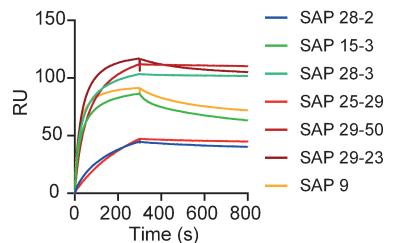
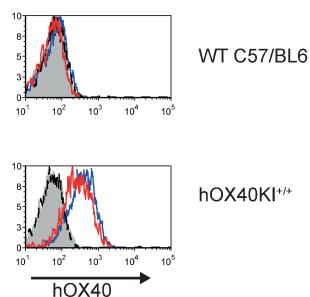


A

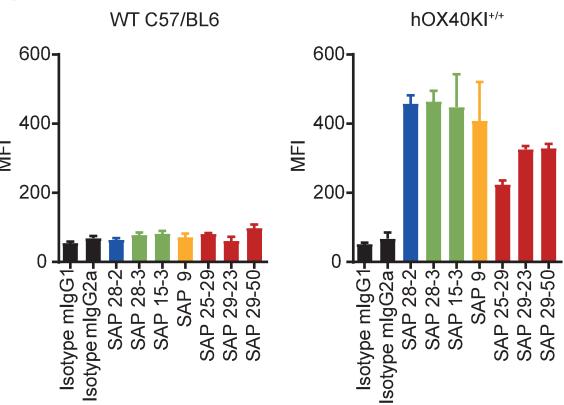
Antibody	Parental Isotype	Domain binding	$k_a$ (M <sup>-1</sup> s <sup>-1</sup> )	$k_d$ (s <sup>-1</sup> )	KD (M)
SAP 28-2	mIgG1	1	$4.2 \times 10^4$	$2.57 \times 10^{-4}$	$6.1 \times 10^{-9}$
SAP 28-3	mIgG1	2	$2.5 \times 10^5$	$0.46 \times 10^{-4}$	$1.84 \times 10^{-10}$
SAP 15-3	mIgG1	2	$14.3 \times 10^4$	$4.68 \times 10^{-4}$	$3.3 \times 10^{-9}$
SAP 9	mIgG2a	3	$50.9 \times 10^4$	$3.98 \times 10^{-4}$	$7.82 \times 10^{-10}$
SAP 25-29	mIgG1	4	$2.56 \times 10^4$	$1.31 \times 10^{-4}$	$5.1 \times 10^{-9}$
SAP 29-50	mIgG1	4	$13.8 \times 10^4$	$0.62 \times 10^{-4}$	$4.49 \times 10^{-10}$
SAP 29-23	mIgG2a	4	$29 \times 10^4$	$1.81 \times 10^{-4}$	$6.24 \times 10^{-10}$



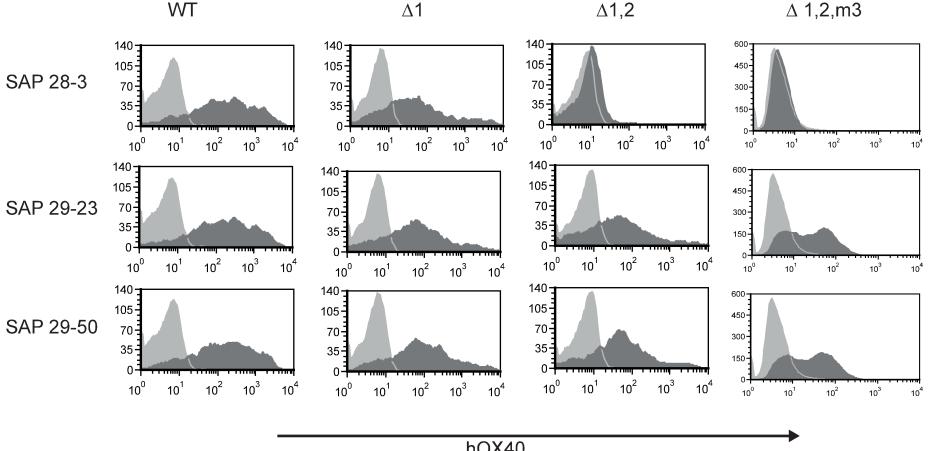
B



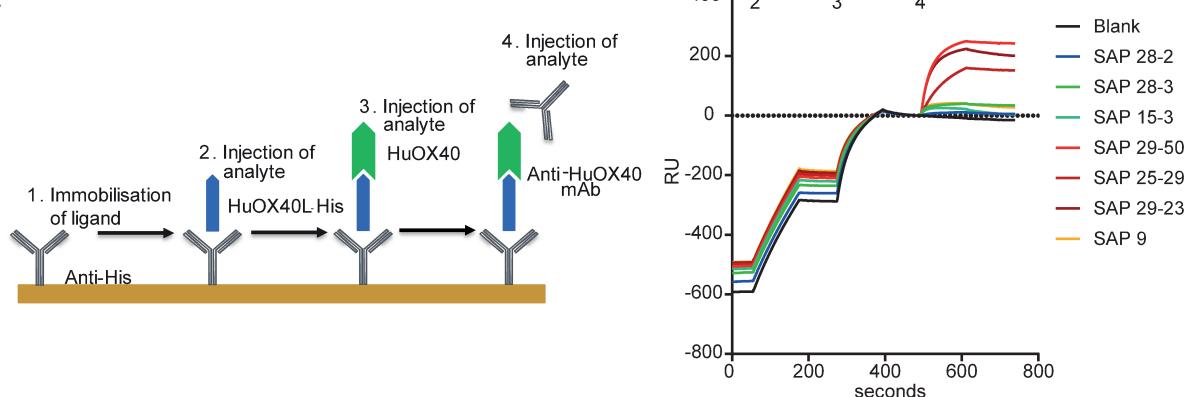
C



D



E



Supplementary Figure 2. Characterisation of anti-hOX40 mAb. A. SPR analysis of anti-hOX40 mAb binding and affinity. B. Binding of anti-hOX40 mAb SAP28-2 mlgG1(blue line) and SAP 29-23 mlgG2a (red line) to activated Tregs (cells activated with  $\alpha$ CD3 (0.1 $\mu$ g/ml) and  $\alpha$ CD28 (5 $\mu$ g/ml) for 24 hours) from WT (top panel) or hOX40KI<sup>+/+</sup> mice (bottom panel). Isotype controls shown as filled grey histogram (mlgG1) and black dotted line (mlgG2a). C. Binding of all parental anti-hOX40mAb to activated Tregs (cells activated as in B) isolated from WT (left bar chart) or hOX40KI<sup>+/+</sup> mice (right bar chart) n=3. D. Binding of FITC-labelled anti-hOX40 mAb to hOX40 domain mutants. E. Schematic and full SPR analysis of anti-hOX40mAb binding in the presence of hOX40L.