

Supplementary Information

FOXO1 forkhead domain mutants in B-cell lymphoma lack transcriptional activity

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Supplementary Figure S1. FOXO1 forkhead domain mutations in B-cell malignancies. Mutations reported in the literature, in COSMIC and in cBioPortal (December 6, 2021). Studied variants are highlighted in gray. CLL: chronic lymphocytic leukemia; DLBCL: diffuse large B-cell lymphoma; BL: Burkitt lymphoma; CNSL: central nervous system lymphoma; FL: follicular lymphoma; PTL: post-transplant lymphoproliferative disorder; MZL: marginal zone lymphoma.

| CDS mutations | AA mutations | Lymphomas | Counts | References |
|---------------------|---------------------|---|-----------------------|--|
| c.455G>A | p.S152N | CLL | 1 | COSM7208266 |
| c.456C>A | p.S152R | DLBCL (2), BL (2), CNSL (1) DLBCL | 5 1 | COSM9213941 Trinh et al, 2013 |
| c.457A>G | p.S153G | DLBCL | 1 | Reddy et al, 2017 |
| c.459C>G | p.S153R | DLBCL | 1 | Trinh et al, 2013 |
| c.486C>G | p.N162K | DLBCL DLBCL | 1 1 | COSM8438787 cBioPortal TCGA-VB-A8QN |
| c.496_588del | p.A166_Y196del | DLBCL | 1 | COSM9863544 |
| c.496G>A | p.A166T | DLBCL | 1 | Reddy et al, 2017 |
| c.497C>T | p.A166V | DLBCL DLBCL DLBCL | 4 2 1 | COSM9866300 Trinh et al, 2013 Reddy et al, 2017 |
| c.497C>G | p.A166G | DLBCL | 1 | Trinh et al, 2013 |
| c.502C>G | p.L168V | DLBCL | 1 | Trinh et al, 2013 |
| c.511A>G | p.K171E | DLBCL | 1 | Trinh et al, 2013 |
| c.514G>A | p.A172T | DLBCL | 1 | Reddy et al, 2017 |
| ns | p.I173M | BL | 1 | Zhou et al, 2019 |
| c.524G>A | p.S175N | DLBCL | 1 | COSM6954168 |
| c.524G>C | p.S175T | DLBCL | 1 | Dubois et al, 2016 |
| ns | p.I175R | BL | 1 | Zhou et al, 2019 |
| c.536_547del | p.K179_L183delinsM | DLBCL | 1 | COSM9863413 |
| c.540_626delinsA | p.L181Efs*7 | DLBCL | 1 | COSM9862360 |
| c.548T>C | p.L183P | DLBCL | 1 | Trinh et al, 2013 |
| c.557T>C | p.I186T | DLBCL | 1 | Trinh et al, 2013 |
| ns | p.I186S | BL | 1 | Zhou et al, 2019 |
| c.567G>A | p.W189* | FL | 1 | COSM1316591 |
| c.568A>G | p.M190V | DLBCL | 1 | Reddy et al, 2017 |
| c.578G>A | p.S193N | DLBCL | 1 | COSM7338533 |
| c.579C>A | p.S193R | FL | 1 | COSM220649 (Morin et al, 2011) |
| c.581T>C | p.V194A | BL | 2 | COSM9213939 |
| c.588C>G | p.Y196* | DLBCL | 1 | COSM9867401 |
| c.592A>T | p.K198* | DLBCL | 1 | COSM9868106 |
| c.595G>C | p.D199H | PTLD | 1 | COSM7338768 |
| c.598A>T | p.K200* | PTLD | 1 | COSM7338760 |
| c.608G>A | p.S203N | FL | 1 | COSM220643 (Morin et al, 2011) |
| c.609C>A | p.S203R | PTLD | 1 | COSM7338756 |
| c.611_614delinsGCTA | p.N204_S205delinsSY | DLBCL | 1 | COSM6986743 |
| c.612C>G | p.N204K | MZL | 1 | COSM9264648 |
| c.614G>A | p.S205N | DLBCL DLBCL | 3 1 | COSM5651478 (Morin et al, 2016) Trinh et al, 2013 |
| c.614G>C | p.S205T | DLBCL (1), PTL (1) SU-DHL5 cell line DLBCL DLBCL BL | 2 1 1 1 1 | COSM7338758 Trinh et al, 2013 Dubois et al, 2016 Zhou et al, 2019 |
| ns | p.S206L | BL | 1 | Zhou et al, 2019 |
| ns | p.A207P | BL | 1 | Zhou et al, 2019 |
| c.648T>A | p.N216K | DLBCL FL | 1 1 | COSM4170502 Pasqualucci et al, 2014 |

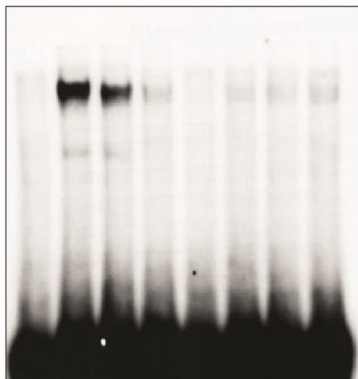
References:

- Morin, R.D., et al., Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*, 2011. 476(7360): p. 298-303.
- Trinh, D.L., et al., Analysis of FOXO1 mutations in diffuse large B-cell lymphoma. *Blood*, 2013. 121(18): p. 3666-74.
- Pasqualucci, L., et al., Genetics of follicular lymphoma transformation. *Cell Rep*, 2014. 6(1): p. 130-40.
- Dubois, S., et al., Next-Generation Sequencing in Diffuse Large B-Cell Lymphoma Highlights Molecular Divergence and Therapeutic Opportunities: a LYSA Study. *Clin Cancer Res*, 2016. 22(12): p. 2919-28.
- Morin, R.D., et al., Genetic Landscapes of Relapsed and Refractory Diffuse Large B-Cell Lymphomas. *Clin Cancer Res*, 2016. 22(9): p. 2290-300.
- Reddy, A., et al., Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma. *Cell*, 2017. 171(2): p. 481-494 e15.
- Zhou, P., et al., Sporadic and endemic Burkitt lymphoma have frequent FOXO1 mutations but distinct hotspots in the AKT recognition motif. *Blood Adv*, 2019. 3(14): p. 2118-2127.

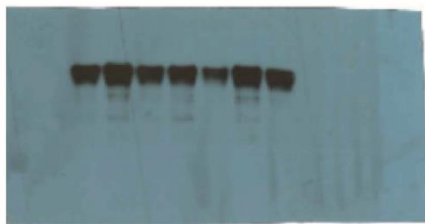
Supplementary Figure S2. Original images of Figure 1C, 3A, 3B.
Unprocessed images of EMSA and western blots.

Original images of Figure 1C.

EMSA. Image detected with a phosphorimager screen overnight.

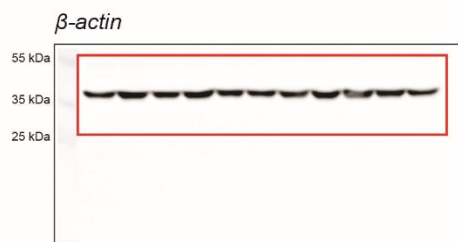
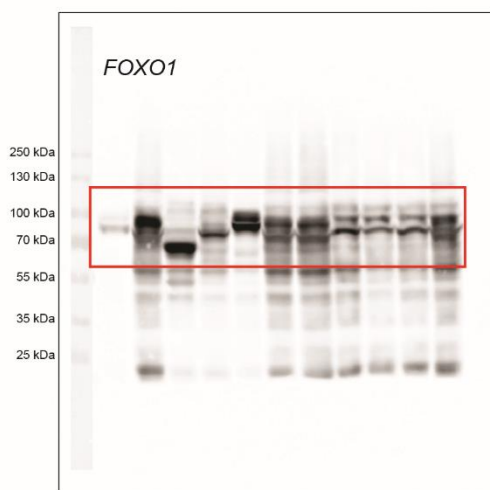


Western blot of FOXO1 expression. Signal detected with "Amersham Hyperfilm ECL". The whole piece of film is shown.

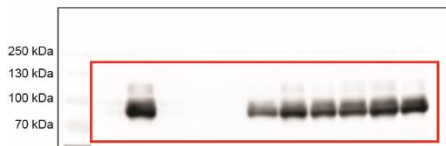


Original images of Figure 3A.

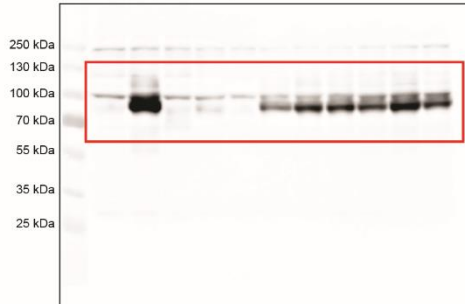
Western blots of phospho- and total FOXO1. Signal detected with FUSION Solo S (Vilber).



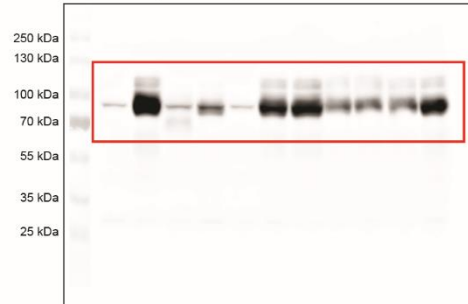
pFOXO1 (Thr24)



pFOXO1 (Ser256)



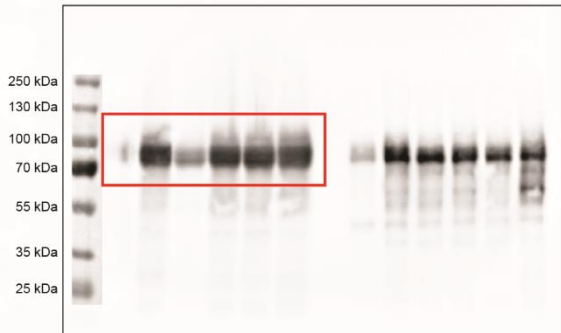
pFOXO1 (Ser319)



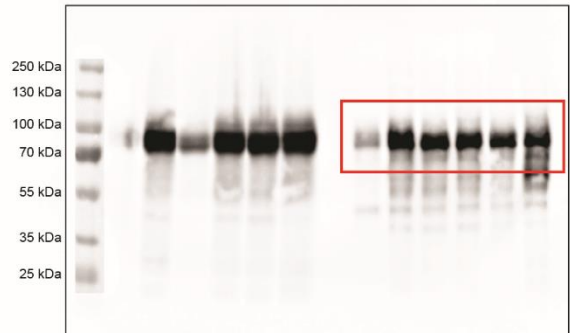
Original images of Figure 3B.

Western blot of phospho- and total FOXO1. Signal detected with FUSION Solo S (Vilber).

FOXO1 (low exposure)



FOXO1 (high exposure)



ATG7 (low exposure)



ATG7 (high exposure)

