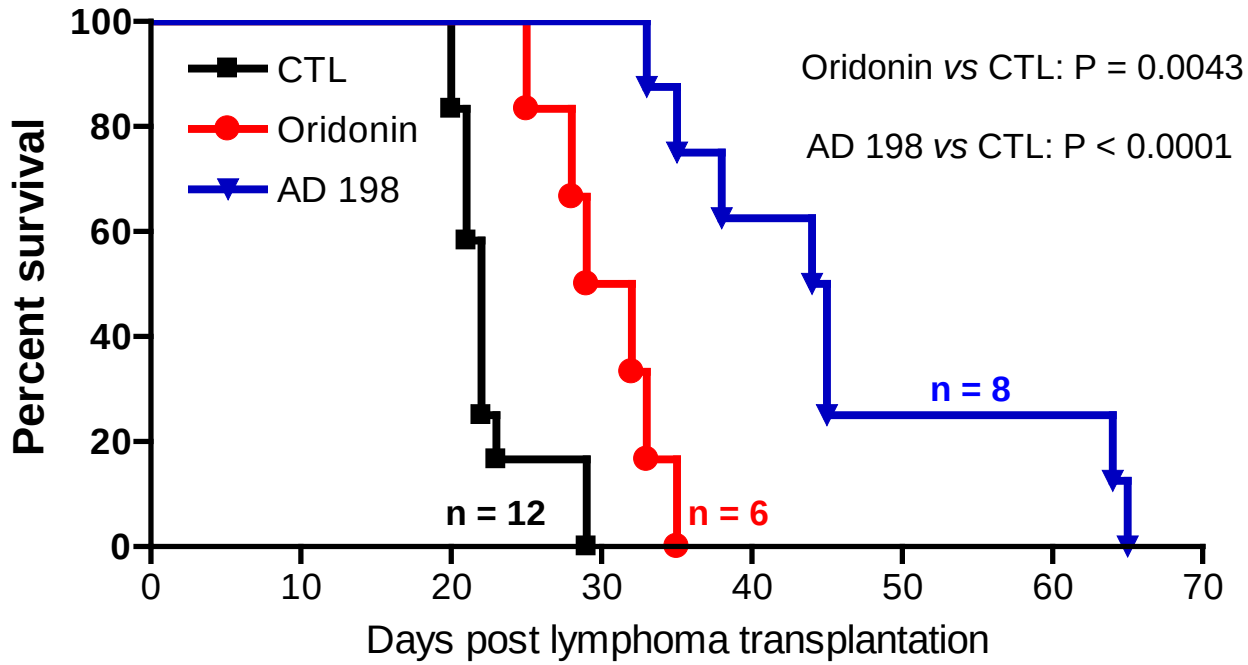
Complex mechanisms of TRAF3 inactivation-mediated oncogenic survival and malignant transformation of B cells



Genetic	**Data analysis**	**RNA-Seq**
Epigenetic	**Global changes**	**ChIP-Seq** H3K27me3 H3K9/14ac
Expression		
mRNA	**160 ↑ and 244 ↓ genes**	**microarray**
microRNA	**Will do**	**microarray**
Metabolic	**Data analysis**	**LC-MS**

Translational study

- **Diagnostic markers: TRAF3 inactivation, Rhbdf1, Sox5, MCC, etc.**
- **Therapeutic targets: NF-κB2, PKCδ, Rhbdf1, PC, MCC, etc.**



Edwards et al., *BMC Cancer* 13:481, p1-20, 2013.

- **To test: PARP inhibitors, PHB2 ligands, etc.**

Acknowledgement

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