HDAC6 and Ubp-M BUZ Domains Recognize Specific C-Terminal Sequences of Proteins†

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Supporting Information

Figure S1. Representative plots showing the binding of FITC-labeled peptides to Ubp-M and HDAC6 BUZ domains (by fluorescence anisotropy). The Ubp-M BUZ domain contained an N-terminal $(His)_6$ tag, whereas the HDAC6 BUZ domain contained an N-terminal GST fusion tag.

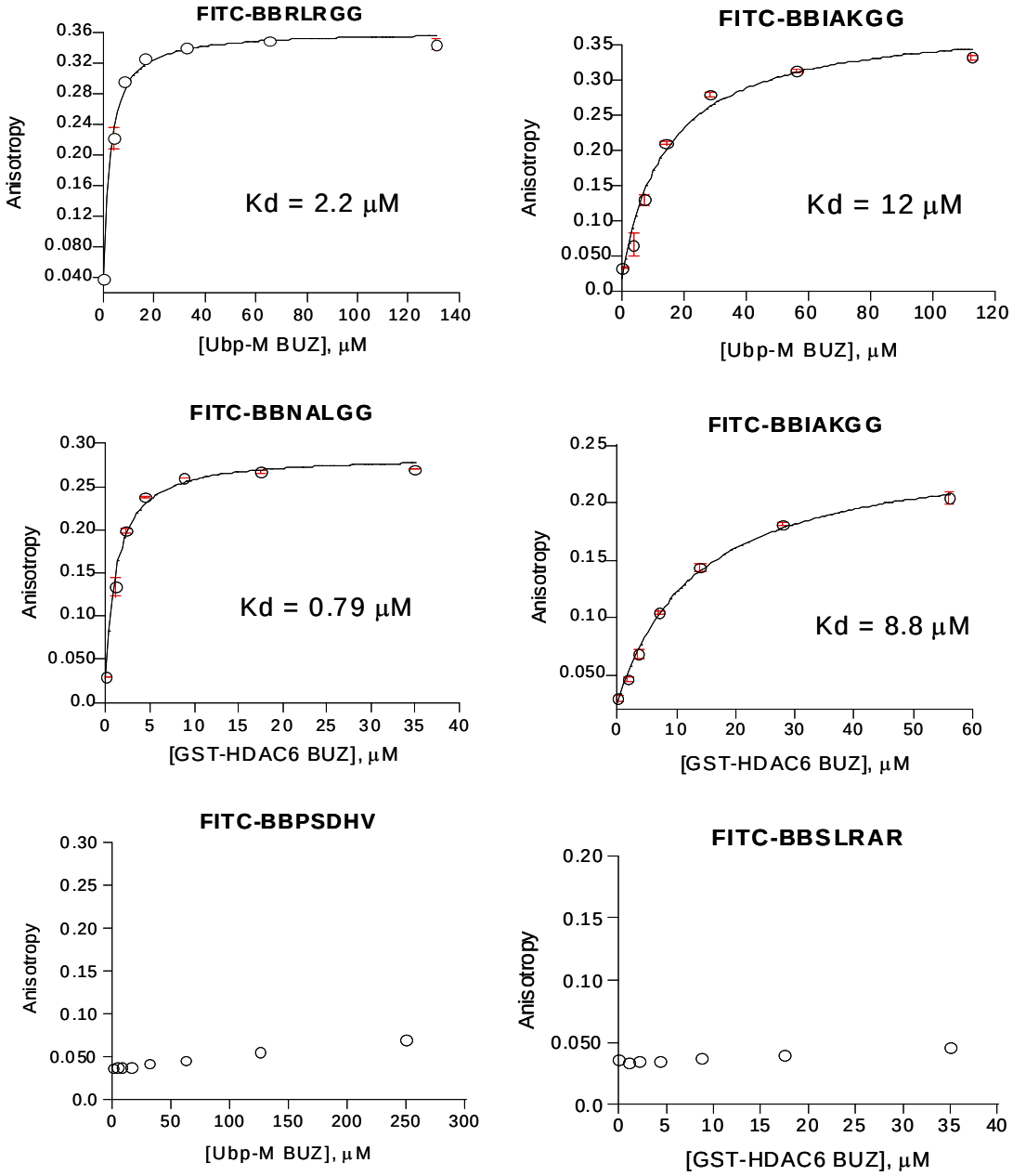

Figure S2. Fluorescence anisotropy assay showing the competition between FITC-labeled peptides (FITC-BBRGMGG) and full-length human ubiquitin for binding to GST-HDAC6 BUZ domain. The peptide and BUZ domain were kept at fixed concentrations (92 nM and 2.6 μM, respectively), while the concentration of ubiquitin was varied (0–30 μM).

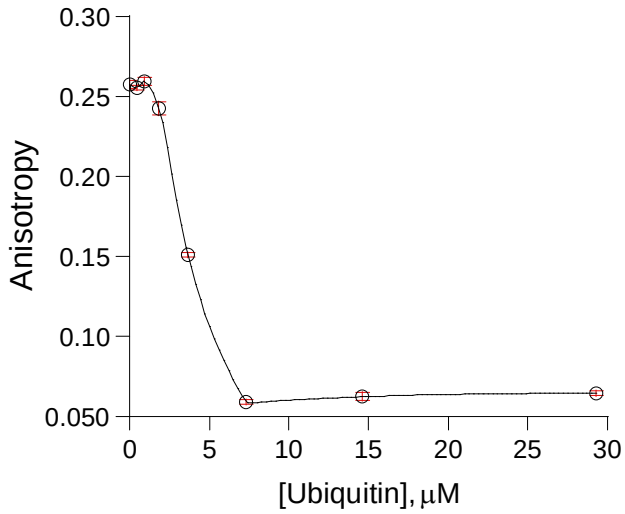

Figure S3. Fluorescence anisotropy assay showing the binding of peptide FITC-BBLQDGF (90 nM in all experiments) to GST. (a) Binding of the peptide to GST-HDAC6 BUZ domain; (b) plot of anisotropy against HDAC6 BUZ domain concentration (no GST tag); (c) Binding of the peptide to GST-Abl2 SH2 domain; and (d) competition between peptide FITC-BBLQDGF (90 nM) and glutathione (0–1000 μM) for binding to GST-Abl2 SH2 domain (33 μM). Taken together, the data indicate that peptide LQDGF does not bind to the BUZ (or SH2) domain with significant affinity, but instead binds to the active site of GST.

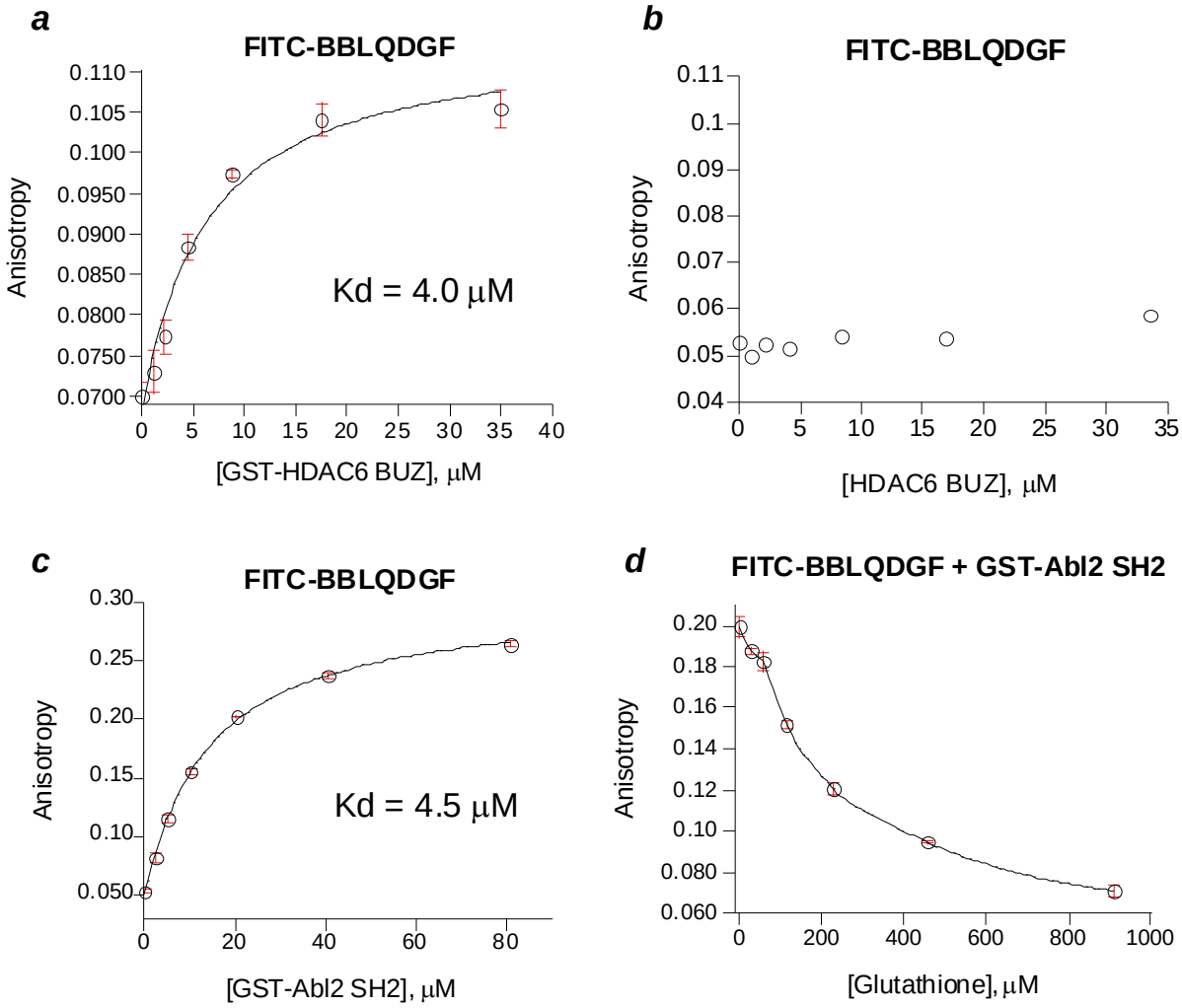

Figure S4. Fluorescence anisotropy assay showing the interaction between the C-terminal peptides of histone H4 (YGFGG) and FAT10 (YCIGG) and Ubp-M and HDAC6 BUZ domains.