

The TLR adaptor protein MyD88 mediates cell sensitivity to HDAC inhibitors through a cytokine-dependent mechanism

Semira Sheikh^{1,2}, Maria New¹, Mina Bekheet¹, Heidi Olzscha¹ and Nicholas B La Thangue¹

¹ Laboratory of Cancer Biology, Department of Oncology, University of Oxford, Old Road Campus Research Building, Old Road Campus, off Roosevelt Drive, Oxford OX3 7FZ, England, UK

² Department of Haematology, Oxford Cancer and Haematology Centre, Oxford University Hospitals NHS Trust, Headington, Oxford OX3 7LE, England, UK
Semira.Sheikh@sjc.ox.ac.uk

Background

Histone deacetylase inhibitors (HDI) are promising new agents for the treatment of haematological malignancy including lymphoma (1).

HDI have potent anti-proliferative effects in cell-based studies; in the clinical setting, however, the picture is quite variable, and the outcome of HDI therapy is complex and difficult to predict (2).

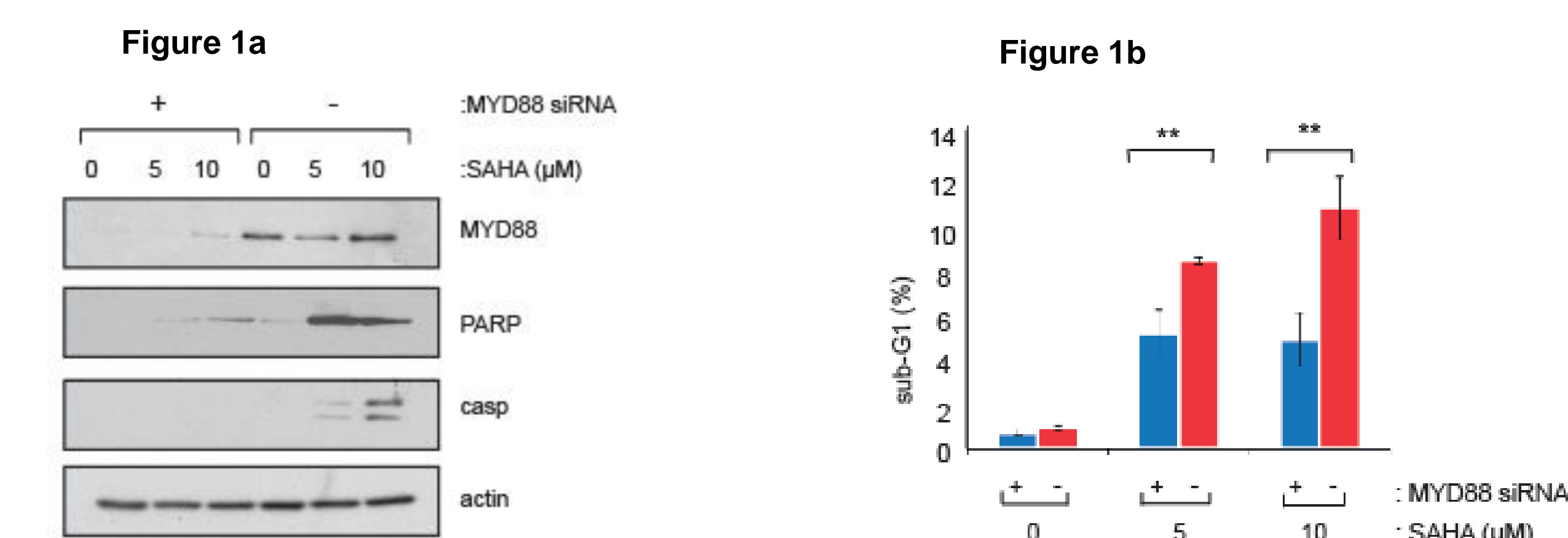
The toll-like receptor (TLR) adaptor protein MyD88 (Myeloid Differentiation Primary Response Gene 88) is expressed at high levels in haematopoietic tissues and has recently been found to be the target for somatic mutation in lymphoid malignancies. In particular, substitution of the amino acid leucine to proline at position 265 (MyD88 L265P mutation) is estimated to be prevalent in over 90% of cases of Waldenstrom's macroglobulinaemia and around 37% of diffuse large B cell lymphoma (DLBCL) of the ABC subtype (3).

An unbiased genome-wide loss-of-function screen identified MyD88 as a key regulator and candidate biomarker which may mediate the susceptibility of cancer cells to HDI (4).

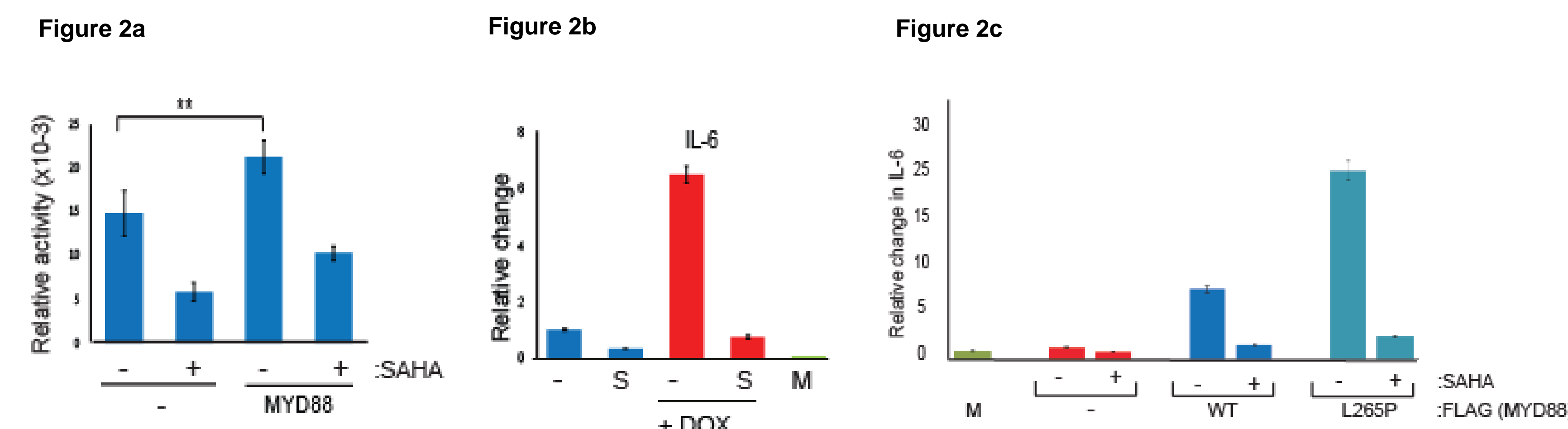
We therefore sought to determine how the expression of MyD88 and its mutant, MyD88 L265P, influences the sensitivity of various cancer cell lines to treatment with an HDI.

Results

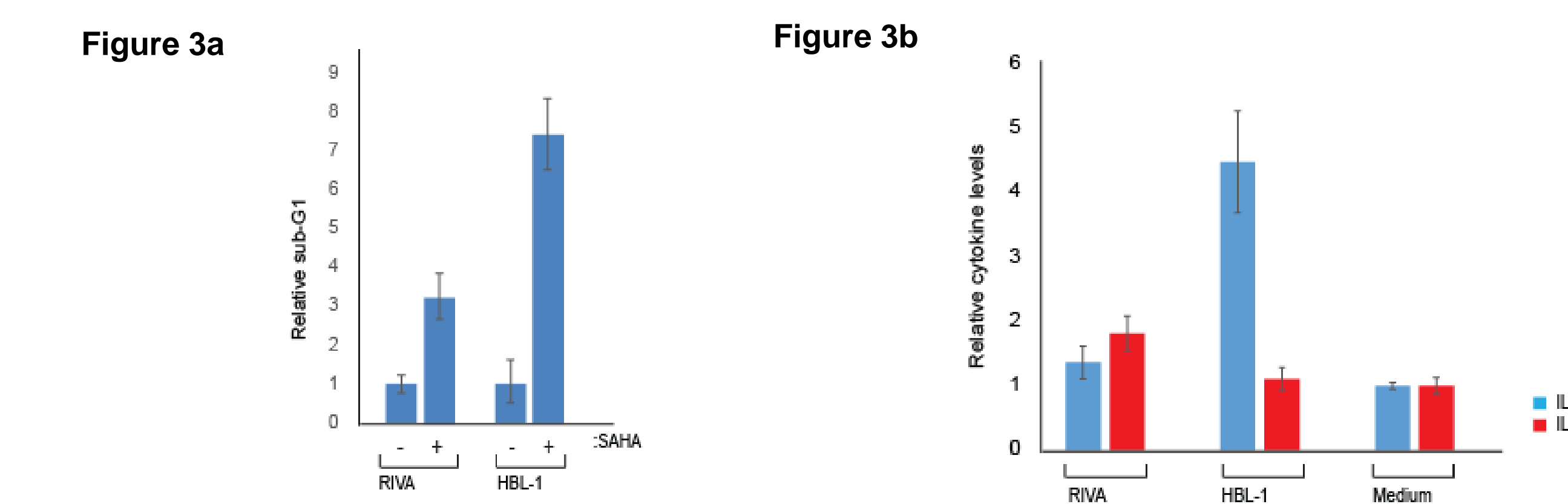
- Short-term siRNA silencing of MyD88 reduced HDI sensitivity of U2OS osteosarcoma cells whereas over-expression of MyD88 and MyD88 L265P led to enhanced sensitivity as assessed by immunoblot (Figure 1a) and FACS (Figure 1b).



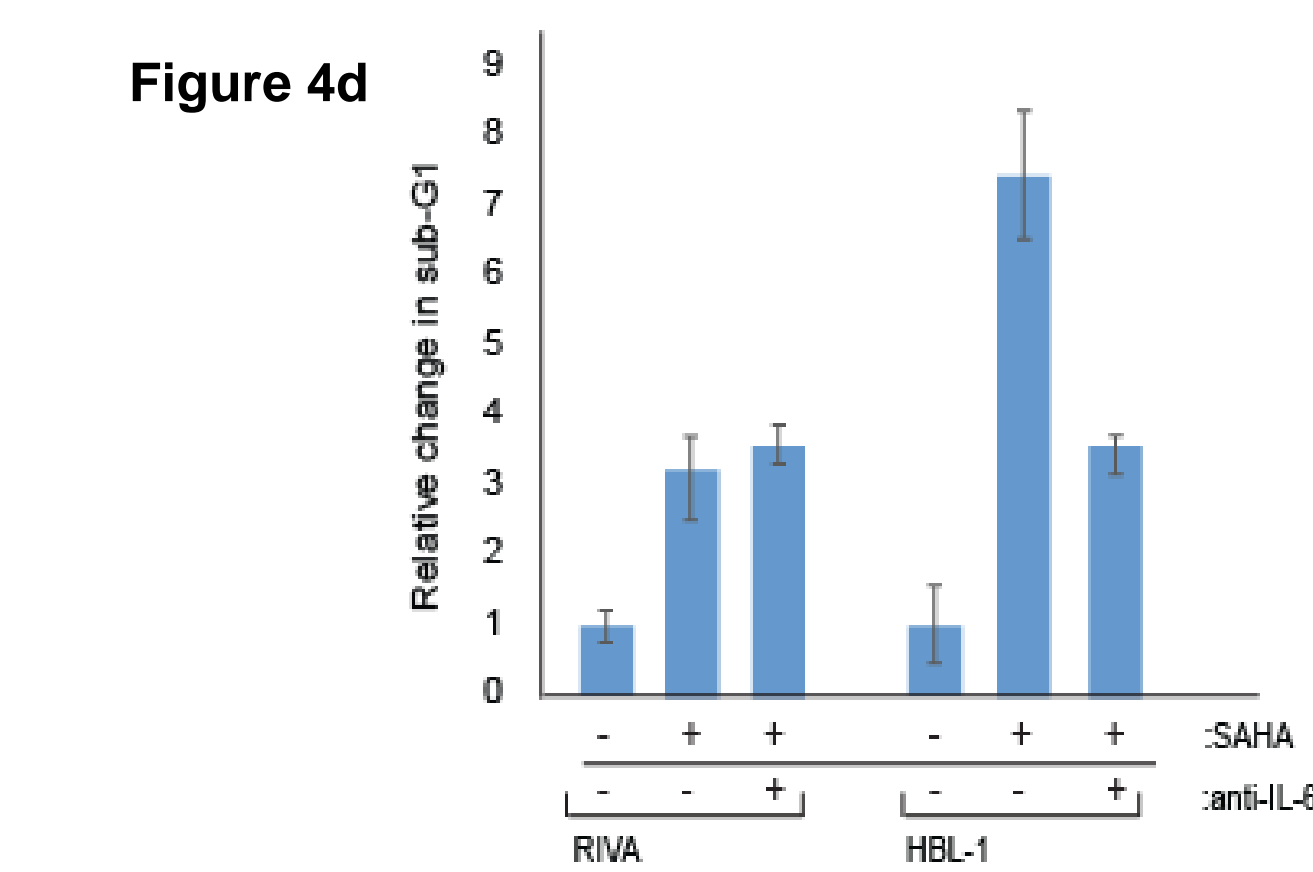
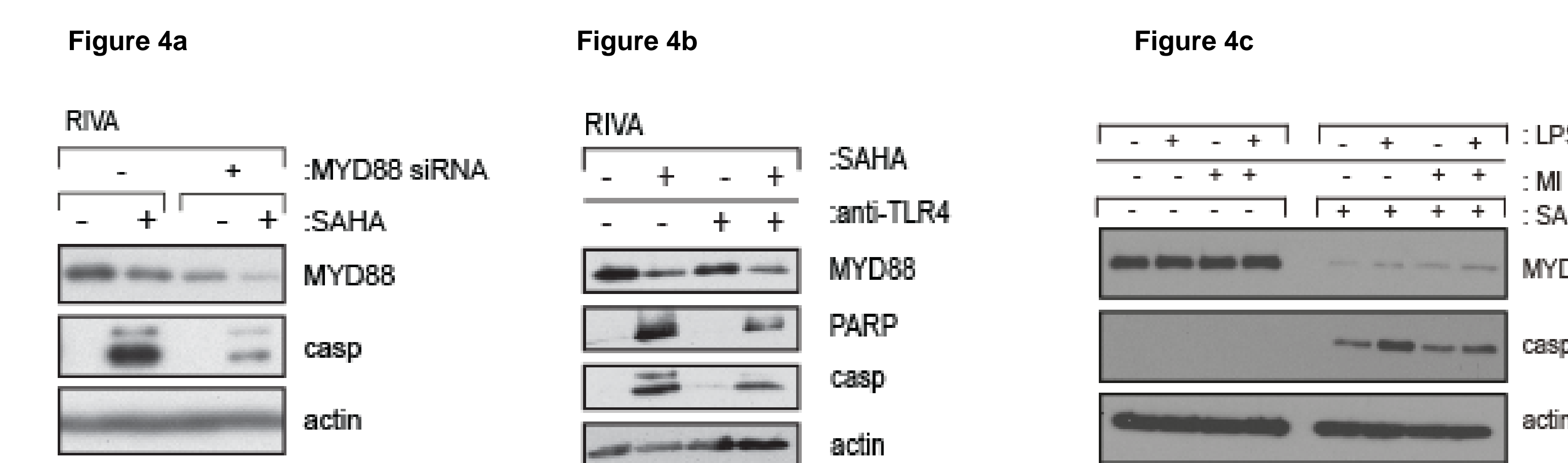
- Transient or stable expression of MyD88 in U2OS cells leads to increased NFκB activity as demonstrated by reporter assay (Figure 2a), and pro-inflammatory cytokine expression, in particular of IL6 (Figure 2b); co-expression of mutated MyD88 L265P augmented this effect (Figure 2c).



- In DLBCL lymphoma cell lines of the ABC subtype, those cells expressing endogenous wild-type MyD88 (RIVA) are less sensitive to HDI treatment compared to those cells carrying a heterozygous MyD88 L265P mutation (HBL-1) (Figure 3a). MyD88-mutated cells again showed higher levels of IL6 expression on cytokine analysis (Figure 3b).



- Transient knockdown of endogenous MyD88 in ABC DLBCL cells resulted in decreased HDI sensitivity (Figure 4a), as did disruption of the TLR/MyD88/NFκB signalling pathway by blocking either the TLR receptor (Figure 4b), MyD88 aggregation (Figure 4c) or IL6 secretion (Figure 4d).



- Immunohistochemical analysis of tumour tissue microarrays from patients with DLBCL showed higher expression of MyD88 compared to patients with prostate or colorectal carcinoma (Figure 5).

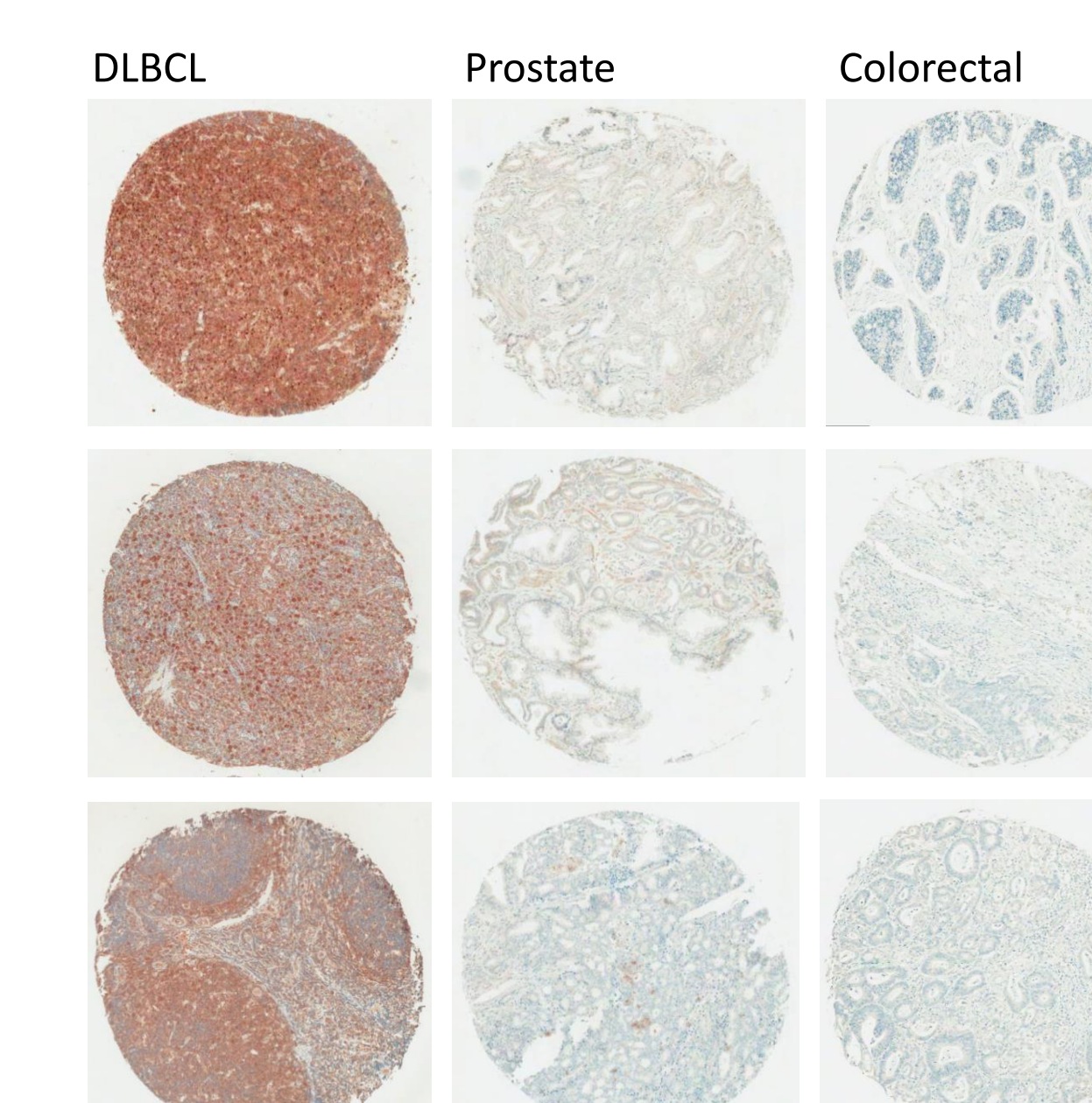
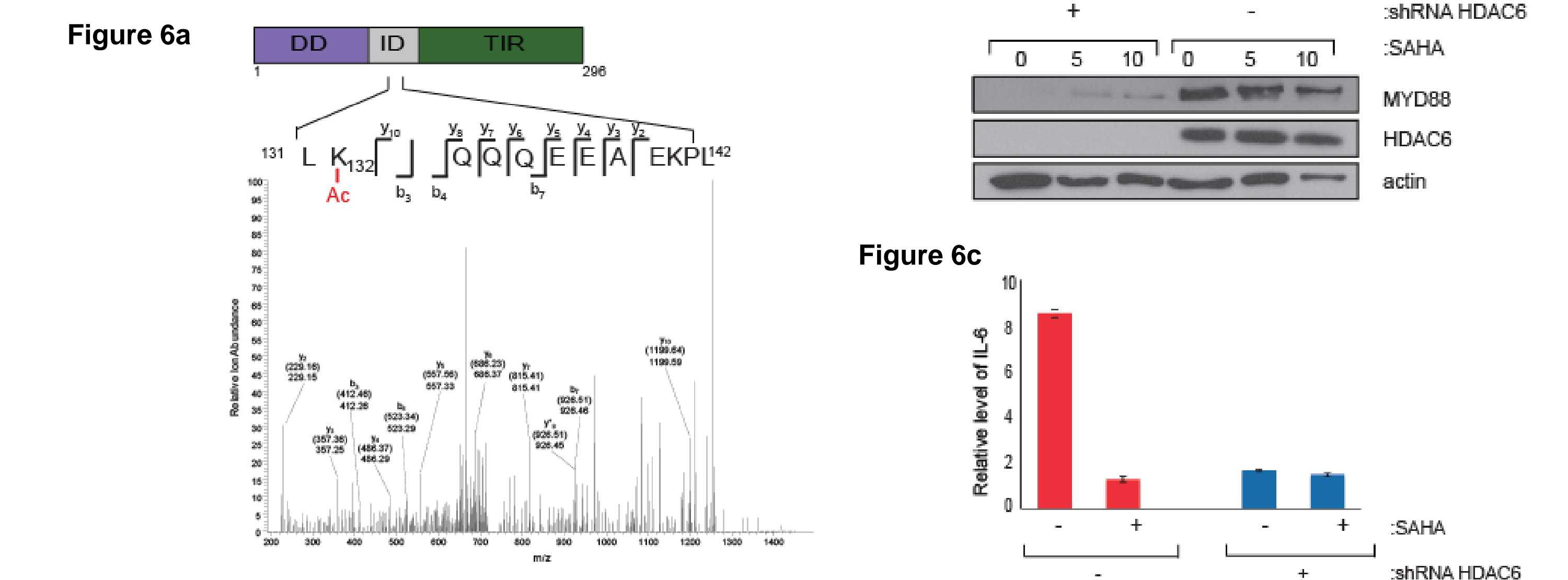


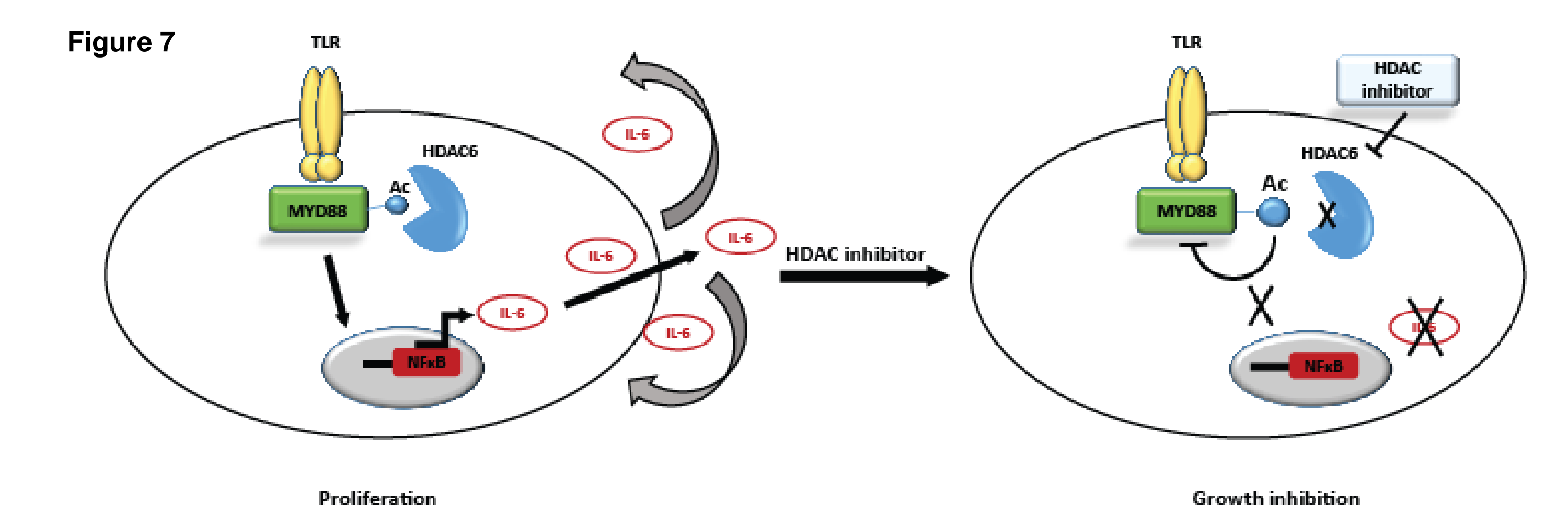
Figure 5

- MyD88 appears to be acetylated at residue K132, as shown by mass spectroscopy (Figure 6a), and a cytoplasmic histone deacetylase such as HDAC6 is a likely candidate for direct deacetylation of MyD88. Silencing HDAC6 reduced MyD88 levels in U2OS cells (Figure 6b) as well as IL6 levels (Figure 6c).



Conclusions

In cultured cells, high levels of MyD88 expression are associated with increased sensitivity to HDAC inhibition, whereas a low level of expression coincides with decreased sensitivity. We hypothesize that MyD88, in its role as adaptor protein in TLR signalling, plays an important role in regulating the expression and autocrine action of proinflammatory cytokines such as IL6 which contribute to the sensitization of cancer cells to HDI (Figure 7). The central role played by MyD88 in this mechanism highlights the importance of TLR signalling and innate immunity, and more generally the inflammatory properties of the extra-cellular tumour microenvironment in mediating the effects of HDAC inhibitors on malignant cells. Therefore, both expression level of MyD88 and MyD88 mutation status could serve as a potential biomarker for HDI sensitivity in patient selection.



References

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For **Methods** please see: New M et al: TLR adaptor protein MyD88 mediates sensitivity to HDAC inhibitors via a cytokine-dependent mechanism. *Cancer Research* 2016 Oct 12.

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Conflicts of interest There are no relationships to disclose.

